Optical coherence elastography for volumetric mechanical microscopy in engineered cell cultures and biological tissues

Nichaluk Leartprapun[a], Yuechuan Lin[a], Colin D. Mackey[a], Audrey Guo[a] and Steven G. Adie[a,b]

CornellEngineering
Nancy E. and Peter C. Meinig
School of Biomedical Engineering

[a]Nancy E. and Peter C. Meinig School of Biomedical Engineering, Cornell University, Ithaca, NY 14853
[b]School of Electrical and Computer Engineering, Cornell University, Ithaca, NY 14853

Background and motivation

- **Cell-extracellular matrix (ECM) biophysical interactions** play an important role in biological processes, including initiation and progression of cancer, stem cell differentiation, morphogenesis, and wound healing.
- Attention has been given to the study of cell-ECM biophysical interactions at the **micro- to mesoscale**, bridging the gap between single molecule biophysics and macroscopic biomechanics.
- Importantly, cell-ECM interactions are **bi-directional** and has been shown to differ in 2D versus 3D, driving the pursuit of studies in the more physiologically relevant **3D engineered cellular systems** and **biological tissues**.
- Although a suit of techniques are available for investigating cellular behavior, the characterization of ECM functional properties is typically limited to **bulk mechanical testing** or, on the other extreme, **atomic force microscopy (AFM)**, which can only probe the 2D surface of the sample.
- **This motivates the development of new imaging tools to enable volumetric characterization of spatially varying mechanical properties at the micro- to mesoscale in both 3D engineered ECM and biological tissues.**
- We develop optical elastography techniques based on mechanical excitation provided by photonic and acoustic forces and detection by **optical coherence tomography (OCT)**.

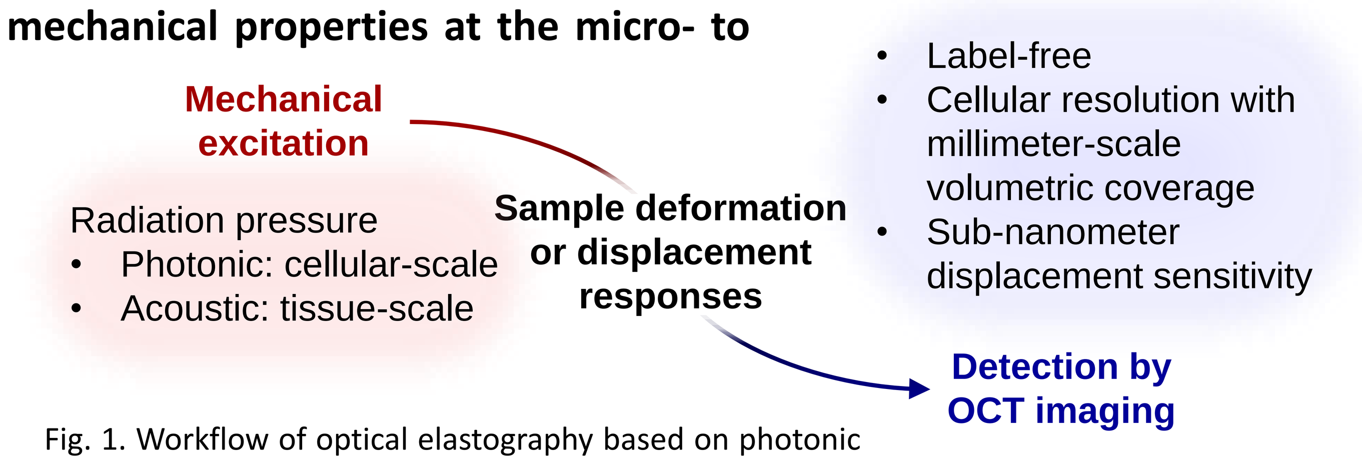


Fig. 1. Workflow of optical elastography based on photonic or acoustic radiation pressure and OCT detection.

