

**Innovations in Encapsulation
RSC Burlington House
12th December 2014**

Microencapsulation using Microfluidics Processes

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www.epigem.co.uk

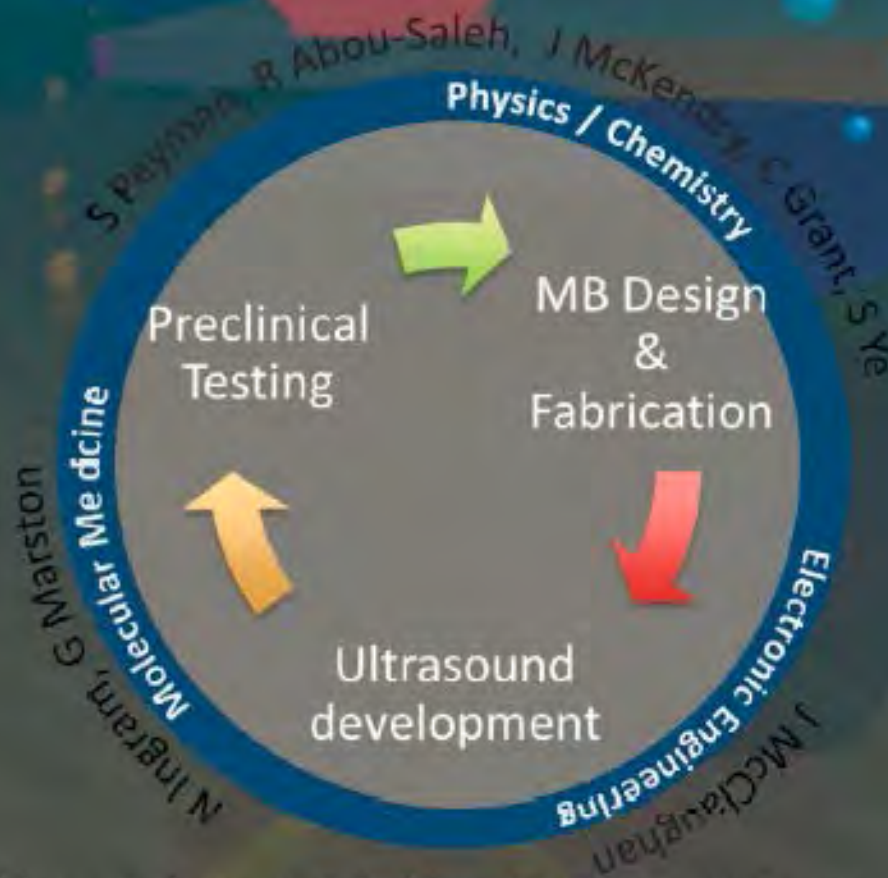


Engineering Theranostic Microbubbles

MB fabrication and
Characterisation
Drug loading

MB response to US
Sonoporation
US aided release

MB targeting
Drug release
PK/PD response



RJ Bushby, L Coletta, K Critchley, SD Evans, JA Evans, S Freear,
B Johnson, P Jones, AF Markham, N Thomson

University of Leeds 2014



Outline

- Microfluidic processes for making phospholipid, microencapsulated, ultrasound contrast agents (gas) for improved imaging of cancers and targeted delivery of drugs
 - Lessons learnt from nature and from microfluidics product development
 - Microbubble generation
 - External transport of liposomes containing drugs and internal transport of oil soluble drugs
 - Ultrasound imaging and targeted release
 - Molecular Targeting
- Summary of “Engineering Theranostic Microbubbles” achievements

Size Considerations

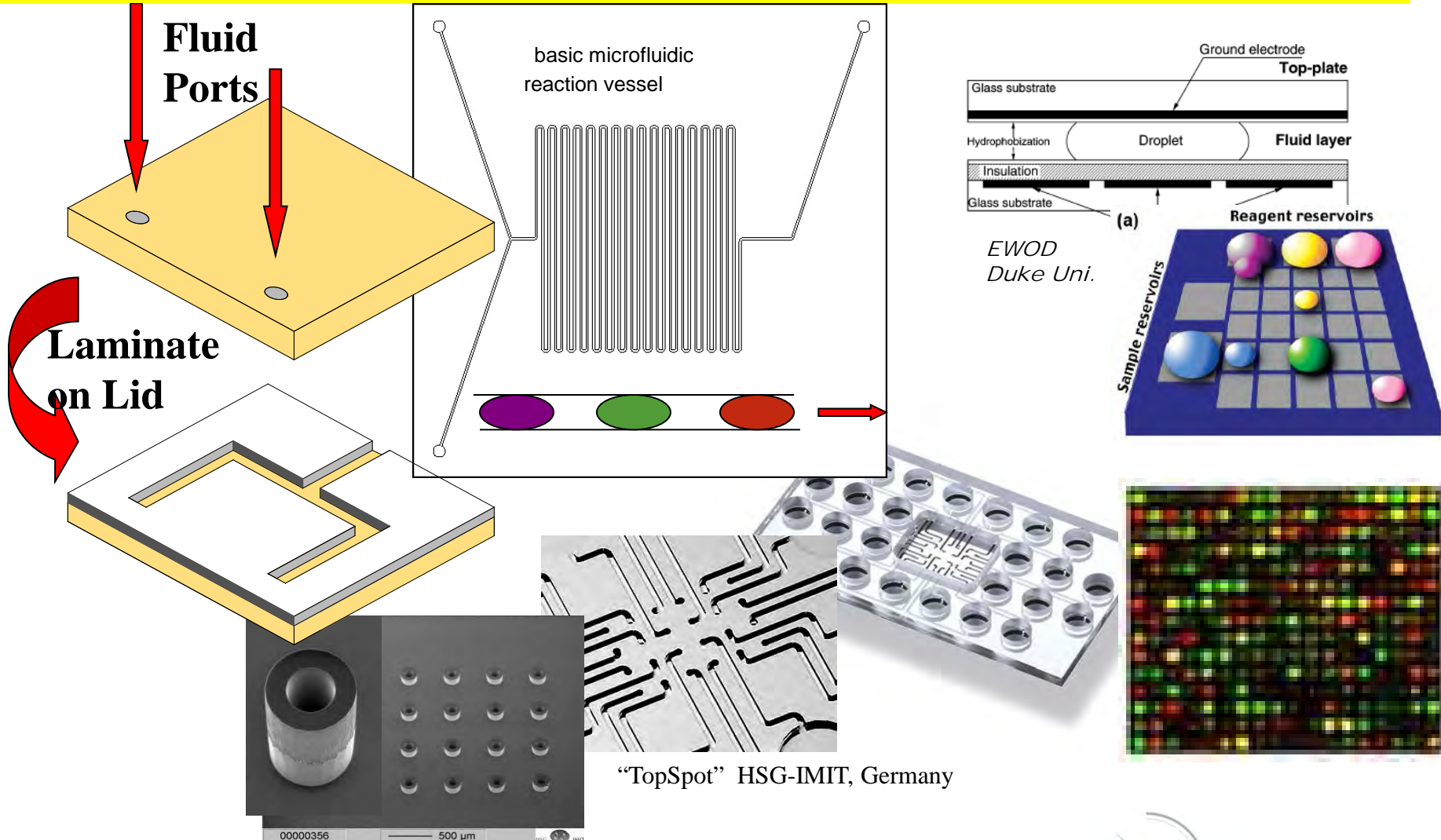
Length (Metres)	Unit	Volume	Reactor	Diffusion Time (secs)	
1	Metre	1000 l	Plant	10^9	
10^{-1}		1 l	Lab	10^7	Chaotic
10^{-2}	cm	1 ml		10^5	Turbulent
10^{-3}	mm	1 μ l	Assay	10^3	
10^{-4}		1 nl		10	Laminar
10^{-5}		1 pl	Cell	10^{-1}	Compartmental
10^{-6}	μ m	1 fl	Organelle	10^{-3}	Self-Organised
10^{-7}			Enzyme		Heisenberg
10^{-8}					
10^{-9}	nm		Molecule		
10^{-10}	Å		Bond		

Blood drop

Ink jet drop

Microfluidic basics – channels, nozzles, wells, arrays

Vertical & Horizontal interconnection in multiple layer boards & film laminates

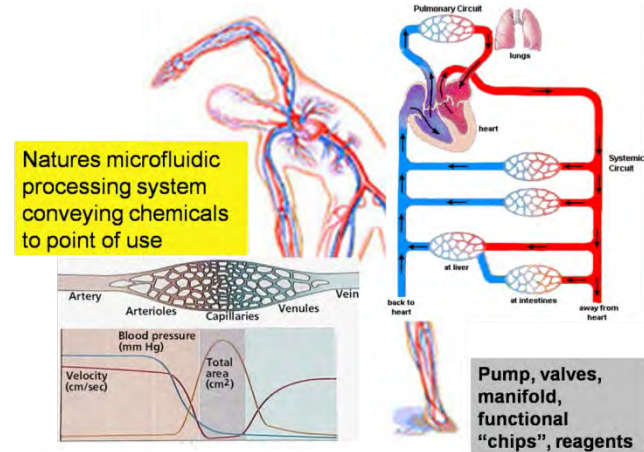
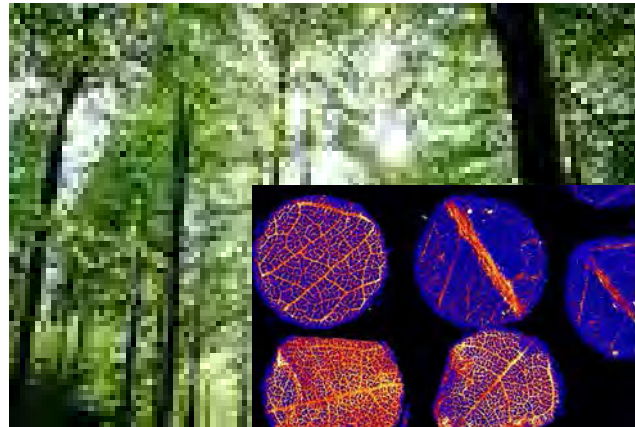


microfluidics e.g. for food (or the clinic etc)

“food fights back”

“indigestible food”

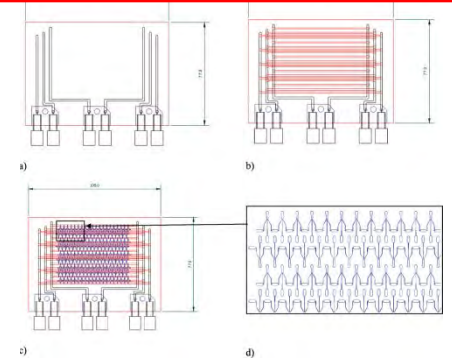
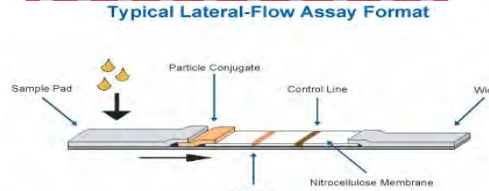
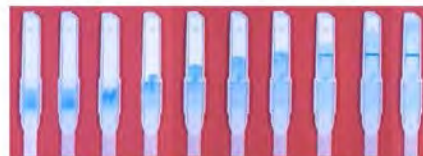
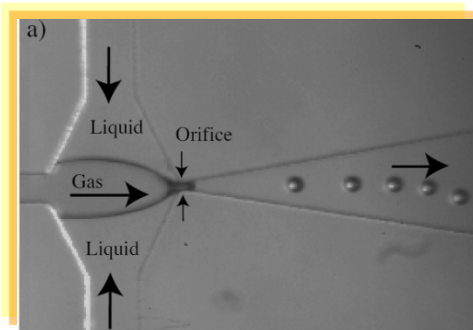
“food nutrition / infection”



