Institution: King's College London		
Unit of Assessment: 9 Physics		
Title of case study: Improving cancer treatments and patient outcomes using Fluorescence Lifetime Imaging Microscopy-Förster Resonance Energy Transfer (FLIM-FRET)		
Period when the underpinning research was undertaken: 2003 - 2018		
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:**
(1) Klaus Suhling (2) Simon Ameer-Beg (3) Borivoj Vojnovic (4) James Levitt (5) Simon Poland	(1) Professor of Physics (2) Professor (3) Professor (4) Microscopy Innovation Centre Manager (5) Research Associate	(1) From 01/09/2003 (2) From 2006 (3) 2007-2015 (20%) (4) From 01/10/2006 (5) From 2010
Period when the claimed impact occurred: 2014 - 2020		
Is this case study continued from a case study submitted in 2014? N		

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

Advanced fluorescence imaging research at King's College London has enabled the development of Fluorescence Lifetime Imaging Microscopy Förster Resonance Energy Transfer (FLIM-FRET) technology viable for drug development and clinical application. Application of FLIM-FRET to tumour biopsies has shown that certain protein combinations predicted either effectiveness or resistance to specific drugs. Thus, FLIM-FRET can help target treatments to the right patients, resulting in reduced healthcare costs, reduced morbidity and improved survival. Clinical studies have validated the technique and informed clinical guidelines for cancer treatment in the USA. Drawn by this technology, Pharma companies AstraZeneca, Daiichi Sankyo, Incyte, Roche and UCB Pharma have funded FLIM-FRET projects to develop and evaluate novel cancer drugs and a spinout company has been created for further commercialisation.

2. Underpinning research (indicative maximum 500 words)

Since 2003 a programme of research has been established at King's concerned with the development of novel fluorescence imaging techniques for application to cell and tissue imaging, involving researchers from the Department of Physics, the Randall Centre for Cell & Molecular Biophysics and the Comprehensive Cancer Centre. This strong cross-college activity in fluorescence imaging development has been brought together through the King's Centre for Biophotonics, established in 2007, leading on more recently to establishment of the King's Imaging Network, and in 2018, creation of the Microscopy Innovation Centre. Imaging of the polarisation, lifetime and spectroscopic properties of fluorescently labelled molecules in biological cells and tissue can yield important information on protein activity, protein-protein interactions, structure, mobility etc. A particular focus has been concerned with research, led by Ameer-Beg, Suhling and Vojnovic, on the development of Fluorescence Lifetime Imaging **(**FLIM), employing time-correlated single photon counting (TCSPC) techniques to determine, at every pixel in an image, the decay rate of fluorescence from fluorophores labelling proteins of interest in biological cells or tissue. In FLIM-FRET, the Förster Resonance Energy Transfer (FRET) between proximal donor and acceptor fluorophores, each labelling the partners of interacting protein pairs, is mapped through the reduction in lifetime of the donor, enabling the visualization and quantification of both the density of receptors and proximity of the donor and acceptor pairs to each other.

In 2004, researchers in the Richard Dimbleby Department of Cancer Research at King's led by Professor Tony Ng, began using FLIM-FRET to analyse specific protein-protein interactions within tumours, to further our understanding of tumour biology with the goal of matching patients to treatments most likely to treat their particular cancer. However, the existing gold standard approach was too slow, too complex, too costly and lacked robustness for analysing a high-volume of samples. To overcome these limitations, King's developed new microscopes and analysis

methods allowing high throughput, accurate assessments of *in vitro* and clinical samples [R1-R3], supported initially by an EPSRC-funded programme concerned with the development of 'optical proteomics' [F1].

To achieve a significant increase of optical detection rates for application to clinical samples, research at King's in new detection and analysis techniques for FLIM has included the development of time-resolved multi-pixel detection techniques for FLIM. Using position-sensitive photon counting detectors, Suhling has implemented camera-based FLIM for total internal reflection fluorescence microscopy to study cell membranes [R4] and lightsheet FLIM to study cell clusters. In particular, since 2010 Ameer-Beg and Suhling have developed a collaboration with the University of Edinburgh concerned with the application of single photon avalanche photodiode array TCSPC detectors for fast frame-rate FLIM with picosecond resolution, supported initially through a BBSRC award [F2] [R5]. Through successive prototyping the latest FLIM-FRET system, called SWARM (SWept ARray scanning Microscope), was completed in 2018 [R6] [P1]. SWARM replaced the single scanning beam with 1024 beams for both excitation and detection. This arrayed approach provided up to a 1024x speed improvement, permitting an increase in the spatial resolution and in the sample acquisition rate.

