



COLLEGE of AMERICAN
PATHOLOGISTS

Next Generation Ex Vivo Digital Microscopy for Anatomic Pathology Practice

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Sandra Camelo-Piragua MD, FCAP
Nick Reder MD, MPH, FCAP

August 24th, 2023

Conflict of Interest

- **The speakers on this webinar will discuss their conflict of interest within their presentations.**

Savitri Krishnamurthy, MD, FCAP

Dr. Krishnamurthy is the vice chair of the Digital and Computational Pathology Committee and is Professor of Pathology at The University of Texas MD Anderson Cancer Center in Houston, TX. She completed her Pathology residency training in New England Medical Center, Tuft's University in Boston followed by fellowship training in Oncologic Pathology at Memorial Sloan Kettering Cancer Center in New York and Cytopathology at the University of Texas MD Anderson Cancer Center.



The CAP Committee hosting this webinar

Digital and Computational Pathology Committee

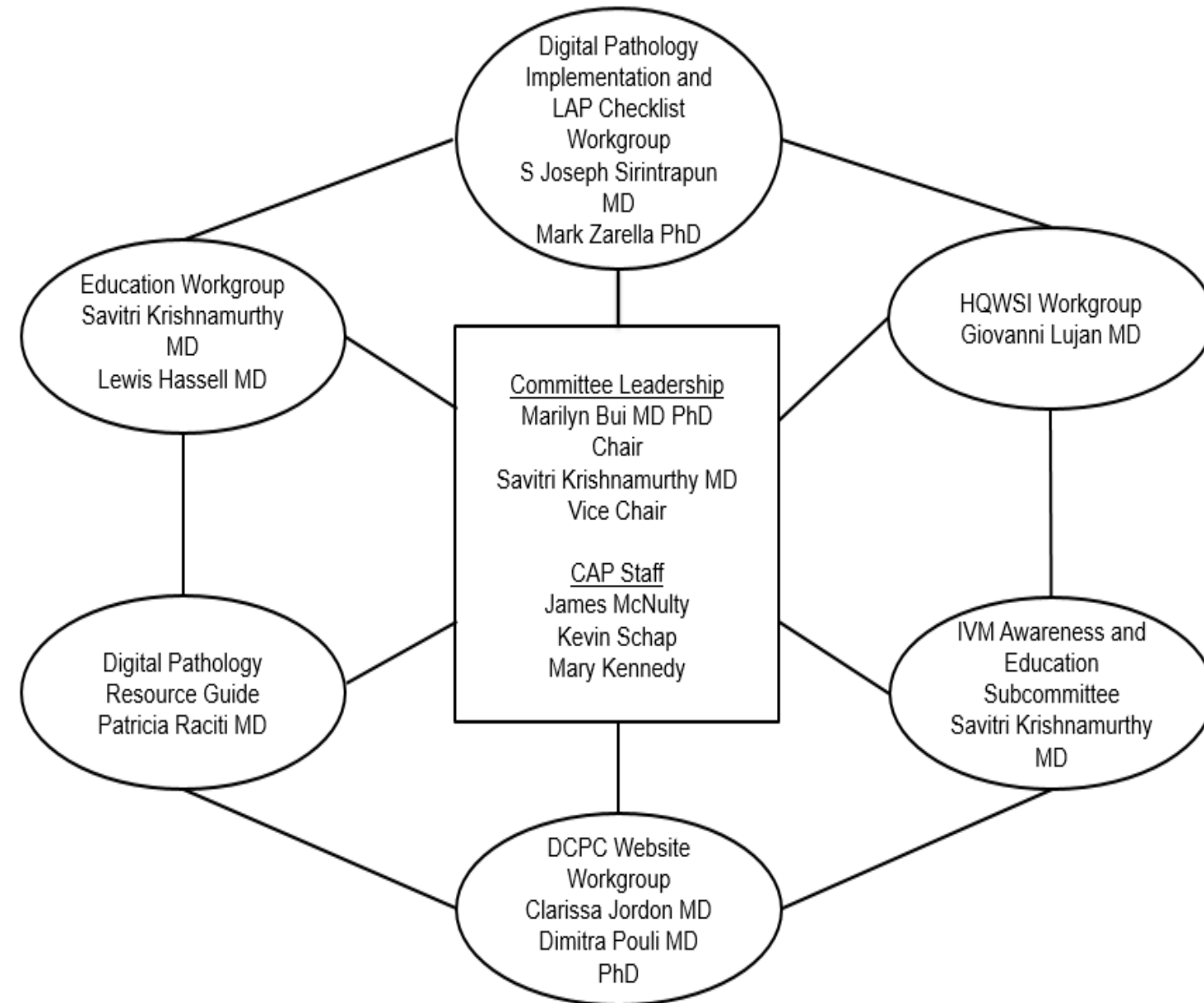
- The charge of the Digital and Computational Pathology Committee (DCPC) is to advance the adoption of digital pathology within the CAP and to serve as a respected resource for information and education for pathologists, patients and the public on the practice and science of digital pathology.

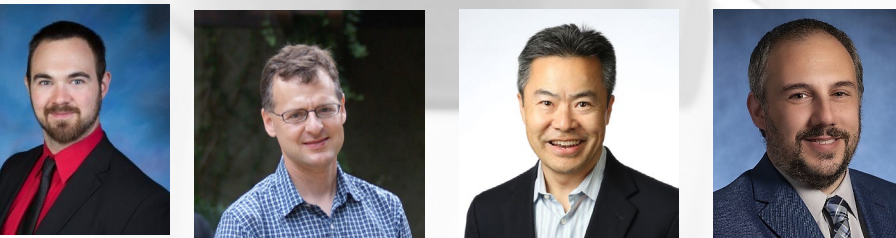
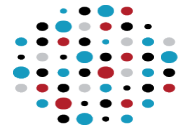
Committee Leadership

- Marilyn Bui, MD, PhD, FCAP Chair
- Savitri Krishnamurthy, MD, FCAP Vice Chair

Digital and Computational Pathology Committee (DCPC)

- Committee structure





Composition of the DCPC

- Pathologists - 24 with variety of specialty interests/niches
- Junior members - 2
- Academic institutions - >18 represented
- Private practice- at least 8 members, some with industry
- Expertise - Informatics, digital pathology use, development, standards, and validation, AI, IVM/EVM, etc.

Webinar agenda

TOPICS

PRESENTERS

Ex Vivo Digital Confocal Microscopy

Dr. Krishnamurthy

Stimulated Raman Scattering Microscopy

Dr. Camelo-Piragua

Light Sheet Microscopy

Dr. Reder

A moderated discussion of audience questions

Dr. Krishnamurthy

Learning Objectives

- To be familiar with the emerging field of optical imaging, including digital modalities with relevance for tissue evaluation.
- Understand the potential of ex vivo digital microscopy tools for several applications related to the practice of Anatomic Pathology.
- Recognize the currently available ex vivo microscopy tools and their advantages and limitations for potential applications.

EX Vivo Digital Confocal Microscopy for Anatomic Pathology Practice

Savitri Krishnamurthy M.D.

Disclosures

Sponsored Research Grant for investigator initiated clinical research:

- **Caliber ID, Inc. (Rochester, NY)**
- **Perimeter Medical Imaging (Toronto, ON)**

Research funding from CPRIT in partnership with Perimeter Medical Imaging

Research funding from NIH/NCI SBIR Grant with PSI

Research funding from Sheikh Khalifa Bin Zayed Al Nahyan Institute of Personalized cancer therapy

Research funding from The University of Texas MD Anderson Cancer Center

No financial interests in the products or companies covered in the talk

Optical Tissue Imaging

Optical sectioning microscopy techniques

Evaluation of tissues requiring minimal or no tissue preparation

- **Inherently digital images**
- **Digital images can be viewed at the site of procurement or remotely**
- **Digital images can be stored, retrieved, and integrated into electronic health records**
- **Digital images amenable to machine/deep learning, development of AI models**

SUITABLE FOR ANATOMIC PATHOLOGY PRACTICE

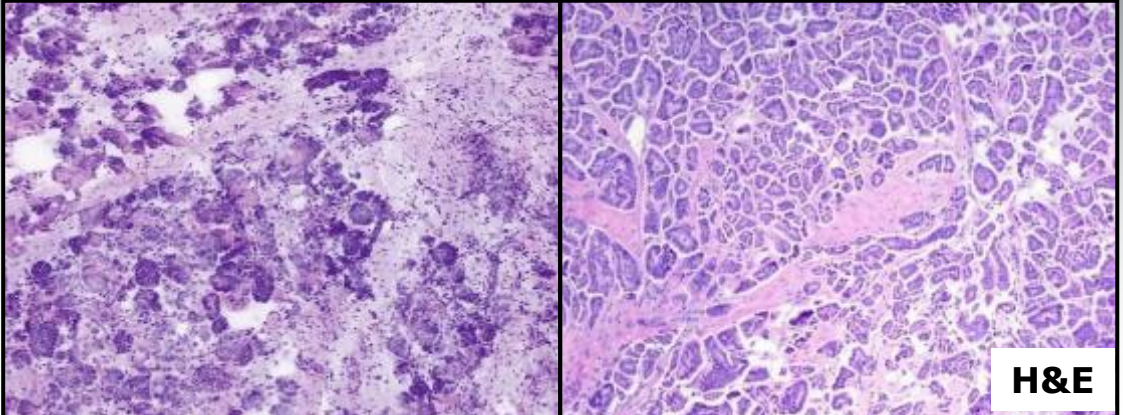
Ex Vivo Tissue Imaging Platforms for Surgical Pathology

Commercially Available Platforms

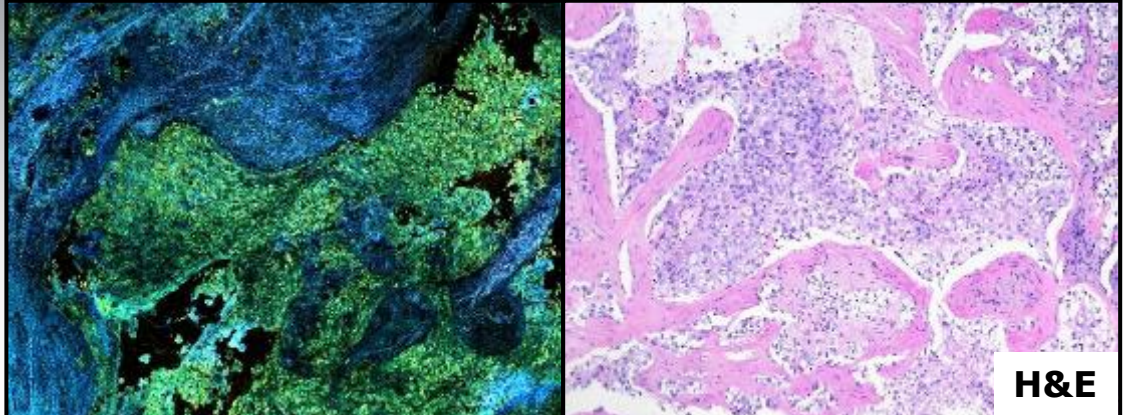
Optical Technique	Labeled	Un-Labeled	H&E Like Images
Fluorescence confocal microscopy	✓	✗	✓
Dynamic full-field optical coherence tomography	✗	✓	✓
Stimulated Raman scatterings microscopy	✗	✓	✓
Light sheet microscopy	✓	✗	✓
Structured illumination microscopy	✓	✗	✓
Optical coherence tomography	✗	✓	✗

EVM Images Using Different Commercially Available Platforms

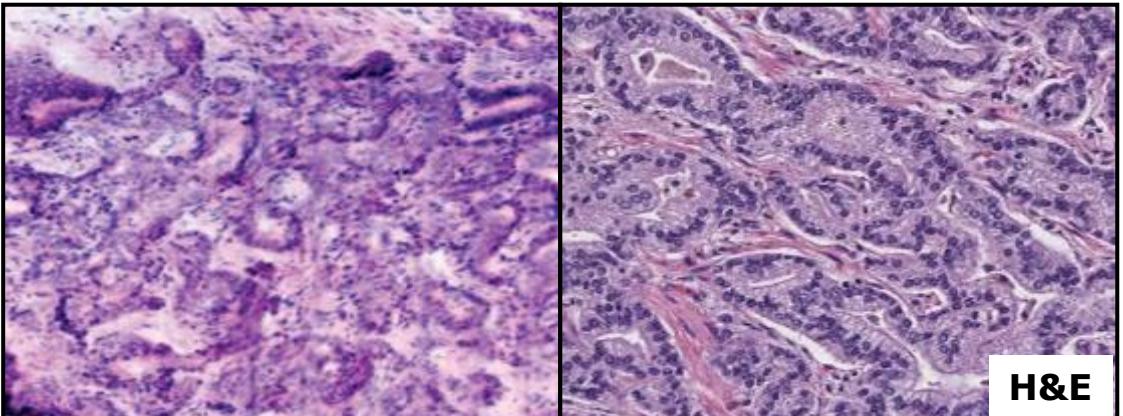
Fluorescence Confocal Microscopy



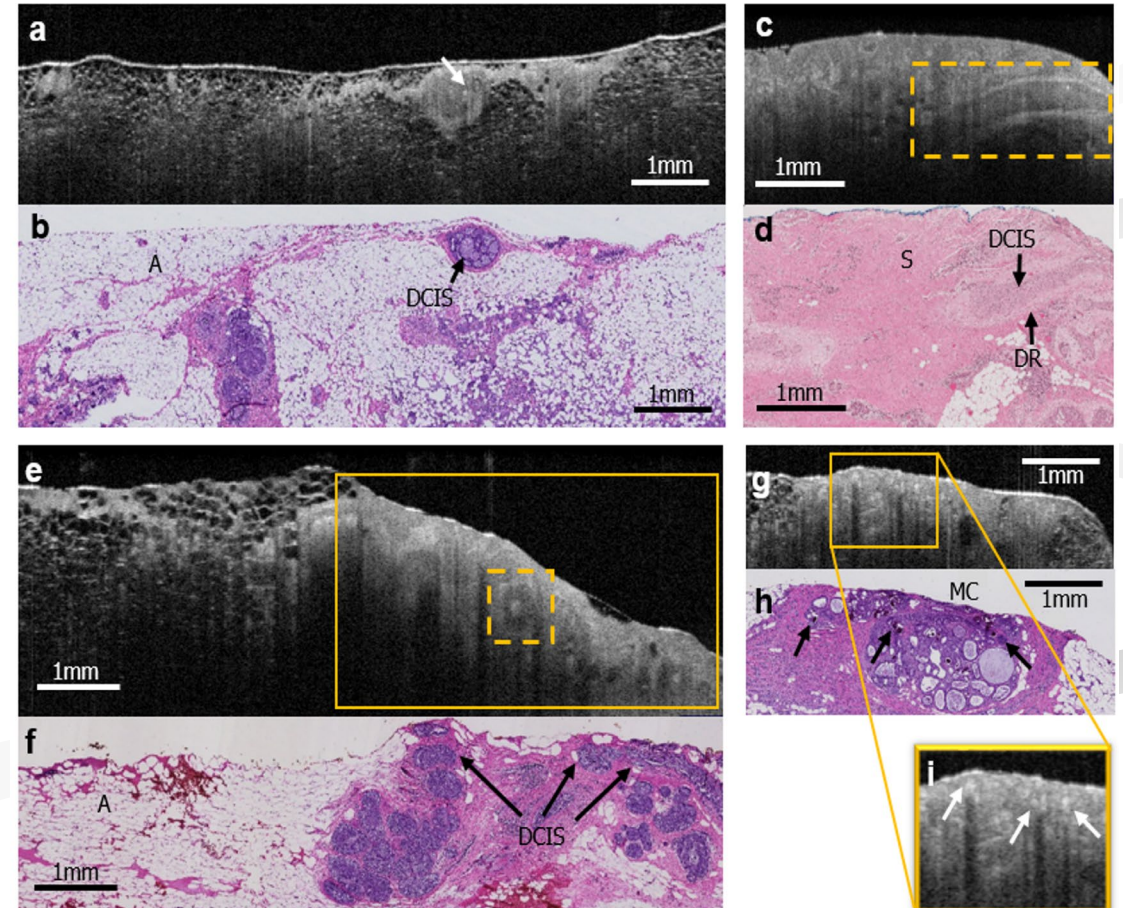
FF – Optical coherence tomography



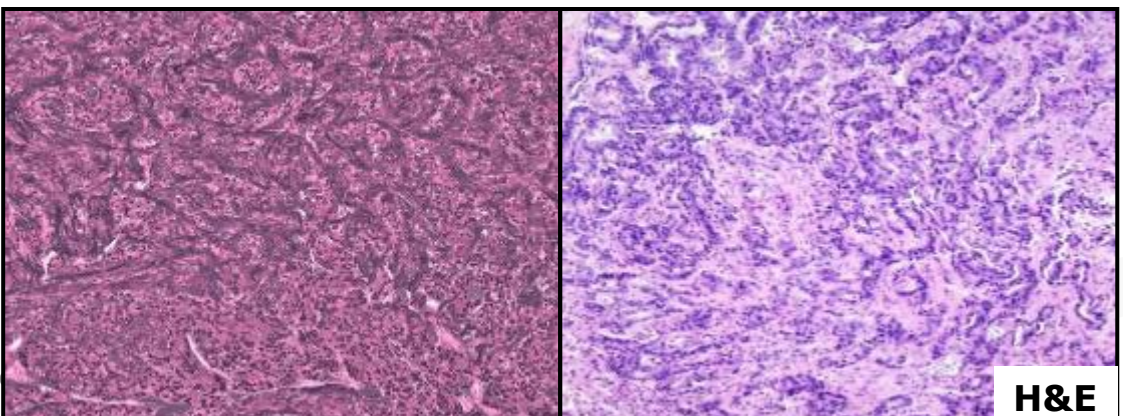
Structured Illumination Microscopy



Optical Coherence Tomography

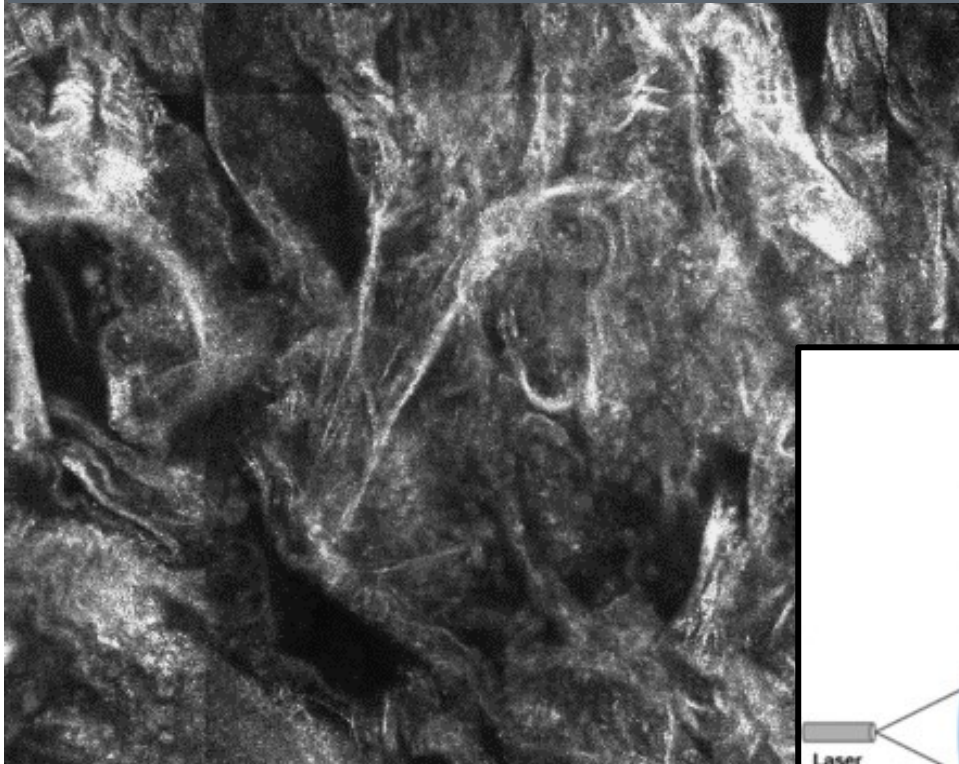


Stimulated Raman Scattering Microscopy



Confocal Microscopy

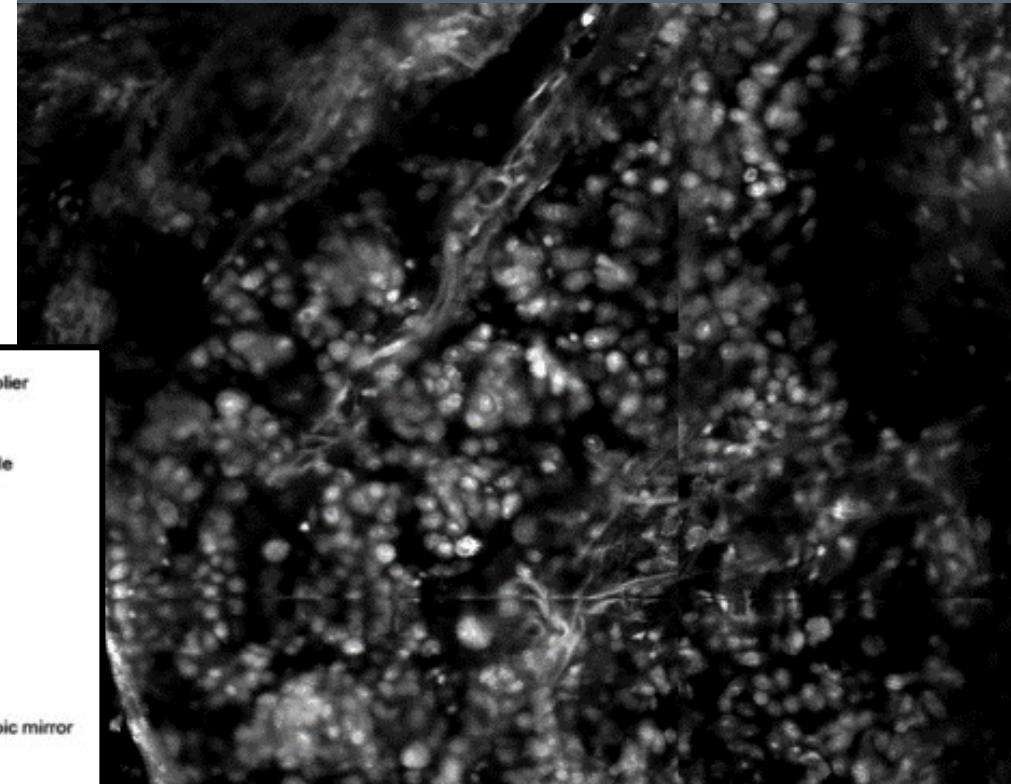
**Confocal Reflectance:
Tissue autofluorescence**



