COLLEGE of AMERICAN PATHOLOGISTS

Applications of In Vivo Microscopy in Surgical Pathology

Speakers:
Babar K Rao MD
Guillermo J Tearney MD, PhD
Wei Chen MD, PhD
Moderator:
Savitri Krishnamurthy MD

Oct. 29th, 2024

Conflict of Interest

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

Savitri Krishnamurthy, MD, FCAP

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

The CAP Committee hosting this webinar

Digital and Computational Pathology Committee

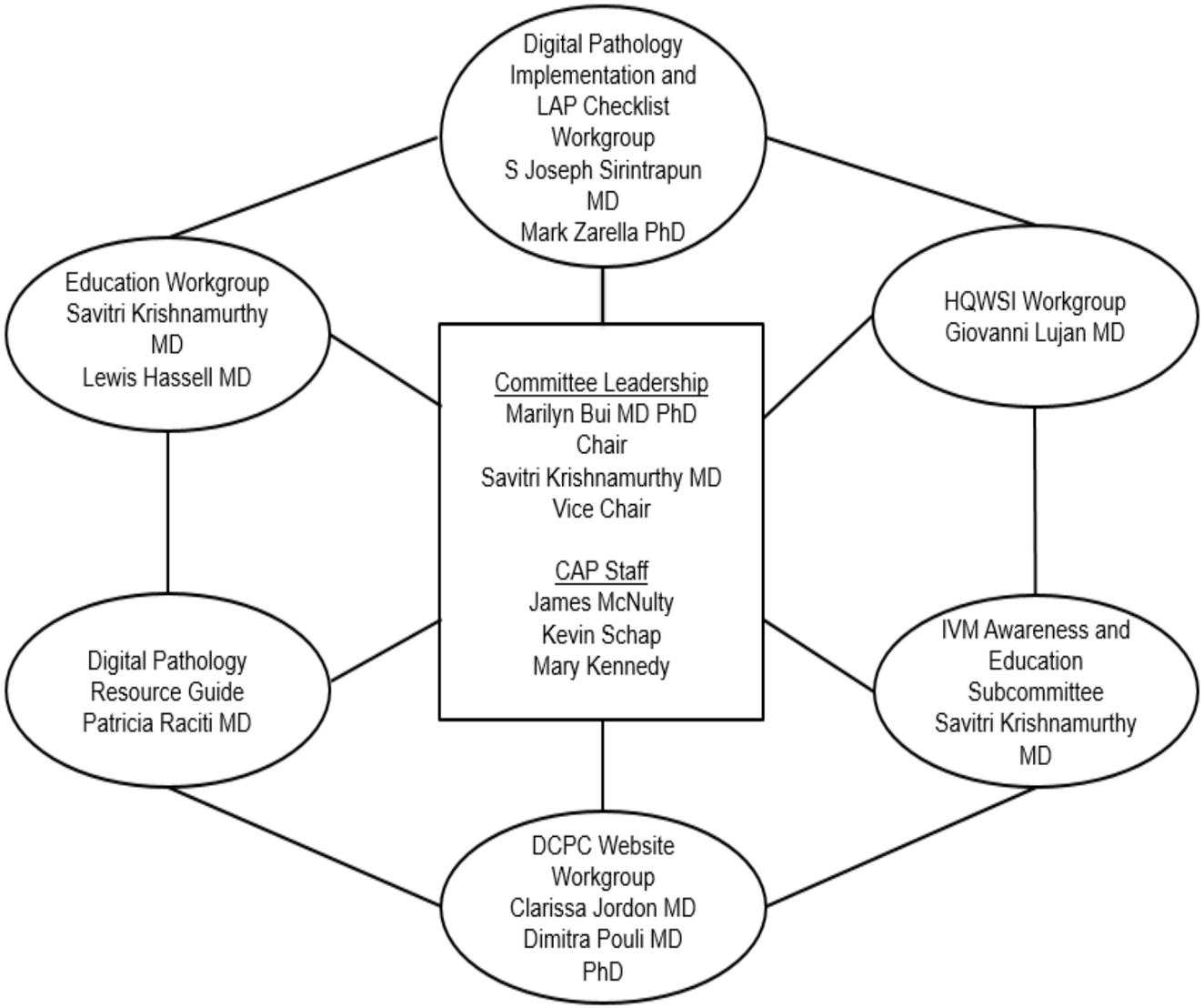
- **The charge of the Digital and Computational Pathology Committee (DCPC) is to advance the adoption of digital pathology within the CAP and to serve as a respected resource for information and education for pathologists, patients and the public on the practice and science of digital pathology.**

Committee Leadership

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

Digital and Computational Pathology Committee (DCPC)

- **Committee structure**



Composition of the DCPC

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

Webinar Agenda

TOPICS	PRESENTERS
IVM of the Skin	Dr. Rao
Application of EUS-nCLE in the Evaluation of Pancreatic Cystic Lesions	Dr. Chen
Gastrointestinal OCT Endomicroscopy	Dr. Tearney
A moderated discussion of audience questions	Dr. Krishnamurthy

Learning Objectives

Learning objectives:

- To learn about the basic principle of in vivo microscopy.
- To recognize the applications of in vivo microscopy in Surgical pathology practice.
- To understand the issues and challenges related to the utilization of in vivo microscopy in Surgical pathology practice

Babar K. Rao, MD, FAAD

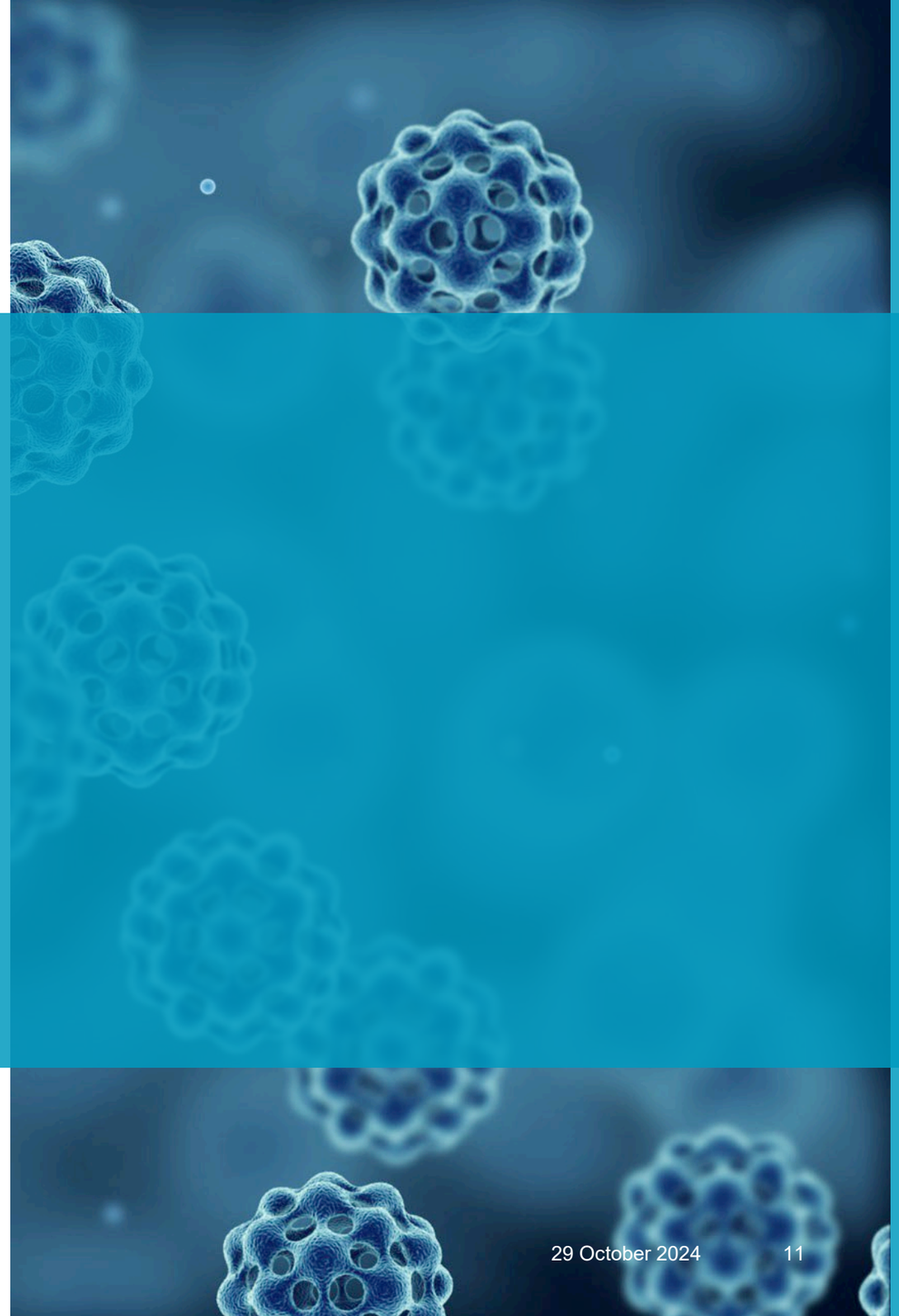
Dr. Rao is a Professor of Dermatology and Pathology at Rutgers Robert Wood Johnson Medical School and a Clinical Associate Professor of Dermatology at Weill Cornell Medical College. He is also President of the American Confocal Group and the President and Founder of Non-Invasive Diagnostic Innovations in Skin (NIDIskin) and EIV Diagnostics. Dr. Rao is board certified in dermatology and dermatopathology. He received his medical degree from Rawalpindi Medical College in Pakistan and completed his residency training in Dermatology at Cornell University Medical Center, where he was chief resident. Additionally, Dr. Rao has trained at University of London, UT Southwestern, New York University, and Rutgers University. His primary research interests include non-invasive diagnostic tools in dermatology, such as Reflectance Confocal Microscopy (RCM), and digital pathology, and he has authored over 200 publications and multiple books on the topics.

IVM of the Skin

Babar K. Rao, MD, FAAD

Conflicts of Interest

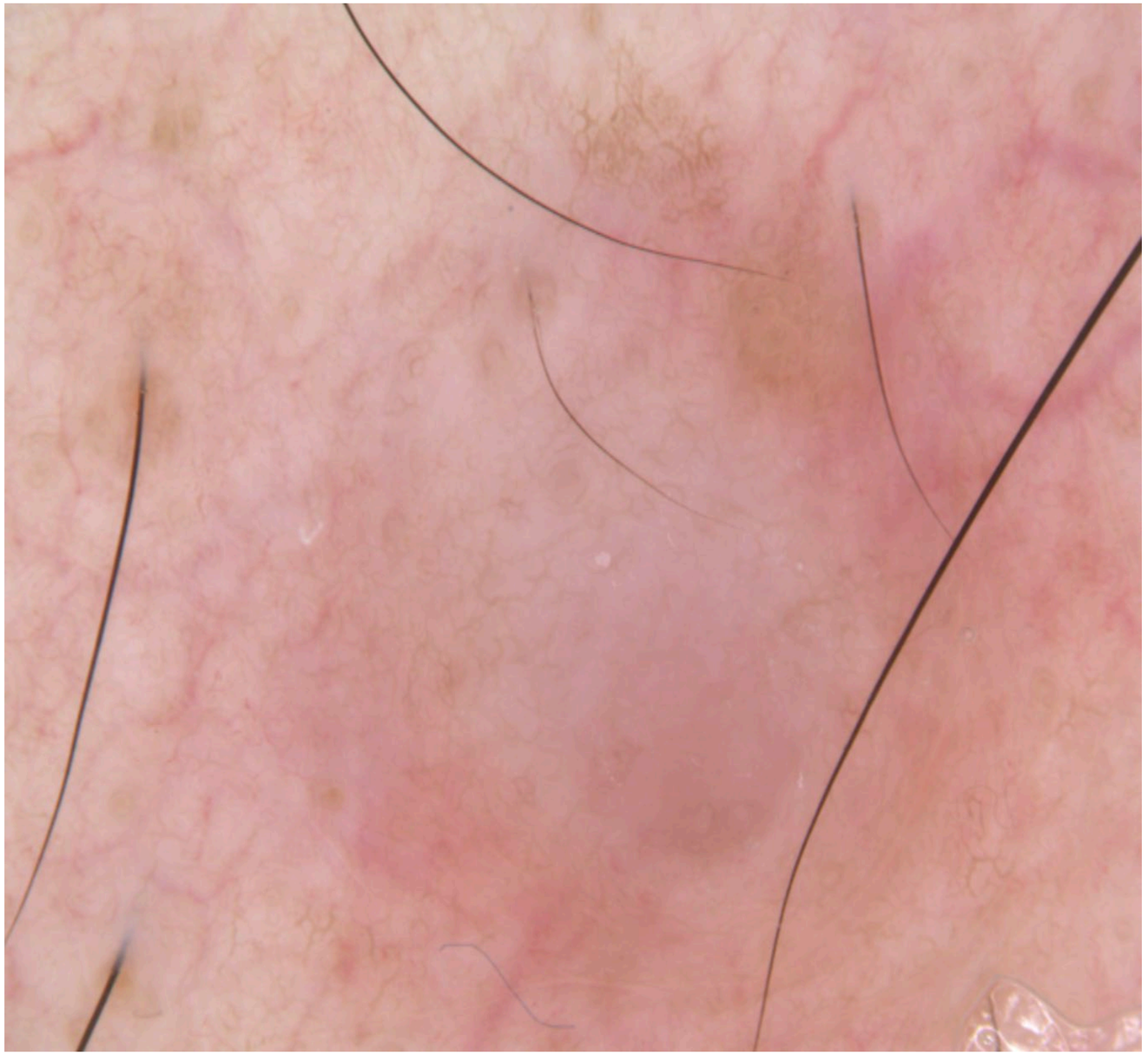
- Founder of NIDI Skin (https://nidiskin.com/)

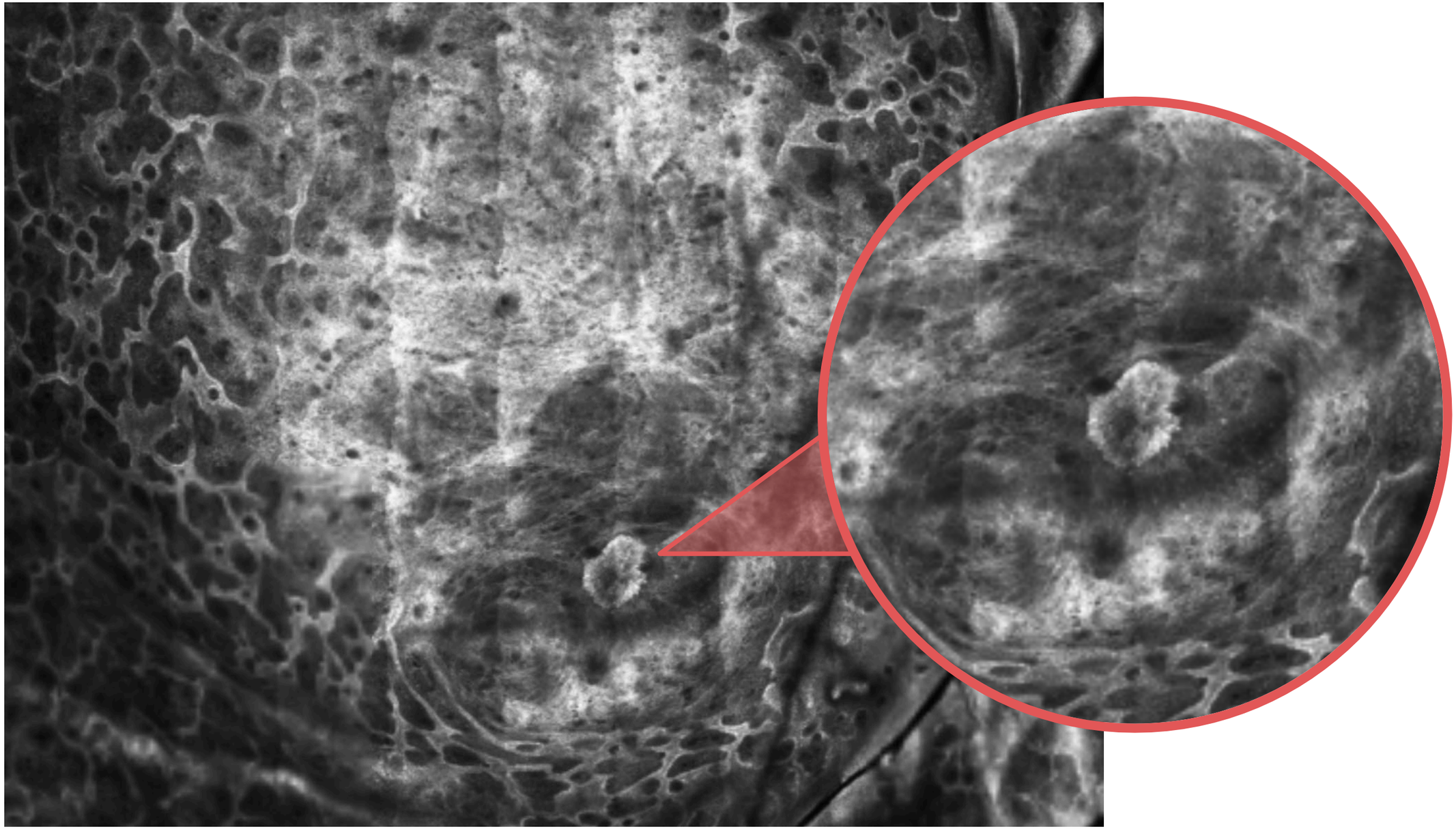


Objectives

- Discuss modalities of in vivo microscopy (e.g. RCM, LC-OCT)
- Discuss case using RCM
- Discuss research using LC-OCT
- Introduce ex vivo confocal microscopy
- Discuss billing and reimbursement of in vivo imaging modalities

In Vivo





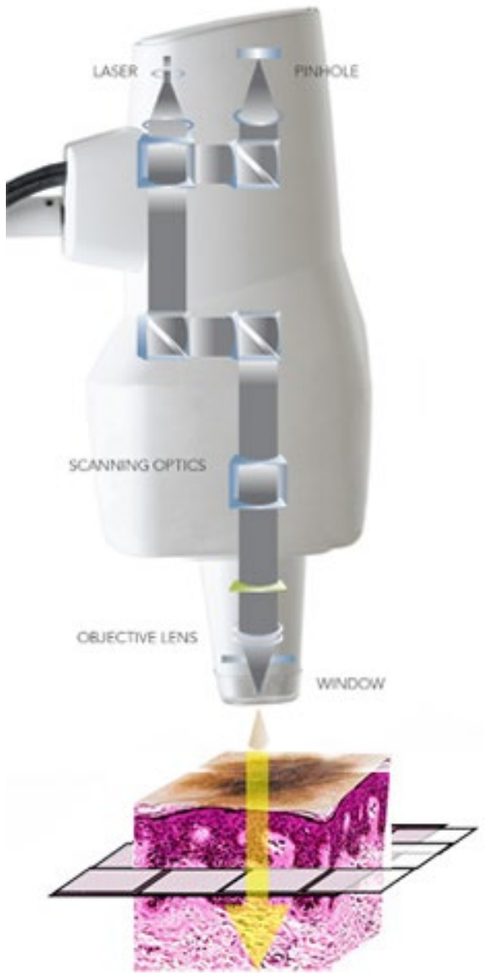
Intro to In Vivo

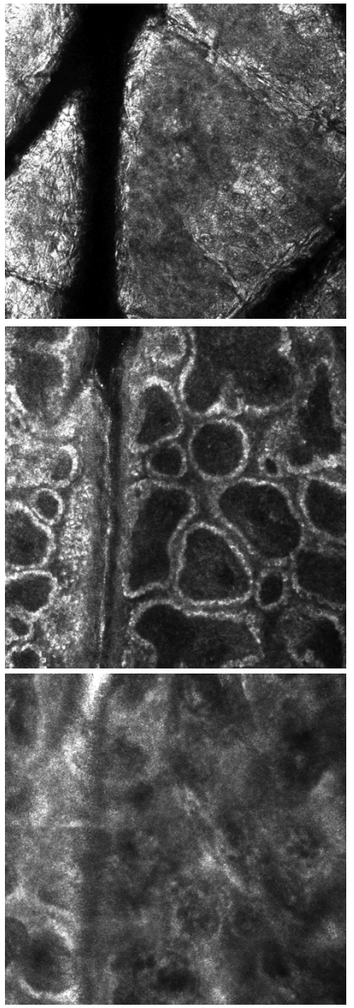
- 5.3 million skin biopsies are performed annually, resulting in $289 million in healthcare costs[2]
- Of the pigmented lesions biopsied, 83.1% are benign or mildly dysplastic (requiring no re-excision)[3]
- Scarring from biopsy/excision has a negative psychosocial impact on patient, but observation leaves patient with anxiety of the unknown…

***Solution?* In Vivo Microscopy**

Intro to Reflectance Confocal Microscopy (RCM)

- *En face* (horizontal), grayscale images
- Captured in vivo with a laser device (830 nm) down to 200-300 µm depth
- Cellular resolution
- High accuracy diagnosis without biopsy, fixation, sectioning, or staining





Reflectance Confocal Microscopy

- FDA 510(k) Cleared in 2008
- Category I CPT Codes in 2016
- >1000 peer-reviewed journal articles focused on RCM skin imaging applications
- >700 installations world-wide in 36 countries

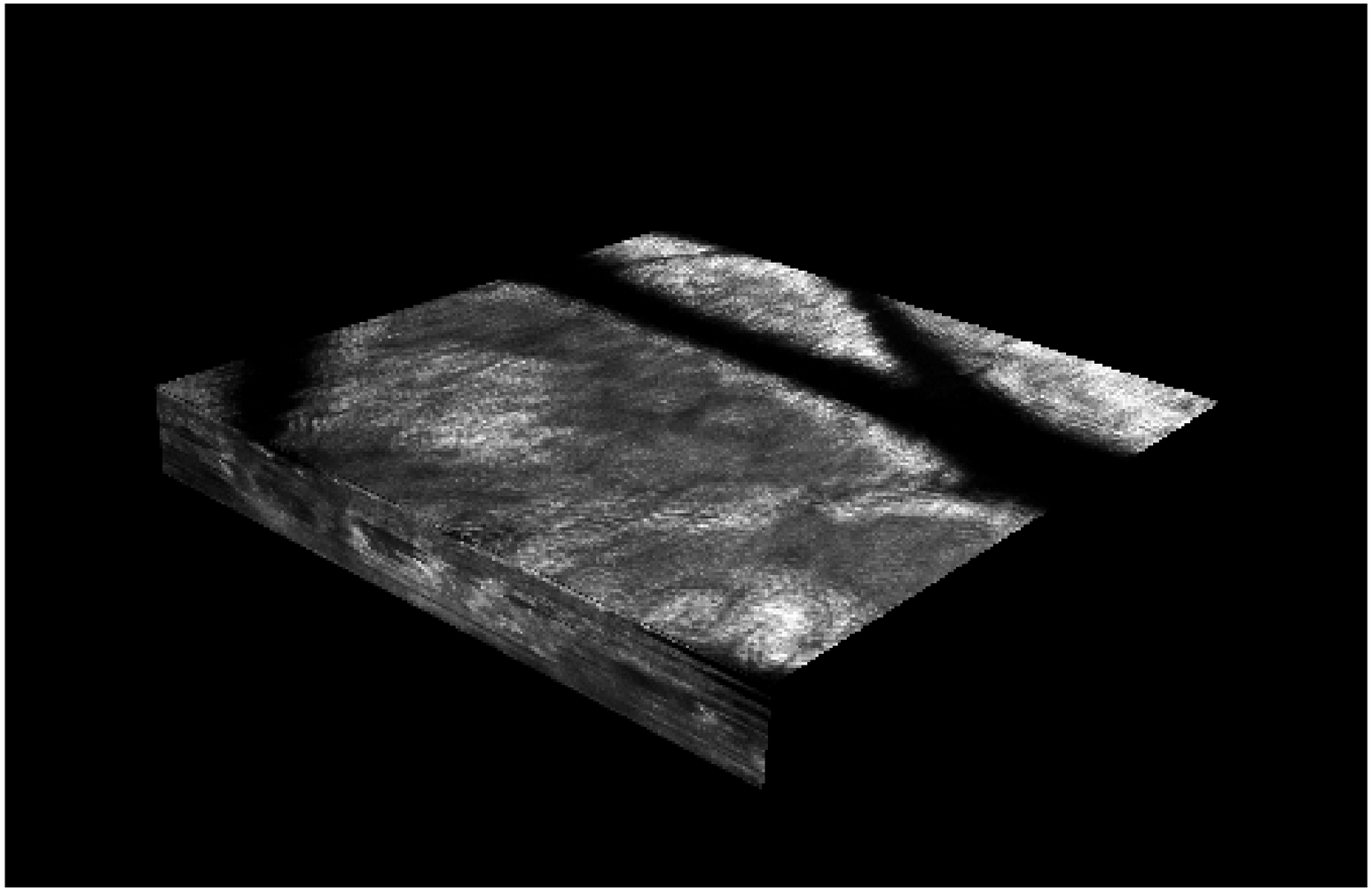
Applications of In Vivo RCM

- Diagnosis & treatment monitoring of melanoma and non-melanoma skin cancers, inflammatory lesions, infestations
- Tumor margin delineation, especially for superficial tumors like lentigo maligna/lentigo maligna melanoma (93% sensitivity, 82% specificity)
- **Melanoma: 92.3 - 100% Sensitivity 69 - 92.4% Specificity**
- **BCC: 66.7 - 100% Sensitivity 78%-100% Specificity**
- **SCC: 100% Sensitivity 75% Specificity**

Guitera P, Pellacani G, Crotty KA, et al. The impact of in vivo reflectance confocal microscopy on the diagnostic accuracy of lentigo maligna and equivocal pigmented and nonpigmented macules of the face. *J Invest Dermatol*. 2010;130(8):2080-2091.

