Perspectives of CARS Microscopy in Life Science Research

Good Vibrations

Dr. Bernd Sägmüller, Leica Microsystems

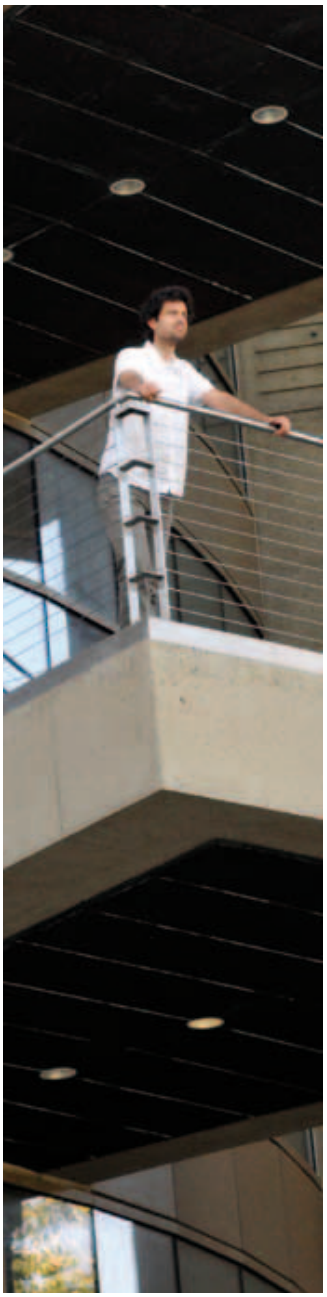

Fig. 1: Eric Potma, Ph.D., assistant professor at the University of California, Irvine, USA

The ability to see the cell's molecular machinery at work has contributed immensely to our understanding of cellular functioning. In recent years, new molecular imaging techniques, such as coherent anti-Stokes Raman scattering microscopy (CARS), have been developed for rapid vibrational imaging of living cells. The intrinsic molecular bond vibrations leave molecular specific fingerprints in the vibrational spectrum. Eric Potma, Ph.D., assistant professor at the University of California, Irvine, USA and his team advance and apply this novel imaging technique for unveiling the molecular secrets of microscopic biological systems.

Professor Potma, how did you come across CARS microscopy?

When I was a graduate student in the Netherlands I did ultrafast spectroscopy. And my adviser suggested I apply those techniques to microscopy. This was a hot trend in the late '90s, when the two-photon microscopy technique was becoming increasingly popular. We also saw a bit of second harmonic microscopy in those days, but not a lot. I was playing with a laser system that had two colors, and thought about doing a pump-probe type of microscopy.

In 1999, I saw Andreas Zumbusch of the University of Konstanz, Germany (then a postdoc in the laboratory of X. Sunney Xie) and Michiel Müller of the University of Amsterdam, the Netherlands, showing their work on CARS microscopy at the FOM conference. I was very impressed with the capabilities and the contrast of CARS microscopy. I immediately went back to my laboratory in the Netherlands and lined up the instrument to do CARS. The next day we got the first CARS signal. I have been doing CARS microscopy ever since.

What was the first sample you used and how did you get involved in biology?

The first sample we looked at was Dictyostelium discoideum, an amoeba cell. We used these cells as model systems to learn about water distribution in cells. We focused on the water band OH-stretching and did dynamic measurements on flushing water through the cells to examine water diffusion in living cells. I was in a physical chemistry department and we focused on biophysical applications in those days. We collaborated with biologists and cell biologists and those people brought us the samples. Ever since it has been a very natural merger of the two fields.

It was great to work with biologists since they brought the real questions to the table, which made us tweak our instruments in such a way that we could see things that actually matter. There is a great collaborative spirit between biological researchers and those more involved in physics.

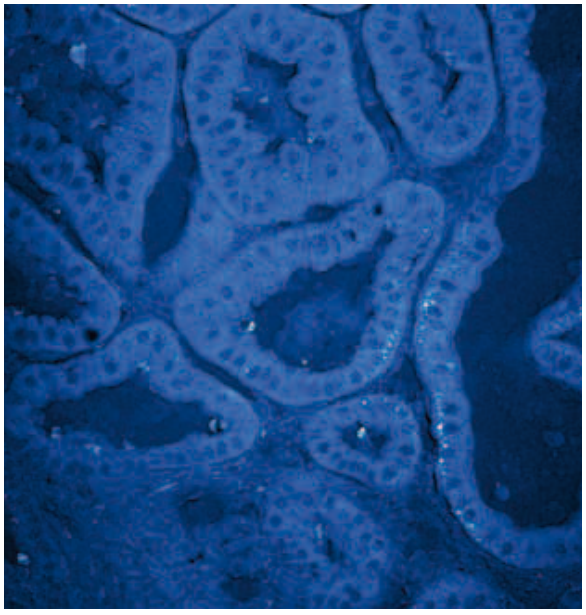

Fig. 2: Kidney tubules: CARS image at 2845 cm^{-1} of the mouse kidney, showing the tubules and intracellular lipid droplets.

