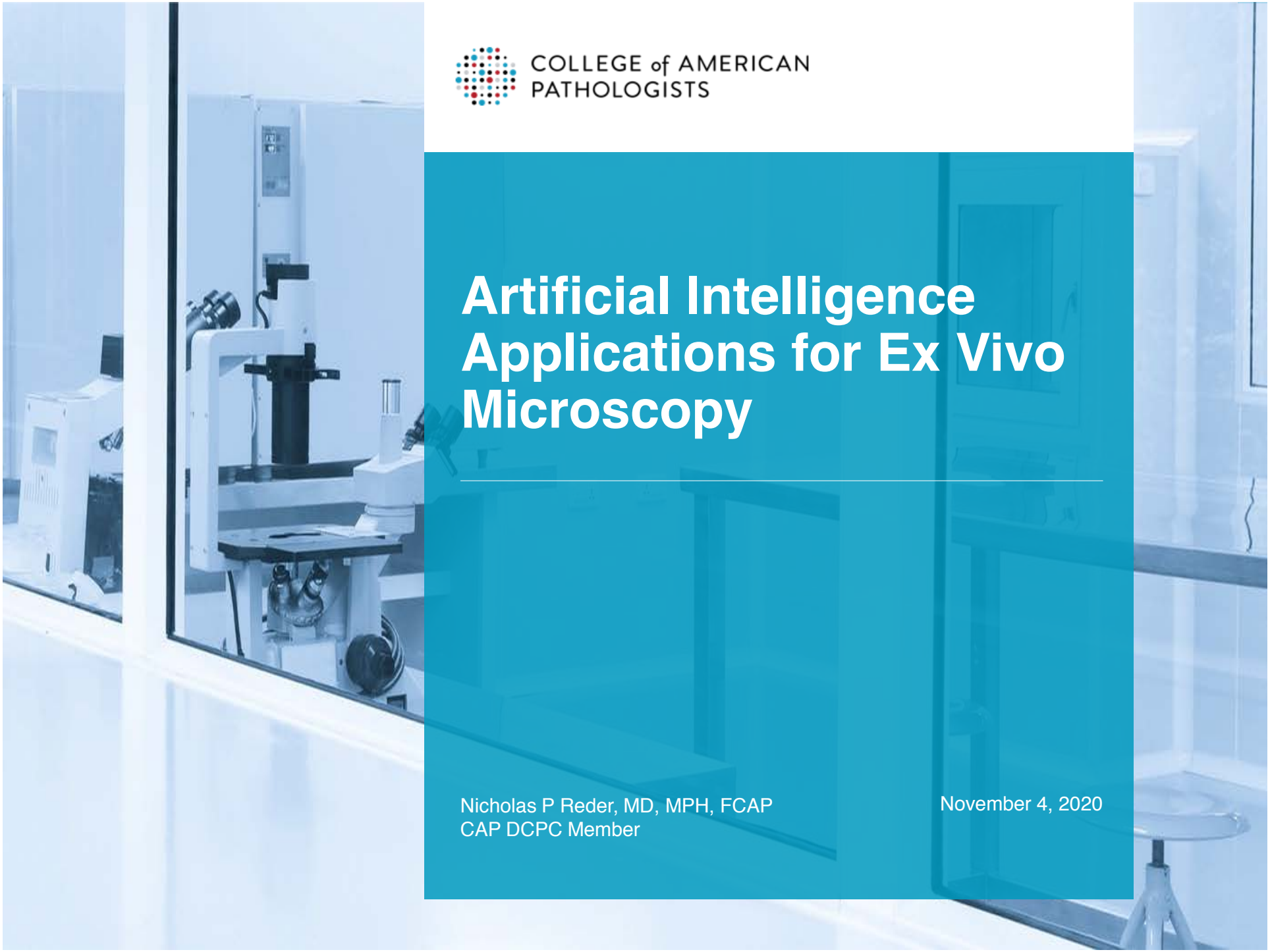

COLLEGE of AMERICAN PATHOLOGISTS

Artificial Intelligence Applications for Ex Vivo Microscopy

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CAP DCPC Member

November 4, 2020

Nicholas Reder, MD, MPH, FCAP

- Acting instructor at the University of Washington with a subspecialty practice in genitourinary pathology.

- Research focuses on using Ex Vivo Microscopy and machine learning to improve prostate cancer diagnostics

- Served on the CAP In Vivo Microscopy Committee since 2016.

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Conflict of Interest Statement

Dr. Reder is a cofounder, CEO, and equity holder in a startup company, Lightspeed Microscopy Inc, that is commercializing open-top light-sheet microscopy. His financial/fiduciary interests have been reviewed and are managed by the University of Washington (UW) in accordance with their conflict of interest policies. He additionally has UW-owned patents that are licensed by LightSpeed Microscopy Inc.

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Outline

- EVM overview
- Clinical applications
- EVM technologies
- Artificial intelligence and EVM
  - Image processing
  - Quantification
  - Molecular inference
- What's next?

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Ex Vivo Microscopy

Definition and background information

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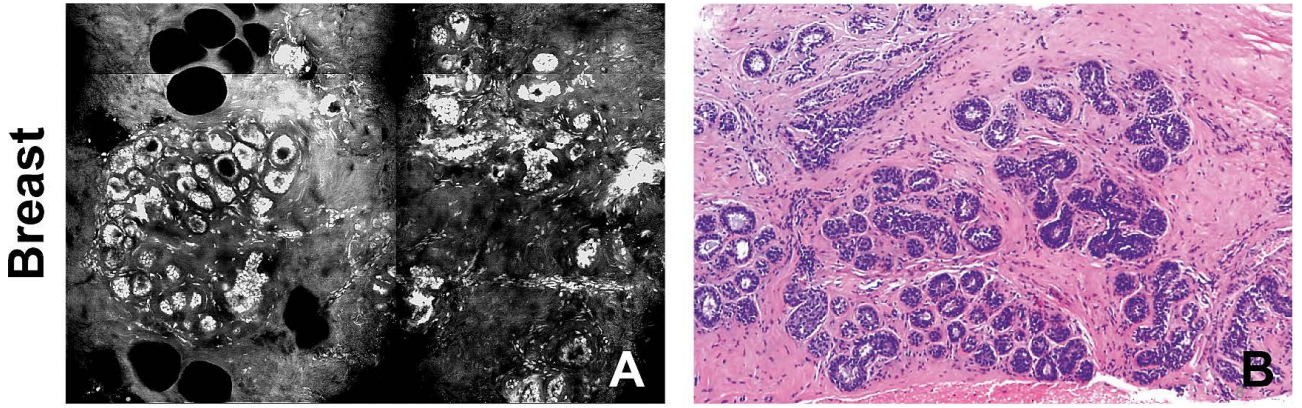
Ex Vivo Microscopy (EVM)

- High resolution images obtained from tissue samples
- Rapid and in real time
- Non-destructive, preserves the tissue
- No tissue processing needed
- +/- exogenous dyes

Images courtesy Savitri Krishnamurthy MD, FCAP:
University of Texas MD Anderson Cancer Center



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Advantages of EVM

- Rapid results
- Compact, benchtop, easy to set up
- Tissue remains intact and undamaged
- Does not interfere with currently utilized tissue processing for histology
- Can visualize tissue in three dimensions
- Allows for enhanced assessment of stroma and blood vessels

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Ex Vivo Microscopy

Clinical applications

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Applications for Pathology Practice

Setting	Pathology Application	Examples of How Pathologist Could Use EVM
Intra-Operative	Margin assessment	Identify tumor margins in breast resections
Intra-Procedural	Needle biopsies & aspirates	Assess adequacy of needle biopsies and aspirates
Gross Examination	Block selection	Identify areas with highest grade lesion in resection for Barrett's Esophagus
Genomic-Molecular Studies	Tissue triaging-selection	Accurately select tumor and conserve tissue without the need for frozen or permanent sections
Biobanking	Tissue triaging-selection	Accurately select tumor and conserve tissue without the need for frozen or permanent section