Alongside the above physics research on the development of FLIM, research by Professor Ton Coolen in the King's Department of Mathematics led to the development of a new Bayesian approach to FLIM analysis (underpinning research reported in a case study for UoA10). Working in close collaboration with Ameer-Beg, Coolen, Suhling and Vojonovic and using the FLIM technology developed at King's, was critical in enabling Ng to use FLIM-FRET for the visualization of epidermal growth factor receptors (EGFR), to show that when two receptors within the EGFR family, such as receptors HER2 and HER3, are proximally close (dimerization), it leads to enhanced tumour growth and that the presence of this dimerization may further predict success or failure of a particular therapy (underpinning research reported in a case study for UoA5).

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

Research articles:

[R1] Matthews, D. R., Carlin, L. M., Ofo, E., Barber, P. R., **Vojnovic, B.**, Irving, M., Ng, T., & **Ameer-Beg, S. M.** (2010). Time-lapse FRET microscopy using fluorescence anisotropy. *Journal of Microscopy*, 237(1), 51-62. DOI: 10.1111/j.1365-2818.2009.03301.x

[R2] Matthews, D. R., Fruhwirth, G. O., Weitsman, G., Carlin, L. M., Ofo, E., Keppler, M., Barber, P. R., Tullis, I. D. C., **Vojnovic, B.**, Ng, T., & **Ameer-Beg, S. M.** (2012). A multi-functional imaging approach to high-content protein interaction screening. *PLoS One*, 7(4), [e33231]. DOI: 10.1371/journal.pone.0033231

[R3] Barber, P. R., Tullis, I. D. C., Pierce, G. P., Newman, R. G., Prentice, J., Rowley, M. I., Matthews, D. R., **Ameer-Beg, S. M.**, & **Vojnovic, B.** (2013). The Gray Institute 'open' high-content, fluorescence lifetime microscopes. *Journal of Microscopy*, 251(2), 154-167. DOI: 10.1111/jmi.12057

[R4] Hirvonen, L. M., Becker, W., Milnes, J., Conneely, T., Smietana, S., Le Marois, A., Jagutzki, O., & **Suhling, K.** (2016). Picosecond wide-field time-correlated single photon counting fluorescence microscopy with a delay line anode detector. *Applied Physics Letters*, 109(7), [071101]. DOI: 10.1063/1.4961054

[R5] **Poland, S. P.**, Krstajić, N., Coelho, S., Tyndall, D., Walker, R. J., Devauges, V., Morton, P. E., Nicholas, N. S., Richardson, J., Li, D. D. U., **Suhling, K.**, Wells, C. M., Parsons, M., Henderson, R. K., & Ameer-Beg, S. M. (2014) Time-resolved multifocal multiphoton microscope for high speed FRET imaging *in vivo*. *Optics Letters*, 39(20), 6013-6016. DOI: 10.1364/OL.39.006013

[R6] **Poland, S. P.**, Chan, G. K., **Levitt, J. A.**, Krstajić, N., Erdogan, A. T., Henderson, R. K., Parsons, M., & **Ameer-Beg, S. M.** (2018). Multifocal multiphoton volumetric imaging approach for high-speed time-resolved Förster resonance energy transfer imaging *in vivo*. *Optics Letters*, 43(24), 6057-6060. DOI: 10.1364/OL.43.006057

Research funding:

[F1] Irving, M., Heintzmann, R., Ng, T., Richards, D., & **Suhling, K.** Optical proteomic technology for *in-situ* analysis of protein interaction networks. EPSRC, 24/10/2005→23/02/2010, GBP1,000,300

[F2] **Ameer-Beg, S.**, Ng, T., & **Suhling, K.** Multiplexed multiphoton fluorescence lifetime microscopy: Real time 3D imaging of protein-protein interactions by FRET. BBSRC, 1/11/2011→31/10/2014, GBP474,306

Patent application:

[P1] **Ameer-Beg, S.**, **Poland, S.**, **Levitt, J.**, & Nedbal, J. (2017). Luminescence Imaging Apparatus and Methods. Application No. PCT/GB2018/051865

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

Background: the opportunity for FLIM-FRET in personalised medicine
Getting the right drug to the right patient allows better outcomes to be achieved sooner. Recent advances in cancer treatment target specific tumour types but only work when the right patients – those with the target tumour type – can be identified. Morbidity and suffering from the side effects from ineffective drugs will be eliminated if patients are matched to treatments most likely to treat their particular cancer. In the UK, an annual total of 156,000 patients have breast, head and neck squamous cell carcinoma (HNSCC), lung and colorectal cancers of which 20% (31,200) would likely benefit from targeted treatment and evaluation, enabled by FLIM-FRET.

The close collaboration between physics, mathematics and biology researchers, alongside the ongoing development at King's of improved FLIM technology and associated biophotonics expertise, has been critical in enabling Tony Ng and co-workers to apply FLIM-FRET technology to clinical cancer samples, leading in turn to the discovery and validation of EGFR dimerization pairs that inferred both drug resistance and drug effectiveness, applied to breast, colorectal, lung and HNSCC cancers. King's research developed in the SWARM system has speed improvements, targeted assays and validation studies making this technology viable for drug development and clinical application, as demonstrated by collaboration agreements with multiple drug companies, formation of a spinout company and early clinical trial results.

