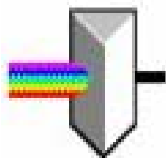
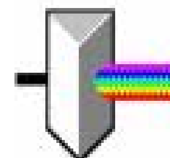


The Fluorescence Imaging Group (FIG) is a newly established group intended for the support and development of fluorescence imaging techniques including (but not limited to) Confocal and Multiphoton microscopy. The group will hold a variety of meetings that will cover issues of relevance to novices as well as experienced microscopists.



From the Editorial Desk.

Brian Jones

Editorially the FIG newsletter will begin to take on a slightly new form in the coming months. This change is due, in a small part, to the departure of Alan Hibbs, see Society & Committee Matters, and a re-shuffle of committee responsibilities. The humble but determined offer from myself, Brian Jones, and Damian Myers to co-edit the newsletter was accepted, after a cheeky nomination to the roles by Ian Harper I should say. Damian and I will endeavor to increase the number of newsletters published and spice up the “feel”, if you will, of the content and look of the layout.

I come to the FIG group via the Baker Medical Research Institute and a rather recent jump into fluorescence microscopy. I have been working within imaging fields since the early 90’s, most of my time spent with machine vision systems but starting out in a laser fusion research lab in Rochester New York. My formal training is from the Rochester Institute of Technology in Imaging and Photographic Technology and I have been in Australia for about five years. And it looks as though I will remain here for quite some time, I am having too much fun!

I am very much looking forward to working with the FIG group to spread the word so to speak. It must have been that rambunctious dinner and too many lovely wines with the FIG committee that sparked my interest. At any rate, it should prove to be a wonderful learning experience and I encourage all forms of response to our editorial decisions by FIG members.

Society & Committee Matters

Due to the departure of Alan Hibbs the committee has a taken on a new form, see below.

Alan Hibbs is on the move! I am currently, accompanied by my family, travelling the world visiting confocal microscope manufacturers, selling manuals, teaching confocal & 2-photon microscopy and carrying out imaging based research. We set off in late May, visiting UCLA in Los Angeles and Bio-Rad USA headquarters in San Francisco. I have now just finished teaching on Jim Pawley's "3-D Microscopy of Living Cells" course in Vancouver, and next month I will begin a 9 month contract lecturing, setting up an imaging facility and carrying out imaging based research at the Philipps University in Marburg, Germany. There are many exciting developments in imaging technologies around the world - I hope to discuss some of these developments through this newsletter over the coming months. Best of luck with your imaging - don't get too frustrated when you're having difficulties - there are lots of others struggling to come to terms with the enormous range of imaging technologies flooding the market!

Alan Hibbs (BIOCON), Peterborough,UK
(ahibbs@biocon.com.au) July 2001.

New Committee Structure:

Ian Harper	Chair	☎ 9905 5635
Mark Lam	Secretary	☎ 9905 1244
Dean Hewish	Treasurer	☎ 9662 7233
Stephen Cody	Speakers / Liaison	☎ 9341 3155
Brian Jones	Newsletter	☎ 9522 4333
Damian Myers	Newsletter	☎ 5226 7383

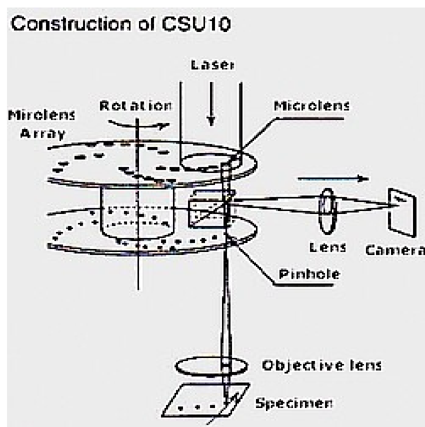
Live Cell Imaging: The UltraView Confocal Microscope at Monash University