Cellular-scale mechanical microscopy of 3D engineered ECM

- An **AFM-like 'poking' in 3D** can be achieved with radiation pressure (force) from a weakly-focused laser beam.
- Acceleration of neutral particles by **photonic radiation pressure** was first demonstrated in 1970 by Arthur Ashkin[1], who was recently awarded the 2018 Nobel Prize in Physics for his pioneering work in optical tweezers.
- **We revisited Ashkin's original work**[2] **and investigated photonic radiation pressure as a mechanism to provide localized mechanical excitation over an extended depth range for 3D mechanical microscopy**[3]**.**

An OCT version of Ashkin's landmark experiment[4]

- Trajectories of polystyrene micro-beads in viscous fluid accelerated by photonic radiation pressure were captured in real-time by OCT imaging.
- Analysis of bead dynamics enabled depth-resolved measurement of photonic radiation-pressure force.
- **Peak force on the order of 0.15 pN/mW can be achieved on polystyrene micro-beads (1.7-μm diameter) in aqueous media.**

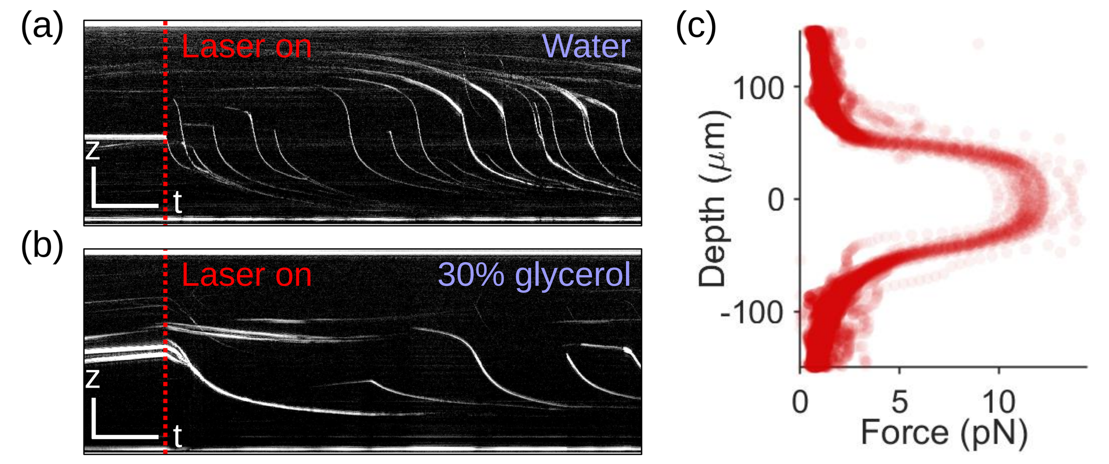


Fig. 2. (a, b) M-mode OCT images of beads accelerated by a laser beam; slower motion is observed in 30% glycerol (higher viscosity). Scale bar: 200 μm, 3 s.[4] (c) Radiation-pressure force on 1.7-μm polystyrene beads as a function of depth w.r.t. the forcing beam focal plane at beam power of 78 mW.

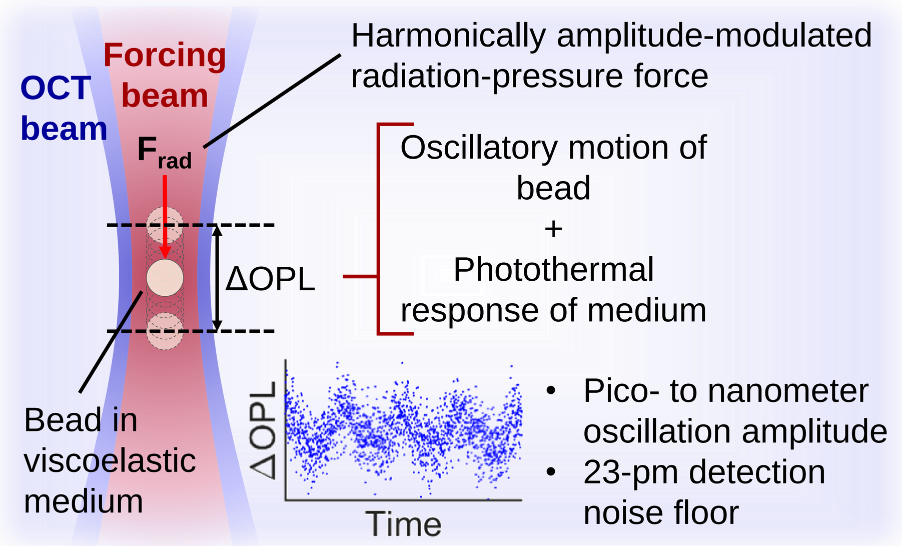


Fig. 3. Principle of PF-OCE

Photonic force optical coherence elastography (PF-OCE)[3]

