Institution: Cardiff University

Unit of Assessment: UoA1

Title of case study:
Discovery and exploitation of fluorescent dyes in healthcare, drug discovery and life sciences

1. Summary of the impact

Cardiff University research in 1997-2008 resulted in the development of a family of novel far-red fluorescent dyes that stain the DNA of cells. The leading live cell dye DRAQ5™ is now utilised in a wide range of laboratory assays, transforming practice in clinical, commercial and research sectors. Smith co-founded the multi-award-winning start-up company Biostatus Ltd in 2001 to undertake product development. Commercial impact post-2008 has been the generation of over $3.2 million in sales revenue enabling job creation, direct funding of UK academic research positions and creation of new technology start-up companies. Used in over 3,500 research, pharmaceutical and clinical organisations, DRAQ™ technology has global reach.

2. Underpinning research

Research on an unmet need: The study of cells and tissues frequently utilizes fluorescent reagents, in particular dyes that can stain nuclear DNA. Live cell DNA-specific dyes are especially valuable since they can indicate cell cycle stage while at the same time preserving cell function. Combining a live cell dye with other fluorescent tags enhances 'multi-colour' analyses on a range of cell imaging and cytometry instruments. Such analyses encompass a wide range of clinical and biomedical laboratory assays in research, diagnostic testing and high-throughput screens. Before 2001, the choice of live cell dyes was highly restricted and required an expensive UV laser for detection or visualisation. Cardiff research focused on this unmet need.

Research at Cardiff University led by Paul Smith (Professor of Cancer Biology; 1995-2013) had discovered the complex intracellular far-red fluorescence patterns of drug-like anthraquinone molecules[3.1]. In 1997 Smith initiated the underpinning research, funded by the MRC and the Joint Research Equipment Initiative, to explore new molecular probes for use in the life sciences. Paul Smith developed a new class of anthraquinone-based DNA binding dyes that could penetrate into the nucleus of living cells, undergo excitation by low cost red-emitting (633 nm) lasers and fluoresce in the 'far red' region of the spectrum. This distinguished them from commonly used visible range fluorescent, making them valuable in a wide range of biomedical applications.

Generating and screening candidate dyes: The research involved the design of dye molecules with rapid penetration and high binding affinity but with low quantum yield to decrease the 'noise' from unbound molecules. In collaboration with Laurence Patterson (Professor; School of Pharmacy, De Montfort University), Paul Smith selected various anthraquinone structures that were synthesised by Patterson and transferred to Cardiff University for all subsequent research. During 1997-2000 Paul Smith utilized cell based imaging and flow cytometry, early on discovering an optimal dye DRAQ5™ capable of entering living cells (from microbes to human tissues) within seconds[3.2,3.3]. A further breakthrough was the discovery that DRAQ5™ could also be excited by conventional blue (488 nm) lasers making it compatible with all flow cytometry platforms[3.2] – a feature not shared by any other vital DNA dye.

Applications, funding & intellectual property:
From 2000-2008, basic research at Cardiff University by Paul Smith and Rachel Errington (Research Fellow, 1998-2003; Lecturer & Senior Lecturer 2003-2011; Reader 2011; Professor 2013-present) created multiple applications for this new class of dye molecules in the life sciences including: lab-on-a-chip applications[3.4] funded by Research Councils UK, Optical Biochips Basic Technology Programme (2003-2007), high resolution timelapse multiphoton imaging[3.5] funded by the Association for International Cancer Research (2000-2003), monitoring drug-DNA targeting and high-content-screening assays funded by the BBSRC (2003-2006 & 2007-2008). Industrial research partnerships between the Cardiff team, Amersham Biosciences PLC & Kinetic Imaging Ltd (2000-2003) established DRAQ5 dye compatibility in cell-based assays using green fluorescent proteins[3.6]. Cardiff-Biostatus Ltd partnerships [BBSRC Small Business Research Initiative grant (2003-2005) & CASE/EPSRC Studentship (2005-8)] led to the further development of

anthraquinone-based dye molecules (including CyTrak™Orange, ApoTrak™ and DRAQ7™) for commercialisation by BioStatus Ltd. The underlying and linked research has generated over £4m of research income. The core technology is protected by US-granted patents (see below) citing Cardiff University's published research.

