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Book of Abstracts

Listed by alphabetical order of presenter's last name

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Analysis of multiple cancer biomarkers using nanomixing-enhanced interfacial biosensing on a multiplexed device

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Interfacial biosensing—the direct sensing of biomolecules at bare metal surface without any surface functionalization requirement—is emerging as a powerful technique for extracting chemical information from biomolecules to inform about the presence of pathological processes like cancer. Yet, such strategy is affected by slow sensing times, and large input concentration which may hinder the wider application of this technique. Herein, we report on a novel concept of an electric field induced nanoscopic flow or nanomixing that significantly increases the adsorption of biomarkers at nanometre distance to the sensing surface, reduces sensing time, and enables the detection of small molecular changes with enhanced sensitivity. To explore nanomixing for interfacial biosening, we developed a multiplex electrochemical microdevice that provides nanomixing and in-situ label-free electrochemical detection of multiple cancer biomarkers on the same device. We present data for the detection of aberrant phosphorylation in EGFR protein and hypermethylation in EN1 gene region. Our method significantly shortens the assay time (from 40 mins and 20 mins to 3 minutes for protein and DNA, respectively), increases the sensitivity by up to two orders of magnitude, and improves detection specificity. We believe that the nanomixing-ficilitated approach will provide significant advancement in the field of biosensing.[1]

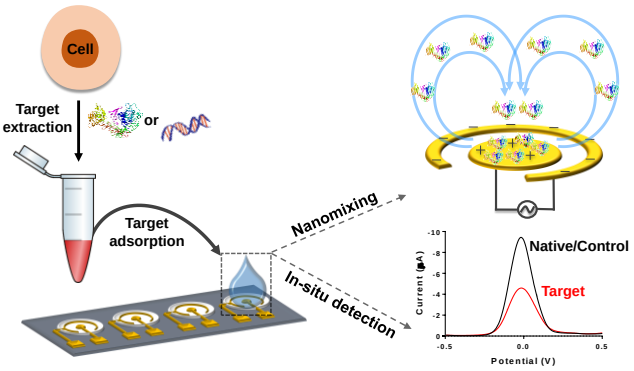

Figure 1. Biosensing workflow for the detection of cancer biomarkers in a miniaturized multiplex platform using electric field-induced interfacial nanomixing.

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N.B.: this work got invitation for back cover of nanoscale journal

Microfluidic Devices Fabricated in Poly (methyl methacrylate) (PMMA) for the separation of Aminoglycoside Antibiotics

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On site therapeutic drug monitoring (TDM) is important to provide a quick and accurate dosing to patients in order to improve efficacy and minimize toxicity. The aminoglycosides such as Amikacin, Gentamicin and Tobramycin are important antibiotics that have been commonly used to treat chronic bacterial infections of the urinary tract, lung and heart. However, these aminoglycosides can lead in vestibular and auditory dysfunction [1]. Therefore, TDM of aminoglycosides is very important due to their ototoxicity and nephrotoxicity [2]. Here, we have developed a hot embossed poly (methyl methacrylate) (PMMA) microfluidic device with a standard cross channel design to separate the aminoglycoside antibiotic drugs such as Amikacin, Gentamicin and Tobramycin. These drugs were separated successfully within 3.5 minutes using this in house make hot embossed PMMA device. In the future, we will adapt the similar separation condition using cross channel design into a device with an electrokinetic size and mobility traps (SMTs)[3] in order to provide on-site TDM of aminoglycoside that will enable more accurate and quick dosing for patients.

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Particle distributions in shear flows: consequences for blood clotting

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Blood clotting is initiated when platelets come into contact with a damaged region of the vessel wall. Although platelets circulate within blood vessels at very low volume fractions, the local concentration of platelets adjacent to vessel walls is high due to the presence of red blood cells within the blood. Red blood cells circulate in an enriched region in the centre of the vessel due to shape and deformability effects, and consequently a cell-free layer forms adjacent to the vessel wall. The high concentration of red blood cells in the centre of vessels causes platelets to "marginate" to regions adjacent to the vessel wall. Without this phenomenon, of which little is known, blood clotting is unable to be initiated. Typical numerical models of multiphase suspensions in micro-channels neglect to account for the presence of inertial lift forces which are significant even at very low Reynolds numbers. This talk will focus on i) a numerical characterisation of inertial lift forces acting on an isolated particle in a shear flow near a wall, and ii) integrating this characterisation into existing numerical models of concentrated suspensions, in order to better understand the conditions under which the cell-free layer develops and subsequent margination of smaller particles occurs.

A Bisulfite-Free Approach for Global DNA Methylation Detection based on Highly Porous Iron Oxide NanoZyme

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Measuring level of DNA methylation in a specific gene (i.e., regional) or whole genome (i.e., global) is of great interest to biology and medicine. However, such information is notoriously difficult to access *via* conventional methodologies as samples typically require cumbersome bisulfite treatment, amplification and readout steps. Herein, we report on a simple, rapid and inexpensive method for the analysis of global DNA methylation. The method avoids the use of conventional bisulfite treatment and amplification steps, and relies on the peroxidase-like activity of a new class of mesoporous iron oxides nanoparticles (NPs).[1] The genomic DNA samples were first extracted from cell-line samples. Following a heat-denaturation step, the samples were directly adsorbed onto the surface of a bare screen-printed gold electrode (SPGE). The 5-methylcytosines (5-mC) present within the surface-bound DNAs were recognized by the 5-mC antibody-functionalized NPs. As NPs exhibit enhanced peroxidase-like activity toward the catalytic oxidation of 3,3′,5,5′-tertamethylbenzidine (TMB), an intense blue-colored product was produced in the presence of H_2O_2 at room temperature.[2] This blue color was converted to a stable, electroactive yellow-colored (diimine) complex by adding the 2.0 M HCl stop solution. The generation of blue color demonstrated the naked-eye observation of methylation in the target genomic DNAs. As the diimine product is also electroactive, it allows the further quantification of DNA methylation level *via* a portable potentiostat on an inexpensive disposable electrode. The assay could successfully detect as low as 10% difference of global DNA methylation level in cancer cell lines with good reproducibility (% RSD = <5%, for n = 3) and specificity. We believe that our assay has significant potential for developing Nanozymes based inexpensive portable devices for global DNA methylation.

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Micropillar arrays as efficient screening platforms: New insight into third phase formation in solvent extraction

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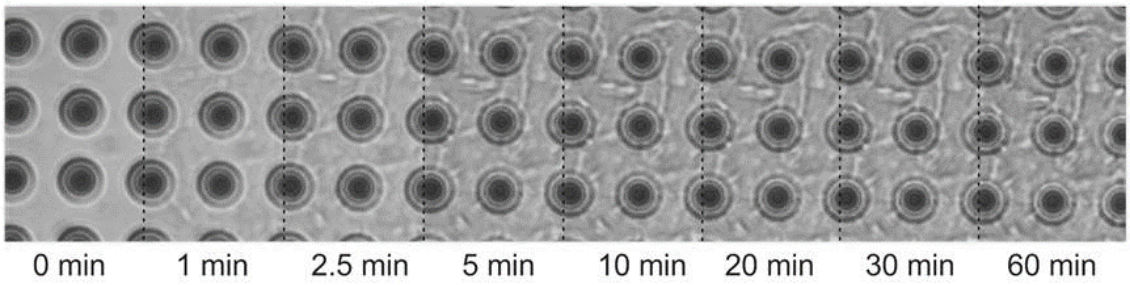
Solvent extraction is an essential technology in mineral processing and allows the separation, purification and recovery of metals from solutions. These processes require fine tuning of process parameters to avoid the formation of an undesired phase – a third phase [1]– in the system, which causes a reduction in efficiency and the addition of cleaning steps. Analysis of possible parameters is still based on bulk shake-out tests, which require large sample volumes, high costs and time, and a well-equipped laboratory. These tests do not allow real-time tracking of third phase formation, limiting our understanding of the triggers and kinetics of the physical chemistry involved. Microfluidic devices offer new insight into these complex extraction processes using small (microliter) sample volumes. Here we present a study of third phase formation in a micropillar array (a “pillar cuvette” [2]). The pillar cuvettes allow real-time investigation of liquid-liquid extractions and third phase formation using sample volumes less than 500 µl. Our focus was the extraction of high-value, industrially-relevant rare earth elements (ytterbium, dysprosium and neodymium) using Cyanex 572. The transparency of the pillar cuvette allowed optical tracking of phase changes and precise determination of the contact time before formation. Exploration of parameters like metal and extractant concentration offers insight into dominant influence parameters, including transport phenomena and metal loading limits for the studied extractant.



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Symmetry Splitting of Impacting Droplets via Surface Patterning

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Impact of liquid droplets on hard surfaces is of importance in many industrial and natural processes. Although water repellence induced by surface patterning is understood in static systems, its influence in dynamic systems is less obvious. Of interest in this study is the quantitative understanding of droplet shape dependence on surface patterning, which has previously been approached qualitatively [1].

This presentation advances on previous work [2] by making improvements to large array microfabrication methods, with the aim to reduce surface defects. Stitching of arrays of microscope images is used as a method to verify the spatial quality of surfaces with regards to defects.

New dynamic wetting outcomes as a function of surface design are of key importance to this study. The main focus of this presentation is on the importance of gas flow through the microstructure, as we aim to explain the emergence of fingers in the outer spreading rim in relation to impact region shape (Fig 1).

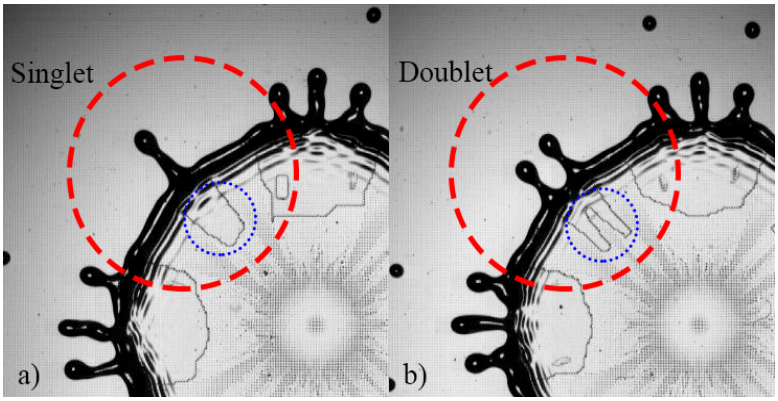

Fig 1: Droplet outcomes on microstructured surfaces consisting of 20µm square pillars with 60µm pitch and 15.8µm height, producing a) a singlet off axis finger, b) a doublet off axis finger.

