Institution: Imperial College London

Unit of Assessment: 5 Biological Sciences

Title of case study: 11 - Ionscope and development of novel scanning Ion Conductance Microscopes

1. Summary of the impact (indicative maximum 100 words)

Researchers at Imperial College London have established a spin-out company called Ionscope Ltd which develops and sells Scanning Ion Conductance Microscopes (SICM). This is a novel technology that can (i) characterise live cells and their derivatives non-destructively during differentiation and development, (ii) correlate biophysical features at unprecedented resolution with detailed transcriptional information on a single cell level, and (iii) steer cell fate by mechanical stimulus. Other high magnification techniques interfere with or kill living cells, whereas SICM is benign, allowing living cells to be studied over long periods, making it a highly desirable technology for all groups working within biomedical research. The technique has application in the study of living processes at nano-scale, which to date has included neurons, heart muscle, kidney, sperm and stem cells. Ionscope Ltd sales since 2009 have totalled [text removed for publication], with the company registering a 20% increase in its revenue over the past 5 years.

2. Underpinning research (indicative maximum 500 words)

Over the past decade researchers at Imperial have pioneered scanning ion conductance microscopy (SICM) of living cells. SICM and a battery of associated innovative methods are unique among current imaging techniques, not only in spatial resolution of living and functioning cells, but also in the rich combination of imaging with other functional and dynamic interrogation methods, and deposition methods.

These methods facilitate the nanoscale study of the organization of molecules on the cell surface and how this changes with time in both healthy and diseased cells. The SICM system uses a micro- or nanopipette to probe and scan the surface of living cells in real time with resolution comparable with Scanning Electron Microscopy. SICM can image very convoluted living cell surfaces at high resolution. This can be used for patch-clamp recording, iontophoretic delivery of reagents, mechanical characterisation and stimulation, simultaneous surface confocal imaging on a millisecond timescale, combined SICM monitoring of chemical reagents and cell respiration.

SICM is based on a scanned nanopipette where the reduction in ion currents is detected as the pipette nears a surface without direct contact. SICM was proposed to study ion current flow through narrow pores but was unreliable and limited to flat polymeric films. In 1997 Y. Korchev and co-workers realised that using very shallow set point (below 1% of ion current drop) SICM can be used for non-invasive imaging of living cell surfaces with high resolution (Korchev et al 1997).

However direct current (DC) feedback was prompted to drift and in 2001 Y. Korchev and co-workers at Imperial College introduced distance modulated feedback control [1]. These led up to the creation of Ionscope in 2004. The technology was used to perform local measurements of proteins in the cell membrane and to map the distribution of single ion channels/receptor [2].

The Imperial researchers' recent advance of "hopping-mode" SICM allows the imaging of very convoluted living cell surfaces at high resolution [3]. Combination of SICM with surface confocal fluorescence [4] and electrochemical [5] measurements brought a new dimension into live functional imaging. This is a "disruptive" technology allowing the organization and function to be probed at a resolution below 20 nm in living cells, which other high resolution techniques cannot do.

Key Researchers:
Professor Yuri Korchev, Imperial College London, Professor of Biophysics, (2005 – present)

Professor Max Lab, Imperial College London, Senior Research Investigator, (2000-present)
Dr Andrew Shevchuk, Imperial College London, Research assistant, (2003-2012)
Dr Pavel Novak, Imperial College London, Research assistant, (2007 – 2013)
Professor David Klenerman, Cambridge University, Professor of Chemistry, (2007 – present)

Ionscope Ltd was founded to commercially exploit the intellectual property surrounding the SICM technology. The underpinning research work was collaboration between Imperial College and Cambridge University. Dr D Klenerman is a scientific inventor on two patents and Cambridge University hold founding shares in Ionscope. Imperial College is the majority shareholder and retains the rights to execute the patents listed in this document (e.g. [6]).

3. References to the research (* References that best indicate quality of underpinning research)

[1] Shevchuk, A. I., J. Gorelik, S. E. Harding, M. J. Lab, D. Klenerman, and Y. E. Korchev, 'Simultaneous measurement of *Ca2+ and cellular dynamics: combined scanning ion conductance and optical microscopy to study contracting cardiac myocytes',* Biophys. J., 81, 1759-1764 (2001). DOI, **77 citations (as at 30/10/13)**.

[2] * Korchev, Y. E., Y. A. Negulyaev, C. R. Edwards, I. Vodyanoy, and M. J. Lab, '*Functional localization of single active ion channels on the surface of a living cell*', Nat. Cell Biol., 2, 616-619 (2000). DOI, **90 citations (as at 30/10/13)**.

