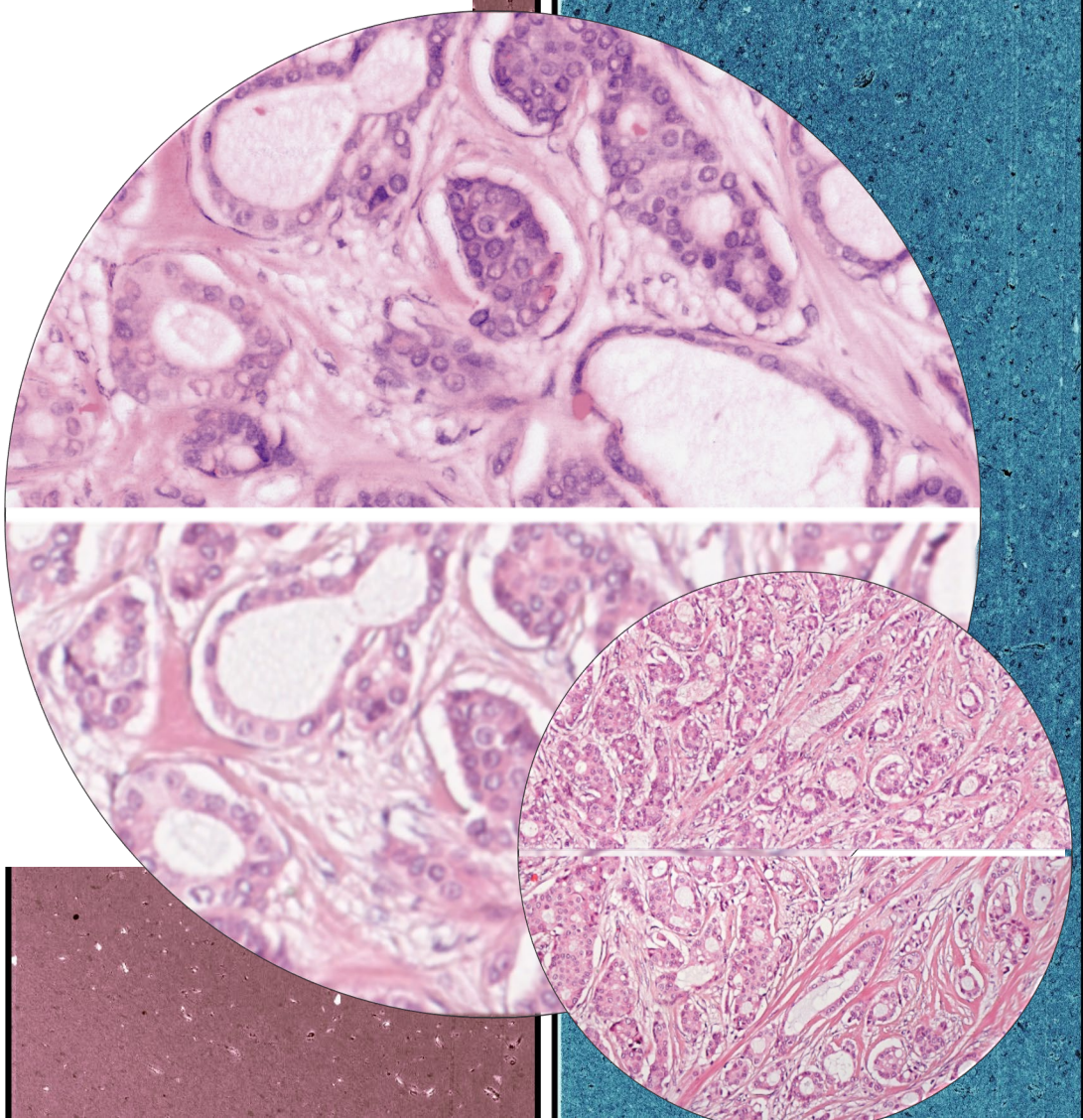
illumiSonics

Introduction to PARS© Technology©2024

Transforming the way the world looks at tissue

JANUARY 2022



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PARS® Technology

illumiSonics created PARS, a revolutionary non-contact, high-resolution, label-free, non-destructive reflection-mode microscope. PARS captures all three light-matter interactions (scattering, absorption, and emission) to give unprecedented 3D images of fresh and living tissues.

Value Proposition

PARS improves cancer patient outcomes, enables cost savings, and simplifies the diagnostics workflow.

3D Microscopic Histo-Pathology Imaging without sample preparation for *In-Situ* Diagnostics. Compresses the 3-14 day workflow into 5 minutes.

Key Messages

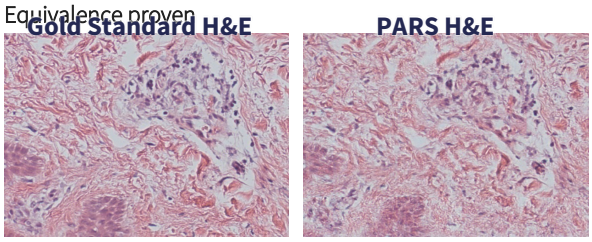
PARS improves cancer patient outcomes. PARS allows diagnostics during surgery, more precise resections, and avoids repeat surgeries. PARS reduces the risks to the patient since the patient spends less time under anesthesia.

