COLLEGE of AMERICAN PATHOLOGISTS

Light-sheet microscopy for 3D pathology

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10/3/2017

Housekeeping

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- **Please ask your questions when you think of them via the “Question box” in your control panel**

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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.**

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Lawrence D. True, MD, FCAP

- **Earned a B.A. from Harvard**
- **Earned MD 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**

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Disclosure

- **Dr. True and Dr. Reder hold a patent and have a start-up company (Alpenglow Optics, LLC) related to their light-sheet microscopy work.**

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Learning Objectives

- **Understand the motivation for 3D pathology**
- **Increase awareness that 3D pathology is possible for the practicing pathologist!**
- **Describe use-cases for 3D pathology**

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Outline

- **Review of light-sheet microscopy** - Dr. Nicholas Reder
- **Motivation for 3D pathology** - Dr. Lawrence True
- **Tissue clarification techniques** - Dr. Nicholas Reder
- **Results** - Dr. Nicholas Reder
- **Summary** - Dr. Lawrence True

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Motivation: pathology has remained unchanged for a century

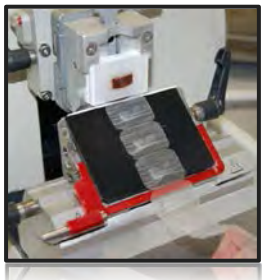
Rapid histology: 4 hours

1. Fix
~30 minutes

2. Process
~2.5 hours

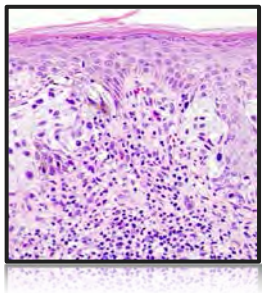
3. Embed
~10 minutes

4. Section
~10 minutes



5. Stain
~40 minutes

Histology



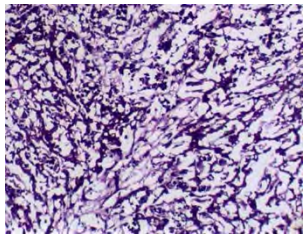
Frozen Section: 10 minutes

1. Freeze
~2 minutes

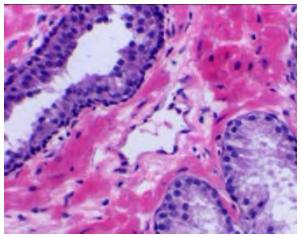
2. Section
~2 minutes

3. Stain
~4 minutes

Histology



or



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Motivation: pathology has remained unchanged for a century

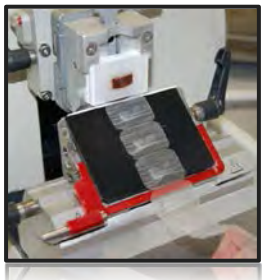
Rapid histology: 4 hours

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

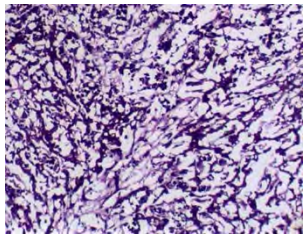
1. Freeze
~2 minutes

2. Section

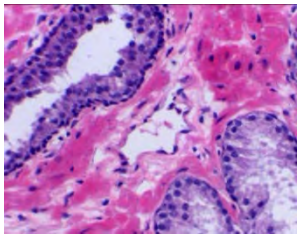
Disadvantages

Destructive
Hazardous
Sampling errors
Variable histology quality

Histology



or



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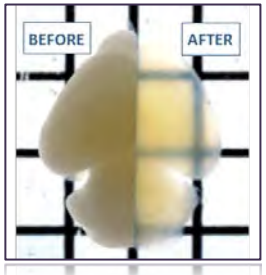
Goal: non-destructive, slide-free, 3D ‘digital’ pathology

***0. Clear**

~5 minutes to overnight



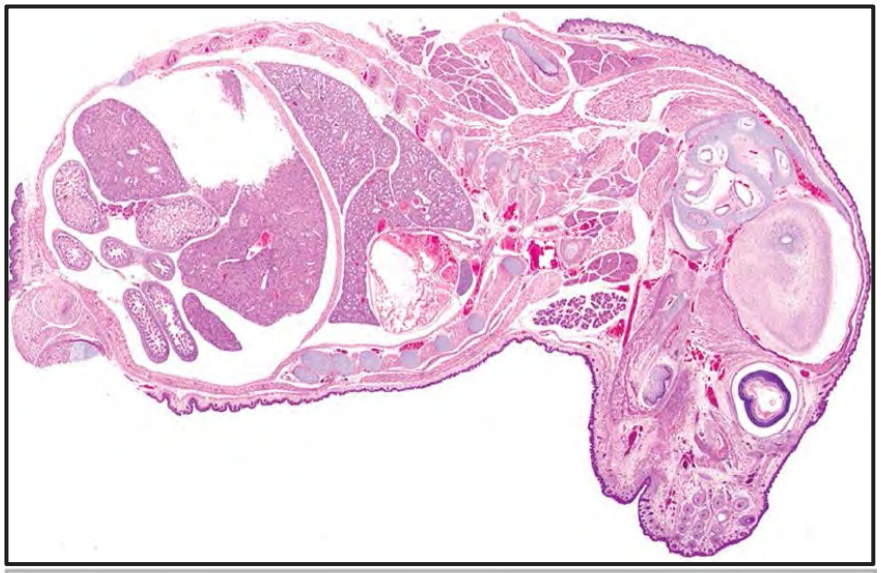
1. Stain

<1 minute

2. Image

<10 minutes

Wide-area ‘digital’ histology



Advantages

Fast
Digital
Non-destructive
Slide-free
Wide-area

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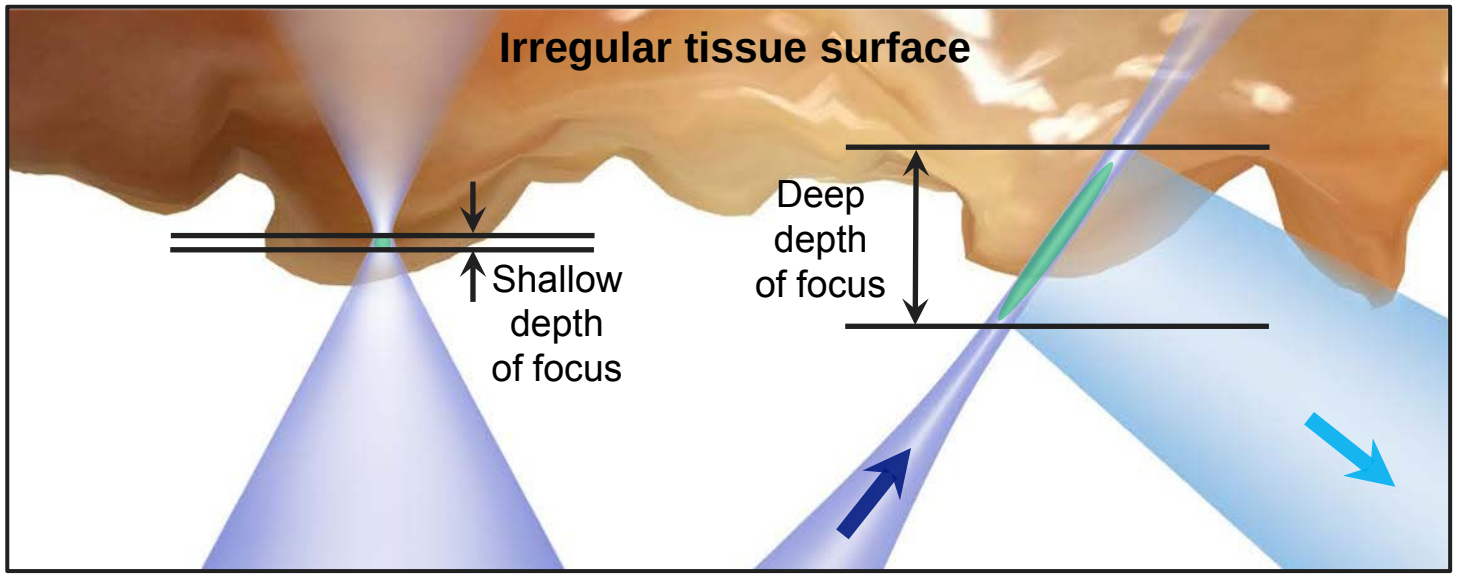
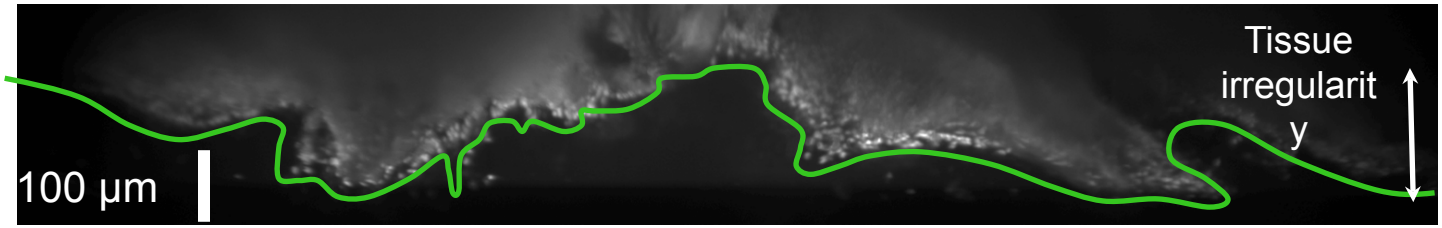
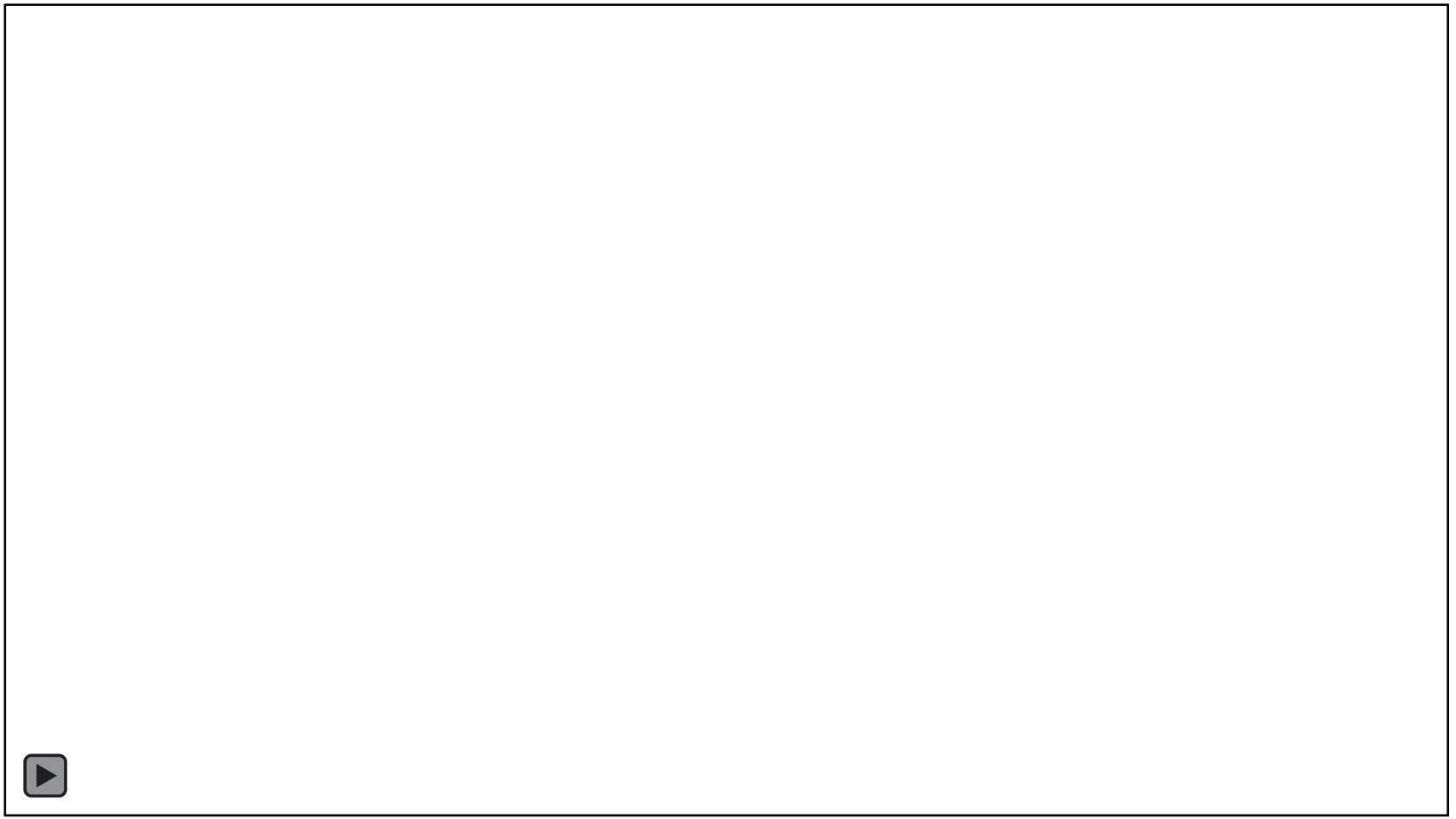
Advantages of LSM for imaging human tissues





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

Light-sheet microscope system demonstration



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Motivation for 3D pathology

- **To more extensively sample a surgical margin**
  - **Radical prostatectomy**
  - **Mastectomy**
  - **Thyroid, follicular lesions (NIFTY)**
  - **Partial nephrectomy**
  - **Ischemic bowel**
- **To more accurately evaluate the structure of cancers**
  - **Gleason score 6 vs. 7 (3+4)**
  - **Satellite lesions of melanoma**
- **To more accurately determine spatial relationships**
  - **Prostate: Is there extraprostatic extension of cancer?**
    How many cancers are there in a prostatectomy?

