Microscopy TODAY

Coming Events

2011

EMAS 2011
May 15–19, 2011
Angers, France
www.emas-web.net

IUMAS-V
May 22–27, 2011
Inchon, South Korea
www.iumas5.org

MSC/SCM Annual Meeting
June 7–10, 2011
Ottawa, Canada

Inter/Micro: 62nd Conference
June 11–15, 2011
Chicago, IL
www.mcri.org/home/section/101/intermicro

Microscopy & Microanalysis 2011
August 7–11, 2011
Nashville, TN

Microscopy Conference MC 2011
August 28–September 11, 2011
Kiel, Germany
www.mc2011.de

ICXOM21
September 5–8, 2011
Campinas, Brazil
icxom21.lnls.br

EMAG 2011
September 6–9, 2011
Birmingham, UK
www.emag-iop.org

FEMMS 2011
September 18–23, 2011
Sonoma County, CA
www.femms2011.llnl.gov

CIASEM 2011
September 25–30, 2011
Mérida, Mexico
www.ciasem.com

Neuroscience 2011
November 12–16, 2011
Washington, DC
www.sfn.org

2012

Microscopy & Microanalysis 2012
July 29–August 2, 2012
Phoenix, AZ

2013

Microscopy & Microanalysis 2013
August 4–8, 2013
Indianapolis, IN

2014

Microscopy & Microanalysis 2014
August 3–7, 2014
Hartford, CT

More Meetings and Courses
Check the complete calendar near the back of this magazine and in the MSA journal *Microscopy and Microanalysis*.

Carmichael's Concise Review

Microscopy is Only Skin Deep

Stephen W. Carmichael
Mayo Clinic, Rochester, MN 55905

carmichael.stephen@mayo.edu

Light optical imaging techniques that don't require labels are attractive for human studies because they are not toxic nor do they perturb the system being studied. Whereas there are several methods available to provide microscopic imaging below the surface of a tissue, they each suffer from limitations, such as low spatiotemporal resolution and a limited number of molecular signatures that can be imaged. Raman spectroscopy offers label-free contrast for major chemical species in tissue, such as water, lipids, DNA, and proteins based on vibrational spectra at light optical wavelengths. However, the Raman signal is very weak, which makes it difficult to image a sample with good temporal resolution. The recent development of stimulated Raman scattering (SRS) microscopy can overcome these limitations as demonstrated by the elegant studies of Brian Saar, Christian Freudiger, Jay Reichman, Michael Stanley, Gary Holton, and Sunney Xie [1]. Saar et al. improved the optics and electronics for the acquisition of the backscattered signal of SRS. In SRS microscopy, the sample is excited by two lasers at different frequencies. When the difference in frequency matches a molecular vibration in the sample, the intensities of the probes change in a predictable manner. These intensity changes are small, but Saar et al. developed a new method to detect them. They chopped one of the laser beams at high frequency (MHz) and detected the intensity change in the other beam, which offered superior sensitivity. The key component was a custom-made all-analog lock-in amplifier with a very short (about 100 ns) response time. The laser probe wavelengths were tuned to match a vibrational frequency of interest and raster-scanned across the sample. Frequencies were tuned to detect CH_2 stretching (primarily for lipids), OH stretching (primarily water), and CH_3 stretching (primarily proteins) vibrations. Imaging of water is of particular interest in studying the transport of water-soluble compounds such as drugs.

SRS microscopy relies on backscattering of the forward-directed signal by the tissue sample. In biological tissues the backscattered signal is too weak to be detected by an objective lens to allow for high-speed imaging. This limitation was overcome by placing a photodetector directly in front of the objective lens and sending the excitation probes through an aperture in the center of the detector. This and other modifications allowed greater collection of backscattered signal to increase the imaging speed by three orders of magnitude!

In a proof-of-concept experiment, Saar et al. studied the penetration of a small-molecule drug (*trans*-retinol, which stimulates collagen synthesis) and showed that *in vivo* it penetrated mouse skin via the hair shaft, one of three hypothesized penetration routes. Similar studies showed the value of SRS microscopy on human skin in a living volunteer (Figure 1). This powerful label-free imaging modality can now be applied to a broad range of problems in whole living organisms. This is likely to play an increasingly important role in clinical diagnostic procedures.

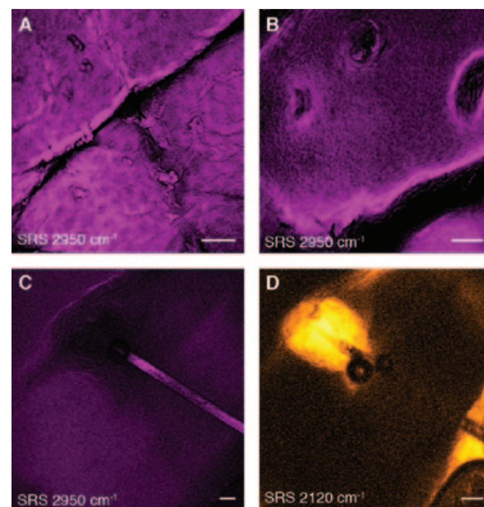

Figure 1: SRS skin imaging in living humans. (A-C) SRS images of the stratum corneum and viable epidermis tuned into CH_3 stretching vibration of proteins (2950 cm^{-1}) showing nuclei of variable size (A and B) as well as a hair (C). (D) SRS image of DMSO penetrating the skin at the same region as (C). DMSO accumulates in the hair shaft. Deuterium labeling was used to create a unique vibration of d6-DMSO at 2120 cm^{-1} for specific imaging. Images are acquired in the epi-direction on the forearm of a volunteer (the principal investigator). Image acquisition time was 150 ms for (A) and (B) and 37 ms for (C) and (D), all with 512 × 512 pixel sampling. Scale: 50 microns [1].

