The Westmead health precinct hosts a cluster of important institutions of medical science — major teaching hospitals and medical research institutes — affiliated with The University of Sydney, including:

**Institute of Dental Research (IDR)** is the oldest named dental institute in the world. Research at the IDR embraces innovative research through broad themes of chronic diseases combined with a deeper understanding of the impact of oral health on the whole body. The IDR’s research strengths include microbial pathogenicity, bioengineering and mucosal pathologies.

**Kids Research Institute (KRI) at The Children’s Hospital at Westmead (CHW)**

KRI and the CHW Clinical School has approximately 300 medical researchers and support staff involved in research that covers a very wide spectrum of childhood diseases, divided into seven major research themes: neuroscience and mental health, cancer, infectious diseases & immunity, population health & health services research, genomic rare diseases, chronic diseases of childhood and clinical sciences.

**The Children’s Medical Research Institute (CMRI)** pioneered microsurgery, immunisations against lethal childhood illnesses and care for premature babies, all of which has improved the lives of countless Australian children over the last 50 years. Today, CMRI is the site of world-leading basic and translational research in the areas of cancer, neurobiology, embryology, genetics and gene therapy.

**The Westmead Institute for Medical Research (WIMR)** is one of Australia’s largest and most productive medical research centres, internationally recognised for its ground breaking work into many of the most significant diseases affecting humankind. WIMR’s more than 300 researchers investigate infectious and immune diseases, cancer and leukaemia, liver and metabolic diseases, eye and brain-related disorders and heart and respiratory diseases.

Westmead Hospital (WH) is a major teaching hospital of The University of Sydney for allied health, dental, medical and nursing students, and is one of Australia’s largest centres for postgraduate training at specialist level in all health fields. Westmead Hospital conducts biomedical, clinical and public health research in hospital-based centres and departments. As such, the research covers a broad spectrum including, but not limited to, laboratory, drug and device trials, and epidemiological studies. Each of these institutions hosts a critical mass of specialised scientists working on diverse, ground breaking research, and all increasingly are offering opportunities for Talented Student Program projects.

For more information on TSP projects at Westmead, contact: Dr Wendy Gold

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Projects on offer for Semester 2, 2017

James Chong (James.chong@sydney.edu.au) and Dr. Leonardo Pasalic (leonardo.pasalic@sydney.edu.au), Institute of Clinical Pathology and Medical Research, Departments of Clinical and Laboratory Haematology, Westmead Hospital

Coronary thrombus architecture and circulating blood cell-derived biomarkers of coronary artery disease (CAD)
Primary and secondary prevention of CAD remain a public health priority. Platelets and monocytes are the crucial cellular determinants of the blood prothrombotic potential, which interacts with the atherosclerotic plaque to initiate thrombosis leading to acute coronary syndromes (ACS). The dynamic process of intracoronary thrombus formation in ACS patients is poorly understood. This project aims to evaluate the utility of the coronary thrombus composition and a range of cell-based biomarkers as novel biomarkers of CAD. The candidate will have an opportunity to work closely with post-doctoral researchers on their choice of research questions:
1. Platelets: A) Is there a platelet functional profile that is predictive of increased risk of de-novo or recurrent ACS? B) What is the role of atherosclerotic milieu, platelet-monocyte interaction and hypoxia in formation of procoagulant platelets? C) Do current antiplatelet agents effectively inhibit platelet procoagulant function?
2. Can we identify potential new agents specifically targeting procoagulant platelets?
3. Can evaluation of circulating blood cell-derived extracellular vesicles and miRNAs improve the established cardiovascular risk scores?
4. Does coronary thrombus architecture correlate with the efficacy of drugs or devices used for coronary reperfusion?
A variety of research techniques (advanced flow cytometry, global coagulation and platelet function assays, genomic and proteomic analysis, microscopy) will be used to analyse samples from healthy volunteers and well-defined patient populations recruited through ongoing clinical and translational studies.

