Università di Roma Tor Vergata

Microbubbles for Theranostics

Diagnosis

Therapy

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The Team

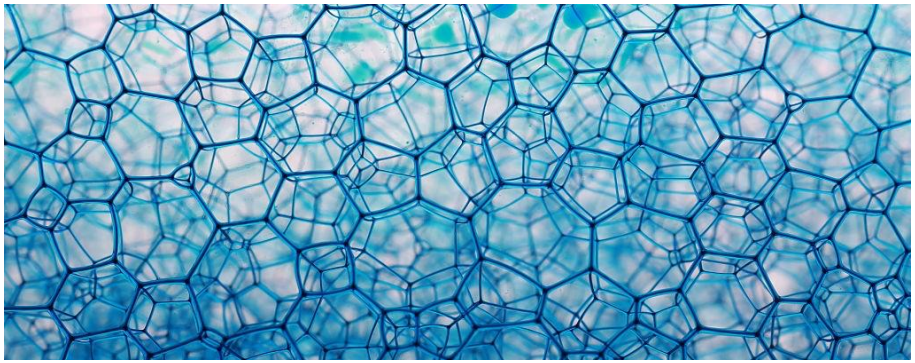
Blow a soap bubble and observe it. You may study it all your life and draw one lesson after another in physics from it.

Lord Kelvin

What is important in bubbles:

1) Surface tension: Energy to create an interface [joule/m^2; N/m]
2) Surface minimization: a single bubble is spherical

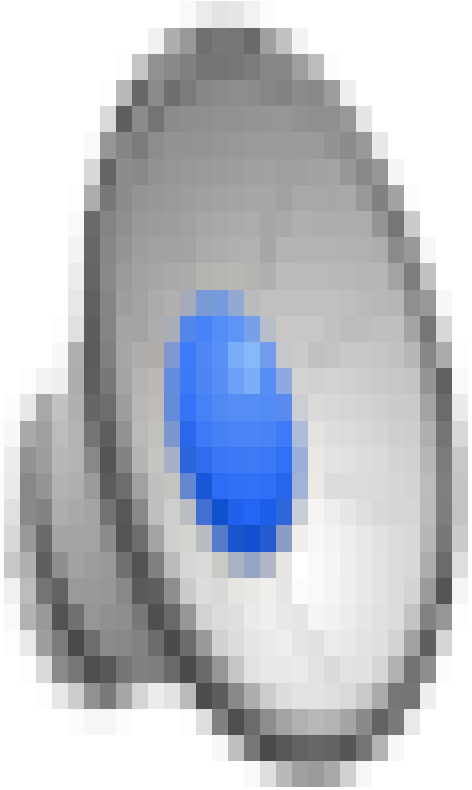
Physicist Joseph Plateau (1801 – 1883) was the first to “observe” that there are only two values of the angles between bubbles: 120 °; 109,5°



In 1976 Jean Ellen Taylor proved the Plateau’s laws (≈1840) by Geometric Measure Theory

....according to the article on “Bubbles” in the Encyclopaedia Britannica (IX Edition, 1878) by James Clerk Maxwell : Leonardo da Vinci, Isaac Newton, Leonhard Euler, J.L. Lagrange, Gaspard Monge, Pierre Laplace, Thomas Young, Carl Friedrich Gauss, Simeon-Denis Poisson, Joseph A.F. Plateau, William Thomson Lord Kelvin, Josiah W. Gibbs studied bubbles....

From Jean Ellen Taylor *Some Mathematical Challenges in Materials Science,* Bullettin of the American Mathematical Society, 2002, *40*, 69 -87.

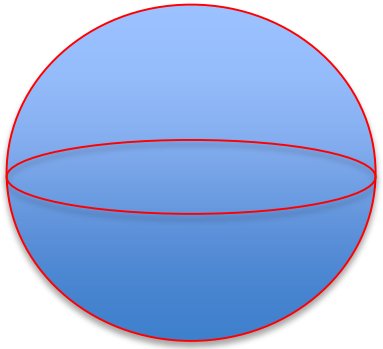


Pressione di Laplace

Tensione superficiale

$$\Delta P = P_{in} - P_{out} = \frac{2g}{R}$$

Raggio della bolla



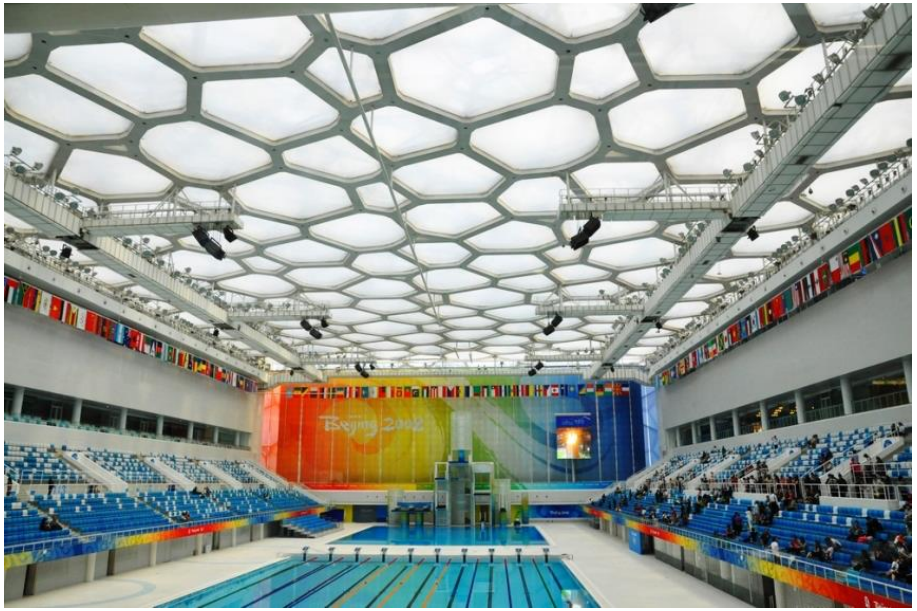
Bubble diameter (µm)	ΔP (atm)
1000	0.00284
3	0.947
0.01	283

Bubbles are everywhere.....

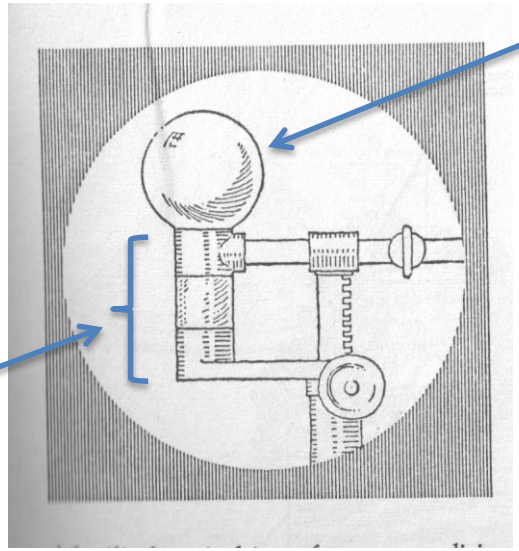
Mura poligonali di Tarawasi in Perù (città Inca, 1450 DC).

Bolle di sapone

Aquatic Sports Center – XXIX Olympic Games (China, 2008)



Curvature of the cylinder

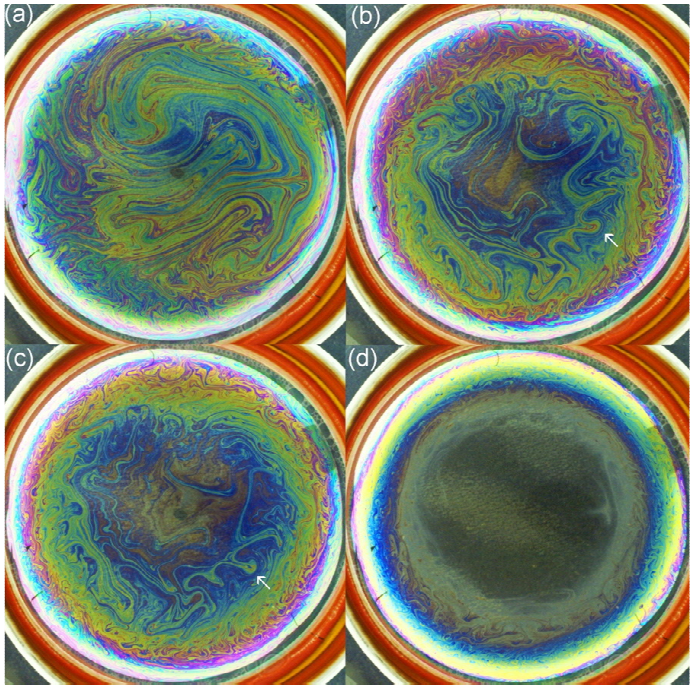


Bubble

cylinder

Curvature of the cylinder from the radius of the associated bubble

Colors:
Bubbles shell is a thin film causing interference pattern with visible light.

