University of Illinois at Urbana-Champaign
Biophotonics Summer School

Optical Coherence Tomography

Technology and Applications

Stephen A. Boppart, M.D., Ph.D.

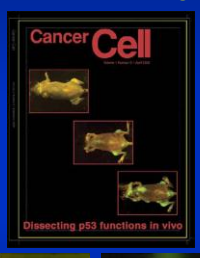
Beckman Institute for Advanced Science and Technology
Departments of Electrical and Computer Engineering,
Bioengineering, Medicine
University of Illinois at Urbana-Champaign

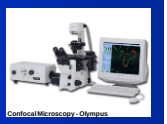
May 24, 2012

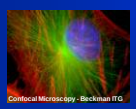
The front line of research is almost always in a fog. It is instruments that function as our eyes, allowing us to gather map-points in the fog, to construct a picture of the world in the form of a map, and ultimately to penetrate those obscuring mists.

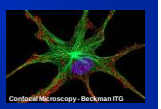
-Stephen S. Hall, Mapping the Next Millennium

Biomedical Imaging









Biomedical Imaging Size Scales

Whole-Body Imaging (meters)

Tissue Imaging (centimeters)

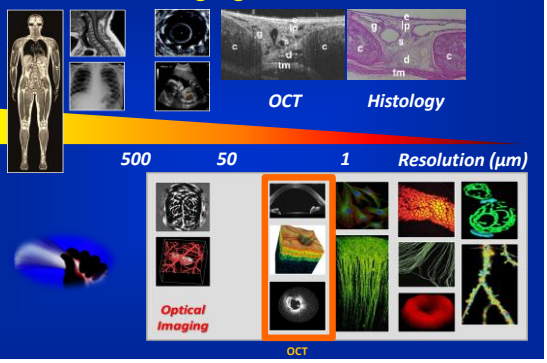
Cellular Imaging (microns)

Molecular Imaging (nanometers)

Molecular Vibrational Imaging (Angstroms)

Goal for Imaging Technology:
Image and diagnose where disease starts

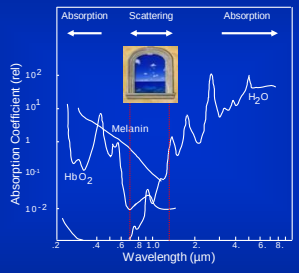
Biomedical Imaging



Comparison of Imaging Modalities

	Resolution (μm)	Field of View In Depth	Cost	Speed	Capability of Probe Imaging
CT	500-1000	Full Body	High	1-2 sec.	Poor
MRI	500-1000	Full Body	Very High	5-10 min.	Poor
US	100-200	10-20 cm	Low	Video Rate	Good
Endoscopy	10-50	Surface Imaging	Low	Video Rate	Excellent
OCT	**2-10**	**2-3 mm**	**Low**	**Video Rate**	**Good**
MPM	**0.2-1**	**50-300 μm**	**Mid**	**Video Rate**	**Poor**

The Biological Window into Tissue



Profio & Doiron, Photochem. Photobiol. 46:591, 1987

Outline

Lecture 1 (today)

- Optical Coherence Tomography (OCT)
- Beam Delivery Instruments
- Morphological & Cellular OCT Imaging
- Spectroscopic OCT
- Applications to Cancer and Primary Care Imaging

Lecture 2 (next week)

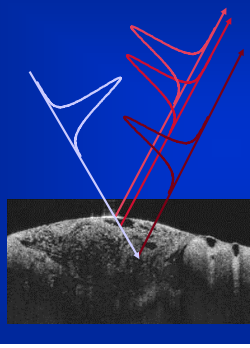
- Molecular OCT Imaging
- Contrast Agents for OCT
  - Scattering, Absorbing, Modulating Probes

OPTICAL COHERENCE TOMOGRAPHY

An Incomplete History of OCT

1980s	Telecom, fiber-optic measurements (OTDR, OFDR)
1990	Optical ranging in tissue
1991	Optical Coherence Tomography (OCT)
1990s	Ophthalmology applications
	Use of longer wavelengths for highly-scattering tissue
	Technology development: Doppler, High-speed/resolution
	Application exploration
1997	Endoscopic OCT in patients
1998	Cellular imaging
1999	Ultrahigh resolution OCT
2000	Barrett's esophagus imaging
2001	Coronary artery imaging
2002	Molecular imaging and contrast agents
2003	Spectral domain OCT techniques
2006	High-speed volumetric OCT (GPUs)
2008	Computational imaging
2010→	Rapid commercialization

Optical Ranging in Biological Tissue



Just like ultrasound, but with invisible light!

Coherence Theory

$G(\tau) = \langle U^*(t)U(t+\tau)\rangle$ Temporal coherence function (Autocorrelation function) where U(t) is a stationary complex random function

$I = G(0)$ Intensity

G(τ) also carries information about degree of correlation (coherence)

$g(\tau) = \frac{G(\tau)}{G(0)} = \frac{\langle U^*(t)U(t+\tau)\rangle}{\langle U^*(t)U(t)\rangle}$ $0 \le |g(\tau)| \le 1$ Complex Degree of Temporal Coherence

$\tau_c = \int_{-\infty}^{\infty} |g(\tau)|^2 d\tau$ Coherence Time

$l_c = c \cdot \tau_c$ Coherence Length

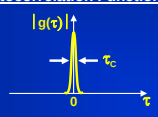
$\Delta\nu_c = \frac{1}{\tau_c}$ Spectral Width

$S(\nu) = \int_{-\infty}^{\infty} G(\tau)\exp(-j2\pi\nu\tau)d\tau$ Power Spectral Density Function

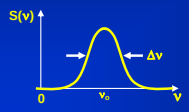
Wiener-Khinchin Theorem

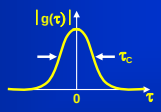
Relationships

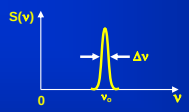
Autocorrelation Functions



Spectral Density Functions



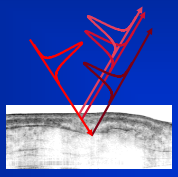


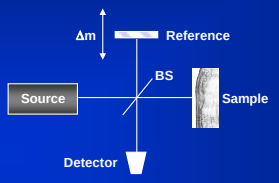


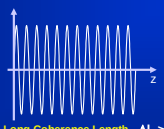
$$S(\nu) = \int_{-\infty}^{\infty} G(\tau)\exp(-j2\pi\nu\tau)d\tau$$

A Fourier-Transform Relationship

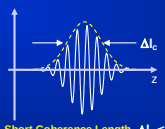
Optical Ranging using Low-Coherence Interferometry







Long Coherence Length $\Delta l_c >> \Delta m$



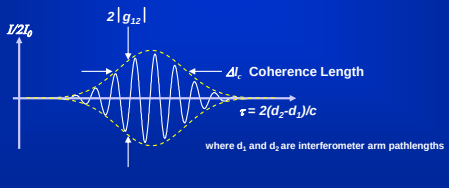
Short Coherence Length $\Delta l_c < \Delta m$

Low-Coherence Interferometry

Two partially coherent waves U_1 and U_2 $\quad I_1 = \langle |U_1|^2 \rangle \quad I_2 = \langle |U_2|^2 \rangle$

Cross-correlation Function: $G_{12} = \langle U_1^* U_2 \rangle$ $\quad$ Normalized: $g_{12} = \dfrac{\langle U_1^* U_2 \rangle}{\sqrt{I_1 I_2}}$

