From the Convenor

Dear Colleagues,

As 2007 swings into high gear, we are pleased to report that our Network continues to flourish and grow. We have almost doubled our membership over the past 6 months and continue to enjoy expansion of the areas of expertise enriching our community.

We offer a warm welcome to all our new members, as well as good wishes for our existing membership. The support and participation of members of our Network has been invaluable, none more so than those members who generously gave their time to contribute to the Committees in 2006.

The AGM was held during the Melbourne workshop on the 3rd February 2007. The success of the day, seen in the high attendance numbers (especially for a Saturday!) and in the relaxed and friendly atmosphere, was a great way to start the year. It was very encouraging to note the continuing steps towards achieving our aims of fostering inter-disciplinary communication in the Fluorescence arena, as well as facilitating increased discussion and partnering between academia and industry.

We would like to express our gratitude to the following members for their contributions over the past year to the FABLS Committees:

**Executive Management Committee:**
Robert Learmonth, Mark Prescott, Carola Thoni, Trevor Smith, Stuart Rumble, Anya Salih, Matthew Phillips;

**Resource Management Committee:**
Mark Prescott, Robert Learmonth, Trevor Smith, Stuart Rumble, Anya Salih, John Harvey, Gerri Springfield.

**Education and Training Committee:**
Robert Learmonth, Ron Clarke, Ian Harper, Meredith Wallwark, Seth Olsen, Pierre Moens.

We would also like to welcome our new committee members for 2007, introduced below. A Strategic Initiative Committee has been set up this year. It will discuss ways in which FABLS or its parts can form groupings to advance large-scale projects and access broader funding opportunities.

We welcome any enquiries about making contact with our membership.

Regards,
Ewa Goldys
Membership

The FABLS membership has grown over the last 3 months by about 50 members to about 260. Members come from 31 national and 41 international research organisations and 18 companies in Australia. Industry people outside the FABLS network are quite interested in hearing that its membership is more than 260 people.

2007 Management Committees

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<td>Ron Clarke (University of Sydney)</td>
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<td>Norman Heckenberg (University of Queensland)</td>
<td>Stephen Cody (Ludwig Institute for Cancer Research)</td>
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<td>Will Hughes (Garvin Institute)</td>
<td>Jin Dayong (Macquarie University)</td>
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<td>Chris Johnson (PerkinElmer Life &amp; Analytical Sciences)</td>
<td>Sarah Ellis (Peter MacCallum Cancer Centre)</td>
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<td>Robert Learmonth (University of Southern Queensland)</td>
<td>Ewa Goldys (Macquarie University)</td>
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<td>Mark Prescott (Monash University)</td>
<td>Peter Karuso (Macquarie University)</td>
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<td>Stuart Rumble (Lastek Pty Ltd)</td>
<td>Tak Kee (University of Adelaide)</td>
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<td>Trevor Smith (University of Melbourne)</td>
<td>Luz Paje (Olympus Australia Pty Ltd)</td>
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<td>Carola Thoni (Leica Microsystems)</td>
<td>Peter Robert (Advanced Labs Pty Ltd)</td>
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<tr>
<td>Ewa Goldys (Macquarie University) - Chair</td>
<td>Lilian Soon (University of Sydney)</td>
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<td>Pierre Moens (University of New England) - Chair</td>
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<tr>
<td>Joerg Enderlein (Forschungszentrum Juelich)</td>
<td>Damian Bird (University of Melbourne)</td>
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<td>Ewa Goldys (Macquarie University)</td>
<td>Warwick Bowen (University of Otago)</td>
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<td>Min Gu (Swinburne University of Technology)</td>
<td>Andrew Clayton (Ludwig Institute for Cancer Research)</td>
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<td>Edwin Yeow Kok Lee (Nanyang Technical University)</td>
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<td>David Sampson (Univ. of Western Australia) - Chair</td>
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Business Liaison

FABLS continues to develop its presence to people from business and government and to establish a system to exchange information for the benefit of both organisations. Recent activities include:

- Dr Bruce Grieve, Syngenta and Manchester University regarding potential opportunities to be involved in University Technology Centre. Syngenta are interested in applications of fluorescence, specifically their possible integration into Syngenta’s core activities in the plant protection and plant breeding. Steve Demeo, Invest Australia, introduced Dr Grieve to FABLS.