- Individual micro-beads embedded in viscoelastic medium is excited by modulated photonic radiation pressure.
- Resulting bead oscillatory response is detected by OCT.
- **Local mechanical properties of the sample at each bead is reconstructed from its mechanical response.**

Microrheological quantification of viscoelastic properties of polyacrylamide gels[5]

- Shear storage (G') and loss (G") moduli were calculated from bead mechanical response via Generalized Stokes-Einstein Relation[3].
- PF-OCE results were compared with shear rheometry (oscillatory test at 20 Hz).

Spectroscopic PF-OCE[6]

- Reconstructing broadband frequency-dependent microrheological properties
- Implying microscale structure dynamics of semiflexible polymer networks over different time scale

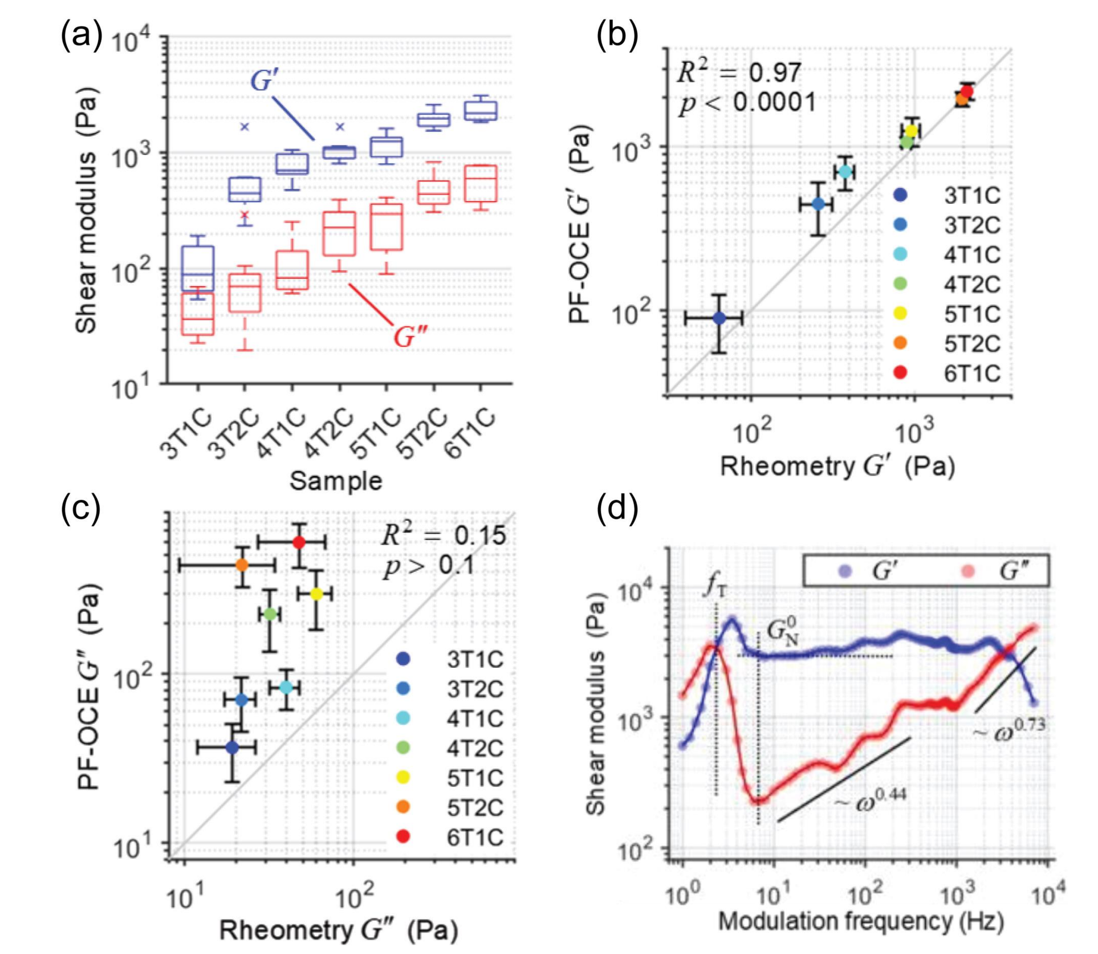


Fig. 4. PF-OCE measurement of polyacrylamide hydrogels. (a) Box plot of shear storage and loss moduli. (b)(c) Comparison against shear rheometry measurements in G' and G"; (d) Spectroscopic PF-OCE

Cellular-scale mechanical microscopy of 3D engineered ECM (cont.)

3D mechanical microscopy of agarose hydrogels

