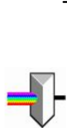
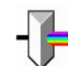
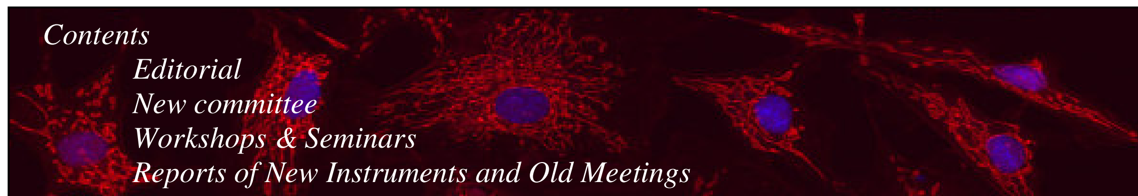


Fluorescence Imaging Group **Newsletter** August 2007

www.FIGroup.org





Editorial

A newsletter should, by its very nature, contain news. The trouble with news is that it dates rather quickly, and following that logic, the more topical the news, the more quickly it will date. This must be the main reason why, since accepting the position of editor of the newsletter in 2006, I have so far refrained from compiling and circulating an actual newsletter. So in an effort to remedy this grave lapse, the current newsletter will cover some of the recent past as well as the near future, thereby restoring colour and fluorescence news (in and around Melbourne) to your stream of consciousness. I trust you will find the news of future activities useful, and strongly encourage you to attend any or all of the several meetings detailed below. Finally, I welcome any news, instrument updates or comments to include in the next newsletter.

Editor: Ian Harper, Monash University
Ian.Harper@med.monash.edu.au

New committee for 2007/2008

As Stephen Cody has stepped down as FIG President due to increasing work commitment, we elected a new President and secretary at the June committee meeting. Many thanks to Stephen for a great effort over the past two years, and we do look forward to his continued participation. We welcome the following new committee members who will add youth, vigour and expand our wine list: Jackie Mills and Cameron Nowell. We are always happy to have anyone with enthusiasm and energy on the committee, so this is an open invitation to you all to participate.

The newly elected FIG committee is:

President:	Sarah Ellis (Peter Mac)
Secretary:	Jackie Mills (Howard Florey)
Treasurer:	Nick Klonis (La Trobe Uni)
Editor:	Ian Harper (Monash Uni)
Members	Stephen Firth (Monash Uni)
	Stephen Cody (Ludwig)
	Damian Myers (Melbourne Uni)
	Andrew Clayton (Ludwig)

Forthcoming Workshops & Seminars

Beyond Imaging * .... 17 Oct 2007

***Next FIG Seminar*: Katherine Gauss. Membrane order in T cells - microscopy approaches to unravel membrane structure.................................... 8 Nov 2007**

Imaging infectious diseases at the molecular, cellular and tissue level * ....31 Jan – 1 Feb 2008

ACMM20 - Conference..................10-15 February 2008

MMI Imaging Workshop (Live Cell) .... April 2008

** see workshop outline below*
*** see report from previous workshop (LCI2006) below*

Beyond Imaging……… Fluorescence and Microscopy-Based Approaches for Examining Macromolecular Interactions in Living Cells, a FIG Mini-Workshop to be held in Conjunction with Department of Biochemistry, La Trobe University

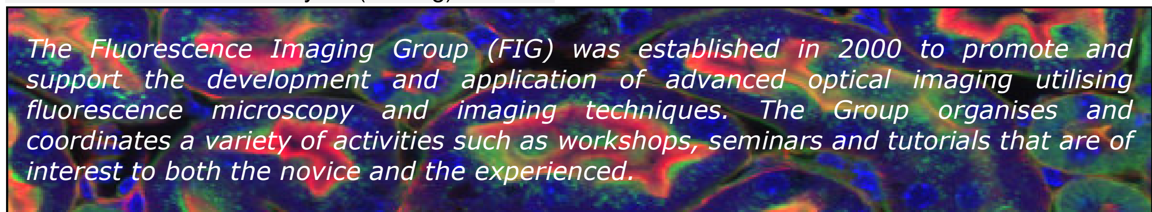
Date: 2:30 - 5 p.m. Wed 17th October, 2007
Location: Biochemistry Seminar Room, Rm 351 Level 3, Phys Sciences 4 Bldg, La Trobe University, Bundoora campus

