COLLEGE of AMERICAN PATHOLOGISTS

In Vivo Microscopy as an Adjunct to Traditional Histopathology

Expanding our View

Lida Hariri, MD, PhD, FCAP
DCPC Webinar Series

November 17, 2021

Lida Hariri, MD, PhD, FCAP

- **Assistant Professor of Pathology and a translational biomedical optics researcher at Massachusetts General Hospital, Harvard Medical School.**
- **Obtained her MD and PhD at the University of Arizona in 2009, with her doctorate in Biomedical Engineering focused on multimodal optical imaging for early cancer detection.**
- **Practicing pathologist at MGH, specializing in pulmonary pathology and research interests focus on the development, translation, and clinical application of high-resolution optical imaging for early detection, diagnosis, and monitoring of pulmonary diseases.**
- **Vice Chair of the CAP Digital and Computational Pathology Committee.**

Disclaimer

- **The CAP does not permit reproduction of any substantial portion of the material in this Webinar without its written authorization. The CAP hereby authorizes attendees of the CAP Webinar to use the PDF presentation solely for educational purposes within their own institutions. The CAP prohibits use of the material in the Webinar – and any unauthorized use of the CAP's name or logo – in connection with promotional efforts by marketers of laboratory equipment, reagents, materials, or services.**
- **Opinions expressed by the speaker are the speaker's own and do not necessarily reflect an endorsement by the CAP of any organizations, equipment, reagents, materials, or services used by participating laboratories.**

Disclosures

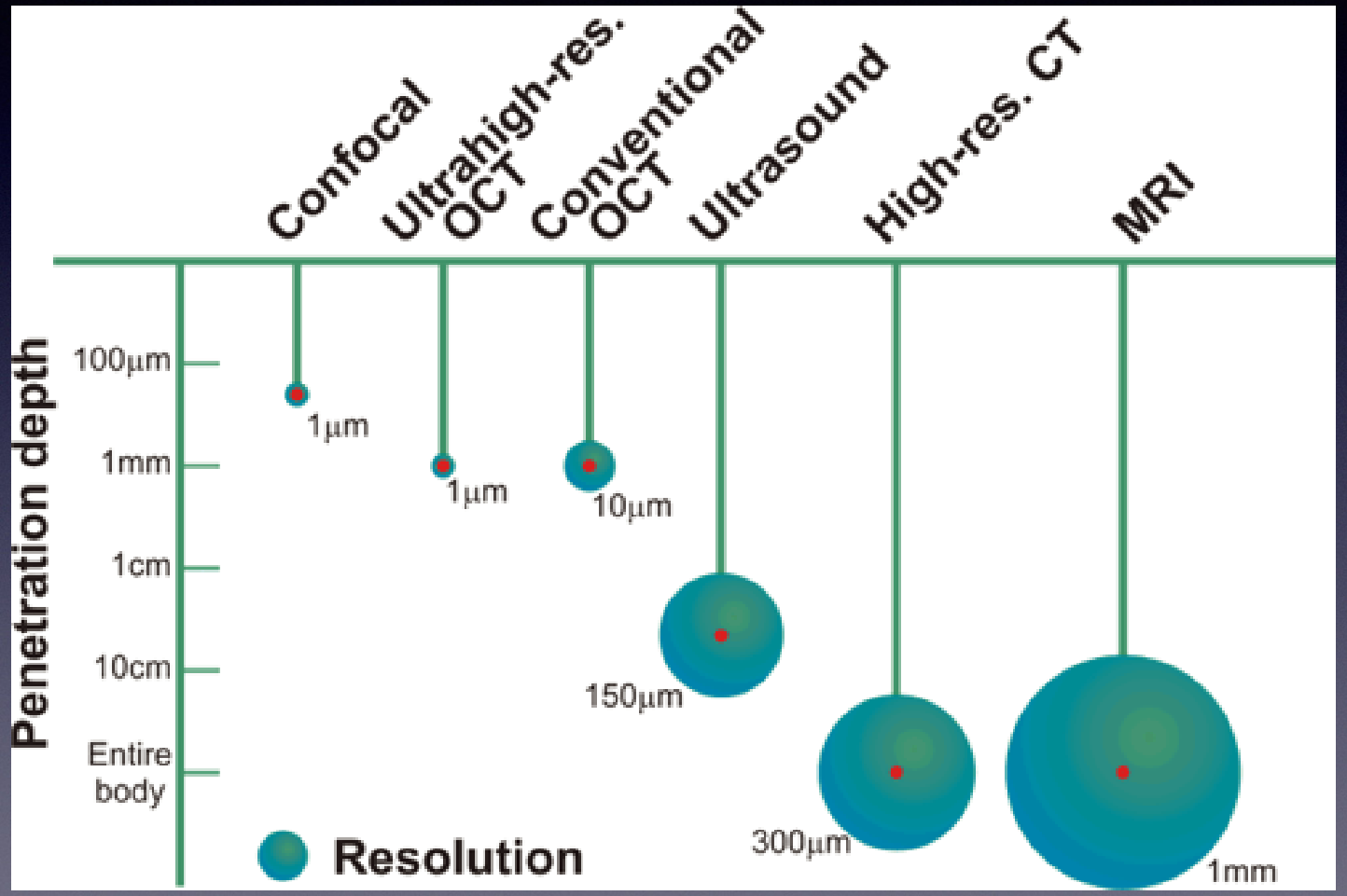
I have the following relevant financial relationships to disclose:

Consultant for Boehringer Ingelheim, Indalo Therapeutics Pliant Therapeutics, and Bioclinica

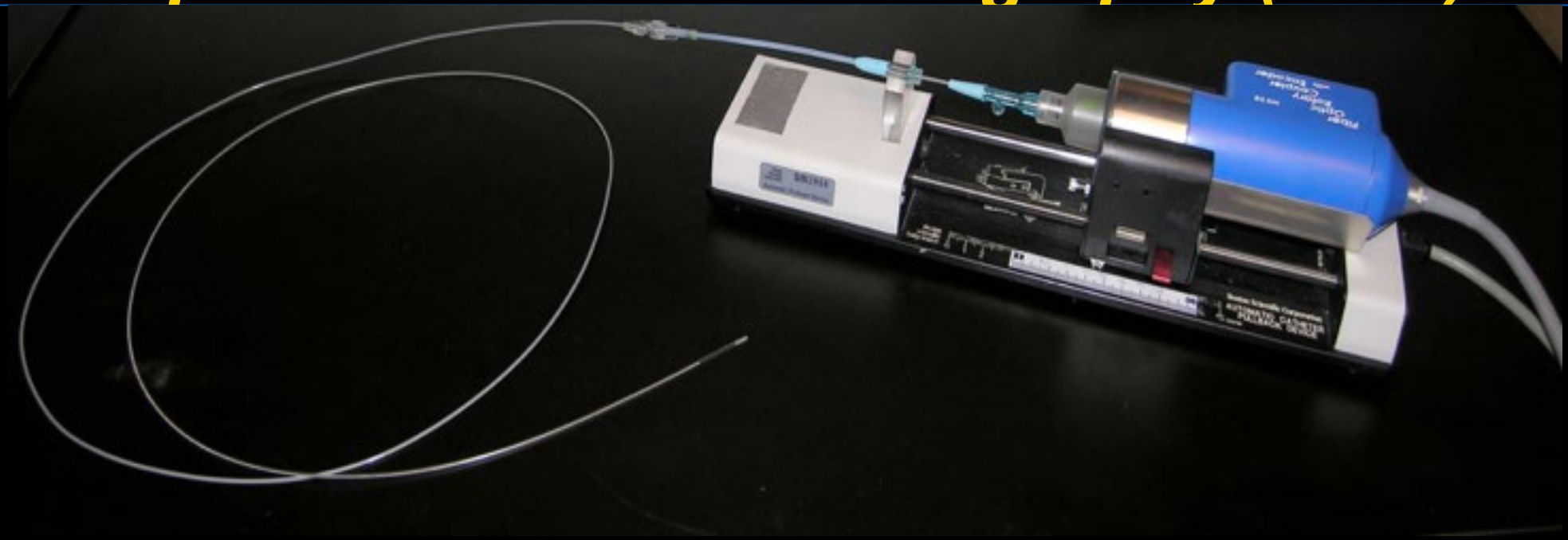
Grant funding from Boehringer Ingelheim

I will not discuss off label use and/or investigational use in my presentation.

High-Resolution Optical Imaging:
Bridging the Radiology/Pathology Divide



Optical coherence tomography (OCT)



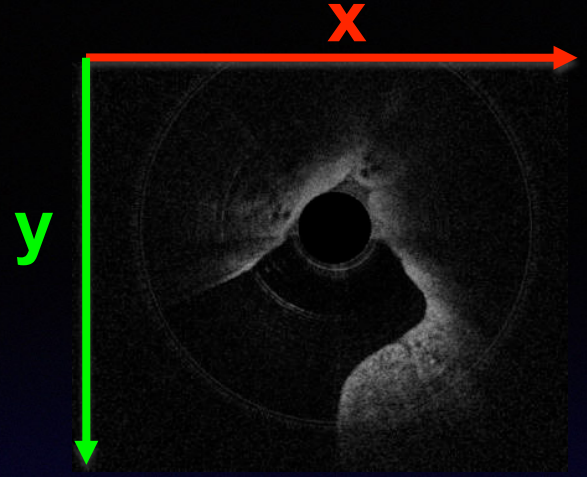
Analagous to Ultrasound

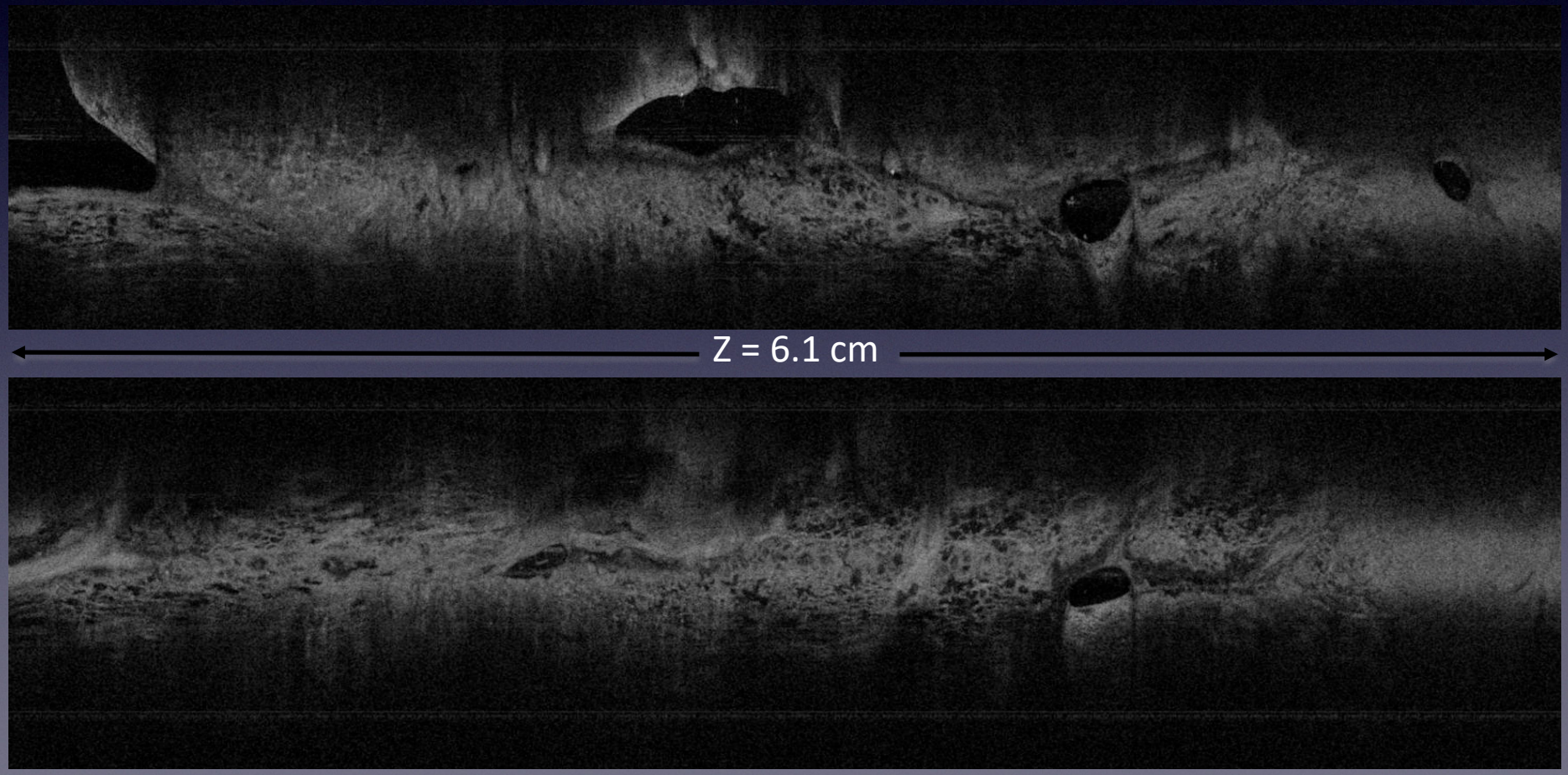
- Cross- sectional (x- z) imaging of tissue structure
- Similar to low power microscopy (4x objective)
- < 10 µm axial resolution (z)
- 10- 30 µm transverse resolution (x)
- < 3 mm penetration depth
- Non- destructive
- No transducing medium

Large Volume Virtual Datasets

To Complement Small Biopsy

Adenocarcinoma





Insufficient biopsy yield in lung cancer is a huge problem

- Need enough tumor for diagnosis AND molecular testing
- Low-risk biopsy techniques suffer from sampling error
  - Miss the targeted nodule → **No Tumor**
  - Biopsy non-diagnostic fibrosis → **Little or no tumor**

Endobronchial Ultrasound

Electromagnetic Navigation

Even with guidance techniques, diagnostic yield still low for lesions < 3.0 cm

OCT in biopsy guidance:
What needs to be assessed?

1) Can OCT tell if the needle is in the nodule?

- Assess whether OCT can distinguish pulmonary nodules from surrounding parenchyma

2) Once in the nodule, can OCT tell if the needle is near tumor?

- Determine if OCT can assess nodule composition to maximize tumor content in biopsies

3) Can OCT quantify tumor in lung nodules?

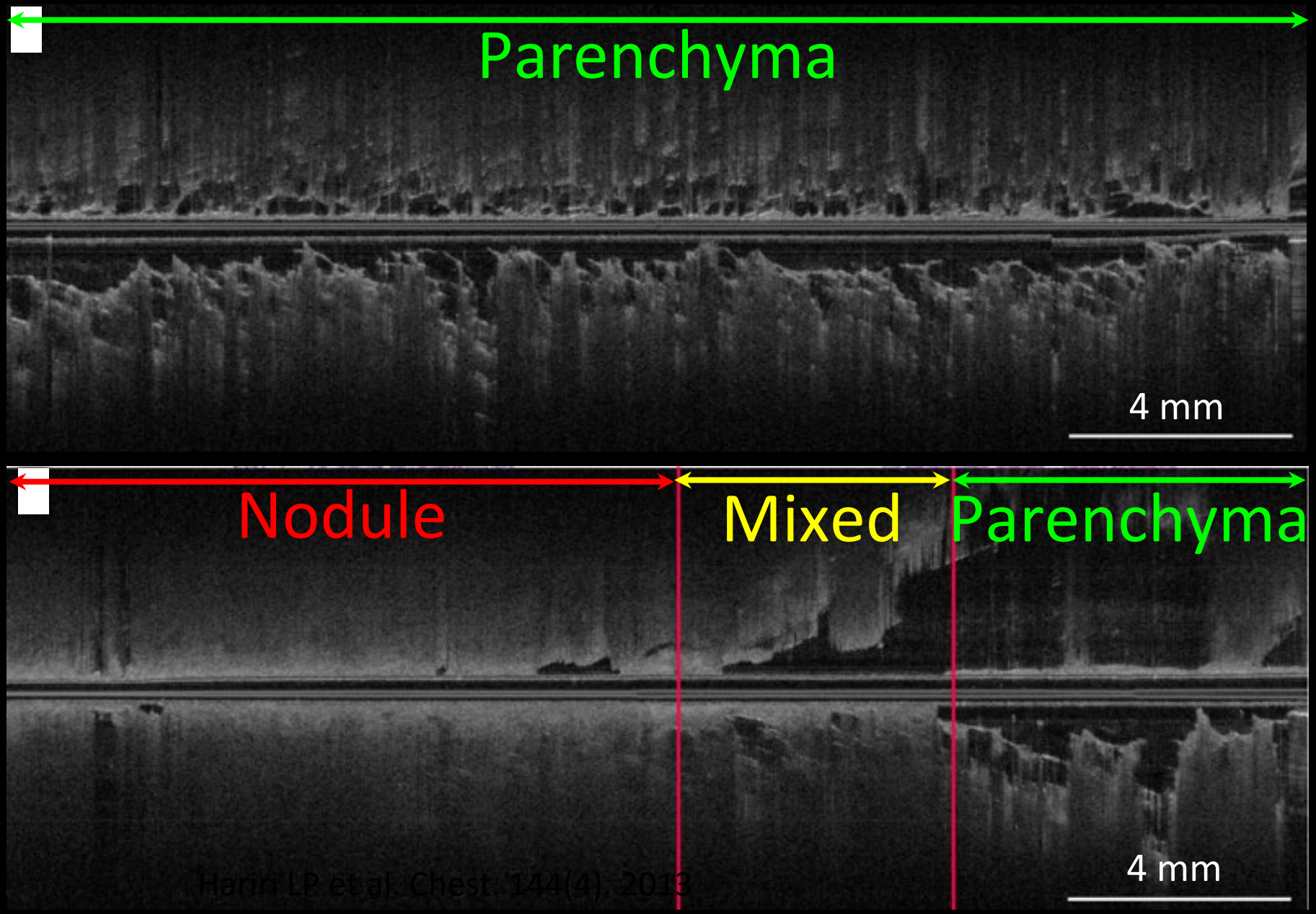
- Rapid assessment of tumor content

Establishing Criteria for Nodule and Lung Parenchyma

- Develop OCT criteria for peripheral nodule and lung parenchyma in ex vivo lung resection specimens
- Validate OCT criteria in a blinded assessment with 6 independent readers
  - Two pathologists, pulmonologists, and OCT experts
  - 15 minute training on criteria
  - Validation Set: 109 ex vivo samples
  - Include a variety of pathology for nodules and parenchyma

Assess: Nodule or Parenchyma

OCT to Target Lung Nodules

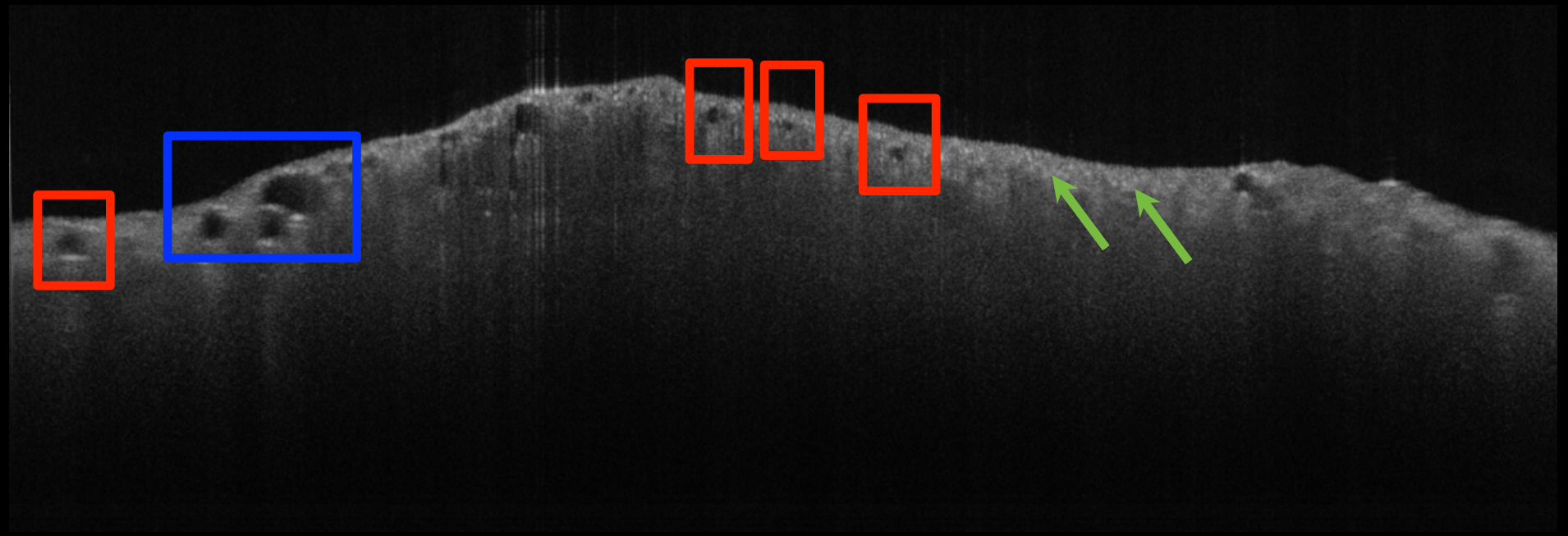


> 95% sensitivity and specificity
in differentiating lung nodules from parenchyma

