"Lighting the Way to Technology through Innovation"

The Institute for Lasers, Photonics and Biophotonics
University at Buffalo

BIOPHOTONICS, NANOPHOTONICS, NANOCHEMISTRY AND NANOMEDICINE

P.N.Prasad

www.photonics.buffalo.edu

Introduction to Biophotonics

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

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3. **Basics of Biology**
4. **Fundamentals of Light-Matter Interactions**
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9. **Biosensors**
10. **Microarray Technology for Genomics and Proteomics**
11. **Flow Cytometry**
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15. **Nanotechnology for Biophotonics: Bionanophotonics**
16. **Biomaterials for Photonics**

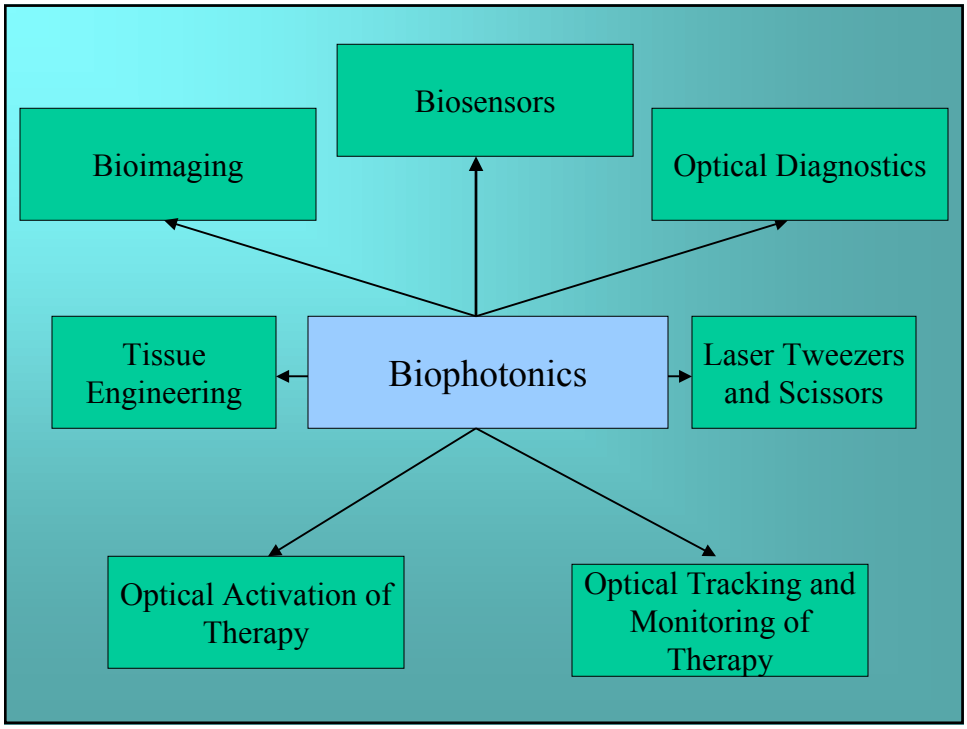
The Institute for Lasers, Photonics and Biophotonics

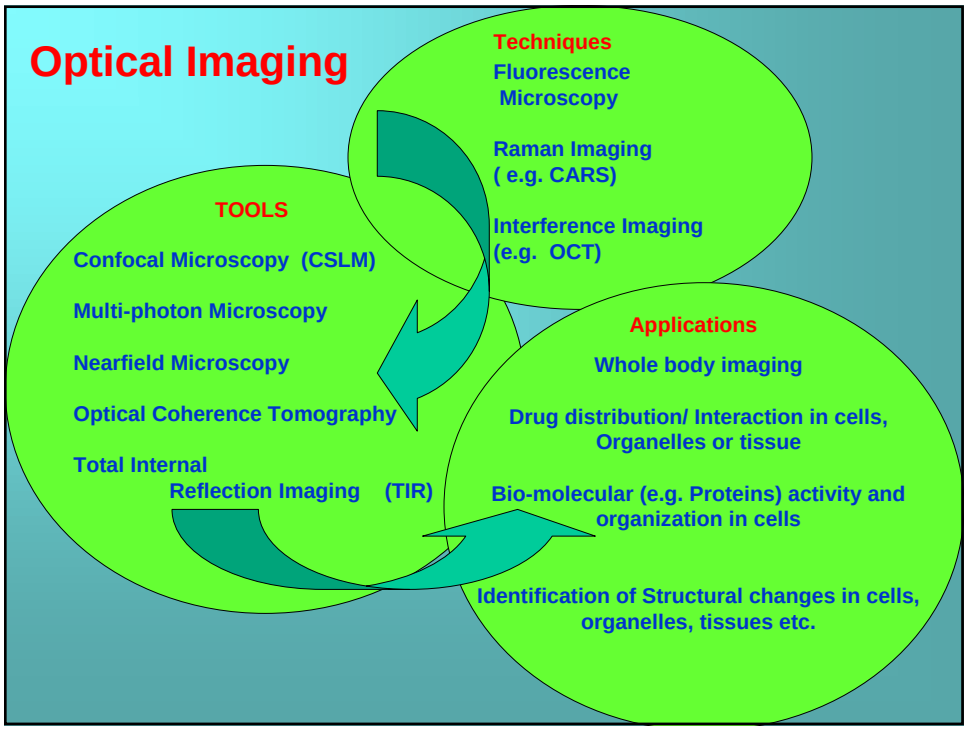
Mission

- **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*


Biosensors
Bioimaging
Optical Diagnostics
Tissue Engineering
Biophotonics
Laser Tweezers and Scissors
Optical Activation of Therapy
Optical Tracking and Monitoring of Therapy


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)

Polarized Fluorescence Imaging :

Fluorescence Life time imaging (FLIM)

Fluorescence

Fluorescence Recovery After Photobleaching (FRAP)

Fluorescence Resonance Energy Transfer (FRET)

Molecular diffusion and Mobility measurements in living cells

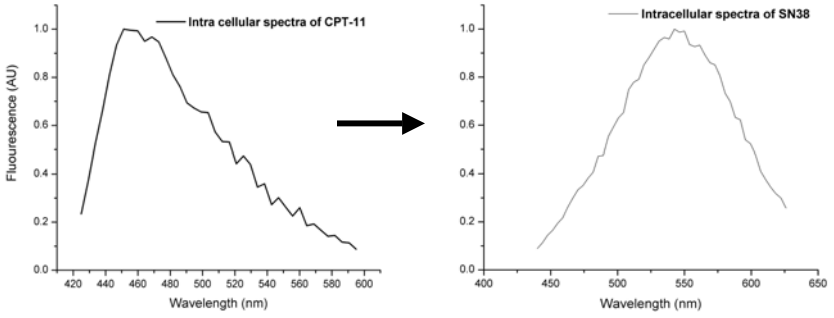
(e.g. Protein mobility and interactions)