- 3D PF-OCE measurement was performed by raster-scanning co-aligned OCT and PF beams to build a volume.
- The sample contained 0.2% agarose (right-side) next to 1% agarose (left-side), forming a mechanical contrast step.
- **Microscale spatial variation in the relative stiffness of the sample can be observed from bead oscillation amplitudes**; larger oscillation amplitude (red) corresponds to softer surrounding.

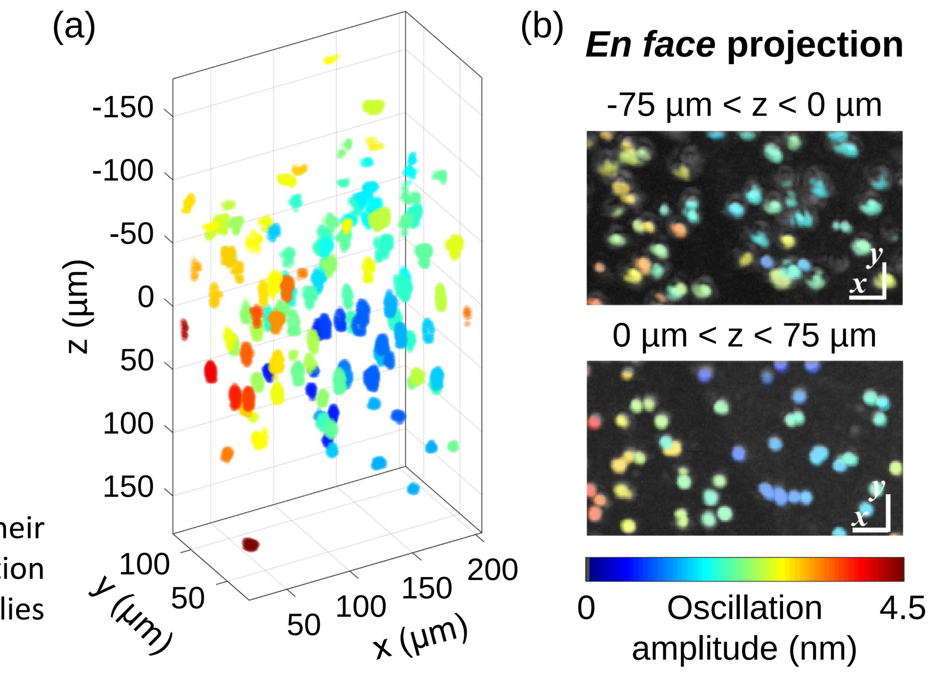


Fig. 5. (a) 3D rendering of beads in agarose hydrogels, color-coded by their oscillation amplitudes (N = 220 beads). (b) *En face* projections of bead oscillation amplitudes overlaid on OCT images. Scale bars: 20 μm. The same color bar applies to both (a) and (b). Depth z = 0 μm corresponds to the focal plane.[3]

Volumetric mechanical characterization of biological tissues

- Non-destructive, non-contact **mechanical 'palpation'** of biological tissues can be achieved with radiation pressure from an ultrasonic beam.
- Focused **acoustic radiation pressure** provides localized mechanical excitation with hundreds of micrometers lateral excitation region and millimeters penetration depth, making it suitable for volumetric measurements at the tissue level.