Hariri LP et al. Chest. 2013

Hariri LP et al. Chest. 144(4). 2013

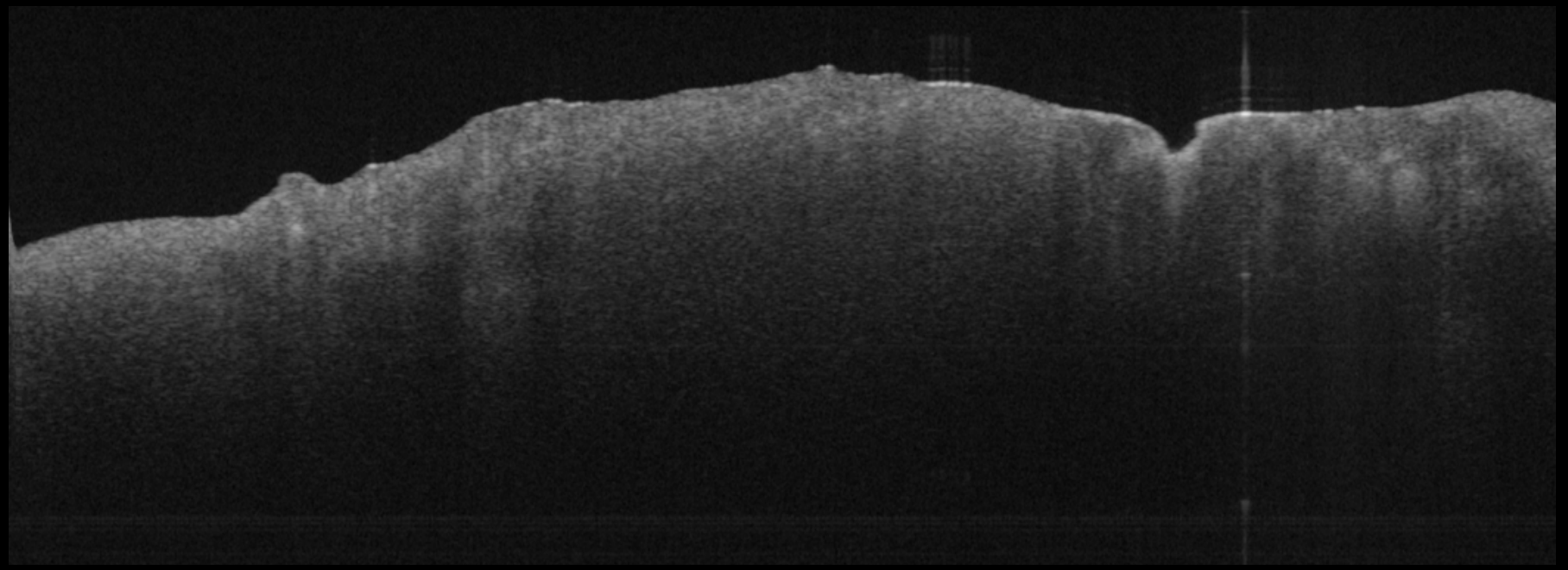
Training Image: Lung Parenchyma



1. Signal void alveoli (Red Boxes)
   - Some enlarged due to emphysema (Blue Box)
2. Evenly spaced high signal intensity specular reflections seen in areas of collapsed lung (Green Arrows)

Hariri LP et al. Chest. 144(4):1261-8. 2013

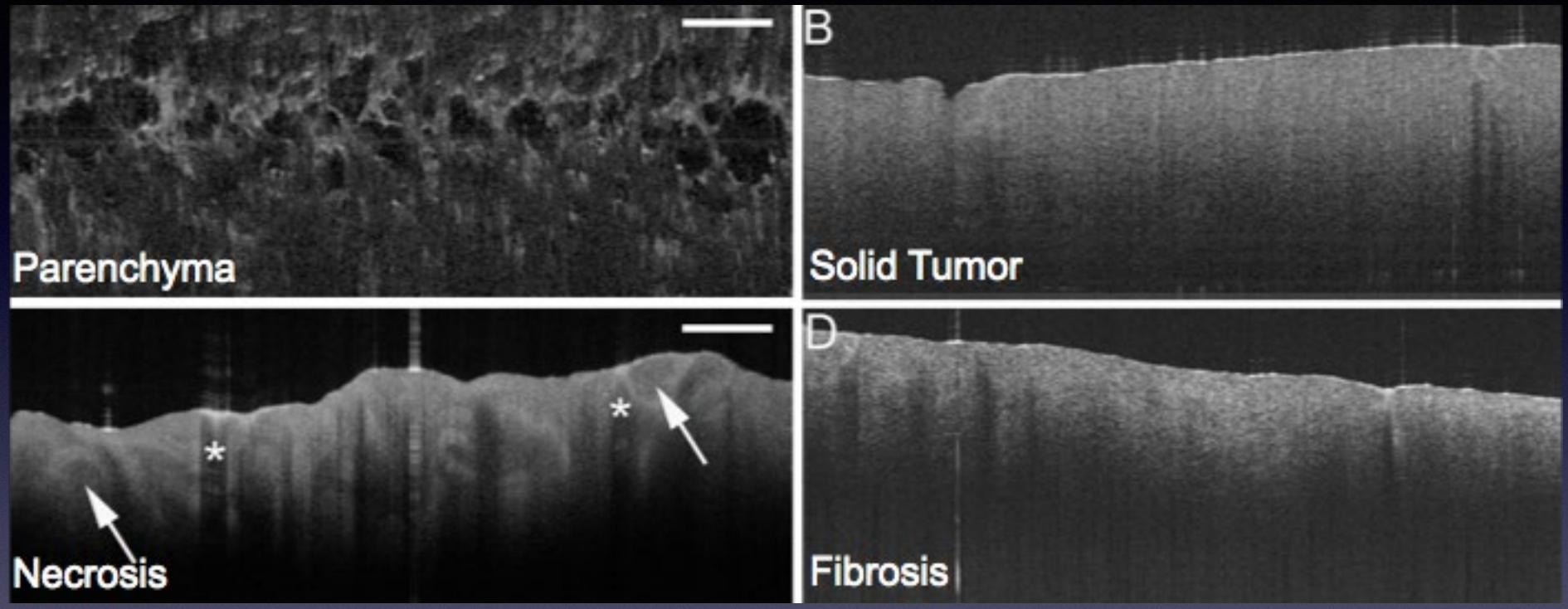
Training Image: Nodule



1. Lack of signal void alveoli
2. Lack of evenly spaced high intensity specular reflections from collapsed alveoli

Hariri LP et al. Chest. 144(4):1261-8. 2013

OCT: Tumor Versus Non-diagnostic Contaminants



Structural OCT can differentiate tumor from:

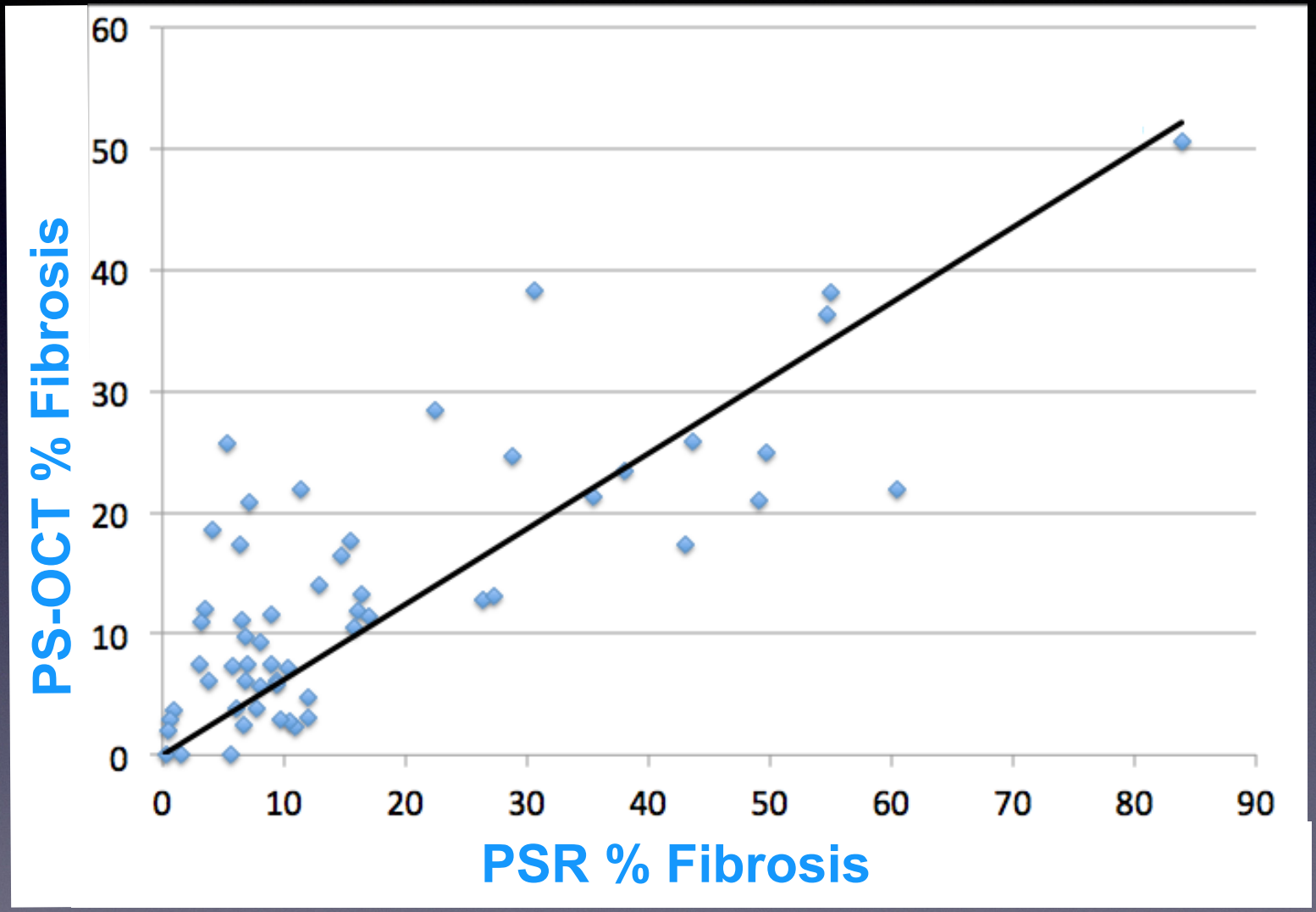
- Airway

- Parenchyma

- Necrosis

<u>Cannot differentiate solid tumor from fibrosis</u>

Quantifying Tumor Fibrosis: PS-OCT vs Picrosirius Red



Correlation (r) = 0.793

Hariri et al. Clinical Cancer Research. 2019

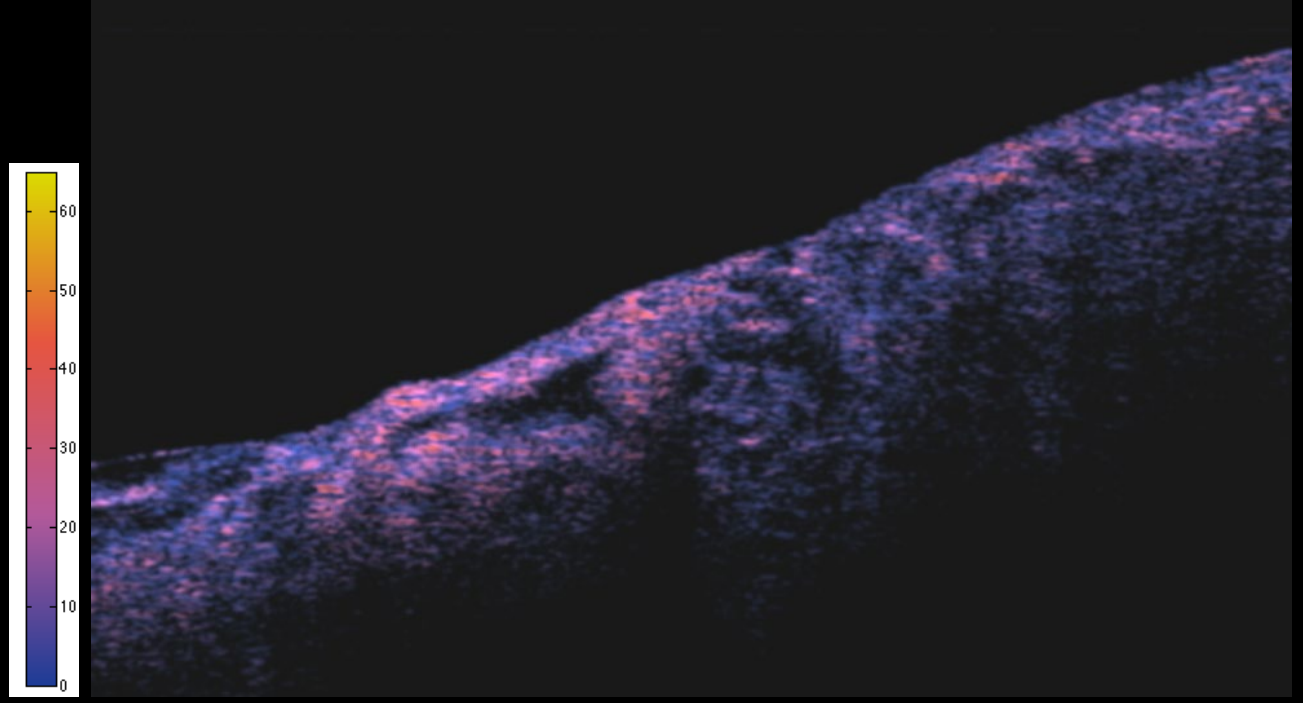
PS-OCT distinguishes low and high tumor fibrosis content

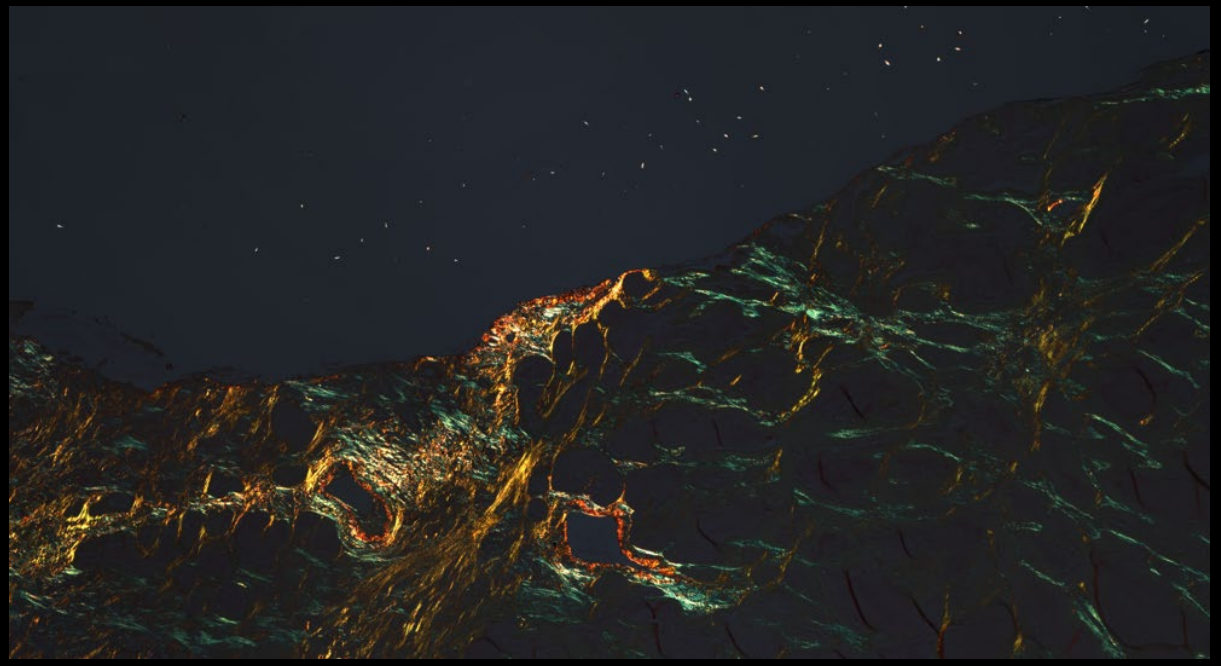
		PS-OCT % Fibrosis		
		≤ 20%	> 20%	Total
PSR % Fibrosis	≤ 20%	40	2	42
	> 20%	3	12	15
	Total	43	14	57

Fisher's exact test

The two-tailed P value is less than 0.0001
The association between rows and columns
is considered to be extremely statistically significant.

Hariri et al. Clinical Cancer Research. 2019

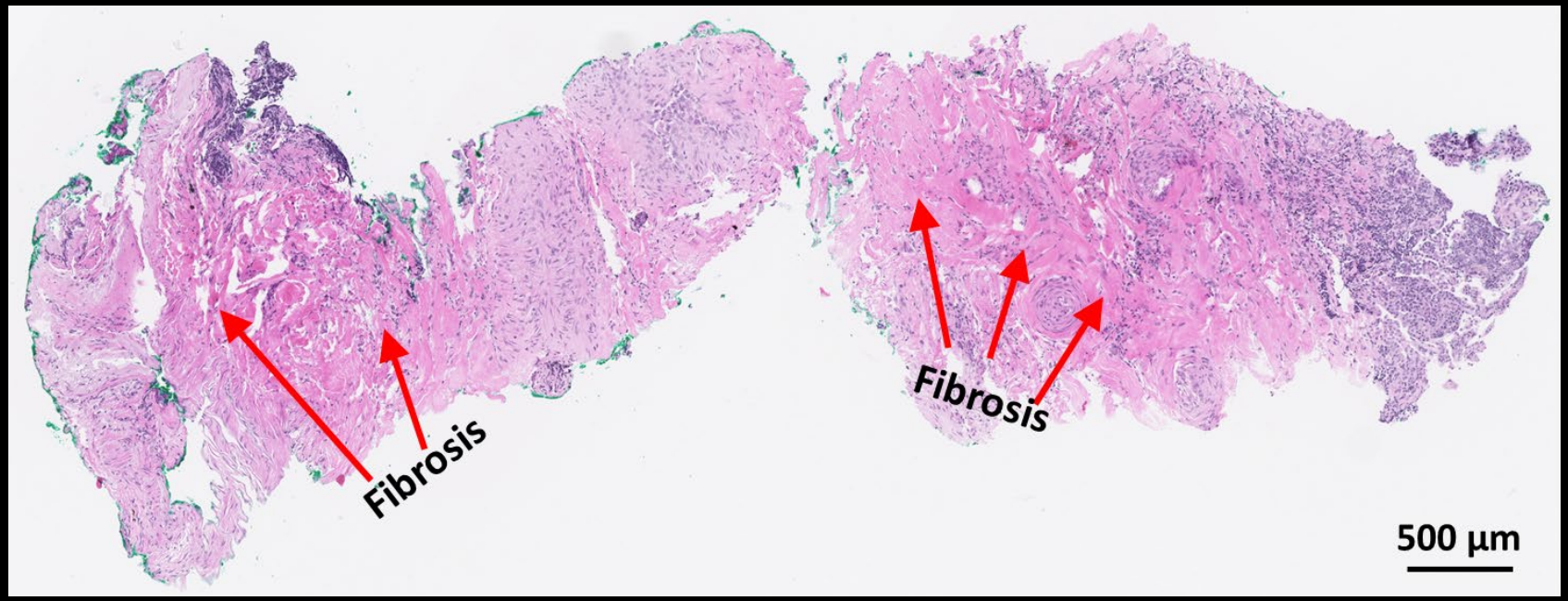




Minimally invasive AdenoCA:
Focus of fibrosis

Hariri et al. Clinical Cancer Research. 2019

Challenges with low tumor yield on core needle biopsies (CNB)



- Presence of fibrosis and atelectatic lung tissue can lead to nondiagnostic/inadequate specimen
- Inadequate sampling leads to delayed diagnosis & repeat biopsy procedures

There is a critical need for rapid, non-destructive rapid method for intraoperative tumor adequacy assessment in CNB

PS OCT to quantify tumor in CNB specimens

- **Birefringence:** measures polarization changes in the light returning from the tissue
- **Degree of polarization uniformity (DOPU):** measures the randomization of the polarization states, leading to depolarization

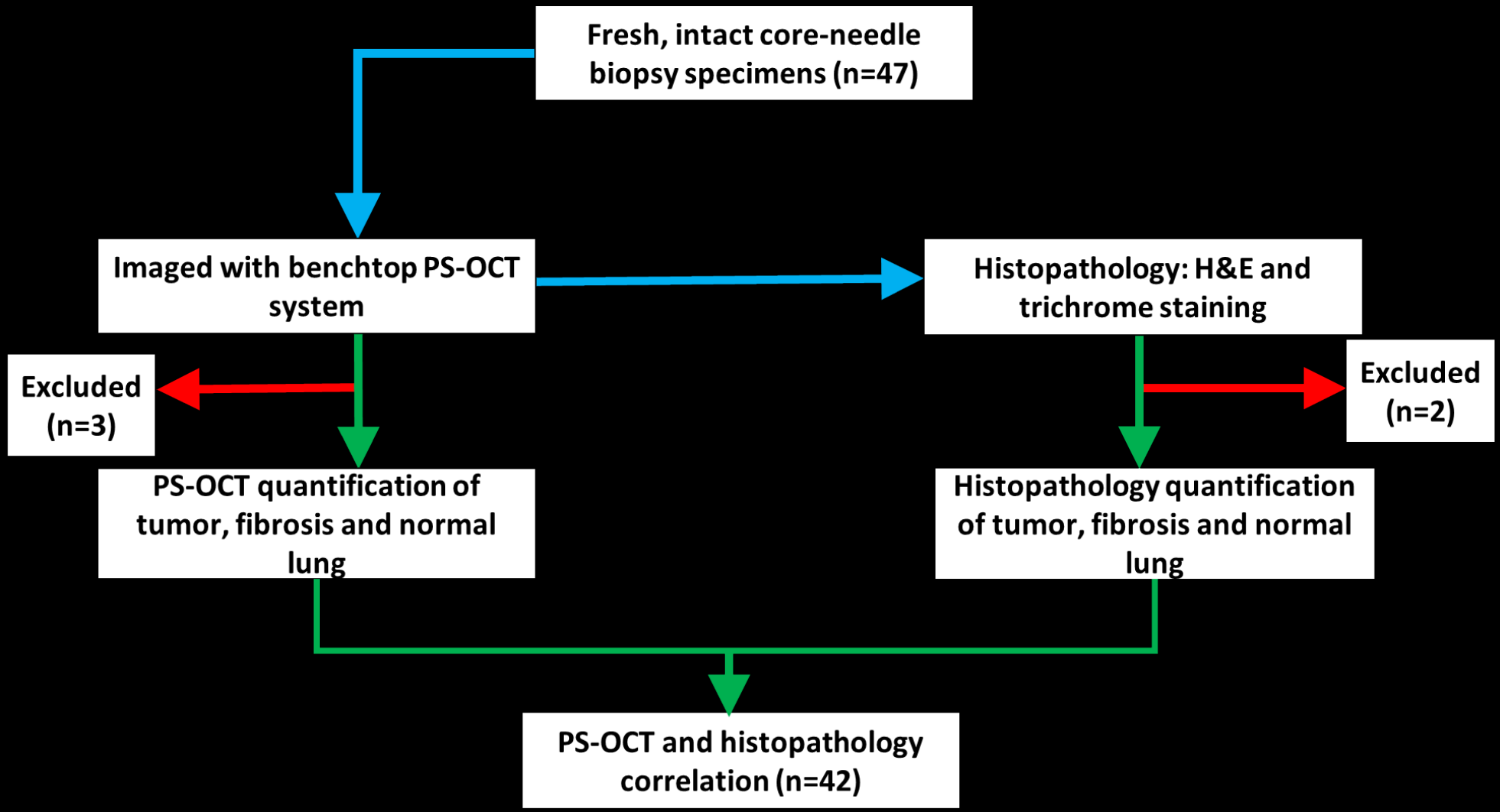
Aims

Aim 1: Use PS-OCT to quantify the amount of tumor, fibrosis and normal lung in core-needle biopsy specimens and compare with matched histopathology in a blinded assessment