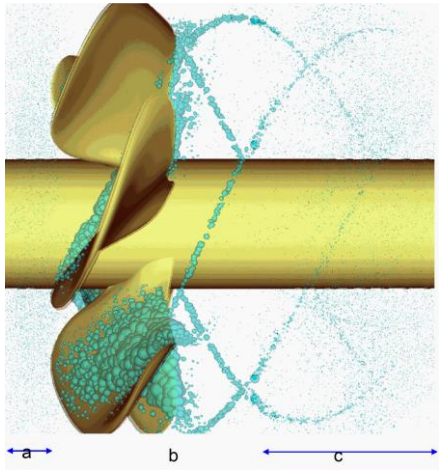


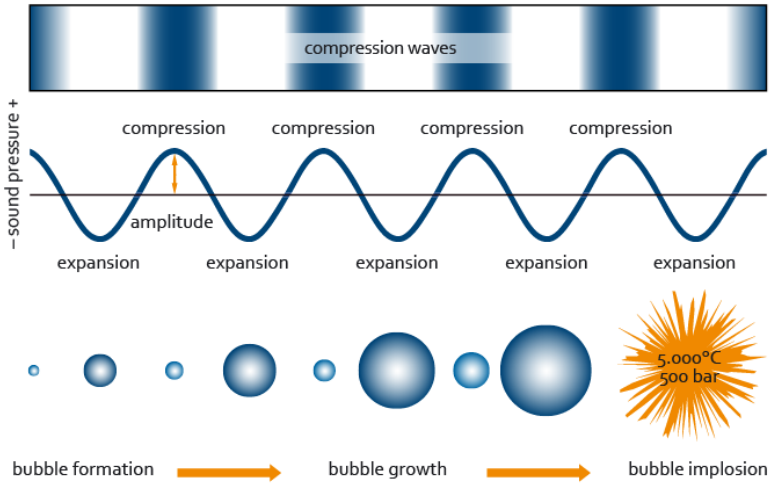
Cavitation & Bubbles

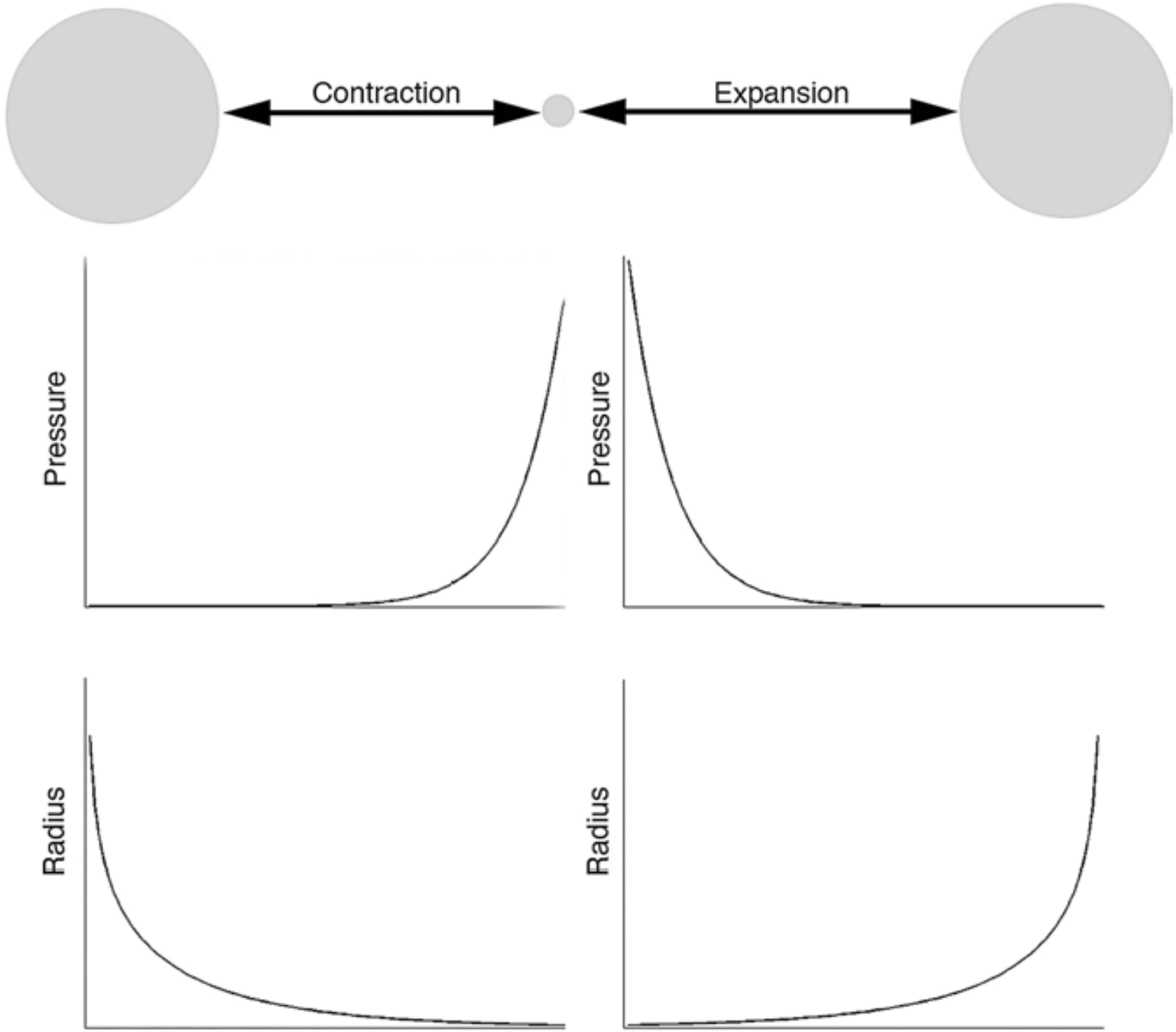
Cavitation: formation of water vapour bubbles in a liquid achieved by a quick pression reduction

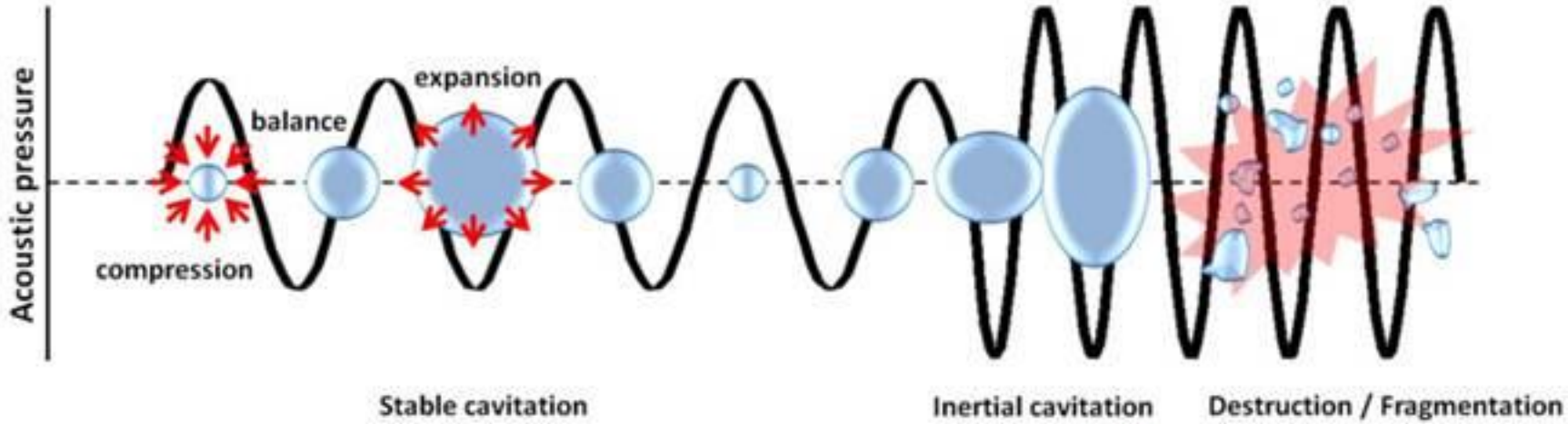
Fast “pressure drop – pressure build up” cycles make bubbles oscillate and eventually blast

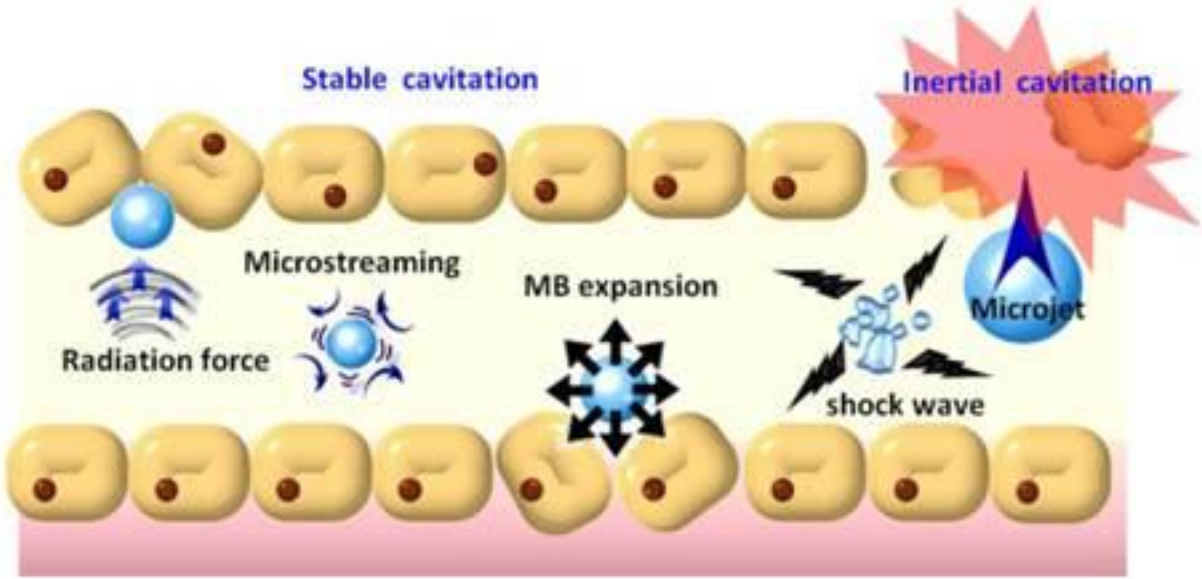
As a propeller spins and generates cavitational bubbles in water.



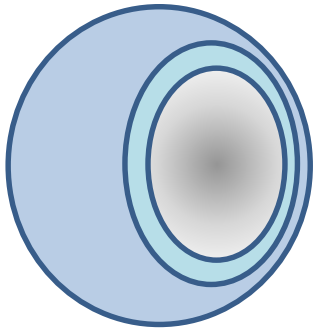


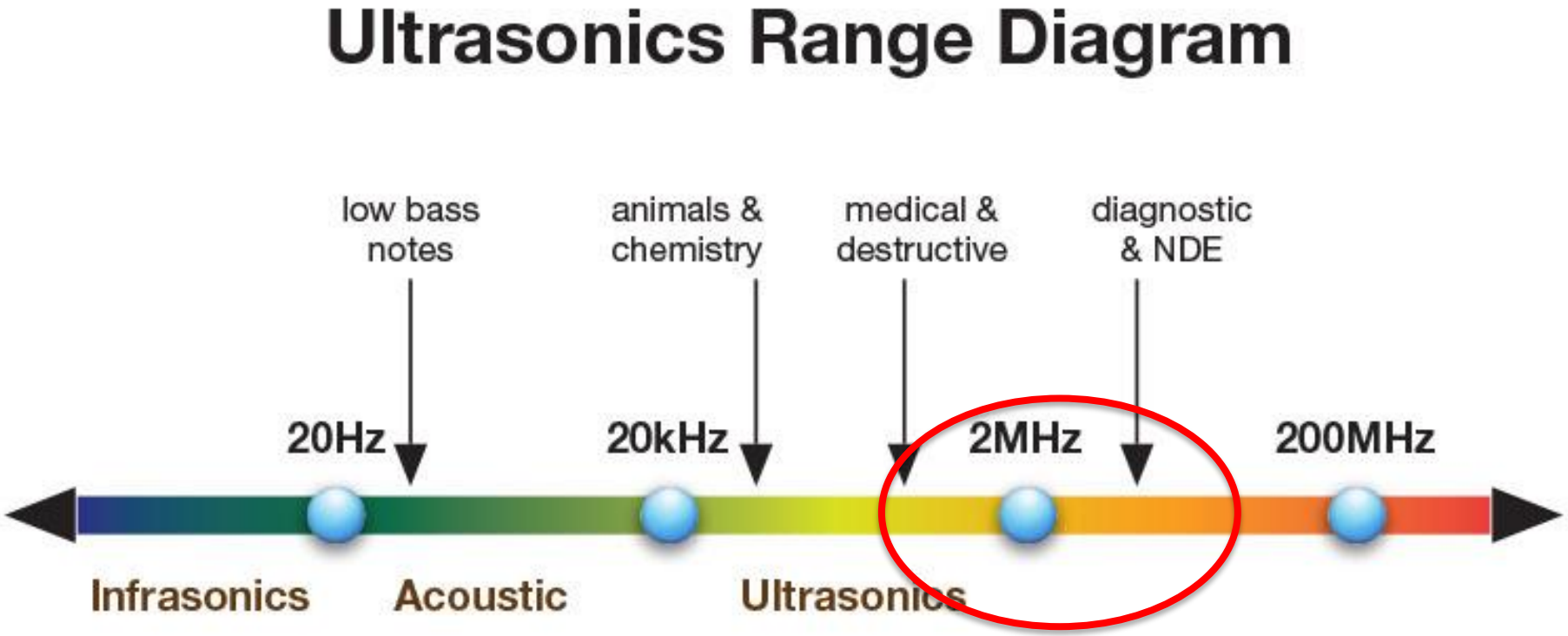

Contraction
Expansion
Pressure
Pressure
Radius
Radius


Acoustic pressure
expansion
balance
compression
Stable cavitation
Inertial cavitation
Destruction / Fragmentation


Stable cavitation
Inertial cavitation
Microstreaming
MB expansion
Microjet
Radiation force
shock wave

Why microbubbles?