Bamini Gopinath (bamini.gopinath@sydney.edu.au), WIMR, Centre for Vision Research.

Profiling retinal structural and blood flow changes in Alzheimer’s disease
This project will use new and improved retinal imaging technology to obtain detailed and integrated retinal image analysis (i.e. multimodal approach) which will provide meaningful information about the brain in health and disease. This research project is imperative in helping to establish the true clinical utility of these imaging tools in dementia. The retina (innermost layer of the eye) is essentially an extension of the brain and being able to directly view the retina makes it highly attractive to observe changes in Alzheimer’s disease (AD). With the continuous advancement in retinal imaging techniques, there remains scope for further exploring the link between retinal pathology and dementia. The project aims to use new sophisticated technology called optical coherence tomography angiography (OCT-A), which allows structural elements of the retina and retinal circulation to be measured with detail. Using OCT-A, changes to the retina will be documented in patients with mild/moderate dementia (assessed using validated tool) and in healthy controls recruited from Westmead eye clinic. Digital retinal photographs will be also taken and photographs will be graded using a validated semi-automated computer assisted program to measure retinal vascular parameters. OCT and retinal vascular measures will be compared between healthy controls and probable AD cases.

Soundappan SVS (s.soundappan@health.nsw.gov.au), CHW.

Review of Paediatric Pre-hospital traumatic cardiac arrest
Pre-hospital traumatic arrests in children are not common but when they occur survival is rare. Resuscitation often continues for prolonged periods in the pre-hospital environment and in hospital. A retrospective review of pre-hospital traumatic cardiac arrests presenting to CHW will be reviewed. A literature review to identify paediatric specific protocols and develop protocol for CHW is planned.
Clara Chow (cchow@georgeinstitute.org.au), The George Institute for Global Health/University of Sydney

Investigating the appropriateness and effectiveness of eConsent forms in low risk clinical trials

This project has a couple of components: clinical, ethical and practical. The student will be required to perform a background review on eConsent, identify barriers and facilitators, liaise with members of the ethics team in regards to elements of the project and look at the various options of obtaining eConsent. eConsent (Electronic-Consent) is an evolving platform for consenting patients using a computer based consent form rather than traditional paper documentation. For this study the student would collaborate with an expert group with experience in creating PISCFs and ethics requirements. The student will compile a background review at the start of the project to provide a description of current research on eConsent, possible applications and the design for a small RCT testing the effectiveness and appropriateness of eConsent using a current PISCF of a low risk clinical trial. The student will be required to look into various options of obtaining eConsent, including RedCap data management, PDF file maker and other options. Finally, they will also be involved in the design of a consumer feedback survey aiming to measure suitable outcome measures such as user-friendliness, participant understanding and time spend to complete the consent process.

Clara Chow (cchow@georgeinstitute.org.au), The George Institute for Global Health/University of Sydney

Customising Text Messaging Program for participants from non-English background

This project is part of a RCT evaluating a text messaging program on lifestyle in people with chronic disease. The student will be required to familiarize themselves with the protocol and identify potential ways of implementing the program in different languages. Ideally this student is fluent in Mandarin or Arabic. Text messaging has been shown to be effective and simple and our group has developed several text message programs that promote lifestyle change. All our programs to date have been delivered in English, however we are keen to explore the option of delivering the messages in other languages, including Mandarin and Arabic. The student will be required to familiarise themselves with the protocol and identify possible ways and barriers of including a text message program in a different language. We aim to implement the program in approximately 25 people and at the completion of the trial we will organise focus groups and in-depth interviews to evaluate the program. The student will collaborate with the team in the design of a small study to test the acceptability of the text message program in a language other than English and assist with the ethics application, writing of study documents and final analysis.