Aim 2: Investigate the potential of PS-OCT to distinguish between biopsies with low tumor vs high tumor content with high sensitivity and specificity

Sreyankar Nandy, PhD

Method


Fresh, intact core-needle biopsy specimens (n=47)
Imaged with benchtop PS-OCT system
Histopathology: H&E and trichrome staining
Excluded (n=3)
Excluded (n=2)
PS-OCT quantification of tumor, fibrosis and normal lung
Histopathology quantification of tumor, fibrosis and normal lung
PS-OCT and histopathology correlation (n=42)

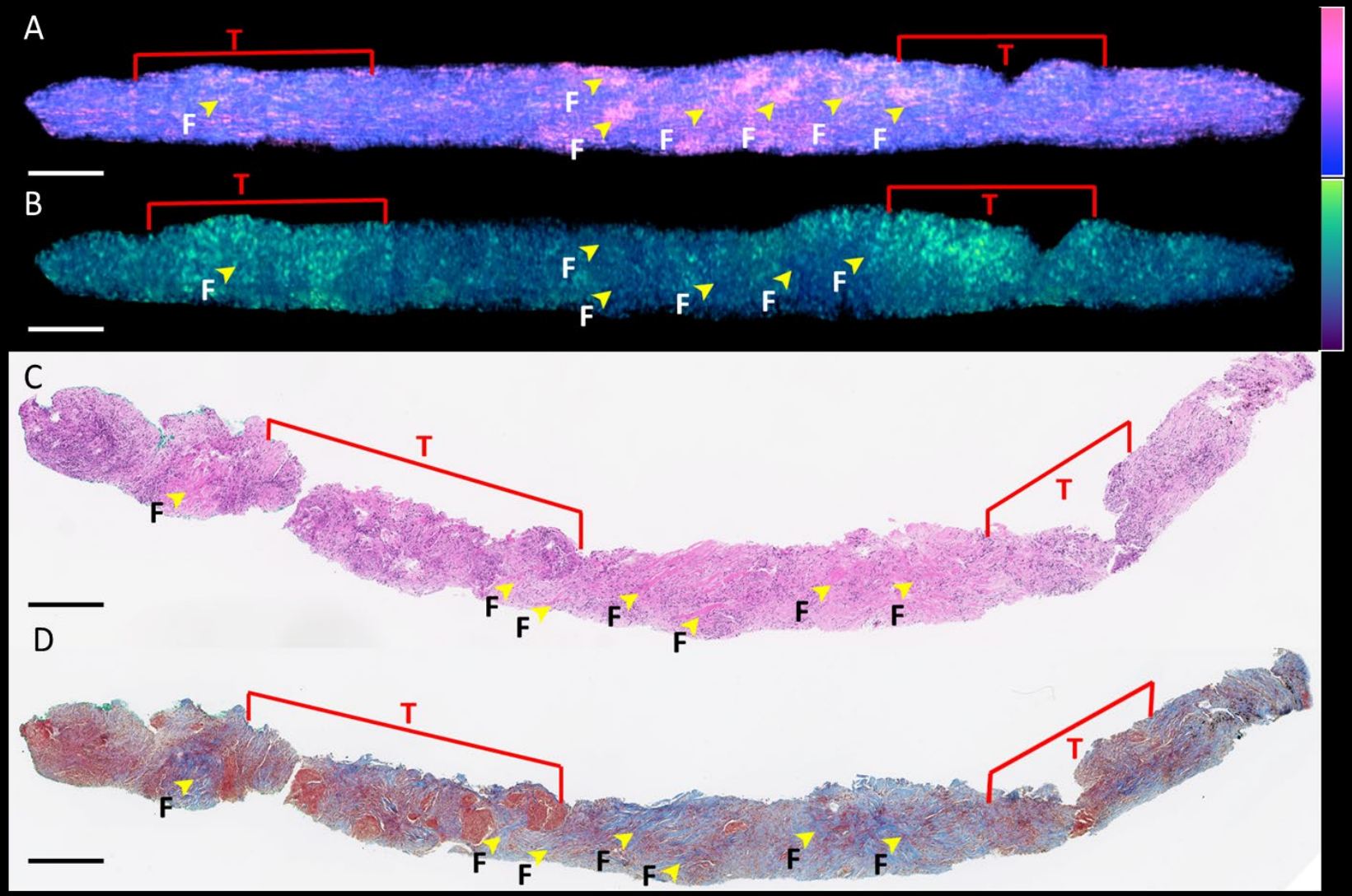
Results

A. PS-OCT birefringence

B. PS-OCT DOPU

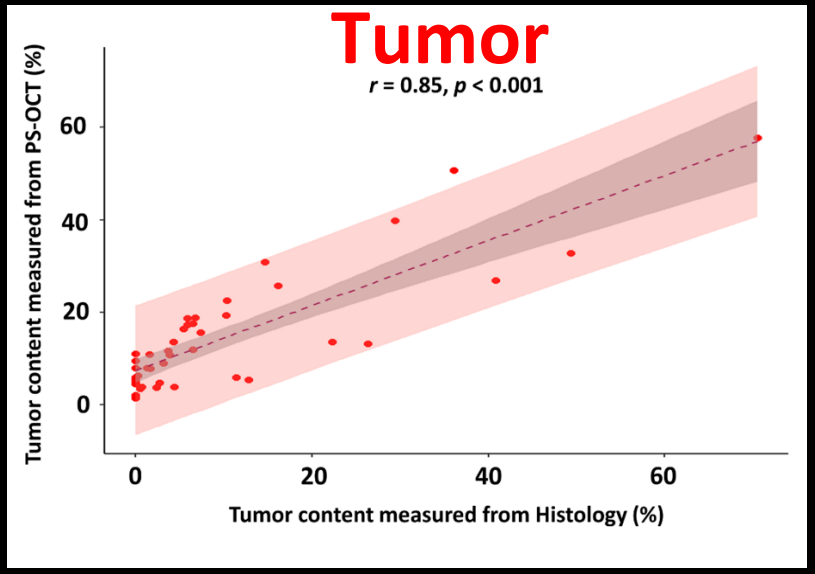
C. Hematoxylin and eosin

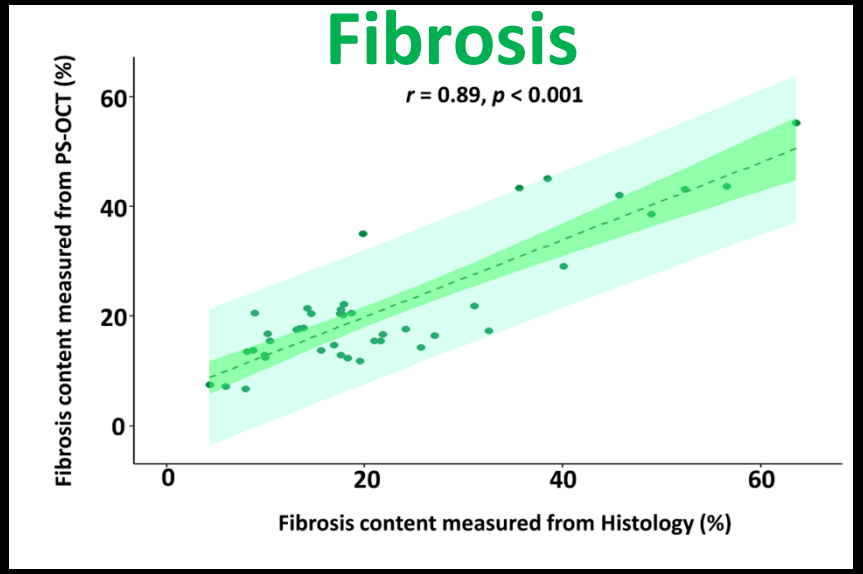
D. Trichrome stain

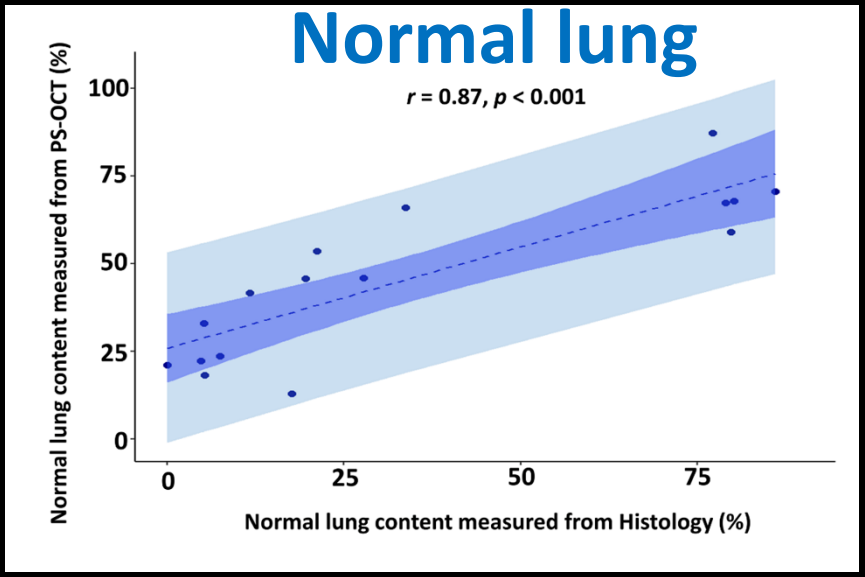


T: Tumor; F: Fibrosis
Scalebar : 500 µm

Results







Fisher's exact contingency table for classification of CNB specimens based on tumor content

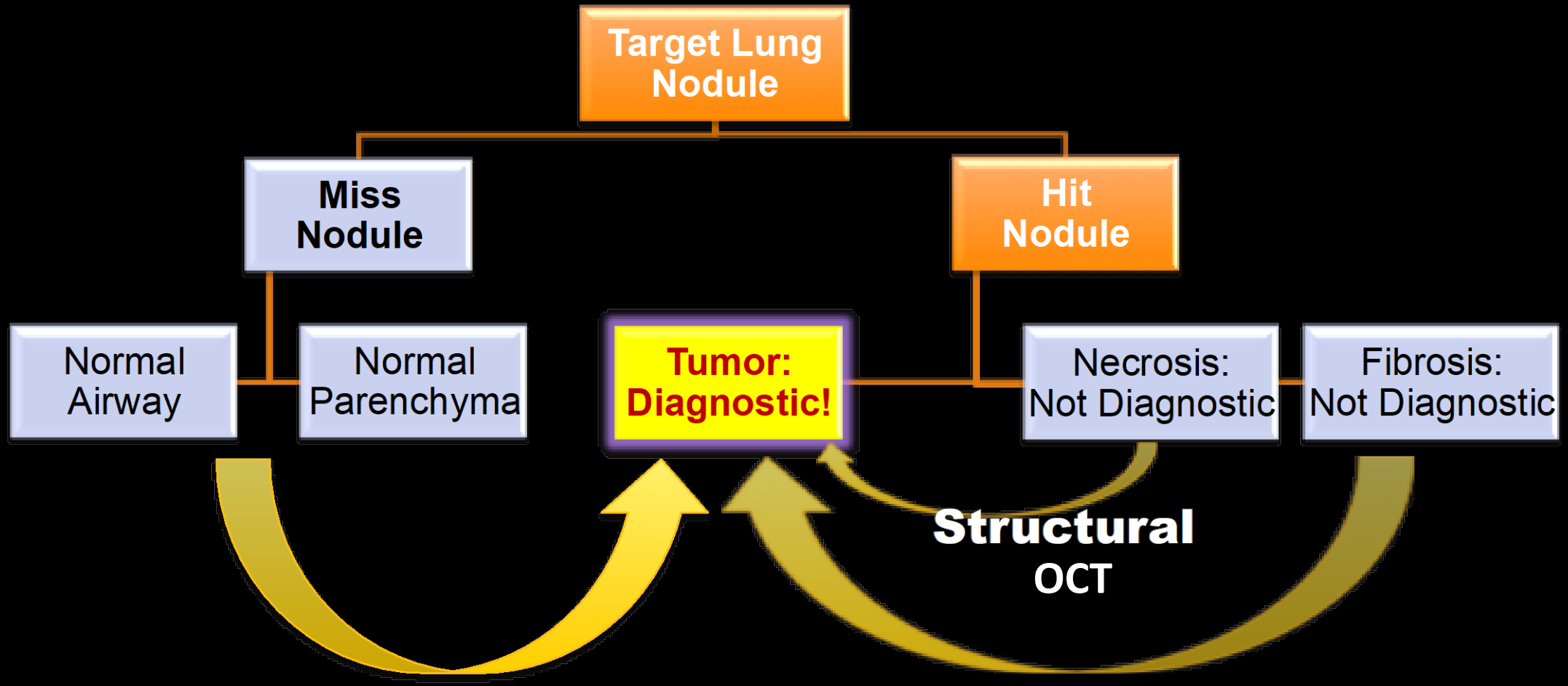
		PS-OCT Tumor Quantification		
Histology Tumor Quantification		≤ 25%	> 25%	Total
	≤ 25%	34	2	36
	> 25%	1	5	6
	Total	35	7	42

Tumor

Sensitivity: 94.4%

Specificity: 83.3%

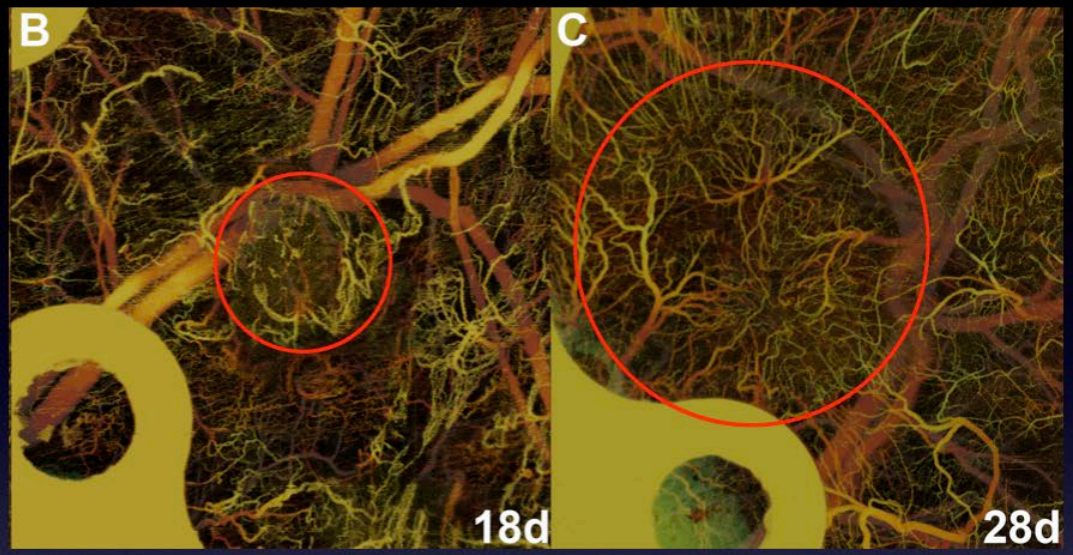
OCT Guided Biopsy of Lung Nodules



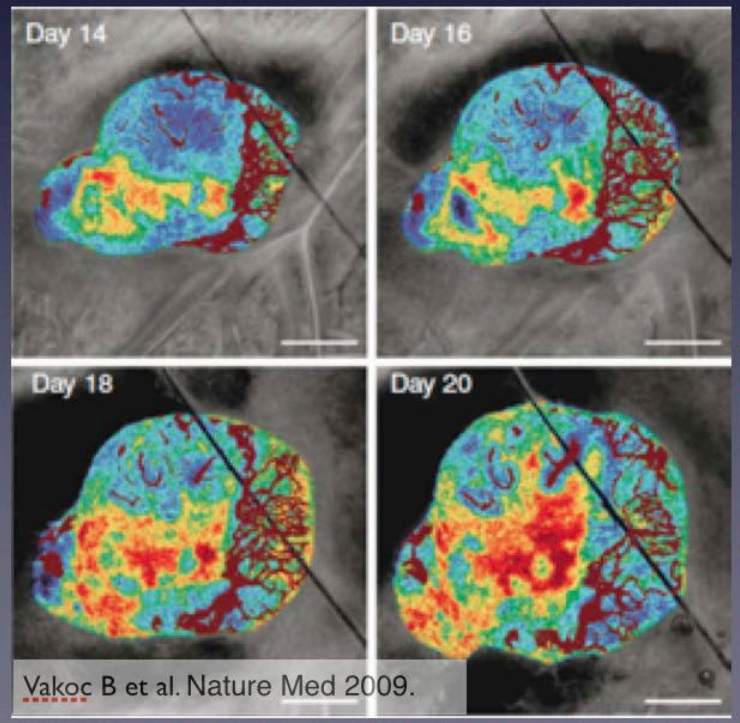
Future Directions in Lung Cancer

- **In vivo study to assess diagnostic yields of transbronchial biopsy with and without OCT guidance**
- **Assess diagnostic capability of OCT + biopsy vs biopsy alone**
- **Can optical imaging provide “virtual tissue” with additional diagnostic information when added to traditional biopsy?**

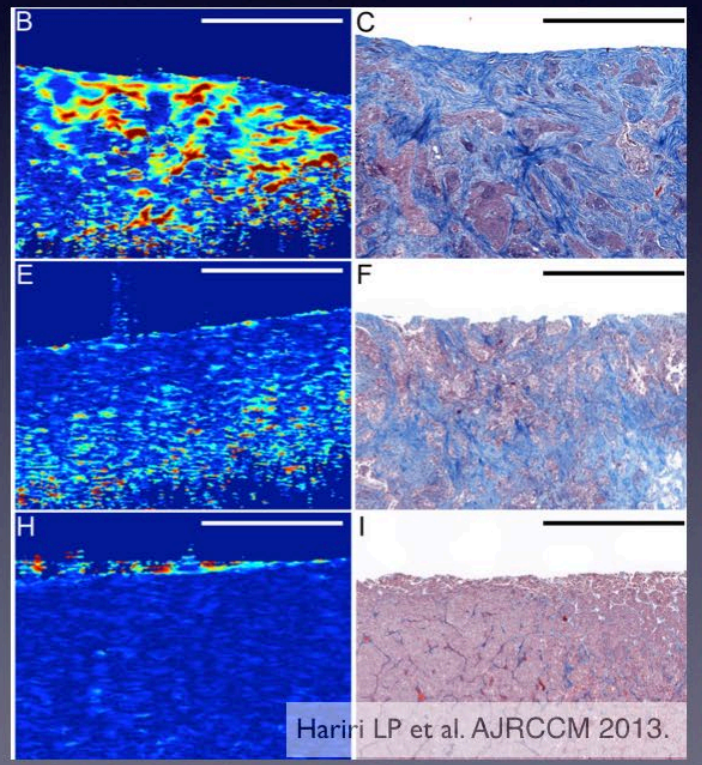
Higher Precision Tumor Measurements Over Therapy



OCT visualizes tumor angiogenesis over time



Optical scattering properties delineate necrotic (red-yellow) and viable (blue-green) tumor regions



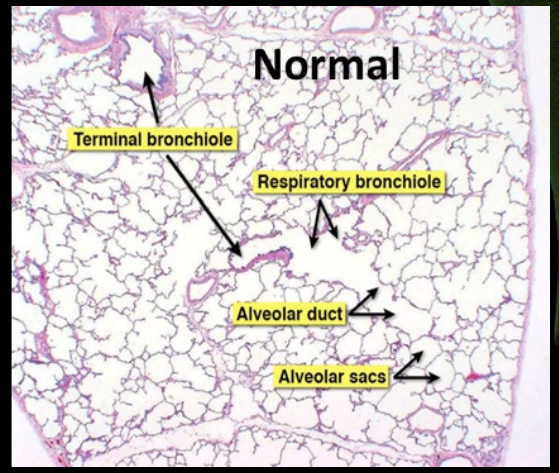
PS-OCT identifies regions of fibrotic stroma in tumor

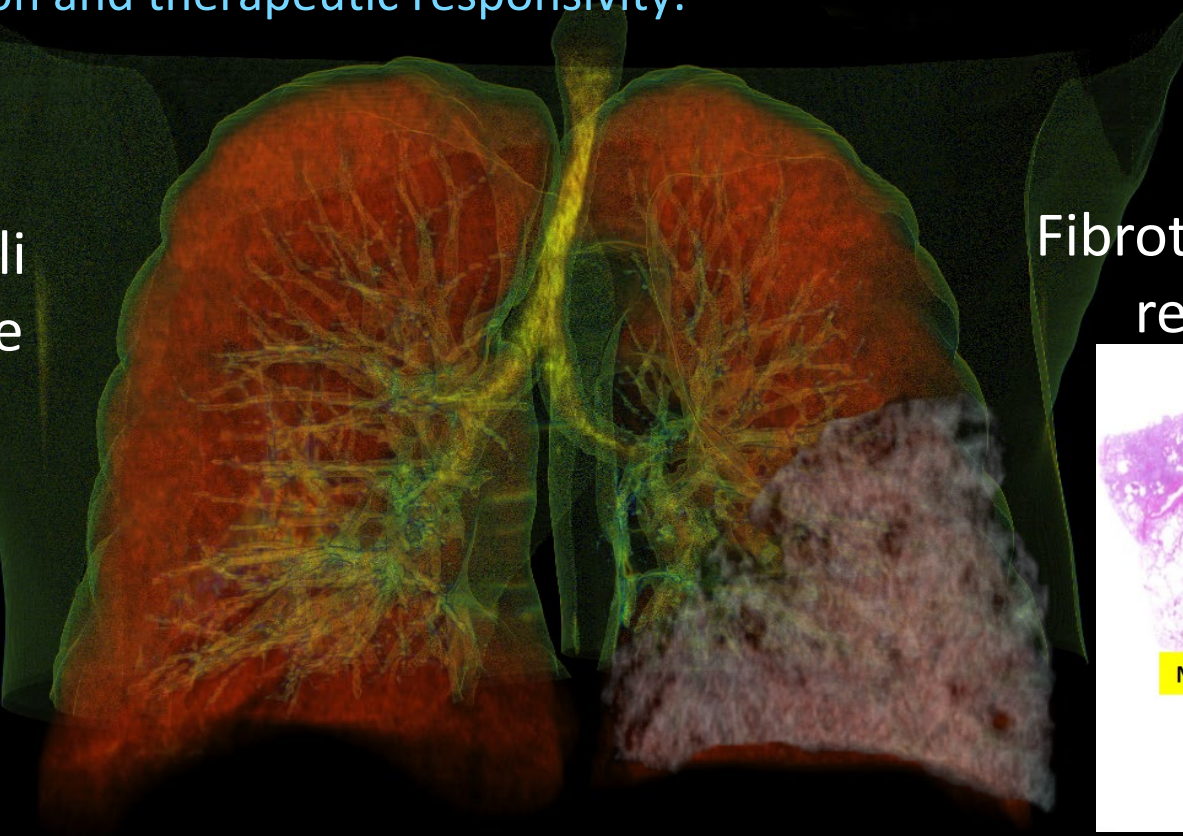
Idiopathic Pulmonary Fibrosis (IPF)

- Chronic progressive fibrosing interstitial lung disease (ILD) of unknown cause ('idiopathic')
- Pathology/Radiology correlate: Usual interstitial pneumonitis (UIP)
- Important to distinguish UIP/IPF from other ILDs for survival implications & therapeutic strategy
- Early diagnosis (especially at the asymptomatic ILA stage) opens the door for early therapeutic intervention, which is essential for maximizing lung function preservation
- The ability to track changes in disease on a microscopic scale over time could allow for assessment of disease progression and therapeutic responsivity.

