University of Illinois at Urbana-Champaign
Summer School on NanoBioPhotonics

Scattering, Absorbing, and Modulating Nanoprobes for Targeted Imaging and Therapy

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Outline

Lecture 1 (last week)
- Optical Coherence Tomography (OCT)
- Beam Delivery Instruments
- Morphological & Cellular OCT Imaging
- Spectroscopic OCT
- Application to Cancer Imaging

Lecture 2 (today)
- Molecular OCT Imaging
- Contrast Agents for OCT
   - Scattering, Absorbing, Modulating Probes

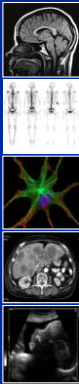## Combine Optics with Multifunctional Agents

Can leverage the portability and multi-dimensional detection of optics with increasingly powerful and versatile agents for point-of-care detection, imaging, and diagnosis?

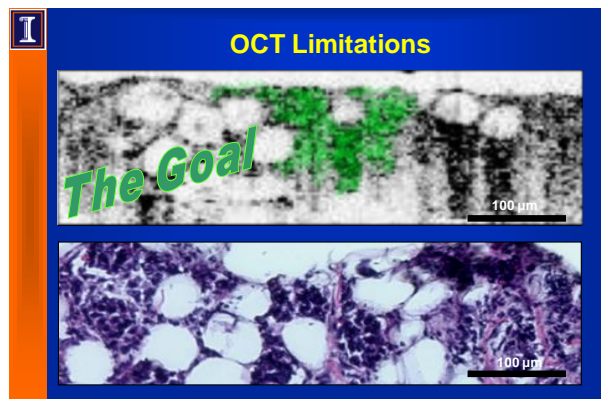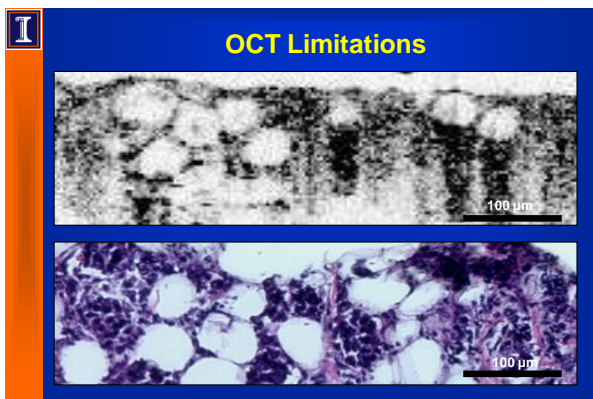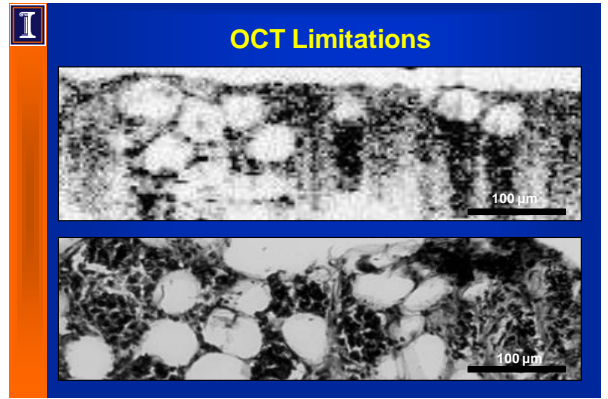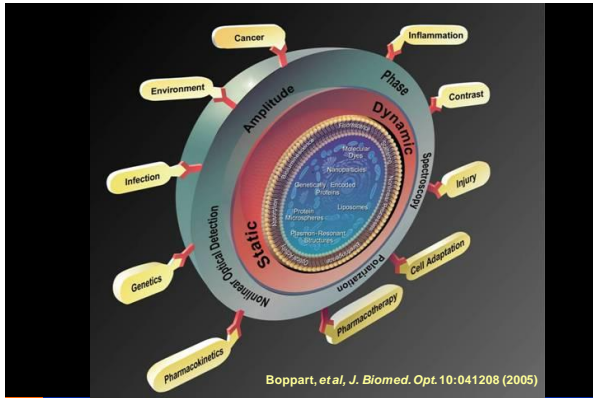|	
Spectroscopic detection	Imaging
Monitoring	Targeting
Drug delivery	Therapy

Halas group   *Nie group*

Contrast in Imaging Modalities

- Magnetic Resonance
   - T_1 and T_2 differences
   - Gadolinium, SPIOs

- Nuclear Medicine
   - Radioactive isotopes
   - FDG in PET

- Optical Imaging
   - Bioluminescence
   - Fluorescence
   - Raman Shifts
   - Scattering / Absorption

