



PHASE CONTRAST TOMOGRAPHY OF
HUMAN TISSUE WITH A LIQUID-METAL
 μ CT X-RAY SOURCE

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Having discovered the existence of a new kind of rays,
I of course began to investigate what they would do....

It soon appeared from tests that the rays had
penetrative power to a degree hitherto unknown.

— Wilhelm Conrad Röntgen [[109](#)]

PHASE CONTRAST TOMOGRAPHY OF HUMAN TISSUE WITH A LIQUID-METAL μ CT X-RAY SOURCE

BY

CHRISTOPHER BOATENG

ENGLISH ABSTRACT

Formalin-fixed paraffin-embedded (FFPE) tissue blocks are widely used in biomedical research, as the paraffin matrix preserves the tissue architecture and allows for precise sectioning for histology. However, this process is labor intensive, destructive, and inherently two-dimensional. Virtual histology using X-ray computed tomography (CT) offers a non-destructive alternative, but imaging FFPE samples has been challenging because paraffin has X-ray interaction properties similar to those of embedded tissue, and conventional microfocus X-ray tubes often lack sufficient flux. As a result, most FFPE imaging has been restricted to synchrotron facilities.

In this thesis, the potential of a liquid-metal jet X-ray source is investigated for laboratory-based imaging of FFPE tissue blocks using X-ray phase-contrast imaging (PCI). Optimization and investigation of system geometry, exposure conditions, empty field correction assessments, source settings, and detector characterization were performed. Performance was evaluated in terms of effective resolution, quantitative metrics such as contrast-to-noise ratio (CNR) and signal-to-noise ratio (SNR), and visual assessment, and compared with results from conventional microfocus sources.

The findings presented indicate that the liquid-metal jet source provides enhanced contrast capabilities at a resolution on the micrometer scale, making it well-suited for CT applications involving FFPE samples. This is primarily attributed to the high-brilliance photon flux that these types of source are able to achieve. The results underscore the applicability of liquid-metal jet sources for imaging histological samples and suggest their role in expanding the reach of laboratory-based X-ray imaging to routine application. This potential can notably be extended when such sources are paired with optimal spectral properties for such application, state-of-the-art detectors, and advanced phase and reconstruction algorithms.

By carefully tuning the source spectrum and pairing with highly efficient detectors, exposure times could be reduced by a large factor, allowing high-throughput imaging and further broadening the applicability and accessibility of virtual histology in laboratory settings.

Keywords: formalin-fixed paraffin-embedded (FFPE), cone beam computed tomography (CBCT), phase-contrast imaging (PCI), propagation based imaging (PBI), liquid-metal jet source, virtual histology, X-rays, Bronnikov-aided correction (BAC), single material object (SMO), modulation transfer function (MTF), noise power spectrum (NPS), Feldkamp-Davis-Kress algorithm (FDK), contrast-to-noise ratio (CNR), signal-to-noise ratio (SNR), Fourier shell correlation (FSC).

FASECONTRAST-TOMOGRAFIE VAN MENSELIJK WEEFSEL MET EEN LIQUID-METAL μ CT RÖNTGENBRON

DOOR
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NEDERLANDSTALIG ABSTRACT

Formaline-gefixeerde, paraffine-ingebbede (FFPE) weefselblokken worden veel gebruikt in biomedisch onderzoek, dit omdat de paraffinematrix de structuur behoudt en nauwkeurige secties voor histologie mogelijk maakt. Dit proces is echter arbeidsintensief, destructief en verzamelde informatie inherent tweedimensionaal. Virtuele histologie met behulp van röntgenstralen en computertomografie (CT) bieden een niet-destructief alternatief aan, maar het in beeld brengen van FFPE-monsters is uitdagend doordat paraffine vergelijkbare interactie eigenschappen heeft als het weefsel, en conventionele microfocus bronnen vaak onvoldoende flux leveren voor dergelijke toepassingen. Daardoor bleef de meeste FFPE-beeldvorming vaak beperkt tot synchrotronfaciliteiten.

In deze thesis wordt het potentieel onderzocht van een liquid-metal jet röntgenbron voor laboratoriumgebaseerde beeldvorming van FFPE-weefselblokken met behulp van fasecontrast (PCI) technieken. Optimalisatie en analyse van de systeemgeometrie, correcties voor flat-fields, broninstellingen en detector karakterisatie werden uitgevoerd. De prestaties werden geëvalueerd in termen van resolutie, kwantitatieve maatstaven zoals contrast-ruisverhouding (CNR) en signaal-ruisverhouding (SNR) en visuele beoordeling. Deze resultaten worden dan verder vergeleken met resultaten bekomen met meer conventionele microfocus bronnen.

De resultaten tonen aan dat de liquid-metal jet bron verbeterde contrast biedt met een resolutie op de micrometerschaal, waardoor deze bijzonder geschikt is voor CT-toepassingen met FFPE-monsters. Dit is voornamelijk toe te schrijven aan de bijzonder hoge helderheid van het fotonenflux die deze bronnen kunnen bereiken. De bevindingen drukken de toepasbaarheid van liquid-metal jet bronnen voor de beeldvorming van histologische monsters uit. Dit potentieel kan aanzienlijk worden vergroot wanneer dergelijke bronnen worden gecombineerd met optimale spectrale eigenschappen voor de routine toepassing, geavanceerde detectors en moderne fase- en reconstructie-algoritmen.

Door het zorgvuldig afstemmen van het bronspectrum en het combineren met zeer efficiënte detectors kunnen de belichtingstijden aanzienlijk worden verkort, wat de toepasbaarheid en toegankelijkheid van virtuele histologie in laboratoriumomgevingen verder vergroot.

Kernwoorden: formaline-gefixeerde paraffine-ingebbede (FFPE), cone beam computertomografie (CBCT), fasecontrastbeeldvorming (PCI), propagatie-gebaseerde beeldvorming (PBI), liquid-metal jet bron, 3D virtuele histologie, röntgenstralen, Bronnikov-aided correction (BAC), single material object (SMO), Modulatie transferfunctie (MTF), Ruisvermogen spectrum (NPS), Feldkamp-Davis-Kress algoritme (FDK), Fourier shell correlatie (FSC), contrast-ruisverhouding (CNR), signaal-ruisverhouding (SNR).

FASEKONTRAST-TOMOGRAPHIE VON MENSCHLICHEM GEWEBE MIT EINER FLÜSSIGMETALL μ CT RÖNTGENQUELLE

VON

CHRISTOPHER BOATENG

DEUTSCHE ZUSAMMENFASSUNG

Formalin-fixierte, paraffineingebettete (FFPE) Gewebelöcke werden häufig in der bio-medizinischen Forschung eingesetzt, da die Paraffinmatrix die Gewebestruktur erhält und präzise Schnitte für die Histologie ermöglicht. Dieser Prozess ist jedoch arbeitsintensiv, destruktiv und die gewonnenen Informationen sind inhärent zweidimensional. Virtuelle Histologie mit Hilfe von Röntgenstrahlen und Computertomographie (CT) bietet eine nicht-destruktive Alternative, doch die Abbildung von FFPE-Proben ist herausfordernd, da Paraffin ähnliche Wechselwerkeigenschaften wie das Gewebe besitzt und konventionelle Mikrofokusquellen für solche Anwendungen oft nicht genügend Fluss liefern. Daher blieb die meiste FFPE-Bildgebung bislang auf Synchrotronanlagen beschränkt.

In dieser Arbeit wird das Potenzial einer Liquid-Metal Jet Quelle für die laborbasierte Bildgebung von FFPE-Gewebelöcken mithilfe von Phasenkontrast-Techniken (PCI) untersucht. Es wurden Optimierungen und Analysen der Systemgeometrie, Flat-Field-Korrekturen, Quelleneinstellungen und Detektorkarakterisierung durchgeführt. Die Leistung wurde in Bezug auf Auflösung, quantitative Kennzahlen wie Kontrast-Rausch-Verhältnis (CNR) und Signal-Rausch-Verhältnis (SNR) sowie durch visuelle Beurteilung evaluiert. Diese Ergebnisse wurden anschließend mit Resultaten konventioneller Mikrofokusquellen verglichen.

Die Ergebnisse zeigen, dass die Liquid-Metal Jet Quelle verbesserten Kontrast bei einer Auflösung im Mikrometerbereich bietet und sich daher besonders für CT-Anwendungen mit FFPE-Proben eignet. Dies ist hauptsächlich auf die besonders hohe Brillanz des Photonenflusses zurückzuführen, den diese Quellen erreichen können. Die Befunde unterstreichen die Anwendbarkeit von Liquid-Metal Jet Quellen für die Bildgebung histologischer Proben. Dieses Potenzial kann erheblich gesteigert werden, wenn solche Quellen mit optimalen spektralen Eigenschaften für Routineanwendungen, hochentwickelten Detektoren sowie modernen Phasen- und Rekonstruktionsalgorithmen kombiniert werden.

Durch die sorgfältige Abstimmung des Quellenspektrums und die Kombination mit hocheffizienten Detektoren können die Belichtungszeiten deutlich verkürzt werden, was die Anwendbarkeit und Zugänglichkeit der virtuellen Histologie in Laborumgebungen weiter erhöht.

Schlüsselwörter: formalin-fixierte Paraffinblöcke (FFPE), Cone Beam Computertomographie (CBCT), Propagation-basierte Bildgebung (PBI), Liquid-Metal Jet Quelle, 3D virtuelle histologie, Röntgenstrahlen, Phasenkontrast-Bildgebung (PCI), Bronnikov-aided correction (BAC), single material object (SMO), Modulationsübertragungsfunktion (MTF), Rauschleistungsdichtespektrum (NPS), Feldkamp-Davis-Kress Algorithmus (FDK), Fourier Shell Korrelation (FSC), Kontrast-Rausch-Verhältnis (CNR), Signal-Rausch-Verhältnis (SNR).

PUBLICATIONS

Throughout this thesis, the core ideas and fundamental concepts developed during this thesis are closely related to the following publications. Some figures, tables, and statements can be traced back to or were inspired by the work in these papers.

- [1] Matthieu Boone. „New imaging modalities in high resolution X-ray tomography.“ eng. ISBN: 9789461971494. dissertation. Ghent University, 2013. URL: <http://hdl.handle.net/1854/LU-4157322> (visited on 12/21/2024).
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STATEMENT ON THE USE OF AI TOOLS

During the course of this thesis, Large Language Models (LLM) such as *Qwen*, *ChatGPT*, *Deepseek*, and *Claude* were used to generate code snippets, identify bugs and errors in codes, refine computational codes used in analysis, refine and improve codes based on feed drafts. LLM tools such as the *Grammarly* and *Writefull's* models built into *Overleaf*, were used to refine draft texts, correct spelling errors, point out punctuation errors, and check grammar effectively. All other contents, research design and implementation, data analysis, and scientific interpretations are my own, and no figure or plot were generated by any diffusion model. The use of AI tools was supplementary, serving to improve efficiency, and did not replace scientific thinking, implementation, or decision making.

Christopher Boateng
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ACRONYMS

CT	computed tomography
CNR	contrast-to-noise ratio
FPD	flat-panel detector
RT	Radon transform
SNR	signal-to-noise ratio
ROI	region of interest
FSC	Fourier shell correlation
PSF	point spread function
MTF	modulation transfer function
OTF	optical transfer function
LSF	line spread function
ESF	edge spread function
JuLiA	Just a Liquid Anode
NPS	noise power spectrum
MSE	mean square error
CTF	contrast transfer function
FWHM	full width at half maximum
FOV	field of view
TIE	transport of intensity equation
MBA	modified bronnikov algorithm
BAC	Bronnikov-aided correction
TEM	transmission electron microscopy
LOS	line of sight
CBCT	cone beam computed tomography
FBP	filtered back projection
FDK	Feldkamp-Davis-Kress algorithm
UGCT	Ghent University Centre for X-ray Tomography
HECTOR	High-Energy CT system Optimized for Research
HBCG	Heart & Brain Centre Göttingen
FFPE	formalin-fixed paraffin-embedded
fps	frames per second
MONA	MOlybdäN Anode
SART	simultaneous algebraic reconstruction technique

SOD	source-to-object distance
SDD	source-to-detector distance
FST	Fourier slice theorem
PCI	phase-contrast imaging
PCT	phase-contrast tomography
SMO	single material object
PBI	propagation based imaging
ODD	object-to-detector distance
FT	Fourier transform
TLE	temporal lobe epilepsy
MRI	magnetic resonance imaging

Part I

INTRODUCTION

INTRODUCTION

1.1 BACKGROUND

Histology, also called microscopic anatomy, is a branch of biology that delves into the microscopic structure of biological tissues [97]. It is derived from the Greek words *histos* (tissue) and *logos* (study) and aims to link the microscopic structure of the tissue to its function [29, 97]. With the development of the light microscope in the early 17th century, histology has emerged as a crucial tool in medical diagnostics. This is particularly significant in the disciplines of pathology and oncology, where it plays an indispensable role.

In the context of histological examination, tissue samples obtained by surgical means or from a post mortem autopsy are sectioned into thin slices, ranging between 4 μm and 10 μm , and examined under a microscope [43]. Prior to sectioning, tissue samples are typically fixed in formalin, dehydrated in a series of ethanol, and cleared in xylene. The resulting fixed tissue is then embedded in paraffin wax, resulting in a formalin-fixed paraffin-embedded (FFPE) sample, which provides support and preserves tissue structure [43].

In clinical and biomedical environments, histology not only exposes the cytoarchitecture of tissues but also allows the diagnosis of diseases and guides the decision making for treatments. The standard histological procedure, in conjunction with tissue and cell-specific staining methods used in histochemical and immunohistochemical techniques, forms the foundation of precision medicine and diagnostics [38, 82, 97]. Despite all the benefits, robustness, and reliability associated with conventional histology, this technique is inherently destructive, requires intensive human labor and effort, and relies heavily on the subjective judgment of a pathologist due to the qualitative rather than quantitative nature of histological assessments. In addition, the use of 2D histological sections provides only limited insight into the 3D architecture of the tissue, which can further introduce interpretational biases and suffers from tissue connectivity.

To overcome these limitations and constraints, X-ray phase-contrast tomography (PCT) has emerged as an advantageous tool for quantitative imaging and fully 3D virtual histology, which is critical for unraveling the physiological and pathological mechanisms of biological tissues at the cellular and subcellular levels. This enhances the understanding of the structure and functional capabilities of biological matter on various scales.

This area of imaging uses advanced methods that involve highly coherent synchrotron radiation, X-ray nano optics [62], along with advanced phase retrieval algorithms [31, 39], to capture three-dimensional slices of tissue microstructures. This approach negates the requirement for microtome sectioning, thus preserving the integrity FFPE samples unlike conventional histology, while simultaneously providing comprehensive volumetric data on tissue morphology.

Given that the workflow is non-destructive, and fully compatible with standard clinical histological processing, correlative studies can be performed. Studies with synchrotron radiation facilities have provided enormous support for the development and

research of neurodegenerative studies, cancer, cardiovascular disease, and more. Ultimately, virtual histology may be used pre-sectioning to guide region of interest (ROI) selection and imaging, within conventional histology.

1.1.1 *Challenges of virtual histology*

Despite all the promising potential, aforementioned advantages and successes, virtual histology of FFPE samples using X-rays still faces challenges, mainly related to imaging requirements in laboratory settings. The surrounding paraffin wax, while suitable for the morphological preservation of tissue structures, exhibits a relatively high absorption index. This characteristic, paired with its naturally low contrast relative to the embedded tissue, presents challenges when FFPE samples are imaged using standard X-ray imaging systems and techniques.

In order to mitigate these contrast limitations, virtual histology of formalin-fixed paraffin-embedded (FFPE) samples necessitates systems capable of providing relatively high photon flux and coherence, and possessing appropriate spectral characteristics. High photon flux is essential to achieve low noise and high signal, leading to adequate SNR and CNR within manageable acquisition times.

Historically, these demanding requirements could only be met at synchrotron facilities because of their intense, highly collimated X-ray beams with exceptionally high brightness and coherence. Coupled with advanced phase algorithms and imaging techniques, these setups have demonstrated success in ROI imaging of FFPE blocks, producing quantitative datasets supporting studies in various biomedical fields. However, reliance on synchrotron facilities is neither guaranteed nor practical for widespread clinical adoption. Synchrotron facilities are known for their high operational cost, limited access, and high level of expertise for operation, making them inaccessible for routine clinical applications. Despite the numerous accolades, the beam width is typically restricted, making imaging of whole FFPE blocks often impractical.

1.1.2 *Promises of virtual histology*

In recent years, there have been significant advancements in laboratory X-ray imaging systems and CBCT, which are providing technological solutions to overcome the limitations traditionally associated with the reliance on synchrotron sources. Modern X-ray sources, detectors, and techniques are providing means to explore imaging regimes and performance, encouraging the exploration of lab-based virtual histology.

With the advent of liquid-metal jet X-ray sources, rotational anode, and advanced focusing optics, relatively high-brilliance X-ray photon beams are gradually becoming more available in laboratory X-ray systems. Simultaneous improvements in detector technology, with micron/nano scale resolution and field of view (FOV), and efficiency such as flat-panel detector (FPD), CMOS, CCD, etc, have improved the detection capabilities for low-contrast biological samples.

The implementation of PBI techniques, such as X-ray PCI, in laboratory settings has further enhanced the visibility of features that would otherwise be difficult to differentiate using conventional imaging methods. Together, these developments suggest that high-quality virtual histology of FFPE samples may be achievable using high-brilliance

lab-based X-ray systems, potentially paving the way for widespread clinical and laboratory adoption.

1.2 OBJECTIVE

The objective of this work is to develop a framework for 3D virtual histology, investigating the feasibility and optimization of a high-brilliance liquid-metal jet system. This study addresses the potential and the gap of laboratory systems that need to be bridged to allow accessibility and routine application. This assessment includes a systematic evaluation of the performance of the laboratory X-ray system and the performance of benchmark experiments on whole FFPE human tissue samples in the realm of X-ray PCI.

1.3 THESIS OUTLINE

The next part of this thesis provides the theoretical foundation for the practical implementation developed in the third part. It introduces the principles of X-ray interaction with matter, from classical electromagnetism to the famous Beer-Lambert law to microscopic models describing interactions such as attenuation and scattering.

The mathematical framework required for computed tomography (CT), specifically phase-contrast tomography (PCT), will be introduced in chapter 3, along with an overview of a variety of phase retrieval methods. In Chapter chapter 3, common unwanted signals in cone beam computed tomography (CBCT), referred to as *artifacts*, are discussed. The final chapter of this part addresses lab-based X-ray generation, spectral characteristics, data correction pipelines, and the working of modern-day X-ray detectors, as well as imaging metrics that will be used throughout this thesis.

Topics covered in this part of the thesis, especially chapter 2, may be familiar to experienced readers and can be omitted without loss of continuity. However, it is recommended as a useful reference and provides an overview of relevant material and a context for the empirical setting in Part III.

The third part of the thesis is dedicated to the empirical realization of concepts introduced in Part II. The first chapter describes the liquid-metal jet system used in this thesis, along with other setups, detector characterization, and data acquisition protocols. The second chapter focuses on optimization strategies and the rationale behind the selection of certain sources and geometric parameters.

The remaining chapters present applications and potential of phase-contrast tomography (PCT) for 3D virtual histology of human tissue. These include imaging of samples from physiological systems such as the central nervous system, circulatory system, respiratory system, and reproductive system. This final section demonstrates the practicality of lab-based FFPE X-ray computed tomography (CT) scanners and hints at improvements that can be pursued in the future.

The very last part of this thesis is the appendix, which contains supplementary material, including the mathematical background of Fourier analysis, image processing in the framework of linear imaging system, and techniques for modeling uncertainties and curve fitting. These materials are not essential for the main body of the thesis and serve as a helpful reference for interested readers.

Part II

PRELIMINARIES

X-RAY INTERACTION WITH MATTER

This chapter provides a brief overview of the interaction of X-ray radiation with matter and general concepts encompassing propagation based imaging (PBI). The current chapter is divided into three main sections. The first section addresses the fundamental principle of photon interaction with matter in the realm of classical electromagnetism and general concepts of PBI. The second section discusses the Beer-Lambert law and its implications in X-ray imaging. The last section delves into the macroscopic description of matter with X-rays or a generic electromagnetic field in the realm of quantum electrodynamics.

2.1 PROPAGATION BASED IMAGING

The well-known Maxwell equations are a set of equations that combine the nature of electric and magnetic phenomena, termed *electromagnetism*. This set of classical equations describes how electric and magnetic fields interact and propagate as waves in space. In differential form, this collection of fundamental laws is most generally given by [28, 51, 98]

$$\nabla \cdot \mathbf{D}(\mathbf{r}, t) = \rho(\mathbf{r}, t) \quad (2.1)$$

$$\nabla \cdot \mathbf{B}(\mathbf{r}, t) = 0 \quad (2.2)$$

$$\nabla \times \mathbf{E}(\mathbf{r}, t) + \frac{\partial \mathbf{B}}{\partial t}(\mathbf{r}, t) = 0 \quad (2.3)$$

$$\nabla \times \mathbf{H}(\mathbf{r}, t) - \frac{\partial \mathbf{D}}{\partial t}(\mathbf{r}, t) = \mathbf{J}(\mathbf{r}, t) \quad (2.4)$$

with $\mathbf{D}(\mathbf{r}, t)$ the electric displacement field, $\mathbf{B}(\mathbf{r}, t)$ the magnetic flux density, $\mathbf{E}(\mathbf{r}, t)$ the electric field, and $\mathbf{H}(\mathbf{r}, t)$ the magnetic induction. The right-hand side of the equation contains terms such as the free charge density $\rho(\mathbf{r}, t)$ and $\mathbf{J}(\mathbf{r}, t)$, the free current density. These equations may be regarded as an effect-cause relationship, with the left-hand side being the effect and the right-hand side the cause. In the absence of matter, the above equations are simplified to the familiar Maxwell equations in free space, which are obtained by the following substitutions

$$\rho(\mathbf{r}, t) = 0, \quad \mathbf{J}(\mathbf{r}, t) = 0, \quad \mathbf{H}(\mathbf{r}, t) \rightarrow \frac{1}{\mu_0} \mathbf{B}(\mathbf{r}, t), \quad \text{and} \quad \mathbf{D}(\mathbf{r}, t) \rightarrow \epsilon_0 \mathbf{E}(\mathbf{r}, t) \quad (2.5)$$

with ϵ_0 and μ_0 , the permittivity and permeability of free space, respectively. In generic media, the above equations should be treated tensorially, since then permittivity and permeability are anisotropic scalar quantities, i.e.

$$D_i(\mathbf{r}, t) = \epsilon_0 \sum_j (\delta^{ij} + \chi_E^{ij}(\mathbf{r}, t)) E_j(\mathbf{r}, t), \quad B_i(\mathbf{r}, t) = \mu_0 \sum_j (\delta^{ij} + \chi_M^{ij}(\mathbf{r}, t)) H_j(\mathbf{r}, t) \quad (2.6)$$

with χ_E^{ij} and χ_M^{ij} the electric and magnetic susceptibility tensors, respectively [36].

Leaving aside special media and polarization effects altogether, while assuming linear isotropic, static, and non-magnetic media, a *scalar wave equation* can be derived [6, 28, 51, 61] from the Maxwell equations (equations (2.1) to (2.4)) with constraints (equation (2.6)), which produces the following

$$(\epsilon(\mathbf{r})\mu_0 \frac{\partial^2}{\partial t^2} - \nabla^2)\Psi(\mathbf{r}, t) = 0, \quad (2.7)$$

where $\Psi(\mathbf{r}, t)$ represents a generic field, which in this case is either a component of the electric field or the magnetic field and is referred to as *wave function*. The wave function $\Psi(\mathbf{r}, t)$, is typically considered a complex function of position ($\mathbf{r}$) and time (t), and thus in possession of an amplitude and phase, i.e. $\Psi(\mathbf{r}, t) = \sqrt{I(\mathbf{r}, t)} \exp(-i\phi(\mathbf{r}, t))$. The differential operator in equation (2.7) is commonly referred to as the *d'Alembert operator* $\square = (\epsilon(\mathbf{r})\mu_0 \frac{\partial^2}{\partial t^2} - \nabla^2)$ such that, in essence, the problem reduces to solving

$$\square E_j(\mathbf{r}, t) = 0, \quad \text{and} \quad \square B_j(\mathbf{r}, t) = 0,$$

with j an index that spans the three components of the electric and magnetic fields.

2.1.1 Spectral decomposition

The wave function can be decomposed into its energy or spectral components by the Fourier theory laid out in chapter A, which yields *spectrum* $\psi(\mathbf{r}, \omega)$. The frequency (ω) here is related to the energy of the field/photon (E_γ) by equation (2.8)

$$E_\gamma = \hbar\omega, \quad (2.8)$$

where $\hbar$ is the reduced Planck constant [89]. The decomposition[51] of the wave function in the spectral domain is explicitly given by equation (2.9). Note that the integral here is for all possible wave energies; however, this can be restricted to a specific bandwidth of energies (ΔE) needed to fully describe the wave field.

$$\Psi(\mathbf{r}, t) = \frac{1}{\sqrt{2\pi}} \int_0^\infty d\omega \psi(\mathbf{r}, \omega) \exp(-i\omega t) \quad (2.9)$$

The deployment of the d'Alembert operator (equation 2.7) on the spectrally decomposed wave function (equation 2.9) gives

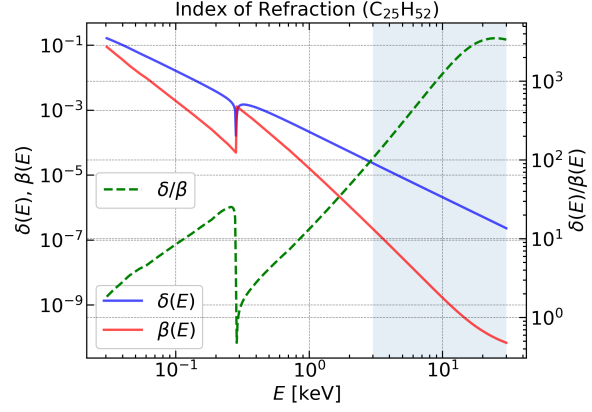
$$\int_0^\infty d\omega [(\nabla^2 + \epsilon(\mathbf{r})\omega^2\mu_0)\psi(\mathbf{r}, \omega)] \exp(-i\omega t) = 0,$$

which is respected if and only if the integrand is zero for all frequencies (ω), i.e.

$$(\nabla^2 + k^2 n_\omega^2(\mathbf{r}))\psi(\mathbf{r}, \omega) = 0, \quad (2.10)$$

where *index of refraction* is defined as $n_\omega^2(\mathbf{r}) = \epsilon_\omega(\mathbf{r})/\epsilon_0$ and after using the vacuum relation $\omega = kc = 2\pi c/\lambda$ [52]. This is the well-known *inhomogeneous Helmholtz equation* [51] and reduces to *Helmholtz equation* in vacuum with $n_\omega = 1$. There exist many solutions to the Helmholtz equation; however, the most common solution is the plane wave solution and the spherical wave solution [51].

Figure 1: Components of refraction of paraffin compound (red, blue) with chemical formula of $C_{25}H_{52}$ and density 0.875 g/cm^3 [11], with respect to the monochromatic photon energy (E) in units of keV, on a logarithmic scale. The fraction of δ to β , shown in green, is orders of magnitude of $10^2 - 10^3$ in the energy range of $3 - 30 \text{ keV}$ (blue region). This data is adopted from the CXRO X-ray database [69].



X-rays, in comparison with other forms of electromagnetic radiation, such as visible light, are characterized by their high energy and short wavelength, allowing them to penetrate matter more effectively. The index of refraction of matter can be expressed as a complex number that marginally deviates from unity [6]. Spelled out, this implies

$$n_{\omega}(\mathbf{r}) = 1 - \delta_{\omega}(\mathbf{r}) + i\beta_{\omega}(\mathbf{r}), \quad (2.11)$$

with the non-trivial real-valued term ($\delta_{\omega}(\mathbf{r})$) the *refractive index decrement* and imaginary-valued term ($\beta_{\omega}(\mathbf{r})$) the *extinction coefficient* of the medium, at frequency (ω) and position ($\mathbf{r}$) [6, 51, 61]. As the name suggests, $\beta_{\omega}(\mathbf{r})$ is responsible for the attenuation of the wave field as it propagates through the medium, while $\delta_{\omega}(\mathbf{r})$ leads to the phase shift of the wave field as it propagates through the medium. Both quantities are related to the composition and structures of the material as the dominant interaction mechanism of X-rays with matter occurs through the interaction with bound electrons and is further discussed in section 2.3. Although both quantities are related to the material's composition, the *refractive index of decrement* ($\delta_{\omega}(\mathbf{r})$) is typically much larger than the *extinction coefficient* ($\beta_{\omega}(\mathbf{r})$) for most biological samples, especially in the hard X-ray regime. As an illustration, fig. 1 shows the magnitude of these non-trivial components for a representative paraffin compound ($C_{25}H_{52}$) with density $\rho = 0.875 \text{ g/cm}^3$ [11], as function of the monochromatic photon energy (E). The ratio of the phase term to the absorbing term, shown in green, is of the order of $10^2 - 10^3$ in the energy range of $3 - 30 \text{ keV}$ [6, 61]. This trend generally applies to materials and suggests contrast formation in propagation based imaging (PBI) and phase contrast imaging, as will be discussed in chapter 3.

2.1.2 Spectrum of plane waves

Although pure plane waves are merely mathematical constructs and do not exist in nature, they are crucial in studying the eigenmodes of systems. Without loss of generality, waves are considered to propagate along the z direction from a plane to $z = 0$ to a parallel plane at $z > 0$. A solution to the Helmholtz equation (equation (2.10)) is thus a plane wave solution of the form

$$\psi^{\text{PW}}(\mathbf{r}, \omega) = \exp(i\mathbf{k}' \cdot \mathbf{r}) \quad (2.12)$$

with $\mathbf{k}'$ the wave vector in the given medium with refractive index $n_{\omega}(\mathbf{r})$ and $\mathbf{k}$ the wave vector in vacuum, i. e. $\mathbf{k}' = \mathbf{k}n_{\omega}$, with modulus $k' = |\mathbf{k}'|$. The distinction between

these two wave vectors is hereafter implicitly understood, and the difference in notation is dropped unless otherwise stated. The wave vector along the optical axis is given by $k_z = \sqrt{k^2 - k_\perp^2}$, with $\mathbf{k}_\perp = (k_x, k_y)$ the wave vector in the plane perpendicular to the optical axis. This allows for an alternative representation of the plane wave solution as

$$\psi^{\text{PW}}(\mathbf{r}, \omega) = \exp(i\mathbf{k}_\perp \cdot \mathbf{r}_\perp) \exp(iz\sqrt{k^2 - k_\perp^2}) =: \mathcal{H}(z) \exp(i\mathbf{k}_\perp \cdot \mathbf{r}_\perp). \quad (2.13)$$

The above derivation provides a framework in which a generic initial monochromatic wave field at $z = 0$ can be propagated to a plane at $z > 0$ by the *space propagator* $(\mathcal{H}(z))$, yielding $\psi(\mathbf{r}, \omega) = \mathcal{H}(z)\psi(\mathbf{r}_\perp, 0, \omega)$. The initial wave field is then further expressed as a decomposition of plane waves with coefficients $\hat{\psi}(\mathbf{k}_\perp, 0, \omega)$, which is the lateral Fourier transform $(\mathcal{F}_\perp)$ of the wave field at $z = 0$, i. e.

$$\psi(\mathbf{r}_\perp, 0, \omega) = \frac{1}{2\pi} \int d^2\mathbf{k}_\perp \hat{\psi}(\mathbf{k}_\perp, 0, \omega) \exp(i\mathbf{k}_\perp \cdot \mathbf{r}_\perp). \quad (2.14)$$

Putting all the above together, the wave field at $z > 0$ can be expressed as

$$\psi(\mathbf{r}, \omega) = \frac{1}{2\pi} \int d^2\mathbf{k}_\perp \mathcal{H}(z) \hat{\psi}(\mathbf{k}_\perp, 0, \omega) \exp(i\mathbf{k}_\perp \cdot \mathbf{r}_\perp),$$

and in terms of operators, is shortened to

$$\psi(\mathbf{r}, \omega) = \mathcal{F}_\perp^{-1} [\mathcal{H}(z) \mathcal{F}_\perp [\psi](\mathbf{k}_\perp, 0, \omega)] =: \mathcal{D}[\psi](\mathbf{r}, \omega),$$

where the *diffraction operator* is defined as $\mathcal{D} := \mathcal{F}_\perp^{-1} \mathcal{H}(z) \mathcal{F}_\perp$.

2.1.3 Paraxial approximation

Considering wave propagation in the *paraxial regime*, where the wave field forms a small angle with the optical axis, or mathematically $k_\perp^2 \approx 0$, with $\mathbf{k}$ denoting the wave vector in vacuum, the free space propagator can be approximated by $\mathcal{H}(z) \approx \exp(izk)$ which further reduces the diffraction operator to

$$\mathcal{D} = \mathcal{F}_\perp^{-1} \exp(izk) \mathcal{F}_\perp = \exp(izk) \mathbb{1}.$$

The paraxial approximation is a common assumption in optics, where wave fronts are assumed to be nearly planar and non-varying in the direction of propagation, as is valid in this work. The deployment of the above diffraction operator in an initial wave field $\psi'(\mathbf{r}', \omega)$ yields the following

$$\psi(\mathbf{r}, \omega) = \mathcal{D}[\psi'](\mathbf{r}, \omega) = \psi'(\mathbf{r}, \omega) \exp(izk). \quad (2.15)$$

This foundation describes the field as a combination of strongly oscillating waves in the direction of propagation ($\exp(izk)$) and a slowly varying envelope function ($\psi'(\mathbf{r}, \omega)$) that transports the information of the wave field. Insertion of $\psi(\mathbf{r}, \omega)$ into the Helmholtz equation yields

$$(\nabla_\perp^2 + \partial_z^2 + k^2 n_\omega^2(\mathbf{r})) \psi'(\mathbf{r}, \omega) \exp(izk) = 0, \quad (2.16)$$

where $\nabla_\perp^2 = \partial_x^2 + \partial_y^2$ is the lateral Laplacian operator in the plane, perpendicular to the optical axis, leading to

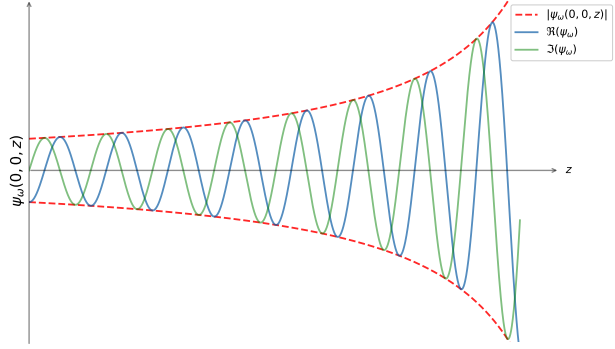
$$(\nabla_\perp^2 + \partial_z^2 + 2ik\partial_z + k^2(n_\omega^2(\mathbf{r}) - 1)) \psi'(\mathbf{r}, \omega) = 0. \quad (2.17)$$

The final execution of equation (2.17) and neglecting the slowly varying term $(\partial_z^2 \psi'(\mathbf{r}, \omega))$, gives a simpler form of the Helmholtz equation, known as *paraxial wave equation* [51, 52, 61].

$$(\nabla_{\perp}^2 + 2ik\partial_z + k^2(n_{\omega}^2(\mathbf{r}) - 1))\psi'(\mathbf{r}, \omega) = 0 \quad (2.18)$$

It is worth noting that the paraxial wave equation and its assumptions deem scattering effects negligible, as the wave field is assumed to be slowly varying in the plane perpendicular to the optical axis. An illustration of the paraxial wave approximation is shown in fig. 2 for a hypothetical wavefront propagating along the optical axis (z) with a parabolic developing envelope function at $\mathbf{r}_{\perp} = \mathbf{0}$.

Figure 2: Illustration of the paraxial wave approximation with a hypothetical wavefront propagating along the optical axis (z). The wave field is described by a parabolic wave and slowly varying in the plane perpendicular to the optical axis. The envelope of the wave field is represented by the red curve, while the real and imaginary components are denoted in blue and green, respectively.



2.1.4 Fresnel diffraction

In case the wave vector along the optical axis is not negligible and is much larger than the lateral wave vector, i.e. $k_z \gg k_{\perp}$, the free space propagator can be approximated via the Taylor expansion of the square root $\sqrt{k^2 - k_{\perp}^2} \approx k - \frac{k_{\perp}^2}{2k}$. The *Fresnel diffraction operator* then becomes

$$\mathcal{D}^{(F)} = \mathcal{F}_{\perp}^{-1} \exp\left(iz\left(k - \frac{k_{\perp}^2}{2k}\right)\right) \mathcal{F}_{\perp} =: \mathcal{F}_{\perp}^{-1} \mathcal{H}^{(F)}(\mathbf{k}_{\perp}, z) \mathcal{F}_{\perp},$$

with $\mathcal{H}^{(F)}(\mathbf{k}_{\perp}, z)$ the *reciprocal Fresnel propagator* in the spatial frequency domain or explicitly

$$\psi(\mathbf{r}_{\perp}, z, \omega) = \mathcal{F}_{\perp}^{-1} \left[e^{iz\left(k - \frac{k_{\perp}^2}{2k}\right)} \mathcal{F}_{\perp}[\psi](\mathbf{k}_{\perp}, 0, \omega) \right] =: \mathcal{D}^{(F)}[\psi](\mathbf{r}_{\perp}, z, \omega).$$

The use of the convolution theorem (section A.1.1.2) and successive algebraic manipulation yield the propagated wave field in the paraxial regime

$$\begin{aligned} \psi(\mathbf{r}_{\perp}, z, \omega) &= \mathcal{H}^{(F)}(\mathbf{r}_{\perp}, z) \star \psi(\mathbf{r}_{\perp}, 0, \omega) \\ &= \frac{e^{izk}}{i\lambda z} \int_{\mathbb{R}^2} d\mathbf{r}'_{\perp} \exp\left(\frac{ik}{2z}(\mathbf{r}_{\perp} - \mathbf{r}'_{\perp})^2\right) \psi(\mathbf{r}'_{\perp}, 0, \omega) \\ &= \frac{e^{izk}}{i\lambda z} \exp\left(\frac{ik}{2z}\mathbf{r}_{\perp}^2\right) \int_{\mathbb{R}^2} d\mathbf{r}'_{\perp} \psi(\mathbf{r}'_{\perp}, 0, \omega) \exp\left(\frac{ik}{2z}\mathbf{r}'_{\perp}^2\right) \exp\left(-\frac{ik}{z}(\mathbf{r}_{\perp} \cdot \mathbf{r}'_{\perp})\right) \end{aligned} \quad (2.19)$$

where $\star$ denotes the convolution operation. The last expression in equation (2.19) insinuates that the propagated wave is proportional to the initial wave field at $z = 0$ multiplied by a phase factor $e^{\frac{ik}{2z} \mathbf{r}'_{\perp}{}^2}$, commonly referred to as the *chirp function* [61]. A closer look at the argument of the chirp function reveals that

$$\frac{k}{2z} \mathbf{r}'_{\perp}{}^2 = \frac{\pi}{\lambda z} \mathbf{r}'_{\perp}{}^2$$

where $\mathbf{r}'_{\perp}{}^2$ in the exponent measures the squared spatial cross section of features in the input wave field. Setting p as the smallest feature of interest during the imaging, the argument of the exponential can be governed by a dimensionless ratio called *Fresnel number* (equation (2.20)) [62].

$$F = \frac{p^2}{\lambda z} \quad (2.20)$$

The Fresnel number, in another sense, quantifies the imaging regime with typical regimes as follows:

- $F \ll 1$: the chirp function has a negligible effect on the propagated wave, and this regime is known as *Fraunhofer diffraction*.
- $F \simeq 1$: the chirp function has a non-negligible effect on the propagated wave, and this is the well-known *Fresnel diffraction regime*. In this regime, different imaging branches can be further distinguished with specific properties given the exact value of F .
- $F \gg 1$: geometric optics-like regime. In this regime, phase contrast tends to zero and amplitude contrast to maximal. This regime is satisfied for short propagation distances and large characteristic feature sizes.

The discussion above is equally correlated with the type of wave front used for imaging. A diverging wave front, such as a spherical wave front, propagates in the near-field regime, with small propagation distances, while a planar wave front propagates in the Fraunhofer regime. The transition from spherical to planar wave is mediated by the paraboloidal fronts and is pictorially depicted in fig. 3, where the spherical wave front transforms into a paraboloidal wave front and finally into a planar wave front at large distances from the divergent source. The imaging branch within the Fresnel regime

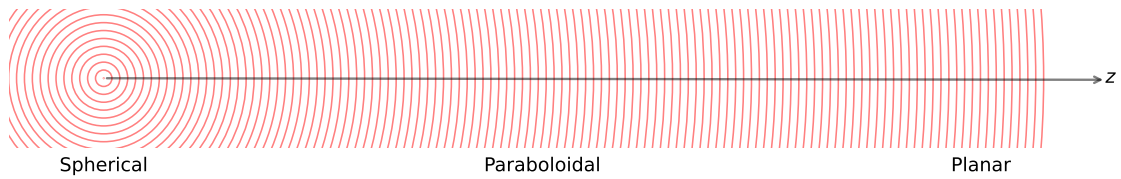


Figure 3: **Illustration of different regimes of wave propagation.** This figure shows how a spherical wavefront propagates and spreads at a distance far from the source into paraboloidal and, lastly, into planar waves.

is what will be further considered in this work, as this is the regime that is typically used in propagation based imaging (PBI) and phase contrast imaging. Alternatively, this implies that X-ray fronts will be considered fairly paraxial with long propagation distances and relatively small effective pixel sizes.

2.1.5 Contrast formation

Interaction of X-rays with matter leads to modulation in the wave field, which leads to measurable quantities that can be detected in the intensity plane and thus is the basis for contrast formation in [PBI](#) and phase contrast imaging. Such modulations are accounted for by the complex refractive index of the material and its mechanism of interaction, which leads to contrast formation in the detector plane, and are discussed in the following. Returning to the paraxial wave equation (equation (2.18)), an expression can be derived for the wave field transmitted through a slab of material of thickness d .

Projection approximation

Given the small magnitude of the non-trivial components of the index of refraction (equation (2.11)), the squared index of refraction can be approximated as

$$n_{\omega}^2(\mathbf{r}) \approx 1 + 2i\beta_{\omega}(\mathbf{r}) - 2\delta_{\omega}(\mathbf{r})$$

through the omission of higher-order terms. Inserting above expression into equation (2.18) and neglecting the lateral Laplacian operator by virtue of a slowly varying envelope function yields

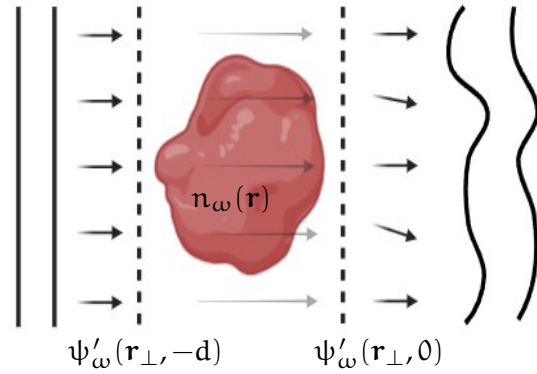
$$(2ik\partial_z + 2k^2(i\beta_{\omega}(\mathbf{r}) - \delta_{\omega}(\mathbf{r})))\psi'(\mathbf{r}, \omega) = 0. \quad (2.21)$$

Integrating equation (2.21) over the thickness of the slab ($-d < z < 0$) in [fig. 4](#) along the optical axis, with the boundary condition that the wave field at $z = 0$ is given by $\psi'(\mathbf{r}_{\perp}, 0, \omega)$, gives

$$\psi'(\mathbf{r}_{\perp}, 0, \omega) = \psi'(\mathbf{r}_{\perp}, -d, \omega) \exp\left(ik \int_{-d}^0 dz (i\beta_{\omega}(\mathbf{r}) - \delta_{\omega}(\mathbf{r}))\right). \quad (2.22)$$

Combination of equations (2.15) and (2.22) yields the wave equation (2.23) directly

Figure 4: Illustration of projection approximation. The wave field is approximated as a plane wave at $z = 0$, and the phase shift and attenuation are accumulated as the wave propagates through the object. The wave field at $z = 0$ is given by the illumination function $\psi'(\mathbf{r}_{\perp}, -d, \omega)$, which is then multiplied by the phase and attenuation terms to yield the wave field at $z = 0$. The figure was created using [Biorender.com](#).



behind the object at $z = 0$ in terms of the wave field at $z = -d$, which is typically referred to as the *illumination function* $\psi'(\mathbf{r}_{\perp}, -d, \omega) = \psi'_0$, and accumulated phase shift and attenuation of the wave field as it propagates through the object [52].

$$\psi(\mathbf{r}_{\perp}, 0, \omega) = \psi'(\mathbf{r}_{\perp}, -d, \omega) \exp(ikd) \exp\left(ik \int_{-d}^0 dz (i\beta_{\omega}(\mathbf{r}) - \delta_{\omega}(\mathbf{r}))\right) \quad (2.23)$$

The illumination function is also referred to as the *probe function* $P(\mathbf{r}_\perp, \omega)$ with additionally the last term denoted as the *object transmission function* $O(\mathbf{r}_\perp, \omega)$ or in other words the wave at the exit plane ($z = 0$) is given by the function

$$\psi(\mathbf{r}_\perp, 0, \omega) = P(\mathbf{r}_\perp, \omega)O(\mathbf{r}_\perp, \omega) \exp(ikd)$$

where $O(\mathbf{r}_\perp, \omega) := \exp(-ik\bar{\delta}_\omega(\mathbf{r}_\perp)) \exp(-k\bar{\beta}_\omega(\mathbf{r}_\perp))$. Here, $\bar{\delta}_\omega(\mathbf{r}_\perp) = \int_{-d}^0 dz \delta_\omega(\mathbf{r})$ and $\bar{\beta}_\omega(\mathbf{r}_\perp) = \int_{-d}^0 dz \beta_\omega(\mathbf{r})$ are also introduced as the *effective refractive index* and *effective extinction coefficient* of the wave field as it propagates through the object, respectively.

$$\phi_\omega(\mathbf{r}_\perp) = -k\bar{\delta}_\omega(\mathbf{r}_\perp) = -k \int_{-d}^0 dz \delta_\omega(\mathbf{r}) \quad (2.24)$$

whereas the intensity of the wave field is given by equation (2.25).

$$I(\mathbf{r}_\perp, 0, \omega) = I(\mathbf{r}_\perp, -d, \omega) \exp(-2k\bar{\beta}_\omega(\mathbf{r}_\perp)) \quad (2.25)$$

Equation (2.25) implies that if the probe function is known, then the projected extinction coefficient can be recovered from the intensity at the exit plane. This is the celebrated *Beer-Lambert law* [6, 61], which is the basis for attenuation-based imaging. Additionally, note that phase information is lost in intensity measurement, a problem widely referred to as *phase problem* in many fields of physics and imaging. This therefore requires additional methods to recover the imaginary part of the wave field, a basis for phase contrast imaging, and will be discussed in chapter 3.

Transport of intensity equation

The transport of intensity equation (TIE) addresses the shortcomings of methods developed previously and provides a formulation of the phase problem. This is achieved by relating the intensity of the wave field, which is measurable, to the phase of the wave field, which is lost in intensity measurements. This method is based on the paraxial wave equation (equation (2.18)) and is derived by expressing the wave field in terms of its intensity and phase. Under certain conditions, this framework leads to the retrieval of the phase from the intensity measurement. The wave field $\psi'_\omega(\mathbf{r})$ is, as mentioned herein, expressed in terms of its intensity $I_\omega(\mathbf{r})$ and phase $\phi_\omega(\mathbf{r}_\perp)$ or explicitly

$$\psi'_\omega(\mathbf{r}) = \sqrt{I_\omega(\mathbf{r})} \exp(i\phi_\omega(\mathbf{r}_\perp)). \quad (2.26)$$

The successive insertion of equation (2.26) into the paraxial wave equation (equation (2.18)) and rearranging terms gives the TIE in the form [51, 52, 61].

$$\nabla_\perp \cdot (I_\omega(\mathbf{r}) \nabla_\perp \phi_\omega(\mathbf{r})) = -k \partial_z I_\omega(\mathbf{r}) \quad (2.27)$$

The transport of intensity equation (TIE) relates the intensity in the detector plane to the phase curvature, which is a measure of local gradients, and the intensity gradient.

It is important to note that in polychromatic imaging, which is typical for lab-based imaging systems, the spectrum of waves is not monochromatic, and therefore the wave field is a superposition of different frequencies. It asserts the measured intensity to be the contribution of all available frequencies, which is expressed as

$$I(\mathbf{r}_\perp, 0) = \int d\omega I_\omega(\mathbf{r}_\perp, 0) S(\omega) D(\omega),$$

where, $S(\omega)$, is the incident spectrum and $D(\omega)$ the detector response [6], containing variable efficiency.

Contrast transfer function (CTF) is also an equivalent method to TIE, with the assumption of weak absorption and also a slowly varying phase, however, it is not discussed here further and more information can be found in the literature [52, 61, 70, 92].

2.1.6 Fresnel scaling theorem

For lab-based computed tomography (CT), with divergent beam, as in the spherical regime (fig. 3), the developed methods are not readily applicable, as the wave field is not parallel and thus requires other treatment. However, developed tools are still relevant as this issue of divergent beam geometries is countered by *Fresnel scaling theorem* [6, 61], which is discussed in the following. Assuming a divergent beam, such as a spherical

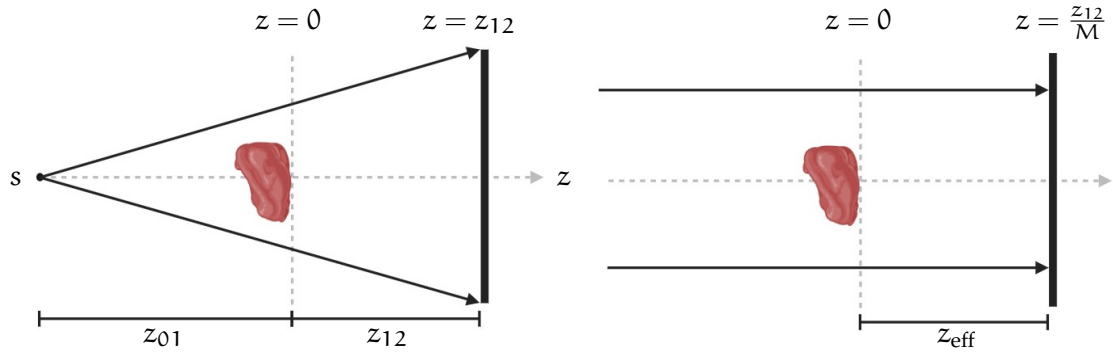


Figure 5: Divergent (**left**) and parallel beam geometry (**right**) as an illustration for the Fresnel scaling theorem. Cone beam and thus divergent beam geometry leads to magnification of the object in the detector plane with $M = (z_{01} + z_{02})/z_{01}$ where z_{01} is the source-to-object distance (SOD) and z_{02} is the source-to-detector distance (SDD). The cone beam geometry is according to the Fresnel scaling theorem equivalent to an effective parallel beam geometry with an effective sample-to-detector distance $z_{\text{eff}} = z_{12}/M$, where z_{12} is the object-to-detector distance (ODD) in the cone beam geometry. Above figures were created using [Biorender.com](https://biorender.com).

wave front as illumination at $\mathbf{r}_0$, and a sample at the origin at a distance z_{01} front source, with z_{01} large enough such that the paraxial approximation holds, the exit wave behind the sample is given by

$$\begin{aligned}\psi(\mathbf{r}_\perp, 0, \omega) &= \frac{\exp(i k |\mathbf{r} - \mathbf{r}_0|)}{|\mathbf{r} - \mathbf{r}_0|} O(\mathbf{r}_\perp, \omega) \exp(i k d) \\ &\approx \frac{\exp(i k z_{01}) \exp(i k \frac{r_\perp^2}{2 z_{01}})}{z_{01}} O(\mathbf{r}_\perp, \omega) \exp(i k d)\end{aligned}$$

which is also referred to as a parabolic wave (fig. 3) [61]. The exit wave behind the sample is propagated to the detector plane at z_{12} from the sample using a Fresnel propagator (section 2.1.4), which eventually leads to

$$\psi_\omega(\mathbf{r}_\perp, z_{12}) = \frac{e^{(i k z_{12})}}{i \lambda z_{12}} e^{(i k \frac{r_\perp^2}{2 z_{12}})} \frac{e^{(i k d)}}{z_{01}} \int_{\mathbb{R}^2} d\mathbf{r}'_\perp O_\omega(\mathbf{r}'_\perp) e^{(i k \frac{r_\perp^2}{2} (\frac{1}{z_{01}} + \frac{1}{z_{12}}))} \exp(-\frac{i k \mathbf{r}_\perp \cdot \mathbf{r}'_\perp}{z_{12}}).$$

From the above expression, an *effective propagation distance* (z_{eff}) and *geometrical magnification factor* (M) can be defined as

$$z_{\text{eff}} := \frac{1}{z_{01}} + \frac{1}{z_{12}} = \frac{z_{12}}{M} \quad \text{with} \quad M = \frac{z_{01} + z_{12}}{z_{01}}, \quad (2.28)$$

respectively. This definition helps to transform the previous expression into its final form

$$\psi_{\omega}(\mathbf{r}_{\perp}, z_{12}) = \frac{e^{(ikz_{12})}}{i\lambda z_{12}} e^{(\frac{ikr_{\perp}^2}{2z_{12}})} \frac{e^{(ikd)}}{z_{01}} \int_{\mathbb{R}^2} d\mathbf{r}'_{\perp} O_{\omega}(\mathbf{r}'_{\perp}) e^{(\frac{ikr'^2_{\perp}}{2z_{\text{eff}}})} \exp\left(-\frac{ik}{z_{\text{eff}}} \left(\frac{\mathbf{r}_{\perp}}{M} \cdot \mathbf{r}'_{\perp}\right)\right),$$

with the intensity in the detector plane denoted as $I_{\omega}(\mathbf{r}_{\perp}, z_{12})$. On the other hand, equation (2.19) from Fresnel diffraction theory learns that the wave field at z_{eff} and lateral position $\mathbf{r}_{\perp}$ obtained with a parallel probe function of the form $P_{\omega}(\mathbf{r}) = e^{(ikz)}/z_{01}$, which leads to the same intensity as in the spherical wave front, is given by the following

$$\psi_{\omega}^F\left(\frac{\mathbf{r}_{\perp}}{M}, z_{\text{eff}}\right) = \frac{e^{(ikz_{\text{eff}})}}{i\lambda z_{\text{eff}}} e^{(\frac{ik}{2z_{\text{eff}}} \frac{r_{\perp}^2}{M^2})} \frac{e^{(ikd)}}{z_{01}} \int_{\mathbb{R}^2} d\mathbf{r}'_{\perp} O_{\omega}(\mathbf{r}'_{\perp}) e^{(\frac{ikr'^2_{\perp}}{2z_{\text{eff}}})} \exp\left(-\frac{ik}{z_{\text{eff}}} (\mathbf{r}'_{\perp} \cdot \frac{\mathbf{r}_{\perp}}{M})\right)$$

with intensity $I_{\omega}^F(\frac{\mathbf{r}_{\perp}}{M}, z_{\text{eff}})$. Relating the two expressions, one can see that the intensity in the detector plane of the two wave fields is related throughout

$$I_{\omega}(\mathbf{r}_{\perp}, z_{12}) = \frac{1}{M^2} I_{\omega}^F(\frac{\mathbf{r}_{\perp}}{M}, z_{\text{eff}}), \quad (2.29)$$

which is the celebrated *Fresnel scaling theorem* [6, 61]. In essence, the consequence of the Fresnel scaling theorem amounts to the fact that a diverging beam, which is typical of lab-based CT systems, can be treated as an effective parallel beam with an effective propagation distance (z_{eff}) and a magnification factor (M). This is shown pictorially in fig. 5 where the point source geometry (**left**) is shown to be equivalent to a parallel beam geometry (**right**) with transformed parameters. The geometrical magnification factor allows for the imaging of objects of varying resolution, as the detector resolution can be reduced to an effective pixel size by choosing z_{01} and z_{12} , which further defines the magnification factor. Analogously, the Fresnel number (F) in equation (2.20) is also modified, resulting in an effective Fresnel number,

$$F_{\text{eff}} = \frac{p_{\text{eff}}^2}{\lambda z_{\text{eff}}} = \frac{1}{M} F, \quad (2.30)$$

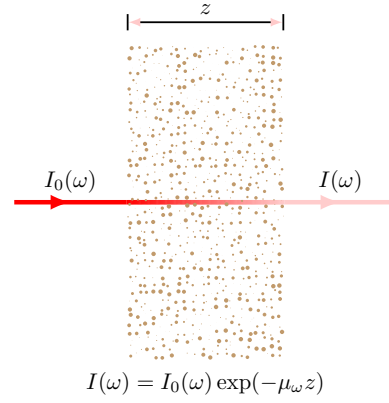
where $p_{\text{eff}} = p/M$ is the effective feature size in the detector plane and F the Fresnel number in parallel-beam geometry.

