COLLEGE of AMERICAN PATHOLOGISTS

Rapid examination of fresh tissue using light-sheet microscopy

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11/7/2017

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**
- **Genitourinary pathology fellow in the University of Washington Department of Pathology.**

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Disclosure

- **Dr. Reder holds a patent and has a start-up company (Alpenglow Optics, LLC) related to light-sheet microscopy work.**

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Learning Objectives

- **Understand the different techniques for examination of fresh tissue**
- **Be able to articulate the strengths and weaknesses of each technique**
- **Describe use-cases for slide-free microscopy of fresh tissue**

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Outline

- **Motivation for slide-free microscopy of fresh tissue**
- **Overview of slide-free microscopy techniques**
- **False-coloring to mimic H&E**
- **Use-cases**
- **Summary**

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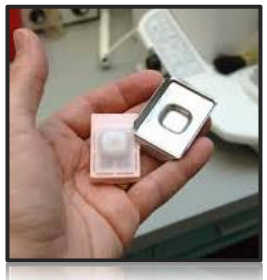
Motivation: pathology has remained unchanged for a century

Rapid histology: 4 hours

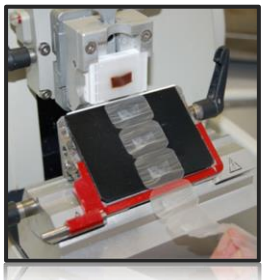
1. Fix
~30 minutes

2. Process
~2.5 hours

3. Embed
~10 minutes

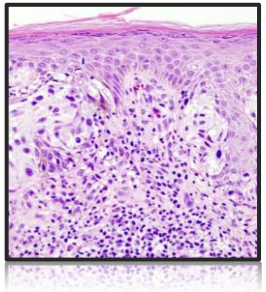


4. Section
~10 minutes



5. Stain
~40 minutes

Histology



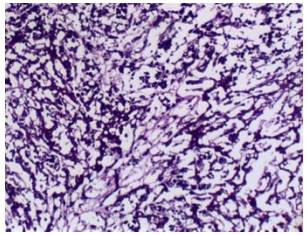
Frozen Section: 10 minutes

1. Freeze
~2 minutes

2. Section
~2 minutes

3. Stain
~4 minutes

Histology



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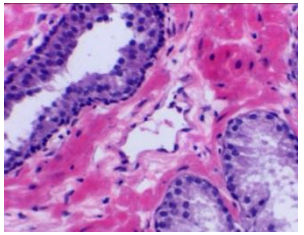

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Motivation: pathology has remained unchanged for a century

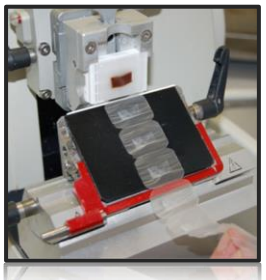
Rapid histology: 4 hours

1. Fix
~30 minutes

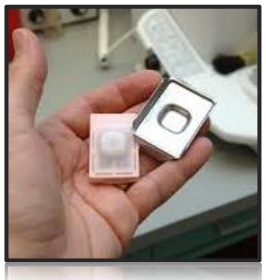
5. St
~40 mi

ess
urs

4. Section
~10 minutes



3. Embed
~10 minutes



Disadvantages

Expensive
Time-consuming
Destructive
Hazardous
Sampling errors

Frozen Section: 10 minutes

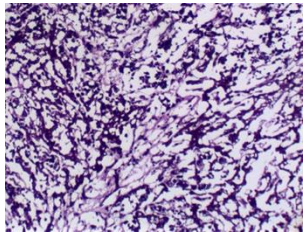
1. Freeze
~2 minutes

2. Section

Disadvantages

Destructive
Hazardous
Sampling errors
Variable histology quality

Histology



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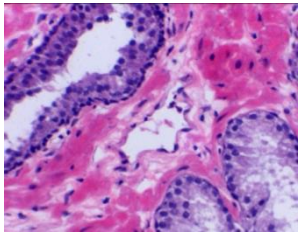

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Goal: non-destructive, slide-free, ‘digital’ pathology

1. Stain
<1 minute

2. Image
<10 minutes

Wide-area ‘digital’ histology

Advantages
Fast
Digital
Non-destructive
Slide-free
Wide-area

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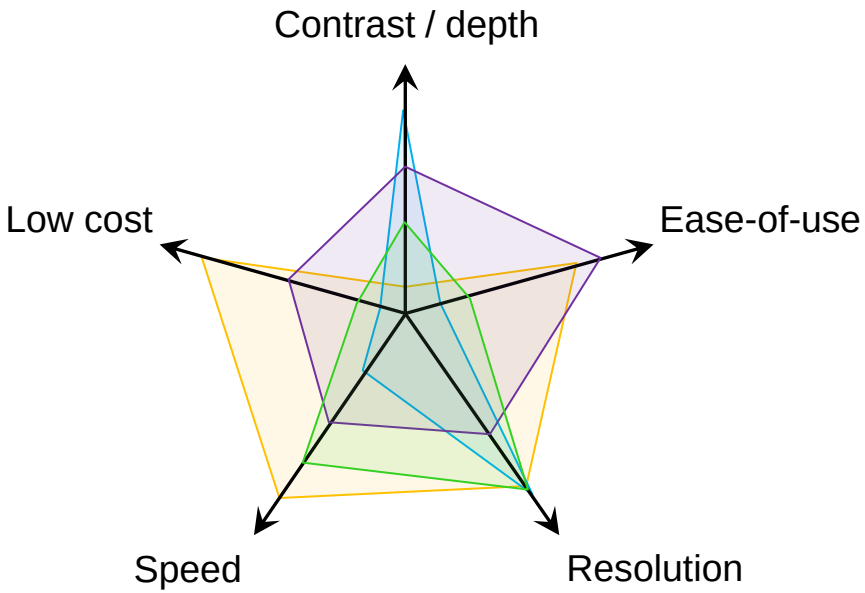
Overall comparison of **fluorescence** microscopy technologies

Surface imaging



Microscopy method

MUSE **Structured-illumination** **Confocal, Nonlinear** **Light-sheet**

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Similarities across all techniques

- Slide-free imaging → Efficient workflow
- Non-destructive → Preservation of tissue for molecular testing
- Fluorescence imaging: need to use topically applied fluorescent dyes, or endogenous fluorophores (autofluorescence)

- Other non-fluorescent techniques can achieve slide-free, non-destructive imaging, but they are beyond the scope of this presentation
  - OCT
  - Photoacoustic
  - Spectroscopy

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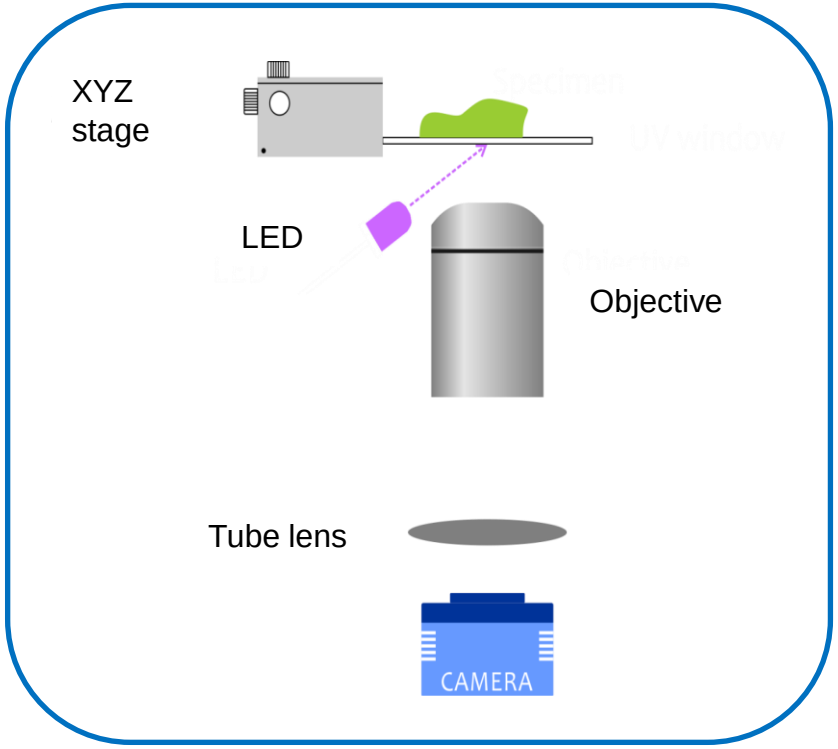
MUSE

- Microscopy with UV Surface Excitation
- UV light has limited penetration (10 microns)
- Advantages
  - Fast
  - Simple
  - Inexpensive
  - High resolution
- Disadvantages
  - Surface imaging only



Fereidouni F, Harmany Z, Demos S, Levenson R. MUSE: Microscopy via UV excitation for rapid histology. In: Photonics Conference (IPC). 2016 IEEE 2016 Oct 2 (pp. 146-147). IEEE.

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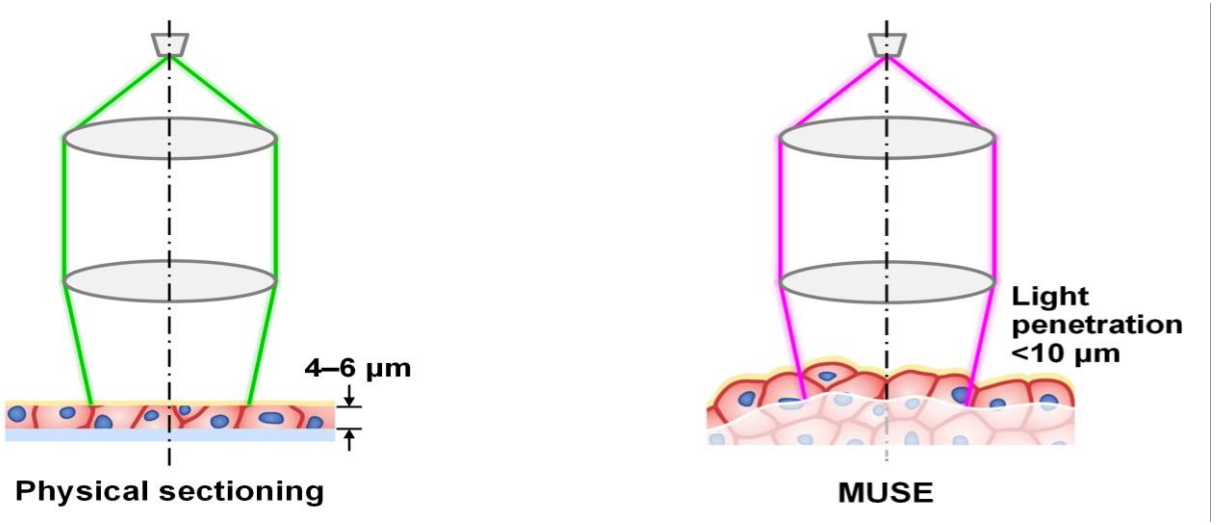
MUSE

How does MUSE produce images?



