University of Illinois at Urbana-Champaign
Summer School on NanoBioPhotonics

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, Internal Medicine
University of Illinois at Urbana-Champaign

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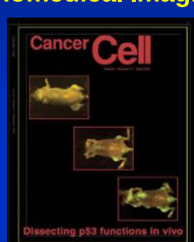
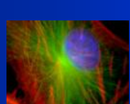
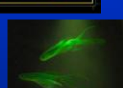
The front line of research is almost always in a fog. It is instruments that function as our eyes, allowing us to gather map-points in the fog, to construct a picture of the world in the form of a map, and ultimately to penetrate those obscuring mists.

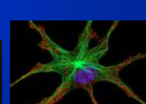
-Stephen S. Hall, Mapping the Next Millennium

Biomedical Imaging

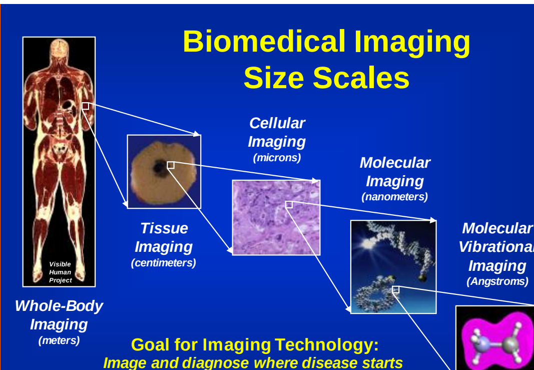






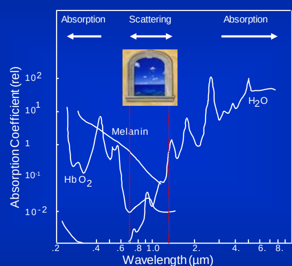


Biomedical Imaging Size Scales



Goal for Imaging Technology:
Image and diagnose where disease starts

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
- Application to Cancer Imaging

Lecture 2 (next week)

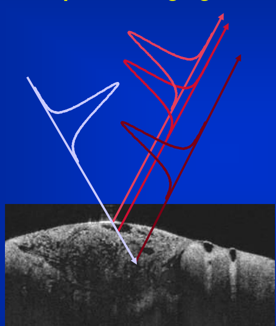
- Molecular OCT Imaging
- Contrast Agents for OCT
  - Scattering, Absorbing, Modulating Probes

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 microscopy

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)

$g(\tau)=\frac{G(\tau)}{G(0)}=\frac{\langle U^*(t)U(t+\tau)\rangle}{\langle U^*(t)U(t)\rangle}$ $0\le|g(\tau)|\le1$ Complex Degree of Temporal Coherence

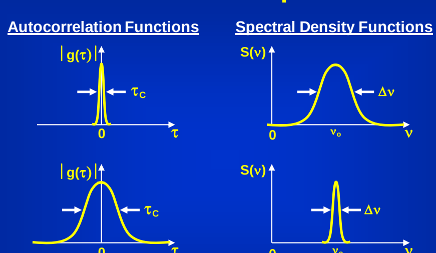
$\tau_c=\int_{-\infty}^{\infty}|g(\tau)|^2d\tau$ Coherence Time

$l_c=c\cdot\tau_c$ 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

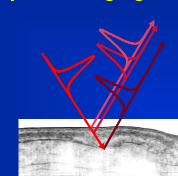
Relationships

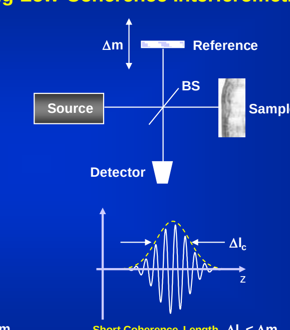


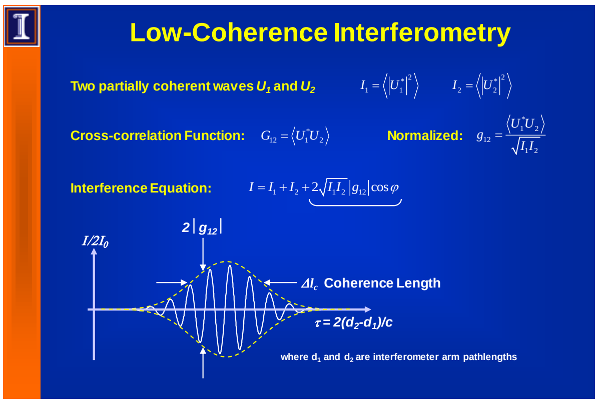
$S(\nu)=\int_{-\infty}^{\infty}G(\tau)\exp(-j2\pi\nu\tau)d\tau$

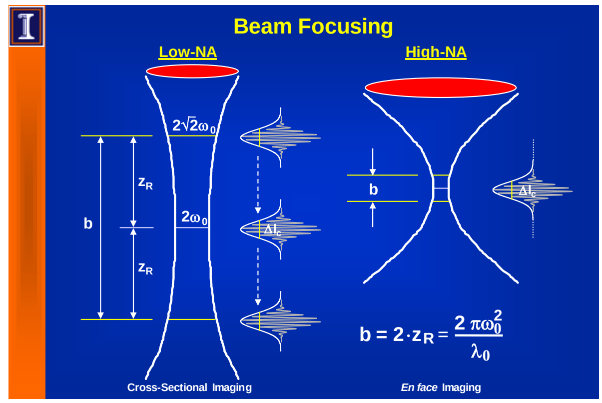
A Fourier-Transform Relationship

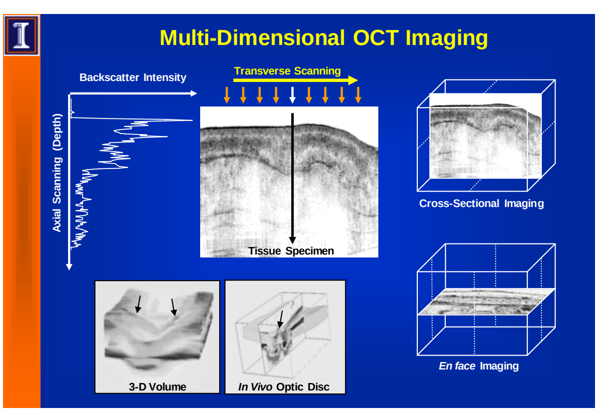
Optical Ranging using Low-Coherence Interferometry

