In Vivo Microscopy as an Adjunct to Traditional Histopathology

Expanding our View

Lida Hariri, MD, PhD, FCAP
DCPC Webinar Series

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- 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.
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Disclosures

I have the following relevant financial relationships to disclose:

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

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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)

Analogous 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

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. 144(4), 2013
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)

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

OCT: Tumor Versus Non-diagnostic Contaminants

Structural OCT can differentiate tumor from:
- Airway
- Parenchyma
- Necrosis

Cannot differentiate solid tumor from fibrosis
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

<table>
<thead>
<tr>
<th>PSR % Fibrosis</th>
<th>PS-OCT % Fibrosis</th>
<th>≤ 20%</th>
<th>&gt; 20%</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>≤ 20%</td>
<td></td>
<td>40</td>
<td>2</td>
<td>42</td>
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<tr>
<td>&gt; 20%</td>
<td></td>
<td>3</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>43</td>
<td>14</td>
<td>57</td>
</tr>
</tbody>
</table>

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
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

Excluded (n=3)

PS-OCT quantification of tumor, fibrosis and normal lung

Histopathology: H&E and trichrome staining

Excluded (n=2)

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

Tumor
Sensitivity: 94.4%
Specificity: 83.3%

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

<table>
<thead>
<tr>
<th>Histology</th>
<th>PS-OCT Tumor Quantification</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 25%</td>
<td>&gt; 25%</td>
</tr>
<tr>
<td>Tumor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 25%</td>
<td>34</td>
<td>2</td>
</tr>
<tr>
<td>&gt; 25%</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Quantification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>35</td>
<td>7</td>
</tr>
</tbody>
</table>

Tumor
Sensitivity: 94.4%
Specificity: 83.3%
OCT Guided Biopsy of Lung Nodules

Target Lung Nodule

- Miss Nodule
  - Normal Airway
  - Normal Parenchyma

- Hit Nodule
  - Necrosis: Not Diagnostic
  - Fibrosis: Not Diagnostic

Tumor: Diagnostic!

Structural OCT
- Differentiate normal elements from tumor

PS-OCT:
- Differentiate fibrosis from tumor
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


Harini LP et al. AJRCCM 2013.
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
Idiopathic pulmonary fibrosis (IPF)

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

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

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

Ref: Jaklitsch et al. Lancet Oncol. 2003

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

- Spatial Heterogeneity
- Honeycomb Change
- Subpleural Fibrosis with Architectural Distortion
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

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

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.
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

Preserved peripheral lung parenchyma

Lattice like regularly spaced alveoli in thin normal interstitial tissue
**Red Arrow:** Honeycomb change in fibrosis, not connected to airway

**Blue Arrow:** Traction bronchiectasis mimicking honeycomb, but connected to airway
EB-OCT of Airway Centered Fibrosis

Traction Bronchiectasis

Distal Hyperinflation

A

B

C

D

F

TB

PBM

ea

5 mm

1 mm

1 mm

1 mm

F

TB & PBM

ea

ea

ea

1 mm
EB-OCT of NSIP

Nandy S*, Raphaely R*..., Hariri LP.
EB-OCT of Emphysema in CPFE

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

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

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

47
Potential ILD diagnostic workflow incorporating EB-OCT

- **HRCT**
  - Definite UIP
  - Not UIP

- **IPF**
  - Probable or Indeterminate UIP
  - Definite UIP

- **EB-OCT**
  - Indeterminate UIP
  - Potential role in diagnosis of ILD
  - If clinically indicated

- **Not IPF**

- **TBLC or SLB**
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
- Spatially heterogeneous
- Honeycombing in dense fibrosis

**Non IPF ILD**
- Non-destructive fibrosis
- Preserved lung architecture

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.

Convolutional Neural Network

Input image tile

Output segmentation map

Markus Herrmann, MD, PhD
MHG Computational Pathology

Sreyankar Nandy, PhD
Post-doctoral fellow
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!

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Resources of Digital and Computational Pathology Committee

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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

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