Ian Harper

In 1883/4 Paul Nipkow, a German physicist and television pioneer, developed a way to send a moving picture by wire. At the time, it was known that selenium was photosensitive, and believed he could use this fact to convert a picture into an electrical signal. All he needed was some way to break up an image into points of light and dark, which could then be changed into electrical

signals using cells made of selenium. To do this, he used a "Nipkow" disk which had a spiral of holes cut into it and positioned so that they could scan every part of an image during a single disk rotation. The light coming from each point would then be focused onto a selenium cell and hence turned into an electrical current. This electrical signal would light up a second light at the other end of the wire. The second light would flicker because the amount of current it received would depend on the brightness of the image being scanned. The light from this light bulb passing through a second disk spinning at the same speed, would then project the picture onto a screen. Thus a type of a mechanical modem (modulator-demodulator) was conceived over 100 years ago.

By 1935 other methods of signal transmission had superseded the scanning disk for TV, but Petran and Hadravsky in 1968 revisited the technology to develop a "real-time" confocal microscope which they called a Tandem Scanning Microscope (TSM). Using a microscope with a rotating Nipkow disk in the light path, the multiple pinholes in the disk generated multiple light spots for imaging a specimen, and since the disk was scanned at high speed, the image could be seen directly or captured by camera. Thus this represented a "real-time" confocal microscope in contrast to the other laser point scanning confocal microscopes, where a computer was used to reconstruct the point images into a full frame image. In the current Nipkow disk based confocal systems, the scan speed may be up to 1800 rpm, one may achieve up to 360 image frames per second and the image can be seen directly or photographed.



One problem with pinholes is that although there are thousands of pinholes in the disk, they only cover a small percentage (< 1%) of the total area and a bright light source is required to produce images. To overcome this problem, a second disk containing a micro-lens array to focus the incoming light onto the pinholes has been adopted, and Yokogawa Electric Corporation manufactures a CSU-10 confocal scanner unit. This design greatly increases the light transmission to the specimen, and causes enough fluorescence to be able to image biological specimens. The UltraView confocal system is an integrated confocal system, incorporates the Yokogawa scanhead, good control and analysis software and a sensitive high resolution CCD camera. There are other Nipkow disk/real-time scanning systems out there: the CSU-10 from Yokogawa, available exclusively from PerkinElmer outside Japan; the CARV system from Atto Instruments, the K2S-BIO from Technical Instruments (which has been around for over 10 years) and the recently developed OptoLine from Klughammer). There probably are a few others I have forgotten to mention, like the slit scanners from Bio-Rad and Meridian. However, the UltraView has become very popular over the past two years as more molecular biologists/biochemists turn to imaging their gene markers, fusion proteins etc. With the need for long term studies (units of hours rather than minutes) in biological systems, the ability to image for extended periods with low phototoxicity/photobleaching is particularly appealing and obviously very important.

An UltraView system is stationed at Monash University in the Monash Micro Imaging Facility where we are currently refurbishing labs to cater for live cell imaging. The UltraView will compliment other confocal systems from Leica & Optiscan, as it is specifically tailored for live cell imaging. Anyone wanting to trial the UltraView is welcome to do so over the next few months, and should contact me directly (details below). Because of the fixed pinholes, the system is designed for use with high resolution (>1.2 NA) lenses only, and hence the sytem is really aimed at high resolution work. Since photobleaching and photodamage effects are greatly reduced, the system is ideal for live cell imaging work over long periods, and the control/analysis software supports 4D (3D +time) and 5D (multichannel 4D) imaging. Essentially, since the channels are obtained by

filter changing and a single detector (camera), rapid imaging (with multiple frames per second) is generally done in single channel mode, and 2-channel imaging is done where acquisition rate is less than, for example, 1 frame per second. In much of our work, rapid scanning is required, and we have managed to obtain up to 7 frames per second. Further references:

1. UltraView:http://lifesciences.perkinelmer.com/areas/cellimaging.asp
2. Access to the system at Monash Micro Imaging: Ian Harper, Building 13C, Monash University, Clayton, VIC 3800, Australia. Email: Ian.Harper@med.monash.edu.au, Tel: 9905-5635 / 0408314168.
   General inquiries: microscopy@med.monash.edu.au

Programme of Meetings

Next Meetings

August 16th, Thurs

"Confocal Imaging, visualization and 3-D surface measurement of small mammalian teeth"
by Alistair Evans, Department of Biological Sciences, Monash University

Seminar room:
Monash, Clayton campus, lecture theatre M1 (Medicine 1), building 13D
Format: 4-5pm Seminar
5pm onwards: Nibbles in Anatomy common-room (Building 13C, first floor) with a tour of confocal and imaging facility, and look at Ultraview for those interested. Sponsored by PerkinElmer.

Parking areas are indicated on map - park in the blue area in W4. Note: For referenced map see: http://www.monash.edu.au/campuses/clayton/map1.html

Alistair Evans is currently undertaking a PhD in the Department of Biological Sciences, Monash University. His project aims to understand the functional morphology of the teeth of insect-eating mammals, in particular, microbats. Engineering principles of tool function have been used to develop functional criteria of these teeth. The new measures of dental function require a three-dimensional understanding of tooth morphology. To fully characterise the shape and function of these small (1-3mm) teeth, Alistair had to develop a means of gathering high resolution, three-dimensional data of the tooth surface. He will discuss confocal imaging of the teeth, with an interesting and well illustrated talk that shows the techniques, quantification and animation of tooth surfaces (as recently described in a paper to be published in the Journal of Microscopy). *Highly recommended.*

September 19th, Wed

"Green eggs and Calmodulin based reporters: 101 uses of Green Fluorescent Protein"
By Dr. David Bowser, Department of Physiology University of Melbourne.

Seminar Room:
CSIRO Division of Health Sciences and Nutrition, 343 Royal Parade Parkville
Format: 3:30pm, drinks and registration
4-5pm, talk, then more drinks etc

David will cover the basics of how to create GFP-tagged proteins and examine protein-protein interactions with mutant GFPs. The majority of the talk will be on the new, exciting technology of GFP-based biosensors such as cameleons, camgaroos, pericams and chlormeleons, and targeting these to organelles, cells or tissues of interest. All topics will be illustrated in relation to their current research projects.
Sponsored by BD Biosciences. http://www.bd.com/

Also Note:

Multi-dimensional Microscopy 2001
Melbourne, Australia
November 25-28

The 3rd Asia-Pacific International Symposium on Confocal Microscopy and Related Technologies.
For more information see the following web site: www.swin.edu.au/optics/cmp/aps2001

Report Back: Seminar Series:

Deconvolution in Microscopy.
A Mini-Symposium.

In February FIG ran a highly successful two day workshop in deconvolution both sessions were well attended with around 65 at the seminars and 20 at the hands-on sessions. We were privileged to hear from two highly experienced international speakers. Prof. Mark Cannell (Auckland Uni., NZ) described the benefits of

applying deconvolution techniques to multiphoton data sets. Mark clearly explained the theoretical benefits of these techniques.
The practical benefits were driven home as he demonstrated how the conjunction of these methodologies enabled the extraction of subresolution, morphological data in the t-system of cardiac muscle. I'm sure most of the biological imaging fraternity present were a little envious of the resolution obtained in his deconvolved images of the t-tubular system, and certainly, all were envious of the deconvolved images of his yacht!

Dr. Jason Swedlow (Dundee Uni., Scotland) explained the benefits of applying deconvolution to widefield fluorescent image data sets. The benefits of the deconvolution algorithm, and the integrated system supplied with the DeltaVison system were explained.

The following day participants were welcome to bring their specimens and try their hand at deconvolution. This session was greatly appreciated by the large numbers that attended bringing with them a variety of specimens.

