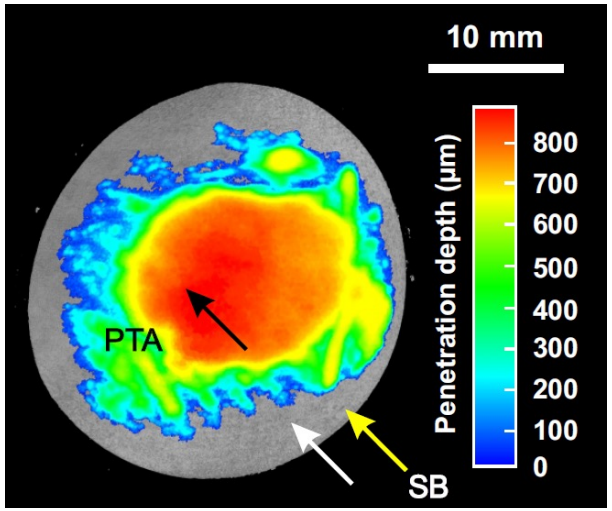
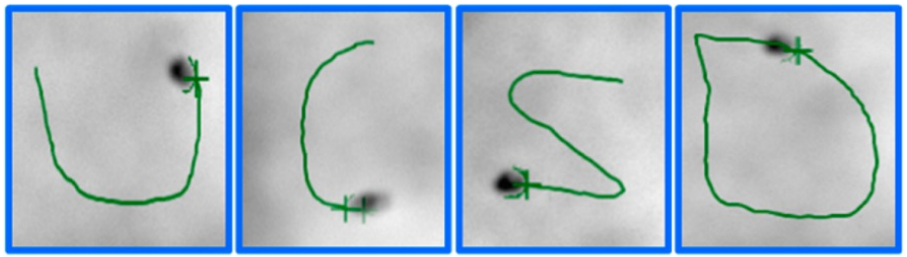
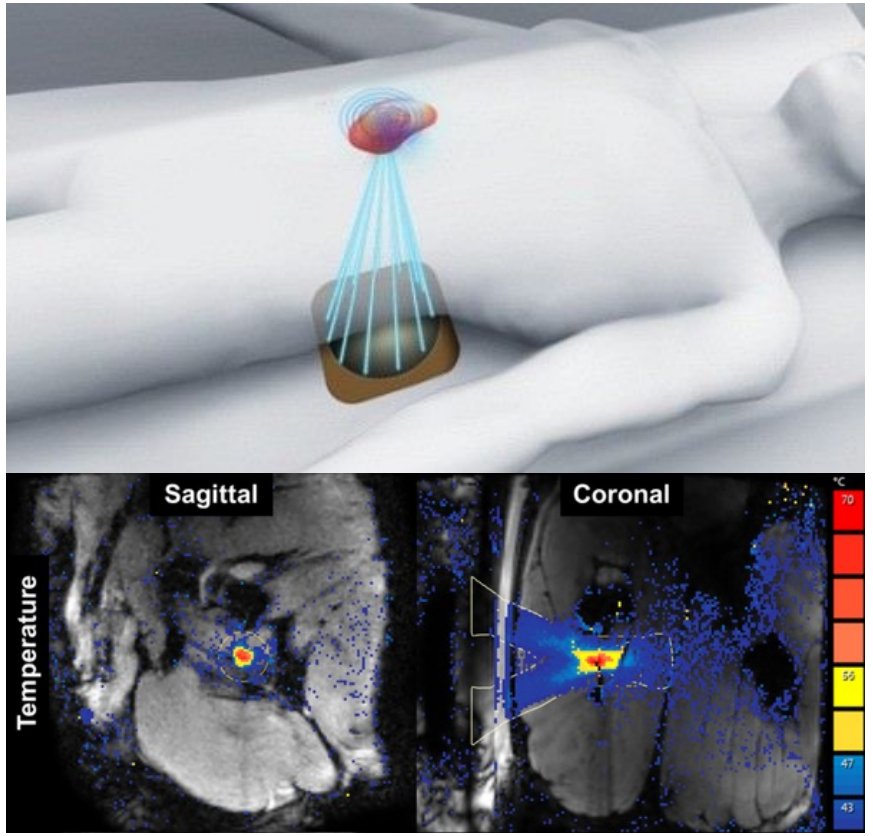
Biomedical Ultrasonics, 5 cr

Heikki Nieminen

13.9.-17.12.2023







Nebulization

Ultrasonic nebulization

- Ultrasonic nebulization is an old technique since 1950's to generate small drug droplets using HIU
- Generates droplets/particles < 5 µm in size
- Especially useful in treating asthma, COPD (chronic obstructive pulmonary disease) or other respiratory diseases
- Small droplets have large reaction area and dissolve quickly
- Claimed advantages: 1. finer droplets than with conventional techniques, 2. high sphericity, 3. uniformity in size

First reported nebulizer

Sales-Girons in 1858

Conventional nebulizer

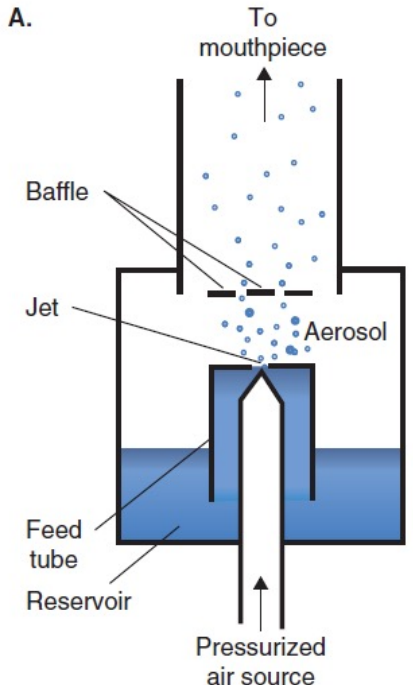
http://www.rtmagazine.com

Ultrasonic nebulizer

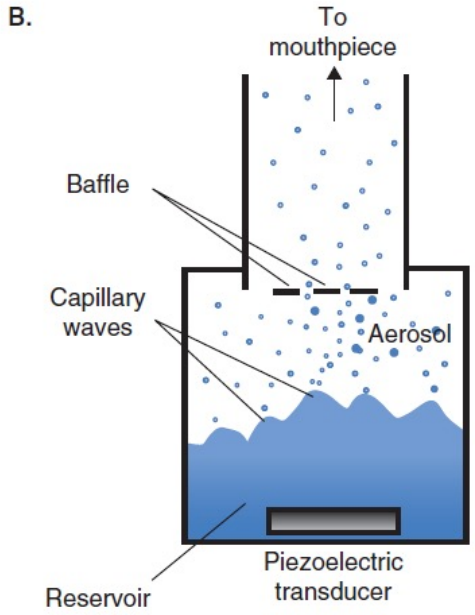
http://www.rmdmediaids.com

Working principle

CONVENTIONAL
Air pressurization & rapid air flow through a nozzle with fluid



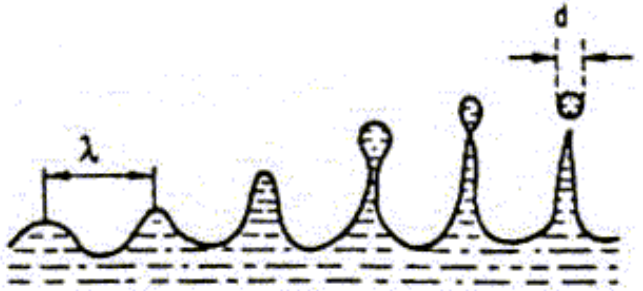
ULTRASONIC
Acoustic radiation force ejects droplets from the liquid surface



Ye et al. 2010: http://users.monash.edu/~lyeo/Dr_Leslie_Yeo/Publications_files/Yeo_Expert%20Opinion%20in%20Drug%20Delivery_7_663-679_2010.pdf

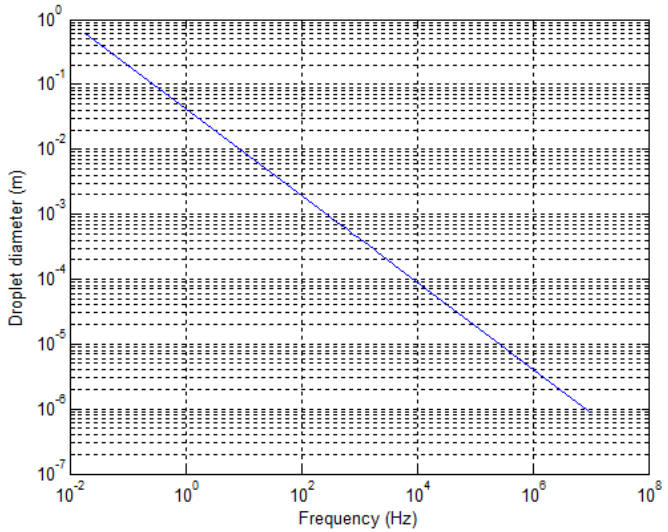
Threshold for nebulization

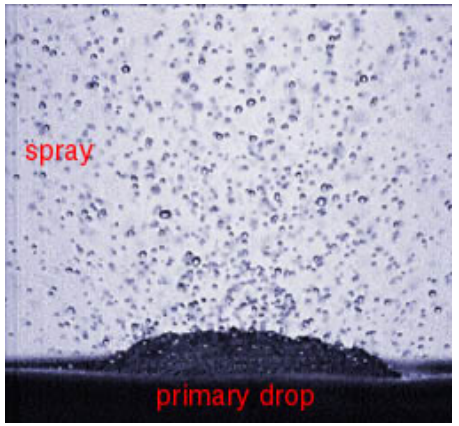
- Sonicating liquid-air interface generates capillary waves at the liquid air surface
- High capillary wave amplitude results in extrusions or ”pinching out” of droplets / liquid jets that generate small droplets



$$d \approx 0.34\left(\frac{8\pi\gamma}{\rho f^2}\right)^{1/3}$$ (median droplet size)

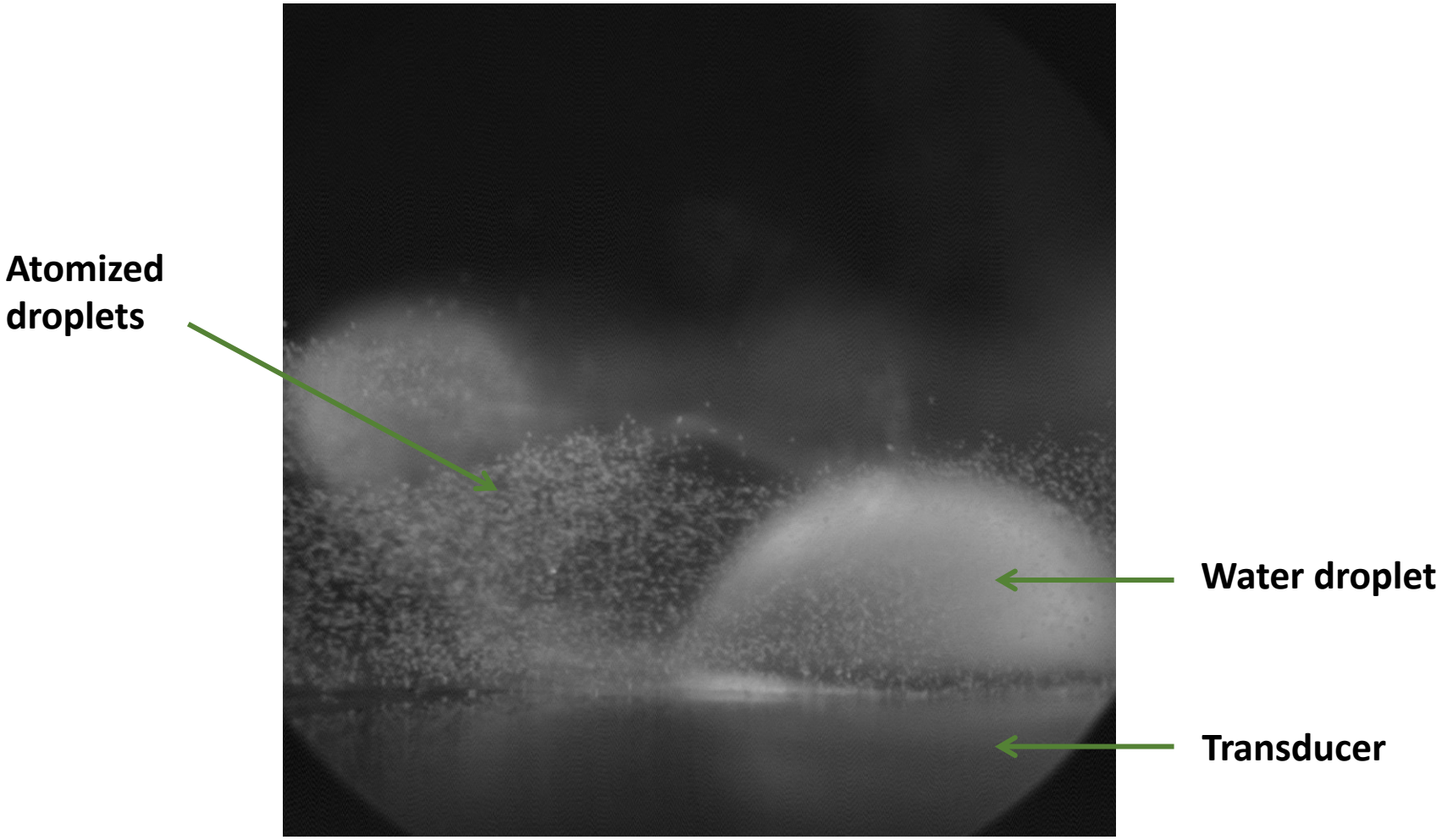
Applies for low flux: 0.1ml/s/cm^2





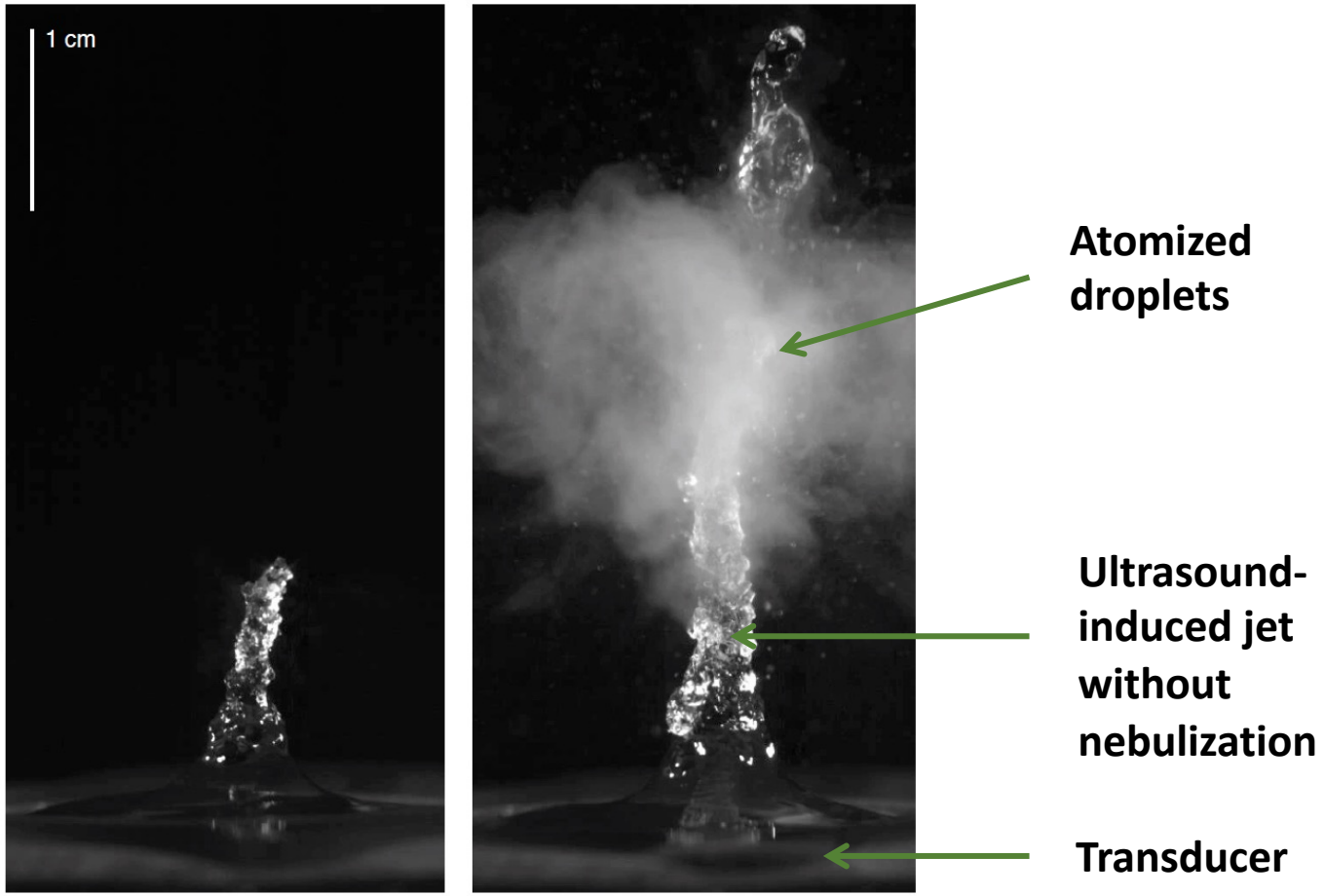
http://www-old.me.gatech.edu/bvukasinovic/VIDA.html

Atomization of a droplet



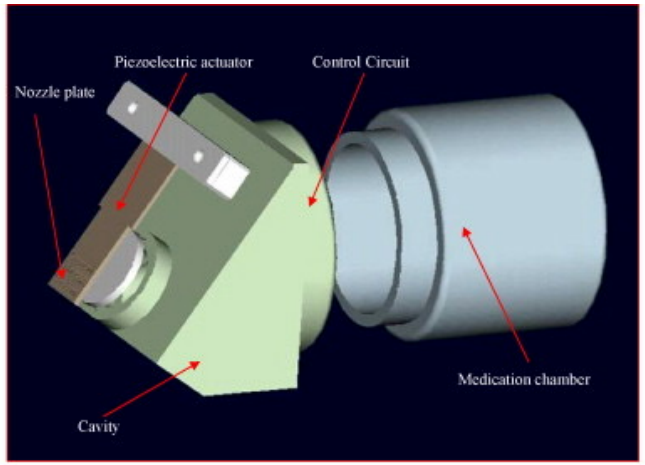
Howard 2010: http://ultrasoundatomization.com/ultrasonic_atomization_thesis_text.pdf

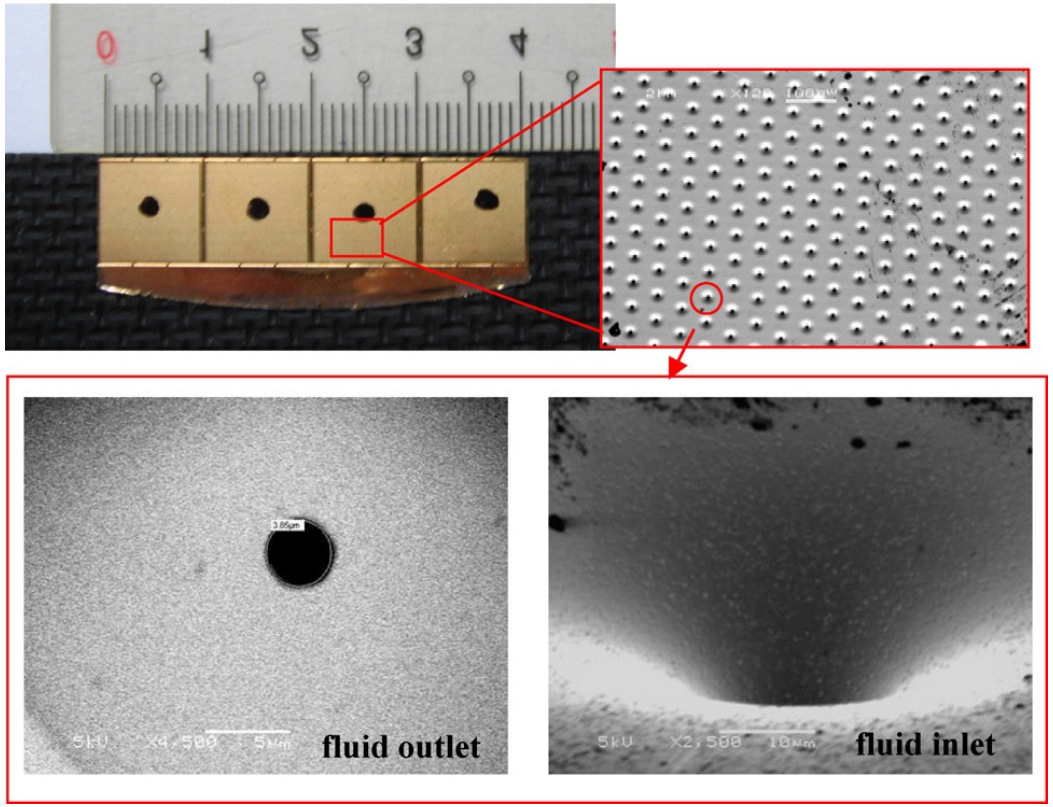
Atomization from a acoustic streaming induced jet (waveguide)



Howard 2010: http://ultrasoundatomization.com/ultrasonic_atomization_thesis_text.pdf