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A step towards fully automated 3D printed Instruments

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The designs of detection devices and flow cells are highly specific depending on the detection type. There is an increasing demand for rapid means of design and fabricating complex detection devices, as this is often a challenging task when using traditional fabrication techniques of subtractive manufacturing. 3D printing as a fast growing group of additive manufacturing techniques is becoming increasingly popular as a simple and convenient alternative. The coupling of computer aided designs with 3D printer allows transformation of digital designs in to physical models which not only speeds up the fabrication process but also makes it practically suitable for simple and quick testing of the different designs and hence speeding up the development process dramatically. This allows for the adaptation of a "fail fast, fail often" approach in optimising designs. The material to create a part or prototype is highly dependent on the 3D-printing technology used, with Fused Deposition Modelling (FDM) providing the widest range of materials including PLA (polylactic acid or polylactide), ABS (acrylonitrile butadiene styrene) and specialised materials including glass reinforced polymers and bioresorbable materials[1]. FDM has been previously used for the creation of interfaces and customised parts to facilitate laboratory experiments[2, 3]. The aim of this project is to design and fabricate next generation miniaturized, low cost instrument applicable in portable microfluidic systems by using FDM 3D printer. Multiple materials will be used to allow one-piece fabrication of all parts of detection device including integrated electroosmotic pumps. This will provide a simple and inexpensive detection system that will be used for on-site pH monitoring of a wide range of environmental samples at various locations.

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Connecting surface force measurements between micro-drops in microfluidic devices and atomic force microscopy

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Attractive interactions between drops in which aggregation, rather than coalescence occur, control processes such as gel formation, fluid microstructure and the deposition of coatings and in formulated products (*e.g.,* food, personal care products, pharmaceutical formulations). These forces are often very system specific and a function of a number of additives or components. This talk will focus on a new method using a microfluidic device to measure the attractive surface forces between drops that arise from multi-component complex fluids including polymers, polyelectrolytes or polymer-surfactant complexes. The outcomes of the interaction force behaviour measured in the microfluidic device will be compared to the detailed Atomic Force Microscopy (AFM) measurements between similar sized drops at the same solution conditions as those used in the microfluidic device. Aspects including the effect of drops size and adsorption time scales in the two different measurement methods will also be discussed.

Real-time and Quantitative Detection of H_2O_2 Concentration by Microfluidic Data-Logger

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A 1.4cm X 3.3cm microfluidic silicon/glass needle-like device that can extract and deliver nanolitre bio samples has been developed (Figure 1a) [1]. The device has seven hydrophilic capillaries at the tip that form a phase-extraction region. This feature can form segmented flows, shown as yellow droplet in Figure 1b). The 50 µm diameter main microchannel is hydrophobic and carries segmented flows shown as blue droplets in Figure 1b). The hydrophilic capillaries can transport the aqueous-phase with nearly zero pressure gradient, but require a 20 kPa pressure gradient for mineral oil to exit because of the surface tension [2]. We have demonstrated the delivery and sampling of nanolitre segmented flows. By recording the fluorescent intensities of FITC segmented samples formed at the tip while the concentration of dye outside the tip is varied, we measured a response time of approximately 5 s. The linear relationship between the recorded fluorescence intensity of samples and the external dye concentration (10 to 40 µg/mL) indicates that this device is capable of quantitative, real-time measurements of rapidly varying chemical signals [1]. We had shown that sampled droplets and generated droplets can be mixed on-board at needle tip [3], illustrated as Figure 1. The sampled H_2O_2 droplets and FBBBE [Fluorescein bis (benzyl boronic ester)] biosensor droplets were mixed on-chip, and a mixing volume ratio of two droplets and corresponding florescence signal change can be recorded. The merged droplets can be transported and stored in the droplet format with temporal and chemical resolution for analysis.

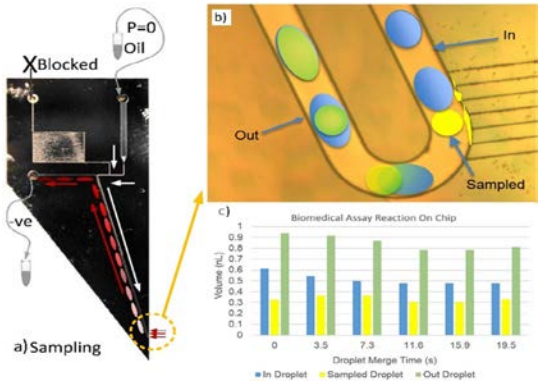

Figure 1 a) Illustration of sampled droplets (yellow) and generated droplets (blue) mixed on board. b) Droplet-merging experiments record. c) Working principle of our needle device for sampling.

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Integrated 3D printed heaters for microfluidic applications

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Temperature control is an important asset to many chemical processes. Using external heat sources for microfluidic platforms can lead to deficient heat transfer both from and to the fluids held within the microchannels, especially when using polymeric materials. Consequently, chemical systems needing external heating such as endothermic or high initiation temperature reactions exhibit long dwell times [1]. Poor performance or low repeatability can also occur due to discrepancies in the interaction between the heating element and the chip.

In recent years, multimaterial 3D printing has enabled significant progress in the development of printed microfluidic platforms with integrated functionality. In this work, we demonstrate the integration of a 3D printed heater within a non-conductive polymeric microfluidic substrate. The desired heating structure (from squared to cylindrical) is printed between two embedded electrodes using conductive graphene based polymer. Meanwhile, microfluidic channels can be printed in any shape through the non-conductive polymer, insulating them from the electric current but close to the heat source. Heating is adjusted through the voltage applied to the embedded electrodes and can reach 100°C at 10V.

Finally, the non-conductive material was replaced by a recently developed heat conductive composite polymer. The synthetic microdiamond composite material (ABS-D) has been recently proved to have increased thermal conductivity [2] and in this case provided better heat dissipation and more even heating.

The integration capability, design flexibility and good performance of the 3D printed heater provide a heating solution for microfluidic applications.

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Nanoaspiration: Development & Applications

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We are developing an experimental technique, nanoaspiration, which uses nanopipettes to sense the mechanical properties of soft nanoparticles. As a first step, the nanoscale sample needs to be located on the substrate using the nanopipette via scanning ion conductance microscopy (SICM), which is a non-contact electrochemical imaging method. A flow of ions is established between an electrode suspended inside the pipette and another in the bulk electrolyte solution. This ion current is impeded when the pipette is brought close to a particle on the surface. This signal is then used as the feedback mechanism to scan a substrate, mapping the topography and local conductivity as the pipette moves over the surface. Built in-house, our SICM instrument was first applied to repeatably map the topography of tunable nanopore membranes (Fig. 1) [1].

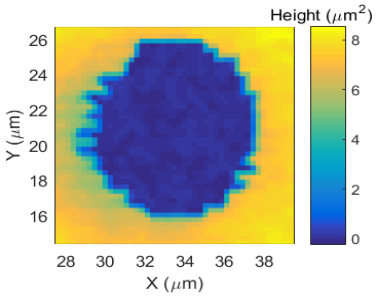

Fig. 1. SICM topography scan of single tunable nanopore (reprinted from [1]).

The eventual aim of our project is to achieve nanoaspiration by combining SICM with micropipette aspiration (MA), a technique used on the micrometre scale to study mechanical properties through optical microscopy by applying pressure through a pipette and aspirating a cell [2]. Our SICM apparatus has been modified to accommodate MA, so that both optical microscopy and the ion current through the pipette can be used as transducing signals for microparticles. By measuring the resistive pulse (i.e. drop in current) as the particle is aspirated, information about its deformation can be obtained. As expected, preliminary results show that as the aspiration pressure is lowered, the time a red blood cell takes to clear the pipette constriction increases. These results indicate that using an ion current signal has promising potential for mechanical transduction.

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A microfluidic device for capture and detection of circulating tumor cells

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Circulating tumor cells (CTCs) are promising biomarkers to determine the stage of cancer patients and its prognosis[1]. These cells are however extremely rare in patients with metastatic cancer in initial stages[2]. Microfluidics provides an unrivalled ability to enrich, isolate and capture cells in microenvironment[3, 4].

We present a hybrid device that uses immuno-affinity and surface functionalization with carbon-based nanomaterials to isolate and capture cells. Results showing the principle and feasibility of this device in bio-fluid processing and the cell capture efficiency of the prostate cancer cell line DU145 will be demonstrated. Preliminary detection results are obtained via fluorescent antibody antigen binding with emission wavelength at 488nm. Integrating this device with novel detection methods of captured cells will further develop this device as a versatile tool for detection of cancer metastasis.

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Microfluidic devices for high throughput investigation of *in vitro* and *in vivo* angiogenesis

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Automated high throughput screening technology has revolutionized our approach to drug discovery, with applications including supporting or inhibiting the growth of new blood vessel networks. However, most currently employed techniques are limited to simple two-dimensional studies in immortalized lines. We thus set out to develop a number of microfluidic platforms that by design could facilitate angiogenesis studies using *in vivo* animal models and *in vitro* human analogues that can be used in robotic systems for large scale drug screens.

Firstly, a 48 well plate format microfluidic fish trap array was developed. This device was designed to use hydrodynamic and inertial forces to trap and orient fish to image their vasculature. These fish were engineered to express GFP in their vasculature nuclei to facilitate real time imaging and quantification during their development. An confocal imaging and image analysis was used to track, identify and quantify regions of vascular development. A panel of drug candidates were screened using this platform.

Secondly, a high throughput compatible human angiogenesis structure was also developed using human cells. Using a variant of soft lithography, several moulding techniques were utilized to fabricate automation-system compatible, microfluidic channels in various hydrogel arrays in a 24 well plate format. Moulds were fabricated through additive manufacturing and were initially seed with HUVEX cells as a proof of concept high through put array. We are currently establishing these system with induced pluripotent stem cell derived endothelial cells and pericytes in a 96 well plate format arrays for large drug screens.