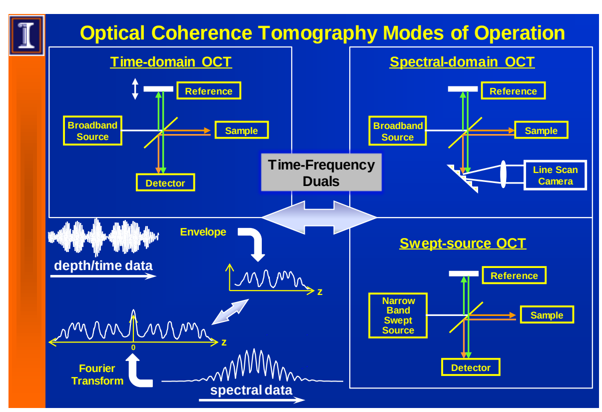





Low-Coherence Interferometry
Two partially coherent waves U_1 and U_2
$I_1 = \langle |U_1^*|^2 \rangle$ $I_2 = \langle |U_2^*|^2 \rangle$
Cross-correlation Function: $G_{12} = \langle U_1^* U_2 \rangle$
Normalized: $g_{12} = \frac{\langle U_1^* U_2 \rangle}{\sqrt{I_1 I_2}}$
Interference Equation: $I = I_1 + I_2 + 2\sqrt{I_1 I_2}\,|g_{12}|\cos\varphi$
$I/2I_0$
$2|g_{12}|$
Δl_c Coherence Length
$\tau = 2(d_2-d_1)/c$
where d_1 and d_2 are interferometer arm pathlengths

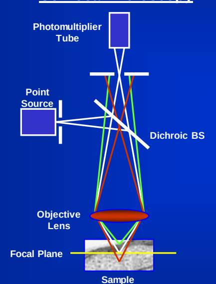

Beam Focusing
Low-NA
High-NA
$2\sqrt{2}\omega_0$
z_R
$2\omega_0$
z_R
b
Δl_c
b
Δl_c
$b = 2 \cdot z_R = \frac{2\pi\omega_0^2}{\lambda_0}$
Cross-Sectional Imaging
En face Imaging


Multi-Dimensional OCT Imaging
Backscatter Intensity
Transverse Scanning
Axial Scanning (Depth)
Tissue Specimen
Cross-Sectional Imaging
3-D Volume
In Vivo Optic Disc
En face Imaging


Optical Coherence Tomography Modes of Operation
Time-domain OCT
Reference
Broadband Source
Sample
Detector
Spectral-domain OCT
Reference
Broadband Source
Sample
Line Scan Camera
Time-Frequency Duals
Envelope
depth/time data
z
z
0
Fourier Transform
spectral data
Swept-source OCT
Reference
Narrow Band Swept Source
Sample
Detector

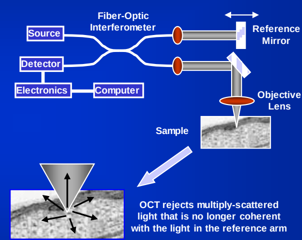
Comparison with Confocal Microscopy

Confocal Microscopy



Spatial Rejection of Out-of-Plane Light

Optical Coherence Tomography

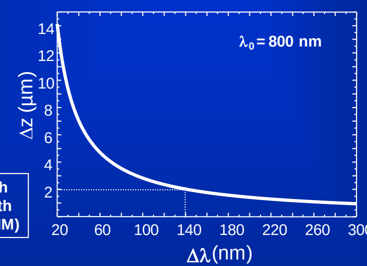


Spatial and Coherent Rejection of Out-of-Plane Light

Optical Bandwidth - Axial Resolution

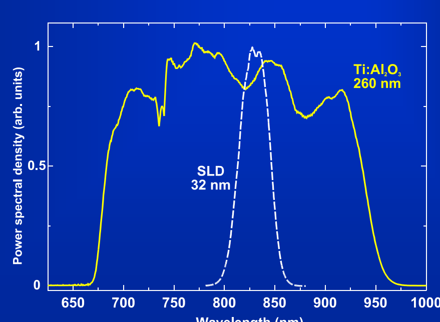
$$\Delta z = \frac{2\ln(2)}{\pi}\frac{\lambda_o^2}{\Delta\lambda}$$

Δz = Coherence length
λ_o = Center wavelength
$\Delta\lambda$ = Bandwidth (FWHM)

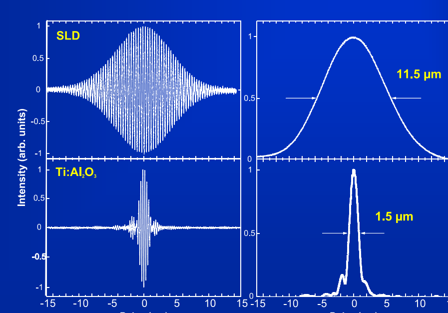


Assumptions: Gaussian spectrum, non-dispersive medium

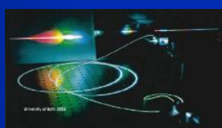
Ultrahigh Resolution OCT System

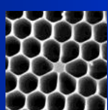


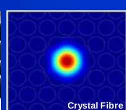
Ultrahigh Resolution OCT System



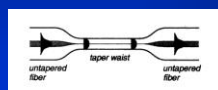
Supercontinuum Generation via Nonlinear Spectral Broadening in Fiber