Thus far, we have focused on the classical interpretation of X-ray interactions with matter, relying on wave front propagation and interaction outcomes. Nevertheless, the microscopic interaction is more intricate, and an introduction to the quantum mechanical perspective is sound, as outlined below.

2.2 X-RAY ATTENUATION AND BEER-LAMBERT LAW

The Beer-Lambert law is a well-known law in physics that describes the attenuation of an electromagnetic wave as it passes through a medium [6, 61]. The law states that

Figure 6: Illustration of the Beer-Lambert extinction law, showing the attenuation of incident radiation $I_0(\omega)$ through a medium of absorbing particles. The transmitted frequency-dependent intensity $I(\omega)$ decreases exponentially on the path length z , following equation (2.25), where μ_ω is the frequency-dependent linear attenuation coefficient (equation (2.32)).



the intensity of light decreases exponentially with the thickness of the medium and its atomic number. The law was derived by August Beer with an alternative derivation provided in section 2.1.5 as a different form is provided again in equation (2.31). The Beer-Lambert law, as already mentioned, serves as a backbone for attenuation-based imaging, as it describes the attenuation of radiation as it passes through a medium and is illustrated in fig. 6.

$$I(\omega) = I_0(\omega) \exp \left(- \int_0^z dz \mu_\omega(\mathbf{r}_\perp) \right) \quad (2.31)$$

$I(\omega)$ in equation (2.31), represents the transmitted energy-dependent intensity of the radiation field in the detector plane and depth z in the medium, $I_0(\omega)$ is the incident intensity of the radiation field, while lastly μ_ω is the frequency-dependent attenuation coefficient. The Beer-Lambert law in equation (2.31) is a special case of the more general form in equation (2.25).

The *attenuation coefficient* (μ_ω) is a measure of the probability of the interaction of the X-ray with the material, which expresses how the radiation field is absorbed/attenuated along its path. This linear attenuation coefficient is material (Z) and energy dependent (E). Intuitively, it is expected that the more matter there is, the higher the chance of interaction and thus attenuation such that μ_ω in equation (2.31) can equivalently be expressed as

$$\mu_\omega \rightarrow \mu(E, Z) = \sum_a \rho_a \sigma_a(E, Z), \quad (2.32)$$

with ρ_a the number density of the atoms of type a and σ_a the total cross section. The cross section expresses the probability that the matter of type a interacts with the radiation field [71, 93]. When there are different mechanisms of interaction, depicted by i , the total cross section is the simple sum of the individual cross sections, i. e.

$$\sigma_a(E, Z) = \sum_i \sigma_{ai}(E, Z).$$

The cross section is attainable through experimental measurement and theoretical prediction rooted deeply within quantum mechanics. These macroscopic mechanisms responsible for the interaction of the radiation field with matter, including attenuation, are briefly discussed in the following section.

2.3 MICROSCOPIC INTERACTION OF X-RAY WITH MATTER

X-ray interaction with matter can be classified into different phenomena depending on the nature of the interaction. Interactions of importance, given the X-ray energy range of 1 – 120 kV, can be classified into *photoelectric effect*, *scattering effects*, under which there is coherent/Thomson and incoherent/Compton scattering. Interactions such as *pair production* and *photo nuclear reaction* demand energies of the order of MeV [6, 61] and thus are not relevant given the energy of the source.

2.3.1 Photoelectric absorption

The *photoelectric effect* is a quantum mechanical interaction of a photon and a bound electron in an atom or molecule [6, 44, 101] and is the main process responsible for the formation of contrasts of X-rays with matter in a low enough energy range. When a photon of energy higher than the binding energy of the electron is incident on matter, the electron gains an excess energy

$$E_e = \hbar\omega - E_b, \quad (2.33)$$

E_b is the binding energy of the electron. The electron then gains an excess kinetic energy and escapes its potential [6, 61]. This electron in such a state is referred to as a *photoelectron*, a process which is also referred to as *photoionization*. Photoionization leads to discrete absorption edges E_i whenever E_γ is large enough to induce the emission of an electron from its bound state, leading to vacancies. These fabricated vacancies may be filled by electrons from different states, releasing the excess energy. This radiation is typically termed *characteristic radiation* as it serves as a tracer for the material. The total cross section of the photoelectric absorption for a given atom (Z) in the K-shell is given by

$$\sigma_{PE}^K(E) = \sigma_{Th_e} 4\sqrt{2}\alpha^4 (m_e c^2)^{\frac{Z}{2}} \frac{Z^5}{E_\gamma^{\frac{Z}{2}}} \quad \text{for } E_\gamma \ll m_e c^2 \quad (2.34)$$

where α is the fine structure constant, σ_{Th_e} natural scattering cross section [61]. Fortunately enough, the K-shell absorption cross section is the dominant contribution to the total photoelectric absorption cross section, particularly for low Z -elements, and equation (2.34) is the meter stick for rough estimations, yet, for practical purposes, the following expression is widely popular [6, 61]

$$\sigma_{PE}(E) \propto \frac{Z^{3-4}}{E^{2-3}}.$$

X-ray photons that do not undergo photoelectric absorption are typically scattered or transmitted through the material. Such scattering processes can be elastic or inelastic and are typically referred to as *Compton scattering* and *Rayleigh scattering*, respectively, as discussed in the following.

2.3.2 Scattering

Elastic scattering involves a process in which the initial energy of the photon (E) is conserved after interaction with an electron, but with an induced change in momentum.

This is typically also referred to as *Rayleigh scattering* and is the dominant scattering process for low-energy photons. In contrast, inelastic scattering (*Compton scattering*) is a process where the interaction causes both a change in the photon's momentum and energy. The energy of a scattered photon (E') after undergoing Compton scattering obeys

$$\frac{1}{E'} - \frac{1}{E} = \frac{1}{m_e c^2} (1 - \cos \theta)$$

such that measurement of the scattering angle (θ) allows one to determine the energy of the scattered photon. *Compton scattering* becomes the dominant form of interaction for very high-energy photons.

The cross section of incoherent scattering, following a lot of assumptions and approximations, can approximately be expressed as

$$\sigma_C = \begin{cases} \sigma_{Th_e} \left(1 - \frac{E_\gamma}{m_e c^2} + \frac{26}{5} \frac{E_\gamma^2}{m_e^2 c^4} \right) & \text{for } E_\gamma \ll m_e c^2, \\ \sigma_{Th_e} \frac{3m_e c^2}{8E_\gamma} \left(2 \ln \left(\frac{2m_e c^2}{E_\gamma} \right) + 1 \right) & \text{for } E_\gamma \gg m_e c^2, \end{cases} \quad (2.35)$$

where σ_{Th_e} is the Thomson scattering cross section for an electron [61]. The contrast resulting from the Compton effect is typically negligible, especially for low-energy photons. This comes from the fact that the probability of the Compton effect is merely dependent on the atomic number (Z).

Summarizing the resulting discussions, the mass attenuation of X-rays in matter, which is the fraction of the linear attenuation coefficient (μ) by the density (ρ), encompassing all the different interactions, is given by

$$\mu(E, Z) = \sum_a \rho_a \sigma_a(E, Z) = \sum_{aj} \rho_a \sigma_{aj}(E),$$

where a sums over the different components/compounds and j over the different contributions, such as the one discussed above.

Given a uniform sample, it is expected that the scattering of X-rays within the sample will occur uniformly and thus contribute as an additive signal to radiographs and reconstructed slices [48]. However, at interfaces, such scattering effects could lead to disturbances. This disturbance can be modeled as a random walk process and can be quantified or empirically quantified. This signal can then be corrected for by subtracting the background scatter signal from the reconstructed slices and the projection images, as, for example, in equation (2.36),

$$I_\omega(\mathbf{r}_\perp, z_{12})_{\text{corrected}} = I_\omega(\mathbf{r}_\perp, z_{12})_{\text{total}} - I_\omega(\mathbf{r}_\perp, z_{12})_{\text{scatter}} \quad (2.36)$$

where $I_\omega(\mathbf{r}_\perp, z_{12})_{\text{total}}$ is the total intensity in the detector plane and $I_\omega(\mathbf{r}_\perp, z)_{\text{scatter}}$ is the background scatter signal.

To finally conclude, the interaction of a free electron with the electromagnetic field and or other charged particles can lead to spontaneous emission of a photon, which in the field of X-ray imaging is typically referred to as *braking radiation* [6, 61]. This is the mechanism, which leads to a continuous spectrum of emitted photons, will be further discussed in later chapters.

The heuristic approach to tomography and its fundamental backbone, the Radon transform (RT), is briefly discussed in this chapter. This chapter focuses on phase retrieval algorithms and computed tomography, and their natural linkage to phase-contrast tomography will become apparent. This chapter mainly discusses three main ideas. The first addresses the fundamentals of phase retrieval, while the second focuses on computed tomography (CT) and phase-contrast tomography.

The focus of the last lies on typical artifacts in CT, which are unwanted signals that require extra pre- and postprocessing techniques, but before diving into the details of phase retrieval and CT, a brief introduction and modeling of phase and transmission contrast, and their discrepancies to conventional tomography is sound.

3.1 IMAGING MODALITIES

Within the realm of X-ray transmission imaging, several subbranches can be identified, such as conventional/absorption-contrast imaging or better attenuation-contrast imaging, and phase-contrast imaging. In projection imaging (radiography), projected images of objects/samples are acquired. While this branch of imaging is sufficient for many assessments, information is inherently limited due to the 2D nature of the images. In contrast, both absorption and phase-contrast tomography provide quantitative 3D information about the intrinsic sample properties. These quantities of interest are the absorption coefficient ($\mu(\mathbf{r})$) and phase distribution ($\delta(\mathbf{r})$) in absorption and phase-contrast imaging, respectively. These imaging quantities are inherently based on intensity measurements, where multiple images at different angles (θ) are taken and reconstructed using mathematical algorithms, such as those spelled out in section 3.3, to obtain a 3D representation of the sample. The main difference between absorption and phase-contrast imaging thus lies in the contrast mechanism, where absorption imaging relies on the attenuation of X-rays, where absorption imaging relies on the attenuation of X-rays, while phase-contrast imaging exploits phase shift of X-rays through the object [6].

For a very long time, absorption imaging has been the go-to method for 3D assessment of objects, especially in very dense materials; however, this method suffers when imaging of weakly absorbing materials, such as biological samples, is required. This shortcoming can be filled by phase-contrast imaging, where the requirement of heavily absorbing materials is relaxed and phase shift is utilized as the imaging quantity.

Figure 7 shows the non-trivial terms of the refractive index of different samples with respect to the photon energy, i. e. the refractive index of decrement $\delta(E)$ and extinction coefficient $\beta(E)$, for different samples. It can be seen that, for increasing photon energy, both quantities decrease, a direct consequence of the reduction in the interaction of X-rays with matter (section 2.3) that leads to meaningful contrast. However, it can also be seen that the refractive index of the different samples is significantly higher than that of the extinction coefficient for all the samples (fig. 7). However, to recover the phase

distribution, the majority of algorithms still depend on the intensity distribution, hence is influenced by the attenuation.

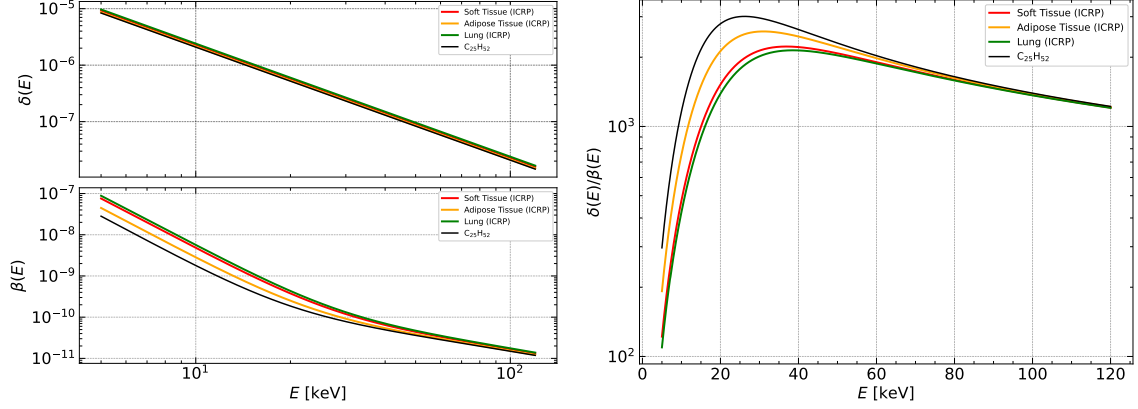


Figure 7: **Left:** refractive index decrement of the different samples with respect to the photon energy (E) on a semi-log scale. The dashed line represents the imaginary part $\beta(E)$ of the refractive index and the solid line the real part $\delta(E)$. **Right:** transmission curves of different samples with respect to the photon energy. The modeling was done with *xraylib* [63] with model parameters listed in table 1.

As phase information is recovered from intensity measurements, an optimal condition for optimizing phase-contrast imaging is to maximize the contrast in the projected images. Consider the schematic in fig. 8, where rays with intensity I_0 are transmitted through the sample (s), paraffin (p) and empty/air (a) layers. The transmitted intensity ($T = \exp(-\int \mu_E(Z) d\ell)$), or alternatively,

$$\tau_A = -\int_{\ell_A} \mu_E(\mathbf{r}) d\ell = \ln\left(\frac{I}{I_0}\right) \quad (3.1)$$

is dependent on the path (ℓ) and the respective attenuation coefficients of the materials in the line of sight (LOS) [6]. With the variance of τ_A given by

$$\text{Var}(\tau_A) = \text{Var}\left(\ln\left(\frac{I}{I_0}\right)\right) = \text{Var}(\ln(I)) + \text{Var}(\ln(I_0)) = \frac{1}{I} + \frac{1}{I_0}, \quad (3.2)$$

where I is the transmitted intensity and I_0 the incident intensity, we are in a position to define the CNR between the line of sight (LOS) intensity values of the rays through the paraffin region and the sample-paraffin region as

$$\text{CNR}_T = \frac{|\ln(T_A) - \ln(T_B)|}{\sqrt{\sigma_A^2 + \sigma_B^2}}, \quad (3.3)$$

according to section 4.5.2, with T_A and T_B being the transmitted intensities through the paraffin and sample-paraffin regions, respectively, and $\sigma^2 = \text{Var}(\ln(T))$. Despite phase effects, the intensities in the detector plane for transmission through the sample (s), paraffin (p), and air (a) layers for the three different paths are given by

$$I_S = I_0 \exp\left(-\int_{\ell_S} \mu_E(\mathbf{r}) d\ell\right) = I_0 T_S, \quad I_P = I_0 \exp\left(-\int_{\ell_P} \mu_E(\mathbf{r}) d\ell\right) = I_0 T_P$$

and $I_F = I_0 \exp\left(-\int_{\ell_F} \mu_E(\mathbf{r}) d\ell\right) = I_0 T_F.$

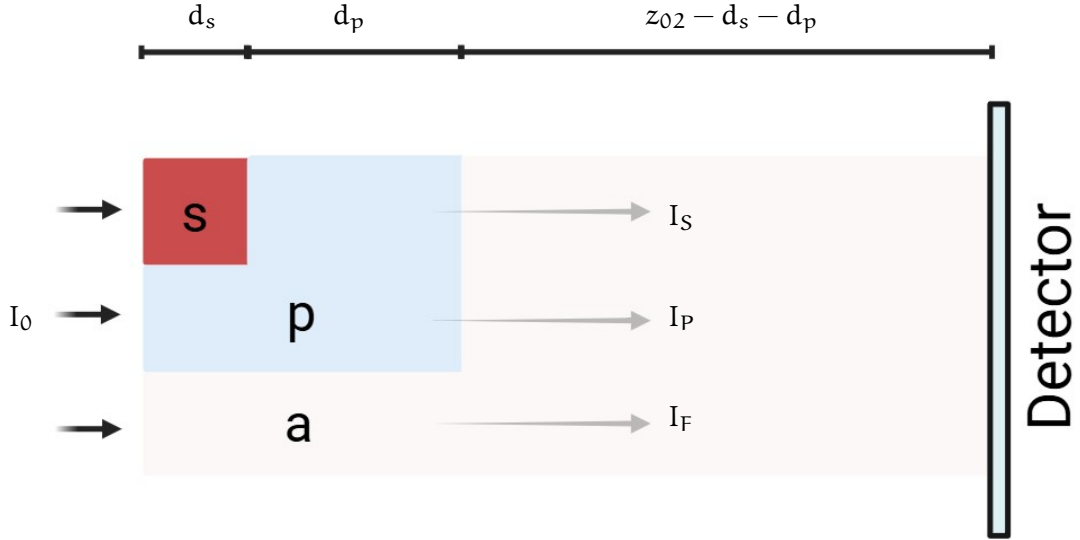


Figure 8: **Illustration of the schematic used to model the contrast-to-noise ratio (CNR) of different samples.** The sample is placed in the beam path with the beam traveling along 3 different paths (empty beam path, sample+paraffin path, paraffin path), and the transmitted intensity is calculated according to Beer-Lambert's law. The model assumes parallel paths and monochromatic rays through the object. The figure was created using [Biorender.com](https://biorender.com).

With successive normalization by the empty beam/flat signal (I_F) according to $T_i = I_i/I_F$, and using equation (3.2), the **CNR** can be rewritten as

$$\text{CNR}_T = \sqrt{I_0} \cdot \frac{|\ln(T_S) - \ln(T_P)|}{\sqrt{\frac{2}{T_F} + \frac{1}{T_S} + \frac{1}{T_P}}}. \quad (3.4)$$

The above metric was evaluated for different sample compositions with an effective size of $d_s = 8 \mu\text{m}$ embedded with an formalin-fixed paraffin-embedded (**FFPE**) block, according to fig. 8 with a thickness of $22 \mu\text{m}$ and a propagation distance of 1500 mm , using the *xraylib* package [63]. The vertical-dashed lines are an indication of important photon energies, which denote the characteristic emission lines of elements, as will be discussed in more detail in section 4.2. All other model parameters are listed in table 1.

Figure 9 shows that the maximum contrast-to-noise ratio (**CNR**), normalized to the incoming fluence, of a standard tissue sample (ICRP) with an effective size of $8 \mu\text{m}$, embedded within a paraffin block with thickness of 22 mm , is reached at a photon energy of approximately 12.9 keV . The curve is skewed towards lower energies, according to fig. 7. Energies below approximately 10 keV are heavily absorbed by the paraffin and air. This makes them unsuitable for imaging **FFPE** blocks due to the increased noise from photon starvation, despite the relatively high refractive index. Not only does the **CNR** decrease for energies lower than approximately 10 keV , but also for energies above 30 keV . The energy level at which the **CNR** reaches its peak, here denoted as E_{opt} , is influenced by the sample thickness. In this respect, the thickness of the different samples is varied proportionally from a feature of a very small **LOS** cross section of size $d_s = 8 \mu\text{m}$ up to sizes filling the whole **FFPE** block. The optimal energy is accessed for the different samples and is plotted in fig. 9 (right). It can be seen that the optimal energy shifts quasi-linearly with respect to the thickness of the sample, with all energy ranges of the

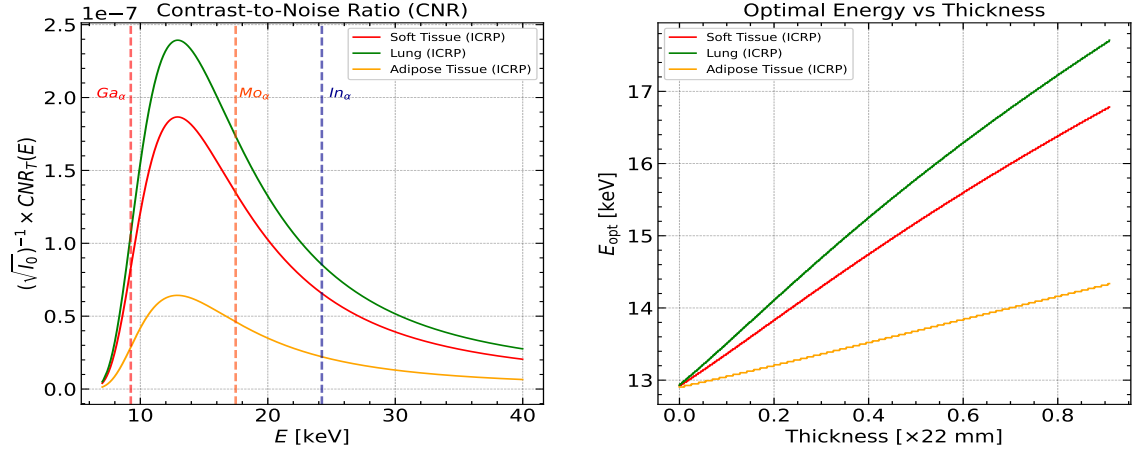


Figure 9: **Left:** CNR for different tissue types as a function of the monochromatic X-ray energy. The transmission CNR is skewed towards lower energies, where the best contrast between the sample and paraffin is reached with low noise. The vertical lines denote characteristic emission energy lines of gallium ($_{31}\text{Ga}$), molybdenum ($_{42}\text{Mo}$) and indium ($_{49}\text{In}$). **Right:** optimal energy (E_{opt}), where CNR_T reaches a maximum, for transmission imaging versus tissue thickness in fraction of the size of the whole block. The optimal energy shifts linearly with respect to the thickness for the different samples. The modeling was done with *xraylib* [63] with model parameters listed in table 1.

different samples covering $E \in [13, 18]$ keV. It is of importance to note that the noise model was based on the assumption of photon statistics such that in real systems with imperfections such as dark noise, read noise, absorption by air, and others, the CNR_T -curve shifts toward higher energies. Not only this, but also thicker FFPE blocks will shift the optimal energy upstream, but the above model gives an idea where the CNR in transmission imaging is maximized. This is desirable in phase-contrast imaging, where the phase information is inferred from the projected intensity distribution.

Sample	Density [$\frac{\text{g}}{\text{cm}^3}$]	Thickness [μm]
Soft tissue (ICRP)	1.0	8
Lung (ICRP)	1.05	8
Adipose tissue (ICRP)	0.92	8
Paraffin	0.875	22×10^3

Table 1: **Samples and corresponding model parameters.** The density and composition of the samples were retrieved from the ICRP database via the *xraylib* package [63]. The paraffin's density was chosen based on literature-reported values, and the thickness corresponding to the typical size dimension of FFPE used in this work.

3.2 PHASE RETRIEVAL

As already hinted multiple times, in phase-contrast imaging (PCI), the phase (ϕ) distribution is the metric of interest. Since the phase is not directly available in intensity measurements (section 2.1.5), it must be accessed through the intensity distribution. Different algorithms, derived with specific assumptions, are available and will be further

Imaging modality	Reconstructed quantity	Algorithm	Method
Transmission imaging	$T(\mathbf{r}_\perp)$	-	-
Absorption-contrast CT	$\mu(\mathbf{r})$	FBP	RT
Phase-contrast CT	$\delta(\mathbf{r})$	FBP	RT

Table 2: **Summary of imaging modalities and their reconstructed quantities.** In particular, the phase-contrast [CT](#) retrieves the phase information of the sample, which is directly related to its density distribution. The algorithms used for the reconstruction of the phase-contrast [CT](#) are based on the principles of Radon transform ([RT](#)) and X-ray transform and the filtered back projection ([FBP](#)), which are further discussed in section [3.3](#).

discussed in this section. The roots of most phase retrieval algorithms, discussed in the chapter, are based on the transport of intensity equation ([TIE](#)) (equation (2.27)), which is rewritten as

$$\frac{I_\omega(\mathbf{r}_\perp, z)}{I_\omega(\mathbf{r}_\perp, 0)} = 1 - \frac{z}{k} \nabla_\perp^2 \phi_\omega(\mathbf{r}_\perp), \quad (3.5)$$

after assuming weakly and homogeneous objects and short propagation distances [[6](#), [61](#), [73](#)]. Here, $I_\omega(\mathbf{r}_\perp, z)$ represents the measured intensity in the detector plane after transmission through the object of interest, $I_\omega(\mathbf{r}_\perp, 0)$ the intensity in the exit plane directly behind the object, $\phi_\omega(\mathbf{r}_\perp)$ is the phase distribution of the object and z the distance between the object and the detector.

3.2.1 Modified Bronnikov Algorithm (MBA)

For weakly absorbent and homogeneous objects, the modified bronnikov algorithm ([MBA](#)) algorithm is used to provide a method for retrieving the phase distribution of the object. Given the near-field [TIE](#) equation (3.5) as a basis, the phase distribution of the object can be retrieved by solving the equation for $\phi_\omega(\mathbf{r}_\perp)$. This can be achieved by use of the pseudo differential operators, i. e. noting that

$$\partial_j^q[\bullet] = \mathcal{F}^{-1}(ik_j)^q \mathcal{F}[\bullet],$$

such that the lateral Laplacian operator $\nabla_\perp^2$ and its inverse can be expressed as

$$\nabla_\perp^2[\bullet] = -\mathcal{F}_\perp^{-1}|\mathbf{k}_\perp|^2 \mathcal{F}_\perp[\bullet] \quad \text{and} \quad \nabla_\perp^{-2}[\bullet] = -\mathcal{F}_\perp|\mathbf{k}_\perp|^{-2} \mathcal{F}_\perp^{-1}[\bullet]$$

with $\nabla_\perp^2 \nabla_\perp^{-2}[\bullet] = [\bullet]$ and $\bullet$ a place holder for any arbitrary function [[6](#)]. The application of the inverse operator to equation (3.5) yields

$$\phi_\omega(\mathbf{r}_\perp) = (2\pi F) \mathcal{F}^{-1} \left(\frac{1}{|\mathbf{k}_\perp^0|^2} \mathcal{F}_\perp \left[\frac{I_\omega(\mathbf{r}_\perp, z)}{I_\omega(\mathbf{r}_\perp, 0)} - 1 \right] \right), \quad \mathbf{k}_\perp^0 = p\mathbf{k}_\perp \quad (3.6)$$

where the reciprocal lateral vector has been rewritten in terms of the feature size/effective pixel size p and F , recognized as the Fresnel number (equation (2.20)). Due to the nature of the derivation of equation (3.6), the phase distribution is disrupted due to the singularity at the origin, i. e. $\mathbf{k}_\perp^0 = 0$. This is addressed using a regularization parameter α such that the singularity is removed in equation (3.7).

$$\phi_\omega(\mathbf{r}_\perp) = (2\pi F) \mathcal{F}^{-1} \left(\frac{1}{|\mathbf{k}_\perp^0|^2 + \alpha} \mathcal{F}_\perp \left[\frac{I_\omega(\mathbf{r}_\perp, z)}{I_\omega(\mathbf{r}_\perp, 0)} - 1 \right] \right) \quad (3.7)$$

The regularization parameter is chosen, typically by virtual inspection, i.e. by evaluation of the filtered image. This is an absorption-dependent parameter, chosen so that high- and low-frequency frequencies are well represented. For low α , the Fourier space filter underrepresents low frequencies, leading to blurring (fig. 10, **third row**). When the α -parameter is chosen relatively too high, the filter suppresses low frequencies, leading to amplified edge enhancement. Thus, given a composite projection image (absorption + phase), α is chosen so that edge enhancements disappear at the cost of blurring. This is illustrated in fig. 10 (**left, first row**), where a normalized projection image is shown containing both absorption and phase information in the form of edge enhancement, manifested by the lateral Laplacian operator. The line profile of the image is shown in fig. 10 (**first row, middle**), where the edge enhancement is visible as discontinuities in the line profile. The histogram of the normalized image is shown in fig. 10 (**right**), where the phase and absorption information can be tracked to the relative counts in the image.

Figure 10 (**second and third row**) shows the phase distribution of the normalized frame using the MBA algorithm for two different choices of the regularization parameter α . The optimal regularization parameter here is $\alpha = 0.15$, while $\alpha = 0.015$ is an overshoot value that leads to excessive blurring. This excessive blurring is apparent in the line profiles of the corresponding phase images.

It is important to note that for polychromatic sources, the α -parameter is to be considered as an effective α_{eff} since this parameter is dependent on the photon energy. In practice, however, this parameter is chosen pragmatically based on visual judgement. Furthermore, since the intensity of the wave in the exit plane ($I_\omega(\mathbf{r}_\perp, 0)$) behind the object is typically not known, it is assumed, in the framework of a weakly absorbing object, hence $I_\omega(\mathbf{r}_\perp, 0)$ is uniform and approximately equal to the flat field (I_0). However, measurement of the wave in the exit plane is possible, although not practical, and might yield more representative phase distributions. This can be achieved by placing the detector right behind the sample, where the phase shifts accumulated in the sample have not yet propagated.

Although MBA is very simple and easy to implement, it has severe limitations, blurring one of the candidates, even if the α -parameter is chosen appropriately. These shortcomings can be addressed by the Bronnikov-aided correction (BAC) algorithm [6, 61].

3.2.2 Bronnikov-Aided Correction (BAC)

The Bronnikov-aided correction (BAC) algorithm constructs the intensity [23, 61] in the exit plane using the measured intensity in the detector plane and thus treats edge enhancements as artifacts. Although this algorithm relies on MBA, it significantly less prone to the assumption of a weakly absorbing and homogeneous object, inherent in the near-field TIE equation (3.5). Furthermore, it helps combat experimental imperfections such as heterochromatic waves and low coherence [73]. Equation (3.5) can be rewritten as

$$I_\omega(\mathbf{r}_\perp, 0) = \frac{I_\omega(\mathbf{r}_\perp, z)}{1 - \gamma \nabla_\perp^2 \phi_\omega(\mathbf{r}_\perp)}, \quad (3.8)$$

with γ a regularization parameter, which is a replacement of z/k , and controls the amount of correction [6, 23, 61]. The BAC algorithm is based on the assumption that the

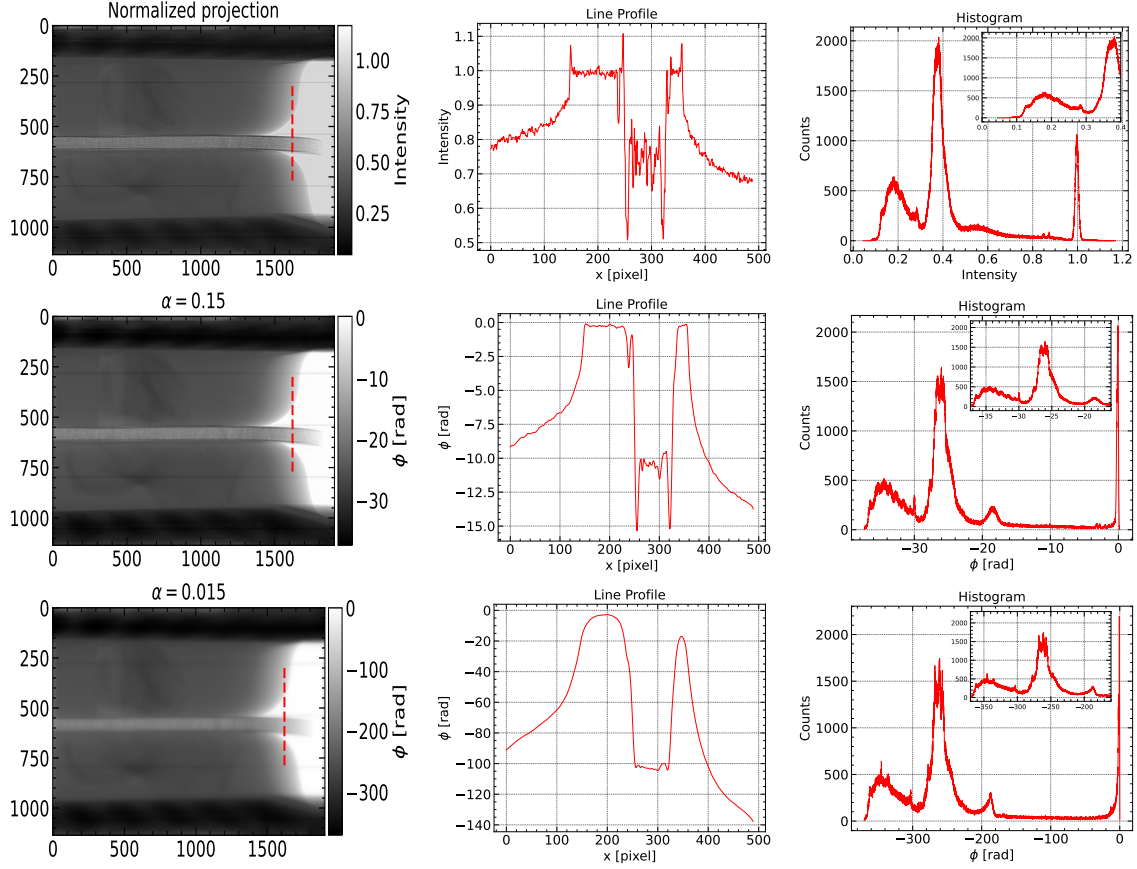


Figure 10: **Analysis of a normalized image, phase images of two FFPE blocks and image parameter selection in the MBA algorithm.** The **top row** presents the raw normalized projection of two FFPE blocks. The projection **left** is a composite image containing absorption as well as phase information. Phase information is represented as an enhancement of edges across materials of differing composition. **Middle:** a line profile of the line in the projected image (**left**) showing the presence of edge enhancement and thus indicating an optimal framework for phase retrieval. **Right:** histogram of normalized image with phase and absorption information. Features in the normalized image can be tracked to the relative counts in the images. The **middle** and **bottom rows** illustrate the retrieved phase distributions on the normalized image using the MBA algorithm with different regularization parameters (α). The **middle row** uses an optimal parameter of $\alpha = 0.15$, where excess edge enhancements are eliminated while retaining small-scale features. The **bottom row** demonstrates the effect of $\alpha = 0.015$, which represents an overshoot state leading to excessive blurring of the structural details.

phase distribution of the object is known, which is not the case in practice. However, this phase distribution can be approximated using the previously discussed [MBA](#) algorithm, such that equation (3.8) becomes

$$I_\omega(\mathbf{r}_\perp, 0) = \frac{I_\omega(\mathbf{r}_\perp, z)}{1 - \gamma \nabla_\perp^2 \phi_\omega^{\text{MBA}}(\mathbf{r}_\perp)}, \quad (3.9)$$

where $\phi_\omega^{\text{MBA}}(\mathbf{r}_\perp)$ is the phase distribution obtained from the modified Bronnikov algorithm (equation (3.7)). The parameter γ is a replacement for z/k , which serves as a regularization parameter to control the amount of correction [23]. By the work of the relation between the phase and attenuation, the desired phase distribution of the object is given by

$$\Theta_\omega(\mathbf{r}_\perp) = \frac{k_\omega}{2} \ln(I_\omega(\mathbf{r}_\perp, 0)), \quad \text{with } k_\omega = \frac{\delta_\omega}{\beta_\omega}, \quad (3.10)$$

assuming a homogeneous material composition, where k_ω is the ratio between the refractive index δ_ω and the attenuation coefficient β_ω . The parameter is, as already anticipated, a function of the photon energy, and in lab-based X-ray imaging with polychromatic radiation it becomes an experimental parameter. The method in equation (3.10) for phase retrieval is only valid in the Compton regime, in certain cases, where the phase and attenuation functions can be considered proportional to each other to the electron density.

Despite the fact that equation (3.10) is capable of phase retrieval using the mentioned phase attenuation duality [6], the [BAC](#) correction in equation (3.9), which effectively removes edge enhancements, is widely preferred and robust. The method is used by first choosing a suitable α parameter low enough that edge enhancements are just blurred out, without erosion of high frequency details, typically by visual inspection [23], as illustrated in fig. 10 and equation (3.7). The amplitude γ -parameter is then chosen such that the blurriness due to the [MBA](#) step is inverted. This is achieved by choosing γ such that the projected image becomes sharper while still avoiding overshoot in edge enhancement. Strictly speaking, the [BAC](#) algorithm is not a phase retrieval algorithm, but rather suppresses edge enhancements. It has been shown to perform very well on biological datasets [61].

3.2.3 Single Material Object (SMO)

One of the most widely used algorithms for phase retrieval, is the so-called single material object (SMO) phase retrieval, also referred to as *Paganin algorithm* [52, 53]. [SMO](#) is based on the assumption that the object of interest is a single homogeneous object with constant refractive index (δ_ω) and extinction coefficient of (β_ω), such that equations (2.24) and (2.25) can be rewritten as

$$\phi_\omega(\mathbf{r}_\perp) = -k\delta_\omega T(\mathbf{r}_\perp), \quad \text{and} \quad I_\omega(\mathbf{r}_\perp, 0) = I_\omega(\mathbf{r}_\perp, -d) \exp(-2k\beta_\omega T(\mathbf{r}_\perp))$$

with $T(\mathbf{r}_\perp)$ the projected line of sight ([LOS](#)) thickness, i.e. $T(\mathbf{r}_\perp) = \int_{-d}^0 dz$. Insertion of the above intensity distribution into the transport of intensity equation ([TIE](#)) equa-

tion (2.27), and use of the Fourier pseudo operators, as well as the above relation between phase and projected thickness, yields the phase distribution (equation (3.11))

$$\phi_{\omega}(\mathbf{r}_{\perp}) = \frac{k_{\omega}}{2} \ln \left[\mathcal{F}_{\perp}^{-1} \frac{\mathcal{F}_{\perp} \left(\frac{I_{\omega}(\mathbf{r}_{\perp}, z)}{I_{\omega}(\mathbf{r}_{\perp}, 0)} \right)}{\frac{k_{\omega} z}{2k} |\mathbf{k}_{\perp}|^2 + 1} \right], \quad \text{and} \quad k_{\omega} = \frac{\delta_{\omega}}{\beta_{\omega}} \quad (3.11)$$

where $\mathcal{F}_{\perp}$ the lateral Fourier transform (FT), $I_{\omega}(\mathbf{r}_{\perp}, 0)$ the intensity in the exit plane of the object. The ratio of the extinction coefficient (β_{ω}) to the refractive index (δ_{ω}), or k_{ω}^{-1} , is here referred to as the *betadelta* value. This constant parameter *betadelta* implies homogeneous and constant material composition while allowing for variation in density, which in the case of FFPE blocks, implies that tissues have the same composition as the surrounding paraffin wax. This is a good approximation for high-energy X-rays, as illustrated in (fig. 7), where both the extinction coefficient and the refractive index of the different samples become very similar to those of paraffin ($\text{C}_{25}\text{H}_{52}$), for increasing photon energy. However, in terms of refractive index, this approximation is also relatively stable for low-energy photons with a small variation in the extinction coefficient in the low-energy range (fig. 7). This results in the choice of *betadelta*-parameter being critical since a certain choice might eliminate or blur out certain features, but in general shows that the *Paganin* phase retrieval algorithm could be instrumental for phase retrieval on formalin-fixed paraffin-embedded (FFPE) blocks.

Although the *Paganin* phase retrieval method and modified bronnikov algorithm (MBA) are fundamentally different, they can be mathematically linked under certain conditions. For weak objects, equation (3.11) can be approximated by first-order expansion ($\ln(x) \approx x - 1$), yielding the MBA algorithm with $\alpha = 4\pi F/k_{\omega}$. However, in practice their performance may differ significantly. While SMO is robust against noise due to its low-pass characteristics, the BAC algorithm often preserves high-frequency details while removing edge enhancement [61].

With all tools now in place, which allow for the retrieval of the phase distribution in a single projected image of the object, attention is drawn to the problem of how this 2D information can be utilized for the 3D reconstruction of the phase distribution of the object. The solution is deeply rooted in the principles of tomography and the Radon transform (RT) or the X-ray transform and is discussed in the following section.

3.3 FUNDAMENTALS OF TOMOGRAPHY

In cone beam computed tomography (CBCT), the goal is the reconstruction of the attenuation function ($\mu(\mathbf{r})$) or phase distribution ($\delta(\mathbf{r})$) in case of absorption-contrast or phase-contrast tomography, respectively, as is briefly overviewed in section 3.1. These two imaging modalities are both based on the same principles and reconstruction algorithms, i.e. the Radon transform (RT) and or the X-ray transform. Assuming monochromatic rays, the Beer-Lambert law can be rewritten as equation (3.1), so that determining the attenuation function ($\mu(\mathbf{r})$) reduces to the computation of the line integrals (τ_{θ}) along paths $\ell(\theta, s)$, at different angles (θ), which can be inverted to recover $\mu(\mathbf{r})$. Likewise for the phase-contrast tomography, where the objective is to retrieve the phase distribution ($\delta(\mathbf{r})$) of the object, the projected quantity is the phase shift $\phi_{\theta}(\mathbf{r}_{\perp})$, which as explained in section 3.2, is inferred from the intensity distribution in the detector plane.

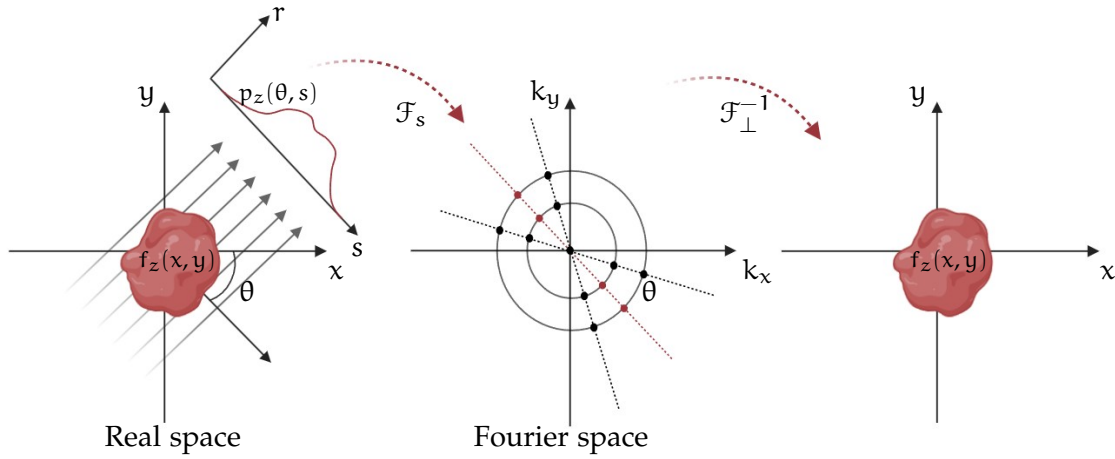


Figure 11: **Coordinate system for the Radon transform.** **Left:** the Radon transform (RT) integrates the function $f(\mathbf{r})$ along straight lines defined by the angle (θ) and the distance (s) from the origin, in respect to the laboratory coordinate system ($\mathbf{r}'$). **Middle:** the object in Fourier space as an illustration of the Fourier slice theorem (FST). **Right:** link between the lateral inverse Fourier transform (FT) and the filtered back projection (FBP), serving an inverse Radon transform (RT). The figures were created using [Biorender.com](https://biorender.com).

The algorithms available for the retrieval of a function ($f(\mathbf{r})$), from projected line integrals, are based on the Radon transform (RT) or the X-ray transform, which are both integral transforms [87].

3.3.1 Radon Transform (RT)

Although the objective function is a three-dimensional function, it is pedagogically useful, for the purpose of illustration, to consider its two-dimensional counterpart, thus restricting the discussion to a certain 2D slice, i.e. $f(\mathbf{r}) \mapsto f_z(x, y)$. Consider the geometric system in fig. 11, where the object is rotated around the z -axis, in the coordinate system $\mathbf{r} = (x, y, z)$. The laboratory system, composed of the detector and the source, is denoted as $\mathbf{r}' = (s, r, t)$, which is, in fact, related to the object's coordinate system by a rotation matrix R_z^θ , defined as

$$\mathbf{r} = R_z^\theta \mathbf{r}'. \quad (3.12)$$

Assuming without loss of generality that the rays in the laboratory system are parallel to the r -axis, the data recorded in a detector element with coordinate s in slice $t = z$ is given by a line integral of the function $f_z(x, y)$, along the r -axis, which, spelled out explicitly, reads

$$p_z(\theta, s) = \int f_z(x, y) dr, \quad (3.13)$$

for an arbitrary orientation (θ) of the object, with the indefinite integral taken along the ray span. Using the rotation matrix equation (3.12) in equation (3.13) yields the Radon transform (RT) ($\mathcal{R}$), which is an integral transform [87] of the function $f_z(x, y)$, and is defined as

$$p_z(\theta, s) = \mathcal{R}[f_z](\theta, s) = \int f_z(s \cos \theta - r \sin \theta, s \sin \theta + r \cos \theta) dr. \quad (3.14)$$

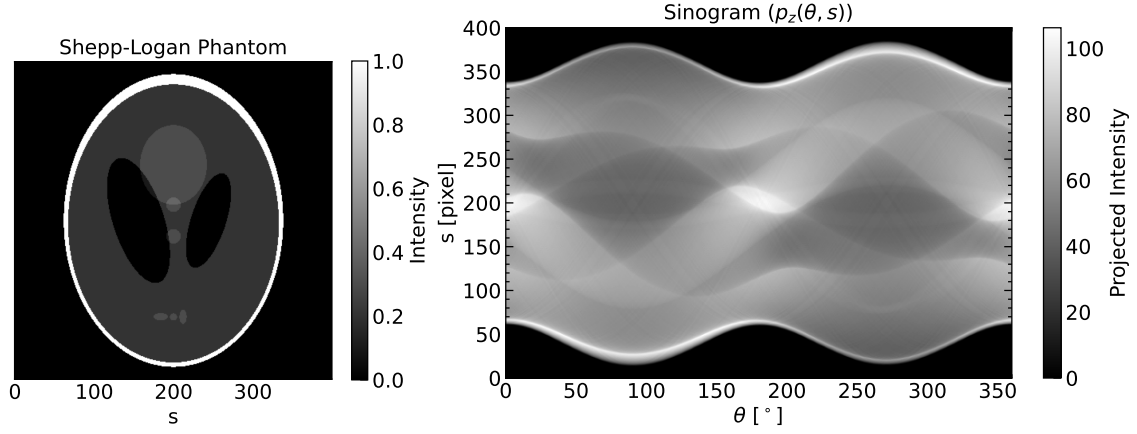


Figure 12: **Illustration of the Radon space of the Shepp-Logan phantom..** The Shepp-Logan phantom (left) and the sinogram (right). The sinogram is a 2D representation of a slice of the Shepp-Logan phantom. The x-axis represents the angle of the projection, while the y-axis represents the integrated pixel values.

Equation (3.14) suggests that the formation of projection images is carried out by a Radon integral transform, such that the search of the objective function $f_z(x, y)$ equates to finding the inverse of the Radon transform, denoted $\mathcal{R}^{-1}$ [61].

The representation of a certain slice ($f_z(x, y)$) of the object in its Radon space is referred to as *sinogram*, which is a 2D representation of the object, where the horizontal axis represents the different angles (θ) of the projection (p), while the vertical axis represents the integrated pixel values along the rays, i.e. s . This is illustrated for the Shepp-Logan phantom (fig. 12, left), where the pixel intensity values are integrated along the vertical axis, which is the r -axis in the RT, for different angles θ . The accumulated intensity along the horizontal axis (s) is then plotted against the angle of projection θ in (fig. 12, right), which is *sinogram*. The sinograms of all the virtual slices of the object, which is a 3D stack, are denoted as $p(\theta, s)$, with the name *sinogram* originating from the fact that point-like density distributions in the object, representable by Dirac delta functions, produce sinusoidal patterns in the Radon space.

3.3.2 Reconstruction

Given the sinogram ($p(\theta, s)$) and thus projections of the object at different angles (θ), for all slices, finding the inverse of the RT is what stands between finding the original object ($f(\mathbf{r})$). Methods for finding the inverse operator are discussed in the following.

Fourier slice theorem (FST)

Taking the FT (chapter A) of the radiograph (equation (3.14)) with respect to the variable s and transforming the coordinates results in

$$\begin{aligned}\hat{p}_z(\theta, k) &= \mathcal{F}_s \mathcal{R}[f_z](\theta, k) \\ &= (2\pi)^{-\frac{1}{2}} \iint dx dy f_z(x, y) \exp(-i(\mathbf{k}\mathbf{n}_\theta) \cdot \mathbf{r}_\perp) \\ &= (2\pi)^{\frac{1}{2}} \mathcal{F}_\perp[f_z](\mathbf{k}\mathbf{n}_\theta) = (2\pi)^{\frac{1}{2}} \hat{f}_z(\mathbf{k}\mathbf{n}_\theta).\end{aligned}$$

The above result is recognized as the lateral Fourier transform (FT) ($\mathcal{F}_\perp[f_z]$) of the objective function ($f_z(r_\perp)$) evaluated at $k\mathbf{n}_\theta = k(\cos\theta, \sin\theta)$, for arbitrary $k \in \mathbb{R}$. This illustrates that the Fourier transform (FT) of the projection with respect to the variable s is a line cut through the $\mathcal{F}[f]$ along $\mathbf{n}_\theta$, a theorem referred to as *Fourier slice theorem* (FST) [1].

Filtered Back Projection (FBP)

The Fourier slice theorem (FST) hints that knowledge of the Radon transform (RT) at set of angles (θ) is sufficient to reconstruct the FT of the original function ($\hat{f}_z$) and hence the original function ($f_z(x, y)$) by employment of the inverse Fourier transform (FT) [6, 61]. This method thus provides a framework for reconstruction by virtue of the inverse FT (chapter A), which is given by

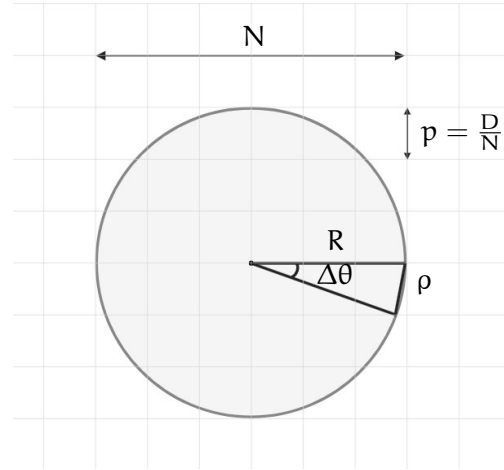
$$\begin{aligned} f_z(x, y) &= (2\pi)^{\frac{1}{2}} \mathcal{F}_\perp^{-1} \mathcal{F}_s \mathcal{R}[f_z](x, y) \\ &= (2\pi)^{-\frac{1}{2}} \int \int dk d\theta \, k \hat{p}_z(\theta, k) \exp(iks) \\ &= (2\pi)^{-1} \int_0^\pi d\theta \left[(2\pi)^{-\frac{1}{2}} \int dk \hat{p}_z(\theta, k) |k| \exp(iks) \right], \end{aligned}$$

where the symmetry $\hat{p}_z(\theta + \pi, k) = \hat{p}_z(\theta, -k)$ has been exploited to rewire the integral [61]. Above method for the retrieval of the function $f_z(x, y)$ involves the filtering of the FT of the projection data $\hat{p}_z(\theta, k)$ with a filter function $|k|$, followed by the back projection, and this method is known as the *filtered back projection* (FBP).

The filter function ($|k|$), which naturally arises from the coordinate transform, can be justified by the need to compensate for the decrease in the density of the data in the Fourier space, as illustrated in (fig. 11, middle). However, different modifications of the filter function ($\varphi(k)$) can be used, accounting for different characteristics at low and high frequencies. Some candidates include the Ram-Lak filter, which is the standard filter function, the Shepp-Logan filter, the Cosine, Hanning, and Hamming filters [6, 9].

Up to this point, it has been implicitly assumed that the rays are parallel; however, as will be discussed in chapter 5, typical lab-CT hosts diverging X-ray sources. The Radon transform (RT) and associated algorithms do not hold in a divergent beam setting where neighboring rays are not independent. For small cone beam angles, the RT and its accompanying methods serve as a good approximation; however, a transition to algorithms that account for the cone beam geometry is necessary whenever this approximation is no longer valid. This transition from parallel beam geometry to cone beam computed tomography (CBCT) is more involved and encompasses sophisticated algorithms such as the ray transform/X-ray transform and a retrieval method such as the Feldkamp-Davis-Kress algorithm (FDK) algorithm [9]. Although FDK will be at the forefront of the reconstruction of the data gathered in this thesis, it will not be discussed in more depth. The interested reader is referred to [1, 9, 27, 61, 74] for a more detailed discussion of the FDK algorithm and the X-ray transform. It is also important to note that during parallel beam CT, an angular range of $\theta \in [0^\circ, 180^\circ]$ is sufficient to reconstruct the objective function, however, in the case of cone beam computed tomography (CBCT), the minimal required angular range is extended to $\theta \in [0^\circ, 180^\circ + \gamma]$ with γ being the angle of the cone beam to avoid missing data [1, 61].

Figure 13: **Illustration of the Nyquist sampling theorem.** Given an object spanning N pixel elements of size p , with a cross sectional dimension of $D = 2R$, the minimal number of projection ($N_{\theta}^{\min}$) within an angular range of 2π is given by the requirement $\rho \leq p = D/N$. The figure was created using [Biorender.com](https://biorender.com).



3.3.3 Sampling theorem

Data gathering is inherently discrete in reality and therefore requires careful selection of data points. This selection of data from a larger set is called *sampling*. *Angular sampling* refers to the measurements/projections within the angular range, i. e. the number of angles taken within a certain angular range, and the way in which this is done. Different angular sampling schemes are available, including continuous and step-and-shoot sampling, etc., each with its own advantages and disadvantages, as illustrated by Cao et al. for SPECT imaging [10]. Moreover, regardless of the angular scheme used, the number of angular samples taken within a certain angular range is of more importance and is directly correlated with the resulting imaging quality. Given an angular range of, say $\theta \in [0^\circ, 360^\circ]$, the minimum number of projections required for accurate reconstruction is given by the Nyquist sampling theorem [56].