Limited penetration of UV light → “Optical section”

Figure courtesy of Dr. Yu “Winston” Wang, University of Washington, Department of Mechanical Engineering

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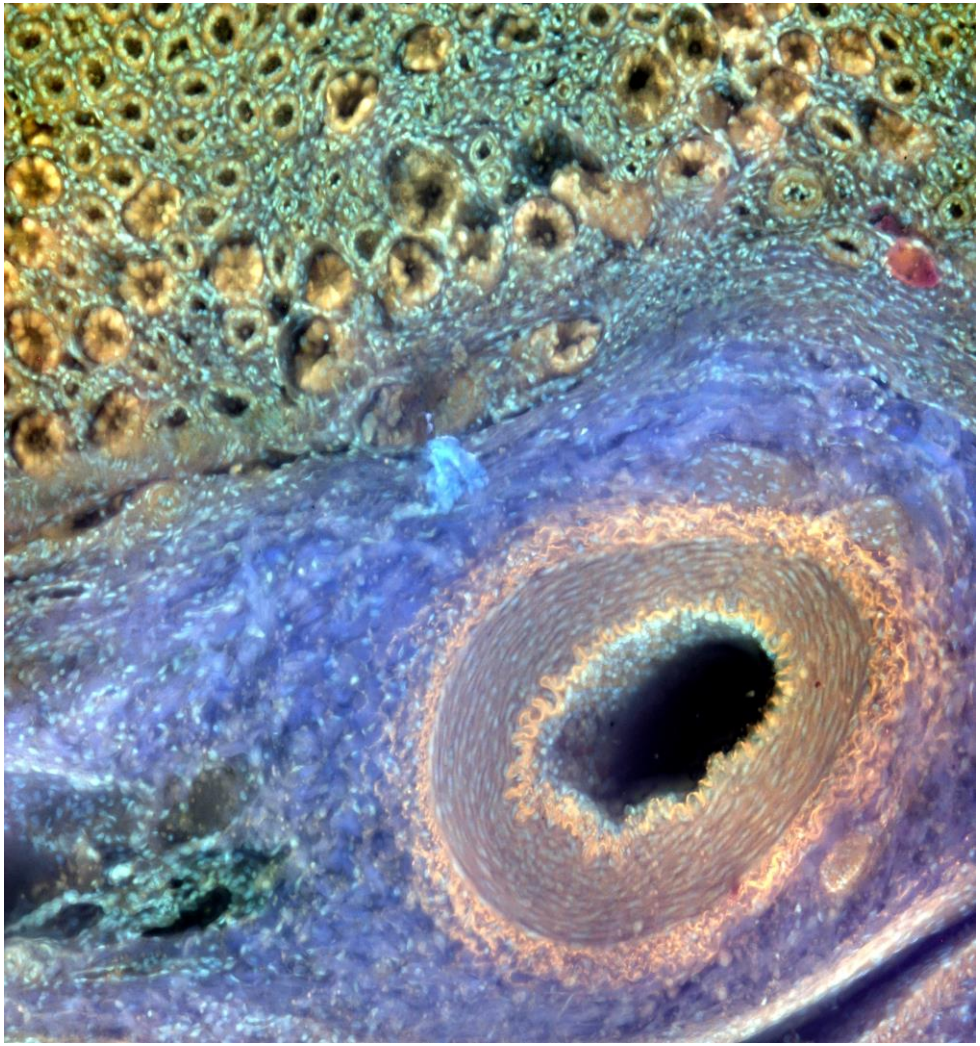
MUSE

Single-excitation source, color CCD camera

- Orange: elastic laminae in artery
- Blue: collagen
- Green, orange and light blue: renal tubules and nuclei



Slide courtesy of Dr. Richard Levenson, UC Davis

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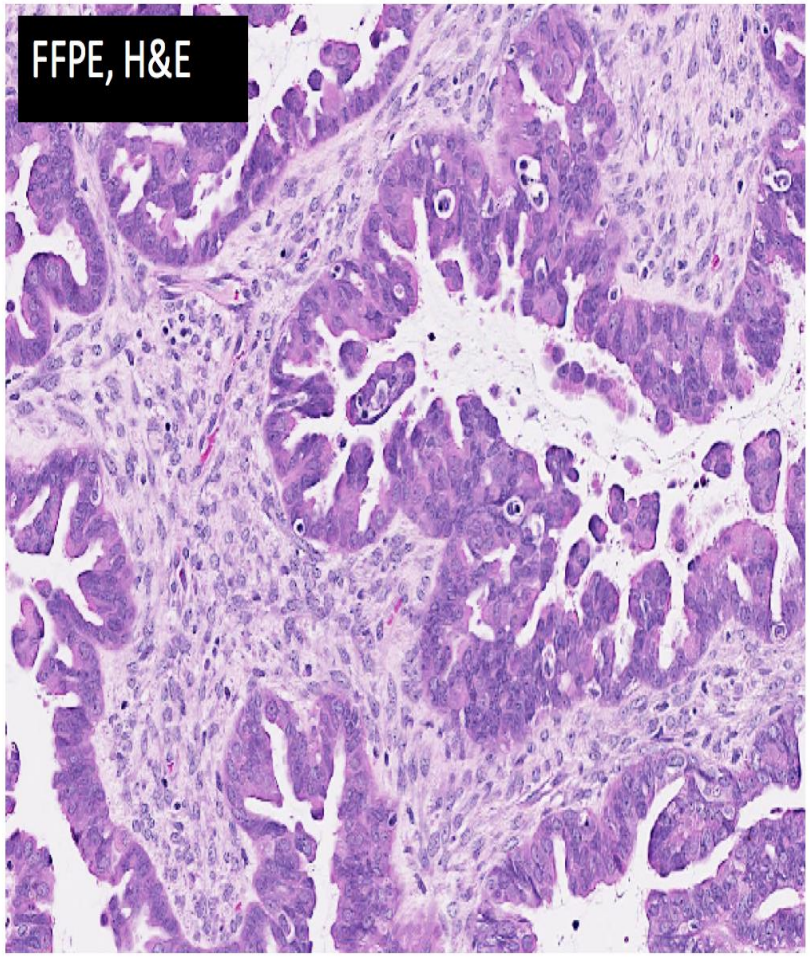
MUSE

SEROMUCINOUS OVARIAN CARCINOMA



Slide courtesy of Dr. Richard Levenson, UC Davis

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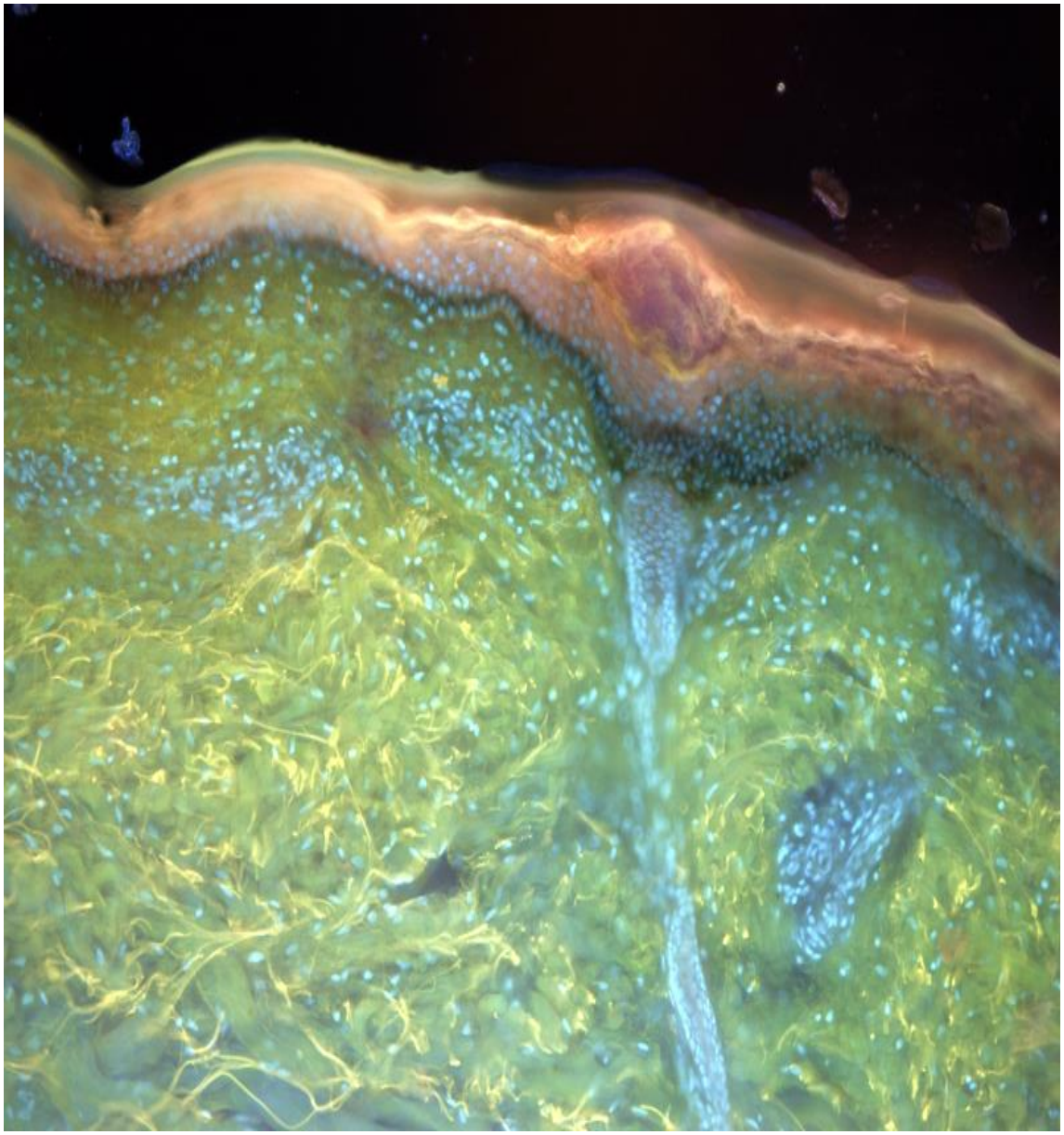
MUSE

- **Example: Skin**
- **Easy distinction between elastin (yellow) and collagen (green)**
- **Usually requires special stains**



Slide courtesy of Dr. Richard Levenson, UC Davis

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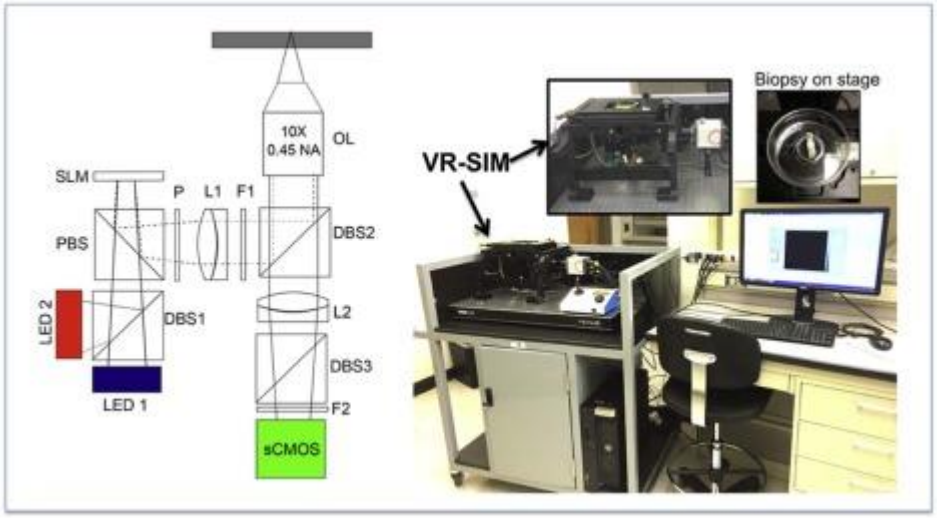
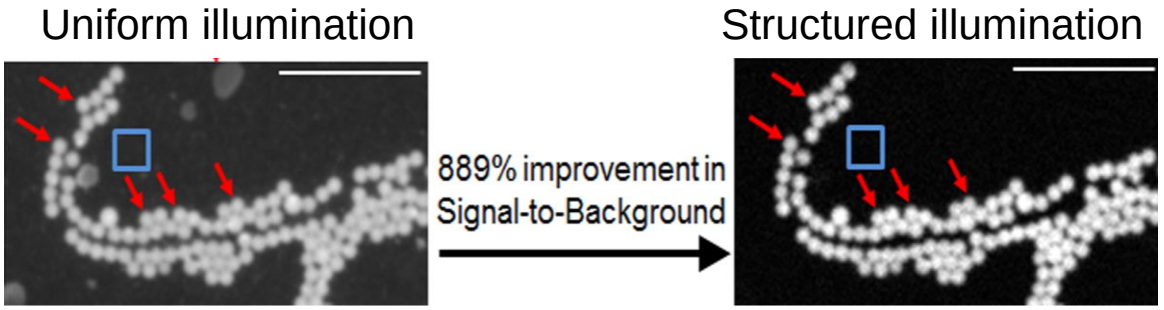
Structured Illumination

- **Structured illumination microscopy**
- **Uses patterned light to improve resolution**
- **Advantages**
  - Fast
  - High resolution



- **Disadvantages**
  - Modest imaging depth
  - Complex optics



Fu HL, Mueller JL, Javid MP, Mito JK, Kirsch DG, Ramanujam N, Brown JQ. Optimization of a widefield structured illumination microscope for non-destructive assessment and quantification of nuclear features in tumor margins of a primary mouse model of sarcoma. *PloS one*. 2013;8(7):e68868.