Source: College of American Pathologists' **In Vivo Microscopy Committee**

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Ex Vivo Microscopy

EVM Technologies

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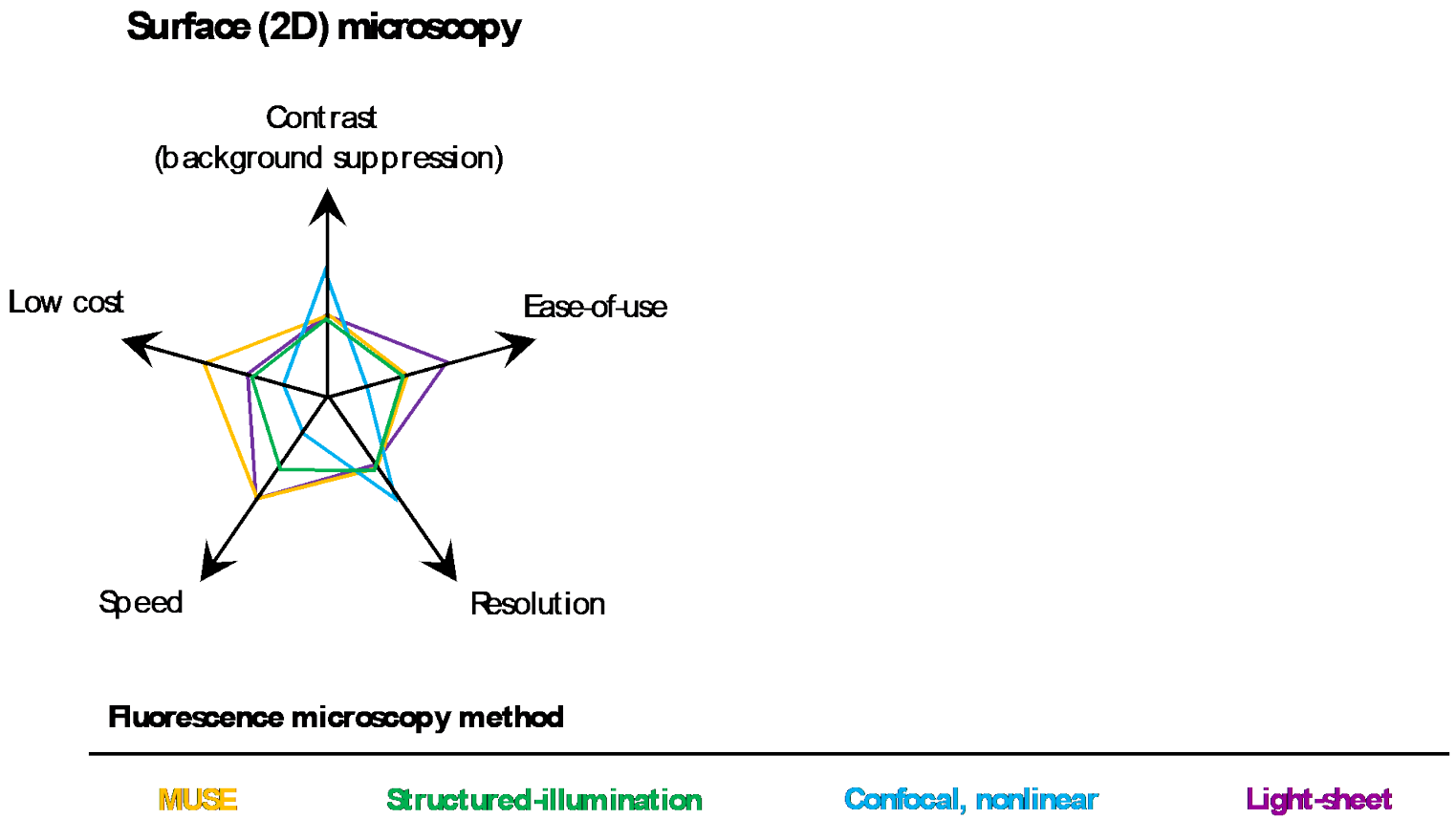
Each EVM technology has tradeoffs



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Incomplete list of emerging EVM technologies

- **Label-free**
  - Stimulated Raman Scattering
- **Fluorescence methods**
  - MUSE
  - Confocal
  - Multiphoton / Two-photon / Non-linear
  - Structured Illumination
  - Light-sheet

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AI and EVM

Image processing

Quantification

Molecular inference

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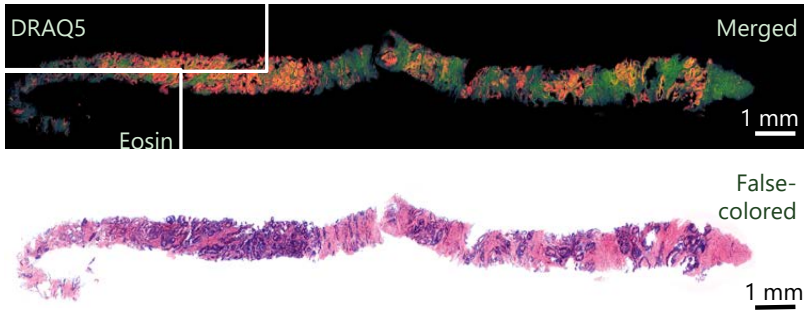
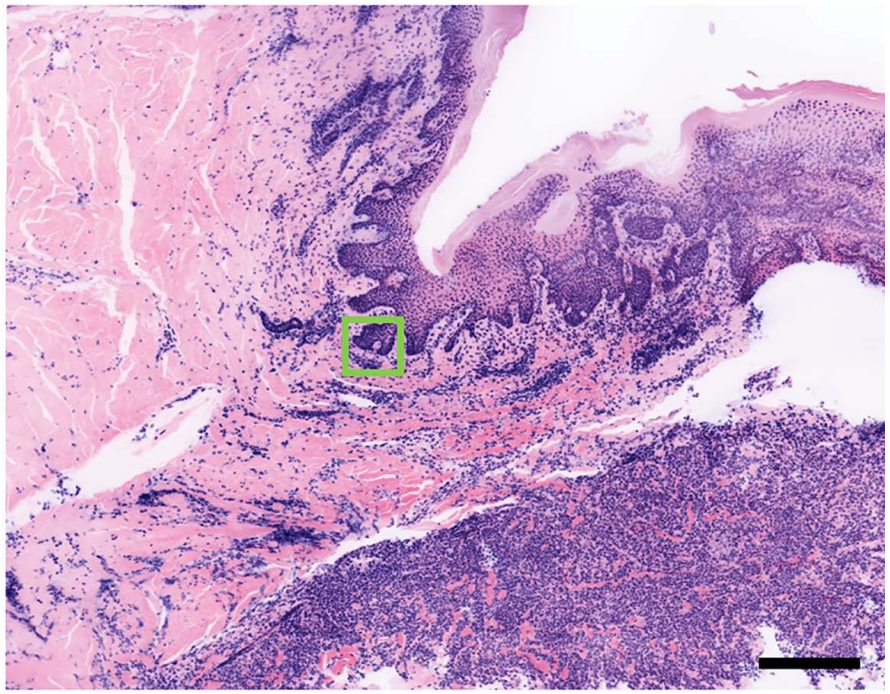
Image processing: Pseudocoloring

Motivation: H&E histology is the current gold standard for disease diagnosis



Prostate sample



Skin melanoma sample

"D&E" fluorescent analog protocol: K.N. Elfer et al., *PLoS ONE* (2016)

False-coloring algorithm: M. Giacomelli et al., *PLoS ONE* (2016)

Automated intensity leveling and false coloring: R. Serafin et al., *PLoS ONE* (2020)

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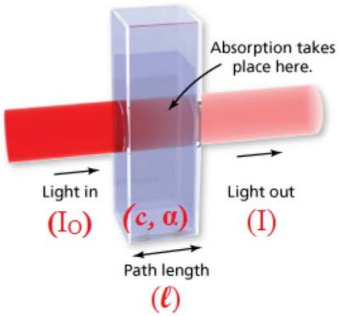
Image processing: Pseudocoloring

