Institution: University of Bristol		
Unit of Assessment: 9) Physics		
Title of case study: Novel atomic force microscopy delivers major benefits for industry and healthcare		
Period when the underpinning research was undertaken: 2000 - 2020		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Mervyn Miles	Professor	08/1989 - 06/2018
Heinrich Hoerber	Professor	09/2005 - 12/2015
Andrew Humphris	Research Fellow	04/2001 - 04/2002
Jamie Hobbs	Research Assistant	11/1996 - 12/2003
Loren Picco	Research Fellow	04/2007 – 09/2018
Oliver Payton	Senior Research Associate	05/2013 - Present
James Vicary	Senior Research Associate	05/2007 - 06/2012
Massimo Antognozzi	Senior Lecturer	10/1999 - Present
Period when the claimed impact occurred: 2014 - 2020		
Is this case study continued from a case study submitted in 2014? No		

1. Summary of the impact

The ability to image rapidly, objects on the nanoscale is crucial for the characterisation of important materials such as thin films, nanoparticles, polymers and biomolecular structures. High-speed atomic force microscopy (AFM) techniques developed at the University of Bristol (UoB), have revolutionized the field of nano-scale imaging by exploiting a super-lubricity effect to enable rapid imaging of large areas of fragile samples. Based on this research, spin-out company Infinitesima, in a collaboration with Zeiss, has developed an essential tool for the repair of photomasks used in the multi-billion-dollar semiconductor industry. Sets of defect-free masks are worth tens of millions of dollars, and most modern integrated circuits are made using photomasks repaired with AFM technology based on Bristol inventions. UoB's AFM research has led to the creation of three further spin-out companies: Bristol Nano Dynamics, NuNano, and Vitamica who are active in areas as diverse as healthcare and instrument manufacture. All four companies together employ 46.5 FTE staff, have an annual turnover of GBP3.5 million and have attracted GBP6.8 million in investment since 2014.

2. Underpinning research

Forging the link between the nanoscale structure of materials and their functional properties is a key aim of many areas of Physics, Materials Science, Life Sciences and Chemistry. AFM plays a central role in realising this goal, permitting the visualization and measurement of nanostructures in a diverse range of systems. Critically, it also provides one of the few methods for micro/nano scale imaging of delicate biological samples such as cells and tissues under physiological conditions.

Background and breakthroughs

AFM uses a mechanical tip mounted on a spring-like cantilever to 'feel' the surface of a nanoscale object and generate an image. Originally, AFM was a slow technique taking from several minutes to an hour to capture each image, with the tip causing damage to fragile samples. Research in the School of Physics at UoB made the discovery (patented in 2005- US6906450B2) that when the AFM tip is moved very rapidly over the sample in liquid, it experiences a super-lubricity effect which significantly reduces friction and damage to delicate samples [1]. This 'gliding mode' also occurs under ambient conditions and even in a vacuum because of a thin layer of adsorbed water on the sample surface. Crucially, such rapid scanning of an AFM tip can be performed without significant loss of measurement resolution because the frequencies involved are well above the low frequency resonances of the cantilever. The ability to scan a sample rapidly with the AFM tip means that it takes three to four orders of magnitude less time to analyse a sample surface than previously [1]. This in turn allows measurements to be taken over larger areas, thereby enabling the study of phenomena that bridge hitherto inaccessible length and time scales. These technical breakthroughs were incorporated in an AFM apparatus via a high-speed scan stage and high-speed data capture [1]. The technique has been developed further by Infinitesima and called Rapid Probe Microscopy (which is incorporated in the Zeiss semiconductor fabrication tool), and by Bristol Nano Dynamics in their line of high-speed AFMs.