- X-Ray Computed Tomography (CT)
   - Differential attenuation
   - Iodinated compounds

- Ultrasound
   - Acoustic impedance
   - Micro-bubbles

Boppart, *et al, J. Biomed. Opt.* 10:041208 (2005)

OCT Limitations

100 µm

100 µm

OCT Limitations

100 µm

100 µm

OCT Limitations

The Goal
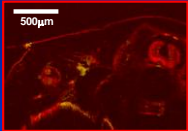
100 µm

100 µm

Slide 1

Contrast Methods in OCT

Functional (Physiological) OCT
- Doppler (blood flow)
- Standard deviation imaging & speckle contrast (movement)
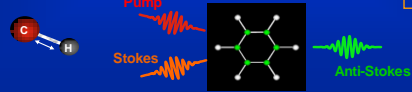- Polarization-sensitive OCT (birefringence)

Endogenous Contrast in OCT
- Spectroscopic OCT (melanin, Hb, HbO_2)
- Second-harmonic generation OCT (collagen, ultrastructure)
- Nonlinear interferometric vibrational imaging (molecular bonds)

500µm

Speckle contrast OCT image of a Xenopus laevis tadpole

Noise advantage to techniques with no background light

Pump
Stokes
Anti-Stokes
C — H

Slide 2

Contrast Methods in OCT

Imaging Exogenous Probes
- OCT does NOT sense fluorescence

Probe Engineering Issues
- Synthesis methods
- Size (scattering ↔ tissue transport)
- Specificity of targeting
- Biocompatibility → minimum detectible concentration < toxic concentration

Detection Engineering Issues
- Background discrimination (*a priori* information needed?)
- Sensitivity

Magnetomotive OCT of Iron-Oxide Nanoparticles

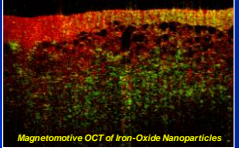**Synergistic Approach**

Detection ⟷ *Synthesis*

Adapt imaging methods for specific probes

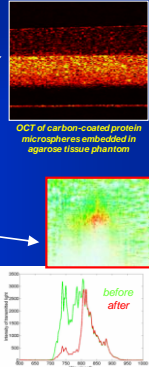Adapt contrast agents for imaging

Slide 3

Probe Detection Techniques in OCT

Exogenous Probe Detection Techniques
- Scattering
  - Protein microspheres (Barton, *today*)
  - Plasmon-resonant nanoshells (Drezek)
- Absorption
  - Plasmon-resonant nanocages (Xia)
  - Plasmon-resonant nanorods (*today*)
- Spectroscopic absorption
  - NIR-absorbing dyes (*today*)
- Transient absorption
  - methylene blue, ICG (Izatt)
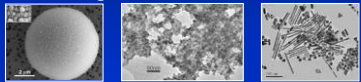- Magnetomotive
  - magnetite nanoparticles (*today*)

Require *a priori* information about sample

OCT of carbon-coated protein microspheres embedded in agarose tissue phantom

before
after

SOCT image of a celery stalk with NIR dye in center (top). Laser spectrum before and after passage through absorbing dye (bottom).
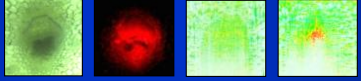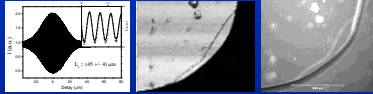
Slide 4

OCT Molecular Contrast Enhancing Techniques

Exogenous

Endogenous

- OCT Contrast Agents:
- Spectroscopic OCT:
- Nonlinear Interferometric Vibrational Imaging (NIVI):

OCT Contrast Agents: Overview

- **Scattering agents**
  - protein microspheres*
  - liposomes

- **Magnetic agents**
  - microsphere-encapsulated ferrofluids
  - nanoparticles* & ferrofluids

- **Spectroscopic agents**
  - NIR absorbing dyes*
  - microsphere encapsulated dyes
  - carbon nanotubes

- **Plasmon-resonant agents**
  - gold* nanorods

*FDA-type analogues

Protein Microsphere Structure

Iron-Oxide Nanoparticles

Surface

Protein Shell (BSA)

Oil Core (Vegetable Oil)

500 nm

1.5 μm

Synthesis of Protein Microspheres

- Titanium Horn
- Stainless Steel Collar
- Gas Inlet /Outlet
- Non Aqueous Liquid
- 5% W/V Bovine Serum Albumin

Sphere size distribution via Coulter counter

Sonication power: 7 W
Sonication time: 3 min
Cell temp: 45-65 °C
Average size 1.4 μm
Yield : 2.6 x 10⁶ μspheres/μL