Preliminary Program:

Fluorescence Detection of Macromolecules During Analytical Ultracentrifugation by Michael Bailey (Melbourne Uni); Practical FRET by Sarah Ellis (Peter MacCallum Cancer Centre,); Fluorescence Correlation Spectroscopy by Nick Klonis (La Trobe University), Image Correlation Spectroscopy as a probe of clustering and dynamics by Andrew Clayton (Ludwig Institute).
See FIG website (www.FIGroup.org) for further details

A Pasteur-Walter and Eliza Hall Institute Workshop: Imaging infectious diseases at the molecular, cellular and tissue level.

Date: 31 January – 1 February, 2008
Location: The Walter and Eliza Hall Institute of Medical Research.
Microscopic imaging techniques like confocal microscopy, multiphoton excitation or wide-field deconvolution have become indispensable tools for the study of biological processes in cells and tissues. We invite you to participate in this Workshop, which will bring together French and Australian scientists and provide a relaxed and



informal forum for the exchange of data and ideas on the exploitation of state of the art imaging technologies to the elucidation of host-pathogen interactions in different infectious diseases from bacteria to parasites.
Topics include cell invasion by bacteria and protozoan parasites, microbial cell-cell interactions, intracellular trafficking and the use of imaging techniques to screen for new drugs. New and evolving technologies include molecular beacons and quantum dots, cryoelectron microscopy and dynamic imaging of cells and tissues.

Contact: *Emanuela Handman*, Chair, Pasteur-WEHI Workshop Organising Committee
http://www.wehi.edu.au/WEHI_Groups/indexworkgroups.php?id=124
Enquiries: enquiries-pasteur2008@wehi.edu.au
Registration:http://www.wehi.edu.au/pasteurworkshop

ACMM20 - Conference 10-15 February 2008 Perth.

The 20th Australia Conference on Microscopy and Microanalysis will be held in Perth in 2008. Use the link below to locate program details and the call for papers (*deadline 31 October*). Preconference workshops include TIRF, correlative microscopy and confocal microscopy. The program includes sessions on Fluorescence Quantitation and Sensing Techniques and their biological applications (FRET, FLIM, Multiphoton etc). see *http://microscopy.org.au/ACMM20/*

Live Cell Imaging workshop, LCI2008 Monash, April 2008

Following the highly successful series of Live Cell workshops (2004, 2006) MMI will hold this event again in 2008. At this stage, we are trying to schedule time in April, but timing depends on lab space availability, so stay tuned, watch this space, and read about the LCI2006 Reports section below. *Ian Harper*

New Facilities

Fluorescence Correlation Spectroscopy at La Trobe University, by Leann Tilley & Nick Klonis

The Biochemistry Department at La Trobe University has recently acquired a Zeiss LSM 510 confocal microscope equipped with a Confocor 3 system for performing fluorescence correlation spectroscopy (FCS). The equipment was purchased through a successful ARC LIEF grant application by Prof. Leann Tilley, (Biochemistry Department), with additional funds from La Trobe University and the Ludwig Institute for Cancer Research.

The FCS system utilises the optics of the confocal microscope to analyse the intensity fluctuations as fluorescent molecules move in and out of the focussed illumination volume (typically ~0.2 fL). These time-dependent intensity fluctuations are used to generate an autocorrolellogram that shows how the intensity is correlated in various time scales. Because large molecules move "slower" than small molecules, their intensity remains correlated on relatively large time scales compared to the smaller molecules. Similarly, the intensity of a fluorescent molecule in a viscous environment such as a biological membrane will remain correlated on larger time scales compared to media of lower viscosity. By analysing the autocorrelation profile it is possible to extract information such as the diffusion coefficient (and hence size) of the molecular entity, the viscosity of the microenvironment and to obtain an independent measure of the average number of molecules in the illumination volume (and hence the concentration).

The FCS system can be used to analyse interactions between macromolecules if they lead to changes in the size of the molecular entity. For example, the change in size arising from the interaction of fluorescently-labelled ligand with protein or from a fluorescently-labelled protein associating with a large macromolecular complex can be quantitated by FCS. More detailed information can be obtained by labelling two components of a macromolecular complex with different fluorescent molecules and examining how the two fluorescence intensities are correlated with each other in time. Indeed cross-correlation analysis permits interactions to be analysed even if they do not lead to a change in size.