Ultrasound nebulizer



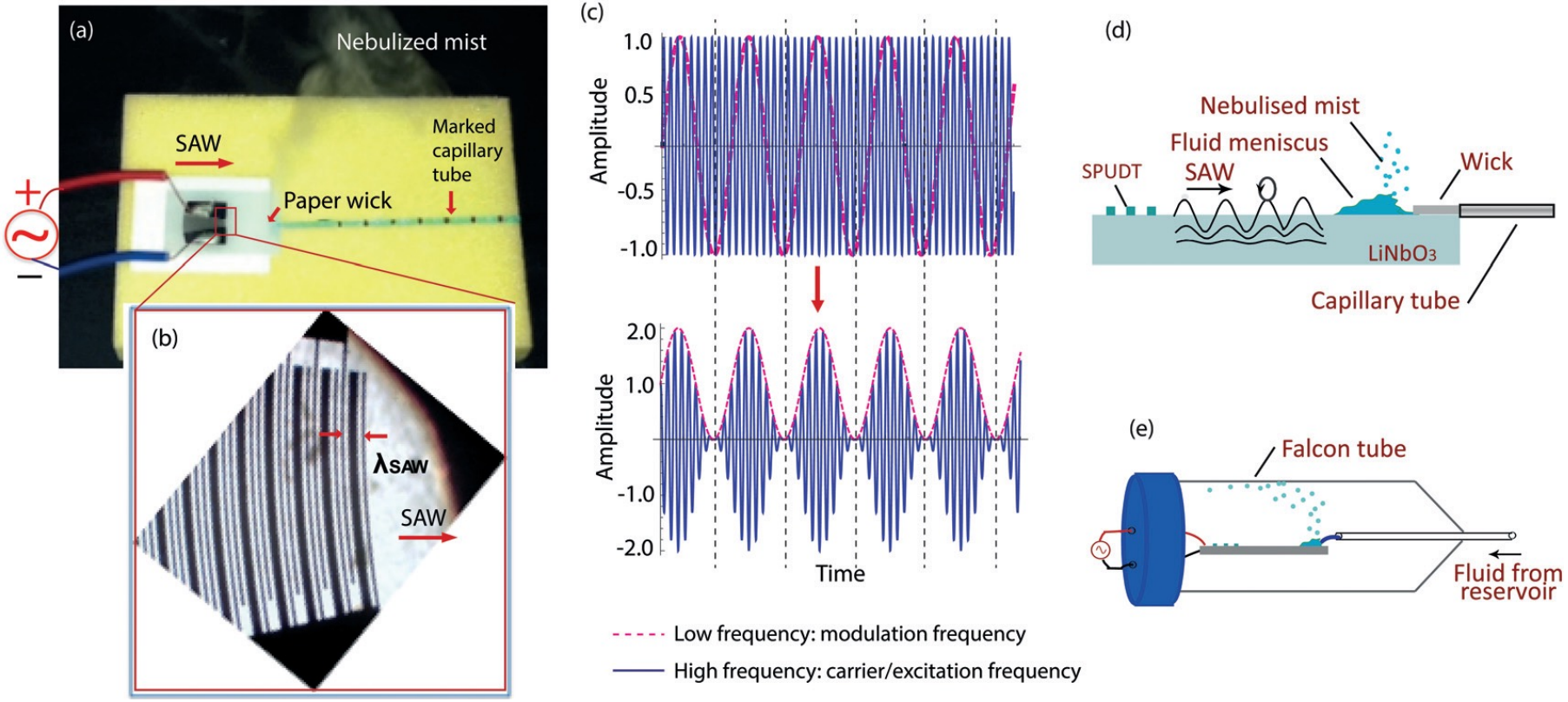


Shen et al 2008:
http://www.sciencedirect.com/science/article/pii/S0924424707008898

Video: Ultrasound nebulizer in action

Shen et al 2008:
http://www.sciencedirect.com/science/article/pii/S0924424707008898

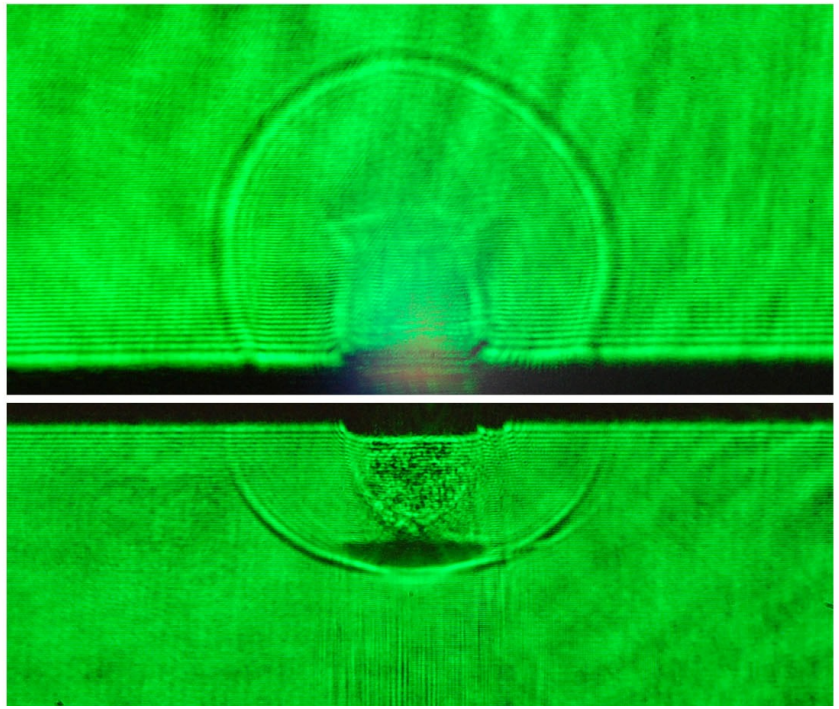
Nebulization with surface acoustic waves (SAWs)



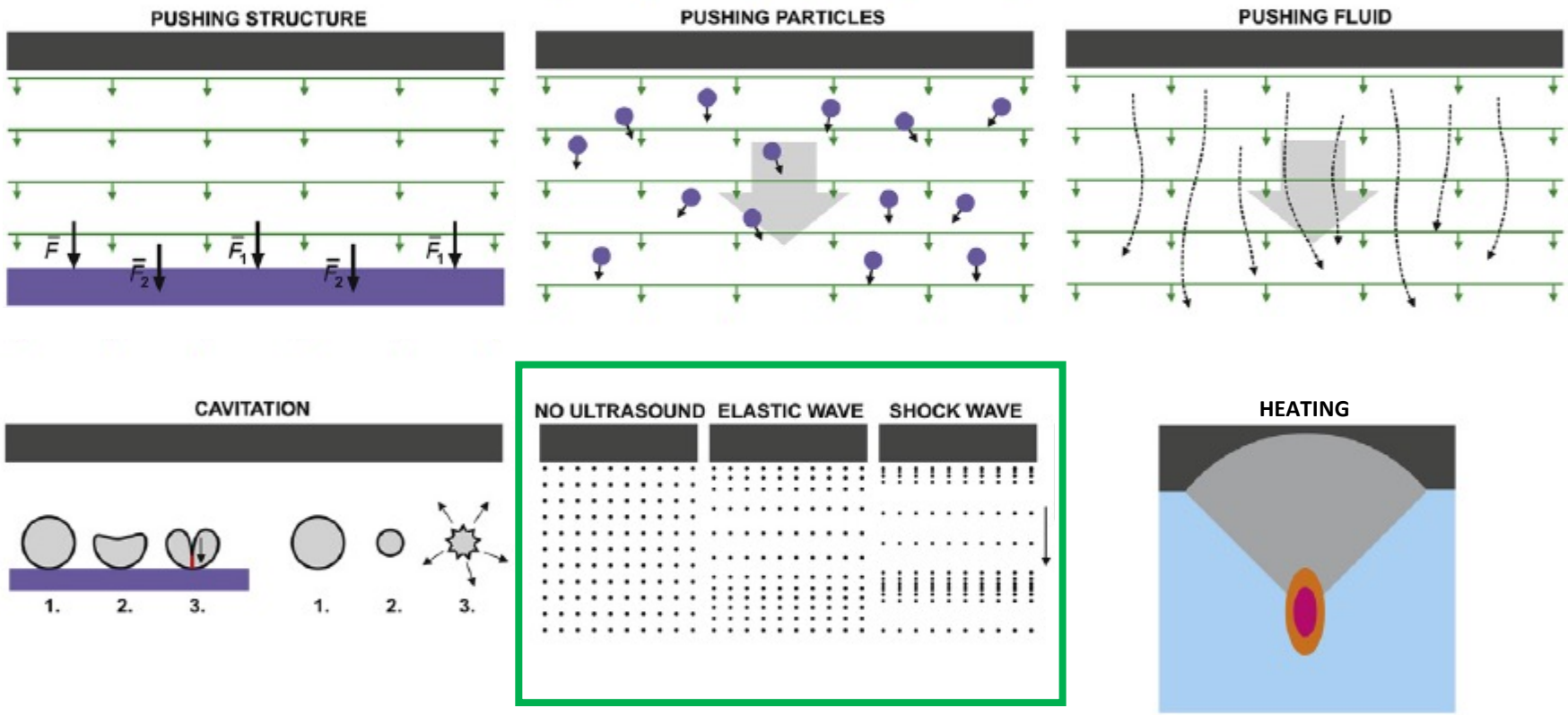
Modulated frequency: 0.5-40 kHz

Rajapaksa et al.2014 http://www.ncbi.nlm.nih.gov/pubmed/24740643

Shock waves

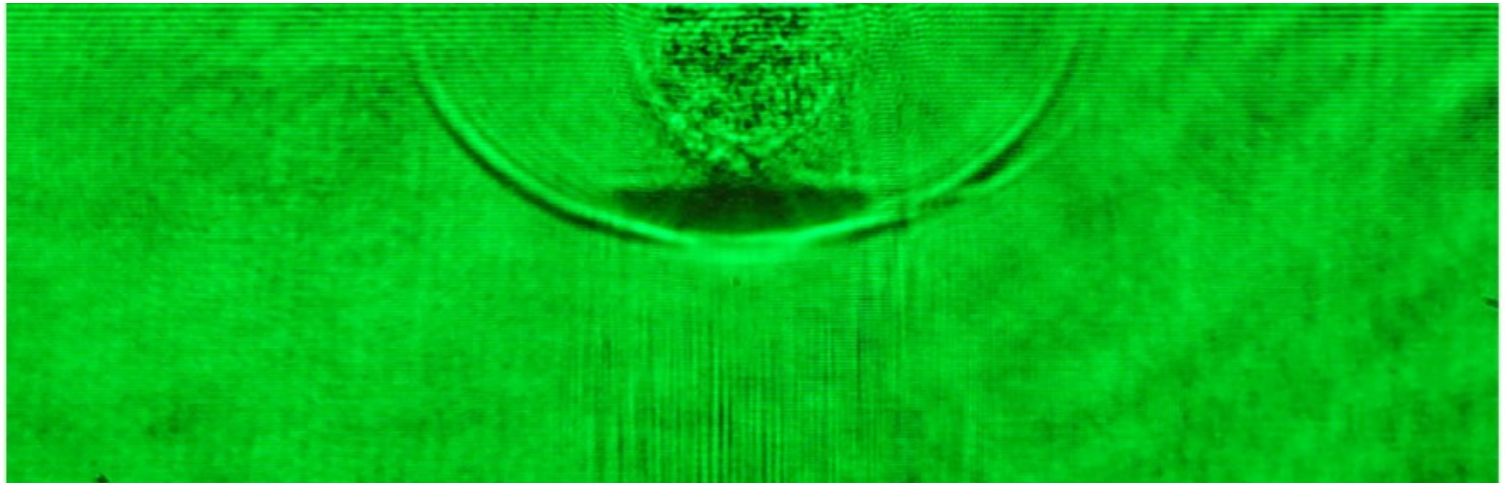


Non-linear ultrasonics



What is a shock wave?

- A travelling discontinuity of density that moves faster than the speed of sound in the material



Strycker et al 2013: http://www.opticsinfobase.org/oe/abstract.cfm?uri=oe-21-20-23772

Shock waves

- Submarines during WWII could not detect enemy ships ➔ reason: Pistol shrimps
  - *e.g.* http://www.youtube.com/watch?feature=player_detailpage&v=XC6I8iPiHT8#t=31s
- Other examples: Bombs, jet fighters, lightning etc
- Shock wave generation in fluid:
  - Non-linear propagation of a pulse
  - Bubble implosion
  - Electric spark
  - Laser-ultrasonic
    - Ablation
    - Plasma generation

Shock waves

- Shock wave evolution

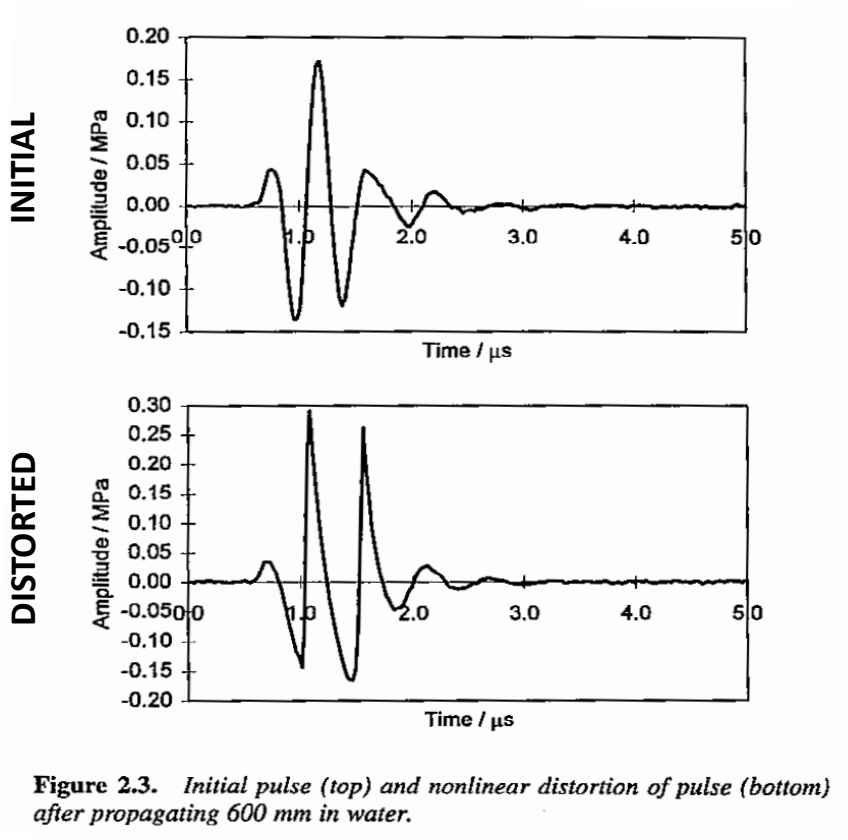
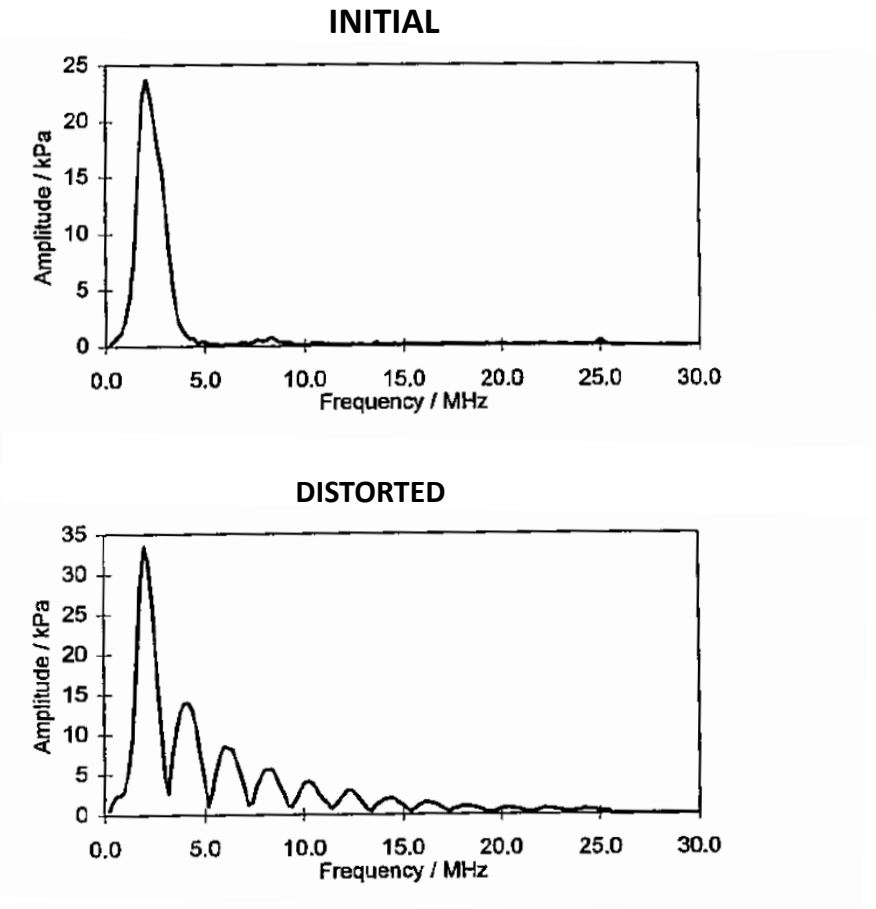


Figure 2.3. *Initial pulse (top) and nonlinear distortion of pulse (bottom) after propagating 600 mm in water.*



Duck et al 1998: Ultrasound in Medicine

Shock waves

Pulse distortion

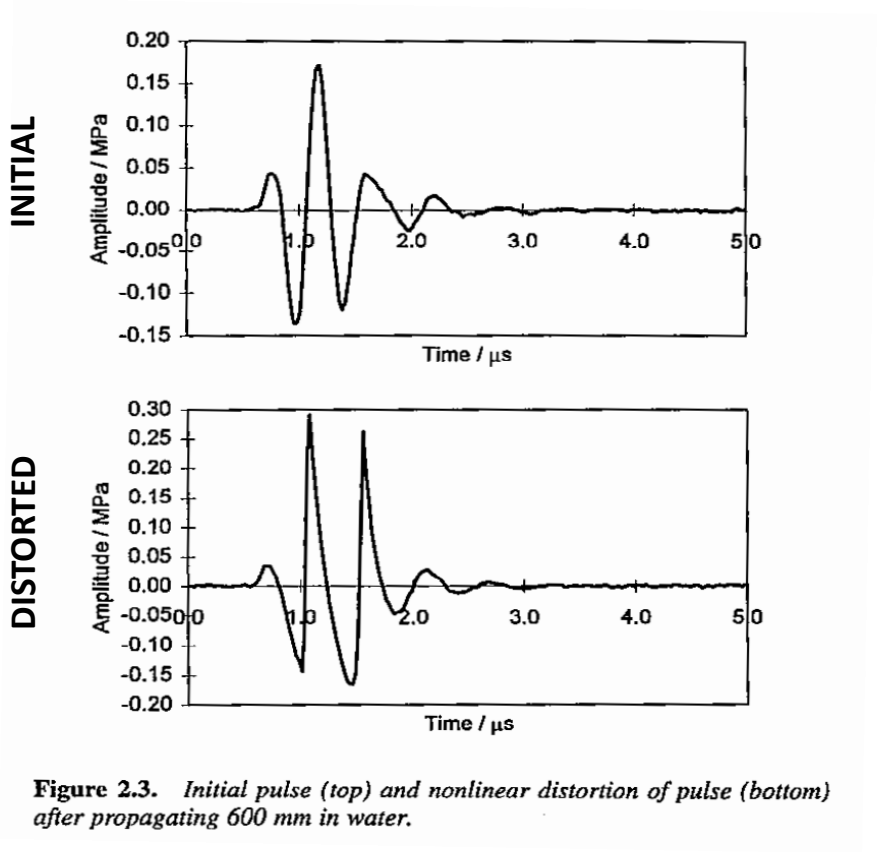


Figure 2.3. *Initial pulse (top) and nonlinear distortion of pulse (bottom) after propagating 600 mm in water.*

Harmonic content with propagation distance

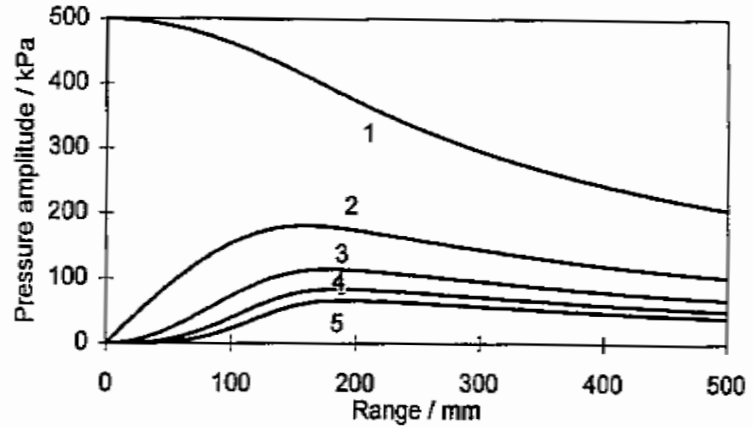
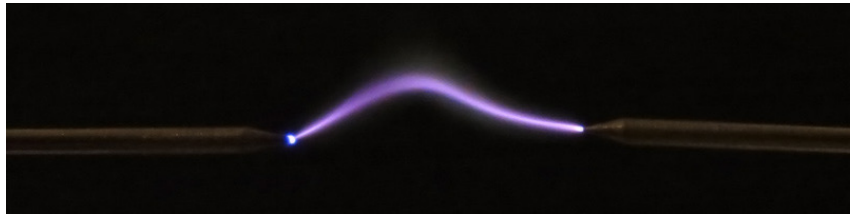