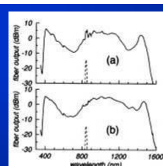






Ranka, *et al*, Optics Letters, 25, 2000





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

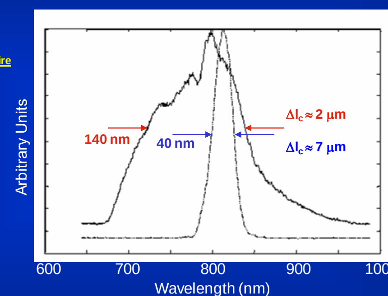
Spectral Broadening - UHNA Fiber

Pump: Titanium:sapphire

$\lambda = 810 \pm 20$ nm
500 mW avg power
$\Delta l_c \approx 7\ \mu m$

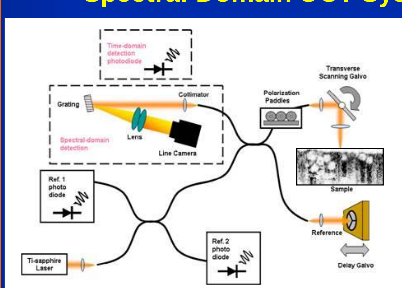
Output: Broadening

$\lambda = 780 \pm 70$ nm
100 mW avg power
$\Delta l_c \approx 2\ \mu m$



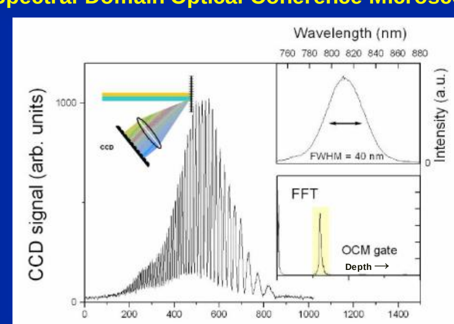
D.L. Marks, *et al*, Optics Letters, 27, 2002

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



System Parameters
Broadband Source
λ_o = 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

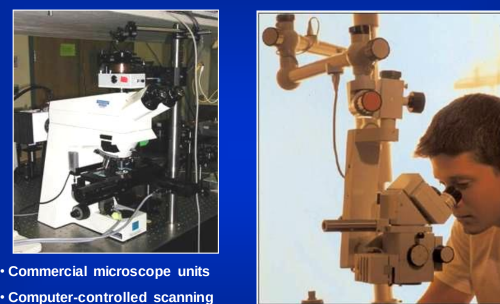
Spectral-Domain Optical Coherence Microscopy



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

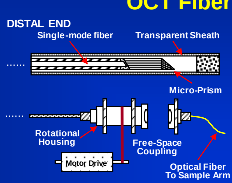
Portable OCT System & Beam Delivery Instruments

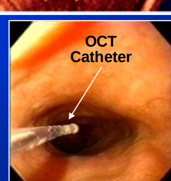
Research and Surgical OCT Microscopes



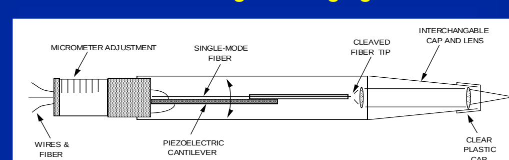
- Commercial microscope units
- Computer-controlled scanning
- Simultaneous OCT/OCM, MPM, CCD

OCT Fiber-Optic Catheter

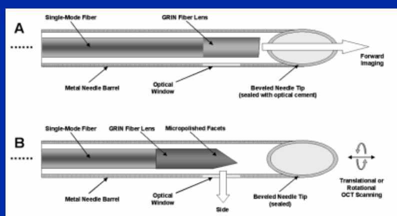


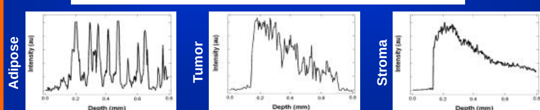


Hand-Held Surgical Imaging Probe



LCI (Axial-Scanning) Needle Probe

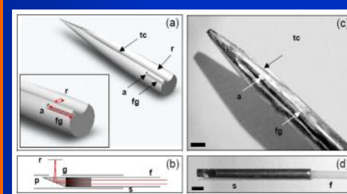


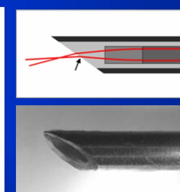


Refractive Index Needle Probes

Fixed Reflector

Reflection Refractometry

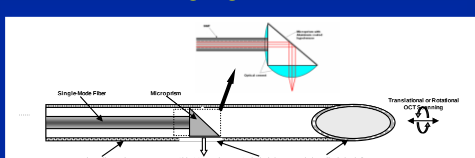


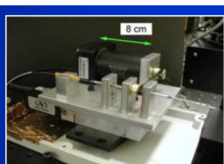


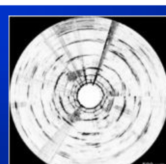
Zysk, *et al.*, Optics Letters 32:385, 2007

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

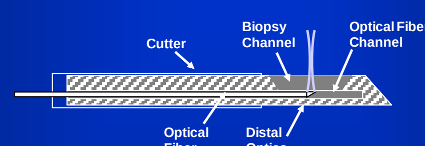
OCT Imaging Needle Probe





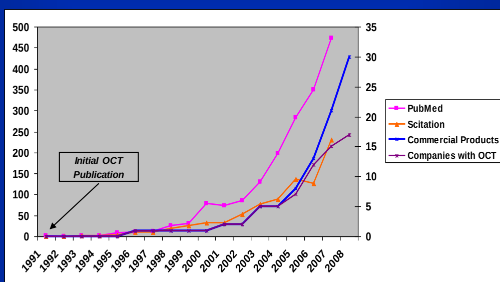


OCT Biopsy Needle



Optical Coherence Tomography

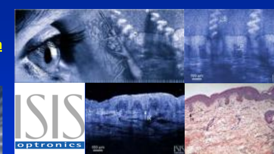
Rapid Development and Application



Zysk AM, Nguyen FT, Oldenburg AL, Marks DL, Boppart SA, J Biomed Optics, 12:051403, 2007

LightLab™

OCT Commercialization



IMALUX™

OCT Parameters

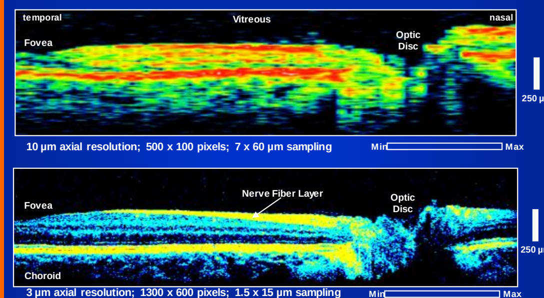
- Non-contact, non-invasive imaging using near-infrared light (800-1500 nm wavelength)
- High-resolution (1-10 μm axial, 1-30 μm transverse)
- High-speed acquisition (4-32 frames per second)
- Deep imaging penetration (2-3 mm) in highly-scattering tissue
- High sensitivity (110 dB signal-to-noise)
- 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)