PARS reduces costs. PARS enables up to 50% higher operating room utilization for surgeons/hospitals and up to 2x throughput for labs.

PARS improves the diagnostic workflow. PARS simplifies logistics since diagnostics are done at point-of-care and the tissue container is automatically traced.

Technical Readiness Level

Equivalence proven



Next Milestones

Q4/2023 FDA Submission Mohs system
Q2/2025 FDA Submission In-Surgery system
Q2/2026 FDA Submission Endoscopy system

Broad Patent Protection

illumiSonics owns all PARS imaging IP

11 US and 3 DE issued patents, 15 additional published and unpublished patents across 8 patent families. IP filled in US, CA, IL, AU, CN, JP, UK and major EU markets.

Scientific & Clinical Validation

>30 peer reviewed publications on PARS imaging technology in high impact journals. Market research with Mohs Surgeons confirms clinic-ready images.

Large Primary Markets

Histology is a >$7 B Addressable Market

PARS is the only label free imaging system to generate histology images directly from fresh tissue. These rapid H&E- diagnostic images will disrupt histopathology sub-markets, intraoperative cancer margin assessment & quality assurance of clinical biopsies.

Better than standard histology: Because PARS measures the total light, it reveals the biology and fine structural detail of lipids, mucin, brain axons and dendrites, fibrin, and collagen which are lost in H&E.

Experienced Management Delivers

Led by Rocky Ganske, CEO with over three decades of senior level corporate, start-up, regulatory & engineering expertise. Direct experience in fundraising, IPO, and corporate deal flow with Fortune 100 & international companies. Jochen Schweizer, COO, previously led the micro-surgery business at Leica/Danaher and Product Management at Zeiss. Vladimir Pekar, PhD, Director of Software Development and AI, has significant background from Philips and Perimeter Medical Imaging AI, and a complementary science team and support staff.

Board

Parsin Haji Reza PhD, Founder; John Mackey M.D., Oncologist, CMO; Kevin Fahey PhD, Past Executive Zeiss & Thermo-Fisher; Bruce Johnson, Founder & Prior CEO Intuit Canada/UK, Chair of Alberta Machine Intelligence Institute.

Business Strategy

We are exploiting PARS platform technology to change the way the world looks at tissue. Our initial strategy targets 3 markets that we will dominate with rapid H&E from fresh tissue, followed by targeted products in the >US$7B Clinical Histopathology market.

First Three Selected Markets We Will Dominate

1st Market: Mohs Skin Cancer Surgery removes the smallest amount of tissue while confirming that all the cancer is removed. Mohs is unique in that the Surgeon, Pathologist and Business Person is the same individual. This is a large defined market ($500M+ in the US alone with 875,000 procedures annually). PARS images resected tissue in 5 minutes, while frozen-section H&E images takes ~30 minutes; will improve treatment efficiency by up to 30% and reduce patient time in office by up to 50%.

2nd Market: In-Surgery Cancer Margin Assessment allows Surgeons and Pathologists to see the cancer in what was removed and what was left behind, while the patient is in the operating room. Providing the same gold standard images in fresh tissue in 5 min. without damaging the tissue. There is >$14B in cost for repeat 2nd surgeries due to Positive Surgical Margins after the initial surgery in the US alone.

3rd Market: Endoscopy provides *in-situ* imaging to determine what needs to be cut before the first excision is made, opening solutions for micro and robotic surgeries (a >$20B market in the US).

Ultimately, PARS imaging digital pathology has the potential to replace the gold standard of formalin-paraffin-embedded H&E diagnosis and handling of resected tissues (a >$7B+ market). The illumiSonics pipeline includes products in Ophthalmology, Pre-Clinical and Clinical Research, Surgical Guidance, and Material Sciences as opportunities for products, partnerships, and licensing.

Established Infrastructure

Extensive lab infrastructure: Human tissue, animal surgery, cell culture, histology, eye imaging, BSL-2 facility, opto-engineering, and an incredible team led by Parsin Haji Reza PhD who discovered and pioneered PARS imaging.

Key Partnerships

EyeStart Ophthalmology, Microsoft®, University of Waterloo PhotoMedicine Labs, Grand River Hospital.

Investment to Date

illumiSonics raised $5.9M in a seed round and has received ~$2M in non-dilutive funding. Seeking "A" round $30M to bring a first histology product, Mohs Surgery, through FDA approval and further support R&D pipeline progression.

What is Histology and Why is it Important?

- Histology is a routine clinical and research lab technique used to study the structure of cells and tissues using a transmission light microscope.
- In medicine, histology includes the microscopic identification and study of diseased tissues, and is critical to cancer diagnosis (*e.g.,* tumor grade, cancer classification), monitoring, prognosis, and developing a treatment plan.
- Histopathology is the Gold-Standard to determine if any cancer remains along the margin of the resected tumor after surgery. This is called a positive surgical margin (PSM) and, if left, can trigger failure of cancer treatments.
- Histology is used for analyzing the effects of different treatments and is invaluable for pharmaceutical testing.
- The current gold standard for histopathology is the assessment of thin, stained tissue sections with a standard transmission light microscope (see page 4).