3. References to the research

3.1 **Smith PJ**, Desnoyers, R., Blunt, N., *et al* Flow cytometric analysis and confocal imaging of anticancer alkylaminoanthraquinones and their N-oxides in intact human cells using 647-nm krypton laser excitation. *Cytometry* 1997;27:43-53 DOI: 10.1002/(SIci)1097-0320(19970101)27:1<43::AID-CYTO6>3.0.CO:2M

3.2 **Smith PJ**, Wiltshire, M., Davies, S., *et al*. A novel cell permeant and far red-fluorescing DNA probe, DRAQ5, for blood cell discrimination by flow cytometry. *J Immunol Methods* 1999;229:131-9 DOI: 10.1016/S0022-1759(99)00116-7

3.3 **Smith PJ**, Blunt, N., Wiltshire M., *et al* Characteristics of a novel deep red/infrared fluorescent cell-permeant DNA probe, DRAQ5, in intact human cells analyzed by flow cytometry, confocal and multiphoton microscopy. *Cytometry* 2000;40:280-91 DOI: 10.1002/1097-0320(20000801)40:4<280::AID-CYTO4>3.0.CO:2-7

3.4 Njoh KL, Patterson LH, Zloh M, Wiltshire M, Fisher J, Chappell S, Ameer-Beg S, Bai Y, Matthews D, Errington RJ, **Smith PJ**. Spectral analysis of the DNA targeting bisalkylaminoanthraquinone DRAQ5 in intact living cells. Cytometry A 2006;69:805-14 DOI: 10.1002/cyto.a.20308

3.5 **Errington RJ**, Ameer-beg SM, Vojnovic B *et al*. Advanced microscopy solutions for monitoring the kinetics and dynamics of drug-DNA targeting in living cells*. Adv Drug Deliv Rev* 2005;57:153-67 DOI: 10.1016/j.addr.2004.05.005

3.6 **Smith PJ**, Marquez, N., Wiltshire, M., et al. Mitotic bypass via an occult cell cycle phase following DNA topoisomerase II inhibition in p53 functional human tumor cells. *Cell Cycle* 2007;6:2071-81 DOI: 10.4161/cc.6.16.4585

Linked research funding 1996-2008

- **1996-1999.** MRC Project Grant G9526470. Development and evaluation of novel flow cytometric assays for anticancer drug induced cell cycle arrest. £220,000. PJ Smith (PI).
- **1997-2000.** MRC/HEFCW Joint Research Equipment Initiative. Two photon confocal imaging. AK Campbell (PI) & PJ Smith £535,428.
- **2000-2003.** Association for International Cancer Research: Research (AICR) Grant ref: 00-292. Title. High resolution timelapse multiphoton imaging of anticancer drugs in living tumour cells: linking single cell pharmacokinetics, tumour cell responses and treatment design through bioinformatics. PJ Smith (PI) & RJ Errington & T Hoy. £115,771.
- **2000-2003.** Amersham Biosciences PLC/Kinetic Imaging Ltd: Research Grant. Cell-based assay development. PJ Smith (PI) & RJ Errington. £123,000.
- **2003-2005.** BBSRC Research Grant SBRI19666. Far-red fluorescent dyes and novel delivery/detection systems for high throughput screening (HTS) and cell-based biotechnology. PJ Smith (PI). £182,000.
- **2003-2006.** BBSRC Grant 75/E19292. The biology of drug targeting: Predictive mathematical modelling of drug impact in complex and dynamic cell populations. PJ Smith (PI) & RJ Errington. £177,392. (A grade evaluation).
- **2003-2007.** Research Councils' UK Basic Technology Research Programme Grant GR/S23483: Optical biochips. PJ Smith (PI), P Blood, SM Ameer-Beg, PM Smowton, H Summers, B Vojnovic, RJ Errington, D Westwood, J Burt, DM Taylor , £2,271,606.
- **2005-2008.** Biostatus Ltd/Cardiff University EPSRC CASE Award RAUU001. Genomic and cell-based evolution of molecular oncotherapeutics. PJ Smith (PI) & RJ Errington, £48,257.
- **2007**. EPSRC HS-LSI feasibility grant EP/E013104/1: Stroboscopic excitation fluorescence lifetime imaging. RJ Errington (PI). £103,000.
- **2007-2008.** BBSRC Research Equipment Initiative Grant BB/E012574/1. Time-based high-content screening. RJ Errington (PI) and PJ Smith. £ 242,000

