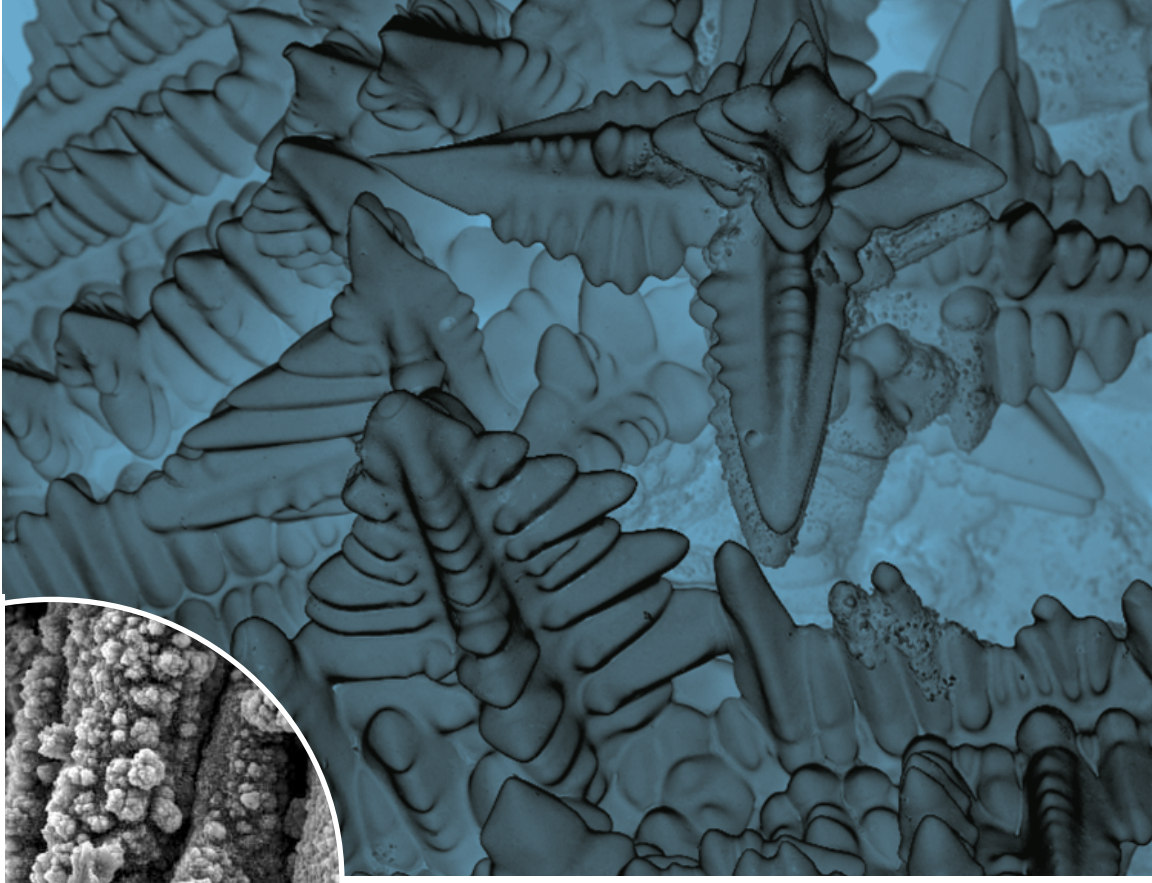

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CARS ENTHUSIASTS GATHER FOR TRAINING CAMP

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In this article, I would like to share a fascinating technology called CARS with Bulletin readers. What do you know about CARS and how does one build the world's fastest CARS? Read on!

"Hello! Congratulations, you've been accepted to attend the Second Annual CARS workshop… there's a good chance that you will see the signs along Storrow Drive that read 'CARS ONLY'. Perhaps you will arrive here by rental car, and if so, you'll probably see how crazy people drive CARS in Boston. Here in Massachusetts, we are CARS enthusiasts!"(1)

WHAT IS CARS?

CARS stands for Coherent Anti-stokes Raman Scattering, one of the 'spin-off' technologies of the multi-photon microscopy (MPM) revolution. Like MPM, CARS is a non-linear optical technique that was made known to the microscopy community in the early 1990's. Both CARS and MPM share similar equipment requirements, such as the use of ultra-fast IR lasers. Although CARS did not get as much attention as MPM during the early years, things are beginning to change now thanks to new technological breakthroughs and increased applications. CARS has rapidly been emerging as a unique imaging tool and from 1998-2005, there was a 500% increase in the number of published papers!