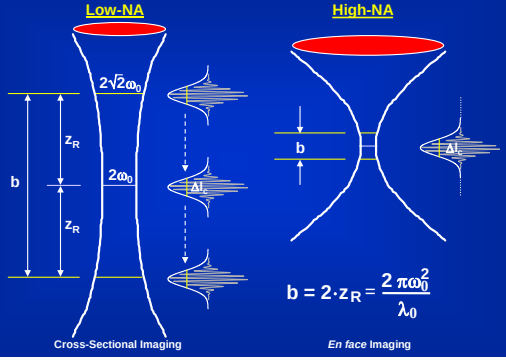
Interference Equation: $I = I_1 + I_2 + 2\sqrt{I_1 I_2}\,|g_{12}|\cos\varphi$



where d_1 and d_2 are interferometer arm pathlengths

Beam Focusing

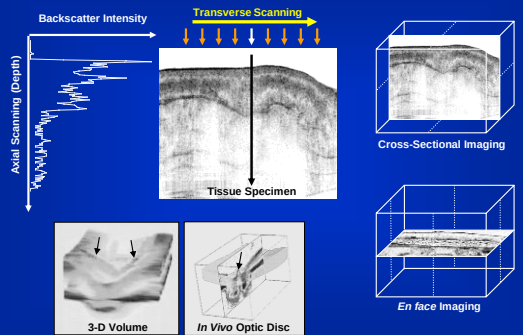
Low-NA $\qquad$ High-NA



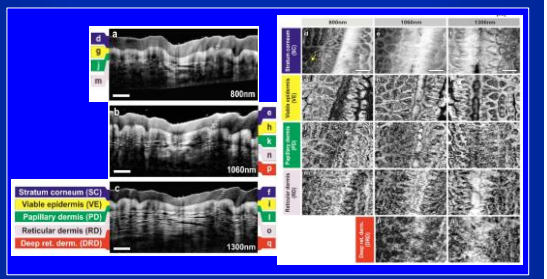
$$b = 2 \cdot z_R = \frac{2\pi\omega_0^2}{\lambda_0}$$

Cross-Sectional Imaging $\qquad$ *En face* Imaging

Multi-Dimensional OCT Imaging

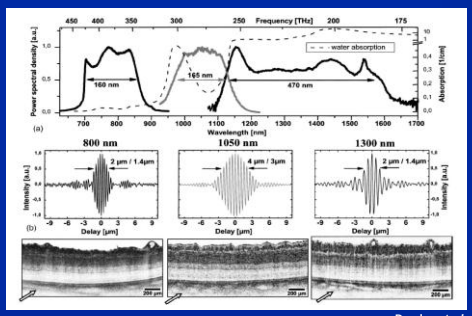


OCT Imaging at Different Wavelengths



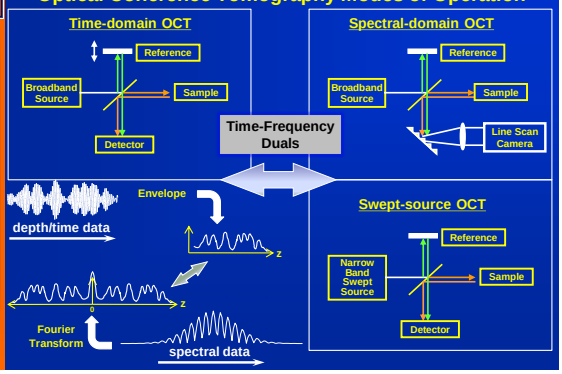
Drexler, *et. al.*

OCT Imaging at Different Wavelengths

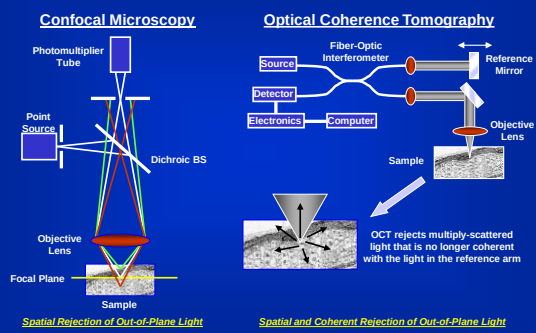


Drexler, *et. al.*

Optical Coherence Tomography Modes of Operation

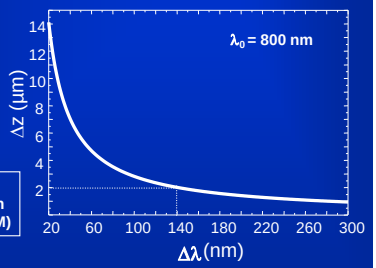


Comparison with Confocal Microscopy



Optical Bandwidth - Axial Resolution

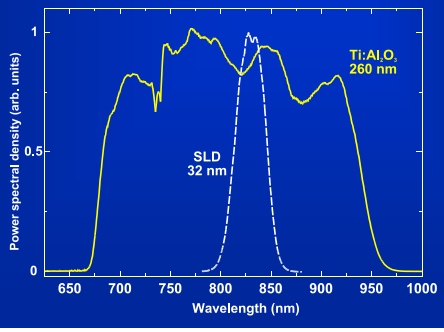
$$\Delta z = \frac{2\ln(2)}{\pi}\frac{\lambda_o^2}{\Delta\lambda}$$



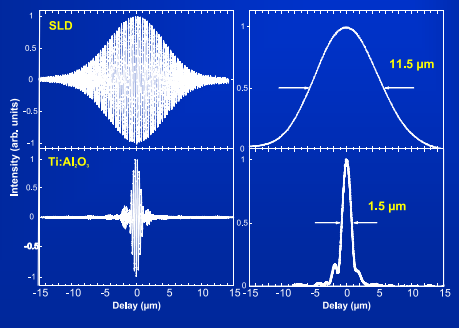
Δz = Coherence length
λ_o = Center wavelength
Δλ = Bandwidth (FWHM)

Assumptions: Gaussian spectrum, non-dispersive medium

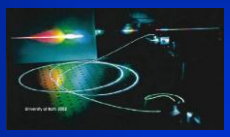
Ultrahigh Resolution OCT System

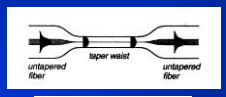


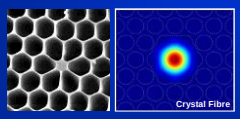
Ultrahigh Resolution OCT System

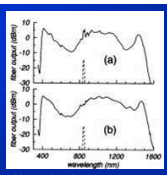


Supercontinuum Generation via Nonlinear Spectral Broadening in Fiber





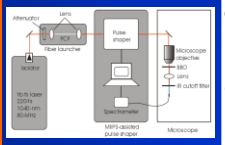




Ranka, *et al*, Optics Letters, 25, 2000

Birks, *et al*, Optics Letters, 25, 2000

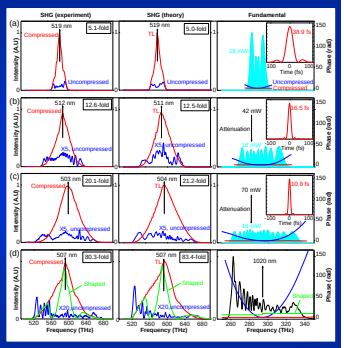
Compression of Fiber Supercontinuum to the Fourier Limit in a High NA Focus



Diode-pumped Yb:KYW laser pumping of a nonlinear PCF with normal dispersion across full spectral range of supercontinuum.

Adaptive phase compensation by Multiphoton Intraphase Interference Phase Scans (MIIPS) – to compress pulse.

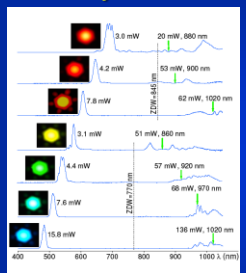
Tunable 10.8-38.9 fs pulses (FWHM), ~1020 nm center wavelength, 76 MHz, 18-70 mW average power.