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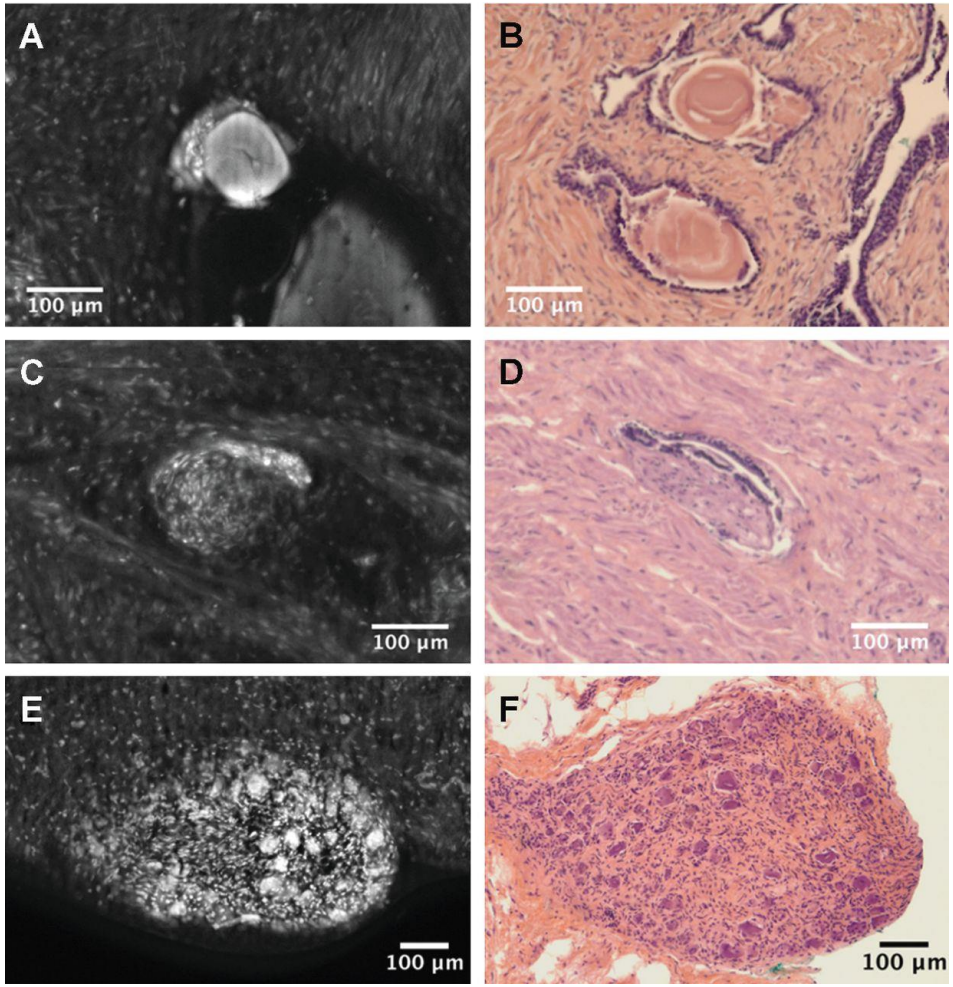
Structured Illumination

Example: Prostate

Easy distinction of benign and neoplastic lesions

High accuracy: AUC of 0.82-0.88



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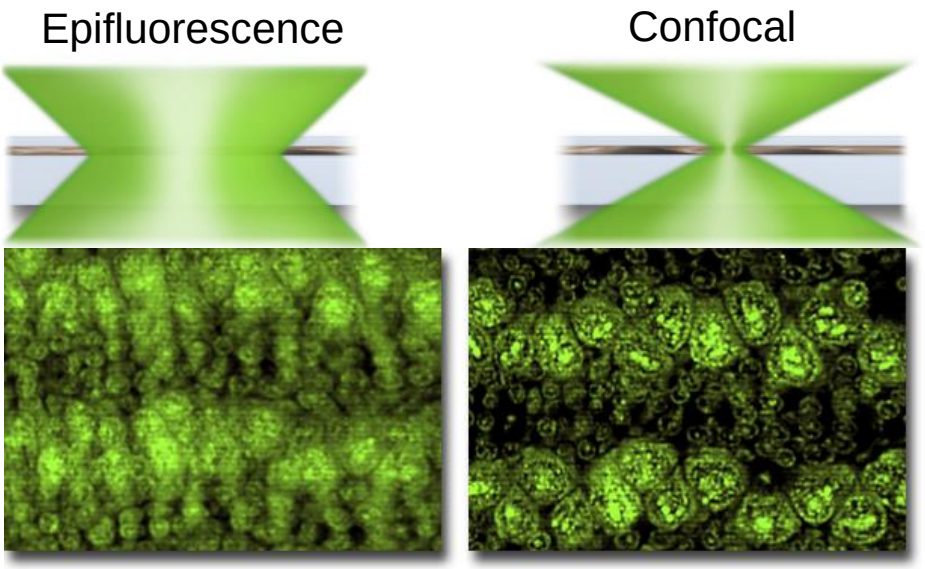
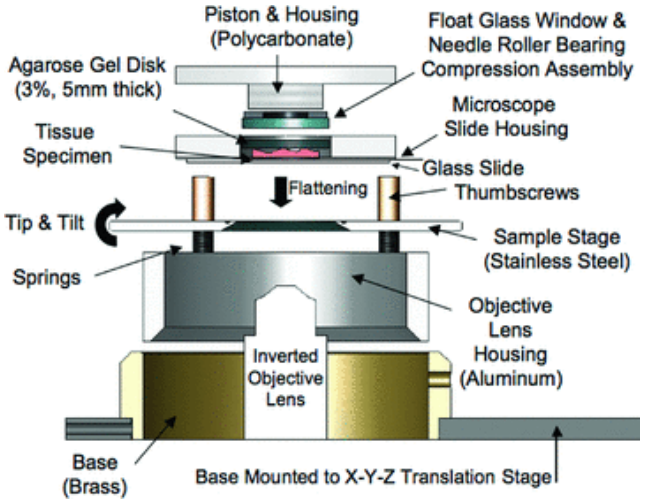
Wang M, Kimbrell HZ, Sholl AB, Tulman DB, Elfer KN, Schlichenmeyer TC, Lee BR, Lacey M, Brown JQ. High-resolution rapid diagnostic imaging of whole prostate biopsies using video-rate fluorescence structured illumination microscopy. Cancer research. 2015;75(19):4032-41.

Confocal

- **Confocal microscopy**
- **Uses a pin-hole to reject out-of-focus light**
- **Advantages**
  - Depth of imaging
  - High resolution
  - Commercially available
- **Disadvantages**
  - Requires elaborate tissue flattening for surface imaging
  - Slow (in 3D) due to point-scanning





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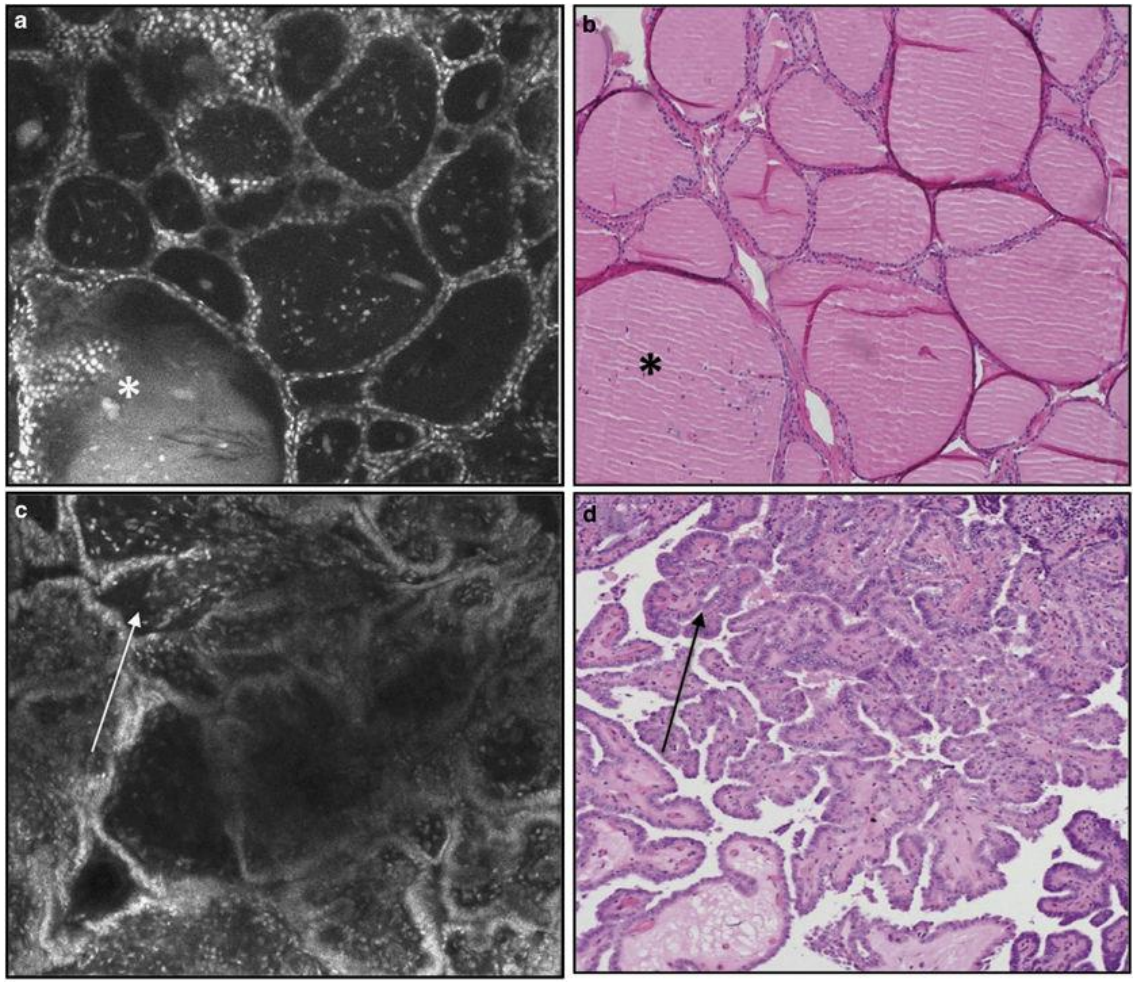
Gareau DS, Patel YG, Li Y, Aranda I, Halpern AC, Nehal KS, Rajadhyaksha M. Confocal mosaicing microscopy in skin excisions: a demonstration of rapid surgical pathology. *Journal of microscopy*. 2009;233(1):149-59.

Confocal

Example: Thyroid

Easy distinction of benign and neoplastic lesions



Ragazzi M, Piana S, Longo C, Castagnetti F, Foroni M, Ferrari G, Gardini G, Pellacani G. Fluorescence confocal microscopy for pathologists. *Modern Pathology*. 2014;27(3):460-71.

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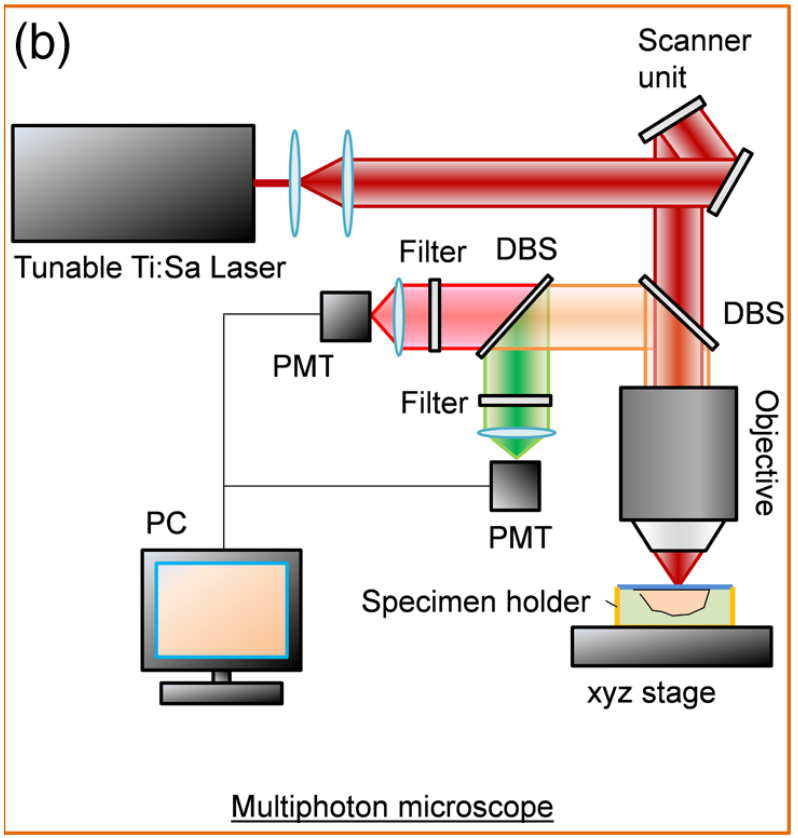
Multiphoton

- **Multiphoton microscopy**
  - Aka two-photon, nonlinear
- **Uses a pulsed laser to achieve precise localization of excitation**
- **Advantages**
  - Depth of imaging
  - Very high resolution
- **Disadvantages**
  - Requires elaborate tissue flattening for surface imaging
  - Slow (in 3D) due to point-scanning
  - Expensive and complex optics



Yoshitake T, Giacomelli MG, Cahill LC, Schmolze DB, Vardeh H, Faulkner-Jones BE, Connolly JL, Fujimoto JG. Direct comparison between confocal and multiphoton microscopy for rapid histopathological evaluation of unfixed human breast tissue. *Journal of Biomedical Optics*. 2016;21(12):126021-.

