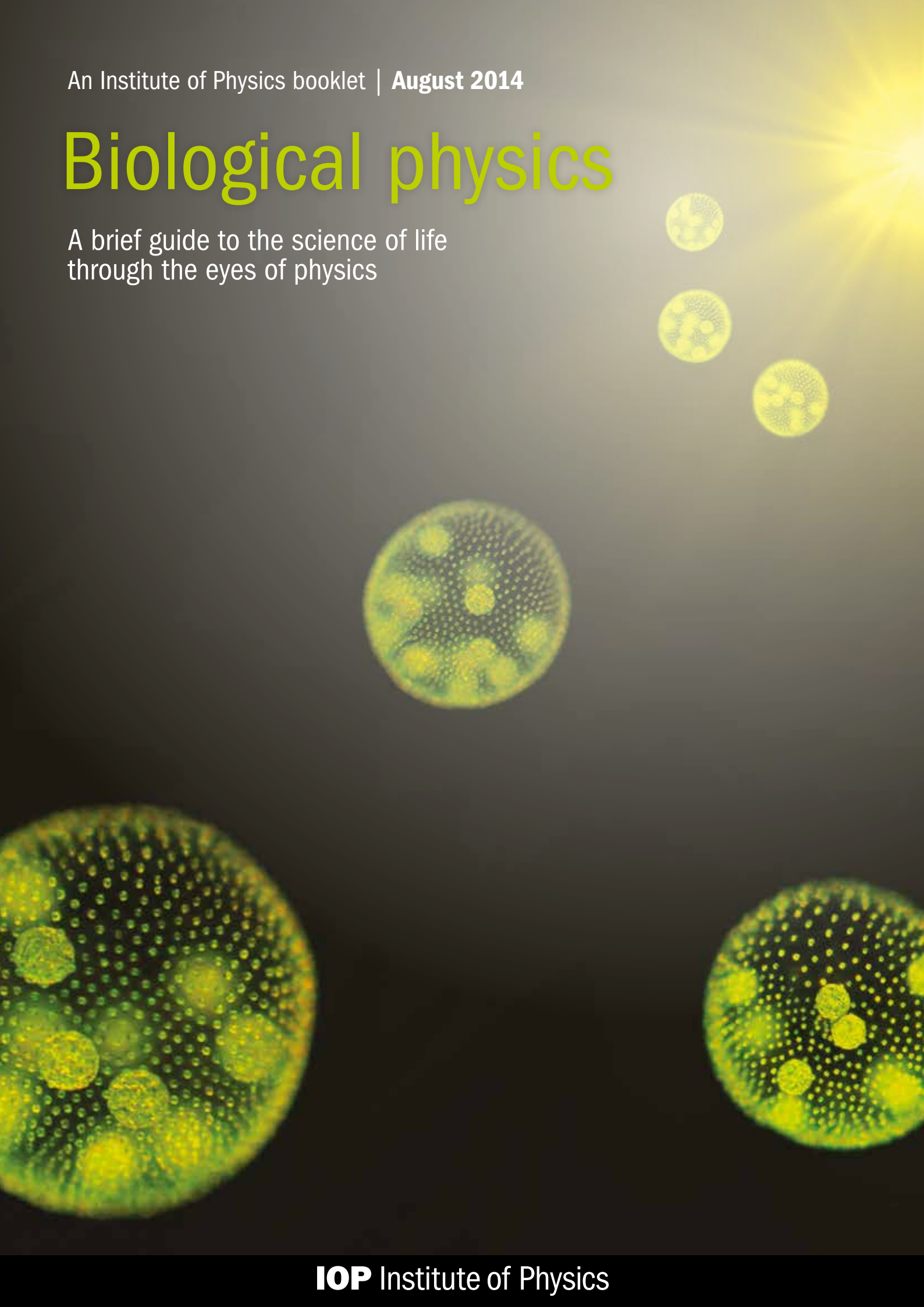
An Institute of Physics booklet | **August 2014**

Biological physics

A brief guide to the science of life through the eyes of physics



IOP Institute of Physics

Front cover image:
Colonies of the phototactic green alga *Volvox carteri* (*Knut Drescher and Ray Goldstein/ University of Cambridge*)

Contents

Foreword

One of the most important characteristics of physics as an intellectual endeavour is that it seeks out meaningful descriptions of the world around us, and the Universe beyond, which can be applied in a context that is as global as possible. The resulting concepts, which are expressed in the language of physics – mathematics – encompass the structure and behaviour of matter and forces at all scales, from the interactions of fundamental particles in atoms to the organisation of galaxies across the Cosmos.

Uncovering these broad unifying principles also provides powerful tools for improving our lives through the new technologies subsequently developed. Fields such as condensed-matter physics (e.g. semiconductors, magnetism, and superconductivity) and optics (lasers, fibre optics, and optoelectronics) have transformed many aspects of our daily lives – from computers and smartphones to supermarket self-checkouts and laser eye-surgery.

Traditionally, the goal of physics was largely seen as reducing our descriptions of nature to the simplest possible. Today, however, physics is increasingly helping us to understand how the complexity of our world is built up. Many everyday phenomena involve a multitude of complex interactions, which can now be investigated by combining universal concepts uncovered by physics with the numerical facility offered by computers.

Nowhere is this becoming ever more apparent than in the field of study known as biological physics. The living systems that have evolved on Earth, such as humans, represent the pinnacle of complexity – at least as far as we know. Yet, we are all made of the same basic building blocks governed by the same basic forces as the stars and galaxies in the sky. A growing number of physicists are now collaborating with biologists, with the aim of providing equally important insights into living processes as they do into the evolution of a star like the Sun, the behaviour of the Higgs boson, or materials that can conduct electricity with no resistance.

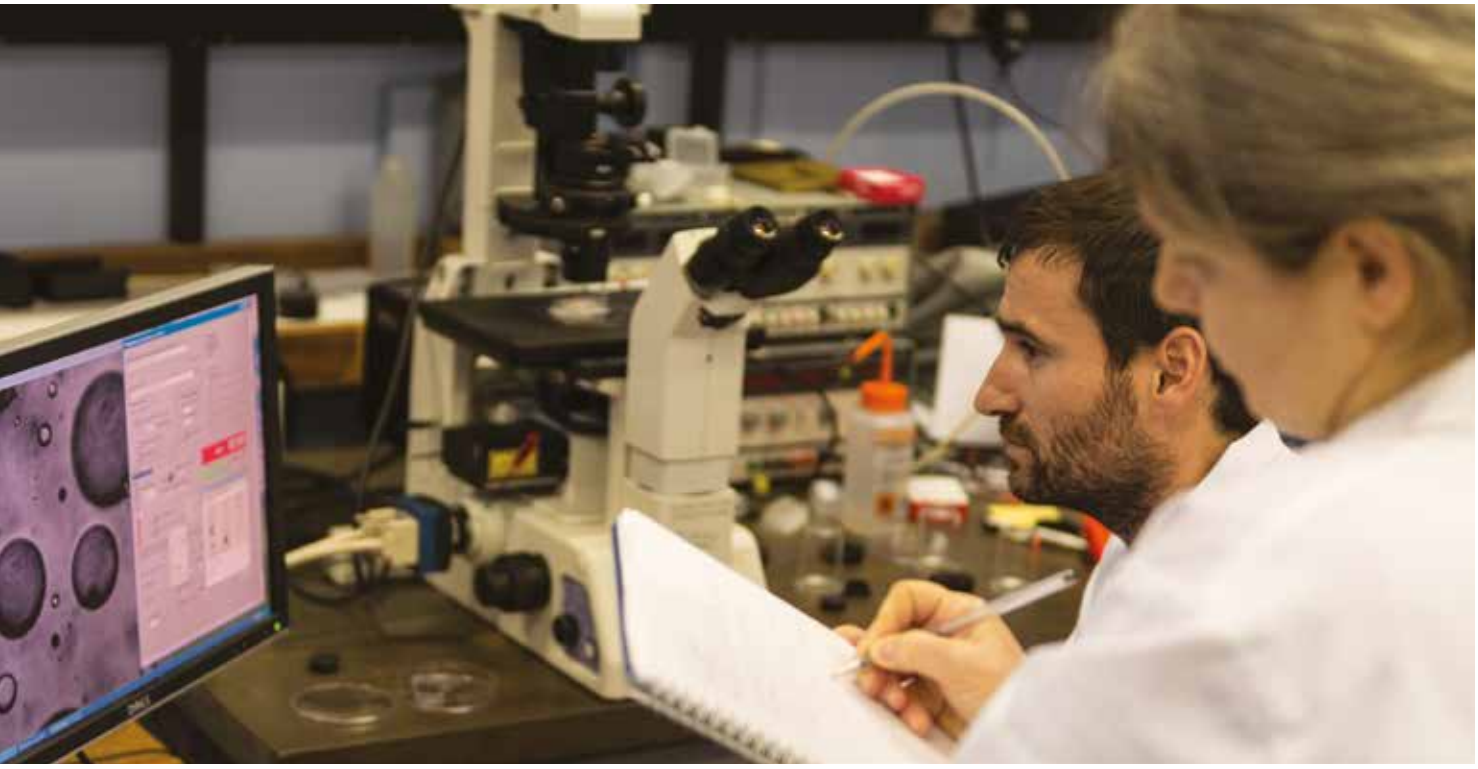
Using well-honed theories underpinning complex behaviour, and developing experimental techniques based in physics, researchers are trying to find useful quantitative descriptions of how living cells, tissues, organs and whole organisms work, which will complement and strengthen the more reductionist approaches currently employed in life-science research – molecular biology, cell biology, genetics and so on. In this way, physicists can uniquely contribute to a better understanding of disease and searches for new medicines, as well as revealing, with more predictive clarity, larger-scale, evolving scenarios such as environmental and ecological change. Biological physics is also helping to underpin the burgeoning subject of synthetic biology – the design of new biologically-inspired devices and systems.

The Institute of Physics has recognised the importance of this exciting new field in establishing www.biologicalphysics.iop.org to help students with an interest in biological physics. Together with the Society of Biology, we also support an active biological physics subject group. Members of the group have helped to put together this booklet that provides a range of examples of the work carried out by biological physicists based in the UK. We hope that these examples show the potential of this field and what it has to offer in terms of providing skills and a stronger knowledge-based platform on which to further biological research, and its applications in the areas of health and the environment.

Professor Paul Hardaker
Chief Executive
The Institute of Physics

Introduction

Biological physics is a new synthesis of physical disciplines, providing fundamental insights into the processes of life



Ray Goldstein
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Of the many revolutions in biology – from Antonie van Leeuwenhoek's discoveries of microorganisms in the 17th century to the elucidation of the genetic code in the last century – the most transformative may yet turn out to be the tremendous expansion of research that is now taking place at the interface between the physical sciences and biology.

There are, indeed, great historical precedents for the impact of physical sciences approaches on biology. One example is the study of electrophysiology, which measures the electrical activities of cells and tissues, and has contributed greatly to an understanding of their behaviour; another is the ground-breaking (and now routine) use of X-ray diffraction to determine the structures of significant functional biological molecules such as DNA and proteins. However, what we are currently witnessing is something altogether different: the wholesale movement of a significant community of physicists, chemists, mathematicians and engineers into a relatively new area known as 'biological physics'.

Evidence of this revolution is apparent across both basic and applied research, from the wave of hiring staff in universities, to the creation of new departments and institutes, and the establishment of academic journals dedicated to this area. At the same time, industry is awakening to the potential that this area offers to innovation, from single-cell sequencing, super-resolution microscopy (p13), and live-cell manipulation technologies to medical diagnostics and the design of novel therapeutic strategies, with an attendant and urgent demand for scientists trained at the PhD level.

