Introduction to Biophotonics
Paras N. Prasad
(John Wiley & Sons, 2003)

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The Institute for Lasers, Photonics and Biophotonics

Mission

- Multidisciplinary Frontier Research in Lasers, Photonics and Biophotonics
  Federal, State and Industrial Support ($26 million)

- Education and Training
  NSF-funded Integrative Graduate Education and Research Training (IGERT)
  NSF-funded Research Experiences for Undergraduates (REU)

- Industrial Collaboration – Co-development, Industrial training, advanced testing

- Technology Transfer - 3 spin off companies (ACIS, Hybrid Technologies and NanoBiotix); Three patents licensed in 2004

- International collaboration – joint research, student exchange, joint workshop

OUR INTERNATIONAL COLLABORATION

- NSF-Funded US-EGYPT Collaboration
  Professor El-Mallawany
  Project: Tellurite Glass, Hybrid Materials

- NSF-SSF Jointly Funded US-SWEDEN Collaboration
  KTH, Stockholm
  Project: Multifunctional Materials/Biophotonics

- Ministry of Education, Japan funded JAPAN-US Collaboration
  Tohoku University, Sendai
  Project: Nanophotonics

- Australian Research Council Supported Research Network
  Macquarie University, Sydney
  Project: Nanomedicine

- DOD Window of Science Program US-JAPAN Pilot Project
  Chitose Institute of Science and Technology, Chitose
  Project: Biomaterials
**Optical Imaging**

**Techniques**
- Fluorescence Microscopy
- Raman Imaging (e.g. CARS)
- Interference Imaging (e.g. OCT)

**Tools**
- Confocal Microscopy (CSLM)
- Multi-photon Microscopy
- Nearfield Microscopy
- Optical Coherence Tomography
- Total Internal Reflection Imaging (TIR)

**Applications**
- Whole body imaging
- Drug distribution/ Interaction in cells, Organelles or tissue
- Bio-molecular (e.g. Proteins) activity and organization in cells
- Identification of Structural changes in cells, organelles, tissues etc.
Fluorescence Imaging Techniques

- Environmental changes inside cells
- Complements FRET technique

Molecular diffusion and Mobility measurements in living cells
( e.g. Protein mobility and interactions )

Fluorescence Life time imaging ( FLIM )
Fluorescence Recovery After Photobleaching ( FRAP )
Fluorescence Resonance Energy Transfer ( FRET )
Polarized Fluorescence Imaging :

Molecular interactions and conformational changes in living cells
( e.g. Protein interactions and conformational changes )

Intracellular Drug Distribution and Activation Monitoring using Confocal Microscopy

Mechanism of action of Topoisomerase-I inhibiting prodrug Irinotecan (CPT-11)

Esterase

[Chemical structures and spectra graphs]

- Intracellular spectra of CPT-11
- Intracellular spectra of SN38

[Graphs showing fluorescence intensity over wavelength]
Study of intracellular conversion of pro-drug irinotecan into active metabolite SN38 using confocal imaging

H460 cells

Ex: 380nm Em: 430nm
30 minutes after incubation

Ex: 560nm
18 hours after incubation

Cellular Mechanism of Chemotherapy

The structure of Chemotherapeutic drug-carrier (LH-RH peptide)-dye conjugate. TPLSM images of MCF-7 cells showing the intake of drug into cell over a time period of 50 minutes.
Spectra profiles of AC&LHTPR treated MCF-7 cell (inside the Nucleus, Cytoplasm and on the Membrane)

Localized spectroscopy was used to identify the localization of a chemotherapeutic drug and one of its component, the carrier protein, inside human cancer cells. The ratio between the two emission at 490nm (From AN152:C625) and the Emission at 590 (From LHRH:TPR) was studied.

Two-Photon FRET Imaging of CFP-YFP pair
Acceptor photobleaching technique estimate FRET efficiency

Cerulean and Venus proteins fused together as a control for FRET expressed in TE671 cells.

\[
FRET_{eff} = \frac{D_{post} - D_{pre}}{D_{post}} \quad \text{for all} \quad D_{post} > D_{pre}
\]

Time correlated single photon counting for lifetime imaging

Applications:
1. Identification of FRET
2. Separation of spectrally overlapping emission from different dyes
**FRAP**

Confocal Microscope

- Lasers:
  - Argon laser (457nm, 488nm, 514nm)
  - DPSS laser (532nm)
  - Ti:Sapphire (740-940nm)

- Time resolution:
  - For FRAP: ~330ms
  - (256x256 images)
  - (<10ms for point scan)