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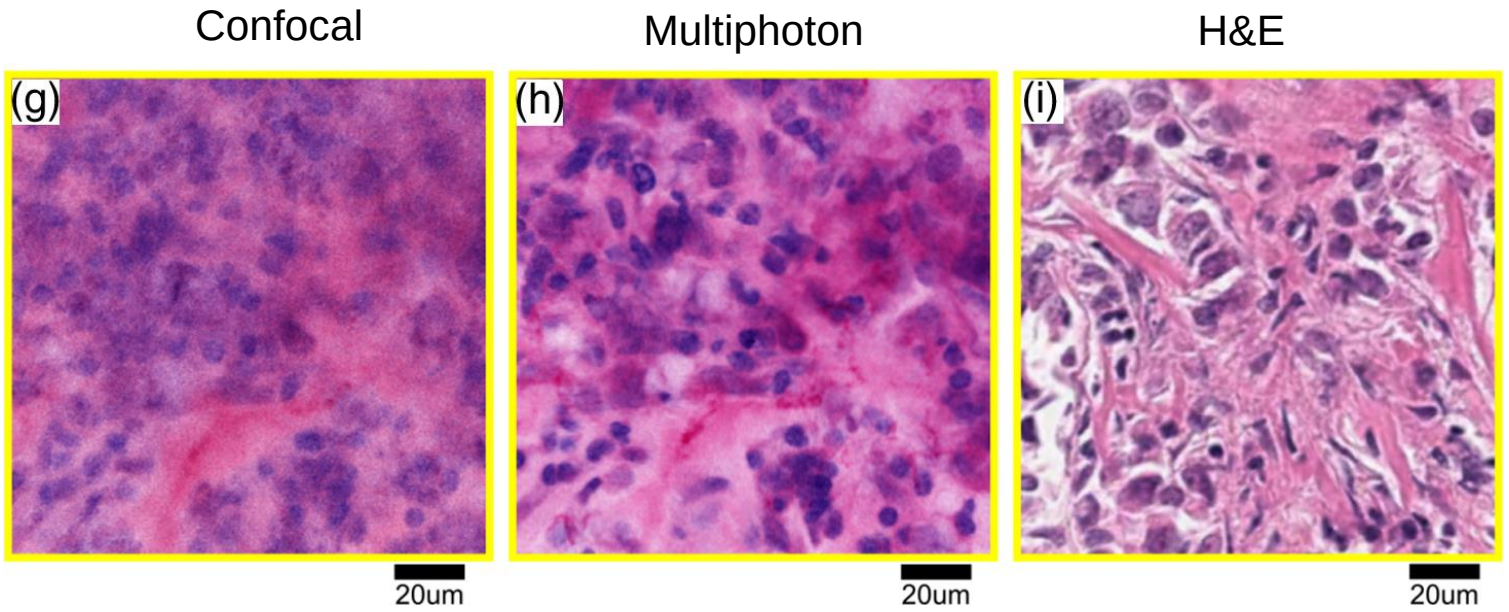
Multiphoton

Example: Breast, invasive ductal carcinoma

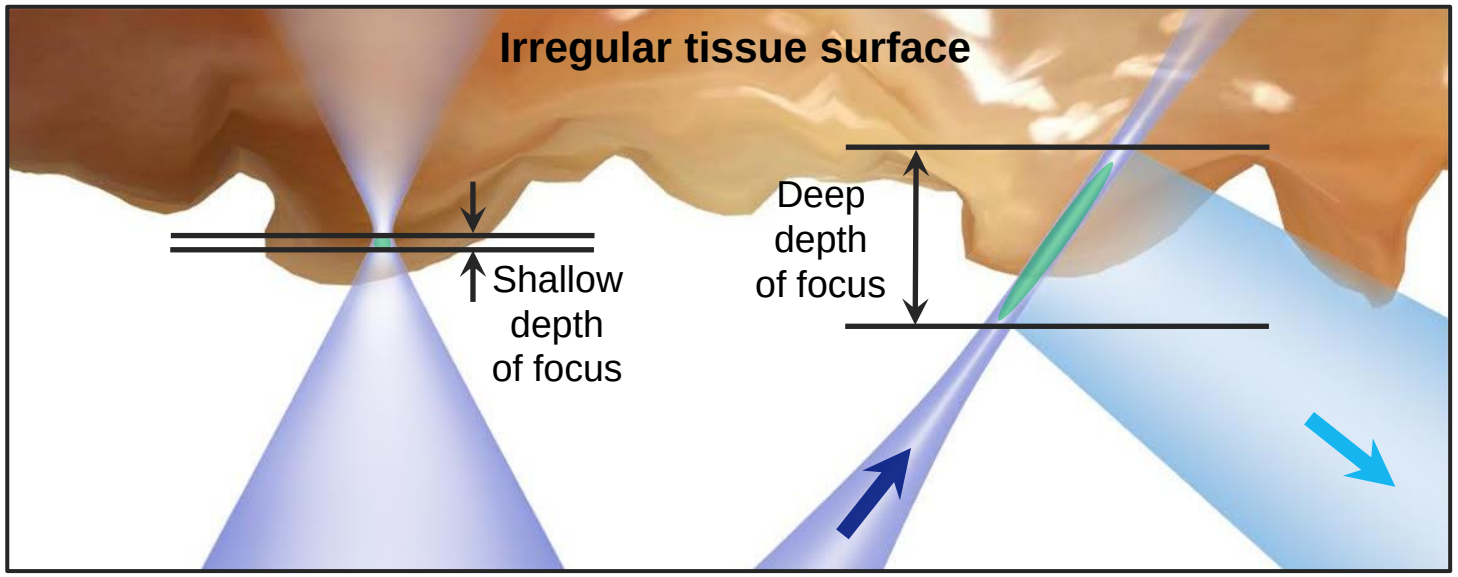
Crisp nuclear detail, easy diagnosis (>94% accuracy)[1]

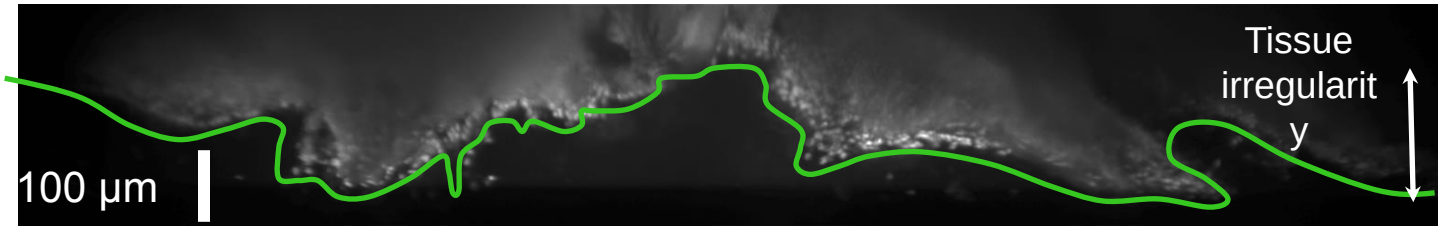


Images: Yoshitake T, Giacomelli MG, Cahill LC, Schmolze DB, Vardeh H, Faulkner-Jones BE, Connolly JL, Fujimoto JG. Direct comparison between confocal and multiphoton microscopy for rapid histopathological evaluation of unfixed human breast tissue. Journal of biomedical optics. 2016;21(12):126021-.

1. Tao YK, Shen D, Sheikine Y, Ahsen OO, Wang HH, Schmolze DB, Johnson NB, Brooker JS, Cable AE, Connolly JL, Fujimoto JG. Assessment of breast pathologies using nonlinear microscopy. *Proceedings of the National Academy of Sciences*. 2014;111(43):15304-9.

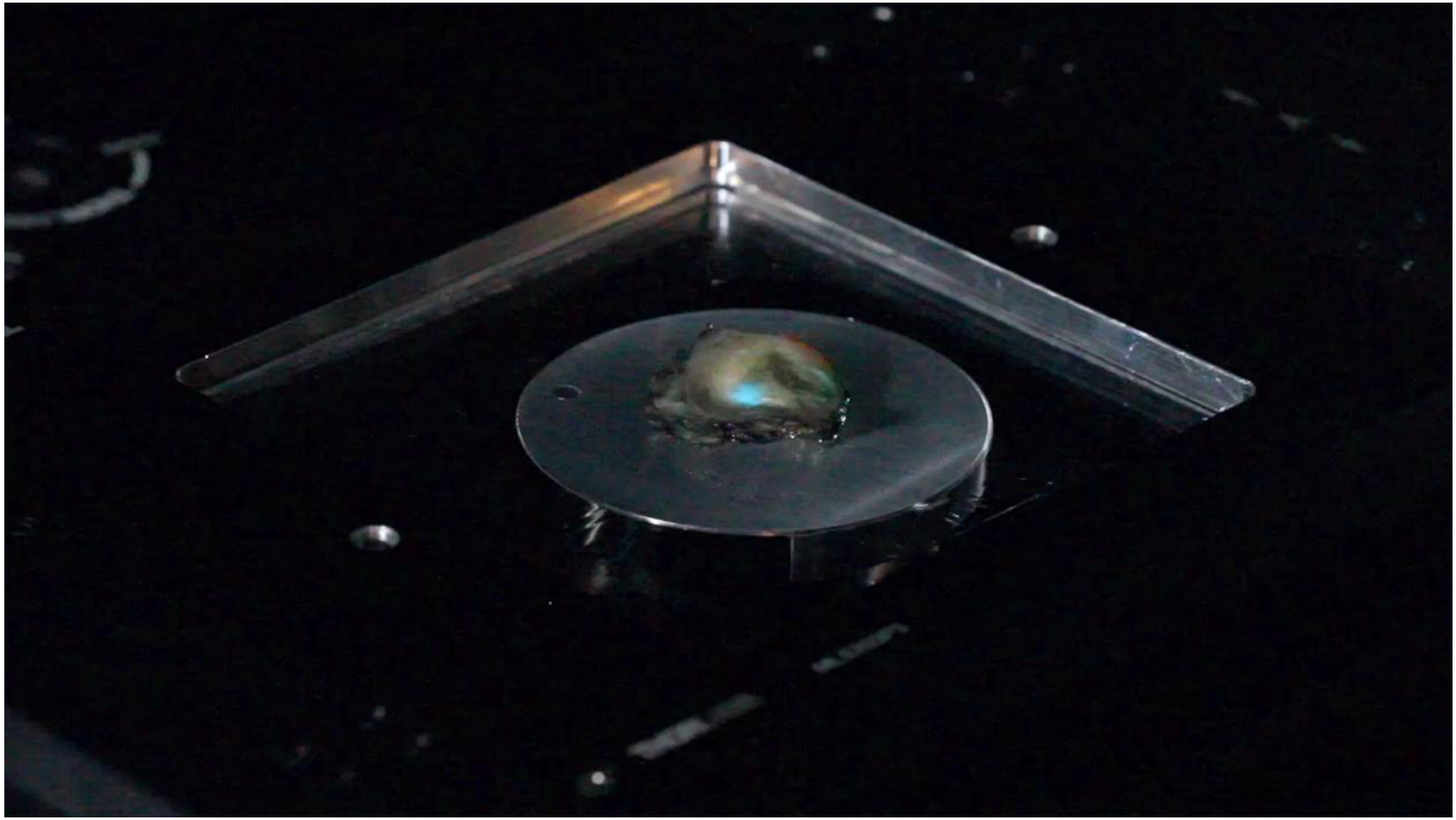
LSM for imaging fresh tissues





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

LSM Demonstration



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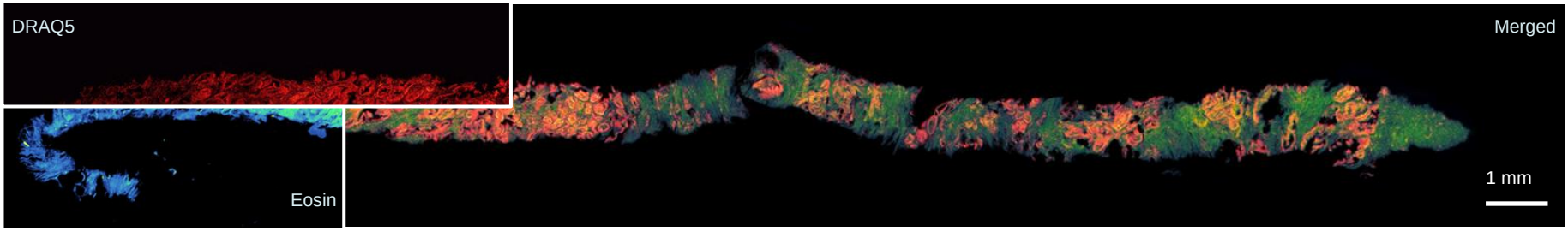
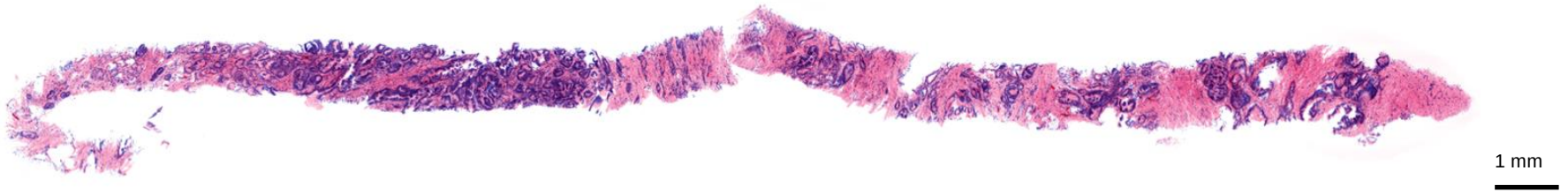
False-color H&E imaging