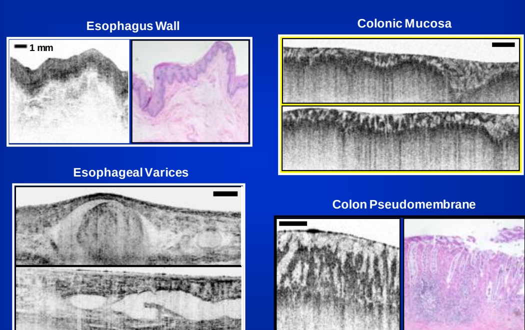
MORPHOLOGICAL IMAGING

Normal vs. Ultrahigh-Resolution OCT of the Papillomacular Axis

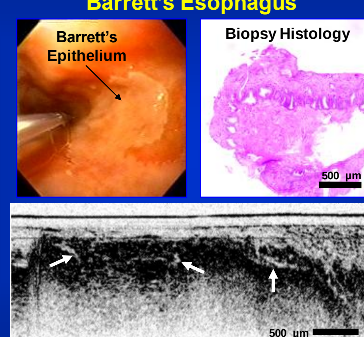


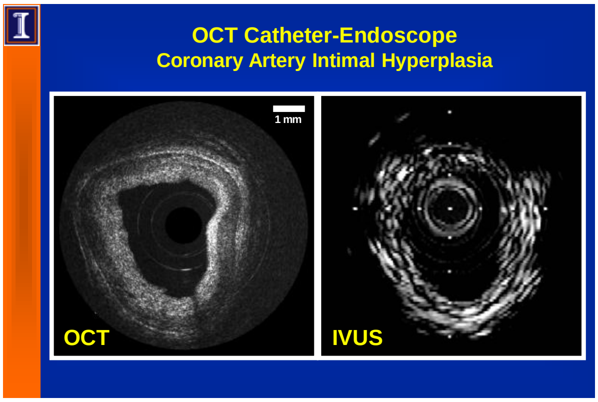
Drexler, *et al.* Nature Medicine 7:502-507, 2001

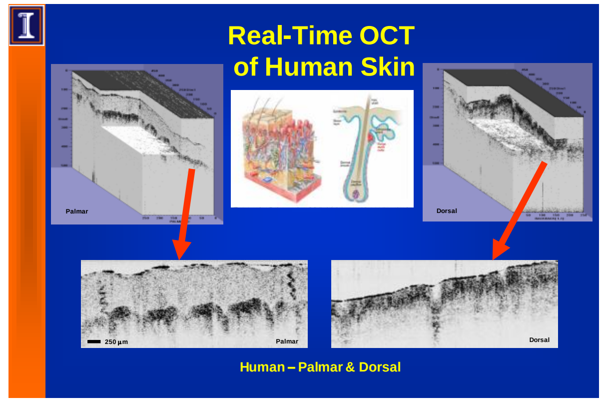
OCT of Human Gastrointestinal Tract Tissues

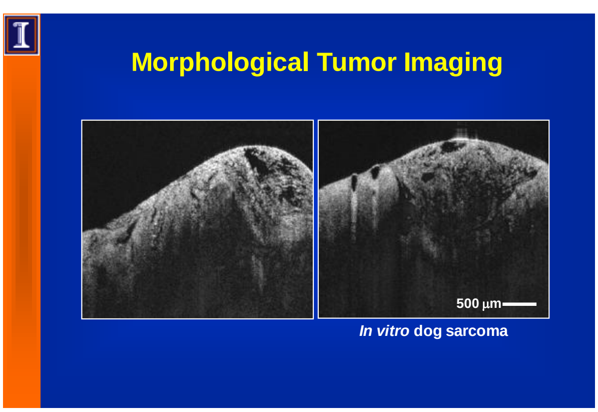


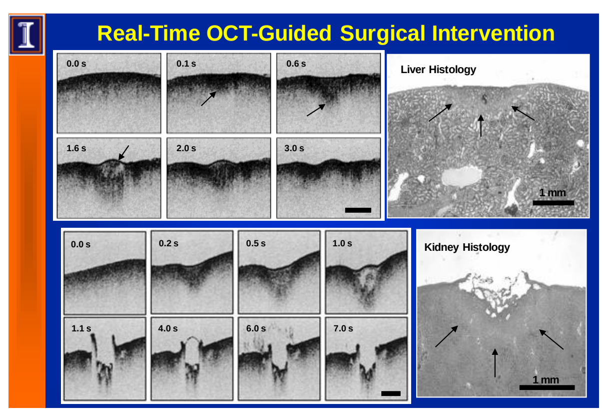
Linear-Scanning OCT Catheter
Barrett's Esophagus




OCT Catheter-Endoscope
Coronary Artery Intimal Hyperplasia
1 mm
OCT
IVUS

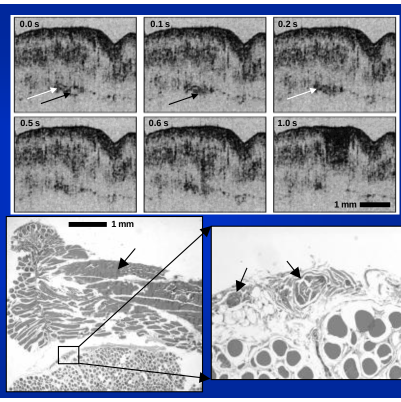

Real-Time OCT
of Human Skin
Palmar
Dorsal
250 µm
Palmar
Dorsal
Human – Palmar & Dorsal


Morphological Tumor Imaging
500 µm
In vitro dog sarcoma


Real-Time OCT-Guided Surgical Intervention
0.0 s
0.1 s
0.6 s
1.6 s
2.0 s
3.0 s
Liver Histology
1 mm
0.0 s
0.2 s
0.5 s
1.0 s
1.1 s
4.0 s
6.0 s
7.0 s
Kidney Histology
1 mm

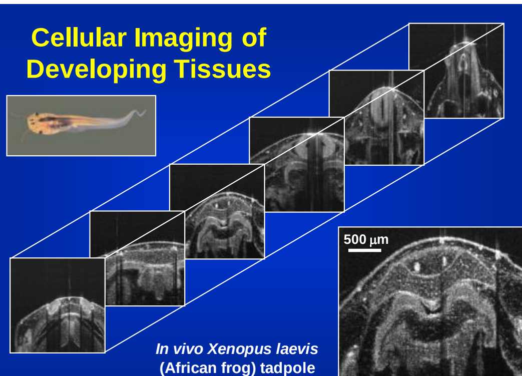
OCT Image Guidance for Selected Vessel Ablation