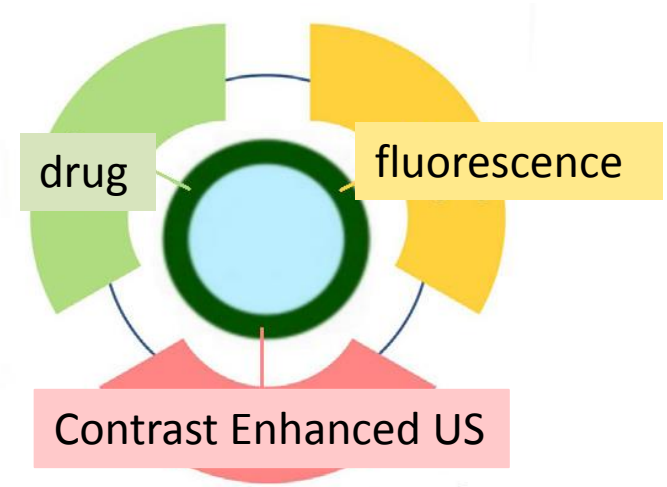


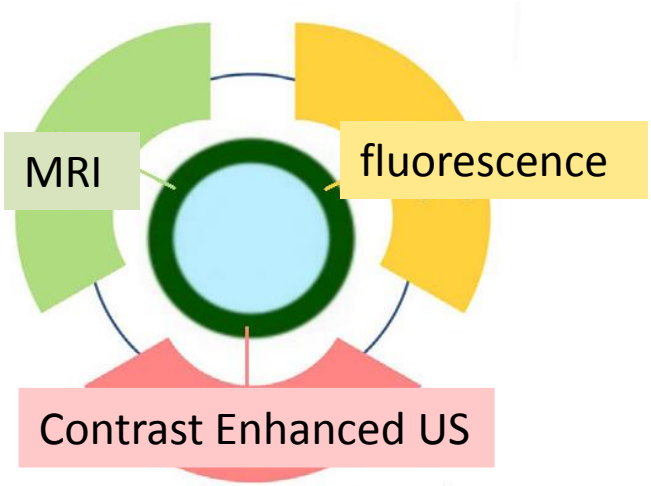
from Wikipedia article on Ultrasound

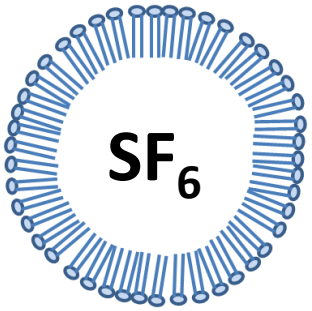
MicroBubbles for Diagnosis and Therapy:

- Lipid shell + stabilizing gas core
- Polymer shell + gas core

Tunable or "smart" microbubbles

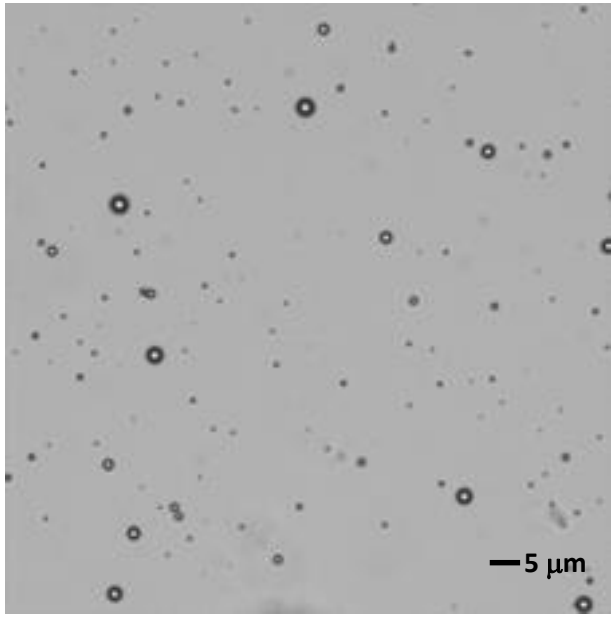




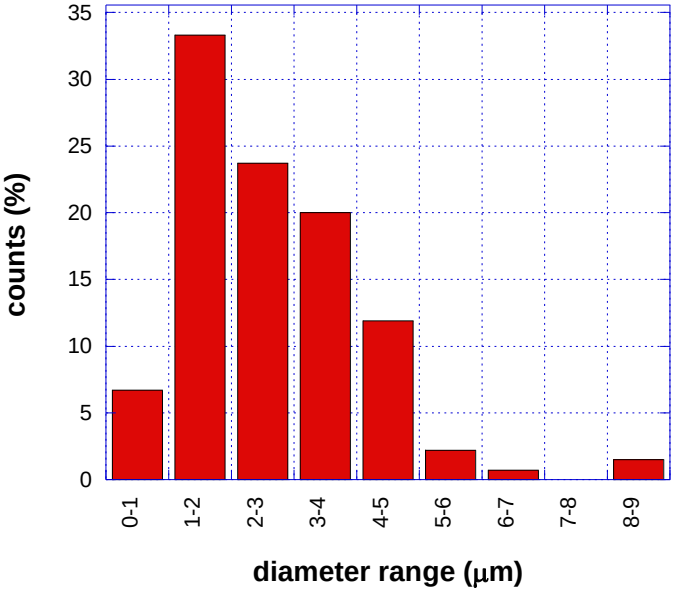


LIPID-based MBs

- ✓ **DSPC:DPPG.Na** = 1:1 (mol/mol)
- ✓ **Poly(ethylene glycol) 4000**
- ✓ **Palmitic acid**



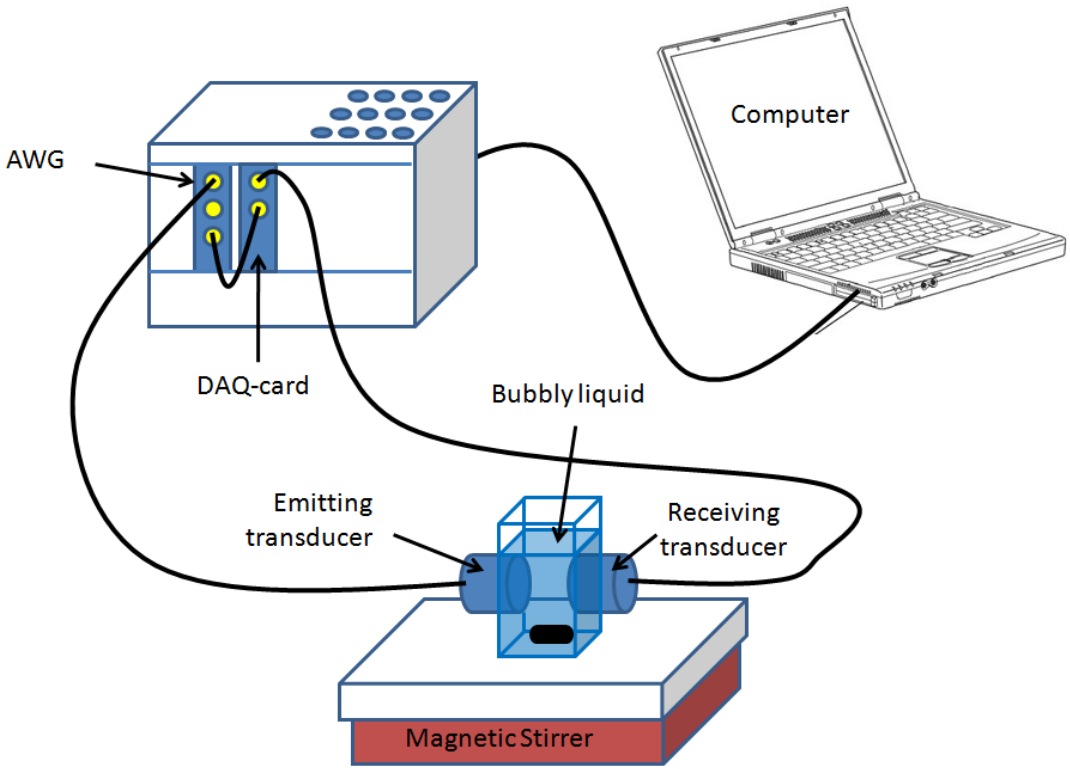
MB yield: ~ 1×10^8 MBs/mL



Mean diameter = 2.7 ± 1.4 μm

Lipid MBs response after exposition to US

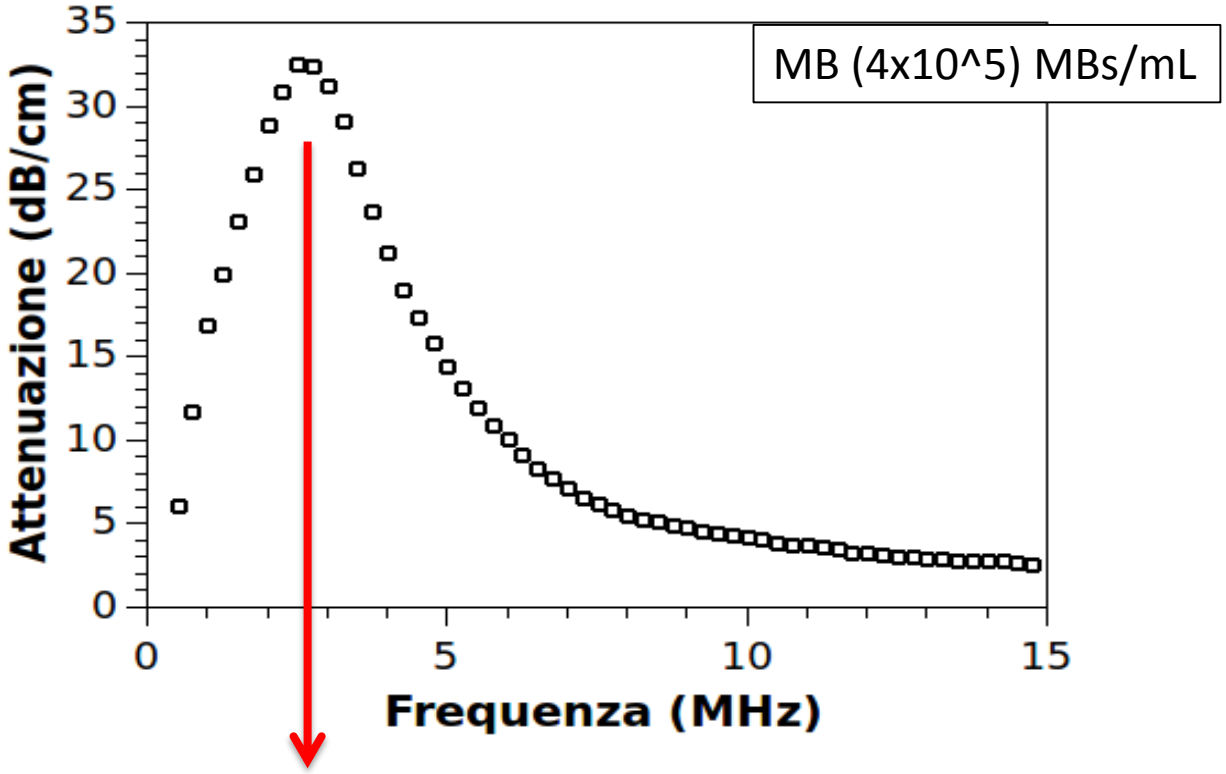
The experimental set-up used for ultrasound transmission experiments through bubbly media



- ✓ flat 10 MHz transducers (Olympus, V311) for emission and reception
- ✓ ultrasonics signals with frequency range: 0.5 to 20 MHz (250 kHz steps)
- ✓ pressure levels below 60 kPa
- ✓ all steps are controlled by LabView (National Instruments).

The received signals were processed by comparison with the signals from a reference medium (saline solution).