Change of approach by multinational pharmaceutical companies in the development and testing of both novel and existing therapeutics for cancer through adoption of FLIM-FRET:
Pharma companies have funded over GBP1,200,000 through collaboration agreements with King's for a number of pre-clinical and clinical projects to develop use of FLIM-FRET to better understand protein dimerization and the impact on treatment effectiveness or resistance. This has allowed these companies to develop drugs targeted to prevent protein dimerization and identify subsets of patients who will respond to their drugs.

- **Daiichi Sankyo** is a global pharmaceutical company based in Japan with annual revenue (2019) of GBP7,000,000,000. The company has been working with Ng since 2014, with application of FLIM-FRET to **three separate compounds in their drug pipeline** [S1], including a phase 2 trial with Patritumab/Cetuximab Combination Therapy in head and neck squamous cell carcinoma. FLIM-FRET analysis as part of this trial provided Daiichi Sankyo with data on how and why a subgroup of patients reacted positively to their drug: The senior Director for Global Oncology R&D describes [S1] the technology as: "*ground-breaking, very scientifically sound, advanced and creative. This technique allows us to better understand the immune system and the drug related outcomes of patients*".
- **Roche Products Ltd** is a Swiss multinational and the world's largest biotech company. As a result of King's EGFR-HER3 dimer development programme, in 2016 Roche took an interest in the technology and funded a study at King's that used FLIM-FRET to validate HER2-HER3 dimerization [S2]. Following this study, the King's team demonstrated that quantification of HER2-HER3 dimerization could guide the stratification of patients with breast cancer towards HER2-directed therapies [S3]. Based on these results a pilot study to measure the HER2-HER3 dimer expression in breast cancer patients receiving HER2 targeted therapies

(NCT04288141) was initiated with recruitment opened from January 2020, which was paused due to COVID-19 but has now recommenced.

- **AstraZeneca** (a FTSE 100 pharmaceutical company) has funded a pre-clinical study to investigate FLIM-FRET as part of a predictive biomarker study for the HER1 inhibitor AZD9291 (Osimertinib). [S4]
- **UCB Pharma** is a global biopharmaceutical company and a top 5 investor in biopharmaceutical R&D in the UK. In 2019 UCB selected King's as a partner in a Phase 1 trial testing their new anti-cancer drug UCB6114 (NCT04393298, 2020-2023). The FLIM-FRET analysis directly contributed to UCB's decision to partner with King's**;** UCB have stated [S5]: "*Looking at combination therapy will help to prioritize assets and translate this to clinic. Identifying the best combinations is important and this technology will help us define this. […] We can see the great potential here on translation to clinical development and enabling us to have a better understanding based on direct data from clinically relevant samples [...] the first project is already underway".*
- **Incyte** is an American pharmaceutical company, with revenue in 2019 of USD2,100,000,000. Incyte have recently selected King's as a partner to use FLIM-FRET to study dimerization of another cancer-associated receptor PD-L1. This study commenced in late 2020 and will reveal the mechanism of action of Incyte's lead anti-PD-L1 compound. [S6]

FLIM-FRET analysis has informed clinical guidelines

The biopharmaceutical drug T-DM1, also known as ado-trastuzumab emtansine, is an antibody-drug conjugate sold under the trade name Kadcyla. T-DM1 treatments, previously only issued to treat patients with breast cancer, are now part of the United States National Cancer Comprehensive Network (NCCN) clinical guidelines for treating HER2 mutated lung cancers [S7] and thus currently impacting patient care. An oncologist at the Memorial Sloan Kettering (MSK) Cancer Center (New York, USA), whose work led to this change to the NCCN guidelines, worked with Tony Ng on the use of FLIM-FRET and considers that "*the clinical data produced and mechanistically supported by FLIM were practice changing in the United States*" [S8]. The MSK oncologist has identified that FLIM-FRET data from a phase 2 trial reported in [S9] was submitted alongside other evidence to the NCCN panel, the outcome of which informed the NCCN guideline change [S7]. These guidelines detail the sequential management decisions and interventions that currently apply to 97% of cancers affecting patients in the United States. They are the recognised standard for clinical direction and policy in cancer care.