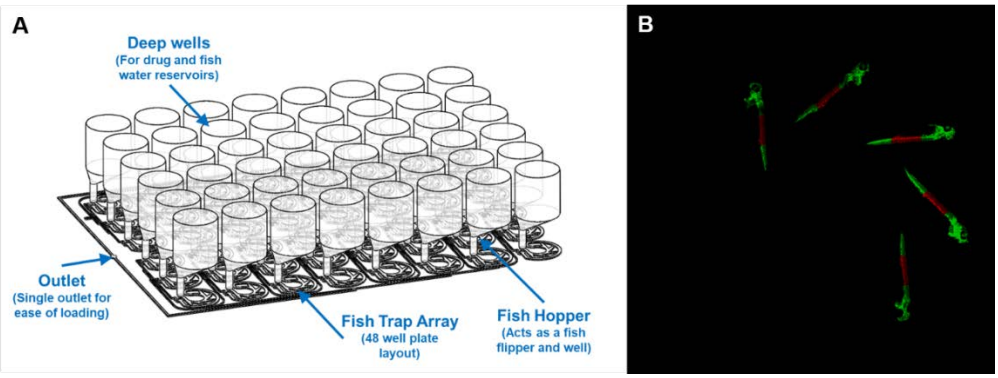

Figure 1: A) 48 well plate layout of the zebra fish trap array. B) Image of live zebra fish (green) with the identification of their trunk vasculature structure (red).

Super resolution and organoid compatible, microfabricated interaction devices for studying sub-cellular single molecule transport and multicellular interactomes

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Traditional cellular and molecular biology rely on standard cell culture ware. These systems are limited regarding separating multiple cell populations to discriminate sub-cellular features. Microfabricated devices can dramatically increase the functionality of these cultures systems, such as the simple axon extension device devised by Taylor *et al.* in 2005[1]. However, a number of limitations remain if one wishes to study the dynamics of interaction and sub-cellular transport at high resolution, largely due to the format and construction of these devices.

We have miniaturized this system further, making it compatible super resolution microscopy techniques like NSTORM, PALM and SIM. This device been used to perform experiments on retrograde transport of single proteins in neurons and their axons[2,3]. We then maximized the area in which axons can grow. Critically, this design now allows for standard molecular biology techniques to be performed on harvested non-stoma cellular components, an impossible task in standard culture ware.

These devices permit only two cell types to interact, which does not reflect typical tissues. We thus next created a multiplexed device that incorporates multiple wells on our “Flower device”. This allows for several super resolution experiments to be carried out on a single population of cells. Alternatively, the interaction between multiple different cell types can be investigated, in 2D or 3D formats (microtissues) in a single chip. We have confirmed that this deice permits the culture of millimetre-scale induced pluripotent stem cell derived neutrospheres in chemically isolated co-culture with both mouse embryonic derived neurons and mouse brain tissue.

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Label free volumetric mapping of thrombus formation and embolism in a microfluidic system

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Thrombus formation is initiated by circulating blood platelets adhesion on a damaged vascular wall. This protective mechanism could lead to the thrombosis due to platelet dysfunction, which can be responsible for the fatal heart attack and stroke. The real time monitoring of thrombus formation under fluidic settings is significant helpful for understanding the fundamental mechanisms that governs the platelet function [1]. Current efforts in monitoring and quantifying platelet function and thrombus formation is almost exclusively relying on fluorescent probes and surface markers [2]. However, the fluorescent imaging requires labelled sample and needs considerable scanning time to get accurate volumetric reconstructions. In this paper, we demonstrate the effectiveness of holographic quantitative phase microscopy (QPM) [3, 4] in identifying multiple morphological parameters of a thrombus (volume, surface area and height) formed over collagen-coated microfluidic channels by exerting a range of shear rates. We tracked entire thrombus volumes change in real-time using anticoagulated platelet-rich plasma (PRP) and observed both growth and contraction trends of thrombi in microfluidic channels, without need for chemical labelling. After that, we also quantified the process of emboli detachment under pathophysiological shear rates. The rapid and direct quantification of an embolizing thrombus can enable the study of events during undesirable vessel occlusion and lead to early diagnosis of acute coronary and venous events.

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The Aggregation of Janus Particles in Flows

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Janus particles with a hydrophobic side and a hydrophilic side are known to form complex arrangements in aqueous solution, including micellar and lamellar-type structures[1], similar to those formed by molecular and macromolecular amphiphiles. Structures can be tuned by controlling the size of the hydrophilic or hydrophobic patch, but it has also been found that structures can be manipulated in shear flow. While the hydrophobic force favours aggregation, torques on individual particles generated by a shear flow can disrupt or destroy the alignment between hydrophobic patches[2]. In this talk we will discuss the forces generated by a flow, including the effect of slip[3,4] at the hydrophobic face of particles. Anisotropic slip, occurring at the hydrophobic face but not the hydrophobic face, will alter the torque due to shear compared to an isotropic particle, but interestingly it can also generate significant torque even in a uniform flow[4]. By considering the balance between these hydrodynamic forces and the hydrophobic force, we discuss the phase diagram of Janus particle aggregates in terms of this anisotropy in slip. In particular, we identify a characteristic shear rate that scales as $\gamma/R\eta$, where γ is the interfacial surface tension on the hydrophobic face, R is the particle radius, and η is the liquid viscosity, as well as a characteristic velocity $\gamma R/b\eta$, where b is the in slip length at the hydrophobic face.

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Anisotropic fluid conduction in periodic obstacle arrays

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We describe anisotropic permeability in microfluidic obstacle arrays such as deterministic lateral displacement (DLD) arrays [1,2]. We demonstrate via experiments and lattice-Boltzmann simulations that subtle array design features cause anisotropic permeability. Anisotropic permeability indicates the microfluidic array's intrinsic tendency to induce an undesired lateral pressure gradient. This can cause an inclined flow and therefore local changes in the critical separation size. Thus, particle trajectories can become unpredictable and the device useless for the desired separation task. Anisotropy becomes severe for arrays with unequal axial and lateral gaps between obstacle posts and highly asymmetric post shapes, such as triangles. Furthermore, of the two equivalent array layouts employed with the DLD, the rotated-square layout does not display intrinsic anisotropy. We therefore recommend this layout over the easier-to-implement parallelogram layout. We provide additional guidelines for avoiding adverse effects of anisotropy on the DLD.

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A versatile microfluidic device for production of ultra-small nanoparticle-encoded microbeads

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Spectrally encoded microbeads are an attractive platform for assay miniaturization and multiplexing in the biological sciences. Compared to organic dye-based commercially available Luminex's xMAP technology, upconversion nanoparticles (UCNPs) are better alternatives due to their photostability, nil background, and single wavelength excitation [1]. However, their translation into clinical research and the pharmaceutical industry has been slow due to the lack of techniques that can produce uniform polymer beads with dimensions below 10μm [2]. Here, we developed a robust microfluidic platform for the production of monodispersed PEG–acrylate polymer microbeads with sizes of a few microns encoded with luminescent upconversion nanoparticles (UCNPs). This capillary microfluidic-based microbeads generator was fabricated by using simply tapered capillaries and epoxy glue. A gradually widening channel was designed to allow optimized UV exposure for photopolymerization and to prevent coalescence. The microbeads exhibited well-defined sphericity and excellent monodispersity with a coefficient of variation (CV) below 3%. The encoded bead synthesis strategy we developed is readily extensible to larger numbers of codes, laying the foundation for future high-throughput multiplexing of biological assays.

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Fluid Dynamics Simulation for New Insight into Microfluidic Systems

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Microfluidic systems are of tremendous technological interest as demonstrated by their use in chemical and biochemical analysis, cell biology and in process intensification. Knowledge of hydraulics behaviour of fluid flow in microchannels leads to accurate predictions of velocity field and pressure drop, which are essential requirements for optimal design of microfluidic system and safe operation. Moreover, simulation data such as instantaneous pressure and velocity fields can be extracted along with a considerable amount of data associated with the motion of a fluid in a microfluidic system. The most broadly used approach to analyse the motion of a fluid within a complex structure is to use computational fluid dynamics to solve the motion of a fluid from a set of governing equations.

Here, we demonstrate the application of simulation in three case studies. In the first case, a simulation study of parallel stream flow was carried out for the scenario of a liquid-liquid multi-stream micro-solvent extraction chip, to study the flow regimes and predict the interface position for a range of flow rates [1]. In the next case, simulation was employed to provide an analysis of the hydrodynamic conditions and geometry optimization in microfluidic post array to induce hydrodynamic flow conditions with microfluidic vortex shedding and deliver mRNA to human pan T cells [2]. And in the last case, simulations in multiphase fluid flows were performed to investigate the phase morphology and confirm the effect of capillary, flow rate ratio, viscosity ratio, width ratio, and inlet angle in microfluidic hydrodynamic focusing [3].

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Fabrication and Characterisation of Nanopipettes

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Extensively used in analytical and electrochemical applications, nanopipettes remain one of the core components in defining the sensitivity of each operation. Notably, the resolution of the scanning ion-conductance microscope (SICM), an instrument capable of imaging the topography of surfaces in electrolyte, is in fact, strongly influenced by the diameter of the nanopipette used for measurement.[1,2] As our fascination with the nanoscale continues to flourish, smaller nanopipettes (see Figure)[3] are increasingly in demand. Therefore, it is imperative to establish a reliable method that accurately characterises the geometry of the nanopipettes as well as assessing the reproducibility of the fabrication protocols. This presentation will demonstrate the most recent results acquired via both imaging and electrochemical characterisation methods for nanopipettes fabricated from a range of pipette laser pulling protocols comprised of various heating temperatures and draw forces.

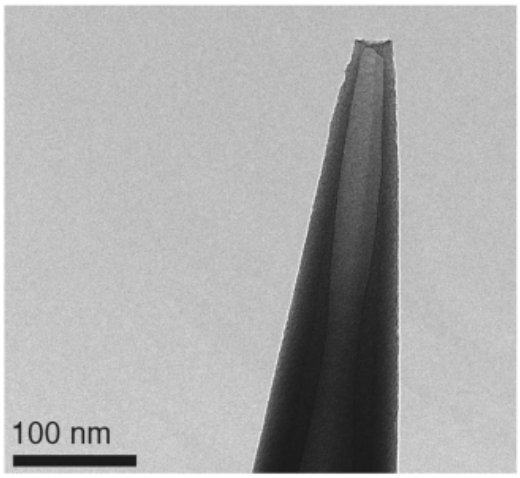

Figure: Transmission Electron Microscopy (TEM) image of a nanopipette, reproduced from Ref. 3.

