



COLLEGE of AMERICAN  
PATHOLOGISTS

# Creating a successful pathology-engineering collaboration

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Nicholas P. Reder, MD, MPH, Adam

5/30/2017

Glaser, PhD, Lawrence D. True, MD,

FCAP, and Jonathan T.C. Liu, PhD

# Housekeeping

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- **The presentation will be recorded and will be available in about 1 week; a pdf of the presentation will be sent to all registrants in about 1 week**
- **All lines are muted during the presentation**
- **Please ask your questions when you think of them via the “Question box” in your control panel**

# Lawrence D. True, MD, FCAP



- **Earned a B.A. from Harvard**
- **Earned M.D. from Tulane**
- **Completed a pathology residency at the University of Colorado**
- **Professor of Pathology, Adjunct Professor of Urology, and lead pathologist for the Genitourinary Cancer Biorepository at the University of Washington**

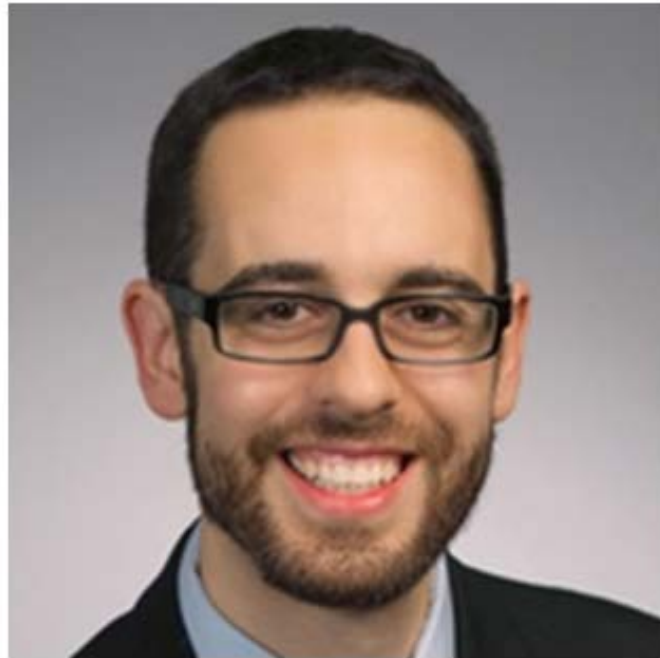
# Adam Glaser, PhD

- **Received a B.S. in biomedical engineering from Tufts University**
- **Ph.D. in Engineering Sciences from Dartmouth College in Dr. Brian Pogue's laboratory**
- **Joined Dr. Jonathan Liu's lab at the University of Washington as a postdoctoral researcher in the summer of 2015.**





# Nicholas P. Reder, MD, MPH



- **Earned a B.S. from the University of Michigan**
- **Earned an M.P.H. in epidemiology from Emory University**
- **Earned MD from Loyola Stritch School of Medicine in 2014**
- **Co-chief resident and a clinical research fellow in the University of Washington Department of Pathology.**

# Jonathan T.C. Liu, PhD

- Received a **B.S.** in mechanical engineering from Princeton
- Earned **Ph.D.** in Mechanical Engineering from Stanford,
- Postdoc from the Molecular Imaging Program at Stanford (MIPS)
- Associate Professor of Mechanical Engineering at the University of Washington and the director of the Molecular Biophotonics Laboratory



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# Disclosure

- **Drs. Glaser, Reder, Liu, and True have nothing to disclose.**
- **They are the co-founders of a start-up company (Alpenglow Optics, LLC) that does not produce, market, or distribute any healthcare goods or services.**



# 3D Light-Sheet Microscopy and Establishment of a Successful Engineering-Pathology Collaboration

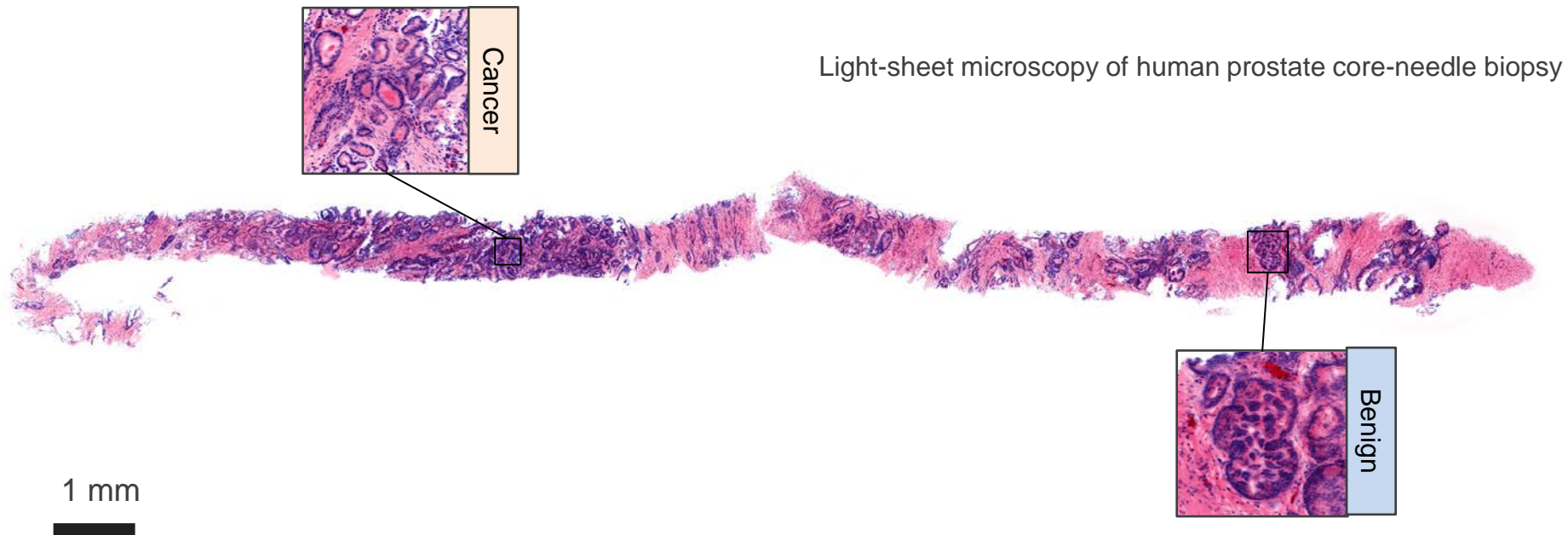
**Adam Glaser<sup>1</sup>, Nicholas Reder<sup>2</sup>,**

**Ye Chen<sup>1</sup>, Erin McCarty<sup>2</sup>, Jeffrey Chia<sup>1</sup>, Chengbo Yin<sup>1</sup>, Peter Wei<sup>1</sup>,**

**Lawrence True<sup>2</sup>, and Jonathan T.C. Liu<sup>1</sup>**

<sup>1</sup> Department of Mechanical Engineering, University of Washington, Seattle, WA

<sup>2</sup> Department of Pathology, University of Washington Medical Center, Seattle, WA



- Introduction - Dr. Larry True
- Technological innovation (3D light-sheet microscopy) - Dr. Adam Glaser
- Clinical applications - Dr. Nicholas Reder
  - 1) Sampling errors (post-operative pathology)
  - 2) Intraoperative guidance (intra-operative pathology)
  - 3) Biopsy grading (“pre-operative” pathology)
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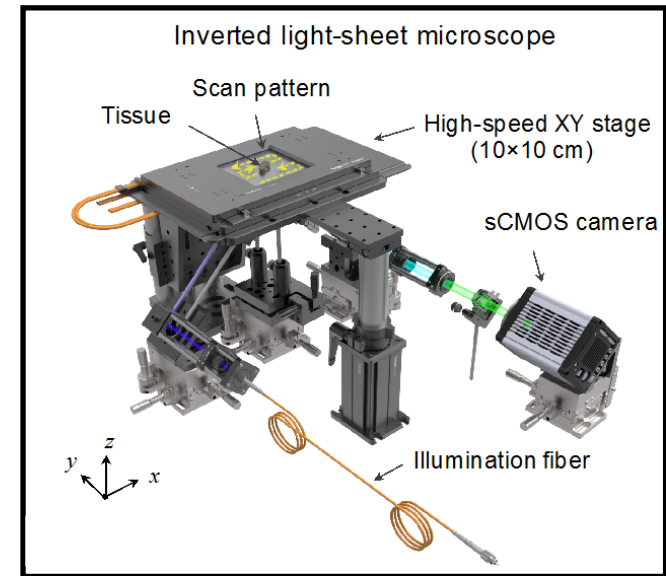


Anton van Leeuwenhoek

1854

Riddell JL  
"On the binocular  
microscope". *Quart J  
Microsc Sci.* (1854)

2016



2014

2015

2016

2017

Adam Glaser

*Postdoc, Mechanical  
Engineering*

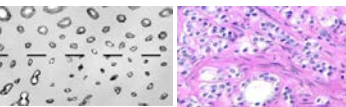
Jonathan Liu

*Assoc. Prof Mechanical  
Engineering*Building a panoramic  
light-sheet microscope

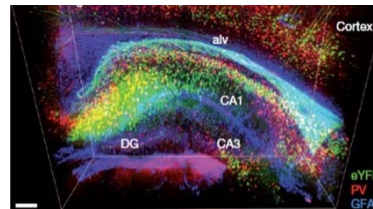
Nick Reder

*Chief resident, Research  
fellow, Pathology*Pathology  
Visions,  
CAP IVM  
Workshop

Larry True

*Professor, Pathology*Prostate Cancer Grade:  
Pattern 3 or 4 ??

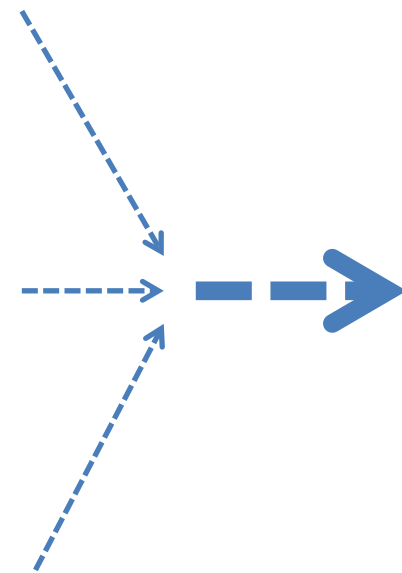
Erin McCarty

*Staff Scientist, Pathology*3-D, multiplex IHC, brain  
Deisseroth group, Nature, '13

**For what biomedical  
applications can 3-D  
light-sheet  
microscopy be used?**

**Can 3-D of  
prostatectomy help  
triage tissue?**

**Can 3-D view of  
prostate resolve  
prostate CA grade  
discrepancies?**







# 3D Light-Sheet Microscopy and Establishment of a Successful Engineering-Pathology Collaboration

UNIVERSITY of WASHINGTON



Nick Reder, MD,  
MPH<sup>#</sup>



Ye Chen,  
PhD<sup>\*</sup>



Adam Glaser,  
PhD<sup>\*</sup>



Jon Liu,  
PhD<sup>\*</sup>



Erin McCarty<sup>#</sup>



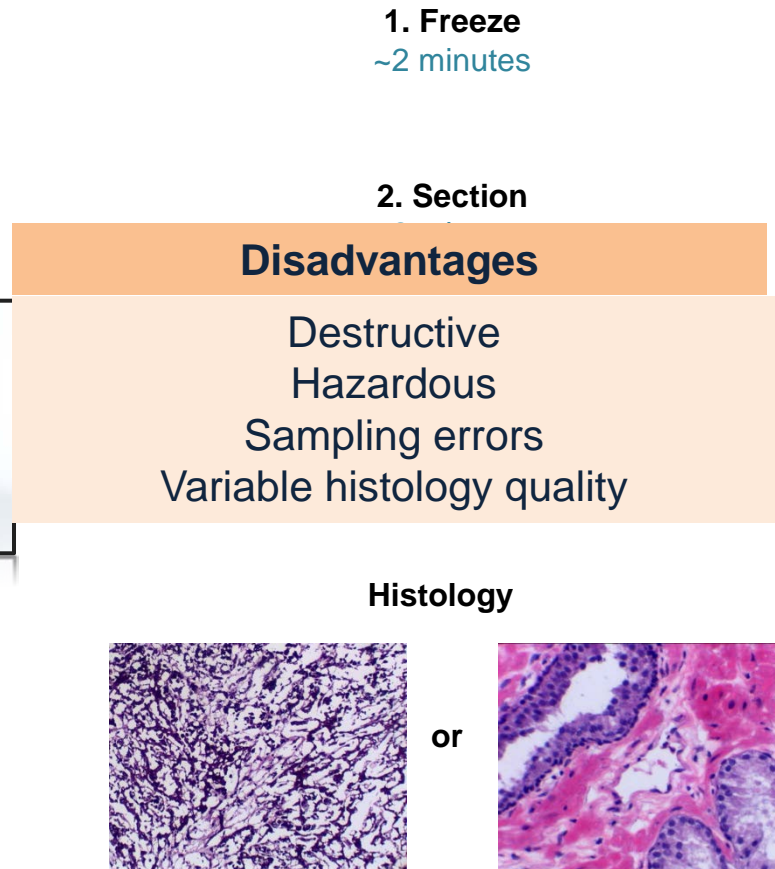
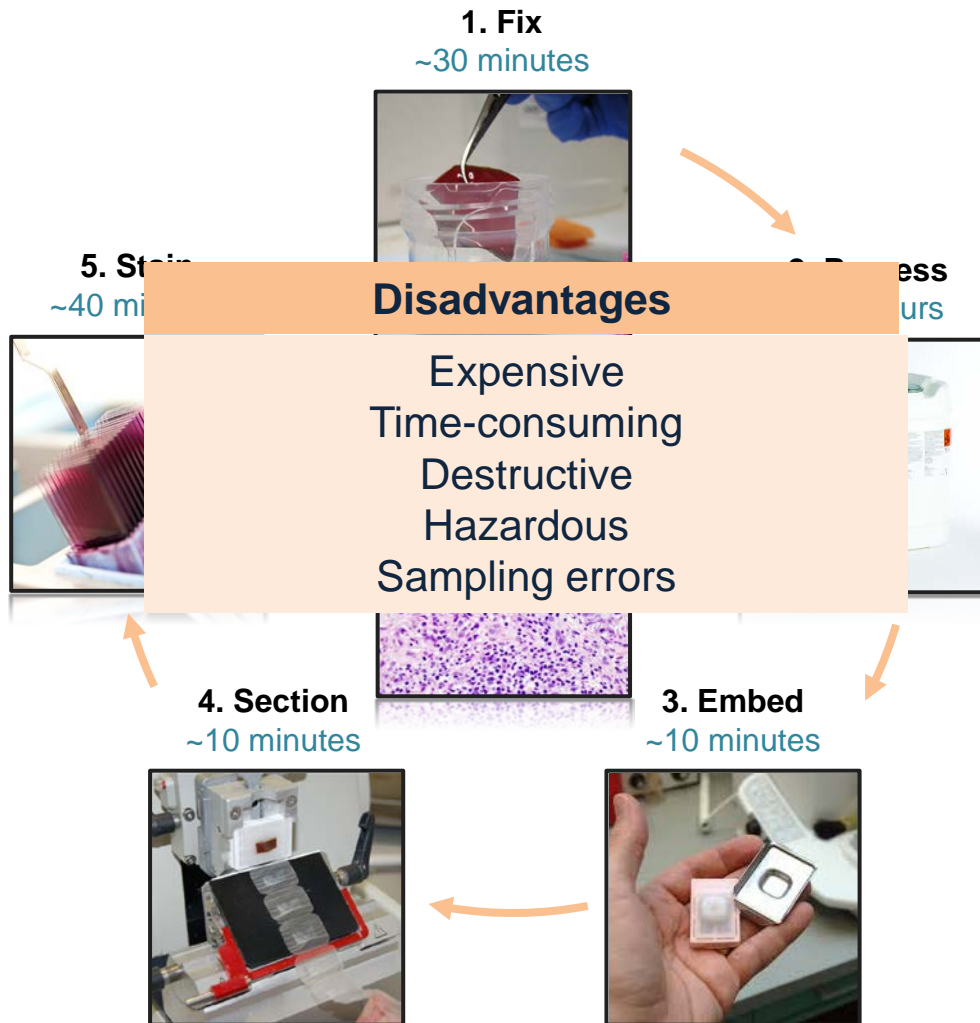
Larry True, MD<sup>#</sup>