Global Cancer Burden:

- Cancer is a leading cause of death globally, responsible for nearly 10 million deaths in 2020. In the next 20 years, the global cancer burden is expected to increase by 47%, amounting to 28.4 million cases by 2040.
- Of the 15 leading causes of death globally, cancer has the highest economic loss worldwide, costing in the trillions globally due to premature death, disability, and direct treatment costs.
- Surgical excision is an integral part of treatment for most solid tumors, with negative 'cancer-free' margins among the strongest indicators of success and good patient outcomes. It is very difficult to determine exactly how much peri-tumoral tissue to remove or to confirm during the procedure if a negative surgical margin has been achieved. Once the surgery is complete, resected samples are sent to the lab for histopathological analysis. The patient is contacted within days or weeks to confirm if all the cancer has been removed.
- Currently, initial cancer resection surgeries leave a positive surgical margin up to 60% of the time. Positive surgical margins frequently require re-excision surgeries (if feasible) and/or additional treatments (*e.g.,* chemotherapy, radiation). This increases health-care costs, health risks, the potential for complications, and extends recovery times while adding physical and psychological stress for patients.
- PARS enhances the standard histological workflow and will transform the diagnostic workflow by providing ***in-situ*** diagnostics using an endoscopic device. PARS *in-situ* diagnostics will enable to surgeon to make an informed surgical plan before making the first incision, saving costs, operating time, reducing patient risks, and providing reliable results immediately rather than in days or weeks. Intraoperative frozen section analysis (see page 4) is costly, pauses surgeries for between ~45-60 minutes for each assessment, during which time the patient is exposed to anesthesia risks, and is not considered definitive (*i.e.,* the gold standard is still required postoperatively to confirm margin status).

Why is PARS ® Important for Histology?

- Rapid histology of fresh tissue is not clinically available, making it very difficult for surgeons to accurately assess, during surgery, if all the cancer has been removed.
- PARS cellular-resolution imaging provides rapid histology-like fresh tissue imaging confirm negative surgical margins *during* resection surgery (i.e., intraoperatively). This would:
    - Drastically reduce re-operation rates (saving costs, lessening risks, and freeing up the operating room and medical staff for other procedures. Meanwhile, the patient can return home knowing their surgery was successful);
    - Shorten operation times (see intraoperative frozen section analysis, page 4), decreasing anesthetic complications while increasing throughput of surgeries per day per operating room;
    - Minimize loss of healthy tissue, improving cosmetic results by allowing the surgeon to pursue a more precise resection.
    - Reduce the need for chemotherapy and/or radiation treatments for positive margins;
    - Provide digital diagnostic quality images for pathologic assessment.
- With the potential for PARS to improve surgical oncology, we have launched a clinical study of PARS histological images to assess tumor margins.

What is Gold Standard H&E?

- The gold standard for cancer diagnosis and postoperative assessment of surgical margins is the histopathologic assessment of formalin-fixed, paraffin-embedded tissue.
- The standard histological workflow requires resection, chemical stabilization with a fixative, embedding in paraffin, slicing, mounting on glass slides, and staining with Hematoxylin and Eosin dyes (H&E), followed by light microscopy qualitative analysis by a trained pathologist (see Figure 1).

Gold-Standard Drawbacks:

- Time- and resource intensive: this process can take from days to weeks before a diagnostic report can be issued. During this time, treatment is delayed.
- Expensive: At a cost of between ~$10 to $60 per slide, with ~300 million slides prepared in the US annually, the gold-standard histology amounts to a several billion-dollar burden.
- Variability in H&E-stained slides can result from staining time, cross-contamination with other dyes, and changes in pH due to storage. Such variability can display as a difference in contrast and requires each pathology lab to undergo regular standardization procedures.
- The standard histopathological workflow limits the amount of resected tissue that can be assessed: only a very small portion of excised tissues can be processed (for breast cancer ~2%). Small samples may not be fully representative and may exclude important and informative tissues; this not only leaves out crucial information, but it may also result in an inaccurate understanding of the full extent of the disease.
- Cannot be used intraoperatively: Due to slide preparation and analysis taking days to weeks to complete, feedback cannot be obtained during surgery. As a result, surgeries are completed, then resected samples undergo a postoperative histological analysis to confirm positive or negative surgical margins.