Normal Lung

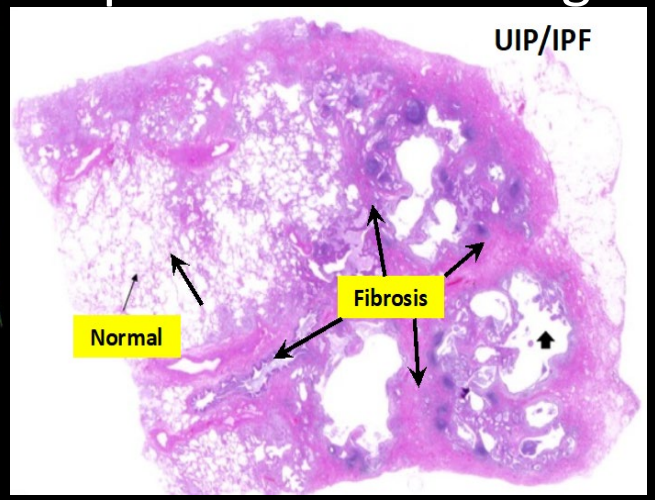
Thin, lattice like alveoli facilitate gas exchange





UIP

Fibrotic/scar tissue gradually replaces normal lung

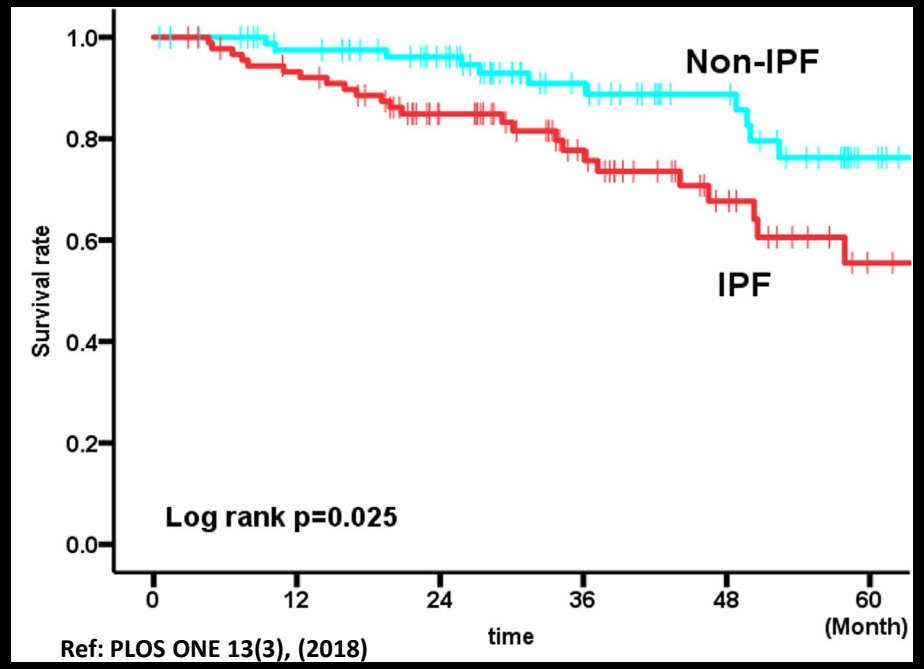


Slide courtesy of Dr. H. Tazelaar, Mayo Clinic

Idiopathic pulmonary fibrosis (IPF)

Prognosis

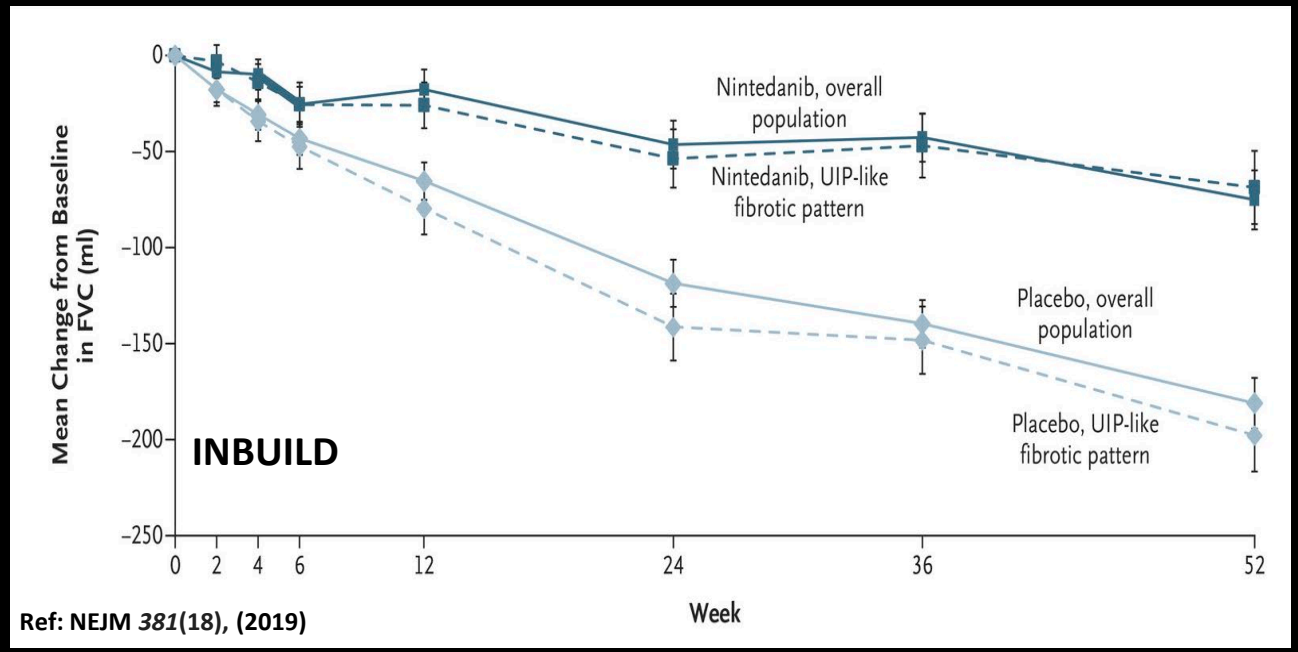
- Worst among all ILDs: 5 yr. survival rate 20-40%
- Prognosis worse than some cancers



Ref: PLOS ONE 13(3), (2018)

Treatment

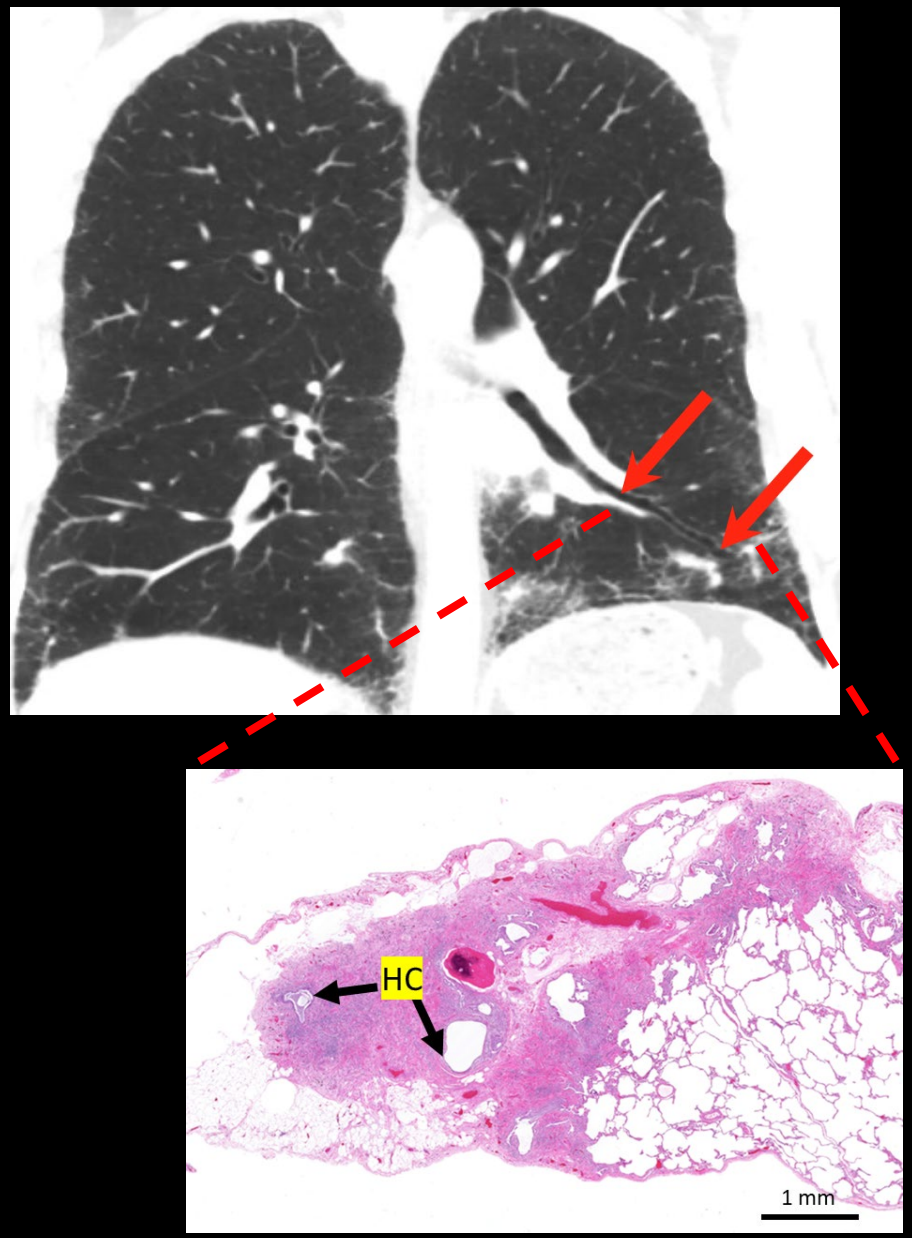
- **UIP/IPF**: Treated with antifibrotics, and immunosuppressants are contraindicated
- **Non-IPF**: Treated with a combination of antifibrotic and immunosuppressants



Ref: NEJM *381*(18), (2019)

Important to distinguish UIP/IPF from other ILDs for survival implications & therapeutic strategy

Diagnostic limitations of ILD Radiology



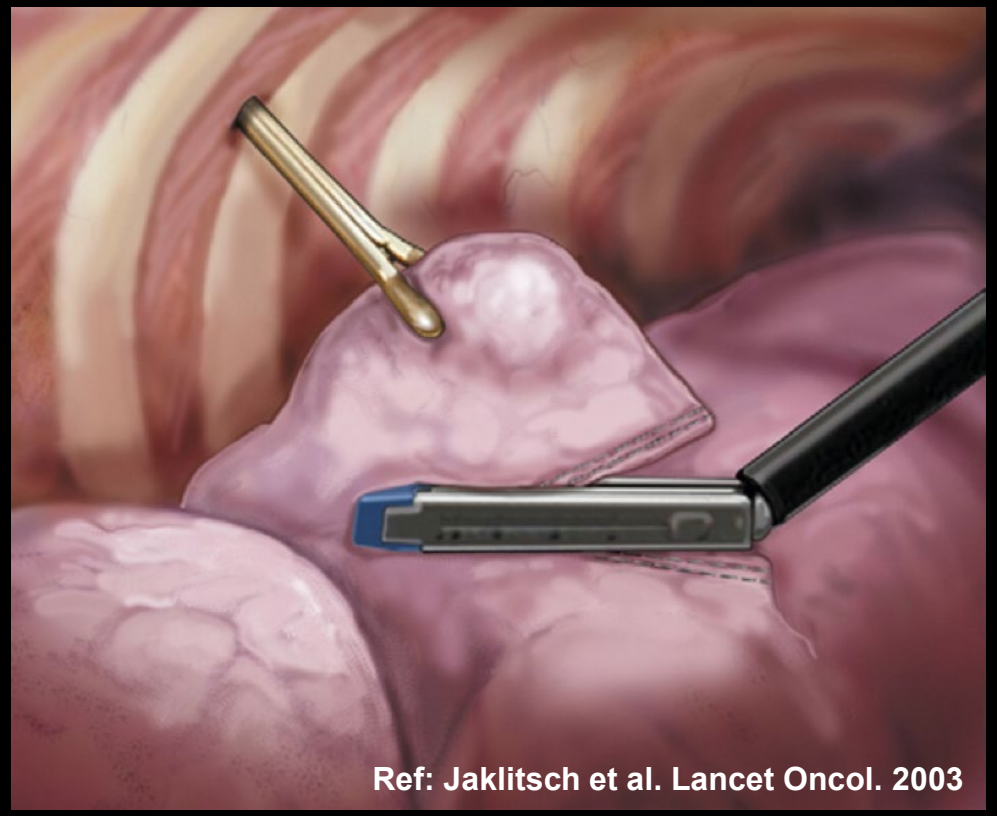
HRCT resolution limitation (~2-3mm) can make it difficult to distinguish microscopic ILD features

- Difficult to identify microscopic honeycombing (< 3 mm)
- Challenging to distinguish honeycombing from mimickers such as traction bronchiectasis or emphysema
- Challenging to distinguish the various ILDs in early-stage

If the patient has low confidence ILD diagnosis

Surgical Lung Biopsy is recommended.

Challenges in ILD histopathology

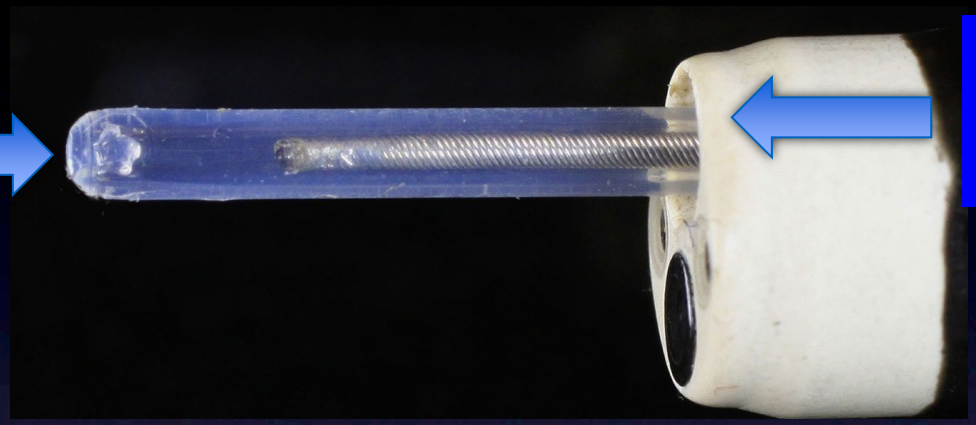


- Invasive surgical procedure
- Increased risk of morbidity and mortality
- Multiple biopsies are required for diagnosis due to disease heterogeneity
- Risks of prolonged hospitalization
- Not recommended in high-risk patients

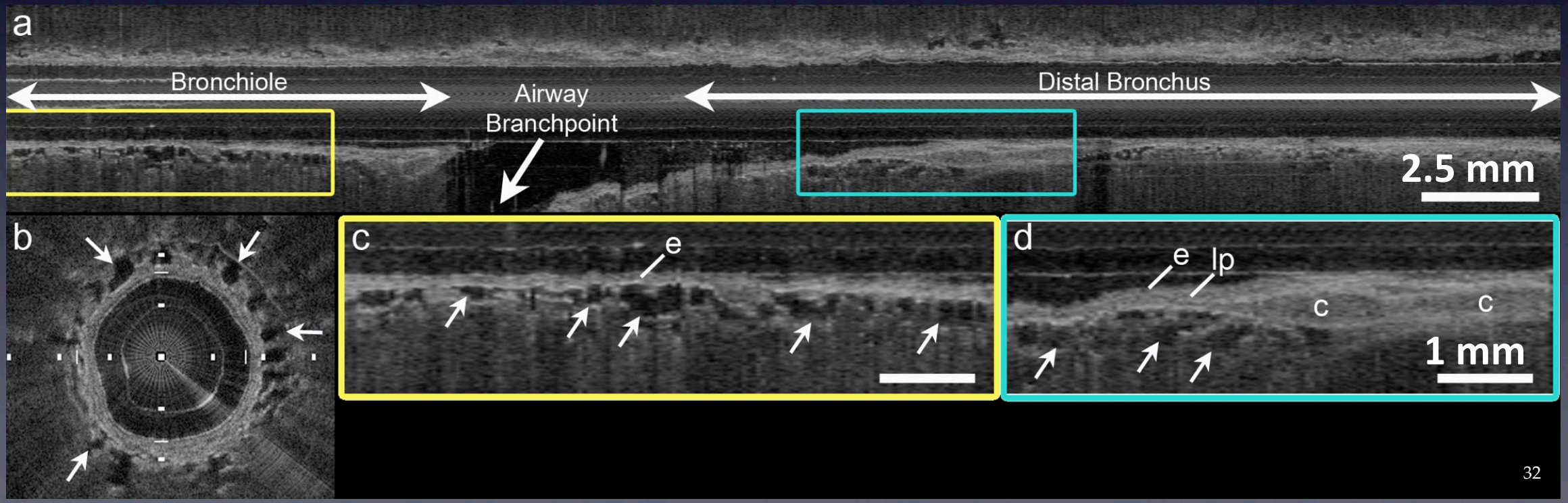
Need for developing minimally invasive microscopic assessment tool for ILD diagnosis

Endobronchial OCT can access the peripheral lung: Can OCT assess peripheral lung disease?