New techniques for studying biological samples

Since the invention of high-speed AFM, the UoB group has capitalized on their complementary skillsets in microfabrication, material science, biophysics and optics to make further important developments. Recognising the potential for using AFM to study biological material, the group continued to develop the methodology, focussing on improving the accuracy of measurements of the cantilever position and on new types of tip for bespoke applications. The UoB group pioneered production methods for very soft cantilevers for fragile samples [2] and led the development of the Scattered Evanescent Wave (SEW) detection method for accurate detection of cantilever position [3]. SEW has been instrumental in developing a rapid antimicrobial susceptibility testing (AST) system. Living bacteria exhibit nanoscale fluctuations (a feature which can be detected by AFM), while dead ones do not. UoB's AST method is known as Sub-Cellular Fluctuation Imaging (SCFI) [4] and involves treating bacteria with a variety of antibiotics and then illuminating them with an evanescent light field. The fluctuations in the surviving bacteria can be detected as modulations in the local scattering of light, allowing identification of successful antibiotics.

Further improvements to AFM speed and accuracy

Recent technical developments have focused on further precision in determining the tip's location and increasing the scanning and video acquisition rate [5,6]. By using an alternative laser-based displacement detection system which locates the laser at the rear of the cantilever, a huge increase in the signal to noise could be achieved, thus enabling scanning at ultra-high rates while reducing the complexity of the microscope [6].

3. References to the research

[1] **Humphris ADL**, **Miles MJ**, **Hobbs JK**. "A mechanical microscope: High-speed atomic force microscopy" *Applied Physics Letters*, 86, 034106 (2005). doi:10.1063/1.1855407

[2] **Vicary JA**, Ulcinas A, **Hörber JKH**, **Antognozzi M.** "Micro-fabricated mechanical sensors for lateral molecular-force microscopy". *Ultramicroscopy* 111, 1547–1552, (2011). doi:10.1016/j.ultramic.2011.08.008

[3] **Antognozzi M,** Ulcinas A, **Picco L,** Simpson SH, Heard PJ, Szczelkun MD, Brenner B, **Miles MJ**. "A new detection system for extremely small vertically mounted cantilevers." *Nanotechnology* 19(38): 384002, (2008). doi:10.1088/0957-4484/19/38/384002

[4] Bermingham CR, Murillo I, Payot ADJ, Balram KC, Kloucek MB, Hanna S, Redmond NM, Baxter H, Oulton R, Avison MB, **Antognozzi M.** "Imaging of sub-cellular fluctuations provides a rapid way to observe bacterial viability and response to antibiotics", *biorxiv*, (2018). doi:10.1101/460139

[5] **Payton OD**, **Picco L**, Scott TB. “High-speed atomic force microscopy for materials science”, *International Materials Reviews* 61(8), 473, (2016). doi:10.1080/09506608.2016.1156301

[6] Mikheikin A, Olsen A, Leslie K, Russell-Pavier F, Yacoot A, **Picco L**, **Payton O**, Toor A, Chesney A, Gimzewski JK, Mishra B, Reed J. “DNA nanomapping using CRISPR-Cas9 as a programmable nanoparticle”. *Nature Communications* 8, 1665, (2017). doi:10.1038/s41467-017-01891-9

References 1-3,5,6 have been published in leading journals in the field. They have accrued significant citations from international groups.

4. Details of the impact

UoB’s innovations in high-speed AFM have provided a step change in terms of the length and timescales over which the properties of nanostructures can be explored effectively. These advances have opened a wide range of potential industrial applications. UoB has an exceptionally strong ecosystem for exploiting such opportunities, and the School of Physics has spun out four innovative and exciting companies based on this research. The largest of these supports the multi-billion-dollar semiconductor industry, while others are active in fields ranging from health care to material science. Below we describe these companies.

Infinitesima: supporting the global semiconductor industry

Infinitesima was co-founded in 2001 by Miles and former Bristol research fellow Andrew Humphris who is the company’s CTO. In 2005, the company used UoB’s high-speed AFM research [1], to develop its Rapid Probe Microscopy (RPM) product. Formation and early growth of the company was described in REF2014. Infinitesima has continued to expand from 12 in 2013 to 30 FTE staff today, has an annual revenue of approximately GBP2.5 million and has attracted ~GBP5.5 million investment.

