

Towards the development of an all-optical, non-contact, photon absorption remote sensing (PARS)
endomicroscope for blood vasculature imaging

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

The need for high-resolution, label-free imaging techniques has spurred the development of advanced endoscopic technologies for real-time tissue characterization. This thesis presents the design, development, and validation of the first forward-viewing, non-contact, all-optical Photon Absorption Remote Sensing (PARS) endomicroscope for *in vivo* vascular imaging. The proposed system is designed to leverage the endogenous optical absorption of hemoglobin to achieve high-resolution contrast, without the use of exogenous labels or acoustic coupling, addressing longstanding limitations of conventional absorption-based and scattering-based imaging modalities. Two prototype designs were developed using image guide fiber (IGF) technology and achromatic graded-index (GRIN) lenses, with systematic de-risking experiments guiding their evolution. The first prototype (P1) achieved a resolution of $\sim 1\ \mu\text{m}$ and signal-to-noise ratio (SNR) of 22 dB, demonstrating the feasibility of high-fidelity PARS imaging within a 1.6mm outer diameter (OD) device footprint. A second design (P2) was introduced to address constraints in working distance and imaging depth for *in vivo* use, trading resolution for improved accessibility in biological tissues.

This work establishes a novel platform for PARS miniaturization and integration with widefield endoscopy, positioning the technology for future applications, including real-time, *in situ* virtual *biopsies*, blood oxygenation measurement, and surgical guidance within internal bodily cavities. The results represent a foundational advancement in the translation of PARS microscopy to clinical settings and lay the groundwork for real-time, high-resolution endoscopic diagnostics.

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Special thanks to GRINTECH GmbH for providing the achromatic objective lens used in the prototype design and for their technical support in optimizing its performance.

Dedication

This work is dedicated to my family, in particular my mother, Charmaine Elien, who has always been supportive of me throughout my pursuit of higher education.

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List of Abbreviations

Abbreviation	Definition
AFI	Autofluorescence Imaging
AFM	Autofluorescence Microscopy
BC	Beam Combiner
CAM	Chorioallantoic Membrane
CF	Carbon Fiber
CEM	Confocal Endomicroscopy
CL	Condenser Lens
DAQ	Data Acquisition
DCF	Double-Clad Fiber
DCFC	Double-Clad Fiber Coupler
DOF	Depth of Focus
EM	Endomicroscopy
ES	Excitation Source
FOV	Field of View
FM	Fluorescence Microscopy
FWHM	Full Width at Half Maximum
GRIN	Graded-Index (Lens)
HD-WLE	High-Definition White-Light Endoscopy
IGF	Image Guide Fiber
LCI	Linked-Color Imaging
LSF	Line Spread Function
NA	Numerical Aperture
NBI	Narrow-Band Imaging
NIR	Near-Infrared
OCT	Optical Coherence Tomography
OL	Objective Lens
OR-PAM	Optical-Resolution Photoacoustic Microscopy
P1	Prototype 1
P2	Prototype 2
PA	Photoacoustic
PAM	Photoacoustic Microscopy
PARS	Photon Absorption Remote Sensing
PBS	Polarizing Beam Splitter
PD	Photodiode
POC	Proof-of-Concept
PRR	Pulse Repetition Rate
PT	Piezoelectric Transducer
QWP	Quarter-Wave Plate
RBC	Red Blood Cell

ROI	Region of Interest
SMC	Single-Mode Core
SNR	Signal-to-Noise Ratio
SO ₂	Blood Oxygen Saturation
TA-PARS	Total Absorption Photon Absorption Remote Sensing
WD	Working Distance
WF	Widefield
WFE	Widefield Endoscopy

1 Introduction

The development of optical imaging technologies has been driven by the need for high-resolution, minimally invasive diagnostic tools capable of providing detailed insights into biological structures and functions. A wide range of imaging modalities have been investigated to address these needs, with particular emphasis on their application in clinical settings. Among these modalities, techniques that exploit endogenous optical contrast, such as optical coherence tomography (OCT) and confocal microscopy, have been extensively studied¹⁻⁶. Despite their widespread use, limitations associated with specificity and lack of functional information have prompted the exploration of alternative approaches. As an alternative, fluorescence-based imaging has been extensively utilized in both research and clinical settings^{7,8}. However, its reliance on exogenous contrast agents presents challenges, including potential toxicity, limited *in vivo* applicability, and the need for targeted delivery. Additionally, fluorescence imaging suffers from inherently low photon yield due to tissue scattering and photobleaching, reducing its effectiveness in deep tissue imaging⁸. These shortcomings have further highlighted the need for imaging modalities that provide label-free operation while maintaining high resolution and specificity. One such approach involves absorption-based imaging modalities, which rely on the optical absorption properties of endogenous chromophores to generate contrast. These modalities have been shown to provide superior specificity for vascular imaging and functional diagnostics when compared to scattering-based techniques⁹. Photoacoustic (PA) imaging has emerged as a promising absorption-based technique, providing high contrast and allowing imaging at depths greater than those achievable with purely optical methods^{10,11}. However, its reliance on acoustic transducers for signal detection introduces limitations, such as the need for direct contact with the sample and challenges in miniaturization for endoscopic applications.

Photon Absorption Remote Sensing (PARS) has been introduced as a promising alternative to overcome these limitations. Originally developed by Haji Reza et al. as a non-contact, all-optical method, PARS can target photothermal and photoacoustic initial perturbations induced by optical modulations to provide non-radiative contrast. Like PA imaging, PARS imaging provides absorption-based contrast, but measures light-scattering modulations instead of acoustic signals. This all-optical approach eliminates the need for transducers, coupling media, or sample contact, enabling smaller devices and potentially higher resolutions. This comprehensive optical absorption microscopy technique has evolved from photoacoustic remote sensing and offers several advantages for clinical applications¹². By eliminating the need for acoustic transducers and enabling label-free imaging, PARS holds significant promise for advancing diagnostic imaging, particularly in endoscopic applications.

There is a growing demand for minimally invasive surgical procedures that has significantly influenced trends in the global healthcare market^{13,14}, with modern endoscopy playing a pivotal role in this shift. Advances in endoscopic technology have enabled safer, less invasive procedures with reduced recovery times, leading to their widespread adoption in fields such as gastroenterology, pulmonology, and urology. As such, endoscopy has established itself as a cornerstone in modern medical diagnostics, offering direct visual access to internal organs and tissues in a minimally invasive manner. It allows for real-time visualization and tissue sampling at the site of interest, making it indispensable for diseases that are otherwise inaccessible through non-invasive techniques. This capability is particularly critical for early detection, monitoring, and treatment of diseases that manifest within internal cavities and organ systems. The ability to combine direct imaging with interventional procedures has enabled endoscopy to address unique diagnostic challenges, such as identifying early-stage cancers, detecting precancerous lesions, and diagnosing inflammatory and

infectious diseases. For example, upper gastrointestinal endoscopy has been pivotal in diagnosing conditions like Barrett's esophagus¹⁵, a precursor to esophageal cancer, while colonoscopy has become the gold standard for colorectal cancer screening¹⁶. Despite the success, significant challenges remain in endoscopy, including the difficulty of imaging submucosal structures, limited specificity in identifying vascular and cellular abnormalities, and the inability to obtain detailed functional information beyond the structural level.

The characterization of blood vasculature has been shown to provide clinically relevant insights into the development of various diseases, including cancer¹⁷, inflammatory conditions¹⁸, and vascular disorders¹⁹. Endoscopy has demonstrated its utility in monitoring pathological activity *in situ* by enabling the detailed visualization of vascular structures. For example, in conditions like Barrett's esophagus, the ability to assess blood vessel morphology and growth patterns is critical for early detection and diagnosis of esophageal cancer²⁰. Similar diagnostic needs exist for other internal pathologies, where direct visualization of vascular changes can inform disease progression and therapeutic outcomes¹⁹. PARS has emerged as a promising modality for imaging blood vasculature in a range of clinical settings^{21–24}. Its ability to provide label-free, high-resolution imaging of vascular structures, combined with its non-contact, all-optical design, positions it as a powerful tool for addressing existing diagnostic challenges. These capabilities align closely with the goals of virtual biopsy techniques, offering the potential to assess vascular and tissue pathology *in vivo* without the need for physical tissue extraction. This motivates the development of a first-of-its-kind PARS endomicroscope device, specifically designed to image blood vasculature *in vivo*. By combining the strengths of PARS imaging with endoscopy, such a device is well-suited to address limitations in current clinical diagnostics, providing high-resolution vascular imaging that enhances the diagnostic and therapeutic value of minimally invasive procedures.

The overarching goal of this thesis is to develop and evaluate a first-of-its-kind PARS endomicroscope prototype that establishes the foundation for PARS miniaturization and integration into clinical endoscopy. Specifically, this work aims to design a compact, forward-viewing, non-contact, and biocompatible device that is compatible with the instrument channels of standard widefield endoscopes. The prototype is engineered to enable high-resolution, label-free imaging of microvasculature and is tested using carbon fiber phantoms as analogs for vascular targets. Through systematic design, proof-of-concept validation, and quantitative performance evaluation, this thesis seeks to demonstrate the feasibility of a flexible PARS imaging platform suitable for future *in vivo* vascular imaging applications.

2 Literature Review

To contextualize the development of a PARS endomicroscope for blood vasculature imaging, the following literature review provides a comprehensive examination of the current landscape. Key imaging modalities are explored, with a focus on their advancements, clinical applications, and limitations. Particular attention is given to absorption-based imaging techniques and their integration into endoscopic systems, highlighting how these approaches address existing challenges while paving the way for innovative diagnostic tools like PARS.

2.1 Brief history of endoscopic devices

The origins of endoscopy can be traced back several centuries, but it was not until the early nineteenth century that the first functional endoscope was developed by Dr. Philipp Bozzini²⁵. Bozzini's device, the Lichtleiter (or light-conductor), addressed two fundamental challenges of earlier designs: inadequate lighting and poor penetration. The Lichtleiter featured an eyepiece for viewing, a candle as a light source, a reflecting mirror to direct both light and the image, and various tubes or specula for insertion into body orifices. Although further work on the Lichtleiter was hindered by Bozzini's untimely death, the foundational principles of his design would go on to influence future advancements in endoscopy. Bozzini is widely regarded as the father of modern endoscopy, as subsequent innovations in the field have built upon the concepts introduced by his pioneering device.²⁵

Nearly two decades after Bozzini's pioneering work, further advancements in endoscopy were made by French physician Pierre Salomon Segalas and American physician John D. Fisher. Segalas

introduced the "speculum urethra-cystique," a device based on the same foundational principles as Bozzini's Lichtleiter²⁵. The speculum, which was designed mainly to inspect the urethra and bladder, was composed of two silver tubes, two metal mirrors, two small candles and an elastic probe. Endoscopy continued to progress in a saltatory fashion, with significant contributions from physicians such as Antonin J. Desormeaux, Julius Bruck, and Maximilian Nitze. The invention of the incandescent light bulb by Thomas Edison in the late 19th century further revolutionized the field, enabling greater illumination and advancing the capabilities of endoscopic devices²⁵.

The discovery of X-rays by Wilhelm Röntgen in 1895 marked a significant milestone in medical imaging, laying the foundation for the field of diagnostic radiology²⁶. Roentgen's groundbreaking work, which was the culmination of decades of research by numerous scientists, shifted the focus of medical diagnostics toward radiographic techniques. This development temporarily diminished interest in the potential of endoscopy as a diagnostic tool. It would take several decades before meaningful breakthroughs would reignite advancements in endoscopic technology.

The introduction of fiber-optic technology revolutionized endoscopy, marking a turning point in the field. In 1957, Basil Hirschowitz along with physicist C. Wilbur Peters and student Larry Curtiss at the University of Michigan, developed a functional fiberscope prototype²⁷. Hirschowitz demonstrated the prototype by successfully passing it through his own esophagus and that of a patient. By 1960, the first production fiberscope was introduced, rendering conventional gastroscopes obsolete²⁷. Fiber-optic technology significantly improved endoscopy by enhancing flexibility, reducing patient discomfort, and enabling safer, more accessible procedures. Notable refinements followed in the late 1960s and 1970s, including wider fields of vision, biopsy and suction channels, and controlled tip deflection. These advancements allowed nearly every region of the gastrointestinal

tract to be explored, establishing fiber-optic endoscopy as a cornerstone of modern medical diagnostics²⁸.

Today, optical fibers remain integral to endoscopic devices, providing high-resolution imaging and advancing the capabilities of minimally invasive procedures. The introduction of fiber optics not only transformed gastrointestinal endoscopy but also set the foundation for innovations across a wide range of endoscopic applications.

1805

MAINZ,
GERMANY

Lichtleiter
developed by
Phillipp Bozzini

1853

PARIS,
FRANCE

Antonin J.
Desormeaux
develops
instrument, coins
term endoscope

1873

VIENNA,
ITALY

Gustave Pierre
Trouvé founds
electro-endoscopy
by developing distal
lighting

1932

MUNICH,
GERMANY

The first semi-
flexible endoscope
developed by
Rudolf Schindler

1957

MICHIGAN,
USA

First known use of
fiber optics in
endoscopy by
gastroenterologist
Basil Hirschowitz

TODAY

Modern
endoscopes work
on same basic
principles

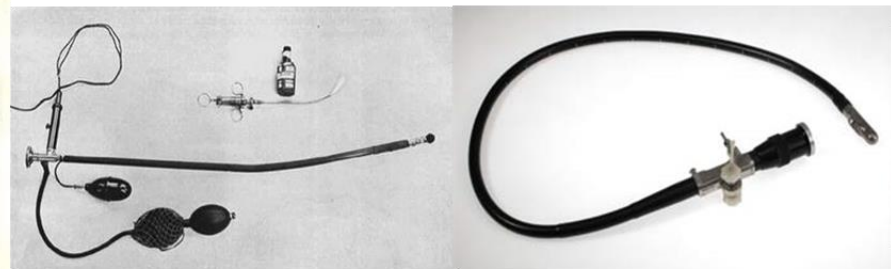
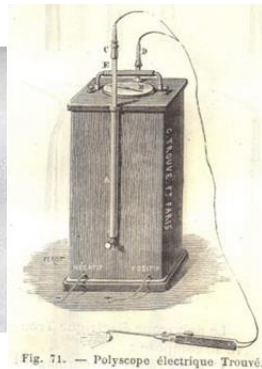
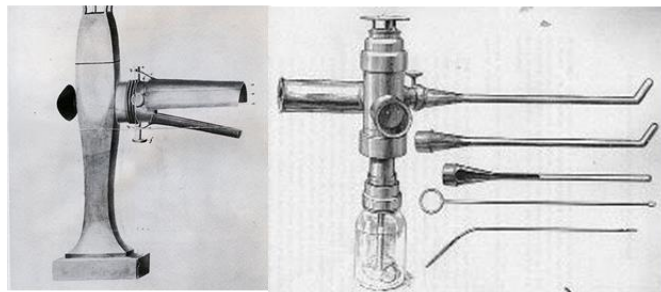


Figure 1: Historical timeline of endoscopy development, focusing on the groundbreaking improvements in design

2.2 Endoscopy market trends and the need for Innovation

Endoscopy is a rapidly growing market. The global endoscopic devices market size was valued at 61.06 billion USD in 2024 and is expected to expand at a compound annual growth rate (CAGR) of 3.79% from 2025 to 2030¹³. One of the major factors boosting the market is the increased adoption of minimally invasive procedures by health care professionals and patients, signaling a move away from traditional open surgeries. Minimally invasive surgical procedures offer increased economic viability, higher patient satisfaction, reduction in hospital stay, and decreased post-surgical complications. In addition, an increased prevalence of gastrointestinal (GI) diseases as well as a rise in demand for endoscopic devices in therapeutic and diagnostic procedures continue to drive future demand in the market.²⁹

Technological advancements, including the integration of high-definition cameras, enhanced light sources, and robotic systems, have further augmented the market by improving precision, control, and visualization in diagnostic and therapeutic interventions. Robotic-assisted endoscopy in particular has gained traction for its ability to enhance maneuverability and precision in complex surgeries.¹⁴ Additionally, the growing preference for biopsies in cancer have fueled the adoption of advanced endoscopic devices. These developments reflect the ongoing innovation within the field, making endoscopy a cornerstone of modern medical diagnostics and treatment.

2.3 Endoscope imaging system configuration and applications

2.3.1 Breakdown of modern endoscope design

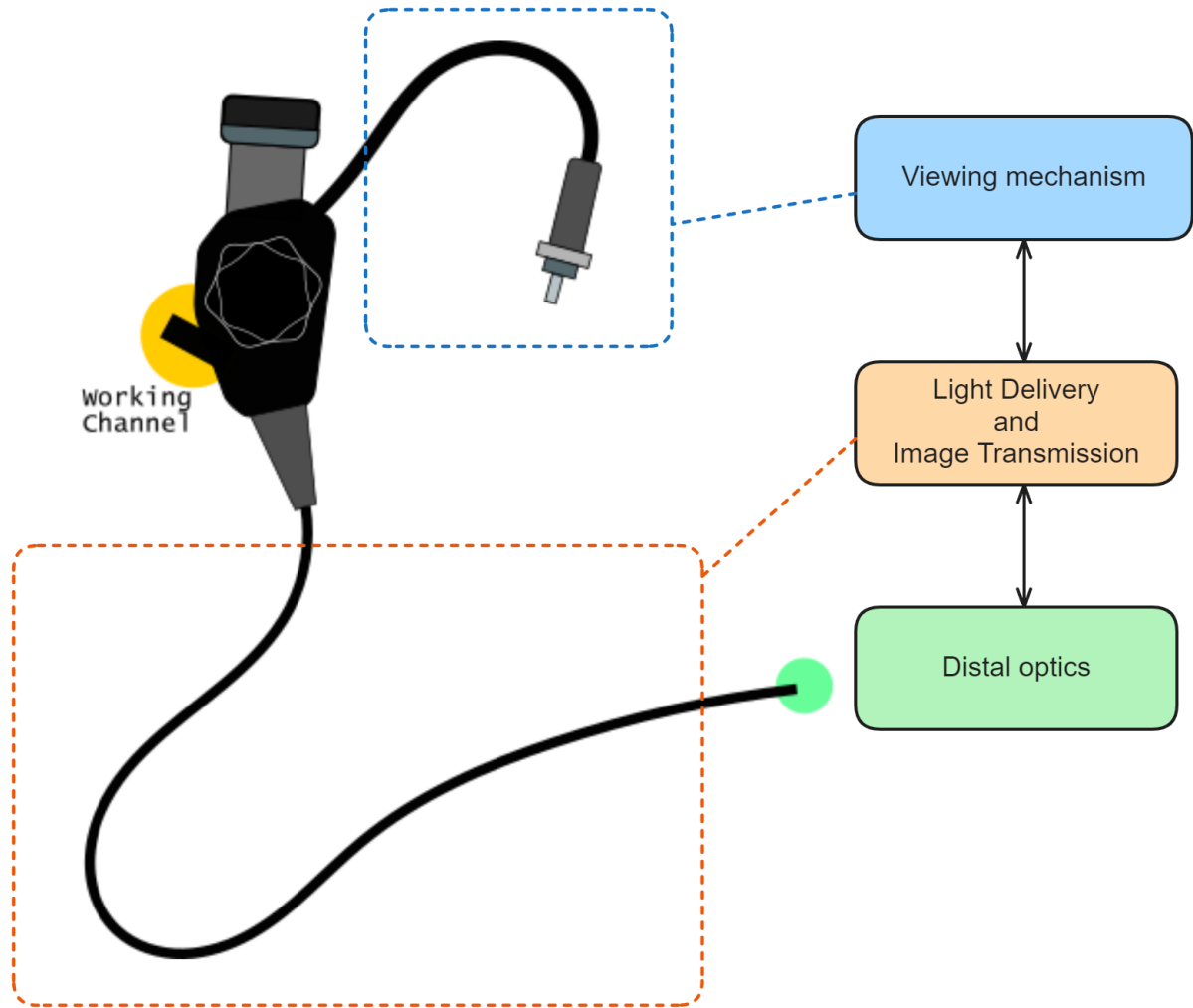


Figure 2: Functional breakdown of modern endoscope designs highlighting the major components

Modern endoscopes are complex instruments composed of several integral components, each serving a specific purpose to facilitate the visualization and manipulation of internal organs and structures. The flexible tube allows navigation through the body's natural pathways with minimal

discomfort. A **light delivery system**, typically using optical fibers, illuminates the area under inspection. An **image transmission** system, often employing fiber optic bundles, transfers visual information coherently from the distal end to the proximal end for viewing. The **distal objective optics**, analogous to a camera lens, determine image characteristics such as field of view, resolution, and depth of focus. A **viewing mechanism**, either an eyepiece or, more commonly, a camera and external monitor, enables clinicians to observe the transmitted images. Additionally, **accessory channels** allow for the insertion of medical instruments or manipulators, facilitating diagnostic and therapeutic interventions during endoscopic procedures. Together, these components make modern endoscopes versatile tools for minimally invasive diagnostics and treatments. The imaging system itself is localized to the light delivery system, the image transfer system and the viewing mechanism (eyepiece, camera) and forms the template for the development of an imaging adapted for endoscopic use.

2.3.2 Endoscope configurations

Endoscopes are available in various configurations to suit specific clinical applications. The most common configuration is the widefield forward-viewing design, which maximizes the field of view (FOV) along the axis of the endoscope. Side-viewing endoscopes, on the other hand, provide an FOV orthogonal to the length of the endoscope, making them ideal for examining regions of interest located perpendicular to the insertion axis. For example, side-view endoscopes are used in procedures such as endoscopic retrograde cholangiopancreatography (ERCP) to directly visualize the biliary tree for the diagnosis and treatment of pancreaticobiliary diseases. Additionally, some endoscopes are designed to provide 360-degree FOVs, enabling comprehensive visualization of the surrounding anatomy in certain specialized applications ³⁰.

In addition to traditional designs, more recent endoscope configurations have been introduced for specialized clinical applications. These include biopsy needle endoscopes^{31–33}, which enable minimally invasive access to deep tissues for diagnostic sampling, and capsule endoscopes³⁴, which allow for non-invasive imaging of the gastrointestinal tract. Furthermore, modular endoscope systems designed for integration into robotic platforms have expanded the range of applications, enhancing precision and control in complex surgical and diagnostic procedures.

Forward-viewing configurations are particularly desirable for clinical applications due to their compatibility with standard endoscopic procedures and their ability to provide a clear view along the axis of insertion. Forward-viewing designs are particularly advantageous for navigating complex anatomical pathways and performing diagnostic and therapeutic interventions with precision. The following review focuses on forward-viewing configurations while briefly addressing other designs as needed.

2.4 Widefield endoscopy

Widefield endoscopy (WFE) is an imaging technique in which the distal optics are optimized for a large field of view (FOV), often at the expense of magnification and resolution. This configuration is designed to serve as a broad "searching tool," allowing clinicians to efficiently flag potential regions of interest (ROIs) for further examination. By providing a comprehensive view of internal structures, WFE is particularly suited for initial screenings in diagnostic procedures.

Modern WFE represents a highly mature technology that integrates real-time video feedback, enhancing its utility for identifying suspicious areas. This real-time capability allows clinicians to dynamically assess and navigate anatomical structures, making WFE an ideal tool for flagging abnormalities during procedures. WFE operates on the principles of brightfield microscopy, where

the sample is illuminated using white light. Contrast is generated based on local variations in light absorption, enabling structural visualization in real time. This method is fundamental to clinical workflows, where the primary goal is to identify areas of concern for targeted intervention.

For example, the gold standard procedure for diagnosing gastrointestinal (GI) cancers typically begins with WFE to flag suspicious ROIs. Tissue samples from these areas are excised using tools fed through the working channel of the endoscope and are subsequently assessed externally through biopsy procedures for definitive diagnosis. This integration of widefield visualization with interventional capabilities highlights the critical role of WFE in modern endoscopy.³⁵

Despite being an integral part of the gold standard for cancer screening and precursor lesion detection in the gastrointestinal (GI) tract, high-definition white-light endoscopy (HD-WLE) has notable limitations. One of its most significant shortcomings is its high miss rate, particularly in high-risk populations such as those with inflammatory bowel disease, Lynch syndrome, or Barrett's esophagus¹⁵. In these cases, lesions are often flat and subtle, making them difficult to detect using WFE's resolution and contrast capabilities. This limitation emphasizes the need for advanced imaging techniques that can enhance lesion detection and improve diagnostic accuracy in vulnerable patient groups.

To address the limitations of widefield endoscopy, advanced imaging techniques such as narrow-band imaging (NBI), autofluorescence imaging (AFI), and linked-color imaging (LCI) have been developed. These methods are integrated into widefield endoscopy systems to enhance lesion detection by providing high-contrast video images, aiding in the identification of cancer-related abnormalities. However, these techniques lack the specificity and resolution required to provide detailed morphological or functional information about tissues and cells, which is essential for accurate diagnosis and staging of diseases³⁶.

