From cells to organs with lasers

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Biophotonics and Laser Science Solutions

Biophotonics in Australia, February 2006

The University Of Queensland Australia

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

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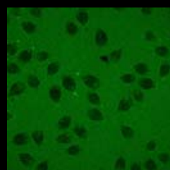
OUR EXPERTISE

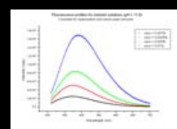
Fundamental Laser Science



Light activated processes

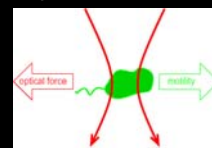
Functional Biopolymer Spectroscopy

Eg. Melanin spectroscopy



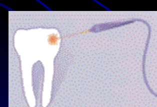
Laser Manipulation

Eg. Laser Tweezers

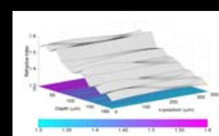


Biomedical & Clinical Applications

Eg. Lasers in Dentistry



Biomedical Imaging



*Plus expertise in tissue engineering……..,**through our Affiliates***

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Examples of Biophotonics projects

- Hyperpolarised Gases for MRI Imaging
- Light activated processes
- Laser micromanipulation and examples of applications

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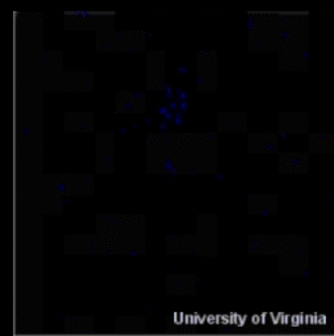
Hyperpolarized Gas MR Imaging

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.

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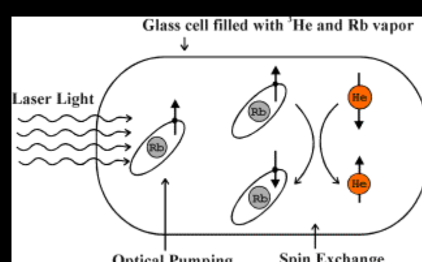
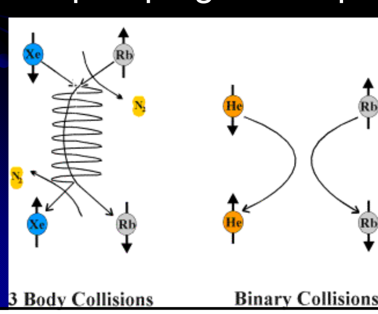
Hyperpolarized Gas MR Imaging

- ^{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.

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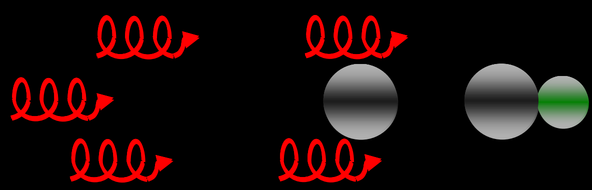
Spin Exchange Optical Pumping (SEOP)



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

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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
- T1 of the vessel
- Magnetic field, photon quality

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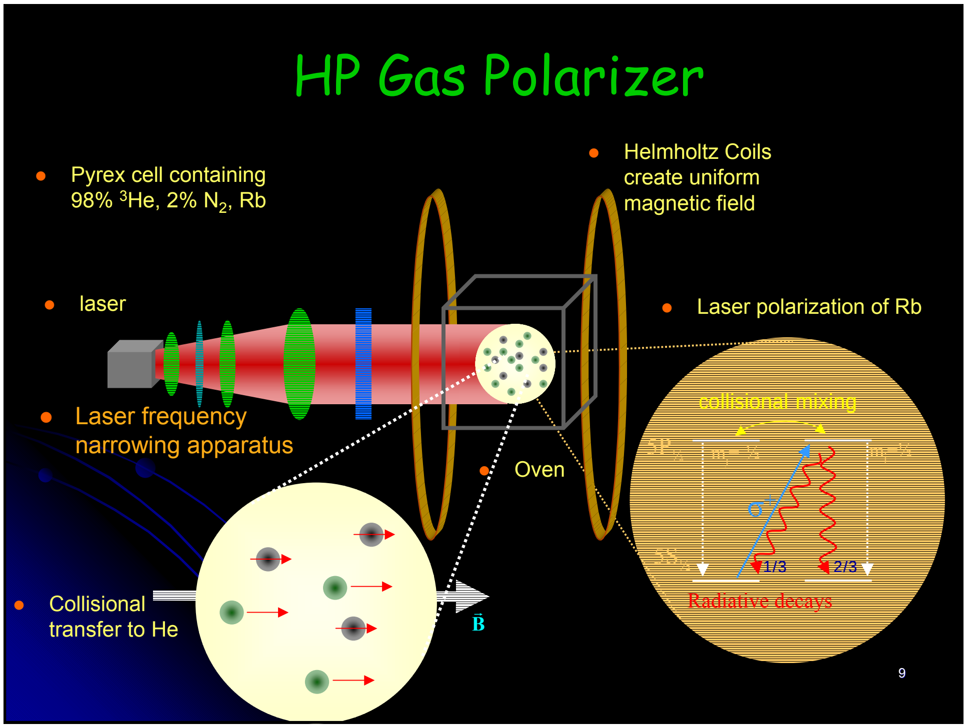

Laser Frequency Narrowing – why bother?