One of the most exciting aspects of the FCS system at La Trobe University is the ability to perform both autocorrelation and cross-correlation measurements in live cells expressing fluorescent proteins. Hence it is possible to measure the size of macromolecular complexes and examine protein-protein interaction within cellular subcompartments of living cells expressing fluorescent proteins. The analysis should allow one to identify whether a protein of interest exists as a monomer or is present as part of a large macromolecular complex within a cell.
Anyone interested in using the instrument or in discussing possible applications should contact Prof Leann Tilley (L.Tilley@latrobe.edu .au) or Dr

Nick Klonis (N.Klonis @ latrobe.edu.au). Note the ***October 17 workshop at La Trobe*** *(above)*.

Slide Imaging at Monash Micro Imaging

While a catchy name or acronym for any novel optical mode or microscope design seems almost a prerequisite for success, the dotSlide (Olympus-SiS) not only has the catchy name but a smart software approach. Recently installed at Monash University (with another at the Peter Mac) the instrument is impressing users. There are many implementations of automated slide scanning (a.k.a. telepathology, digital histology, “virtual microscopy” etc), but one of the significant improvements in dotSlide has to be the image handling and experience of the virtual slides that are produced. This is not to downplay the great optics, and precise image tiling (if correctly calibrated) but the “google-earth“ feel is what really impresses researchers: one can easily pan, scroll, zoom and inspect the tiled image in a very responsive way, and you can also overlay different image modes (fluorescence and brightfield, or different resolutions) and also annotate. All of these features instigated us to take on the instrument, with the agreement that full automation of fluorescence microscopy would be implemented over 2007, so that our researchers would be able to capture not only large anatomical structure or whole organs, but also large populations of fluorescently labelled cells. Confocal imaging suffers all too often from people gathering insufficient cell data, or being too subjective in what they capture. We believe that the instrument can function as a fluorescence cytometer as well, complimenting high resolution data with cell population data on the same samples. Whole slides (up to 4 slides in the standard slide holder format, or 50 slides in the slide loader) can be imaged automatically, and viewed remotely as well, using a choice of 2x-60x lenses, so you can essentially select the resolution you require and walk away. Supported by the newly established online Datastore (currently being rolled out in the Faculty of Medicine), researchers will be able to image at the highest resolution knowing that this information will be available for review (remotely and in conference mode if necessary), and easily handled for later analysis. *Ian Harper*

Optical Projection Tomography (OPT)

An OPT system was recently installed in Prof John Bertram’s lab, Department of Anatomy and Cell Biology, Monash University, and is now in almost daily operation. The instrument, which was reviewed in FIGNews March 2004, utilises both transmitted white light and fluorescence to recreate 3D volume. Resolution of the system is around 5 x 5 x 5µm at best, but fluorescence intensity, resistance to photobleaching, absorbtion, scattering and depth invariable determine the effective detection and resolution for any specific sample. While most work is done on specimens cleared in an organic solvent (usually BABB), there are reports of imaging live specimens in water[1].

Reports on Workshops and FIG activities

Ludwig Scientific Imaging Workshop

The inaugural “***Ludwig Scientific Imaging Workshop-Microscope to Publication***” was held May 16 - 18, 2007. Tony Burgess (Director, Ludwig Institute for Cancer Research or LICR), noting a general lack of knowledge regarding the preparation of images for publication amongst scientists, was the driving force behind the workshop. Demand for enrolments was very high - the hands-on component of the workshop was oversubscribed within 36 hours of initial advertising. The workshop was a great success: 45 scientists enrolled, 29 of whom enrolled in the full hands on section of the workshop. A “lectures only” enrolment category, and extra microscopes and computers were added to help meet the demand. Topics raised during the workshop on scientific ethics of imaging, have been discussed recently at an international level in *Nature* and on the confocal microscopy listserver[2] indicating the importance of this type of workshop. Articles published in *Journal of Cell Biology*[3] and written by one of the workshop speakers, Mike Rossner have been the impetus to this extremely important debate.