- Ink jet print head
- High throughput droplet generation

Capillary flow with extra push & pull pumping

The Patient / Consumer - manifolded array of microfluidic devices



There is a view regarding Microfluidics:

Q. “not good for making large quantities & channels / nozzles block”

A. depends on the purpose and precautions taken

Blocking or embolism

- Human life requires 5 litres of blood continuously processed for 80 yrs – blockage by gas bubbles, fouling and particles can occur.
 - Note the elegance of the kidney for QC
- In the case of gases it is sensible to keep volumes to less than a millilitre and the particle size to 1-10 μm or less than the pipe OD typically 100 μm s

Throughput (as nature does it)

- Decentralised synthesis & formulation - uses large arrays of fast synthesis microreactors - manifolded together using even faster self assembled / self repairing microfluidic architectures: purpose is highly specific function delivery using “particles” (packaged molecules)

Helping nature using Theranostic Microbubbles produced using similar microfluidic processes seems a better solution than current methods – lower volumes by targeting - controlled bubble size

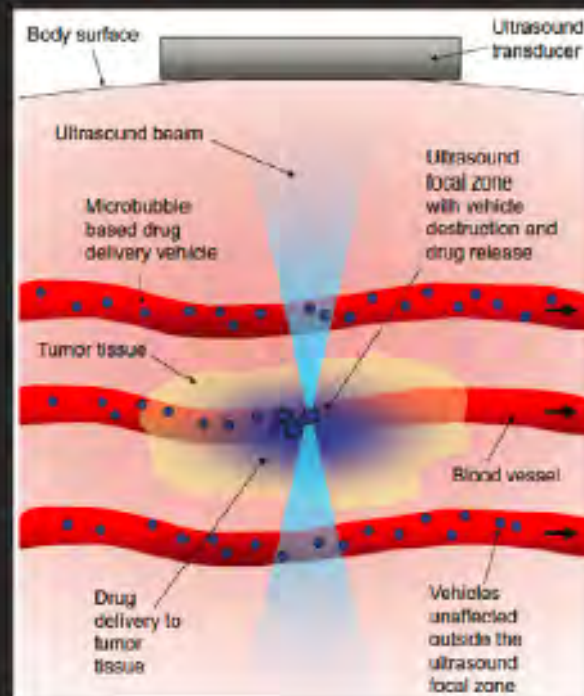
Theranostic Microbubbles

Diagnostic microbubbles



VISUALSONICS

Therapeutic Capability



Ibsen et al
Drug Design, Development
and Therapy 2013, 7, 375-388

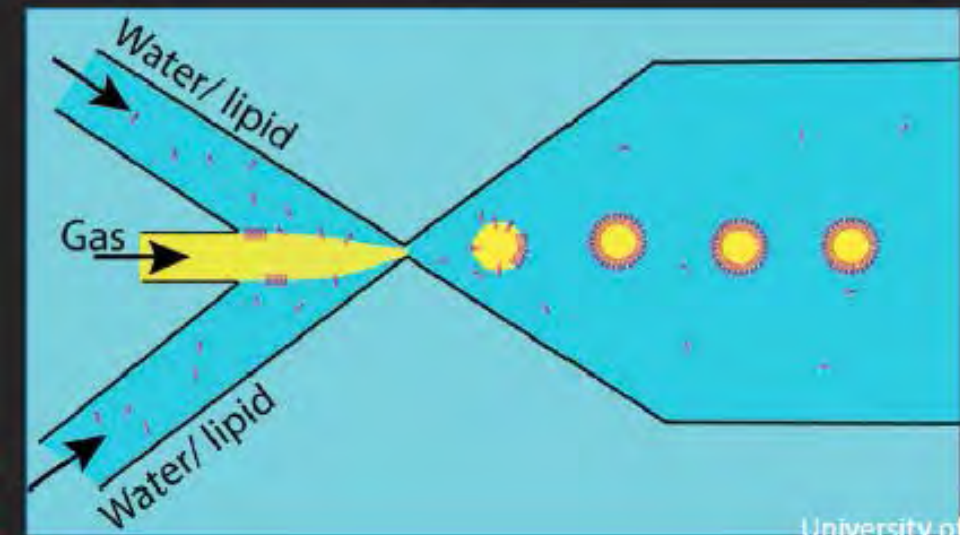
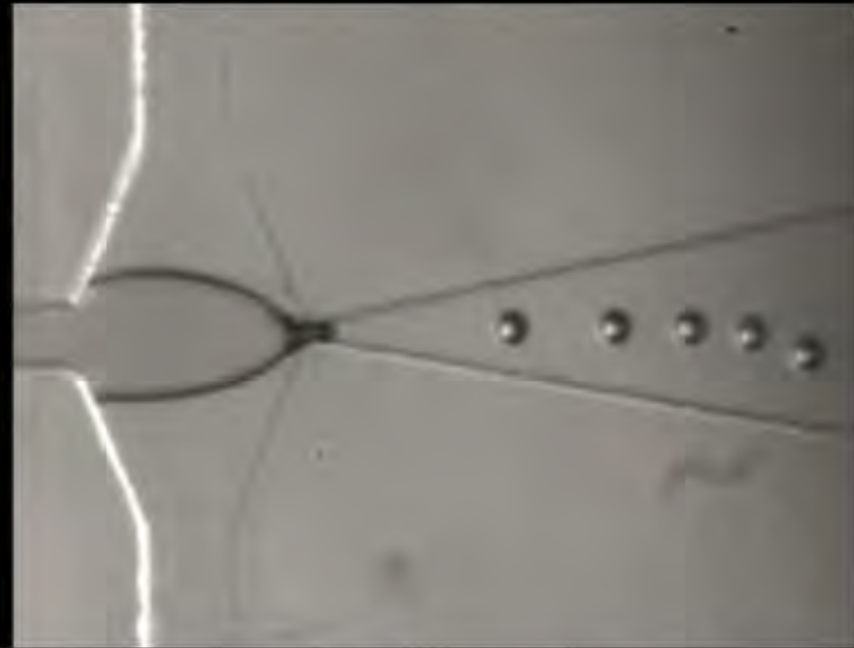
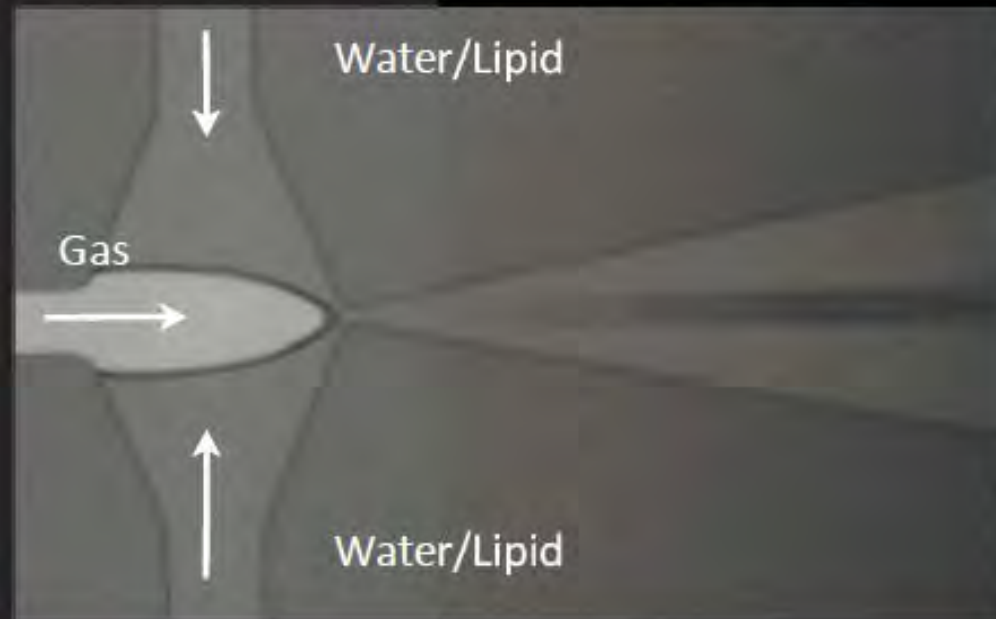
Reflection

Soft tissue- S.tissue	1-2%
Soft tissue - bone	50%
Soft tissue - gas	99%

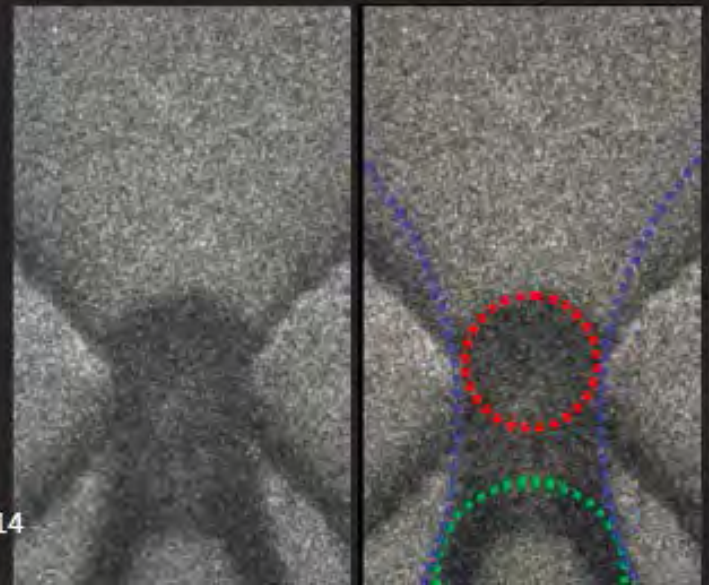
2-8 μm MB allows safe navigation within vessels and capillaries.

