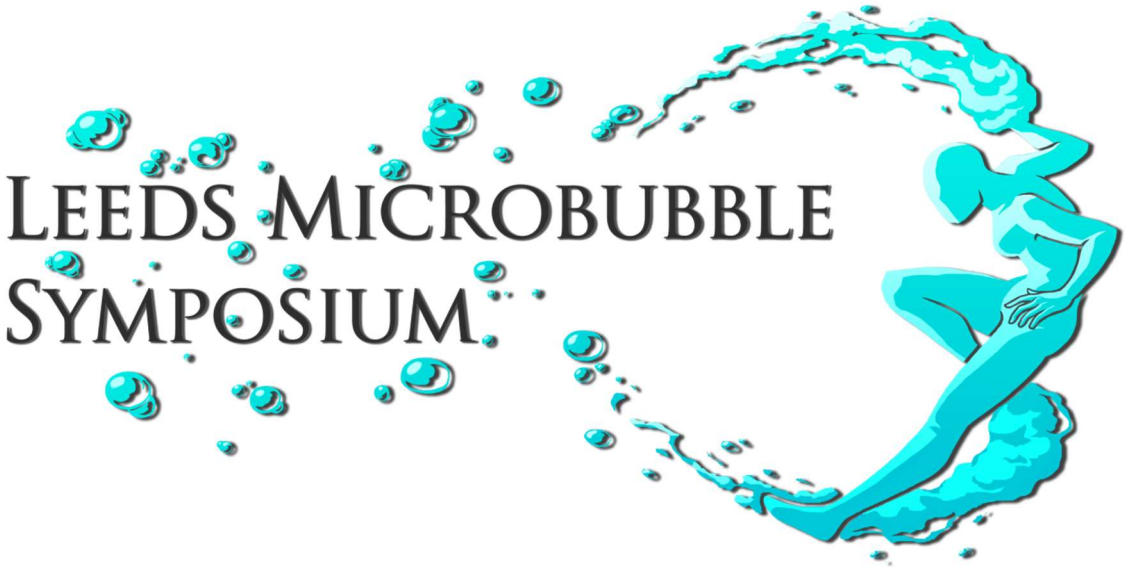
# Microbubble Symposium:

## Fabrication, Characterisation and Translational Applications

## 18 & 19th July 2016

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|---|---|---|
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| 09:35 – 09:55 | Guillaume Lajoinie | Microbubble generation using laser-activated microcapsules for contrast and therapy |
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| 11:20 – 11:55 | Steven Lind | On the computational modelling of microbubble dynamics |
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**Session 3: Microbubble Translational Applications – Chair: Louise Coletta**

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|---|---|---|
| 13:30 – 14:05 | Ingo Stoffels | Indocyanin green and multispectral optoacoustic imaging can replace radioactive lymphoscintigraphy and histology in sentinel lymph node detection and determination |
| 14:05 – 14:40 | Spiros Kotopoulis | Low-intensity ultrasound- and microbubble-mediated cancer therapy: from lab-to-clinic |
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| 16:40 – 17:00 | Sir Alex Markham | Closing remarks |

**Session 1: Microbubble Fabrication and Characterisation**

**Oral Presentation:** David Goertz

**Title:** Considerations for potentiating the effects of anticancer agents with microbubbles

**Abstract:**
Substantial efforts are being directed towards investigating the use of ultrasound stimulated microbubbles to potentiate the effects of anticancer agents in tumours. In many cases the paradigm being considered is that employed in other applications- to permeabilize microvessels with the objective of promoting uptake into the extravascular space. It has also been shown that, at relatively low intensities, microbubbles can induce a rapid shutdown of the tumour vasculature in preclinical models. Indeed, we have shown that with increasing transmit pressure there is first a phase where extravasation is promoted whereas at higher levels competing 'antivascular' effects become dominant and extravasation is inhibited. These antivascular effects are accompanied by tumour growth inhibition but eventually there is a degree of flow recovery along with the resumption of tumour growth. This parallels the effects observed for small molecule vascular disrupting agents, which achieve their most effective antitumour effects when combined with chemotherapy. We have previously shown that antivascular microbubble effects can induce potent antitumour effects when coupled with taxanes or cyclophosphamide. In recent work we have expanded this to show potentiated antitumour effects with the antigangiogenic agent Sorafenib and an immunotherapeutic agent. This work was conducted using conventional agent formulations such as Definity or equivalents and using exposure levels (1 MHz transmit, peak negative pressures ~1.3-1.6 MPa) that produce inertial cavitation. It will also be discussed how antivascular effects can be achieved with a submicron microbubble formulation. This general approach is of relevance to a range of anticancer agents whose activity is not inhibited by their ability to extravasate from the tumour blood supply.

**Session 1: Microbubble Fabrication and Characterisation**

**Oral Presentation:** Xiaochen Ma

**Title:** A facile synthesis of shell-stabilized microbubbles using surface-thiolated bovine serum albumin with the Traut's reagent

**Abstract:**
The short lifetime of proteinaceous microbubbles produced using conventional sonication method has hindered their applications in clinical practice, food industries, and water treatment. In this study, we aimed to synthesize stable proteinaceous microbubbles and to demonstrate their potential utilizations in different aspects. Our model protein, bovine serum albumin (BSA), was treated with 2-iminothiolane hydrochloride (Traut's reagent) to convert primary amines to thiols prior to the synthesis of microbubbles. The optimal ratio of Traut's reagent to BSA was determined to be 20× molar excess.
Microbubbles produced with BSA treated with Traut's reagent (BSA-SH MBs) were initially concentrated at median sizes of 0.5 µm and 2.5 µm. The 0.5 µm portion quickly vanished, and the 2.5 µm portion gradually shrank to ~ 0.6 µm within six days and became stabilized afterwards. Free unbound thiols and primary amines on the surface of
BSA-SH MBs identified with Fourier transform infrared (FTIR) spectroscopy and X-ray photoelectron spectroscopy (XPS), imply the possibility of further surface modification.
Based on a measured isoelectric point (IEP) of 4.5, successful adsorptions of BSA-SH
MBs on alumina, silica, and gold surfaces in different pH environments were carried out with a quartz crystal microbalance with dissipation monitoring (QCM-D). At pH 6, the negatively charged BSA-SH MBs were adsorbed onto the alumina surface by virtue of electrostatic interaction; analogously, at pH 4, the adsorption of the positively charged
BSA-SH MBs on the silica surface was confirmed. A greater extent of the adsorption of
BSA-SH MBs on the gold surface was attributed to the strong gold-thiol bond. A common anticancer drug, doxorubicin (DOX), was successfully loaded onto the as synthesized
BSA-SH MBs and thermally released with the aid of a temperature-sensitive poly (N-isopropylacrylamide)-co-(acrylic acid) shell.

