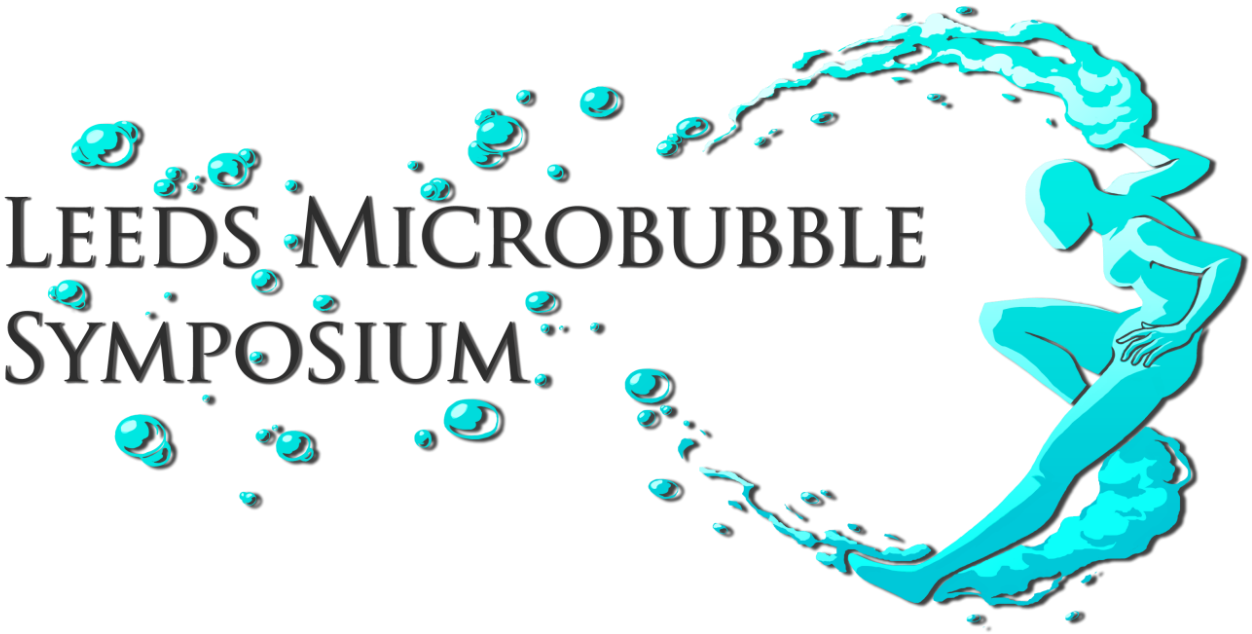

Microbubble and Nanobubbles:

From Fundamentals to Application

17th - 19th July 2023

Sir William Henry Bragg Building, University of Leeds
Woodhouse Lane, Leeds, LS2 9JT

Abstract Booklet

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Monday 17th July 2023

12:30 – 13:30	**Registration**	
13:30 – 13:40	Welcome (Bragg 2.37)	Stephen Evans
SESSION 1	**Microbubble Fundamentals** (Bragg 2.37)	**Chair: Stephen Evans**
13:40 – 14:20	Monodisperse bubbles: formation, characterization, application.	Michel Versluis
14:20 – 14:35	Acoustic microstreaming of bound targeted microbubbles undergoing shape oscillations at 1 to 1.5 mhz frequencies	Hongchen Li
14:35 – 14:50	Surface wettability effects on heterogeneous inertio-thermal vapour bubble growth	Patrick Sullivan
14:50 – 15:15	An eulerian multiscale multimaterial framework for the modelling of solid liquid gas interactions in capped bubbles drug delivery and bacteria incapacitation configurations.	Andreas Papoutsakis
15:15 – 15:45	***REFRESHMENTS***	**Bragg GR.18**
15:45 – 16:10	*Poster blitz*	
16:10 – 16:25	Effects of pluronic f68 addition on the coalescence, stability and acoustic behavior of monodisperse microbubbles produced at room temperature	Yuchen Wang
16:25 – 16:50	Dependence of sonoporation efficiency on microbubble size: an in vitro monodisperse microbubble study	Tim Segers
16:50 – 17:30	Experimental and numerical investigation of the size-dependent behavior of microbubbles and nanobubbles in response to focused ultrasound: insights into optimization of applications	Amin Jafarisojahrood
17:30 – 19:00	Drinks and Posters	Bragg GR.18

Tuesday 18th July 2023

SESSION 2	**NanoBubbles / NanoDroplets** (Bragg 2.37)	**Chair: Sally Peyman**
09:30 - 10:10	Nanobubbles – an update on formulation and new applications	Agata Exner
10:10 - 10:25	Characterising the chemical and physical properties of phase-change nanodroplets	Weiqi Zhang
10:25 - 10:50	Interaction and stability of nanobubbles and pre-nucleation calcium clusters during ultrasonic treatment of hard water.	Michael Coey
10:50 - 11:05	Characterisation of nanobubbles: utilizing their optical and physical properties	Damien Batchelor
11:05 - 11:35	***REFRESHMENTS***	**Bragg GR.18**
11:35 - 12:15	Nanobubble cavitation dynamics	Duncan Dockar
12:15 - 12:40	Ultrasound-mediated theragnostic nanobubbles with a langmuir's multilayer polymeric shell as controlled drug delivery system for topical administration	Roberta Cavalli
12:40 - 13:40	***LUNCH***	***Bragg GR.18 / Atrium***
13:40 - 14:20	Polyhedral perfluorocarbon nanodroplets that phase-shift into polyhedral nanobubbles	Marie-Pierre Kraft
14:20 - 14:45	Unexpected nanobubble generation by microfluidic microbubble collapse	Michael Kolios
SESSION 3	**Microbubbles: Towards Translation** (Bragg 2.37)	**Chair: James Mclaughlan**
14:50 - 15:30	Controlling microbubble–mediated drug delivery: influence of microbubble type and cell morphology	Klazina Kooiman
15:30 - 16:00	***REFRESHMENTS***	**Bragg GR.18**
16:00 - 16:15	Enhancing microbubble binding to staphylococcus aureus biofilms through multi-targeting	Kristian Hollingsworth
16:15 - 16:55	Ultrasound targeted microbubble destruction mediated chemo-sonodyamic therapy for the treatment of solid tumours.	John Callan
16:55 - 17:10	Developing an in-vitro adaptive therapy model for colorectal cancer	Joseph Fox
17:10 - 17:35	Lipid-shell microbubbles with air core.	Alexander Klibanov
18:00 – 20:30	***Symposium Dinner***	Great Woodhouse Room, University House

Wednesday 19th July 2023

SESSION 4	**Microbubbles: Preclinical / Translation** (Bragg 2.37)	**Chair: Steve Freear**
09:30 - 10:10	Development of preclinical contrast-enhanced ultrasound imaging to identify and image sentinel lymph nodes in a cancerous mouse model	Helen Mulvana
10:10 - 10:35	Acoustic cluster therapy; an update on research and evaluation	Jeff Bamber
10:35 - 10:50	A microfluidic PDAC culture model for investigating microbubble-mediated gemcitabine delivery	Delanyo Kpeglo
10:50 - 11:20	***REFRESHMENTS***	**Bragg GR.18**
11:20 - 12:00	Microbubbles and focused ultrasound to non-invasively deliver drugs to the brain	Sophie Morse
12:00 - 12:25	Nanodroplets preparation characterisation and applications	Maria Thanou
12:25 - 12:50	Brain focused ultrasound the next steps	Wladyslaw Gedroyc
12:50 – 13.00	**Closing remarks**	**Prof Sir Alex Markham**

Session 1	**Microbubble Fundamentals**
Oral Presentation	**Michel Versluis,** University of Twente
Title	**Monodisperse bubbles: formation, characterization, application.**

Abstract

The use of coated microbubbles as ultrasound contrast agents has been investigated for medical applications in both imaging and therapy. Clinically available contrast agents contain microbubbles with a size distribution typically ranging from 1 to 10 μm in diameter. Because of the size-dependent resonance frequency of microbubbles, only a fraction of the polydisperse size distribution will resonate with the ultrasound pulse and produce strong scattering. It has been shown experimentally that contrast generation can be greatly enhanced through the use of monodisperse microbubble suspensions. The size-distribution of commercial agents can be narrowed-down by microsieve filtration, decantation, centrifugation or, on chip, by microfluidic fractionation or acoustic sorting. Microbubble suspensions can also be directly produced with a well-controlled and narrow size distribution in microfluidic flow-focusing devices, at production rates exceeding a million bubbles per second. Monodisperse bubbles are a great research tool for a wide range of applications that we will discuss, including improved backscatter with deep tissue imaging potential, more efficient sonoporation, non-invasive pressure measurements, and super-resolved flow imaging. Pushing the precision of microbubble-enhanced applications even further will require going beyond monodispersity. We will therefore close with initial considerations on monoacousticity based on careful optical and acoustical characterization of monodisperse bubble suspensions.

