From cells to organs with lasers

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Biophotonics

- Biophotonics is the science of generating and harnessing light (photons) to image, detect and manipulate biological materials

- At the interface of physical, biological and medical sciences
  - The Institute for Lasers, Photonics and Biophotonics, University at Buffalo
  - NSF Centre for Biophotonics, Science and Technology, UC Davis
  - Bio-X, Stanford
  - Division of Biophysics and Imaging, Ontario Cancer Institute
  - Beckman Laser Institute, Irvine
OUR EXPERTISE

Fundamental Laser Science

Functional Biopolymer Spectroscopy
Eg. Melanin spectroscopy

Laser Manipulation
Eg. Laser Tweezers

Biomedical & Clinical Applications
Eg. Lasers in Dentistry

Biomedical Imaging

Plus expertise in tissue engineering, through our Affiliates

Examples of Biophotonics projects

- Hyperpolarised Gases for MRI Imaging
- Light activated processes
- Laser micromanipulation and examples of applications
Dynamic images of the human lung during inhalation and expiration of $^3$He

The use of hyperpolarized $^3$He and $^{129}$Xe for imaging air spaces and certain tissues in humans.

Traditional MRI techniques derive images from hydrogen. In places such as the lungs where hydrogen is not so abundant, imaging is difficult using these techniques.

- $^3$He and $^{129}$Xe polarisation processes are both based on the spin exchange optical pumping technique.

Time necessary to hyperpolarize the noble gas as well as the amount of gas produced and the process used to collect it is different.
Spin Exchange Optical Pumping (SEOP)

- SEOP method can be used for both noble gases
- Same Rb absorption line $\rightarrow$ same laser $\lambda = 794.6$ nm
- Otherwise, process is quite different for $^3$He and $^{129}$Xe

Variables for SEOP

- Gas Pressure
  - Affects the Rb absorption line
  - Changes laser requirements
- Temperature - Rb number density
- Gas mixture
  - Determined by collisional cross-section
- Time in the Spin Exchange region
  - $T_1$ of the vessel
  - Magnetic field, photon quality
**HP Gas Polarizer**

- Pyrex cell containing 98% $^3\text{He}$, 2% $\text{N}_2$, Rb
- Helmholtz Coils create uniform magnetic field
- Laser
- Laser frequency narrowing apparatus
- Oven
- Collisional transfer to He

**Laser Frequency Narrowing - why bother?**

Maximize $^3\text{He}$ Polarization Rate

Maximize spin exchange collisions with Rb

Density of species

Maximize Rb polarization

Quality of the laser light
- Polarization
- Spectrum

Laser power
- Absolute Power
- Spectral power
Improving Spectral Power Density

Free running laser spectrum spans 3-5 nm

Rb absorption ~0.5 nm

Putting all the laser power in a narrow band would increase spectral power density and thus Rb polarization rate

Centre $\lambda$ (Rb resonance, 794.7 nm)

$^3$He : progress

- Produced HP $^3$He
- Achieved ~15% polarization on first attempt
Images

- The cell

15% polarisation

Imaging

balloon
rat's lung
Light modified fluorophore transporters

- The interaction of light with some organisms can drive the transport of solutes across the cellular membrane.

- The transport of these solutes may be measured by:
  - patch clamping methods
  - optical microscopy
  - epifluorescent bright field microscopy
  - imaging of calcium ions using two photon excitation at an electronic resonance and monitoring the fluorescence emission of the ion.

A hybrid of single-photon and multi-photon imaging

- Decoupling of the membrane transporter and the excitation of fluorescence from the fluorophore.

- The system allows simultaneous and independent activation of the light induced membrane transport and imaging of the fluorophore.
Uptake of the fluorophore

Confocal Microscopy, light-activated pump action of cells

Justin Ross

More from the Confocal

The localisation of Rhodamine 123 in G. trophozoites

Justin Ross

5 µm
A hybrid of single-photon and multi-photon imaging

Spectral Response of light induced uptake of Rh123 by Giardia

10
Influx and Efflux of Dye from Giardia

- Time (s)
- Dye Concentration (uM)
- Concentration Change (uM/s)
- Dye Concentration in Cell
- Rate of Dye Influx
Optical micromanipulation - using light to make:

- Laser tweezers
- Laser scissors
- Laser catapult
- Laser screwdriver
- Optical spanner (wrench)

(Simpson et al, Opt Lett, 1997)

Optical torque wrench measures torque as it is applied.