Beer-Lambert Equation

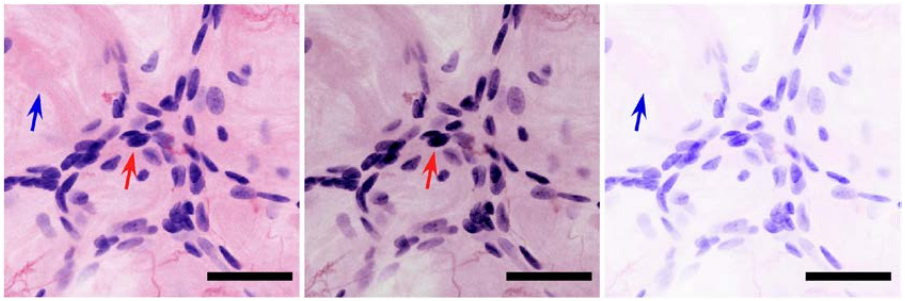


$$A = log_{10}(\frac{I_0}{I}) = \varepsilon cl$$

$$A = \varepsilon cl$$

MacNamara. https://www.youtube.com/watch?v=VWHt4MBWBmc

False H&E coloring



Giacomelli et al. 2016

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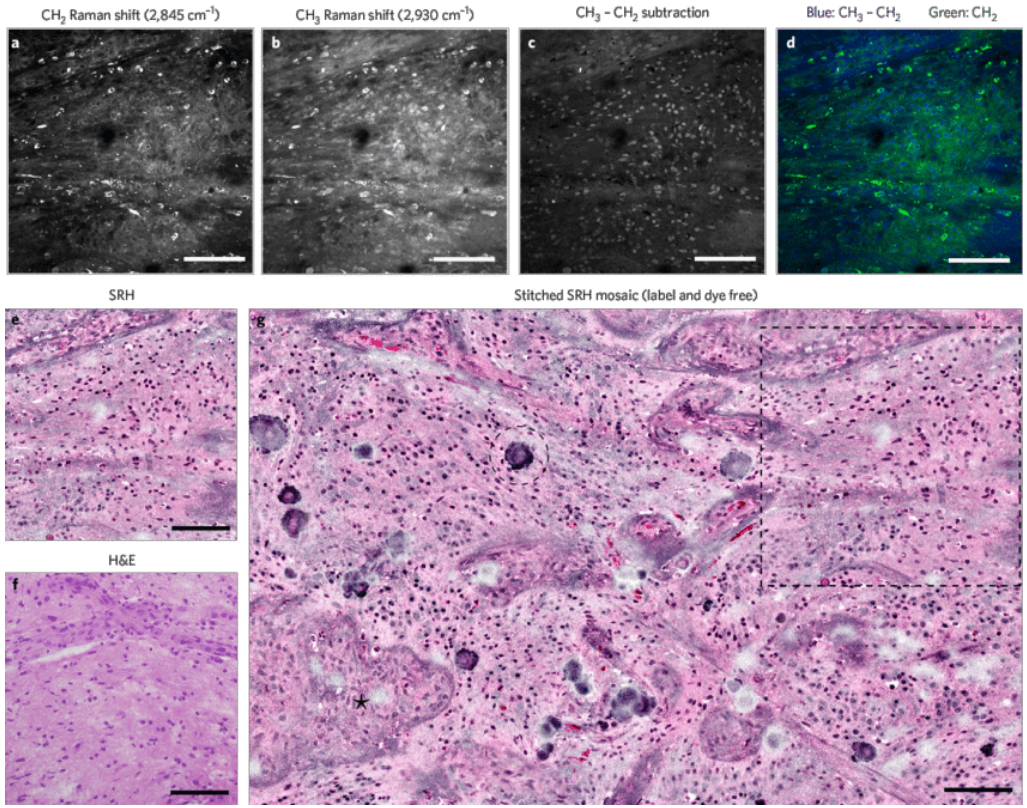
Image processing: Pseudocoloring



Orringer et al. 2017.

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Image processing: Pseudocoloring

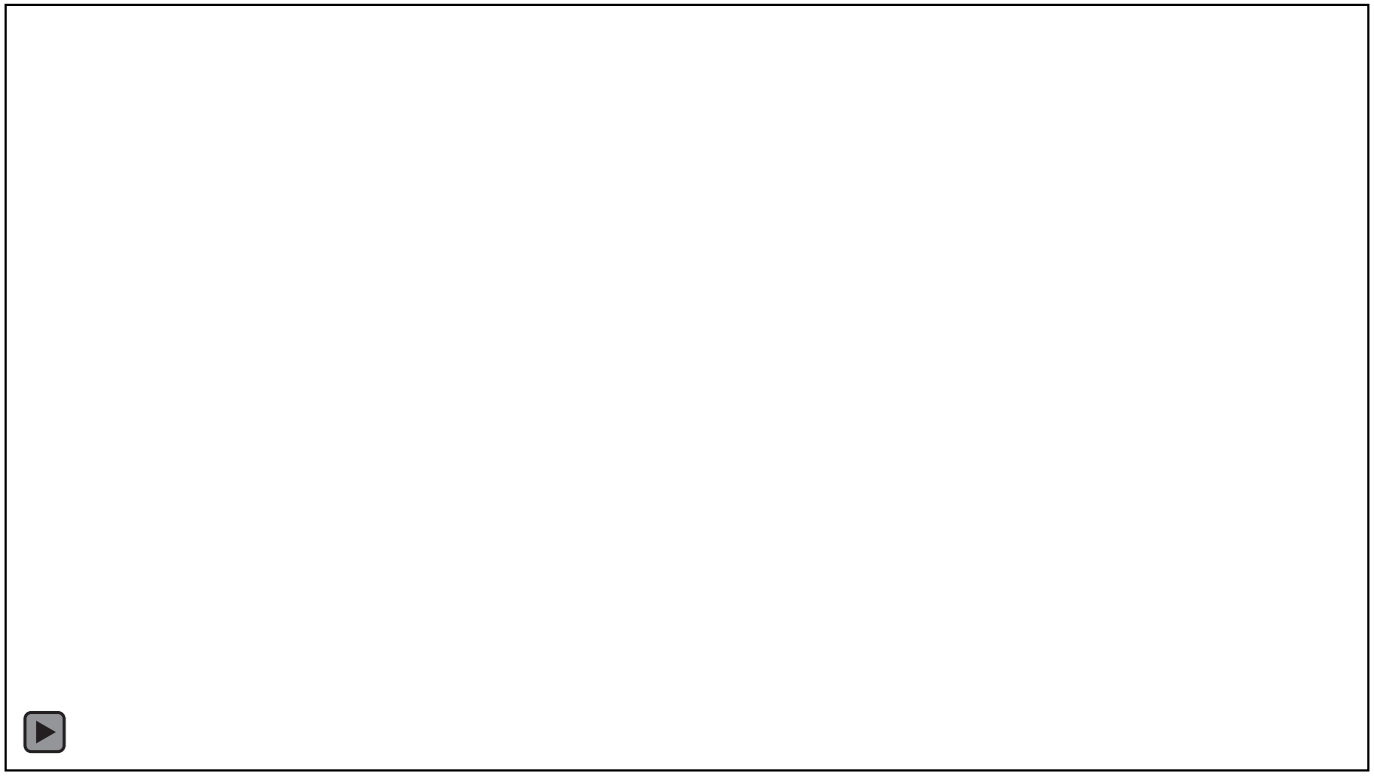

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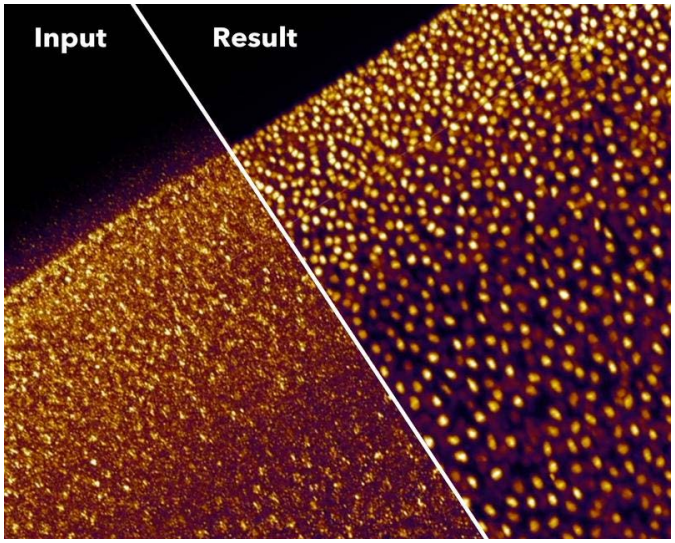
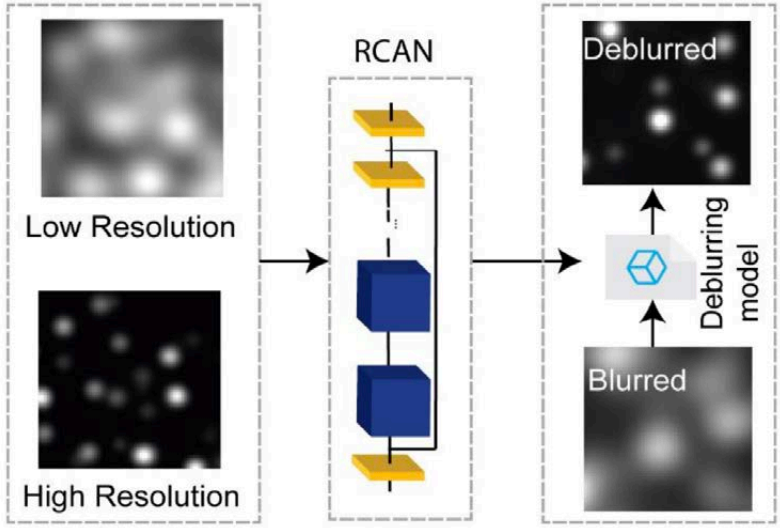
Image processing: Noise reduction