Session 1	**Microbubble Fundamentals**
Oral Presentation	**Hongchen Li,** Erasmus MC
Title	**Acoustic Microstreaming of Bound Targeted Microbubbles Undergoing Shape Oscillations at 1 to 1.5 MHz Frequencies**

Abstract

Insonification of microbubbles initiates shape oscillation and generates microstreaming, yet a comprehensive understanding of these complex dynamics remains elusive. This study aims to investigate the frequency-dependent shape oscillation and microstreaming profiles of monodisperse biotinylated lipid-coated microbubbles targeted to a wall, utilizing both experimental and numerical approaches. A microscope with two coupled Shimadzu HPV-X2 ultra-high-speed cameras was used to observe microbubble shape oscillation (at 5 Mfps) and microstreaming (at 10 kfps) using 500 nm polymer beads. Monodisperse biotinylated microbubbles (9.2±0.5 μm diameter; produced by flow-focusing device Horizon) bound to a streptavidin-coated glass were insonified at three frequencies (1, 1.25, or 1.5 MHz), along with acoustic pressures ranging from 100 to 500 kPa for 20,000 cycles. A refined microbubble shape-instability model was implemented and demonstrated satisfactory performance (88% accuracy, 14 out of 16 cases) in predicting the surface mode for a given microbubble size and ultrasound parameters. Both the experimental and modeling results indicated that higher ultrasound frequencies induced higher-order surface modes (up to mode 5 at 1.5 MHz and 500 kPa). This study also revealed a radial microstreaming pattern for each microbubble, characterized by an average velocity magnitude ranging from 0.01 to 0.015 m/s. Notably, the shape oscillation of microbubbles under high acoustic pressures resulted in a substantial increase in velocity magnitude within a 10 μm radius from the microbubble center. Furthermore, this significant velocity increase was accompanied by opposite flows (i.e. towards and away from the microbubble), indicating a strong mixing and circulation, especially in the direction perpendicular to the wall. Overall, the findings of this study can provide a better understanding of MB-mediated theranostics through better prediction and control of microbubble shape oscillation and microstreaming.

This project received funding from the European Research Council under the European Union's Horizon 2020 research and innovation program [grant agreement 805308].

Session 1	**Microbubble Fundamentals**
Oral Presentation	**Patrick Sullivan,** University of Edinburgh
Title	**Surface Wettability Effects on Heterogeneous Inertio-Thermal Vapour Bubble Growth**

Abstract

The formation of heterogeneous vapour bubbles is a widely studied phenomenon due to its importance to two-phase thermal management systems and turbomachinery performance. However, the role that the solid surface plays in determining the growth of the bubble is still poorly understood. Currently, theoretical understanding of heterogeneous vapour bubble growth is limited to hemispherical bubbles or completely spherical bubbles next to a surface. We have previously developed an inertio-thermal model to accurately predict homogeneous vapour bubble growth from the nanoscale to the macroscale [1]. By accounting for the effect of the surface on both the spherical cap geometry of the nanobubble and on the available thermal energy, we extend our model to capture how surface wettability influences heterogeneous vapour bubble growth. With comparisons to Molecular Dynamics simulations, we show our heterogeneous inertia-thermal model captures the enhanced nanobubble growth rate on less-wetting surfaces, which generally decreases as wettability increases. This trend continues until we see the formation of a non-evaporating layer beneath the bubble, at which point the surface nanobubble's contact angle tends to a fixed value, and the resulting growth rate becomes the limiting case for the most-wetting cases.

Session 1	**Microbubble Fundamentals**
Oral Presentation	**Andreas Papoutsakis,** University of Herdfordshire
Title	**An Eulerian multiscale multimaterial framework for the modelling of solid liquid gas interactions in capped bubbles drug delivery and bacteria incapacitation configurations.**

Abstract

Drug delivery by collapsing capped bubbles and bacteria incapacitation involves the interfacial interaction between the gaseous and the liquid phases in a bubbly environment in the proximity of soft mater constituting the microorganisms and the soft membrane of capped microbubbles. In these multi-material configurations, the different materials and phases interact across a dynamic network of interfaces, i.e.: Fluid-Gas and Fluid-Vapour interfaces, Gas-Gas shocks, Cracks and Boundary Layers, which introduce fine temporal and spatial scales [1]. Traditionally, multi-material configurations are dealt by the weak coupling of Lagrangian domains and are solved independently. The coupling is achieved by Immersed boundary approaches or Arbitrary Eulerian Lagrangian methods. Recently, Fully Eulerian approaches like the Diffused Interface Method (DIM) have been introduced towards a unified model [2]. In the simulation framework suggested here we utilise a newly developed Diffused Interface Method (DIM) that allows for the modelling of all participating phases in a unified Eulerian framework that can still model the full elastic stress tensor for the fluid phase. In the DIM approach, each material is modelled with an individual Equation of State, including extended EOS for the solids accounting for the full stress tensor. DIM has the advantage of dealing with large deformations, within a single mesh, modelling compressibility effects and normal and transverse waves and flow discontinuities [3].

[1] A. Papoutsakis, et. al An efficient Adaptive Mesh Refinement (AMR) algorithm for the Discontinuous Galerkin method: applications for the computation of compressible two-phase flows. JCP 363 399-427, 2018. [2] S. Richard, C. Pantano. Diffuse-Interface Capturing Methods for Compressible Two-Phase Flows. Annu. Rev.Fluid Mech. 105-130, 50(1) 2018. [3] E. Koukas, A. Papoutsakis, M. Gavaises. Numerical investigation of shock-induced bubble collapse dynamics and fluid–solid interactions during shock-wave lithotripsy. Ultrasonics Sonochemistry. Volume: 95, 2023.

Session 1	**Microbubble Fundamentals**
Oral Presentation	**Yuchen Wang,** Erasmus MC
Title	**Effects of Pluronic F68 addition on the Coalescence, Stability and Acoustic Behavior of Monodisperse Microbubbles Produced at Room Temperature**

Abstract

Producing monodisperse microbubbles (mMBs) at room temperature using a flow focusing device is challenging because of mMBs coalescence, limiting their use. Previous study (Roger et al., 2013) demonstrated that 82.7 mol% Pluronic F68 (PF68) reduces mMB coalescence; however, the assessment of size stability was limited to 10 minutes post-production, and acoustic characterizations were not conducted. Hence, this study investigates the effects of PF68 on mMBs coalescence, shelf stability and acoustic behavior. The microfluidic platform Horizon was used to produce mMBs at room temperature with a 6 µm on-chip radius and C4F10 gas core. The lipid coating consisted of DSPC and DPPE-PEG5000 (9:1 mol ratio; 20 mg/ml) with 0 (0PF-MB), 7.2 (7.2PF-MB), 10 (10PF-MB) or 30 (30PF-MB) mol % of PF68. Coulter Counter Multisizer 3 was employed to evaluate the shelf stability by measuring the mMB size distribution (number-weighted) over 7 days. To measure acoustic attenuation, 1-5 MHz ultrasound was transmitted with 12-cycle pulses at acoustic pressures ranging from 10-150 kPa. Stiffness was calculated by applying a linearized Rayleigh-Plesset equation to the observed resonance at 10 kPa and the peak size of the mMBs. On-chip coalescence was observed for the 0PF-MB, but not when PF68 was added. Two h post production, the 30PF-MB dissipated suggesting instability. In contrast, 7.2PF-MB and 10PF-MB, having a peak radius of 2 µm, remained stable for up to 7 days. With increasing the pressure from 10 to 150 kPa, the resonance frequency of both 7.2PF-MB and 10PF-MB exhibited a decrease from around 3 to 2.7 MHz. Both 7.2PF-MB (0.81 N/m) and 10PF-MB (0.86 N/m) exhibited similar stiffness. These results show that coalescence-free, shelf stable (at least 7 days) and acoustically responsive mMBs can be produced at room temperature when Pluronic F68 is added to the lipid solution.

Funding by NWO (VIDI-project no.17543) is gratefully acknowledged.

Session 1	**Microbubble Fundamentals**
Oral Presentation	**Tim Segers,** University of Twente
Title	**Dependence of sonoporation efficiency on microbubble size: an in vitro monodisperse microbubble study**

Abstract

Sonoporation is the process where intracellular drug delivery is facilitated by ultrasound-driven microbub- ble oscillations. Several mechanisms have been proposed to relate microbubble dynamics to sonoporation including shear and normal stress. The present work aims to gain insight into the role of microbubble size on sonoporation and thereby into the relevant mechanism(s) of sonoporation. To this end, we measured the sonoporation efficiency while varying microbubble size using monodisperse microbubble suspensions. Sono- poration experiments were performed in vitro on cell monolayers at a fixed ultrasound frequency of 1 MHz while the acoustic pressure amplitude and pulse length were varied at 250, 500, and 750 kPa, and 10, 100, and 1000 cycles, respectively. Sonoporation efficiency was quantified using flow cytometry by measuring the FITC-dextran (4 kDa and 2 MDa) fluorescence intensity in 10,000 cells per experiment to average out inherent variations in the bioresponse. Using ultra-high-speed imaging at 10 million frames per second, we demonstrate that the bubble oscillation amplitude is nearly independent of the equilibrium bubble radius at acoustic pressure amplitudes that induce sonoporation (≥ 500 kPa). However, we show that sonopo- ration efficiency is strongly dependent on the equilibrium bubble size and that under all explored driving conditions most efficiently induced by bubbles with a radius of 4.7 µm. Polydisperse microbubbles with a typical ultrasound contrast agent size distribution perform almost an order of magnitude lower in terms of sonoporation efficiency than the 4.7-µm bubbles. We elucidate that for our system shear stress is highly unlikely the mechanism of action. By contrast, we show that sonoporation efficiency correlates well with an estimate of the bubble-induced normal stress.

Session 1	**Microbubble Fundamentals**
Oral Presentation	**Amin Jafarisojahrood,** Toronto Metropolitan University
Title	**Experimental and numerical investigation of the size-dependent behavior of microbubbles and nanobubbles in response to focused ultrasound: Insights into optimization of applications**

Abstract

Microbubbles (MBs) and recently nanobubbles (NBs) are being explored in therapeutic ultrasound (US) applications such as drug delivery. Smaller agents such as NBs provide advantages such as penetrating through leaky tumor vasculature. However, their efficacy in applications has been a subject of debate and controversy. To provide fundamental insight on this subject, the size size-dependent acoustic response of these agents was investigated.