9 cm PCF – 1050-NEG-1, NKT Photonics
60X / 0.9-NA Objective, LUMPlanFLIR, Olympus

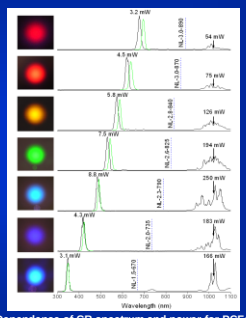
Tu, *et al*., Optics Letters, 36:2315, 2011

Optical Frequency Up-Conversion by Supercontinuum-Free Widely-Tunable Fiber-Optic Cherenkov Radiation



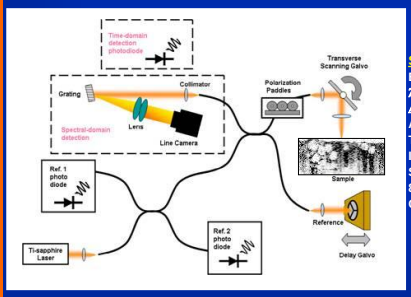
Dependence of CR spectrum and power on pump-wavelength and input power for 8.3 cm NL.1.8-845 and 7.2 cm NL-1.7-770 PCFs.

Tu and Boppart, Optics Express, 17:9858, 2009
Tu and Boppart, Optics Express, 17:17983, 2009



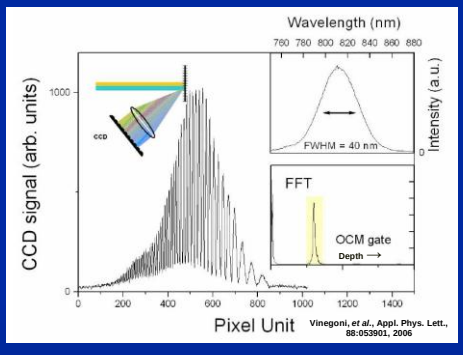
Dependence of CR spectrum and power for PCFs with varying ZDWs and 1020 nm pump powers.

Fiber-based Time-Domain OCT and Spectral-Domain OCT Systems



System Parameters
Broadband Source
λ_0 = 800 nm or 1300 nm
$\Delta\lambda$ = 80-150 nm
Axial Res. = 2-3 μm
Trans Res. = 5-10 μm
Incident Power = 5 mW
SD-OCT: 29 kHz acq.
8 Hz (1000x1000 pix)
Continuous display rate

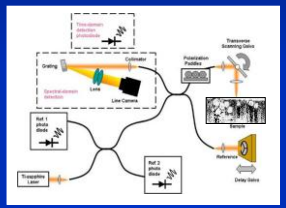
Spectral-Domain Optical Coherence Microscopy



Vinegoni, *et al*., Appl. Phys. Lett., 88:053901, 2006

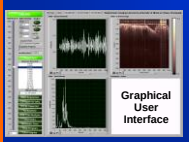
Portable & Modular OCT Imaging System

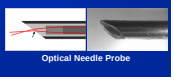
Portable OCT



Surgical Microscope

Catheter-Endoscope

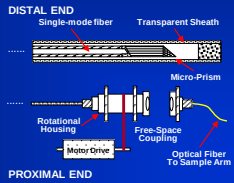


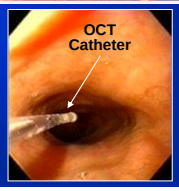


Optical Needle Probe

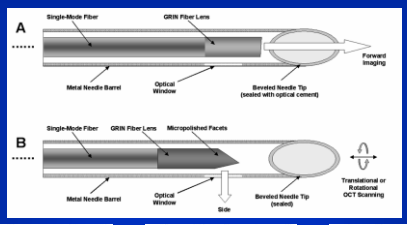
- ► Compact portable clinical system
- ► User-friendly software interface
- ► Modular beam delivery devices

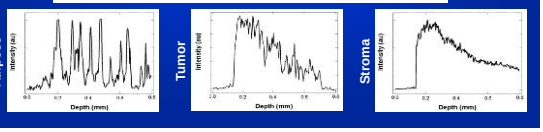
OCT Fiber-Optic Catheter





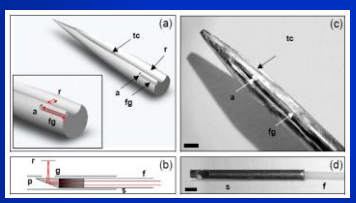
LCI (Axial-Scanning) Needle Probe

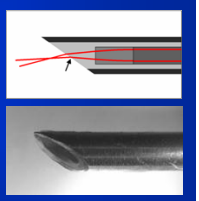




Refractive Index Needle Probes

Fixed Reflector | **Reflection Refractometry**

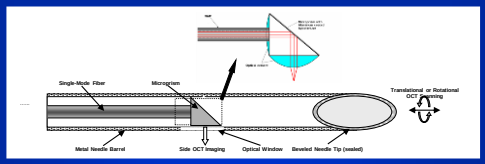


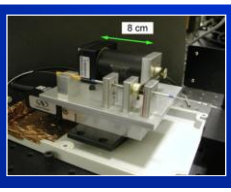


Zysk, *et al.*, Optics Letters 32:385, 2007

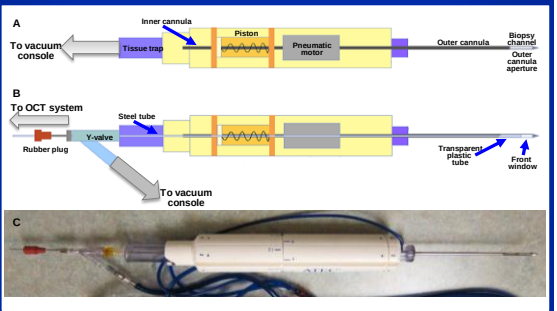
Zysk, *et al.*, Optics Express 15:4787, 2007

OCT Imaging Needle Probe

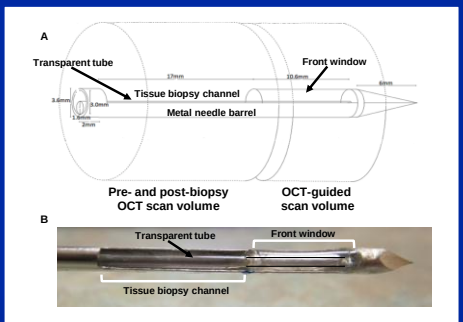




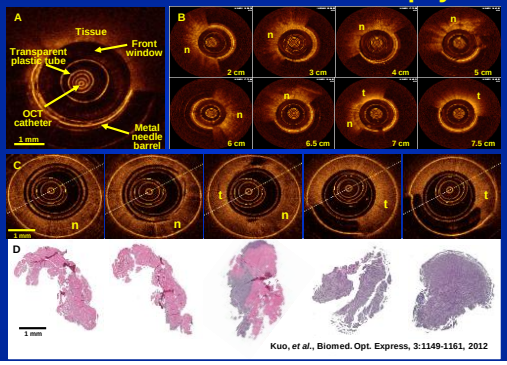
Modified Vacuum-Assisted Core-Needle



Core-Needle: Tip Details



OCT-Guided Core-Needle Biopsy

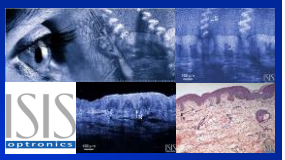


Kuo, *et al.*, Biomed. Opt. Express, 3:1149-1161, 2012

OCT Commercialization

LightLab™

Optical Coherence Tomography



DIAGNOSTIC PHOTONICS

Bioptigen

IMALUX

ThorLabs, Inc.

Commercial OCT Systems

OCT Parameters

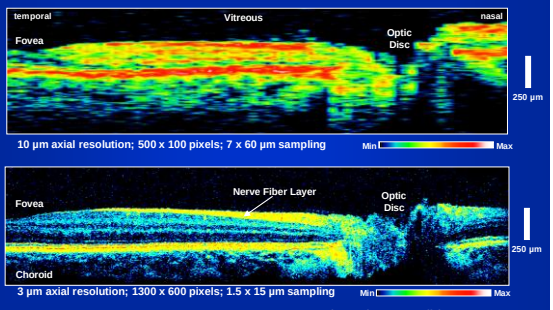
- Non-contact, non-invasive imaging using near-infrared light (800-1500 nm wavelength)
- High-resolution (1-10 μm axial, 1-30 μm transverse)
- High-speed acquisition (4-32 frames per second)
- Deep imaging penetration (2-3 mm) in highly-scattering tissue
- High sensitivity (110 dB signal-to-noise)
- Compact, fiber-optic design readily integrated with optical instruments (microscopes, catheters, endoscopes)
- Doppler OCT, Spectroscopic OCT, Molecular imaging

What is Needed for Imaging and Diagnostics using OCT?