Molecular interactions and conformational changes in living cells

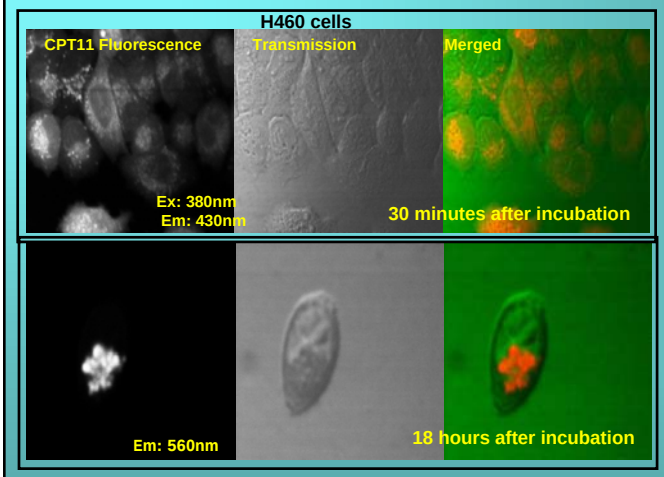
(e.g. Protein interactions and conformational changes)

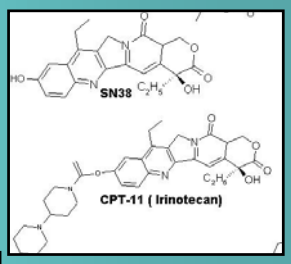
Intracellular Drug Distribution and Activation Monitoring using Confocal Microscopy

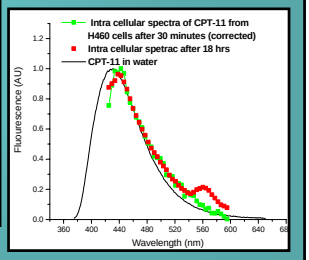
Mechanism of action of Topoisomerase-I inhibiting prodrug Irinotecan (CPT-11)



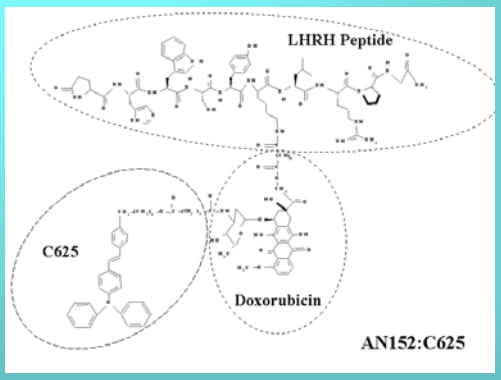
Study of intracellular conversion of pro-drug Irinotecan into active metabolite SN38 using confocal Imaging

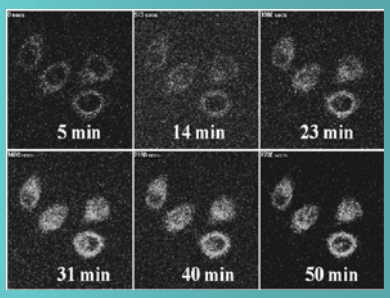






Cellular Mechanism of Chemotherapy

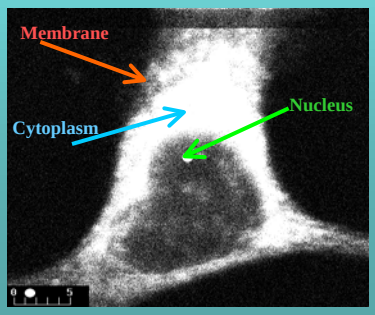


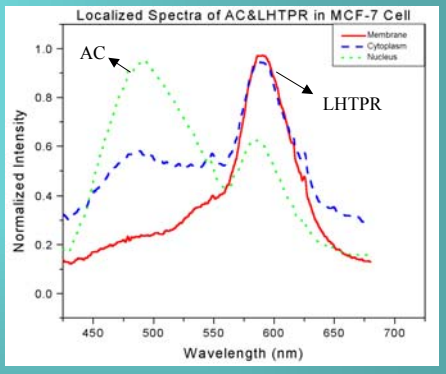


The structure of Chemotherapeutic drug-carrier (LH-RH peptide)-dye conjugate. TPLSM images of MCF-7 cells showing the intake of drug into cell over a time period of 50 minutes.

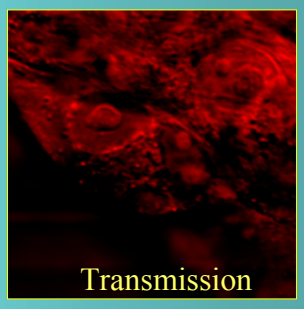
Spectra profiles of AC&LHTPR treated MCF-7 cell (inside the Nucleus, Cytoplasm and on the Membrane)

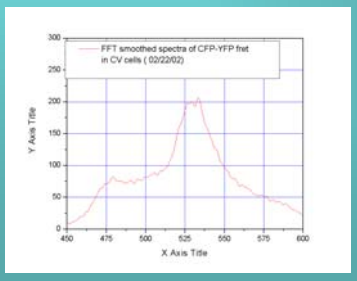
Localized spectroscopy was used to identify the localization of a chemotherapeutic drug and and one of its component, the carrier protein, inside human cancer cells. The ratio between the two emission at 490nm (From AN152:C625) and the Emission at 590 (From LHRH:TPR) was studied.