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



False-colored



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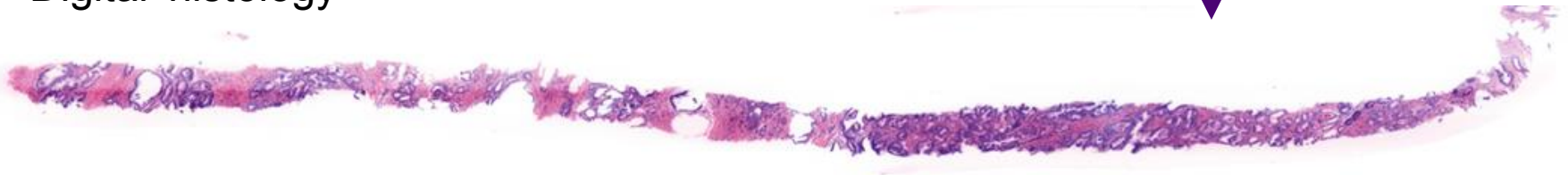
False-color H&E imaging

Nuclear stain (DRAQ5, λ_{ex} = 660 nm, λ_{em} = 680 nm)

‘Digital’ histology

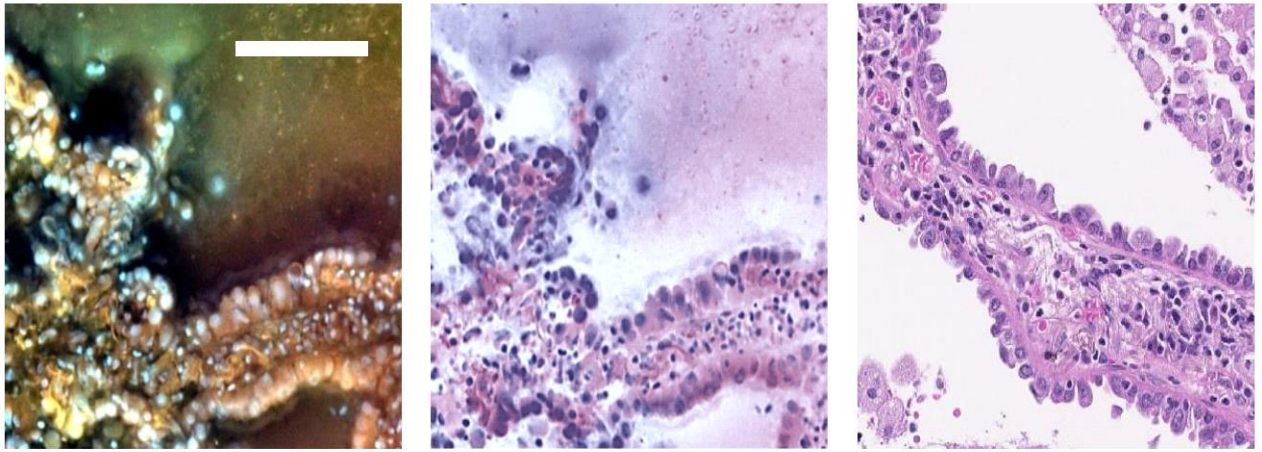


Cytoplasmic stain (Eosin, λ_{ex} = 488 nm, λ_{em} = 500 nm)

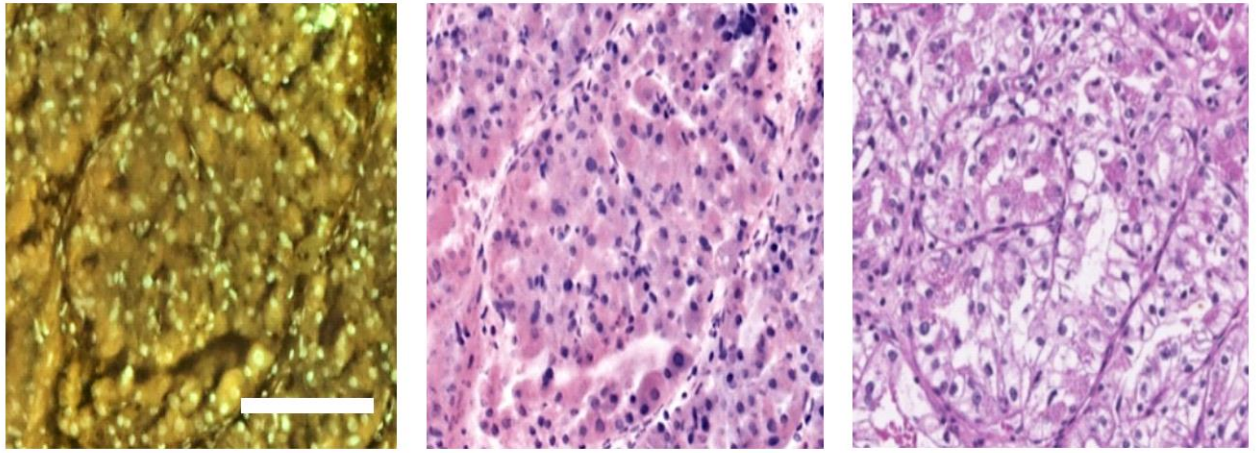
False-color H&E imaging

MUSE H&E

Adenocarcinoma Lung



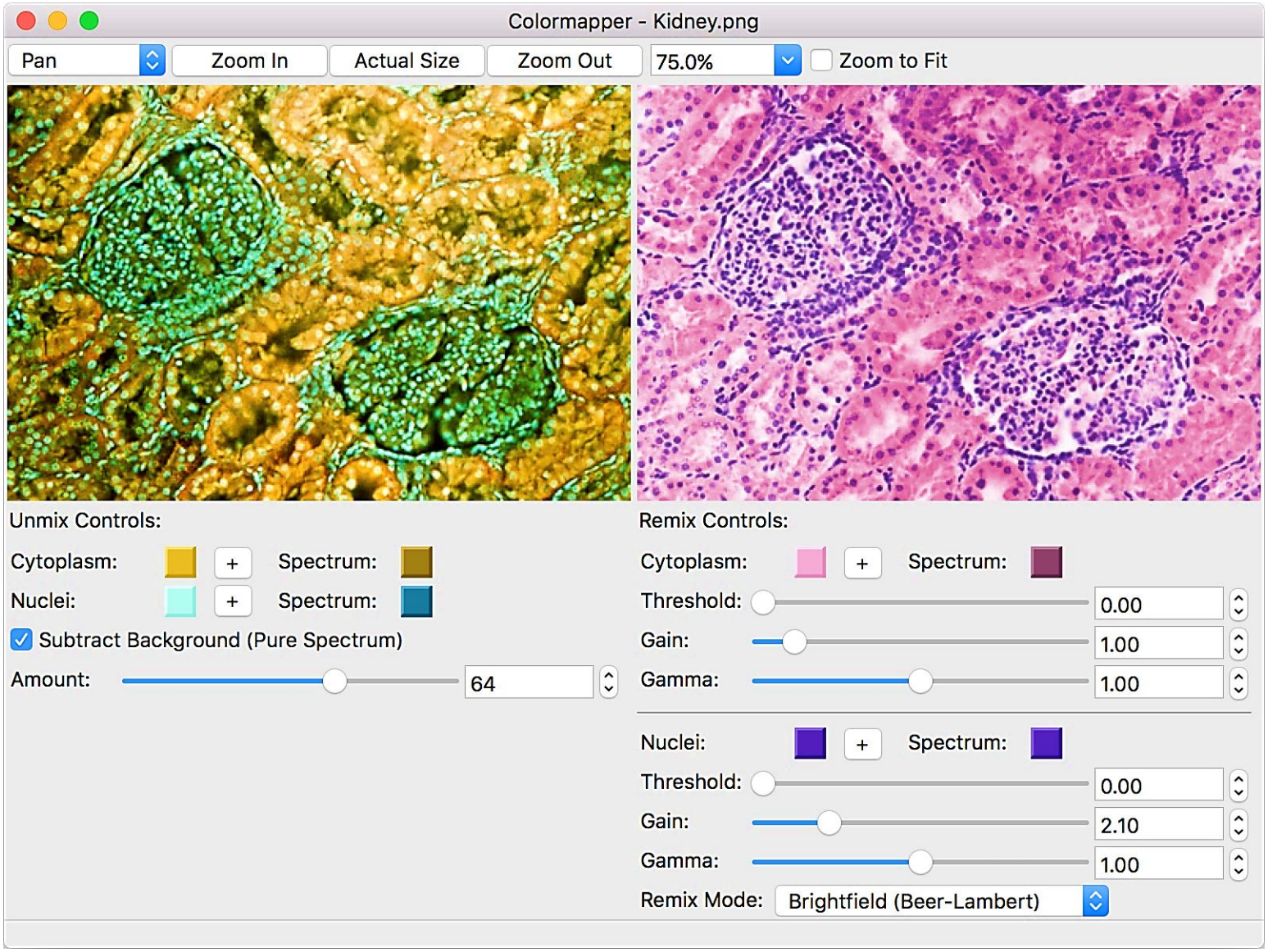
Clear cell carcinoma Kidney



H&E GUI – Tune the stain to your liking

MUSE

Kidney



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Slide courtesy of Dr. Richard Levenson, UC Davis

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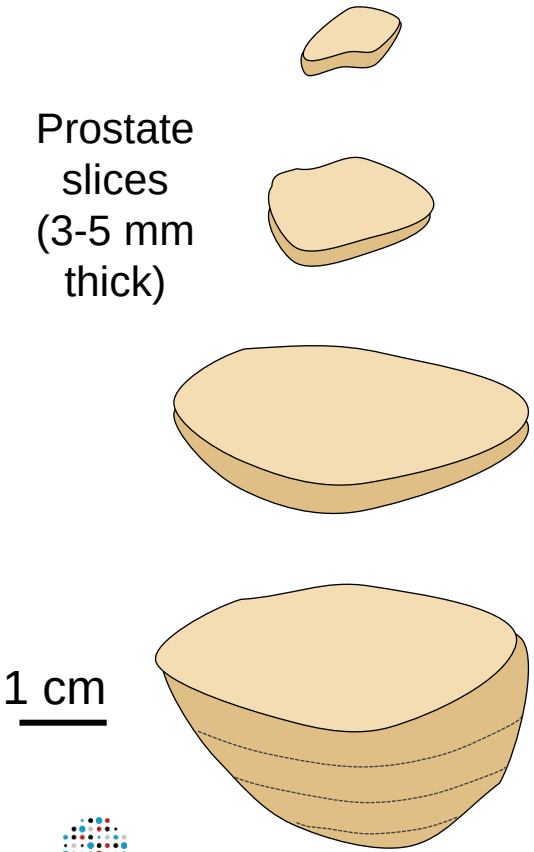
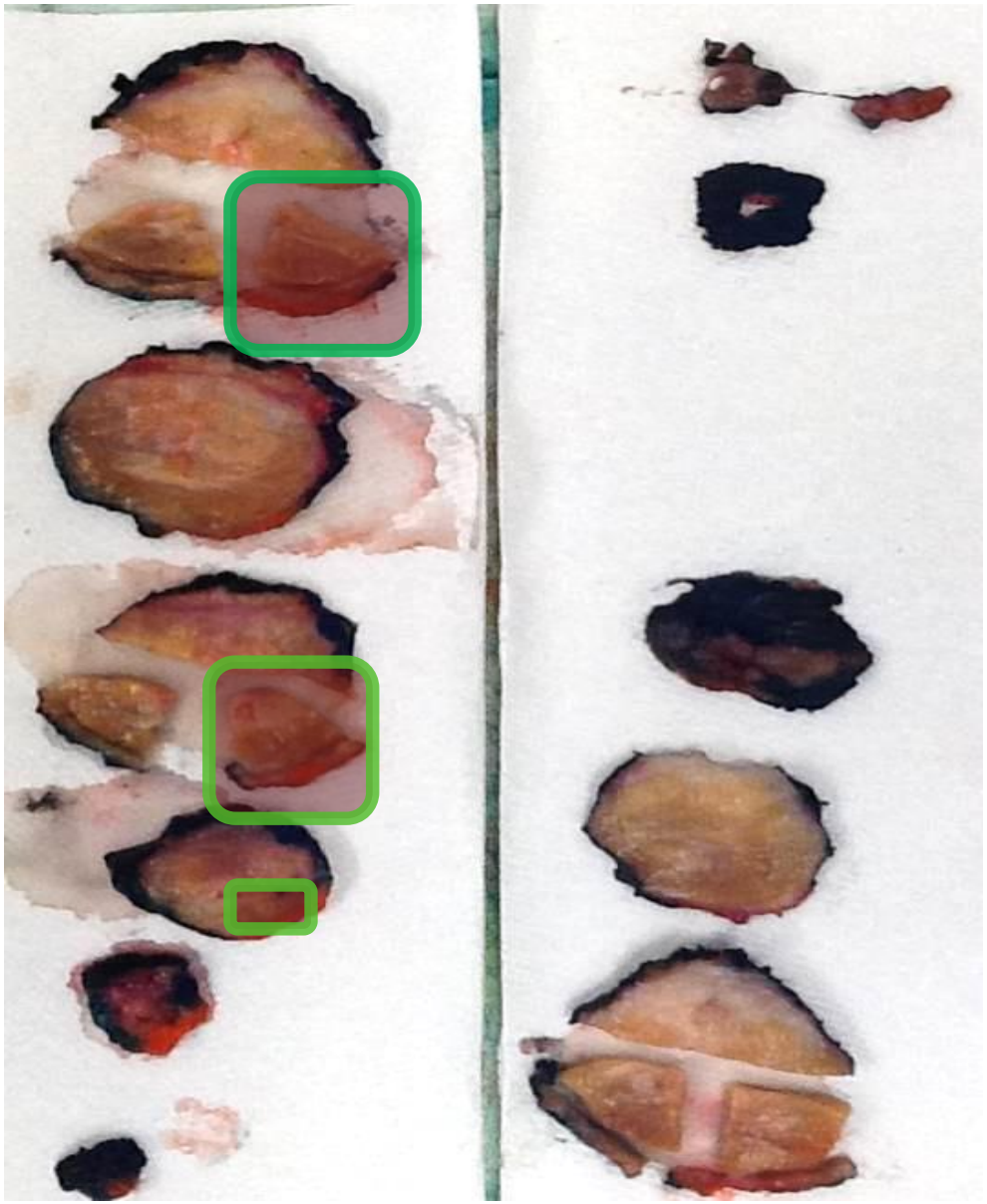
Use-case: Post-operative triaging of fresh tissue