## Session 1: Microbubble Fabrication and Characterisation

**Oral Presentation:** Margaret Wheatley

**Title:** Designing microbubbles for molecular oxygen delivery to hypoxic tumors

**Abstract:**

Hypoxia in tumors inhibits sensitivity to radiation and chemotherapy. We studied the delivery of oxygen to tumors using an ultrasound-sensitive microbubble (MB) platform developed at Drexel and Thomas Jefferson Universities consisting of a mixed surfactant shell (Span and water soluble vitamin E) surrounding either oxygen ($SE61_{O2}$) or nitrogen ($SE61_{N2}$). Oxygen release kinetics were measured using an Oxy Lite 2000 bare fiber $pO_2$ probe. In vitro ultrasound used a Sonix RP scanner with a PA4-2 probe in power Doppler mode. Samples in 100 ml degassed saline were triggered over 20 minutes (readings obtained every 30s). *In vivo* proof of concept (two mice with MDA-MB-231 breast tumor xenografts) introduced the probe into the tumor via 21G catheter. Flash-replenishment imaging at the fiber tip was performed using a Vevo 2100 scanner in nonlinear imaging mode at 18 MHz during IV injection of 0.05 ml of agent. Partial oxygen pressures were recorded every 5s until returned to baseline. Release profiles were compared to untriggered $SE61_{O2}$, and triggered $SE61_{N2}$. Two ml of $SE61_{O2}$ triggered with ultrasound elevated oxygen partial pressures of 100 ml of degassed saline 13.8 mmHg more than untriggered bubbles and 20.6 mmHg more than triggered nitrogen-filled bubbles. *In vivo* controls produced no discernible increase in oxygen partial pressure except for a brief (25s) 5.6 mmHg increase in one animal. Ultrasound triggered $SE61_{O2}$ resulted in a 30.4 mmHg increase in one tumor, with elevated tumor oxygen levels lasting over 4 minutes, and an increase of 27.4 mmHg, with elevated tumor oxygen levels lasting 1.7 minutes in the second. We conclude that in vivo elevation of tumor oxygenation levels using $SE61_{O2}$ appears feasible but highly tumor dependent.

*In vitro* acoustic response from three additional freeze-dried MBs with different shell compositions was also tested. The first (A) originally containing sulfur hexafluoride ($SF_6$), had a phosphatidylserine shell, the second (B) was composed of galactose micro-particles and palmitic acid (PA) from an air core, the third (C) from a perfluorocarbon (PFC) bubble had a mixed phospholipid/PA shell with a polyethylene glycol stabilizer. After removal of the primary filling gas, oxygen was introduced into the MB vials under vacuum. *In vitro* acoustic testing was completed in a tank setup (37°C) with a single element 5 MHz transducer (0.45 MPa peak negative pressure with a pulse repetition frequency of 100 Hz). The sample was continuously stirred. Enhancement as a function of dosage was also compared between MBs with an SE61 shell fabricated with either PFC ($SE61^{PFC}$) or $SF_6$ ($SE61^{SF6}$) primary filling gas. The average peak enhancement for the MBs A, B, and C was 2.8 ± 2.5 dB, 5.0 ± 1.5 dB and 5.0 ± 1.1 dB, respectively ($P<0.0001$) and for $SE61^{PFC}$ and $SE61^{SF6}$ was 15.1 ± 1.4 dB and 8.6 ± 1.9 dB ($P<0.0001$), while the half-life was 6.1 ± 1.6 min and 0.76± 0.2 min respectively ($P<0.0001$).

MBs comprised of a surfactant/vitamin E shell appeared to be superior at encapsulating oxygen compared to MBs with lipid or galactose shells. In addition, using PFC as the primary filling gas resulted in superior oxygen MBs when compared to $SF_6$. Thus, formulation D initially fabricated with PFC appears suitable for future therapeutic oxygen delivery applications *in vivo*.

**Session 1: Microbubble Fabrication and Characterisation**

**Oral Presentation:** John Callan

**Title:** Microbubble-sensitiser conjugates for use in the sonodynamic therapy treatment of pancreatic cancer

**Abstract:**
Tumour hypoxia represents a major challenge in the effective treatment of solid cancerous tumours using conventional approaches. As oxygen is a key substrate for Photo- / Sono-dynamic Therapy (PDT / SDT), hypoxia is also problematic for the treatment of solid tumours using these techniques. We have developed an oxygen-loaded microbubble ($O_2$MB) platform for the targeted treatment of pancreatic cancer using both sonodynamic therapy (SDT) and antimetabolite therapy. $O_2$MB were prepared with either the sensitiser Rose Bengal ($O_2$MB-RB) or the antimetabolite 5-fluorouracil ($O_2$MB-5FU) attached to the microbubble (MB) surface. The MB were characterised with respect to size, physical stability and oxygen retention. A statistically significant reduction in cell viability was observed when three different pancreatic cancer cell lines (BxPc-3, MIA PaCa-2 and PANC-1), cultured in an anaerobic cabinet, were treated with both SDT and antimetabolite therapy compared to either therapy alone. In addition, a statistically significant reduction in tumour growth was also observed when ectopic human xenograft BxPC-3 tumours in SCID mice were treated with the combined therapy compared to treatment with either therapy alone. When tumours were treated with the $O_2$MBs and ultrasound a significant reduction in HIF-1α expression was found compared to tumours treated with perfluorobutane loaded MBs. Collectively, these results illustrate not only the potential of combined SDT/antimetabolite therapy as a stand-alone treatment option in pancreatic cancer, but also the capability of $O_2$-loaded MBs to deliver $O_2$ to the tumour microenvironment in order to enhance the efficacy of therapies that depend on $O_2$ to mediate their therapeutic effect.

## Session 1: Microbubble Fabrication and Characterisation

**Oral Presentation:** Jeff Bamber

**Title:** Microbubbles, nanoparticles and phase change agents in cancer research

**Abstract:**
This presentation provides an update on projects that are or have been running at the Institute of Cancer Research, some jointly with Phoenix Solutions Oslo, NTNU Trondheim, Imperial College London and the University of Oxford.
Acoustic cluster therapy (ACT): With Sontum et al and de Lagrange Davies et al, in Oslo and Trondheim respectively, we have been exploring ACT for overcoming the problem that drugs produce unacceptable side effects at doses that would be fully effective for cancer treatment. In the ACT concept the drug is either co-injected with, or carried within, liquid microdroplets that are electrostatically bound to microbubbles. When such clusters are exposed to even quite low MI (e.g. < 0.4 1-10 MHz) diagnostic ultrasound the microdroplets vapourise to 20-30 µm bubbles that stop microvascular blood flow for 5-10 minutes, preventing the drug from washing out and encouraging it to extravasate. Extravasation is enhanced by a low frequency (300-500 kHz) delivery-insonification that induces controlled oscillations of the large bubbles which, unlike microbubbles, generate large shear forces and are in continuous direct contact with the vessel wall. Parallel independent preclinical trials have been conducted in Trondheim and Sutton, using different experimental setups and different preclinical tumour models. Signal processing software has been developed to identify the stationary large bubble echoes after vapourisation and to correct dynamic contrast ultrasound (DCE-US) movies for mouse respiratory motion, providing ACT guidance information. Time intensity curves thus generated for the moving (non-vapourised) and stationary bubbles demonstrate the presence of both components. ACT has been found to significantly enhance the tumour uptake of near infrared dye 800CW™, as shown by fluorescence imaging, and to markedly increase therapeutic efficacy of paclitaxel and nab-paclitaxel when treating human prostate adenocarcinoma xenografts.
Combining microbubbles with multispectral optoacoustic tomography (MSOT): We have developed a novel method of co-registering MSOT and DCE-US images, demonstrating in various subcutaneous cancer models in mice that across regions within a tumour, an inverse relationship typically exists between microbubble arrival time and blood oxygen saturation, and that some regions show no haemoglobin signal despite having functioning vasculature on DCE-US. It appears that MSOT and DCE-US yield complementary information, and a combined approach should improve our understanding of angiogenesis and hypoxia.
Novel contrast agents and imaging methods: Gold nanorods have been synthesised of specific length-width aspect ratio and conjugated with selected antibodies to explore potential for multiplexed molecular imaging using MSOT. A clinical multispectral optoacoustic scanner was constructed at ICR and phantoms that mimicked in vivo imaging conditions were used to show that cells from various preclinical tumour models displayed optoacoustic spectral contrast for strength of expression of diagnostically important receptors that was highly correlated with expression as seen by flow cytometry. With Tang et al in London and Stride et al in Oxford, preliminary work is underway on both dye and nanoparticle coated microbubbles, and phase change droplets, as optoacoustic contrast agents. Potential has also been explored for dual wave imaging, and acoustic relativistic modifications developed of the theory for high frequency ultrasonic observation of microbubbles and phase change agents activated by ultrasound.