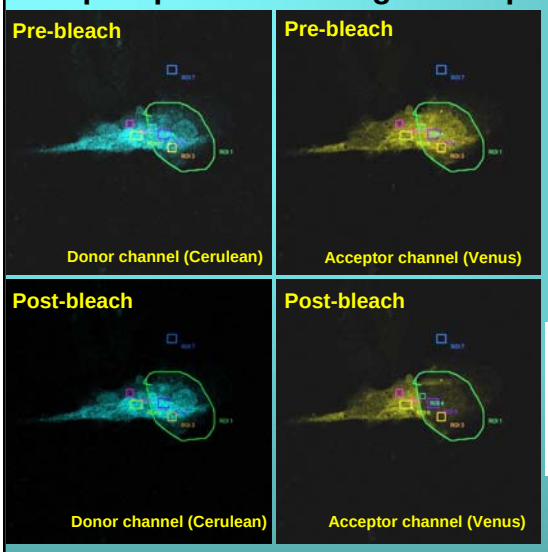


Two-Photon FRET Imaging of CFP-YFP pair





Acceptor photobleaching technique estimate FRET efficiency

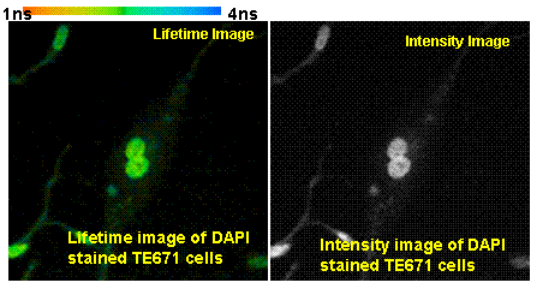


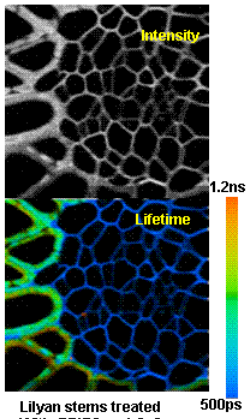
ROI	ROI_1	ROI_2	ROI_3	ROI_4	ROI_5
D pre	55.95	138.89	103.12	116.77	62.81
D post	80.80	193.97	153.05	181.18	57.10
A pre	56.93	119.90	89.86	121.76	58.88
A post	17.11	30.51	24.47	28.73	56.95
FRETeff (%)	30.75	28.40	32.62	35.55	0.00

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



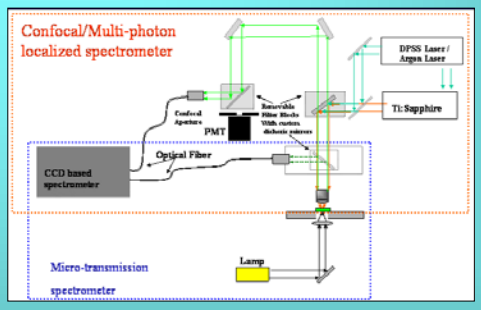


Lilyan stems treated With TRITC and Cy3 (Fixed tissue)

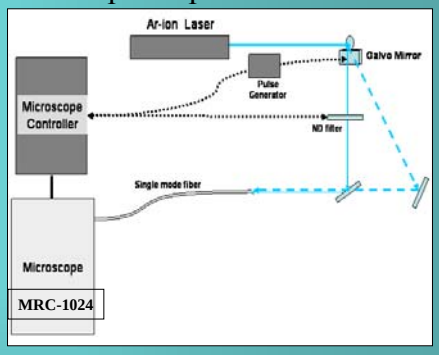
Applications: 1. Identification of FRET
2. Separation of spectrally overlapping emission from different dyes

FRAP

Confocal Microscope



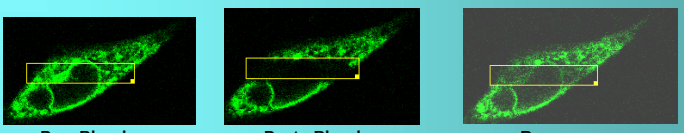
Frap Setup at ILPB



Lasers	**:**	**Argon laser (457nm, 488nm, 514nm)**
	:	**DPSS laser (532nm)**
	:	**Ti:Sapphire (740-940nm)**
Time resolution For FRAP	**:**	**~330ms (256x256 images) (<10ms for point scan)**

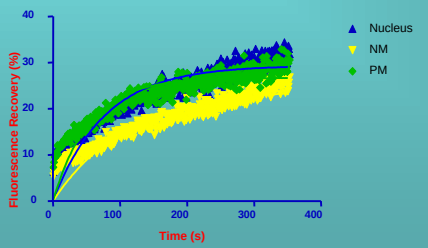
FRAP technique

FGFR1



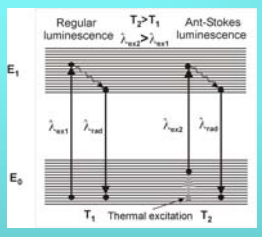
Pre -Bleach **Post -Bleach** **Recovery**

	Nucleus	Nuclear Membrane	Plasma Membrane
$t_{½}$ (s)	53.78	76.52	37.63
95% Confidence	50.82 to 57.10	71.70 to 82.03	35.55 to 39.97

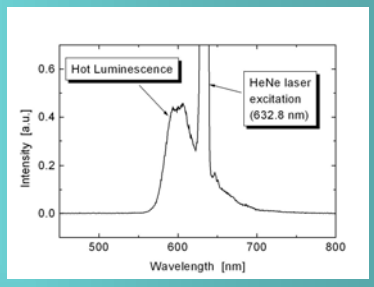


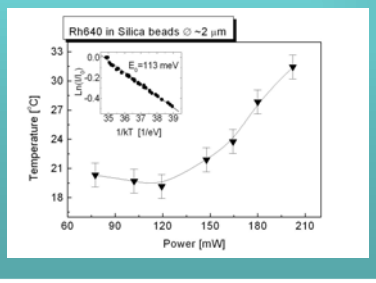
S. Dunham, H.E. Pudavar, M. Stachowicak & P.N. Prasad, Unpublished results.

Anti-Stokes Fluorescence Confocal Microscopy For Thermal Imaging (Temperature Mapping)

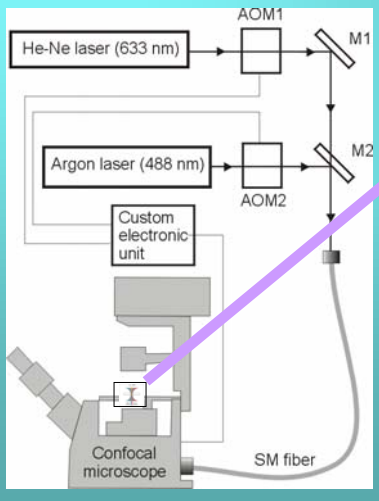


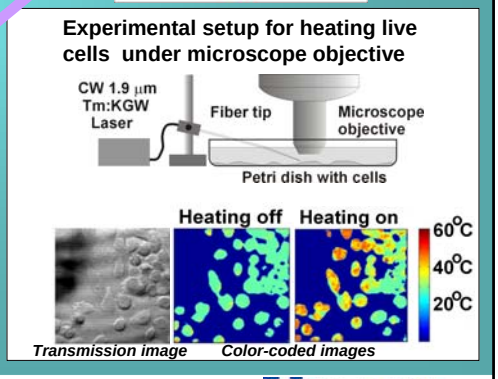
$$I \sim \exp\left(\frac{kT}{E_0}\right)$$





Anti-Stokes Fluorescence Confocal Microscopy For Thermal Imaging (Temperature Mapping)

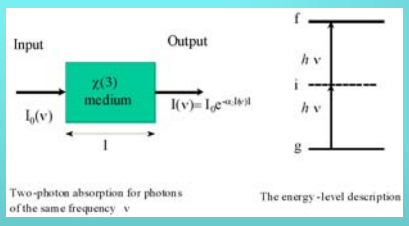




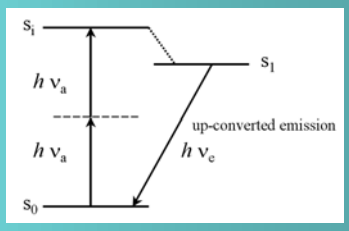
Nonlinear Optical Techniques

- Second harmonics Imaging
  - membrane dynamics
  - excitation at ν, signal at 2ν
- CARS Imaging
  - vibrational imaging
  - excitation at ν_p and ν_s, signal at $2\nu_p - \nu_s$ with Raman resonance at $\nu_p - \nu_s$