Numerical simulations were carried out by solving the Marmottant model for volume matched bubble sizes of 4, 2, 1 and 0.4 um in response to 1 MHz US sonication with pressures between 120-1000 kPa. The pressure-dependent attenuation and the total acoustic power (TAP) were calculated. In house -made MBs were size isolated to batches with 4, 3, 2 and 0.45um sizes. They were volume matched and their acoustic response were recorded in 500um channel phantoms. Agents' behavior was also investigated optically with (High-speed, 5Mfps) inside smaller micro-channels.

Numerical results show that the TAP and attenuation of the agents are size dependent. Bigger MBs have a stronger response at lower pressures. However, smaller agents exhibit a size dependent pressure threshold behavior above which both their attenuation and TAP grow stronger than their bigger counterparts. The attenuation and TAP of the 0.4um NBs, become respectively more than double and two orders of magnitude larger than the bigger agents. The experimental results qualitatively confirm the numerical simulations. Agents exhibit a size size-dependent pressure threshold for acoustic activity. The TAP of the NBs is lower (~66times) than the rest of the agents below 500kPa. However, above 500kPa, their response is stronger due to the sudden increase in NB activity (~3.6 times more than Definity). The pressure threshold of the oscillations may be used to reduce pre-focal attenuation and activity, while the stronger focal response may have potential in enhancing the therapeutic effects.

Session 2	**NanoBubbles / NanoDroplets**
Oral Presentation	**Agata Exner,** Case Western Reserve University
Title	**Nanobubbles – An Update on Formulation and New Applications**

Abstract

Gas-core nanoparticles (also called nanobubbles or ultrafine bubbles) which have a diameter of ~100-500 nm, have gained momentum as a robust contrast agent for molecular imaging and treatment of cancer using ultrasound. Nanobubble (NB) behavior is distinct from conventional microbubbles (MB) especially in tissues exhibiting vascular hyperpermeability. This is due to the smaller volume, 3-5 order of magnitude higher particle density per imaging voxel and > 5-20 fold extended stability in vivo. NB can provide superior tumor detection, identify biomarkers on the vasculature and on tumors cells, and improve the efficiency of drug delivery. However, development of new NB applications have been limited by complex preparation and lack of NB-specific imaging protocols. This presentation will discuss our work recent work on streamlined NB formulation using a mini-extruder setup, development of NB-CEUS imaging biomarkers and using the unique NB capabilities for detection and drug-free treatment of prostate cancer.

Session 2	**NanoBubbles / NanoDroplets**
Oral Presentation	**Weiqi Zhang**, King's College London
Title	**Characterising the chemical and physical properties of phase-change nanodroplets**

Abstract

Phase-change nanodroplets have attracted increasing interest in recent years as ultrasound theranostic nanoparticles. They are smaller compared to microbubbles and they may distribute better in tissues (e.g. in tumours). They are composed of a stabilising shell and a perfluorocarbon core. Nanodroplets can vaporise into echogenic microbubbles forming cavitation nuclei when exposed to ultrasound. Their perfluorocarbon core phase-change is responsible for the acoustic droplet vaporisation. However, methods to quantify the perfluorocarbon core in nanodroplets are lacking. This is an important feature that can help explaining nanodroplet phase change characteristics. In this study, we fabricated nanodroplets using lipids shell and perfluorocarbons. To assess the amount of perfluorocarbon in the core we used two methods, 19F-NMR and FTIR. To assess the cavitation after vaporisation we used an ultrasound transducer (1.1MHz) and a high-speed camera. The 19F-NMR based method showed that the fluorine signal correlated accurately with the perfluorocarbon concentration. Using this correlation, we were able to quantify the perfluorocarbon core of nanodroplets. This method was used to assess the content of the perfluorocarbon of the nanodroplets in solutions over time. It was found that perfluoropentane nanodroplets lost their content faster and at higher ratio compared to perfluorohexane nanodroplets. The high-speed camera showed that these nanodroplets have similar cavitation with commercial microbubbles., Ultrasound imaging also demonstrated they can have similar contrast to microbubbles when tested in phantoms. Nanodroplet characterisation should include perfluorocarbon concentration assessment as a critical information for their development in vitro and in vivo.

Session 2	**NanoBubbles / NanoDroplets**
Oral Presentation	**Michael Coey,** Trinity College Dublin
Title	**Interaction and stability of nanobubbles and pre-nucleation calcium clusters during ultrasonic treatment of hard water.**

Abstract

A series of eight water samples ranging in hardness from 0 to 332 mg/L CaCO3 were sonicated for periods of 5' - 45' with an ultrasonic horn. The conductivity, temperature, zeta potential, composition and pH of the water were analysed, together with the crystal structure of any calcium carbonate precipitate. Quasi-stable populations of bulk nanobubbles are characterized by a ζ-potential of -35 to -20 mV in Millipore and soft water, decaying with a half-life of 80 h. After sonicating the hardest waters for about 10', they turn cloudy when the temperature of the water reaches 45°C. The ζ-potential then jumps from -10 mV to +20 mV and remains positive for several days. From analysis of the change of conductivity of the hard water before and after sonication it is estimated that 37 ± 5% of the Ca2+ was originally present in nanoscale prenucleation clusters (DOLLOPs) which decorate the nanobubbles formed in the early stages of sonication. Heating and charge screening in the nanobubble suspension causes the decorated nanobubbles to collapse or disperse, leaving amorphous calcium carbonate, the precursor of aragonite. When the precipitate sediments, the ζ-potential falls to back to -5 mV. Sonicating the supernatant increases its conductivity and pH, but there is no further precipitation or development of zeta potential. Our investigation of the correlation between nanobubble production and Ca2+ ion agglomeration spans hardness and composition ranges of natural waters and shows the sonication method for introducing nanobubbles is viable for hard water only if the water is kept cold.

Session 2	**NanoBubbles / NanoDroplets**
Oral Presentation	**Damien Batchelor,** University of Leeds
Title	**Characterisation of Nanobubbles: Utilizing their optical and physical properties**

Abstract

Accurate characterization of nanobubble (NB) physical properties, as well as their size and concentration, is challenging and may limit their translation into clinical use. The submicron nature of NBs limits accuracy of conventional microscopy techniques, while common light scattering methods fail to distinguish between subpopulations present in NB samples (i.e., bubbles and liposomes) whilst also unable to identify the presence of a gas-core. Firstly, we demonstrate a simple method to distinguish between NBs and liposomes based on their differing optical properties, using a commercially available nanoparticle tracking analysis (NTA) system (NanoSight NS300). This technique was used to characterise NB populations of different sizes and investigate how size influences their stability. Contrary to predictions from the Laplace pressure, larger NBs did necessarily demonstrate enhanced stability compared to smaller NBs. Based on a combination of stability measurements at either matched NB concentration or measured at varying dilutions, we hypothesized that inter-bubble distance and free lipid concentration both play an important role in governing NB lifetime. Secondly, we utilize the reduced density of the bubble gas core to distinguish them from non-bubbles (i.e. liposomes) using Quartz Crystal Microbalance with Dissipation (QCM-D) as the change in resonance frequency of the QCM-D crystal is directly proportional to the coupled mass. Microbubbles (MBs) and liposomes were attached to a supported lipid bilayer formed on the QCM-D crystal using dual-anchored cholesterol-DNA tethers. It was found that the attachment of MBs lead to a decrease in the magnitude of frequency shift compared to liposomes alone, likely due to their reduced density. Interestingly, it the dissipation of the system increased for MBs compared to liposomes, hypothesized to be due to the increased stiffness of MBs as well as an increased number of binding sites per particle.

Session 2	**NanoBubbles / NanoDroplets**
Oral Presentation	**Duncan Dockar,** University of Edinburgh
Title	**Nanobubble Cavitation Dynamics**

Abstract

Gas bubbles can be stimulated to grow, oscillate, and violently collapse, through exposure to acoustic waves, in the phenomenon known as cavitation. While cavitation at the macroscale has long been a problem for increased wear and pitting on solid surfaces, for example in turbomachinery, cavitation at the microscale has many beneficial applications, such as increased efficiencies in waste-water treatment, non-invasive and targeted cancer treatment and diagnosis, and precision surface cleaning. Recently, there has been increasing interest in employing nanobubbles in these applications for further improved targeting, since their sub-micron sizes allow them to enter intricate micro-fluidic networks that microbubbles are too large reach, such as in MEMS devices and fine biological structures. However, classical cavitation models, including the Blake threshold and Rayleigh–Plesset equation, typically assume spherical bubbles in the bulk and are unable to predict dynamics for surface nanobubbles, a type of spherical-cap shaped bubble that can exist in diffusive equilibrium when pinned on patterned or rough solid surfaces, and which have been speculated to act as the long-theorised nanoscale nuclei for cavitation. Through the use of high-fidelity Molecular Dynamics (MD) simulations, my research overviews the cavitation dynamics of both surface and bulk nanobubbles, by examining their size and shape, non-ideal and non-equilibrium gas behaviour, and other fluid properties that dominate at the nanoscale, such as viscosity and electrostatic effects. I propose new theoretical models for their cavitation threshold, natural frequency, thermal/mechanical oscillatory behaviour, and shock-induced collapse, and briefly discuss the future direction of nanobubble cavitation research.