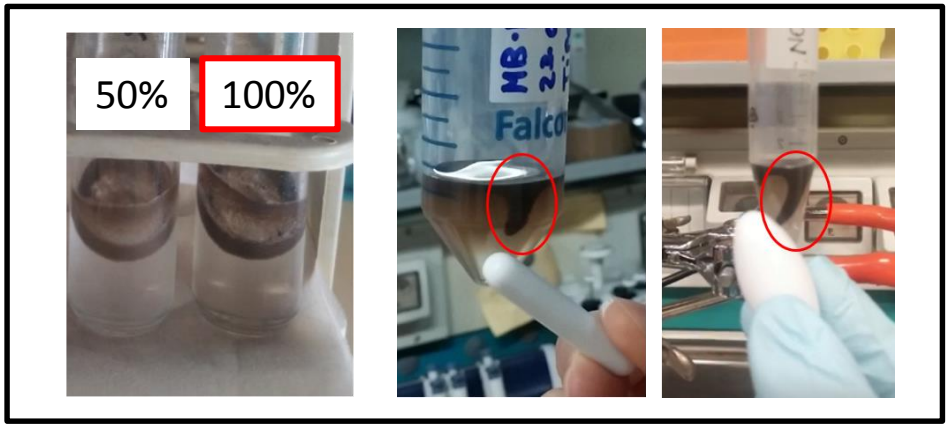
Attenation vs. Ultrasounds

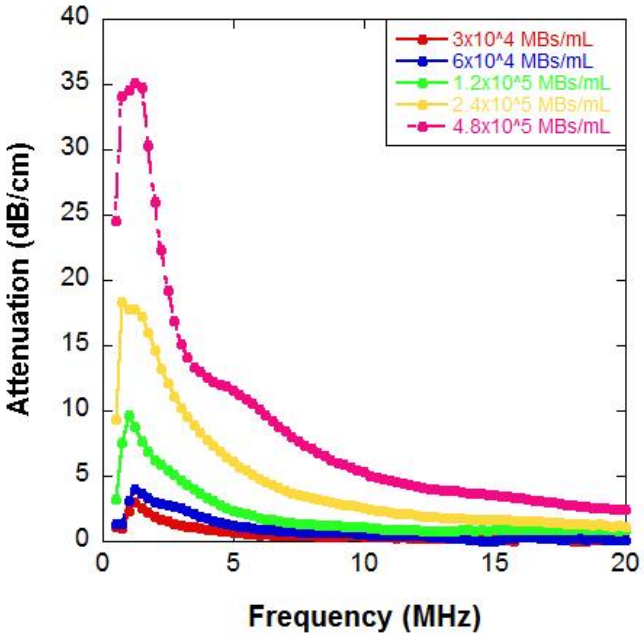


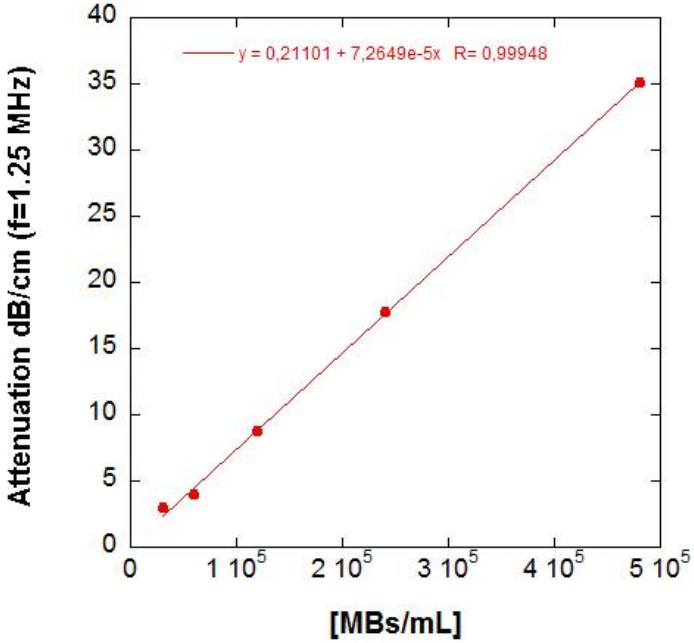
Resonance frequency

MBs+Iron Oxide NPs for Magnetic Resonance Imaging

MB cake is reconstituted in a suspension of the EMA-approved SPIONs

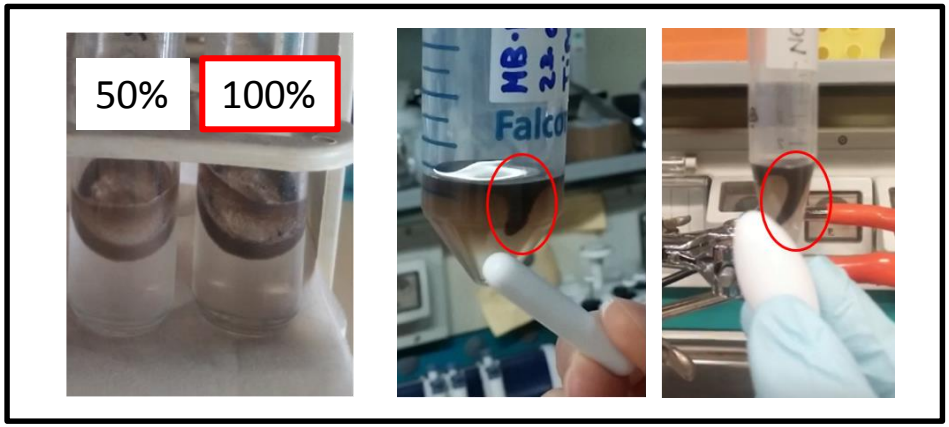


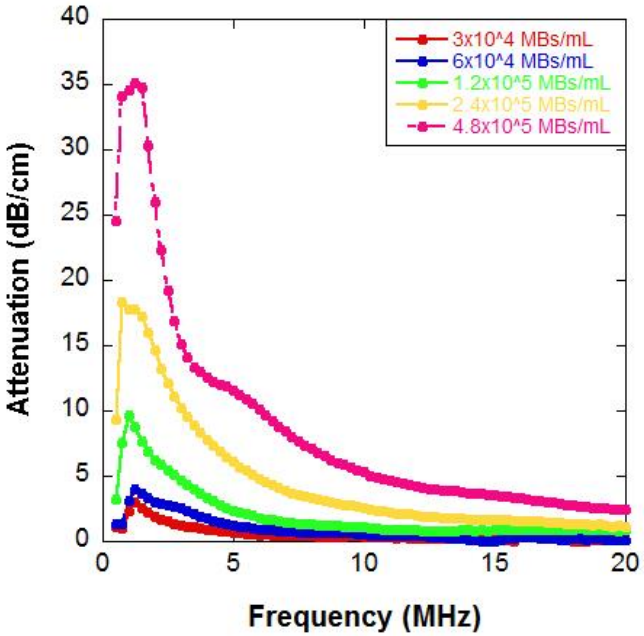


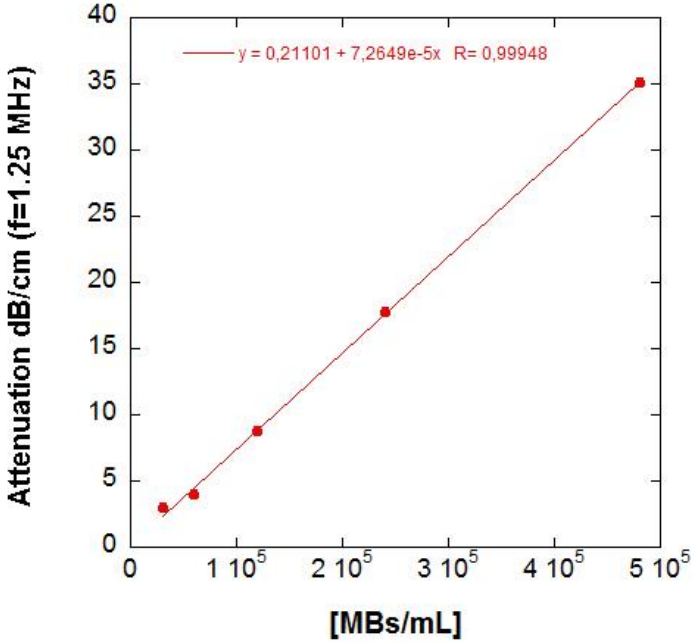


MBs+Iron Oxide NPs for Magnetic Resonance Imaging

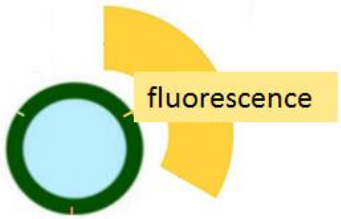
MB cake is reconstituted in a suspension of the EMA-approved SPIONs







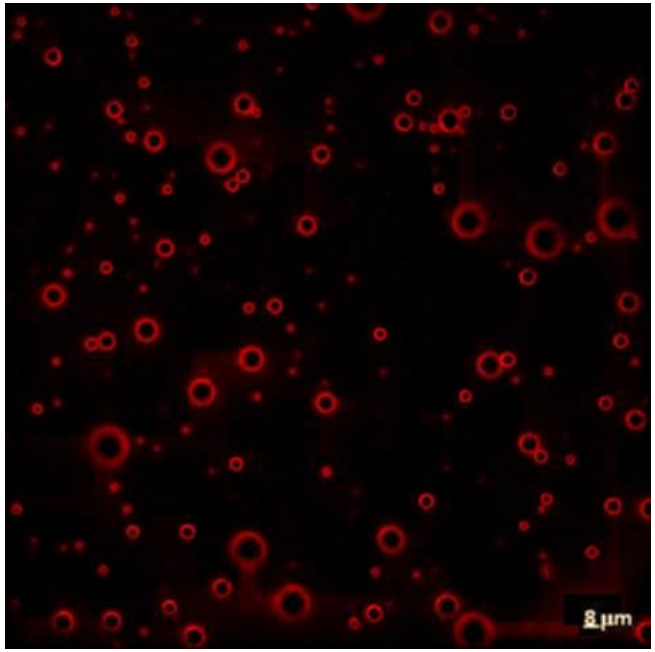
MBs for Fluorescence Imaging



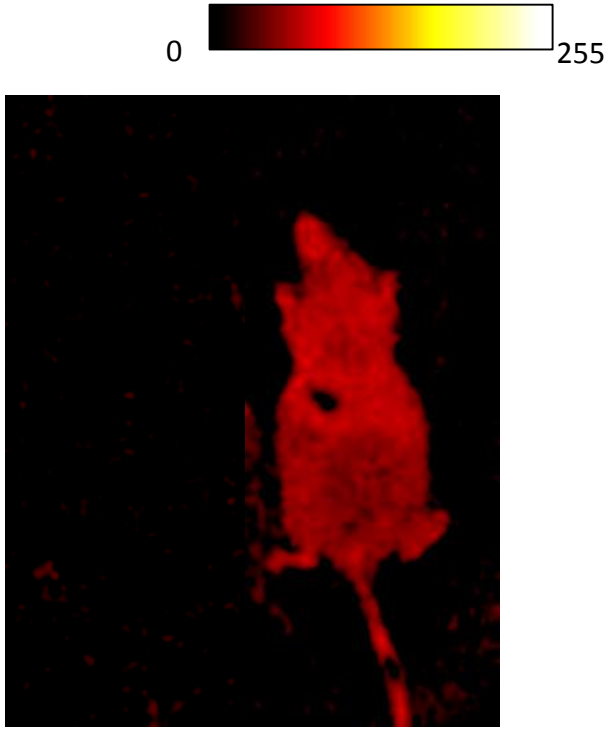
MB cake is reconstituted in a Indocyanine Green (ICG, FDA/EMA approved Pulsion Medical System)

ICG is a Near Infrared fluorophore for deep tissue imaging

25 mg lipid MB cake
5 mL ICG 1 mg/mL
SF_6 gas



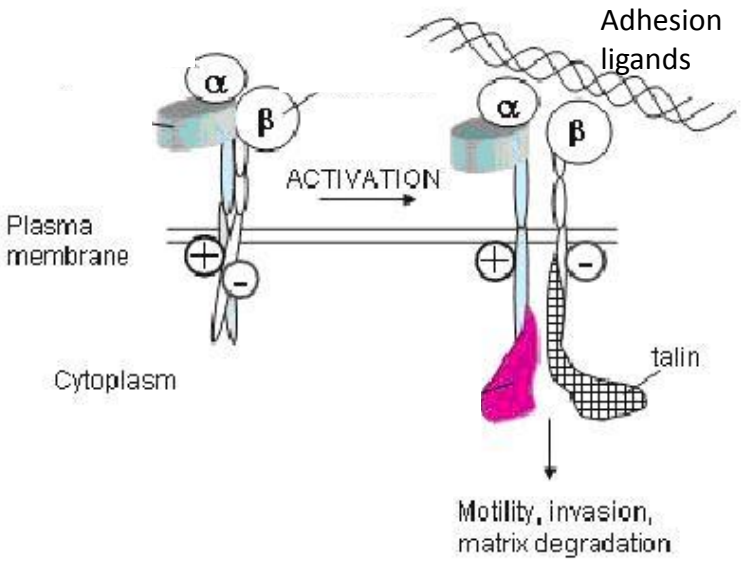
Fluorescence Micr, 60x
NIR fluorescence detection