785 nm

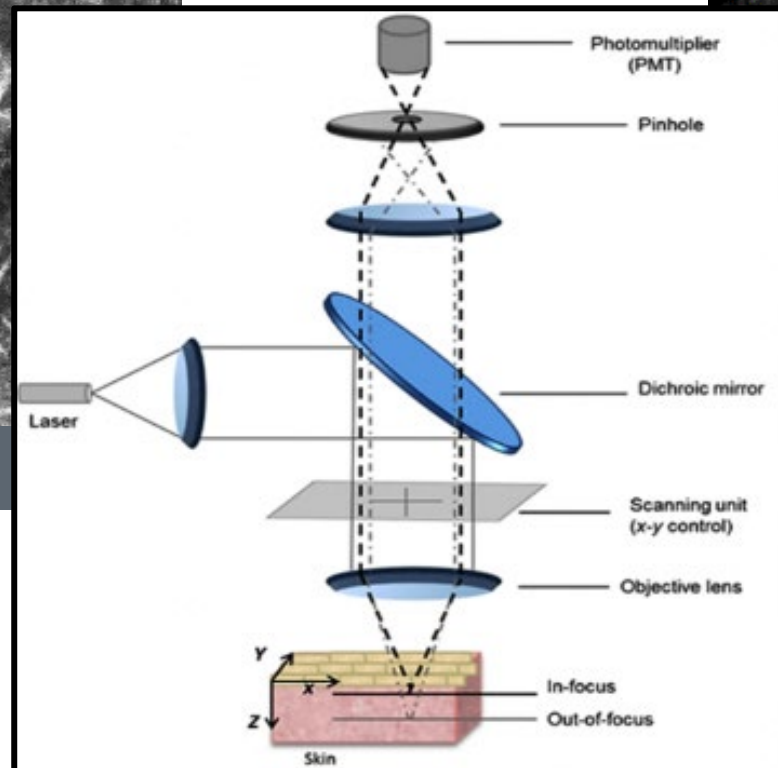
**Axial Resolution
5.0 μ m**

**Confocal Fluorescence:
With contrast agents**

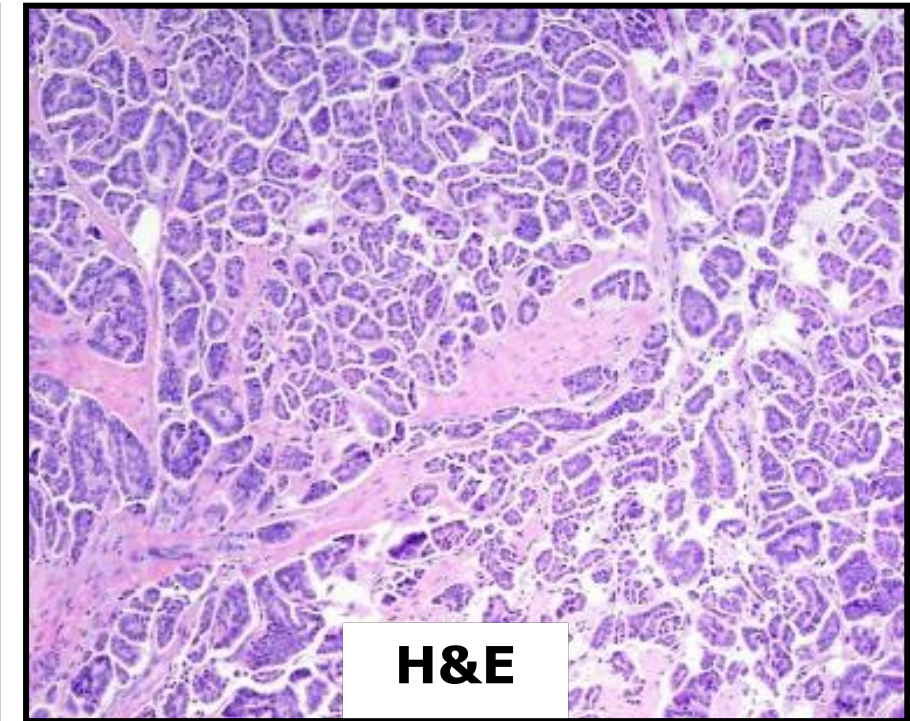
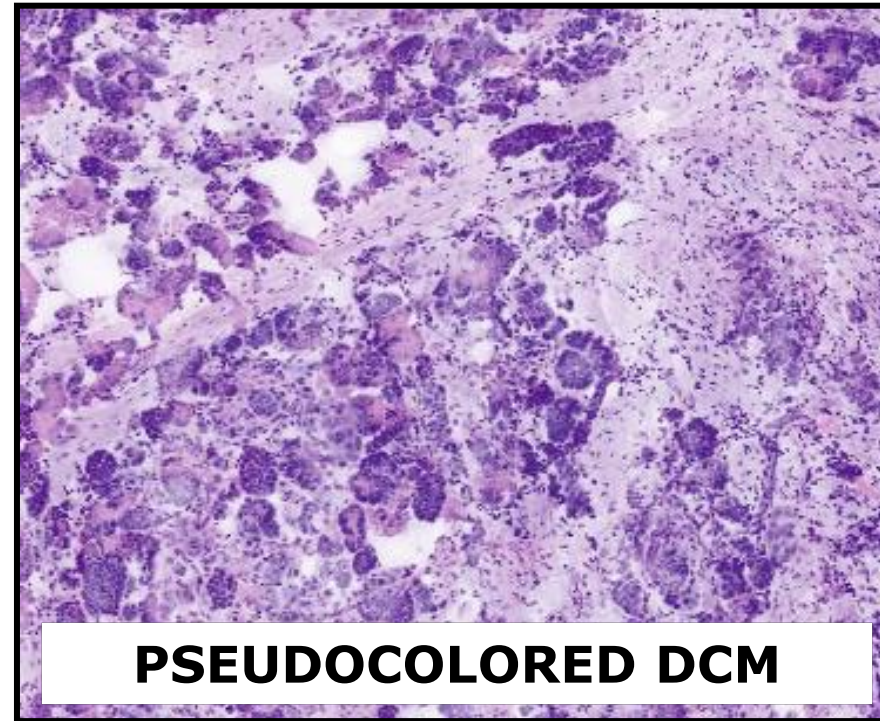
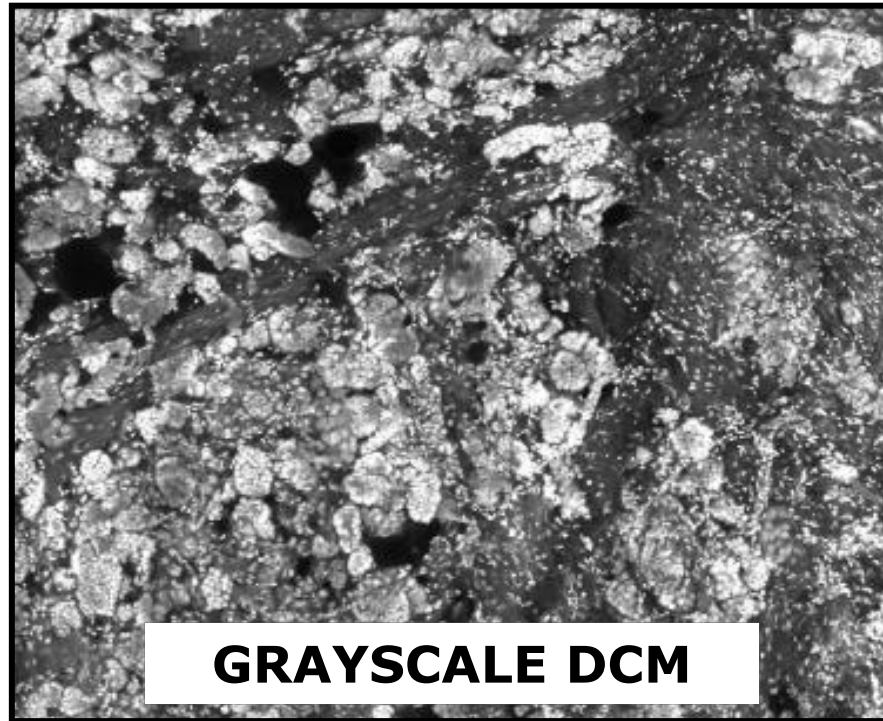


Acridine orange : 488 nm

**Depth of penetration
200 μ m**



Ex Vivo Digital Confocal Microscopy (Ex Vivo DCM)



- **DCM can be used for real-time tissue evaluation of fresh tissue : core biopsies, endoscopic biopsies and tissue fragments that are prepared as frozen sections for intraoperative evaluation.**
- **Margin assessment of small skin specimens such as those obtained from Moh's surgery, small skin excisions, neurosurgical specimens, margins or representative tissue sections of surgical excisions can be performed.**
- **Cytopathology specimens**

Ex Vivo Digital Confocal Microscopy

POTENTIAL APPLICATIONS FOR ANATOMIC PATHOLOGY PRACTICE

Real time bedside Evaluation of Core needle biopsy/fine needle aspiration biopsy

Intraoperative evaluation of small fragments of tissues in lieu of or as an adjunct to frozen sections

Intraoperative evaluation of margins of surgical specimens

Evaluating donor tissue suitable for transplantation

Procuring high quality tissue for Biobanking

Ex Vivo Digital Confocal Microscopy

Most Frequently Used Optical imaging technique for Ex Vivo Microscopy

Skin Specimens
Moh's Surgery
Basal Cell Carcinoma
Diagnosis
Margin Assessment

Non-skin specimens from almost all organs
Tissue Recognition
Specific Diagnosis

Sensitivity	~ 96%	~ 96%
Specificity	~ 99%	~ 97%

Imaging platform: Custom built or commercially available platform (Vivascope 2500, Caliber Inc. Rochester, NY; Histolog Scanner, SamanTree, Switzerland,)

Majority of studies investigated the role of DCM for evaluation of skin specimens

Longo C et al. Br J Dermatol. 2018
Krishnamurthy S et al Archives of Pathol Lab Medicine 2018
Puliatti S et al. BJU 2019

Prospective EVM Clinical Studies

Two different Confocal Microscopy Platforms

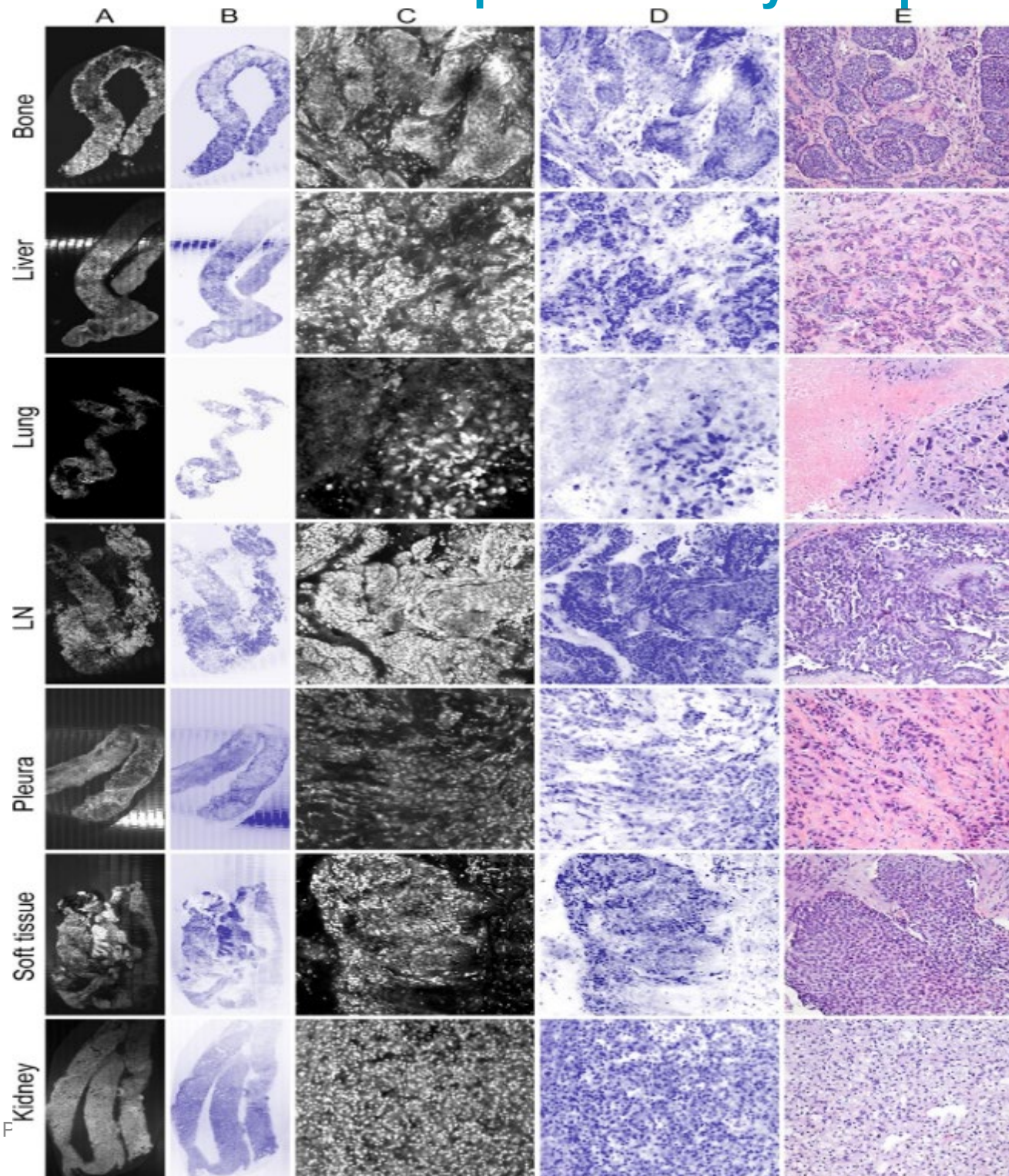
Moh's surgery specimens excised for Basal Cell Carcinoma

COMPARISON OF DIGITAL CONFOCAL MICROSCOPY WITH FINAL HISTOLOGY

	Italian Study Margins = 753 (Patients : 127)	German Study Margins = 544 (Patients : 148)
Sensitivity	79.8%	73%
Specificity	95.8%	96%

Real Time Bed Side Evaluation of IR-CNBs in the Radiology Suite :

Prospective study 105 patients



Acquisition of DCM images in mean of 7 minutes (3-13 min)

DCM images of optimal quality in 96.2 % cases

Tissue integrity preserved for subsequent H&E and ancillary testing

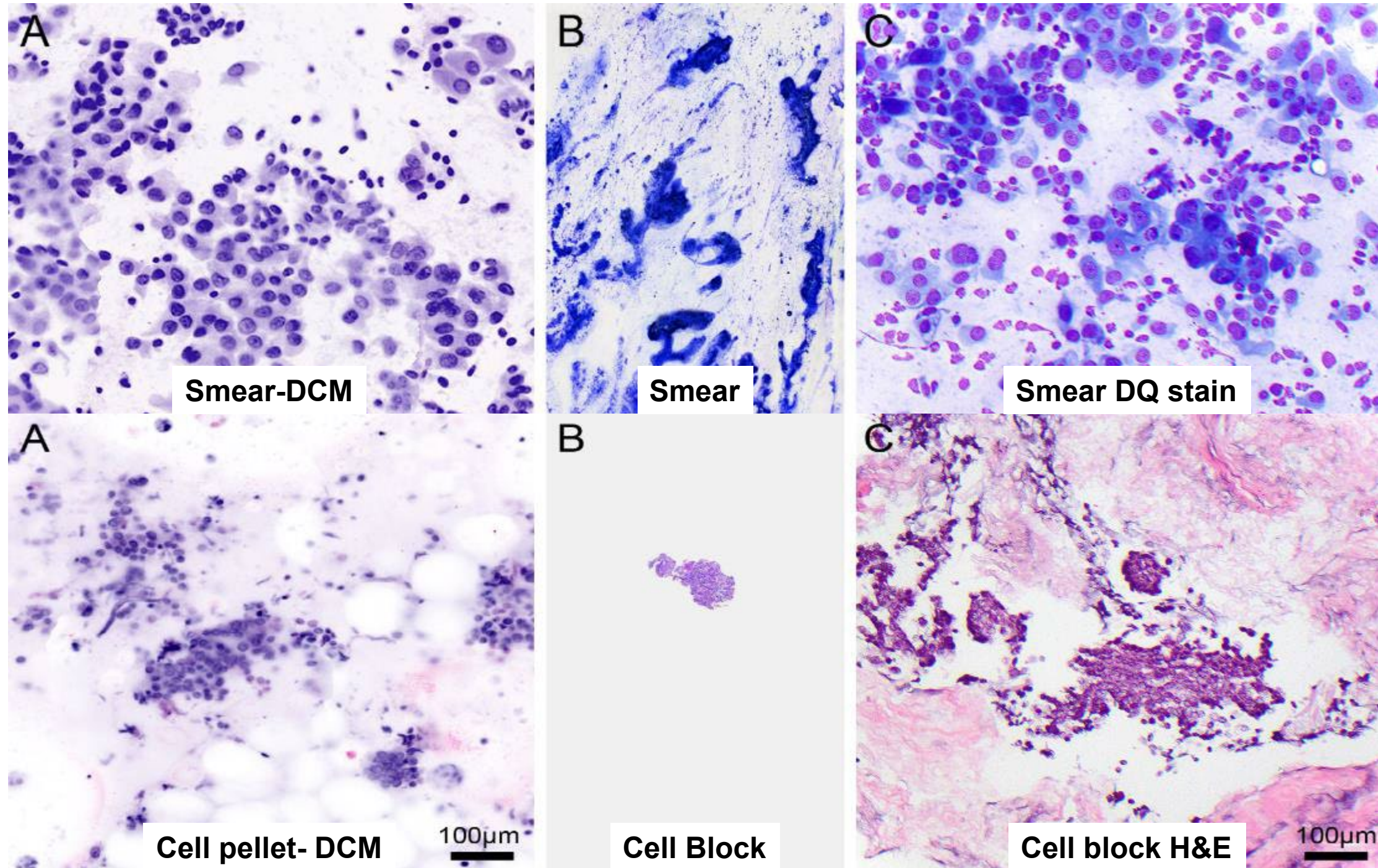
DCM images accurately interpreted by 2 pathologists in 101/105 (96.2%)

Real Time Bedside Evaluation of Interventional Radiology (IR)-guided Core Needle Biopsy (CNB) Using Ex Vivo Digital Confocal Microscopy

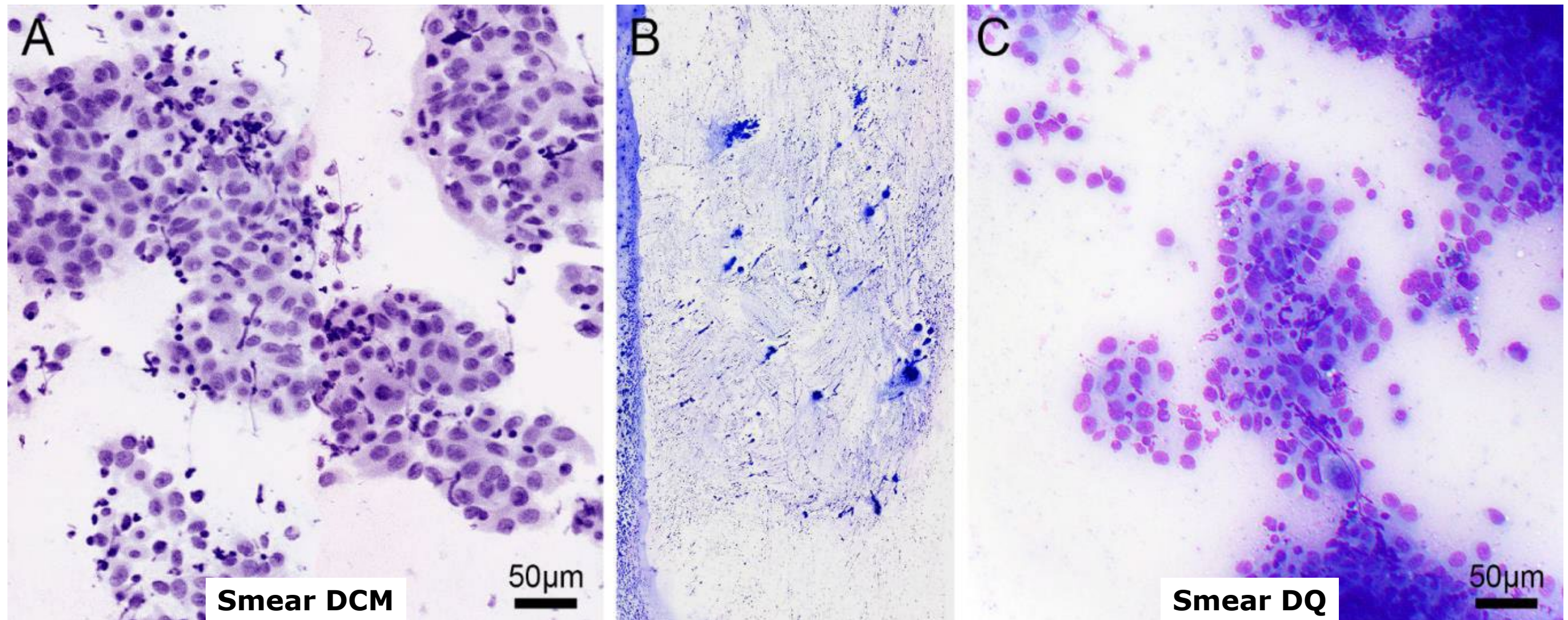
- **First prospective study showing real time bed side evaluation of IR-CNBs in Radiology suite**
- **DCM can be used with high accuracy in a range of medical settings for procuring IR-CNBs**
- **Rapid evaluation of tissue can facilitate the acquisition of high quality CNBs in 1 hospital visit**

PRACTICE CHANGER FOR MEDICAL FIELD

Digital Confocal Microscopy for Cytopathology Specimens



Ex Vivo Digital Confocal Microscopy of Smears



Fine needle aspiration smear of metastatic breast carcinoma in liver

Ex Vivo Digital Confocal Microscopy for Cytopathology

- **Ability to evaluate smears/cell pellets rapidly for cytomorphological examination**
- **Conserving all material to prepare cell block/triage for ancillary molecular testing**
- **Integrity of cellular material will be preserved**
- **Two prospective studies including 25 and 81 patients using EUS-FNB Pancreas specimens showing substantial agreement with H&E cell block diagnosis**

NEXT GENERATION DIGITAL CONFOCAL MICROSCOPY

Revolutionary change for the field of Cytopathology

Krishnamurthy and Ban, Modern Pathology 2021
Stigliano et al Gastrointestinal Endoscopy 2021
Amendoeira et al eBioMedicine 2022

Ex Vivo Digital Confocal Microscopy

Margin assessment of Robot-assisted Radical Prostatectomy

Two prospective studies

- **10 patient study with 100% concordance of intraoperative DCM diagnosis with permanent sections**
- **50 patient study, 96 margins**
- **Agreement between DCM and frozen section diagnosis was 80%**

Sensitivity	86% vs. 93%
Specificity	96% vs. 99%

**CAN BE A FASTER ALTERNATE TO NeuroSAFE
(Neurovascular structure adjacent frozen sections)**

Ex Vivo Digital Confocal Microscopy

Prospective studies for Evaluation of Breast specimens

- **23 patient study of US-guided core needle biopsies (CNB) for evaluating tumor cellularity in patients with Inflammatory Breast carcinoma. Moderate agreement of DCM with final pathology (k-0.48)**
- **24 patient study of breast CNB
Substantial agreement between DCM and final pathology(k-0.61)**
- **HIBISCUSS Project : High resolution imaging for breast carcinoma detection in ex vivo breast conservation surgery specimens by Histolog scanner (ultra fast fluorescence confocal microscope)
181 patient study, interpreted by 7 surgeons and 2 pathologists
Acquisition of image in 8-10 minutes
Accuracy of diagnosis of pathologists 99.6% vs 98% for surgeons**

Dobbs J et al, Breast Cancer Research and Treatment, 2015

Elfgren C et al, Diagnostic Pathology, 2019

Conversano A et al, BJS Open 2023

Ex Vivo Digital Confocal Microscopy

Applications in Transplant Pathology

- **DCM can serve as an alternate to frozen section for evaluation of donor liver biopsy for liver transplantation**
- **DCM could be a game changer in transplantation pathology**

Applications for Biobanking

- **DCM can ensure representative tissue procurement in prostate cancer biobanking**
- **Tissue suitable for downstream analysis**

Ex Vivo Digital Confocal Microscopy

NEXT GENERATION DIGITAL MICROSCOPY TOOLS

Feasibility

**Clinical
studies**

**Standard of
Care**

Digital frozen sections

Slide free digital histopathology

Real time bedside histopathology

CURRENTLY INVESTIGATIONAL

Ex Vivo Digital Confocal Microscopy For Anatomic Pathology Practice : Current Status

- **Feasibility studies and limited prospective clinical studies indicates promising potential of ex vivo digital confocal microscopy for utilization in Anatomic pathology clinical practice**
- **Need for more prospective clinical studies involving more pathologists to facilitate incorporation into clinical practice**
- **Establishment of billing code based on utilization**
- **Emerging Artificial Intelligence algorithms based on ex vivo digital confocal microscopy images**

PROMISING NEXT GENERATION DIGITAL MICROSCOPY TOOL

Sandra Camelo-Piragua, MD

Dr. Camelo-Piragua is Professor of Pathology and Neuropathology Fellowship Program Director at the University of Michigan, in Ann Arbor, MI. She completed her AP/CP training at Baystate Medical Center, Western Campus of Tufts Medical School, Springfield, MA and her Neuropathology Fellowship at Massachusetts General Hospital, Harvard Medical School, Boston, MA.

Dr. Camelo-Piragua is a Clinical Neuropathologist with interest in digital pathology, robotic microscopy, ex-vivo microscopy, and implementation of machine learning algorithms for digital imaging analysis in histopathology.



Intraoperative Digital Imaging: Stimulated Raman Scattering (SRS) Microscopy

Sandra Camelo-Piragua, M.D.