Session 2	**NanoBubbles / NanoDroplets**
Oral Presentation	**Roberta Cavalli,** University of Turin
Title	**Ultrasound-mediated theragnostic nanobubbles with a Langmuir's multilayer polymeric shell as controlled drug delivery system for topical administration**

Abstract

Ultrasound (US)-mediated nanobubbles (NBs) are formed by a gas core and coated with a shell and they are an attractive drug delivery system thanks to their biocompatibility and versatility. US-mediated systems can bypass biological barriers following sonoporation (the partial permeabilization of cell membranes following the formation and oscillation of nanobubbles once exposed to US) increasing drug biodistribution and their localization can be monitored in real time. Here we developed polymeric coated NBs with high stability and able to provide a sustained drug release kinetics, by optimizing both the design composition of the shell and the choice of the ultrasound parameters. Langmuir's multilayer technique was used to coat the NBs and exploit the interaction between a phospholipid layer and a chitosan layer to reduce the surface tension and consequently promote stability. For this purpose, we developed a series of oil/water nanoemulsions where nanodroplets can be vaporized after insonation, by modifying the composition and the concentration of the materials used for the shell. The physico-chemical properties of the system were optimized: the formulation obtained with a layer of Epikuron® (3% w/v) with palmitic acid (1% w/v) solution and a layer of low molecular chitosan (1,5% w/v) showed the best average size, polydispersity index and surface tension. The NBs were loaded with Curcumin as a model drug. The frequency and Intensity of US were optimized to provide a sustained and slow drug release: the frequency of 2 MHz and the intensity of 2 W/cm2 showed lack of burst release and a sustained release of 14% of Curcumin after 6h. To conclude we were able to optimize the design of Langmuir's multilayer polymeric shell NBs to obtain a drug delivery system that could be exploited for topical administration following US exposure.

Session 2	**NanoBubbles / NanoDroplets**
Oral Presentation	**Marie-Pierre Kraft**, University of Strasbourg (CNRS)
Title	**Polyhedral Perfluorocarbon Nanodroplets that Phase-Shift into Polyhedral Nanobubbles**

Abstract

The potential of perfluorocarbon (PFC)-stabilized microbubbles (MBs) for contrast ultrasound (US) diagnosis, therapy (focused US-mediated drug delivery and therapeutic energy delivery), and for sensitization of oxygen-dependent therapies (radio- and chemotherapy, dynamic photo(sono)therapy) is being intensively investigated [1, 2]. The micrometric size of the MBs that confines them to the vascular system and their short life-time in the circulation limit the scope of their uses. These issues can be circumvented by injecting nanodroplets (NDs, a nanoemulsion) of a liquid PFC that is subsequently vaporized to give birth to MBs by applying US pulses once their target (e.g., a tumor) is reached. Here, we report that a series of new amphiphilic oligo(ethylene glycol) alkylated dendrons [3] used in combination with phospholipids strongly stabilize perfluorohexane (F-hexane) NDs, as assessed by dynamic light scattering (DLS) (Fig. 1a). Remarkably, we found that the F-hexane NDs are not spherical immediately after preparation, but polyhedral, as demonstrated by cryogenic transmission electron microscopy (Cryo-TEM). Over time, the faceted NDs convert slowly into spherical ones (Fig. 1b), a phenomenon investigated by micro-differential scanning calorimetry (micro-DSC). Notably, the shelf stability of the F-hexane NDs is directly linked with the existence of the facets. Incorporation of the dendrons inhibits the faceted-to-spherical ND conversion, thus increasing stability dramatically (Fig. 1a, red curve). The interactions between DPPC and dendrons spread as Langmuir monolayers were investigated using the molecular area additivity rule. The potential of these faceted PFC NDs for acoustic droplet vaporization to MBs is discussed. The interest of polyhedral nanostructures, such as droplets and vesicles based on fluorinated compounds, is also presented from a fundamental viewpoint [4].

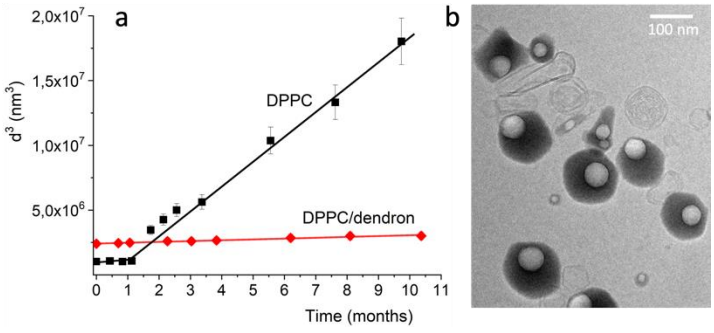

Figure 1: a) Variation of *F*-hexane droplet volume versus time; dipalmitoylphosphatidylcholine (DPPC, black)-stabilized *F*-hexane nanoemulsions start to become destabilized after ~1 month, which corresponds to their conversion to spherical morphologies. By contrast, nanoemulsions stabilized by DPPC/dendron combinations (molar ratio: 25:1) remain stable for at least 10 months. b) Cryo-TEM image showing polyhedral *F*-hexane nanodroplets (black) and nascent nanobubbles (white).

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Session 2	**NanoBubbles / NanoDroplets**
Oral Presentation	**Michael Kolios**, Toronto Metropolitan University
Title	**Unexpected nanobubble generation by microfluidic microbubble collapse**

Abstract

Several groups have produced monodisperse microbubbles using microfluidics techniques. We have previously shown a microfluidic design that shrinks lipid-stabilized microbubbles from O(100) to O(1) μm in diameter by utilizing vacuum channels adjacent to the microfluidic flow channels to extract air from the microbubbles. The bubbles shrink until an equilibrium size is reached, determined by the concentration and type of molecules stabilizing the gas-liquid interface. In this work, we exploit another unexpected shrinkage mechanism and control the solution lipid concentration and microfluidic geometry to make monodisperse nanobubbles (NB) without vacuum channels. We show that a critical initial microbubble diameter exists, above and below which the bubble shrinkage dramatically changes. Microfluidic microbubbles generated with an initial diameter larger than the critical bubble diameter (CBD) shrinks to a stable diameter. However, microbubbles that are initially smaller than the CBD experience a sudden contraction into nanobubbles of a consistent final diameter, whose size is an order of magnitude below what is found with microbubbles with a larger initial diameter (final diameter ~ 150 - 250 nm). Electron microscopy and resonance mass measurement methods are used to quantify the NB size and uniformity and probe the CBD's dependence on the lipid concentration. The final NB size is consistent with theoretical predictions based on the Helfrich model used to describe the NB free energy of curvature. This microfluidics platform suggests a robust technology for making monodisperse nanobubbles.

Session 3	**Microbubbles: Towards Translation**
Oral Presentation	**Klazina Kooiman,** Erasmus MC
Title	**Controlling microbubble–mediated drug delivery: influence of microbubble type and cell morphology**

Abstract

Ultrasound-activated microbubbles can be used to locally deliver drugs [1]. However, the microbubble–cell–drug interaction is not fully understood, which is hindering controlled and optimal therapeutic outcomes. With the use of a custom-built Nikon A1R+ microscope coupled to an ultra-high-speed camera [2], we have been simultaneously studying the cellular response to ultrasound-activated microbubbles in micrometer and microsecond resolution. Endothelial cells in a 100% confluent monolayer were used to assess sonoporation, tunnel formation, cell-cell contact opening and F-actin remodeling upon ultrasound-activated microbubble treatment (2 MHz, 200 kPa PNP, 10 cycles). Depending on the microbubble type, cells either internalized microbubbles (aVβ3-targeted; CD31-targeted) or not (non-targeted, isotype control). The internalized microbubbles had a significantly damped oscillation. At the same time, the internalized aVβ3-targeted microbubbles had a lower sonoporation threshold than the other microbubble types: excursion amplitude of 0.7 µm (n = 70) versus 0.9 µm (n=158). Smaller membrane pores and less tunnel formation was observed for the internalized microbubbles in comparison to non-internalized microbubbles [3]. With monodisperse microbubbles (monoMBs), produced by the Horizon microfluidic device, the cellular responses could be tuned: 47% sonoporation with tunnel formation for 1.25 µm radius monoMBs versus 50% reversible sonoporation for 1.5 µm radius monoMBs. Yet, there was no clear relationship between microbubble excursion amplitude and cell-cell contact opening. When endothelial cells were transfected with fluorescent cytoskeleton F-actin, remodeling of the F-actin was only seen upon sonoporation. F-actin remodeling consisted of disruption only in case of irreversible sonoporation, disruption and recovery in case of reversible sonoporation, or for F-actin stress fibers disruption and recoiling resulting in cell-cell contact opening. In conclusion, these findings are further unraveling the microbubble-cell-drug interactions.

References: [1] Kooiman et al., Ultrasound Med Biol 2020, 46:p.1296; [2] Beekers et al., Ultrasound Med Biol 2019, 45:p.2575; [3] Beekers et al., J Control Rel 2022, 347:460.

Session 3	**Microbubbles: Towards Translation**
Oral Presentation	**Kristian Hollingsworth,** University of Leeds
Title	**Enhancing microbubble binding to Staphylococcus aureus Biofilms through multi-targeting**

Abstract

Biofilm-associated infections caused by Staphylococcus aureus pose significant challenges in healthcare settings due to their resistance to conventional antimicrobial therapies. Targeted drug delivery systems utilizing microbubbles offer a promising approach to combat biofilms. This project explores the potential of utilizing multiple targets within staphylococcal biofilms to enhance the binding efficiency of microbubbles. Staphylococcus aureus biofilms are complex structures composed of various components, including proteins, lectins, the peptidoglycan layer, polysaccharides, and extracellular DNA. These diverse components provide potential targets for specific binding of therapeutic agents or diagnostic agents. By targeting multiple components simultaneously, it is hypothesized that the binding efficiency of microbubbles to staphylococcal biofilms can be improved. This study presents the successful targeting of proteins within biofilms using affimers, engineered non-antibody binding proteins. The research also explores the combination of affimer-based targeting with other methods to enhance biofilm targeting efficiency.