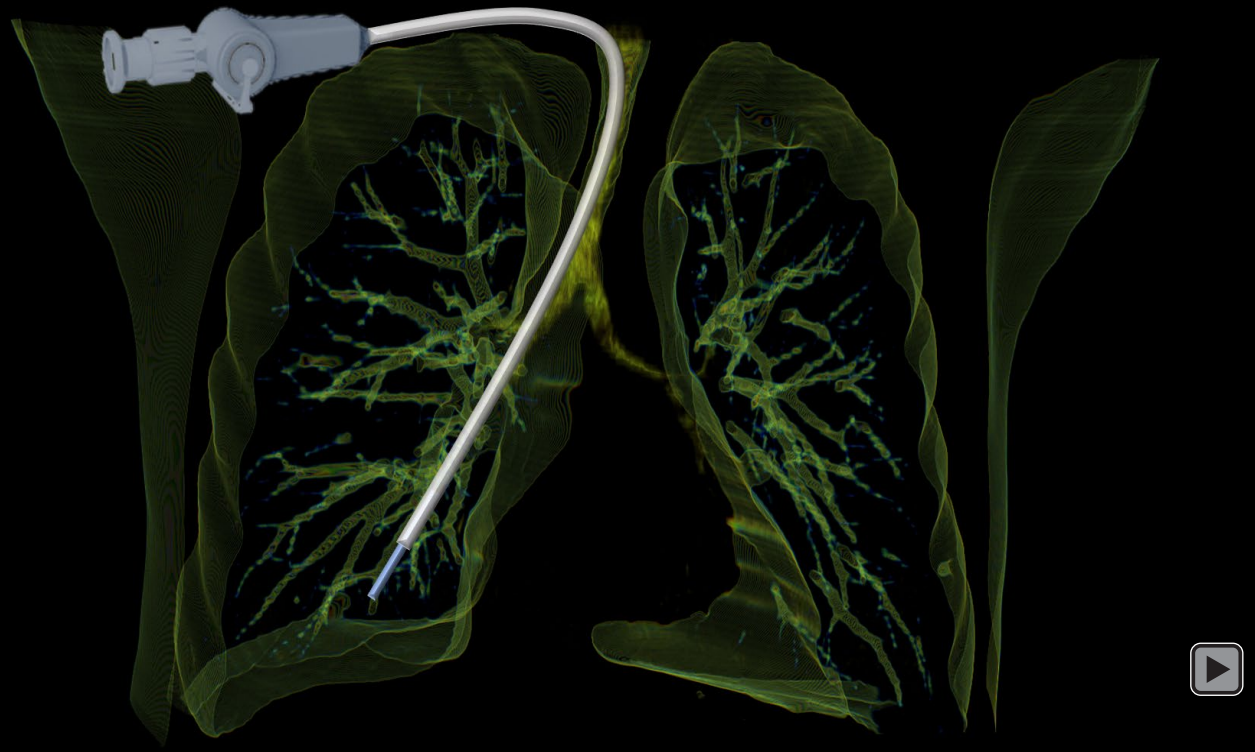
OCT catheter 1.6 mm OD



Bronchoscope working channel



Endobronchial OCT



- Catheter is passed through the bronchoscope working channel to the subpleural lung
  - 8-10 cm of volumetric imaging within 1-2 minutes/site
    - Able to image multiple distinct anatomic sites

Determine whether EB-OCT can provide a rapid, low-risk, non-surgical method for microscopic diagnosis of ILD

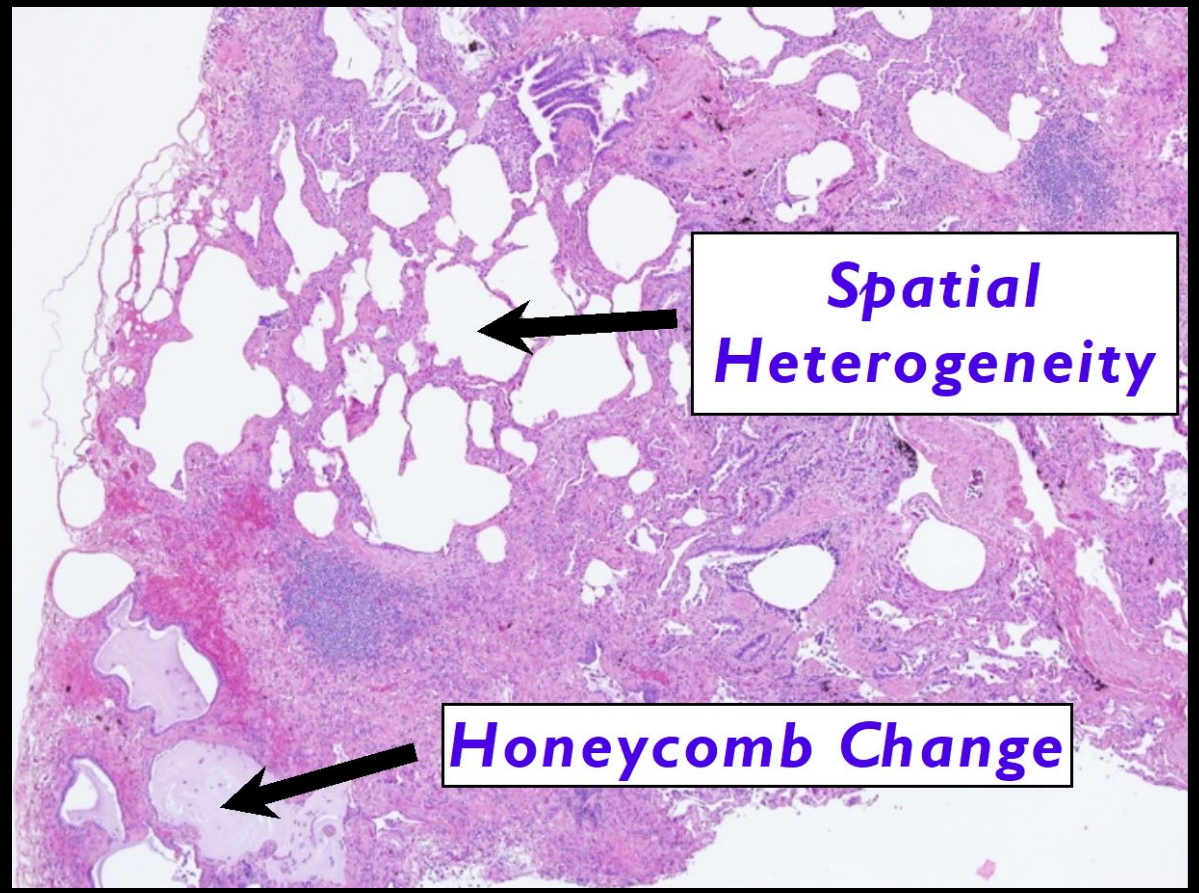
Develop and validate EB-OCT features of ILD

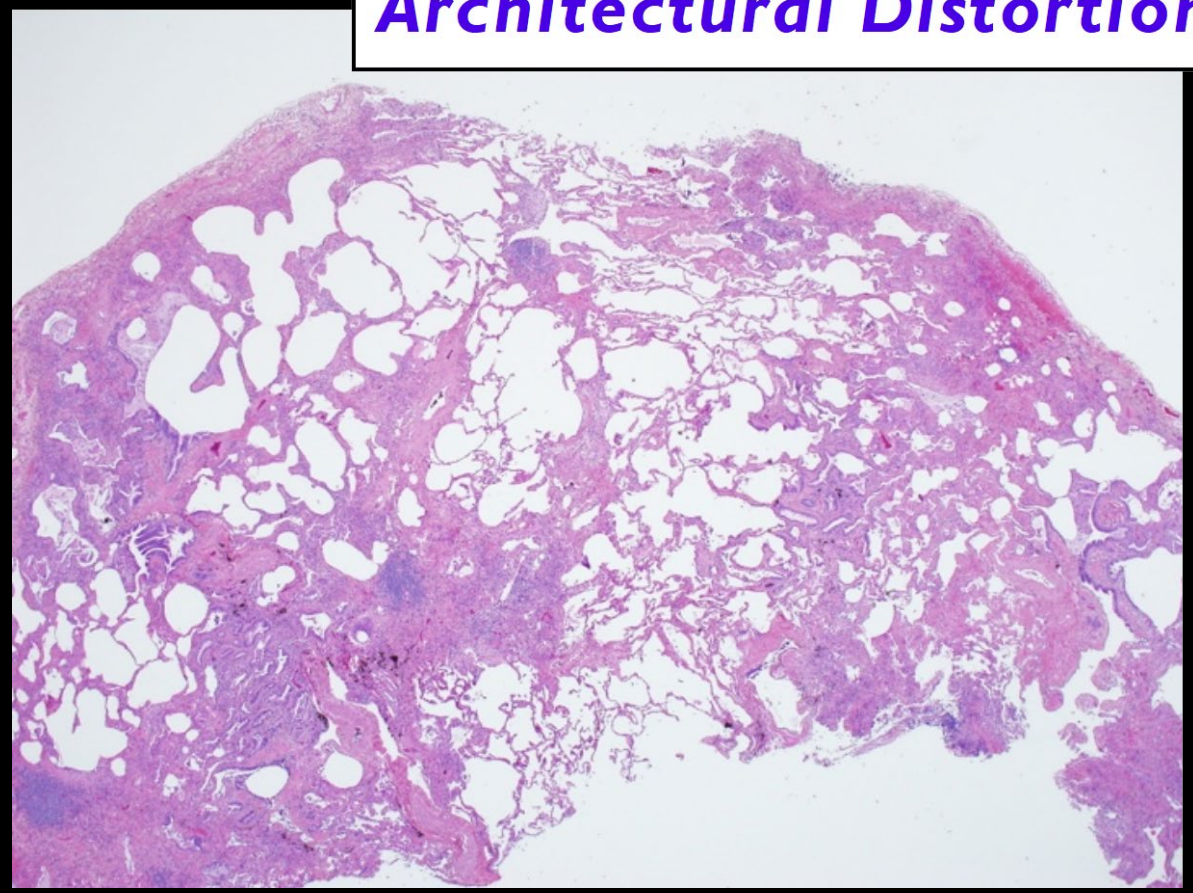
Identify microscopic ILD features from *ex vivo* EB-OCT imaging and validate against matched histology (freshly resected lung samples including wedge biopsies, transplant & autopsy specimens)

Conduct a prospective diagnostic accuracy study in ILD patients undergoing SLB

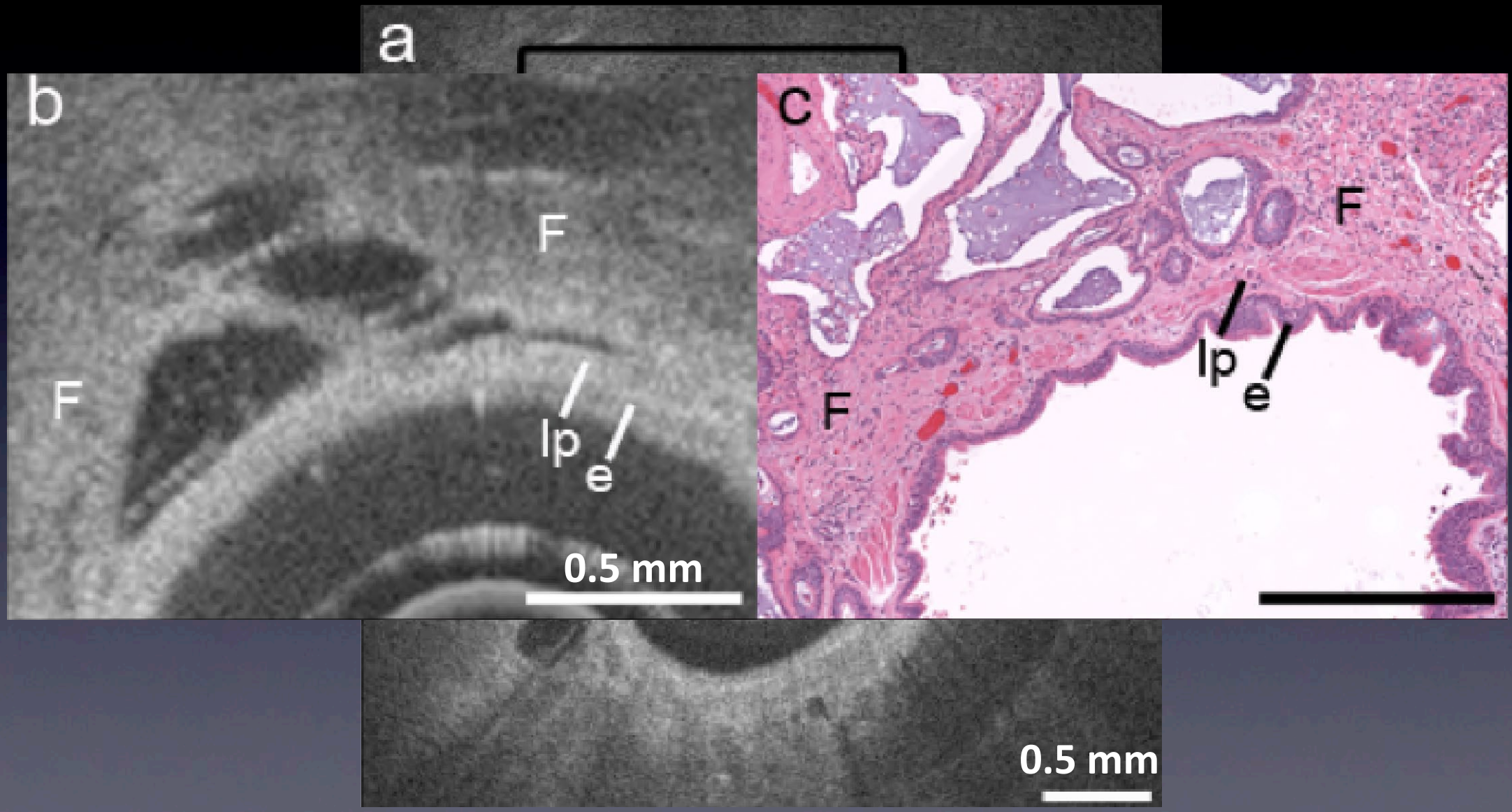
Conduct a study in patients undergoing SLB for ILD diagnosis to determine whether OCT can identify microscopic features of UIP/IPF and distinguish from other ILDs

Microscopic Features of UIP/IPF





Endobronchial OCT in IPF Lung: Peripheral Fibrosis and Microscopic Honeycomb



Diagnostic Accuracy of EB-OCT in ILD

Prospective study to determine whether EB-OCT can accurately diagnose ILD

- Patients with suspected ILD undergoing diagnostic surgical lung biopsy (SLB)
- Performed EB-OCT imaging during bronchoscopy before SLB
  - Based on areas of abnormality on recent HRCT
  - Approx 4-8 OCT sites per patient, including in upper, mid, and lower lobes
  - Each OCT site has an 4-8 cm long imaging pullback
- After imaging, patients underwent surgical lung biopsy per clinical care
- Independently compare EB-OCT against histopathology and clinical follow-up diagnosis

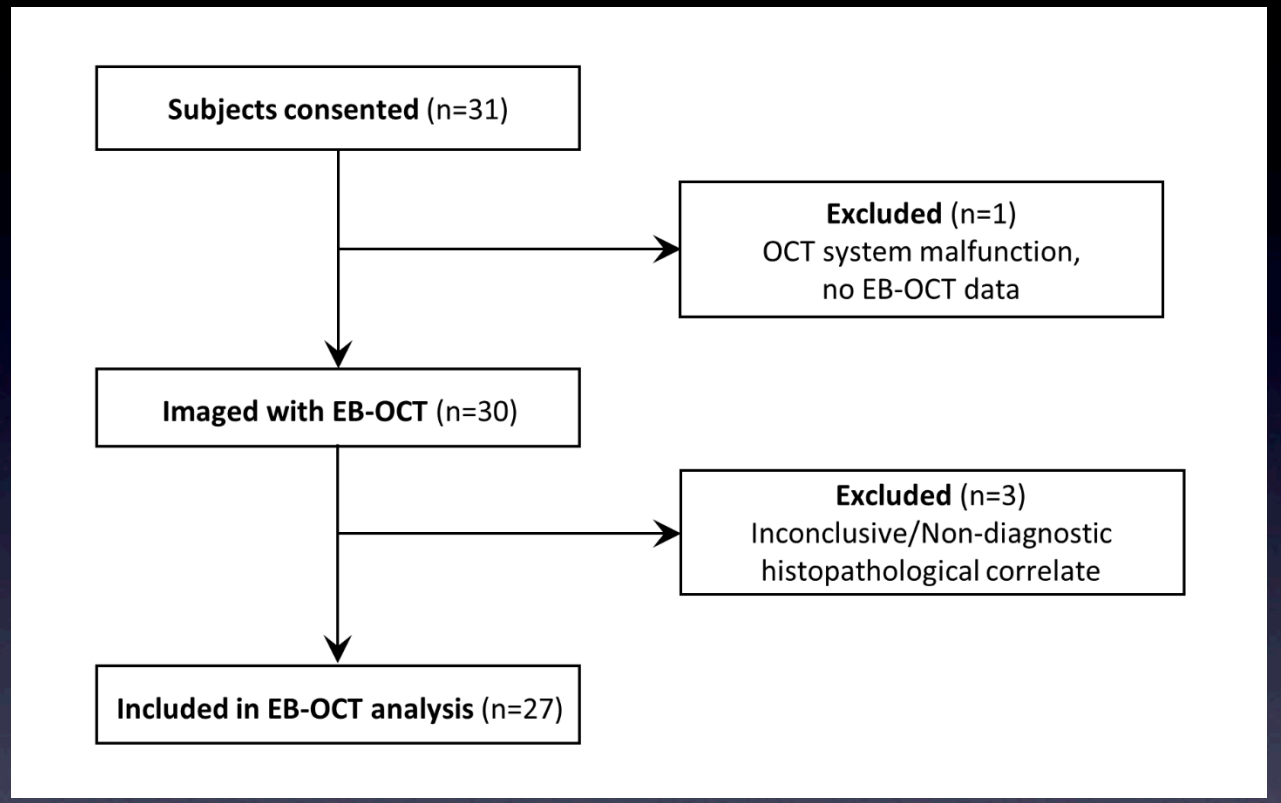
Sreyankar Nandy, PhD

Rebecca Raphaely, MD

Nandy S*, Raphaely R*…. Hariri LP. AJRCCM. In Press. 2021.

Diagnostic Accuracy of EB-OCT in ILD

- EB-OCT interpreted by pathologist with expertise in ILD and OCT, blinded to histology: UIP, NSIP, ACF, or mixed ACF/UIP
- Histopathology interpreted by two independent pathologists, blinded to OCT data/interpretation. If discrepancy, read by 3rd pathologist and majority diagnosis rendered.
- Clinical follow-up diagnosis obtained from EMR from patient's treating pulmonologist after systematic review of all available data



- Primary Outcome: EB-OCT sensitivity/specificity for histopathologic UIP and clinical IPF
- Secondary Outcome: Agreement between EB-OCT and histopathologic ILD pattern diagnosis

Nandy S*, Raphaely R*…. Hariri LP. AJRCCM. In Press. 2021.

EB-OCT in ILD patients

- Average of 6 EB-OCT imaging sites per patient (range 1-9 sites)
- Up to 8.7cm long pullback lengths per ROI (average 4cm, SD 1.5cm)

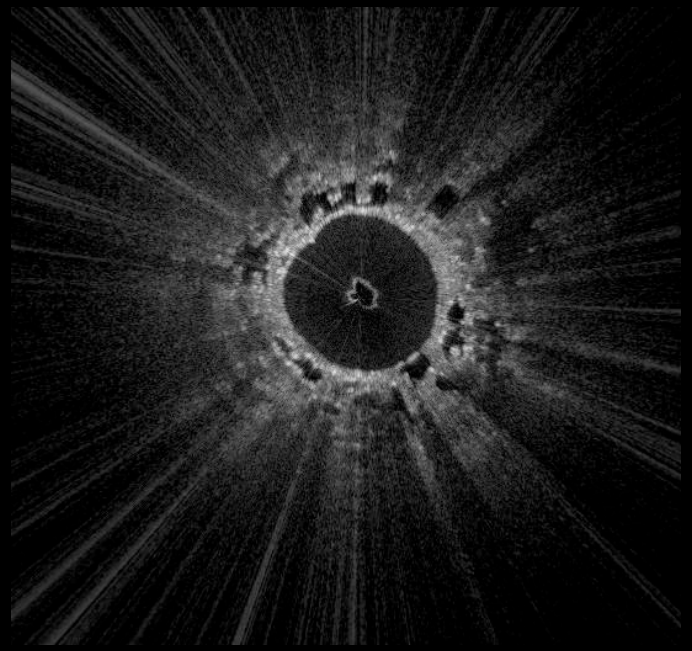
- Of 27 patients, the histopathologic diagnosis on SLB was:
  - 12 were diagnosed as UIP (44.5%)
  - 3 as mixed UIP/ACF/NSIP (11.1%)
  - 1 as ACF (3.7%)
  - 7 as mixed ACF/NSIP (25.9%
  - 3 as NSIP (11.1%)
  - 1 as DIPNECH with carcinoid tumorlet (3.7%)

- All patients diagnosed with UIP on SLB had a clinical follow-up diagnosis of IPF.
- All patients diagnosed with any other pattern had a clinical follow-up diagnosis of non IPF ILD.

Nandy S*, Raphaely R*…. Hariri LP. AJRCCM. In Press. 2021.