An intuitive and geometric consideration of this theorem can be easily understood by considering fig. 13, where an object of size $2R$ is projected onto N pixel elements of size p . The distance between two neighboring points on the circle is given by $\rho = R\Delta\theta$, with $\Delta\theta = 2\pi/N_{\theta}$ the angular sampling, where N_{θ} is the number of projections within $[0^\circ, 360^\circ]$. The requirement for accurate reconstruction and no loss of information is that the distance between neighboring points should be at most that of the size of the pixel, i. e. $\rho \leq p = D/N$, leading to

$$\frac{2\pi R}{N_{\theta}^{\min}} \leq \frac{D}{N} \Rightarrow N_{\theta}^{\min} \geq N\pi. \quad (3.15)$$

or equivalently, $\Delta\theta \leq 2/N_{\theta}^{\min}$. In case the requirement stated in equation (3.15) is not met, the reconstruction will suffer from loss in resolution and, in particular, radially towards the edge of the reconstructed slices. Even though equation (3.15) dictates the theoretical minimum number of projections, in practice, however, one can easily get away with fewer projections, for example $N_{\theta}^{\min} \approx N$, due to inherent blurring such as vibration, source spot size, blurring originating from reconstruction etc, deeming above requirement less valid.

3.4 ARTIFACTS

Computed tomography (CT)-artifacts are unwanted signal that shows up in reconstructed data. This unwanted signal originates from different mechanisms, such as movement artifacts, reconstruction error, detector errors, and/or incomplete data. A few examples of such artifacts that are of importance here, such as beam hardening artifacts, ring artifacts, streaking, and motion artifacts, are elaborated on below.

3.4.1 Beam hardening

Accustomed to the type of material and penetration depth, X-rays of certain energies are likely to be totally or partially blocked from reaching the detector, leading, in other words, to absorption. This phenomenon can be totally blamed on the polychromaticity of typical lab-CT sources, typically the *braking radiation*. As the X-ray beam passes through a sample, it becomes ‘harder’, in the sense that low-energy X-rays become more easily attenuated by the sample, such that the mean energy of the X-ray shifts to higher energies, which in turn results in lower attenuation coefficients. Beam hardening artifacts in reconstructed slices can be recognized as dark streaks and are more pronounced in CBCT, where, automatically, X-rays traverse a longer path through the sample, compared to parallel beam geometry, due to the diverging rays. One side effect of this is the so-called *cupping artifacts* [48], which leads to an apparent increase of the attenuation of the X-ray beam through the center of the reconstructed object. Cupping artifacts in CT reconstructed data manifest as a radial gradient in otherwise uniform gray values from the center of the reconstructed slices to the boundary of the reconstructed slices, as illustrated with a uniform cylindrical object in fig. 14. Given this cylindrical and uniform object, the LOS distance through the center of the sample is longer than the surrounding path, so that soft/low-energy X-rays through the center are relatively more absorbed than rays through the outer layer of the object with relatively shorter line of sight (LOS). This process induces a gradient in the refractive index within the slice in the outward direction. Only when this type of artifact is accounted for does the reconstructed slice reflect the attenuation or phase distribution of the sample.

Cupping artifacts can be mitigated whenever one finds that certain lower-energy X-ray photons are not contributing to the final image and thus can be eliminated by the employment of X-ray filters. Care in the selection of X-ray filters should also be taken into account in order not to reduce the photon counts of interest in the imaging.

3.4.2 Ring artifacts

A second type of CT artifact that appears in reconstructed slices originates from defective pixels in imaging detectors [48]. These defective pixels can be hot or dead pixels. The hot and dead pixels, and in general all pixels deviating from linearity, manifest themselves as stationary objects in the projected images or as straight lines in the sinogram. These errors, after reconstruction, show up as concentric circles. Ring artifacts can be suppressed by cooling the detector to a lower temperature in the case where thermal excitations of the detector element are dominant. However, when residual ring artifacts persist, post-processing ring removal algorithms as elaborated in [22, 66, 113] can be applied to eliminate these artifacts. Techniques such as dithering, as shown in [15], have

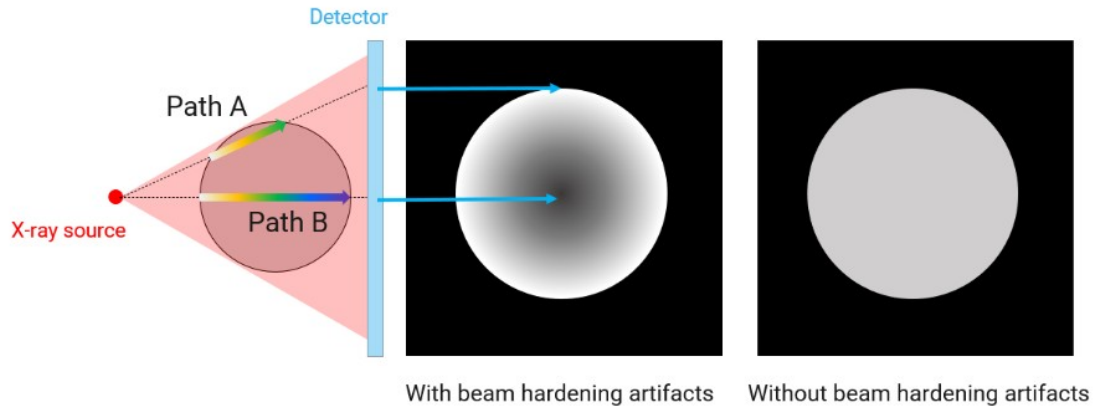


Figure 14: **Cupping artifact.** Schematic of the process leading to beam hardening artifacts or concretely *cupping artifacts*. The X-ray beam is attenuated more in the center of the sample than in the rays passing through the edges. The right-most image shows a reconstructed slice without cupping artifacts. This image is adapted from [3].

been shown to suppress ring removal artifacts and increase resolution while depressing noise in reconstructed data. Other candidates include application of median filter on projection images, filtering in Fourier space, neural networks, etc. [26].

In the course of this thesis, the ring artifacts will be removed by using the *ringremove* function incorporated in the *hotopy* package [40]. The algorithm invites a choice in the desired ring removal method, level, and spread parameter. For our purpose, the *wavelet* ring removal method was always chosen since this immediately yields desirable results.

Figure 15 shows a region of interest (ROI) of a reconstructed slice of a human FFPE bronchus sample and a slice after application of the ring removal algorithm. The figures in the first column of fig. 15 are both the raw reconstructed slice of the sample in a certain chosen ROI with no ring removal applied. The second column figures in fig. 15 both correspond to application of the *wavelet* and *additive* ring removal method, respectively, as implemented in the *hotopy* package [40]. The difference images of both corresponding ring-corrected slices and the raw slices are shown in the third column of the respective figures, on a difference color map. It can be seen that the rings in this slice are fairly well removed, however, it has been observed that the *wavelet* method has the tendency to remove signal from the tissue region, which is not desired. This can be seen in the images in the second column of fig. 15, where in certain ROI in the tissue region, where dominant ring artifacts were removed, there are induced ring artifacts from the ring removal process.

It is concluded, opposed to the *wavelet* method, that the *additive* method performs better in the removal of the rings without erosion of the signal from the tissues region. However, this algorithm does not always perform well in all slices. In general, it was found that the parameters of the algorithms can be adjusted to the user's preference, so that the *additive* method was eventually chosen as the standard for ring removal in the course of this work.

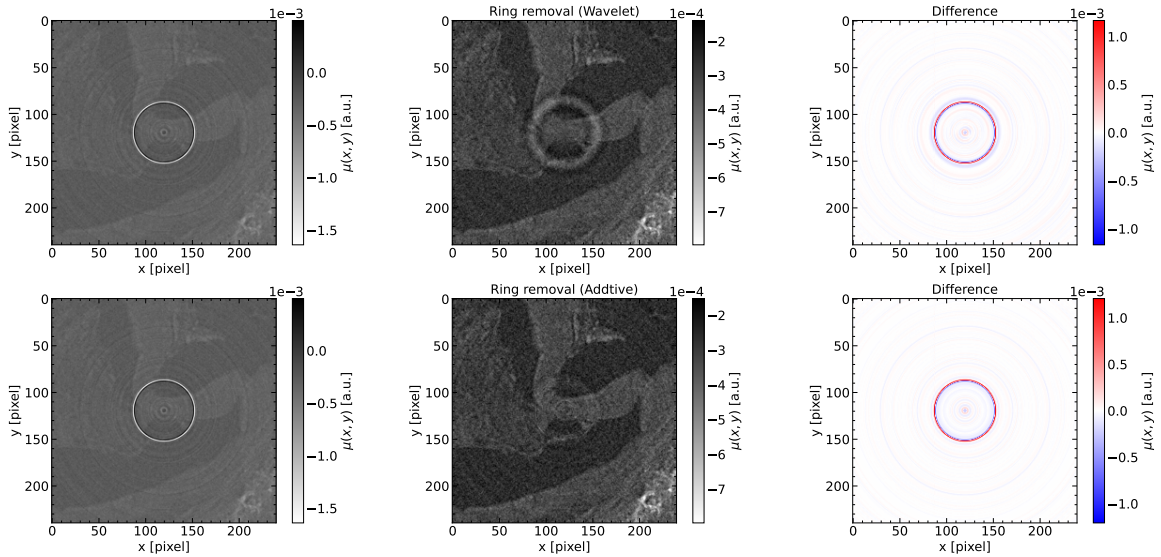


Figure 15: Ring artifact reduction using the *wavelet* and *additive* method in the **first** and **second** row respectively, as implemented in *hotopy* package [40]. The **first column** shows a reconstructed slice of a human *FFPE* bronchus sample with no ring removal applied. The **middle column** shows the reconstructed slice after application of the respective ring artifact reduction, while the last column image is the difference between the two images on a difference color map. The ring artifacts are clearly visible in the left-most images and are significantly reduced in the middle images as a result of the application of the algorithm. The *wavelet* algorithm has the tendency to remove and overcorrect the signal from the tissue region, while the *additive* method has a moderate effect on the tissue region.

3.4.3 Streak artifacts

Highly dense regions or absorbing materials, such as bone or materials with high atomic number composition, within the sample, here the tissue, or a sudden change in the material property with respect to the surrounding paraffin background, lead to streak artifacts. This leads to streaks of intensity near the interface between these materials, for example, at the interface of air-paraffin, dense material-paraffin, or bone-tissue, as well as the bone-paraffin interface.

3.4.4 Motion artifacts

Motion during acquisition induces a change in features with respect to time, i.e. $f(\mathbf{r}, t)$, leading to blurred or totally unrecognizable structures. This change in the objective function can be caused by the movement of the sample itself, vibration of the setup, source drift during the acquisition, etc. In case the motion effect occurs in a non-excessive manner, the reconstructed slices are deemed to be blurred due to the mingling of features in the sinograms originating from different sources/rays. In case the motion artifacts are excessive, then the reconstructed slices do not reflect the desired signal. Motion artifacts, in case predictable and non-excessive, can be corrected for by post-processing realigning algorithms and/or refitting of the sinogram using advanced algorithms. The acquisition of reference frames from a set of angles can also be per-

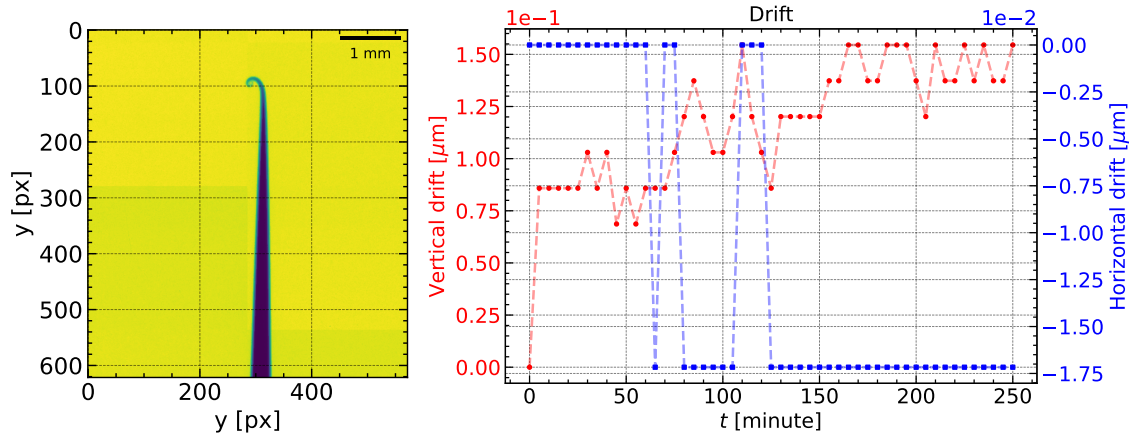


Figure 16: **Analysis of motion of stationary needle as a function of time.** The drift (right) is measured by correlation of consecutive radiographs of a stationary (left) needle using the phase correlation algorithm as implemented in [40]. The drift was initially measured in pixels, and the time is measured in minutes; however, the knowledge of the effective pixel size ($p_{\text{eff}} = 9.26 \mu\text{m}$) and magnification yields the effective drift in micrometers.

formed at the start/end of the tomographic scan and correlated with the projection images. Reshifting of the projected images allows mitigation of the motion artifacts.

To quantify the degree of motion at JuLiA setup, a stationary object (fig. 16, left) with a defined edge was imaged for a period of approximately 4 hours in a well-defined geometry, with $z_{01} = 160 \text{ mm}$, $M = 10.80$, $p_{\text{eff}} = 9.26 \mu\text{m}$ and $V_0 = 40 \text{ kV}$. The gathered projected images were corrected for dark- and flat-field effects. The images were then successively correlated with each other, resulting in a shift in position over time. The results of the analysis for the setup are shown in fig. 16, where it is apparent that the horizontal drift is minimal compared to the vertical drift. Yet still, the vertical drift and the total drift are relatively low, an indication of stability, thus deeming post-processing motion artifacts unnecessary.

3.4.5 Scattering artifacts

As briefly mentioned in section 2.3.2, deviation of X-ray photons from their original path leads to a false detection of incremental information in other pixels. This process is expected to occur uniformly with the bulk of the FFPE blocks, such that scattering within the sample leads to an incremental change in the detected intensity distributed evenly across all slices. However, near the interface between the paraffin-cassette and the paraffin-air interface, pronounced artifacts become apparent, likely arising from a combination of scattering, and cone beam artifacts. This makes the first few slices of the reconstructed data less informative. Additionally, as noted in equation (2.36), one can correct scattering within the bulk of the FFPE block by modeling and measuring the scattered signal and subtracting this from the slices or the projected images of the signal, for e. g. by employing an anti-scatter grid. Scattering artifacts within the formalin-fixed paraffin-embedded (FFPE) blocks manifest as streaking artifacts, similar to those already explained above. Given that the main focus is to optimize the acquisition of FFPE with lab-CT setups, scattering corrections will not be further discussed.

X-RAY GENERATION AND DETECTION

X-rays are a form of electromagnetic radiation with wavelengths ranging between 0.01 to 10 nanometers (nm), corresponding to photon energies between 100 eV and 250 keV. As all forms of electromagnetic rays, they can be produced when charged particles are decelerated, leading to the so-called *bremsstrahlung* (or *braking radiation*) or during a two-level process, producing *characteristic radiation*.

This chapter is divided into three main branches. The first discusses the generation of X-rays in lab-CT and different X-ray tubes. The second focuses on the spectrum of typical lab-CT X-ray sources, with emphasis on the *bremsstrahlung* and characteristic radiation. The last section discusses the detection of X-rays, including common examples of X-ray detectors and their operations.

Finally, the concluding two sections of this chapter cover the topic of signal correction for solid-state detectors as well as the imaging metrics employed throughout the course of this thesis.

4.1 GENERATION OF X-RAYS

The fundamental principles underlying X-ray generation in a lab-CT X-ray source can be tracked down to the same physical principles. However, variations in different additive techniques, often imposed to mitigate certain limitations, lead to different types of X-ray sources with different characteristics. The most common examples relevant to this thesis are discussed in the following.

4.1.1 Coolidge tube

While a lot more efficient techniques for generating X-rays exist, the simplest and most popular method for producing X-rays is a version of the one that Wilhelm Conrad Röntgen [83] used himself during his discovery of this penetrative radiation. An example of such a tube is shown in fig. 17 with the schematic in fig. 18 depicting the principle of such a tube in detail in two different geometries, i. e. *transmission* and *reflection*. This conventional tube for X-ray generation is referred to as a *Coolidge tube*, which is a vacuum tube containing a cathode and an anode [108]. The cathode is typically made of a filament that is heated to encourage the thermionic emission of electrons. The thermionic electrons are then accelerated by applying a peak voltage (V_0) across the anode and the cathode. The accelerated electron beam is focused with a series of electromagnetic lenses on the anode, which is typically made of high atomic number materials such as tungsten ($_{74}\text{W}$) and molybdenum ($_{42}\text{Mo}$), allowing for the conversion of the kinetic energy of the electrons into X-ray photons upon impingement through the processes called *characteristic emission* and *bremsstrahlung* (*braking radiation*) as briefly discussed in section 2.3. Acceleration as well as deceleration of electrons with orbital electrons, nuclei, etc., through electromagnetic interaction leads to the emission of a continuous spectrum of X-ray photons, called *bremsstrahlung* [71]. This continuous spectrum reaches a maxi-

mal energy (E_0) or equivalently a minimal wavelength (λ_0), dictated by the *Duane-Hunt law* [91], and reads as

$$\lambda_0 = \frac{hc}{E_0} = \frac{1240}{E_0[\text{keV}]} \text{ nm}, \quad \text{where } E_0 = eV_0 \quad (4.1)$$

with h , c , and e being Planck's constant, the speed of light in vacuum, and the elementary charge, respectively. Equivalently, this means that the broadness of the spectrum and the overall flux density ($\Phi \propto V_0^2$) are fully determined by the acceleration voltage (V_0), while the number of emitted X-ray photons is heavily dependent on the number of emitted thermionic electrons and thus on the anode current (i).

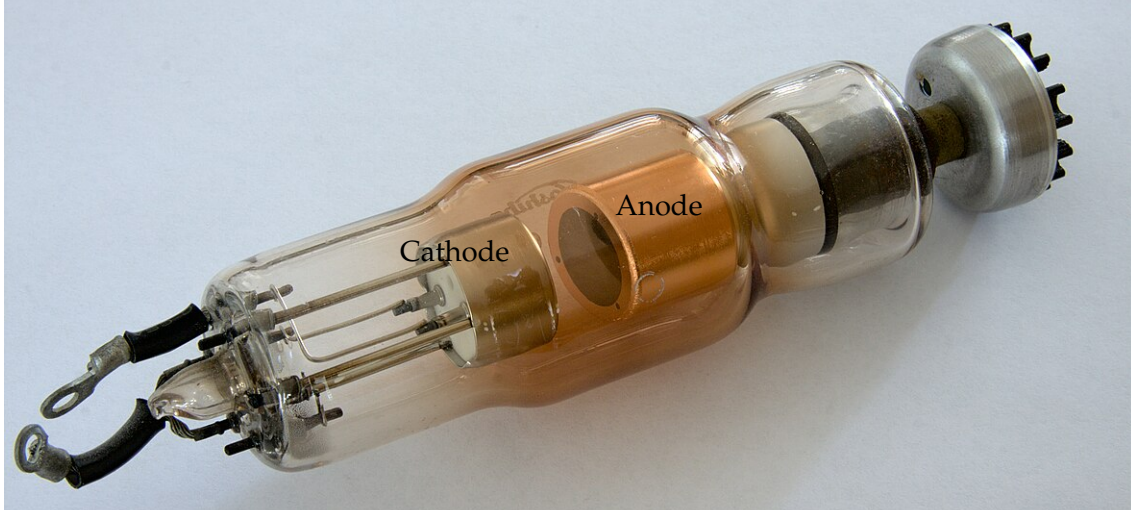


Figure 17: **Conventional X-ray tube** [108]. The anode is encapsulated in a copper sleeve, with the whole circuit embedded in a vacuum tube to improve efficiency.

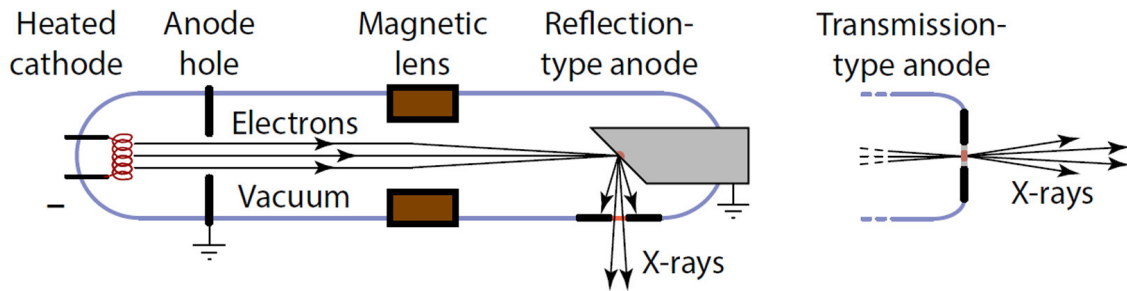


Figure 18: **Schematic of a conventional X-ray tube/solid-anode tube**, also known as **Coolidge tube**. The anode can be either in a transmission or reflection geometry, depending on the application and needs. The cathode is typically made of a filament, which can be heated by the application of a current, which encourages thermionic emission of electrons. This image is adapted from [5].

The observed spectrum of typical *bremsstrahlung*, for energies less than 100 keV, can be approximated by the *Kramer's law* [84, 91], that is

$$I_B(\lambda)d\lambda = K \left(\frac{\lambda}{\lambda_0} - 1 \right) \frac{d\lambda}{\lambda^2}, \quad (4.2)$$

where K is a material-dependent constant and λ_0 is the minimum wavelength of the *bremsstrahlung*, as given by equation (4.1). On top of the continuous spectrum, a typical

lab-CT spectrum is also superposed with peaks at certain energies (ε_j), defining the anode material, and is referred to as *characteristic radiation*.

Characteristic lines arise from the interaction of accelerated electrons with the inner shell electrons of the anode material (section 2.3.1). If the initial energy of the electron is sufficient to eject an electron from the atom, i.e. to cause ionization and leave a vacancy, then an electron from a higher shell may occupy this vacancy. The excess energy of this electron is then released as electromagnetic radiation, with energy (ε_j), i.e. $\varepsilon_j = E_i - E_f$, where E_i and E_f are the initial and final energy levels, respectively, of the electron. Alternatively, the excess energy can also be released through immediate emission of fluorescent photons.

Due to the stochastic nature of the interaction and the requirement for the incident photon to be high in energy, the characteristic radiation is heavily dependent on the peak acceleration voltage (V_0). In fact, characteristic radiation is only emitted if the incident electron has enough energy to overcome the binding energy of the inner shell electron. For a sufficiently high acceleration voltage, the intensity of the characteristic radiation can be modeled as [108]

$$I_e(E) \propto \begin{cases} Z(E - \varepsilon_j)^m & , E \geq \varepsilon_j \\ 0 & , E < \varepsilon_j \end{cases} \quad (4.3)$$

which is typically orders of magnitude larger than the *bremsstrahlung*, again given a sufficient incident peak energy E_0 , with $m \approx 3/2$, and Z the atomic number of the anode material.

Theoretically, and as already insinuated by equations (4.1) and (4.2), there is no upper limit on the wavelength (λ) of X-rays, or equivalently lower limit on the X-ray energy (E) of X-rays that can be generated through *bremsstrahlung*, thus suggesting a divergence for ($\lambda \rightarrow \infty$). However, this has in reality never been met as ultra-soft X-rays are easily self-absorbed by the components and anode material. Additionally, the generated X-ray beam, typically perpendicular to the initial electron beam, then passes through a window made of a lower atomic material such as beryllium ($_4\text{Be}$), aluminum ($_{13}\text{Al}$), etc, which further ensures the absorption of low-energy unwanted X-ray photons. As an illustration, the continuous spectrum based on equation (4.2) is simulated for two peak voltages (V_0), and is plotted in fig. 19 (left).

It is of importance to note that only a small fraction, typically less than 1% of the total energy [108], of the initial kinetic energy of the electron beam is invested in the generation of X-ray photons, meaning that approximately 99% of the initial energy is dissipated as heat at the focal point of the anode material. Generated heat thus makes the cooling of the anode a necessary task in order to avoid damage and increase longevity.

Although Coolidge tubes and all other related tubes are, due to their simplicity, easy to design and produce at low cost, they are highly limited in terms of their maximum operating power and intensity due to the above-mentioned heat load [108]. This invites the need for more advanced X-ray tubes, such as those described hereafter.

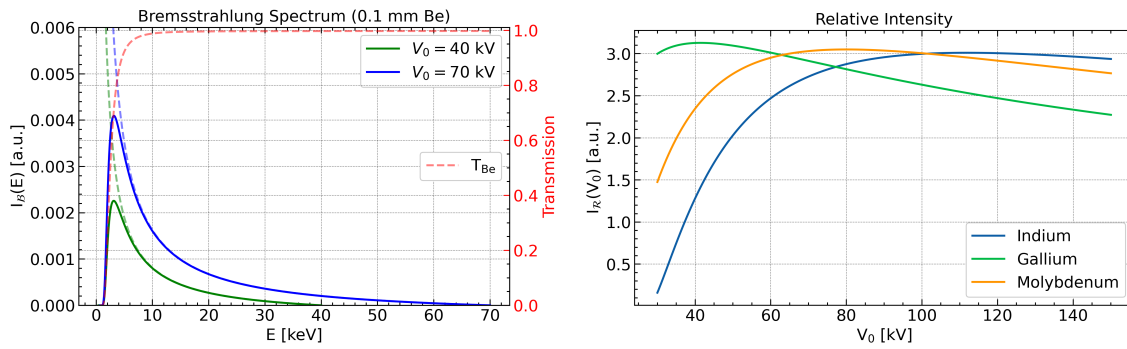


Figure 19: **Left:** relative intensity of *bremsstrahlung* for two peak voltages according to equation (4.2). This continuous spectrum diverges for low energies, denoted by dotted lines, and shielded with low-energy absorbing material (0.1mm Be). These spectra were modeled using Kramer's law. **Right:** the relative intensity of the characteristic radiation to the total intensity of the *bremsstrahlung* is shown equation (4.6) in arbitrary units for the main characteristic radiation of galinstan. The relative intensity reaches a maximum for an acceleration energy of $E_0 = 4\mathcal{E}_j$, which for gallium is reached at 41.42 keV.

4.1.2 Rotating anode

Rotating anode sources have a rotating anode around their central axis, such that the heat generated by the impingement of the electron beam is distributed over the entire surface of the anode. This type of anode allows for higher power, intensity, improved performance, and enhanced heat dissipation, unlike the conventional Coolidge tubes described in section 4.1.1.

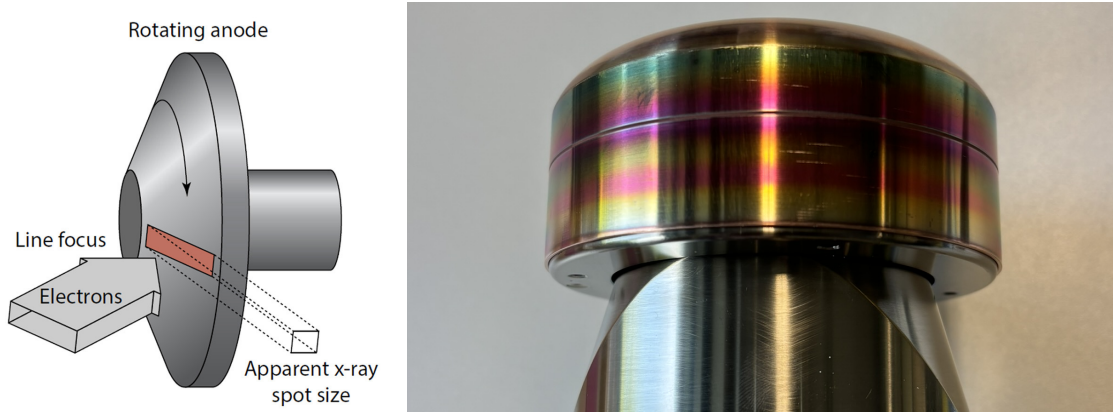


Figure 20: **Rotating anode X-ray system.** **Left:** schematic of a rotating anode [5]. The anode is typically made of a high atomic number material and is rotated at high speed to aid the distribution of dissipated heat by the electron beam and to improve longevity while allowing high-power X-ray generation. **Right:** a picture of a rotating anode of TONI setup, where damage has been induced at the line focus.

Figure 20 (left) shows a schematic of a rotating anode of a typical X-ray tube. It can be seen that the energy deposited by the high-intensity electron beam creates a dent (fig. 20, right) over a long period. The positioning of the electron beam can be controlled by electron optics, allowing the user to shift the focal spot on the anode.

Rotating anodes can be considered as a bridge between conventional solid anodes and more advanced liquid-metal jet anodes, as they both aim to improve heat dissipation and allow for higher X-ray output. The latter, more advanced type of X-ray source is discussed in the following subsection.

4.1.3 Liquid-metal jet anode

In a liquid-metal jet anode, the solid anode, as in sections 4.1.1 and 4.1.2, is replaced by a liquid-metal jet, which now acts as the target. The liquid-metal alloy is composed of different materials that are liquid at room temperature and is continuously pumped through a circulatory system under near-vacuum conditions (pressure of the order of 10^{-5} – 10^{-7} mbar), allowing for continuous cooling of the anode. The liquid is regenerated by the continuous flow, making it more resistant to damage and wear, which leads to sources with higher X-ray brilliance potential while maintaining a small focal spot (s). A schematic of the operation of a liquid-metal jet anode X-ray tube is shown in fig. 21, where an incident electron beam on the circulating liquid-metal alloy leads to the emission of X-rays. The whole circuit is embedded in a vacuum tube to improve efficiency and prevent oxidation of the liquid-metal and other inner components.

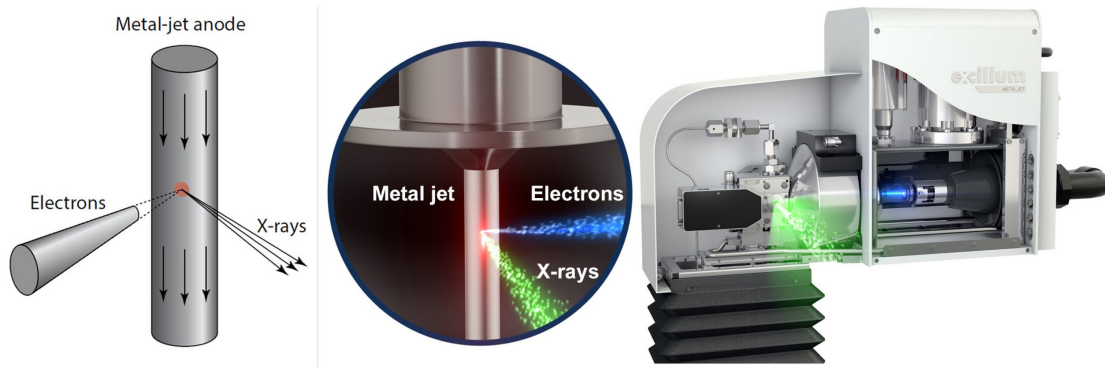


Figure 21: **Schematic of a liquid-metal jet anode X-ray tube.** The liquid-metal jet is pumped through a system at high speed and is continuously cooled. Focusing the electron beam on the liquid-metal jet thus leads to emission of characteristic and *bremsstrahlung* spectra. The above schematic is adapted from [5].

Experiments conducted in this thesis utilize a liquid-metal jet anode X-ray tube, benefiting from the high X-ray brilliance and small micro-focal spot, and are described in more detail in section 5.1.1.

4.2 SPECTRUM OF X-RAYS

Kramer's law (equation (4.2)) can conversely be expressed in terms of energy, resulting in

$$I_B(E)dE = I(\lambda) \left| \frac{d\lambda}{dE} \right| dE = K \left(\frac{E_0}{E} - 1 \right) dE, \quad (4.4)$$

where K is a material-dependent constant. Figure 19 (left) presents a simulation of such a broad spectrum for two peak voltages. The weighted *bremsstrahlung* average intensity yield is given by

$$I_{\mathcal{B}}^{\text{tot}} = \int_0^{E_0} E I_{\mathcal{B}}(E) dE = \frac{KE_0^2}{2} \propto E_0^2 \quad (4.5)$$

and thus proportional to the peak voltage squared. Hence, the combination of equations (4.3) to (4.5) shows that the relative intensity of the characteristic radiation to the total intensity of the *bremsstrahlung* is

$$I_{\mathcal{R}} = \frac{I_{\mathcal{C}}}{I_{\mathcal{B}}^{\text{tot}}} \propto \frac{(E_0 - \mathcal{E}_j)^m}{E_0^2}, \quad (4.6)$$

where $\mathcal{E}_j$ is the critical energy or the binding energy required for the emission of characteristic radiation. From equation (4.6), we learn that the relative intensity reaches a maximum for an acceleration energy of $E_0 = 4\mathcal{E}_j$. Figure 19 shows the simulated relative intensity of the characteristic radiation to total yield in *bremsstrahlung* for the main characteristic radiation line of gallium ($_{31}\text{Ga}$), indium ($_{49}\text{In}$) and molybdenum ($_{42}\text{Mo}$) with respect to energy. It can be seen that $I_{\mathcal{R}}$ reaches a maximum for a peak energy of $E_0 = 4\mathcal{E}_j$ that for $\text{Ga}\alpha_{\alpha}$ is reached at 41.42 keV.

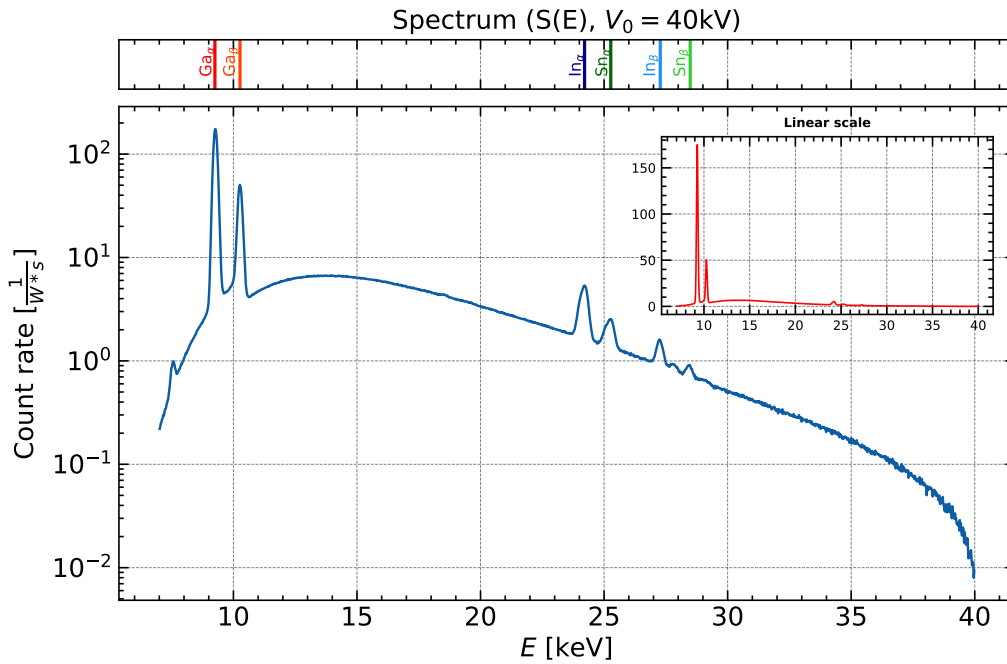


Figure 22: **Spectrum of JuLiA setup** (section 5.1.1) both on a semi-log scale (main) and on a linear scale (inset) for a peak voltage of $V_0 = 40$ kV. Characteristic lines such as that of gallium ($_{31}\text{Ga}$) K_{α} -edge at 9.251 keV and indium ($_{49}\text{In}$) at 24.210 keV originating from the gallium alloy are clearly visible and indicated. The superimposed broad spectrum is the *bremsstrahlung*. This spectrum was measured with a X-123 Si-PIN detector from Amptek with a resolution of 139 – 260 eV at 5.9 keV [110].

An example of such a spectrum is shown in fig. 22 for a peak voltage of $V_0 = 40$ kV. The X-ray spectra was recorded using an Amptek X-123 spectrometer with a Si-PIN detector (section D.1.1). The characteristic peaks originating from components in galinstan are clearly above the continuous broad spectrum and are indicated. This spectrum is

of the Just a Liquid Anode (JuLiA) instrument and is elaborated and discussed in section 5.1.1. The superimposed broad spectrum is indicative of the *bremsstrahlung*, and for a peak voltage of $V_0 = 40$ kV, is of almost the same magnitude as the characteristic lines stemming from the indium and tin in galinstan. It can be seen that the characteristic K_α and K_β radiation of gallium, here labeled Ga_α and Ga_β , respectively, are orders of magnitude higher than the *bremsstrahlung*, not only due to the ratio of gallium in galinstan (section 5.1.1) but also due to the peak voltage well above the critical energy of these lines, as discussed in the previous paragraph.

4.3 DETECTION OF X-RAYS

The X-ray detector is a critical component of any tomographic imaging system, responsible for capturing attenuated photons and quantifying them. It plays an important role in reconstructed data, such as noise. Its performance directly impacts the accuracy and reliability of the final results of the radiographs or reconstructed slices. The following provides an overview of the working principles of X-ray detectors in general and flat-panel detector (FPD) that will be utilized in this thesis.

4.3.1 Basic working principle

X-ray detectors operate by converting incident X-ray photons into quantifiable results, which can be achieved through direct detection and indirect detection [6]. Upon interaction of an X-ray photon with the detector material, it deposits energy through processes such as *photoelectric absorption*, *Compton scattering*, etc., depending on the dynamics as already elaborated in section 2.3.

In direct detection, the detector material immediately generates charge carriers (electrons and holes) [6, 107], from the absorbed X-ray photons. These charge carriers are then subsequently measured by readout electronics.

In contrast to direct X-ray detection, indirect X-ray detection utilizes a scintillator material (e.g., cesium iodide CsI, amorphous-silicon), which converts the X-rays into visible light. These visible photons are then detected by a photodiode array or other optical sensors, which convert the light into signals that can be stored and manipulated digitally. The choice of scintillator material is crucial and is chosen on the basis of the desired properties and required efficiency. Figure 23 shows a schematic of the operation of such an indirect detection system, where incoming X-rays are incident on a scintillator layer (blue) and converted to visible light, which is then detected by the integrated photodetector array (IC).

Flat Panel Detectors (FPD)

In essence, a FPD operates similarly to all other indirect X-ray detectors and is thus constructed of a scintillator material that converts X-rays into visible light and is read out by photodetectors. However, FPD makes use of stack/multiple layers (row and column) of such individual indirect detectors, which are arranged in a two-dimensional array, allowing simultaneous detection of X-rays over a large area [9]. This is in such a manner to maximize the *fill factor* (f), which is the ratio of the area of the active detector elements to the total area of the detector. This design allows for the manufacturing

Figure 23: **Schematic layout of the working of an indirect X-ray detector.** The X-ray photons are converted into visible photons by a scintillator layer, which is then detected by an array of photodetectors. The photodetectors convert the visible light into electrical signals. Image adapted from [34].

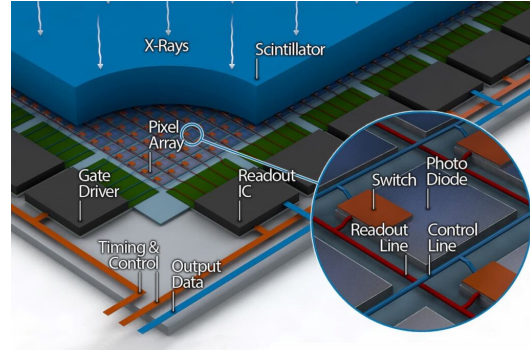
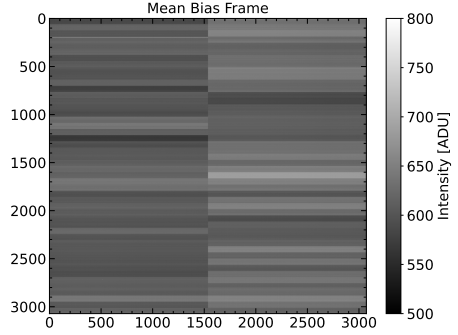


Figure 24: **Panels in a FPD:** illustration of the individual sub-detector panels within a flat panel detector. Each sub-panel is composed of a scintillator and readout electronics, arranged over a large area, with a high fill factor.



of highly efficient X-ray detectors and large detectors. As shown in the average bias frame in fig. 24, the bias voltage applied to the detector elements makes it even possible to visualize the different panels of the detector array. The characterization of the FPD mainly used in this thesis will be discussed in the following chapters.

4.4 SIGNAL PROCESSING

During imaging, a general recorded intensity ($I'(n, m)$) by X-ray detectors is contaminated by unwanted signals, through a different process, popularly referred to as *noise*. Such an intensity frame, called *raw frame*, can be expressed as

$$I'(n, m) = I_B(n, m) + I_D(n, m) + I_F(n, m) \cdot I(n, m) + \sigma(n, m), \quad (4.7)$$

where $I_B(n, m)$ is the *bias signal*, $I_D(n, m)$ the *dark current*, $I_F(n, m)$ the *flat-field* and $\sigma(n, m)$ the *noise*, with (n, m) or alternatively $(r_\perp)$ being the pixel coordinates. In the following, the pixel coordinates are dropped for brevity, and all operations are considered pixel-wise unless otherwise stated. The raw frame is thus a combination of the desired signal (I) and unwanted signals (I_B , I_D , I_F , σ). Different contributions of the raw image are dependent on the exposure time (t) of the detector, so that equation (4.7) can be rewritten as

$$I'(t) = I_B + I_D(t) + I_F(t) \cdot I(t) + \sigma(t). \quad (4.8)$$

Unwanted signals may be of *stochastic/random* or *deterministic/systematic* in nature. While *deterministic noise*, as the name implies, is predictable and therefore can be corrected for, *random noise* (σ), on the contrary, is completely stochastic, implying that it cannot be predicted or corrected. Correction of deterministic noise is achieved by modeling *calibration frames* and is discussed in the following section.

4.4.1 Calibration frames

Bias frames

Even though the bias signal (I_B) is typically not dealt with in X-ray imaging, it is an unwanted signal that is present in all raw frames and exposes the noise characteristic of a detector. The bias signal is intrinsically due to the manner in which a solid-state detector converts an analog signal to a digital signal. Since intensity readout must physically be a positive integer, detector readout electronics are given an artificial floor voltage, which manifests itself as a floor signal in the output. However, due to thermal excitations in electronics and small changes in these floor voltages, this frame also exhibits a degree of uncertainty, called the *read noise* (σ_R) of the detector, which is stochastic in nature.

The bias frame (I'_B) is temperature independent and can be accessed by taking a frame with an exposure length of zero seconds ($t = 0$, equation (4.8)), or the shortest exposure length the detector allows, in the absence of X-rays. Bias images (I'_B), i. e. $I'_B = I_B + \sigma_R$, with I_B the true bias signal, can be used to access the read noise (σ_R) of a detector. This is done by subtracting multiple bias frames from each other and accessing the standard deviation (section B.2.1.2). Figure 24 shows an example of an average bias frame, which not only exposes the sub-panels within a FPD, but also the subtle differences in the bias signal of the sub-panels are apparent.

Dark field

Dark current (I_D) can be defined as the signal/current flowing through a detector when there are no incident X-ray photons, and thus no photoelectrons, for a non-zero exposure time ($t > 0$ s). Thermal excitation of charge carriers (electrons) in detector readouts leads to this false detection of photoelectrons and is the cause of dark current. This signal hence defines a floor level, which is to be exceeded in order to detect a photoelectron.

The thermal excitation of electrons is temperature (T) and can, as a result, be suppressed by cooling of the detector. However, this stochastic Poisson process is also exposure time dependent (t), leading to a dark noise of the form

$$\sigma_D(t) = \sqrt{I_D \cdot t}. \quad (4.9)$$

An unwanted signal of this nature also depends on other properties of the pixel, such as its position in the detector matrix. A dark frame is acquired by taking a frame with desired exposure length (t) without incident X-ray photons, i. e.

$$I'_D(t) = I_D \cdot t + I_B + \sigma_D(t) + \sigma_R. \quad (4.10)$$

To access the true dark current, the dark frame must still be corrected for the bias signal as discussed in section 4.4.1. In contrast to the bias signal, which is already included within dark frames equation (4.10), dark frames themselves play a significant role in radiograph calibration, as will be elaborated upon in the following discussion.

Empty-beam correction

X-ray projection images are also affected by variation in pixel-to-pixel gain (efficiency) in sensitivity and beam inhomogeneity. This multiplicative factor is known as *flat-field* (I_F) and represents the detector and system response.

A flat frame is typically acquired by taking a frame with the sample removed (empty-beam) with the same source, geometric, and detector settings. This frame is also infused with unwanted signals, as discussed above, and is given by

$$I'_F(t) = I_F(t) + I_D \cdot t + I_B + \sigma_F(t) + \sigma_D(t) + \sigma_R. \quad (4.11)$$

Even if corrected for other fixed-pattern signals, a flat-field frame still possesses noise which is attributed to the photon arrival statistics, which is Poisson distributed. This type of noise, known as *photon shot noise*, is dependent on the number of photoelectrons detected (n) such that $\sigma_n^2 = n = \eta \cdot t$, where η represents the photoelectron count rate.

In general, the total effective noise (σ) in any arbitrary frame of an X-ray detector can be expressed as

$$\sigma_{eff} = \sqrt{\sigma_n^2 + \sigma_D^2 + \sigma_R^2} = \sqrt{\eta \cdot t + I_D \cdot t + \sigma_R^2} \quad (4.12)$$

with η the number of detector photons per time, I_D the dark current, and σ_R the read noise. With these tools all in place, it can now be noted that an arbitrary frame can be corrected for dark signal and inhomogeneities by the following manual

$$I_j(\mathbf{r}_\perp) = \frac{I'_j(\mathbf{r}_\perp) - I'_D(\mathbf{r}_\perp)}{I'_F(\mathbf{r}_\perp) - I'_D(\mathbf{r}_\perp)}, \quad (4.13)$$

where an arbitrary projection image ($I'_j(\mathbf{r}_\perp)$) is first corrected by a dark field ($I'_D(\mathbf{r}_\perp)$) of the same exposure length acquired at the same detector settings and then corrected by the dark-corrected flat-field, again of the same exposure length. The above method for flat-field correction will in the following be referred to as conventional flat (**CF**) correction. Note that in the last, the notation of the pixel coordinates ($\mathbf{r}_\perp$) has been made explicit.

Dynamic flat-field correction

Traditional flat-field correction (equation (4.13)) might not produce satisfactory results in tomography. Specifically, in scenario of imaging **FFPE**, the standard flat-field only corrects the frame with respect to air. Employing dynamic flat-field correction can be beneficial in improving contrast and signal gain by normalizing the sample's attenuation against the paraffin background [54, 86, 112].

This empty-beam correction is approached here in the following different ways

- In the first approach, paraffin-embedded tissue is treated as a slow absorbing and homogeneous medium, such that after correction for beam inhomogeneities and detector efficiency according to equation (4.13), with I'_F taken in air, projection images ($I_\theta(\mathbf{r}_\perp)$) are averaged to obtain a single representative paraffin-flat field ($I_F^*(\mathbf{r}_\perp)$). All projection images are then further normalized by this average flat-field image, i. e.

$$I_\theta(\mathbf{r}_\perp) \rightarrow \frac{I_\theta(\mathbf{r}_\perp)}{I_F^*(\mathbf{r}_\perp)}, \quad \text{with} \quad I_F^*(\mathbf{r}_\perp) = \frac{1}{N} \sum_{\theta}^{N_\theta} I_\theta(\mathbf{r}_\perp). \quad (4.14)$$

The aforementioned approach for flat-field correction will henceforth be referred to as *slowly varying background flat (SVBF)* correction.

- In the second approach, flat-field correction by paraffin signal is carried out by first considering the conventional correction (equation (4.13)) and then considering the idea of a slowly varying envelope. In this respect, projection images are corrected for by the construction of an *eigen-flat* (I_F^θ) for each projection as

$$I_\theta(\mathbf{r}_\perp) \rightarrow \frac{I_\theta(\mathbf{r}_\perp)}{I_F^\theta(\mathbf{r}_\perp)}, \quad (4.15)$$

where $I_F^\theta(\mathbf{r}_\perp)$ is obtained by selection of region of interests **ROIs** from the initial projection image in a paraffin-only region and averaging over the selected regions. This average **ROI** is then used to fabricate a uniform frame with the same mean intensity ($I_F^\theta(\mathbf{r}_\perp)$), for each projection angle (θ), and hence the name *eigen-flat (EF)* correction.

Different and simpler approaches to the above-mentioned considerations may also be considered and will be illustrated in the following chapters.

4.5 IMAGE METRICS

There are many metrics to access the quality of an image or even compare two images. Some of the most relevant metrics in the literature for resolution, noise, and signal estimates are defined and briefly elaborated upon in the following.

4.5.1 Signal-to-noise-ratio (SNR)

The signal-to-noise ratio (**SNR**) is a measure of signal intensity and noise in an image. It is defined as the ratio of the mean intensity ($\bar{I}_A$) of a **ROI**, indexed by A , to the standard deviation of noise in the region (σ_A). Mathematically, **SNR**, is given by

$$\text{SNR} = \frac{\bar{I}_A}{\sigma_A}, \quad (4.16)$$

serving as a simple measure of the quality of the image in terms of available signal. The **SNR** is sensitive to the selected **ROI** such that during the comparison of images, the same **ROI** must be selected to minimize bias.

Furthermore, when comparing scans of different set parameters, etc., the objective assessment of the **SNR** requires taking into account the independent variables. The **SNR** can be related to the scanning parameters as

$$\text{SNR} \propto \sqrt{N} \propto \sqrt{\Phi \times t} \propto \frac{\sqrt{t}}{z_{02}} \quad (4.17)$$

where N is the number of projections/photon counts, Φ the fluence, t the exposure time and source-to-detector distance (**SDD**) (z_{02}) ($\Phi \propto z_{02}^{-2}$) (section B.2). In particular, it is of interest to express the **SNR** (equation 4.17) in terms of an effective **SNR**, i.e. SNR_0 , which depends on other independent factors such as X-ray filters, sample composition, integrated parameters, etc.

$$\text{SNR} \equiv \text{SNR}_0 \frac{\sqrt{t \cdot i}}{z_{02}} \quad (4.18)$$

The **SNR** automatically favors data with higher photon counts and consequently lower noise. However, this metric alone is not sufficient to evaluate the quality of the reconstructed slices. In this account, the **CNR** is a more favorable metric, providing not only the amount of signal and noise but also how visible features are with respect to the background.

4.5.2 Contrast-to-noise ratio (CNR)

For simple evaluation of scan qualities up to artifact and resolution assessment, the **CNR** in its standard definition will mainly be the quantity of merit to be considered. It is a metric that quantifies the amount of contrast of a feature with respect to a certain reference, typically the background signal. The **CNR** in its simplest form is defined as

$$\text{CNR} = \frac{|\bar{I}_A - \bar{I}_B|}{\sqrt{\sigma_A^2 + \sigma_B^2}}, \quad (4.19)$$

with $\bar{I}_A$ and $\bar{I}_B$ being the mean signal in the region of interest (**ROI**) A and B, respectively, and σ_A and σ_B the noise in the respective **ROI**. A higher **CNR** indicates high contrast ($|\bar{I}_A - \bar{I}_B|$) and visibility of the features against the background signal and or low noise.

Similarly to the discussion in section 4.5.1, the **CNR** is also subjectively sensitive to the selection of a **ROI**, and hence **ROI** must be maintained during the comparison of images. Similarly, **CNR** may be subjected to normalization conditions discussed in equations (4.17) and (4.18) by virtue of the discussion of image operations discussed in sections B.2 and B.2.1.

4.5.3 Fringe Visibility

A specific form of the Michelson contrast, termed *visibility*, defines the fringe enhancement as a measure of the quality of the fringe pattern or coherence. In other words, it serves as an experimentally observable indicating how coherent the system is for phase-contrast imaging. This unitless metric ($\mathcal{V}$) ranging from 0 to 1, is defined as

$$\mathcal{V} = \frac{I_{\max} - I_{\min}}{I_{\max} + I_{\min}} \quad (4.20)$$

where $I_{\max}$ and $I_{\min}$ denote the maximum and minimum measured intensity [61]. This simply implies that a well-optimized X-ray system will possess a visibility close to 1, while a poorly tuned and non-coherent system will have a contrast close to 0.

4.5.4 Fourier shell correlation (FSC)

The Fourier shell correlation (**FSC**) is a metric that aids in the evaluation of the fidelity of two data sets and particularly resolution measurement when talking in terms of images of reconstructed volumes [61, 78]. It is defined as the normalized cross-correlation of shells between two signals, f_j and f_l in Fourier space, i.e. $\hat{f}_j$ and $\hat{f}_l$. Assuming that the data being dealt with is discrete, the **FSC** is then defined as

$$\text{FSC}(k_r) = \frac{\sum_k^{k_r} \hat{f}_j(k) \cdot \hat{f}_l^*(k)}{\sqrt{\sum_k^{k_r} |\hat{f}_j(k)|^2 \cdot \sum_k^{k_l} |\hat{f}_l(k)|^2}}, \quad (4.21)$$

where $k = \sqrt{\mathbf{k} \cdot \mathbf{k}}$ refers to the frequency in Fourier space and k_r the shell radius [78]. Equation (4.21) implies that $\text{FSC}(k_r) = 1$, if $\hat{f}_j$ and $\hat{f}_l$ are identical, while $\text{FSC}(k_r) = 0$ if they are completely uncorrelated.

The FSC is subjected to a half-bit threshold curve to define a scale where the data maintains correlation [77], and thus serving as a criterion for resolution estimate. It mathematically expressed as

$$\sigma_{1/2}(k_r) = \frac{0.2071 + 1.9102/\sqrt{n(k_r)}}{1.2071 + 0.9102/\sqrt{n(k_r)}}, \quad (4.22)$$

with $n(k_r)$ the number of data points within the shell at radius k_r . The frequency at which the FSC intersects this curve, is defined as the resolution of the data [77].

Part III

3D VIRTUAL HISTOLOGY

EXPERIMENTAL SETUP AND METHODOLOGY

In this chapter, an overview of the experimental setups, detectors, and methodologies used throughout this thesis is provided. The purpose of this chapter is thus to describe the components and conditions under which tomographic data were acquired, and pave the way for how these data were gathered in the first place, as well as the context for the analysis and results presented in the following chapters.

5.1 X-RAY SETUPS

5.1.1 *JuLiA*

The main experimental setup, here referred to as *JuLiA*, is a liquid-metal jet anode source [35] manufactured and assembled by Excillum at Institut für Röntgenphysik (IRP). Unlike conventional X-ray tubes, where the anode is solid, this setup features a circulating liquid anode, which offers the potential for achieving relatively high brilliance in a laboratory environment, as discussed in section 4.1.3. The *JuLiA*-setup consists of a microfocus X-ray tube [35] utilizing a galinstan metal alloy, a mixture of gallium (Ga), indium (In) and tin (Sn) at relative concentrations of 68.5%, 21.5%, and 10%, respectively. The galinstan anode is continuously regenerated through circulation at a pressure of 190 bar. In addition, it is kept under near-vacuum conditions to prevent residuals and instabilities.