Session 3	**Microbubbles: Towards Translation**
Oral Presentation	**John Callan,** Ulster University
Title	**Ultrasound targeted microbubble destruction mediated Chemo-SonoDyamic Therapy for the treatment of solid tumours.**

Abstract

As part of our ongoing research programme in the area of pancreatic cancer, we have demonstrated that combining established antimetabolite or taxane chemotherapy with the less familiar approach known as Sonodynamic Therapy (SDT) was significantly more effective at reducing the size of pancreatic tumours in mice, than treatment using either therapy alone. In SDT, a sensitiser drug is activated by low-intensity ultrasound (US), in the presence of molecular oxygen, to generate cytotoxic levels of reactive oxygen species (ROS). The attraction of SDT when compared to conventional cancer treatments is that both the sensitiser and low-intensity ultrasound treatment are harmless on their own, but when combined together, produce a potent cytotoxic effect. To target delivery of this chemo-sonodynamic therapy treatment to pancreatic tumours, we have utilised microbubbles as a delivery vehicle. Microbubbles are gas filled lipid-stabilised bubbles 1-3 μm in diameter and are approved for use in diagnostic ultrasound. When microbubbles are loaded with drugs, ultrasound targeted microbubble destruction (UTMD) enhances drug concentration at the tumour site. In addition, microbubble induced cavitation can enhance the dispersion of drugs in tissue thus improving their effectiveness in the treatment of solid tumours. In this presentation, recent results obtained for the efficacy of UTMD mediated chemo-sonodynamic therapy in pancreatic and prostate cancer tumour models will be presented.

Session 3	**Microbubbles: Towards Translation**
Oral Presentation	**Joseph Fox,** University of Leeds
Title	**Developing an In-Vitro Adaptive Therapy Model for Colorectal Cancer**

Abstract

Adaptive therapy applies evolutionary principles to the way cancer is treated. In this regime, treatment is only administered after sensitive tumor cells have been permitted to recover, with the theory of using sensitive cells to generate survival competition, subduing the proliferation of resistant cells. Our group has been working towards the development of an ¬in vitro adaptive therapy model for colorectal cancer. The work consists of two main components: i) the development of a treatment timing feedback mechanism and ii) the development of a targeted drug delivery system. Towards the first goal, we have been developing a lateral flow assay to monitor the expression of Carcinoembryonic Antigen (CEA), a prognostic biomarker produced by human colorectal cancers (CRC). For lateral flow assays, nanoparticles with high surface-area-to-volume ratios or rough surface topologies can boost lateral flow assay performance. Hence, to enhance assay sensitivity we have developed and applied novel gold nanoparticle morphologies, such as 2D gold nanosheets (AuNS), gold nanotapes (AuNT) and gold nanopinecones (AuNPC). Our results show AuNPC offer improved CEA detection when compared to control assays using spherical gold nanoparticles (AuNP). Towards the second aim, we have studied microbubbles (MBs) and ultrasound (US) as a targeted drug delivery system. We have investigated the delivery of liposomal vitamin C to colorectal cancer cells lines in vitro and shown therapeutic efficacy is enhanced when the drug payload is delivered using the MB+US system. By combining these two studies, we aim to construct the first in vitro adaptive therapy model system.

Session 3	**Microbubbles: Towards Translation**
Oral Presentation	**Alexander Klibanov,** University of Virginia
Title	**Lipid-shell microbubbles with air core.**

Abstract

Three out of four microbubble formulations currently used in clinical practice consist of a phospholipid shell that encases a poorly soluble fluorinated gas core. Use of fluorinated gases (perfluoropropane, perfluorobutane, or sulfur hexafluoride) is necessary to extend the particle stability, both in vitro and in vivo. However, air-filled research-grade microbubble formulations exist that demonstrate excellent storage stability and in vivo circulation time. As an example, poly-butyl cyanoacrylate (PBCA) microbubbles are widely tested in preclinical setting. Therefore, there might be interest in preparing lipid-based formulation of air-based microbubbles. In this study we attempted to investigate storage stability as well as in vivo circulation time of air-filled microbubbles, formulated with "traditional" distearoylphosphatidylcholine (DSPC, C18) and lipids with longer chain lengths: dibehenoylphosphatidylcholine (DBPC, C22) and dimontanylphosphatidylcholine (DMOPC, C28). The working hypothesis was that longer-chain lipids would increase microbubble shell thickness and thus improve stability and circulation residence time, without the need for a low-solubility gas core. Microbubbles were prepared in the aqueous micellar saline medium that contained phosphatidylcholine and PEG stearate. Resulting microbubbles were sized by flotation at normal gravity to remove the largest particles. The resulting bubble concentration was determined by Coulter counter. In vivo circulation time was assessed by ultrasound imaging of mouse kidney in a non-destructive contrast mode. Animals were under isoflurane/medical air anesthesia during the course of the study. Air-filled microbubbles prepared from DSPC were not stable on storage and disintegrated overnight. Microbubbles prepared from DBPC and DMOPC demonstrated better storage stability and efficient manufacturing. However, their circulation residence time, as was assessed by ultrasound imaging, was at least an order of magnitude shorter than the standard decafluorobutane-based microbubbles. In conclusion, the use of long-chain phospholipid formulations, while improving storage stability, in itself does not drastically improve microbubble lifetime in the bloodstream in vivo.

Session 4	**Microbubbles: Preclinical / Translation**
Oral Presentation	**Helen Mulvana,** University of Strathclyde
Title	**Development of Preclinical Contrast-Enhanced Ultrasound Imaging to Identify and Image Sentinel Lymph Nodes in a Cancerous Mouse Model**

Abstract

Bowel cancer is the fourth most common cancer in the UK. Treatment is dominated by major surgery because current imaging modalities cannot accurately determine lymph node involvement or vascular invasion. Lymph nodes (LNs) are believed to be the first organs targeted by colorectal cancer cells detached from a primary solid tumor because of their role in draining interstitial fluids. Better detection and assessment of these organs have the potential to help clinicians in stratification and designing optimal design of oncological treatments for each patient. Whilst highly valuable for the detection of primary tumors, CT and MRI remain limited for the characterization of LNs. B-mode ultrasound (US) and contrast-enhanced ultrasound (CEUS) can improve the detection of LNs and could provide critical complementary information to MRI and CT scans; however, the European Federation of Societies for Ultrasound in Medicine and Biology (EFSUMB) guidelines advise that further evidence is required before US or CEUS can be recommended for clinical use. Moreover, knowledge of the lymphatic system and LNs is relatively limited, especially in preclinical models. In this work, we have created a mouse model of metastatic cancer and utilized 3D high-frequency ultrasound to assess the volume, shape, and absence of hilum, along with CEUS to assess the flow dynamics of tumor-free and tumor-bearing LNs in vivo. The aforementioned parameters were used to create a scoring system to predict the likelihood of a disease-involved LN before establishing post-mortem diagnosis with histopathology. Preliminary results suggest that a sum score of parameters may provide a more accurate diagnosis than the LN size, the single parameter currently used to predict the involvement of an LN in disease.

Session 4	**Microbubbles: Preclinical / Translation**
Oral Presentation	**Jeff Bamber,** Institute of Cancer Research and Royal Marsden
Title	**Acoustic cluster therapy; an update on research and evaluation**

Abstract

Acoustic cluster therapy (ACT) employs intravenously injected clusters of microbubbles and microdroplets which circulate stably until activated by diagnostic ultrasound when they are seen on the images to arrive at a treatment site such as a tumour. Activation involves microdroplet vaporisation, due to the ultrasonic energy absorbed by the microbubbles within clusters to form 20 40 µm bubbles which lodge in the tumour capillaries. Subsequent insonation with low-frequency (~ 500 kHz) low-pressure (MI ~ 0.2) ultrasound modulates these ACT bubbles in contact with the vascular endothelium to elicit various biomechanical effects, until they dissolve after 5 10 minutes. This presentation reviews ACT research in cancer treatment since last presented at the Leeds Microbubble Symposium in 2018. ACT has now been demonstrated to improve the efficacy of four chemotherapeutics in four tumour models, and provided ACT-imaging biomarkers that predict therapeutic outcome in individual tumours. The first-in-man (phase I/II) clinical trial is in progress, to date demonstrating no adverse effects due to ACT. Initial data also provides anecdotal examples of how response of colorectal cancer liver metastases to standard of care chemotherapy is improved by ACT. Finally, ongoing work aims to determine whether ACT exhibits synergism with immunotherapy based on three potential mechanisms: a) enhanced delivery of checkpoint inhibitors (ICI), b) improved trafficking of immune cells and c) stress induced gene expression to favourably alter the tumour immune microenvironment. Preliminary results in B16 melanoma and 4T1 triple negative breast cancer models are yielding a wealth of data on imaging biomarkers, flow cytometry, RNA sequencing and immunohistochemistry, suggesting that ACT has complex and heterogeneous impacts on perfusion, ICI efficacy, immune cell movement and gene expression, and could enhance treatment of some tumours. Work is ongoing to understand how best to exploit the observed effects.