Maximize ^{3}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

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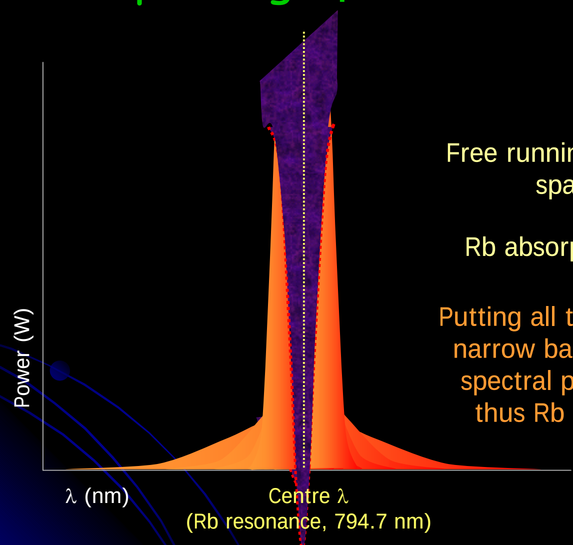
Improving Spectral Power Density



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

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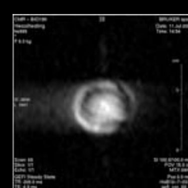
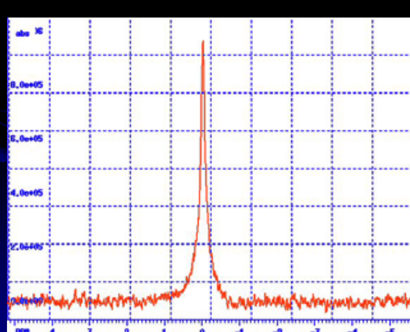
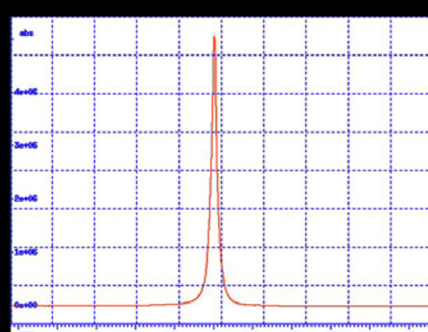
^3He : progress



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





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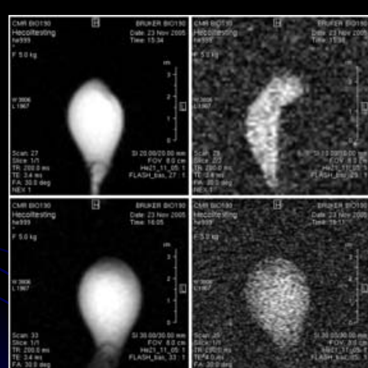
Images

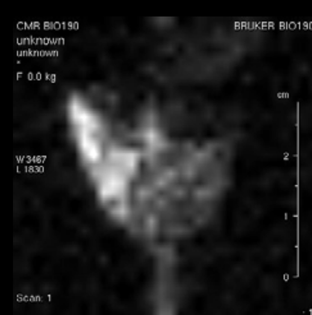
- The cell

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
  - by 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.

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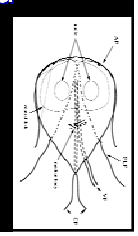
A hybrid of single-photon and multi-photon imaging

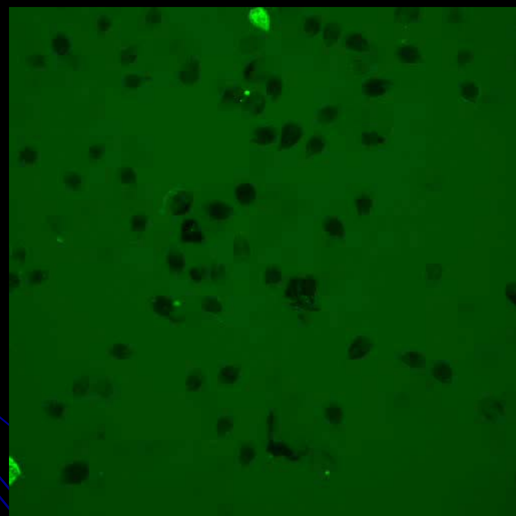
- Decoupling of the membrane transporter and the excitation of fluorescence from the fluorophore.

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



Giardia

Uptake of the fluorophore



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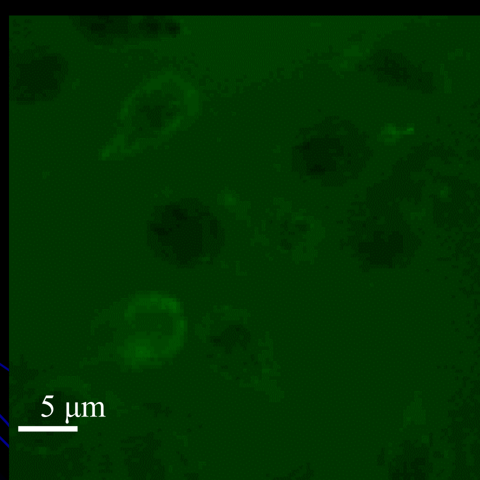
Confocal Microscopy, light-activated pump action of cells

More from the Confocal



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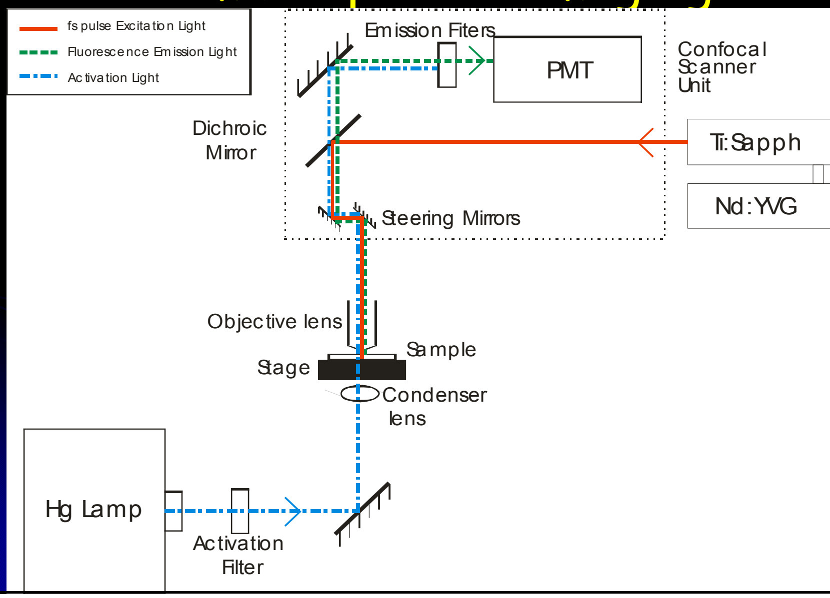
The localisation of Rhodamine 123 in Geardia trophozoites

