Could you begin by outlining the research objectives of your Scanning Probe Microscopy (SPM) group at the University of Sheffield, UK?

JH: My group works on the development of atomic force microscopy (AFM) techniques and their application to synthetic polymer and biological systems. From an instrument development perspective, we are primarily interested in improving resolution towards molecular and submolecular resolution in ambient conditions, along with increasing imaging speed to allow observation of molecular-scale processes in real time. We apply these techniques to understand how polymers crystallise, with the aim of linking properties and function to molecular architecture. We also collaborate extensively with biologists and medical researchers to understand the relationship between structure, properties and, in some cases, disease in living systems.

Your work on AFM feeds into biological physics. Which research activities are you currently focused on and how do they interlink?

JH: I started as a polymer physicist and became interested in AFM because of its potential to answer specific polymer questions. I began to develop the technique further to improve that capability and have recently branched out into biological systems because the methods we use have clear application there.

NM: I started working with Jamie as a PhD student and am interested primarily in instrument development. I developed the torsional tapping technique towards the end of my PhD, and have been working with Jamie for the last four years as a research associate, most recently on a large multidisciplinary grant: Low-Dimensional Chemistry. By combining our interests in what the instrument is ultimately capable of, with a desire to answer specific scientific challenges in polymer and biological physics, we have been able to tackle more difficult questions.

In biological physics, we have worked with microbiologists to study the cell wall of Staphylococcus aureus (as in MRSA), discovering how the cells use the architecture of their cell walls to determine where to divide. Understanding how the bacterial cell wall works is important for the future development of new antibiotics.

What are your individual backgrounds and how do your research interests converge?

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Sight beyond sight

The Scanning Probe Microscopy group at the University of Sheffield, UK, is developing a groundbreaking technique that allows polymers and living systems to be imaged with true molecular resolution.

FROM POLYSTYRENE TO DNA, polymers play a vital role in everyday life. Yet, while these fascinating macromolecules have been studied extensively, there is a great deal to learn regarding their fundamental properties and how these depend on what the molecules themselves are doing. Whereas information about the average behaviour and distribution of polymers is currently available, microscopy techniques have not attained the resolution necessary to observe individual chains that are not isolated on a surface or highly and regularly ordered. This is a clear deficiency because some researchers believe it is precisely these local variations that hold a controlling influence over many polymers’ properties.

To address this need, the Scanning Probe Microscopy (SPM) group at Sheffield University in the UK is developing innovative SPM technology that is shedding new light on the way that synthetic and naturally occurring macromolecules organise. Led by Professor Jamie Hobbs, the group was established to develop atomic force microscopy (AFM) technology and apply it to the study of both synthetic polymer and biological systems. Dr Nic Mullin, a postdoctoral research associate in the SPM group, has been particularly instrumental in helping to develop a valuable microscopy technique over the past few years – torsional tapping atomic force microscopy (TTAFM) – which represents an important breakthrough in the study of a range of physical and biological processes.

BIological developments

Hobbs has recently expanded his investigations into applying AFM techniques to living systems in order to explore medically relevant biological problems. Current projects include investigating cancer metastasis and finding out whether ‘stem-like’ cancer cells have a unique mechanical phenotype. He is also running projects on bacteria, in which the structure and function of the cell wall are being analysed, with the ultimate aim of developing new antibiotics – particularly for Staphylococcus aureus and another human pathogen, Clostridium difficile. Most recently, Hobbs’ team has brought together AFM with super-resolution optical microscopy to observe how the wall of Escherichia coli is made in a way that directs its own growth: “Our work on the bacterial cell wall with Professor Simon Foster has really helped redefine our understanding of this structure, which is vital for cell viability,” Hobbs recounts.

In 2011, Hobbs collaborated on a paper that characterised the surface of various endospores using the TTAFM technique. The structure, based on electron cryomicroscopy, informed a functional model of the spore surface and

Professor Hobbs, you are co-founder of Infinitesima, a company that develops high-speed AFM tools for the silicon fabrication process control market, and an active consultant for a number of polymer companies. Does close collaboration with industry facilitate more rapid developments?

JH: Working with industry can really drive innovation forward. Helping Infinitesima develop their product for market forced us to delve much deeper into how high-speed AFM works than we would have otherwise, and this was remarkably fruitful scientifically as well because the level of understanding we gained has led us to new research ideas. Similarly, working with the polymer industry gives us access to samples, questions and challenges that we would otherwise lack: it really is a two-way process.

Why does understanding polymer crystallisation represent such a significant scientific challenge and how do you plan to further develop this understanding?