Session 4	**Microbubbles: Preclinical / Translation**
Oral Presentation	**Delanyo Kpeglo,** University of Leeds
Title	**A microfluidic PDAC culture model for investigating microbubble-mediated gemcitabine delivery**

Abstract

We present a microfluidic pancreatic ductal adenocarcinoma (PDAC) culture model, encompassing PDAC's biophysical barriers to therapeutics, to test microbubbles (MBs)-mediated drug delivery approach. There are many promising new drugs and delivery methods in development against PDAC, the most prevalent pancreatic cancer with poor prognosis. However, there remains a fundamental knowledge gap to translate drug studies to the clinic: a rigid fibrotic stroma, blocking drug penetration into the tumour and resulting in treatment failure. The high densities of cells and extracellular matrix (ECM) proteins form a rigid mass and high interstitial pressure, leading to vasculature collapse and a fibrotic, hypoxic, mechanically stiff stroma with reduced interstitial flow, critical to drug delivery to cells. Despite this, most pre-clinical drug tests are performed on cellular models without these biophysical characteristics central to ineffective drug delivery. Microfluidics provides a means to develop adequate culture models, mimicking tumour biophysical features with appropriate flow conditions, and allows accurate test beds to assess new drugs and emerging delivery approaches such as MBs-mediated drug delivery. The cavitation and collapse of the micron-sized phospholipid-shelled gas bubbles with ultrasound (US) generates shock waves and exert forces in the local ECM and cell membranes to increase drug uptake. Our microfluidic PDAC model features co-culture of PANC-1 with PSCs, the fibroblasts responsible for over-producing collagen for the rigid fibrotic stroma. Off-chip investigation found a 21-day culture required to develop the stiff PDAC stroma (~1kPa). Translated on-chip, immunostaining of our model found a collagenous matrix by day 21 of culture, leading to increased mechanical rigidity, reduced interstitial flow, and a hypoxic environment. When the chemotherapeutic gemcitabine was delivered with US-activated MBs, gemcitabine effectiveness increased by ~15%. This work demonstrates the importance of modelling disease biophysical characteristics to adequately test drugs and the use of MBs-mediated drug delivery to successfully deliver drugs to cells.

Session 4	**Microbubbles: Preclinical / Translation**
Oral Presentation	**Sophie Morse,** Imperial College London
Title	**Microbubbles and focused ultrasound to non-invasively deliver drugs to the brain**

Abstract

The blood-brain barrier protects our brain from harmful substances. However, it also prevents over 98% of drugs and imaging agents from entering, including those that could treat and help diagnose currently incurable brain diseases, such as brain tumours, Alzheimer's and Parkinson's disease.

Focused ultrasound, in combination with microbubbles, is a non-invasive technology that can temporarily and locally open the blood-brain barrier, allowing drugs and imaging agents to enter the brain. These microbubbles only oscillate when they reach the region where the ultrasound is focused, creating mechanical forces that allow the blood-brain barrier to temporarily open and for agents to finally enter the brain.

In this talk, I will show how very short pulses of ultrasound and microbubbles can non-invasively and safely deliver a variety of compounds to targeted regions of healthy and diseased murine brains, including liposomes and small-molecule drugs. This technology opens up immense opportunities for novel drugs to reach desired brain regions and for powerful drugs that have failed clinical trials due to the blood-brain barrier, to be retested.

Session 4	**Microbubbles: Preclinical / Translation**
Oral Presentation	**Maria Thanou**, Kings College London
Title	**Nanodroplets preparation characterisation and applications**

Abstract

Phase-changing sonoresponsive materials have attracted substantial attention in biomedical applications for both tumour imaging and therapeutic purposes due to their unique response to ultrasound. As ultrasound is applied at different frequencies and powers, nanodroplets have been shown to cavitate by the process of acoustic droplet vapourisation (ADV), causing the development of mechanical forces which promote sonoporation through cellular membranes. This allows drugs to be delivered efficiently into deeper tissues where tumours are located. Research has focused on the mechanism of cavitation and their applications in biomedical fields. However, the chemistry of the nanodroplet components or the method of preparation has not been thoroughly investigated. Materials and preparation methods of nanodroplets vary and there is limited structure activity relationship consideration. Herewith, we suggest a series of physicochemical methods that can help selection of suitable materials for the preparation of the nanodroplets and the monitoring of their properties.

Session 4	**Microbubbles: Preclinical / Translation**
Oral Presentation	**Wladyslaw Gedroyc,** Imperial NHS Trust
Title	**Brain Focussed ultrasound the next steps**

Abstract

This talk, describes the current status and future development of Brain focused ultrasound beyond its current state. I will describe the current utilisation of the 660 kHz transducer, and how it is being used in the treatment of tremor.

I will also describe the future expansion of this technology in using lower frequency transducer at 2:20 khz and how this will be utilised in the treatment of brain tumours and potentially other complex brain conditions.

Hopefully the talk with explain how the current and future development of this approach will radically change the paradigm for the treatment of many neurological diseases.

Poster Presentations

Number	Name	Title
1.	Monica Argenziano	Chitosan-shelled nanobubbles as Glypican-3 targeted nanomedicine for Hepatocellular carcinoma treatment
2.	Hannah Bargh-Dawson	Evaluation of murine head and neck carcinoma models for determining response to ultrasound-stimulated microbubbles combined with radiotherapy
3.	Kathryn Burr	Targeted delivery of antibiotics using proteins that bind to bacterial biofilms
4.	Hamidreza Hassanloo	Molecular Dynamics Simulation of Nanobubble Formation: Unraveling the Impact on Thermophysical Properties
5.	Amin Jafarisojahrood	Influence of the ion density of the medium on the shell elasticity and viscosity of lipid-coated microbubbles
6.	Anjali Lad	Microbubbles for Tackling Antimicrobial Resistance
7.	Chloe McClenaghan	Phase-shift nanoemulsions for the targeted treatment of pancreatic cancer
8.	Carmel Moran	Characterisation of multimodal contrast agents for lymph node imaging

Poster Number 1	**Monica Argenziano,** University of Turin
Title	**Chitosan-shelled nanobubbles as Glypican-3 targeted nanomedicine for Hepatocellular carcinoma treatment**

Abstract

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related death. Most patients with HCC are diagnosed at an advanced tumor stage, in which systemic treatments are minimally effective and do not generally improve patient survival. The high levels of Glypican-3 (GPC3) detected in HCC and the absence or very low levels in normal and non-malignant liver make it a promising target for cancer treatment strategies. Polymeric nanobubbles (NBs) have been proposed for anticancer drug delivery [3]. The aim of this work was the development of GPC3 targeted polysaccharide-shelled NBs loaded with idarubicin (IDA) to treat HCC. The NB formulation was purposely tuned to load IDA in decafluoropentane core and to conjugate the anti-GPC3 antibody 4A1 to the NB shell. Moreover, fluorescent-labelled NBs were prepared binding cyanine 5.5 to the shell of NBs. The NB formulations were in vitro characterized determining their physico-chemical parameters, the morphology, the loading capacity, drug release kinetics and the stability. The cytotoxic activity of NBs was evaluated by MTT assay on an HCC-like cell line, HUH7 cells. Moreover, biodistribution studies and therapeutic efficacy evaluation were carried out on GPC3 expressing HCC xenograft murine model. Stable NB nanosuspensions with an average diameter of about 400 nm, low polydispersity index and positive surface charge were obtained. The NBs were able to load IDA with a good encapsulation efficiency and release it with a slow and prolonged in vitro release kinetics. Blank NBs demonstrated high biocompatibility, whereas IDA loaded NBs showed cytotoxicity on HUH7 cells in vitro. A higher accumulation at the tumor site of the targeted NBs was found in in vivo biodistribution studies. Moreover, IDA-NBs increased mice survival compared to free drug. In conclusion, the targeted NBs were a biocompatible delivery system with promising results for HCC treatment once loaded with a chemotherapeutic agent.

Poster Number 2	**Hannah Bargh-Dawson,** Institute of Cancer Research
Title	**Evaluation of murine head and neck carcinoma models for determining response to ultrasound-stimulated microbubbles combined with radiotherapy**

Abstract

Stereotactic radiotherapy (SRT) has shown clinical success in several cancer types; however, its use in treating head and neck cancers is limited by the risk of delivering high doses to the surrounding vital normal tissues. Vascular injury is reported to enhance tumour responses to SRT through the inhibition of DNA damage repair. As a result, vascular targeting strategies have been proposed as potential radiosensitisation agents, aiming to de-escalate the radiation dose required to provoke vascular-mediated enhancement of tumour cytotoxicity. Extensive pre-clinical research supports the use of ultrasound-stimulated microbubbles (USMB) as mechanically acting radiosensitisation agents, however, further exploration of the mechanism of action and optimisation of USMB therapy is required to fully exploit the therapeutic potential. Here, we (a) assess the suitability of pre-clinical head and neck models for use in future studies evaluating USMB as radiosensitisation agents and (b) pilot the combination of USMB and radiotherapy in vivo. Two syngeneic murine oral carcinoma models (MOC1 and MOC2) were characterised in terms of their vascularity, using multimodality imaging (dynamic contrast-enhanced ultrasound and photoacoustic imaging), and histology, and their sensitivity to a single radiation dose of 8 Gy (a dose commonly used in SRT). Both models were well vascularised at the target treatment volume and exhibited tumour growth delays of 15 and 7 days, for MOC1 and MOC2 tumours respectively in response to 8 Gy, relative to the controls. The characterisation established that both models are suitable for future investigations. In addition, preliminary tumour growth results from the pilot study combining USMB and radiotherapy were encouraging and have prompted larger, ongoing studies in which multimodality imaging, histological analysis, tumour growth and immune profiling, will be used to assess head and neck tumour responses to treatment.