- **Morphological Imaging**
  - High resolution
  - Beam-delivery systems
  - Real-time acquisition
  - Surgical guidance
- **Cellular Imaging**
  - Ultrahigh resolution
  - Contrast enhancement
- **Molecular Imaging**
  - Contrast agents
  - Spectroscopic OCT
  - Nonlinear Interferometric Vibrational Imaging (NIVI)

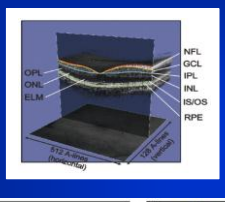
MORPHOLOGICAL IMAGING

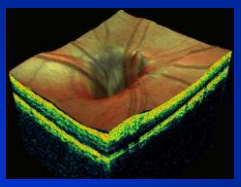
Normal vs. Ultrahigh-Resolution OCT of the Papillomacular Axis

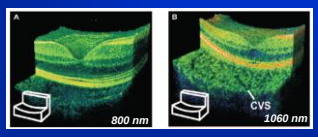


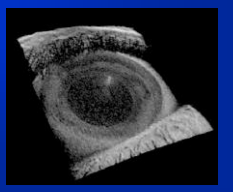
Drexler, *et al.* Nature Medicine 7:502-507, 2001

Ophthalmic Imaging



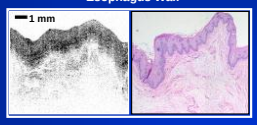




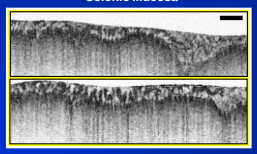


OCT of Human Gastrointestinal Tract Tissues

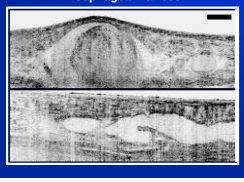
Esophagus Wall



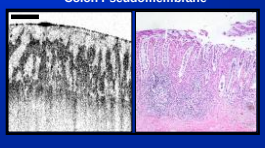
Colonic Mucosa



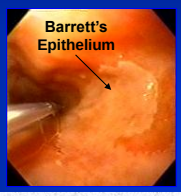
Esophageal Varices



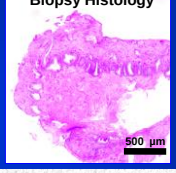
Colon Pseudomembrane

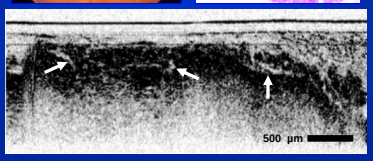


Linear-Scanning OCT Catheter Barrett's Esophagus

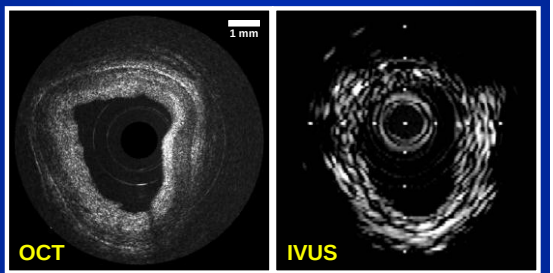


Biopsy Histology

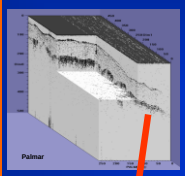


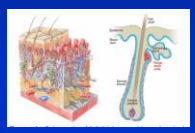


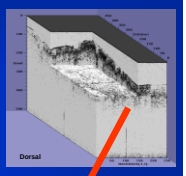
OCT Catheter-Endoscope Coronary Artery Intimal Hyperplasia

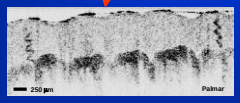


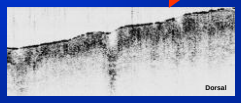
Real-Time OCT of Human Skin





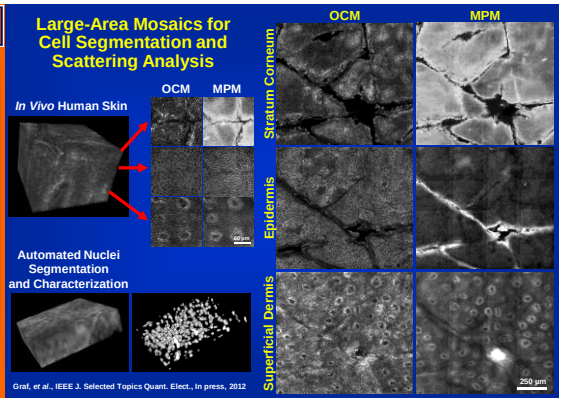






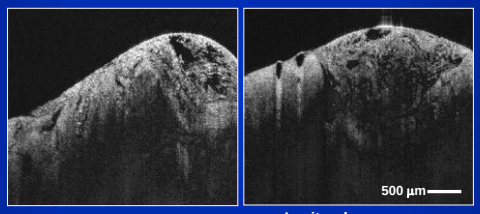
Human – Palmar & Dorsal

Large-Area Mosaics for Cell Segmentation and Scattering Analysis



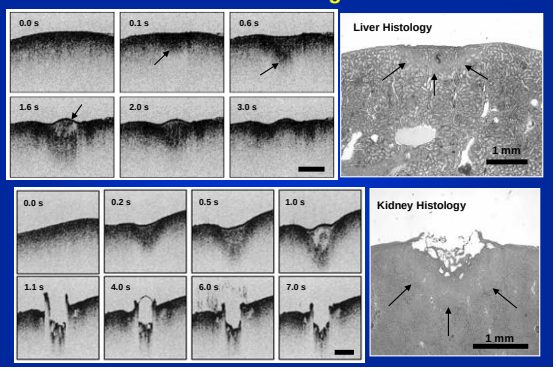
Graf, *et al.*, IEEE J. Selected Topics Quant. Elect., In press, 2012

Morphological Tumor Imaging

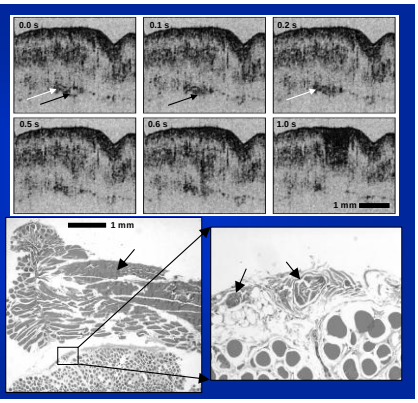


In vitro dog sarcoma

Real-Time OCT-Guided Surgical Intervention



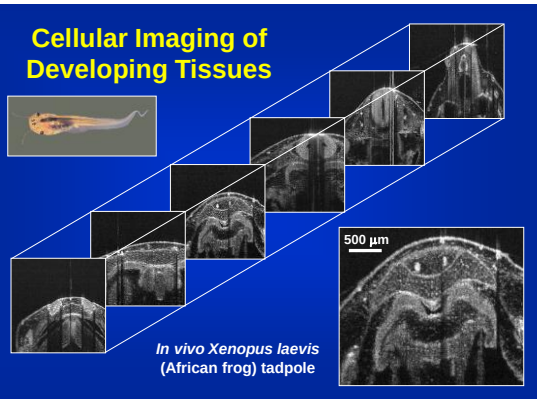
OCT Image Guidance for Selected Vessel Ablation