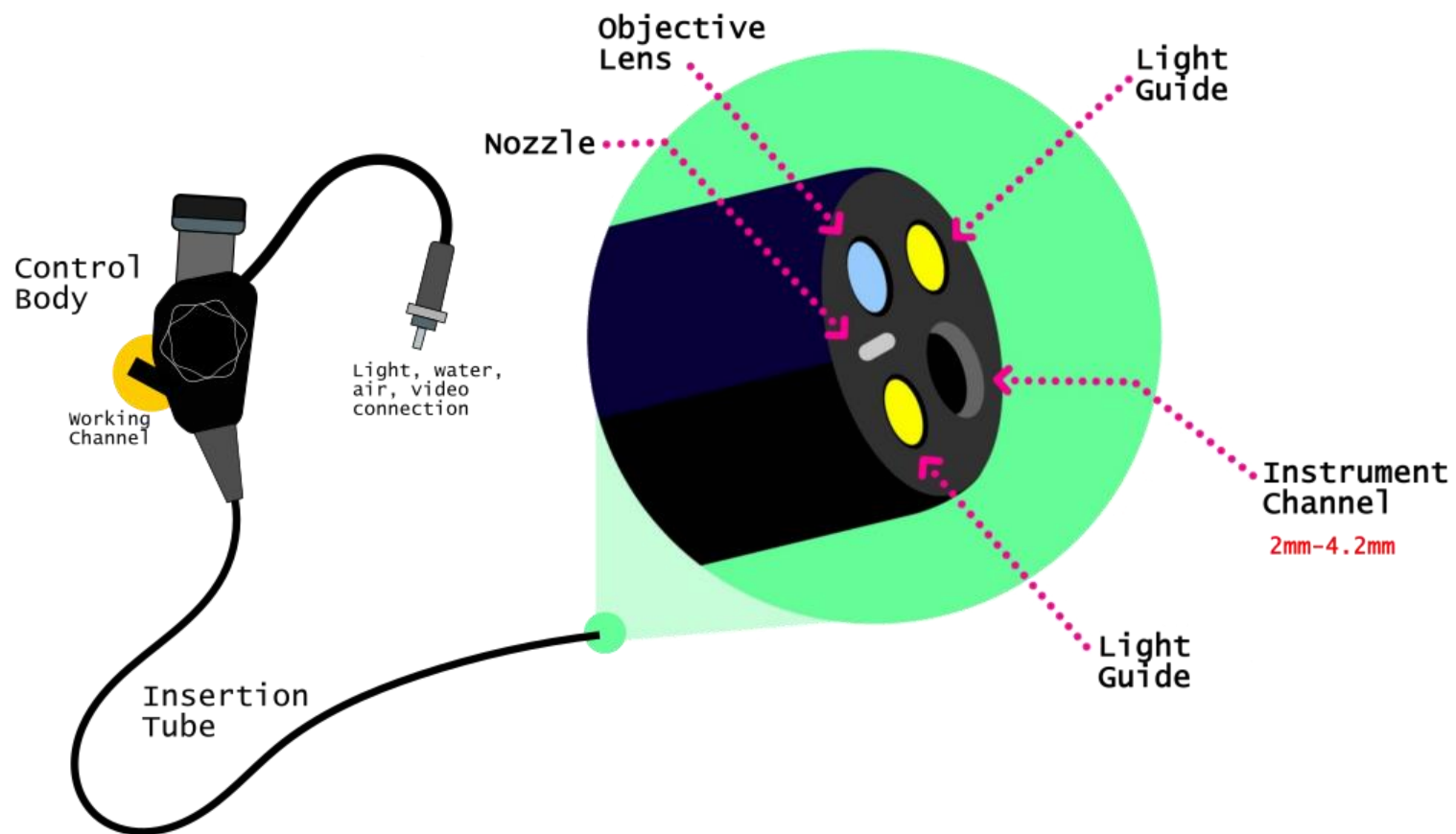


Figure 3: Simple illustration of a widefield endoscope with working channel and distal-end configuration. This illustration shows the typical instrument channel inner diameter, which ranges from 2mm – 4.2mm.

2.5 Endomicroscopy and the movement toward Virtual Biopsies

Endomicroscopy (EM) is a general term used to refer to any endoscopic technique capable of acquiring structural and/or functional information non-invasively, while achieving cellular or subcellular resolution that surpasses the capabilities of traditional endoscopic technologies ¹⁵. EM devices are typically designed with compact footprints, often only a few millimeters in diameter, enabling their use in minimally invasive procedures. These systems provide significant advantages, including improved image resolution, enhanced contrast, deeper tissue penetration, and the ability to report on biochemical and molecular changes that cannot be discriminated by conventional endoscopic methods ³⁶.

Towards Virtual biopsies

The integration of advanced imaging techniques with minimally invasive surgeries has significantly expanded the scope of modern diagnostics. Robotic-assisted surgery, for example, has facilitated the widespread adoption of minimally invasive procedures across various surgical specialties ³⁷. Despite these advancements, limitations remain, including low resolution and the inability to provide molecular or biochemical information critical for diagnosing many major diseases. As the medical field continues to move toward minimally invasive procedures, the demand for practical EM techniques has grown significantly. EM addresses these limitations by enabling *in situ* imaging of tissues with cellular or subcellular resolution, offering real-time diagnostic capabilities during invasive clinical procedures.

For GI cancers, detection, characterization, and staging remain the diagnostic pillars, with *ex vivo* histology as the gold standard. An ideal endoscopic technique should combine widefield “red-flag” screening for detecting suspicious regions with microscopy-level imaging *in situ* for tissue characterization and staging. This integrated approach encapsulates the term **virtual biopsy** and

allows for immediate and detailed evaluation of malignancies without requiring *ex vivo* tissue processing. Endomicroscopy facilitates "virtual biopsies", offering an alternative to conventional biopsies for the diagnosis and staging of prominent diseases like gastrointestinal (GI) and breast cancers. EM technologies, such as confocal laser endomicroscopy, nonlinear endomicroscopy, and HiLo microscopy, have led to the development of commercial products from companies like Mauna Kea Technologies, Pentax, and Optiscan, highlighting the demand for such devices in clinical settings. These devices enable the acquisition of high-resolution, real-time images of tissue *in vivo*, attempting to bridge the gap between widefield screening and histological analysis.^{38,39}

2.6 Technical review of EM technologies

Despite the advancements in high-definition white-light endoscopy (HD-WLE) over the past decade, its unparalleled ability to detect dysplasia has yet to be surpassed by competing technologies. With image clarity exceeding one million pixels, HD-WLE remains a powerful tool for broad screening. However, its lack of spatial resolution limits specificity and contributes to high false positive rates, particularly in detecting high-grade dysplasia and early adenocarcinoma¹⁵. While multi-modal platforms have demonstrated higher targeted detection rates compared to HD-WLE, they too suffer from the inherent limitations of widefield imaging technologies, including limited resolution and specificity.

To overcome the challenges faced by HD-WLE, the development of high-resolution imaging techniques has gained momentum, propelled by advances in fiber-optic technology, miniaturized optics, and mechanical systems. These advancements have facilitated the emergence of clinically viable EM technologies that leverage modalities such as confocal laser microscopy (CM), optical coherence tomography (OCT), fluorescence microscopy (FM) – including non-linear techniques

(two-photon-, three-photon- microscopy), autofluorescence microscopy (AFM) – and photoacoustic microscopy (PAM). Endoscopic devices that leverage these techniques offer improved resolution and contrast, addressing limitations of conventional widefield imaging.

Among these technologies, absorption-based modalities are particularly notable for their ability to achieve subcellular resolution, deeper imaging depths, and high specificity, enabling real-time imaging that provides information resembling traditional histopathology. The interpretation of images generated by these systems relies on detailed morphological criteria, including epithelial structure, cellular features (e.g., cell size and arrangement), and vascular patterns (e.g., size, leakage, and tortuosity). These criteria allow for more precise characterization of tissues, offering significant potential for *in vivo* diagnostics.

The following review provides a detailed examination of the most prominent EM technologies currently under investigation, focusing on how they address challenges inherent to widefield endoscopy.

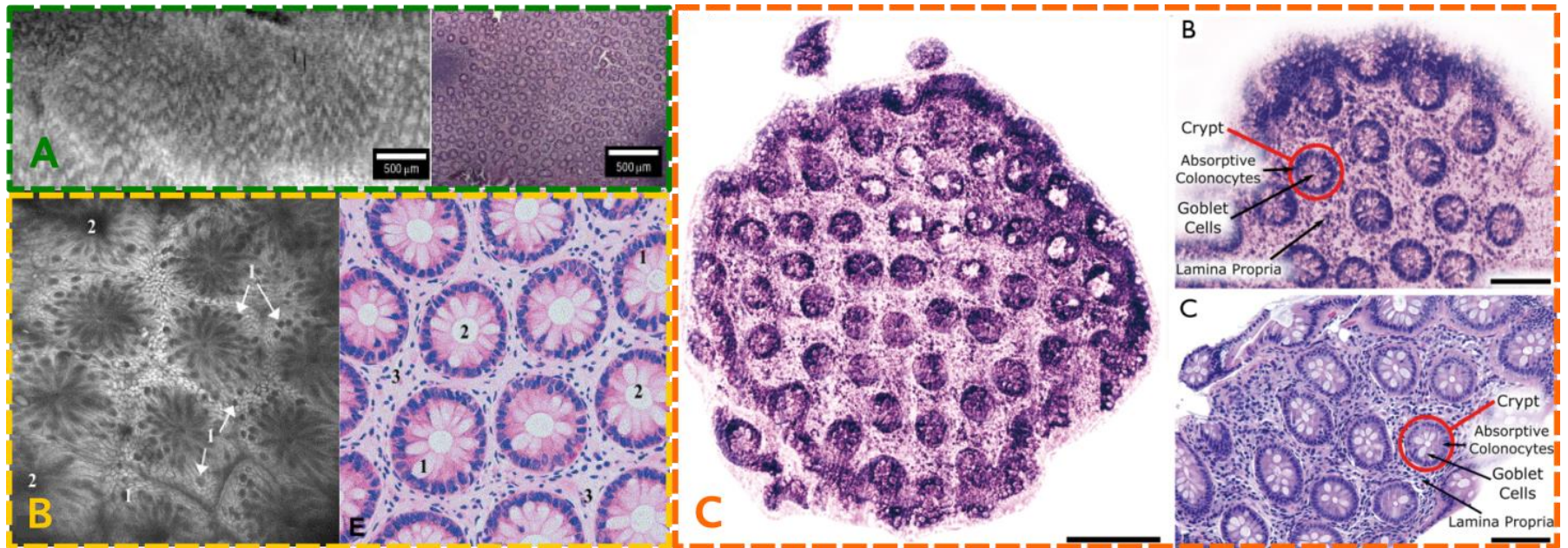


Figure 4: Comparison of images captured by various endomicroscopy technologies. A (green): En face image of ex vivo human colonic tissue sample⁴⁰. *Left* – OCT image averaged over the mucosal layer. *Right* - representative human colon histology with hematoxylin and eosin stain. B (yellow): Single scan confocal images (FOV = 500 × 500 μm) of the normal human colon (mucosal aspect)⁴¹. *Left* - Confocal image of descending colon mucosa. Topical application of acriflavine strongly stained the superficial cells only. *Right* - Tissue section cut parallel to the tissue surface for comparison with the en face view of the confocal image data. C (orange): Comparison of bright field H&E stained colonic mucosa and PARS images of unstained colonic mucosa⁴². *Left* - Brightfield H&E image of a normal colonic mucosa in sectioned formalin fixed paraffin embedded (FFPE) form. Scale bar: 100 μm; *Top Right* - wide field of view PARS image of unstained sectioned FFPE normal colonic mucosa biopsy. Scale bar: 200 μm; *Bottom right* - small field PARS image of unstained sectioned FFPE normal colonic mucosa biopsy. Scale bar: 100 μm. Artificial H&E-like coloring has been applied to the PARS images.

2.6.1 Scattering-based Endomicroscopy

Scattering-based endomicroscopy technologies have emerged as powerful tools for *in vivo* imaging, leveraging the interaction of light with tissue to generate high-resolution images. Techniques such as optical coherence tomography (OCT) and confocal microscopy (CM) are among the most well-established approaches. These modalities rely on the scattering properties of tissue to provide morphological information at microscopic scales, making them suitable for real-time diagnostic applications. The following paragraphs explore the principles, advancements, and clinical applications of OCT and confocal EM, highlighting their strengths and limitations in addressing the challenges of endoscopic imaging.

Confocal Microscopy

Confocal microscopy (CM) integrates confocal scanning microscopy with flexible endoscopy, offering insights into the morphology and function of tissues during clinical procedures. CM achieves cellular-level resolution, allowing clinicians to see tissue morphology and cellular structure. This capability has proven particularly valuable in the evaluation of gastrointestinal diseases, such as inflammatory bowel disease and celiac disease, where histological confirmation is crucial ^{43,44}. Its versatility even extends to applications in oncology, where it facilitates the early detection and differentiation of malignancies, including head and neck cancers ⁴⁵. Additionally, the portability and adaptability of CM systems have allowed their use across a range of clinical scenarios, from routine gastrointestinal endoscopy to specialized procedures for conditions such as graft-versus-host disease

⁴⁶.

Optical Coherence Tomography

Optical Coherence Tomography (OCT) has emerged as a prominent imaging modality for *in vivo* endoscopy, offering high-resolution visualization of biological tissues with greater depth and detail than traditional methods. OCT achieves micrometer-scale resolution and millimeter-scale imaging depth, making it particularly effective for real-time visualization of subsurface structures ^{2,3}. Recent advancements in nano-optic and micromotor endoscopic probes have further expanded the capabilities of OCT systems, enabling high-speed imaging applications in areas such as gastrointestinal and pulmonary diagnostics ^{47,48}. These innovations have enhanced the adaptability of OCT across a range of anatomical regions, including the gastrointestinal tract, airways, and vascular systems, demonstrating its versatility in diverse clinical scenarios ^{1,49}. Additionally, OCT has shown improved sensitivity and specificity in detecting neoplastic and pre-cancerous lesions, offering diagnostic accuracy that surpasses traditional imaging modalities ^{50,51}.

Limitations of scattering-based EM

Scattering-based endomicroscopy techniques, such as Confocal Endomicroscopy (CEM) and Optical Coherence Tomography (OCT), face several shared limitations that restrict their clinical utility. Scattering modalities suffer from relatively shallow imaging depths, with CEM being limited to superficial tissue layers and OCT capable of imaging only a few millimeters below the surface. This constraint hinders their applicability for visualizing larger or deeper structures ^{52,53}. In addition, these techniques often lack diagnostic sensitivity and specificity, necessitating multimodal integration with complementary imaging techniques, such as fluorescence microscopy, to provide additional structural and functional data ^{53,54}. This limits their utility in certain patient populations and for characterization for prevalent diseases. Furthermore, detailed images produced by these systems,

while valuable, often require a high level of training and experience to evaluate, which can slow down the diagnostic process and introduce variability in clinical outcomes ⁴.

2.6.2 ABSORPTION BASED EM

Absorption-based microscopy modalities utilize the absorption of light as they interact with a sample, providing contrast based on the absorption properties of the specific material or marker. This approach offers distinct advantages over scattering-based methods, as it directly measures the absorption of specific wavelengths, enhancing sensitivity to chemical composition, density variations, and molecular structures. Absorption-based techniques excel in applications where fine detail and contrast differentiation are critical, such as in detecting subtle changes in biological tissues, identifying material compositions, or visualizing nanoscale structures. By focusing on the intrinsic absorption characteristics of samples, absorption-based methods can achieve superior resolution and contrast in complex or heterogeneous environments, often enabling insights that scattering-based methods overlook. The following sections detail the prominent absorption-based technologies that have been explored in research and adapted for EM applications, outlining the major advantages and disadvantages for clinical use.

Fluorescence microscopy

Fluorescence microscopy (FM) in its various forms has become one of the dominant methodologies in biological imaging due to its single molecule selectivity using targeting labels and capability for high resolution imaging. By exploiting the specific excitation and emission properties of fluorescent dyes and markers, FM enables the targeted investigation of biological structures and

functions with high specificity and sensitivity. This has enabled a wide range of experimental observations, including molecular interactions in cells and tissues, as well as tissue morphology^{55,56}.

In endoscopic applications, FM provides detailed contrast of tissues, allowing for the characterization of pathologies that are challenging to distinguish using conventional imaging techniques and facilitating more precise diagnostics and intraoperative decision-making. Its integration with advanced optical systems, such as confocal microscopy, optical coherence tomography and photoacoustic microscopy has further expanded its versatility, allowing deep tissue imaging with minimal invasiveness^{57,58}. Fluorescence microscopy can provide molecular and functional insights, allowing clinicians to observe dynamic processes at cellular resolution. Its compatibility with various fluorophores, including targeted molecular probes, enhances diagnostic specificity and facilitates the visualization of specific biomarkers⁵⁹. Furthermore, FM's integration into endoscopic platforms has significantly improved its applicability, offering real-time feedback during procedures, such as in the identification of intramucosal bacteria in inflammatory bowel disease⁵⁷ and in fluorescence-guided cancer surgery⁶⁰.

Limitations of Fluorescence Microscopy

Despite these strengths, FM has inherent limitations. It often relies on external fluorescent dyes or probes, which can pose potential toxicity or immune risks⁶¹. Autofluorescence from endogenous tissue components can interfere with the detection of targeted signals, reducing clarity and specificity⁶². Moreover, the imaging depth of conventional FM is restricted, particularly in scattering tissues, though advancements in multiphoton techniques have partially mitigated this limitation⁴³.

Autofluorescence Microscopy

Autofluorescence microscopy (AFM) is an imaging technique that leverages naturally occurring fluorescence in biological tissues. AFM eliminates the need for external fluorescent markers, making it a non-invasive, label-free approach to imaging. AFM offers several advantages over FM, including reducing the risk of adverse reactions due to its ability to provide high-contrast imaging without the need for exogenous agents. This makes it advantageous for clinical applications where minimizing patient risk is paramount⁶³. Moreover, its reliance on intrinsic fluorescence allows for the detection of metabolic changes, which can serve as biomarkers for diseases like cancer or inflammatory conditions⁶⁴⁻⁶⁶.

Limitations of AFM

Despite its advantages, AFM is not without limitations. Autofluorescence on its own does not provide enough useful information to challenge the gold standard and enable biopsy. Regardless of its promising label-free, structural and metabolic imaging potential and minimal regulatory hurdles in *in vivo* applications, ubiquitous tissue autofluorescence is under-exploited owing to the phototoxicity associated with the high-power ultraviolet excitation needed for signal generation. Moreover, the natural autofluorescence of tissues can vary widely between individuals and tissue types, which may complicate signal interpretation⁶⁷. Autofluorescence signals are often weaker compared to those produced by external fluorescent dyes, leading to reduced sensitivity in some applications⁶⁸. Additionally, the shallow imaging depth of AFM limits its effectiveness for visualizing deeper tissue structures.

Non-linear Multiphoton

One strategy to overcome some of the major shortcomings of FM and AFM is to introduce non-linear imaging techniques. FM and AFM generate contrast from light-matter interactions, in which the elementary process involves a single photon, and which therefore depends linearly on the incident light intensity. Nonlinear techniques are fundamentally different in that they use 'higher-order' light-matter interactions involving multiple photons for contrast generation. The nonlinear nature of these interactions leads to qualitatively new imaging properties ⁶⁹.

The most widely used non-linear process in biological imaging is fluorescence excitation by two-photon absorption. Two photons arrive 'simultaneously' (within ~ 0.5 fs) at a molecule combine their energies to promote the molecule to an excited state, which then proceeds along the normal fluorescence-emission pathway ⁷⁰. This process occurs only at the focal point where photon densities are highest, minimizing out-of-focus excitation and photodamage. Two-photon microscopy is particularly effective for imaging tissues at depths of up to 500–800 μm , surpassing the capabilities of traditional fluorescence microscopy due to its use of near-infrared (NIR) light, which penetrates deeper into tissues with reduced scattering and absorption ⁶⁹.

Similarly, three (or more) photons can combine to cause excitation. Three photon absorption has been leveraged to achieve significant improvements in excitation localization (leading to higher resolutions deeper in tissue), increasing the signal to background ratio and improving the axial sectioning capability over two-photon microscopy (2PM) ⁷¹. Three-photon microscopy (3PM) outperforms 2PM in depth penetration, particularly in highly scattering tissues, by achieving better signal-to-noise ratios at greater depths. While 2PM typically reaches depths up to 500–800 μm , 3PM extends this limit beyond 1 mm ⁷².

Non-linear, multiphoton fluorescence techniques have been miniaturized to create endomicroscopy devices and have produced promising results. Unsurprisingly, the most promising and successful of these implementations has been two-photon endomicroscopy (2PEM), which has been successfully utilized in a range of applications including for cancer detection to identify and characterize malignant lesions ⁷³, for detection of early-stage abnormalities in the GI tract ⁷⁴ and for imaging of neural and vascular networks *in vivo* ⁶⁹.

Limitations of non-linear multiphoton

Non-linear, multiphoton endoscopy techniques face several limitations that hinder their broader clinical adoption. A major drawback is the complexity and cost of the required instrumentation; these methods rely on femtosecond pulsed lasers that are expensive and difficult to maintain, creating barriers to widespread use in resource-constrained settings ^{69,73–76}. Additionally, the bulky nature of non-linear systems complicates their integration into compact, portable endoscopic devices, making them less practical for routine procedures compared to simpler linear imaging techniques ⁷². Another limitation is the relatively slow imaging speed, as the need for precise beam scanning and photon detection results in slower image acquisition rates, which is not ideal for real-time diagnostics ⁷⁷. These techniques also depend on external fluorescent markers to achieve adequate signal contrast and continue to face issues of localized phototoxicity and photobleaching with prolonged imaging.

Photoacoustic Microscopy

Photoacoustic Microscopy (PAM) combines the principles of optical imaging and ultrasound detection, providing high-resolution imaging based on the optical absorption properties of tissues. This hybrid technique uses short laser pulses to induce thermoelastic expansion in tissues, which

generates ultrasonic waves detected by transducers. PAM is increasingly integrated into endoscopic platforms for *in vivo* imaging, offering unique insights into structural, functional, and molecular tissue characteristics.

PAM excels in high-resolution imaging of vascular structures, oxygen saturation, and other functional biomarkers deep within tissues, surpassing many conventional optical methods ⁷⁸. Its ability to differentiate tissue types based on optical absorption contrasts, such as hemoglobin concentration, makes it invaluable for cancer diagnosis, cardiovascular assessments, and monitoring metabolic activities ⁷⁹. Unlike fluorescence-based systems, PAM does not require exogenous labels, relying instead on endogenous chromophores like hemoglobin and melanin for imaging. Its label-free imaging approach eliminates concerns related to photobleaching and toxicity, which are challenges in fluorescence microscopy. Additionally, the combination of optical and acoustic resolution extends its effective imaging depth compared to traditional optical microscopy. The integration of PAM with endoscopy has expanded its clinical applications, enabling minimally invasive imaging of internal organs, including the gastrointestinal tract, bladder, and lungs ^{80,81,81,82}. Studies have demonstrated its capability to achieve subcellular resolution *in vivo*, which is critical for detecting early pathological changes ⁸³.

Limitations of PAM

PAM faces several limitations for endoscopic imaging. The biggest challenge is miniaturization – the integration of PAM into endoscopic devices requires complex miniaturization of laser sources and ultrasound detectors, increasing system cost and complexity ⁸². Furthermore, the reliance on acoustic signal detection limits its resolution compared to purely optical methods like confocal or two-photon microscopy. Lastly, PAM's imaging depth, while superior to fluorescence-based systems, is constrained to a few millimeters due to light scattering and acoustic attenuation in tissues.

Photon Absorption Remote Sensing

Photon absorption remote sensing (PARS) is a new and exciting modality that has shown great potential in biomedical applications. In PARS, a short-pulsed excitation laser (on the order of nanoseconds or shorter) is incident on a sample that is absorbing at that wavelength. The absorption of excitation elicits a combined thermal and pressure perturbation in the exposed volume, generating a corresponding modulation in the local optical properties (see *figure 5*). A secondary probe beam co-focused with the excitation beam captures the absorption induced modulations as changes in backscattering intensity. These backscatter modulations are directly correlated to the local non-radiative absorption contrast^{84,85}. Unlike photoacoustic methods, rather than relying on the response to propagate through the sample before detection, the PARS mechanism instantaneously detects the induced modulations at the excited location⁸⁵. As a result, PARS offers non-contact operation, facilitating imaging biological regions which would otherwise be impractical to image with contact-based photoacoustic methods. Previously, PARS has demonstrated label-free absorption contrast of hemoglobin, DNA, RNA, lipids, and cytochromes, in specimens such as chicken embryo models, resected tissue specimens, and live retina and murine models, as well as functional imaging of oxygenation saturation in tissue^{21,23,24,86,87}.

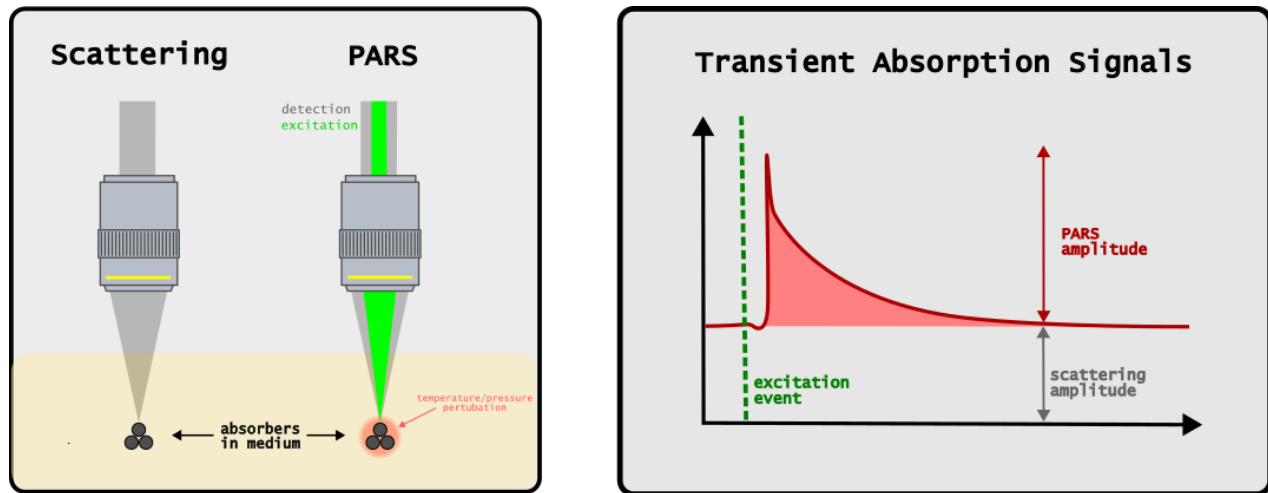


Figure 5: Illustration of the PARS mechanism showing the perturbation of a continuous wave scattering beam as a pulse of light interacts with an absorber. *Left*: illustration of event; *Right*: transient signals measured from scattering beam

PARS represents a promising diagnostic tool due to its versatility in imaging a wide range of tissue and cellular structures using absorption contrast. This makes it ideal for enabling virtual biopsies by providing high specificity in the characterization of malignant tissues. Its all-optical signal generation and detection scheme offers significant advantages for endomicroscopy applications, offering better compatibility with current widefield endoscopy technologies compared to photoacoustic devices. This work marks the first endeavor to adapt the PARS mechanism for endoscopic applications, with an initial focus on imaging blood vasculature *in vivo*. The research presented in this work paves the way for further miniaturization of PARS microscopy systems, allowing for applications such as widefield- and needle- compatible endomicroscopy, and addressing the critical need for virtual biopsy techniques in clinical settings.