Poster Number 3	**Kathryn Burr,** University of Leeds
Title	**Targeted delivery of antibiotics using proteins that bind to bacterial biofilms**

Abstract

Staphylococcus aureus is a versatile human pathogen and a significant cause of infected indwelling medical devices such as catheters and prosthetic valves. The ability of S. aureus to infect these sites is mediated by biofilm formation, a multi-layered, aggregated community of bacteria embedded within a protective matrix. Biofilms provide protection from environmental stresses, enhance tolerance to antimicrobials, influence bacteria to enter a dormant 'persister' state and cause chronic infections. Treatment failure frequently occurs for biofilm-related infections, highlighting the need for improved therapeutic strategies. Microbubbles are micron-sized gas bubbles encased by a lipid, polymer or protein shell that are traditionally used as contrast agents for ultrasound imaging, however there is interest in their use for treating bacterial infections. The aim of the BETATRON project is to target microbubbles encapsulated with an antimicrobial peptide to localise at biofilms and subsequently treat the infection using ultrasound-mediated drug release. My project addresses the first objective of the BETATRON project, to evaluate the binding interactions of an Affimer library raised against S. aureus biofilms in order to identify which Affimers would be the optimal targeting agents. Affimers are small, heat-stable proteins with antibody-like hypervariable loops conferring binding specificity. Preliminary work investigating the use of Affimers as biofilm targeting agents demonstrated significantly enhanced binding of microbubbles to S. aureus biofilms (Caudwell et al., 2022). This showed that the Affimer A-ClfA1 bound specifically to ClfA on the surface of S. aureus, a cell wall-anchored adhesin involved in the initial stages of biofilm formation. However, the remaining library of Affimers are uncharacterised. My work attempts to elucidate ligands for the remaining Affimers using a biotin-Neutravidin 'pull-down' assay to identify proteins 'pulled down' from a mixture of S. aureus cell wall protein by biotin-labelled Affimers. Subsequently, the binding interactions of potential ligands can be investigated using isothermal titration calorimetry.

Poster Number 4	**Hamidreza Hassanloo,** Brunel University London
Title	**Molecular Dynamics Simulation of Nanobubble Formation: Unraveling the Impact on Thermophysical Properties**

Abstract

Thermophysical properties of fluids, such as specific heat capacity, viscosity, density, etc., are essential for their successful application in various industrial contexts. Nanobubbles, characterized by their size smaller than 1 µm, have garnered considerable interest in a range of industrial and chemical applications that can affect the thermophysical properties of fluids and their applications. They find utility in diverse areas such as electrochemical reactions, aquaculture, water treatment, biomedical engineering, and energy/power engineering. These tiny gaseous cavities hold promise for enhancing process efficiency, enabling advanced treatments, and advancing technological innovations across multiple fields. Molecular dynamics simulation serves as a powerful tool for researchers to investigate complex phenomena that deviate from traditional continuum theories. It offers a unique approach to explore and understand intricate molecular-level dynamics, allowing for the study of systems that exhibit non-continuum behavior. This study utilized molecular dynamics (MD) simulations to investigate the dynamic behavior of nanobubbles formed by dispersing nitrogen, oxygen, and carbon dioxide gases in dodecane. The focus was on understanding the effects of these nanobubbles on the thermophysical properties of the samples. The interactions between the atoms in the liquids were described using the Condensed-Phase Optimized Molecular Potentials for Atomistic Simulation Studies (COMPASS) force field. Additionally, the behavior of the gas atoms was captured using the Lenard-Jones potential. The study's findings revealed the formation of nanobubbles by nitrogen and oxygen gases in dodecane, while no nanobubbles of carbon dioxide were observed. Furthermore, it was observed that the formation of NBs through the dispersion of gas enhances the specific heat capacity of the samples by facilitating stronger molecular collisions due to increased attractive forces. The resulting enhancement leads to improved heat storage and transfer capabilities when compared to samples lacking nanobubbles.

Poster Number 5	**Amin Jafarisojahrood**, Toronto Metropolitan University
Title	**Influence of the ion density of the medium on the shell elasticity and viscosity of lipid-coated microbubbles**

Abstract

Correct measurement of the shell properties of coated microbubbles (MBs) is essential in understanding and optimizing their response to ultrasound (US) exposure parameters in diagnostic and therapeutic ultrasound. MBs are surrounded by blood; however, the influence of the medium charges on the MB properties is poorly understood. This study aims to measure the medium charge interactions with MB shell by measuring the frequency-dependent attenuation in mediums of varying charge density. In house Lipid-coated MBs with C3F8 gas core were prepared. The MBs were isolated to a mean size of 2.35um using differential centrifugation. MBs were diluted in distilled water (DW), PBS1x and PBS10x. The frequency-dependent attenuation of the MBs solutions was measured using an aligned pair of PVDF transducers with a center frequency of 10MHz in the linear oscillation regime (7kPa pressure amplitude). The shell properties of the MBs were estimated by fitting the linear equation to experiments. Using a pendant drop tension meter, the surface tension of mm-size bubbles was measured inside DW, PBS1x and PBS10x. The frequency of the peak attenuation were 13, 7.5 and 6.25MHz in DW, PBS1x and PBS-10x, respectively. The attenuation peak increased by ~140% with increasing ion density. MBs' estimated shell elasticity decreases by 64% between DW and PBS-1x and 36% between PBS-1x and PBS-10x. Reduction in shell stiffness is in qualitative agreement with drop surface tension measurements in Fig.1d. The shell viscosity reduced by ~40% between DW and PBS-1x and by 42% between PBS-1x and PBS-10x. The reduction in the stiffness and viscosity are possibly due to the formation of a densely charged layer around the shell, further reducing the effective surface tension on the MBs. This effect may be utilized in enhancing the MB oscillations amplitude and a better understanding of the interaction of the magnetic fields with MBs during MRI-guided applications.

Poster Number 6	**Anjali Lad,** University of Leeds
Title	**Microbubbles for Tackling Antimicrobial Resistance**

Abstract

The dependence and overuse of antibiotics when treating infections have led to the proliferation of antimicrobial resistance (AMR) in society. Staphylococcus aureus (S.aureus) is a bloodstream infection that has already demonstrated resistance in the 50s and 60s to the antibiotic methicillin and penicillin. S.aureus forms biofilms a community of microbial cells that are part of an extracellular matrix, which can grow on medical devices and internal organ surfaces. Biofilms protect the bacteria making it harder for drugs to penetrate and for treatment. Alongside this, the increase in AMR has led to the need to find alternative methods and drugs to treat this type of infection. Microbubbles (MBs) are micro-sized gas bubbles commonly used as contrast agents and drug delivery vessels to treat tumours. Research has moved to using MBs alongside ultrasound, to penetrate biofilms allowing for more targeted drug delivery and destruction of biofilms. Layer-by-layer (LbL) assembly has become an increasingly popular technique that has evolved from flat surfaces to structures such as MBs. The technique involves the build-up of sequential materials on a surface through interactions including electrostatic interaction. The use of LbL assembly for drug delivery has allowed increased drug loading and easier manipulation of properties. Using a LbL assembly technique, a novel Antimicrobial Peptide (AMP), which has been shown to have adverse effects on S.aureus biofilms, will be loaded around the lipid-covered microbubble in order to be delivered to the biofilm and burst using ultrasound. However, the AMPs has been seen to be broken down by enzymes in the bloodstream before reaching the site of the infection. Therefore, it is proposed the AMP will be protected by a polyelectrolyte. This will allow multilayers to be created around the MB increasing the drug loading and then be burst with ultrasound, delivering the drug to the biofilm.

Poster Number 7	**Chloe McClenaghan,** Ulster University
Title	**Phase-shift nanoemulsions for the targeted treatment of pancreatic cancer**

Abstract

In 2018, pancreatic cancer was the 4th major cause of cancer related death in Europe. Currently, the only curative intervention for pancreatic cancer is surgical resection, however, late-stage diagnosis results in approximately 20% of patients being eligible for this procedure. Therefore, neoadjuvant chemotherapy improves survival rates by reducing tumour burden prior to surgery, increasing eligibility to undergo surgery. Phase-shift nanoemulsions (PSN's) are comprised of lipid or polymer coated nanoparticles, encapsulating a low boiling point perfluorocarbon (PFC) gas. The encapsulated PFC will induce a phase-shift, from liquid to gas, upon appropriate ultrasound (US) stimulation. Drug functionalised lipids incorporated into the PSN's formulation would enable a highly site-specific drug delivery at the tumour site whilst reducing off-target toxicities associated with conventional chemotherapy. The large inter-endothelial gaps in the tumour vasculature make nano-sized drug carriers ideal for passive tumour targeting, potentially benefitting from the enhanced permeability and retention effect (EPR). After tumour accumulation, US irradiation of the tumour initiates vaporisation of the PFC, converting the PSN to microbubbles (MB). The cavitation effects associated with the PSN to MB conversion and subsequent inertial cavitation could enhance drug dispersion within the tumour tissue. Gem PSN's comprised of a functionalised phospholipid derivative of gemcitabine (Gem) were initially prepared as liposomes. Perfluoropentane (PFP) was added, and mechanical agitation encapsulated the PFP, producing PSN's. PSN size was determined using Malvern Nano ZetaSizer. The PSN to MBs phase-shift was initiated using low-intensity US and resultant Gem-MB size and number were determined using optical microscopy and MATLAB software. Drug-loaded PSNs were successfully prepared and underwent a phase shift when exposed to appropriate ultrasound conditions. The cavitation effects associated with the PSN to MB conversion and subsequent inertial conversion could enhance drug dispersion within the tumour tissue. The next step will involve testing the PSN in animal models of pancreatic cancer.