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Microfluidic Third Phase Screening in Rare Earth Solvent Extraction Systems

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Rare earth elements are indispensable for many high-tech applications but notoriously difficult to purify. Separation and purification of rare earths usually relies on solvent extraction but their chemical and physical similarities demand many extraction stages, specific extractants, and fine tuning of pH.[1] At times, extraction conditions lead to an undesired 'third phase',[2] which is immiscible in both aqueous and organic phases and causes a reduction in process efficiency, additional cleaning steps, and increased costs. Effective process design and operation requires careful parameter screening. This is usually achieved by bulk-scale shake-out tests, wasting large volumes of sample, reagent, and time. Here, we report a microfluidic screening test for third phase formation, suitable for industry process development and optimisation. We demonstrate third phase screening of three rare earths (Yb, Dy and Nd) for the extractant Cyanex 572[3]. The method permits exploration of a wide range of parameters, while reducing the organic volume by three orders of magnitude.

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Emergent Properties of Janus Spheres: Experiments

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Janus spheres are colloidal particles with two distinct hemispheres [1]. This asymmetry contributes towards interesting emergent properties for clusters of Janus spheres. Our interest in these emergent properties is driven by two factors. Firstly, there are untested simulation results suggesting that Janus sphere clusters can form interesting phases [2]. Secondly, the asymmetry of Janus spheres suggests that the aggregation process could be controlled. One potential mechanism for controlling self-assembly involves the self-orientation of Janus spheres in flow. This is the subject of our current research relating to the dynamics of individual Janus spheres with slip boundary conditions [1].

In preliminary experiments, free diffusion studies have been conducted to measure the diffusion coefficients of 800 nm and 600 nm diameter silica spheres. Control silica spheres were compared with hydrophobic (silane coated) silica spheres (see Figure). Initial results have indicated there was no detectable difference between the diffusivity of control and silane coated silica beads. Further experimental studies are required to establish whether slip-induced dynamics can be used to control the clustering behavior of Janus spheres.

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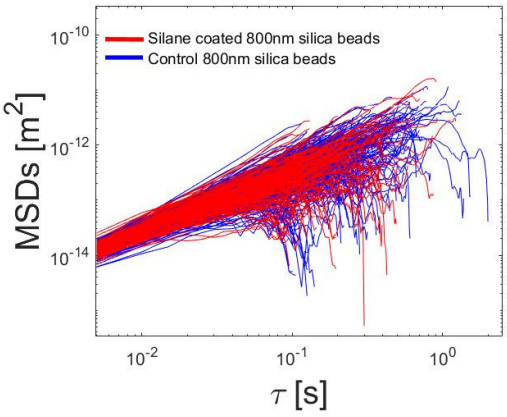

Figure: Mean square displacements (MSDs) as a function of time for 800nm silica spheres

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Inertial separation of hydrogel microdroplets by size

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Hydrogel droplet microfluidics has been demonstrated for a variety of applications in biochemistry, biology, and medicine, including single-cell and single-molecule analysis, directed molecular evolution, and detection of cellular secretion. The ability to prepare hydrogel droplets with high monodispersity can lead to better synchronous populations and more quantitative assays. A variety of methods have been reported for sorting common types of droplets, such as water-in-oil (W/O) and oil-in-water (O/W) emulsions, however, there have been few reports about the separation of hydrogel droplets in microfluidic devices.

Here, we describe the use of inertial microfluidics [1] for passive, continuous, and high-throughput separation of hydrogel droplets by size. The separation is achieved due to size-dependent inertial equilibrium lateral positions (Figure 1). We found that hydrogel droplets containing microalga *Euglena gracilis* (*E. gracilis*) shrink due to cell growth, while empty hydrogel droplets retain their size. Moreover, we demonstrated the inertial separation of cell-laden and empty hydrogel droplets varying in size. After sorting, we were able to recover cells from hydrogel droplets by melting without significantly affect cell viability. By combining with genetic engineering and active sorting techniques, we expect that this platform would be used as a powerful tool for directed evolution.

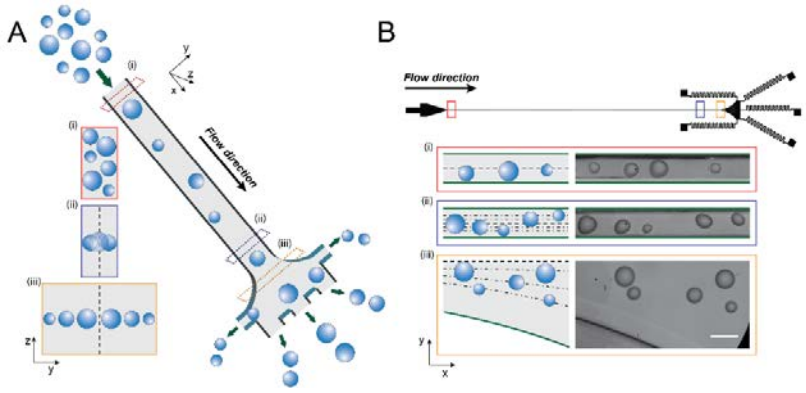

Figure 1 Separation of hydrogel droplets due to size-dependent inertial focusing equilibrium positions. (A) A schematic view of separation principles. (B) A top view (upper) of the microchannel structure. Schematics (left) and experimental images (right) of the droplet lateral distributions. Scale bar = 100 μm.

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Thermal flow sensor for micro-velocimetry and sub surface flow measurements

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AC resistance thermometry is a thermal characterisation technique for solid materials [1]. It has also been adapted for liquid measurement. The technique measures a materials thermal response as it changes with measurement depth. The measurement depth is controlled by the electrical frequency driving the thermometer. The electrical response of the thermometer is linearly coupled to the thermal response of the system. A fabricated resistance thermometer was sealed inside a microfluidic channel. An applied liquid flow increases thermal conduction away from the thermometer. The fluid velocity varies over the height of the channel (Poiseuille flow). A sweep of electrical frequency gives the thermal response as it varies across the flow field. This experiment aims to measure flow velocity varying with height in microfluidic systems. This technique is often referred to as velocimetry. Existing velocimetry techniques function by the addition of particles to the fluid. The flow velocity can be measured by microscopy or by Doppler shift. An AC resistance thermometry approach can measure in-situ, without the addition of particles. This is an advance for velocimetry techniques.

We present experimental measurements showing that the sensor can measure volumetric flow rate. COMSOL simulations agree well with experiment. Extended simulations support that the sensor can measure the velocity profile of flowing fluid.

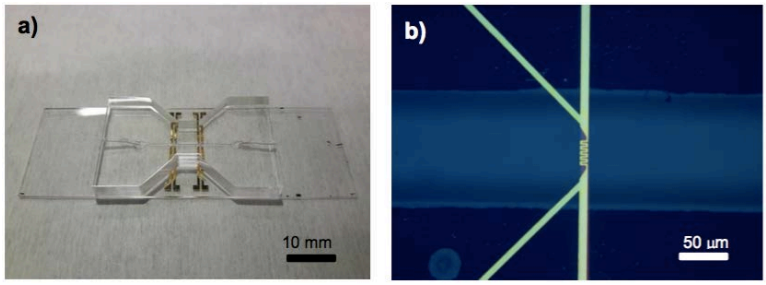

Figure 1: a) PDMS microfluidic chip, plasma bonded to patterned electrodes. b) The serpentine resistance thermometer inside the 125 micrometer channel.

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“Snail mail separations” – a portable, battery-powered and solvent-less platform for electrophoresis

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The recent trend in personalized medicine –where therapeutic conditions are tailored for each individual – requires the development of affordable, user-friendly medical diagnosis devices. This is particularly important for sample collection in remote areas with limited access.[1] However, samples collected at home still need to be sent to a laboratory for analysis. Careful and adequate storage is very crucial in order to preserve the quality of the samples as transportation time might be varied.[2] In an attempt to improve this step, we present a portable electrophoresis platform, without liquid reagents, that can perform electrophoretic separations of small molecules from dried blood spots while being sent through the mail. The key to the platform is a polymer inclusion membrane (PIM) consisting of Cellulose Triacetate (CTA), 2-Nitrophenyl Octyl ether, and 1-Ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide ([EMIM][NTf_2]). A 5-cm, “dry-to-touch” PIM strip was assembled into portable device equipped with two 12V batteries in a plastic housing. The separation of Berberine from dried blood spot (DBS) without further treatment while being transported through the mail system from one university campus to the laboratory at another campus demonstrates the through-mail capabilities.

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Fluid flows during drops coalescence triggered by the presence of surfactant

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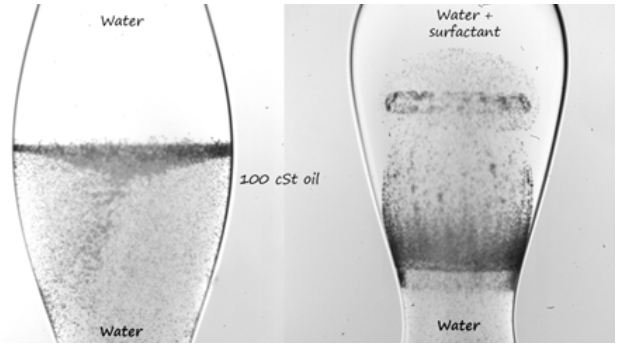
What will happen upon merging of miscible drops that have different composition? Typical investigation of coalescence involves same drops, however merging of dissimilar drops, being of different size and/or composition remains and open question. This study provides a thorough understanding of the merging process immediately after contact of surfactant-laden and surfactant-free aqueous drops in surrounding oils. Numerical simulations provide a deeper insight into the liquid redistribution during the merging and the results are in good agreement with the experimental data.

It is observed that the surfactant-free drop intrudes into the surfactant-laden drop in the form of a penetrating jet and mixing patterns within the coalescing drops are due to the force imbalance caused by capillary pressure difference and surfactant-induced Marangoni stresses; the intensity of the convective bulk motion is also influenced by the viscosity of the outer phase. Observed spontaneous interfacial flow due to Marangoni stresses can be potentially used for the interesting structures formation, such as aqueous drop inside aqueous drop.