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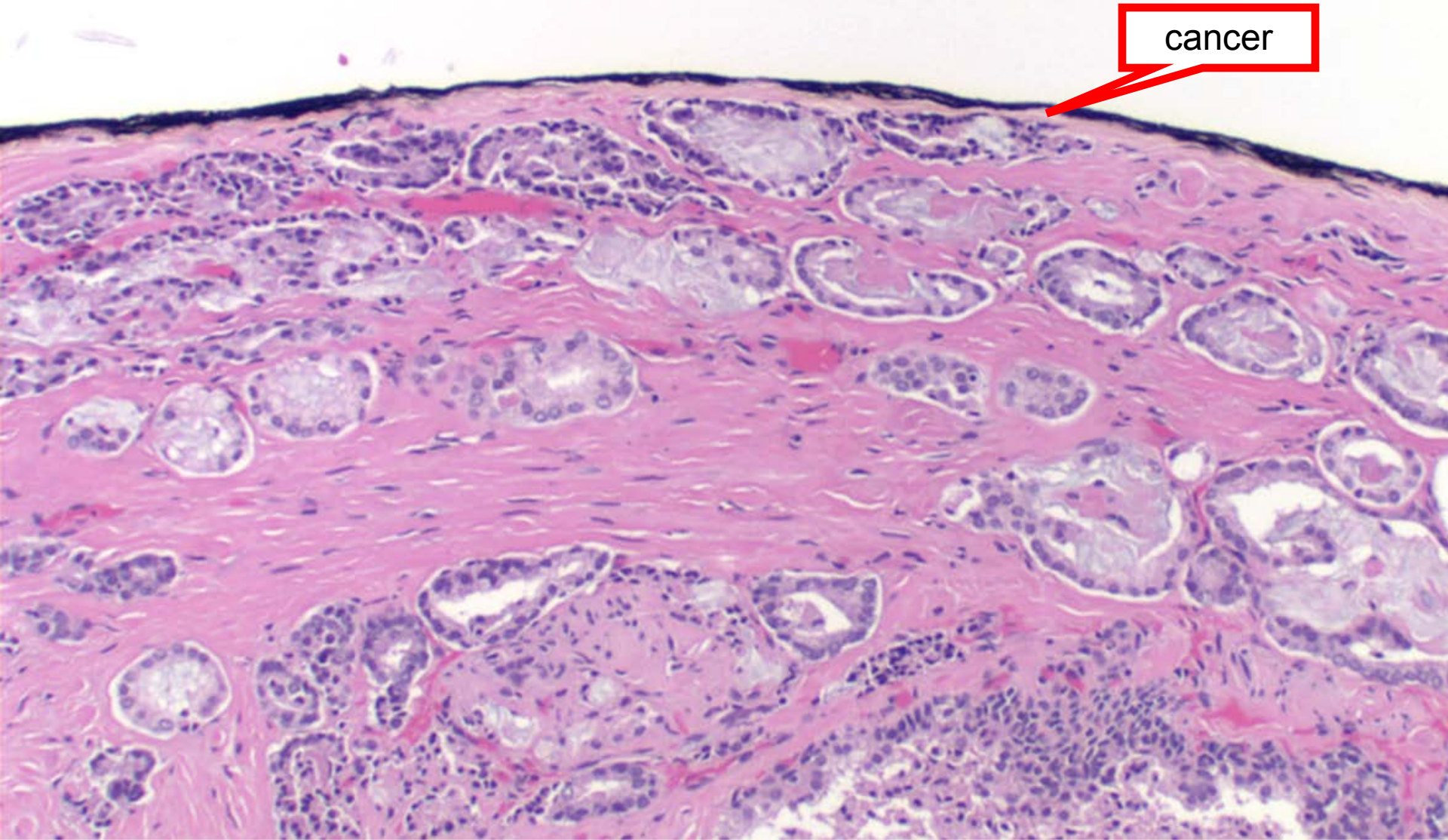
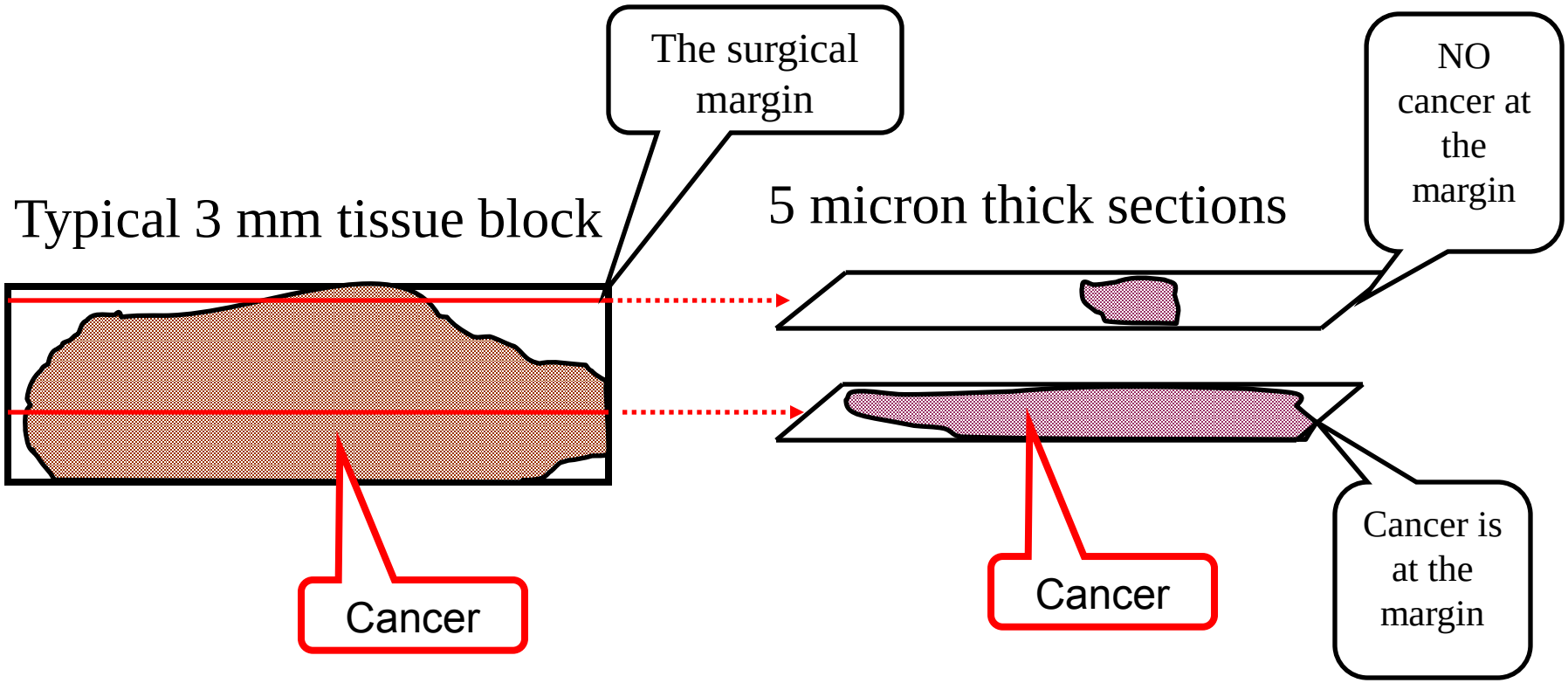
Prostatectomy: Negative margin

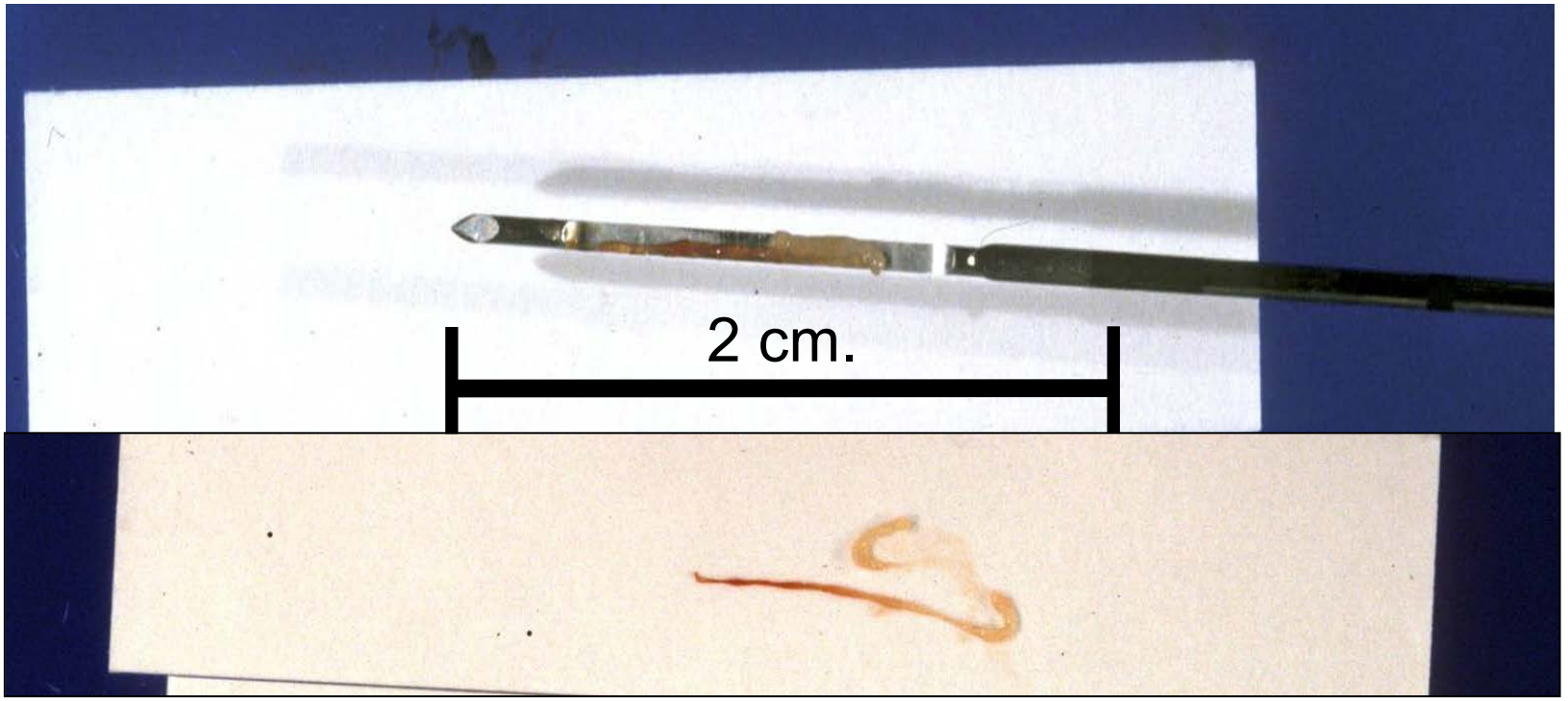
Is cancer at the margin deeper in the block of tissue?



Is cancer at a margin deeper in the block of tissue?



Example prostate core-needle biopsy



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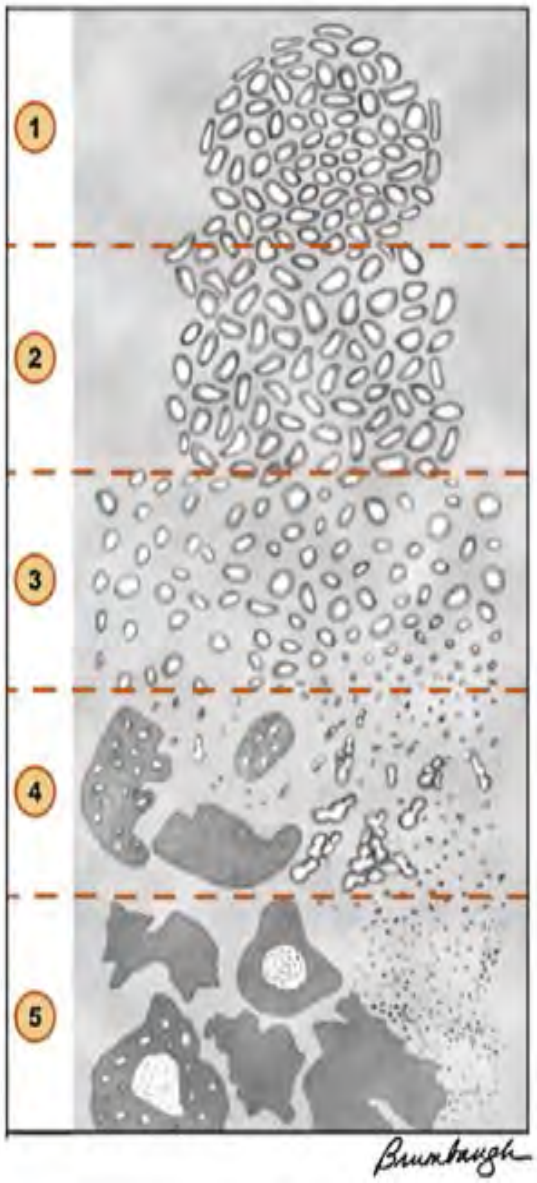
Gleason grading system