Poster Number 8	**Carmel Moran,** University of Edinburgh
Title	**Characterisation of multimodal contrast agents for lymph node imaging**

Abstract

Contrast-enhanced magnetomotive ultrasound (CE-MMUS) aims to combine two forms of contrast agents, superparamagnetic iron oxide nanoparticles (SPIONS) and microbubbles (MBs), to improve early detection of lymph node metastasis in colorectal cancer. Simulation studies indicate SPION-MBs may yield larger tissue displacements than SPIONS alone, improving sensitivity to metastatic stiffness. We present detailed characterisation of each agent alongside tissue mimicking material (TMM) validation studies utilising a catalyst polymerising hydrogel to trap both MBs and SPIONs for detailed investigation. Target-ready (TR) MBs (MicroMarker, Fujifilm Visualsonics) with streptavidin binding sites were reconstituted (3 x 109 MBs/mL) and added to biotinylated SPIONs to create SPION-MBs. Concentration, polydispersity, mean diameter and verification of SPION loading were obtained with nanoparticle tracking analysis (NTA). Mean SPION loading per MB was further validated through titration and nuclear magnetic resonance spectroscopy (1H NMR; Agilent/Varian VNMR-S, 500MHz). Polyacrylamide (PAAm) hydrogel inclusions (θ 5 mm x 12.5 mm) of each agent (0.6mg/ml SPIONS ± 50µL of TR-MBs or SPIONS + saline), held in PVA cubes (25 mm3) were subject to alternating magnetic field (0.14T, 4 & 20 Hz) and imaged (Vevo 3100, 40 MHz). RF signals were post processed in MATLAB to recover MMUS displacement. Successful conjugation of SPIONs to MBs was confirmed when over 90% of particles had diameter 1385 nm, without free SPIONs detected. 1H NMR spectroscopy of biotinylated SPIONs added in increasing ratios to TR-MBs established T2 relaxation time to determine maximum loading capacity showing saturation at 1:3. Knowing the ratio of these two contrast agents is vital to ensure complete conjugation of all the SPIONS to the MM before fabrication and incorporation in the TMM material. PAAm hydrogel was fabricated and preliminary data shows the displacement values for the PAAm containing SPIONs-B or SPIONs-B + TR-MM. It can be noted that SPIONs-B + TR-MM display a trend towards higher displacement especially for the 20Hz case, though further investigation is required for optimisation and progression to preclinical work.

Recommended Restaurants and Pubs:

City Centre:

Bundobust – Vegetarian Indian Street Food £
https://bundobust.com/locations/leeds/

Rudy's Pizzeria – Neapolitan Style Pizza £
https://www.rudyspizza.co.uk/pizzerias/newstationst

Sarto – Fresh, handmade pasta ££
https://sartopasta.uk/

Phranakhon – Thai tapas ££
http://phranakhon.co.uk/phranakhon-leeds/

Tapped – Pizza and micro-brewery £
https://tappedleeds.co.uk/

Nation of Shopkeepers – Restaurant and bar serving pizza, burgers etc. £
https://www.anationofshopkeepers.com/#/

Ambiente – Spanish Tapas ££
https://www.ambiente-tapas.co.uk/leeds

Tharavadu – Keralan/Indian restaurant ££
https://www.tharavadurestaurants.com/

Zaap Thai – Thai Restaurant/Bar £
https://zaapthai.co.uk/locations/leeds/

Crowd of Favours – Classic Pub Food £
https://crowdoffavours.co.uk/

Belgrave Music Hall – Bar/Pub with roof terrace, serving Pizza and Burgers £
https://www.belgravemusichall.com/

Tattu – Contemporary Chinese Cuisine £££
https://tattu.co.uk/locations/leeds-chinese-restaurant/

North Brew Co Tap Room – Craft beers and Bao Buns £
https://info.northbrewing.com/venue/sovereign-street/

The Brunswick – Pub serving burgers and fried chicken £
https://www.thebrunswick.co.uk/

The Head of Steam – Classic Pub Food £
https://www.theheadofsteam.co.uk/bars/leeds-park-row

Headingley

Zaap Thai - Thai Restaurant/Bar £
https://zaapthai.co.uk/locations/headingley/

Rudy's Pizzeria – Neapolitan Style Pizza £
https://www.rudyspizza.co.uk/pizzerias/headingley

De Baga – Traditional Indian Cuisine ££
https://www.debaga.co.uk/

Fat Hippo – Independent Burger Restaurant £
https://fathippo.co.uk/locations/headingley-leeds/

Kuala Lumpur Café – Malaysian Cuisine £
https://www.klcafeleeds.co.uk/

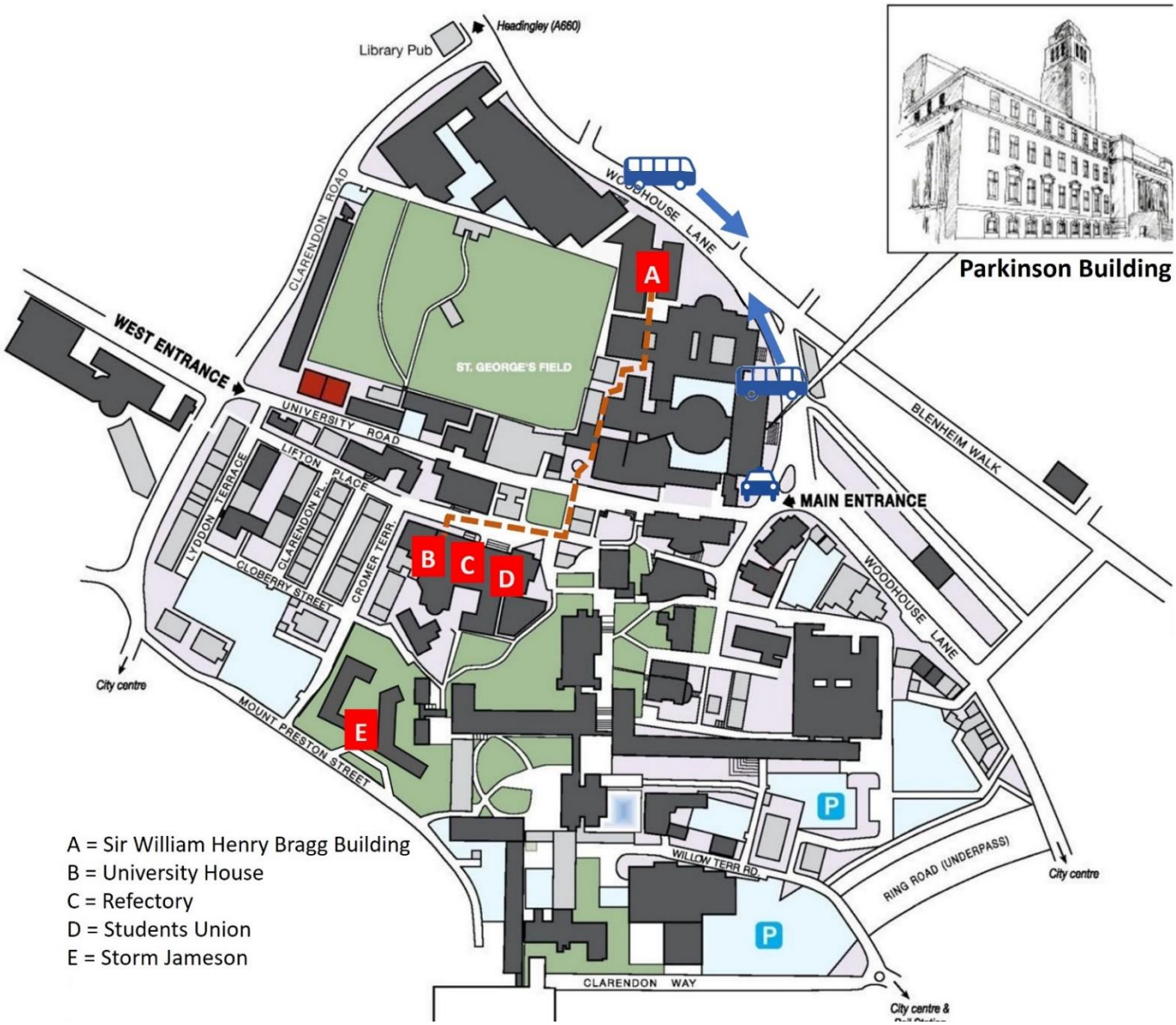
Signature – Mediterranean Restaurant ££
https://signaturebarandrestaurant.com/

Cat's Pyjamas – Vibrant Indian Street Food £
https://catspjs.co.uk/

Santorini Bar and Grill – Greek and Turkish Cuisine ££
https://santorinileeds.co.uk/

No. 14 North Lane – Contemporary British Cuisine £££
https://no14northlane.co.uk/

Map of University of Leeds:



Breakfast (8-9am) is available in Refectory (C) – for those staying in Storm Jameson on room only rate.

Bars in Students Union (D) – Old Bar & Terrace Bar – open until 10pm

Taxis ( ) are available from front of Parkinson Building

Buses ( ) are available from front of Parkinson Building to Headingley (1, 1B, 6, 8, 27, 28) and Weetwood Hall (1, 6, 8, 28) or from opposite Bragg building into Leeds City Centre.

Walking – City Centre is 10-15 minutes, Headingley is 25-30 minutes.