Institution: Imperial College London

Unit of Assessment: 9 – Physics

Title of case study: B9-3 Commercial impact of fluorescence lifetime imaging (FLIM) technology

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 Paul French, Dr Christopher Dunsby, Dr James McGinty, Prof Mark Neil	Prof of Physics, Reader in Biomedical Optics, Senior Lecturer, Prof of Photonics	PF: 1986-2020 CD: 2003-2020 JM: 2006-2020 MN: 2002-2020

Period when the claimed impact occurred: 2013 - 2020

Is this case study continued from a case study submitted in 2014? Y

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

Having demonstrated the first FLIM instrument to utilize solid-state ultrafast laser technology in 1997 [REF impact case 2014], the Photonics team at Imperial developed a multidimensional fluorescence imaging technology platform enabling FLIM for clinical diagnostic imaging of disease, preclinical imaging of disease progression and assays of protein interactions in cell biology and drug discovery. This stimulated significant commercial sales of FLIM equipment with many research laboratories purchasing components to replicate our capabilities. From 2013, this included sales of gated optical intensifier (GOI) technology from Kentech Instruments Ltd ([redacted from public version]) and ultrafast supercontinuum sources (USS) from Fianium Ltd (now NKT Photonics, ~£2.25M in 2019 alone).

2. Underpinning research (indicative maximum 500 words)

Fluorescence lifetime imaging (FLIM) maps the variation in fluorescence lifetime across a field of view. Fluorescence lifetime measurements temporally analyse the decay of emission from fluorophores and can distinguish and quantify different molecular (fluorophore) species and local molecular environments, including molecular interactions. FLIM microscopy was first demonstrated in ~1990 in a laser scanning confocal microscope but its uptake was limited for decades to a few laboratories, partly due to instrumentation complexity, the lack of user-friendly software and a paucity of use-cases. From 2000, the Photonics team led by French developed robust FLIM microscopes and other instruments, particularly focussing on wide-field time-gated FLIM that provided faster imaging than laser scanning techniques and demonstrated a range of applications for FLIM. For drug discovery, they implemented rapid time-gated FLIM (up to video rate **[2]**), which they applied to fluorescence resonance energy transfer (FRET) readouts of cell signalling processes, progressing to automated high throughput FLIM/FRET multiwell plate assays for drug discovery **[5]**. With colleagues from Life Sciences and Medicine at Imperial, they also pioneered the application of wide-field time-gated FLIM to endoscopy **[3]** and optical tomography for biomedical applications, including the study of disease mechanisms, cancer diagnosis **[1,4]** and preclinical imaging.

Time-gated FLIM requires ultrashort pulsed lasers for excitation and time-resolved imaging detectors. The Photonics team pioneered the use of ultrafast supercontinuum sources (USS) for tunable pulsed excitation, including the first use of USS for FLIM, using a home-built USS **[2]** and later using commercial USS from Fianium Ltd, which they applied to both wide-field time-gated FLIM and laser scanning microscopy with time-correlated single photon counting (TCSPC).

They also worked with Kentech Instruments Ltd to evaluate and improve Kentech's gated optical image intensifier (GOI) detectors for FLIM and they developed a robust time-gated FLIM platform integrating the Kentech GOI that they extended to a range of FLIM modalities, including wide-field and spinning disc confocal FLIM **[5]**, endoscopy **[1]** and optical projection tomography (OPT) **[5]**. They also combined FLIM with hyperspectral imaging and tunable (USS-based) excitation, thereby resolving fluorescence emission with respect to three spectroscopic and three spatial dimensions. While the Photonics team primarily applied FLIM technology to biomedical research, including for clinical diagnosis, live cell studies, automated high content analysis (HCA), and preclinical imaging of murine and zebrafish models of disease, they also applied it to diamond photophysics with De Beers UK.

The Photonics team worked to understand and optimise time-gated FLIM in terms of the FLIM data acquisition and analysis. They implemented global fitting with time-gated FLIM, which enables quantitative FLIM of complex fluorescence decay profiles to be acquired much faster (and with lower phototoxicity) than conventional FLIM where data is analysed on a pixel-wise basis. They also developed the first FLIM data analysis software ("*FLIMfit*") incorporating global fitting that was sufficiently fast to be practical (requiring seconds to minutes), which they released as open source software **[6]**. *FLIMfit* combined with time-gated FLIM using USS+GOI technology provides a means for rapid FLIM, including for FRET assays of protein interactions for, drug discovery and clinical imaging. They also developed an open approach to FLIM instrumentation, which now provides a new commercial channel for FLIM and multidimensional fluorescence imaging, and they extended this to low-cost, modular hardware and software for many advanced microscopy modalities (www.openScopes.com).