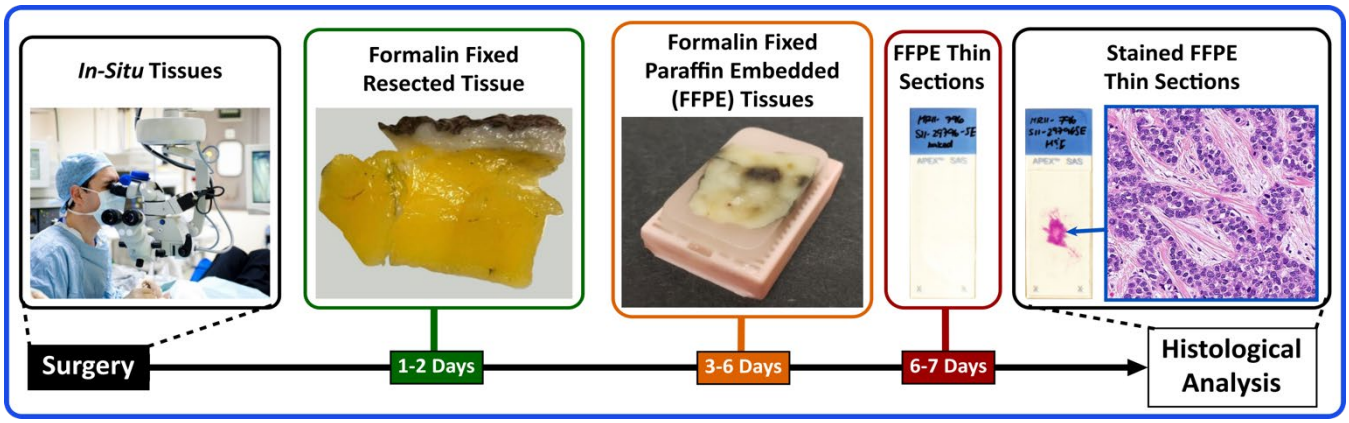


Figure 1: This outlines the processing workflow and average timeline to perform the gold standard histological analysis on resected tissue specimens.

Gold Standard for Intraoperative Margin Assessment:

Frozen Section Analysis (FSA) is the gold-standard for assessing surgical margins intraoperatively. Resected tissues are embedded, cooled, frozen, sliced, stained with H&E, and mounted for histological analysis. Each assessment takes ~45-60 minutes, prolonging operating times, cost, and increasing anesthesia risks. Additional rounds compound these issues. Freezing introduces artefacts, rendering histological interpretation difficult. Only a portion of the margin can be assessed, potentially decreasing margin determination accuracy and contributing to the likelihood of leaving PSM. FSA has a false negative rate up to 36%.

How Does PARS Work?

- PARS focuses a picosecond scale pulsed excitation laser into biological tissues to generate radiative relaxations (*i.e.,* optical emissions), non-radiative relaxations (*i.e.,* heat and pressure), and scattering effects in the sample.
    - Absorbed photons are captured by chromophores and converted into different forms of energy that are emitted from the sample as non-radiative and radiative relaxations, while scattered photons continue moving and interacting with other portions of the sample.
    - Non-radiative relaxations are recorded using a secondary confocal interrogation beam co-focused and co-scanned with the excitation spot, enabling temperature and pressure changes to be detected at the source. These changes are registered as modulations in backscattering intensity, which are then directly correlated to the local non-radiative absorption contrast.
    - The unperturbed backscatter (pre-excitation event) simultaneously captures the optical scattering contrast.
- PARS is the only modality visualizing all three contrasts.
- PARS can create H&E images that include detail of lipids, mucin, brain axons and dendrites, fibrin, and collagen which are traditionally lost in the processing required for H&E.
- Molecular PARS (mPARS) is being developed to image and quantify specific biomarkers for molecular pathology. By enabling concurrent molecular and histologic imaging, mPARS will provide immunohistology in the future.

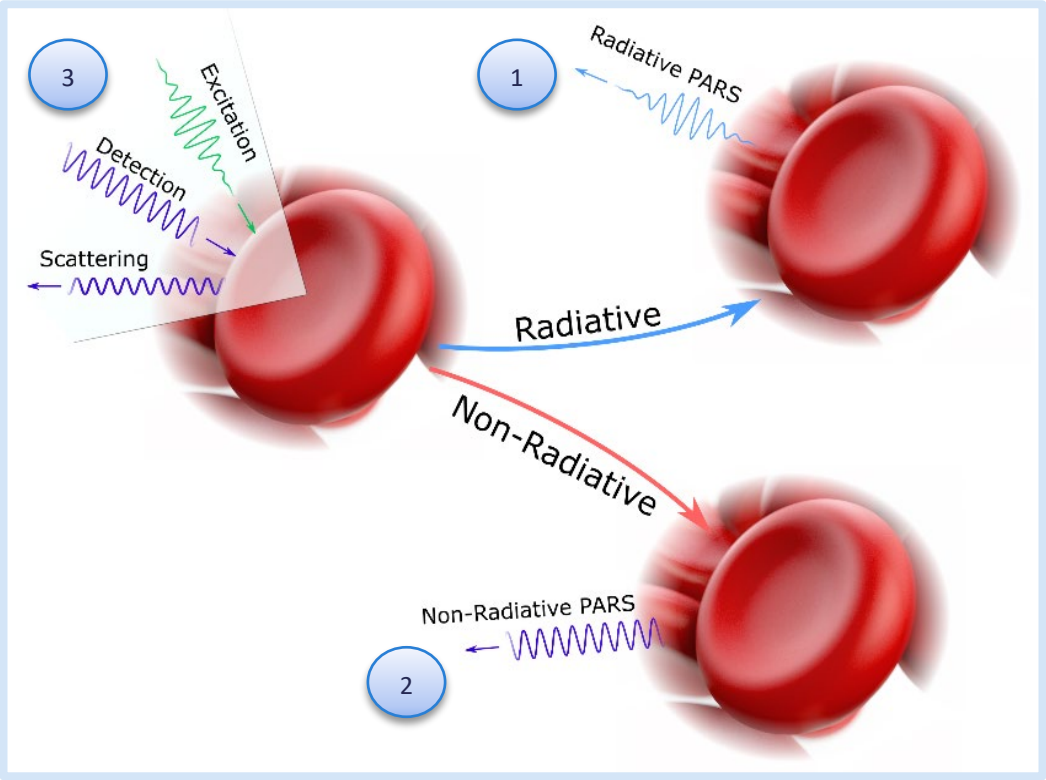


Figure 2. Three PARS contrast mechanisms generated by the excitation laser.
1. The radiative absorption pathway captures optical emissions attributed to radiative relaxation (*i.e.,* optical emissions).
2. Non-radiative relaxation leads to heat and pressure induced modulations, both of which cause back-reflected intensity variations in the detection beam.
3. The local scattering contrast is captured as the unmodulated backscatter (pre-excitation pulse) of the detection beam.
PARS captures all three dimensions simultaneously with a single photodiode.

