Institution: Imperial College London

Unit of Assessment: 9 Physics

Title of case study: P6 - Label Free Intrinsic Imaging LFII: A New Platform for Separation Science

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

Work since 1993 at Imperial College has led to a novel generic approach to Separation Science called: Label Free Intrinsic Imaging (LFII) - with applications in high throughput proteomics, metabolomics, analytical chemistry, health care diagnostics and genomics. LFII was commercialised by Imperial spin-out deltaDOT Ltd in Nov 2000. Sales of LFII products by deltaDOT have been made to various sectors including pharmaceutical, analytical and diagnostic companies. The average annual turnover of deltaDOT Ltd in the REF period was >£600k per annum and the net worth and total assets in 2012 were £569,595 and £808,027, respectively. The deltaDOT technology has been adopted by the US multi-million dollar DARPA-funded [Defense Advanced Research Project Agency] rapid-vaccine development programme. In 2009 an affiliate company, deltaDOT QSTP-LLC, was formed in Qatar, developing a world-class proteomics research and testing facility in Doha also entirely based on the LFII technology. Since its formation deltaDOT QSTP-LLC has generated total revenues of >$10M.

2. Underpinning research (indicative maximum 500 words)

The LFII system combines UV absorption, photodiode array detection, automated fluidic handling and digital signal processing to map the space-time trajectories of bioanalytes and chemicals in capillaries. The underpinning research relates to the signal-to-noise enhancing image processing algorithms and the methodology associated with analysis of very large data sets taken from each molecule as it traverses each pixel at a specific time point. This allows a space-time correlation called vertexing to be achieved. Vertexing enables a vastly increased signal to noise ratio to be acquired allowing for molecular imaging that exceeds traditional single-detector absorption imaging techniques and for molecules that have significant UV absorption it allows for a label free methodology creating a superior performance over conventional capillary electrophoresis.

The pattern recognition and track-finding tools, known as vertexing, were developed by S Margetides and J Hassard, working within the High Energy Physics (HEP) group at Imperial, for tracking and identifying high energy particles at CERN. The work was written up as a conference paper in 1993 [1].

The underpinning research that resulted in the LFII technology for molecular detection has been captured in a series of patents, originating from academics within the Physics department published from 1996 to 2004 [P1-P3]. LFII generates massive data files, which need to be processed, integrated, visualised and interpreted in real time. The development of this type of analysis was supported during the 2001-2005 period by the EPSRC grant [G1].

Colling and Hassard generalised the vertexing methodology, calling the technique the 'Generalised Separation Transform', (GST). The method is capable of finding and separating objects in one or more dimensions by correlating peaks in velocity space. It was patented in 1996, as a method of identifying individual substances within a mixture of substances. The mixture travels past a spaced series of detectors, each being arranged to produce a signal representative of a characteristic of the mixture as it passes. Readings are taken repeatedly from each detector which allows individual substances to be identified within the mixture according to the peaks they create in velocity space [P1].

The method was developed further by J Hassard, S Hassard (both Imperial staff) and A Mainwood (Kings College) with specific application to biological molecules where the molecules of interest are good UV absorbers. This was patented in 2003 [P2].

D Sideris (HEP group member) refined the vertexing concept using information typically available

at the analyte injection point (for example in a Capillary Electrophoresis separation tool) and was able to improve the signal-to-noise considerably. This was patented by Sideris in 2004 (along with William Sanders, John Hassard and Stuart Hassard) [P3]. In 2004 deltaDOT built the world's first label-free sequencer in 2004 and much intellectual property was generated from this point forward by the company at the incubator site at Imperial College.

Enhancement of the fundamental capability of label free multipoint UV detection was demonstrated for double stranded DNA molecules of different lengths using the LFII technology in 2009 [2].