Higher grade associated with worse prognosis

Gleason grade = primary pattern + secondary pattern

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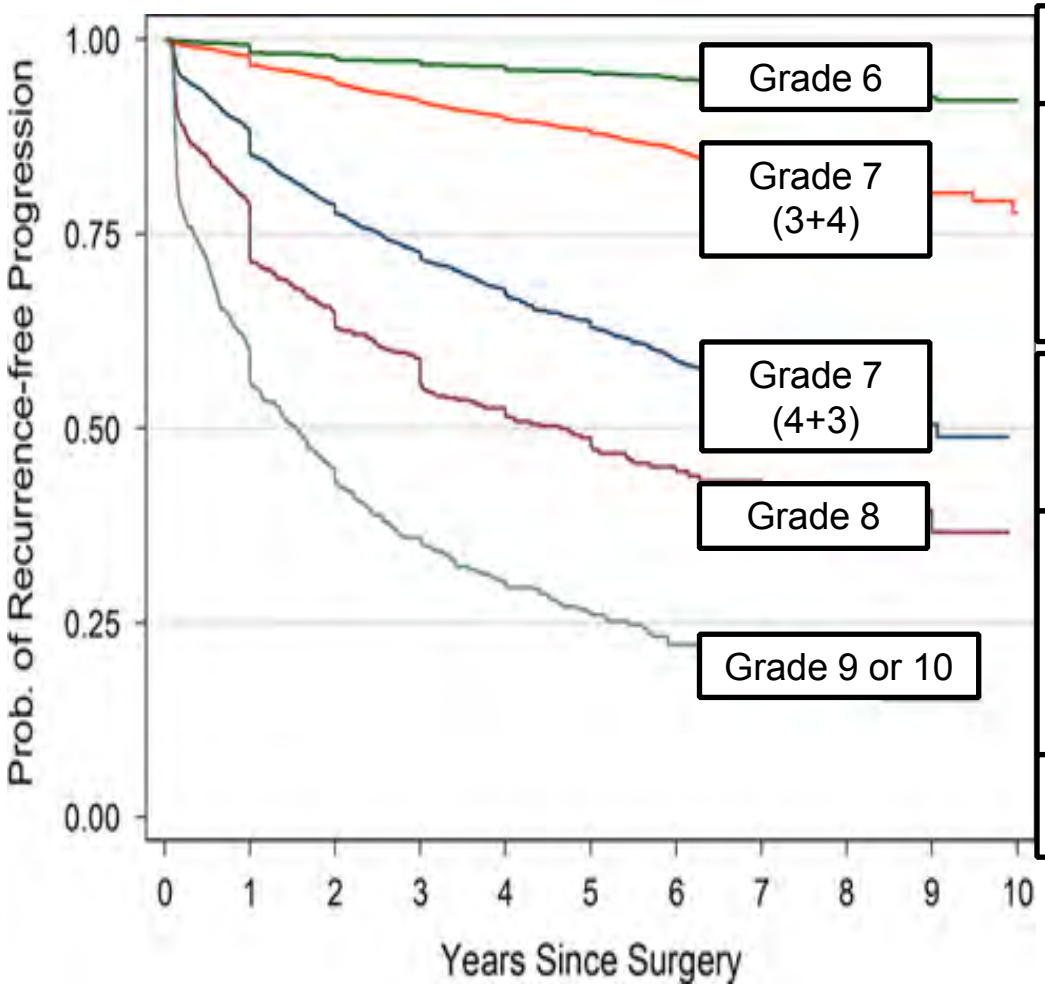
Gleason score is used clinically



Active surveillance
Either active surveillance or Intent-to-Cure Therapy
Intent-to-Cure Therapy
Intent-to-Cure Therapy ± Neoadjuvant Therapy
Neoadjuvant Therapy

Active surveillance: Periodic monitoring and biopsies
Intent-to-Cure therapy: Surgery vs. Radiation (Seed implants/brachytherapy or External beam radiation therapy)
Neoadjuvant therapy: Chemotherapy before Intent-to-Cure therapy

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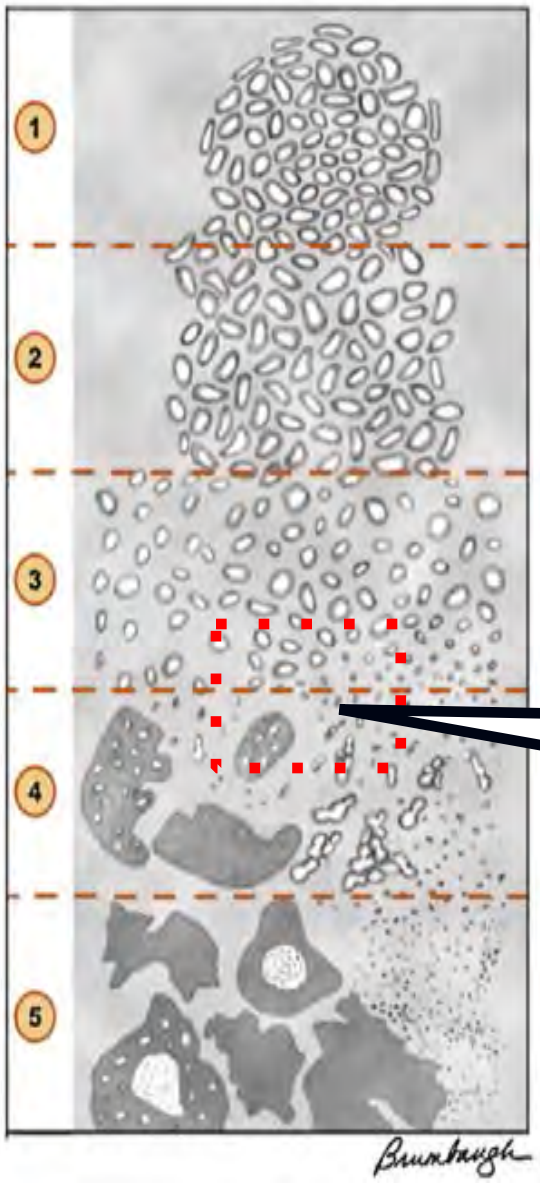
Difficulties in Gleason grading



A source of inter-pathologist variance in grading

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

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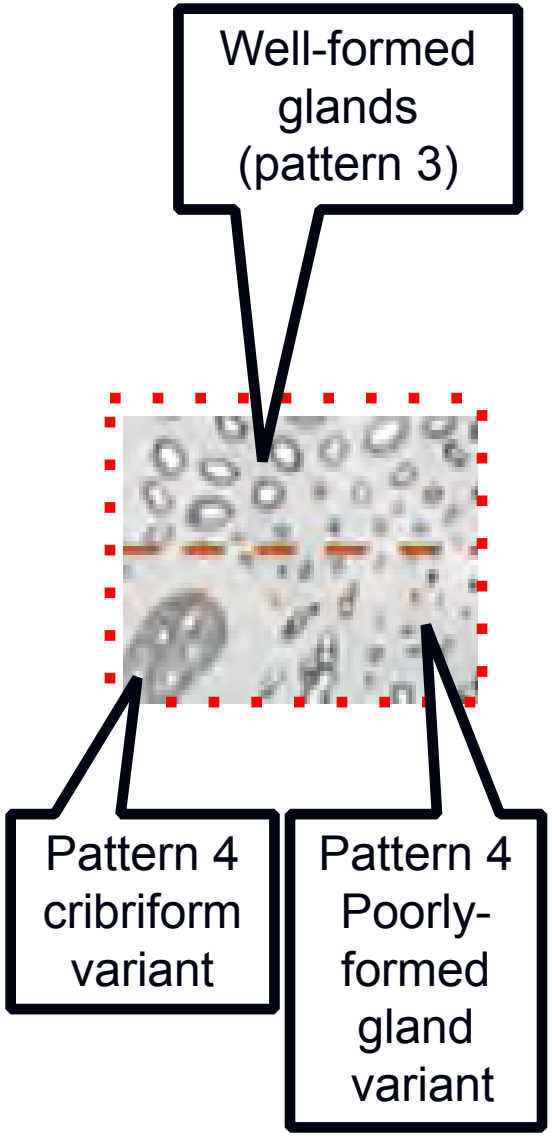
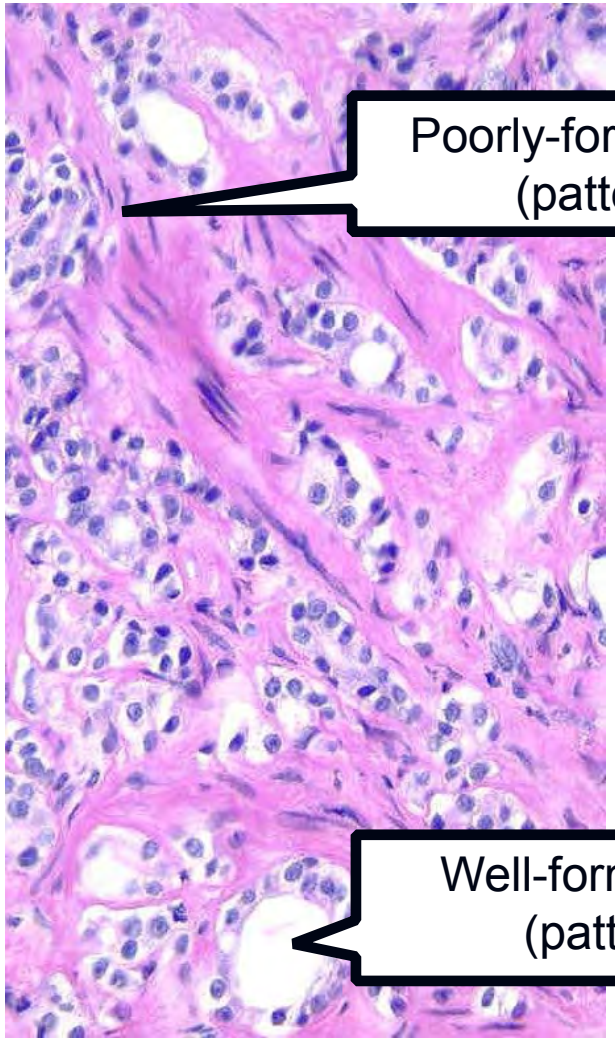
Difficulties in Gleason grading





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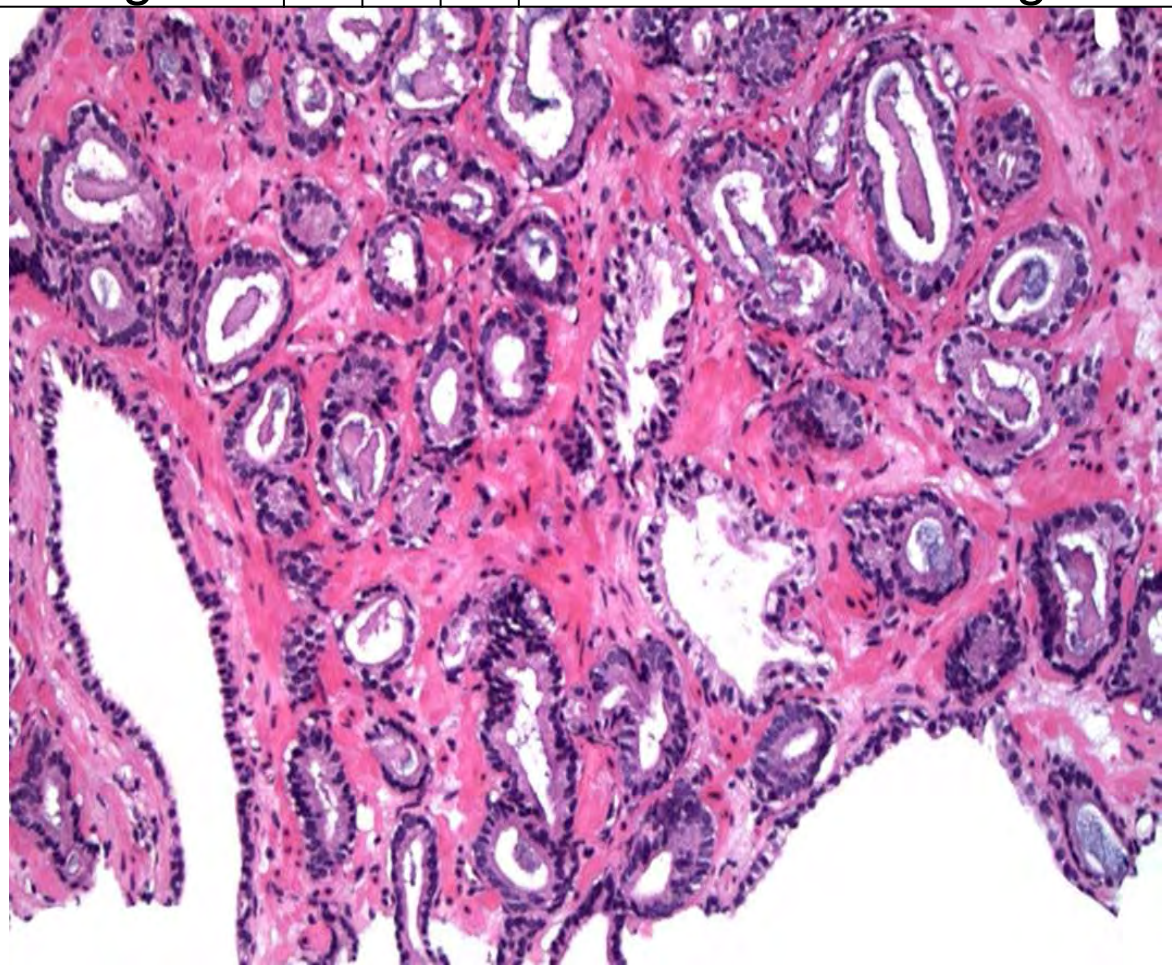
Consensus: Gleason pattern 3

Gleason score	**6**	**7**	**>7**	***comment***
# of pathologists	7			Well formed cancer glands



No consensus: Gleason pattern 3 versus 4

Gleason score	6	7	>7	*comment*
# of pathologists	4	3		Poorly formed glands vs. tangential sections of well-formed glands????