What Does PARS® Offer?

PARS acquires H&E-like diagnostic quality images from fresh tissues in a single acquisition:

- PARS microscopy is an all-optical, fast, deep, label-free, non-contact, cellular-resolution, reflection-based optical imaging technique that gives 3D multilayered diagnostic-quality histopathology in the same H&E format that pathologists are already trained in.
- PARS can image anything that absorbs light. By leveraging radiative and non-radiation relaxations as well as optical scattering, PARS can visualize most light-matter interactions, providing morphological details and visualizing a wide range of light absorbing molecules, including DNA, RNA, lipids, hemeproteins, collagen, and fibrin.

PARS does not require contact with the sample:

- An important feature that distinguishes PARS from conventional photoacoustic modalities is that it operates fully non-contact, allowing it to visualize the optical absorption and optical scattering contrasts in suspended media without any acoustic coupling (*e.g.,* water or ultrasound gel) and without the need for a transducer.
- PARS can be used in clinical applications where conventional photoacoustic imaging cannot. This includes where contact is impractical (*e.g.,* during surgery, ophthalmology) or where the working space (*e.g.,* surgical microscope) and footprint (*e.g.,* endoscope) are limited.

PARS is non-destructive:

PARS has successfully visualized tissue structure in unstained tissue slides, frozen sections, tissue blocks, and even fresh bulk tissues without affecting further processing. PARS uses a low-power dosage that is below ~3.8mJ/cm^2 to ensure that PARS is non-destructive to tissues and can be used at every step of the histological workflow, facilitating clinical adoption and easy integration into existing practices.

PARS can image thick tissue samples:

- PARS employs a reflection-mode architecture which enables it to provide rapid cellular structure visualizations of optically thick samples.
- This capability makes it possible to image freshly resected tissue without sectioning and enables *in-situ* clinical applications (e.g., *in-situ* surgical microscope, endoscopy).

PARS does not require labels or dyes:

- PARS does not require the use of labels (*e.g.,* dyes) since it directly images the endogenous absorption of tissue. Because it is label-free, it can be used safely and quickly *in-situ* and intraoperatively.
- If labelling is present, PARS will image labelled tissue as well.

PARS is capable of 3-Dimensional Imaging and Optical Sectioning:

PARS can perform 3D imaging and optical sectioning. PARS provides 5µm virtual slices up to 100µm below the surface, which is the equivalent of ~20 prepared slides, saving time and resources.

PARS is capable of Real-Time Feedback:

- Unlike conventional photoacoustic modalities, with PARS, speed is not limited by the time it takes for sound waves to reach the sample surface where they can be detected by a transducer. Instead, real-time feedback is possible because PARS uses a detection laser to visualize chromophores right at the source.

PARS© Histology:

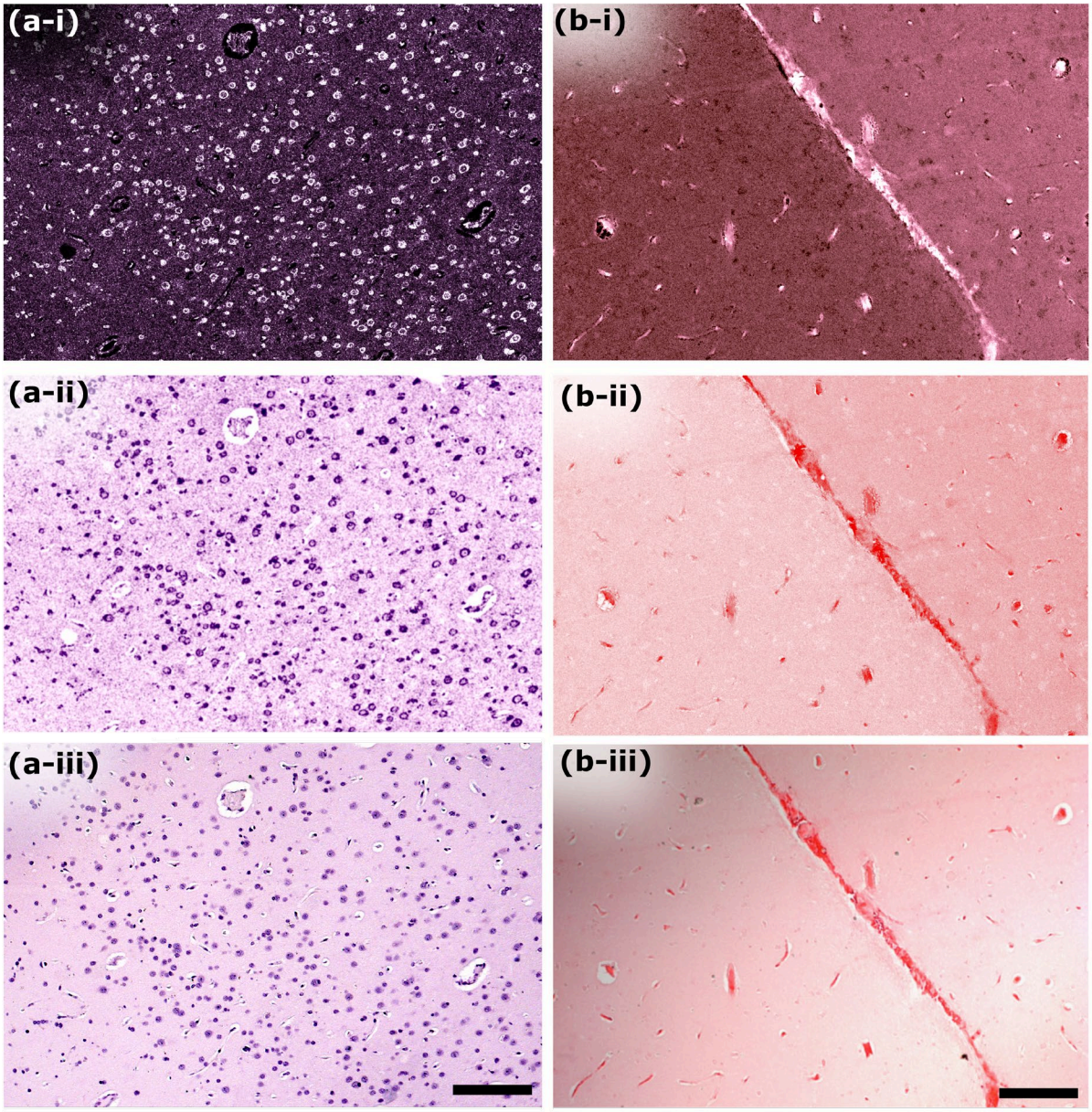


Figure 3: Comparison of PARS non-radiative and radiative contrast to Hematoxylin and Eosin staining.
(a-i) PARS non-radiative absorption contrast, highlighting predominantly nuclear structures.
(a-ii) PARS non-radiative absorption contrast, with reproduction of hematoxylin color mapping. A false colored version of the image presented in (a-i).
(a-iii) The same section of tissue, stained with hematoxylin stain only, and imaged with a brightfield microscope. This provides a one-to-one comparison with non-radiative PARS.
(b-i) PARS radiative absorption contrast, highlighting predominantly extra-nuclear structures (i.e., collagen, elastin. NADPH).
(b-ii) PARS radiative absorption contrast, with reproduction of eosin color mapping. A false colored version of the image presented in (b-i).
(b-iii) The same section of tissues, stained with eosin stain only, and imaged with a brightfield microscope. This provides a one-to-one comparison with radiative PARS.

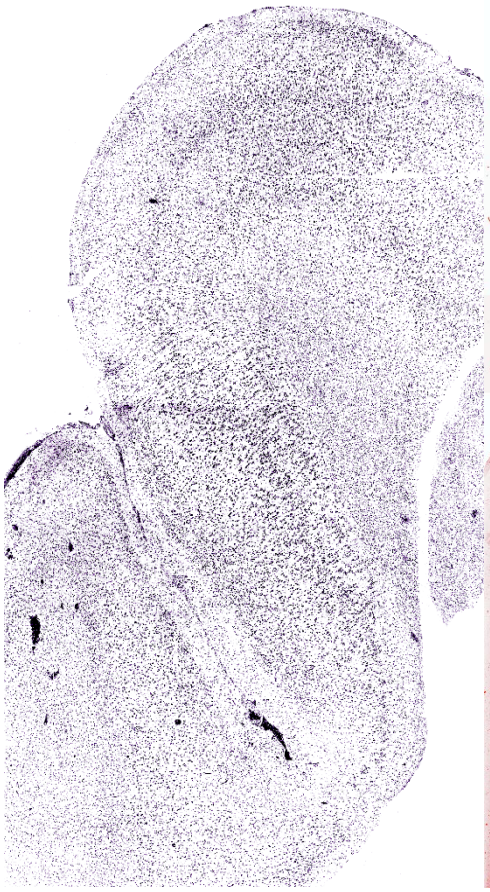


Figure 4: PARS image shows non-radiative contrast imaging of a thin section of FFPE human brain tissue, providing visualizations similar to hematoxylin (H) staining. Image is artificially colored to represent hematoxylin staining contrast.