Key researchers:
From 1994-2000 all LFII work was academic in nature and took place solely at Imperial by Imperial staff. From 2000-2013, LFII work has been a mixture of commercial and academic, and until 2009 was exclusively undertaken in the Imperial incubator and sites. Since 2009, the LFII work has continued to be driven by Imperial personnel. In addition to the lead researcher J Hassard, Imperial researchers include S. Margetides, F. Pereira, and D. Colling (all of HEP).

Key contextual information about this area of research.
HEP analysis required the isolation and identification of particles through their 2D or 3D space and 3D or 4D space-time correlations. Three central driving issues in that analysis method that were all transferred over to the LFII platform were the painstaking attention to the identification, minimisation and removal of inherent biases, the very high throughput necessary, and the very small scales involved. All of these gave HEP technology a head start in biotechnological and microfluidic separations, where small really is beautiful.

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

Papers and patents:

[1] J.F. Hassard and S. Margetides, "*K0s Finding Efficiencies in Increasing Luminosities*", Proceedings of the Workshop on B Physics at Hadron Accelerators, Snowmass, Colorado, June 21 – July 2, p279 (1993) [Available here]

[2] * Pereira, F., Hassard, S., Hassard, J. and de Mello, A, "*CE of dsDNA in low-molecular-weight polyethylene oxide solutions*", Electrophoresis, Volume 30, Issue 12, pages 2100–2109 (2009). DOI.

[P1] * '*Molecular imaging*', WO1996035946 A1/US Patent 6103533 A, Inventors: David Colling, John Hassard, Publication date: 14-11-1996, Applicant: David Colling, John Hassard, Imperial College London.

[P2] * '*Molecular imaging*', US6613210 B1/WO9635946, Inventors: John Hassard, Stuart Hassard, Alison Mainwood, Publication date: 02-09-2003, applicant: Imperial College

[P3] "*Method and apparatus for separating biological molecules*", US2004100268 A1, Inventors Stuart Hassard, John Hassard, Giles Sanders, Dimitrios Sideris, published 27-05-2004, [N.B. applicants were the named inventors & deltaDOT]

Grants:

[G1] GR/R67750/01, 'Discovery Net: An e-Science Test Bed for high Throughput Informatics', 01/10/01-31/03/05, £2,082,704, PI: Y Guo (Imperial College London, Department of Computing) [N.B. LFII component of grant was ~£400k]

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

deltaDOT Ltd was spun out of Imperial College London via Imperial Innovations in 2000 and consists of a team of scientists and engineers dedicated to the advancement of analytical techniques using patented methods and instrumentation in High Performance Capillary Electrophoresis (HPCE). deltaDOT designs and produces HPCE instruments which are marketed worldwide. The offices and laboratories are based in the London Bioscience Innovation Centre (LBIC) [A]. The average annual turnover of deltaDOT Ltd from 2008-2012 was £640.2k per annum, with turnover sales consisting of "*sales of instruments and from service fees, contract research and analytical work*", with much of these sales representing exports [B].

The HPCE analytical instruments are designed, manufactured and marketed by deltaDOT Ltd. These instruments use deltaDOT's proprietary imaging and analysis technology – LFII. HPCE and LFII applied to a Capillary Electrophoresis (CE) analytical system offers dramatically improved discovery power in comparison to other conventional CE systems. HPCE is applied in Life Sciences research, drug testing, diagnostics and bio-pharmaceutical manufacturing. The technology is used in detection, separation and analysis of proteins, nucleic acids, carbohydrates, viruses and bacteria, chemicals and a wide range of other analytes [A]. In 2012 deltaDOT Ltd signed an agreement with the major distributor HORIBA UK for sales in the UK and Eire [L]. HORIBA UK currently offers the HPCE-512 from deltaDOT Ltd [C].

deltaDOT has raised over $12M equity (e.g. [D]) over the last 15 years. It has been profitable since 2010, after a Buy-in Management Buy-Out led by Hassard and Nigel Stokes, an Imperial alumnus. The latest Annual Accounts submitted to Companies House for the year up to 31/12/2012 reported 'cash at bank' of £257,601, 'net worth' of £569,595 and 'assets' worth £808,027 [E]. All of the revenues generated by deltaDOT Ltd are based on Imperial HEP research [B].