Ultrasound properties can control the release of therapeutic drugs – carried on MBs

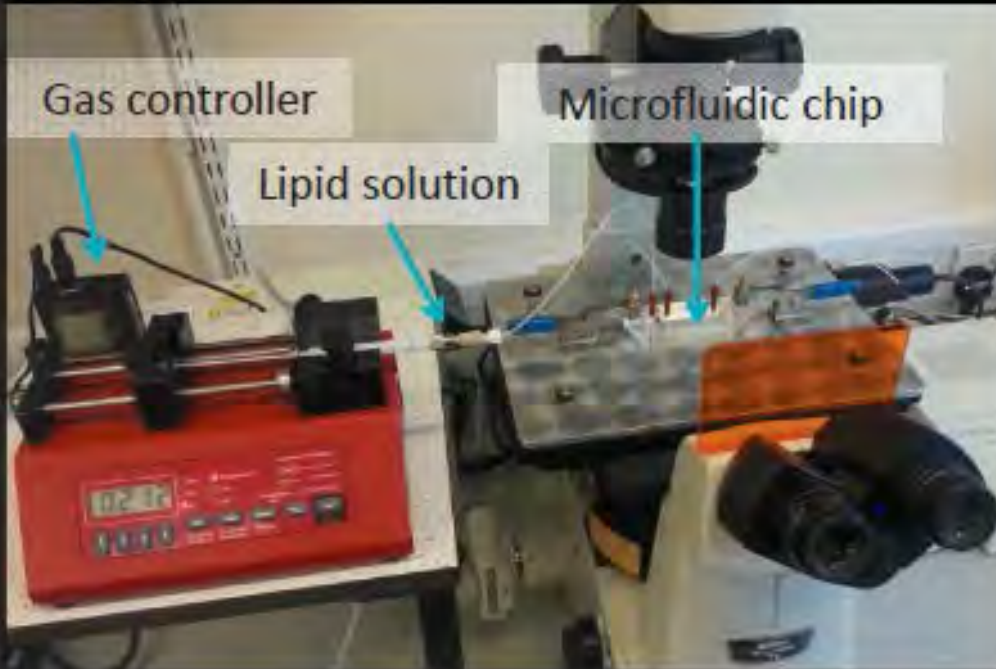
Microfluidics



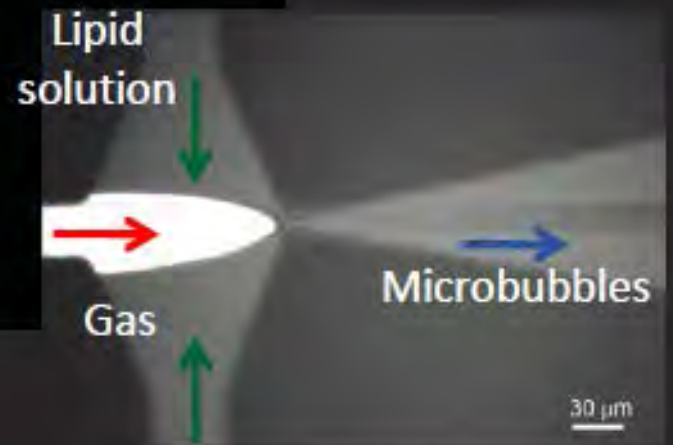
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Microfluidic Rig



A quad-channel microfluidic chip



'Nozzle' inside microfluidic chip

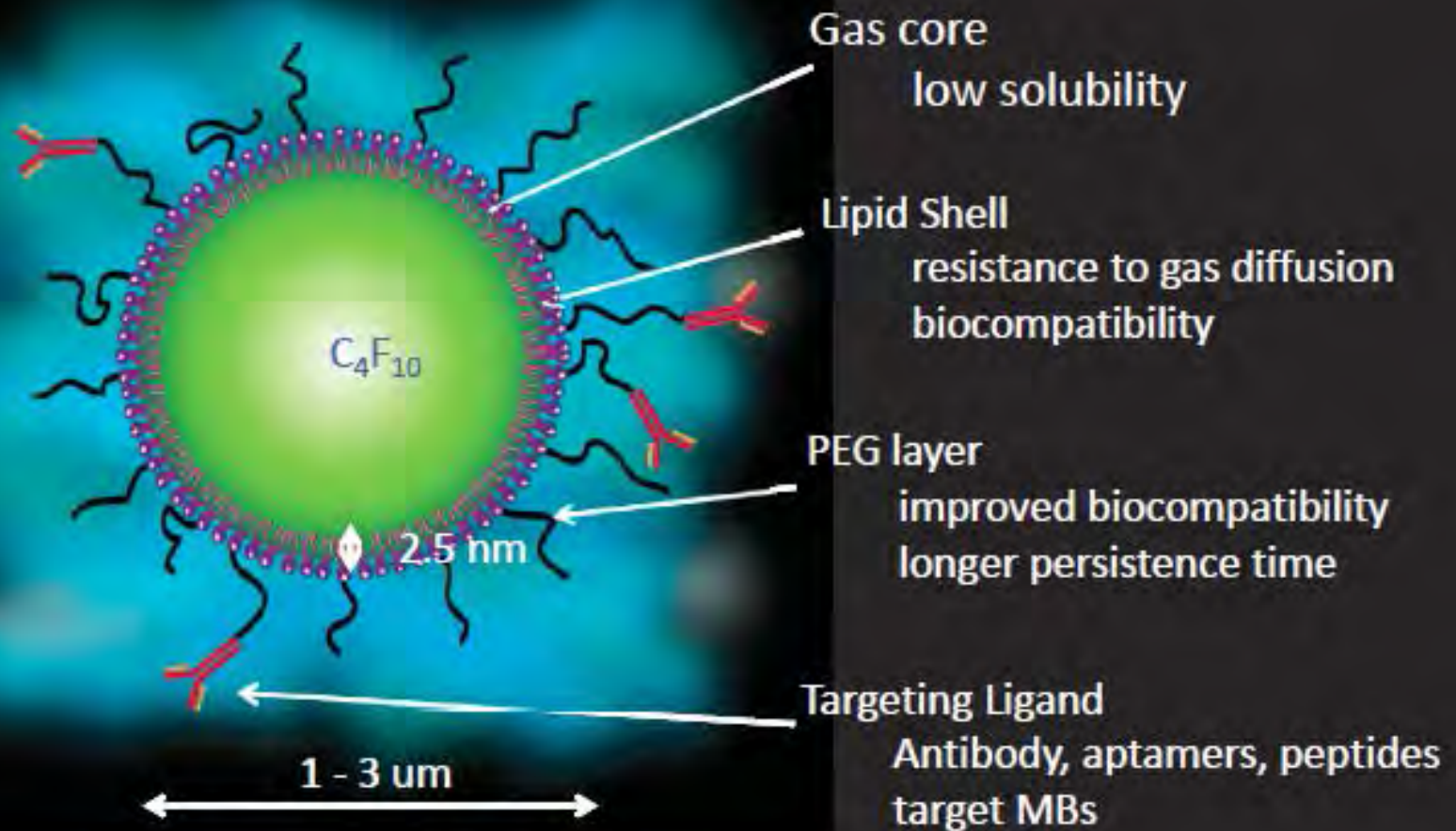
Plug n play chips

Easy to configure

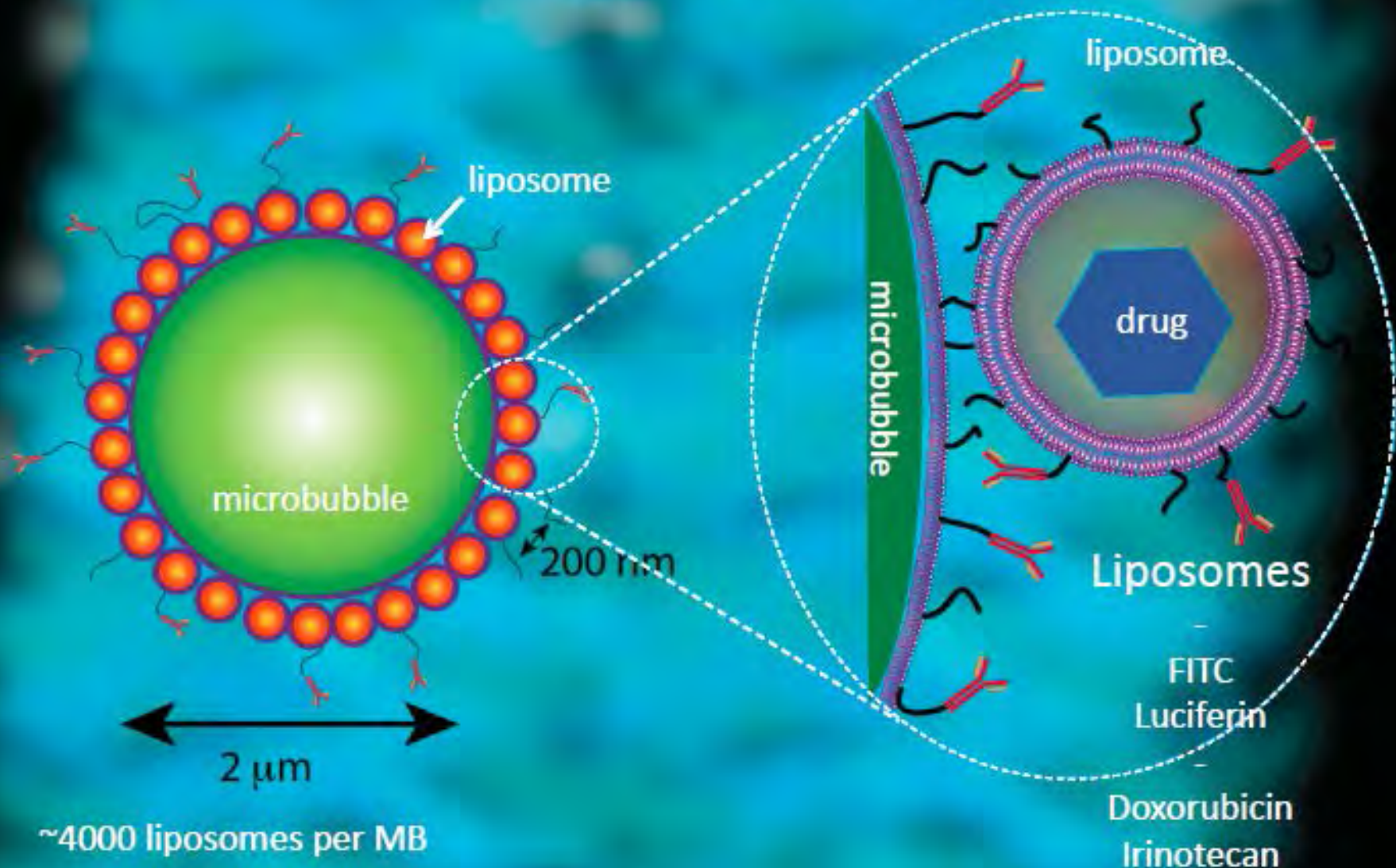
Reproducible



Construction of Diagnostic Microbubble



Drug Loading

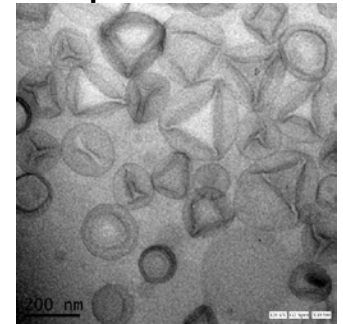


Therapeutic Payload

- Liposomes are generated by extrusion through a 200 nm membrane.

