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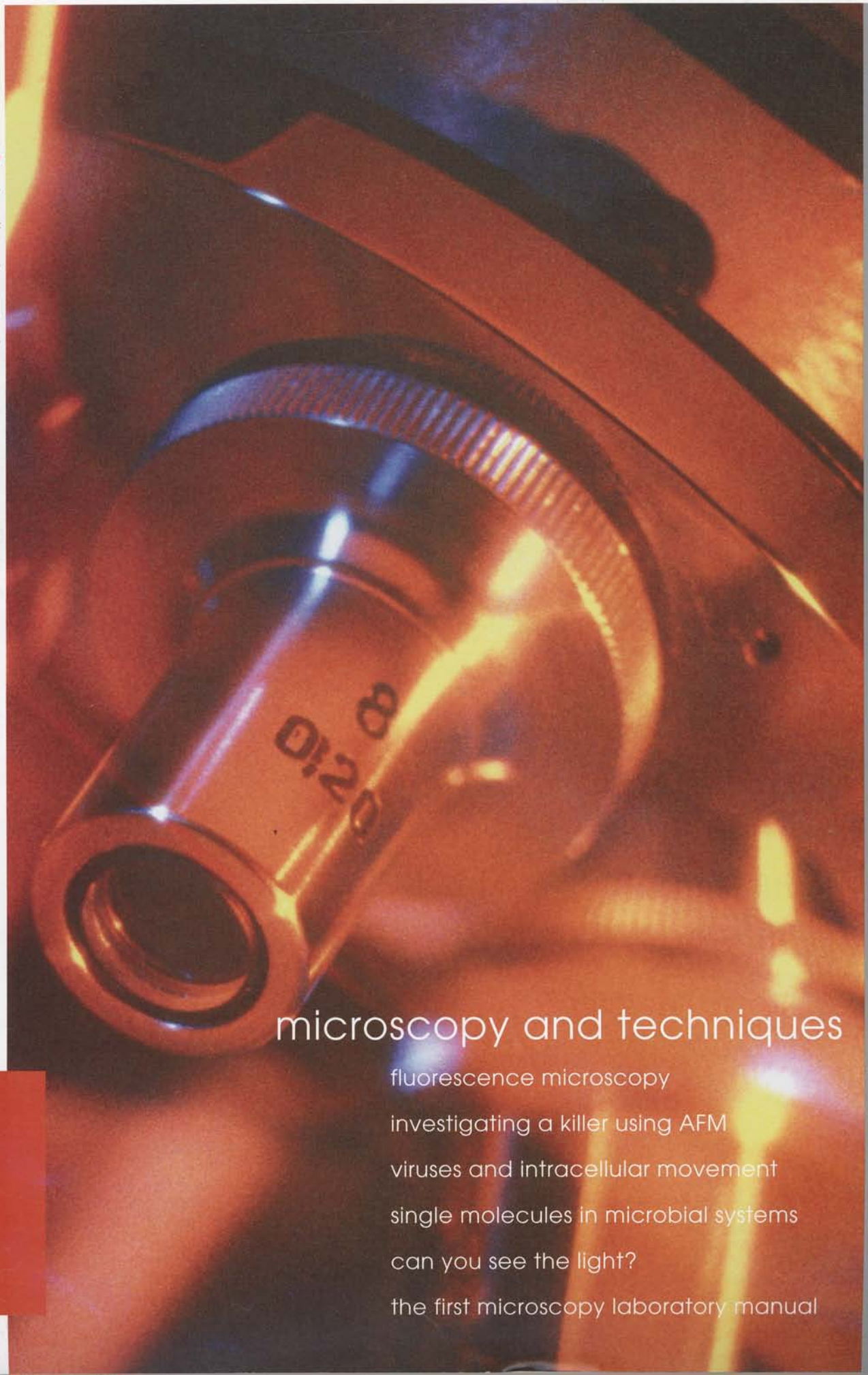

microscopy and techniques

fluorescence microscopy

investigating a killer using AFM

viruses and intracellular movement

single molecules in microbial systems

can you see the light?

the first microscopy laboratory manual

contents

vol33(3)

regular features

other items

articles

Cover image Microscope lenses. *Tony Craddock / Science Photo Library*

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news

Under the microscope

The theme of this issue is imaging. It includes articles describing some of the secrets of microbial cells now being revealed thanks to new technological developments in microscopy, plus a look back at a pioneer in the use of microscopes who worked when the existence of micro-organisms was just being discovered.

Another feature gives sound advice on what to look for when obtaining equipment in this field. As an enhancement to this article, for the first time *Microbiology Today* is making movies available with the web version. To view these check out **www.sgm.ac.uk/pubs/micro_today/** Your feedback on this extra feature is welcome as we will try to provide more animations if readers like them.

See more at York

The imaging theme is also continued in a two-day symposium at the SGM meeting at York University in September. It takes place on 13 and 14 September and will include sessions on fluorescence and confocal laser scanning microscopy, scanning probe microscopy, and electron (cryo) microscopy and electron crystallography. Between the scientific sessions there will be demonstrations of techniques and equipment from a range of suppliers.

Small groups of delegates will be able to attend short presentations and also see the goods on display in the trade exhibition. These workshops have been scheduled so that everyone attending the meeting will be able to drop in. There will be something of interest to all, no matter what their specialist field in microbiology. See **www.sgm.ac.uk/meetings/MTGPAGES/York06.cfm** for full details of the scientific programme and the workshops.

SGM wins second silver medal at Chelsea


THE ROYAL HORTICULTURAL SOCIETY

SILVER MEDAL

CHELSEA FLOWER SHOW 2006

Awarded to Society for General Microbiology

For an exhibit of Information on plant diseases

IN THE LINDLEY RANGE

The SGM's display on microbial plant diseases at the RHS Chelsea Flower Show in May attracted the attention of thousands of visiting gardeners. It also pleased the judges, as you can read on p. 132.

New science champion for Scotland

SGM member **Professor Anne Glover** of Aberdeen University has been appointed Chief Scientific Adviser for Scotland. She has been seconded to the Scottish Executive to provide independent advice to ministers, take the lead on co-ordinating science policy and work closely with the science community. Anne Glover was an elected member of SGM Council from 1995 to 1998.

Address Book 2006

A new edition of the Address Book, containing a list of the names and contact details of Society members and other useful information about SGM is being compiled and will be distributed with the November issue of *Microbiology Today*.

Please let the Membership Office have any changes to your address, telephone/fax numbers or email details as soon as possible, but not later than **11 August**. Send them to **members@sgm.ac.uk**

Also, if anyone wishes to have their details omitted from the Address Book and has not already notified the Society, they should do so immediately.

Microbiology lost for words

The Editorial Board of *Microbiology* has been concerned that many articles in the journal were longer than they really needed to be. Articles in *JGV* are already written to strict length limits, and the Editorial Board of *IJSEM* has succeeded in recent years in reducing the average length of articles dramatically through a consistent policy of encouraging conciseness. As an encouragement to brevity, Council has agreed that *Microbiology* articles of up to 4,500 words (including legends, but excluding references) and eight tables or figures will be acceptable, but those exceeding these limits will be subject to a charge of £75 (plus the inevitable VAT where applicable) for every additional 500 words or part thereof and every additional display item. This policy will come into effect for articles submitted from **1 October 2006**. The level of charge will be assessed on the accepted version of the manuscript.

AGM 2006

The AGM of the Society will be held on **Tuesday 12 September** at the meeting at York University. Agenda papers, including reports from Officers and Group Conveners and the accounts of the Society for 2005, are in the separate booklet distributed to all members with this issue of *Microbiology Today*.

Staff news

Congratulations to Staff Editor **Stefan Sidorowicz** on his marriage to Helen Willis on the 3 June at All Saints Church, Sedgley, West Midlands. Helen is a PA for a construction company and over 100 people attended the wedding. The reception was held at the Beacon Institute for the Blind, Wolverhampton, and was followed by a disco.

We are very sorry to announce the death of **John Brimelow**, the former Managing Editor of *Journal of General Virology*, who worked on SGM journals from 1985 to 2005. John leaves a wife, Isobel, and two children.

SGM Council

May Meeting Highlights

Outgoing and New Council Members

Professor Peter Andrew, Leicester, **Professor Jeff Cole**, Birmingham and **Professor Jeff Errington**, Newcastle, will soon complete their terms on Council, and their input and contributions were gratefully acknowledged. There were three nominations so an election was not required: the following will become members of Council at the AGM in September: **Professor Michael R. Barer**, University of Leicester, **Dr Richard M. Hall**, GlaxoSmithKline, Harlow and **Dr Catherine O'Reilly**, Waterford Institute of Technology, Ireland.

SGM Prizes 2007

Council was reminded that nominations are invited for the SGM Prizes 2007: the Fleming Award, the Peter Wildy Prize for Microbiology Education, the Colworth Prize Lecture and the Fred Griffiths Review Lecture (see *Microbiology Today*, May 2006 or the SGM website for details). The closing date is **30 September 2006** and the decisions will be made by Council at their November meeting.

SGM Annual Report 2005

Officers presented their draft Annual Reports for 2005 to Council. These were accepted and a copy of the entire Annual Report is enclosed with this issue of *Microbiology Today*.

Membership

Council was encouraged to learn that a campaign to enrol new members from different areas of microbiology is underway. The health and diversity of its membership are among the core strengths of the Society.

Biosciences Federation

The Chief Executive of the Biosciences Federation (BSF), **Dr Richard Dyer**, was welcomed by Council. He gave a short presentation about the aims, business plan and management of the BSF and answered questions from members. The BSF was founded in 2002 and wishes to be an authoritative voice of the biological sciences community with a big impact on policy and decision making at government level. This it intends to do with the help of its member organizations but without duplicating their efforts. SGM has been a member of BSF since it was founded, and Council was pleased to offer an increased level of support for the next three years, provided certain conditions were met.

***Ulrich Desselberger**, General Secretary*

News of Members

Congratulations to Elected Member of Council **Petra Oyston** who has been made visiting professor in the Department of Infection, Immunity and Inflammation at the University of Leicester.

The Society notes with regret the following deaths:

Dr Sidney R. Elsden, President of the SGM 1969–1972, on 29 April 2006, aged 91. An obituary will appear in the next issue of *Microbiology Today*.

Dr David M. Meredith, St James' University Hospital, Leeds (member since 1980).

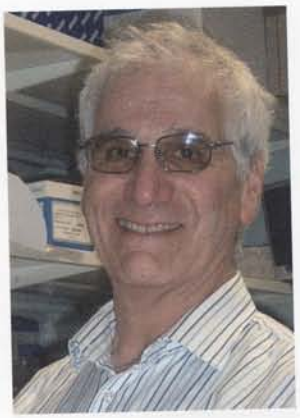
Geoffrey Talbot Banks (1933–2006), a member since 1965. He will be remembered as a lecturer and fermentation technologist at the Department of Biochemistry at Imperial College, London. After a spell at Glaxo in Barnard Castle in the early 1960s, Geoff joined Ernst Chain's team at Imperial College, where he worked *inter alia* on interferon production, publishing several papers in *Nature*. He retired from Imperial College in the early 1990s, settling with his wife, Mary, in the Vale of the White Horse.

Alexandra Schwenger, a postgraduate student at the University of Aberdeen, died tragically in a car accident in April (member since January 2006).

New President

Professor Robin Weiss

Robin A. Weiss is Professor of Viral Oncology at University College London. Robin graduated in Zoology at UCL in 1961, and gained his PhD in 1969. His first research was on the effects of high natural radioactivity on rat populations in Kerala, India. However, he has spent most of his research career studying retroviruses, including the discovery of endogenous genomes transmitted in a Mendelian manner while still a research student with Michael Abercombie. He worked briefly in Jan Svoboda's laboratory in Prague in 1968/9 and spent 2 years as a postdoc with Peter Vogt in the USA, before establishing a laboratory at the Imperial Cancer Research Fund in 1972 investigating retroviral oncogenes. While he was Director of the Institute of Cancer Research, London, 1980–1989, he investigated human T-cell leukaemia virus and HIV. He made significant contributions to our early understanding of HIV and AIDS, which included the identification of CD4 as the HIV receptor, neutralizing antibodies to HIV, and the demonstration that 'Slim' disease in Uganda really was AIDS. More recently, he has conducted research on pig retroviruses as a potential infection hazard in xenotransplantation, and on the aetiology of AIDS-associated malignancies, particularly Kaposi's sarcoma herpesvirus.



Thus Robin's interests span infectious disease and cancer. Indeed, a recent veterinary student in his lab, Claudio Murgia, has completed a study on how a cancer cell has evolved into a cellular parasite that is sexually transmitted among dogs and has spread globally, disregarding transplantation barriers. Robin also takes an interest in the history of microbiology and says that he much enjoyed the recent 'historical' issue of *Microbiology Today*. He has written a new slant on Robert Koch as the 'grandfather of cloning' (*Cell*, 2005, **123**, 359), and a book review on the Nobel Prizes in infectious diseases (*Nat Med*, 2006, **12**, 605).

Robin was elected to EMBO in 1977, and to the Royal Society in 1997. He was awarded the 1st International Beijerinck Prize of the Royal Netherlands Academy of Arts and Sciences in 2001 (SGM member David Baulcombe was awarded the 2nd Beijerinck Prize in 2004). He retires this year as Chair of the governing body of the BBSRC

New Group Conveners

Clinical Virology
Professor Judy Breuer

Judy is Professor of Virology at Barts and the London Medical School, Queen Mary College London and head of the Centre for Infectious Disease. Having qualified in Medicine, she trained in Microbiology and Virology at Kings College and St Mary's Hospitals. Following research at NIMR, she was appointed as Senior Lecturer in Clinical Virology at Barts and the London. Her research interests include the molecular epidemiology of Varicella zoster virus (VZV) and the Oka strain vaccine, as well as the investigation of viral determinants of VZV virulence. Professor Breuer is head of Clinical Virology and of the Health Protection Agency Varicella Zoster Reference Service. She is also actively involved in the establishment of clinical academic training programmes in microbiology and virology.

Eukaryotic Microbiology
Dr Alastair Goldman

I obtained my first degree in Genetics at QMC London in 1986. After a spell as a research assistant, I did my PhD, with Maj Hultén, centred on meiosis, which continues to be my main area of interest. Using the new technology of FISH, I analysed chromosome pairing and segregation in human male meiosis. In 1993 I joined the lab of Michael Lichten at NIH where I crossed the species barrier to work on yeast, concentrating on the interaction between meiotic chromosome pairing and recombination. I then took up a lectureship in the Dept of Molecular Biology and Biotechnology at Sheffield. The main emphasis of work in my lab revolves around the genetic influences on the mode and detail of DNA double-strand break repair during meiosis. We also undertake work on proteomic and cytological aspects of meiosis in yeast.

Institute for Animal Health, and as President of the British Association of Cancer Research. Otherwise, he says, he would not have time to devote to the SGM.

Robin writes: 'I have been a member of the SGM since 1968 and it really is a privilege to take the Presidency following Hugh Pennington's able leadership. Although there are now fewer university departments specifically dedicated to microbiology, the discipline remains as important as ever. Microbiology pervades our environment, it lies at the core of the biotech industry, and of course, novel and re-emerging pathogens of plants, animals and humans continue to threaten our welfare. The SGM will therefore build on its mission in education, research and policy advice.'

'We must continue to attract young members and there will be challenges for our magnificent portfolio of research journals. I saw no better evidence of the SGM's vibrancy than the recent meeting at the University of Warwick. At one point I felt split three ways between the fascinating sessions on retroviruses, on the early evolution of eukaryotes, and on the metagenome. Microbiology never ceases to amaze me in its diversity and complexity. To paraphrase Samuel Johnson: *When a person is tired of microbiology he is tired of life, for there is in microbes all that life can afford.*'

Irish Branch
Dr Evelyn Doyle

I am a Senior Lecturer in the School of Biology and Environmental Science at UCD. I completed my BSc in Microbiology in the National University of Ireland, Galway and then came to UCD as a PhD student working on high-maltose-producing amylases under the supervision of Liam Fogarty and Renee Kelly. After 4 years working as a postdoc, I joined the staff of the Department of Industrial Microbiology at UCD and was Head of Department from 2002 to 2005. I have retained an interest in microbial enzymes, but the main focus of my research is microbial degradation of xenobiotics and bioremediation. I am particularly interested in linking specific microbial populations to biodegradation in natural ecosystems. I have been a member of the Irish Branch since 1983 and served on the committee twice.

Prize Lectureship

Peter Wildy Prize for Microbiology Education

Professor Liz Sockett

Not just germs – bringing bacteria to life

Liz comes from Newcastle on Tyne. She did her BSc in Biochemistry & Microbiology at Leeds and a PhD in Microbiology at UCL. She followed this with a postdoc in the genetics of photosynthetic bacteria with Sam Kaplan at University of Illinois and then set up a molecular analysis of motility in photosynthetic bacteria at Judy Armitage's lab at Oxford. Liz was appointed to the Botany Department at Nottingham University in 1991 to set up and run an interfaculty degree in Applied Microbiology. She has always enjoyed lecturing and has taught some 3,000 students.

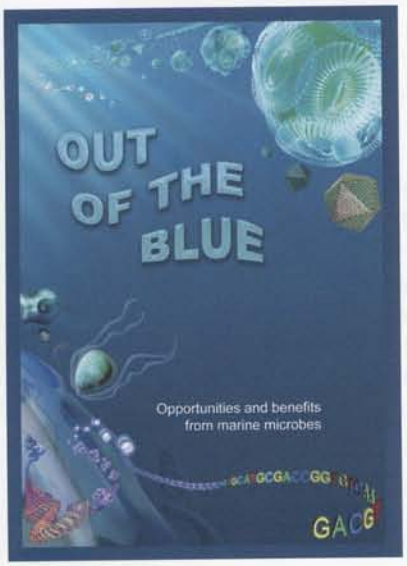
In 1999 Liz moved to the Institute of Genetics where she teaches bacterial genetics, biochemistry and evolution. Since the move, Liz's group has flourished. In collaboration with researchers around the world they have discovered the roles of motor, switch and stator proteins in the minute flagellar engines of photosynthetic bacteria, engineered flagellar filaments with specific designs and studied flagellar assembly in bacterial pathogens.

Liz's lab has also become internationally known for work on another swimming bacterium, the predator *Bdellovibrio bacteriovorus*. Liz's talk will describe its amazing lifestyle and ability to kill other bacteria without harming humans; it may one day be a living antibiotic.

Her teaching and research contributions have been recognized and she was SGM Education Officer from 1998 to 2002. She was appointed Review Chair of the 2005 International 'BLAST' Conference on Bacterial Locomotion and sits on the Wellcome Trust's Basic Immunology and Infectious Disease Panel. Liz believes that science should be made accessible to all. She is Disability Liaison Officer and Welfare Officer for her School. She has worked with the Royal National Institute for the Blind New College, making practical genetics accessible to visually impaired students, is on the Executive of the Science Learning Centre East Midlands and a regular speaker promoting science to the public and in schools.

Marine microbes star in DVD

'Small in size, big in importance' is the microbiologists' mantra. Marine bacteria, archaea, viruses and single-celled plants and animals are not only crucial in making the Earth habitable, but their genetic diversity provides an immense resource that we are only just beginning to use, for novel natural products and processes. *Out of the Blue*, a new DVD by the UK Natural Environment Research Council, will help to get that message across to a wider audience. 'TV programmes such as *Planet Earth* ignore the real action', explained Phil Williamson, the DVD's co-director. 'Admittedly, microbes are difficult to film, and the public only thinks of them as primitive organisms that are agents of disease. But they have a life of their own, with social behaviour, sophisticated biochemistries and immense ecological importance. Without them, neither polar bears nor humming birds would last for long.'



Narrated by Quentin Cooper, *Out of the Blue* is produced for the NERC BlueMicrobe knowledge transfer network (www.bluemicrobe.com) by Panache Productions, Oxford. Bioprospecting, molecular technologies, cell signalling, biofilms and the versatility of viruses are covered and illustrated by computer-generated imagery of microbial processes. Copies of the 35 minute DVD (playable on PCs and PAL DVD players) are available from **p.williamson@uea.ac.uk**. Initial distribution is at no cost; however, if a reprint is needed, a no-profit charge may be made.

Careers Conferences 2006

4 November	University of Manchester
18 November	University of Edinburgh
2 December	University of Reading

Aimed at life science under- and postgraduate students, each conference includes widely varying talks on career choices and further training, plus a small exhibition by a range of organizations. A CV review service is also available by prior arrangement. SGM is involved in organizing the events and will have a stand in the exhibition. Cost £12, to include refreshments and lunch. Details and a booking form are available at **www.bsf.ac.uk/careers/careersconf.htm**

Grants

Overseas schemes

The deadline is **13 October 2006** for receipt of applications to the following schemes:

- International Research Grants
- International Development Fund
- Watanabe Book Fund

Student schemes

Postgraduate Student Meeting Grants

Applications for a grant to attend the SGM's York meeting (11–14 September) must be received by **8 September 2006**.

President's Fund research visits

Four applications were successful in the last round; the second round of applications closes on **13 October 2006**.

Elective grants

Funding for medical/dental/veterinary students to work on microbiological projects in their elective periods. The closing date for applications is **27 October 2006**.

Other schemes

Seminar Speakers Fund

The Fund supports talks on microbiological topics in departmental seminar programmes. Applications will be dealt with on first come, first served basis during the academic year.

Education Development Fund/ PUS Awards

Grants are available to members for projects intended to lead to an improvement in the teaching of any aspect of microbiology relevant to education in the UK. Funding is also available for small projects to promote the public understanding of microbiology, such as workshops, talks, demonstrations, leaflets, activities at science festivals.

Applications will be considered on a first come, first served basis during the calendar year.

Retired Member Conference Grants

Retired members are reminded that they may now apply for a grant to attend one SGM conference each year. The award covers en-suite accommodation and the Society dinner, up to a maximum of £250.

Applications for grants to attend the SGM meeting at York University are now invited.

SGM has a wide range of grant schemes to support microbiology. See **www.sgm.ac.uk** for details and closing dates.

Any enquiries should be made to the Grants Office, SGM, Marlborough House, Basingstoke Road, Spencers Wood, Reading RG7 1AG (t 0118 988 1821; f 0118 988 5656; e grants@sgm.ac.uk).

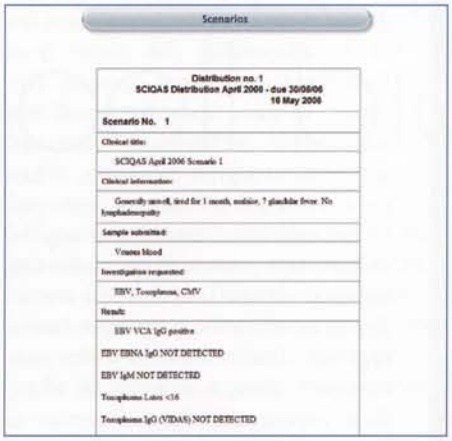
The UK Clinical Virology Network (CVN)

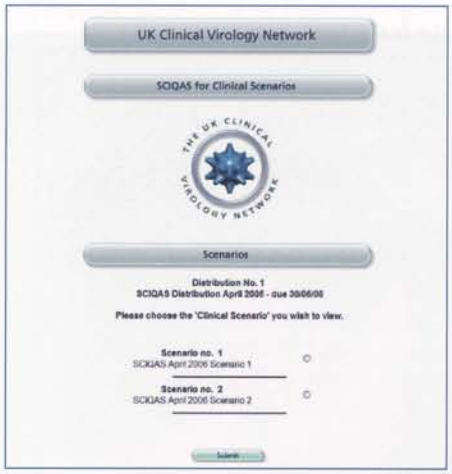
The CVN has around 30 member virology laboratories. The CVN website (www.clinical-virology.org) contains a wealth of information on virological topics and a message board. Additionally, the network operates a web-based continuing education tool called SCIQAS.