A hybrid of single-photon and multi-photon imaging



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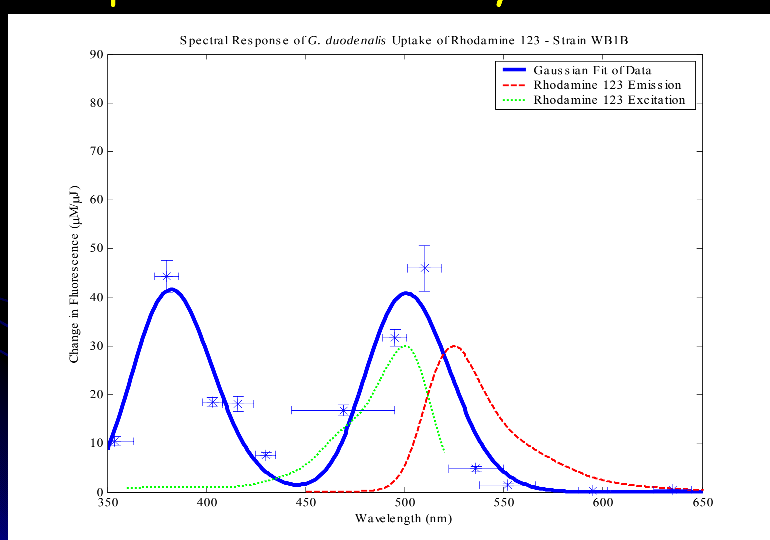
Spectral Response of light induced uptake of Rh123 by Geardia



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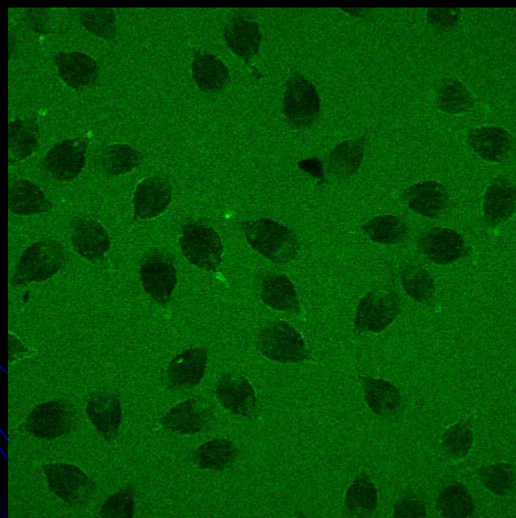
Influx and Efflux



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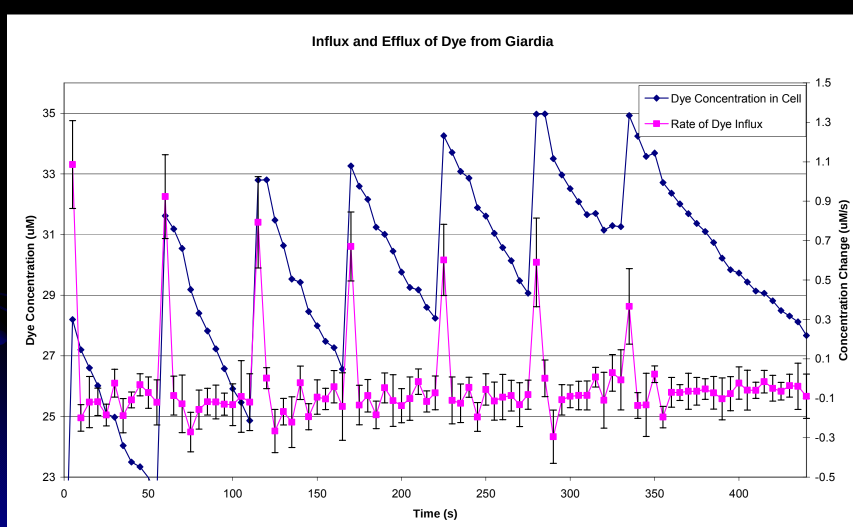
Influx and Efflux



Optical micromanipulation - using light to make:

- Laser tweezers

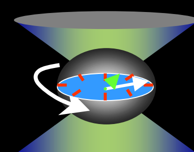
- Laser scissors

- Laser catapult

- Laser screwdriver

- Optical spanner (wrench)

(Simpson et al, Opt Lett,1997)



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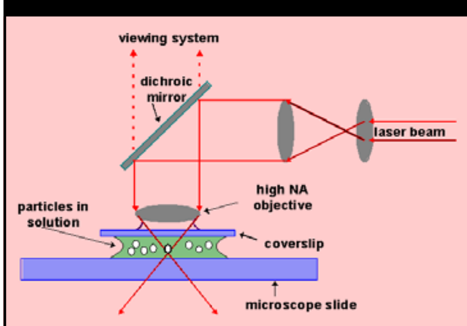
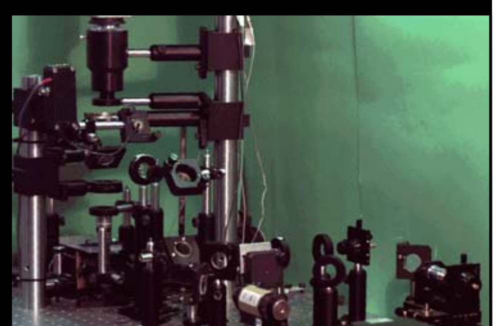
optical torque wrench measures torque as it is applied

