

# SENSOR TECHNOLOGIES FOR LIQUID BIOPSIES

INNOVATION SANDPIT

15-18 JULY 2018



Together we will beat cancer



CANCER  
RESEARCH  
UK

EPSRC

Engineering and Physical Sciences  
Research Council



Science & Technology  
Facilities Council

# AIMS

As part of Cancer Research UK's (CRUK) wider strategy in early detection research, we have recognised that this nascent field benefits from bringing together expertise not only from cancer biology, but other disciplines, leveraging the best minds and most novel concepts emerging from chemistry, physics, engineering, mathematics and computer science. To this end, we are partnering with the Engineering and Physical Sciences Research Council (EPSRC) and the Science and Technology Facilities Council (STFC) to deliver innovative workshops that develop new multidisciplinary and revolutionary research ideas.

We brought together research communities from CRUK, EPSRC, and STFC for the first Early Detection Innovation Workshop from 15-18 July 2018 at The Oxford Belfry, Oxfordshire, focusing on new research to develop and improve sensor technologies for liquid biopsy.

24 participants including cancer biologists, clinicians, bioinformaticians, analytical chemists, technologists, and physicists took part in the sandpit – 11 from CRUK and 13 from EPSRC and STFC communities.



Research conducted as a result of this event is relevant to a range of new approaches and sensor technologies for early detection of cancer markers, including (but not limited to):

- How to collect bio-specimens cost-effectively with minimal stress to a patient, while preserving critical information and ensuring that the sample taken is truly representative?
- How to process specimens robustly and at high throughput? Will specimens need to be preserved for transport or tested on site?
- What are the relevant markers and molecules? Can we have a generic 'cancer test' or do we need to be more specific?
- How to develop and try out sensors and sample processing technologies for new analytes?
- How to develop representative model systems to test and optimise sensors?
- How to improve sensitivity of sensors for the relevant markers and molecules?
- How to test and implement the technologies in the clinic?

# THE WORKSHOP PROCESS

The Director acts as the leader of the event and guides the process from a scientific content perspective. The Director works closely with the Mentors, guiding them as they interact with the participants and plays a key role in the funding decisions.

The Mentors act as real-time 'peer reviewers' but with a much more creative role. At the start of the event, their job is to encourage new ideas by asking questions, highlighting ideas that seem exciting, and making connections between participants and to the wider body of knowledge. The Mentors' role changes towards the end of the event, when they have to adopt a more critical perspective and assist with the funding decisions.



The sandpit process can be broken down into several stages:

- defining the scope of the challenge;
- sharing understandings of the challenge and expertise;
- evolving common languages and terminologies amongst people from a diverse range of backgrounds and disciplines;
- breaking down preconceptions of researchers and stakeholders;
- taking part in break-out sessions focused on challenges, using creative thinking techniques;
- capturing outputs in the form of highly innovative feasibility study proposals;
- a funding decision on those proposals at the sandpit, using "real time" peer-review.

## FEEDBACK FROM PARTICIPANTS:

### OVERALL:

"A GOOD FORMAT AND A GREAT OPPORTUNITY. A LOT LEARNT THAT WILL INFLUENCE MY RESEARCH AND FUTURE COLLABORATIONS."

### ON THE OPPORTUNITY TO WORK ACROSS DISCIPLINES

"MULTI-DISCIPLINARY WORKING IS A CORE STRENGTH OF THE WORKSHOP WITH A STRONG BLEND OF RESEARCH FROM MULTIPLE DISCIPLINES. THERE COULD HAVE BEEN ADDITIONAL CLINICAL RESEARCHERS PRESENT SO THAT ADDITIONAL OPTIONS TO FOCUS ON AN ARRAY OF AREAS OF CANCER COULD HAVE BEEN EXPLORED."

### ON REAL-TIME PEER REVIEW:

"BEING ABLE TO PITCH THE IDEAS TO THE GROUP AND MENTORS SEVERAL TIMES AND GET IMMEDIATE FEEDBACK BEFORE DEVELOPING THE IDEA FURTHER WAS REALLY INVALUABLE. I WISH ALL SCIENCE FUNDING WAS LIKE THIS!"

