Euroson 2005

Geneva 25-27 September

USWNetwork Workshop

12:15-14:00 27 September 2005

Ultrasonic Standing Wave (USW) technology and Life Science applications

Geneva Pelexpo Congress and Exhibition Centre
Route François-Peyrot 30
1218 Grand Saconnex- Geneva- Switzerland

Room A
Wine and Cheese provided

Preliminary Programme -

12:20-12:40	Terence Coakley & Despina Bazou (Cardiff University, Wales, UK) Particle and cell manipulation in USW traps.
12:45-13:05	Martin Wiklund (Royal Institute, Sweden) TBA
13:05 – 13:25	Martyn Hill (Southampton University, England, UK) Manipulation of cells in USW
13:30- 14:00	Short presentations (3 min presentation + 2min discussion) **Abstract call now open.**

Further details:
Euroson 2005 conference
http://www.euroson2005.org/default.htm
USW Network workshop: **Deadline for abstract submissions: Friday 15th August 2005 Email you…**

http://www.ucl.ac.uk/medicine/hepatology-rf/research/usw-net/

EPSRC Engineering and Physical Sciences Research Council

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Manipulation of Cells in Ultrasonic Standing Waves

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Ultrasonic Standing Waves (USWs) exert radiation forces on particles or second phase fluids within the standing wave. Although these forces are small they offer a useful tool for manipulating micron scale particles of near neutral buoyancy, and have attracted particular interest for handling cells. Resonators designed to establish sub-wavelength standing waves in the low MHz frequency range are of a size that offers the potential for use within micro total analysis systems (μTAS), or "Lab on a Chip" devices.

This presentation discusses the characteristics of radiation forces, including axial, lateral, and inter-particle effects. The characteristics of resonators used to generate the standing waves are also discussed paying particular attention to half and quarter wave sub-wavelength systems. Several ways in which the forces have been used to manipulate cells are outlined, including:

- filtration, based on either cell agglomeration and sedimentation or using flow-through techniques
- trapping cells or cell agglomerates and holding them against a flow.
- forcing cells against surfaces, enabling enhancement of biosensor sensitivity.
- cell fractionation based on differential force fields.

Particle and Cell Manipulation in an Ultrasound Standing Wave Trap

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Cells or micro particles in non-cavitating ca. 1 MHz ultrasound standing wave field are driven by radiation force to a pressure node plane and then, in an appropriately designed field, concentrated within that plane to form a 2-d aggregate within 1 min. The aggregation process can be observed and video recorded microscopically through the glass reflector of the standing wave resonator. The morphology of latex bead aggregates thus formed can be experimentally modulated from hexagonally-ordered closely packed aggregates to increasingly dendritic forms by increasing the ionic strength of the suspending phase. This short-range (nm scale) electrostatic control of aggregate form is consistent with classical colloid aggregation theory. The inter-particle acoustic forces are small compared to electrostatic repulsion as particle separation decreases to the 10s of nm level that is typical of the range of molecular adhesive receptors on animal cell surfaces.

Rapid molecular responses of cells to contact in the non-intrusive ultrasound trap has been shown for surface receptors cadherin and NCAM, for intracellular F-actin and the gap junction protein Cx43. Immuno-fluorescence labelling of these components showed adherens junction formation within 30 min and functional gap junction communication within 60 min for a cultured neural cell line and for freshly isolated primary chondrocytes respectively. It was shown, in a protein-synthesis pulse inhibition experiment, that the protein synthesis required for gap junction formation occurred in the trapped chondrocytes. The overall results show that the trap functions only to facilitate, not drive, surface receptor interaction and that intracellular processes normally triggered by such interaction proceed also in the otherwise passive sound trap.

Ultrasonic manipulation in microsystems for cell handling and biochemical analysis

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Ultrasonic standing-wave (USW) radiation forces can be used for particle manipulation, separation and enrichment. We have developed two different miniaturized USW systems, a microtiter plate USW system and a lab-on-chip USW system. The goal is to use the devices for ultrasensitive biochemical analysis and for gentle handling of single cells in microchannels.