Typical Laser Tweezers setup





Can trap and manipulate high index particles in water

Typical: 1μm radius, 1pN/mW

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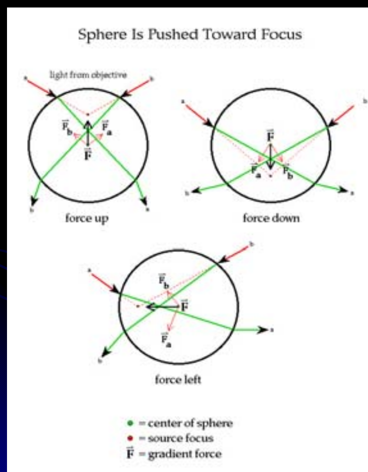
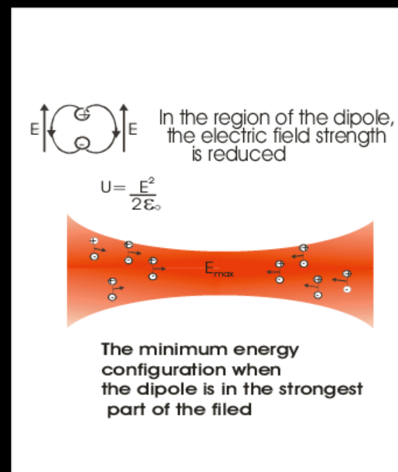
Optical Trapping

- Ray optics model
- Force on a point dipole





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Force results from exchange of momentum with beam

Optical Force

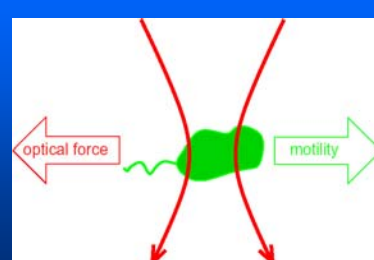
$$F = \frac{nP}{c} Q_{opt}$$

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

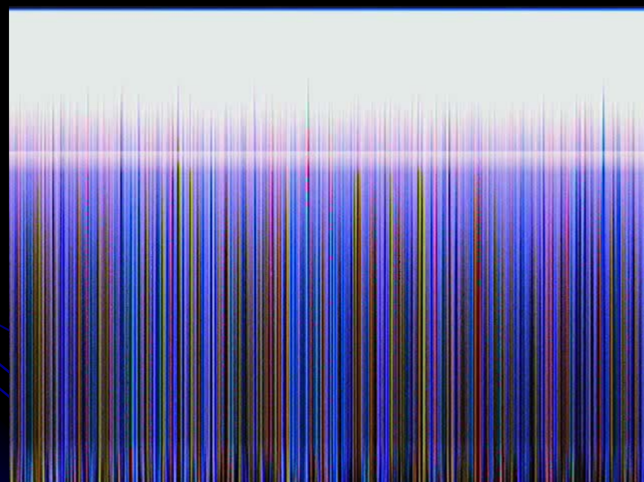
$$\mathbf{F_{drag} = 6\pi\eta a v_{max}}$$

trap against viscous drag or movement

$$Q_{trans} = 6\pi\eta v_{max} a \frac{c}{nP}$$



2 mm polystyrene spheres



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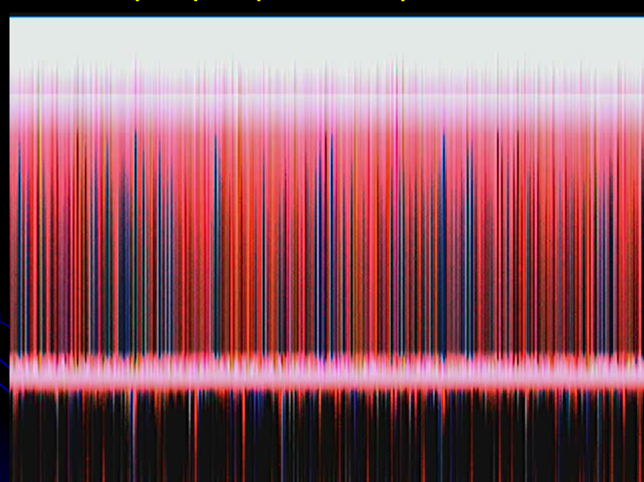
Trapping and manipulation of 2 µm polystyrene spheres



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Trapping of a macrophage

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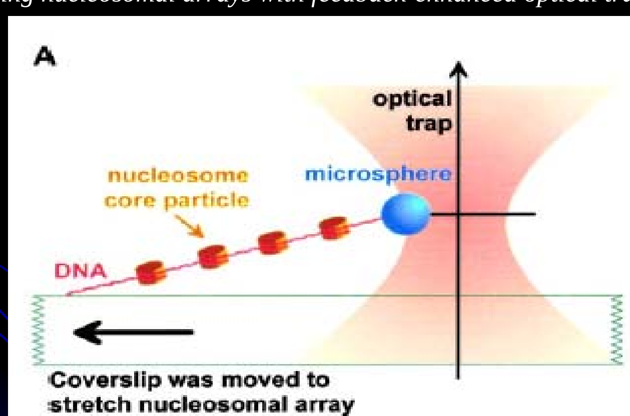
Studies of the dynamic structure of individual nucleosomes

(DNA organised into nucleosomes)

Stretching nucleosomal arrays with feedback-enhanced optical trap.