### MOST VALUABLE LESSON:

"THE IMPORTANCE OF MAINTAINING AN OPEN MIND IN COLLABORATIVE RESEARCH, EMBRACING NEW IDEAS, AND THE FANTASTIC POTENTIAL OF ON-THE-SPOT THINKING IN GENERATING NEW IDEAS!"



WORKSHOP DIRECTORS



Dr Nitzan Rosenfeld  
Group Leader CRUK  
Cambridge Institute and  
Chief Scientific  
Officer of Inivata



Dr Anneke Lubben  
University of Bath



Professor Hywel Morgan  
University of Southampton



Dr James Flanagan  
Imperial College London



Billy Boyle  
CEO of Owlstone Medical  
and member of CRUK's  
Early Detection Research  
Committee.  
Billy attended the final  
pitches and served on the  
funding panel.

WORKSHOP SPEAKERS



Dr David Guttery,  
University of Leicester,  
introduced liquid biopsy and  
the clinical challenges of  
implementing the technique.



Professor Josephine Bunch,  
National Physical Laboratory,  
spoke about the challenges  
of multi-disciplinary team  
science.



Dr Kate Ronanye,  
Science and Technologies  
Facilities Council,  
presented exemplars  
of research innovation  
leading to translation  
commercialisation.

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# FUNDING AWARDED

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On the final day of the workshop, each group presented their research idea. The Funding Panel, comprising the workshop Director, the Mentors, and Billy Boyle awarded the best proposals up to £100,000 each to support the subsequent pilot and feasibility studies.

We funded four projects, commencing autumn 2018 for a period of 12 months.

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## PROJECT 1:

### Epigenetic enrichment of circulating tumour DNA to enable deep profiling for cancer early detection – Team Epienrich

The team will investigate whether enrichment of a liquid biopsy sample is possible by concentrating nucleosomes, then removing DNA bound to them.

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#### Group members & Institutions:

Charlie Massie (University of Cambridge),  
Pedro Estrela (University of Bath),  
Francesco Crea (Open University),  
Nicholas Leslie (Herriot Watt University),  
Paul Milner (University of Leeds),  
Tingting Zhu (University of Oxford).

#### Aims

- Establish SOPs for ctDNA enrichment directly from plasma using chromatin epitopes.
- Develop a pattern recognition framework to quantify ctDNA enrichment.
- Test new workflows for high-resolution size-based ctDNA enrichment.
- Build capacity and line-of-sight using low-cost Affimers and fluidic device tests.
- Apply combined enrichment methods to clinical samples to test specificity/sensitivity.

#### Background

To detect aggressive cancers at an early, treatable stage we will need accurate, low cost, minimally invasive tests. Circulating tumour DNA (ctDNA) analysis offers great potential in this area due to its high analytical specificity, but applications in cancer early detection are currently limited by low signal-to-noise ratio and limited ctDNA copies. We aim to bypass these limitations by selectively enriching tumour DNA and using broad genomic analysis to improve detection in early cancers.

## Methods

Plasma cell-free DNA is nucleosomal, carrying the chromatin epitopes from the cell of origin. Several histone modifications are enriched in tumour cells and ctDNA is frequently shorter than somatic cell-free DNA. This provides an opportunity to selectively enrich ctDNA directly from plasma and to perform genome-wide analysis, bypassing the sampling limits of single locus assays.

The team will optimise chromatin enrichment methods using validated antibodies (Volition) and qPCR/CNA in preclinical models. In parallel they will systematically profile histone modifications to identify targets for ctDNA enrichment (Volition NuQ). They will develop methods for pattern recognition signal enrichment to quantify ctDNA signals and benchmark enrichment methods.

To build capacity and line-of-sight for clinical application the EpiEnrich team will develop low-cost, high-affinity Affimers targeting chromatin epitopes and benchmark these using validated antibodies. Repurposing established methods, they will fractionate cell-free DNA and identify ctDNA enriched fractions (qPCR/CNA and signal enrichment analysis). To provide line-of-sight to clinical implementation they will explore the feasibility of ctDNA enrichment on a fluidic device.