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Liquid marble-based digital microfluidics – a new platform for miniature labs

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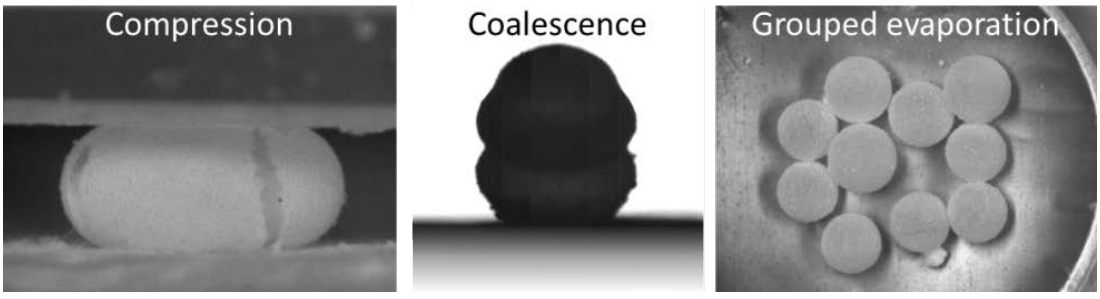
The liquid marble (LM) is a microliter-sized droplet coated with hydrophobic powder. A LM possesses useful properties that allow it to function as a self-contained, open-format digital microfluidic system. Its ease of production and versatility in handling techniques promoted the use of LM's in numerous fields, most notably in three-dimensional cell culture applications. LM's have been used to culture tumour cells, spheroids, as well as cryopreservation of mammalian cells without cryoprotectants[1]. LM's can even be applied in high-throughput PCR or cell stretching. A LM is created simply by rolling a droplet on a powder bed and then transferred onto a solid or liquid surface for further manipulation. LM's infused with cells and growth media can be incubated or mechanically agitated. LM's can be moved across various surfaces, acoustically levitated, spun, squeezed, coalesced and split. Our previous work focuses on the various manipulation techniques such as magnetic actuation[2] and self-propulsion via Marangoni solutocapillary flow[3]. Currently, we are expanding our work to investigate the fundamental mechanics of a LM to develop them into miniature labs capable of cell culture and three-dimensional manipulation. Our studies include investigating the LM under compression, coalescence of binary LM's as well as the behaviour of grouped marbles at elevated temperatures for PCR applications.



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A microfluidic gradient generator to simulate the oxygen microenvironment in cancer cell culture

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The lab model used to represent cancer during drug discovery and testing does not exhibit many of the important properties that are present in cancer *in-vivo*. It is well known that the simplest lab models, cell monolayers on plates/flasks, react differently to anti-cancer drugs compared to more complicated models[1]. Low oxygen (hypoxia) is an important and common difference between simple cancer models and tumours *in-vivo* because of their rapid growth and lack of functional vasculature[2], and is also associated with poor prognosis and resistance to drugs[3]. We present a system capable of exposing a monolayer of cancer cells to a cross-stream oxygen gradient between hypoxia (<5%) and hyperoxia (>70%) using a re-sealable y-shaped microchannel and gas-control system. The gradient is measurable in real-time using an integrated thin-film oxygen sensing substrate, which consists of a polystyrene matrix with incorporated oxygen-sensitive fluorescent dye. The dye exhibits a linear response and high contrast ($I_0/I_{100} = 12$) within the range of interest[4]. To demonstrate the applicability of the platform, the expression of a hypoxia inducible factor (HIF-1) in *Ishikawa* human endometrial cancer was characterised after 1.5 hours in the system. This platform provides a valuable tool for not only the culture of cancer cells in a more in-vivo-like microenvironment, but allows separation of the cells from the microfluidic system after fixation for immunofluorescence and other biological characterisation techniques.

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Liquid manipulation in a functionalised ‘pillar cuvette’

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Manipulation of micro- and nano-volumes of liquid offers advantages in the study of biological, chemical, and physical processes. Multiple reactions can be observed simultaneously to screen the effects of reagent concentration, stoichiometry temperature, and/or cell type. Our group has previously developed the ‘pillar cuvette’, which creates a small (< 1 μL) volume liquid film over a relatively large area (several mm^2) and is suitable for absorbance spectrophotometry [1, 2] and studying crystallisation [3]. The transparent cuvettes rely on wicking for liquid movement through the pillar array. [4] In this study, we create patterned liquid films by exploiting sharp boundaries in surface chemistry and dislocations in the array, which guide the liquid during filling. Open ‘channels’ and ‘wells’ can be prepared without precision liquid handling. The physical behaviour and potential for using pillar cuvettes for analytical chemistry and screening is discussed.

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South Australian Node of the Australian National Fabrication Facility Supports Lab-on-a-Chip and Micro/Nanofluidics Science

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The South Australian Node of the Australian National Fabrication Facility (ANFF-SA) is a state-of-the-art micro/nanofabrication facility specialising in design and engineering of miniaturized fluidic chip technologies. Lab-on-a-chip technology we provided are able to integrate with functional interfaces, integrated electrodes, and optical structures for sensing, separation, and synthesis capabilities in the health, defence, resources sectors. Fabrication in a variety of materials is available in-house: silicon, borosilicate glass, quartz, various polymers, and metal. The Node's experienced team can help with all steps of prototyping, from simulation to fabrication, characterisation and testing, as outlined below.
ANFF-SA provides services on modelling and simulation of micro-scale fluid flow to gain a detailed understanding of fluid behaviour in complicated geometries to shorten the design-to-prototype time and optimise the device performance.

The ANFF-SA facilities support fabrication of precise patterns on glass/silicon by using direct-write (maskless) and masked UV-photolithography, coupled with wet/dry etching to form the geometrical structures. Furthermore, the chips can be sealed by using thermal compression process or adhesive to achieve same/hetero- material bonding. ANFF-SA can also embed metal electrodes or pattern other surface layers in these devices for the use in sensing, manipulation, and heating/cooling. Where lithography is not applicable, ANFF-SA offers expertise in nano-precision CNC machining and can prepare master structures for embossing or injection moulding. Direct machining of complex geometries can be achieved in PMMA, PTFE, Al, Ti, stainless and tool steel, with feature accuracy down to micron levels.

Many characterisation tools are available to users. Micro/nano x-ray computed tomography provides non-destructive 3D imaging of low and high atomic number materials, composites, polymers and biological samples with resolution down to 50 nm (depending on the sample and preparation). Access to the latest image processing software allows calculation of pore size, continuity calculations, and density.

The highly specialised and professional technical team is experienced working with both industry and academics and, where possible, training and hands-on access is encouraged. You are welcome to access the facility by contacting the author of this paper or the Facility Manager (Simon.Doe@unisa.edu.au).

Drop Impact of Non-Newtonian Fluids on Patterned Polymer Surfaces

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Interactions of fluids with micro-patterned surfaces are important for numerous microfluidic applications such as surgical endoscopes, biological fluids, MEMS, and applications related to cell growth platforms.[1-3] In recent times, profound developments in high speed photography and microfabrication have led to detailed investigation of dynamic interactions between fluids and micro-patterned surfaces (Figure 1).[1,2] Much interesting work has been conducted on drop impacts of Newtonian fluids, primarily focused on water.

Drop impact of high viscosity and non-Newtonian fluids on micro-patterned surfaces is of significant additional interest due to common use of such fluids in industrial and biological processes.[2,3] High velocity impacts lead to several interesting phenomena which will be studied alongside collaborators. In this research work, aqueous solutions of (for example) glycerol and cornflour will be used in drop impact experiments on micropillar arrays. The effect of different micropillar patterns (for example rectangular and hexagonal patterns) will also be studied. The impacts will be analysed and compared with results for water.

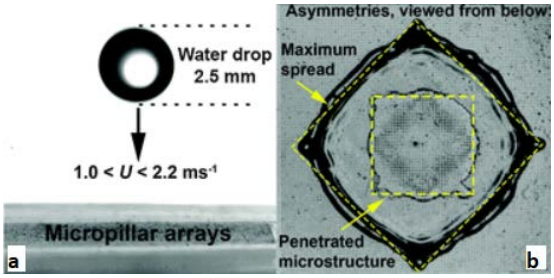

Figure 1: Water drop impacts on a patterned surface viewed a) from the side and b) vertically. Reproduced from Ref. 1.

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Effects of viscoelasticity on particle sorting through a straight microchannel with trapezoidal cross section

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Inertial microfluidics has been a flourishing area of research in the past decade for particle and cell sorting due to its capability to precisely position microparticles; however, the underlying mechanisms especially in non-Newtonian fluids is poorly understood. To date, several studies have been performed to enhance the mechanism of particle focusing within microchannels[1, 2] by altering channel geometry, flow rate and fluid viscosity. Previously, researchers have considered fluid viscosity to be Newtonian, while recently, using non-Newtonian solutions has drawn attention via its viability to improve particle sorting by applying an extra viscoelastic force, which alters the equilibrium positions[3]. There have been very few studies conducted to observe the behavior of microparticles' movement in non-Newtonian fluids especially within trapezoidal geometry. Therefore, the present paper demonstrates this phenomenon within two different straight trapezoidal microchannels for particle sizes of 5.1 and 9.7 µm. Our results reveal that 5.1µm particles can be focused utilizing 1500 ppm PEO (poly (ethylene oxide), M_w=2000 KDa, Sigma- Aldrich) solution for a wide range of Reynolds number (Re), while remain dispersed in Newtonian solution for almost all values of Re. For 9.1 µm particles, by decreasing the cross-sectional area of the channel and increasing viscoelasticity, more accurate focusing was achieved. Furthermore, effects of all the dominant forces in both Newtonian and non-Newtonian solutions are explained to shed a better light on the mechanisms of microparticle lateral movements.

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Molecular Dynamics Simulations of Janus Particles

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Janus particles have attracted much interest because of the way that they interact with each other, and self-assemble into coherent phases and higher-order structures. While most molecular dynamics studies simulate Janus particles using soft-sphere potentials to observe their self-assembled structures, we apply hard-sphere potentials to study the dynamics of slip-asymmetric Janus particles in a fluid. Previous theoretical predictions show that in a Newtonian fluid flow if a Janus particle has an asymmetric slip boundary condition, a torque is generated on it, and our molecular dynamics simulations have verified that.[1, 2] Moreover, the results indicate that the magnitude of torque on the particle depends on the angle (θ) between the asymmetry and the flow (Figure, left).[2] As a result, a uniform Newtonian flow can exert enough torque to orientate a Janus particle in the direction of the flow (Figure, right).[1] Therefore, self-assembled structures of Janus particles may be unstable in uniform flows. We will present our latest results investigating the effect of exerted torque on the formation and stability of the self-assembled structures and orientation of Janus particles in a uniform fluid flow.