4. Details of the impact
Commercial Impact Of New Business Creation With Established Viability:

Background: The invention of DRAQ5™ led to the creation in 2001 of BioStatus Ltd (by Smith, Patterson and CEO Stefan Ogrodzinski; http://www.biostatus.com/aboutus.asp) to co-develop the dye technology, gain world-wide patent protection and develop a route for sustainable commercial impact. A significant outcome was the creation of a new market for far-red molecular probes (primary and commercially-sensitive corroboration information from BioStatus Ltd CEO; Factual Statement)[5.1,5.2]. Between 2002 and 2008, BioStatus Ltd engaged academic opinion leaders and commercial organisations with a focus on flow cytometry - winning two *New Technologies Initiative Awards* in 2005 and funding research at 4 UK universities (Cardiff, Swansea, Nottingham and Bradford). By 2009 BioStatus Ltd had secured granted DRAQ patents[5.3]. Biostatus Ltd remains privately owned and in 2009 moved its direct sales and product development operations into its own 2,500 sq ft office & laboratory facility in Shepshed, Leicestershire[5.1].

New product commercialized with revenue generation: In the impact period, DRAQ5™ alone has an accumulated earnings of $3.2m, >95% arising from export income, returning royalties of >$180K[5.1,5.2] with a conservative estimate of ~5 million sample assays performed to-date.

New linked start-ups funded by revenue from the core dye technology: In 2008 Biostatus Ltd created Biosuspensions Ltd (www.biosuspensions.com Register No.06780280) to progress its new drug and probe delivery technology and in 2009 created the award-winning Oncotherics Ltd (www.oncotherics.com; Register No.06940617) to progress a new anticancer drug based on its accumulated company expertise in molecular probe chemistry.

Global reach of the innovation: To extend commercial global reach, from 2008 BioStatus established a widening network of key distributors including: ThermoFisher (2009-), e-Bio (now Affymetrix; 2009-) and AbCam (2010-). By 2013, the reach of the technology to its research constituency was evidenced by its incorporation into research practice world-wide in over 500 academic centres (source: client management database Biostatus Ltd)[5.1,5.2]. By March 2013 the widening significance and intensity of the influence of DRAQ5™ was evidenced by its application featuring in 158 peer-reviewed articles (101 post-2008; source: SCOPUS) and a wider referencing of the use of DRAQ5™ as a standard reagent in 3,060 text articles (2,340 post-2008; source: Google Scholar). More recently, by October 2013 SCOPUS reported 925 Journal references for DRAQ5™ and 338 patent applications exploiting DRAQ technology published world-wide.

Recognition of impact: Successful translation of research through to product impact was recognised by the award of the *2012 Royal Society of Chemistry Teamwork in Innovation Award* to Smith, Patterson, Errington and Biostatus Ltd, "For worldwide exploitation and impact of novel fluorescent molecular probes and cell detection technologies in drug discovery, clinical diagnostics & the life sciences" [5.4].

Job creation: BioStatus Ltd is an ISO 9001 company, currently employs 8 people and supports employment indirectly though contracted activities for legal, synthesis and business support services[5.1,5.2].

Impact On Business And New Medicines Discovery Sector Through Adoption Of A New Technology:

Early partnerships with antibody suppliers (Cell Signalling Technologies Inc, USA; www.cellsignal.com), major instrument developers (Amnis Corporation; now EMD Millipore) and high-throughput assay developers (Norak Biosciences, USA) led to validation for multi-colour microscopy and imaging cytometry with product adoption by March 2008[5.1,5.2].

DRAQ5™ (product overview video: http://www.biostatus.com/SearchResults.asp?Cat=1889) is now used extensively on discovery imaging platforms with beneficiaries being: GlaxoSmithKline, the world's leading pharmaceutical company (GSK ranks #1, in the 2012 Access to Medicine Index), other major drug companies (Roche, Bayer Schering & Takeda/Nycomed) and contract high-throughput screening organisations (eg Odyssey Thera) involved in new medicine development for pharmaceutical clients. Here the commercial impact has been a simplification of cell identification routines in imaging-based screens, cost-reduction/well, verified compatibility with

multiple green fluorescent protein reporter-based assays[3.6] and reduced attrition of naturally fluorescent drug candidates in screens[5.1].

