High-Throughput Chemical Imaging In Living Cells

Ji Qi, Pratik Motwani, Jack Wolfe & Wei-Chuan Shih
Department of Electrical & Computer Engineering
University of Houston

Taylor Weiss & Timothy Devarenne
Department of Biochemistry & Biophysics
Texas A&M University

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 microscope 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.

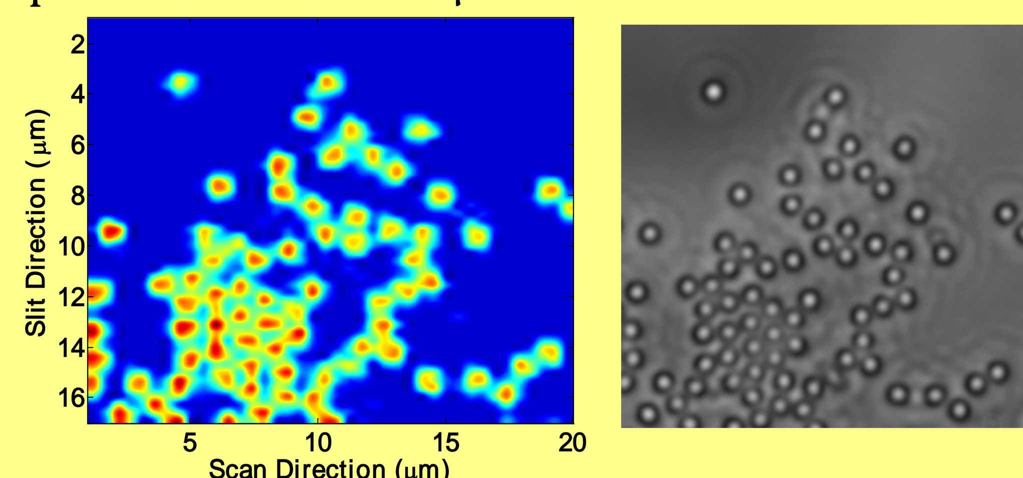
We present *in situ* 2D label-free chemical profiling in living cells based on high-throughput line-scan Raman microscope. We demonstrate the instrument capabilities in terms of spatial and spectral resolution, scanning speed and overall throughput using polystyrene beads and microalgae cells.

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 images.

This method has the potential to be applied to high-throughput screening of multiple intra-cellular chemical constituents and structural information for living cells.

Line-Scan Raman Microscopy System

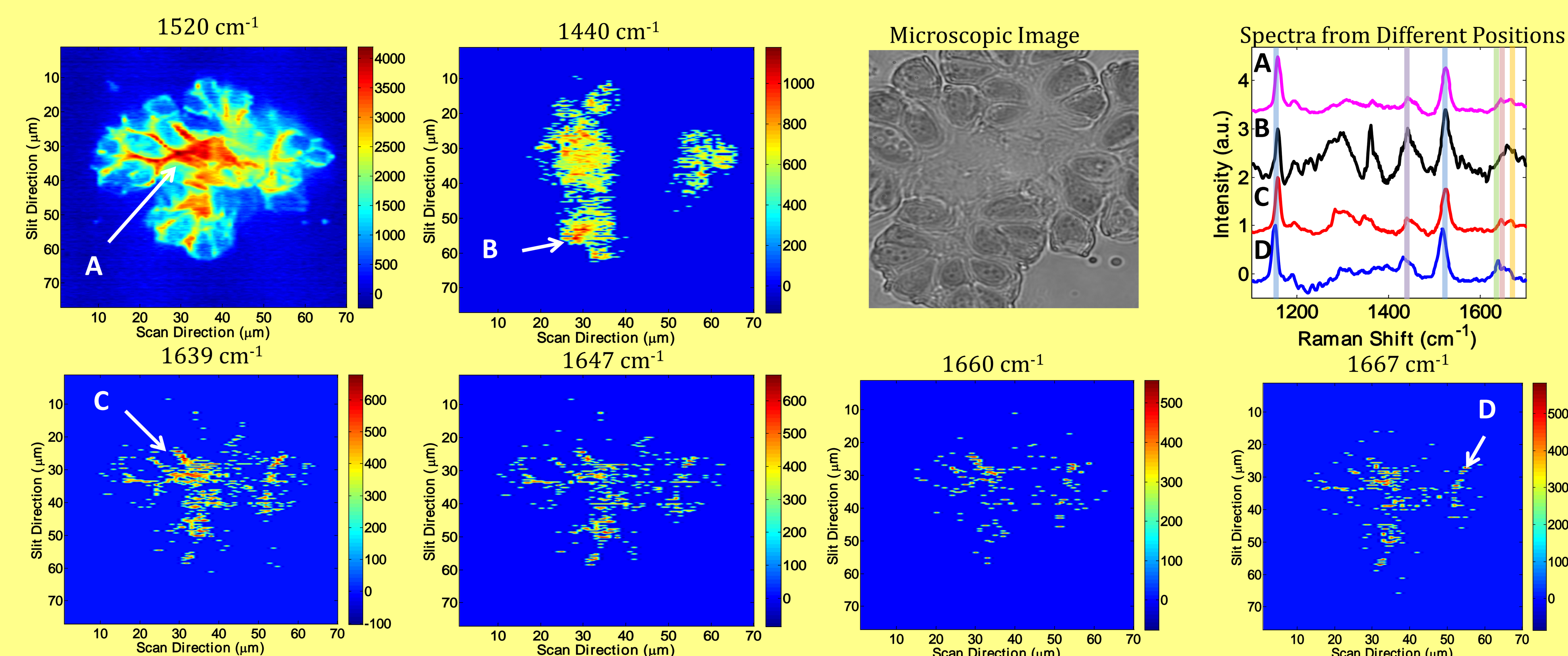
- uniform power distribution along a 100µm line
- power density approaching damage threshold
- mapping 100 × 100µm^2 area < 5min using 750ms laser dwelling time at each scan step
- ~400× increase in mapping throughput
- spatial resolution <1µm



