DE-FG02-07ER15875

Title of Original Proposal:

Coherent Anti-Stokes Raman Scattering Spectroscopy of Single Molecules in Solution

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Executive Summary

During this funding period, we have developed two breakthrough techniques. The first is stimulated Raman scattering microscopy, providing label-free chemical contrast for chemical and biomedical imaging based on vibrational spectroscopy. Spontaneous Raman microscopy provides specific vibrational signatures of chemical bonds, but is often hindered by low sensitivity. We developed a three-dimensional multiphoton vibrational imaging technique based on stimulated Raman scattering (SRS). The sensitivity of SRS imaging is significantly greater than that of spontaneous Raman microscopy, which is achieved by implementing high-frequency (megahertz) phase-sensitive detection. SRS microscopy has a major advantage over previous coherent Raman techniques in that it offers background-free and readily interpretable chemical contrast. We demonstrated a variety of biomedical applications, such as differentiating distributions of omega-3 fatty acids and saturated lipids in living cells, imaging of brain and skin tissues based on intrinsic lipid contrast, and monitoring drug delivery through the epidermis. This technology offers exciting prospect for medical imaging.

The second technology we developed is stimulated emission microscopy. Many chromophores, such as haemoglobin and cytochromes, absorb but have undetectable fluorescence because the spontaneous emission is dominated by their fast non-radiative decay. Yet the detection of their absorption is difficult under a microscope. We use stimulated emission, which competes effectively with the nonradiative decay, to make the chromophores detectable, as a new contrast mechanism for optical microscopy. We demonstrate a variety of applications of stimulated emission microscopy, such as visualizing chromoproteins, non-fluorescent variants of the green fluorescent protein, monitoring lacZ gene expression with a chromogenic reporter, mapping transdermal drug distributions without histological sectioning, and label-free microvascular imaging based on endogenous contrast of haemoglobin. For all these applications, sensitivity is orders of magnitude higher than for spontaneous emission or absorption contrast, permitting nonfluorescent reporters for molecular imaging.

Comparison of the actual accomplishments with the goals of the project

Although we did not accomplish the original goal of detecting single-molecule by CARS, our quest for high sensitivity of nonlinear optical microscopy paid off in providing the two brand new enabling technologies.

Summarize project activities for the entire period of funding

Both techniques were greatly benefited from the use of high frequency modulation for microscopy, which led to orders of magnitude increase in sensitivity. Extensive efforts have been made on optics and electronics to accomplish these breakthroughs.

Publications resulted from DE-FG02-07ER15875

Freudiger, Christian W.; Min, Wei; Saar, Brian G.; Lu, Sijia; Holtom, Gary R.; He, Chengwei; Tsai, Jason C; Kang, Jing X.; Xie, X. Sunney "Label-Free Biomedical Imaging with High Sensitivity by Stimulated Raman Scattering Microscopy," *Science*, **322**, 1857-1861 (2008)

Min, Wei; Lu, Sijia; Chong, Shasha; Roy, Rahul; Holtom, Gary R.; Xie, X. Sunney "Imaging chromophores with undetectable fluorescence by stimulated emission microscopy" *Nature*, **461**, 1105-1109 (2009).

Lu, Ju; Min, Wei; Conchello, Jose-Angel; Xie, X. Sunney; Lichtman, Jeff W. "Super-Resolution Laser Scanning Microscopy through Spatiotemporal Modulation" *Nano Letters*, **9**, 3883-3889 (2009).

Min, Wei; Xie, X. Sunney; Bagchi, Biman "Role of conformational dynamics in kinetics of an enzymatic cycle in a nonequilibrium steady state" *J.Chem.Phys.*,**131**, 065104 (2009).

Min, Wei; Lu, Sijia; Rueckel, Markus; Holtom, Gary R.; Xie, X. Sunney "Near-Degenerate Four-Wave-Mixing Microscopy" *Nano Letters*, **9**, 2423-2426 (2009).

Inventions resulted from DE-FG02-07ER15875

Harvard Case No.	Inventors	Title	Date reported to government by Harvard OTD
3303	Xie, Xiaoliang Sunney Freudiger, Christian W. Min, Wei	Raman Gain/Loss Microscopy	7/30/08
3407	Xie, Xiaoliang Sunney Freudiger, Christian W. Min, Wei	Mapping chemical species by stimulated Raman scattering spectral Imaging with high frequency modulation	5/1/09
3409	Xie, Xiaoliang Sunney Min, Wei Lu, Ju Lichtman, Jeffrey W.	Microscopy Imaging System and Method Employing Laser Scanning Structure Illumination to Double the Spatial Resolution of Fluorescent and Non-Flourescent Optical Microscopy	12/19/08
3414	Xie, Xiaoliang Sunney Min, Wei Lu, Sijia	Near-Degenerate Four-Wave-Mixing Microscopy	1/5/09

3434	Xie, Xiaoliang Sunney Min, Wei Lu, Sijia	Microscopy Imaging System and Method Employing Stimulated Emission Spectroscopy as a Contract Mechanism	4/3/09
3512	Xie, Xiaoliang Sunney Freudiger, Christian W. Saar, Brian G. Young, Geoffrey S. Lu, Sijia Min, Wei Kesari, Santosh Xu, Xiaoyin	Method and means for providing in situe histology based on Coherent Raman Imaging (CRI)	7/2/09
3541	Xie, Xiaoliang Sunney Min, Wei Lu, Sijia Saar, Brian G. Freudiger, Christian W.	Microscopy imaging system and method employing multi-photon excited electronic or vibrational photothermal effect as contrast mechanisms	7/21/09