A central role for physics

Biological physics lies at the intersection of many disciplines. The reason is because the processes of life are vastly complex, involving many kinds of interactions over a very wide range of length, energy and time-scales. In particular, at the cellular and sub-cellular scale, biological behaviour is 'noisy' and 'stochastic' involving myriads of random motions and interactions. The outcomes are nonlinear and nonlocal – in other words, small changes at one location can result in large changes elsewhere. The traditional fields associated with physics – chemical physics, engineering, statistical physics, kinetic theory, optics, continuum mechanics, fluid dynamics,

computational science, and control theory – provide the methods and associated intellectual rigour to probe such behaviour in a quantitative and predictive way.

In this sense, biological physics shares many features with astrophysics, which in its entirety also considers complex and dynamical phenomena occurring on a wide range of length, energy and time-scales. Unlike astrophysics, though, biological physics need not rely solely on observations from afar. Indeed, it is the close connection between theory and experiment that is the hallmark of the field, to the point that many individual researchers explore both the fundamental ideas underpinning the subject as well as conducting experiments guided by the theoretical predictions.

▲ Pairs of *Volvox* alga colonies waltz around each other in a hydrodynamically-bound state. Studying the underlying physics gives clues to the emergence of multicellular organisms in evolution (*Knut Drescher and Ray Goldstein/University of Cambridge*)

Applying the physical concepts

Besides the technical, instrumental contributions that physics and physicists have historically made in the field, and which continue to be important, there is also a conceptual approach that is new to the study of living systems. The extent to which physical properties were discussed in biological textbooks and research articles in the past has often been limited to equilibrium quantities such as (free) energies, with rarely a mention of the kinds of concepts and descriptions common to the disciplines of physics – for example, forces and flows, and theoretical descriptions based on symmetries and scaling laws. It is largely through the efforts of physicists that phenomenological and fundamental approaches based on these concepts are starting to be the norm in the field. Most significantly, physicists are contributing as much to the framing of questions as they are to the search for answers.

The broader methodologies of physics are now being used to characterise processes at scales from the molecular and cellular level, through tissues, organs, whole organisms – and even ecologies and their evolution. Systems involving the dynamics of many particles, networks of interactions, and self-regulating systems with feedback, are particularly amenable to the techniques of physics.

The advantages of collaboration

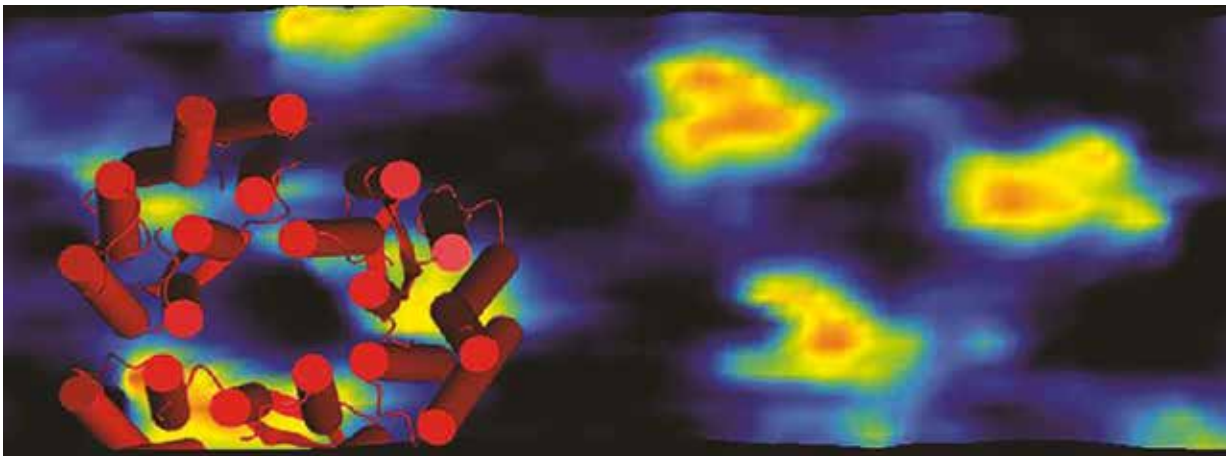
Physics in turn also benefits from the input of associated biological disciplines (biochemistry, molecular biology, genetics and so on) through the collaborative research programmes that increasingly characterise modern biology. Indeed, the rapid development of biological physics has been accompanied by a great upsurge in direct collaborations between physicists and life scientists. They work together on research, write joint papers, jointly mentor PhD students, and apply for funding as a team.

Through such collaborations, it is becoming clear that not only can physics help biology, but physics itself has much to learn from biology. For example, biological systems can provide challenging test-beds for developing fundamental theories, which can then be exploited in other fields of physics.

The following pages give us, through a wide range of case studies spanning biology at all scales, an exciting overview of the state of biological physics in the UK. It truly is thriving, with a compelling international presence and a remarkable degree of interdisciplinarity.

The nanomechanics of biomolecules

Uncovering the stresses and strains in biological nanostructures that control how they behave



▲ AFM image showing the highly dynamic physiological structure of the membrane protein, bacteriorhodopsin, contrasted with the superimposed static crystal structure *(Kislon Voïtchovsky and Sonia Contera/University of Oxford)*

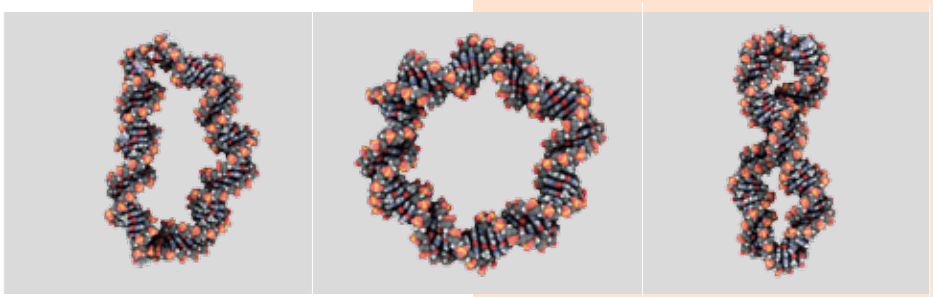
Exploring the complex interactions involving the molecules of life – DNA, RNA and proteins, and the larger assemblies they form – is essential to understand gene expression, metabolism and the cell life-cycle. Experimental and theoretical approaches based in physics enable the forces and fluctuations that shape their dynamics and functionalities to be measured. The behaviour of both single biomolecules and the broader molecular machinery of the cell can be probed.

How stiff is a protein?

Proteins provide the workforce of the cell. They are long-chain molecules that fold into a characteristic 3D shape that governs their function. The overall stiffness, elasticity and adhesive properties of the folded protein contribute to their roles in processes such as translocating other molecules across a cell membrane, or in muscle function. Lorna Dougan of the University of Leeds measures the force needed to make a protein unfold by tethering single model protein molecules onto a gold surface and attaching them to the tip of a tiny flexible cantilever of an atomic force microscope (AFM, opposite). As the AFM tip is moved away, the protein stretches out and unfolds, and the cantilever bends. Its deflection is then measured with a laser and photodetector. The forces needed to cause unfolding can be measured and, in combination with theoretical models, the protein's "energy landscape" uncovered. Dr Dougan's research group hopes to use this technology to quantify the stability of proteins from organisms evolved to cope with extreme conditions of heat, cold or salinity (extremophiles), with the hope of understanding better how life evolved in extreme environments (or could evolve on other planets) and to design new biomaterials based on artificial extremophile proteins.
Sonia Contera at the University of Oxford also investigates protein nano-mechanics by using an AFM cantilever to push and pull proteins that are bound within a cell membrane. Membrane proteins are key to molecular recognition – the signalling between cells, and also transport processes in and out of cells (p10). Not surprisingly, they are a key target for drug design. However, membrane proteins are difficult to crystallise and thus study with X-rays. Furthermore, to obtain a firm handle on their all-important dynamic behaviour, they need to be studied in their natural environment at the membrane interface. Dr Contera's team is now able to map the mechanics of protein structures embedded in cell membranes using high-speed AFM. Her aim is to provide what will be crucial information for successful drug design, nanomedicine and tissue engineering.

Wind-up DNA takes control

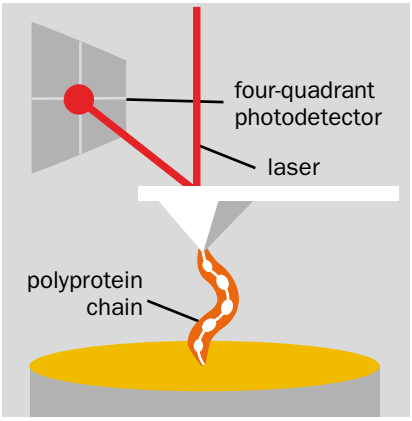
Mechanical forces are also important in storing DNA and regulating gene expression. In bacteria, the very long DNA double helix is held under torsional stress so that it forms elaborate "supercoils" like a twisted telephone cable. It can then be compacted enough to fit into a cell nucleus or a bacterial cell. In all organisms, when each DNA sequence making up a gene is transcribed into



▲ Models of circular, supercoiled DNA during gene transcription: the left shows it under-wound; the centre shows it relaxed; and the right-hand model, overwound

the complementary RNA sequence, the double helix must be unwound to allow the enzyme, RNA polymerase, to "walk" along the chain and synthesise the corresponding RNA strand. This causes the DNA to be over-wound ahead of the transcription site and under-wound behind it. The build-up of over-winding eventually prevents further transcription until enzymes relieve the stress by cutting and reforming the double helix.

However, it now seems likely that changes in the DNA supercoiling itself alter levels of transcription by influencing the binding of the proteins involved. Until recently, this hypothesis could not be tested because experiments investigating the structures of protein–DNA complexes were carried out only on linear DNA segments. Sarah Harris and colleagues at the University of Leeds, in collaboration with biological researchers at the John Innes Centre in Norwich, have taken up the challenge by studying protein recognition in samples of circular DNA, which is supercoiled, and comparing laboratory results with model calculations based on classical thermodynamics. They believe that DNA actively participates in regulating gene expression.



