Institution: University of Strathclyde		
Unit of Assessment: B9 - Physics		
Title of case study: Market-leading fluorescence products for global multidisciplinary markets		
Period when the underpinning research was undertaken: 2000-2019		
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:**
Prof David Birch	Professor	01/09/1978 – present
Dr Olaf Rolinski	Senior Lecturer	14/04/1994 – present
Dr Yu Chen	Reader	02/04/2007 – present
Period when the claimed impact occurred: August 2013 – December 2020		
Is this case study continued from a case study submitted in 2014? No		

1. Summary of the impact

Strathclyde fluorescence lifetime research continues to contribute to growth in the commercial success of spin-out scientific equipment manufacturer HORIBA Jobin Yvon IBH Ltd (HORIBA-IBH). The company develops products exploiting the technical, operational and cost advantages of high repetition rate semiconductor light sources and photon detectors for markets spanning life sciences, healthcare, chemistry, nanotechnology, and solar energy. Recent expansion of its facilities in Glasgow has supported growth in HORIBA's leading 16% share of the USD152,000,000 global fluorescence spectroscopy market in 2013 to 22% in 2020. Recently the company introduced rapid fluorescence lifetime imaging microscopy (FLIM) for the study in real time of live cells and transient species which is impacting on a range of medical applications. Strathclyde and HORIBA's collaboration was recognised through Institute of Physics Awards in 2019 and 2020.

2. Underpinning research

Context

Fluorescence studies are key analytical techniques across many research and commercial applications, providing detailed information about both the emitting substance and its local environment. Beyond academic research, they are key tools in industrial biomedical research and diagnostics, the pharmaceutical industry, and in environmental monitoring. In particular, fluorescence lifetime techniques, which are independent of fluorophore concentration, photo-bleaching, excitation intensity fluctuations, scattered light, and excitation and detection wavelengths, provide particular performance and implementation benefits compared with intensity-based techniques, resulting in robust and easily calibrated analytical tools. An extensive body of time-resolved fluorescence research, undertaken over the past two decades by Strathclyde's Photophysics Group led by Prof David Birch, has spanned fundamental research studies of fluorescence mechanisms, development of novel instrumentation, and the implementation of techniques to a range of biomedical research and healthcare applications.

Advances in fluorescence instrumentation

Research into the application of newly emerging light sources to time-resolved spectroscopy has enabled the replacement of unstable spark flashlamps or complex and expensive mode-locked short-pulse laser systems with high repetition rate semiconductor light-emitting diode (LED) and laser diode sources, resulting in smaller, more reliable and more cost-effective systems. This, in turn, has extended the performance and usability of systems, thus expanding the market by opening-up a wide range of applications to non-specialist users.

One key research achievement was the development of LED sources, and associated drive electronics, operating at MHz repetition rates, representing a significant improvement on previous LEDs operating at ~10kHz. An inexpensive, miniaturized and portable blue-green indium gallium nitride (InGaN) LED source and drive electronics produced ~1.9ns pulses at up to 10MHz. This LED source was shown to be ideal for faster acquisition of fluorescence decay data using time-correlated single-photon counting (TCSPC). Experiments confirmed agreement with previous results obtained with a Ti:Sapphire laser, but at around 1% of the capital and running costs [R1].

The capabilities of the LED approach were extended through collaborative research with IBH Ltd (a spin-out from Strathclyde co-founded in 1977 by Prof David Birch and later incorporated into HORIBA as its TCSPC Centre of Excellence). This led to the development of UV LED sources that enabled, for the first time, the routine and inexpensive excitation of protein intrinsic fluorescence decay using a semiconductor source, widening opportunities for solvated protein structural studies that had been unfulfilled since fluorescence was first observed from amino acids in the 1950s. The resulting paper reported excitation of the amino acid tyrosine at 280nm [R2], and subsequent joint publications with IBH addressed the other two fluorescent amino acids tryptophan and phenylalanine.

More recent research has focussed on fluorescence lifetime imaging microscopy (FLIM) which uses fluorescence lifetime for contrast rather than fluorescence intensity as used in conventional microscopy. Conventional FLIM is limited by its slow rate of sequential scanning of a sample. However, recent collaboration between Strathclyde, Edinburgh University and HORIBA-IBH on single-photon avalanche diode (SPAD) timing arrays has greatly enhanced the multiplexed data acquisition rates of FLIM [R3] and led to a new product, the FLIMera. When combined with developments in software and firmware, a 192 x 128 pixel array, implemented in 40nm complementary metal-oxide-semiconductor (CMOS) technology, with individual pixel timing, has resulted in image capture times of 15s, a 64-fold improvement on previous commercial scanning systems. This has enabled live cell imaging at video scanning rates of 30 frames per second.