Dr Monica Miranda-Saksena (monica.saksena@sydney.edu.au) and Christopher Denes (cden1166@uni.sydney.edu.au), WIMR, Centre for Virus Research

Investigating the role of the Arp2/3 complex in HSV-1 infection

In this project, a TSP student will attempt to develop and express a mutant recombinant herpes simplex virus type 1 (HSV-1) envelope protein in E. coli. The project will involve PCR mutagenesis, bacterial transformation, plasmid preparation, DNA sequencing, affinity purification, SDS-PAGE and Western blotting. HSV-1 glycoprotein E (gE) is an envelope protein involved in the egress of the herpesvirus from the cell, contributing to its cell–cell spread. Previous work has generated mutant forms of gE fused to the common affinity tag GST. The student will perform PCR mutagenesis to mutate codons in the gE cytoplasmic tail in order to produce a mutant protein following expression. The project will then involve the purification and collection of the mutant plasmid from its E. coli host and its subsequent analysis through sequencing and analytical gel electrophoresis. Successful mutant colonies will be expanded and the collected DNA used to transform a bacterial expression system which will be induced to express the GST-tagged protein which will be evaluated by Western blotting. This plasmid will be used to further evaluate the hypothesised role in egress of specific and/or putative sorting motifs in the cytoplasmic tail of gE.
Professor Jennifer Byrne (jennifer.byrne@health.nsw.gov.au), Children’s Cancer Research Unit, KRI

Identifying the misuse of nucleotide sequence reagents within biomedical research publications

Incorrect published research results waste resources directed towards scientific research, slow research translation, and reduce broader trust in science and use of the scientific method. This project investigates the hypothesis that incorrect use of nucleotide sequence reagents represents an underestimated source of error in the biomedical research literature. Our work has previously described a cohort of highly similar pre-clinical cancer research publications characterized by the frequent incorrect description and experimental use of nucleotide sequence reagents (such as gene knock down targeting sequences and PCR primers). To reliably and efficiently identify publications that include incorrect descriptions and use of nucleotide sequence reagents, this project will test and optimise a semi-automated tool to identify misused reagents within published papers. The project involves manual analysis of cohorts of publications that describe the use of nucleotide sequence reagents, and then cross-checking these results with those from automated screening. This will lead to suggested improvements in the automated method which can be engineered by collaborators in France. Publications that describe misidentified nucleotide sequence reagents can then be analysed to identify common features that may help to explain their origins.

David van der Poorten (David.vanderpoorten@sydney.edu.au), Westmead Hospital, Gastroenterology Department

Pathogenesis and outcomes of NAFLD and NASH in patients pre and post Bariatric Surgery

One student needed to perform a literature review and write a review article relating to the incidence, pathogenesis and outcomes of NAFLD and NASH in patients undergoing Bariatric surgery. Guaranteed authorship in a review paper at the end of the project, and first authorship if the work is of an appropriate standard. The project will involve searching pubmed, medline, Cochrane and other appropriate online resources and collating all the literature available on Nonalcoholic Fatty Liver Disease (NAFLD) and Nonalcoholic Steatohepatitis (NASH) in patients undergoing Bariatric surgery. Cross sectional and longitudinal studies will be included. The papers will then be discussed with the supervisor and a framework for a review article created. The student will then prepare and write the article with assistance of the supervisor and other experts in the field in preparation for a large prospective study of Bariatric patients at Westmead, Blacktown and Hospital for Specialist Surgery Norwest. If appropriate the student can later assist and be involved with the trial itself. The review article will be published in an Obesity, Surgery or Hepatology Journal with the student guaranteed authorship, and first authorship if the work is of an appropriate standard.

Bamini Gopinath (bamini.gopinath@sydney.edu.au), WIMR, Centre for Vision Research.