In vitro rat skeletal muscle



CELLULAR IMAGING

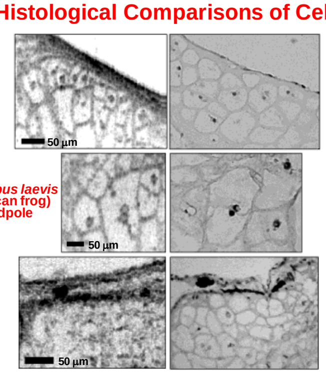
Cellular Imaging of Developing Tissues



In vivo Xenopus laevis (African frog) tadpole

Histological Comparisons of Cellular Imaging

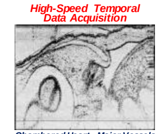
Xenopus laevis (African frog) tadpole



Three-Dimensional Spatial Data Acquisition

Respiratory Tract - Gills

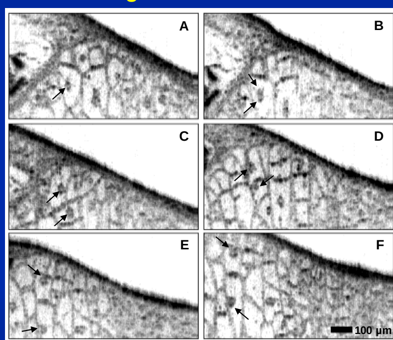
High-Speed Temporal Data Acquisition



Chambered Heart - Major Vessels

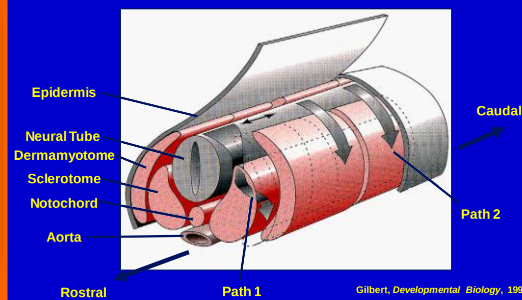
Boppart, *et al.*, Nature Medicine, 4:861, 1998

Tracking Mitosis in Real-Time



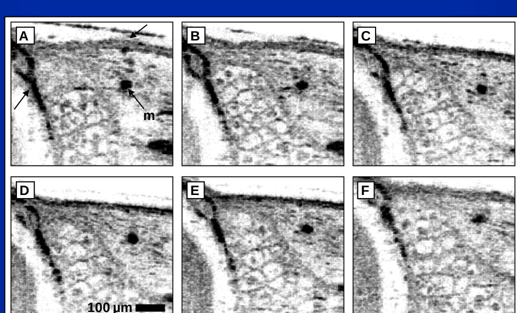
Boppart, *et al.*, Nature Medicine, 4:861, 1998

Neural Crest Cell Migration



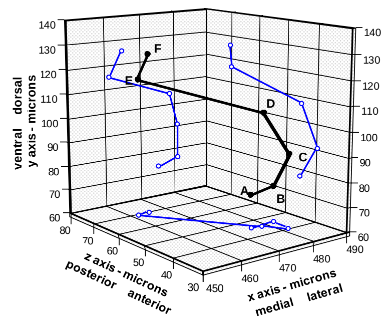
Gilbert, *Developmental Biology*, 1994

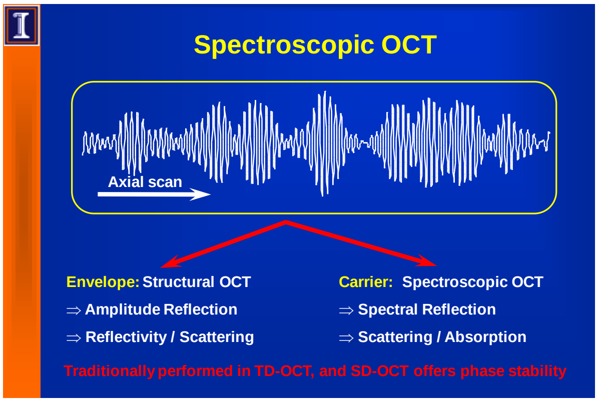
Tracking Cell Migration in Real-Time

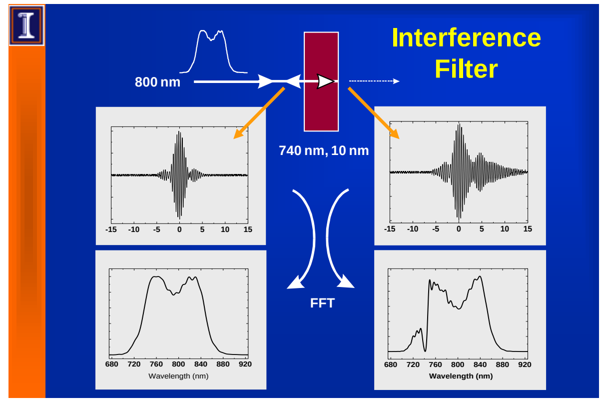


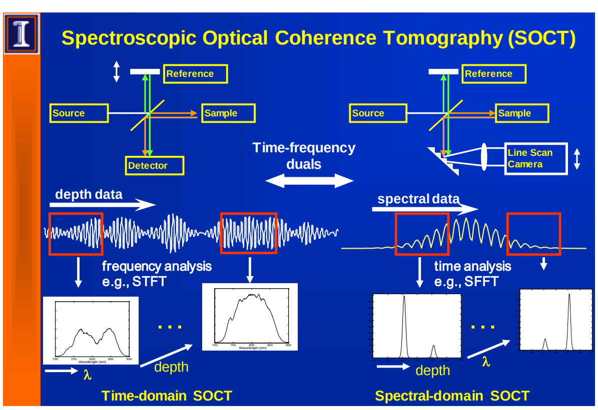
Boppart, *et al.*, Nature Medicine, 4:861, 1998

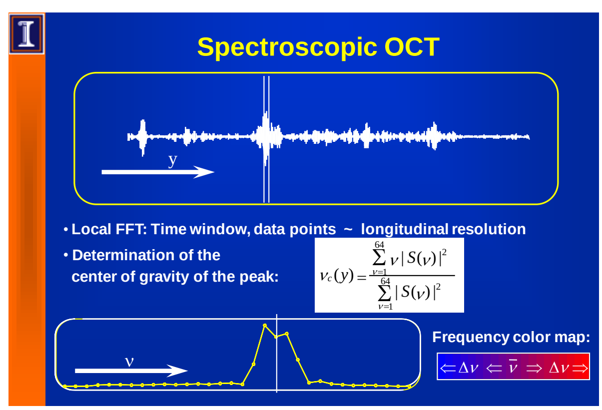
Three-Dimensional Tracking of Melanocytes