University of Washington Departments of Pathology <sup>#</sup>, Mechanical Engineering <sup>\*</sup>

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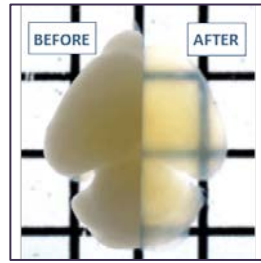
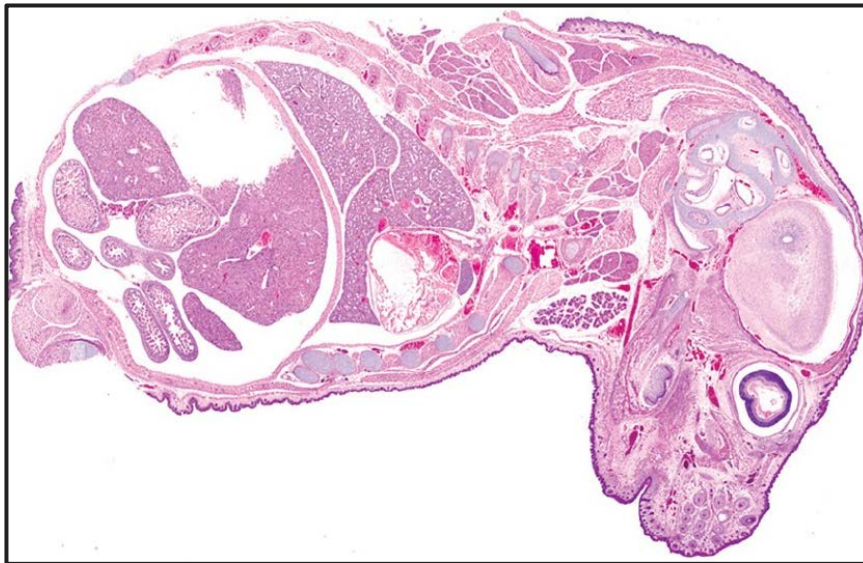
## Rapid histology: 4 hours

## Frozen Section: 10 minutes

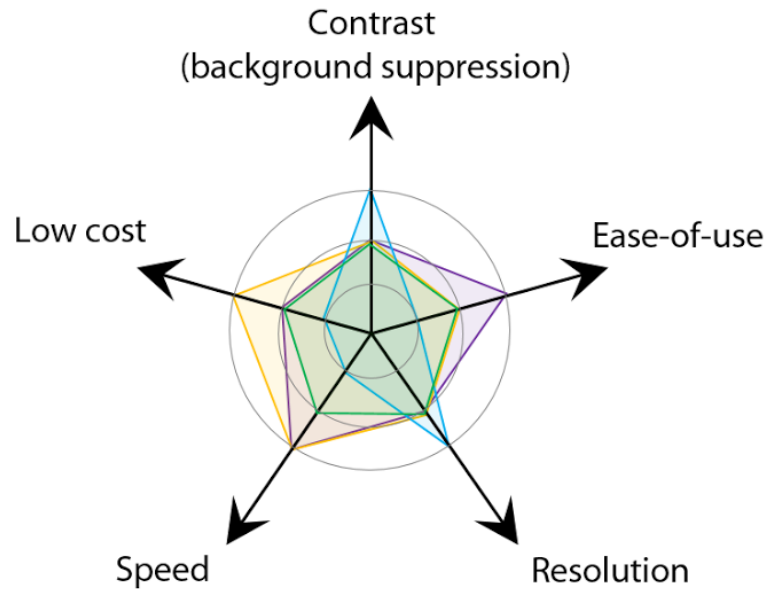
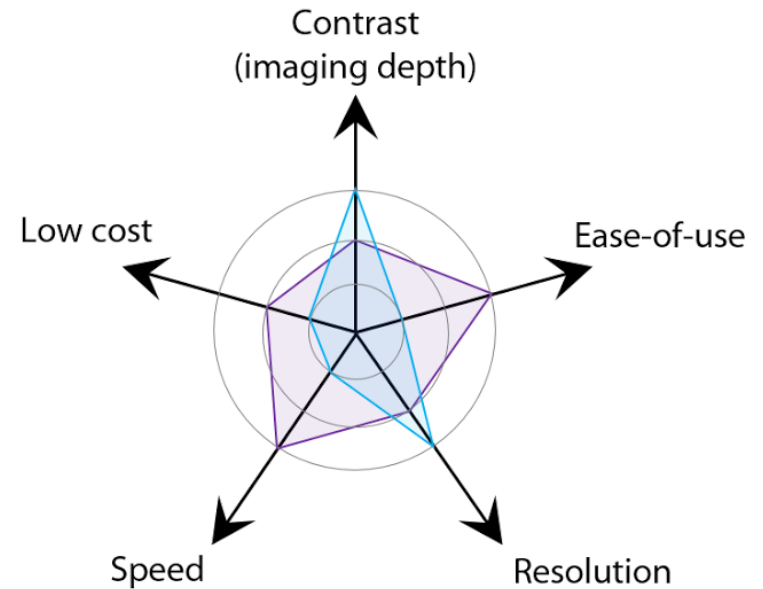


**\*0. Clear**

~5 minutes to overnight

**1. Stain**  
<1 minute**2. Image**  
<10 minutes**Wide-area 'digital' histology****Advantages**

Fast  
Digital  
Non-destructive  
Slide-free  
Wide-area

**a Surface microscopy****b Volumetric microscopy****Fluorescence microscopy method**

MUSE

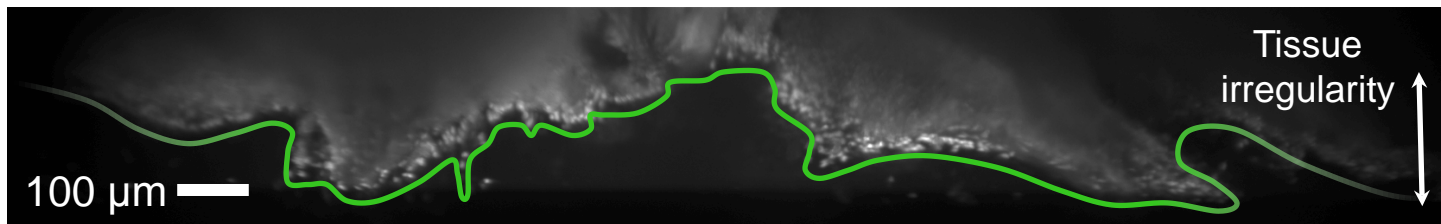
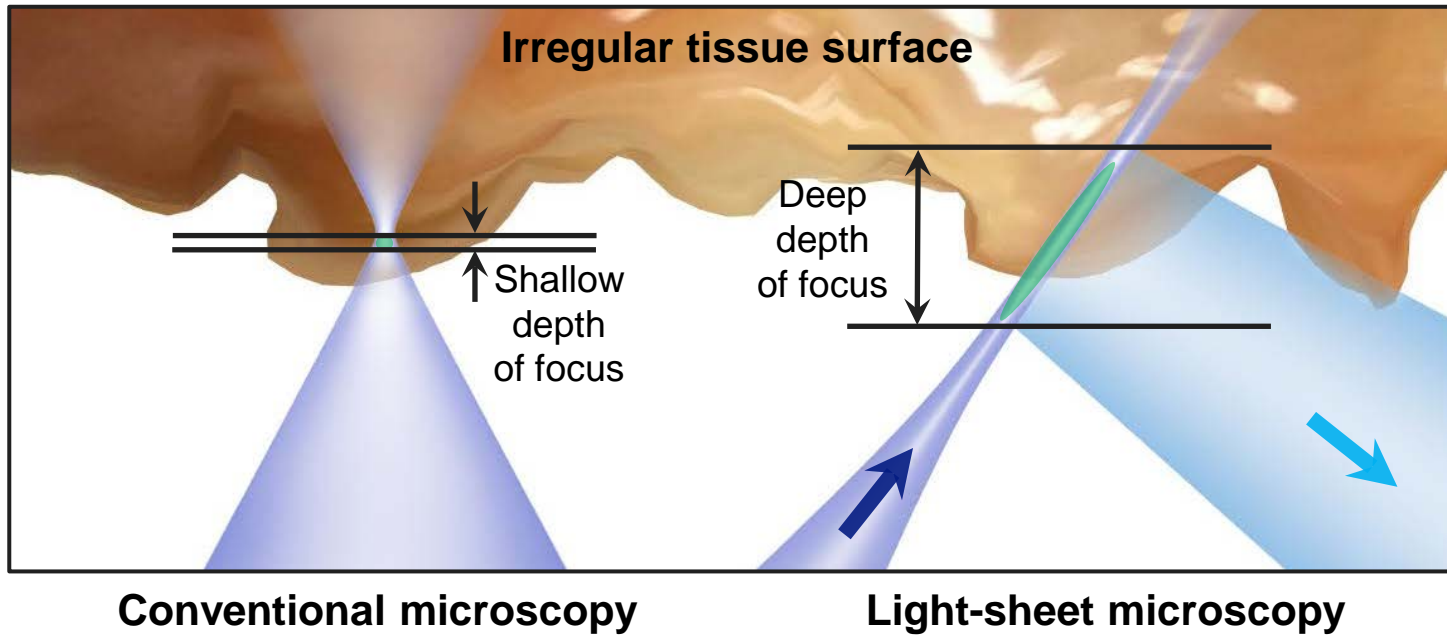
Structured-illumination

Confocal, nonlinear

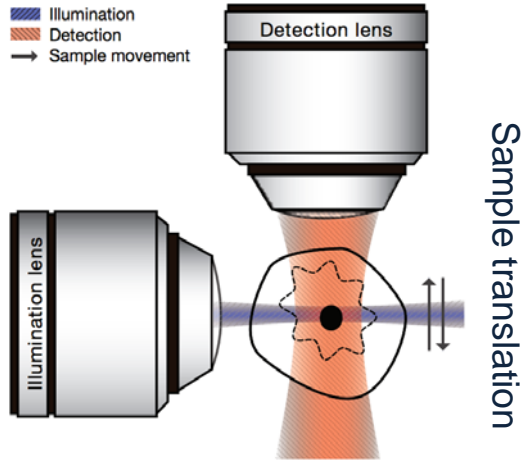
Light-sheet

3D information from LSM enables easy alignment/surface extraction for 2D imaging, and is unrivaled for 3D imaging capabilities.



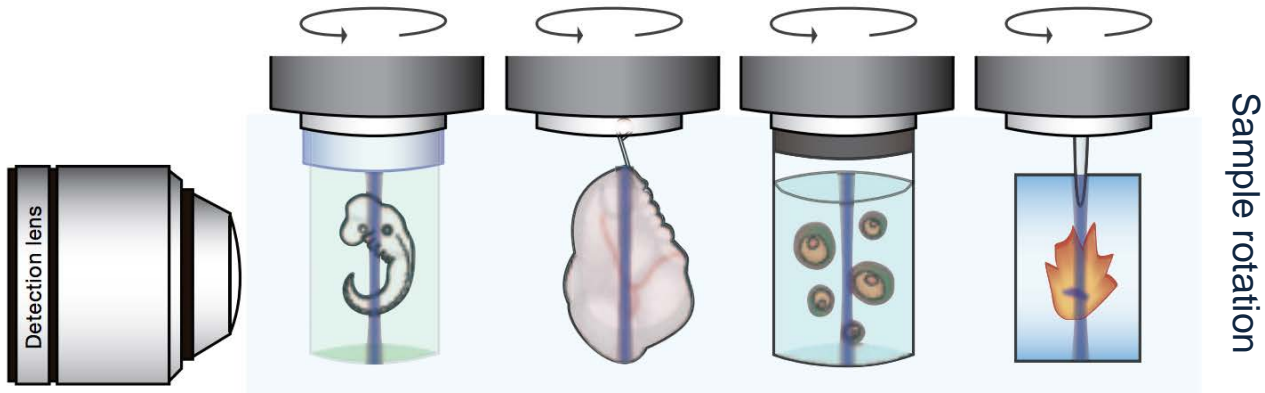


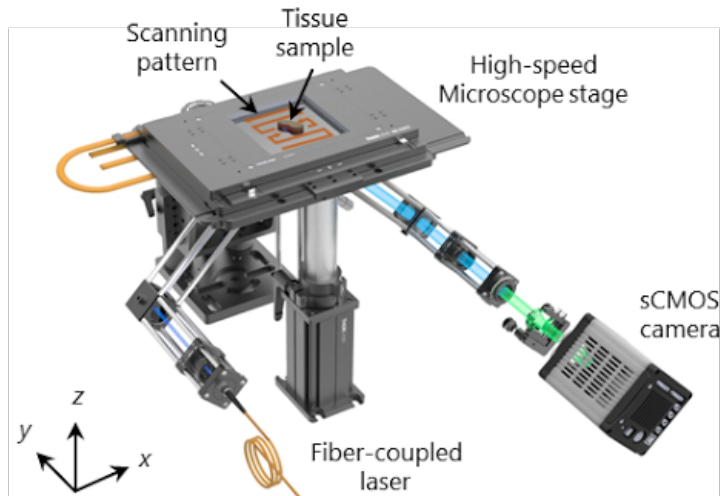
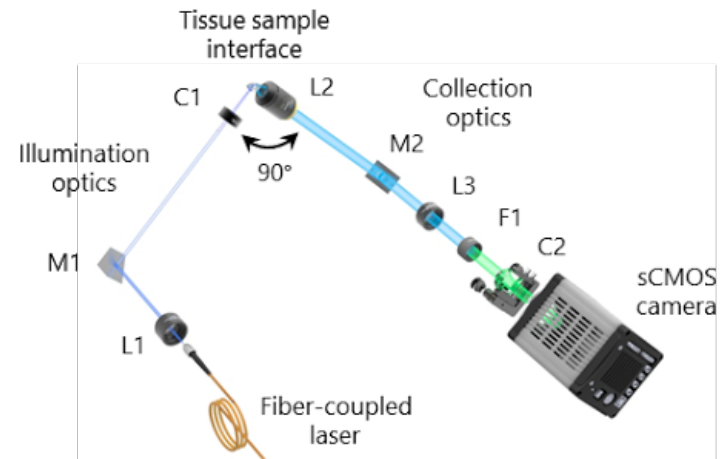
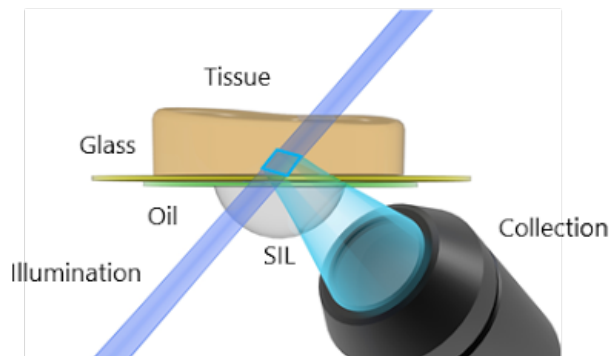
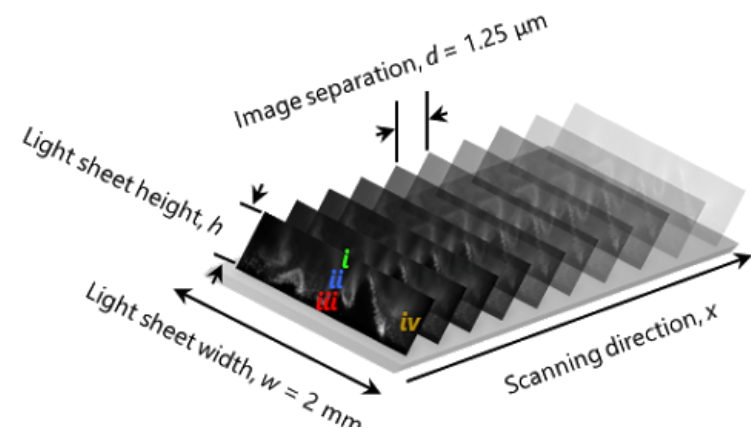
**Advantage:** LSM rapidly images a 3D volume, within which an irregular tissue surface may be digitally extracted and imaged over a range of depths