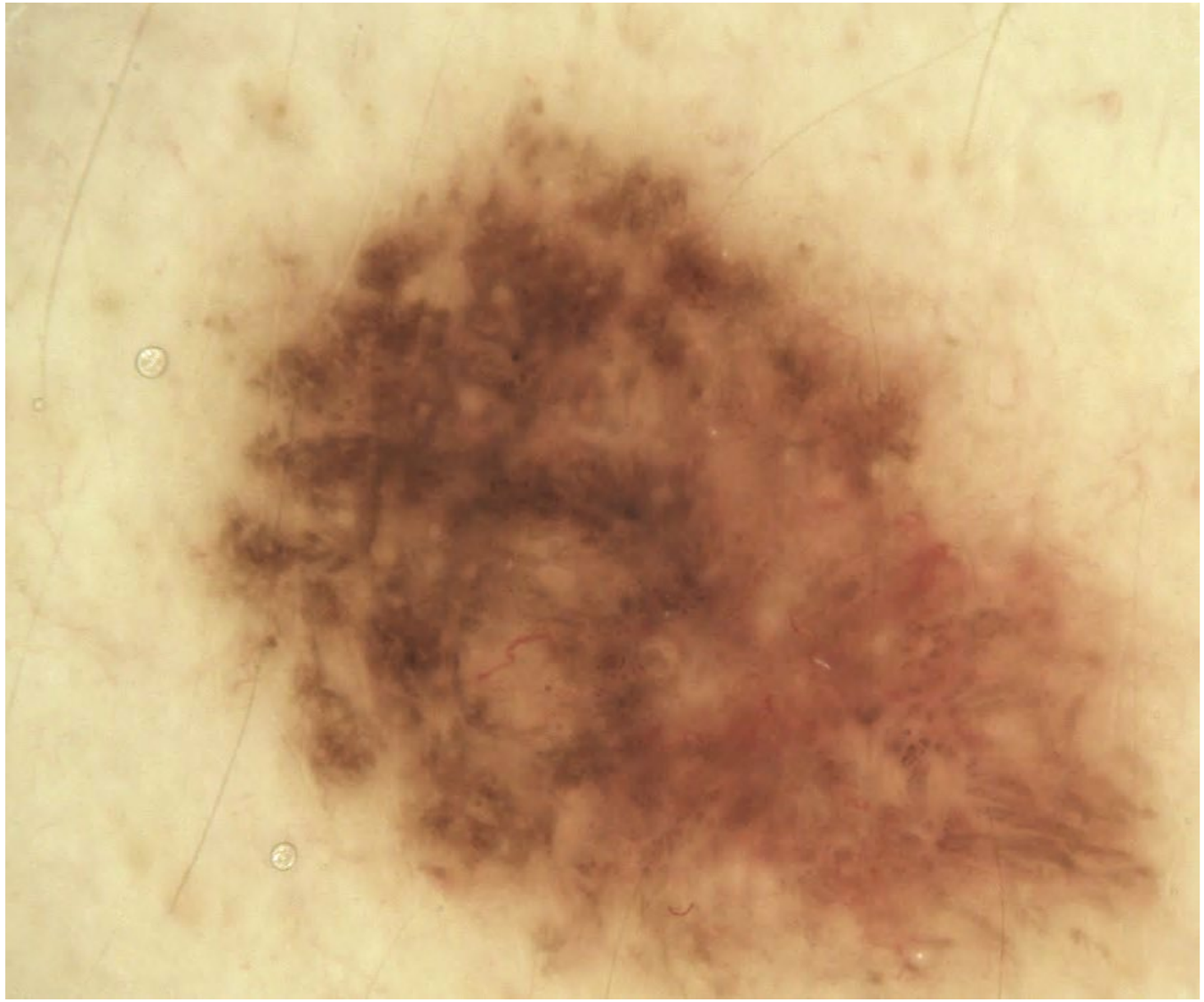
Edwards, S. J., Osei-Assibey, G. , Patalay, R. , et al. Diagnostic accuracy of reflectance confocal microscopy using VivaScope for detecting and monitoring skin lesions: a systematic review. Clin Exp Dermatol. 2017; 42: 266-275.

3D Reconstruction Video

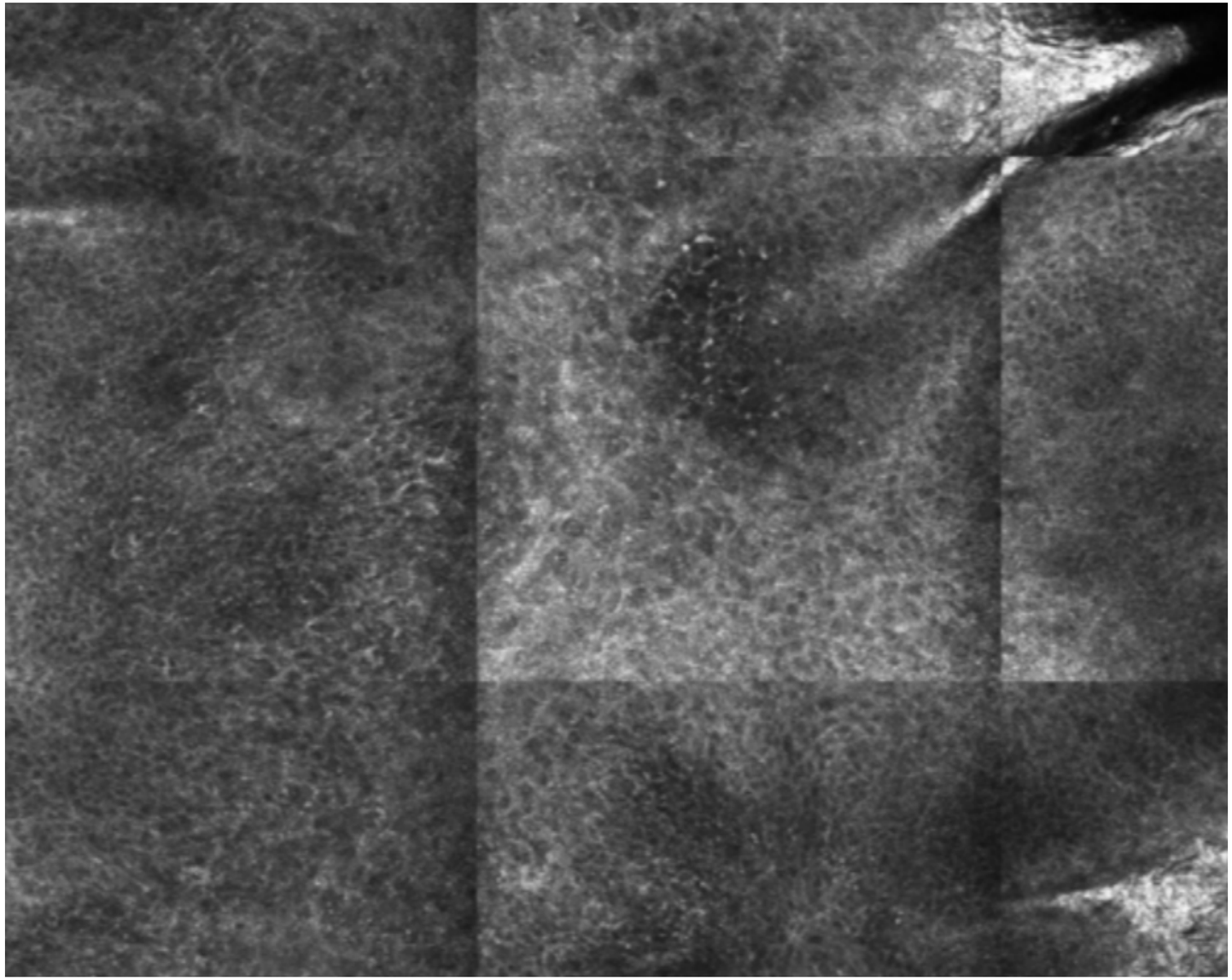


Practice Case

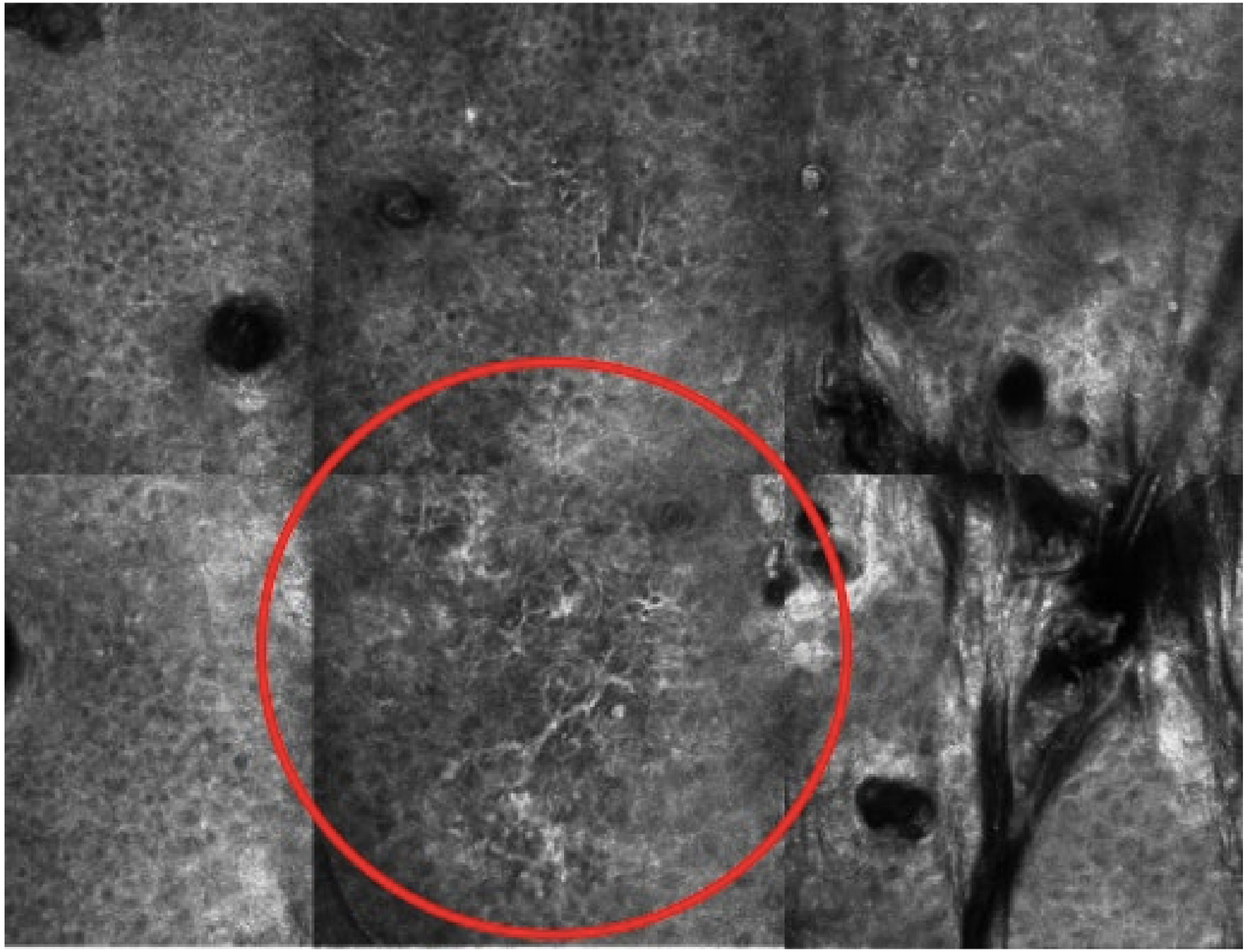
Dermoscopy



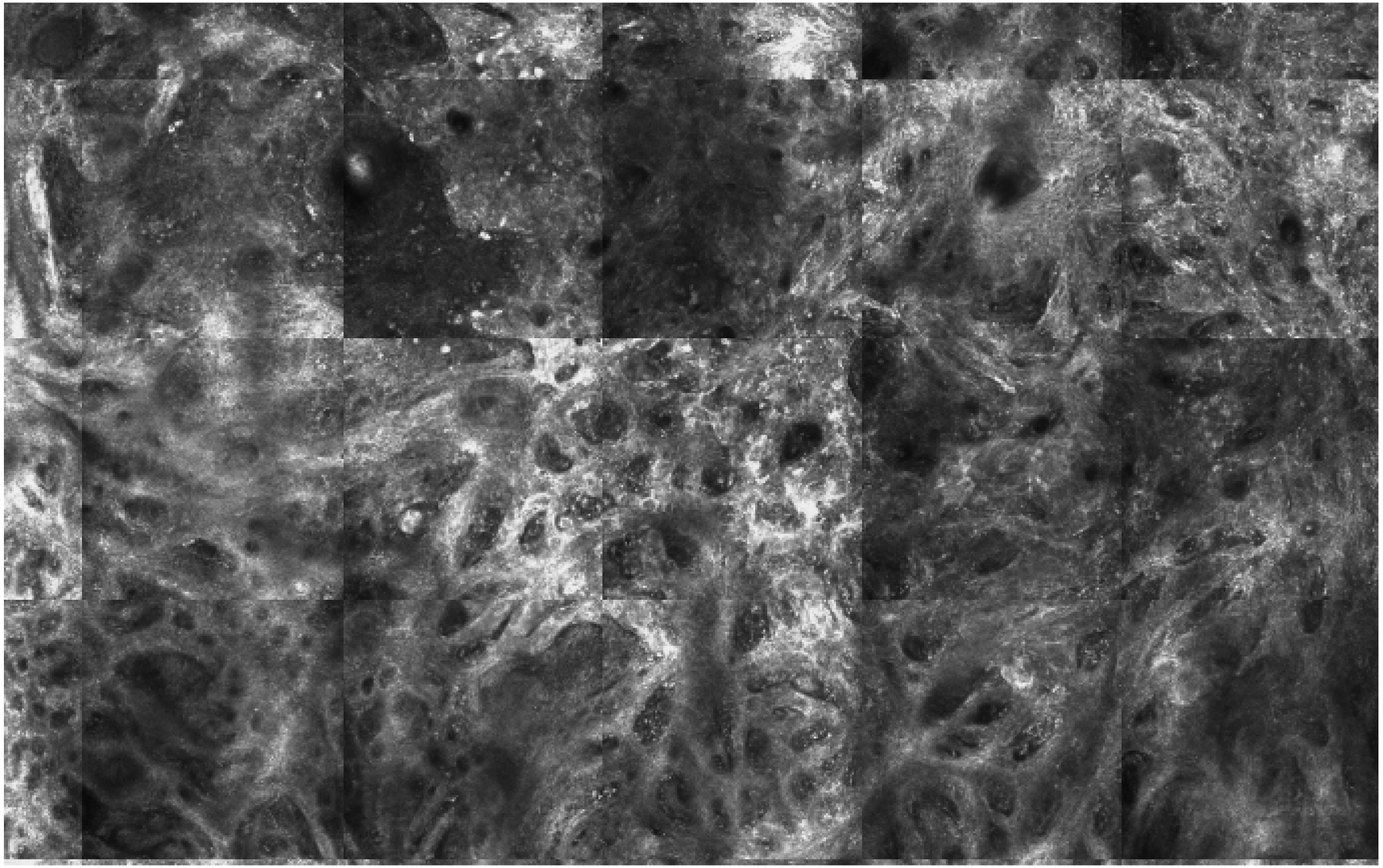
Irregular Honeycomb



Dendritic & Roundish Cells

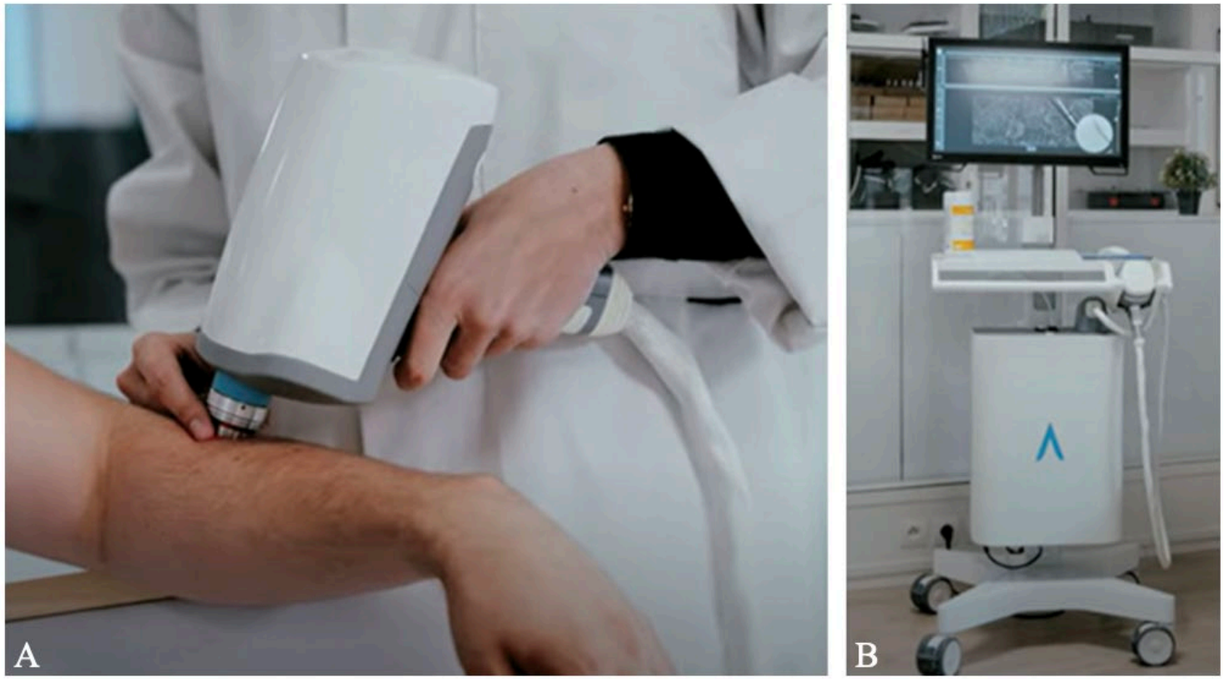


DEJ Disarray



Dx: Melanoma

Practical Uses of LC-OCT

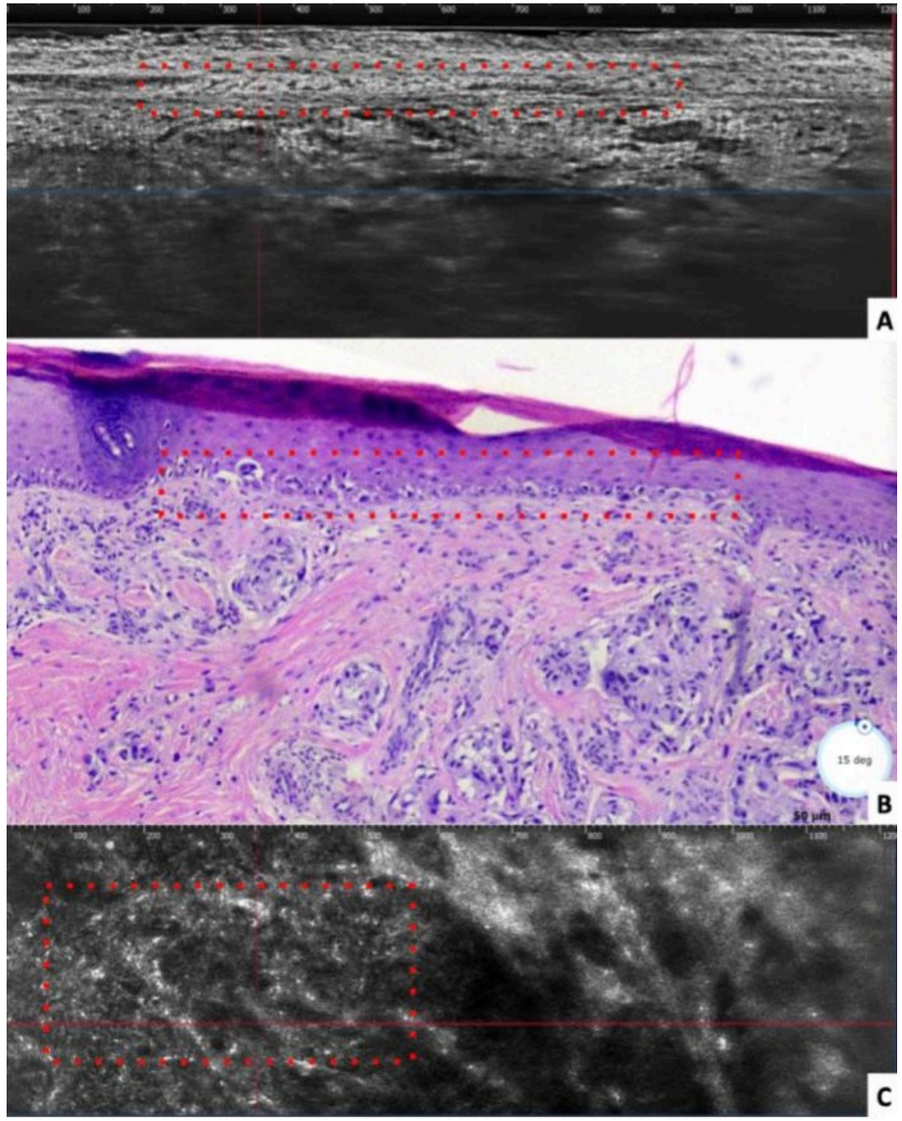


Ogien J, Tavernier C, Fischman S, Dubois A. Line-field confocal optical coherence tomography (LC-OCT): principles and practical use. Ital J Dermatol Venerol. 2023 Jun;158(3):171-179. doi: 10.23736/S2784-8671.23.07613-2.