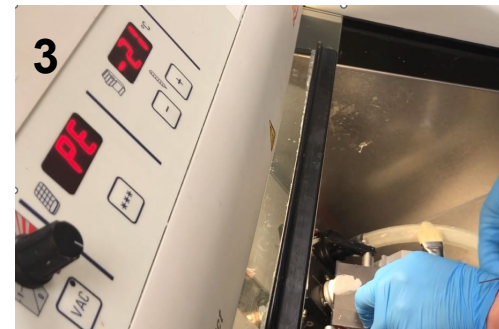
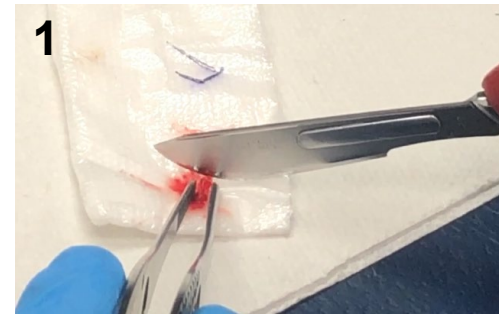
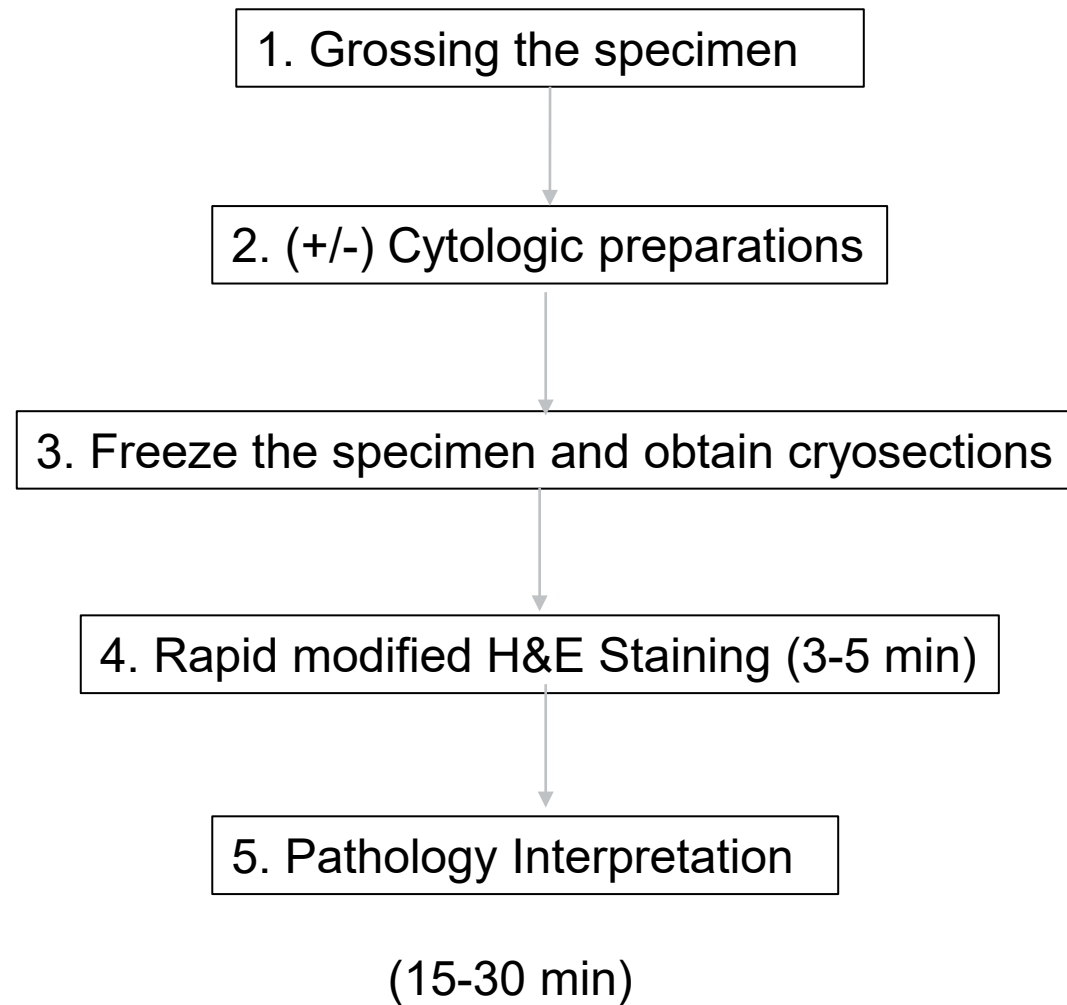
Intraoperative Digital Imaging using SRS Microscopy

- Objectives of intraoperative consultation (IOC)
- Current IOC workflow
- Major challenges in Neurosurgical IOC
- SRS principle
- SRS workflow in IOC
- Examples of surgical specimens imaged with SRS

Objectives of intraoperative consultation

- **Secure procurement of lesional tissue**
- Is the specimen diagnostic?
- Does the specimen explain the clinical and radiologic presentation?
- Is there sufficient material for final diagnosis?
- Is the specimen representative for additional testing (banking, trial enrolment, molecular, etc.)?
- **Provide sufficient information to guide surgery**
- Is the surgeon in the lesion?
- Is this a resectable lesion?
- Is maximal tumor resection needed/desired?
- Will the type of lesion determine intraoperative treatment? (e.g., are margins clear?)

Current intraoperative consultation workflow

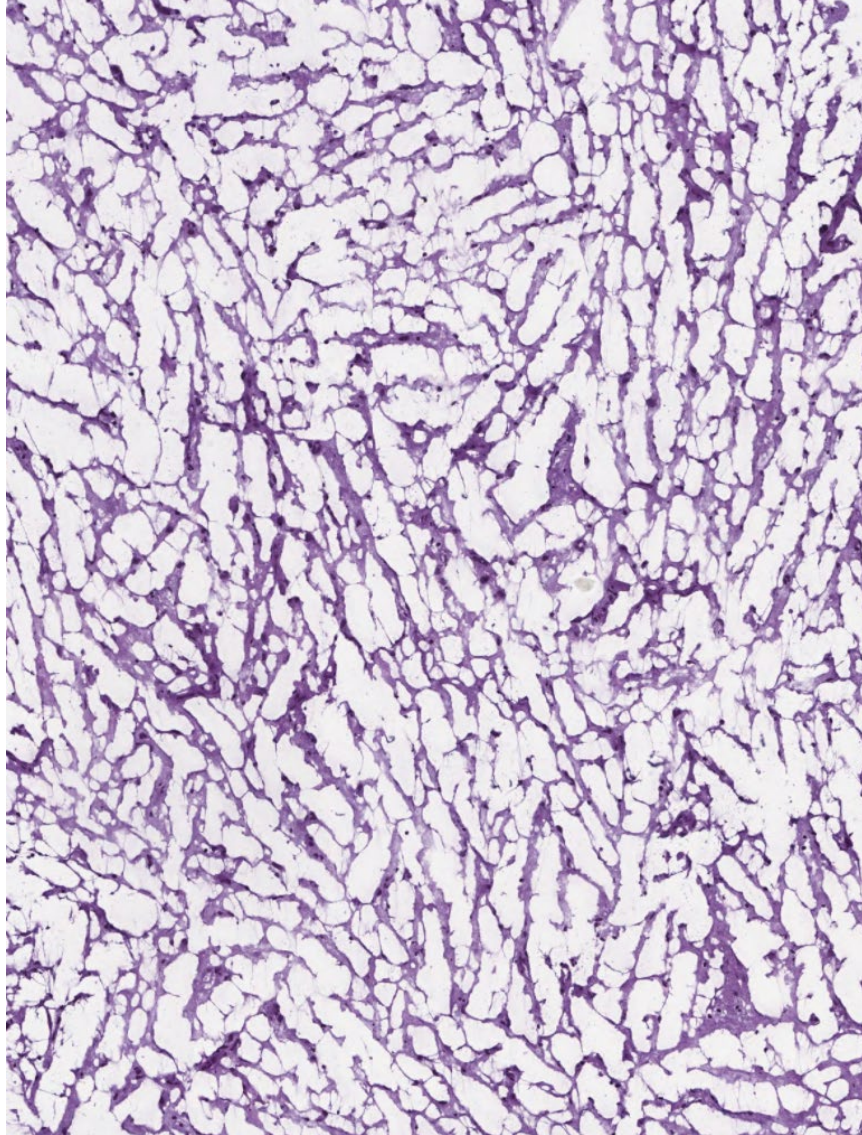


Major challenges in Neurosurgical IOC

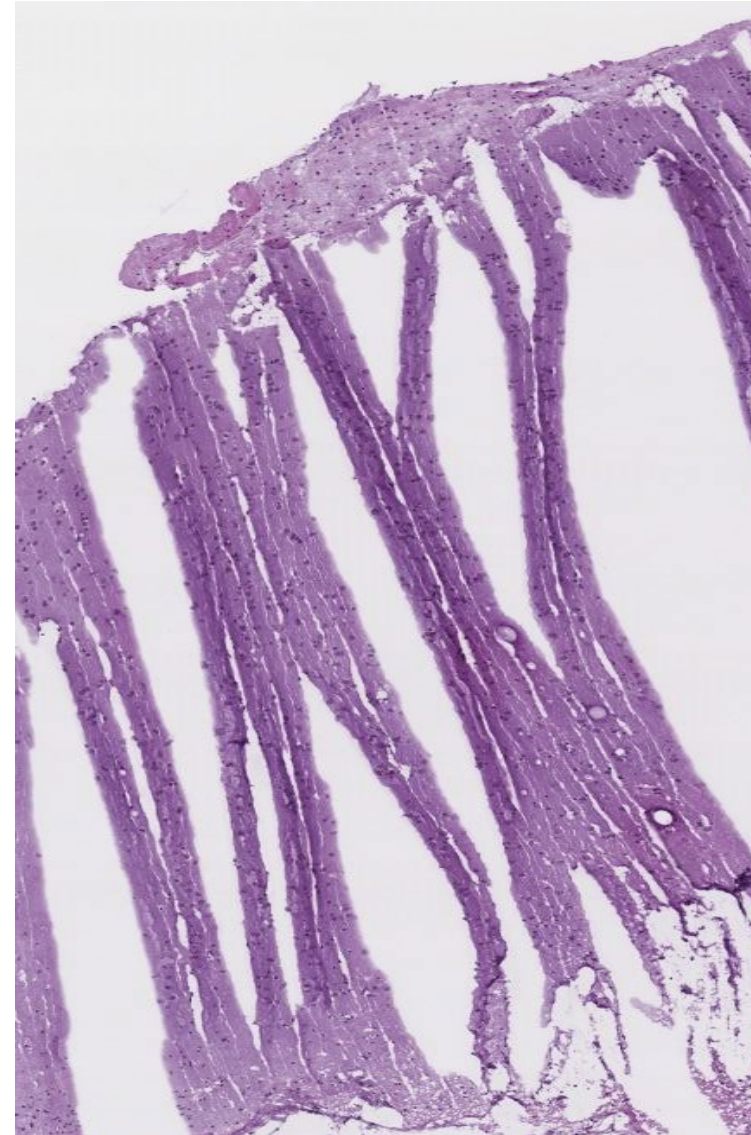
- **Tissue is often small, requires expert technique for sectioning**
- **High pressure from Neurosurgery: “Real State” is very valuable**
- **Re-biopsy has major risk (bleeding, sensitive areas)**
- **Freezing artifact may affect interpretation**
- **Current workflow is relatively time consuming**
- **Not all institutions have board certified neuropathologists**
- **General pathologists are not 100% comfortable with neurosurgical specimens**