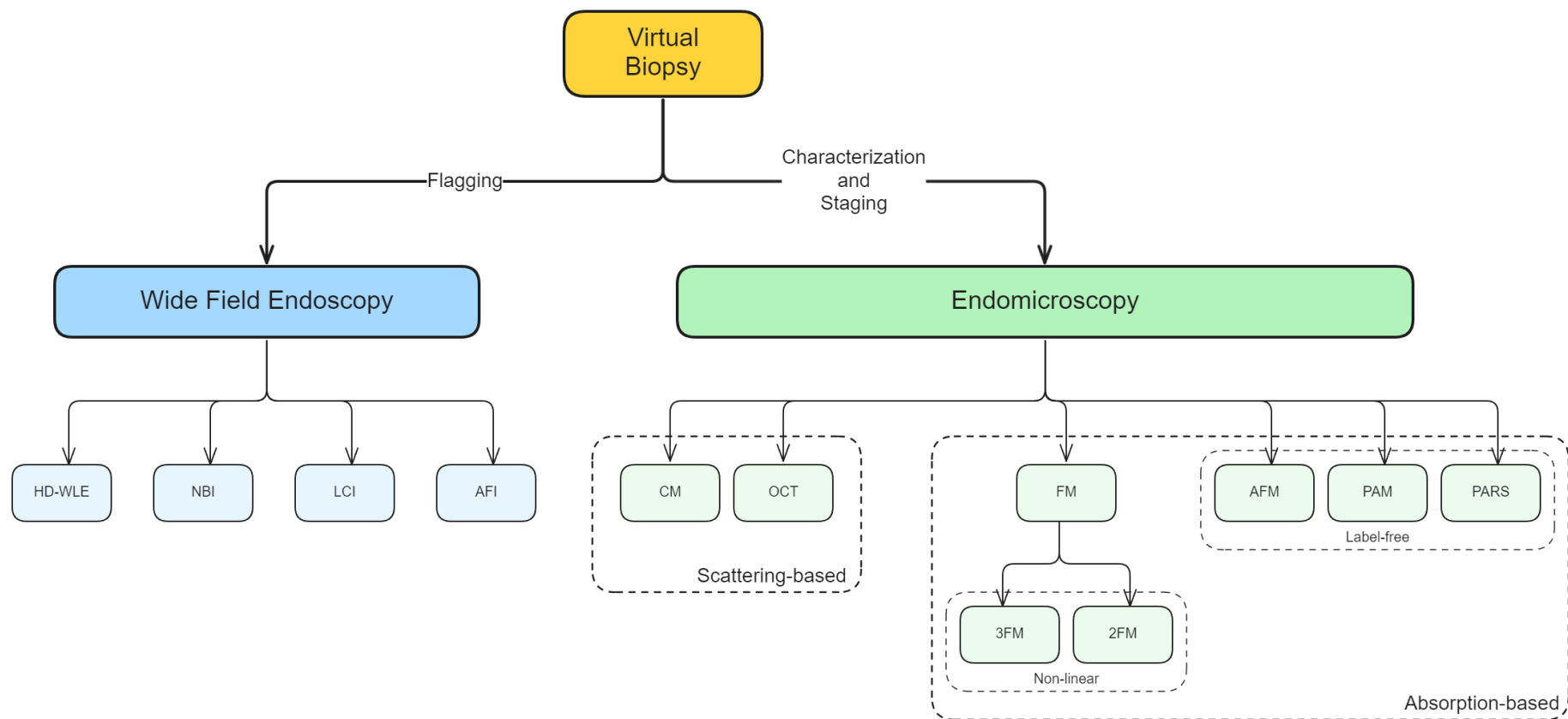


Figure 6: Enabling endoscopic technologies for virtual biopsies, highlighting the major scattering-based and absorption-based technologies, including label-free modalities

2.7 Summary

There remains an increasing demand for effective early-stage disease detection, particularly for gastrointestinal cancers, that has driven the development of minimally invasive imaging solutions that enhance the safety and diagnostic yield of biopsy procedures. Endomicroscopy technologies address this need by enabling *in situ* imaging of tissues with cellular or subcellular resolution and offering real-time diagnostic capabilities during invasive clinical procedures. This has created a demand for practical endomicroscopy techniques that can provide comprehensive diagnostic information.

PARS has shown promise as an effective diagnostic tool due to its versatility in imaging a wide range of tissue and cellular structures using absorption contrast. A PARS endomicroscopy device can enable *in situ* imaging of tissues with cellular or subcellular resolution, offering real-time diagnostic capabilities during invasive clinical procedures. Despite successful demonstrations of PARS in benchtop configurations, its translation to a miniaturized endoscopic form factor remains unrealized, limiting its clinical applicability, representing a major gap.

PARS requires a sophisticated signal generation and detection scheme that relies on high imaging performance across the entire FOV. Successful implementation relies on developing a system design that is optimized for excellent chromatic performance, high transmission efficiencies, and high image resolution, to achieve the required performance for adequate structural and functional image acquisition. This presents unique challenges for the endoscopic form factor – the device requires flexible and efficient multiwavelength light delivery, an achromatic distal objective with high collection efficiency, and sufficient intensity damage threshold to handle the required optical densities for PARS signal generation. These challenges must be met before a clinically viable PARS endomicroscopy device is realized.

The work in the following sections lays the foundation for PARS miniaturization. This prototype is designed to target microvasculature and be compatible with modern, widefield endoscopes. As

such, the prototype must be compact, forward viewing, and non-contact, as well as biocompatible and water resistant. The prototype device is evaluated for performance using representative carbon fibre phantoms to simulate performance on microvasculature.

Below is a table that includes quantified metrics that will be used to evaluate the PARS endomicroscope prototype designs.

Metric	Target	Driving Constraint
SNR	>40dB	Generally accepted criteria for excellent SNR for reliable quantification in biomedical imaging ⁸⁸
Resolution	<2 μ m	To target capillaries which range from 5-10 μ m in diameter ⁸⁹

Table 1: Driving performance metrics for PARS EM prototype design

3 Design and Methods

3.1 Design of an EM device

It is important to lay out the functional distinctions between endomicroscopy (EM) and traditional wide-field (WF) endoscopy, to put into context the utility and constraints of EM devices. EM can be thought of as an add on technology than complements WF endoscopy's flagging capabilities to acquire information beyond what is achievable with WF endoscopy alone. Endomicroscopes stand in contrast to WF endoscopes by possessing capabilities that exceed that of WF endoscopy, providing complementary information that can lead to improved clinical outcomes. Regardless of the distinctions, endoscopes in general work on similar principles, and most endoscope designs can be analyzed using a simple functional breakdown (*Figure 7*). There are many configurations of EM devices, a list that continually grows with imagination. For the purposes of this research, I will limit the scope of analysis of endoscope designs to devices that are all-optical, non-contact, and forward-viewing. The general endomicroscopy design can be decomposed based on functionality. Functional capabilities of EM are contrasted against WF endoscopy in the following sections to highlight the advantages of EM and limitations of current WF endoscopy in clinical application.

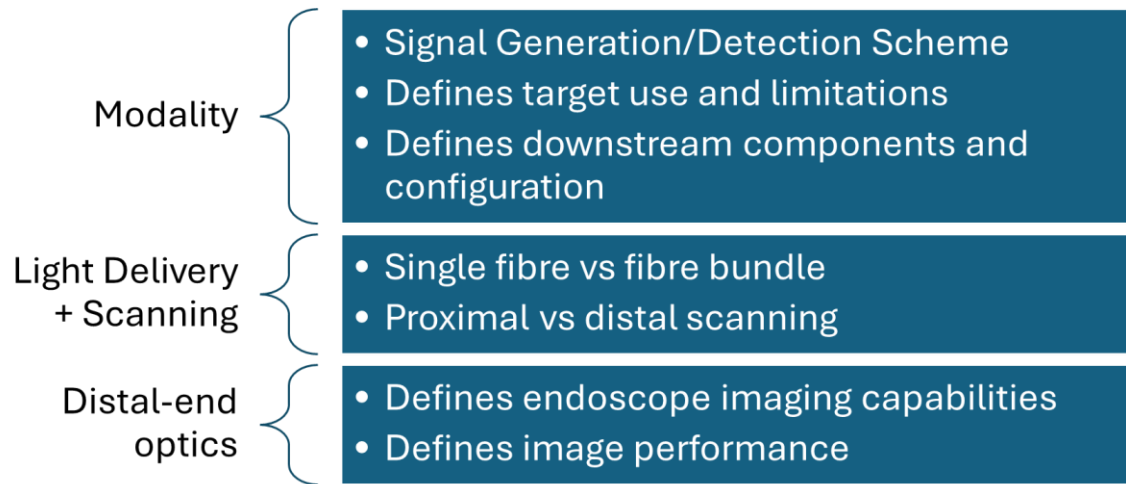


Figure 7: All-optical, forward-viewing, non-contact endoscope breakdown

3.1.1 Modality

The method by which an endoscope generates information (i.e. contrast mechanism) is what is referred to as modality. This is the basic working principle that results in signal generation and defines the clinical use and functional limitations of the device. Regarding the design of an endoscopic device, imaging modality significantly influences the component selection and overall conceptual design of an endoscopic device. Aspects such as design wavelength(s), light delivery component selection and distal-end design is largely constrained by the selected imaging modality.

WF vs. EM: WF endoscopy utilizes color contrast to flag abnormalities within otherwise healthy tissue. This contrast mechanism requires a broadband light source, thus requiring all downstream components to be compatible with a broadband detection scheme (broadband optical components, chromatic aberration correction ect.). On the other hand, EM devices typically rely on specialized signal generation and detection schemes, which utilize a select few wavelengths to provide tissue discrimination capabilities, providing additional information beyond WF capabilities.

3.1.2 Light delivery and scanning mechanism

Endoscope devices must address a fundamental challenge: maintaining efficient light delivery while achieving the flexibility needed to navigate narrow body cavities. Signal losses can occur at every bend and interface in the optical path, making the preservation of light transmission efficiency particularly challenging in flexible designs. In addition, a mechanism for image formation must be implemented that is compatible with the light delivery method of choice, adding another layer of complexity to the design. These competing requirements of flexibility, signal preservation, and imaging capability form the basis of design constraints for light delivery and scanning mechanisms in endoscopes. The most common approach to overcoming these constraints is through the use of image guide fibres (IGFs), which are coherent fiber bundles capable of transmitting high-resolution images over long distances while maintaining acceptable signal losses even when flexed. These fibers are used in both medical and industrial applications to effectively guide and transmit images while maintaining signal integrity. IGFs are the most utilized light delivery and image formation scheme in modern endoscope design, a fact that is reflected in its common implementation by major endoscope manufacturers⁹⁰. In contrast to IGFs, single fibres can be utilized to achieve light delivery and image formation. Single fibre implementations transmit light through a single channel and use sophisticated distal scanning mechanisms to raster scan ROIs and interpolate images from point data^{76,81,91–93}. To maintain small footprints, scanning is most often achieved by small-scale piezoelectric scanners.

WF vs. EM: Given the simplicity and ease of integration, IGFs are used ubiquitously in WF endoscopy. WF endoscope systems often compromise resolution for larger field of views (FOVs), longer depth-of-fields (DOFs) and higher video rates by employing optical systems that acquire entire ROIs frame by frame, and as such, generally do not require scanning mechanisms to construct an image. Spatial discretization is generally handled by the image array of a camera either at the distal

end or the proximal end, with the latter requiring a light delivery method to transmit information across the endoscope. Given the advantages of IGFs for use in WF endoscopy, single fibre implementations are virtually unheard of. On the other hand, EM implementations often require tight focus requirements and high-resolution image formation, and are accompanied by narrower FOVs, and shallower DOFs. These factors require the ROI to be confocally raster scanned to form images. As a result, EM implementations typically require a scanning mechanism, which is determined by the light delivery component: IGFs are coupled with proximal scanning, while single fibres are coupled with distal scanning. Single fibre light delivery and distal scanning has shown to be highly effective for EM applications. This arrangement greatly simplifies coupling and light transmission, and the fibre core can be uniquely tailored to the modality requirements of the system, which helps improve signal fidelity. IGFs can also provide the means for high resolution scanning by raster scanning the proximal face of the array. Each fibre (or group of fibres) within the array can be accessed sequentially, satisfying the confocal (or quasi-confocal) condition necessary for high resolution microscopy.

3.1.3 Distal end optics

The distal end optics of an endoscope ultimately determines the imaging capabilities and image performance. Apart from the metrics of interest previously discussed (FOV, DOF, resolution), efforts must be made to reduce optical aberration, optimize transmission efficiency and maximize light gathering, all of which can be optimized at the level of distal optic design. One of the main challenges in designing distal end optics for endoscopes is maintaining a small footprint without sacrificing imaging performance. In addition, materials used must be biocompatible and optics must be configured to be water resistant.

WF vs. EM: To improve the flagging capabilities of WF endoscopes, distal optical systems are designed to maximize FOV and DOF at the expense of resolution. Systems are also designed to reduce chromatic aberrations since broadband white light is generally used for illumination. Wide FOV, high f-number compound lens systems are generally sufficient for use in WF endoscope devices. In contrast, as discussed previously, endomicroscopy aims to maximize resolution and distal optical systems are designed to do so at the expense of FOV and DOF. Furthermore, design constraints for EM devices require appreciably smaller distal footprints for integration alongside WF endoscopes. This presents a challenge: light gathering ability is inversely proportional to f-number. Small diameters require proportionally small focal lengths, resulting in short working distances and high NA devices being leveraged for EM. These factors make the design, fabrication and successful application of distal end optical systems for EM difficult to realize. Given these challenges, graded index (GRIN) lenses make suitable components for distal end optical lens design, and this suitability is reflected in literature [2]. GRIN optics allow the design of inexpensive optical systems that combine high optical quality with compactness and simplicity. To add, cylindrical GRIN lenses have an ideal form factor for alignment with other cylindrical optical components such as IGFs and combine well with compound microlens systems for aberration control.

3.1.4 Functional requirements and design constraints for PARS EM

The development of a PARS endomicroscopy system is considered a significant advancement in medical imaging technology. In this approach, the high-resolution capabilities of optical microscopy are combined with the non-contact, label-free advantages of photoacoustic imaging. The design of such a system must be carefully balanced to achieve optimal performance while meeting the stringent requirements of clinical use. In this section, the key functional requirements and design

constraints that guide the development of the PARS endomicroscopy system are outlined. These considerations are derived from the need for compatibility with existing widefield endoscopy techniques, the specific demands of the PARS mechanism, and the technical challenges of creating a tool capable of providing high-quality, *in vivo* imaging of blood vasculature and tissue microstructures.

Below is a summarized table of the PARS EM system requirements:

Category	Requirement	Description
Compatibility with WF Endoscopy	Forward Viewing	Enables viewing in the direction of insertion
	Non-contact	Allows imaging without direct tissue contact
	All-optical	Drives compact size, flexibility and straightforward integration
	Small Footprint	Outer diameter < 2mm to fit into instrument channel
PARS Mechanism	Target Blood Vasculature	Excitation wavelength aligns with hemoglobin absorption peak
Light delivery	Multispectral	Capable of transmitting multiple wavelengths.
Distal optics	Finite-Finite Conjugate	Fixed magnification. Object and image plane at finite distance.
	Achromatic Objective Lens	Optimized for multiple wavelengths
Image Quality	High Resolution	1-2 μ m resolution for detailed tissue imaging and cellular-level visualization
	High SNR	SNR >15dB. Enables contrasts beyond that achievable by WF. Increases efficiency, reduces likelihood of tissue damage.
Biocompatibility	Biocompatible	Safe for use in biological tissues

Category	Requirement	Description
	Water-resistant	Protected against fluids during use

Table 2: PARS Endomicroscopy System Requirements Summary

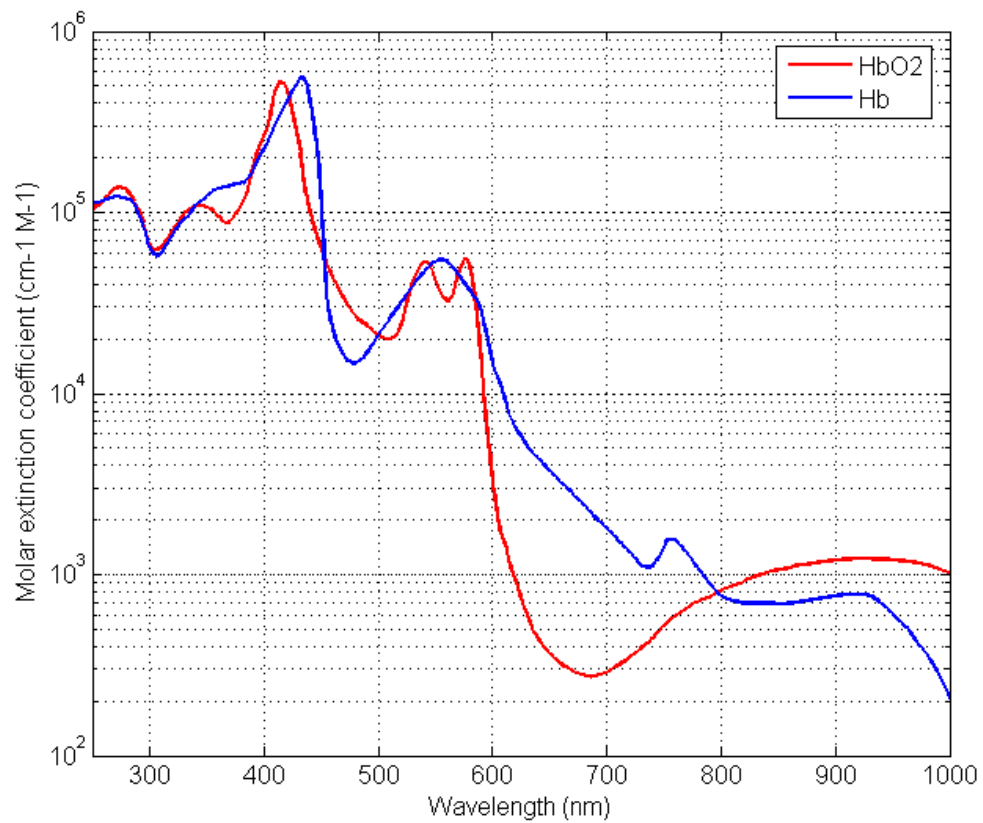


Figure 8: Absorption spectra of oxygenated (HbO₂) and deoxygenated (Hb) hemoglobin

3.2 PARS EM Concepts

3.2.1 PARS EM Design Concepts

While the chosen modality significantly influences the high-level design and requirements of a PARS endomicroscopy (EM) device, the light delivery and scanning mechanism are the primary drivers of the overall concept. These elements represent the core structural components of the design and ultimately determine the image acquisition process.

Two main concepts were considered for the development of a PARS EM device:

1. Image Guide Fiber (IGF) with Proximal Scanning:

- Utilizes a bundle of optical fibers
- Proximal end is raster scanned
- Spatial information is preserved through the IGF array
- Requires a high-performance distal objective lens
- Objective lens requirements: high resolution, achromatic performance, minimal aberrations across the image plane

2. Double-Clad Fiber (DCF) with Distal Scanning:

- Single fiber implementation
- Consists of inner core, inner cladding, and outer core
- Requires a miniaturized distal scanning mechanism
- Often utilizes a piezoelectric element, with the distal end of the fiber acting as a cantilever for scanning
- Allows for application-specific optimization of fiber design

Both concepts must address the PARS requirement for exciting and interrogating a small volume at a time, necessitating a raster scanning approach. The choice between these concepts involves trade-offs in design complexity, image quality, and system miniaturization. Successful

implementations of both approaches have been demonstrated in literature, with each offering unique advantages and challenges in the context of PARS endomicroscopy.

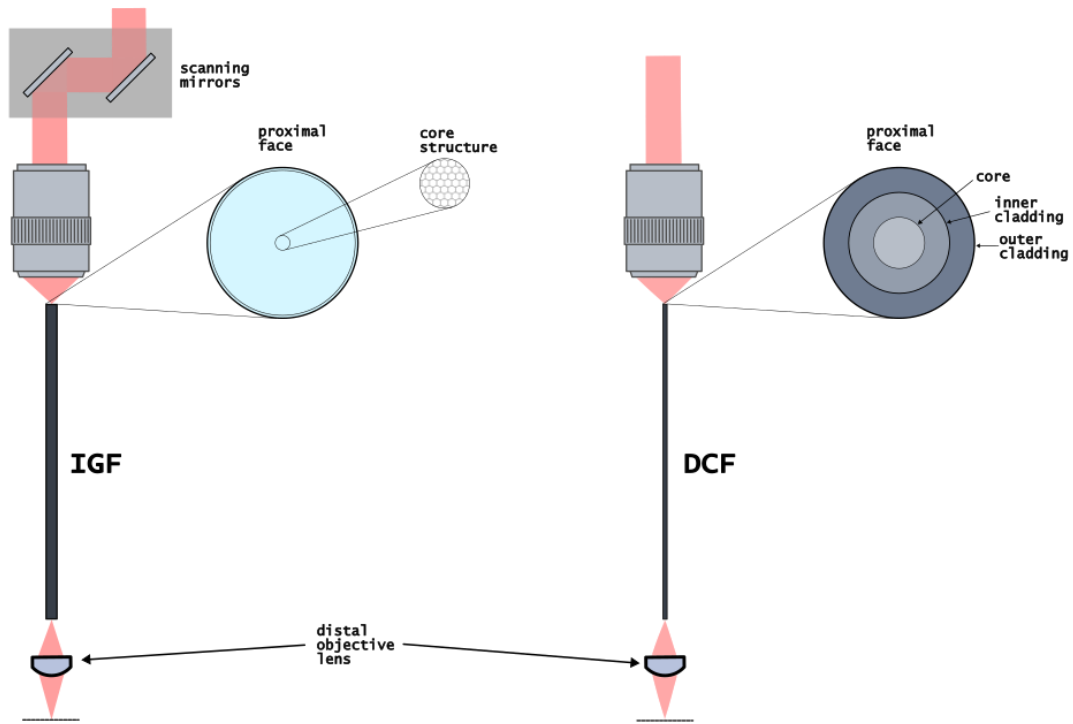


Figure 9: Comparison of Image Guide Fiber (IGF) and Double-Clad Fiber (DCF) configurations in endoscopic systems. The IGF's multiple core structure and the DCF's concentric core-cladding arrangement are highlighted in cross-sectional views.

3.2.2 Comparison of IGF and DCF for PARS EM

Following the overview of the two main concepts for PARS EM design, we now examine the advantages and disadvantages of each approach in detail.

Image Guide Fiber (IGF)

In evaluating the two main concepts for PARS EM design, the Image Guide Fiber (IGF) and Double-Clad Fiber (DCF) approaches each present unique advantages and challenges. The IGF, leveraging mature fiber bundle technology, offers a relatively simple implementation with high-resolution imaging capabilities. Its multiple fibers transmit individual pixels, enabling detailed imaging within a compact design suitable for endoscopic applications. The ability to directly couple the distal end to objective lenses using optical adhesives further simplifies the design. Additionally, IGFs demonstrate robustness through minimal heat generation, good biocompatibility, and water resistance. However, IGFs are not without drawbacks. They are more prone to breakage than single fibers, with repairs often requiring costly full replacements. The proximal scanning method, necessary for image formation, risks damage due to scanning the light-insensitive cladding. IGFs also face limitations in chromatic performance and maximum resolution, the latter being determined by fiber spacing and arrangement. Precise alignment of the entire fiber array presents another challenge, affecting coupling efficiency and overall performance.

Double-clad Fiber (DCF)

On the other hand, the DCF approach offers superior versatility through its dual-core design, allowing optimization for specific wavelengths and applications. This configuration enhances signal-to-noise ratio by separating light delivery and collection paths, while also improving collection efficiency, particularly beneficial for imaging in diffuse media. DCFs generally have a higher damage threshold during scanning due to their single-core design. However, the DCF approach introduces significant design complexity, requiring a miniaturized distal scanning mechanism. This necessity not only increases overall complexity but also poses challenges in maintaining a small footprint due to the

supporting components required for the scanning mechanism. The development process for a DCF-based system is more demanding, requiring optimization of both the scanning technique and objective lens design. Unlike IGFs, DCFs cannot be directly coupled to objective lenses, potentially introducing parasitic reflections and reducing efficiency. Maintaining water-resistance and biocompatibility with moving parts presents additional challenges in DCF implementations.