Content aware image restoration



Weigert et al. 2018

RCAN



Chen et al. 2020

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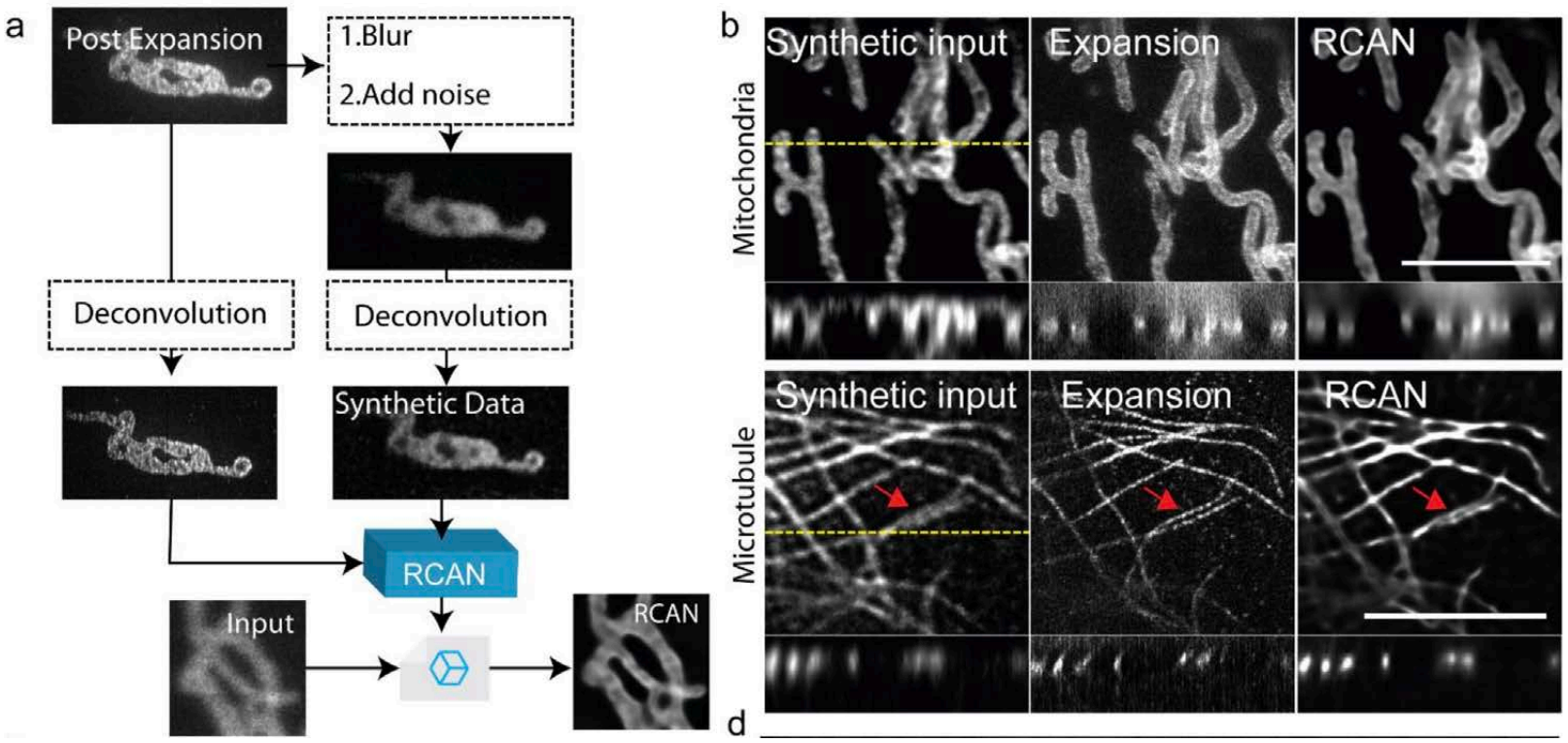
Image processing: Improving resolution



Chen et al. 2020

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Quantification

- In general, EVM images are amenable to quantification, similar to WSI
- Quantification with EVM images can outperform WSI

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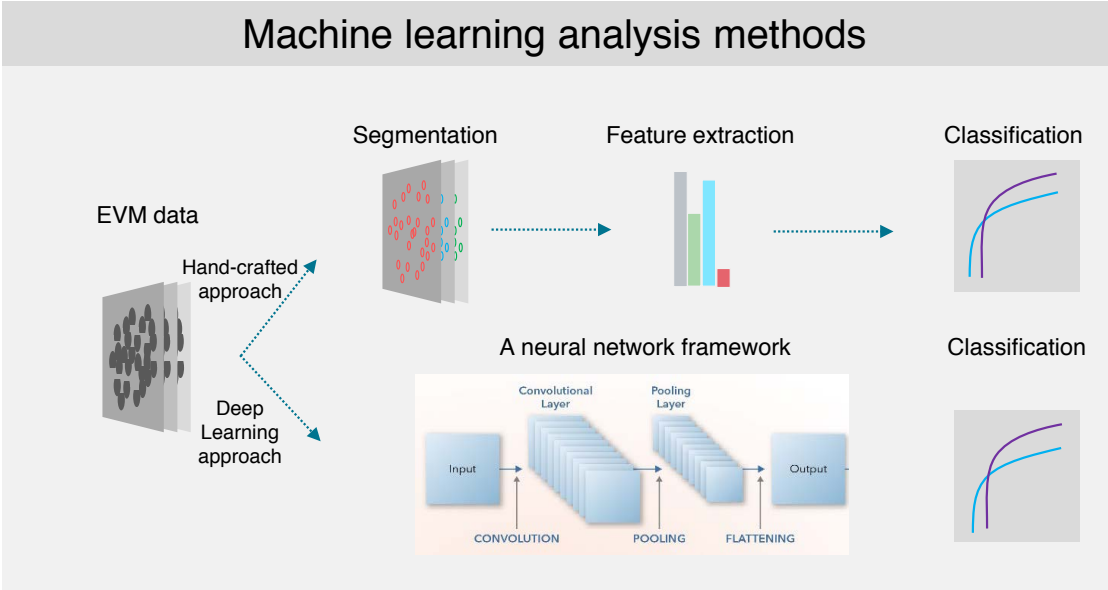
Quantification



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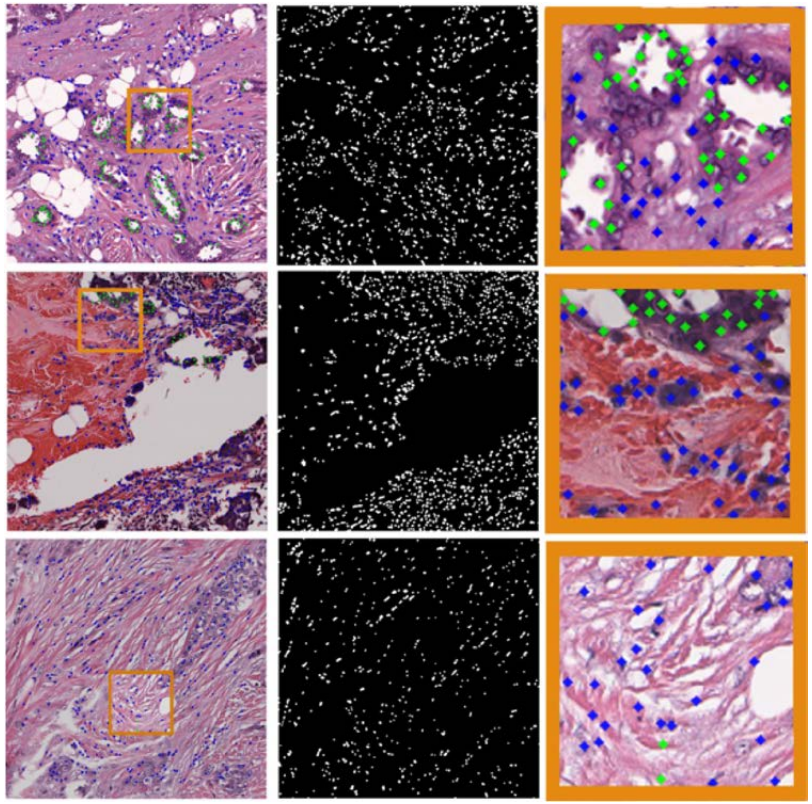
Quantification



Inference

Romo-Bucheli et al. 2016.

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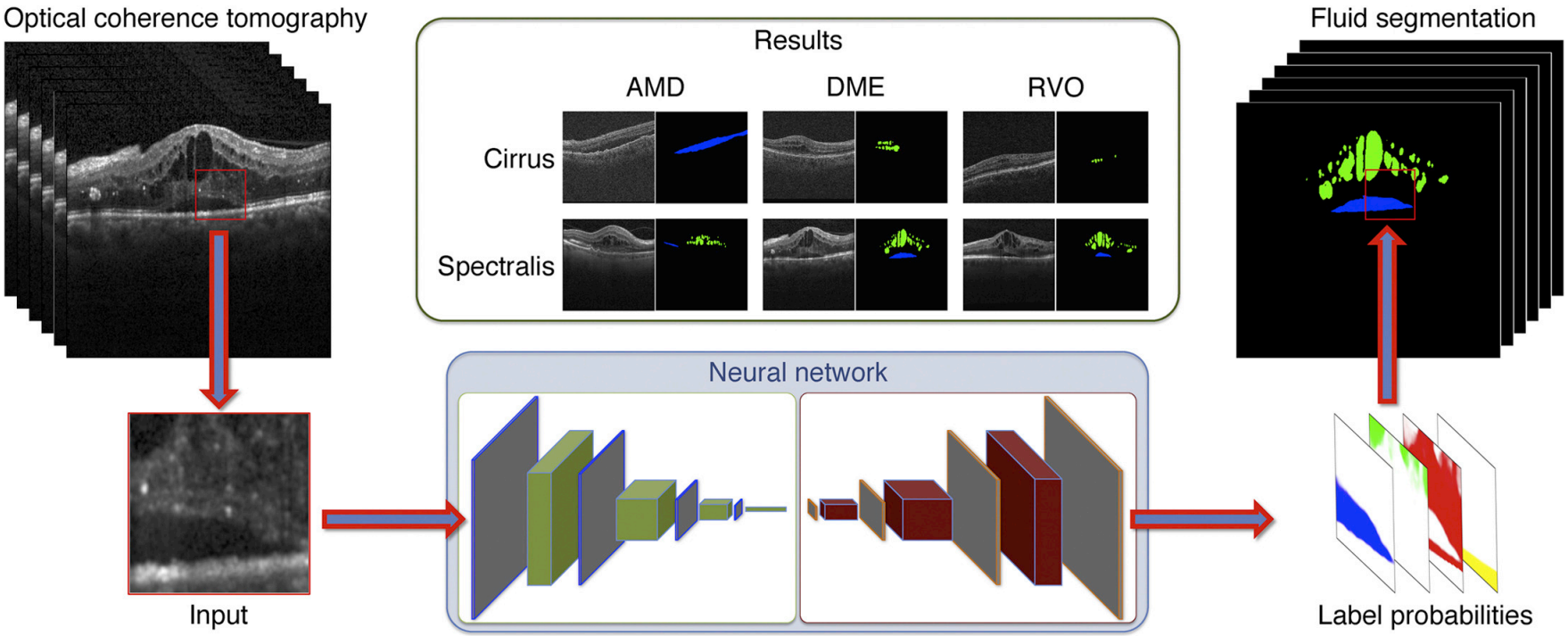
Quantification – EVM example



