Nanotechnology and ultrasound

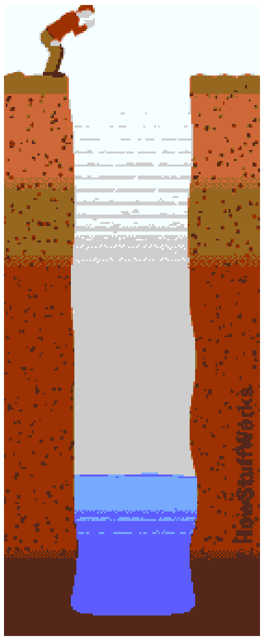
Prof. Theo Pavan

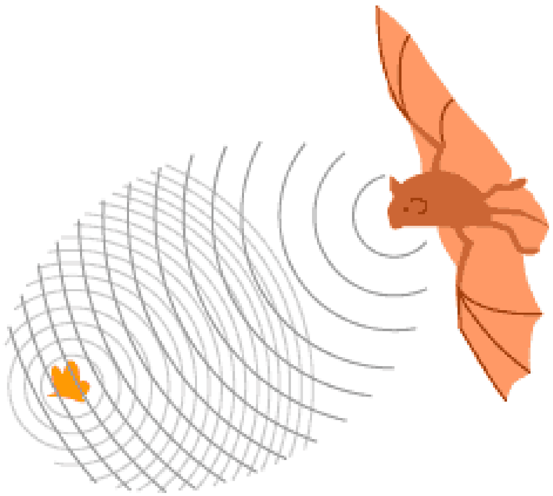
Outline

- Quick introduction about ultrasound imaging
- Contrast enhanced ultrasound with microbubbles
- Molecular images with microbubbles
- Nanobubbles
- Magnetomotive ultrasound
- Therapeutics

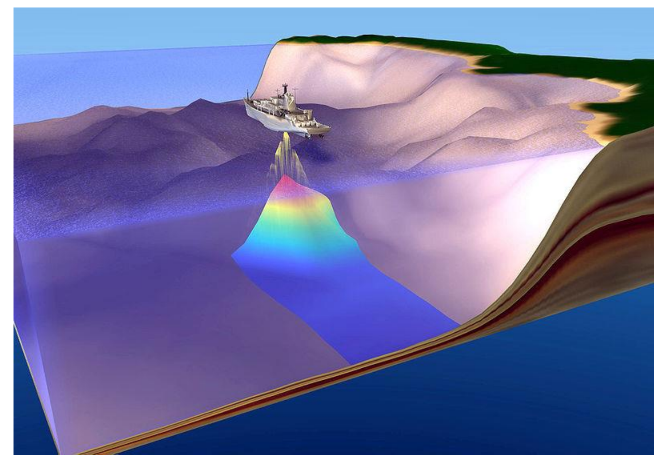
Ultrasound

Audible sound





Ultrasound ~ kHz

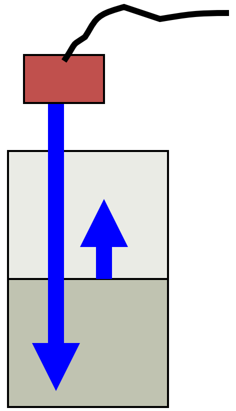


- **Medical ultrasound 1 to 15 MHz.**

Ultrasound


A ultra-sonografia, ou ecografia, é um método diagnóstico que aproveita o eco produzido pelo som para ver em tempo real as reflexões produzidas pelas estruturas e órgãos do organismo
Aparelho de ultra-som
Transdutor
Eco
Onda incidente
Freqüência de 2 até 14 Mhz
Ecografia
Os ecos gerados são interpretados através de computação gráfica. Quanto maior a freqüência, maior a resolução obtida

Impedância acústica (Z_a)



O eco só surge quando o feixe de ultrassom passa por dois meios com diferentes impedâncias.

$$Z = \rho . v$$

- **Z - impedância acústica**
- **ρ - densidade do meio**
- **V - velocidade do som nesse meio**

Impedância acústica

Body Tissue	Acoustic Impedance (10^6 Rayls)
Air	0.0004
Lung	0.18
Fat	1.34
Liver	1.65
Blood	1.65
Kidney	1.63
Muscle	1.71
Bone	7.8

Rayl → kg/(m².s)

Homenagem a Lord Rayleigh

Tipos de espalhamento

Vamos classificar os espalhadores em 3 classes:

Classe 1:

• Causado por concentração de uma ou mais dezenas de espalhadores por célula de resolução; **ka<<1** (produto do número de onda pelo raio do espalhador).
• É difusivo**.**
• Origem dos **speckles**. Agregados; efeitos combinados.

Classe 2:

• Causado por espalhadores com concentração de unidades por célula de resolução.
•Espalhadores dão origem a espalhamento difrativo (acho que inventei esse termo☺)**.**
•Espalhadores são independentes e distinguíveis.

Classe 3:

• Espalhadores dão origem a espalhamento especular. **ka>>1.**
• Associado aos limites de órgão e vasos calibrosos.

Tipos de espalhamento

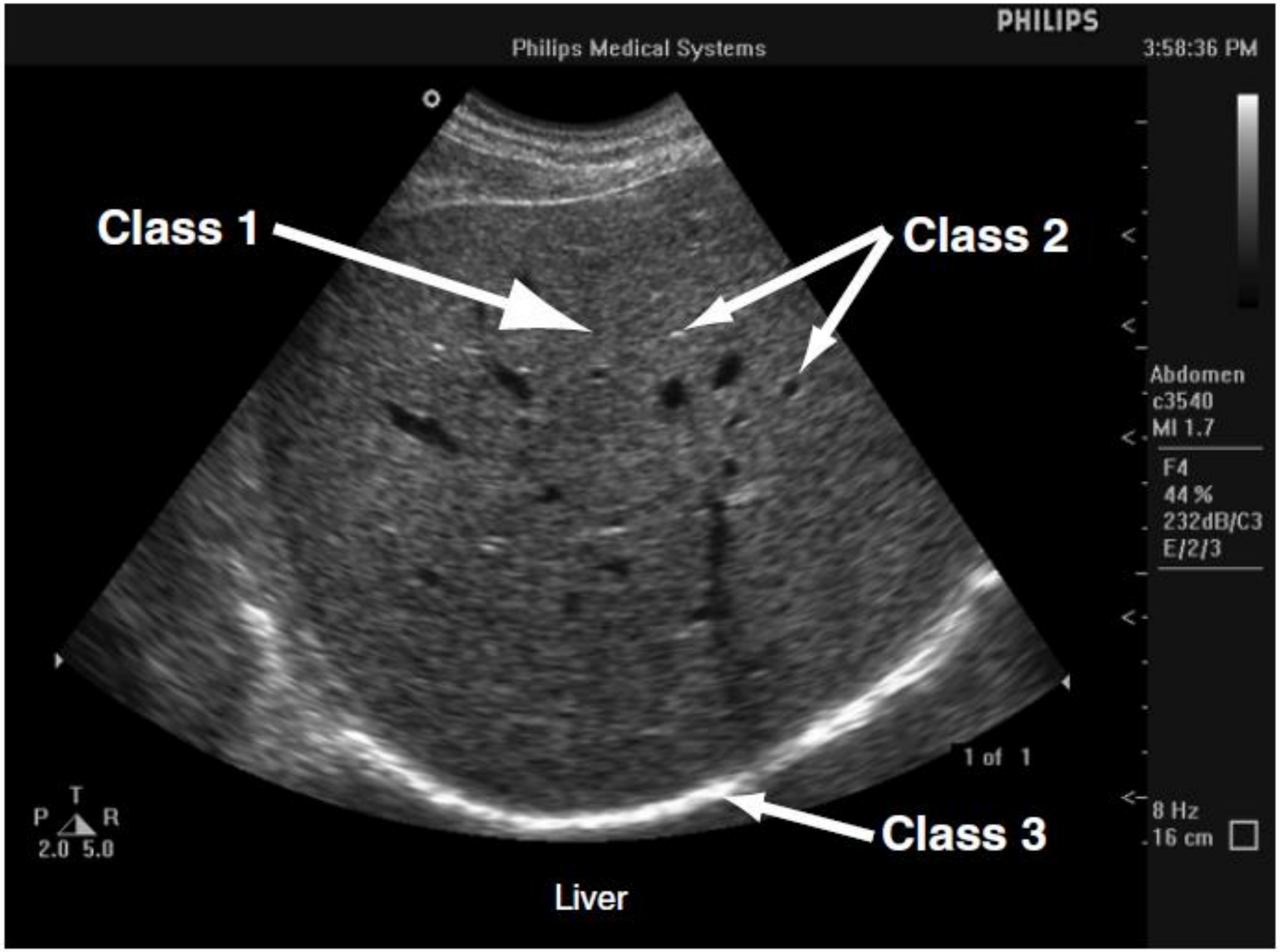


Figure 9.1 Ultrasound image of a liver showing four types of scattering effects (courtesy of Philips Medical Systems).

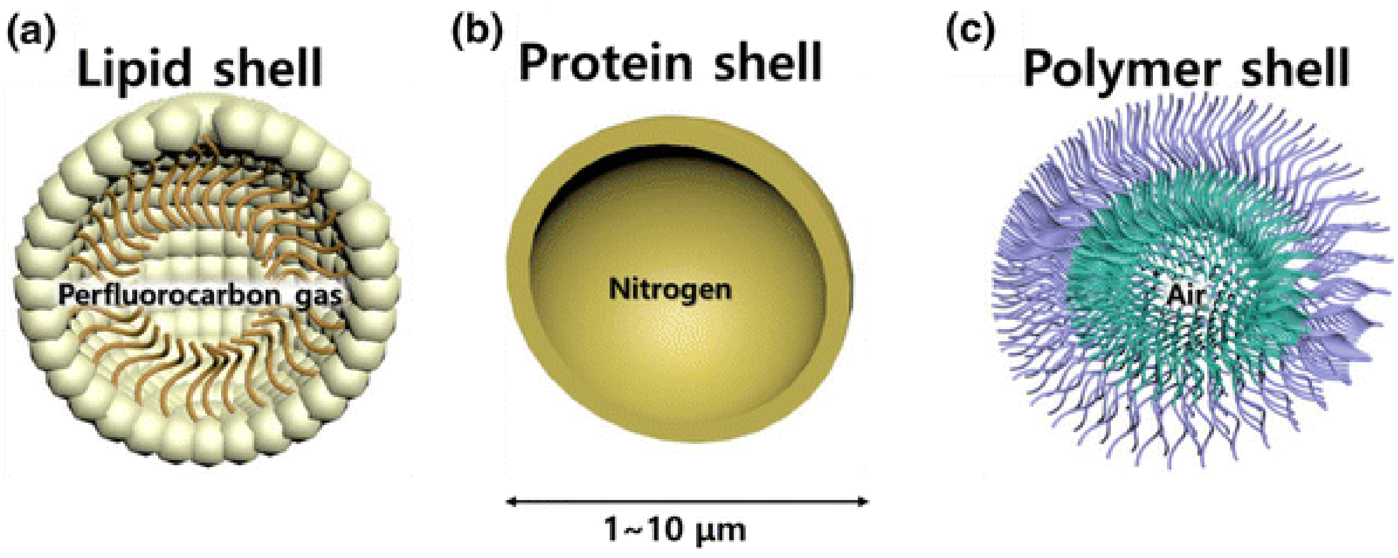
Contrast-enhanced ultrasound

- Contrast-enhanced ultrasound can be used to image blood perfusion in organs, measure blood flow rate in the heart and other organs, and for other applications.
- Commercially available contrast media are gas-filled microbubbles that are administered intravenously to the systemic circulation.
- Microbubbles have a high degree of echogenicity (the ability of an object to reflect ultrasound waves).

https://en.wikipedia.org/wiki/Contrast-enhanced_ultrasound

Microbubble

- Microbubbles generally consist of a shell that surrounds a core gas. Materials that often comprise microbubble shells include lipids, proteins, and polymers. Air, nitrogen, and perfluorocarbon are typically used as the core gas.



Biomed. Eng. Lett. (2017) 7:59–69 DOI 10.1007/s13534-017-0016-5

Microbubbles

- The microbubbles typically range from 1 to 10 μm diameter (red blood cell diameter is 8 μm).
- Permits unhindered passage from the peripheral injection site through the pulmonary vasculature with subsequent entrance into the left heart chambers and access to the systemic circulation.

Microbubble

- When a gas bubble is insonified by a US wave, it generates two kinds of responses.

Large Difference in Acoustic Impedance → **Increase Wave Scattering**

Frinking et al. Ultrasound in Med. & Biol., Vol. 26, No. 6, pp. 965–975, 2000

Microbubble

- More importantly, however, when the bubble size is much smaller than the wavelength of the US wave, it is forced into volume pulsation (for a 3-MHz US wave, the wavelength in water is 0.5 mm).