In the first application, an ultrasonic transducer is combined with a commersially available 96-well microtiter plate for particle enrichment in bulk samples[1]. The device is tailor-made for the imaging properties of a confocal microscope, resulting in two-dimensional particle rearrangement matching the confocal laser-scanning plane. The system is developed for a separation-free immunoassay based on antibody-coated beads and fluorophore-labelled tracer antibodies.

In the second application, a flow-through lab-on-chip system is developed with the aim to allow manipulation of single cells and single particles. Flexible manipulation of both single cells as well as of large cell groups is possible by combining USW manipulation with dielectrophoretic manipulation. Such manipulation is important for cell programming by surface imprinting of cells by, e.g., particles with individually tailored macromolecular landscapes on their surfaces[2]. We will present preliminary experimental results and videosequences.

[1] M. Wiklund, J. Toivonen, M. Tirri, P. Hänninen and H. M. Hertz, J. Appl. Phys. **96**, 1242 (2004).

[2] More information available at http://www.cellprom.net.

Ultrasonic Particle Manipulation using Plate Vibration Excitation

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The contactless movement of micro-particles and cells to known locations within a fluid volume is of interest in the fields of microtechnology and life sciences. A device which can position such inhomogeneities suspended in a fluid at multiple locations is described and modelled. The device consists of a thin fluid layer contained between two surfaces (e.g. glass plates or in a channel etched into a silicon wafer). One of the surfaces is vibrating, excited e.g. by piezoelectric elements. The result is a pressure field throughout the fluidic volume. When an inhomogeneity in a fluid is exposed to an ultrasonic field the acoustic radiation force results; this is found by integrating the pressure, retaining the second order terms, over the surface of the field and taking the time average. Thus, due to the presence of a pressure field in the fluid in which the particles are suspended, a force field is created. The particles are then collected at the locations of the force potential minima. In the device described here, the force field is used to position particles (e.g. polymer spheres, cells, etc.) into lines or at points. The locations of the particles are predicted by using a finite element model of the system. The experimental and modelling results, presented here, can be seen to compare well.

Keywords: particle, resonance, positioning, non-linear, finite element

A resonant tubular Ultrasound system for cell disruption

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Ultrasonic microbial cell disrupters operating at around 20 kHz are often physically large and, due to significant heating, are unsuitable for small sample volumes where biochemical integrity of the extracted product is required. Development of an ultrasonic cell disruptor based on a 63.5 mm diameter; 6.5 mm thick tubular transducer with a half wavelength steel insert is described here.

The aim was to create a compact ultrasonic system for rapid cell disruption in small volume samples in a high intensity acoustic cavitation field with minimal temperature rises. A 1-Dimensional (1-D) transfer matrix model designed for planar piezoelectric standing wave resonators was applied to an axial cross section of the tubular device to predict frequencies of mechanical resonance in the sample volume associated with maximum acoustic pressure. Admittance measurements identified frequencies of electrical resonance. Saccharomyces cerevisiae breakage efficiency was twice as great, in terms of protein released per dissipated Watt, at the mechanical resonance predicted by the model compared to those at the electrical resonance frequencies. The results form a basis for rational design of a compact tubular cell disruptor using numerical modelling prior to construction.

The efficiently cooled, compact cell disruptor was employed to treat bacterial endospores to increase detectable antigens in suspension for direct enzyme-linked immunosorbent assay (ELISA) measurement. Bacillus subtilis var.niger (BG) spores were used as a stimulant for Bacillus anthracis. Sonication for 30 s was sufficient to increase ELISA detection sensitivity 20-fold. Analysis of sonicated spores revealed release of antigen into suspension from the spore coat, which could be detected using immobilised antibody on the Resonant Mirror biosensor, an evanescent optical sensor.

USW Network Workshop, Euroson 2005. 27th September 2005. Room A

About the USW Network

http://www.ucl.ac.uk/medicine/hepatology-rf/research/usw-net/

The USW Network has five major aims:

- To establish research collaborations in Ultrasonic standing wave technology that will lead to novel advances in Life Sciences.
- To disseminate information on properties and performances of USW fields, for exploitation in a wide variety of applications.
- To involve industrial partners for production of devices created for existing USW and offshoot applications.
- To bring together Life Sciences interests in USW to create a truly multidisciplinary field.
- To inform powerful research grant applications leading to further development of the technology, and its transfer to solve problems in Biology.

Contact the USW Network

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