Saline ICG-MBs

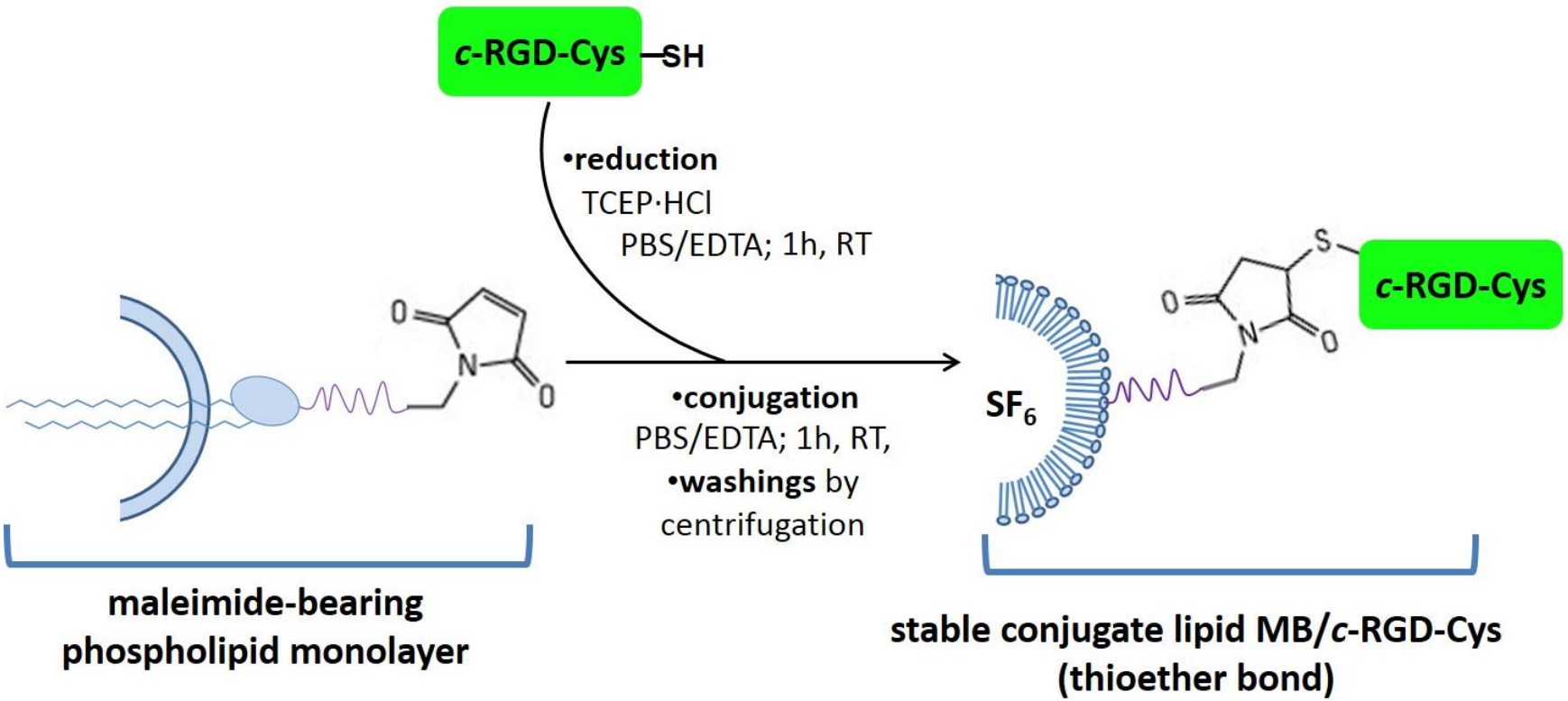
25 min post-injection ICG 1mg/kg

Cyclo(Arg-Gly-Asp-D-Phe-Lys)
RGD motif



fibronectin

LIPID-based MBs for Cancer Targeting



2%

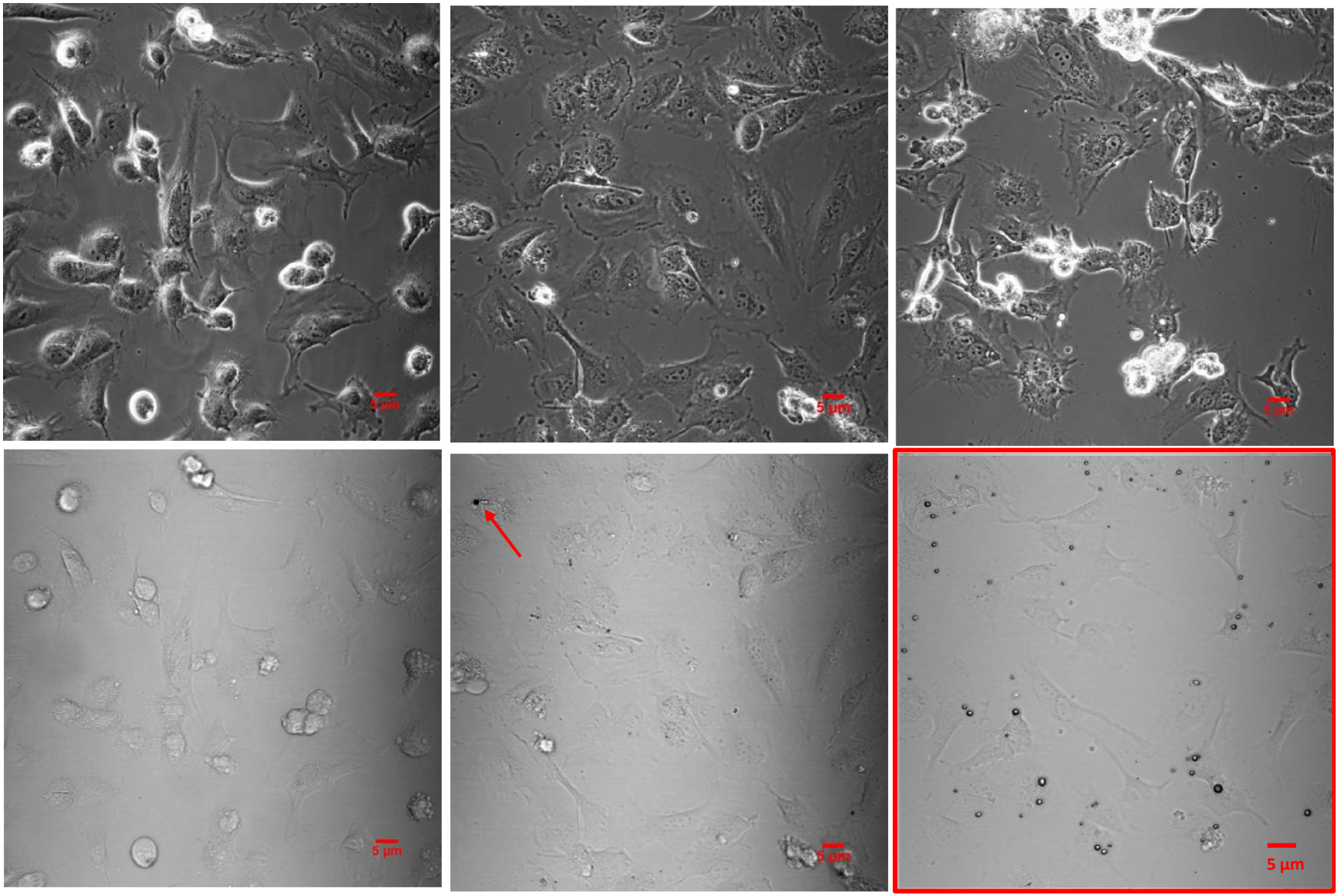
HUVEC+Naked MBs

HUVEC+ MB 10 RAD

HUVEC+ MB 10 RGD

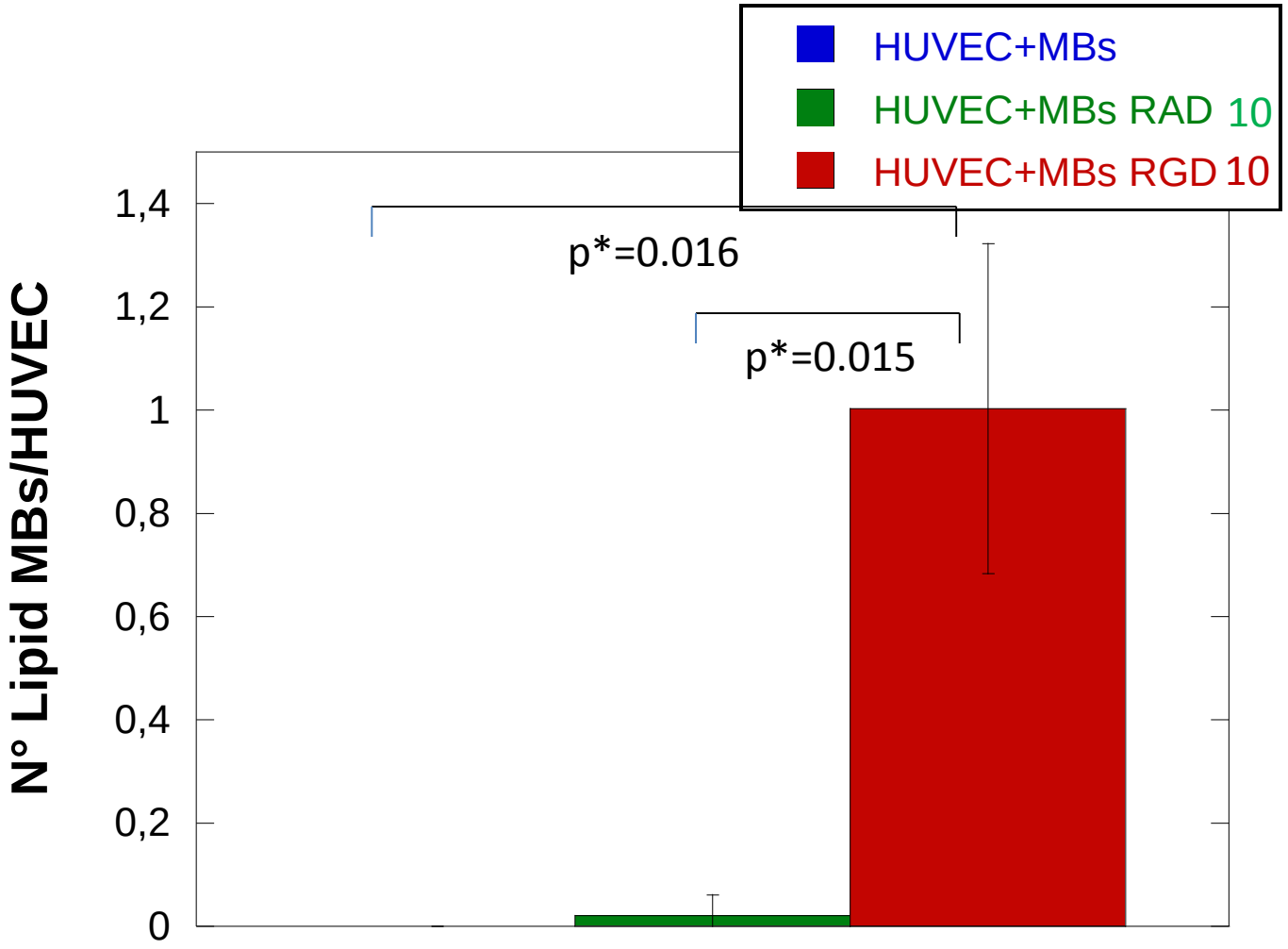
Transmission+ Phase Contrast

Transmission



40x LD

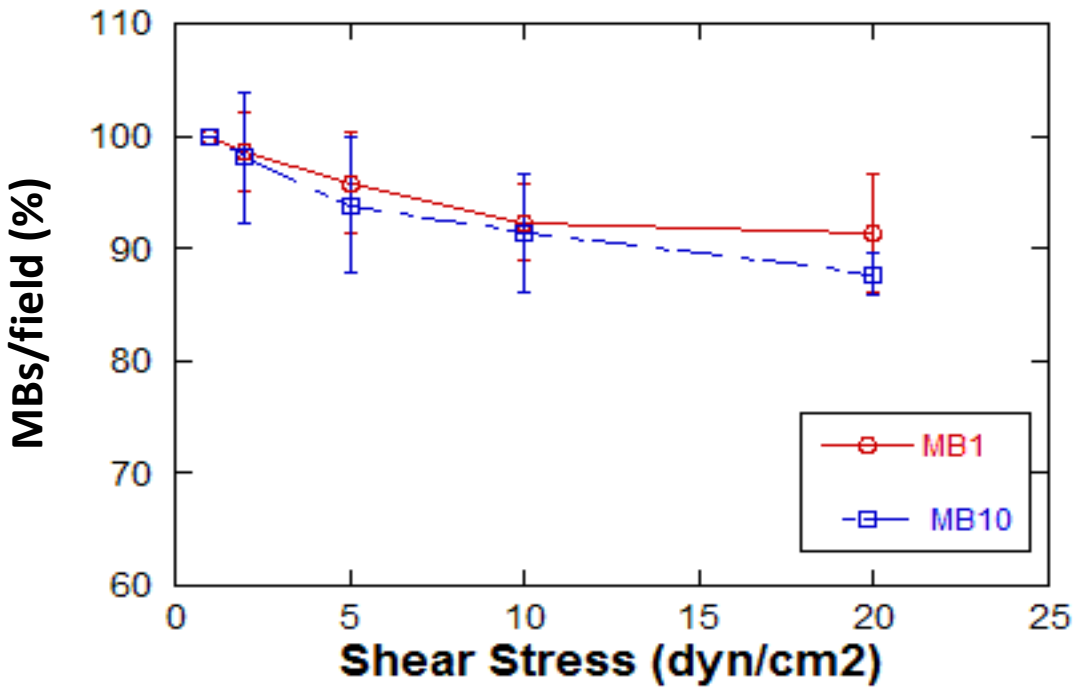
Static bioadhesion of HUVEC with lipid MBs for tumour (αVβ3 integrin) targeting



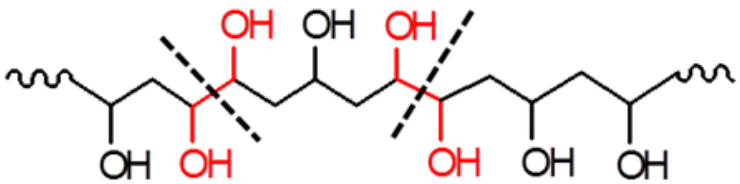
Dynamic bioadhesion of HUVEC with lipid MBs for tumour (αVβ3 integrin) targeting

Detachment Evaluation

3 minutes flow at increasing shear stress

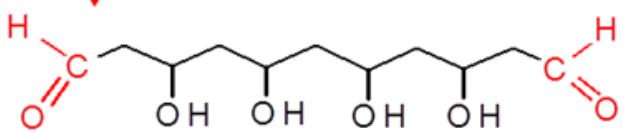