In vitro rat skeletal muscle

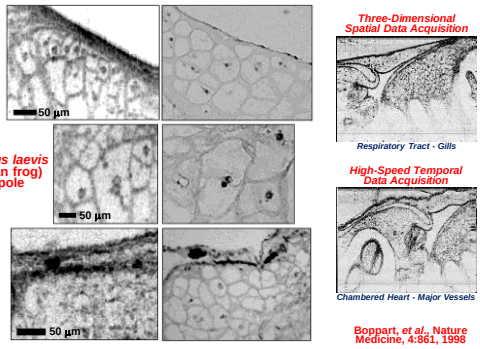
CELLULAR IMAGING

Cellular Imaging of Developing Tissues



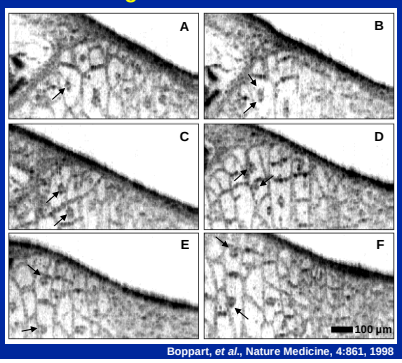
In vivo Xenopus laevis (African frog) tadpole

Histological Comparisons of Cellular Imaging



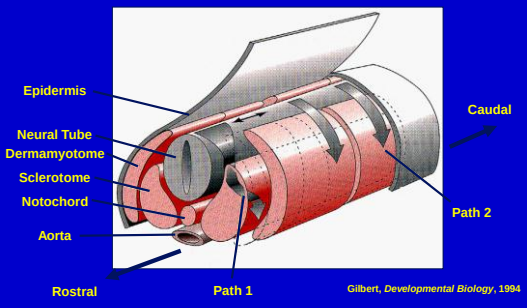
Boppart, *et al.*, Nature Medicine, 4:861, 1998

Tracking Mitosis in Real-Time



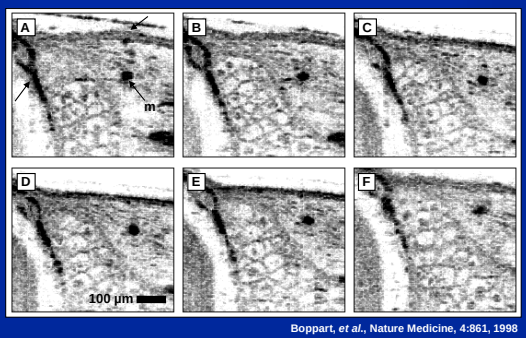
Boppart, *et al.*, Nature Medicine, 4:861, 1998

Neural Crest Cell Migration



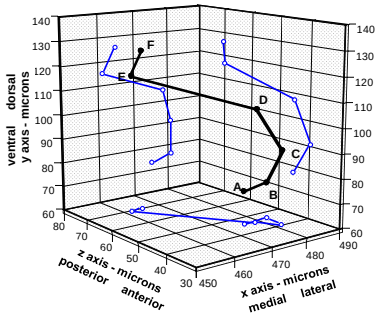
Gilbert, *Developmental Biology*, 1994

Tracking Cell Migration in Real-Time

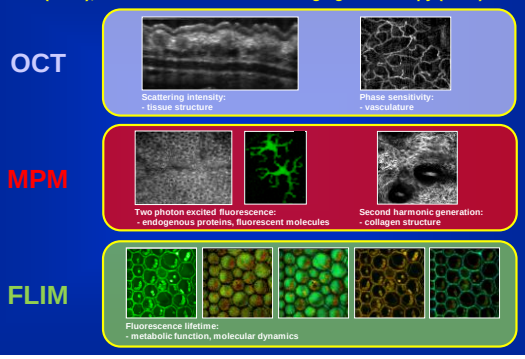


Boppart, *et al.*, Nature Medicine, 4:861, 1998

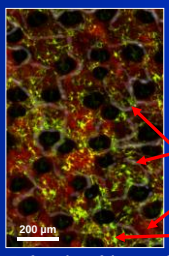
Three-Dimensional Tracking of Melanocytes



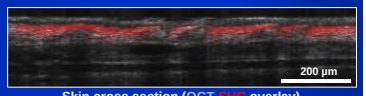
Integrated optical coherence tomography (OCT), multiphoton microscopy (MPM), and fluorescence lifetime imaging microscopy (FLIM)



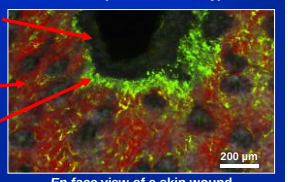
Co-Registered OCT and MPM Images from GFP Bone Marrow Transplanted Mouse Skin



En face view of the superficial dermis
(OCT variance-SHG-GFP overlay)

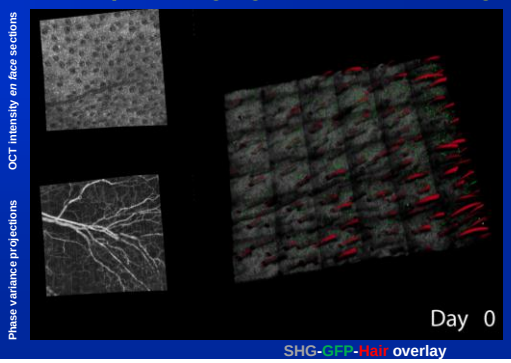


Skin cross section (OCT-SHG overlay)



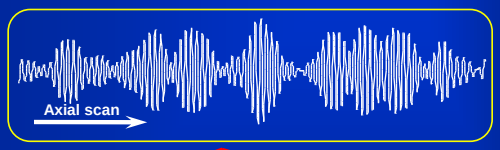
En face view of a skin wound
(OCT intensity-SHG-GFP overlay)

Time-Lapse Imaging of Wound Healing



SHG-GFP-Hair overlay

Spectroscopic OCT

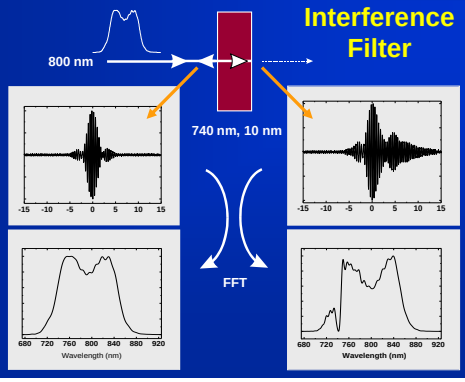


Envelope: Structural OCT
⇒ Amplitude Reflection
⇒ Reflectivity / Scattering

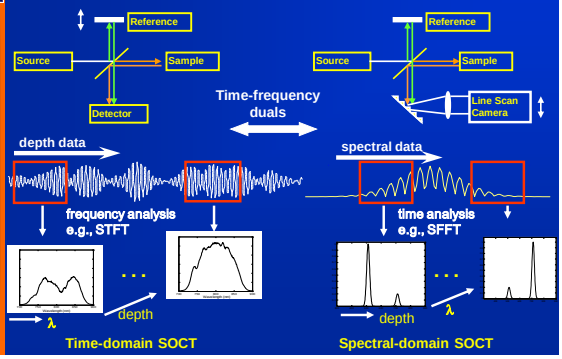
Carrier: Spectroscopic OCT
⇒ Spectral Reflection
⇒ Scattering / Absorption

Traditionally performed in TD-OCT, and SD-OCT offers phase stability

Interference Filter



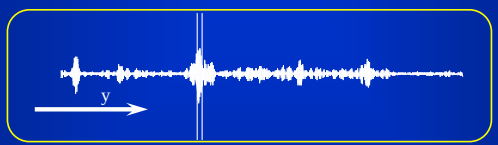
Spectroscopic Optical Coherence Tomography (SOCT)



Time-domain SOCT

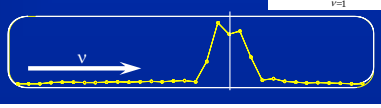
Spectral-domain SOCT

Spectroscopic OCT



- Local FFT: Time window, data points ~ longitudinal resolution
- Determination of the center of gravity of the peak:

$$\nu_c(y) = \frac{\sum_{\nu=1}^{64} \nu\,|S(\nu)|^2}{\sum_{\nu=1}^{64} |S(\nu)|^2}$$

