Raman and Fluorescence Imaging with Polymer Dot for Cell Biology

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Abstract

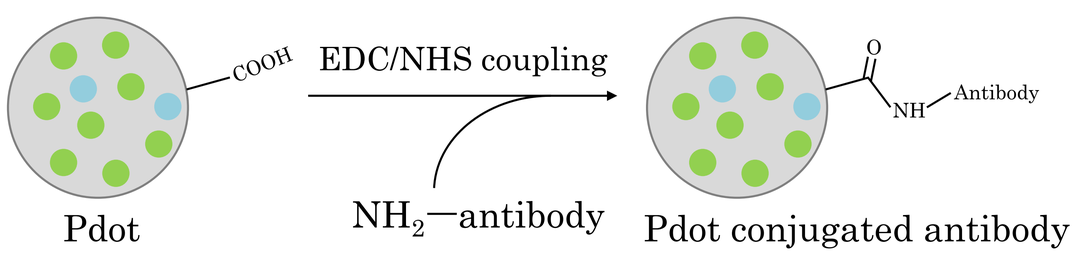
Raman scattering is an emerging contrast mechanism for biological imaging due to its narrow spectral bandwidth. However, the sensitivity is many orders of magnitude lower than that of fluorescence. Fluorescence offers extremely high sensitivity, but suffers from broad absorption and emission spectra. Since Raman and fluorescence are complementary to each other, we developed conjugated polymer-based nanoparticles (Pdots) as an imaging probe for both Raman and fluorescence for cellular imaging. The Raman-active vibrating groups in the Pdots that are electronically resonant to the π-conjugation system produced highly enhanced Raman scattering signals. When the Pdots were irradiated with 532 nm laser near the absorption maximum of the π-conjugated small molecule, the Pdot produced highly enhanced Raman signal for the vibrational modes at 1200-1800 cm^{-1}, while emitting high far-red fluorescence to the sensitivity level of detecting single particles. By conjugating the carboxylic groups on the Pdots and the amine groups on proteins such as avidins and antibodies, we labeled specific proteins in the cell with Pdots and performed molecular-specific cell imaging experiments. Imaging probe with both fluorescence and Raman-activity is a unique and powerful tool that combines high multiplexing of Raman and single-particle sensitivity of fluorescence.

Properties of Pdots using Donor-Acceptor combination

➢ Various Donor-Acceptor combinations

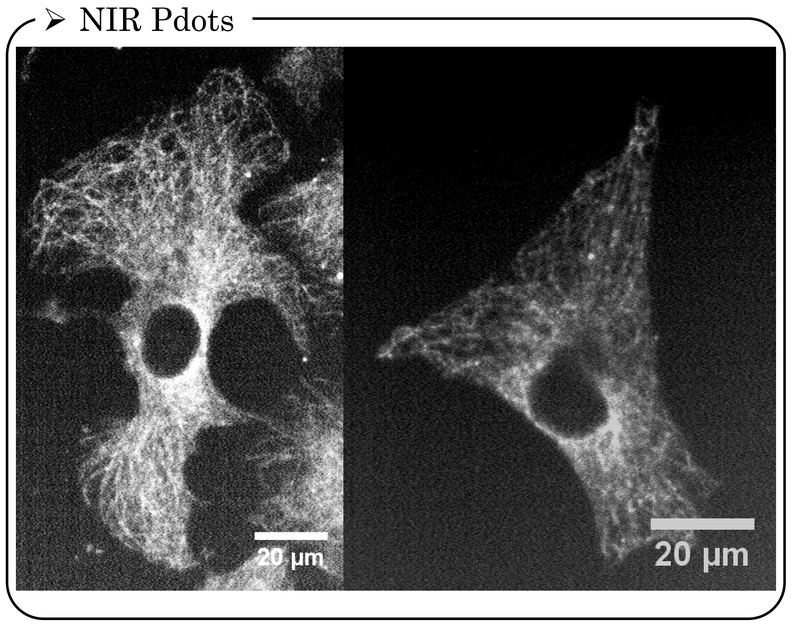
Pdots contain highly π-conjugated small molecules composed of electron donor and acceptor groups. Also the absorption region of Pdots can be adjusted from green to orange. These Pdots represent the emission region of far-red at ~ 700 nm band.