3.2.3 Qualitative Risk Assessment

Below is a summary of the advantages and disadvantages of IGFs and DCF designs for PARS EM.

Image Guide Fiber (IGF)

Advantages:

- Simple implementation: Mature technology with well-established manufacturing processes.
- High-resolution imaging: Multiple fibers transmit individual pixels, enabling detailed imaging.
- Compact design: Small footprint suitable for endoscopic applications.
- Direct coupling: Distal end can be directly coupled to objective lenses using optical adhesives.
- Robustness: Minimal heat generation, good biocompatibility, and water resistance.

Disadvantages:

- Fragility: More prone to breakage than single fibers, with costly repairs often requiring full replacement.

- Damage risk: Proximal scanning involves scanning the cladding, which risks damage due to energy absorption in the cladding layer, resulting in fiber degradation.
- Limited chromatic performance: Designed for broadband light, potentially limiting performance at specific wavelengths.
- Resolution limitations: Maximum resolution determined by fiber spacing and arrangement.
- Alignment challenges: Efficient coupling requires precise alignment of the entire fiber array.

Double-Clad Fiber (DCF)

Advantages:

- Versatility: Dual-core design allows optimization for specific wavelengths and applications.
- Improved efficiency: Separate light delivery and collection paths enhance signal-to-noise ratio.
- Higher damage threshold: Single-core design reduces risk of damage during scanning.
- Enhanced collection efficiency: Outer cladding collects backscattered radiation, improving imaging in diffuse media.
- Potential for advanced functionality: Can be combined with DCF couplers for more complex diagnostic capabilities.

Disadvantages:

- Design complexity: Requires a miniaturized distal scanning mechanism, increasing overall complexity.

- Size constraints: Supporting components for the scanning mechanism can increase device footprint.
- Development challenges: Necessitates optimization of both scanning technique and objective lens design.
- Coupling inefficiencies: Cannot be directly coupled to objective lenses, potentially introducing parasitic reflections.
- Environmental protection: Maintaining water-resistance and biocompatibility with moving parts is challenging.

To summarize the comparison, a qualitative risk matrix has been developed:

Metric	Qualitative Risk Assessment	
	IGF Risk Level	DCF Risk Level
SNR	Med	Med
Efficiency	Med	Med
Design Complexity	Low	High
Design Flexibility	Med	Low
Damage Threshold	High	Low
Image Quality	Low	Med
Footprint	Low	Med

Table 3: Summary of risk assessment comparing the IGF and DCF designs for PARS EM

This matrix highlights the trade-offs between the two approaches, with IGF offering simplicity and robustness at the cost of some performance limitations, while DCF provides potential for higher performance but with increased complexity and design challenges.

3.2.4 Selection of IGF Approach for PARS EM Prototype

After careful consideration of the advantages, disadvantages, and associated risks of both IGF and DCF approaches, the Image Guide Fiber (IGF) was ultimately selected for the PARS EM prototype. This decision was based on several key factors:

Design Simplicity: The IGF approach offers a less complex design, facilitating easier integration with existing PARS benchtop systems. Its inherent flexibility and biocompatibility align well with endoscopic applications.

Optical Coupling Efficiency: The flat distal face of the IGF allows for straightforward coupling with distal optics, including GRIN lenses. This coupling efficiency helps mitigate risks associated with SNR, overall system efficiency, and damage thresholds.

Compatibility with Existing Endoscopes: The small footprint of the IGF system ensures compatibility with standard widefield endoscope working channels.

Image Quality Preservation: IGFs are designed to transfer images from the proximal to distal end with minimal degradation. The structure of IGFs, including core spacing and the number of cores, can be optimized for the high-resolution imaging required in endomicroscopy.

Risk Mitigation: While the IGF approach has its challenges, particularly in terms of damage threshold and chromatic performance, these risks were deemed more manageable compared to the complexity introduced by the DCF approach.

This selection balances the need for high-quality imaging with practical considerations of design simplicity and compatibility with existing systems, positioning the IGF-based PARS EM prototype as a promising platform for further development and testing. To help derisk the IGF concept, a derisking experiment was designed to show proof-of-concept (POC) before moving forward to the prototype design.

3.3 Proof-of-concept (POC) experiments

Following the selection of the Image Guide Fiber (IGF) approach for the PARS EM prototype, a series of proof-of-concept (POC) experiments were designed and conducted. These feasibility studies aimed to systematically de-risk the PARS-IGF concept and validate its potential for endomicroscopy applications. To maintain practicality and cost-effectiveness, the experimental designs utilized an off-the-shelf IGF bundle combined with readily available optical components. This approach allowed for rapid prototyping and iterative testing while closely mimicking the intended final design.

3.3.1 IGF Specifications

For the POC experiments and the subsequent prototype design, the FIGH-30-650S fibre bundle from Fujikura was used ⁹⁴. See below the specifications of the fibre bundle.

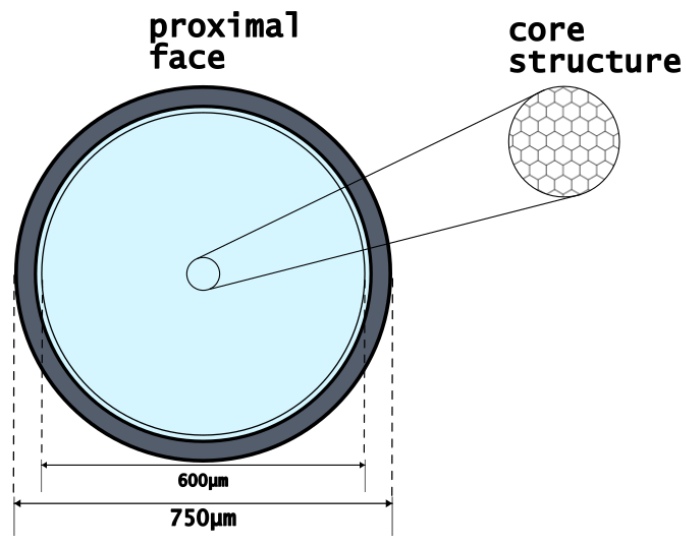


Figure 10: Simplified diagram of the proximal face of the IGF illustrating core structure and cross-sectional dimensions of fibre

Parameter	Value
Fibre length	2m
number of cores	30,000
Core size	~2µm
Core spacing	~3.5µm
Image circle diameter	600µm
Outer diameter	750µm

Table 4: Summary table showing specifications of IGF used in POC and prototype work

By utilizing this specific IGF configuration, the performance capabilities and limitations of the PARS-IGF concept under realistic conditions could be assessed.

3.3.2 POC Roadmap

The main goal of the POC experiments was to gain insights that would inform the subsequent development of the full prototype. Carbon fibres were used as a blood vasculature phantom in experiments due to its high absorption and similar size. A simple roadmap was developed to guide the progression of experiments. Each experiment provides more confidence for the next experiments by derisking some aspect of the conceptual design. The roadmap consists of four key experiments:

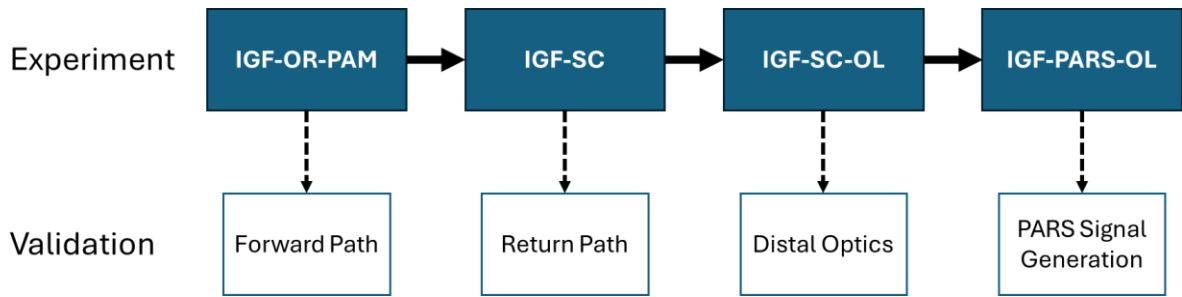


Figure 11: Proof-of-concept roadmap

IGF-OR-PAM: Validates the optical forward path of the system and ensures that the IGF has the capacity to propagate the light energy necessary to generate photoacoustic signals. These signals are detected by a traditional OR-PAM setup that utilizes a piezoelectric transducer.

IGF-SC: Validates the optical return path of the IGF. Ensures that the IGF design can generate a scattering image, which requires light to be transmitted and collected through the IGF. This builds on the previous experiment and validates the detection path in the PARS signal generation scheme.

IGF-SC-OL: Validates and helps determine the requirements for the distal optics. Gives indication of expected imaging performance and helps to understand the different parameters that affect image quality.

IGF-PARS-OL: Validates the full PARS signal generation. Combines the lessons learned from the previous experiments to generate PARS signal. Integrates excitation and detection schemes and helps to understand the parameters affecting efficient PARS generation using the IGF, including coalignment and achromatic performance as well design constraints and path to optimization.

Each step in this progression builds upon the previous one, systematically validating different aspects of the PARS endomicroscopy design.

3.3.3 POC Experimental Designs and Methodologies

IGF-OR-PAM System design and methodology

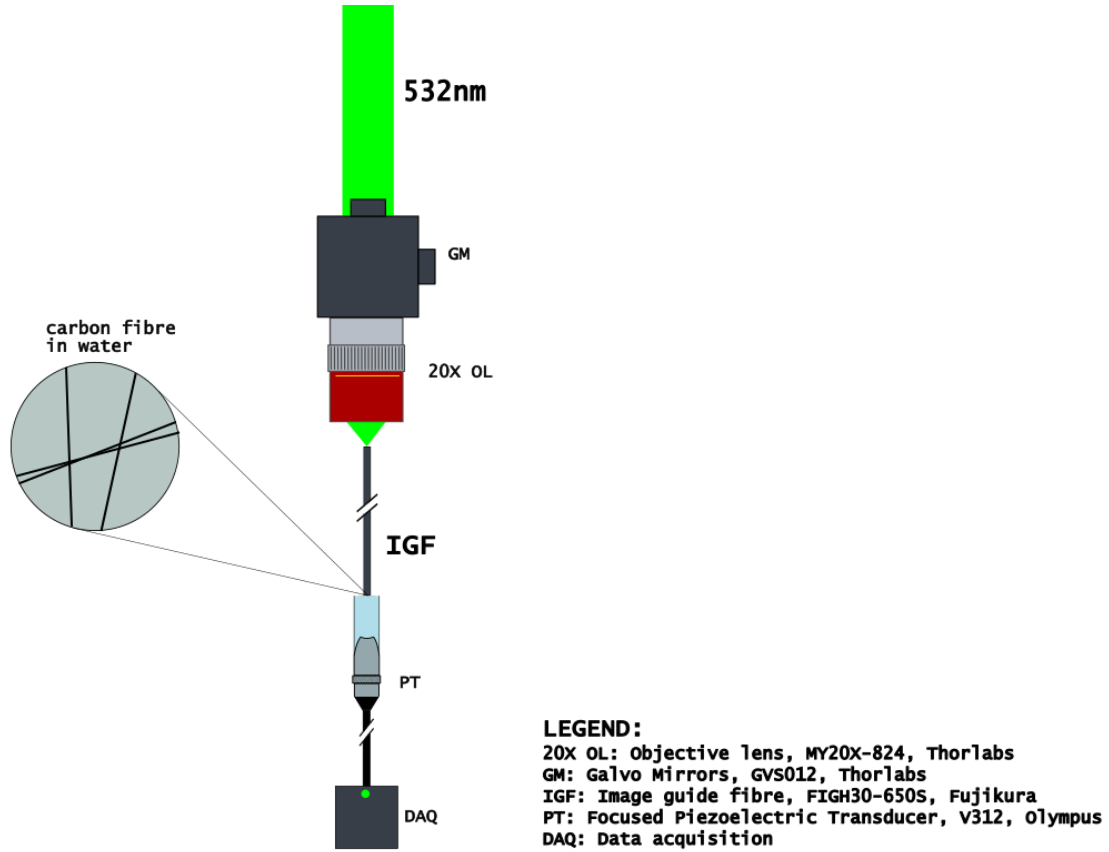


Figure 12: IGF-OR-PAM System Design

This experiment aims to validate the forward optical path of the IGF concept using the proposed excitation wavelength. As shown in *Figure 13*, the design elements include:

- 532nm excitation wavelength source, intended for use in the final system to target blood vasculature

- Galvo mirror (GM) for scanning, allowing testing of coupling across the fiber area and identify and derisking any coupling challenges
- 20X objective lens with high NA and small spot size to ensure efficient coupling
- Piezoelectric transducer (PT) for detection of generated photoacoustic waves, with a central frequency of 10MHz and a focal length of 1 inch
- Data acquisition module (DAQ) to acquire and analyze signals from the PT

Methodology:

1. Align the excitation beam on the benchtop to maximize coupling efficiency in fiber cores across the IGF.
2. Suspend carbon fibers in a small water bath at the focal distance of the focused transducer.
3. Scan the excitation beam across the proximal face of the IGF using the galvo mirrors.
4. Acquire signals from the transducer using the DAQ for evaluation.

IGF-SC System design and methodology

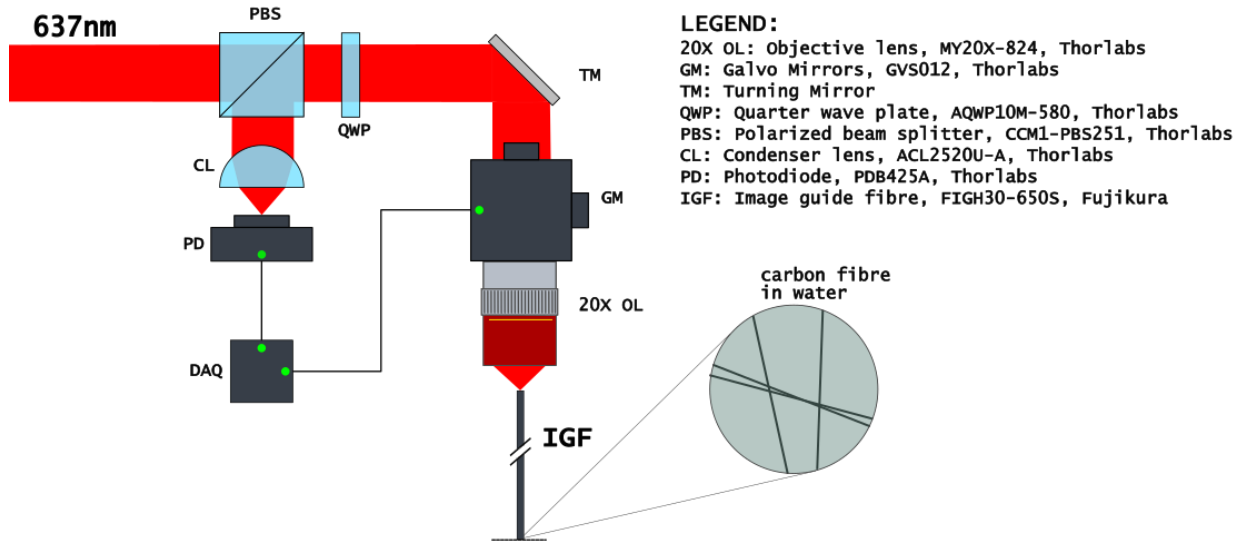


Figure 13: IGF-SC System Design

This experiment aims to derisk the return path for the IGF concept using a detection wavelength. As illustrated in *figure 14*, the experimental setup includes:

- 632nm detection source, chosen to be close to the excitation wavelength to mitigate chromatic effects along the optical path
- Galvo mirror (GM) for scanning, revealing unknowns with image acquisition including optimal scanning patterns and frequencies for spatial sampling
- PBS + QW plate forming an optical isolator to separate forward and backward paths while rejecting parasitic reflections from the proximal face to increase SNR

- Condenser lens (CL), photodiode (PD), and DAQ for collection of backscatter and data acquisition

Methodology:

1. Align the detection beam on the benchtop to maximize coupling efficiency in fiber cores across the IGF.
2. Place carbon fibers in a small amount of water on a glass slide.
3. Scan the detection beam using an optimized pattern across the proximal face of the IGF using the galvo mirrors.
4. Acquire signals from the GM and PD using the DAQ and construct images.

IGF-SC-OL System design and methodology

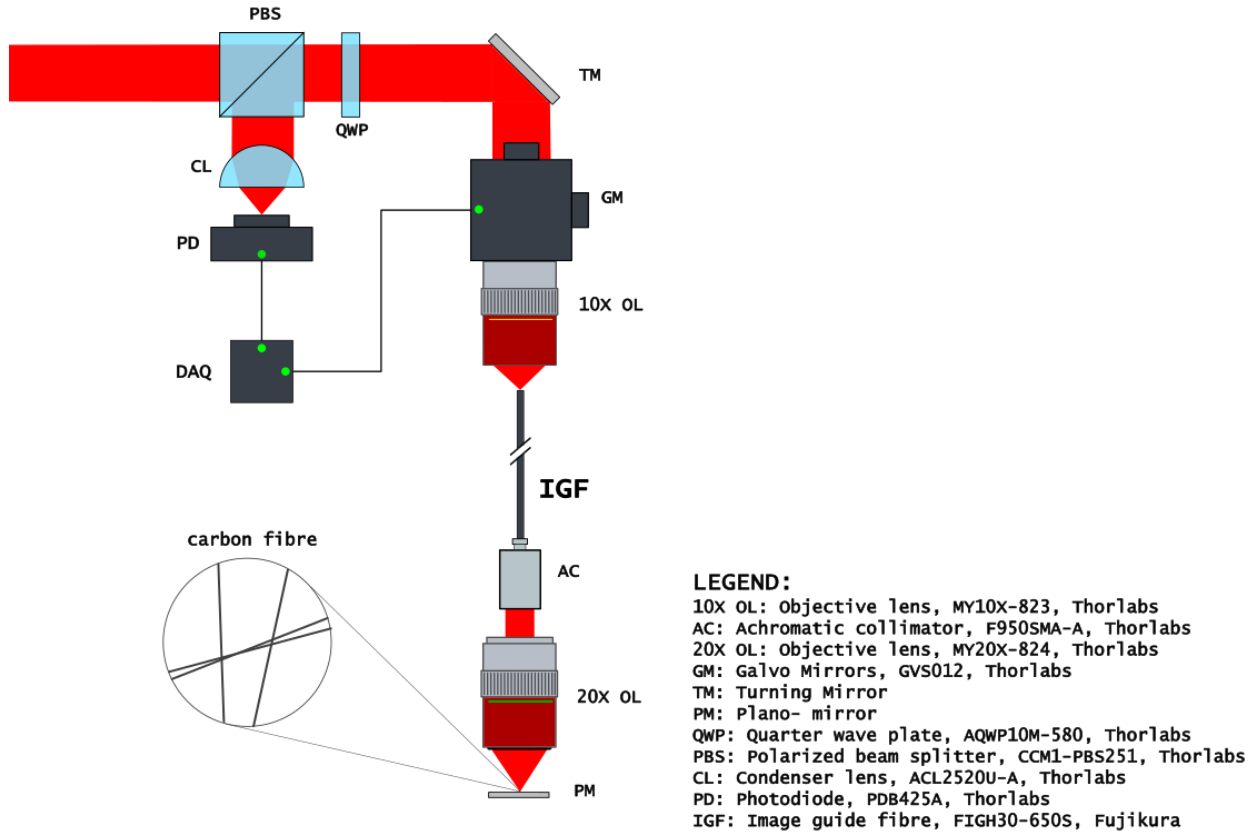


Figure 14: IGF-SC-OL System Design

This experiment validates a scattering microscope using a free-space objective lens design at the distal end. *Figure 15* shows the experimental setup, which builds upon the previous setup with the following additions:

- Achromatic collimator (AC) to collimate the multimode output from the fiber cores and for integration with downstream optics (infinity corrected)

- 10X proximal objective lens (10X OL) to provide coupling to the fiber at the proximal end
- 20X distal objective lens (20X OL) with high NA for improved light gathering and higher resolution

Methodology:

1. Align the detection beam on the benchtop to maximize coupling efficiency in fiber cores across the IGF.
2. Place carbon fibers in a small amount of water on a glass slide.
3. Scan the detection beam using an optimized pattern across the proximal face of the IGF using the galvo mirrors.
4. Acquire signals from the GM and PD using the DAQ and construct images.

IGF-PARS-OL System design and Methodology

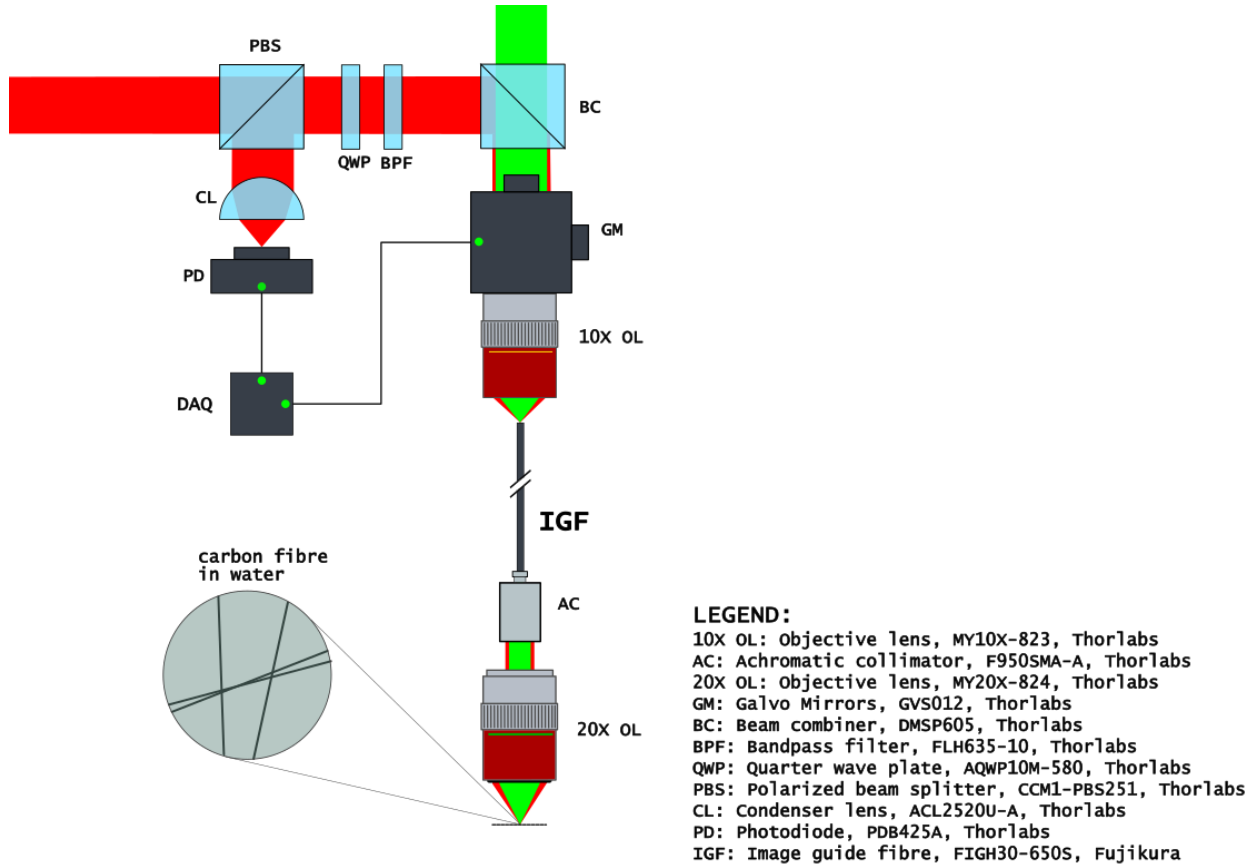


Figure 15: IGF-PARS-OL System Design

This final experiment builds on the previous setups to detect the first PARS signals through IGF on a carbon fiber phantom, demonstrating the feasibility of using IGF for an endomicroscope prototype design. As shown in *Figure 16*, the experimental setup integrates excitation and detection paths:

- 632nm detection beam and 532nm excitation beam

- 10X OL (proximal), 20X OL (distal), GM, PBS, QWP, CL, PD, and DAQ as described in previous experiments
- Beam combiner (BC) in the form of a dichroic mirror to co-align excitation and detection beams

Methodology:

1. Co-align the excitation and detection beams on the benchtop to maximize coupling into the same fibers in the IGF.
2. Place carbon fibers in a small amount of water on a glass slide.
3. Scan both beams using an optimized pattern across the proximal face of the IGF using the galvo mirrors.
4. Acquire signals from the GM and PD using the DAQ and construct images.

3.4 PARS Prototype I (P1) EM Design

Based on lessons learned in POC work, a PARS EM design was conceptualized for evaluation in further experiments (including *in vivo* trials). The design can be separated into benchtop and endoscope parts of the design.