Brent D. Brower-Toland et al. PNAS, 2002

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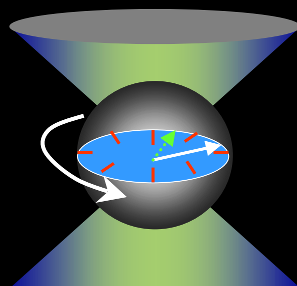
Rotating Tweezers

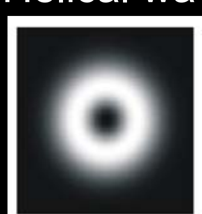
Creating torque and measuring it as it is applied



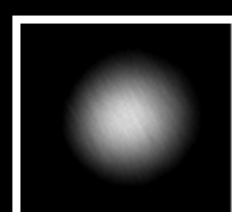
- Asymmetric particles in laser beam
- Special laser beams
- Multiple traps

Optical Angular Momentum I

Helical wavefront ('orbital' a.m.)



- 'optical vortex'
- 'singular beam'
- 'Gauss-Laguerre' GL_{pl}



Gaussian beam

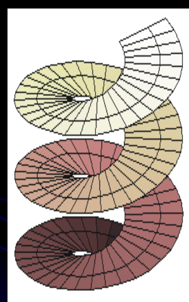
$l = 1$

AM = *integer* x $\hbar$ per photon

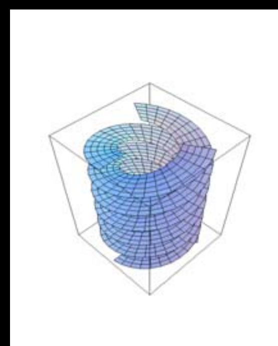
Phase singularity on axis, dark spot
generated by laser, or phase plate or hologram

Optical Angular Momentum – 'orbital'

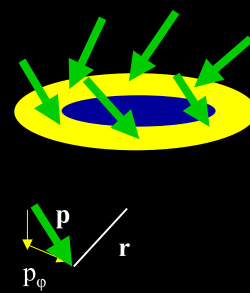
Helical wavefront



l = 1



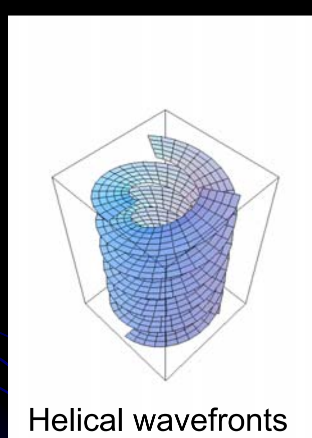
l = 3



L= r x p

Associated with spatial distribution

Laser beam with orbital a.m.



l = 3

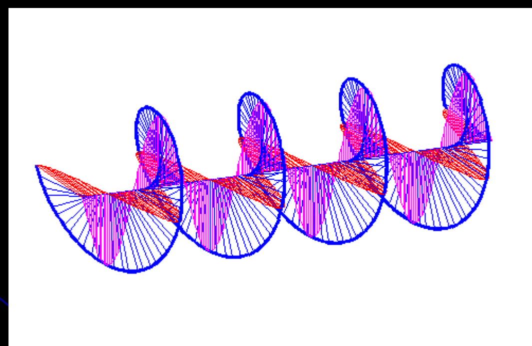
Absorbing particle

He *et al*., *PRL (1995)*

Not useful because of heating

Optical Angular Momentum II

Spin



Circularly polarised light…….ħ per photon (spin) 35

Transfer of AM to a waveplate

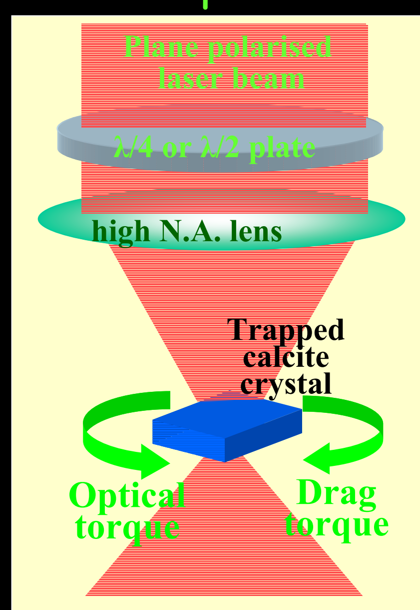
• $CaCO_3$ particles in H_2O are 3-D trapped in polarised light

• 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

alignment

Optical axis of crystal aligns to electric field vector

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Calcite crystal rotates in circularly polarised light

Friese et al, Nature 394, *348-350, 1998*

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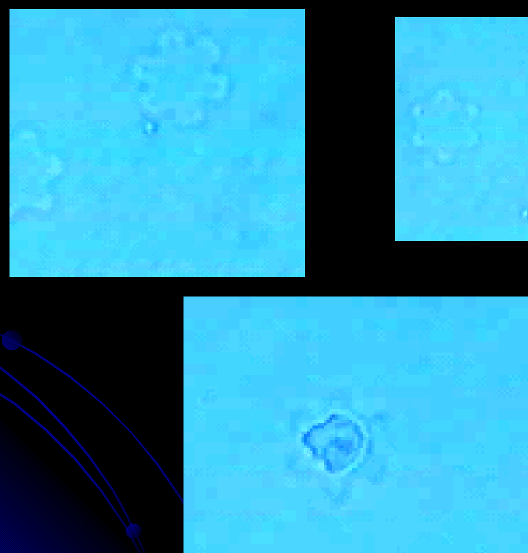
All optically driven micromachine elements