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

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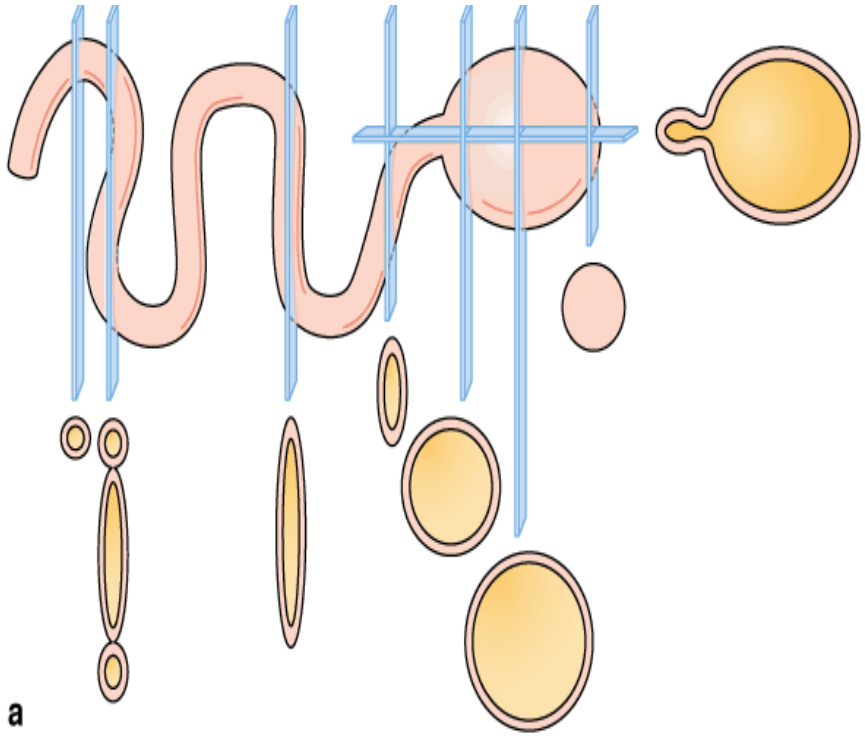
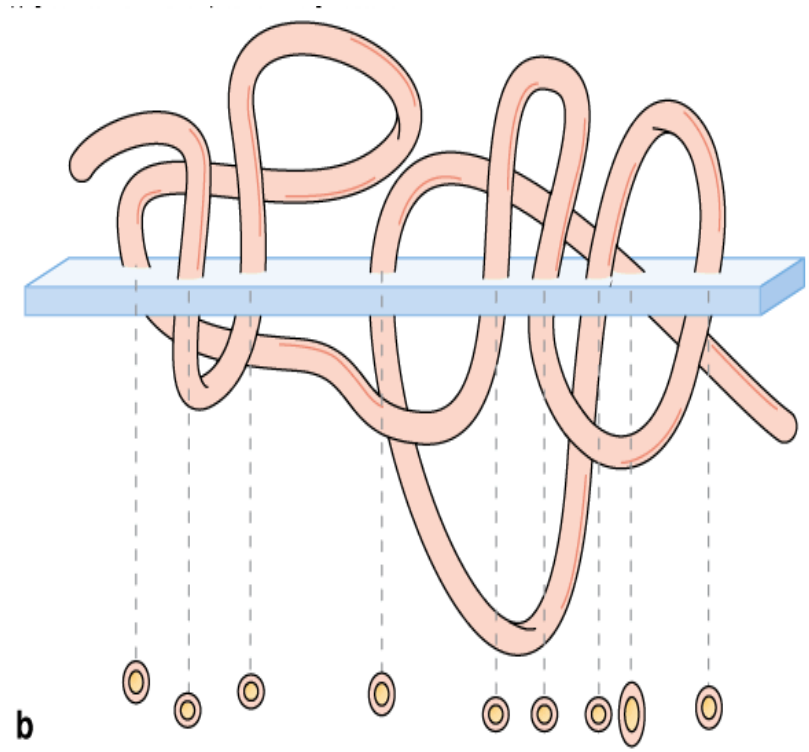
Theoretical model of tangentially sections glands





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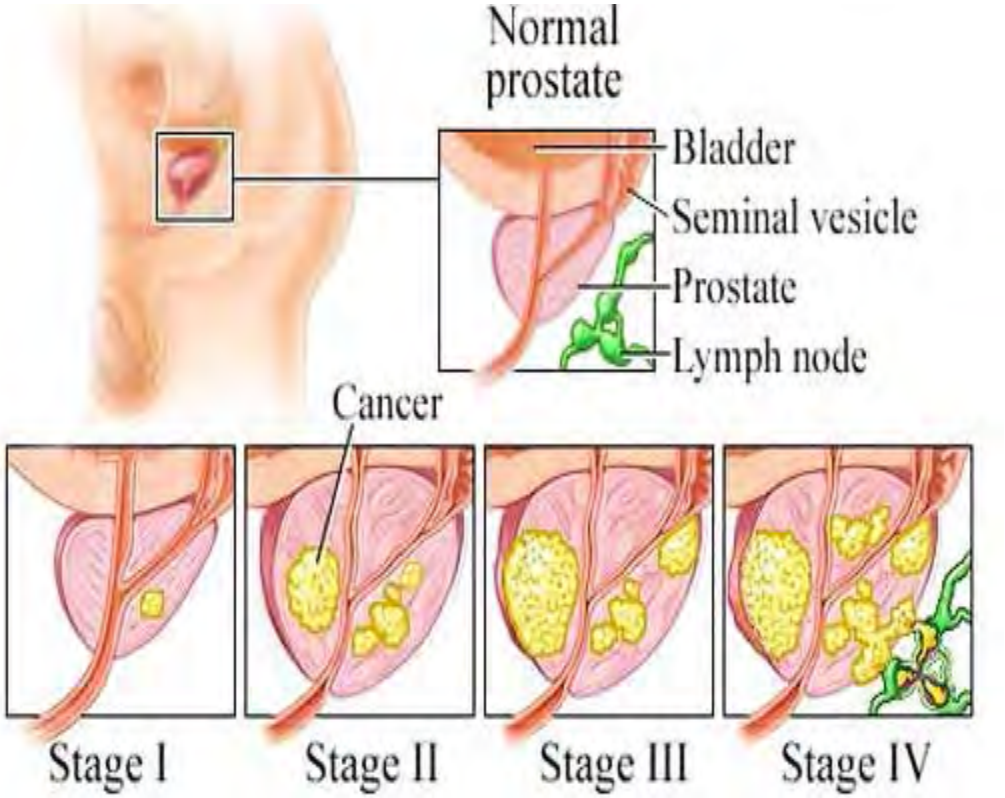
Sampling and relationships



Important tumor parameters affected by sampling

- Size
- Grade
- Extent
- Margins

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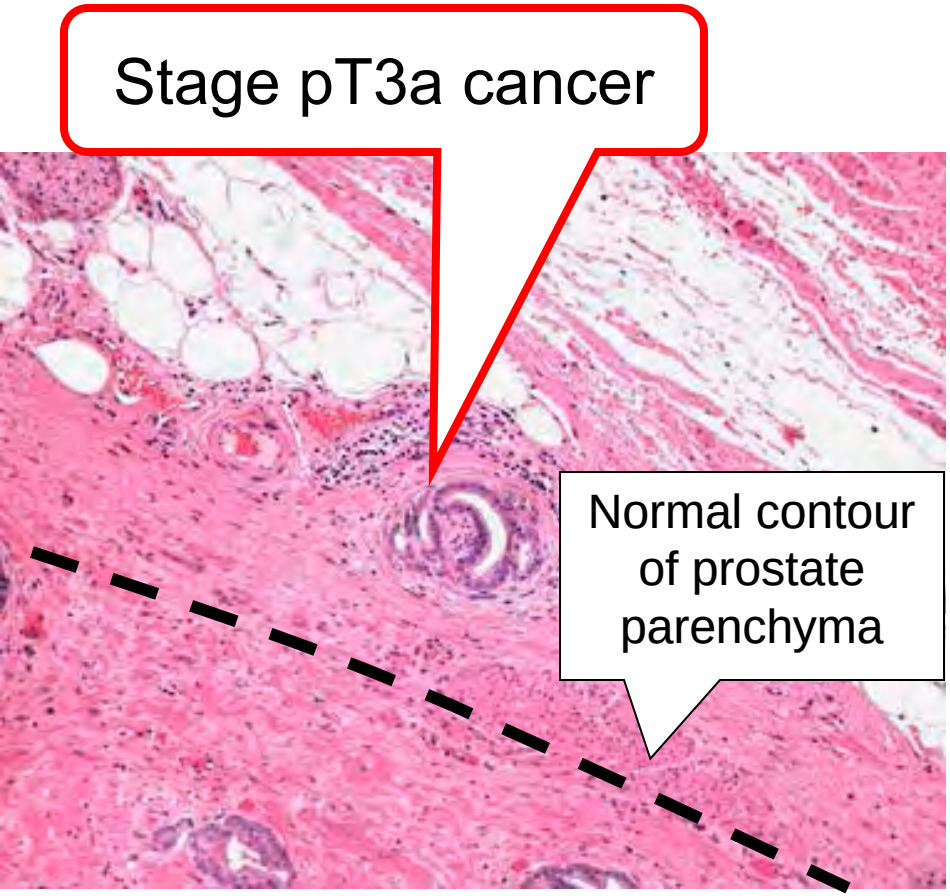
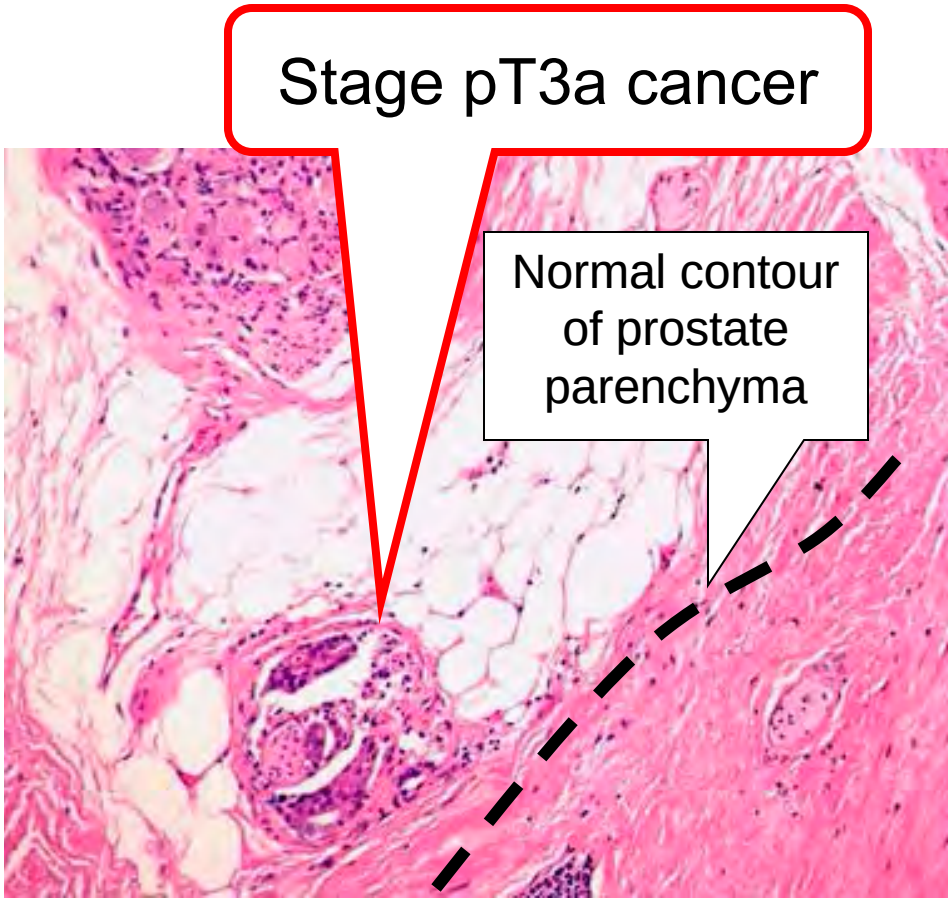
Extraprostatic extension (focal)