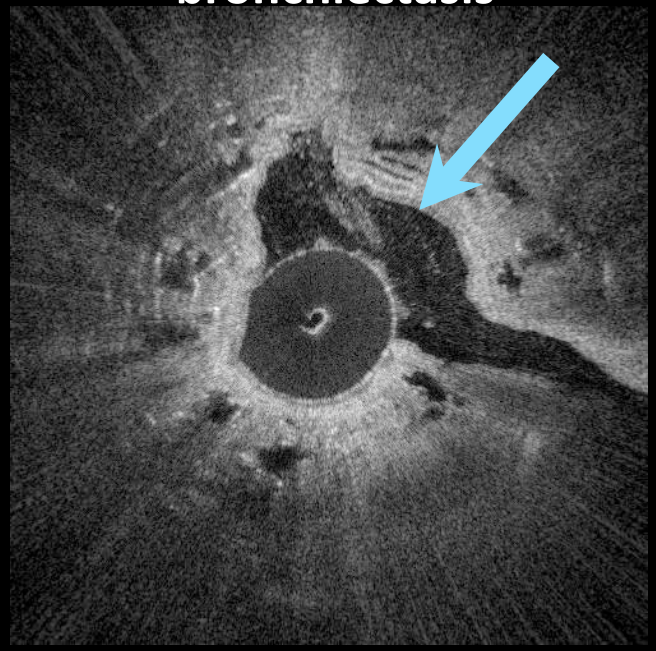
EB-OCT in ILD lung

Normal Lung



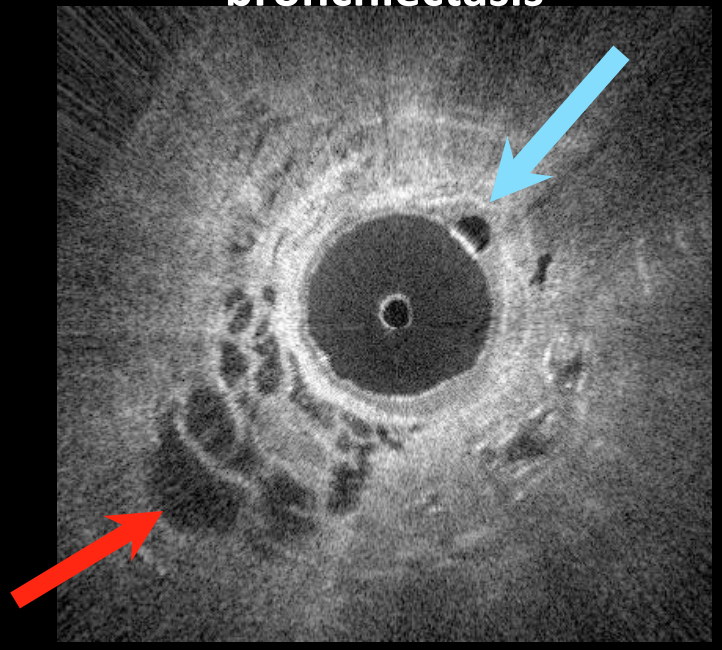
Lattice like regularly spaced alveoli in thin normal interstitial tissue

Non-destructive airway centered fibrosis with traction bronchiectasis



Blue Arrow: Traction bronchiectasis with fibrosis mimics honeycomb, but connected to airway

UIP: Destructive fibrosis with HC and traction bronchiectasis

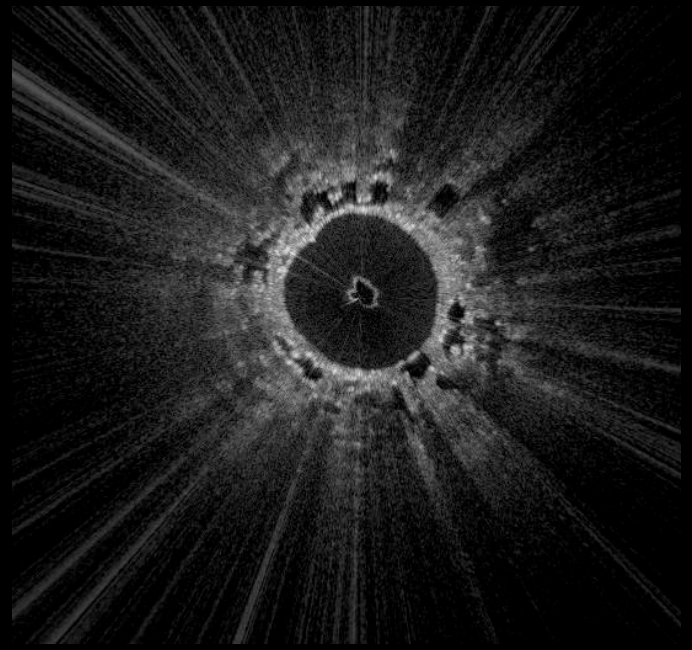


Red Arrow: Honeycomb change in fibrosis, not connected to airway

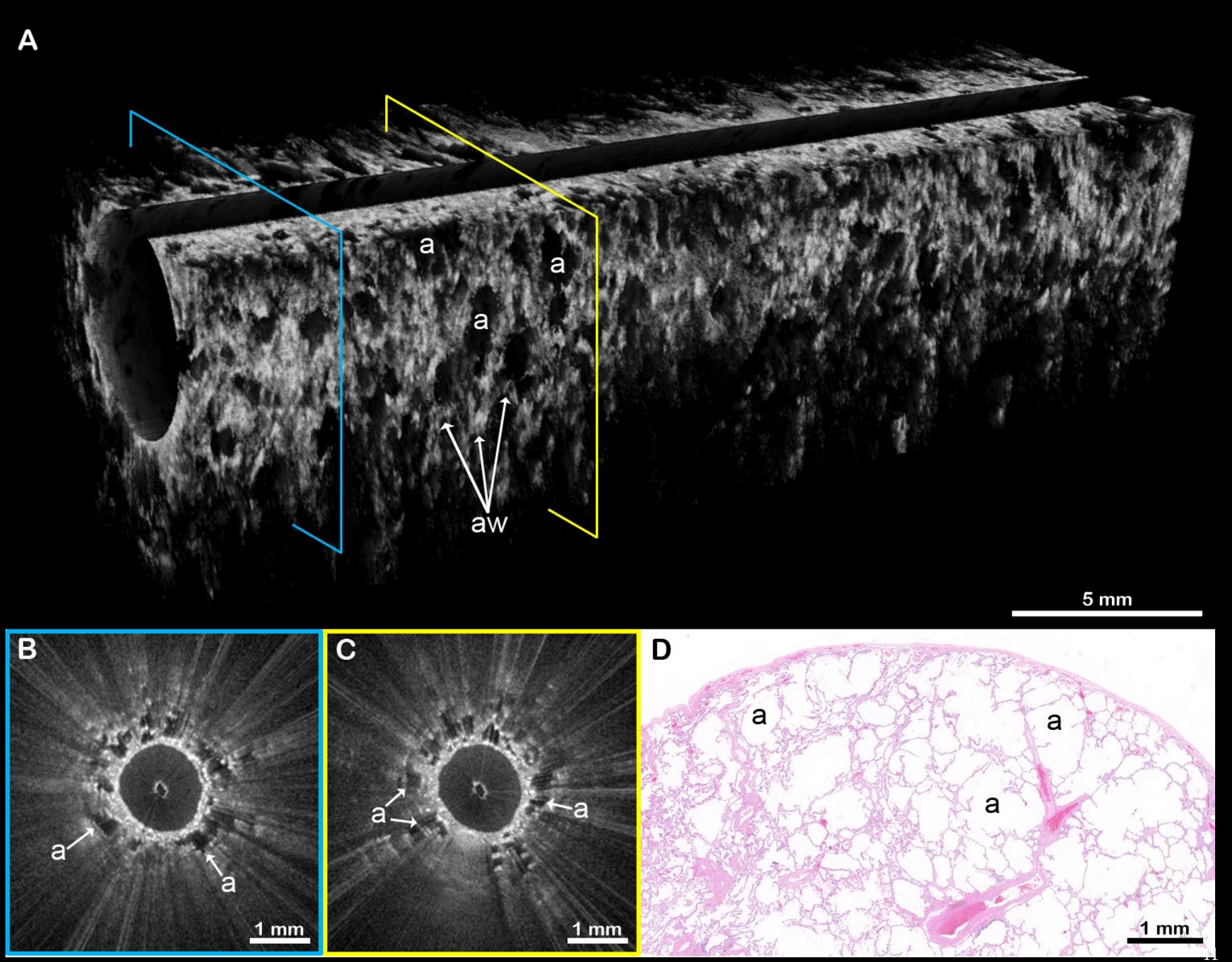
Blue Arrow: Traction bronchiectasis mimicking honeycomb, but connected to airway

Nandy S*, Raphaely R*…. Hariri LP. AJRCCM. In Press. 2021.

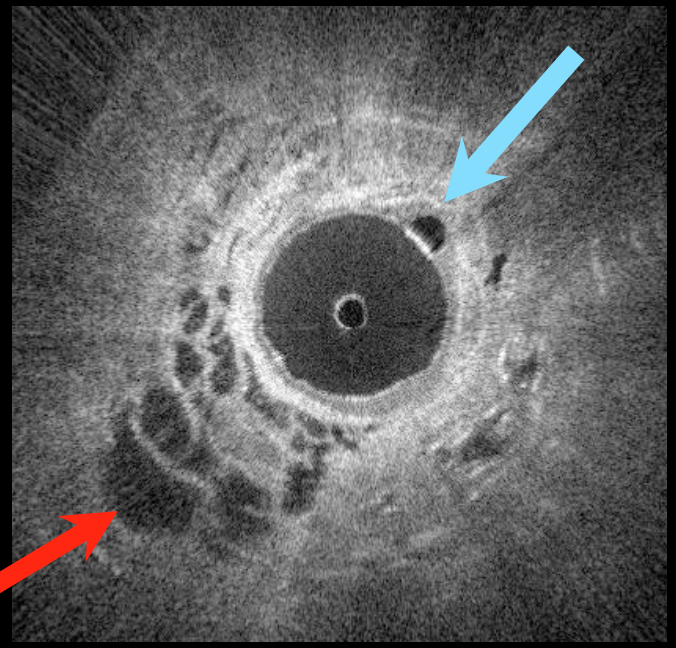
Preserved peripheral lung parenchyma



Lattice like regularly spaced alveoli in thin normal interstitial tissue

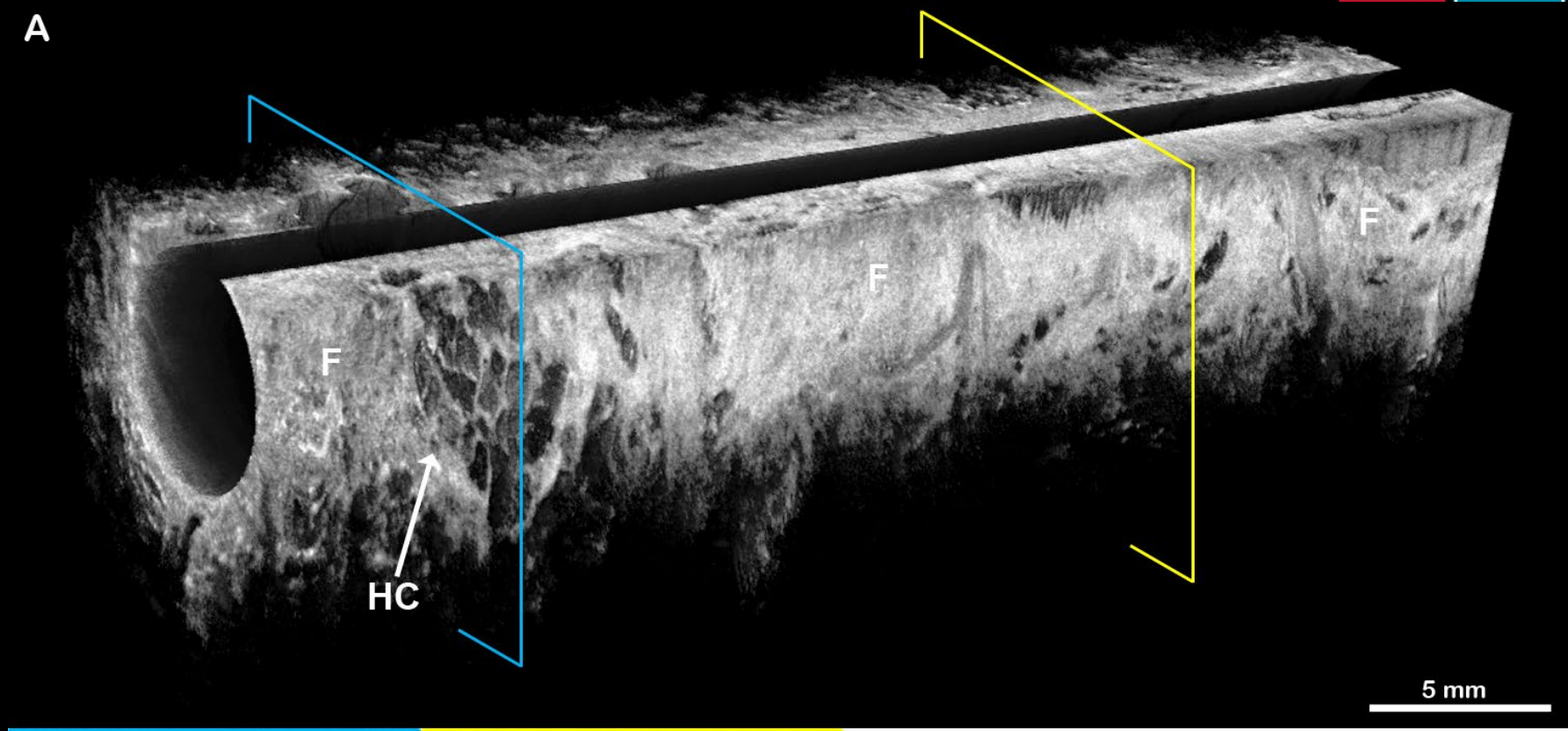


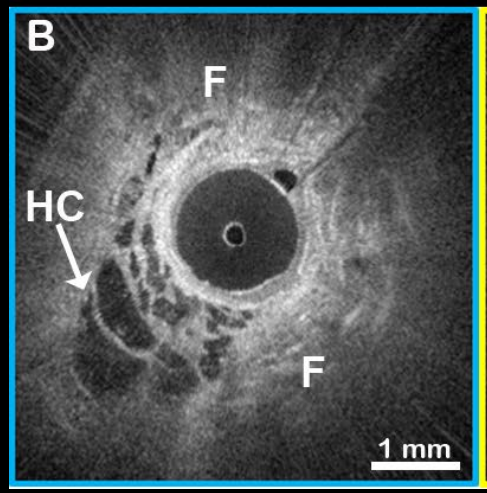
EB-OCT of UIP / IPF

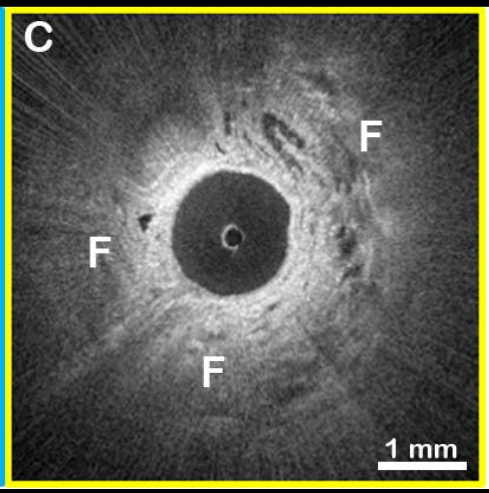


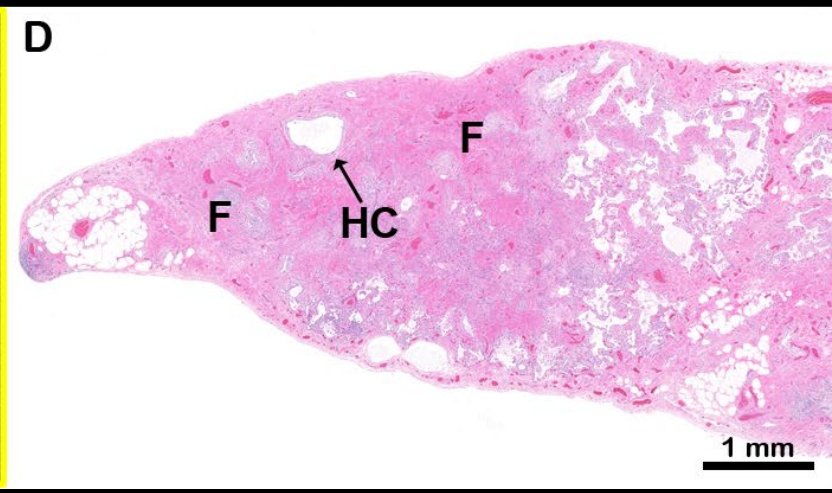
Red Arrow: Honeycomb change in fibrosis, not connected to airway

Blue Arrow: Traction bronchiectasis mimicking honeycomb, but connected to airway



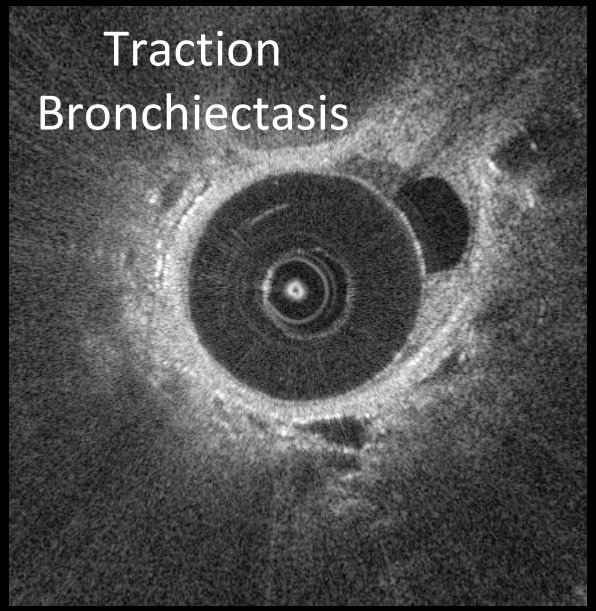


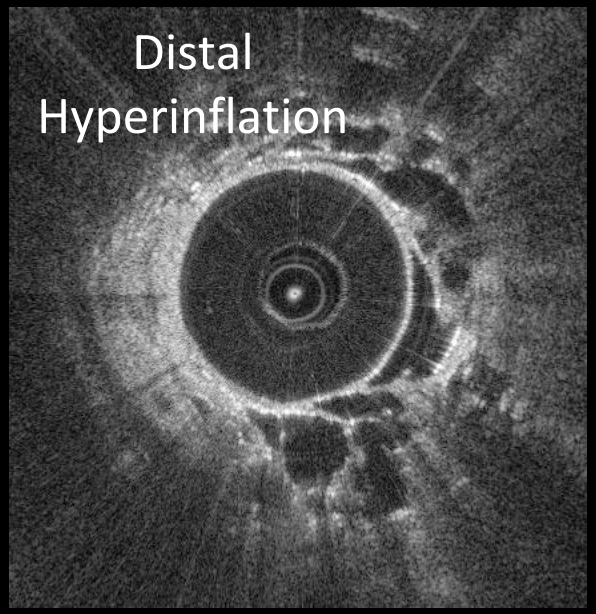


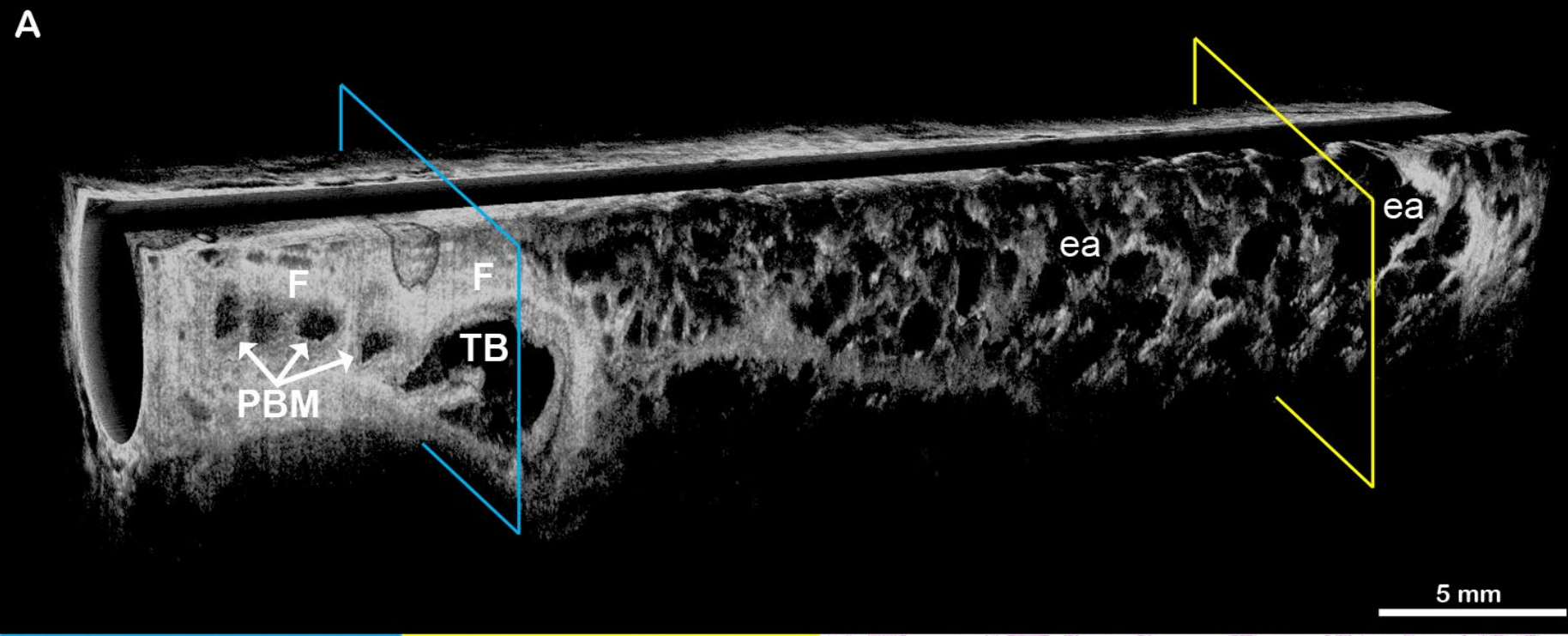


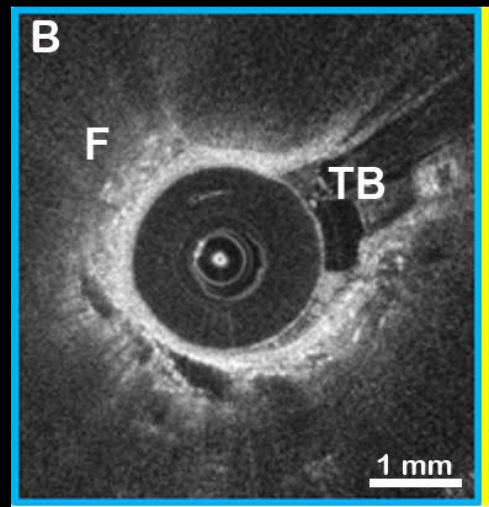
Nandy S*, Raphaely R*…. Hariri LP. AJRCCM. In Press. 2021.