Freezing Artifacts

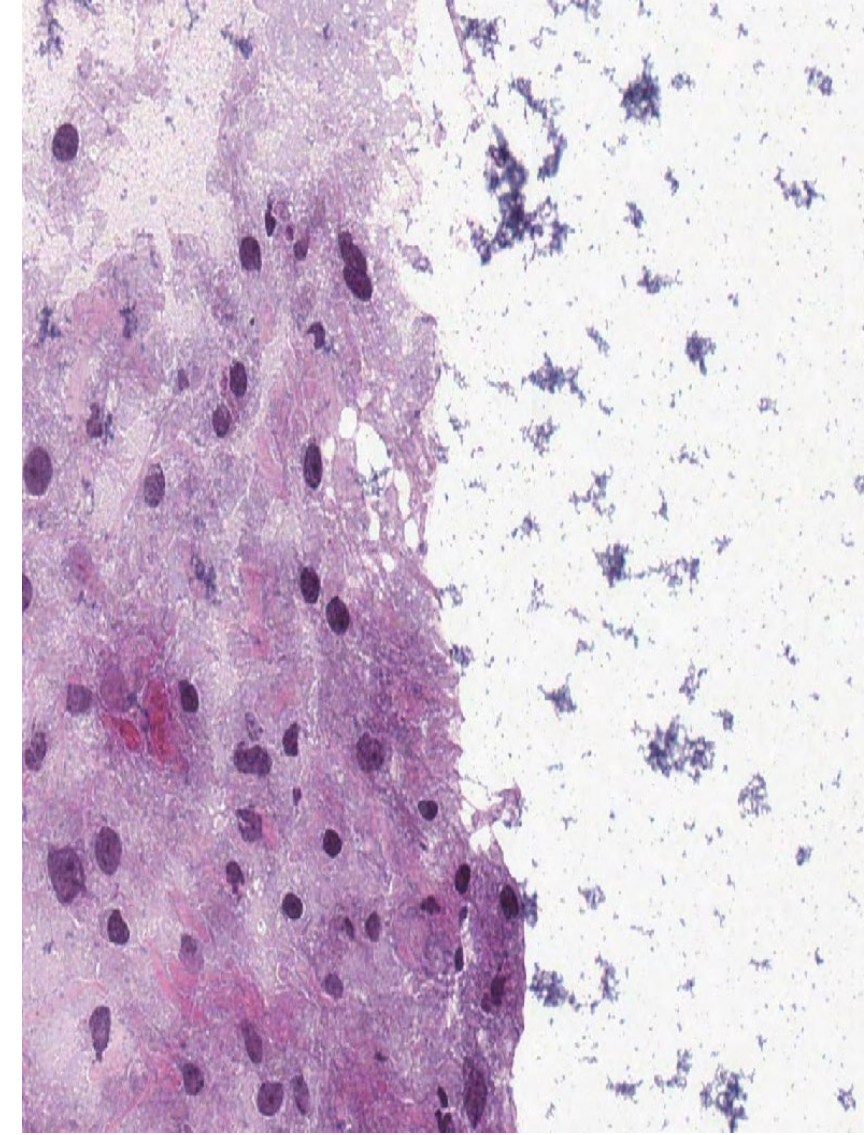
Ice Crystals



Tissue folding/Knife artifact

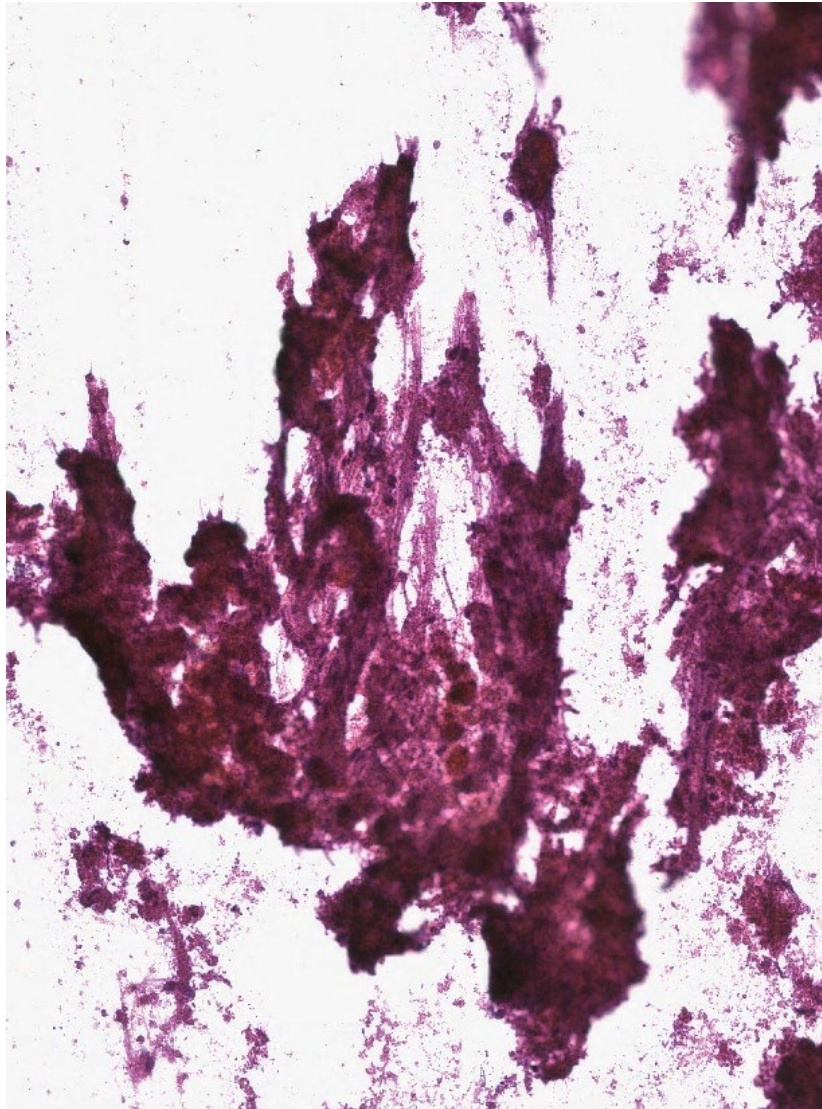


Stain Precipitate

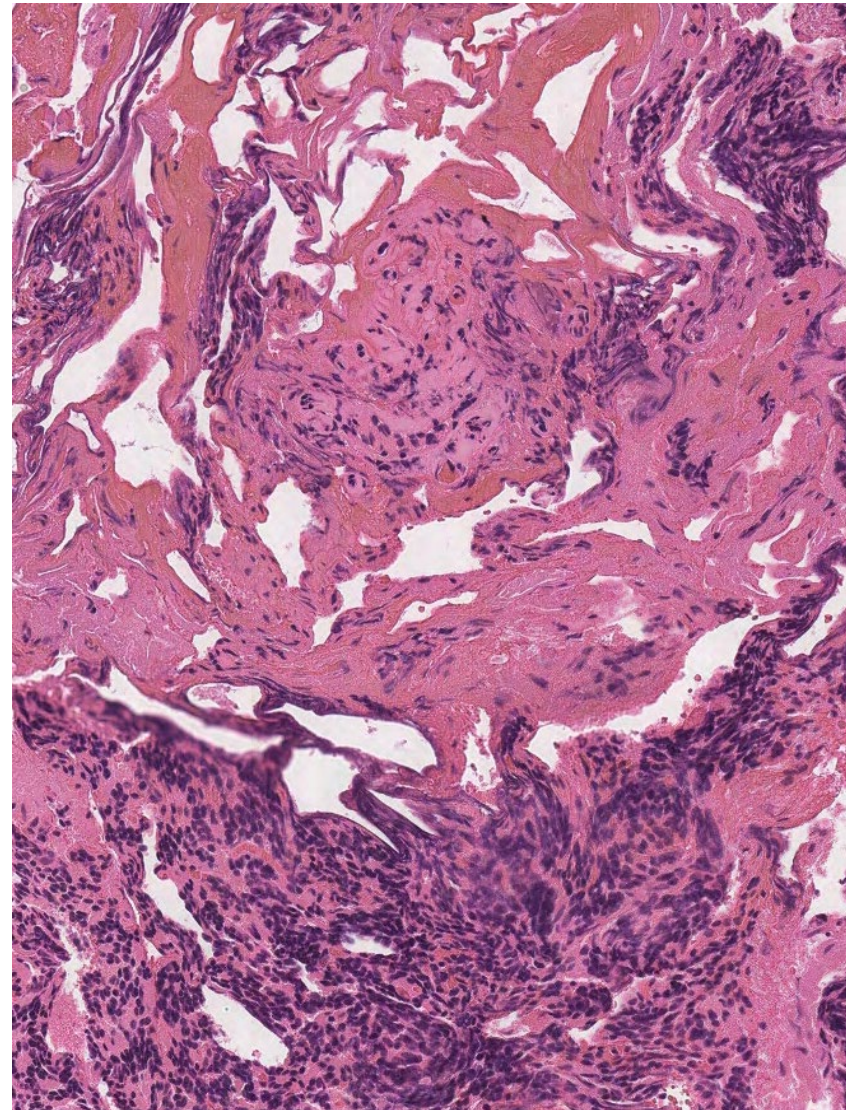


Artifacts

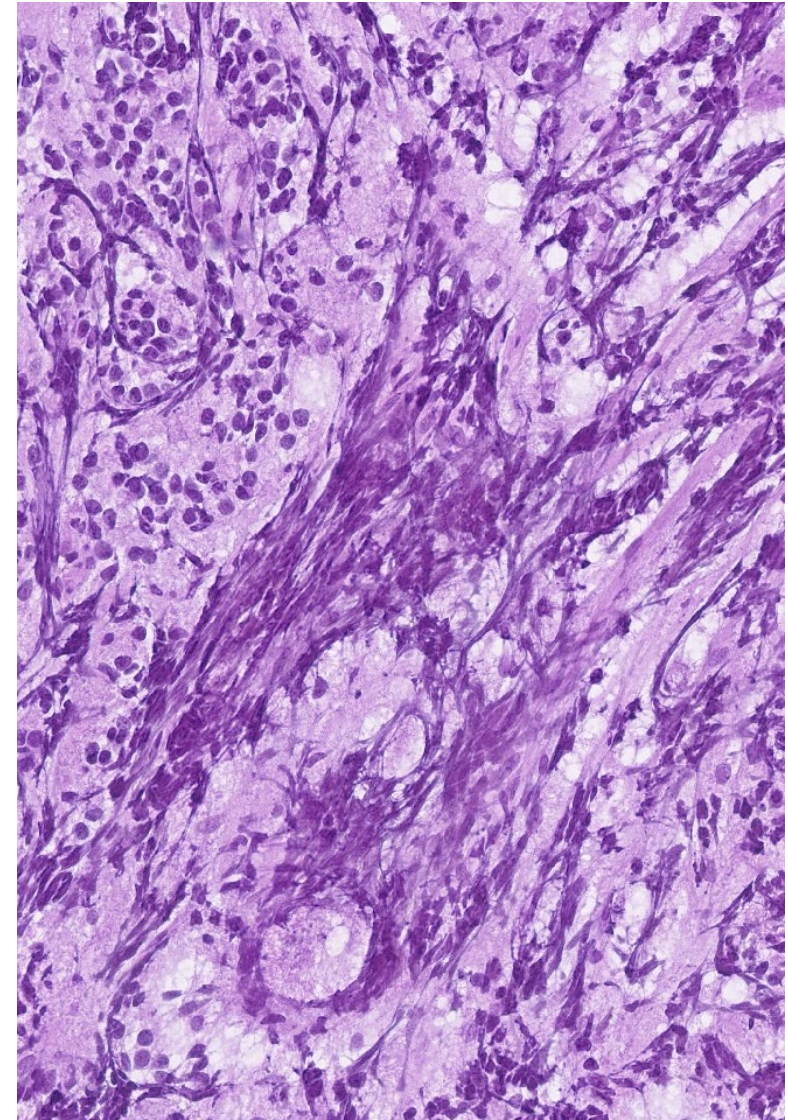
Thermal artifact Smear



Thermal artifact Frozen



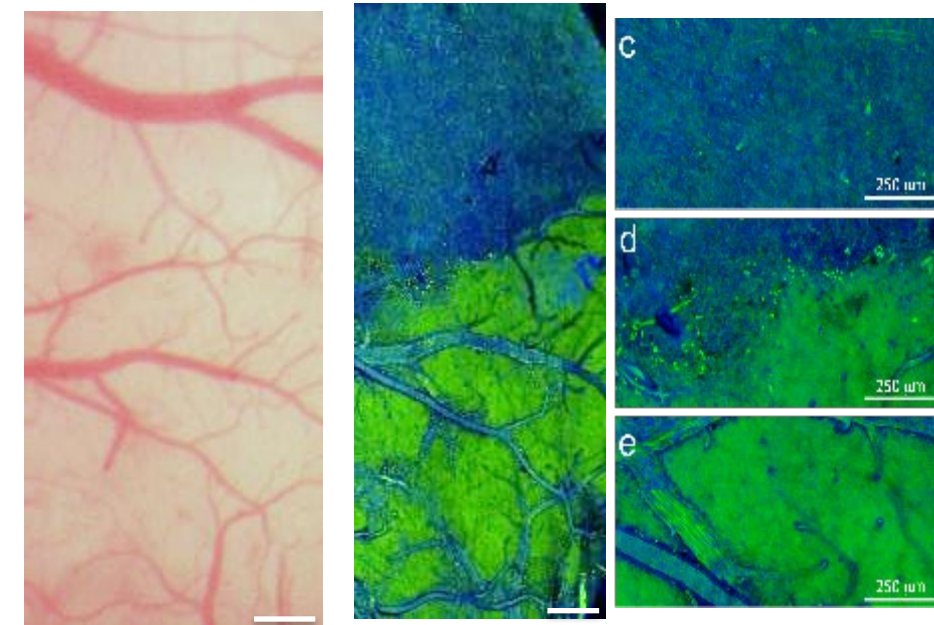
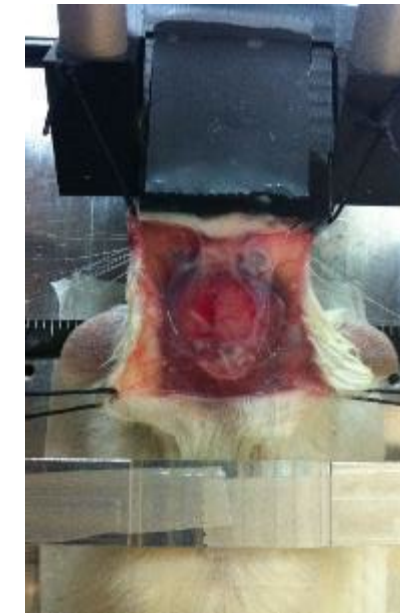
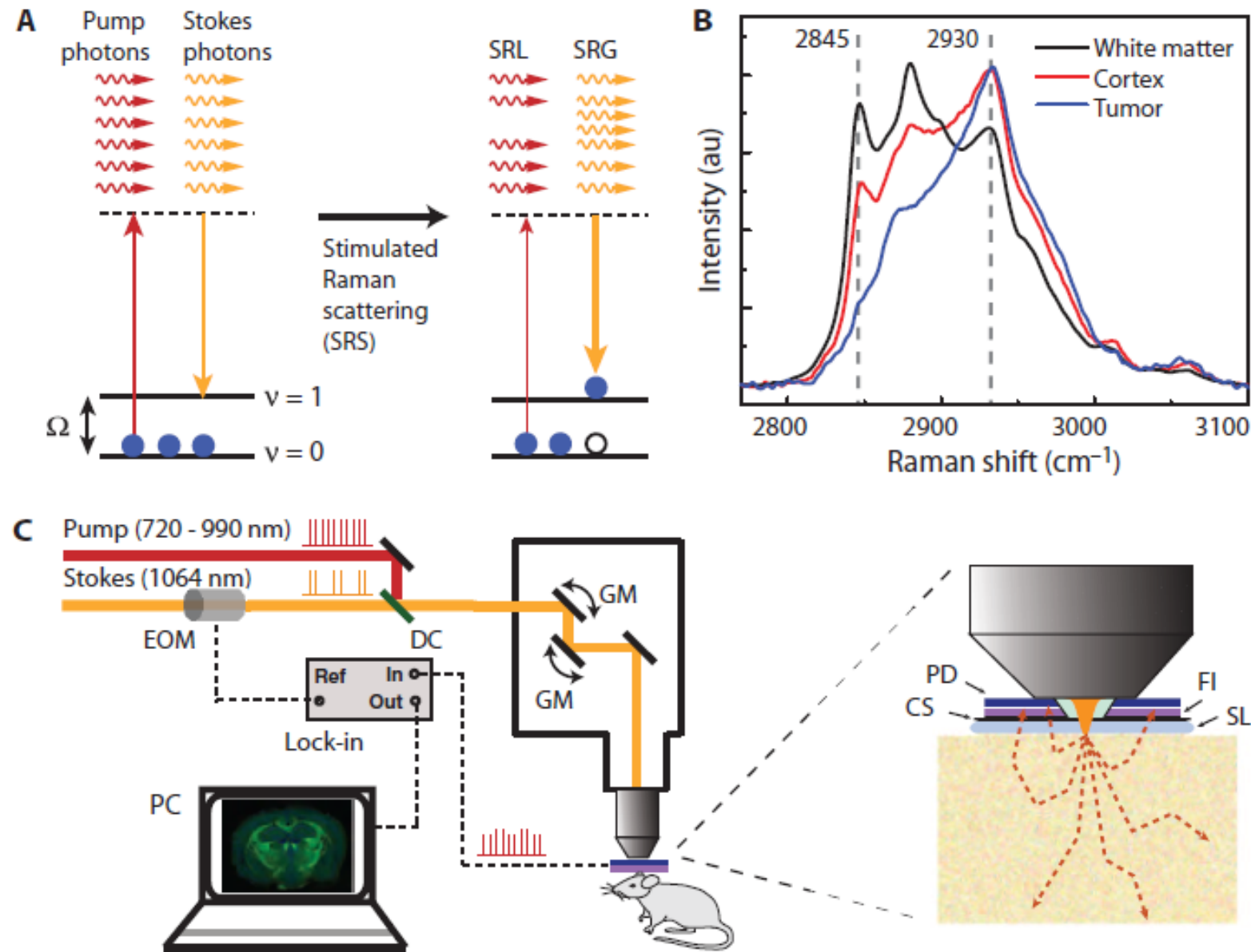
Crush artifact



What can we do?

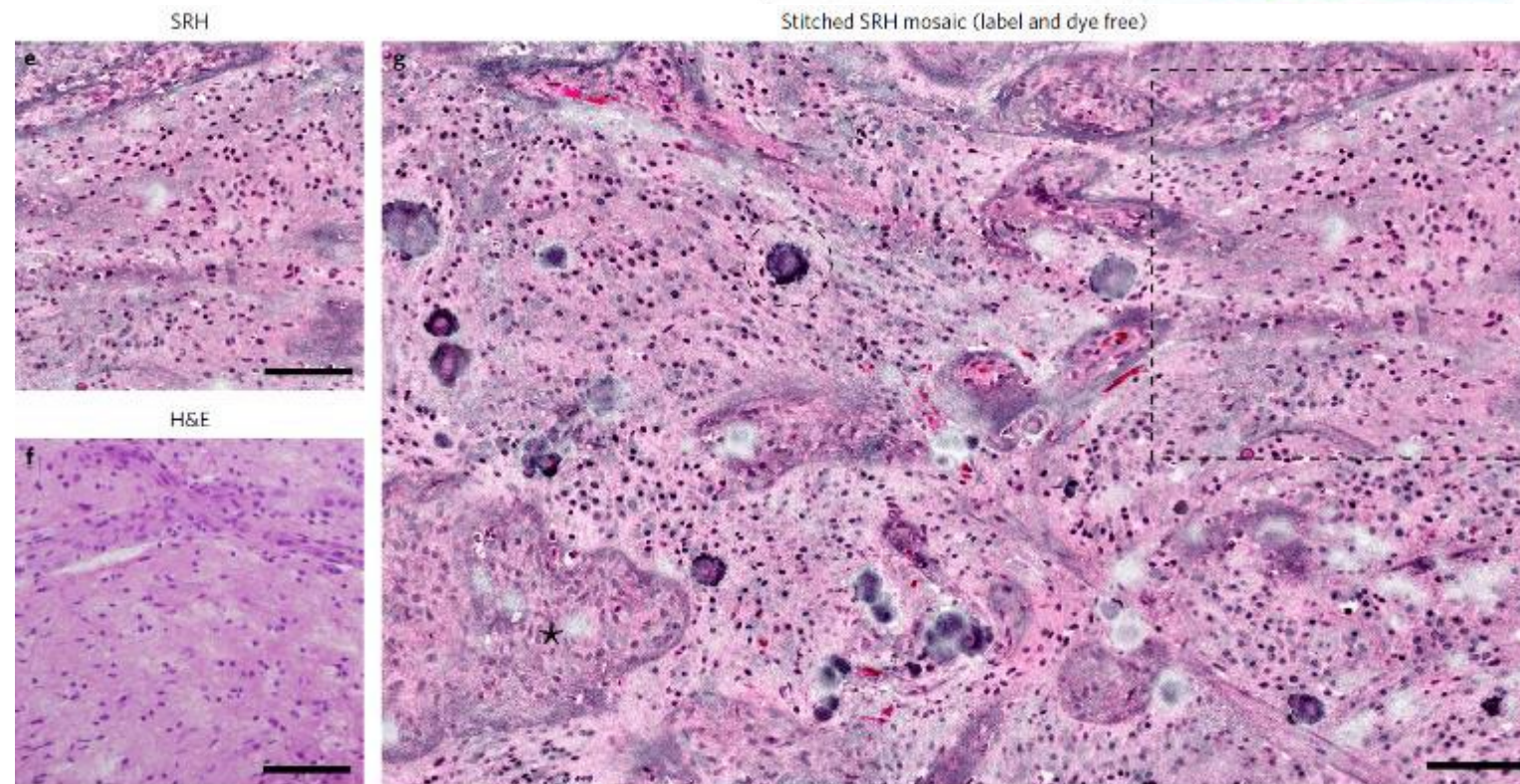
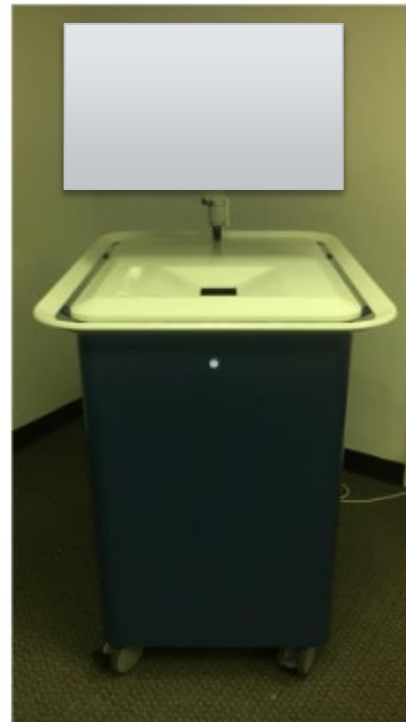
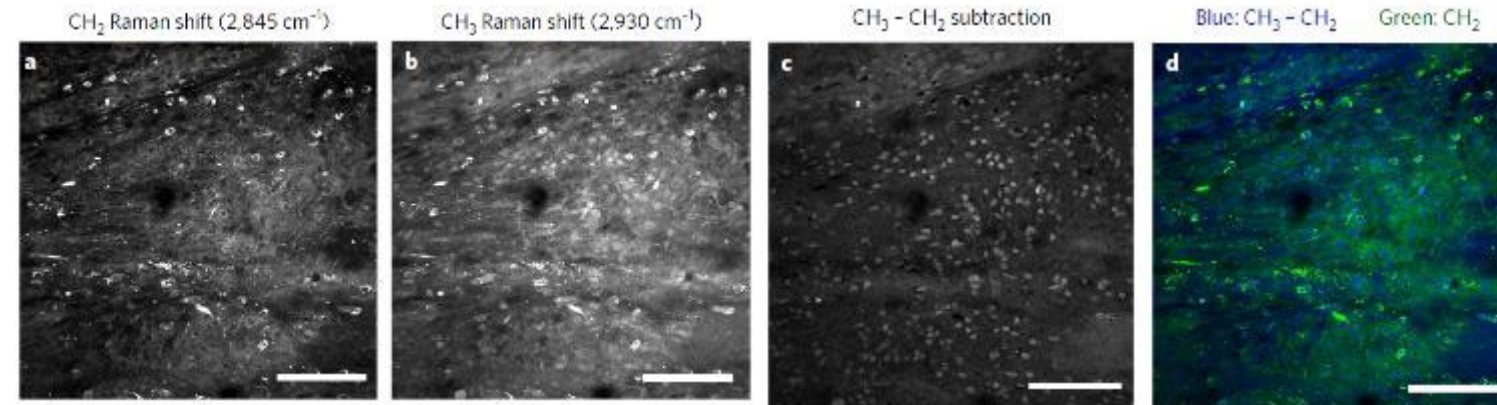
Let's explore other alternatives!

Stimulated Raman Scattering Microscopy



Ji et al *Sci Trans Med* 2013

Stimulated Raman Scattering Microscopy



Stimulated
Raman
Histology

SRH

Orringer, et al. *Nature Biomedical Engineering* 1,0027 (2017)

SRS/SRH intraoperative consultation workflow

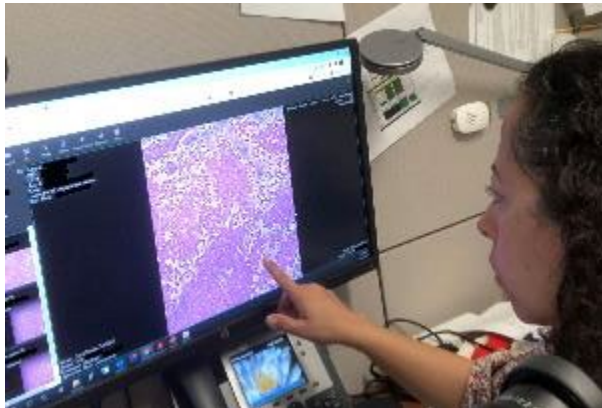
Tissue procurement by NSx



Non-destructive image acquisition



Image Interpretation



(<3 min)

Rapid intraoperative histology of unprocessed surgical specimens via fibre-laser-based stimulated Raman scattering microscopy

Daniel A. Orringer^{1*}, Balaji Pandian¹, Yashar S. Niknafs¹, Todd C. Hollon¹, Julianne Boyle¹, Spencer Lewis¹, Mia Garrard¹, Shawn L. Hervey-Jumper¹, Hugh J. L. Garton¹, Cormac O. Maher¹, Jason A. Heth¹, Oren Sagher¹, D. Andrew Wilkinson¹, Matija Snuderl^{2,3}, Sriram Veneti⁴, Shakti H. Ramkissoon^{5,6}, Kathryn A. McFadden⁴, Amanda Fisher-Hubbard⁴, Andrew P. Lieberman⁴, Timothy D. Johnson⁷, X. Sunney Xie⁸, Jay K. Trautman⁹, Christian W. Freudiger⁹ and Sandra Camelo-Piragua^{4*}

Survey Results

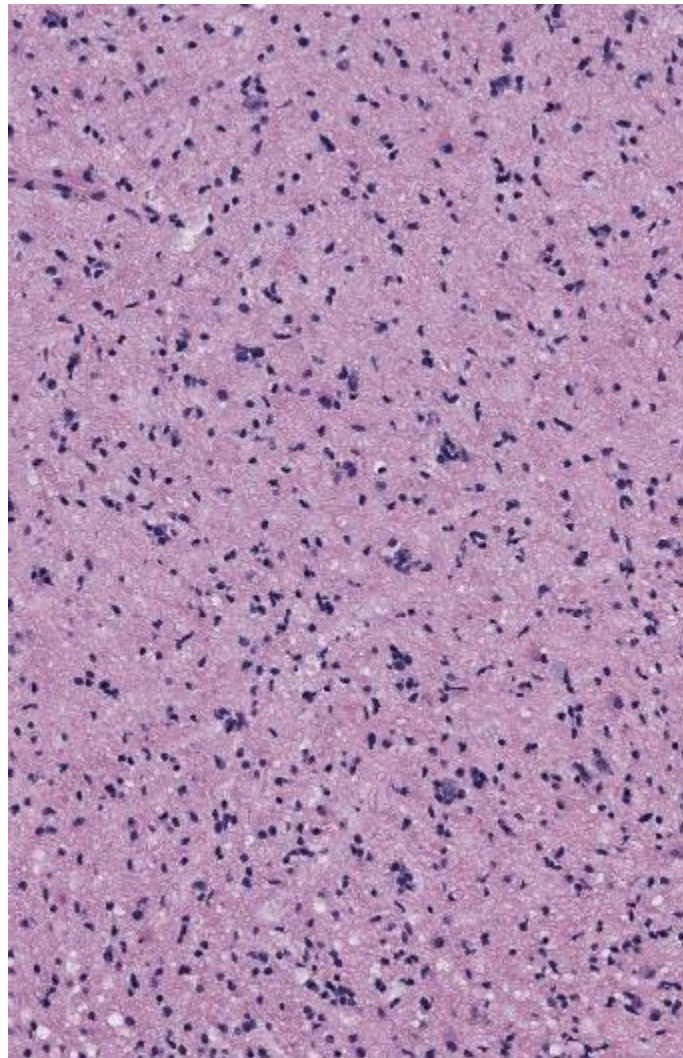
Specimen Type	Imaging Modality	NP1		NP2		NP3		Combined Accuracy
		✓	⊘	✓	⊘	✓	⊘	
Normal	SRH	4	1	5	0	5	0	93%
	H&E	3	2	5	0	5	0	86%
Glial Tumor	SRH	14	1	12	3	13	2	86.6%
	H&E	14	1	14	1	15	0	95.5%
Non-Glial Tumor	SRH	10	0	10	0	10	0	100%
	H&E	10	0	9	1	10	0	96.6%
Total	SRH	28	2	27	3	28	2	92.2%
	H&E	27	3	28	2	30	0	94.4%
Combined Accuracy		91.6%		91.6%		96.6%		94.4%
Concordance (K)		0.924		0.855		0.923		

✓ = Correct ⊘ = Incorrect NP = Neuropathologist

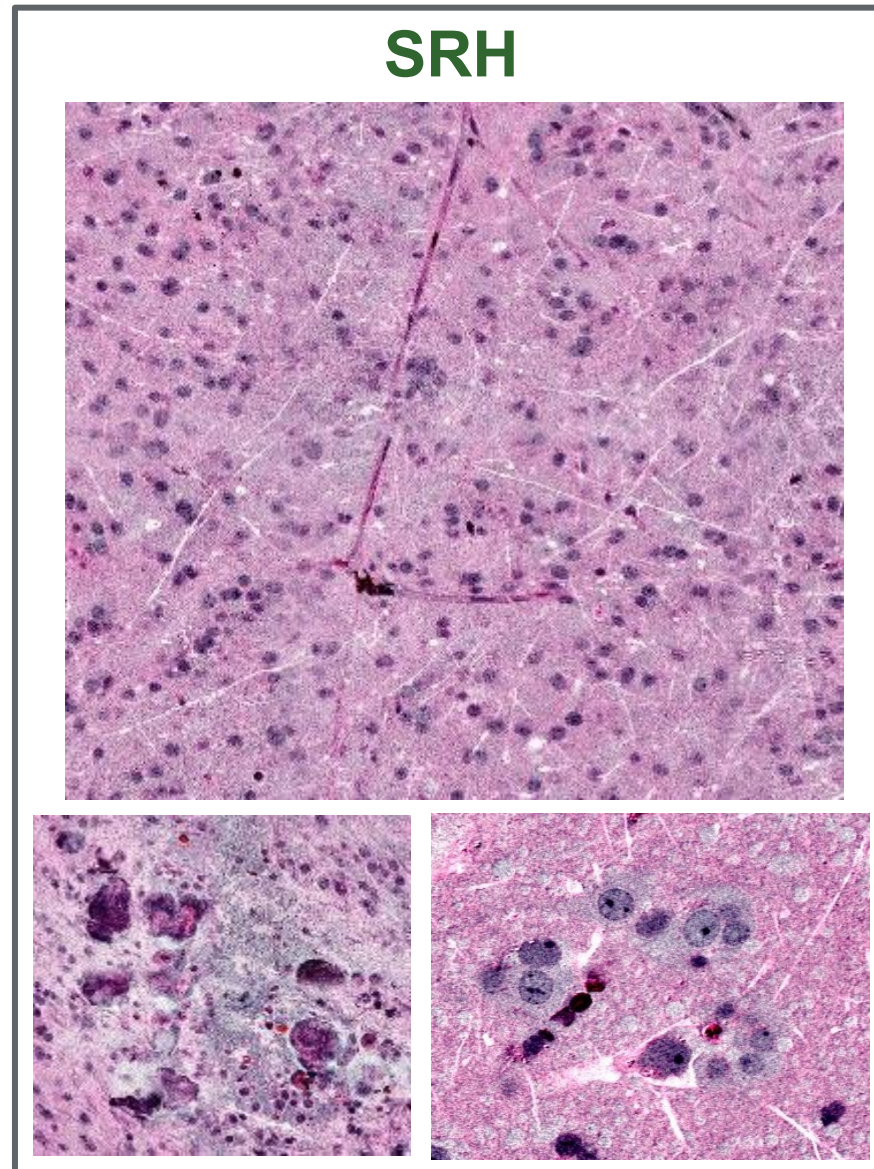
Examples of surgical specimens imaged with SRS

Oligodendroglioma, CNS WHO Grade 2

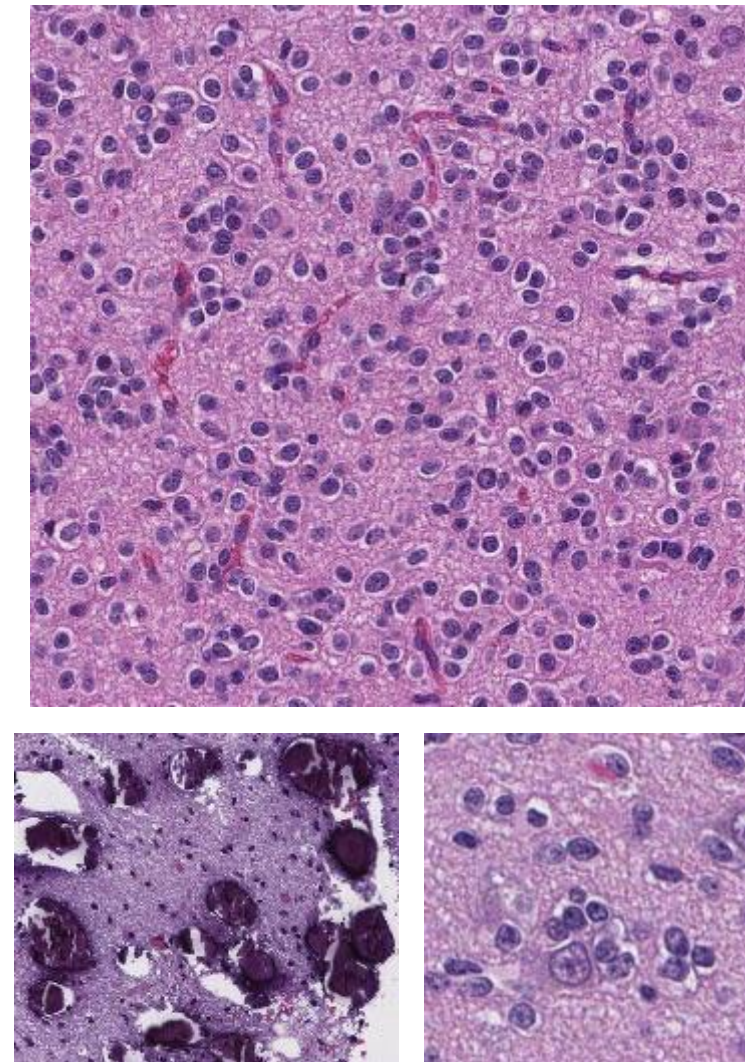
CHE: Frozen



SRH

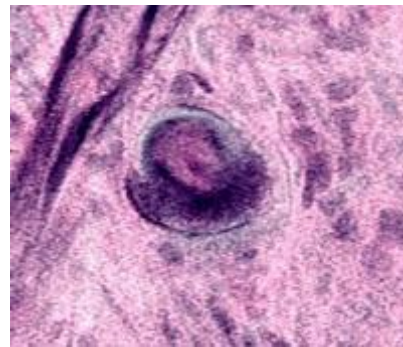
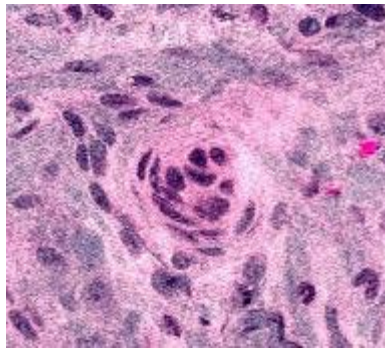
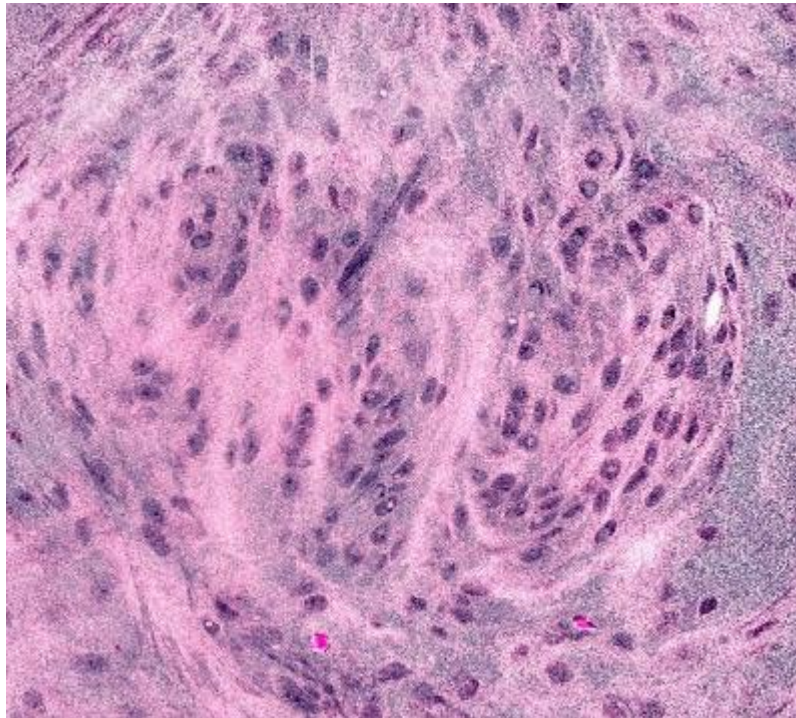