[3] * Novak, P., C. Li, A. I. Shevchuk, R. Stepanyan, M. Caldwell, S. Hughes, T. G. Smart, J. Gorelik, V. P. Ostanin, M. J. Lab, G. W. Moss, G. I. Frolenkov, D. Klenerman, and Y. E. Korchev, '*Nanoscale live-cell imaging using hopping probe ion conductance microscopy*', Nat. Methods, 6, 279-281 (2009). DOI, **102 citations (as at 30/10/13)**.

[4] * Nikolaev, V. O., A. Moshkov, A. R. Lyon, M. Miragoli, P. Novak, H. Paur, M. J. Lohse, Y. E. Korchev, S. E. Harding, and J. Gorelik, '*β_2-adrenergic receptor redistribution in heart failure changes cAMP compartmentation*', Science, 327, 1653-1657 (2010). DOI, **95 citations (as at 30/10/13).**

[5] Takahashi, Y., A. I. Shevchuk, P. Novak, Y. Murakami, H. Shiku, Y. E. Korchev, and T. Matsue, '*Simultaneous noncontact topography and electrochemical imaging by SECM/SICM featuring ion current feedback regulation*', J. Am. Chem. Soc., 132, 10118-10126 (2010). DOI, **56 citations (as at 30/10/13).**

[6] **Patent**, WO 2008/015428, '*Scanning ion conductance microscopy for the investigation of living cells*', Applicant: Iionscopt Ltd, Y. Korchev, M.J. Lab, D.P. Sanchez-Herrera, Inventors: Y. Korchev, M.J. Lab, D.P. Sanchez-Herrera, Filing date: 1/8/07, Publication date: 7/2/08

Grants:

[G1] BBSRC, BB/D020875/1, 'Development of the next generation of sicm for live cell imaging', PI: Y Korchev, 01/04/07-31/03/10, £336,524

[G2] BBSRC, C19021, 'Study of ion channels in sub-cellular structures using automated high resolution scanning patch clamp', PI: Y Korchev, 13/10/03-12/10/06, £281,688

[G3] BBSRC, E12931, 'Simultaneous fluorescence and topographic imaging of live cells: towards a new and general method for functional imaging', PI: Y Korchev, 07/06/00-04/10/03, £263,532

4. Details of the impact (indicative maximum 750 words)

Professor Korchev established Ionscope in 2004 with Prof David Klenerman to exploit advances in scanning ion conductance microscopy [A]. The main aim of the spin-out company was to supply fully assembled SICM instruments to the biological community allowing them access to this technology. Both Imperial College and Cambridge University have shares in Ionscope and the intellectual property developed is incorporated into the company. Imperial College is a major shareholder in the company and Professor Korchev is non-executive Chief Scientist of Ionscope [B].

In addition to patent [6], Imperial College retains the rights to execute the following patents:

WO 2000/063736	Optical microscopy and its use in the study of cells

WO 2002/102504	The production of molecular arrays
WO 2002/077627	Patch-clamping and its use in analysing subcellular features
WO 2007/042776	Modulators of the purinergic signalling pathway for treating sodium homeostatsis, hypertension and aldosteronism
WO/2009/095720	Scanning ion conductance microscopy

Ionscope currently employs 4 members of staff [C]. In 2007 it secured £680k in investment from individual investors belonging to the Cambridge Angels Group [D] and was awarded the University Spin-Out award at The Engineer Technology and Innovation Awards [E]. This award recognises excellence in a business formed within the last five years to commercially exploit a technical innovation, product, process or centre of expertise originally conceived within a university. Ionscope currently has operations in nine countries: UK, USA, China, Japan, Germany, France, Canada, Mexico, Australia and Taiwan [F]. In the UK it is based at the Melbourne Science Park outside Cambridge.

[text removed for publication]

Ionscope's first product was called ICnano, and provided a platform for high resolution imaging of living cell membranes, and soft or fragile surface features without making contact with the surface of the sample. Since 2008, 3 variants of the instrument have been sold internationally.

In 2012 the company introduced the ICnano 2000 series [I]. This is a new generation of microscopes based on the recently developed "hopping-mode" SICM (2009) with the advanced controller which includes an embedded processor, field-programmable gate array (FPGA) with digital signal processing (DSP) functionality. The ICnano2243 [J] is the top range in the ICnano 2000 series and combines both sample scanning and probe scanning in a single scan head. This gives the advantages of large lateral scan range and fast probe scanning and can outperform the most of assays developed at Imperial College.

The research and products developed are considered transformative and a novel disruptive technology that will redefine this commercial arena. Ionoscope Ltd currently has six novel products that are marketed internationally [K]. SICM microscopy was originally developed for biological applications. However, it has other applications in broader fields such as electrochemistry. The SICM microscope is now used in energy research in characterizing and improving our understanding of how and why lithium ion batteries fail [L].