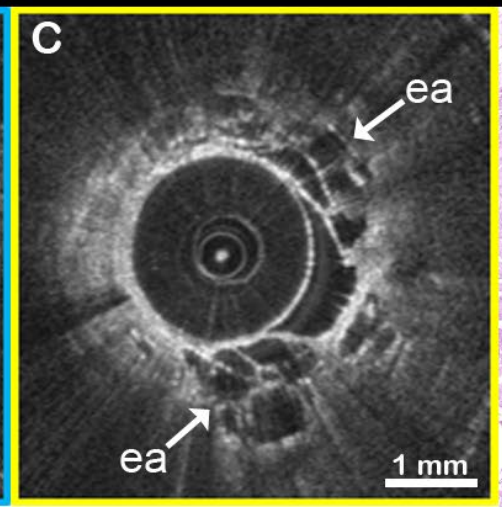
EB-OCT of Airway Centered Fibrosis

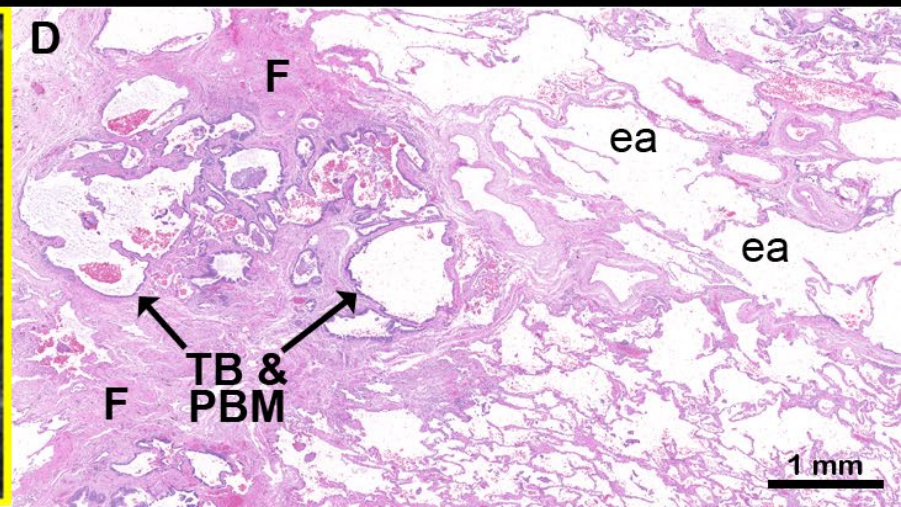




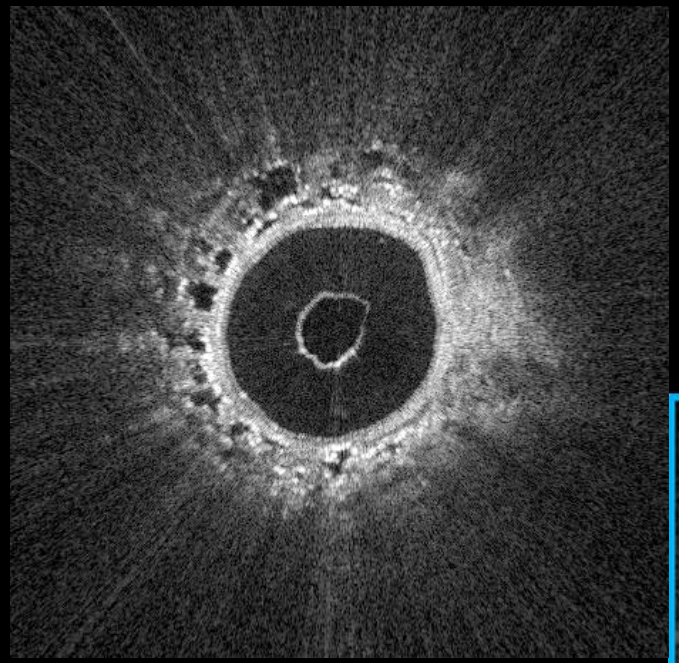


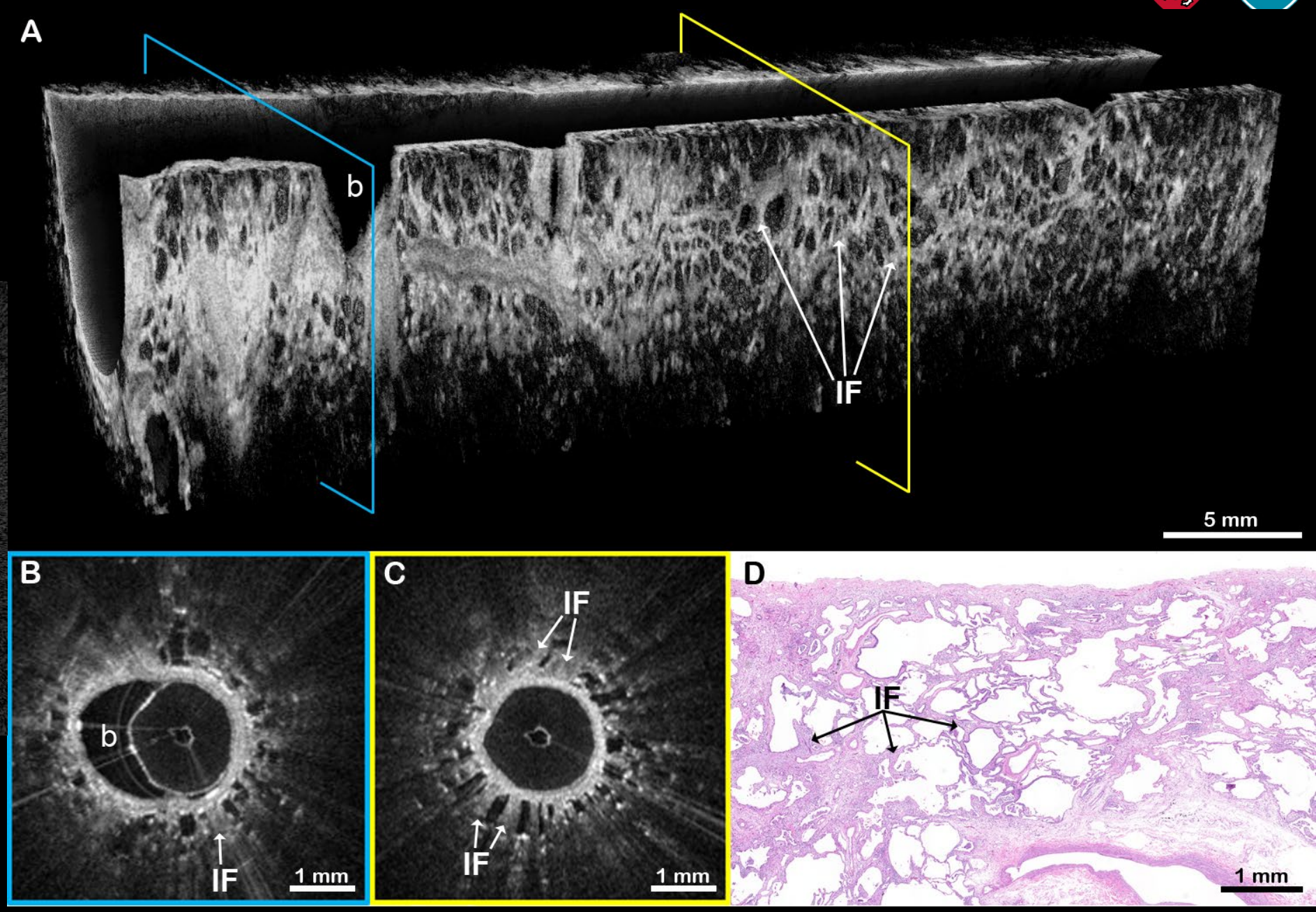






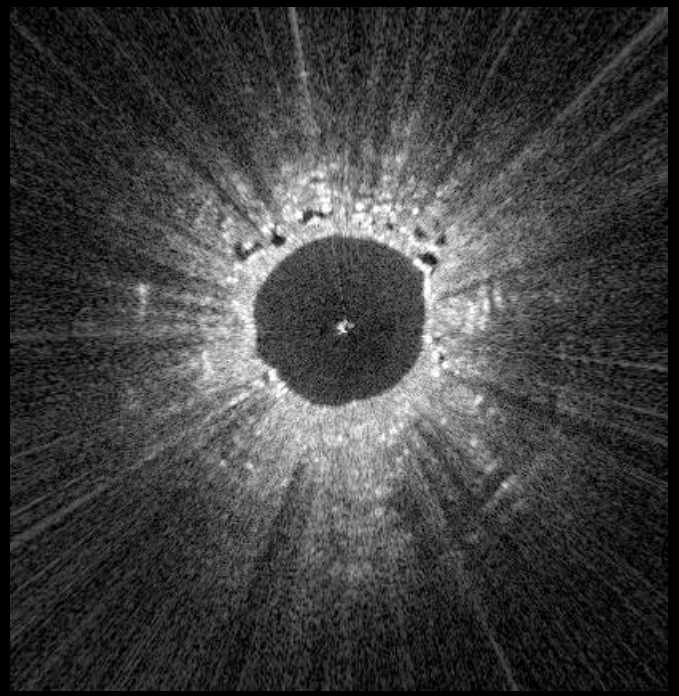
EB-OCT of NSIP

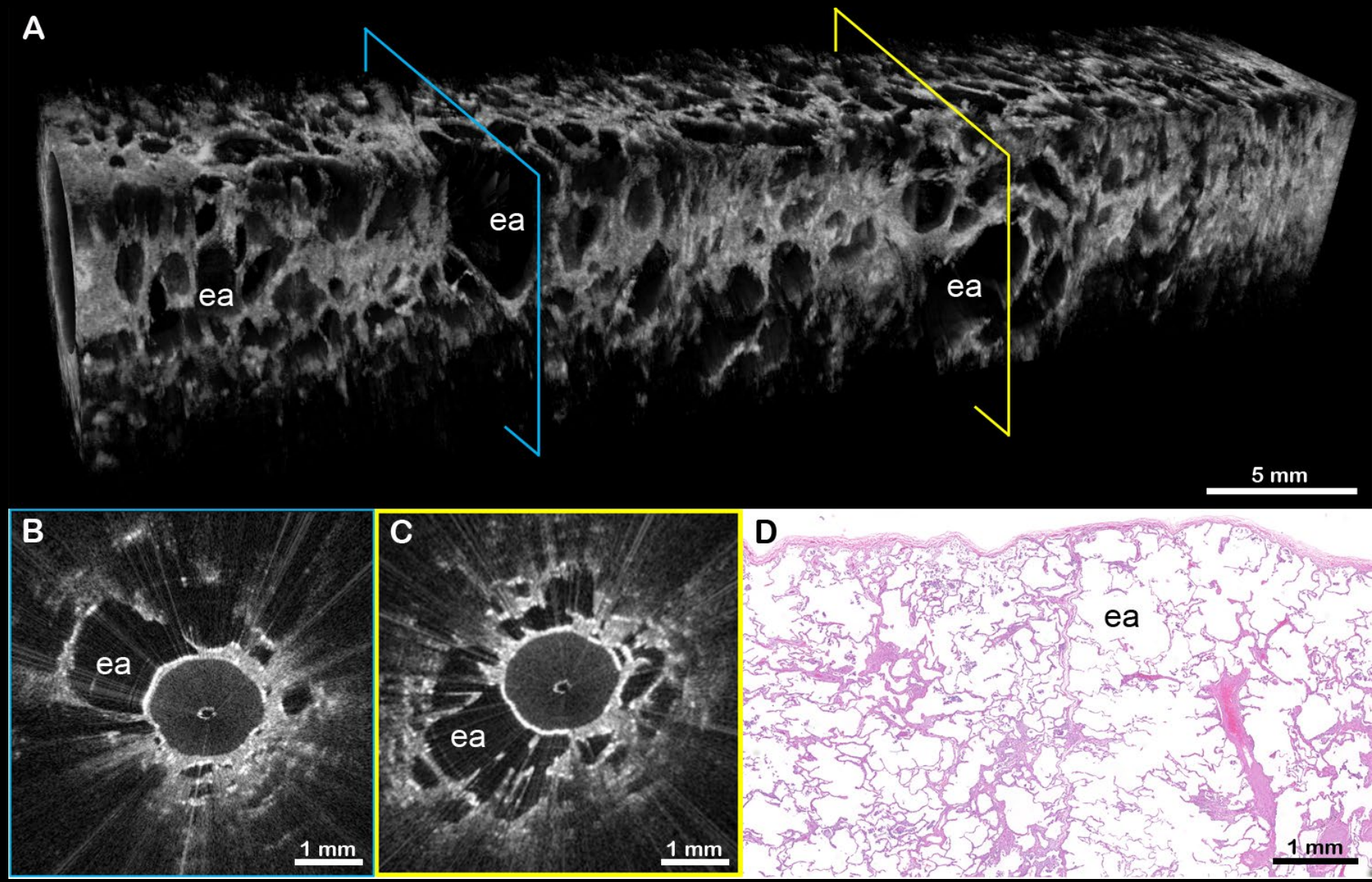




Nandy S*, Raphaely R*…. Hariri LP. AJRCCM. In Press. 2021.

EB-OCT of Emphysema in CPFE





Nandy S*, Raphaely R*…. Hariri LP. AJRCCM. In Press. 2021

Diagnostic accuracy of EB-OCT in ILD

EB-OCT diagnosis of UIP against SLB

Sensitivity and specificity for UIP on histologic on SLB were 100%

(95% CIs: 75.8% to 100% and 79.6% to 100%, respectively)

EB-OCT diagnosis of UIP against clinical follow-up diagnosis

Sensitivity and specificity for IPF on clinical follow-up diagnosis were 100%

(95% CIs: 75.8% to 100% and 79.6% to 100%, respectively)

EB-OCT diagnosis of ILD fibrosis pattern (UIP, NSIP, ACF, or mixed)

High agreement with histologic ILD fibrosis pattern, weighted kappa: 0.87

(95% CI: 0.72 to 1)

Nandy S*, Raphaely R*…. Hariri LP. AJRCCM. In Press 2021.

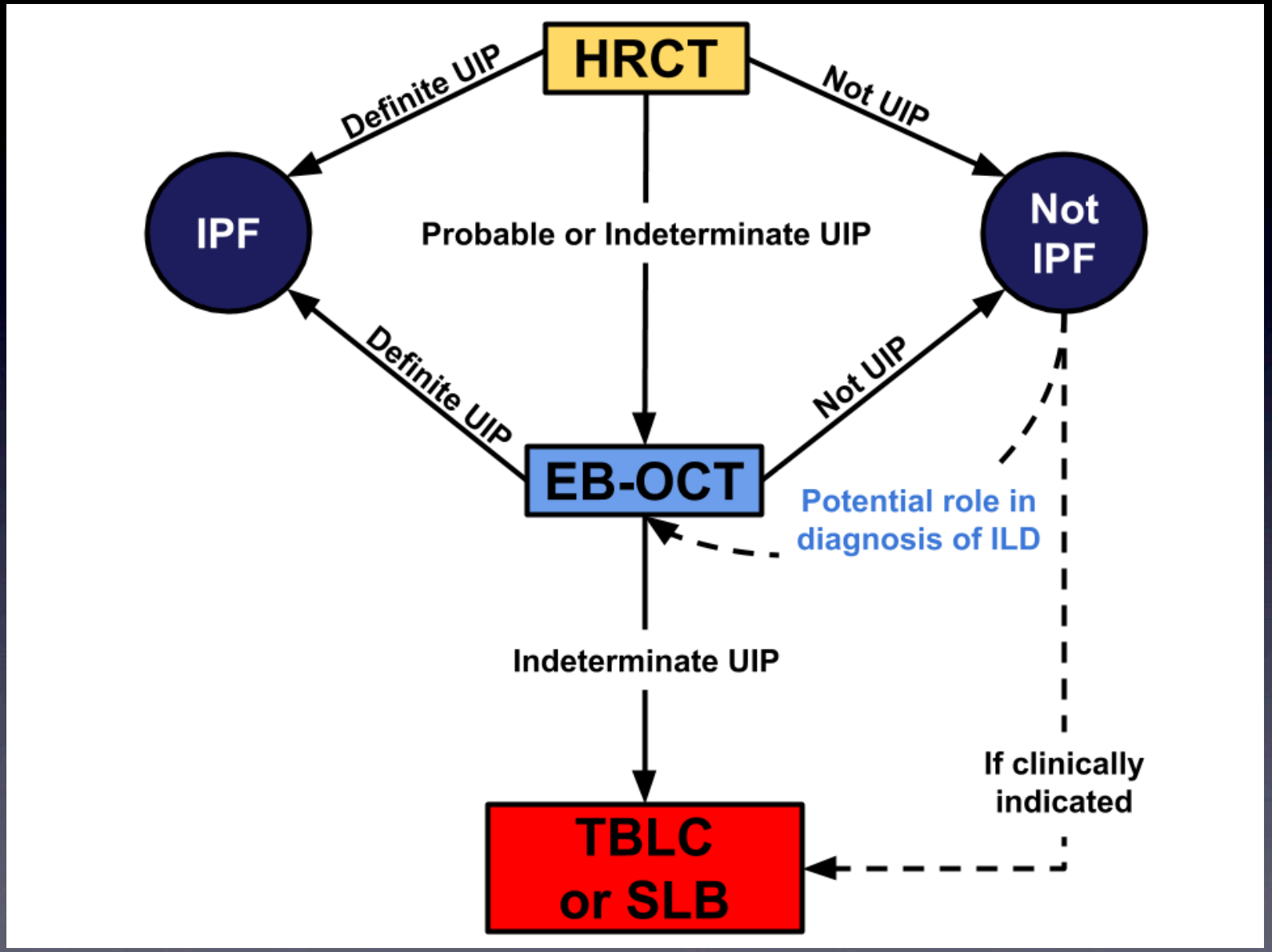
Results

Validation testing with novice, external EB-OCT readers

- 3 ILD pathologists underwent a 3-hour training session with expert OCT reader
- 50% data for training and 50% for testing (equal proportion of each ILD diagnosis)
- Following the training session, the novice pathologist readers were asked to independently evaluate the test dataset
- Provide a single diagnosis of UIP or non-UIP ILD pattern for each subject

EB-OCT Reader	No. of Cases	Sensitivity (95% CI) (%)	Specificity (95% CI) (%)	PPV (95% CI) (%)	NPV (95% CI) (%)
Novice EB-OCT reader 1	13 (6 UIP/7 non-UIP ILD)	100 (54.1–100)	100 (59.0–100)	100 (54.1–100)	100 (59.0–100)
Novice EB-OCT reader 2	13 (6 UIP/7 non-UIP ILD)	100 (54.1–100)	100 (59.0–100)	100 (54.1–100)	100 (59.0–100)
Novice EB-OCT reader 3	13 (6 UIP/7 non-UIP ILD)	66.7 (22.3–95.7)	100 (59.0–100)	100 (39.8–100)	77.8 (40.0–97.2)

Potential ILD diagnostic workflow incorporating EB-OCT



Next steps: Diagnostic study

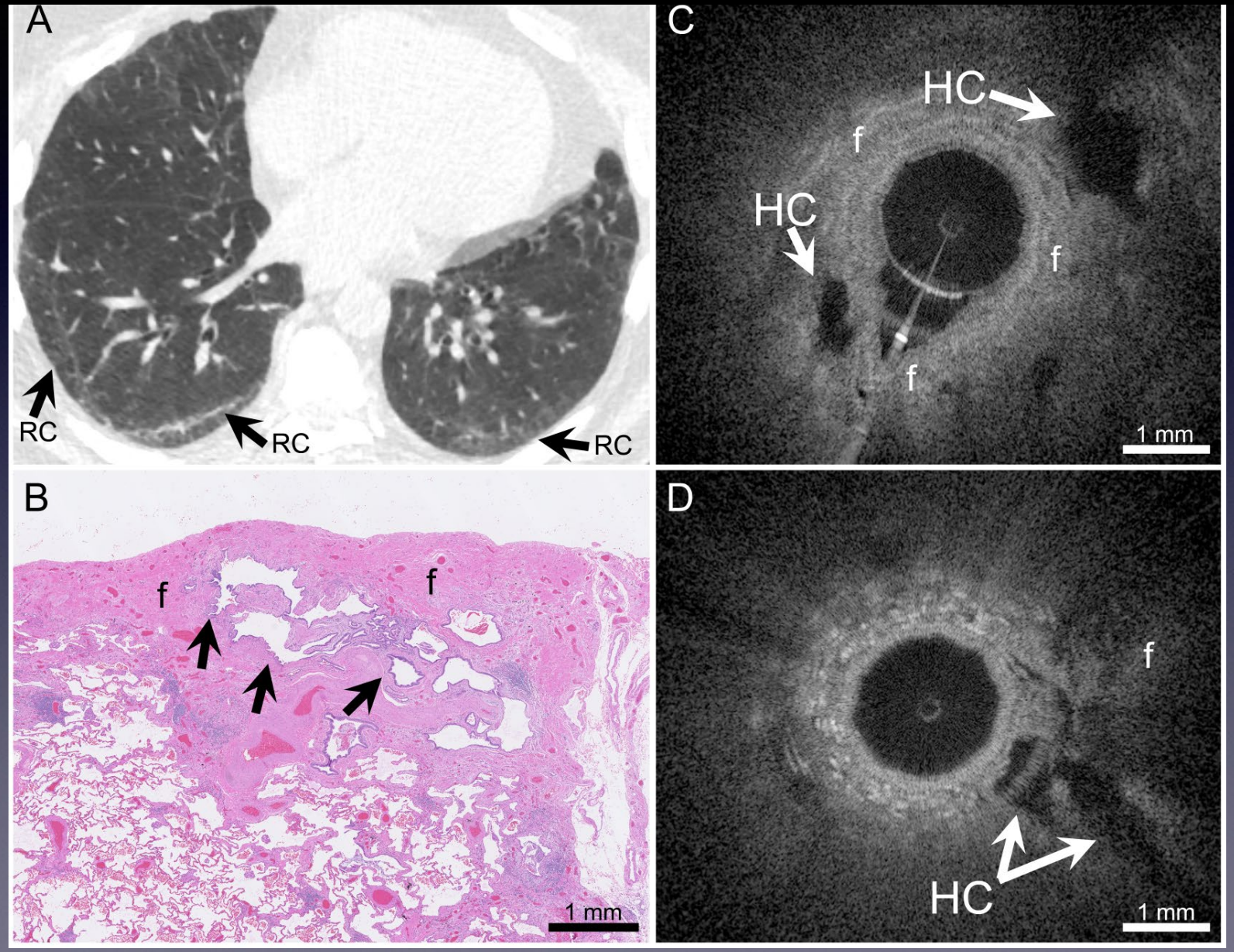
- Need to conduct a larger-scale, multicenter study to further validate our findings
- Continue studies at MGH with Thoracic Surgery and Interventional Pulmonary
- Will be starting a 2nd site at Beth Israel Deaconess Medical Center very soon
- Planning to expand to additional sites within the next 1-2 years