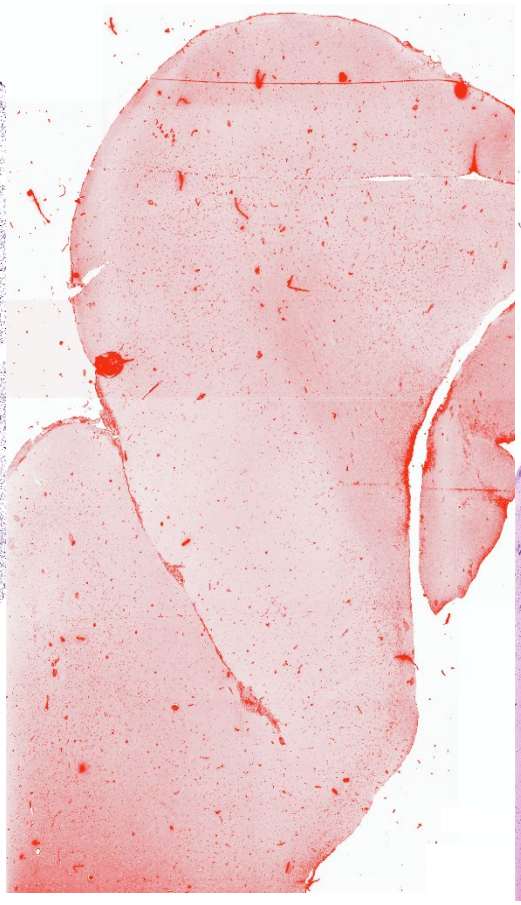


Figure 5: PARS image shows radiative contrast imaging of a thin section of FFPE human brain tissue providing visualizations similar to eosin (E) staining. Image is artificially colored to represent eosin staining contrast.

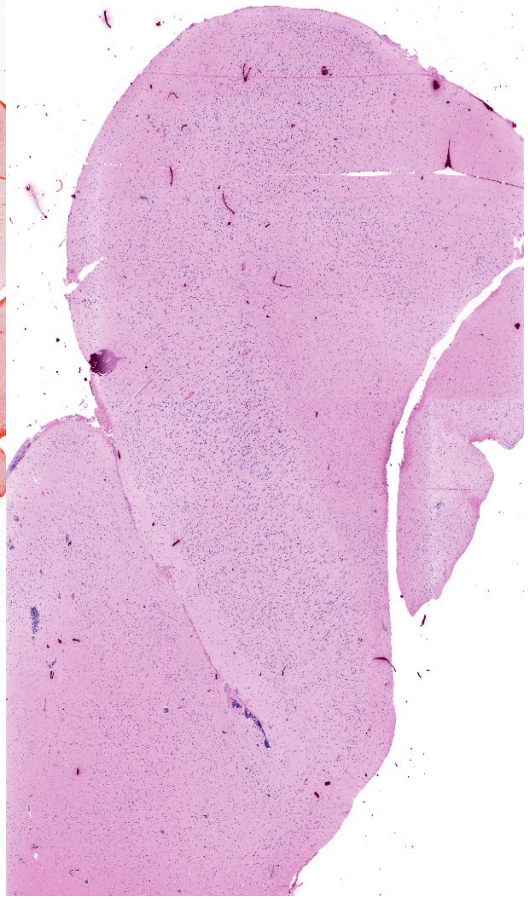


Figure 6: PARS image shows a reproduction of both H&E staining of a thin section of FFPE human brain tissue.

With PARS microscopy, we can virtually stain tissues using UV light and color match them with hematoxylin (H) and eosin (E). We no longer need to go through the rigorous tissue processing and staining workflow to achieve gold standard H&E images.

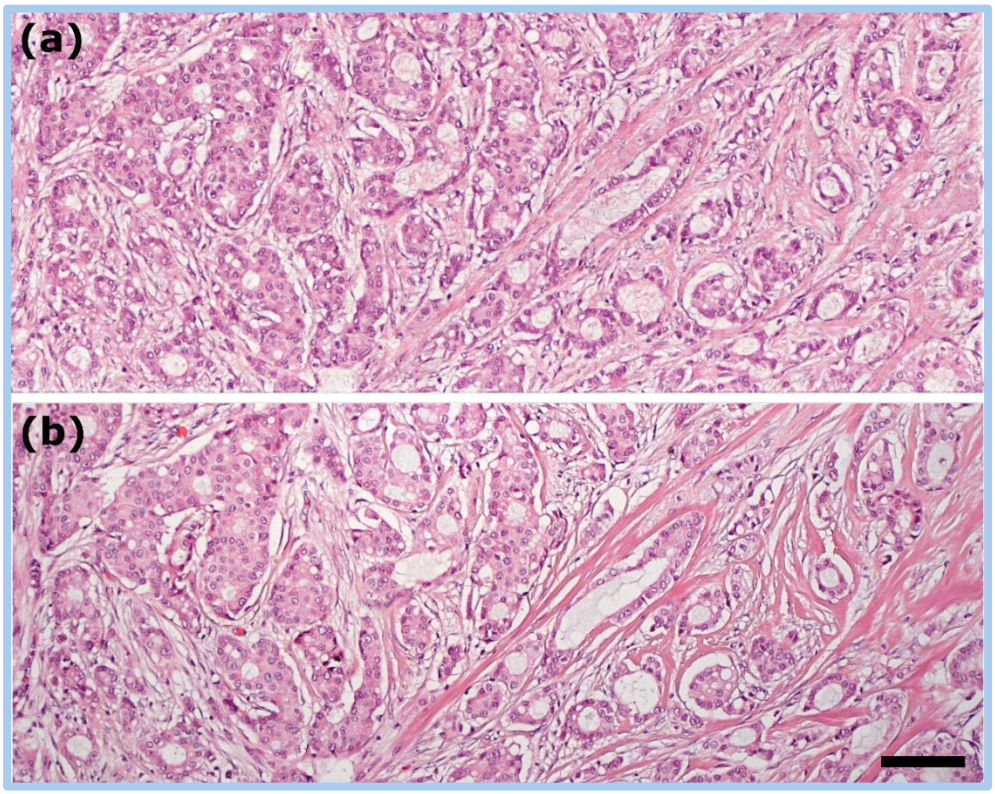


Figure 7: One-to-one comparison of (a) PARS H&E virtual emulation and (b) gold-standard H&E staining of the same thin section of resected human breast tissue imaged under a brightfield microscope. Scale Bar: 100 µm.