Interplay between diet, retinal pathology and coronary artery disease: innovative strategies to address information gaps

The Australian Heart Eye Study (AHES) is an internationally unique cohort of symptomatic cardiac patients with quantitative coronary angiography and retinal photography data available. This project involves the preparation and detailed analysis of in-depth dietary data in order to investigate the interplay between diet, retinal pathology and coronary artery disease. The NHMRC-funded Australian Heart Eye Study (AHES) is an internationally unique study that was designed specifically to investigate the association between retinal microvascular signs and indices of coronary artery disease (CAD) extent and severity. The primary purpose of AHES was to provide new knowledge on the potential value of retinal microvascular changes as prognostic markers of CAD risk, and whether there is a role for non-invasive retinal imaging of CAD suspects that could add to or precede coronary angiography. We have detailed nutrition information collected from a subset of AHES participants. The aim of this project is to complete the coding and cleaning of the AHES dietary data and to conduct in-depth analysis of dietary intakes and links with retinal pathology and CAD indices. These study findings could have important flow-on benefits for cardiovascular research and development of targeted preventive strategies.
CRICOS 00026A

Golo Ahlenstiel (Golo.ahlenstiel@sydney.edu.au), WIMR, Storr Liver centre
The Role of Lambda Interferons in Immune Cell Migration to Sites of Inflammation
We aim to examine the direct effects of interferon lambdas on the trafficking and retention of immune cells, particularly monocytes and macrophages, into inflamed tissue in chronic viral infections and autoimmune disease. Interferons lambda 1, 2 and 3 (IFNL) are a family of cytokines that are expressed in response to bacterial and viral infection. Unlike other interferons, IFNLs are expressed in specific tissues like the liver, lungs and gastrointestinal tract, suggesting that they have a specific immune role that remains unidentified. We have recently demonstrated that a single nucleotide polymorphism (SNP) with the IFNL3 gene can predict an individual’s ability to clear hepatitis C virus (HCV).

Unlike acute infection, where IFNLs are beneficial, chronic immune activation can result in over-activation of IFNL signalling pathways, which seems to further exacerbate the inflammatory response leading to tissue damage. Using state of the art methods such as RNA sequencing (RNAseq), flow cytometry and immunofluorescence from blood and tissue biopsies we plan to study immune cell trafficking into different organs in the context of chronic inflammation such as chronic hepatitis and inflammatory bowel disease. The aim is to elucidate how IFNL drives immune cell migration and trafficking, the trafficking molecules involved, and immune cells responsible for the pro-inflammatory role of IFNLs.

Golo Ahlenstiel (Golo.ahlenstiel@sydney.edu.au), WIMR, Storr Liver centre
Generation of human colonoids to examine gut immune responses
We aim to optimise a protocol to generate colon organoids from colon resections performed at Westmead Hospital. Following the establishment of colonoid lines, we aim to test the immune response to bacterial/viral ligands and interferon lambdas. The human colon epithelium is a complex immunological niche, bathed on one side by billions of bacteria and viruses, and the other by a mass of resident immune cells. Due to this close association, the intestinal immune system generally demonstrates a dampened response to luminal contents that prevents excessive immune reactions. While genetic factors play a key role in the development of inflammatory bowel syndromes (IBS) like Crohn’s disease and colitis, there is also an environmental component that remains poorly understood. We aim to develop a colon organoid model using human colon resections to further examine how a dull colonic immune response can become excessive in the case of IBD. This requires optimising growth factor supplementation, small molecule inhibitor concentration and other culture conditions to establish a continuous culture system. Once optimised, we will be able to perform co-culture with immune cells and subsequent treatment with bacterial/viral ligands as well as inflammatory cytokines such as interferon lambda.