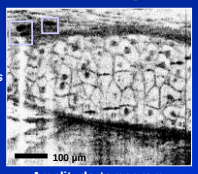


Frequency color map:

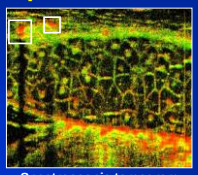
$\Leftarrow \Delta\nu \Leftarrow \bar{\nu} \Rightarrow \Delta\nu \Rightarrow$

In Vivo Spectroscopic OCT

Xenopus laevis (African frog)

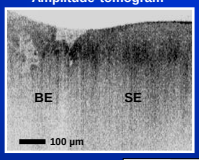


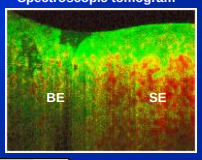
Amplitude tomogram



Spectroscopic tomogram

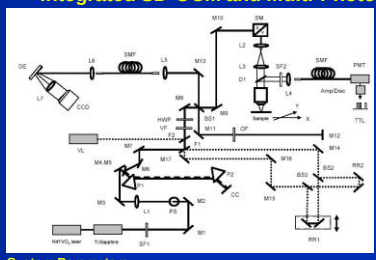
Human Barrett's Esophagus

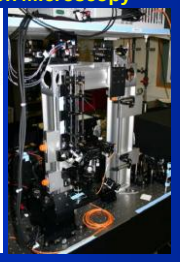




λ_{short} λ_{long}

Multi-Modality Microscopy
Integrated SD-OCM and Multi-Photon Microscopy



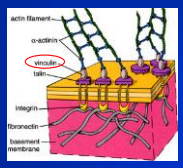


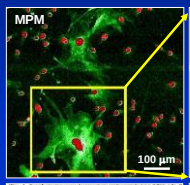
System Parameters

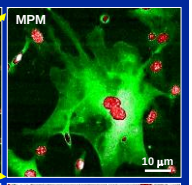
Broadband Ti:saph, λ_0 = 800 nm, $\Delta\lambda$ = 60 nm, Incident Power = 1-2 mW
Objective: Olympus 20X, 0.95 NA, water immersion, long working distance (2 mm)
OCM: Axial PSF = 1.6 µm, Trans Res = 0.9 µm, ΔL_c = 3.4 µm, SNR = 97 dB
MPM: Axial Res = 800 nm, Trans Res = 500 nm
Acquisition Time = Limited by scanning, SNR, image size: 1000 x 1000 pix, 600 x 600 µm (sec-min)

Vinegoni, *et al.*, Appl. Phys. Lett., 88:053901, 2006

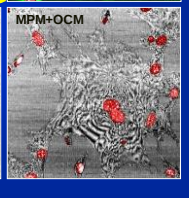
MPM-OCM GFP-Fibroblasts







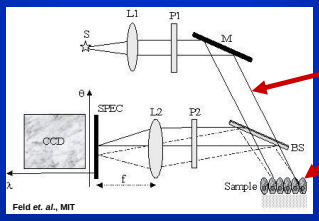
Phase Contrast



Light Scattering Spectroscopy (LSS)

LSS is a functional analysis method with applications in:

- Particle sizing
- Tissue characterization
- Early tumor detection



Loose focusing (no transverse resolution)

Little depth resolution

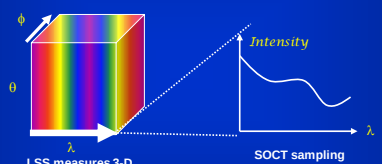
Feld *et. al.*, MIT

LSS is for functional analysis, less for functional imaging

Spectral-Scatttering for OCT

Combine LSS and OCT to get:

- Spatial resolution
- Functional analysis

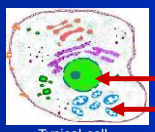


LSS measures 3-D scattering cube

SOCT sampling of the 3-D cube

SOCT only detects wavelength dependent back-scattering, but in a spatially-resolved way, with deeper penetration.

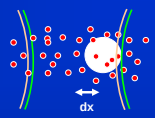
Heterogeneous Scatterers

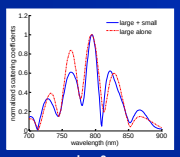


Typical cell

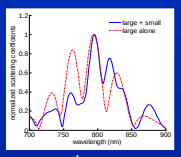
Nucleus

Mitochondria

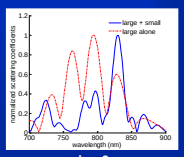




dx = 0 (centered)



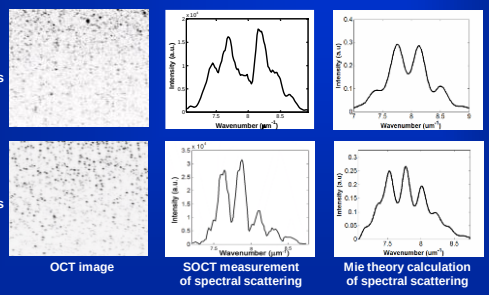
dx = w_0 (off-centered)



dx = $2w_0$ (very off-centered)

Xu, *et al.*, Optics Express 13:5450, 2005

Size Determination of Microspheres



OCT image | SOCT measurement of spectral scattering | Mie theory calculation of spectral scattering

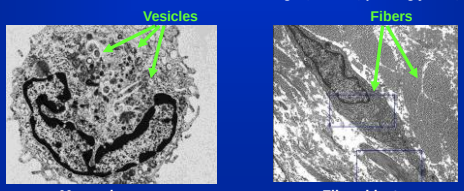
Can be potentially used for measuring the sizes of biological scatterers

Complications with Cells

Cells are never homogeneous spheres!

Major scatterers are:

- Cell membrane
- Nucleus
- Organelles such as mitochondria, ER, Golgi, Lysosome, etc.
- Cellular & extracellular matrix such as collagen, fibrillin, proteoglycans, etc.

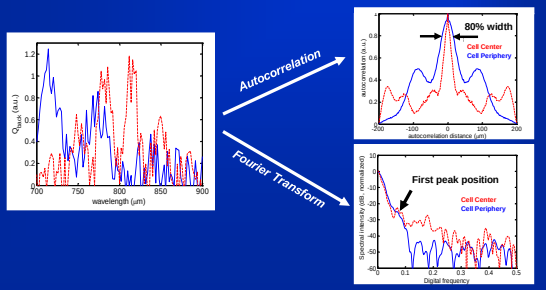


Macrophage (Biodic) | Fiberoblast (Biodic)

Cells contain many scatterers of different sizes and shapes.

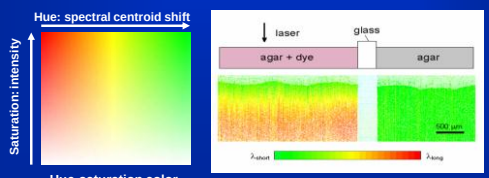
Spectral Analysis of SOCT Data from Cells

Instead of a clean modulation measured from microspheres, spectral data from cells are complex. Can use spectral analysis methods to extract features, e.g., frequency analysis or correlation analysis



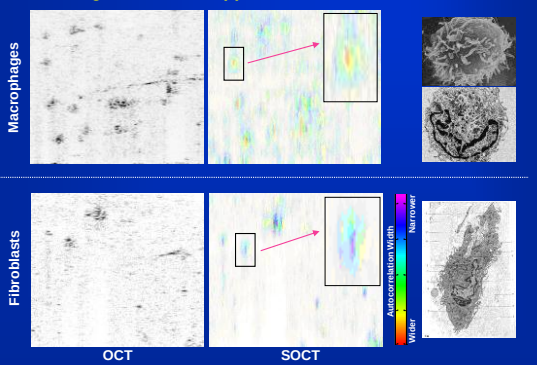
SOCT Hyper-Spectral Display

- **Time-frequency analysis of OCT signal generates 3-D data**
  - Two geometry dimensions and one spectral dimension
- **To display SOCT image: HSV color scale**
  - Hue: spectral analysis, *e.g.*, autocorrelation width
  - Saturation: backscattering intensity



Hue-saturation color space | SOCT of dye phantom

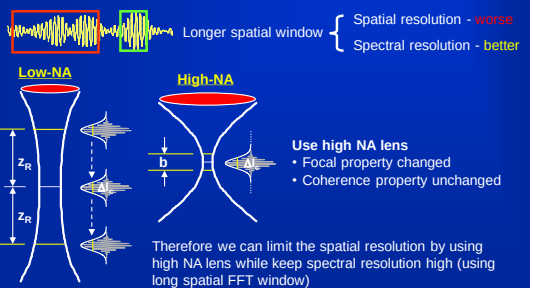
Scattering-Mode SOCT Application: Cell Characterization



OCT | SOCT

Time-Frequency Resolution With OCM

Time-frequency uncertainty principle states that the time resolution and frequency resolution cannot both be high.