◀ The force needed to unfold the 3D structure of a protein can be measured by attaching it to an AFM needle

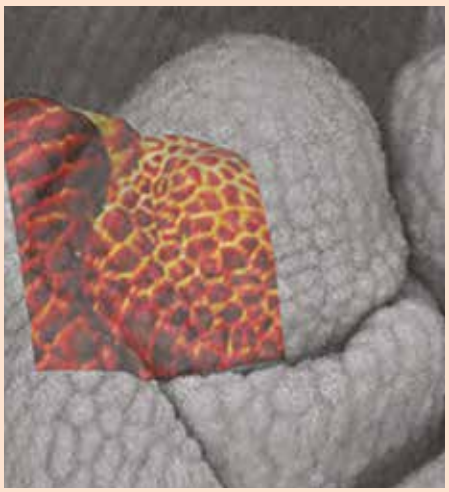
Atomic force microscopy

Applications of research

- Ecology
- Astrobiology
- Biomaterials
- Antimicrobials
- Agriculture
- Biofuels
- Drug design
- Nanomedicine
- Tissue engineering

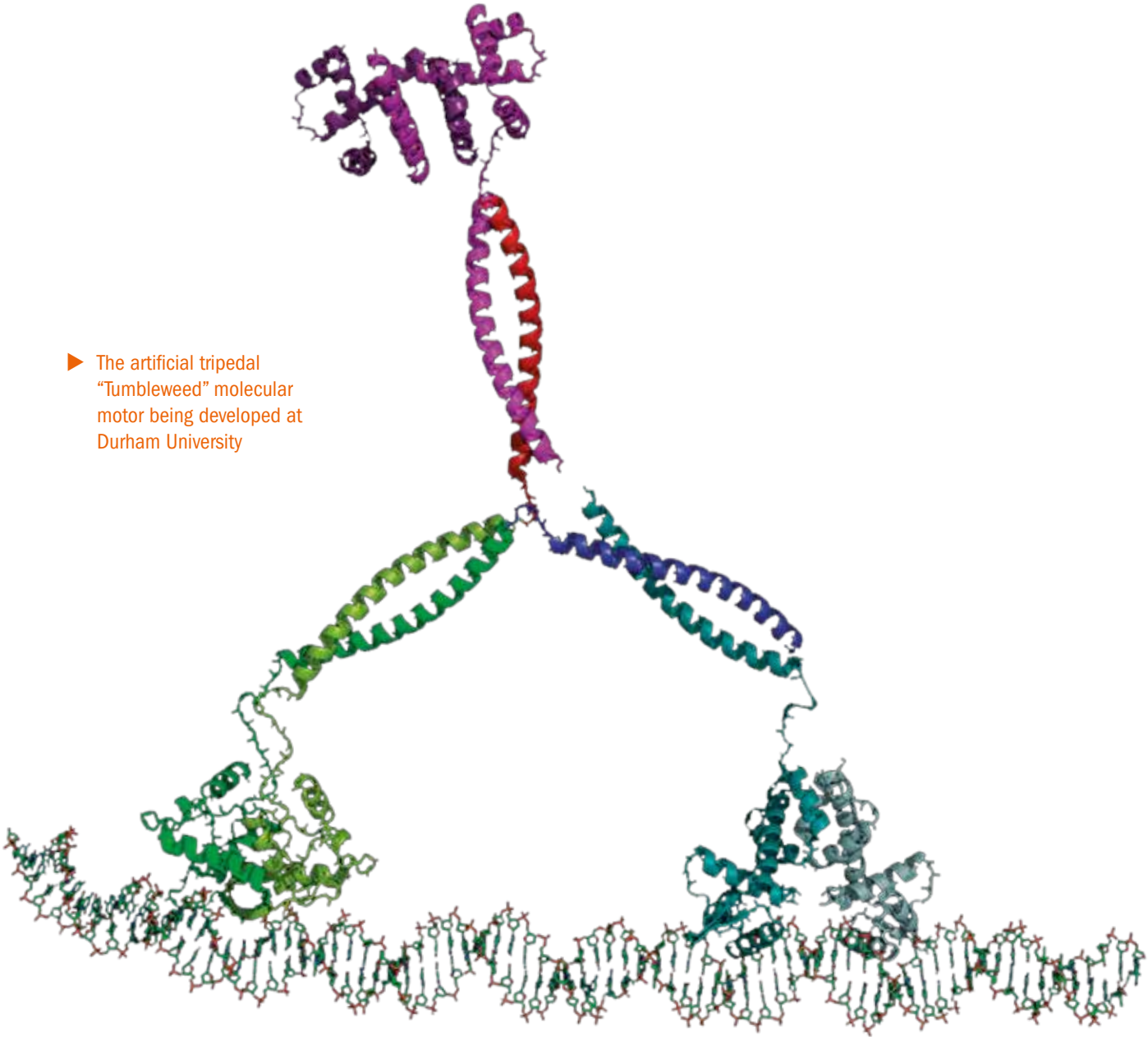
The AFM, in which the sharp tip on the end of a flexible cantilever is used to create a nanometre-scale image by "feeling" across the surface of a sample, can also be used to measure the mechanical properties of molecules and tissues, in particular stiffness and stickiness.

▼ An electron micrograph (grey part) of a developing tassel flower in maize, overlaid with an AFM 'stiffness' map (red–yellow) showing the elasticity for the various cells. The actively elongating lower part is more elastic than the centre part of the flower



Nanomechanical tester
Siobhan Braybrook in the Sainsbury Laboratory at the University of Cambridge uses the AFM as a 'nanomechanical tester' to probe how plant cells grow and produce characteristic plant shapes and branching patterns. She has measured the changes in elasticity in cells in the growing tip of a plant, relating them to chemical modification in the structural polysaccharide pectin found in cell walls. Such studies have considerable significance in developing more robust crops and biofuels.

Combination techniques
Jamie Hobbs at the University of Sheffield has been combining AFM imaging and force measurements with super-resolution fluorescence microscopy (p13) to map the architecture of bacterial cell walls, which are primarily constructed from a giant, interconnected molecule known as peptidoglycan. How the peptidoglycan is organised influences cell growth and controls cell shape, and, as it is not found in humans, is the target of many common antibiotics. Using an AFM, he can follow the structural changes that happen during the growth of living bacteria and their attack by antibiotics, helping the future development of new antimicrobials.



▶ The artificial tripedal "Tumbleweed" molecular motor being developed at Durham University

THE MOLECULES OF LIFE AT WORK 2

Molecular motors

Specialised molecules provide the cell's mechanical power

The living cell is like a factory; products are made in specific locations and then have to be moved around. Although transport can occur simply through random motion, if the distances are more than a few micrometres, then motorised assistance becomes essential. This is provided by "molecular motors", which consume chemical fuel (provided by the energy-rich molecule adenosine triphosphate, ATP) and convert it into mechanical energy.

Nano-sized hauliers

Rob Cross at the University of Warwick heads a team that has been studying a family of molecular robots called kinesins, which haul molecular cargo between the centre of the cell and its periphery, by walking in a stepping motion along a dense network of protein filament tracks called microtubules (p12). By isolating a single kinesin molecule and attaching it to a transparent plastic bead that is trapped by a laser beam, the force the molecule exerts as it tries to walk out of the beam can be measured. In this way, a mechanical fingerprint of the molecule is obtained. In cells, teams of multiple kinesins work continually to organise the track network and deliver molecular cargos to their correct destinations. The Warwick research group is trying to unpick the traffic dynamics of the whole system, which is in constant flux and involves complex

self-organising interactions between multiple motor molecules. This requires computer simulations of the dynamics combined with experiments on kinesins that have been re-engineered to change their behaviour.

Nano cogs and wheels

A fascinating rotating molecular motor is to be found propelling the whip-like protrusions of swimming bacteria. It consists of a series of concentric protein rings around a central protruding corkscrew filament, which rotate. A separate, outer series of rings anchored to the cell wall creates the torque, driven by an electromotive force generated by the flow of ions. Richard Berry at the University of Oxford has been analysing the mechanism and measuring the torque generated. Employing a genetically engineered system in which the rotation was slowed down, he could see the individual steps of the process using single-molecule methods such as tracking with fluorescent beads under a microscope or using optical trapping (opposite).

Synthesising soft machines

These experiments demonstrate that molecular motors and the substrates to which they bind are in constant flux, with the molecular components continually falling out and being replaced. Nevertheless, researchers are attempting to create molecular-sized engines that mimic biological systems. Beth Bromley at Durham University has been developing a concept for an artificial tripodal protein motor called the Tumbleweed that can roll along a DNA track, with the aim of understanding the principles of developing such designs.

DNA is a strong candidate as a building block for designing synthetic nano-sized machinery, which is what Andrew Turberfield at Oxford is exploring. DNA is relatively easy to synthesise and readily programmable because the four base pairs of DNA bind to each other in predictable ways. DNA strands can be joined to form 3D structures, creating motors, tracks, and fuels. DNA nanostructures can even be used to control protein motors like kinesin to create a molecular shuttle. Artificial DNA devices also have the potential to process information.

Optical trapping

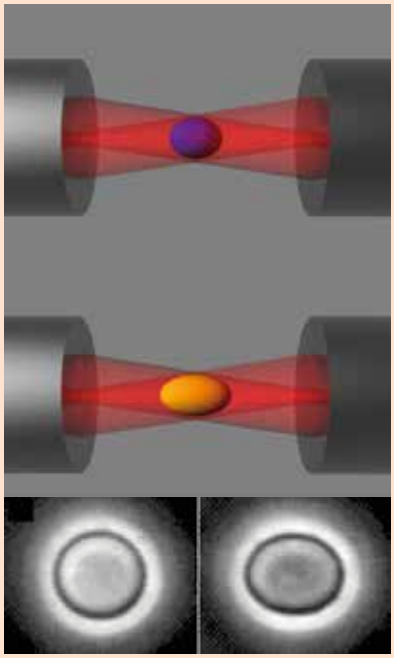
Light pressure exerted by focused laser beams can be used to measure the forces generated by single molecules and aspects of cell mechanics.

The optical stretcher

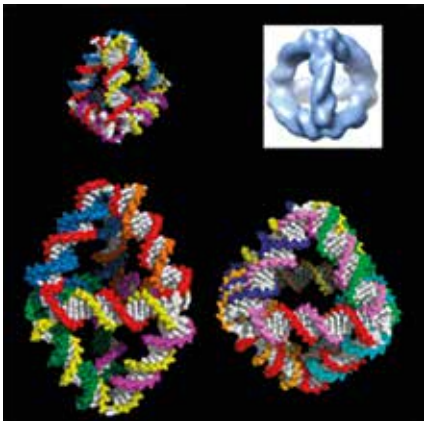
Jochen Guck, working in several laboratories including the Cavendish Laboratory at the University of Cambridge, has developed optical trapping into an automated high-throughput process to measure the stiffness or pliability of cells. Two laser beams trap cells suspended in a flowing liquid and then deform them. He has found that cancer cells taken from a patient can be identified and sorted according to their softness. Dr Guck is now applying the technology to the study of the mechanical properties of a variety of tissues, with the aim of characterising the changes associated with, for example, blood disorders, nerve regeneration and scarring.

Optical trapping microscopy

Kishan Dholakia and his group at the University of St Andrews have been applying optical trapping to whole cells, in particular blood and stem cells – the cells that then differentiate into specialised cells. Using an "optical lattice" created with laser beams, living cells can be arranged in arrays and even sorted. In this way, it is possible to study how individual stem cells are influenced by the cells around them, thus providing clues about the process of tissue differentiation. Further work with colleague, Frank Gunn-Moore, has explored the use of pulsed laser light to create transient nano-pores in various cells to allow delivery of therapeutic agents. The laser creates a pore that heals with no long-term cell damage but survives long enough for material to enter the cell interior.