SCIQAS

SCIQAS presents the user with a web-based interface to virology clinical scenarios. Six distributions per year will be provided; each will contain two scenarios. Each is a real clinical case submitted for inclusion by one of the CVN's specialist virology laboratories. SCIQAS' primary aim is to provide participants with a convenient method of augmenting their knowledge of clinical virology and increasing their ability to accurately interpret clinical information.

On login, users are asked to choose a scenario from the current distribution (right). The relevant scenario is displayed, including clinical information on the patient in question, the sample submitted and assays performed upon it. An example scenario is displayed below.





The user then inputs their clinical interpretation of these data; simultaneously they may state that additional samples ought to be taken or further assays performed on those samples already sent to the virology laboratory.

After the SCIQAS user has submitted their response, they are sent a confirmatory email message automatically. This can be used as evidence for the Royal College of Pathologist's CPE scheme that the distribution has been completed. Each completed distribution is worth two points. After the submission deadline, a summary of user responses and an evidence-based expert response will be provided on the site. Simultaneously a forum is opened to allow user discussion of issues raised by the scenario.

Registration

SCIQAS registration may be gained in two ways. (1) CVN Membership is granted automatically to staff of any CVN member laboratory. If you are a member of one of these, but don't yet have a CVN username and password, please contact Graeme O'May (graeme.o'may@northglasgow.scot.nhs.uk). Staff of CVN member laboratories (see the website for a full list) are already members of the CVN and so are eligible to take part in SCIQAS. (2) For those not already CVN members, a CVN Personal Membership can be purchased at a cost of £50 per annum. CVN Personal Members enjoy all the benefits of membership, including participation in SCIQAS, use of the message board and access to the resources on the CVN site.

Elected Fellows of the Royal Society

The following microbiologists have been elected as new Fellows of the Royal Society.

Nicholas J. White, OBE, Professor of Tropical Medicine, University of Oxford for outstanding contributions towards an understanding of the pathogenesis, treatment and control of tropical diseases.

Joseph S.M. Peiris, Professor of Microbiology at the University of Hong Kong for his elucidation of human viral infections causing respiratory disease.

Carl R. Woese, Professor of Microbiology at the University of Illinois has been appointed a New Foreign Member of the Royal Society. He is distinguished for his discovery of a third major division of life, the *Archaea*, that ranks equally with bacteria and eukaryotes.

Immunofluorescence methods, together with GFP and digital imaging, are providing a revolutionary new view of the subcellular organization of bacteria, as **Jeff Errington** describes.

▼ Technician using a fluorescence microscope. *CC Studio / Science Photo Library*

Bacterial cells are remarkable in many ways. They carry out a huge and diverse range of biochemical reactions and are capable of growing in an amazing range of environmental conditions. One secret of their success lies in the adaptability that comes from their rapid growth and division. This comes, in turn, from their small size and simple architecture, compared with cells of higher organisms. When the electron microscope was developed in the mid-20th century, and applied to bacteria, it confirmed the notion that bacterial cells are simple. There was no sign of the complex, membrane-bound organelles that characterize eukaryotes. However, there is a sense in which these experiments did a disservice to bacteria, because they emphasized all of the things they were missing – cytoskeleton, membrane-bound organelles, particularly a nucleus. Meanwhile, some bacteria, such as *Escherichia coli* and *Bacillus subtilis*, became great experimental work-horses because of their rapid and easy growth and excellent genetics. Many scientists began to view them essentially as 'bags of enzymes' – wonderful for biochemical studies and as tools for molecular biology, but of little interest from the perspective of cellular organization.

This view changed dramatically in the mid-1990s when the renewed application of immunofluorescence methods to bacteria, together with the advent

Fluorescence microscopy as a research tool in bacterial cell biology

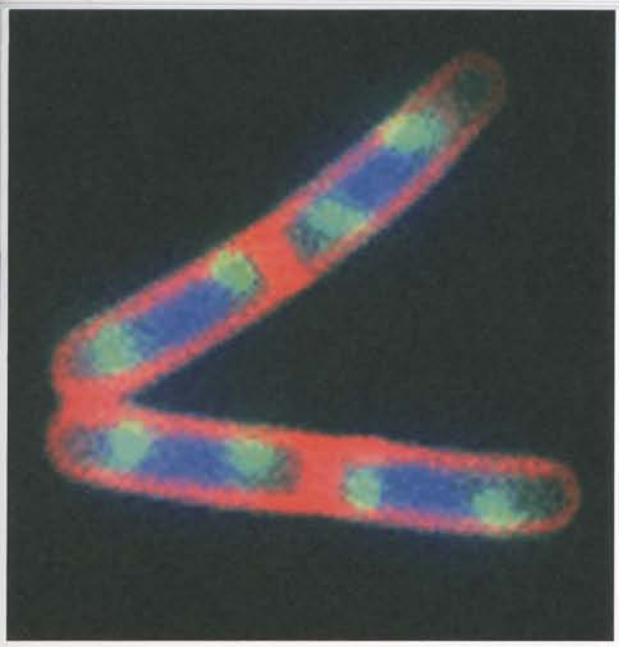
of green fluorescent protein (GFP) and digital imaging, provided a revolutionary new view of the subcellular organization of bacteria. These developments founded what was effectively a new field of 'bacterial cell biology' and provided a powerful suite of methods and approaches with which to probe fundamental aspects of the workings of bacterial cells.

Powerful new technologies

Fluorescence microscopy had been applied sporadically to bacteria in various ways for 30 years or more. However, in the early 1990s it entered the mainstream with the successful application of immunofluorescence methods to intracellular proteins in bacteria, followed soon after by the harnessing of GFP. At around the same time, the development of affordable CCD cameras with high sensitivity and spatial resolution, together with digital image processing packages, made it much easier to capture the faint fluorescence from bacterial cells.

Immunofluorescence methods essentially involve the use of a fluorescently labelled antibody to identify the location of the target protein. A major advantage of the method is that it can be done with completely wild-type cells, provided a specific antibody is available. In principle, the location of the fluorescent antibody reports the position of the native protein. Alternatively, the cells can be genetically engineered to add a short (~10 aa) epitope tag to the target protein, allowing it to be detected by any of a number of readily available monoclonal antibodies. The main disadvantage of this method arises from the need to get large antibody molecules into the cell. To achieve this, the cells need to be permeabilized. Since this would normally result in destruction of the cells, they first need to be gently fixed. But this is tricky because too much fixation makes the cells impenetrable. Therefore, the whole process is technically demanding. Inevitably, the problems of access of the fluorescent label and degradation of cellular structure mean that artefacts and even complete failure of detection are not uncommon. Finally, since the cells are obviously killed by the process, artefacts can arise from cell death and there is no question of being able to follow movement or changes in localization over time.

GFP is an intrinsically fluorescent protein derived from a jellyfish, *Aequoria victoria*. The big advantage of GFP labelling is that it can be done in native, unperturbed cells. Moreover, the cells can be imaged repeatedly, offering the possibility of collecting optical sections through cells, or obtaining a time lapse series. Although GFP has had a revolutionary effect on research across biology (of which more below) there are some caveats and limitations to its use. The main disadvantage arises from the fact that the cells need to be genetically engineered in some way, to produce a fusion of GFP and the target protein. Many, perhaps most, fusion



◀ *Bacillus subtilis* cells carrying a GFP fusion to the SpoOJ chromosome segregation protein. This protein sticks to regions that flank the origin of replication of the circular chromosome and forms spots or 'foci' (green) that mark the origin region. The spots duplicate soon after chromosome replication begins, as soon as there are two copies of the origin region. These then move rapidly apart towards opposite poles of the cell. The chromosome (blue), occupies most of the cell and the foci lie at each end of the DNA mass. The cell outline is stained red with a membrane dye. Two pairs of cells are shown. The cells are about 2 μm long. *Dr Heath Murray*

proteins are not completely wild-type in behaviour, because adding the 27,000 Da GFP moiety can mess up the target protein's activity in any one of a number of different ways. It is therefore important to check that the fusion is functional, by testing for its ability to sustain the function of the normal gene, where possible. If the protein is not fully functional, any pattern of localization should be treated with caution. In our experience the two most common problems that arise are: first, aggregation of the GFP fusion to form 'inclusion bodies', often in the form of a single bright spot located near the pole of the cell; second, loss of a specific pattern because of saturation, due to the fusion protein being overexpressed relative to the native protein.

A good way to mitigate being misled by this kind of problem is to construct GFP fusions to both the N and C terminus of the target protein. Sometimes only one of them is functional, and it is not uncommon for the two fusions to give different results! In our experience with hundreds of fusions, the N-terminal fusions have a slightly better success rate, which is unfortunate because they are somewhat more difficult to construct (the *gfp* gene has to be placed between the coding and upstream regulatory sequence of the gene).

Although GFP is often used as a reporter of subcellular localization, it can also be used as a reporter of the timing or cell-specificity of gene expression. Fusing *gfp* to the regulatory sequences of one's favourite gene provides a way of identifying where and when the gene gets turned on. This can be very informative in multicellular situations, such as in biofilms, or to study genes turned on under any kind of stochastic control.

A myriad of patterns

GFP fusions have now been made to many thousands of bacterial proteins. An ever-increasing multitude of different localization patterns has been described. Some of the results have been of a unifying nature. For example, it is now clear that transcription occurs in the core of the cell and translation in the periphery, just as in eukaryotes, even though there is no nuclear membrane demarcating these zones in bacteria. DNA replication occurs in specialized 'replication factories', just as in eukaryotes. Localization experiments have also been crucial in demonstrating that bacteria have all three elements of the eukaryotic cytoskeleton, tubulin (FtsZ), actin (MreB) and intermediate filament proteins (crescentin), with critical roles in the cell cycle and cell morphogenesis. Among the surprises have been the discovery of proteins that rapidly oscillate, from pole to pole, or round and round the cell, and which are involved in mapping the size and shape of the cell for the purposes of DNA equipartition or accurate selection of the mid-cell site for cell division.

Overall, the results show that bacteria have an astonishing and completely unexpected level of complexity. The patterns being distinguished today probably represent the tip of the iceberg, because resolution is currently confined by the physical limitations of fluorescence in the visible light range. A greater level of sophistication probably awaits discovery when emerging technologies that can overcome the current limits of resolution become available to bacterial cell biologists.

Jeff Errington FRS
Director of the Institute of Cell and Molecular Biosciences, Faculty of Medical Sciences, University of Newcastle, Newcastle upon Tyne NE2 4HH, UK (e jeff.errington@ncl.ac.uk). Jeff is currently setting up a world's first 'Centre for Bacterial Cell Biology' to reflect the importance of this new field for microbiology.

Further reading

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Investigating a bacterial killer using atomic force microscopy

The lifestyle of the bacterial predator *Bdellovibrio bacteriovorus* bears a striking resemblance to the creatures in the *Alien* movies. Propelled by its long polar flagellum, this nanoscale killer swims about randomly until it encounters a prey bacterium. When it finds and identifies a prey cell, the bdellovibrio predator burrows through the outer membrane and takes up residence in the prey periplasm, sealing off the outer membrane behind it. The bdellovibrio excretes a variety of enzymes to digest the prey cell, growing larger and larger as it breaks down and assimilates the prey cytoplasm. At this stage in the life cycle the growing predator protected by the carcass of its prey is called a bdelloplast. When the prey cytoplasm is totally devoured, the predator cell divides into progeny cells that lyse the outer membrane and swim away to find some new cell to consume.

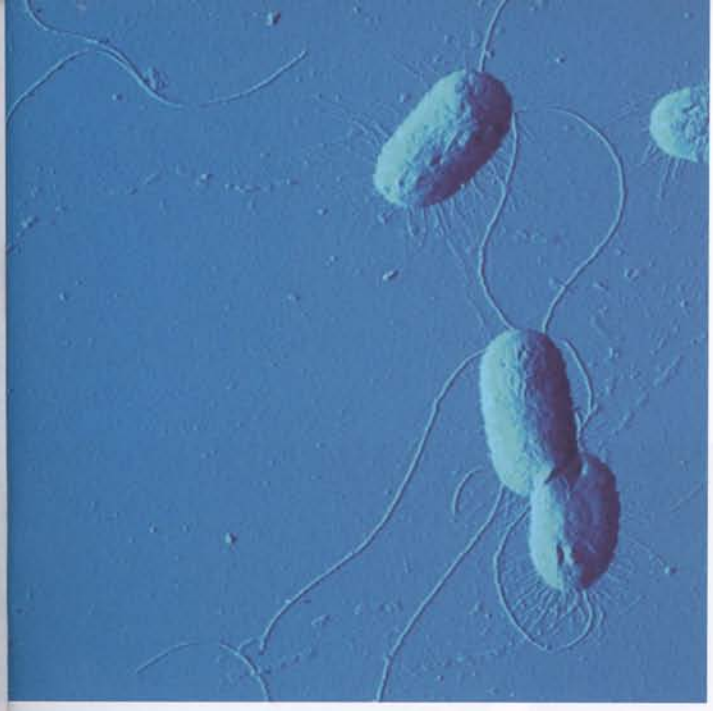
B. bacteriovorus consumes a wide variety of Gram-negative prey, but is harmless to other bdellovibrios, Gram-positive bacteria and eukaryotes. Because of this broad yet specific spectrum of target organisms, the role of bdellovibrios in the environment and potential applications in industry, agriculture and biodefence are of particular interest. In the past couple of years, we have explored the predator's behaviour at interfaces and surfaces with an eye towards examining its role in biofilms and other complex microbiological communities. Atomic Force Microscopy (AFM) has proved especially useful in exploring the interaction of bdellovibrios with prey in these environments.

Using AFM

With AFM, a small, pointed tip is scanned across the surface of a sample. Small deflections and alterations in the behaviour of this tip are then used to generate an image of an object's shape and contours. In general, AFM can resolve features on the nanometre to micron scale. With soft microbiological samples such as our predator and prey cells, we have used AFM to resolve small structures on individual cells, such as scars, flagella and pili, as well as to study groups of bacteria at once (Fig. 1).

AFM provides high-resolution information about the surface of a sample, so we might not expect it to reveal anything about the interior of a cell. However, it turns out that we can in fact learn a lot about the interior of bacterial cells as well. For example, we can clearly distinguish the growing bdellovibrio predator inside of the periplasm of an invaded prey cell. The shrunken ball of cytoplasm also can be discerned (Fig. 2).

Thus AFM provides a useful complement to traditional forms of microscopy, given that its resolution is better than light microscopy, but that samples require minimal or no preparation compared to electron microscopy. It is important



to note, however, that the cells of interest must sit on a solid surface to be imaged. Given the importance of surfaces and interfaces to bacterial growth, this requirement has provided little difficulty and also new areas for exploration.

Studying communities

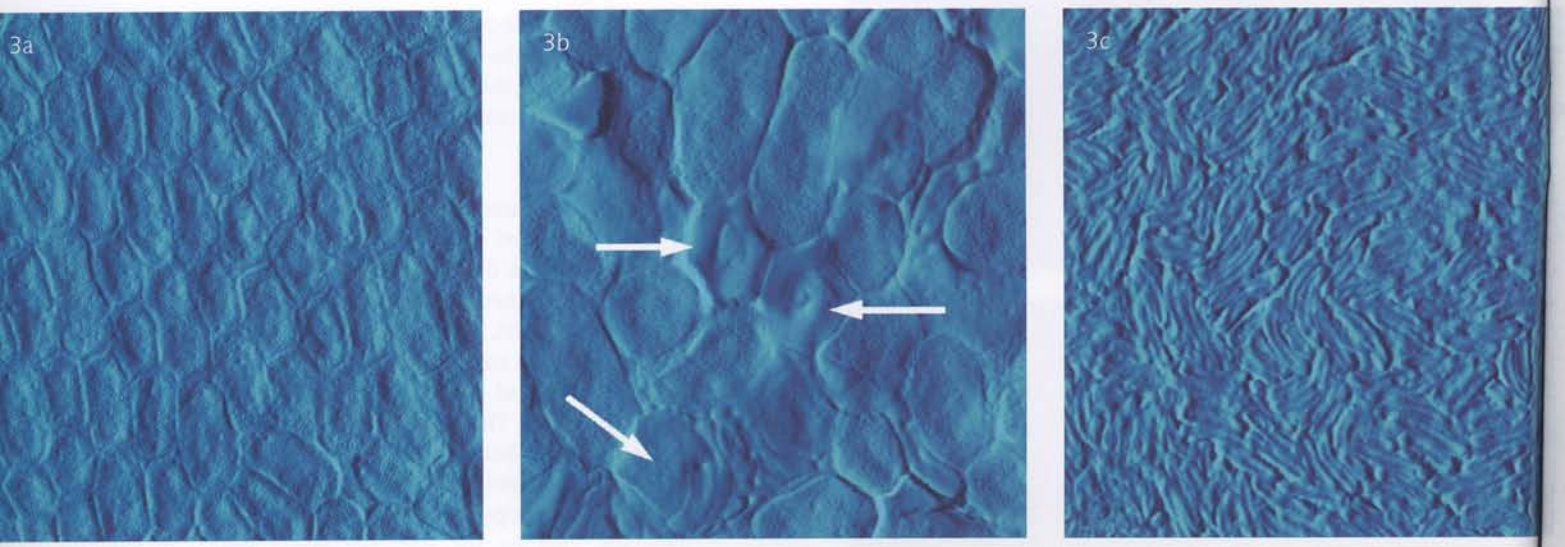
We initially grew simple bacterial communities on sterile polycarbonate filters placed on top of agar medium plates, an environment perhaps best described as a hydrated air–solid interface. Both prey and non-prey organisms, including several strains of *Escherichia coli*, *Aquaspirillum serpens*, *Micrococcus luteus*, *Ensifer adhaerens* and mutant host-independent *Bdellovibrio*, grow robustly, and divide and spread outward across the surface of the filter. The cells can be easily imaged by AFM right on the filter without modification.

Interestingly, when bdellovibrio predators are mixed with a Gram-negative prey population, they thrive in these surface communities. Within the first few hours after deposition onto the filter, only prey cells are seen, but soon the first bdelloplasts appear. The predators and bdelloplasts multiply and within a few days the bdellovibrios completely dominate the surface, forming a continuous lawn of large, well-fed predators. No prey cells are left

▲ Fig. 1. AFM image of *E. coli* ZK1056 on a glass surface. With this technique, bacteria can be imaged without staining or drying under vacuum, and structures such as individual flagella and pili can easily be resolved. *Modified from Núñez et al., (2005) Methods Enzymol 397, 258, with permission from Elsevier.*

◀ Fig. 2. Despite the fact that AFM is by nature a surface technique, the curved shape of the *Bdellovibrio* predator is clearly visible growing inside the ruined, rounded carcass of the prey cell. *Modified from Núñez et al., (2005) Colloids Surf B 42, 267, with permission from Elsevier.*

Atomic force microscopy is a powerful technique for studying biological surfaces and molecular structure. In this article **Megan Núñez** and **Eileen Spain** discuss the application of AFM to investigate the interaction between predator bdellovibrios and their prey.



▲ Fig. 3. *Bdellovibrio* predators devour *E. coli* prey on a surface. (a) Initially, the prey cells divide to cover the filter surface densely. (b) After 1–2 days, *Bdellovibrio* predators and bdelloplasts begin to appear. The bdelloplasts, such as the three indicated by arrows, can be distinguished from the unmolested prey cells by a rounded shape, a smooth surface texture, a shrunken cytoplasm and a distended periplasm inhabited by the oblong, coiled predator cells. (c) Within 3–5 days, the surface of the filter is entirely covered by the oblong, vibrioid predators. *Fig. 3b is modified from Núñez et al. (2003) Biophys J* **84**, *3383, with permission from the Biophysical Society.*

► Fig. 4. AFM images of bdellovibrios attacking model biofilms. Unlike staining methods, AFM allows us to distinguish normal prey cells from predators, bdelloplasts and cell debris. (a) The biofilm-forming *E. coli* strain ZK1056 attaches to a glass surface submerged in liquid and forms communities there. (b) In this interfacial environment the predator bdellovibrios attack and devour prey cells. *Modified from Núñez et al., (2005) Colloids Surf B* **42**, *266–268, with permission from Elsevier.*

alive except for a few refugees found at the outside edges of the bacterial community (Fig. 3).

The astounding success of bdellovibrios in these surface communities raises several interesting questions. In particular, we wonder about the nature of the air–filter interface. How thick is the layer of liquid above the filter? How do the bdellovibrios hunt on a surface? Do they swim or just wiggle? It is believed that the force generated by the movement of the flagella and the collision with the prey cell is important for penetration of the prey cell, but how much force can be generated on a flat surface within a tightly packed community? Bdellovibrios clearly dominate these communities, consuming all of the prey cells and growing to extraordinary lengths, unlike in the wild or even in liquid medium in the laboratory. Is their success due to the benefits of a 2-dimensional (as opposed to a 3-dimensional) search? Do they receive additional nutrients by 'grazing' on cell debris?

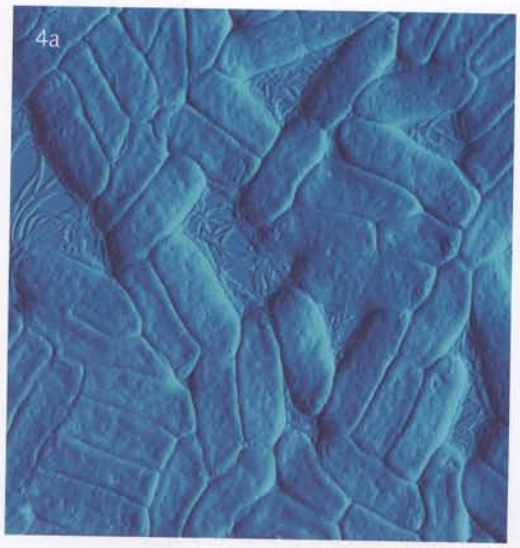
Bdellovibrios in biofilms

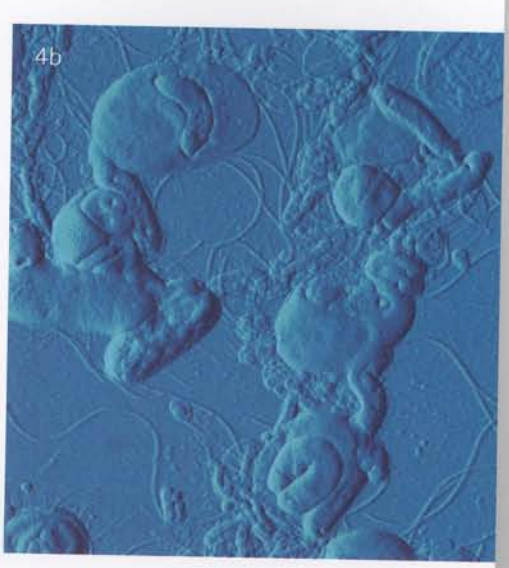
Given the success of bdellovibrios in hunting and devouring prey communities on the hydrated air–solid interface of a filter over a culture plate, we began to wonder how successfully the predator would hunt within other interfacial environments, in particular within biofilms. We prepared simple model biofilms by growing a 'biofilm-forming' strain of *E. coli*, ZK1056, on glass coverslips half-submerged in growth medium. These *E. coli* prey cells formed a dense, matted film on the glass at the air–liquid interface. AFM revealed that these cells adhere to the glass in clumps, utilizing multiple pili and flagella as well as a layer of excreted sticky extracellular material. As on the filters we found that the predators hunted quite successfully within this surface community, devouring most or all of the cells and leaving little behind but debris, even when the prey cells had 24 hours to establish themselves before the predator was added. Within these simple biofilms, protective features, such as excreted extracellular materials and compact clumps of cells, do not seem to protect the prey cells from the bdellovibrio predators. Neither do slow-growing prey escape the predator, as they might escape antibiotics. Only in more concentrated, sugary growth medium can the prey cells reach equilibrium with the predators (Fig. 4).