## Problems

Designed for imaging small, transparent samples  
Human tissues can be large, and are highly scattering

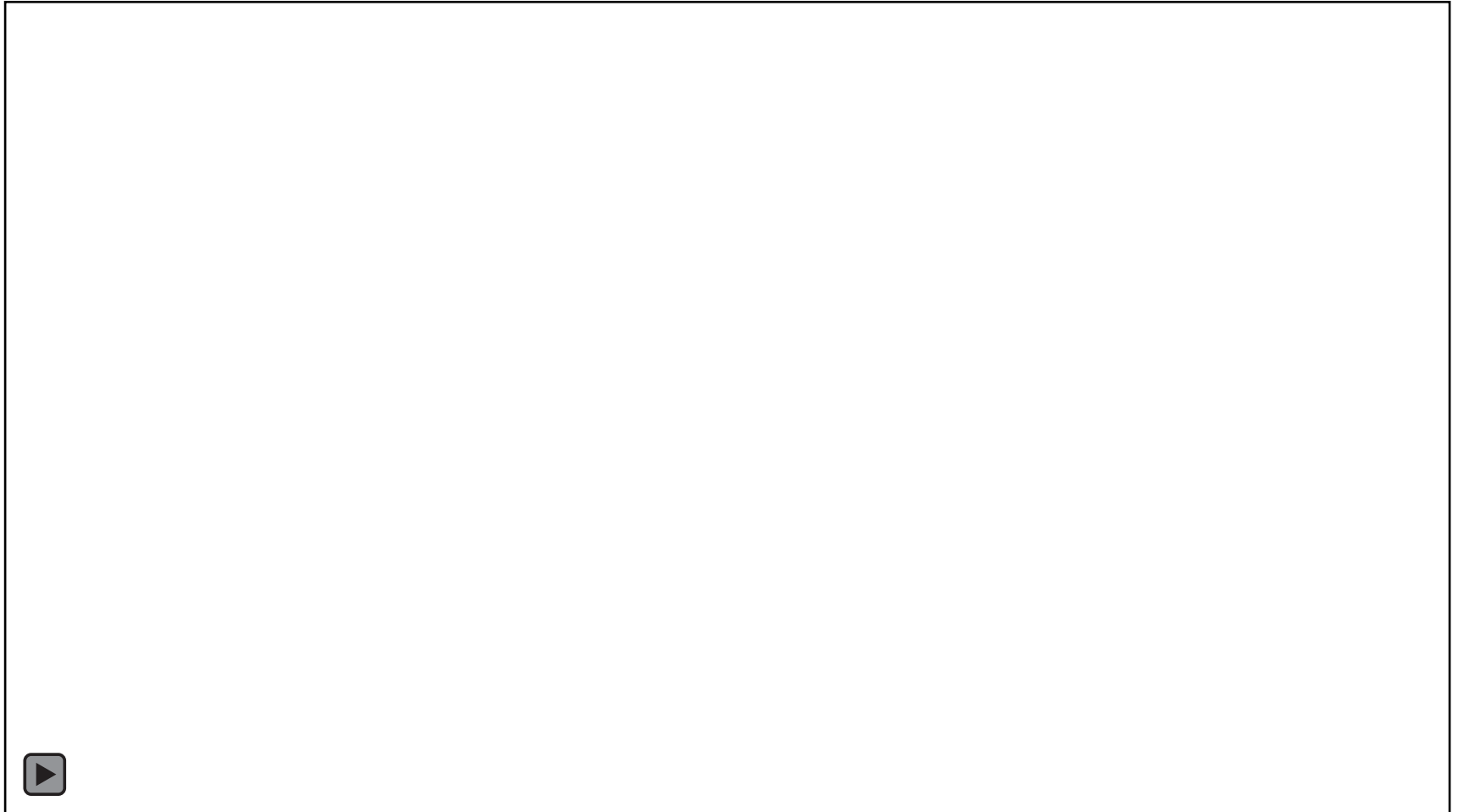


**a Panoramic light-sheet microscope****b Illumination and collection optics****c Tissue sample interface****d Scanned imaging and field points within the light sheet**



# Light-sheet microscope system demonstration

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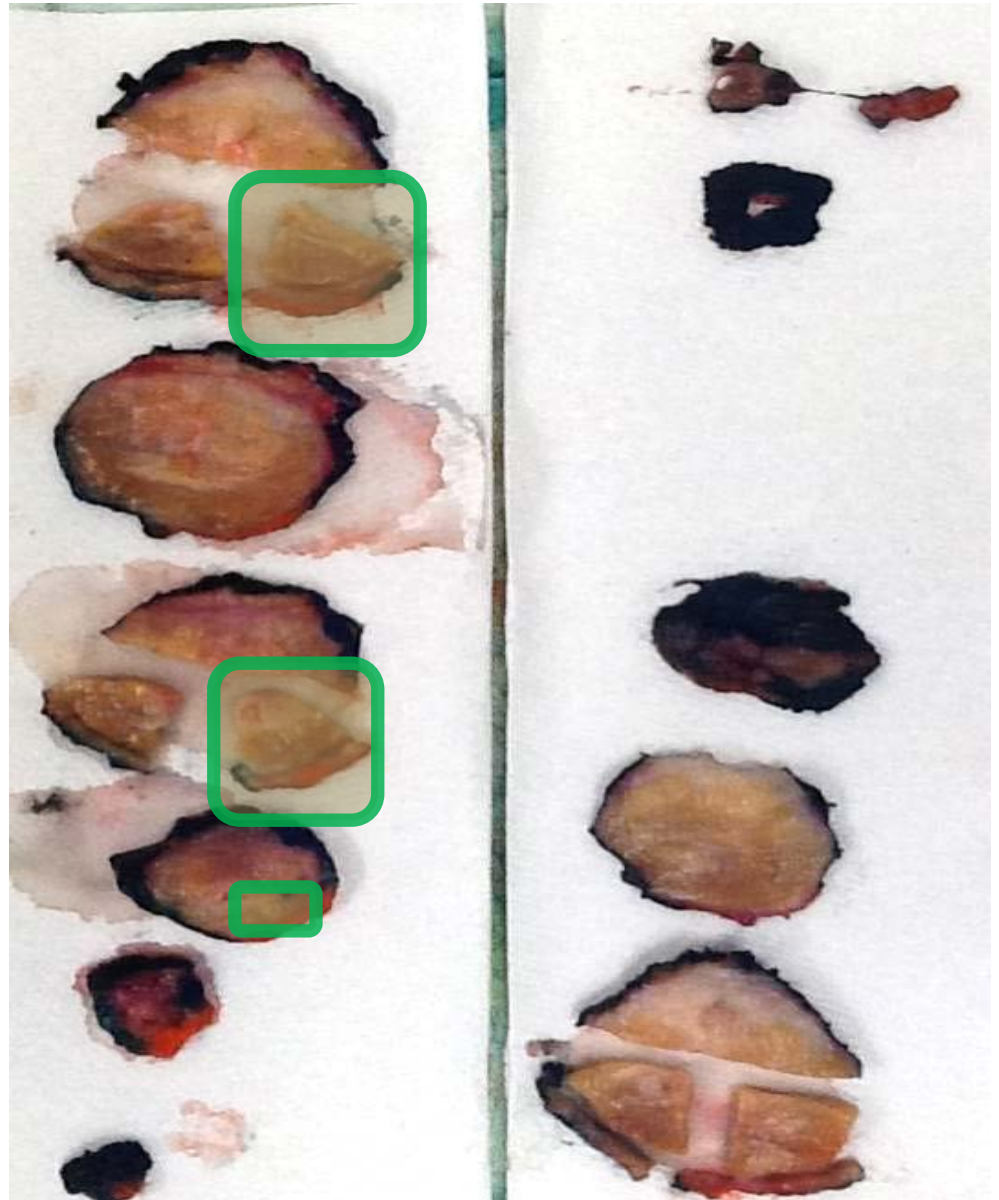
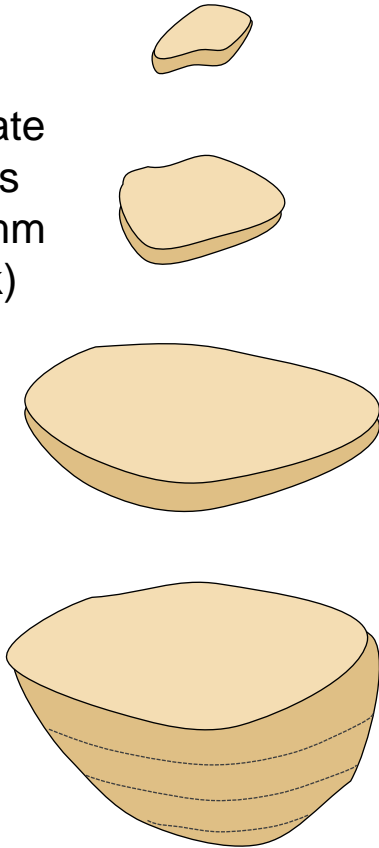
## Unmet clinical need #1: Triaging of non-essential tissue

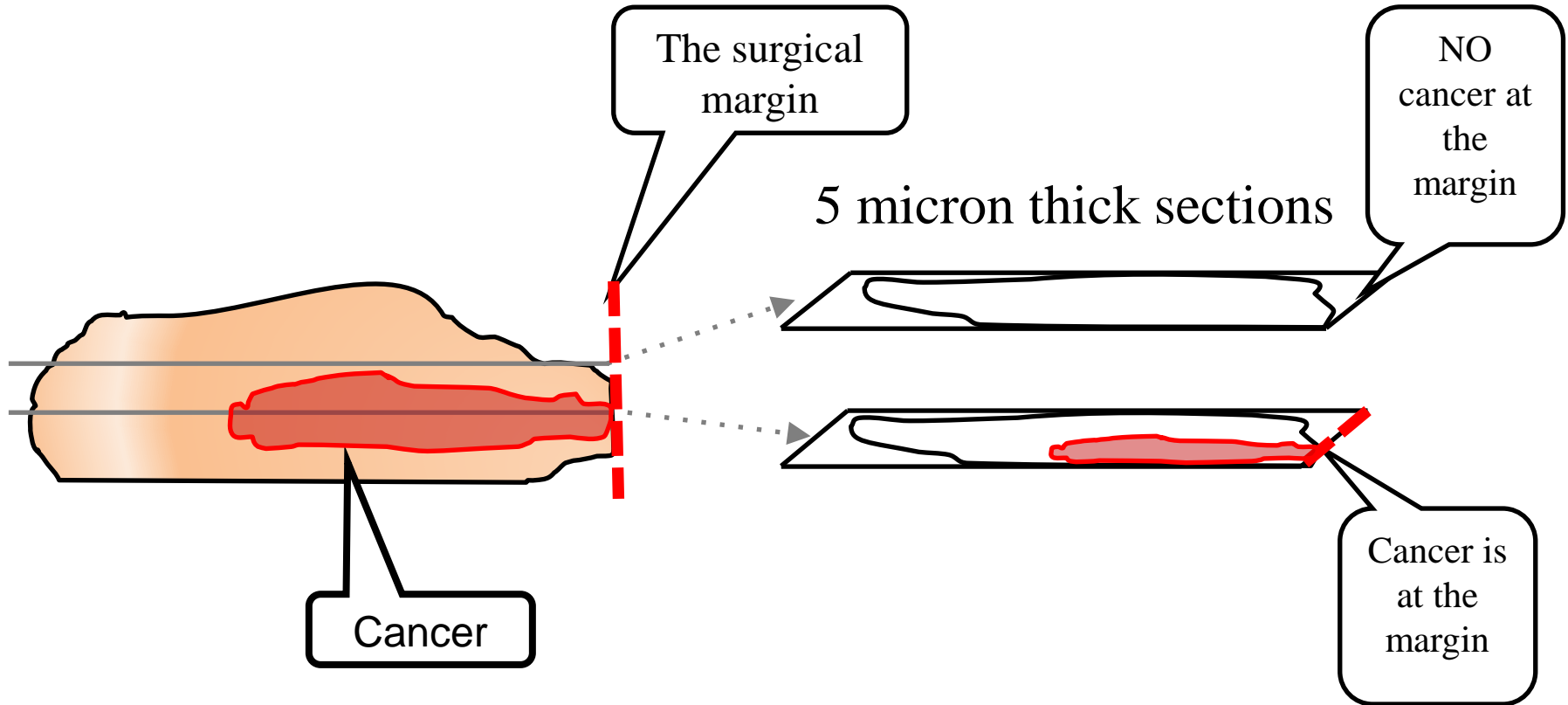
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# Unmet clinical need #1: Triaging of non-essential tissue from prostatectomy specimens

## Radical prostatectomy

Prostate  
slices  
(3-5 mm  
thick)





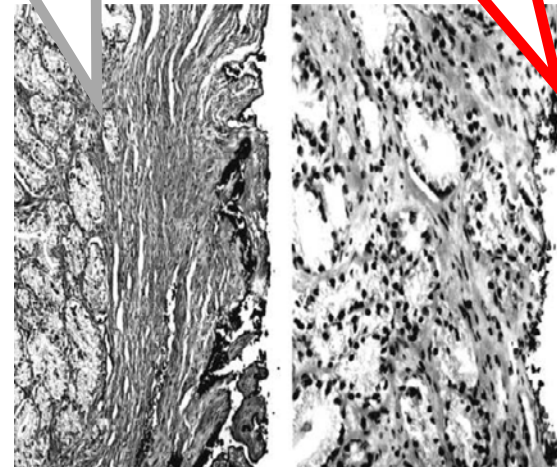
# Complete Histologic Serial Sectioning of a Prostate Gland with Adenocarcinoma

Peter A. Humphrey, M.D., Ph.D.