Polymer shelled Microbubbles



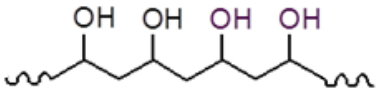
PVA

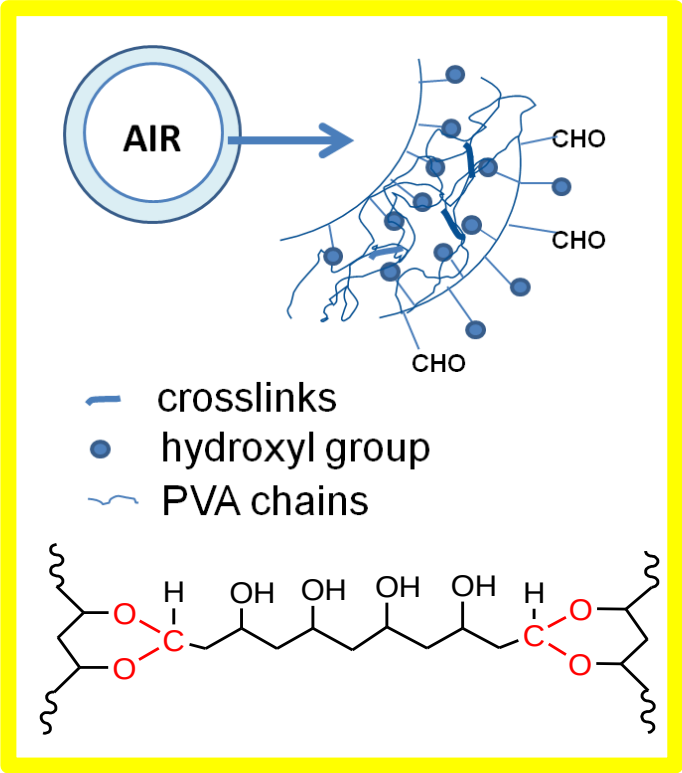
selective oxidation



oxidized PVA

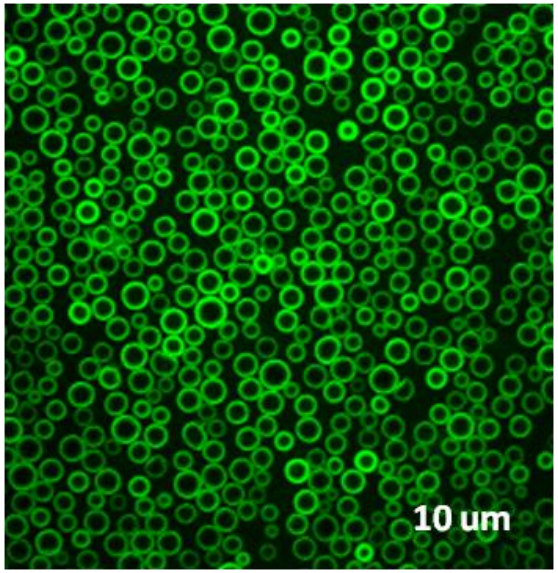
homogenization: foaming + crosslinking

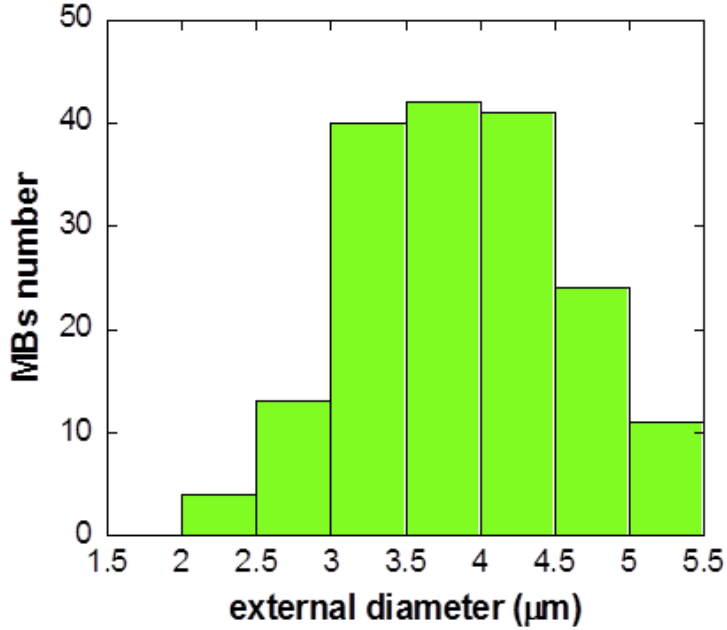


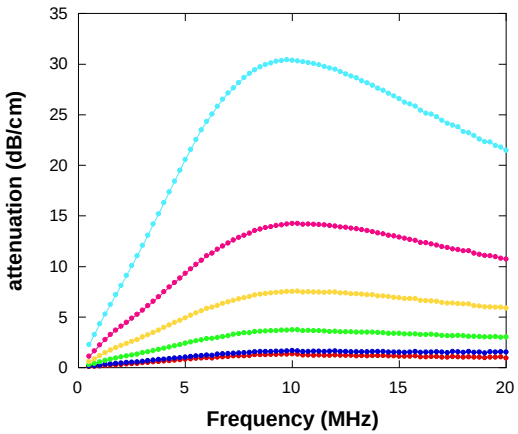


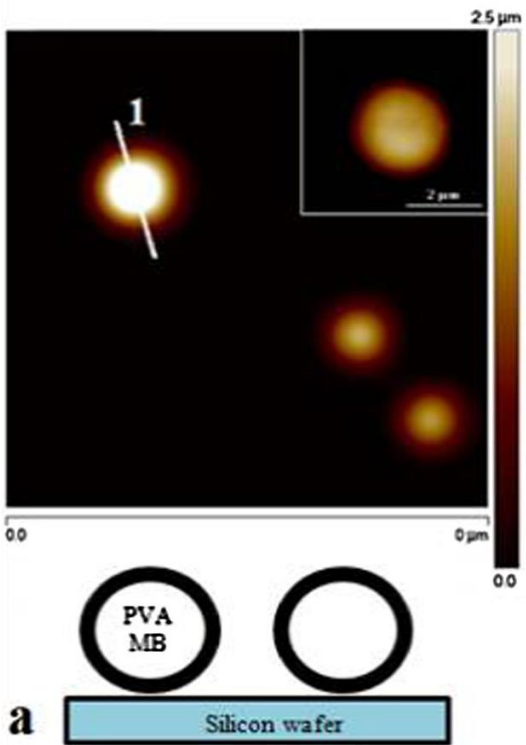
crosslinked PVA

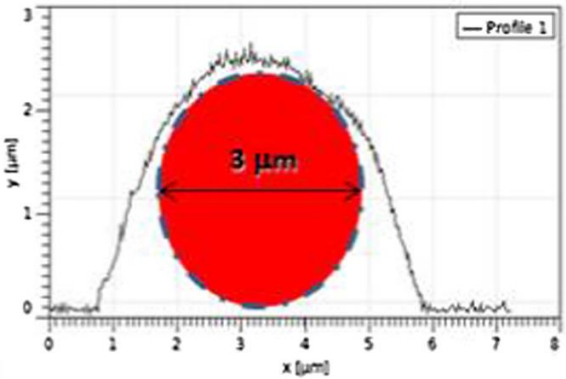
MBs formation

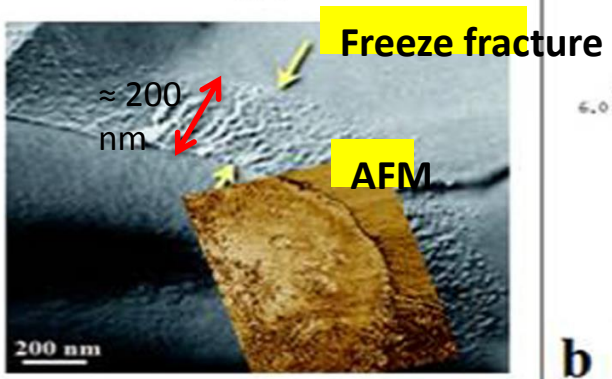

10 um

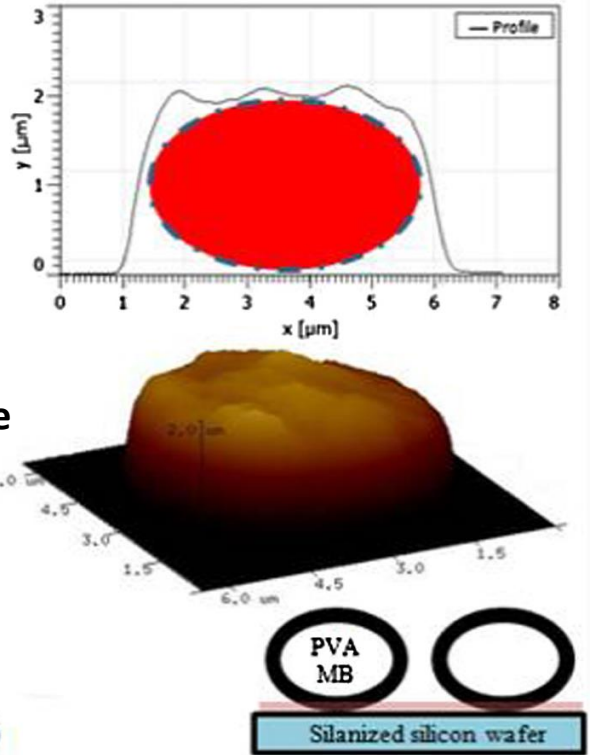

MBs number
external diameter (µm)
0
10
20
30
40
50
1.5
2
2.5
3
3.5
4
4.5
5
5.5


attenuation (dB/cm)
Frequency (MHz)
0
5
10
15
20
25
30
35


2.5 µm
1
0 µm
0.0
PVA
MB
a
Silicon wafer


Profile 1
3 µm
y [µm]
x [µm]


