High-Throughput Chemical Imaging In Living Cells

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Introduction
Confocal Raman microscopy has been a powerful tool for in vivo biological analysis because 2D chemical distribution in a cell can be obtained. The bottleneck of current point-scan confocal Raman microscopy is the long acquisition time owing to the small intrinsic spontaneous Raman scattering cross-section and additional photo-bleaching step required by intense fluorescence background. Increasing laser intensity is certainly a way to shorten acquisition time. However, there is an upper limit for cell damage concerns. By exploiting massively parallel acquisition, line-scan is the most effective solution to increase throughput.

We present in situ 2D label-free chemical profiling in living cells based on high-throughput line-scan Raman microscope. We demonstrate the instrument capabilities in terms of spatial and spectral resolution, scanning speed and overall throughput using polystyrene beads and microalgae cells. We also combine other label-free techniques based on elastic light scattering and surface enhanced Raman spectroscopy (SERS) into our system. In elastic light scattering mode, measurement of the scattering spectrum or angularly resolved scattering pattern can yield detailed information about the cell structure. In SERS mode, enhanced Raman spectra from cell surface are collected. All modalities share the same collection path in our system. This guarantees perfect overlay of structural and chemical imaging. This method has the potential to be applied to high-throughput screening of multiple intra-cellular chemical constituents and structural information for living cells.

Whole Cell Chemical Imaging – Raman Microscopy
We demonstrate our Raman chemical imaging capability using microalgae cells which are representative living cells currently we have. Carotenoids and different lipids can be clearly identified. Most carotenoids (1155 cm⁻¹, 1520 cm⁻¹), saturated lipid (1440 cm⁻¹) and liquid hydrocarbon oils (1639 cm⁻¹-1670 cm⁻¹) are in the cell membranes and colony extracellular matrix. 1639 cm⁻¹, 1647 cm⁻¹, 1647 cm⁻¹ bands are specific for indicating methylated liquid hydrocarbons (C₃₂, C₃₃, C₃₄).

Structural Profiling – Elastic Light Scattering Microscopy
In the elastic light-scattering microscopy mode, optical path length difference (OPL) from reference plane can be calculated using a slab model. We have observed OPL dependent scattering spectra from 10μm polystyrene beads with known refractive index ~1.57. At the center location of the polystyrene bead as indicated by the Raman map, we calculated a thickness of 9.8μm from the elastic light-scattering spectrum; 3.5μm is calculated from the halfway location between the center and the circumference.

Cell Surface Chemical Imaging – Surface Enhanced Raman Microscopy
Surface chemical information can be obtained by surface enhanced Raman spectroscopy because only Raman peaks from the surface of the cell are enhanced. We have developed an uniformly dense SERS substrate with enhancement factor of 7.5×10⁶ measured from benzethiol self assembled monolayer.

Conclusion
We present a high-throughput chemical imaging system based on line-scan Raman microscope. The system is integrated with elastic light scattering microscopy and surface enhanced Raman microscopy techniques. The Raman mode is able to obtain chemical information from a whole cell, the SERS mode can measure chemical composition from cell surface and the elastic light scattering mode is capable to provide structural information or refractive indices of the cell. The instrument is well-suited for studying in vivo biochemical processes without staining or labeling the cells.

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Reference