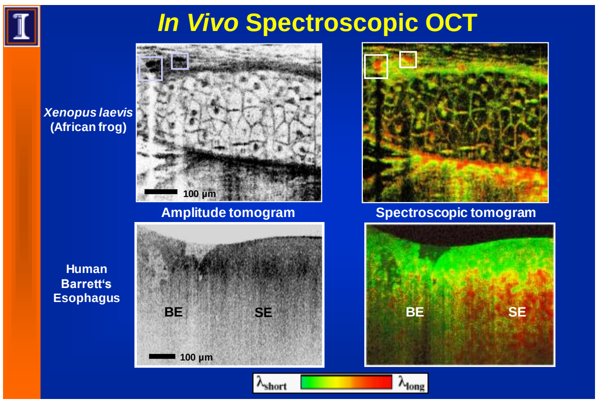


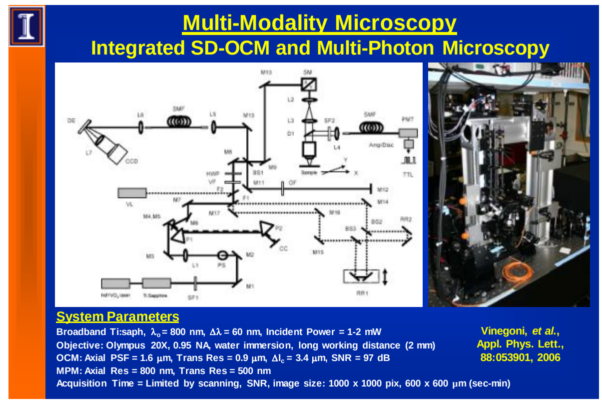

Spectroscopic OCT
Axial scan
Envelope: Structural OCT
⇒ Amplitude Reflection
⇒ Reflectivity / Scattering
Carrier: Spectroscopic OCT
⇒ Spectral Reflection
⇒ Scattering / Absorption
Traditionally performed in TD-OCT, and SD-OCT offers phase stability

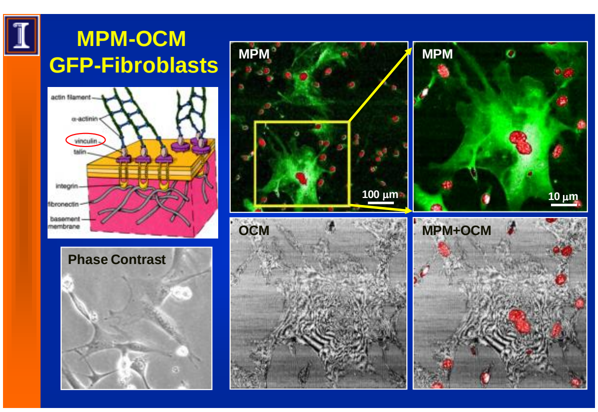

Interference Filter
800 nm
740 nm, 10 nm
FFT
-15 -10 -5 0 5 10 15
680 720 760 800 840 880 920
Wavelength (nm)

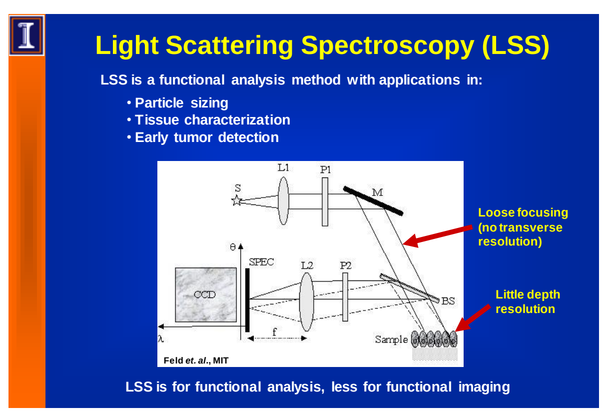

Spectroscopic Optical Coherence Tomography (SOCT)
Reference
Source
Sample
Detector
Time-frequency duals
Line Scan Camera
depth data
spectral data
frequency analysis e.g., STFT
time analysis e.g., SFFT
depth
λ
Time-domain SOCT
Spectral-domain SOCT


Spectroscopic OCT
y
• Local FFT: Time window, data points ~ longitudinal resolution
• Determination of the center of gravity of the peak:
$\nu_c(y) = \frac{\sum_{\nu=1}^{64} \nu |S(\nu)|^2}{\sum_{\nu=1}^{64} |S(\nu)|^2}$
ν
Frequency color map:
⇐Δν ⇐ $\bar{\nu}$ ⇒ Δν⇒


In Vivo Spectroscopic OCT
Xenopus laevis (African frog)
100 µm
Amplitude tomogram
Spectroscopic tomogram
Human Barrett's Esophagus
BE
SE
100 µm
BE
SE
λshort
λlong

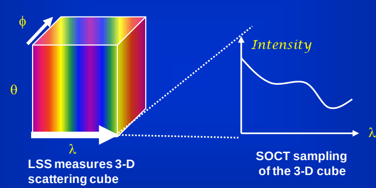

Multi-Modality Microscopy
Integrated SD-OCM and Multi-Photon Microscopy
System Parameters
Broadband Ti:saph, λo = 800 nm, Δλ = 60 nm, Incident Power = 1-2 mW
Objective: Olympus 20X, 0.95 NA, water immersion, long working distance (2 mm)
OCM: Axial PSF = 1.6 µm, Trans Res = 0.9 µm, Δlc = 3.4 µm, SNR = 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
actin filament
α-actinin
vinculin
talin
integrin
fibronectin
basement membrane
Phase Contrast
MPM
100 µm
MPM
10 µm
OCM
MPM+OCM


Light Scattering Spectroscopy (LSS)
LSS is a functional analysis method with applications in:
• Particle sizing
• Tissue characterization
• Early tumor detection
L1
P1
S
M
θ
SPEC
L2
P2
CCD
BS
λ
f
Sample
Feld et. al., MIT
Loose focusing (no transverse resolution)
Little depth resolution
LSS is for functional analysis, less for functional imaging

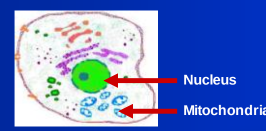
Spectral-Scatttering for OCT

Combine LSS and OCT to get:
- Spatial resolution
- Functional analysis

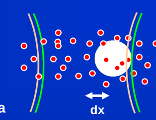


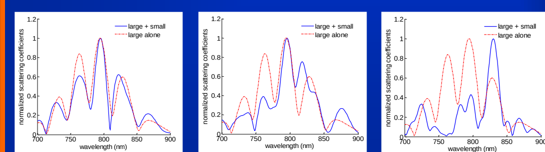
SOCT only detects wavelength dependent back-scattering, but in a spatially-resolved way, with deeper penetration.