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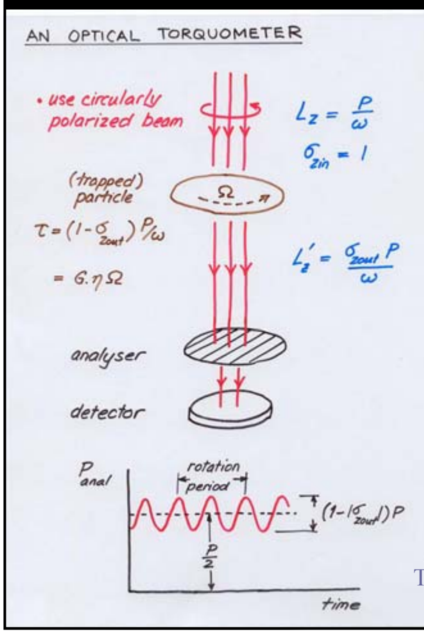
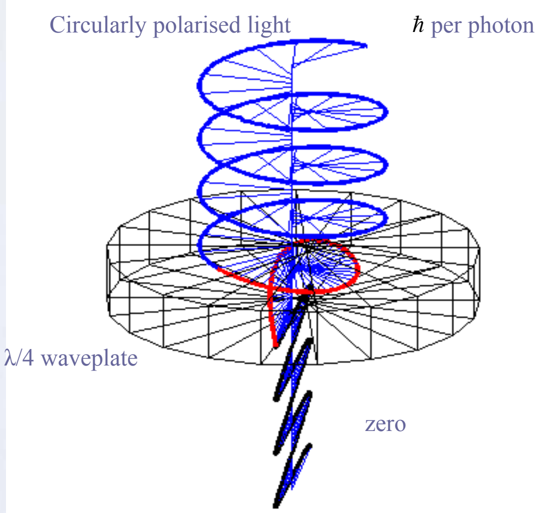
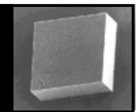
Friese et al, Appl. Phys. Lett. 78, 1, 2001

Micro-rheology





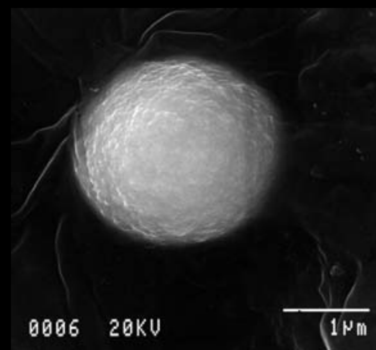
Try to maintain circular symmetry to avoid orbital A.M.



Making better probes

Spherical $CaCO_3$ Crystals

optical image



SEM image

Better probe particles
- smooth rotation
- 3D trapping

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Alexis Bishop et al, PRL 2004

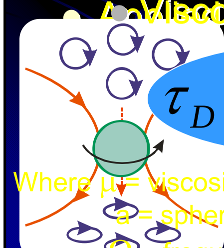
Theory

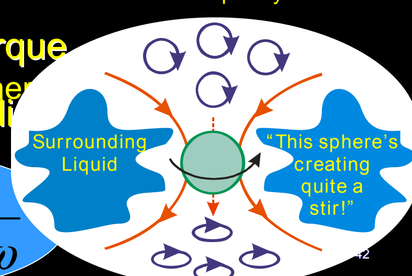
- Viscosity => ratio of stress to strain $\mu = \frac{\alpha}{\dot{\gamma}}$ Where α = shear stress, $\dot{\gamma}$ = shear rate
- Applied Torque $\frac{\Delta\sigma P}{\omega}$ Where $\Delta\sigma$ = change in polarisation, P = laser power, ω = optical frequency
- We measure viscosity by applying a stress to the fluid using a sphere rotated by circularly polarised light
- Drag Torque $8\pi\mu a^3\Omega$ Where μ = viscosity of liquid, a = sphere's radius, Ω = frequency of rotation
- The response of the fluid ... torque
- Applied Torque = Drag Torque
- Viscous drag torque

$$\tau_D = 8\pi\mu a^3\Omega$$

$$\mu = \frac{\Delta\sigma P}{8\pi a^3\Omega\omega}$$

Where μ = viscosity of surrounding liquid
a = sphere's radius
Ω = frequency of rotation





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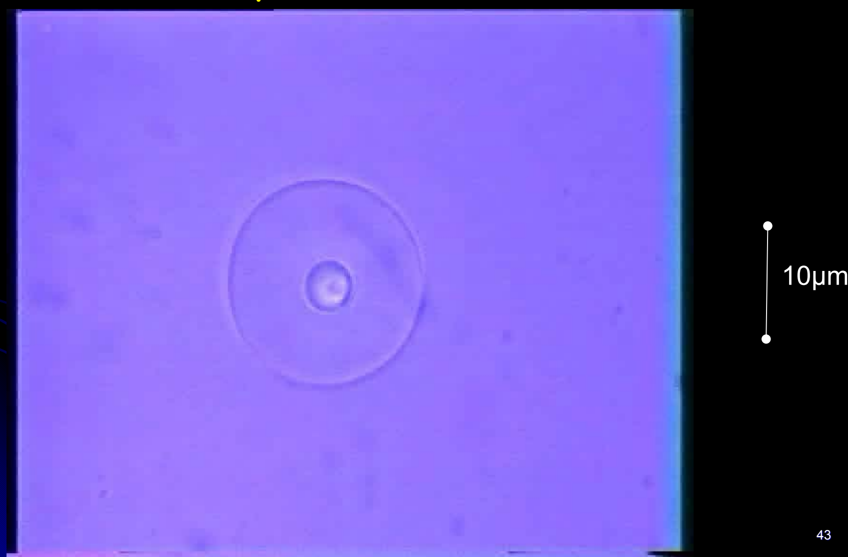
$CaCO_3$ sphere spinning inside an artificial liposome (15μm dia)



Alexis Bishop et al, PRL 2004

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Four vaterite spheres rotating inside an artificial liposome