➢ Role of High-Intensity Ultrasound (20 kHz)
  - Formation of micro-emultion
  - Chemical cross-linking with superoxide from water sonolysis
  - Control microsphere size (50 nm - 15 μm)

SEMs of iron-oxide nanoparticle colloid in core or in embedded monolayer in albumin microsphere shell
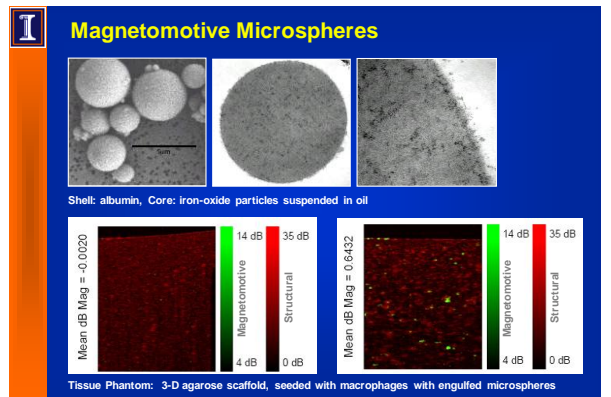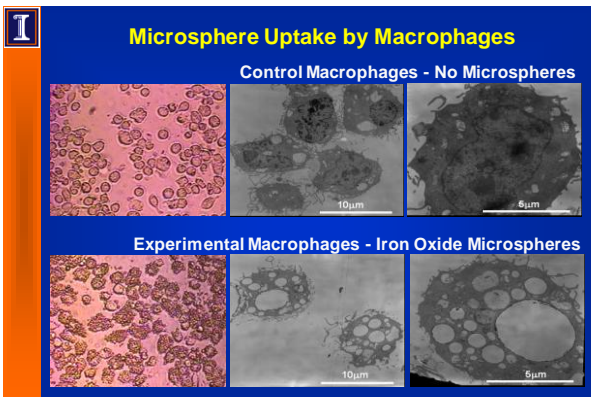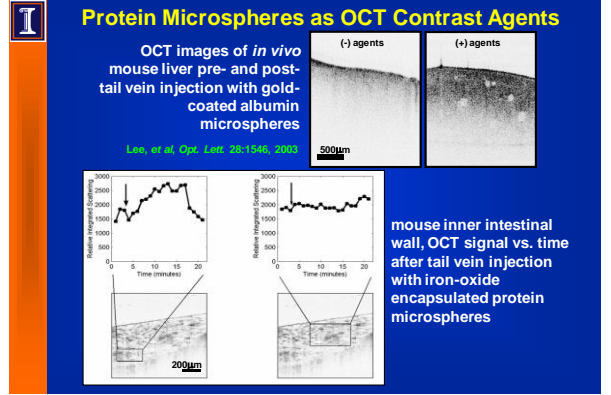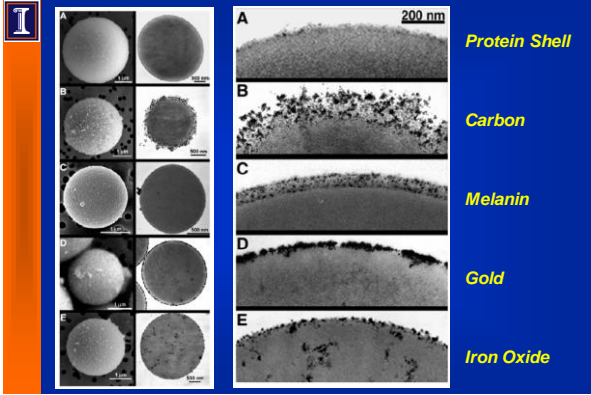

1μm

0.25 μm

OCT Microsphere Contrast Agents

- Nanoparticles embedded in shell or encapsulated
- Synthesis via high-intensity ultrasound
- Diameters typically ~ 2μm
- Biocompatible

Shell: albumin, Core: iron-oxide particles suspended in oil

Engineered Microsphere Combinations

Protein Shells	Inner Cores	Surface Modifications
Albumin	Air, O₂, N₂, Ar	PEG
Hemoglobin	Vegetable oils	Fluorescein
Pepsin	Water	Iron oxide colloid
Immunoglobulins	Organic liquids	Immunogobulins
Lipase	Acetoacetate	Folate
Peroxidases	Fluorocarbons	Gd complexes
Modified Myoglobin	Iodinated agents	Monoclonal Antibodies
	Gd complexes	Gold
	Ferrofluids	Carbon
		Melanin

Panels labeled A–E with scale bars; right column labeled: **Protein Shell**, **Carbon**, **Melanin**, **Gold**, **Iron Oxide**; 200 nm scale bar.