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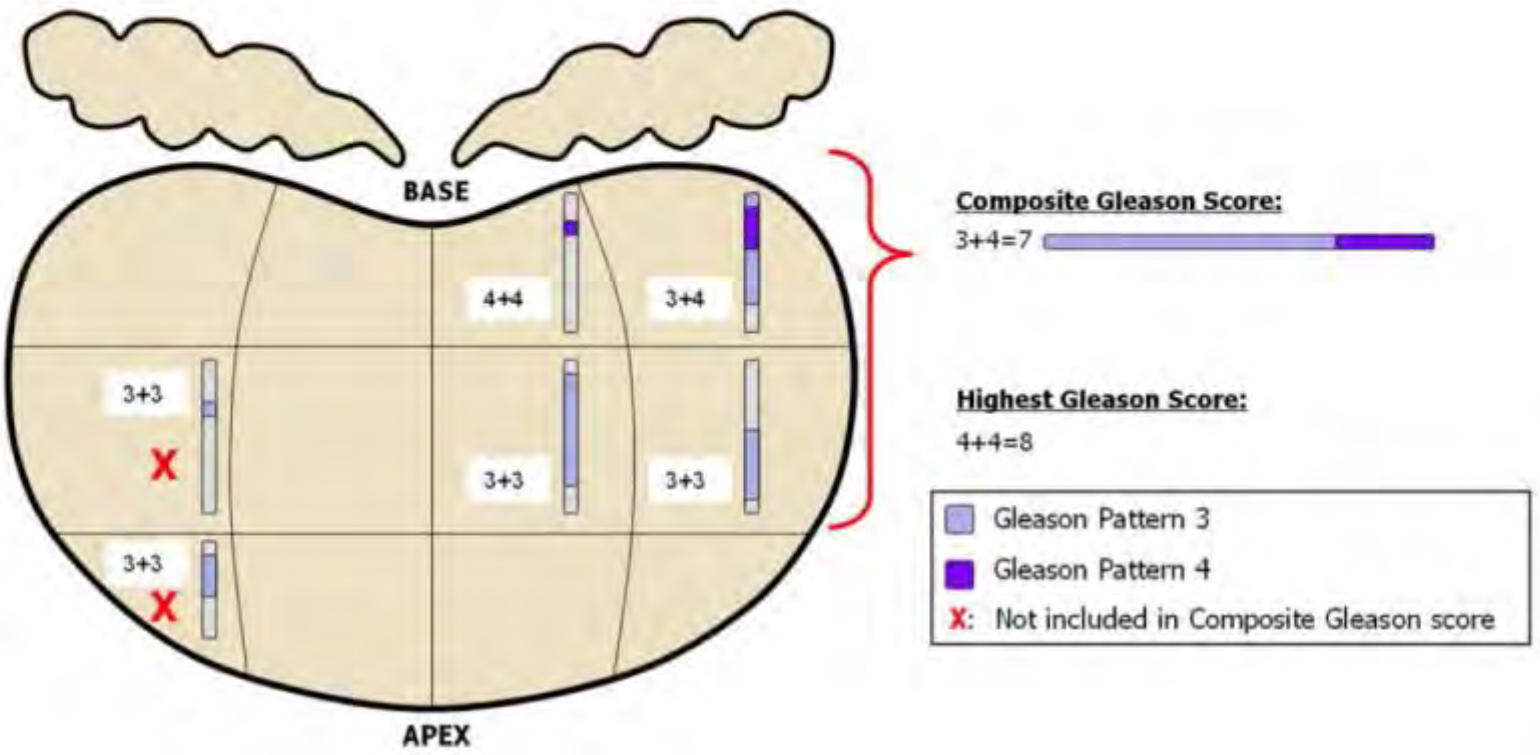
Spatial Relationships: Prostatectomy

Are these 2 separate cancers?



Am J Surg Path 2015 Sep;39(9):1213-8 PMID: 26274028

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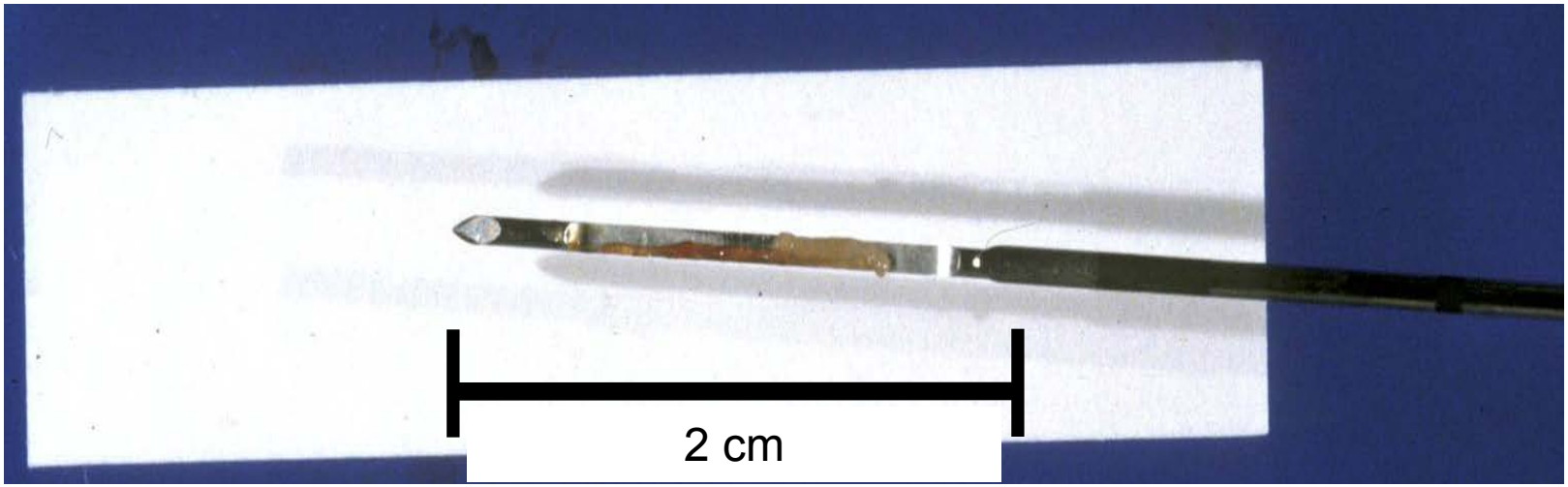
Prostate core needle biopsy (fresh and opaque)



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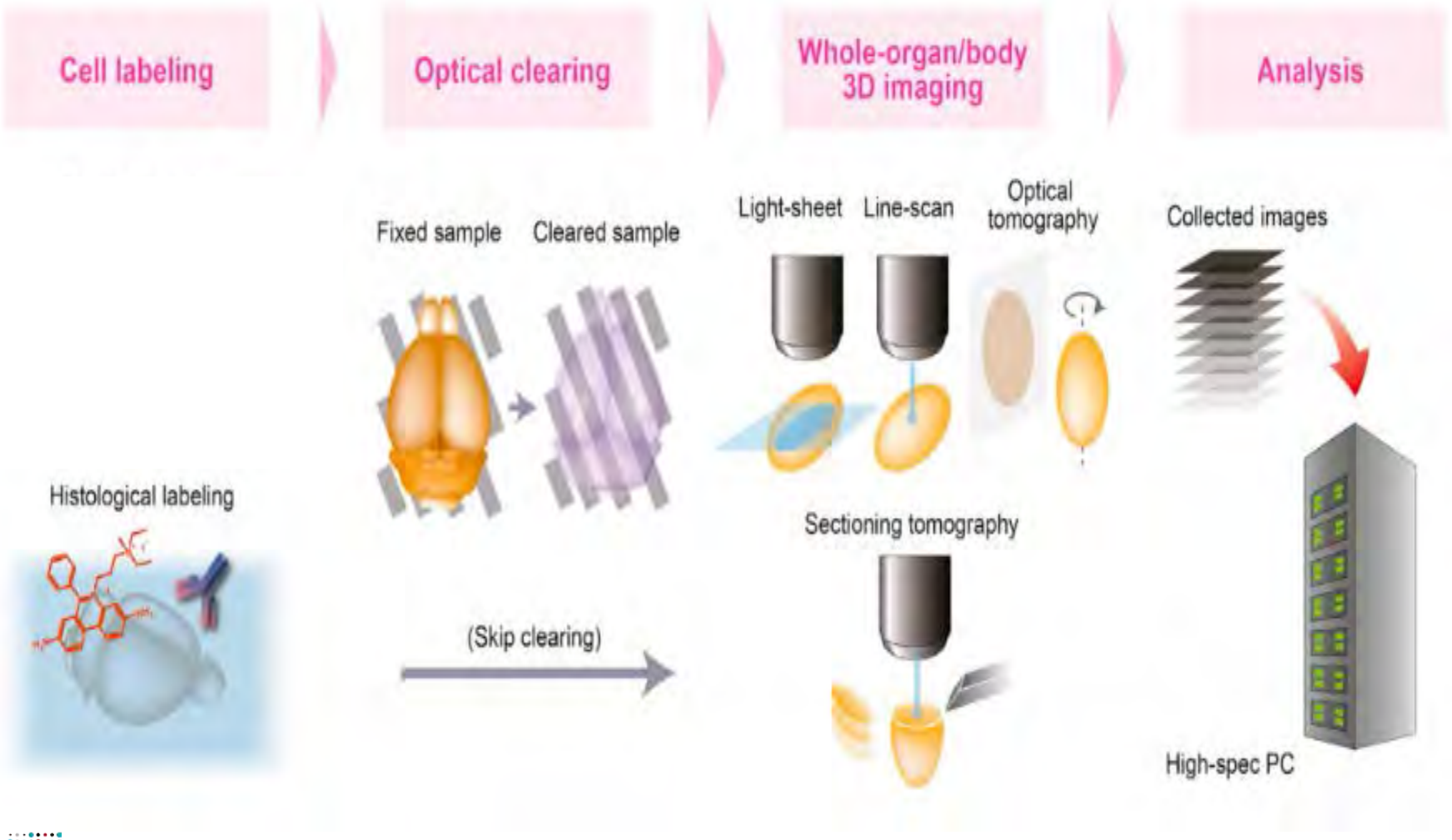
3D imaging workflow



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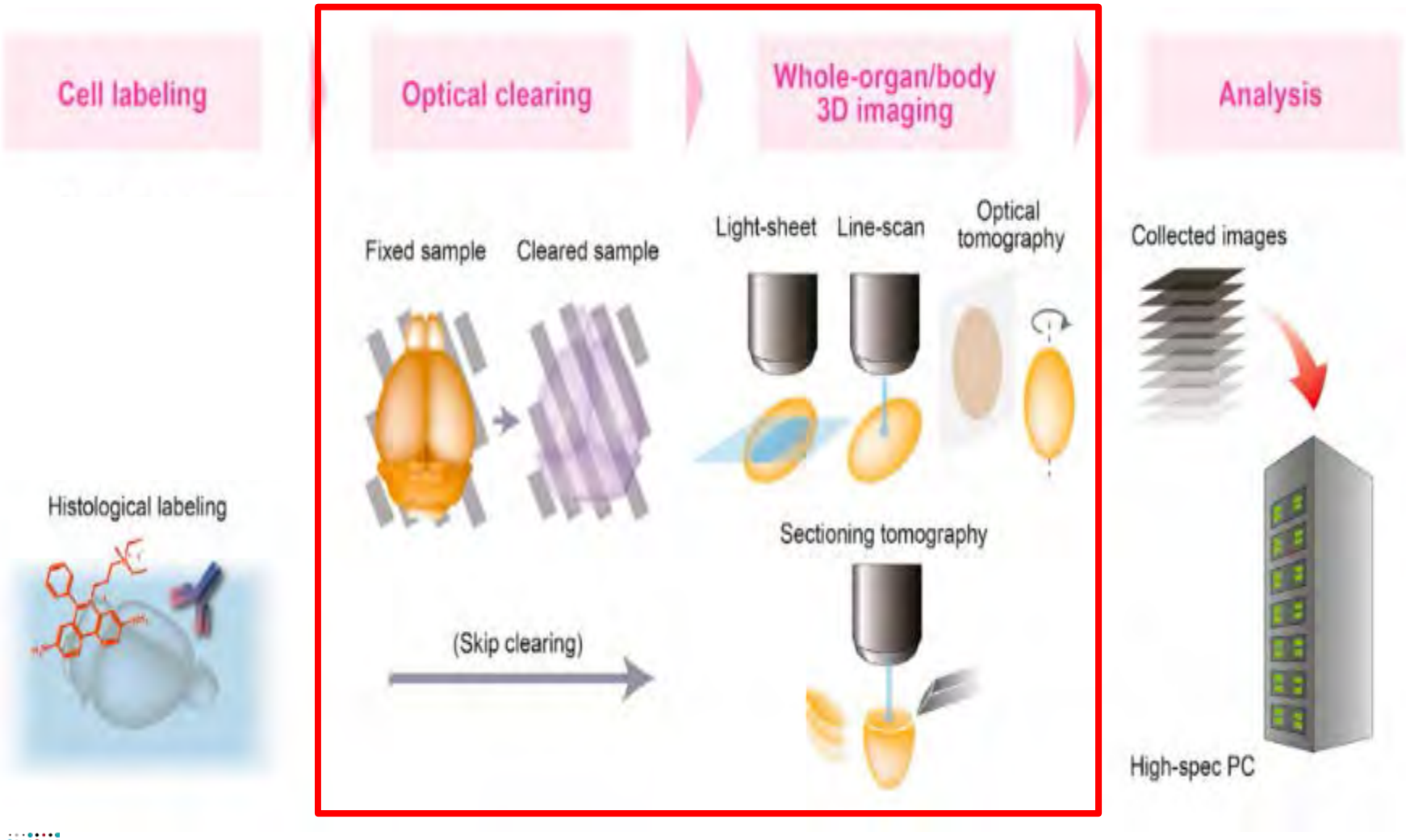
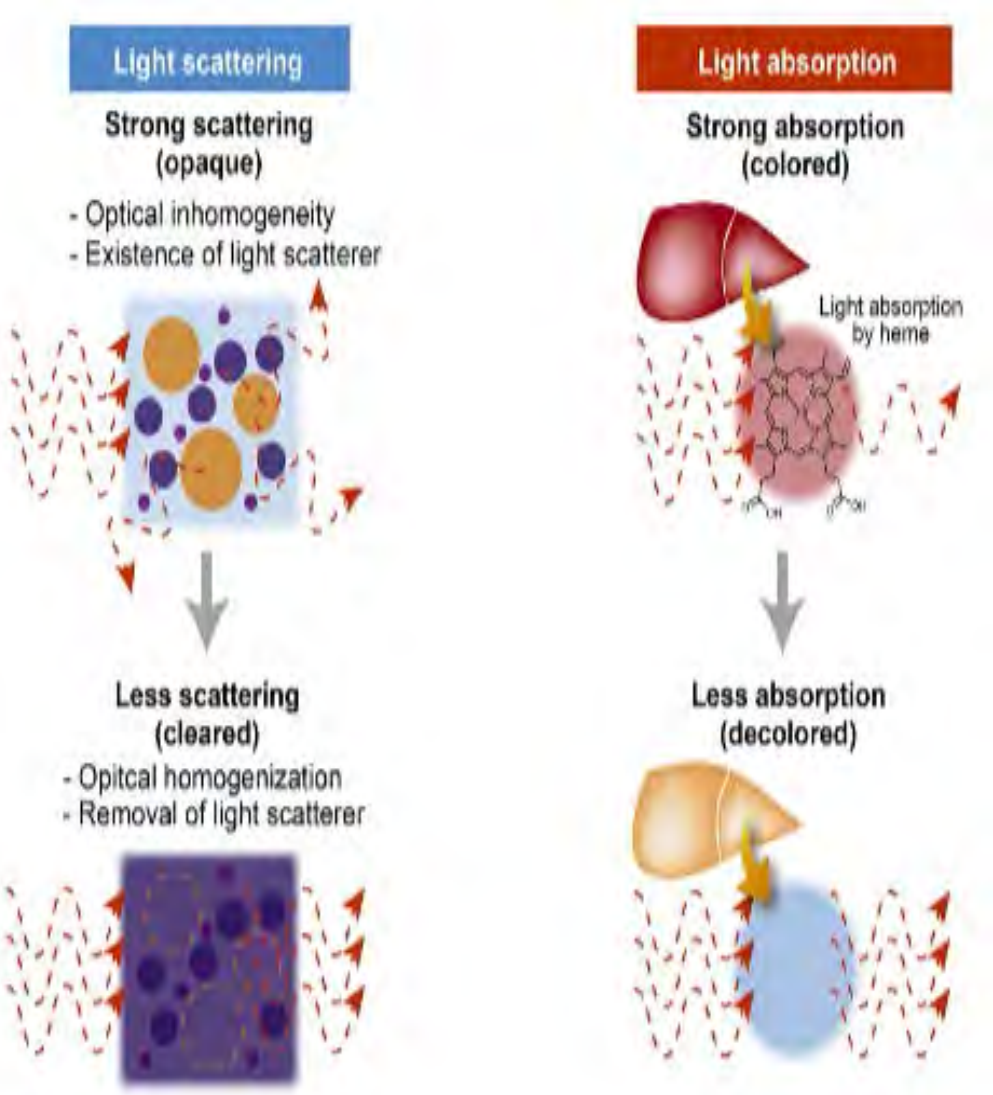
Susaki EA, Ueda HR. Whole-body and whole-organ clearing and imaging techniques with single-cell resolution: toward organism-level systems biology in mammals. *Cell Chem Biol*. 2016;23(1):137-57.