Ionscope's Scanning Ion Conductance Microscope (SICM) products are in use today across the globe, providing answers to difficult measurement problems in topographic scanning. In the past three years the number of publications using Ionscope technology has more than doubled reflecting an increasing interest in adopting SICM technique in different fields of research [M]. ICnano products are used in cellular biology to investigate membrane processes at the nano-scale. In addition to its function as an imaging tool, ICnano products have been integrated with other measurement techniques, including: fluorescence microscopy, for combined topography and florescent marker detection electrophysiological measurements; for topography guided patch-clamp recordings and measurement of ion channels in non-biological membranes. The nanopipette used as probe can be exploited to allow: nano-deposition, for local application of chemicals; and pipette pressure control, for local force application and displacement measurement [N].

In November 2012 Ionscope welcomed international attendees to its first Scanning Ion Conductance Microscope (SICM) User Group Meeting held in Cambridge (UK) [6]. At the meeting current and future developments in the company's range of products for SICM were described by Chris Moore, the former CEO of Ionscope. The included a new scan head for sample or probe scanning, hopping mode raster scanning, high-speed Z-control, topography-guided positioning and an environmental chamber [6]. The meeting demonstrated the wide adoption of the SICM technology, with delegates discussing applications as varied as: (i) studying ion channel distribution and function in renal epithelia and neuroblastoma cells, (ii) the combined use of SICM and patch-clamp to study the clustering of sodium channels in cardiac Myocytes, (iii) the

application of SICM to the analysis of membrane structure and second messenger compartmentation in cardiomyocytes, and (iv) applications in plant science to reveal the structure of the anti-fungal spider web on strawberry leaves, and platelet structures on pea leaves [O].

5. Sources to corroborate the impact (indicative maximum of 10 references)

[A] Ionscope History, http://www.ionscope.com/company/history/ (archived at https://www.imperial.ac.uk/ref/webarchive/c3f on 5/11/13)
[B] Ionscope People, http://www.ionscope.com/company/people/ (archived at https://www.imperial.ac.uk/ref/webarchive/d3f on 5/11/13)
[C] Applications Scientist, Ionscope Ltd
[D] 'Ionscope raises funds', Imperial Innovations press release, 7/8/07, http://www.imperialinnovations.co.uk/news-centre/news/ionscope-raises-funds/ (archived at https://www.imperial.ac.uk/ref/webarchive/v2f on 4/11/13)
[E] Ionoscope spin out of the year, http://www.theengineer.co.uk/news/ionscope/university-of-cambridge/imperial-college-london/300350.article (archived at https://www.imperial.ac.uk/ref/webarchive/f3f on 5/11/13)
[F] Ionscope Contacts, http://www.ionscope.com/contact (archived at https://www.imperial.ac.uk/ref/webarchive/g3f on 5/11/13)
[G] Extract from Ionscope Ltd annual accounts, 2009-2010 (available from Imperial on request)
[H] Extract from Ionscope Ltd annual accounts, 2011-2012 (available here Imperial on request)
[I] Ionscope 2000 series, http://www.ionscope.com/products/in-2000-series/ (archived at https://www.imperial.ac.uk/ref/webarchive/h3f on 5/11/13)
[J] Ionscope ICnano2243, http://www.ionscope.com/products/icnano2243/ (archived at https://www.imperial.ac.uk/ref/webarchive/j3f on 5/11/13)
[K] The Ionoscope now has six novel products that are marketed internationally, http://www.ionscope.com/products (archived at https://www.imperial.ac.uk/ref/webarchive/k3f on 5/11/13)
[L] Stimulation and development of new markets and technologies, http://www.energyfrontier.us/newsletter/201204/diving-lithium-ion-batteries (archived at https://www.imperial.ac.uk/ref/webarchive/l3f on 5/11/13); http://web.anl.gov/energy-storage-science/news/sicm.html (archived at https://www.imperial.ac.uk/ref/webarchive/m3f on 5/11/13); http://www.mrs.org/s11-abstract-l/ (archived at https://www.imperial.ac.uk/ref/webarchive/n3f on 5/11/13)
[M] Publications citing Ionscope technology, http://www.ionscope.com/research/publications/ (archived here)
[N] Ionscope Applications, http://www.ionscope.com/research/ (archived at https://www.imperial.ac.uk/ref/webarchive/p3f on 5/11/13)
[O] Ionoscope users meeting, http://www.microscopy-analysis.com/sites/default/files/magazine_pdfs/mag%20SICM_Cambridge_April2013.pdf (archived here)