Acoustic radiation force optical coherence elastography (ARF-OCE)

- Harmonic mechanical excitation is directly applied to tissue sample via modulated acoustic radiation pressure.
- Resulting axial oscillatory displacement of each tissue voxel is detected by OCT.
- Dynamic ARF-OCE offers two types of measurements:
  - **High-resolution mechanical contrast imaging** produces uniaxial strain elastogram with 100-μm lateral mechanical resolution.
  - **Quantitative shear wave imaging** enables model-independent reconstruction of viscoelastic properties with lateral resolution limited by the shear wavelength[7].

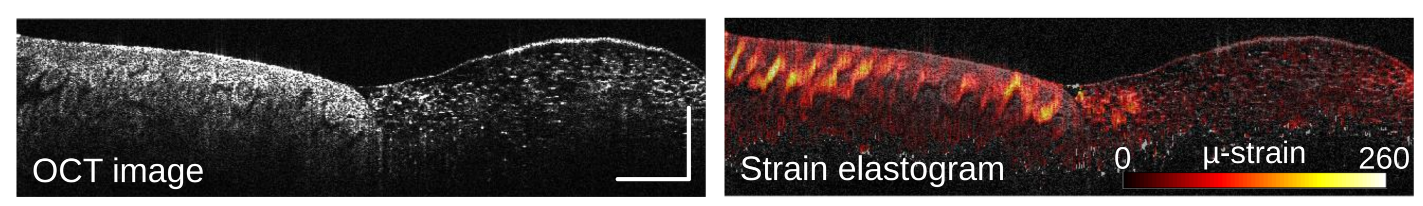


Fig. 6. Structural OCT image and strain elastogram in murine intestinal and connective tissues obtained with ARF-OCE high-resolution mechanical contrast imaging. Scale bar: 500 μm.