Protein Microspheres as OCT Contrast Agents

OCT images of *in vivo* mouse liver pre- and post-tail vein injection with gold-coated albumin microspheres

Lee, et al, Opt. Lett. 28:1546, 2003

(−) agents   (+) agents

500μm

mouse inner intestinal wall, OCT signal vs. time after tail vein injection with iron-oxide encapsulated protein microspheres

200μm

Microsphere Uptake by Macrophages

Control Macrophages - No Microspheres

10μm   5μm

Experimental Macrophages - Iron Oxide Microspheres

10μm   5μm

Magnetomotive Microspheres

Shell: albumin, Core: iron-oxide particles suspended in oil

Mean dB Mag = −0.0020

Mean dB Mag = 0.6432

14 dB   35 dB
Magnetomotive   Structural
4 dB   0 dB

Tissue Phantom: 3-D agarose scaffold, seeded with macrophages with engulfed microspheres

Integrin-Targeted Microspheres

RGD-Targeting of Integrin Receptors
Hetero-dimeric trans-membrane receptor
Cell attachment, survival, migration, proliferation, tumorgenesis, metastasis

Over 25 known integrin receptors
Most recognize small tri-peptide RGD sequence (arginine-glycine-aspartic acid)

Integrins overexpressed in tumor cells, angiogenesis, atherosclerosis $(\alpha_v\beta_3)$

Fluorescence microscopy of RGD peptides and Nile Red microspheres

HT29 Human Colon Tumor Cells

RGD Microspheres | HT29 Cells | HT29 Cells Unlabeled µspheres
HT29 Cells KKKRGDKKK - µspheres | HT29 Cells RGDKKKKKK - µspheres | HT29 Cells KKKKKKRGD - µspheres

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In Vivo Integrin-Targeted Microspheres
MNU-rat model, RGD-Nile Red Microspheres

In vivo fluorescence | In vivo fluorescence | Cryosectioned liver | Cryosectioned liver DAPI co-stain
Resected liver | Resected kidneys | HUVEC cells (+) | SKBR-3 cells (-)

Cell lines	RGD coated microspheres	Non-coated microspheres
HUVEC	+++	+
SKBR-3	+ / -	-
Macrophages	+	+ / -
Empty cover slip	+	+ / -

Background-Free Contrast Agent Imaging

Magnetically-susceptible agents:

- Human tissue not ferromagnetic
- Displacement or rotation induced by B
- Modulate optical scattering
- B switched on & off with successive depth scans – difference image

5µm

M-mode of magnetite particle in agarose with modulated B-field

transverse displacement | axial displacement | rotation of anisotropic agent

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Motivation: Targeted Imaging Contrast in OCT

Contrast agents figures of merit:
- NIR scattering or absorption
- Background discrimination
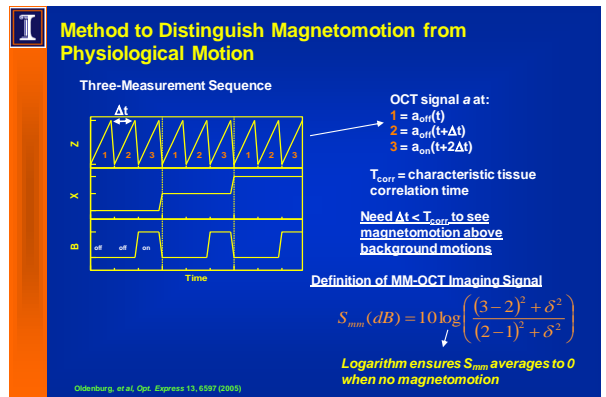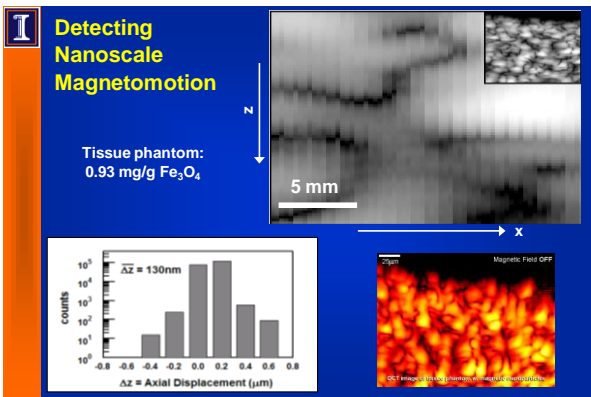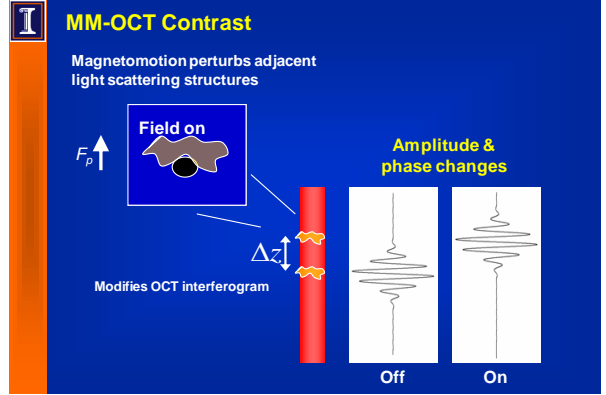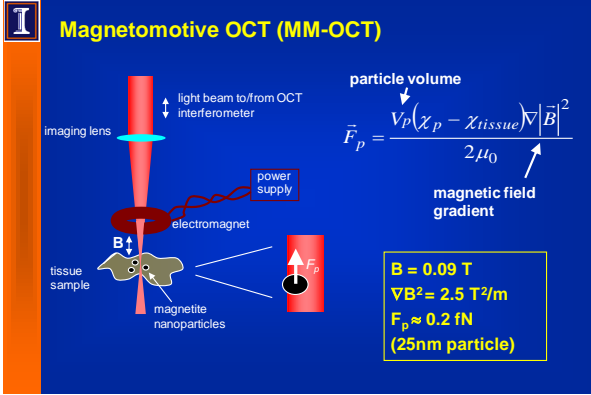- Biocompatibility
- Size < 200 nm
- Molecular specificity