CARS builds on the principles of Raman Microscopy (RM) in detecting the vibrational signatures of molecules. However RM only works well in a static environment. The need for extremely high laser power and long integration times makes RM unsuitable to be used on living cells and tissues. CARS takes advantage of two collinearly overlapping laser beams (one as the Pump beam and the other as the Stokes beam) to generate an anti-Stokes signal. When the frequency difference between the Pump and Stokes is tuned to the vibrational frequency of the subject molecule, a strong CARS signal is generated, providing what is known as the vibrational contrast for CARS microscopy (which is several orders of magnitude higher than the traditional Raman signal). In short, CARS contrast results from the non-linear interaction between two synchronized laser sources beating in resonance at the vibrational frequency of the subject molecule. In a CARS microscope, this phenomenon occurs only at the focussed spot where the excitation intensities are the highest. Hence, CARS microscopy has intrinsic optical sectioning capability. Since the contrast is vibrational specific and unrelated to fluorescence detection, sensitivity is not affected by the presence of auto-fluorescence in the sample. The technique also has high chemical selectivity as molecules usually have their own specific vibrational signature. Without photo-bleaching effects, CARS is highly desirable for the non-invasive 3D analysis of molecules in live cells and tissues.

LEARNING THE NUTS AND BOLTS OF CARS

The ability to track and image molecular interactions on unstained live cells is indeed a powerful technique; something I have always been keen to learn about. The opportunity came last summer when I attended the CARS workshop organized by Prof. Sunney Xie here on campus. It was a three day event exploring the underlying princi-

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ples of CARS. Theory (morning lectures) and hands-on training (afternoon and evening labs) were both emphasized. There were 24 attendees from eight different countries and from diverse backgrounds (from academia to industry, engineers to biologists, etc.).

Day 1 – Finding the first light: The introductory lectures covered the in-depth theory of CARS and laser designs. The very first lab involved taking the spontaneous Raman spectrums of polystyrene beads and yeast cells. Peaks representing lipids and DNA were identified on the yeast spectrum. Later on, we started exploring the construction of both laser scanning microscopes and ultra-fast lasers. We also explored the mechanisms used to synchronize the lasers for CARS. Since we worked in small groups and there were four machines, there were ample opportunities for each person to learn how to manually tune, align and set mode-lock for the lasers. Making these adjustments for CARS is more complicated than that for MPM because one has to adjust both the Pump and the Stokes beams and ensure that their pulses are synchronized. Finally, each group had to be able to collect a MPM image of polystyrene beads and find the CARS signal before being dismissed for the day. Figure 1 shows the optical setup of one of the systems used in the lab.

Day two – Beyond the basics: The lectures concentrated on analyzing various imaging properties of CARS (eg. contrast mechanisms) and how one would apply such knowledge in optimizing CARS. Strategies such as "Forward-detection (F-CARS)", "Epi-detection (E-CARS)" and Polarization-sensitive detection (P-CARS) were discussed. Specifically, F-CARS is useful in probing larger objects while E-CARS is sensitive in smaller objects. P-CARS can be used to probe molecular orientation and to suppress the non-resonant background. We also learned why pico-second pulses give a better signal to noise ratio than femto-second pulses. During the afternoon lab session, the instructor reiterated all of the important imaging theories discussed during the lectures and allowed participants to experience first-hand how such parameters affect the outcome of the final image. We also experienced the fun/agony of trying to put the system back to work after the instructor intentionally disrupted the tuning and alignment of the machine before