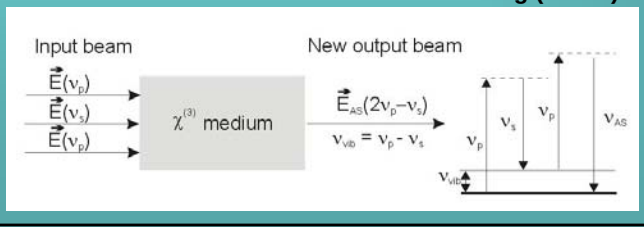
A two-photon absorption for photons of the same frequency, ν, (only one laser beam of intensity ν). (ii) the energy-level description



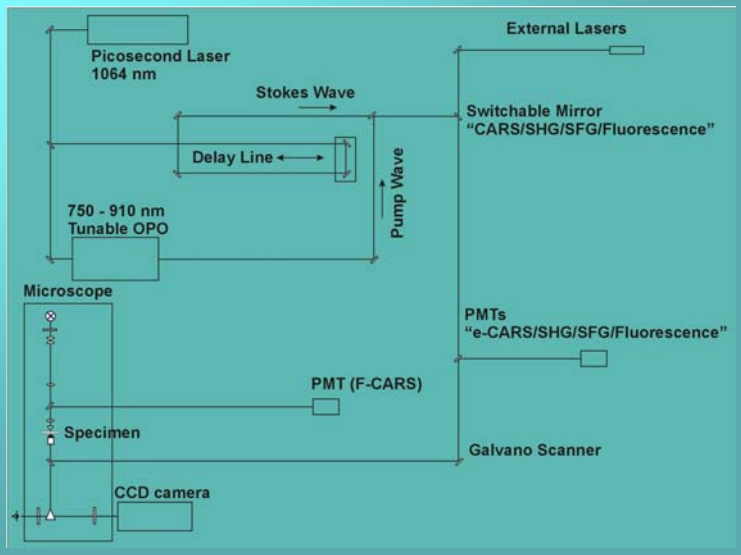
Two-photon pumped up-conversion emission



Coherent Anti-Stokes Raman Scattering (CARS)

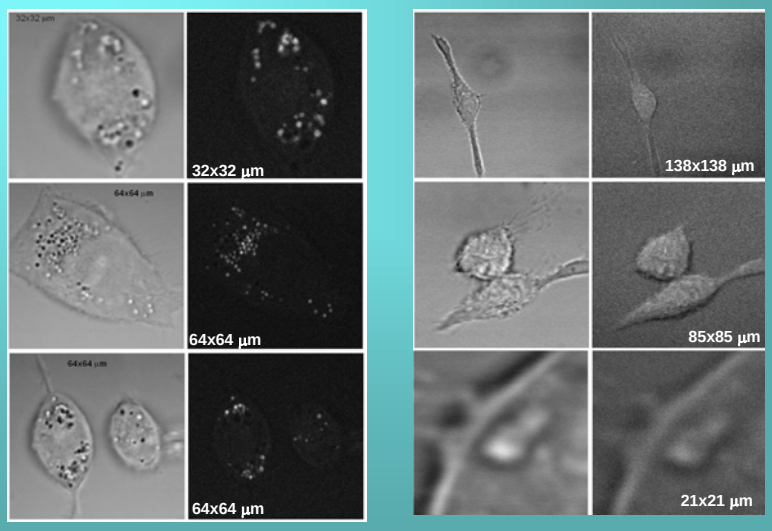


ILPB CARS/SHG/SumFrequencyGeneration Microscopy Setup



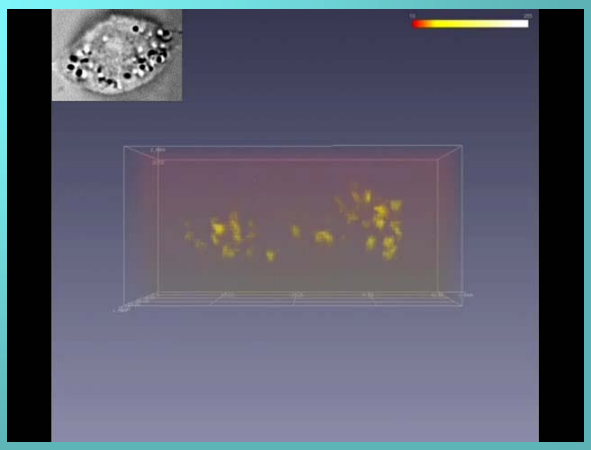
CARS imaging of living cells

Mia PaCa 2 **HeLa**



Transmission (λ=1064nm) **CARS (2845 cm^{-1})** **Transmission (λ=1064nm)** **CARS (1670 cm^{-1})**

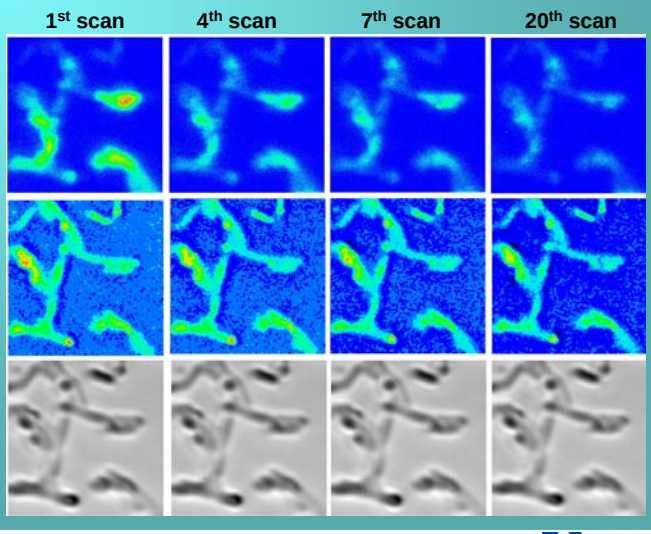
3D CARS imaging of lipid droplets in Mia PaCa 2