- Vertical mode assesses lobular structures, the DEJ, and the position of cellular atypia in the epidermis
  - Useful for diagnosing BCC, SCC, AK, or inflammatory lesions
- Horizontal mode analyzes the regularity of the keratinocyte network and dendritic cells
- 3D mode is best for atypical melanocytic lesions

Visualizing Amelanotic Melanoma

Vertical and Horizontal Views vs. H&E

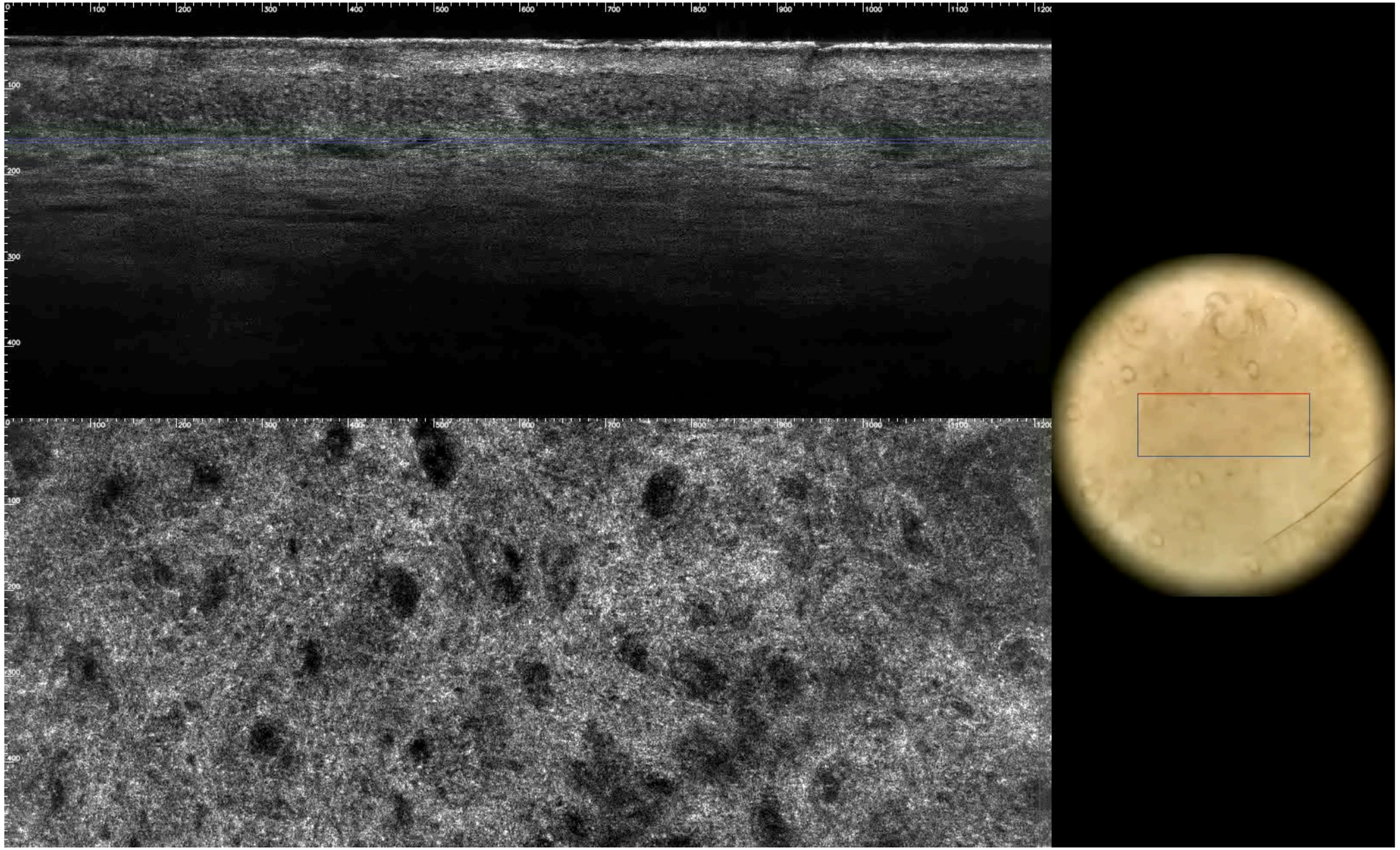


A. LC-OCT Vertical view, melanocyte nests displayed varying sizes and shapes, unevenly distributed at the dermal-epidermal junction (red dotted box)

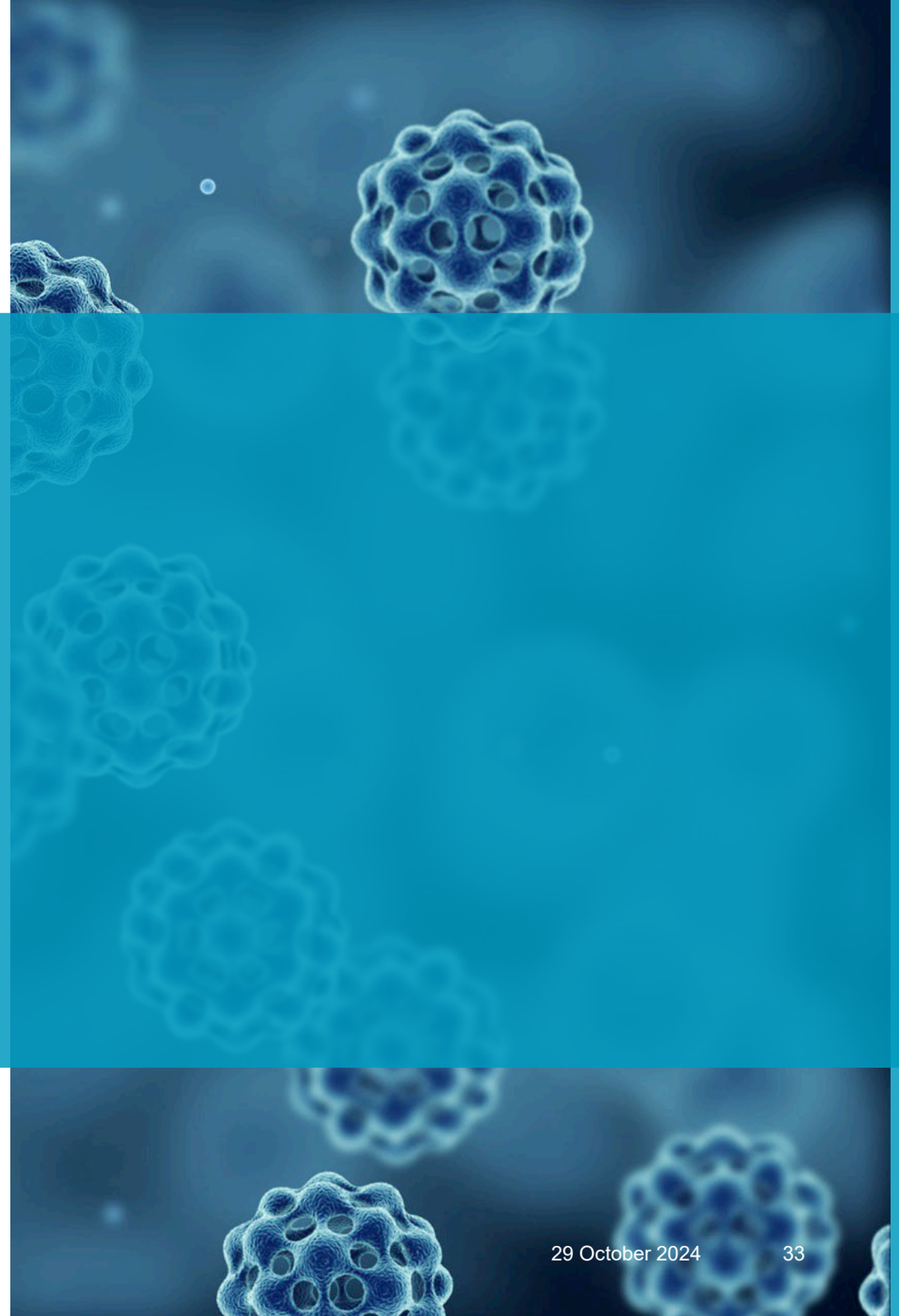
B. H&E 20x showing Melanocyte nests displayed varying sizes and shapes, unevenly distributed at the dermal-epidermal junction (red dotted box)

C. LC-OCT Horizontal view, large atypical nests of pagetoid cells with dendritic protrusions (red dotted box)

Collagen Response to Various Treatments



Thank You!



Wei Chen, MD, PhD, FCAP

Dr. Chen is a member of the Digital and Computational Pathology Committee. She is an Associate Professor and Director of Pancreaticobiliary Pathology at The Ohio State University in Columbus, OH. She completed both her Pathology residency and a fellowship in Gastrointestinal and Hepatopancreaticobiliary Pathology at The Ohio State University.

Application of EUS-nCLE in the Evaluation of Pancreatic Cystic Lesions

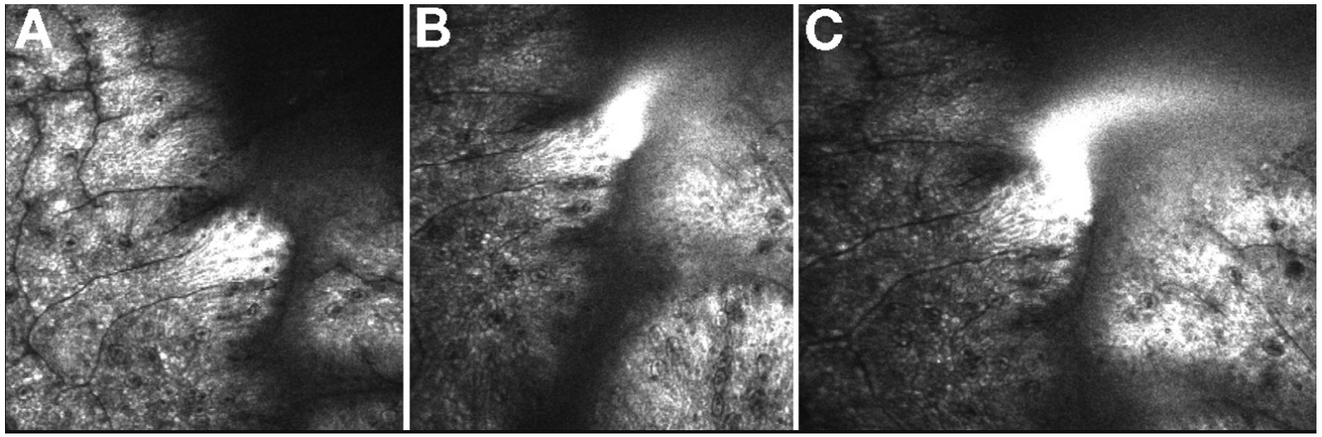
Wei Chen, MD, PhD, FCAP

29 October 2024

Table of Contents

In Vivo Microscopy in GI Pathology

- Real-time endomicroscopic imaging without biopsy
  - Changes invisible on fixed tissue
- Useful in assessing Barrett's esophagus, colon (IBS, IBD, polyps), pancreatic cysts, etc
  - Non-neoplastic
  - Neoplastic



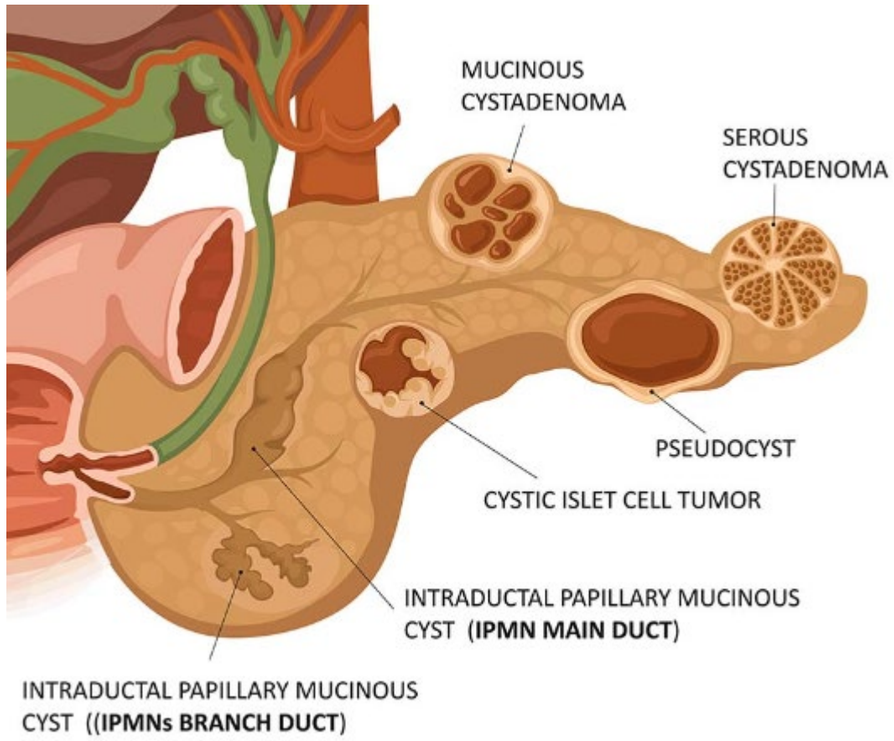
Leaky gut: CLE of duodenal mucosa demonstrates mucosal damage after food challenge. The intact epithelium (*A*) starts to break its continuity (*B*), forming a leak with eruption of fluorescein into the gut lumen (*C*).

Fritscher-Ravens Gastroenterology 2014

Introduction:
Pancreatic Cystic Lesions & EUS-nCLE

Pancreatic Cystic Lesions (PCLs)

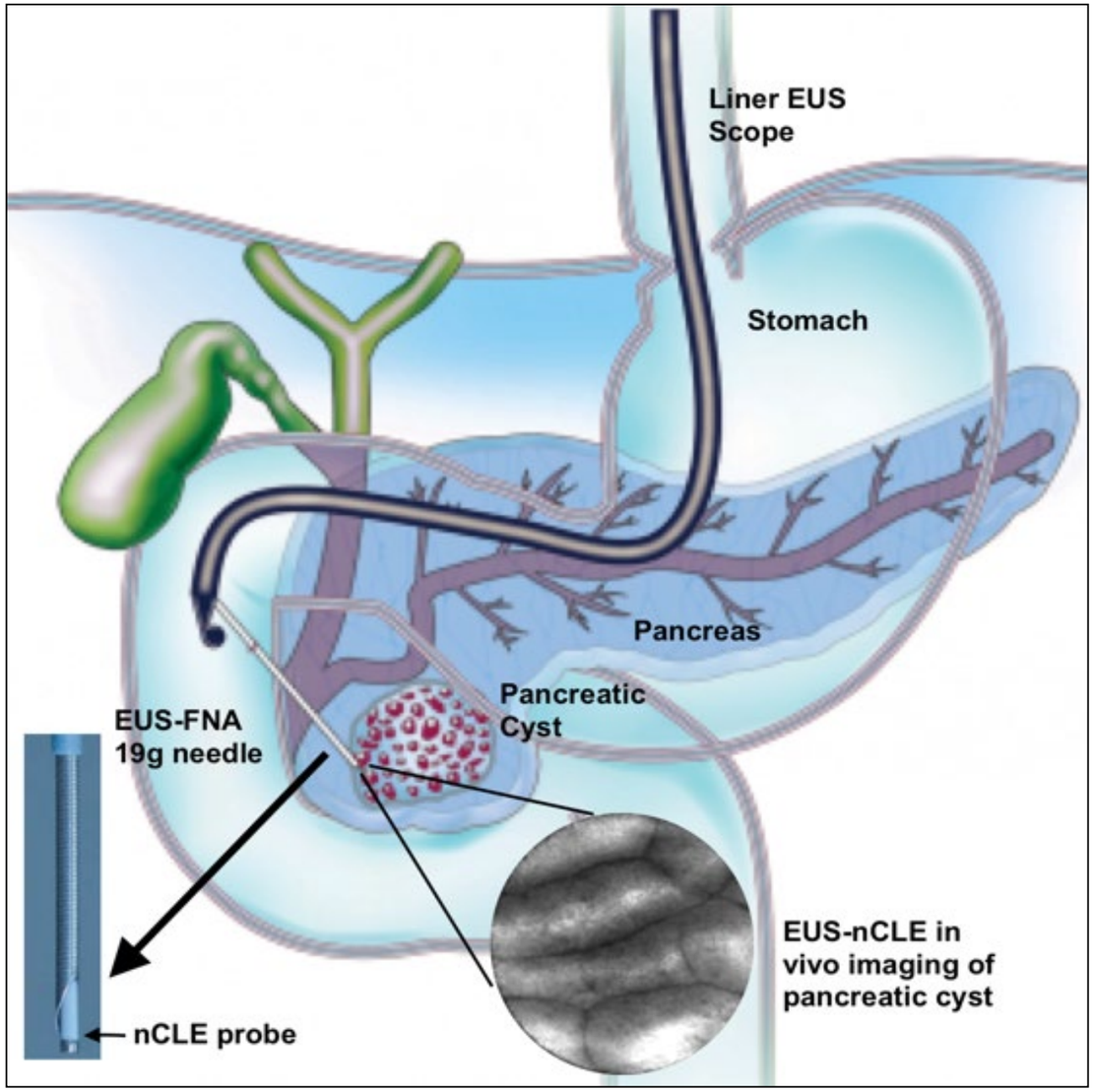
- **Importance:**
  - Incidental PCLs ~15% and increase with age
  - Benign, premalignant, and malignant
  - Accurate diagnosis and risk stratification crucial
- **Challenges:**
  - Traditional imaging (CT/MRI) and EUS-FNA have limitations
  - Cytology has low sensitivity; imaging alone lacks specificity
- **EUS-nCLE**
  - Clinical-grade equipment commercially available
  - Fully reimbursed!
  - High diagnostic accuracy



Kimura Int J Pancreatol 1995; Zerboni Pancreatology 2019; Wu Clin Endosc 2024; Gardner Gastroenterology 2024

EUS-nCLE

- Endoscopic Ultrasound Guided Needle-based Confocal Laser Endomicroscopy
- A miniprobe advanced through a 19-gauge EUS-FNA needle
- In vivo real-time imaging
  - IV fluorescein
  - Contrasts vasculature and tissue structures
  - Specifications:
    - Field of view 0.325mm (LM: 60x)
    - Resolution 3.5µm (RBC: 7µm)
    - Depth of observation 40-70µm



EUS-nCLE of PCL, head of pancreas

Image courtesy of Dr. Somashekar G. Krishna

Wu Clin Endosc 2024

EUS-nCLE of Pancreatic Cystic Lesions (PCLs)

- **How much % of PCLs at OSU are evaluated by EUS-nCLE?**
  - **>3cm, ~95%**
  - **2-3 cm, ~50% depending on the need**
  - **<2 cm, none (does not impact mgmt.; cyst fluid analysis/NGS study)**

- **How much % of the cyst epithelium is typically covered by nCLE?**
  - **Uncinate 30-40% - most difficult due to scope position**
  - **Head, neck, tail 40-60%**
  - **Body 80%**

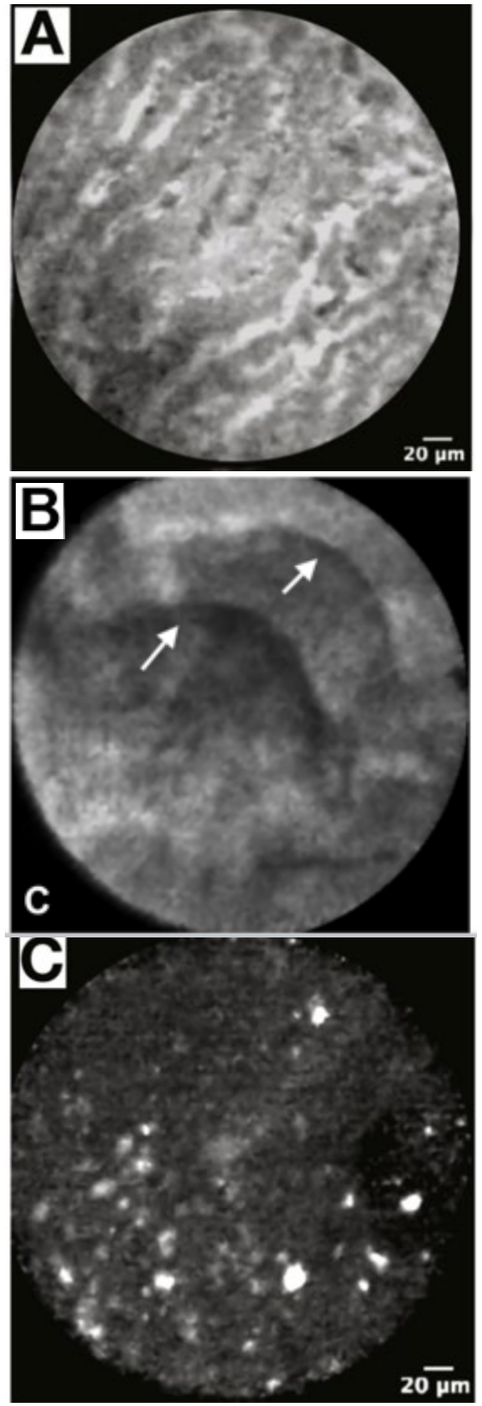
EUS-nCLE Evaluation of PCLs

- **Pros:**
  - **Real-time diagnosis**
    - **Immediate classification and risk stratification**
    - **Even when fluid/tissue analysis is inconclusive**
  - **Unique in vivo features (vascular pattern)**
- **Cons**
  - **Unable to cover the entire cyst epithelium**
  - **Possible post-procedural pancreatitis**
    - **2-3%, mild pancreatitis, no reported fatality to date**
  - **Cost and expertise**

Napoleon Endosc Int Open. 2020; Wu Clin Endosc 2024.