- Dr David Topping, CSIRO’s Food Futures Flagship (drawing together resources in cereal genetics, plant genetics plant breeding, agronomy, plant physiology and food engineering), regarding
research alliances with CSIRO. David also has an involvement in preventative health flagship. A number of collaborative projects adopting greater use of fluorescence were identified.

- Dr Bruce Cornell, Ambri Ltd regarding approaching private industry and research collaboration opportunities. Bruce is a director of the Medical Device Industry Association (MDIAA), which has a membership of about 50 companies.

- Sue Evans, Ausbiotech identifying how FABLS can maximise its membership with Ausbiotech organisation. Examples include newsletter distribution, seminar event notification, and articles for their journal. FABLS is a member of Ausbiotech.

- Dr Christopher Armstrong, NSW Office for Science and Medical Research, regarding research collaboration and funding programs. OSMR are willing to support large-scale research projects.

- Ausbiotech 2006 Conference Five new posters, a new brochure, a CD of the one-page research descriptions were created to exhibit research expertise FABLS. Company members of FABLS Leica and BTFbio also took part. Discussions regarding the FABLS network (future collaboration, membership, general interest in the network and what we did) were held with over 50 research and industry representatives.

Good News Story: A Win for Jin

A new technique for detecting rare waterborne pathogens like *Giardia* and *Cryptosporidium* in moving fluids, developed by Dayong Jin, Macquarie University PhD student, may one day be used to help detect HIV in the developing world.

Studying at Macquarie University’s Centre for Lasers and Applications, Jin has developed a cheap, yet fast and accurate device for counting the number of *Giardia* cells in a fluid stream.

“It’s currently quite hard for medical researchers to find the exact number of contaminated blast cells in blood,” he says. “Because we can look at 10,000 cells per second, and find rare pathogens like *Giardia* or *Cryptosporidium*, we can perhaps use it in other areas like clinical diagnosis.”

The technique he employed – known as Time-gated Luminescence Flow Cytometry – involves ‘tagging’ any offending microorganisms with fluorescent molecules, which glow after being excited by a laser or LED light and can be counted in real-time under a high-powered microscope.

His technique recently collected him two of the five Awards at the 3rd Annual MQ Innovation Awards in November 2006 i.e. the Invention Disclosure Award and the Postgraduate Innovation Award, defeating entrants from diverse fields.

Now Jin has travelled to the US, supported by funding from the Fluorescence Applications in Biotechnology and Life Sciences (FABLS) Network, in order to find out if the technique could be successfully applied to detecting HIV in the bloodstream of people in the developing world.

At Purdue University, he has received training in medical areas and presented his work to international scholars and biotech companies at three major conferences, leading to a number of opportunities to further expand and apply the research. These include: NIH Funding with Prof. J. Paul Robinson (Purdue Uni); as well as, US Department of Homeland Security and Defence Funding for the development of a time-gated luminescence imaging flow cytometry technique in collaboration with AMNIS, Newport Instruments & Phoenix Flow System.

Jin acknowledges the support of Professor Jim Piper and Dr Russell Connally, at Macquarie.
As in previous newsletters, a number of review articles are presented below. The articles are brief version of the one-page project descriptions provided by the researchers. All the researchers (contact details provided) are interested to discuss applications of their technology with commercial partners and interested academics.