The MSK oncologist states: *"There has been a historical reluctance to accept HER2 targeted therapies outside of breast and gastric cancers due to two decades of failed clinical trials. FLIM has helped motivate a shift in mindset for both investigators and funding agencies such as NIH based on our preliminary data and the ability to analyze trial data to gain mechanistic insights. With this change in direction of research funding and interest by pharma, FLIM has opened up potential new areas of promising research for cancer patients."* [S8]

Clinical studies have validated the technique and demonstrated that the FLIM-FRET diagnostic technology leads to improved outcomes for a range of cancer patients:

- In a phase 2 clinical trial in HNSCC sponsored by Daiichi Sankyo (NCT02633800, 2015-19, see above), FLIM-FRET enabled identification of EGFR:HER3 dimers from head and neck cancer patient serum exosomes and was predictive of treatment response to the drug patritumab within 3 weeks, after a single treatment cycle. [S10]
- One of the challenges of targeted, personalised therapy is identifying the correct patients who will benefit. Retrospective FLIM-FRET analysis of samples from a phase 3 colorectal trial (NCT00008060, 2000-2003) was able to identify a sub-group of ~10% of the colorectal cancer patients with lower HER2:HER3 dimers that showed significant clinical improvement in response to a therapy globally adding cetuximab to oxaliplatin; this is in contrast to the original trial, which had shown no significant benefit to this combination therapy. 44/398 patients who were identified as benefiting from having EGFR-targeted therapy added to standard chemotherapy had a significantly longer progression-free overall survival (difference = 221 days) [S11]. This demonstrates the application of FLIM-FRET to identify patients with HER2-

HER3 dimers provides previously unappreciated insight into those who benefit from this treatment.

- Breast cancer patient biopsy samples analysed by FLIM-FRET showed EGFR-HER3 and HER2:HER3 dimers predicted treatment resistance and likelihood of metastatic relapse.[S3] Based on further work which found that HER2-HER3 dimerization may render HER2+ breast cancer patients less responsive to HER2-targeted treatment trastzumab, the King's team worked with the MSK to determine whether this effect would also be seen in HER2-positive lung cancer patients (NCT02675829) – *"Outside of breast and gastric cancers, HER2 wasn't being pursued as a drug target but it was hypothesized that its therapeutic targeting may have more widespread benefit [...] FLIM technology provides us with a tool to gain better mechanistic understanding of ADC response as several pharma companies are focusing their clinical development of ADCs to targeting HER2 mutations with active dimers."* (MSK oncologist [S8]). Their findings demonstrated that HER2 inhibitors were further enhanced by co-treatment with a HER2-HER3 dimerization inhibitor, resulting in improved patient outcomes. [S12]

Company creation

The need for commercialisation of FLIM-FRET has led to formation of a spinout company, **Nano Clinical Ltd.** to secure regulatory approval, conduct definitive validation and make FLIM-FRET widely available. A patent was filed in 2017 [P1] and Nano Clinical was established in 2019; a CEO has been recruited to begin the fund-raising process. [S13]

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

[S1] Testimonial - Senior Director, Global Oncology R&D, Daiichi Sankyo, Inc.

[S2] KCL-Roche contract

[S3] Cancer Research UK press release, Imaging technique could help focus breast cancer treatment, 07-07-2016

[S4] KCL-AstraZeneca contract

[S5] Testimonial - Head Translational Medicine Immunology, UCB Pharmaceuticals

[S6] KCL-Incyte contract

[S7] NCCN Flash eBulletin Newsletter Update - NCCN Guidelines, NCCN Compendium NCCN Templates & NCCN Radiation Therapy Compendium for NSCLC

[S8] Testimonial - Consultant Medical Oncologist, Thoracic Oncology and Early Drug Development Service, Memorial Sloan Kettering Cancer Center

[S9] Li, B., […], & Scaltriti, M. (2018). P1.13-43 Molecular and imaging predictors of response to ado-trastuzumab emtansine in patients with HER2 mutant lung cancers: An exploratory phase 2 trial. *Journal of Thoracic* Oncology, 13(10), [S599]. DOI: 10.1016/j.jtho.2018.08.900

[S10] Ng, T., […], & Coolen, A. (2018). The use of exosome and immune profiling to analyze a phase 2 study on the addition of patritumab or placebo to cetuximab and a platinum agent for recurrent/metastatic head and neck cancer patients. *Journal of Clinical Oncology*, 36(15_suppl), [6043]. DOI: 10.1200/JCO.2018.36.15_suppl.6043

[S11] Barber, P. R., […], & Ng, T. (2019). HER2-HER3 heterodimer quantification by FLIM-FRET and patient subclass analysis of the COIN colorectal trial. *JNCI: Journal of the National Cancer Institute*, 112(9), 944-954. DOI: 10.1093/jnci/djz231

[S12] Li, B., […], & Scaltriti, M. (2020). HER2-mediated internalization of cytotoxic agents in ERBB2 amplified or mutant lung cancers. *Cancer Discovery*, 10(5), 674-687. DOI: 10.1158/2159-8290.CD-20-0215

[S13] Testimonial - CEO, Nano Clinical Ltd.