Figure 2.2. *Fundamental and second to fifth harmonics for a nonlinear plane wave in water ($f_0 = 3.5$ MHz, $P_0 = 500$ kPa, $\Gamma = 38$).*

Duck et al 1998: Ultrasound in Medicine

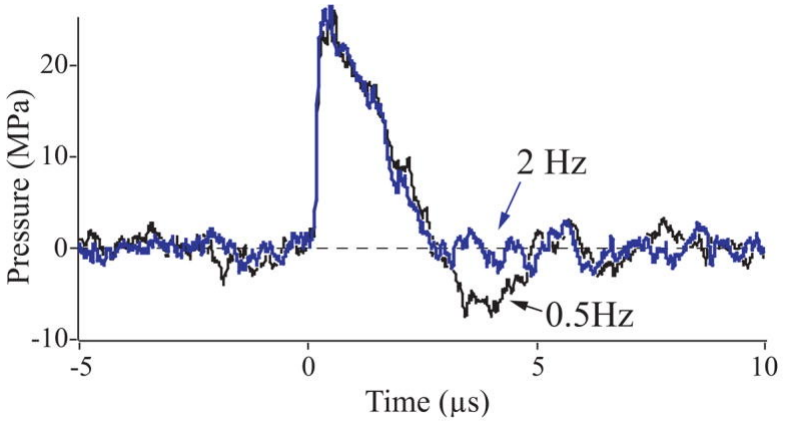
Sound generation with plasma spark

Spark gap



- Spark gap in air or fluid generates plasma ➔ plasma expands ➔ generates sound/cavitation

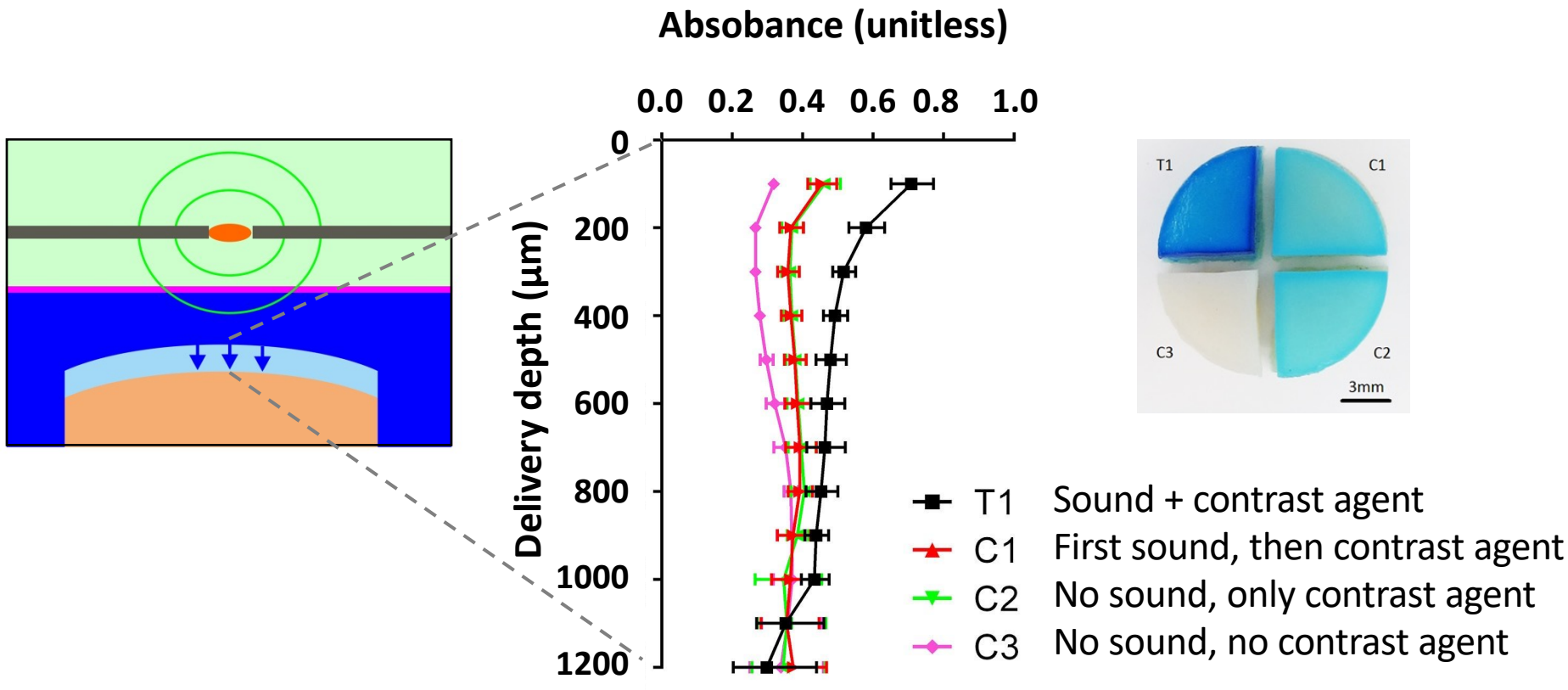
Shock wave signals from lithotripter at different PRF (0.5 or 2 Hz)



Pishchalnikov et al 2006:
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2442574/

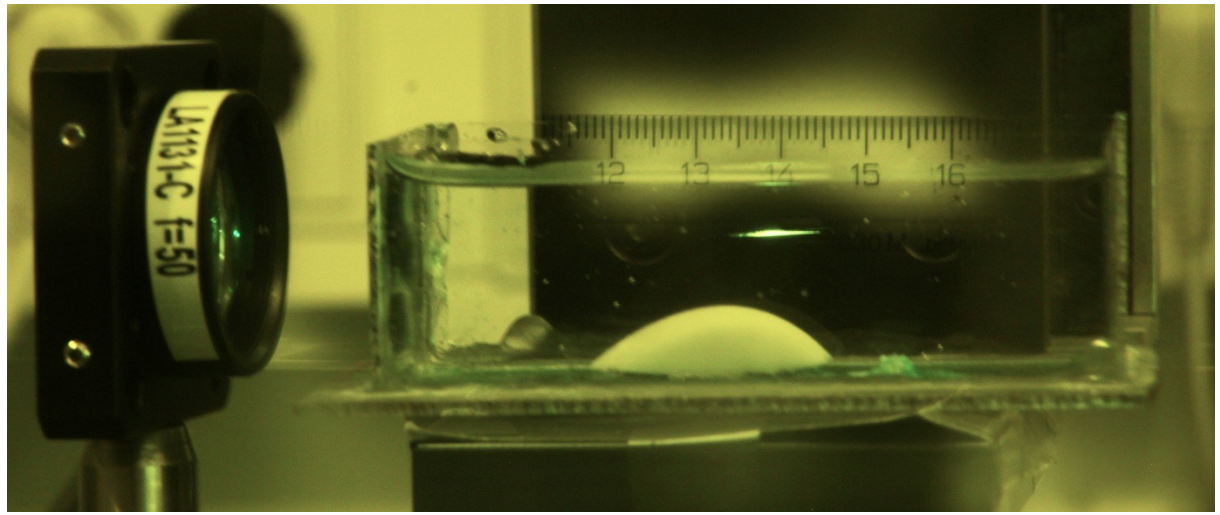
Shock wave drug delivery

- **Delivery of methylene blue (320 Da) to a depth of 0.8 mm in 5 minutes.**



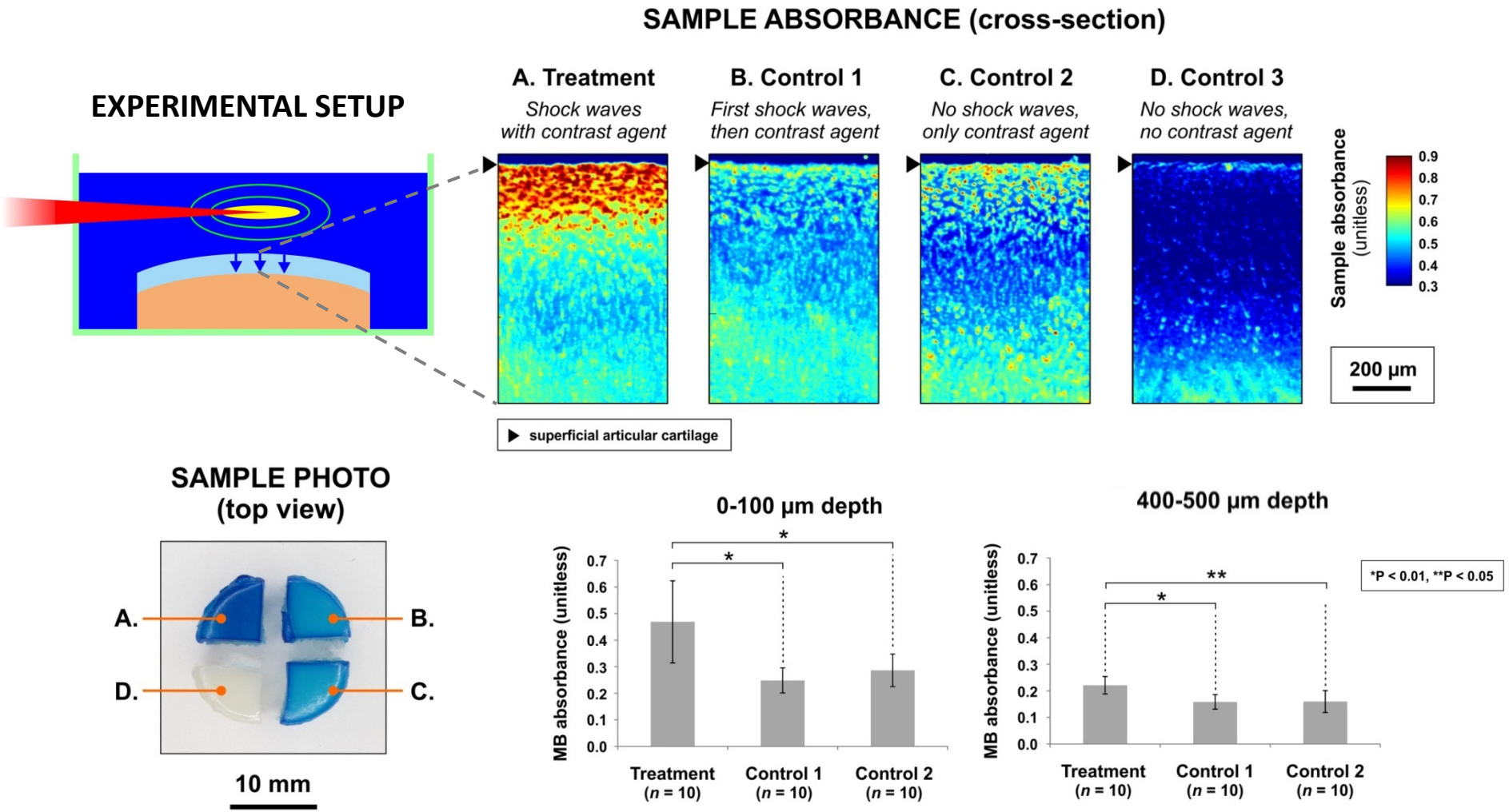
Laser-ultrasonic plasma generation

- Laser pulses can be exploited for ultrasonic drug delivery by generating sound by them by means of:
  - thermoelastic expansion
  - ablation
  - plasma generation (*perhaps the most interesting for ultrasonic drug delivery*)



Laser-ultrasonic delivery

- Delivery of methylene blue (320 Da) to a depth of 500 µm (11 min)



Nieminen et al. doi: 10.1038/s41598-017-04293-5.

Femtosecond laser plasma generation

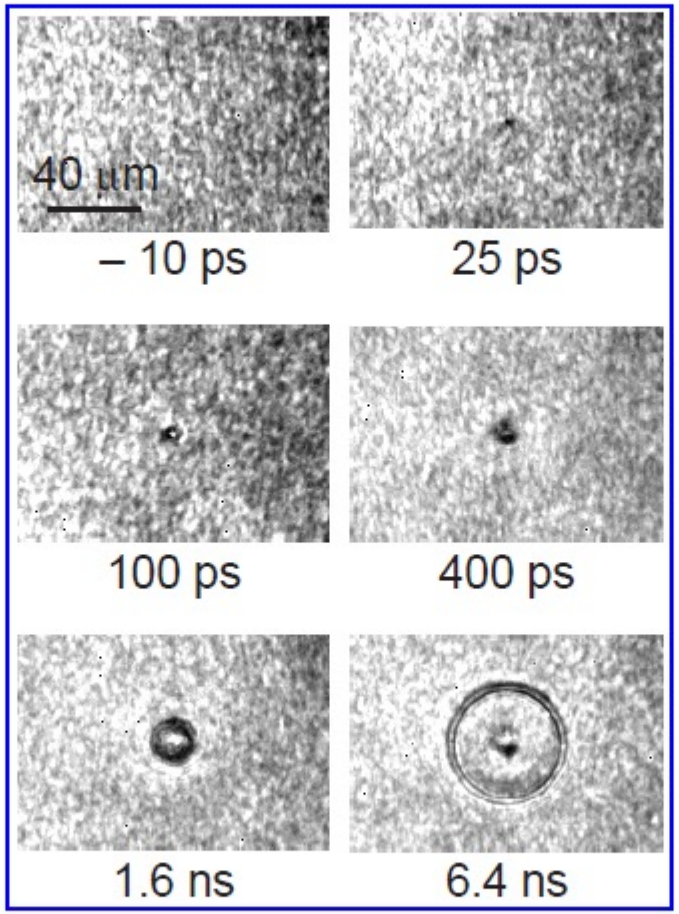
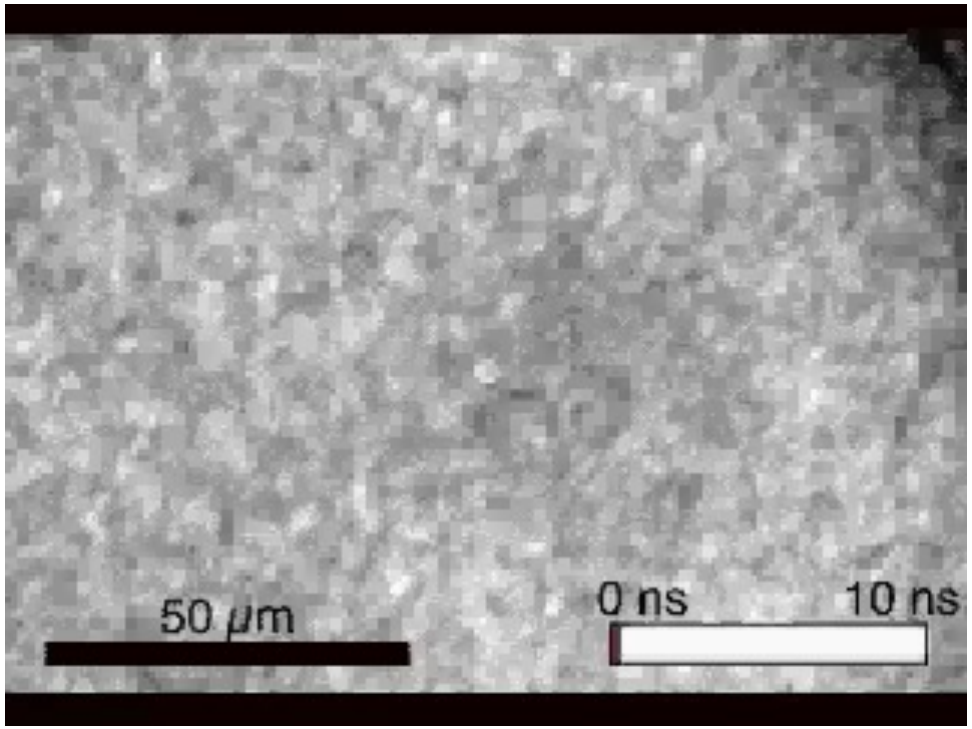


Fig. 2 Images of femtosecond laser-induced breakdown in water obtained for various time delays using the setup shown in Fig. 1. A corresponding quicktime movie shows the first 10 ns of expansion. One second of the movie shows 1 nanosecond of the dynamics.

Video



Schaffer et al 2002: http://www.opticsinfobase.org/oe/abstract.cfm?uri=oe-10-3-196

Femtosecond laser pulse at air-water interface

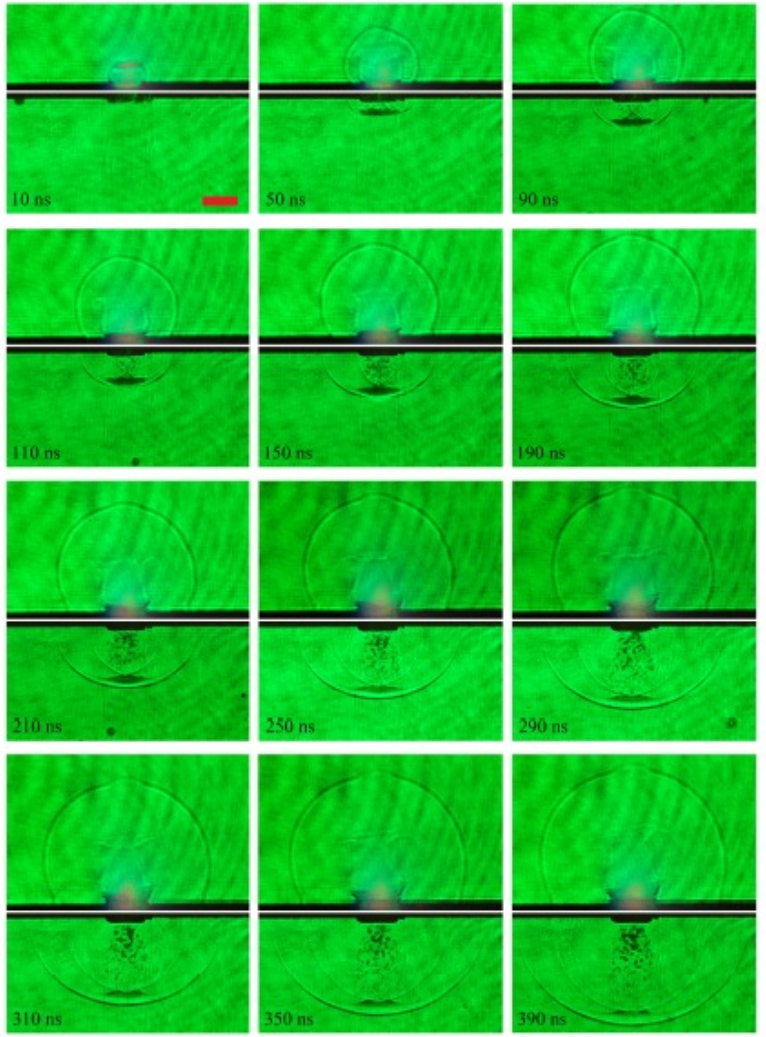


Fig. 3. Temporal evolution of shockwaves in air and water using pulses with about 2.2 mJ energy and 1.25×10^{15} W/cm^2 peak on-axis intensity. The red scale bar in the upper left corner is 200 μm in length.

Strycker et al 2013: http://www.opticsinfobase.org/oe/abstract.cfm?uri=oe-21-20-23772

Femtosecond laser pulse at air-water interface

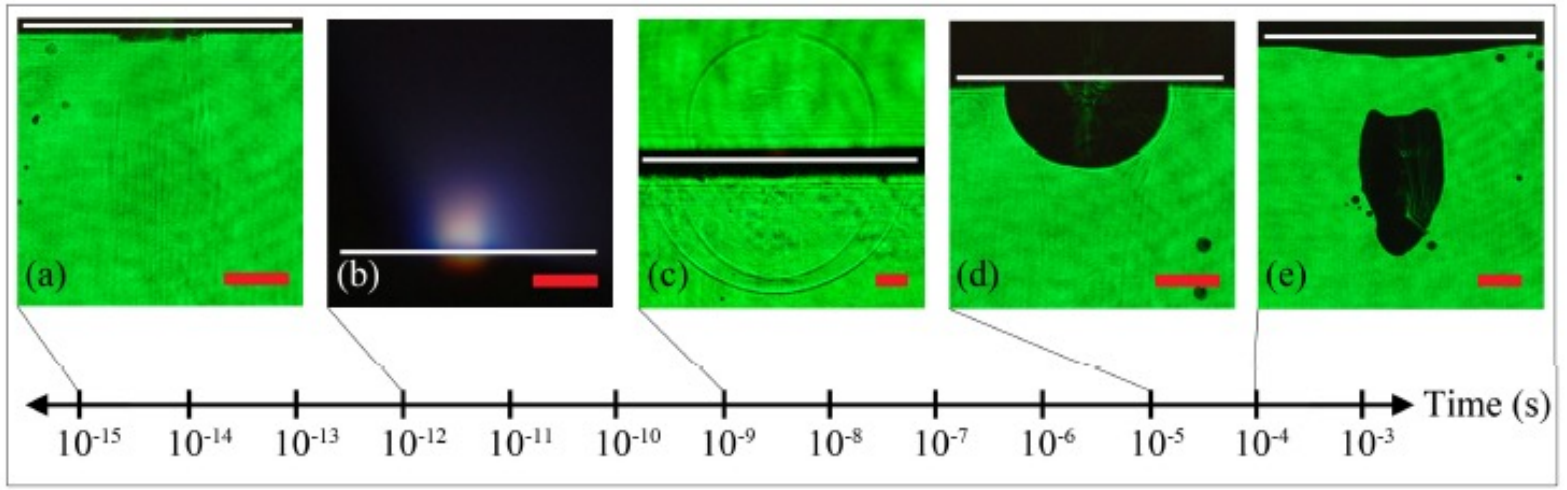


Fig. 2. Outline of events induced by femtosecond laser pulses of few millijoule energies incident on an air-water interface. The red scale bars in the lower right corners are 200 μm in length. The white lines show the approximate position of the air-water interface, with air above and water below. Each picture is shown to be associated with an approximate time scale on which the phenomenon may first begin to be observed. (a) Ionization, plasma generation, and electron-ion thermalization at and beneath the surface, corresponding to the slightly darker region of about 200 μm width in the center of the picture. (b) Plasma expansion from the surface and emission of light. This image was taken without the use of the 532 nm probe pulse. (c) Generation of shockwaves both above and below the surface. Notice the two shock fronts generated within the water sample. (d) Cavity formation at the surface. (e) Cavity closure and bubble formation.