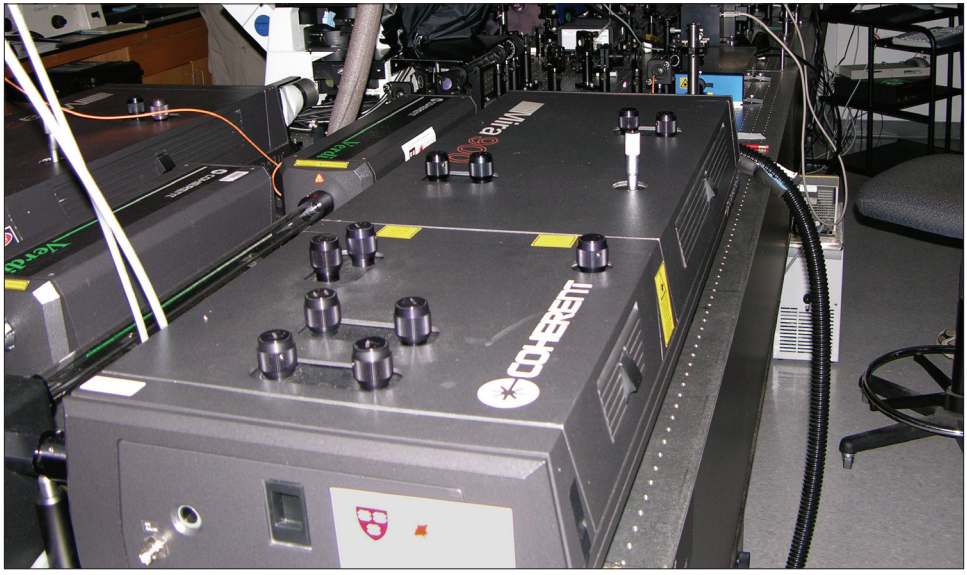

Figure 1. One of the CARS microscopes used in the lab. Seen in the foreground are two electronically synchronized Ti:sapphire lasers (Coherent Mira-900) operating as a Master and Slave pair.

he handed it to us. The assignment for the day was to collect a high resolution CARS image of unstained cells (Figure 2).

Day three – Putting CARS to the test: The morning lectures featured prominent scientists who use CARS in "real-life" biomedical research. A practical example was the talk given by Zemin Yao, a fellow Canadian from the University of Ottawa. Yao used a combination of EM and CARS techniques to demonstrate how fish oil lowers the triglyceride levels in our blood. Another example was the work of Charles Lin (Massachusetts General Hospital) who studies transdermal molecular transport and leukocyte-endothelial interaction. His microscope is custom built which incorporates a polygonal scanner to achieve a fast video-rate (probably is the world's fastest CARS microscope). The microscope can be used to collect *in-vivo* images in single-photon (confocal), multi-photon and CARS modes. The original design (without CARS features) was described in a recent paper (2). Based on the images presented in his talk, the CARS images compared favorably with those from two-photon in terms of resolution and imaging depth. After lunch, participants had a full day of applications in the lab. Assignments included "imaging single lipid bilayers", "thick tissue imaging", "monitoring lipid droplet trafficking in adrenal cortical cells" and "imaging the uptake of exogenous lipids by macrophages". The latter two experiments were carried out on live cells and time lapse movies were collected. The workshop wrapped up at the end of the day with a wine and cheese reception, just in time for me to return to Toronto for Canada Day.

The workshop was truly a hands-on learning experience. For those who wanted to run their own samples, there was also an extra morning set aside for them. Although some of the theoretical materials might have been too heavy for those who do not have an engineering background, I found this short-coming to be easily compensated for by the guidance of the very patient instructors who were willing to walk us through each step of the labs. Most of the difficult-to-understand materials were finally made clear after the hands-on sessions. Many attendees came with the goal of learning to build their own systems. Although I did not think I would ever have that opportunity, I still came away with the confidence that if I ever will collaborate with someone who uses CARS, I will be ready and know exactly what it is all about.

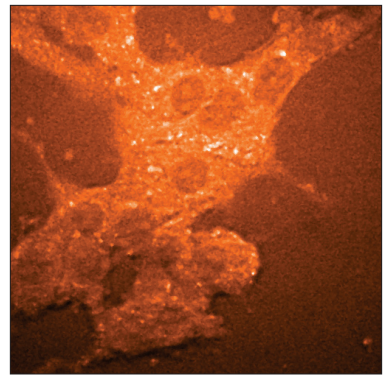

Figure 2. CARS image of unstained L1 NIH fibroblast cells showing triglyceride-containing lipid droplets. (The stretch vibration of CH bonds in lipids provides strong CARS signals).

Notes: To learn more about CARS and its latest applications, readers can read the following two papers from the Xie's group (3, 4) or visit their web site (5). The site also provides a link to past and future workshop information.

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