Whereas the initial set of 19 sections revealed margin positivity in two separate slides, serial sectioning of the blocks revealed positive margins in two additional blocks, where the initial slide from the block exhibited a negative margin (Fig. 4). In both blocks with the new positive margin, carcinoma approached to within less than 1 mm of the inked margin in the initial histologic section, but variable degrees of sectioning into the block were required to identify the new positive margin. In one block, the

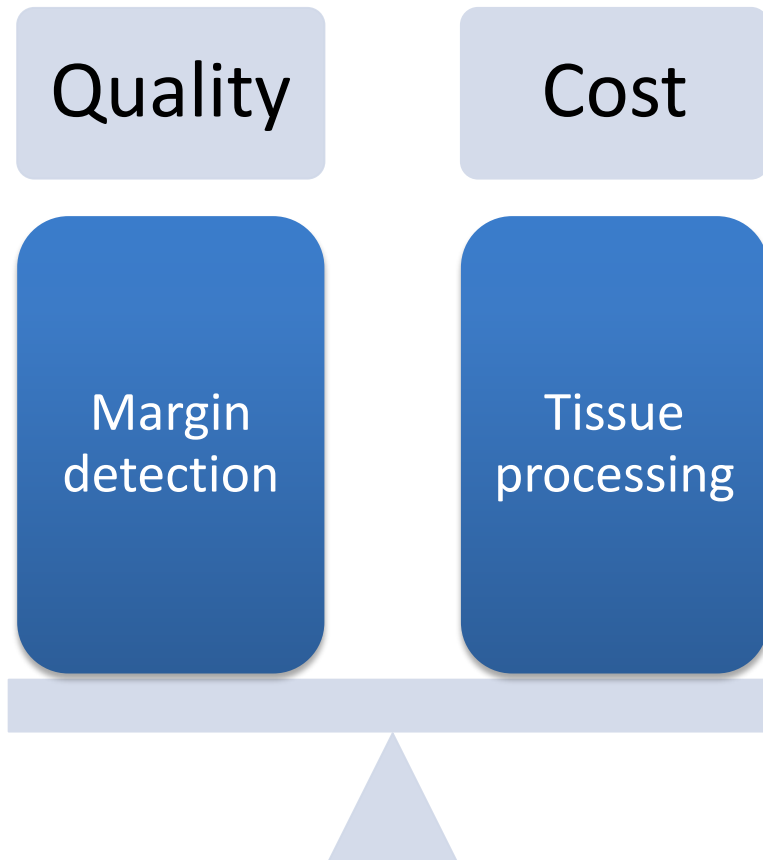
Initial section:  
cancer free of  
margin

Deeper  
level: cancer  
at margin

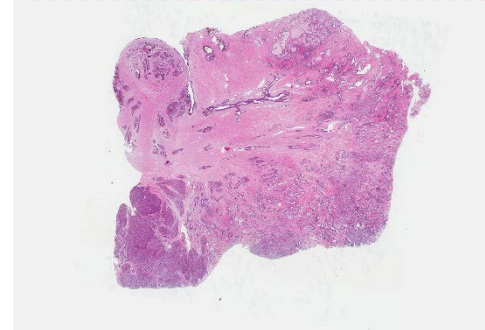
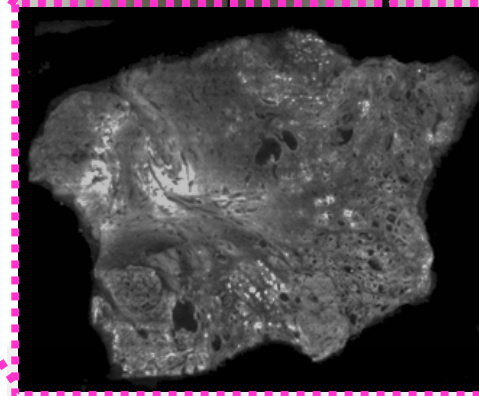
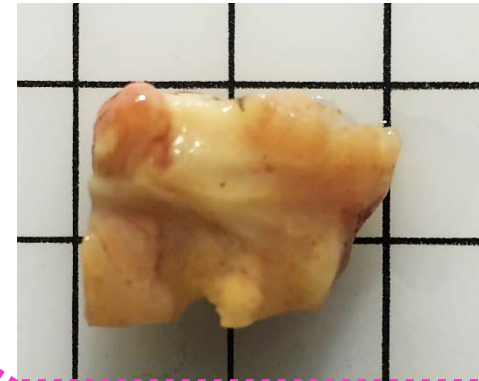
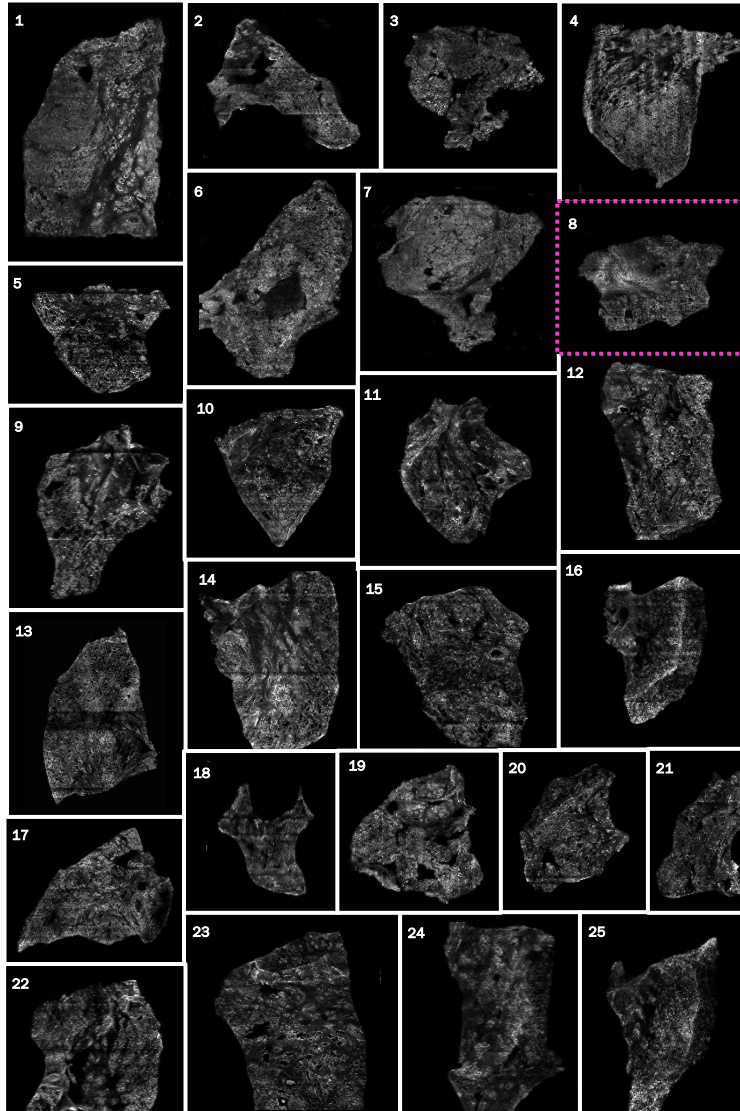


Humphrey PA, Am J Surg Pathol  
1993;17:468

**Pathology laboratories encounter a double-edged sword:**  
Increased sampling detects more positive margins but increases laboratory costs

**Goal:**

Provide a “sneak peek” of histology to inform decision on whether to “triage” tissue for full pathology work-up



Fresh  
prostate  
slice



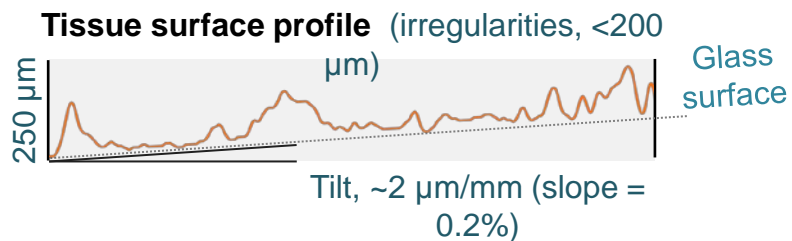
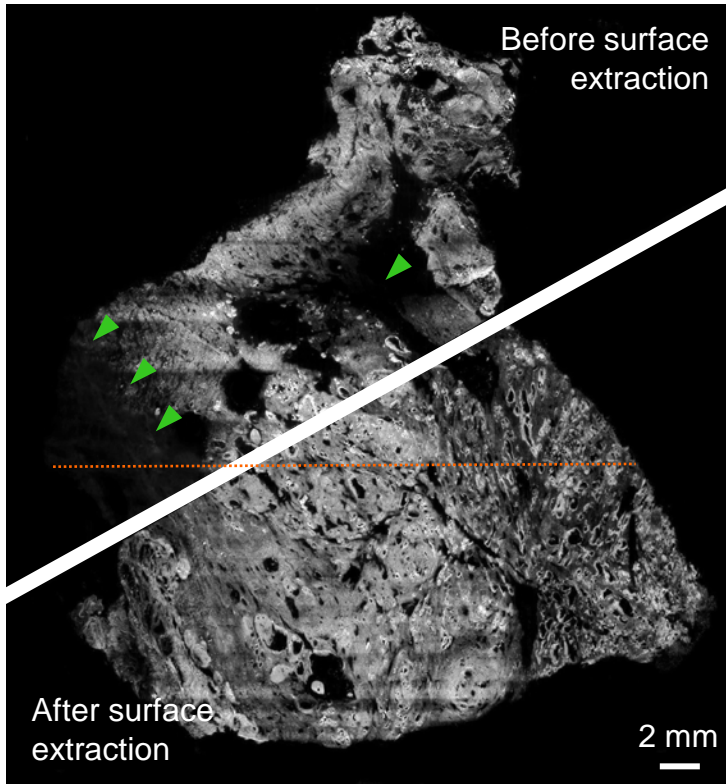
LSM  
image



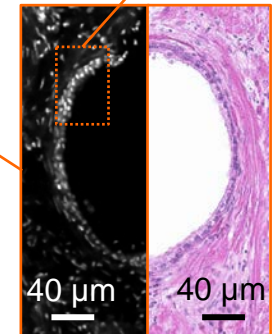
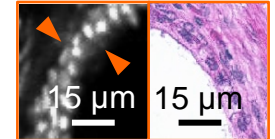
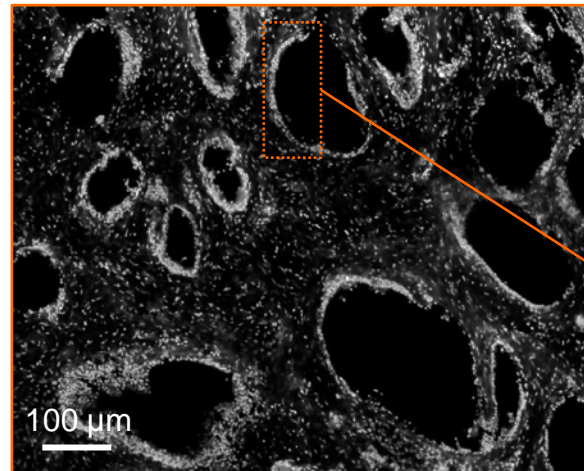
FFPE  
H&E  
slide



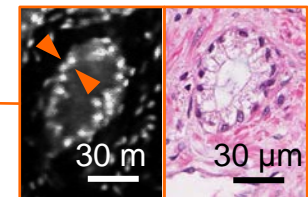
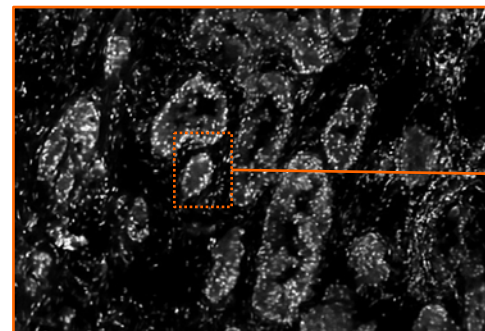
## Light-sheet microscopy of prostate tissue



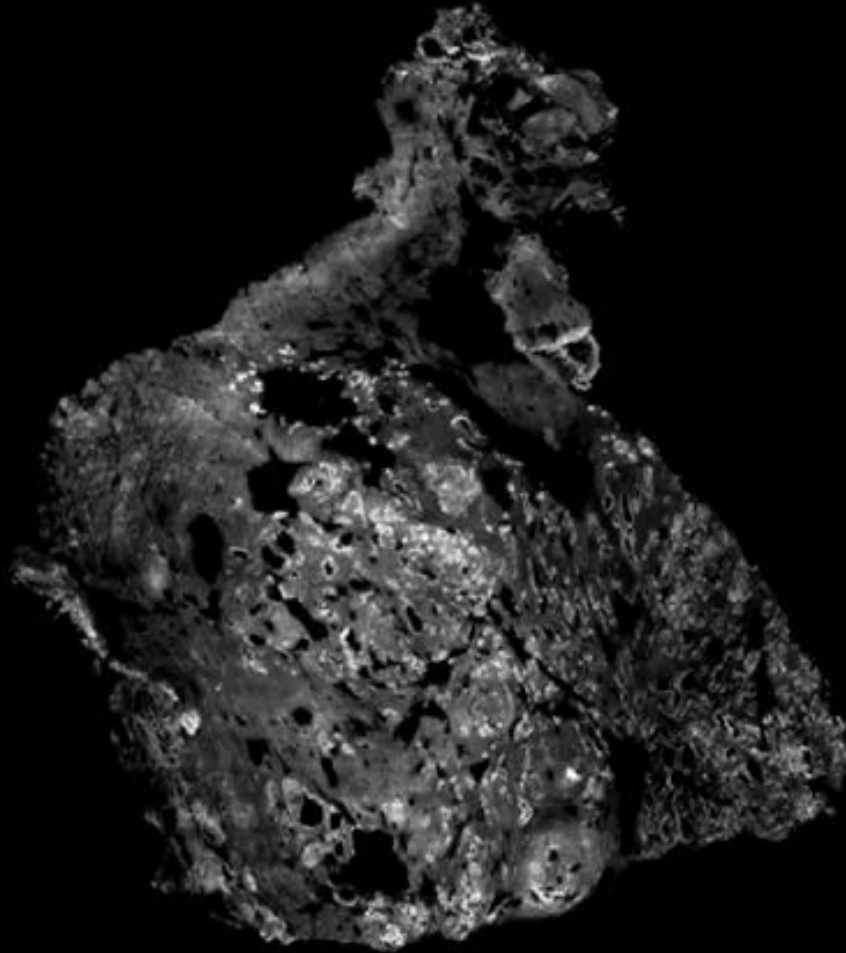
## Normal prostate glands



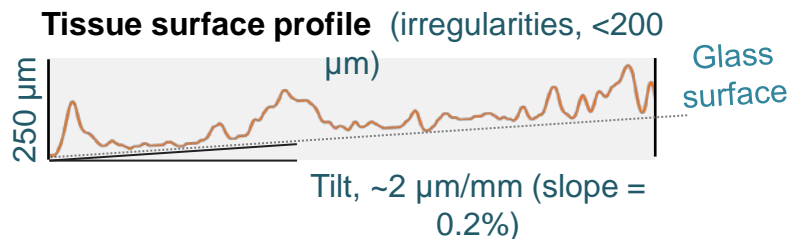
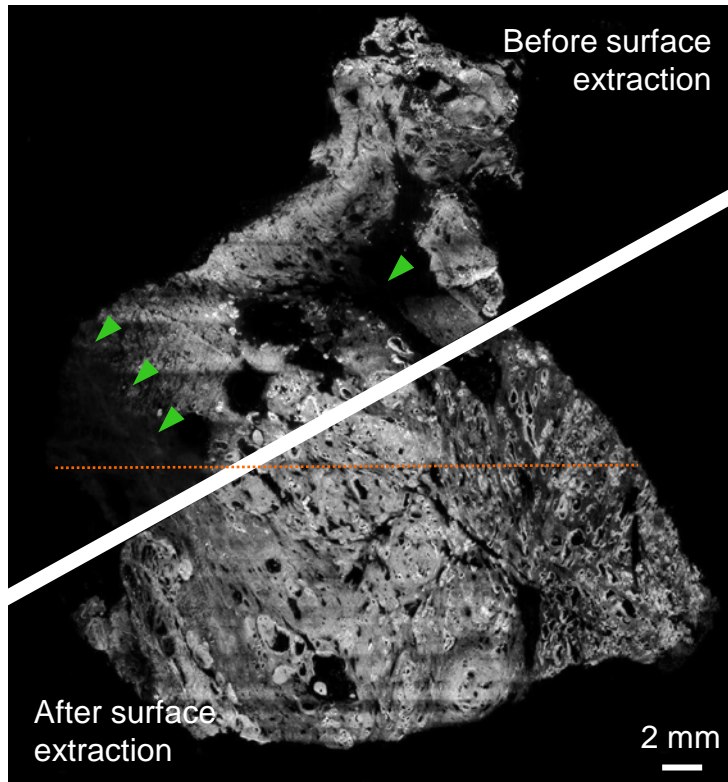
## Prostate adenocarcinoma