In 2015, following partnership between Infinitesima and optical design and engineering company ZEISS, the MeRit neXT was launched [A, B]. This is the semiconductor industry’s standard tool for the repair (i.e., correction of defects) of photomasks and the RPM plays a key role in the repair. The lithographic manufacture of integrated circuits relies on large numbers of photomasks (up to 80 for a typical high-end microprocessor). Owing to the intricacies of their production, each mask can be worth up to USD1 million [C]. However, with feature sizes of modern integrated circuits often being less than 10nm, photomasks are highly prone to defects and errors during manufacture. Defective photomasks are useless as they will produce inoperable integrated circuits. It has now become practically impossible to produce the current feature sizes used in the production of state-of-the-art integrated circuits without repairing the photomasks.

MeRit neXT’s importance stems from the fact that it is the sole repair tool on the market that can add and remove photomask material. The MeRit neXT contains the Infinitesima RPM as a pivotal component [B]. *“The RPM enables applications like repair verification, 3D feature analysis and repair of Chromeless Phase Shifting Lithography as well as buried multilayer defects on EUV masks”. “The new ZEISS MeRiT neXT is a paradigm shift in classical mask repair. Low energetic electrons finally enable the repair of smallest defects through straight local interaction with no collateral damage and an outstanding imaging quality at the same time.”* stated the Product Manager at ZEISS [B]. The CTO of Infinitesima and inventor of the RPM said “*The RPM’s throughput is 10-100 times faster than standard AFM technology.... In combination with its sub-nanometre resolution, this has been one of the key factors to be selected by ZEISS*” [B,C]. No other technology provides 3D information with the same resolution and scalable throughput [C]. It therefore allows the rapid repair of photomasks necessary to maintain high production rates of integrated circuits.

All the major integrated circuits manufacturers (including systems at Intel, Samsung, Toshiba) use the MeRit neXT [C], *"Essentially all photomask sets for microprocessors using feature sizes below 100nm have been through a Zeiss MeRiT neXT system, which since 2015 contains the Infinitesima RPM technology, making our RPM a vital instrument in the production of modern micro-electronic devices"* [C]. Total integrated circuit sales in 2018 were valued at greater than USD400 billion. Sales of the MeRiT neXT gross over GBP5 million for Infinitesima [C], contributing to a cumulative turnover of GBP8.5 million for high-speed AFM products since 2014. Infinitesima currently employs approximately 30 FTE.

NuNano: producing bespoke AFM probes

Key to AFM performance are the physical parameters of the cantilever tip. UoB innovations in cantilever and tip fabrication [2] have been commercialised through NuNano, founded in 2011 by Vicary, Hoerber and Miles. NuNano produces top quality tailor-made probes with market leading tolerances which are sold to both the academic sector and industry. The company sells 20 types of probe across three main categories: semiconducting (Silicon), conducting (Platinum coated), and ultra-soft varieties (Silicon Nitride). These together permit a wide range of studies, with the ultra-soft variety being particularly suitable for biological material. Since 2017, the company has entered a new growth phase. There has been GBP800,000 of private equity investment, a new patent for assembly technology and a new commercial service, taking total revenue in 2019 to GBP108,000 and staff to 8 FTE. NuNano have sold approximately 8,500 devices worldwide in the current REF period [D].

Bristol Nano Dynamics: Imaging services for industry and healthcare

Bristol Nano Dynamics (BND) was founded in 2015 by Picco and Payton with the aim of capitalising on UoB's more recent AFM research [5,6]. The company manufactures high speed AFMs for rapid, high-resolution mapping and nanoscale surface characterisation. Their instruments allow nanoscale imaging over millimetre to centimetre sized areas or, alternatively, imaging an area of ~25μm^2 at video frame rate. The latter permits observation of dynamic surface processes occurring (or initiating) at the nanoscale. BND's AFMs permit new insight into, for example, the onset of corrosion or crack initiation in metals, novel 2d material manufacturing, and even watching a virus infect an algal cell.