Is the emphasis shifting back to physics?

CARS became popular on the biology side, but you can probe anything else with this particular type of contrast. You can probe vibrational and electronic features as well. We are looking at carbon nanotubes, and trying to understand the propagation and coherent evolution of primary excitations. We also study the nonlinear optical properties of plasmonic structures on a microscopic level. CARS is a great way to perform these applications. So, it is finding its way into material science as well.

How can CARS microscopy give us insight into fundamental cellular processes?

The real benefit of CARS microscopy is that you look at molecules just the way they are. There is no need to put labels on them, no need to dress them up in a certain way to make them fluorescent. That's really where the technique is advantageous. You can look at all molecules that have a good Raman signature and so there are a couple of biomolecular candidates that can be easily visualized using the CARS microscope.

Any question dealing with lipid metabolism is where CARS can make a difference, as well as any question dealing with the mobility of water molecules, membrane dynamics, and variations in protein density distributions. And CARS holds great promise for following extrinsic agents like drug molecules or any molecular compound with a strong vibrational signature in tissue. This is great, since these molecules are typically hard to visualize otherwise, as they cannot always be labeled. Usually they are too small – if you label them you don't get them into the cell or you change their functionalities.

With CARS we image such targets at a rate that is much faster than conventional vibrational imaging. We are talking about imaging in real-time, which is important for imaging all things biological, like living cells and tissues in vivo – these are the situations where CARS microscopy really helps.

Where would you focus with CARS in life science research?

There is a very important research direction that aims at visualizing endogenous molecules in living animals. The CARS microscope is great because of its speed, so you can monitor molecules in real-time. For instance, people have used CARS to look at myelin degradation – which is a way to investigate diseases like multiple sclerosis. There is no other way you could do this; visualizing myelin in real time in a living animal is really difficult. CARS is the only avenue for people to do this.

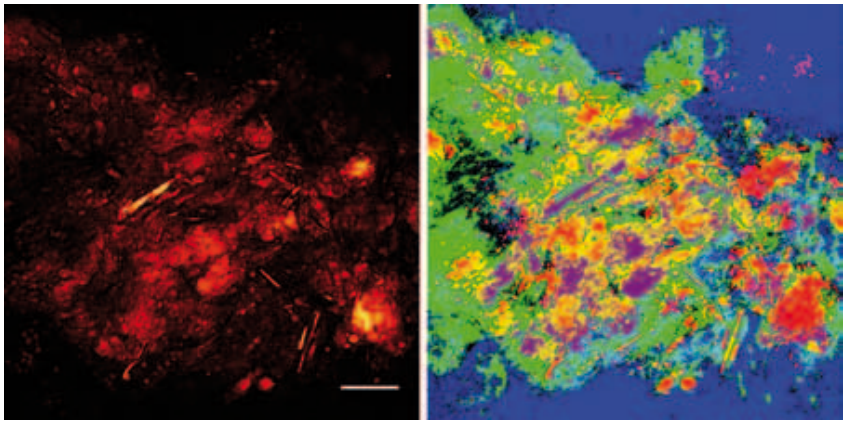

Fig. 3: AortaPCA: This is a CARS image of an atherosclerotic lesion in a mouse aorta. The left panel shows a regular CARS image of the lesion at 2845 cm^{-1}, highlighting the lipophilic components. The right panel shows a spectral decomposition (principal component analysis) of the CARS signal in the 2750–3050 cm^{-1} range, showing that different areas in the image correspond to different lipophilic compounds. Each color in the image corresponds to a different CARS spectrum. Scale bar is 50 microns.

Another example is the use of CARS for skin imaging: attempts are under way to do this with a system that is currently optimized such that you can put your arm under a microscope and look at tissue morphology and abnormalities. This has direct implications for improving human health.

What does a commercial CARS system need to have to be successful in the market?