Matloob Khushi (mkhushi@cmri.org.au), CMRI
Identification of correlation and novel binding patterns for transcription factors in liver cancer cell line (HepG2)
Many cellular functions are mediated by sequence-specific DNA-binding proteins, called transcription factors (TF), that regulate gene expression. Transcription factor binding to DNA, either alone or as part of a complex with other proteins, is influenced by epigenetic modifications. Perturbation of cofactor binding plays a role in many diseases, including but not limited to cancer. Therefore, the characterization of transcription factor binding sites across the genome using next-generation technologies has been a focus of research in recent years. Much of these data are now available in public repositories but is under-utilised. Therefore, in this project, we aim to apply bioinformatics and statistical tools to effectively collate, query, and mine public data repositories of transcription factor binding data for liver cancer to unveil novel, biologically relevant discoveries. The data for various transcription factors and histone modifications have been sourced and processed. Now we will apply various bioinformatics techniques to study correlation among these factors. A survey of literature will also be performed.
Pierre Osteil (posteil@cmri.org.au), CMRI
Deciphering early cell fate commitment by 3D computational image analysis
This project will aim at deciphering early commitment of embryonic cells into endoderm layer. To achieve this goal we use embryonic stem cell that we differentiate in vitro. These cells contain different reporter genes specific to the endoderm germ layer. The student will start with imaging these aggregates in 3D by using newly developed microscopy. Then, image analysis will be required to assess the intensity of fluorescence of each fluorescent protein and be achieved by computational programming through different languages (ImageJ (Java), R (C++) and bash). Gleaned data will enrich our knowledge on early cell fate choice and can be easily transposed to the proper embryo. The student will work closely with a PostDoc researcher and the computational work will be done in close interaction with a Bioinformatician from our group.

Dr Karen Scott (Karen.scott@health.nsw.gov.au), The CHW Clinical School, Sydney Medical School
Helping young people assess the trustworthiness of online health information
A plethora of online health information is available on the Internet, social media and software applications (apps). Health information can enable adolescent patients and their parents or carers to participate in healthcare decision-making with health professionals using reliable evidence and motivate them to adhere to treatment. However, many young people do not know how to determine the trustworthiness of online health information. Due to the unregulated nature of the Internet, this leaves many young people vulnerable to accessing inaccurate information on potentially critical or fatal health conditions, developing inaccurate or biased health beliefs, or being susceptible to the influence of groups with self-serving interests. Our previous TSP project found young people often rely on their parents for help, but in our research with parents we found they also need help to assess trustworthy online health information. The aim of this research is to understand how young people determine trustworthiness in online health information. We will also trial MedCred, an online rating tool we developed with Macquarie University students to help young people determine the trustworthiness of online health information. The research questions are: 1) How do young people determine trustworthiness in online health information? 2) Does the MedCred online rating tool help them determine trustworthiness? 3) How do young people evaluate the effectiveness and usability of MedCred? Findings of the research will help the researchers identify young people’s needs so they can improve educational strategies, such as MedCred. This research will be conducted using semi-structured interviews and observations. It will be conducted with adolescent patients at The Children’s Hospital at Westmead. Qualitative data will be analysed using thematic analysis.

Wendy Gold (wendy.gold@Sydney.edu.au), KRI, Westmead Children’s hospital
Integrative neuroscience: Data mining for Rett syndrome
Rett syndrome is a neurodevelopmental disorder for which there is no cure. Despite many ‘omic’ studies being conducted, very few target genes/proteins have been identified that are consistently dysregulated. This lack of discordance could be due to differences between mouse models, tissues, developmental time points and ‘omic’ approaches taken. However we cannot rule out the possibility that this could also be due to the inability for us to comprehensively compare and mine these data sets.
Rett syndrome is a neurodevelopmental disorder mostly cause by mutations in the transcription factor MECP2. Patients with Rett syndrome are physically and intellectually disabled and to date, there is no cure. Despite many transcriptomic, proteomic and metabolomic studies being conducted on Rett syndrome patient cell lines and mouse models, very few target genes/proteins have been identified that are consistently dysregulated. This lack of discordance could be due to differences between mouse models, tissues, developmental time points and ‘omic’ approaches taken. However we cannot rule out the possibility that this could also be due to the inability for us to comprehensively compare and mine these data sets.
Kavitha Kothur (Kavitha.kothur@health.nsw.gov.au), Neurology, CHW

Economic evaluation of gene panel approach versus traditional investigations in children with epilepsy