**Application of luminescent nanodiamonds to intracellular imaging**

Functional intracellular imaging on the molecular level has been enabled by application of optical labels, which tag specific molecular sites to enhance their visibility on the non-specific cellular background. The optical labels can be either ultrabright scatterers, e.g. plasmon nanoparticles, or fluorophores, e.g. fluorescent dyes and quantum dots. Photostability, low cross-section, and toxicity of existing optical labels limit the scope of optical imaging, especially in the context of tracking individual molecules in the cells.

An alternative optical label, termed luminescent nanodiamond (LND) features a nitrogen-associated vacancy defect. The luminescence properties of LND, including its lifetime and near-infrared emission band, ensure the LND high visibility on the cell morphological and autofluorescence background. The scattering properties of highly refractile diamond nanocrystals render them visible in the cells. LND is extremely photostable. In virtue of their carbon composition, nanodiamonds are biologically compatible, and their bioconjugation is straightforward. We will report results of our collaborative research on production, characterization of luminescent nanodiamonds, and LND-assisted intracellular imaging.

Dr Zvyagin (zvyagin@physics.uq.edu.au) at the University of Queensland have developed a LND surface biofunctionalisation protocol, which should allow them to perform specific labeling of cell structures, first, *in vitro*, finally, *in vivo*. A method to produce true luminescent nanometer-sized nanodiamonds with desirable colour centres has been developed. Biological problems, such as long-time virus tracking in the cell, will be addressed using LNDs making use the new optical labels merits.

The researcher would like to collaborate with people to demonstrate the background-free optical imaging of LNDs and to test LNDs for tracking of single biological molecules such as proteins

**Development of correlative biomolecular imaging methods**

Correlative microscopy, by using combined light-, probe-, laser- and electron microscope techniques have become increasingly important for the analysis of the structure and function of cells and tissues. New concepts and progress in structural and molecular cell biology have been discovered thanks to improving correlative microscopy techniques that continues to rely predominantly on advances in new, three-dimensional (*X*, *Y* and *Z*) visualization techniques. It is clear from the literature that the development of correlative imaging methods has not come to an end yet and that soon the time dimension (*X*, *Y*, *Z* and *t*) will be added by bridging the time resolution gap by using rapid transfer systems.

Dr Filip Braet from the Electron Microscopy Unit at the University of Sydney (filip.braet@emu.usyd.edu.au) is developing straightforward approaches to combine fluorescent and electron microscopic information on the same cell.

The research is combining imaging methods in which a fluorescence signal of a small molecule is reconciled with a signal from the electron microscope and developing novel sample preparation protocols for correlative biomolecular imaging at the cellular and molecular level.

Dr Braet would like to collaborate to further develop his method to combined fluorescence and scanning electron microscopy method for retrieving simultaneous cytoplasmic and topographic information.
Fluorescent Chelate: Advancing new technology in the fluorophores industry

Immunofluorescence techniques are widely employed, for the specific detection of microorganisms and to identify regions of interest in histopathology samples. Typical fluorescent lifetimes ($\tau$) of most fluorophores are in the nanosecond regime, for example, Fluorescein has $\tau$ of 4 nanoseconds. A luminescent chelate (BHHST) developed by Dr. Russell Connally (rconnall@ics.mq.edu.au) at Macquarie University belongs to a class of compounds that exhibit exceptionally long luminescent decay lifetimes when bound to trivalent europium ions. The persistent luminescence of the chelate makes the compound valuable for use in time-gated luminescence studies.

BHHST has improved properties in aqueous solutions compared to the parent molecule, BHHCT. This new fluorescent compound is intensely luminescent and has been used for the preparation of immunoconjugates that have proved successful in time-gated luminescence studies of Cryptosporidium and Giardia. The BHHST chelate offers a number of key benefits allowing simplified preparation, improved storage stability, formation of more stable immunoconjugates, ten times enhanced Signal to Noise Ratio (SNR), and luminescence lifetime greater than 500 microseconds.