Gallium has a characteristic peak K_α at 9.25 keV, and indium at 24.22 keV. The characteristic radiation of tin occurs at 25.27 keV, however, this contribution is barely visible as it almost of the same yield as the *bremsstrahlung*. In the current configuration, the source can be operated at acceleration voltages of up to 70 kV, although in principle the maximum is 160 kV with a power of 100 W. It is generally used at 40 kV or 70 kV, with the latter offering more yield to characteristic radiation at the cost of a broad spectrum. The spectrum of *JuLiA* at an acceleration voltage of 40 kV is shown in fig. 22. This was measured with an Amptek X-123 X-ray detector at $z_{01} \approx 2$ m, power setting of 2 W and an accumulation time of approximately 4 hours. The photon counts in each energy bin were divided by the real time, accounting for the dead time of the detector, and the power yielding a count rate per Watt.

The X-ray beam exits the source through a thin beryllium window with a half beam angle of $\gamma = (6.55 \pm 0.01)^\circ$, allowing for tomography with tunable geometric magnification in the diverging beam setting (section 2.1.6). This geometry is illustrated in fig. 26, where the sample (FFPE) is imaged at SOD with the detector plane at SDD, leading to an effective pixel size with a geometric magnification.

The X-ray system is operated using a manual user interface through *SPEC* commands, along with custom-built *MATLAB* functions. The *SPEC* [13] commands are used to control the motorized stages, the detector stages, as well as detectors, and a list of useful commands is given in section D.4. However, the source is controlled via a graphical user interface provided by Excillum [5].

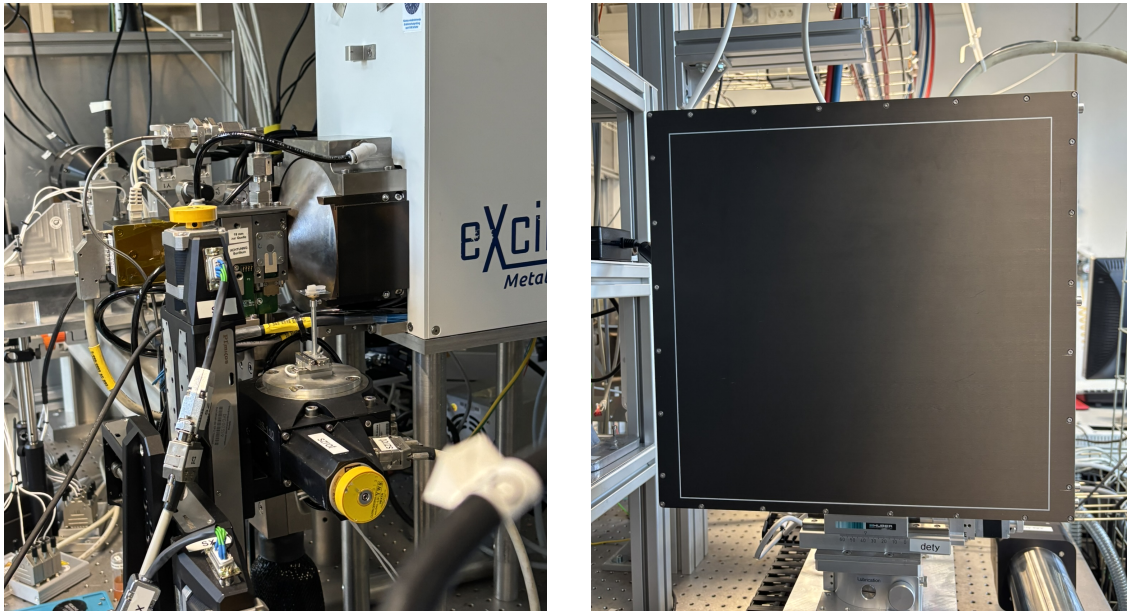


Figure 25: **JuLiA** source and Varex flat-panel detector (FPD). **Left:** motorized stages consisting of a rotation stage, 3 translation stages (sx, sy, sz), and additional high precision stages cx and cy, allowing for precision alignment of the sample. **Right:** front view of the Varex detector on motorized stages, used for imaging at the **JuLiA** and MOlybdäN Anode (**MONA**) setup.

5.1.2 *EasyTom*

The **EasyTom** system is a user-friendly laboratory-CT scanner at Heart & Brain Centre Göttingen (**HBCG**), manufactured by RX Solutions [58], and forms part of the Institut für Röntgenphysik (**IRP**) network of CT systems. The system consists of a conventional X-ray tube with a tungsten anode, a motorized sample stage, and detector stages, allowing for precise positioning on the sample and detector. It hosts a highly efficient PaxScan2520DX-I FPD from Varex Imaging, featuring a CsI scintillator and a native pixel size of $127\ \mu\text{m}$ (table 3). Unlike the Varex 3131Z detector, as will be discussed below, the PaxScan2520DX-I FPD has a relatively smaller field of view (FOV) of $(244 \times 196)\ \text{mm}^2$.

The **EasyTom** setup consists of a nanofocus and a microfocus X-ray source, allowing for imaging with a peak voltage of up to 240 kV, covering a wide range of resolutions and FOV. The setup is mounted on an active anti-vibrational table, which mitigates artifacts introduced by motion from external sources. The control of the stage, source, and acquisition equipment is managed through an XAct graphical user interface provided by [58]. The tomograms were reconstructed using the native XAct software [59]. An image of the **EasyTom** setup is shown in fig. 27.

5.1.3 *MONA*

The **MONA** set-up is a laboratory-CT system with a solid-anode Molybdenum (Mo) X-ray source, within the **JuLiA**-lab at Institut für Röntgenphysik (**IRP**). Like the **JuLiA** setup, the **MONA** has rotational and translational degrees of freedom for positioning and rotation of the sample as well as the detector. It operates at a maximum voltage of 60 kV and a

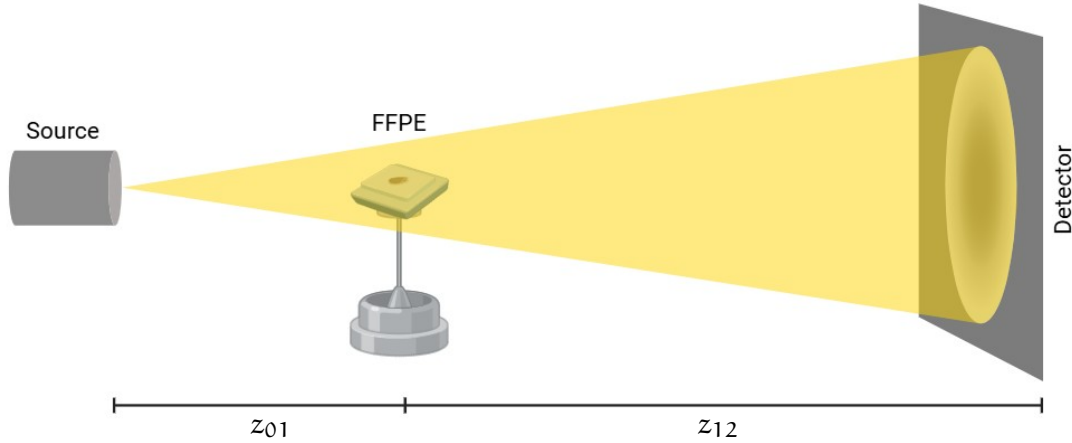


Figure 26: **Illustration of the geometry setup of the JuLiA source.** The source emits a cone beam of X-rays with a beam half-angle of $\gamma = (6.55 \pm 0.01)^\circ$. The source is mounted on a motorized stage, which allows for precise alignment of the source with respect to the sample and detector. The sample is placed on a rotational stage, which allows for tomographic imaging. The detector is mounted on a translational stage, which allows for precise positioning of the detector with respect to the sample and source. Above figure was created using [Biorender.com](https://biorender.com).

maximum power of 4.2 W. This setup can host various detectors, however, in this thesis, it is specifically paired with the Varex detector (section 5.2). An overview of the MONA system is shown in fig. 28.

The X-ray emission spectrum of Molybdenum (Mo) was simulated using *xraylib* at an acceleration voltage of $V_0 = 50$ kV and is depicted in fig. 29. The spectrum shows characteristic K_α and K_β emission lines at 17.48 keV and 19.60 keV, respectively, superimposed on the continuous *bremsstrahlung* background, which is of magnitude lower. Like the JuLiA-setup, the X-ray system is operated by *SPEC* commands along with custom-built *MATLAB* functions, and reconstruction of tomograms is performed using tools from the *hotopy* package [40].

5.1.4 HECTOR

HECTOR [42], short for High-Energy CT system Optimized for Research, is a lab-CT system developed at Ghent University Centre for X-ray Tomography (UGCT) and centered around an XWT-240 xray source by X-RAY WorX [111]. It consists of a conventional X-ray tube equipped with a cooled tungsten anode, allowing for a maximal operating voltage of 240 kV and a power of 350 W. The setup is equipped with a large FOV flat-panel detector (VAREX XRD 4343) and motorized linear and rotational stages, allowing for a large degree of freedom in the positioning of the sample and detector. The degree of freedom allows for a large SOD distance of up to 2 m [42]. These characteristics, along with the small spot size of 3 – 4 μm , allow for achieving optimal spatial resolution of 3 μm [6, 42].

Figure 30 shows the setup HECTOR system with the source, detector, and sample stage. A close-up image of the source and the sample stage is shown in fig. 30b.

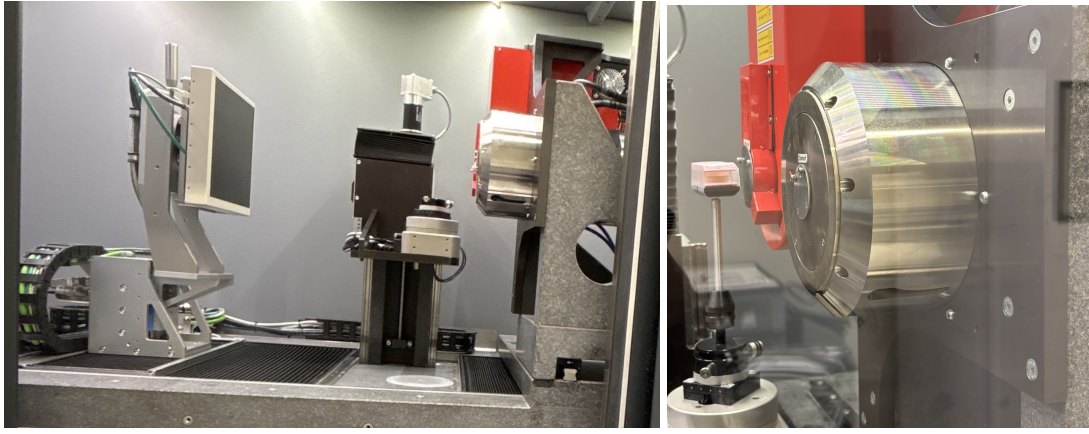
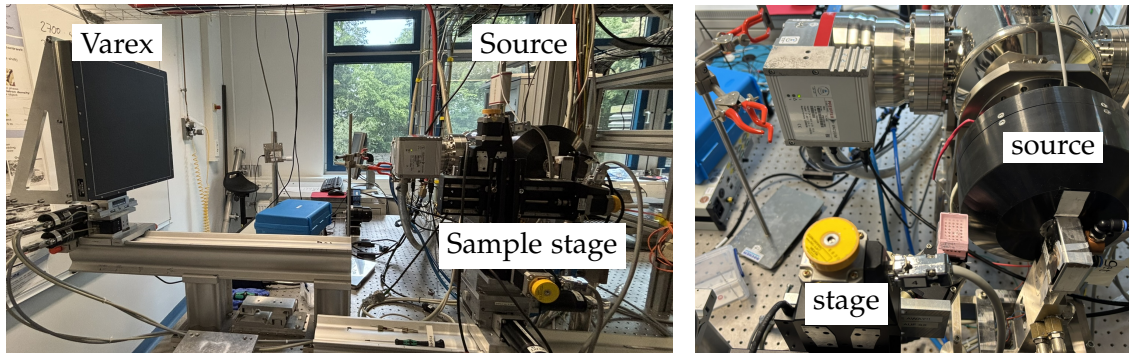


Figure 27: **EasyTom X-ray setup** consisting of two conventional high-energy X-ray tubes, a translational and piezo-equipped sample stage, and a FPD. **Right:** close-up image of the X-ray tube and sample stage of the **EasyTom** instrument at **HBCG**.



(a) Setup of **MONA** consisting of a conventional X-ray tube, sample stage and the Varex detector. (b) X-ray tube and sample stage of the **MONA** setup.

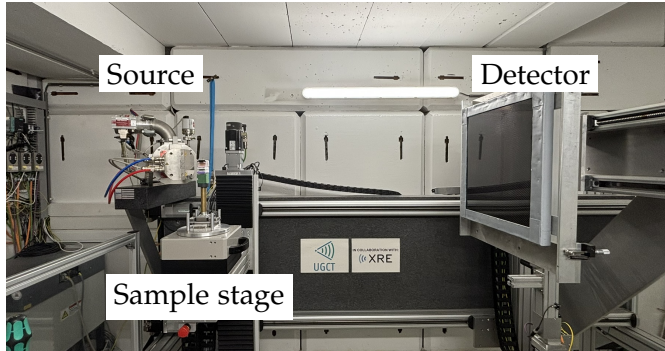
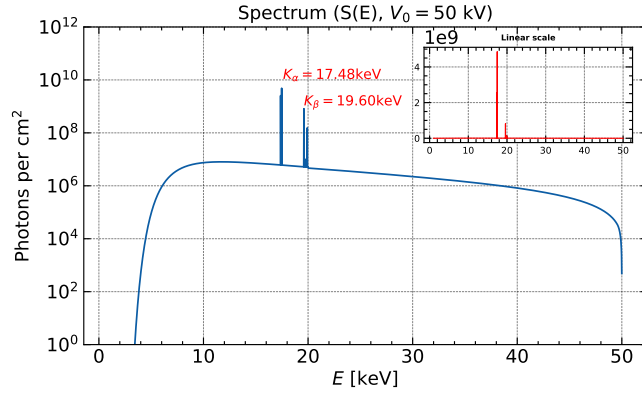
Figure 28: **MONA setup**. Figure 28 shows the setup of the **MONA** system consisting of a molybdenum X-ray tube, sample stage, and coupled with the Varex FPD. Figure 28b shows the head of the molybdenum ($_{42}\text{Mo}$) tube.

5.2 DETECTOR AND ASSESSMENT

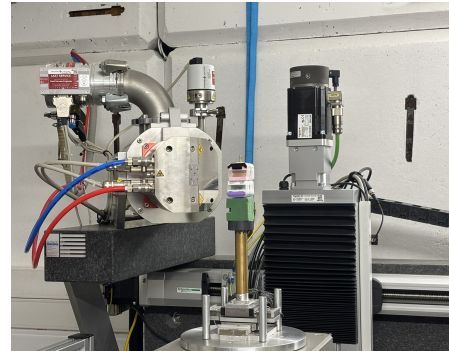
The Varex flat-panel detector (FPD) mainly used in this work is briefly discussed, and performance evaluations are performed. The detector, hereinafter referred to as Varex, is an FPD manufactured by Varex Imaging Corporation. It has a native pixel pitch of $p = 100 \mu\text{m}$ and a total of (3072×3072) pixels², resulting in a large FPD with an active area of $(307 \times 307) \text{ mm}^2$. The readout electronics are coupled to a high brightness cesium iodide ($\text{CsI-700 } \mu\text{m}$) scintillator layer for X-ray to visible light conversion and operate with a 16-bit dynamic range with a frame rate of 60 – 120 fps (unbinned- 2×2 binned) [32, 65]. The high dynamic range enables high sensitivity over a wide range of exposure levels. The technical properties of the detector are summarized in table 3 and the front view of the detector is shown in fig. 25 (right).

Assessment of detector characteristics is necessary as part of the successful integration of the detector into the tomographic environment. Detector characteristics such as noise properties, linearity, and resolution metrics are assessed and discussed in this section.

Figure 29: **Simulated X-ray spectrum of Molybdenum (Mo) source** at accelerating voltage of 50 kV on a semi-log scale. Characteristic lines $K_\alpha = 17.48$ keV and $K_\beta = 19.60$ keV emission lines are labeled. The **inset** shows the spectrum on a linear scale. The spectrum was simulated using *xraylib* package [63].



(a) Setup of **HECTOR** consisting of a conventional high-energy tube, a translational and piezo-equipped sample stage, and a flat-panel detector.



(b) Close-up image of the X-ray tube and sample stage of the **HECTOR** instrument at **UGCT**.

Figure 30: **Images of the HECTOR setup.** The image on the **left** shows the setup of the High-Energy CT system Optimized for Research (**HECTOR**) system consisting of a conventional high-energy tube, a translational and piezo-equipped sample stage, and a flat-panel detector. The image on the **right** shows the source of the **HECTOR** system with a tungsten anode.

5.2.1 Noise assessment

Read noise (σ_R) is the noise introduced by electronics during sensor readout as discussed in section 4.4.1.

$$\sigma_{R_{ij}} = \sqrt{\frac{\text{Var}(I_{B_i} - I_{B_j})}{2}} \quad (5.1)$$

To access the read noise of the Varex detector, a series of 17 bias frames with a very short exposure time ($t \leq 1/60$ s) were taken. The pairs of two bias frames, I_{B_i} and I_{B_j} , were then formed from this set, resulting in a total of 136 pairs. Given a certain pair, say I_{B_m} and I_{B_n} , the standard deviation of these residual bias frames was then calculated, in a central **ROI** with a size of (1000×1000) pixels², and divided by $\sqrt{2}$ or equivalently following equation (5.1).

To further minimize statistical influences, the mean of the different $\sigma_{R_{ij}}$ was calculated to produce a single value for the read noise (σ_R). The spread of all the values is taken as an uncertainty on this read noise value.

Table 3: **Technical specification of detectors used in this work at different setups.** The table shows the active area, pixel size, and frame rate for the unbinned matrix, sensor matrix, and scintillator (thickness) type of each detector.

Detector	Varex	PaxScan2520DX-I	XRD 4343
Active area [mm ²]	307 × 307	244 × 195	432 × 432
Pixel size [μm]	100	127	150
Dynamic range [bit]	16	16	16
Frame rate [fps]	60	30	15
Sensor matrix [pixels ²]	3072 × 3072	1920 × 1536	2880 × 2880
Scintillator - [μm]	CsI -700	CsI	
Model	Azure 3131Z	PaxScan2520DX-I	XRD 4343

This analysis results in a read noise of $\sigma_R = (3.539 \pm 0.006)$ ADU, and is represented graphically in fig. 31, where it is regarded as a constant in time, thus independent of the exposure time (t_s). This implies that each time the sensor is read out, the detector elements will exhibit an uncertainty of σ_R solely due to the readout process.

Unlike read noise, dark noise is time-dependent as was discussed in section 4.4.1. To quantify the dark current noise, similarly to the read noise analysis, a series of 17 dark frames were taken for each exposure time, with exposure times varying from $t_s = 0.01$ s to the maximum exposure time $t_s = 1$ s. As before, for a certain exposure time, pairs of dark frames were formed and the standard deviation of these residual dark frames was calculated in a certain ROI with a size of (1000×1000) pixels². The spread and mean standard deviation were also calculated, yielding a single value of total noise ($\sigma(t)$) and spread (σ_σ) for each exposure time.

This analysis was repeated for all the other sets of frames and for different exposure times. Since dark noise is temperature dependent, all frames were captured under normal working conditions, i. e. detector at room temperature, and uncooled.

It is important to note that the total noise (σ) results from the contributions of read noise (σ_R) and dark noise ($\sigma_D(t)$) section 4.4.1, such that the total noise is given by

$$\sigma(t) = \sqrt{\sigma_R^2 + I_D \cdot t}, \quad (5.2)$$

where σ_R and I_D are the fitting parameters. The results of the fit yield $I_D = (0.18 \pm 0.04)$ ADU²/s and $\sigma_R = (3.548 \pm 0.002)$ ADU.

The result of both analyses is pictorially represented in fig. 31, where the read noise and its spread are shown in red, the dark noise scattered in green, and the total noise in blue. The total noise is fitted to a power law model equation (5.2) and is shown as a solid line in blue. It is evident that the results of the fit from equation (5.2) are in good agreement with the read noise inferred from the bias analysis. Additionally, it can be seen that the dark noise is of magnitude smaller than the read noise, as can be seen by the magnitude of the second y-axis, such that in a normal tomographic setting, the noise can be considered photon-shot noise-limited. An exemplary bias frame is depicted in fig. 32a, with its histogram shown in fig. 32b. The histogram is centered on $\mu = 616$ ADU and is slightly skewed to the right with a standard deviation of $\sigma = 3.92$ ADU. The first four moments of the distribution are given in the legend of

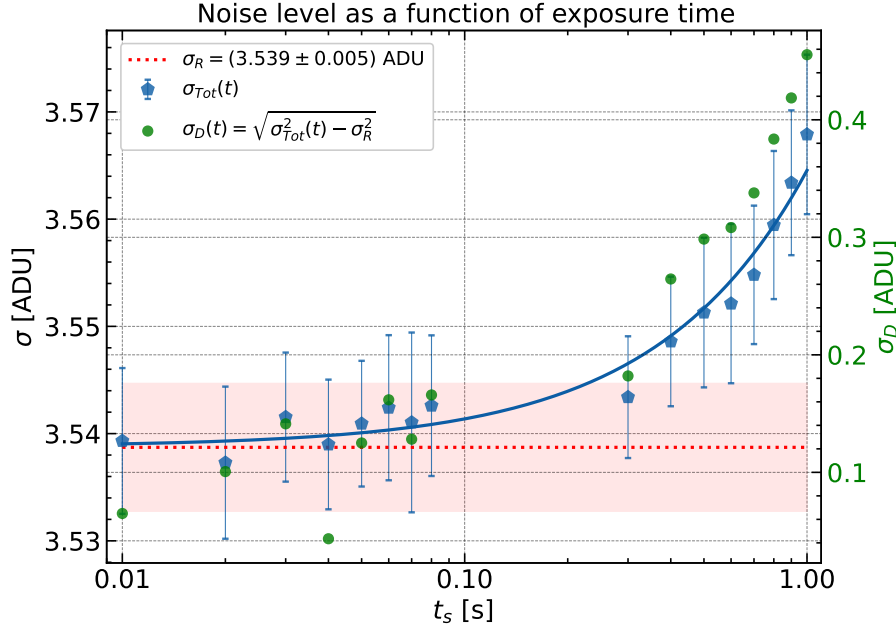


Figure 31: **Noise assessment of the FPD as a function of the exposure time, on a semi-log scale.**

The noise is given in terms of the standard deviation in units of ADU/counts. In red is the read noise (σ_R), measured with bias frames, which is assumed to be independent of the exposure time, with the red-shaded region indicating the standard deviation around σ_R . The blue scattered data represents the noise of the dark frame, which is a combined effect of the read noise and electronic noise (σ_R). The fit to this data is given by a power law (equation (5.2)). The green scattered data, scattered on the second y-axis, is the noise introduced solely by the thermal activity in the sensor. This contribution is relatively smaller than the read noise.

fig. 32b. Thus, a meaningful exposure setting during acquisition is such that features yield a minimal signal of $\mu = 616$ ADU. The script used for the analysis is provided in section D.3.2.

Noise power spectrum (NPS)

Until now, all the focus has been on the dimensional noise characteristics of the Varex FPD in the absence of signal. However, it is also informative to assess the noise characteristics in 2D, in the presence of a signal. In this regard, the noise characteristics of the detector in the frequency domain are examined.

This is achieved with noise power spectrum (NPS) which here is defined as [12, 37]

$$\text{NPS}(k_x, k_y) = \frac{\Delta p_x \Delta p_y}{N} \sum_{n=1}^N \left| \mathcal{F}[I_F(x, y) - \bar{I}_F(x, y)] \right|^2 \quad (5.3)$$

where $\mathcal{F}$ denotes the FT (chapter A), I_F a flat-field image, Δp_i the pixel size (p) in the i -th direction and N the number of region of interest (ROI) used for the analysis. The fully theoretical reasoning and motivation are provided by Kuhls-Gilcrst et al.[37].

In this regard, 17 flat frames are acquired with the Varex detector at fixed z_{02} , with source setting of $V_0 = 40$ kV and $I_0 = 1.431$ mA and an exposure time of $t = 800$ ms. The flat-field images are acquired with the source on and the detector exposed to an

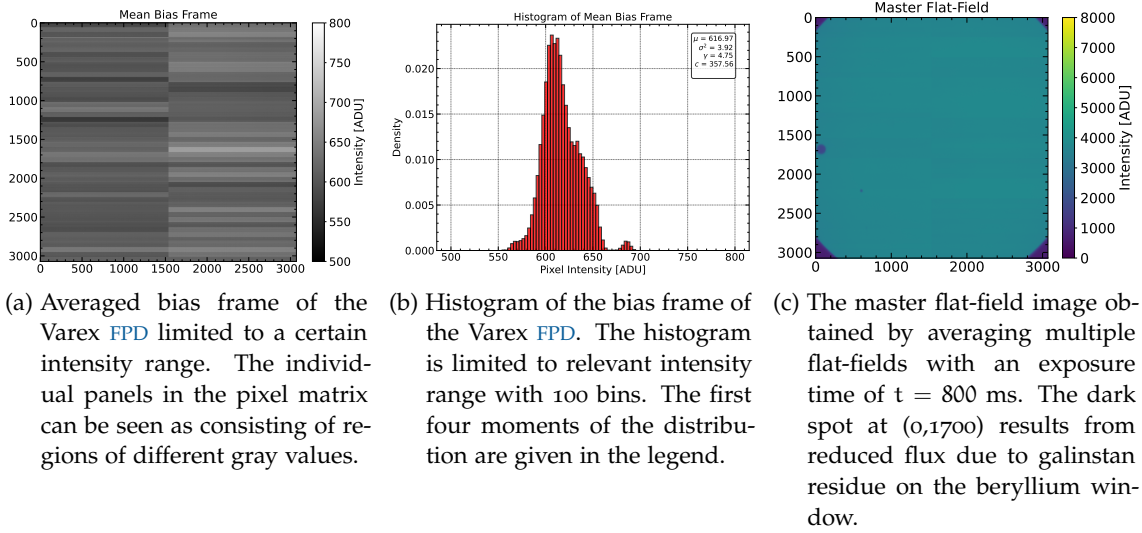


Figure 32: Analysis of the bias frames and master flat-field of the Varex FPD for read noise and linearity assessment.

X-ray beam, so that almost the whole active area of the detector is uniformly illuminated (fig. 32c). The flat-field images are then averaged to produce a master flat-field image ($\bar{I}_F$). The residual of an individual flat-field image (I_F) is then calculated and a central ROI of size (1024×1024) pixels² is selected. This ROI is further split up into 16 non-overlapping ROI and the analysis is repeated for all remaining flat fields leading to 17×16 ROI of residual frames. All frames are transformed into the frequency domain, the magnitude of the coefficients is calculated and a mean is taken for all the different 272 ROIs.

A set of 16 ROI in NPS is then taken and averaged over. Incidentally, this is repeated for all the remaining flat fields. Figure 33 (left) shows a NPS of the Varex FPD as a function of the spatial frequency for an exposure time of $t = 800$ ms.

It can be seen that the NPS is not uniform but decreases with increasing spatial frequency. This suggests that noise at low frequencies is more structured than high-frequency noise, with a smooth transition. This is also shown in the radial NPS in the middle and right figure of fig. 33 on a semi-log and linear scale, respectively. A Lorentzian fit to the radial NPS of the form, $P(k) = P_0 + p/(1 + (\frac{k}{k_c})^n)$, reveals a roll-off frequency of $k_c = k_{Nyq}/4.59$, with k_{Nyq} the Nyquist frequency. It is evident that below the knee of the Lorentzian curve, i.e. $k < k_c$, the noise is predominantly flat on the log scale and hence approximately white(uncorrelated). However, above the knee of the curve, the power in the noise decreases sharply to the floor level. The remaining fit parameters are given in the caption in fig. 33. Alongside to this smooth transition, there are two dominant peaks superimposed on the NPS, with one at $k = 0$ and the other at high frequency. The former is as expected, from all noise contributions, while the latter is an indication of structured noise. This can be recognized as an effect that originates in the subpanels of the detector, as can be seen, for example, by restriction of the flat-field intensity range or by looking at the bias frame (fig. 32a).

Noting that the NPS is dependent on the measured signal, the NPS can further be split into two contributions, the additive noise due to the electronics of the system and the

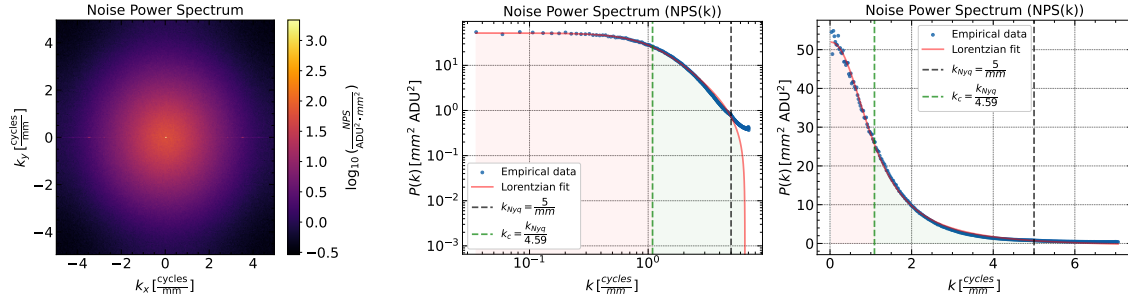


Figure 33: NPS analysis showing (left) the 2D power spectrum in frequency space, (center) the radially averaged noise power spectral density on a log-log plot, scattered data, Lorentzian fit, and (right) the same data on a linear scale emphasizing the low-frequency behavior. The corresponding fit parameters are $P_0 = -0.88$, $p = 52.83$, $k_c = 1.09$ cycles/mm and $n = 2.27$.

quantum noise due to the photon flux. According to Kuhls-Gilcrist et al. [37], equation (5.3) can be rewritten as

$$\text{NPS}(k_x, k_y) = \Delta p_x \Delta p_y \left[\bar{g} \frac{M^2(k_x, k_y)}{A_s(k_x, k_y)} + \frac{g_4}{F_p} \right] \bar{I}_F + \text{NPS}_W(k_x, k_y) \quad (5.4)$$

where M is the modulation transfer function (MTF) of the system (section B.1.2), A_s the Swank noise [37], F_p the fill factor, g_4 the electron-to-digital conversion gain, and $\bar{g}$ the gain of the system. The term $\text{NPS}_W(k_x, k_y)$ represents the white noise contribution, and $\bar{I}_F$ is the mean signal of the flat-field. Conversely, this can be rewritten as

$$\text{NPS}(k_x, k_y) = q(k_x, k_y) \bar{I}_F + \text{NPS}_W(k_x, k_y)$$

such that finding the electronic and quantum noise contributions amounts to a 2D linear regression problem. In this regard, the above analysis of the NPS was performed for 7 different exposure times ranging from 400 ms to 950 ms. The corresponding flat-field was corrected with corresponding dark frames, and the mean signal was calculated in a centered ROI of size (1024×1024) pixels².

A 2D linear regression was performed to extract the white noise (NPS_W) and the quantum noise contribution (q). The results of this analysis are shown in fig. 34, where the 2D spectral distribution of the contributions of white noise and quantum noise is given in the first row, with their respective radial profiles shown in the second row. The white noise can be considered flat/uniform across all spatial frequencies. Irregularities can be observed at low frequencies, which can be attributed to the lack of constraint on the fit parameters. However, for mid- to high frequencies, this noise can be considered flat/uniform, indicative a uniform distribution of noise power across all spatial frequencies. Quantum noise, on the other hand, shows a smooth pattern with characteristics to the initial NPS. The radial profile of the quantum noise shows a smooth transition from low to high frequencies, with fewer or no irregularities. It can be recognized that the radial quantum noise profile shows a resemblance to a typical MTF of a system.

As a matter of fact, the MTF can be extracted from the quantum noise by virtue of equation (5.4), recognizing that

$$M(k_x, k_y) = \sqrt{\frac{A_s(k_x, k_y)}{\bar{g}} \left(\frac{q(k_x, k_y)}{\Delta p_x \Delta p_y} - \frac{g_4}{F_p} \right)}.$$

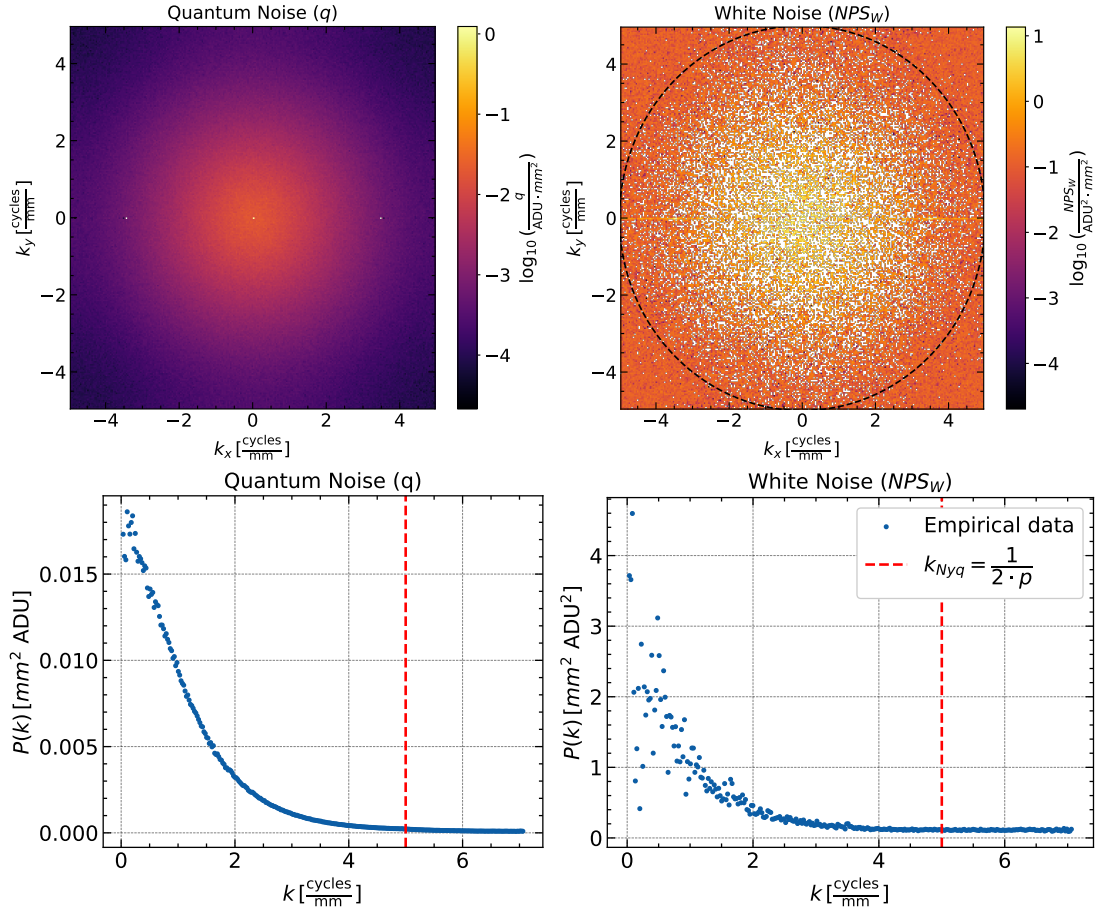


Figure 34: **NPS analysis.** **Top** figures show the 2D power spectra in frequency space with their respective radial profiles shown in the **second** row. The white noise remains relatively uniform with minor irregularities, while the quantum noise shows the expected smooth behavior. The data beyond the Nyquist frequency is not reliable.

Knowledge of the different gain and fill-factor and direct evaluation gives the **MTF** of the system. Since these are not readily available, a regression was performed using Gaussian mixture models after construction of a one-dimensional Swank model [37].

The resulting normalized **MTF** of the system is shown in fig. 35 (**right**) as a function of the radial spatial frequency. The Nyquist frequency is marked by a dotted line at 0.5 cycles/mm. The **MTF** falls off steadily from low to high frequencies, where it still retains a significant performance of approximately 0.1 near the Nyquist frequency. It is important to note that this presampled **MTF** represents the detector frequency response under the specific beam setting and exposure setting.

5.2.2 Linearity

The linearity of the detector response is crucial for quantitative imaging applications. As a by-product of the noise assessment, the linearity of the detector becomes readily available.

Here, a centered **ROI**, with size (1024×1024) pixels², of dark-corrected flat-field images is analyzed to assess the linearity of the Varex detector. The mean ($\bar{I}_F$) and standard

deviation in the ROI are calculated and plotted against the respective exposure time (t_s). In this respect, 10 master flat-field and 5 dark-field images were acquired at each exposure time to improve the statistics, and the script used for the analysis is provided in section D.3.3.

The results are shown in fig. 35 (left), where the analysis illustrates the mean pixel value ($\bar{I}_F$), within the ROI, as a function of exposure time (t_s), demonstrating a linear relationship. The scattered data aligns well with a linear model, which is shown in red, with a standard deviation of 3σ shown in the shaded region (chapter C). It can be seen that the standard deviation of the scattered data increases with the exposure time, which is expected from Poisson statistics (B.2).

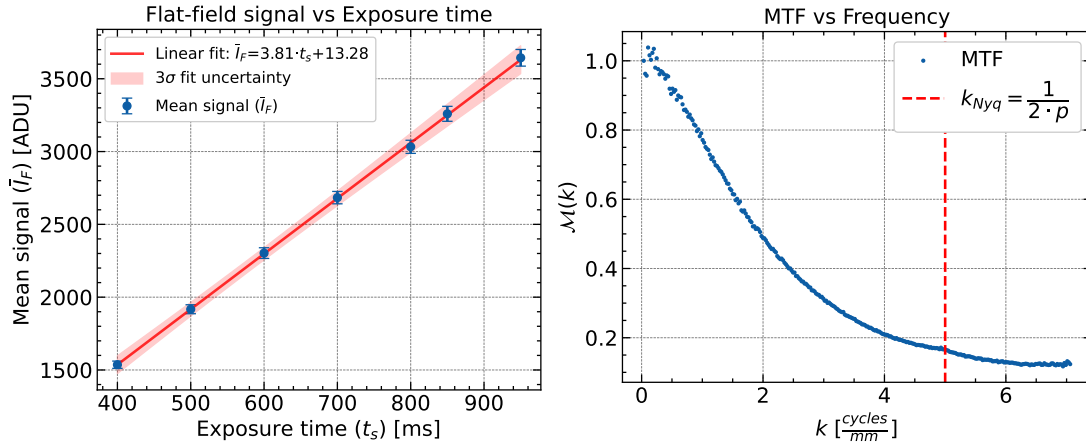
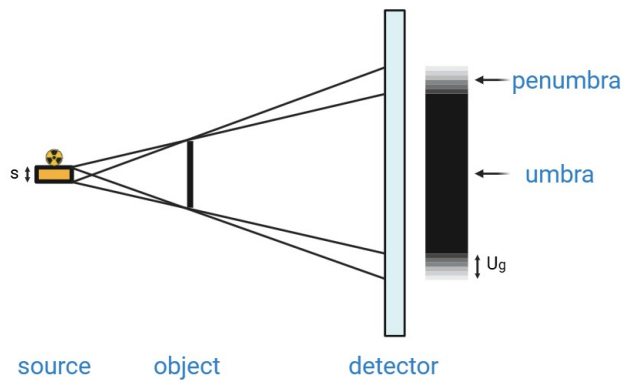


Figure 35: **Left:** flat-field signal response as a function of exposure time (t_s), showing a linear relationship. The slope and intercept are given with a 3σ uncertainty region shaded in red. **Right:** modulation transfer function (MTF) versus spatial frequency, showing the roll-off of imaging system performance with increasing frequency. The red dashed vertical line indicates the Nyquist frequency. The script used for this analysis is provided in section D.3.3

Figure 36: **Illustration of geometrical unsharpness caused by the finite X-ray source size (s).** Rays diverging from different points of the source lead to blurring on the detector plane, especially at the edges of the object. The visual comparison on the right shows an ideal projection (*umbra*) versus one affected by blurring (*penumbra*) due to finite spot size (s). The figure was created using [Biorender.com](https://biorender.com).



5.2.3 Resolution assessment

The spatial resolution of an imaging system fundamentally determines its ability to distinguish fine structural details and directly affects the quality of data. In microfocus X-ray systems, the resolution is limited by the focal spot size (s), detector blurring, magnification (M) and as well as sample-induced blurring. A resolution assessment is therefore sound and justified to ensure that the system meets the requirements for high-resolution imaging tasks.

Blurring due to the finite spot size (s), as mentioned above, can be seen by considering a diverging beam geometry as illustrated in fig. 26, where the sample is placed at z_{01} and the detector plane in z_{02} . This leads, as discussed in section 2.1.6, to a geometric magnification (M) of the system, which is defined as the ratio of the SDD to the SOD as given by

$$M = \frac{z_{02}}{z_{01}}.$$

Equivalently, this implies that the native pixel size of the detector reduces to an effective pixel size ($p_{eff} = p/M$) as noted in section 2.1.6. The smallest features that can be resolved, are therefore limited by the effective pixel size (p_{eff}). However, a second limiting factor is due to the finite spot size (s) of the X-ray source.

Consider fig. 36, which is a schematic representation of fig. 26, where a finite spot size (s), in the course of imaging of an object, leads to a penumbra effect which contributes to additional blurring at the edges of objects. In general, the total blurring (σ_{tot}) of the system is given then given by the quadrature sum of all contributions (equation (5.5)).

$$\sigma_{tot} = \sqrt{\left(\frac{M-1}{M}\right)^2 s^2 + \left(\frac{p}{M}\right)^2 + \sigma_O^2} \quad (5.5)$$

Neglecting the blur due to the sample (σ_O), the optimal magnification factor (M^*) such that the contributions from different sources are balanced, is given by the equation

$$\frac{\sigma_D}{\sigma_s} = 1 \Rightarrow M^* = 1 + \frac{p}{s},$$

so that a smaller spot size (s) favour a larger optimal magnification (M^*) and directly smaller effective pixel size (p_{eff}).

In this regard, the spatial resolution of the JuLiA-setup was characterized using a sharp tantalum (Ta) knife edge, as shown in fig. 37 (left), and the slanted-edge MTF method [41] to asses this limit.

The high atomic number ($Z = 73$) material of tantalum provides strong attenuation and a sharp intensity transition. The edge was positioned at a small angle relative to the pixel grid of the detector to enable pixel-to-subpixel sampling. A radiograph with an effective pixel size of $p_{eff} = 8.33 \mu\text{m}$ with a geometric magnification (M) of 12, at an acceleration voltage of 40 kV and power of 57.2 W, was acquired. The edge was placed at a distance of $z_{01} = 125 \text{ mm}$ from the detector and multiple projections were acquired to suppress the noise. The projections were corrected with respective dark and flat-field images.

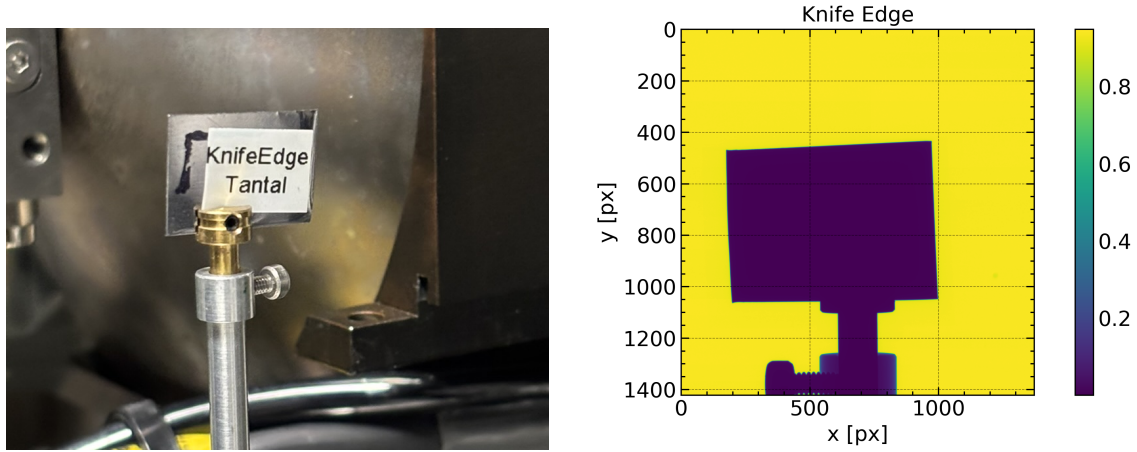


Figure 37: **MTF analysis.** **Left:** tantalum (Ta) knife edge used for MTF measurements. The high-Z edge of the tantalum and relative sharpness provide a well-defined interface suitable for measurement of the edge spread function (ESF) and subsequent calculation of the MTF of the imaging system. **Right:** projection of a tantalum knife edge on the Varex detector (fig. 25, right). The projection of the knife edge is taken at an angle $\theta \approx 0^\circ$ at $z_{02} = 1000\text{mm}$ with a geometric magnification (M) of 5. The source was operated at 40 kV and 1.431 mA.

The ESF (chapter B) was measured by taking a line profile perpendicular to the edge and averaging over a width of 30 adjacent lines. The resulting line profile was fitted to an error function of form equation (5.6), to achieve a smooth transition.

$$e(x) = a \cdot \text{erf}\left(\frac{x - x_0}{\sqrt{2}\sigma}\right) + b \quad (5.6)$$

The ESF was differentiated to obtain the line spread function (LSF), representing the system's response, and was successively examined in Fourier space.

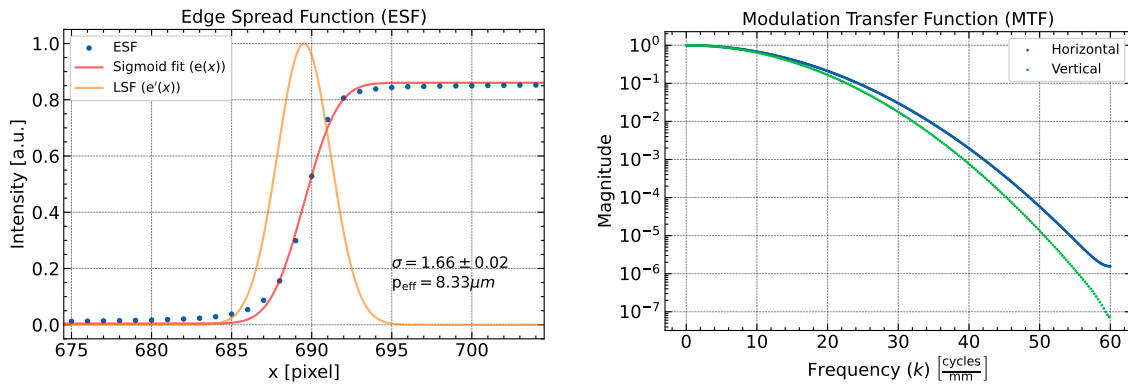


Figure 38: **Left:** The ESF of the knife edge projection scattered in blue and corresponding fit to an error function (red). **Right:** The MTF of the system obtained from the Fourier transform (FT) of ESF. The MTF is given both in the vertical and horizontal direction in blue and green, respectively. Discrepancy in the two directions is due to the anisotropic nature of the source, such that the spot size in the vertical direction is larger than that in the horizontal direction.

The results of the analysis are shown in fig. 38, where the ESF is shown on the left and the MTF on the right. The MTF is given in both the horizontal and vertical directions,

with the discrepancy in the two directions due to the anisotropic nature of the source, such that the spot size in the vertical direction is larger than that in the horizontal direction. The analysis reveals a standard deviation of $\sigma_x = 1.66 \pm 0.02$, which implies a blur of $\sigma_x \cdot p_{\text{eff}} = (13.83 \pm 0.16) \mu\text{m}$. This suggests that even with an effective pixel size of $p_{\text{eff}} = 8.33 \mu\text{m}$, the system is able to resolve features down to approximately $14 \mu\text{m}$, which is sufficient for most biological applications. A complementary visual

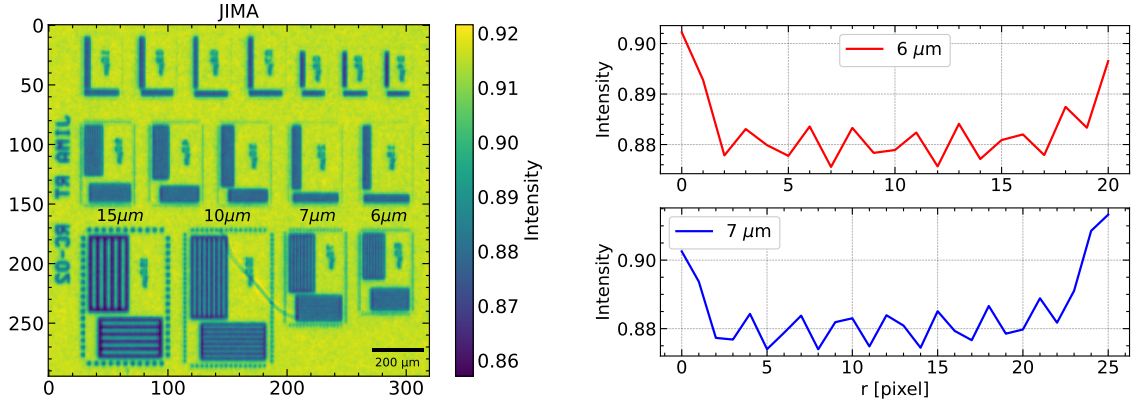


Figure 39: **JIMA resolution test pattern analysis for spatial resolution assessment.** **Left:** Flat-field corrected X-ray projection of the JIMA test pattern showing line pair groups with different spatial resolutions denoted. **Right:** line profiles extracted perpendicular to the line patterns, averaged over 10 parallel lines, for the $6 \mu\text{m}$ and $7 \mu\text{m}$ line pairs.

assessment of the spatial resolution relies on the use of a JIMA pattern that, unlike the knife edge, does not suffer from irregularities in the edge profile. In this regard, a JIMA pattern was used to assess the spatial resolution of the imaging system. The JIMA pattern is a standard test pattern consisting of a series of lines with varying spacings, allowing for a quick and unbiased resolution assessment (section D.1.2). Here, a JIMA RT RC-02 pattern was placed at a distance of $z_{01} \approx 78 \text{ mm}$ and the Varex detector at $z_{02} \approx 1700 \text{ mm}$, leading to an effective pixel size of $p_{\text{eff}} = 4.58 \mu\text{m}$. Multiple projections were acquired for averaging to suppress the noise and successively corrected for dark and flat signal.

The corrected frame is shown in fig. 39, where $15 \mu\text{m}$ line pairs is clearly visible. However, the resolution is limited for line pairs below $10 \mu\text{m}$, which can be seen in fig. 39, both on the radiographs and on the extracted line profiles. As previous scenario, the effective resolution is above the effective imaging pixel size.

Consequently, the geometric settings were constrained by the physical dimensions of the FFPE and by the degrees of freedom available for positioning the sample and detector. For standard scanning, samples were placed at $z_{01} = 120 \text{ mm}$ and $z_{02} = 1500 \text{ mm}$, resulting in an effective pixel size of $8 \mu\text{m}$ and a magnification factor of $M = 12.5$. This is sufficient to resolve cellular structures, as well as imaging of the whole FFPE block. The large propagation distance was chosen on the grounds that it has a large area of the detector illuminated and the large propagation distance z_{eff} , as will further be discussed in section 6.1.

5.3 IMAGING PROTOCOLS

5.3.1 Exposure time

Exposure time (t_{exp}) is a key parameter in retaining high **SNR** and **CNR** in the projected images as well as reconstructed slices. A longer exposure generally increases the number of detected photoelectrons, thereby increasing the **SNR**, **CNR**, as well as the general image quality, but also increases the scan duration and radiation dose.

The exposure time here is typically chosen so that the mean signal is well above the bias/dark noise floor section 5.2. Typically, this will be chosen so that the mean of the histogram is positioned towards the right-hand side of the dynamic range of the detector. In other words, a general rule of thumb is to opt for a mean flat-field signal of around 70% of the dynamic range. For a 16-bit detector, this exposure time is chosen such that the mean of the flat-field is around 40000 – 50000 ADU.

For relatively noisy projected images, multiple averages will also be taken per projection to further suppress the noise and enhance the signal.

Effective pixel size

The effective pixel size represents the smallest effective detector sampling and characterizes the feature size in projection images. Accurate determination of the effective pixel size, or alternatively the **SOD** and **SDD**, is essential for quantitative analysis and reconstruction. The method used to determine the effective pixel size at the **JuLiA** and **MONA** setups is based on measuring the distance between two points in a projection image.

For this purpose, a tantalum knife edge is used as a well-defined sharp edge. Radiographs of the knife edge are taken at a certain **SOD** and swept perpendicular to the beam at different motor positions. Figure 40 shows normalized projection images of a tantalum knife edge at two different positions perpendicular to the beam. The images are

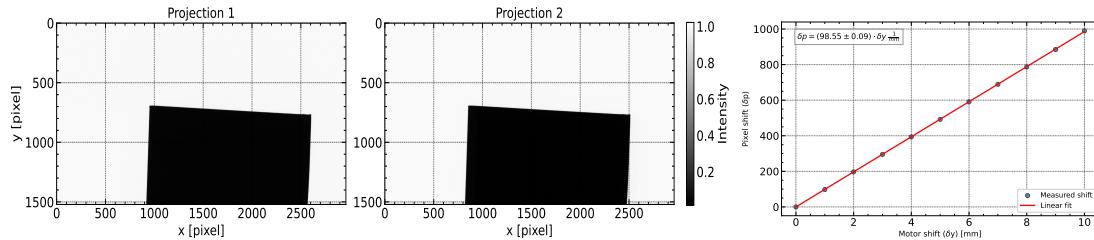


Figure 40: Projection images of a tantalum knife at different positions perpendicular to the beam (**left** and **middle**). **Right:** motor shift versus the pixel shift of the projection images. The pixel shifts are determined by the cross-correlation algorithm. The linear fit to the data is given by the red line with the data in blue. The inverse of the slope of the fit is the effective pixel size of the system.

then correlated with each other using a cross-correlation algorithm, which yields a pixel shift between the images. A linear fit of form $\delta p = a \cdot \delta y$ to the image shifts reveals the effective pixel size, according to, i. e. $p_{\text{eff}} = 1/a$. An example of such a fit is showcased in fig. 40 (**right**). This previously described method can also be accomplished through the execution of the `mat_pixelsize` (D.4) function implemented in **MATLAB**, which can be run in the **SPEC** terminal.

Rotation axis alignment

Alignment of the rotation axis is crucial for artifact-free tomographic reconstruction due to geometry [35]. Before tomography, a transmission electron microscopy (TEM) grid [106] is centered in the center of the FOV using combinations of sz , cy and or sy . A projection image of the grid is taken at two anti-posed angles, typically 0° and 180° . Two projection anti-posed images, given that the rotation axis is centered, should be identical if one of the projected images is inverted around the rotation axis. However, in case there is a misalignment of the rotation axis, the two images will not be mirror images of each other. In the latter case where the rotation axis is shifted by Δ , in units of pixels or thus a shift of 2Δ between the two images. The rotation axis can be aligned by movement of the stage sy by an amount of $\Delta * p_{eff}$ in the opposite direction.

This misalignment (Δ) can be calculated using the cross-correlation algorithm [61, 72] or equivalently by minimizing the mean square error (MSE) of the images and or by visual inspection.

Figure 41 shows a projection of a TEM grid on a misaligned rotation axis (**left** and **middle**). The two images are not mirror images of each other and hence are misaligned. However, the residual image (**right**) shows that the two images are identical, in the center, except for discrepancies towards the edge of the image after a shift of 200 pixels. Discrepancies towards the edge of the difference image can be attributed to difference in effective magnification in a cone beam geometry.