Routine diagnostic evaluation of refractory epilepsy in infants and children is extremely challenging in clinical practice as it involves performing a large number of metabolic and imaging investigations to identify the cause. Many of these tests require hospital admission and general anesthetics, which are costly and have health risks. Genetic causes are important cause of epilepsy and identification of genetic causes using early use of gene panel testing avoids diagnostic odyssey thereby reducing need for investigations and costs associated with it. Several studies on targeted gene panels of 35–265 genes have been reported in the literature with diagnostic yields varying between 10% and 48.5%. There is limited information available on cost benefits of doing the gene panel approach versus traditional evaluation in children with epilepsy. Identification of cost benefits would be helpful for planning diagnostic approach in children with epilepsy thereby facilitating economic use of limited resources. Research Aim: To compare the cost of investigations in children with epilepsy who underwent early gene panel testing with those evaluated with traditional investigations.

Methodology: Using epileptic encephalopathy database, we identified 105 children who underwent gene panel testing using MPS testing between January 2014 and September 2016 at The Children's Hospital at Westmead (CHW). The yield of genetic testing was 28.5%. We performed preliminary analysis in 19 patients and cost of evaluation was cheaper using gene panel approach compared to traditional evaluation. We are planning to extend the study to analyse all patients who underwent gene panel testing. All patients underwent range of diagnostic evaluation prior to enrolling in gene panel testing. A retrospective review of medical records will be performed to identify diagnostic tests and the procedures patients underwent and will be grouped into 4 categories as discussed below: 1) metabolic tests including CSF studies (first line and second line), 2) neuroimaging, 3) admissions and procedures, 4) presurgical evaluation including video telemetry monitoring for surgical indications, PET and SPECT and 5) other genetic testing. The costing information on investigations will be accessed through the clinical costing centre in Management Support Analysis Unit at our institute as done before with preliminary analysis. The cost of investigations will be compared in the patients that were investigated with gene panel approach with those that underwent traditional investigation. We hope that this study will identify the cost effective approach to investigation of patients with epilepsy.

Naisana Seyedasli (Naisana.seyedasli@sydney.edu.au), Discipline of Life Sciences, Faculty of Dentistry, Westmead Hospital

Real-time Study of Cell Cycle Dynamics in Human Epithelial Carcinoma

Cell Cycle heterogeneity in tumours has a key role in their differential response to environmental stimuli and chemotherapy drugs. This project will use a real-time fluorescent sensor to monitor cell cycle dynamics during the course of tumour’s exposure to metabolic stress and chemotherapy to find novel connections between cell cycle states and cancer cell behaviours. The concept of tumour cell heterogeneity has attracted a large degree of attention as a source of differential tumour behaviour in response to stimulants and drugs. A key aspect of cellular heterogeneity is cell cycle asynchrony; cells in the tumour reside at different phases of the cell cycle but more importantly, different subpopulations show distinct signatures of cell cycle profiles and growth dynamics conferring selective response and behavioural profiles during the course of tumour growth and treatment. In this project, epithelial carcinoma cells will be stably transfected with a previously designed and validated “double-fluorescent cell cycle sensor” to mark distinct phases of the cell cycle during cellular response to different environmental stimuli. Stably-transformed carcinoma cells will be treated in forms of tumour spheres, or in vivo xenografts and cell cycle dynamics will be imaged and analysed. Treatments will include chemotherapy drugs, and a model of metabolic stress (hyperglycemia).
Three dimensional characterization of canalicular fragmentation in apoptotic endothelium