Application of fluorescence instrumentation advances to healthcare

Study of early stage Alzheimer's disease: Alzheimer's disease is thought to be triggered by beta-amyloid ($A\beta$) aggregating to form cytotoxic oligomers in the brain. These had previously been widely studied only in the later stages of development, with the limitation that the extrinsic biochemical probes used perturb the very local structure under study. The research described in [R2] enabled, for the first time, the critical early stages of aggregation of $A\beta$ to be studied in native form using the fluorescence of $A\beta$s single tyrosine. As a result, elements of the causes of Alzheimer's and potential therapies can now be studied in vitro at a molecular level without the distortions to the local environment caused by extrinsic probes [R4].

Developing a smart sensor for diabetes management: A long-term collaboration with King's College London School of Medicine and Guys Hospital on in vivo glucose sensing for diabetes management using fluorescence lifetime sensing has led to an improved performance non-invasive Concanavalin A- based smart sensor for serum glucose. The sensor utilises transdermal red light excitation, which enhances signal fidelity by minimising the fluorescence of endogenous species [R5]. This demonstrated the digital advantages of laser diode TCSPC in a non-invasive sensor compared with analogue frequency domain lifetime measurement used previously, and encouraged diabetes researchers to consider TCSPC as a method of data acquisition.

Improved FLIM for medical diagnostics and surgery: Rapid FLIM with SPAD arrays and 100MHz pulsed laser diode sources have also been investigated for new methods of fluid biopsy for cancer screening based on the Strathclyde group's development of a cancer biopsy using gold intracellular nanoprobes. This detects a tumour cell when a messenger RNA cancer biomarker releases a plasmonically-quenched dye bound to a complementary single-stranded DNA hairpin. This increases the fluorescence intensity and lifetime, thereby uniquely identifying the cancer gene of interest [R6]. A 2020 Strathclyde-HORIBA-IBH joint publication in Meas. Sci. Technol. demonstrated the FLIMera's capability for real-time tumour margin estimation, thus bringing FLIM to fluorescence-guided surgery.

3. References to the research (Strathclyde affiliated authors in **bold**)

R1 **O'Hagan W.**, **McKenna M.**, **Sherrington D.**, **Rolinski O.**, and **Birch D**. (2002). 'MHz LED source for nanosecond fluorescence sensing.' Measurement Science and Technology 13: 84-91. https://doi.org/10.1088/0957-0233/13/1/311 [FWCI: 3.5]

R2 **McGuiness C.**, Sagoo K., McLoskey D. and **Birch D**. (2004). 'A new sub-nanosecond LED at 280 nm: application to protein fluorescence.' Measurement Science and Technology 15: L19-22. https://doi.org/10.1088/0957-0233/15/11/L02 [FWCI: 7.01]

R3 Henderson R., Johnston N., Mattioli F., Della Rocca F., **Chen H.**, **Li D**., Hungerford G., Hirsch R., Mcloskey D., **Yip P**. and **Birch D**. (2019). 'A 192×128 Time Correlated SPAD Image Sensor in 40-nm CMOS Technology.' IEEE Journal of Solid-State Circuits 54: 1907-1916. https://doi.org/10.1109/JSSC.2019.2905163 [FWCI: 5.47]

R4 **Amaro M.**, **Birch D**. and **Rolinski O**. (2011). 'Beta-amyloid oligomerisation monitored by intrinsic tyrosine fluorescence.' Physical Chemistry Chemical Physics 13: 6434-6441. https://doi.org/10.1039/c0cp02652b [FWCI: 1.24]

R5 **McCartney L.**, **Pickup J.**, **Rolinski O**. and **Birch D**. (2001). 'Near-infrared fluorescence lifetime assay for serum glucose based on allophycocyanin-labelled concanavalin A.' Analytical Biochemistry 292: 216-221. https://doi.org/10.1006/abio.2001.5060 [FWCI: 1.72]

R6 **Zhang Y.**, Guoke W., **Yu J**., **Birch D**. and **Chen Y**. (2015). 'Surface plasmon enhanced energy transfer between gold nanorods and fluorophores: application to endocytosis study and RNA detection.' Faraday Discussions 178: 383-394. https://doi.org/10.1039/C4FD00199K [FWCI: 1.34]

Notes on the quality of research: The field-weighted citation impact (FWCI) at 02/02/2021 for each of the above publications is noted alongside each reference. These demonstrate that the publications, representative of the extensive body of research over the past two decades, have had higher than average influence on the academic field. Since 2000 the research has been supported by some GBP9,000,000 of research funding, including a multimillion pound EPSRC and SFC award for Nanometrology for Molecular Science, Medicine and Manufacture (Chen, Birch, 01/08/2008-01/11/2017, GBP3,122,501) and industrial funding from HORIBA-IBH (approximately GBP400,000). More recently, the work has been supported through QuantIC, the UK Quantum Technology Hub. EPSRC highlighted diabetes work with KCL [**R5**] as 1 of 5 excellent outcomes in the 2011 EPSRC review of Science & Innovation Awards and in EPSRC Impact Case Study 36. David Birch's contributions have been recognised by the 2017 HORIBA Lifetime Achievement Award for innovative research leading to commercial success, and the award of the 2020 Institute of Physics (IOP) Dennis Gabor Medal and Prize for pioneering the UK fluorescence lifetime industry. The FLIMera camera [**R3**] won the 2019 IOP Business Innovation Award.