Frinking et al. Ultrasound in Med. & Biol., Vol. 26, No. 6, pp. 965–975, 2000

Frequency dependence – free air bubble

The resonant frequency for a free air bubble (a bubble without a shell), f_r, is related to the radius of the bubble, a , by:

$$f_r = \frac{1}{2\pi a}\sqrt{\frac{3\gamma P_0}{\rho_w}}$$

γ is the ratio of the specific heats at constant pressure and at constant volume of gas and equals 1.4 for air

P_0 is the hydrostatic ambient pressure and equals $1.013 \cdot 10^5$ Pa or $1.013 \cdot 10^6$ dyn/cm^2 at 1 atm

ρ_w is the density of the surrounding medium, e.g., water

K. Kirk Shung, Diagnostic Ultrasound: Imaging and blood flow measurements, 2006

Frequency dependence – free air bubble

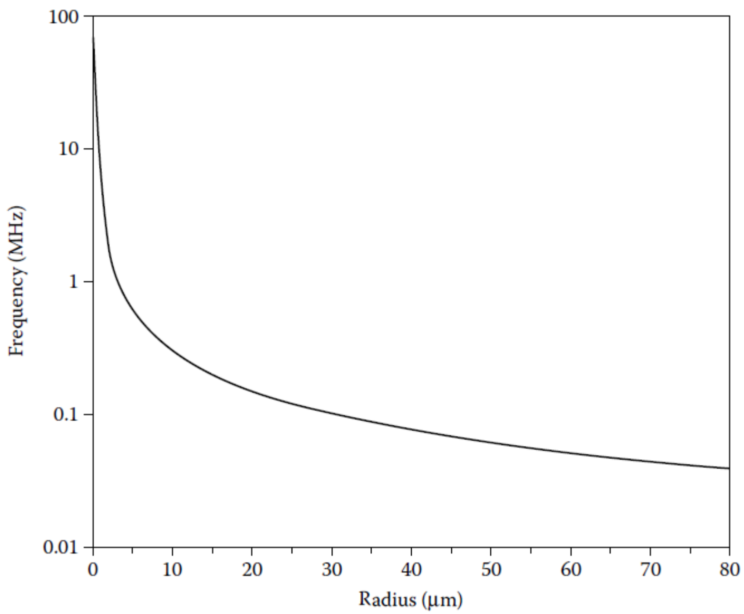


FIGURE 7.1 Calculated resonance frequency vs. radius of a free air bubble.

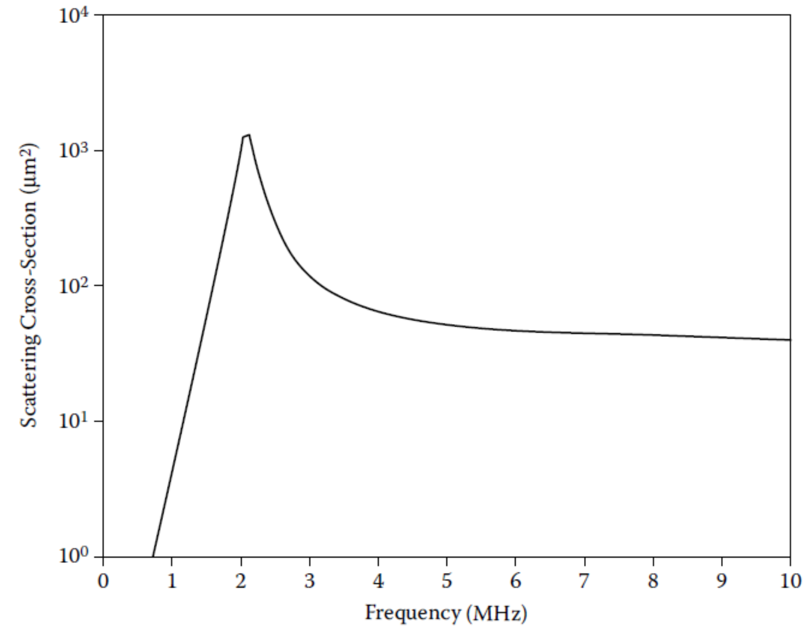


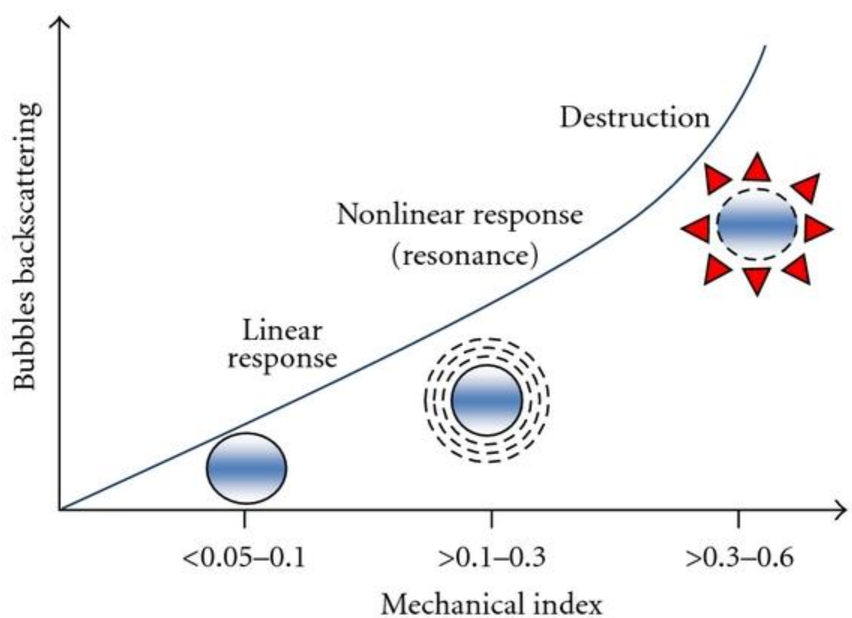
FIGURE 7.2 Calculated scattering cross-section for a free bubble of 1.7-μm radius.

The shell composition is also a key determinant for microbubble's physical properties as well as their acoustic behavior and imaging time.

K. Kirk Shung, Diagnostic Ultrasound: Imaging and blood flow measurements, 2006

MB – US interaction

- The response of a gas bubble to a US wave depends on the acoustic pressure amplitude and can be divided into three regimens.



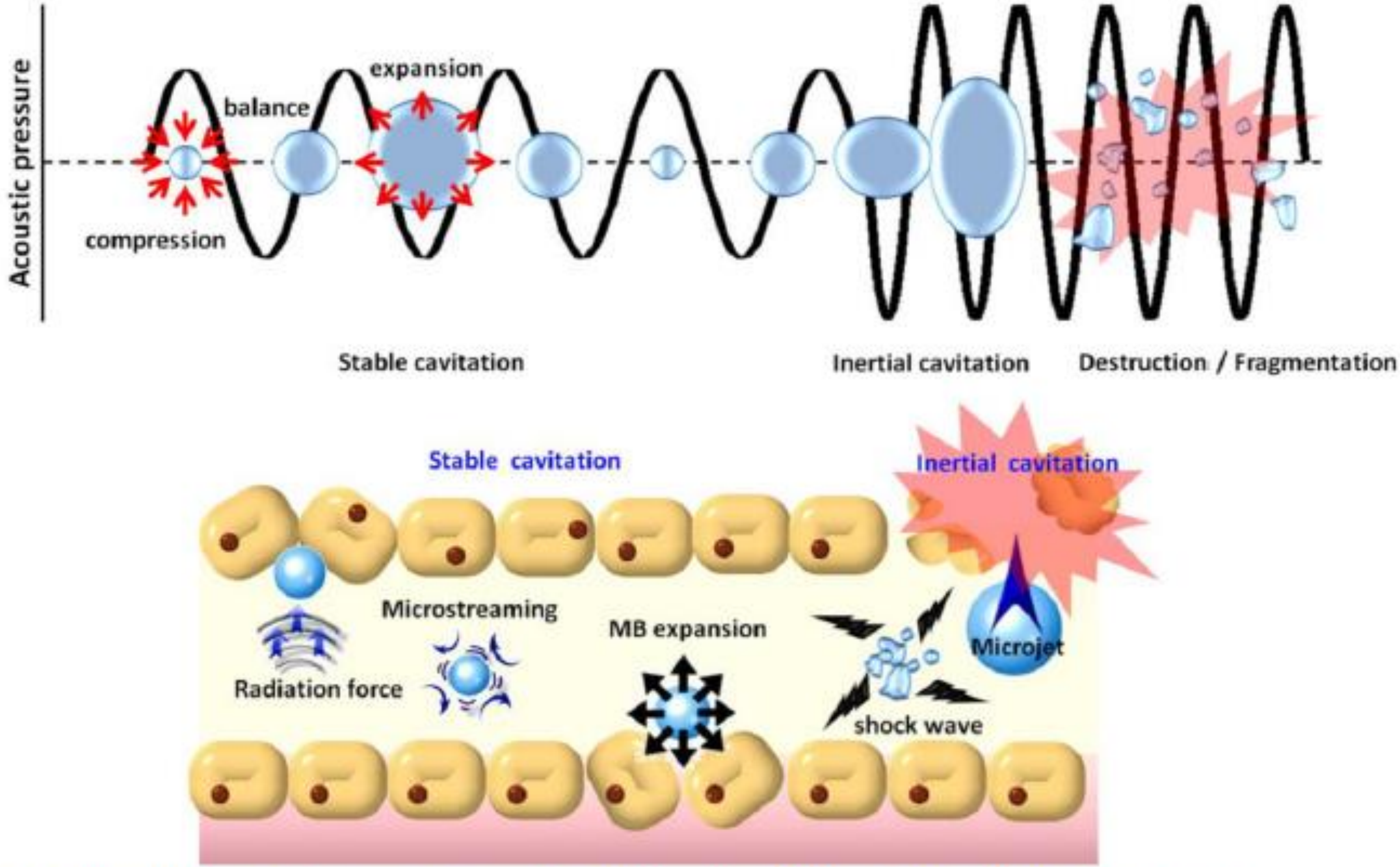


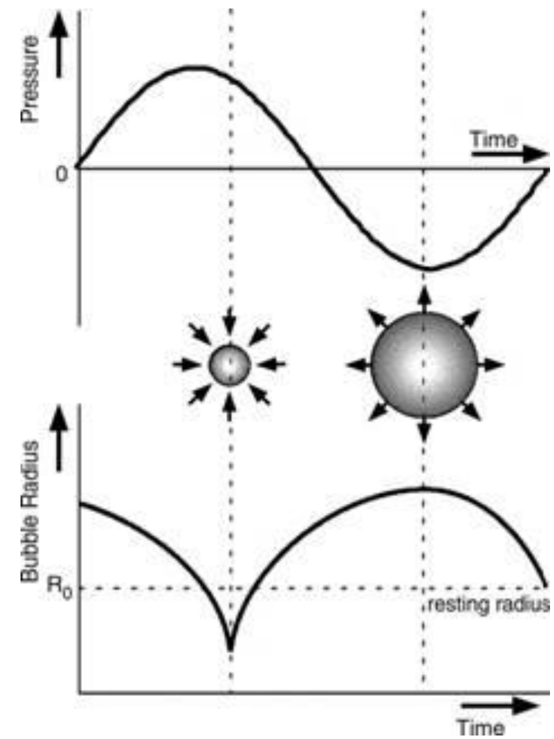
Fig. 2. Physical mechanisms underlying the biological effects induced when microbubbles are excited by ultrasound energy.

Lui et al. Theranostics 2014, Vol. 4, Issue 4

Nonlinear effect

- For higher amplitudes, compression generally retards relative to expansion and nonlinearity occurs.
- Bubble size is not linearly related to the applied acoustic pressure.

Because of the finite compressibility of the entrapped air.



Frinking et al. Ultrasound in Med. & Biol., Vol. 26, No. 6, pp. 965–975, 2000

http://bme240.eng.uci.edu/students/08s/chiashel/results.htm

Nonlinear effect

- Bubble vibration contains second and higher multiples of the transmitted frequency.
- The backscattered signal from the bubble not only contains the fundamental (transmitted) frequency, but also harmonic frequencies, most notably at twice the fundamental frequency.