Heterogeneous Scatterers



Typical cell

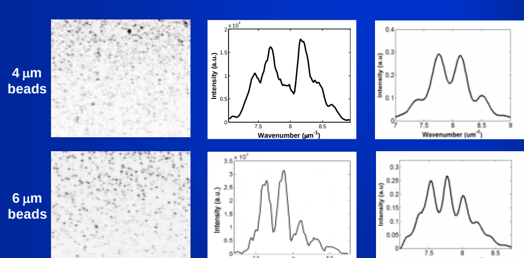




dx = 0 (centered) | dx = w_0 (off-centered) | dx = $2w_0$ (very off-centered)

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

Size Determination of Microspheres



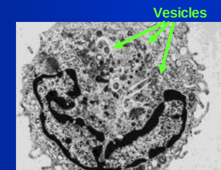
OCT image | SOCT measurement of spectral scattering | Mie theory calculation of spectral scattering

Can be potentially used for measuring the sizes of biological scatterers

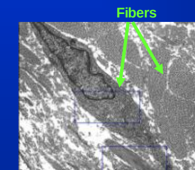
Complications with Cells

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)

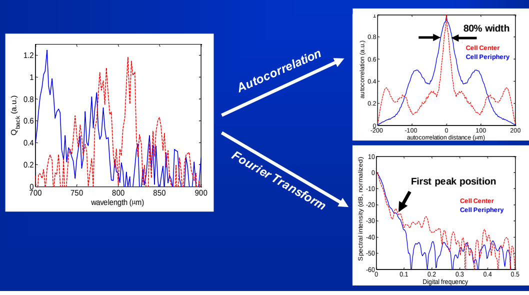


Fiberoblast (Biodic)

Cells contain many scatterers of different sizes and shapes.

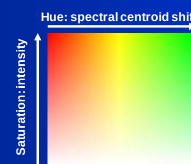
Spectral Analysis of SOCT Data from Cells

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

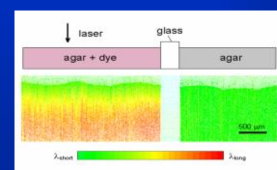


SOCT Hyper-Spectral Display

- 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

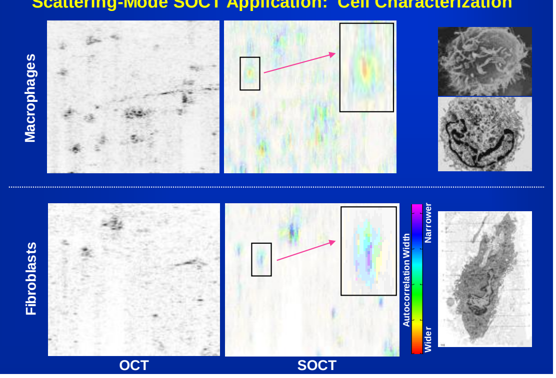


Hue-saturation color space



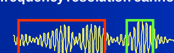
SOCT of dye phantom

Scattering-Mode SOCT Application: Cell Characterization

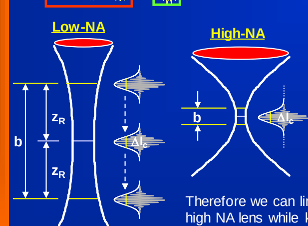


Time-Frequency Resolution With OCM

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



Longer spatial window { Spatial resolution - worse; Spectral resolution - better }



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

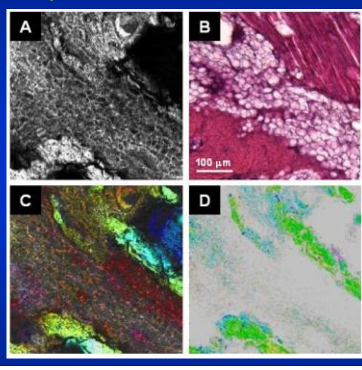
B) H&E Histology

C) Metameric SOCM

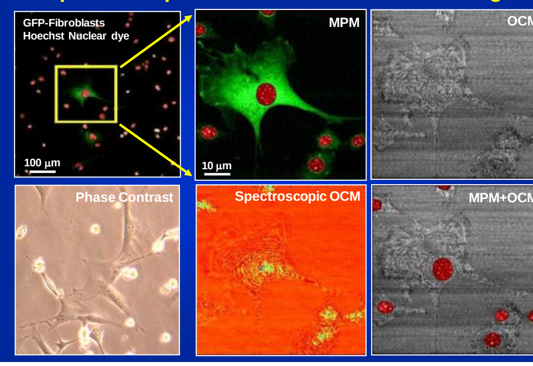
D) LSS SOCM

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 <u>molecular</u> 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