In addition to the specific cases outlined below, sales of LFII instruments by deltaDOT Ltd have been to various sectors and include (i) five global pharmaceutical companies, (ii) a global analytical company, (iii) a US diagnostic company, and (iv) UK and US universities [B].

2009 saw the formation of the affiliate company, **deltaDOT QSTP-LLC**, in Qatar. The company was launched as a joint venture between the Qatar Science and Technology Park (QSTP) and deltaDOT Ltd [F]. The company developed a 500-square-meter world class proteomics research and testing facility in Doha which was the Arab emirate's first such facility [G]. QSTP invested $15 million in the project. $5 million of that was an equity investment in deltaDOT Ltd, which provided staff, expert support and an exclusive regional licence to its technology. The remaining $10M set up the proteomics facility at QSTP [B, C, F]. Discussing the investment, Dr Eulian Roberts, Managing Director of QSTP, said: "*We hope that this new venture will result in ground breaking findings in the field of Proteomics which will then find applications locally and internationally. The establishment of deltaDOT QSTP-LLC also falls in line with the Qatar National Vision 2030 which is for Qatar to be an active centre in the fields of scientific research and intellectual activity*" [F].

Since its formation deltaDOT QSTP-LLC has generated revenues of $1.7M, $3.5M, $3.7M and $1.8M per annum for the years 2009-2012 respectively. John Hassard has been the CEO of deltaDOT QSTP-LLC during that time. In July 2012 deltaDOT Ltd sold its 20% stake in deltaDOT QSTP–LLC for circa £550,000 [B]. As is the case with deltaDOT Ltd, all the IP used by deltaDOT QSTP-LLC is based on the research from the HEP group at Imperial College [B]. A two-way IP pipeline agreement exists between deltaDOT Ltd and deltaDOT QSTP-LLC [B].

Since 2008, examples of the use of the LFII system produced by deltaDOT include:

Defence Advanced Research Project Agency (DARPA)
deltaDot Ltd's LFII, in the form of the Peregrine HPCE system, was adopted by the DARPA-funded Accelerated Manufacture of Pharmaceuticals (AMP) programme, and by the Defense Threat Reduction Agency's rapid vaccine programme [H, I]. The Peregrine was adopted as a competing analytical tool for one of the several Rapid Vaccine development programmes under the aegis of the AMP programme, working with a consortium which included Dow Chemicals and Xcellerex. Only "*deltaDOTs analytical technology went through to the next stage*" [J]. The rapid vaccine programme was established by the US Department of Health and Human Services (HHS) to enhance the nation's emergency preparedness against emerging infectious diseases and "*deltaDOT's technology was adopted as one of the few non-US contributors to the program*" [J]. The LFII approach was praised for its "*adaptability across a range of targets" and for "allowing rapid analysis of contaminants, protein post-translational modifications, as well as allowing a highly quantitate, unbiased, sensitive and reproducible analysis over a broad dynamic range in both molecular weight and concentration*". The AMP programme is "*required to generate 50 million vaccine doses within 120 days of identifying a threat's genome*" and the "*Quality Control provided*

by the LFII approach is uniquely suited to this demanding task" [J].

In 2010, as part of this programme, "*Xcellerex, Inc. and Pfenex Inc. announced…that the companies, along with deltaDOT Ltd. and BioPharm Services have successfully demonstrated the production of purified swine flu H1 hemagglutinin (California strain) in 42 days starting from the amino acid sequence of the protein*" [I]. During that period the product quality was "*shown to be fully within the specifications set out by the Defense Threat Reduction Agency (DTRA) under its Accelerated Manufacturing of Pharmaceuticals contract*" with the results of the programme providing "valuable tools for biodefense and pandemic response" [I]. As part of the programme "*analytical capability was supplied by deltaDOT*" [I].