Use high NA lens
- Focal property changed
- Coherence property unchanged

Therefore we can limit the spatial resolution by using high NA lens while keep spectral resolution high (using long spatial FFT window)

Scattering-Mode Spectroscopic OCM: Tissue Contrast Enhancement

Contrast based on size of scatterers (cells, nuclei)

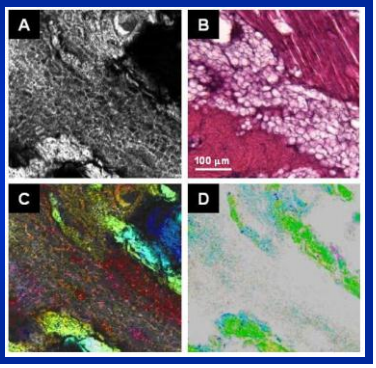
A) OCM

B) H&E Histology

C) Metameric SOCM

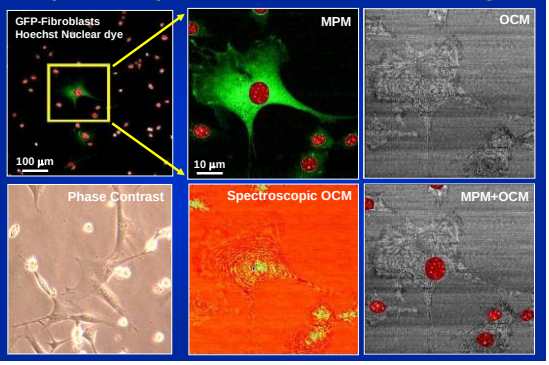
D) LSS SOCM

Rat mammary tissue: Adipose and Muscle



Xu, *et al.*, Opt. Lett., 31:1079, 2006

Spectroscopic OCM for Cell Scatterer Sizing



Cancer Facts

- Cancer is a molecular disease of DNA
- More than 1.4 million new cancer cases per year resulting in ~ 23% of all deaths in the United States, a rate second only to heart disease
- Early identification improves patients prognosis
- Interventional decisions require accurate diagnosis and positive identification of the tumor margins
- Diagnosis not necessarily definite and quantifiable due to the limitations associated with conventional biopsies and currently available imaging techniques

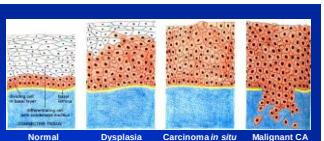
Cancer Progression

Where can imaging aid diagnosis?

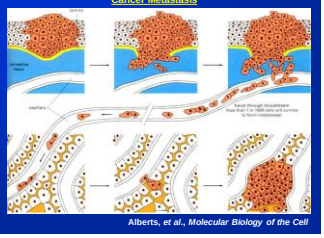
- Morphological changes
- Cellular proliferation
- Cell mitotic index
- Cell N/C ratio
- Growth beyond basal lamina
- Tracking metastatic cells
- Molecular indicators
- Molecular precursors

Gold standard:
Biopsy with histology

Diagnostic Trends:
Morphology to molecules
Histology to *in vivo*

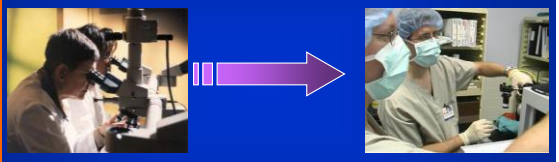


Cancer Metastasis



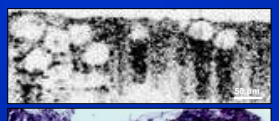
Alberts, *et al.*, *Molecular Biology of the Cell*

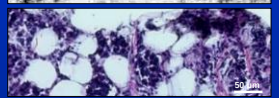
Intra-Operative and Intra-Procedure Optical Biopsy of Breast Cancer



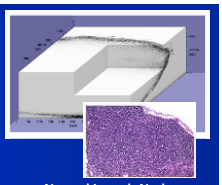
Change the Paradigm for Diagnosis

Comparison of Human Breast Tissue and Lymph Nodes with Corresponding Histology

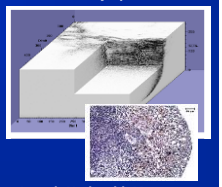




Invasive Ductal Carcinoma



Normal Lymph Node

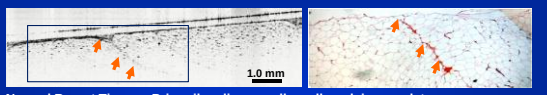


Lymph Node with Metastases

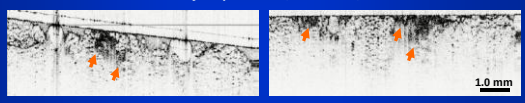
Boppart, et al., Breast Cancer Research and Treatment, 2004
Luo, et al., Technology in Cancer Research and Treatment, 2005

Translational Imaging Research Protocol

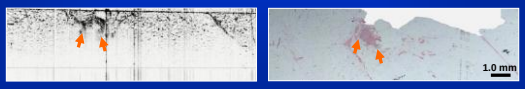
Intra-Operative Imaging Results



Normal Breast Tissue: Primarily adipose cells, cell nuclei, vasculature

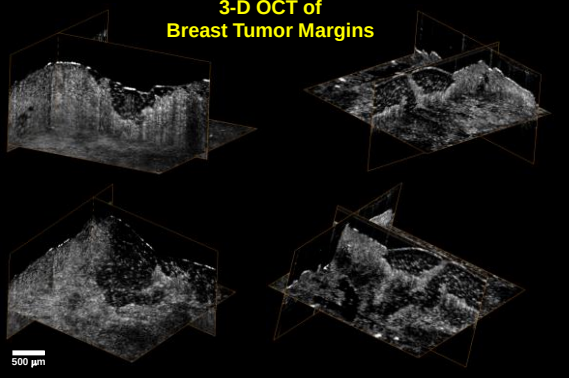


Tumor margin with non-adipose features: Left: DCIS Right: DCIS & IDC

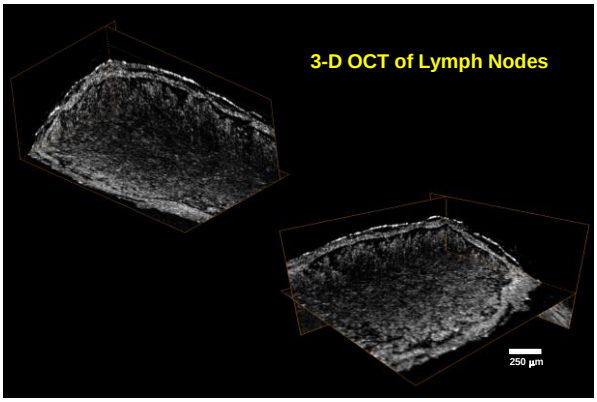


Isolated Tumor Focus (DCIS): Left: OCT Right: Histology

3-D OCT of Breast Tumor Margins



3-D OCT of Lymph Nodes



Intra-Operative Tumor Margin Assessment Study Results

- 38 patients (17 training set, 20 study set, 1 excluded)
- Undergoing lumpectomy for biopsy-diagnosed breast cancer
- 15 DCIS, 2 infiltrating DC, 9 invasive DC, 1 LCIS, 1 invasive papillary, 2 invasive mammary carcinoma, 1 atypical ductal hyperplasia
- ~ 10 OCT/site, 1-2 sites/specimen, marked for histological correlation