Magnetic Contrast Agents
- Externally controllable!
- High magnetic susceptibility χ
- Overlap with MRI contrast agent technology

$$\chi_{Fe_3O_4} \sim 1$$
$$|\chi_{tissue}| < 10^{-5} \Rightarrow \text{Contrast of up to } 10^5\,!$$

Ocean Nanotech | Sigma-Aldrich

Carboxyl-terminated magnetite (Fe_3O_4) nanoparticles

50nm

Magnetomotive OCT (MM-OCT)

light beam to/from OCT interferometer

imaging lens

power supply

electromagnet

B

tissue sample

magnetite nanoparticles

F_p

particle volume

$$\vec{F}_p = \frac{V_P(\chi_p - \chi_{tissue})\nabla|\vec{B}|^2}{2\mu_0}$$

magnetic field gradient

B = 0.09 T
∇B² = 2.5 T²/m
F_p ≈ 0.2 fN
(25nm particle)

MM-OCT Contrast

Magnetomotion perturbs adjacent light scattering structures

F_p

Field on

Δz

Modifies OCT interferogram

Amplitude & phase changes

Off   **On**

Detecting Nanoscale Magnetomotion

Tissue phantom: 0.93 mg/g Fe₃O₄

Z

X

5 mm

$\overline{\Delta z}$ = 130nm

counts

10^5
10^4
10^3
10^2
10^1
10^0

-0.8 -0.6 -0.4 -0.2 0.0 0.2 0.4 0.6 0.8

Δz = Axial Displacement (μm)

Magnetic Field OFF

Method to Distinguish Magnetomotion from Physiological Motion

Three-Measurement Sequence

Z

X

B

Δt

off   off   on

Time

OCT signal a at:
1 = $a_{off}(t)$
2 = $a_{off}(t+\Delta t)$
3 = $a_{on}(t+2\Delta t)$

T_{corr} = characteristic tissue correlation time

Need $\Delta t < T_{corr}$ to see magnetomotion above background motions

Definition of MM-OCT Imaging Signal

$$S_{mm}(dB) = 10\log\left(\frac{(3-2)^2 + \delta^2}{(2-1)^2 + \delta^2}\right)$$

Logarithm ensures S_{mm} averages to 0 when no magnetomotion

Oldenburg, et al, Opt. Express 13, 6597 (2005)

Tissue Phantoms

Optically & mechanically simulate tissue
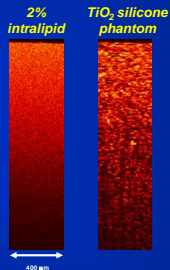
Optical:
TiO$_2$ microparticles 4 mg/g
($\approx$ 2% intralipid)

Mechanical:
silicone matrix w/ tailored elasticity
(10:1 PDMS + GE-RTV 615)

Magnetic:
Fe$_3$O$_4$ nanoparticles 20-30 nm
(Sigma-Aldrich #310069)