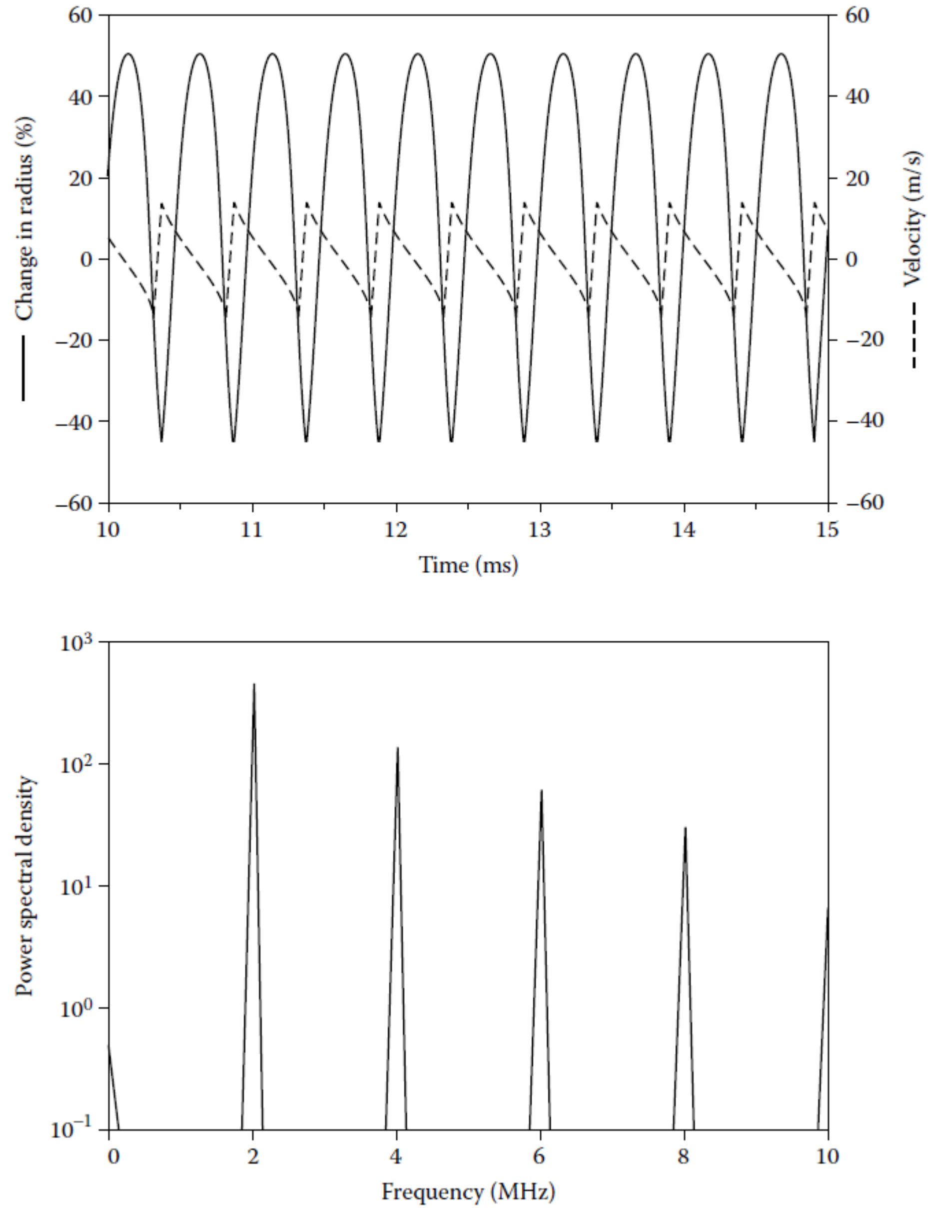
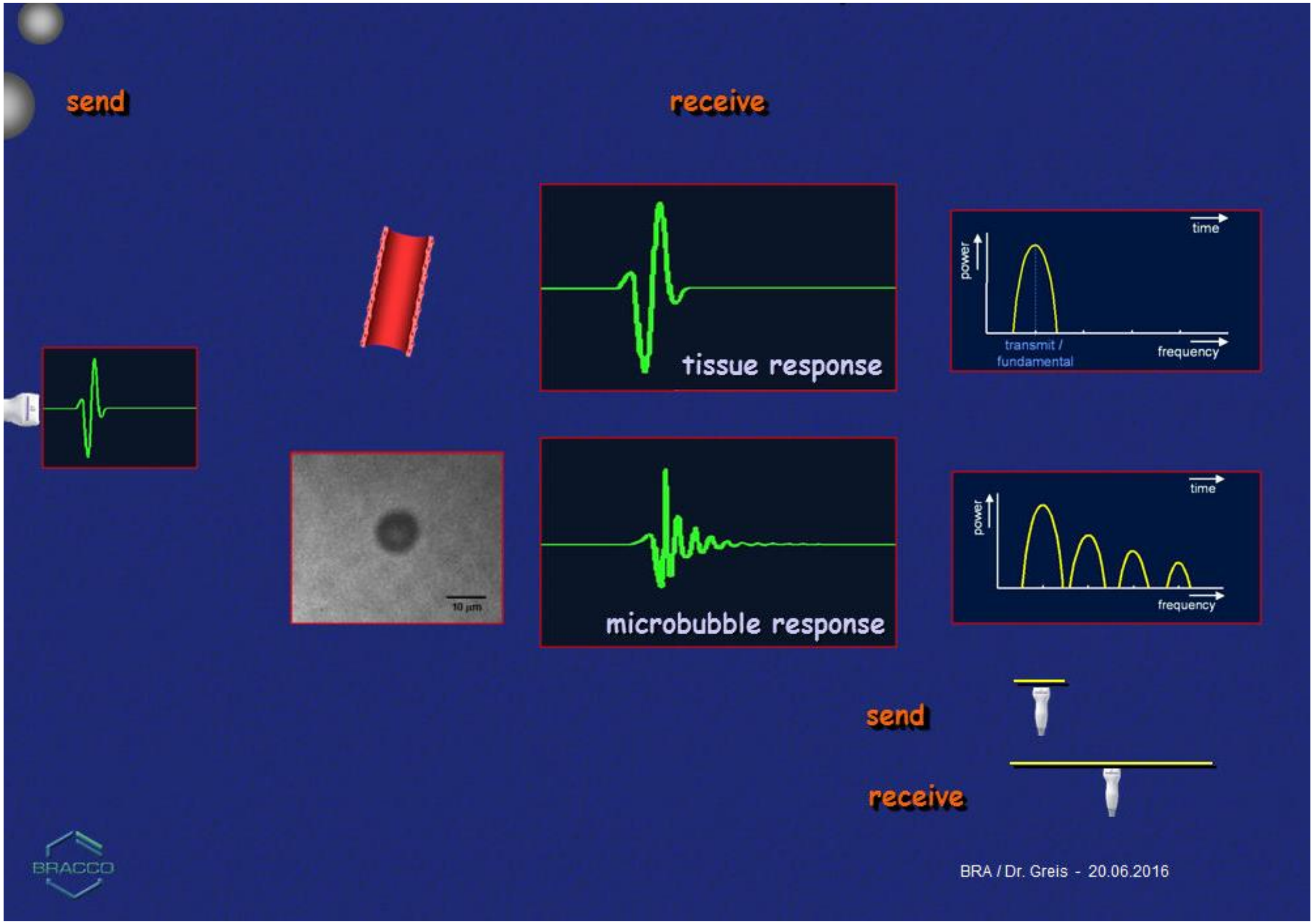


FIGURE 7.5 Response of an air bubble driven at its resonant frequency by an ultrasonic wave of 40 kPa. Top: changes in radius and velocity of bubble wall. Bottom: corresponding power spectra.

Microbubble



http://bme240.eng.uci.edu/students/08s/chiashel/results.htm

http://www.contrastultrasound-modality.com/contrast-ultrasound/what-microbubble/microbubbles-non-linear-oscillation

Harmonic imaging

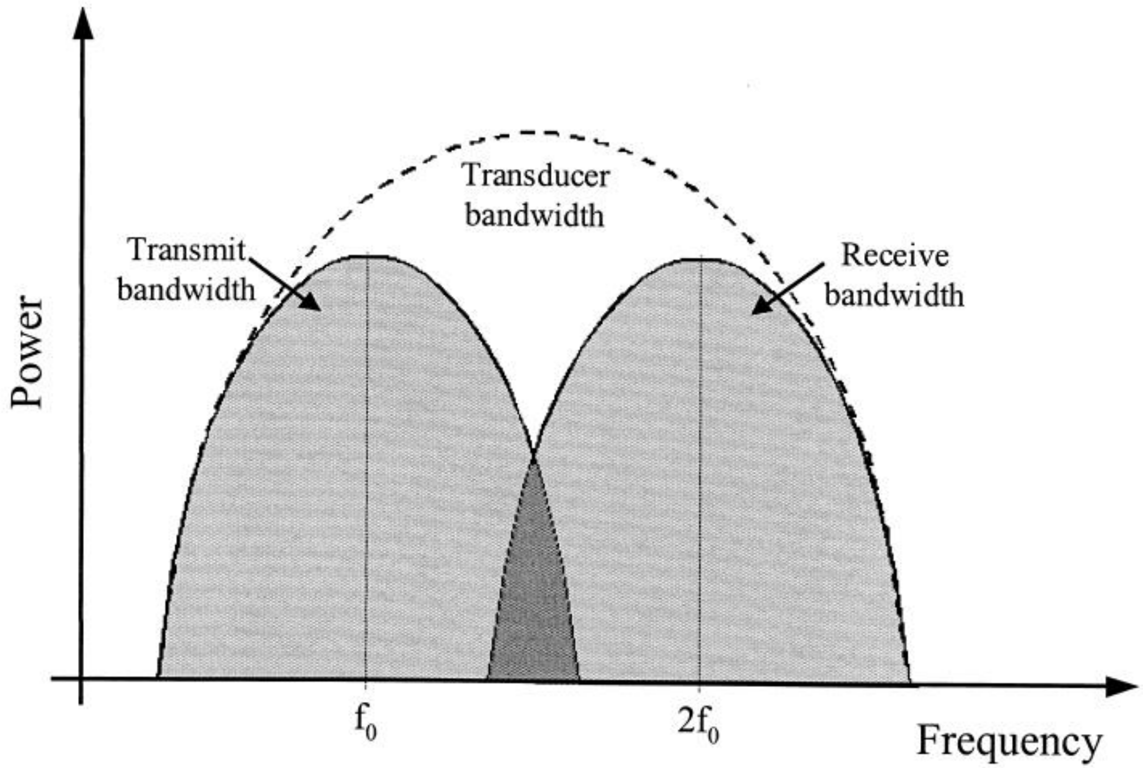
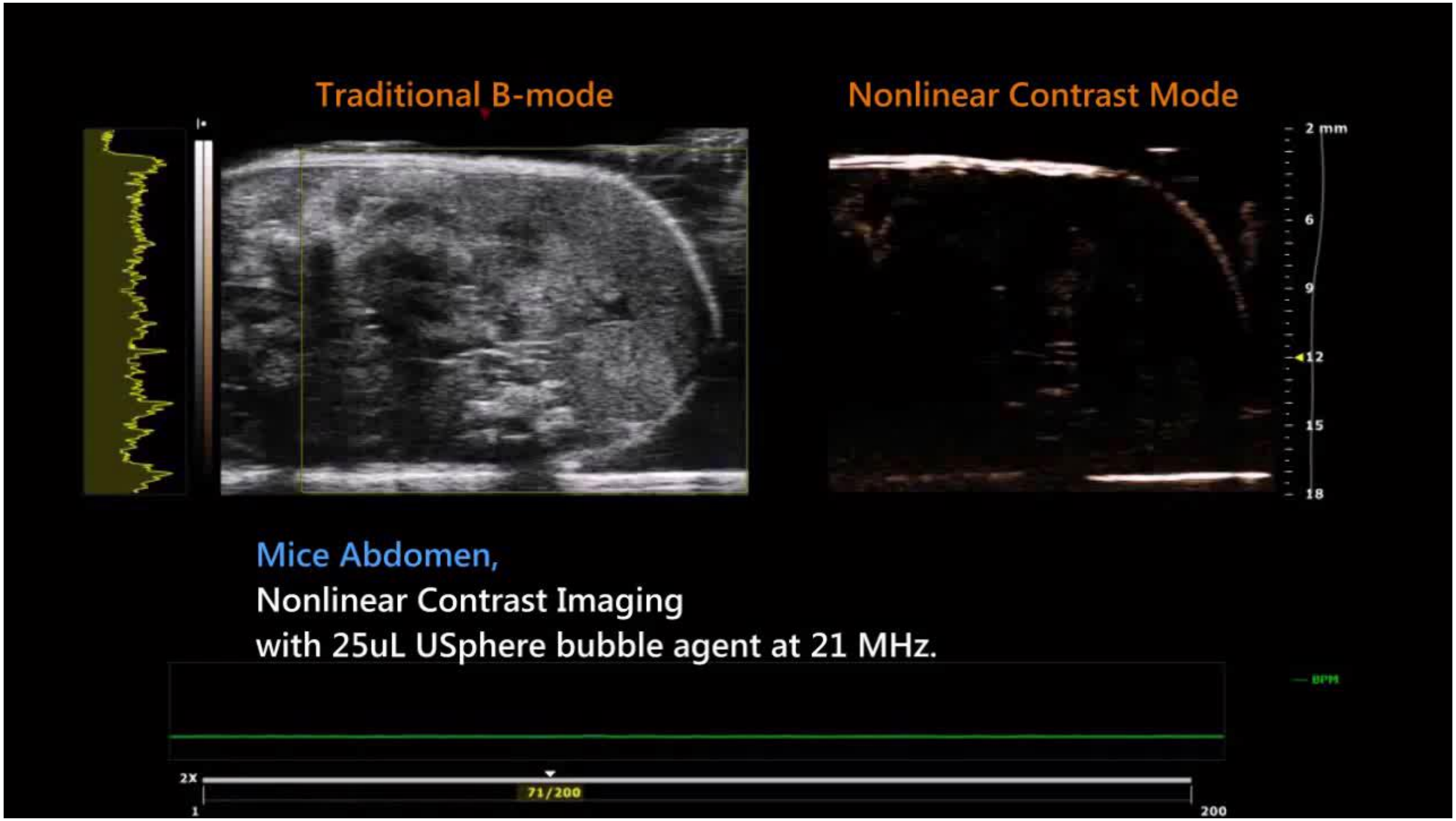


Fig. 2. Overlap between transmit (f_0) and receive ($2f_0$) passbands (dark grey area) results in a residual signal of the fundamental image in the filtered harmonic image.

Harmonic vs B-mode



Example

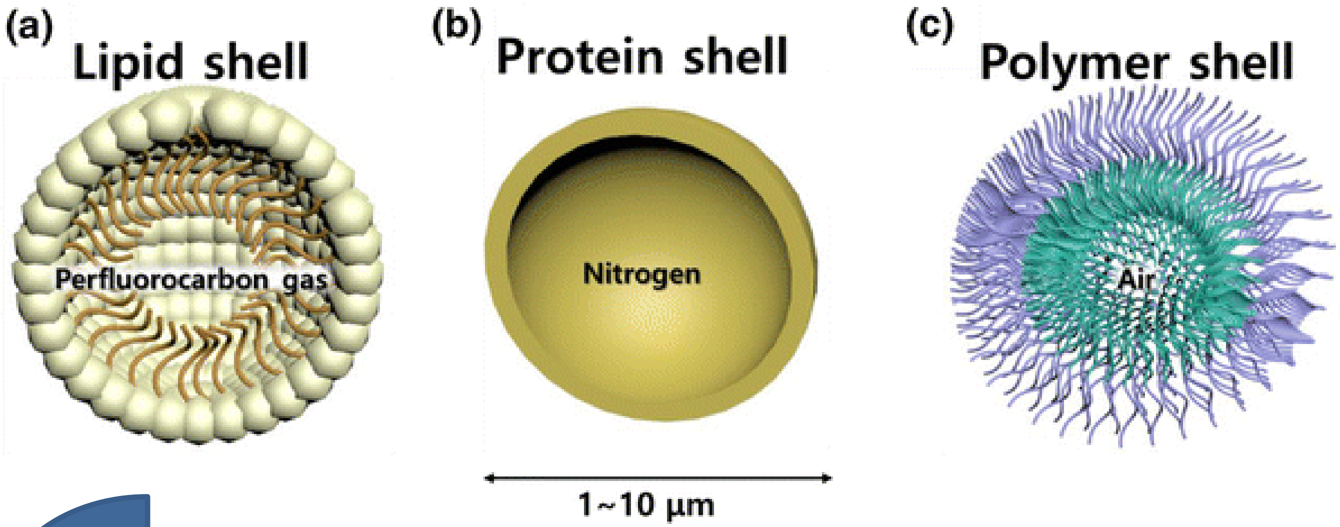
Focal Nodular Hyperplasia with contrast SonoVue

Current status

- The first commercial UCAs became available in the 1980s and included Echovist (1982) and Levovist (1985), which were available in Europe, Japan, and Canada.