Strycker et al 2013: http://www.opticsinfobase.org/oe/abstract.cfm?uri=oe-21-20-23772

Shock-wave ”shooting” of tungsten particles into rat liver

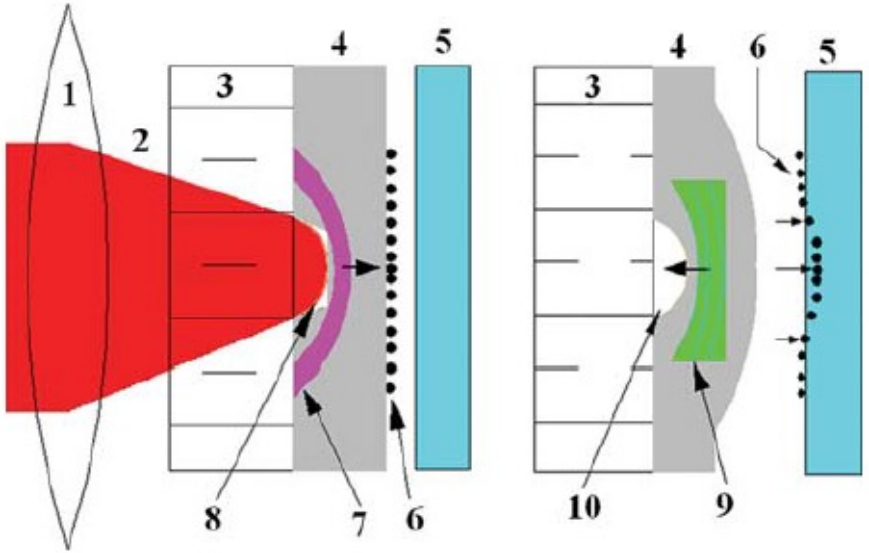


Fig. 2 Illustration of laser ablation assisted particle acceleration (*1* Lens. 2 Laser beam. *3* Glass overlay. *4* Foil. *5* Target. *6* Particles. *7* Shock wave. *8* Confined ablation. *9* Expansion wave. *10* Microcrater due to ablation)

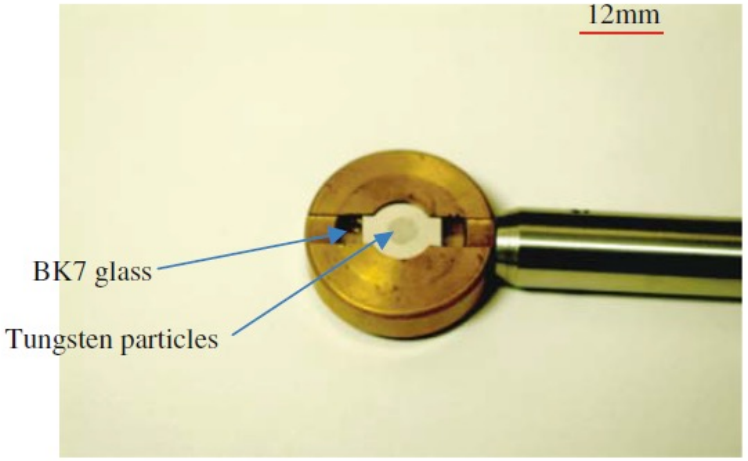
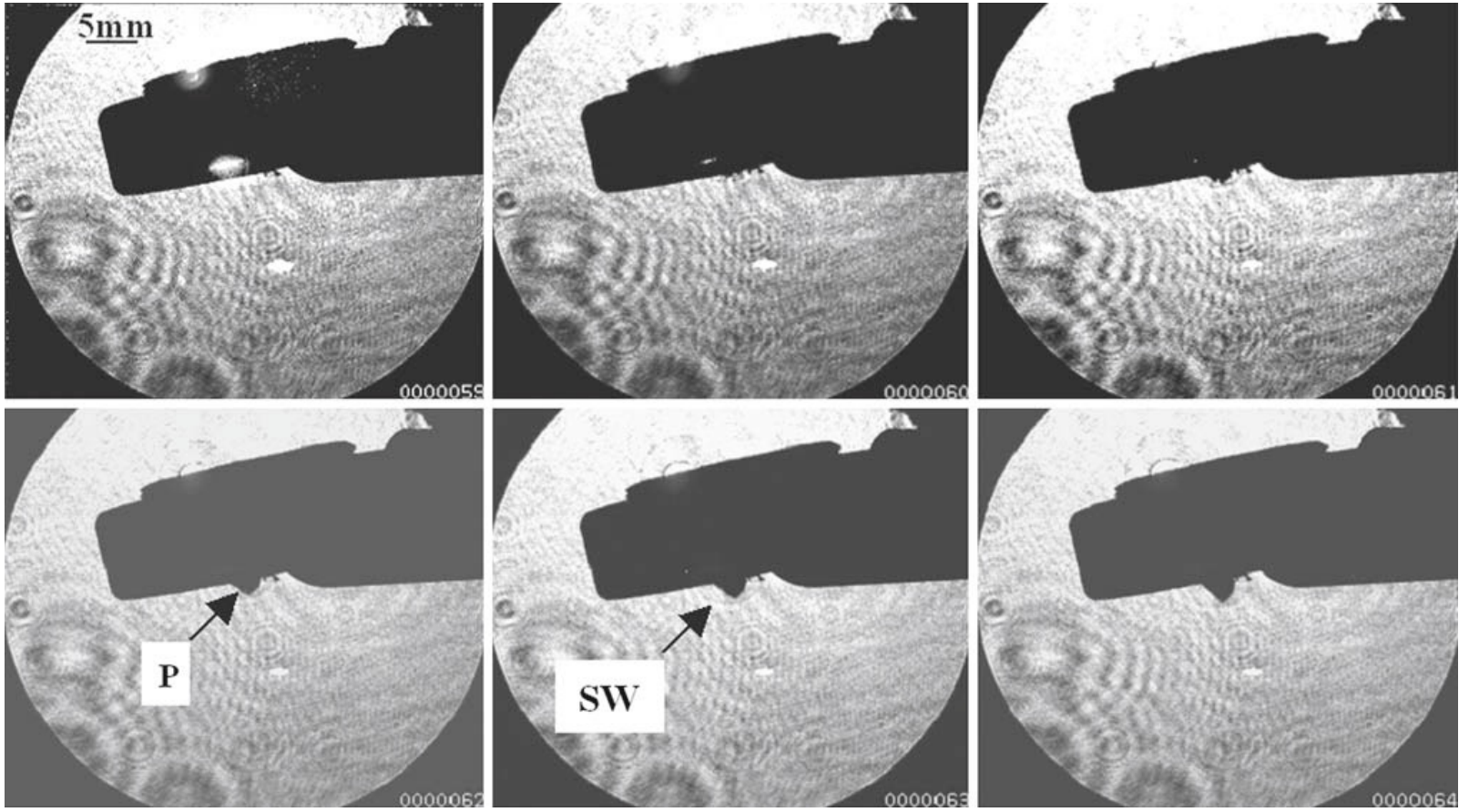


Fig. 3 A layer of 1 μm size tungsten particles deposited on a 100 μm thick Al foil

Menezes 2008: http://link.springer.com/article/10.1007/s00193-008-0163-9

Shock-wave ”shooting” of tungsten particles into rat liver



P = particle cloud, SW = shock wave, 1 µs interval between high-speed camera frames

Menezes 2008: http://link.springer.com/article/10.1007/s00193-008-0163-9

Shock-wave ”shooting” of tungsten particles into rat liver

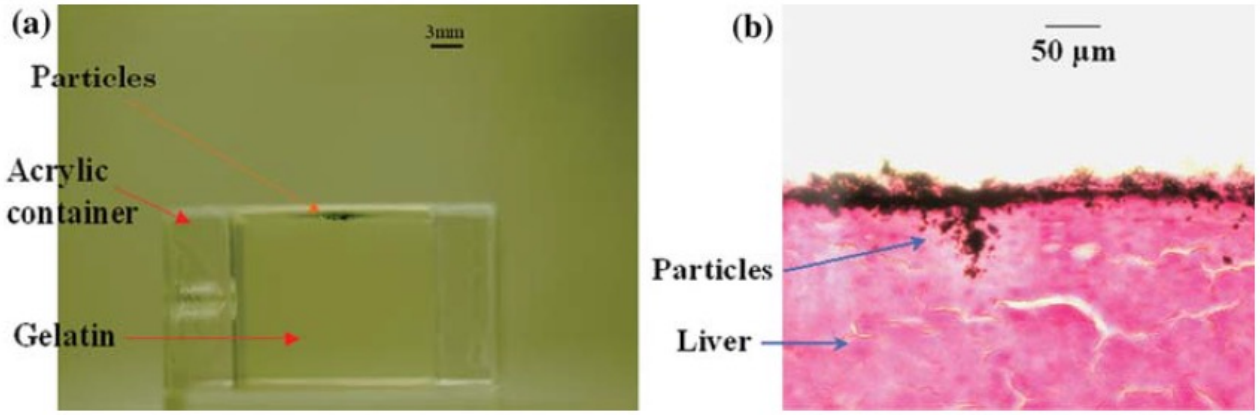
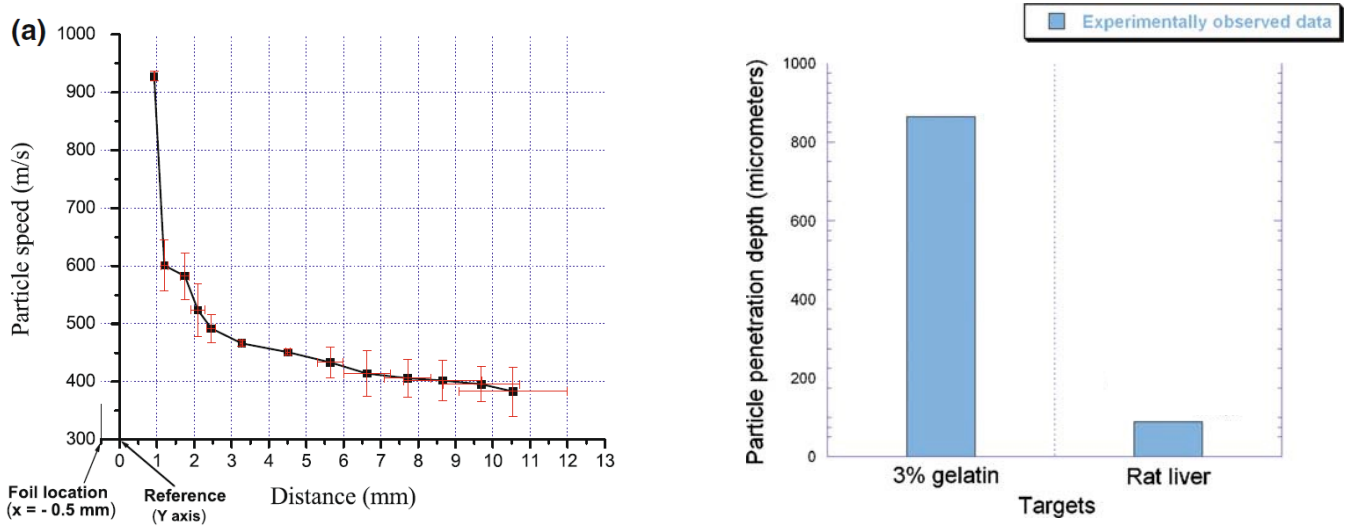


Fig. 8 1 µm tungsten particles delivered into **a** 3% gelatin and **b** liver tissue of Sprague Dawley male rat



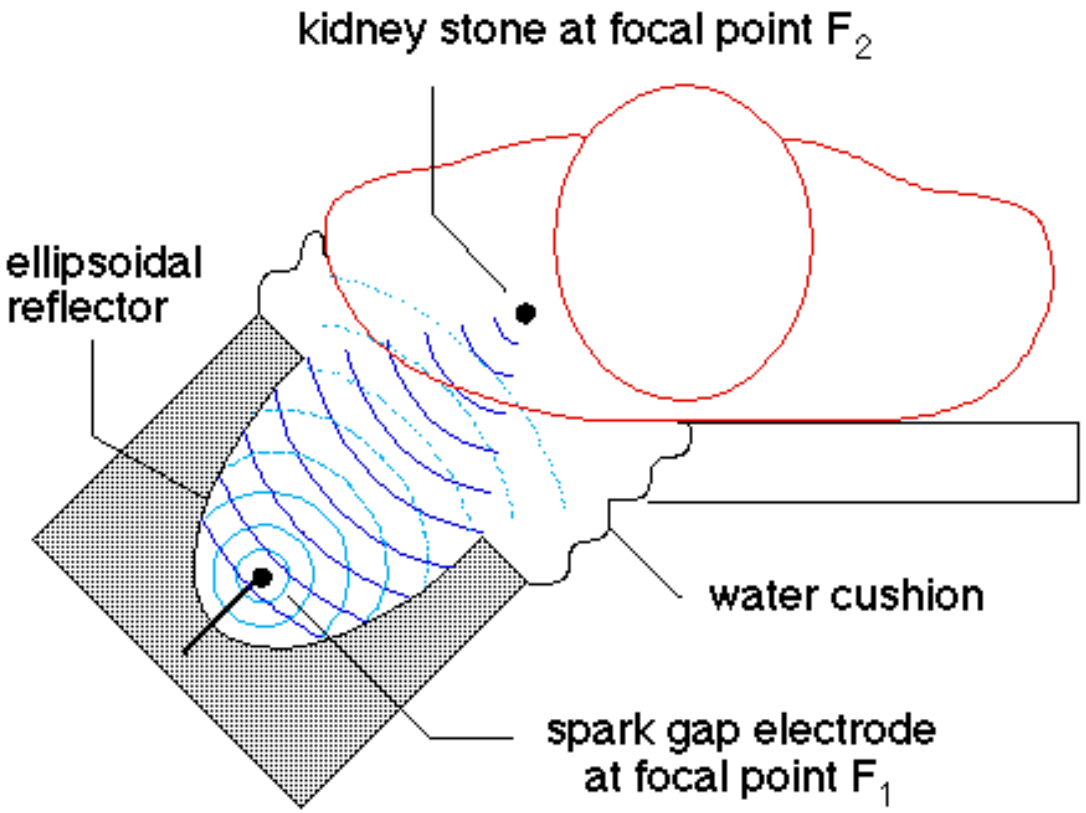
Menezes 2008: http://link.springer.com/article/10.1007/s00193-008-0163-9

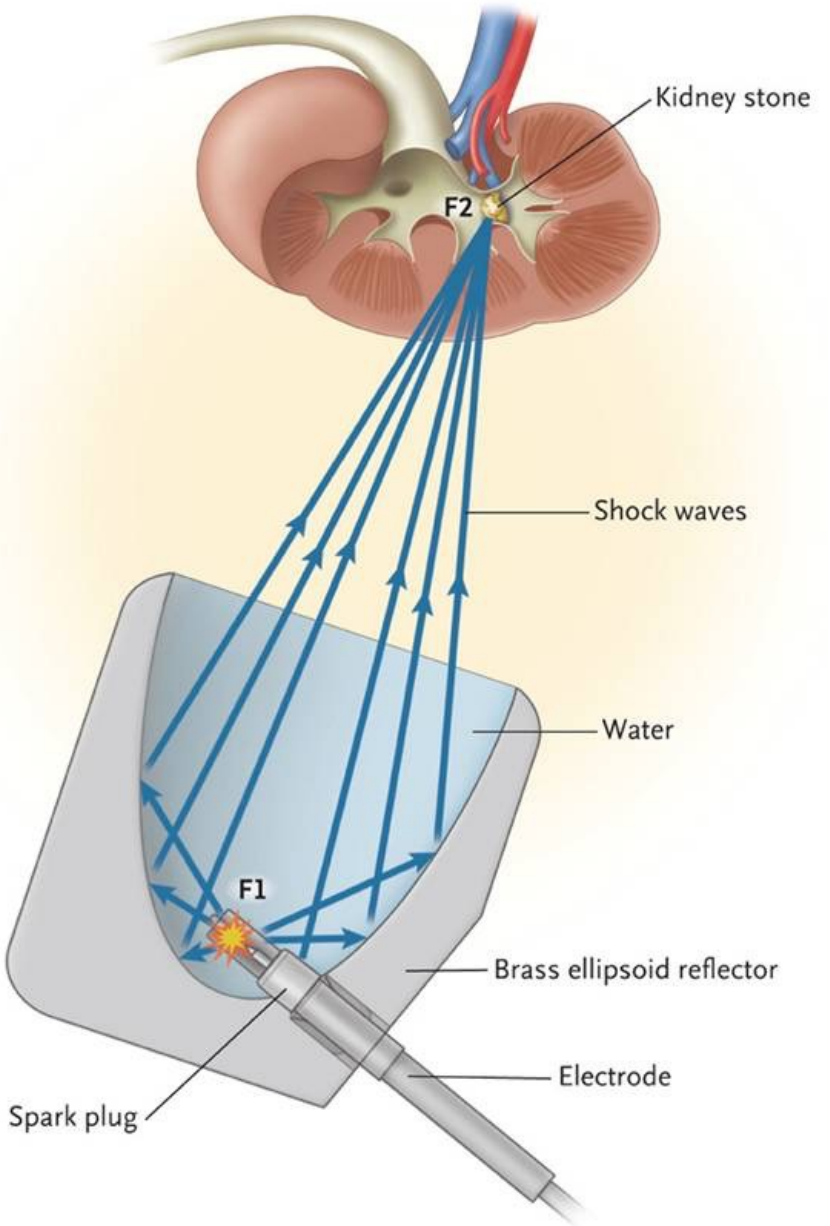
Breaking the bone

FIG. 4. Complete fractures of the bones occurred after 10,000 shock waves at maximum energy density (0.60 mJ/mm^2). This photograph shows two femurs.

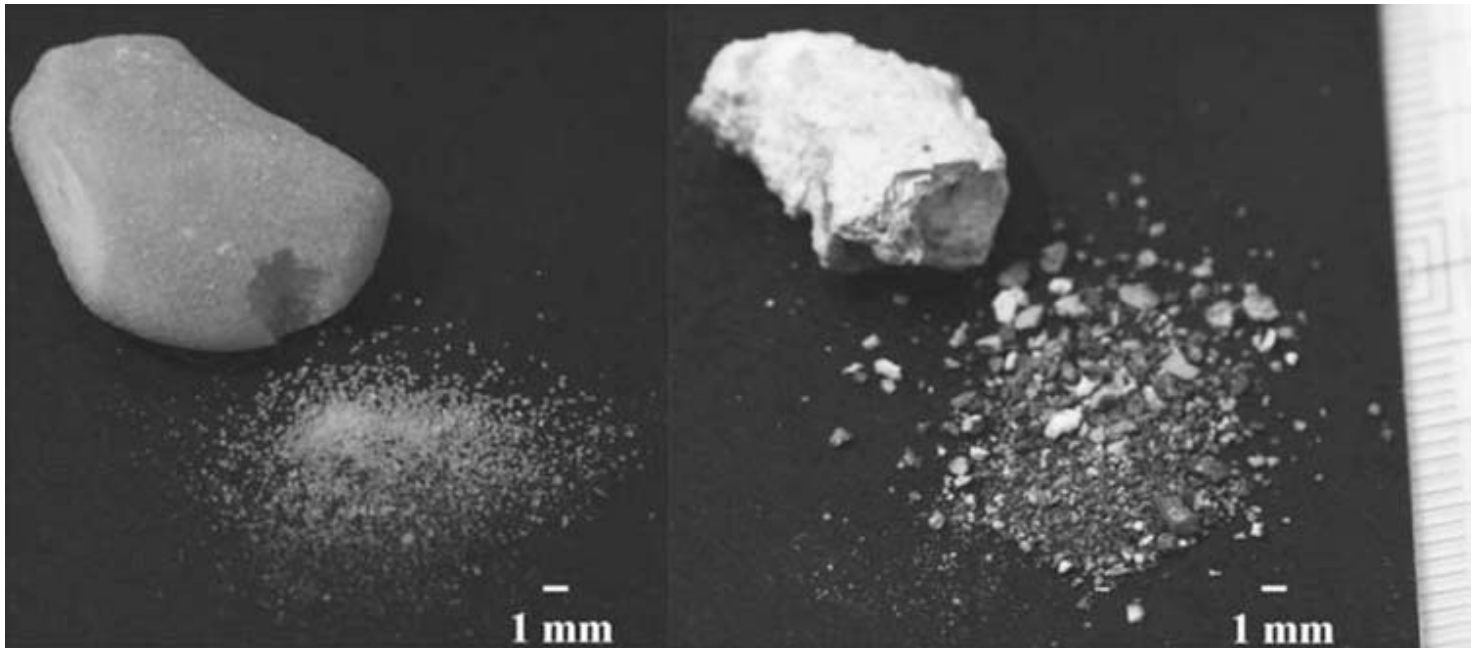
Kaulesar Sukul et al 2002: http://www.sciencedirect.com/science/article/pii/S0022480483710085

Lithotripsy





Breaking calculi with shock waves



Yoshizawa et al. 2009: http://link.springer.com/article/10.1007/s11517-009-0471-y