Answer questions:
- Dependence of MM-OCT signal on:
  - magnetic field strength B
  - magnetic particle concentration
  - optical scattering

- Sensitivity of MM-OCT

OCT images

2% intralipid   *TiO$_2$ silicone phantom*

400 µm

Predicted Scaling of MM-OCT

SQUID magnetometry

Sigma 20 nm magnetite

Field during MMOCT

Force induced by magnetic field:

$$\vec{F}_p = V_p \left(\vec{M} \bullet \nabla \right) \vec{B} \propto \nabla \left| \vec{B} \right|^2 \text{ (low field)}$$

$$\propto \nabla \left| \vec{B} \right| \text{ (saturated)}$$

Displacement in elastic medium:

$$\Delta z \propto \frac{\rho F_p}{E}$$ ← Elastic modulus

OCT signal change caused by displacement:
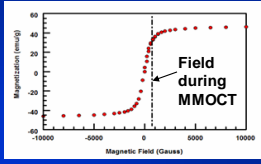
$$\Delta a_{mm} \propto s \left| \Delta z \right|$$

Speckle-averaged scattering

Predicted Scaling Law:
$$\Delta a_{mm} \propto s^{n_s=1} \rho^{n_\rho=1} B^{n_B=1 \text{ or } 2}$$

Detection sensitivity ~220 ppm
(Theoretical ~2 ppm)
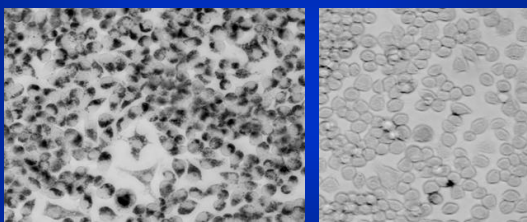
Oldenburg, et al., *Opt. Express* 13, 6597 (2005)

Magnetomotive OCT in Practice

Macrophages (*J774A.1*)

Ocean Nanotech
20 nm magnetite, 0.15 mg/mL overnight exposure   Control
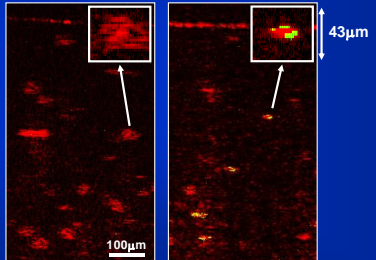
Trypan blue → no loss of viability (> 99%)

Cell Labeling with Magnetic Optical Contrast Agents

Macrophages (*J774A.1*) in three-dimensional agarose gel scaffold

■ **Structure**
■ **Magnetic**

microscopy of magnetite-exposed macrophages in scaffold

50µm

Control cells   **Cells with ~50% uptake of magnetite**

43µm

100µm

Oldenburg, *et al.*, Optics Letters, 30:747, 2005

MM-OCT in Practice

Macrophages suspended in 1.5% agarose scaffold

OCT Signal (dB) 0 — 20
MMOCT Signal (dB) 0 — 15

Magnetic | Control

100 µm

Oldenburg *et al, Opt. Lett.* **30**, 747 (2005)

In Vivo MM-OCT

Xenopus laevis tadpole

(-) AGENTS
(+) AGENTS

Prussian blue stain

Oldenburg, *et al*, *Optics Express*, 13:6597, 2005

a
(-) agents, dorsal
500 µm

b
(-) agents, ventral

c
(+) agents, dorsal

d
(+) agents, ventral

Structural OCT Signal (dB) 0 — 30
Magnetomotive OCT Signal (dB) 0 — 18

Magnetomotive OCT of *in vitro* chicken skin

No magnetic agents | Injected magnetic nanoparticles

Structural OCT
MM-OCT
Combined

Structural OCT Signal (dB) 0 — -30
Magnetomotive OCT Signal (dB) 20 — 0

200 µm

S. A. Boppart, A. L. Oldenburg, C. Xu, D. L. Marks, *J. Biomed. Opt.*, 10:041208, 2005

Magnetomotive OCT *In Vivo*

Imaging magnetic nanoparticles in a carcinogen-induced rat mammary tumor

OCT Signal
Magnetic Signal

Control tumor | Magnetite-injected tumor

100 µm

Magnetomotive OCT *In Vivo*

Delivery via tail-vein injection

Control Injected — Nanoparticle Injected

Structural OCT | Magnetomotive OCT | Structural OCT | Magnetomotive OCT

200 μm

Rat spleen distribution of magnetic nanoparticles euthanized 2 hours after tail vein injection.