We earlier discovered that apoptosis in vascular endothelial cells is unique, in that the plasma membrane undergoes extreme invagination to form an extensive network of canaliculi. The current project is to firstly fully characterize canalicular fragmentation by serial section scanning electron microscopy, and to then explore the effects of canalicular fragmentation on cell stiffness by atomic force microscopy. When we first observed ‘canalicular fragmentation’, we felt it likely that this was a mechanism for reducing micro-embolism and micro-thrombosis. We have published data supporting this idea, but despite this, little remains known of this process or it’s true biological function. Recent developments in three dimensional reconstruction by serial section scanning electron microscopy, as well as in atomic force microscopy, raise the possibility for more complete characterization of canalicular fragmentation, both with regard to structural features and the functionality of this process. Human endothelium will be cultured, and apoptosis induced by combined serum starvation and deprivation of adhesion. Cells will be harvested and embedded for serial sectioning with scanning electron microscopy, and in-silico three dimensional reconstruction used to properly define the morphology of canalicular fragmentation in individual cells. Dependent on time, atomic force microscopy will be used to correlate canalicular fragmentation in individual cells with cell stiffness.

Cross-species analysis of serum albumin with reference to the possible anti-apoptotic domain for endothelial cells

We earlier published that serum albumin, the major blood protein, contains a cryptic domain that mediates anti-apoptotic activity in vascular endothelial cells. The current project is to perform a structural analysis across species to help identify the likely active protein domain. Endothelial apoptosis is important for microvascular remodeling during growth, development, wound healing and cancer, and probably plays an important role in atherosclerosis and both diabetic and hypertensive vasculopathy. Our earlier work demonstrated a potent anti-apoptotic activity in serum albumin for vascular endothelium, and data indicates a likely putative active anti-apoptotic protein domain. The current project is to further explore the possible location of this protein domain by structural analysis across species, on the assumption that more highly conserved domains are more likely to responsible for the activity under study. This will involve comparisons of serum albumin protein sequence data, as well as structural three-dimensional analysis using SPDB protein viewer software. In this way, amino acids will be considered with regard to their type, as well as with reference to their surface topology. This will form the basis for further work using molecular expression systems to explore the mechanisms for the anti-apoptotic activity.
We have discovered that fibroblasts exchange cytoplasm with cancer cells, with the effect that the recipient cancer cells have altered shape, migration and proliferation. The current study will use single cell tracking of time-lapse recordings, to determine the persistence or otherwise of these changes following multiple cell divisions. We have discovered significant exchange of cytoplasm between fibroblasts and malignant cells, and have further data demonstrating correlation with malignant cell phenotype. Our data is, however, primarily from pooled cells isolated after FACS sorting, and this is difficult because pooled cell data obscures important events at the single cell level. To overcome this, we will perform fluorescence time-lapse experiments in which individual cells are tracked over a prolonged period of time. We will use a highly sophisticated cell tracking MATLAB script for this work, to relate the individual history of discrete cells, to their changing phenotype. In particular, changes in cancer cell size, circularity, migration velocity and division rate will be correlated with the timing and extent of uptake of fibroblast cytoplasm. The fate of daughter cells will be followed over multiple generations, determining the role of accumulating fibroblast label over generations, and also establishing the persistence or otherwise of phenotypic changes. This will substantially advance understanding of the biological significance of this newly discovered cellular process.

There is increasing interest in the relationship between mechanical cell stiffness and cellular functions. We recently published 'stiffness fingerprints' as a nuanced Atomic Force Microscopy method for cell stiffness characterization. This project will use stiffness fingerprints at the single cell level, to examine the relationship between individual cell height and cell stiffness. Cell stiffness appears to play a role in malignant cell metastasis and invasion. We recently published a novel approach to characterization of cell stiffness by atomic force microscopy, that generates 'stiffness fingerprints'. Our purpose in developing this nuanced method for stiffness evaluation, was to examine the possible role of cell stiffness in inter-cellular exchange of cytoplasm, a previously unrecognized biological process. Our more recent data, suggests that cells with low stiffness are more likely to receive cytoplasm from exchange partners. In addition, we have found that exchange is correlated with increased cell height. The question that emerges from our current data, is if cultured cells that have greater cell height, also have inherently lower cell stiffness, or if the exchange process is responsible for the increased cell height seen. The current study will examine this question, by determining stiffness fingerprints for a range of cultured cells, correlating cell height with stiffness at the single cell level.