Commercial lithotripter

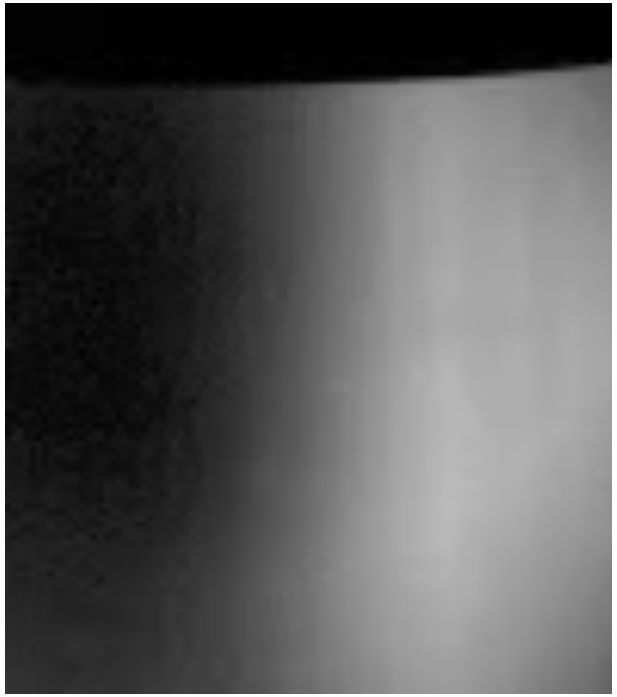
https://www.youtube.com/watch?v=qnVqSAX-GIU

Burst wave vs. shockwave lithotripsy

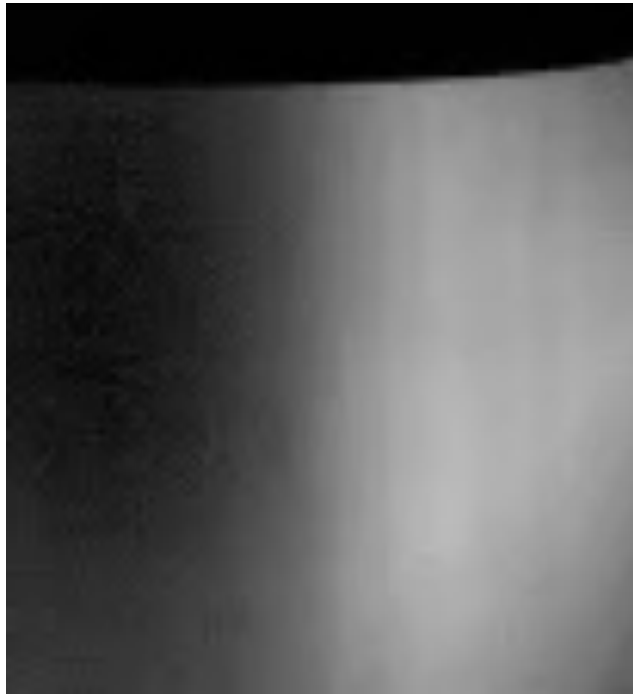
https://www.youtube.com/watch?v=8EJ9vqLsI_Y

Shock wave produced by a collapsing cavitation bubble

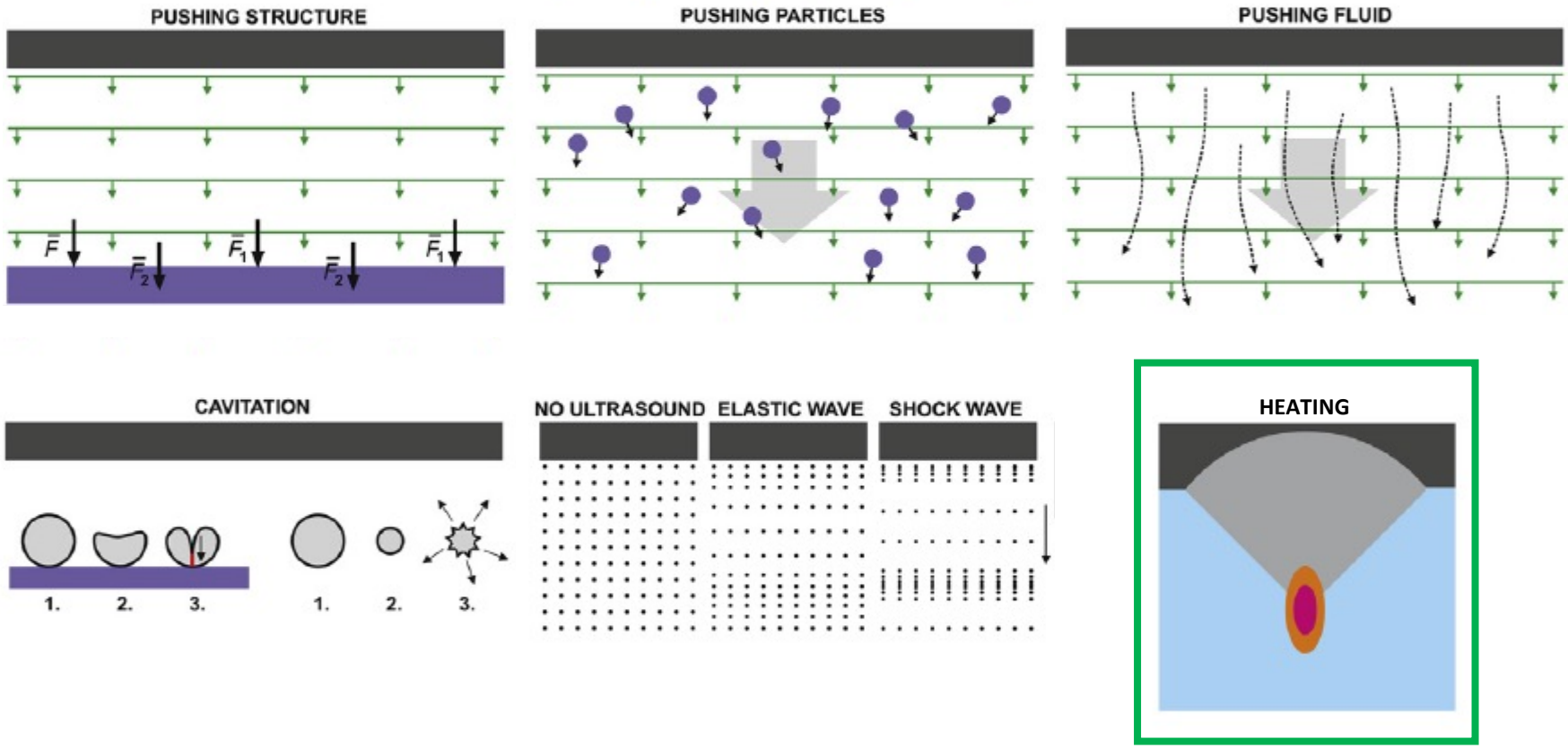
No cavitation



Cavitation

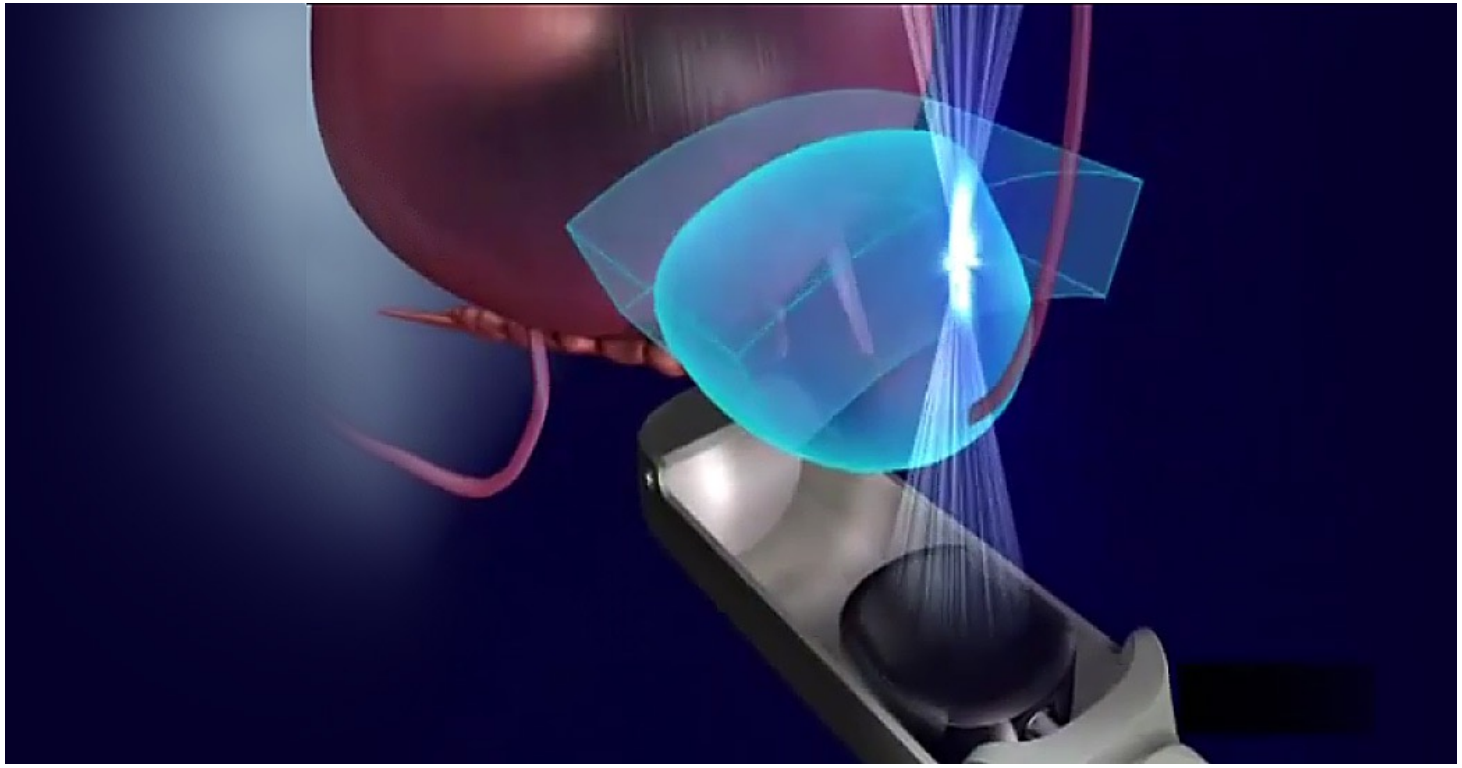


(Non-linear) ultrasonics



Thermal therapies

Thermal ablation by high-intensity focused ultrasound (HIFU)



Ultrasound focused into the target region, where sound is absorbed as heat and heat induces damage to the targeted tissue.

Thermal dose

- Thermal dose:

$$TD_{43}(\mathbf{r},t) = \int_0^t R^{43-T(\mathbf{r},\tau)} d\tau$$

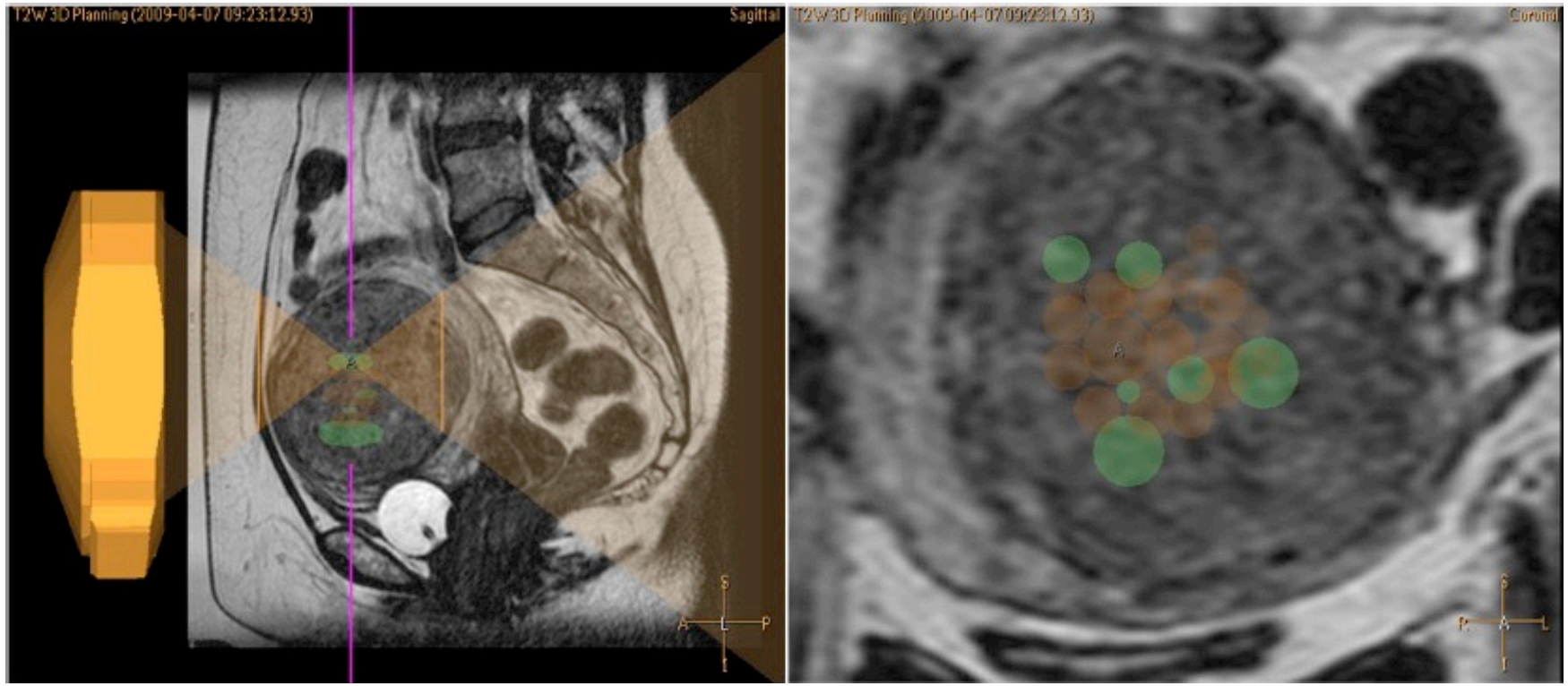
where $R = 0.25$ when temperature, $T(\mathbf{r},t)$, is less than $43^{\circ}\mathrm{C}$ and $R = 0.5$ at temperature $43^{\circ}\mathrm{C}$ or higher.

- Describes the dose due to temperature
- Keeping tissue in 43^{o}C for 240 minutes destroys destroys the tissue
- Keeping tissue in 55^{o}C for few seconds destroys destroys the tissue
- Thermally induced damage is strongly a threshold-like phenomenon ➔ tissue near the focus is destroyed, while leaving adjacent tissues intact.

Applications

- Thermal ablation: destroying thermally tissue by denaturing proteins
- Hyperthermia: elevating temperature of tissue to enhance drug therapy (influencing the cellular repair mechanisms)
- Drug release: releasing drugs from thermally sensitive microcapsules

Thermal ablation by HIFU: Uterine fibroid



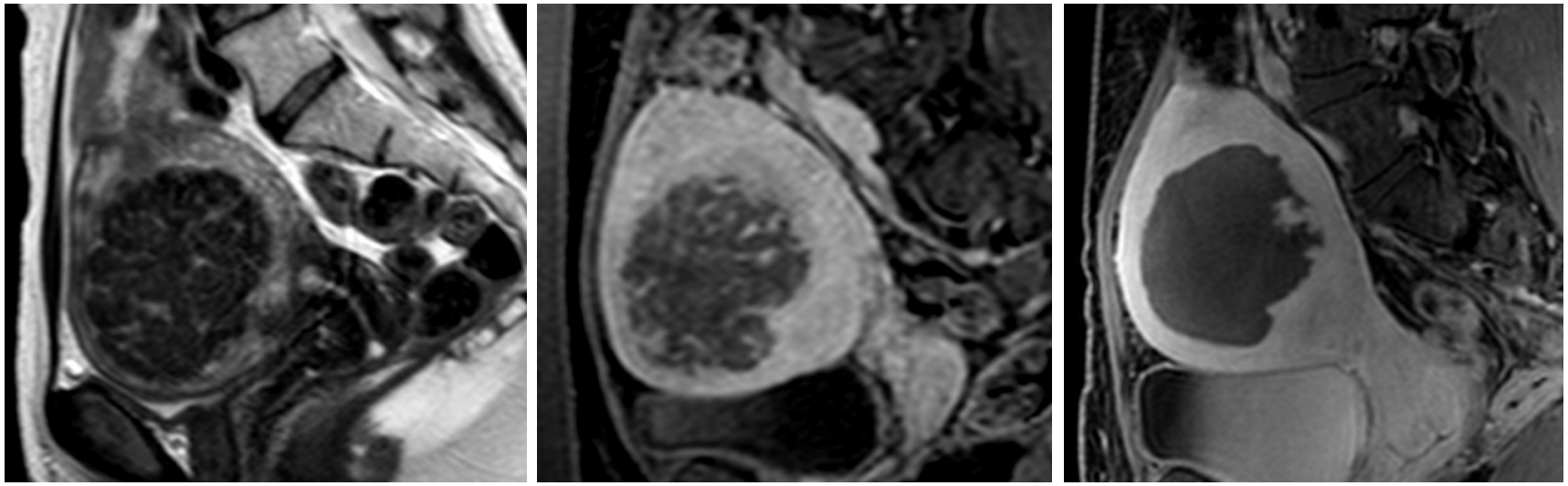
From lectio praecursoria of Dr. Matti Tillander, Aalto University

Thermal ablation by HIFU: Uterine fibroid

Planning (T2)

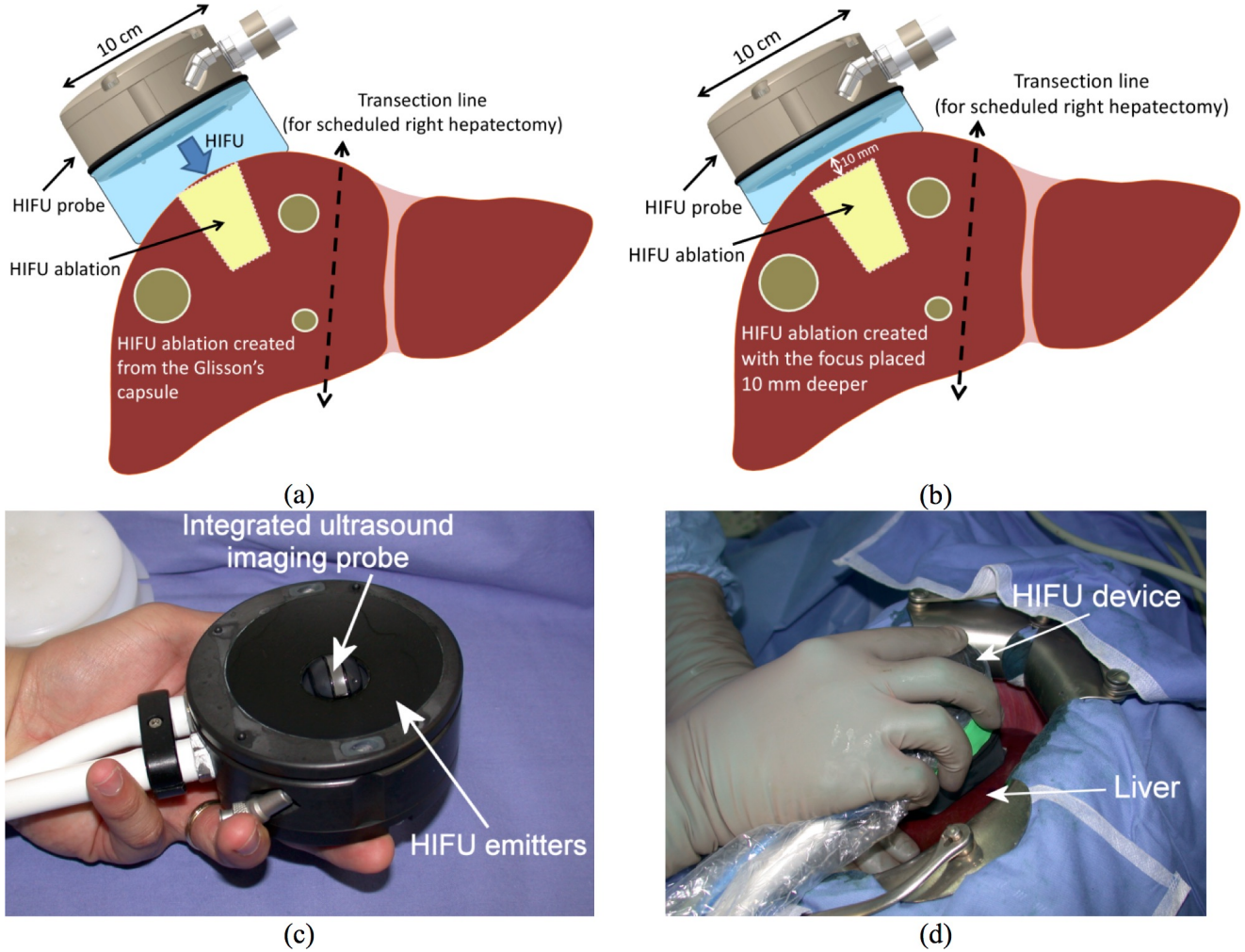
0 day post-treatment (T1, CE)

0 month post-treatment (T1, CE)