**Session 1: Microbubble Fabrication and Characterisation**

**Oral Presentation:** Marie-Pierre Krafft

**Title:** Fluorocarbon gases for effectively recruiting and immobilising bioactive molecules, proteins, polymers and particles at the air/water interface

**Abstract:**
We have developed new perspectives in the use of fluorocarbon gases for controlling the adsorption, spreading and organization, and hence, properties of different classes of both fluorinated and non-fluorinated compounds at various aqueous phase/gas phase interfaces. The components of the interfaces include phospholipids, proteins (albumin, fibrinogen, hydrophobin), fluorinated hypoxia biomarkers (nitrosoimidazoles), polymers (poloxamers) and nanoparticles.1-4
In particular, we have recently demonstrated that attractive fluorine-fluorine interactions can arise between a fluorocarbon gas, perfluorohexane (F-hexane), and CnF2n+1-labelled compounds, present in the sub-phase, across a phospholipid monolayer, thus allowing recruitment and immobilisation of some fluorinated compounds within the phospholipid layer.4 This new phenomenon allowed preparation of microbubbles loaded with a C2F5-labelled hypoxia biomarker (EF5, Collab. V. Gouverneur, Oxford), with potential applications in diagnosis. EF5, when radio-labelled with 18F, is in clinical trials for the early diagnosis of tumours using positron emission tomography. Further recent results show that nanoparticles (e.g. nanodiamonds) can also be efficiently recruited at an air/water interface using F-hexane (Collab. M. Dubois, Clermont-Ferrand). Also noteworthy is the fact that F-hexane could stabilize microbubbles with a shell made of only the water-soluble copolymer, Poloxamer 188, as the sole surfactant (Collab. D. Koyama, Kyoto).
Controlling the kinetics of adsorption and the structural organization of these compounds at interfaces self-assembled from phospholipids or other surfactants have potential applications in microbubble-mediated drug delivery and ultrasound imaging, as well as for the design of more efficient lung surfactant substitutes.5, 6 The development of these topics should hopefully benefit from the input of the Microbubble Consortium, in the friendly atmosphere of the Microbubble Symposium.

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5. M. P. Krafft, Soft Matter, 2015, 11, 5982.
6. M. P. Krafft, J. Fluorine Chem., 2015, 177, 19.

## Session 1: Microbubble Fabrication and Characterisation

**Oral Presentation:** Adam Churchman

**Title:** Forming oil layer inside microbubbles through single step microfluidics, towards hydrophobic drug delivery

**Abstract:**
This paper reports a new and simple one-step method for the formation of hydrophobic drug delivery capsules with echogenic properties, termed oil layer inside microbubbles (OLI-MBs).

A large number of new, high potency anti-cancer drugs that have shown promising results in vitro exhibit poor water solubility, which makes them difficult to deliver in vivo, resulting in reduced drug efficacy and even toxicity. As a result there is an urgent need to develop novel systems for the in vivo delivery and release of hydrophobic drugs. The conventional MB architecture has been adapted through the incorporation of an oil layer, between the gas core and lipid shell, to allow hydrophobic drugs to be contained and protected in the inner oil layer before undergoing site-specific, triggered release using an US destruction pulse.

OLI-MB structures have been constructed previously using three-phase flow-focused (FF) microfluidics, where gas, oil and lipid solution were brought together in a pinch-off regime. However, control of three phases on-chip is often unstable and requires careful consideration of oil viscosity, limiting the range of suitable oils.

We present a new method for OLI-MB formation utilizing simple FF microfluidics and nanoparticle self-assembly. A solution of highly stable ~150 nm lipid (POPC) stabilized oil (squalane) nanodroplets were used as the aqueous phase in a FF device to pinch off MBs of C4F10 gas. During MB production the oil nanodroplets were found to self-assemble at the gas-water interface. To reduce the energy of the system further, the oil nanodroplets spread to form a thin layer of oil-lipid around the gas core. OLI-MB structures were produced at clinically relevant diameters (< 8 µm) of 5.9 +/- 0.7 µm at a rate of ~$5x10^3$ bubbles/s. In order to visualize the thin oil layer, a hydrophobic fluorophore (DiI) was added to the oil and OLI-MBs were trapped in microfluidic particle traps so that any free nanodroplets could be washed away. As the fluorophore was in the oil fraction only, a fluorescent shell around the MB confirmed the presence and stability of the oil.

Nanodroplet spreading was confirmed on mechanically agitated 10-40 µ;m OLI-MBs, made using fluorescence resonance energy transfer (FRET) enabled nanodroplets, and confocal microscopy. Here, donor (DiO) loaded nanodroplets and acceptor (DiI) loaded nanodroplets were mixed before OLI-MB formation. FRET occurred only at the gas-water interface, confirming that the oil from both nanodroplet suspensions must have mixed on the MB surface to allow energy transfer.







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## Session 2: Microbubble Ultrasound Characterisation

**Oral Presentation:** James Choi

**Title:** Novel short-pulse ultrasound sequences for enhancing ultrasound drug delivery across capillaries

**Abstract:**
Acoustic cavitation – volumetric oscillations of a bubble driven by an acoustic field – is a mechanical force used in therapeutic ultrasound. It dissolves clots, delivers drugs into cells and across capillaries, and releases drugs from carriers; but it also causes haemorrhage, elicits immune responses, and damages untargeted tissue. Producing the correct type of cavitation while avoiding unwanted modes is essential for an effective and safe technique. Here, we investigated how ultrasound drives cavitation, primary and secondary Bjerknes forces and how the use of rapid short pulse (RaSP) sequences can improve control of cavitation dynamics.

We performed a multi-dimensional analysis of microbubble dynamics – defined by the type, magnitude, distribution, and duration of cavitation for both conventional (long pulses of >10 ms) and RaSP sequences. Microbubbles (SonoVue) flowing within an 800-µm diameter tube were exposed to ultrasound (PRP: 146-900kPa, PRF: 0.62-10kHz, PL: 5-50,000 cycles) while a linear array passively captured radiated emissions. The microbubble dynamics were characterized using passive acoustic mapping and spectral analysis. A subset of parameters were analyzed using a high-speed microscope (>5,000 fps). To understand microbubble dynamics in capillaries, we simulated a capillary bed with flowing microbubbles and tracked their distribution during sonication.

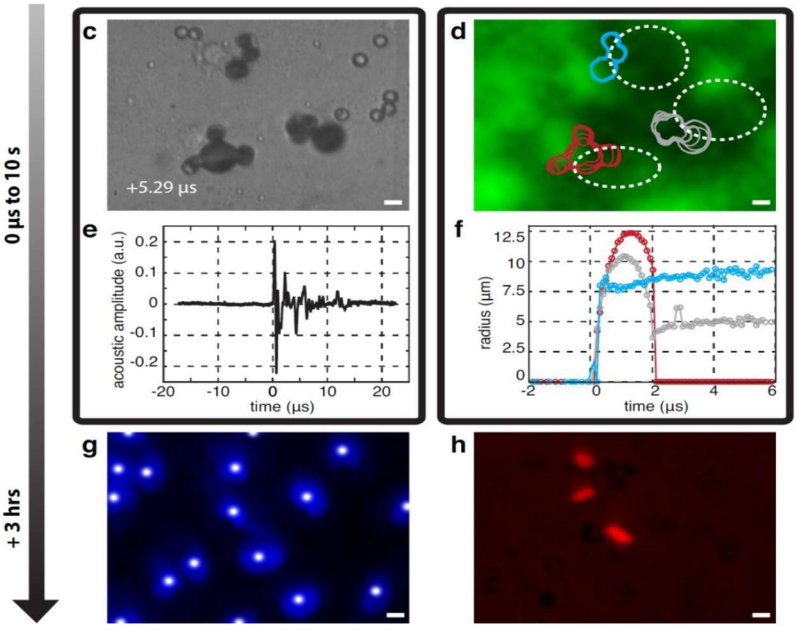
Cavitation persisted 5 times longer during short-pulse, low-pressure exposure than a 100-ms long pulse. High pressures and long pulse lengths generated high magnitude inertial cavitation during the first millisecond, which rapidly decreased because of microbubble destruction. Cavitation activity was biased upstream as new microbubbles entered the focal volume. High-speed microscopy revealed rapid displacement, clustering, and coalescence at these parameters. Low pressures RaSP resulted in a more consistent magnitude and distribution of cavitation throughout the sequence. High-speed microscopy revealed reduced clustering rates and displacements. Simulations revealed similar findings in capillaries. Our RaSP sequences has the potential to improve applications by enhancing therapeutically relevant cavitation dynamics and eliminating unwanted mechanical stress.