Part of Raman Mapping of 1µm Polystyrene Beads from 100 × 100µm^2 area using 1001cm^{-1} (Aromatic Ring Breathing) and Microscopic Image

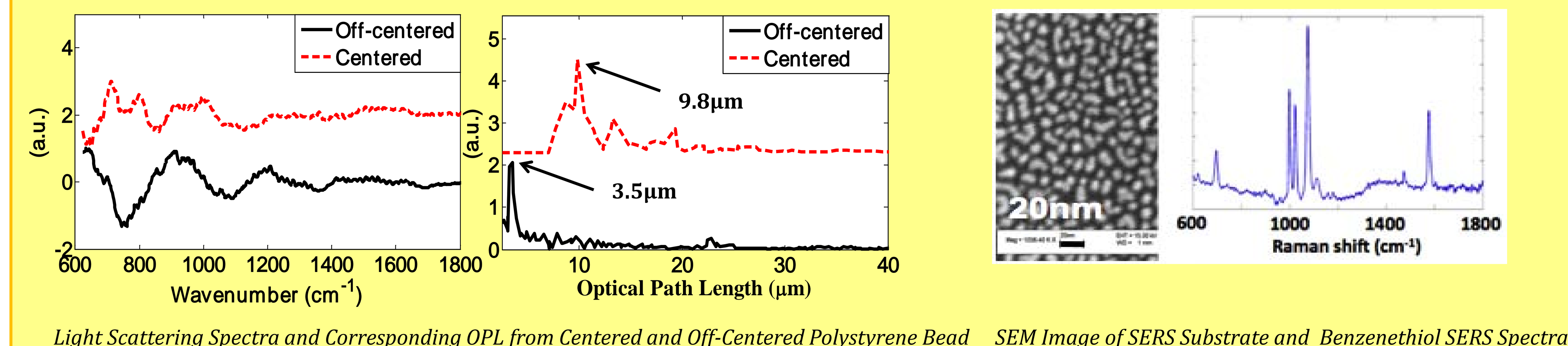
Whole Cell Chemical Imaging – Raman Microscopy

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 (1155cm^{-1}, 1520cm^{-1}), saturated lipid (1440cm^{-1}) and liquid hydrocarbon oils (1639cm^{-1}-1670cm^{-1}) are in the cell membranes and colony extracellular matrix. 1639cm^{-1} ,1647cm^{-1},1647cm^{-1} bands are specific for indicating methylated liquid hydrocarbons (C_{31},C_{32},C_{33} and C_{34}).



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.



Light Scattering Spectra and Corresponding OPL from Centered and Off-Centered Polystyrene Bead

SEM Image of SERS Substrate and Benzenethiol SERS Spectra

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 7.5×10^5 measured from benzenethiol 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.

Acknowledgement

Wei-Chuan Shih thanks the Cullen College of Engineering at the University of Houston for his start up funding.

Reference

[1] Kenneth A. Christensen, Michael D. Morris, 'Hyperspectral Raman microscopic imaging using Powell lens line illumination', App. Spec, vol. 52, no.9, 1998.

[2] Keisaku Hamada, Satoshi Kawata, 'Raman microscopy for dynamic molecular imaging of living cells', J. Biomed. Opt., vol. 13, Jul/Aug. 2008.

[3] Y.Y. Huang, E.M. Terentjev, 'Micro-Raman spectroscopy of algae: composition analysis and fluorescence background behavior', Biotech & Bioeng, vol. 105, no. 5, 2010.

[4] Taylor L. Weiss, Timothy P. Devarenne, ' Raman spectroscopy analysis of Botryococcene hydrocarbons from the green microalga Botryococcus braunii', J. Biol. Chem, vol. 285, no. 42, 2010.

[5] Pin Wang, Yang Liu, 'Spatial-domain low-coherence quantitative phase microscopy for cancer diagnosis', Opt. Lett., vol. 35, no. 51, 2008

[6] Adam D. McFarland, Richard P. Van Duyne, 'Wavelength-scanned surface-enhanced Raman excitation spectroscopy', J. Phys. Chem, vol. 109, no. 22, 2005.