Other uses of AFM

Thus far we have primarily exploited the imaging functions of the atomic force microscope to investigate *Bdellovibrio* predation. Notably, other investigators

AFM provides a useful complement to traditional forms of microscopy, given that its resolution is better than light microscopy, but that samples require minimal or no preparation compared to electron microscopy.





have used AFM in different and elegant ways to explore their favourite microbes. Some have probed structures of subcellular and molecular assemblies on cell surfaces and freeze-fractured membranes, including porins, S-layers and the purple membranes of *Halobacterium*. Others have elucidated the effects of environmental factors and antibiotics on cellular integrity by repeatedly poking microbial cells with the AFM tip to measure the cells' physical properties. Other groups have quantitatively measured forces of adhesion between specific cells, surfaces and molecules. Another interesting approach to biological exploration with AFM is to measure the real-time dynamics of cellular processes such as division, motility, contraction or 'breathing' occurring in solution or at a hydrated interface. This kind of measurement has been used successfully with eukaryotic cells, but less frequently with prokaryotes.

Finally, there are many variations of scanning probe microscopy, including AFM, and also electrochemical force microscopy and magnetic force microscopy that may have some applications to microbiological systems. We expect to employ some of this large array of techniques to learn more about the ways in which *Bdellovibrio* finds and consumes its prey.

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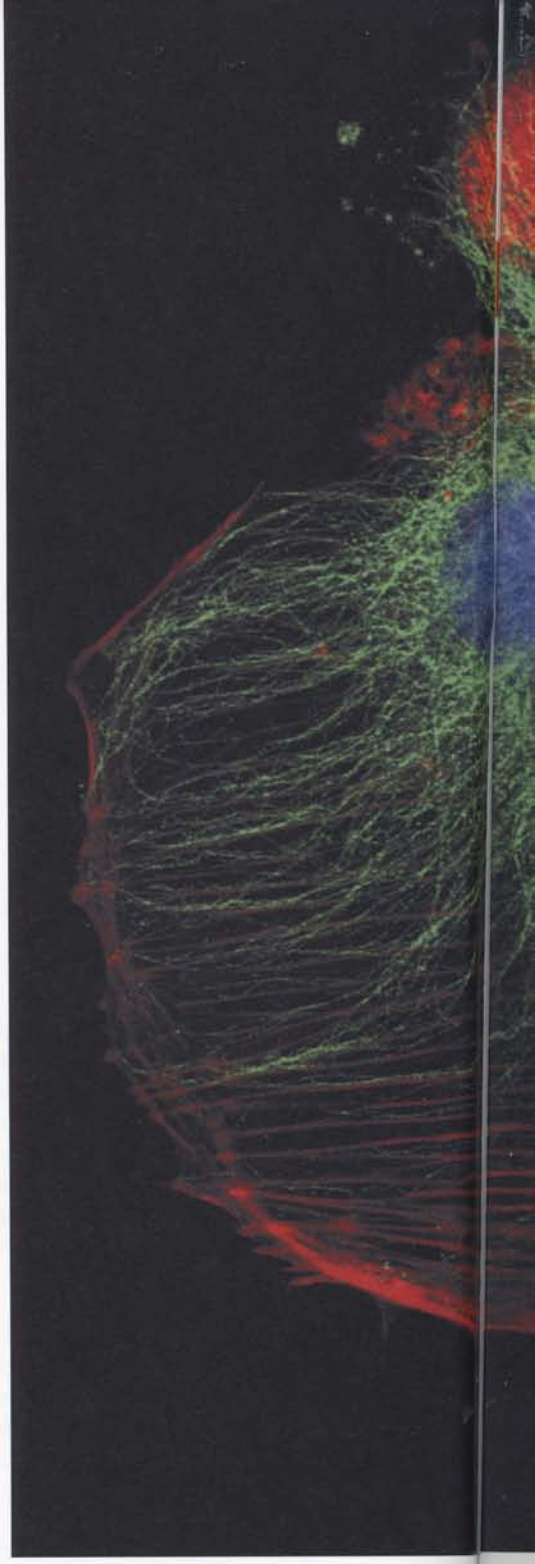
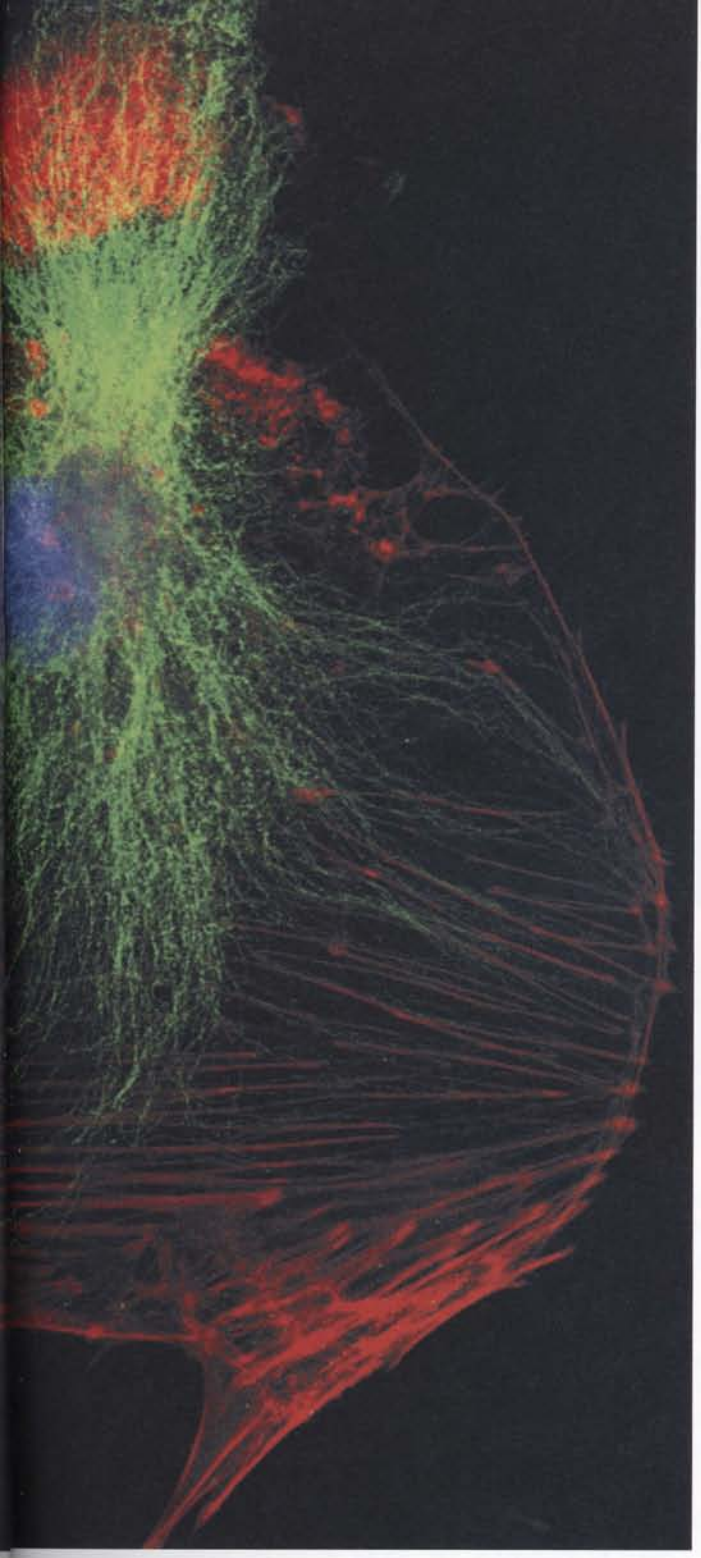


How do viruses, which have no independent means of locomotion, manage to move within and between the cells of their host? **Tom Wileman** uses fluorescence microscopy to find out.

Viruses and intracellular movement

The discovery of a dead Whooper swan infected with the pathogenic H5N1 strain of avian influenza on the shores of Scotland in April 2006 illustrated how viruses can move globally in a very short period of time. As pandemics develop, viruses are distributed over long distances by hosts such as migratory birds, insect vectors and unwitting airline passengers, or passively by rivers and other water systems. All viruses, however, start life inside cells, and recent work is unravelling how they exploit intracellular trafficking pathways at the beginning of what can become a global migration.

Viruses are obligate intracellular parasites and need to enter the cytoplasm to gain nucleotides and amino acids necessary for genome replication and capsid synthesis. Efficient replication often requires transport of the virus through the cytoplasm to specific intracellular compartments. Since viruses have no independent means of locomotion, it has been a challenge to work out how they find their way across these relatively long distances. The cytoplasm is crowded with organelles and cytoskeleton, and hypothetical calculations suggest that viruses would move very slowly if they had to rely on passive diffusion. It would take at least 200 years, for example, for herpesvirus capsids to travel just 1 cm along an axon from sensory nerve endings to their favoured site of replication in the nucleus of the cell body. Interestingly, recent discoveries made using microscopes to observe the movement of fluorescent



◀ Immunoflourescence microscopy reveals the nucleus (blue) microtubules (green), and actin filaments and stress fibres (red). *Bill Rennie, br@brp.co.uk*

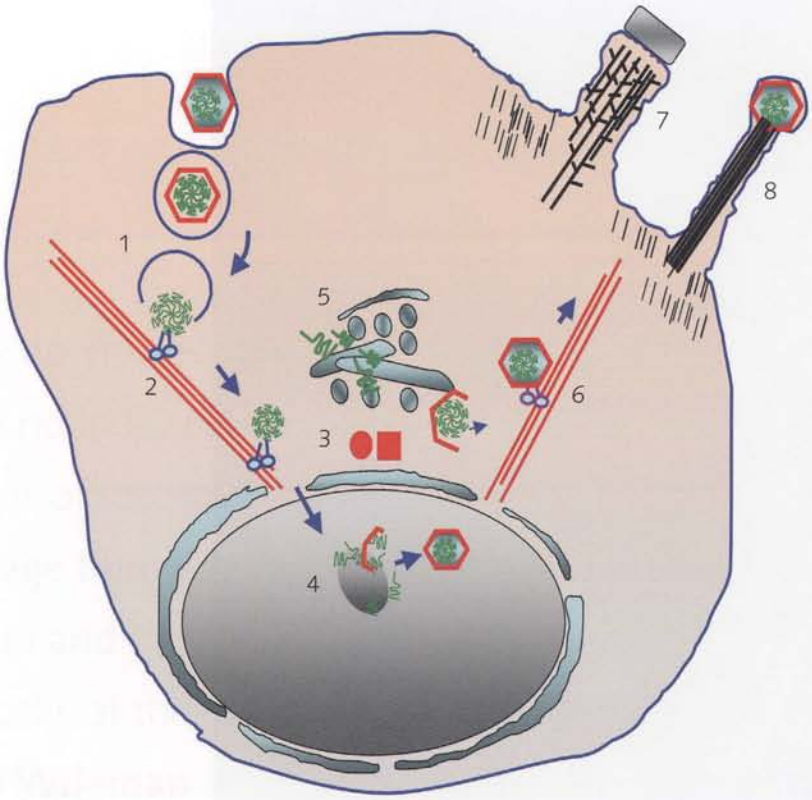
viruses in living cells show that they use the cytoskeleton to move within, and between, cells.

Observing fluorescent viruses in living cells

Green fluorescent protein (GFP) technology was first used in the mid-1990s to track and quantify the movement of cellular proteins. GFP from the jellyfish *Aequorea victoria* was fused to proteins of interest, and the natural fluorescence of GFP allowed the proteins to be tracked in real time in living cells. Systematic mutagenesis of GFP has produced brighter and more stable GFPs, and fluorescent proteins of different colours, allowing several proteins to be tracked at the same time. The fusion of GFP and its variants to viral structural proteins has made it possible to follow the movement of viruses in living cells. The fluorescent structural proteins are either expressed directly from viral genomes by generating recombinant viruses, or expressed in cells prior to infection, and incorporated into viruses during assembly. The trafficking of viral genomes can also be studied by fusing GFP to appropriate DNA- and RNA-binding proteins. Incorporation of GFP into herpesviruses has been particularly successful, and viruses with red capsids and green tegument proteins have been imaged as they travel within neurones in culture. It is not always easy to produce GFP-labelled viruses, and it has proved particularly difficult to engineer naturally fluorescent icosahedral viruses where capsid geometry is constrained. In some cases viruses are labelled using fluorescent chemicals before they are added to cells, and this has been used to follow the cell entry of adenovirus and influenza virus.

Viruses use microtubules and molecular motor proteins to move within cells

Many DNA viruses replicate in the nucleus, while others, such as poxviruses and many positive-strand RNA viruses, replicate in the cytoplasm in virus factories, or rearranged cellular membranes called 'virioplasm'. These structures are invariably close to the microtubule-organizing centre (MTOC) which anchors microtubules close to the nucleus. Early studies showed that the replication of many viruses was blocked if microtubules were disrupted, suggesting that transport down microtubules towards the MTOC was important for them to reach sites of replication. Recent live-cell imaging experiments have confirmed that this is true and provided further information about dynamics of virus movement, and the proteins involved. Live-cell analyses



► This page. Summary of viral movement through a cell. Viral core particles leave endosomes (1), bind to microtubules and dynein motors (2), and are transported to the MTOC (3). Virus replication occurs in the nucleus (4) or in cytoplasmic virus factories (5). Newly assembled viruses bind kinesin motors and move along microtubules to the cell cortex (6). Some viruses polymerize cortical actin and leave cells on actin comets (7) or filopodia (8). *T. Wileman*

► Opposite page. *African Swine Fever virus* associates with microtubules. Virus particles (red) moving through the cytoplasm align along microtubules (green). *Reproduced with permission from Jouvenet et al. (2005) J Virol* **78**, *7990 (American Society for Microbiology)*

are now available for adenovirus, *Adeno-associated virus 2*, influenza virus, HIV, *Vacciniavirus*, *African swine fever virus* and herpesviruses.

Viruses align on microtubules and move at speeds varying between 1 and 4 $\mu m\ s^{-1}$. Many viruses show complex bidirectional movement, making it difficult to understand how they get from one end of the microtubule to the other. Careful analysis has shown that run lengths towards the centre of the cell predominate, resulting in preferential movement towards the MTOC. Once virus replication and assembly are completed, it is important for many viruses to embark on the reverse journey to the cell surface. Again live-cell imaging has shown that viruses move along microtubules, this time towards the plasma membrane, and in some cases viruses can switch from one microtubule to another.

Cells use two kinds of ATP-fuelled motor protein to move organelles and other cargoes along microtubules. The dynein motors move cargoes inwards towards the MTOC while the kinesin motors move in the opposite direction towards the plasma membrane. Analysis of the sequences available for virus genomes made it unlikely that viruses encode their own motor proteins, and since they move at the same speed as cellular cargoes, it was suspected that viruses hijacked cellular motors to move in cells. It is now known that viruses use dynein motors to reach sites of replication in the cell interior, and that after replication and assembly new viruses switch to kinesin motors to reach the cell surface. How viruses recruit the motors, switch them on and off and exchange one for another are key areas of current research.

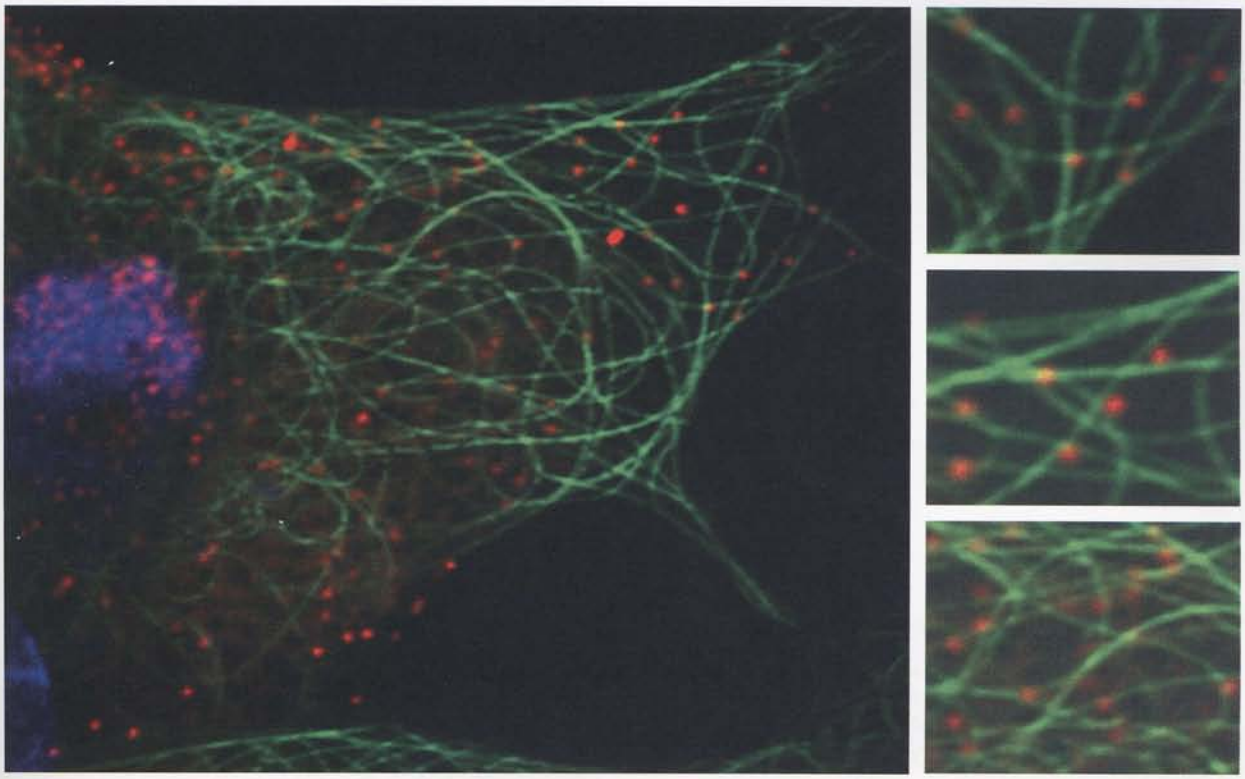
Viruses can use actin to leave cells

Viruses delivered to the cell periphery by kinesin motors are deposited into the cell cortex just below the plasma membrane which contains high concentrations of actin. Polymerization of cortical actin allows cells to produce extensions called lamellipodia and filopodia which are used for cell movement. Some viruses (and several bacteria) use the power generated by the polymerization of this rich source of actin to generate extensions and to move into neighbouring cells. *Vacciniavirus*, for example, fuses with the plasma membrane but remains attached to the outside of the cell where it signals the polymerization of a comet-shaped actin tail which propels the virus away from the cell surface. *African swine fever virus*, a virus related to poxviruses, is also delivered to the actin cortex by kinesin motors where it stimulates actin filament formation. The filaments are bundled beneath the virus and carry the virus away from the plasma membrane at the tip of filopodia. Understanding how viruses induce localized polymerization of actin at the cell surface is not only of interest to virologists. The strong similarity between virus-induced projections and the lamellipodia and filopodia used for cell motility allows the study of virus motility to provide valuable insight into how cells migrate, for example during recruitment of lymphocytes to sites of infection, metastasis of cancer cells and outgrowth of neurones.

The future of virus motility

Advances in microscope technology, such as increased sensitivity and improved imaging rates, coupled with more stable fluorescent probes, will allow single virus particles to be

The study of virus motility will provide valuable insight into how cells migrate, for example during metastasis of cancer cells.



tracked in greater detail. It is anticipated that dual and triple labelling studies coupled with fluorescence energy transfer (FRET) and fluorescence recovery after photobleaching (FRAP) microscopy will be used to follow the exchange of specific motor proteins as the viruses bind to (or change direction on) microtubules, or switch to actin-based motility. It will also be important to determine how cellular signalling proteins regulate virus motility, and how viruses switch them on and off. Work over the last year has shown that movement of herpesviruses along microtubules can be reconstituted *in vitro*. Such systems will allow virus movement to be analysed in biochemical and biophysical terms. This will tell us how much force is required to move a virus along microtubules, and how many motors are needed.

It is important to remember that many viruses pose a serious threat to mankind and it is hoped that studies on virus motility can lead to new antiviral drugs. One such drug, the c-Abl tyrosine kinase inhibitor Gleevac, was shown recently to prevent the spread of *Vacciniavirus* and coxsackievirus B by preventing actin mobilization at the cell periphery. This raises the hope that in the future we will be able to stop viruses in their tracks in cells, before they start their global migrations.

