Institution: King's College London		
Unit of Assessment: 10 Mathematical Sciences		
Title of case study: Improving cancer treatments and patient outcomes using Bayesian fluorescence lifetime estimation and patient subclass analysis		
Period when the underpinning research was undertaken: 2003 - 2018		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s): Anthonius Coolen	**Role(s) (e.g. job title):** Professor	**Period(s) employed by submitting HEI:** 01/09/1995 – 31/03/2020
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)

Novel methods of lifetime estimation and patient subclass analysis developed at King's College London, alongside research on advanced optical imaging technology for the visualisation of complex protein-protein interactions, have shown in tumour biopsies that certain protein combinations predict either effectiveness of or resistance to specific drugs. These methods help target treatments to the right patients, resulting in reduced healthcare costs, reduced morbidity and improved survival. Clinical studies have validated the methods 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 projects to develop and evaluate novel cancer drugs.

2. **Underpinning research**

Research since 2000 at King's College London led by Professor Anthonius (Ton) Coolen has been concerned with the development of new mathematical methods for the study of heterogeneous many-variable systems, complex non-equilibrium processes and survival data analysis using the tools of statistical mechanics [R1]. In particular, from 2005 Coolen developed an interdisciplinary research programme at King's in collaboration with Professor Tony Ng and colleagues in the Faculty of Life Sciences & Medicine at King's, initially supported by an EPSRC Springboard Fellowship [F1] concerned with the transfer of new mathematical techniques developed by the disordered systems community to the study of complex cellular networks, for application in cancer research [F2].

In 2004, researchers in the Richard Dimbleby Department of Cancer Research at King's, led by Ng, began using the combination of Fluorescence Lifetime Imaging microscopy and Förster Resonance Energy Transfer (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 biochemical analysis methods, along with novel methods of data analysis, allowing high throughput accurate assessments of *in vitro* and clinical samples.

The fluorescence intensity from a given ensemble of molecules decays over time according to an exponential function, which can be characterised by the fluorescence lifetime (time to decay by 1/*e*). Fluorescence Lifetime Imaging (FLIM) involves the determination, at every pixel in an image, of the fluorescence lifetime from fluorophores labelling proteins of interest in biological cells or tissue. The lifetime of the fluorophore is affected if a protein to which a fluorophore is attached forms complexes with other proteins, thereby allowing the FLIM-FRET technique to obtain a spatially resolved image of complex formation involving the protein in question. Researchers from the Department of Mathematics at King's developed a novel Bayesian model to extract the fluorescence lifetime from a fluorescence emission [R2]. The Bayesian approach allows the use of more complex models of the emission profile and model selection. Additionally, it is straightforward to extract additional measurements and to incorporate prior knowledge in the

Bayesian framework. By building these modelling methods into FLIM, powerful tools for biological research are created.

In collaboration with Ng and others, Coolen's research in survival analysis has allowed FLIM-FRET technology to visualize receptor pairings using patient subclass analysis, leading to improved matching of treatments based on patient's specific tumour biology. The research built on Coolen's earlier work on model selection in survival analysis. In particular, covariate dimension reduction is essential to be able to extract meaningful conclusions from large biomedical data sets. King's researchers developed a Bayesian method, which extends the Gaussian process latent variable model to incorporate survival outcomes [R3].

A major breakthrough in the data analysis was enabled by the development at King's of a Bayesian latent class model for competing risks [R4]. Competing risks can lead to both cohort heterogeneity and informative censoring, so that the common proportional hazards assumption is violated. The new method simultaneously models all risks and so exploits the cohort information more fully than traditional analyses. Bayesian model selection is used to obtain the optimal cohort substructure and for parameter estimation. This allows estimation of relative frailties, covariate associations and baseline hazards for each latent class and for each risk.

Working in close collaboration with Coolen, and using the FLIM technology developed at King's (underpinning research reported in a case study for UoA9), was critical in enabling Ng to use FLIM-FRET for the visualization of epidermal growth factor receptors (EGFR). This showed that two receptors within the EGFR family, such as receptors HER2 and HER3, being proximally close (dimerization) leads to enhanced tumour growth and that the presence of this dimerization may further predict success or failure of a particular therapy [R5] (underpinning biological sciences research reported in a case study for UoA5).

In a retrospective FLIM-FRET analysis of the MRC COIN trial, Bayesian latent class modelling was used to detect baseline hazard heterogeneity from the 550 patients in the trial. This gives a cohort stratification, with the number of latent classes driven by Bayesian model selection methods. Patients were retrospectively assigned to latent groups according to maximum a posteriori class membership probabilities. This allowed the identification of a subclass of 11% of patients who could benefit from treatment with the drug cetuximab. The Bayesian model selection showed that the identification of this subclass depending on the FRET score and 8 other covariates. Further analysis indicated insufficient evidence for any other subgroups. [R6]

3. **References to the research**

Research articles:

[R1] Replica analysis of overfitting in regression models for time-to-event data; Coolen, ACC., Barrett, JE., Paga, P. & Perez-Vicente, C. J., 17 Aug 2017, In: Journal of Physics A and EPL (Europhysics Letters); DOI: 10.1088/1751-8121/aa812f.