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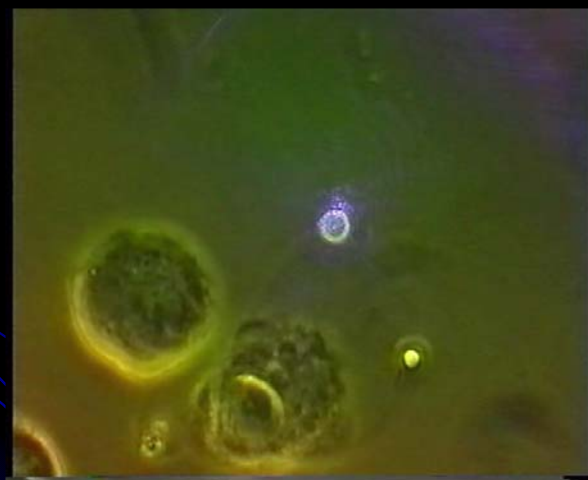
Laser tweezers, scissors and wrench



Gregor Knöner

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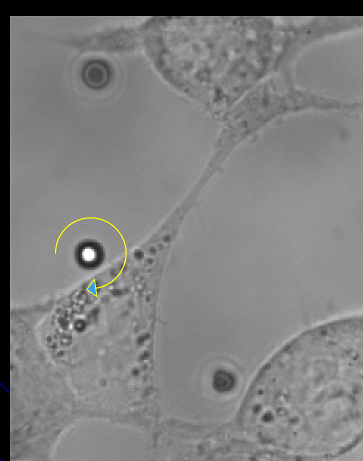
Fluid-Coupled MechanoTransduction

Flow-Coupled Shear Stress



Elliot Botvinick, Gregor Knöner et al

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Optically driven micromachines

Simon Parkin

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Optically driven micromachines

Gregor Knöner

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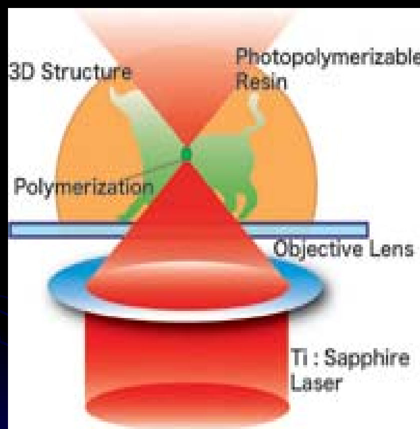
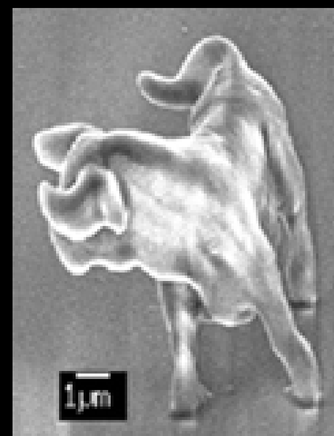
Two-photon photopolimerization



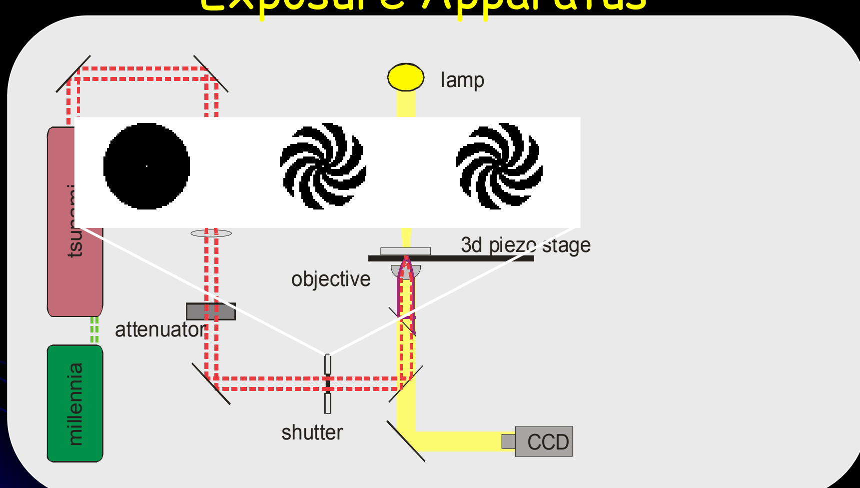
Two-photon initiated photopolymerization for three-dimensional fabrication



SEM image of a micro-bull sculpture.

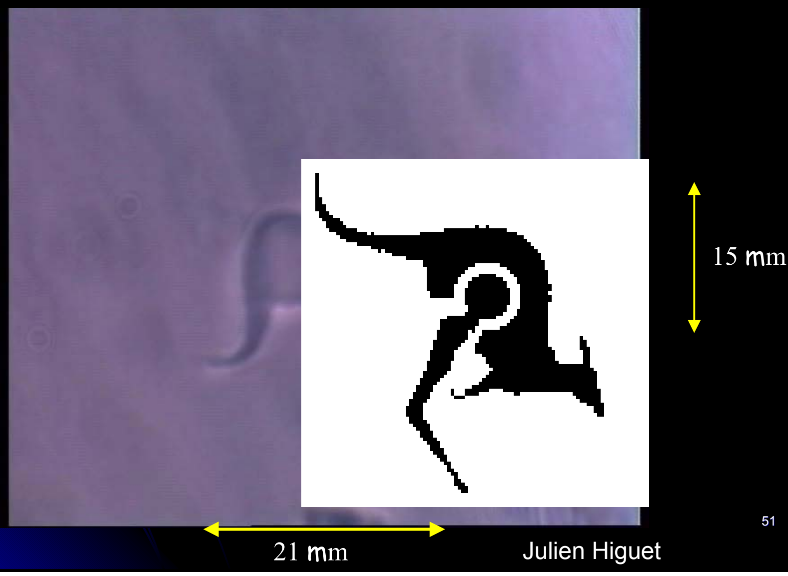
Kawata, Japan 2001

Exposure Apparatus



- subsequent production and trapping
- Ti:Sapphire laser, 1 W, 80fs, 780nm for production of structure