▲ A cell caught in an optical laser trap can be stretched to measure its softness



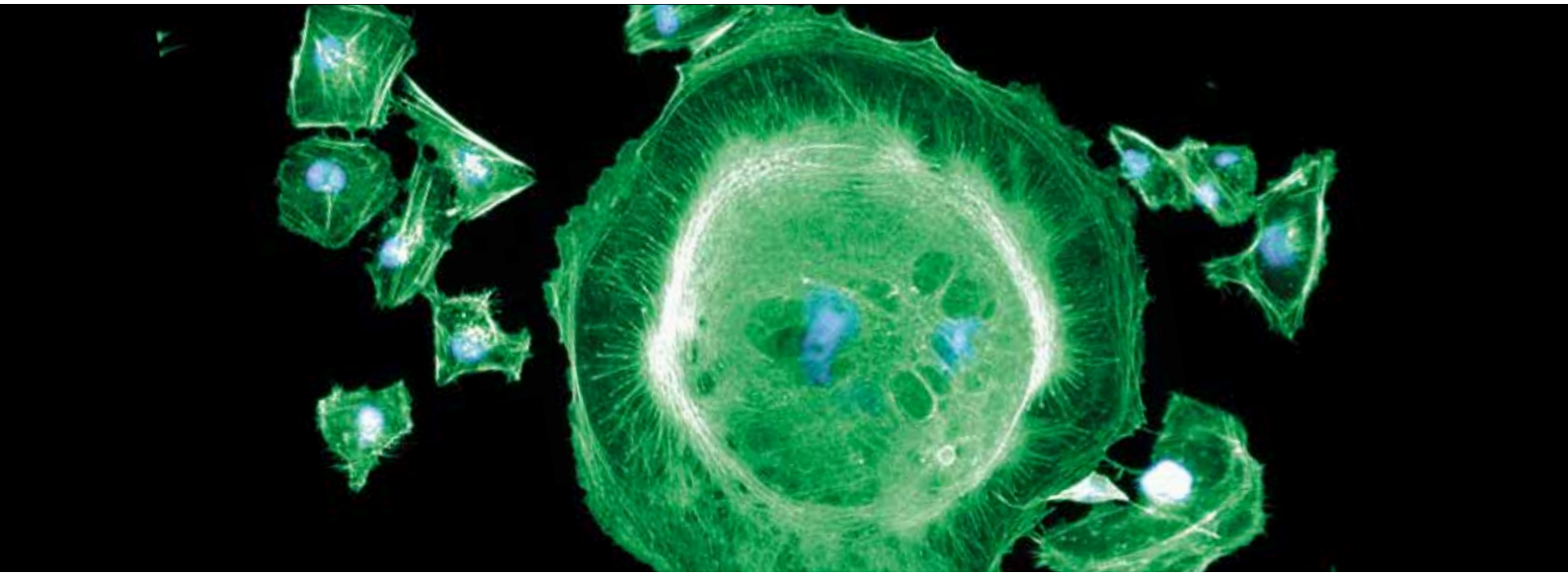
▲ Artificial DNA structures can be used to make nano-machines and even computing devices

Applications of research

- Genetics
- Diagnosis of disease
- Tissue repair and nerve regeneration
- Drug design and delivery
- Synthetic biology
- DNA computers
- Nanocomposites and nanodevices

The life and times of a cellular citadel

Physics can explore how the two major molecular frameworks of the cell – the cell membrane and the cytoskeleton – behave mechanically and dynamically, and so control the key processes of life



▲ HeLa cells, an "immortal" cell line used in biological research

The living cell is like a vast self-organising urban community that is maintained by the input and generation of energy and materials. Its complex and fluid structure contains thousands of different types of molecules organised into subcellular components whose behaviour is driven by weak molecular and mechanical forces. For example, phospholipids – molecules with a phosphate head and hydrocarbon tail – assemble into 2D bilayers that form the outer thin membrane of the cell, as well as providing the compartmental framework for specific functional structures (organelles) such as the nucleus. In eukaryotic cells, these structures are anchored by the protein-based cytoskeleton, which not only gives the cell its shape and mechanical strength but also acts as a transport highway (p12).

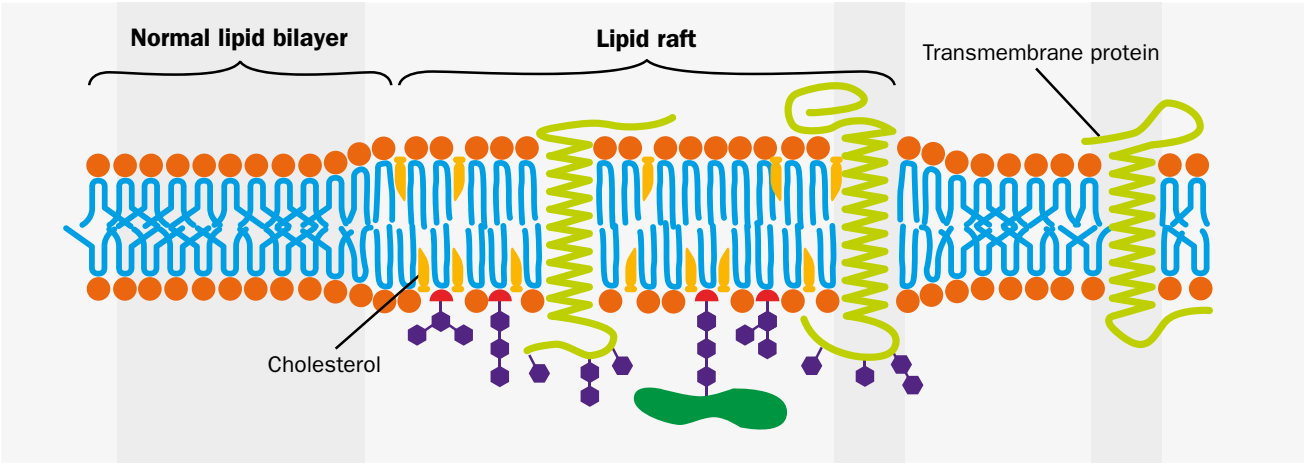
Cell membranes as gate-keepers

Cell membranes are composed of about 300 different kinds of lipids in which are embedded hundreds of proteins. This highly dynamic 2D molecular association serves as a selective barrier between the outside and inside of the cell. Chemical and electrical gradients across the membrane actively regulate which molecules or ions can pass through. These include nutrients, molecules or ions involved in signalling, and inevitably pathogens. Because the membrane is very flexible, the forces controlling its curvature play a crucial role in cell division and cell mobility.

Membrane lipid rafts

During the past decade, researchers have realised that membranes show specific localised behaviour in their fluid makeup. They appear to host tiny, gel-like regions, nanometres across and enriched in specific lipids. These so-called membrane rafts are not well understood, but seem to be associated with the engulfing and secretion (endo- and exocytosis) of materials and cell-signalling reactions. They appear to recruit particular membrane proteins, but how the rafts form and tether the proteins is still unknown, which is being investigated both theoretically and experimentally.

Using the methods of statistical thermodynamics, Matthew Turner at the University of Warwick has been modelling the role of the rafts in the endo- and exocytosis cycle, as they form and break up due to the random fluctuating forces generated by cytoskeleton filaments. The filaments are attached to the membrane, and their pushing and pulling alter the local curvature of the membrane so as to induce mixing and de-mixing of the lipid composition.



▲ A lipid raft sitting in a cell membrane is an active micro-domain of lipids and proteins

Membrane interactions

The changes in the distribution of regions of intense electrical fields in the membrane are also key to understanding how molecules bind to the membrane and penetrate its structure. Paul O'Shea at the University of Nottingham is part of a multidisciplinary team including Imperial College London and the Universities of Cambridge, Durham and Leeds, which is studying the cell membrane. The researchers have developed technologies for visualising how molecules interact with the cell surface. Fluorescent molecules, which are sensitive to changes in electric charge, are attached to the phospholipids in the membrane. When molecules such as proteins or drugs bind to the membrane, they alter its electrostatic potential, which in turn affects the amount of fluorescence observed with a microscope (p13). The technique is sensitive enough to pick out tiny changes in electronic distribution within the rafts and thus provide information about changes in composition.

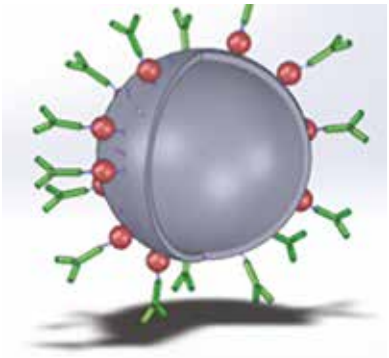
The ease with which incoming molecules bind to the membrane can be measured, and this is important in understanding infection and disease processes. Lipid rafts are thought to be targeted by the HIV virus. Images show viruses binding to these micro-domains rather than the more fluid parts of the membrane. There is also evidence that the rafts attract the small prion proteins associated with Alzheimer's disease. Professor O'Shea is also developing a new method of screening drug activity by creating layers of artificial membranes that mimic a drug's route through the human body. This research has the potential to reduce the need for testing in animals.

Membrane's traffic controllers

Other important components of the membrane are the many proteins that straddle the lipid bilayer. They act as transducers, relaying signals and materials between the inside and the outside of the cell. Membrane-bound proteins are one of the main targets for developing new drugs. However, they are difficult to study because they lose their all-important 3D shape when separated from the membrane, and are extremely hard to crystallise for classical structure determination with X-ray diffraction.

Steve Evans' research group at the University of Leeds has been preparing protein crystals sitting in their membranes tethered on a piece of glass. The challenge is to create an artificial system that mimics the natural one as closely as possible. These artificial bio-membranes also have potential as diagnostic sensors with a sensitivity and selectivity that could not be obtained with a current alternative technology, based on chemically treated silicon chips.

Artificial lipid vesicles are also potential vehicles for imaging and drug delivery. Professor Evans is developing lipid-coated gas micro-bubbles, which show up strongly in ultrasound scans, to target tumours. Attaching drug molecules or personalised antibodies to the micro-bubbles could provide the next generation of

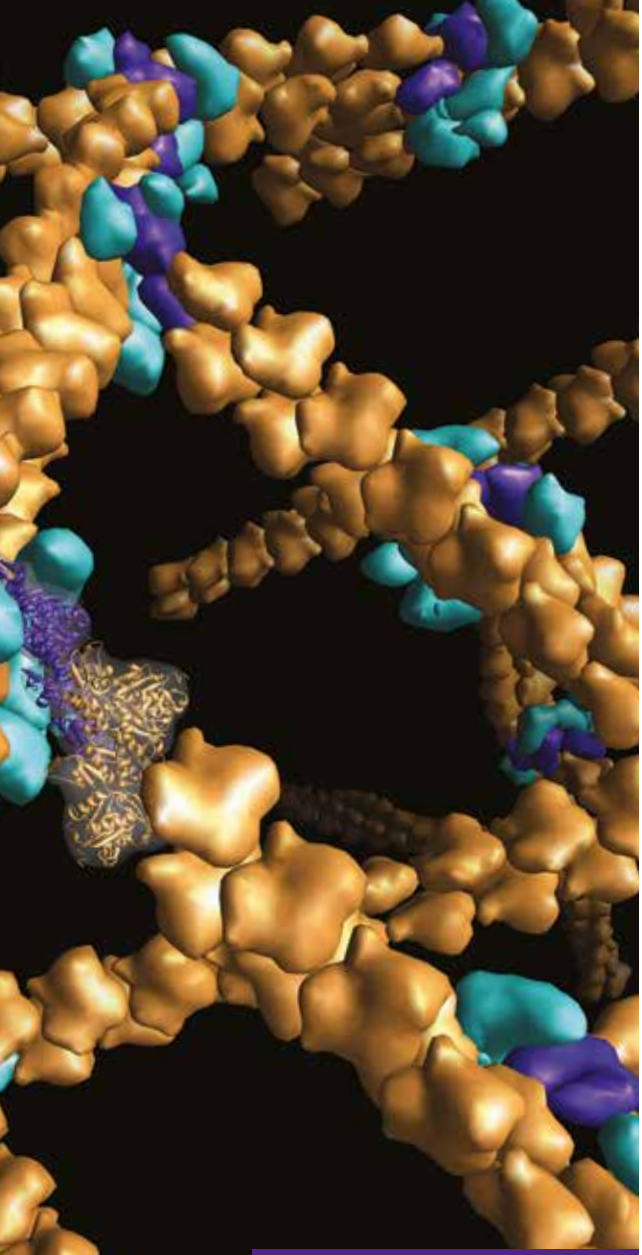


▲ Lipid-coated gas micro-bubbles can be used as drug-delivery agents

safe chemotherapeutic agents. Once the micro-bubbles have reached the tumour, a low-frequency, high-ultrasound pulse will be used to burst the bubbles and release the drug. Such an approach will help to minimise the adverse side-effects associated with many chemotherapy agents, since the drugs will be released only at the tumour site, allowing much smaller doses to be used.