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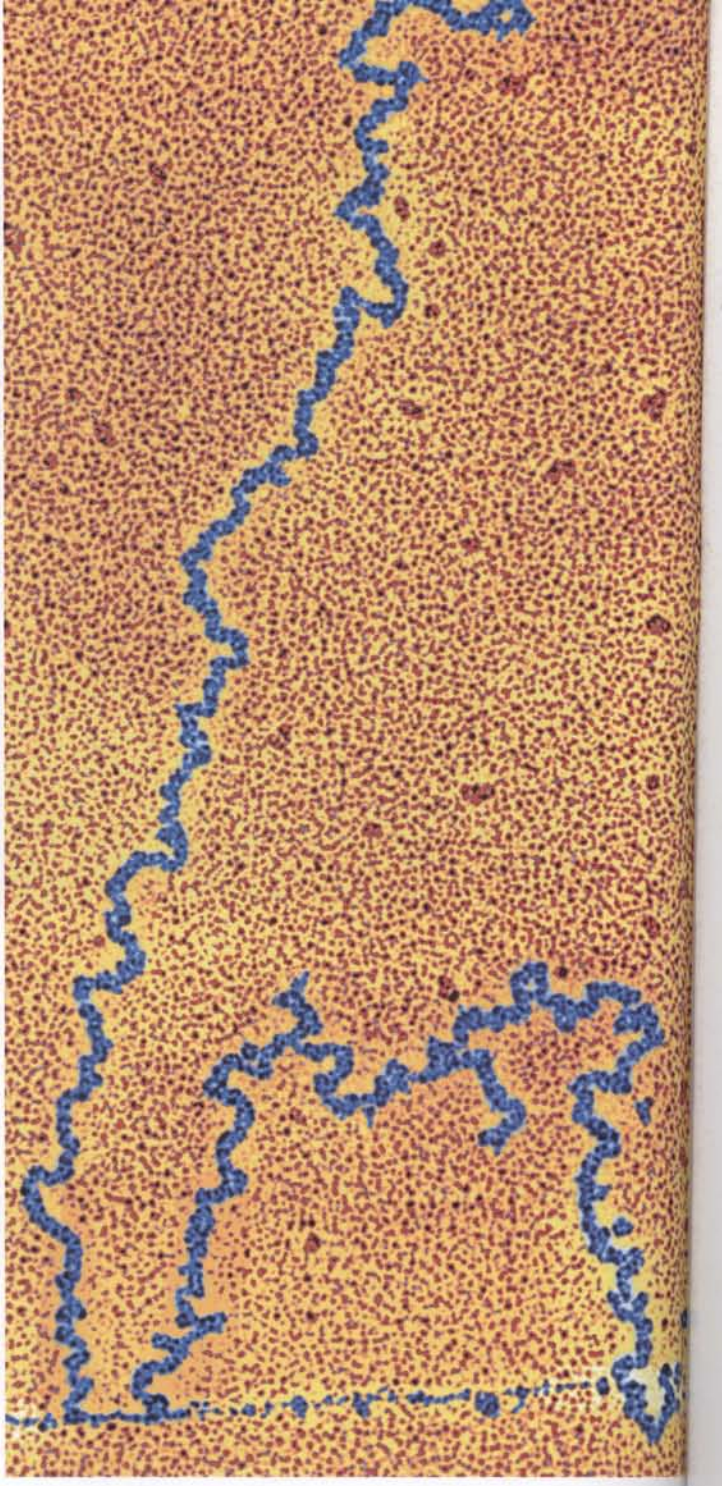


▶ Coloured transmission electron micrograph of transcription complexes from *Escherichia coli* on DNA (blue horizontal line). During DNA transcription, complementary mRNA strands (blue vertical lines) are synthesized. *Dr Elena Kiseleva / Science Photo Library*

Recent advances in imaging technologies have enabled researchers to pinpoint individual molecules in living cells, as **Christoph Baumann** describes.

In 1683 Antony van Leeuwenhoek published the first microscopic description of bacteria, which he isolated from the plaque between his own teeth. He acquired the images using a simple magnification device with a hand-ground lens. This important milestone highlights a common occurrence in the microbiological sciences – significant advances in our understanding of the microscopic world are often linked closely to technological developments. In the last three decades, advances in laser and light detection technologies have allowed us to quantify the small displacements, and associated forces, generated by a single enzyme molecule. This article aims to make clear the need for single-molecule studies on microbial systems, and to highlight some recent milestones in this area.

The unfamiliar world of a single molecule

The nature of the 'nanoscopic' environment where an enzyme must function is very different to the macroscopic world in which we live. Imagine standing in a crowded room where people are standing elbow-to-elbow and trying to move across the room while the floor beneath you is jiggled violently. Your attempts to move in a directed way will be scrambled by frequent collisions with your neighbours. This resembles the effects of 'thermal noise' (Brownian motion) and molecular collisions on single molecules, which together drive the process of diffusion in a liquid by pushing molecules in random directions. This is a good approximation of the environment where enzyme molecules must function and the physical obstructions to movement that they must overcome.

At first glance, the 'noisy' environment inside a cell appears an inhospitable place for any molecular process. Furthermore, thermal noise increases with temperature, thus it is amazing that certain microbes can flourish under extremely hot conditions. In fact, it is thought that an enzyme can use the energy released from a chemical reaction to bias the thermal forces acting on it and drive mechanical motion.

Studying single molecules in microbial systems

Here, two key bacterial enzyme complexes are used to show how single-molecule-level investigations have advanced our understanding of 'nanoscopic' motion: *Escherichia coli* RNA polymerase and the bacterial flagellar motor. These examples are typical of two classes of 'molecular motors', which are characterized by their ability to couple a favourable chemical reaction to directed 'linear' and 'rotary' motion, respectively. Using state-of-the-art physical techniques, the motion of these tiny motors can be observed in real time and provide new insight into their mechanisms. Advances in fluorescent imaging have allowed single molecules to be observed in living cells. Recent work on gene expression at the single-cell level is used as an example of this approach.

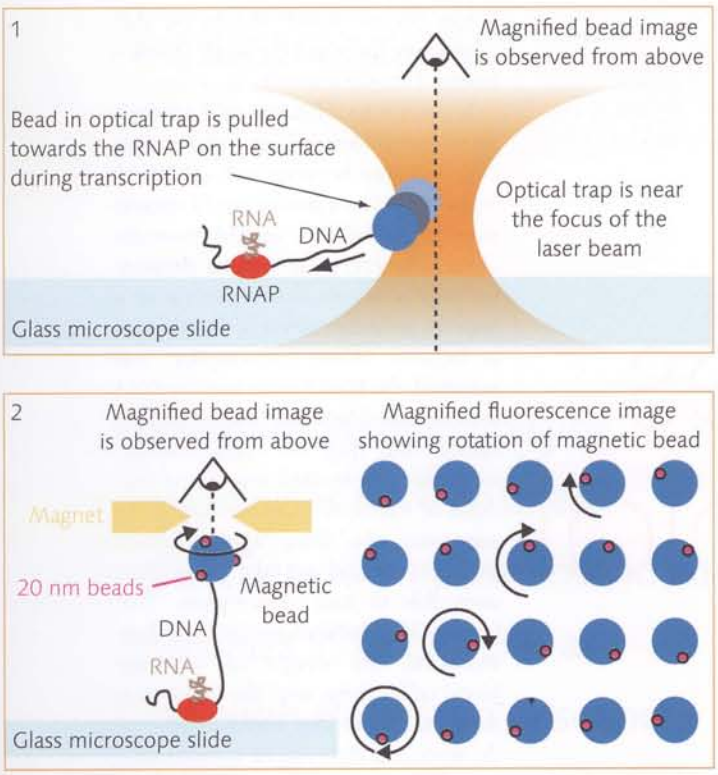
Mechanics of gene transcription

Transcription of a gene into RNA is a vital biological process as it is the first committed step in gene expression. The enzyme responsible for transcription in bacteria, RNA polymerase (RNAP), must move along the DNA template. Due to its relatively large physical size, an RNAP molecule may pull the DNA through its active site during transcription, rather than moving along the DNA and pushing through the crowded cytoplasmic environment. If this occurs, RNAP should be able to generate force and do 'work' (work = force × distance moved).

To determine if RNAP could do work, researchers tethered an appropriate DNA template between an *E. coli* RNAP immobilized on a surface, and a micron-sized bead held in optical tweezers. Optical tweezers use photon pressure to 'trap' a micron-sized particle at a spot in solution where a laser beam is focused. When transcription was restarted, the RNAP pulled on the DNA tether and displaced the bead held in the optical trap (Fig. 1). These displacements were converted into linear velocities of ~10 bp s^{-1}, or ~3.4 nm s^{-1}. By increasing the force against which the RNAP must act, the researchers were able to stall transcription. This force, ~25 picoNewtons (pN), is equivalent to the weight of ~25 red blood cells. These were the first experiments to show that RNAP is able to do work and thus can be considered a 'molecular motor'.

A simple model for RNAP movement along the DNA template might predict that this molecule should move in discrete ~0.34 nm steps, which are equivalent to the distance between two base pairs. Recent technological improvements have allowed this discrete stepping to be observed at the single-molecule level. The steps observed are consistent with *E. coli* RNAP moving forward 1 bp for every ribonucleotide triphosphate incorporated into the growing RNA chain. This result implies RNAP couples polymerization directly to forward translocation along the DNA template, which is consistent with a Brownian ratchet type model.

Advances in single-molecule detection will continue as new technologies from the physical and chemical sciences are applied in the biological sciences.



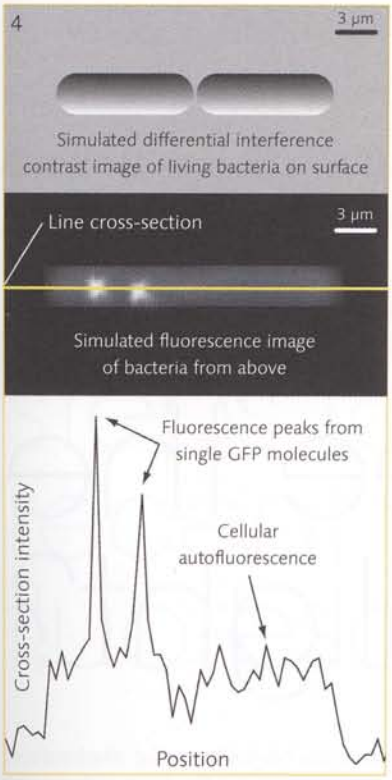
▲ Figs 1–3. Determination of the ability of RNAP to do work by watching it pull on a DNA molecule (1), measurement of the ability of RNAP to rotate a DNA molecule and thus track along the DNA helix (2), and observation of discrete mechanical steps of a bacterial sodium-driven chimaeric flagellar motor (3). *C. Baumann, based on images in the Further reading articles*

How does RNAP track along the DNA helix?

The DNA duplex is composed of two complementary strands running in opposite directions, which coil around a common axis to form the DNA double helix. To determine if *E. coli* RNAP follows a helical path along the DNA template, researchers used a similar experimental geometry as described above. However, instead of using optical tweezers to trap the bead, an iron magnet was used to hold a micron-sized magnetic bead and stretch the tethered DNA template above a glass surface, i.e. magnetic tweezers. The magnetic bead was labelled with small (20 nm) fluorescent beads, allowing the rotation of the large bead to be followed by viewing its magnified image from above by fluorescence microscopy (Fig. 2). If the surface-immobilized RNAP followed the right-handed helical path of the DNA template, the bead should rotate once for every ~10 bp traversed (number of base pairs per helical turn of DNA). Indeed, the bead was observed to rotate clockwise at a rate ≤1 DNA turn s^{-1} during transcription, which was consistent with the linear velocity (~10 bp s^{-1}) measured in the optical tweezers experiments. The ability of the RNAP to rotate the bead, and by association the DNA, also showed that it could produce torque just like a macroscopic motor.

Bacterial propulsion

Many bacteria are able to propel themselves through their liquid environment using flagella. The rotary motor responsible for this propulsion is known as the bacterial flagellar motor – it consists of ~10 torque-generating units arranged like a static bearing around the rotor shaft and anchored in the bacterial cell wall. The flagellar motor can be fuelled by either proton-motive force or sodium-motive force, whereby the inward flux of ions across the cytoplasmic membrane, and down an electrochemical gradient, drives rotation. The flagellar motor should undergo discrete mechanical steps; however, its high speed and predicted small step size had prevented the resolution of these steps. Using a bacterial strain expressing a 'slower' sodium-driven chimaeric flagellar motor, and high-speed video recording of a 200 nm fluorescent bead attached to an intrinsically sticky flagellar filament (Fig. 3), researchers have been able to visualize individual steps for the first time. 13.7° steps were observed with typically 26 steps per revolution. The number of steps observed



▲ Fig. 4. Using laser-induced fluorescence and cell microscopy to follow the production of a fluorescent protein (GFP) localized on the inner bacterial membrane in real time. *C. Baumann, based on an image in a Further reading article*

per revolution is consistent with the number of FliG molecules thought to exist in the torque-generating rotor. This work begins to shed light on how electrochemical gradients are coupled to directed motion and highlights how recent technological advances enable new milestones in the single-molecule field to be achieved.

Gene expression at the single-cell level

All biological events are dependent on the collision of one or more molecules. The expression level of a protein in a cell is dependent on several collisional complexes leading to 'active' complexes, e.g. one or more transcriptional activators binding to RNAP and the promoter DNA, and a ribosome binding and translating the RNA transcript. In addition, most genes in bacteria exist in only one copy, which further reduces the probability of a collision. These collisions are driven by thermal forces, which are random or stochastic events. As a result, gene expression can often be random too.

The expression level of a protein is usually determined by averaging a population of cells, which masks its random nature. By combining laser-induced fluorescence and cell microscopy (Fig. 4), the production of a fluorescent protein (GFP) localized on the inner bacterial membrane could be followed in real-time in individual cells. Single-molecule detection of fluorescent proteins in live bacterial cells removes the aforementioned ensemble averaging. The GFP gene was under the control of a repressed *lac* promoter, thus protein production represented leaky gene expression. This work showed that each gene expression event, or mRNA molecule, was translated to yield approximately four protein molecules per molecule of mRNA. By allowing low-copy-number proteins to be imaged in real time, this approach allows the effects of stochasticity on fundamental biological processes to be investigated directly.

Future prospects

The last decade has seen great advances in the areas of single-molecule detection and manipulation. These advances will continue as new technologies from the physical and chemical sciences are applied in the biological sciences. Such developments will allow greater temporal and spatial resolution at both the single-molecule and single-cell levels.

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Can you see the light?

While recent advances in imaging technologies have had a dramatic impact upon biology, the technical challenges and baffling array of options have tended to restrict these technologies to a limited number of *cogniscenti*. This is particularly true for imaging microbes where low intensity signals have conspired with the need for considerable magnification to present a formidable challenge. However, some straightforward changes to standard fluorescence microscopes can now satisfy this obsessive desire to maximize sensitivity and resolution.

Imaging platform: spinning, scanning – how convoluted can it be?

Laser scanning confocal microscopes (LSCMs) have a reputation as benchmark instruments for high-resolution imaging. However, they require very bright samples and most microbiologists have no need to take advantage of their ability to illuminate a constrained slice in thick sample. Although LSCMs are not ideal for imaging bugs, a second type of confocal microscope, the spinning disk microscope (SDM), is. Instead of scanning consecutive points in a series of lines across a field to build up an image line by line that is the hallmark of LSCM, SDMs simultaneously monitor 356 points which reduces off-site bleaching and combines with further technical advantages to give arguably the best temporal and spatial resolution available. However, SDMs are very expensive and rely upon bright lasers for illumination. Imaging multiple wavelengths invariably means using multiple lasers, further raising prices. Traditional, 'widefield' fluorescence microscopy, on the other hand is versatile, affordable and now sensitive enough to deliver top quality imaging. It works by bathing samples in light of a specific wavelength that is selected by the use of dedicated filters from a universal white light source. The secret is to ensure that as much of the light that leaves the specimen gets to the camera as possible.

▲ Microscope lenses. *David Parker / Science Photo Library*

Enhancing an existing widefield system

1. Light path: provision

The most easily justified modification to an existing widefield system is to upgrade the light source from the traditional mercury bulbs to a tungsten/xenon system. As well as being brighter than mercury bulbs, tungsten/xenon systems are much cheaper to run (£0.35 per hour vs. £1.20 per hour). Thus, they not only boost sensitivity, but pay for themselves over a 2- to 3-year period, and then save money after that. A final perk is that they overcome the common pitfall of uneven illumination that has plagued the use of mercury sources. The xenon/tungsten systems use a fibre optic cable that scrambles the image of the bulb to deliver an even illumination that is invaluable for quantification.

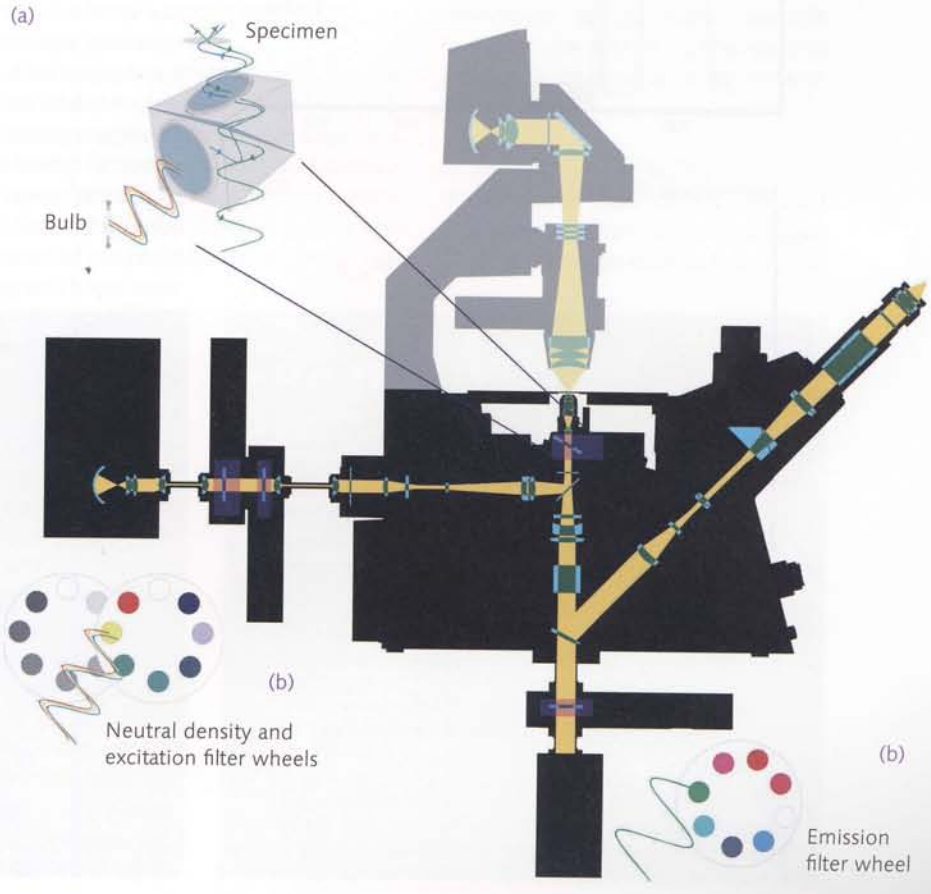
2. Light path: transmission

When passing through, or bouncing off, an optical element some light is lost, eroding both the intensity and quality of the image. While the optical elements in microscope

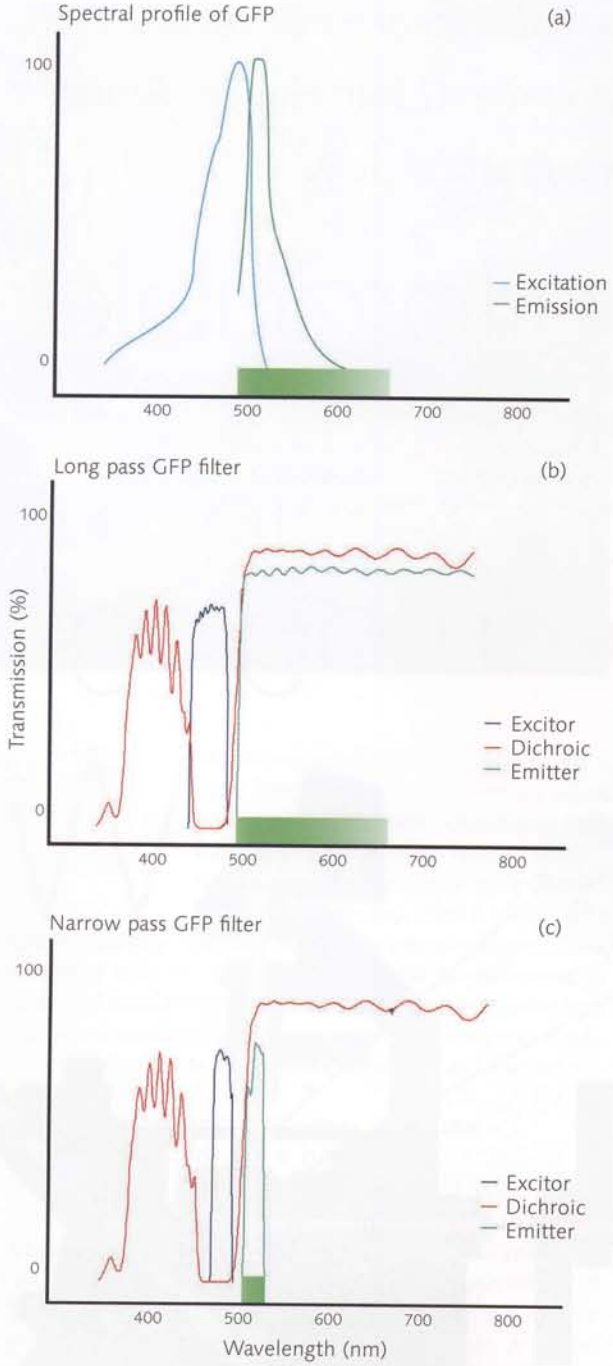
Amazing findings can be made using fluorescence microscopy, but how do you choose the right equipment? **Iain Hagan**, **Agnes Grallert** and **Steve Bagley** give some practical help.

Simple and affordable steps to exploit recent advances in fluorescence imaging

bodies have been reduced to a minimum, the demands of certain disciplines mean that objective lenses can contain a considerable array of additional elements. Pathologists and neurobiologists often want to see high-power magnification images of entire fields that are free from distortion and in sharp focus from one side to the other. This technically challenging demand is met by the inclusion of correcting elements to counteract distortions at the edge of a field of view. However, these extra elements reduce transmission. This cost is too great for microbiologists who would happily tolerate distortion at the periphery of a field that is often tens of cells wide, as the benefit of neglecting this correction is enhanced transmission. In other words because commercial suppliers offer a range of lens types for a range of



▶ Fig. 1. A cartoon showing the two options for filtration of light in a widefield microscope system. (a) Most systems use filter cubes that sit between the objective and the eyepiece; however, the use of filter wheels (b) enables rapid switching from one channel to another. *Steve Bagley*



▲ Fig. 2. Filtration of GFP emission by long and narrow band pass filters. (a) The excitation (blue) and emission (green) spectra of GFP. The green bar indicates the breadth of fluorescence emission that is fully sampled by the long pass (b) but not the narrow pass filter (c). *Steve Bagley*

► Fig. 3. Labelling living *S. pombe* cells with two fluorescent lectins facilitates the ultimate live cell imaging control – three distinct cell types imaged alongside each other. Red, cell type 1; green, cell type 2; black, cell type 3; blue, chromatin (Hoescht 33342). *Agnes Grallert*

applications it is vital to know what type of lens you are using. Does it have a lot of correction or maximal transmission?

The key term is the Numerical Aperture (NA) of an objective. The higher the NA, the higher the transmission. Quite fortuitously for microbiologists, a form of microscopy called total internal reflection microscopy (TIRF) that demands the highest NA possible has recently gained in popularity, inducing every commercial supplier to produce objectives with extremely high NAs. The catch is an extremely shallow depth of field – however, the size of microbes comes to our rescue again as depth of field is rarely a problem for a microbiologist. A further catch is that you are likely to be told by the sales representative that these lenses are only useful for TIRF and you should not try them! However, persist! Demand a loan for a trial run! A switch from a 100× 1.3 to a 100× 1.45 doubled the intensity of our yeast images.

Many microscopes have optional internal magnification lenses on sliders or wheels that can provide further magnification between the objective and the eyepiece/camera. Beware, these lenses can severely erode image quality and should be used with utmost caution.

3. Light path: wavelength selection

Fluorochromes are excited at one wavelength and emit light at a second, lower energy (longer) wavelength. Thus paired excitation and emission filters that select the appropriate wavelengths for a particular fluorochrome lie at the heart of any widefield microscope. Most fluorescence microscopes have filters paired up, either side of an appropriate (dichroic) mirror to reflect and transmit the appropriate wavelengths in specific blocks between the light source objective and eyepiece/camera (Fig. 1a). Alternatively, and more expensively, filters can be mounted on independent filter wheels to enable more rapid changes in wavelengths (50 ms vs 900–2000 ms for changes of internal filters) and to isolate the moving parts from the body of the microscope to minimize vibration (Fig. 1b). Whichever system is available it is important to understand the range of filters at your disposal.