3.4.1 PARS EM benchtop system

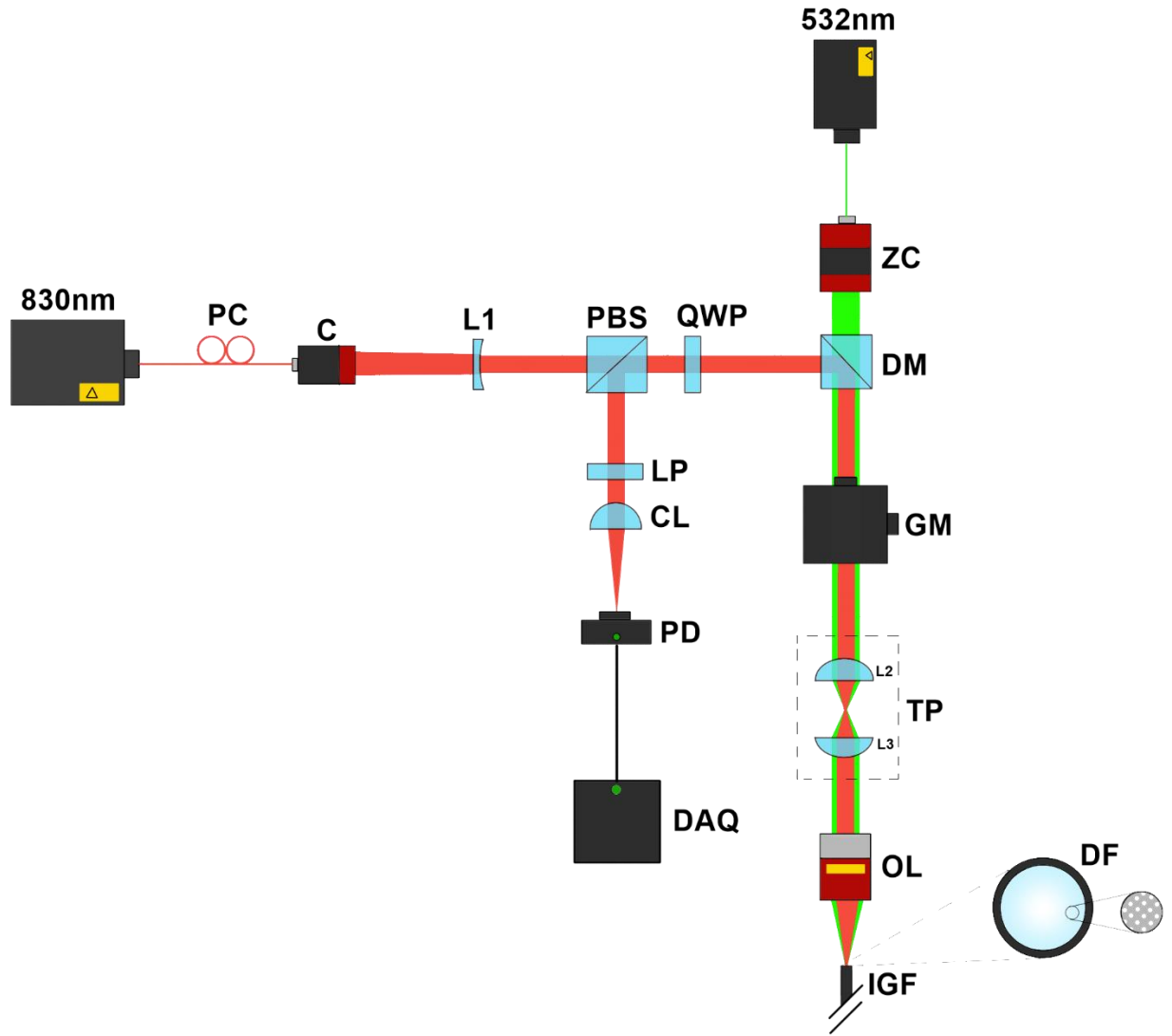


Figure 16: PARS EM Benchtop System Design

The PARS EM system included a modified PARS benchtop system similar to those reported in previous publications^{24,84}. The PARS EM system features an 830nm linearly polarized probe beam (SLD830S-A20W, Thorlabs) coupled with a pulsed 532nm excitation source (GLPN-532-QCW, IPG

Photonics), ideal for haemoglobin contrast. The detection wavelength was selected to optimize the chromatic performance of an achromatic GRIN lens system integrated at the distal end of the endoscope. Included in the benchtop design is a polarised beam splitter (PBS) and quarter-wave plate (QWP) combination, configured to reject unwanted reflections off the proximal face of the IGF. An avalanche photodiode (APD430A2, Thorlabs) is leveraged to detect scattering light from the distal end of the endoscope. Images are acquired by raster scanning the proximal face of the IGF using a 2D galvo mirror system, while coupling is achieved using a 20x objective lens (M Plan Apo NIR 20x, Mitutoyo).

3.4.2 PARS P1 Endoscope

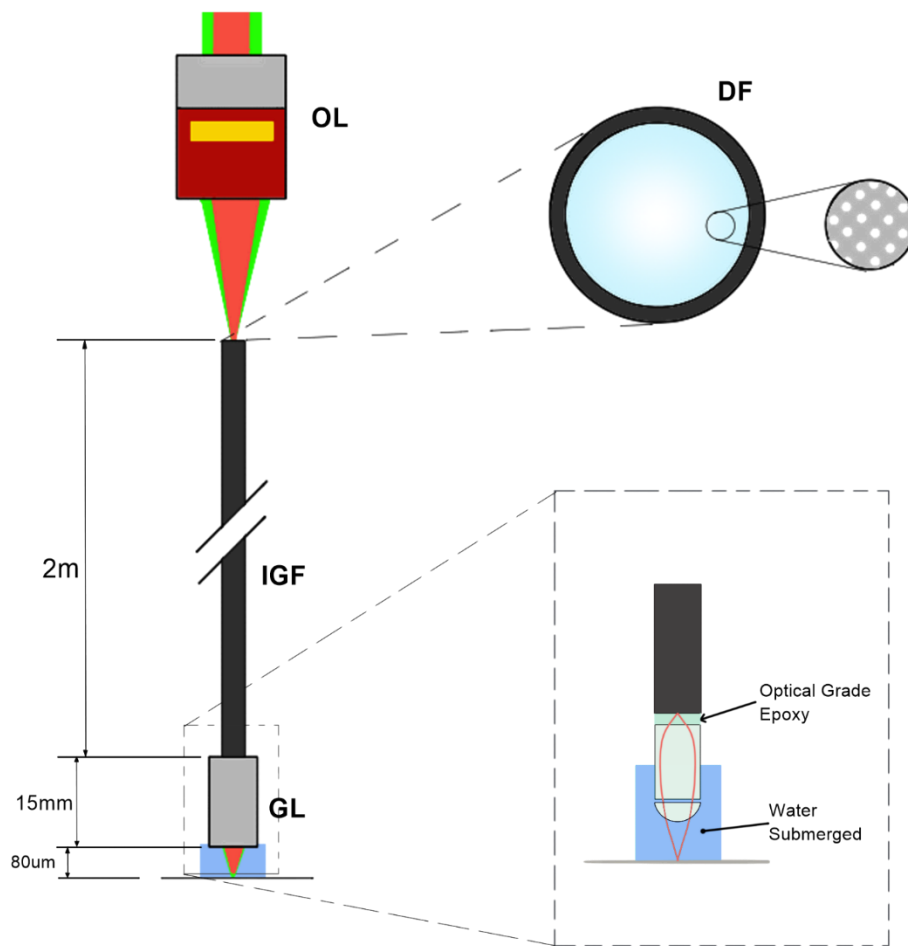


Figure 17: PARS P1 Endomicroscope Design

The endoscope designs utilize a 2m long image-guide fibre (IGF), consisting of 30,000 silica cores all within a 600- μm diameter image circle (FIGH-30-650S, Fujikura) as described previously

(Figure 11). For the P1 design, the distal end of the IGF is glued directly to a high-NA, achromatic GRIN lens system using optical-grade adhesive¹ (GT-MO-080-032-ACR-VISNIR-08-00, Grintech). The distal objective lens assembly has a maximum outer diameter (OD) of 1.6mm and a length of 15mm. The endoscopic imaging system has a working distance of 80 μ m in water, a distal-end NA of 0.75, and an image magnification of 2.3, giving the endoscope a working FOV of 260 μ m.



Figure 18: PARS P1 EM distal end showing critical dimensions of GRIN lens assembly

¹ In a later design, the adhesive used in the P1 prototype was modified to address damage threshold concerns. See discussion for more details on this modification.

Parameter	Specification
Detection wavelength	830nm
Excitation wavelength	532nm (pulsed)
Total Length	2m
Maximum OD	1.6mm
Rigid Length	15mm
Working Distance	80 μ m in water
Numerical Aperture	0.75
Magnification	2.3
Field of view	260 μ m
Number of pixels (cores)	30,000
Projected resolution ²	1-2 μ m

Table 5: P1 PARS EM Design Specifications Summary

3.4.3 Distal objective performance

The distal objective represents a critical component in the PARS EM device design, significantly influencing overall system performance. Efficient PARS signal generation relies heavily on achieving confocal excitation and detection beams at the object plane. To evaluate and optimize

² Based on IGF core spacing and distal objective magnification

this performance, the chromatic behavior of the objective lens system was examined across the visible to near-infrared spectrum.

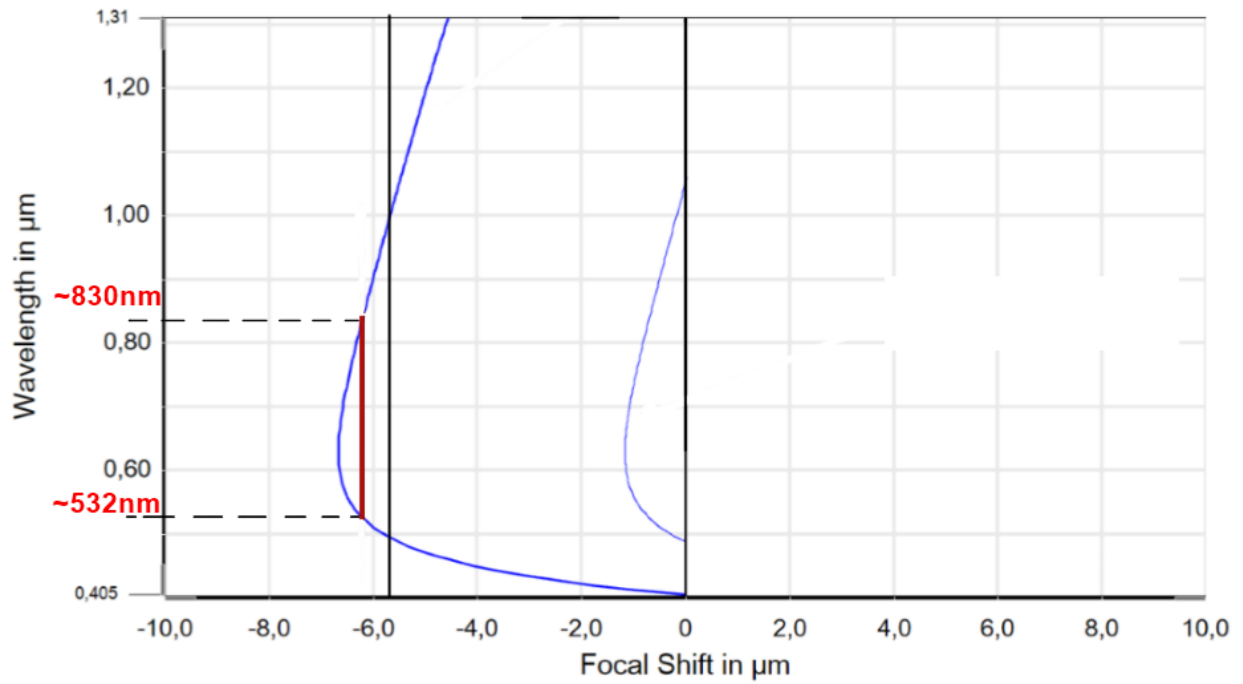


Figure 19: Focal shift vs. wavelength plot showing achromatic theoretical performance of the P1 PARS EM distal objective lens

Figure 20 illustrates the theoretical chromatic focal shift of the P1 PARS EM distal objective lens across various wavelengths. The GRIN objective demonstrates optimized performance within the 488-1064nm wavelength range, exhibiting minimal focal shift between the excitation and detection wavelengths. This characteristic was leveraged in the system design by selecting 830nm for the detection wavelength, which provides excellent confocal performance when paired with the 532nm excitation beam. This wavelength combination, falling well within the optimized range of the GRIN

objective, enhances PARS signal generation efficiency through improved beam coalignment at the object plane.

3.5 PARS Prototype II (P2) EM Design

Following the development of the P1 prototype, a second design (P2) was developed to address specific challenges encountered with the initial prototype. The high numerical aperture (NA) and short working distance (WD) of the P1 design presented significant limitations for *in vivo* trials

(discussed in detail in the Discussion section). While the P2 design maintains the same benchtop system and IGF configuration as P1, it features a substantially modified distal objective lens design.

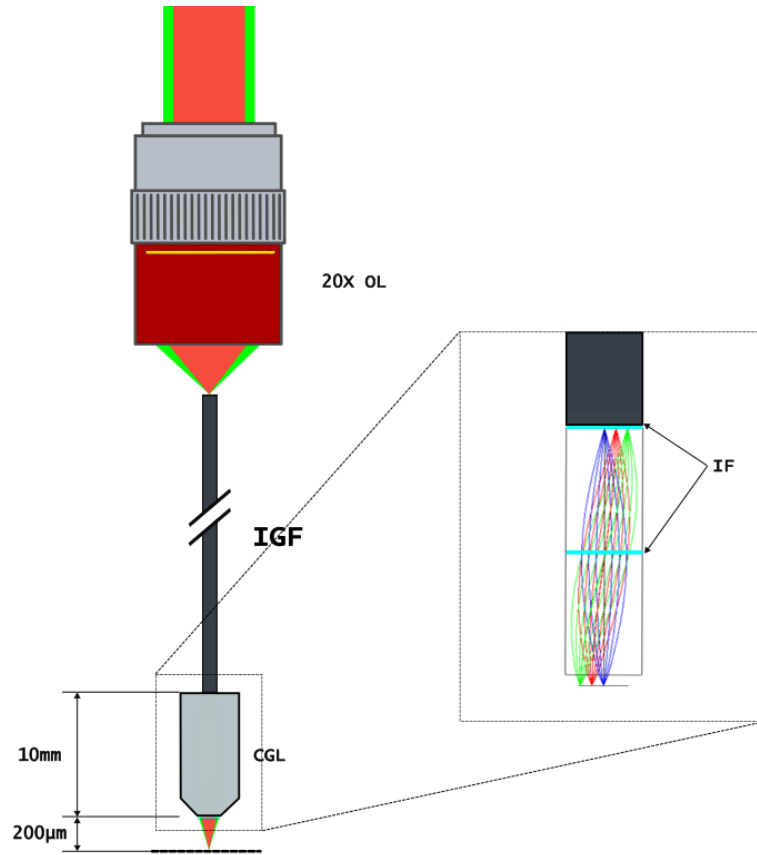


Figure 20: PARS P2 Endomicroscope Design

Just like the P1 design, the P2 design utilizes the same a 2m long image-guide fibre (IGF), consisting of 30,000 silica cores all within a 600-µm diameter image circle (FIGH30-650S, Fujikura). The distal objective was a custom GRIN doublet composed of off-the-shelf GRIN lenses (GT-LFRL-100-023-50, Grintech). A custom stainless-steel sleeve was designed and machined to house the doublet, and index-matching fluid (IF) was used to reduce unwanted reflections from boundaries

along the optical path. The distal objective lens assembly as a maximum OD of 3.18mm and a length of 10mm. The design has a working distance of 230 μ m in water (200 μ m in air), a distal NA of 0.3, and an image magnification of 1, resulting in a working FOV of 600 μ m.



Figure 21: PARS EM P2 prototype distal end assembly.

Below is a table summarizing the PARS EM P2 variant specifications:

Parameter	Specification
Detection wavelength	830nm
Excitation wavelength	532nm (pulsed)
Total Length	2m
Maximum OD	3.18mm
Rigid Length	10mm
Working Distance	230 μ m in water
Numerical Aperture	0.3
Magnification	1
Field of view	600 μ m
Number of pixels (cores)	30,000
Projected resolution ³	$\sim$ 8 μ m

Table 6: P1 PARS EM Design Specifications Summary

³ Based on mean RMS radius of excitation wavelength at image plane from optical simulation

3.6 Comparison of P1 and P2 Designs

For reference, below is a comparison between the P1 and P2 designs, particularly the objective lens performance specifications:

Parameter	P1 Specification	P2 Specification
Maximum OD	1.6mm	3.18mm
Rigid Length	15mm	10mm
Working Distance	80 μ m in water	230 μ m in water
Numerical Aperture	0.75	0.3
Magnification	2.3	1
Field of view	260 μ m	600 μ m
Projected resolution	$\sim$ 1 μ m	$\sim$ 8 μ m

Table 7: Comparison summary of P1 and P2 design specifications

The P2 design represents a significant departure from P1 in several key parameters. While sacrificing some resolution, it offers substantially improved working distance and field of view, making it more suitable for *in vivo* applications. These trade-offs and their implications for different applications are examined in detail in the Discussion section.

4 Results

The results presented in this section represent the first successful demonstration of PARS imaging through a miniaturized, flexible endomicroscope architecture. Building on the proof-of-concept (POC) experiments, two successive prototype devices (P1 and P2) were developed and tested to evaluate imaging performance under conditions approximating clinical use. These prototypes were specifically designed to meet the key requirements for endoscopic integration: forward-viewing, non-contact operation, label-free contrast, and high spatial resolution. The P1 prototype, which utilizes an achromatic GRIN objective, achieved a measured resolution of $1\mu\text{m}$ and a signal-to-noise ratio of 22 dB (158:1), when imaging carbon fiber phantoms in a water. The P2 prototype was developed in response to limitations encountered during *in vivo* trials with the P1 design, particularly related to working distance and field-of-view. By trading some resolution for greater working distance and a wider field of view, the P2 prototype achieved a resolution in the range of $7\mu\text{m}$, which remains suitable for imaging microvasculature, including small vessels such as capillaries.

Collectively, the results demonstrate that the PARS mechanism is compatible with endoscopic architectures and can deliver high-resolution, label-free imaging using a fully optical, forward-viewing configuration. The following findings represent a critical milestone toward the realization of *in vivo* PARS endomicroscopy for virtual biopsy and high-specificity diagnostics.

4.1 Proof-of-Concept (POC) Results

To guide the development of a functional PARS endoscope prototype, a series of proof-of-concept (POC) experiments were conducted to systematically address key design risks. Each experiment was tailored to de-risk a specific aspect of the PARS endoscope design, focusing on optimizing the excitation and detection pathways, enhancing signal efficiency, and improving image quality. The lessons learned from these POC experiments informed critical design elements of the final prototype, establishing a foundation for successful endoscopic imaging within a miniaturized, flexible form factor.

4.1.1 IGF-OR-PAM Results

The IGF-OR-PAM experiment served as an initial validation step for the prototype design, focusing on de-risking the optical coupling efficiency and forward excitation path. The primary aim was to demonstrate the feasibility of generating photoacoustic (PA) signals through the image-guide fiber (IGF) by placing its distal end in direct contact with a carbon fiber target. This test allowed for qualification of the excitation pathway, assessing whether sufficient optical power could be coupled through the IGF to generate reliable PA signals.

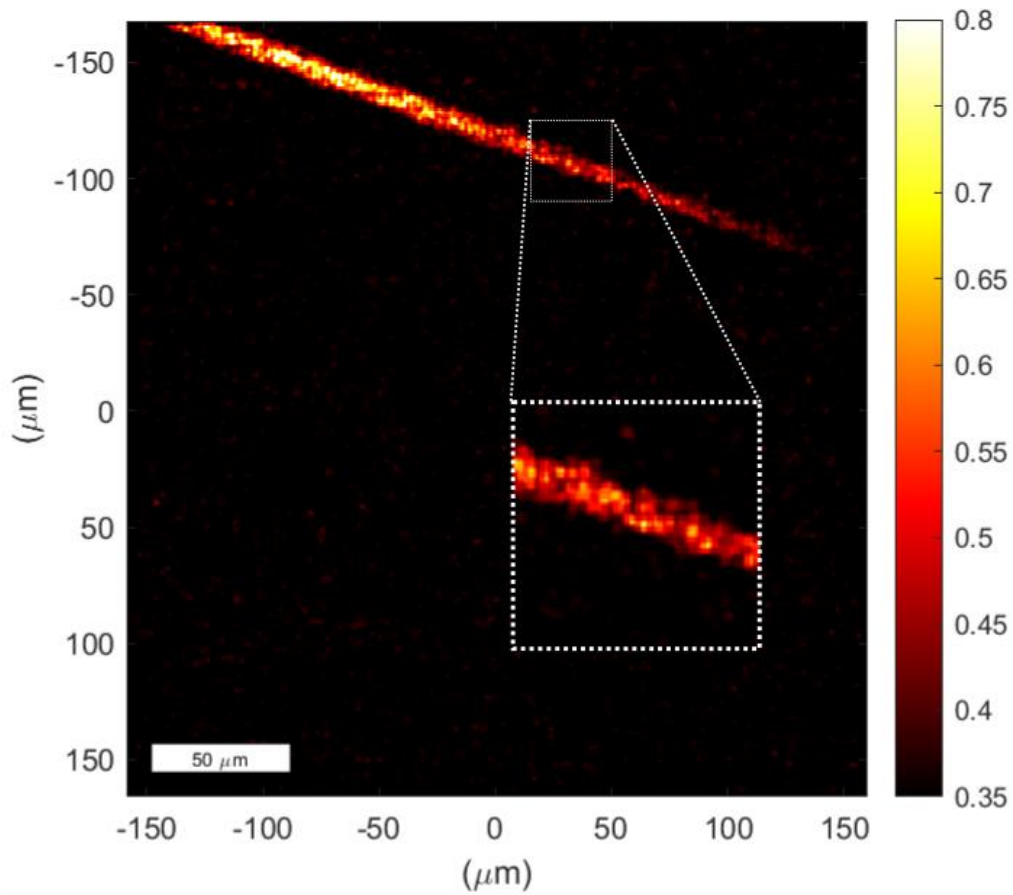


Figure 22: Optical resolution photoacoustic microscopy image obtained by scanning IGF proximal face and placing distal face in contact with carbon fibres. The array pattern from the IGF can be seen in the intensity of the resulting image (inset).

Figure 23 shows an image acquired during the IGF-OR-PAM experiment. The intensity pattern of the IGF cores is clearly visible, highlighting the structure of the fiber array. Signals were generated by placing the distal end of the IGF in contact with the carbon fibers suspended at the cylindrical focus of a transducer. The overlay of the IGF array onto the carbon fiber target is evident in the

resulting image, with interpolated intensity values extracted from the peak-to-peak amplitudes of the generated PA signals.

Significance of Results

The results from the IGF-OR-PAM experiment demonstrate that the IGF can support the fluences required to generate photoacoustic (PA) signals on carbon fiber targets. Importantly, the fluence levels necessary for PA signal generation are comparable to those required for PARS signal generation⁸⁴. This similarity establishes a critical link between the IGF-OR-PAM experiment and the development of the PARS endoscope, confirming that the IGF is capable of meeting the energy density requirements for both mechanisms.

This finding provides strong evidence that the IGF is a viable candidate for integration into the PARS endoscope prototype. The ability to efficiently couple sufficient optical power through the IGF for signal generation de-risks one of the critical aspects of the optical pathway and establishes a reliable foundation for further development.

4.1.2 IGF-SC Results.

Following the IGF-OR-PAM experiment, the IGF-SC experiment was conducted to evaluate the return path and signal collection efficiency without the use of distal optics. The primary aim was to assess the scattering signal generated at the distal end of the IGF and identify potential risks associated with signal-to-noise ratio (SNR), particularly the impact of parasitic reflections and low signal discrimination.

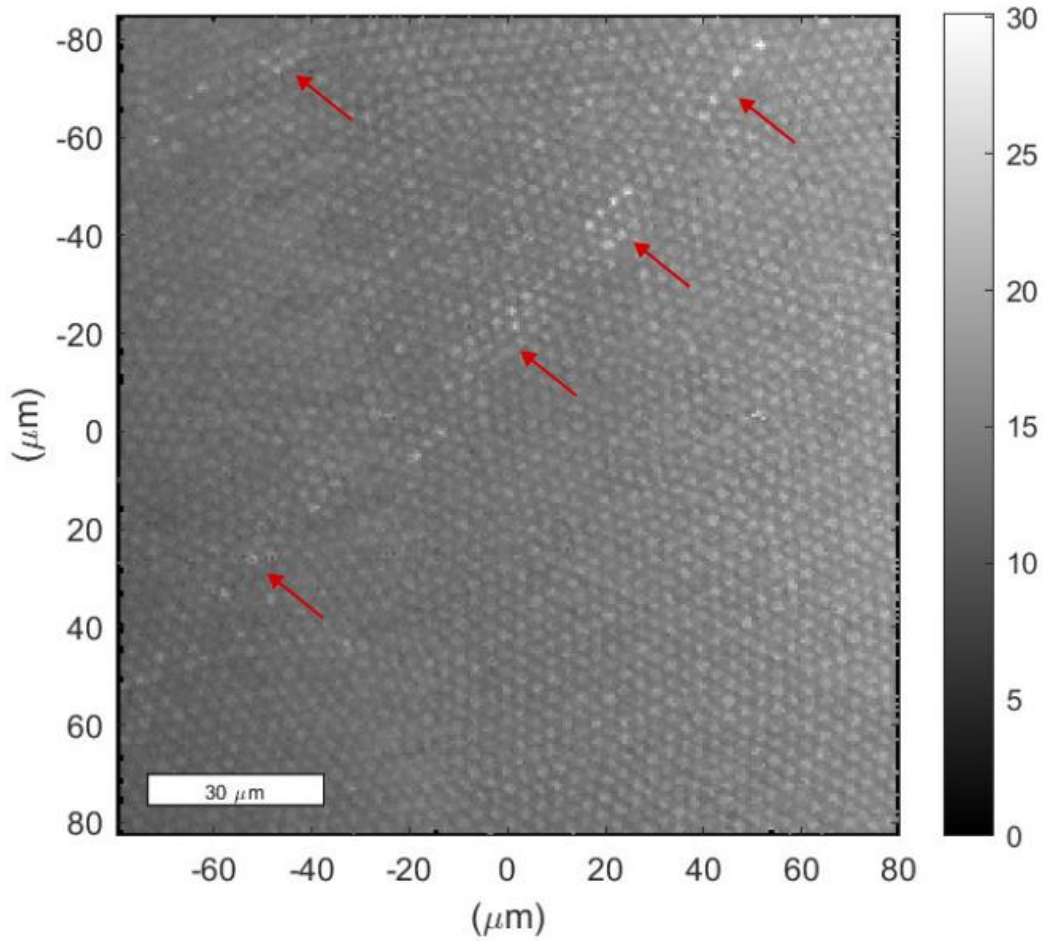


Figure 23: Small FOV scattering image of carbon fibres through IGF (indicated by red arrows). Carbon fibres are placed in contact with the distal face of the IGF with no distal optics.