Staining time: 20 sec.  
Imaging time: ~8 min  
Tissue size: ~3.4x3.6 cm  
Resolution: 1.25  
 $\mu\text{m}/\text{pixel}$



24 tissue samples

- 12 benign
- 12 carcinoma

Sensitivity: 0.92

Specificity: 0.92

\*Detected 2 cases of positive margins missed on 2D section

### Take-away points:

- Successful technological solution
- Not an ideal problem-solution fit due to cost/benefit ratio

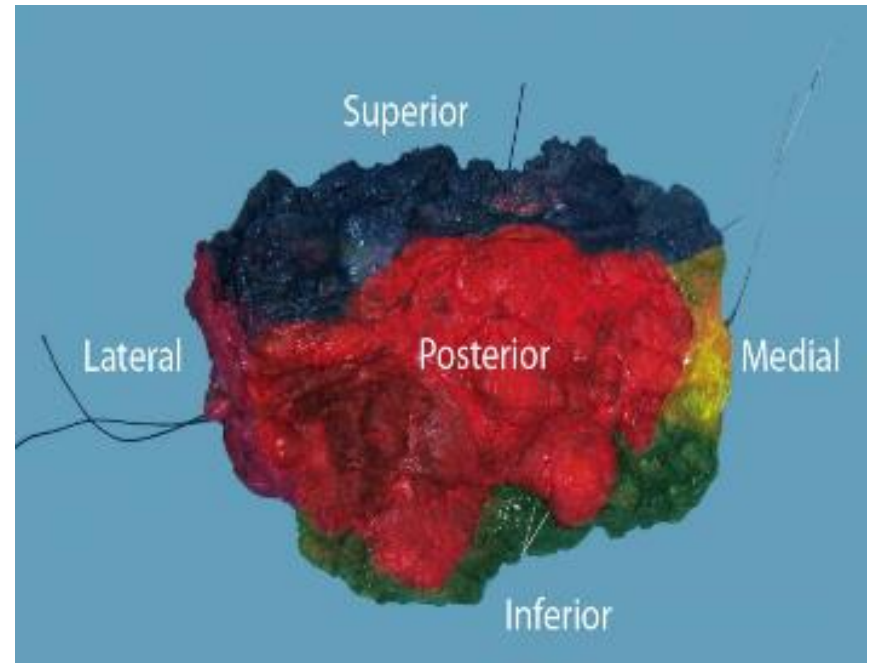


## Unmet clinical need #2: Intraoperative imaging

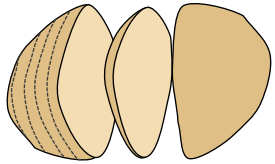
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# Unmet clinical need #2: Intraoperative pathology consultation

- Medical problem: Lumpectomies for breast cancer result in positive margins in 20-50% of cases, leading to re-operations.
- Re-operations are costly to the healthcare system, to the patient, and cause undue stress.
- Our solution: Slide-free 3D light-sheet microscopy for rapid margin assessment



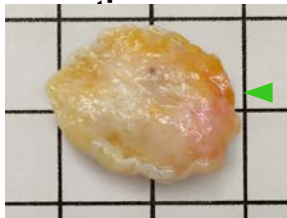
## Lumpectomy



1 cm

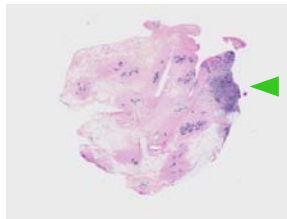
Breast slice  
(3-5 mm thick)