	Histology (Positive)	Histology (Negative)	Total	
OCT (Positive)	9 (TP)	2 (FP)	11	PPV = 82%
OCT (Negative)	0 (FN)	9 (TN)	9	NPV = 100%
Total	9	11	20	
	Sensitivity = 100%	Specificity = 82%		

Nguyen, et al., Cancer Research, 69:8790, 2009

Intraoperative Imaging Platform based on OCT and ISAM: Interferometric Synthetic Aperture Microscopy Computed Imaging of Histology

DIAGNOSTIC PHOTONICS

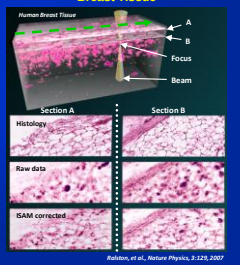
Interferometric Synthetic Aperture Microscopy (ISAM)

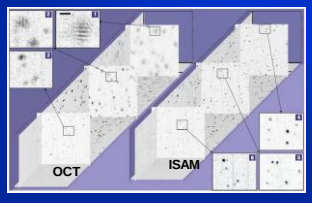
OCT ISAM

1 μm-size TiO_2 particles in 3-D scaffold

- Computed real-time 3-D microscopic image formation technique
- Solution to inverse scattering problem for coherence microscopy
- Spatially-invariant resolution, equivalent to that at focal plane
- Eliminates compromise b/w transverse resolution and depth-of-field
- Broad applications including biomedical imaging and diagnostics

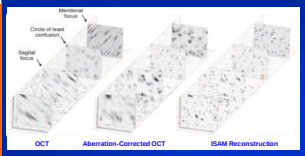
Breast Tissue



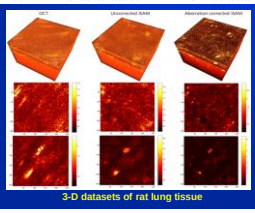


Ralston, et al., Nature Physics, 3:129, 2007

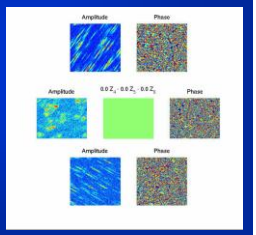
Computational Adaptive Optics



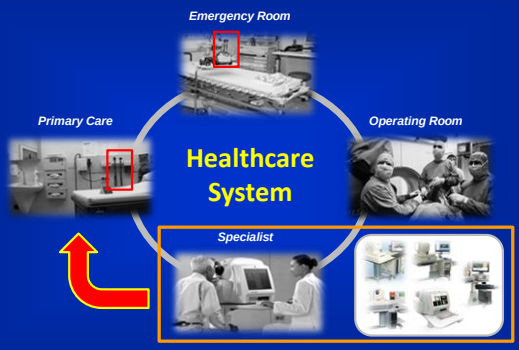
Correcting system and specimen-induced aberrations using complete amplitude-phase retrieval in coherence imaging



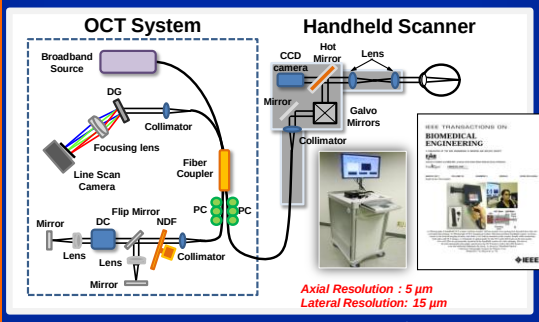
3-D datasets of rat lung tissue



Adie, et al., Proc. Natl. Acad. Sci., 2012



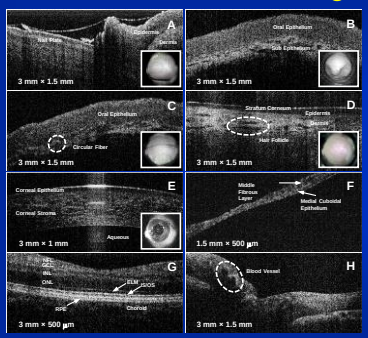
Portable OCT System

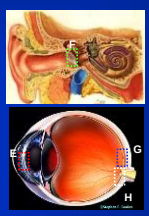


Axial Resolution : 5 μm
Lateral Resolution: 15 μm

Jung, *et. al.*, IEEE TBME 58:741, 2011

In vivo OCT and Video Images (Human)





(A) nail fold (B) uvula
(C) gum (D) arm (E) cornea
(F) tympanic membrane
(G) retina around fovea
(H) optic nerve head

Jung, *et. al.*, IEEE TBME 58:741, 2011

Conclusions

- OCT technology is robust and versatile.
- OCT has the potential to detect disease at all size scales:
  - *Morphological Imaging*
  - *Cellular Imaging*
  - *Molecular Imaging*
- OCT techniques have the potential as novel diagnostic methods for many areas in healthcare.
  - *Image-guided biopsy and surgery*
  - *Intraoperative tumor & margin assessment*
  - *Primary Care Imaging*

Outline

Lecture 1 (today)

- Optical Coherence Tomography (OCT)
- Beam Delivery Instruments
- Morphological & Cellular OCT Imaging
- Spectroscopic OCT
- Applications to Cancer and Primary Care Imaging

Lecture 2 (next week)

- Molecular OCT Imaging
- Contrast Agents for OCT
  - Scattering, Absorbing, Modulating Probes

Acknowledgments

Beckman Institute
for Advanced Science and Technology
Biophotonics Imaging Laboratory
Stephen Boppart, M.D., Ph.D.

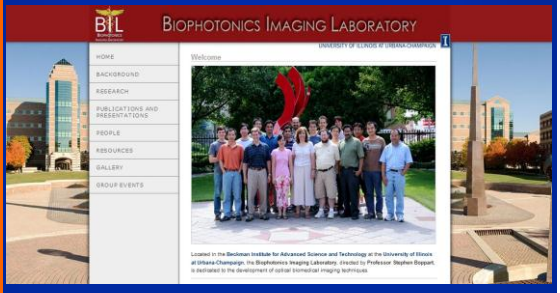
Graduate Students
Adeel Ahmad, M.S.
Vasilica Crecea, M.S.
Ben Graf, M.S.
Joanne Li
Yuan Liu, M.S.
Guillermo Monroy
Cac Nguyen, M.S.
Nathan Shemonski, M.S.
Fredrick South
Jonathan Sun

Undergraduate Students
Zita Hubler
Jessica Hsu
Eric Kuo
Shreya Prakash
Jonathan Rasio
Wolfgang Rubrecht

Research Coordinator
Darold Spillman

Research Scientists
Steven Adie, Ph.D.
Eric Chaney
Woonggyu Jung, Ph.D.
Jongsick Kim, Ph.D.
Marina Marjanovic, Ph.D.
Haohua Tu, Ph.D.
Chao Wang, Ph.D.
Youbo Zhao, Ph.D.

Clinical Collaborators (Carle Foundation Hospital, Provena Medical Center):

Medical Oncology:	Kendrith Rowland, M.D.	Patricia Johnson, M.D., Ph.D.
Surgical Oncology:	Partha Ray, M.D.	Uretz Oliphant, M.D.
Otolaryngology:	Michael Novak, M.D.	John Brockenbrough, M.D.
Pathology:	George Liu, M.D.	Krishnan Tangella, M.D.

biophotonics.illinois.edu