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Oblique Impact of a Droplet on a Textured Surface

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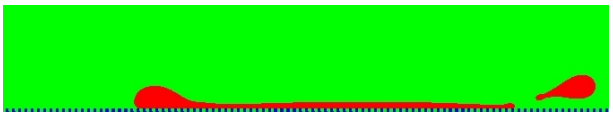
The impact of droplets has been intensively studied over the past because of its prevalence in a number of industrial applications such as inject printing or spray coating but also because it encompasses some of the most difficult modelling challenges in fluid mechanics such as a free surface, a wetting front, or topography changes. Recently, Josserand and Thoroddsen [1] have conducted an exhaustive review of the research related to the dynamics of wetting after impact on smooth and rough surfaces. Better understanding how the droplet wets the solid surface after impact is critical to obtain a better control in practical applications. For example, one may wish to avoid lamella break-up and the production of satellite droplets post-impact in the application of pesticide on foliage.

In spite of its obvious practical relevance, the problem of the oblique impact of a droplet on a textured substrate has not to date been investigated in a rigorous and systematic way. The goal of this study is to investigate the conditions under which a spreading lamella of an oblique drop impact onto a textured substrate breaks-up and generates a satellite droplet. To provide a greater understanding of the relation between break-up in the lamella with the control parameters, we have developed a two-dimensional multiphase Lattice Boltzmann code following the Shan-Chen model [2]. Numerical results demonstrate that the impact angle, impact velocity and wettability of the substrate influence the occurrence of the break-up in the lamella.



Multi-Phase Lattice Boltzmann simulation of the break-up in the lamella of an oblique drop impact onto a textured substrate.

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Models for the Bead Mobility Technique: a Droplet-Based Viscometer

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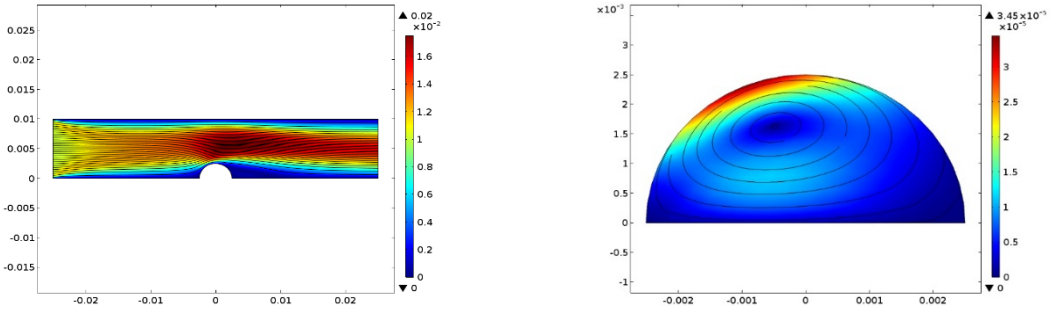
Better understanding the properties of organic aerosols (OA) is attracting increasing attention because of the important role they play in climate change. The viscosity of OA has been shown to range from liquid to solid/semi-solid across the range of atmospheric relative humidity. A method known as the "bead-mobility technique" has been developed by Renbaum-Wolff et al. [1] to quantify the viscosity of an atmospheric particle over a range of atmospherically relevant humidities. The method is based on the assumption that the strength of the flow recirculation inside a droplet placed in a shear flow is related to the droplet viscosity. This work presents a simple analytical model which predicts the internal flow in the droplet and provides a correlation relating the strength of the flow in the droplet to its viscosity. The validity of this analytical model is assessed by comparing the analytical results with a corresponding two-phase flow simulation with a moving mesh which captures the motion of the interface. The ability of the analytical model to reproduce experimental data reported in [1] is also quantified. The good agreement between the analytical model and the experimental data confirms that the droplet velocity provides a useful proxy to estimate the droplet viscosity for small liquid samples for which standard viscometry techniques do not apply.



Flow streamlines and contours of velocity magnitude for the outer flow (left-hand-side) and the inner flow (right-hand-side)

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Microfluidics Slurry Handling Platform for Ion Sensing

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A microfluidic device based on laminar flow is presented for on-line monitoring of small inorganic ions during mineral processing. The concept was demonstrated previously in H-filter [1] and hydrodynamic filtration [2] in closed microfluidic systems. Here, we expand the concept to applications where the extraction stream inside the microchannel is in contact with the bulk sample slurry under turbulence.

Laminar flow can be easily maintained in microfluidics under low flow rates (Reynold's numbers <1). Under these conditions, ions and particles will move across an aqueous-aqueous interface by diffusion only as mixing is negligible. Small ions will diffuse faster and to longer distances than particles. This difference can be utilized to efficiently extract small ions while avoiding the blocking of the microchannels and fouling of detector by particles.

Here, we demonstrate the extraction of iron (III) ions from acidic solutions into an extraction stream containing 100mM thiocyanate ions. The rapid complex formation enables colorimetric detection at 490nm. The effects of flow rate, extraction time and sample stirring were studied along with theoretical simulations. We achieved at least 18% extraction of iron (III) at flow rate of 3mL/hr and stirring at 500rpm. The linear range was 0.2 – 1.2 mM (R^2 = 0.998). With further optimization, this device can be employed for on-line monitoring of several ions during mineral processing to aid making timely decisions and consequently increasing profits while minimizing the cost and environmental impact. Regarding workers' safety, automation will facilitate remote monitoring especially when processing highly toxic or radioactive slurries.

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Nanoparticles Based Novel Nanomachineries for Cancer Diagnostics

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The development of user-friendly, rapid and low-cost diagnostic methods for the point-of-care analysis of circulating disease biomarkers has the potential to transform health care to many millions people both in the developed and developing countries. Recent advances in sequencing and proteomics technologies have now given rise to a large number of potentially useful genetic, epigenetic and other novel molecular biomarkers for the development of new diagnostic methods for various diseases including cancer. Despite these great input from biotechnology and bioengineering fields, significant technical challenges for achieving point-of-care diagnostic methods are yet to be overcome. This is partly due to the lack of sensitive, specific, rapid and low-cost detection methods. We have recently developed several strategies to isolate, amplify and quantitatively display the molecular targets in cancer patients. These includes new electrochemical and optical methods for detecting DNA modifications (i.e., methylation), gene-fusion, circulating-free miRNA, circulating tumor-DNA, exosomal miRNA, protein biomarkers, and humoral immune response (autoantibody).[1-6] This presentation shall review some of these developments, highlighting their applications into the clinics.

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The rolling and slipping of droplets on superhydrophobic surfaces

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The leaves of many plants are superhydrophobic, a property that may have evolved to clean the leaves by encouraging water droplets to bead up and roll off. Superhydrophobic surfaces can also exhibit reduced friction and liquids flowing over such surfaces have been found to slip in apparent violations of the classical no-slip boundary condition. Here we introduce slip into a model for rolling droplets on superhydrophobic surfaces and investigate under what conditions slip might be important for the steady state motion. In particular, we examine three limiting cases where dissipation in the rolling droplet is dominated by viscous dissipation, surface friction, or contact line friction. We find that in molecular dynamics simulations of droplets on ideal superhydrophobic surfaces with large effective slip lengths, contact line dissipation dominates droplet motion. However, on real leaves, droplet motion is likely to be dominated by viscous shear, and slip, for the most part, can be neglected.

Leaf-on-a-chip: Artificial or Art?

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Phyllosphere microbiology is the study of microorganisms residing on leaf surfaces. Such studies are of importance to increase understanding of plant microbiology, for instance, human enteropathogenic bacterial contamination of leaves or foliar diseases. For our leaf-on-a-chip, we are developing fabrication processes to replicate leaf surface topography and nutrient delivery systems. We are using Arabidopsis thaliana as our model leaf, which is a well-established model system for microbial ecology. Arabidopsis leaves are inherently fragile and possess intricate structures - trichome and stomata. Thus, leading us to develop a fabrication process that minimizes damage to the leaf integrity during replication, whilst improving replica fidelity. The fidelity of the replica leaf surfaces was evaluated with optical microscopy, AFM (**Fig. 1a**), and SEM. To highlight the use of our replica leaf surfaces for phyllosphere microbiology, we investigated: (1) their hydrophobic properties, and (2) the behaviour of bacteria on the replica surfaces. In addition, for nutrient pathways we are working on replicating the leaf nervature system into microfluidic channels (**Fig. 1b)**; and investigating adding fluid transporters into our replicas, towards a leaf-on-a-chip for phyllosphere studies.

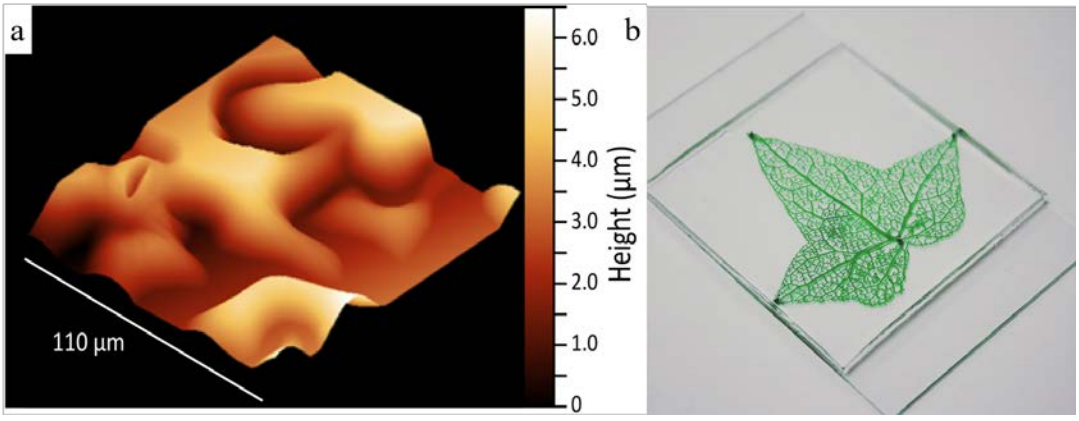

Figure 1. (a) AFM Image of *Arabidopsis thaliana* PDMS Replica, and (b) Microfluidic Channels of Ivy Nervature.