## Fresh breast



5 mm

## H&amp;E

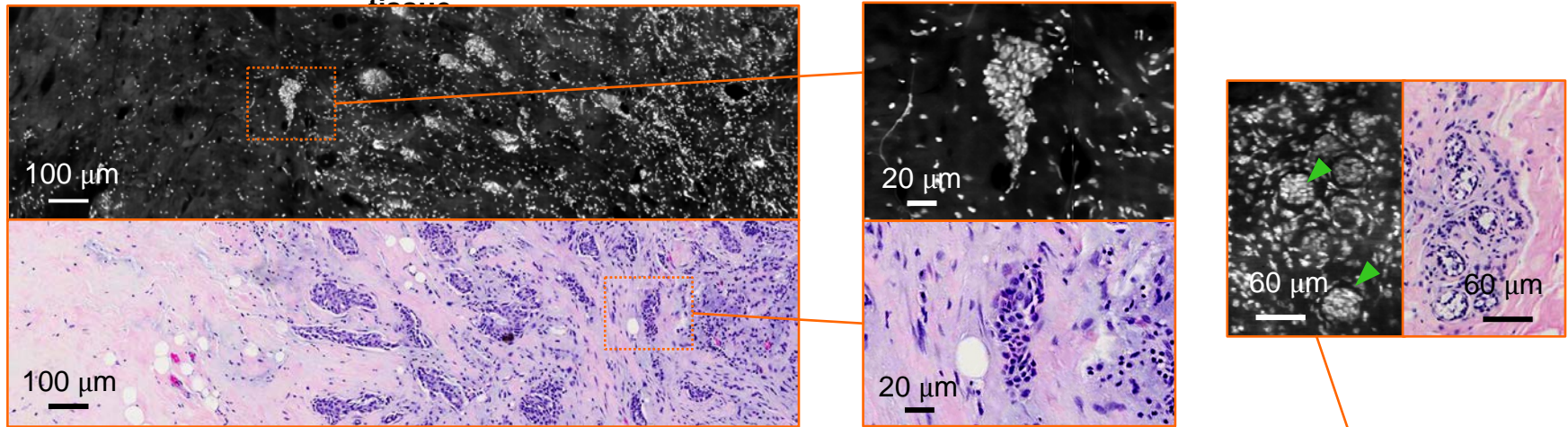


5 mm

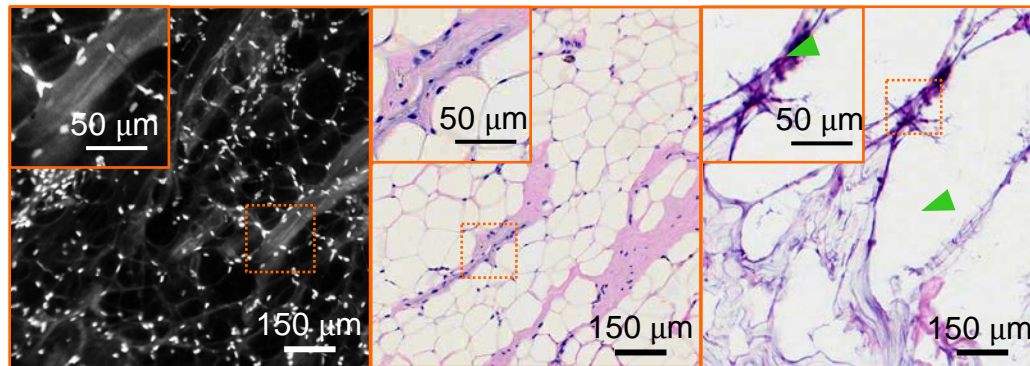




## Invasive ductal carcinoma with adjacent normal breast tissue



## Adipose tissue

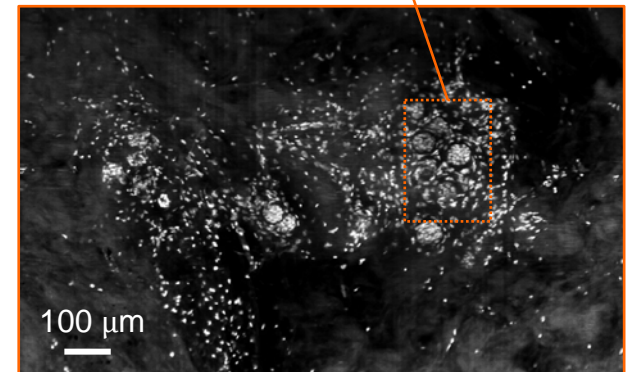


Light-sheet microscopy of fresh breast tissue

Formalin-fixed paraffin-embedded section

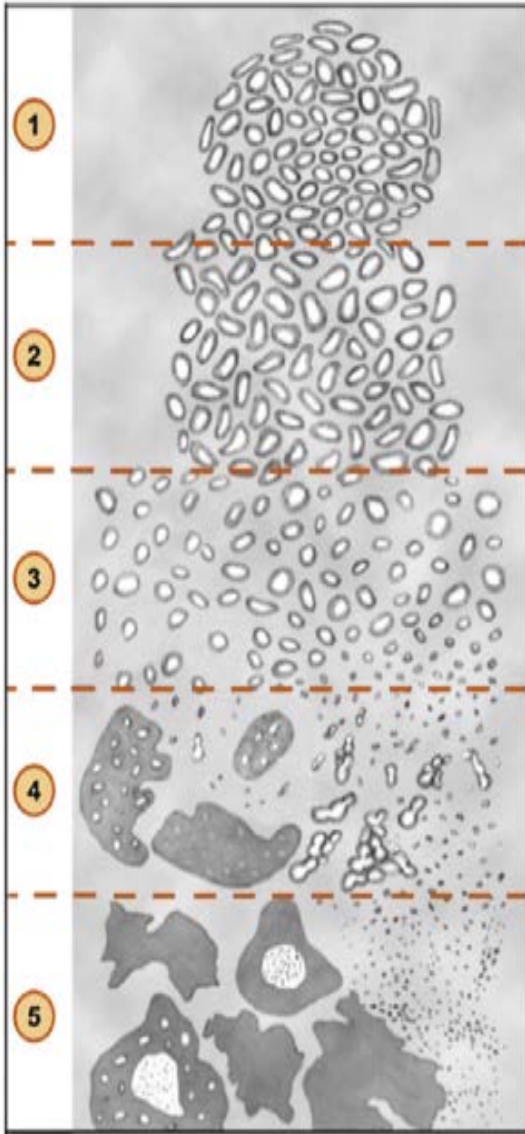
Frozen tissue section

## Benign breast lobules



# Unmet clinical need #3: 3D pathology of prostate core needle biopsies

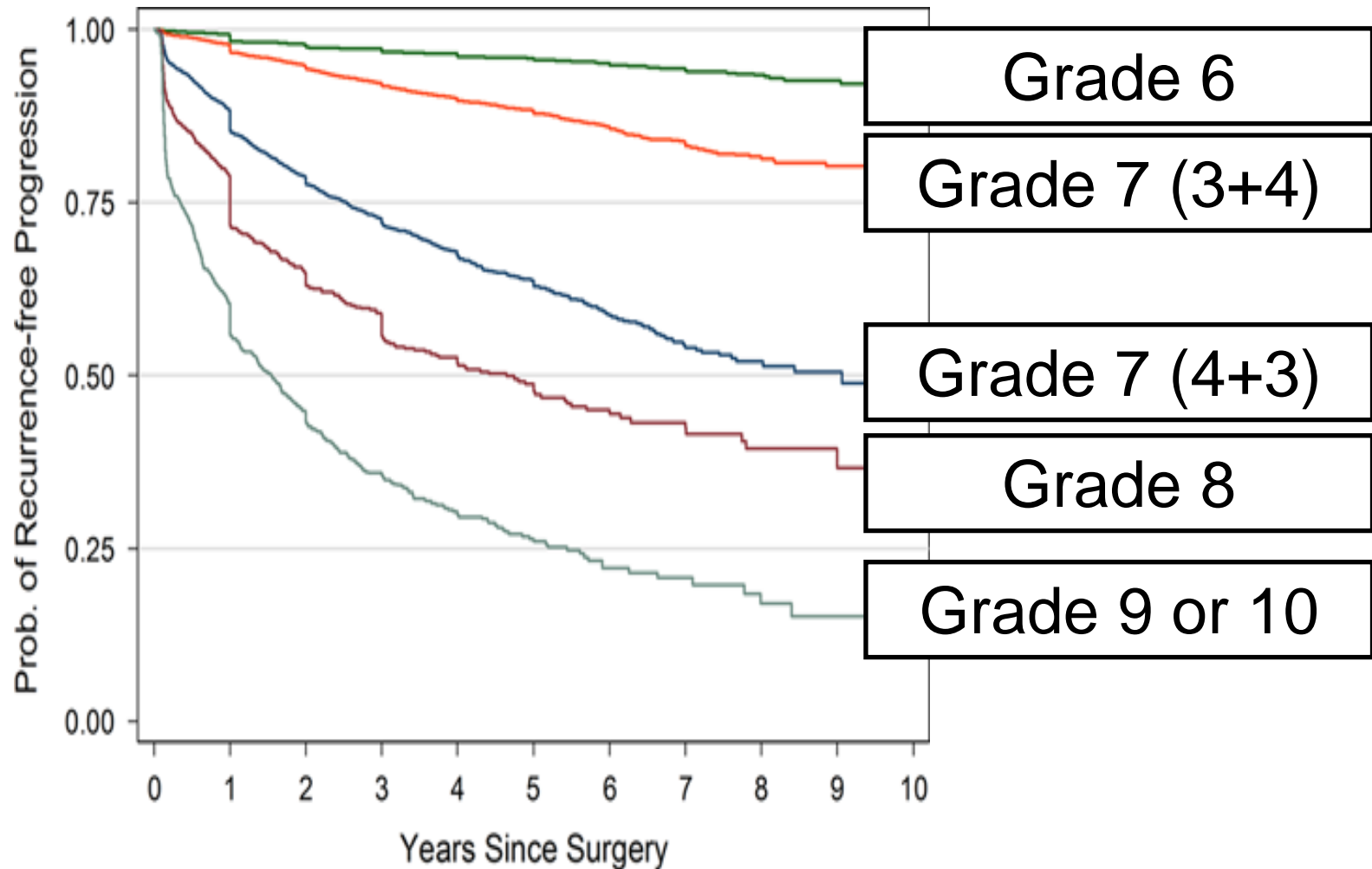


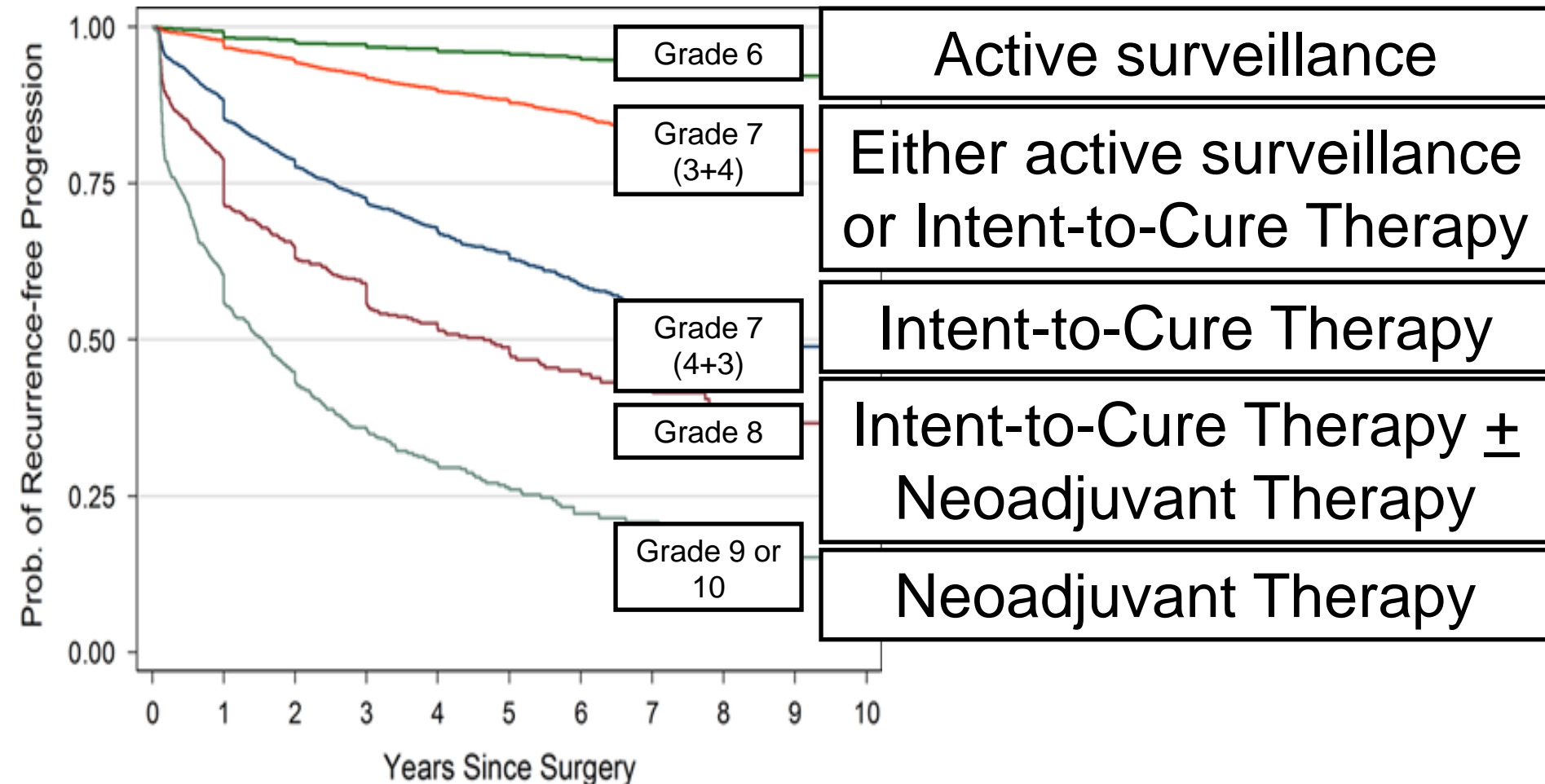


**Higher grade associated with worse prognosis**

Gleason grade = primary pattern + secondary pattern

*Burnbaugh*

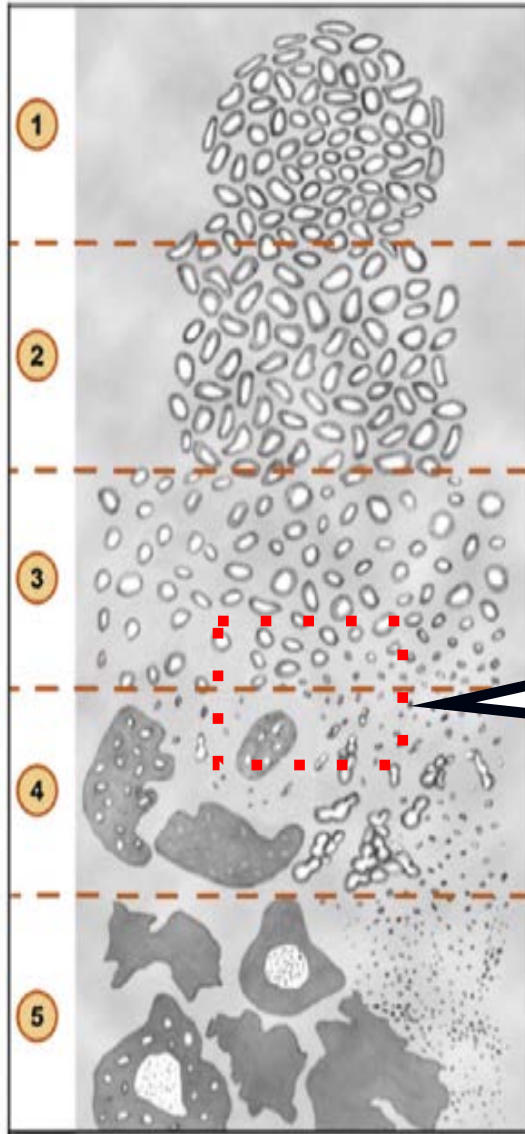




**Active surveillance:** Periodic monitoring and biopsies

**Intent-to-Cure therapy:** Surgery vs. Radiation (Seed implants/brachytherapy or External beam radiation therapy)

**Neoadjuvant therapy:** Chemotherapy prior to Intent-to-Cure therapy

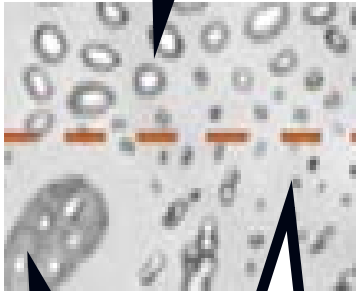


## Problematic aspect of grading

Distinguishing tangential sections of pattern 3 glands from poorly formed glands (pattern 4 glands)

*Burns*

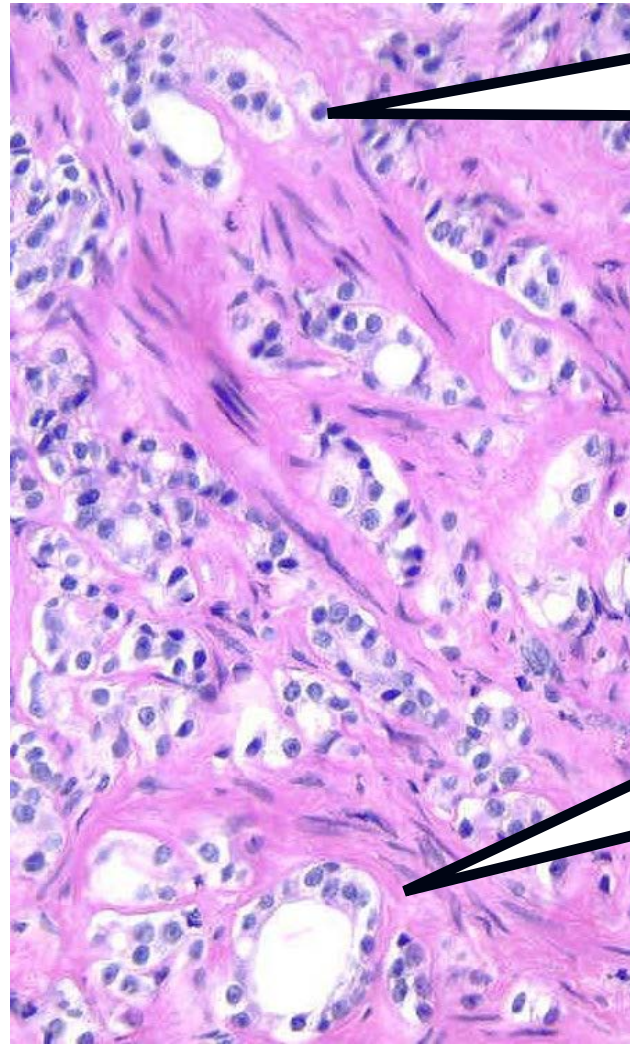
Well-formed  
glands  
(pattern 3)



Pattern 4  
cribriform  
variant

Pattern 4  
Poorly-  
formed gland  
variant

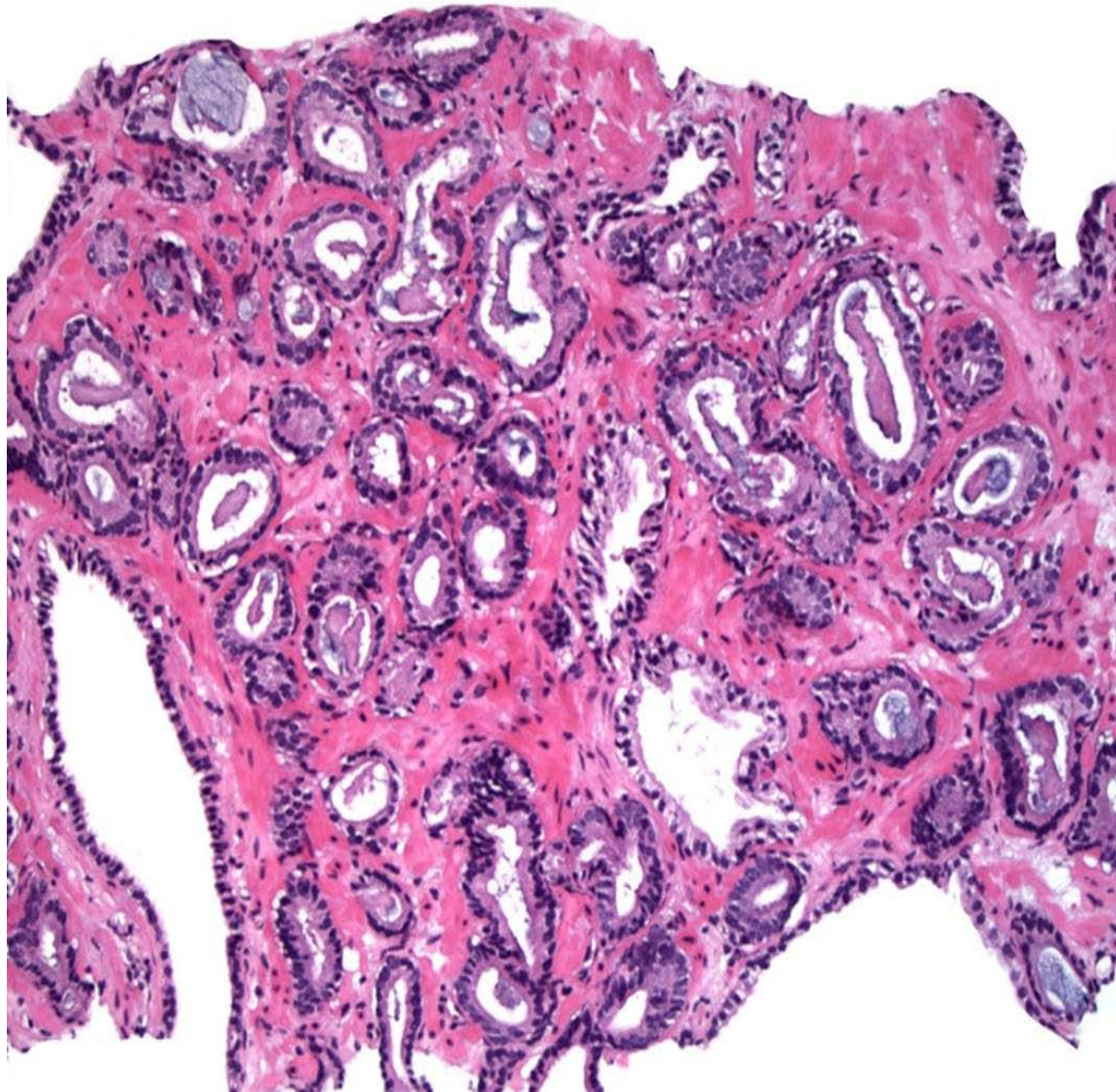
Gleason grade  
7 (4+3)

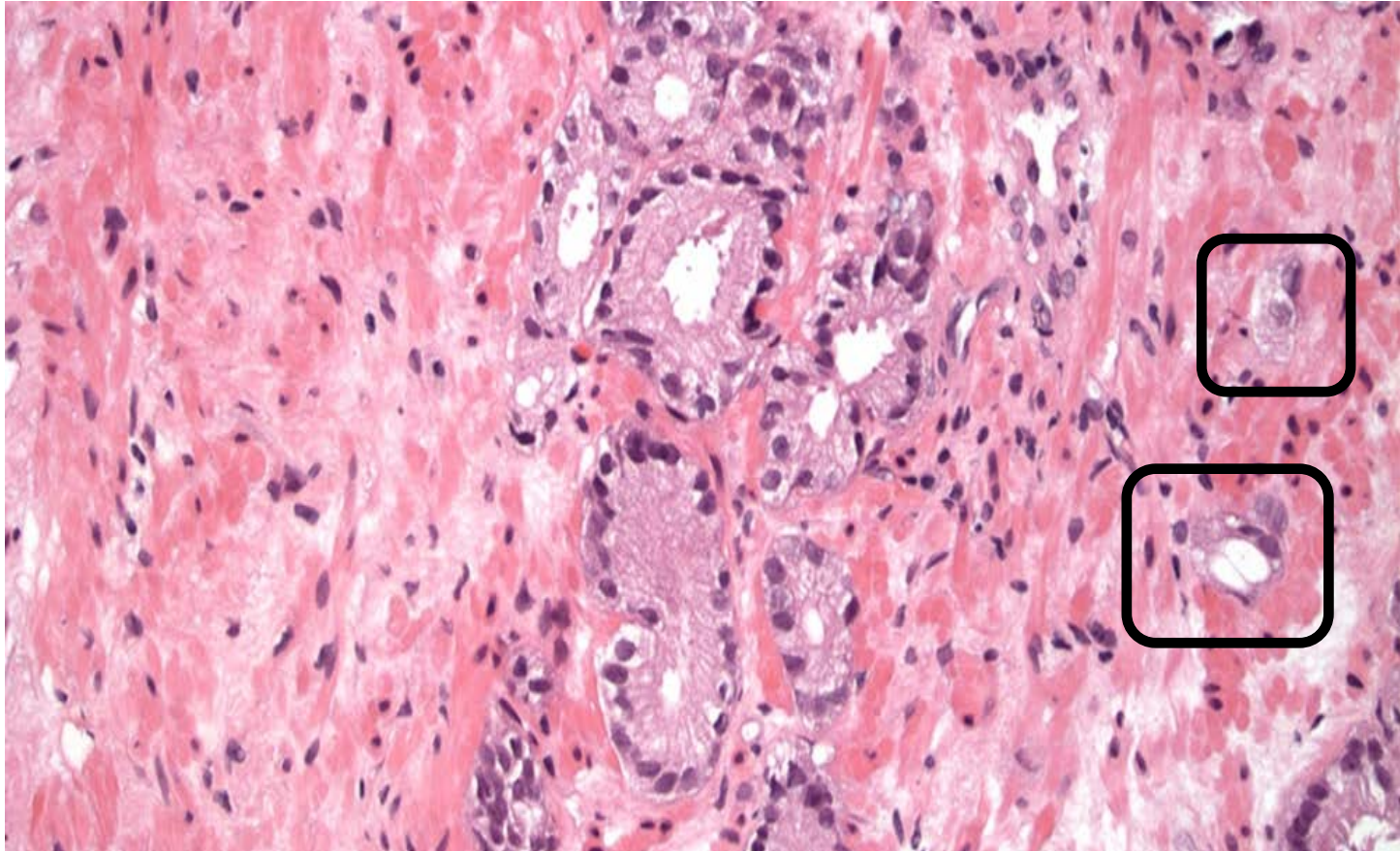


Poorly-  
formed  
gland  
(pattern 4)