BHHST has a number of potential business applications, including in situ direct labelling of organisms with immunoconjugate, conjugation of fluorophore to protein, time gated fluorescence techniques (ELISA, confocal microscopy, and high through put screening in drug discovery) and comparative hybridisation/DNA microarray techniques.

Rapid identification techniques for useful biomolecules

The gene cassette metagenome is a very large and diverse group of mobile genes that are available to a wide variety of microorganisms through the processes of horizontal gene transfer. Research to date indicates that gene cassettes largely contain genes of adaptive significance, so allowing organisms containing appropriate gene cassettes to survive environmental challenges. Gene cassettes have already been shown to produce proteins that confer antibiotic resistance, heavy metal resistance, UV protection and a variety of other specialised adaptive functions.

Professor Hatch Stokes (hstokes@rna.bio.mq.edu.au), A/Prof M Gillings and Carolyn Michael have developed a variety of molecular techniques to identify, categorise, characterise, express and manipulate gene cassettes. These include mass environmental screening for different types of gene cassettes as well as providing data on their relative abundances. In addition, enrichment techniques that enhance the recovery of those gene cassettes responding to specific stressors are under development.

These techniques will allow the rapid isolation of individual and synergistic groups of gene cassettes with specific biological functions related to particular challenges. These gene cassettes could then be utilised to produce gene products that may have significant therapeutic, industrial or environmental applications.

Collaboration is sought to undertake field trials of existing methods, to develop new methodologies for the analysis and assembly of customised gene cassette systems, and to commercialise the technology.
**Latest Equipment Update**

**Fluorescent protein-based biosensors - Invitrogen**

**what it is...**
Invitrogen introduces the new Molecular Probes™ Premo™ product line, combining the stability of genetically encoded ion indicators and environmental sensors with the efficiency of BacMam delivery\(^1\). The first in a series of products, the Premo™ Cameleon Calcium Sensor is a no-wash, non-organic dye indicator for intracellular calcium signal measurements. Based on the YC3.60 version of a GFP-based sensor family developed by Tsien, Miyawaki, and co-workers,\(^2,3\) the binding of four Ca\(^{2+}\) ions to a calmodulin-M13 moiety induces a conformational change (Figure 1). This change brings the cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) domains closer, allowing fluorescence resonance energy transfer (FRET) to occur.

**what it offers...**
- Genetically encoded calcium sensor for iterative assays
- Ratiometric, FRET-based detection for quantitative results
- Highly efficient delivery system for large-scale cell transfection and batch freezing
- Easy-to-use and efficient delivery system

Learn more about the Premo™ Cameleon Calcium Sensor
Contact: mail.australasia@invitrogen.com, or Barry Lynch at c-barry.lynch@invitrogen.com

**STED - Superresolution Microscope - Leica Microsystems**

Stimulated Emission Depletion Microscopy, or STED microscopy, is a method that overcomes the diffraction limit of conventional fluorescence confocal laser scanning microscopes and fluorescence widefield optical microscopes by reducing the active area of a pulsed excitation spot by a superimposed, pulsed and tightly synchronized ring-shaped beam, which depletes fluorescence before it is emitted.

As a result, STED microscopy approaches molecular resolution, which offers breakthrough potentials for almost all aspects of bio-medical research.

STED microscopy has been invented by Prof. Stefan Hell of the Max Planck Institute for Biophysical Chemistry, Germany. With this invention, he has won the German Future Award 2006.

What is new about this technique is the fact that the resolution of microscope images is no longer limited by the wavelength of the light. The attainable resolution is purely a question of technical design. Resolutions of 20 nanometers have already achieved. As protein complexes are in the 10-200 nanometer range, this microscope has the potential to probe life on a molecular scale soon and obtain more accurate information on diseases.

The principles of STED microscopy are licensed for commercialization to Leica Microsystems CMS GmbH. In 2007, Leica Microsystems will introduce a commercial STED system based on the Confocal and Multiphoton System Leica TCS SP5.