Applications of research

- Drug design (Alzheimer's disease, HIV)
- Screening drug activity in the body
- Chemotherapy
- Drug delivery
- Diagnostic sensors



The cellular scaffolding

The cytoskeleton provides the cell with a dynamic structural framework and shape, and is key to transport, mobility and cell division

Eukaryotic cells are filled with three types of protein filaments – actin filaments, microtubules and intermediate filaments – in the form of an elaborate mesh. This is the cytoskeleton. It interacts with cell membranes, providing the scaffold that controls the shape of the cell, its mechanical strength and ability to move; it also plays the central part in transporting materials and organelles, and in cell division and differentiation. Like the cell membrane, the cytoskeleton is a dynamic structure in which the basic building blocks are constantly being unassembled and re-assembled as required to control cell adhesion and mobility.

The actin scaffold

Actin filaments, which are continually formed and re-formed from actin subunits, control many dynamic processes such as muscle contraction and cell motility. Close interactions with the cell membrane allow the actin scaffold to help cells move and adapt their responses to exterior and interior changes. Understanding its physical properties is an important target in tackling a number of diseases including cancer and coeliac disease. The latter is characterised by a reduction in size of the tiny protrusions of bundles of actin called microvilli from the cells lining the gut, which inhibits the absorption of nutrients. Using AFM (p7) and fluorescence microscopy (p13), Steve Evans' group at Leeds is aiming to prepare simple synthetic cells built from a lipid membrane containing actin scaffolds to investigate how microvilli form and are affected by the behaviour of domains and proteins in the cell membrane (p11) that associate with them.

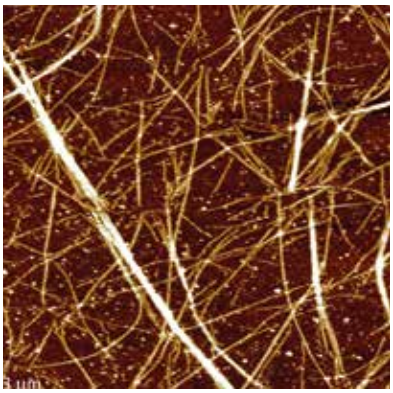
Microtubules divide and organise

Thomas Surrey at the London Research Institute is studying the dynamical organisation of another cytoskeletal component, microtubules. These are larger cylindrical structures built from tubulins. Like actin, they do many jobs, organising cell layout and generating the forces needed for swimming – in the case of sperm and some single-cell organisms, for example. They also rapidly form complex assemblies that control the separation of chromosomes in cell division.

Dr Surrey is building a simple cytoskeleton built of microtubules and motor proteins (p8). Using a theoretical model developed by colleagues that predicts how the components interact, his aim is to understand at a quantitative level the factors affecting the organisation of the cytoskeleton and the dividing process. Matthew Turner at Warwick is also modelling how cell division is regulated by the forces developed through microtubule dynamics.

Applications of research

- Synthetic biology
- Antiviral drugs
- Animal-free testing of drugs
- Drug delivery
- Treatment of cancer and coeliac disease



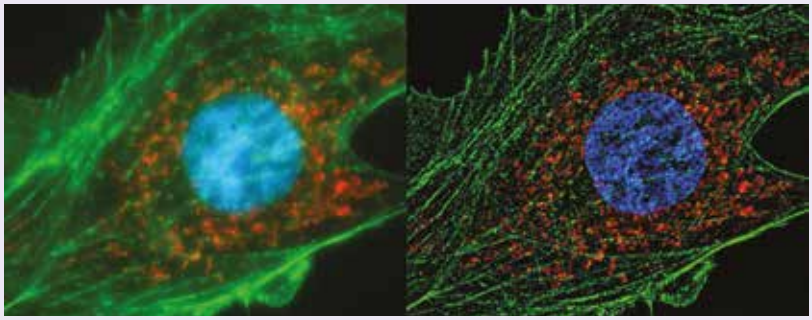
◀ An actin cytoskeleton created at a model lipid membrane and imaged using AFM. The white dots are single proteins that assemble to form the helical filaments just 7 nm wide. These filaments can be cross-linked or bundled to control the mechanical properties of the scaffold *(Steve Evans and George Heath/University of Leeds)*

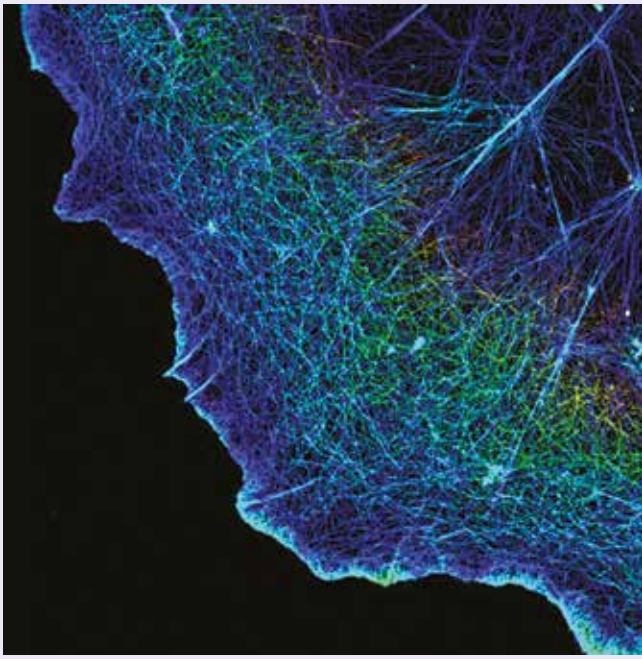
ADVANCED MICROSCOPY 1

Molecules light up under the microscope

Physicists have developed novel optical microscopies that allow cellular nanostructures and even individual biomolecules to be imaged

Much groundbreaking experimental work in molecular and cell biology has depended on the development of measurement techniques, including novel optical microscopy. Resolution in conventional microscopy is limited by the wavelength of visible light (called the diffraction limit) resulting in the blurring of detail below this scale-length. Molecular dimensions and intermolecular distances are much smaller (typically less than 10 nm); nevertheless, techniques have been developed to overcome this limitation. By attaching small flourescent molecules it is now possible to probe the interactions of individual biomolecular structures, and monitor their motions and changes in conformation within living cells.





▲ A super-resolution image of a human cell showing actin filaments (green), mitochondria (red) and DNA (blue) with and without super-resolution *(Muthugapatti Kandasamy/University of Georgia)*

Fluorescence resonance energy transfer (FRET) spectroscopy

FRET spectroscopy is a well-established technique, in which the distance between two molecular structures or sites only a few nanometres apart can be measured. Both structures are labelled with fluorescent probes that can couple to each other, exchanging energy with an efficiency that depends very sensitively on their separation. Measurement of the changes in the relative intensities of fluorescence emitted by the two probes can be used to work out the distance between them extremely accurately.

Total internal reflection fluorescence (TIRF) microscopy

Another method that allows measurements of individual fluorescing molecules employs the tiny 'evanescent' glow (extending over a depth of about 100 nm) from a glass surface at which laser light has undergone total internal reflection. Only fluorescent molecules sitting in a very thin layer within range of this short-range subtle illumination will reveal their presence by lighting up – allowing them to be observed without being swamped by background fluorescence from other cellular components.

Super-resolution microscopy

In recent years, fluorescence imaging techniques have become ever more sophisticated, taking advantage of advanced laser techniques to bypass the diffraction limit and image single molecules.

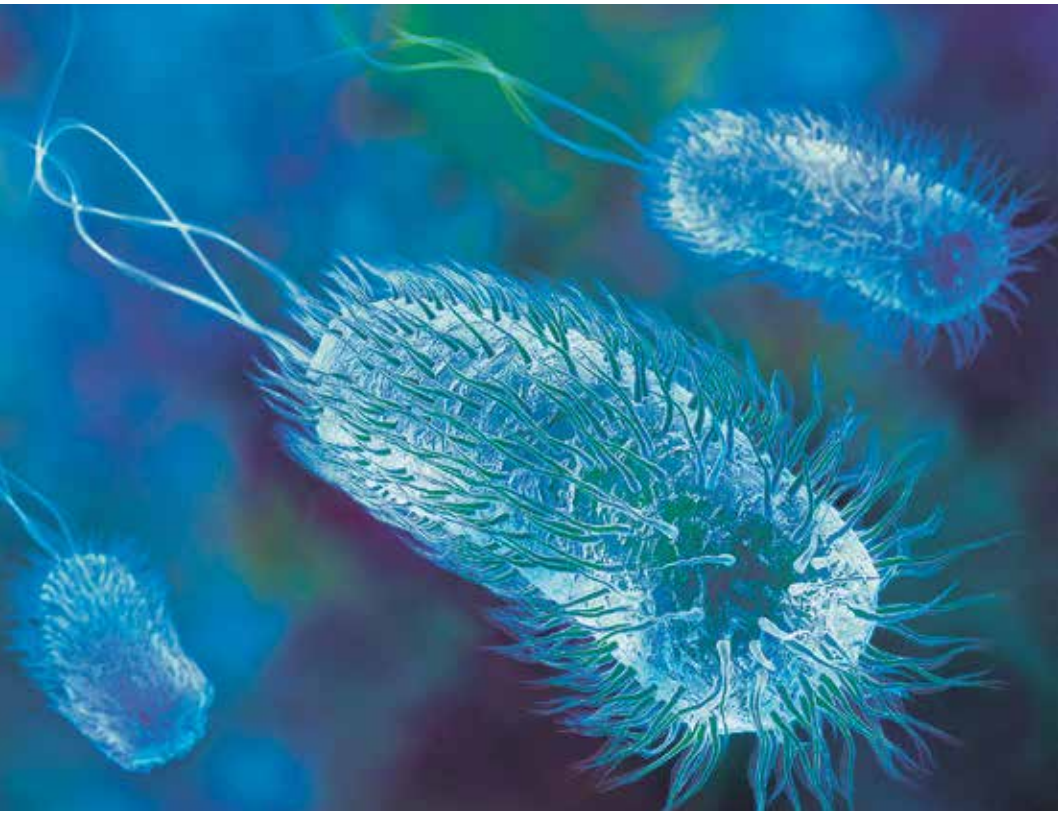
Stimulated emission depletion (STED) microscopy was the first so-called super-resolution method. It employs two lasers, one to excite and thus turn on the fluorescent probes, and a second to switch them off. The second laser beam is usually doughnut-shaped, and overlays the circular spot made by the first laser beam so that only molecules in a tiny central portion remain excited and become visible. The whole sample is scanned in this way to reconstruct a complete image.

Another set of techniques developed in the past few years cleverly takes advantage of the inherent randomness of the fluorescence process. In stochastic optical reconstruction microscopy (STORM) and photo-activated localisation microscopy (PALM), only a tiny proportion of fluorescent molecules in a sample is randomly excited at any one time. Although the image of each molecule is a 'fuzzy blob', the fact that they do not overlap means that the position of the molecule itself can be computed with high accuracy before its fluorescence becomes deactivated. The process is repeated thousands of times and the positions of all molecules combined to give a computer-generated image with nanometre resolution.

Swimming microbes

Living cells are constantly in motion – changing shape, dividing and often physically moving

A simple three-bead model showing the stroke cycle for a swimming cell with two flagella ▶



▲ *E. coli* employs a rotatory molecular motor to power its motion via beating flagella

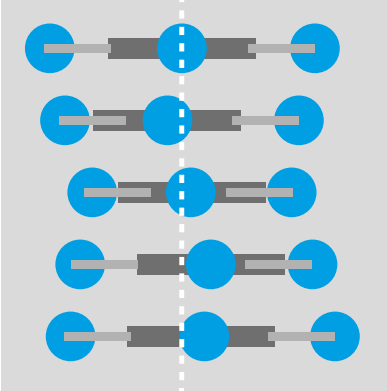
Uncovering the dynamical behaviour of living cells is essential to understanding processes such as growth and development, and the spread of disease. So how does movement happen at the cellular level and how are the underlying processes regulated? Physics has the theoretical and experimental tools to demonstrate how these mechanisms lead to large-scale emergent behaviour such as coordinated movement.