Impact On Health Through Delivering Improvements To The Analysis Of Cells

New reagents for clinical diagnostics: DRAQ technology has had the impact of delivering improvements to the accuracy of diagnostic assays in a wide range of flow cytometry applications while providing previously unattainable information or cost-savings in work flows. An early trial in 2004 showed that DRAQ™ technology was readily adoptable by regional flow cytometry clinical laboratory services employing cost-saving and automated cytometers[5.5]. DRAQ5 is a validated reagent in multiple applications, typical examples are: improved rare cell detection in clinical diagnosis[5.6, 5.7], more accurate myeloid to erythroid precursors ratio determinations and a simplified workflow in bone marrow analysis for haemato-oncology[5.8], and improved detection of nucleated erythroblasts[5.9].

Evidence of widespread adoption in clinical assays: DRAQ5™ is featured directly in 29 independent medical publications (source: SCOPUS search < DRAQ5>) providing evidence of the role of DRAQ5™ in improving different clinical assays as a generic probe but for multiple cell types – typical examples being: DRAQ5™ permitting the optimization of multi-colour approaches for the clinical analysis of cancer cells, DRAQ5™ increasing the speed of assays by allowing single-step processing of clinical samples such as bone marrow aspirates, DRAQ5™ simplifying assays by allowing nucleated cells to be identified in blood and bone marrow samples. Further DRAQ5™ introduces a new cell descriptor to enhance accuracy of diagnosis in lymphoproliferative disorders and plasma cell neoplasias. DRAQ5™ enables for the first time the simple extraction of proliferation index of specific bone marrow cell compartments – an important feature linked to patient outcomes. The core technology also encompasses new derivatives. For example DRAQ7 detects cell viability and is to be supplied in 2013 as a validated probe[5.10] by Beckman Coulter Inc – a leading in vitro diagnostics flow cytometry company. DRAQ7 is being evaluated further for an improved version of the ISHAGE protocol for Paroxysmal Nocturnal Hemoglobinuria diagnostics and for the standardization of flow cytometry for myelodysplastic syndromes.

5. Sources to corroborate the impact

5.1 **Factual Statement**: CEO Biostatus Ltd (Provides corroboration of company activities)

5.2 **Contact Person:** CEO BioStatus Limited (Source corroboration of financial and client information)

5.3 **Combined pdf document of US patents**: 6,468,753 2002; 7,060,427 2006; 7,605,280 2009: Smith PJ, Patterson LH; BioStatus Limited, assignee; title: Anthraquinone and its derivatives (backs up claims of commercial impact). <http://patft.uspto.gov/netacgi/nph-Parser?Sect1=PTO2&Sect2=HITOFF&p=1&u=%2Fnetahtml%2FPTO%2Fsearch-bool.html&r=0&f=S&l=50&TERM1=Anthraquinone+and+its+derivatives&FIELD1=TI&co1=AND&TERM2=Biostatus&FIELD2=ASNM&d=PTXT>

5.4 **Scientific Organisation website**: http://www.rsc.org/ScienceAndTechnology/Awards/TeamworkinInnovation/2012-Winner.asp (backs up claims of recognition of impact)

5.5 **Research document**: Luider J, Cyfra M, Johnson P & Auer I. Lab Hematol. 2004;10(2):102-8 (backs up claims of new reagents for clinical diagnosis).

5.6 **Research document**: Kraan, J., et al. Journal of Thrombosis and Haemostasis 10.5 (2012): 931-939 (backs up claims of new reagents for clinical diagnosis).

5.7 **Research document**: Swerts, K., et al. Clinica chimica acta 379.1 (2007): 154-157 (backs up claims of new reagents for clinical diagnosis).

5.8 **Research document**: Allan, R.W., et al. Am J Clin Path 129.5 (2008): 706-713 (backs up claims of new reagents for clinical diagnosis)

5.9 **Research document**: A van de Geijn, G-J, et al. Cytometry Part A79.9 (2011): 694-706 (backs up claims of new reagents for clinical diagnosis)

5.10 **Research document**: Akagi, J et al. Cytometry A (2013) 83(2): 227-34 (backs up claims of adoption for clinical assays)**.**