EUS-nCLE: Image Interpretation
Different Features than Histology!

- IV fluorescein / contrast
- Highlight blood vessels
  - Classification: Diagnostic vascular patterns
  - Risk stratification: Increased density/complexity in HGD/Ca
- Negative image of tissue structure
  - Dye gradually leaks out into surroundings
  - Epithelial contours - black lines
- Auto-fluorescent structures: Macrophages



Krishna SG. Clin Gastroenterol Hepatol. 2020; Krishna SG, Gastrointest Endosc. 2020

Two Main Tasks When Evaluating PCLs

- **Classification**
  - **Mucinous:**
    - **20% PDAC associated with mucinous cysts**
    - **Intraductal papillary mucinous neoplasm (IPMN)**
    - **Mucinous cystic neoplasm (MCN)**
  - **Non-mucinous:**
    - **Serous cystadenoma (SCA)**
    - **Pseudocyst**
    - **Pancreatic neuroendocrine neoplasm (pNEN)**
    - **Solid pseudopapillary neoplasm (SPN)**
- **Risk Stratification**
  - **60% resected IPMNs have only LGD on histology**
  - **Low vs. High risk**
  - **Surveillance vs. Surgical resection**

Classification of PCLs by EUS-nCLE

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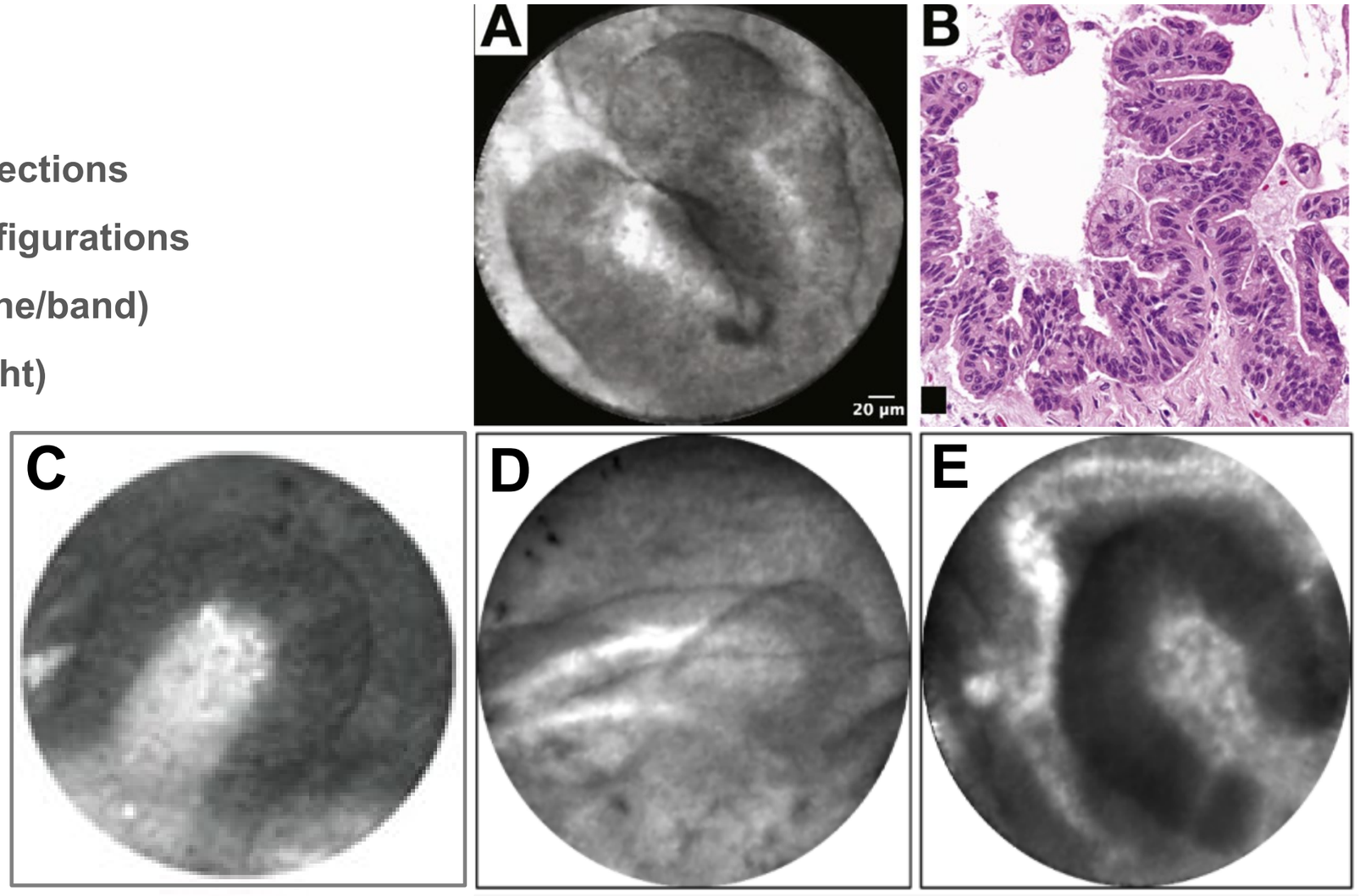
EUS-nCLE: Intraductal Papillary Mucinous Neoplasm (IPMN)

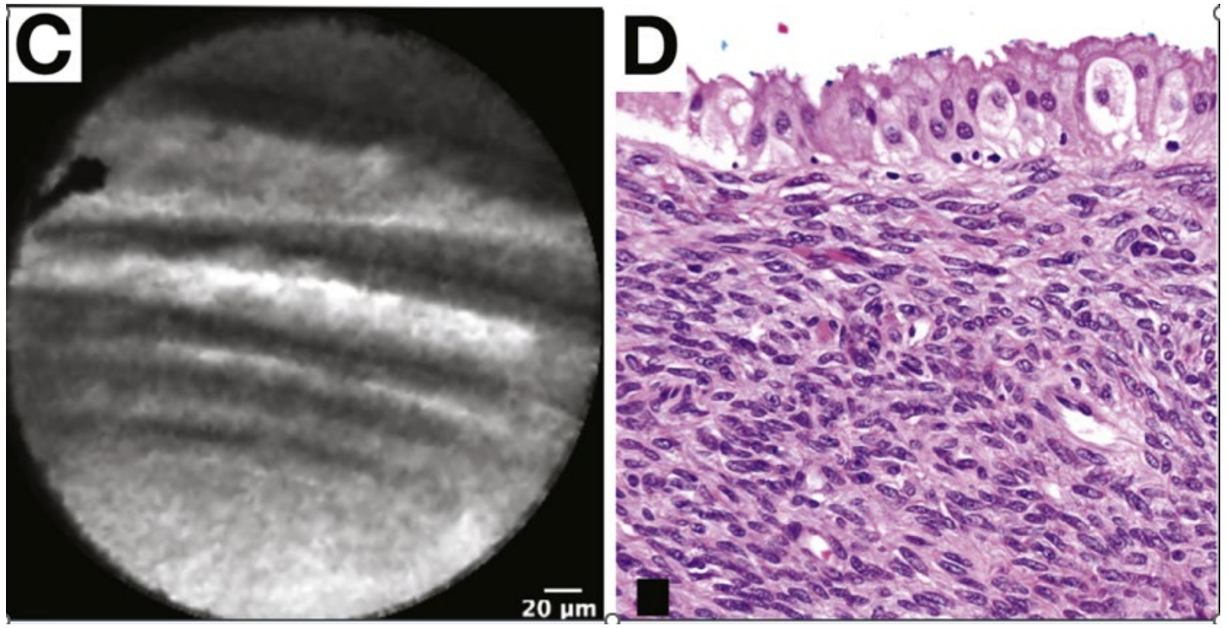
- **nCLE Features:**
  - Finger-like papillary projections
    - Various shapes & configurations
  - Outer epithelium (dark line/band)
  - Inner vascular core (bright)



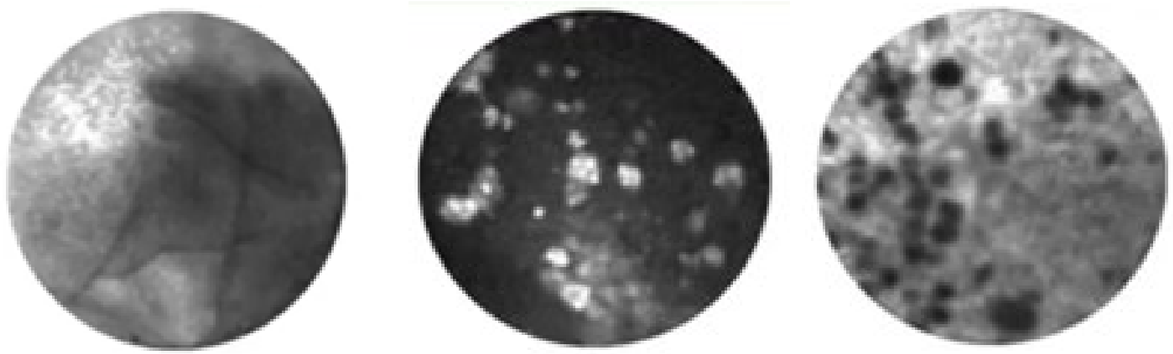
Krishna SG. Clin Gastroenterol Hepatol. 2020; Chen Diagnostics 2022

EUS-nCLE: Mucinous Cystic Neoplasm (MCN)

- **nCLE Features:**
  - Horizon-type epithelial bands
    - Single or multiple layers
  - No papillary conformation
  - ± Auto-fluorescent macrophages and floating inflammatory cells



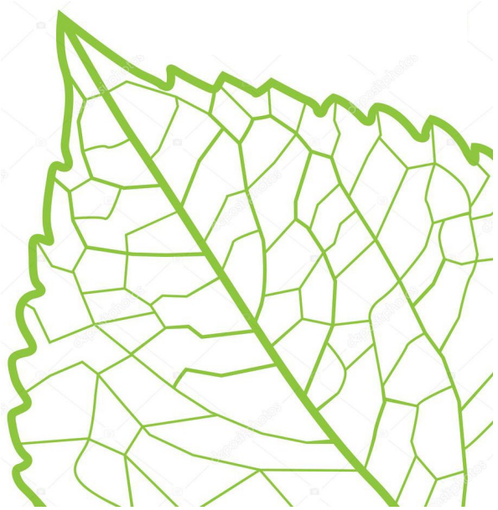
Krishna SG. Clin Gastroenterol Hepatol. 2020

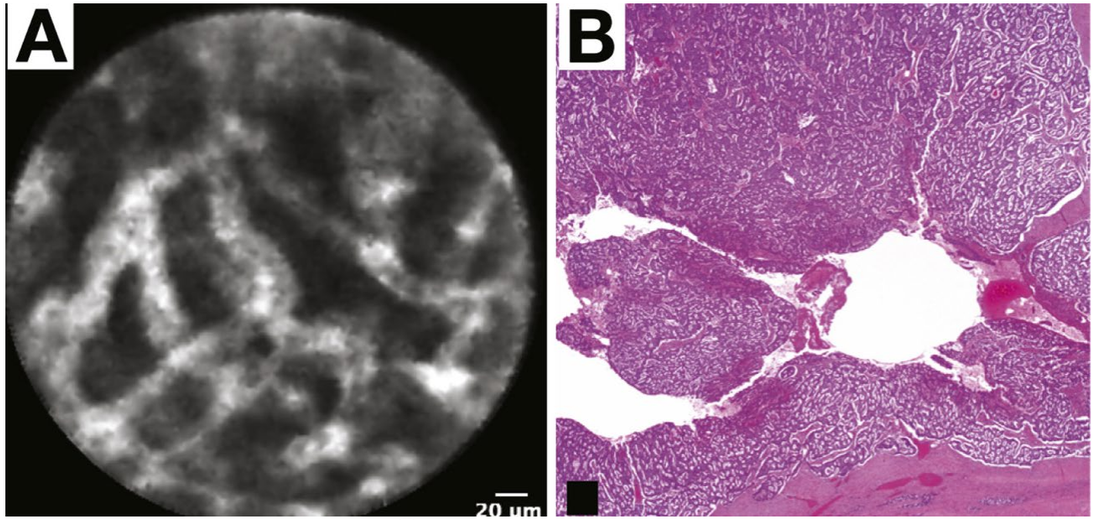


Images courtesy of OSU Gastroenterology and Dr. SG Krishna

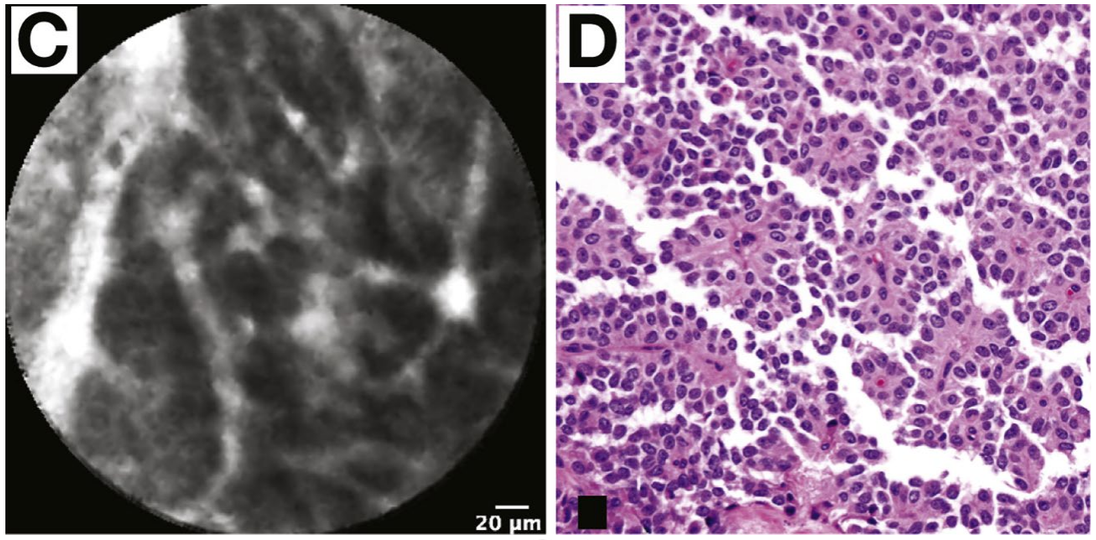
EUS-nCLE: Cystic pNEN and SPN

- **nCLE Features:**
  - **Cystic NET:**
    - **Leaf vein pattern**
    - **Dark clusters (trabeculae) of cells separated by vascular stroma**
  - **Solid pseudopapillary neoplasm (SPN)**
    - **Similar to NET**





Cystic NET

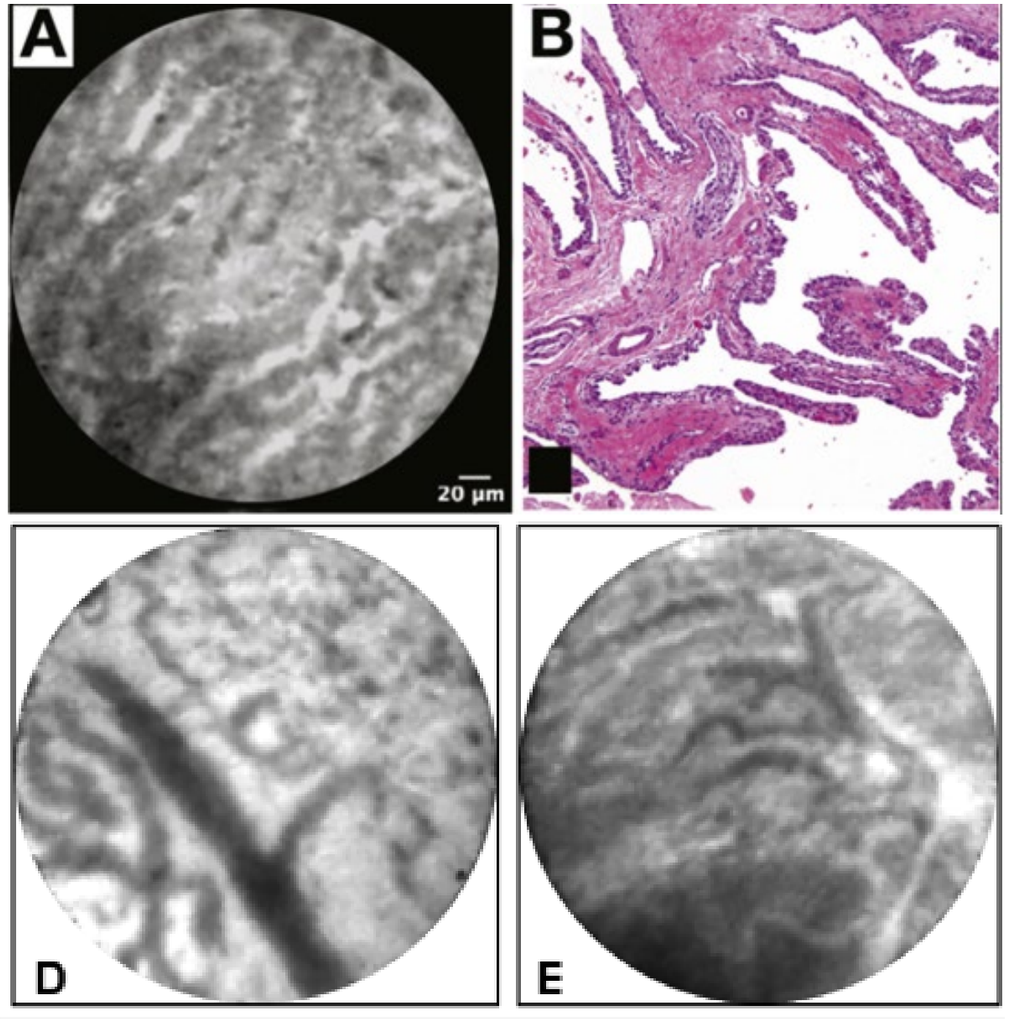


SPN

Krishna SG. Clin Gastroenterol Hepatol. 2020

EUS-nCLE: Serous Cystadenoma (SCA)

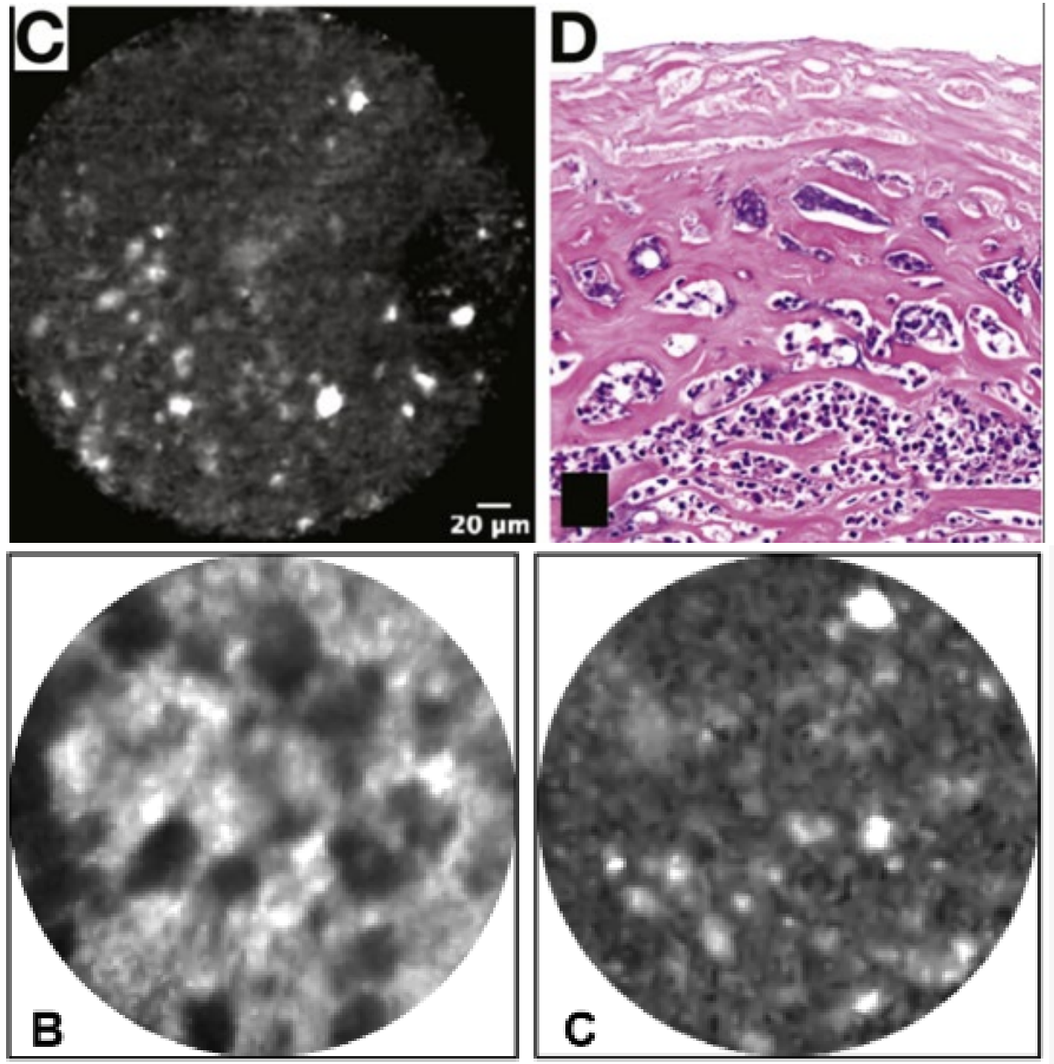
- **nCLE Features:**
  - Subepithelial, parallel or interconnected, network of blood vessels
    - Fern pattern of vascularity
    - Superficial vascular network
  - Flow of RBCs within the capillaries