References

[1] BG Saar, CW Freudinger, J Reichman, CM Stanley, GR Holtom, and XS Xie, *Science* 330 (2010) 1368–70.
[2] The author gratefully acknowledges Drs. Sunney Xie and Brian Saar for reviewing this article.

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doi:10.1017/S1551929511000368

https://doi.org/10.1017/S1551929511000368 Published online by Cambridge University Press

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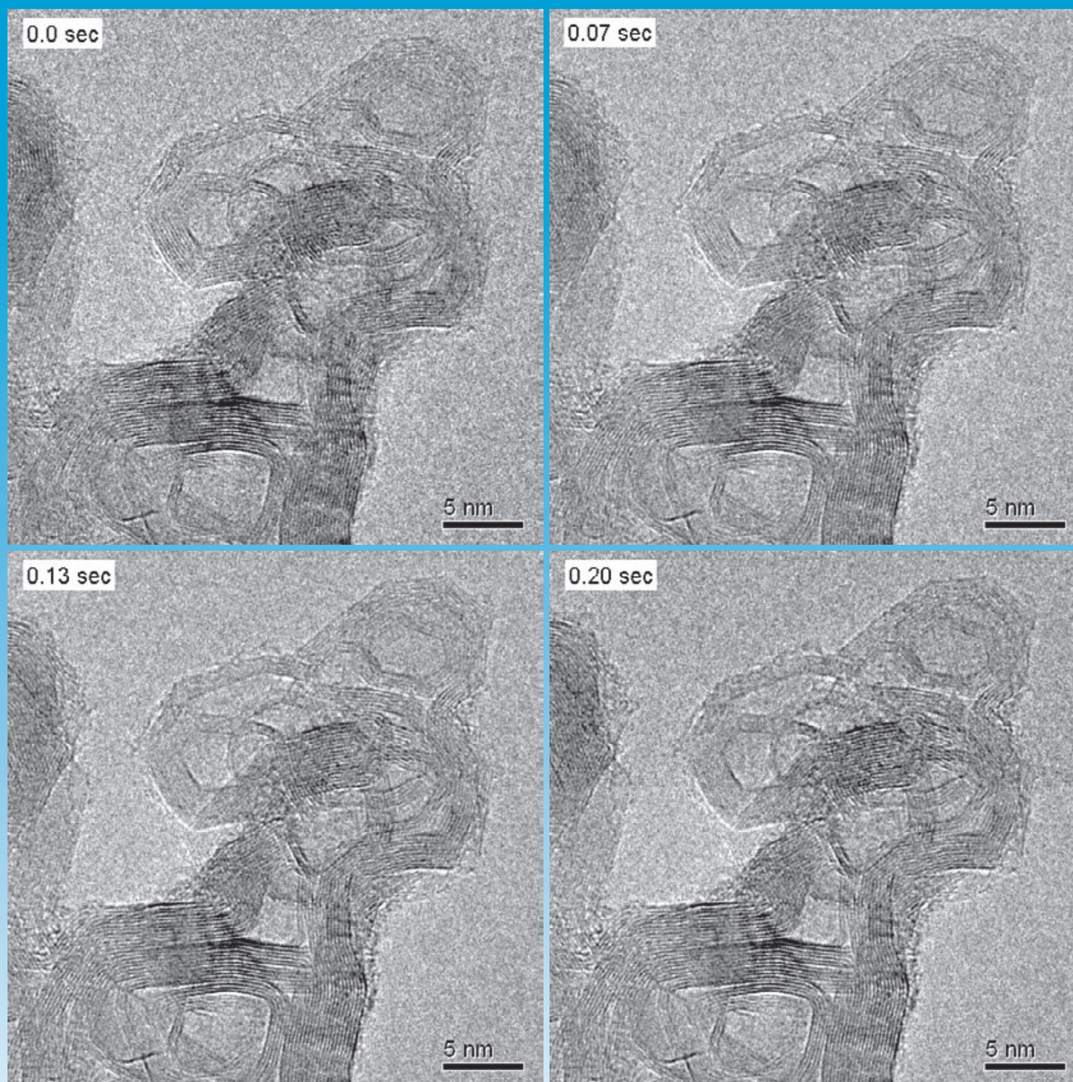

Individual frames of a captured digital streaming video sequence of graphitized carbon sample with Gatan UltraScan® 1000XP CCD camera on a 200 kV TEM. The camera was operated at 16 fps. TEM magnification = 400 kx.

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