Bacterial sensing

Robert Endres at Imperial College London has been analysing how bacteria and other cells sense and then swim towards nutrients, or away from toxins or cells of the immune system that would engulf them. Bacterial cells such as *Escherichia coli* can infer the concentration gradient of the relevant molecules from the small numbers that randomly reach specific receptors on various parts of the cell surface. The sensing occurs against a noisy background of other molecules hitting the cell surface and reactions going on inside the cell. Using a theoretical model that calculates gradients by comparing positions of particles absorbed on a spherical surface, Dr Endres was able to estimate the minimum concentration of molecules needed for sensing tiny differences in gradients from one side of a cell to the other. The model accurately explained what had been seen in biological experiments. Such precise predictions are useful for understanding all kinds of molecular sensing such as those governing immune responses and embryo development.

Run-and-tumble cells

E. coli moves by means of a rotary molecular motor (p8) attached to corkscrew-like protrusions called flagella that aid swimming. A complex biochemical network in the cell sends signals that modulate which way the motor rotates: counter-clockwise rotation corresponds to smooth swimming in which the flagella bundle and rotate together, but a switch to clockwise rotation produces a tumbling motion where the flagella are out of synchrony so causing the bacterium to change direction. Linking the rate of switching between these behaviours to the signals provided by molecular sensors provides an efficient search strategy, as it allows the microbe to explore all the space around it when responding to chemical sensory input.

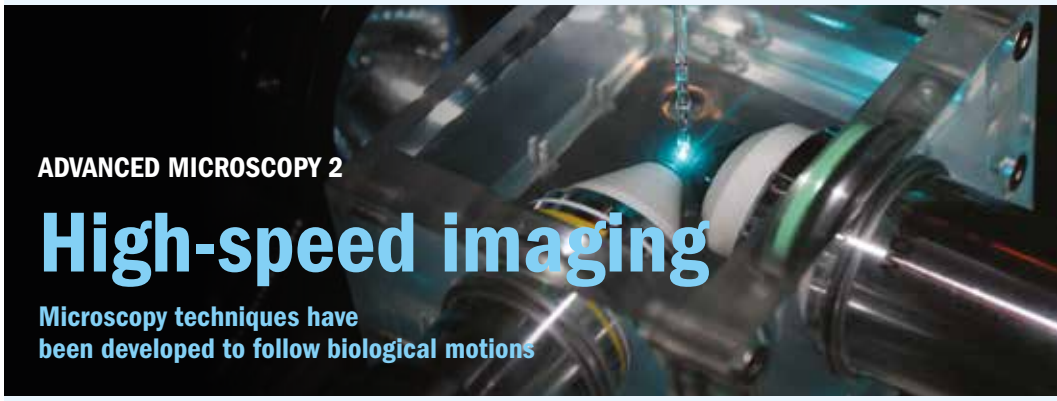


Because the signal transduction depends on random molecular collisions, the level of signalling fluctuates and is noisy. Dr Endres was able to characterise mathematically how the signal was passed to the motor, and describe how microbes are able to respond quickly and precisely.

Ray Goldstein's research group at the University of Cambridge was interested in seeing if eukaryotic cells with flagella behaved similarly. Using high-speed imaging and a 3D tracking system, they monitored the dynamics of the pairs of flagella of a spherical alga, *Chlamydomonas reinhartii*. They then analysed the observations using a theoretical model based on the dynamics of the motion in a thick fluid, taking into account the viscous drag and the inherent probabilistic nature of the signal input. To their surprise, they found the same kind of run-and-tumble behaviour. Ramin Golestanian at the University of Oxford has considered whether the trigger for this kind of behaviour was biochemical or mechanical. By modelling the alga as three attached beads – the cell and the two flagella – with a simple stroke pattern, the running-and-tumbling emerges naturally from noisy hydrodynamic interactions between the two rather elastic flagella and the surrounding liquid.

Applications of research

- Immunology
- Growth
- Tissue and embryo development
- Bacterial infection



ADVANCED MICROSCOPY 2

High-speed imaging

Microscopy techniques have been developed to follow biological motions

Particle image velocimetry (PIV)

Elucidating the collective motions of structures inside a cell, and of cells themselves, is crucial to understanding living processes such as metabolism, reproduction and infection. To study such biological flows, physicists have adapted imaging methods originally developed to track the flow of liquids and particles in engineering materials and systems. PIV visualises flows in fluids by adding tracer particles that are then illuminated and tracked with a charge coupled device (CCD) camera.

Ray Goldstein at Cambridge employs PIV to follow the motions of freely swimming algae with millisecond timing. It can also be applied to visualise the flow patterns inside cells, such as cytoplasmic streaming. Streaming within the cytoplasm is thought to be driven by the motor proteins that control the microtubule framework (p12), and are responsible for distributing nutrients and repositioning organelles during changes such as cell division.

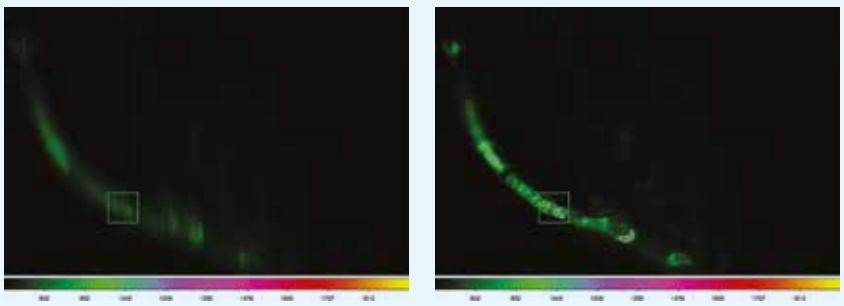
Differential dynamic microscopy

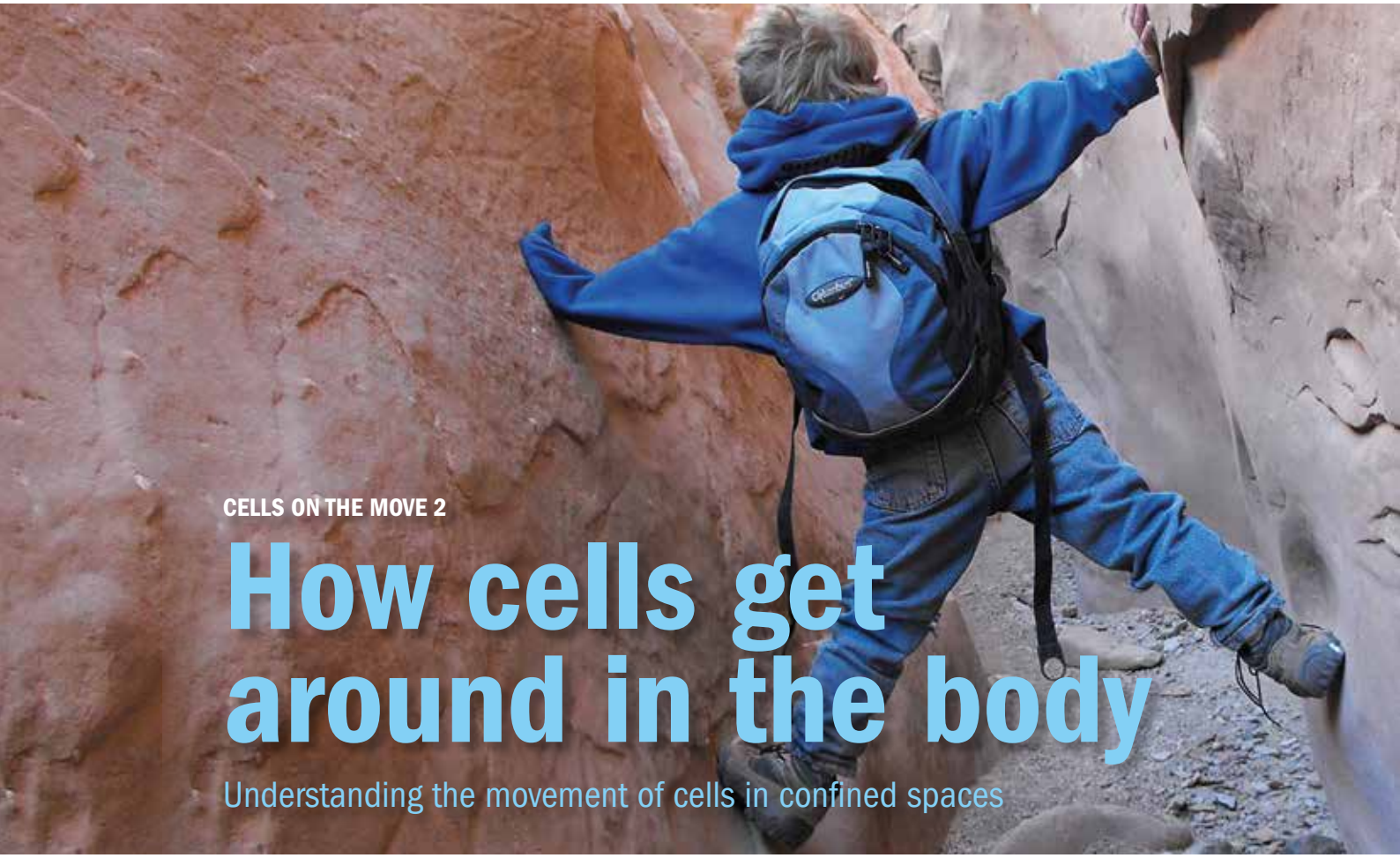
Wilson Poon at the University of Edinburgh has developed a microscopic technique for measuring the motility of bacteria and other microorganisms using low-resolution, white-light microscopy. By monitoring how successive images in a time-sequence relate to each other and fitting the data to mathematical models, his team can determine the swimming speed distribution of some 10 000 microorganisms in a matter of minutes. This method can be applied to understanding how pathogenic bacteria invade the protective lining of the human respiratory and digestive systems, and so cause disease.

Single-plane microscopy with adaptive optics

John Girkin at Durham University has developed a technique to image in 3D the beating heart of a zebra-fish – a model used in developmental biology. Thin layers of the sample are selectively illuminated in sequence, in combination with a technique called adaptive optics – in which a computer-controlled flexible lens can compensate for distortions caused by irregularities in the tissue. Images are taken at regular intervals to build up a description of how the heart develops over 48 hours, or how it repairs itself after damage. The method can be used to track the movement of individual cells during development of tissues, for example, an eye lens or neurons in the brain.

▼ Images of a zebra fish beating heart with and without adaptive optics





CELLS ON THE MOVE 2

How cells get around in the body

Understanding the movement of cells in confined spaces

In biological tissues, many processes involve single cells moving about in confined environments. Examples are the white blood cells that home in on bacteria and eat them, cells that move to seal a gap in a wound, and more ominously, cancer cells that migrate in the body and set up secondary tumours. For a theoretical physicist, all these processes can be treated in essentially the same way from a dynamics point of view. They have shown that some confinement can dramatically affect the behaviour of moving cells.

Rock climbers

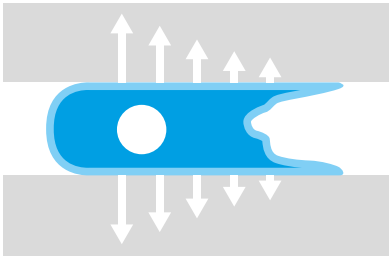
Rhoda Hawkins at the University of Sheffield applies basic physical principles to describing cell motility in confined spaces. Cells move using self-generated contractile stress caused by many molecular motors walking along actin filaments in the cell (p12). Experiments with cells on glass surfaces show that they move by making a flat protrusion at the front that sticks to the surface while the contractile stress pulls the back part off. The process depends upon the growth of the cytoskeleton filaments via polymerisation in the direction of movement and the adhesion to the surface breaking in response to the stress. In a confined environment, however, the mechanism appears to be different, and Dr Hawkins has developed a model in which the polymerising actin alone allows the cells to move by pushing against the confining walls – in the same way as a rock climber, in a technique called chimneying, pushes against opposing rock faces to gain friction.

