Raman and Fluorescence Imaging with Polymer Dot

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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 Pdots with π-conjugated small molecules were prepared by utilizing poly(styrene-maleic acid) as a matrix that offers low toxicity, high photostability and easiness of surface functionalization. In the Pdots, the Raman-active vibrating groups that are electronically resonant to the π-conjugation system produced highly enhanced Raman scattering signal. When the Pdots were irradiated with 532 nm laser near the absorption maximum of the π-conjugated small molecule, the Pdots 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. 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.

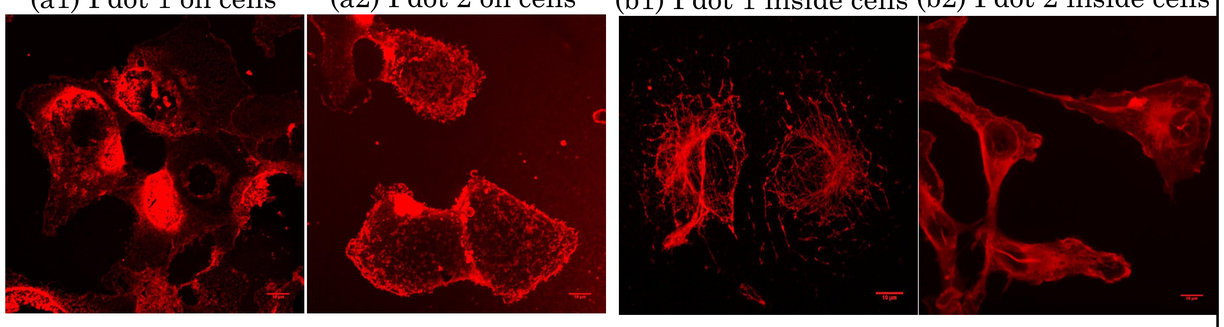
Properties of Pdots using Donor-Acceptor combination

➢ Various Donor-Acceptor combinations

Due to the electron donor and accceptor groups linked in Pdots, Pdots contain highly π-conjugated small molecules. 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.

Fluorescence images of Pdots in COS-7 cell

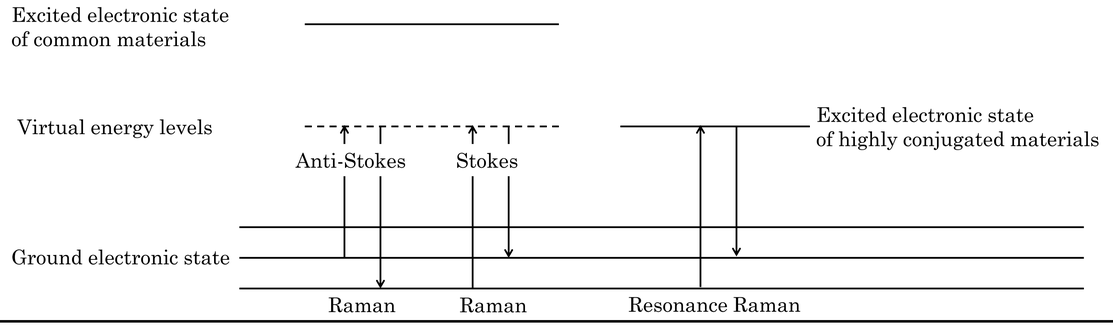
(a1) Pdot 1 on cells (a2) Pdot 2 on cells (b1) Pdot 1 inside cells (b2) Pdot 2 inside cells



Confocal fluorescence images of COS-7 cells with Pdots with excitation at 640 nm. (a1-a2) Pdots were sprayed on the surface of cells. (b1-b2) Pdots penetrated into the permeated cells. Scale bar : 10 μm.

Resonance Raman Spectroscopy

The main advantage of RR spectroscopy over non-resonant Raman spectroscopy is the large increase in intensity of the bands in question (by as much as a factor of 10^6). This allows RR spectra to be obtained with sample concentrations as low as 10^{-8} M.