Typical Laser Tweezers setup

Can trap and manipulate high index particles in water

Typical: 1µm radius, 1pN/mW
**Optical Trapping**

- **Ray optics model**

- **Force on a point dipole**

Force results from exchange of momentum with beam

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**Optical Force**

\[ F = \frac{nP}{c} Q_{opt} \]

- \( n \) - refractive of the trapped object,
- \( P \) - incident laser power
- \( Q_{opt} \) - efficiency factor

\[ F_{\text{drag}} = 6\pi\eta v_{\text{max}} \]

trap against viscous drag or movement

\[ Q_{\text{trans}} = 6\pi\eta v_{\text{max}} a \frac{c}{nP} \]
2 mm polystyrene spheres

Trapping and manipulation of 2 µm polystyrene spheres
Trapping of a macrophage

Studies of the dynamic structure of individual nucleosomes (DNA organised into nucleosomes)

Stretching nucleosomal arrays with feedback-enhanced optical trap.

Brent D. Brower-Toland et al. PNAS, 2002
Creating torque and measuring it as it is applied

- Asymmetric particles in laser beam
- Special laser beams
- Multiple traps

Optical Angular Momentum I

Helical wavefront (‘orbital’ a.m.)
- ‘optical vortex’
- ‘singular beam’
- ‘Gauss-Laguerre’ $GL_{p\ell}$

$AM = integer \times \hbar$ per photon

Phase singularity on axis, dark spot$_{12}$
generated by laser, or phase plate or hologram
Optical Angular Momentum - 'orbital'

Helical wavefront

\[ l = 1 \quad l = 3 \]

Associated with spatial distribution

Laser beam with orbital a.m.

Helical wavefronts

Absorbing particle

He et al., PRL (1995)

Not useful because of heating
Optical Angular Momentum II

Spin

Circularly polarised light \ldots \hbar \text{ per photon (spin)}

Transfer of AM to a waveplate

- CaCO$_3$ particles in H$_2$O are 3-D trapped in polarised light
- They either rotate continuously or align to a particular orientation
- In linear light, their orientation is controllable
- In elliptical light, their rotation frequency is controllable.

Friese et al., Nature 1998
Transfer of angular momentum of light

Optical axis of crystal aligns to electric field vector

Calcite crystal rotates in circularly polarised light

All optically driven micromachine elements

Friese et al., Appl. Phys. Lett. 78, 1, 2001

Micro-rheology

Circularly polarised light

\( \lambda/4 \) waveplate

Try to maintain circular symmetry to avoid orbital A.M.
Making better probes
Spherical CaCO$_3$ Crystals

Better probe particles
- smooth rotation
- 3D trapping

Alexis Bishop et al., PRL 2004

Theory

- Viscosity \( \alpha = \frac{\text{stress}}{\text{strain}} \) or \( \frac{\alpha}{\gamma} = \mu \) where \( \alpha \) = shear stress, \( \gamma \) = shear rate, \( \mu \) = viscosity of liquid
- Applied Torque \( \Delta \sigma = P \omega / \tau \) where \( \Delta \sigma \) = change in polarisation, \( P \omega / \tau \) = rate of shear, \( P \) = laser power, \( \omega \) = optical frequency
- Drag Torque \( \tau_D = 8\pi \mu a^3 \Omega \) where \( \tau_D \) = viscous drag torque, \( \mu \) = viscosity of liquid, \( a \) = sphere’s radius, \( \Omega \) = frequency of rotation
- Viscous drag torque \( \tau_D = 8\pi \mu a^3 \Omega \) where \( \mu \) = viscosity of liquid, \( a \) = sphere’s radius, \( \Omega \) = frequency of rotation

This sphere is creating quite a stir!
CaCO$_3$ sphere spinning inside an artificial liposome (15µm dia)
Laser tweezers, scissors and wrench

Fluid-Coupled MechanoTransduction
Flow-Coupled Shear Stress
Optically driven micromachines

Simon Parkin

Optically driven micromachines

Gregor Knöner
Two-photon photopolymerization

Two-photon initiated photopolymerization for three-dimensional fabrication

SEM image of a micro-bull sculpture.

Kawata, Japan 2001

Exposure Apparatus

- subsequent production and trapping
- Ti:Sapphire laser, 1 W, 80fs, 780nm for production of structure
Two-photon photopolymerization at UQ

Microhologram for Lab-on-Chip
- on axis phase hologram
  → transfer of angular momentum to linear polarized Gaussian beam

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**Angular momentum transfer**

- polystyrene spheres, \( d = 2.1 \ \mu m \)
- incident beam: Gaussian, linear polarized

*linear polarized beam*
*\( \lambda/4 \) waveplate*
*circularly polarized spin angular momentum*
*transfer to anisotropic particle*

*TEM\(_{00}\)*, linear pol.
*phase hologram*
*LG\(_{04}\)*, helical wavefront
*orbital angular momentum*
*transfer to anisotropic particle*
Setup for Microhologram Rotation

- microhologram
- rotating beads
- collection lens

Rotating beads with Microhologram

- incident laser beam: linearly polarized, Gaussian
UQ Laser tweezers team

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• Staff:
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  Martin Dienstbier
  Dr. Alexis Bishop

Thank you for your attention !!!

Rotation using orbital AM  LG_{02} doughnut beam