Schlegl et al. 2018.

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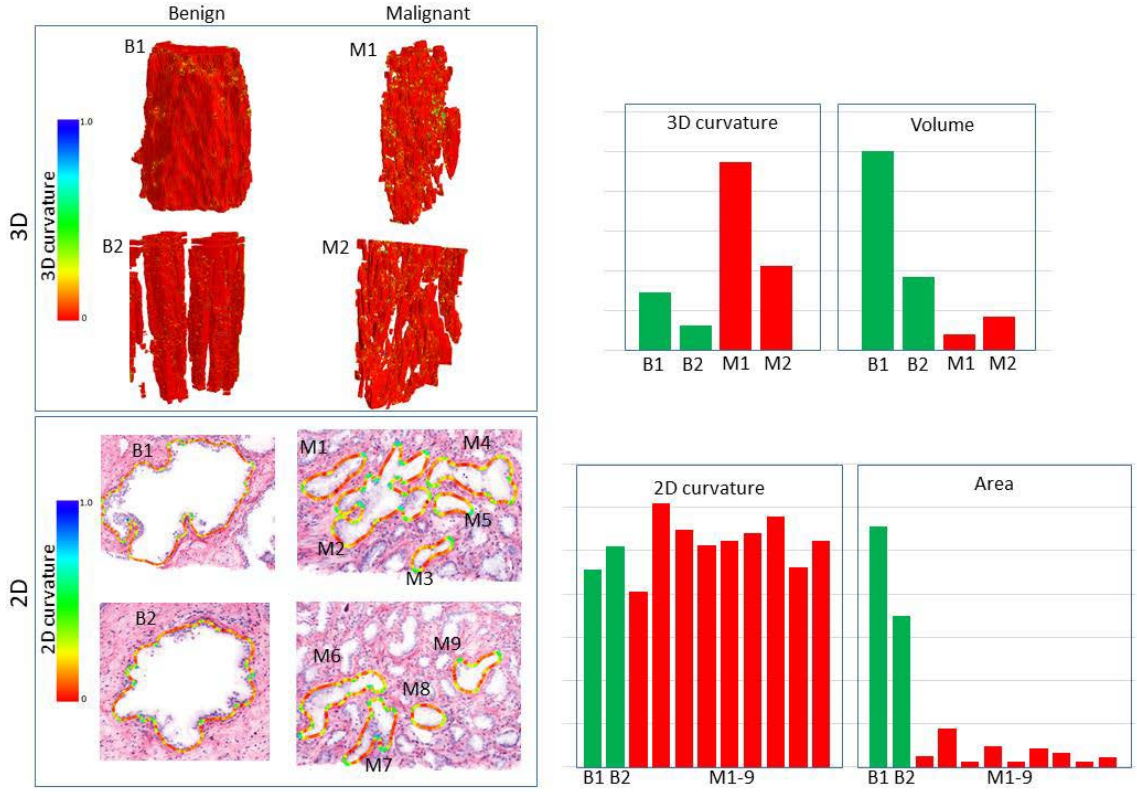
Quantification – EVM example



Koyuncu et al. 2020.

3D light-sheet microscopy

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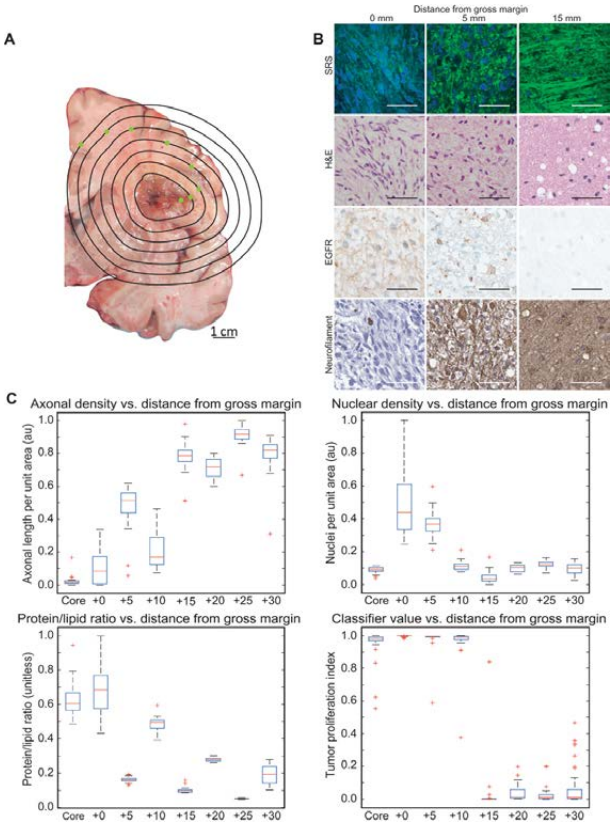
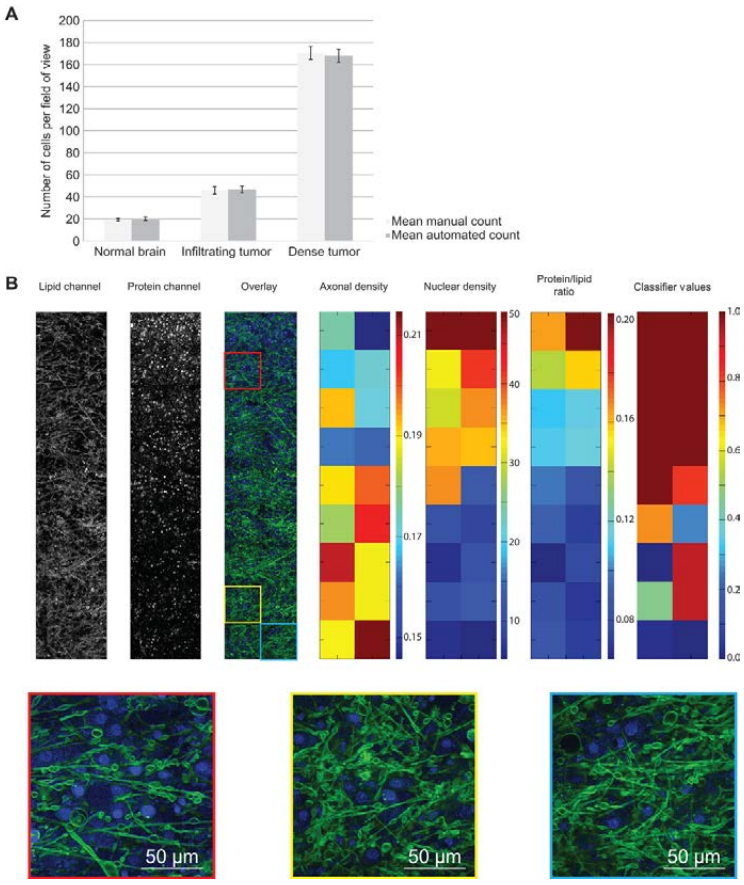
Quantification – EVM example



Ji et al. 2020.