Krishna SG. Clin Gastroenterol Hepatol. 2020; Chen Diagnostics 2022

EUS-nCLE: Pseudocysts

- **nCLE Features:**
  - **Dark background due to lack of vascular interstitium**
  - **Floating auto-fluorescent macrophages or dark clumps of inflammatory debris**



Krishna SG. Clin Gastroenterol Hepatol. 2020; Chen Diagnostics 2022

Risk Stratification of IPMNs by EUS-nCLE

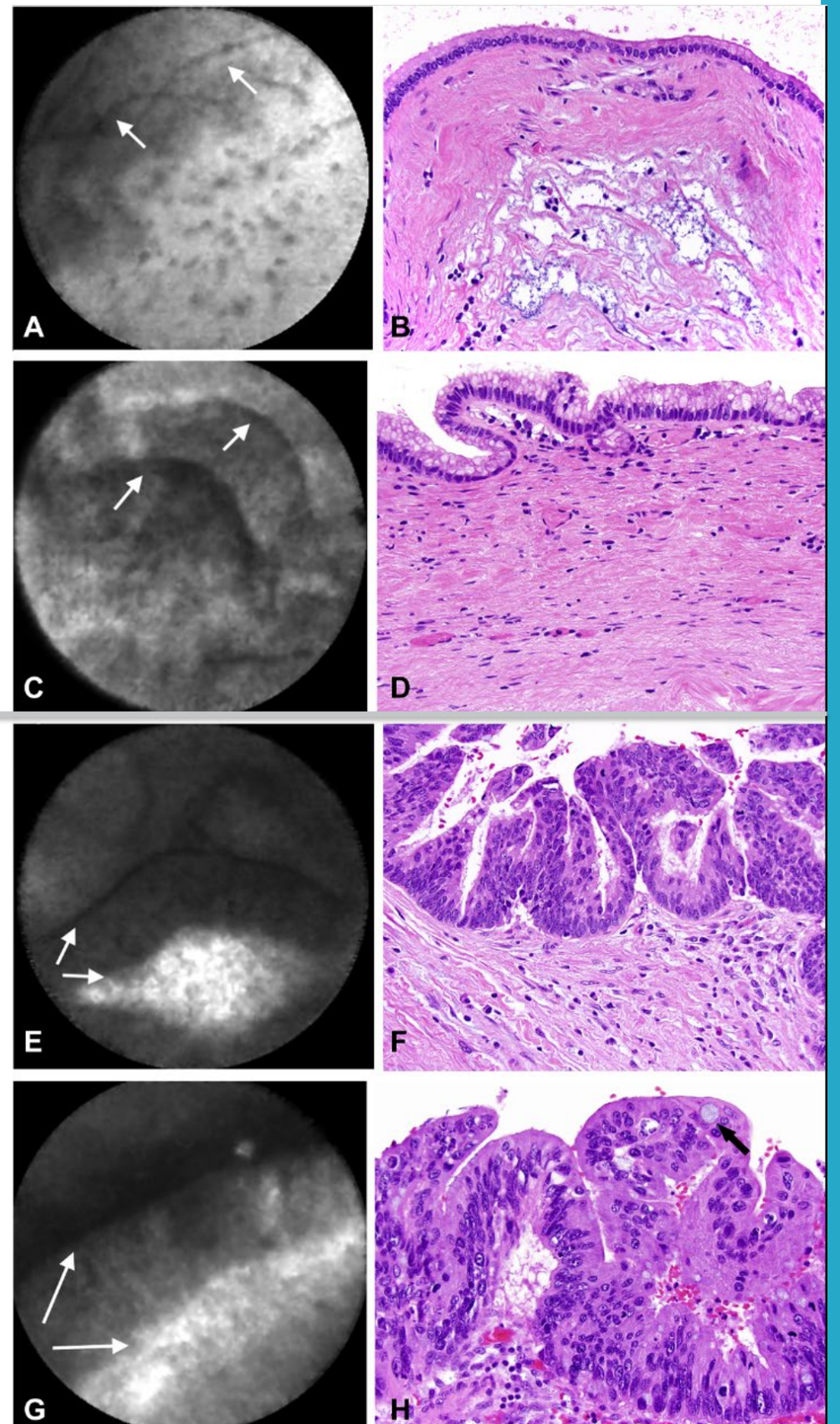
IPMN: Risk Stratification by nCLE

- Low-grade dysplasia (LGD)
  - Small, scalloped papillae
  - Thin, translucent epithelial lines
  - Rope-ladder vascularity
  - Pauci-cellular background

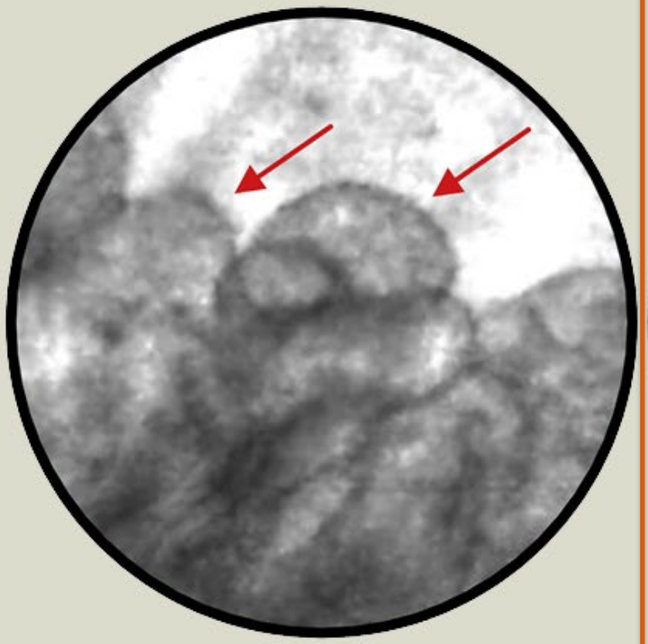
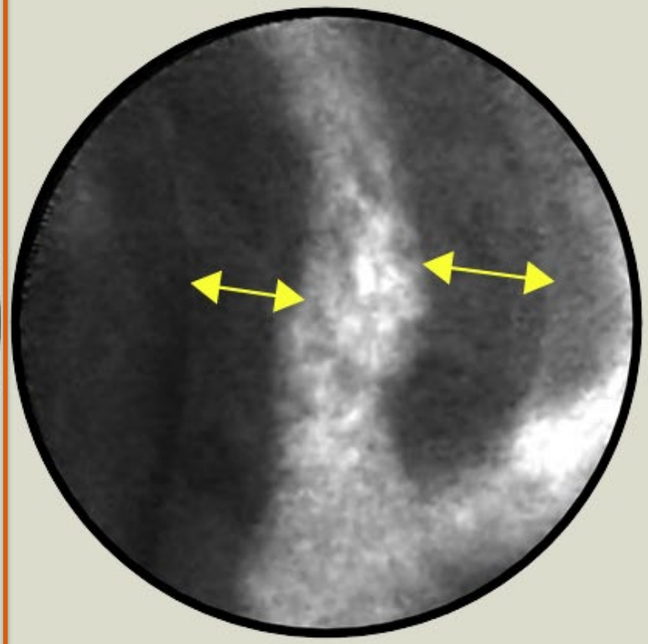
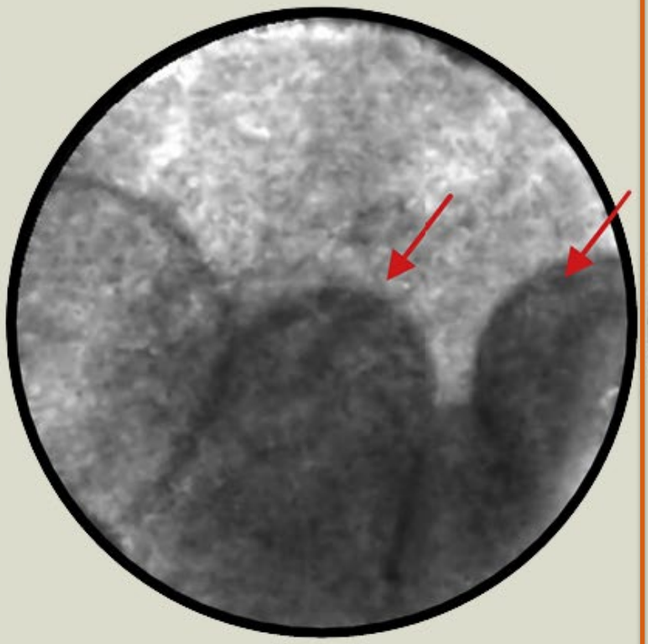
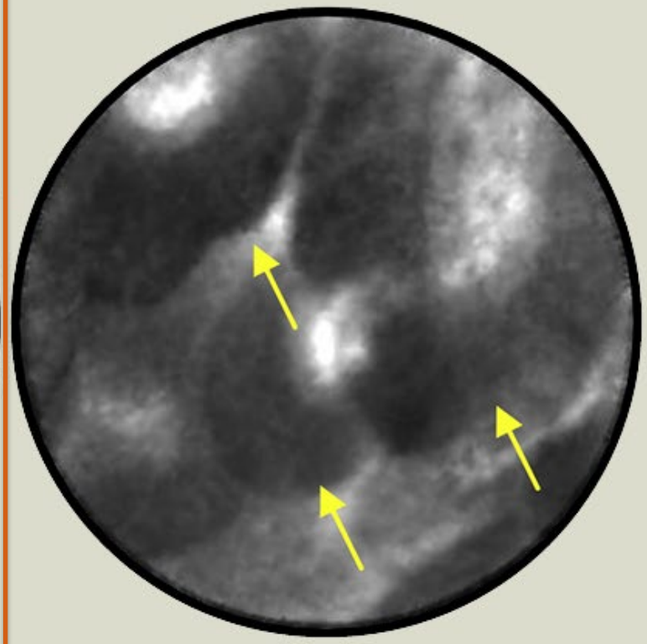
- High-grade dysplasia (HGD)
  - Large, irregular papillae
  - Thick, dark epithelial bands
  - Complex vascularity
  - More cellular background

LGD

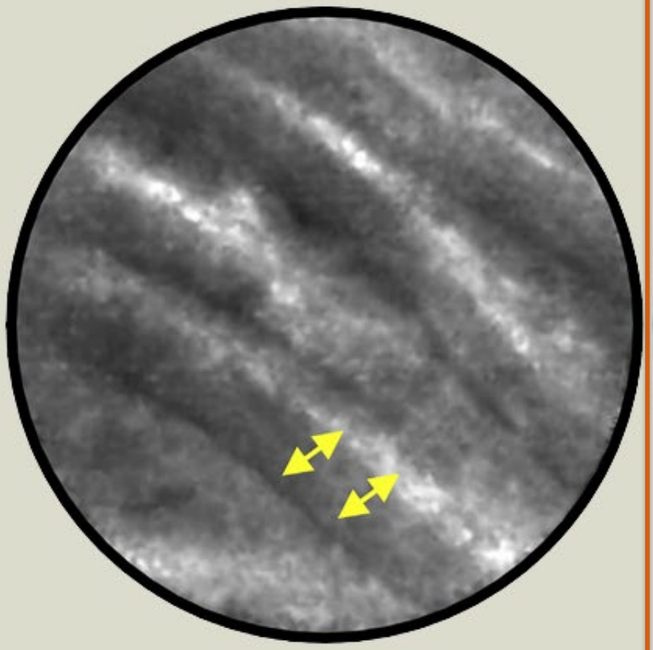
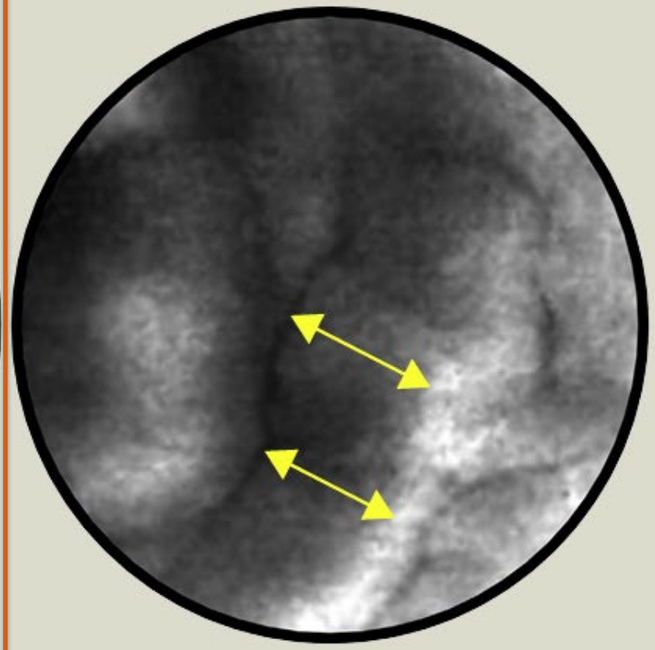
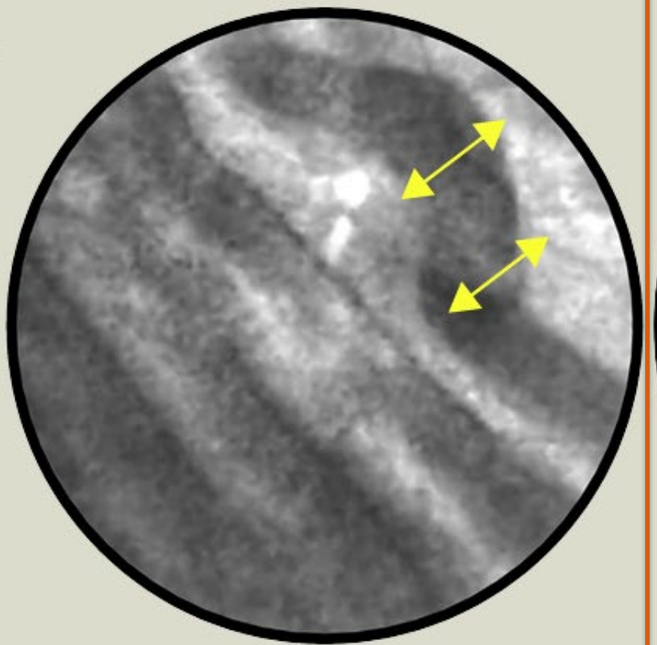
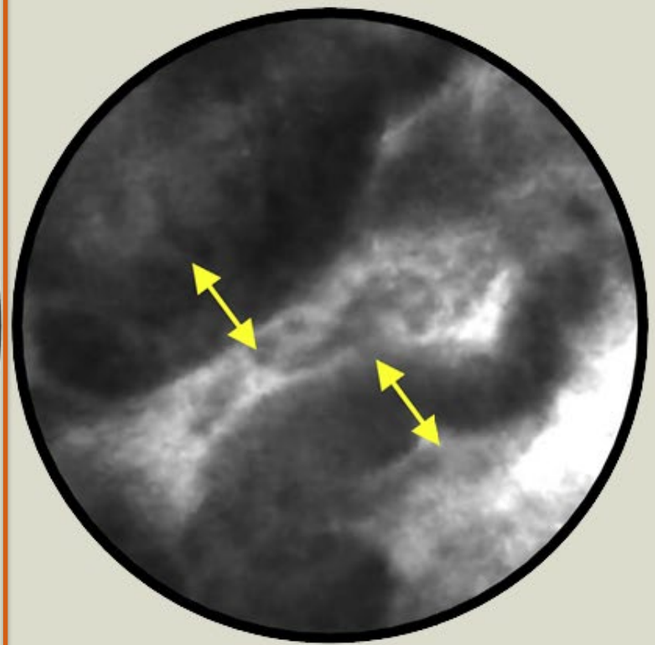
HGD



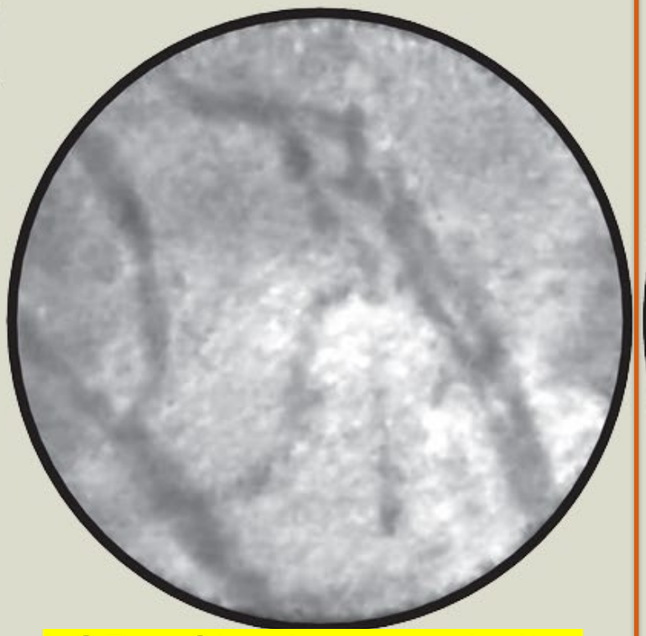
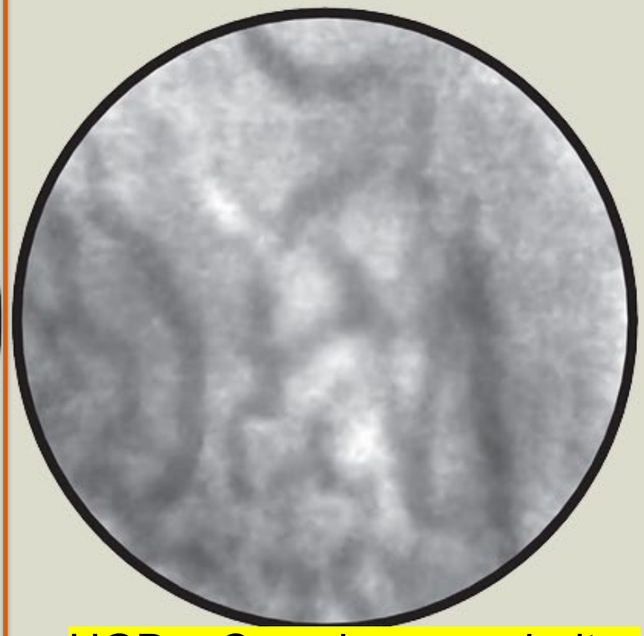
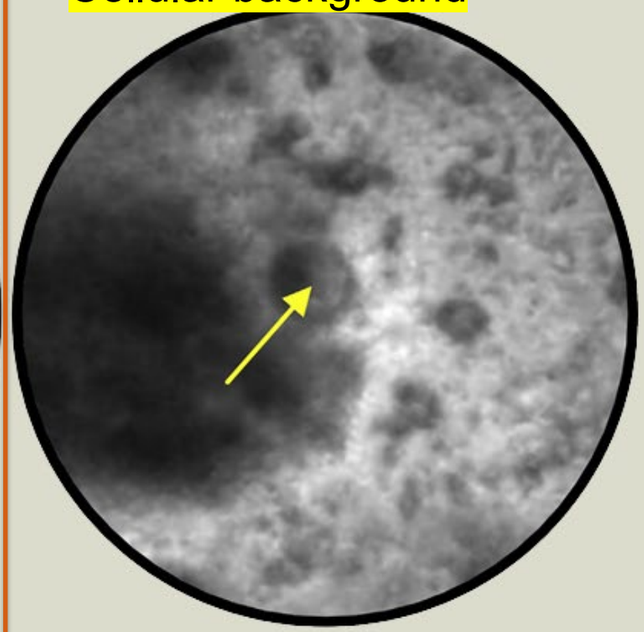
Krishna SG, Gastrointest Endosc. 2020

Variable	Low-grade dysplasia (LGD)	High-grade dysplasia or adenocarcinoma (HGD-Ca)
Size of the papillae. IPMNs with LGD have smaller sized papillae with narrow outer epithelium and inner vascular core (*red arrows*); in contrast, HGD-Ca lesions show large papillae with thick outer epithelium (*yellow double arrows*)		
	LGD = Small, scallop papillae	HGD = Larger, crowded papillae
Density of papillae. IPMNs with LGD have lower density of papillae (*red arrows*); in contrast HGD-Ca lesions demonstrate crowding of large and thick papillae (*yellow double arrows*)		

Krishna SG, Gastrointest Endosc. 2020

Variable	Low-grade dysplasia (LGD)	High-grade dysplasia or adenocarcinoma (HGD-Ca)
Thickness of the papillary epithelium (papillary width, μm). A papillary structure has the outer epithelium (*yellow double arrows*) and an inner vascular core. Increased thickness of the epithelium correlates with cellular stratification in progressive dysplasia		
	LGD = Thin, translucent epi <10μm	HGD = Thick, dark epi 30-40μm
Darkness of the papillary epithelium (pixel intensity). The nuclei of cells appear dark during nCLE, consequent to nuclear stratification, increased nuclear/cytoplasmic ratio, and loss of polarity. The papillary epithelium (*yellow double arrows*) appears darker in HGD-Ca		

Krishna SG, Gastrointest Endosc. 2020

Variable	Low-grade dysplasia (LGD)	High-grade dysplasia or adenocarcinoma (HGD-Ca)
Vascularity. IPMNs with LGD have more organized rope-ladder type vascularity, whereas those with HGD-Ca can reveal high density of blood vessels		
Cellularity. The background of IPMNs with LGD has sparse cellularity, whereas those with HGD-Ca can reveal large cells (yellow arrow) with prominent nuclei		

LGD = Simpler vascularity, Pauci-cellular background

HGD = Complex vascularity, Cellular background

Krishna SG, Gastrointest Endosc. 2020

Diagnostic Accuracy of EUS-nCLE

- Excellent accuracy superior to SOC
- For mucinous cysts
  - EUS-nCLE : Sensitivity 85% Specificity 99%
  - EUS-FNA: Sensitivity 54-63% Specificity 88-93%
  - ICG Fukuoka: Sensitivity 56% Specificity 94%
- Future: AI-assisted interpretation of EUS-nCLE images:
  - Screening (selection of high-yield frames), classification, risk stratification
  - 1st iteration of an AI model (Machicado et al. 2021)
    - N = 35 IPMNs
    - Sensitivity 83%; Specificity 88%

Konjeti J Clin Gastro 2020; Machicado Gastrointest Endosc. 2021; Jiang Biomimetics 2023; Gardner Gastroenterol 2024.