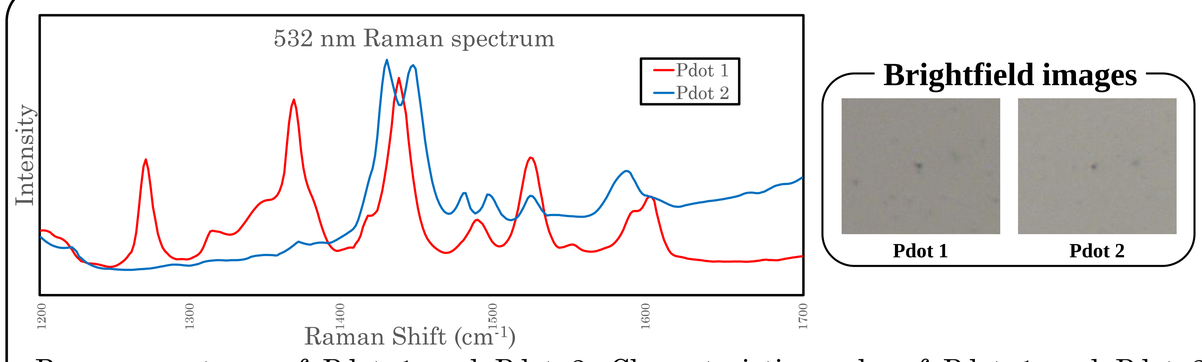


Future directions

➢ Goal : Multiplexed barcoded Raman imaging of cellular proteins and RNA

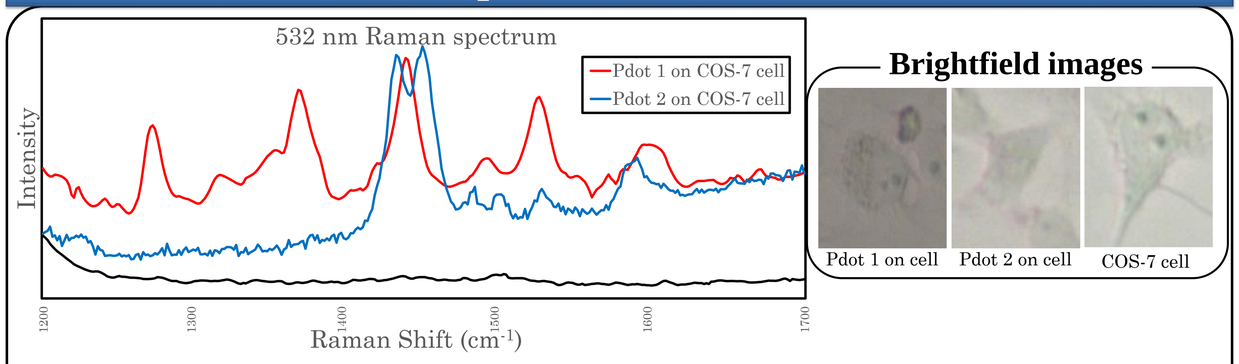
1. Synthesis and screening other Pdots with similar chemical structures to make a library of Raman barcodes in 1200-1700 cm^{-1}.
2. Barcoded Raman imaging of specific peaks by Raman mapping of Pdots on cell surface.
3. Molecular-specific labeling with Pdots. The surface of Pdots can be modified with DNA to make it bind to DNA-conjugated antibody. Then, the DNA-labeled Pdots will be used for labeling specific proteins in cells.

Raman spectra of Pdots



Raman spectrum of Pdot 1 and Pdot 2. Characteristic peaks of Pdot 1 and Pdot 2 appeared after 1200 - 1700 cm^{-1}. Therefore, we confirmed that the π-conjugated Pdots have characteristic peaks at low Raman intensities due to resonance effect. The above images are bright-field images of each spectrum.