4. Details of the impact

Co-founded by David Birch and incorporated in 1977 as a spin-out from Strathclyde, IBH Ltd was acquired by multinational company HORIBA in 2003 to form HORIBA Jobin Yvon IBH Ltd (HORIBA-IBH), bringing together IBH's leading technology in pulsed fluorescence lifetime systems and HORIBA steady-state fluorescence systems to produce a joint product line [**S1**]. The success of the IBH acquisition helped trigger HORIBA's 2014 acquisition of Photon Technology Inc. David Birch served as IBH Chairman from 1977 to 2003 and subsequently as HORIBA-IBH Director of Science and Technology. Through this route, the body of research described has:

- Expanded the commercial success of HORIBA-IBH, through new products, improved product performance, and wider application of fluorescence lifetime instrumentation across a number of sectors but particularly in underpinning healthcare
- Enabled economic growth, including contributing to HORIBA achieving and maintaining the leading position in the global fluorescence spectroscopy market
- Facilitated global multidisciplinary research through enhanced capabilities for users
- Supported fluorescence research communities globally, with international training workshops and the founding of a fluorescence journal

Impact 1: New and improved products

Strathclyde's research has been a major influence on HORIBA-IBH's product development [**S2**] and has enabled the company to introduce fluorescence products, both optical source components and full systems, utilising semiconductor optical sources that combine lower cost, improved reliability, enhanced repetition rate (up to 100MHz) and better spectral coverage than the previously used spark discharge sources. **R1** and **R2**, which described these developments, were noted in world-leading expert Joseph Lakowicz's 2006 *Principles of Fluorescence Spectroscopy (3rd Edition)* as '*perhaps the most important development for TCSPC since 2000*.'

The *NanoLED* range of sources, with repetition rates up to 1MHz, was introduced from 2000, with the UV version launched in 2004, followed by the *DeltaDiode* range in 2013. At launch, the *DeltaDiode's* repetition rate of up to 100MHz, combined with pulse durations of ~100-200ps, was the highest available in the field. Improved time resolution enables lifetimes down to 5ps to be measured with TCSPC [**S3**], facilitating a better understanding of transient dynamics in solids, liquids, colloids and polymers on the nanometre scale. The reduction in timing electronics dead time to 10ns combined with the 100MHz *DeltaDiode* has enabled fluorescence decays to be measured in ms rather than seconds [**S3**], opening up application in the analysis of transient samples. The main commercial and application benefits have been achieved through integration of these semiconductor sources in complete fluorescence lifetime systems such as the present range of *DeltaFlex*, *DeltaPro* and *DeltaTime* dedicated lifetime systems, introduced around 2013 and hybrid lifetime versions of the *Fluorolog* and *FluoroMax* steady-state fluorimeters [**S1**].

Compared with previous technologies and systems, these products are very user friendly with largely turnkey operation, benefiting from features such as a touchscreen software interface as well as the ease of use and reliability of the semiconductor sources. This has significantly widened market appeal by attracting non-specialist users, with HORIBA-IBH fluorescence products now employed across healthcare, life sciences, pharmaceutical, material sciences, nanotechnology and energy research, in both academic and commercial settings.

Recent product development has resulted in a fully integrated spectroscopy-microscopy laboratory suite incorporating the single-photon avalanche diode (SPAD) fluorescence lifetime imaging microscopy (FLIM) research described in [**R3**]. *FLIMera*, the HORIBA-IBH new SPAD FLIM molecular movie camera, launched at Photonics West in February 2020, enables, for the first time, video rate live cell FLIM, benefiting applications such as cancer screening and fluorescence guided surgery. A leading expert in fluorescence from Texas Christian University (TCU) has described this work as '*ground-breaking …. this new contribution has the potential to revolutionize biomedical imaging, enabling truly live imaging of various cellular processes*' [**S4**]. When awarding the FLIMera its 2019 Business Innovation Award, the Institute of Physics described it as '*game changing technology*' [**S5**].