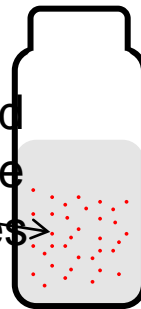
Payload	Mean Diameter (nm)
Empty	204 ± 1.3
Doxorubicin	182 ± 2.8
Luciferin	194 ± 2.5
Irinotecan	198 ± 4.6

TEM of Irinotecan Liposomes



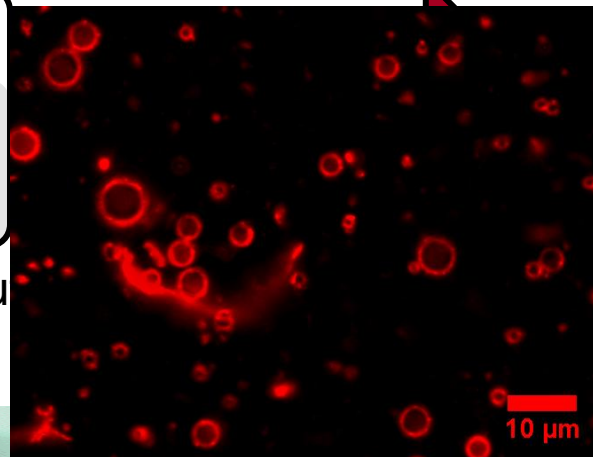
A 'one-pot' approach to therapeutic microbubble production

Fluorescent images showing liposomes and antibodies bound to the outside of liposomes

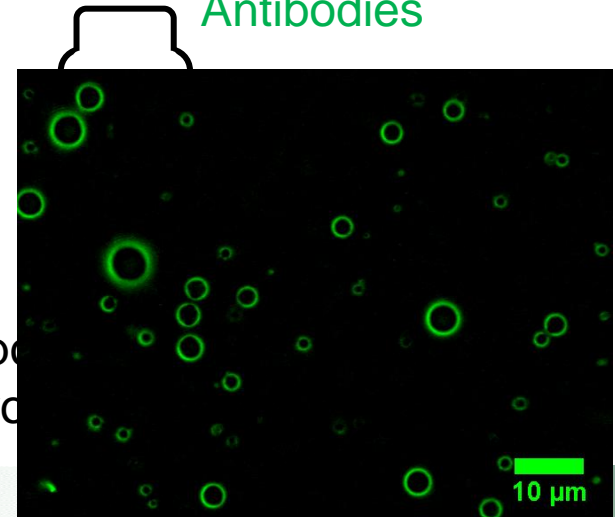


Lipid solution

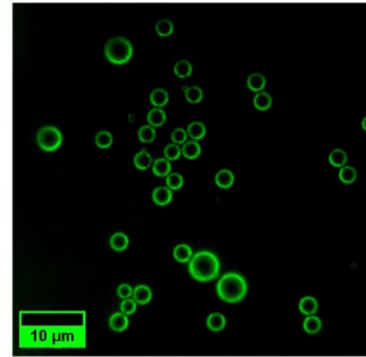
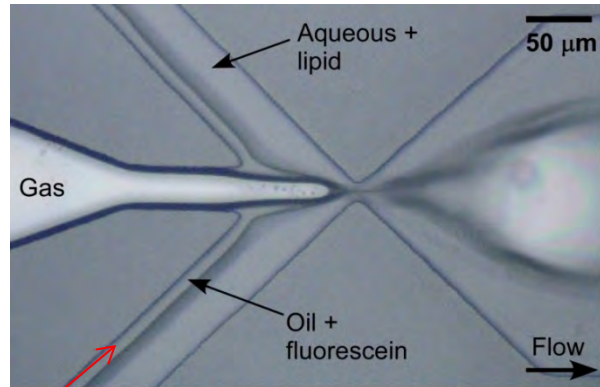
Liposomes



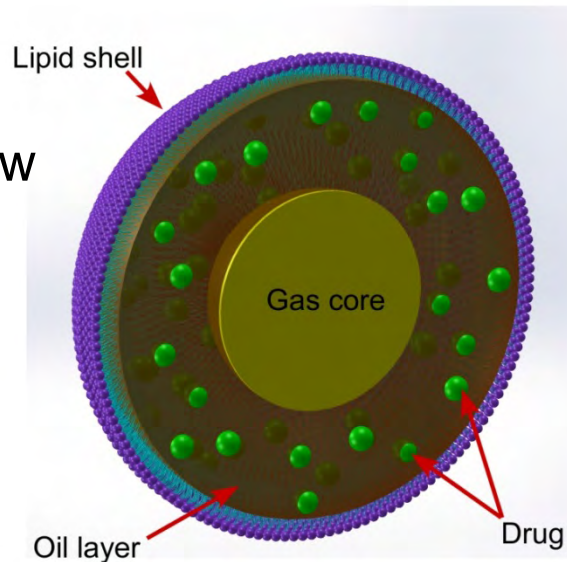
Antibodies



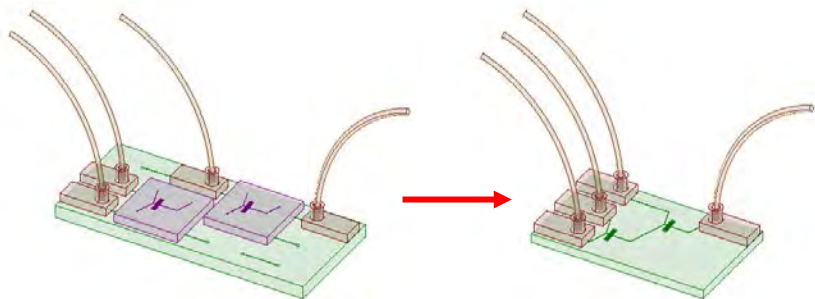
Encapsulation of oil soluble ingredients (flavours, nutraceuticals, drugs etc) within microbubble using oil layer



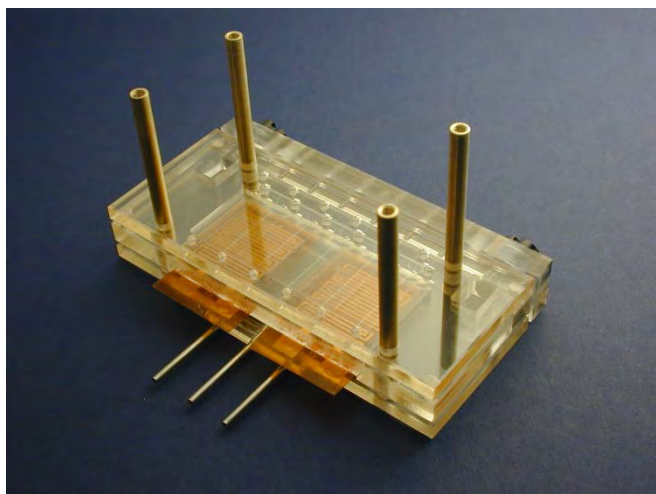
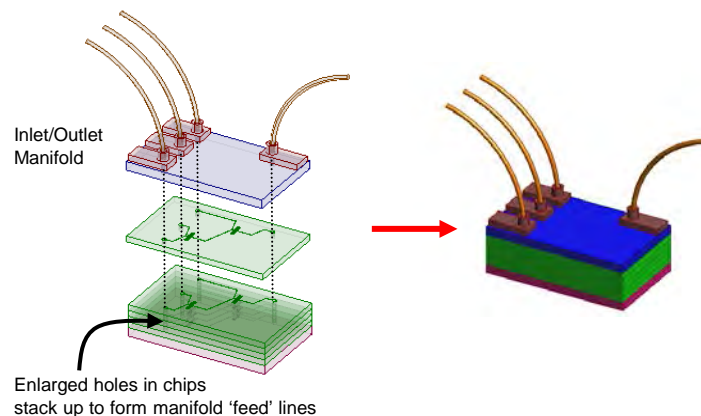
Note: use of laminar flow



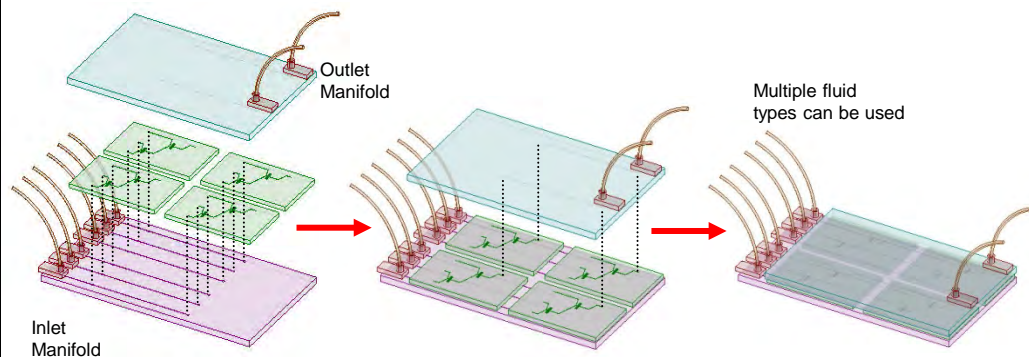
Modular systems including for high throughput applications



3D Stacking



In-Plane Tiling

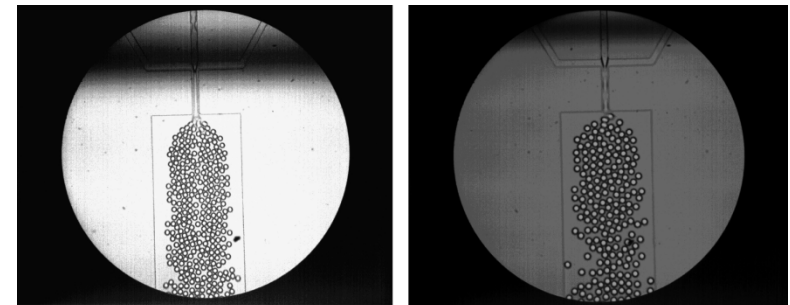
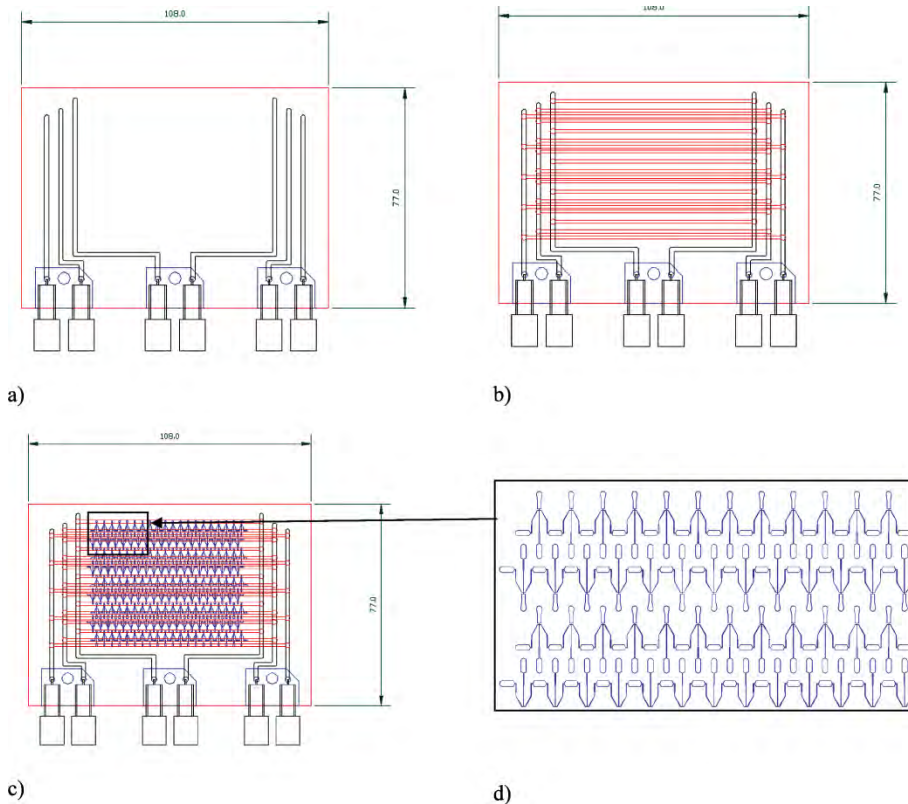


Increasing throughput

Case history: Parallel integration of monodisperse droplet generators
(scale up by scale out)

180 devices in a ladder network layout

- Droplet formation rate:
 - >100Hz, 8000 $\mu\text{l}/\text{min}$, 21 μm (CV,5%)
 - monodisperse w/o water in oil
 - applications: drug delivery and consumer goods