Magnetomotive Optical Coherence Elastography

A-scan

amplitude — 100 μm

- Optical and mechanical properties of phantom similar to those of tissue
- Magnetic nanoparticles homogeneously dispersed in sample volume

Optical scatterers:
TiO_2 microparticles 4 mg/g (avg. size 1 μm)

Mechanical simulator:
silicone matrix with tailored elasticity (10:1 PDMS + GE-RTV 615)

Magnetic agents:
Fe_3O_4 nanoparticles 20-30 nm (Sigma-Aldrich #310069); mass concentration 2.5 mg/g

MM-OCE Transients

A-scan
amplitude — 100 μm

- f_{Bmod} = 6.67 Hz; duty cycle = 33%
- Camera line rate = 1 kHz, M-mode imaging

B — off / on — 0

M-mode amplitude image

Sample depth

B on | B off

Z — 0 | 100 | 200 | 300 | 400 | 500 | Time (ms)

vertical scale bar = 0.3 mm

Magnetomotive Optical Coherence Elastography

A-scan
amplitude — 100 μm

- evidence of underdamped oscillations in sample when magnetic impulse exerted:

B off ⇒ on

M-mode imaging ⟹ a(t), φ(t)

$$y(t) = Ae^{-\gamma t}\cos(\omega t - \delta) + C$$

- damping coefficient ⟺ viscosity
- oscillation frequency ⟺ elasticity

$\gamma = 82.86$ s^{-1}

$\omega = 450.9$ rad/s $\Rightarrow f = \dfrac{\omega}{2\pi} = 72$ Hz

B — off / on — 0
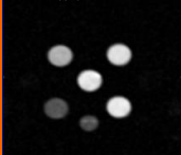
Magnetic Nanoparticle MRI Contrast
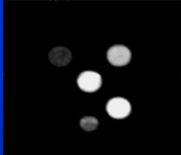
Negative T_2 contrast due to particle magnetization

→ **same figure of merit as in MM-OCT!**

MRI of agarose phantoms dosed with Ocean Nanotech 20 nm magnetite
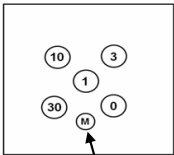
T_{echo} = 11 ms T_{echo} = 50 ms   Dose in ppm
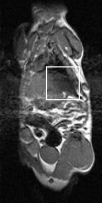
Marker

Spin-echo MRI, 4.7T Sisco, $T_{repetition}$ = 4s

Collaboration with B. Odintsov, Beckman Imaging Center
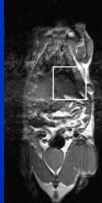
In Vivo Magnetic Nanoparticle MRI Contrast

MNU rat mammary tumor model, non-targeted agents

Negative T_2 contrast due to particle magnetization
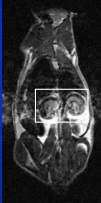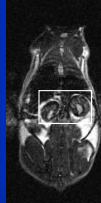
(-) agents   (+) agents   (-) agents   (+) agents

Spin-echo MRI, 4.7T Sisco, $T_{repetition}$ = 4s

Collaboration with B. Odintsov, Beckman Imaging Center

Protein Microspheres as an MM-OCT Contrast Agent

(-) Microspheres   (+) Microspheres

5% Agarose Gel
- (-) 0.074 dB (n = 11)
- (+) 1.130 dB (n = 11)
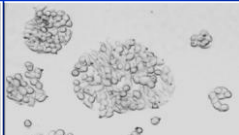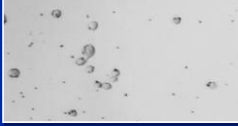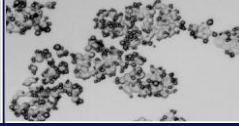
Macrophage Uptake
- (-) 0.018 dB (n = 11)
- (+) 0.411 dB (n = 11)

***In Vivo* Intra-Tumor Injection**
- (-) 0.002 dB (n = 21)
- (+) 0.598 dB (n = 21)

200 µm

Cell Studies
Targeting Specificity

Non-Targeted   RGD-Targeted

CRL - 4010 ($\alpha_v\beta_3$-)
Human Mammary Epithelium

HT - 29 ($\alpha_v\beta_3$+)
Human Colon Adenocarcinoma

Animal Studies (Atherosclerosis)

- **Control Rat: Normal Diet**
- **MM-OCT Results: Mean dB Mag = 0.027 dB (n=17)**

Animal Studies (Atherosclerosis)

- **Atherosclerotic Rat**
  - High Fat, High Cholesterol Diet
  - Euthanized at 10 Weeks on Diet

