Cellular imaging using Nano-Materials

A Case-Study based approach
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Agenda

• Discuss a few papers
• Explain a couple of new imaging techniques and their benefits over conventional imaging
• New avenues
GOLD NANO-PARTICLES IN BIOLOGY: BEYOND TOXICITY TO CELLULAR IMAGING
Plasmon

Plasmons
Jellium model in metals

Discrete positive nuclei → Approximated by continuous, immobile positive charge distribution
Free Electron cloud

Jellium

Displacement → Coulomb Restoring Force
Plasmon Oscillation!

Collective, coherent oscillation of charge
Photographs of aqueous solutions of gold nanospheres (upper panels) and gold nanorods (lower panels) as a function of increasing dimensions. Corresponding transmission electron microscopy images of the nanoparticles are shown; all scale bars ) 100 nm.

The difference in color of the particle solutions is more dramatic for rods than for spheres. This is due to the nature of plasmon bands (one for spheres and two for rods) that are more sensitive to size for rods compared with spheres. For spheres, the size varies from 4 to 40 nm (TEMs a-e), whereas for rods, the aspect ratio varies from 1.3 to 5 for short rods (TEMs f-j) and 20 (TEM k) for long rods.
Optical spectra of gold nanoparticles of various shapes, showing positions of the plasmon bands.

The sphere diameter is 8 nm; aspect ratio of the short rods is 3; aspect ratio of the long rods is 20.
THE EXPERIMENT

• Gold nanorods were added to thin collagen films and subsequently plated with neonatal cardiac fibroblasts, the cells responsible for depositing and modifying the ECM.

• Positional displacements of the gold nanorods, generated by the traction forces applied by the cells through their attachments to the ECM, were tracked.

• Material response under loading is observed using gold nanorods.

• Displacement is measured in this case in terms of strain.

• Image correlation analysis is used.
Two Photon Luminescence (TPL)

• Normal optical absorption
  \[ \frac{dl}{dx} = -\alpha l \rightarrow l(x) = l_o e^{-\alpha x} \]
  \( \alpha \) has units of \( \text{cm}^{-1} \)

• Two-photon (non-linear) absorption
  \[ \frac{dl}{dx} = -\beta l^2 \rightarrow l(x) = \frac{l_o}{1+l_o\beta x} \]
  \( \beta \) has units of \( \text{cm/Watt} \)
Theory - Maria Göppert-Mayer, 1929
Experimental observation – 1961

Probability of excitation ($W$) $\sim$ (Intensity)$^2$
$W \sim (I \text{[photons/cm}^2\text{/s]})^2$
Advantages of TPL:

• Optical sectioning (automatically confocal)
• High axial resolution
• Minimized out-of-focus absorption
• Minimized out of focus photobleach/photodamage
• High penetration depth
Fluorescently stained cardiac fibroblasts (top left); scale bar 100 μm. Gold nanorods are present but are not visible by fluorescence. In the same sample but imaged by dark-field optical microscopy, gold nanorods (top right) scatter orange-yellow light. Contour plots are of (bottom left) horizontal strain, $\varepsilon_{xx}$, and (bottom right) vertical strain, $\varepsilon_{yy}$. The colored scale bar goes from compressive strain of -0.12 (purple) to tensile strain of 0.15 (red). The circled region shows that where there was extensive cell movement that deformed the matrix, significant vertical ($\varepsilon_{yy}$) and horizontal ($\varepsilon_{xx}$) strain occurred.
Prospects for the future

• attractive alternative to traditional organic fluorescent dyes
  – they do not photobleach outside the focal volume,
  – they can absorb throughout the visible and NIR,
  – they can be nontoxic under certain experimental conditions.

• specific detection, imaging, and therapy for very particular target cells (e.g., certain cancers, bacteria).
TWO-PHOTON LUMINESCENCE IMAGING OF CANCER CELLS USING MOLECULARLY TARGETED GOLD NANORODS
The Experiment

• Gold nanorods synthesised
• Conjugated with anti-EGFR antibody
• A line of skin cancer cells known to over express EGFR was used to make tissue phantoms
• Non specific antibody was used as a control
• TPAF and TPL were used to image the cells – at varying depths
Two-photon images of cancer cells placed on a coverslip from a cell suspension. (a) TPAF image of unlabeled cells. (b) TPL image of nanorod labeled cells. Imaging required 9 mW of excitation power in unlabeled cells to get same signal level obtained with only 140 μW for nanorod labeled cells, indicating that TPL from nanorods can be more than 4,000 times brighter than TPAF from intrinsic fluorophores. (c) TPL image of non-specifically labeled cells.
Two-photon imaging of cancer cells embedded in a collagen matrix at increasing depths. 
(a) TPAF imaging of unlabeled cells and (b) TPL imaging of nanorods labeled cells. Both samples required the same excitation power increase of 26% at each 20μm depth increment to maintain constant emission intensity.
THREE-DIMENSIONAL HARMONIC HOLOGRAPHIC MICROCOPY USING NANOPARTICLES AS PROBES FOR CELL IMAGING
Dynamic light scattering measurement of 90-nm BaTiO3 particles colloidal suspension.