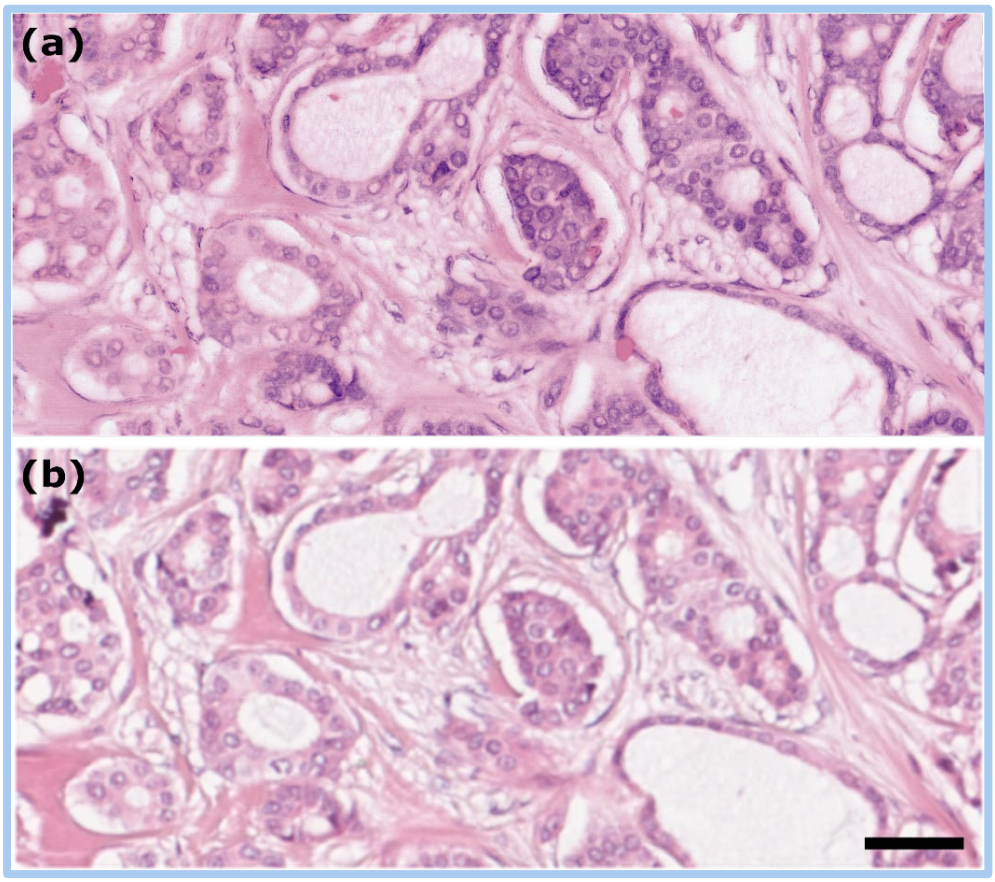


Figure 8: One-to-one comparison of PARS reproduction of H&E staining and traditional H&E staining in thin sections of resected human breast tissues. (a) PARS H&E image (b) Same section of tissues imaged under a brightfield microscope following H&E staining. Scale Bar: 50 µm.

PARS vs. Competition

Method	Contrast	Resolution (nm)	Imaging Depth (mm)	Non-Contact	Label-Free	H&E-like Specificity	FFPE Section	FFPE Block	Fresh Tissue (thick)	*In-Situ*
MUSE	Fluorescence	~1000	<0.05	✓	X	✓	✓	X	✓	X [a]
LSM	Fluorescence	2000-3000	<0.2	✓	X	✓	X	X	✓	X [a]
SRS	Molecular Vibration	<500	<0.1	✓	✓	✓	✓	X	X [b]	X
OCT	Optical Scattering	~10000	2-3	✓	✓	X	✓	✓	✓	✓
UV-PAM	Optical Absorption	700-1200	<1.0	X	✓	✓	✓[c]	✓[c]	✓[c]	X
PARS	Radiative and Non-radiative Relaxations, Optical Scattering	250-700	<2.5	✓	✓	✓	✓	✓	✓	✓

a. Requires the deparaffinization of FFPE Tissue Block to apply fluorescence stains. This process destroys the tissue block.
b. Requires tissue to be squeezed into a thin specimen, typically 120 µm in thickness.
c. Requires samples to be submerged in water.

Resources:

1. PARS PROOF OF ENDOSCOPY PAPER
2. PARS IMAGES BRAIN TISSUE AND BRAIN CANCER
3. PARS IMAGES FRESHLY RESECTED UNPROCESSED RODENT TISSUES
4. PARS SECOND GENERATION VIRTUAL HISTOLOGY
5. PARS IMAGES BACK OF THE EYE WITHOUT CONTACT
6. PARS IMAGES FRONT OF THE EYE WITHOUT CONTACT