Freeze fracture
≈ 200
nm
AFM
200 nm

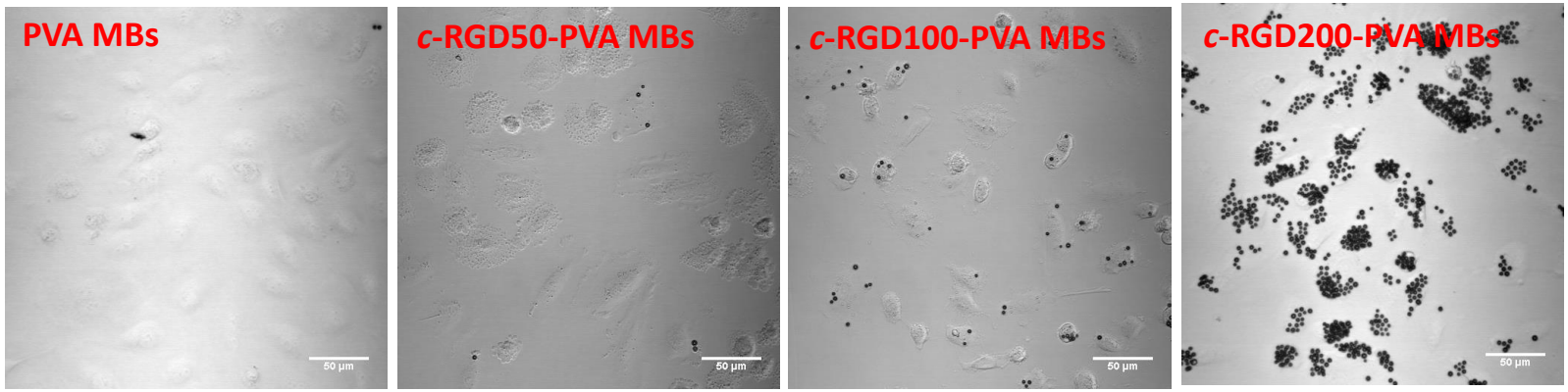

Profile
y [µm]
x [µm]
PVA
MB
b
Silanized silicon wafer

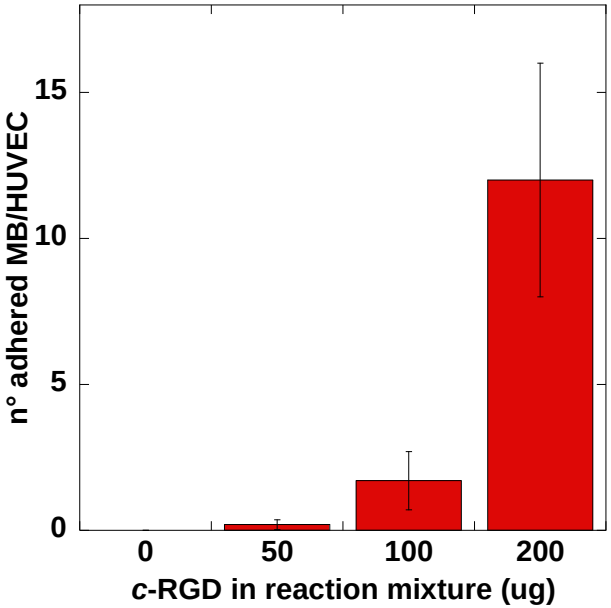
New concept:
A lipid or polymer nanodroplet (liquid core)

Ultrasounds (US)

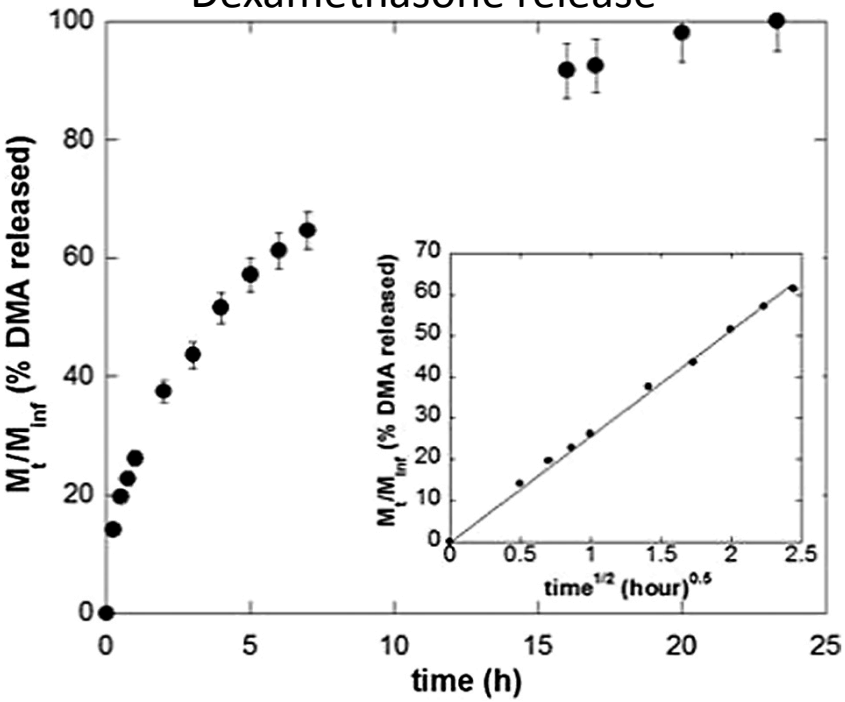
Acoustic droplet vaporization (ADV)

