Optical tweezers -assisted measurements of elastic light scattering

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Motivation

• Light scattering phenomenon is present when using optical methods to study turbid materials and tissues
• Good theoretical models are needed
• It is important that models can be verified with measurements

• Reduction in scattering?
  – Optical clearing, i.e. Reractive index matching
Modeling vs. measurement

It is important that theoretical models can be verified with experiments.

Figure. Modeling (left) and measurement (right) for different size of polystyrene spheres.

http://www.philiplaven.com/mieplot.htm
Some existing theoretical models

Modeling of light scattering from RBC is more demanding


Simulation and modeling papers:
• How to measure light scattering from a single particle or red blood cell or several cells at different orientations?
Principles of optical trapping

- Axial force
- Gradient force

Microscope
Objective
High numerical aperture is needed.
How the fix the position of RBC in optical trap?

- Two beam optical tweezers system
  - Two Gaussian beams
  - Elliptical and Gaussian beams

Red blood cells in elliptical optical tweezers

M.Sc. Thesis, Antti Kauppila, Physics Department, University of Oulu, Finland (2009)
Optical tweezers setup

Figure. Optical tweezer setup with Gaussian and elliptical tweezers.
Light scattering measurements

Setup

- A He-Ne laser (05-LHP-151, Melles Griot), 5 mW
- Vertical polarization was used
- Cylindrical cuvette (shortened version of Helma 540.115)
- Amplified photomultiplier tube (PMT) (Thorlabs PMM02)/ PMT H9305-04 (Hamamatsu) and photon counting (Stanford Research Systems)
- A motorized rotation stage (Standa 8MR190-2-28)

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Figure. Red blood cells in elliptical tweezers during measurement.

No scattering is allowed from the background medium
Sample preparation

- RBCs were diluted in a filtered PBS solution
- The PBS solution was filtered three times using a 0.2 μm filter (GELMAN Acrodisc 13 CR PTFE)
- Polystyrene spheres were diluted in a filtered distilled water
- Glycerol/Water mixture was filtered three times
- Low background signal is a key issue
Measurements from single particles and RBCs

Arrow shows the direction of the incoming light.

Two point tweezers, light scattering measurements from double spheres and RBCs in different positions

Figure A. Single RBC in point and elliptical optical tweezers (a), the same RBC illuminated with a He-Ne laser (b), two cells in elliptical optical tweezers (c), and two RBCs illuminated with a He-Ne laser (d). Arrows show direction of the incident laser light. The scale bar is 10 µm.
Figure B. Measured scattering patterns from one and two RBCs. (Cells are shown in Fig. B.)
Figure. Two RBCs in rim-on position (a), near-field intensity image of scattering light from two cells (b) and measured light scattering signals (c). Arrow shows direction of the incident laser light. The scale bar is 10 µm.
Matching of refractive indices

Adding of optical clearing agent

Scattering is reduced
Now we study this effect at a single particle level
Optical clearing at single particle level, theory

Modeling of light scattering of a polystyrene sphere with two different refractive indices for the background medium.

RI of Sphere = 1.59
RI of background = 1.33
RI of 20% glycerol = 1.3542
Wavelength = 632.8 nm
Sphere size: 23.25 µm
Measured light scattering patterns from a sphere in two different background medium with different refractive indices.

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Discussion

• Problems in measurements
  – Background scattering signal increases, when some clearing agent is added to the PBS or water (e.g. glucose, dextran)
  – This makes the measurement challenging
  – High optical power is used in optical clearing measurements to keep the particles in place because matching of refractive indices decreases trapping efficiency and induce buoyancy
Conclusions

• Light scattering measurements were demonstrated from:
  – Single particles
  – Single RBC at different orientations
  – Two spheres
  – Two cells at different orientations

• Refractive index matching and the effects on light scattering was shown at a single particle level
• Scattering phase functions of 23.25 µm polystyrene spheres show a clear shift in the position of intensity maxima at angle range of 90-120 degrees
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