Fluorophore emission spectra generally have a strong peak that trails off towards the red end of the spectrum (GFP in Fig. 2a). To distinguish between the signals from two different fluorochromes in a single specimen, two combinations of excitation and emission filters are required to select the appropriate portion of the spectrum to excite and observe one fluorochrome while excluding those that would excite/observe the signal from the other. Two issues arise here. The more restrictive the filtering, the more sophisticated is the coating on the glass needed to generate the filter. The more complex the coating, the less light is transmitted. Second, the selection of the narrow bands of the spectrum that are required to see multiple wavelengths as separate signals in one sample excludes the long tail in the emission spectrum of the fluorochrome and so effectively throws away up to

20 % of the signal from the sample. Therefore, if only one fluorochrome is to be imaged, 'long pass' filter sets (Fig. 2b), that need less coating and lack an upper cut off, enabling the full tail of the spectrum to be captured, give much better signals than the restricted and heavily coated 'narrow band pass' filters that permit selective imaging of different fluorochromes in a single sample (Fig. 2c). If you are just interested in GFP in an experiment, a long pass filter set will boost intensity by up by 20 %. Furthermore, the technology used to generate filters has been revolutionized in recent years so that simply replacing old filter sets with newer alternatives (splutter-coated filters) can increase the brightness by 20–30 %.

One aspect of light sampling that is often overlooked is the type of dichroic mirror used. Just like filters, the greater the demands placed upon the specificity of the mirror, the more sophisticated are the coatings, so less light is transmitted. Thus, the selection of a dedicated dichroic for a particular wavelength can also considerably boost transmission.

4. Detection: cameras

Cameras take us away from this article's theme of 'easily affordable' adaptation. However, changes in CCD technology that are transforming the quality of cameras in high-street stores are also transforming microscopy. For microbiologists, the key issue is the quantum efficiency of a CCD chip – literally, how much light arriving at the surface will be converted into an electrical read out. A technology in which light signals are converted into a stream of electrons has pushed quantum efficiencies of chips to greater heights. Switching to an 'electron multiplying CCD' camera has revealed signals that we could not detect with the previous generation of chips.

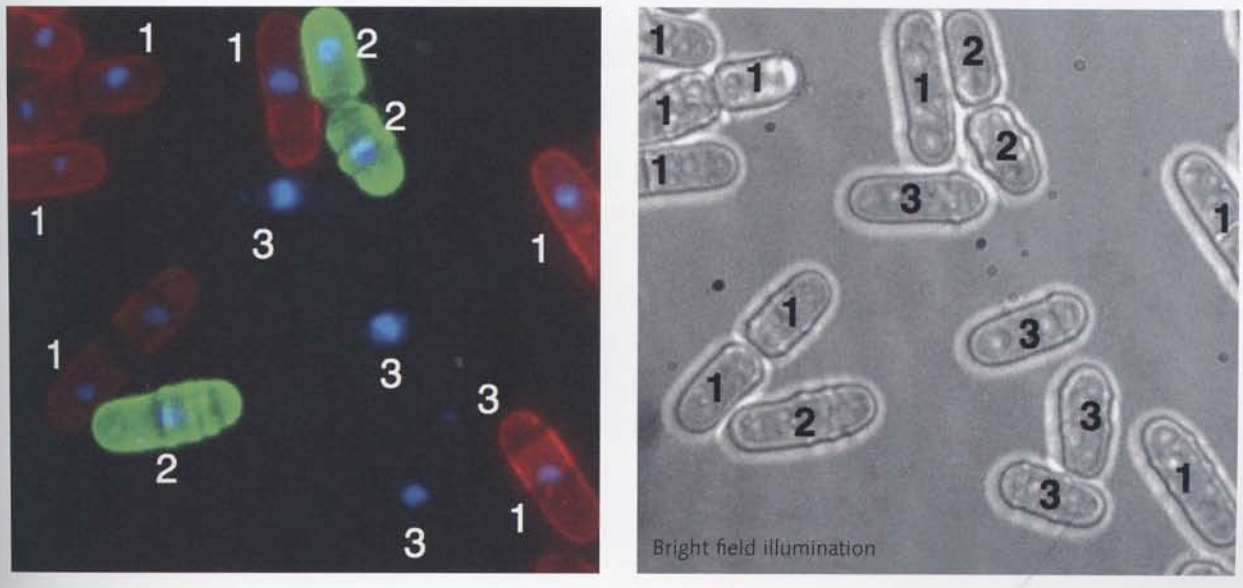
Some straightforward changes to standard fluorescence microscopes can now satisfy the obsessive desire to maximize sensitivity and resolution

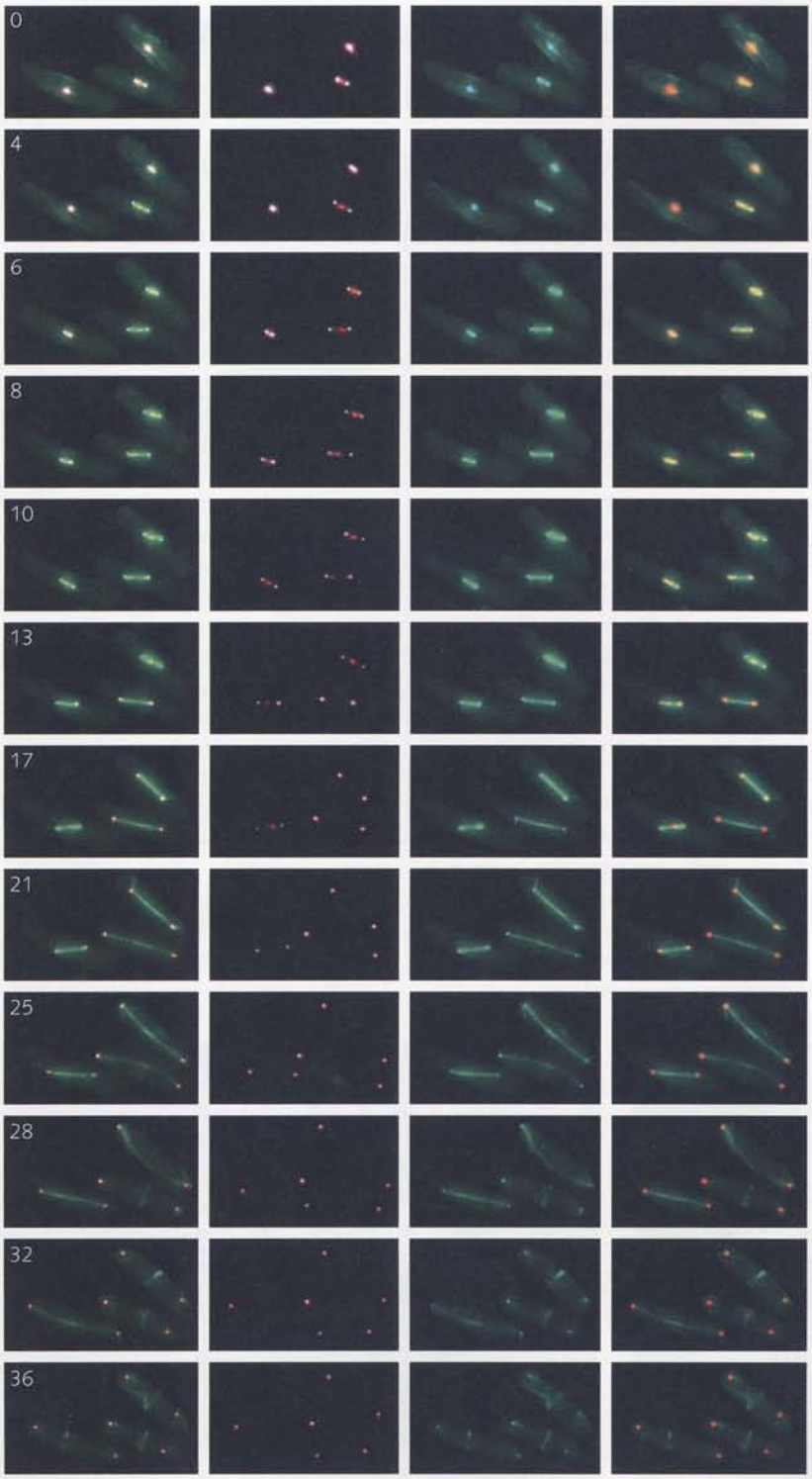
5. Optimal sample control: staying alive

Environmental control can be as critical as the optimization of the light path. For example, encasing the stage in a heated Perspex box might not be sufficient to give accurate temperature control. The metal of the objective lens is connected to the microscope body that lies outside the box. It can therefore act as a 'heat sink' that reduces the temperature in the one place where you do not want it changed – the field of view. This problem can be overcome with accurate and highly versatile commercial systems that control the temperature of the coverslip surface (for example, by heating a transparent coating of metal) and the temperature of the objective with a heating collar. A number of micro-perfusion chambers can be used to maintain nutrient supplies or deliver drugs or toxins on cue. Cells can be mounted on a range of transparent gas-permeable matrices to ensure the correct aerobic/gaseous conditions are maintained over extended periods.

6. Wall to wall imaging

Microbial cell walls can be exploited in a variety of ways to assist imaging. Coating coverslips in ligands that bind





▲ Fig. 4. Three colour images of dividing *S. pombe* cells in which the centromeres are marked by *cnp1.cherry*, the spindle poles with *sid4.tomato* and microtubules with *atb2.GFP*. A movie of this file is linked to this article on the *Microbiology Today* website (www.sgm.ac.uk). Green, tubulin; red, centromere; blue, spindle pole body. *Agnes Grallert*

cell-wall components, such as poly-L-lysine or lectins, is a simple way of ensuring a sample stays put during image capture. This is particularly important if medium has to be changed via perfusion. Another simple trick is the selective labelling of one cell type in a single field of view with a fluorochrome before they are mounted side by side in the same field. Thus, a mutant strain can be observed in exactly the same conditions as the wild-type control (Fig. 3).

Is it worth it?

A modest equipment grant of around £12,500 can transform an existing microscope system for imaging GFP to deliver some top notch images (light source, £3,000–4,000; specialized objective, £5,000–7,000; dedicated filter/dichroic set, £800). A quick trip to another lab that has the technology in place can determine just how great a difference can be made and whether it will be of any use. Invariably the gains obtained with these modest adjustments eventually provide justification to granting bodies for a new camera and more sophisticated image processing, leading to the ability to visualize three or more proteins for extended periods (Fig. 4). There has never been a better time to dispel the folklore surrounding imaging and have a go.

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A supplement with further tips to negotiate imaging are linked to this article on the Microbiology Today *website at* ***www.sgm.ac.uk***

Using microscopy to enhance undergraduate teaching

Microbiology undergraduates can be inspired by the use of microscopy and digital imaging, as **Eleanor Landy** describes.

▶ Students using digital eyepieces to visualize *Penicillium* on the laptop screen. *E. Landy*

With the outcomes of Student Course Experience Questionnaires, League Table Scores, External Advisor Comments, Internal Exam Boards and Boards of Studies, Student Assessment of Module Reports and a national downturn in applicant numbers for microbiology degree programmes, we all have to take stock of what both our potential applicants and current students expect from microbiology at an undergraduate level.

Electronic tools are available to us in the marketplace that we can manipulate to improve the student course experience – but the question is where to start?

This September the Level One Microbiology Experience at Surrey was transformed. For the first time, digital eyepieces that connect microscopes to laptops and the university network recorded live organisms *in situ*. To enhance the introductory practical microbiology module, where we felt the need to progress student learning from drawing to electronic recording, we also provided a Virtual Learning Environment (VLE).

For biological science groups engaged in teaching and research, microscopy is fundamental to the microbial and cellular sciences. Thus, the School of Biomedical and Biomolecular Sciences (SBMS) have invested in this technology to provide a unique, cutting edge student experience across the School. Microscopy and digital imaging are moving at a fast pace, and the ability to project what one sees down the microscope onto a PC screen and manipulate or share this data is key for teaching in the biological sciences. Previously, SBMS undergraduate students drew by hand the images that they saw and only lecturers or researchers had access to digital imaging equipment which they demonstrated to students. Practical biological teaching at Surrey has been revolutionized by providing our students with the tools and hands-on skills that they will need when graduating via this new technology.

With the introduction of the eyepieces, students can now email the images captured in class to their undergraduate accounts (and to the lecturer simultaneously), as well as print out or save images of the micro-organisms that they study for use in their lab reports, posters, seminars or revision notes. The students can then compare their images with prepared images and resource materials in the VLE for use during reflective practice exercises, pre-assessment revision and discussion boards for group work exercises.

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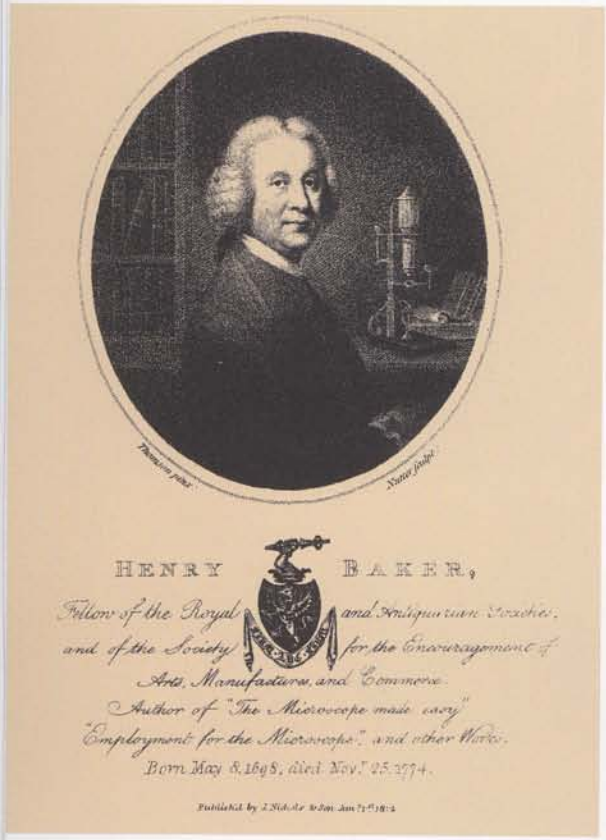


▲ Portrait of Henry Baker. A stipple engraving by William Nutter published in 1812 and taken from an earlier oil painting by William Thomson. Baker was variously described as upright, benevolent, patient, homely, quietly spoken – and short-sighted (not surprising given all those hours looking down microscopes). The instrument on the table in the background is one of John Cuff's double microscopes.

Henry Baker: author of the first microscopy laboratory manual

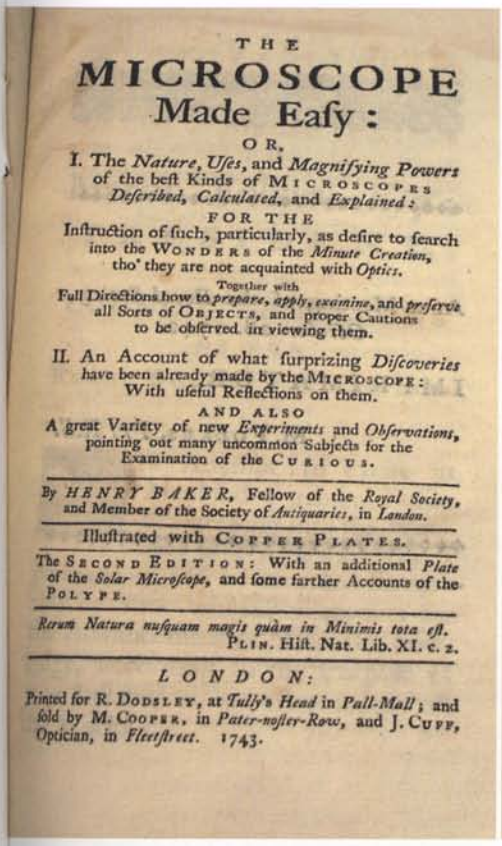
Henry Baker (1698–1774) was a typical 18th century polymath: natural historian, poet, translator of Molière, editor of a literary periodical and prolific correspondent. He was a co-founder of the Royal Society of Arts and a Fellow of the Royal Society of Antiquaries. Baker's influence on the development and popularization of the microscope was considerable and he wrote three books; the first, *The Microscope Made Easy*, was a best seller. In 1741 Baker was elected a Fellow of the Royal Society and played a prominent role in its activities for 30 years. The Bakerian Lecture was founded as a result of a bequest in his will.

The Microscope Made Easy

'Assert nothing till after repeated experiments and examinations, in all lights, and in all positions. Truth alone is the matter that you are in search after; and if you have been mistaken, let not vanity seduce you to persist in your mistake.'

This is Baker's cautionary advice in *The Microscope Made Easy* published in November 1742. In setting out the reasons for writing the book Baker berates previous users of the microscope for giving the impression that only those with skill and learning could benefit from it. The only needs are *'good glasses, good eyes, a little practice, and a common understanding, to distinguish what is seen; and a love of truth, to give a faithful account thereof.'*

Part I describes simple and compound microscopes and trumpets the qualities of those made by his friend John Cuff. There is guidance on how to prepare specimens and how to calculate their size. Part II is a beginner's guide to microscopy and laboratory manual. It gives instructions on making infusions of pepper, hay, oats and wheat, and tells what to expect when these are studied under the microscope. 'Animalcules'



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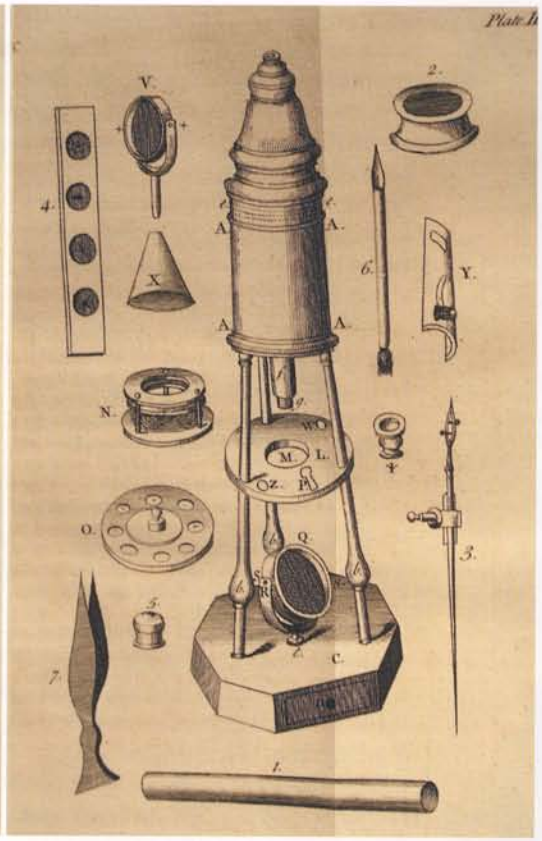


Henry Baker played a big part in popularizing the microscope in the 18th century.

Richard Burns, Henry's 'great x6' grandson, describes the life and activities of his illustrious ancestor.

seen in rain, ditch and pond water are illustrated and there are descriptions of blood, muscles, bones and nerves, semen, the louse, fleas and spiders, and how to kill insects with tobacco oil and mercury. One chapter contains a rag-bag of observations ranging from the behaviour of ants to snow-flakes. All come (literally) under the microscope.

Baker refers frequently to the earlier discoveries of Hooke, Swammerdam, Leeuwenhoek and others. But he goes further and expresses his views on many of the controversial issues of the day. For example, spontaneous generation is dismissed ('*Nothing seems now more contrary to reason ... that dead corrupting matter, and blind uncertain chance, should create living animals.*'), but preformation gets the thumbs up ('*The growth of animals and vegetables seems to be nothing else but the gradual unfolding and expansion of their vessels*'). There are even some thoughts about relativity ('*Our ideas of matter, space, and duration are meerly comparative*').

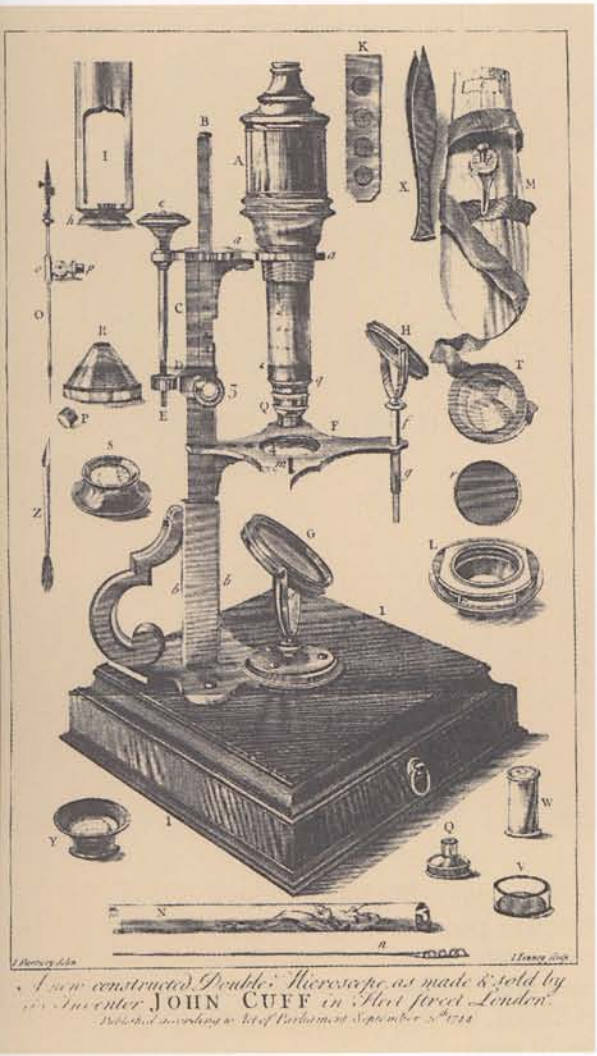
Towards the end of the book Baker compares the beauty of natural (bee sting, silkworm web) and man-made objects (lace, works of art). To him the artefacts come a distant second: '*Our finest miniature paintings appear before this instrument as meer dawbings, plaistered on with a trowel, and entirely void of beauty.*'

1000 copies of *The Microscope Made Easy* were printed and sold so rapidly that it was reprinted in April 1743. Soon other microscopical studies were published, but this book had opened up the market.

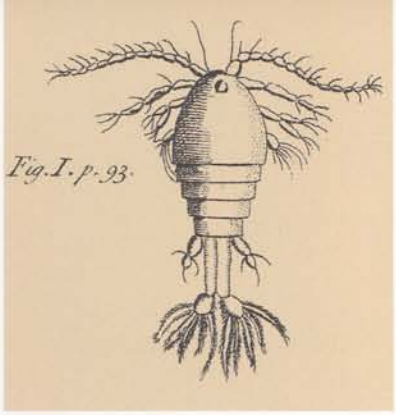
Eleven years later, in *Employment for the Microscope*, Baker humbly advises critical assessment of the book's contents and further study, and declares that his target audience is wide ('*many of both*

▲ Left. Title page of *The Microscope Made Easy* (2nd edn, 1743). A total of six English editions were published from 1742 to 1785 as well as Dutch, French and German translations. The book sold for 5 shillings (about £30 today) and Baker received the equivalent of approximately £5,000 for the first edition and half that for subsequent editions.