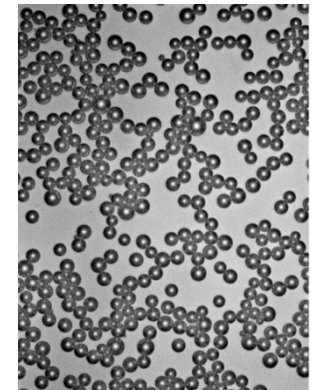
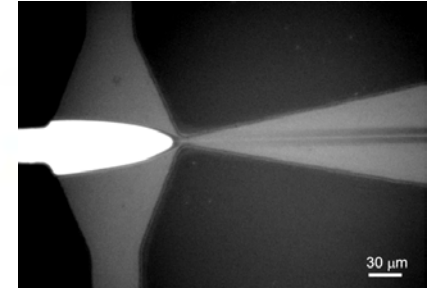
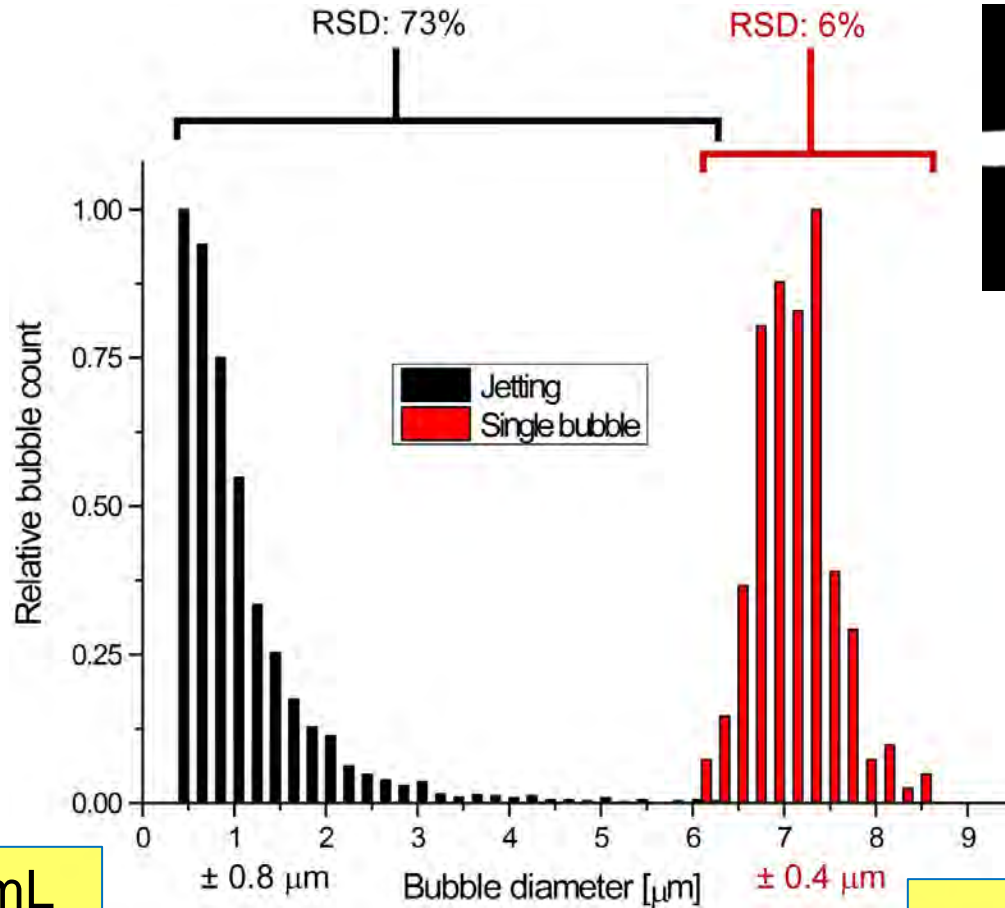
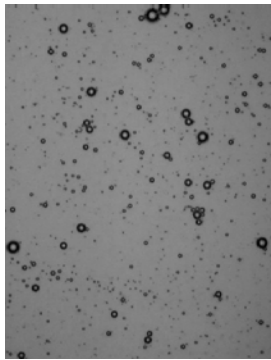
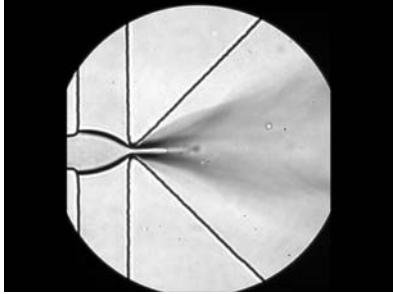


-a.

b.

Georgios Tetradis-Meris et al , *Ind. Eng. Chem. Res.*, **2009**, 48 (19), pp 8881–8889