Barriers to the Adoption of EUS-nCLE

- High upfront cost
- Steep learning curve: Technical dexterity + Interpretation nCLE images
  - Median 38 cases to attain expertise in EUS-nCLE
  - To prevent post-procedural pancreatitis:
    - <10 min intracystic needle time
    - Limiting scope movement
  - Image interpretation challenges:
    - Fast-moving images
    - Interobserver disagreement in image interpretation exists even among experts
- Not currently incorporated into guidelines
    - Need randomized controlled trials and large prospective studies

Machicado Pancreatology 2022; Machicado Gastrointest. Endosc 2023; Krishna Shreyas Cancers 2024; Wu Clin Endosc 2024

Take Home Messages

- EUS-nCLE provides a valuable tool for real-time in vivo microscopic evaluation of PCLs
- New biomarker to enhance diagnostic accuracy and aid in risk stratification
- Potential to reduce unnecessary surgeries and improve patient outcomes
- Commercially available and fully reimbursed

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Gastrointestinal OCT Endomicroscopy

An Update

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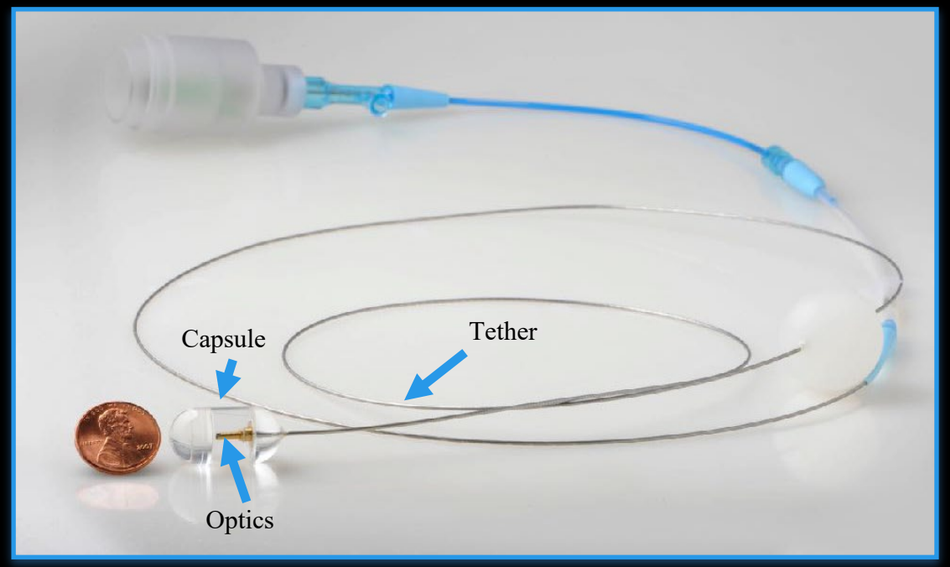
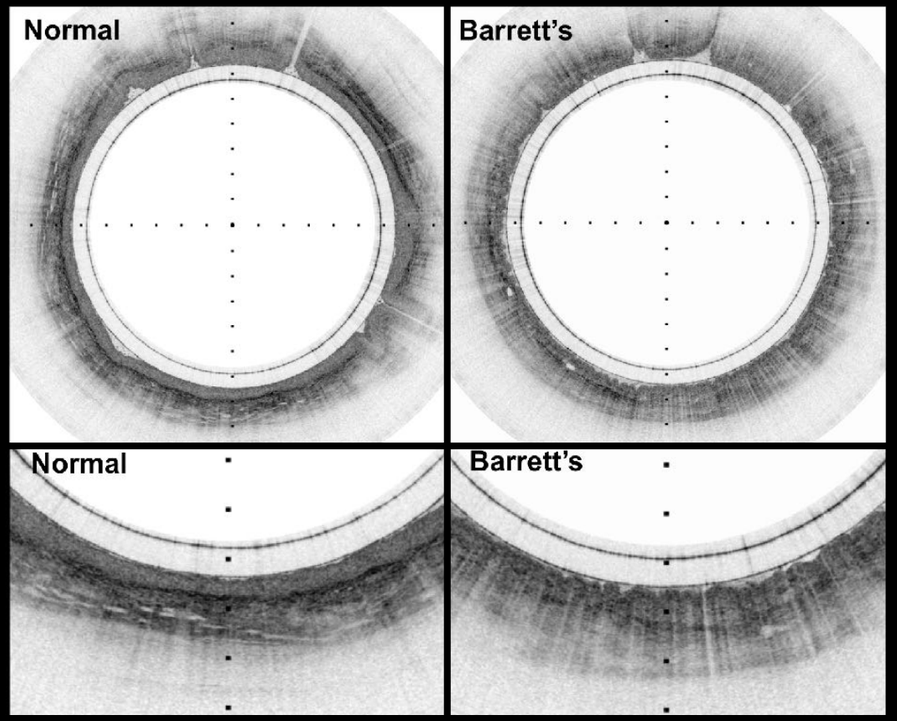
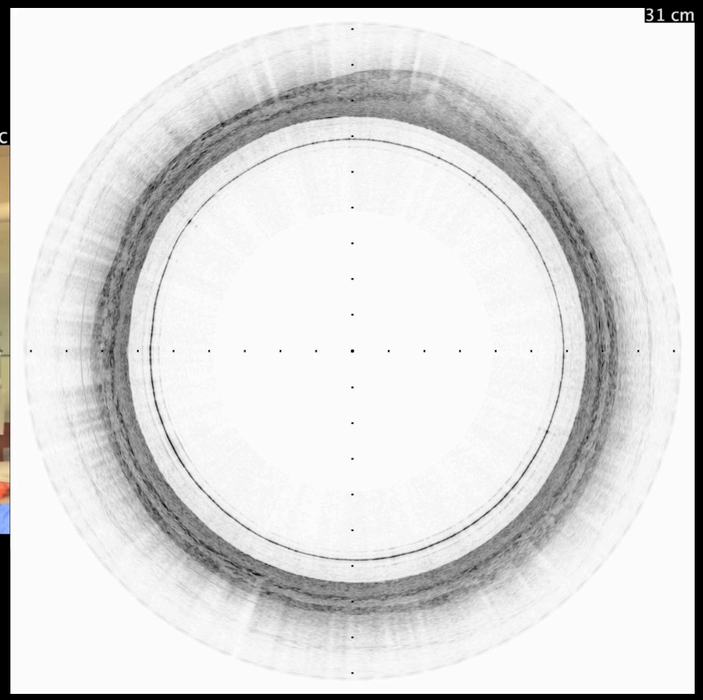
Major Stock Shareholder/Equity	Spectrawave
Royalty Income	NinePoint, Terumo, MIT, Nidek, Abbot Vascular, iLumen, Heidelberg Engineering
Ownership/Founder	Spectrawave
Intellectual Property Rights	MIT, MGH
Other Financial Benefit	None

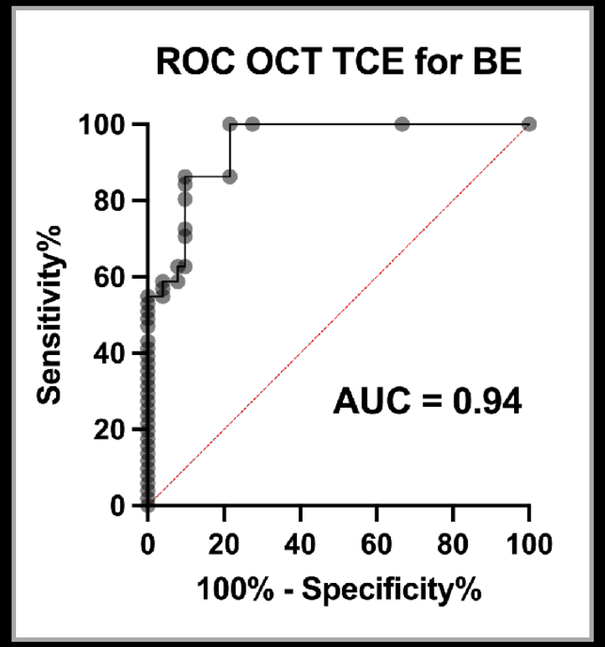
OCT Tethered Capsule Endomicroscopy (OCT-TCE)





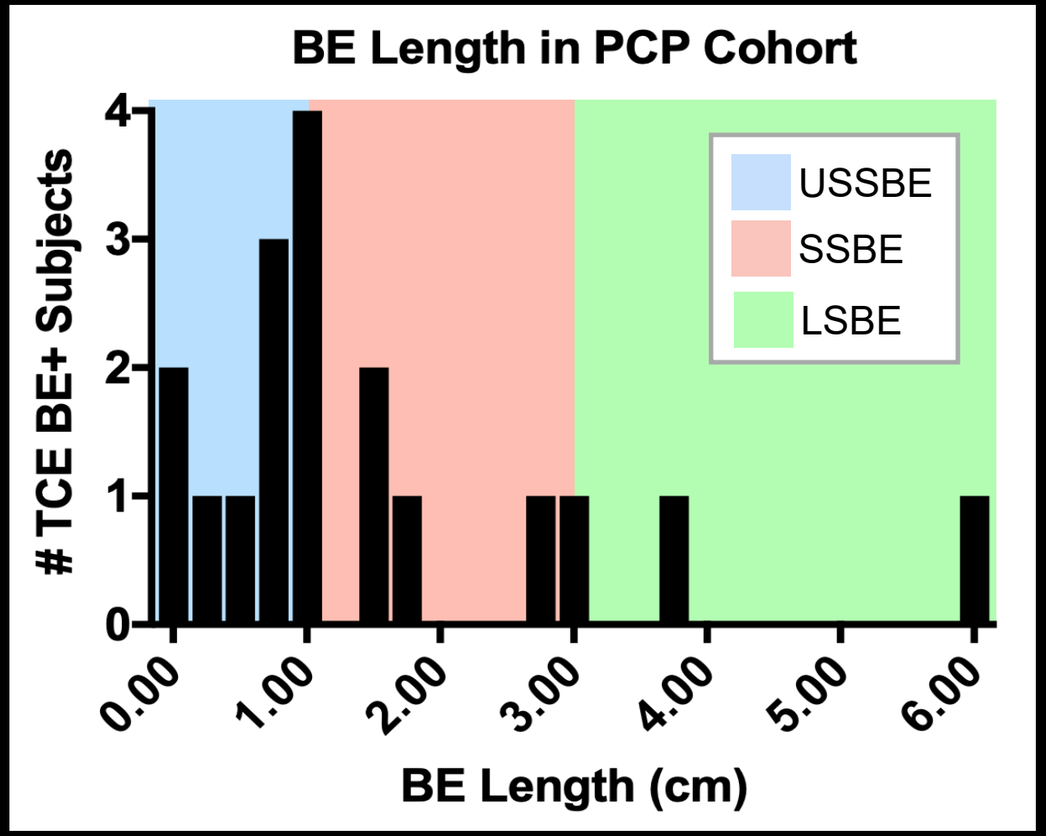


Validation



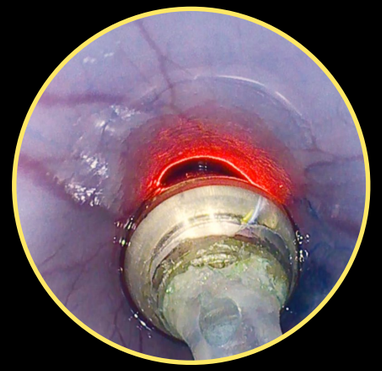
Esophageal OCT Capsule Clinical Study Summary – MGH Primary Care

n=173; BE Prevalence: 16.4% ± 6.9%

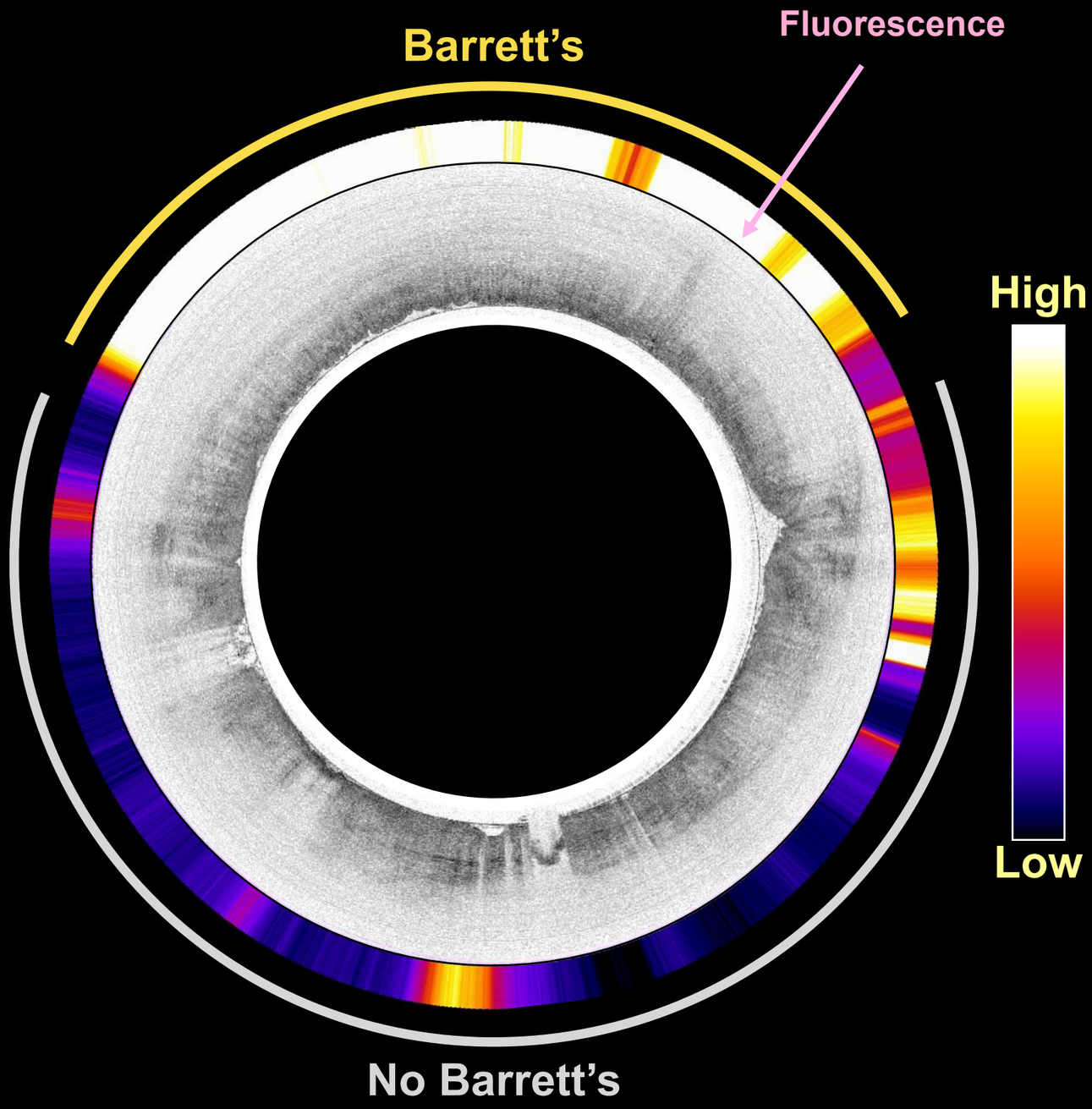


First-in-human OCT and Fluorescence Tethered Capsule Barrett's Detection

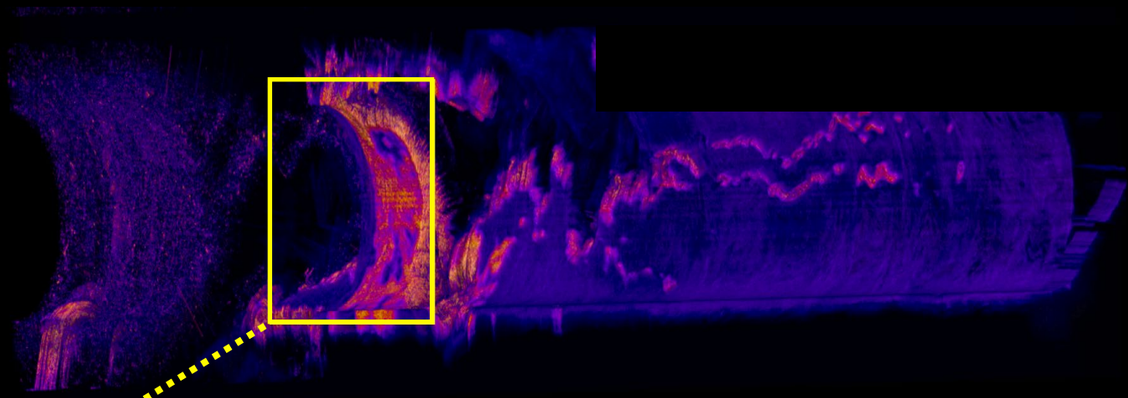
Methylene Blue Slurry Swallow

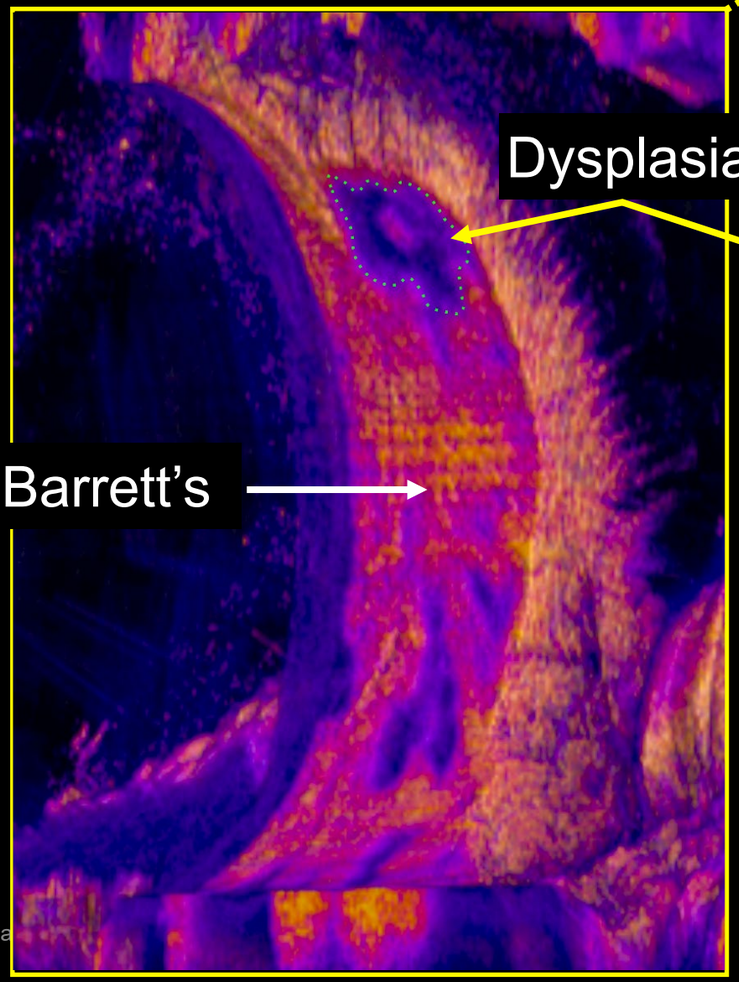


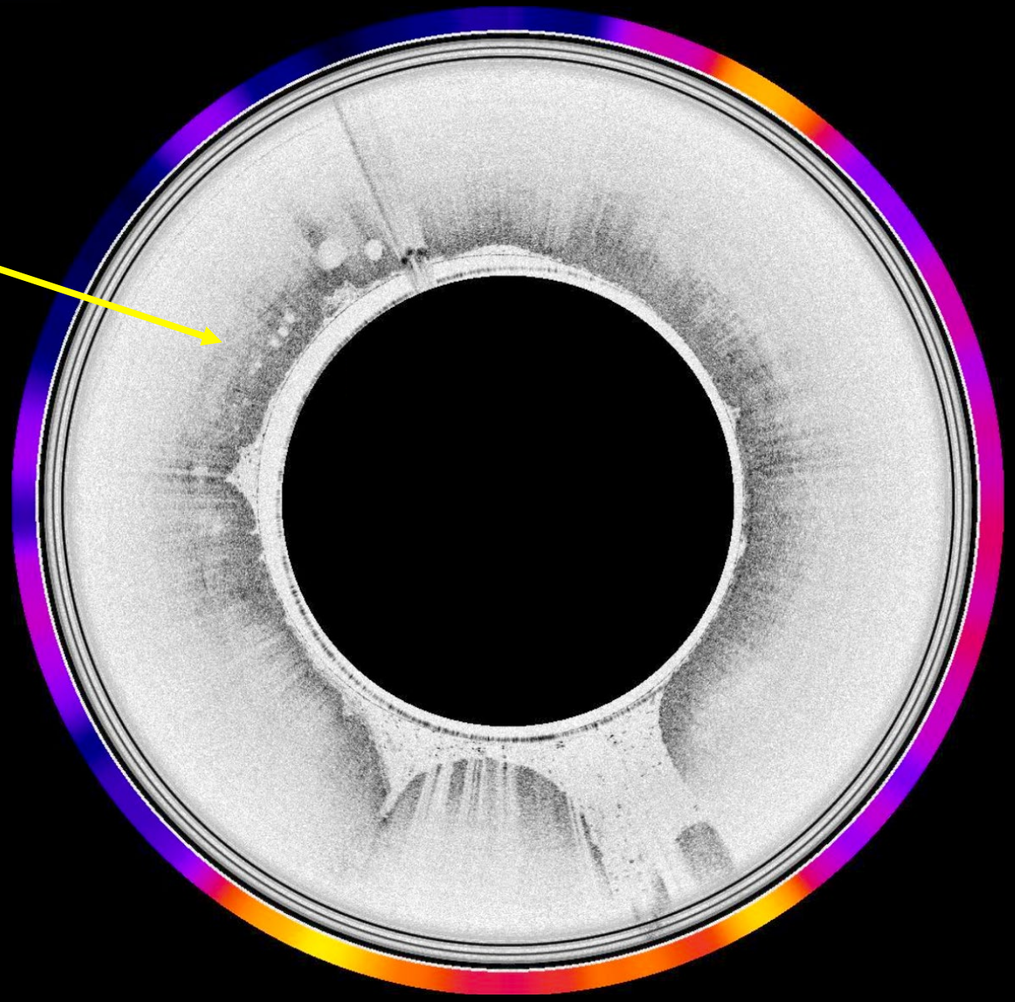
Capsule in the esophagus



Finding Barrett's Dysplasia with Fluorescence-OCT Tethered Capsule







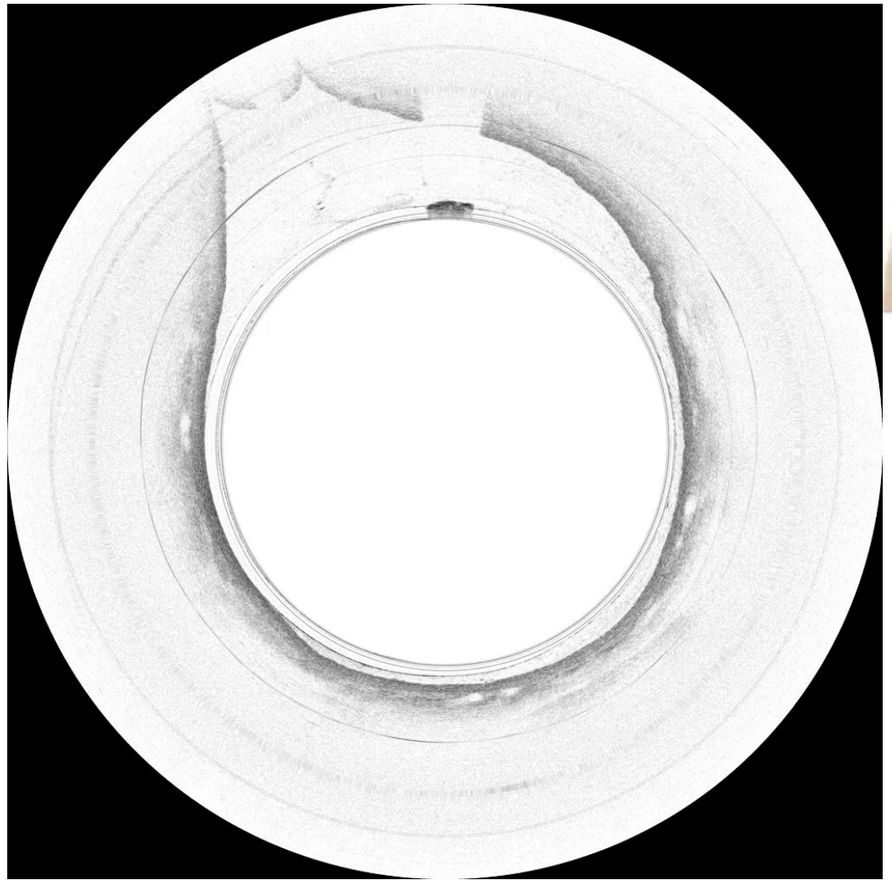
Retrograde OCT TCE (R-TCE)