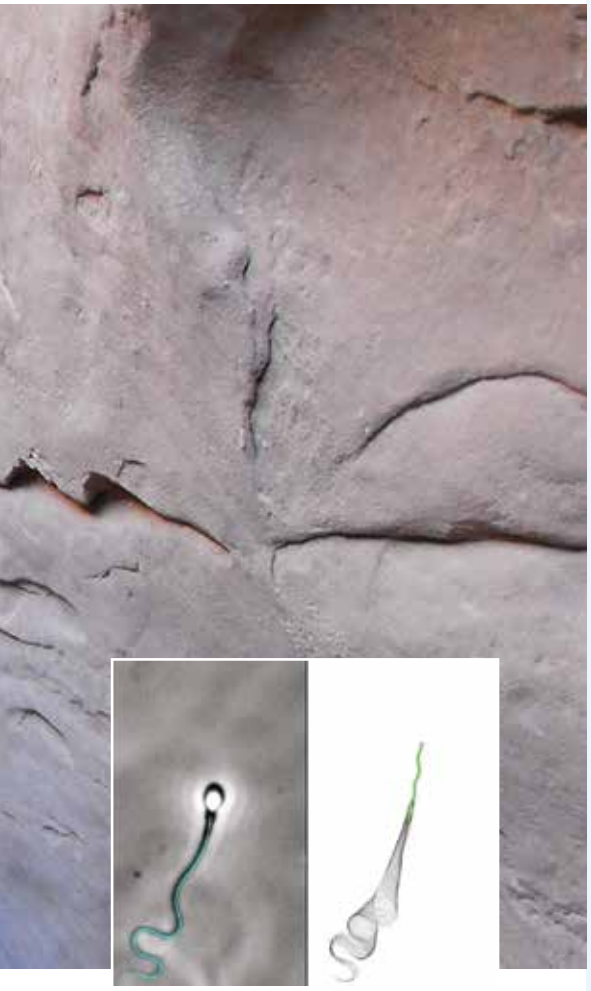
Channel swimmers

Of particular interest to medical scientists is the migration of sperm within the female reproductive system. Clinical diagnostics typically examine only gross cell movements in fluids and chambers, which do not reflect the confined micro-architecture and highly viscous fluids inside the body. David Smith at the University of Birmingham has been working with the Centre for Human Reproductive Science, Birmingham Women's Hospital, applying mathematical modelling techniques to capture how the sperm's flagellum moves and regulates its path along a micro-channel filled with a fluid of the same viscosity as semen. The model indicated that fluid-dynamical effects brought the sperm close to the walls of the channel.

Subsequent experiments in collaboration with engineers at the University of Warwick using a micro-channel maze with curved corners showed that the apparently random behaviour of a sample of sperm can be directed by shaping these walls, allowing motile cells to be organised on a micro-device, without the need

▼ Cells can move in the body by pushing against confining walls, just as rock climbers climb up a narrow gap – a technique called chimneying





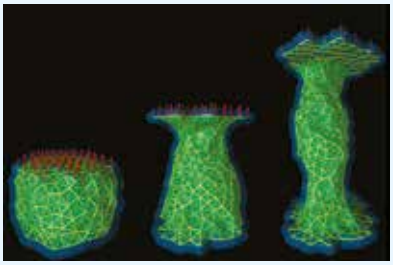
▲ Studying the mechanics of sperm motility is extremely important in understanding infertility

for external intervention. The model is now being used to understand how energy is transported and generated along the sperm tail during different types of motility, which is of interest as a potential target for treatment. Studying the mechanics of sperm motility with a physics perspective is important for understanding infertility and for designing microfluidic devices for IVF.

Applications of research

- Understanding the immune system, infections and metastasis
- Embryo development
- Wound healing
- Fertility and IVF

The role of physics theory



▲ A computer simulation of a single cell undergoing slow mechanical stretching. The cytoskeleton adapts to internal stresses to allow the cell to more than double its length and avoid rupture *(Sebastian Sandersius and Tim Newman/ University of Dundee)*

Physics can offer a global approach to understanding dynamic and complex behaviour using the tools of mathematics and computing

The search for universal principles that characterises the discipline of physics can offer useful explanations for biological behaviour based on a deep understanding of the fundamental properties of matter. Cells are complex many-body systems whose behaviour is modified by many factors including viscous flow and the random motion of molecules both inside and outside the cell: any large-scale fluid-mechanical behaviour is modified or even driven by myriads of small fluctuations. Living systems are continually being transformed by flows of energy and matter: the system is never in equilibrium and there is always "free energy" available to do work. Non-equilibrium behaviour leads to the formation of self-organising large-scale patterns and self-regulating behaviours that are transiently stable: life shows exactly these characteristics at all scales. The precise mathematical language of physics can provide powerful approaches to explain the intricate dynamics of phenomena such as cell movement and offer crucial quantitative predictions for microbiological experiments. The approach adopted is to establish the simplest mathematical model that can be applied to describe the physical system – it might be based on Newton's laws combined with statistical theory used to describe many interacting particles moving randomly. If the equations of the model become too complicated to solve, then computer methods can be used to make quantitative predictions about what would be expected to be observed in the laboratory or in the clinic.

The extracellular matrix

The mechanical properties of tissues such as cartilage and blood vessels are central to their function. These tissues contain very few cells, and such properties derive from an extensive extracellular matrix composed of the fibrous proteins collagen and elastin embedded in a visco-elastic gel. A major challenge in biological physics is to understand the ways in which the physical properties of the extracellular matrix change with ageing and the onset of diseases such as arthritis, hypertension and diabetes. Peter Winlove at the University of Exeter is seeking to relate these changes to specific components of the extracellular matrix and to develop structurally-based models of tissue micromechanics. He uses a microscopic technique developed in the past two decades, which can, for the first time, reveal the organisation of cells and matrix fibres in living tissues. Molecules such as elastin are intrinsically fluorescent and may be stimulated by infrared light. Using sophisticated spectroscopy, he can map the chemical composition of cell membranes and thereby investigate structural changes associated with mechanical loading.

The emergence of multicellularity

How single cells become organised into multicellular organisms and how they differentiate into specialised cells are major questions in biology

Traditional biological research into multicellular behaviour has mostly involved unravelling the genetics and biochemical interactions underlying cell signalling, adhesion processes and self-organisation. However, another valuable starting point that physicists are now exploring is the larger-scale physical driving forces that favour emergent cooperative behaviour between single cells.

Collaborating micro-swimmers

A dense suspension of bacteria shows turbulent-like behaviour, with swirls and jets forming and decaying. Very similar flows are seen in other active systems, on widely varying length- and time-scales, from suspensions of microtubules and molecular motors, to agitated granular matter, schools of fish, and flocks of birds. Julia Yeomans at the University of Oxford is trying to understand how far the same theoretical ideas can be applied to these different systems. This work has potential application to the design of artificial cells and swimming micro-machines.

Beating cilia

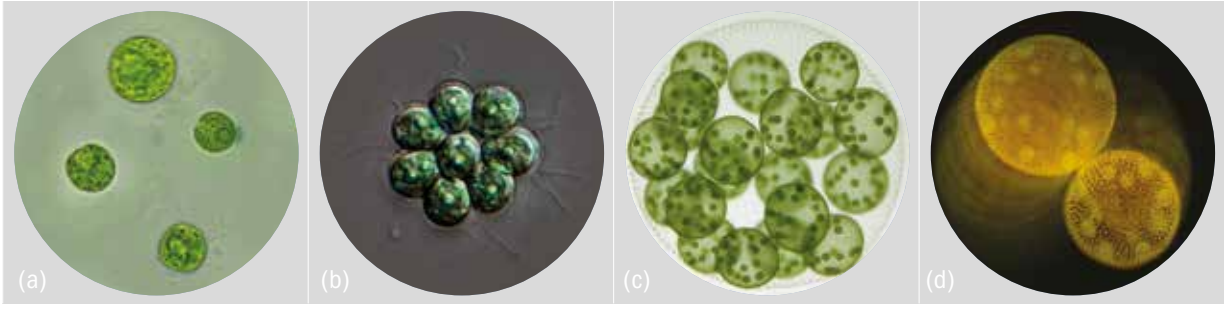
Ramin Golestanian at Oxford has also been analysing the cooperative behaviour of cells with flagella or cilia tethered on a flat surface, by describing them as groups of objects with rotating tails. Their motion may be uncoordinated, or may settle into synchronous wave-like motions in both time and space. Such behaviour is significant in understanding the ecology of bacterial films. It is also relevant to physiological processes involving cells with beating cilia which, for example, move eggs along in the Fallopian tubes and sweep mucus out of the lungs.

The rotational fluid flows driven by carpets of cilia may also play a part in determining the right–left positions of organs such as the heart and liver, by controlling the flow of nutrients and chemical signals. Working with biologists studying development in mouse and zebra-fish embryos, David Smith at Birmingham has been analysing the complex, small-scale fluid flows around cilia, demonstrating the dependency of flow direction on their length and distribution.

A multicellular waltz

The coordinated motion of cells in a series of related green algae is being investigated by Ray Goldstein at Cambridge in an effort to find out how multicellular behaviour might have evolved. Using a combination of fluid dynamical models and experiments in which the 3D motions of organisms in fluid suspensions were tracked, he compared the behaviour of three species of algae: a single-cell organism (*Chlamydomonas*); another related species that clusters into small conglomerations of cells; and *Volvox carteri*, which forms well-defined spherical colonies of cells. These colonies consist of a hollow sphere comprised of thousands of flagellated cells, and encapsulating a few specialised cells without tails, which are responsible for the colony's reproduction. They have no central nervous systems yet behave in a coordinated way. The division into two types of cell could represent the first evolutionary step towards multicellularity.

Professor Goldstein has been investigating the fluid flows that cause *Volvox* to spin (hence its name) and cluster at surfaces, with pairs of



▲ Related species of alga exist either as single cells (a), clusters (b), or spherical colonies of cells (c), which also bind in dancing pairs (d)

colonies waltzing around each other in closely bound harmony. Hydrodynamic flows and lubrication forces appear to drive this dance and probably enhance the reproductive phase of the colony's life-cycle. Another intriguing aspect is the colony's collective attraction to light; its motion and spinning frequency are hydrodynamically tuned to give the maximum photo-response.

Microbial communities

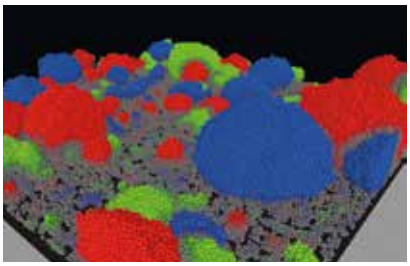
In the early evolution of life, the first cells to act together in concert were probably communities of bacteria and other microbes growing on surfaces, and collectively secreting a polymeric matrix – slime – that provides structure and protection. The ecologies of such biofilms can become complex, involving hundreds of different species of different single-cell organisms, each with a different role. They behave, in many ways, like a multicellular organism. Rosalind Allen at the University of Edinburgh has been studying the spatial structures formed by bacterial colonies in biofilms through computer simulations in which bacteria are represented by spheres that grow and push each other out of the way.

Dental plaque

The earliest recognised biofilm was dental plaque, which, as well as being the most accessible biofilm relevant to human health, is also the cause of the two most widespread diseases globally, tooth decay and gum disease. David Head at the University of Leeds is collaborating with biologists and clinicians to model the ecological change in the film triggered by excessive sugar intake that then causes plaque to produce acid, resulting in tooth decay. His computer model also includes the biofilm's response to mechanical forces, allowing a broad range of medical and environmental problems to be investigated, including the spread of pathogenic bacteria in water systems.