The ability to view life in nanometer dimensions opens the door to an understanding of intracellular life processes which was never thought possible before and which may lead to revolutionary discoveries on the subject of how diseases originate.

Learn more about STED, contact...
Fluorescent cell stains - FLUOROtechnics

LavaCell™ is a new fluorescent cell stain that provides a simple solution to intracellular imaging of live and fixed cells. LavaCell is based on epicocconone, a water soluble, uncharged, low molecular weight fluorophore that readily permeates cells. LavaCell only becomes fluorescent on entry to cells and enables staining without permeabilisation or washing steps. LavaCell is excitable by violet, blue and green light and exhibits a long Stokes’ shift making it suitable for most platforms and idea for multiplexing with other fluorophores.

LavaDigest™ is a simple fluorescent assay that provides real-time monitoring of proteolysis. Monitoring proteolysis is important in many areas ranging from digestibility studies in the food industry to ensuring complete protein digestion prior to MS analysis in proteomics. LavaDigest does not modify proteins during their analysis enabling subsequent down-stream analysis by MS, HPLC, immuno-staining, etc. LavaDigest is ideal for high throughput proteomic applications where ensuring complete digestion is important.

LavaPep is a new, fast, sensitive and robust fluorescent-based assay, to quantify peptides. It is compatible with downstream proteomics processes such as mass spectrometric peptide mass fingerprinting (PMF). It offers over 3-orders of magnitude linear dynamic range with sensitivity of less than 100ng/mL. LavaPep requires no heating or reduction steps and results are available within 1 hour. Samples can be analysed on a wide range of fluorescence-based instruments and the assay is robust to detergents, DTT, Urea, etc

LavaPurple™ Total Protein Stain - Gel Stain is the most sensitive fluorescent stain available for the detection of proteins in IEF and SDS-PAGE gels. LavaPurple is compatible with Mass Spec, Edman sequencing and functional analysis. LavaPurple staining results in very low background fluorescence and no speckling. LavaPurple Total Protein Stain - Blot Stain is the most sensitive and simple to use fluorescent protein stain for nitrocellulose and PVDF membranes. Stained proteins can be analysed by techniques such as Edman sequencing, Mass Spec, and functional analysis. One product, LavaPurple Protein Stain, can be used for all gel and blot staining applications.

For more information contact Duncan Veal (sales@fluorotechnics.com) or visit www.fluorotechnics.com

FABLS Website

The number of people accessing FABLS website is growing. You will notice from the figure below that about twice as many people accessed the site in 2006 in comparison to 2005. If the trend for the first month of 2007 continues, the hits on the website could double again in 2007. You can access this information and more including who is looking at the website on
Information about the network and its members is now going to a large audience. You are encouraged to use it and contribute and take advantage of its potential networking opportunities. A number of additions have been made, including the capability statements and project descriptions provided by members. While it has been some time coming, a new home page will be launched this month to give it a new look.

**Fluoro2006 Workshop**

The Fluoro2006 Workshop on use of optical spectroscopic and microscopic techniques in biological and biomedical research, was held at Coffs Harbour 2-6 October 2006.

Forty four participants attended the workshop which was presented to by 6 scientists with support from 10 company technical representatives.

Contributing scientists were Prof Enrico Gratton (University of California Irvine), Prof Dave Jameson (University of Hawaii), Prof Ken Jacobson (University of North Carolina), Dr Pierre Moens (University of New England) and A/Prof Rob Learmonth (University of Southern Queensland) and Prof Brett Hambly (University of Sydney).

Lectures and practical sessions included basics of fluorescence, instrumentation, light and fluorescence microscopy, fluorescent labelling, Förster resonance energy transfer (FRET), fluorescence correlation spectroscopy (FCS), raster image correlation spectroscopy (RICS) and fluorescence lifetime imaging (FLIM), and 3-D particle tracking.

