The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL:

PROJECT DETAILS	
Project Title:	**Photoacoustic imaging for the optimisation of CAR T-cell cancer therapy of soft-tissue tumours – metrology and system development**
Short Project Title:	**Metrology for photoacoustic imaging of CAR T-cell cancer therapy**
SUPERVISORY TEAM	
PhD registered at:	University of Surrey
Application webpage:	https://www.surrey.ac.uk/centre-vision-speech-signal-processing/phd-study.
Primary Supervisor(s):	Prof. Jeff Bamber (Institute of Cancer Research) Dr. Lucia Florescu (University of Surrey)
Associate Supervisor(s):	Dr. Anant Shah (National Physical Laboratory) Dr. Astero Klampatsa (Institute of Cancer Research)
Secondary Supervisor:	Dr. Emma Harris will act as the Secondary Supervisor for Institute of Cancer Research purposes.
Lead contact person for the project:	Prof. Jeff Bamber
DIVISIONAL AFFILIATION	
Primary Division:	Radiotherapy and Imaging
Primary Team:	Ultrasound and Optical Imaging
PROJECT PROPOSAL	

BACKGROUND TO THE PROJECT

This is one of two projects offered to develop photoacoustic imaging for the optimisation of CAR (chimeric antigen receptor) T-cell cancer therapy. This student will be registered at the University of Surrey within the Centre for Vision Speech and Signal Processing (https://www.surrey.ac.uk/centre-vision-speech-signal-processing) but will carry out most of their experimental work at the Institute of Cancer Research (ICR) in Sutton within the Division of Radiotherapy and Imaging (https://www.icr.ac.uk/our-research/research-divisions/radiotherapy-and-imaging), and will spend at least three months with the National Physical Laboratory (NPL) in Teddington (https://www.npl.co.uk/). Further technical advice will come from iThera Medical (https://www.ithera-medical.com/), the company that manufactures the multispectral optoacoustic tomography (MSOT™) equipment that will be used for photoacoustic imaging in the project.

In 2018, NHS England and the FDA in the USA approved the availability of CAR T-cell therapy for cancer patients. The therapy involves removal of the patient's T cells from the blood, followed by their genetic modification to

express CARs that specifically target cancer cells, and re-infusion of the cells back into the patient. The access to this "living drug" therapy has set the scene for major development in the field of personalised medicine, with more than 800 CAR T-cell therapies now being investigated in clinical trials (ClinicalTrials.gov).

Preclinical assessment of the efficacy, pharmacokinetic profile and toxicity profile of CAR T cells is of critical importance in the development and optimisation of such therapies, prior to clinical trials. This is particularly so for application to the treatment solid tumours (Klampatsa et al 2017, Martinez et al 2019, Sacchetti et al 2019). Ideally, this requires an ability to noninvasively image and track the CAR T cells in vivo. Current imaging approaches (optical, PET, SPECT, MRI) have limitations such as low tissue-penetration depth, poor sensitivity, poor quantification, inadequate resolution, lack of 3D information, or a sensitivity to CAR T cells that fades with time.

The two students will work on related projects which aim to develop and evaluate the use of photoacoustic imaging for determining the biodistribution of the CAR T cells *in vivo*. Photoacoustic imaging is an exciting relatively new biomedical imaging method which employs an ultrasound scanner in combination with a pulsed laser to create high-resolution 3D images of the optical absorption-properties of tissues and cells (Attia et al 2019, Wang and Yao 2016). The CAR T cells will be genetically modified to express proteins that are detectable using photoacoustic imaging. Both projects are multidisciplinary, but the emphasis of this project is in the physics, engineering and quantitative metrology aspects of the new technology development and evaluation, whereas the other project focuses more on the biological aspects.

PROJECT AIMS

- Develop the methodology for photoacoustic quantification of numbers of tumour and/or CAR T cells transfected for various reporter genes and assess its performance in tissue mimicking phantoms.
- Validate the impact of the method, relative to uncorrected photoacoustic imaging, using selected reporter genes and tumour models to determine sensitivities and linearities for cell number quantification *in vivo*.
- Explore the application of the method to the dynamic quantification of CAR T-cell numbers as they penetrate tumours to be treated.
- Evaluate whether alternative ultrasound array configurations and noise reduction methods have the potential to improve performance for CAR T-cell tracking *in vivo*.

RESEARCH PROPOSAL

This is an exciting multidisciplinary convergence-science project involving development of a novel non-invasive imaging approach for cell tracking *in vivo* and will require the use of biological techniques applied in gene transduction, photoacoustic imaging and computational modelling. This high-level and multi-faceted skill set will be developed over the duration of the PhD and represents excellent training in research techniques and methodology. The need to develop creative solutions for addressing challenges in the project, in liaison with researchers at the ICR, the NPL, the University of Surrey, will provide outstanding doctoral training.

The project will begin by learning ultrasound, optical and photoacoustic physics and practical methods, cell culture, transduction, CAR-T cell fluorescence-activated cell sorting and other techniques.

The student will then focus on tissue mimicking phantom studies and the measurement challenge in the project. These will involve absolute quantification of the cells, so that the *in-vivo* photoacoustic images are calibrated in terms of cell numbers, with well-assessed uncertainties validated through cross-technique comparisons. Early publishable data will arise from developing with the University of Surrey a theoretical model for quantification of the photoacoustic signal corrected for system dependent factors, and testing this for quantification of cell numbers; a standardised photoacoustic imaging phantom being developed at NPL as a part of the International Photoacoustic Standardisation Consortium (IPASC), will be used for these *in-vitro* studies, modified as appropriate for varying the optical and acoustic propagation variables for which the theoretical model is designed to provide a correction.