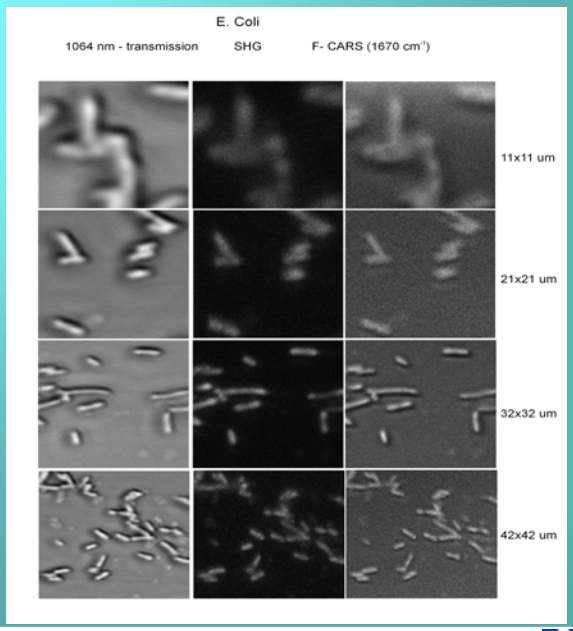
32x32x20 μm scan volume (z-step 0.5 μm)
Resonance: 2845 cm^{-1} (CH-aliphatic stretch)
Scanning time: 20 s

CARS imaging versus TPE imaging

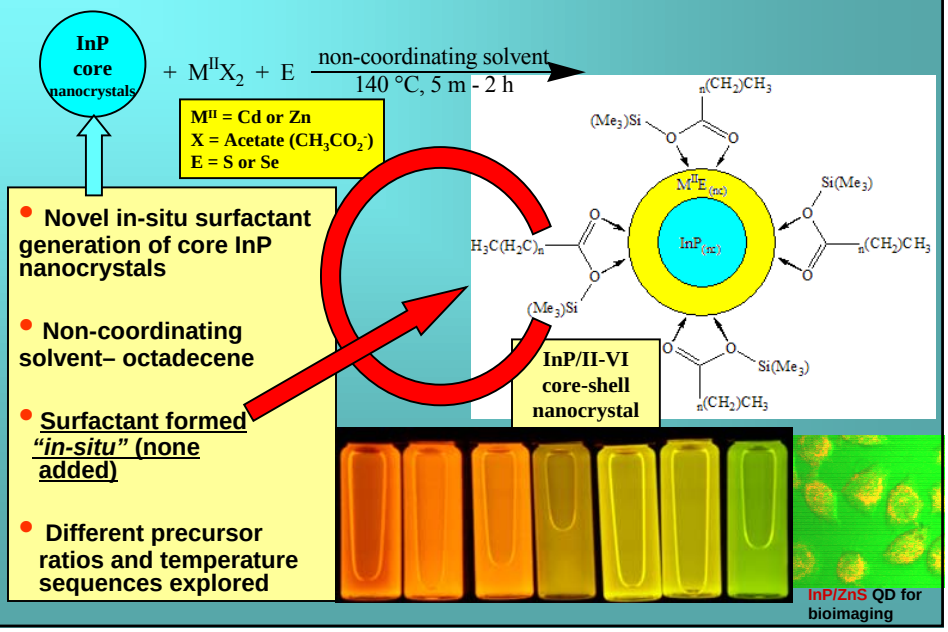
E. Coli 21x21 μm scanning area, time sequence in 3 channels



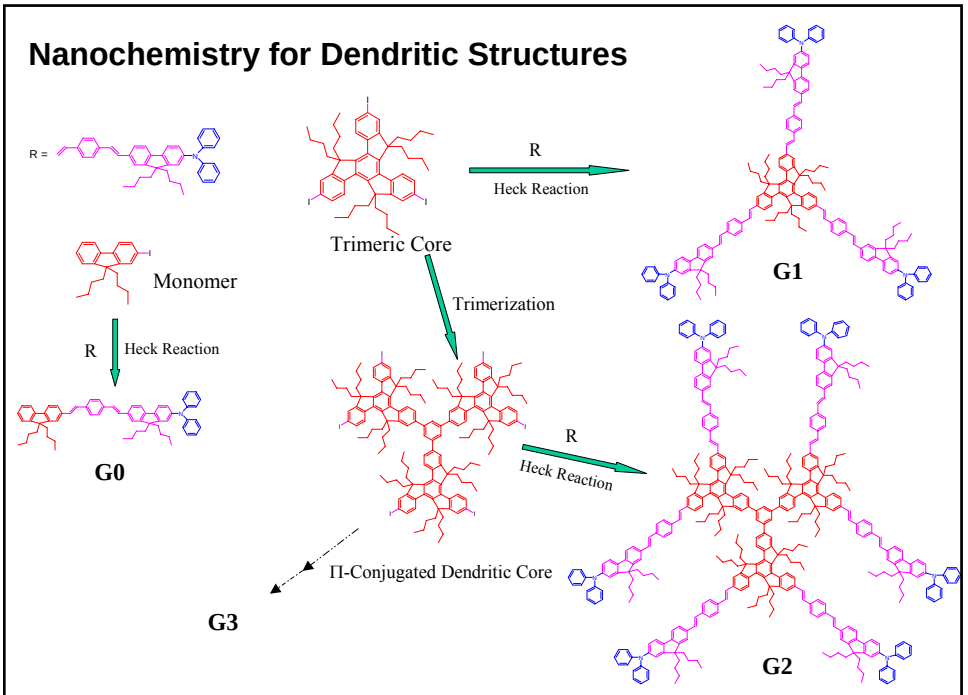
CARS imaging versus SHG imaging



Nanochemistry for Nanostructures

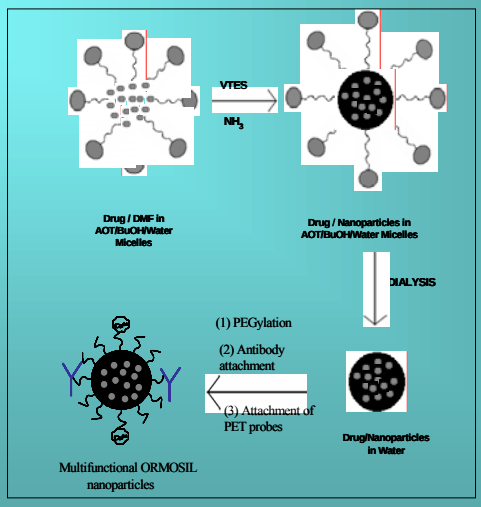


Nanochemistry for Dendritic Structures



Enhanced multi-photon absorption

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



NANOPHOTONICS

Paras N. Prasad

(John Wiley & Sons, April 2004)