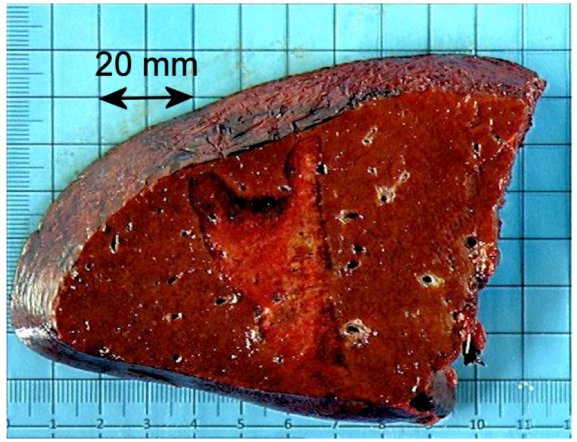
From lectio praecursoria of Dr. Matti Tillander, Aalto University

Thermal ablation by HIFU: Liver

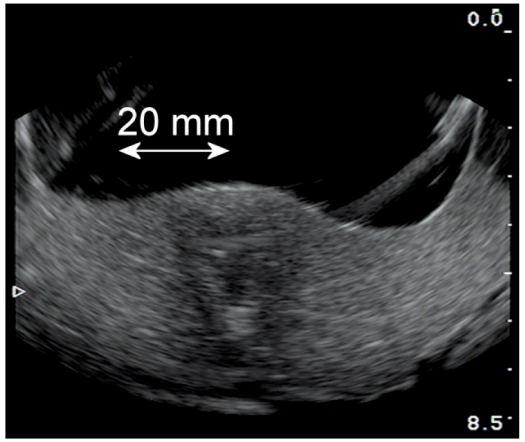


Dupré et al 2015: https://doi.org/10.1371/journal.pone.0118212

Thermal ablation by HIFU: Liver



(a)



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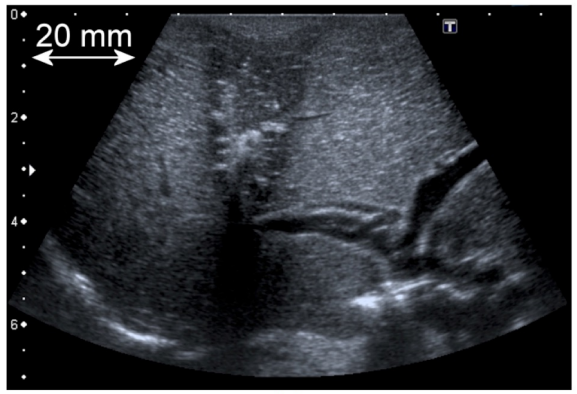
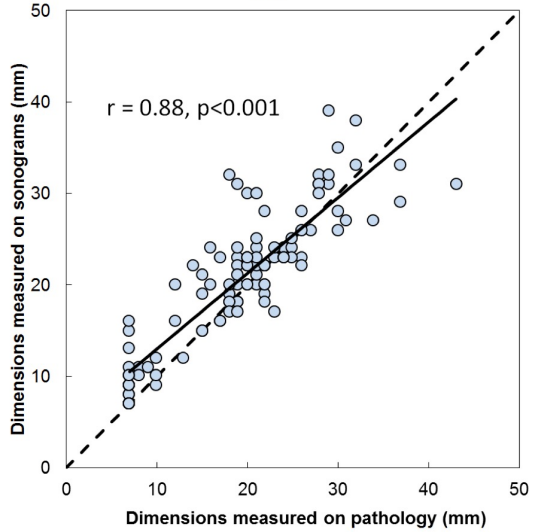


(c)



(d)

Dupré et al 2015: https://doi.org/10.1371/journal.pone.0118212

Hyperthermia

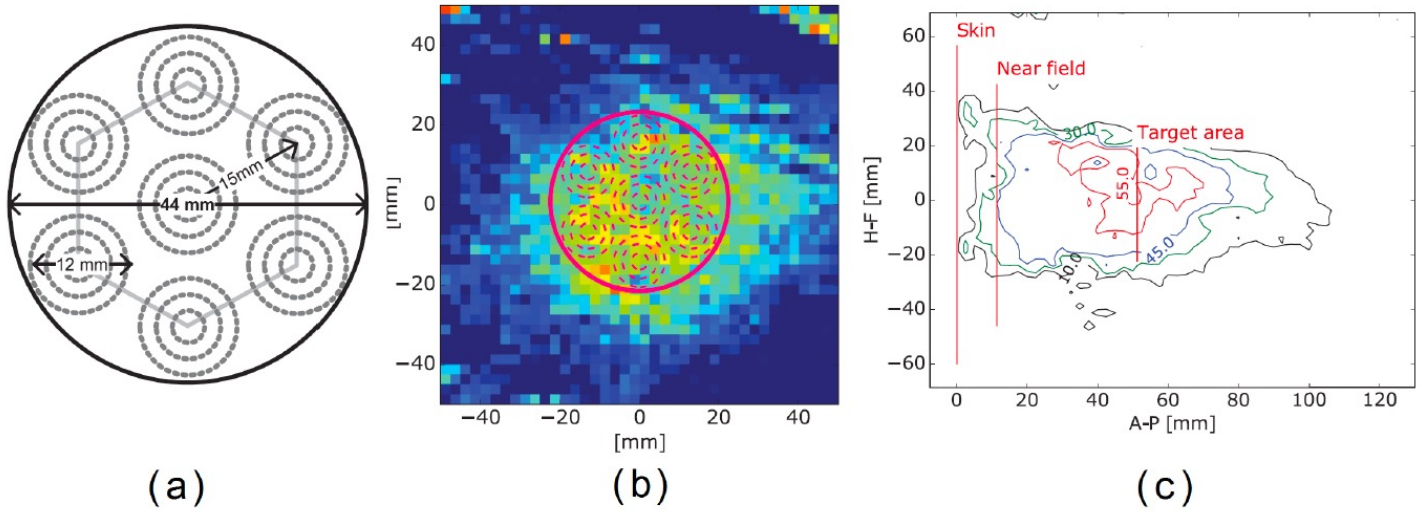


Figure 4.1. (a) A schematic representation of multiposition sonication cells in a plane parallel with the beam axis. The sonication is divided into seven transducer positions arranged on a hexagonal grid with one position in the middle. In each focal position, steering is used. (b) An example of measured focal-plane temperature map in *in-vivo* porcine muscle tissue during heating (sonication cell grid overlayed in magenta). (c) Beam axis view of one hyperthermia sonication. The contours represent the minutes the area was within hyperthermia temperature range (41–45°C) during sonication. (Modified from Publication I)

Tillander 2017: https://aaltodoc.aalto.fi/handle/123456789/25038?locale-attribute=fi

Hyperthermia

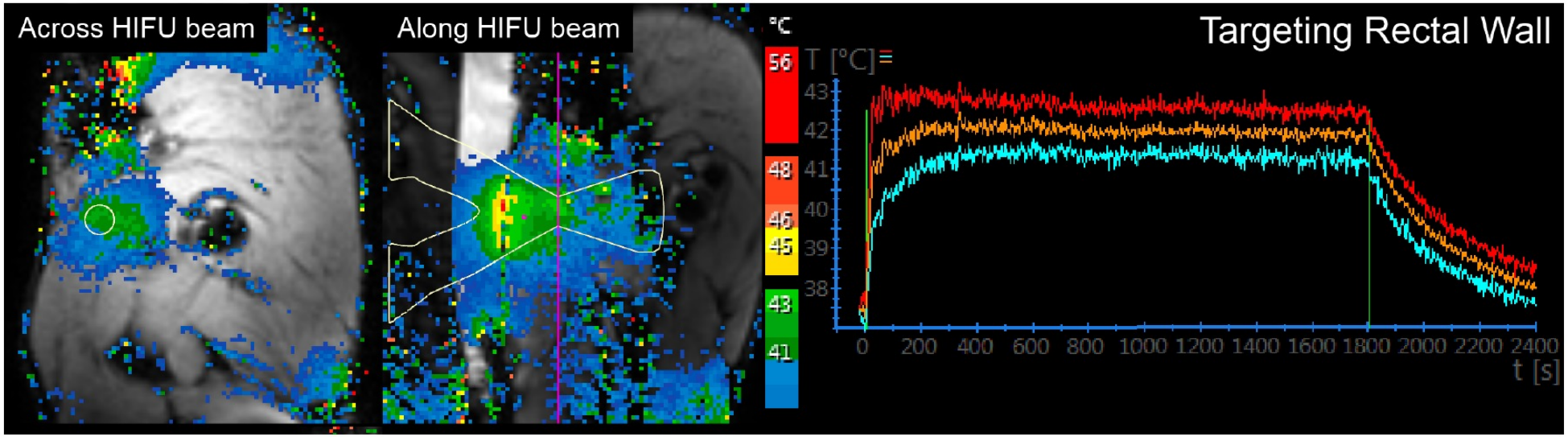
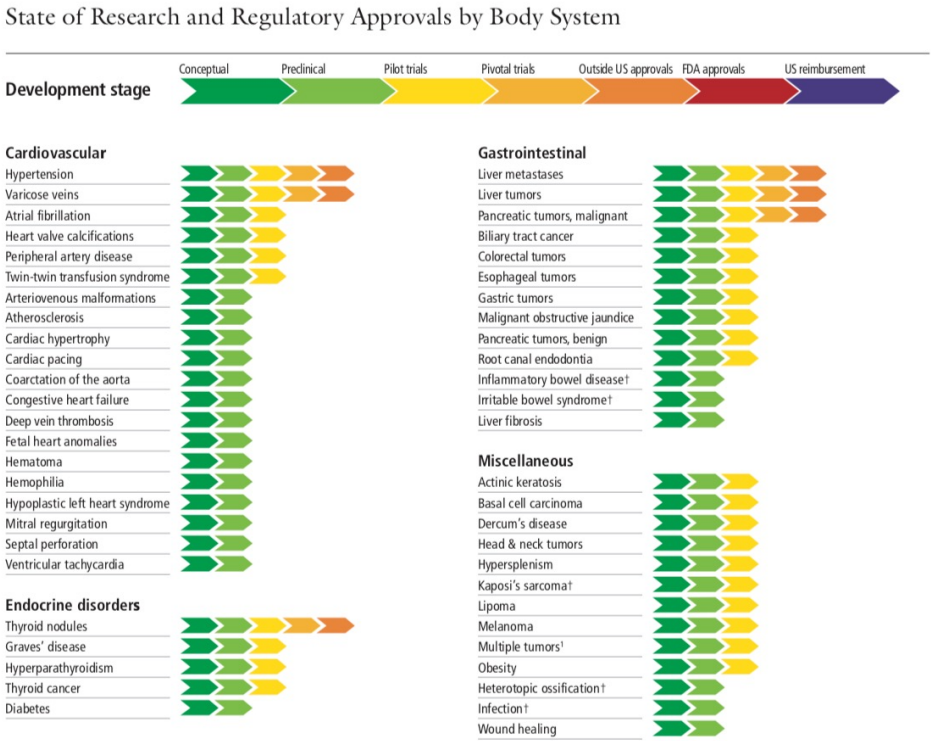


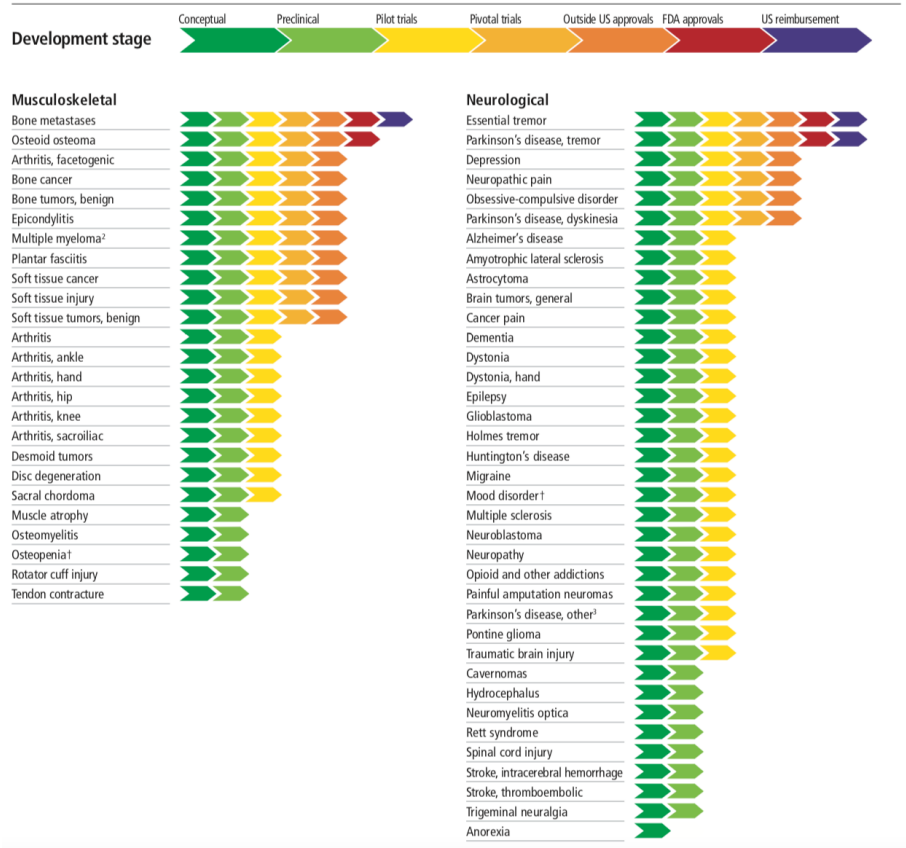
Figure 4.2. Coronal (left) and sagittal (center) temperature maps for a hyperthermia sonication near the porcine rectal wall. The plot on the right shows the temperature evolution of spatial average, spatial T10, and spatial T90 temperatures for the target area at focal depth. (Modified from Publication II)

Tillander 2017: https://aaltodoc.aalto.fi/handle/123456789/25038?locale-attribute=fi

APPLICATIONS

HIFU applications status (FUS Foundation)

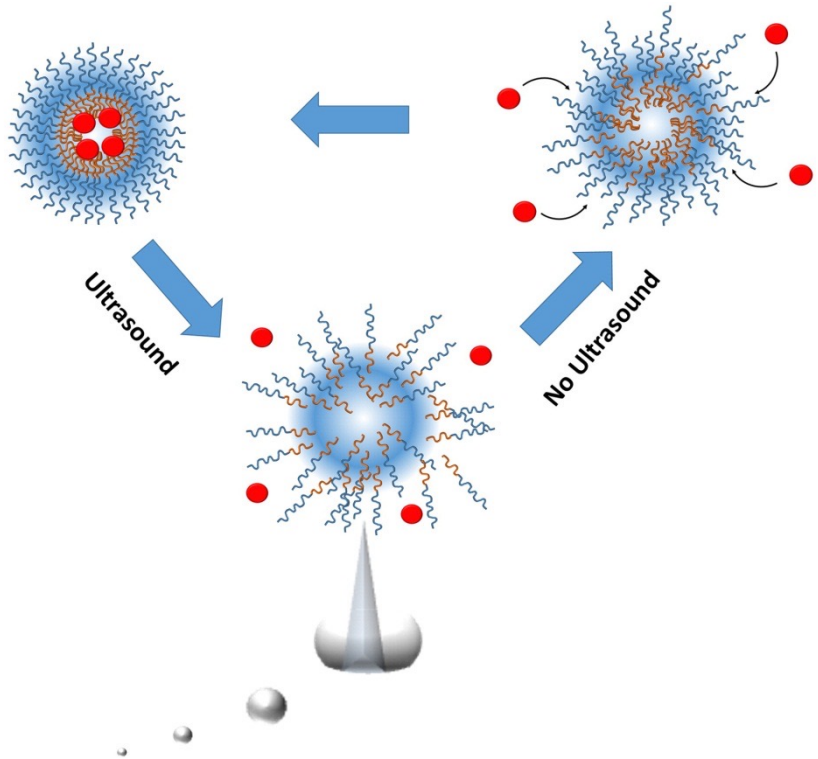




Treatment of the essential tremor

https://www.youtube.com/watch?v=ZCPeswPaUvM

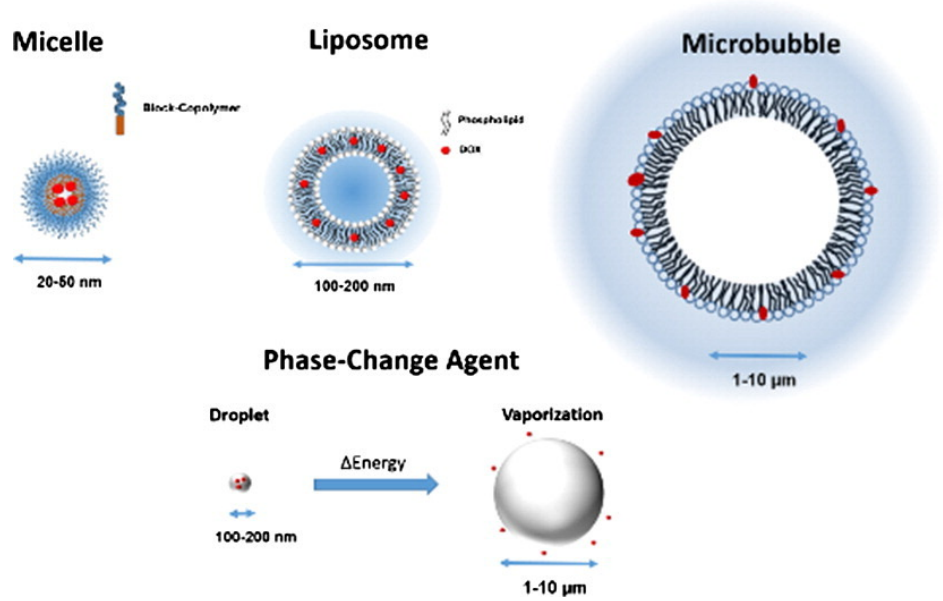
Ultrasonic drug delivery



Sirsi & Borden 2014: http://www.sciencedirect.com/science/article/pii/S0169409X13002962

Ultrasound triggered drugs

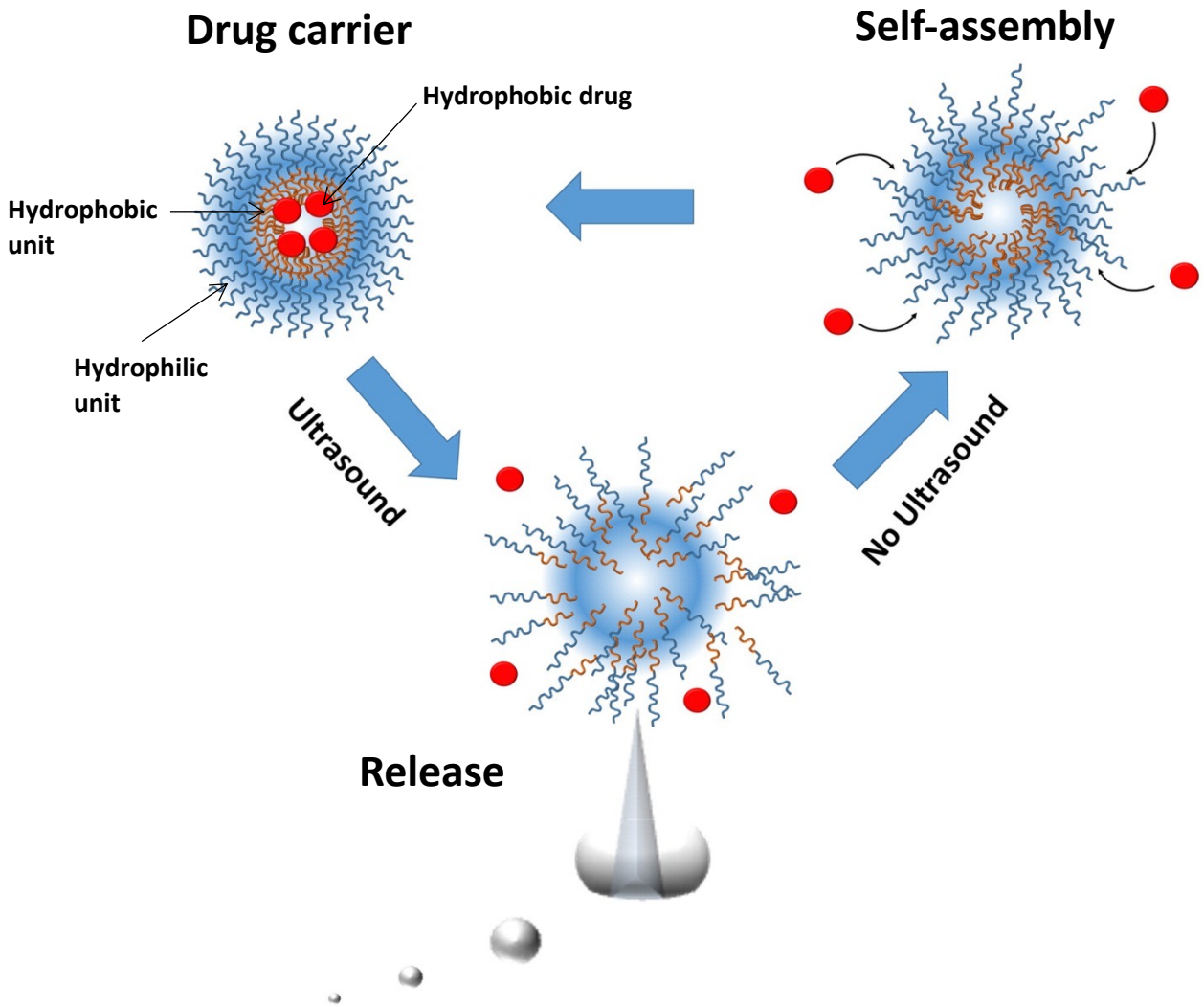
- In the following we deal with drug delivery systems that can be used for ultrasonic drug delivery
- These systems can be categorized to the following:
  - Nanocarriers
    - Micelles
    - Liposomes
  - Microbubbles
    - Soft-shelled microbubbles
    - Hard-shelled microbubbles
  - Nanocarrier-microbubble hybrids
  - Microbubble-loaded hydrogels
  - Phase change agents
  - Advanced systems