Leading Sites of New Cancer Cases and Deaths – 2009 Estimates

Estimated New Cases*		Estimated Deaths	
Male	Female	Male	Female
Prostate 192,280 (25%)	Breast 192,370 (27%)	Lung & bronchus 88,900 (30%)	Lung & bronchus 70,490 (26%)
Lung & bronchus 116,090 (15%)	Lung & bronchus 103,350 (14%)	Prostate 27,360 (9%)	Breast 40,170 (15%)
Colon & rectum 75,590 (10%)	Colon & rectum 71,380 (10%)	Colon & rectum 25,240 (9%)	Colon & rectum 24,680 (9%)
Urinary bladder 52,810 (7%)	Uterine corpus 42,160 (6%)	Pancreas 18,030 (6%)	Pancreas 17,210 (6%)
Melanoma of the skin 39,080 (5%)	Non-Hodgkin lymphoma 29,990 (4%)	Leukemia 12,590 (4%)	Ovary 14,600 (5%)
Non-Hodgkin lymphoma 35,990 (5%)	Melanoma of the skin 29,640 (4%)	Liver & intrahepatic bile duct 12,090 (4%)	Non-Hodgkin lymphoma 9,670 (4%)
Kidney & renal pelvis 35,430 (5%)	Thyroid 27,200 (4%)	Esophagus 11,490 (4%)	Leukemia 9,280 (3%)
Leukemia 25,630 (3%)	Kidney & renal pelvis 22,330 (3%)	Urinary bladder 10,180 (3%)	Uterine corpus 7,780 (3%)
Oral cavity & pharynx 25,240 (3%)	Ovary 21,550 (3%)	Non-Hodgkin lymphoma 9,830 (3%)	Liver & intrahepatic bile duct 6,070 (2%)
Pancreas 21,050 (3%)	Pancreas 21,420 (3%)	Kidney & renal pelvis 8,160 (3%)	Brain & other nervous system 5,590 (2%)
All sites 766,130 (100%)	All sites 713,220 (100%)	All sites 292,540 (100%)	All sites 269,800 (100%)

*Excludes basal and squamous cell skin cancers and in situ carcinoma except urinary bladder.

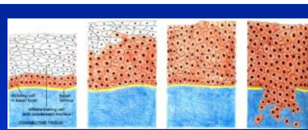
Cancer Progression

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

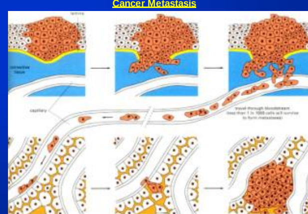
Gold standard:
Biopsy with histology

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



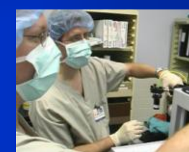
Normal Dysplasia Carcinoma *in situ* Malignant CA

Cancer Metastasis



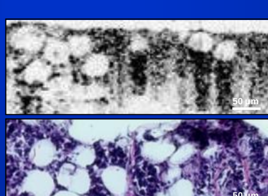
Alberts, et al., Molecular Biology of the Cell

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

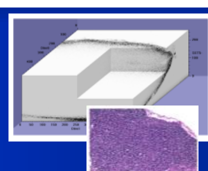


Change the Paradigm for Diagnosis

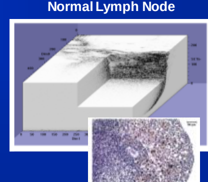
Comparison of Human Breast Tissue and Lymph Nodes with Corresponding Histology



Invasive Ductal Carcinoma



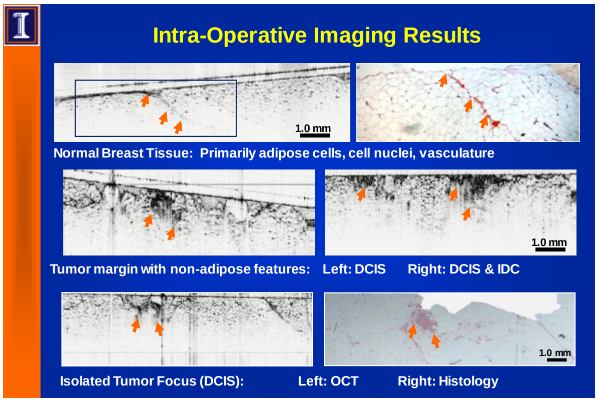
Normal Lymph Node

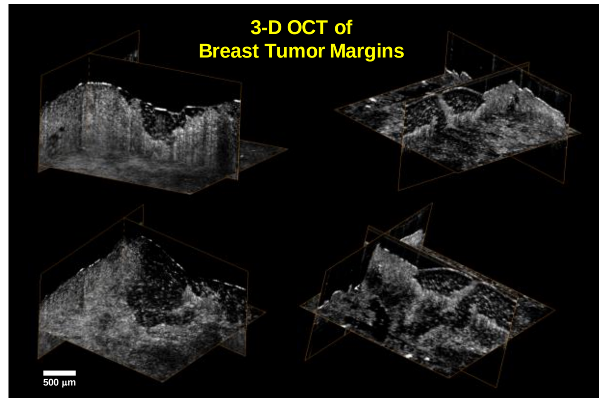


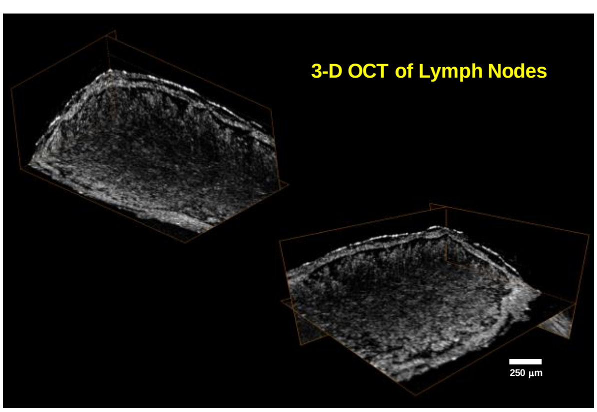
Lymph Node with Metastases

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

Translational Imaging Research Protocol







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 detection, guidance, and diagnosis of cancer.
  - *Image-guided biopsy and surgery*
  - *Intraoperative tumor & margin assessment*
  - *Intraoperative staging*

Outline

Lecture 1 (today)

- Optical Coherence Tomography (OCT)
- Beam Delivery Instruments
- Morphological & Cellular OCT Imaging
- Spectroscopic OCT
- Application to Cancer Imaging

Lecture 2 (next week)

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

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Leon Liang, M.S.
Cac Nguyen
Freddy Nguyen
Sunghwan Shin

Research Scientists
Steven Adie, Ph.D.
Joe Geddes, Ph.D.
Zhi Jiang, Ph.D.
Renu John, Ph.D.
Woonggyu Jung, Ph.D.
Marina Marjanovic, Ph.D.
Utkarsh Sharma, Ph.D.
Haohua Tu, Ph.D.

Research Specialists
Eric Chaney, Darold Spillman

Clinical Collaborators:

Medical Oncology:	Kendrith Rowland, M.D.	Patricia Johnson, M.D., Ph.D.
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