Microbial evolution

The growth and evolution of microbial communities are also extremely important in studies of global carbon cycling and extinction events. The major mass extinction that marked the boundary between the Permian and Triassic eras, more than 250 million years ago, is associated with fossils containing high sulfide concentrations. This was probably the result of a sudden growth spurt in sulfur-reducing bacteria stimulated by the generation of hydrogen from fermenting bacteria feeding on dead organic material in the oceans. Dr Allen has been trying to simulate this kind of ecosystem in experiments with microbial microcosms based on pond sediment, in which she tracks how the microbial populations grow over time. She then compares the outcomes with computer simulations. So far, she has found that large populations all evolve in the same way but small ones can change in diverse ways.

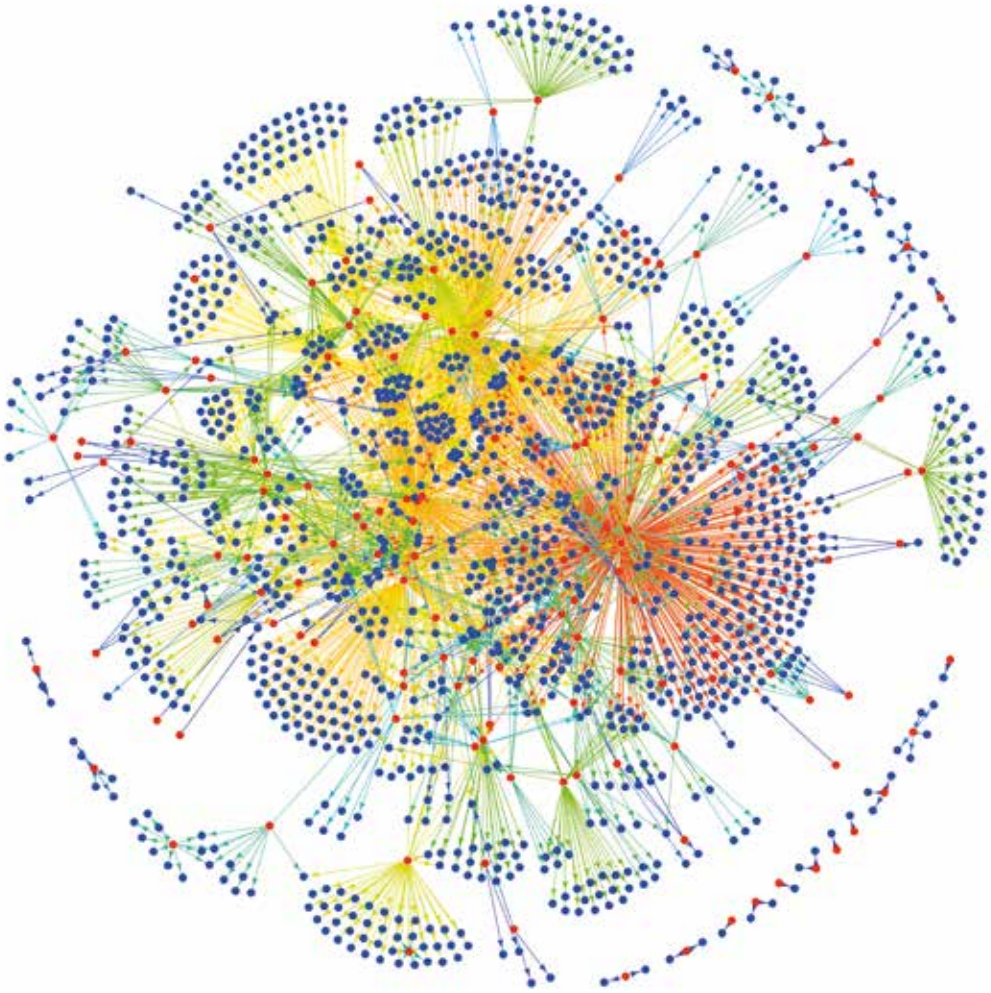


▲ A snapshot from a computer simulation of a microbial biofilm consisting of three species (red, green and blue), where each species inhibits the growth of other species nearby. Cells affected by this growth inhibition are shaded grey

▼ Coloured scanning electron micrograph (SEM) of the surface of a Fallopian tube, showing non-ciliated (green) and ciliated (orange) cells. Microvilli (small protrusions) cover the surface of the secretory, non-ciliated cells *(Steve Gschmeissner/Science Photo Library)*

Applications of research

- Evolution
- Embryo development and growth
- Infection and oral healthcare
- Nanotechnology



◀ The gene regulatory network of the *E. coli* bacterium

COMPLEX SYSTEMS 2

The big picture

From cells to whole organisms, physicists can model the outcomes emerging from the behaviour of genetic networks and biochemical pathways in a complex living environment

The huge advances in molecular biology and genetic mapping over the past few decades have transformed our knowledge of how living processes work. Current understanding is largely based on unpicking individual molecular interactions, demonstrating the causative links between biochemical pathways – and, in the case of medical research, uncovering where they go awry.

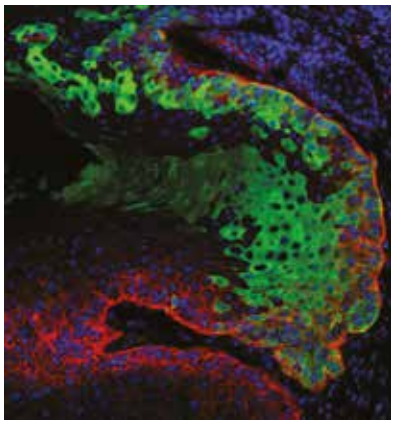
However, it has become increasingly clear that the large-scale structure and behaviour of organisms cannot be understood simply in terms of a linear chain of molecular reactions; they are a manifestation of the broad collective behaviour of a network of interacting systems across a hierarchy of scales. Furthermore, the emergent mechanisms are ultimately a result of a group of inherently random microscopic motions and reactions happening within a sea of other random changes, driven by a similarly fluctuating input of energy.

Decisions, decisions

The randomness of gene expression was noted some years ago by Peter Swain at the University of Edinburgh in experiments in which the amounts of protein expressed in cells were measured optically using fluorescent labelling (p13), and shown to vary randomly over time and from cell to cell. Environmental signals, such as nutrient levels or hormones, may also fluctuate. So how does the decision-making underlying cellular responses and behaviour work? Professor Swain believes that probability and decision theory can quantitatively describe the sensing of external signals by cells, the transduction of these signals and any resulting gene regulation. Using a microfluidic setup, he is testing these ideas by investigating the real-time response of individual yeast cells to fluctuating sugar levels, to see if they can advantageously infer the current state of their environment and even predict future states. This kind of work is still in its infancy but has important implications for exploring poorly-understood biological phenomena such as infection and antimicrobial resistance.

Proliferate or differentiate?

Another important response is that of stem cells to their local environment or "niche". Stem cells are defined by their capacity to replicate and give rise to daughter cells that are more differentiated, leading to the development and patterning of particular tissues. Stem cells also maintain tissues that are constantly being renewed, such as blood, skin and the lining of the gut. An important regulatory decision they have to make is whether to proliferate or differentiate. Ben Simons at the University of Cambridge has been applying so-called lineage-tracing methods based on transgenic mouse models, whereby the fate of a stem cell and all its progeny can be traced over time. By analysing the ensemble behaviour of these "clonal" lineages, he has elucidated patterns of random stem-cell fate that are conserved across different tissues and organisms.



▲ Stem cells and their progeny can be traced using genetic labelling, and can show how cancer cells proliferate, as in this mouse tumour *(Ben Simons/University of Cambridge)*

These quantitative studies have already provided insights into mechanisms of uncontrolled growth in skin tumours.

Keeping the show on the road

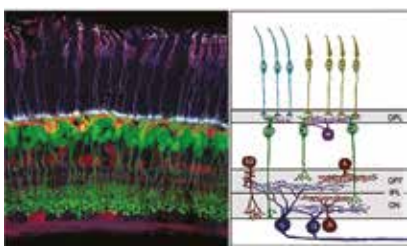
Tim Newman and colleagues at the University of Dundee have also been investigating how regulatory genetic networks provide cell robustness in a fluctuating environment. Using a mathematical approach taken from economic theory, they showed that the stability of the networks does not depend on the strength of the individual links but only on the way the network is wired, and that networks are robustly buffered against the effects of mutations rewiring the network. In cancer cells, these buffered networks are broken, but only by a handful of genes. Targeting these genes so that the buffered network is restored could provide a route to new cancer therapies. On the negative side, he has also shown, through probability theory, that early cancer metastasis may be driven by rare random events that would be hard to treat using targeted drug therapies.

Plant computers: digital and analogue

Not all responses to the environment are directly encoded in genetic sequences. Martin Howard at The John Innes Centre, Norwich, has been mathematically analysing the dynamics of seasonal flowering. In plants, for example, Arabidopsis, the relevant gene controlling flowering is repressed by winter cold via dynamic chemical modifications of the histone proteins that are wrapped around a plant's DNA. Together with his experimental collaborators, Professor Howard has demonstrated that this repression acts through a bistable switch in each cell. This "epigenetic memory" system is thus digital, with winter cold modulating the fraction of cells in which the bistable switch is flipped and the appropriate flowering gene repressed.

It is unclear whether other processes in biology happen in an analogue or digital fashion. Intriguingly, Professor Howard and experimental collaborators have shown that the way plants regulate their night-time consumption of starch is actually an analogue computation. After sunset, when plants cannot utilise energy from the Sun, they instead gradually break down the starch supplies built up during sunlight hours. However, the way in which this is achieved is remarkably precise, such that the starch levels always run out at around dawn. This requires the plant to perform a kind of arithmetic division computation between the amount of starch present and the time-to-dawn, with the latter information provided by the plant's internal circadian clock. Professor Howard has proposed that this computation can be simply performed only using rates of change that are analogue, an insight that could be important in synthetic-biology applications.

▼ Coloured generic labelling can follow how stem cells differentiate *(Ben Simons/University of Cambridge)*



▲ Many birds such as starlings fly in closely coordinated flocks. How do they do it?

Flocking together

Many animals gather together in swarms or flocks, most likely for protection from predators. Anyone who has watched a "murmuration" of starlings swoop in unison across the sky will wonder how each bird coordinates its movement with its neighbours within the dense cloud. Matthew Turner at Warwick believes that each bird can sense the surrounding opacity, in particular the boundaries between light and dark within the flock. Using a model based on a 2D projection of what each bird can see, he is able to explain how whole flocks can respond rapidly.

Applications of research

- Infection and antibiotic resistance
- New cancer therapies
- Spread of cancer
- Agriculture and horticulture
- Synthetic biology

Contacts and further information

Acknowledgements
This booklet was prepared under the guidance of Professor Ray Goldstein (University of Cambridge), Professor Andrew Turberfield (University of Oxford), and Dr Sarah Harris (University of Leeds). Many researchers provided information; however, only a limited proportion of research in biological physics carried out in the UK could be mentioned here. The IOP thanks all the researchers who helped with the preparation of this booklet.

Further information (Websites)
The IOP Biological Physics Group supports the UK research community in all fields of biologically inspired physics: www.iop.org/activity/groups/subject/bp

biologicalphysics.iop.org is the IOP website for the teaching of biological physics: www.biologicalphysics.iop.org

Understanding the Physics of Life is an EPSRC Grand Challenge Network that aims to bring physicists and biologists to work together to tackle the challenge of integrating understanding from single molecules to systems in biology: www.physicsoflife.org.uk/

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Design: h2o creative

Published: August 2014

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