[R2] Robust Bayesian Fluorescence Lifetime Estimation, Decay Model Selection and Instrument Response Determination for Low-Intensity FLIM Imaging; Rowley, M. I., Coolen, ACC., Vojnovic, B. & Barber, P. R., 29 Jun 2016, In: PLOS One. 11, 6, e0158404; DOI: 10.1371/journal.pone.0158404.

[R3] Covariate dimension reduction for survival data via the Gaussian process latent variable model; Barrett, J. & Coolen, ACC., 1 Nov 2015, In: Statistics in Medicine. 35, 8, p. 1340-1353; DOI: 10.1002/sim.6784.

[R4] A latent class model for competing risks; Rowley, M., Garmo, H., Van Hemelrijck, M., Wulaningsih, W., Grundmark, B., Zethelius, B., Hammar, N., Walldius, G., Inoue, M., Holmberg, L. & Coolen, ACC., 24 Feb 2017, In: Statistics in Medicine; DOI: 10.1002/sim.7246.

[R5] HER2-HER3 dimer quantification by FLIM-FRET predicts breast cancer metastatic relapse independently of HER2 IHC status; Weitsman, G., Barber, P. R., Nguyen, L. K., Lawler, K., Patel, G., Woodman, N., Kelleher, M. T., Pinder, S. E., Rowley, M., Ellis, P. A., Purushotham, A. D., Coolen, ACC., Kholodenko, B. N., Vojnovic, B., Gillett, C. & Ng, T., 9 Aug 2016, In: Oncotarget. 7, 32, p. 51012-51026; DOI: 10.18632/oncotarget.9963.

[R6] HER2-HER3 heterodimer quantification by FRET-FLIM and patient subclass analysis of the COIN colorectal trial; Barber, P. R., Weitsman, G., Lawler, K., Barrett, J., Rowley, M., Rodriguez-Justo, M., Fisher, D., Gao, F., Tullis, I. D. C., Deng, J., Brown, L., Kaplan, R., Hochhauser, D., Adams, R., Maughan, T. S., Vojnovic, B., Coolen, ACC. & Ng, T., 9 Sep 2020, In: Journal of the National Cancer Institute. 112, 9, p. 944-954: DOI: 10.1093/jnci/djz231

Research grants:
[F1] Coolen, T., 'Modelling and analysis of integrated metabolic, proteomic and genetic regulatory networks', EPSRC, 01/09/2007→31/08/2009, GBP113,793.00.

[F2] Coolen, T., Ng, T., Parker, P., Santis, G., Ameer-Beg, S., 'Supra-molecular rules in signalling networks: A single-molecule comparative study in cells and tissues', BBSRC, 1/09/2009 → 31/08/2014, GBP2,289,392.00.

[F3] Coolen, T., Ng, T., Gillett, C., O'Doherty, M. & Parker, P., 'ImagInt - HER Imaging and Molecular Interaction Mapping in Breast Cancer', EC European Commission, 01/05/2011 → 30/04/2015, GBP489,125.00.

[F4] Van Hemelrijck, M., Coolen, T. & Enting, D., 'New quantitative tools for precision medicine: overfitting correction in multivariate Cox analysis', CRUK, 01/01/2019 → 30/11/2021, GBP143,004.17.

4. Details of the impact

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) could benefit from targeted treatment and evaluation, enabled by FLIM-FRET and associated patient subclass analysis.

The Bayesean lifetime data analysis developed by Coolen has been incorporated as a standard feature in the TRI2 FLIM analysis software employed by the King's team and has been used routinely in FLIM-FRET studies, providing in particular a distinct advantage when photon counts are low, enabling more rapid FLIM acquisition of large sample arrays with the required level of accuracy. Alongside the ongoing development at King's of improved FLIM technology, the close collaboration with Coolen and the survival analysis developed by him 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. The diagnostic platform developed through King's research, incorporating Bayesian fluorescence lifetime data analysis, 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 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 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 and the modelling incorporated in it has allowed these companies to develop at speed and at scale 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. Since 2014 the company has applied 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 and the

associated survival 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 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*". [S1]

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 COVID19 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 and modelling 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. [S6] This study commenced in late 2020 and will reveal the mechanism of action of Incyte's lead anti-PD-L1 compound.

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 [S8]: *"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."*

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 the survival analysis, 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. In the retrospective FLIM-FRET analysis of samples from a phase 3 colorectal trial (NCT00008060, 2000-2003), the patient subclass analysis 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 the probability 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]

5. **Sources to corroborate the impact**

[S1] 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] 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] Consultant Medical Oncologist, Thoracic Oncology and Early Drug Development Service, Memorial Sloan Kettering Cancer Center.

[S9] 2018 P1.13-43 Molecular and imaging predictors of response to ado-trastuzumab emtansine in patients with HER2mutant lung cancers: An exploratory phase 2 trial

[S10] 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

[S11] HER2-HER3 Heterodimer Quantification by FLIM-FRET and Patient Subclass Analysis of the COIN Colorectal Trial

[S12] HER2-Mediated Internalization of Cytotoxic Agents in ERBB2 Amplified or Mutant Lung Cancers