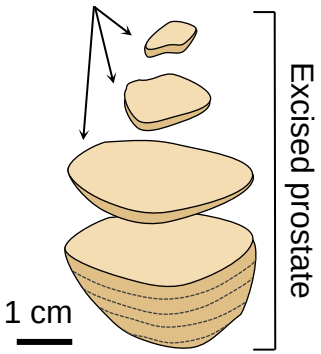
Radical prostatectomy



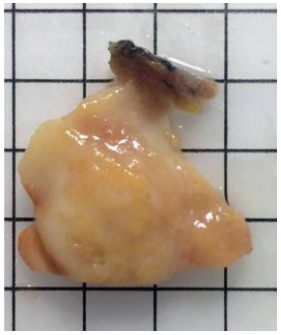


Use-case: Post-operative triaging of fresh tissue

Prostate slices (3-5 mm thick)



Fresh prostate slice



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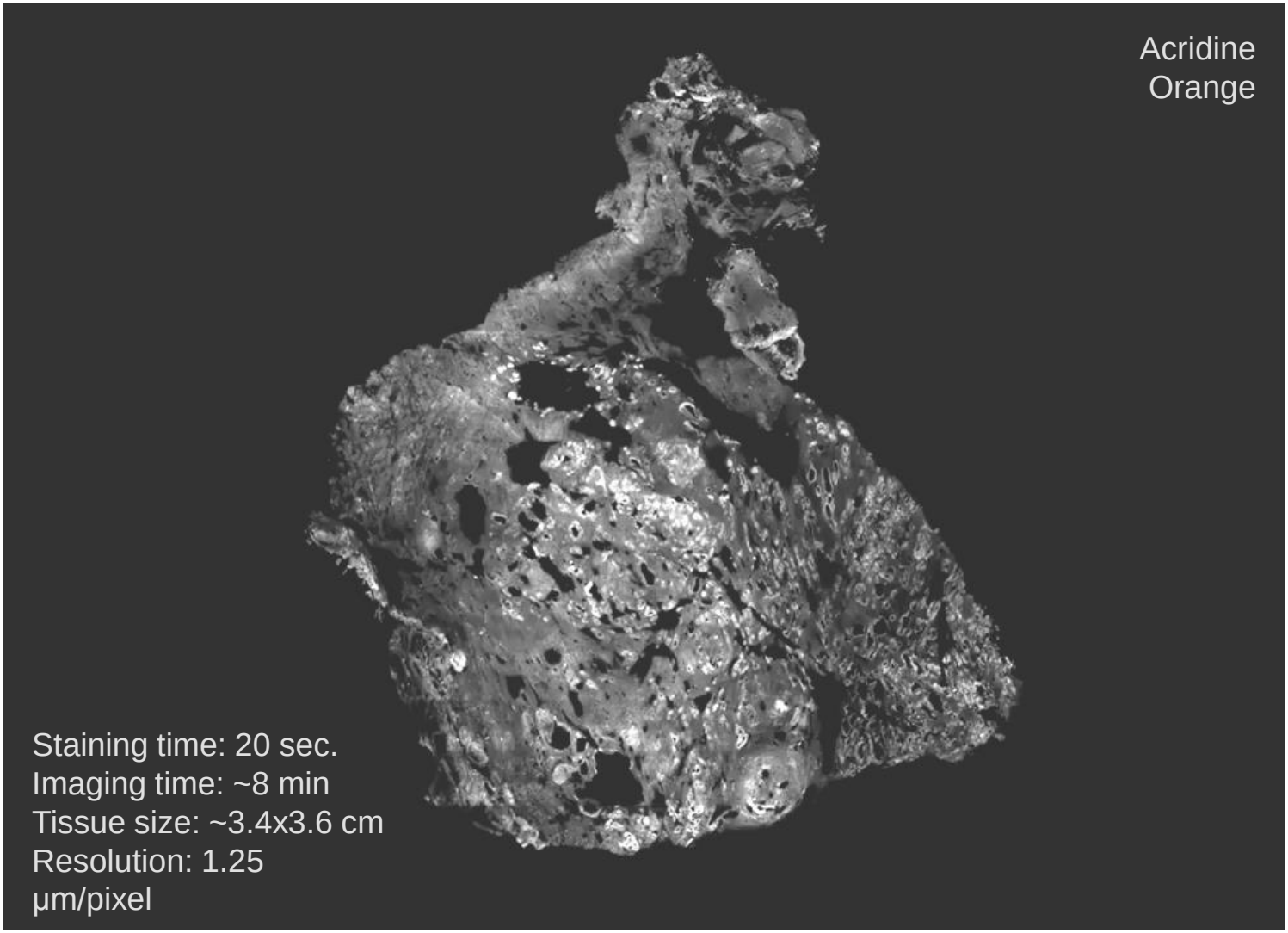
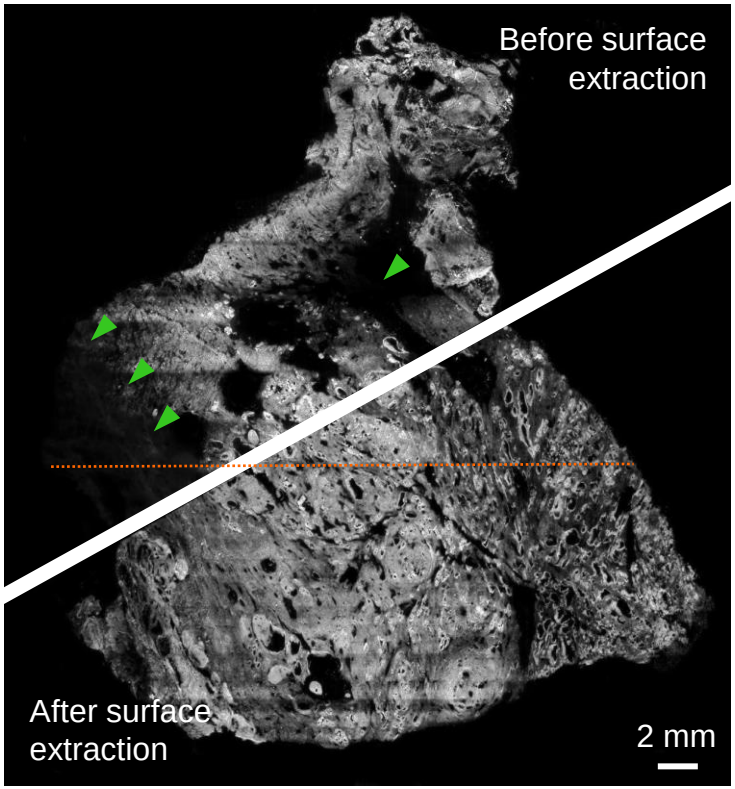
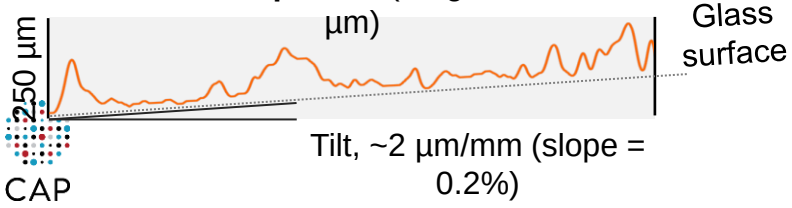


Use-case: Post-operative triaging of fresh tissue

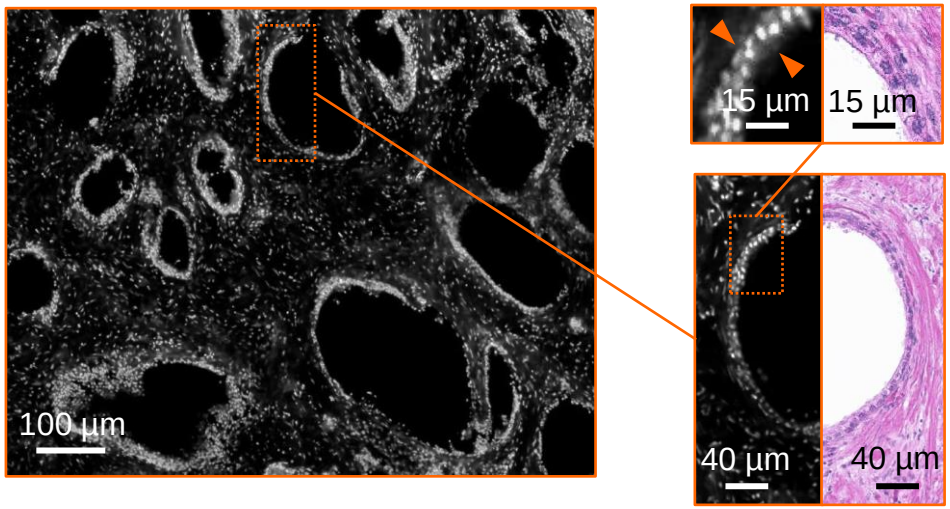
Light-sheet microscopy of prostate tissue



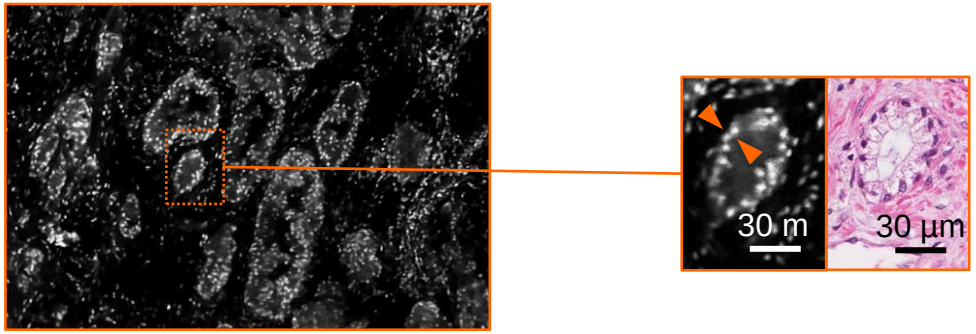
Tissue surface profile (irregularities, <200 µm)