▲ Right. The double reflecting microscope. This is described by Baker as an improvement on John Marshall's turn of the century great double microscope made initially by Edmund Culpeper in the 1720s and then by Edward Scarlet in the late 1730s. The body is supported by three brass pillars mounted on a wooden base. There are five objective lenses that can be screwed to the nose piece (*g*). The brass plate (O) fits onto the fixed plate (L) and contains holes to carry specimens; ivory discs are used as the background for dark coloured objects and ebony discs for light coloured objects. Concave glasses retain liquids containing 'animalcules'. The lens (V) is attached at W to direct candle light or sunlight down onto opaque objects. Y is a fish-plate in which the blood circulation in the tail of a fish could be observed. Glass tubes (*1*) are used to immobilize frogs, newts, etc., for observation. Insects are trapped in the glass cell (*2*).



▲ John Cuff's new double microscope, made in 1744 following advice from Henry Baker who wrote *'when examining daily the configurations of saline substances, the legs were continual impediments to my turning about the slips of glass ... Pulling the body of the instrument up and down was likewise subject to jerks, which caused a difficulty in fixing it exactly at the focus ... Mr Cuff, the optician, applyed his thoughts to fashion a microscope in another manner, leaving the stage intirely free and open by taking away the legs* (the popular Culpeper microscopes had a tripod support), *applying a fine threaded screw to regulate and adjust its motions'*. These polished brass instruments sold for 7 guineas – today you would be lucky to acquire one for less than £6,000!



▲ Top of page. Drawings of copopods and polyps from *The Microscope Made Easy*. First described by Leeuwenhoek, polyps were regarded by many as the missing link between plants and animals.

sexes who have not had the advantage of a learned education'). There is much information on insects, polyps, rotifers, nematodes and fungal spores as well as detailed accounts of Baker's own painstaking studies of inorganic and organic salts and how these led him to suggest mechanical improvements to the microscope. [The major optical advance, the adoption of the achromatic lens to reduce distortion, came much later.] In fact, crystals were Baker's first scientific love (*'their variety and beauty no words or language can possibly express'*) and for this he received the Copley gold medal, putting him on a list of 18th century 'giants': Franklin, Priestley and Volta.

Romance, poetry and the Royal Society

Baker's lucrative 'day job' was as a speech therapist and in 1724 he was teaching in Stoke Newington when its most famous resident, Daniel Defoe, invited him to call. Despite a 40 year age difference, the two hit it off and Baker became a regular visitor. Soon, however, the highlight was afternoon tea with Defoe's daughters and Henry found himself falling in love with the youngest, Sophia, but it was almost four years before increasingly acrimonious negotiations with Defoe were resolved and the pair were married.

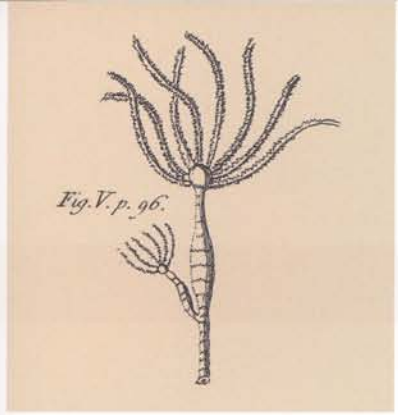
During this time, Baker launched and edited *The Universal Spectator* and published the much reprinted *The Universe. A Philosophical Poem Intended to Restrain the Pride of Man*. This is a long and somewhat reverential tract about the number and diversity of living forms. It is apparent that Baker was already familiar with the power of the microscope.

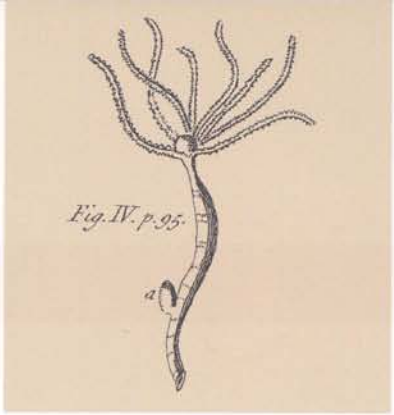
'Extend thy narrow sight: consult with art;
And gladly use what helps it can impart;
Each better glass with larger field display,
And give thee fields of life, unthought of, to survey.'

In 1740 Henry's influential friends elected him a Fellow of the Society of Antiquaries and, the following year, to the Royal Society. His citation reads *'A Gentleman well versed in Mathematicks and Natural knowledge, particularly eminent for his great Skill and happy Success in teaching persons born deaf and consequently Dumb to Speak (having improved upon that great Invention of the late famous Dr Wallis) Author of a very beautifull Poem called the Universe, with many Curious Notes regarding Natural History, and one who hath communicated Some usefull papers to the Royal Society, being desirous to become a Member of the Same, is recommended by us as a Candidate well deserving that honour.'* The six proposers included the heavyweights Hans Sloane (President), Martin Folkes (Sloane's successor) and Cromwell Mortimer (Biological Secretary).

Baker's 80 plus communications to the Royal Society between 1740 and 1769 are a mixture of the good, the bad and the ugly. They are mostly short papers and letters. Many appear trivial and reports about the weather, avalanches and minor earthquakes are common. One of the few substantial papers is a description of the Society's collection of 26 Leeuwenhoek microscopes. Baker calculates their magnifying power (the best was about ×160) and concludes that by using these Leeuwenhoek could not possibly have seen the detail that is shown in many of his drawings. He carefully dodges the issue of whether the Dutchman exaggerated by concluding that experience, good eyesight and more powerful lenses would account for the discoveries. We can't check this because the microscopes have long since disappeared.

In 1743 Baker published his second book *'An Attempt Towards a Natural History of the Polype'*. In it he records his attempts to repeat the experiments of Abraham Trembley who, 4 years





earlier, had cut up fresh water polyps (*Hydra vulgaris*) and found that the parts regrew to form entire animals. This news excited the scientific establishment, because of the debate about the distinction between animals and plants (only plants grow from 'cuttings' but only animals move and eat worms), as well as the natural philosophers who were puzzled as to how the 'soul' of the polyp was distributed among the progeny. Baker was amazed by what he saw as he chopped the hydra into pieces.

In the late 1740s Baker's interests turned to electricity (the hot topic of the day) and he reported experiments that were thought to show its beneficial medical qualities.

The friend and the cheat

Henry Baker's most prolific correspondent was William Arderon, an obsessive collector of objects and obscure facts who wrote about what he had seen and heard. In return Baker kept his pen pal up to date with events at the Royal Society and life in London, and describes his editing activities, of which two are noteworthy.

In the mid 1740s, the surviving plates for Robert Hooke's classic *Micrographia* were cleaned and the missing ones re-cut. Baker tells Arderon that he is: '*overlooking the press which a little work of mine has just now passed through. I call it Micrographia Restuarata or the Copper Plates of Dr Hook's wonderful discoveries by the microscope reprinted and fully explained.*' Baker says that he has asked the booksellers to price it as low as possible to attract customers and convert them to the wonders of microscopy.

In August 1749 Baker informs Arderon that he is involved in editing Benjamin Wilkes' *The English Moths and Butterflies* and pays Wilkes a wonderful backhanded compliment: '*indefatigable in his observations, and faithful in miniting down every particular, but for want of learning quite incapable of writing a book.*' But there is no mention of Baker's involvement when this beautifully illustrated book was published, although some lines on butterflies from *The Universe* are included.

George Adams was a prominent instrument maker who could see what *The Microscope Made Easy* was doing for the sales of Cuff's instruments and in 1746 published *Micrographia Illustrata* in which he describes the merits of his own microscopes. However, Adams had plagiarized sections of *The Microscope Made Easy* and Baker was incensed. He wrote to his friends warning them of the '*notorious robbery*' by '*an ignorant but impudent fellow*' and urging them not to buy the book.

The end

Henry Baker died in November 1774 and was buried alongside his wife in the churchyard at St Mary le Strand. In his will he left £500 to the Royal Society (in addition to the Bakerian lecture bequest of £100), but most of his considerable fortune went to his grandson, William. The Bakerian lecture ('*for an oration or discourse to be spoken or read yearly by some one of the Fellows of the Society on such a part of natural history or experimental philosophy*') was first given in 1775. Early speakers included Cavallo, Davy and Faraday and many of the greatest scientists of the day, including Rutherford, Herschel, Fox Talbot, Maxwell and Hoyle, have delivered the lecture.

Would Henry Baker be remembered if not for the eponymous lecture and footnotes in the many Daniel Defoe biographies? There is no doubt that *The Microscope Made Easy* was widely read not only by natural historians, but also writers, poets and thinkers of the day. Baker's purpose in writing the book was to popularize the use of the microscope and instill in others a curiosity for objects previously invisible to the naked eye. These aims were achieved. Henry Baker was once described as '*a philosopher of little things*', which can be interpreted in two ways: a man who thought deeply and wrote extensively about the minute objects he examined under the microscope or one who spent his life collecting and explaining essentially trivial observations. The former definition is the accurate one.

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Further reading

Baker, H. & others (1740–1768). Papers published in the *Philosophical Transactions of the Royal Society*. *JSTOR – The Scholarly Journal Archive* (www.jstor.org).

Potter, G. (1932). Henry Baker, FRS (1698–1774). *Mod Philol* 29, 310–321.

Turner, G. L'E. (1974). Henry Baker, FRS: founder of the Bakerian lecture. *Notes Rec Roy Soc* 29, 53–79.

Victoria and Albert Museum Forster Manuscripts. The collection includes four volumes of Baker's correspondence with William Arderon and his autobiographical memoranda.

Woodruff, L.L. (1918). Baker on the microscope and the polype. *Sci Mon* 7, 212–226.

Schools Membership costs only £10 a year. Benefits include *Microbiology Today*, advance copies of new teaching resources and discounted fees on SGM INSET courses. To join see www.sgm.ac.uk/membership. Enquiries: education@sgm.ac.uk or go to www.microbiologyonline.org.uk for full details of resources and activities.

Under the microscope

Using a microscope in school to look at microbes can be very rewarding but is not as difficult as it seems if you follow **John Grainger**'s handy hints.

▲ Young schoolgirl using a microscope. *Richard Bailey / Science Photo Library*

The setting up of a microscope is a basic skill that is rarely mastered in schools. Only when it is done properly can the smaller end of the diversity of life be fully appreciated and microscopy be put to many other uses, such as aiding identification, measuring growth and checking for contamination. The magnifying power of a microscope is important but, particularly when looking at microbes, remember that the amount of detail seen is determined by its resolving power. The sub-stage condenser plays a crucial role in achieving good resolution.

Hints

Adjust the iris diaphragm to achieve optimum balance between definition and glare. Do not control light intensity by moving the sub-stage condenser, the position of which should be to focus the light on the specimen. Re-adjust the iris diaphragm for each objective lens.

For looking at wet mounts of living specimens of protozoa, algae, moulds and even yeasts, the low power objective lens (×10) is often adequate, but also necessary for locating and centering on an area of interest before turning to the high power objective lens (x40). Without altering the focus, turn to the high power lens and then finely re-focus.

Use the oil immersion objective lens for examining stained preparations of bacteria. Put one drop of immersion oil onto the preparation; a coverslip is not required. Remove the slide and wipe the oil immersion lens clean after use.

More detailed information on microscopy, including observing and preparing mounts for observing different types of micro-organisms, making smears and staining procedures is available in *Basic Practical Microbiology: A Manual* (email education@sgm.ac.uk to obtain a copy).

John Grainger is Chairman of the Microbiology in Schools Advisory Committee (MISAC) and co-delivers SGM's basic practical microbiology courses for teachers and technicians. He can be contacted via SGM HQ.

SGM aims to promote microbiology education in its broadest sense. National Science Week (NSW) provides an ideal opportunity to raise the profile of our subject. **Joy Perkins** describes some events that were supported by a grant from the Society's PUS Fund.

National Science Week in Aberdeen

Shedding light on the amazing secret world of soil microbes

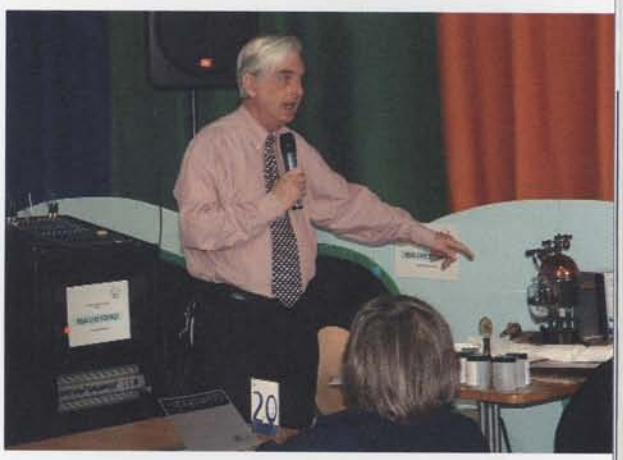
Professor Ken Killham delivered the NSW lecture on this topic at Aberdeen University on 16 March 2006. He provided a fascinating insight into the tiny organisms that live underground. The event was attended by Higher and Advanced Higher Biology pupils from local schools. It also featured a competition with GIANT Microbes™ as prizes. These are educational cuddly toys modelled on viruses and bacteria.

Ken explored *The Good, The Bad & The Ugly* of soil microbes. *The Good* focused on a range of beneficial plant–microbial associations such as mycorrhizal systems for nutrient capture and the use of microbes to clean up persistent organic pollutants in soil. *The Bad* investigated the deadly stomach bug *E. coli* O157:H7 and its presence in Scottish cattle, as well as *the very bad* endospores of the soil bioterrorism bug *Bacillus anthracis*. *The Ugly* covered devastating plant pathogens, including winter wheat root rot. At the end of the talk the audience was reminded that the soil sustains the planet – providing our food and fibre, regulating our water and air quality, supporting trees for timber and fuel, and cleaning up most of our pollution. The lecture was also peppered with interesting facts, for example, in a single hectare of land the biomass of microbes equates to 30 sheep! Professor Killham's enthusiasm was infectious and the event was a huge success with visiting pupils.

The three Ps – Perkins, Porter and Pennington – also helped to raise the profile of microbiology at the NSW *Science Discovery Day*. The excellent venue for this event was Satrosphere, Aberdeen's hands-on science centre, and it was organized by the Aberdeen Branch of the British Association for the Advancement of Science (BA). The event was only made possible by the generosity of BP who covered admissions charges for 800+ visitors.

At this family-orientated day I delivered a workshop called *Trees, plants and microbes – getting to the root of the issue*. Visitors found out about soil microbes through a range of hands-on activities. They discovered how tiny organisms in soil help trees and plants grow and investigated beneficial root–microbe interactions. Examples of friendly mycorrhizal fungi from the roots of orchids and a pine tree were also on display. Drag and drop computer exercises were very popular with visitors of all ages. Pete Jeffels from the Learning Technology Unit at the University used Flash Macromedia software to design mushroom-based activities.

In the Satrosphere café, Professors Andy Porter and Hugh Pennington contributed to the *Real Live Science* programme. As visitors relaxed and enjoyed a bite to eat, scientists gave short presentations. Andy described the human immune system and how it fights diseases. Hugh, using the memorable title *Bird 'flu! MRSA! We're all doomed!?*, provided a lively account of Sir Alexander Ogston, a famous graduate from Aberdeen University. In the 1880s Ogston discovered and worked on *Staph. aureus* and researched the cause of hospital infections.



Many thanks to SGM for funding both events and Aberdeen University staff (too many to mention individually) for their support in raising the profile of microbiology during NSW 2006.

Joy Perkins
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If you are going out into the community to promote microbiology, a grant from the SGM's PUS Fund can provide up to £1,000. See ***www.sgm.ac.uk/grants*** *for further details of the scheme. We can also supply ideas for activities and provide goodies to give away, as well as tell you what is safe to do! Contact* ***education@sgm.ac.uk***

▲ Professor Hugh Pennington at the 'Real Live Science' initiative.

What's new?

Science in School – www.scienceinschool.org

This is a new European journal to promote inspiring science teaching. It covers the whole range of science, highlighting the best in teaching and cutting edge research. It is available in print in English and online in several different European languages.

To receive an alert when an issue is published, send an email with the subject *Subscribe to Science in School* to **scienceinschool@embl.de**

Where do medicines come from?

ABPI has put together this new resource for primary schools, designed to be used with interactive white boards and computers. It is in three parts:

- where do medicines come from? – Powerpoint presentation of stories for KS1 and KS2 with follow-up activities
- how do we get medicines in our bodies? – a series of animations
- posters – A2 size to be used at different points in the primary curriculum.

See **www.abpischools.org.uk** for details and to download web versions.

2006 MISAC Competition MRSA today

SGM sponsored the 18th MISAC competition for secondary schools. This year's topic was chosen because of the serious problems caused in hospitals by MRSA (methicillin-resistant *Staphylococcus aureus*) and their extensive coverage by the media. Therefore, it was no surprise that the competition to write a newspaper feature on a hospital outbreak of MRSA proved to be very popular. It generated nearly 700 entries and involved more than 800 students from 105 schools and colleges drawn from England, Wales, Scotland and Northern Ireland. Pleasingly, this year there were more entries than usual from the GCSE Group, with numbers still holding up well in the 11–14 age range.

The judges looked particularly for attention to the guidance given to entrants on the writing of a news story, including preparing the headline to catch the reader's attention, structuring the story to maintain interest while conveying essential information, and the appropriate use of pictures, diagrams and scientific terms. Other important features were evidence of scientific merit, the use of entrants' own words rather than text downloaded from the web, and an appreciation of the importance of bringing out human and local interest.

Sue Assinder and Janet Hurst, representing SGM, joined the Chairman and other members of MISAC for the judging at Marlborough House. This year the judging panel also benefited from the expertise of Alexandra Blair, Education Correspondent on *The Times* newspaper. Many entries impressed the judges with their high quality, but unfortunately a notable proportion were excluded from consideration because they did not adhere to the competition rules, particularly by using the format of a factsheet instead of that of a newspaper article.

The winner of the 11–14 age group was Eleanor Tayler of the Abbey School, Reading; Emma Pascall of Durham High School came first in the GCSE group. Further details of the winners are available on the web (www.microbiologyonline.org.uk/misac) and a selection of the entries will be on display on the SGM stand at the ASE annual meeting at the University of Birmingham (4–6 January 2007).

Each school entering the competition received a pack of microbiology teaching resources and every student was sent a certificate of entry.

Next year's competition on *Salmonella: from farm to fork*, will be sponsored by the Society for Applied Microbiology and an entry form will be downloadable from the MISAC website (see above) in September. MISAC is grateful to all its sponsors for enabling the competition to take place each year.

◀ The MISAC judging panel at SGM HQ in April 2006. From left to right: Peter Fry, John Tranter, Sue Assinder, Janet Hurst, John Grainger and Alexandra Blair.

letter

The world in miniature: sealed ecosystems

Dear Dr Thomas

In connection with the article under the above title in the May edition, I am reminded that when visiting the microbiologists at the University of Hawaii at Manoa in 1980 I was shown something similar. They had placed samples of marine materials in rather larger sealed containers and kept them in daylight for several years. The containers showed cycles of photosynthesis, death and renewal. One or two had been opened and sampled (under extreme containment in case anything dangerous might have evolved), but the only significant fact that I now remember is that the opened vessels revealed that their contents had developed a higher level of oxygen in the air over the liquid than is found in the normal terrestrial atmosphere. I do not know if this work is still ongoing, but I sincerely hope so, as it offers (as your article demonstrates so well) a fascinating insight into how systems can self-maintain when one might expect that they would have long-since collapsed, being so relatively small.

I hope that such work is relevant to the question of survival in such difficult environments as the pools located beneath thousands of feet of Antarctic ice. I am sure that it must be relevant to the dynamics of the pockets of cyanobacteria permanently trapped in the ice present as 'pools' (well, it must have been once, it was so flat) in the hills above McMurdo Base, Ross Island, Antarctica.