Finally, they will quantify ctDNA enrichment using the combined ctDNA enrichment methods on metastatic patient plasma samples, and test ctDNA detection rates in a cohort of 100 diagnostic plasma samples.

## How the results of this research will be used

By developing new experimental methods for liquid biopsies and computational methods to assess ctDNA enrichment, they will advance liquid biopsy early detection research. In addition they'll produce detailed prostate cancer ctDNA datasets (nucleosome profiling, size distributions).

Successful ctDNA enrichment assays will be validated in large-scale cohorts with line-of-sight to prospective clinical trials, to evaluate EpiEnrich as a diagnostic test.



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## PROJECT 2:

### ExoPop: Extracellular Vesicle Profiling for early detection of leukaemic progression – Team ExoPop

The team will aim to define content signatures of extracellular vesicles by using a current to pop them, capturing their contents on specially designed probes and analysing the signals.

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#### Group members & Institutions:

David Carter (Oxford Brookes University),  
Bhavik Patel (University of Brighton),  
Beth Psaila (University of Oxford),  
Tingting Zhu (University of Oxford),  
Nicholas Turner (De Montfort University).

#### Background

Extracellular vesicles (EVs) are cargo-carrying lipid-enclosed vesicles released by all cells. As tumours form, the behaviour of the cells is deregulated, and this is reflected by changes in the cargo they put into the EVs they release. These EVs reach the circulation, so they could be used as biomarkers to detect the presence of cancer.

Two potential barriers to using EVs as cancer biomarkers exist: determining the specific

differences in cargo between 'normal' and 'tumour' EVs requires large-scale and expensive profiling experiments; most methods for profiling these differences do not measure individual EVs, but instead rely on extracting total content from billions of EVs, meaning that small numbers of 'tumour EVs' cannot be easily detected above the background. Team ExoPop propose to overcome these issues using a novel approach to detect differences from the content of individual EVs that does not require prior knowledge of what these differences are.

#### Aims

The aim of this project is to test whether this approach could be used to detect specific EV 'fingerprints' for different cell types, including those of cancer cells.



### Methods

Plasma cell-free DNA is nucleosomal, carrying the chromatin epitopes from the cell of origin. Several histone modifications are enriched in tumour cells and ctDNA is frequently shorter than somatic cell-free DNA. This provides an opportunity to selectively enrich ctDNA directly from plasma and to perform genome-wide analysis, bypassing the sampling limits of single locus assays.

The team will optimise chromatin enrichment methods using validated antibodies (Volition) and qPCR/CNA in preclinical models. In parallel they will systematically profile histone modifications to identify targets for ctDNA enrichment (Volition NuQ). They will develop methods for pattern recognition signal enrichment to quantify ctDNA signals and benchmark enrichment methods.

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### How the results of this research will be used

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## PROJECT 3:

### Optically detected magnetic resonance (ODMR) for early cancer detection – Team Quantum Leapers

The team will build a new platform for metabolomics that uses a diamond sensor, where imperfections in the diamond lattice excite electrons to create spectra similar to NMR.

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#### Group members & Institutions:

Melissa Mather (University of Nottingham), Philippe Wilson (De Montfort University), Mangesh Thorat (Queen Mary University of London), Jacqui Shaw (University of Leicester), Victoria James (University of Nottingham).

#### Background

This work takes a 'quantum leap' towards development of a novel sensing platform for detection and quantification of metabolites in biological fluids for early detection of cancer. Biological fluids are rich in analytes that have tremendous potential to signal the early onset of cancer. Metabolites stand out as excellent candidates particularly as cancer cells are significantly more active metabolically than noncancer cells. Reports also show specific metabolites occur at altered concentrations in cancer patients as compared to healthy individuals. Tailored sensing platforms with the necessary sensitivity and specificity to

characterise metabolites at low concentration are needed to capitalise on the potential metabolites in biological fluids have as early markers of cancer. In this project a quantum sensing technology, namely an electron associated with the negative charge state of a Nitrogen Vacancy (NV) in a diamond crystal, will be used to address the above challenge. In practice, changes in the chemical environment in the proximity of a diamond substrate will be sensed through the acquisition of optically detected magnetic resonance (ODMR) signals.