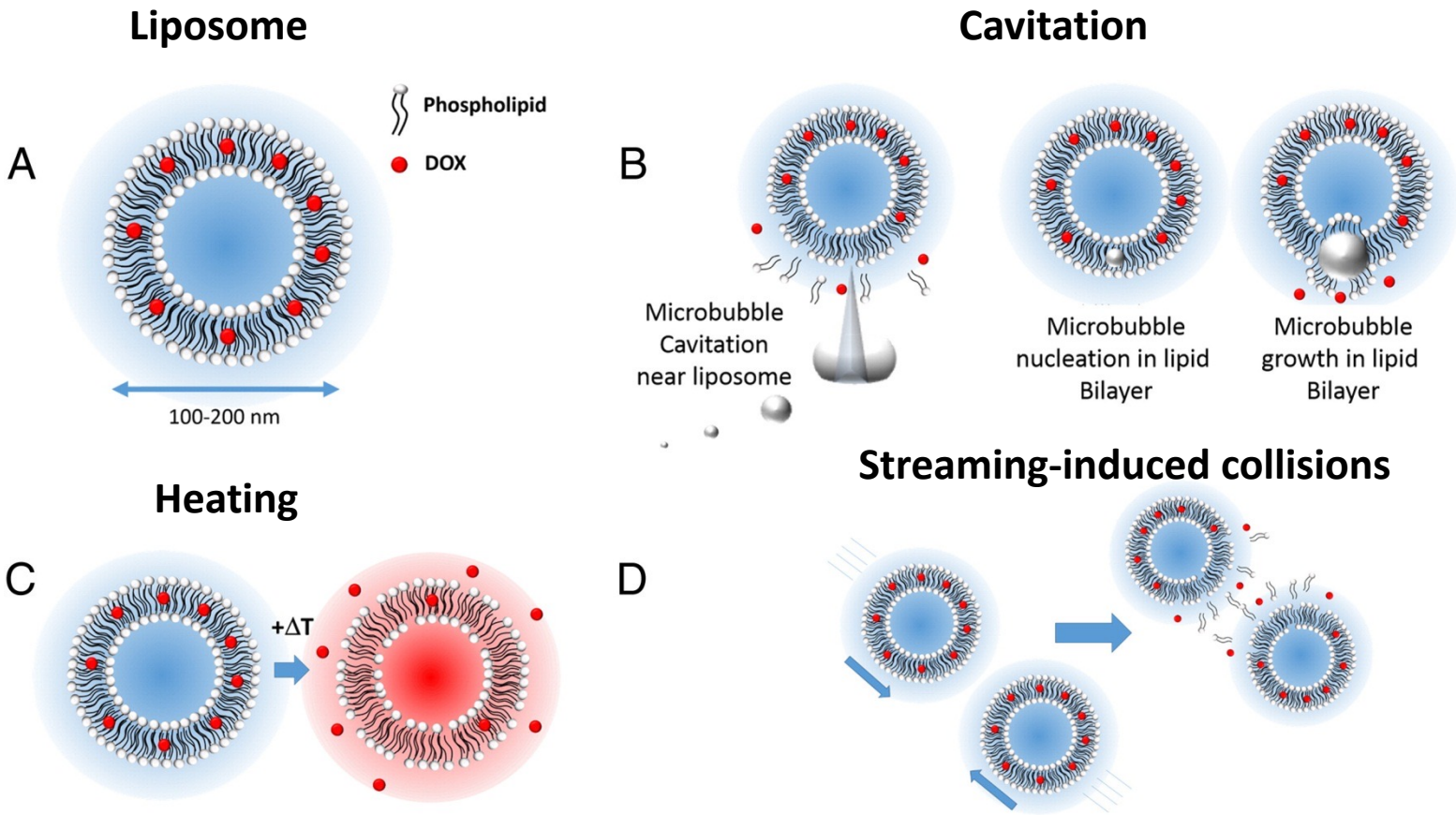
Sirsi & Borden 2014: http://www.sciencedirect.com/science/article/pii/S0169409X13002962

Nano-carriers: Micelles

- Composed of amphilic molecules
- Relatively easy to make due to self-assembly above critical concentration
- Micelles are typically 10-100 nm in diameter
- **Ultrasonic release from micelles mainly associated with cavitation, although hyperthermia plays a role with thermosensitive micelles**
- Release typically conducted kHz range although studies at MHz range also exist



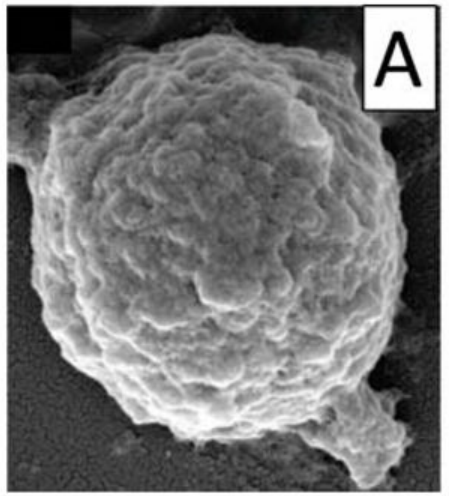
Nano-carriers: Liposomes



- Liposomes typically 100-200 nm in diameter
- Drugs loaded to 1. the phospholipid bilayer or 2. aqueous core
- **Release mechanisms: 1. cavitation, 2. heat** (phase transition above physicological temperature ➔mild hyperthermia) **and 3. acoustic streaming**

Micro-bubbles as drug carriers

- Micro-bubbles used commonly used as vascular contrast agents
- Responsive to external driving pressure (ultrasound)
- Size typically 1-10 µm
- Structure:
  - Gas-filled core
  - Stabilizing shell:
    - Polymer
    - Lipid
    - Protein



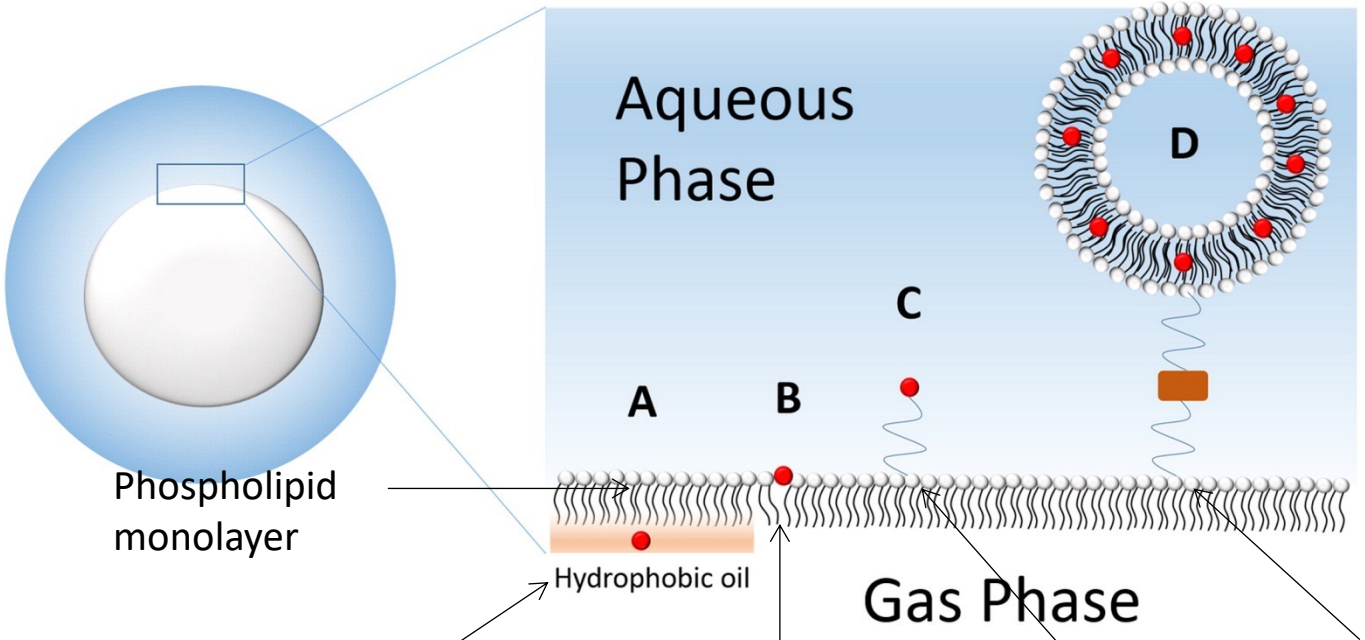
1 µm micro-bubble with a protein shell

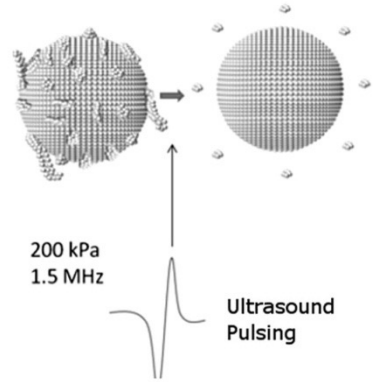
Sirsi & Borden 2009: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2889676/pdf/nihms204772.pdf

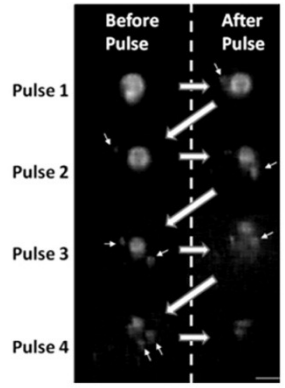
Micro-bubbles: soft-shelled bubbles

- Microbubbles used in ultrasonic drug delivery are commonly soft-shelled, *i.e.* they have a thin shell made of phospholipids or proteins
- Compliant shell ➔ sensitive to the driving pressure ➔ stable or inertial cavitation may occur

Micro-bubbles: soft shell







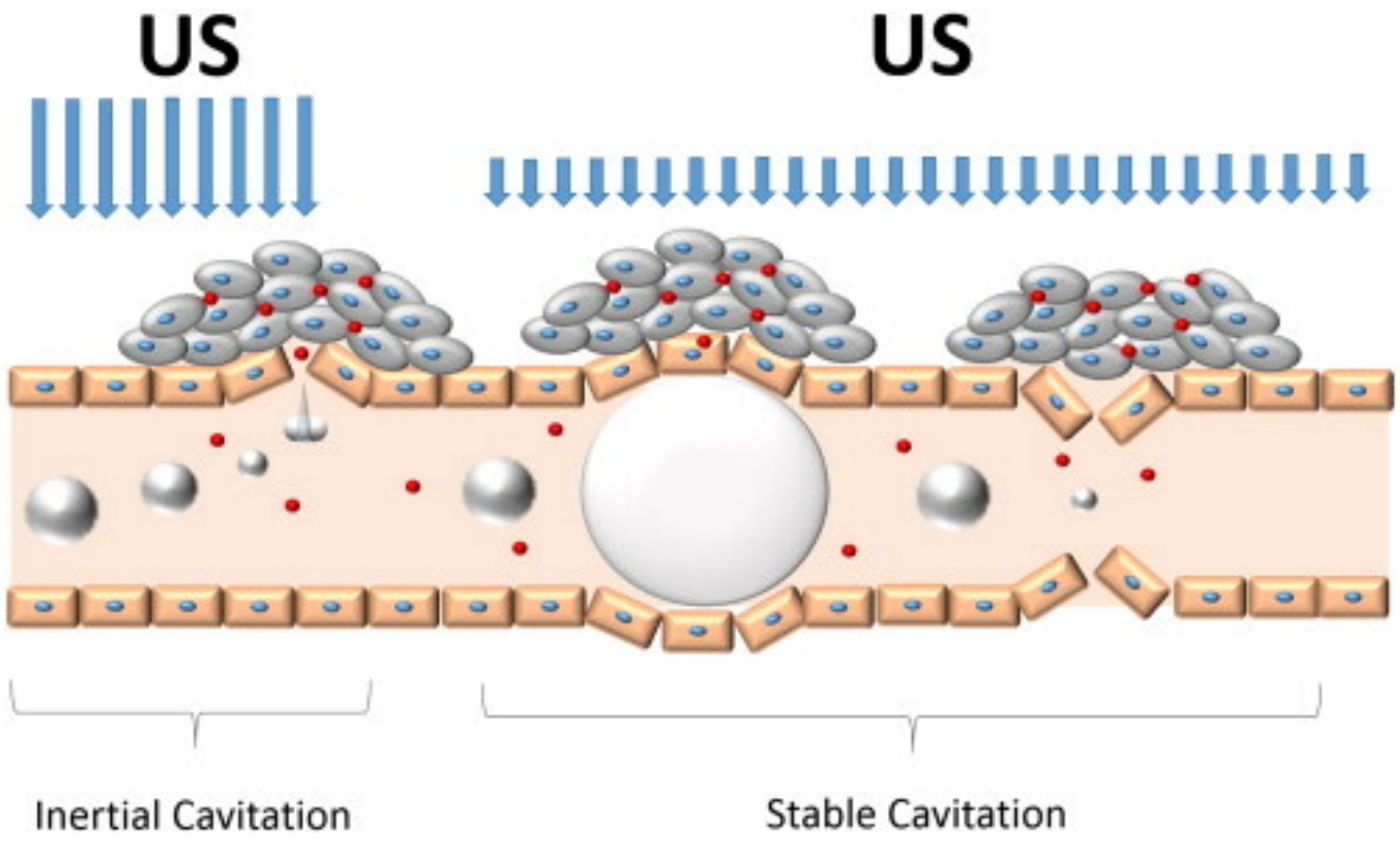
Loading hydrophobic oil with the drug

Incorporating the drug in the phospholipid monolayer

Tethering the drug to the phospholipid monolayer

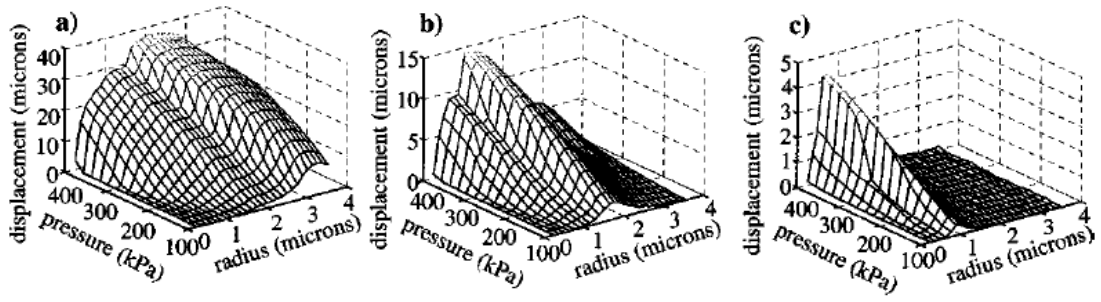
Loading the drug into nanocarriers (e.g. liposome) tethered to the bubble

Extravasation by micro-bubbles



Bubble translation

20 cycle pulse



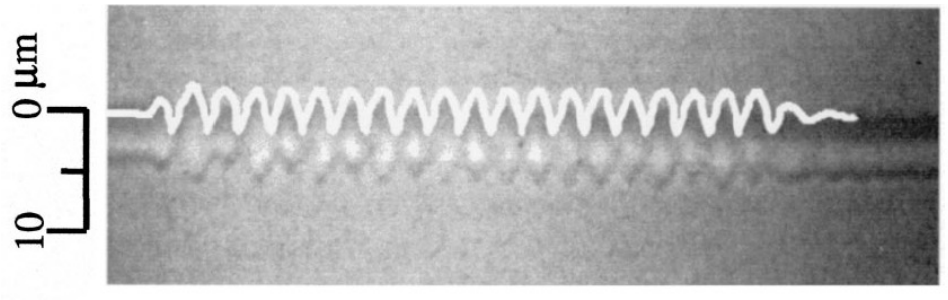
1 MHz **2.25 MHz** **5 MHz**

Max. translation:

- Maximized at bubble resonance

2.25 MHz, 100 kPa

Initial radius (microns)	Radiation force (Newtons)	Maximum radius (microns)	$\langle R - R_o \rangle$ (microns)
0.5	4.8×10^{-9}	0.55	0.00
1.38	4.0×10^{-6}	1.8	0.09
1.63	1.07×10^{-5}	2.3	0.15
2.88	3.60×10^{-6}	3.2	0.01
3.50	2.43×10^{-6}	3.7	0.00



Medical ultrasound may generate micro-bubble speed as high as 0.5 m/s (5 um distance in 20 cycles of 380 kPa, 2.25 MHz ultrasound).

Dayton et al. http://www.ncbi.nlm.nih.gov/pubmed/12430830

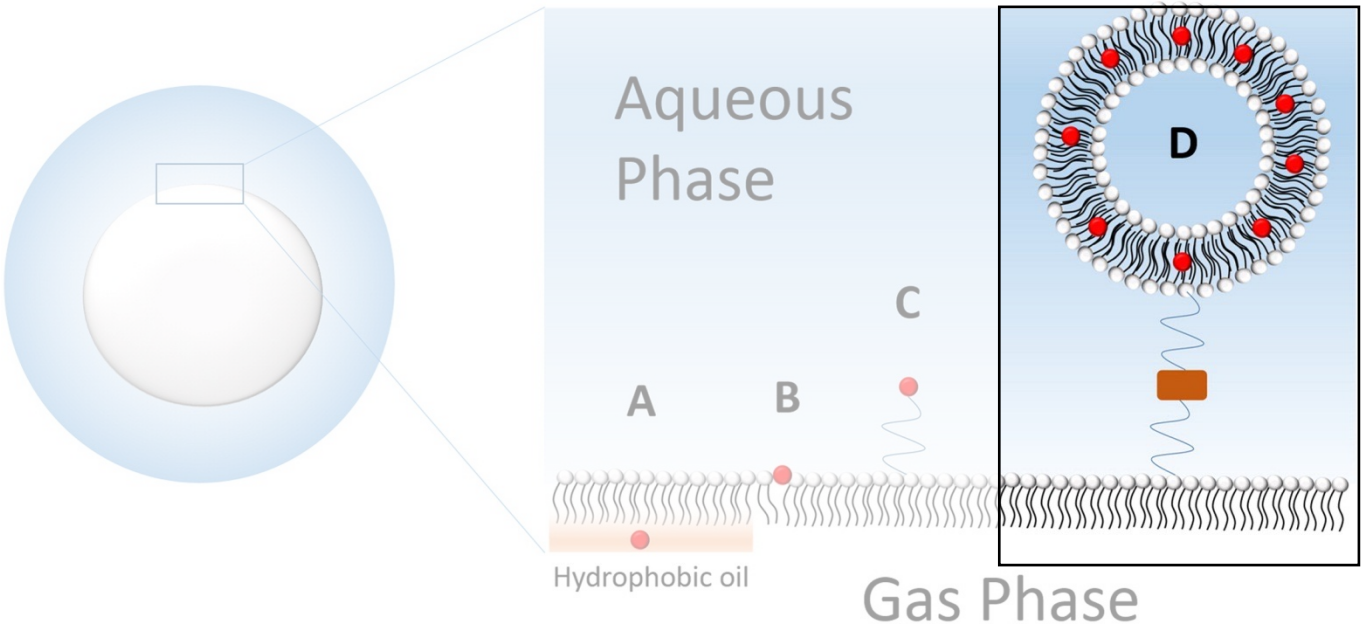
Micro-bubbles: hard-shelled bubbles

- Hard shell ➔ negligible radial oscillation due to low compliance of the shell
- High pressure (>1 MPa) cracks the shell
  - ➔ gas expands and is released
  - ➔ potentially propels the vehicle to vessel walls & contributes to extravasation

➔ *Hard-shelled microbubbles are typically pressure sensitive*

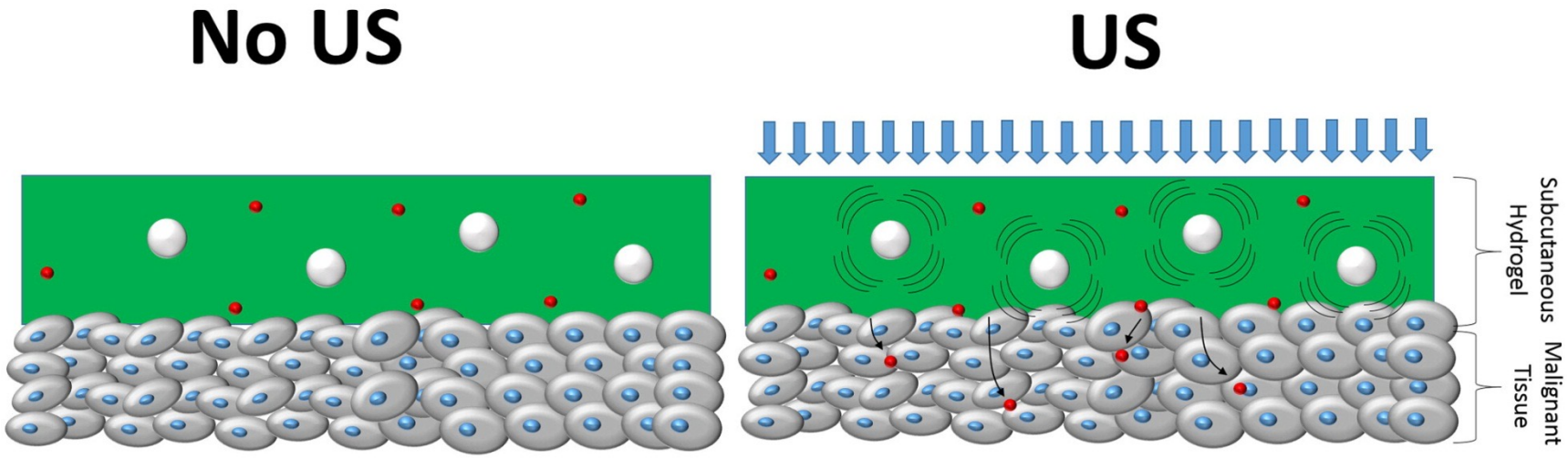
Nanocarrier-microbubble hybrids

- Drug-containing liposomes, micelles or nanoparticles are attached to the microbubble
- High-loading capacity (10^3-10^4 liposomes per bubble)