---

**FRAP technique**

**FGFR1**

<table>
<thead>
<tr>
<th></th>
<th>Nucleus</th>
<th>Nuclear Membrane</th>
<th>Plasma Membrane</th>
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<tbody>
<tr>
<td>$t_1$ (s)</td>
<td>53.78</td>
<td>76.52</td>
<td>37.63</td>
</tr>
<tr>
<td>95% Confidence</td>
<td>50.82 to 57.10</td>
<td>71.70 to 82.03</td>
<td>35.55 to 59.97</td>
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Anti-Stokes Fluorescence Confocal Microscopy
For Thermal Imaging (Temperature Mapping)

Experimental setup for heating live cells under microscope objective
Nonlinear Optical Techniques

- Second harmonics Imaging
  - membrane dynamics
  - excitation at \(\nu\), signal at \(2\nu\)

- CARS Imaging
  - vibrational imaging
  - excitation at \(\nu_p\) and \(\nu_s\), signal at \(2\nu_p - \nu_s\) with Raman resonance at \(\nu_p - \nu_s\)

A two-photon absorption for photons of the same frequency, \(\nu\), (only one laser beam of intensity \(I\)). (ii) the energy-level description

Two-photon pumped up-conversion emission

Coherent Anti-Stokes Raman Scattering (CARS)
ILPB CARS/SHG/SumFrequencyGeneration Microscopy Setup

CARS imaging of living cells

Mia PaCa 2

Transmission ($\lambda=1064$ nm)  |  CARS (2845 cm$^{-1}$)

HeLa

Transmission ($\lambda=1064$ nm)  |  CARS (1670 cm$^{-1}$)
3D CARS imaging of lipid droplets in Mia PaCa 2

32x32x20 μm scan volume (z-step 0.5 μm)
Resonance: 2845 cm⁻¹ (CH-aliphatic stretch)
Scanning time: 20 s

CARS imaging versus TPE imaging

E. Coli 21x21 μm scanning area, time sequence in 3 channels

1st scan | 4th scan | 7th scan | 20th scan
---|---|---|---
TPE YO-PRO-1 dye (green)
CARS (1670 cm⁻¹)
Transmission (1064 nm)
CARS imaging versus SHG imaging

E. Coli
1044 nm - transfection SHG F - CARS (1670 cm⁻¹)

1x11 µm
2x21 µm
3x32 µm
4x42 µm

The Institute for Lasers Photonics and Biophotonics
Nanochemistry for Dendritic Structures

Monomer → Heck Reaction → Trimer Core → Heck Reaction → Trimerization → II-Conjugated Dendritic Core

G0 → R → Trimeric Core → G1

G2 → G3 → Enhanced multi-photon absorption

Nanochemistry for multifunctional ORMOSIL nanoparticles
(targeted optical/PET Imaging, photodynamic therapy, and gene delivery)

(1) PEGylation
(2) Antibody attachment
(3) Attachment of PET probes

Multifunctional ORMOSIL nanoparticles

Drug/DFP in AOT/BuOH/Water Micelles

Drug/Nanoparticles in AOT/BuOH/Water Micelles

Cu64

DIALYSIS

VTES

NH3

Drug /DMF in AOT/BuOH/Water

Micelles

Drug/Nanoparticles in Water
NANOPHOTONICS
Paras N. Prasad
(John Wiley & Sons, April 2004)

SUMMARY OF CONTENTS
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13. Nanophotonics for Biotechnology and Nanomedicine
14. The Market Place for Nanophotonics

NANOBIOPHOTONICS

Size Dependent Optical Transitions

Optical Interactions

NANOSCALE CONTROL OF OPTICAL FUNCTIONS

Excitation Dynamics

Manipulation Of Phonon Induced Relaxations
Nanoscopic Perturbation on Nonradiative Processes
Nanoscopic Intermolecular Energy Transfer (FRET)
QUANTUM DOTS: Manifestations of Quantum Confinement

• Size Dependent Optical Properties
• Increase of Oscillator Strength
• New Intraband Transitions
• Increased Exciton Binding
• Increased Transition Probability in Indirect Band Gap Semiconductors (relaxation of \( k \)-selection)

Quantum Dots for Bioimaging

Focus: II-V quantum dots
Merits: • Longer wavelength emission
  • Molecular materials (less toxic?)
Our Approach: Nanochemistry for one pot rapid synthesis of highly monodispersed core-shell structure, using novel precursors and non-coordinating solvents.
Folate Receptor mediated nanoparticle delivery


Core-Shell (III-V) quantum dot semiconductor nano-particles for multiphoton imaging

Two photon excited emission from quantum dots

Two-photon image of KB cells treated with QD-FA for 6 h.
Aggregation-Enhanced Fluorescence and Two-Photon Absorption in Nanoaggregate and Organically Modified Silica Nanoparticles of Novel 9,10-Bis[4'-aminostyryl]styryl]anthracene Derivatives

Synthetic scheme for preparation of organically modified silica nanoparticle (OSNP): i. Injection of partially hydrolyzed and sol-gel condensed BDSA-Si in NH4OH/NMP. ii. Immediate formation of fluorescent aggregate. iii. Further condensation within the aggregate. iv. Dialysis against water.