Ashok Muniappan, MD
MGH Thoracic Surgery

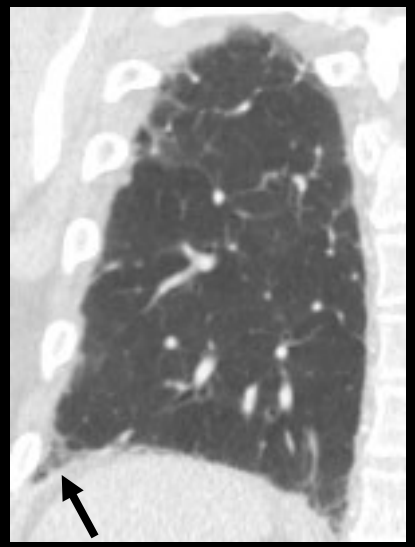
Colleen Keyes, MD
MGH Interventional Pulmonary

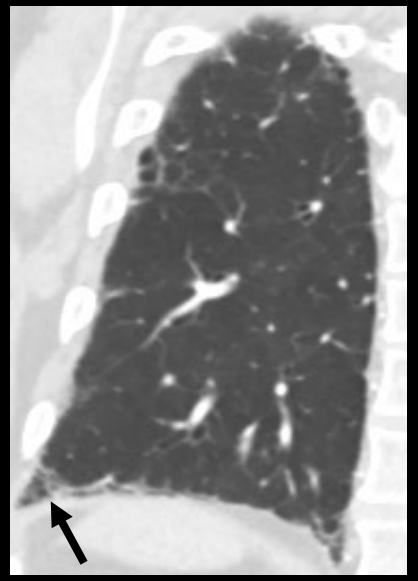
Adnan Majid, MD
BIDMC Interventional Pulmonary

EB-OCT in asymptomatic, incidental interstitial lung abnormalities (ILA) for early detection of microscopic progressive fibrosis

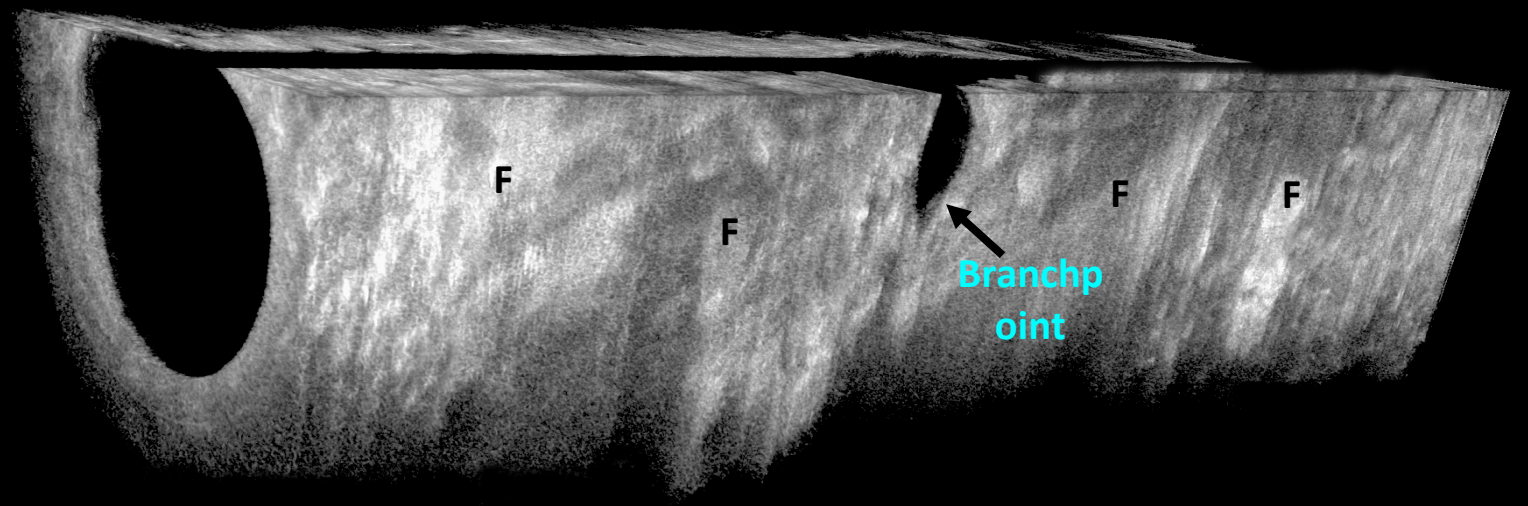


EB-OCT to detect microscopic disease progression in IPF over time outside HRCT and PFT capabilities

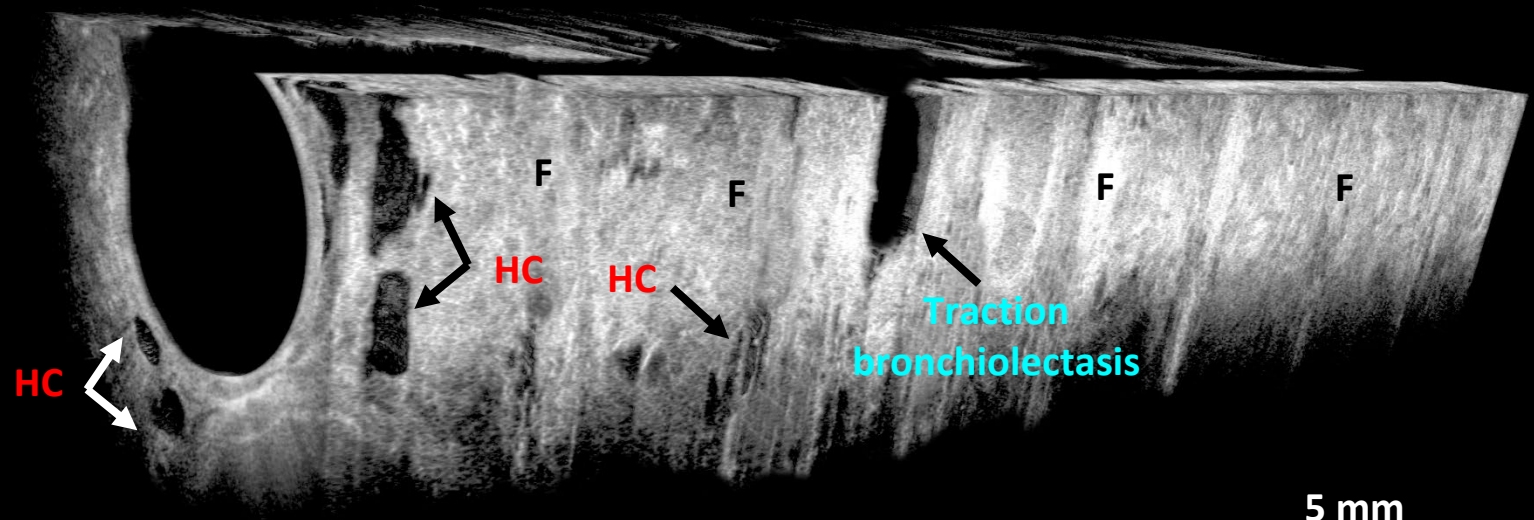




Baseline EB-OCT at diagnosis



EB-OCT at 2 year follow-up: 5% FVC decline



Sarita Berigei, BS
Research Technician

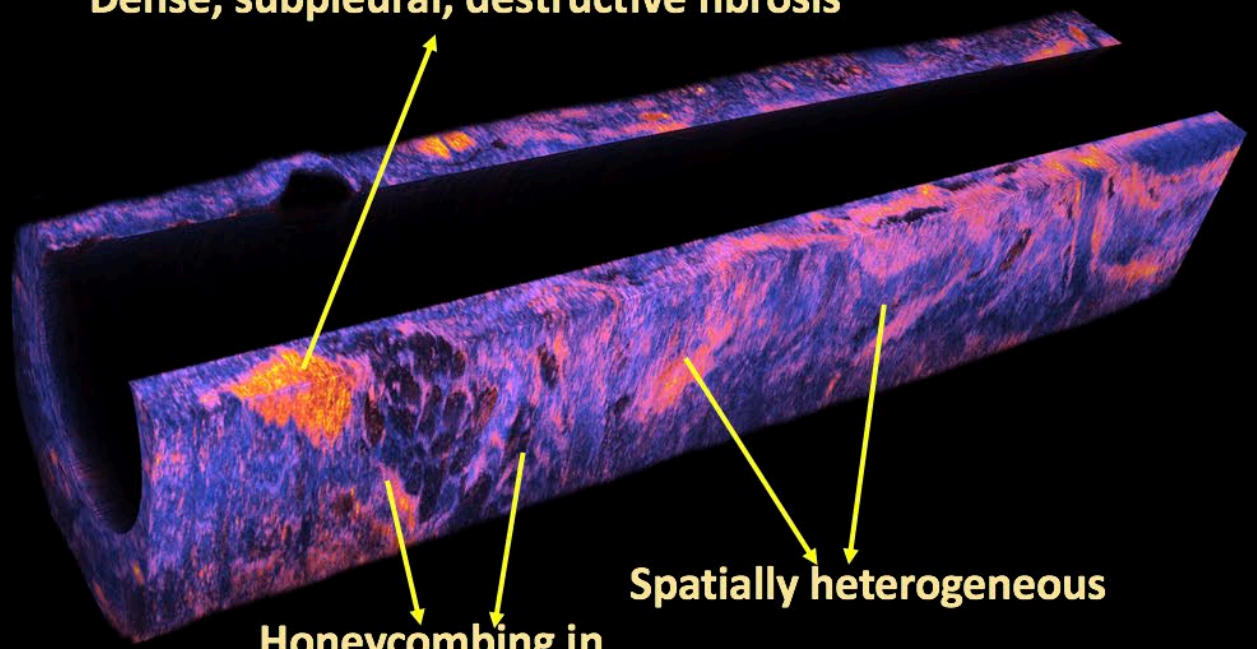
Bess Flashner, MD
BIDMC/MGH DPCCM

Colleen Keyes, MD
MGH DPCCM IP

Polarization-sensitive OCT to detect birefringence from fibrosis

UIP/IPF

Dense, subpleural, destructive fibrosis

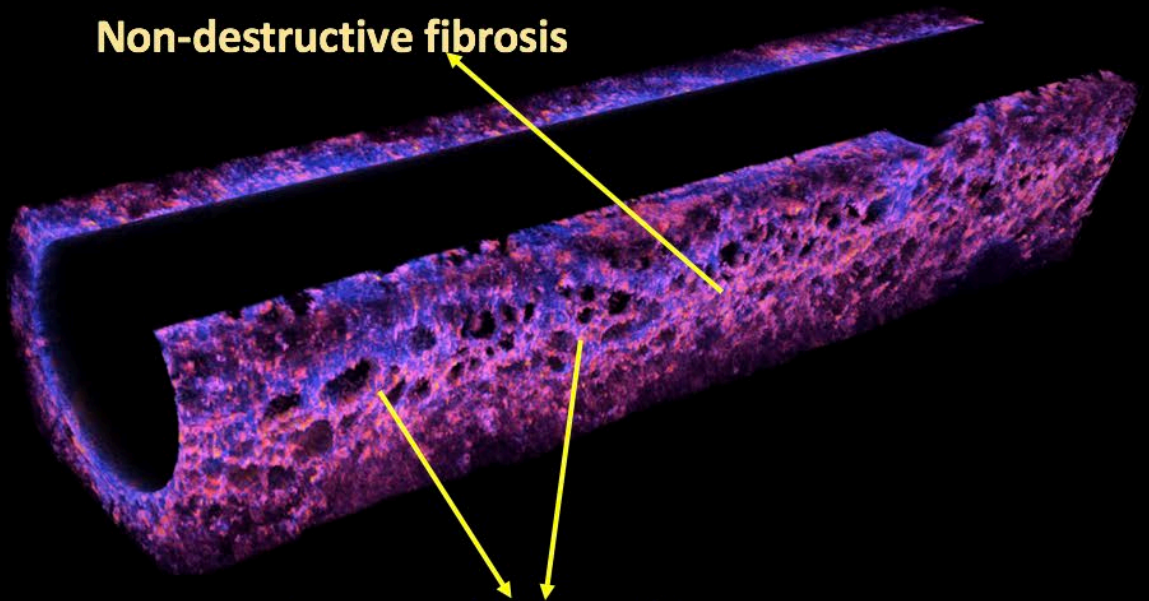


Honeycombing in dense fibrosis

Spatially heterogeneous

Non IPF ILD

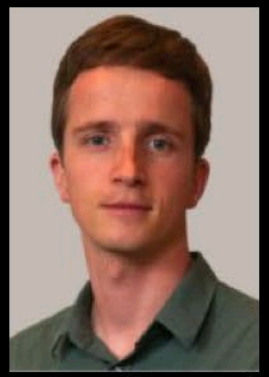
Non-destructive fibrosis



Preserved lung architecture

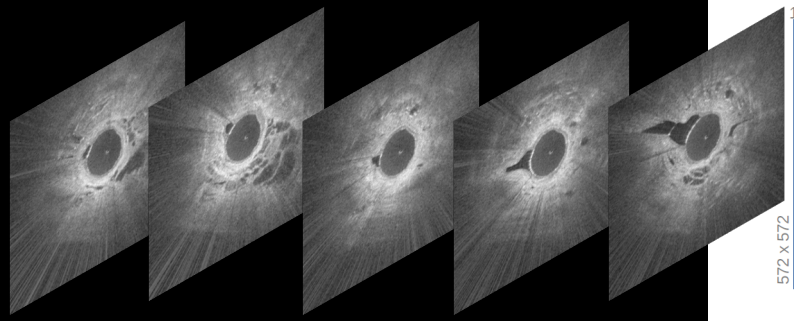
0.5 cm

0 90

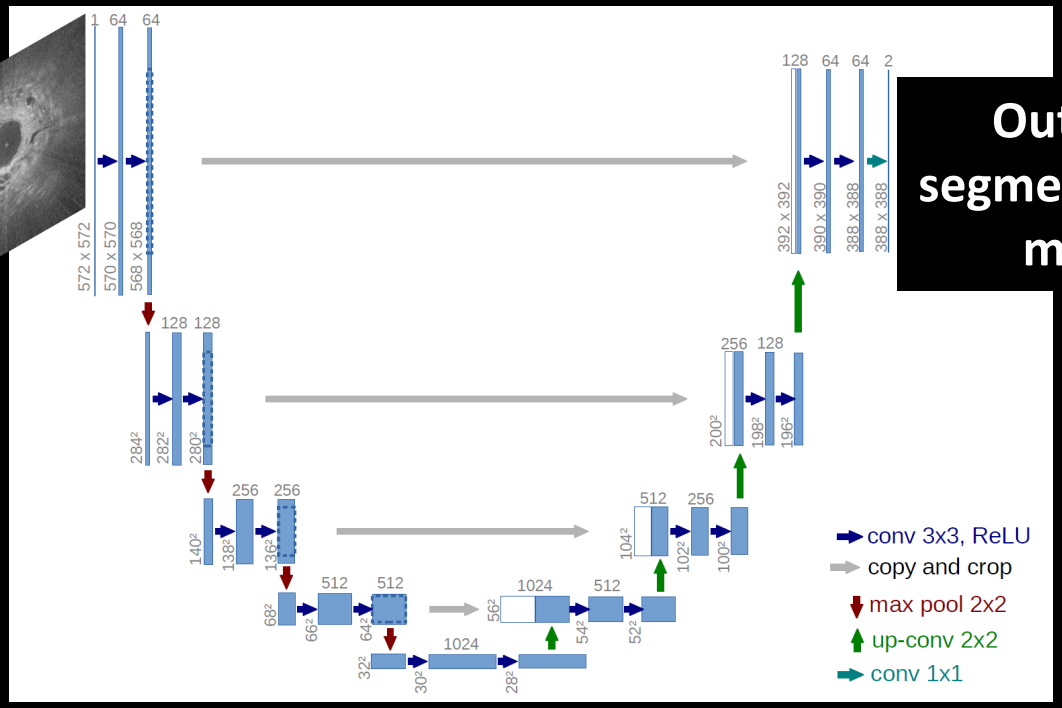


Brett Bouma PhD Martin Villiger, PhD
MGH Wellman Center for Photomedicine

Deep Learning Convolutional Neural Networks: Computer-aided diagnosis and feature quantification as disease biomarker

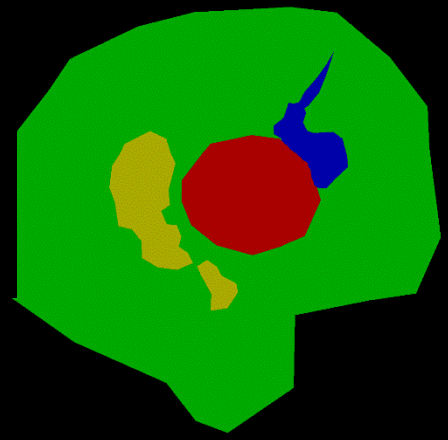


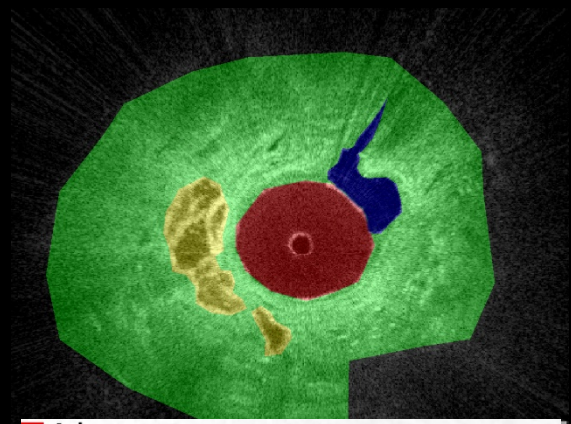
Input image tile



Convolutional Neural Network

Output segmentation map





Sreyankar Nandy, PhD
Post-doctoral fellow

Markus Herrmann, MD, PhD
MGH Computational Pathology

IVM needs a defined expert: Pathologists

- In radiology, many clinicians can assess CT scans but that does not make them radiologists
- Similarly, many clinicians may use and interpret IVM
- IVM is in essence a form of microscopy, and as such pathologists are the obvious choice as IVM experts

Thanks!

Hariri Lab
Sreyankar Nandy, PhD
Rebecca Raphaely, MD
Arash Dadkhah, PhD
Bess Flashner, MD
Benjamin Roop, BS
Sarita Berigei, BS

Suter Lab
Melissa Suter, PhD
David Adams, PhD
Margit Szabari, MD, PhD

MGH Pathology
Markus Herrmann, MD, PhD
Mari Mino-Kenudson, MD
Angela Shih, MD

Wellman Center
Brett Bouma, PhD
Martin Villiger, PhD
Photopathology

MGH Pulmonary and Critical Care
Benjamin Medoff, MD
Andy Tager, MD
Colleen Keyes, MD
Corey Hardin, MD, PhD
Barry Shea, MD
Mamary Kone, MS
Lloyd Liang, BS
Caroline Fromson, MS

MGH Institute for Innovation in Imaging (i^3)
Peter Caravan, PhD

Brown/Rhode Island Hospital
Maria Garcia-Moliner, MD

Beth Israel Deaconess Medical Center
Adnan Majid, MD
Sidhu Gangadharan, MD
Mike Kent, MD
Paul VanderLaan, MD

Funding
NIH K23HL132120; R01HL152075;
NIH P41EB015903; Boehringer Ingelheim

MGH Thoracic Surgery
Yolanda Colson, MD
Ashok Muniappan, MD
Harald Ott, MD
Hugh Auchincloss, MD
Michael Lanuti, MD
Chris Morse, MD
Henning Gaissert, MD
Cameron Wright, MD
Lana Beal-Schumacher, MD
John Wain, MD

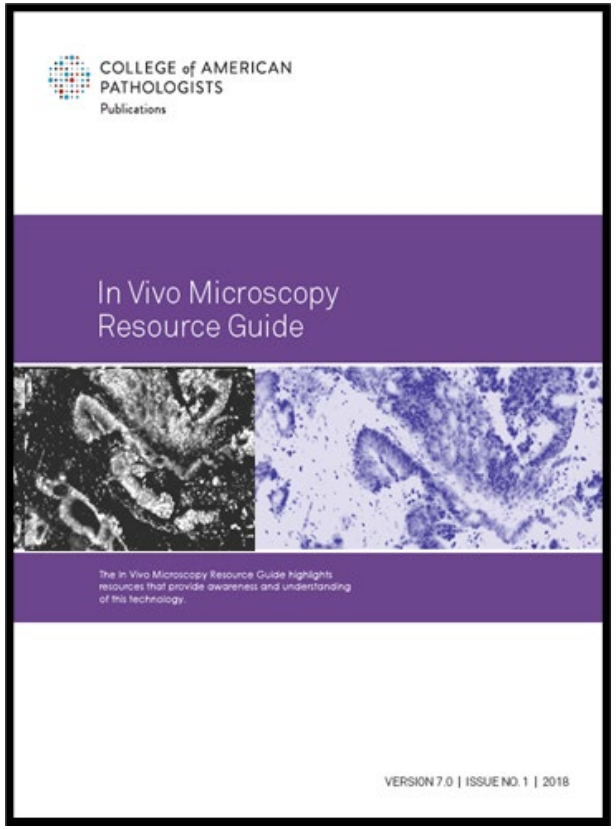
MGH Radiology
Amita Sharma, MD

MGH Biostatistics Center
Nora Horick, MS

Mayo Clinic, Scottsdale
Tom Colby, MD
Max Smith, MD

Resources of Digital and Computational Pathology Committee

- **List resources**
  - **SPECs**
  - **Resources Guides**
  - **Topic Center Pages & AI pages**
  - **AI@CAP.ORG email address**



THANK YOU!

Thank you for attending our webinar "In Vivo Microscopy as an Adjunct to Traditional Histopathology: Expanding our View" by Lida Hariri, MD, PhD, FCAP. For comments about this webinar or suggestions for upcoming webinars, contact AI@cap.org

NOTE: There is no CME/CE credit available for today's complimentary webinar. The recording of the presentation will be sent out in about 1 week.

COLLEGE of AMERICAN PATHOLOGISTS