**Session 2: Microbubble Ultrasound Characterisation**

**Oral Presentation:** Guillaume Lajoinie

**Title:** Microbubble generation using laser-activated microcapsules for contrast and therapy

**Abstract:**
Traditionally, photoacoustic contrast is generated by the differences in light absorption by the diverse tissues. Subsequently, photoacoustic imaging is efficient at imaging blood vessel lying close to the skin surface. The use of differential absorption also provides information on oxygenation levels of the blood for example. Applying the technique to the assessment of diseases and imaging deeper structures, however, requires the use of contrast enhancement by means of nano or microagents. The first ones, usually metal nanoparticles, present the interest of being small enough to extravasate and can be tuned to absorb near-infrared light, thereby allowing for deeper imaging by making use of the biological window.
The contrast generation by such agents remains nonetheless limited, and their potential toxicity, a concern. In order to address these issues, we designed and characterized different types of light-absorbing microcapsules that, upon laser irradiation, generate microbubbles together with acoustic waves that were shown to be potentially orders of magnitude stronger those generated by gold nanoparticles. The stronger absorption of these agents would also allow for deeper tissues imaging and its polymeric nature for easy functionalization. The absorption of these dye based particles be tuned to any wavelength, and the generated microbubbles can also be used for therapeutic application in cells. Finally, the resulting stable microbubbles can be used for acoustic contrast enhancement, leading to a potent multimodal agent.



**Session 2: Microbubble Ultrasound Characterisation**

**Oral Presentation:** Massimo Mischi

**Title:** Quantitative contrast-enhanced ultrasound imaging for cancer localization

**Abstract:**
In Westerns countries, one in four people die because of cancer. Prostate Cancer (PCa) is the most common form of cancer in men; yet the available diagnostic options are limited by the lack of reliable imaging solutions. Dynamic Contrast-Enhanced UltraSound (DCE-US) is a diagnostic tool that is suitable for analysis of the vascularization, by imaging an intravenously-injected bolus of ultrasound contrast agents. The localization of angiogenic vascularization associated with cancer growth is of particular interest for the diagnosis of aggressive cancer. In the past few years, methods for the analysis of the convective-dispersion process of ultrasound contrast agents have shown promise to localize angiogenesis. Several dispersion estimators have been introduced and evaluated with dynamic 2D and, more recently, 3D acquisitions. However, none of the estimators could provide an independent estimation of dispersion due to the ambiguity between convection and dispersion.

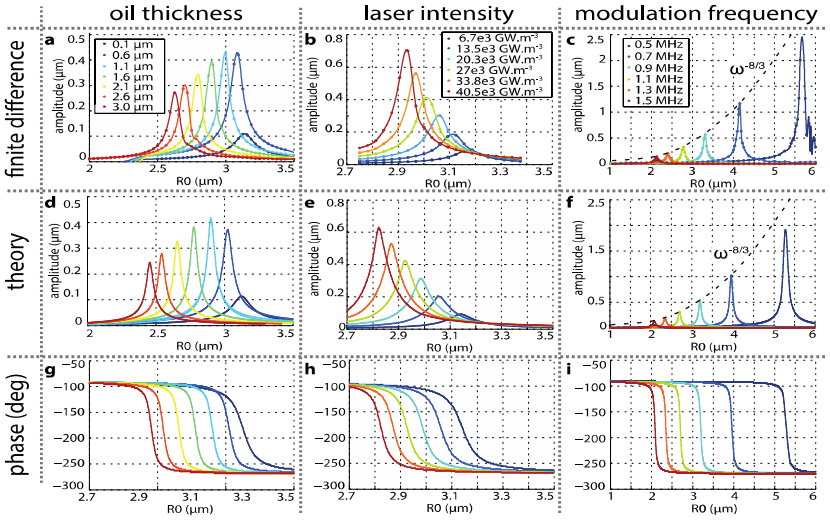
More recently, a new method has been introduced that provides the separate estimation of convection and dispersion by identification of the local (linear) dilution system. To this end, model-based parameter estimation is employed. The method also permits generating maps of the Péclet number (Pe), a physics parameter characterizing the dilution system. Clinical evaluation using data recorded from 25 patients at the Academic Medical Center University of Amsterdam (the Netherlands) and Jeroen Bosch Ziekenhuis in ‘s-Hertogenbosch (the Netherlands) is promising; this method can be applied effectively to DCE-US, and is able to locally characterize the hemodynamics, yielding promising results for prostate cancer localization. In parallel to these studies, use and modeling of ultrasound contrast agents that are targeted to angiogenic expressions (BR55, Bracco Suisse), such as the vascular endothelial growth factor, are being tested for cancer localization in several animal models. The proposed approach enables the assessment of the binding/unbinding kinetics of the agent, showing promise for future applications aimed at prostate cancer localization. In general, the proposed methods for angiogenesis imaging are not specific to prostate cancer only, and future extension to other types of cancer can also be envisaged.

**Session 2: Microbubble Ultrasound Characterisation**

**Oral Presentation:** Michel Versluis

**Title:** Laser-driven resonance of light-absorbing ultrasound contrast microbubbles

**Abstract:**
The sensitivity of ultrasound imaging is greatly enhanced by the use of microbubble contrast agents through resonant volumetric oscillations. While the increased acoustic contrast is of prime interest for perfusion imaging of organs, microbubbles until now have limited benefit in terms of specificity for ultrasound imaging. Original strategies are required to tackle this difficulty that rely on loading functional targeting ligands onto the microbubble encapsulation. In parallel, another type of wave is used in biomedical imaging that shows great specificity in its interaction with tissue, namely light. This advantage is put to use in photoacoustic imaging where absorbed laser light is converted into a measurable acoustic signal.
Here we present a novel ultrasound contrast agent designed to also make use of the superior specificity of laser light. The acoustic agent consists of a gas core encapsulated by an oil layer containing an optically absorbing dye. The resulting laser light absorption can then be used to heat up the gas and drive the system into resonance, thereby generating ultrasound. Combining finite difference simulations and ultra high-speed imaging led to a quantitative physical description of the optical and thermal interactions in the system resulting in the efficient generation of acoustic waves in the MHz range. A range of physical bubble parameters are investigated, in particular those related to the thickness and composition of the light absorbing oil layer. This new generation of contrast agents will open up new applications in medical diagnostic and therapeutic imaging.