3. References to the research (indicative maximum of six references)

[1] Fluorescence lifetime imaging of unstained tissues: early results in human breast cancer, P. J. Tadrous, J. Siegel, P. M.W. French, Dr S. Shousha, El-Nasir Lalani and G. W.H. Stamp, J Pathol 199 (2003): 309–317; https://doi.org/10.1002/path.1286 (102 citations)

[2] An electronically tunable ultrafast laser source for fluorescence imaging including fluorescence lifetime imaging microscopy, C. Dunsby, P. M. P. Lanigan, J. McGinty, D. S. Elson, J. Requejo-Isidro, I. Munro, N. Galletly, F. McCann, B. Treanor, B. Önfelt, D. M. Davis, M. A. A. Neil, and P. M. W. French, J Phys D: Appl. Phys. 37 (2004) 3296-3303; https://doi.org/10.1088/0022-3727/37/23/011 (84 citations)

[3] High-speed wide-field time-gated endoscopic fluorescence-lifetime imaging, J. Requejo-Isidro, J. McGinty, I. Munro, D.S. Elson, N. Galletly, M.J. Lever, M.A.A. Neil, G.W.H. Stamp and P.M.W. French, Opt Lett, 29 (2004) 2249-2251; https://doi.org/10.1364/OL.29.002249 (61 citations)

[4] Wide-field fluorescence lifetime imaging of cancer,
J. McGinty, N. P Galletly, C. Dunsby, I. Munro, D. S Elson, J. Requejo-Isidro, P. Cohen, R. Ahmad, A Forsyth, A. V Thillainayagam, M. A. A. Neil, P. M W French and G.W Stamp, Biomedical Optics Express, 1 (2010) 627-640; https://doi.org/10.1364/BOE.1.000627 (60 citations)

[5] FLIM FRET technology for drug discovery: automated multiwell plate high content analysis, multiplexed readouts and application *in situ,*S. Kumar, D. Alibhai, A. Margineanu, R. Laine, G. Kennedy, J. McGinty, S. Warren, D. Kelly, Y. Alexandrov, I. Munro, C. Talbot, D. W. Stuckey, C. Kimberly, B. Viellerobe, F. Lacombe, E. W.-F. Lam, H. Taylor, M. J. Dallman, G. Stamp, E. J. Murray, F. Stuhmeier, A. Sardini, M. Katan, D. S. Elson, M. A. A. Neil, C. Dunsby and P. M. W. French, ChemPhysChem 12 (2011), 627-633, https://doi.org/10.1002/cphc.201000874 (51 citations)

[6] Rapid global fitting of large fluorescence lifetime imaging microscopy datasets, S.C. Warren, A. Margineanu, D. Alibhai, D.J. Kelly, C. Talbot, Y. Alexandrov, I. Munro, M. Katan, C. Dunsby and P.M.W. French, PLoS ONE 8 (2013) e70687; https://doi.org/10.1371/journal.pone.0070687 (96 citations)

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

During the development and application of the robust wide-field time-gated FLIM technology, the Imperial Photonics team worked with industrial partners, including, AstraZeneca, De Beers, GE Healthcare, GSK, Karl Storz, Kentech Instruments, PerkinElmer and Pfizer, who directly co-funded the research through CASE PhD studentships, contributions to BBSRC (BB/M006786/1 IPA award, £508,815, 2015-2018) and EPSRC grants (EP/IO2770X/1, Healthcare Partnership award, £1,174,248, 2011-2015) and industry-focussed projects funded by a DTI Beacon Award (QCBB/C/012/0007C, £1,042,493, 2002-2006) and a DTI/TSB Technology award (CHBT/007/00030 (100297, £735,000, 2006-2011) for which we worked with GE Healthcare to build a prototype using the Fianium USS and Kentech GOI technology for automated multiwell plate assays.