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Quantification – EVM example (in 3D)

Courtesy of Lightspeed Microscopy

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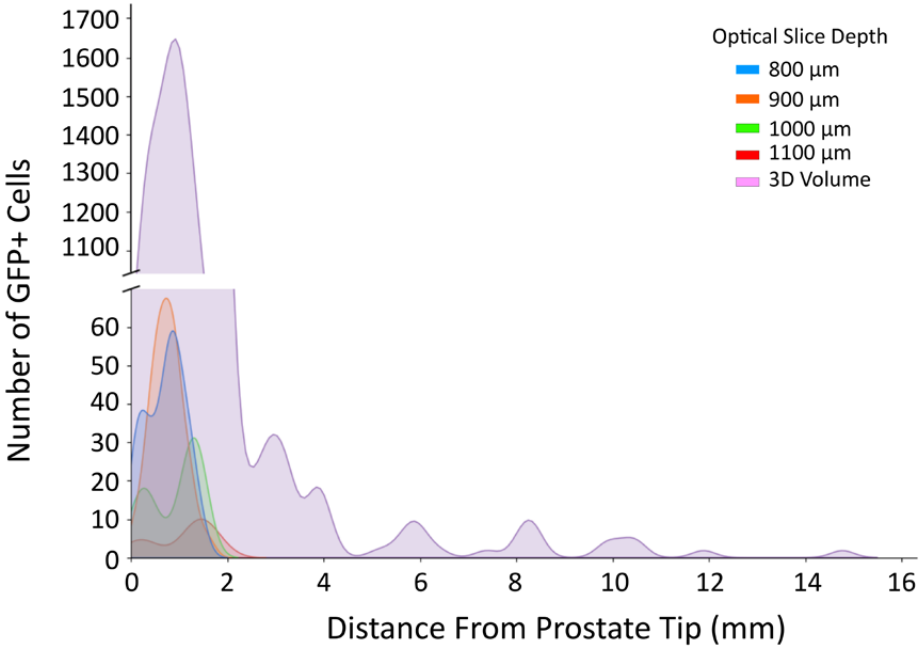
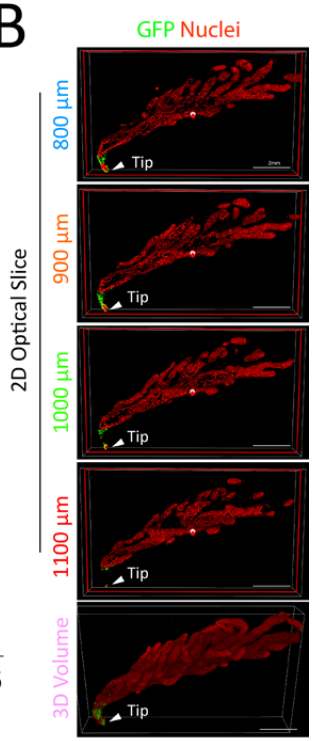
Quantification – EVM example (in 3D)

A



B



	Total Cells	Cells closer than 0.2 mm	Cells further than 1.25 mm
800 µm slice	60	12 (20.0%)	4 (6.7%)
900 µm slice	57	3 (5.3%)	3 (5.3%)
1000 µm slice	33	6 (18.2%)	14 (42.4%)
1100 µm slice	13	4 (30.8%)	9 (69.2%)
3D volume	1629	144 (8.8%)	355 (21.8%)

Conclusions:

- AI and automation enables quantification across massive imaging volumes
- There is large variability in cell counts depending on the sectioning depth

Courtesy of Lightspeed Microscopy

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Molecular inference

- Cutting-edge machine learning techniques enable the inference of molecular markers (ER/PR, p53, etc.) from images
- This application is furthest away from the clinic and will require extensive validation

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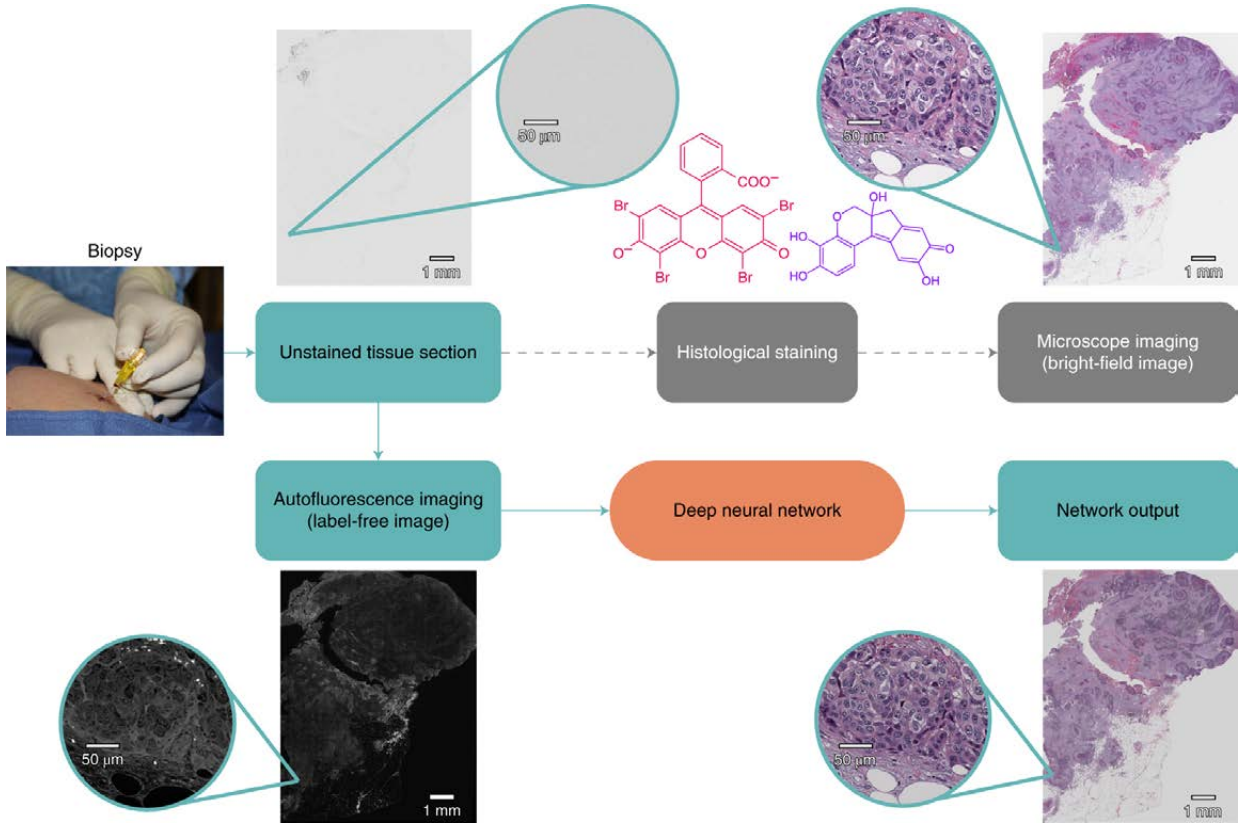
Molecular inference: Label-free



Rivenson et al. 2019.

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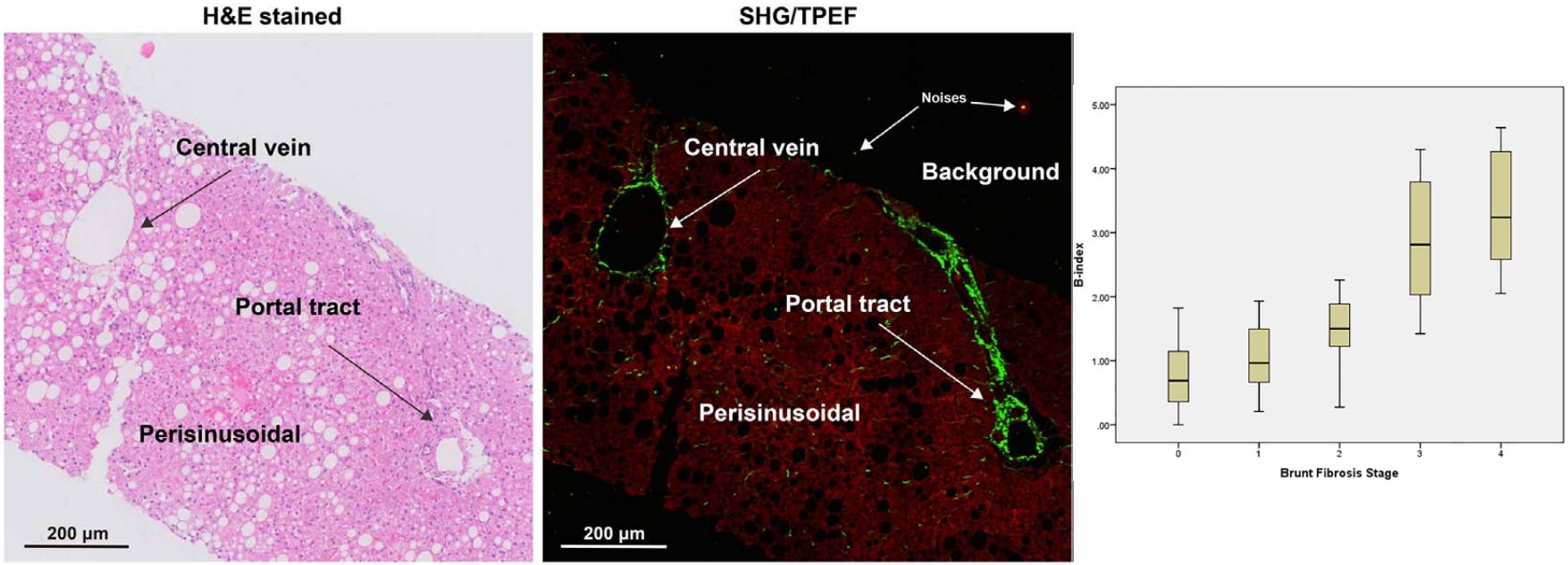
Molecular inference: Label-free



Chang et al. 2018.