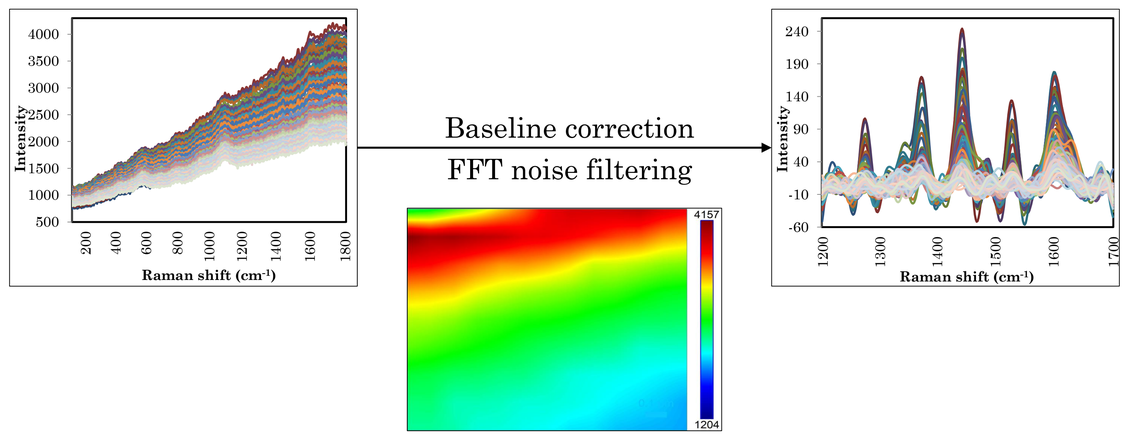
Raman spectra of Pdots on cell



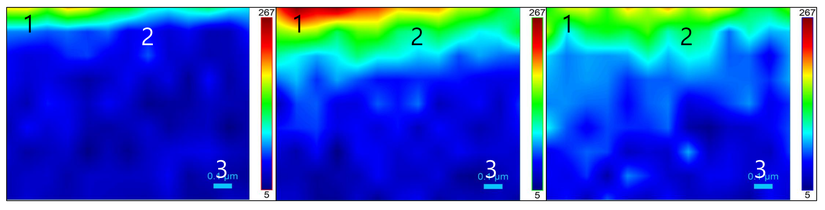
Raman spectrum of COS-7 cells. Pdot 1 on COS-7 cell (Red spectrum and left image), Pdot 2 on COS-7 cell (Blue spectrum and middle image), and COS-7 cell (Black spectrum and right image). The spectrum of COS-7 cell is too low for perturbing the characteristic Raman peaks of Pdots at 1200 - 1700 cm^{-1}. The multiple cell-free Raman peaks may be utilized as spectral barcodes for encoding each Pdot in multiple dimensions.

Raman mapping of Pdot 1 film

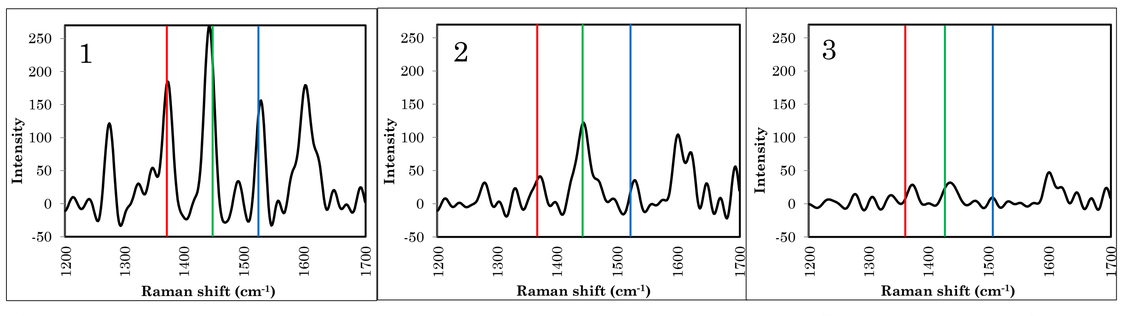
➢ Raman mapping of Pdot 1 film



- Raman mapping image at 1800 cm^{-1} (middle), raw spectra (left) and corrected spectra (right). Because of fluorescence emission of Pdot, fluorescence background appeared in all spectra and interfered with the Raman spectra. We corrected the Raman spectra in 1200 ~ 1700 cm^{-1} using line-based baseline correction and Fourier transform noise filter for correcting the artifact form fluorescence. Scale bar : 0.1 μm



- Raman mapping images at 1373.56 cm^{-1} (left image, red line in the spectrum below), 1443.47 cm^{-1} (middle image, green line in the spectrum below), 1531.46 cm^{-1} (right image, blue line in the spectrum below). Scale bar : 0.1 μm



- Raman mapping cursor spectrum at points denoted as 1 (left), 2 (middle), 3 (right). Since the film lies in the top of the field and the film is absent at the bottom of the field, the peaks of Pdot become smaller and the peaks disappear as the cursor is moved from top to bottom.