We are grateful to Tom Donnelley from Applied Precision Instruments and Jason Swedlow who shared their expertise generously.

We are grateful to Applied Precision Instruments, (www.api.com/products/bio/deltavision.html) for sponsoring the mini-symposium, including the expenses for two international speakers. Special thanks go to Tom Donnelley from Applied Precision for his support initially and throughout the visit.

Membership.

Membership of the FIG is open to everyone and we strongly urge everyone from young students or graduates to those more experienced in digital and fluorescence imaging to join the group. FIG will endeavor to provide a forum for those wishing to learn, apply or continue development of fluorescence imaging technologies in the widest sense possible. We will endeavor to provide interesting and informative talks, seminars and workshops, and where possible to interact with technology developers and suppliers to our mutual benefit. Membership fees for individuals are nominal, in place only to cover costs of administration and provide a small reserve to expand our operation. Membership is only $20.00 for full members and $10.00 for registered students. Please use the membership form attached to the end of this newsletter to apply for membership.

So why the FIG should I join ?

1. Become part of a scientific and technology network sharing resources, training and expertise.
2. Gain access to news, other information and local resources (eg, a resources database detailing users, expertise & equipment) is under construction.
3. Gain entrance to seminars and regular tutorial meetings.
4. Receive informative newsletters.
5. Attend an annual conference (from 2001 onwards).
6. Learn about and understand the latest technology and research applications.

We also have a category of membership for commercial or corporate members. Contact details for supporting companies will be listed in each newsletter.

SPEAKERS

Please note: we are always interested in locating speakers with interesting, novel or just plain challenging applications involving fluorescence imaging techniques. We are also seeking sponsors for each meeting.

Diffracted Perspectives:

Strong opinions, useful hints, calumny/traducement, fantastic assumptions. If members want to contribute, this is the place. Send comments to "Diffracted Perspectives" brian.jones@baker.edu.au

"There is no science without fancy, and no art without facts"

Vladimir Nabokov, c. 1966
Russian-American novelist 1899-1977

"In science it is common practice to break down and destroy material in order to gain new knowledge. The artist does not wring new knowledge from the material but re-interprets it, making the existing material visible in a different form."

Cornelia Parker, c. 1996, Postmodern Artist.

Cornelia Parker, *Cold Dark Matter*, 1991.
Photo: Hugo Glendinning.

Cornelia Parker began this piece with an inoffensive garden shed, illuminated from within and stuffed with a collection of acquired clutter. The shed was located in a field. The Army set explosives off within the shed. The contents were violently scattered, laboriously re-collected and then re-configured in the above fashion.

The above image and text was taken from the following:
Siân Ede (Ed.). (2000). Strange and Charmed: Science and the Contemporary Visual Arts. United Kingdon: Calouste Gulbenkian Foundation. ISBN 0 903319 87 X
For topics relating to Science & Art collaborations see the following:

The Arts Catalyst http://www.artscatalyst.org/
The Sciart Consortium http://www.wellcome.ac.uk/
CAMAC (Center d' Art, Marnay Art Centre) http://www.camac.org/accueil_a.htm
Leonardo, The International Society of the Arts, Sciences and Technology http://mitpress.mit.edu/e-journals/Leonardo/

Corporate News: Products and Info

We will gladly run advertisements for companies under paid advertisement scheme. Adverts will only be accepted for items relating to the interests of the group. Please inquire from the committee about advertising costs for quarter, half page, or full page). Our mailing list is well over 100 professionals covering most major research and tertiary education institutions.

Membership Application

[ ] $20 Ordinary member
[ ] $10 Student member
[ ] corporate sponsor

Surname	Given Names	Title
Institute/Department		
Address		
Town/Suburb	State	Post Code
Phone	FAX	
Email		

Please send completed form & payment to:
The Fluorescence Imaging Group
c/o Dr. Dean Hewish,
CSIRO HSN, 353 Royal Parade, Parkville 3052