The course was co-ordinated by Stephen Cody, (LICR), Andrew Runting (Head IT, LICR) and Pierre Smith (Imaging & IT Manager, Surgery, Melbourne University). Lecturing Faculty included Stephen Cody, John Bertram (Monash University), Sarah Ellis (Peter MacCallum Cancer Center), Glen MacDonald (University of Washington), Felix Margadant (National University of Singapore &

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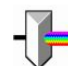
[1] *Visualizing Plant Development and Gene Expression in Three Dimensions Using Optical Projection Tomography. K. Lee, J. Avondo, et al. (2006) PLANT CELL 18, 2145-2156.*

[2] *http://listserv.acsu.buffalo.edu/archives/confocal.html*

[3] *http://www.jcb.org/cgi/reprint/166/1/11.pdf*



University of Sydney), Mike Rossner (Managing Editor, Journal of Cell Biology), Janna Taylor (Biomedical Illustrator, University of Melbourne) and David Vaux, (LaTrobe University). The workshop emphasized acquiring images in a scientifically valid way, and then handling and preparing those images for publication so that they retain their scientific integrity. There was an introduction to the physiology (& bias) of the human visual system, current microscope platforms, working with digital images, display, storage, compression, projection and preparation of images for publication.

The mornings consisted of 3-4 lectures and demonstrations, while afternoons were occupied with “hands on” laboratory sessions to give the participants practical experience with the equipment and techniques. Each day finished with a panel based question and answer session on the day’s topics. Four student scholarships were awarded to subsidise accommodation and travel expenses for travelling students. The lectures and practical sessions were highly successful with very positive feed back from the participants. A nominal enrolment fee of $50:00 was collected from those attending the full workshop and $20 from those wishing to attend just the lectures. Lunch, morning and afternoon tea as well as the conference dinner was funded from enrolments and the commercial sponsorships.

Significant **support** for the workshop was provided by LICR (including the purchase of 5 new microscopes for the course), FABLS (funding for travel and accommodation for two of the speakers, plus administration costs) and GTAC (facilities and IT support). Other sponsors were Coherent Scientific, BD Biosciences, and Molecular Devices and Journal Cell Biology.

Stephen Cody (LICR)

[Editors comment: This workshop represents another innovative scientific service contributed by the Victorian science community and both Tony Burgess and Stephen Cody are to be commended for their vision and efforts].

LCI 2006

The second Live Cell Imaging workshop (LCI2006) was run from 3-8 December at Monash University. Convened by Dr Ian Harper and Monash Micro Imaging, the aim of the course was to provide live cell imaging training for biologists who already use microscopy. Lectures covered brightfield and fluorescence microscopy, and the various forms of 3D microscopy (deconvolution, confocal, TIRF and multiphoton microscopy) and fluorescence applications (FRET, FRAP etc). These were all backed by practical work on a platform of 14 instruments provided by MMI and the major microscope manufacturers. The course was heavily subscribed, but restricted to 37 participants: together with 5 instructors, 9 assistants and 20 company representatives, this made for a considerably larger contingent (71) than when it was last run in 2004 when there were 40 in total. Company support was excellent and the course would not have been possible without such a commitment, some companies providing up to 5 support staff for the week. FABLS was also a significant sponsor of the event.

The 14 instruments provided a strong platform for teaching, demonstrating and group access, and for the first time, an image processing component was also included. Image processing for live imaging is somewhat complicated, and its inclusion will need to be carefully evaluated as there was insufficient time to cover all required aspects appropriately – future inclusion via an additional short course will be considered for future workshops. A major powerfailure (campus wide) halfway through day 1 (30 min before the first practical) resulted in rapid reorganisation: day 2 lectures were brought forward into the afternoon and evening, and fortunately we found premises in the local Halls of Residence. The remainder of the week proceeded relatively calmly, and in general the course was reviewed by most as a success, by some as a rave, and by some as “rather difficult”. For future courses, numbers will be further restricted, and ensuring that all instruments are fully capable of facilitating the imaging undertaken in the practical sessions. Practical activities, as with the 2004 workshop, will be conducted along the lines of student practicals, so that participants are encouraged to fully participate and present results.

Ian Harper, Convener, LCI 2006.