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Two-stage microfluidic scheme for high-throughput retention of mammalian cells

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A growing trend in biopharmaceutical industry involving the continuous cell perfusion process requires an efficient and reliable cell retention scheme. The current filtration systems suffer from the issue of membrane clogging, limiting the efficiency and productivity of the process[1]. Some cell retention devices based on inertial microfluidics offer better reliability, however their efficiency reduces for higher cell concentrations[2]. Herein, we report the development of a two-step membrane-less microfiltration system for retention of the mammalian cells for high cell concentrations (~30-50million cells/mL).

As the first step of the cell retention system, a miniaturised hydrocyclone was developed that could separate the Chinese hamster ovary (CHO) and HeLa cells with macroscopic volume processing rates (~200 mL/min). At this stage more than 50% of the cells were isolated from the spent media with minimal effect on the viability. The remaining cells exiting through the overflow of the device enter the multiplexed system spiral microchannel where the more than 90% of the cells were recovered. The new integrated system proposed is thus ideal for continuous and high throughput cell retention and for development of cell perfusion system for biomanufacturing process.

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Laser parameters and solvent treatment improvements for CO2 laser ablated Poly(methyl methacrylate) microchannels

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Fast, simple microfluidic prototyping is important for the initial development and testing of different microfluidic platforms. One of the main methods of fast prototyping is the ablation of micro-channels on thermoplastics, such as polymethylmethacrylate (PMMA), with commercially available CO2 lasers followed by thermal bonding. However, this technique has several drawbacks, such as the poor quality of the ablated channels, thermal bonding issues and reported solvent-based treatments to improve the quality of the channels are unreproducible. Here we present a systematic study of channels ablated in PMMA by a continuous wave CO2 laser, detailing the effect of the laser power and scan-speed on the channel dimensions and quality. Furthermore, we developed a new solvent treatment that reliably improves the quality of the microchannel and leaves negligible residual solvent on the PMMA surface. Finally, a microfluidic prototype was fabricated as a proof-of-concept that our technique can be used concurrent with 3-layered microfluidic designs keeping the optical properties of the PMMA whilst improving the thermal bonding strength by a factor of four compared to the untreated sample.

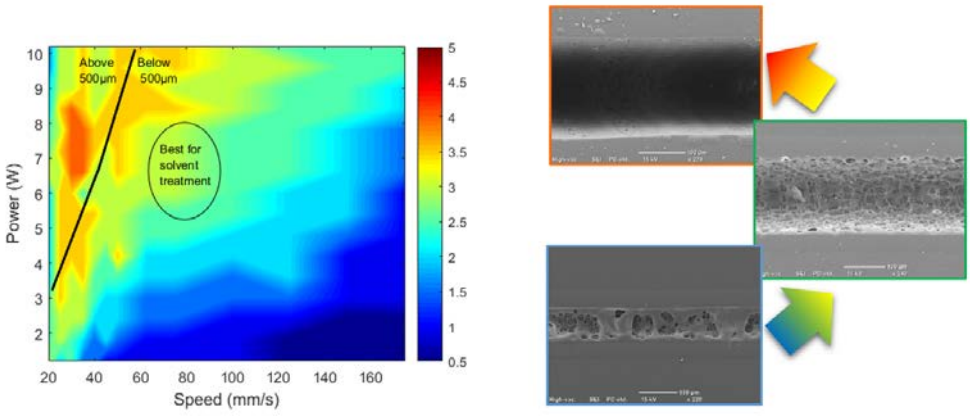

Figure 1 – (left) Qualitative map of the quality of the untreated bottom of the channels (0.5-5, lowest to highest quality). It displays the area where the solvent is most effective being that the channels are not too deep, and have enough quality to be developed in smoother channels. (right) The qualitative scale of the untreated channels being shown as images, where the bottom SEM photo shows an image threshold where the errors increase.

Lab-on-a-chip Platforms Enabled by Novel Magnetorheological Elastomer Microactuators

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Microfluidic systems enable rapid diagnosis of diseases, biological analysis, drug screening, and high-precision materials synthesis. And yet, shrinking a whole laboratory to miniaturized lab-on-a-chip (LoC) system is still challenging as complicated processes need to be conducted on a single chip. Conventional microfluidic systems are microfabricated monolithically on a single platform and their operations often rely on bulky expensive external equipment. This restricts their applications outside of research laboratories, and prevents development and assembly of truly versatile and complex systems. Here, we present novel magnetorheological elastomer (MRE) microactuators including pumps and mixers using an innovative actuation mechanism without the need of delicate elements such as thin membranes [1]. Modularized elements are realized using such actuators, which can be easily integrated and actuated using a single self-contained driving unit to create a modular, miniaturized, and robust platform. We demonstrated that our MRE microactuators are able to offer unique advantages including simple fabrication process, low cost, and low power consumption, while being capable of achieving high pumping or mixing performance in microchannels with an excellent controllability. Most importantly, the operation of multiple MRE microactuators can be conducted onto a single miniaturized platform driven by a set of permanent magnets, making our system reconfigurable that enables a versatile and multifunctional system simply by choosing different combinations of actuators and microchannels. This facilitates the development of a modular microfluidic platform that has the potential to achieve complex LoC systems for applications such as micro-total analysis systems (μTAS), chemical synthesis, and the studies of cellular/molecular biology.

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MicroAngelo Technique: 3D Sculpting of Nanofilms by Modulation of Thermocapillary Forces

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At age 17 and with only a hammer and chisel, Michelangelo in 1492 sculpted one of the most beautiful marble reliefs ever known, the Madonna of the Steps. Millimeter variations in stone thickness imbue the surface of this masterpiece with an ethereal quality. Modern photolithographic techniques are also a marvel in their own right wherein exposure to patterned UV fields yields feature sizes below 100 nm. Remaining challenges involve fabrication of non-planar shapes and mitigation of surface roughness from multiple etching steps needed to reveal final shapes. Here we explore the foundations of a novel one-step, etch-free, 3D microscale patterning technique called MicroAngelo that can produce curved shapes with ultra-smooth surfaces. This technique relies on thermal sculpting of molten thin films by spatiotemporal modulation of thermocapillary forces projected onto the gas/liquid or liquid/solid interface by conduction via patterned thermal fields. Final shapes rapidly solidify in-situ once the thermal fields are removed. We'll first review interesting properties of the dynamical behavior of the moving interface equation obtained from linear, weakly non-linear and fully nonlinear analysis, Lyapunov studies, Cahn-Hilliard analogy, parametric resonance, resolution limits and proximity corrections. We'll then review decades long efforts by researchers worldwide to uncover the primary mechanism responsible for 3D patterning and recent fabrication of thin film micro-optical arrays. Altogether, these findings advocate for a new generation of thin film lithographic techniques by which surface force patterns can be programmed to sculpt entire micro-optical circuits in one step.

Using Fiber Optic Particle Plasmon Resonance Biosensors to Determine Nervous Necrosis Virus in Fish Pond Water

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Fiber optic particle resonance (FOPPR) biosensors have been useful tools to determine a variety of biological samples including viruses with superior detection limits than using conventional ELISA methods. In addition, without using tedious procedures required in ELISA protocols, FOPPR method can minimize contamination interferences and obtain accurate results in timely fashion. FOPPR biosensor is potentially a suitable virus detection device to implement in fish farms. In Taiwan, aquaculture products such as groupers suffer tremendous loss because of ineffective disease prevention efforts. The infected signs are not detected in the early stage before severe outbreak to cause uncontrollable consequences. Therefore when the virus level is low, using highly sensitive FOPPR biosensors to monitor pond water is a promising means to improve the effectiveness of aquatic product disease prevention by taking water treatment actions.

We have also developed on FOPPR assays to determine nervous necrosis virus (NNV), which cause massively high mortality rate (~90%) in larva and juvenile stages of grouper species. Using coat proteins of NNV as standards in 2% salt water, the detection limit of 100 ng/mL is obtained. This limit is nearly 2 orders of magnitude lower than that using ELISA assays and adequate to indicate the severance level of NNV outbreak. Ground meat of infected juvenile grouper was diluted with saline to prepare simulated water samples. The comparison determination data between using FOPPR and quantitative PCR methods are reported. We will also discuss the monitoring of NNV level in infected water and in treated water using charcoal-based filtration.

Microfluidics for the Evaluation of Immobilised Liquid Surface Coatings

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Immobilised liquid surface coatings are a promising technology for medical device applications. They can prevent adhesion of protein and cells and therefore reduce biological fouling events such as blood clots (thrombosis) and microbial biofilm formation (biofouling) (Fig 1). Effective evaluation of their biocompability and longevity in the context of their application is essential for their successful clinical translation. However, many *in vitro* methods do not provide sufficient data with regards to their *in vivo* or clinical performance. Microfluidic based techniques provide a greater level of control of the *in vitro* experimental conditions and could be utilised in screening studies and evaluation of efficacy. We used microfluidic based assays to evaluate the recently developed liquid surface coatings. We coated microfluidic channels with the tethered-liquid perfluorocarbon (TLP) coating and evaluated the thrombogenicity over time[1]. Furthermore, we evaluated the stability of the liquid layer over varying times and under varying flow rates[2]. Together with well-established organs-on-chips methodologies, these types of assays could be expanded to evaluate a range of parameters for medical device translation.

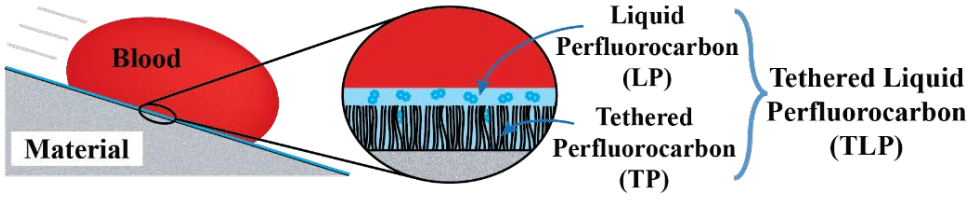

Fig 1. Schematic of TLP showing the tethered perfluorocarbon (TP) bound to a substrate with the liquid perfluorocarbon (LP) adhered, repelling blood[1].

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Ferrofluid Drop Impacts in a Non-Uniform Magnetic Field

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Impacts of magnetic fluid (ferrofluid) droplets in a downward-increasing magnetic field were examined using high-speed photography [1]. Novel behaviour was seen both before and after their impact with a flat surface.

Falling or suspended ferrofluid droplets tend to elongate along the magnetic field direction. In this non-uniform field, elongation was seen to occur asymmetrically, with a spike forming in the direction of increasing field as a droplet fell.