Two-photon photopolimerization at UQ



Julien Higuet

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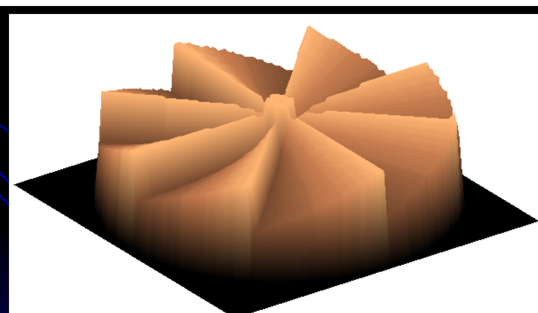
Microhologram for Lab-on-Chip

- on axis phase hologram

  → transfer of angular momentum to linear polarized Gaussian beam



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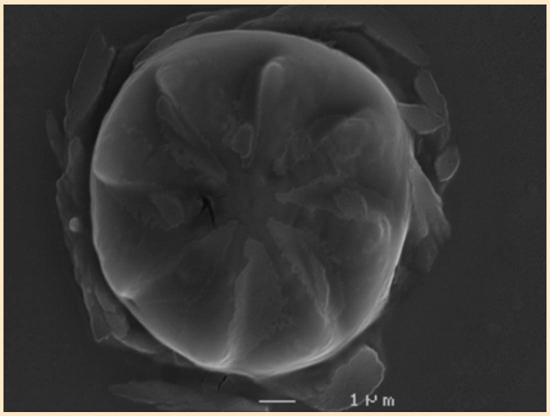
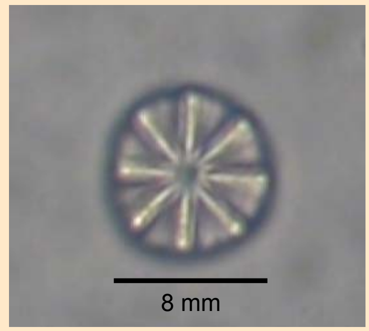
SEM of phase hologram





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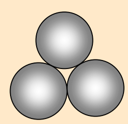
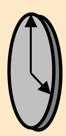
Angular momentum transfer

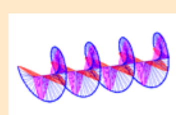
- polystyrene spheres, d = 2.1 μm
- incident beam: Gaussian, linear polarized



linear polarized beam
no angular momentum

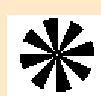


λ/4 waveplate

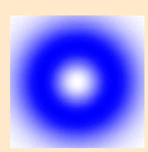


circularly polarized
spin angular momentum
→ transfer to anisotropic particle

TEM_{00}, linear pol.
no angular momentum



phase hologram



LG_{04}, helical wavefront
orbital angular momentum
→ transfer to anisotropic particle

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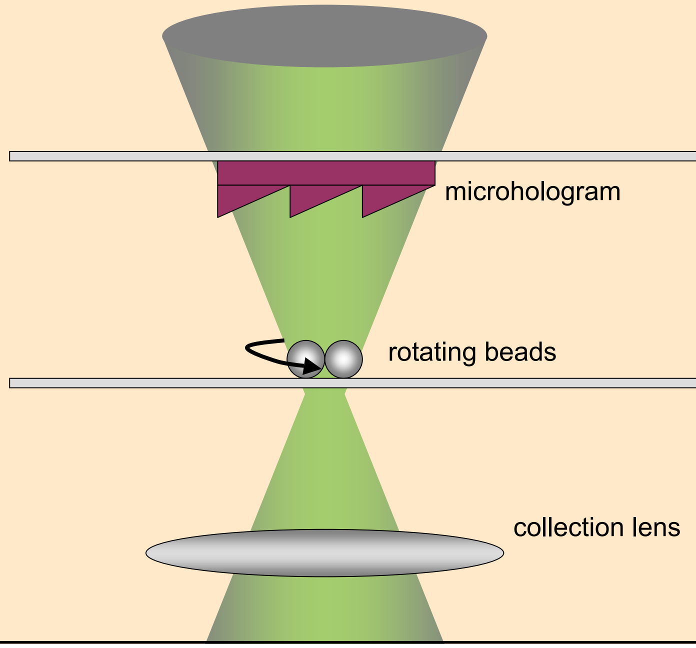
Setup for Microhologram Rotation



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Rotating beads with Microhologram

- incident laser beam: linearly polarized, Gaussian

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UQ Laser tweezers team

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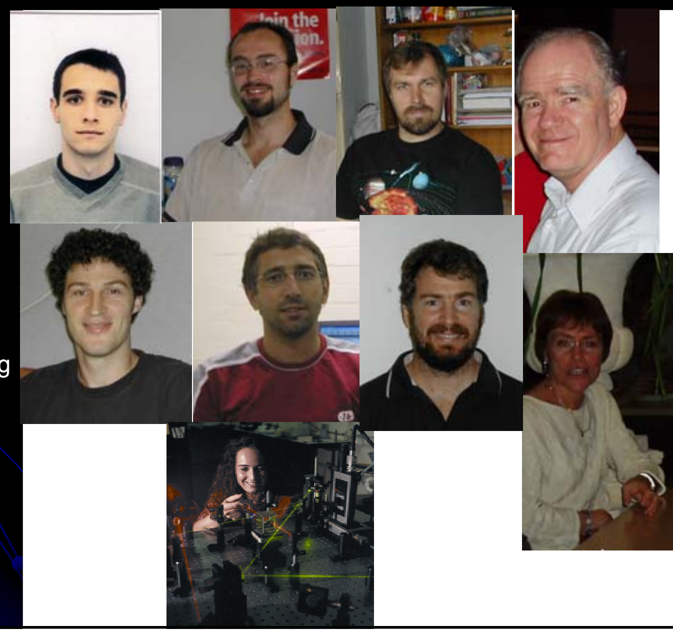

Thank you for your attention !!!

Simon Parkin

Rotation using orbital AM

LG_{02} doughnut beam

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