3D imaging workflow



Susaki EA, Ueda HR. Whole-body and whole-organ clearing and imaging techniques with single-cell resolution: toward organism-level systems biology in mammals. *Cell Chem Biol*. 2016;23(1):137-57.

Tissue clearing



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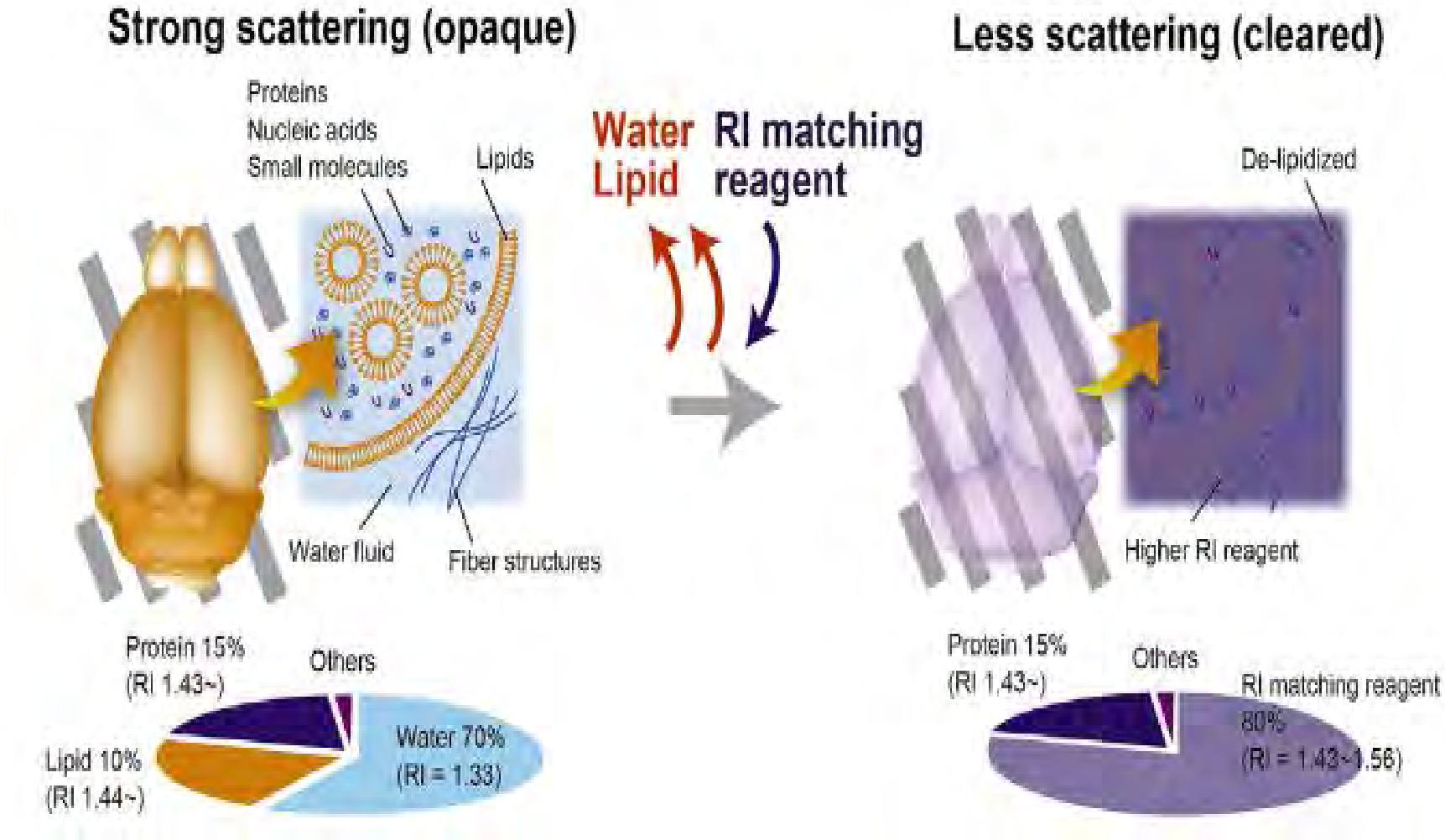
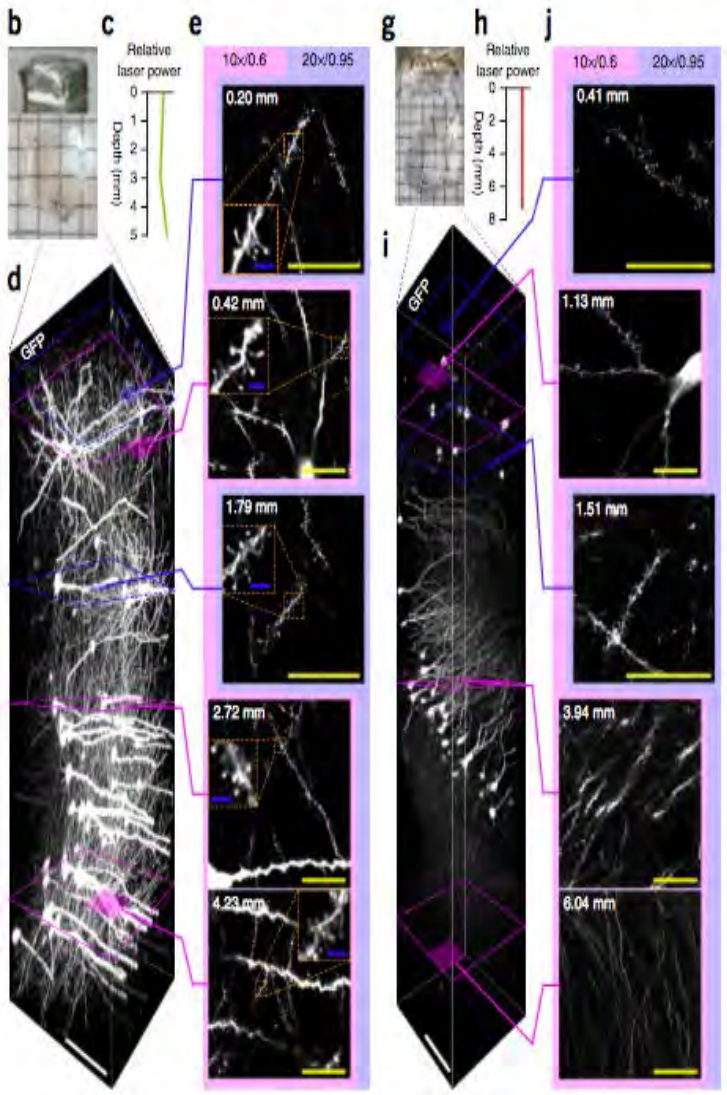
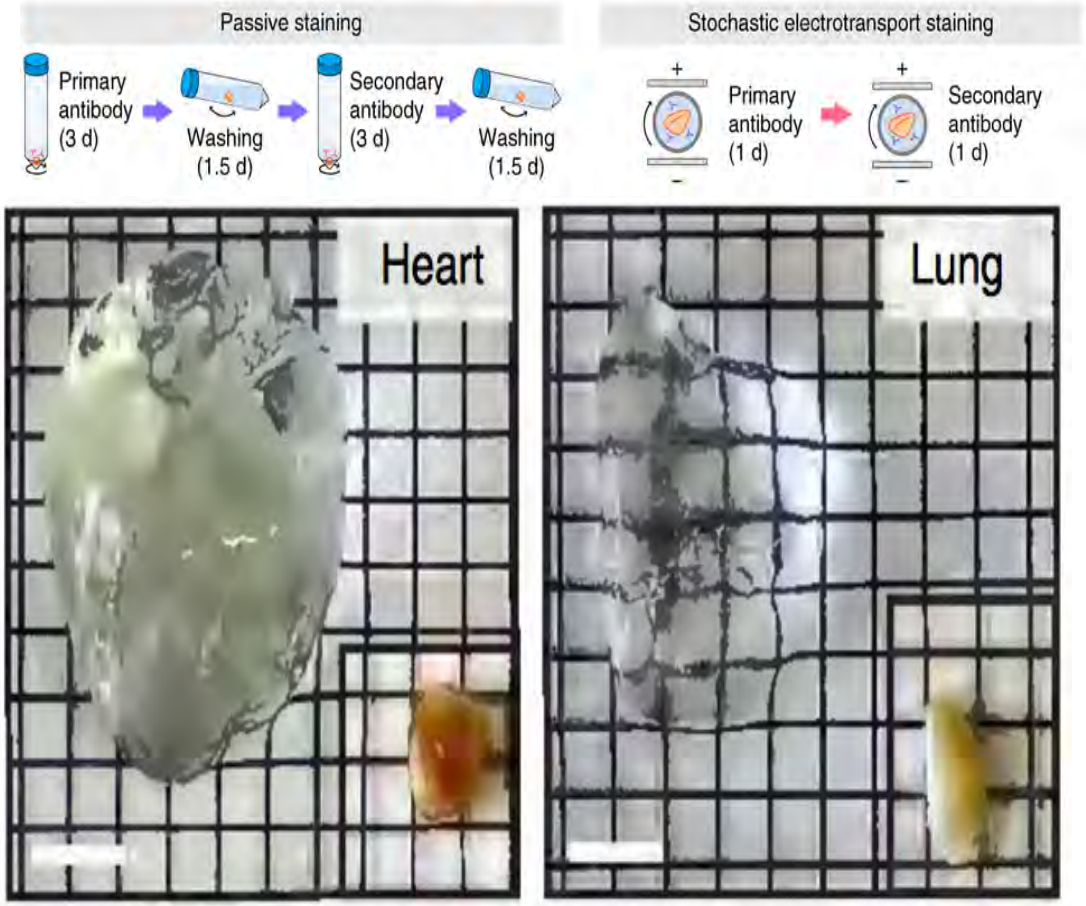
Tissue clearing



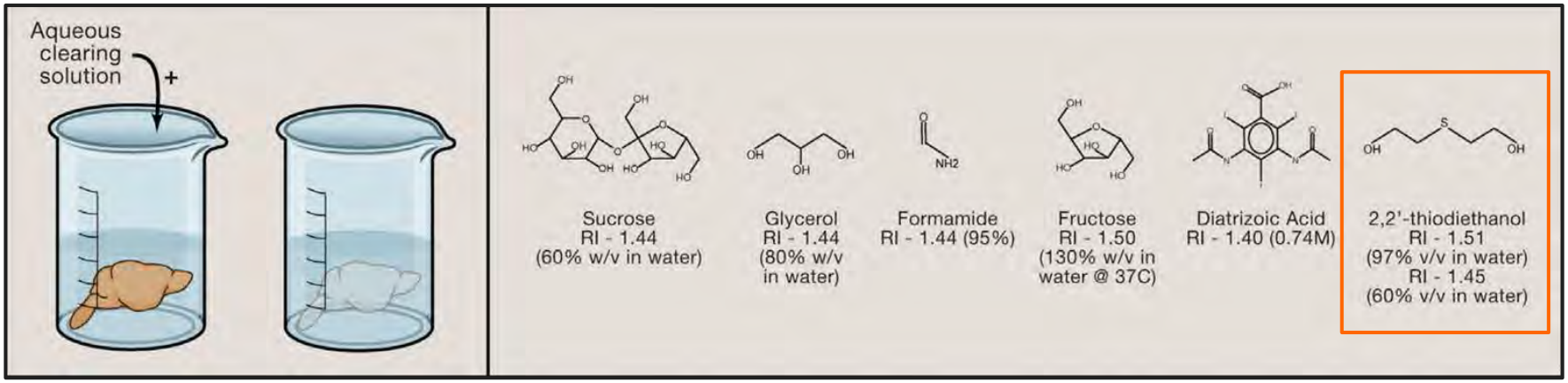
Elaborate tissue clearing protocol





Ku T, et al. Multiplexed and scalable super-resolution imaging of three-dimensional protein localization in size-adjustable tissues. *Nat Biotechnol*. 2016;34(9):973-81