EDC/NHS coupling

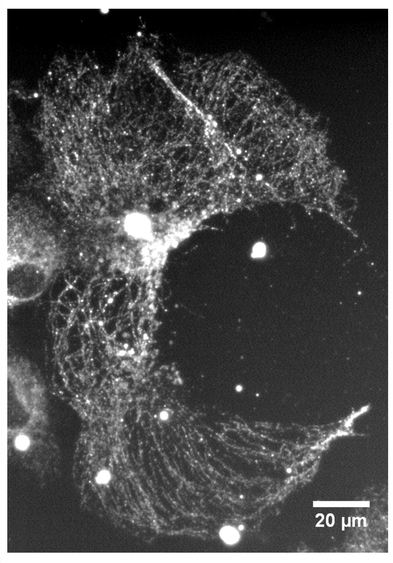


β-tubulin images of Pdots conjugated Antibody

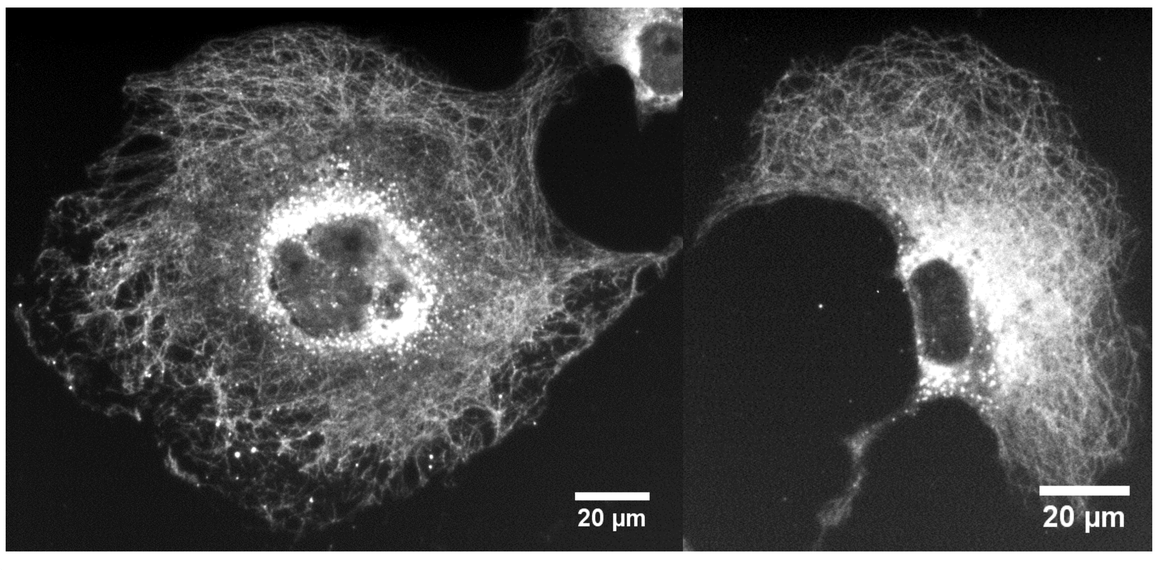
➢ NIR Pdots



➢ Pdot 2

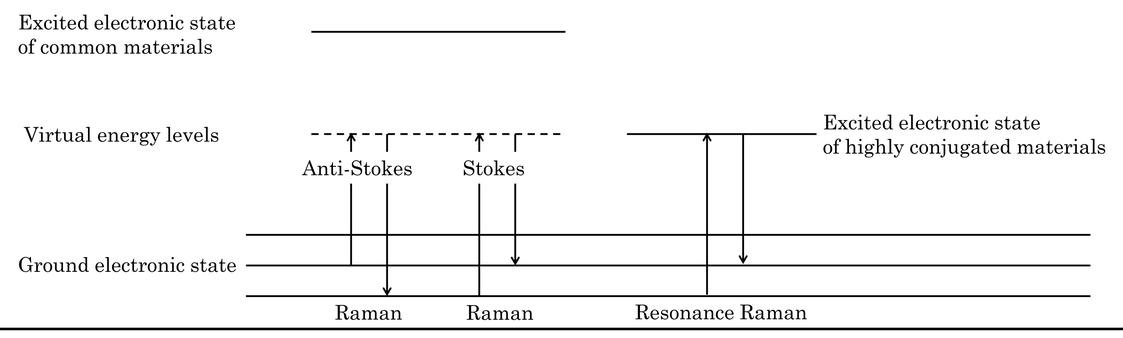


➢ Pdot 1

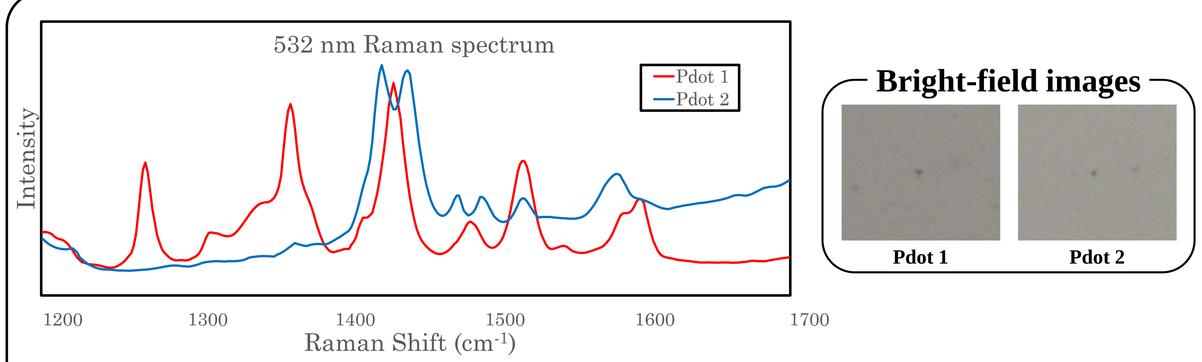


Resonance Raman Spectroscopy

The main advantage of resonant Raman scattering over non-resonant Raman is the large increase in intensity of the bands of interest (by about a factor of 10^6). This allows sensitive detection of Raman-active probes.