Impact 2: Economic impact, market leading product sales

HORIBA-IBH's product innovation has led to considerable and ongoing economic success for the business. Sales figures presented here are obtained from reputable and independent market reports from Strategic Directions International (SDI) [**S6**], supplemented by market share information provided by the company [**S2**]. From SDI data on the global fluorescence spectroscopy market (wider than the lifetime fluorescence market), HORIBA corporately has the largest market share, growing from 16% of USD152,000,000 i.e. USD24,300,000 (04-2013) in 2013 to a projected 22% of USD186,000,000 i.e. USD40,900,000 (04-2020) in 2020 (nearest competitor at 13% market share). Turning to the global lifetime fluorescence segment of the market, this is consistently the fastest growing segment and SDI consistently record HORIBA as the major player in the segment. [Text removed for publication] This growth is also evidenced by the HORIBA-IBH's annual reports which show net assets growing from GBP465,000 to GBP725,000 over the REF period, a growth of 56% [**S7**].

As a result of sales growth, HORIBA-IBH, which undertakes all of HORIBA's lifetime fluorescence product design, manufacturing and sales support functions in Glasgow, moved to new premises, increasing its space in 2015 from 4,800sq ft to 6,660sq ft. [Text removed for publication] HORIBA-IBH is part of HORIBA Scientific which internationally expanded into new premises in New Jersey, USA, in 2018 by 90% to 132,000sq ft.

Impact 3: Facilitating global multidisciplinary research and development

HORIBA-IBH's market reflects the international nature of the fluorescence spectroscopy market which is approximately evenly split across North America, Europe and Asia with China the fastest growing market [**S6**]. The company has over 1000 customer sites world-wide which span industry, government laboratories and academia [**S2, S4**] reflecting the market sector breakdown across pharma/biotech (41%), applied (27%), public sector (20%), industrial (12%) sectors [**S6**]. Experts in the field and users describe HORIBA-IBH products as '*a major leap in technology*' that provide '*outstanding performance combined with ease of use*' and are '*used by leading laboratories all over the world*' [**S4**].

Impact 4: Supporting fluorescence research communities, industrial and academic

Strathclyde and HORIBA's roles at the forefront of the sector have led to a range of activities supporting communications across academia and industry, and providing training to increase fluorescence R&D capacity internationally; these include:

- First launched in 2009 and sponsored by HORIBA (enabling nominal cost or free registration), FluoroFest is a series of international hands-on training workshops. Five FluoroFest workshops have taken place around the world since August 2013, consistently attracting around 100 delegates from industry and academia [**S2, S8**].
- A Photophysics CPD course was launched at Czech Technical University, Prague, in 2013, when it ran over 6 days, and rerun in 2017 over 3 days, each with approximately 25 attendees
- Institute of Physics Publishing (IOPP), launched the journal Methods and Applications in Fluorescence (MAF) in 2013, with David Birch as founding co-editor in chief, a position he still holds. IOPP's decision to launch the journal was based in part on their strong connection to Strathclyde's Photophysics Group through the latter's publications in IOPP journals [e.g. **R1**, **R2**]. An associated MAF international conference series, launched biennially in 1989 and annually in 2019, is now the world's largest fluorescence conference. Both the journal and conference are heavily supported by industry and HORIBA is always a major sponsor.

5. Sources to corroborate the impact

S1 Collated web content from HORIBA website:
   a. Manufacturing and Assembly in the United Kingdom. https://bit.ly/3jZEycY
   b. Lifetime Fluorescence Spectrofluorometers. https://bit.ly/3qyGPyt
   Both accessed 13 Oct 2020

S2 Supporting statement from Global Product Line Manager, Fluorescence Division, HORIBA Scientific, USA (27/04/2020)

S3 Birch D, Hungerford G, McLoskey D, Sagoo K and Yip P (2019) 'Instrumentation for Fluorescence Lifetime Measurement Using Photon Counting.' Chapter 2 pgs 110-116 in: *Fluorescence in Industry*. Springer Series in Fluorescence. Bruno Pedras (ed.) Vol 16.

S4 Supporting statements from HORIBA equipment users:
   a. Director, Center for Fluorescence Technologies & Nanomedicine, Texas Christian University, USA (21/12/2018)
   b. Professor of Chemistry, Durham University, a UK customer of HORIBA-IBH (14/01/2019)
   c. Professor of Physical Chemistry, Brown University, USA and Czech Technical University, Prague, Czech Republic (28/04/2020)

S5 Institute of Physics Awards: Business Innovation Awards 2019 (https://bit.ly/3qWloGJ); Dennis Gabor Award 2020 (https://bit.ly/2NGpzbL, accessed 11 Mar 2021)

S6 Strategic Directions International Inc. Global Assessment Reports, The Laboratory Analytical and Life Science Instrumentation Industry, 13th edition (2014) pages 283 - 286. and 14th edition (2017) pages 328 - 332

S7 HORIBA-IBH balance sheets 2013-2019 from published annual accounts.

S8 FluoroFest international training workshop http://www.fluorofest.com/ accessed 13 Jan 2021