#### Aims

This work aims at engineering a sensing platform compatible with biological fluids analysis; designing and implementing sensing regimes to attain the sensitivity and specificity needed to identify key metabolites in biological fluids; and forming a protocol for extraction of key features from the ODMR data.



## Methods

The key objectives are to:

- Identify candidate metabolites with predictive power for early detection of cancer.
- Develop and optimise a NV diamond sensor for detection of metabolites in liquid.
- Establish a protocol for extraction of key metabolites from ODMR signals.
- Detect metabolites in biologically relevant fluids using ODMR, validated by NMR.
- Identify actionable metrics from ODMR and NMR signals.
- Produce a 'blueprint' of a point of care NV diamond sensor for liquid biopsies.

## How the results of this research will be used

Achieving these objectives will help the team assess the potential the NV diamond sensing technology has to reliably capture signatures from metabolites with sufficient sensitivity to detect disease.

This work could ultimately lead to changes in cancer screening. In the case of breast cancer, screening could potentially be carried out using nipple aspirate that would be accessible to a wide range of the population. The proposed method could be applicable not just to early detection of breast cancer but also to other cancers, particularly those that are metabolism-related or where proximal sample quantities are limited or diluted.



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## PROJECT 4:

### TEP-eDx: Tumour-educated platelets for early cancer diagnosis – Team TEP-eDx

The team will aim to identify and sort tumour educated platelets from blood.

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#### Group members & Institutions:

Beth Psaila (University of Oxford),  
Chris Gregory (University of Edinburgh),  
Kenith Meissner (Swansea University),  
Henkjan Gersen (University of Bristol).

#### Background

Earlier detection greatly improves outcomes for aggressive cancers. Liquid biopsy – the non-invasive sampling of cancer cell-derived biomarkers from peripheral blood – is showing great promise in early detection of cancers. Current approaches focus on circulating tumour cells (CTC), cell free DNA (cfDNA) and extracellular vesicles (EVs), which are technically challenging to isolate and insufficiently sensitive. Platelets, a highly abundant cell in circulating blood, alter their content and biomechanical profile in the context of malignancy (“tumour-educated platelets” – TEPs). Circulating platelets sequester nucleic acids and cancer cell-derived products from plasma, tumour cells and EVs, and thereby may act as easily accessible, physiological ‘sentinels’ of malignancy.



### Aims and Methods:

In this project, we aim to:

- validate the utility of TEPs over cfDNA, CTC and EVs in accurate detection and molecular profiling of cancer by comparing the cancer-specific gene rearrangements detected in these individual compartments within samples from healthy donors and patients with early and frank blood cancers;
- study in vitro and in a mouse model the mechanism for transfer of cancer biomarkers to platelets. EVs as mediators of tumour-platelet cross-talk will be examined, focusing on the role of EVs from apoptotic tumour cells;
- determine the distinguishing morphological and biomechanical properties of TEPs vs. normal platelets and platelets activated by non-malignant causes e.g. inflammation using high-throughput imaging flow cytometry (ImageStream) and Brillouin spectroscopy to measure platelet elasticity/deformability.

### How the results of this research will be used

These parameters will be used to inform the design of a novel TEP sensing and isolation device. This one-year project will catalyse new interdisciplinary collaborations between clinicians, scientists and bioengineering experts. The proposed studies will validate TEPs as an enticing platform for cancer diagnostics that have great potential to facilitate molecular profiling of tumours, enabling personalised medicine and improved outcomes.

This work will form the basis of a future application for a larger-scale project to build a novel TEP isolation/sensor device. It is anticipated that the results of these investigations will open several avenues of potentially high-impact discovery science, and early collaboration with industry will be sought to assist with target prioritization and feasibility of approach.