Upon impact, ferrofluid droplets show rich dynamic behaviour depending on velocity and magnetic field. Normal splash behaviour, including crown instabilities gives way to the formation of magnetic instabilities [2] (see figure). These instabilities then move around the surface in a quasi-predictable manner, and may coalesce or divide before reaching an energetically stable configuration. The final equilibrium shape is not constant, but strongly dependent on impact conditions as well as magnetic field.

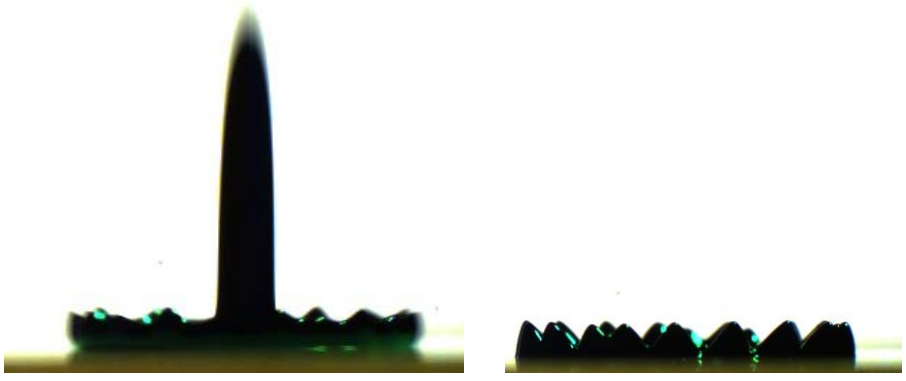

Figure: Ferrofluid droplet in 0.20 T magnetic field at the surface, at 3.5 ms (left) and 15ms (right) after initial impact with glass, after falling from height 94mm. Crown instabilities form during impact (left) and develop into magnetic instabilities (right).

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Imparting mosquito repellent property to fabric through silver nano particles biosynthesised from citronella oil

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This work is an approach to produce a mosquito repellent fabric by eliminating bacterial growth on textile with the use of silver nano particles (AgNP) and the repellence of mosquitoes through microencapsulation technique. The biosynthesis of AgNP were achieved from an aqueous solution of $AgNO_3$ using the bio-reduction process by citronella oil extract. The formation of AgNP was confirmed through several different methods such as colour of the AgNP, Ultra-Violet Visible Spectrum (422nm) and the FT-IR analysis for the dried biomass of lemon leaves before and after the reduction. The standard industrial binder "Aprocat tow" was used with AgNP to implant it to fabric. The wash durability of the finished fabrics was evaluated at four intervals – 5, 10, 15 and 20 washes. It was found that the AgNP implanted fabrics exhibit excellent antimicrobial activities against for four different bacteria namely *Staphylococcus Aureus*, *E. Coli*, MRSA and *Pseudomonas aeruginosa* after several times of washing treatment. Repellent activity was assessed by exposure of a human hand and arm covered with the treated textiles to female mosquitoes. Fabrics treated with microencapsulated citronella presented a higher and longer lasting protection from mosquitoes compared to fabrics sprayed with an ethanol solution of the essential oil, assuring a repellent effect higher than 90% for three weeks. Therefore this is a simple, low cost, scalable and reproducible method of obtaining encapsulated citronella oils for textile application. This is the first report on the mosquito repellent activity of the AgNP synthesized by citronella oil extract.

Drug screening of cancer cell lines and human primary tumors using droplet microfluidics

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Precision Medicine in Oncology requires tailoring of therapeutic strategies to individual cancer patients. Due to the limited quantity of tumor samples, this proves to be difficult, especially for early stage cancer patients whose tumors are small. In this study, we exploited a 2.4 × 2.4 centimeters polydimethylsiloxane (PDMS) based microfluidic chip which employed droplet microfluidics to conduct drug screens against suspended and adherent cancer cell lines, as well as cells dissociated from primary tumor of human patients. Single cells were dispersed in aqueous droplets and imaged within 24 hours of drug treatment to assess cell viability by ethidium homodimer 1 staining. Our results showed that 5 conditions could be screened for every 80,000 cells in one channel on our chip under current circumstances. Additionally, screening conditions have been adapted to both suspended and adherent cancer cells, giving versatility to potentially all types of cancers. Hence, this study provides a powerful tool for rapid, low-input drug screening of primary cancers within 24 hours after tumor resection from cancer patients. This paves the way for further technological advancement to cutting down sample size and increasing drug screening throughput in advent to personalized cancer therapy.

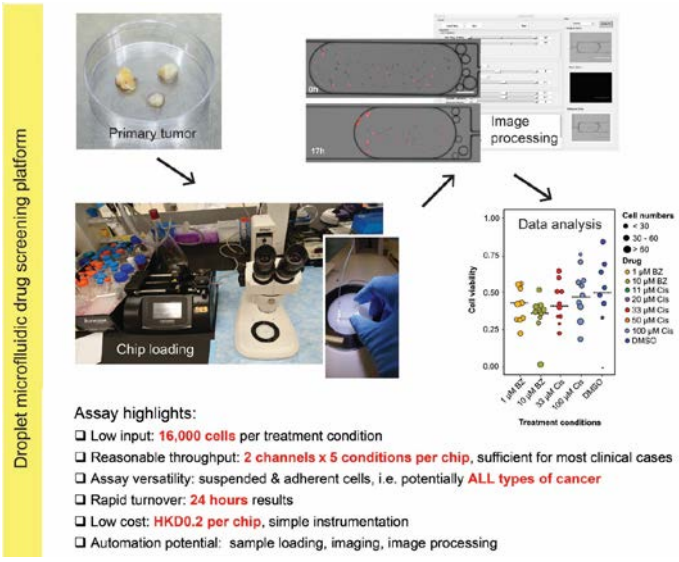

Figure 1. Overview of droplet microfluidic drug screening platform. (Top) Procedures of the assay include: (i) primary tumor dissociation, (ii) chip loading followed by 18-24 hours incubation, (iii) imaging acquisition and processing, and (iv) data analysis and graph plotting. (Bottom) Highlights of this assay was depicted.

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Optofluidic Platform for High-throughput Screening of Leach Chemistry

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We demonstrate an optofluidic screening platform for studying thiosulfate leaching of Au in a transparent microchannel. The approach permits in situ (optical) monitoring of Au thickness, reduced reagent use, rapid optimization of reagent chemistry, screening of temperature, and determination of the activation energy. The results demonstrate the critical importance of the (1) preparation and storage of the leach solution, (2) deposition and annealing of the Au film, and (3) lixiviant chemistry. The density of sputter deposited Au films decreased with depth resulting in accelerating leach rates during experiments. Atomic leach rates were determined and were constant throughout each experiment. Annealing above 270 °C was found to prevent leaching, which can be attributed to diffusion of the chromium adhesion layer into the Au film. The optofluidic analysis revealed leach rates that are sensitive to the stoichiometric ratio of thiosulphate, ammonia and copper in the leach solution, and optimized for 10 mM $CuSO_4$, 1 M $Na_2S_2O_3$ and 1 M NH_4OH. The temperature dependence of the leach rate gave an apparent activation energy of ~ 40 $kJ.mol^{-1}$, based on Arrhenius' relationship.

Acoustic Enhancement of Intracellular Delivery for Ex Vivo Therapeutics

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Recent advances in gene editing/therapy have highlighted the potential of ex vivo techniques to treat many diseases. Considerable challenges however remain in the ability not just to insert therapeutic agents into cells whilst retaining high levels of cellular viability, but also to ensure that they are not lysed within the cell. Physical methods (e.g., electroporation, sonoporation, etc.), for example, allow efficient translocation of therapeutic cargo into the cell through the formation of pores in the cell membrane. This, however, afflicts some damage to the cells, leading to poor viability. Biochemical methods, in contrast, rely on carriers such as nanoparticles, vesicles or viruses to facilitate greater endocytotic take-up. This pathway nevertheless results in the concentration of the internalised cargo within the endosomal regions, ending up in the lysosomes where they are degraded, Strategies that allow escape from the endosomal recycling path into the cytoplasm are therefore required for nuclear targeting.

We show that exposure of cells to high frequency (>10 MHz) sound waves enhances the uptake of nanoparticles, molecules and nucleic acids by several-fold, whilst retaining very high viabilities (>97%). This is because the high frequency excitation does not induce pore formation but rather temporarily disrupts the membrane lipid structure, thus increasing its permeability sufficiently to allow the therapeutic agent to diffuse through it. The effect, is however, transient such that the lipid structure immediately returns to its original state upon removal of the excitation. Such immediate recovery of the cell is the reason for the high cell viability. As this internalisation mechanism does not involve endocytosis, we observe the therapeutic cargo to be distributed throughout the cell instead of being localised within the endosomes/lysosomes, thus facilitating a greater possibility for entry to the nucleus and hence transfection. Indeed, with siRNA delivery, we observe a two-fold knockdown in the gene expression.

Inertial microparticle manipulation by sheath flow-enhanced secondary flow in slanted groove microchannel

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In the regime of inertial microfluidics, secondary flow is widely adopted to alter and optimize the equilibrium position and focusing performance of microparticles. In this work, we proposed a double-layer microchannel with slanted groove structures to generate continuous transverse secondary flow for the microparticle manipulation. Moreover, a sheath flow was introduced to enhance the performance of microparticle manipulation by the structure-generated secondary flow, especially for the relatively small size microparticle. The effects of flow rate and ratio between the sheath and sample flow were investigated exhaustively within a large range (the ratio was from 1 to 7 and the flow rate was from 200 to 700μl/min). The captured microparticle fluorescent trajectories demonstrated that the 4.8μm microparticle could be focus or manipulate effectively at different flow rate and ratio conditions. And larger 9.9 and smaller 2.9μm microparticles could be also guided to the equilibrium position as well at corresponding different modified flow rate and ratio. Meanwhile, a plasma extraction with the undiluted whole blood was conducted in this microchannel to prove the practical functionality of this method. The results achieved show that the purity of plasma extracted could reach up to ~99% in a single process validated by the flow cytometry and hemocytometer, when the ratio of sheath flow and sample flow applied was ~6 and the total flow rate was ~700μl/min. In conclusion, the sheath flow-enhanced secondary flow microparticle manipulation method offers stronger manipulation ability of smaller-size or higher-concentration microparticle comparing with the conventional inertial microfluidics method, which is great potential in the biological and diagnostic assays.