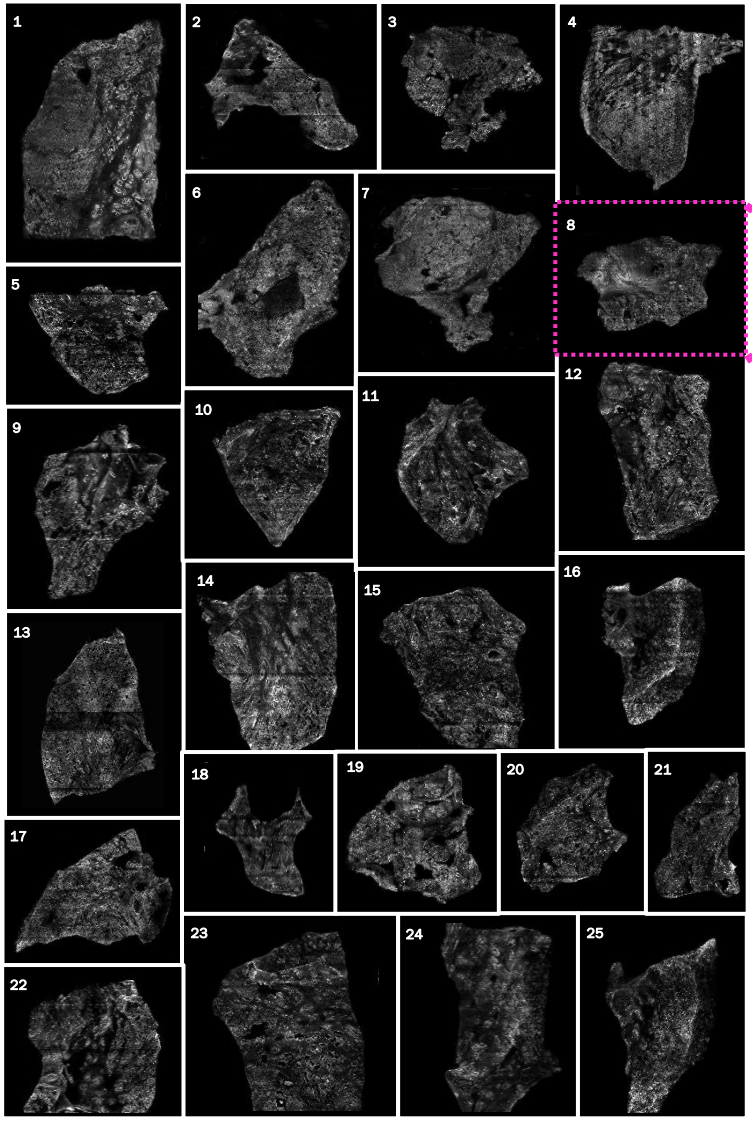
Normal prostate glands



Prostate adenocarcinoma



Clinical correlation study



Fresh prostate slice

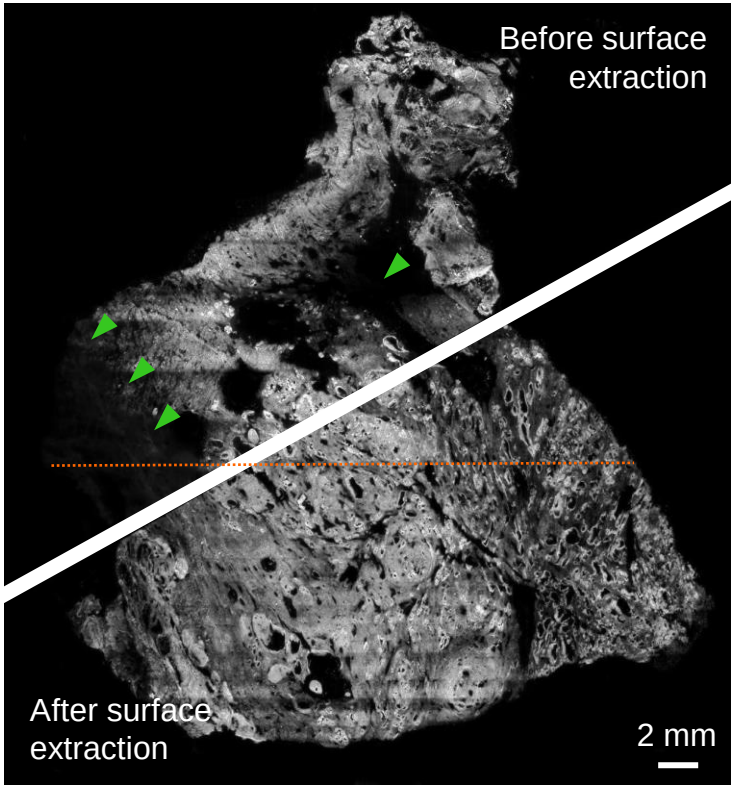
↓

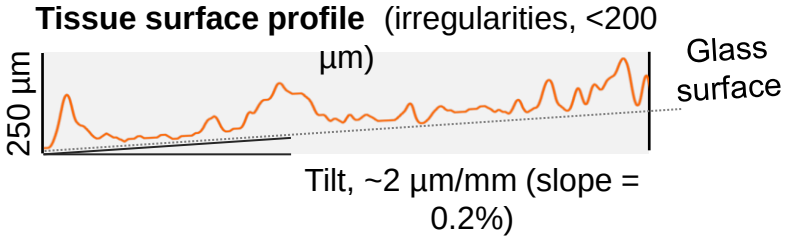
LSM image

↓

FFPE H&E slide

Clinical correlation study





24 tissue samples

- 12 benign
- 12 carcinoma

Sensitivity: 0.92
Specificity: 0.92

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

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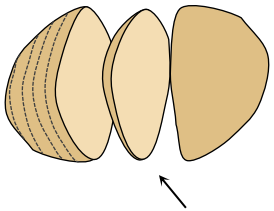
Intra-operative imaging of breast tissue

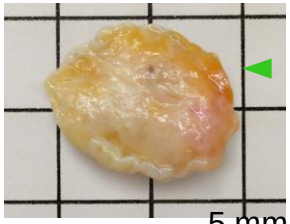
Lumpectomy



Breast slice
(3-5 mm thick)

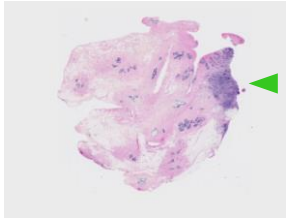
1 cm

Fresh breast tissue



5 mm

H&E



5 mm

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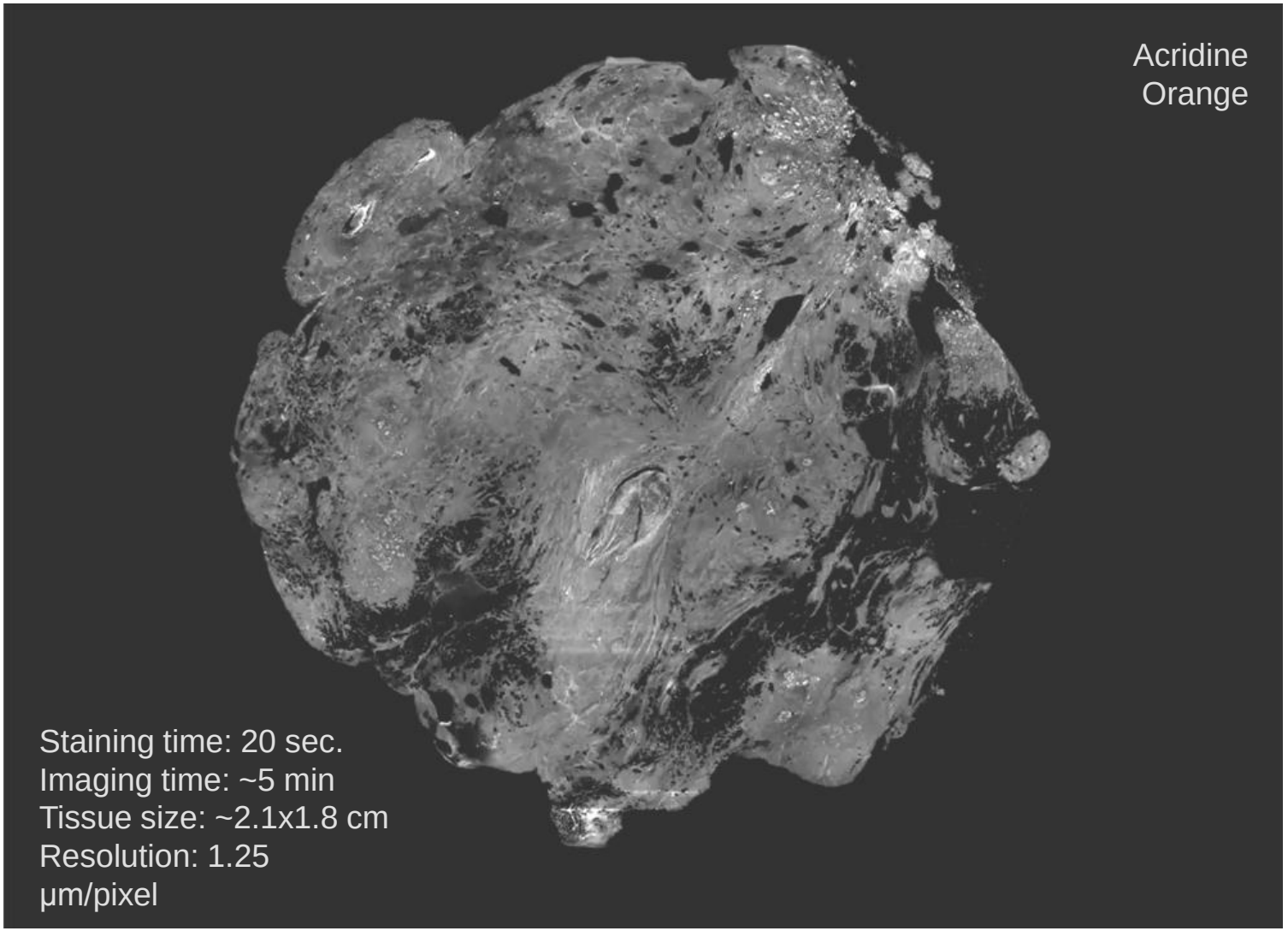
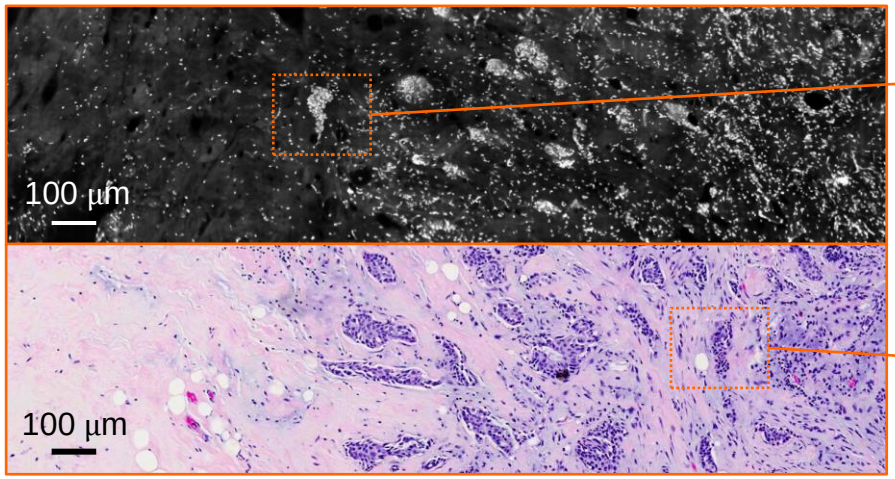
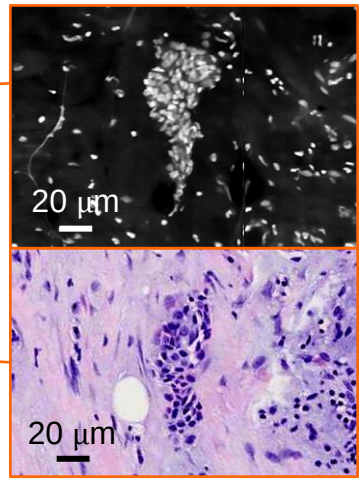
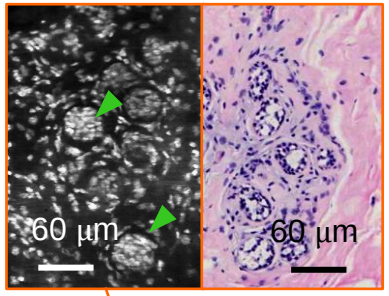


Intra-operative imaging of breast tissue

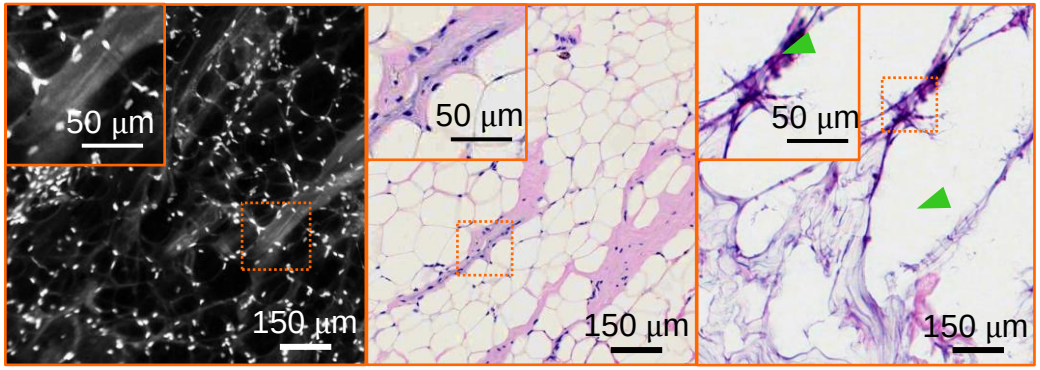
Invasive ductal carcinoma with adjacent normal breast tissue