Rapid tissue clarification for 3D pathology



TDE

For core needle biopsies, clearing achieved in ~15 minutes

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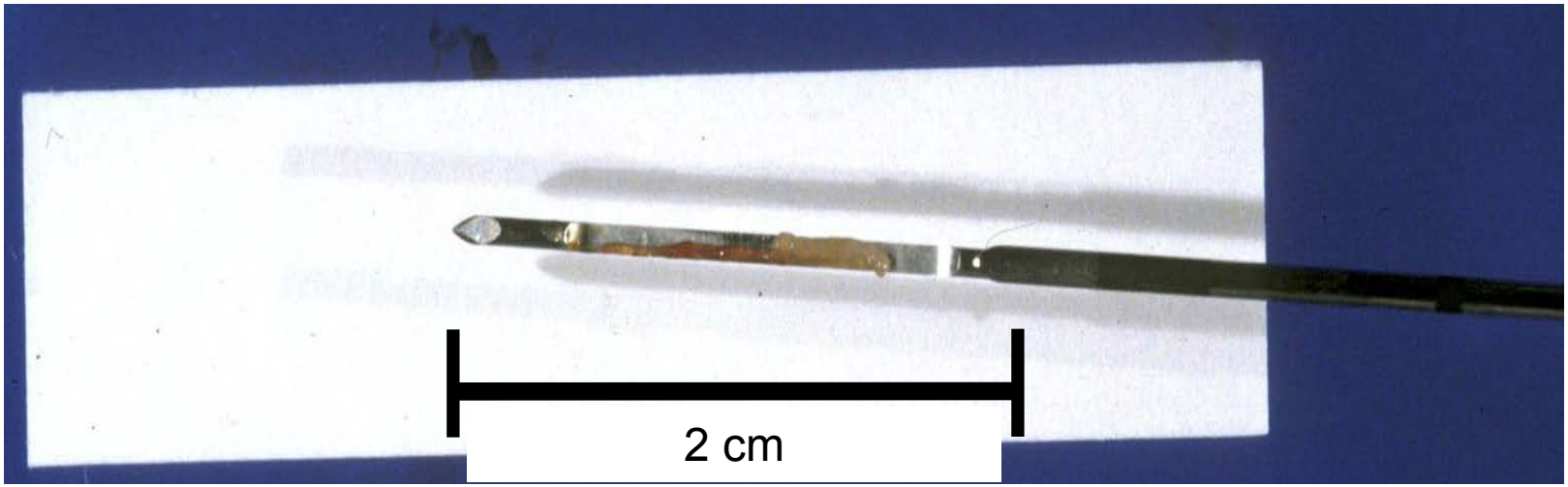
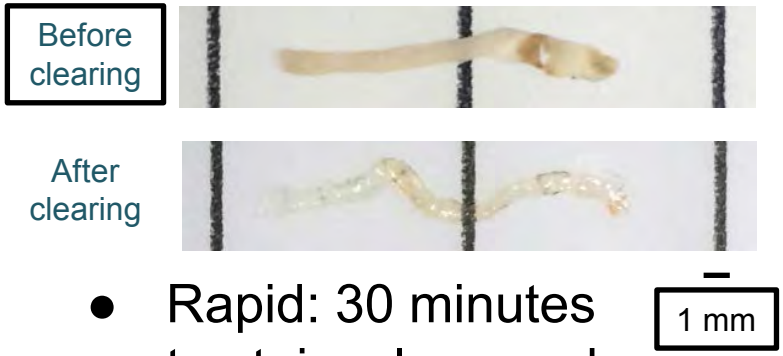
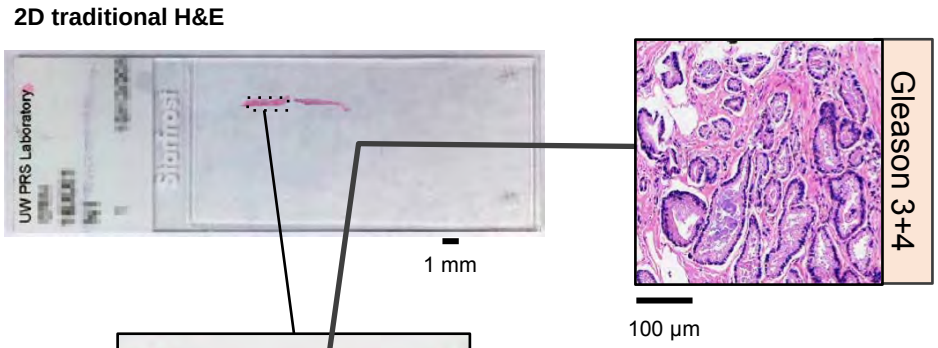
Prostate core needle biopsy procedure







- Rapid: 30 minutes to stain, clear, and image
- Does not alter H&E

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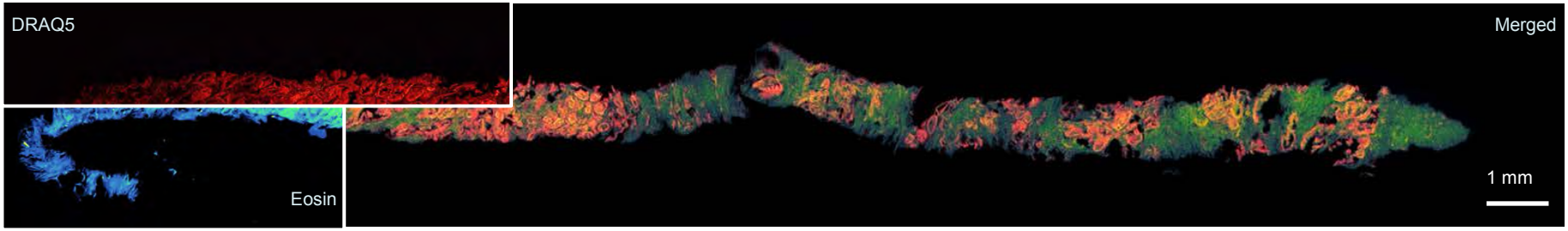
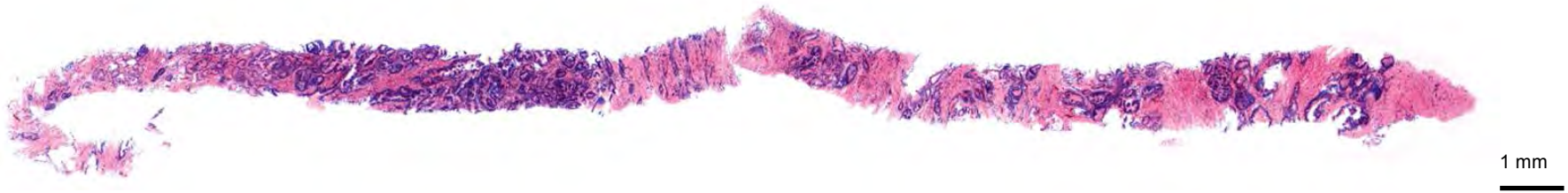
False-color H&E imaging

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



False-colored



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Example false-colored H&E result

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

‘Digital’ histology

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

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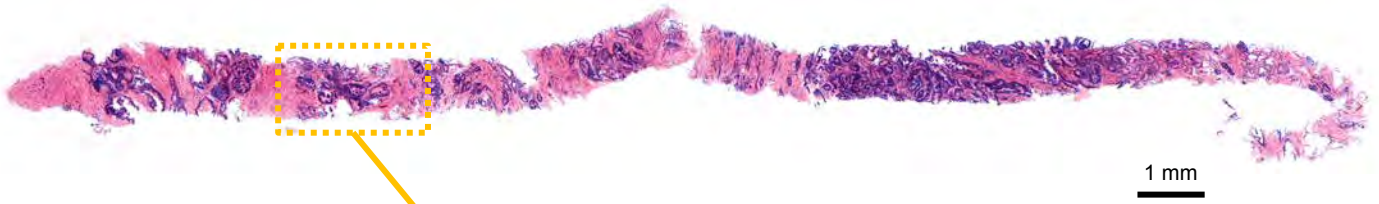
Volumetric imaging of human prostate core-needle biopsy



Glaser et al, *Nature Biomedical Engineering.* 2017
Profiled on Dr. Francis Collins NIH Director's Blog

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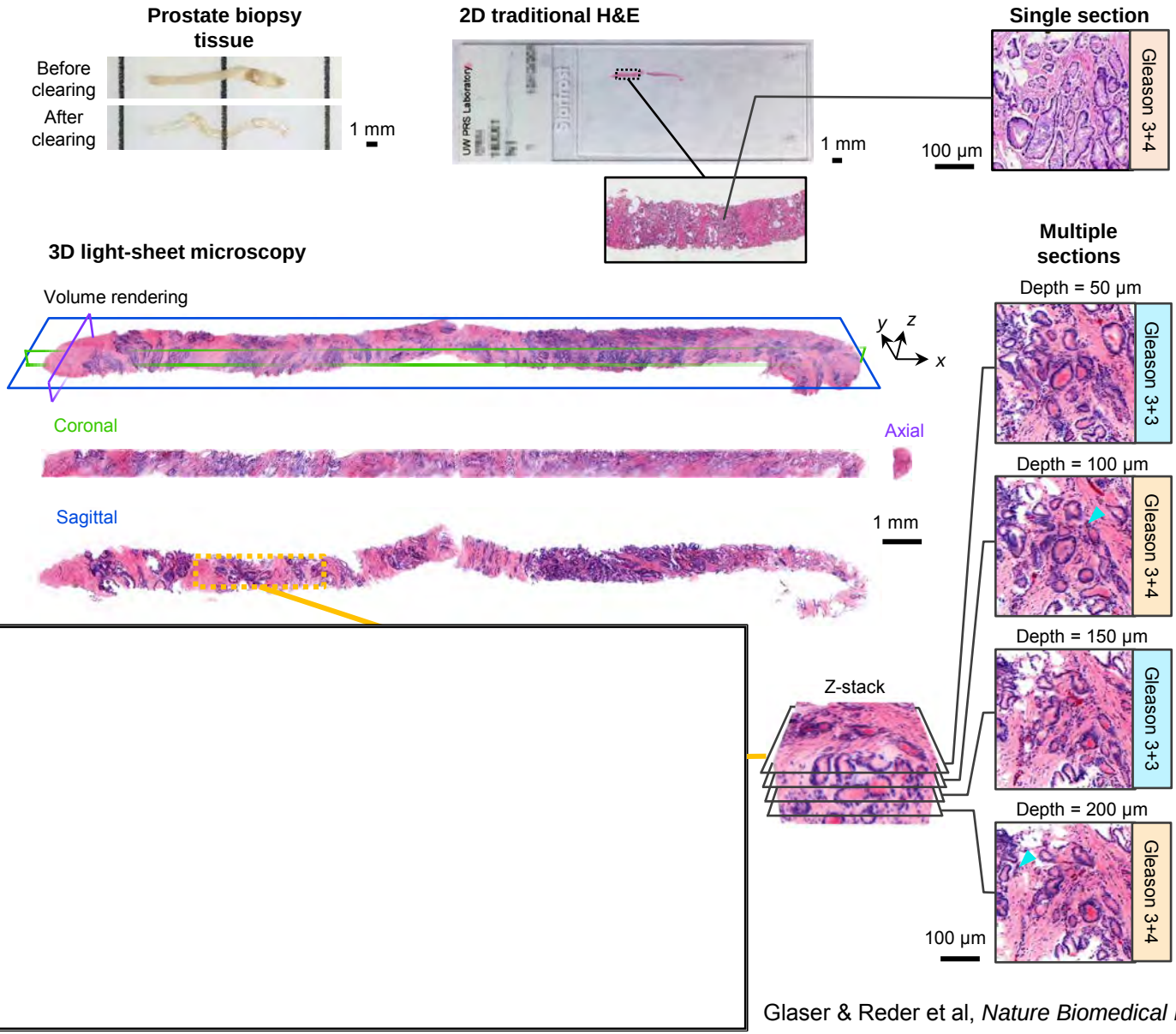
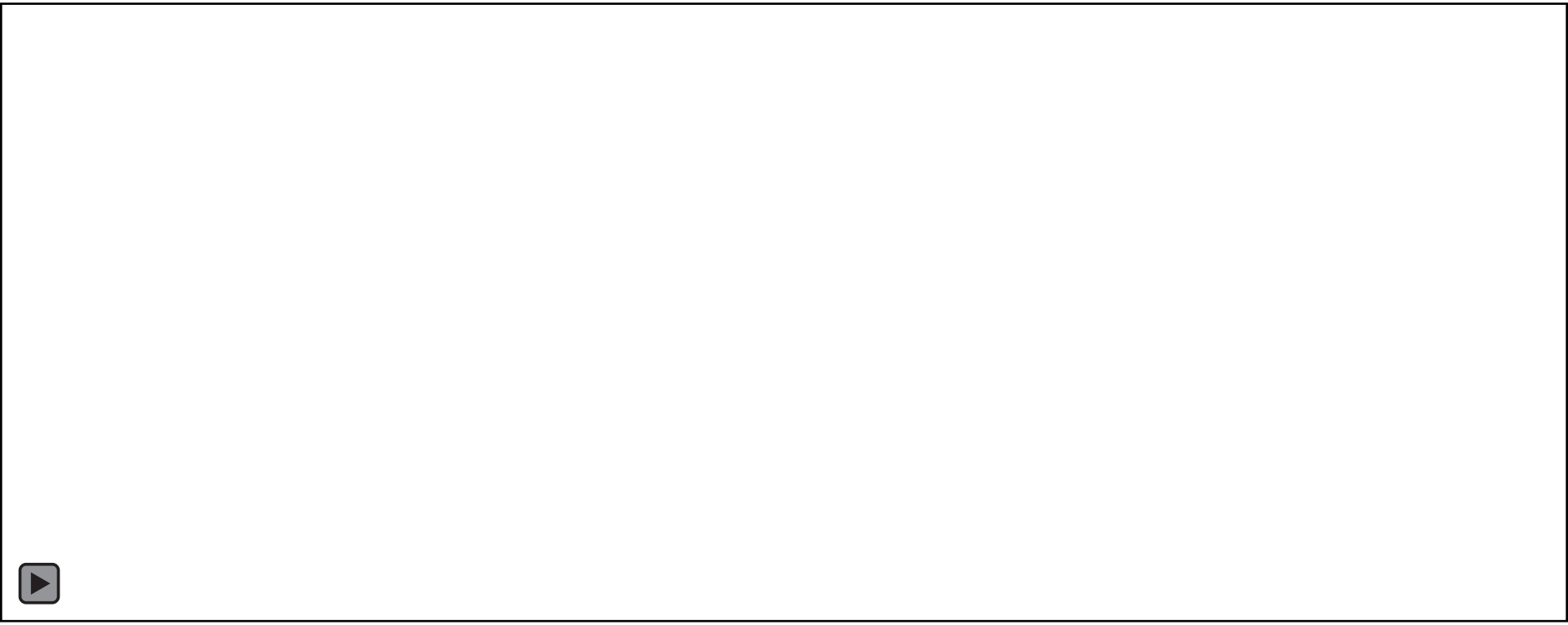
Voumetric imaging of human prostate core-needle biopsy