CHE: FFPE

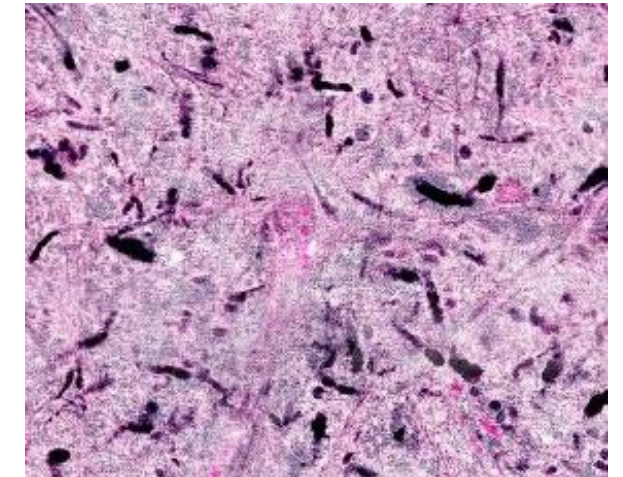
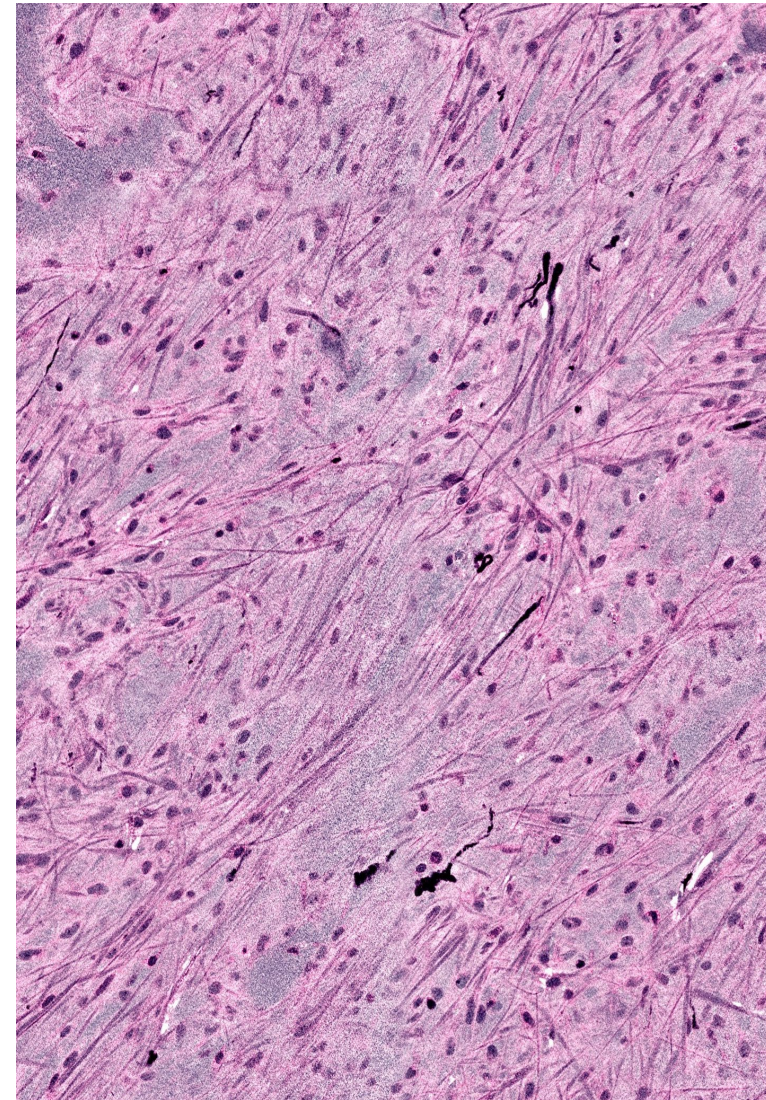


Examples of surgical specimens imaged with SRS

Meningioma

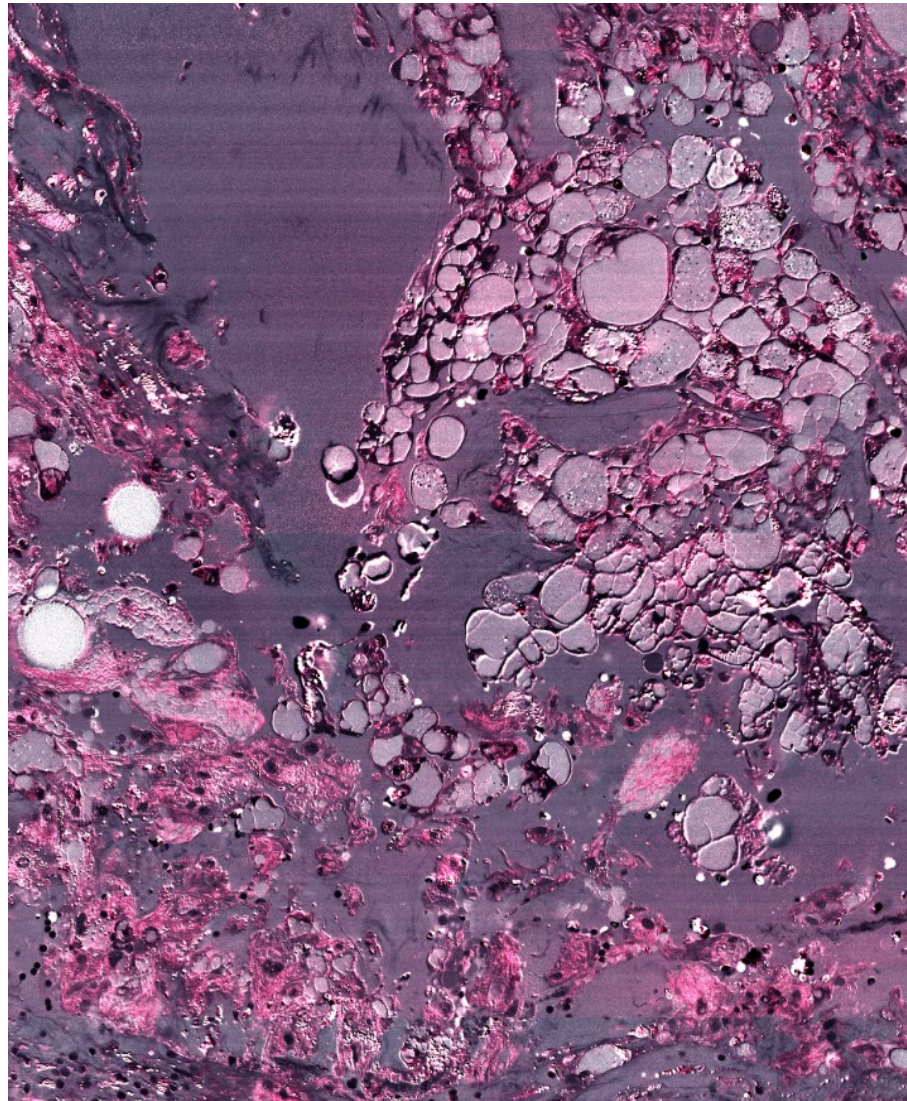


Pilocytic Astrocytoma

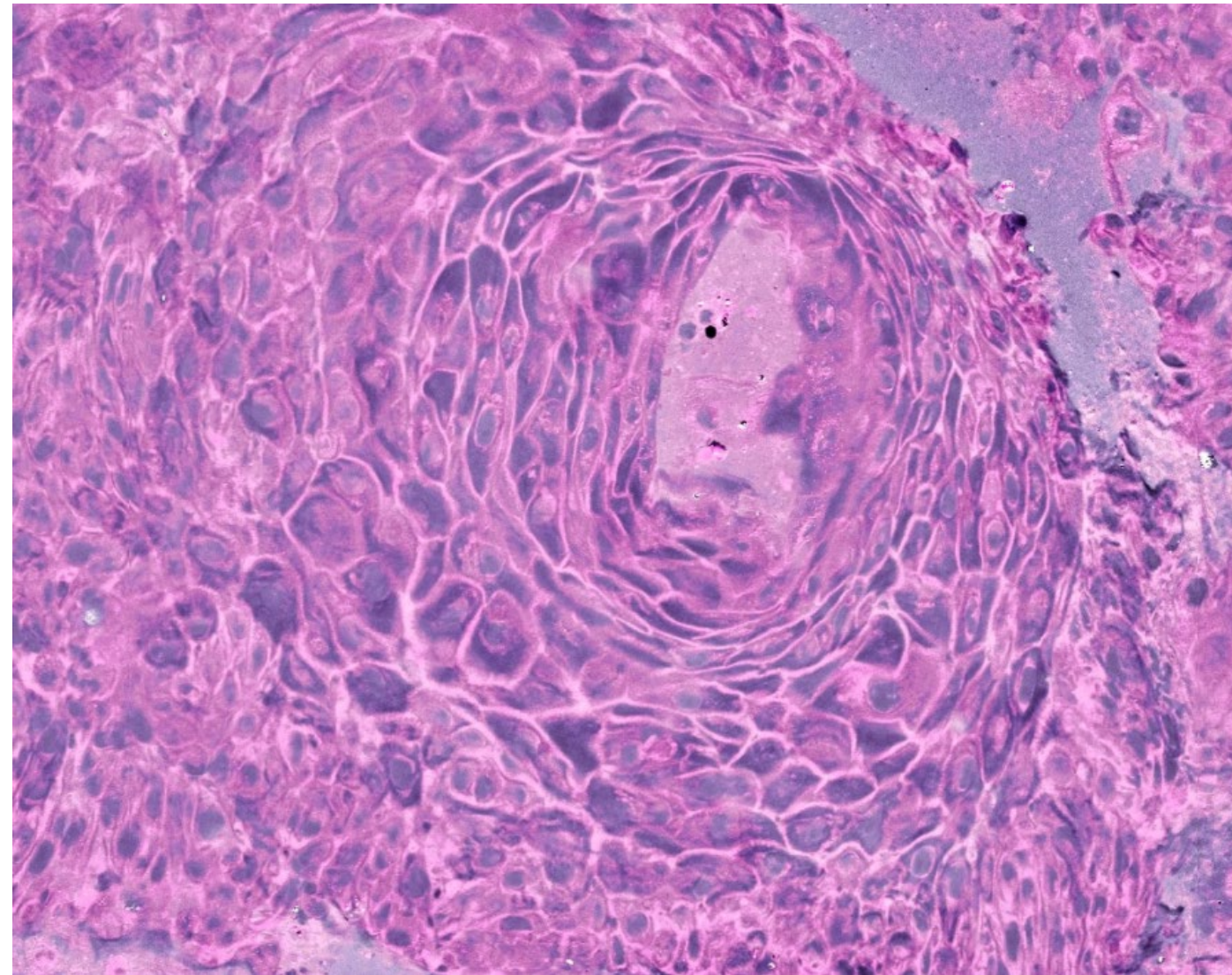


Examples of surgical specimens imaged with SRS

Chordoma

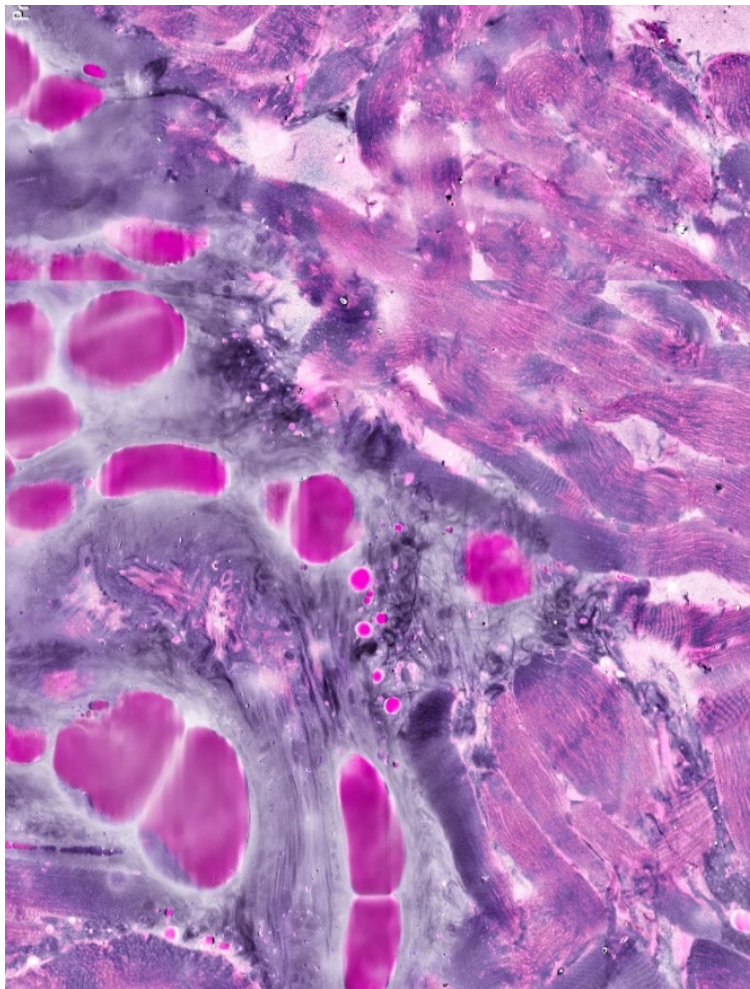


Squamous Cell Carcinoma

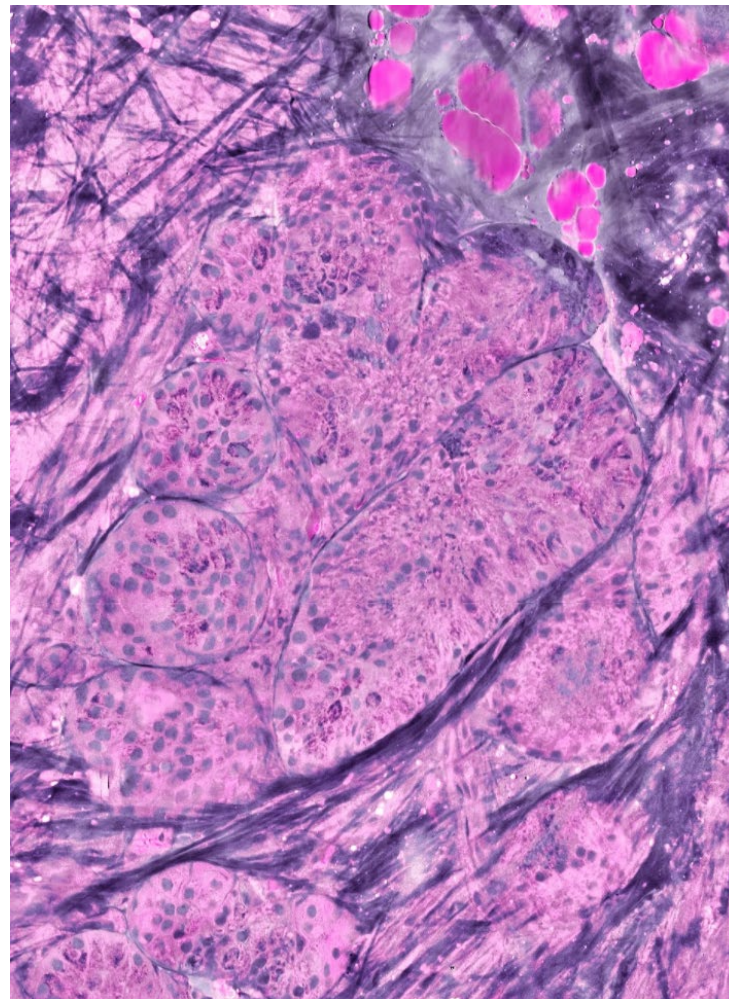


Examples of surgical specimens imaged with SRS

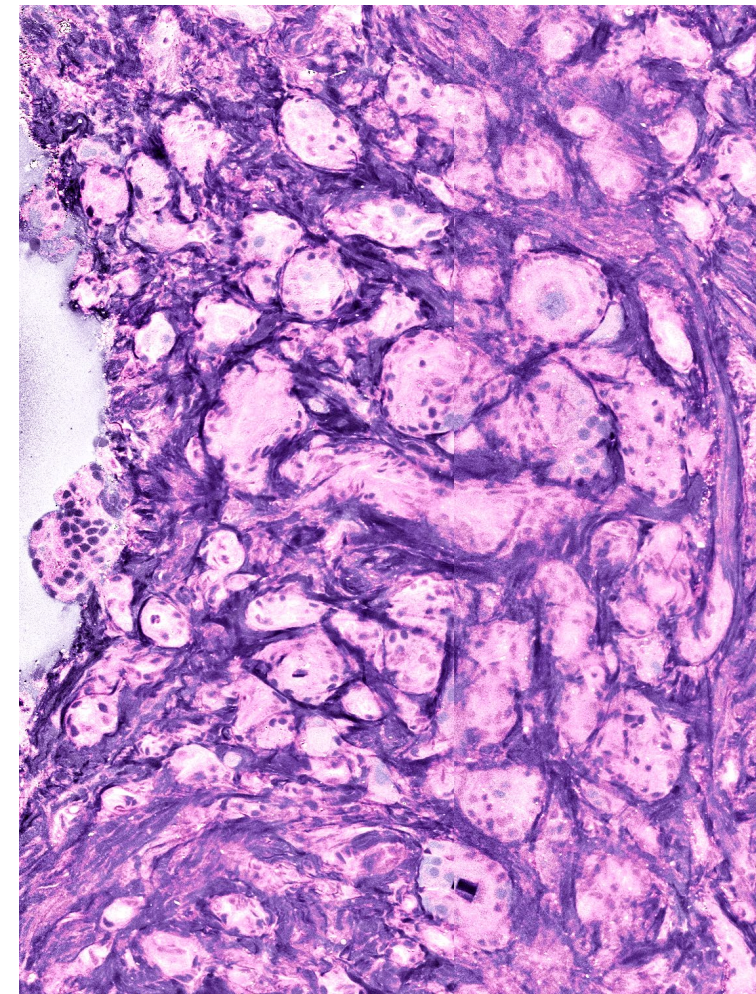
Adipose Tissue,
Muscle



Salivary
Gland



Prostate
Biopsy

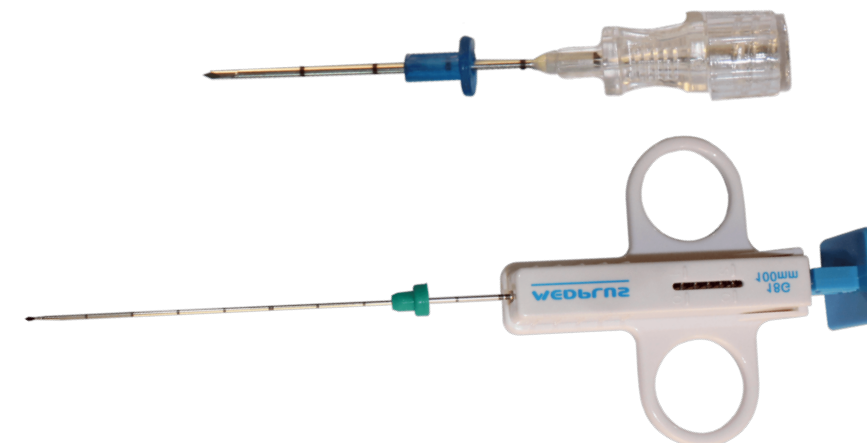


Courtesy NYU

Current Areas of Investigation and Application

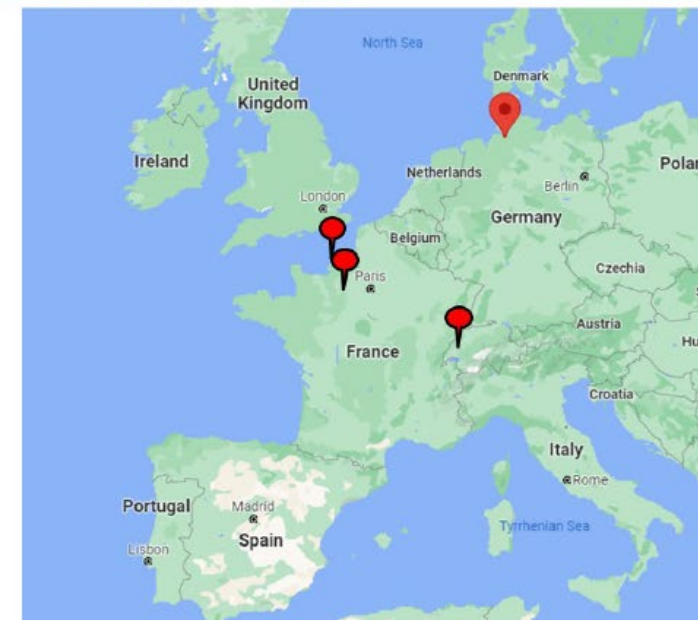
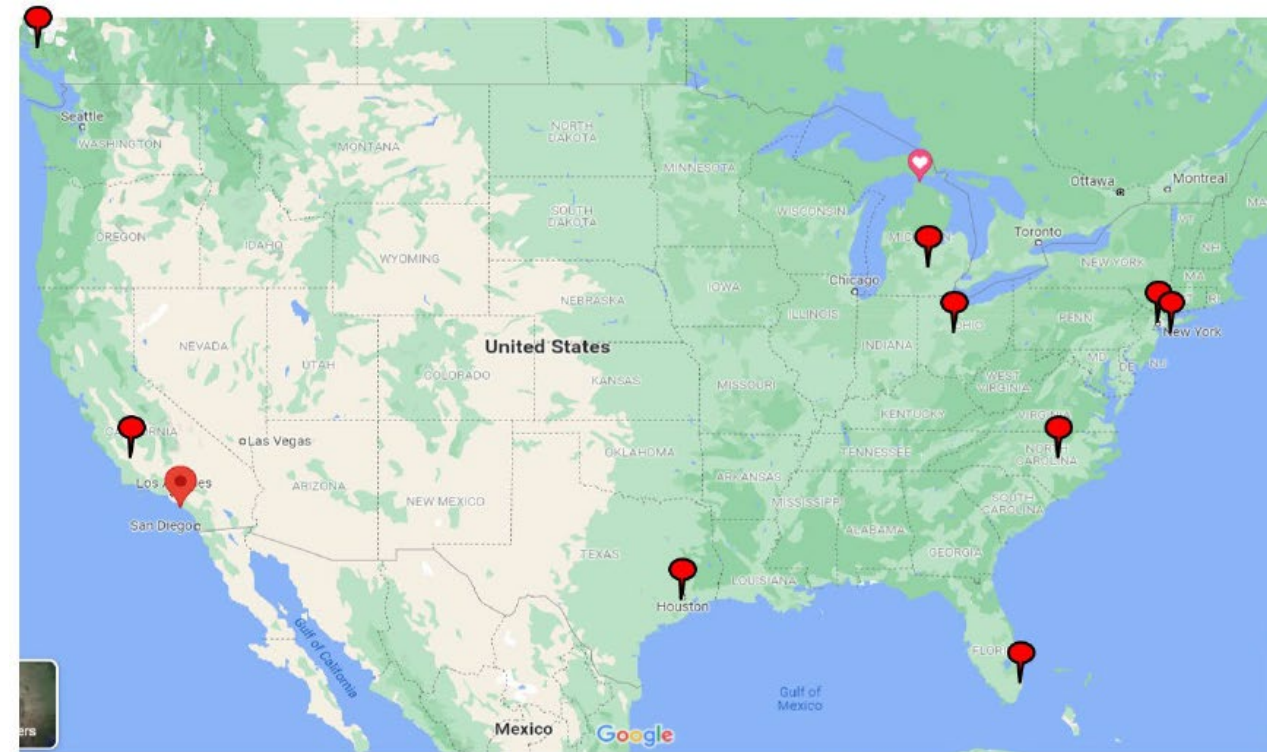
Technology good for any tissue the size of a grain of rice (endoscopic biopsies, core needle biopsies, etc.).

