Microscopic Biophotonics *in vivo*

Xingde Li, PhD

Biomedical Engineering,
Electrical and Computer Engineering,
and Oncology
Johns Hopkins University

Group Website: *bit.bme.jhu.edu*

Disclosure

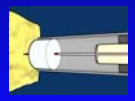
I serve as a paid consultant/scientific advisor for and/or hold equity/shares in **MicroTech LLC, and Insight Photonics Inc**.

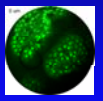
All opinions expressed and implied in the above activities are solely those of Dr. Xingde Li and do not represent or reflect the views of the Johns Hopkins University or the Johns Hopkins Health System.

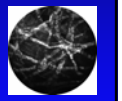
Outline

OCT:
Architecture morphology;

Two-photon:
Subcellular microstructure with biochem / metabolic info



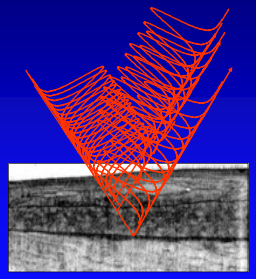




- Basic working principles & some recent developments
- Representative/potential applications

Optical Coherence Tomography (OCT)

(Optical Analog of Ultrasound)

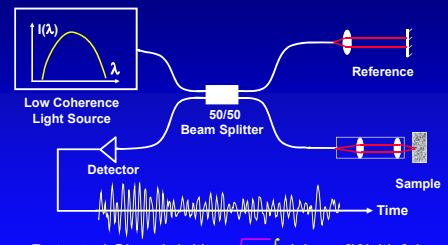


Catching Optical Echoes

- High resolution (1-10 μm)
- High speed (1,000 fps)
- Noninvasive
- Portable and compact
- Contrast: mismatch in refractive index
- Depth: 0.5 – 2.5 mm

→ Optical Histology

Schematic of an OCT System



Detected Signal: $I_k(t) \sim \sqrt{I_{Ref}} \int r(z) \exp[i2k(t)z]dz$

OCT Resolution

- Transverse:

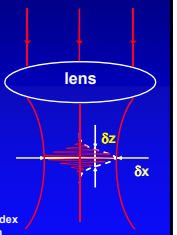
$$\delta x = \frac{2\lambda}{\pi}\frac{1}{NA} = 1\text{-}20\ \mu m$$

NA: numerical aperture

- Axial:

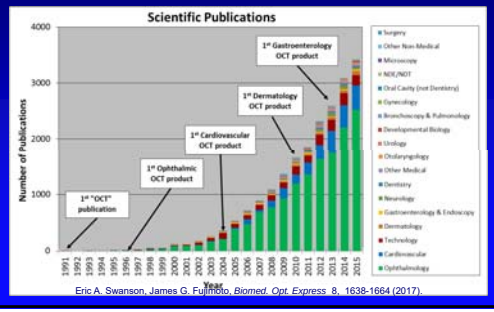
$$\delta z = \frac{c * \tau}{2n} = \frac{2\ln(2)}{n\pi}\frac{\lambda_0^2}{\Delta\lambda} = 1\text{-}10\ \mu m$$

τ: pulse width; n: tissue refractive index
λ_0: center wavelength; $\Delta\lambda$: spectral bandwidth



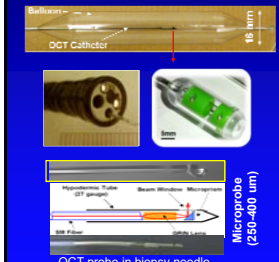
Axial and transverse resolutions are independent

OCT Applications

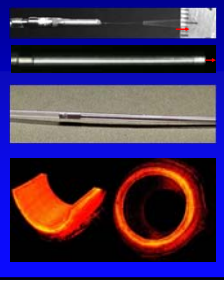


Eric A. Swanson, James G. Fujimoto, *Biomed. Opt. Express* 8, 1638-1664 (2017).