JH&NM: Polymers are long chain molecules that have to reorganise considerably during crystallisation. This leads to very high levels of complexity and means that simple concepts based on equilibrium properties rarely apply. The crystal structure is also constrained to nanometre-length scales, and parts of the molecules never manage to crystallise, leading to the ‘semi-crystalline’ structures that result in some of their unique physical properties.

This high level of complexity opens up lots of scientific challenges – both fundamentally and for analytical techniques such as microscopy, which aim to reveal the relationship between the molecular building blocks and larger-scale structures. In the future, we aim to combine our torsional tapping technique with our fast-scanning know-how in order to follow processes at molecular resolution as they happen in polymer samples: this would represent a real breakthrough.
INTELLIGENCE
STUDYING HOW POLYMER CHAINS ORGANISE USING TORSIONAL TAPPING ATOMIC FORCE MICROSCOPY

OBJECTIVES
• To use torsional tapping atomic force microscopy (TTAFM) to reveal, by direct imaging, the conformation of individual molecules in semi-crystalline polymer samples
• To see, at true molecular scale, the impact of chemical heterogeneity and particulate additives on crystal structure
• To develop the technique further to enable its uptake as a general method within the polymer industry

KEY COLLABORATORS
JPK Instruments AG
Innovia Films
Sheffield Polymer Centre

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CONTACT
Jamie Hobbs
Professor of Physics
Department of Physics and Astronomy
University of Sheffield
Hicks Building, Hounsfield Road
Sheffield, S3 7RH, UK
T +44 114 22 24532
E jamie.hobbs@sheffield.ac.uk

PROFESSOR JAMIE HOBBS followed a PhD in Polymer Physics from the University of Bristol, UK, and postdoctoral work alongside Professor Andrew Keller, with a five-year EPSRC Fellowship in 2001. In the same year, he co-founded Infinitesima Ltd, a high-speed AFM company. He moved to the University of Sheffield, UK, as a lecturer in 2003 and was promoted to full Professor in 2013.

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has received significant attention recently. This work, in collaboration with Professors Per Bullough and Anne Moir, has opened up a new route for unravelling the structure of bacterial spores, and is important for the fight against pathogens such as Anthrax.

In terms of the microscopy, many of the challenges faced in these biological projects are similar to those found in polymer physics, as the materials in both are relatively soft, have strongly temperature- and environment-dependent properties, and simple concepts like equilibrium are not always relevant: “The big difference experimentally is the added complexity of living systems,” adds Hobbs. “A major surprise has been just how little we know about so many of the things that kill us.”

Collaboration with other scientists has been vital to the group’s success. Combining Hobbs’ research background as a polymer physicist with the expertise of biological scientists has resulted in new biological questions coming to the fore, and the need for instrumentation to develop in novel ways.

PUSHER THE BOUNDARIES
The TTAFM technique has already revealed some surprising findings, and Hobbs and Mullin have been able to image, for the first time, phenomena that have been predicted but never directly observed: “One example is chain folds in polyethylene,” highlights Hobbs. “Since the 1950s, polymer chains were predicted to fold back on themselves during crystallisation, forming the thin crystals that we can see with electron microscopy and AFM. Exactly how they do this, and exactly what shape the folds have, has been the subject of argument ever since. We have directly imaged such a chain fold for the first time.”

Another recent advance made by the SPM team includes demonstrating that it is possible to cut open bulk samples with a cryomicrotome and still image at an individual chain level. This opens up many exciting possibilities for understanding the structure of more complex polymer systems that are frequently used today, such as blends, multilayers and particle-reinforced samples.

LOOKING TO THE FUTURE
The group’s most recent project is focused on semi-crystalline polymers. Using TTAFM, the researchers aim to obtain governing principles on processes that occur at the chain level of polymers and demonstrate the technology is effective so that it can be used more widely. Once TTAFM is proven and widely accepted, the benefactors will extend beyond the realms of polymer science and nanotechnology, to biological science and bio-nanotechnology.

The SPM group has already made great progress in developing AFM instruments to obtain beautiful images and answer fundamental questions about how polymer chains organise, yet they are keen to keep pushing the TTAFM instrument to the next level: “We can see molecules as chains, but the chemical detail is still lacking, and disordered areas are harder for us to visualise,” Hobbs states. “We have ideas about how to approach this, but it is a substantial challenge to see everything on a surface with atomic-level detail.”

Looking ahead, Hobbs aims to continue close collaboration with industry and maintain a flexible approach to modifying his technology and research methods, allowing the team to image a greater range of samples. In future, the researchers hope the technique will be used to help solve real industrial problems that ultimately have an impact on people’s everyday lives.

A phase image showing polyethylene molecules in a crystal at the surface of a thick film. A chain fold can be seen in the centre of the image. Scale bar 5 nm.

Sample courtesy of Ashley Cadby.