Microspray vs Monodisperse generation

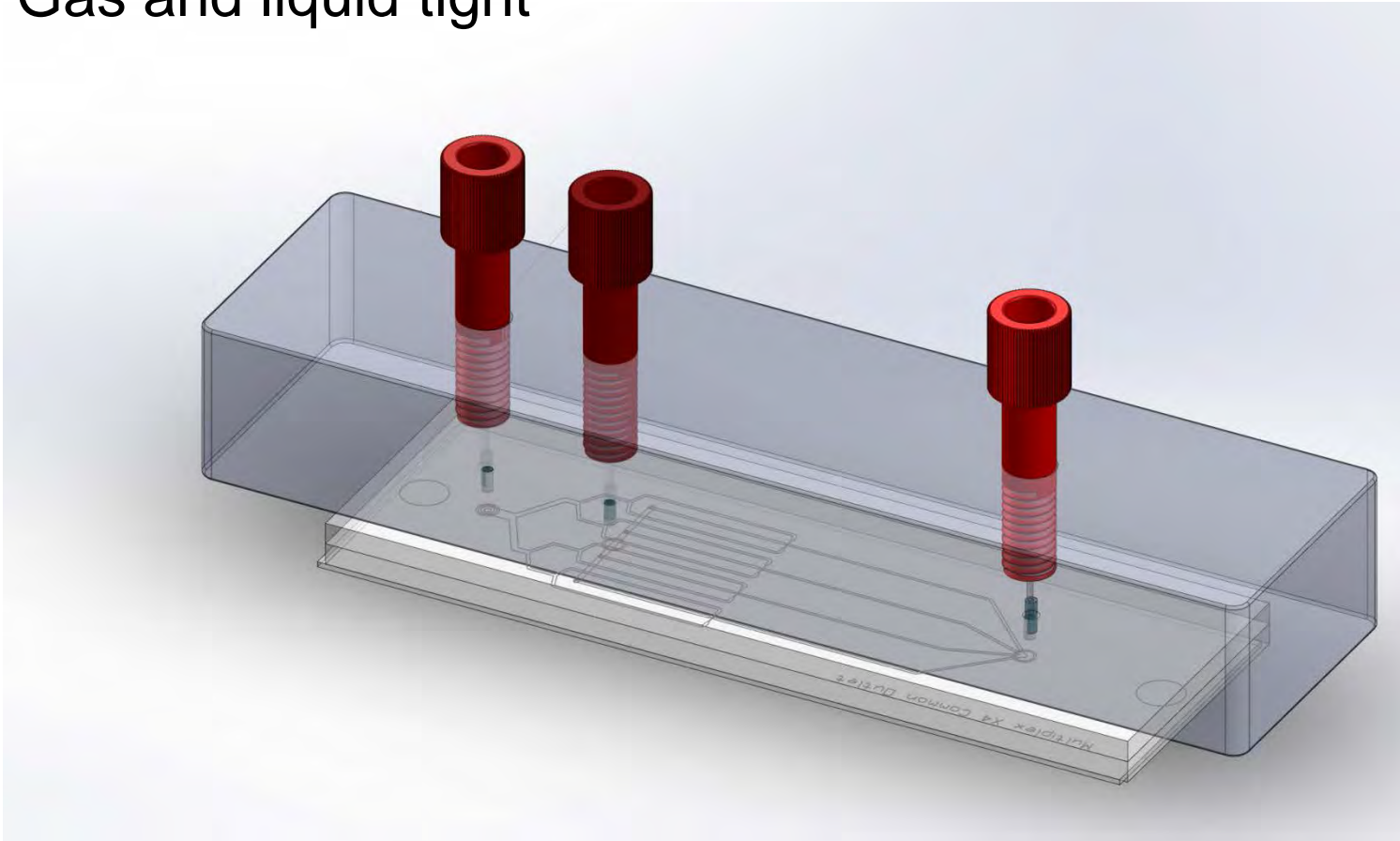


- $10^8 - 10^9$ MB /mL
- 6 – 10 min prep time

- $10^6 - 10^7$ MB /mL
- ~ 60 min prep time

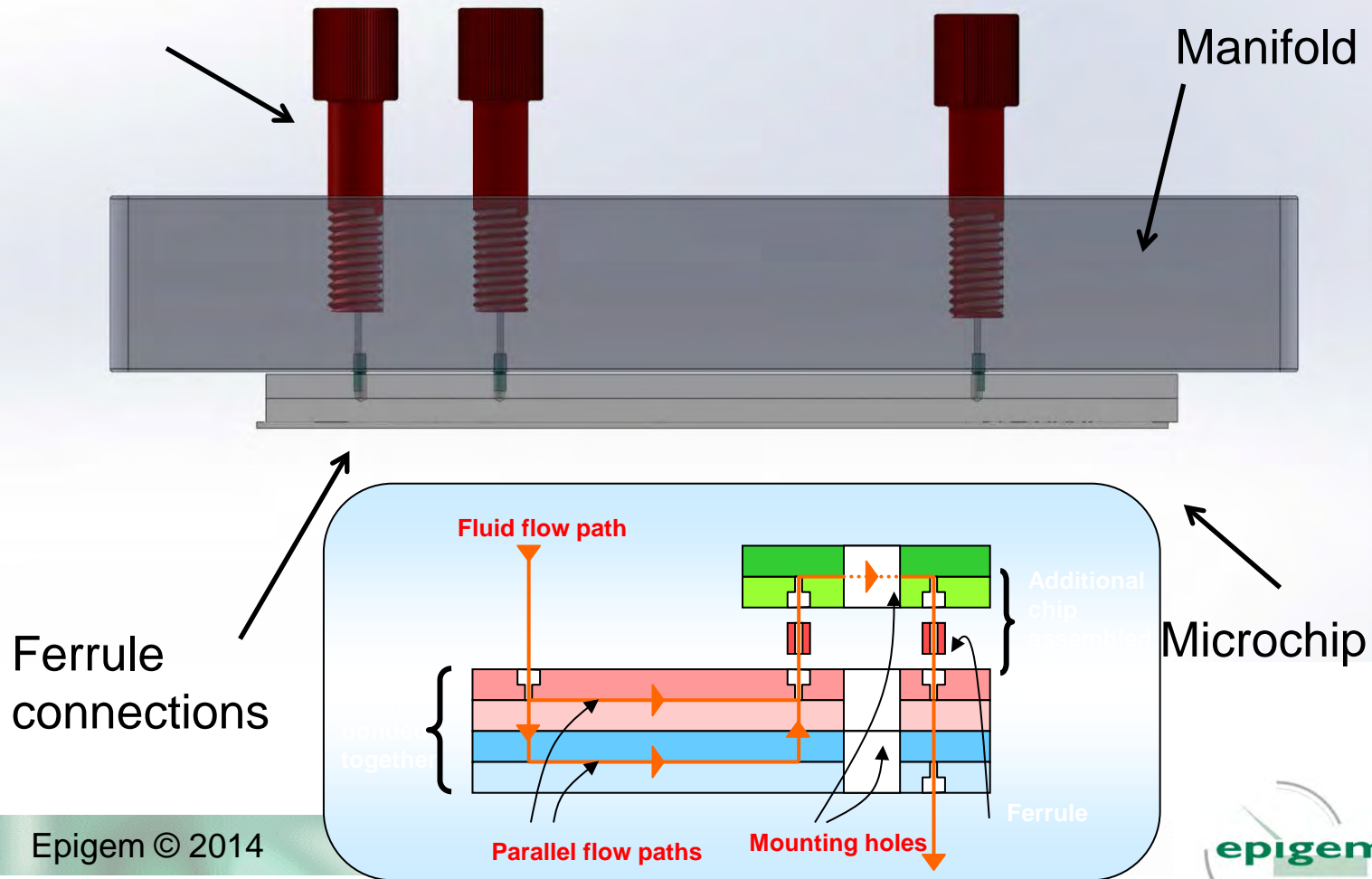
The Microbubble Generator

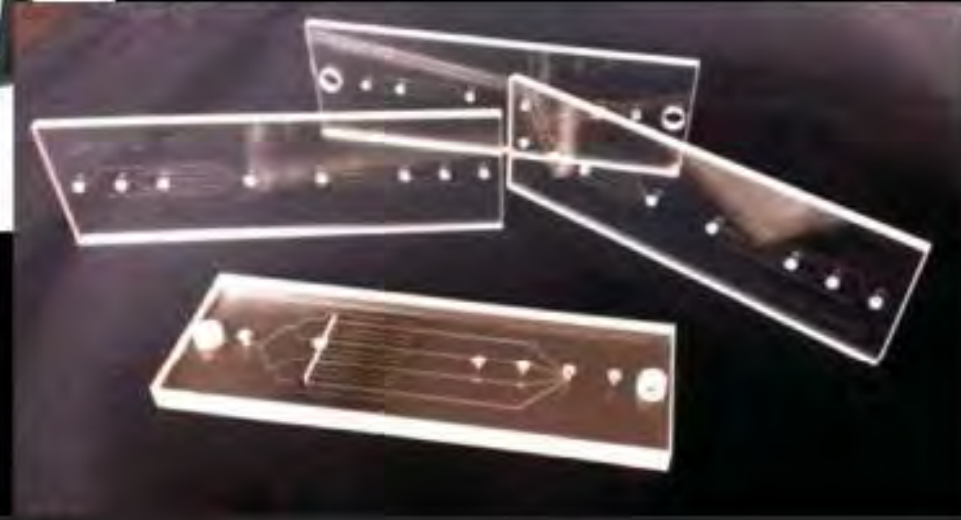
- Chip inserted and assembled for use in < 30 seconds
- Gas and liquid tight



The Microbubble Generator

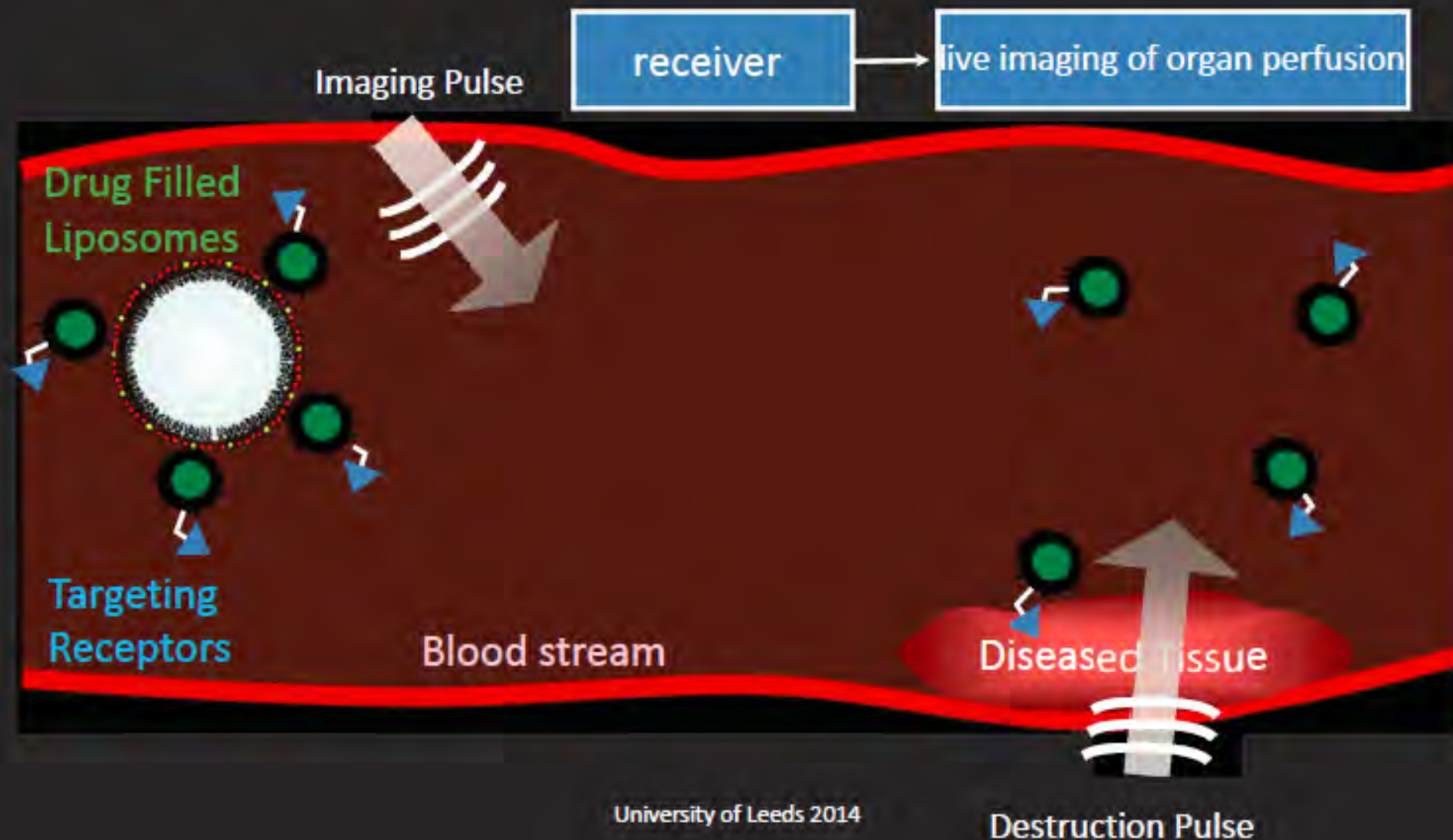
Fluidic interfacing for 'plug and play' operation





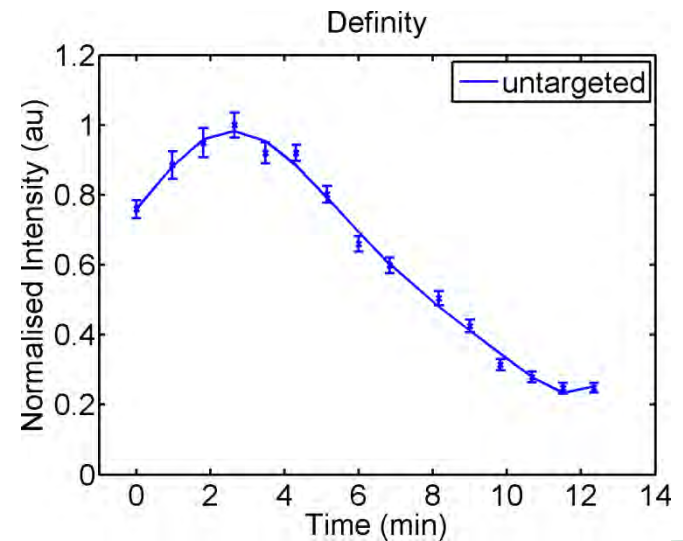
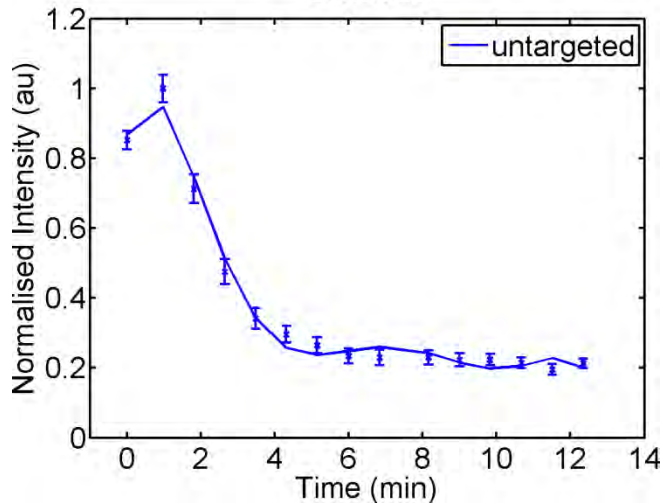
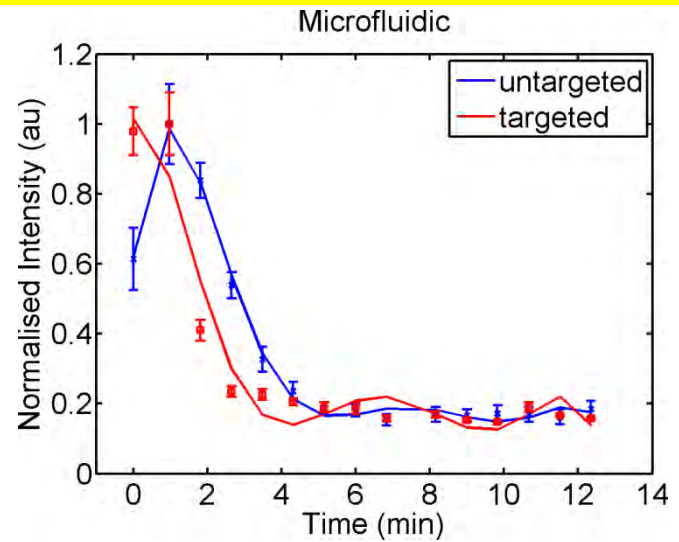
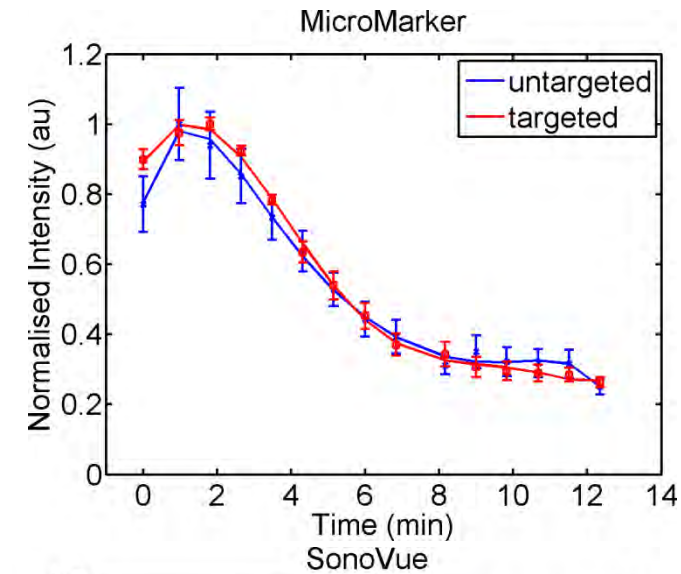
- 2nd Generation bubble instrument
- Portable, plug and play design to fit all microchips
- Double pumps for triple phase systems (gas, oil, water)
- Compatible for monodisperse and micro-spray production