Adipose tissue

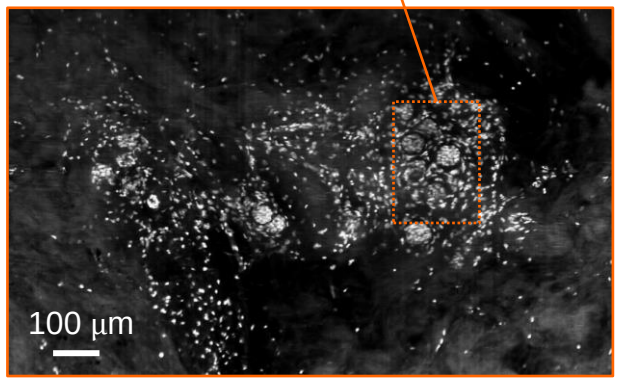


Light-sheet microscopy of fresh breast tissue

Formalin-fixed paraffin- embedded section

Frozen tissue section

Benign breast lobules



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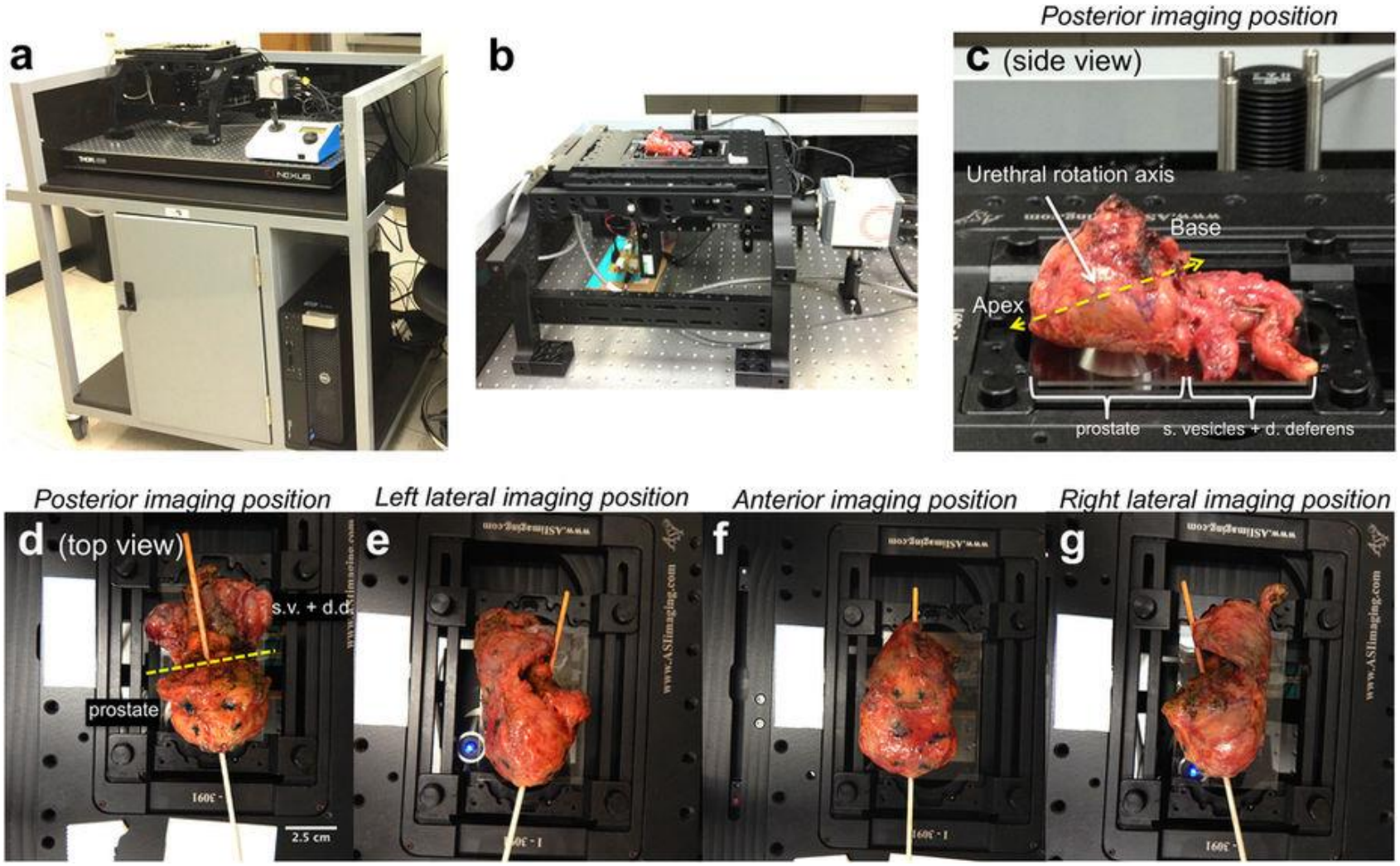
Intra-operative imaging of prostatectomies - SIM



Wang M, Tulman DB, Sholl AB, Kimbrell HZ, Mandava SH, Elfer KN, Luethy S, Maddox MM, Lai W, Lee BR, Brown JQ. Gigapixel surface imaging of radical prostatectomy specimens for comprehensive detection of cancer-positive surgical margins using structured illumination microscopy. *Scientific reports*. 2016;6:27419.

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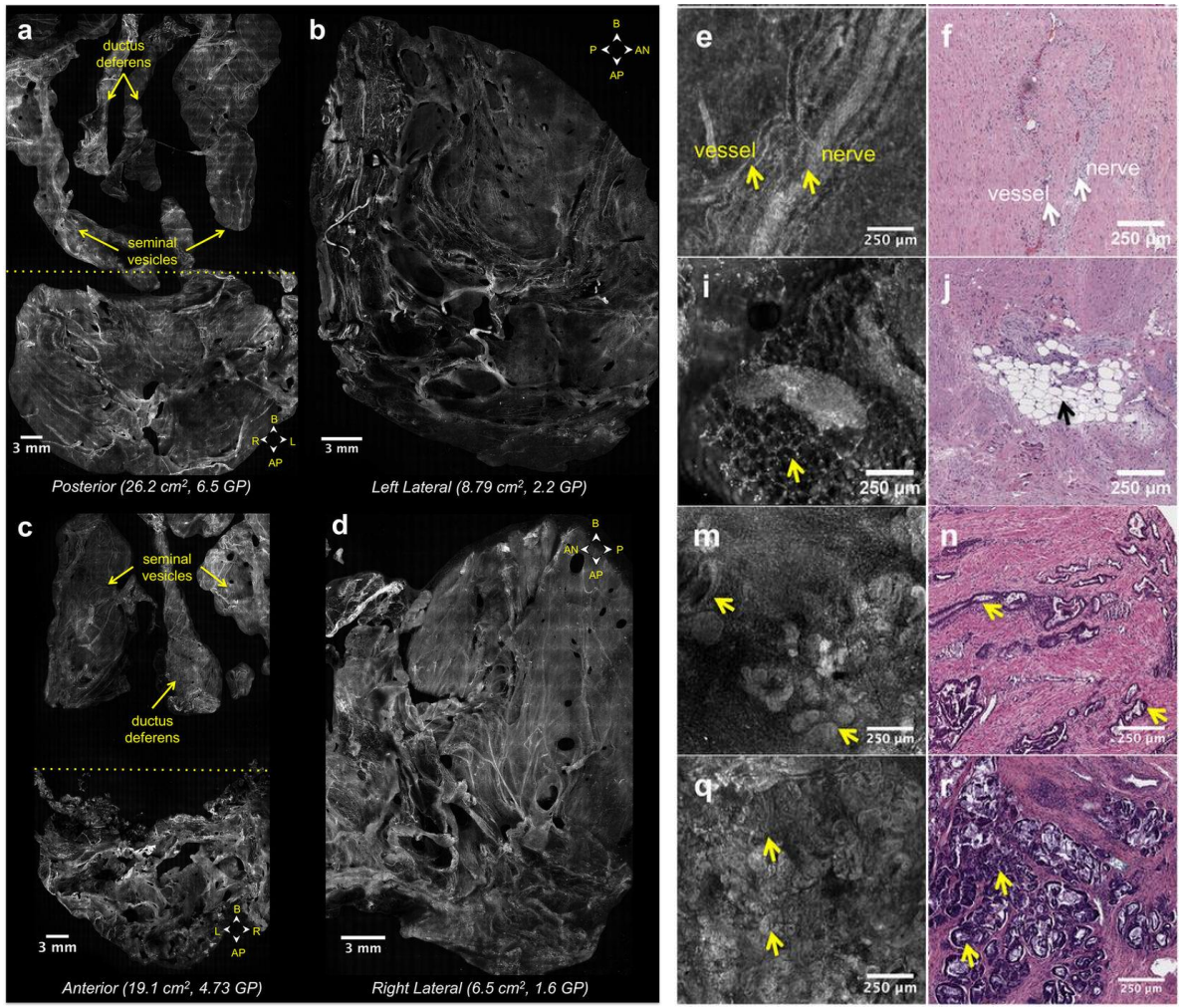
Intra-operative imaging of prostatectomies - SIM

Imaging of entire surface of radical prostatectomy specimen

~60 cm^2, ~15 minutes

Identification of key structures – nerves, adipose, carcinoma, benign glands



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Outline

- **Motivation for slide-free microscopy of fresh tissue**
- **Overview of slide-free microscopy techniques**
- **False-coloring to mimic H&E**
- **Use-cases**
- **Summary**

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Summary

- Slide-free imaging → Efficient workflow
- Non-destructive → Preservation of tissue for molecular testing
- Digital → Use digital pathology tools for quantification, annotation, etc.
- Multiple options (MUSE, SIM, Confocal, MPM, LSM)

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Summary (continued)

- Use-cases
  - Triaging of large surgical specimens
  - Triaging of small biopsies
    - Permanent digital record
    - Entire specimen can be sent fresh for molecular studies
  - Intraoperative imaging
    - Large surface areas
    - Fatty tissue

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Acknowledgements

UW Mech Engin
Dr. Jonathan Liu Lab
Dr. Adam Glaser
Ms. Ye Chen
Dr. Yu “Winston” Wang
Mr. Peter Wei
Mr. Chengbo Yin

UW Pathology
Dr. Nick Reder
Dr. Lawrence True
Ms. Erin McCarty

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

UW CoMotion
Forest Bohrer
Ken Myer
Mike Connolly

UC Davis (MUSE)
Dr. Richard Levenson

NIH / NCI - Pacific Northwest Prostate Cancer SPORE P50CA97186
NIH / NIDCR – R01 DE023497
NIH / NCI – R01 CA175391
Department of Defense Prostate Cancer Research Program
NIH / NCI - PO1 CA163227
UW Royalty Research Fund
ITHS Collaboration Innovation Award
UW CoMotion Innovation Award
Gordon and Betty Moore Foundation - Data Science Environments Project Award
Alfred P. Sloan Foundation Award

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Upcoming Webinars

DATE	TOPIC	SPEAKER(s)
12/5	Role of Reflectance Confocal Microscopy in Skin Inflammations	Babar K. Rao, MD

Register for upcoming & archived webinars:
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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 > Resources and Publications**

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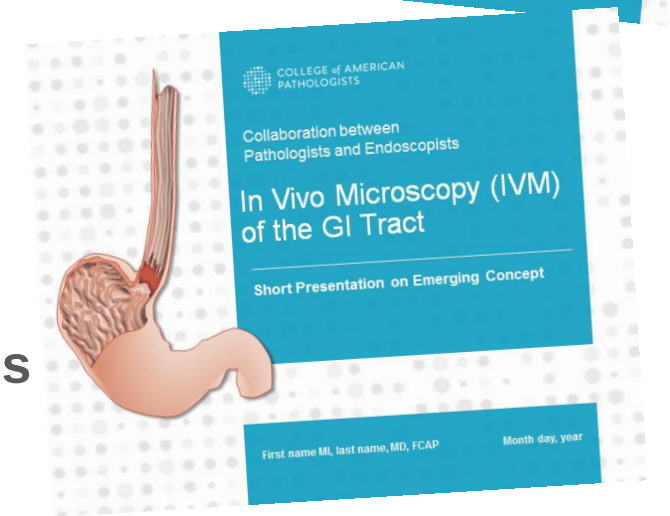
IVM Short Presentations on Emerging Concepts (SPECs) – see handout

- **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 > Resources and Publications



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THANK YOU!

- **Thank you for attending our webinar "Rapid examination of fresh tissue using light-sheet microscopy" by Nicholas P. Reder, MD, MPH.**
  - **For comments about this webinar or suggestions for upcoming webinars, contact 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.**

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