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7. **Processing and Characterization of Nanomaterials**
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9. **Nanocomposites**
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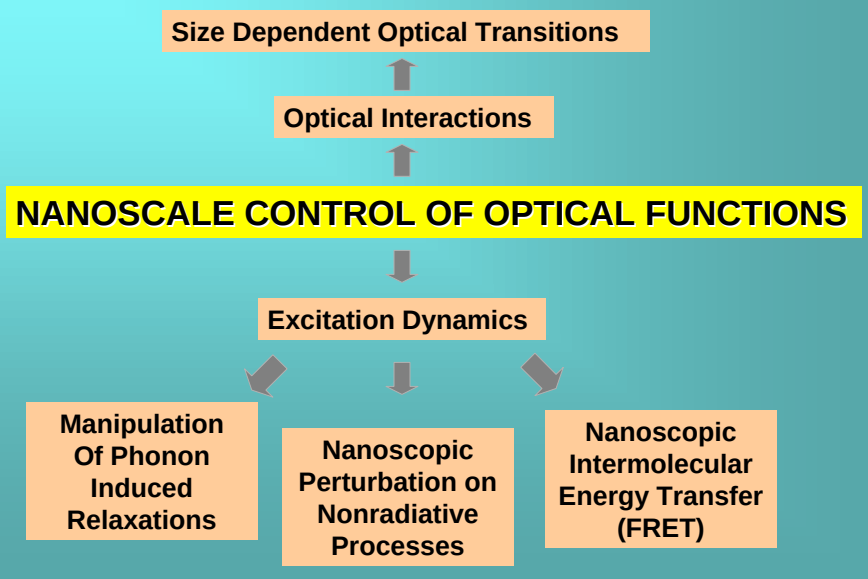
11. **Nanolithography**
12. **Biomaterials for Nanophotonics**
13. **Nanophotonics for Biotechnology and Nanomedicine**
14. **The Market Place for Nanophotonics**

NANOBIOPHOTONICS



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)

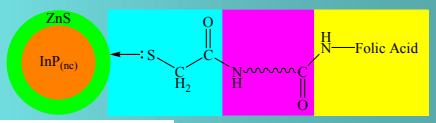
Quantum Dots for Bioimaging

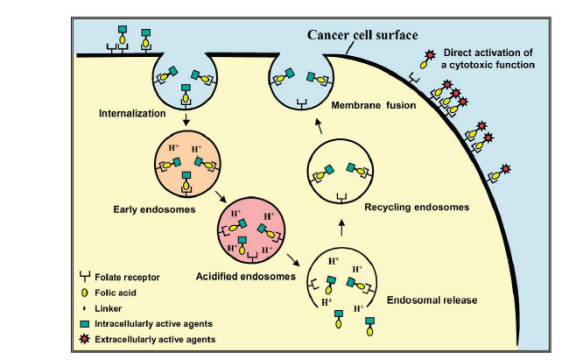
Focus: III - V quantum dots

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

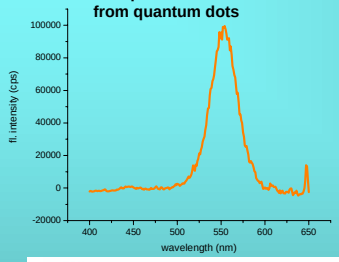


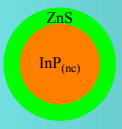


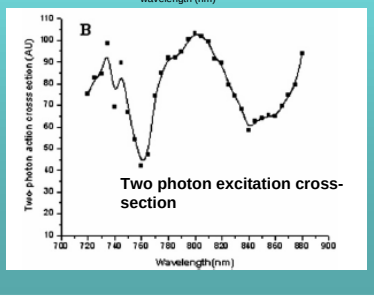
Y . Lu ,P.S . Low I Advanced Drug Delivery Reviews 54 (2002) 675– 693

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

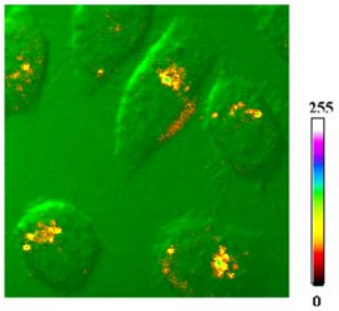
Two photon excited emission from quantum dots



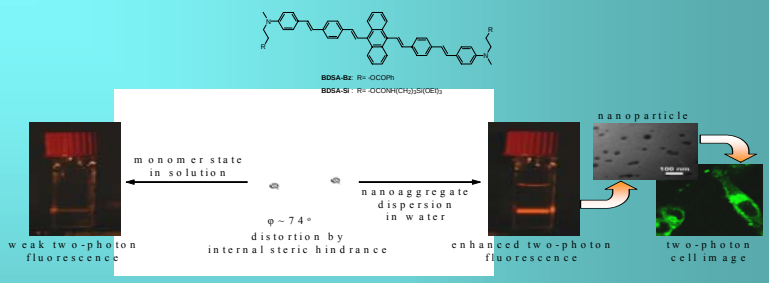


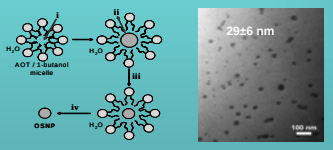


Two-photon image of KB cells treated with QD-FA for 6 h.



Aggregation-Enhanced Fluorescence and Two-Photon Absorption in Nanoaggregate and Organically Modified Silica Nanoparticles of Novel 9,10-Bis[4-(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 NH_4OH/NMP. ii. Immediate formation of fluorescent aggregate. iii. Further condensation within the aggregate. iv. Dialysis against water

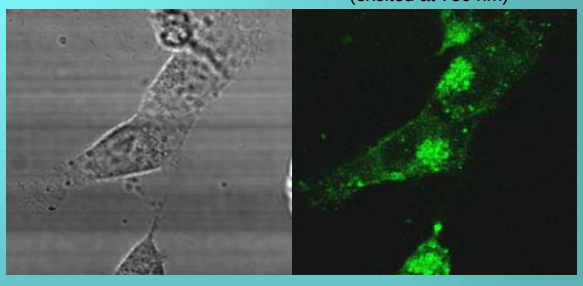
S.Kim, A.Baev and P.N.Prasad, Journal of American Chemical Society (submitted)

Two-photon fluorescence image of HeLa cells

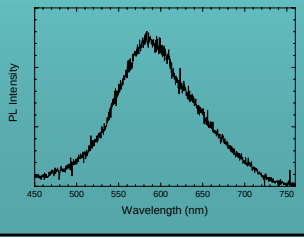
(stained with ORMOSIL nanoparticles of BDSA-Si)

Transmission

Two-Photon Fluorescence
(excited at 780 nm)