Well-  
formed  
gland  
(pattern 3)

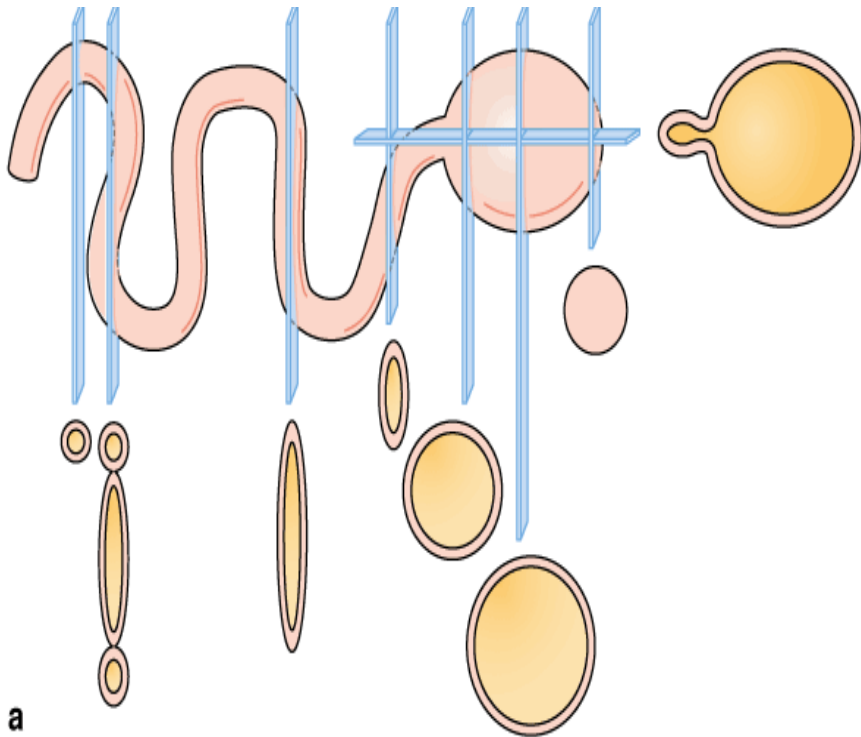




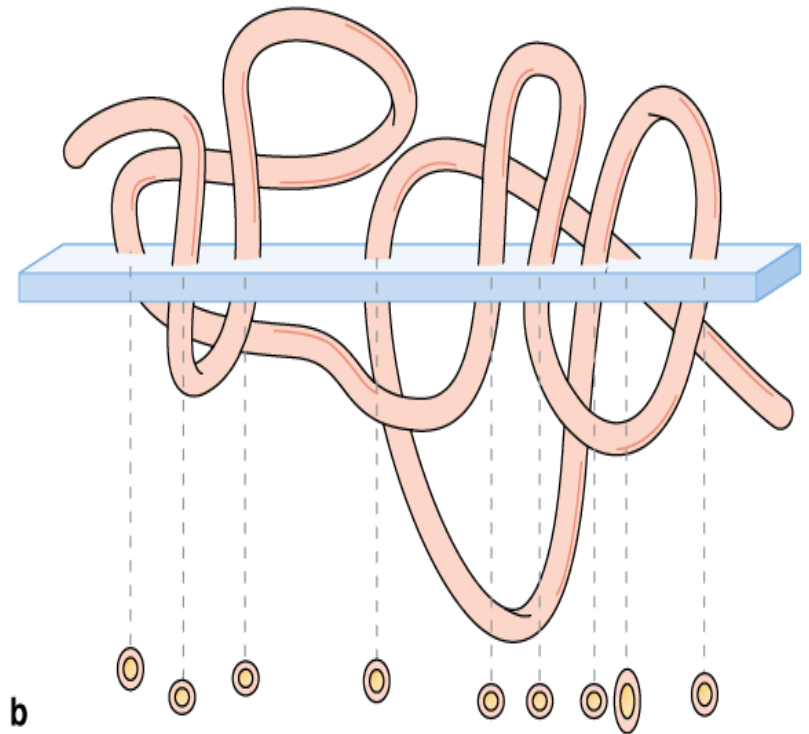


**Are the small glands Gleason pattern 4 (poorly formed gland variant) or tangential sections of pattern 3 glands?**





Source: Mescher AL: *Junqueira's Basic Histology: Text and Atlas, 12th Edition*: <http://www.accessmedicine.com>  
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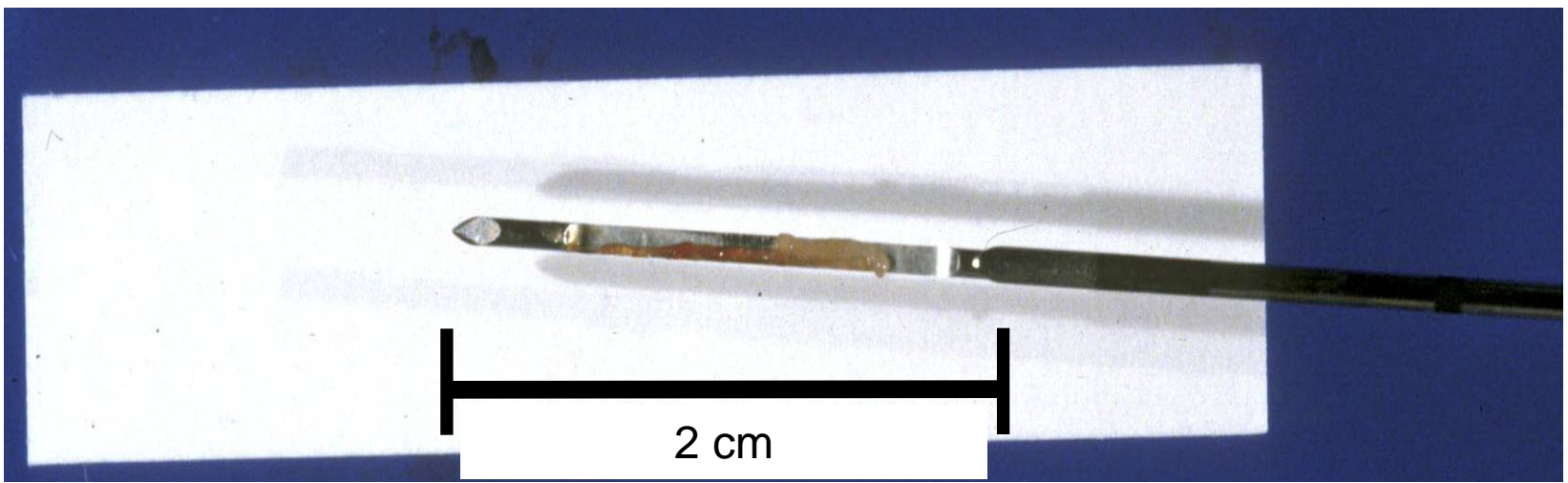


Source: Mescher AL: *Junqueira's Basic Histology: Text and Atlas, 12th Edition*: <http://www.accessmedicine.com>  
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# Prostate core needle biopsy (fresh)

UNIVERSITY of WASHINGTON



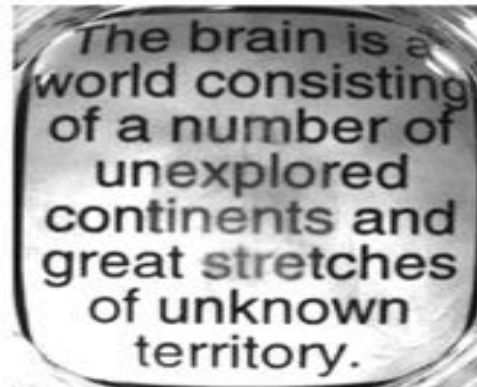
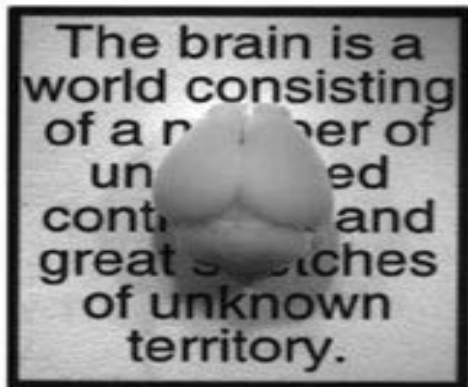
Aqueous clearing solution +

<chem>C12OC(O[C@@H]1O[C@@H](O[C@@H]3OC(O)[C@H](O)[C@@H]3O)CO)O2</chem>	<chem>OCC(O)CO</chem>	<chem>NC=O</chem>	<chem>C1OC(O)[C@H](O)[C@@H](CO)O1</chem>	<chem>CC(=O)N1C=CC(=O)N(C)C1=O</chem>	<chem>OCCSCCO</chem>
Sucrose RI - 1.44 (60% w/v in water)	Glycerol RI - 1.44 (80% w/v in water)	Formamide RI - 1.44 (95%)	Fructose RI - 1.50 (130% w/v in water @ 37C)	Diatrizoic Acid RI - 1.40 (0.74M)	2,2'-thiodiethanol RI - 1.51 (97% v/v in water) RI - 1.45 (60% v/v in water)

Before

After

**TDE**



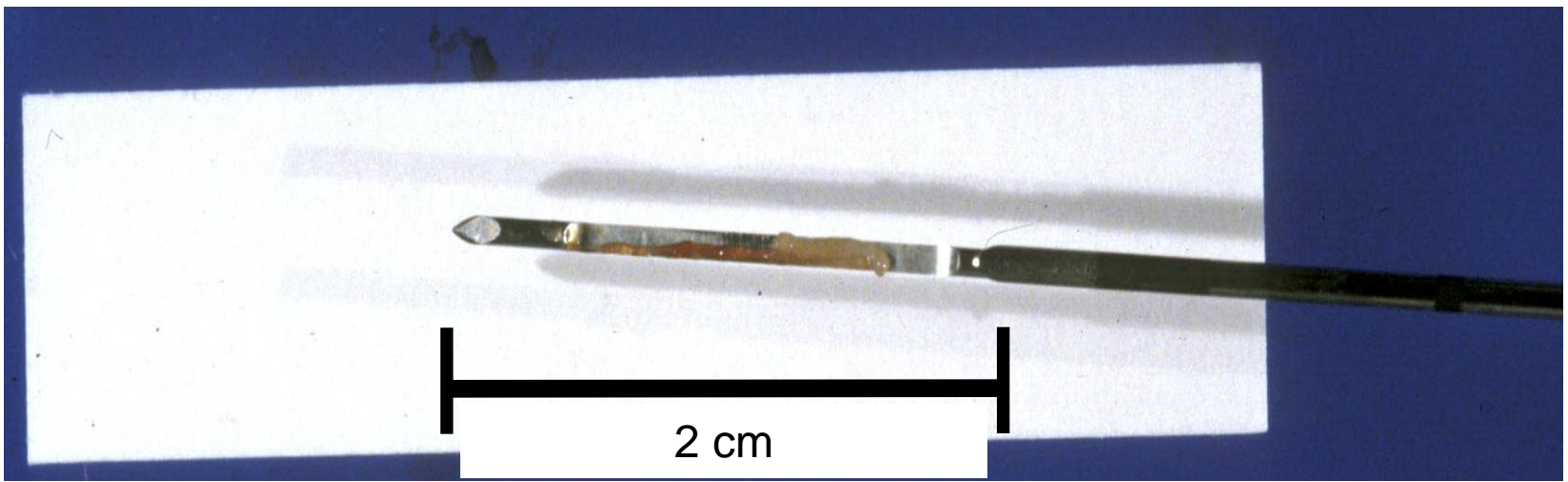
2 days

**For core needle biopsies, clearing achieved in ~5 minutes**



# Prostate core needle biopsy procedure

UNIVERSITY of WASHINGTON



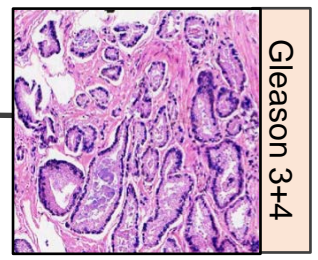
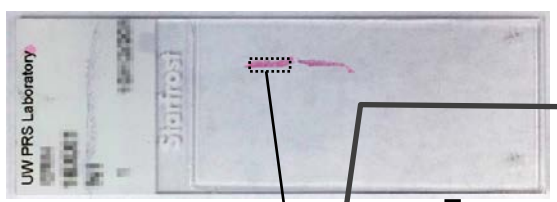
Before clearing