- Prostate bx
- Kidney bx
- Pancreas Bx
- Breast Bx
- Pulmonary
- Margin assessment of head and neck
- Adequacy assessment
- Banking
- Allocation of tissue for molecular testing



Current Areas of Investigation and Application

- USA
- Canada
- Europe
(Research and Clinical)



Intraoperative Digital Imaging Stimulated Raman Scattering Microscopy

- **Non-destructive.**
 - Uses fresh, unlabeled, unprocessed tissue.
 - Minimal tissue handling. Does not required expert staff.
 - After digital image is acquired, the tissue can be used for standard histopathology/molecular.
- **Avoids freezing artifact.**
- **Rapid image acquisition (<3 min)**
- **Digital image can be stored, analyzed on site or remotely**
- **Ideal for small specimens**

Nick Reder, MD, MPH, FCAP

Dr. Reder is the CEO of Alpenglow Biosciences and a clinical acting instructor at University of Washington with a subspecialty practice in genitourinary pathology. He received a B.S. in biochemistry from the University of Michigan, an M.P.H. in epidemiology from Emory University, and an M.D. from Loyola University Chicago before receiving his pathology training at University of Washington, where he completed an anatomic pathology residency followed by a genitourinary pathology fellowship. He has served on the CAP In Vivo Microscopy Committee since 2016. While in residency, Dr. Reder won the Castleman Award for his research in 3D light-sheet microscopy. Dr. Reder's research focuses on using light-sheet microscopy and machine learning to improve clinical diagnostics and accelerate drug development.



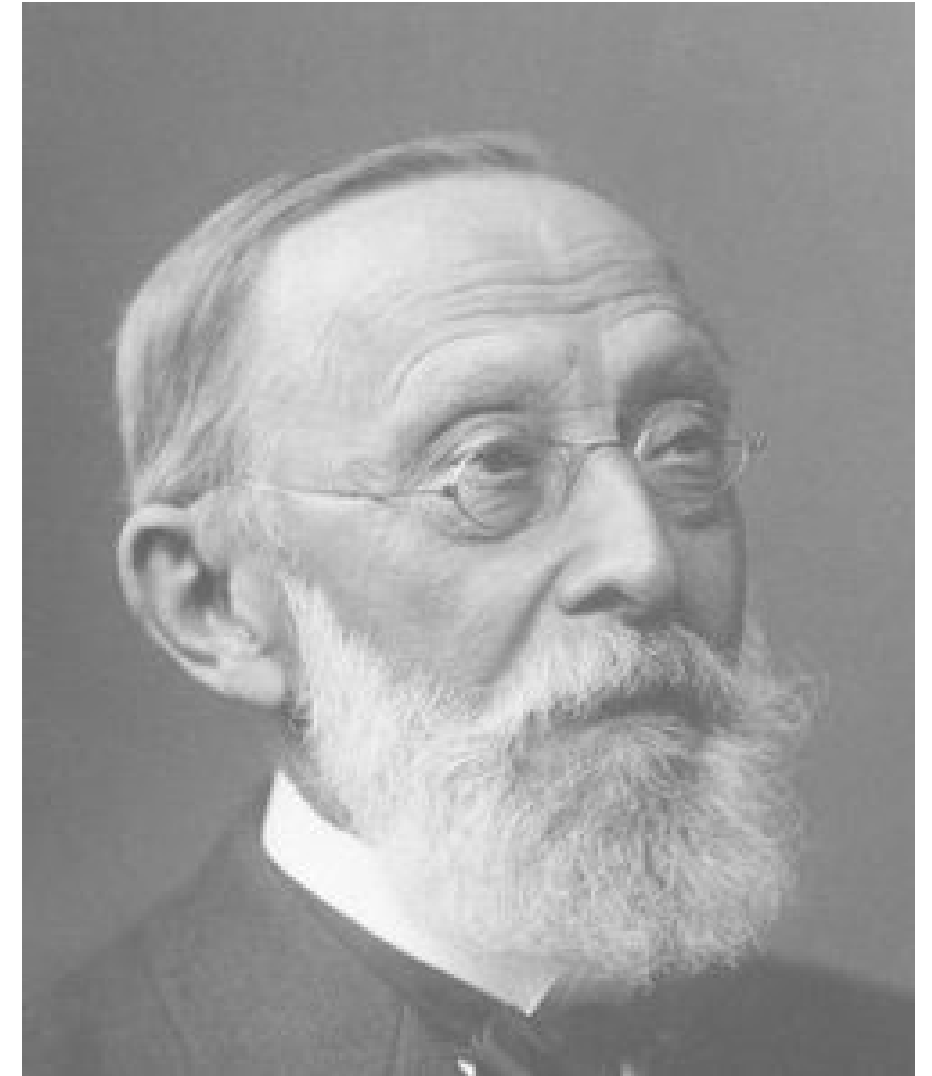
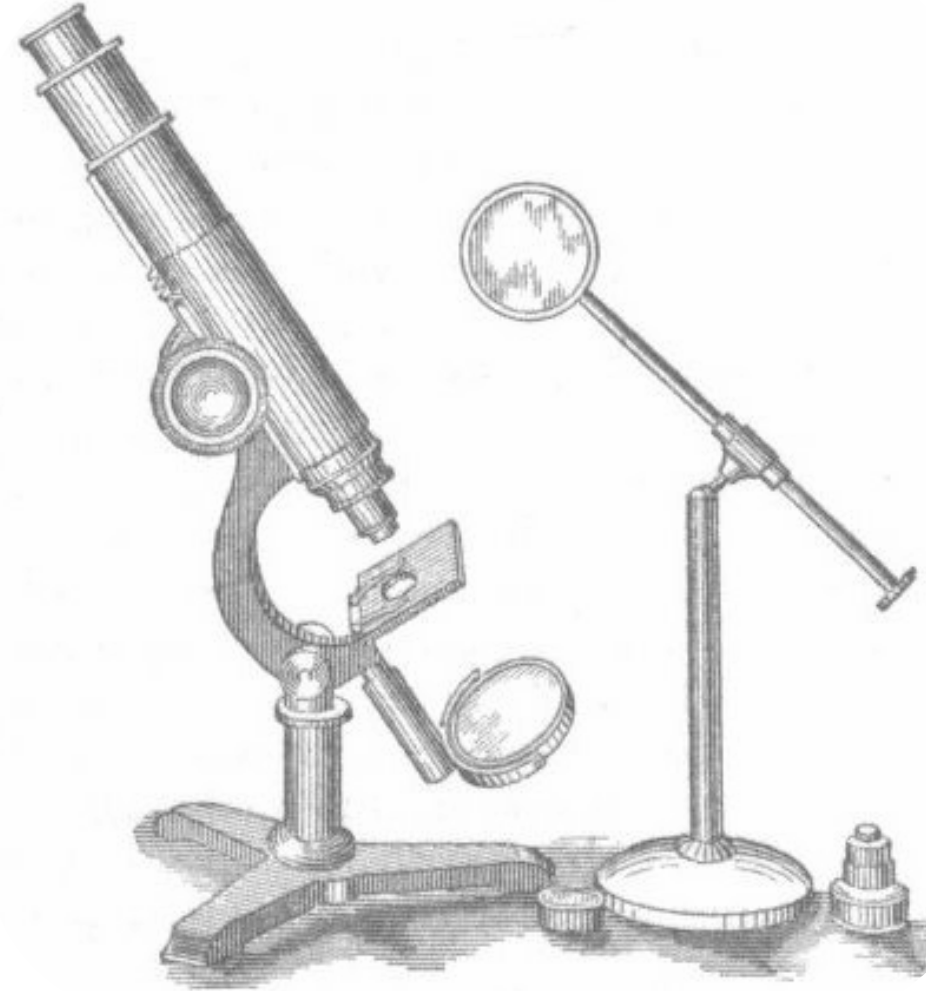
Light Sheet Microscopy

Nicholas Reder, MD MPH

Conflicts of Interest

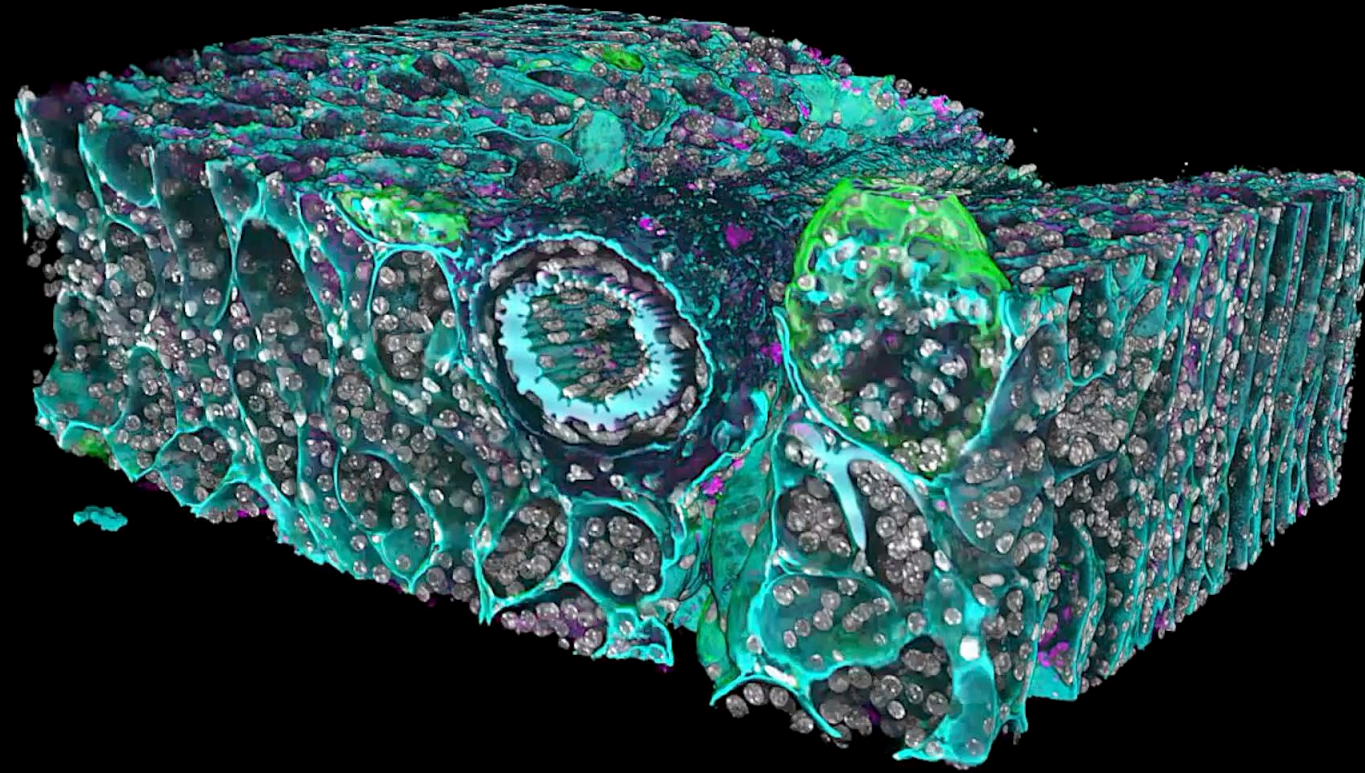
Dr. Reder is an employee, shareholder, and board member of Alpenglow Biosciences

The problem



Pathology (i.e. microscopy of tissue) is **essential**, but uses **archaic technology** that gives an incomplete, biased 2D view of 3D tissue structures

The Solution: 3D tissue imaging



DAPI
WGA-lectin
Coll IV
Podxl

3D spatial biology

2D to **3D**, analog to **digital**, image entire tissues, and generate richer data for AI analysis

Analog



Slide-based 2D pathology

2D
Analog
Fraction of tissue
Consumes tissue
Labor intensive
Subjective variability
Manual

3D spatial biology

AI-powered 3D spatial biology

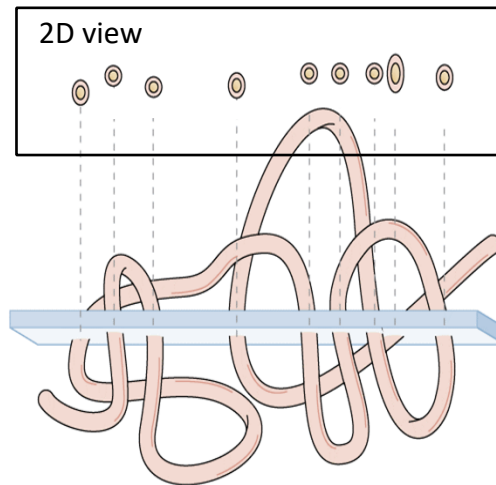
3D → more and better data
Direct to Digital → enhanced workflow
Entire tissue → understand the whole story
Preserves tissue → improved molecular assays
Minimal labor → laboratory efficiency
Objective → Consistent results
Automated → Scalable and repeatable



3D spatial biology is critical

Many tissue analyses benefit from 3D, especially in 3 key areas

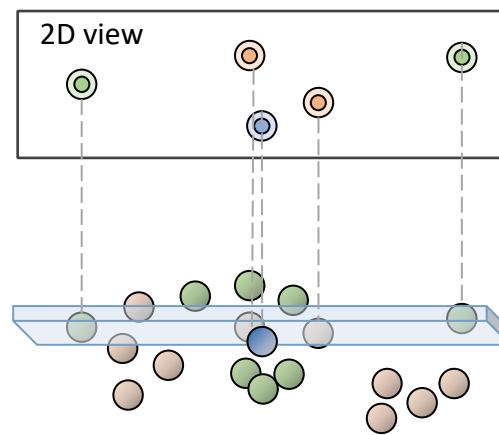
Convolved shapes



Vasculature, neurons & fibrosis

NASH, other fibrosis, neuroscience, ophthalmology, organoids

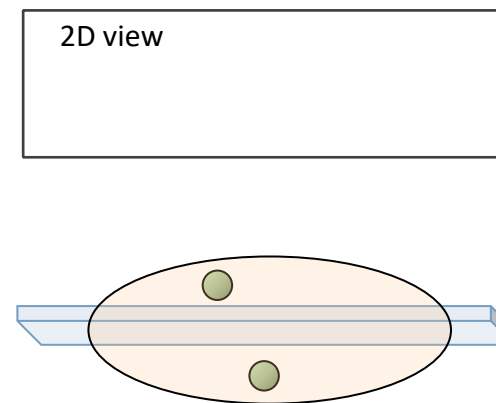
Complex distributions



Spatial relationships between cell populations

Immuno-oncology, immunology, metabolic disease

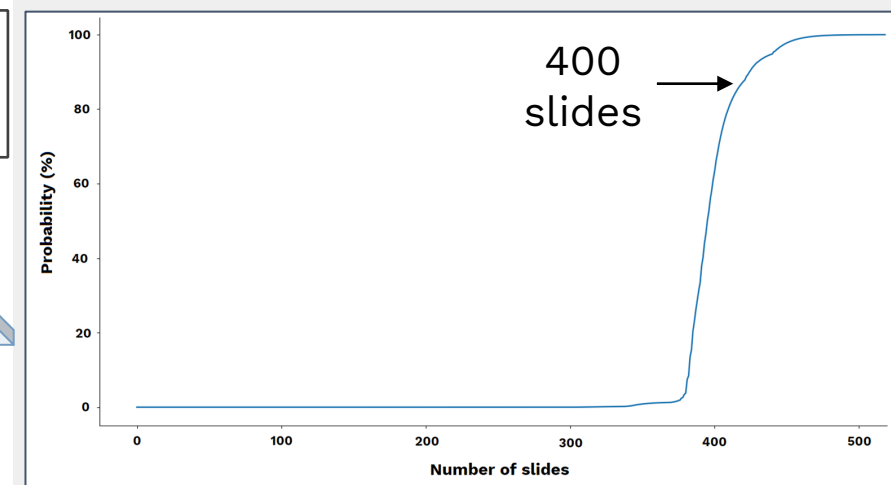
Sparse objects



Rare cell populations in large volumes of tissue

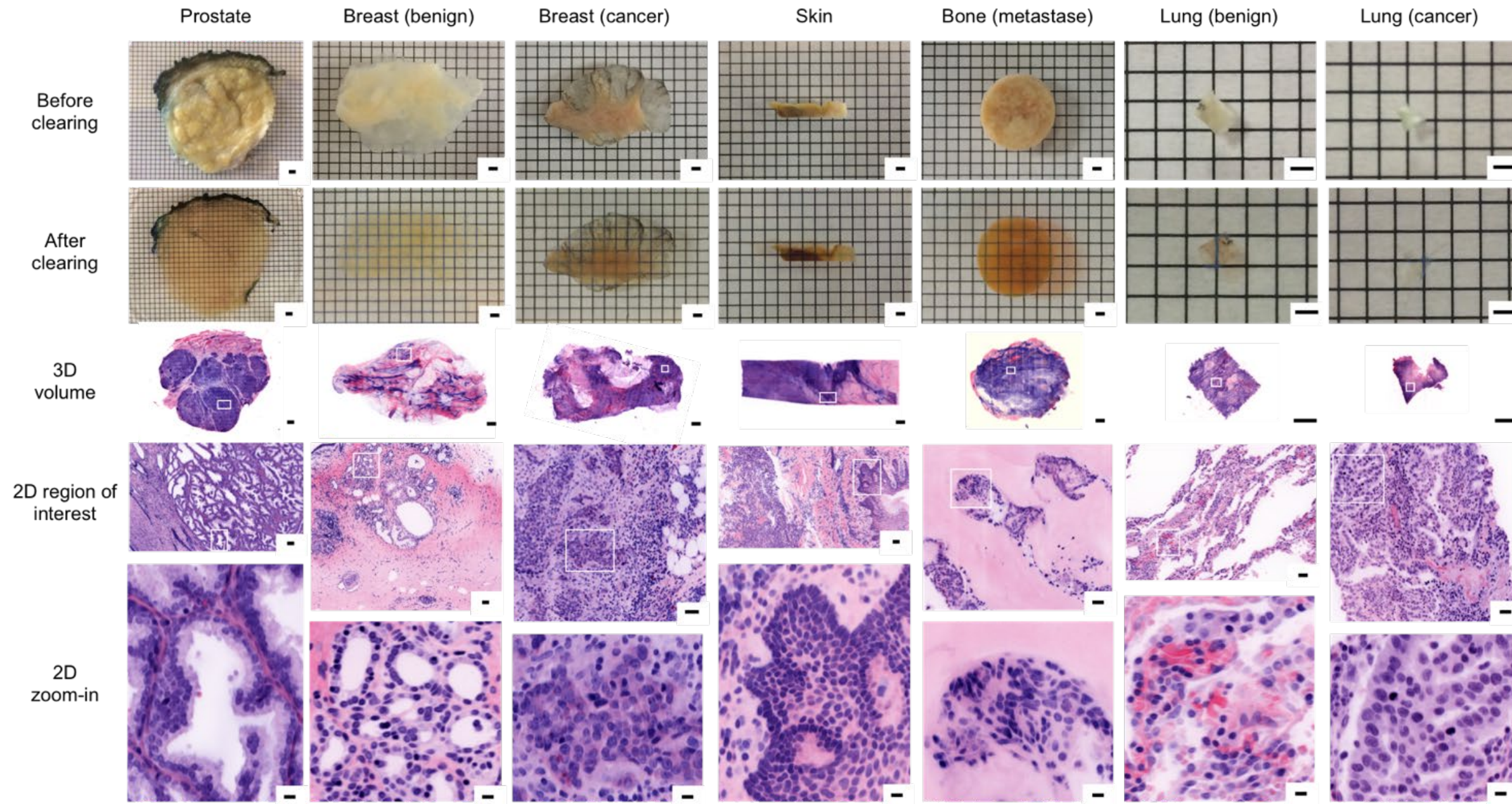
Gene therapy, CRISPR, CAR-T

Replicating 3D insights with 2D slides



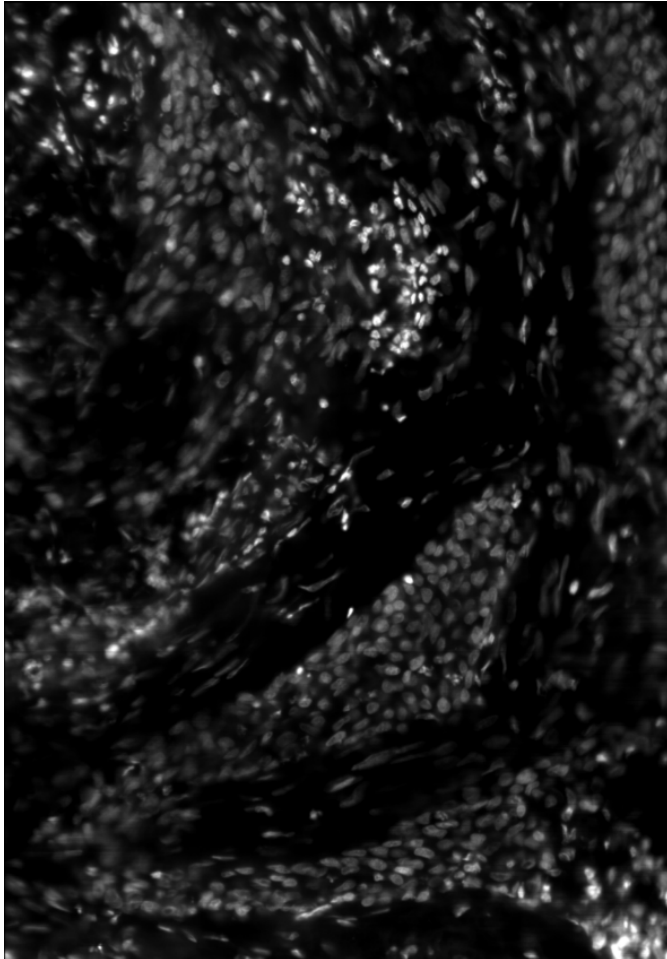
It would take **400 slides, 2+ wks** of lab time, and **\$100k+** in cost to get the same answer in 2D