Multi-photon microscopy, second harmonic generation, machine learning quantification of fibrosis in NASH samples

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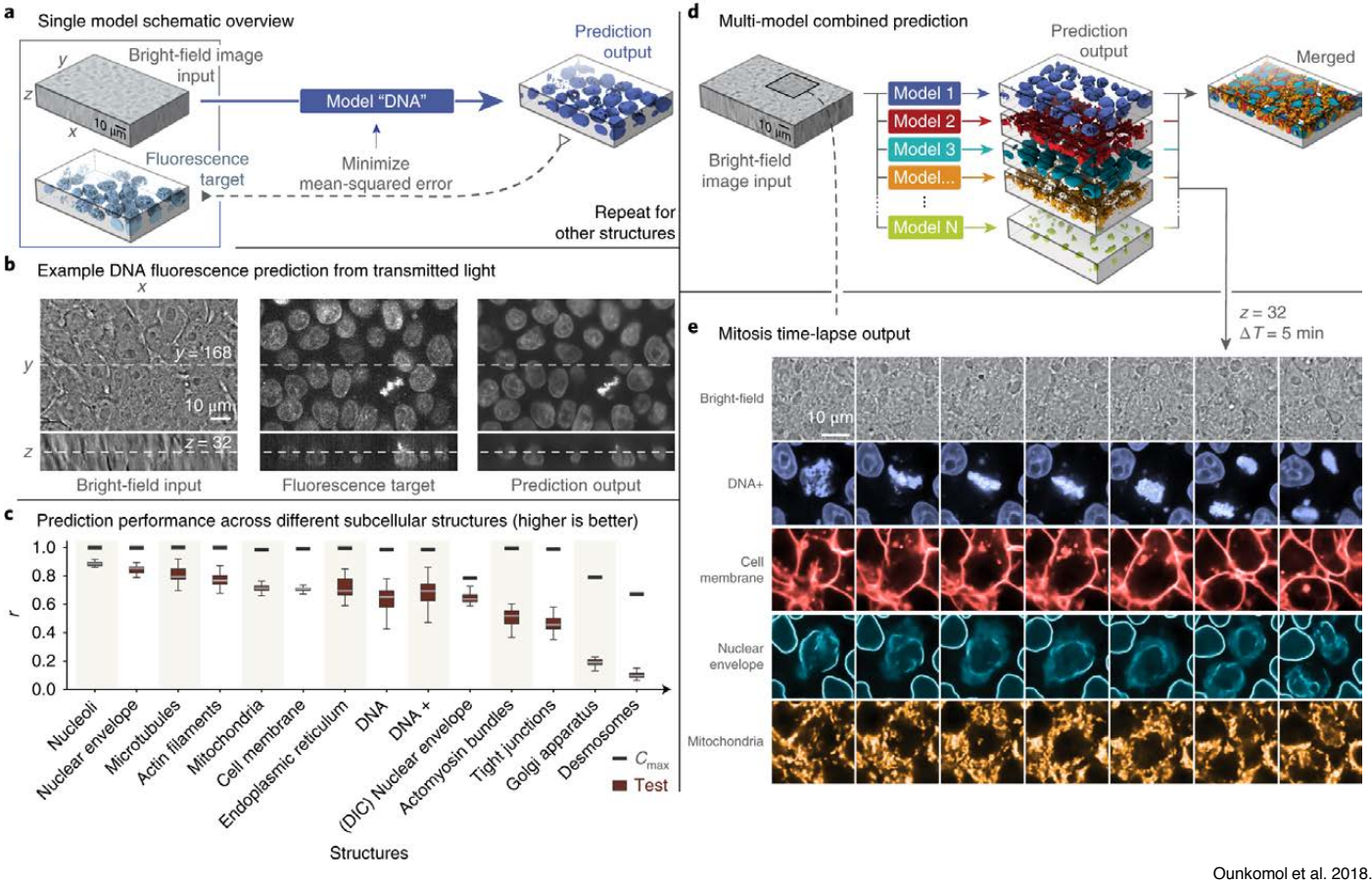
Molecular inference: Label-free



Ounkomol et al. 2018.

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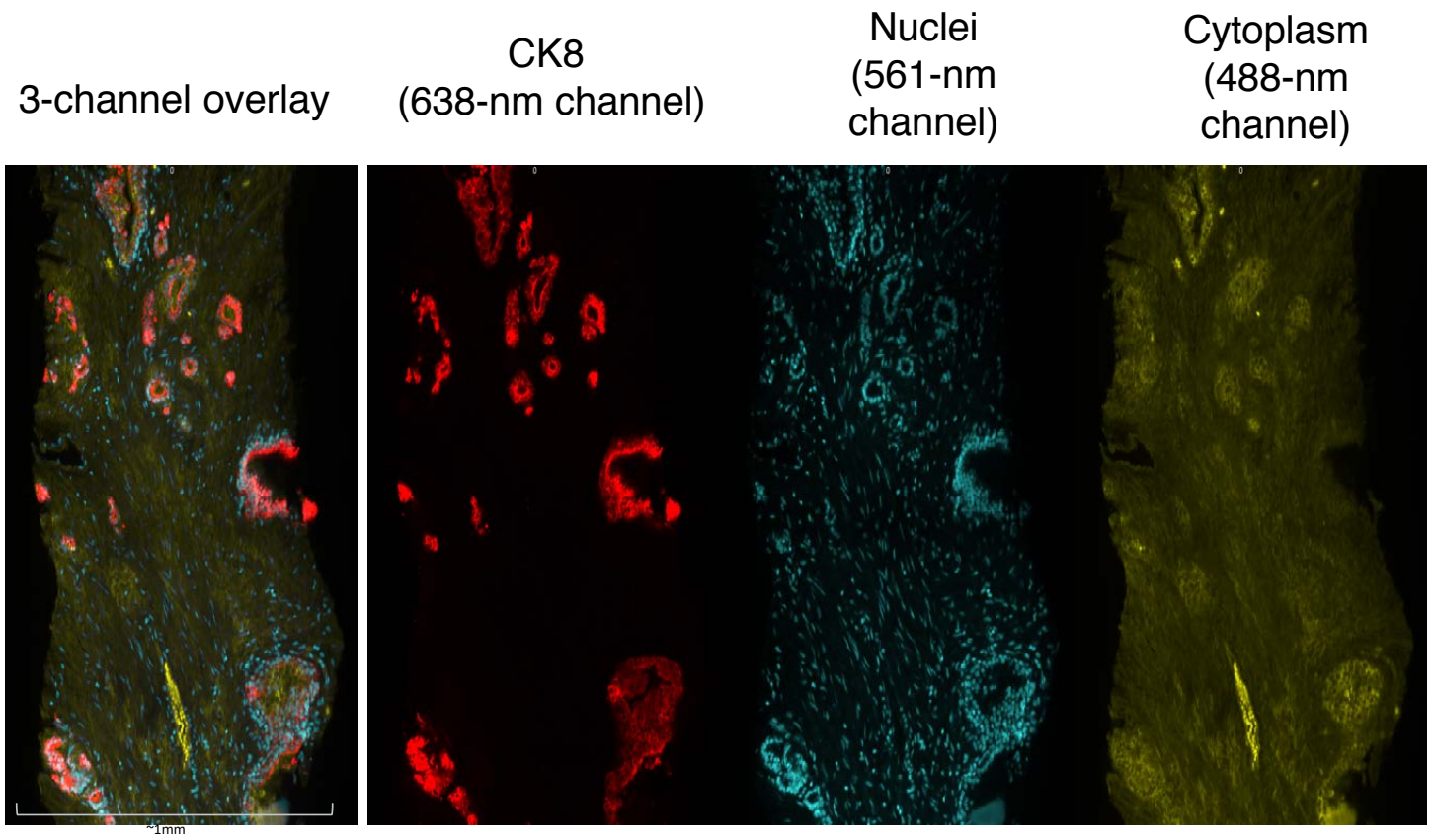
Molecular inference: “IF sans IF”



Xie et al. In preparation.