S.Kim, A.Baev and P.N.Prasad, Journal of American Chemical Society (submitted)

Two-photon fluorescence image of HeLa cells
(stained with ORMOSIL nanoparticles of BDSA-Si)

Transmission Two-Photon Fluorescence
(excited at 780 nm)

Localized fluorescence spectrum from the cytoplasm
Nanomedicine

A vision for future health, utilizing cross-fertilization of nanotechnology and biology. Nanoparticles and nanostructures are used to produce:

- Multiple intracellular probes for basic understanding of cellular interactions and dynamics
- Early detection of diseases or infections
- Therapy to target only the diseased sites with minimal collateral damage
- Many therapies in tandem to produce multimodal treatment
- Minimally invasive diagnostic and therapy
- Real-time monitoring of drug action or therapy

Multimodal Imaging

Enhanced Contrast MRI

In vivo fluorescence imaging
A NANOPARTICLE PLATFORM FOR NANOMEDICINE

Nanocontrol of Excitation Dynamics

Photodynamic Therapy

PDT Drug (P)

\[ ^1P^* \] PDT drug in singlet state

\[ ^3P^* \] PDT drug in triplet state

Type I process

\[ ^3P^* + H_2O \rightarrow HO^- + H_2O_2 \]

Type II process

\[ ^3P^* + O_2 \rightarrow ^1P + ^1O_2^- \]

Oxidation of cellular components

cytotoxicity
Structure of ORMOSIL nanoparticles

Triethoxyvinylsilane (VTES), precursor used for synthesis of the nanoparticles

Nanoparticle collocalization of a photosensitizer with heavy atom in an ORMOSIL: triplet yield increase as a result of the external heavy atom effect

External heavy atom effect

Enhanced Intersystem Crossing

Enhanced Singlet Oxygen Generation

ORMOSIL nanoparticle with coencapsulated photosensitiser HPPH and I$_2$

T.Ohulchansky, D.Bharali, and P.N.Prasad, unpublished
I$_2$ inside nanoparticles influences on the absorption and emission of the encapsulated HPPH as well as on generation of $^{1}$O$_2$

Changes in HPPH fluorescence with the increase in I$_2$ concentration inside particles

$^{1}$O$_2$ generation by HPPH manifested by $^{1}$O$_2$ phosphorescence

Harvesting of two-photon excitation for two-photon photodynamic therapy

Energy transfer to photosensitizer

IR excitation (TPA)

$^{1}$O$_2$

Singlet O$_2$

Generation

In collaboration with Frechet Research Group
University of California, Berkeley

Co-encapsulation of the two-photon absorbing donor and singlet oxygen generating acceptor in the ORMOSIL nanoparticles

Gene Therapy

Therapeutic Approaches
- Gene replacement – single gene defects, discrete cell populations
- Suicide genes – eliminate specific cells or cell functions (i.e., cancer cells)
- Protective genes – express protective gene product (i.e., viral/bacterial infections)
- Immune stimulation – stimulate host immune response (i.e., DNA vaccines)
- Cell Marking – for autologous bone marrow transplant, cancer

Applications – virtually all human diseases
- e.g., vascular, cancer, genetic disorders (diabetes, cystic fibrosis, parkinson etc), neurodegenerative, autoimmune, immunodeficiency syndromes, diabetes, microbial diseases, etc.
Optically Trackable ORMOSIL Nanoparticles for Gene Delivery (FRET)

In Vitro Uptake and Transfection of Cells by ORMOSIL/DNA Nanoparticles

Cellular Uptake of DNA loaded ORMOSIL nanoparticles and subsequent translocation of DNA into the nucleus of the cell

Expression of eGFP in cells transfected with eGFP ORMOSIL nanoparticles vector
Gene transfer into neural Stem/Progenitor cells
*In vivo* Imaging of EGFP Expression

PNAS 2005 102: 11539

Opportunities in Biophotonics

- *In vivo* Bioimaging, Spectroscopy, and Optical Biopsy
- Nano-Biophotonic Probes
- Single Molecule Biofunctions
- Multiphoton Processes for Biotechnology
- Real-Time Monitoring of Drug Interactions
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The Institute for Lasers, Photonics and Biophotonics
University at Buffalo

BIOPHOTONICS, NANOPHOTONICS,
NANOchemistry and Nanomedicine

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www.photonics.buffalo.edu