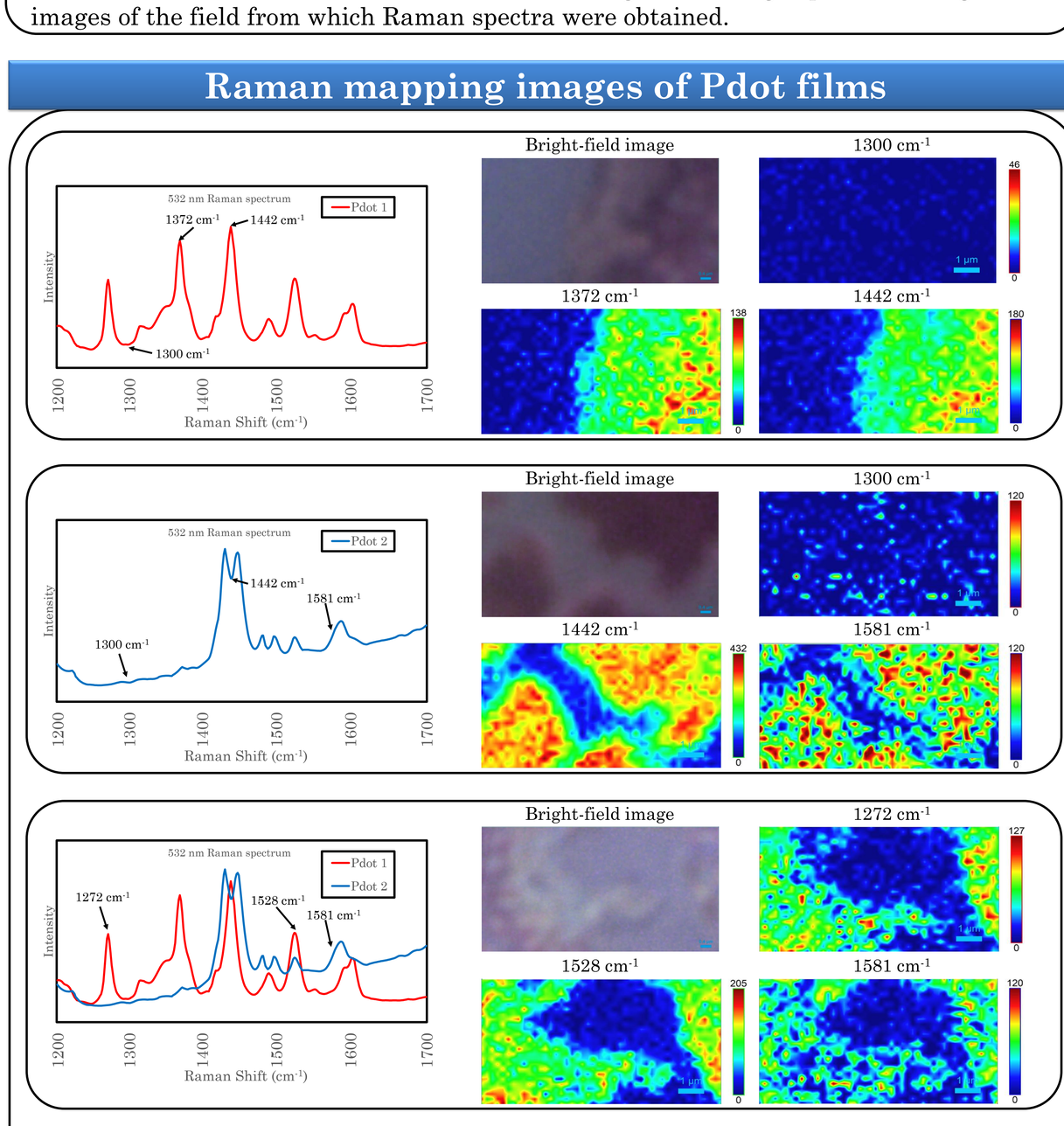


Raman spectra of Pdots



Raman spectrum of Pdot 1 and Pdot 2. Characteristic Raman peaks appeared in 1200 - 1700 cm^{-1}. We found that the π-conjugated Pdots produced high Raman signal by using 532 nm excitation due to resonance effect. The images in the right panel are bright-field images of the field from which Raman spectra were obtained.

Raman mapping images of Pdot films



(Left) Raman spectra of Pdot 1 and Pdot 2 film samples. (Right) Bright-field image & Raman mapping images at specific wavenumber.

Future directions

➢ Long-term Goal : Multiplexed barcoded Raman imaging of cellular proteins

1. Synthesis and screening other Pdots with similar chemical structures to make a library of Raman barcodes in 1200-1700 cm^{-1}.
2. Barcoded Raman mapping by using multivariat curve resolution (MCR) analysis over Raman spectra in the spectral range of 1200-1700 cm^{-1}.
3. Molecular-specific labeling with Pdots. The surface of Pdots can be modified with DNA to make it bind to DNA-conjugated antibody. Then, the DNA-labeled Pdots will be used for labeling specific proteins in cells.