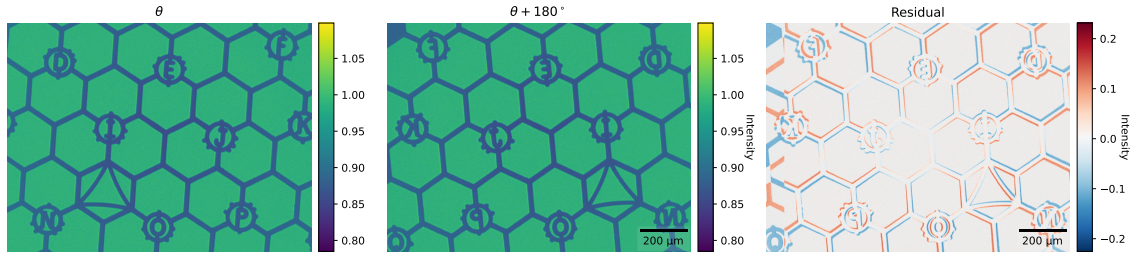


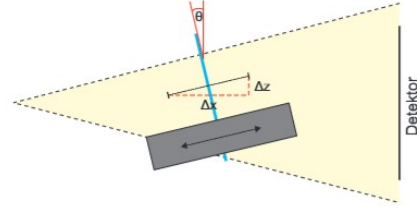
Figure 41: **Rotation axis alignment using a TEM grid.** **Left:** Projection image of a TEM grid at $\theta = 0^\circ$. **Middle:** projection at $\theta = 180^\circ$. **Right:** residual image of the two images, in inverse RdBu color scheme, after a horizontal shift of 200 pixels. The scale bar represents a distance of 200 μm .

An automated procedure for the alignment of the rotation axis is implemented in the MATLAB environment, and can be started by using the syntax `mat_align_rotaxis` function in the terminal. The script acquires two projection images at θ and $\theta + 180^\circ$ and computes the shift required to align the rotation axis (D.4).

Nick angle alignment

The nick angle (ϑ), commonly known as the pitch angle, plays a crucial role in the accurate reconstruction of 3D images. This refers to the angle between the rotation axis of the sample and the direction of the X-ray beam, as shown in fig. 42. Proper alignment of the nick angle is thus required to minimize artifacts such as elongation and blurring. Depending on the effective voxel size, the effect might be negligible or non-significant. However, in high-resolution imaging or imaging of extended samples, attention has to be paid to the alignment of this pitch angle. The alignment of the nick angle was performed through an iterative procedure and is as follows.

Figure 42: **Illustration of a tilt (nick angle) in the rotation axis of the CT system.** The rotation axis is not perfectly perpendicular to the detector, resulting in a tilt angle ϑ with respect to the beam direction. This misalignment leads to a shift Δx across the detector as a function of depth Δz along the beam path. Such geometrical misalignments can introduce artifacts in the reconstructed volume and must be corrected during CT alignment. The illustration is adapted from [35, 73].



- The use of a well-defined and highly contrasted sample, such as a TEM grid, is desirable. A certain feature of interest is centered in the FOV of the detector.
- The sample is translated parallel to the beam direction by a distance Δx , and the vertical position of the feature of interest is recorded as y_1 .
- The sample is then relatively rotated by 180° , and the vertical position of the feature of interest is again recorded as y_2 . If the rotation axis is perfectly parallel to the detector plane (i.e. $\vartheta = 0^\circ$), the vertical position of the feature of interest should remain identical, such that i. e. $y_1 = y_2$.

However, in case y_1 and y_2 are not identical, then the nick angle is misaligned by a factor given by

$$\vartheta = \arcsin \left(\frac{(y_1 - y_2)p_{\text{eff}}}{2\Delta x} \right)$$

with p_{eff} the effective pixel size. The angle of nick alignment is then achieved by vertical translation of the detector stage by an amount of $\Delta z = z_{02} \tan(\vartheta)$, where z_{02} is the source-to-object distance (SOD). Iteratively going through step 3 and the remaining processes ensures the correct alignment of the pitch angle. Similar to before, this procedure can be automated using the `mat_align_nickangle` (D.4).

5.3.2 Reconstruction

Tomograms constructed at the JuLiA and MONA setups are reconstructed using custom *Python* scripts with useful functions inherited from the *hotopy* package [40]. The manual and open-source philosophy of reconstruction provides independence and options to freely manipulate the data to your liking. The reconstruction method used in this work is the Feldkamp-Davis-Kress algorithm (FDK) in CBCT setting (section 3.3.1). For the sake of completeness, a detailed example of a reconstruction script can be found in section D.3.1.

Unlike the scripts for JuLiA and MONA setups, scans conducted at the HECTOR setup at UGCT are processed and reconstructed using the reconstruction software *Octopus* [80]. The *Octopus* software is a powerful, versatile and user-friendly tool for tomographic reconstruction, allowing for various reconstruction algorithms such as SART [104], FBP, and others.

The *XAct* software [59] is used for the 3D reconstruction of projected images performed with the *EasyTom* setup at *HBCG*. *XAct* is a commercial software package for tomographic reconstruction and analysis, provided by RX Solutions [59], allowing for fast and user-friendly reconstruction of *CT*-data as well as advanced post-processing correction of artifacts in a cone beam setting.

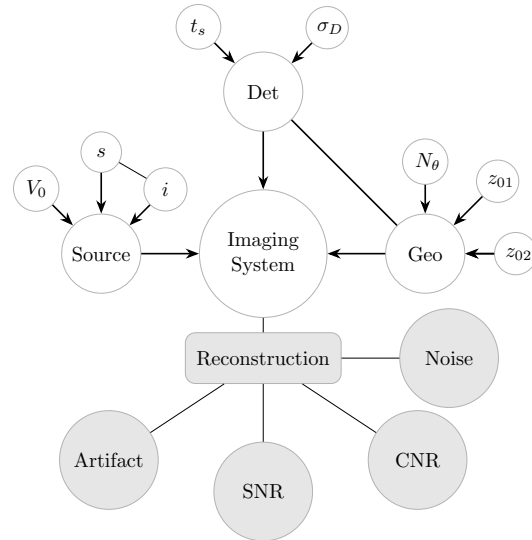
OPTIMIZATION OF μ CT IMAGING

Building on the imaging principles and independent parameters established in the previous chapters, attention is now turned to the systematic optimization of the parameters specific to the sample.

The optimal use of an X-ray CT and choice of sample-specific parameters is of crucial importance. For optimal imaging, appropriate parameters are to be selected based on the imaging goal and the characteristics of the sample. Although there are a lot of general guidelines, such as, for example, provided by Du Plessis et al. [25], there are still diverse parameters that can be tuned and optimized. These parameters can be summarized into different categories, such as source, geometry, detector, and reconstruction parameters.

Figure 43 is, for instance, a graphical representation, summarizing a set of parameters that all interact within the imaging system, contributing to the quality of the final images. Source parameters include peak voltage (V_0), current (i), and focal spot size (s), although it is generally known that the peak voltage is also correlated with the spot size. Geometric parameters such as the number of angular projections (N_θ), SDD (z_{02}) and SOD (z_{01}) are also crucial for the optimization process, since these also affect the magnification, effective pixel size, exposure time, etc.

Figure 43: **Graphical model of independent parameters and observables in X-ray CT imaging system.** Input parameters can be divided into three categories: source, geometric, and detector influence. The source parameters include the peak voltage (V_0), the current (i), and the focal spot size (s). Geometric parameter encompasses the number of angular projections (N_θ), SDD (z_{02}) and SOD (z_{01}). Blurring (σ_D) due to MTF, which is typically fixed, the exposure time (t_s) is another input parameter originating from the detector. The observables of the resulting image, i.e. images metric are as SNR, CNR, noise, and artifacts.



In this chapter, different parameters and degrees of freedom that have not yet been explored will be broken down and tested empirically. Parameters that are not sample-specific or have a non significant influence on the resulting outcome are chosen based on common best practices.

6.1 VISIBILITY

For the assessment of the performance of the X-ray system for phase-contrast imaging, the visibility (4.20) of edge enhancements is evaluated.

In this regard, projection images of an empty (no tissue) paraffin block, with a LOS thickness of 22 mm, were taken for fixed $z_{02} \approx 1700$ mm but varying z_{01} or equivalently varying effective propagation distance (z_{eff}). Multiple averages were taken to suppress the noise while yielding a sufficient statistic. The fixed pattern noise was then corrected for using the corresponding dark and flat frames. Given the corrected images, a line profile perpendicular (fig. 44, label **A**) to the paraffin-air interface (fig. 44, **left**) is taken over 60 neighboring pixels and averaged to produce a less noisy line profile. The visibility close to the fringe interface, by virtue of equation (4.20), was calculated using the maximum (I_{max}) and minimum intensity (I_{min}) values at the fringe interface. The maximum of the absolute LSF (B.6), as shown in fig. 44 (**right**), i. e. $\max\{|LSF|\}$ was then used as a measure of the sharpness of edge enhancement.

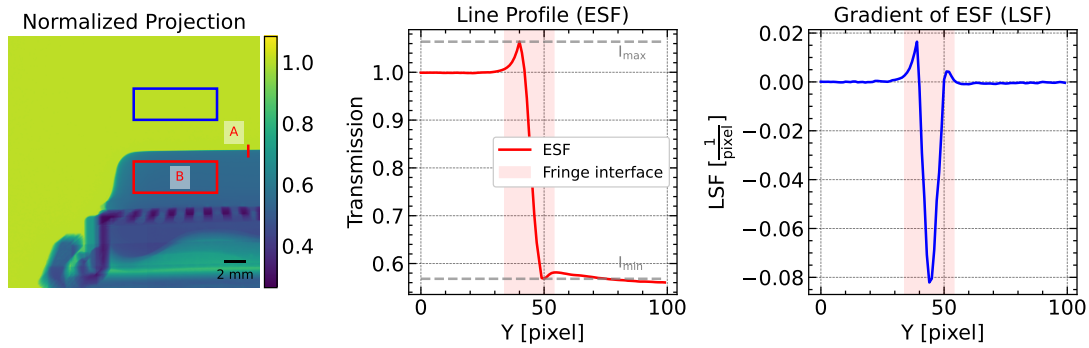


Figure 44: **Visibility analysis.** A normalized projection image (**left**) of an empty paraffin block, imaged at a peak voltage of 70 kV, with indicated line profile (**A**) and region of interest (**ROI**) for analysis of transmission and edge effects. The **ESF** (**middle**) indicated by **A** shows the fringe effect at the paraffin-air interface. The **LSF** (**right**) of the line profile (**A**) is computed using the gradient tool in *numpy* [30].

The results of this analysis are depicted in fig. 45, where in the **left** figure, the visibility ($\mathcal{V}$) as a function of the effective propagation distance (z_{eff}), for two the different peak voltage settings of $V_0 = 40$ kV and 70 kV. The trend is monotonically increasing with respect to (z_{eff}), in line with section 2.1.4, and is higher for the lower energy setting (40 kV) compared to the higher energy setting (70 kV). This confirms that decoherence is enhanced at high energy setting, where the broad spectrum due to the tail of *bremsstrahlung* increases. A similar trend is also observed for the analysis of the line spread function (**LSF**), where again the computed metric monotonically increases with the effective propagation distance. An example script used for this analysis is given in section D.3.4.

Above analysis suggests that the optimal effective propagation distance for phase contrast imaging is chosen with z_{eff} as large as possible. In this sense, source-to-detector distance (**SDD**) was chosen as large as possible to maximize the effective illuminated area of the detector, while maintaining relatively short exposure times. source-to-object distance (**SOD**) was then adjusted based of ground of section 5.2.3, where the magnification was chosen such that the whole **FFPE** is in the **FOV** of the detector and maintaining

effective pixel size around $8\ \mu\text{m}$ (section 5.2.3). Figure 45 (right) shows the transmission

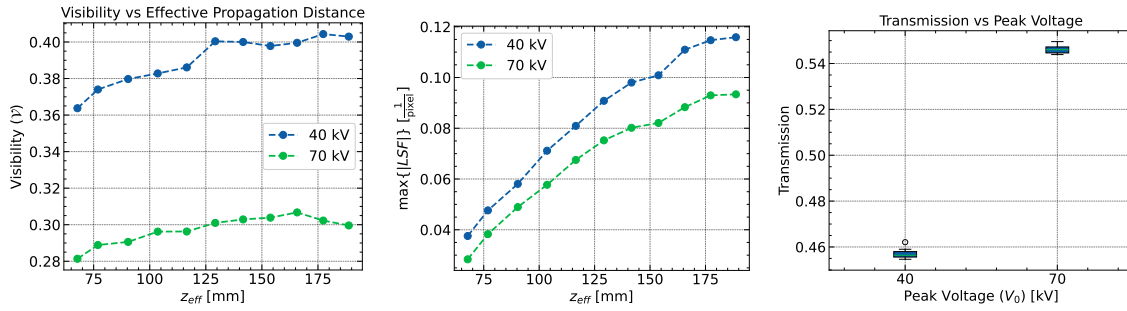


Figure 45: **Left:** visibility (4.20) as function of z_{eff} , for voltage settings of 40 kV and 70 kV. The visibility is higher for the lower energy setting (40 kV) as opposed to the higher energy setting (70 kV). However, the trend is monotonically increasing with respect to effective propagation distance. **Middle:** the maximum value of the absolute LSF as a function of the effective propagation distance for different voltage settings. A monotonic increasing trend with respect to the effective propagation distance is apparent. **Right:** boxplot of the transmission coefficient attained for a voltage of 40 kV and 70 kV. This is attained by measurement of the mean value of a ROI well inside the paraffin block.

coefficient as a function of the peak voltage. This was accessed by measurement of the intensity values within rectangular ROI (fig. 44, region B), within the empty paraffin block. The transmission coefficient is higher for the higher energy setting (70 kV) as opposed to the lower energy setting (40 kV). This is expected as higher X-ray photons are more penetrative than soft X-ray photons.

Before proceeding, a note on the mounting configuration of the FFPE blocks is warranted. As reported by Tajbakhsh et al., the shape of FFPE blocks gives an extra degree of freedom in the mounting of the sample [68]. This can either be *horizontal* (H) or *vertical* (V) as is graphically represented in fig. 46. The vertical positioning here is such that the surface normal of the paraffin wax is perpendicular to the rotation axis (along z-axis), while in the horizontal positioning, the surface normal of the paraffin wax is parallel to the rotation axis fig. 46.

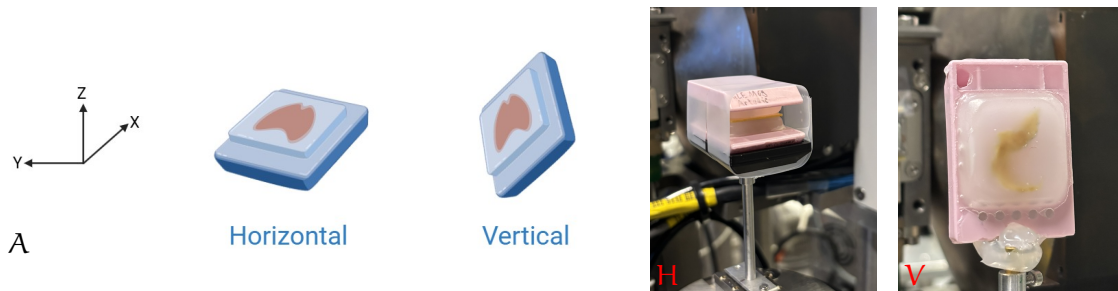


Figure 46: **Mounting configurations for FFPE blocks in the beam path.** Figure A shows the coordinate system of the setup with the beam traveling in the x-direction and rotation axis along the z-axis. The different mounting configurations are also illustrated, both pictorially and schematically. The choice of mounting not only affects the signal-to-noise ratio (SNR) and contrast-to-noise ratio (CNR) of the reconstructed image but also artifacts.

The vertical configuration (fig. 46, V) yields a higher SNR and CNR compared to the horizontal configuration for the same scan settings. This is attributed to the relatively shorter LOS thickness of the paraffin wax in the vertical mounting in comparison to that in the horizontal position [68]. However, the horizontal configuration allows for stacking of multiple FFPE blocks in the beam path and is therefore beneficial, as it enables imaging of multiple samples in a single scan. This mounting is thus the preferred option and will further be used as the standard unless otherwise stated. In this geometry, FFPE blocks are stacked on top of each other with an X-ray transparent layer inserted in between (fig. 46, H). This is to prevent scattering at the interface and leakage of information from the slices of the opposing blocks. Here, a double-sided tape with a thickness of 2 mm proved to be sufficient.

6.1.1 Voltage

Beaming back to the analysis above, a tomographic scan was conducted at the two available voltages (40 kV and 70 kV). In this respect, a scan of two stacked FFPE blocks (**bronchus** and **artery**) were imaged using the JuLiA setup, with $z_{02} = 1500$ mm, $z_{01} = 106.66$ mm and a source current of 1.431 mA, resulting in source power of 57.24 W and 100.17 W respectively. The tomographic scan was performed with the Varex detector, yielding an effective pixel size of $7.07 \mu\text{m}$. A total of 1500 angular projections were taken over an angular range of 360° , with 6 projections per angle. Each projection consists of an exposure length 2s, amounting to a total exposure time of 5 hours. For calibration, 15 dark frames were taken at the end of the acquisition, while 30 flat frames were taken before and after the tomographic scan. The tomograms were reconstructed using the FDK algorithm, implemented in *hotopy*, and the BAC algorithm was chosen as the phase retrieval algorithm of choice. To keep the data intact, a subtle ring removal algorithm was applied using the *additive* method section 3.4.2.

Figure 47 shows a reconstructed slice of a FFPE of a human artery at two different peak voltages. The reconstructed slices show high fidelity with each other with minor differences; however, the lower energy (40 kV) setting is visibly noisier than the higher energy setting (70 kV). Computation of the CNR and SNR (equations (4.16) and (4.19)), using a uniform ROI in the tissue and paraffin, reveals that the high peak energy setting has the overall better metrics. This is illustrated in 48, for the two different samples. This can be attributed to complex interplay of factors including the higher photon energy from the *bremsstrahlung* spectrum and the overall higher source power.

Further analysis of the ESF along the paraffin-tissue interface (fig. 47, label L) reveals that the magnitude of the step ($|\mu_S - \mu_P|$) is more pronounced for the lower energy setting (40 kV) as opposed to the higher energy setting (70 kV). This is depicted in fig. 49 and indicates that the lower energy setting is more sensitive to the contrast difference between paraffin and tissue, while the higher energy setting exhibits a higher CNR and SNR mainly due to the increased photon energy, lower attenuation, and hence less noise. On the other hand, a fit to the scattered data in fig. 49 reveals that the standard deviation (σ) of the edge response is smaller for the higher energy setting (70 kV) as opposed to the lower energy setting (40 kV).

Furthermore, a line profile through the center of an empty slice (fig. 50, left), reveals the intensity profile through the paraffin region. This is shown in fig. 50 (right), where it can be seen that the two profiles are uniform in the center region with slight gradi-

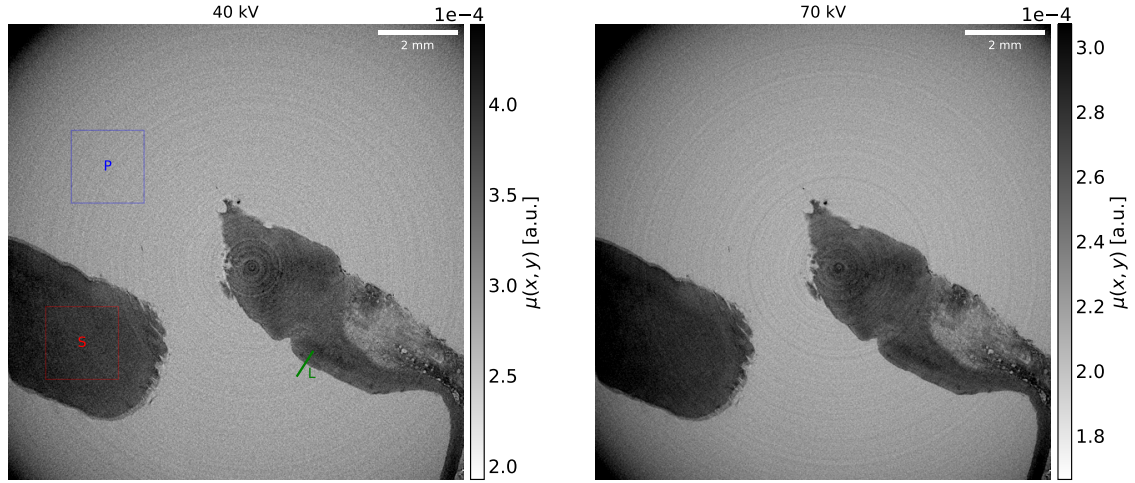


Figure 47: Virtual slices of a **human artery** embedded in paraffin, at two different peak voltages. **Left:** reconstructed slice at a peak voltage of 40 kV. **Right:** reconstructed slice at a peak voltage of 70 kV. The slice looks very similar, however, for the higher energy setting (70 kV), the noise level is less as opposed to the lower energy setting (40 kV). Image metrics such as the SNR and CNR are also higher in the lower energy setting. These metrics were evaluated using ROI denoted on the left slice with the red ROI taken in the sample region (s) and the blue ROI taken in the paraffin region (p). A ESF (L) is taken along the paraffin-tissue interface.

ents towards the edges. This hints at beam hardening, which is less pronounced for the higher energy setting. However, visually, this does not translate into a significant difference in the reconstructed slices.

From the above discussion, it is concluded that for imaging of FFPE blocks at the JuLiA setup, the higher energy setting is the overall optimal source setting for contrast and noise performance, while maintaining short scan times. This setting benefits mainly from samples that have high contrast with paraffin, such as tissues infiltrated with contrast agents. However, since a lower-energy setting is more sensitive to the contrast difference between the paraffin and tissue, it is the optimal source voltage in the case where the inherent contrast between the tissue and paraffin is low, while maintaining a longer exposure time.

6.2 PHASE CONTRAST ALGORITHM

In the previous section, the choice of the phase retrieval method was based on common knowledge. However, the choice of the phase retrieval method can significantly impact the quality of the outcome as discussed in section 3.2.

In this regard, a tomographic scan of the same sample above (stack of **bronchus** and **artery**) was performed at source setting of $V_0 = 40$ kV and $I = 1.431$ mA. Geometric setting was determined by $z_{02} = 1500$ mm and $z_{01} = 150$ mm, resulting in an effective pixel size of $p_{eff} = 8$ μ m. The sample was sampled over an angular range of 2000 projections spanning 360° , with 7 s exposures per angle, accumulated over 2 frames. This gives a total scan time of approximately 7.78 hour. Calibration included 15 dark frames and 30 flats (before and after acquisition). Reconstructions were again performed with the FDK algorithm and the different phase retrieval methods (SMO, BAC and none) were

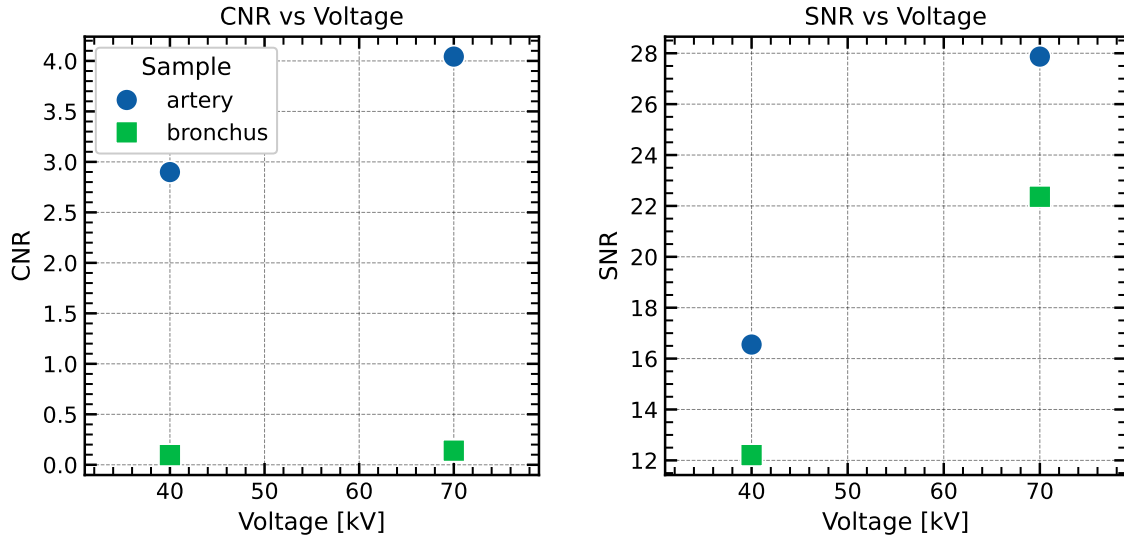
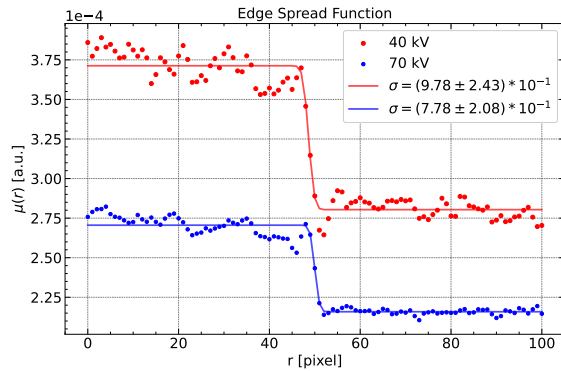


Figure 48: **Left:** the contrast-to-noise ratio (CNR) with respect to the accelerated voltage for two formalin-fixed paraffin-embedded (FFPE) samples. The CNR is higher for the higher energy setting (70 kV) as opposed to the lower energy setting (40 kV) by a factor of ≈ 1.5 . **Right:** the signal-to-noise ratio (SNR) with respect to the accelerated voltage. The SNR is similarly higher for the higher energy setting (70 kV) as opposed to the lower energy setting (40 kV) with an increment of around 100%.

Figure 49: A slice along the paraffin-tissue interface revealing the difference in the edge response $|\mu_S - \mu_P|$. The empirical data are represented by the scattered points, while the continuous line is a sigmoid fit. It is observed that the spread (σ) of the response curve is slightly smaller for the higher energy setting (70 kV) as opposed to the lower energy setting (40 kV).



applied. Explicit evaluation of the modified bronnikov algorithm (MBA) phase retrieval algorithm was deemed unnecessary as it structurally similarly SMO, yet performs poorly in the presence of absorption.

Figure 51 illustrates the influence of the phase retrieval algorithm on the quality of the reconstructed data. This is shown for two different slices for the two respective samples in the respective row. When there is no phase contract algorithm applied, features remain sharp and visually appealing, but the contrast between tissue and paraffin is relatively poor, making subtle boundaries difficult to identify. Additionally, edge enhancements show up in the reconstructed slice, as is indicated in the first column in section 3.2 with the red arrow. Employment of the BAC algorithm enhances contrast and removes edge enhancements at paraffin-tissue interfaces, while improving visibility of the tissue. The Paganin algorithm (SMO), on the other hand, provides the strongest increase in contrast, suppressing noise in the tissue region and background intensity. However, this algorithm tends to blur high-frequency features and fine features sec-

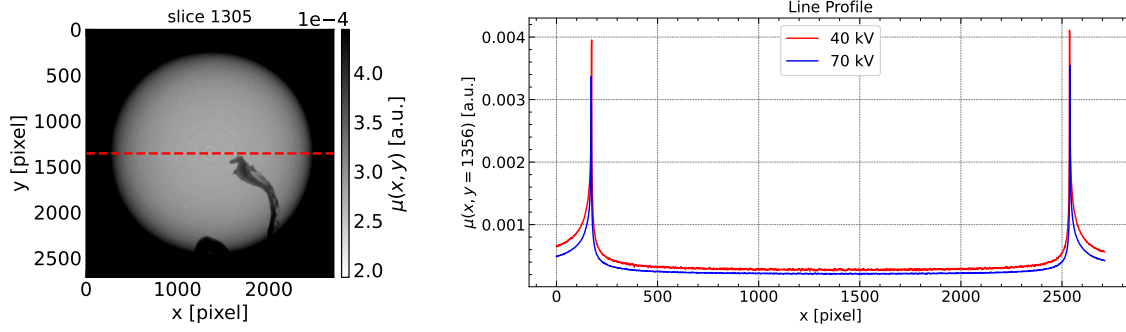


Figure 50: **Left:** A slice through the reconstructed volume of a human artery embedded in paraffin wax. The red dotted line depicts an edge spread function (ESF) through the center of the slice, while this line profile is projected on the **right**. The low-frequency artifacts surrounding the reconstructed slices result from ROI/local tomographic reconstruction [61].

tion 3.2. This is quantified in fig. 52, where the CNR, SNR, and noise fraction (noise in paraffin ROI to that of the tissue) are computed in a selected ROI (fig. 51). The SMO algorithm yields a higher CNR and SNR compared to the BAC, with also a significantly lower noise fraction in regard to when no phase retrieval algorithm is performed.

Overall, this highlights a trade-off between contrast enhancement and preservation of spatial resolution, and can be judged based on the specific requirement. The SMO algorithm is recommended in cases where noise suppression and soft tissue contrast are prioritized, and especially in cases where very small-scale features are not of interest. In contrast, the BAC algorithm provides a more balanced outcome, maintaining edge sharpness while still improving contrast. This makes the BAC method the more robust choice for quantitative analysis, especially in situations where the resolution of small-scale structures is important. Even visually, it is clear that phase retrieval methods are superior to the case where no phase algorithm is applied, justifying the use of phase algorithms in phase-contrast imaging (PCI) of FFPE blocks.

6.3 EMPTY-BEAM CORRECTION

As discussed in section 4.4.1, the correction of the empty beam is essential for an accurate representation of the acquired data. In this regard, different methods that were discussed in addition to the conventional empty-beam correction method are tested here.

The tomographic scan discussed in the previous section (section 6.2) is used for this analysis, with the relevant parameters summarized in table 5. The projection images were initially corrected using the conventional method (CF) (equation (4.13)). In this framework, projection images are corrected for the empty-beam effect after dark-field correction. The results show normalized projection images (I_0) as shown in fig. 53 (A).

In the framework *slowly varying background* (SVBF) flat correction, the projection images (I_0) were further divided by the mean of all the projection images (I_0) as discussed in equation (4.14).

Lastly, for the *eigen-flat* (EF) method, the projection images (I_0) were corrected by the mean of the four ROI (fig. 53, A) taken in the paraffin region of the two FFPE blocks, for each projection angle (θ). These four ROI were chosen so that they are well inside

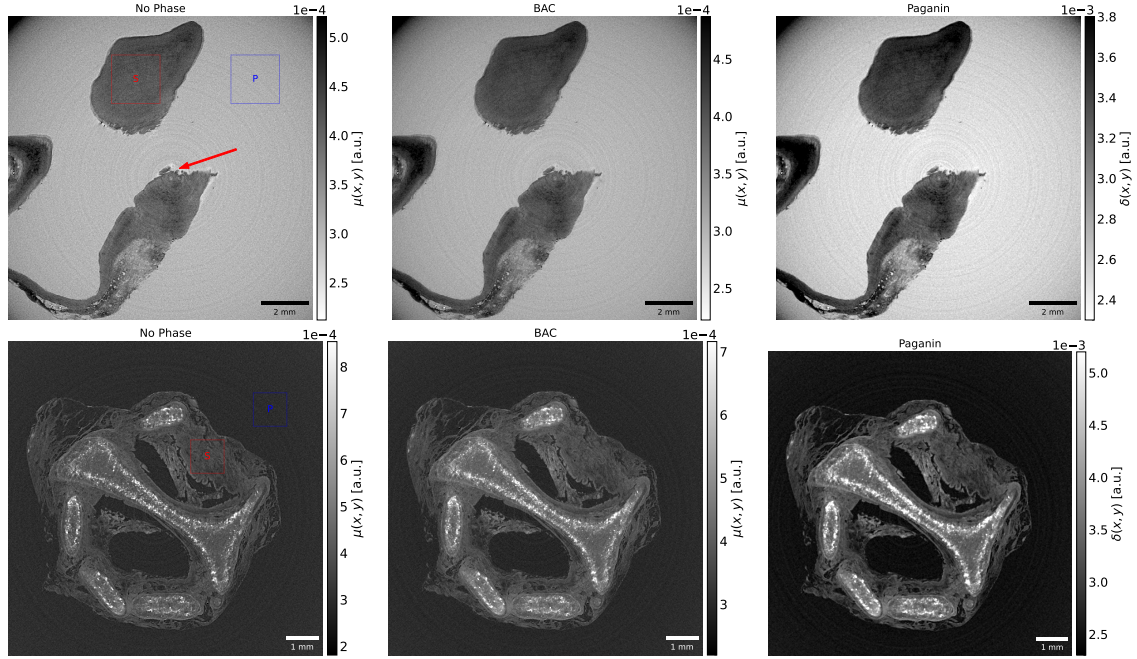


Figure 51: Phase-contrast imaging (PCI) of two FFPE blocks with an effective pixel size of $8\ \mu\text{m}$. The first row shows reconstructed slices of an **artery** while the second row shows a **human bronchus**. The slices in respective columns are reconstructed with different phase retrieval algorithms. The first column shows reconstruction with **no phase retrieval** applied, while the second and last columns employ the **BAC** and **SMO** algorithm, respectively. The **SMO** algorithm yields better **SNR** and **CNR**, trailed by the **BAC** algorithm. However, the **SMO** algorithm leads to slight blurring, which reduces the overall sharpness/resolution. This makes the **BAC** algorithm the more robust algorithm. The labeled **ROI** serves as a reference for computation of **SNR** and **CNR**. Slices are subjected to automatic contrast adjustment for visualization purposes.

the paraffin region and outside any tissue structures. This is achieved by analyzing the sinogram. The mean value of the average **ROI** was then calculated and used to generate a synthetic full empty frame. Then, all projections (I_θ) were divided by their corresponding *eigen-flat*. Figure 53 (B) shows the corrected projection after evaluating the *eigen-flat* correction. Visual inspection reveals that this does not lead to any major changes, although it is observed that the gray values are shifted as expected. In all these schemes, the reconstruction parameters were kept consistent, and **SMO** was chosen as the phase retrieval algorithm. For analysis, the reconstructed data were then binned by a factor of 2 in all dimensions, leading to an effective pixel size of $p_{\text{eff}} = 16\ \mu\text{m}$.

Figure 54 shows the results of the above analysis. Here, a slice of the **bronchus-FFPE** is shown for each empty-beam correction scheme. A slice of the bronchus sample is shown in the first row, with the other rows depicting different **ROI** for each correction scheme. Each column corresponds to a different correction scheme in the order of conventional flat-field (**CF**), *slowly varying background* (**SVBF**), and *eigen-flat* field (**EF**), respectively.

It is observed that the different correction schemes do not lead to a significant improvement in signal over the conventional method (**CF**). In contrast, these correction schemes erode very subtle features in the data, as can be seen in the different **ROI**. Especially, the *slowly varying background* (**SVBF**) method overcorrects the signal in the paraffin/background-tissue interfaces, manifesting as valleys/hills of the low/high in-

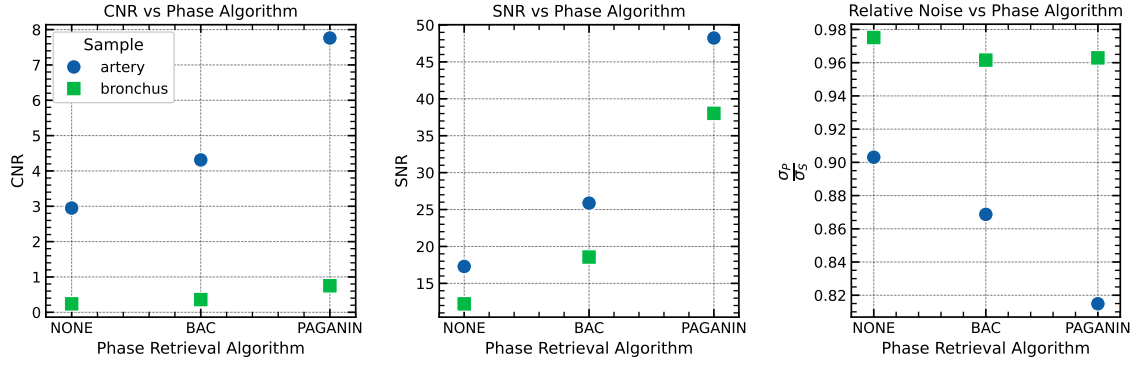


Figure 52: Relation between the different phase retrieval algorithms and image metrics. The **left figure** and **middle figure** show the **CNR** and **SNR** as a function of the phase retrieval algorithm, respectively. The **right figure** shows the noise fraction - the noise in the paraffin ROI to that of the tissue - as a function of the phase retrieval algorithm. The Paganin algorithm (**SMO**) yields a higher **CNR** and **SNR** compared to the **BAC** while also yielding a lower noise fraction. However, **SMO** tends to blur high-frequency features such that **BAC** is a more preferred choice in case overall performance is required.

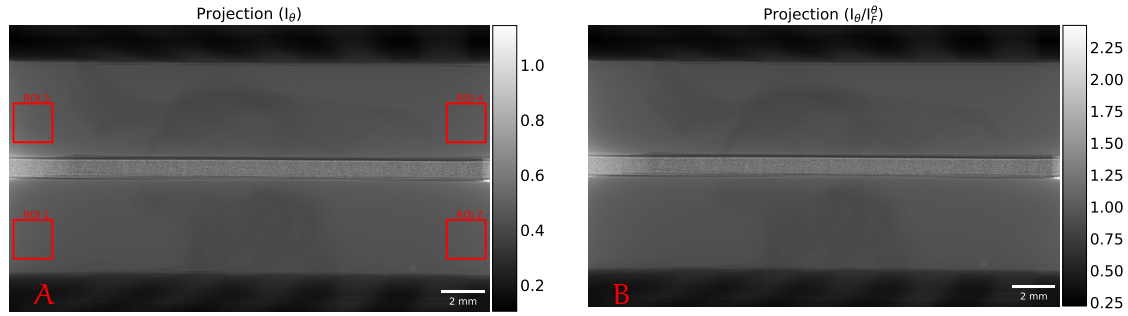


Figure 53: **Empty beam correction schemes**. **Left (A)**: projection image of two **FFPE** blocks corrected using flat-field relative to air. The 4 **ROI** for the empty-beam correction in the scheme of *eigen-flat* are denoted in red. **Right (B)**: projection image of the same sample but corrected with respect to the eigen-background, shifting the gray values.

tensity region. This can be seen in the reconstructed slice, where the background tissue region appears mottled. This overcorrection has a significant effect in regions where the tissue has a localized structure and is therefore not spread out over a larger area. This can be understood as the averaging of projection frames leads to a bias in regions where there are more or fewer than average tissues present in the **LOS**, leading to under- or over-representation.

However, visual inspection reveals that the **SVBF** correction leads to a more uniform background, less fluctuation in the background, and fewer ring artifacts in the background.

The *eigen-flat* method (**EF**), on the other hand, exhibits similar characteristics to that of the **SVBF** method, resulting in a more uniform background and reduced ring artifacts, by visual inspection. However, as noted above, this shifts the intensity distribution and erodes very subtle features in the data as well. However, this scheme does not cause any overcorrection at the tissue and paraffin interface, as was the case in the **SVBF** method. Large-scale structures are preserved and appear less noisy with slight contrast enhancement.

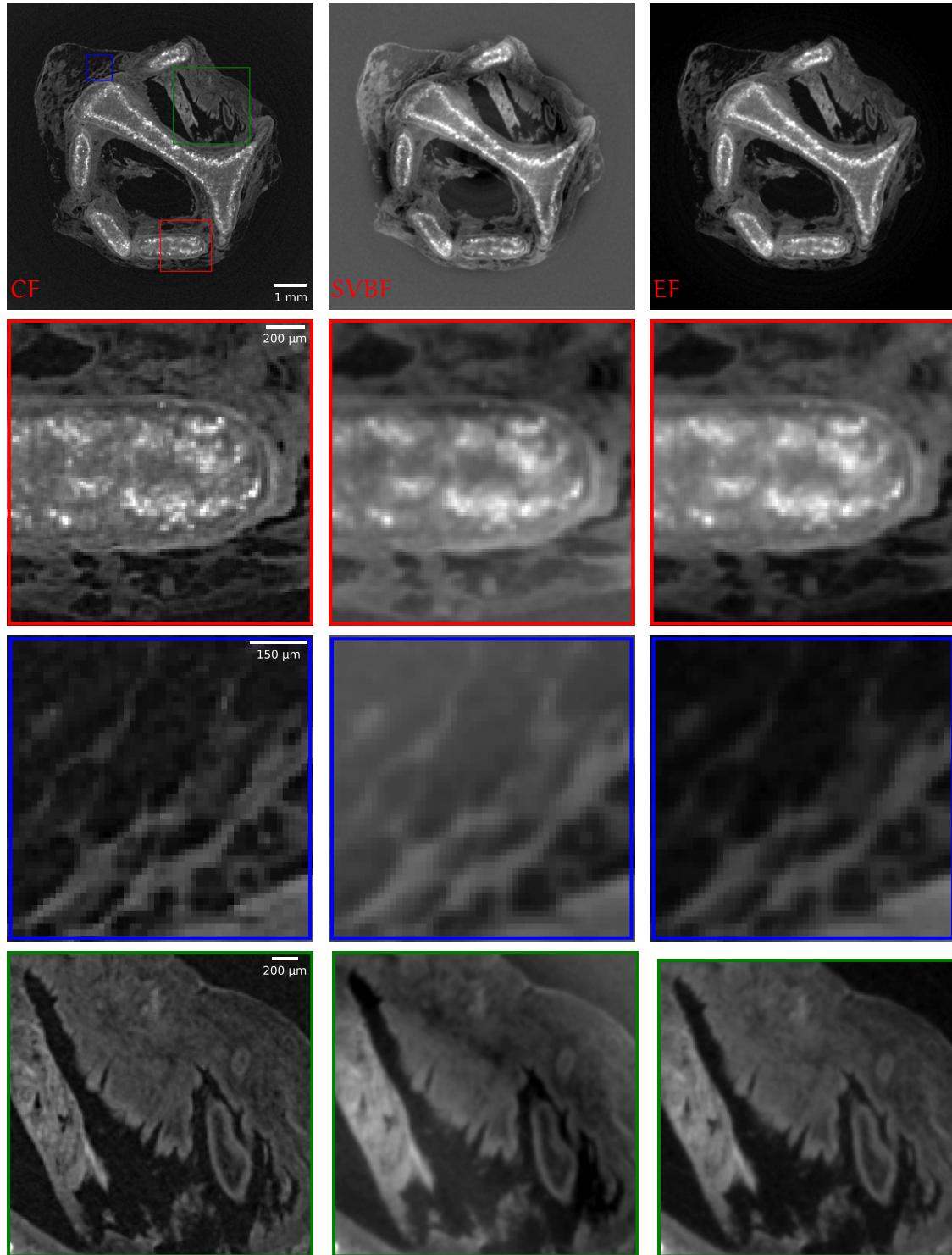


Figure 54: **Flat-field correction schemes analysis for the bronchus-FFPE.** The first row shows a slice of the bronchus sample for each correction scheme, with a scale bar of 1 mm. The second, third, and fourth rows depict different ROI of the sample with corresponding color coding. These ROI are of different scales, showing different characteristics of the samples in the respective empty-beam correction scheme. The different columns represent the different correction schemes, with CF, SVBF, and EF the conventional, slowly varying, and *eigen-flat* correction schemes, respectively.

The combination of these methods and discussions insinuates the potential for a hybrid approach, where the flat frame taken in empty paraffin could lead to a better contrast and representation of the sample. This can, in particular, be beneficial in the case where the tissues of interest are embedded in circularly symmetric paraffin blocks. In this case, a manual flat in paraffin could be taken and used to correct all scans. This symmetry eliminates the need for a framework of varying background. On top of that, the spectrum of X-rays passing through this flat field in all projection angles, as well as the sample, will be identical to the effects of the sample.

It is of importance to note that the above analysis also exhibits an inherent bias toward the correction schemes, as the choice of the consistent phase retrieval parameters in the SMO also influences the results. For a complete evaluation of the correction schemes, a more systematic evaluation of the phase retrieval parameters and their impact on the results and visibility should be considered, as briefly discussed in section 3.2.3.

6.4 TRANSMISSION ASSESSMENT

In all previous discussions, it was taken for granted that the JuLiA source is optimal for imaging of FFPE blocks. However, a more thorough assessment of the transmission of the X-ray beam through paraffin is of interest, as this can provide insights into the optimal source settings for imaging of FFPE blocks. The transmission of the X-ray beam through paraffin was briefly discussed in section 6.1, where the integrated transmission through an empty paraffin block followed automatically from the analysis. In this respect, the transmission of paraffin samples is assessed spectrally.

Spectral assessment

The transmission of the X-ray beam through a sample is given by the Beer-Lambert law (equation (2.25)). It is evident that for an optimal balance between noise and contrast, the transmission should be neither too low nor too high. However, spectrally, it is of interest to assess the spectrum of X-rays after transmission through the sample.

In this regard, a pure paraffin block with a thickness of $\Delta x = 22$ mm was placed in the beam path, and the spectrum was recorded at an acceleration voltage of 40 kV with a X-123 Si-Pin detector from Amptek [110]. This detector was placed at SDD of approximately 2.5 m and the paraffin block at $z_{01} = 150$ mm. The source was operated at a current of 0.05 mA, resulting in a source power of 2 W. The spectrum was recorded over a time frame of approximately 4 hours.

The resulting counts were normalized by power and real time, resulting in the spectrum shown in fig. 55, where the raw spectrum (without sample/filter) is shown in blue and the spectrum after passing through the paraffin block is shown in green. As discussed, the dominant peaks in the spectrum are the K_α line of gallium at 9.25 keV. However, it is observed that after transmission through the paraffin, this peak intensity is heavily attenuated, as can be seen on the linear scale.

The division of the two spectra yields the transmission spectrum of the paraffin block and thus of the FFPE blocks, as shown in fig. 56 (left). The transmission is observed to be very low for energies below 10 keV, while it increases rapidly for energies above 10 keV. The transmission curve is subjected to a model (chapter C) of the form

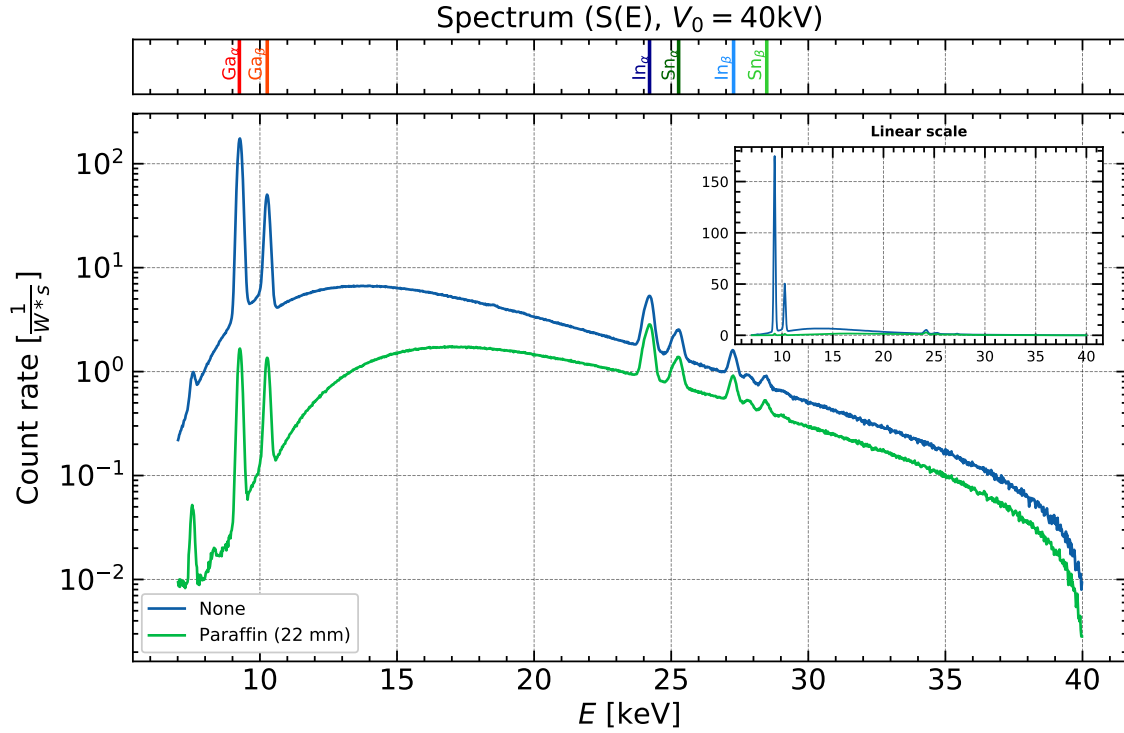


Figure 55: X-ray energy spectrum showing intensity versus photon energy (E) in keV at an acceleration voltage of 40 kV. The main plot displays the spectrum of the raw spectrum (no sample/filter) on a semi-log scale. The spectrum in green depicts the X-ray spectrum after passing through a paraffin block with a LOS thickness of 22 mm. The corresponding transmission spectrum is further discussed in fig. 56.

$$T(E) = \frac{S}{S_0}(E) = \exp(-\mu_m(E)\rho\Delta x) + b, \text{ with } \mu_m(E) = \mu_0 \sum_{n=0}^3 a_n \left(\frac{E_\alpha}{E}\right)^n,$$

where b serves as a baseline offset that accounts for the background signal, dimensionless components a_n account for the relative contribution to the attenuation coefficient with respect to energy E_α and $\mu_m(E_\alpha) = \mu_0$ in units of cm^2/g . Here, this energy is chosen as the characteristic energy of the K_α indium line at $E_\alpha = 24.22$ keV. This fit model is photon-statistic informed by imposing a noise model ($\sigma_T = \sqrt{T}$) on the transmission coefficients and the outcome of the above analysis is summarized in table 4 and subsequently shown in fig. 56 (left) in red, with the 1σ deviation shown as a shaded area. It is observed that a_1 is relatively smaller than a_3 and $a_2 = 0$, which implies that photoelectric absorption is the dominant effect in paraffin wax for the energy range below 40 keV (section 2.3).

The spectral assessment reveals that contrast formation of FFPE with a LOS thickness of 22 mm with the JuLiA source, is mainly due to the non-dominant contributions from the *bremsstrahlung* and the indium characteristic lines. The paraffin transmission spectrum is hereto modeled using the *xraylib* package [63] for different LOS thickness and is shown in fig. 56 (right).

Compared to a LOS thickness of 22 mm, a paraffin block 2 mm thick shows a significantly different transmission spectrum, with increased transmission at lower energies. This indicates that smaller paraffin samples would be the ideal imaging candidates for the JuLiA source. A paraffin sample with a thickness of 8 mm is not ideal but is less

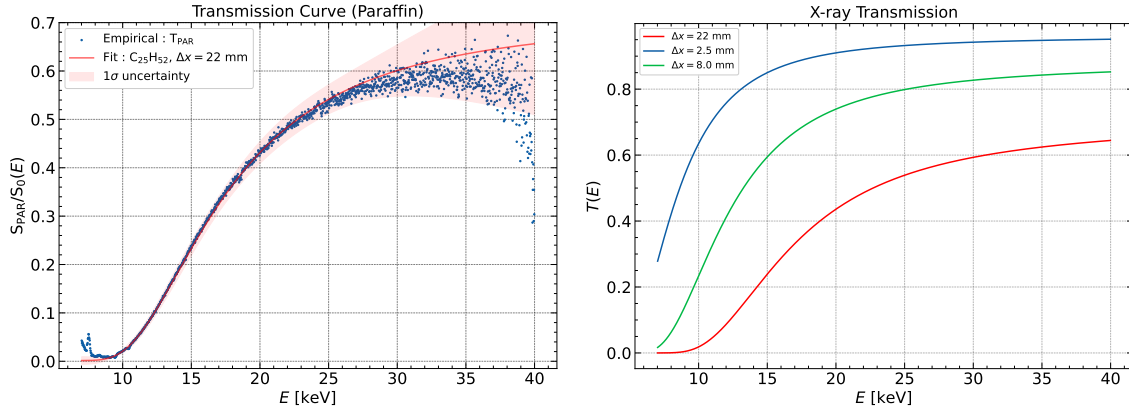


Figure 56: **Spectral assessment of paraffin blocks.** **Left:** Transmission coefficient as a function of the mono-chromatic X-ray energy for a paraffin block with a [LOS](#) thickness of 22 mm (blue). A corresponding fit model is given in red with 1σ deviation shown as a shaded area in red. **Right:** spectral transmission of paraffin with different thicknesses. The red curve is representative of the thickness of [FFPE](#) used in this work, while the green curve represents a paraffin block with a thickness of 8 mm. Smaller paraffin punches of around 2 mm would be ideal for imaging applications at [JuLiA](#) source, as shown in blue.

attenuating than the sample with a [LOS](#) thickness of $\Delta x = 22$ mm. In this line, a region of interest (ROI) imaging of [FFPE](#) tissue block is considered.

Table 4: Summary of the fit of parameters of the paraffin transmission model in fig. 56 (left). The fit parameters hint that the photoelectric absorption is the dominant effect in the paraffin wax for energy range below 40 keV.

b	μ_0 [$\frac{\text{cm}^2}{\text{g}}$]	a_0	a_1	a_2	a_3
0.00148	0.333	0.5	0.113	0.0	0.373

6.5 REGION OF INTEREST IMAGING

In light of the previous analysis, it is reasonable to consider region of interest (ROI) imaging of the [FFPE](#) blocks. In this regard, a biopsy punch of 8 mm diameter is used to extract a ROI from the bronchus sample.

This is achieved by heating the [FFPE](#) block at 40°C for 10 minutes to soften the wax, followed by the use of a biopsy punch to extract the sample. The sample is then carefully glued to a 3D printed sample pin using an instant adhesive and subsequently mounted on the sample stage for imaging. This is shown in fig. 57, where a full [FFPE](#) block of a human bronchus is shown in **left**, while the biopsy punch of 8 mm diameter is shown in **middle** and a corresponding projected image is shown in **right**. A tomographic scan with consistent source, geometry, and reconstruction parameters was performed as discussed in section 6.2 and summarized on the table 5.

The reconstructed slice of the biopsy punch is shown in fig. 58 on the left, with a slice from a whole [FFPE](#) block on the right for comparison. The latter was subjected to a circular ROI for visualization. It is visually observed that the image quality of the

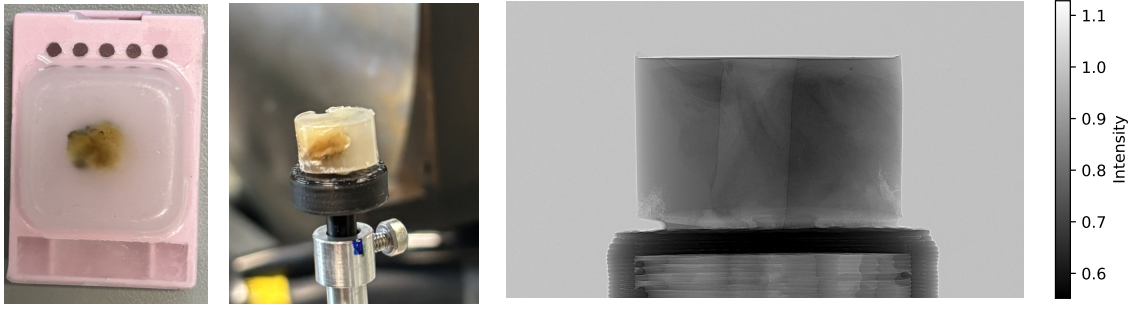


Figure 57: **Region of interest imaging.** **Left:** A full **FFPE** blocks of a human bronchus. **Middle:** A biopsy punch of a human bronchus embedded in paraffin wax with a diameter of 8 mm with a projected image shown in **right**.