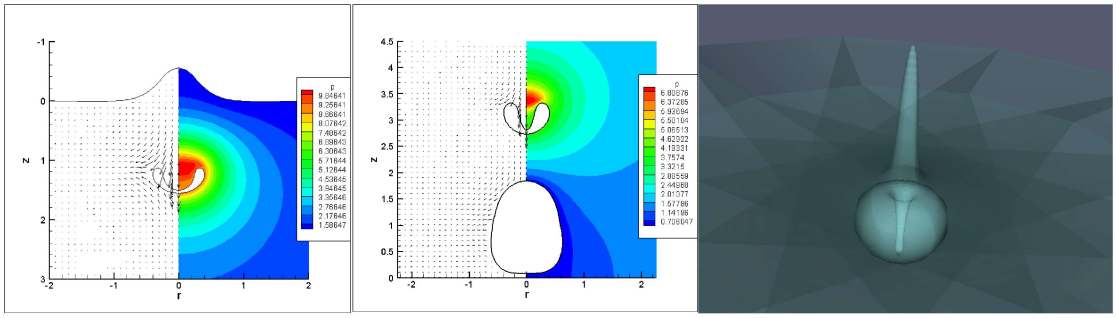


**Session 2: Microbubble Ultrasound Characterisation**

**Oral Presentation:** Steven Lind

**Title:** On the computational modelling of microbubble dynamics

**Abstract:**
Techniques in computational fluid mechanics have much to offer in providing insight into the dynamics of microbubbles in sonoporation and related processes. This talk reviews recent work undertaken by the author and collaborators in the modelling of bubble dynamics for biomedical applications, particularly sonoporation and drug delivery. Investigations include studies on the role of viscoelasticity in the ambient fluid and adjacent tissue, surface tension at the bubble shell, the effect of magnetic fields on magnetic-particle seeded bubbles, and the role of idealised shockwave interaction. Though the computational models remain somewhat idealised, the results to date are promising and have been validated against simple in vitro experimentation as well as available theoretical results. Some example computational results are shown below. Figure 1a shows a snapshot of the profile of a single bubble with associated pressure contours and velocity vectors during dynamics adjacent to a viscous membrane (originally at z=0). Figure 1b presents an example of two-bubble behaviour, where jetting in the lower bubble is prevented by the presence of the upper bubble (which is seen to jet in this instance). Figure 1c shows a 3D rendering of bubble collapse and jetting near a highly deformable membrane, which also jets in response to bubble dynamics. The talk concludes with discussion around current modelling and computational challenges in sonoporation and possible avenues for future research.



(a) (b) (c)

Figure 1. Computational results for a selection of bubble collapse problems: (a) Bubble collapse near a deformable viscous membrane (b) Two-bubble collapse with jet prevention (c) 3D bubble collapse near a highly deforming membrane.







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**Session 3: Microbubble Translational Applications**

**Oral Presentation:** Ingo Stoffels

**Title:** Indocyanin green and multispectral optoacoustic imaging can replace radioactive lymphoscintigraphy and histology in sentinel lymph node detection and determination

**Abstract:**

*Background*

Sentinel lymph node (SLN) staging is included in various cancer guidelines. This complex, time- and cost-intensive procedure requires radioactive tracing. In addition excluding metastasis in draining lymph nodes by noninvasive imaging could spare many patients a SLNE. Multispectral optoacoustic tomography (MSOT) could help overcome the drawbacks of SLN using its potential to detect and stage noninvasively utilizing nonradioactive tracer.

*Methods*

Eighty patients were enrolled in a cross-sectional trial aiming to detect SLNs by MSOT in vivo. We administered ICG peritumorally as an exogenous contrast agent with distinct absorption spectra in the near infrared, and then used a two-dimensional MSOT detector to image inguinal, cervical and axillary SLNs. Pulse-echo ultrasound images of the lymph nodes were taken using a 2D MSOT detector in combination with an experimental integrated ultrasound imaging device. The specific ICG signal simultaneously acquired by MSOT could then be overlaid onto the anatomical reference image based on ultrasound contrast. MSOT determination of SLN status was directly compared to conventional pathological analysis of the SLNs.

*Results*

The primary efficacy endpoint in the trial was the concordance of SLN identified by lymphoscintigraphy and those identified with hybrid ultrasound/MSOT imaging by detection of ICG. All lymph node basins identified in lymphoscintigraphy from the 80 patients were also detected by MSOT. All SLNs visualized by lymphoscintigraphy and SPECT/CT (n = 163) were also ICG-marked and detected by MSOT, yielding a concordance rate of 100%. In addition MSOT identified cancer-free SLNs in vivo without a single false negative (133 total lymph nodes), with 100% sensitivity and 54.4% specificity.

*Conclusions*

ICG labeling and hybrid ultrasound/MSOT imaging proved to be an excellent approach for SLN detection, eliminating the need for radioactive tracers. Furthermore we can determine the SLN status in melanoma patients noninvasively.

**Session 3: Microbubble Translational Applications**

**Oral Presentation:** Spiros Kotopoulis

**Title:** Low-intensity ultrasound- and microbubble-mediated cancer therapy: From lab-to-clinic

**Abstract:**

*Background*

Experimental research using ultrasound in combination with microbubbles to induce or improve delivery has snowballed in the past decade. A majority of this work focuses on the use of high-intensity ultrasound to induce inertial cavitation to enhance the therapeutic effect. Here we demonstrate the use of low-intensity ultrasound to induce similar effects.

*Methods*

The work present here will cover application from microbubble simulation, high-speed imaging, in-vitro and in-vivo experiments, and results from a Phase I clinical trial.

*Results & Discussion*

To date our results show that low-intensity ultrasound has the ability improve the therapeutic efficacy from in-vitro all the way into clinical trials. Nevertheless, this field is only in its infancy requiring substantial research to fully understand the biological interactions and further improve its efficacy.

**Session 3: Microbubble Translational Applications**

**Oral Presentation:** Klazina Kooiman

**Title:** Microbubbles for high-frequency contrast enhanced ultrasound imaging

**Abstract:**
Although high frequency ultrasound is gaining attention in various applications [1], there has not been enough attention to specific ultrasound contrast agents (UCAs) dedicated to such frequencies (>15 MHz). Moreover, the composition of commercially available UCAs for high frequency contrast enhanced ultrasound imaging (hfCEUS) is largely unknown, while shell properties have been shown to be an important factor for their performance [2,3]. The aim of our study was to produce UCAs in-house for hfCEUS. The performance of the in-house produced UCAs was compared to MicroMarker (FujiFilm VisualSonics Inc.) using the Vevo2100 high frequency preclinical scanner (FujiFilm VisualSonics Inc.). Twelve different UCAs, type A-L, were made by either sonication or vial mixing. The main coating lipid was either DSPC or DPPC (59.4 or 92.4 mol%) with the addition of DSPE-PEG2000 (4.9 or 7.6 mol%); for the sonication UCAs PEG40-stearate (35.7 mol%) was also added. The gas core consisted of C4F10. UCAs made by vial mixing resulted in microbubbles with smaller diameters (mean ~1 µm) than sonication (mean ~2 µm). UCA type L had the most similar size distribution to MicroMarker (mean 1 µm). Non-linear in vitro hfCEUS (transmit 30 MHz, subharmonic pulse inversion, MS250 probe, 10 % power) showed that two UCA types, F and L, performed similar to MicroMarker. Both F and L type UCA were made by vial mixing where F had DSPC as main coating lipid, while this was DPPC for L. F type UCA also performed similar to MicroMarker in vivo in pigs with non-linear imaging (transmit 18 MHz, amplitude modulation, MS250 probe, 10% power) regarding the mean contrast intensity within the kidney (n=7), but L type UCA did not as shown in Fig. 1. This is likely due to the instability of L type UCA in vivo. Interestingly, the signal from UCA type F was higher than MicroMarker in the larger vessels in the upper part of the renal cortex, whereas MicroMarker gave a higher signal in the microcirculation, suggesting different non-linear behaviours of the UCAs. In conclusion, our study reveals the importance of both in vitro and in vivo testing when characterizing the performance of in-house made UCAs. F type UCA is suitable for hfCEUS as it performs similar to MicroMarker.
References: [1] Foster et al, Interface Focus 2011; 1: p. 576; [3] Helfield et al, Ultrasound Med Biol 2012; 38: p. 846; [2] van Rooij et al, Ultrasound Med Biol 2015; 41: p. 1432.