BND currently has an annual turn-over of over GBP1 million and employs 5.5 FTE [E]. Customers include the National Physical Laboratory and Plymouth Marine Laboratory in the UK, Virginia Commonwealth University (USA), and the Institute for Basic Science in South Korea. UoB purchased two instruments which are run as a TRAC facility and used to test materials for Airbus, AWE, EDF Energy, Sellafield, National Nuclear Laboratory (NNL) and Rolls-Royce who have together funded three PhD students for this purpose [F]. NNL employ the unique capabilities of the high-speed AFM to develop new insights into radiation damage at the atomic scale over statistically relevant large sample areas and to understand corrosion in real-time in liquid environments [E]. Its importance in this regard was acknowledged by the 2015 NNL Innovation Award. The facility has clocked up over 1,000 hours of industry funded research and sample analysis [F].

In collaboration with Virginia Commonwealth University (VCU), BND has developed a test for the genetic mutations responsible for Acute Myeloid Leukaemia which is diagnosed in over 23,000 people annually in the US and UK alone [G]. VCU have patented the test [H], which relies on the BND microscope's ability to detect tiny changes in the length of genetic molecules, "*with greater speed, accuracy, and sensitivity than existing molecular diagnostic methodologies*." [G]. The Bristol development is allowing improved identification of patients who can benefit from targeted life-saving therapies. A successful pilot clinical trial of this diagnostic test was recently completed; in a double-blind study the technique correctly identified the Leukaemia gene (FLT3/ITD mutation) if it was present in the patient's blood sample [G]. In the words of Prof Jason Reed, Massey Cancer Center at VCU *"Testing for pathological DNA structural changes is a $1 billion plus market annually [in the US alone], the largest fraction being from blood cancers. Roughly 20% of these patients cannot be properly diagnosed because current DNA diagnostic technologies deliver equivocal results at an unacceptable rate.... our method, making use of high-speed AFM technology addresses these issues."* [G].

Vitamica: rapid diagnostics improves targeting of antibiotics

"In the UK alone, failure of antibiotics to treat urinary infections has fatal consequences for almost 5,000 people and costs the NHS almost GBP1billion a year in hospitalisations" [I, J]. Current tests to identify bacteria take 24-72 hours which is too slow to guide the choice of initial antibiotic. Vitamica produces an instrument which uses Bristol's SCFI method [5], and which will reduce the test time to one hour. Vitamica's CEO said: *"…we have produced promising results for a range of antibiotics commonly used to treat UTIs. We now need to extend this testing to bacteria in other samples such as blood where sepsis is a real threat to life."* [J] Vitamica was founded in 2018, with Antognozzi as CTO, and their instrument is currently being readied for market. They have so far raised over GBP500,000 investment and employ 3 FTE [J].

In summary, high-speed AFM technology developed at the University of Bristol has generated 46.5 FTE jobs, attracted GBP6.8 million in investment, and made vital contributions across a range of commercial and academic settings. It has enabled the progress towards ever smaller microchip circuitry, it has provided unique capability for addressing industrial problems, and it is helping to meet key diagnostic challenges in healthcare.

5. Sources to corroborate the impact

[A] ARC InterCapital (2015). Infinitesima Ltd announces major RPM design win with Zeiss

[B] ZEISS (2015). Press Release: ZEISS pursues new techniques in ebeam mask repairs

[C] Infinitesima Ltd (2020). Supporting Letter – Chief Technology Officer

[D] NuNano Ltd (2020). Supporting Letter – Managing Director

[E] Bristol Nano Dynamics (2020). Supporting Letter – Director

[F] i) UoB TRAC facility (2020). Supporting Letter – Director
ii) EDF (2020). Supporting Letter – Engineering & Structural Integrity Representatives

[G] Virginia Commonwealth University (2020). Supporting Letter

[H] Reed *et al.* (2019). US Patent: US20190352710A1

[I] NIHR report (2016) Point-of-care testing for urinary tract infections

[J] Vitamica Ltd (2020). Supporting Letter – Chief Executive Officer