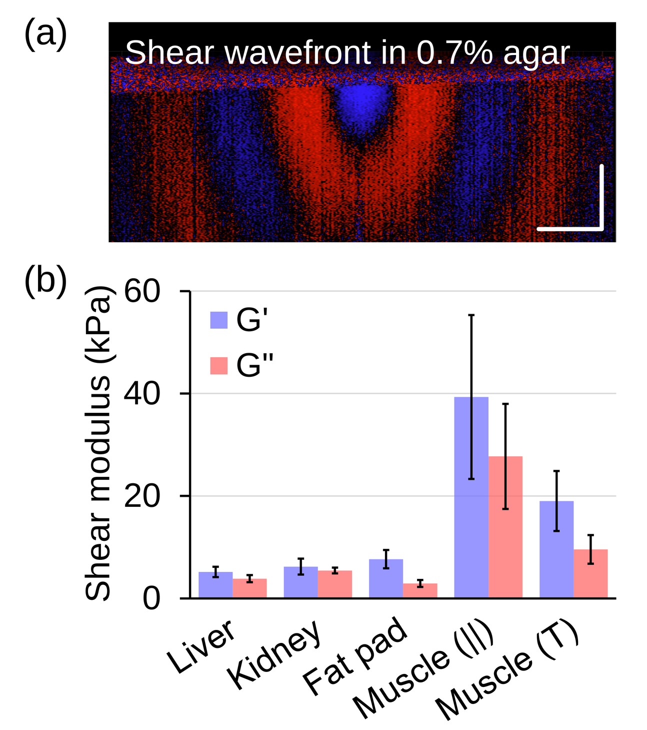
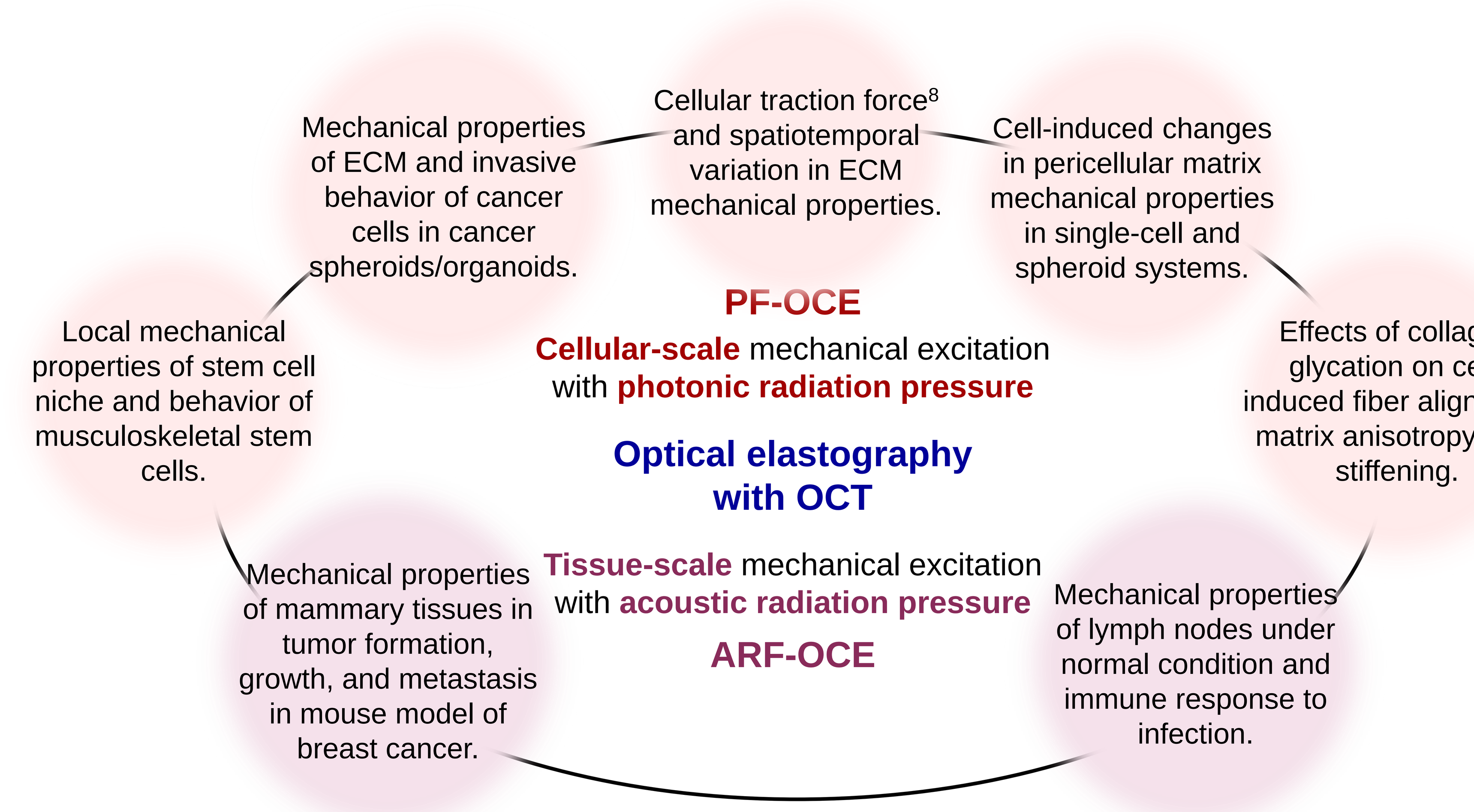


Fig. 7. (a) Snapshot of propagating 1250-Hz shear wavefront measured by ARF-OCE. Scale bar: 500 μm. (b) Shear storage and loss moduli in *ex vivo* murine tissues measured by quantitative shear wave imaging.[7]

Future outlook



1. Ashkin, A. *Phys. Rev. Lett.* **24** (1970); **2.** Ashkin, A. et al. *Opt. Lett.* **11** (1986); **3.** Leartprapun, N. et al. *Nat. Commun.* **9** (2018). **4.** Leartprapun, N. et al. *Opt. Express* **26** (2018). **5**. Leartprapun, N. et al. *Opt. Express* **27** (2019). **6**. Lin, Y. C. et al. *Opt. Lett*. (in press). **7.** Leartprapun, N. et al. *Proc. SPIE 10053*, **10053** (2017). **8.** Mulligan, J. A. et al. *Sci. Rep.* **9** (2019). [Green text indicates the publication in our lab]