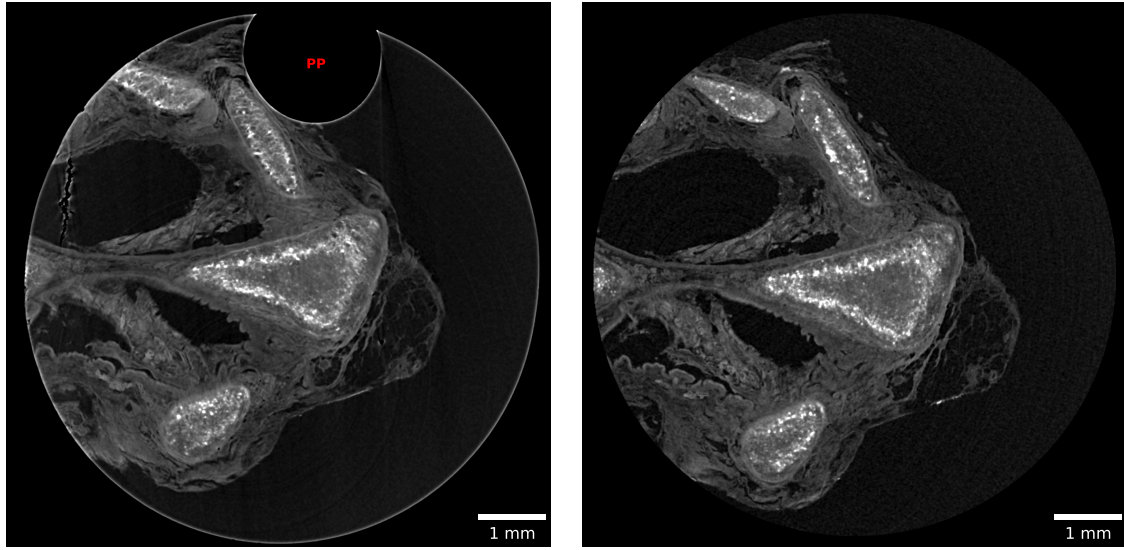


Figure 58: **Reconstructed slices of the biopsy punch (left) and the whole **FFPE** block (right).** The **ROI** (left) labelled **PP** is a 2 mm which was extracted for the larger **FFPE** for high resolution imaging. Very fine tissue structures are visible with improved signal-to-noise ratio (**SNR**) in the biopsy punch.

biopsy punch is better than that of the whole block, with finer details and less noise. Although very fine tissue structures are visible in both reconstructed slices, the signal in the biopsy punch is comparatively stronger with better connectivity. The **SNR** and **CNR** were computed in a uniform **ROI** (fig. 58) for both samples, and the results are summarized in fig. 59. It is observed that both metrics are significantly higher for the biopsy punch sample compared to the whole tissue block, confirming the visual impression.

6.6 SPECTRAL TAILORING AND BEAM PRE-HARDENING

It is evident from previous discussions that the lower energy X-ray photons are beneficial for the signal and the overall contrast of **FFPE** as discussed in sections 3.1, 6.1.1 and 6.5. However, these lower photons are also heavily absorbed, reducing the most dominant peak to the *bremsstrahlung* yield (figs. 55 and 56). Therefore, the use of X-

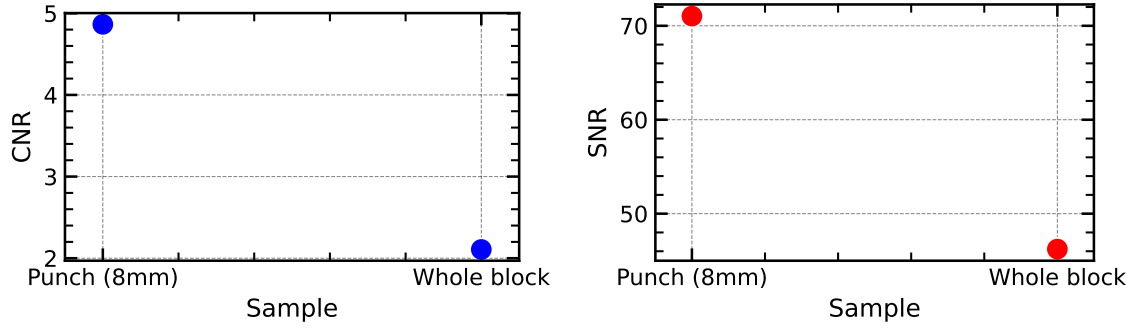


Figure 59: **Comparison of CNR and SNR values between punch biopsy samples and whole tissue blocks.** Both metrics demonstrate superior image quality in smaller punch biopsy specimens compared to larger whole tissue block samples.

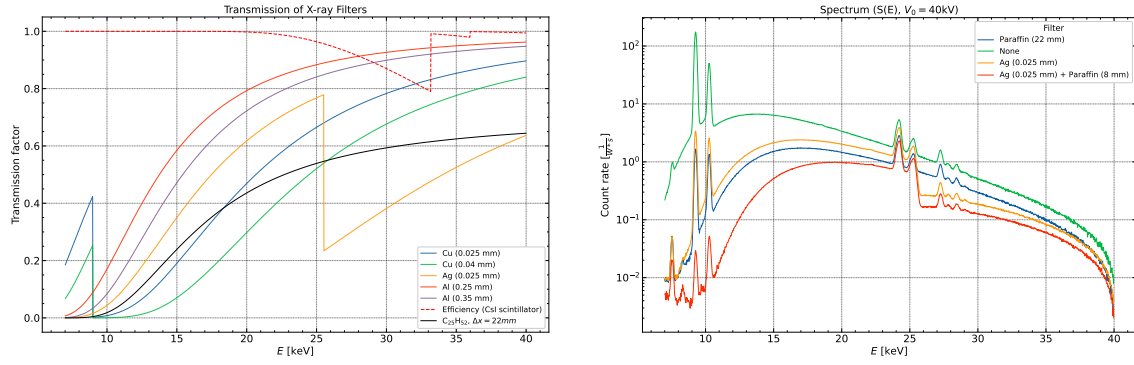


Figure 60: **Left:** transmission coefficient of different X-ray filters versus the energy. The red curve is the absorption factor of the cesium iodide (CsI) scintillator with a thickness of 500 μm . **Right:** measured spectra of JuLiA source for different filters on a semi-log scale. Characteristic lines of gallium K_{α} -edge at 9.251 keV and indium at 24.210 keV are clearly visible in the raw spectrum. Reduction of low-energy photons due to the filter is also very apparent and dominant. The spectra were measured with a X-123 Si-Pin detector from Amptek [110].

ray filters to tailor the spectrum in a broad spectrum is a good idea. Filtering of the spectrum can be described mathematically by

$$S(E) = S_0(E) \prod_j^n \exp(-\mu(E, Z_j) \Delta x_j)$$

with S_0 the initial spectrum and Δx_j the thickness of the filter. The X-ray filters are placed in the beam path at the exit window of the source. The spectrum of some candidate filters used here was measured and is shown here in fig. 60 (right) with modeled transmission in fig. 60 (left).

Tomographic scans were performed with different filters of different thicknesses and combinations. The filters were chosen with the aim of mainly attenuating the continuous spectrum while retaining the characteristic radiation of indium (In). The bronchus-artery FFPE blocks were imaged $z_{01} = 120$ mm, with a current and peak voltage of 1.431 mA and 40 kV or 70 kV respectively. The tomographic scan was performed with the Varex detector at $z_{02} = 1500$ mm, resulting in an effective pixel size of 8 μm in the angular range of 360° with a total of 2000 angular projections. The duration of the

Table 5: **Experimental settings for the different measurements at different setups.** Scans conducted at the JuLiA setup and MONA were imaged with the Varex detector. The respective source settings and exposure times of the other setups were based on common practice, while geometric parameters were chosen such that a similar effective pixel size of approximately $8\text{ }\mu\text{m}$ was maintained, while imaging full FFPE blocks.

Parameter	JuLiA	EasyTom	MONA	HECTOR
V_0 [kV]	40	100	60	80
i [mA]	1.431	0.1	0.07	0.125
P [W]	57.24	10.00	4.20	10.00
t_s [s]	7.0	2.5	14.0	3.0
t_{tot} [s]	28000	18000	56000	10500
z_{01} [mm]	120.00	46.39	99.20	85.18
z_{02} [mm]	1500.00	735.00	1076.20	1244.36
M	12.50	15.84	10.85	14.78
p_{eff} [μm]	8.00	8.02	9.22	10.15
N_p	2	5	1	1
N_D	15		15	40
N_F	30		30	80
N_θ	2000	1440	4000	3500
Angular range	$[0 - 360]^\circ$	$[0 - 360]^\circ$	$[0 - 360]^\circ$	$[0 - 360]^\circ$
Phase retrieval	SMO	SMO	SMO	SMO
$(F, \frac{\beta}{\delta})$	(1, 0.06)		(1, 0.06)	
Reconstruction	hotopy[40]	Xact [59]	hotopy[40]	Octopus[80]
Detector	Varex 3131Z	PaxScan2520DX-I	Varex 3131Z	Varex XRD 4343

exposure was maintained at 7 s or 14 s per projection, and depending on the signal, multiple projections per angle were taken to increase the overall signal.

For calibration, 15 dark frames were taken at the end of the acquisition, while 30 flat frames were taken before and after the tomographic scan. The tomograms were reconstructed using the FDK algorithm, and contrary to previous scans, the BAC algorithm was chosen here as the preferred phase retrieval algorithm. Subtle ring removal artifact was applied using the *additive* method (section 3.4.2).

The reconstructed slices of all the scans for different filters were benchmarked against the baseline scan (table 5). However, these scans did not show any significant improvement in image quality compared to the baseline. In contrast, these scans exhibited a slight degradation in image quality, particularly in noise levels. Therefore, these are not shown here.

Figure 61 shows the CNR and SNR as function of the X-ray filter. These metrics were accessed in the same slices. It is observed that for both samples (bronchus, artery), the CNR and SNR are highest in the case of the silver ($_{47}\text{Ag}$) filter with a thickness of $25\text{ }\mu\text{m}$, at an acceleration voltage of 70 kV. This is trailed by the use of a $25\text{ }\mu\text{m}$ silver filter and a combination with aluminum ($_{13}\text{Al}$), but requiring twice the exposure time.

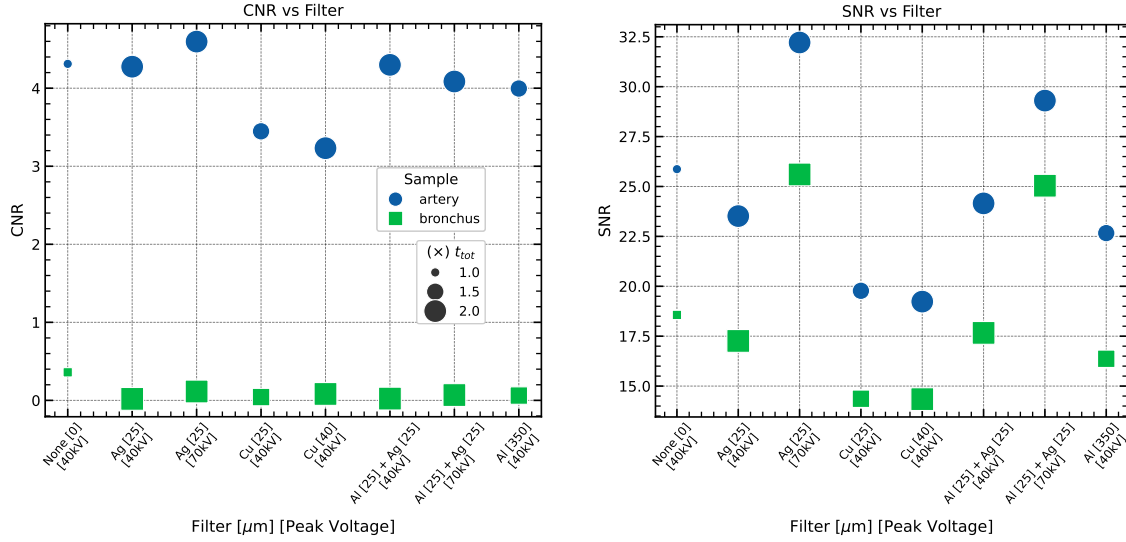


Figure 61: **Left:** scattered **CNR** measurements as a function of the filter for the two different samples. The cross section of the scattered point represents a multiple of the total exposure time of the baseline scan (**NONE**) with $t_{\text{tot}} = 28000$ s. Similarly, the figure on the **right** represents **SNR** as a function of the filter.

As noted above, the introduction of the filter does not lead to a significant change in the outcome of the reconstruction. This is attributed to the fact that the **FFPE** sample itself absorbs mainly all low-energy photons such that the introduction of the filter has a minor to no effect on the overall image quality. In this regard, it is recommended for imaging of whole **FFPE** with the **JuLiA** setup to not use any filter at all, as this does not lead to any significant change in image quality while requiring more exposure time. This can also be seen by considering the effective **CNR** and **SNR** measurements as a function of the filter for two different samples, as shown in fig. 62. In addition to whether the images look visually appealing or not, this reveals that the use of a copper ($_{29}\text{Cu}$) filter, at an acceleration voltage of 40 kV, leads to the worst image quality in terms of both **CNR** and **SNR** metrics, while not filtering at all, is the most optimal choice.

6.7 COMPARISON OF IMAGING RESULTS ACROSS DIFFERENT CT-SETUPS

In order to unravel the potential of phase-contrast imaging (**PCI**) of **FFPE** samples and their different imaging modalities, it is of interest to scan across different setups and compare their results. In this light, studies on the **FFPE** samples were performed not only using the **JuLiA** setup but also the **HECTOR** and **EasyTom** setup at **UGCT** and **HBCG** respectively, as well as the **MONA** setup.

Experimental setup and methods

Scans at the different setups, as lengthily discussed in section 5.1, were performed using the same samples to ensure comparability of the results, while the geometric and source settings were chosen according to common practice.

Scans at the **JuLiA** setup were performed with a $z_{01} = 120$ mm and a $z_{02} = 1500$ mm, with a current and peak voltage of 1.431 mA and 40 kV, respectively, and without

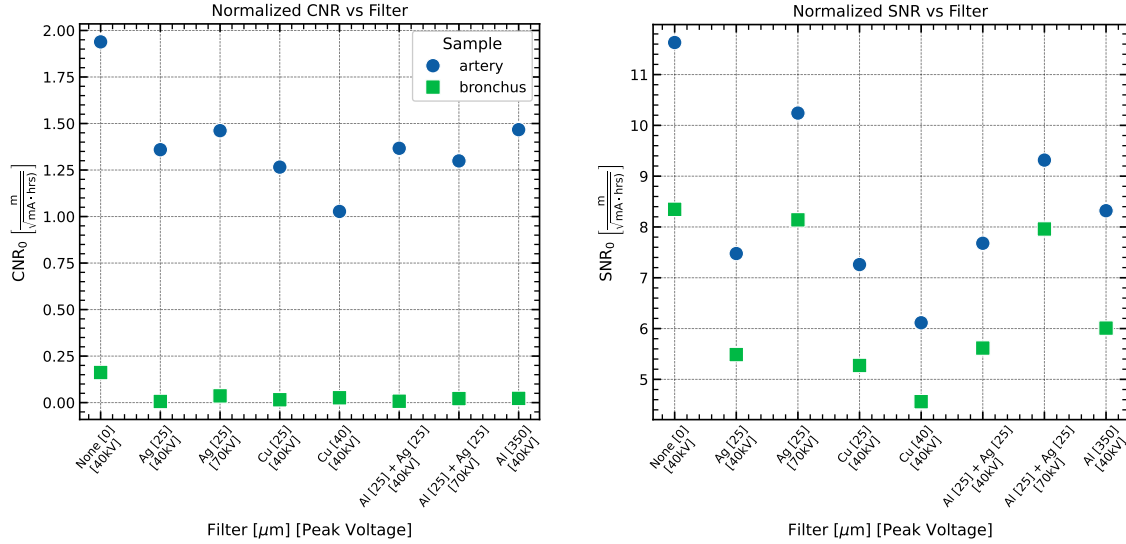


Figure 62: **Left:** effective CNR measurements as a function of the filter for two different samples. Similarly, the **right** figure gives SNR as a function of the different setups. The peak voltage that was used for the measurements is also indicated.

any filter. The tomographic scan was performed with the Varex detector, yielding an effective pixel size of $8 \mu\text{m}$ over an angular range of 360° with a total of 2000 angular projections. Each projection had an exposure time of 7 s, and 2 frames per angle were accumulated, resulting in a total exposure time of 28000 s. For calibration, 15 dark frames were acquired at the end of the scan, while 30 flat frames were taken before and after the tomographic scan. The tomograms were reconstructed using the FDK algorithm, with the single material object (SMO) algorithm applied for phase retrieval. In addition, a subtle removal of ring artifacts was applied using the *additive* method implemented in the *hotopy* package [40].

Due to the relatively low brilliance of the other setups, a consistent and comparable effective pixel size was maintained, while other geometric parameters were adjusted to allow for PCI of full formalin-fixed paraffin-embedded (FFPE) blocks within reasonable exposure times.

For the EasyTom setup, scans were performed with the sample placed at $z_{01} = 46.39 \text{ mm}$ and detector at $z_{02} = 735 \text{ mm}$, yielding an effective pixel size of $8.02 \mu\text{m}$. The tomographic scan was performed with the PaxScan2520DX-I flat-panel detector (FPD) over an angular range of 360° with a total of 1440 angular projections. Each projection had an exposure time of 2.5 s, and 5 frames per angle were accumulated to improve photon statistics. The total exposure time was 18000 s. The tomograms were reconstructed using native Xact software [59].

The Varex detector was paired with the MONA setup for the tomographic scans of the FFPE blocks. The samples were placed at $z_{01} = 99.2 \text{ mm}$ from the source, with the FPD placed at $z_{02} = 1076.2 \text{ mm}$. The source was operated at 0.07 mA and 60 kV without a filter. A single projection per angle was acquired with an exposure time of 14 s. A total of 4000 projections were taken over 360° , resulting in a total exposure time of 56000 s, which is double that of the JuLiA scan (i.e. 28000 s). For calibration, 15 dark frames and 30 flat frames were used. Reconstruction was performed using the FDK algorithm combined with the single material object (SMO) algorithm. In short,

the scans at the [MONA](#) setup followed the same reconstruction workflow as the [JuLiA](#) setup, differing only in the source settings and the exposure time.

Finally, the [HECTOR](#) setup was used to scan the same samples with $z_{01} = 85.18$ mm and $z_{02} = 1244.36$ mm. No X-ray filter was used and the source was operated at 80 kV and 10 W. The tomographic scan was performed with the XRD 4343 [FPD](#), yielding an effective pixel size of $10.15 \mu\text{m}$ over 360° with a total of 3500 projections. Each projection had an exposure time of 3 s, with single frames acquired per angle, giving a total exposure time of 10500 s. For calibration, 40 dark frames and 80 flat frames were used. The tomograms were reconstructed using Octopus software [80] and the Paganin ([SMO](#)) algorithm, with minimal to no post-processing applied. A summary of all relevant parameters is given in [table 5](#).

It is important to note that comparing these acquisitions is non-trivial due to differences in exposure settings and reconstruction workflows. While the [JuLiA](#) and [MONA](#) setups allow for a direct comparison of source effects (as they share a detector and reconstruction pipeline), the results from the [EasyTom](#) and [HECTOR](#) systems are less trivial to evaluate due to their inherently different reconstruction algorithms, exposure parameters, and detectors. Consequently, the comparison is based visual judgement and evaluates the image quality of each system configuration.

Results and discussion

Reconstructed slices of the [FFPE](#) samples from different setups are presented in [fig. 63](#). Here, only virtual slices of the bronchus and artery samples are shown. The first row shows the reconstructed slices obtained with the [HECTOR](#) setup. The first and second columns correspond to slices of the artery sample, while the last column represents the bronchus. The second row shows the reconstructed results using the [JuLiA](#) setup, followed by the [EasyTom](#) and [MONA](#) setups in the third and last rows, respectively. The reconstructed slices are shown on either a gray- or inverted gray-scale map, depending on the visibility of features, and all scans were subjected to automatic contrast adjustment for visualization purposes.

In general, the brilliance of the [JuLiA](#) source provides an optimal environment for high-quality imaging of [FFPE](#) samples. Scans conducted with the [JuLiA](#) and [MONA](#) setups are visually more appealing and exhibit superior imaging performance compared to the others, regardless of imaging time. The corresponding [CNR](#) and [SNR](#) values for the different setups are shown in [fig. 64](#), where the cross section of the data points indicates the total exposure time and the color encodes the type of sample. It is observed that the [CNR](#) from the [JuLiA](#) setup is significantly higher than those from the other setups, with the exception of [MONA](#).

The improved performance can be attributed to the high brilliance and relative flux of low-energy photons in the [JuLiA](#) setup, which promote better signal acquisition and enhanced contrast. By comparison, the [EasyTom](#) and [HECTOR](#) setups yield higher [SNR](#), but with lower [CNR](#) and less visually pleasing reconstructions. Their higher [SNR](#) arises from the dominance of high-energy photons, which improve signal strength but reduce contrast. The [MONA](#) setup stands out by providing a good balance between signal and contrast, and thus provides superior photon economy for [FFPE](#) imaging. This is consistent with the analysis in sections 3.1 and 6.4, where it was shown that low-energy photons are crucial for contrast formation, while high-energy photons mainly contribute

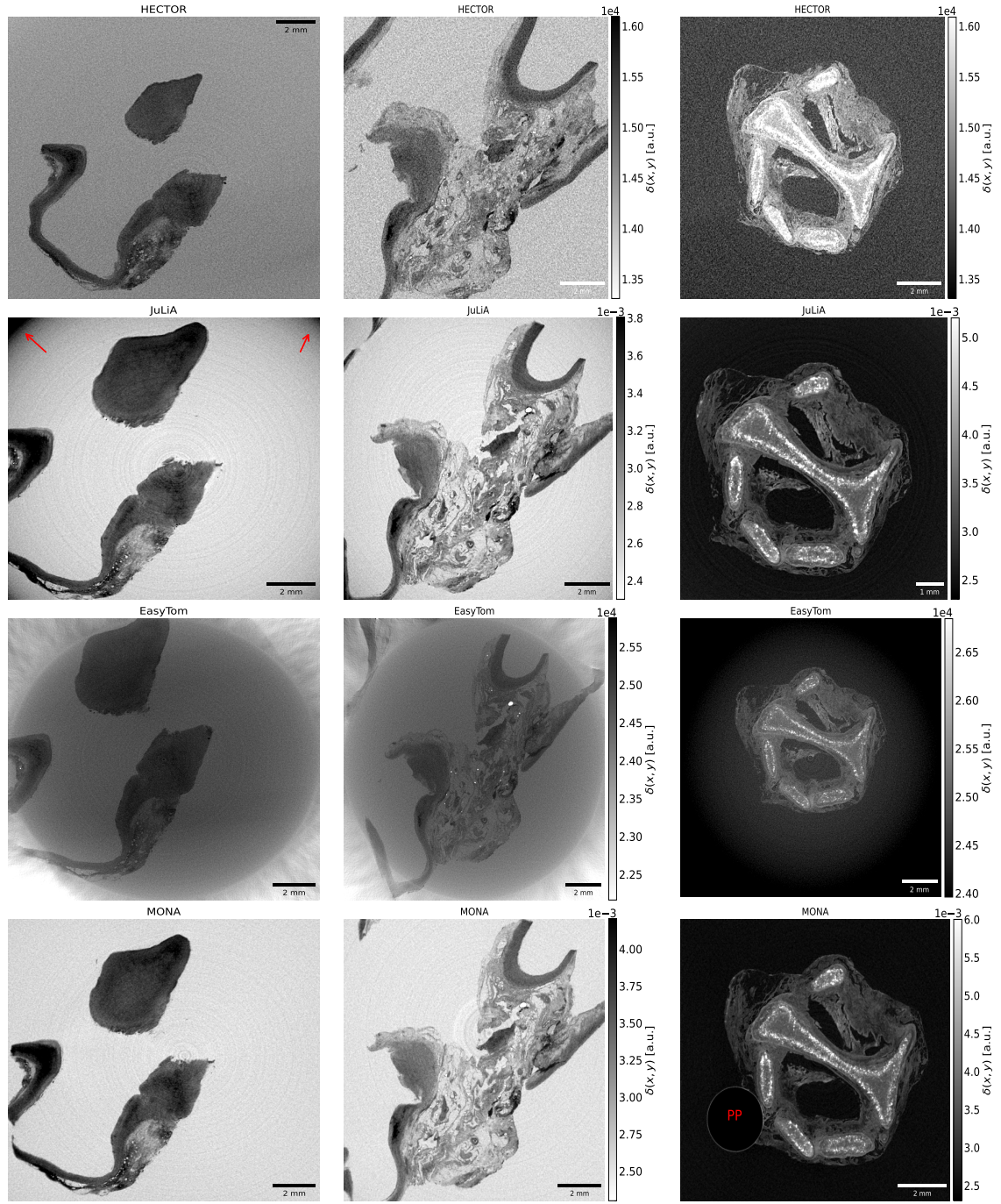


Figure 63: **Reconstructed slices of a human artery and bronchus FFPE samples, imaged using different setups with different source characteristics.** The first row shows the reconstructed slices of a FFPE sample at the HECTOR setup. The first and second columns are representative slices of the artery sample, while the last column is that of the bronchus. The second row shows the reconstructed results using the JuLiA setup, followed by the EasyTom setup and MONA setup, respectively, in the third and last row. The effective pixel size of all the scan was held relatively constant around $8\ \mu\text{m}$. The reconstructed slices are shown on a gray or inverted gray scale map and are also subjected to automatic contrast adjustment. The ROI labeled PP in the last row and last column is a circular ROI that was extracted for high-resolution imaging. The dark regions, indicated with the red arrows, in the JuLiA artery slice, are artifacts due to ROI/local tomography [61]. Similar artifacts are likewise visible in the EasyTom artery slice.

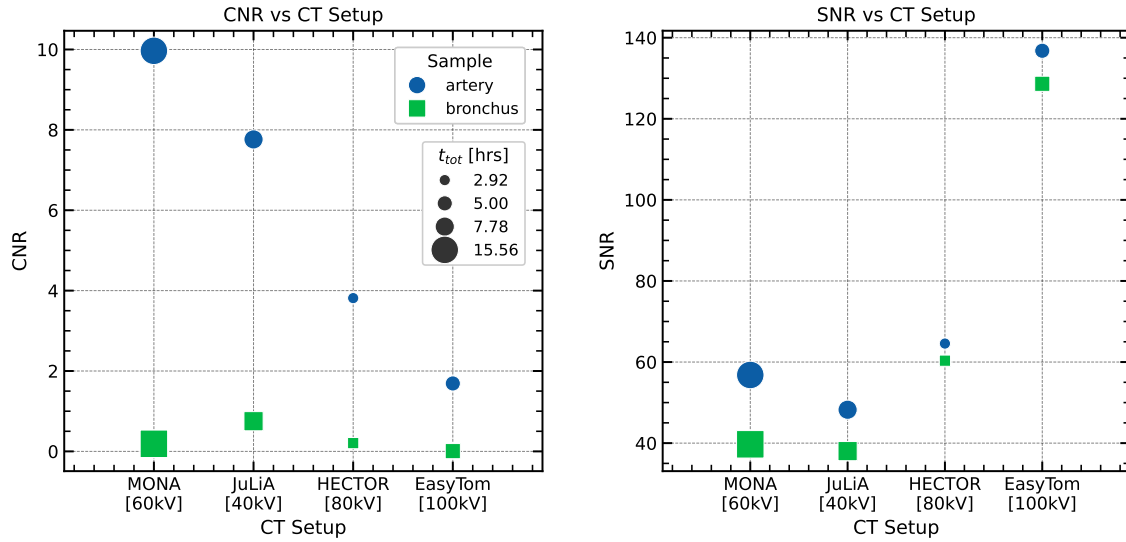


Figure 64: **Left:** measured CNR as function of the different setups, in similar ROI in the respective samples. The cross section of the scattered point depicts the total exposure time in hours. The JuLiA and MONA setups show higher CNR and SNR values compared to the other setups. Similarly, the **right:** figure gives SNR as a function of the different setups. The peak voltage at which the scans were conducted is also indicated under the respective setups.

to signal strength. The MONA spectrum achieves this balance through its molybdenum K_{α} line at 17.48 keV, which is expected to penetrate the FFPE relatively more effectively (fig. 56): not too low to be fully absorbed, and not too high to reduce contrast. In contrast, the dominant K_{α} line of gallium is heavily attenuated by the sample, thereby contributing relatively less to the overall contrast (fig. 55) and signal.

This conclusion is further supported by the normalized metrics in fig. 65, where both CNR and SNR have been corrected for exposure time and SDD, i.e. $\text{SNR} \propto \sqrt{N} \propto \text{SNR}_0 \cdot \sqrt{i \cdot t} / z_{02}$, and equivalently for the CNR. The MONA setup achieves the highest normalized CNR, confirming its advantage in contrast gain, while setups dominated by high-energy photons (such as EasyTom and HECTOR) yield higher SNR.

In conclusion, contrast gain at the JuLiA setup can be attributed to its continuous *bremsstrahlung* spectrum and the contribution of the indium K_{α} line at 24.2 keV. These results highlight the importance of a source spectrum that balances low- and high-energy photons. For optimal imaging of FFPE samples, the source should ideally have a dominant spectral peak in the range of 15 – 25 keV. High-brilliance sources such as liquid-metal jets, especially with indium as the dominant anode material, or conventional and rotating anode sources with molybdenum ($_{42}\text{Mo}$), would be an ideal candidate, combining short exposure times with strong signal leading to high contrast gain.

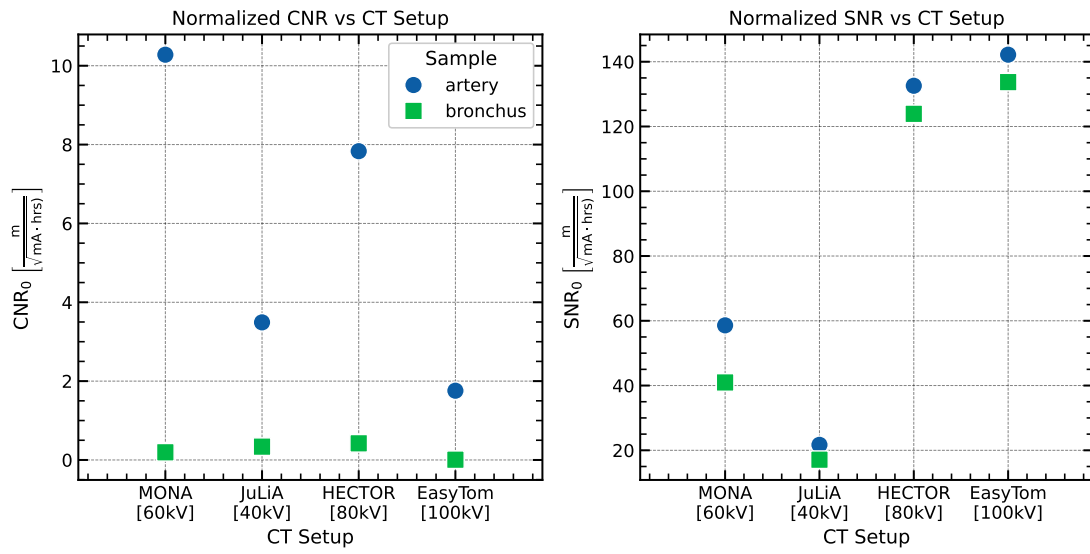


Figure 65: **Effective image metrics.** The **SNR** and **CNR** have been normalized, accounting for the difference in exposure time and **SDD**. **Left:** the **MONA** setup shows a higher normalized **CNR** compared to the other setups, indicating better performance in terms of contrast gain, which is here the metric of merit. **Right:** the **SNR** is high for setup with dominant high energy photon.

3D VIRTUAL HISTOLOGY

Having established and discussed the optimization of X-ray imaging parameters in the previous chapter, this chapter focuses on the outcome and limitations of imaging of various formalin-fixed paraffin-embedded (FFPE) biological samples with the JuLiA setup. Here, tomograms of various FFPE biological samples, which were acquired, will be presented and briefly discussed. These data sets allow for an exploration of anatomical structures in situ, preserving the tissue structure while allowing for quantitative analysis and elimination of the labor-intensive procedure in classical histology. Special attention is paid to biological samples that originate from different systems of the human body, such as the central nervous system, circulatory system, respiratory system, and reproductive system.

Before demonstrating the capabilities propagation based imaging (PBI) of FFPE sample with the JuLiA setup, a brief overview of the FFPE preparation process is justified and briefly discussed in the following.

7.1 MATERIALS AND METHODS

Throughout this work, FFPE blocks were mainly obtained from collaborating research institutions *Medizinische Hochschule Hannover (MHH)*. MHH manages tissue samples and patient information in accordance with the respective rules and regulations. A few of the samples were obtained from the *Universitätsklinikum Hamburg-Eppendorf (UKE)* and *Oslo Universitysykehus*. These institutions also follow strict ethical guidelines in their respective practices. All samples were collected for research purposes, with ethical approval ensured, and patient data fully anonymized. These are further documented with additional optical images in section D.2.

The making of a histology block involves intricate steps that each require attention and precision. The process begins with the collection of the tissue sample, which is then fixed in formaldehyde, also referred to as formalin (CH_2O). The fixed tissue is then dehydrated in a series of ethanol ($\text{CH}_3\text{CH}_2\text{OH}$) baths with increasing concentrations of ethanol, followed by a clearing process in xylene ($(\text{CH}_3)_2\text{C}_6\text{H}_4$). The tissue is then embedded in paraffin wax, which solidifies at room temperature. This can be categorized into fixation, dehydration, clearing, and embedding, as shown in fig. 66, and a more elaborate description is given below [57, 60].

Fixation

After careful dissection of the tissue of interest, the sample is immersed in fixative. Tissue sections are incubated in fixative formalin (formaldehyde), which penetrates tissue, cells, and discontinues cellular processes by cross-linking proteins, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA), thus preserving the structural components of the tissue. The duration of formalin fixation is critical and varies with the type and size of the tissue, with optimal fixation times ranging generally from 6 to 24 hours or longer.

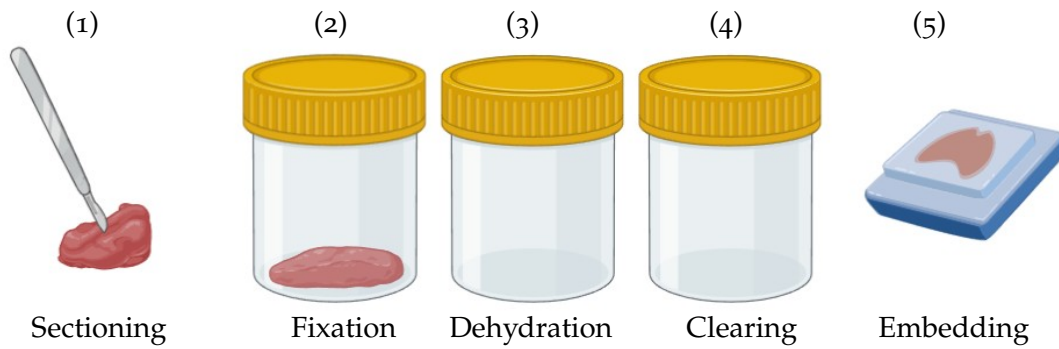


Figure 66: **Schematic illustration of the formalin-fixed paraffin-embedded (FFPE) preparation process.** The tissue is first fixed in formalin (2) to preserve the structural components, followed by dehydration (3) in a graded series of ethanol with increasing concentration. This is followed by clearing in xylene (4), allowing for complete infiltration of paraffin wax. The tissue is then embedded (5) in paraffin wax, which solidifies at room temperature, providing structural support for sectioning and imaging [43]. Above illustration was created using [Biorender.com](https://biorender.com).

This ensures hardening and thorough preservation while preparing the tissue for further processing. This step is followed by the dehydration process, which is essential for the preservation of tissue structure [43, 57, 60].

Dehydration

After fixation, the tissue is dehydrated to allow complete infiltration of the tissue by paraffin wax. The water content in the specimen during this process is removed because of its insolubility in paraffin wax. Therefore, the sample is dehydrated by repeatedly immersing the tissue in a graded series of ethanol solutions with increasing concentration. The dehydration process is a slow process and requires careful monitoring to avoid rapid or uneven dehydration, which can cause tissue distortion. Immersion of the specimen immediately in high-concentration ethanol is not recommended, as it can lead to tissue shrinkage and distortion.

Xylene replacement of ethanol

Clearing of the specimen is still necessary as ethanol and water are immiscible. The clearing solvent, which should be fully miscible with both ethanol and paraffin wax, is used to displace ethanol in the tissue and to remove a substantial amount of fat from the tissue ('clearing'). This allows for the full penetration of the paraffin wax into the tissue. Xylene ($(\text{CH}_3)_2\text{C}_6\text{H}_4$) is a commonly used clearing agent, but a solvent such as isopropanol ($\text{C}_3\text{H}_7\text{OH}$) is often used as an alternative, which is a relatively less toxic solution. A typical clearing step involves immersing the tissue in a series of clearing solvents for relatively short periods, typically ranging from 30 minutes to 1 hours per step.

Embedding

After clearing, the tissue is embedded in hot and melted paraffin at approximately 60°C. Then, this is cooled in a mold of choice and solidifies, providing structural support for the tissue. After solidification, the block on the histology cassette, which serves as a support, can be easily removed from the mold.

7.1.1 Imaging protocol

All results provided of FFPE samples were imaged using the liquid metal μ CT X-ray source (JuLiA) with a focus on achieving high contrast, cell resolution, and low noise. The final results were obtained at a source peak voltage of 40 kV and a current of 1.4310 mA. Already discussed, this serves as a sweet spot with relatively high resolution and high contrast gain for imaging the whole block with the Varex detector. Unless otherwise stated, geometric parameters such as SOD and SDD will be set at 120 mm and 1500 mm, respectively. This allows for an effective pixel size of 8 μ m. For the tomograms, a total of 2000 angular projections were acquired and an exposure time of 7 s is maintained. The Feldkamp-Davis-Kress algorithm (FDK) implemented in the *hotopy* toolbox [40] is used for reconstruction with the Paganin algorithm (SMO), the phase retrieval method.

7.2 HIPPOCAMPUS - TLE003

The nervous system consist of the brain, spinal cord and nerves and serves as the body by coordinating information [21]. It regulates complex behaviors and processes such as memory and regulation. This system can be categorized into two main parts, which is the central nervous system and the peripheral nervous system [21]. The former is made up of the brain and the spinal cords. The role of the brain is to function as the control center, processing sensory information, regulating and coordinating responses.

The hippocampus, a part of the brain's limbic system, plays a crucial role in memory and spatial navigation as well as the responsibility of learning. The limbic system consist of sets of brain structures that are responsible for smell regulation, emotions, memories as and autonomic behaviors. The limbic system is mainly relied on hippocampus to play the most significant role and the dentate gyrus. The hippocampus provides one with the awareness of the environment while playing a key role in short- and long term memory formation. It is situated on the left and right side of the brain, within the temporal lobefig. 67 (left).

Figure 67 (right) spells out the anatomical structure of the limbic system with inclusion of the hippocampus, amygdala, cingulate gyrus, fornix and mammillary body. The hippocampus is a curved structure that is located in the medial temporal lobe of the brain. It is divided into two main parts: the dentate gyrus and the Cornus Ammonis (CA) region, which is further subdivided. It is involved in various functions such as memory consolidation, spatial navigation and emotional regulation and also known to be involved in the formation of new neurons.

Temporal lobe epilepsy (TLE) belongs to the family of focal onset seizures that hinder brain function. It is a long-term disorder characterized by recurrent seizures due to abnormal electrical activity induced by damaged brain cells [46]. The most common

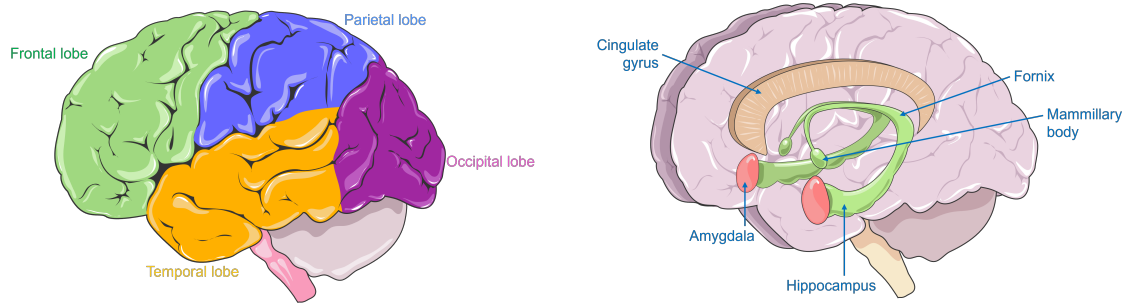


Figure 67: **Left:** view of the human brain segmented into major lobes. **Right:** anatomical structure and substructures of the limbic system including the hippocampus, amygdala, cingulate gyrus, fornix and mammillary body. These substructures are all involved in memory and emotional processing. Above figures are adapted from [64].

tracers of TLE is temporal changes in awareness, consciousness, sensations, emotion, and disruptive muscle jerking [19]. TLE is the most common focal onset epilepsy, and 80% of TLE cases are mesial (medial), arising from the inner (medial) part of the temporal lobe that may involve the hippocampus, gyrus, or amygdala [16, 45]. On top of this, it has been demonstrated that patients with TLE exhibit decreased hippocampal functional connectivity ipsilateral to the epileptogenic focus and a compensatory increase in the function of the hippocampus contralateral to the epileptogenic focus [45]. The duration of the epilepsy disease is further correlative with these connectivity changes [45] and even though epilepsy in general cannot be cured, there are many available treatments and antiseizure medications [19]. Nevertheless, the cause of up to approximately 70% cases of epilepsy remains unknown [19], and therefore a comprehensive study of surgical hippocampal tissues removed from epilepsy patients is warranted to shed light on the disease mechanisms.

In this context, a FFPE sample of the hippocampus from an anonymized TLE patient was obtained and imaged. This imaging step is part of a correlative study where magnetic resonance imaging (MRI) datasets of *ex vivo* hippocampal samples are obtained, followed by paraffin embedding (section 7.1) for preservation. A tomographic scan on the whole FFPE sample is then performed at micrometer scale resolution. Thereafter, ROI imaging of the FFPE sample is conducted, by taking multiple biopsy punches and imaging these sample at high resolution at a synchrotron facility. The different datasets acquired at different scales are then correlated to provide a comprehensive understanding of diseases and tissue structure.

In the following, an exemplary scan of such a sample (fig. 83i) is conducted and briefly presented. This sample was acquired from *Universitätsklinikum Hamburg-Eppendorf (UKE)*. It was scanned according to the protocol spelled out in section 7.1.1, with the exception of an exposure time of 8 s per projections. This thus leads to a total exposure time of 8.89 hours at an effective pixel size of 8 μm .

An axial slice of the reconstructed dataset is depicted in fig. 68 with corresponding virtual ROIs. These ROI highlight different features that can be recognized within the structure on different scales. A uniform region is recognized by the red region while the green and blue ROIs correspond to more complex structures. In the blue ROI, different layers of the sample can be identified. These two layers corresponds to the white and gray matter, labelled B and A. The green ROI zooms in into a non uniform regions

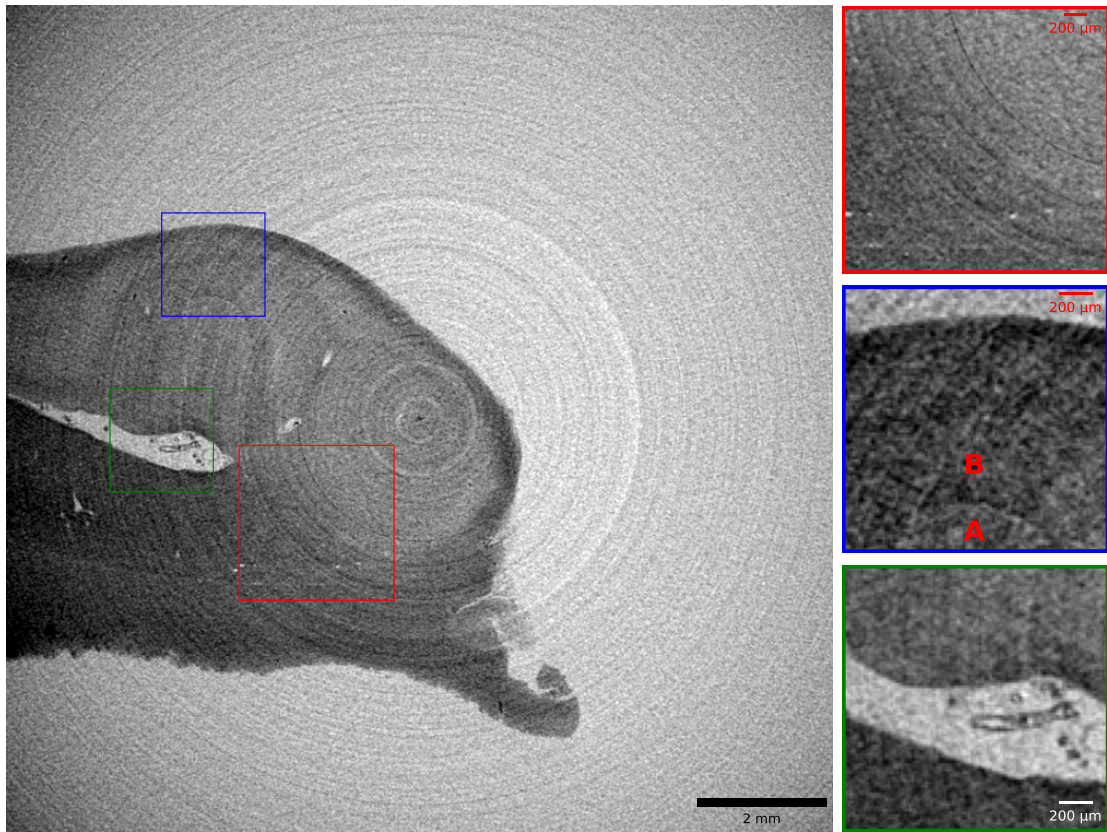


Figure 68: **Axial slice of a human hippocampus of a patient with temporal lobe epilepsy (TLE).** The figure shows a virtual slice through the data set obtained with the JuLiA setup. Within the slice, distinct anatomical features of the sample can be recognized. Certain ROI are highlighted, indicating areas of interest. The blue ROI exhibits a uniform density, while the red ROI shows increased contrast at the interface between two of more different layers. The green ROI highlights a region with a heterogeneous texture and very fine features. The respective scale bar are given in the corresponding subfigures and figure.

with very fine structures. Further studies on the fine structures can be obtained by ROI imaging.

7.3 ARTERY - GLE 1169

The cardiovascular system forms the central transport network of the human body, ensuring the circulation of blood, oxygen, nutrients, hormones, and waste products. It is composed of the heart, which functions as the central pump, and a network of blood vessels including arteries, veins, and capillaries.

Arteries, which branch from the aorta, are blood vessels responsible for transporting oxygen-rich blood from the heart to all tissues of the body. They play a critical role in the systemic distribution of oxygen, nutrients, and signaling molecules [20]. Structurally, arteries are tubular conduits with thick muscular walls and thin connective tissue layers, enabling them to withstand and regulate high-pressure blood flow.

Arterial imaging provides valuable insights not only for anatomical characterization but also for disease modeling and biomedical research. This is particularly relevant

when conventional imaging modalities such as ultrasound, angiography, echocardiography, or MRI lack the resolution or penetration depth required to capture fine-scale histological structures.

In this work, an unidentified arterial segment was imaged following the protocol described in section 7.1.1. An axial slice of the reconstructed dataset, shown in fig. 69, reveals distinct histological features including a dense vascular wall and finer substructures within the lumen region. Several regions of interest (ROI) are highlighted and color-coded. For example, the blue ROI contains characteristic artifacts arising from incomplete paraffin embedding, observed as voids caused by trapped air pockets.

To further assess reproducibility, a second scan was performed under identical source settings, geometry, and reconstruction parameters. The resulting datasets were quantitatively compared using the Fourier shell correlation (FSC), as implemented in the *hotopy* toolbox [40]. The script for carrying out this analysis is provided in the supplementary materials (section D.3.5). The FSC measures the correlation between two independent reconstructions at spatial frequencies (section 4.5.4). The FSC curve for the arterial data set is shown in fig. 69. Based on the half-bit threshold criterion, the estimated resolution is 20.49 μm , indicating that the structural features remain reliably resolvable up to this scale.

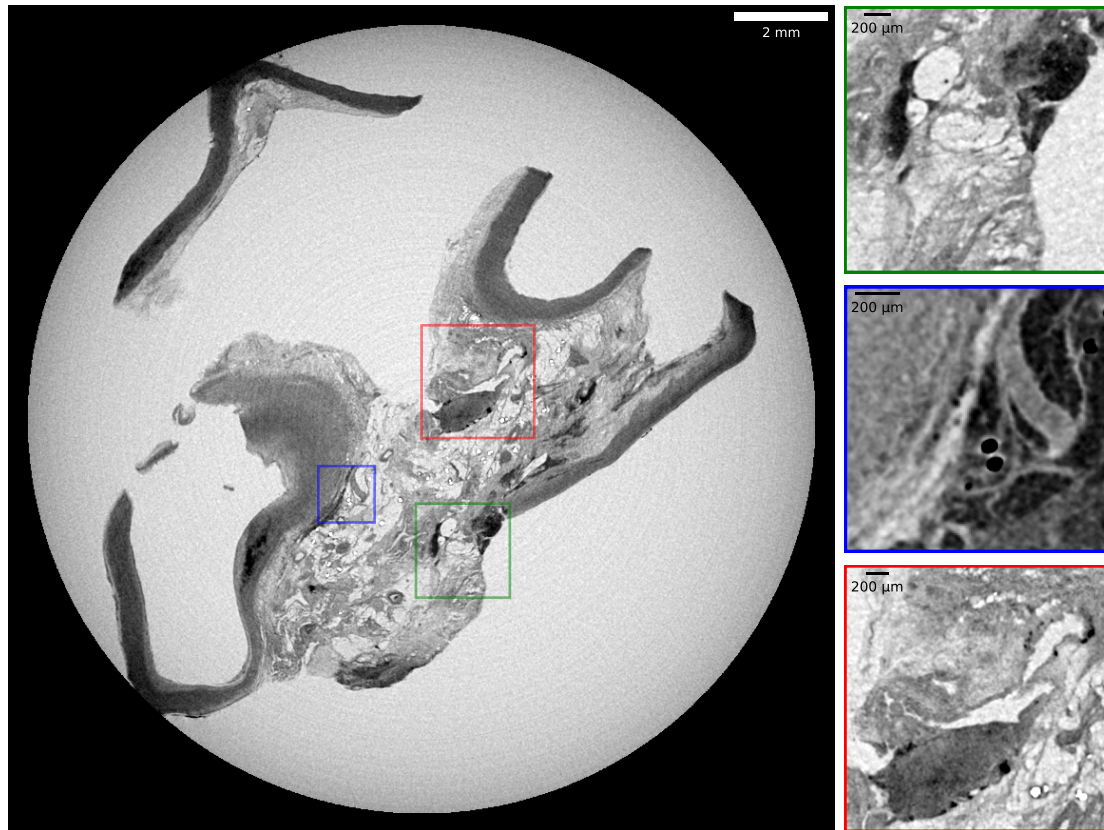


Figure 69: **Axial slice of a human artery embedded in paraffin.** This figure illustrates the multiscale structural features of an artery. Several ROIs are highlighted, corresponding to distinct histological regions. The blue ROI indicates embedding-related artifacts, visualized using an inverted grayscale colormap to enhance contrast. Scale bars are included in all corresponding subfigures.

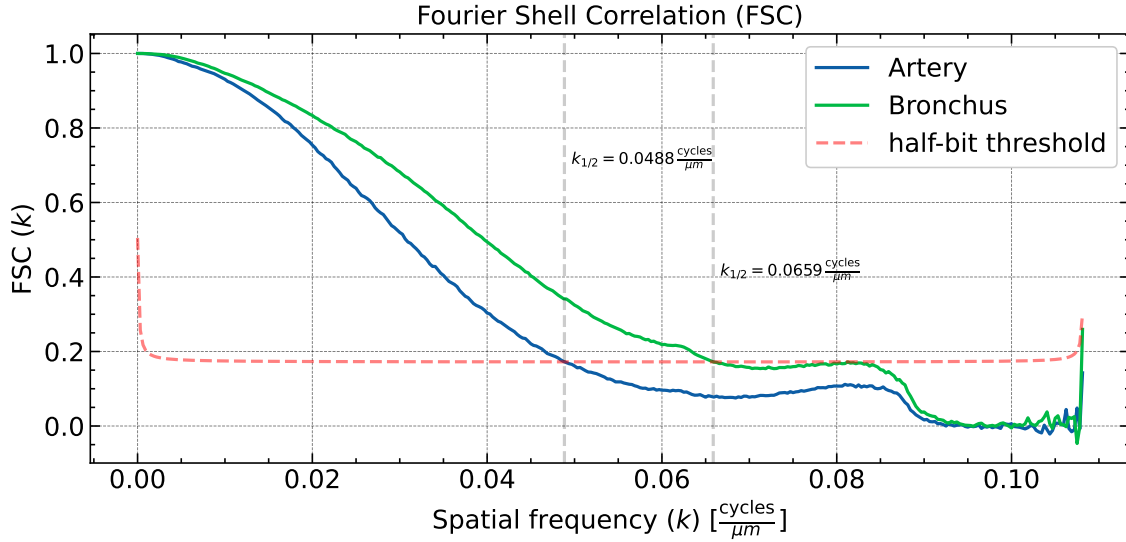


Figure 70: **Fourier shell correlation (FSC) curves for artery and bronchus datasets.** The FSC quantifies the correlation between two independent reconstructions as a function of spatial frequency. The blue and green curves correspond to the artery and bronchus datasets, respectively. The half-bit resolution thresholds are marked in red, with the resolution estimate for each dataset indicated by the vertical dashed gray lines. The script for performing the FSC analysis is provided in section D.3.5.

7.4 BRONCHUS - GLE 1169

The respiratory system consists of a branching network of small airways, trachea, bronchi, etc, that conduct and exchange gases, enabling respiration. Beginning at the trachea, as shown in fig. 71, the airways branches into the bronchi and bronchioles, and finally terminate in the alveolar ducts and sacs (fig. 71, right) where respiration occurs [8]. Each part of the respiratory system has distinct structure, function and characteristics. In the following subsections, representative examples of respiratory samples such as the bronchus, bronchioles, and trachea are presented to highlight the capabilities of the JuLiA setup for imaging FFPE samples.