Microbubbles (gas core): US effective





Dexamethasone release



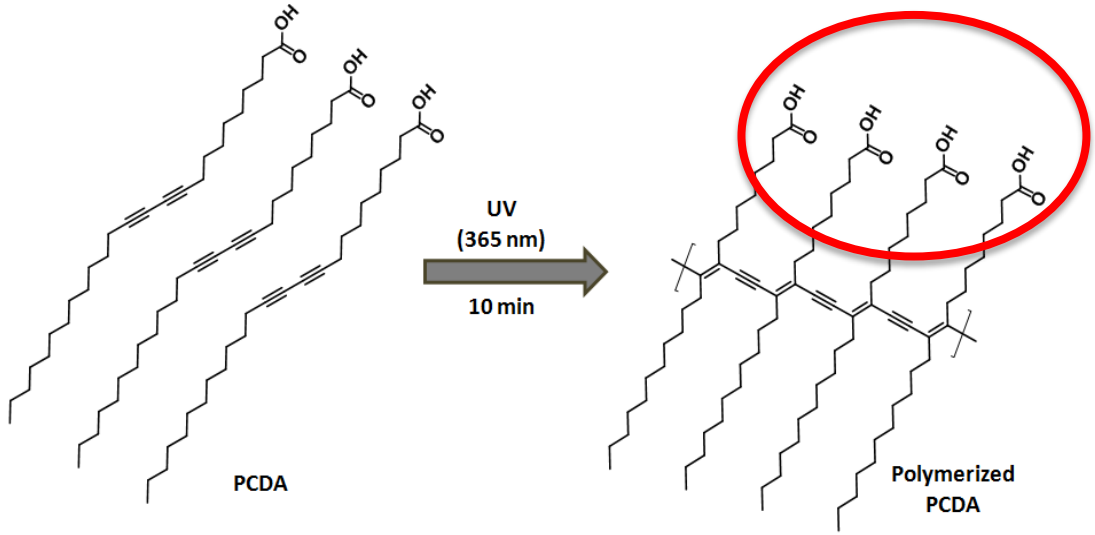
$$\frac{M_t}{M_{inf}} = kt^n$$

n=0.5 release according to Fick' law

Y. Toumia

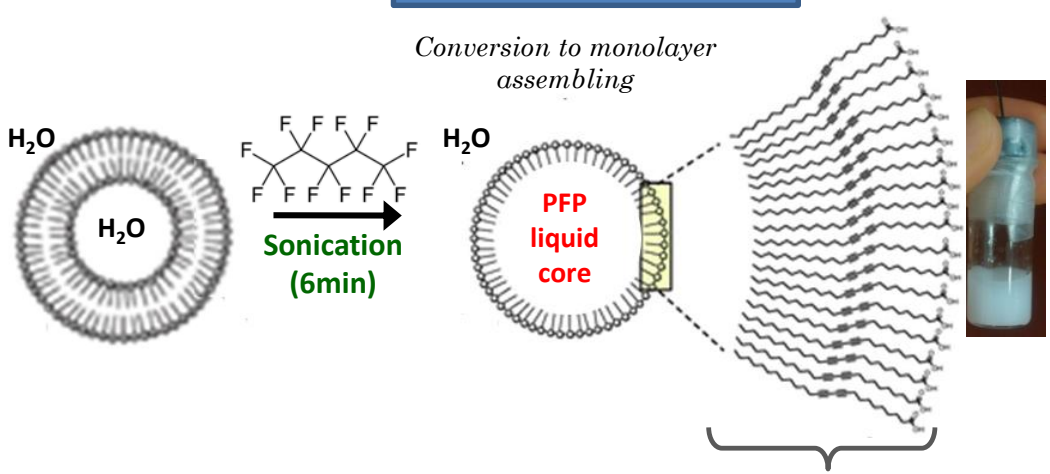
Polymer - lipid Microbubbles

Poly-PCDA droplets Preparation Procedure

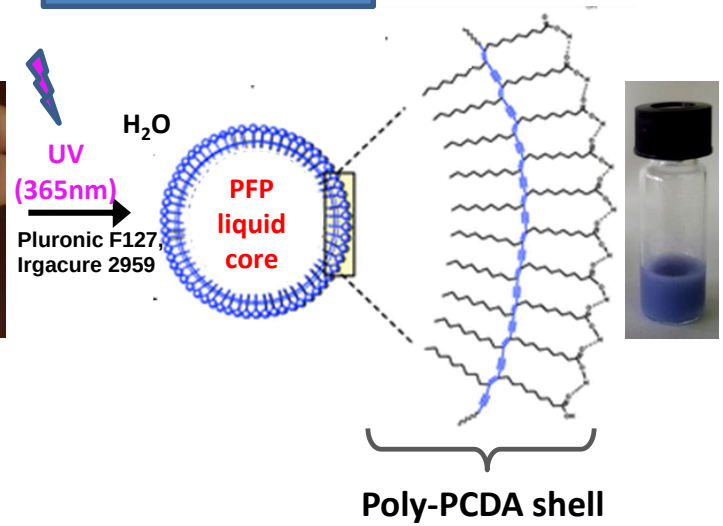


PCDA-PFP self assembly

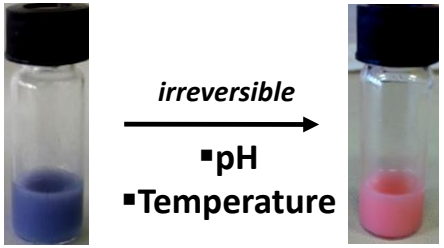
Conversion to monolayer assembling

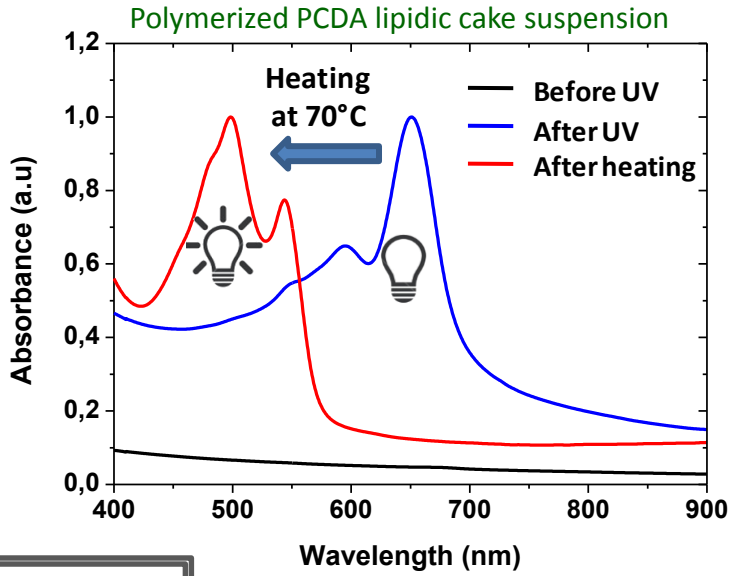


Shell crosslinking

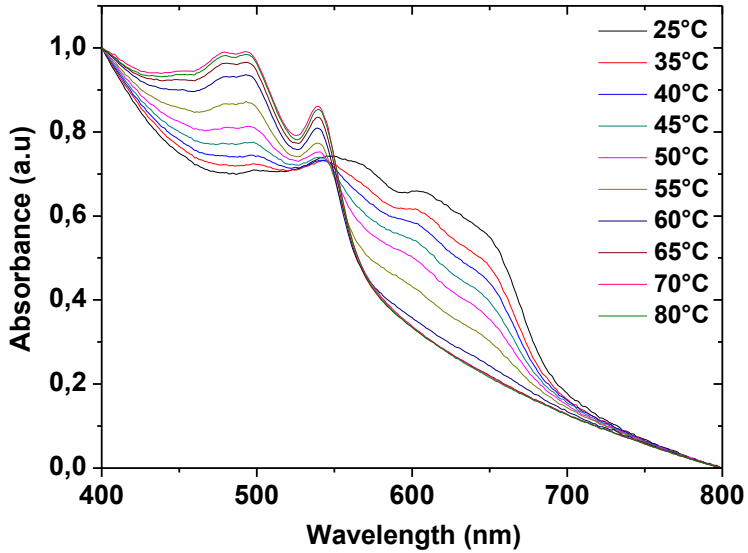


Optical properties of Poly-PCDA droplets

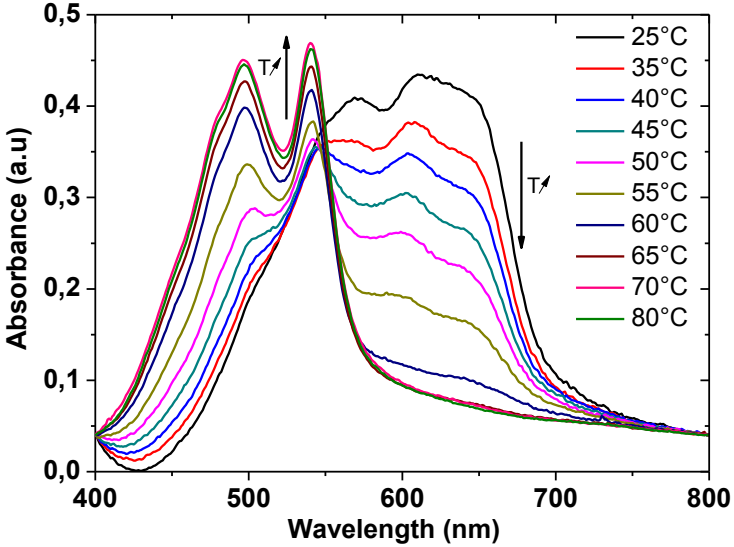




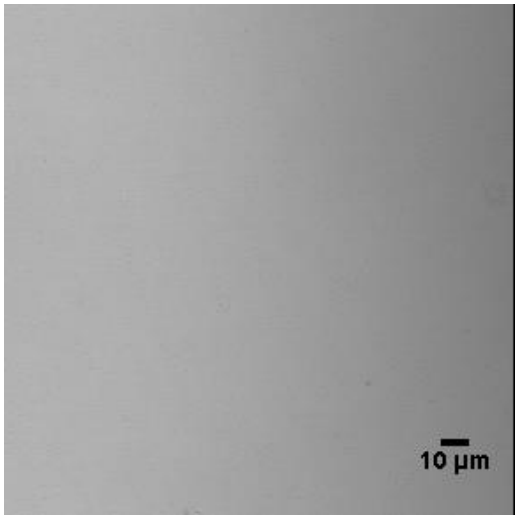
Normalized spectra-without blank correction



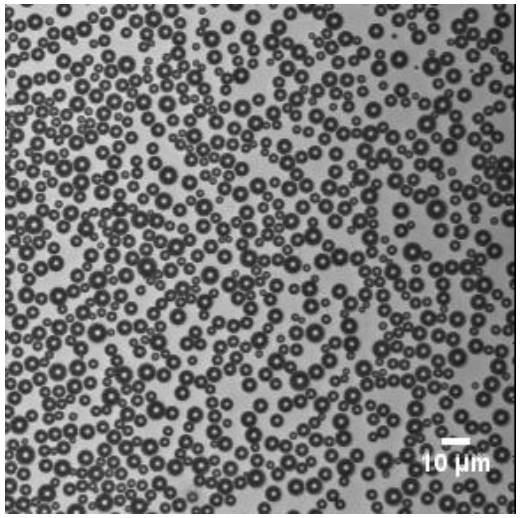
Normalized spectra-Blank subtracted



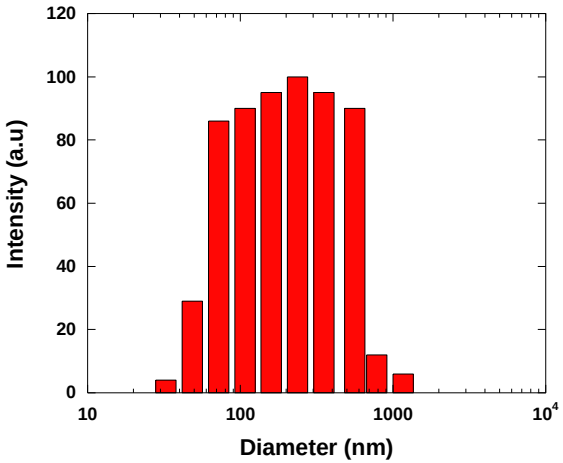
Acoustic Droplet Vaporization

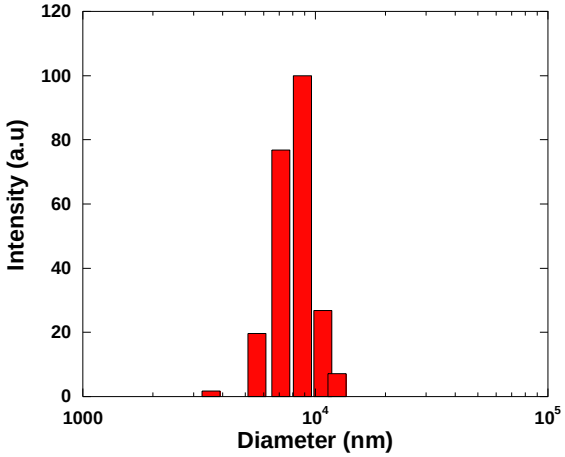


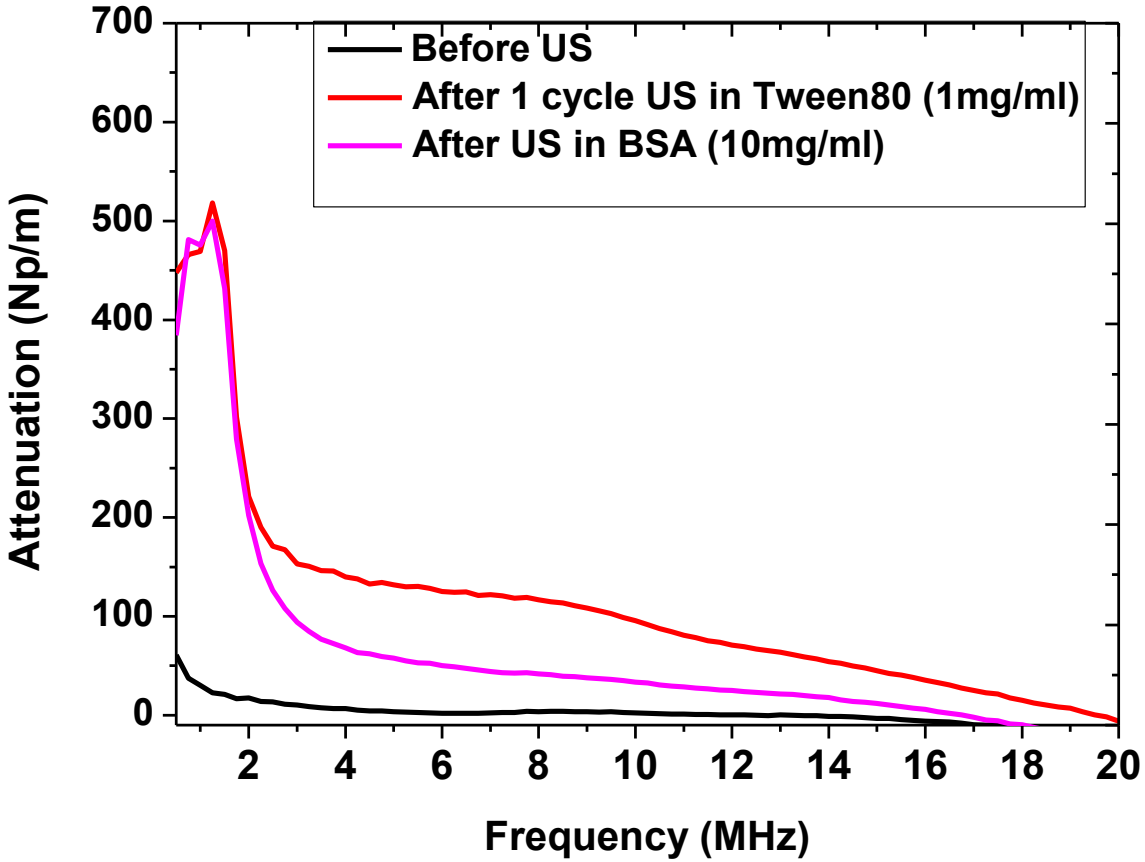
Transmission microscopy (x40)



Transmission microscopy (x40)







The presence of surfactant results in more efficient ADV and significantly much higher acoustic attenuation

Ringraziamenti

2007 - 2010

2010 - 2013

2013 - 2018

Grazie a tutti voi per l'attenzione !!