Technology: Tissue clearing



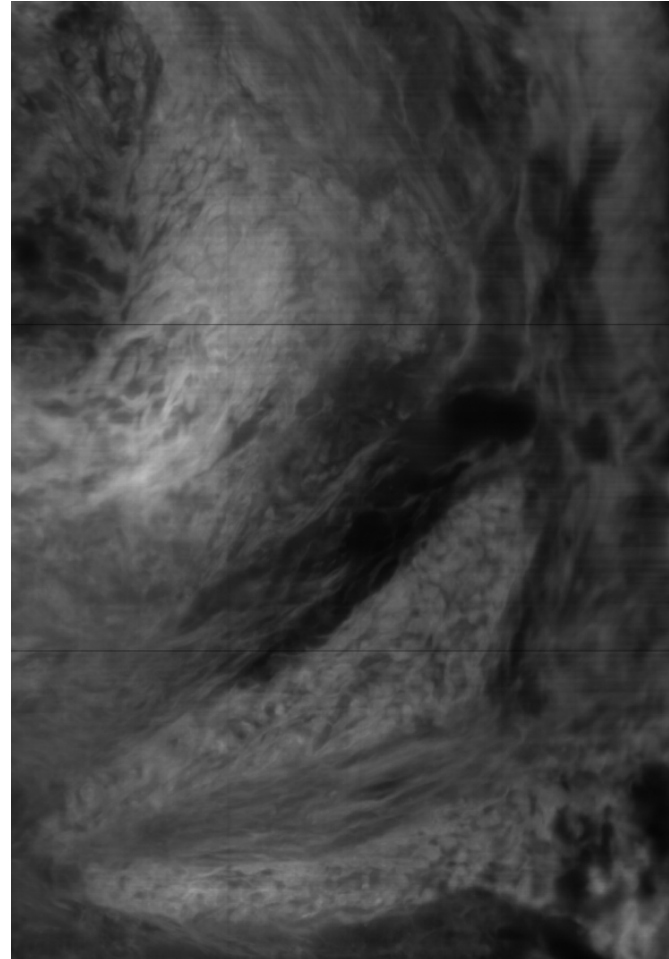
Technology: Computational H&E

TOPRO3: Nuclear

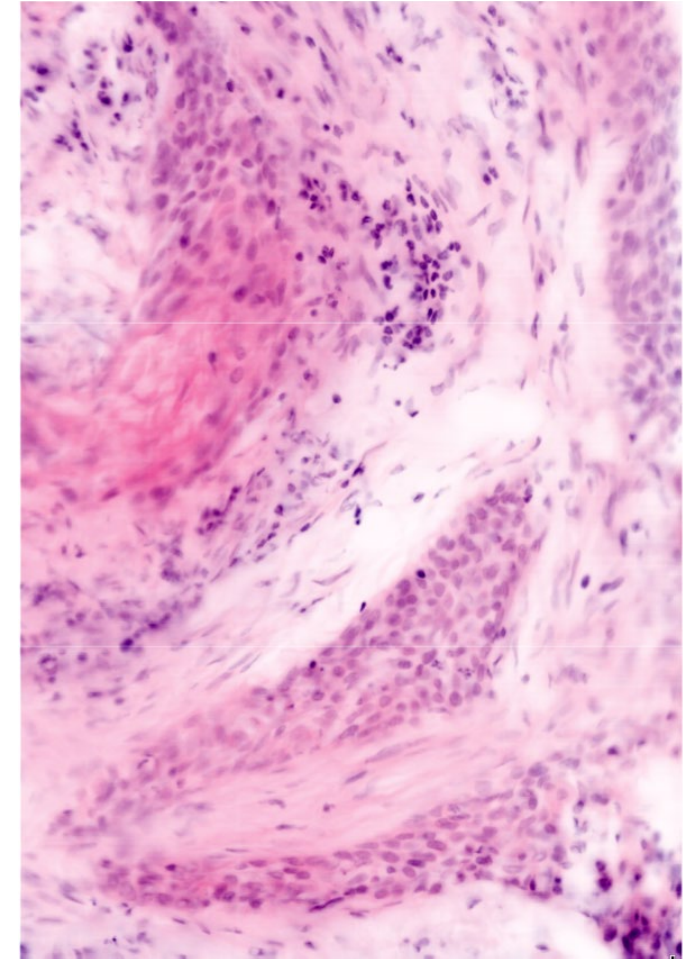


+

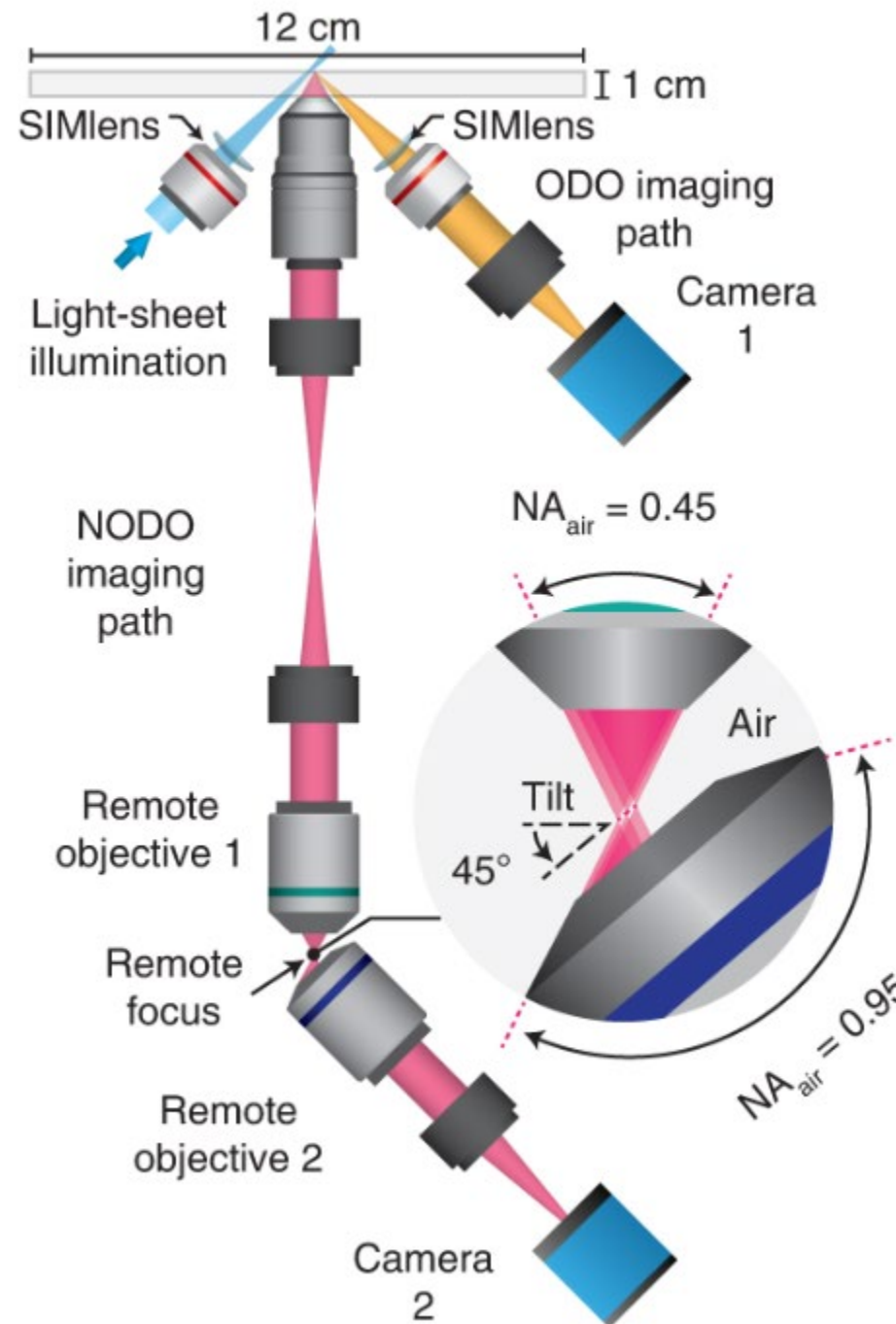
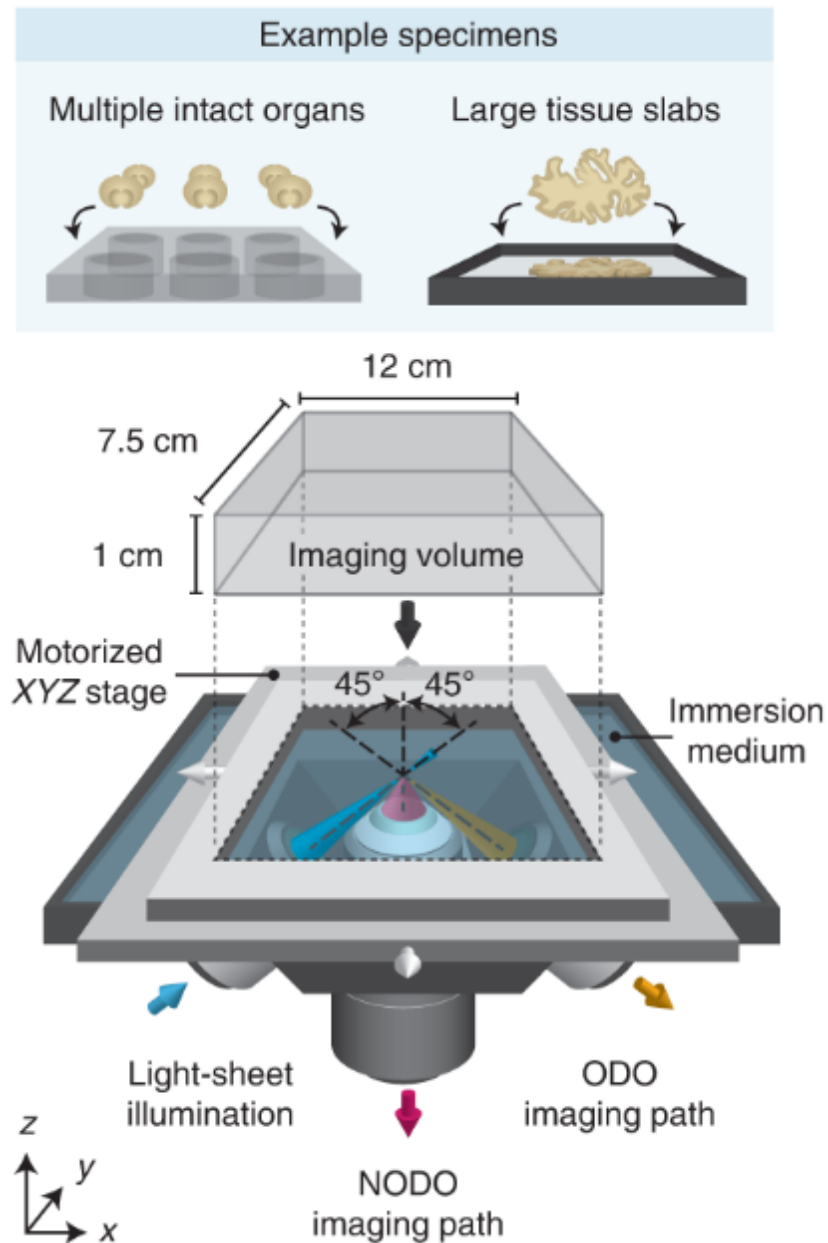
Eosin: General protein



Computational H&E



Technology: Hybrid open-top light-sheet microscope



Benefits of Hybrid OTLS

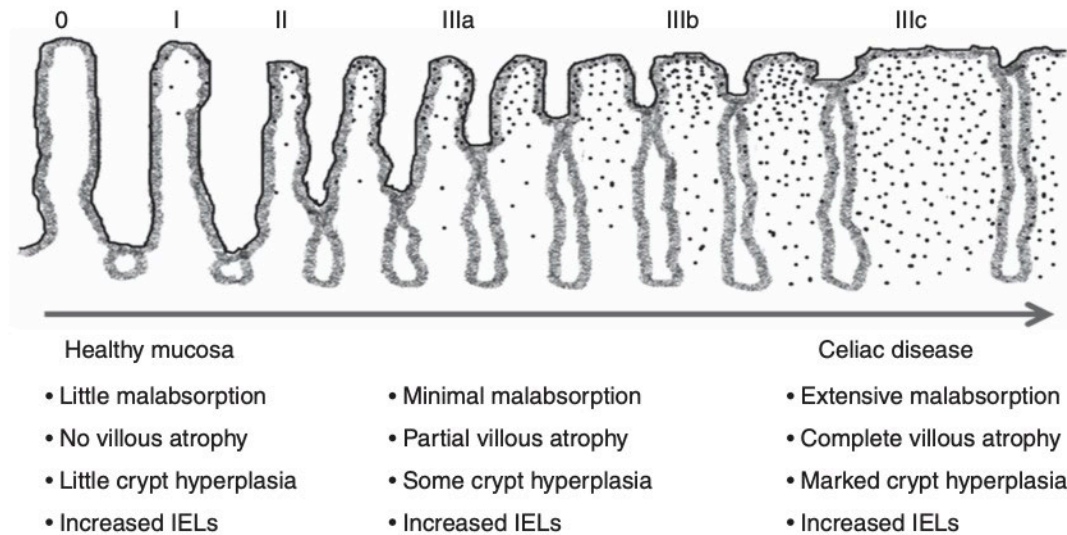
- Scout and Zoom
- Large sample area up to 12 cm x 7.5 cm x 1 cm
- High-throughput and automated multi-sample imaging of 12 core biopsies, 96 well plates, and other uses

Clinical use-case: Celiac disease

- Presented by Dr. David Simmons at DDW 2023

Challenges in Celiac disease pathology

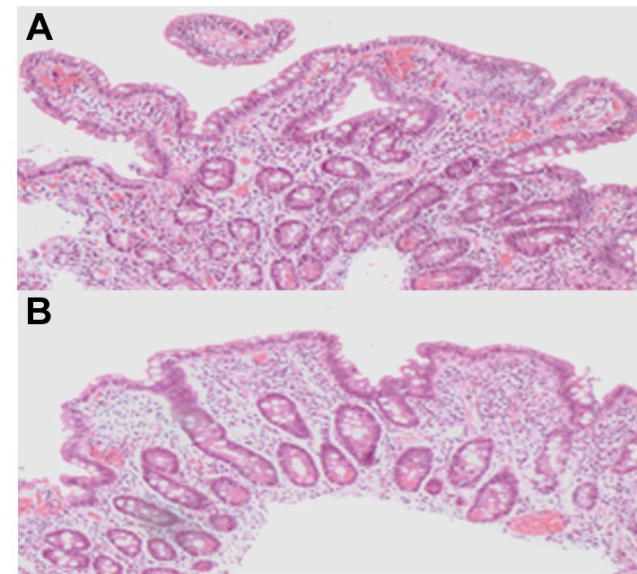
Theory: Marsh classification



Categorization of a spectrum of biology

Adelman DC et al. Am J Gastroenterology. 2018

Practice



2D sections of complex 3D objects
Sampling error
Subjective interpretation

Taavela J et al. PloS one. 2013

Variable results

Parameter	Kappa
Villous height : Crypt depth	0.39-0.72
Intraepithelial Lymphocytes	0.49-0.85

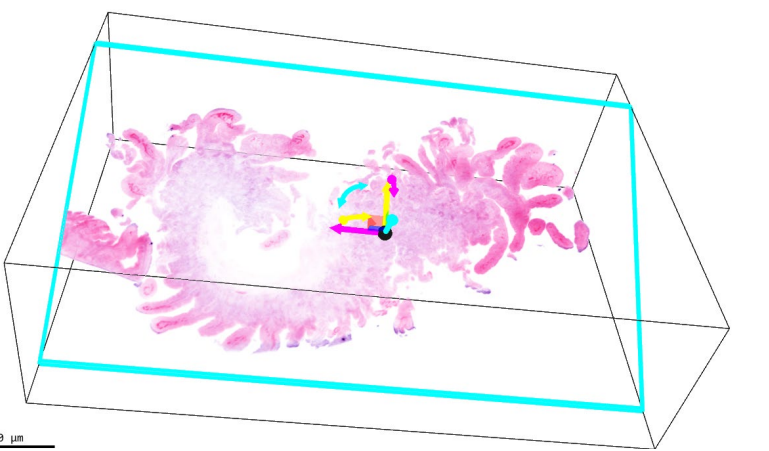
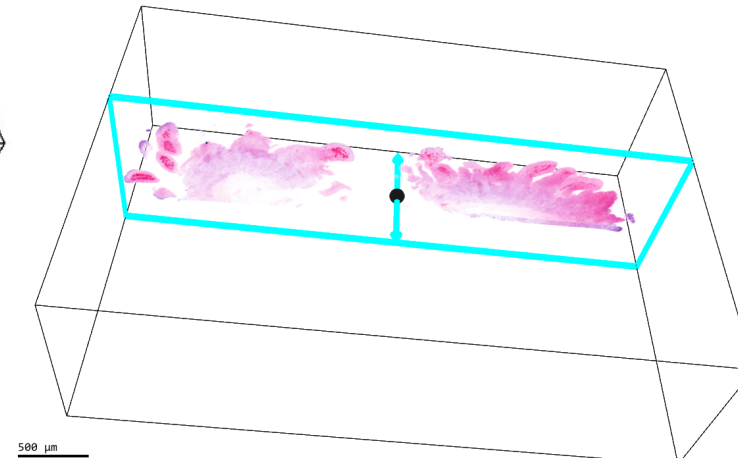
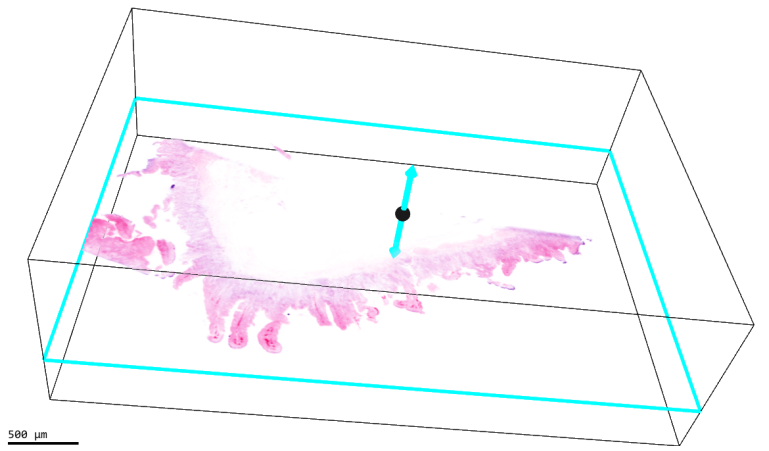
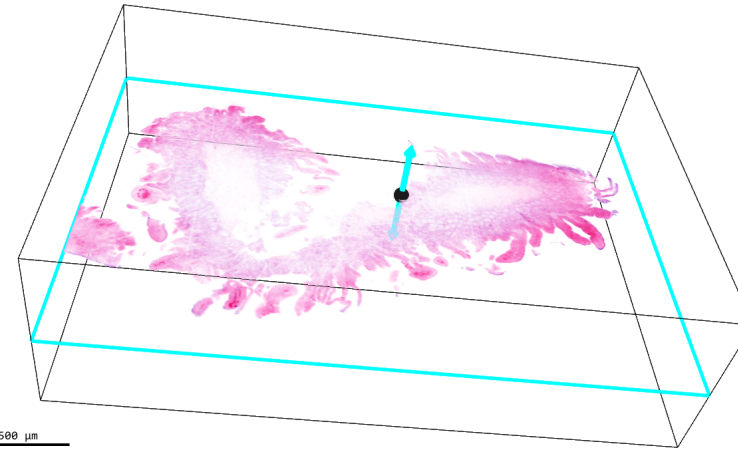
Inter-rater variability
Unpredictable diagnosis and
clinical trial readouts

Picarelli A et al. Scandinavian Journal of Gastroenterology. 2014

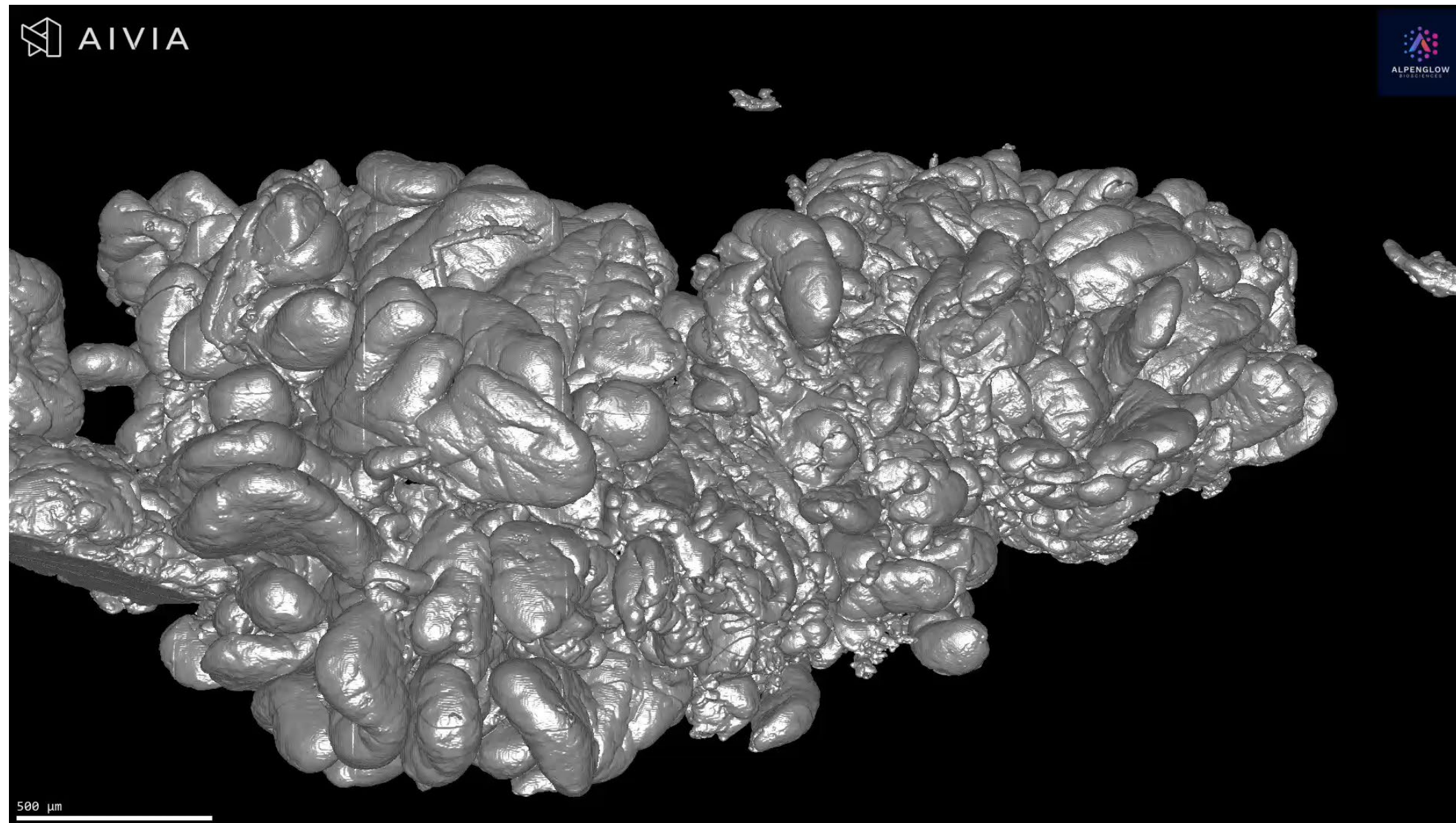
3D imaging of duodenal biopsy



Plane of sectioning influences 2D results



Boundary detection in 3D



Results

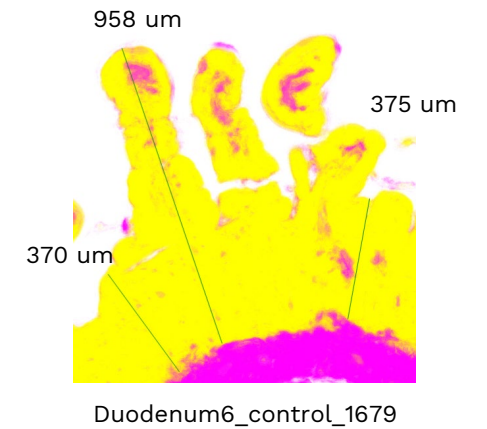
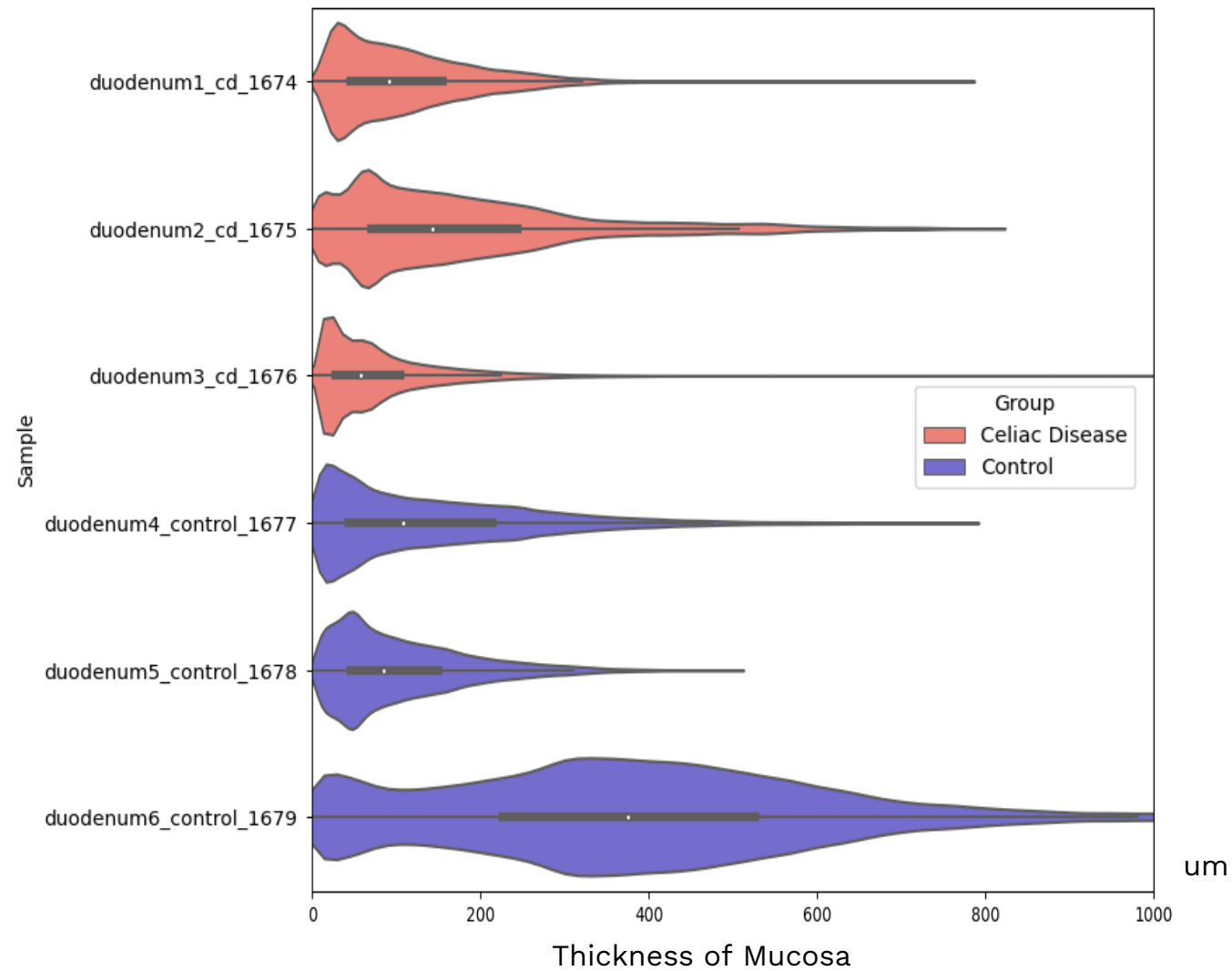
Celiac disease samples