## Session 3: Microbubble Translational Applications

**Oral Presentation:** Ine Lentacker

**Title:** Sonoprinting: the importance of microbubble loading for the ultrasound mediated cellular delivery of nanoparticles


### Abstract:

The amount of drug-loaded microbubbles for ultrasound triggered drug delivery applications is vastly expanding. Although several studies have shown the potential for drug-loaded microbubbles to enhance nanoparticle uptake in cells, until now very little quantitative information is available on the biophysical microbubble-cell interaction mechanisms leading to drug release and uptake [1]. The relevant phenomena involve a cascade of events: release of drugs during microbubble oscillations, subsequent transport of the released material and, finally, drug delivery to cells [2]. This study is an effort to unravel the complete chain of events at a series of timescales, ranging from seconds, to milliseconds, down to tens of nanoseconds.

First, lipid-coated microbubbles were loaded with fluorescent, 100nm polystyrene beads as model nanoparticles via avidin-biotin interaction. We used real-time confocal microscopy to image the loaded microbubbles and cells during exposure to ultrasound. The recordings revealed that nanoparticle-loaded microbubbles directly deposited the nanoparticles in patches onto the cell membrane, a process that we termed 'sonoprinting' (Figure 1 and 2) [3]. This phenomenon resulted in the delivery of large amounts of nanoparticles onto the cellular membrane cells and it is suggested not to be correlated with the creation of cell membrane pores and enhanced endocytosis, mechanisms that have been reported before to be primarily responsible for ultrasound-controlled drug delivery in cells.

The use of high-speed fluorescence imaging (Photron) and ultra-high-speed imaging (Brandaris128) to study nanoparticle release and transport at different acoustic settings revealed that at lower acoustic pressures, drug-loaded microbubbles transported the released drugs away from the cells through microstreaming, analogous to earlier work by Luan et al. [4] and Lajoinie et al. [5]. In contrast, at higher acoustic pressures and longer ultrasound pulses, microbubbles were rapidly translated, while being driven by primary acoustic radiation forces. As a result, drugs that were released in the microbubble surroundings were dragged along with the travelling microbubble. Eventually this transport led to the deposition of the drugs in elongated patches onto the cellular membrane, confirming the origin of the sonoprinting mechanism.


***References***


[1] Lentacker et al., Advanced Drug Delivery Reviews, 2014. 72:p49-64.; [2] Kooiman et al., Advanced drug delivery reviews, 2014. 72: p28-48.; [3] De Cock et al., Biomaterials, 2016. 83:p297-307.; [4] Luan et al., Ultrasound in Medicine & Biology, 2014. **40**(8): p. 18341846.; [5] Lajoinie G., *Ultrasound contrast agents: bubbles, drops and particles*, 2015, University of Twente.


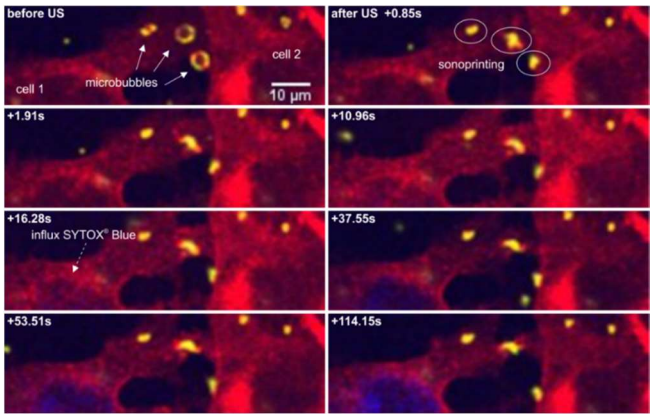


**Figure 1. Sonoprinting by nanosphere-loaded microbubbles.** Series of confocal images depicting three nanosphere-loaded microbubbles (arrows) that deliver their nanospheres to the cell membrane (sonoprinting) upon ultrasound exposure (t=0). Cell 1 shows influx of SYTOX® Blue (dashed arrow), indicating cell membrane poration, while this is not observed in cell 2. Cells were exposed to pulsed ultrasound with an acoustic pressure of 300 kPa (pulse length 10 cycles, PRP 0.01 s, duration 0.19 s). The cell membrane is labeled in red with CellMask™ Orange Plasma membrane Stain, while green fluorescent 100 nm nanospheres are attached to the microbubble shell.

## Session 3: Microbubble Translational Applications

**Oral Presentation:** David Jayne

**Title:** Clinical challenges in colorectal disease and applications for microbubble technologies

**Abstract:**
Colorectal disease represents a large healthcare burden and includes common and diverse diseases, such as colorectal cancer, colitis, and incontinence. The management of these diseases has not changed appreciably over the past decades and there is a need to apply new technologies in order to make a step-wise change in patient care. This presentation will discuss the clinical challenges in colorectal disease with specific reference to where the application of micro bubbles could make a difference. This includes enhanced imaging and diagnosis, disease stratification, and treatment of colorectal cancer, with emphasis on prophylactic therapies targeted at minimal residual disease to prevent local cancer recurrence. Consideration will also be given to general disease processes, such as assessment of blood flow to normal and diseased tissues, and the delivery of non-cancer therapeutics, for example in slow-release pain control post-surgery.







UNIVERSITY OF LEEDS

**Poster Presentations**

| Number | Name | Title |
|---|---|---|
| **1** | Anastasia Alataki | Developing therapeutic microbubbles for enhanced delivery of epigenetic drugs for breast cancer |
| **2** | Alexia Alexandraki | Epigenetic drug delivery using targeted microbubbles as a novel therapeutic approach for the treatment of colorectal cancer |
| **3** | Hugo Christenson | Cavitation in confinement |
| **4** | Kevin Critchley | Engineering gold nanomaterials with near-infrared absorption for optoacoustic imaging and photothermal therapy in for the treatment of cancer. |
| **5** | Georg Feichtinger | Ultrasound-responsive gene-activated matrices (gams) for osteogenesis using matrix-assisted sonoporation (mas) |
| **6** | Stephen Maughan | Using the innovative technique of mems resonant mass measurement to characterize microbubbles |
| **7** | Victoria Mico | Microbubble-nanodroplet composites for hydrophobic drug delivery |
| **8** | Luzhen Nie | Ultrafast contrast enhanced 2d ultrasonic velocity mapping |
| **9** | Christopher Rowlatt | Microbubble-cell interaction using the spectral element marker particle method |
| **10** | Adeel Shafi | Development of fluorescent, targeted, ultrasonic contrast microbubbles for preclinical in-vivo optical and ultrasonic imaging |

**Poster Number 1:** Anastasia Alataki

**Title:** Developing therapeutic microbubbles for enhanced delivery of epigenetic drugs for breast cancer

**Abstract:**
Although technologies continue the advancement of several detection and treatment methods, breast cancer (BC) still remains the most common cancer type in the UK. There is evidence that epigenetic inactivation of genes, which are vital for normal cell growth, is involved in early stages of cancer initiation and contribute to cancer progression. Epigenetics refers to external modifications to DNA that turn genes "on" or "off". These changes do not alter the DNA sequence, but they affect how cells "read" genes, instead. These alterations are potentially reversible, therefore, re-activation of genes can result in reduction of tumour or sensitisation to other anti-cancer therapies, in response to epigenetic drugs. Nevertheless, the drug dose required to detect an effect in patients as well as the off-site toxicity can compromise their use.
The aim is to use targeted microbubbles (MBs) and low drug doses in order to enhance the drug delivery both in cell culture and in a orthotopic BC mouse model. Ultrasound (US)-mediated drug delivery using MBs is a non-invasive approach for localised drug administration, which can temporarily increase the cell membrane permeability (sonoporation) and cellular drug uptake. Moreover, it is proposed that a targeted drug delivery system could, also, increase drug delivery to the tumour vasculature and tumour tissue.
As part of the project, effects of an epigenetic drug, called decitabine, have been tested on BC cells, revealing restoration of expression of specific biomarkers. A flow assay was performed and verified a specific binding of microbubbles conjugated with the VEGFR2-targeted antibody in VEGFR2+ mouse endothelial cells compared with an isotype control. Currently, a system called Ultrasound Array Research Platform (UARP) is being used in order to determine any benefit of epigenetic drug delivery after US transmission in cells under flow conditions. Next, we are aiming to investigate the anti-tumour effects of decitabine in the BC orthotopic mouse model using targeted free or drug-loaded MBs and guided US versus free drug delivery alone.