Several companies supported the workshop by providing resources and staff to assist in the practical sessions; Lastek and Jobin Yvon Horiba, Leica Microsystems, Shimadzu Oceania, Varian, ISS, BMG Labtech, Edinburgh Instruments and Invitrogen.

The University of New England and FABLS managed the administration and promotion of the workshop. Scholarships were provided 25 postgraduate students - FABLS (20), Australian Society for Biochemistry and Molecular Biology, (3), and the Australian Society for Biophysics, (2).
Short Courses

The EM Unit and the Australian Key Centre for Microscopy and Microanalysis

Sydney University run a range of courses in light microscopy, electron microscopy, specimen preparation and microanalysis. Courses available in the first half of the year are listed below. All courses will be held again in the second half of the year. There is a fee attached with each course. Enrolments close two weeks in advance.

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<td>Biological Specimen Preparation, TEM &amp; SEM</td>
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<td>Materials Specimen Preparation, TEM &amp; SEM</td>
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<td>Stereology</td>
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<td>Image Analysis</td>
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<td>TEM of Crystalline Materials</td>
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<td>Research Methodology</td>
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<tr>
<td>Microscopy of Biomolecular Processes</td>
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More information about the next 2 courses scheduled:

• **Introductory Microscopy & Microanalysis - March 12–22**
  An introduction to the fundamental principles of optics and the related principles of spectroscopy that are commonly used in microscopy and microanalysis. Students are introduced to a variety of imaging and analysis techniques and their role in both biotechnology and the technology of materials, as relevant to laboratory-professionals and researchers. An emphasis on light-optical microscopy and related imaging modes is developed.

• **Biological Specimen Preparation, TEM & SEM - March 26–30**
  A theoretical background and practical knowledge of basic routine specimen preparation techniques for electron microscopy. It covers biological specimen preparation for TEM, including fixing, embedding, sectioning, drying, coating and staining techniques. An introduction to cryotechniques and immuno methodologies is included. All aspects of specimen preparation for SEM are also covered.

For more information, including course fees, contact courses@emu.usyd.edu.au.

**FISH Course – University of Queensland – 16th – 18th April 2007**

The use of rRNA-targeted Fluorescence in situ Hybridisation (FISH) is now widely accepted as the least biased method for identifying and enumerating populations of microorganisms within a large variety of environmental samples - including sludge, seawater, freshwater, intestinal/skin flora, plant root/leaf epiphytes, rumen, etc.

There will be theoretical knowledge and practical sessions on sample handling, preparation and fixation, FISH preparation - expert tips and tricks, fluorescence microscopy and interpretation, image analysis and cell enumeration, and optimising and interpreting your own samples.

*3-day laboratory-based course with handbook and CD for $650**
ARB Workshop – University of Queensland – 11th – 13th April 2007

ARB operates using large cumulative databases – therefore, the introduction and alignment of new sequences continually contributes towards all future phylogenetic analysis, including FISH probe and PCR primer evaluation and design. Starting with a Greengenes database can even eliminate the need for time-consuming sequence retrieval and alignments, although ARB can easily import and autoalign almost any sequence from NCBI. ARB has incorporated some of the newest and most sophisticated tree-building algorithms for both DNA and protein alignments.

Participants learn theoretical knowledge and practical ARB skills with:

• web-based bioinformatics and multiple sequence alignment
• tree constructions and phylogenetic analysis
• probe & primer design & evaluation

With Handbook and CD, all-inclusive in a 3-day interactive workshop for $550**
Combination FISH and ARB $1000

Contact Sandra Hall, 07 3346 7209 email: s.hall@uq.edu.au

Your Contributions to the Newsletter

We hope to instigate lots of interest in the newsletter content, and would like the readers to also be the editorial providers. Some of the sorts of things we would like to include are

• interesting contacts, (we need to build our national database)
• equipment available for collaborative (and consultative) projects
• latest equipment updates from industry
• 'good news stories'

Compiled by David Tayler
dtayler@ics.mq.edu.au