Yours sincerely

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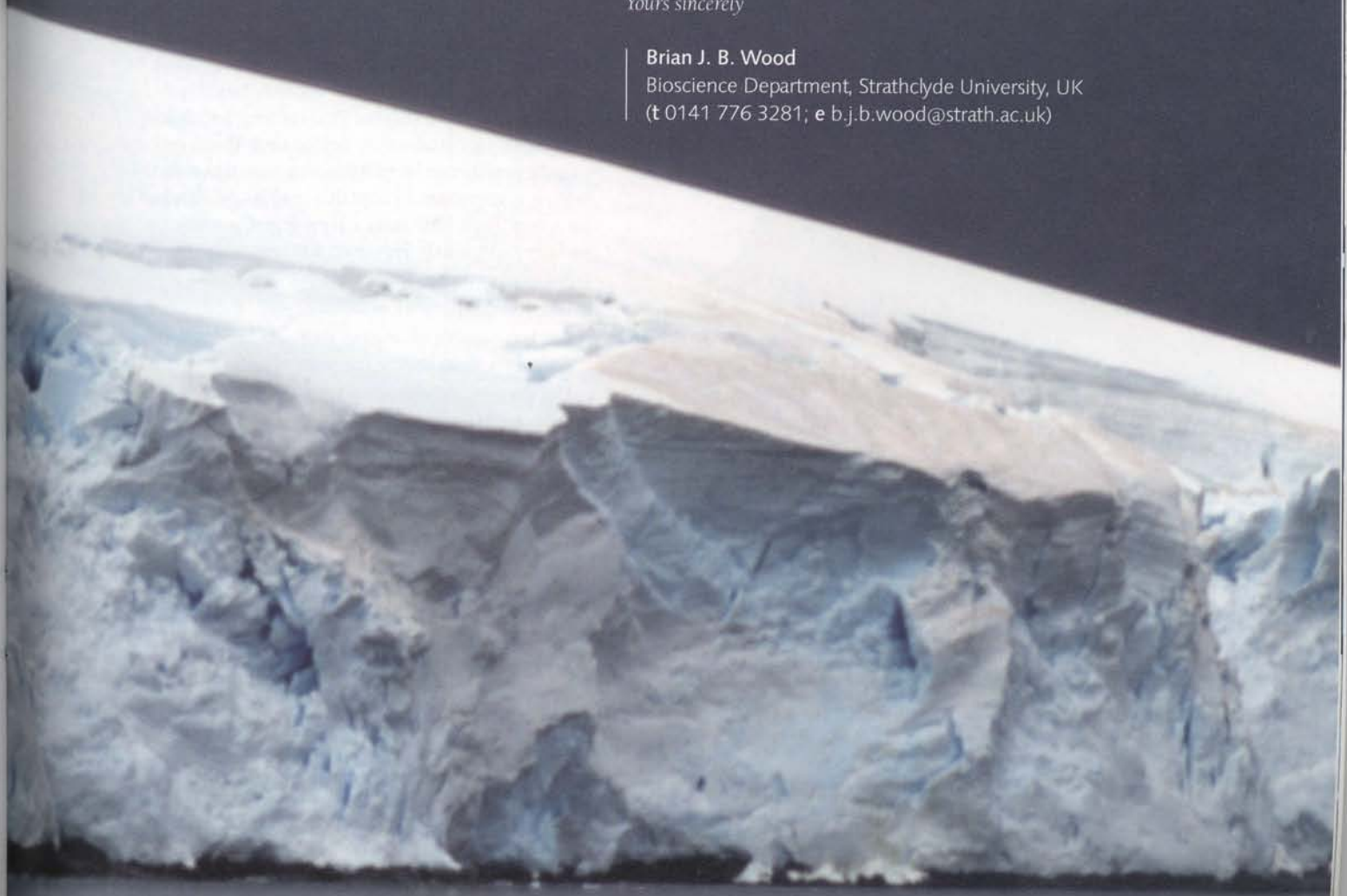
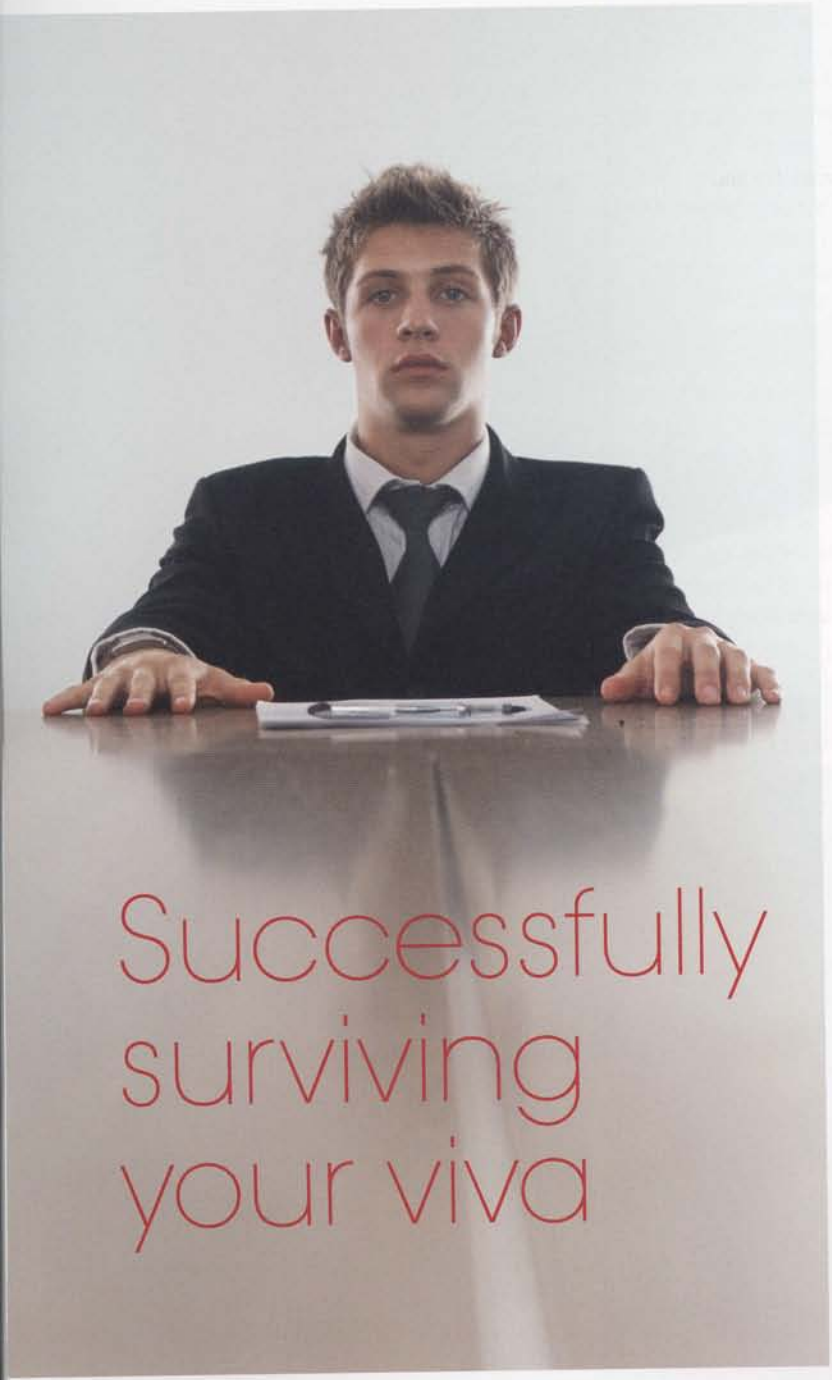
(**t** 0141 776 3281; **e** b.j.b.wood@strath.ac.uk)



An ice peninsula in Antartica. *Michael Pura (www.michaelpura.com)*

gradline

Gradline aims to inform and entertain members in the early stages of their career in microbiology. If you have any news or stories, or would like to see any topics featured, contact **Jane Westwell** (e j.westwell@sgm.ac.uk).



Successfully surviving your viva

It may be the height of summer, but for some UK and Eire based postgrads the rapidly approaching autumn brings thesis submission deadlines and, of course, the viva. Some of you may have pushed thoughts of the final hurdle to the back of your minds - but don't leave them there too long. Despite the great mystique that still surrounds the viva (who hasn't heard tall tales of marathon 5 hour ordeals?) information is available to PhD candidates from individual universities, websites and books, not to mention supervisors. To start you off, we offer a few words of general advice. Obviously, for specific information about PhD examining procedures, you must check out your own institution's regulations and talk to your supervisor to find out what to expect on the big day.

The viva is the final stage of PhD training and is an opportunity for students to defend their thesis and show that they really can be independent researchers in their field. It is important to note that there is no standard format for a viva. Each PhD and each examiner is unique so styles do vary enormously. However, all vivas aim to answer a number of common questions:

> Did you do the work yourself?
>
> Are you capable of original thinking i.e. did you have your own ideas during the research?
>
> Did you write the thesis yourself?
>
> Can you identify the value of your own work and put it into context of the wider field of research?

The examiners

The examining panel usually consists of an external examiner (chosen carefully by the supervisor – sometimes with input from the candidate) and an examiner from within your department who has not had any involvement with your project. Some universities also require an independent chair to be present and the supervisor may sometimes attend (by invitation) to (usually silently!) support the candidate. Before the viva, the examiners must write reports on the thesis according to university guidelines or using a pro-forma. Although the examiners may have formed an opinion of the research, this can be changed during the course of the viva. After the viva, it is usual for both examiners to have to complete a joint report/pro-forma. You might want to ask your supervisor to show you these forms to see what the examiners are being asked to say about you.

The questions

Examiners will ask a range of questions designed to obtain different kinds of information. Good examiners should not be aggressive but they will ask quite searching and comprehensive questions which can typically be about your 'journey', the research context, your methodology, your results and conclusions.

Journey questions give the examiner a feel for your personal voyage of discovery.

> Why did you choose this field of study?
>
> What was the most significant challenge you have faced in this work?
>
> Which aspects did you enjoy the most?
>
> If you could go back and do it all again what would you do differently?

Context questions test whether you can put your work into the wider research/societal context.

> Which techniques do you wish you had been able to use?
>
> How would you apply your findings to…?
>
> Tell me more about the work of X and Y and how it relates to your findings in Figure 4.2
>
> What are the implications of your findings to public health policy…?

Challenging questions make you defend your position.

> Do you really mean that…?
>
> I can think of at least three alternative explanations for Figure 5.3 – why should yours be correct?
>
> Your data in Table 6 contradicts that of Xxxxx *et al.* (2004) – who should we believe?

Your responses

In your answers you must demonstrate that you can put your work into context and you must have justified critical opinions on the value of your work

If you encounter aggression or hostile questions, try to work out why this is happening. Sometimes probing questions on a weaker area of your thesis can seem very intimidating. Maybe your examiner is trying to make you see another point of view. Perhaps your examiner has an aggressive speaking style. Stay calm and defend your position rationally. If necessary you should concede some points or agree to disagree. The chair and/or internal examiner are there to ensure fair play.

Most vivas last between 1.5 and 3 hours. At the end, it is common practice for the candidate to leave the room during the examiners' discussion. This can be nerve-wracking and you may prefer to spend this time alone, although some people do line-up some supportive company. Once called back into the room there are a number of possible outcomes. Not all of these are available at every institution.

> *PhD awarded – no corrections* (unusual but by no means impossible)
>
> *PhD awarded subject to minor corrections* (usually approved by internal examiner within a set time period)
>
> *PhD not awarded but candidate may submit a revised thesis to both internal and external examiners* (sometimes known as major corrections)
>
> *PhD not awarded but candidate must resubmit a revised thesis and also undergo a second viva*
>
> *Recommendation that a lower degree is awarded*
>
> *Candidate advised to revise thesis and submit for a lower degree*
>
> *No degree awarded and no opportunity to submit for lower degree*

Other tips for success

A successful PhD candidate shows excellence in both thesis presentation and performance during the viva. There are a number of things you can do to ensure your own success:

> If feasible, publish your work before writing the thesis – it always helps to have been subject to positive peer review.
>
> Keep reading the literature after you submit your thesis; people do publish all the time and you don't want to be caught on the hop.
>
> Work out how to summarize succinctly what you did, why you did it, what you found and why this is important.
>
> Prepare a list of questions that are likely to come up – then think about your answers.
>
> Look critically at your thesis and if you spot errors or weaknesses don't try to hide them, be ready to discuss them with your examiners.
>
> Don't dig yourself into a hole! Be honest and admit to not knowing answers to questions.
>
> Recognize the strengths of your work too.
>
> Research your examiner – what are their interests? Do they have any biases? Bear these in mind during the viva.

Good luck!

Jane Westwell
External Relations Office

Bob Rastall
School of Food Biosciences,
University of Reading

Further reading

How to survive your viva (R. Murray, 2004, OUP, ISBN 0-335-21284-0) offers a detailed insight to the viva process including questioning styles and suggests strategies for success.

The doctoral examination process: a handbook for students, examiners and supervisors (P. Tinkler & C. Jackson, 2004, OUP, ISBN 0-335-213057) – information, advice, real-life accounts and case studies based on a review of the examining process in the UK.

◀ *Peter Cade / Getty Images*

hotoffthepress

Science writer **Meriel Jones** takes a look at some recent papers in SGM journals which highlight new and exciting developments in microbiological research.

Rabies in the air

Johnson, N., Phillpotts, R. & Fooks, A.R. (2006). Airborne transmission of lyssaviruses. *J Med Microbiol* **55**, 785–790.

Rabies is rightly feared, but measures to reduce its incidence have been very successful within Europe, especially in the UK. However, the *Rabies virus* (RABV) that causes typical rabies is only one member of the genus *Lyssavirus*. Others also cause disease in mammals, including fatal illness in people. These viruses are found in bats, although the animals are unlikely to bite humans. However, they are considered to be a new public health risk, maybe linked to the few cases of rabies, including one of a bat conservationist in the UK, where the route of infection has never been confidently identified.

One concern is whether lyssaviruses can cause infection from breathing an aerosol. This could come from the large amount of bat urine and guano around bat colonies in caves and other poorly ventilated spaces. The risk would depend on how much virus was in the air and how well it survived, as well as whether it could actually cause an infection after passing through the surface of the respiratory tract. As a first step in evaluating any risk, researchers from the UK at the Veterinary Laboratories Agency, Weybridge, and Dstl, Porton Down, have been testing whether *Lyssavirus* aerosols can cause disease in mice. They used strains of both RABV and also *European bat lyssavirus 2* (EBLV-2), isolated from a Daubenton's bat that causes a rabies-like disease. Some mice became unwell after either virus was inoculated into their noses and aerosols of RABV over a 100-fold range were able to cause illness. This provided convincing evidence that the rabies virus can be transmitted by these unusual routes.

However, more encouragingly, the mice that had breathed an aerosol of EBLV-2 showed no signs of any illness, suggesting that contact with the virus from bats poses a lesser risk.

BSE – no prions detected in milk

Everest, S. J., Thorne, L. T., Hawthorn, J. A. & others (2006). No abnormal prion protein detected in the milk of cattle infected with the bovine spongiform encephalopathy agent. *J Gen Virol* **87**, 2433–2441.

One niggling question about BSE is whether it can be carried in milk. There is no evidence that milk can transmit BSE, but there is also no evidence that it cannot. Now, for the first time, a long-term collaborative study by scientists within the UK's Veterinary Laboratories Agency and Agricultural Development and Advisory Service has given reassurance that the most sensitive biochemical tests currently available cannot detect the infectious agent within milk from infected cows.

One of the reasons why it has taken so long to find this out is to do with the difficulty of detecting infection. BSE is caused by a protein, called a prion, adopting an abnormal shape. The protein is present in all cattle and laboratory detection techniques were initially unable to distinguish the normal and abnormal prion forms. Prion levels are very low in most tissues. The first tests for infective material were bioassays that involved injecting samples into mice and then observing how their behaviour and brain structures changed. In late 1999 the first of several rapid, non-animal tests became available. These are now used to detect prions but have not been used to test milk.

◀ *Veronique Leplat / Science Photo Library*

Milk is a difficult material to test for BSE. All tests require appropriate negative and positive controls, but there is no naturally infectious milk to use as a positive control. Milk is predominantly water, but the researchers were certain that any prions would be within the very small amount of cow cells also present in milk. They concentrated these cells for tests and used samples spiked with authentic BSE prions to estimate sensitivity. They used two tests, based on different physicochemical principles, so they could assess the results for random variation generating false-negatives and false-positives.

The researchers screened 541 milk samples from groups of cows over several pregnancies that were BSE-free, or had been fed one dose of either 100 or 1 g of BSE brain homogenate. Statistical analysis of results from 22 of the samples suggested that prions might be present. However, eight of these were from BSE-free animals and due to the random scatter associated with any biochemical assay. Some further false-positives were observed using the confirmatory test because of a cross-reaction between a reagent in one of the tests and something in milk collected within the first week post-calving.

The end result of this study is that the researchers have not identified BSE prion protein in the cellular fraction of milk from cattle incubating BSE using tests at their limits of detection. This concurs with the current understanding of the pathogenesis of BSE and gives further support for the idea that milk does not transmit prions.

Bacteria solve a problem for wasps

Kaltenpoth, M., Goettler, W., Dale, C., Stubblefield, J.W., Herzner, G., Roeser-Mueller, K. & Strohm, E. (2006). '*Candidatus* Streptomyces philanthi', an endosymbiotic streptomycete in the antennae of *Philanthus* digger wasps. *Int J Syst Evol Microbiol* **56**, 1403–1411.

Insects have a love–hate relationship with microbes. Researchers have recently discovered that the European beewolf wasp (*Philanthus triangulum*) uses bacteria to solve a problem caused by their nesting habits. The females build nests in soil provisioned with paralysed honeybees. Young beewolves spend the winter within cocoons in the burrow, emerging the next summer to start their own nests. However, the dampness underground poses a constant risk that bacteria or fungi will colonize the young wasps. This danger is averted because the wasps incorporate symbiotic bacteria into the cocoons that are presumed to produce antifungal compounds. The bacteria live within glands in the antennae of female wasps and are secreted into the nest just before egg-laying. To follow up their discovery of this very close relationship, the researchers wanted to know whether it occurred in all wasp species within the genus *Philanthus* and the bacteria involved.

Zoologists in Germany and the USA collaborated with researchers from South Africa to obtain females from 32 *Philanthus* and closely related species. However, the bacteria defied all attempts to grow them in the laboratory. The researchers had to rely on the evidence of microscopy and molecular methods to identify the bacteria. Electron microscope pictures of sliced antennae showed that specialized glands within the antennae were packed tightly with long fine strands. This filamentous form of growth is characteristic of actinomycete bacteria and agreed with the results of molecular tests. PCR was used to identify the presence of a single species of acinomycete bacterium, from the genus *Streptomyces*, within antennae of females from all the *Philanthus* species. Almost complete 16S rRNA gene sequences from bacteria in each wasp species were recorded. The high degree of similarity among the sequences suggested that the bacteria had been transmitted from mother to offspring for many generations, maybe even from the origin of this relationship between beewolves and *Streptomyces* 26–67 million years ago.

Colonization of buried plastic

Sabev, H.A., Handley, P.S. & Robson, G.D. (2006). Fungal colonization of soil-buried plasticized polyvinyl chloride (pPVC) and the impact of incorporated biocides. *Microbiology* (2006) **152**, 1731–1739.

It is difficult to imagine modern life without plastics. A particular benefit comes from their stability and long-lived nature, sometimes enhanced by including biocides within the plastic. One of the most common plastics consist of mixtures of polyvinyl chloride with plasticizers, fillers and stabilizers (pPVC). The downside is that plastic rubbish survives for a very long time in landfill sites or as an eyesore in the environment. Surprisingly, there have been very few studies on how pPVC is degraded by micro-organisms. However, researchers from the University of Manchester have now investigated how microbes colonize abandoned plastics, with intriguing results.

The authors buried sheets of pPVC incorporating biocides in fields and in a forest in the Vitosha mountains near Sofia in Bulgaria. Every few months they retrieved some of the sheets and tested both the physical state and the microbes living on them. After 10 months the thread-like strands of fungi formed a dense network all over the sheets, with matching furrows showing how secreted enzymes had eaten away the plastic. The plastics containing biocides were less thickly coated, but when the tensile-strength characteristics were measured, all the samples had been weakened by the microbes.

The researchers focused on the fungi and discovered 92 different types on the plastics, which they considered an underestimate. Some were species of *Penicillium* or *Trichoderma*, but many were unidentifiable, even using molecular tests. When they tested the ability of individual fungi to make use of either of two plasticizers, the researchers discovered that growth increased over the first 4.5 months, but then decreased. They speculated that initially there was selection for strains able to feed from the unfamiliar plasticizer, but that cell debris and by-products then allowed other fungi to grow on the plastic surface.

The value of this research is that it provides a baseline for the ways in which plastics are degraded in the environment. The impact of the different biocides on this process provides ideas for modifying plastics to be either more or less biodegradable, depending on their intended uses.

► pPVC before (top) and after (bottom) soil burial. *Geoff Robson, University of Manchester*

▲ Female European beewolf (*Philanthus triangulum*) secreting symbiotic '*Candidatus* Streptomyces philanthi' from specialized antennal glands and applying them to a brood cell within the soil. *Erhard Strohm, University of Regensburg, Germany*

Society wins silver medal for the second year running at the RHS Chelsea Flower Show

Gardening enthusiasts at the 2006 RHS Chelsea Flower Show were keen to learn how some microbes can harm their garden plants. The Society's exhibit, 'Plants and microbes – a deadly duel', also impressed the judges, who awarded it a silver medal in the Lindley Range.

▲ The SGM stand at the 2006 RHS Chelsea Flower Show with *Microbiology Today* Editor Gavin Thomas on duty. *Ian Atherton, SGM*

In the garden there is an unseen and ongoing battle between plants and microbes such as fungi, bacteria and viruses. Disease-causing microbes are always in the environment, ready to attack garden plants when the opportunity arises. Fine powdery residues, grey furry coatings, strange growths and slimy exudates are signs of microbial attack.

SGM staff designed, put together and manned the display for the *Lifelong Learning* section in the Great Pavilion. The backdrop explained various aspects of microbial infections in garden plants, including information on how microbes attack plants, how diseases spread, how the plants protect themselves from infection and how gardeners can help in the fight for healthy plants.

The display also included a wide range of shrubs, perennials, annuals, herbs and fruit bushes set out in attractive containers in a courtyard scene, which were all labelled with their susceptibility to diseases caused by bacteria, viruses and fungi.

A handout giving more detailed information about plant pathogens was available for interested visitors to take away.

This exhibition was in complete contrast to last year's display where the Society had highlighted the beneficial effects that microbes can have in the garden – in the form of plant–root associations with rhizobial bacteria and mycorrhizal fungi.

It was another huge undertaking by the Society and many thanks are due to the staff and also members of the SGM who helped to man the stand from 8am to 8pm each day.

Over the six days of the show, 3,000 leaflets were distributed and the stand was viewed by many thousands of interested gardeners, including members of the Royal Family!

Janet Hurst
Faye Stokes
SGM

Famelab

FameLab is a national competition to find the UK's best new talent in science communication and this year's competition finalists included two microbiologists – a virologist and a mycologist.

National heats were held during March and April at Newcastle, Swansea, Edinburgh, London and Belfast. Each entrant had only 3 minutes to impress the judges with an interesting and exciting presentation that was both scientifically accurate and engaging to a non-scientific audience. Successful contestants went through to the afternoon round to present a different 3 minute talk – this time with a public audience present!

Two finalists were selected from each heat and these went on to complete a weekend masterclass in science communication – working with TV producers, comissioning editors, journalists, science communicators and media trainers. During the class, each finalist recorded a 99 second podcast on one of the following three subjects:

Quantum mechanics

What makes octopuses different to other sea life apart from their legs?

As water is made of hydrogen and oxygen – why doesn't it burn?

Karl Byrne is currently in the final year of his PhD at Queen's University Belfast, where his project involves looking for novel therapies for viral exacerbations of Chronic Obstructive Pulmonary Disease. He would like to make science more accessible to non-scientists and entered the competiton after some persuation from a friend.

A mycologist studying for a PhD in the fungal affects of rotting wood at the University of Newcastle upon Tyne, Steve Robertson was always scientifically inclined and wants more science programmes on TV and radio.

The final was held on 10th June at the Cheltenham Science Festival, where each finalist gave a 5 minute presentation of a contemporary science topic that was different from those given during the heats. Unlike previous rounds, where only the judges selected the best, during the final, the public audience had to be impressed, as they were involved in choosing the winner, Jonathan Wood, a biologist turned Deputy Editor of *Materials Today*. The winner of the 2006 competetion received £2,000, and will also get the chance to pitch an idea to Channel 4.

Faye Stokes
Public Affairs Administrator

FameLab (www.famelab.org) is an initiative of Cheltenham Science Festival in partnership with NESTA and is supported by Pfizer, the Daily Telegraph, Channel 4, Research Councils UK, the British Council, Enable Interactive and Silicon 19.

Microbiology and the media

At a recent meeting Hugh Pennington looked at how microbiology stories get into the media and gave some tips on how microbiologists could publicize their own research.

The media are interested in stories that have human interest. When it comes to microbiology, journalists favour lurid diseases that could affect anybody, especially the young or old. They particularly like it if they have a disease that has caused the death of a named individual. In 1994 a television company ran a story and issued a press release on necrotizing fasciitis '*the bug that eats you alive*' after there had been a cluster of cases in Gloucestershire. This was picked up by both the paper and electronic press in the UK and worldwide, and over a relatively short period a huge number of stories were featured on this subject. In fact, as Hugh pointed out, journalists were much better at finding stories on necrotizing fasciitis than microbiologists! With the launch of the National Lottery the media soon lost interest in necrotizing fasciitis and the story was dropped. Surprisingly, during this period the number of cases of the disease had not increased. This was an example of a completely media-driven story.

When scientists are asked to comment on a story that has appeared in the press, Hugh believes that it is always worth responding so that you are:

not accused of a cover-up at a later date

protected from unfair comments from journalists

It is important that the comment is accurate, professional, not overly optimistic and made without delay. It is essential that the scientist stays away from silly media stunts such as the government minister feeding a beef burger to his daughter during the BSE crisis.