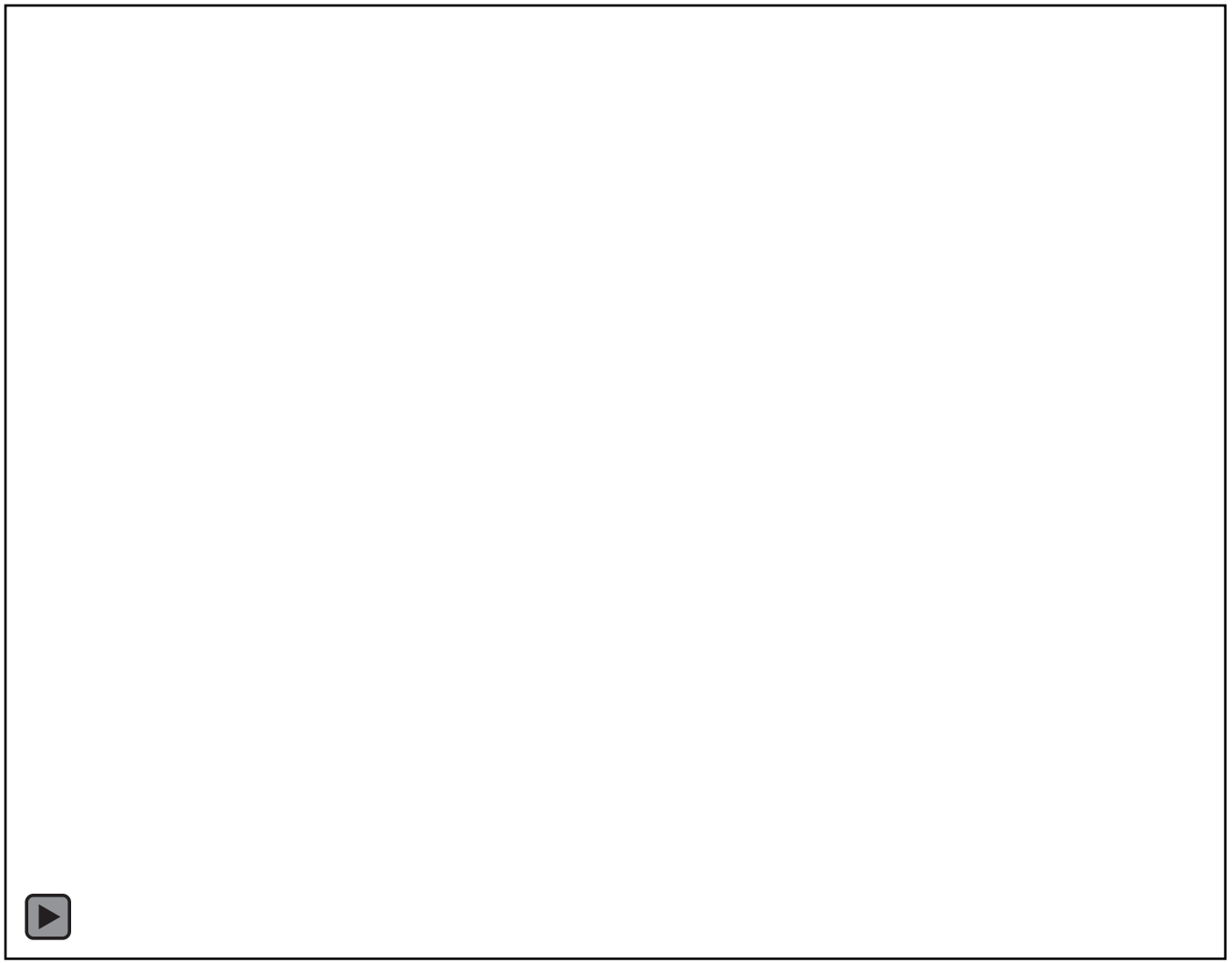
Glaser & Reder et al, *Nature Biomedical Engineering* (in press)

3D pathology of a prostate core needle biopsy



Glaser AK, et al. Assessing the imaging performance of light sheet microscopies in highly scattering tissues. *Biomed Opt Express*. 2016;7(2):454-66.

3D pathology – prostate glandular structure



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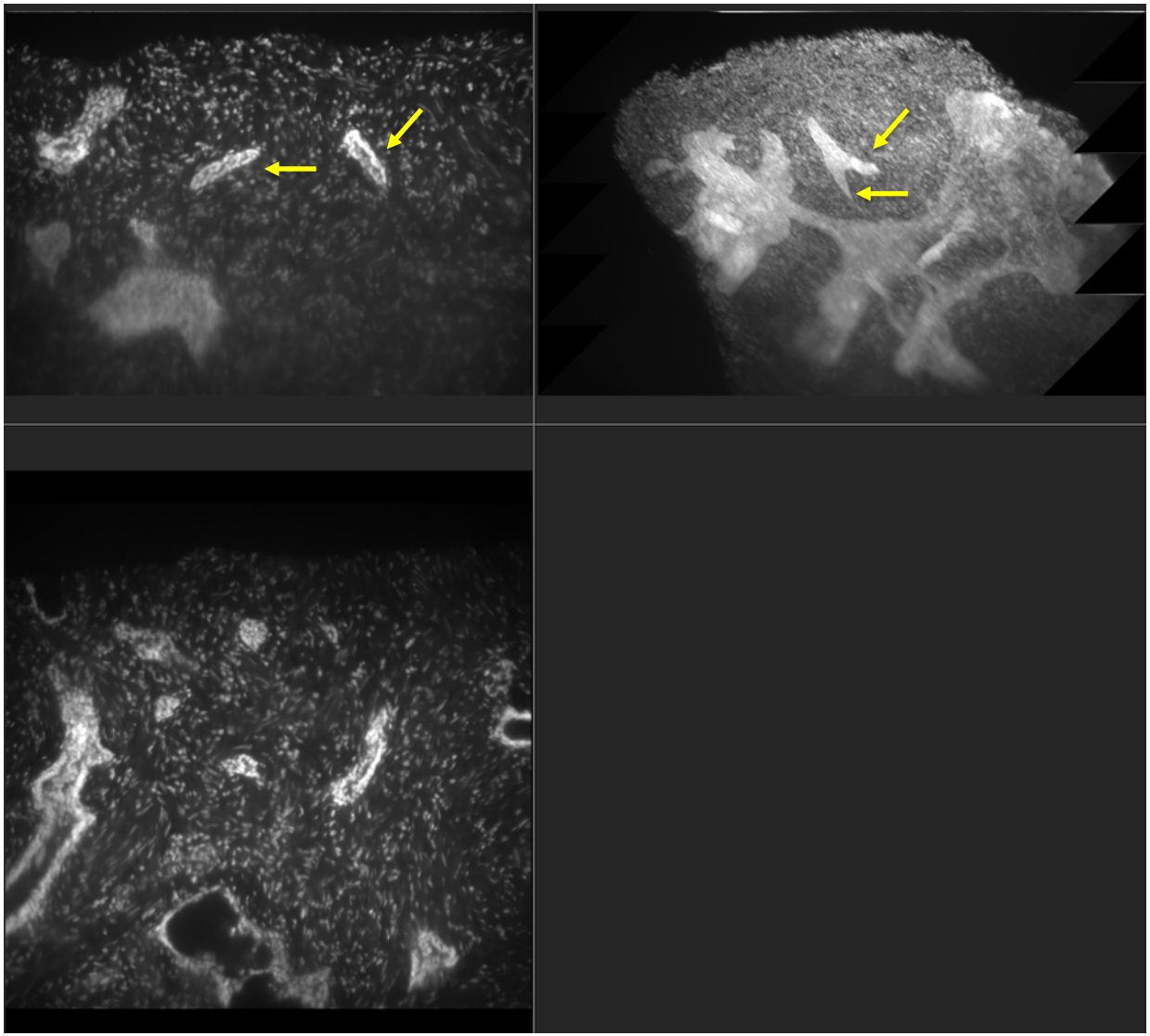
3D pathology – prostate glandular structure



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3D pathology – 3D IHC

- **3D CK8 IHC of mouse prostate: highlights 3D glandular topology**

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Outline

- **Review of light-sheet microscopy** - Dr. Nicholas Reder
- **Motivation for 3D pathology** - Dr. Lawrence True
- **Tissue clarification techniques** - Dr. Nicholas Reder
- **Results** - Dr. Nicholas Reder
- **Summary - Dr. Lawrence True**

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Clinical applications of 3D microscopy

- **Better pathology using 3D**
  - **Reduced sampling errors**
  - **Non-destructive comprehensive 3D imaging**
  - **3D Immunofluorescence phenotyping**

- **Advantages over conventional pathology:**
  - **Speed and cost (fresh unprocessed tissue)**
  - **Non-destructive (allows downstream molecular diagnostics of tissue)**

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Challenges of 3D pathology

- **1. Image processing**
  - **Mosaicking**
  - **Segmentation**
  - **Deconvolution**
- **2. Image presentation**
  - **Data storage**
  - **Compression**
  - **Visualization**

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Challenges of 3D pathology (continued)

- **3. Image interpretation**
  - **How do we interpret 3D data?**
  - **Computer-aided diagnosis (CAD)**
  - **Machine learning / automated interpretation**

- **4. Clinical acceptance, FDA approval, and reimbursement**

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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)
11/7	Rapid examination of fresh tissue using light-sheet microscopy	Nicholas P. Reder, MD, MPH

Register for upcoming & archived webinars:
www.cap.org > Calendar > 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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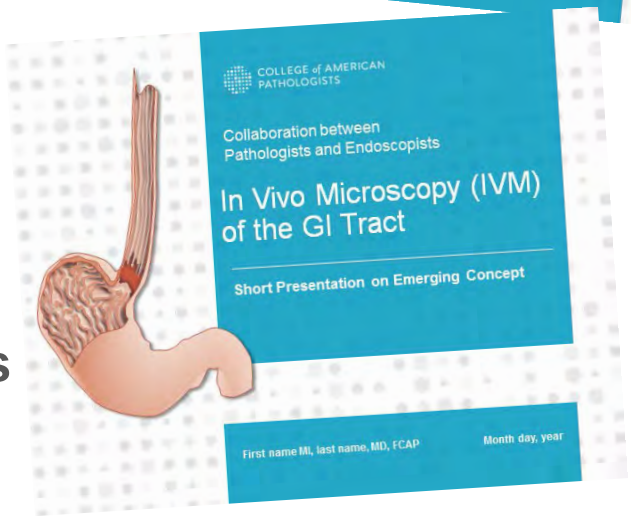
IVM Short Presentations on Emerging Concepts (SPECs) – see handout

- **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 (NEW)**

Access them www.cap.org > Resources and Publications



CAP17 The Pathologists' Meeting – IVM Highlights

- Visit the IVM Table at the **Fellowship Fair** to find out about IVM and EVM fellowships that you can participate in
- Learn about CAP's in vivo microscopy resources and talk with fellow members who are pioneering these technologies at the **CAP's IVM Resources 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**

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IVM Topic Center Page on CAP.ORG

- **Check the IVM Topic Center for continued updates and for all your IVM resources**

  www.cap.org > Search for “IVM Topic Center”

THANK YOU!

- **Thank you for attending our webinar "Light-sheet microscopy for 3D pathology" by Nicholas P. Reder, MD, MPH and Lawrence D. True, MD**

  - **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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