- Albunex, the first commercial agent approved by the US - FDA was subsequently released in the USA in 1994. It is an albumin-coated and air-filled microsphere.

Current status



- PFC provides more stable MB because it is not soluble in water.
- Longer lifetime (minutes).

Current status

TABLE 1 | Ultrasound contrast agent that have/had been clinically approved.

Name	First approved for clinical use	Shell material	Gas	Application (examples)	Producer/distributor	Countries
Optison	1998	Cross-linked serum albumin	Octafluoropropane	Left ventricular opafication	GE healthcare, Buckinghamshire, UK	US, Europe
Sonazoid	2007	Phospholipid	Perfluorobutane	Myocardial perfusion, liver imaging	GE healthcare, Buckinghamshire, UK/ Daiichi Saniko, Tokyo, JP	Japan, South Korea
Lumason/SonoVue	2001/2014	Phospholipid	Sulphurhexafluoride	Left ventricular opafication, microvascular enhancement (liver and breast lesion detection)	Bracco diagnostics, Milano, Italy	US, Europe, China
Definity/Luminity	2001/2006	Phospholipid	Octafluoropropane	Echocardiography, liver/kidney imaging (Canada)	Lantheus medical Imaging, North Billerica, MA	North America, Europe (approval filed)
Imagent/Imavist	2002, withdrawn	Phospholipid	Perfluorohexane, Nitrogen	Echocardiography, heart perfusion, tumor/blood flow anomalies	Schering AG, Berlin, DE	US
Echovist	1991, withdrawn	Galactose microparticles	Air	Right heart imaging	Schering AG, Berlin, DE	Germany, UK
Levovist	1995, withdrawn	Galactose microparticles, palmitic acid	Air	Whole heart imaging, doppler imaging	Schering AG, Berlin, DE	Canada, Europe, China, Japan
Albunex	1993, withdrawn	Sonicated serum albumin	air	Transpulmonary imaging	Molecular Biosystems Inc., San Diego, CA, USA	Japan, US

Molecular imaging with microbubbles

Molecular analyses are achieved by coupling specific ligands to the bubbles' shell, which bind to marker molecules in the area of interest.

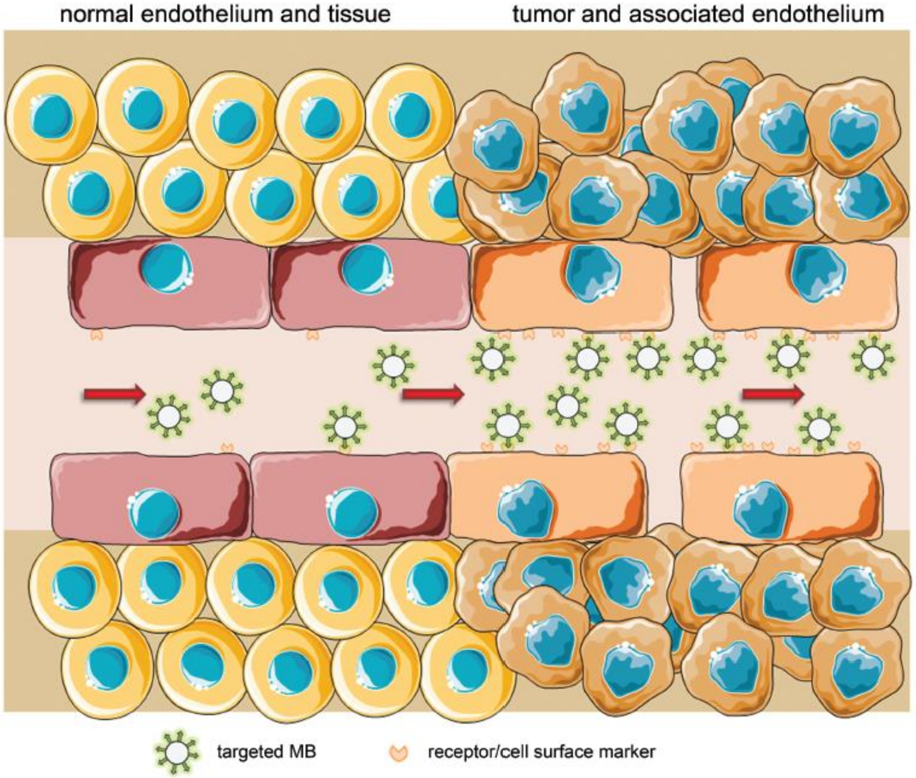
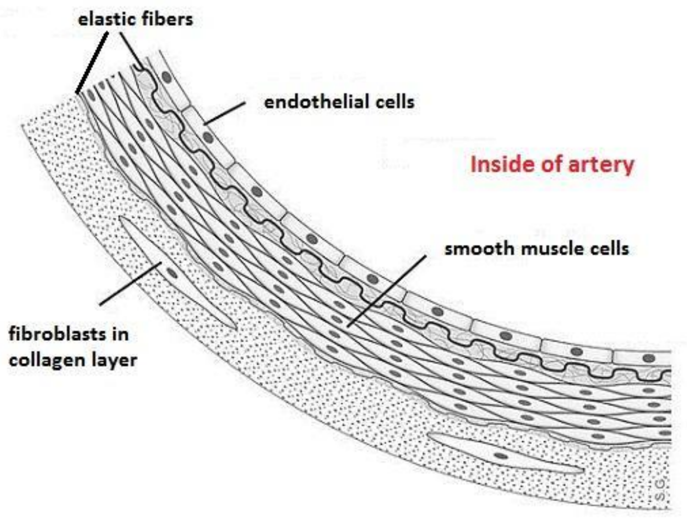


FIGURE 2 | Active targeting by coupling of ligands to MB that bind to structures overexpressed or exclusively expressed on tumor endothelium (schematic illustration, not drawn to scale).

Molecular imaging with microbubbles

- Active targeting requires specific surface modification.
- Since MB are limited to the vascular compartment, their targets need to be expressed on the luminal side of endothelial cells in pathological environments

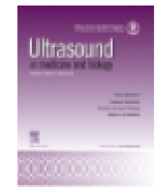


Exemplo de aplicação

ELSEVIER

Ultrasound in Medicine & Biology

Volume 41, Issue 1, January 2015, Pages 197-207



Original Contribution

Molecular Ultrasound Imaging Using Contrast Agents Targeting Endoglin, Vascular Endothelial Growth Factor Receptor 2 and Integrin

Ingrid Leguerney[*], Jean-Yves Scoazec[†], Nicolas Gadot[†], Nina Robin[‡], Frédérique Pénault-Llorca[‡], Steeve Victorin[*], Nathalie Lassau[*]

- The expression levels of three tumor angiogenesis biomarkers (endoglin, integrin and VEGFR2) in a murine melanoma model xenografted in nude mice were evaluatesd.

- Three different types of functionalized microbubbles were used.

Nanobubble

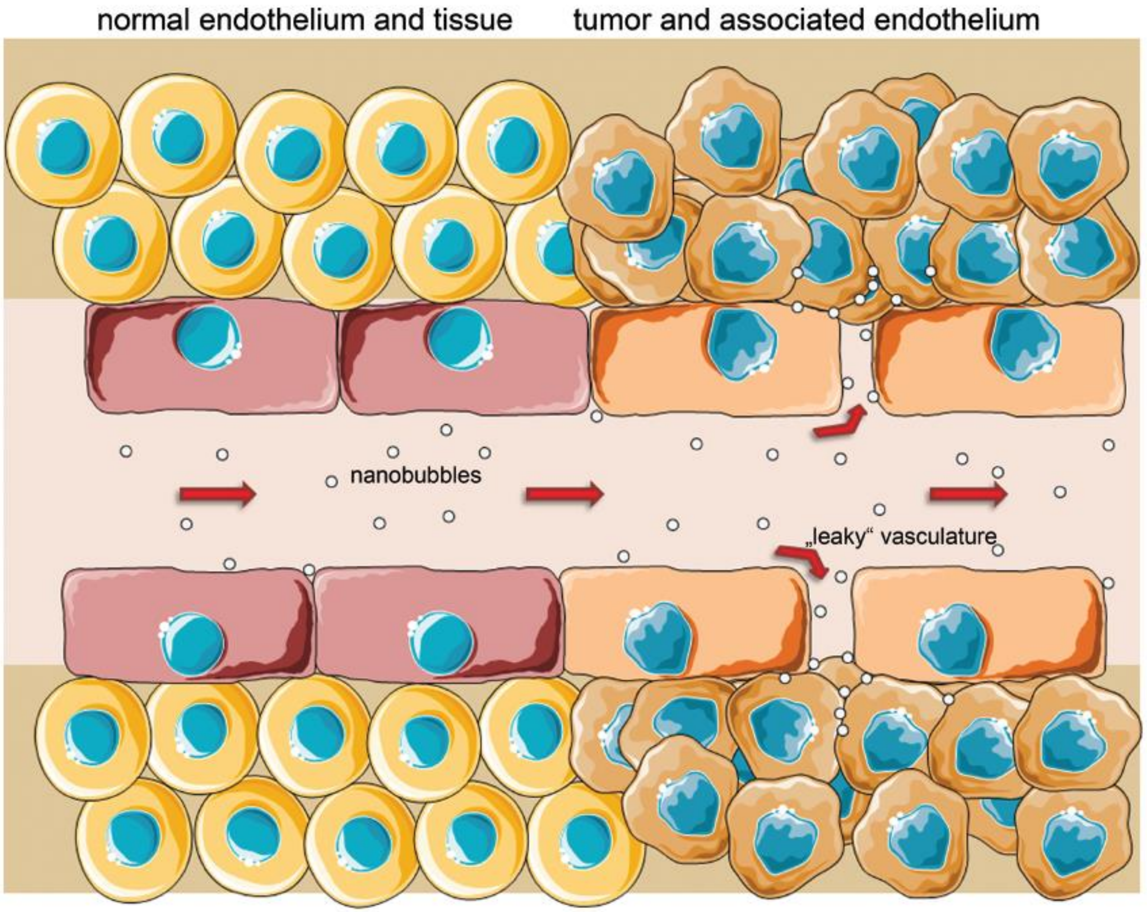


FIGURE 3 | Passive targeting is enabled by 'leaky' vessels with fenestrae up to several 100 nm in tumor-associated endothelium and a poor lymphatic drainage, increasing both likelihood and retention time of nano-sized particles in the interstitium (EPR effect). After extravasation, NB/particles could also actively target specific surface molecules on cancer cells (schematic illustration, not drawn to scale).

International Journal of Nanomedicine

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ORIGINAL RESEARCH

Nanobubbles for enhanced ultrasound imaging of tumors

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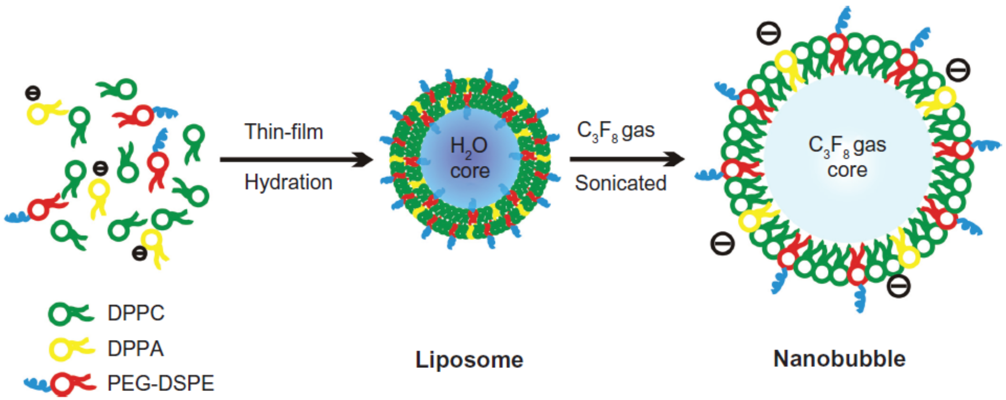


Figure 1 Formation and structural transitions of nanobubbles for ultrasonic imaging and tumor targeting.
Abbreviations: DPPA, 1,2-dipalmitoyl-sn-glycero-3-phosphate; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; PEG-DSPE, 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[biotinyl(polyethylene glycol)2000].