This collaborative research and development resulted in prototype instruments and joint publications, impacting industry by informing their product development and marketing strategies and raising awareness of the potential of wide-field time-gated FLIM and its practical implementation. The Photonics team's papers co-authored with industry include, e.g. https://doi.org/10.1364/OE.15.015656 ; https://doi.org/10.1111/j.1365-2133.2008.08577.x ; https://doi.org/10.1088/0022-3727/42/13/135103 ; https://doi.org/10.1002/cphc.201000874 ; https://doi.org/10.1002/jbio.201200185 ; https://doi.org/10.1063/1.4973917; https://doi.org/10.1088/2050-6120/ab4eac.

The >65 papers and many international conference presentations (including ~145 invited talks/lectures) from the Photonics team helped raise awareness of FLIM, encouraging other laboratories to implement their own instruments based on our prototypes using products from Fianium and Kentech, which increased their commercial sales, most of which are outside the UK. For example, NKT Photonics (previously Fianium UK) report: "*… your work highlighting the potential of fibre-laser based supercontinuum sources for FLIM helped us establish ourselves as a leading provider of this technology*" **[A]**, and Kentech report: "*Your world-leading research pioneering the biological and medical applications of time-gated FLIM, […], created significantly increased demand in our HRI products across our international market, with our customers frequently wanting to purchase HRI technology in order to do similar work*" **[B]**.

Fianium Ltd (now part of NKT Photonics) was founded in 2003 to sell fibre laser-based technology and USS rapidly became their main product. They note that our work *"… had a significant influence on our product development resulting in a new generation of 'WhiteLase' supercontinuum lasers …*" **[A]**, reporting that this was Fianium's most successful laser from its release in 2012 until the acquisition of Fianium by NKT Photonics in 2016. We demonstrated new applications of the Fianium USS technology – including wide-field and optically sectioned FLIM, hyperspectral FLIM, time-gated optical tomography and single point time-resolved spectrofluorometry – and NKT Photonics note that our publications stimulated global demand for their USS products, which are incorporated in other commercial FLIM microscopes (e.g. from Leica **[C]**) and are also sold for imaging and medical applications representing ~15% of their direct scientific sales , amounting to ~£15M in 2019 alone **[A]**. NKT Photonics note that "*Most of our customers reference the pioneering work undertaken at Imperia*l" and they use our work as a reference for potential customers, including on their website **[D]**.

Since 2012, Kentech Instruments Ltd, who manufacture a range of specialised, custom-built electronics and imaging equipment, including GOI for FLIM **[E]**, report [redacted from public version] sales of FLIM systems based on gated optical image intensifier technology since 2012 **[D]**, most of which were through LaVision GmbH in Germany who incorporated the Kentech GOI in their "Picostar" product. The Photonics team collaborated continuously with Kentech from 2000, providing feedback on the performance of their GOI technology and discussing advances to increase its capability and impact. Kentech noted that the interaction with Imperial had directly led to improvements in their technology: "… *such as the enhanced magnetic focussing and improved intensifier response - technologies that we developed specifically for your highly innovative research work*" **[D]**. Kentech also note that "*The thorough characterisation and application of these developments in the novel FLIM technologies you have published have significantly contributed*

to our commercial success" **[D]** and go on to express their enthusiasm for the open source approach to FLIM being pioneered by the Photonics team.

Since 2013, and following the development of a prototype instrument with PerkinElmer/GE Healthcare, AstraZeneca, GSK and Kentech Instruments Ltd that was funded by the DTI/TSB ("*Ultrafast photonics for fluorescence imaging and time-resolved assays*" £735,000, 2006-2010), CHBT/007/00030), it became clear that the major commercial market for FLIM technology was laboratories that would assemble their own instruments from commercially available components if they could access the appropriate software and know-how. Accordingly, supported by an internal EPSRC Impact Acceleration Account ("*Translating automated FLIM-HCA to drug discovery, systems biology and basic research*", £124,591, 2013-2015) the Photonics team developed complete open source FLIM instruments **[F]** based on wide-field time-gated imaging using the (Fianium) USS and (Kentech) GOI technologies that can be controlled using open source FLIM data acquisition software based on *MicroManager* that are available from the Photonics website with a downloadable list of hardware components **[F]**. These FLIM instruments can utilise the Photonics team's world-leading open source FLIM analysis software (http://www.flimfit.org/, downloaded at least 3,964 times since 2016). For automated FLIM high content analysis (HCA), the Photonics team published a video guide (http://www.jove.com/video/55119) to make it straightforward for other laboratories to implement their own FLIM HCA instrumentation, e.g. for FLIM/FRET assays, using the NKT Photonics and Kentech technologies.