Images were acquired using the experimental setup described in *figure 14*, which employed a detection wavelength of 633 nm. Scanning was performed over a small area of the IGF's proximal face ($160 \times 160 \mu\text{m}^2$), while the distal end of the fiber bundle was placed in contact with carbon fibers. No distal optics were included during this acquisition. The resulting image clearly reveals the IGF

core structure, with scattering signals from the carbon fibers only ~ 1.5 times greater than the signal from the IGF cores, indicating poor SNR.

Significance of results

The IGF-SC experiment demonstrated that a scattering signal could be successfully returned from the distal end of the IGF, effectively de-risking the return coupling pathway. Despite the poor signal-to-noise ratio (SNR) observed, the experiment confirmed the feasibility of signal detection through the IGF without distal optics, marking an important step forward in validating the overall optical path.

However, the results also highlighted significant risks associated with low SNR, primarily caused by parasitic reflections from the IGF interfaces. This poor signal discrimination emphasizes the critical need to design a prototype system that mitigates these reflections while improving return signal efficiency. Addressing these challenges in subsequent prototypes will be essential to achieving the robust performance required for practical endoscopic imaging.

4.1.3 IGF-SC-OL Results

Building on the findings of the IGF-SC experiment, the IGF-SC-OL experiment introduced distal optics into the optical path to evaluate their impact on image formation and overall performance. The primary objective was to validate both the forward and return optical pathways when a distal lens system is employed and to assess the imaging performance using known targets. This experiment provided key insights into the challenges introduced by distal optics and their role as a critical component in the prototype design.

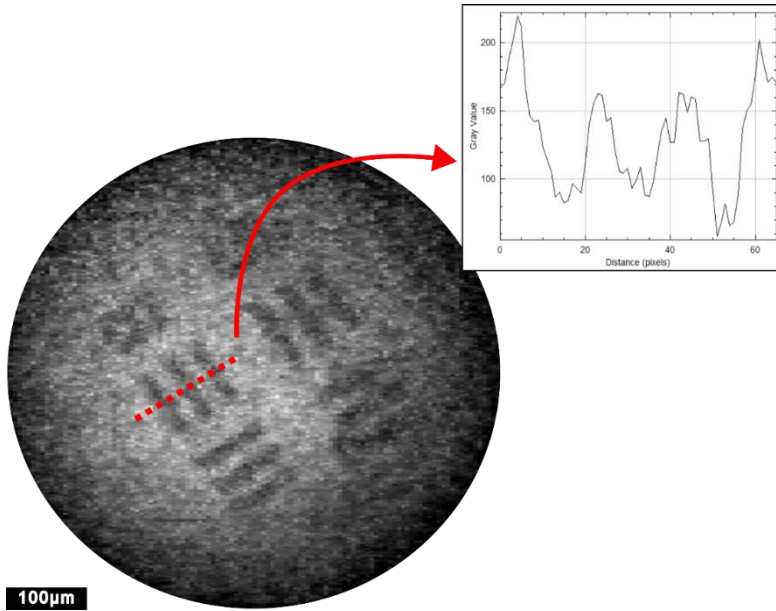


Figure 24: Scattering image acquired using IGF-OL-SC setup of USAF target showing bar target (group 4, element 5, 6). Inset shows line profile across G4/E6, highlighting contrast levels.

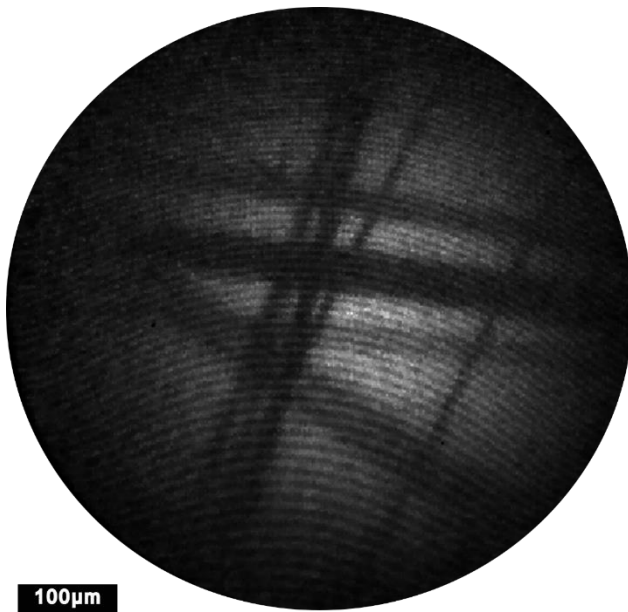


Figure 25: Scattering image acquired using IGF-SC-OL experimental setup of carbon fibres in front of mirrored surface.

The results of the IGF-SC-OL experiment were obtained using the experimental setup described in *figure 15*. Two different targets were employed to evaluate the capabilities of the system. The configuration of the distal optics provided a field of view (FOV) of 600 μm , corresponding to the image circle of the IGF. Images of a USAF resolution target (R1DS1N1, Thorlabs) were acquired to assess the imaging performance of the setup, with a focus on line pair (lp) group 4, element 6, which has a spatial frequency of 28.5 lp/mm.

Additionally, images of carbon fibers were captured to evaluate the system's performance on the intended target. A mirrored surface was used to back the carbon fibers, enhancing contrast due to their low reflectivity in the visible range (typically 0.15–0.25 for multiple fibers ⁹⁵) and addressing the challenges posed by parasitic back reflections.

Both sets of images revealed an intensity roll-off toward the perimeter of the IGF FOV, attributable to the imaging performance of the distal lens system. This roll-off underscores the necessity of a fully optimized distal objective lens to ensure uniform performance across the entire working FOV. The findings highlight key areas for optimization in the scattering path and provide valuable guidance for mitigating performance bottlenecks in subsequent designs.

Significance of results

The IGF-SC-OL experiment highlights several critical challenges in the imaging performance of the PARS endoscope system. The introduction of distal optics into the optical path significantly increases the system's complexity and represents a significant bottleneck for device performance. The results emphasize the necessity of a highly optimized distal objective lens for the PARS application, as this component is central to addressing major risks, including signal-to-noise ratio (SNR), optical efficiency, damage thresholds, and image quality.

Despite the limitations observed in the IGF-SC-OL setup, including intensity roll-off across the field of view, the experimental setup served as an acceptable design to carry forward into the final proof-of-concept experiment to validate the IGF concept for PARS endoscope prototyping.

4.1.4 IGF-PARS-OL Results

The IGF-PARS-OL experiment represents a significant milestone as it demonstrates the first PARS images captured using an IGF-based setup with integrated distal optics. The primary objective of this experiment was to validate the system's ability to generate and detect PARS signals. This experiment builds upon the findings of previous tests, integrating all key components of the optical path in a configuration suitable for endoscopic applications.

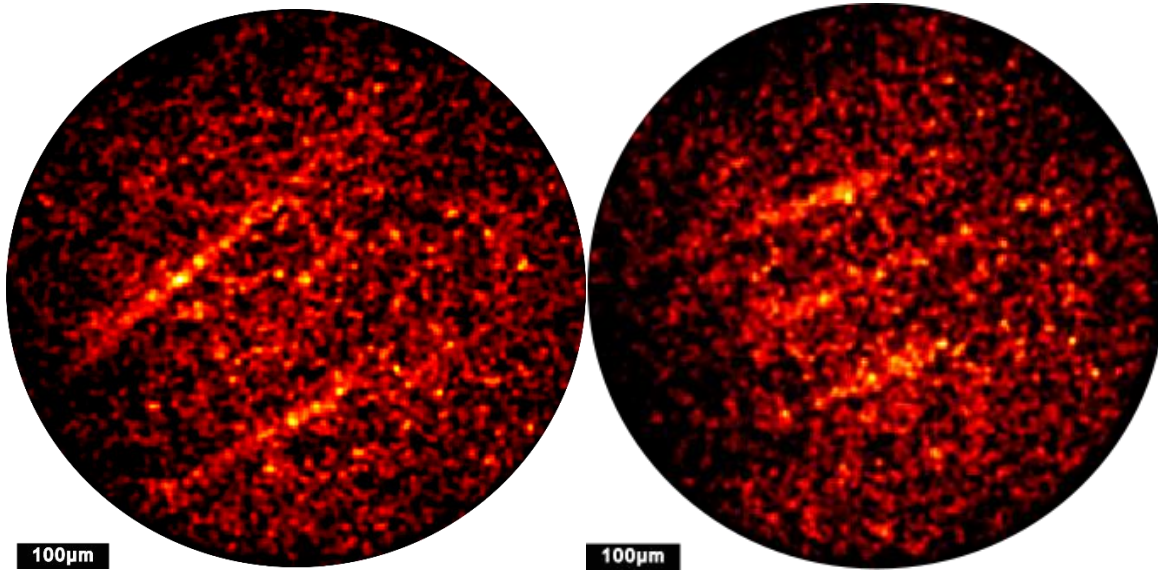


Figure 26: PARS images acquired using the IGF-PARS-OL experimental setup.

In this experiment, PARS images were acquired by calculating the min-max amplitude of the PARS signal at each raster point and interpolating the values across the field of view (FOV). To mitigate the impact of parasitic reflections from optical boundaries, the signal floor was elevated

during image processing. As shown in the resulting images, the POC system successfully resolved single strands of carbon fibers. The images also revealed limitations, including a low signal-to-noise ratio (SNR) and an intensity roll-off across the FOV. These findings are consistent with the scattering results observed in earlier experiments, further highlighting the need for optimized distal optics and improved light path efficiency to enhance system performance.

Significance of Results

The IGF-PARS-OL experiment marks a significant milestone in the development of the PARS endoscope. Despite the limitations of the proof-of-concept (POC) device, including low signal-to-noise ratio (SNR) and intensity roll-off, these results represent the first successful acquisition of PARS images through a system designed with a form factor adaptable to handheld and endoscopic applications. This achievement demonstrates the feasibility of using the IGF concept to generate and detect PARS signals, providing a template for prototype development.

The findings highlight key areas for improvement, particularly in addressing the challenges posed by parasitic reflections and light path inefficiencies. Enhancing the performance of the distal optical lens system, with a focus on reducing reflections and maximizing light collection efficiency, will be critical to achieving reliable and high-quality imaging. Additionally, ensuring a high numerical aperture (NA) within the constraints of the device's maximum diameter is essential for generating efficient signals and optimizing overall performance. These results establish a clear pathway for advancing the PARS endoscope toward practical, real-world applications.

4.1.5 POC summary

Based on the proof-of-concept (POC) work, here's a summary of the primary lessons learned, and risks addressed for the endoscope design leveraging an image-guide fiber (IGF):

Experiment	Goal	Outcome	Items de-risked/Risks Identified	Mitigation Strategies
IGF-OR-PAM	generate photoacoustic (PA) signals using the IGF	Successful generation of photoacoustic signals through IGF	Items de-risked: - excitation coupling efficiency across working FOV - damage threshold of IGF for PARS signal generation	- Match benchtop objective NA to IGF NA to maximize coupling efficiency (20x OL and appropriate diameter)
IGF-SC	assess signal return path and scattering performance without distal optics	Successful collection of scattering signals through IGF	Items de-risked: - Return path and detection scheme Risks identified: - Low signal return efficiency in IGF with no distal optics - High parasitic reflections and proximal and distal optical boundaries of IGF	- Optical isolator design needed to reject proximal reflections, index-matching at distal face to reduce back-reflections - Magnification to reduce artifacts from IGF core structure to improve imaging performance

Experiment	Goal	Outcome	Items de-risked/Risks Identified	Mitigation Strategies
			- Magnification necessary for high resolution images	
IGF-SC-OL	Validate forward and return path with distal optics; test imaging performance with a distal objective lens using known targets.	Successful image formation using distal objective lens configuration	Items de-risked: - Outgoing and return path, specifically return coupling and image formation with distal optics Risks identified: - Distal objective represents imaging performance bottleneck: aberrations, transmission efficiency, parasitic reflections all major risks	- Highly optimized distal objective lens that has high aberration control, transmission efficiency and collection efficiency necessary for viable design
	Capture the first PARS images with a form factor adaptable to endoscopy	Successfully obtained PARS images of carbon fibers	Items de-risked: - PARS signal generation through IGF with distal optics Risks identified - Low SNR due to low inefficient PARS signal generation and parasitic back reflection	- Achromatic distal OL design - Optimized detection path efficiency and backscatter collection - Minimize parasitic reflections by index matching optical boundaries

Table 8: Summary of items derisked/identified and resulting mitigation strategies from POC experiments

The POC work collectively addressed critical risks related to signal efficiency, damage thresholds, SNR, and image quality. Ensuring an optimized distal optical design, managing parasitic reflections, and achieving high collection efficiency within the form factor constraints are essential steps moving forward. These lessons directly inform the design elements needed for a viable prototype capable of high-quality *in vivo* PARS imaging.

4.2 P1 PARS Endoscope Results

Building on the findings of the proof-of-concept experiments, the P1 PARS endoscope represents the first fully integrated prototype designed for high-resolution, label-free imaging. This prototype incorporates key design improvements to address challenges identified during the POC studies, including strategies to mitigate parasitic reflections, optimize light path efficiency, and enhance signal-to-noise ratio (SNR). The following results evaluate the performance of the P1 endoscope, focusing on its imaging capabilities and the effectiveness of implemented design enhancements.

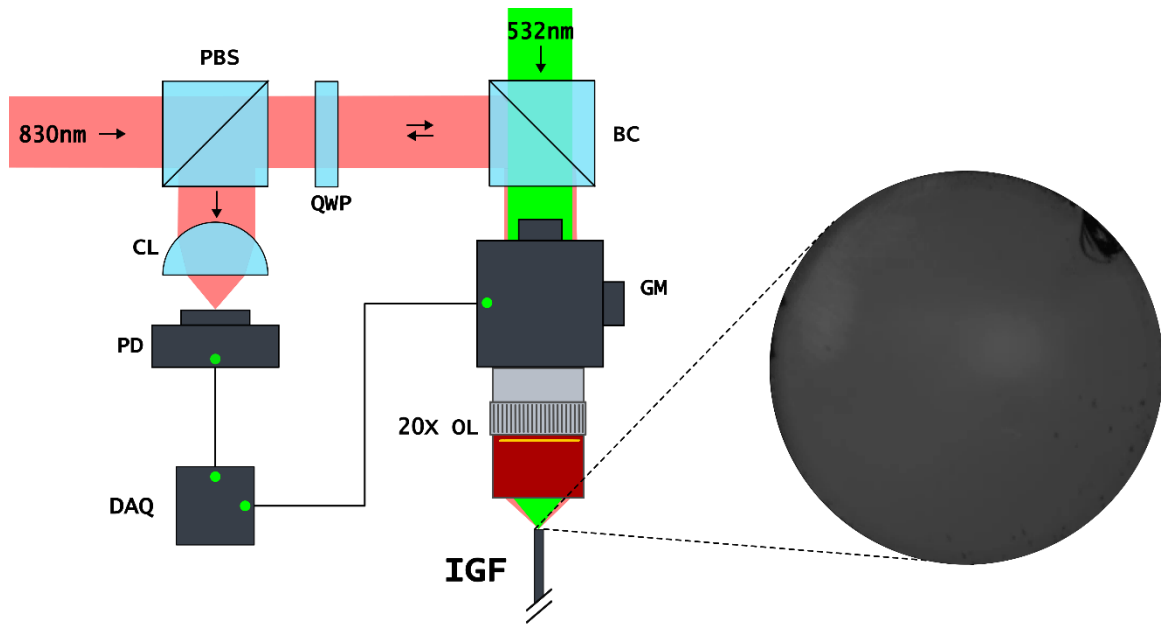
4.2.1 Scattering path optimization

A key risk identified during the proof-of-concept (POC) work was the low signal-to-noise ratio (SNR) caused by parasitic reflections from optical boundaries. To address these concerns, several mitigation strategies were implemented in the design of the P1 endoscope to optimize the scattering path, which is critical for both SNR and PARS signal generation efficiency.

Proximal reflection rejection

One of the primary strategies involved the configuration of an optical isolator to reject reflections from the proximal face of the IGF. The optical isolator was designed using a polarized beam splitter (PBS) combined with a quarter-wave plate, ensuring that polarized light reflected from the proximal face (which undergoes a phase inversion) is rejected. Since the IGF cores are not polarization-maintaining, the scatter return from the distal end was randomly polarized. As a result, 50% of the backscatter intensity is collected by the photodiode, while the remaining 50% is rejected. This trade-off was deemed acceptable, as it eliminated the impact of proximal reflections, a significant source of parasitic reflection. To compensate for the sensitivity loss caused by the

rejection of proximal reflections, an avalanche photodiode was incorporated into the system design to amplify the intensity of the returning signal, improving overall sensitivity. The effectiveness of these mitigation strategies is demonstrated in the images below, which highlight the improvement in scattering images when proximal reflections are successfully rejected.



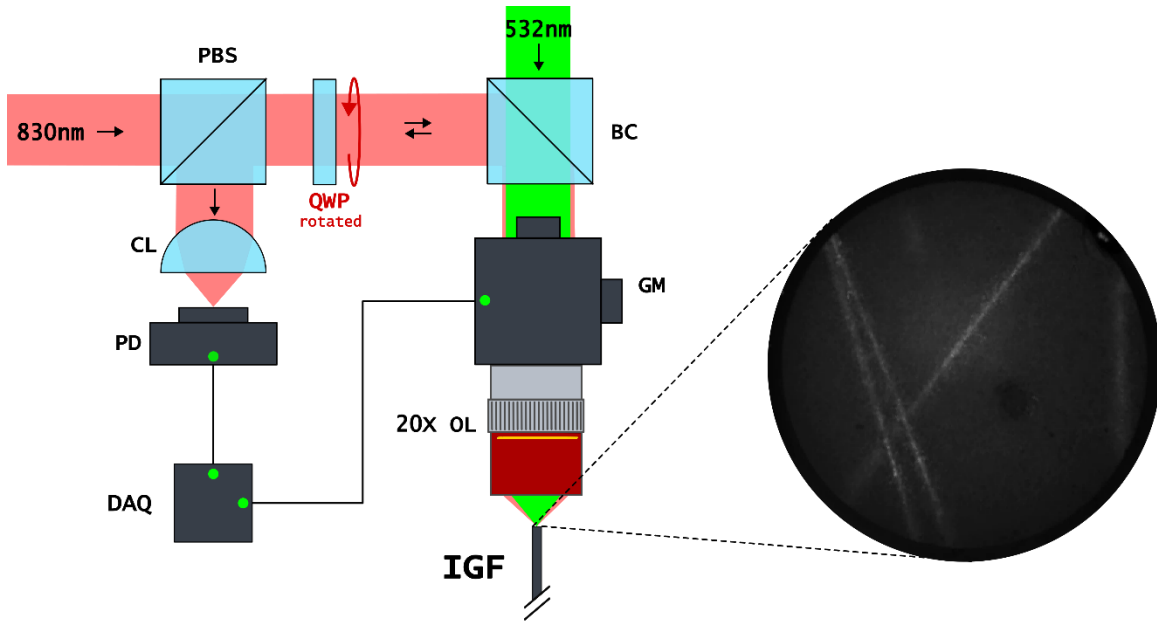


Figure 27: Illustration showing the improvement in scattering images from proximal rejection scheme.

The images above illustrate the effectiveness of the implemented optical isolator in rejecting proximal reflections, which are a significant source of parasitic noise in the system. By carefully rotating the quarter-wave plate (QWP), all reflections from the proximal face of the IGF were rejected, allowing for a substantial improvement in scattering performance. The working field of view (FOV) of the endoscope is 260 μ m.

As observed in the images, the rejection of proximal reflections leads to a marked enhancement in scattering signal quality. This improvement is critical for the PARS endoscope's application, as highlighted during the proof-of-concept (POC) work. Scattering performance is directly tied to signal-to-noise ratio (SNR) and overall image quality, making it a foundational component for achieving efficient and reliable PARS imaging.

Distal end submersion

To further optimize the scattering path and enhance signal-to-noise ratio (SNR), the distal end of the endoscope was submerged in water during imaging. This approach aimed to minimize optical reflections at the interface between the distal objective lens and the surrounding medium by closely matching their refractive indices.

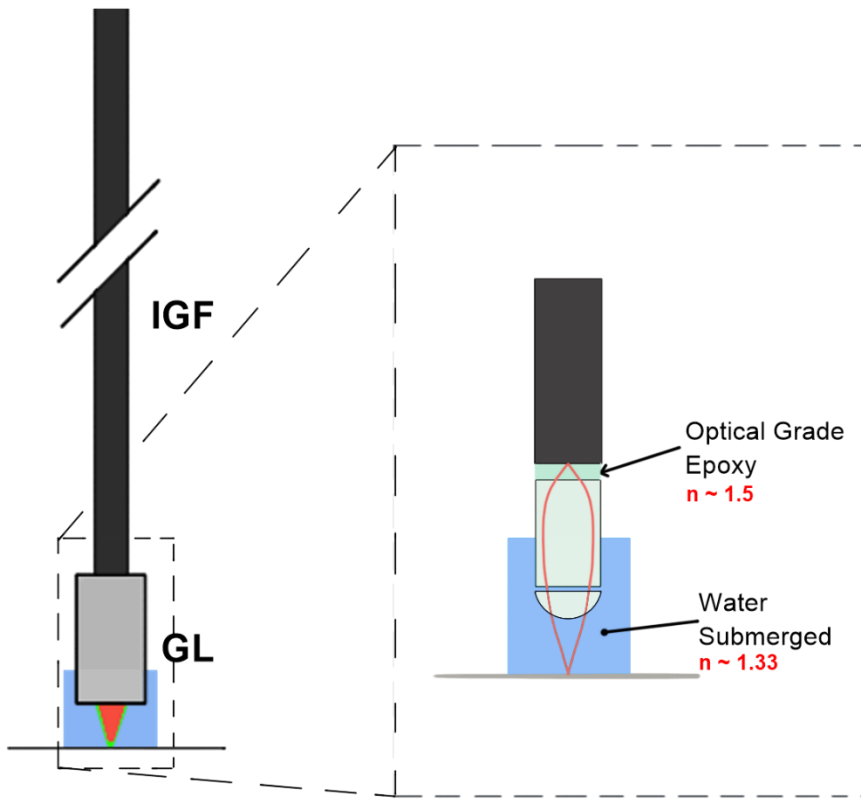


Figure 28: Optical path at distal end of PARS P1 device showing index-matched boundaries.

The illustration above depicts the distal end of the P1 endomicroscope, highlighting the key optical path elements. Index matching was employed at each boundary to minimize refractive index differences and reduce optical reflections. Submerging the distal end in water addresses reflections at

the final interface between the objective lens and the object plane. This effectively "closes the loop" on index matching for the optical path, reducing parasitic reflections and enhancing overall system efficiency.

The images below demonstrate the significant improvement in signal-to-noise ratio (SNR) achieved by submerging the distal objective in water, underscoring the importance of this approach for optimizing endoscopic imaging performance.

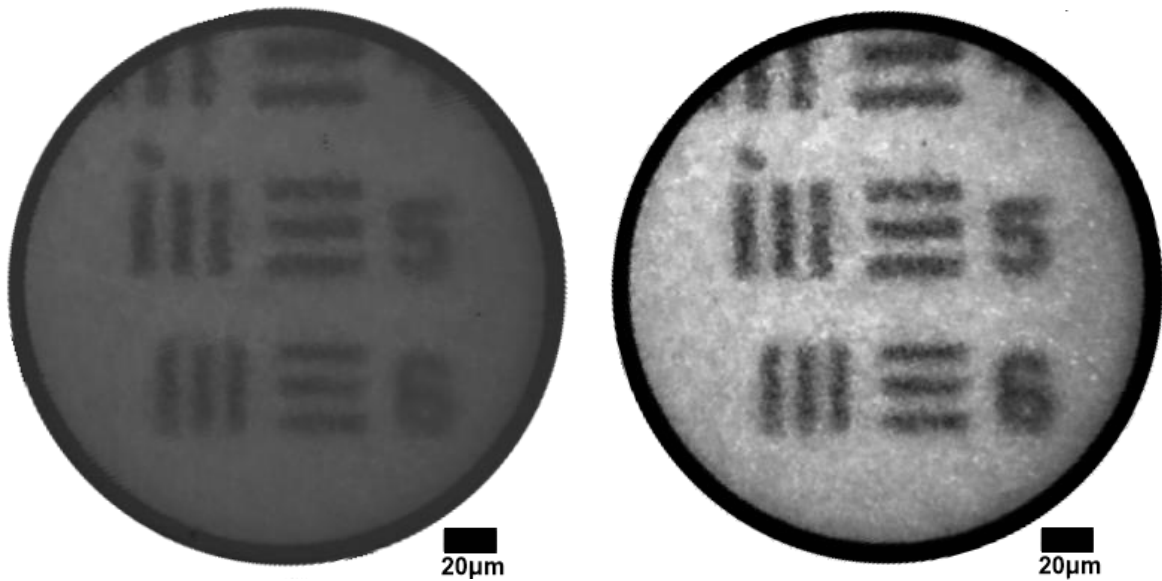


Figure 29: Left: Scattering image of USAF target (group 5, element 5,6) in air. Right: Scattering image of the same region of USAF target with distal end submerged in water.