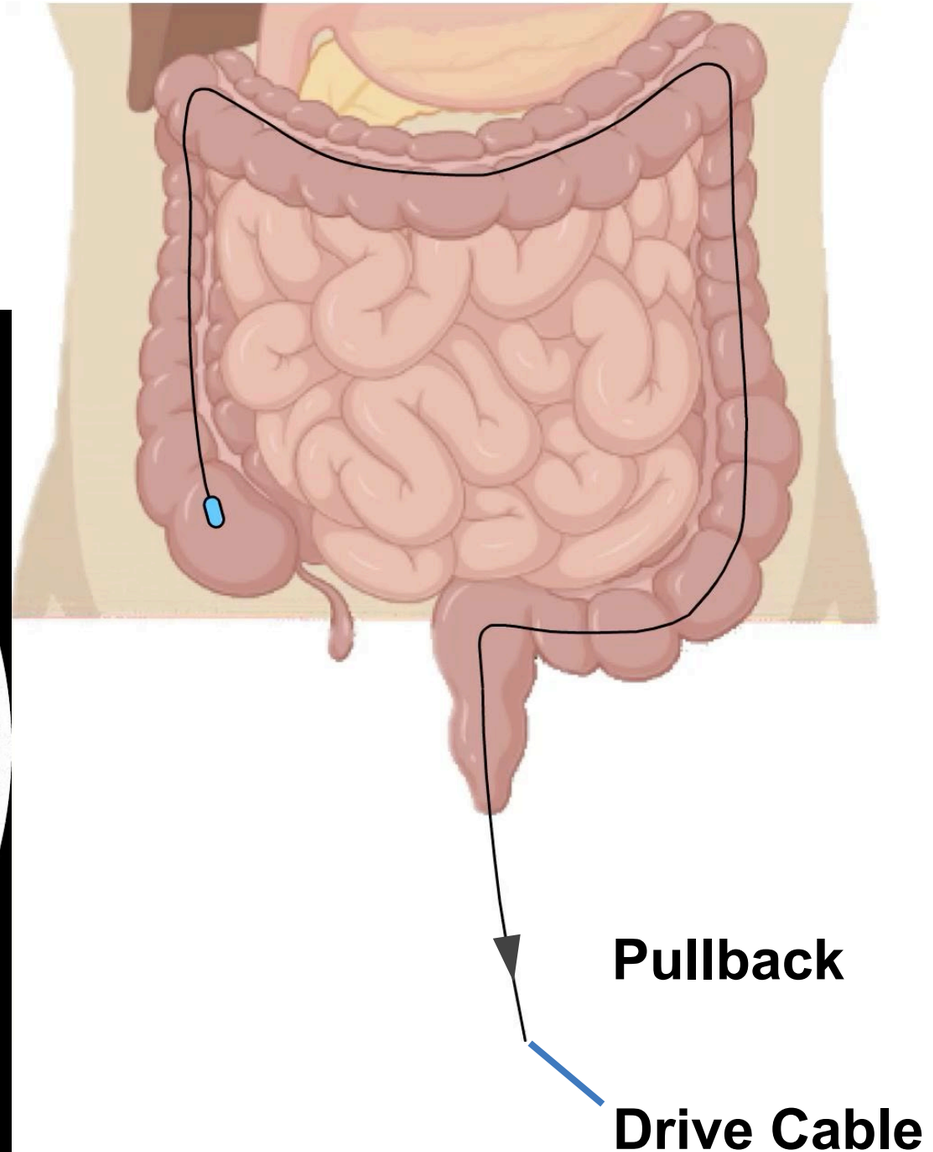
Microscopic imaging of entire colon

- No sedation, less expensive
- Automatic - objective
- Fewer missed lesions

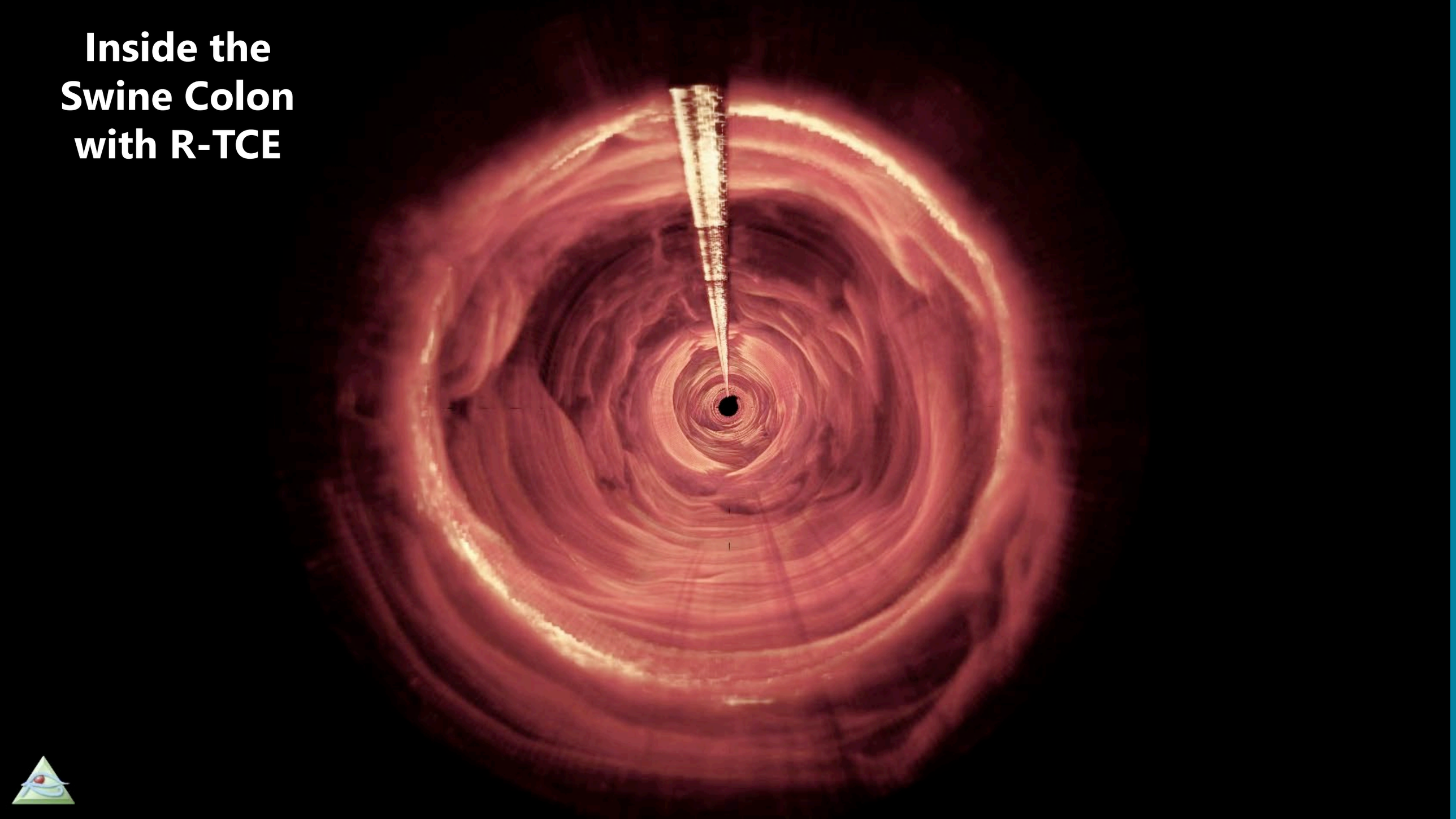
Retrograde OCT TCE (R-TCE)

Cross-sectional OCT Images of Colon



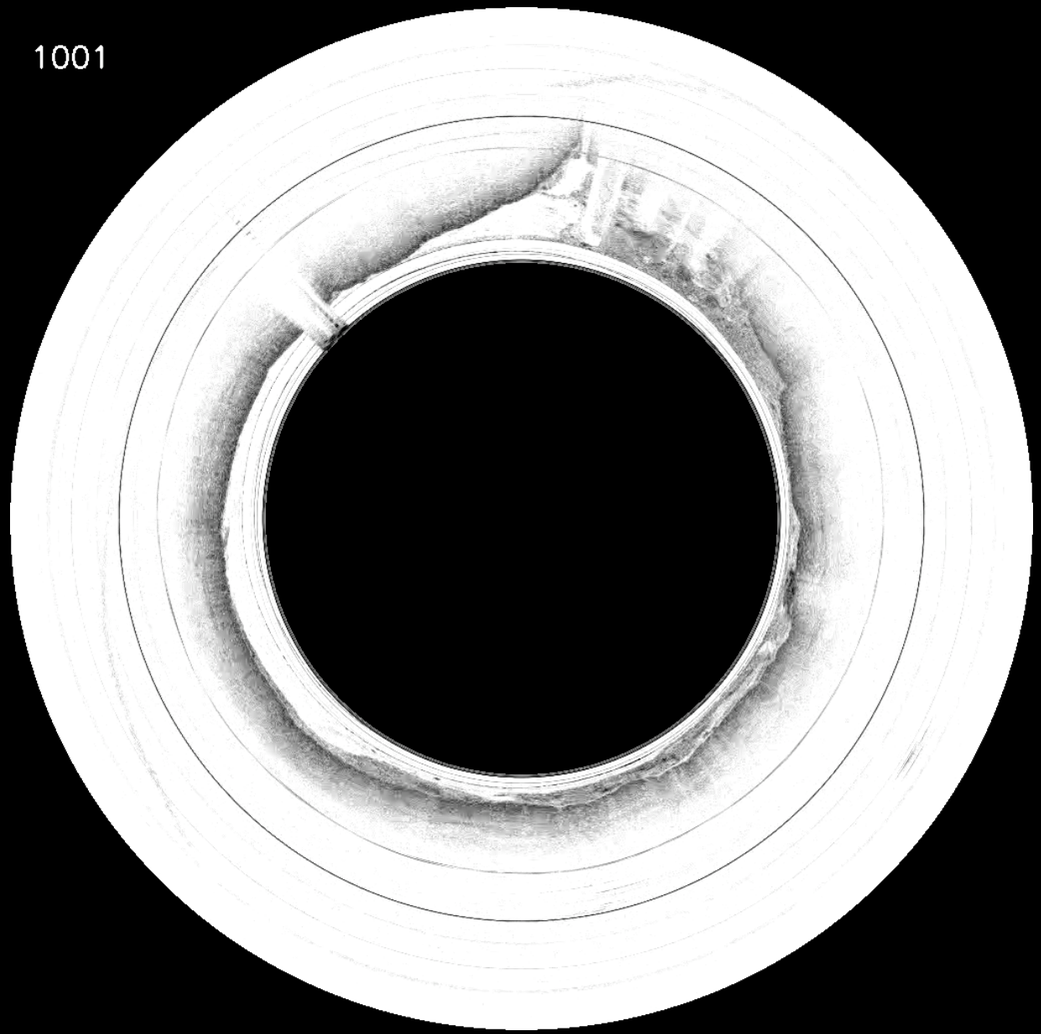


Inside the Swine Colon with R-TCE

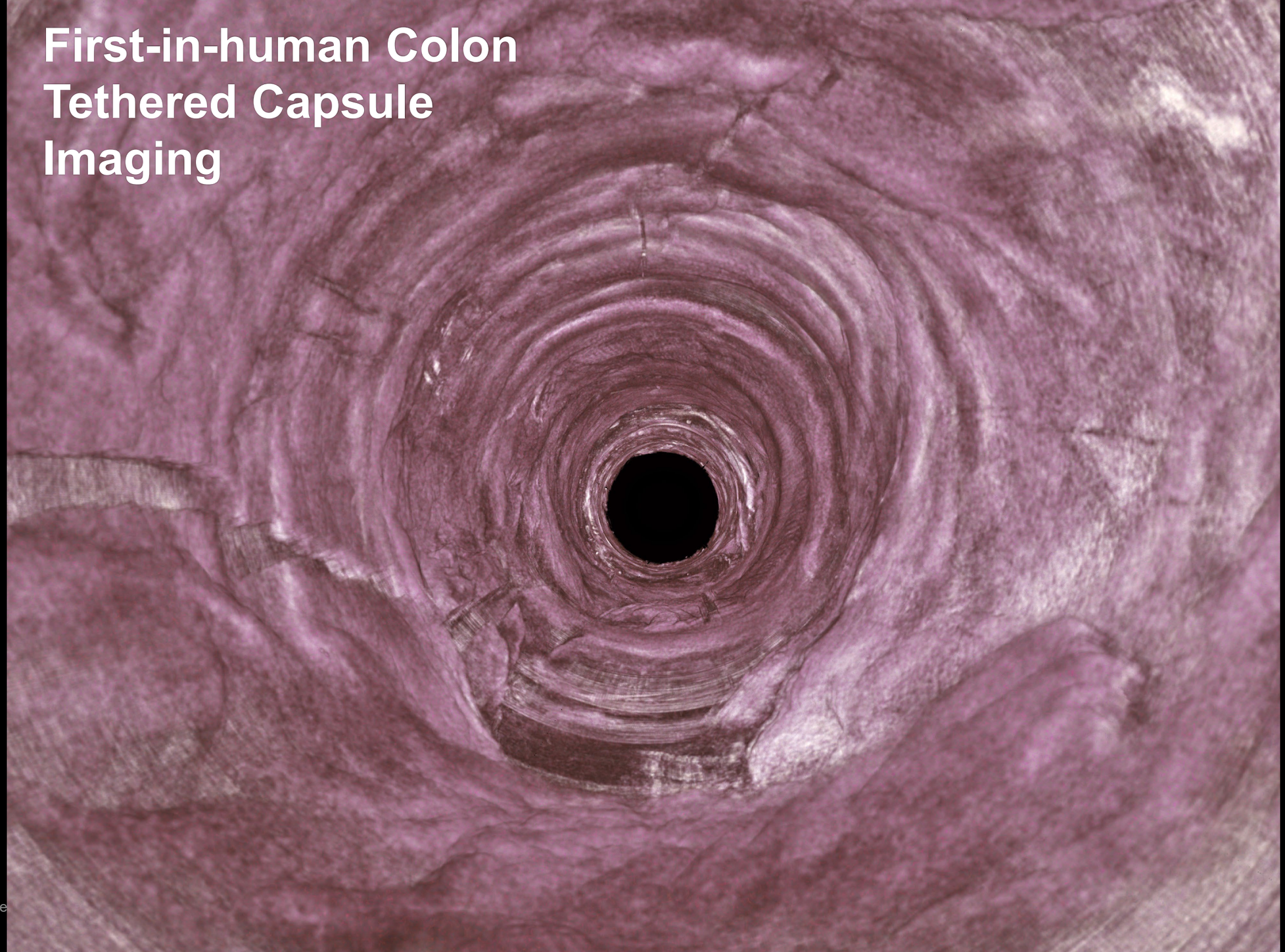


R-TCE First In Human Study

- n=5 unsedated healthy subjects
- Enema prep (2)
- Flex sig before and after
- R-TCE from rectum→splenic flexure
- Tolerability questionnaire after



First-in-human Colon Tethered Capsule Imaging



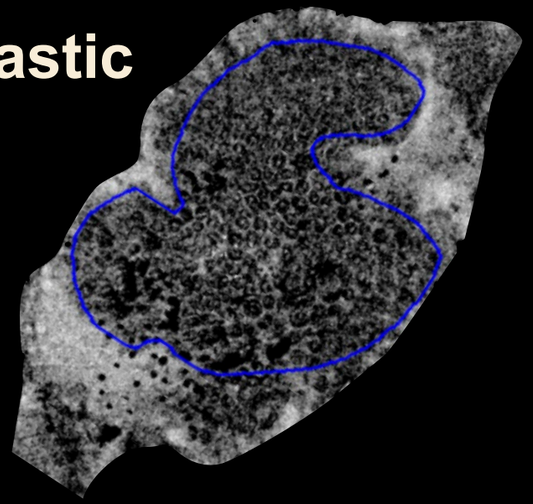
Tolerability Questionnaire

Scores: 1→least discomfort; 10→most discomfort)

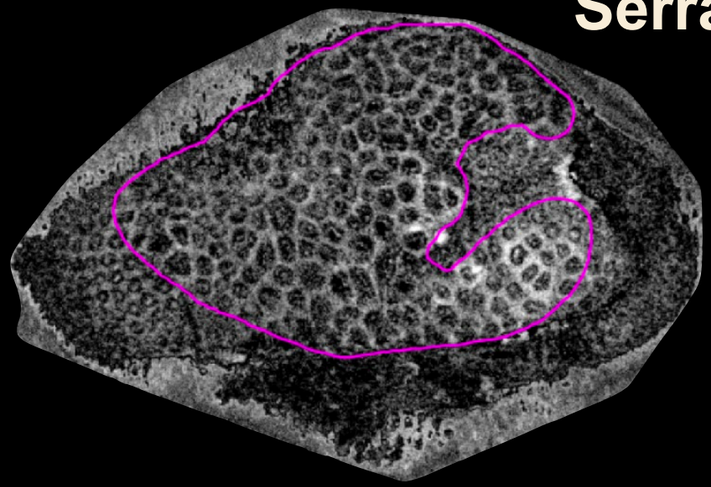
Question	R-TCE	Flex Sig
Insertion	2.8±1.3	2.4±1.0
Advancement	2.4±1.0	3.0±1.1
Pullback	2.6±1.0	2.8±1.5
Removal	2.0±1.3	2.0±0.9
Overall procedure	2.2±0.7	2.5±0.8
Prefer R-TCE vs. flex sig	n=5	0
Prefer R-TCE vs. colonoscopy	n=5	0