The Photonics team recently extended this open source FLIM platform to a more general open (hardware and software) modular microscopy platform (www.openscopes.com) **[G]** for industry and biomedicine as well as academic research, specifically intended to benefit scientists in ODA countries. In partnership with Cairn Research Ltd, we developed the *openFrame* microscope system **[G]** to enable industry to develop new products and academic laboratories to develop or implement advanced microscope modalities. An *openScopes* FLIM module based on Kentech technology is now commercially available via Cairn Research Ltd **[H]**, establishing a new commercial channel for time-gated FLIM. For laboratories wishing to build their own FLIM instruments, the *openScopes* website links to the Photonics team's FLIM open source resources **[F]**.

In addition to the translation of the technology into industrial application and sales, this FLIM research at Imperial impacted industry, including at Base4, De Beers, Genentech, Rutherford Appleton Laboratories, Scientifica, Smiths Industries, Thorlabs (Sweden), and the NHS, via their recruitment of PhD students and research associates from the Photonics FLIM technology development programme. This includes De Beers, who are exploring the application of FLIM and ultrafast supercontinuum technologies to characterise luminescence from natural, treated and synthetic diamonds. De Beers have benefitted from access to the Photonics team's expertise and ultrafast super-continuum/FLIM based instrumentation to characterise specific diamond samples and to develop internal projects. They note that one of our former PhD students trained in FLIM techniques "*provided a high level of technical ability in the area of applied optics and has played a pivotal role in the design and development of instrumentation based on a number of different approaches*" **[I]** and note that "*the use of fluorescence lifetime-based instruments could have a significant impact on the diamond gemstone market.*" **[I]**. And to "*quantify and map emission from defects in diamond-based quantum computing components (including for quality assurance) and for monitoring growth processes for synthetic diamond*" **[I]**.

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

[A] Letter from Sales Manager, NKT Photonics (formerly Fianium Ltd), 5th January 2021. Letter confirms the significant impact that Imperial's pioneering research on the development of FLIM technology has had on sales of their supercontinuum devices. FLIM has become a major application of Fianium's technology.

[B] Letter from Managing director, Kentech Instruments Ltd, 6th January 2021. Letter confirms Imperial's role in the development of FLIM technology and their sales of GOI and HRIs.

[C] Web page on Leica Microsystems describing FLIM/FRET that cites our work: https://www.leica-microsystems.com/science-lab/flim-fret-and-biosensors-versatile-tools-for-biomedical-research/ (Archived here)

[D] Web page on NKT Photonics website referring to Imperial FLIM applications of supercontinuum: https://www.nktphotonics.com/lasers-fibers/cases/multidimensional-fluorescence-imaging-metrology-using-tunable-super-continuum-sources/ (Archived here)

[E] Web page on Kentech Instruments Ltd listing FLIM as application of GOI and HRI (a specific model of time-gated optical intensifier) referencing Imperial work: http://kentech.co.uk/index.html?/&2 [click on "FLIM" in sidebar] (Archived here)

[F] Web page on open source time-gated FLIM shared by the Photonics Team to enable other laboratories to assemble their own FLIM systems including links to software and downloadable parts list: https://www.imperial.ac.uk/photonics/research/biophotonics/instruments--software/fluorescence-microscopy/wide-field-time-gated-flim/ (Archived here)

[G] Web page of openScopes presenting mlow-cost, modular, open source microscopy components including the openFrame and openFLIM showing the FLIM module referencing the FLIM resources of the Photonics team at Imperial https://www.cairn-research.co.uk/product/fluorescence-lifetime-imaging-module/ (Archived here)

[H] Web page of Cairn Research Ltd showing the FLIM module referencing the FLIM resources of the Photonics team at Imperial https://www.cairn-research.co.uk/product/fluorescence-lifetime-imaging-module/ (Archived here)

[I] Letter from Senior Manager Physics & IP, De Beers Technologies UK, outlining impact of Photonics FLIM research, including recruitment of former PhD student and impact on research for gemstone applications and quantum computing applications (via Element 6).