- **MM-OCT Results:**
  - Mean dB Mag - All Images = 0.329 dB (n=17)
  - Mean dB Mag - Images with Focal Magnetic Signal = 0.931 dB (n=5)
  - Mean dB Mag - Images with no MM Signal = 0.077 dB (n=12)

Animal Studies (Atherosclerosis)

MM-OCT   **H&E Histology**

Mag dB = 2.218 dB

Spectroscopic OCT

Envelope: Structural OCT

$\Rightarrow$ Amplitude Reflection

$\Rightarrow$ Reflectivity / Scattering

Carrier: Spectroscopic OCT

$\Rightarrow$ Spectral Reflection

$\Rightarrow$ Scattering / Absorption

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

Spectroscopic OCT Detection of Contrast Agents
Analyze spectrum of backscattered light in a depth-resolved way

Basic Idea:

1
2

Cuvette containing dyes absorbing shorter wavelength

laser
transmitted through NIR-absorbing dye

Intensity of transmitted light

Intensity (a.u.)

1
2

120 nm FWHM

Wavelength (nm)
600 700 800 900 1000

Dye Concentration Optimization

laser glass
agar + dye   agar

spectral centroid of SOCT (nm)

absorption(l/mmol·cm)
Laser spectrum
dye absorption
laser spectrum (a.u.)

American Dye Source, Inc.

peak absorption (1/mm)

- peak absorption
- spectral centroid by SOCT
- calculated spectral centroid

dye concentration (µg/ml)

Xu, *et al.*, Opt. Lett., 29:1647, 2004

Dye-Based Contrast Agents

Sensing absorption through Spectroscopic OCT (via centroid shift)

Celery cross-sectional images:

Control   **NIR-absorbing dye + rhodamine**

SOCT   **SOCT**   **Fluorescence Microscopy**   **Brightfield Microscopy**

250µm

0 nm $\Delta\lambda_c$ **+15 nm**

Vascular tubular system

Xu, *et al.*, Opt. Lett., 29:1647, 2004

Plasmon-Resonant Au Nanorods as OCT Contrast Agents

Tunable surface-plasmon (SP) modes in Au nanorods:

Link and El-Sayed,
J. Phys. Chem. B **1999**, *103*, 3073

50 nm

absorbance (a. u.)

aspect ratio R

wavelength λ / nm

Longitudinal SP
(I_{max})

Au

Transverse SP

Alex Wei, Purdue Univ.

Modified Mie calculations of longitudinal SP in Au nanorods as a function of aspect ratio:

van der Zande et al, *Langmuir* **2000**, *16*, 451

2.5 4 6 9 10 12.5

wavelength (nm)
400 800 1200 1600

Magnetic Plasmon-Resonant Gold Nanorods

Extinction Spectrum of GNRs

Ti:saph Laser Spectrum

Synthesis of IONP-GNR Hybrids Using EDC/Sulfo-NHS (APL1-142).

Magnetic Gold Nanorods

Oldenburg, *et al.*, J Mat Chem, 2009

H & E Histology

Two-Photon Luminescence

TEM

Asymmetric Single-Walled Carbon Nanotube/Iron Oxide Nanoparticle Complexes

Choi, *et al.*, Nanoletters, 7:861-867, 2007

Summary and Conclusions

- Optical scattering-based images (OCT) frequently exhibit poor inherent contrast

- Novel scattering, absorbing, and modulating contrast enhancing techniques have the potential to improve the diagnostic ability of these imaging techniques

- Significant potential for therapeutic applications

- *Exogenous Contrast Agents*
  - Scattering microspheres & liposomes
  - Magnetically-modulated agents
  - Absorbing chemical dyes
  - Plasmon-resonant nanorods

- *Endogenous Spectroscopic Detection*
  - Spectroscopic OCT (Absorption & Scattering)
  - Nonlinear Interferometric Vibrational Imaging (NIVI)
  - CARS, SHG, THG

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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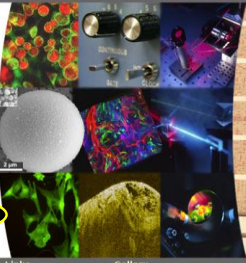

BIOPHOTONICS IMAGING LABORATORY

Home Technology Research Publications Presentations Academics Prof. Boppart News

Located in the Beckman Institute for Advanced Science and Technology at the University of Illinois at Urbana-Champaign, the Biophotonics Imaging Laboratory, directed by Professor Stephen Boppart, is dedicated to the development of optical biomedical imaging techniques.

Optical coherence tomography (OCT) is the core technology used within the lab. OCT is promising for the early detection and diagnosis of various pathologies and can also be used in many types of biological research.

biophotonics.illinois.edu

Personnel Links Gallery Facilities