These skills and data will contribute to the first-year report and transfer viva assessment of the student. After completion of any necessary unfinished components to the work, papers will be submitted to peer-reviewed journals, and the findings will be presented at appropriate international/national conferences.

The student will then undertake training and receive certification for skills including those for growing subcutaneous and orthotopic tumours and *in-vivo* imaging. The project will continue with *in-vivo* extensions of the year-1 studies, using tumour models and reporter genes selected from the companion project. Minimum tumour size (i.e., number of cells in a localised collection expressing the reporter gene) for photoacoustic visibility for several models *in vivo* will be established and tumour growth monitored, with and without corrections using the theoretical model, evaluated in comparison with callipers (subcutaneous only), ultrasound imaging and fluorescence imaging using an IVIS™ scanner in the frequently encountered situation that the photoacoustic signal-generating proteins are also fluorescent. Further publications and presentations are expected at this stage.

The performance of photoacoustic imaging for studying the whole-body biodistribution of CAR-T cells, including determining the extent to which they penetrate the target tumours, will be studied as a function of time after infusion, using *in-vivo* optical (IVIS™) imaging for dynamic comparison. After termination of *in-vivo* studies at an appropriate time (to be determined), tumour and various organs and tissues will be sampled, histological sections prepared, and fluorescence microscopy and NPL's photoacoustic microscope used to validate the cell numbers predicted by ICR's *in-vivo* photoacoustic imaging system. The effect on quantification of theoretical modelling-based correction for system-dependent factors will again be studied. Opportunities will exist for comparisons of CAR T-cell imaging performance using the standard preclinical MSOT™ two-dimensional ring ultrasound array with imaging using a three-dimensional hemispherical cup array (Deán-Ben et al, 2013), and for evaluating the improvement provided by advanced noise and clutter reduction techniques that we have previously explored (Leow et al, 2019, Petrosyan et al, 2018). Further publications are expected at this stage.

To Apply for this Project:

Applications for this project must be made through the University of Surrey. For additional information about studying for a PhD within the Centre for Vision Speech and Signal Processing at the University of Surrey, and for online application details, see https://www.surrey.ac.uk/centre-vision-speech-signal-processing/phd-study. For enquiries contact Mrs Nan Bennett (N.Bennett@surrey.ac.uk) indicating your interest in this project and including your CV with qualification details (copies of transcripts and certificates).

LITERATURE REFERENCES

Attia ABE, Balasundaram G, Moothanchery M, Dinish US, Bi R, Ntziachristos V, Olivo M. A review of clinical photoacoustic imaging: Current and future trends. Photoacoustics.16:100144, 2019.

ClinicalTrials.gov. https://clinicaltrials.gov/ (2021, accessed 5 February 2021)

Deán-Ben XL, Razansky D. Portable spherical array probe for volumetric real-time optoacoustic imaging at centimeter-scale depths. Opt Express;21(23):28062-71, 2013.

IPASC, International Photoacoustic Standardisation Consortium. https://www.ipasc.science/ipasc.science/

Klampatsa A, Haas AR, Moon EK, Albelda. Chimeric antigent receptor (CAR) T cell therapy for malignant pleural mesothelioma (MPM). Cancers;0:115, 2017.

Leow CH, Bush N, Stanziola A, Braga M, Shah A, Hernández-Gil J, Long N, Aboagye E, Bamber J, Tang M-X. 3D microvascular imaging using high frame rate ultrasound and ASAP without contrast agents: development and initial in vivo evaluation on non-tumour and tumour models. IEEE Trans Ultrason Ferroelec and Frequ Contr; 66(5):939-948, 2019.

Martinez M, Moon EK. CAR T cells for solid tumors: new strategies for finding, inifiltrating and surviving in the tumor microenvironment. Front Immunol;10:128, 2019.

Petrosyan T, Theodorou M, Bamber J, Frenz M, Jaeger M. Rapid scanning wide-field clutter elimination in epi-optoacoustic imaging using comb LOVIT. Photoacoustics;10:20-30, 2018.

Sacchetti B, Botticelli A, Pierelli L, Nuti M, Alimandi M. CAR-T with license to kill solid tumors in search of a winning strategy. Int J Mol Sci;20:1903, 2019.

Wang LV, Yao J. A Practical Guide to Photoacoustic Tomography in the Life Sciences. Nat Methods. 28;13:627–638, 2016.

CANDIDATE PROFILE

Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1)

Pre-requisite qualifications of applicants: e.g. BSc or equivalent in specific subject area(s)	BSc in Physics/Engineering/Quantitative Biology (1st/2i), optionally Masters with a biomedical application.
Intended learning outcomes:	• Knowledge of advanced and novel biomedical imaging techniques, including ultrasound and photoacoustic imaging, photoacoustic microscopy and methods for improving signal-to-noise ratio. • In-depth developmental experience and skills in medical image data and signal processing. • Photoacoustic phantom design, construction and use. • Knowledge of mathematical modelling and simulation methods as applied to correcting for confounding variables in photoacoustic signal quantification. • Knowledge and skills in the planning and execution of preclinical in-vivo hypothesis-testing experiments, and corresponding statistical analysis methods, to evaluate various cancer imaging strategies in the context of CAR-T cell therapy. • The skills necessary to become an independent self-directed research scientist, including the design of hypotheses and the planning and execution of studies to test them.

ADVERTISING DETAILS

Project suitable for a student with a background in:	☑ Biological Sciences ☑ Physics or Engineering ☐ Chemistry ☐ Maths, Statistics or EpidemiologLy ☐ Computer Science ☐ Other (provide details)
Keywords:	**1.** quantitative cancer imaging biomarkers
	2. adoptive cell immunotherapy
	3. photoacoustic/optoacoustic/ultrasound imaging
	4. reporter gene imaging
	5. cell tracking
	6. physics/engineering/biology/convergence science PhD London