Caliber biotherapeutics

Over the past 3 years Caliber Biotherapeutics, a US-based a biotechnology company with a mission to develop and commercialize protein-based therapeutics that improve outcomes for patients with cancer and other diseases, has been using the deltaDOT Ltd HPCE instrument (HPCE-512). It has been used in (i) reduced and native antibody analysis as part of their antibody-biobetter project and to be used in future antibody-conjugate projects, (ii) Nicotine analysis at picomolar levels, (iii) analysis of pesticides down to very low levels, (iv) batch-to-batch analysis of products, for demonstrate consistency. As a result Caliber Biotherapeutics is determined that deltaDOT's technology is useful for providing *"in-process" analytical information that is useful for bioprocessing*" and as a result will be purchasing further deltaDOT Ltd instruments for QA/QC use in its manufacturing processes. Calilber Biotherapeutics also praised the reliability of product [K].

Oil & Gas sector

Since 2010 LFII has been used for profiling in the oil and gas sector [J], namely with Qatar Petrolium and Total E&P, where a programme to build real-time bespoke oil-head tools is underway, funded by Middle East and North Africa (MENA)-based O&G companies.

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

[A] deltaDOT website, http://www.deltadot.com/ (archived at https://www.imperial.ac.uk/ref/webarchive/1pf on 8/8/13)
[B] Letter from Managing Director, deltaDOT Ltd, 12/9/13 (available on request)
[C] HORIBA UK HPCE-12 product brochure (available here)
[D] thepharmaletter.com, 'deltaDOT raises $10.5M in private financing', 07/4/06, http://www.thepharmaletter.com/file/28126/deltadot-raises-105m-in-private-financing.html (archived at https://www.imperial.ac.uk/ref/webarchive/2pf on 8/8/13)
[E] Company Check, http://companycheck.co.uk/company/03949707/DELTADOT-LIMITED/company-summary (archived at https://www.imperial.ac.uk/ref/webarchive/3pf on 8/8/13)
[F] UKSPA news, 'QSTP and deltaDOT Ltd partner to develop world class proteomics research and development centre', 16/6/09, http://www.ukspa.org.uk/news/content/2481/ (archived at https://www.imperial.ac.uk/ref/webarchive/vsf on 24/9/13)
[G] Genoneweb.com, 'DeltaDot-QSTP JV to Create Qatar's First Proteomics Facility', 18/6/09, http://www.genomeweb.com/proteomics/deltadot-qstp-jv-create-qatars-first-proteomics-facility (archived at https://www.imperial.ac.uk/ref/webarchive/4pf on 8/8/13)
[H] 'Xcellerex, Dowpharma, Biopharm, and deltaDot Win $11M Technology Development Contract', 24/3/09, http://www.genengnews.com/gen-news-highlights/xcellerex-dowpharma-biopharm-and-deltadot-win-11m-technology/51735090/ (archived at https://www.imperial.ac.uk/ref/webarchive/ypf on 8/8/13)
[I] 'Xcellerex, Pfenex Inc. and Collaborators Complete Successful Test: Rapid Production of Swine Flu H1 Hemagglutinin', 18/5/10, http://www.fiercepharma.com/press-releases/xcellerex-pfenex-inc-and-collaborators-complete-successful-test-rapid-production-sw-0#ixzz2IELKu5AI (archived at https://www.imperial.ac.uk/ref/webarchive/zpf on 8/8/13)
[J] Letter from Vice Chancellor, Federal and State Relations, Texas A&M University, 10/6/13 (available from Imperial on request)
[K] Letter from Director, Research & Development/Quality Laboratories, Caliber Biotherapeutics, LLC, 5/8/13 (available from Imperial on request)