Localized fluorescence spectrum from the cytoplasm

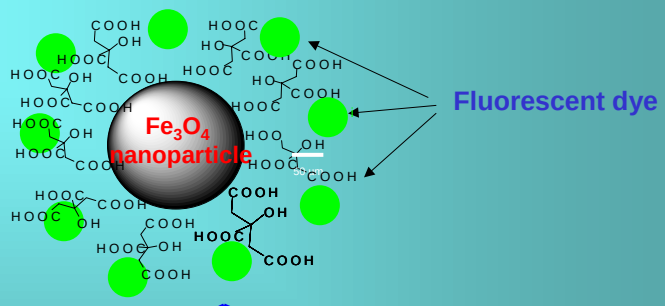


Nanomedicine

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

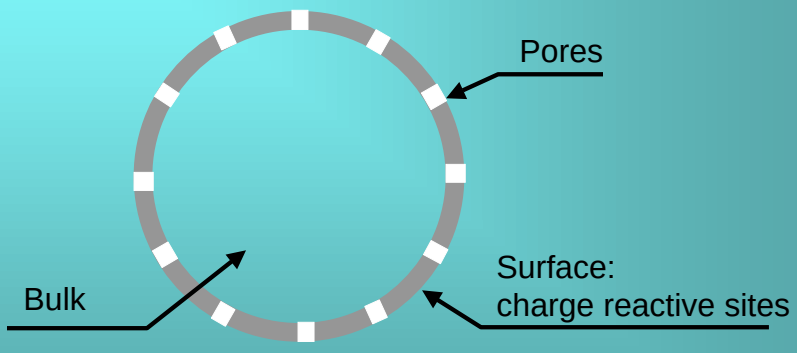
- Multiple intracellular probes for basic understanding of cellular interactions and dynamics
- Early detection of diseases or infections
- Therapy to target only the diseased sites with minimal collateral damage
- Many therapies in tandem to produce multimodal treatment
- Minimally invasive diagnostic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

Multimodal Imaging



Enhanced Contrast MRI ↔ **In vivo fluorescence imaging**

A NANOPARTICLE PLATFORM FOR NANOMEDICINE



(silica based nanoparticle)

Nanocontrol of Excitation Dynamics

Photodynamic Therapy

PDT Drug (P)

↓ *Light absorption*

$^1P^*$ PDT drug in singlet state

↓ *Intersystem crossing*

$^3P^*$ PDT drug in triplet state

Type I process

$^3P^* + H_2O \rightarrow HO\bullet$

H_2O_2

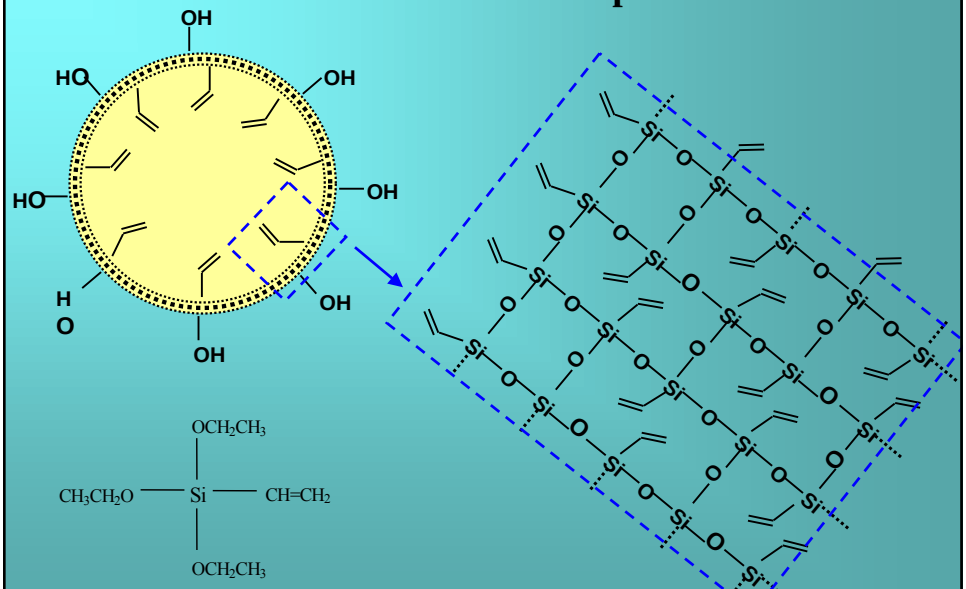
Type II process

$^3P^* + {}^3O_2 \rightarrow {}^1P + {}^1O_2^*$

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

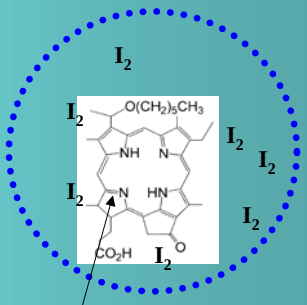
External heavy atom effect

↓

Enhanced Intersystem Crossing

↓

Enhanced Singlet Oxygen Generation

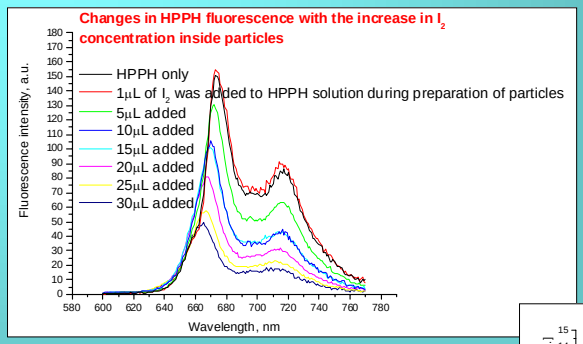


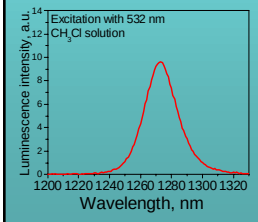
Photosensitizer HPPH

ORMOSIL nanoparticle with coencapsulated photosensitiser HPPH and I_2

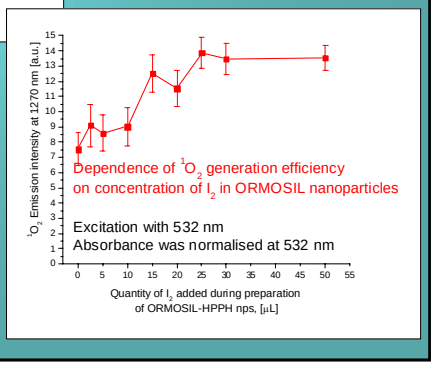
T.Ohulchanskyy, D.Bharali, and P.N.Prasad, unpublished

I_2 inside nanoparticles influences on the absorption and emission of the encapsulated HPPH as well as on generation of $^1O_2$