An axial and complementing sagittal slice as well as corresponding ROI of the bronchus sample is depicted in fig. 72. This sample was imaged with the JuLiA setup according to protocol in section 7.1.1. The axial slice obtained with the JuLiA setup reveals clear morphological features, including the airway lumen, connective tissue structures of varying density, and small cellular components that appear as dense white dots. Regions of interest are selected to highlight these distinct features. The red ROI marks the hyaline cartilage and its surrounding tissue layers, where chondrocytes are clearly visible as bright white spots embedded in lacunae within the intercellular matrix. Additional ROIs emphasize connective tissues with distinct structures. Notably, a circular ring artifact (denoted by ar) is present, caused by the absence of X-ray photons in certain part in the projections due to a residue within the X-ray source. Furthermore, regions indicated as A and C exhibit differences in gray levels between adjacent slices. These variations are pronounced near the cassette of the FFPE blocks, where scattering effects become significant. Overall, the combined perspectives highlight both the fine structural detail and the imaging limitations encountered in the dataset.

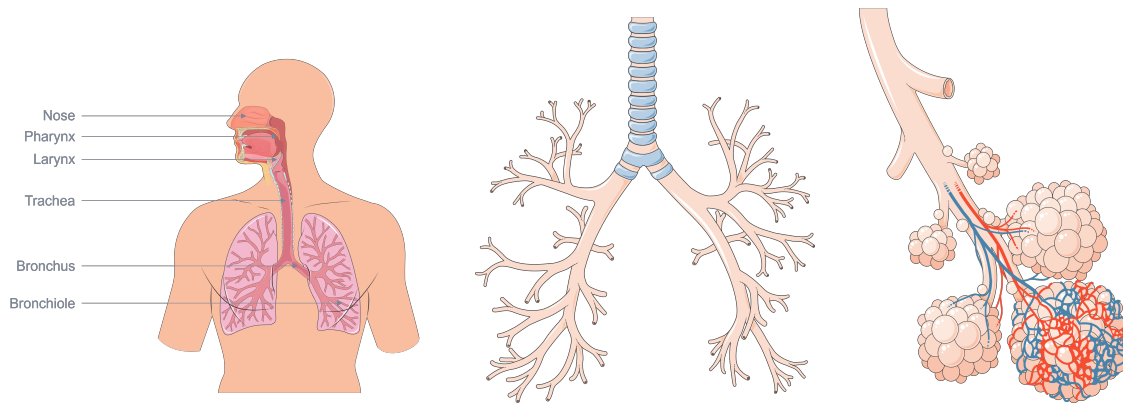


Figure 71: **Anatomical illustrations of the human respiratory system.** **Left:** major components of the upper and lower respiratory system including the nasal cavity, larynx, trachea, bronchi, and bronchioles, which guide airflows. **Middle:** detailed view of the tracheobronchial tree, showing bifurcations of the trachea into the main bronchi and bronchial branches. **Right:** render of the intrapulmonary airways, highlighting the alveolar ducts and sacs where respiration occurs. Above figures are adapted from [64].

Similar to the method applied here above (section 7.3), an additional scan with same parameters was performed for correlation analysis (fig. 70). The analysis reveals a threshold frequency of $k_{1/2} = 0.0659$ cycles/mm which translates to a spatial resolution of $15.12 \mu\text{m}$, which is supportive of the fact that chondrocytes are vaguely discernible and shows up as bright spots within the hyaline cartilage (fig. 72).

7.5 BRONCHIOLES - GLE 1169

The main bronchi further branches to the bronchioles (*kleine atemwege*), as illustrated in fig. 71 [85]. Bronchioles are smaller airways that branch off from the bronchi [8]. Respiratory bronchioles are the first structures where gas exchange/respiration takes place [8]. Unlike the bronchi, bronchioles have a diameter less than 2 mm and lack lymph nodes, subepithelial glands, cartilages of any shape [8]. Here a bronchioles sample embedded in paraffin is scanned with the JuLiA setup using the standard imaging settings in section 7.1.1 and the data was binned by a factor of 2. Virtual slices of the bronchioles sample is reconstructed from the acquired projection images and a slice is depicted in fig. 73 with annotated ROI. The axial slice reveals the structures of the sample. The red ROI highlights a region containing a trapped air pocket which induces a faint fringing artifact. The green and blue ROIs highlight regions containing very fine and dense tissue structures. Sac-like structures can also be identified within the slice.

7.6 TRACHEA - GLE 1169

The trachea, or windpipe, is a long U-shaped tube that serves a joint of the larynx and the lungs, as is shown in fig. 71. It is part of the tracheobronchial tree and serves as passage for air through the nose, the larynx and the via the bronchi (section 7.4).

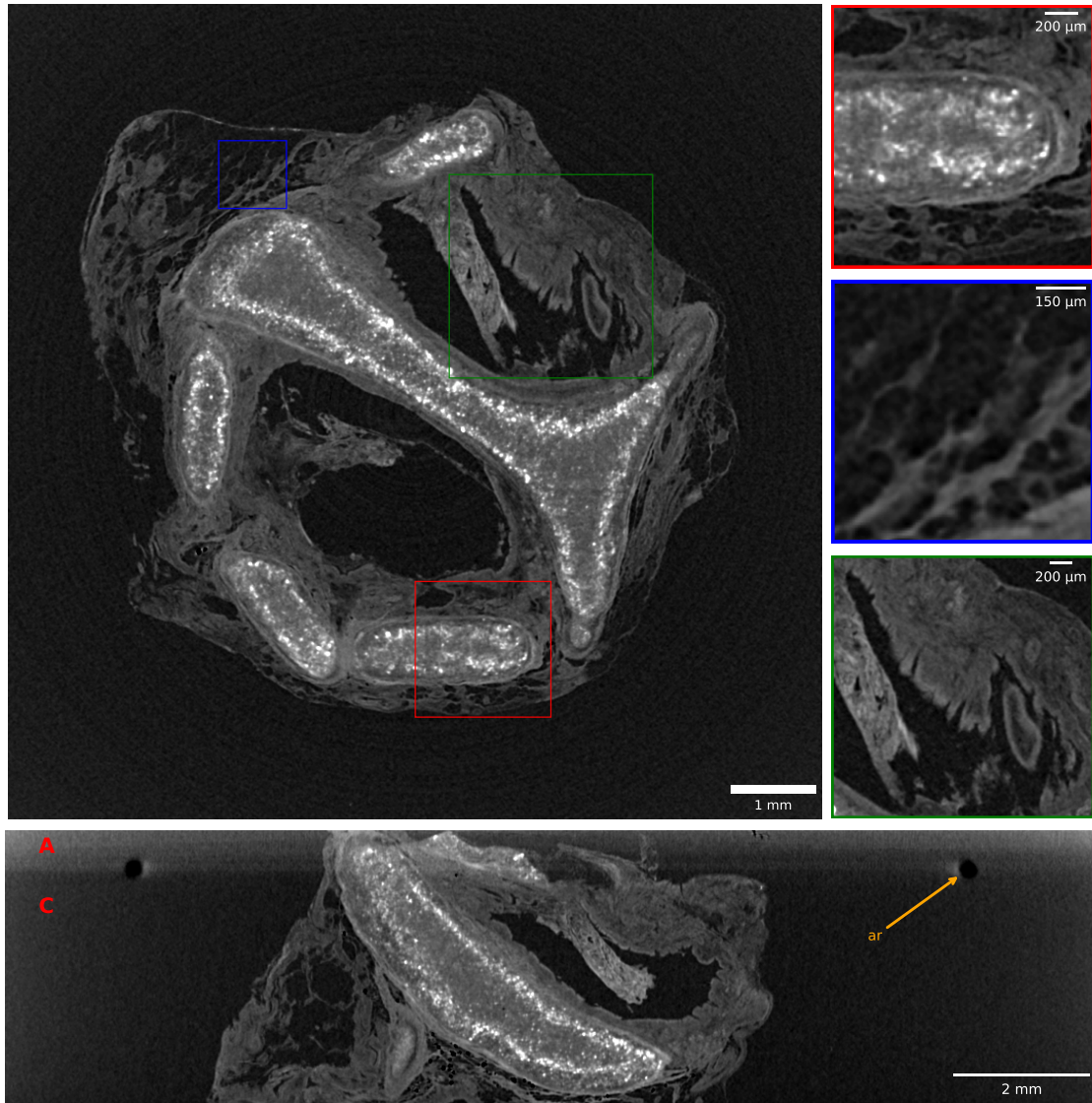


Figure 72: **Axial and sagittal slice of a bronchus FFPE sample.** The main figure show an axial slice of the bronchus dataset obtained with the **JuLiA** setup. Different morphological structures of the sample can be identified such as the region for the passage of air, very fine and connective tissue structures and very small cell that shows up as dense dots (white). The red **ROI** highlight a region showing the hyaline cartilage and surrounding tissue and tissue walls. Within the hyaline cartilage, distinct cellular arrangements can be observed, illustrating the complex architecture of the bronchial tissue. Chondrocytes show up as bright white spots within the hyaline cartilage. These structures surrounded by the lacunae, which further stored in the intercellular matrix. The green and red **ROIs** highlight connective tissues with very different characteristics. A sagittal slice, shown below the main figure, yields other perspectives of the sample. This also reveal artifacts that were induced by different effects. The dark spot denoted by **ar** is a circular ring artifact induced by the absence of X-ray photon in the projection images due to a residue inside the source. The regions indicated here **A** and **C** exposes region between the slice with different baseline gray value. Slices in the vicinity of **A** are close to the cassette of the **FFPE** blocks, which induces unwanted scattering effects.

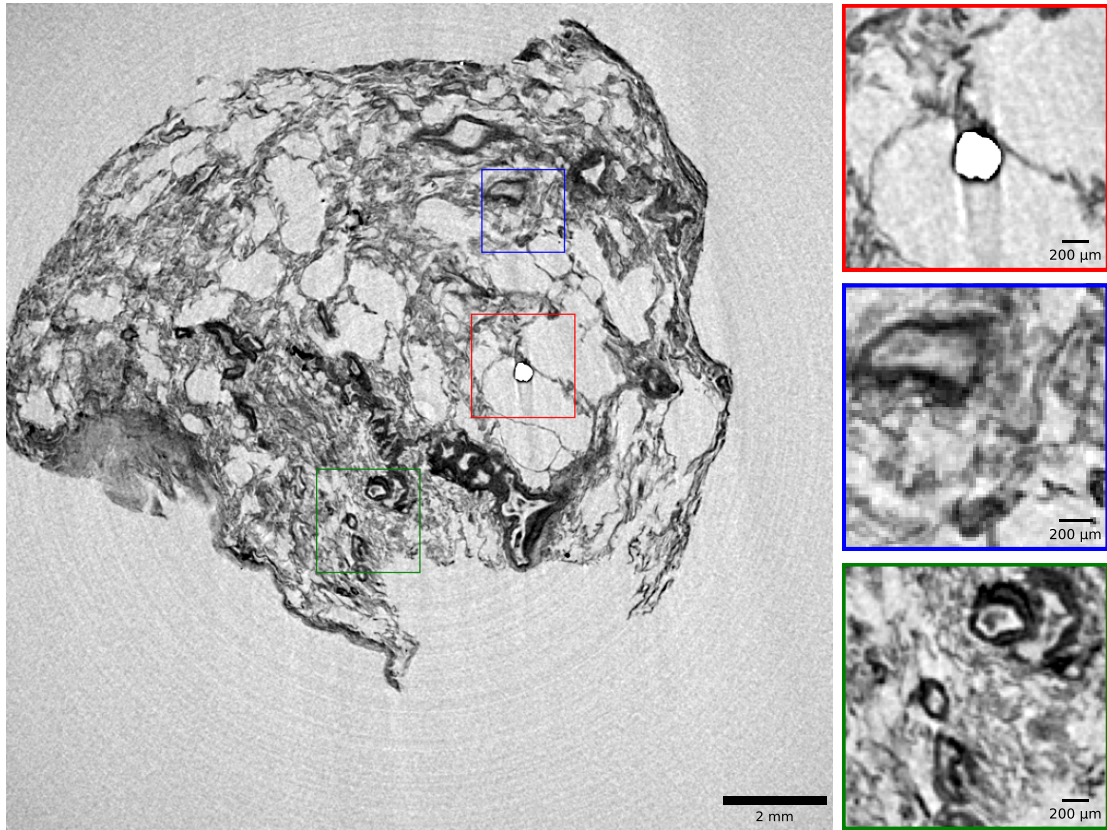


Figure 73: **Axial slice of a human bronchioles (small airways) FFPE sample.** The axial illustrates the complex and fine structure of the sample. The red ROI highlights a region containing an air pocket which induces a faint fringing artifact. The green and blue ROIs highlight regions containing very fine tissue structures. Very dense regions within the slice can also be identified as well as respiratory ducts. The alveoli can be recognized as sac-like structures in the slice. Corresponding scale bar is given in the respective figures.

The sample was scanned in the horizontal position according to the methods elaborated in section 7.1.1. The dataset was then binned by averaging by a factor of (2) in all dimensions resulting in an effective voxel size of 16 μm .

Figure 74 shows an axial slice of a human trachea embedded in paraffin. This sample has a compact and well defined structures with features varying in a large amount of scale. Uniformly dense tissue regions are well small features can be recognized and representative ROIs has been selected as illustration. More importantly, residue of cartilage can be observed within the sample, serving as a hindrance not only to the contrast but also introducing smearing and streaking artifacts. High energy photons such as at the HECTOR were able to penetrate this dense region, revealing some substructures within this calcified (cartilage) region. However, this happens at the cost of loss in contrast in the tissue of interest. This illustrates that sample preparation and dissection of ROIs need to be streamlined and optimized in case the scanning of FFPE block with laboratory-CT system is the ultimate purpose.

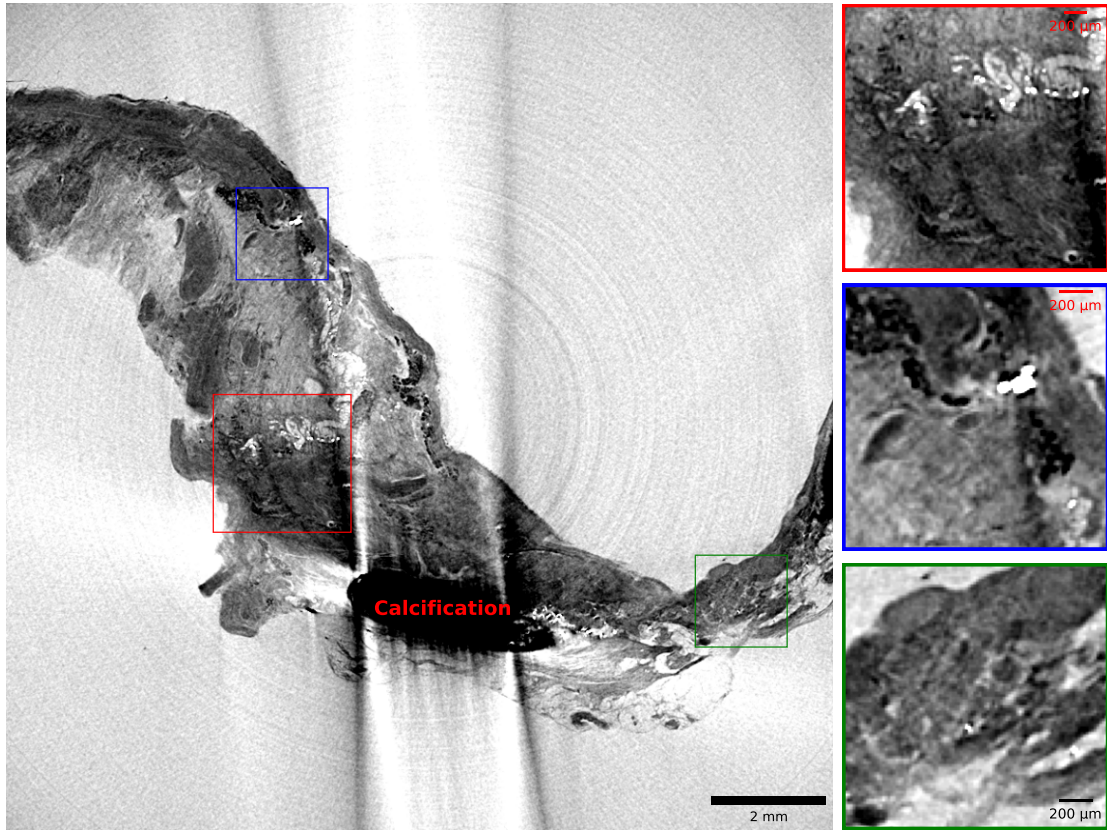


Figure 74: **Axial slice of a trachea** sample. Above figure shows a dataset obtained with the **Julia** setup. The overall morphology of a part of the trachea, including connective tissue structures is visible. **ROIs** have been highlighted to illustrate distinct features. The different **ROIs** highlight various characteristics of the tissue, ranging from fairly homogenous to very fine cellular structures. Notably, a very dense region in the slice labelled **calcification**, manifests as a highly absorbing region, leading to disruptive smearing and streaking.

7.7 OLZ-GLE 1169 AND OLP-GLE 1169

In this subsection, two additional samples that were scanned in the course of the study are briefly presented. These are believed to be originated from the lung but were not conclusively identified. Consequently, the label that were given to these samples is here given explicitly, without further description, and the data should be interpreted with caution. The two datasets presented here, referred to as OLZ-GLE 1169 and OLP-GLE 1169, are depicted in figs. 75 and 76. These data sets were scanned according to the standard source and geometric settings in section 7.1.1. This results in an effective pixel size of 8 μm . However, the data shown here is an binned version, resulting in a voxel size of 16 μm and consequently subjected to automatic contrast adjustment. These samples exhibit very distinct features, including varying degrees of tissue density and structure. It is observed that the quality of the visibility slightly degrades towards the edge of the **FOV**. This can be attributed to invalid assumptions in the reconstruction. Since the **FFPE** sample are large, it is expected that geometric assumptions such as source-to-object distance (**SOD**) and source-to-detector distance (**SDD**) may vary and slightly misjudgment by the reconstruction algorithm.

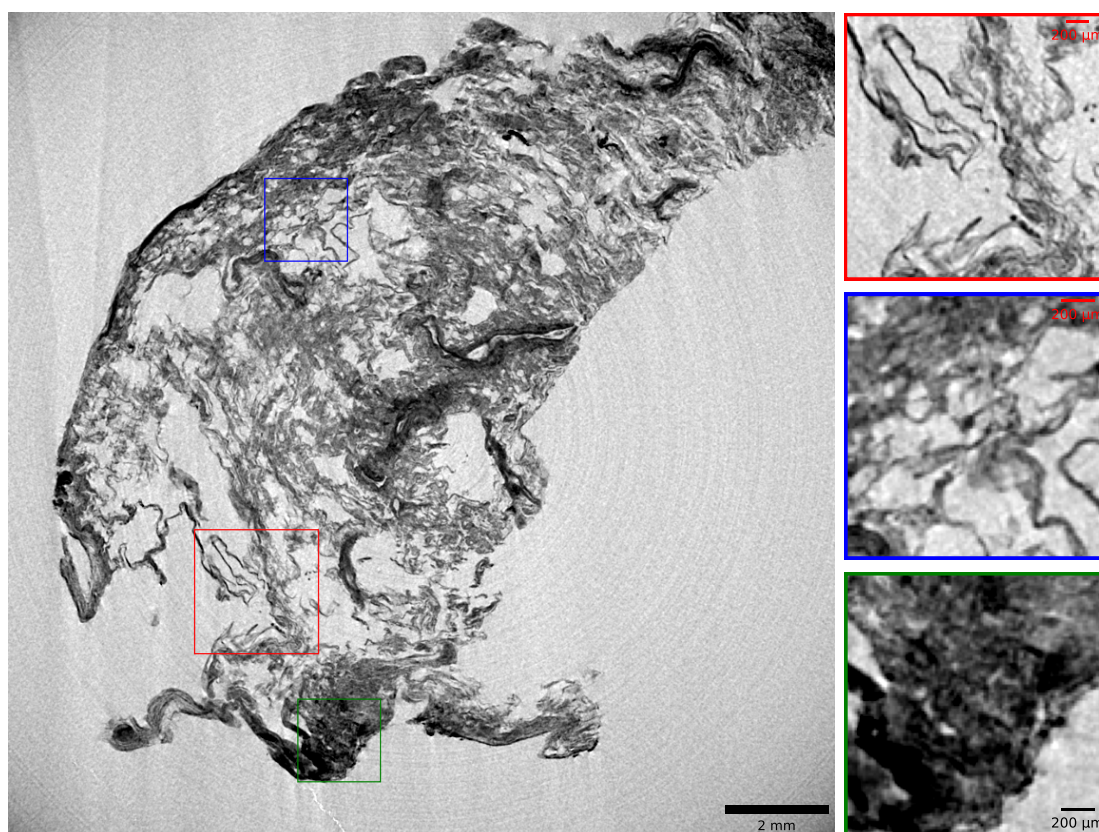


Figure 75: **Virtual slice of the OLZ-GLE 1169 FFPE sample.** Different ROIs are highlighted, illustration structures of different characteristics. Loss in quality of the data towards the edge of the FOV is visible. This can be attributed to invalid assumptions in the reconstruction.

As can be seen in fig. 76, the OLP-GLE 1169 sample also exhibits a rich network of tissues. Certain regions are indicated by the highlighted ROIs, showcasing the sample's complex morphology. Streaking artifacts due to calcified structures are present. As noted here above, a slight atrophy in the image quality towards the edge of the FOV is observed, which can be attributed to similar reasons as mentioned for the OLZ-GLE 1169 sample.

7.8 PLACENTA - MCDA-GA 3676 SPFAA-DYPAV / MCDA-GA 3072 SPFAA-DYPVV

The placenta is a temporary organ that develops from the blastocyst shortly after implantation [18, 102]. It plays a crucial role in supporting the developing fetus by providing nutrients, oxygen, and exchanging waste products between the mother and the fetus [14, 18, 102]. The placenta is composed of both maternal and fetal tissues, forming intricate structures that allows for efficient nutrient transfer while also serving as a barrier to protect the fetus from harmful waste [18]. The placenta is typically disc-shaped and is attached to the uterus, with the umbilical cord connecting it to the fetus, as is shown in fig. 77 (labelled A).

The umbilical cord is a soft (fig. 77), tortuous structure with a smooth outer covering of amnion and is composed of two arteries and a vein [7]. The umbilical arteries trans-

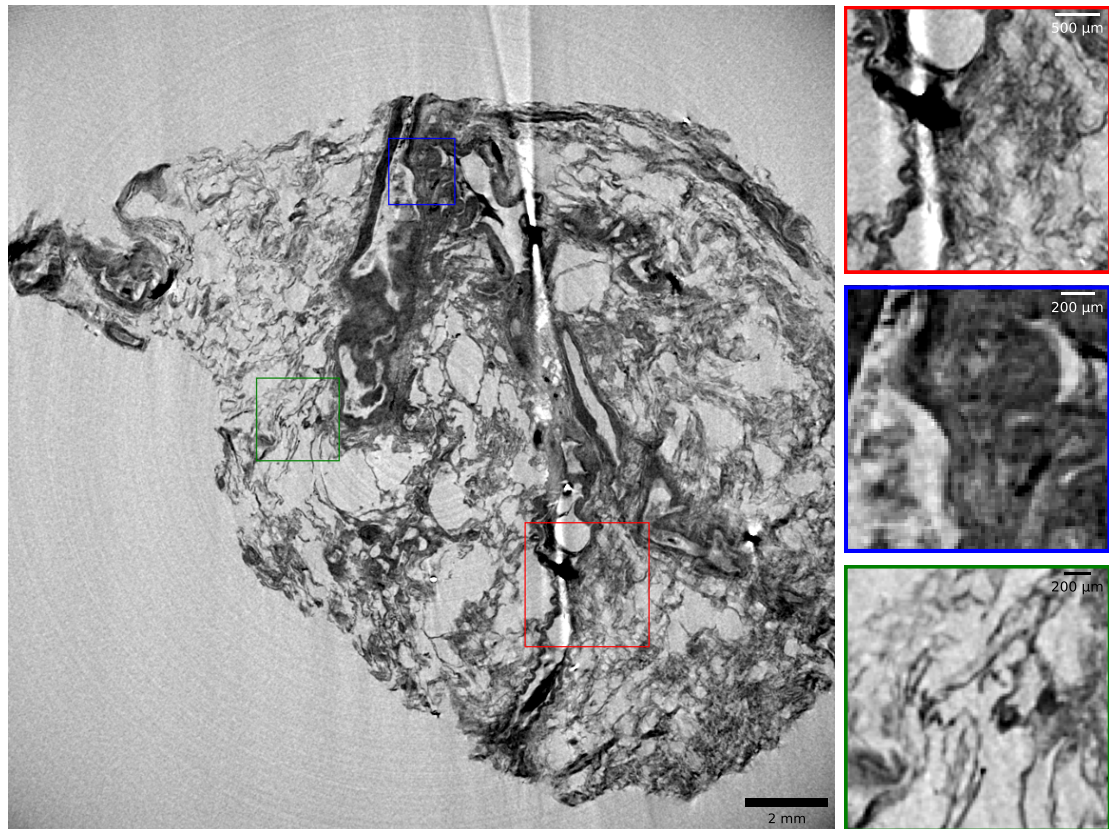


Figure 76: **Virtual slice of the OLP-GLE 1169 FFPE sample.** Different ROIs are highlighted, illustration structures of different characteristics. Loss in quality of the data towards the edge of the FOV is subtle but apparent, especially when zoomed in. This may be attributed to invalid assumptions regarding the reconstruction, including both the geometry and geometric parameters.

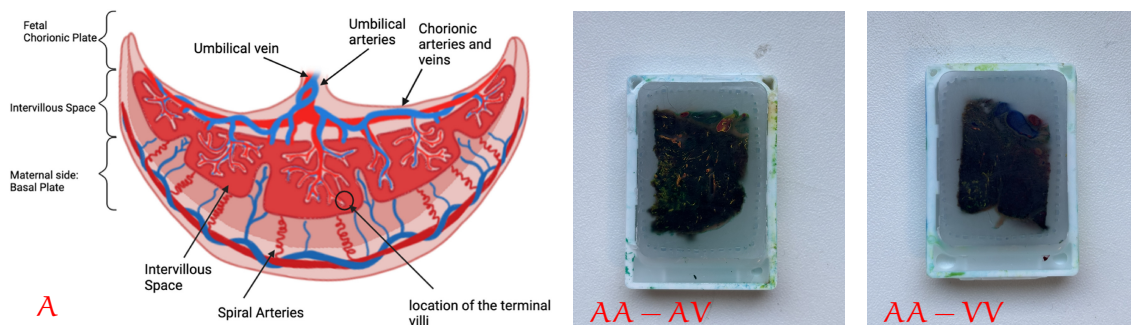


Figure 77: **Illustration of the placental structure and placenta FFPE samples.** Left: anatomical overview of the human placenta identifying the different levels of structure. Structure such as the umbilical vein and arteries are annotated. This illustration is adapted from Chappell et al. [14]. Middle (spfAA-dypAV): dyp AV placenta FFPE sample. Right (spfAA-dypVV): dyp VV placenta FFPE sample.

port deoxygenated blood and waste products from the fetus to the placenta while the umbilical vein transport oxygenated blood, hormones, etc to the fetus [4, 7, 18]. Light can be shed upon the vascular characteristics and arrangements by the study sections of the umbilical cord. Here, sections of joints, commonly referred to as anastomoses [81,

90], of both artery-to-artery (AA) and vein-to-vein connections (**dyp VV**), as well as artery-to-artery (AA) and artery-to-vein (**dyp AV**) are imaged.

These samples during the fixation process were infused with contrast agent to enhance imaging contrast and further embedded according to the normal procedure. As illustration, these are shown in fig. 77 (labelled **VV** and **AV**). The samples were scanned with the **JuLiA** setup according to the protocol in section 7.1.1, but with $z_{01} = 150$ mm. This results in an imaging effective pixel size of $10\ \mu\text{m}$. However, the dataset shown here is an binned version, by a factor of 2, resulting in a voxel size of $20\ \mu\text{m}$ and consequently subjected to automatic contrast adjustment. Figure 78 show a reconstructed

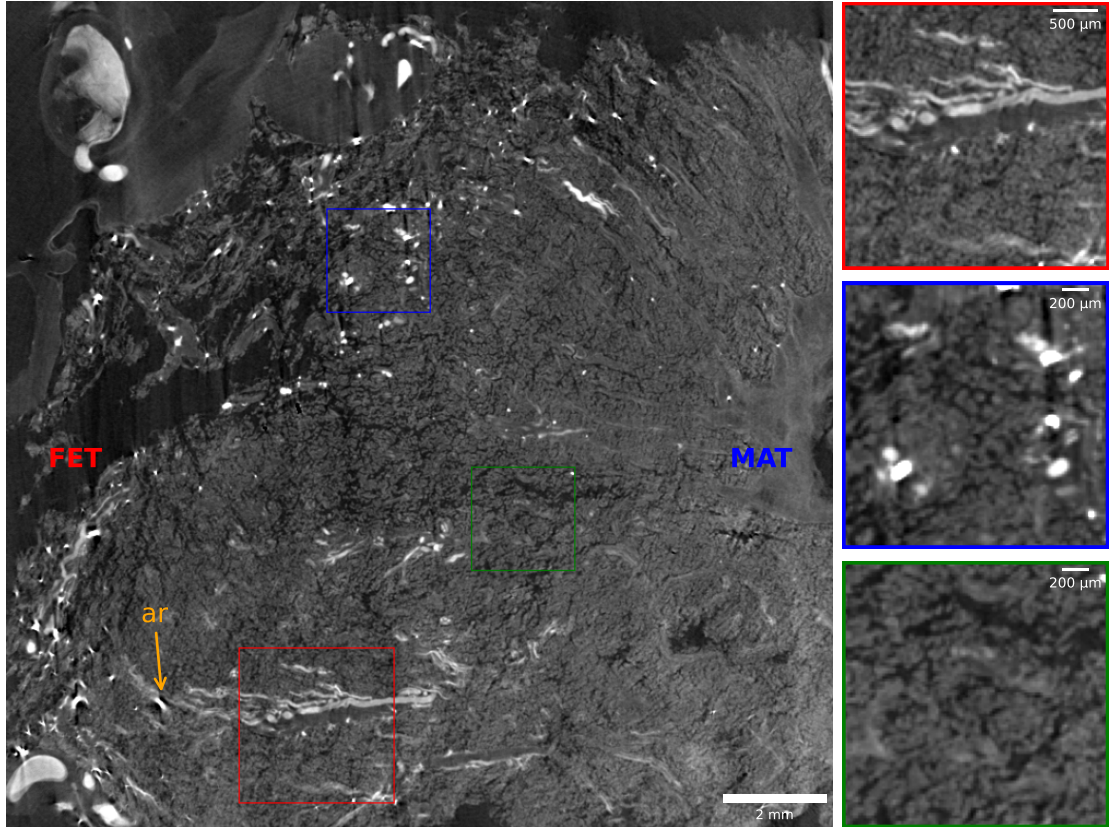


Figure 78: **Axial slice of the dyp VV placenta FFPE sample.** Above figure shows a dataset obtained with the **JuLiA** setup with an binned voxel size of $20\ \mu\text{m}$. Morphological parts of the sample can be identified. The maternal (**MAT**) and fetal (**FET**) sides of the placenta have been labelled accordingly. White dots/spots in the slice is representative of contrast agent infused in blood vessels such as shown in the blue ROI. The red ROI samples out a region with very fine structures. Candidate of smearing and streaking artifacts around blood vessel is also highlighted in the orange (**ar**). This type of artifacts are more pronounced towards the edge of the sample, which could be indicative of propagation of reconstruction artifacts and geometric assumptions.

slice of the **dyp VV** placenta FFPE sample where the maternal and fetal side of the slice are indicated. Vascular structure such as blood vessels, filled with contrast agent, can be identified. This shows up as very bright spots/region (white) as is illustrated in the blue ROI. The slice exhibit a subtle, homogenous structure with very fine channels. This is for example zoomed in on the green ROI, indicative of microscopic structures which is recognized as the villous tree within the intervillous space.

CT-inherent artifact such as smearing around the blood vessels is also observed and an example is denoted by **ar**. These high resolution distortion are pronounced towards the **FOV** of the slice, hinting of geometric artifacts and inaccuracies in the reconstruction. As noted previous times, this could be attributed to geometric invalidation in the reconstruction package due to the relative sample size.

A virtual slice of the **dyp AV** placenta **FFPE** sample is shown in fig. 79, with the fetal and maternal sides indicated. This sample shows a slight different architecture to the **dyp AV** sample (fig. 78). They both share a commonality of exhibition fairly uniform structures. However, the fine structures here are more pronounced and has filamentous characteristics. The red **ROI** highlights the microscopic fine structure of the chronic villi, where channels within this structure can be identified. The green region on the contrary showcases a region of slice blood vessel (white) with other vessels warped around it.

Unlike in the previous in fig. 78, the smearing of features towards the edge of the **FOV** are masked out a bit and thus less apparent due to the large scale of the structures present in the slice. Streaking artifacts due to excessive absorption of the X-ray by the contrast agent is also observed, however, less disturbing. Additionally, holes in the paraffin blocks it self, as can be seen fig. 77 (**left** and **right**), induces an additional intensity oscillation across the slice, in the horizontal direction.

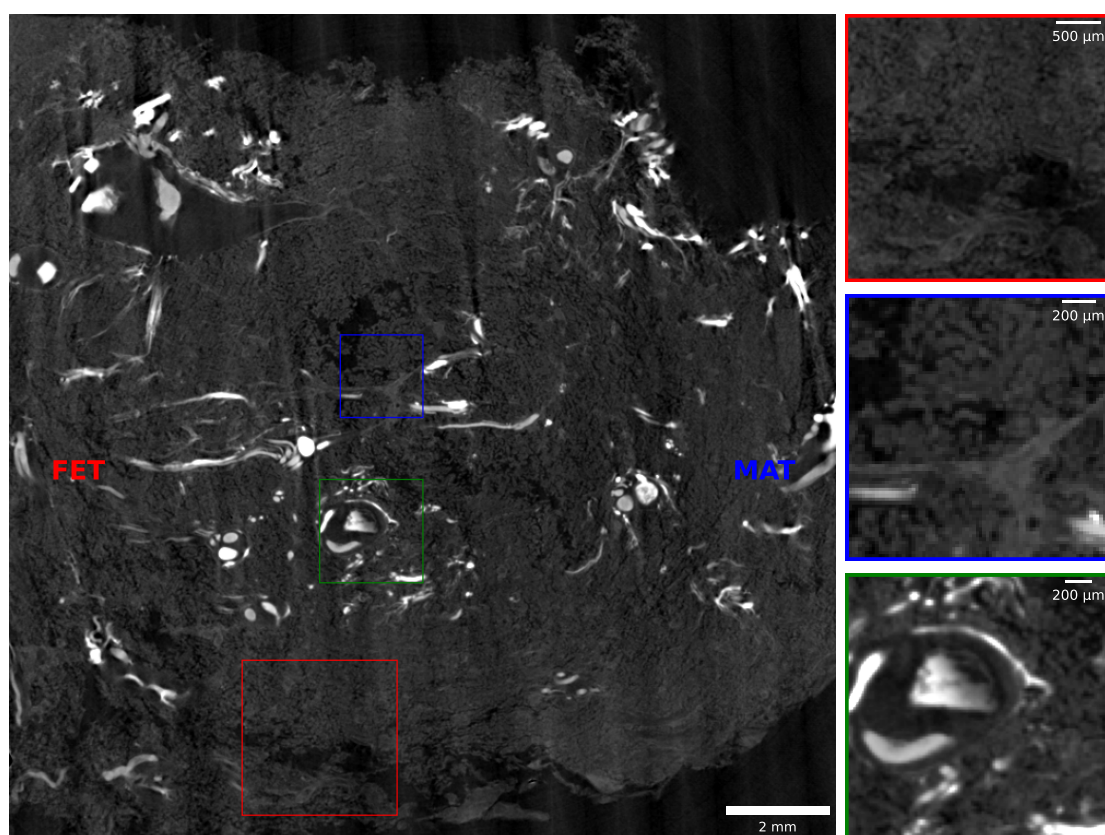


Figure 79: Axial slice of the dyp AV placenta **FFPE** sample. Above figure shows a dataset obtained with the **JuLiA** setup with an binned voxel size of $20\ \mu\text{m}$ with a respective scale bar of $1\ \text{mm}$. Morphological parts of the sample can be identified. The maternal (**MAT**) and fetal (**FET**) sides of the placenta have been labelled accordingly. High contrast region (white) in the slice is representative of contrast agent infused in blood vessels zoomed in the green **ROI**. The red **ROI** samples out a region with brittle like structures.

CONCLUSIONS

In trailing chapters, a systematic optimization of the JuLiA setup for propagation based imaging (PBI) of FFPE samples was carried out with the aim of achieving high phase contrast gain images characterized by low noise, micrometer resolution, visibility, and high SNR. This study focused on several key aspects of the CT system, including the calibration of projection data, optimisation of source parameters, filtering and spectral assessment. Together, these steps results in a guideline for reproducible imaging conditions, enhanced contrast in FFPE samples as lengthly discussed and illustrated in chapter 7. The findings provides a comprehensive overview of the imaging of FFPE samples with the JuLiA setup while hinting at potential improvements that can be treaded in future studies.

A guideline for imaging of whole FFPE samples, as extensively discussed in the previous chapter, using the JuLiA setup, in its current state, is as follows:

- Imaging at lower acceleration voltage setting of $V_0 = 40$ kV is recommended for high contrast-to-noise ratio (CNR), even though the higher voltage setting ($V_0 = 70$ kV) leads to lower noise, especially when the contrast between the biological tissue and the paraffin is low.
- Horizontal mounting of FFPE is recommend, especially imaging of multiple samples, however the vertical mounting is beneficial in case one interests lies achieving high signal-to-noise ratio (SNR) and contrast-to-noise ratio (CNR), given that the sample thickness is smaller.
- Due to the relative high dynamic range and low noise of the Varex detector 5.2, prioritizing smaller angular sampling steps is beneficial for improving image quality than acquiring multiple images per angle. Here, $N_\theta = 2000$ projections were enough to achieve high quality images, however, a higher number of projections may be required and is recommended for larger samples.
- The use of X-ray filters, as discussed in section 6.6, does not leads to any significant improvement in image quality and may increase scan times.
- Single material object (SMO) algorithm provides better image quality, SNR and CNR, compared to other methods of phase retrieval (section 6.2) methods but the Bronnikov-aided correction (BAC) algorithm yields optimal balance between signal and visibility of low resolution features.

A summary of general parameters and source settings for imaging of whole FFPE samples is given in table 5 for the JuLiA setup in its current state. Region of interest (ROI) imaging of smaller biopsy punches of FFPE samples can also be conducted at the JuLiA setup with similar guidelines and parameters. Alternatively, imaging of smaller FFPE samples with higher magnification is also possible, however, this may require different geometric configuration and or mounting.

Imaging of FFPE samples can also be conducted at the MONA setup, again with similar guideline as provided here above. However, the MONA setup at its current state, requires further tuning and stability measures should be considered.

FUTURE OUTLOOK

Several opportunities for further improvement of the setup and methodologies are identified in the following:

- The implementation of advanced reconstruction algorithms and phase retrieval methods such as SART and CTF based phase retrieval algorithms can further enhance image quality and eliminate reconstruction artifacts [2, 31, 92, 104].
- Infusion of different contrast agents of the sample prior to embedding can enhance the visibility of specific tissue structures.
- Full assessment of empty-beam correction with respect to the paraffin wax may lead to improved image quality.
- Removal of geometric constraints that limit the setup's magnification capabilities permits a greater degree of freedom. In this regard, employing a smaller sample stage enables smaller SOD, thus allowing a decrease in the SDD. This configuration ensures that the same effective pixel size is maintained while simultaneously enhancing signal quality due to the reduced SDD.
- Enhancing the penetrative nature of the rays by adapting the anode material of the JuLiA setup may lead to reduced noise and improve CNR and SNR as well as reduced exposure time (sections 6.4 and 6.7). A liquid alloy anode with a higher concentration of indium ($_{49}\text{In}$) could be an alternative at the JuLiA setup. Another option is a high-brilliance source that has the characteristic spectrum of molybdenum ($_{42}\text{Mo}$) or similar.

Part IV

APPENDIX

The chapters in this appendix discuss supplementary material that augments the main body of the thesis. It includes a discussion of the Fourier transform ([FT](#)), imaging metrics and operations, linear imaging systems, and selected empirical results. Additionally, it provides technical specifications for the detectors and instruments used, as well as relevant scripts that were used for reconstruction and analysis.

FOURIER ANALYSIS

A.1 THEORY OF FOURIER TRANSFORMATION

Fourier analysis provides a tool of decomposing a generic waveform into sinusoidal waves and is a specific form of an integral transform [50, 55, 95, 99]. The Fourier transform (FT) of a generic function $\phi(\mathbf{r})$ in a d -dimensional space is defined as

$$\mathcal{F}[\phi](\mathbf{k}) := (2\pi)^{-\frac{d}{2}} \int_{\mathbb{R}^d} d^d \mathbf{r} \phi(\mathbf{r}) \exp(-i\mathbf{k} \cdot \mathbf{r}), \quad (\text{A.1})$$

where $\mathbf{k}$ is a d -dimensional vector representative of the frequency vector in the Fourier space and $(\cdot)$ the dot product. [24] This integral transform thus maps a function from its original domain (often time or space) to the frequency domain, providing insights of the frequency components [95, 99]. In terms of operators, this can be written as $\hat{\phi}(\mathbf{k}) = \mathcal{F}[\phi](\mathbf{k})$ where $\hat{\phi}(\mathbf{k})$ is the Fourier operator of $\mathcal{F}$. Conversely, the inverse Fourier transform ($\mathcal{F}^{-1}$) is given by

$$\mathcal{F}^{-1}[\hat{\phi}](\mathbf{r}) := (2\pi)^{-\frac{d}{2}} \int_{\mathbb{R}^d} d^d \mathbf{k} \hat{\phi}(\mathbf{k}) \exp(+i\mathbf{k} \cdot \mathbf{r}), \quad (\text{A.2})$$

such that

$$\mathcal{F}^{-1}\mathcal{F}[\phi] = \mathcal{F}^{-1}[\mathcal{F}[\phi]] = \phi. \quad (\text{A.3})$$

The Dirac delta distribution $\delta_d(\mathbf{r})$ is implicitly defined as [94]

$$\delta_d(\mathbf{r}' - \mathbf{r}) = \begin{cases} \infty, & \mathbf{r}' = \mathbf{r} \\ 0, & \mathbf{r}' \neq \mathbf{r}. \end{cases} \quad (\text{A.4})$$

This impulse function is such that it is zero everywhere except at the point $\mathbf{r}' = \mathbf{r}$ where it is infinite in such a way that the integral expression of equation (A.4) and a generic test function $\phi(\mathbf{r}')$ yields the function value ϕ at $\mathbf{r}' = \mathbf{r}$, i. e.

$$\phi(\mathbf{r}) = \int_{\mathbb{R}^d} d^d \mathbf{r}' \phi(\mathbf{r}') \delta_d(\mathbf{r}' - \mathbf{r}). \quad (\text{A.5})$$

Equation (A.3) in combination with the definition of the Fourier transform and its inverse leads to an explicit definition of the Dirac delta function in the framework of Fourier analysis, i. e.

$$\phi(\mathbf{r}) = (2\pi)^{-\frac{d}{2}} \int_{\mathbb{R}^d} d^d \mathbf{k} \left[(2\pi)^{-\frac{d}{2}} \int_{\mathbb{R}^d} d^d \mathbf{r}' \phi(\mathbf{r}') \exp(-i\mathbf{k} \cdot \mathbf{r}') \right] \exp(+i\mathbf{k} \cdot \mathbf{r}).$$

A quick shuffle of the normalization factors and change the orders of integration leads to

$$\phi(\mathbf{r}) = \int_{\mathbb{R}^d} d^d \mathbf{r}' \phi(\mathbf{r}') \left[(2\pi)^{-d} \int_{\mathbb{R}^d} d^d \mathbf{k} \exp(-i\mathbf{k} \cdot (\mathbf{r}' - \mathbf{r})) \right].$$

The comparison of above result to the definition of the Dirac delta distribution in equation (A.5) leads to an explicit definition of δ_d as

$$\delta_d(\mathbf{r}' - \mathbf{r}) = (2\pi)^{-d} \int_{\mathbb{R}^d} d^d \mathbf{k} \exp(-i\mathbf{k} \cdot (\mathbf{r}' - \mathbf{r})). \quad (\text{A.6})$$

A.1.1 Elementary properties

The FT exhibits a couple of elementary but yet very important properties that are worth noting. These properties are shortly outlined below

A.1.1.1 Linearity

Given an arbitrary set of functions (ϕ_i) with cardinality N and indices i , i.e. ϕ_i and constant coefficients α_i , a new function (ψ) can be constructed by the superposition

$$\psi = \sum_i^N \alpha_i \phi_i \quad (\text{A.7})$$

The Fourier pair of this new function $(\hat{\psi})$ becomes

$$\hat{\psi} = \mathcal{F}[\psi] = \mathcal{F}\left[\sum_i^N \alpha_i \phi_i\right] = \sum_i^N \alpha_i \mathcal{F}[\phi_i] = \sum_i^N \alpha_i \hat{\phi}_i \quad (\text{A.8})$$

where $\hat{\phi}_i$ denotes the FT of ϕ_i [95].

A.1.1.2 Convolution and correlation theorem

Given two generic functions $f(\mathbf{r})$ and $g(\mathbf{r})$ in a d -dimensional space, the convolution is a binary operation $(\star)$ on two functions that is defined as

$$h(\mathbf{r}) = (f \star g)(\mathbf{r}) = \int_{\mathbb{R}^d} d^d \mathbf{r}' f(\mathbf{r}') g(\mathbf{r} - \mathbf{r}'). \quad (\text{A.9})$$

The convolution operation is commutative, i.e. $f \star g = g \star f$ and associative, i.e. $f \star (g \star h) = (f \star g) \star h$. Another important property of the convolution operation is the correlation theorem which states that the Fourier transform of a convolution of two functions is the pointwise product of their Fourier transforms [67, 75]. This is mathematically given by

$$\mathcal{F}[f \star g] = \mathcal{F}[f] \mathcal{F}[g], \quad (\text{A.10})$$

or alternatively $\mathcal{F}[fg] = \mathcal{F}[f] \star \mathcal{F}[g]$.

IMAGE PROCESSING AND METRIC

This following material covers the fundamentals of signal processing in the realm of linear systems, image arithmetics, and image metrics such as signal-to-noise ratio (SNR) that are extensively used and implicitly implied throughout this thesis.

B.1 LINEAR IMAGING SYSTEMS

Imaging systems in this thesis is considered *linear*, shift invariant, and collectively referred to as *linear shift-invariant imaging (LSI)* systems [61]. LSI systems are bound by the following requirements

- Linearity, which mathematically can be formulated as

$$\mathcal{H}[\psi] = \mathcal{H}\left[\sum_i \alpha_i \phi^i\right] = \sum_i \alpha_i \mathcal{H}[\phi^i], \quad (\text{B.1})$$

where ψ is a superposition of functions (ϕ_i) with coefficients α_i . The operator $\mathcal{H}$ is therefore a linear operator that represents the mapping of the input function (ϕ) to the output ($\mathcal{H}[\psi]$), i. e. or the optical system.

- Shift invariance: this requirement asserts that whenever the input function is shifted, i. e. $\phi(\mathbf{r}) \rightarrow \phi(\mathbf{r} - \mathbf{r}_0)$, then the image (output) of this translated input function is also expected to be shifted following

$$s(\mathbf{r}) = \mathcal{H}[\phi(\mathbf{r})] \rightarrow s(\mathbf{r} - \mathbf{r}_0) = \mathcal{H}[\phi(\mathbf{r} - \mathbf{r}_0)]. \quad (\text{B.2})$$

The above discussion allows an explicit definition of the linear operator ($\mathcal{H}$) as a convolution (equation (B.3)) of the input function (ψ) and the point spread function (PSF) (h) [61].

$$s(\mathbf{r}) = \mathcal{H}[\phi(\mathbf{r})] = (h \star \phi)(\mathbf{r}) = \int_{\mathbb{R}^d} d^d \mathbf{r}' h(\mathbf{r}' - \mathbf{r}) \phi(\mathbf{r}') \quad (\text{B.3})$$

A shift-invariant system is thus is completely described by the PSF, which determines how the input function is related to the output via the convolution.

B.1.1 Optical transfer function (OTF)

The optical transfer function (OTF) is in general a complex number, defined as the normalized FT of the PSF [61, 100]

$$\text{OTF}(\mathbf{k}_\perp) = \frac{\hat{h}(\mathbf{k}_\perp)}{\hat{h}(\mathbf{0})} \in \mathbb{C}. \quad (\text{B.4})$$

The above expression can simply be written as $\text{OTF}(\mathbf{k}_\perp) = M(\mathbf{k}_\perp) \exp i\phi(\mathbf{k}_\perp)$, where $M(\mathbf{k}_\perp)$ depicts the MTF and the second factor is the phase modulation function.

B.1.2 Modulation transfer function (MTF)

The modulation transfer function (MTF), defined in section B.1.1 is a real-valued function given by norm of the OTF [61]

$$M(\mathbf{k}_\perp) = \left| \frac{\hat{h}(\mathbf{k}_\perp)}{\hat{h}(0)} \right| \in \mathbb{R} \quad (\text{B.5})$$

The MTF expresses the modulation in the output to the modulation in the input. In other terms, it expresses the way in which the contrast of the input function undergoes modulation as it is converted to the output by the imaging system. The MTF of an imaging system is given by the product of the MTF of the individual components of the

system, i.e. $M_{\text{tot}}(\mathbf{k}_\perp) = \prod_i^N M_i(\mathbf{k}_\perp)$ [61]. This implies that the MTF with the lowest magnitude and thus the poorest quality determines the performance of the system.

The MTF can be measured in many different ways. The most simple way is measuring the PSF and subsequently calculate the modulus of the FT of the PSF. This is simply done by imaging a point absorber in a X-ray CT. Alternatively, one can proceed to the measurement of the LSF (LSF(x)), which is defined through

$$\begin{aligned} \phi(\mathbf{r}_\perp) &= \delta(\mathbf{r}_\perp) \rightarrow i(x, y) = \text{LSF}(x) \\ \text{LSF}(x) &= \int dx' dy' \delta(x') h(x - x', y - y') = \int dy' h(x, y - y') \end{aligned} \quad (\text{B.6})$$

However, the measurement of the ESF is a more practical approach in probing the PSF of a given system. This later function is given by measuring a sharp fabricated edge that can be parameterized with a Heaviside function ($H(x)$) [96]. The piecewise function for a Heaviside function is given by equation (B.7) here, which for a 2D case is shown in fig. 80 [96].

$$H(x) = \begin{cases} 1, & x \geq 0 \\ 0, & x < 0 \end{cases} \quad (\text{B.7})$$

The edge spread function ($E(x)$) is then given by the convolution of the object ($\phi = H$),

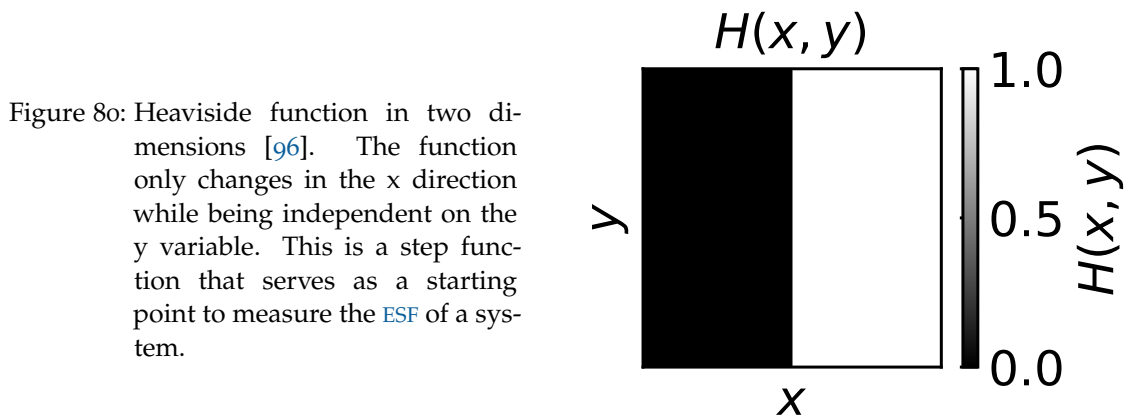


Figure 80: Heaviside function in two dimensions [96]. The function only changes in the x direction while being independent on the y variable. This is a step function that serves as a starting point to measure the ESF of a system.

here the Heaviside function, with the PSF of the system. This is mathematically given by

$$E(x) = H(x) * h(x, y) = \int dx' dy' h(x - x', y - y') H(x'). \quad (\text{B.8})$$

In all practical purposes, equation (B.8) is parameterized by the error function $e(x) = a \cdot \text{erf}((x - x_0)(\sqrt{2\sigma})^{-1}) + b$ with a full width at half maximum (FWHM) of $\sqrt{8 \ln 2} \sigma$.

B.2 IMAGE OPERATION

A scalar image (i) can be described as an array $i(n, m)$ or, equivalently, as

$$i : N \times M \rightarrow I \quad (\text{B.9})$$

where I is the intensity value range i.e. $I = \{0, 1, \dots, I-1\}$ and $(n, m) \in (N, M)$ that denote the indices/position of the individual pixels, with $N = \{0, 1, \dots, N-1\}$ and analogously for M . The number of elements in N and similarly M , represents the total number of pixels in each direction [61].

Thus, a grayscale image can be denoted by $i(n, m)$ or i_{nm} , and such arrays can be subjected to operations that apply to matrices in this space. Such basic image operation are, for instance, addition (+) of two more images, subtraction (−), division, as well as multiplication of two images. A couple of these basic image manipulations will be discussed in the following section.

B.2.1 Image arithmetic

Some of the fundamental image operations as briefly mentioned in section B.2 are elaborated here. These operations form the core for the basic of all calibration steps and thus require some attention.

B.2.1.1 Addition

In a situation where deterministic noise effects in a frame are well corrected, leaving only random noise, in case a series of uncorrelated images (i_j), each with a random noise level (σ_j), are added together, then this summation operation yields a final image

$$i = \sum_j i_j \quad \text{with} \quad \sigma^2 = \sum_j \sigma_j^2.$$

The SNR of the new image is then given by

$$\text{SNR}_+ = \frac{i}{\sigma} = \frac{\sum_j i_j}{\sqrt{\sum_j \sigma_j^2}}$$

It should be observed that although the signal (i) continues to increase in the process, so does the noise level (σ) so that in the limit where the Poisson noise dominates over the read noise, i.e. $\sigma_{\text{shot noise}} \gg \sigma_R$ and $\sigma_{\text{shot noise}} = \sqrt{i}$ [103], the SNR becomes

$$\text{SNR}_+ \approx \frac{i}{\sqrt{\sum_j i_j}} = \sqrt{i}.$$

This implies a relatively slow increase in SNR with increasing signal. Thus, collecting more signals is desirable only if the statistical noise level of the individual frames (σ_R) is well below the Poisson noise.

Considering specifically the scenario where N sub-frames of the same signal are taken under similar conditions and averaged over, the signal of the different frames will be relatively similar $i_l \approx i_k \forall k, l$ as well as their noise level. The addition of these frames together gives a [SNR](#) of

$$\text{SNR}_{+N} = \frac{\sum_j i_j}{N \sqrt{N^{-2} \sum_j \sigma_j^2}} \approx \frac{N \cdot i_j}{N \sqrt{N^{-2} \cdot N \cdot \sigma_j^2}} = \frac{i_j}{\sqrt{N^{-1} \cdot i_j}} = \frac{N}{\sqrt{N}} \cdot \text{SNR}_j,$$

which simplifies to

$$\text{SNR}_{+N} = \sqrt{N} \cdot \text{SNR}_j, \quad (\text{B.10})$$

with SNR_i the [SNR](#) of an individual frame (j). The results in equation (B.10) show that the averaging of N sub-frames of images taken under similar conditions yields a total [SNR](#) of $\sqrt{N}$ multiplied by the [SNR](#) of a single sub-frame SNR_j . In this way, it is shown that statistical noise in such a system can not be mitigated, but averaging similar images yields a total image with a better [SNR](#).