When trying to promote your science to the press Hugh Pennington stressed the importance of getting across your points to the media in a succinct and memorable way, the message must be given using no more than three bullet points. Other sound advice included:

produce a press release

speak only to known journalists

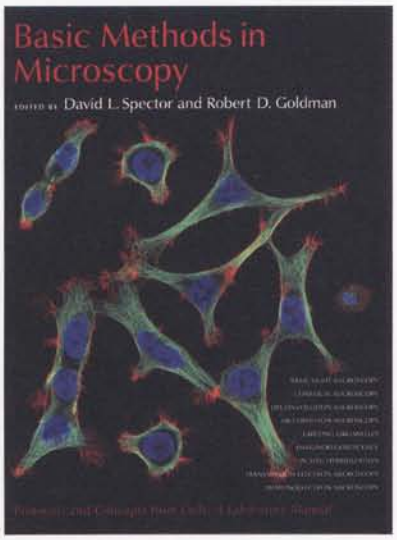
use media departments in universities

attend media training courses such a those run by the SGM

Dariel Burdass
SGM

reviews

If you would like your name to be added to our database of book reviewers, please complete the book reviewer interests form on the SGM website. A classified compendium of reviews from 1996 to the present is also available on the website.



Basic Methods in Microscopy

By D.L. Spector & R.D. Goldman
Published by Cold Spring Harbor Laboratory Press Europe (2005)
£45.00 pp. 375
ISBN 0-87969-751-2

The Cold Spring Harbor Laboratory Manuals grow from the best ground, the fertile interaction between top scientists and promising students in the intense laboratory course setting. Other manuals on imaging and microscopy from this series (*Live Cell Imaging: A Laboratory Manual* and *Imaging in Neuroscience and Development: A Laboratory Manual*) affirm the excitement of cellular research arising from the convergence of molecular and optical advances, while providing tools and cautionary tales sufficient for practical results.

The *Basic Methods in Microscopy* manual provides good introductions to optical methods and cell labelling techniques and many protocols for reference by the general biological microscopist. Unfortunately, segregation of this material away from the topic of live cell research has resulted in a step back from the cutting edge and a more prosaic scope and presentation. However, there are important advances in the microscopy of fixed materials, which often are the best approach for many research questions. The chapter on three-dimensional microscopy (Confocal microscopy, deconvolution, and structured illumination methods) by John Murray bucks the general trend. It conveys the delight and frustration of optical analysis of cell structure beyond the cover slip/sample interface. His exposition of the interaction of resolution and contrast over the axial dimension provides a valuable starting place for those dealing with microbiology in the real world. The theory and application of a broad range of 3D technologies are compared in a way that can help us make practical decisions about our research strategies. The 'Tips' for reliable imaging and the 'Troubleshooting guide' are full of golden advice for users of scanning confocal microscopes.

Multiphoton microscopy and multiplex-spectral analysis are also technologies on the advancing edge of cell biology with exciting applications. However, it is difficult to see a unifying concept in the optical technology or in the biological application that would allow these subjects to be covered in one chapter. The result is a narrow treatment of each technology confined largely to a particular microscope platform. It lacks the practical and theoretical detail that might help new users do this kind of microscopy. A chapter on fluorescence resonance energy transfer (FRET) was notable in its absence as this is one powerful sub-micron technology that does not depend upon live cell dynamics. Energy transfer can be used for *in situ* analysis of molecular association and can exploit the immense variety of fluorescence labelling techniques when applied to permeablized tissues.

The rest of the manual is conventional in its content and approach. The introductory chapter on optics covers the theory that is needed to analyse the problems and solutions raised in the rest of the manual. Unfortunately, this is a very big task for one chapter and the information may be too condensed to be accessible without considerable background. The wealth of specific reagents that permit localization of different molecules and structures in cells are covered in later chapters, including non-immunological, immunological and nucleic acid hybridization-based techniques. Several chapters cover protocols specific to eukaryotic model systems, including yeast, *Drosophila*, *Caenorhabditis elegans* and cell culture, but not bacteria, protists or common parasites. The final chapters venture into electron microscopy.

In summary, this book is a useful reference for a teaching laboratory or a microscopy facility with very general interests in a variety of microscopy techniques primarily for fixed tissues. But, to some extent, it is a useful appendix to its more exciting companion volume on live cell microscopy.

***Roger Phillips**, University of Sussex*

Cell Imaging Techniques Methods and Protocols

Edited by D.J. Tattjes & B.T. Mossman
Published by Humana Press (2005)
US$125.00 pp. 512
ISBN 1-58829-157-X

This 21-chapter volume is complementary to most other literature available on the market as it provides detailed descriptions of and protocols for a plethora of microscopy and related methods to study tissues, cells and macromolecules. By doing so, the volume covers (i) different types


of light, (ii) scanning probe and (iii) single particle electron microscopy techniques, including second-harmonic imaging, laser capture microdis-section, fluorescence *in situ* hybridization, laser cytometry, calcium imaging and near-field scanning optical microscopy. In terms of the systems under study, most of the chapters focus on animal tissues in a pathology/medical setting. Particularly useful, but rare, are the chapters on evaluating the performance of laser confocal microscopes and microarray laser scanners. Regarding the former, the chapter by Robert M. Zucker discusses on almost 60 pages (!) the various quality assessment aspects in an easily accessible manner and provides practical instructions on how to test individual parameters. This comprehensive and well rounded book is suitable for institutional or personal purchase, novices and experts, and it is certainly a must-read for all serious confocal microscopists and imaging facility managers. Unfortunately, the 19 colour plates are not embedded in the text, but constitute a 12-page insert between chapters 13 and 14. Other than that: a state-of-the-art account packed with valuable and accurate information.

***Andreas Holzenburg**, Texas A&M University*

Microscopy Techniques Vol. 95

Edited by E.J. Rietdorf
Published by Springer-Verlag GmbH & Co. KG (2005)
£168.50/US$289.00 pp. 319
ISBN 3-54023-698-8

Light microscopy has returned to fashion through the synergy of green fluorescent protein and a clutch of techniques involving some permutation of lasers, mathematics and acronyms, which offer the prospect of watching biological molecules at work inside living cells. This volume provides an introduction to these techniques, edited from the European Molecular Biology Laboratory, but also using experts from elsewhere. Chapters cover spinning disk, total internal reflection, fluorescence correlation, lifetime and recovery, deconvolution and tracking movement. If all these terms are alien then this is probably not the book for you. It is a series of robust and well written descriptions of the bases of the methods (yes, including equations) and the strengths and weaknesses of each. Physics tends to stay true, so this volume will be a longer-term reference for anyone who wants to understand how these methods actually work. In this respect it is excellent.

***John Armstrong**, University of Sussex*

Physical Principles of Electron Microscopy

By R.F. Egerton
Published by Springer-Verlag GmbH & Co. KG (2005)
£42.50/US$69.95 pp. 122
ISBN 0-38725-800-0

This book comprises a concise introduction to the fundamental physical concepts of electron microscopy and related analytical techniques from a predominantly material sciences/ engineering perspective. The concepts are well explained and illustrated, and in addition, the author offers a helpful introduction to microscopy as a whole, covering even scanning probe and X-ray microscopy, thereby comparing and contrasting the different microscopic approaches. With regards to light and electron optical systems one can, for instance, learn about why chromatic aberration causes shorter wavelengths to result in longer focal lengths with electrons, but shorter focal lengths with light optics. The text includes interesting historical tidbits and also alludes to more recent developments like time-resolved electron microscopy and monochromators. The sections on specimen preparation (almost exclusively focused on materials) are presented as brief but informative overviews, while the chapter on vacuum systems lacks a little substance and up-to-date information. Scroll pumps, for instance, are not even mentioned. In conclusion, this book serves as an ideal supplemental theory textbook for students, researchers and educators who already have a basic understanding of the operation of an electron microscope and do not seek hands-on information and/or are beginners with a biological background. It is suitable for institutional or personal purchase.

***Andreas Holzenburg**, Texas A&M University*

Reviews on the web

Reviews of the following books are available on the website at www.sgm.ac.uk/pubs/micro_today/reviews.cfm

Optical Imaging and Microscopy
Mycoplasmas: Molecular Biology, Pathogenicity and Strategies for Control
Microbe
Bergey's Manual of Systematic Bacteriology Volume Two: Part A, B & C 2nd edn
Dictionary of Parasitology
Molecular Microbial Ecology
Microbiology of Fresh Produce
Landmark Papers in Yeast Biology
The Biomedical Scientist as Expert Witness
Aflatoxin and Food Safety
Biofilms, Infection, and Antimicrobial Therapy
Biodefense Research Methodology and Animal Models
Pocket Guide to Extended-Spectrum β-Lactamases in Resistance
Microbial Biotechnology in Agriculture and Aquaculture
DNA Repair and Mutagenesis, 2nd edn
Parvoviruses
Probiotics in Food Safety and Human Health
Antimicrobial Resistance in Bacteria of Animal Origin
Algae Anatomy, Biochemistry, and Biotechnology
Cell Death during HIV Infection
The RNA World, 3rd edn
Cytomegaloviruses: Molecular Biology and Immunology
Adeno-associated Virus Vectors for Gene Therapy
Rinderpest and Peste des Petits Ruminants

obituary

Marler Thomas Parker (27.10.**1912**–25.2.**2006**)

M. T. (Tom) Parker, one of the inspirational figures of post-war medical microbiology in addressing our increasing concerns about staphylococcal and other healthcare-associated infections, died on 25 February 2006, aged 94. His work on *Staphylococcus aureus* – initially the type 80/81 epidemic strain that caused severe hospital infections in the 1950s and later the increasing antibiotic resistance amongst hospital isolates leading to the MRSA problems of the 21st century – is as relevant to medical practice today as when he led the Public Health Laboratory Service (PHLS) Cross-Infection Reference Laboratory through the 1960s and 1970s.

Tom was born on 27 October 1912 in North Walsham, Norfolk, and obtained his senior schooling at Paston Grammar School (1923–1931). He went on to Downing College, Cambridge, as an exhibitioner, reading Natural Sciences with specialism in Pathology (1931–1934), gaining first-class grades in both parts of the Natural Sciences Tripos. He then completed his medical studies at Charing Cross Hospital, London, qualifying MB, BChir (Cantab.) in 1937, and continuing there as a House Physician. In 1938, he began a Studentship in Pathology at Charing Cross Hospital Medical School and gained the Diploma of Bacteriology (London) with distinction in 1939 from the London School of Hygiene and Tropical Medicine under the exacting standards of W. W. C. Topley and G. S. Wilson.

The Second World War interrupted his academic studies. He held strong anti-fascist convictions and he enlisted in the armed forces at the outbreak of war in 1939. Through to 1945, he served in the Royal Army Medical Corps as a specialist pathologist, initially in the UK and then in India and Burma with the rank of Major. He made lasting friendships in India with memorable colleagues including James Rhind, Jerry Morris and Reg Passmore. He had graphic stories of his experiences in Lucknow and Calcutta and in his travels when on leave, with sad accounts of the terrible famine and the diseases that he witnessed at first hand. In Assam, his hospital unit supported Field-Marshal Slim's advance and he was especially proud of his laboratory's record in ensuring prompt diagnosis and effective treatment of the many patients who developed the debilitating infections that went with military operations in difficult conditions in a tropical climate. His unit was then shipped to Rangoon, arriving on the day after the Japanese retreat, and he was faced with establishing services in a ransacked infirmary that had even lost its water taps.

Early in 1946, on his return to civilian life, he joined the newly established PHLS under the directorship of G. S. (later Sir Graham) Wilson. Tom's first PHLS job was as Director of the Area Public Health Laboratory at Carmarthen, but in 1948 he moved to Manchester as Consultant Microbiologist and Director of the Regional Public Health Laboratory. There, his knowledge and experience were quickly acknowledged and he was recruited to deliver classes in the Manchester University Dip. Bact. course as a specialist teacher. His research at the time started to focus on *S. aureus* as a cause of severe hospital infections and in 1956 his thesis on *S. aureus* earned him a Cambridge Doctorate of Medicine.

In 1961, he was appointed Director of the Cross-Infection Reference Laboratory at the Central Public Health Laboratory, Colindale, London, a post he held with distinction until his retirement in 1978. Dr Parker was pivotal in his role as Director in bringing together the staphylococcus and streptococcus reference laboratories working on the qualitative differences in the pathogenic potential of these organisms. Throughout the 57-year history of the PHLS, this laboratory played a crucial role in investigating and understanding healthcare-associated infections, particularly those caused by *S. aureus* and β-haemolytic streptococci, and helping to devise preventive strategies that remain at the heart of the current Department of Health programme to combat MRSA. Tom had directed the laboratory for over half of its first 30 years. During that time many collaborations were established with colleagues and centres overseas which formed the foundation of the international links that remain to this day.

Tom's unassuming personality, integrity, wide range of expertise, and his friendly and helpful manner endeared him to all his colleagues and his many friends. It was inevitable that his help and influence would be widely sought within the discipline of medical microbiology, particularly with reference to staphylococci, streptococci and aspects of bacterial cross-infection, and he gave his time unstintingly to students, trainees and senior colleagues alike. He served on a host of international committees, and his contributions to WHO Working Groups, and his Short-Term WHO Consultantships in the Sudan, India and Burma (1974, 1976, 1980), all testify to the high regard in which he was held abroad. He had numerous collaborators across the world. His daughter Judith recalls a stream of visitors to the family home at Radlett in the 1960s, including Lewis Wannamaker (Minnesota), Theo Poon-King (Trinidad) and Hugh Dillon (Birmingham, AL, USA), but there were many, many more. He was a Founder Fellow of the Royal College of Pathologists (FRCPath 1964), was appointed a Corresponding Member of the Deutsche Gesellschaft für Hygiene und Mikrobiologie (1978), and was President of the Hospital Infection Society from 1984 to 1988. In 1983, he was awarded Honorary Membership of the Pathological Society of Great Britain and Ireland in particular recognition of his contribution to the Society's journals for almost 30 years. In turn, Tom's care for members of his staff is epitomized by his support for Winston Maxted, a colleague in his laboratory at Colindale, who was awarded an Honorary Doctorate from Leiden in recognition of his work on streptococci.

The day-to-day responsibilities of a hospital laboratory in relation to public health were exemplified by Tom's work in Carmarthen and Manchester, and are reflected in the requirement from the current Chief Medical Officer (England) that all microbiology laboratories should fulfil their public health responsibilities. In addition to aspects of hospital cross-infection that constantly required attention, there were innumerable urgent requests to identify the agents of infectious diseases and to check their antibiotic sensitivities or otherwise to guide treatment options. Tom carried all of these responsibilities with exemplary calmness and real ability.

One of Tom's greatest legacies to medical microbiology was his remarkable service as an editor of, and a significant contributor to, our journals and to Topley and Wilson's authoritative text on the *Principles of Bacteriology and Immunity*, which latterly became *Microbiology and Microbial Infections*. He worked tirelessly and with daunting commitment in these roles. For the 8th edition of the textbook in 1990, for which he and Leslie Collier were the two General Editors, he personally checked each of more than 2,600 pages of text produced by 125 authors from around the world. He worked on five editions of this book (our 'professional bible' over the years) from 1964 to 1998. Those of us who knew Tom's standards in relation to his editorial work, with the *Journal of Pathology and Bacteriology* and subsequently (for nearly 20 years), with the *Journal of Medical Microbiology*, found him a demanding and impressive colleague. We admired him hugely and it was quite impossible to thank him as he waved away our expressions of gratitude with a rare smile and a slight flourish of his pipe. He was instrumental in setting the standard for scientific rigour and accuracy of expression in the journal. He expected those standards of his colleagues, but was also prepared to spend endless time guiding would-be editors as well as helping innumerable authors (especially those for whom English was not their native language) to re-work their submitted articles into worthy scientific presentations. He created the rehabilitation part of his role as rejection and rehabilitation editor of the *Journal of Medical Microbiology* as an important teaching commitment and many authors benefited from 'distance learning tutorials' over the re-working of their papers – a hard act to follow.

Tom and his wife Beryl were married in October 1938. Their son David was born in 1940 and their daughter Judith in 1950. Tom's wider interests were reading, gardening, classical music and opera. He was, however, a dedicated family man and always found time, between all of the other activities for hill-walking with the family, for beach days with sandcastles and fossil-hunting, and for picking blackberries or visiting stately homes.

After moving to Beckenham in 1987, he started on yet another garden and took up serious studies of Italian and Philosophy at Birkbeck College, only withdrawing from Philosophy classes when, in his late eighties, he felt that his essays were no longer up to standard. Beryl died in 1996 and, latterly, Tom moved to Sunrise at Frognal House, Sidcup, where he made new friendships and found an alpine garden needing his attention. He had an extended illness with surgery in December 2004, but he returned to independent living in Sunrise with his beloved books, his paintings, his music and his view of the garden that he had made. He died peacefully in Queen Mary's Hospital, Sidcup. At the funeral, his son, Professor David Parker, talked warmly of his father as a marvellous inspiration and mentor. This is abundantly true for everyone who met Tom or who worked with such a remarkable man.

J. Gerald Collee
Edinburgh

Brian I. Duerden
Department of Health, London

Androulla Efstratiou
Health Protection Agency, London

Professor J.R. Quayle FRS (18.11.**1926**–26.2.**2006**)

John Rodney (Rod) Quayle was born and grew up in Mold, North Wales. Following his graduation in Chemistry from University College of North Wales, Bangor in 1946 he did a PhD with Professor E.D. Hughes, FRS in physical organic chemistry. His obvious talents were recognized with a senior research award from the Department of Scientific and Industrial Research and by Professor A.R. (later Lord) Todd who picked him to study the chemistry of blood pigments in Cambridge where he, unusually, took a second PhD in 1951. It was his research on photosynthesis with Professor Melvin Calvin at Berkeley that ignited his career in microbial C1 metabolism.

It was with Calvin that he published the classic paper on the carboxylation of ribulose bisphosphate to phosphoglycerate in cell extracts of *Chlorella* in 1954. Since then he had been recognized universally as being the godfather of the subject who had tutored and inspired many of us with his knowledge and insight which was far broader than carbon metabolism. This lasted right up to the late 1980s when his appointment as Vice-Chancellor of the University of Bath in 1983 somewhat curtailed his active involvement in the subject, although he did Chair the British National Committee for Microbiology (1985–1990). Nevertheless, it did not diminish his interest and role as being adviser, confidante and unraveller of some of the more complex issues of C1 metabolism. Indeed he was in demand as a plenary speaker long after his move to Bath.

Rod returned from Calvin's lab in 1955 with a brief foray into pyrethrum insecticides at the Tropical Products Institute in London moving swiftly to Sir Hans Krebs' laboratory in 1956 to continue his passion for the metabolism of C1 and C2 compounds when he collaborated with Hans (now Professor Sir) Kornberg and showed that bacterial growth on acetate involved the glyoxylate cycle. He used his experience in photosynthesis from Calvin's lab with labelled compounds to set out evaluating the metabolism of methanol, formate and carbon dioxide in bacteria. His work led to the discovery of the serine pathway and, from studies with methane-oxidizing bacteria, the ribulose monophosphate cycle that paved the way for the discovery of a variety of cycles and pathways in C1-utilizing bacteria and yeasts. He also had a significant role to play in the elucidation of a cyclic pathway of formaldehyde oxidation (the prevailing routes were linear), the dihydroxyacetone pathway of formaldehyde incorporation in yeast and the identification that the oxidation of methane to methanol in methanotrophs proceeded via a monooxygenase using dioxygen rather than water as the source of oxygen in the methanol.

Much of this work was done during his tenure as senior lecturer (1963–1965) and then Professor (1965–1983) at Sheffield University. Rod's pioneering work was recognized by his election as a Fellow of the Royal Society and the award of the CIBA Medal and Prize of the Biochemical Society, both in 1978. He served as President of the SGM from 1990 to 1993 and was awarded honorary doctorates from the Universities of Göttingen (1989), Bath (1992) and Sheffield (1992).

But most of all Rod will be remembered by most who knew him as the voice of reason, a serious intellect, generous in his advice and help in bringing a compassionate and sympathetic understanding of anyone's problems, be they be personal or scientific. His valedictory lecture at the 1995 symposium on microbial growth on C1 compounds in San Diego was typical of the man, in which he highlighted all the achievements since the first symposium 22 years earlier in Edinburgh and played scant attention to his own discoveries, even though these had influenced nearly every facet of C1 metabolism for over 30 years. In retirement he kept a strong interest in local area health authority matters and was a member of the Board of the Bath Festivals Trust, the Bristol Exploratory and member of the Council of the Bath Institute of Medical Engineering.

He is survived by his wife Yvonne and children (Susan and Rupert).

Howard Dalton, DEFRA

comment

Open option for SGM journals

The Wellcome Trust has joined other bodies in imposing open access conditions on authors of journal papers reporting research they have funded. SGM has had to develop a strategy to combat this threat to the Society's publishing income, as **Ron Fraser** and **Robin Dunford** explain.

The Wellcome Trust has notified its grant-holders that they must deposit articles arising from work the Trust has funded in PubMed Central and make them freely accessible within 6 months of publication. The policy is already operative for all grants awarded since 1 October 2005, and applies to work from earlier grants from 1 October 2006. The SGM journals on HighWire have access-controlled embargo periods of 12 or 24 months after publication, and these are seen as important for the maintenance of library subscription income. Clearly the Trust's proposals, as they stand, could lead to a potential loss of income, and put our Wellcome Trust-funded authors in a difficult position in reconciling the Trust's requirements with their copyright agreement with SGM. In 2005, the proportion of papers acknowledging Trust funding were 3.5 % for *Microbiology*, 1.1 % for *Journal of Medical Microbiology*, 5.9 % for *Journal of General Virology*, and 0 % for *International Journal of Systematic and Evolutionary Microbiology*.

The Trust has indicated its willingness to pay publication charges for authors, in return for enhanced online access to their papers, and has already reached agreement with Blackwell Publishing, Oxford University Press and Springer, amongst others. The Trust requires that, where a publication charge is paid, the article must be made open access at the time of publication.

SGM Treasurer's Committee has investigated the financial consequences of various 'what if' scenarios, such as whether librarians will cancel subscriptions as increasing amounts of content become freely available at the time of publication. These matters and others related to the scientific objectives of the journals have been discussed with the Editors-in-Chief and Editorial Boards. A recommendation from Treasurer's Committee, that authors should be offered optional open access at the time of publication, for a fee of £1,500 per article plus VAT where applicable, was approved by SGM Council at its meeting on 5 May. This fee is broadly in line with those being charged by many other publishers, especially after allowing for the fact that SGM journals do not have page charges or charges for colour figures. The Wellcome Trust has already provided publication funds to its 'top 30' universities, and authors can also apply directly to the Trust. Funded articles will be made open access at the time of publication on the SGM journal sites at HighWire, and authors of such articles will be permitted to post the published version on PubMed Central, again at the time of publication. Of course, there is nothing to stop authors with other sources of funding from choosing to go down the immediate open access route with us too.

At the time of writing, we still await final policy statements on open access publishing for work funded by Research Councils UK, the US National Institutes of Health and the European Union, amongst others. These may or may not pose threats to our journal business model, and further elaboration of the author-side payment model may be necessary. The objective will be to maintain the flow of high-quality submissions, while helping authors comply with their funding agencies' requirements, but maintaining the financial stability of the journals. This is important for Society members and for the science of microbiology, as it is largely the income from journal publishing that enables SGM to support its scientific meetings, numerous grant schemes, and educational and outreach activities.

Ron Fraser
Executive Secretary

Robin Dunford
Journals Manager