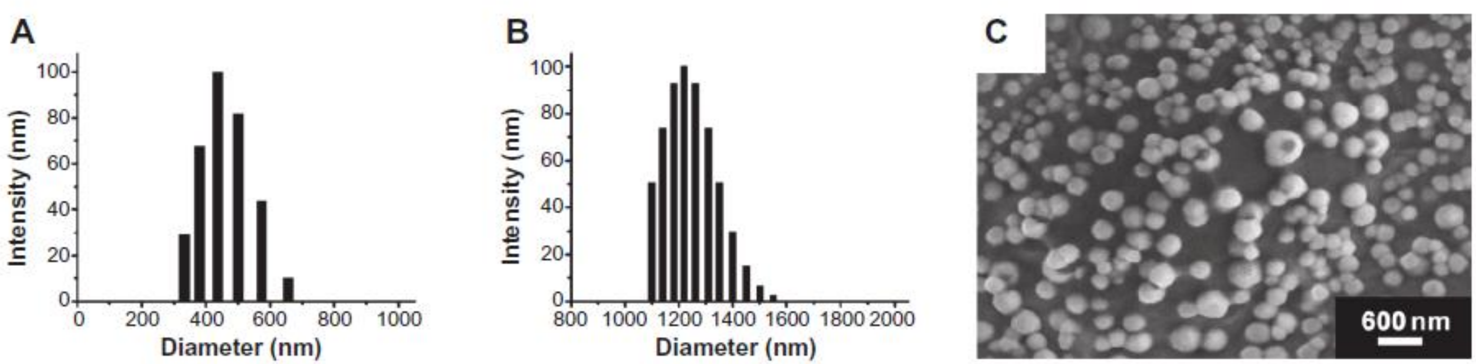
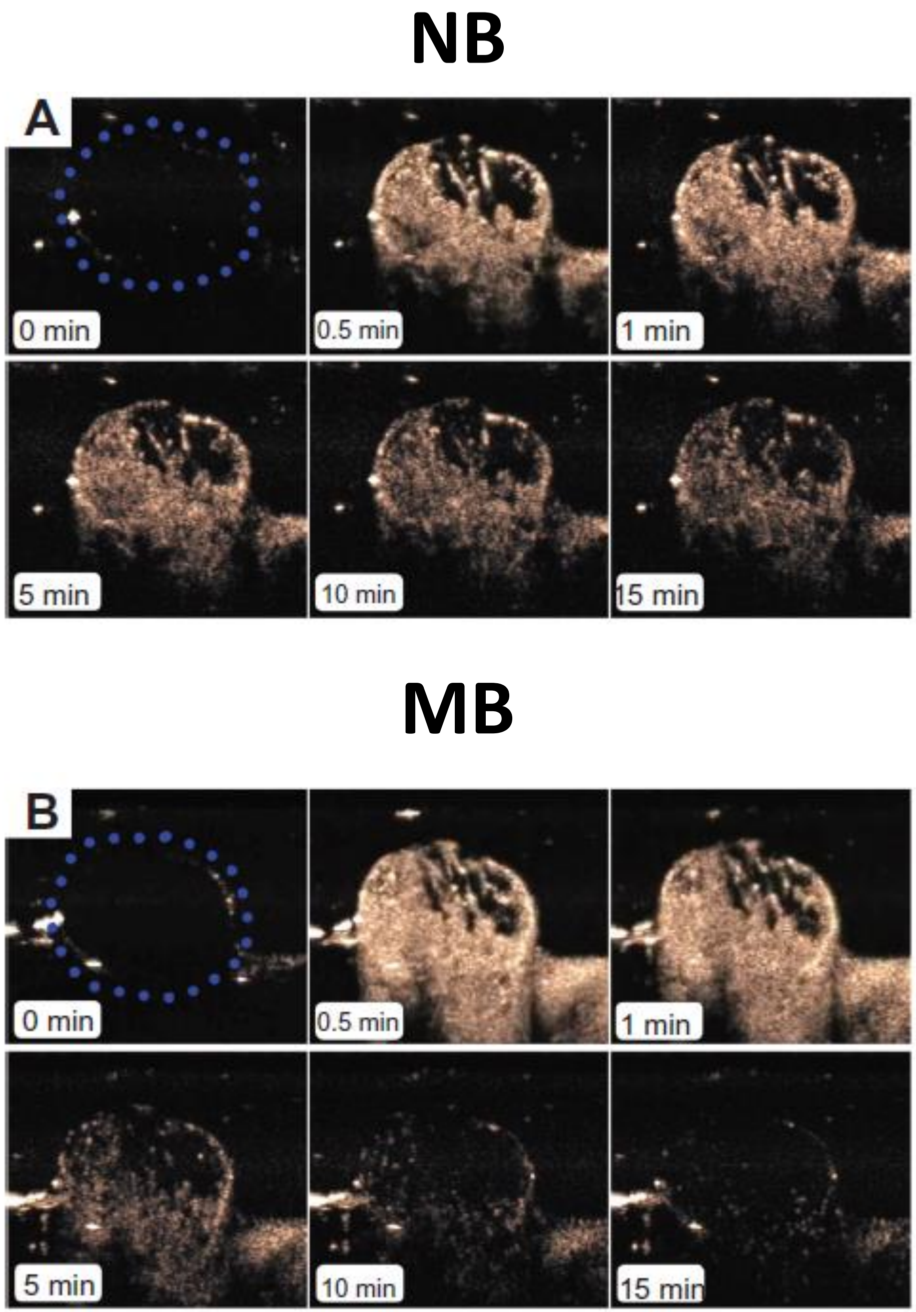


Figure 3 Particle size and morphology of the nanobubbles. The diameter distribution was measured using dynamic light scattering in the nanobubbles (**A**) and microbubbles (**B**). The surface morphology of the nanobubbles was visualized using scanning electron microscopy (**C**).



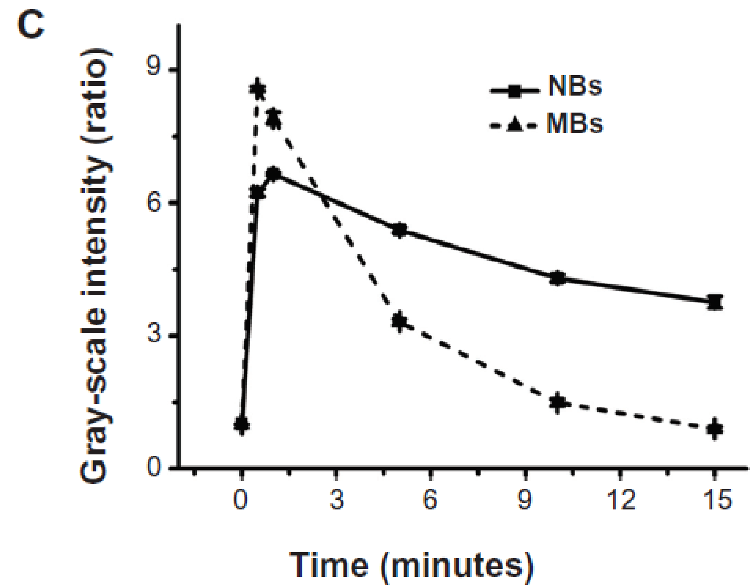


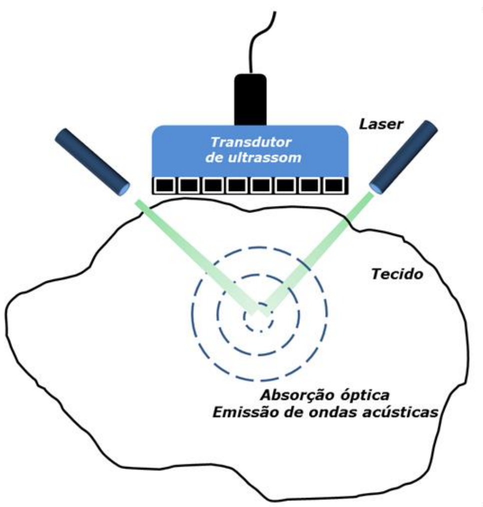
Figure 7 In vivo passive tumor targeting. Representative subcutaneous tumor images before (blue dotted line) and after the injection of nanobubbles (NBs) (**A**) compared with microbubbles (MBs) (**B**) at various time points (0, 0.5, 1, 5, 10, and 15 minutes). The corresponding time–intensity curve of tumor enhancement after injection of the contrast agent (**C**).

Can ultrasound imaging detect Nanoparticles?

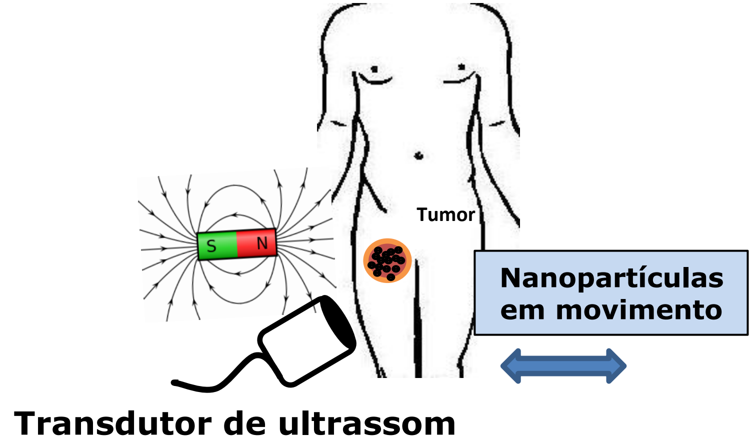
Óptica e Ultrassom

Magnetismo e Ultrassom

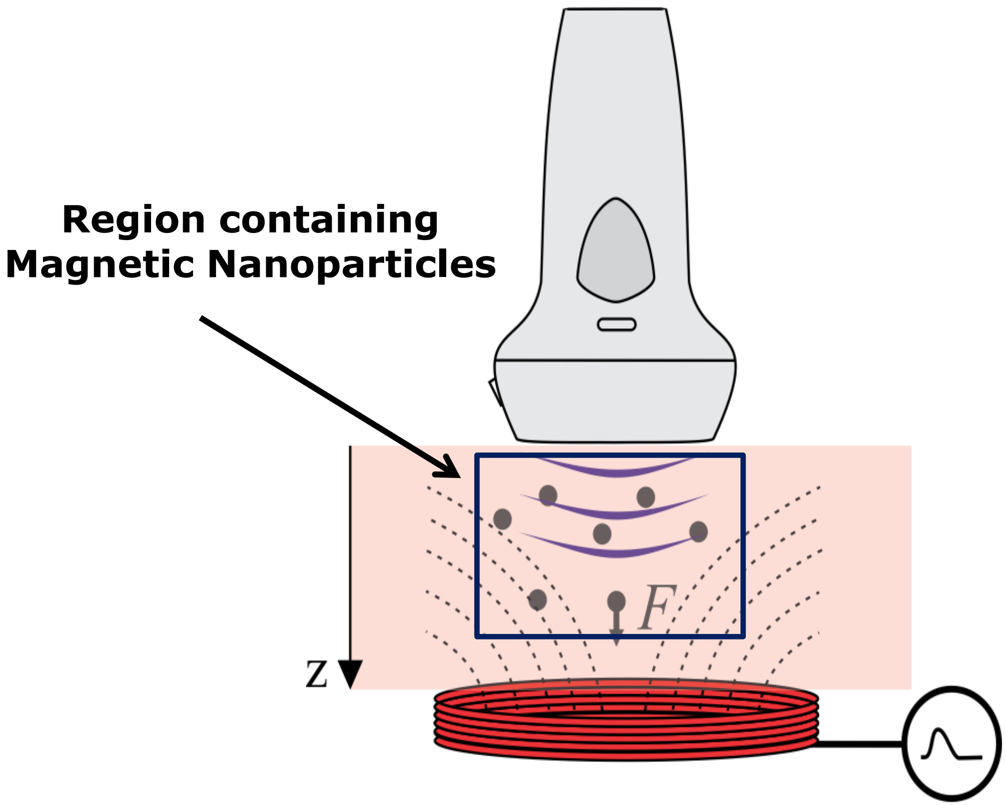
Imagem fotoacústica



Magnetoacustografia



Magnetomotive Ultrasound (MMUS)



$$F(z) = \chi V H \frac{dB_z}{dz}$$

F → magnetic force, χ → susceptibility of MNP, and *V* is the volume of MNP.