Such a system needs to be easy to operate. People want to focus on the applications, they want to start an experiment first thing in the morning. They don't want to fiddle with lasers and microscope settings. Ease of use is very important. This includes biological applications, people with medical research pro-

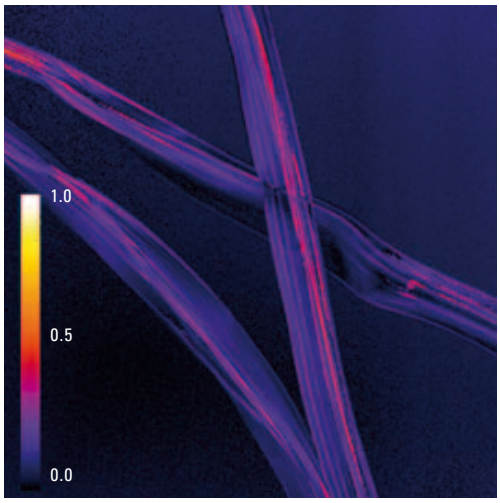

Fig. 4: Rayon anisotropy: CARS anisotropy image of rayon fibers. The anisotropy was determined at the 2880 cm^{-1} methylene stretching mode of rayon. The brightness in the image corresponds to the degree of orientation of the methylene mode in the fiber.

jects who want to look at their samples. The system must be very user friendly to tap into a huge number of user groups.

You need to be able to automatically tune to several laser wavelengths, and also address wider portions of the spectrum for deeper chemical analysis …

… which you are currently taking to its next stage, such as hyperspectral imaging with multivariate analysis?

You can combine that naturally. Once you have a hyperspectral data stack you can improve the data extraction using multivariate analysis – so I believe it's a powerful resource. Anything that can automate data extraction will attract a larger pool of users. Currently, it's confined to academic users with a lot of experience; optical and engineering expertise is essential to operate these systems.

What is your vision of CARS microscopy for the next 20 years?

I think we can anticipate that with CARS the same thing will happen as with confocal imaging. CARS is the latest edition of contrast methods. You want to inspect your samples, look at molecules, have depth-resolved imaging and do it with the least amount of perturbation to the sample – no harsh treatment or staining protocols – this is especially true for living systems.

So, having a system that adds this type of contrast to the microscope is an enormous step forward. It clearly widens the current landscape of scientific investigation. You can see more than was previously possible. It enables you to do more and to work on key applications. Just one breakthrough application is a tremendous success in itself.

For instance, people would have never foreseen the impact of being able to directly visualize lipid metabolism, an essential process in our bodies that was previously difficult to study without the use of perturbing labels.

By creating such new research avenues, CARS is a true asset to the label-free imaging approach. Other applications are waiting to be discovered. It is inevitable that CARS will continue to have a major impact in the biological and material sciences. It's the way forward.

Professor Potma, thank you for the interview.

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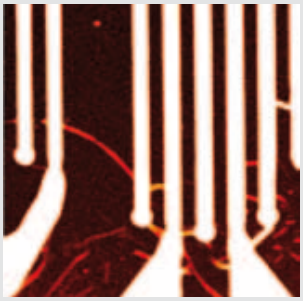

Fig. 5: Nanotubes: Electronic CARS signal from carbon nanotubes between Ti electrodes. The wider white lines correspond to the electrodes.

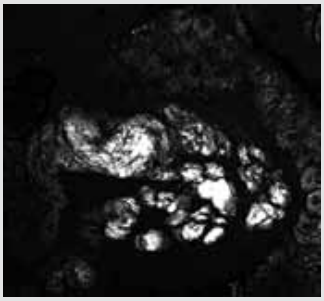

Fig. 6: Meibomian: Transversal cross section through a mouse meibomian gland. CARS signal was measured at 2845 cm^{-1} to visualize the lipids in the gland. The center portion of the image corresponds to the duct of the gland, whereas the peripheral regions show the lipid rich meibocytes.

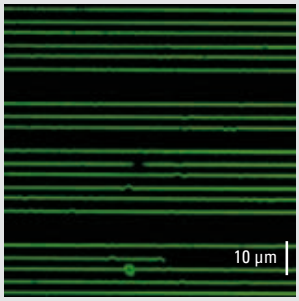

Fig. 7: Nanowires: Electronic CARS image of semiconducting CdSe nanowires using ps laser pulses. CdSe wires are 330 nm wide, 60 nm high and were lithographically fabricated on a glass coverslip.