

## **Best practice: Teaching biologists to get the best out of their confocal microscopes**

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It is safe to say that every week thousands of biologists use confocal microscopes. Although much of this work is done on fixed specimens, an increasing number of workers are looking at living specimens, often in studies that follow cells over long periods of time. Although it is possible to get usable results from dead specimens without using “best practice” this is seldom true when viewing living cells because in this case, one must get the structural information without interfering with cellular physiology. Because physiology is disrupted by phototoxicity that is, in turn, generally proportional to the number of fluorescent excitations, one can only succeed if one knows how to get the best image using the fewest fluorescent excitations.

It is often the case, that those research biologists with the background and skill to design and interpret living-cell micro-experiments that are both informative and reproducible and can be carried out while the cell is being observed in 3D on the stage of the microscope have never needed to understand the optics, photochemistry, information theory, and image processing needed to perform optimal confocal microscopy.

In 1996, a number of the authors of the Handbook of Biological Confocal Microscopy decided to put together an intensive summer course to bridge this gap, at the University of British Columbia, in Vancouver Canada. Although this course is in many ways an outgrowth of earlier courses at Woods Hole in North America and at EMBL-Heidelberg, it did have a slightly different slant. To begin with, work started before the students arrived. Not only were the textbooks sent out accompanied by homework questions but student groups of 4-5 were formed and given a faculty advisor so they could plan live-cell experiments to be carried out at UBC. We hoped that, by being introduced early, students would be more likely to learn from each other when they finally met up in Vancouver. Secondly, rather than teaching diffraction/optics, Poisson statistics and sampling theory separately, we have always tried to teach them as a set of interacting variables, emphasizing that, while there was no “right answer,” for a given experiment, there is usually an optimal way to set the laser power, and the size of the pinhole and the pixel. Another unusual feature was the prominence given to lectures about the structure and function of the dyes used in fluorescence microscopy. Finally, from the beginning, the course was designed so that each student spent a lot of time (28- 31 hours) working in a group of 3-4 on one of 9 to 13, 3D microscopy workstations. Some of this time was spent working on projects designed by the faculty to demonstrate important lessons about living-cell microscopy. The remainder was available for projects designed by group members and their advisor, often on cells they brought from home.

In recent years, new lectures have been added on TIRF and FLIM and on the advantages to be gained by always deconvolving any raw 3D microscopy data before either viewing or measuring it. This June, the 3D Microscopy of Living Cells course will celebrate its 10<sup>th</sup> anniversary. To the over 300 students and almost 40 faculty members who have contributed so far: A big thank you! And to those students who have started their own courses: Congratulations!