**Poster Number 2:** Alexia Alexandraki

**Title:** Epigenetic drug delivery using targeted microbubbles as a novel therapeutic approach for the treatment of colorectal cancer

**Abstract:**
Colorectal cancer (CRC) is the third leading cause of cancer related deaths worldwide. Despite the therapeutic advancements, many CRC patients die due to secondary liver metastases as well as chemoresistance. It is now well established that epigenetic alterations play a significant role in tumour development and progression. The concept of epigenetics refers to any changes in gene activity without affecting the primary DNA sequence. Consequently, therapeutic agents that can potentially reverse those changes and restore crucial cellular processes are gaining great interest in the clinical setting. Use of epigenetic drugs has shown to reinforce the effectiveness of secondary drug treatment resulting in increased drug anti-tumour effects. Epigenetic drugs including decitabine and azacytidine are DNA methyl-transferase (DNMT) inhibitors FDA approved for the treatment of haematological malignancies. However, their use for the treatment of solid tumours such as CRC has proven to be a bit more challenging. Reasons associated with this include lack of drug stability due to aqueous hydrolysis, degradation within the liver resulting in reduced cellular drug uptake while side effects including myelosuppression in patents are also observed resulting in treatment cessation in certain cases.

Consequently, our pre-clinical studies aim to use epigenetic therapeutic agents including decitabine for treating CRC using targeted, ultrasound (US)-triggered microbubble drug delivery system. Microbubbles are currently used as contrast agents and as drug delivery vehicles targeted to VEGFR2 within the tumour vasculature. By encapsulating epigenetic drugs within liposomes attached to microbubbles we are aiming to improve drug stability, prevent drug degradation and increase therapeutic efficacy within the tumour. For this purpose, in vitro assays have been developed using genes that have shown to be switched off or low expressed in CRC cell lines while their expression is restored following epigenetic drug treatment. Such epigenetic biomarkers will be utilized as an indication of successful epigenetic drug delivery using targeted US-triggered microbubbles in CRC xenograft mouse models. Ultrasound will be used to visualized targeted microbubbles while an ultrasound de struction pulse will be applied to burst the bubbles resulting in drug release at a sufficient dose at the tumour targeted region. Our aim is to utilise the potential of using epigenetic drug treatment of CRC whilst increasing therapeutic potential with minimal adverse effects for the patients.

**Poster Number 3:** Hugo Christenson

**Title:** Cavitation in confinement

**Abstract:**
Bubble formation in water occurs during boiling, when the vapour pressure of the water reaches atmospheric pressure, and during cavitation, when the external pressure decreases below the vapour pressure. In both cases, however, the formation of visible bubbles is almost invariably due to the growth of pre-existing, microscopic bubbles. Microscopic bubbles in bulk are typically stabilised by surface active substances; on surfaces they may also be stabilised in hydrophobic surface cracks or by contact-line pinning. True nucleation of a bubble in water is a very rare event and in bulk requires a tension (negative pressure) in excess of 1000 atm. Even hydrophobic surfaces, which provide transient stabilisation of existing bubbles, do not promote their ready nucleation. However, simple surface energy considerations show that a thin film of water between two hydrophobic surfaces is metastable towards replacement by vapour or dissolved gas. From the 1980s scattered o
Observations with the surface force apparatus and similar instruments have shown that bubbles will under some conditions nucleate between two hydrophobic surfaces in water [1]. Despite some evidence that this is true nucleation without the involvement of pre-existing bubbles, it appears as if little systematic work has been carried out. While the relationship of bubble or cavity stability to thermodynamic quantities like surface energies and contact angles is straightforward, little is known about the kinetic factors involved in the nucleation of a cavity during a dynamic process such as separation of two surfaces from contact. There is hence scope for much discussion and speculation!

**Poster Number 4:** Kevin Critchley

**Title:** Engineering gold nanomaterials with near-infrared absorption for optoacoustic imaging and photothermal therapy in for the treatment of cancer

**Abstract:**
Gold nanoparticle have high extinction coefficients in the near-infrared making them excellent photoacoustic imaging agents. However, there are several important aspects to consider when engineering gold nanoparticles for photoacoustic applications: control of size, optical properties, cytotoxicity, biodistribution, and clearance. In our study, Au nanotubes with controllable length and absorption in the near-infrared (NIR) region have been exploited for applications as in vivo photoacoustic imaging contrast agents. A length-controlled synthesis has been developed to fabricate Au nanotubes (NTs) with well-defined shape, with inner void and open ends, high crystallinity, and tunable NIR surface plasmon resonance. A coating of poly(sodium 4-styrenesulfonate) (PSS) endows the nanotubes with colloidal stability and low cytotoxicity. The PSS-coated Au NTs have the following characteristics: i) cellular uptake by colorectal cancer cells and macrophage cells, ii) photothermal ablation of cancer cells using single wavelength pulse laser irradiation, iii) excellent in vivo photoacoustic signal generation capability and accumulation at the tumor site, iv) hepatobiliary clearance within 72 h post intravenous injection. These results demonstrate that these PSS-coated Au NTs have the ideal attributes to develop their potential as effective and safe in vivo imaging nanoprobes, photothermal conversion agents, and drug delivery vehicles. To the best of knowledge, this is the first in vitro and in vivo study of gold nanotubes.

**Poster Number 5:** Georg Feichtinger

**Title:** Ultrasound-responsive gene-activated matrices (GAMs) for osteogenesis using matrix-assisted sonoporation (MAS)

**Abstract:**
Gene-activated matrix (GAM)-based therapeutics for tissue regeneration are limited by efficacy, the lack of spatiotemporal control and availability of target cells, which impact negatively on their translation to the clinic. Here we describe an advanced ultrasound-responsive GAM containing target cells that facilitates matrix-assisted sonoporation (MAS) to induce osteogenic differentiation. Ultrasound-responsive GAMs consisting of fibrin/collagen hybrid-matrices containing microbubbles, bone morphogenetic protein BMP2/7 co-expression plasmids together with C2C12 cells were treated with ultrasound either in vitro or following parenteral intramuscular implantation in vivo. Using direct measurement for alkaline phosphatase activity, von Kossa staining and immuno-histochemical analysis for osteocalcin expression, MAS-stimulated osteogenic differentiation was confirmed in the GAMs in vitro 7 days post treatment with ultrasound. At day 30 post-treatment with ultrasound, ectopic osteogenic differentiation was confirmed in vivo using X-ray microcomputed tomography (μCT) and histological analysis. Osteogenic differentiation was indicated by the presence of ectopic bone structures in all animals treated with MAS when compared with controls. In addition bone volumes in this group were statistically greater than those in the control groups. This novel approach of incorporating a MAS capability into GAMs could provide a minimally invasive means of stimulating in situ transgene delivery with enhanced spatiotemporal control for osteoinductive gene-based therapies.