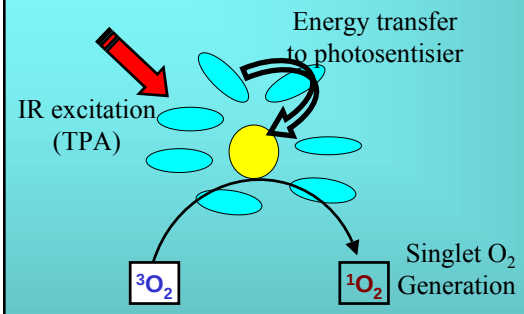


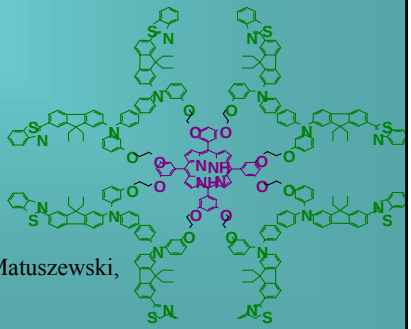


$^1O_2$ generation by HPPH manifested by $^1O_2$ phosphorescence



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



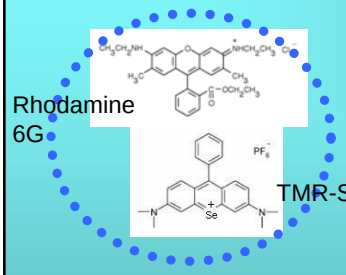


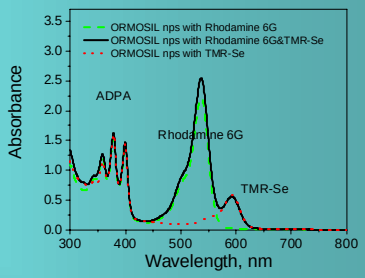
In collaboration with Frechet Research Group

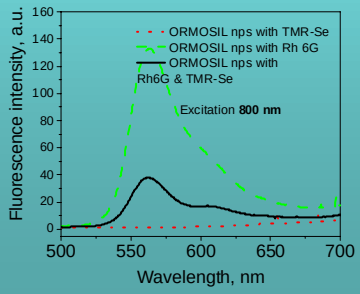
University of California, Berkeley

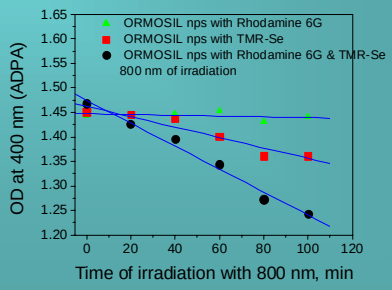
W.R. Dichtel, J. M. Serin, C.Edder, J.M. J. Fréchet, M.Matuszewski, L.-S. Tan, T. Y. Ohulchanskyy,. and P. N. Prasad, *JACS* 2004, **126**, 5380.

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









Gene Therapy

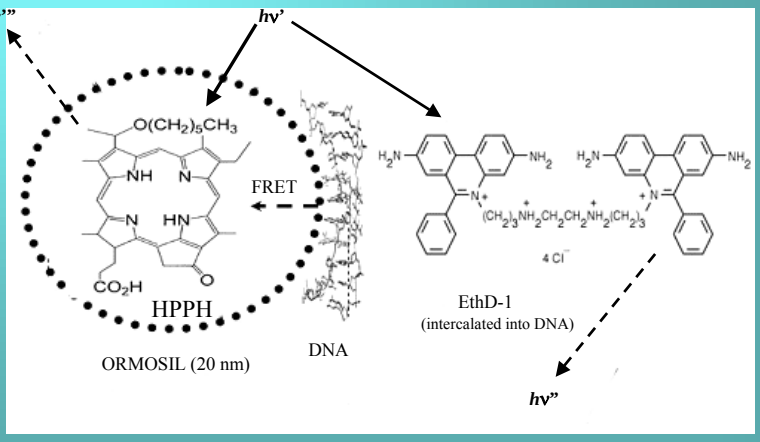
Therapeutic Approaches

- **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*

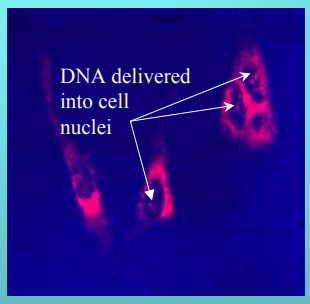
e.g., vascular, cancer, genetic disorders (diabetes, cystic fibrosis, parkinson etc), neurodegenerative, autoimmune, immunodeficiency syndromes, diabetes, microbial diseases, etc.

Optically Trackable ORMOSIL Nanoparticles for Gene Delivery (FRET)

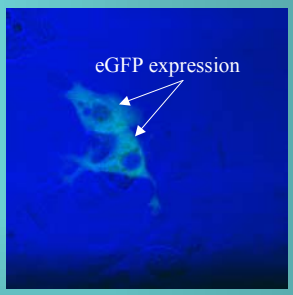


I. Roy, T. Y. Ohulchanskyy, D. J. Bharali, H. E. Pudavar, R. A. Mistretta, N. Kaur, and P. N. Prasad. *PNAS 2005*, 102 : 279

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

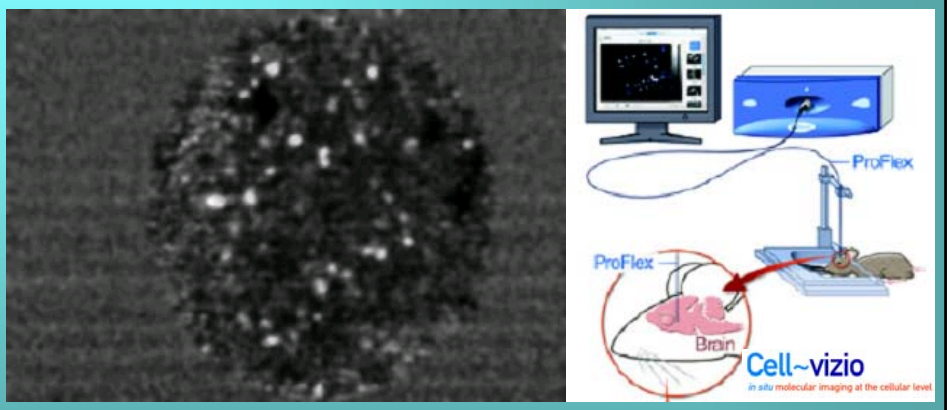


Cellular Uptake of DNA loaded ORMOSIL nanoparticles and subsequent translocation of DNA into the nucleus of the cell



Expression of eGFP in cells transfected with eGFP ORMOSIL nanoparticles vector

Gene transfer into neural Stem/Progenitor cells
***In vivo* Imaging of EGFP Expression**



D. J. **Bharali**, I. Klejbor, E.K. Stachowiak, P. Dutta, I. Roy, N. Kaur, E. J. Bergey, P. N. Prasad, and M. K. Stachowiak
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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- Dr. A.Kuzmin
- Dr. D. Bharali
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- Dr. S. Kim
- Dr. I. Roy
- Ms. H. Huang

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- Prof. M. Stachowiak
- Dr. A. Oseroff
- Dr. R. Pandey
- Dr. J. Morgan

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BIOPHOTONICS, NANOPHOTONICS, NANOCHEMISTRY AND NANOMEDICINE

P.N.Prasad

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