4.2.2 Excitation path optimization

Efficient PARS signal generation relies on precise optimization of the excitation at the object plane. It is critical that the excitation beam is tightly focused, as this increases the excitation energy density at the object plane, resulting in more rapid and localized heating and pressure events, which in

turn enhance signal generation^{21,84,85}. Additionally, the excitation and detection schemes must be as co-aligned as possible to maximize system efficiency.

To facilitate alignment and optimization for PARS signal generation, a scattering microscope was implemented along the excitation path. This tool was instrumental in optimizing the endomicroscope, ensuring that the excitation focus was properly aligned with the detection path. The diagram below illustrates the setup of the excitation-based scattering microscope, which was used as a tool for system alignment and overall performance improvement.

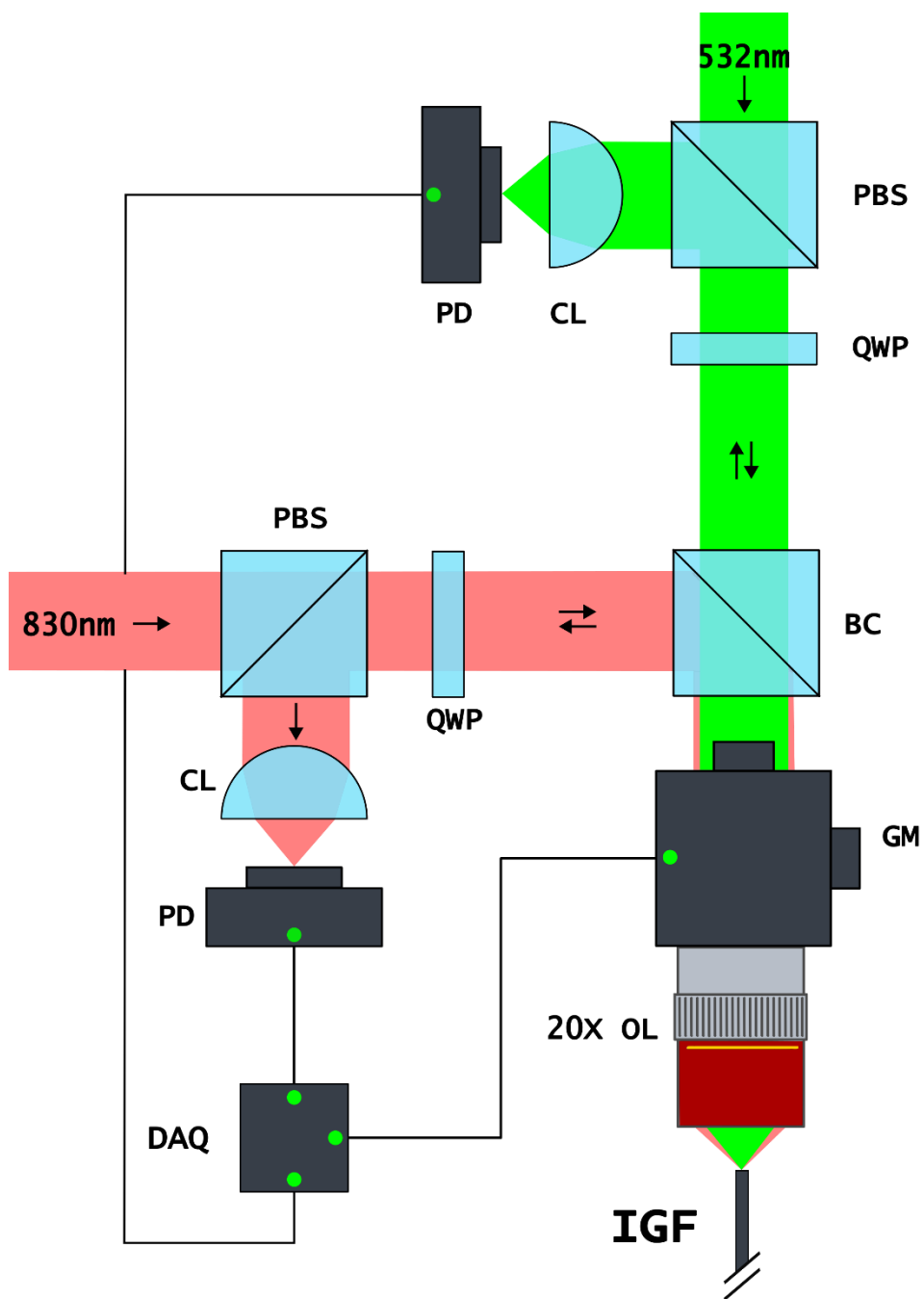


Figure 30: PARS benchtop microscope system with both detection- and excitation- based scattering microscopes implemented.

Below are simultaneous images acquired using the detection and excitation scattering microscopes, showing near perfect coalignment of both schemes.

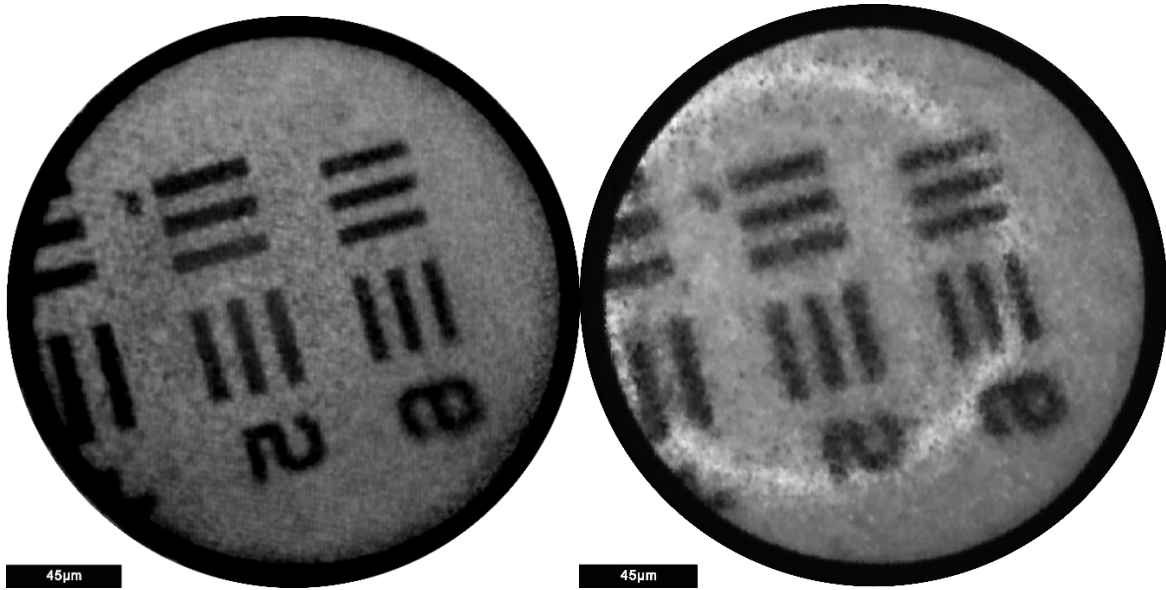


Figure 31: 1-1 excitation and detection scattering images of USAF target (group 5, element 5,6). Left: 532nm excitation scattering image. Right: 830nm detection scattering image.

4.2.3 P1 Endoscope CF Images

Following system optimization, images of carbon fibers were acquired using the system configuration described in *figure 18*. These images represent a significant milestone, as they are the first acquired using the PARS EM device in a configuration that meets the requirements outlined at design conceptualization for integration with widefield endoscopy applications.

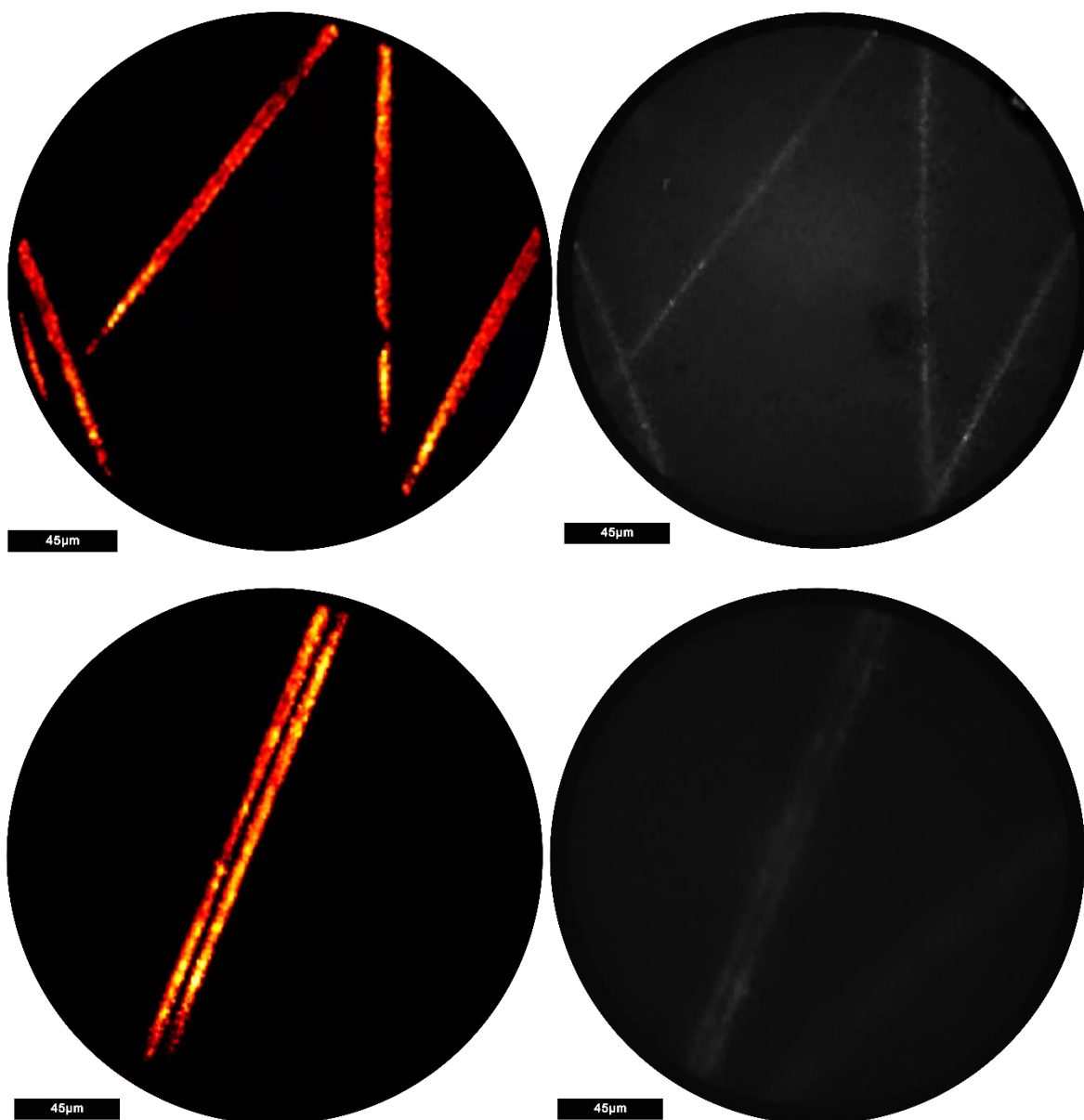


Figure 32: 1-1 PARS and 830nm Scattering images of carbon fibres submerged in water. *Left:* PARS images, *Right:* 830nm Scattering images.

Figure 33 presents images acquired using the P1 endomicroscope setup described in *figure 17*. Signals were generated by carefully aligning the distal end of the endoscope to position the carbon fibers, submerged in water, within the working distance of the distal objective lens. The proximal face

of the IGF was raster-scanned, and the resulting raster points were interpolated to construct the images.

The peak-to-peak (pk-pk) amplitude of the generated photoacoustic (PA) signals was extracted and used to generate the intensity values in the final interpolated images. Images were captured using an average excitation pulse of 4nJ at the object plane. Notably, the IGF array structure is absent in the reconstructed images, demonstrating the effectiveness of the distal lens design and the interpolation method in producing high-quality images. These results highlight the potential of the P1 endomicroscope design for producing clear, high-resolution images in a practical endoscopic configuration.

4.2.4 P1 Endomicroscope Performance

The performance of the P1 endomicroscope (EM) was quantified using the acquired carbon fiber images, with a focus on two key metrics: resolution and signal-to-noise ratio (SNR). These metrics are critical for evaluating the imaging capabilities of any endomicroscope and ensuring its suitability for clinical and research applications.

Resolution

The image analysis conducted on the carbon fiber data is presented below.

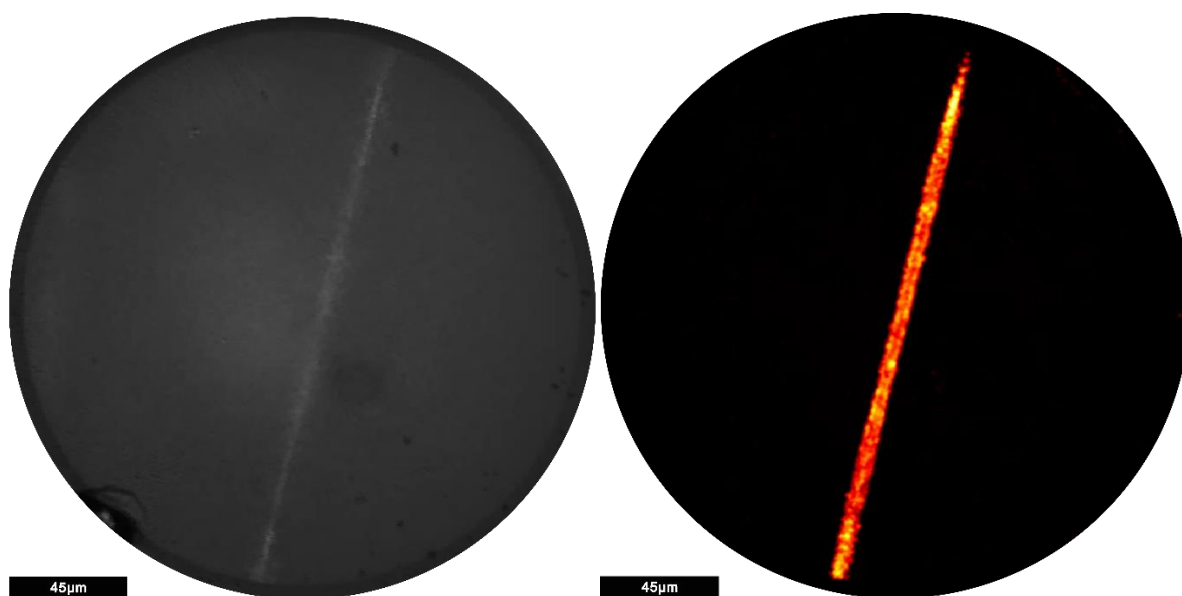


Figure 33: Image of carbon fibre acquired using the P1 design for resolution evaluation

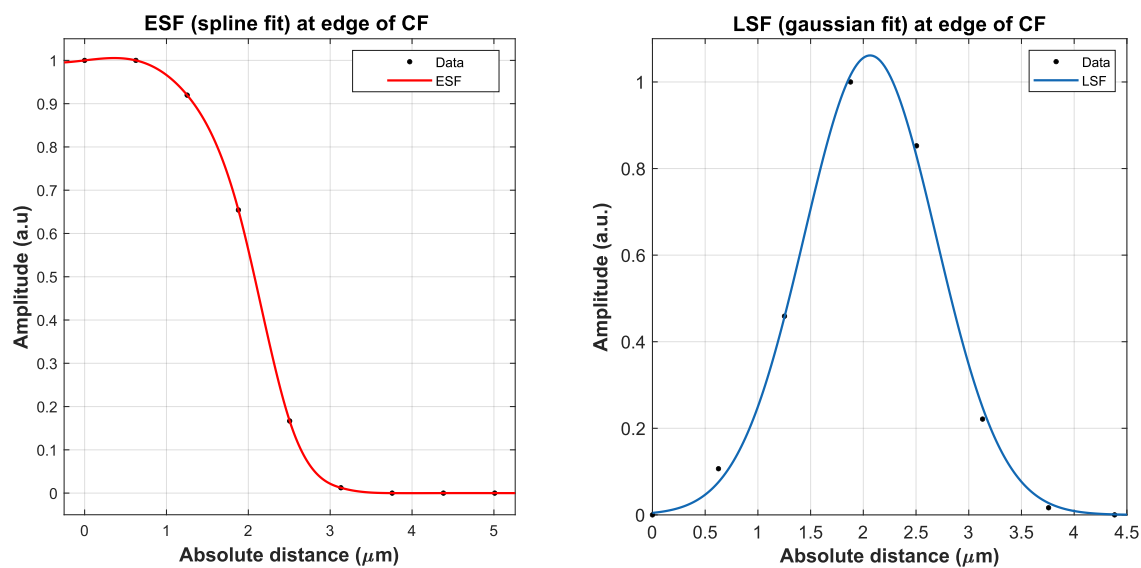


Figure 34: Plot of ESF (left) and LSF (right) and resulting fits of the transitional boundary on the edge of a PARS CF image taken with the P1 EM device.

The resolution of the P1 endomicroscope was estimated using a line profile taken perpendicular to the edge of a carbon fiber, resulting in the edge spread function (ESF). The derivative of the ESF was computed to produce the line spread function (LSF), which was then fitted to a Gaussian. The full width at half maximum (FWHM) of the Gaussian fit was extracted and used to estimate the resolution of the device.

Based on this method, the resolution of the P1 device was calculated to be 1.04 μm . This value is consistent with the resolution estimated based on the array spacing and the magnification of the distal objective lens, validating the accuracy of the measurement approach and the optical design of the system.

Signal-to-Noise (SNR)

The SNR of the P1 endomicroscope was calculated using the peak noise of the background signal and the mean signal intensity of the carbon fiber in the acquired image. By applying the general expression for calculating image SNR⁹⁶, the SNR was determined to be 158:1 (22dB). Both the resolution and SNR achieved by the P1 prototype on carbon fibers are highly promising and indicate the system's potential for *in vivo* applications. The measured resolution meets the requirements outlined during the initial development phase, while the SNR significantly exceeds the thresholds recommended for adequate discrimination in biomedical imaging^{97,98}. These results underscore the viability of the P1 endomicroscope design for high-resolution, label-free imaging in practical scenarios.

4.2.5 Summary of P1 Endoscope Performance

The table below summarizes the performance of the P1 PARS device:

Specification	Value
General Design	Forward viewing, non-contact, all-optical, multispectral, biocompatible, water-resistant
Footprint	1.6mm maximum OD
Target chromophore	Hemoglobin (532nm excitation wavelength)
Resolution	1 μ m
SNR	22dB (158:1)

Table 9: Summary of P1 EM specifications

4.3 P2 PARS Endoscope Results

The P2 endoscope was developed to address shortcomings observed during *in vivo* imaging trials using the P1 design, particularly in imaging the blood vasculature of a chorioallantoic membrane (CAM) model. The modifications in the P2 design aimed to overcome these challenges by incorporating a lower numerical aperture (NA) and longer working distance distal objective, trading off imaging performance for potential useability *in vivo*. Additionally, the P2 design included features that allowed for reworking the distal components in the event of damage. The design process, outlined in Section 3.5, relied on optical modeling using OpticStudio to ensure adequate performance. To qualify the P2 design, scattering and PARS images were acquired, with the P1 results serving as a

baseline for comparison.

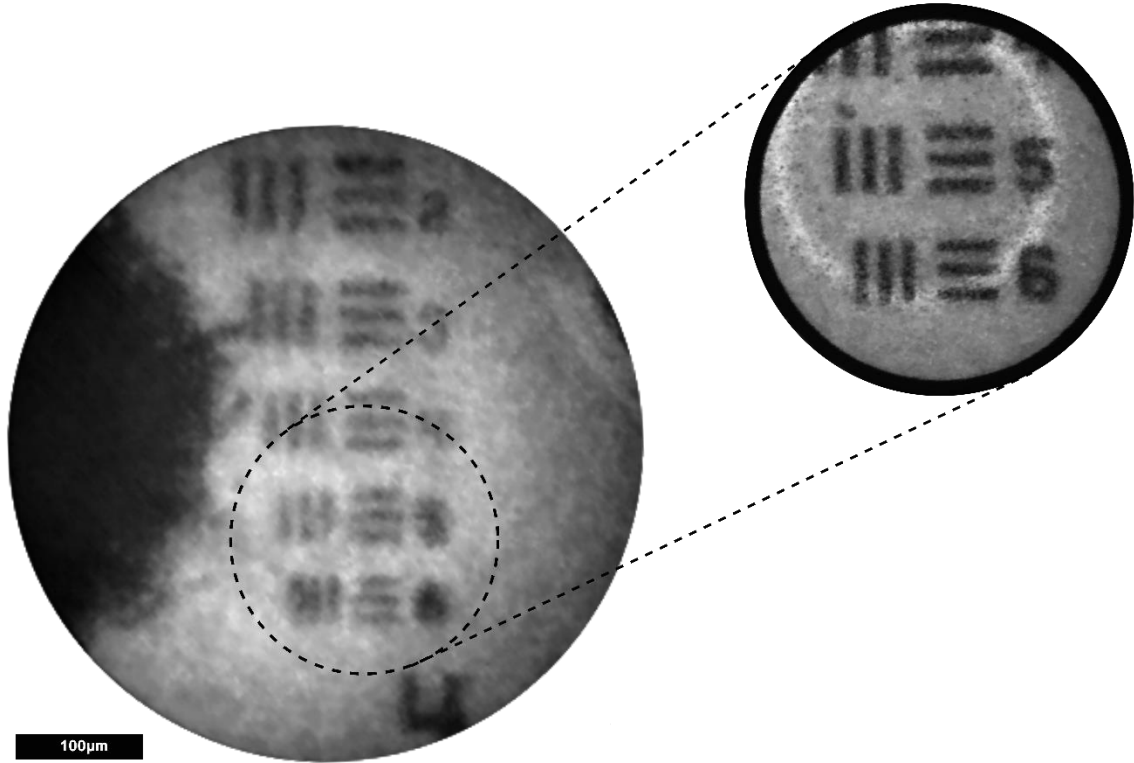


Figure 35: Scattering image of USAF target (group 5, element 5/6) from PARS P2 endomicroscope design. Inset: P1 scattering image for comparison

Figure 36 shows a scattering image of a USAF resolution target (group 5, element 5/6) acquired using the P2 endomicroscope. For comparison, an inset of the P1 scattering image is included. The P2 design demonstrated a resolution of approximately 10 μm , consistent with the predicted value based on optical modeling of the distal objective lens. The image highlights the P2 device's increased field

of view (FOV) compared to the P1, achieved at the expense of resolution. This trade-off was intentional to address identified shortcomings during *in vivo* imaging.

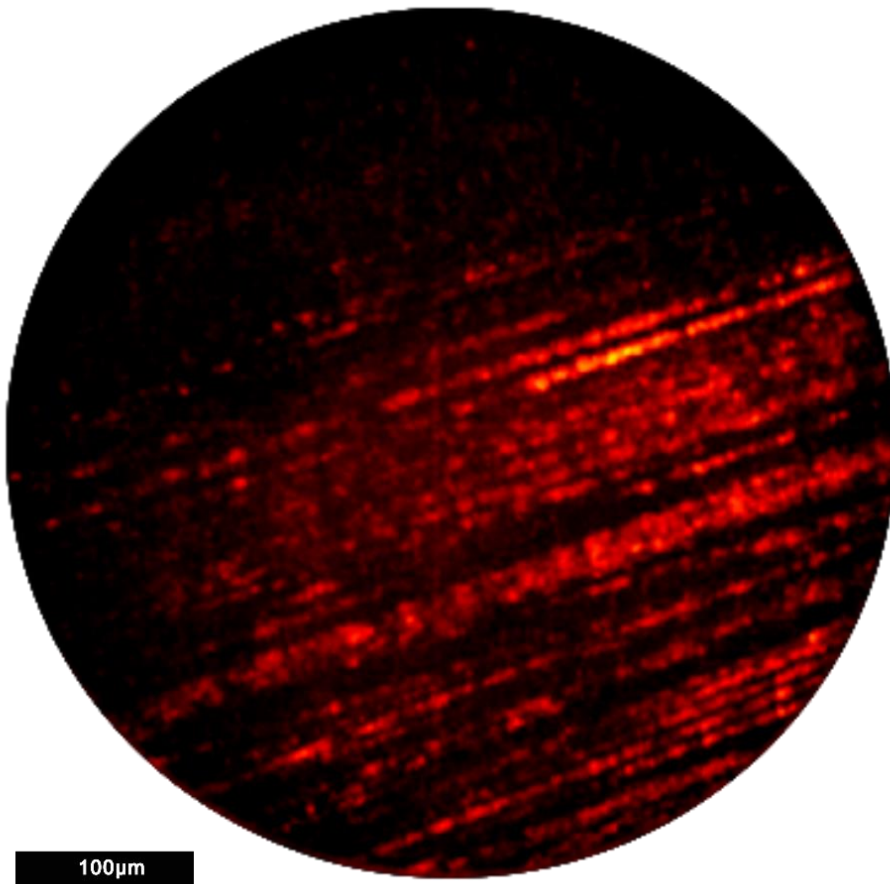


Figure 36: PARS CF images acquired from the P2 EM device showing individual carbon fibre strands showing the design has the capability to resolve down to 7µm.