	Mean	Std dev
duodenum1_cd_1674	110.4	83.4
duodenum2_cd_1675	178.9	145.0
duodenum3_cd_1676	79.3	76.8
Average	122.8	114.1

Control samples

	Mean	Std dev
duodenum4_control_1677	144.4	128.3
duodenum5_control_1678	106.3	81.4
duodenum6_control_1679	392.2	242.2
Average	214.3	208.1

p-value from T-test for mean length of groups is 0.38



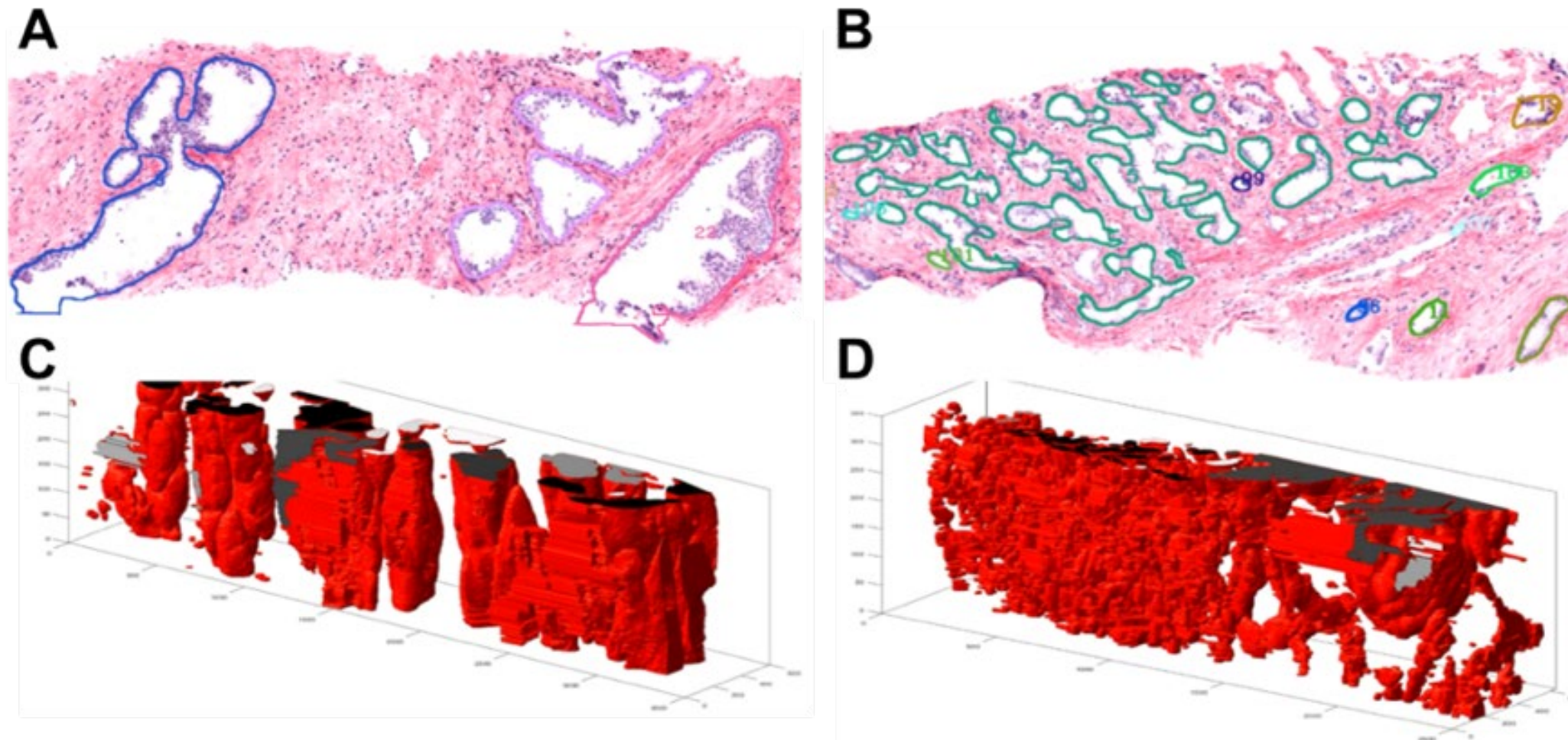
Clinical use-case: Prostate cancer

- Xie et al. Cancer Research. 2022.

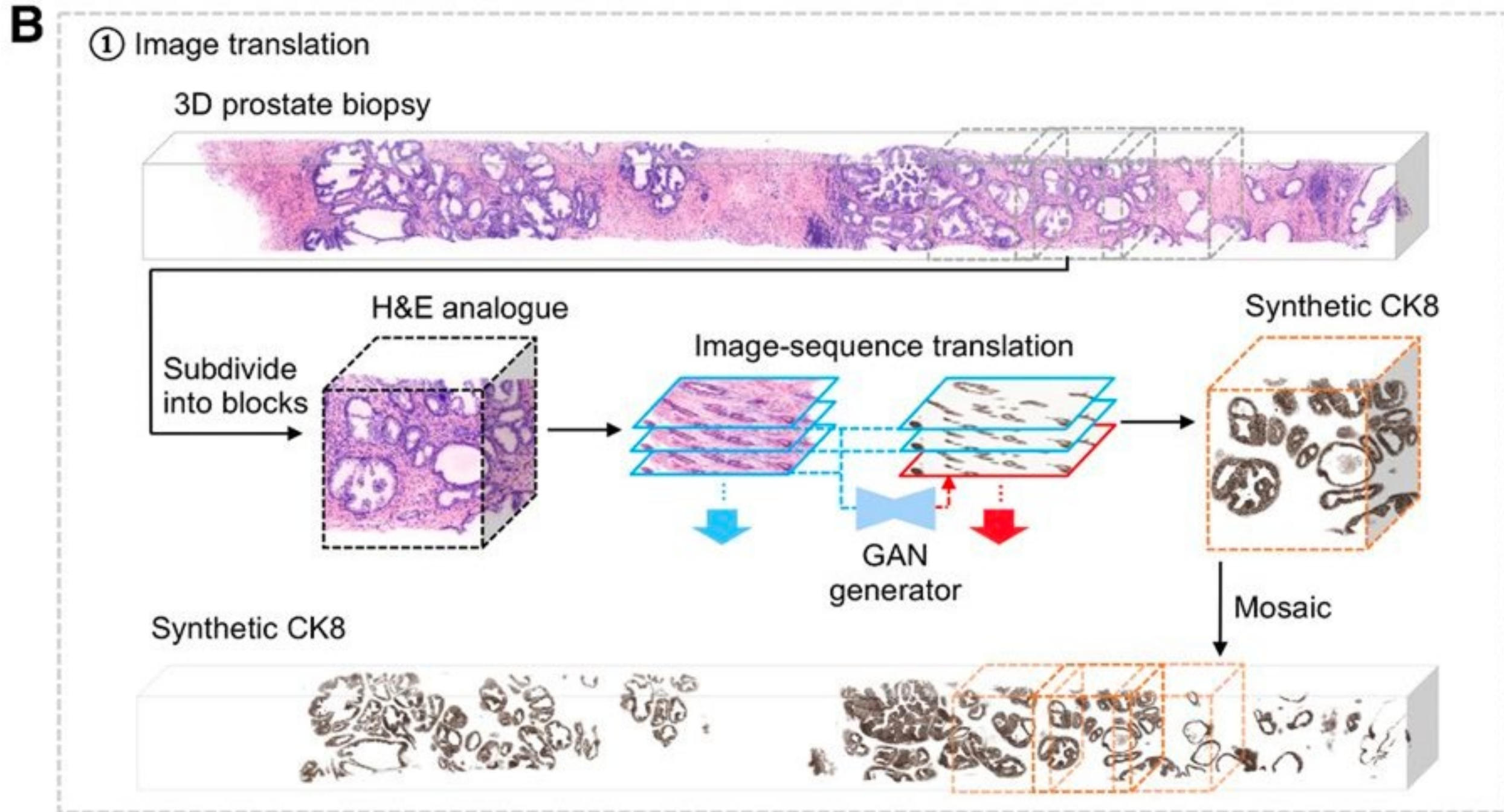
3D imaging of prostate biopsies

We must extract useful info from the 3D datasets

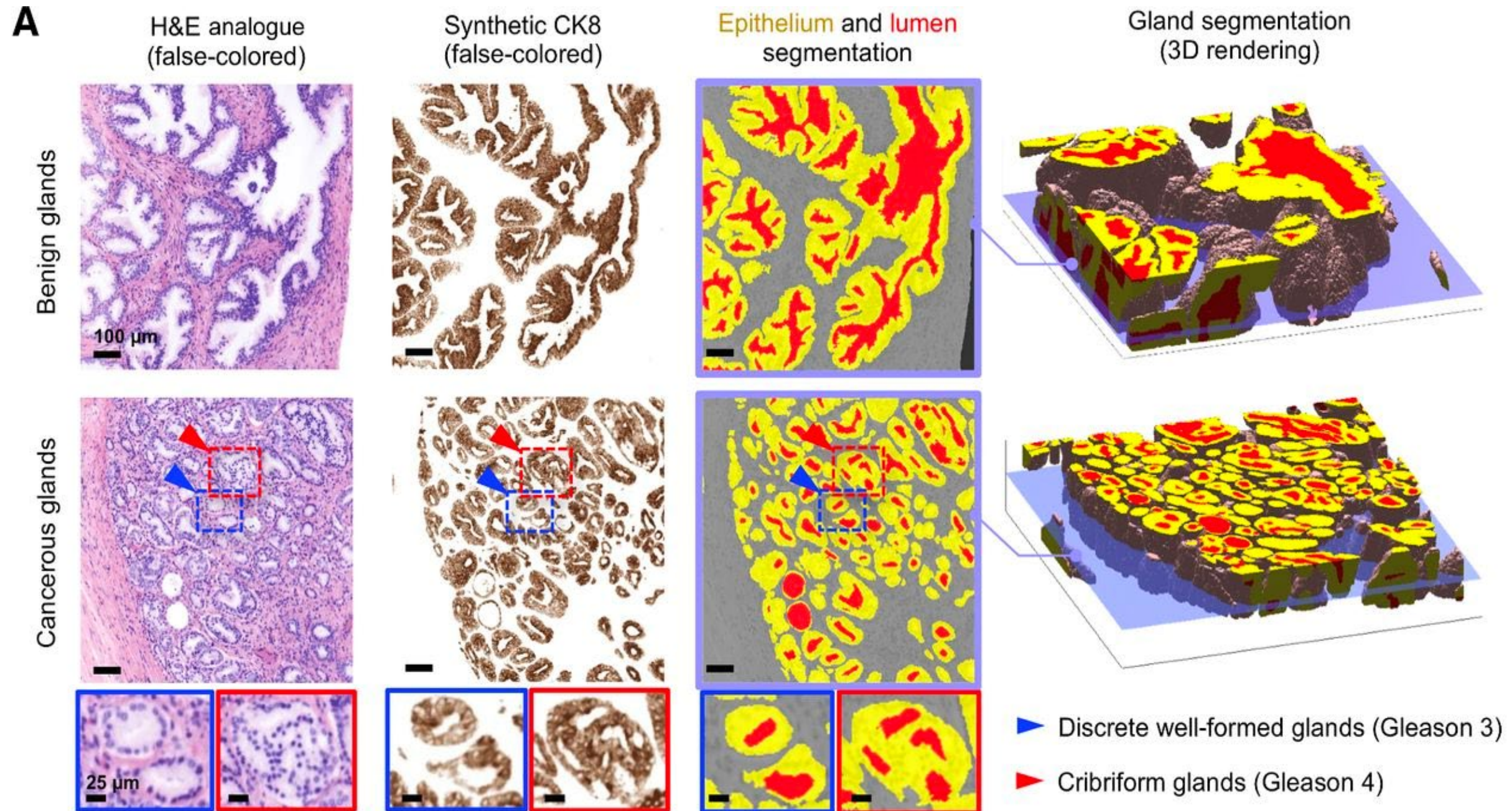
These datasets required 10+ hours to annotate!



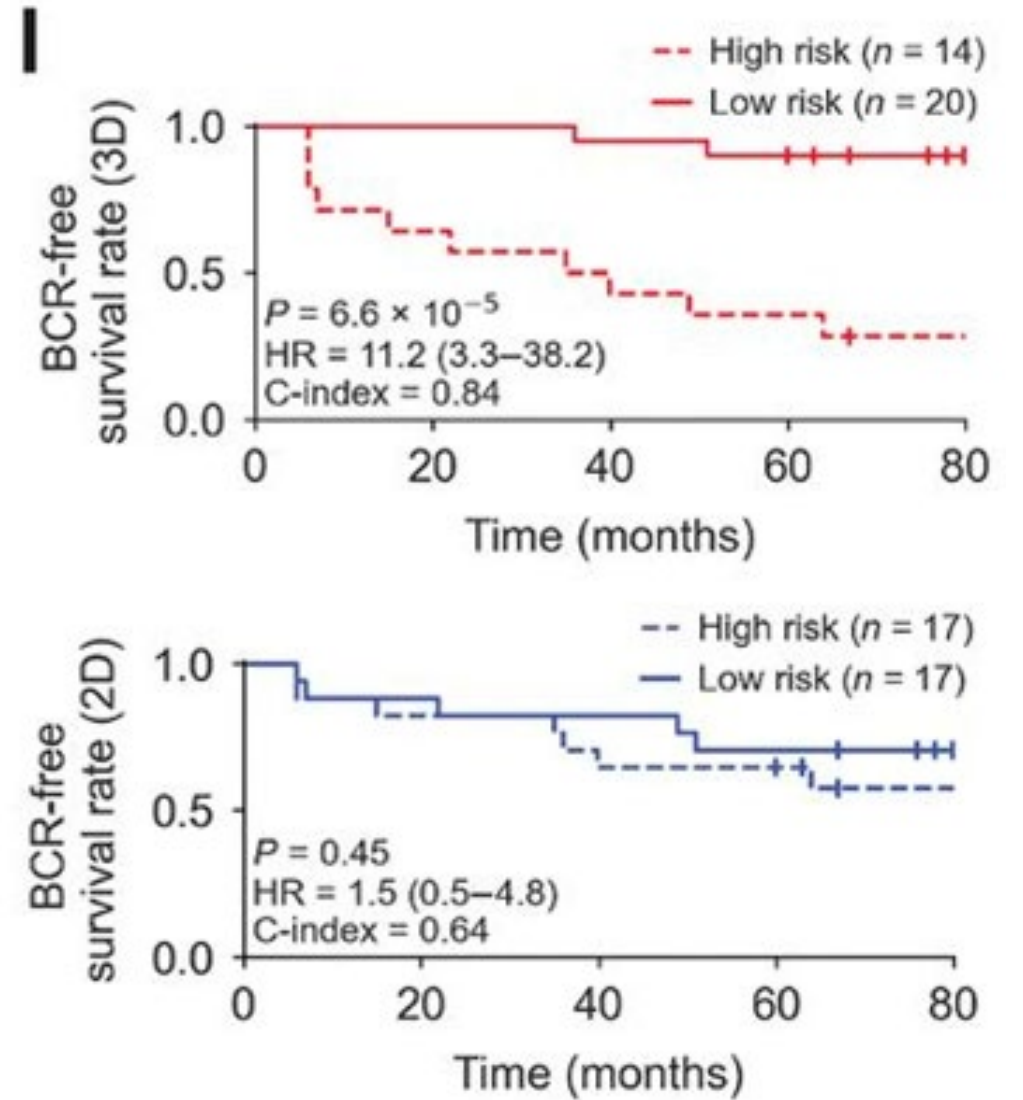
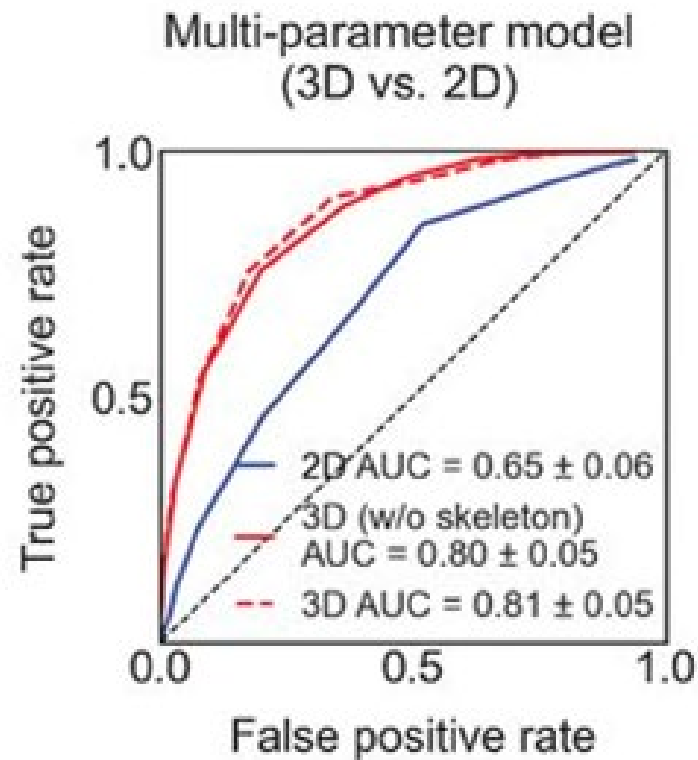
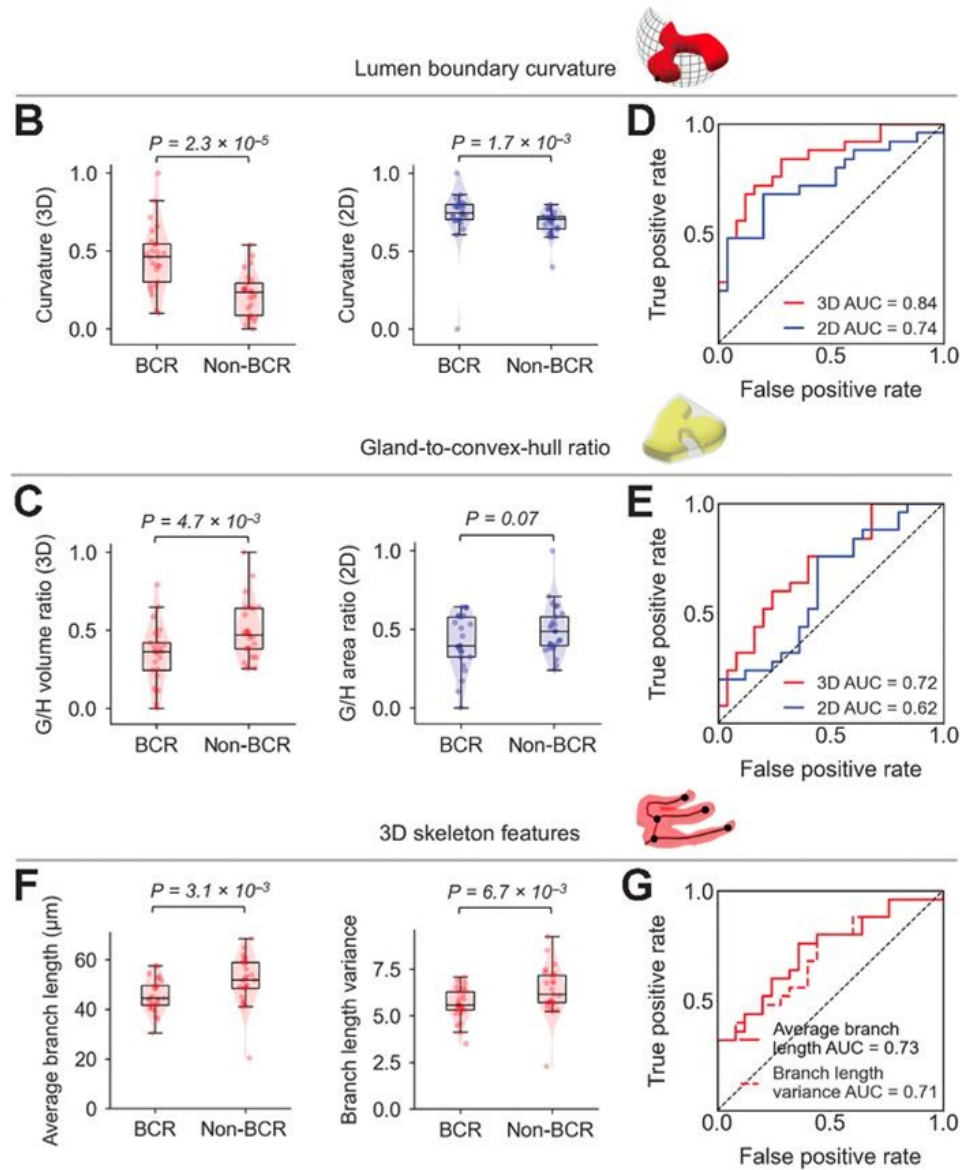
Generative AI to assist in segmentation



Gland, lumen, and stroma segmentation



3D outperforms 2D in prognostication



Summary of 3D pathology

Strengths

- Full 3D imaging of tissue samples
- No sectioning (labor, non-destructive)
- Natively digital technology
- Compatible with most fluorescent labels
- Superior AI analysis compared with WSI analysis
- Can be used before or after FFPE embedding (prelim dx or ancillary assay)

Limitations

- Fluorescence only
- Prolonged time (now decreasing)
- Antibody staining currently requires 1-2 weeks
- Big data requires IT infrastructure
- Additional validation needed
 - Non-interference with downstream assays
 - Morphologic difference with traditional H&E
- Differences in reagents
 - TO-PRO-3 vs. DAPI or hematoxylin
 - Ethyl cinnamate vs paraffin

Questions

Thank You !

The DCPC will be producing more digital pathology educational content in 2023.

- In addition to webinars the committee will produce podcasts on digital pathology implementation and will create a digital pathology frequently asked questions (FAQ) section for our updated and enriched website.

- [DCPC Website](#)

We are also updating the Digital Pathology Resource Guide. Please reach out if you are interested in assisting with this effort.

To become a DCPC member please apply during the upcoming committee appointment cycle.



COLLEGE of AMERICAN
PATHOLOGISTS