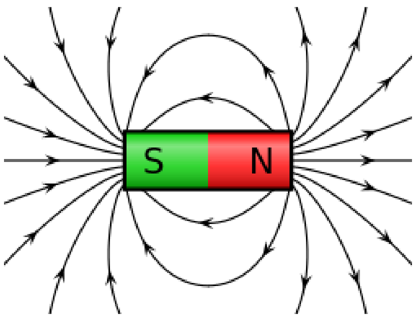
S. A. McAleavey, et. al.*IEEE Transactions on Biomedical Engineering, 2003*

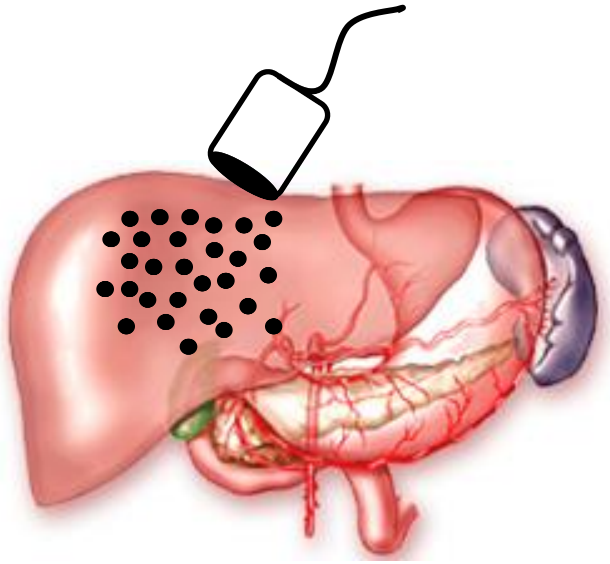
J. Oh, et. al., *Nanotechnology, vol. 17, p. 4183, 2006*

A. C. Bruno, et al; IEEETUFFC, 2013

Magnetomotive ultrasound

Ferromagnetic or superparamagnetic particles are displaced by an external magnetic field gradient





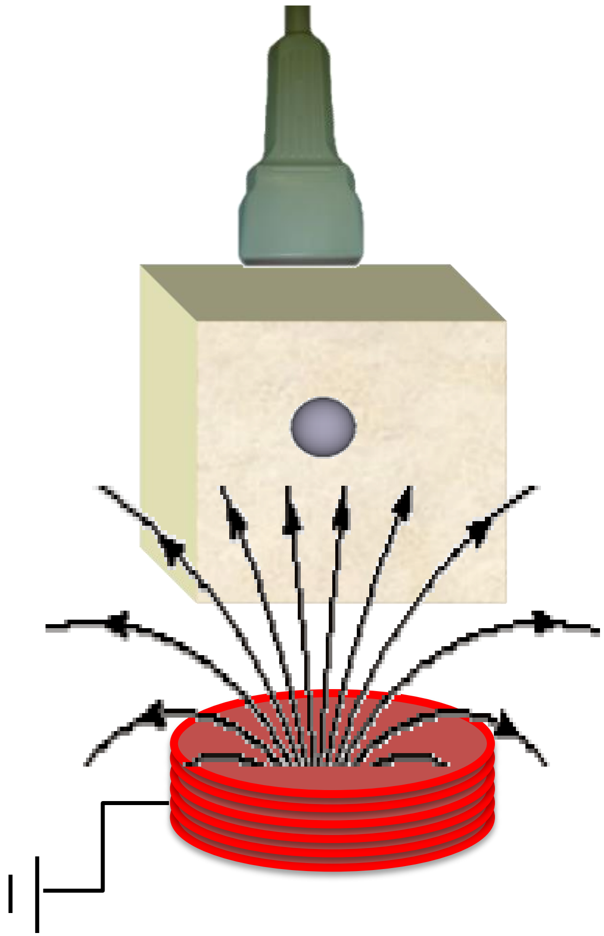
Magnetic field

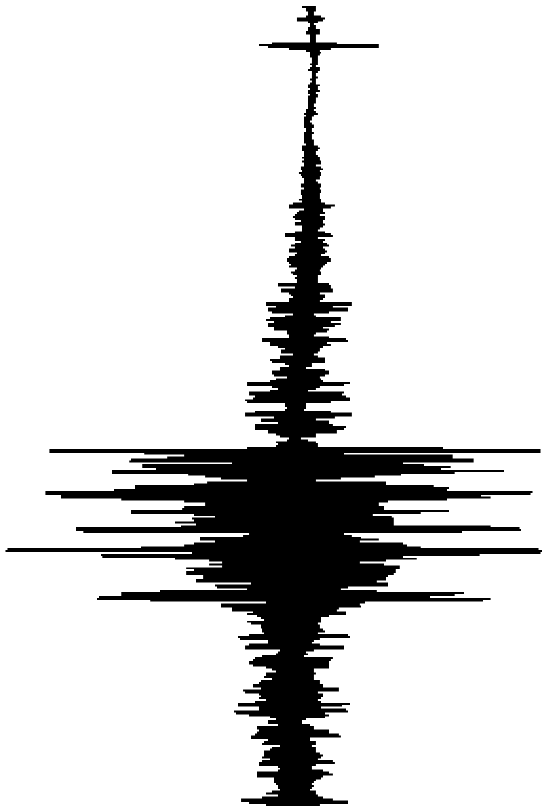
Pulsed | **CW**

Resulting movement is evaluated using ultrasound

S. A. McAleavey, et. al.*IEEE Transactions on Biomedical Engineering, 2003*

Magnetomotive ultrasound





In-vivo study

Ingestion of a test food marked with a magnetic material

⬇

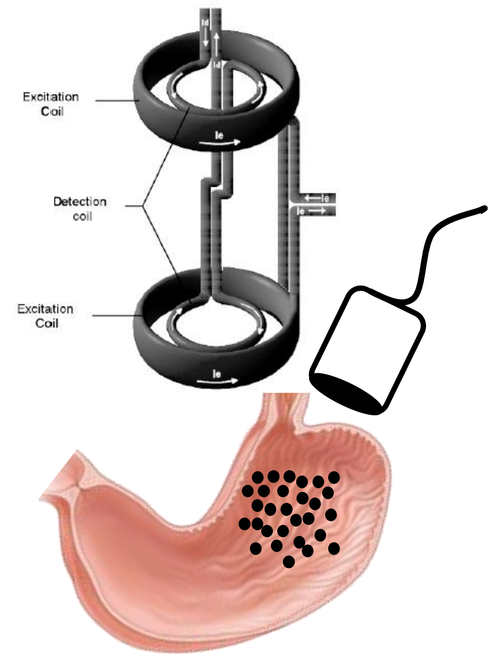
Study gastrointestinal motility

Biosusceptometry → lower spatial resolution

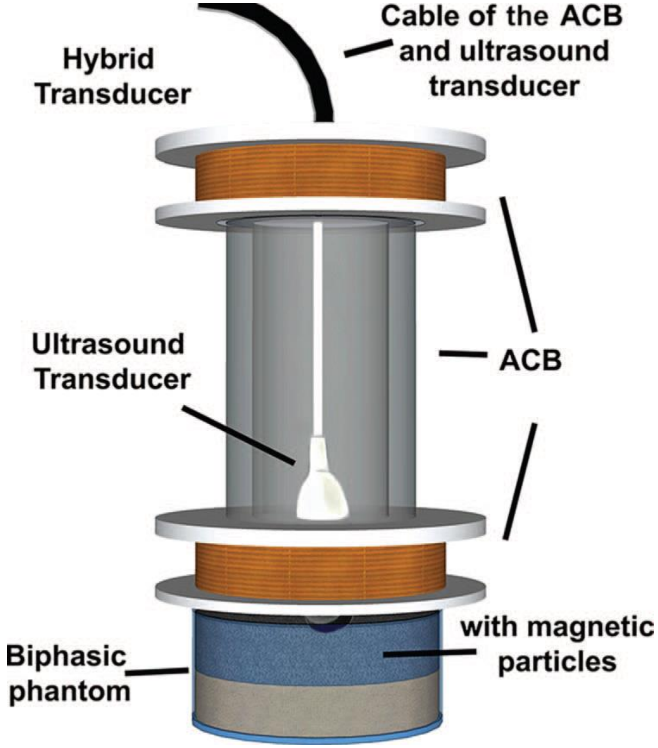
Magneto motive ultrasound

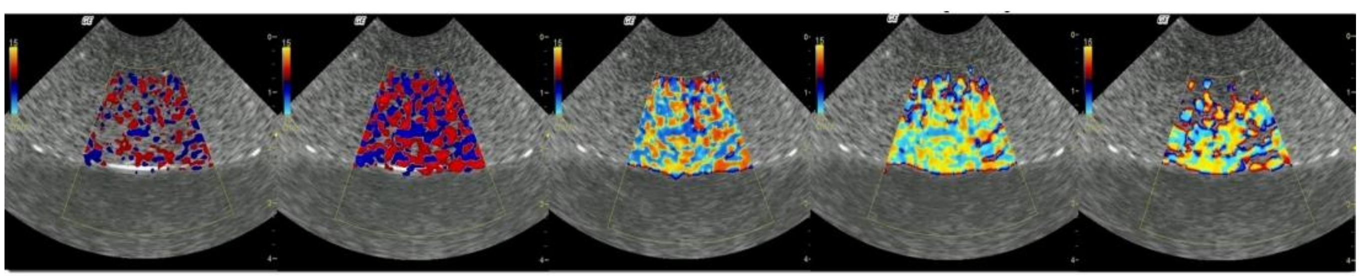
⬇

Spatial information during biosusceptometry



A. C. Bruno, D. T. Sampaio, T. Z. Pavan, O. Baffa and A. A. O. Carneiro, IEEETUFFC, 2015

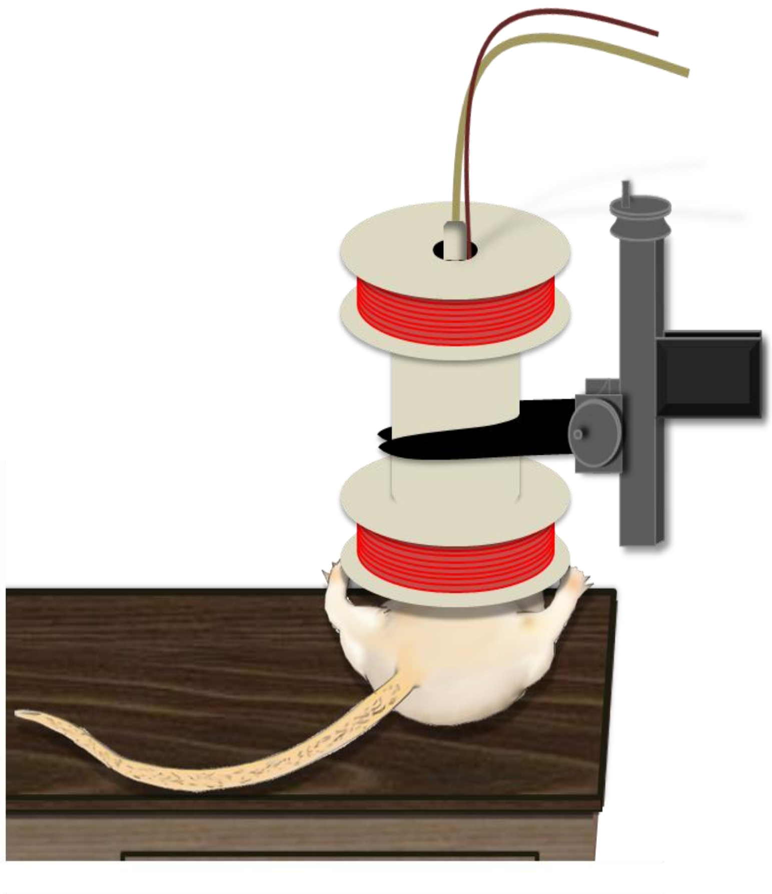




100 Hz 150 Hz 200 Hz 250 Hz 300 Hz

A. C. Bruno, T. Z. Pavan, O. Baffa and A. A. O. Carneiro, IEEETUFFC, 2013

In vivo viability study



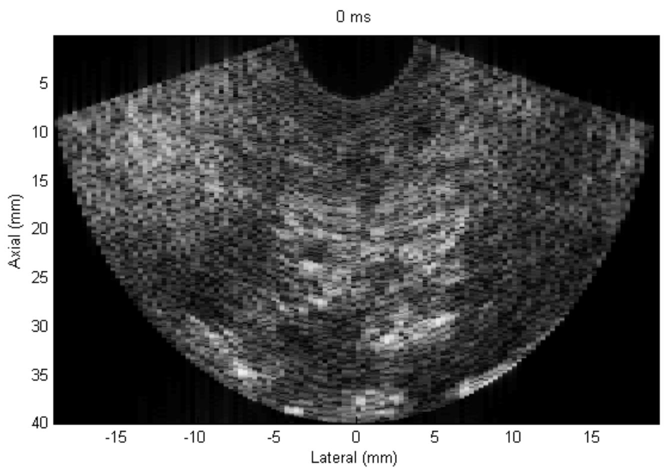
Four male Wistar rats (weighting 300–350 g)

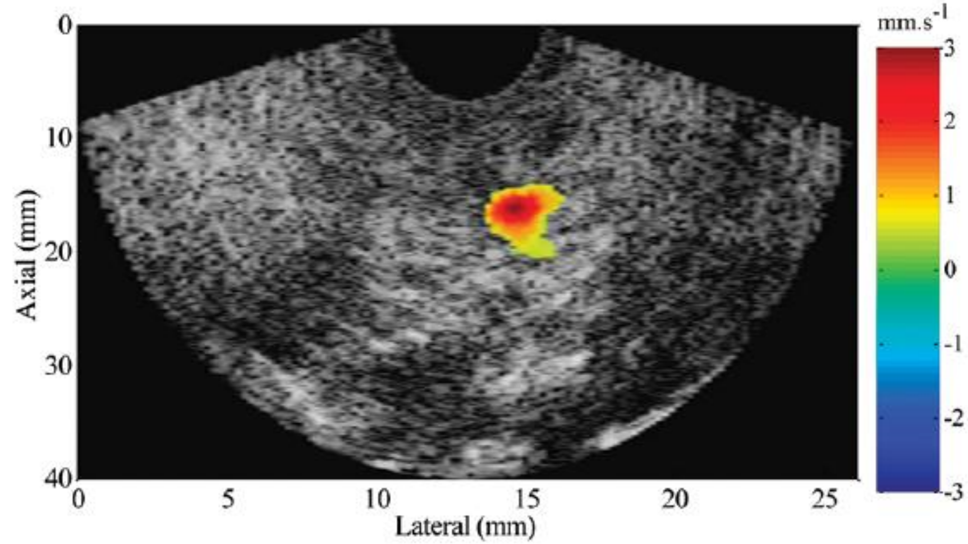
A gavage needle was used to deliver the meal directly into the stomach

Meal →ferrite particles (diameter between 37 and 70 μm) mixed with yogurt.