Figure 37 presents PARS images of carbon fibers acquired using the P2 endomicroscope. The P2 design successfully resolved single carbon fibers within a bundle, achieving resolutions between 7–10 µm. The average pulse energy used during acquisition was 28nJ. These results confirm the device's capability to resolve features comparable in size to blood vessels, such as capillaries, making the resolution suitable for vascular imaging applications.

Although the P2 endomicroscope was tested for *in vivo* application on CAM models, these efforts were also unsuccessful. Further elaboration on these challenges and their implications is provided in the discussion section.

5 Discussion

5.1 Significance of the work

It is very important not to understate the significance of this work. The PARS EM devices represent the first adaptation of photon absorption remote sensing to a form factor suitable for endoscopic applications. The development of the PARS EM devices also serves as proof-of-concept for the miniaturization of PARS technology, showing potential for integration into various clinical procedures. The devices present specific advantages over widefield endoscopy and other endomicroscope designs for vascular imaging.

The PARS mechanism can target chromophores by providing absorption-based, label-free contrast, addressing limitations of scattering-based imaging techniques, which often lack the specificity required for diagnosing many diseases^{21–24,84–87,99}. The PARS images presented in this work demonstrate improved SNR and object discrimination compared to scattering-based images, providing tissue characterization and diagnostic information that current endoscopic technologies cannot achieve.

The PARS EM devices are designed as all-optical, forward-viewing, and non-contact systems. Unlike photoacoustic (PA) endomicroscopes, which are side-viewing due to the use of opaque transducers, the PARS devices are forward-viewing, making them compatible with widefield endoscopes. The P1 device, with an outer diameter of 1.6 mm and a rigid length of 15 mm, is one of the smallest absorption-based endoscope designs reported in the literature^{32,93,100}. This compact design allows it to function as an add-on technology within standard widefield endoscopes, or as a standalone tool in minimally invasive procedures requiring small device footprints, such as a forward-looking guidance probe for large core biopsies, making biopsy procedures safer and more effective for cancer diagnoses^{32,101}. The non-contact design further broadens its capabilities for *in situ*

application, as it allows for imaging where physical interaction is not desirable, such as areas with burns or open wounds.

The IGF used in this study supports wavelengths in the 400 nm to 2 μ m range, enabling the selection of excitation wavelengths to target specific chromophores. This adaptability makes the design suitable for applications such as SO₂ measurements, for which PARS benchtop microscopes have already been shown to be an effective tool (cite). Furthermore, image guides have been developed that are capable of supporting UV wavelengths, such as Fujikura's FIGR- series IGFs, which is capable of 200 nm transmission. This expands the device's potential to PARS-based histology applications including total-absorption PARS (TA-PARS) (cite), further opening the door to clinical applications, most notably virtual biopsies (cite).

In particular, the performance of the P1 device is notable, with a resolution on the order of 1 μ m, which is amongst the highest resolutions demonstrated in literature (cite). This allows the device to resolve biological structures down to the cellular level, including blood and brain cells (cite). The device also demonstrated an SNR of 158:1 (22 dB) on carbon fibre, which shows promise towards the acquisition of highly discriminated biological images. If realized, these capabilities would provide functional imaging performance that exceeds what is currently achievable with widefield endoscopy.

5.2 Shortcomings of the EM Prototypes for *in vivo* application

5.2.1 Distal OL performance considerations

While the P1 design demonstrated impressive performance on carbon fiber samples, it faced significant challenges during *in vivo* trials. Attempts to image blood vasculature in CAM models were unsuccessful, primarily due to limitations in the distal optics, optical configuration, and system design. The distal objective lens of the P1 endoscope has a short working distance (80 μm in water), a consequence of its high numerical aperture (NA) and small lens diameter. This working distance constraint arises from the geometric relationship between NA, working distance (WD), and lens diameter:

$$\text{NA} \approx \frac{D}{2 \cdot \text{WD}}$$

(1)

The high NA of the objective lens improves imaging performance, resolution, and collection efficiency, but it also restricts the working distance. Larger diameter optics could address this issue, but the device's footprint constraints place an upper bound on the diameter, necessitating the trade-off. For shallow samples, or samples where the aqueous layer can be penetrated (like carbon fiber submerged in water), the working distance is practical. However, *in vivo* imaging of CAM vasculature presents difficulties due to target depths that exceed the working distance the device. In CAM models, arteries and veins are typically 100–200 μm below the surface, with capillaries extending to depths of 300 μm (cite). These depths exceed the P1's device capabilities. This limitation prompted the development of the P2 iteration, which sacrifices imaging efficiency for a longer working distance.

To add, the P1's high NA also poses a significant challenge for the PARS signal generation *in vivo*. PARS relies on excitation energy absorption within the focal volume, which depends on the focal depth (DOF) and spot size of the OL design:

$$w = \frac{1.22\lambda}{2NA}$$

(2)

$$DOF = \frac{2n\lambda}{NA^2}$$

(3)

The equations above represent general approximated relationships between various optical parameters in a diffraction limited system, where w is the diffraction limited spot size, λ is the wavelength of light, NA is the numerical aperture, DOF is the depth of focus and n is the refractive index of the medium.

PARS relies on the absorption of excitation energy by the target to produce a response. The energy absorbed is directly proportional to the density of the target molecules within the excited volume. The excitation volume is a function of DOF and spot size, both of which are inversely proportional to NA (equation 2, 3). With a spot size of $1\mu\text{m}$, and an NA of 0.75 for the P1 prototype, the DOF is approximated to be around $2.5\mu\text{m}$ and the resulting working volume around $2\mu\text{m}^3$. Typically, this is not a concern for targets with a high density of absorbers (as is the case with carbon fibre) but becomes problematic when considering a medium with a lower density of absorbers, as is the case with aqueous blood. The blood within a CAM contains red blood cells (RBCs) at concentrations comparable to those found in adult avian blood. The proportion of blood volume occupied by blood cells is expressed as the hematocrit and is typically in the range of 35-50% in avian species (cite). Given the fact that the excitation volume is appreciably smaller than the average

RBC volume (diameter of $\sim 7\mu\text{m}$) means there is at most a 50:50 probability of exciting the chromophore of interest within the working FOV. This further complicates blood vasculature imaging using the P1 device.

5.2.2 Proximal rejection scheme

The proximal rejection scheme implemented in the benchtop design is highly effective at reducing parasitic reflections and improving endomicroscope performance. However, the implementation also results in 50% of the return light being rejected. The IGF cores are not polarization-maintaining, and this ensures that the return light is randomly polarized. While this trade-off is necessary to suppress reflections from the proximal face, losing 50% of the return signal is less than ideal. A more optimal approach would involve separating the forward and reverse propagating light paths without sacrificing signal, such as implementing an optical circulator into the benchtop design.

5.2.3 Damage threshold limitations

One major risk identified during conceptualization and proof-of-concept work was poor efficiency leading to suboptimal performance. All sensing devices have operating ranges, with the lower bound determined by the sensitivity and noise of the system, and an upper threshold, defined by damage thresholds or power limitations. For the PARS endomicroscope prototypes, the upper threshold is determined by the lowest damage threshold component along the optical path. Efforts during design were made to optimize transmission efficiency and reduce the required power necessary for achieving the desired performance to minimize the risk of damage. However, during

testing of the P1 device, signs of degradation were observed at pulse energies as low as 0.1 μJ at 3ns pulse widths (PW), and 60kHz pulse repetition rates (PRR). This threshold is well below the experimentally determined damage threshold of the IGF, which is upwards of 1 μJ pulse energies at the similar PW and frequencies. The observed damage patterns, consistent with stress birefringence, suggest failure at the glue interface between the IGF and the distal lens optics. This issue was confirmed by the endomicroscope vendor and addressed by replacing the original epoxy (EPO-TEK 301-2) with an alternative epoxy (EPO-TEK 310) with higher damage thresholds, as reported in literature (cite). This modification increased the theoretical upper threshold of the endoscope system, enabling the use of higher pulse energies during experiments.

5.2.4 Multimode propagation, IGF

Lastly, the multimode nature of the IGF introduces an additional challenge. IGFs are designed to be multimode, enabling them to support a wide range of wavelengths and improve coupling efficiency across the bundle. Multimode fibers also allow for the delivery of higher optical power, which is advantageous for imaging applications. However, multimode propagation can become a bottleneck for high-resolution imaging systems. A beam intensity profile that deviates from an ideal Gaussian increases the beam propagation ratio (M^2) leading to a larger minimum spot size thus reducing the focusing quality of the optical system. *Figures 38 and 39* below show the beam profiles of 532 nm and 830 nm light output from the IGF cores.

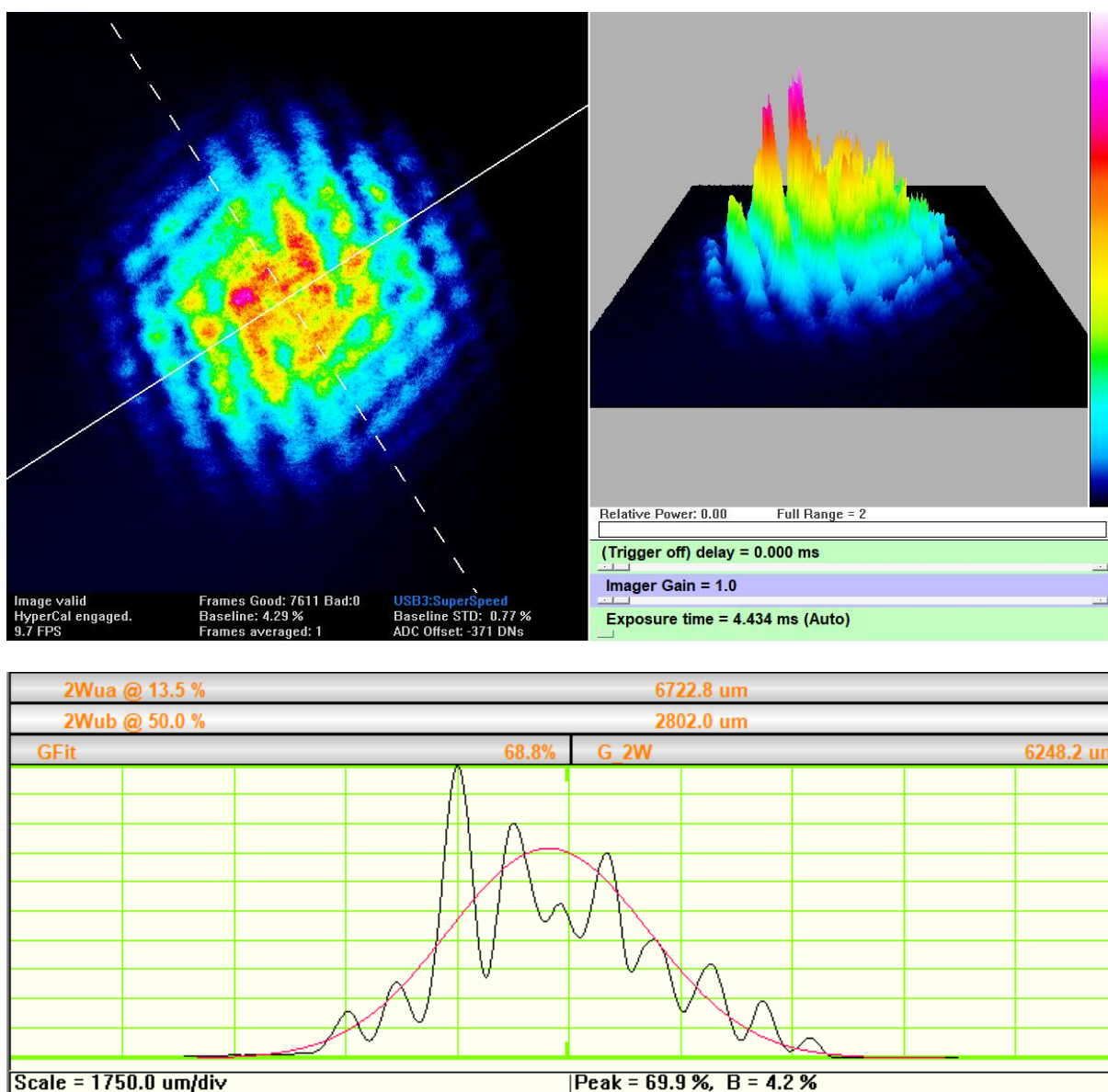


Figure 37: Beam profile images of 532nm output from IGF core

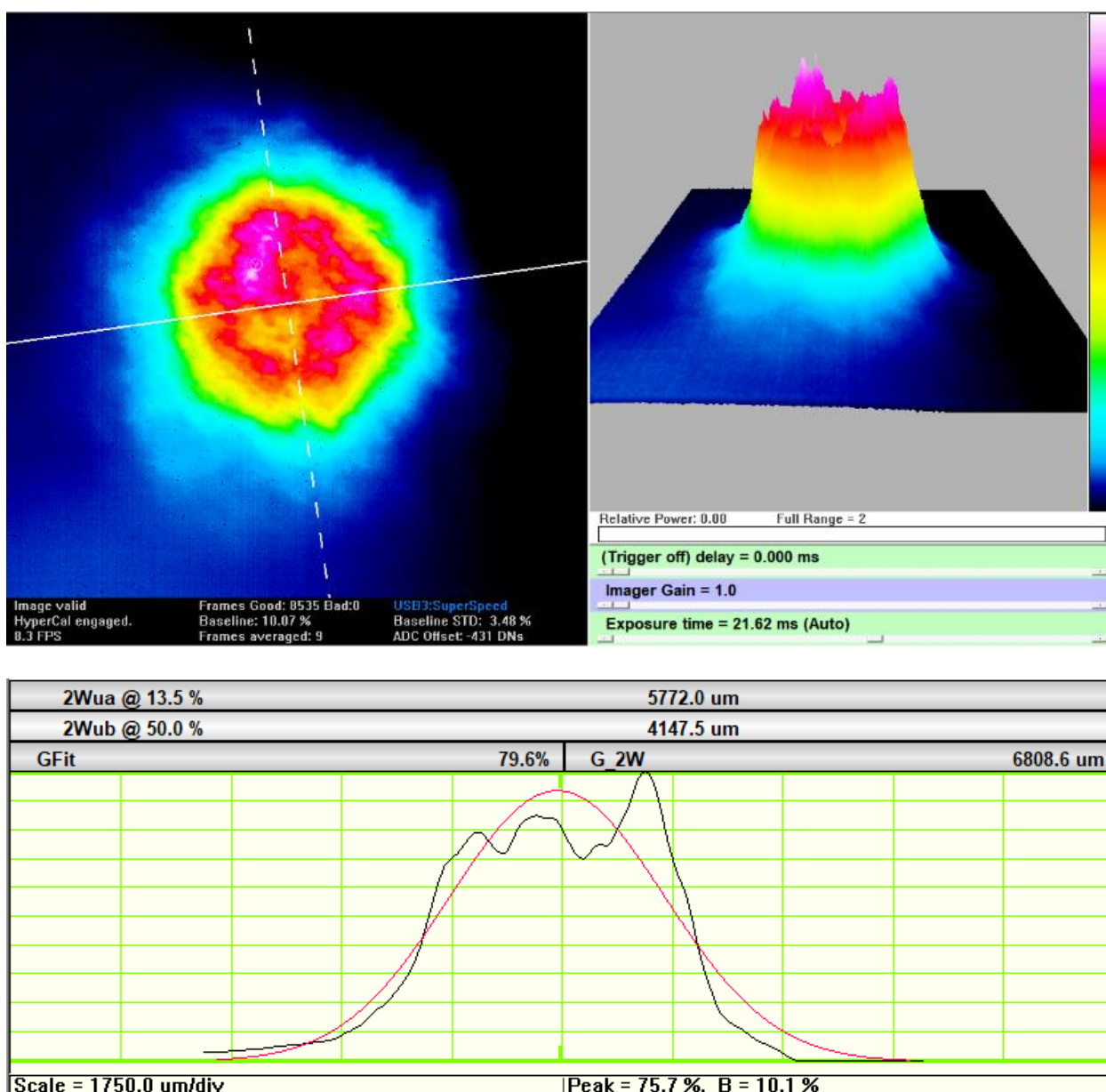


Figure 38: Beam profile images of 830nm output from IGF core

The 1D and 2D intensity profiles reveal significant deviations from an ideal Gaussian distribution in at the output of the IGF, particularly for 532 nm output, which suffers from beam degradation due to the presence of higher-order modes. This deviation adversely impacts the spot size

and imaging performance at the object plane, which imposes a limitation on the IGF PARS devices. Implementing a design that utilizes a single-mode fibre for beam propagation would help mitigate the adverse effects of higher-order modes on PARS signal generation and overall imaging performance.

5.3 PARS DCF Concept

To address the shortcomings of the IGF-based PARS EM design for blood vessel imaging, an alternative concept utilizing a double-clad fiber (DCF) is proposed. This design introduces several features to optimize efficiency, improve imaging quality, and provide additional clinically relevant information. The DCF-based concept is outlined below, along with its key design elements.

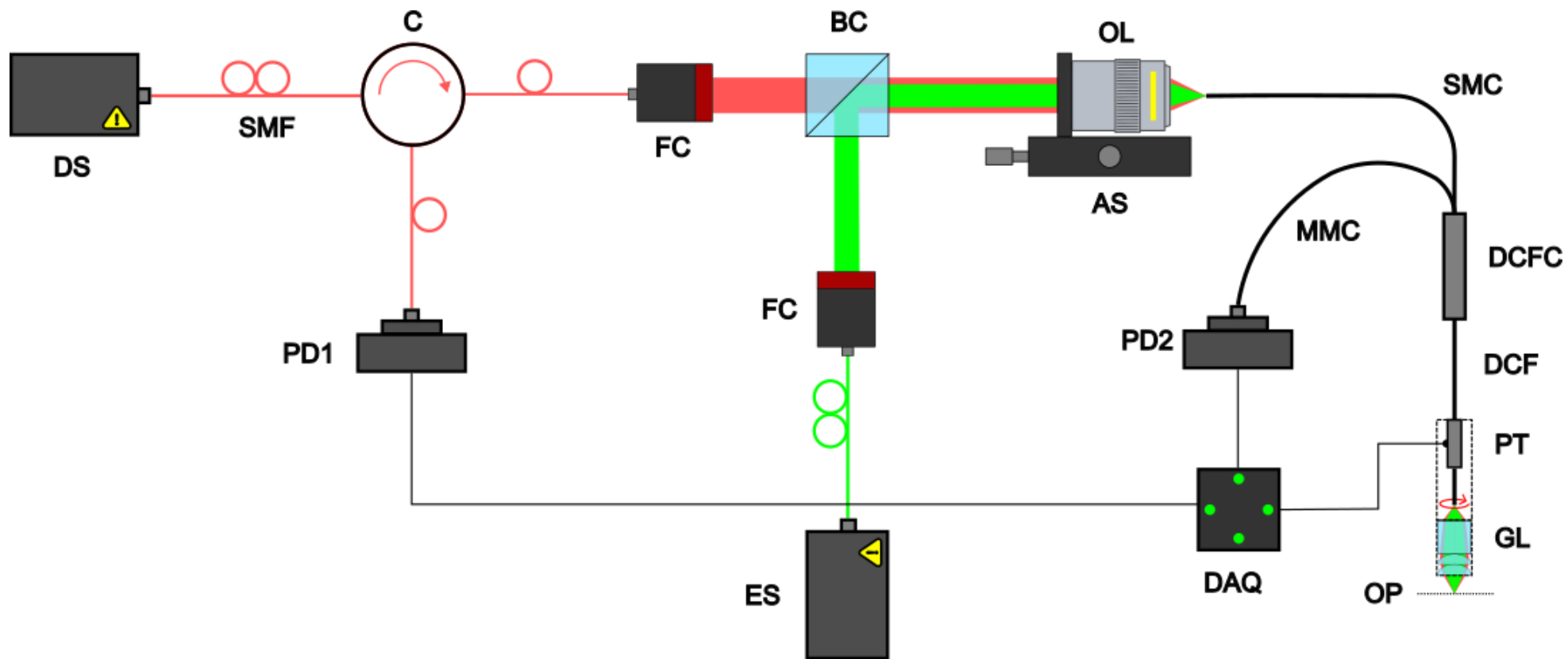


Figure 39: PARS EM design using double-clad fibre in combination with coupler to target blood vessels

The proposed DCF configuration features a circulator to separate forward and backward-propagating specular reflections collected through the single-mode core (SMC), enabling detailed, high-resolution imaging. Additionally, a DCF coupler is incorporated to separate diffuse backscatter collected through the higher-NA multimode outer core. This dual-channel approach optimizes efficiency and provides two streams of complementary information that can be combined to deliver clinically meaningful insights. The design also includes a distal scanning mechanism, driven by a piezoelectric tube (PT), with a GRIN lens objective assembly for optimized PARS imaging performance. Similar configurations have been explored in the literature and shown to provide proven solutions for endoscopic imaging^{93,102,103}.

5.3.1 Optimized DCF PARS Design for blood vascular imaging

Excitation and Detection Sources

The excitation source (ES) for the design concept is flexible, and the system can be designed around a suitable wavelength that optimizes performance. For example, the DCF PARS design allows for excitation wavelengths in the 400–450 nm range that can target the Soret region, where hemoglobin absorption is maximal, and scattering length is shortest (cite). For detection, wavelengths in the NIR region (800–900 nm) are ideal due to their minimal absorption in water and hemoglobin and the availability of low-cost, compatible optical components. Shorter detection wavelengths are generally preferred for their higher resolution capability, while the 800–900 nm range provides a practical optimization for imaging performance.

Double-Clad Fiber Coupler

The DCF and DCF coupler (DCFC) offer a sophisticated solution for multiwavelength light transmission and collection. The single-mode core (SMC) transmits the forward-propagating

excitation and detection beams, maintaining high beam quality essential for high-resolution imaging.

Backward-propagating light is separated by the DCFC based on the NA of the reflections:

- **Specular backscatter** is collected in the single-mode core for detailed imaging.
- **Diffuse backscatter** is collected by the multimode outer core, which has a higher NA, improving overall collection efficiency and capturing deeper scattering signals.

This dual-path design provides valuable information about the sample, with diffuse and specular signals being detected separately and compared for further characterization.

Image Formation and Focusing

Images are formed by raster scanning the cantilevered distal end of the fiber using a piezoelectric tube. The distal optics consist of a GRIN lens objective assembly similar to that used in the IGF-based design, with performance further optimized for *in vivo* imaging based on the limitations of the IGF PARS trials. This overall approach to image formation, although more complex, has been validated in previous research as a robust solution for PARS image acquisition (cite).

Beam path and coupling

An achromatic objective lens (OL) in combination with an alignment stage (AS) is used to couple both excitation and detection beams into the single-mode core. A dichroic mirror acts as a beam combiner (BC) to separate and combine the excitation and detection wavelengths, similar to the IGF design. A circulator (C) separates forward and backward-propagating detection beams, offering a robust fiber-based architecture with minimal losses. Finally, the backscattered light is detected by two separate photodiodes (PD1, PD2), enabling the formation of two complementary images for detailed sample characterization.

The proposed DCF-based design addresses critical limitations of the IGF configuration for *in vivo* imaging, including improved efficiency through multimode collection of diffuse backscatter, single mode detection propagation and output and the use of a circulator to separate forward and backward detection propagation. By combining dual-channel imaging with optimized excitation and detection configurations, this design represents a significant step toward improved performance in vascular imaging applications.

6 Conclusion

This thesis presents the first successful demonstration of PARS imaging through a flexible, miniaturized endomicroscope device designed for integration into clinical endoscopy systems. By achieving forward-viewing, non-contact, label-free imaging within a <2 mm form factor, this work establishes a foundational platform for the clinical miniaturization of PARS and significantly advances the field of absorption-based optical endomicroscopy.

The PARS endomicroscope prototypes, P1 and P2, validate the compatibility of the PARS mechanism with flexible image-guiding fiber architectures. Notably, the P1 design demonstrated an impressive resolution of $\sim 1\ \mu\text{m}$ and a signal-to-noise ratio of 22 dB (158:1) on carbon fiber phantoms — performance metrics that rival or exceed those reported in state-of-the-art optical endomicroscopy systems. These results confirm the feasibility of high-resolution PARS imaging through IGFs and open new avenues for absorption-based imaging in scenarios where conventional scattering-based or contact-dependent modalities fall short.

This work also confronted key challenges. These included limited working distances associated with high-NA optics, reduced excitation volumes due to multimodal beam propagation in IGFs, and damage thresholds imposed by distal adhesive interfaces. In response, the P2 prototype was developed with a lower NA and extended working distance, trading resolution for increased *in vivo* usability. Design iterations and mitigation strategies are discussed in detail, offering a robust technical roadmap for future device development.

Importantly, the results highlight the potential for advanced clinical applications. The design supports multispectral imaging and can be adapted for blood oxygenation measurements (SO_2) by targeting hemoglobin's Soret band, as demonstrated in previous publications²⁴. With the development of a UV-capable configuration, *in situ* PARS-based virtual histology becomes possible^{22,99,104},

expanding this platform's utility to tissue classification, surgical guidance, and even brain imaging^{23,87,104}. These directions represent compelling paths forward, particularly as PARS imaging continues to evolve toward video-rate acquisition and real-time diagnostic feedback¹⁰⁵.

In summary, this thesis lays the foundation for a new class of endomicroscopic devices by demonstrating, for the first time, PARS imaging through a clinically relevant form factor. It establishes a benchmark for resolution and SNR in label-free vascular imaging and offers a blueprint for translating this technology to practical *in vivo* diagnostics. Continued development and validation, particularly in live animal models and eventually human trials, will be critical in realizing the clinical impact of PARS endoscopy.

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