B.2.1.2 Subtraction

Equivalently for the subtraction ($-$), one imagines a series of uncorrelated images i_j , subtracted from each other and each with a random statistical noise matrix of σ_j . This yields a residual image $i = i_1 - \sum_{j \neq 1} i_j$. When only random noise is considered, this operation yields $\sigma^2 = \sum_j \sigma_j^2$ with a [SNR](#) of

$$\text{SNR}_{-} = \frac{i}{\sigma} = \frac{i_1 - \sum_{j \neq 1} i_j}{\sqrt{\sum_j \sigma_j^2}}$$

As observed before, even when the signals are subtracted from each other, the noise still adds up. This mechanism may lead to image erosion, for example, when two similar images are subtracted from each other. Alternatively, it gives a method to compute the noise level of a sensor, the so-called *subtraction method* and this analysis is relevant for dark frame correction.

B.2.1.3 Division

Considering the situation where all the deterministic noise effects in a certain frame are well corrected, leaving only random noise or uncorrelated noise, the signal of two divided images is then given by

$$i = \frac{i_1}{i_2}. \quad (\text{B.11})$$

By this operation, it is meant that the signal of the two images is divided pixel by pixel. The noise level of the new image is given by

$$\sigma = \frac{i_1}{i_2} \sqrt{\frac{\sigma_1^2}{i_1^2} + \frac{\sigma_2^2}{i_2^2}} = i \sqrt{\frac{1}{\text{SNR}_1^2} + \frac{1}{\text{SNR}_2^2}}. \quad (\text{B.12})$$

The [SNR](#) of the resulting image is given by

$$\text{SNR} = \left(\frac{1}{\text{SNR}_1^2} + \frac{1}{\text{SNR}_2^2} \right)^{-\frac{1}{2}} \quad (\text{B.13})$$

which suggests that the [SNR](#) of the resulting image is highly dominated by images with the lowest [SNR](#).

MODEL FITTING AND UNCERTAINTY ESTIMATION

Consider an objective function denoted as ψ , which is a function of variables $\mathbf{x}$ and parameters $\boldsymbol{\vartheta}$, chosen in such a way that the objective function $\psi(\mathbf{x}; \boldsymbol{\vartheta})$ aligns closely with the empirical data set underlying denoted by $\mathbf{y}$, i. e.

$$\mathbf{y} = \psi(\mathbf{x}; \boldsymbol{\vartheta}) + \varepsilon, \quad (\text{C.1})$$

with ε quantifying the noise characteristics. The model function $\psi(\mathbf{x}; \boldsymbol{\vartheta})$ can be non-linear or linear in nature of the parameters $\boldsymbol{\vartheta}$ and may as well be some physically informed model that may be derived from first principles and or a phenomenological model that is constructed to the data. In the former case, the model could, for example, be the attenuation coefficient, transmissivity with a well-defined functional form on the energy, while in the latter case, one makes use of a set of basis functions, such as the polynomials, sinusoidal basis, splines, ReLU etc [47]. Given a linear model,

$$\psi(\mathbf{x}; \boldsymbol{\vartheta}) = \sum_j \vartheta_j \phi_j(\mathbf{x}) \quad (\text{C.2})$$

with features (ϕ_j) and the Gaussian noise model $\varepsilon \sim \mathcal{N}(0, \sigma^2)$, the fit parameters $\boldsymbol{\vartheta}$ are estimated by minimizing the cost function or equivalently the χ^2 -function [47], which is given by

$$\chi^2 = \sum_i \left(\frac{y_i - \psi(\mathbf{x}_i, \boldsymbol{\vartheta})}{\sigma_i^2} \right)^2. \quad (\text{C.3})$$

This task in this thesis was performed using the Levenberg-Marquardt algorithm [88] as implemented in the `scipy.optimize.curve_fit` function [79]. The standard error on the fit parameters (σ_{ϑ_j}) is given by the square root of the diagonal elements of the covariance matrix, which is obtained from the `curve_fit` function [79].

Uncertainty in the predicted objective function is obtained by propagation of error in the fit parameters using the Jacobian of the model function according to [47, 49]

$$\sigma_\psi = \sqrt{\sum_j \left(\frac{\partial \psi(\mathbf{x}; \boldsymbol{\vartheta})}{\partial \vartheta_j} \sigma_{\vartheta_j} \right)^2}. \quad (\text{C.4})$$

An α -confidence interval for the predicted values can then be obtained using the Student's t distribution [105] or, alternatively, a z uncertainty band given by $[\psi - z\sigma_\psi, \psi + z\sigma_\psi]$.

SUPPLEMENTS

D.1 INSTRUMENTATION

D.1.1 X-123 Amptek detector

Table 6 shows the key specification of the Amptek [110] X-123 Si-PIN for the measurement of X-ray spectra in this thesis. The data originates from Amptek [product page](#) and the key technical data sheet of the detector. Figure 81 shows the detector efficiency curves on different scales [110] from the deposition of photon energy due to the photoelectric effect. Both figures above combine the effects of transmission through the Beryllium window (including the protective coating) and interaction in the silicon detector. The low-energy portion of the curves is dominated by the thickness of the beryllium window, whereas the high-energy portion is dominated by the thickness of the active depth of the Si detector. Depending on the window chosen, 90% of the incident photons reach the detector at energies ranging from 2 to 3 keV. Depending on the detector chosen, 90% of the photons are detected at energies up to 9 to 12 keV [110].

Table 6: **Technical specifications** of the Amptek X-123 Si-PIN X-ray spectrometer [110].

Detector type	Si-PIN photodiode (thermoelectric cooled)
Scintillator thickness - active area	500 μm - 6 mm^2
Energy resolution	139 – 260 eV FWHM @ 5.9 keV
Optimum energy range	1 – 40 keV
Window material	Beryllium (${}_4\text{Be}$) - 25 μm (1 mil)

D.1.2 JIMA test chart

The JIMA RT RC-02 (JIMA-[JuLiA](#)) is a micro-resolution test chart for X-ray imaging produced by the Japan Inspection Instruments Manufacturers' Association (JIMA) [33]. It is here used to evaluate the resolution of the [JuLiA](#) setup in a well-defined geometry.

The sample consist of a ${}_{14}\text{Si}$ base with an absorbing 1 μm tungsten (${}_{74}\text{W}$) and 1.1 μm SiO_2 layer deposited on top. A cross section of the test chart is depicted in fig. 82. The test chart contains groups of slits with varying widths ranging from 0.4 to 15 μm , as detailed in table 7 and depicted in fig. 39.

D.2 FFPE SAMPLES

This appendix contains the optical images of all [FFPE](#) samples used for study in this thesis. Each image is labeled with its corresponding sample code/reference to ensure traceability with the experimental results reported in the main text. Figures 83a to 83f

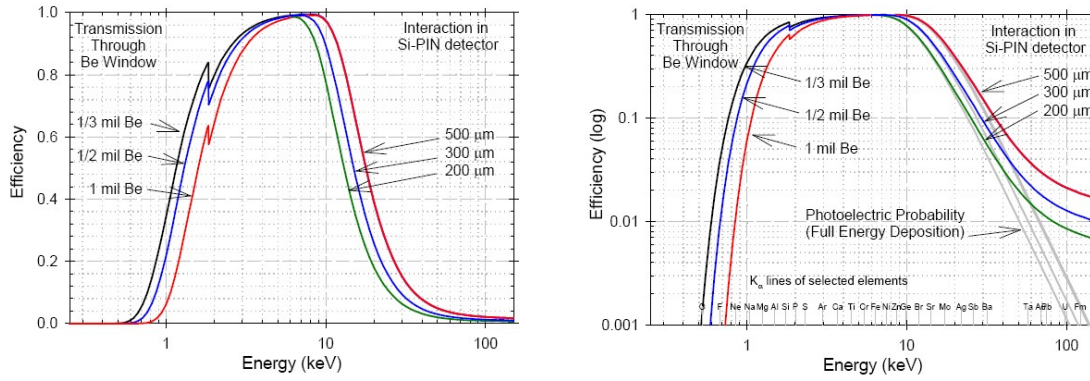


Figure 81: **Efficiency curves of the X-123 detector.** The left figure shows the intrinsic full energy detection efficiency for the X-123 Si-PIN detectors on a linear scale. This efficiency corresponds to the probability that an X-ray will enter the front of the detector and deposit all of its energy inside the detector via the photoelectric effect. **Right:** Shows the probability of a photon undergoing any interaction, along with the probability of a photoelectric interaction which results in total energy deposition, on a log-log-scale. As shown, the photoelectric effect is dominant at low energies but at higher energies above about 40 keV the photons undergo Compton scattering [110]. Above figures and description are fully adapted from [110].

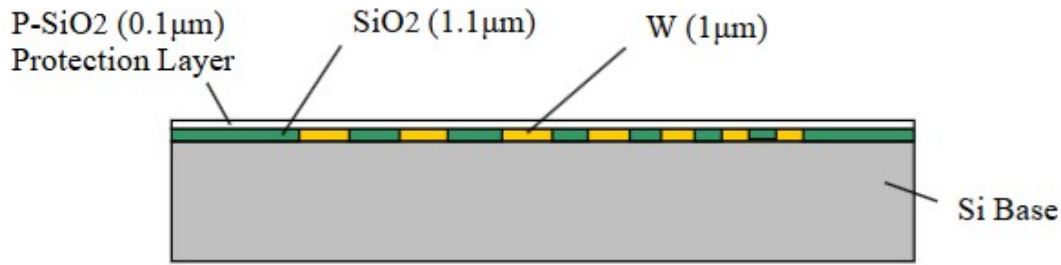


Figure 82: **Cross section of the JIMA RT RC-02 test chart** [33]. This test sample is made of a ^{14}Si base with a $1\text{ }\mu\text{m}$ tungsten (^{74}W) and $1.1\text{ }\mu\text{m}$ SiO_2 layer deposited on top. Above schematic is adapted from [33].

show images of the FFPE samples attained from *Medizinische Hochschule Hannover (MHH)*. These samples are embedded in paraffin for histological analysis with a square paraffin block of side length of approximately 2.2 cm.

The placenta samples (figs. 83g and 83h) are embedded in paraffin blocks and were attained from *Oslo Universitetsykehus*. These samples originates from twin placentas analyzing the anastomoses between the two placental circulations. Figure 83g shows a sample with an artery-vein anastomosis, while fig. 83h shows a sample with a vein-vein anastomosis. The samples are labeled as MCDA-GA 3676 and MCDA-GA 3072, respectively.

The hippocampus sample (fig. 83i) originates from the *Universitätsklinikum Hamburg-Eppendorf (UKE)*. The sample was extracted from a patient with temporal lobe epilepsy (TLE) [17] and is labeled as TLE003.

Table 7: Specifications of the JIMA RT RC-02 resolution test chart [33].

Chip - ($_{14}\text{Si}$ -base)	$5 \times 5 \times 0.06 \text{ mm}^3$
Absorbing material	Tungsten ($_{74}\text{W}$) layer - $1 \text{ }\mu\text{m}$
Protection layer	P-SiO ₂ - $0.1 \text{ }\mu\text{m}$
Slit widths	(0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 10.0, 15.0) μm . Each group contains 7 except the 15 μm which has 5 lines.

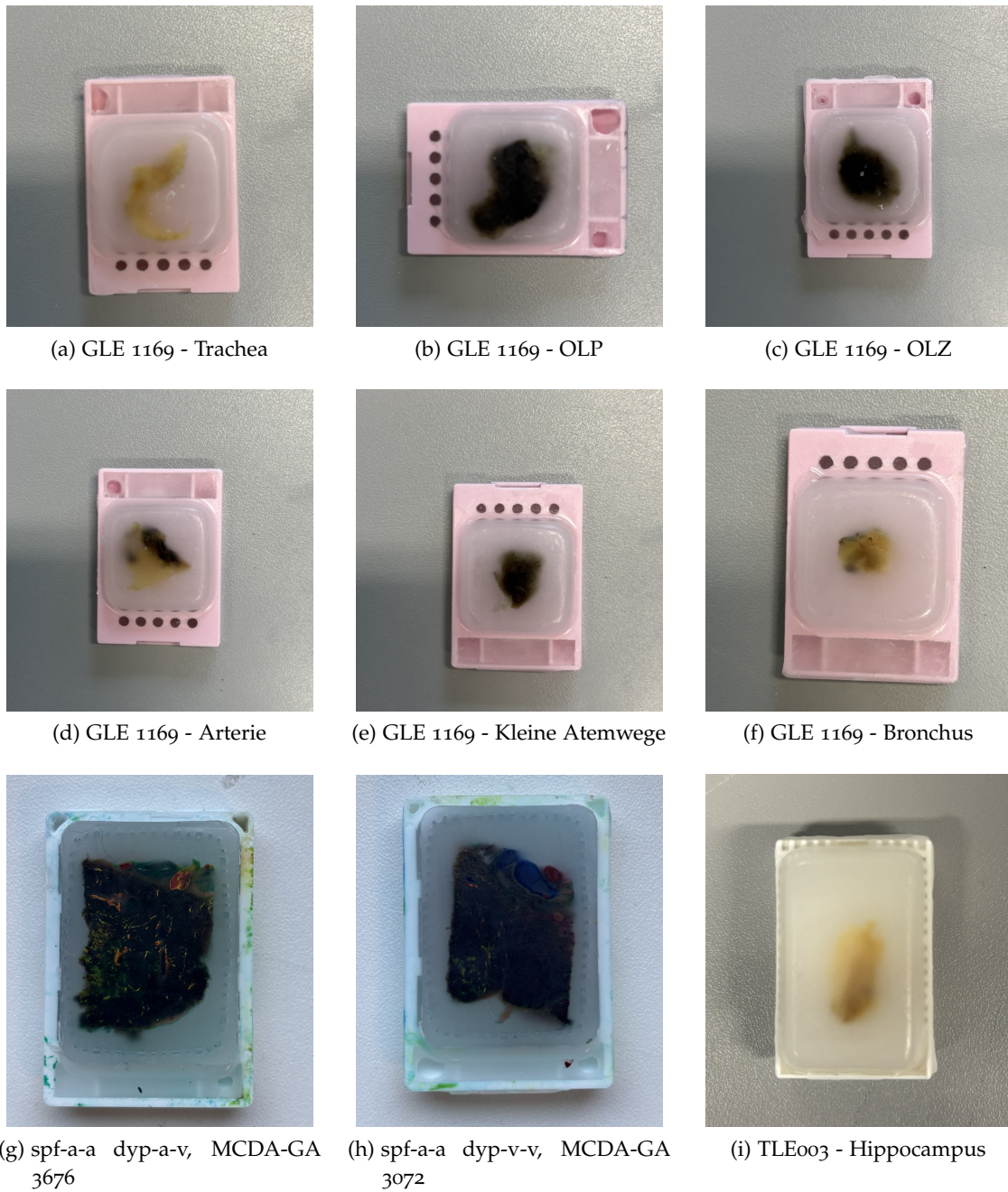


Figure 83: **Images of FFPE samples used in this thesis.** The samples listed in figs. 83a to 83f come from *Medizinische Hochschule Hannover (MHH)*, figs. 83g and 83h from *Oslo Universitetsykehus*, while the sample in fig. 83i comes from *Universitätsklinikum Hamburg-Eppendorf (UKE)*. Each image is labeled with its corresponding sample code to ensure traceability with the experimental results.

D.3 SUPPLEMENTARY SCRIPTS AND CODES

D.3.1 *Reconstruction script*

A comprehensive example script for the full reconstruction of datasets obtained from [JuLiA](#) and [MONA](#) is presented below, including detailed comments that clarify each step of the process. This script provides insight into reading the acquired projection images, performing preprocessing steps such as dark- and flat-field correction, alignment, phase retrieval, and the actual reconstruction of the tomographic dataset using the [FDK](#) algorithm. The script also includes visualization tools for inspecting the raw, sinogram, and reconstructed data, as well as for removing ring artifacts. The script is modular and can be adapted to different datasets and experimental parameters by changing the input parameters accordingly. This script relies mainly on tools inherited from *hotopy* [40], *ASTRA* toolbox [76] and *numpy* [30].

Tomographic reconstruction of datasets taken at [JuLiA](#)

```
import sys
sys.executable
```

Imports

```
import numpy as np
import os, math, json
from tqdm import tqdm
import hotopy
import xraylab.collections.tomotools as tt
import warnings
warnings.filterwarnings("ignore")
from pathlib import Path
import h5py, tiffio
import matplotlib.pyplot as plt
# matplotlib setup
plt.rcParams["figure.figsize"] = (10,6)
plt.rcParams["figure.autolayout"] = True
plt.rcParams["image.interpolation"] = "none"
plt.rcParams["image.cmap"] = "gray"

detector_px = {"varex": 100e-6}
```

Write here the instrument, filename and scan parameters

```
# ----- INPUT -----
pars = dict(
    instrument = 'julia', # name of the instrument
    run = '2025_08_04',
    specfile = 'Bronchus_8mm_punch_40kV_57W', # name of the folder/scan
    detector = 'varex', # name of the detector
    ct = 7, #exposure time
    geometry = dict(
        z01 = 120e-3, # in m, Distance Source Sample
        z02 = 1500e-3, # in m, Distance Source Detector
        angular_range = 350, # in deg
        px = detector_px["varex"], # in m, native pixel size of the detector
```



```

    ),
    scan_structure = dict(
        pattern = 'eted', # z.B. "eted", e: empty, d: dark, t: tomo
        num_empty = 30, # number of empty images
        num_dark = 15, # number of dark images
        num_angles = 2000+1, # number of angles
        accumulations = 2,
        defino_first = 1,
        i_tomo = 1, # which tomoblock in pattern to reconstruct
    ),
)
pars['geometry'].setdefault('M', pars['geometry']['z02'] /
    pars['geometry']['z01'])
pars['geometry'].setdefault('px_eff', pars['geometry']['px'] /
    pars['geometry']['M'])
pars['geometry']['num_angles'] = pars['scan_structure']['num_angles']

# determine relevant image numbers
def get_sequence_numbers(pattern, i_tomo):
    def find_nth_substr(string, substring, n):
        start = string.find(substring)
        while start >= 0 and n > 1:
            start = string.find(substring, start+1)
            n -= 1
        return start

    # i_seq counts sequences starting at 1 (not 0)
    if "i_tomo" in pars["scan_structure"]:
        i_seq_tomo = find_nth_substr(pattern, "t",
            pars["scan_structure"]["i_tomo"])
    else: # take first tomo
        i_seq_tomo = pattern.find("t")+1
    i_seq_empty_pre = pattern.rfind("e", 0, i_seq_tomo+1)
    i_seq_empty_post = pattern.find("e", i_seq_tomo)
    i_seq_dark = pattern.find("d")
    return i_seq_tomo, i_seq_empty_pre, i_seq_empty_post, i_seq_dark

pattern = pars["scan_structure"]["pattern"]
block_length = dict(
    e=pars["scan_structure"]["num_empty"], d=pars["scan_structure"]["num_dark"],
    t=pars["scan_structure"]["num_angles"]*pars["scan_structure"]
    ["accumulations"]
)
defino_block = np.cumsum([pars["scan_structure"]["defino_first"]] +
    [block_length[letter] for letter in pattern])
i_seq_tomo, i_seq_empty_pre, i_seq_empty_post, i_seq_dark =
    get_sequence_numbers(pattern, pars["scan_structure"]["i_tomo"])

print(" "*13+"### scan structure ###")
for i_seq, letter in enumerate(pattern):
    notice = {
        i_seq_tomo: "tomo ----->",
        i_seq_empty_pre: "empty_pre -->",

```

```

        i_seq_empty_post:"empty_post ->",
        i_seq_dark:      "dark ----->",
    }.get(i_seq, "")
    print(f"{notice:13s} {i_seq} - {letter} first image: {defino_block[i_seq]}")
    defino_tomo = np.arange(defino_block[i_seq_tomo], defino_block[i_seq_tomo+1])
    defino_empty_pre = np.arange(defino_block[i_seq_empty_pre],
                                defino_block[i_seq_empty_pre+1])
    defino_empty_post = np.arange(defino_block[i_seq_empty_post],
                                  defino_block[i_seq_empty_post+1])
    defino_dark = np.arange(defino_block[i_seq_dark], defino_block[i_seq_dark+1])

```

Determine the correct image numbers

```

pars["crop_y"] = (850, 1150) # top and bottom
pars["crop_x"] = (400, 800) #left and right

crop_2d = np.s_[pars["crop_y"][0]:-pars["crop_y"][1] or None,
                pars["crop_x"][0]:-pars["crop_x"][1] or None]
img_dir =
    Path(f".../juliaLab/{pars['run'][:4]}/{pars['instrument']}/{pars['run']}/
         detectors/{pars['detector']}/{pars['specfile']}")

prefix=f"{img_dir}/{pars['specfile']}"
def read_image_hdf5(i_img):
    file = h5py.File(f"{prefix}.h5", 'r') # Open the file
    Images = file['ct.0000']['source.0000']
    image = Images[f'data_{i_img:06d}'][0]
    return np.fliplr(np.rot90(image, k=3))[crop_2d]

read_image = read_image_hdf5
def show_image(image, ax=None, percentiles=(1, 99), **kwargs):
    if ax is None:
        fig, ax = plt.subplots()
    else:
        fig = ax.get_figure()
    kwargs.setdefault("vmin", np.percentile(image, percentiles[0]))
    kwargs.setdefault("vmax", np.percentile(image, percentiles[1]))
    im = ax.imshow(image, **kwargs)
    fig.colorbar(im, ax=ax)

raw0 = read_image(defino_tomo[0])
empty0 = read_image(defino_empty_pre[-1])
dark0 = read_image(defino_dark[0]) if len(defino_dark)>0 else 0
prj0 = ((raw0-dark0) / (empty0-dark0)).astype(np.float32)
prj0[~np.isfinite(prj0)] = 0

for defino, title in [(defino_empty_pre, "empty_pre"), (defino_tomo, "tomo"),
                      (defino_empty_post, "empty_post"), (defino_dark, "dark")]:
    if len(defino) == 0:
        continue
    fig, axes = plt.subplots(1, 2, figsize=(6,2))
    ax = axes[0]
    show_image(read_image(defino[0]), ax)
    ax.set_title(f"{title}[0]")

```

```
ax = axes[1]
show_image(read_image(defino[-1]), ax)
ax.set_title(f"{title}[-1]")
```

Read all images of your dataset according to the scan type

```
from concurrent.futures import ThreadPoolExecutor, as_completed,
    ProcessPoolExecutor
#parallel read function
def read_single_image_by_args(args):
    i, pathh = args
    with h5py.File(pathh, 'r') as f:
        img = f['ct.0000']['source.0000'][f'data_{i:06d}'][0]
        img = np.fliplr(np.rot90(img, k=3))[crop_2d]
    return img

p = f"{prefix}.h5"
def par_read_sequence(pathh, indices):
    args = [(i, pathh) for i in indices]
    with ProcessPoolExecutor() as executor:
        images = list(tqdm(executor.map(read_single_image_by_args, args),
            total=len(indices)))
    return np.stack(images)

dark = par_read_sequence(p, defino_dark).mean(0, dtype=np.float32) if
    len(defino_dark)>0 else 0

raws = par_read_sequence(p, defino_tomo) - dark
#average acquisitions recorded at the same angle
num_accum = pars['scan_structure']['accumulations']
if num_accum > 1:
    num_angles = pars['scan_structure']['num_angles']
    if raws.shape[0] == num_accum * num_angles:
        tmp = np.zeros((raws.shape[0]//num_accum, *raws.shape[1:]),
            dtype=np.float32)
        for i_angle in tqdm(range(raws.shape[0]//num_accum)):
            tmp[i_angle] = np.median(raws[i_angle * num_accum:(1+i_angle) *
                num_accum], axis=0)
        raws = tmp
    del tmp
else:
    raise UserWarning(f"Has this cell already been executed?\n
        {raws.shape=}\n{num_accum=}\n{num_angles=}")

if len(defino_empty_pre) > 0:
    empty_pre = par_read_sequence(p, defino_empty_pre).mean(0, dtype=np.float32)
    - dark
if len(defino_empty_post) > 0:
    empty_post = par_read_sequence(p, defino_empty_post).mean(0,
        dtype=np.float32) - dark

#Remove outliers from flats
from xraylab.image.filter import remove_outliers
def process_empty(defino_empty):
```

```

if len(defino_empty) > 0:
    empty = par_read_sequence(p, defino_empty).mean(0, dtype=np.float32) -
    dark
    empty, mask = remove_outliers(empty)
    print(f"empty: {mask.sum()} pixels corrected")
    return empty

with ThreadPoolExecutor() as executor:
    empty_pre, empty_post = executor.map(process_empty, [defino_empty_pre,
    defino_empty_post])

```

Flat frames correction

```

projs = np.empty((len(rows), *raw0.shape), dtype=np.float32)
w = np.linspace(1, 0, len(rows), dtype=np.float32) #weights

def process_projection(i):
    if 'empty_pre' in globals() and 'empty_post' in globals():
        this_empty = (w[i] * empty_pre + (1-w[i]) * empty_post)
    elif 'empty_pre' in globals() and 'empty_post' not in globals():
        this_empty = empty_pre
    elif 'empty_pre' not in globals() and 'empty_post' in globals():
        this_empty = empty_post
    return rows[i] / this_empty

with ThreadPoolExecutor() as executor:
    results = list(tqdm(executor.map(process_projection, range(len(projs))),
    total=len(projs)))
projs = np.array(results, dtype=np.float32)

```

Remove outliers from projections

```

def process_projection(i, projs):
    projection = projs[i]
    proj_corr, mask = remove_outliers(projection, filter="lowpass_mean",
    inplace=True)
    num_fixed = mask.sum()
    return proj_corr

def process_projections(projs): #parallel executor
    with ThreadPoolExecutor() as executor:
        corrected_projs = list(tqdm(executor.map(lambda i:
        process_projection(i, projs), range(len(projs))), total=len(projs)))
    return np.array(corrected_projs, dtype=np.float32)

projs = process_projections(projs)

```

Binning

```

import torch
pars["binning"] = 1
if pars["binning"] != 1:
    projs = torch.nn.functional.avg_pool2d(torch.as_tensor(projs),
    kernel_size=pars["binning"]).numpy()
projs.shape

```

Sinogram

```
show3d(projs.transpose(1, 2, 0))
del raws #release memory
```

Phase retrieval MBA - single slice

```
from hotopy.phase import ModifiedBronnikov
pars["phase_reco"] = dict()
pp = pars["phase_reco"]
pp["algorithm"] = "MBA"
pp["fresnel_number"] = 1
pp["alpha"] = 0.0581

def MBA_reco(alpha, F=1):
    get_phase_MBA = ModifiedBronnikov(projs[0].shape, F, alpha)
    return get_phase_MBA(projs[0])
show_image(MBA_reco(pp["alpha"]))
s = 100
s_start, s_end = 600, 800
proj_test = MBA_reco(0.0581)
profile = "v" #v for vertical line profile, else horizontal
fig, axs = plt.subplots(1, 2, figsize=(12, 5))
axs[0].imshow(proj_test, cmap='gray')
if profile == "v":
    line_profile = proj_test[s_start:s_end, s]
    axs[0].vlines(x=s, ymin=s_start, ymax=s_end, color='red', linestyle='--')
else:
    line_profile = proj_test[s, s_start:s_end]
    axs[0].hlines(y=s, xmin=s_start, xmax=s_end, color='red', linestyle='--')
axs[0].set_title('Test Projection')
axs[1].plot(line_profile, color="red")
axs[1].set_title(f'Line Profile at s={s}')
axs[1].set_xlabel('X [pixel]')
axs[1].set_ylabel('Intensity')
plt.tight_layout()
plt.show()
```

BAC - single slice

```
from hotopy.phase import BronnikovAidedCorrection
pars["phase_reco"] = dict()
pp = pars["phase_reco"]
pp["algorithm"] = "BAC"
pp["alpha"] = 0.0581
pp["gamma"] = 0.89

def BAC_reco(alpha, gamma):
    get_phase_BAC = BronnikovAidedCorrection(projs[0].shape, alpha=alpha,
                                              gamma=gamma)
    return -get_phase_BAC(projs[0])
show_image(BAC_reco(pp["alpha"], pp["gamma"]))

proj_test_MBA = BAC_reco(pp["alpha"], 0.89)
fig, axs = plt.subplots(1, 2, figsize=(12, 5))
show_image(proj_test_MBA, axs[0])
if profile == "v":
```

```

    line_profile_MBA= proj_test_MBA[s_start:s_end, s]
    axs[0].vlines(x=s, ymin=s_start, ymax=s_end, color='red', linestyle='--')
else:
    line_profile_MBA = proj_test_MBA[s, s_start:s_end]
    axs[0].hlines(y=s, xmin=s_start, xmax=s_end, color='red', linestyle='--')
axs[0].set_title('Test Projection')
axs[1].plot(line_profile_MBA)
axs[1].set_title(f'Line Profile at s={s}')
axs[1].set_xlabel('X position')
axs[1].set_ylabel('Intensity')
plt.tight_layout()
plt.show()

```

Phase retrieval for all images

```

if pp["algorithm"]=="BAC":
    get_phase = BronnikovAidedCorrection(projs.shape[1:], alpha=pp["alpha"],
    gamma=pp["gamma"], device="cuda")
else:
    raise NotImplementedError()
phase_recos = -get_phase.apply_stack(projs, chunksize=5).cpu().numpy()

from torch.cuda import empty_cache
empty_cache()

```

Paganin - single slice

```

from hotopy.phase import Paganin
betadelta = 0.06
fnums = np.array(1)
shape = projs[0].shape

pars["phase_reco"] = dict()
pp = pars["phase_reco"]
pp["algorithm"] = "PAGANIN"
pp["fresnel_number"] = fnums
pp["betadelta"] = betadelta
paganin = Paganin(shape, fnums, betadelta=betadelta,device="cuda")
phase_paganin = paganin(projs[0])
show_image(phase_paganin)

```

Phase retrieval for all images

```

get_phase = Paganin(shape, fnums, betadelta=betadelta,device="cuda")
phase_recos = get_phase.apply_stack(projs, chunksize=5).cpu().numpy()

from torch.cuda import empty_cache
empty_cache()

```

```

# Phase cross correlation
from hotopy.image.filter import gaussian_bandpass2_real
from hotopy.image.transforms import imshift
from skimage.registration import phase_cross_correlation as pcc

# Read images for 0 degrees

```



```

img0 = phase_recos[0]
angular_range = pars["geometry"]["angular_range"]
numangles = pars["scan_structure"]["num_angles"]
def check_angle_for_pcc(angular_range):
    if angular_range != 180 and angular_range != 360:
        proj180 = int(np.round(180/angular_range * numangles,0))
        print(f'The angular range is not 180 or 360 degrees. Projection number
        for 180 will be calculated manually: {proj180}.', )
        return proj180

proj180 = check_angle_for_pcc(pars["geometry"]["angular_range"])

# Read image for 180 degrees
if pars["geometry"]["angular_range"]==180:
    img180 = np.fliplr(phase_recos[-1])
elif pars["geometry"]["angular_range"]==360:
    img180 = np.fliplr(phase_recos[int(len(phase_recos)/2)])
else:
    img180 = np.fliplr(phase_recos[proj180])

def flt(x):
    return gaussian_bandpass2_real(x, 4, 6)

fig, (ax1, ax2) = plt.subplots(1, 2, figsize=(14,5), layout="constrained")
plt.suptitle('Make sure the pictures look the same', fontsize=18)
ax1.imshow(flt(img0))
ax2.imshow(flt(img180))
plt.show()

shift = pcc(flt(img0), flt(img180), normalization=None, upsample_factor=50)[0]
def apply_shift(i):
    return imshift(phase_recos[i], shift=[0, -shift[1]/2], order=1,
    prefilter=False)

with ThreadPoolExecutor() as executor:
    projs_shift = list(tqdm(executor.map(apply_shift, range(len(phase_recos))),
    total=len(phase_recos)))
check_pcc = True
projs_shift = np.array(projs_shift)

from copy import deepcopy
import hotopy.astra as hastra
pars["tomo_reco"] = deepcopy(pars["geometry"])
tp = pars["tomo_reco"]
tp["px_eff"] *= pars["binning"]
tp["px"] *= pars["binning"]
angles = np.linspace(0, np.deg2rad(tp["angular_range"]), tp["num_angles"])
sinogram = phase_recos

def reco_sino(i_sino, shift_sino=0, imagesShifted = False, ringRemoval = False):
    if (imagesShifted==False):
        sino = phase_recos[:, i_sino]
    else:

```

```

sino = projs_shift[:, i_sino]
if(ringRemoval==True):
    sino = rr.ringremove(sino, **ringremoval_pars)
fanflat = hastra.fanflat(sino.shape, -angles, z01=tp['z01'], z02=tp['z02'],
dx=tp['px'], sino_pad=tp["sino_pad"])
if(imagesShifted==False):
    fanflat.correct_shift(shift_sino)
return fanflat(sino[:,None,:])[0]

```

Determine geometric parameters

```

tp["sino_pad"] = 200
i_top = math.ceil(0.28 * phase_recos.shape[1])
shift_top = -85.51
i_bottom = math.floor(0.895 * phase_recos.shape[1])
shift_bottom = -87.93
tp['global_shift'] = (shift_top + shift_bottom)/2
tp['global_rot'] = np.rad2deg(np.arctan2(i_top - i_bottom, shift_top -
    shift_bottom) - np.pi/2)
pars['global_shift'] = tp['global_shift']
pars['global_rot'] = tp['global_rot']
print(f"{tp['global_shift'] = } px")
print(f"{tp['global_rot'] = } deg")

roi = np.s_[:, :]
show_image(reco_sino(i_top, shift_top)[roi], percentiles=(15, 90))
show_image(reco_sino(i_bottom, shift_bottom)[roi], percentiles=(15, 90))
check_pcc=False

A = np.linspace(-84, -88, 30)
l = []
for i in A:
    l.append(np.std(reco_sino(i_top, i)[roi]))
l = np.array(l)

# Plot noise vs shift (refinement of shift)
plt.figure(figsize=(8, 5))
plt.plot(A, l, marker='o', linestyle='--', color='blue')
plt.xlabel('Shift (pixels)')
plt.ylabel('noise')
plt.title('noise vs Shift')
plt.grid(True)
min_idx = np.argmax(l)
plt.axvline(A[min_idx], color='red', linestyle='--', label=f'Max noise at
    shift={A[min_idx]}')
plt.legend()
plt.tight_layout()
plt.show()

```

Ring removal, Test parameters

```

import hotopy.tomo as rr
tp["sino_pad"] = 100
i_center = math.ceil(0.4 * phase_recos.shape[1])
ringremoval_pars = dict(type="additive", smoothing = "median", filter_size = 10)

```

```

proj_ring = reco_sino(i_center, tp['global_shift'], imagesShifted =
    check_pcc, ringRemoval=True)
proj_control = reco_sino(i_center, tp['global_shift'], imagesShifted =
    check_pcc, ringRemoval=False)

view = 500
roi = np.s_[int(proj_ring.shape[1]/2)-view:int(proj_ring.shape[1]/2)+view,
    int(proj_ring.shape[0]/2-view):int(proj_ring.shape[0]/2+view)]
plt.figure(figsize=(14,5))
plt.subplot(121)
plt.title('Without ring removal')
plt.imshow(proj_control[roi])
plt.colorbar()
plt.subplot(122)
plt.title('With ring removal')
plt.imshow(proj_ring[roi])
plt.colorbar()
plt.show()

```

Ring removal for all projections

```

tp["ringremoval"] = ringremoval_pars
if check_pcc: # if phase cross correlation has been used to shift the images
    ring_recos = rr.ringremove(projs_shift, **ringremoval_pars)
else:
    ring_recos = rr.ringremove(phase_recos, **ringremoval_pars)

```

Reconstruct all slices

```

import astra
import hotopy.astra as hastra
from importlib import reload
reload(hastra)
fdk = hastra.fdk(ring_recos.shape[-2:], angles, z01=tp["z01"], z02=tp["z02"],
    dx=tp["px"])
if not check_pcc: # if phase cross correlation has been computed, then the
    following lines are not necessary
    fdk.correct_shift(shift = (-tp['global_shift'], 0), reference_geom=None)
    fdk.rotate_detector(angle_deg = tp['global_rot'], reference_geom=None)
    fdk.load_proj(ring_recos)
else:
    fdk.load_proj(ring_recos)
tomo_reco = fdk()

```

Save result

```

save_predir = Path(f".../juliaLab/{pars['run'][:4]}/
    {pars['instrument']}/{pars['run']}/analysis/{pars['specfile']}
    /reconstruction_python/")
save_name = "Bronchus_8mm_punch_40kV_57W_PAG.tif"
save_dir = f"{save_predir}/{save_name}"
if not os.path.exists(save_predir):
    os.makedirs(save_predir)
if os.path.isfile(save_dir):
    print("File already exists!")

```

```

else:
    tiffiffle.imwrite(save_dir, tomo_reco)
    if os.path.isfile(save_dir):
        print(f"Saved {save_name} successfully!")
        print(save_dir)
    else:
        print("Something went wrong.")
tt.dicttoh5(
    dict(
        tomo_reco = tomo_reco,
        tomopars = pars,
    ),
    'Bronchus_8mm_punch_40kV_57W_PAG.h5', # filename
    mode='w-', # w-: do not overwrite
)

```

D.3.2 Dark and readnoise assesment

The following script demonstrates how the the read noise and dark noise of the Varex detector were assessed as discussed in section 5.2. It utilizes libraries different *Python* packages for reading, processing and analyzing the data, including *h5py* for handling HDF5 files, *CuPy* for GPU-accelerated computations, and *Pandas* for data manipulation.

```

import numpy as np
import cupy as cp
import seaborn as sns
import matplotlib.pyplot as plt
import h5py
import pandas as pd
from scipy.ndimage import rotate
import itertools

path = ".../julia/2025_01_22/.../testdetector.h5"

# Basic functions
def load_image(file, num):
    Images = file['ct.0000']['source.0000']
    image = Images[f'data_{num:06d}'][0]
    return cp.rot90(cp.asarray(image), k=3)

def display(file, num, vmin=0, vmax=800):
    Img = load_image(file, num)
    plt.imshow(cp.asnumpy(Img), vmin=vmin, vmax=vmax, cmap="gray",
        aspect="auto")
    plt.colorbar()
    plt.grid(False)
    plt.show()

def average_images(file, first, last):
    num_images = last - first
    image_shape = file['ct.0000']['source.0000'][f'data_{first:06d}'][0].shape
    images = cp.empty((num_images, *image_shape), dtype=cp.float64) # Use CuPy

```

```

    for i, num in enumerate(range(first, last)):
        images[i] = load_image(file, num)
    return cp.mean(images, axis=0)

file = h5py.File(path, 'r')

bias_images = [load_image(file,i) for i in range(247,247+17)] # i indexes the
                    image
combination = itertools.combinations(bias_images, 2)

Sigmar = []
for bias1, bias2 in combination:
    bias1 = cp.array(bias1,
        dtype=cp.float64)[roi_start:roi_end,roi_start:roi_end]
    bias2 = cp.array(bias2,
        dtype=cp.float64)[roi_start:roi_end,roi_start:roi_end]
    Sigmar.append(cp.std(bias1 - bias2))

sigmar = cp.array(Sigmar)/cp.sqrt(2) #divide by sqrt(2) to get the std of a
                    single image
sigma_meanr = cp.mean(sigmar) # mean read noise
sigma_stdr = cp.std(sigmar) # error on the mean read noise
print(sigma_meanr,sigma_stdr)

dark_images001 = [load_image(file,i) for i in range(267,267+17)]
combination = itertools.combinations(dark_images001, 2)

Sigma = []
for dark1, dark2 in combination:
    dark1 = cp.array(dark1,
        dtype=cp.float64)[roi_start:roi_end,roi_start:roi_end]
    dark2 = cp.array(dark2,
        dtype=cp.float64)[roi_start:roi_end,roi_start:roi_end]
    Sigma.append(cp.std(dark1 - dark2))

sigma = np.array([i.get() for i in Sigma]) / np.sqrt(2)
sigma_mean = np.mean(sigma)
sigma_std = np.std(sigma)

dF = pd.DataFrame({
    "Label": ["dark001"],
    "Time" : 0.01,
    "sig": [sigma_mean],
    "sigma_sig": [sigma_std],
    "sig_dark": [np.sqrt(np.abs(sigma_mean**2-sigma_meanr.get()**2))]
})

dF
"""Repeat analysis for remaining dark frames"""

```

D.3.3 Noise power spectrum assessment (NPS)

The following *Python* scripts provides insight into the calculation and analysis of the NPS for the Varex detector, according to the methodology provided by Kuhls-Gilcrst et al. [37] and discussed in section 5.2.1.

```

from scipy.optimize import curve_fit
from cupyx.scipy.fft import fft2, fftshift
import scipy.stats as stats

px = 100e-3 #pixel size in mm

Master_flat = average_images(file,617,617+17)
Residual_flats = [load_image(file,i)-Master_flat for i in range(617,617+17)]
Residual_FFT_shift = [fftshift(fft2(residual)) for residual in Residual_flats]

def NPS_batch(Residual_flats):
    """Takes in a list of 2D numpy arrays (Residual flats) and returns a list
    of 2D NPS, kfreq, phi and phi_mean"""
    delta = 256
    center = 1536
    Delta = 512

    nps_2d_total = cp.zeros((delta,delta))
    for ii in range(len(Residual_flats)):
        Image = Residual_flats[ii]
        Image = Image[center-Delta:center+Delta:center-Delta:center+Delta]
        for i in range(4): #section the image into 16 subregions
            for j in range(4):
                subregion = Image[i*delta:(i+1)*delta, j*delta:(j+1)*delta]
                subregion = fftshift(fft2(subregion))
                nps_region = px**2*cp.abs(subregion)**2/(delta**2)
                nps_2d_total += nps_region #accumulate NPS of all subregions

    nps_2d = nps_2d_total/(4*4*len(Residual_flats)) #mean NPS of all subregions
    C = nps_2d.shape[0]//2
    #compute and store mean NPS value in the central region
    phi = nps_2d[C-((delta)//6):C+((delta)//6),C-((delta)//6):C+((delta)//6)]
    phi_mean = cp.mean(phi)
    npix = nps_2d.shape[0]
    kfreq = np.fft.fftfreq(npix, d=px) #frequency
    return nps_2d, kfreq, phi, phi_mean

def NPS_regression_gpu(container,mean_container):
    """Performs linear regression on the computed NPS data using GPU with cupy.
    return slope (A) and constant (B) which are both 2D arrays. The input
    container is a list of 2D numpy arrays (NPS) and mean_container is a list
    of mean values master flat fields."""
    epsilon = 1e-12
    # Move data to the GPU
    container_gpu = cp.array(container)
    X_gpu = cp.array(mean_container)

```



```

X_mean = X_gpu.mean()
Y_mean = container_gpu.mean(axis=0) # Mean over the exposure axis

# Compute slope (A) and constant (B) for all (kx, ky)
numerator = cp.sum((X_gpu[:, None, None] - X_mean) * (container_gpu -
Y_mean[None, :, :]), axis=0)
denominator = cp.sum((X_gpu[:, None, None] - X_mean) ** 2, axis=0)
denominator = cp.where(denominator == 0, epsilon, denominator)
A = numerator / denominator
B = Y_mean - A * X_mean
B = cp.maximum(B, 0.0)
return A, B

def Radial_NPS(Image):
    """Computes the radial average of a 2D NPS(Image). Returns the radial
    frequencies (kvals) and the corresponding averaged NPS values (Abins)."""
    npix = Image.shape[0]
    px = 100e-3 #native pixel size in meters
    kfreq = np.fft.fftfreq(npix, d = px) # frequency in um^-1
    kx, ky = np.fft.fftshift(np.meshgrid(kfreq, kfreq))
    k = np.sqrt(kx**2 + ky**2).flatten()

    nps = Image.flatten()
    k_max = np.max(k)
    kbins = np.linspace(0, k_max, 300)
    kvals = 0.5 * (kbins[1:] + kbins[:-1])
    Abins, _, _ = stats.binned_statistic(k, nps, statistic = "mean", bins =
kbins)
    kvals = kvals[1:] #exclude the zero frequency
    Abins = Abins[1:]
    return kvals, Abins

M = [735, 745, 755, 765, 775, 785, 617] #first image numbers of flat fields
N = [471, 488, 506, 523, 540, 557, 540] #first image numbers of dark fields
Nps_container = []
Mean_container = []
Sig_container = []

for u in range(len(M)):
    i = M[u]
    l = N[u]
    Delta = 512
    center = 1536
    Master_flat = average_images(file,i,i+10)
    Master_dark = average_images(file,l,l+5)
    Residual_flats = [load_image(file,j)-Master_flat for j in range(i,i+10)]

    B = Master_flat - Master_dark #correct flat for dark field
    mean_dn =
B[center-Delta:center+Delta:center-Delta:center+Delta].get().mean()
sig = B[center-Delta:center+Delta:center-Delta:center+Delta].get().std()
Mean_container.append(mean_dn) #mean signal of the flat field
Sig_container.append(sig) #standard deviation of the flat field

```

```

Mean_container = np.array(Mean_container) #convert to np array
Sig_container = np.array(Sig_container)

nps, kfreq, phi, phi_mean = NPS_batch(Residual_flats)
Nps_container.append(nps)

t = np.array([400,500,600,700,850,950,800]) #list of exposure times in ms

from scipy.optimize import curve_fit
def linear_model(t, a, b):
    return a * t + b

popt, pcov = curve_fit(linear_model, t, Mean_container, sigma=Sig_container,
    absolute_sigma=True)
a_fit, b_fit = popt
perr = np.sqrt(np.diag(pcov)) # uncertainties on the fit parameters
print(f"Fit results: a = {a_fit:.3f}  $\pm$  {perr[0]:.3f}, b = {b_fit:.3f}  $\pm$  {perr[1]:.3f}")

t_fit = np.linspace(np.min(t), np.max(t), 500)
fit_curve = linear_model(t_fit, *popt)
fit_std = np.sqrt((t_fit**2)*pcov[0,0] + pcov[1,1] + 2*t_fit*pcov[0,1])

# Plot
plt.figure(figsize=(6, 5))
plt.errorbar(t, Mean_container, yerr=Sig_container, fmt='o', label=r'Mean
    signal ( $\overline{I}_F$ )', capsize=4)
plt.plot(t_fit, fit_curve, 'r-', label=rf'Linear fit:
     $\overline{I}_F = \{a\_fit:.2f\} \cdot t_s + \{b\_fit:.2f\}$ ', alpha=0.8)
plt.fill_between(t_fit, fit_curve - 2*fit_std, fit_curve + 2*fit_std,
    color='r', alpha=0.2, label=r'3 $\sigma$  fit uncertainty')
plt.xlabel(r'Exposure Time ( $t_s$ ) [ms]')
plt.ylabel(r'Mean signal ( $\overline{I}_F$ ) [ADU]')
plt.title('Flat-Field signal vs Exposure Time')
plt.legend(fontsize=12)
plt.grid(True)
plt.tight_layout()
plt.show()

aa, bb = NPS_regression_gpu(Nps_container, Mean_container) #aa: quantum noise,
bb: white noise
kvalsbb, Abinsbb = Radial_NPS(bb.get())
kvalsaa, Abinsaa = Radial_NPS(aa.get())

```

D.3.4 Visibility analysis

In the following, an exemplary *Python* script that was used for visibility analysis is briefly given and commented.

```

import h5py
import numpy as np

```

```

with h5py.File('../Visibility_projs_70kV.h5', 'r') as f: #load corrected
    dataset (projection images)
    projs = [f[key][()] for key in f.keys() if key.startswith('projs_')]
    projs = np.array(projs, dtype=np.float32)

image = projs[6] #select a projection image

y_min, y_max = 1050 , 1150
x_min_roi, x_max_roi = 1200, 2000
y_min_roi, y_max_roi = 1200, 1500

x_max_flat, x_min_flat = 2000, 1200
y_min_flat, y_max_flat = 500, 800
roi = image[y_min_roi:y_max_roi, x_min_roi:x_max_roi]
roi_flat = image[y_min_flat:y_max_flat, x_min_flat:x_max_flat]

t = 30 #half width of the vertical slice
x_profile = 2300
vertical_slice = image[1050:1150, x_profile-t:x_profile+t+1]
vertical_profile = vertical_slice.mean(axis=1) #edge spread function (ESF)

grad = np.gradient(vertical_profile) #line spread function (LSF)
edge_idx = np.argmax(np.abs(grad)) #compute the location of the peak LSF

w = 10 #half width of the window around the edge
window = vertical_profile[edge_idx - w : edge_idx + w] #window around the edge
I_max, I_min = np.max(window) , np.min(window) #max and min intensity
V = (I_max - I_min) / (I_max + I_min) #visibility
M_ = np.abs(grad).max() #peak LSF
mean_roi = np.mean(roi) #mean transmission in the ROI
std_roi = np.std(roi) #standard deviation in the ROI
std_flat = np.std(roi_flat) #standard deviation in empty ROI
V, M_, mean_roi, std_roi, std_flat

```

D.3.5 Fourier shell correlation (FSC)

The following *Python* script demonstrates how the FSC between two independently acquired tomography datasets, as discussed in section 4.5.4 was computed. This script uses the *hotopy* library for calculating the FSC as well as *matplotlib* and *numpy* to visualize and handle the results.

```

import numpy as np
import h5py
import matplotlib.pyplot as plt
from hotopy.image.stats import fourier_shell_correlation as FSC

pathA = ".../julia/2025_02_19/.../Bronchus_Arterie_40kV_57W_BAC.h5"
pathB = ".../julia/2025_02_20/.../Bronchus_Arterie_40kV_57W_run2_BAC.h5"

with h5py.File(pathA, 'r') as f: #first dataset
    #load the reconstructed tomography dataset
    tomo_reco = f['tomo_reco']

```

```

tomo_reco_scanA = tomo_reco[:]

with h5py.File(pathB, 'r') as f: #second dataset
    tomo_reco = f['tomo_reco']
    tomo_reco_scanB = tomo_reco[:]

#crop a square ROI within the reconstructed circular FOV
tomo_reco_scanA_crop = tomo_reco_scanA[:,500:-500,500:-500]
tomo_reco_scanB_crop = tomo_reco_scanB[:,500:-500,500:-500]

#pick out 3D ROIS of the respective sample
Arterie_A = tomo_reco_scanA_crop[815:1215,:,:)
Arterie_B = tomo_reco_scanB_crop[815:1215,:,:)

Bronchus_A = tomo_reco_scanA_crop[250:650,:,:)
Bronchus_B = tomo_reco_scanB_crop[250:650,:,:)

#Tape_A = tomo_reco_scanA_crop[705:755,:,:)
#Tape_B = tomo_reco_scanB_crop[705:755,:,:)

#compute the fourier shell correlation using hotopy
freq, fsc, nfreq, half_bit = FSC(Arterie_A, Arterie_B)
freqB, fscB, nfreqB, half_bitB, hbfreqB = FSC(Bronchus_A, Bronchus_B)

#geometric magnification
M = 1500/120
p_eff = 100/M #microns

#computing half-bit threshold crossing
i_max = int(np.argwhere(fsc < half_bit)[0, 0])
slc = slice(i_max - 1, i_max + 1)
freq_half_bit = np.interp(0, np.flip(fsc[slc] - half_bit[slc]), (freq[i_max],
    freq[i_max - 1]))

i_maxB = int(np.argwhere(fscB < half_bitB)[0, 0])
slcB = slice(i_maxB - 1, i_maxB + 1)
freq_half_bitB = np.interp(0, np.flip(fscB[slcB] - half_bitB[slcB]),
    (freqB[i_maxB], freqB[i_maxB - 1]))

#plotting the results
plt.figure(figsize=(10,5))
plt.plot(freq/p_eff, fsc, label='Artery')
plt.plot(freqB/p_eff, fscB, label='Bronchus')
plt.plot(freq/p_eff, half_bit, label='half-bit threshold', linestyle =
    'dashed', color = 'red', alpha = 0.5)
plt.axvline(x=freq_half_bit/p_eff, color='k', alpha= 0.2, linestyle = 'dashed')
plt.text(freq_half_bit/p_eff+0.0008, 1 * 0.7, r'$k_{1/2} = $
    0.0488\,\frac{\text{cycles}}{\mu m}$', color='k', rotation=0)
plt.axvline(x=freq_half_bitB/p_eff, color='k', alpha= 0.2, linestyle = 'dashed')
plt.text(freq_half_bitB/p_eff+0.0008, 1 * 0.4, r'$k_{1/2} = $
    0.0659\,\frac{\text{cycles}}{\mu m}$', color='k', rotation=0)
plt.xlabel(r"Spatial frequency ($k$) [ $\frac{\text{cycles}}{\mu m}$ ]")
plt.title("Fourier Shell Correlation (FSC)")

```

```
plt.ylabel(r"FSC ($k$)")
plt.legend()
plt.tight_layout()
#plt.savefig('fscbronchusartery.png', dpi=300)
#plt.savefig('fscbronchusartery.pdf')
plt.show()
```

D.4 LIST OF FUNCTIONS AND COMMANDS

Table 8 provides an overview of the frequently used *MATLAB* and *SPEC* functions. The functions integrate with *SPEC* to enable alignment procedures, acquisition, calibration, etc. Each function is designed with specific input parameters to control motor movements, exposure times, and acquisition parameters [13, 35].

Table 8: Summary of useful and frequently used *MATLAB-SPEC* functions at [JuLiA](#) and [MONA](#) [13, 35].

Function	Syntax	Description
<code>newrun</code>	<code>newrun</code>	creates a new folder in the saving directory and initializes a a folder for the appropriate detector
<code>newfile</code>	<code>newfile filename</code>	creates a new folder with the name filename in the current run directory
<code>ct</code>	<code>ct (time)</code>	acquires a projection image with an exposure length of time seconds.
<code>(u)mvr</code>	<code>(u)mvr motor value</code>	moves motor relatively to position value.
<code>(u)mv</code>	<code>(u)mv motor value</code>	moves motor absolutely to position value.
<code>mat_align_rotaxis</code>	<code>mat_align_rotaxis rotmotor time</code>	aligns the rotation axis in the center of the detector by acquiring images at 0° and 180° for time seconds and aligning them; the effective pixel size has to be known for this function to work properly.
<code>mat_pixelsize</code>	<code>mat_pixelsize motor pos1 pos2 nsteps time</code>	a perpendicular motor is moved from pos1 to pos2 in nsteps steps and images are acquired for time seconds; subsequently, the shift between the images is determined and together with the physical step size the effective pixel size can be calculated
<code>mat_align_nick</code>	<code>mat_align_nick motor range time</code>	command for the alignment of the pitch angle of the rotation axis; the motor is moved relatively by $-\text{range}/2$ and $\text{range}/2$ and images are acquired for time seconds at both positions
<code>tomo_setup</code>	<code>tomo_setup</code>	sets up a tomographic scan with the specified pattern, number of empty and dark images, number of angles, number of accumulations per angle and exposure time
<code>tomo_scan</code>	<code>tomo_scan file-name</code>	starts the tomographic scan with parameters defined in the setup phase and saves the projections according to the specified filename

Part V

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