Targeted, Triggered Release



Lifetime

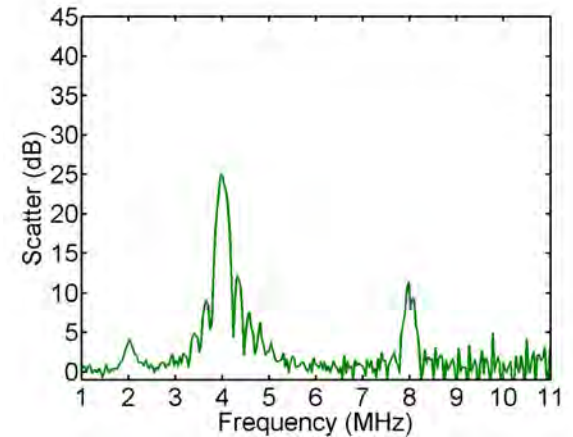
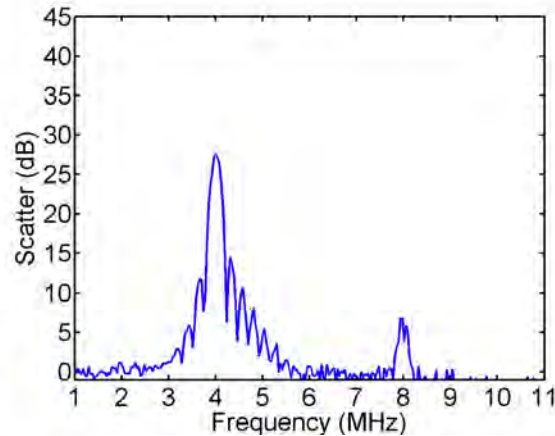
Comparing commercial and microfluidic microbubbles



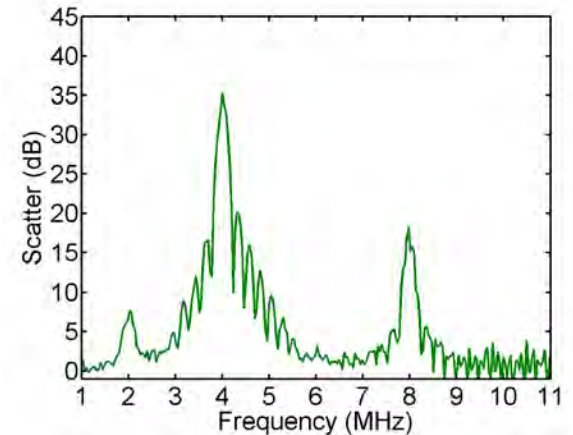
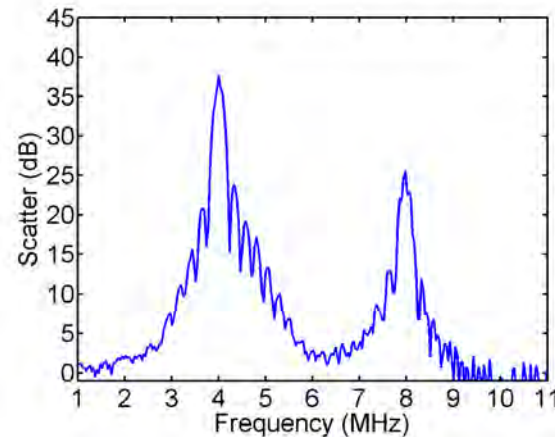
Microbubble Characterisation

- Acoustic characterisation of microbubbles with and without liposomes

50 kPa



100 kPa



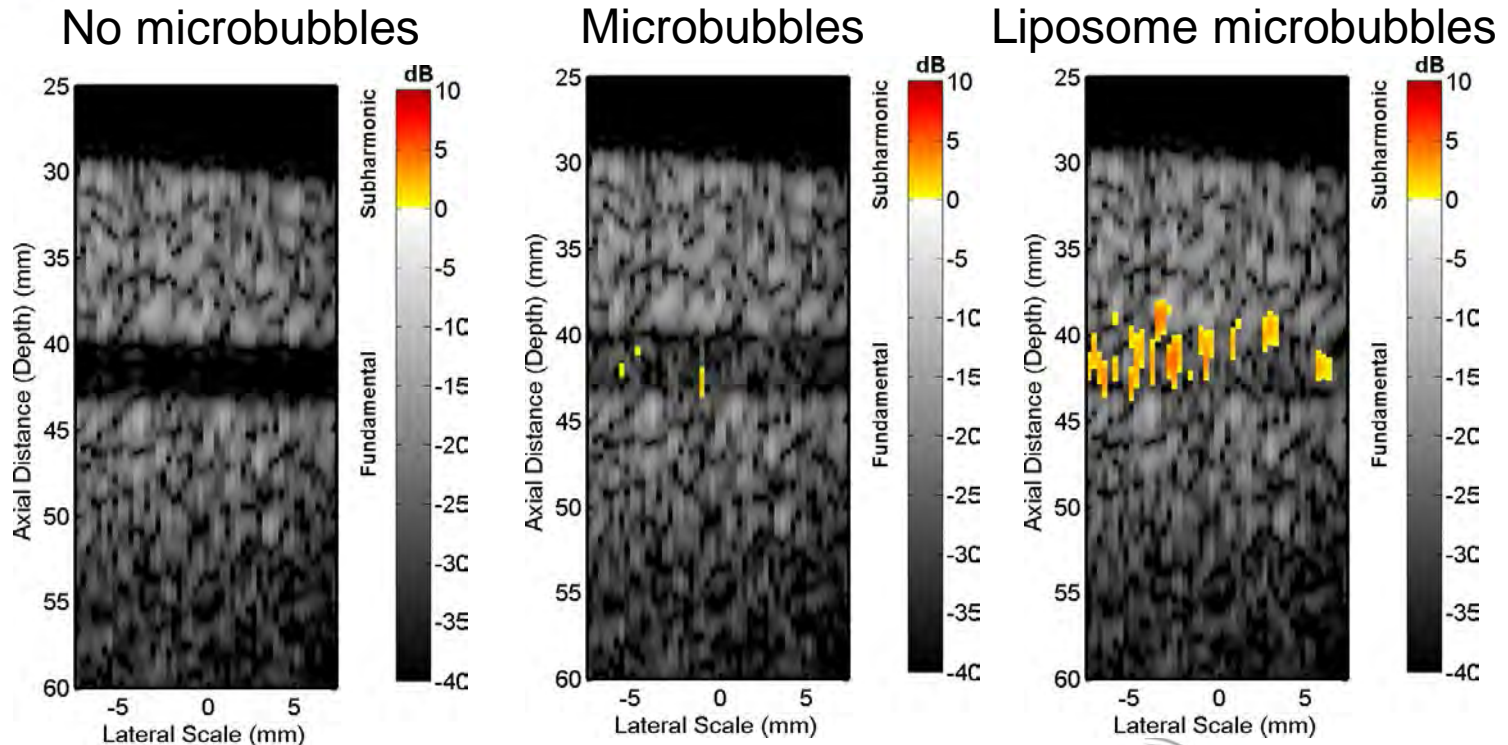
No liposomes attached

Liposomes attached

Microbubble Characterisation



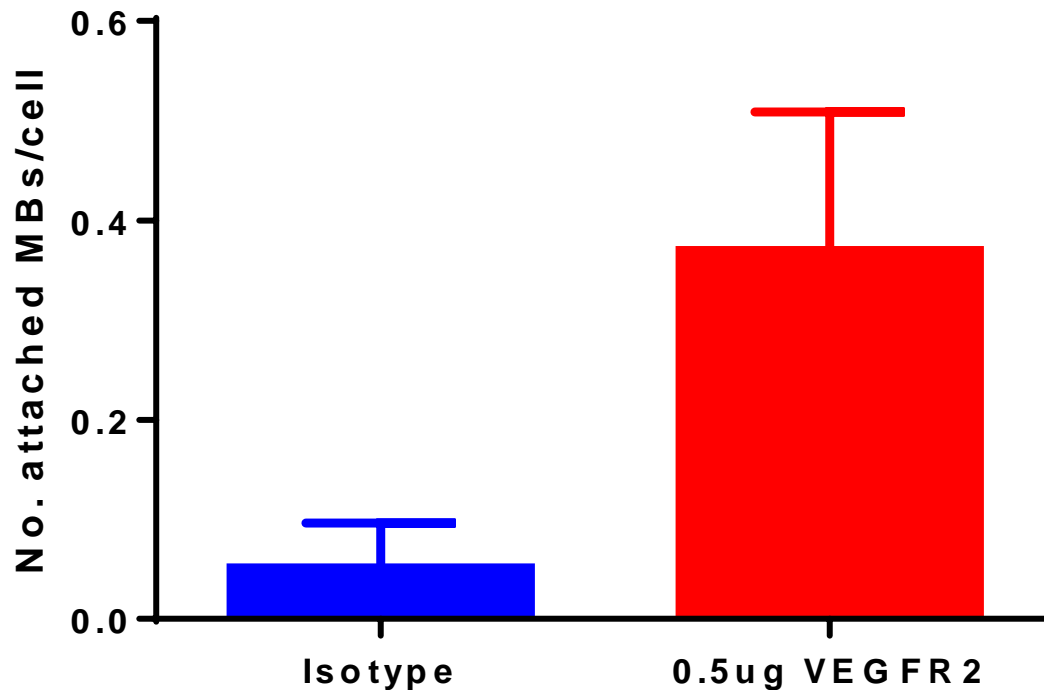
Ultrasound Array Research Platform (UARP).
Imaging in a tissue-mimicking flow phantom,
with a diagnostic probe (L3-8 40EP).