μ_s z-projections

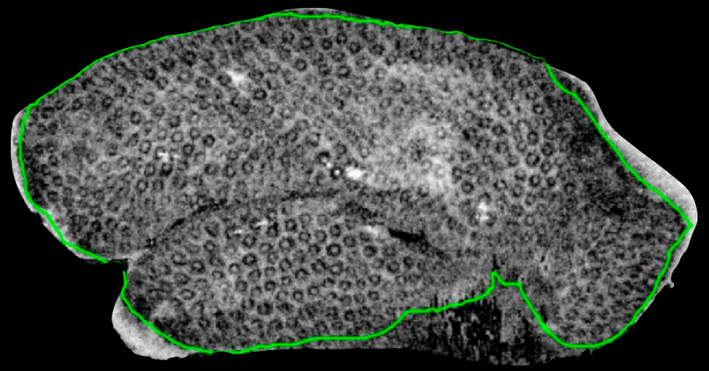
Hyperplastic



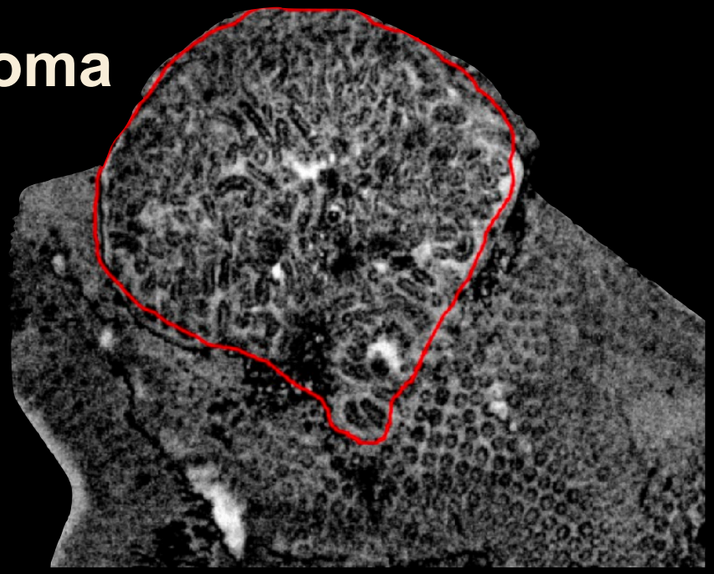
Serrated



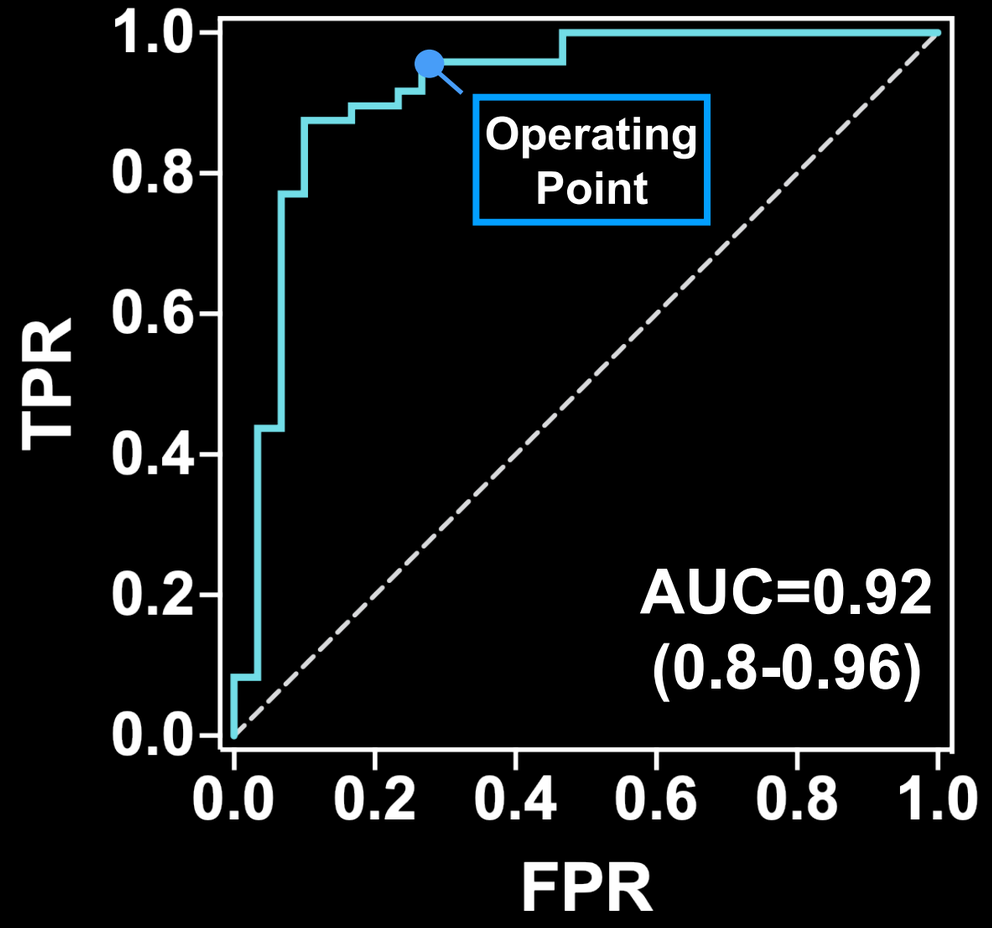
Normal



Adenoma



Prospective Classification

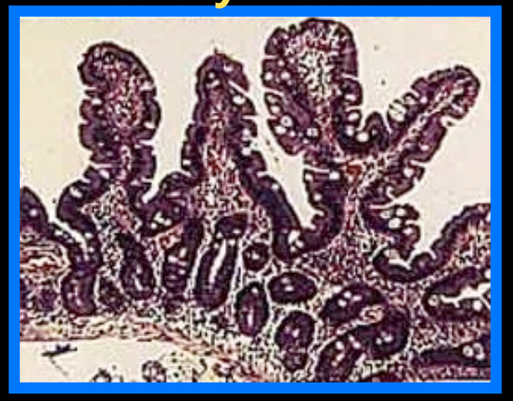


	Value	95%CI
Sens	96%	86%-99%
Spec	74%	54%-88%
PPV	85%	76%-91%
NPV	92%	74%-98%
Acc	87%	78%-94%

Met ASGE PIVI thresholds

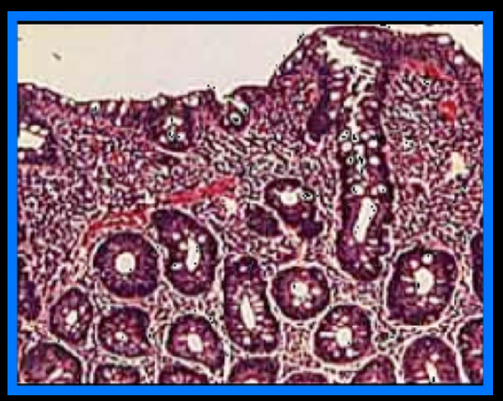
Environmental Enteric Dysfunction (EED)

Healthy Intestine

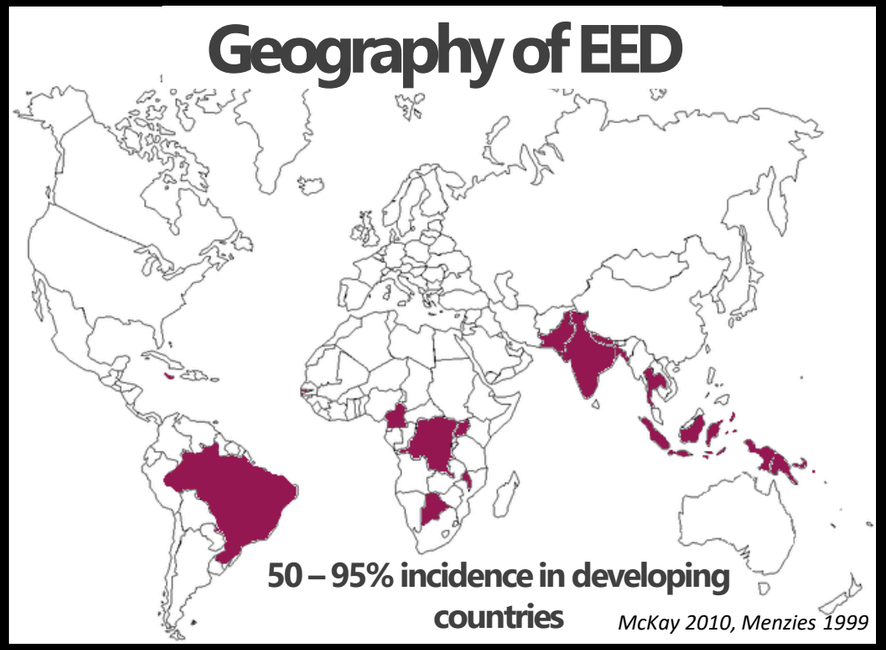


- Healthy villi
- Intact barrier function

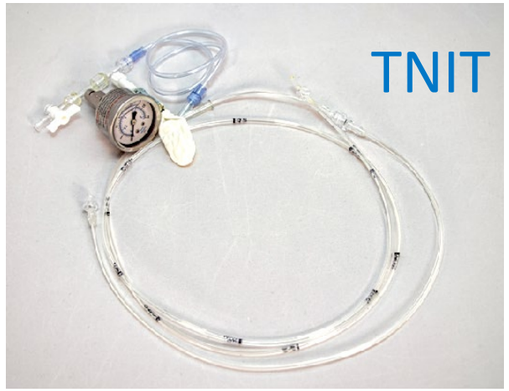
EED

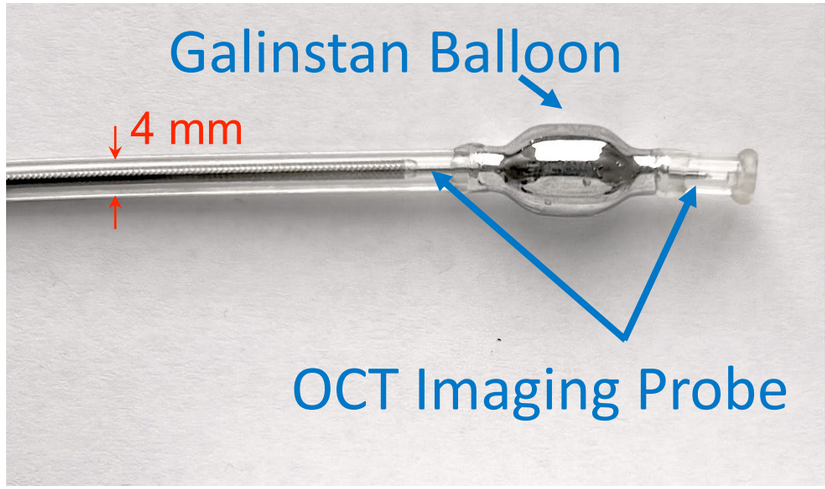


- Villous blunting
- Inflammation
- Translocation
- Loss of barrier function

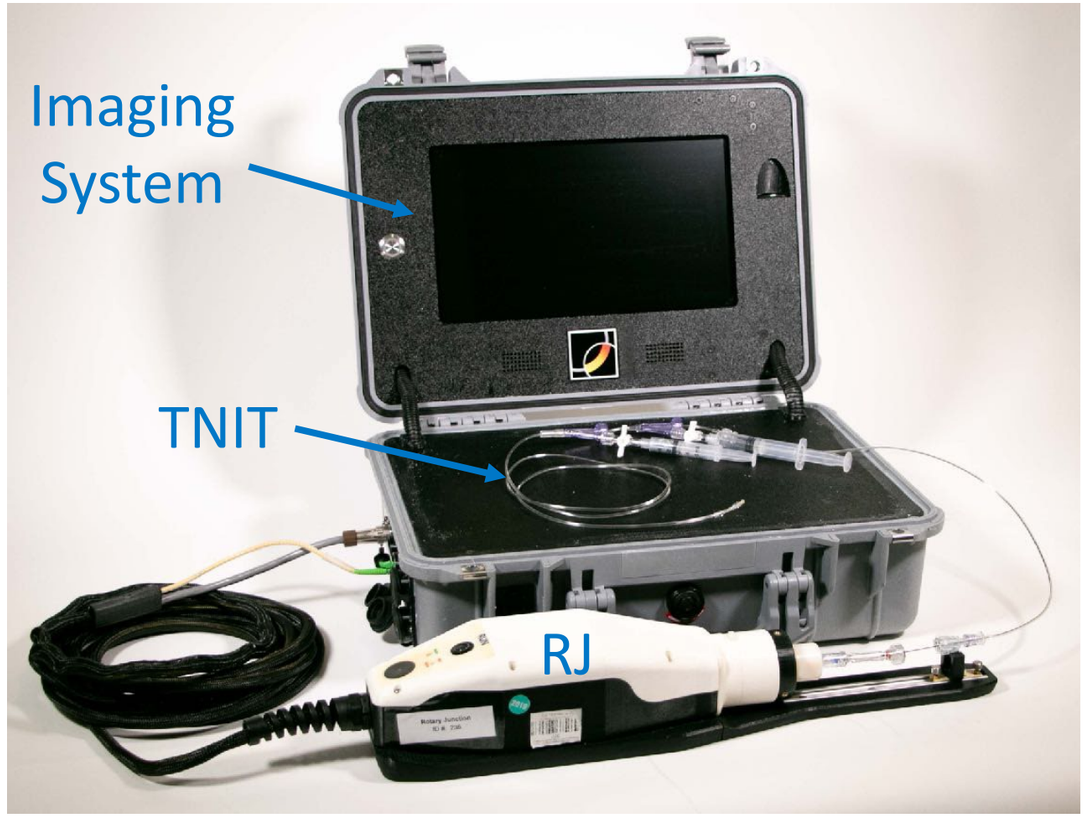


Transnasal Introduction Tube (TNIT)





- Inserted transnasally without sedation
- Cross-sectional OCT conducted using a rotating imaging probe inside TNIT's lumen
- Galinstan-filled balloon facilitates crossing the pyloric sphincter into the duodenum
- OCT imaging of the duodenum



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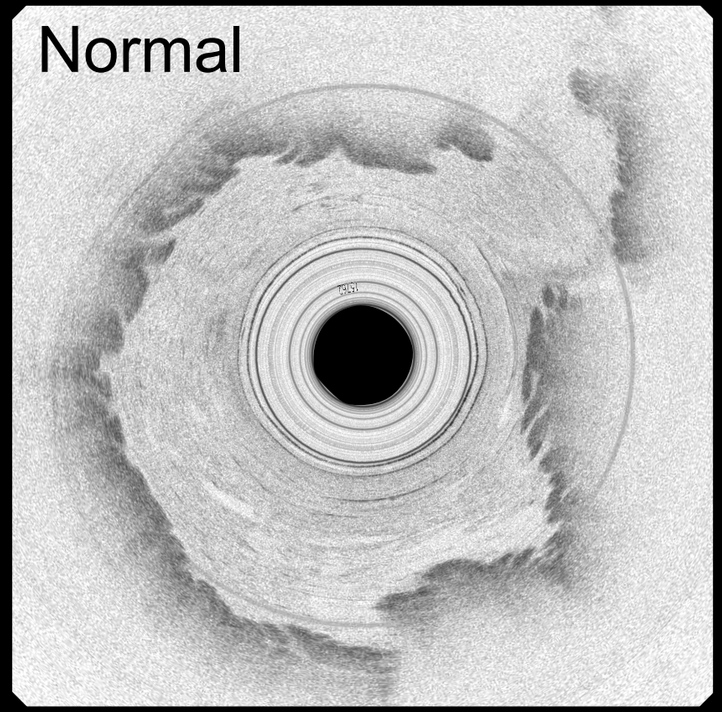
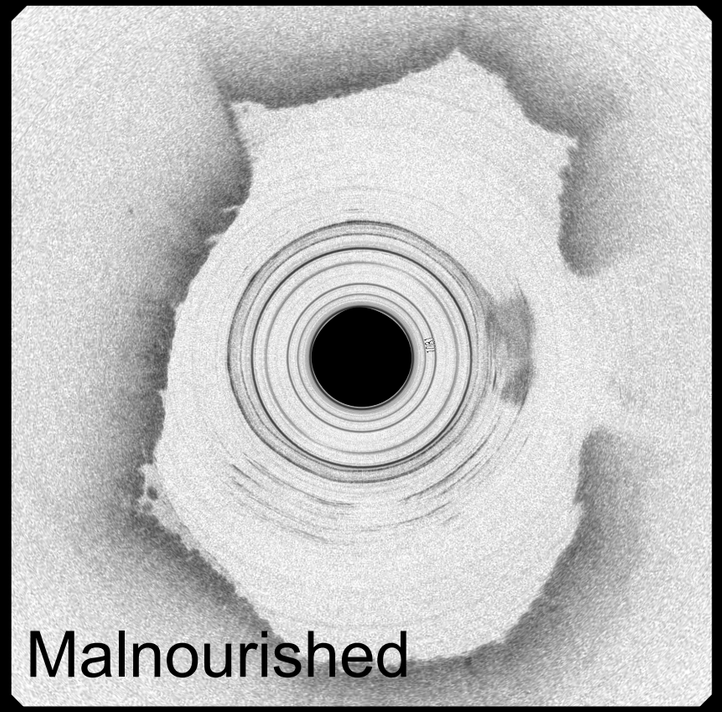
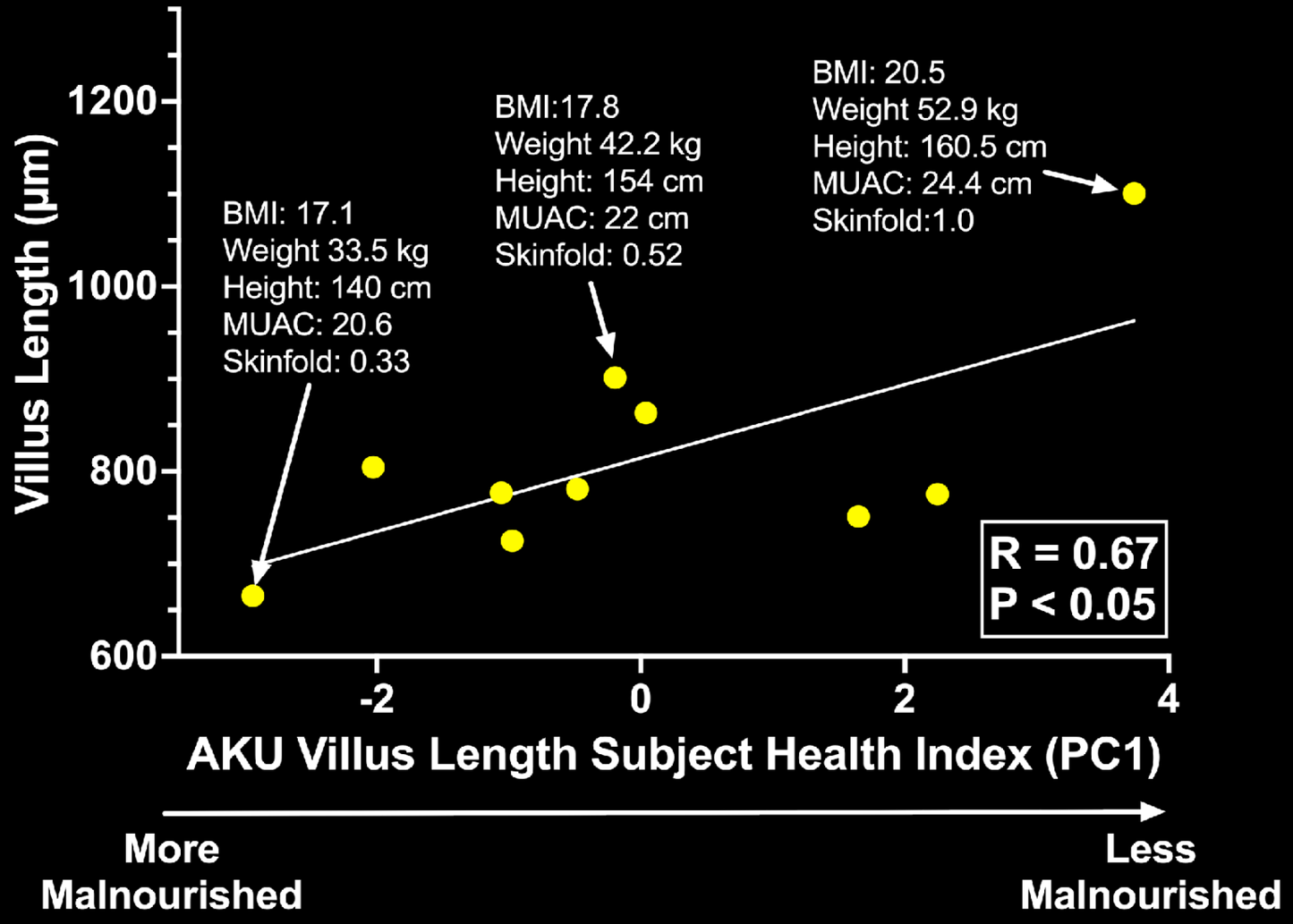
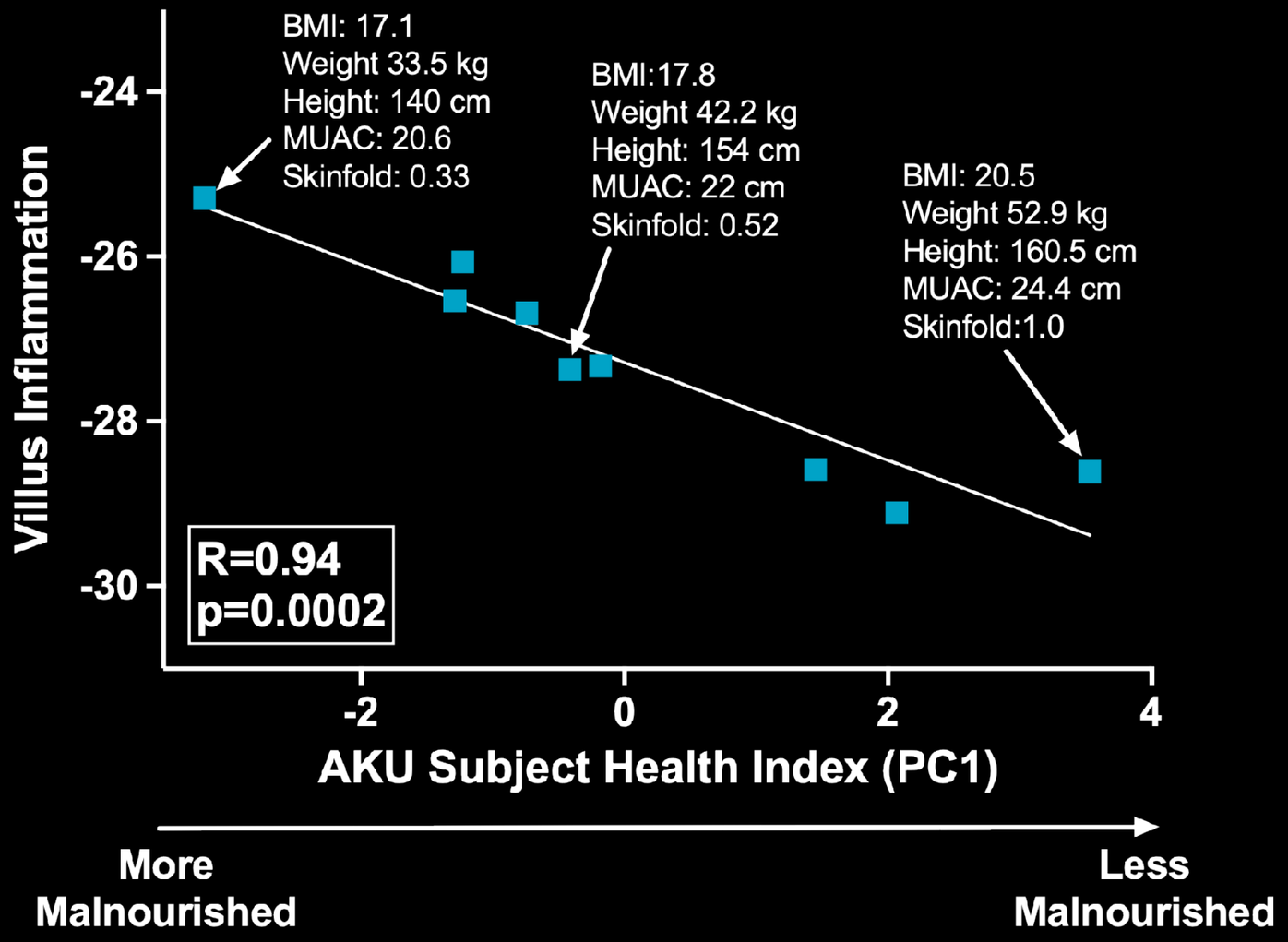




AKU Subject Villus Length



AKU Subject Villus Inflammation



Summary

- OCT TCE has been used in over 1000 patients, demonstrating high accuracy for BE and a high BE prevalence in the general population
- OCT-FL TCE first in human study shows potential for improving the detection of BE dysplasia
- OCT R-TCE for colonic screening
- OCT TNIT imaging of the duodenum can quantify duodenal villous morphology and demonstrate a relationship with subject health status
- Promise for clinical application of OCT endomicroscopy for a variety of important gastrointestinal disorders

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Questions

Thank You !

The DCPC will be producing more digital pathology educational content in 2025.

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

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

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

COLLEGE of AMERICAN PATHOLOGISTS