**Poster Number 6:** Stephen Maughan

**Title:** Using the innovative technique of mems resonant mass measurement to characterize microbubbles

**Abstract:**
Microbubbles used as contrast agents for ultrasound imaging and targeted drug delivery require accurate size control for improved performance. Here we present a novel method for the characterisation of bubbles which provides buoyant mass, size and count information. Additionally, the method enables differentiation of particles of different densities such as lipid droplets and perfluorocarbon encapsulated gas bubbles using the innovative technique of resonant mass measurement (RMM). This technology utilises a suspended MEMS microchannel resonator (cantilever) which allows single particles to transit across the resonator and be individually characterised. Particles passing through the resonator alter the resonant frequency by an amount proportional to the buoyant mass of the particle, which can then be translated into mass, size or surface area. This change in resonant frequency of the cantilever is monitored through an optical based method. RMM therefore enables particle size to be determined as well as particle count in a single measurement.

When evaluating bubble particle size distribution, it is difficult to determine the identity of the particles; whether the particles are bubbles, lipid droplets or solid particle contaminates. In this work we utilize resonant mass measurement to enable this differentiation. With this technique it is also possible to evaluate the efficiency of encapsulated bubble production. Utilizing optimized instrument pressure conditions, we present data showing the impact of microbubble preparation variables via the mechanical agitation method upon the microbubble size and count.

Using RMM for microbubble characterisation enables quantitative assessment of bubble size distribution and count, but critically, also provides differentiation by buoyant mass to discriminate other particles in the sample.

**Poster Number 7:** Victoria Mico

**Title:** Microbubble-nanodroplet composites for hydrophobic drug delivery

**Abstract:**
Microbubbles have been extensively used as contrast agents in diagnostic ultrasound imaging, and have demonstrated to be promising theranostic agents given their dual therapeutic and diagnostic attributes. A number approaches for microbubble drug loading have been proposed, which so far have been limited to hydrophilic drugs. However hydrophobic drugs comprise a high percentage of new pharmaceutical entities. These drugs often show promising results against malignancies such as tumours in vitro, but their hydrophobicity makes them difficult to disperse in aqueous solutions and therefore difficult to be delivered in vivo.

We propose a hybrid microbubble-nanodroplet architecture for the delivery of hydrophobic drugs. This drug delivery system exhibits the ultrasound imaging properties characteristic of the microbubbles, but also incorporates the hydrophobic drug delivery capabilities of oil-based nanodroplets. The composites assembly via the biotin-NeutrAvidin link chemistry, in a two-step process on-chip. We have successfully assembled different species of LONDs to the MB surface, and have studied the stability of the composites at 37°C. Furthermore, we demonstrate the formation of these architectures combining two nanodroplet species, towards combination treatments.

**Poster Number 8:** Luzhen Nie

**Title:** Ultrafast contrast enhanced 2D ultrasonic velocity mapping

**Abstract:**
The advent of ultrafast ultrasound imaging proved beneficial for capturing transient clinical phenomena which is never readily achievable before. Ultrafast imaging can achieve thousands of frames per second by sacrificing the transmit focusing where the resolution is recovered later by receive beamforming techniques. Acquisition of thousands of frames real time could potentially improve the vascular disease diagnosis and give great insight into progression monitoring of vascular stenosis. The high frame rates comes with a cost of lower penetration depth and poor signal to noise ratio (SNR) due to the lack of transmitting focus in ultrafast imaging. However, when contrast agents are introduced into the blood stream the image SNR becomes high enough to detect individual microbubbles. Methods have already been developed to improve image quality with microbubbles both for pre-clinical and clinical research benefiting from a large amount of information gathered by ultrafast imaging.

Key words: Velocity mapping, bubble tracking, tracking algorithms, error correction

A wall-less tissue mimicking flow phantom was built with varying vessel diameters to generate turbulent flow with clinically relevant flow rates. A 128-element linear array transducer with center frequency of 5 MHz was connected to the Leeds Ultrasound Array Research Platform II (UARP II) to perform ultrafast plane wave imaging. Transient flow patterns were achieved by processing consecutive RF images. The first cross correlation map calculated between two same-size sub-windows was multiplied by the correlation table obtained from the same area but in a second successive pair of RF images. Given sub-pixel correlation peak offsets in corrected correlation maps and the time interval between adjacent RF images, a more accurate quantification of 2D blood flow is acquired in conjunction with ultrafast imaging.

Regarding the emerging super-resolution ultrasound imaging, tracking single microbubbles in combination with high frame-rate imaging will underpin microvascular flow imaging with an unprecedented high resolution. Drug delivery and sonoporation efficiency can be also monitored by using ultrafast ultrasound imaging techniques. And efforts will be allocated to quantify micro-flow more accurately and productively in the future.

**Poster Number 9:** Christopher Rowlatt

**Title:** Microbubble-cell interaction using the spectral element marker particle method

**Abstract:**
The spectral element marker particle (SEMP) method is a high-order numerical scheme for modelling multiphase flow where the governing equations are discretised using the spectral element method and the (compressible) fluid phases are tracked using marker particles. Thus far, the method has been successfully applied to two-phase problems involving the collapse of a two-dimensional bubble in the vicinity of a rigid wall (S. Lind, T. N. Phillips. Bubble collapse in compressible fluids using a spectral element marker particle method. Part 1. Newtonian fluids. International Journal for Numerical Methods in Fluids. 70 (9), 2012, 1167-1187). In this poster, the SEMP method is extended to include a third fluid phase before being applied to a simplified model of (micro)bubble-cell interaction with the aim of gaining initial insights into the flow mechanisms behind sonoporation and microbubble-enhanced targeted drug delivery. Preliminary results show a build up of nor mal stress at the top of the cell which then spreads out along the cell interface. Similar behaviour is also seen for the membrane shear stress, primarily caused by the bending of velocity streamlines around the cell. The observation that normal and shear stresses propagate along the cell interface following microbubble interaction may offer a hydrodynamical reason for the non-localised blebbing phenomenon seen as a cell membrane recovers post bubble/ultrasound interaction (R. S. Leow, J. M. F. Wan, A. C. H. Yu. Membrane blebbing as a recovery manoeuvre in site-specific sonoporation mediated by targeted microbubbles. Journal of The Royal Society Interface. 12 (105), 2015, 20150029).

**Poster Number 10:** Adeel Shafi

**Title:** Development of fluorescent, targeted, ultrasonic contrast microbubbles for preclinical in-vivo optical and ultrasonic imaging

**Abstract:**
Ultrasonic contrast agents have been developed in an attempt to improve visualisation of the vasculature. Ultrasonic contrast agents, known as microbubbles, have the ability to mix freely with red-blood-cells due to their similar size. Their physical characteristics (gas encapsulated by a thin phospholipid shell) result in increased scattering and reflection of ultrasonic waves which can enhance the ultrasound signal from vascular structures. Moreover, investigations into microbubbles behaviour under different insonation conditions has highlighted their potential in molecular imaging.

To understand microbubble behaviour; optical investigations will be carried out in-situ with Atomic Force Microscopy, a relatively new technique in the biomedical field in an attempt to further the understanding of the mechanical and nanostructural properties of the in-house microbubbles produced. Further analysis will look at designing and manufacturing fluorescent-targeted microbubbles for dual modal investigation by combining the Atomic Force Microscope with Fluorescent Microscopy. Additional in-vitro analysis using the Atomic Force Microscope will be conducted to further the understanding of the binding potential of microbubbles to cellular targets. In-vivo and in-vitro research will be made possible using the in-house fluorescent-targeted microbubbles to assess their potential in intravascular imaging in preclinical, small animal studies.

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