Molecular Targeting

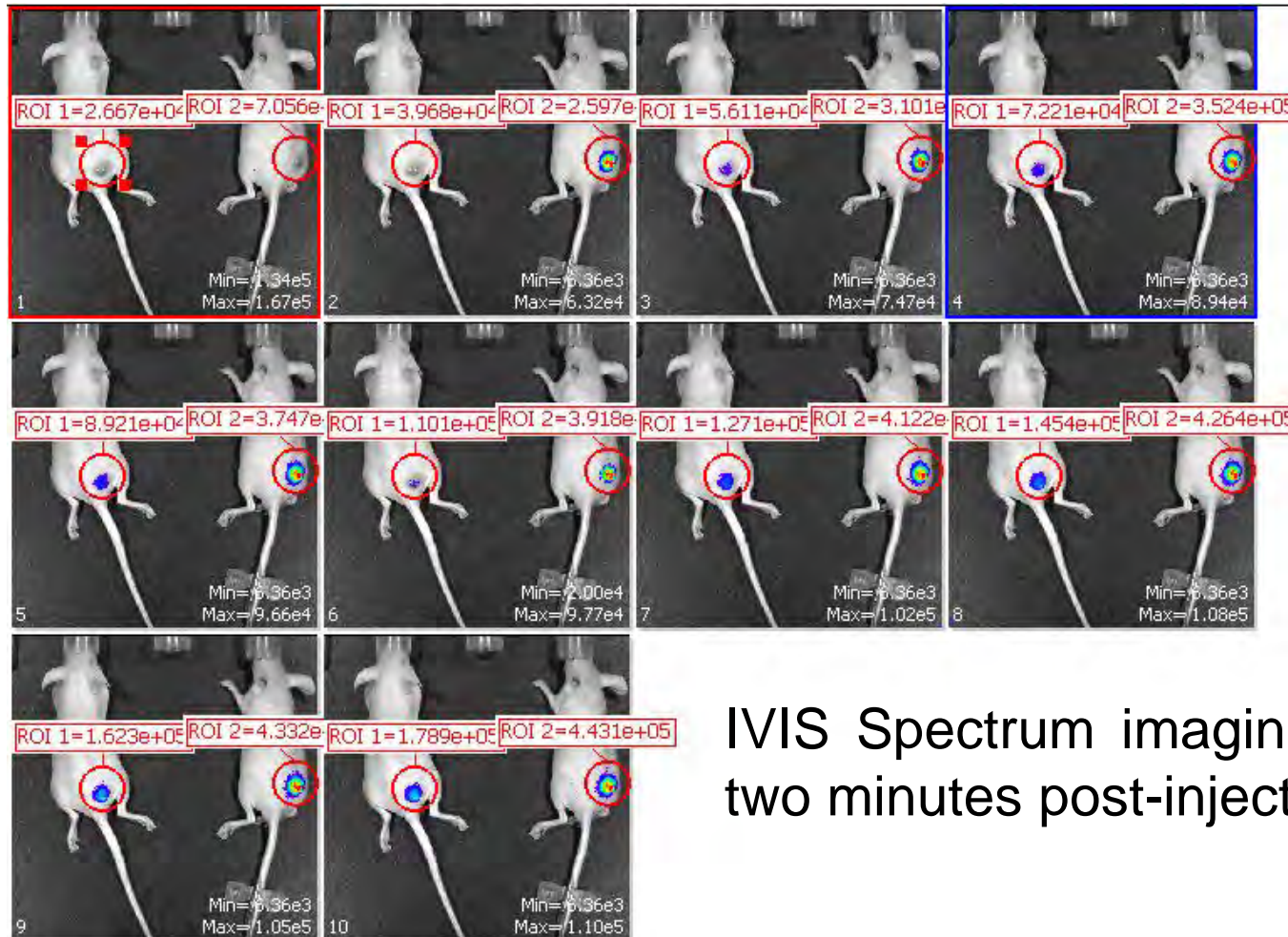
In vitro flow assay to check for microbubble binding to mouse endothelial cells.

Flow Assay



Molecular Targeting

In vivo of luciferin filled liposomes alone and bound to microbubbles.



IVIS Spectrum imaging every two minutes post-injection.

Selection of “*Engineering Therapeutic Microbubbles*” EPSRC Healthcare Partnership Project achievements

- Development of a new mF ‘spray-regime’ capable of producing MBs of clinically relevant sizes and concentration & enhanced throughput using arrays of DGs
- Production of three prototype instruments with excellent reproducibility across multiple users / locations capable of producing monodisperse MBs.
- Improved *in vivo* MB lifetimes, over commercially available imaging-MBs.
- Demonstration that mF MBs are stable and non-toxic, can be targeted, and bind to endothelial cells and tumour cells under flow conditions *in vitro* and *in vivo*.
- Demonstration of the addition of therapeutic payloads, Irinotecan, Doxorubicin, as well as fluorophores (Luciferin, Qdots and dyes) in the form of antibody labelled pegylated-liposomes.
- Demonstration of improved uptake in human colorectal cancer xenografts of luciferin as a model drug when targeted to tumour vasculature via VEGFR2 antibody.

Thank you for your attention

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For further information

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Website: www.dur.ac.uk/soft.matter/cdt/

www.facebook.com/softmattercdt

The basics

- Established in 1994
 - Spin-out from ICI Advanced Materials
- >200 person years of experience
- >200 satisfied customers
- >£5m of asset investment
- Strategy 2013-17
 - product development to production
 - vertical integration to the clinic
 - printed electronics and microfluidics USPs

Epigem introduction

linking the molecular domain to the macroscopic world via micro and nanotechnology

- EPIGEM is a highly specialised polymer microengineering SME supplying microfluidic, optoelectronic and microsystem markets.
- This ranges from high resolution flexible circuit boards and specialty coated /microstructured films for display touch screens through to microfluid devices, modules and systems for instrumentation and medical devices.
- EPIGEM was a Europractice / Integramplus Microsystems service provider and operated the Fluence Microfluidics Centre within the UK's Micro and Nano Technology Network.
- EPIGEM are ISO9001:2008 and MNT Quality Mark certified and "Investors in people" accredited.

OR....

- Microfluidics for **producing** - **printing, synthesis, extraction**
 - Oil / air/ water emulsions / double emulsions
 - Blood fractionation (rare anaemias “Commitment”)
- Microfluidics for **diagnosing and therapy**
 - Ultrasound contrast agents for cancer theranostics
 - Active / latent TB differentiation (“Mimic”), paraTB PCR (“MilkED”)
- Microfluidics for **monitoring** food safety “Symphony” (Milk), “Aquavalens” (Water)

Microfluidics

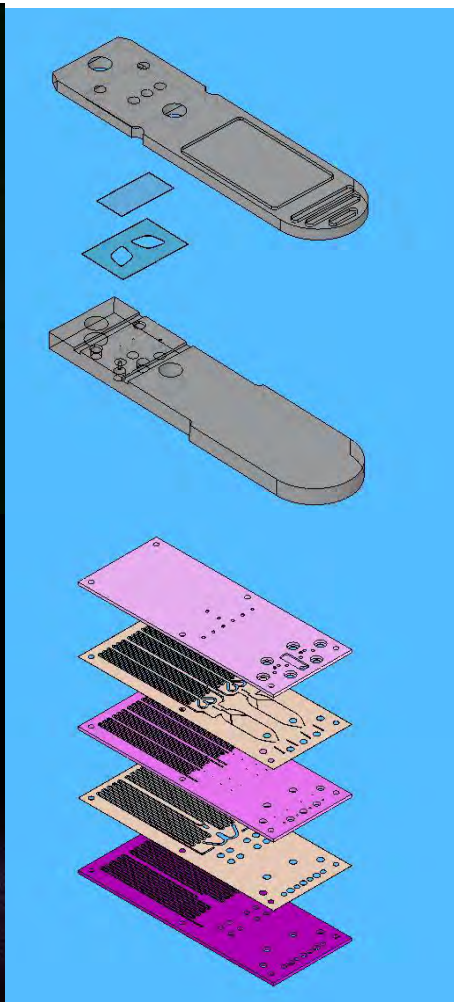
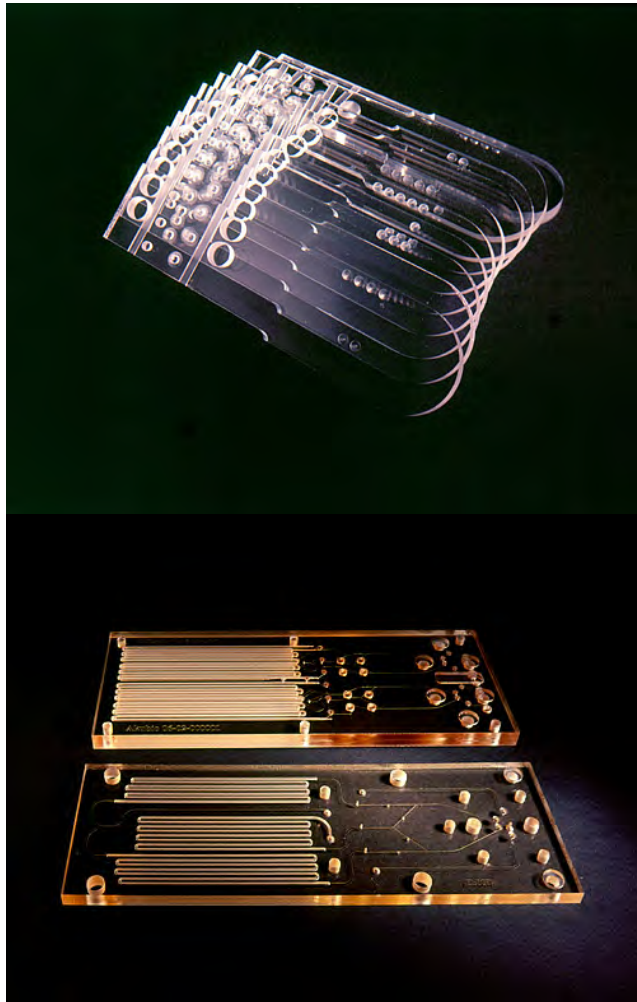
- Droplet generation processes becoming increasingly sophisticated for producing bubbles, micro and nanoparticles with complex chemistries, surfaces and interiors and for encapsulation
- High volume manufacturing processes are increasingly using microfluidic “printing” processes (Facilities at Epigem, CPI Catapult)
- Multilayer (>10), multifunctional manufacture with interconnection in fluidic, electrical and other domains (acoustic, magnetic, biological) and associated packaging (reversible microgaskets for sensor to fluid sealing and creation of microfluidic environments for cells etc)

Epigem Limited Location

Redcar - where the chemical industry meets the North Yorkshire Moors National Park



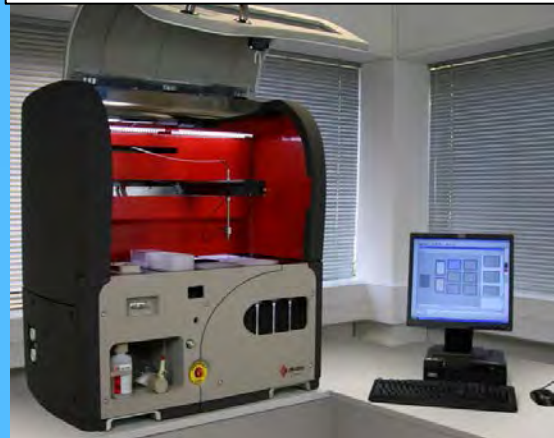
Disposable sensor, integrated fluidics instrument board and high throughput instrument



**Labels free acoustic detection
automated 24+ hours
unattended operation.**

**Minimised sample preparation
using valve controlled
microfluidic processor board**

**Disposable sensor with
electrical and fluidic
connections**



What we need from NHS

- We think that theranostic microbubbles will be delivered via the hospital pharmacist.
Are we right?
- We are seeking feedback on issues regarding the use of theranostic microbubbles and help from ultrasound and pharmacy experts
Instrument(s) specification?
- We are extending our interaction with clinicians into other areas where Epigem core technology looks relevant like electrophysiology

Microfluidics

- In simple terms it is miniature plumbing - pipes (channels) but also nozzles, membranes & droplets on surfaces.
- It is nature's key technology for transporting life supporting liquids over long distances whilst preserving cellular / micro and nano dimensions – think plants and trees, lungs, blood stream, glands, things that spit, etc
- It is printing & sprays in all forms (drug, scent delivery to product fab.)
- It is a key technology in analytical instrumentation - high throughput, small volumes and portable diagnostics and environmental sensing
- It enables new product forms to be synthesised and processes to be intensified - heat input / output (PCR), energy conservation etc
- It enables both product miniaturisation and large area film / sheet manufacturing via “printing”

Throughput by numbering up or scale out

- Plant: manifold together 500,000 microreactors continuous flow @ 10 $\mu\text{l}/\text{min}$ produces 0.3 t/hr synthesised product
- Gravure film coating: 400,000 off 2nl wells / roller 150mm diameter x 300 mm width dispenses @ 1m/min micron coat thickness
- Ink jet printing: 40 μm dia. drops contains 40 pl for coating a page @ 600 dpi. Arrays of print heads for posters to 2.5m print width