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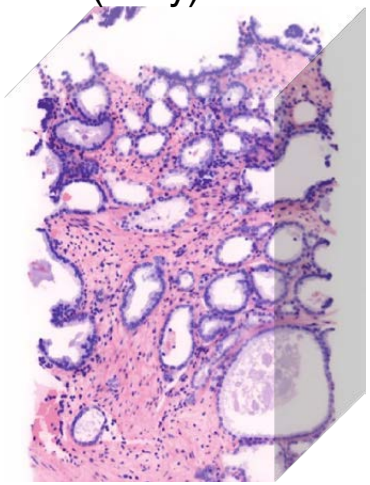
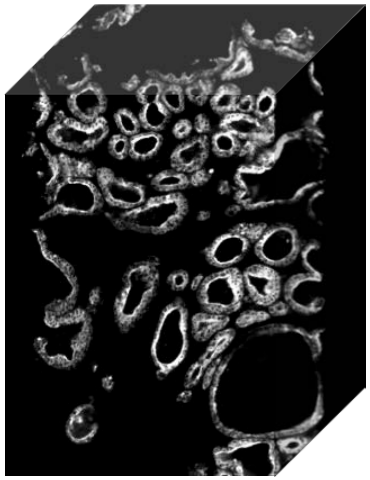
Molecular inference: Label-free

H&E analog
(easy)

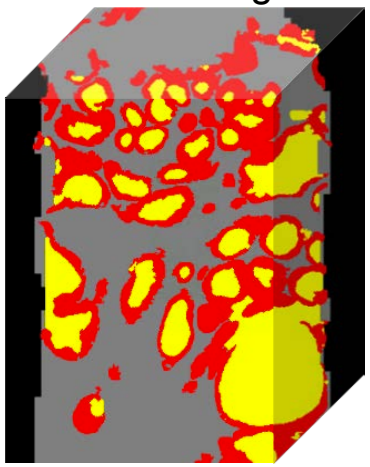


(false-
colored for
visualization)

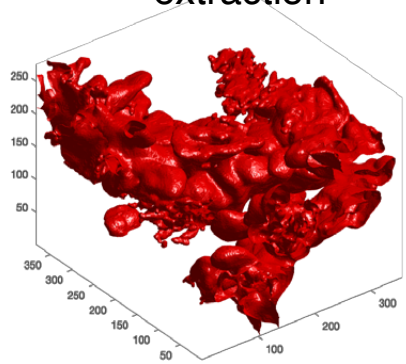
CK8 immunofluorescence
(technically challenging)



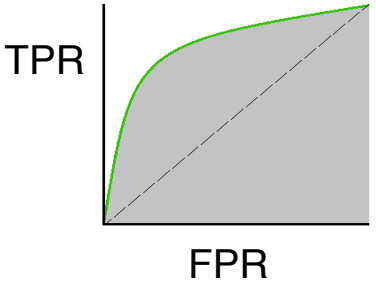
Gland segmentation



Glandular
morphological
feature
extraction



Clinical outcome
prediction



Xie et al. In
preparation.

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Ex Vivo Microscopy

What's next?

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How does EVM fit into the future of pathology?

- Low risk applications in the near term
  - Biobanking
  - Enrichment of tissue for genetic sequencing
  - Adequacy
- Time-limited, high impact applications in the intermediate term
  - Intraoperative consultation
- In the long term, why ever make a glass slide?

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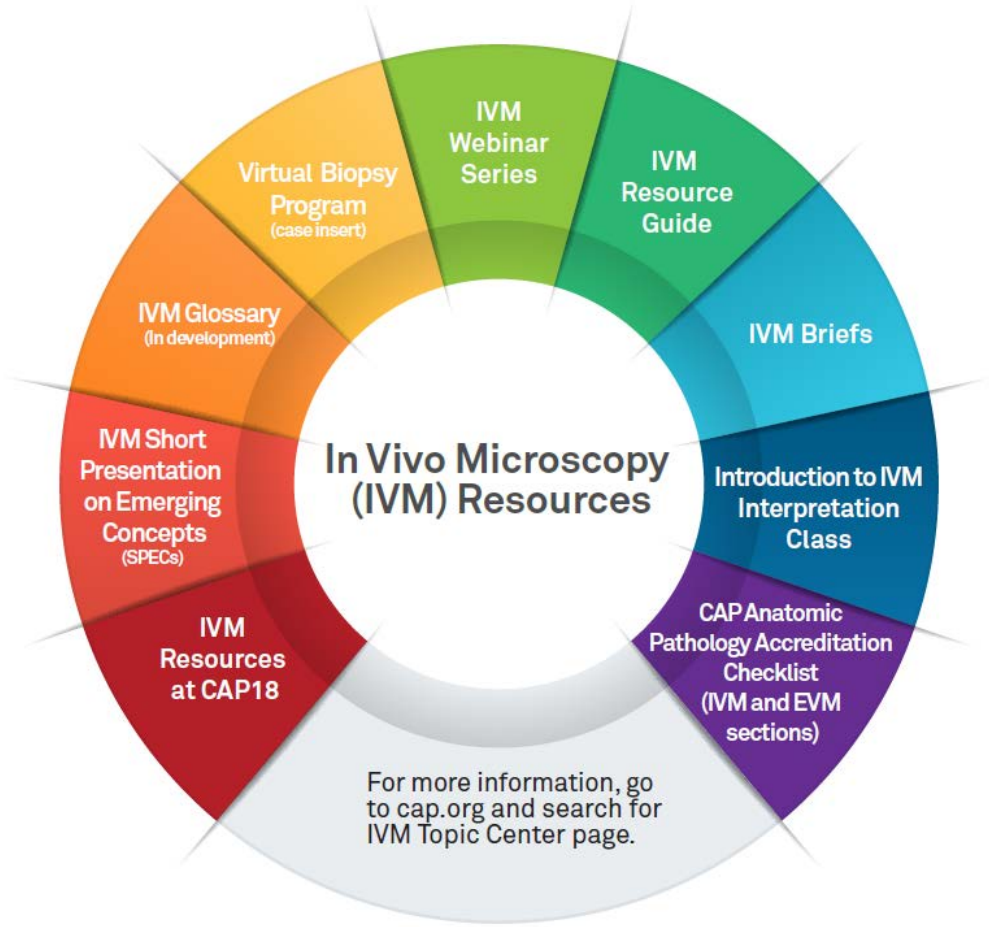
IVM Resources at CAP



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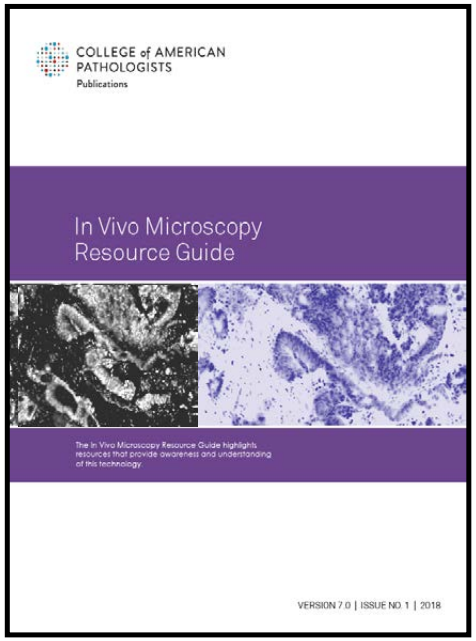
The CAP In Vivo Microscopy Resource Guide – see handout

- **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 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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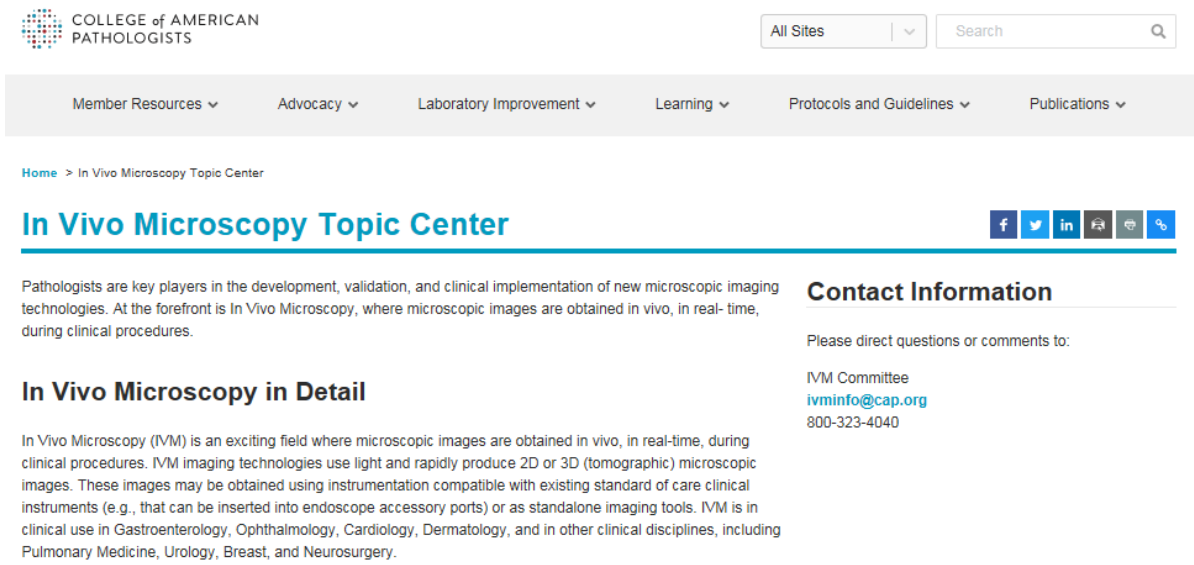
IVM Topic Center Page on CAP.ORG

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

  www.cap.org > Search for "IVM Topic Center"



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THANK YOU!

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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 about 1 week.

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