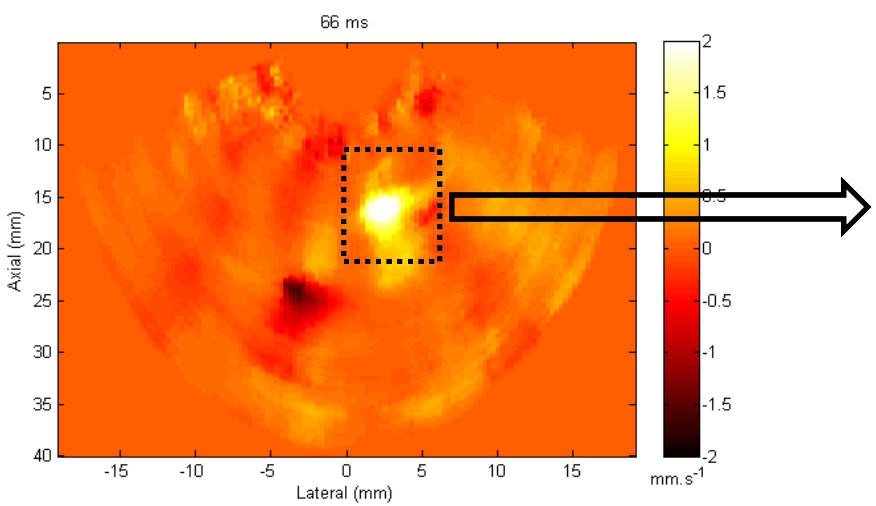
A. C. Bruno, D. T. Sampaio, T. Z. Pavan, O. Baffa and A. A. O. Carneiro, IEEETUFFC, 2015

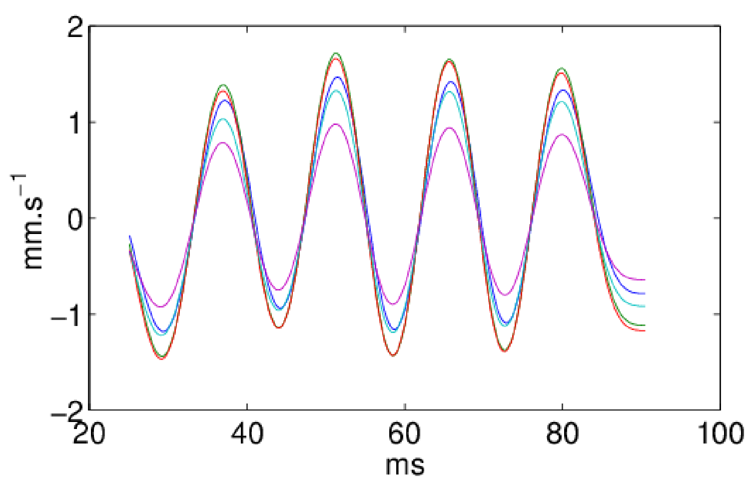
In vivo viability study



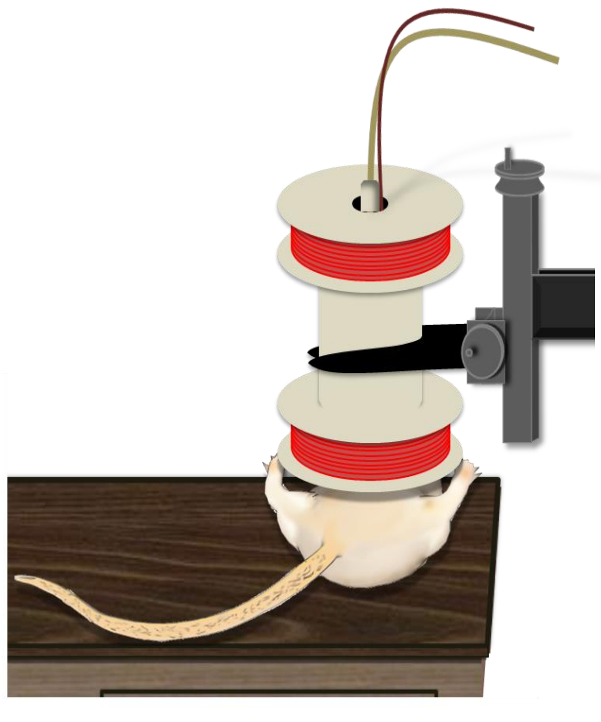


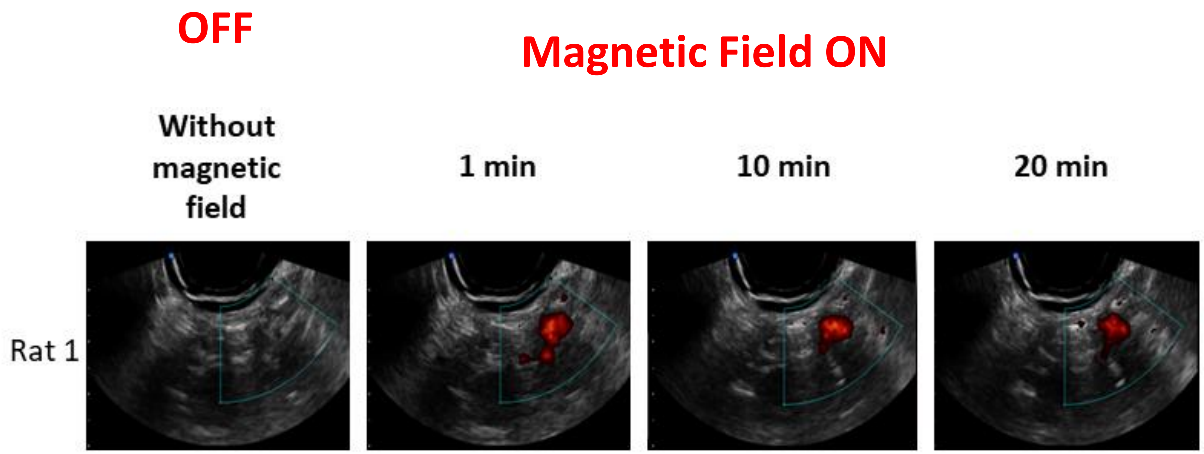
Magnetic Field ON
Displacement map

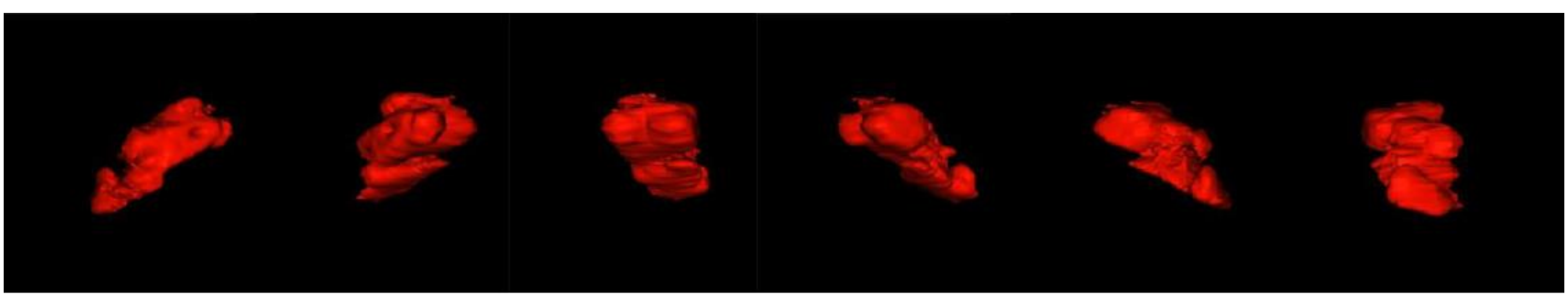




In vivo viability study



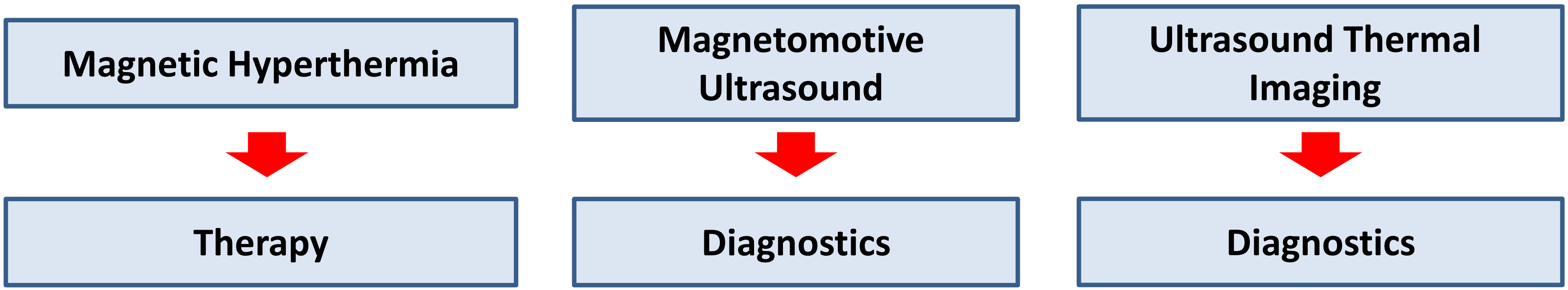




Theranostic Platform

- Diagnostic and therapeutic procedures using a single platform.
- Great potential to improve the efficiency of cancer treatments.
  - The so-called personalized medicine.
- For example: a single magnetic nanoparticle (MNP) composition can act as a contrast agent and a heating mediator.
- Development of instrumentation with theranostic purposes.

Theranostic Platform



Magnetic nanoparticle hyperthermia

- Conversion of the electromagnetic energy to heat;
- Great potential to selectively induce cell death in cancerous tissue;
  - Nanoparticle → heat mediator;
- It can increase the efficiency of radiotherapy and chemotherapy.

Therapeutic purpose: Magnetic Hyperthermia

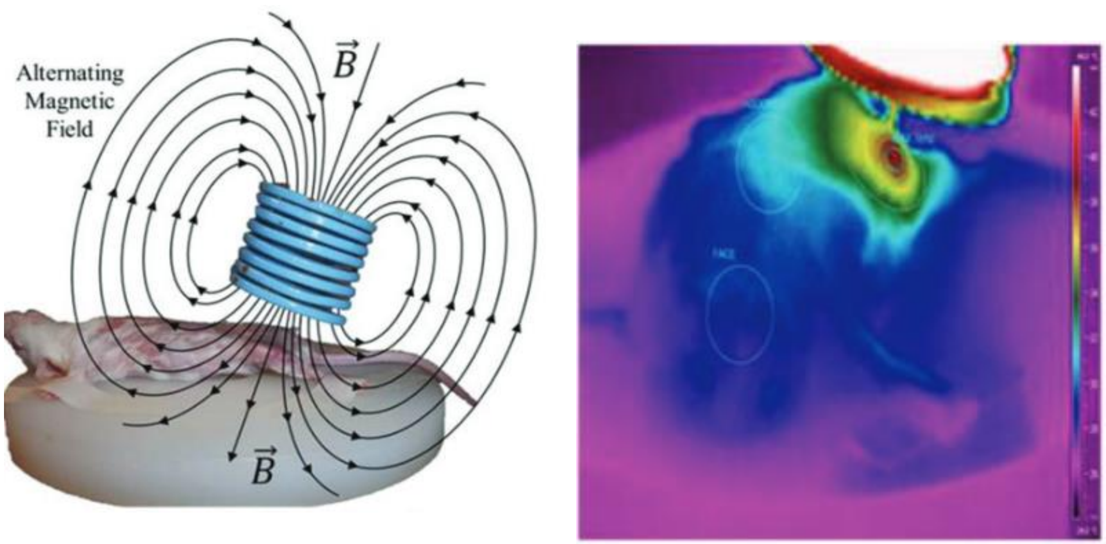
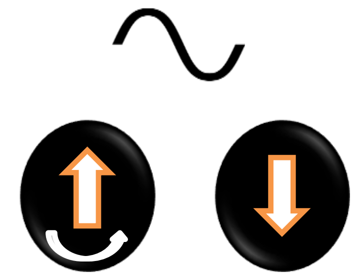
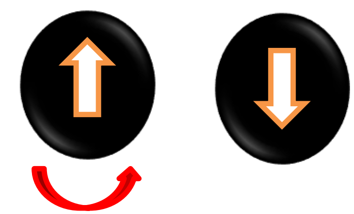


Figure from Rodrigues, Harley F., et al. "Real-time infrared thermography detection of magnetic nanoparticle hyperthermia in a murine model under a non-uniform field configuration." *International Journal of Hyperthermia* 29.8 (2013): 752-767.

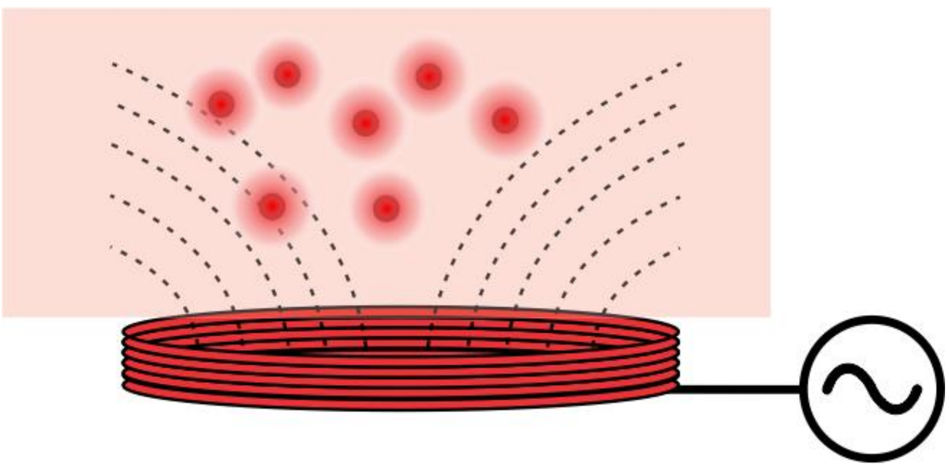


Néel relaxation



Brownian relaxation

Magnetic nanoparticle hyperthermia



Radiofrequency
100 – 500 kHz

$$P = \frac{1}{2}\mu_0\chi_0\omega H^2 \frac{\omega\tau}{1+(\omega\tau)^2}$$

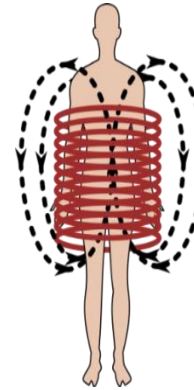
P → dissipated power, χ_0 → static susceptibility, and τ is the relaxation time.