Inset: Scanning electron microscope image of a single 90-nm BaTiO3 particle.

SHRIMPS: Second Harmonic Radiation IMaging Probes.
Second Harmonic Generation (SHG)

When a nanocrystal of non-centrosymmetric structure is optically excited at a fundamental frequency, it emits the optical signal at the exact doubled frequency. Only materials with crystalline structures lacking a center of symmetry are capable of efficient SHG.

When imaged at the second harmonic frequency, SHRIMPs provide an effective mechanism of contrast between the markers and the generally unstructured or isotropic biological microenvironment.
Advantages of SHG

• Unlike fluorescence, the process of SHG does not involve electron energy transition and therefore has nonradiative energy loss.
• SHRIMPs do not bleach over time and emit a stable, nonsaturating signal with a femtosecond-scale response time.
• This also results in the flexibility of tuning the wavelength of the SHG signal by changing the excitation wavelength accordingly.
• The coherent nature of the SHG signal is also a main advantage, providing a possibility to detect the second harmonic signal with interferometric optical techniques
Power dependence of the SHG signal from BaTiO₃ nanoparticles in double logarithmic scale. The squares are measured results and the solid line is the linear fit with the slope of 2.2.

The inset shows the SHG optical spectrum centered at 400.6 nm with a full width half maximum of 5 nm.

Experimental parameters: 15fs laser pulses at 800nm from a Ti:sapphire oscillator.
Polarization dependent SHG response measured from an isolated BaTiO3 nanocrystal. Dots: experimental data. Line: Theoretical calculation based on the second order susceptibility of a bulk BaTiO3 crystal oriented at $\phi_0 = 90$ degree and $\theta_0 = 30, 50, 70, 90$ degree respectively. The red arrow in the polar diagram indicates the projection of the c-axis of the nanocrystal on the XY plane.
Spatial resolution of the H2 microscope measured by imaging an isolated BaTiO3 particle.

a) SHG intensity image measured by a conventional microscope.
b) Holographic reconstructed intensity image at the object plane.
c) Cross section of a stack of holographic reconstructed intensity images along the axial direction. The scale bars are 2 μm.

Single SHRIMP detection with the H2 microscope
Images of SHRIMPs embedded in PDMS.

(a)-(d): SHG images of SHRIMPs on four different planes, focusing by moving the optics with a conventional microscope.

(e)-(h): Holographic reconstructed images of SHRIMPs on four corresponding planes, focusing by digital reconstruction. The relative depths of these four planes are 0, 9.4, 17.2, and 20.3 μm respectively. Scale bars are 2 μm.
3D imaging of the SHRIMPs in HeLa cells

HeLa cells were incubated for 24 hours at 37 oC with 30-nm BaTiO3 particles that have been stabilized with aminomethylphosphonic acid.
Images of SHRIMPs non-specifically labeling HeLa cells (a) Superposition of the bright field transmission image of a HeLa cell (in red) and the SHG image of SHRIMPs (in green) taken by a conventional microscope. Six SHRIMPs clusters assigned with numbers were under analysis. Some of them cannot be seen clearly because they are out of focus. (b)-(d) H2 reconstructed images at three different planes with relative depths of 0 μm, 3.12 μm and 6.24 μm respectively. The white arrows show the SHRIMPs that are on focus, while the gray arrows show the SHRIMPs that are out of focus. The SHRIMP labeled with number 4 is a big cluster so that it is bright both in (b) and (c). (e) Normalized intensity line profiles of the six clusters when they are on focus through digital reconstruction. The scale bars are 5 μm.
New Frontiers

• Unique properties of SHRIMPS
  – long-term observation without photobleaching
  – flexibility in excitation wavelength,
  – coherent signals for 3D imaging,
  – ultrafast response time

• SHRIMPs are not replacements for fluorescent markers

• New modalities – polarization dependence… can rotation be monitored?
THE END
Some advantages of 2-photon excitation  versus one-excitation in confocal microscopy

Better Light collection efficiency.

Multi-photon excitation confines fluorescence excitation to a small volume at the focus of the objective. Photon flux is insufficient in out-of-focus planes to excite fluorescence. **No confocal pinhole is needed.** All fluorescence (even scattered photons) constitutes useful signal.

Photobleaching and photodamage are limited to the zone of 2P excitation and do not occur above or beyond the focus.

Larger penetration depth. IR photons travel deeper into tissue with less scattering and absorption comparing to visible photons. **Scattering $1/\lambda^4$!**

In practice - approximaterly 2 times larger penetration depth.

**Much smaller background from impurity fluorescence when IR laser is used in comparison with VIS or UV light.**

2 photon excitation spectra are usually very broad. Therefore, one laser source can be used for many different dyes having different fluorescence wavelengths. No chromatic aberration problems.