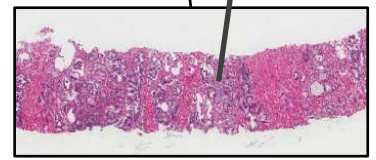
After clearing



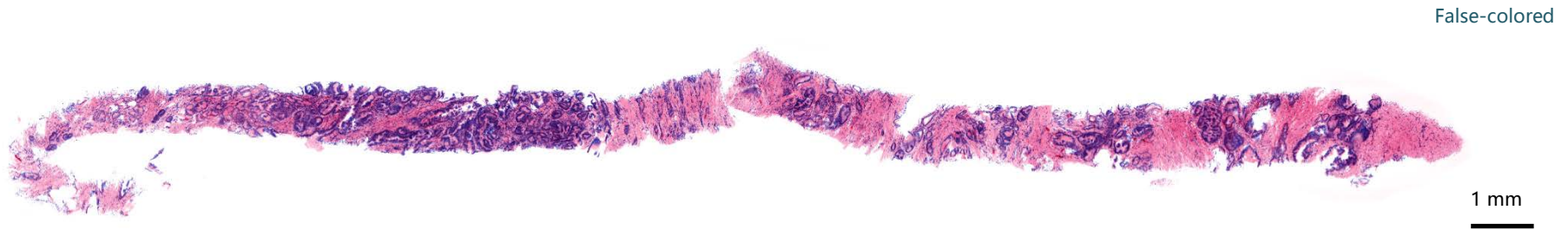
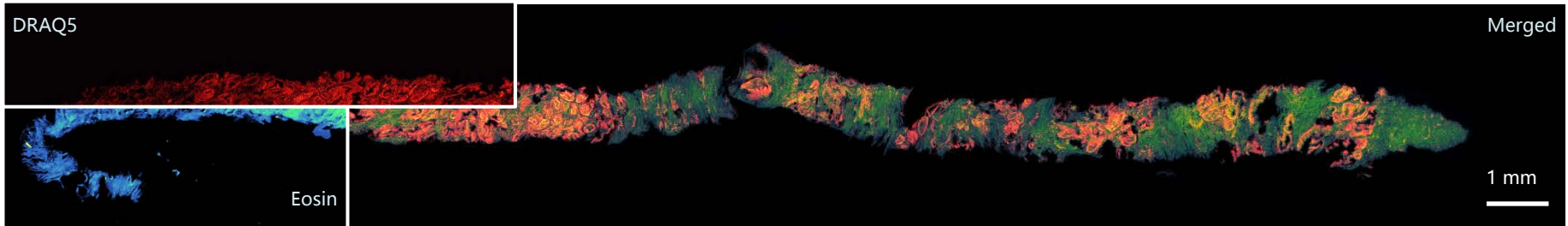
2D traditional H&E



100 μm

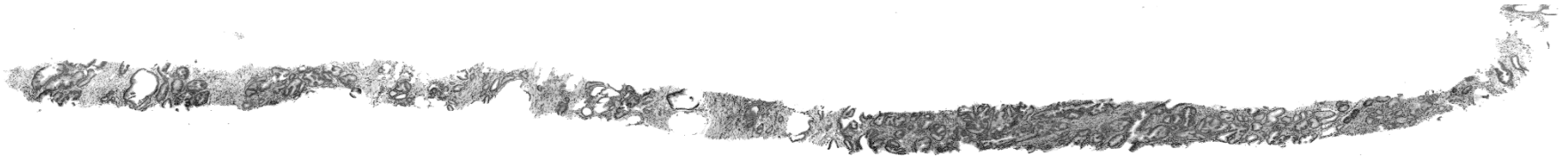


## DRAQ5 and Eosin dual-channel fluorescent staining and imaging of human prostate core-needle biopsy

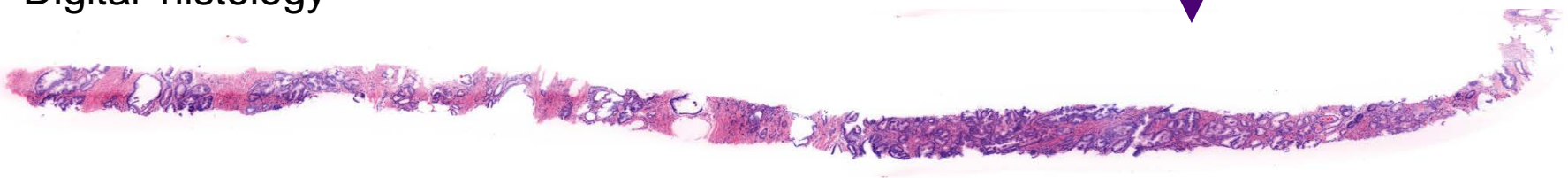




Nuclear stain (DRAQ5,  $\lambda_{ex} = 660 \text{ nm}$ ,  $\lambda_{em} = 680 \text{ nm}$ )

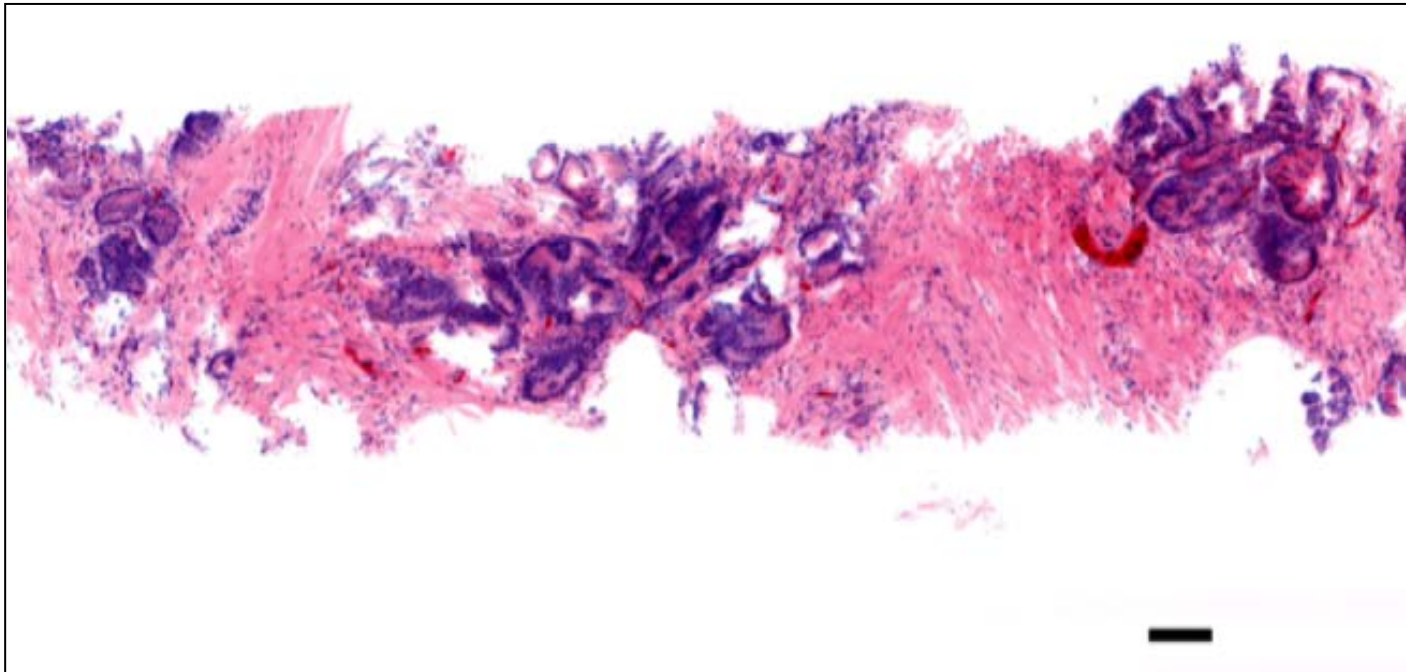
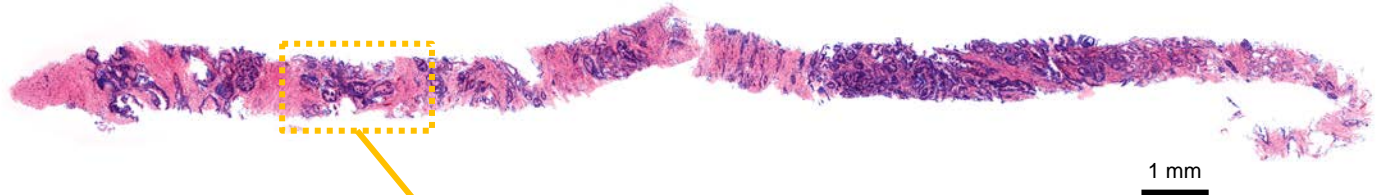


'Digital' histology



Cytoplasmic stain (Eosin,  $\lambda_{ex} = 488 \text{ nm}$ ,  $\lambda_{em} = 500 \text{ nm}$ )









- Introduction - Dr. Larry True
- Technological innovation (3D light-sheet microscopy) - Dr. Adam Glaser
- Clinical applications - Dr. Nicholas Reder
  - 1) Sampling errors (post-operative pathology)
  - 2) Intraoperative guidance (intra-operative pathology)
  - 3) Biopsy grading (“pre-operative” pathology)
- **Summary and tips for successful collaborations** - Dr. Jonathan Liu



High impact  
Slow adoption

## Post-operative digital pathology - “Triaging”

- Improve efficiency and costs

## Real-time intraoperative guidance

- Breast, prostate, skin, lymph nodes...

## Diagnostic and prognostic 3D pathology of biopsies

- Reduced sampling errors
- Non-destructive comprehensive 3D imaging
- 3D Immunofluorescence

## Advantages over conventional pathology:

1. Speed and cost (fresh unprocessed tissue)
2. Comprehensive 3D pathology with reduced sampling errors
3. Non-destructive (allows downstream molecular diagnostics of tissue)

## 1. Image processing

- Mosaicing
- Segmentation
- Deconvolution

## 2. Image presentation

- Data storage
- Compression
- Visualization

## 3. Image interpretation

- Computer-aided diagnosis (CAD)
- Machine learning / automated interpretation

## 4. Clinical acceptance, FDA approval, and reimbursement

## 1. Attend Meetings

- CAP
- Pathology Visions - resident travel award
- USCAP
- SPIE Photonics West

## 2. Contact an engineer at your institution

- You do not have to be an expert in optics
- Image interpretation
- Specimen acquisition

## 3. Background knowledge

- CAP in-vivo microscopy resource guide is a good start
- Journal publications (check lab websites)



## 1. Contact an engineer at your institution

- You do not have to be an expert in optics
- Image interpretation
- Specimen acquisition

## 2. Hands-on feedback

- Key to our collaboration
- Bidirectional working relationship, mutually beneficial

## 3. Background knowledge

- CAP in-vivo microscopy resource guide is a good start
- Journal publications (check lab websites)

## 1. We need your help the most!

- Essential for translation
- Ex vivo microscopy will not succeed without clinical adoption by community pathologists

## 2. Contact an engineer working on a project that interests you

- You do not have to be an expert in optics
- Image interpretation
- Ease-of-use / practical feedback

## 3. Background knowledge

- CAP in-vivo microscopy resource guide is a good start
- Journal publications (check lab websites)

## UW Seattle

Dr. Yu “Winston” Wang  
 Dr. Adam Glaser  
 Dr. Steven Leigh  
 Dr. Danni Wang  
 Ms. Ye Chen  
 Ms. Soyoung Kang  
 Mr. Peter Wei  
 Mr. Chengbo Yin  
 Dr. Matt Wall  
 Dr. Qian Yang  
 Dr. Nick Reder  
 Dr. Lawrence True  
 Ms. Erin McCarty  
 Dr. Suzanne Dintzis  
 Dr. Sara Javid

## Illinois Institute of Tech.

Dr. Ken Tichauer  
 Ms. Clover Xu



NIH / NIBIB – K99/R00 EB008557

NIH / NIDCR – R01 DE023497

NIH / NIBIB – R21 EB015016

NIH / NCI – R21 CA215561

NIH / NCI – R01 CA175391

UW Royalty Research Fund

ITHS Collaboration Innovation Award

UW CoMotion Innovation Award

Safeway / Breast SPORE Developmental Award (Fred Hutch)

## UW eScience

Dr. Ariel Rokem  
 Dr. Amanda Tan  
 Dr. Rob Fatland

## UW CoMotion

Forest Bohrer  
 Ken Myer  
 Mike Connolly

## Memorial Sloan-Kettering Cancer Center

Dr. Milind Rajadhyaksha  
 Mr. Sanjee Abeytunge  
 Dr. Melissa Murray

## Barrow Neurological Institute

Dr. Nader Sanai (Neurosurgery)

# Upcoming Webinars

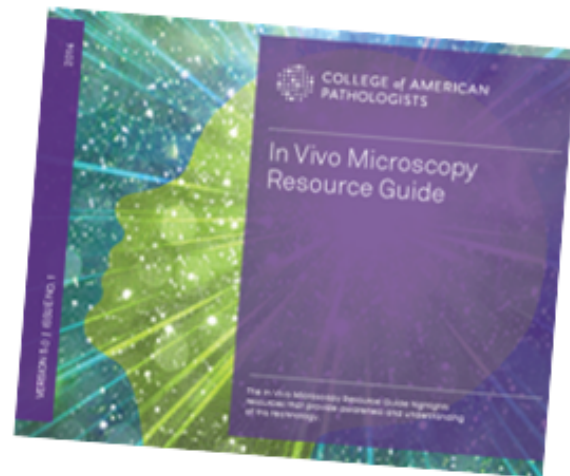
<b>DATE</b>	<b>TOPIC</b>	<b>SPEAKER(s)</b>
7/18	IVM and Inflammatory Intestine	Gary Tearney, MD, PhD, FCAP
10/3	Light-sheet microscopy for 3D pathology	Nicholas P. Reder, MD, MPH Lawrence D. True, MD
11/7	Rapid examination of fresh tissue using light-sheet microscopy	Nicholas P. Reder, MD, MPH

Register for upcoming & archived webinars:  
[www.cap.org](http://www.cap.org) > Calendar > Webinars



# The CAP In Vivo Microscopy Resource Guide – see handout

- The IVM resource guide highlights current IVM articles and other resources that assist in understanding and potentially adopting IVM and EVM
  - Printed guides are available for members (\$39) and non-members (\$69)
  - The digital copies of all four Resource Guides are a complimentary member benefit
  - Access them [www.cap.org](http://www.cap.org) > Resources and Publications





# IVM Short Presentations on Emerging Concepts (SPECs) – see handout

- IVM SPECs are:
  - Short PowerPoints, created for pathologists
  - Useful for educating pathologists colleagues about IVM and GI specialist on the role and value of pathologists in IVM
- IVM SPEC Topics:
  - In Vivo Microscopy (IVM): A New Role for Pathologists
  - IVM of the GI Tract
  - Ex Vivo Microscopy (EVM): A New Tool for Pathologists

Access them [www.cap.org](http://www.cap.org) > Resources and Publications



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# Introduction to In Vivo Microscopy Interpretation Workshop 2017 – see handout

**September 16, 2017, 8-4 pm in Chicago at The James Hotel**

- **Complimentary workshop!** Seminar topics:
  - Explain IVM image terminology
  - Demonstrate ex vivo optional imaging for tissue evaluation and surgical pathology practice
  - Explain latest IVM technologies and image acquisition modalities with a focus on GI, skin, and lung
  - Demonstrate familiarity with IVM image interpretation criteria
  - Demonstrate ex vivo optional imaging for tissue evaluation and surgical pathology practice
  - Explain the role of pathologists in IVM programs
  - Explain IVM reimbursement opportunities



CAP

- Register today [surveymonkey.com/r/IVMWorkshop17](https://www.surveymonkey.com/r/IVMWorkshop17)

# CAP17 The Pathologists' Meeting – IVM Highlights

- Learn about CAP's in vivo microscopy resources and talk with fellow members who are pioneering these technologies at the **CAP's IVM Committee Booth** in the Exhibit Hall
- Sign up for the complimentary breakfast workshop **Justifying the Introduction of Emerging Technologies into a Pathology Department: How to Develop a Business Plan**
- Register at [www.cap.org/cap17](http://www.cap.org/cap17)



# THANK YOU!

- Thank you for attending our webinar **“Creating a successful pathology-engineering collaboration.”**
  - For comments about this webinar or suggestions for upcoming webinars, contact [ivminfo@cap.org](mailto:ivminfo@cap.org)
  - NOTE: There is no CME/CE credit available for today’s complimentary webinar. The pdf of the presentation will be sent out in a week.