Catheter/Endoscope/Capsule for Imaging Internal Organs



OCT probe in biopsy needle



2G Endoscopic OCT:

Ultrahigh Resolution, Better Contrast

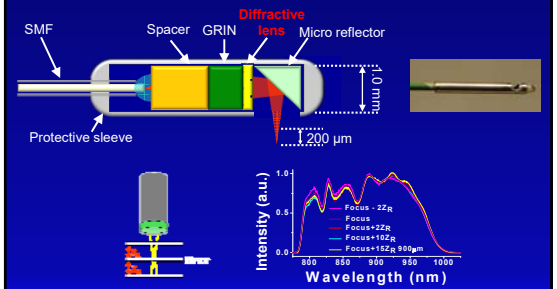
- For the past, endoscopic OCT imaging has been performed with a **1,300 nm** light sources.

  with an axial resolution ~ 8 – 20 um

  Axial Resolution: $\delta z \sim \frac{\lambda_c^2}{\Delta\lambda}$

- **Moving from 1300 nm → 800 nm**
  To improve resolution 3X and **imaging contrast**

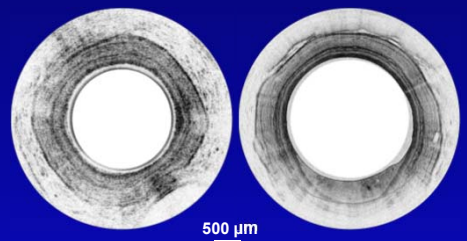
Diffractive OCT Endoscopy – Ultrahigh Res



Xi, *et al.*, ***Optics Letters*** 39(7), 2014; ***Photonics Spectra***, June, 2014; Yuan, *et al.*, ***Optics Letters*** 41(2), 2016.

Image Quality Comparison

(guinea pig esophagus *in vivo*)



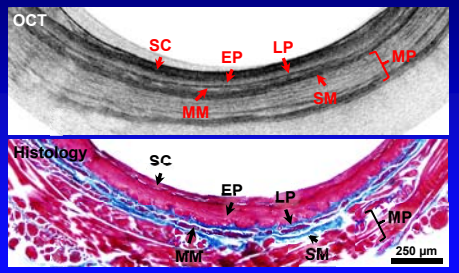
500 μm

1300 nm endoscope
9.5 μm axial resolution

800 nm diffractive endoscope
2.5 μm axial resolution

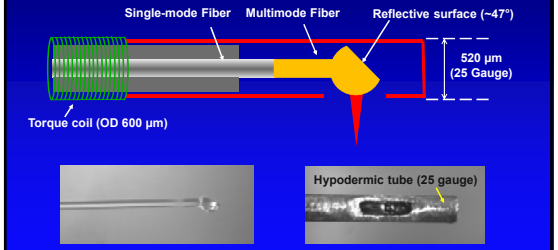
In vivo OCT versus Histology

(guinea pig esophagus)



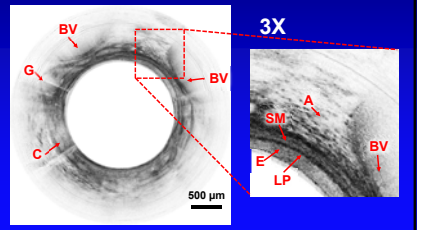
SC: Stratum Corneum; EP: Epithelium, LP: Lamina Propria, MM: Muscularis Mucosa, SM: Submucosa, MP: Muscularis Propria
J. F. Xi, A. Q. Zhang, Z. Liu, W. X. Liang, L. Y. Lin, S. Yu, and X. D. Li, ***Optics Letters*** 39(7), 2014.

Achromatic Ultrahigh-resolution OCT Microprobe (Φ250-500 um)



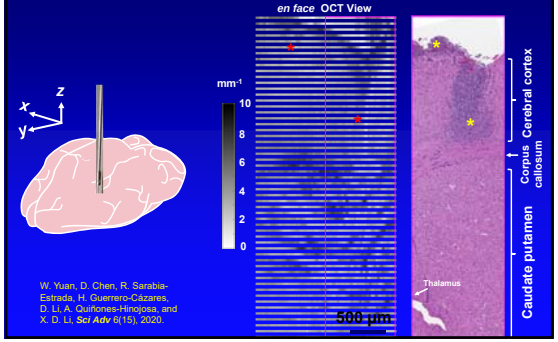
W. Yuan, R. Brown, W. Mitzner, L. Yarmus, and X. D. Li, ***Nat. Communications*** 8(1), 2017.

