Broadband Coherent Anti-Stokes Raman Scattering Microscopy (μCARS) for Noninvasive Imaging of Cell Phenotype

Marcus.T. Cicerone, Sapun H. Parekh, Young J. Lee, Khaled Aamer
Polymers Division, National Institute of Standards and Technology, Gatihersburg, MD 20899.

Statement of Purpose: Rational development of tissue scaffolds requires a clear understanding of cellular responses to the environmental cues provided to the cells by the scaffold construct. Many of these responses, particularly those involving differentiation are generally detected using invasive assay techniques such as RT-PCR or histological methods, so that direct spatio-temporal connections between environment and continuing cell behavior cannot be made. A convenient and non-invasive technique for real-time measurements of cellular physiology during the process of in-vitro culturing would open up exciting possibilities for detection of cellular response and facilitate a leap forward in tissue engineering by allowing the rapid discovery of causal relationships between scaffold characteristics and the cell behaviors they elicit.

Spontaneous Raman scattering studies have shown that phenotypic and activity changes in cells are accompanied by chemical changes that can be detected by vibrational spectroscopy. However, spontaneous Raman scattering is too slow and inefficient to be used for noninvasive imaging. We are developing broadband coherent anti-Stokes Raman scattering microscopy (μCARS), a nonlinear optical microscopy that provides chemical sensitivity and spatial resolution needed to track phenotype and activity changes under imaging conditions that are compatible with continuous interrogation of live-cells.

Methods: We have developed a multiphoton Raman imaging microscope (broadband μCARS) that allows us to obtain vibrational spectra of cells and materials in 50 ms or less at power levels that do not damage cells. This makes possible high resolution 3D chemical images of cells and materials. We apply multivariate analysis techniques to the chemical maps of cells that we obtain in order to find statistically significant changes in chemical distributions associated with differentiation.

Results: A significant complication of CARS is that a nonresonant background (NRB) accompanies the desired signal. The NRB can easily overwhelm the weak vibrational fingerprint signal from cells. We have developed an approach to analysis of the CARS spectra that allows us to reliably extract the resonant component of the spectrum, and perform meaningful analysis of the spatial distribution of chemical components within the cell. Figure 1 shows spectra taken from fixed cells and the surrounding medium, both before and after signal processing. After processing, the cell spectra clearly show features characteristic of nuclear and membrane regions. Figure 2 shows chemical maps of two fixed cells, at different chemical resolution. Statistically based multivariate analysis methods allow us to distinguish regions of the cell that contain chemically distinct structures such as the nucleus, mitochondria, cytosol, etc.

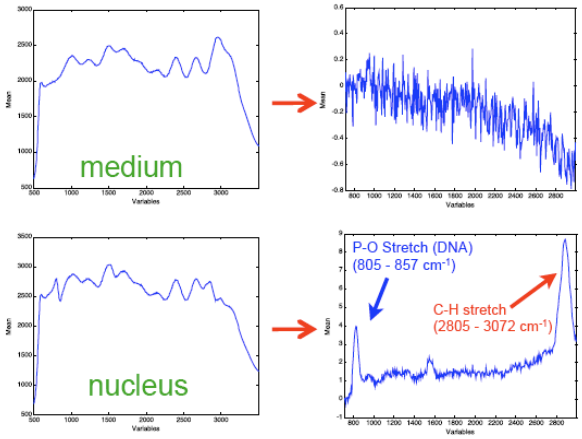

Fig 1: μCARS spectra of unlabeled L929 fibroblasts: Left - unprocessed spectra, primarily nonresonant. Right - spectra after processing and subtracting the spectral contribution of the medium.

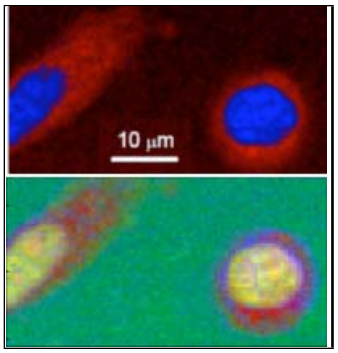

Figure 2. CARS pseudo-color images of unlabeled L929 fibroblasts: Top - binary just showing nucleus and cytosol. Bottom - shows finely graded chemically distinct regions of cell - including organelles in perinuclear region.

Conclusions: We are able to obtain full spectral images from cells and tissues, and expect to use this spectral information to characterize and then noninvasively track phenotype changes in pluripotent cells. Broadband CARS microscopy is well on its way to becoming a powerful tool for high information content non-invasive imaging of live cells and tissue constructs *in-situ*.

References:

Liu, Y., Lee, Y. J. and M. T. Cicerone (2009). " Broadband CARS Spectral Phase Retrieval Using a Time-Domain Kramers-Kronig Transform." Optics Letters (in press).

Kee, T. W. and M. T. Cicerone (2004). "Simple approach to one-laser, broadband coherent anti-Stokes Raman scattering microscopy." Optics Letters **29**(23): 2701-2703.