Challenges for magnetic hyperthermia

Biocompatibility of the nanoparticles

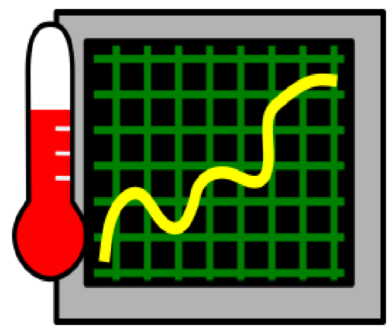
Heating efficiency of the magnetic nanoparticles

Safety limitation of the applied magnetic field



Large scale magnetic field

Localizing the nanoparticles within the tissues



Temperature monitoring during the therapeutic procedure

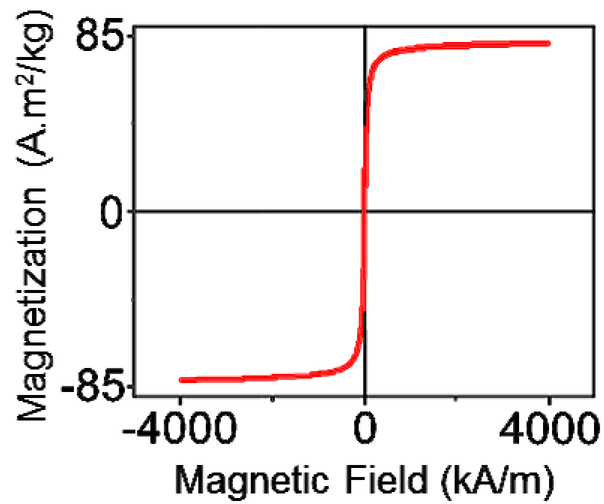
Temperature monitoring: Ultrasound Thermal Strain Imaging

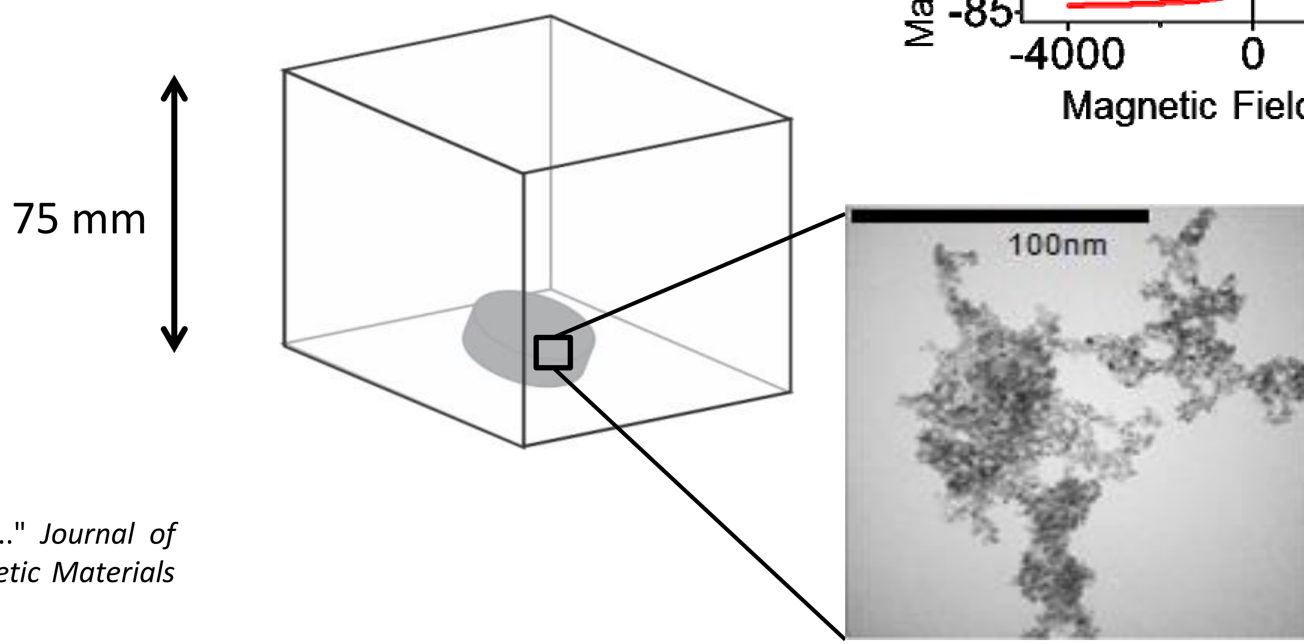
- **Temperature-dependent changes in the speed of sound;**
- Non-invasive thermometry;
- Compatible with magnetic hyperthermia.

Seo, C. H., Shi, Y., Huang, S.-W., Kim, K., & O'Donnell, M. (2011). Thermal strain imaging: a review. *Interface Focus*, *1*(4), 649–664.

Phantom with inclusion of magnetic nanoparticles

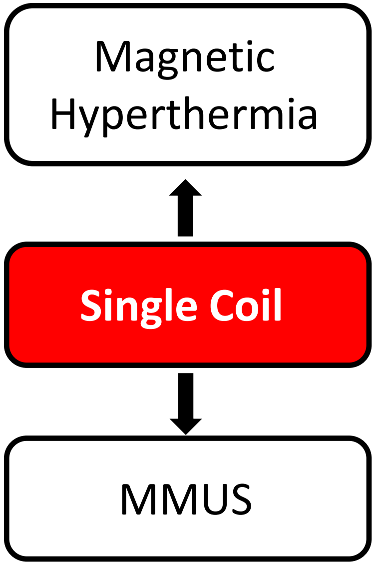
- Gelatin phantom
- MNPs diameter: 12 nm
- Saturation magnetization: 83 $A.m^2/kg$
- MNPs concentration: 20 mg/cm^3
- MNPs: $Zn_{0.1}Fe_{0.9}Fe_2O_4$

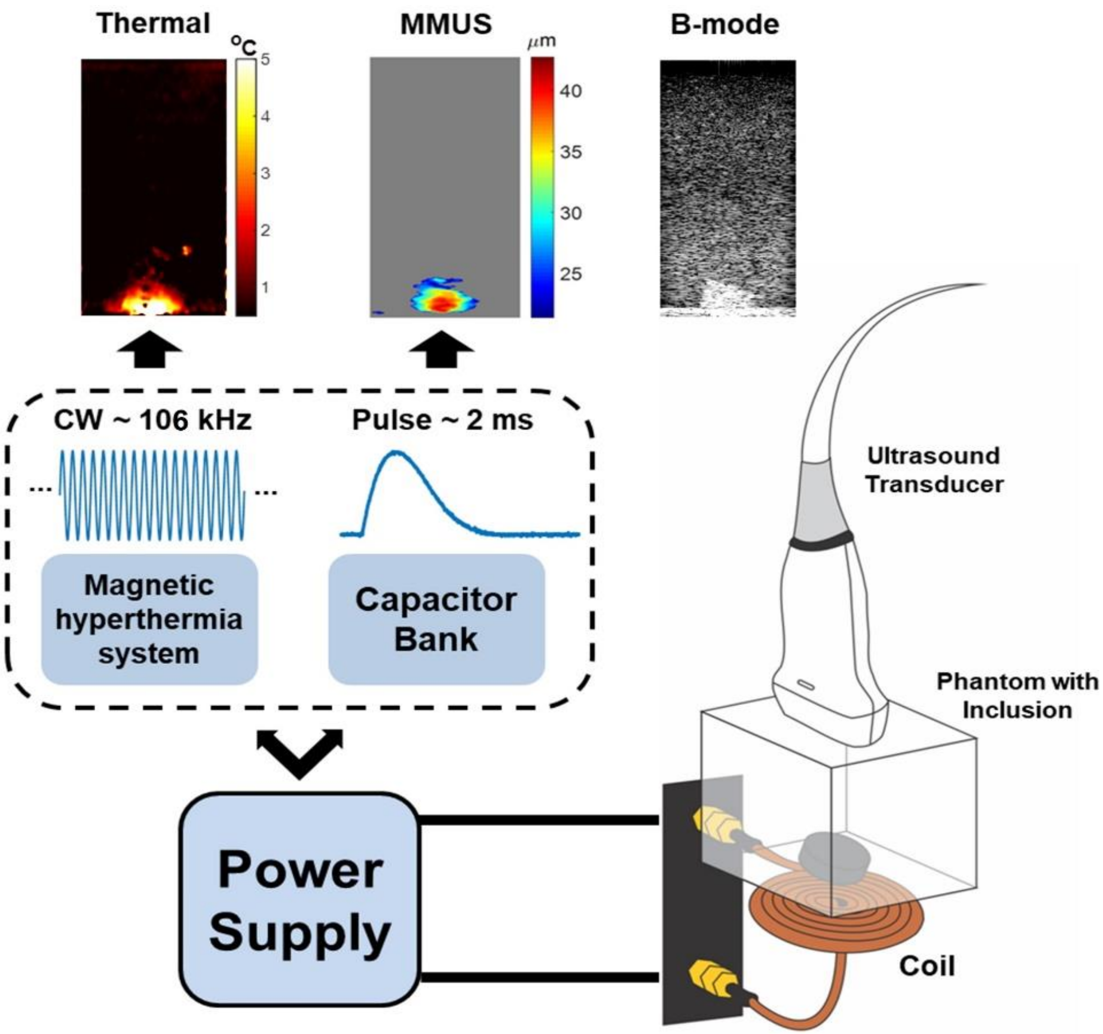




Hadadian, Yaser, et al.." *Journal of Magnetism and Magnetic Materials* 465 (2018): 33-43.

Developed Theranostic Platform

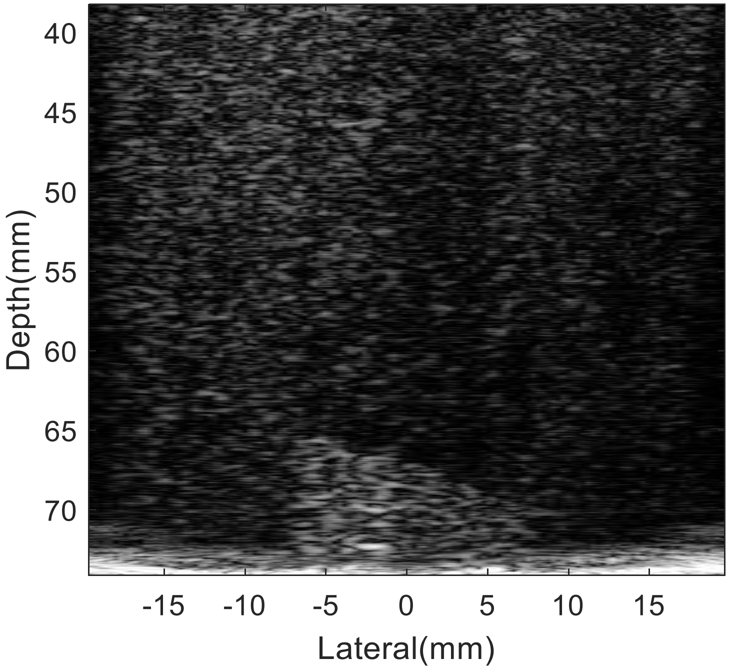




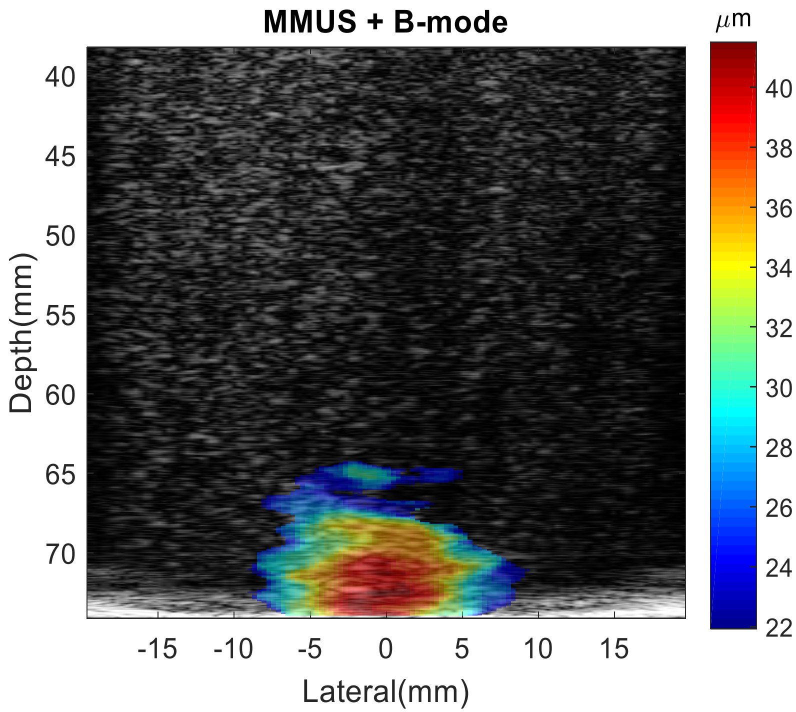
Hadadian et al., IEEE-TBE, 2020

Magnetomotive Ultrasound

B-mode



MMUS



Ultrasound Thermometry