In vivo Imaging of Human Airway (smooth muscle)



A:Alveoli
E: epithelium
LP: lamina propria
SM: smooth muscle
C: cartilage
G: gland
BV: blood vessel

In vivo OCT Needle Imaging in Mouse Brain Tumor

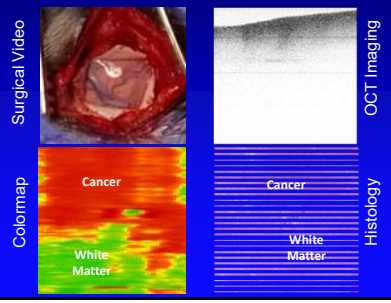


W. Yuan, D. Chen, R. Sarabia-Estrada, H. Guerrero-Cazares, D. Li, A. Quiñones-Hinojosa, and X. D. Li, ***Sci Adv*** 6(15), 2020.

Feasibility Study in Patient *in vivo* (Hopkins and Mayo Clinic)

C. Kut, K. Chaichana, J. Xi, S. Raza, X. Ye, E. McVeigh, F. Rodriguez, A. Quiñones-Hinojosa, and X. D. Li, ***Sci Trans Med*** 7(292), 2015.
W. Yuan, C. Kut, W. Liang, and X. D. Li, ***Scientific Reports*** 7, 2017.

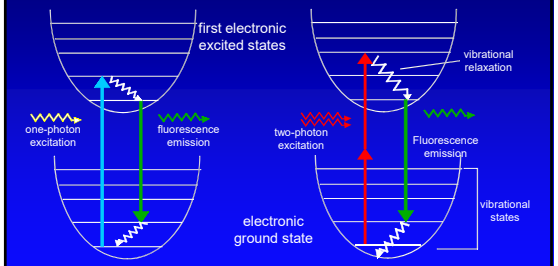
Real-Time OCT Imaging and Color-Mapping during Brain Surgery



Outline

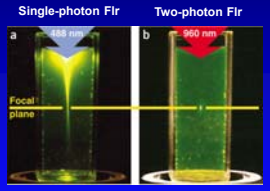
- OCT Endomicroscopy for Mesoscale Structural Imaging
- **Two-photon and SHG Endomicroscopy for Subcellular Biochemical/Molecular Imaging**

Single-photon vs Two-photon Fluorescence



Single-photon excitation Two-photon excitation (fs pulses)

Two-photon Fluorescence Imaging



$I_{Flr} \propto I_{Ex}$ $I_{Flr} \propto I_{Ex}^2$

Adapted from: W. R. Zipfel, R. M. Williams, and W. W. Webb, ***Nature Biotechnology*** 21(11), 2003.

Two-photon Fluorescence (and SHG) Imaging

- **Superb spatial resolution** (optical sectioning w/o pinhole)
  Requiring two or multiple photons arriving at the same spot and being absorbed at the same time
- **Better penetration**
  Using NIR excitation light
- **Full field fluorescence collection**
- .....

Great tool for studying biology with subcellular resolution (NADH, FAD, Noncentrosymmetric structures such as collagen network / ECM etc.)

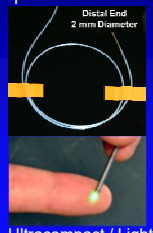
→ **Label-free imaging at submicron resolution**

Two-photon Microscopy → Endomicroscopy

Bench-top Microscope

Indispensable research tool
Expensive ($0.5 million)

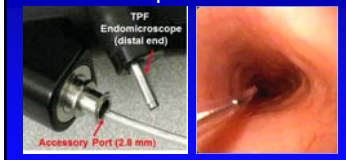
Fiber-optic Endomicroscope



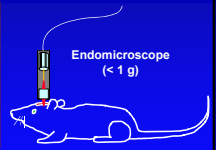
Flexible, Ultracompact / Lightweight
Enabling, Affordable, Turnkey

Applications

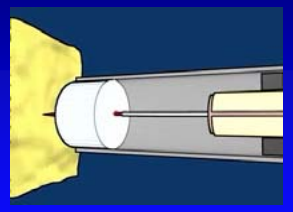
Endoscope-deliverable



Head-mountable

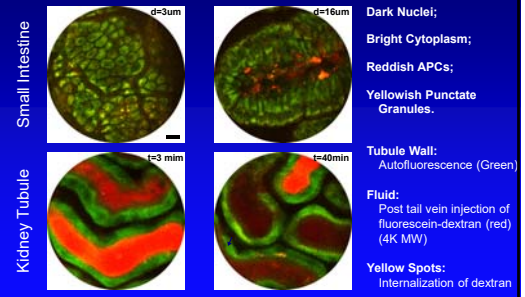


Two-photon Endomicroscopy



M. T. Myaing, D. McDonald, and X. D. Li, ***Optics Letters*** 31(8), 2006;
Y. Y. Zhang, M. L. Akins, K. Murari, J. F. Xi, M.-J. Li, K. Luby-Phelps, M. Mahendroo, and X. D. Li, ***PNAS*** 109(32), 2012;
H.-C. Park, H. Guan, A. Li, Y. Yue, M.-J. Li, H. Lu, and X. D. Li, ***Optics Letters*** 45(13), 2020.
W. X. Liang, G. Hall, B. Messerschmidt, M.-J. Li, and X. D. Li, ***Light: Science & Applications*** 6, 2017.

In vivo Mouse Imaging



Dark Nuclei;

Bright Cytoplasm;

Reddish APCs;

Yellowish Punctate Granules.

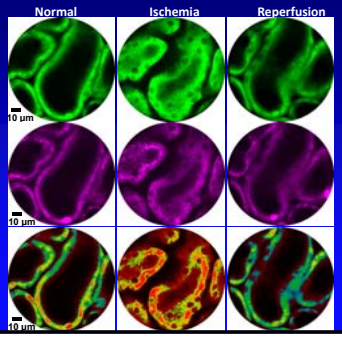
Tubule Wall:
Autofluorescence (Green)

Fluid:
Post tail vein injection of fluorescein-dextran (red) (4K MW)

Yellow Spots:
Internalization of dextran

W. X. Liang, G. Hall, B. Messerschmidt, M.-J. Li, and X. D. Li, ***Light: Science & Applications*** 6, 2017.

Metabolic Imaging *in vivo* (mouse kidney)



Speed:
~2.5 fps

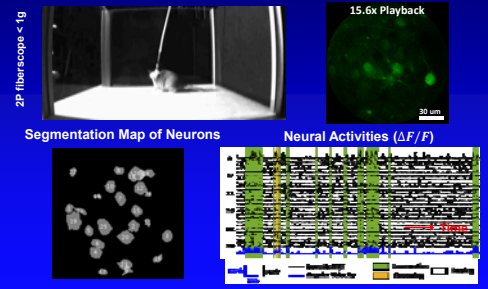
Redox Ratio

$$= \frac{\text{FAD}}{\text{FAD+NADH}}$$

Low High

W. X. Liang, G. Hall, B. Messerschmidt, M.-J. Li, and X. D. Li, ***Light: Science & Applications*** 6, 2017.

Neuron Activity Imaging in Freely-moving Mice

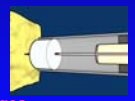
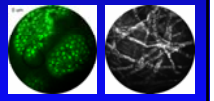


A. Li, H. Guan, H. C. Park, Y. Yue, D. Chen, W. Liang, M.-J. Li, H. Lu, and **X. D. Li**, ***Optica*** **8(6)**, 2021.

Summary

OCT:
- architecture
- morphology
- blood flow/vessel
- lack molecular info


Two-photon:
- subcellular imaging
- metabolic info
- regulatory challenges

Exciting field with many challenges to overcome such as high-speed, signal and image processing.

Acknowledgements

Funding Supports

National Institutes of Health:

R01CA200399 (Li and Quiñones-Hinojosa)
MTEC-16-02-BMI-09 (Kao, Kanold, Li)
R01CA247595 (Vargas, Li, Liang)

Bisciotti Foundation Translational Award
(Li and Park)

Team Work

Team Members

Graduate Students (PhD, MD/PhD):
- Wenxuan Liang
- Ang Li
- Honghua Guan
- Carmen Kut

Postdoctoral Fellows:
- Dawei Li
- Gunnsteinn Hall
- Scott Yuan

Research Associate:
- Hyeon-Cheol Park

Collaborators

Johns Hopkins University
- Radiology: Dr. Z Bhujwalla
- Pulmonary Medicine: Drs. R Brown, L Yarmus
- Pathology: Drs. K Li, H Zhang, D Nauen
- Neurosurgery: Dr. C Bettegowda
- Neuroscience: Dr. D Bergles
- Gastroenterology: Dr. S Yu

GWU: Dr. H Lu
Mayo Clinic: A Quiñones-Hinojosa
Corning Inc.: Dr. MJ Li

Thank You!

Group Website: *bit.bme.jhu.edu*

Virtual Group Party (2020-08-28)

Summer Getaway (before)

Email Me: xingde@jhu.edu