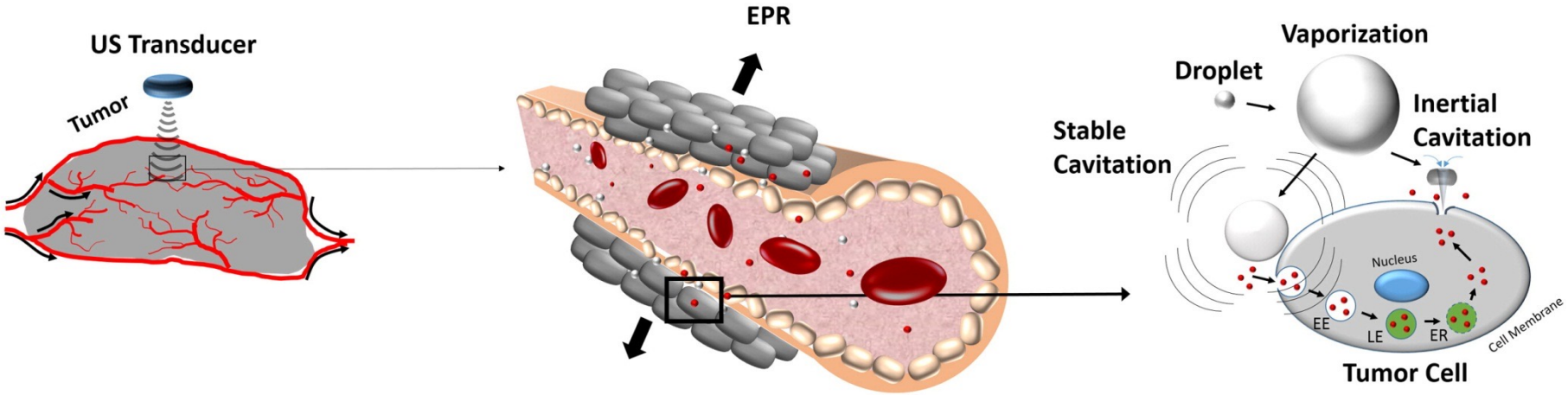


Microbubble-loaded hydrogels

- Drugs & microbubbles embedded into hydrogel
- Enables drug-release-on-demand implants



Phase-change agents

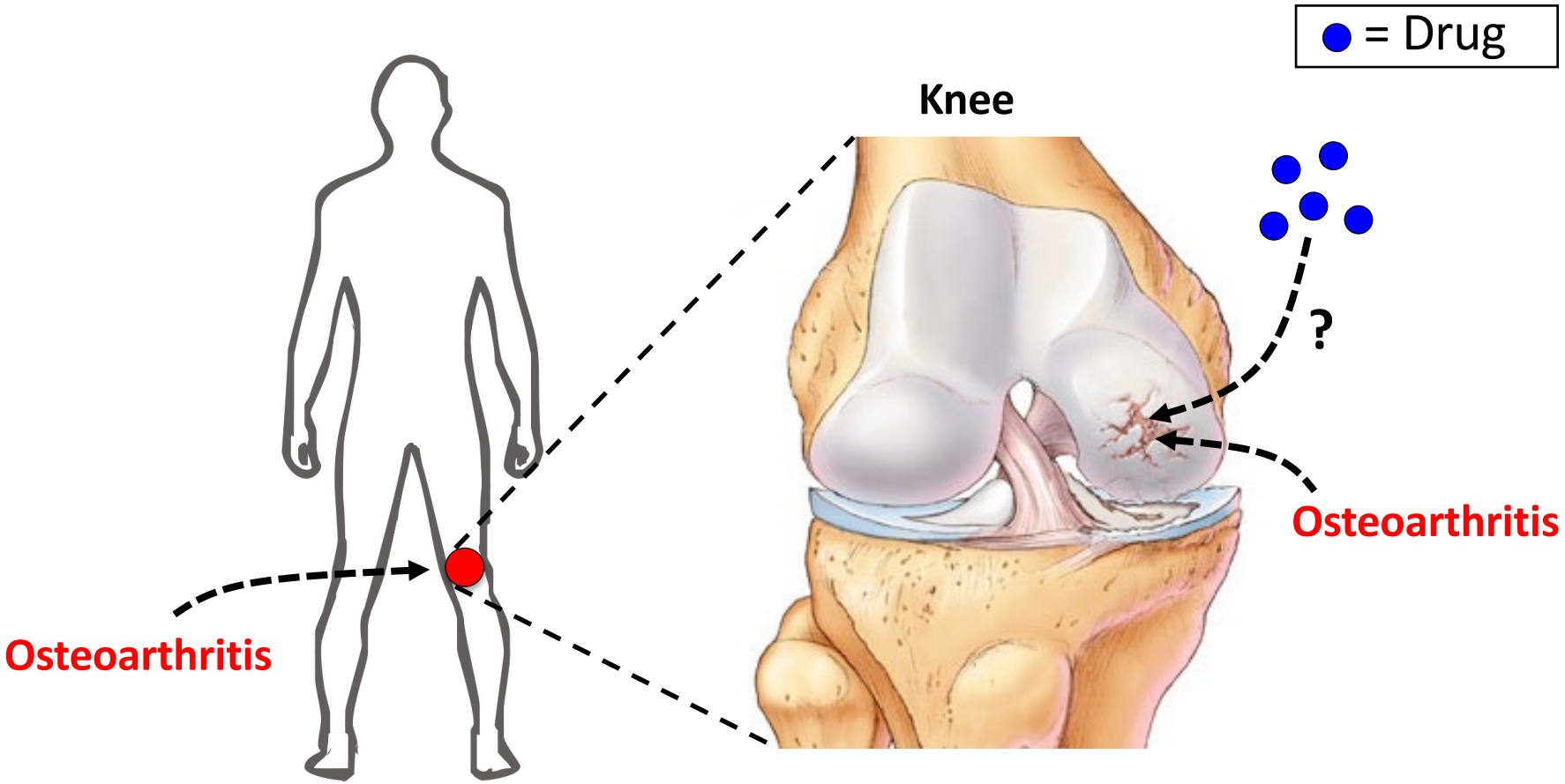


- Micelle contains thermally ”sensitive” liquid containing the drug ➔during the positive phase of the driving sound, the droplet is heated and vaporized
- Gas bubble interacts by means of cavitation to deliver the drug to the target (cell)
- Directed propulsion can be achieved (> 6 m/s)
- Simultanously with drug delivery therapy feedback can be achieved with ultrasound imaging.

Sirsi & Borden 2014: http://www.sciencedirect.com/science/article/pii/S0169409X13002962

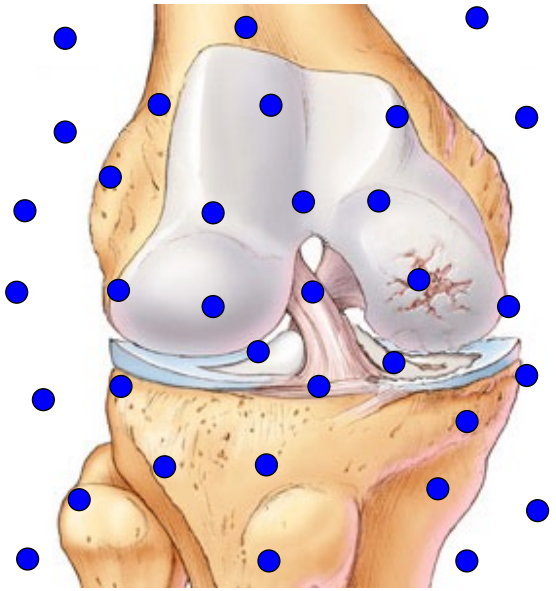
Ultrasonic drug transport

Drug transport by ultrasound



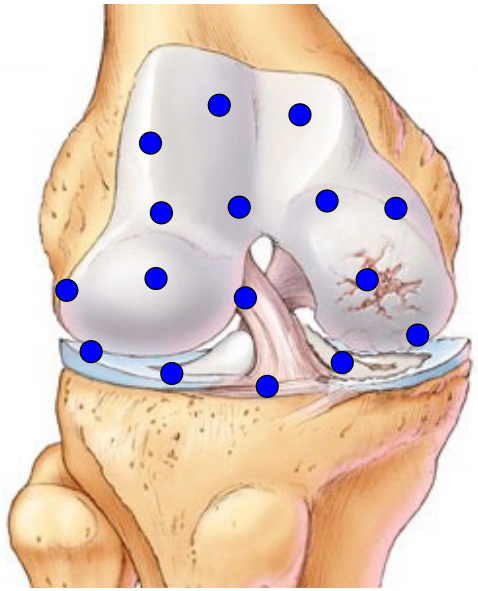
1.

Systemic delivery



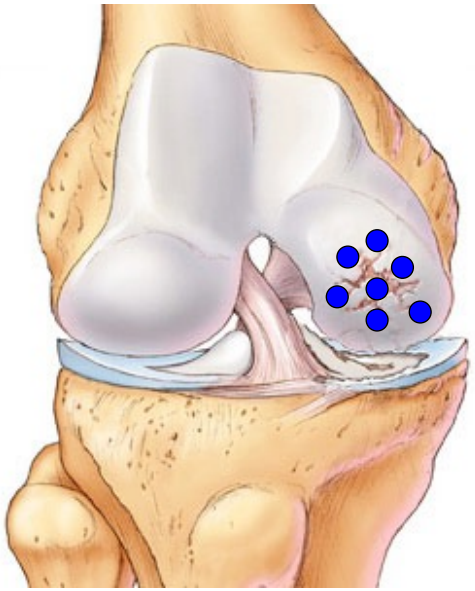
2.

Localized therapy



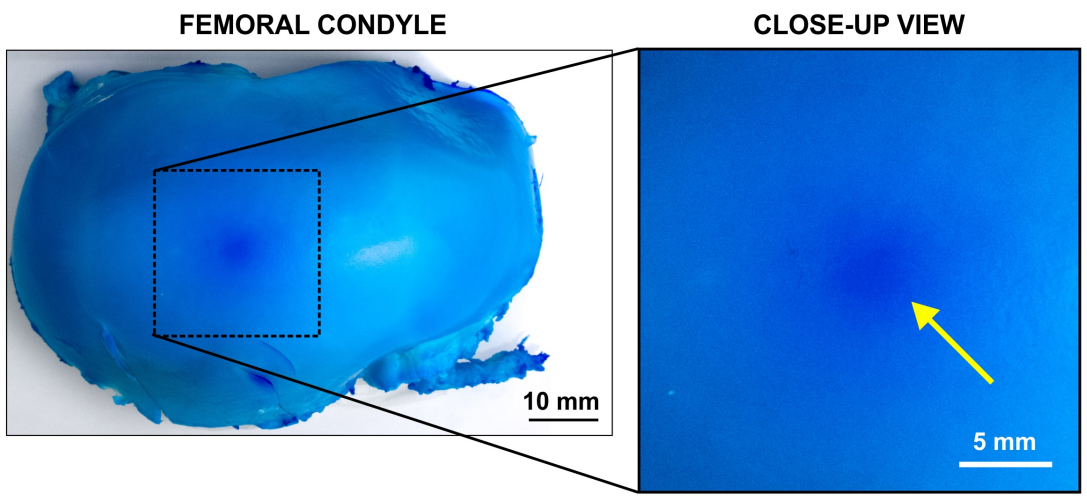
3.

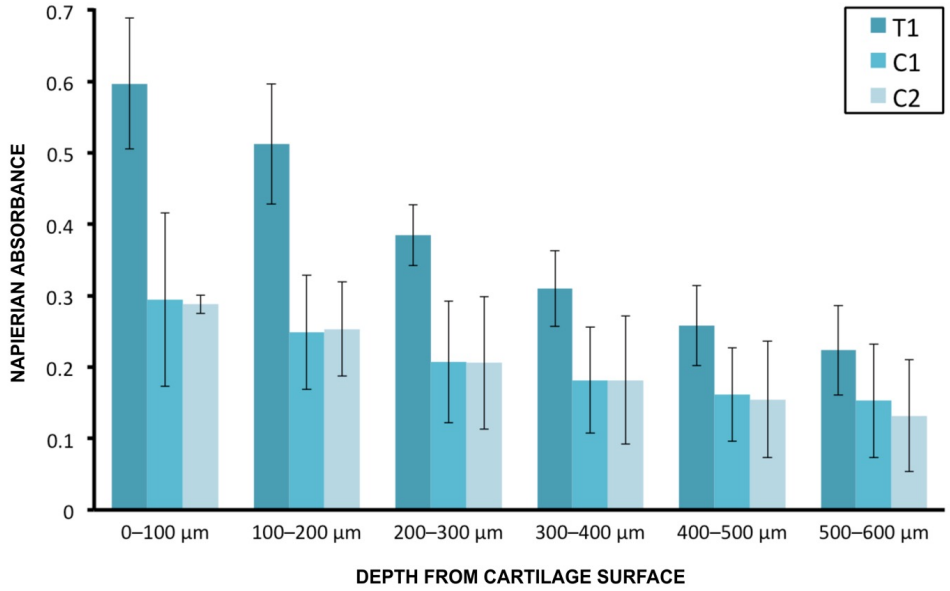
Targeted delivery



● = drug

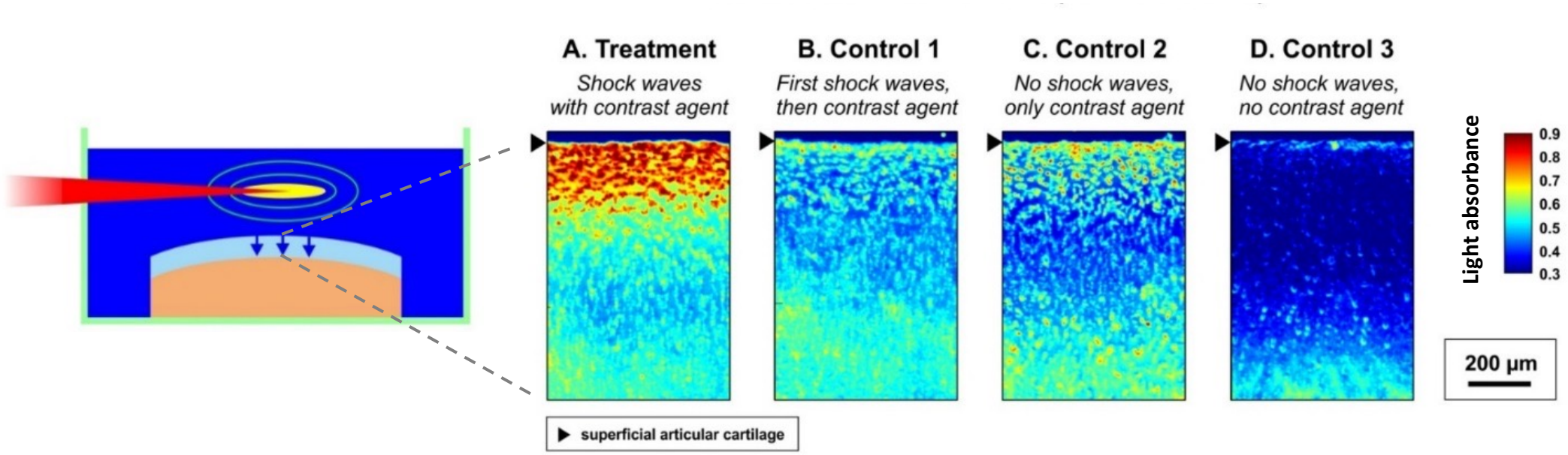
Drug transport by focused ultrasound





Nieminen et al. 2018 (Manuscript)

Laser-ultrasonic drug transport

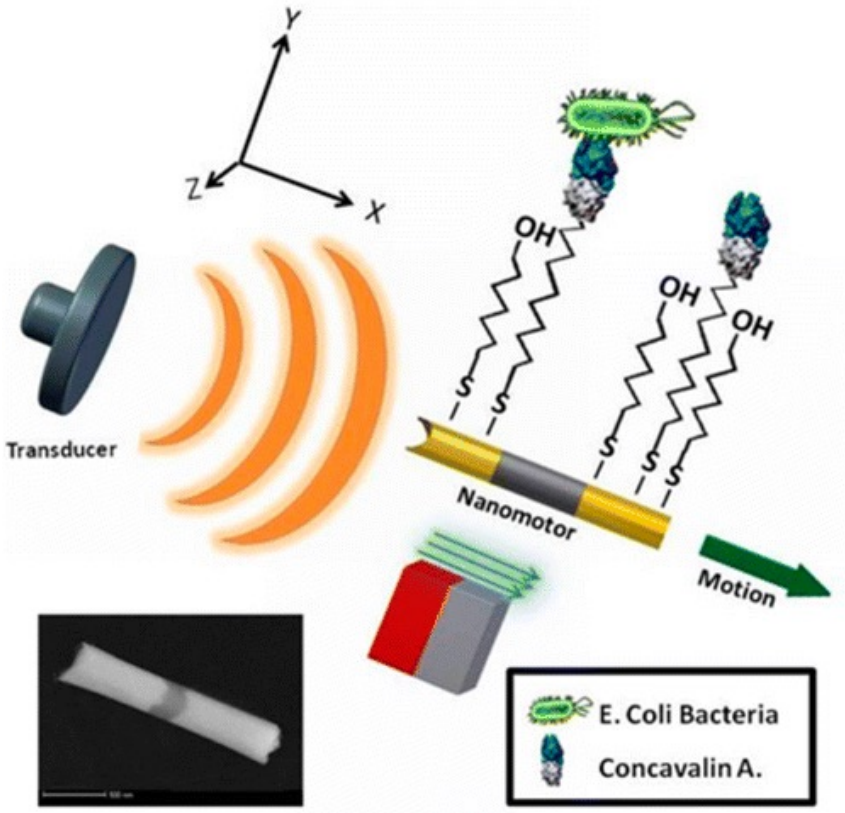


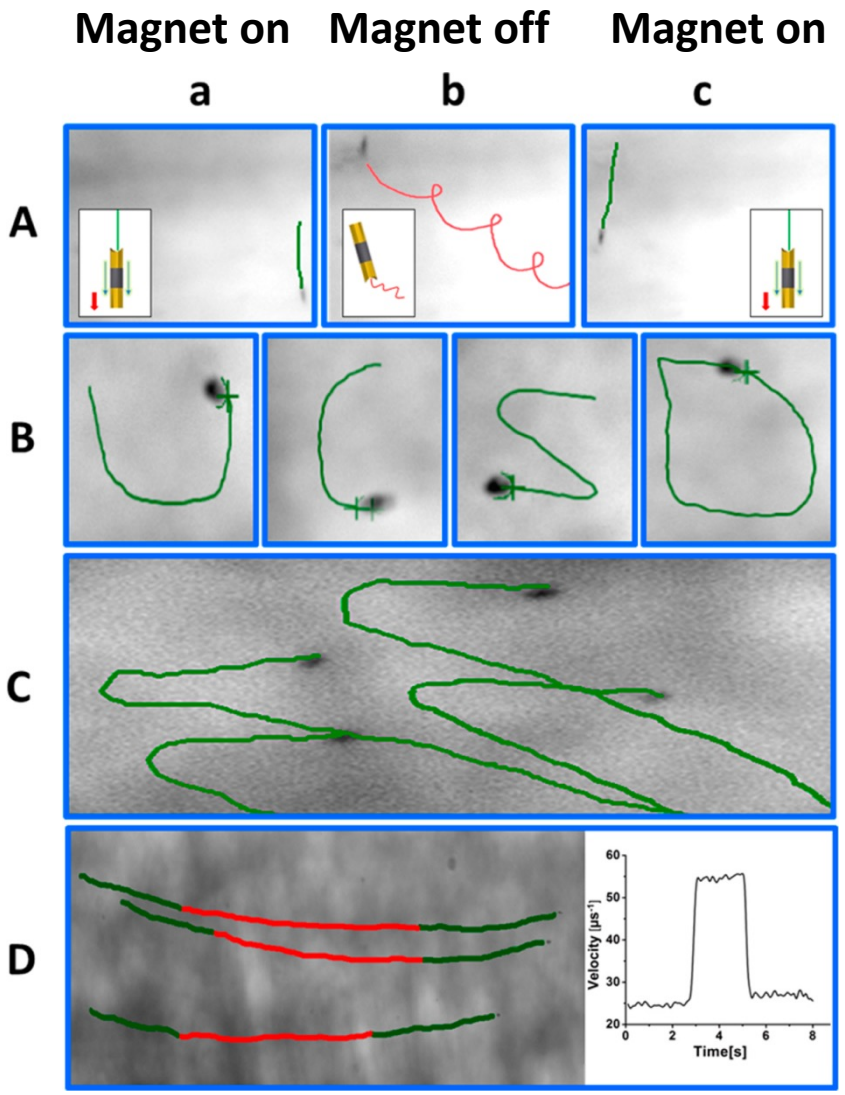
Nieminen et al. 2017. DOI: 10.1038/s41598-017-04293-5.

Ultrasonic microvehicles

Deterministic functionalized transportation

- Almost all delivery approaches are statistical
- Deterministic delivery system:

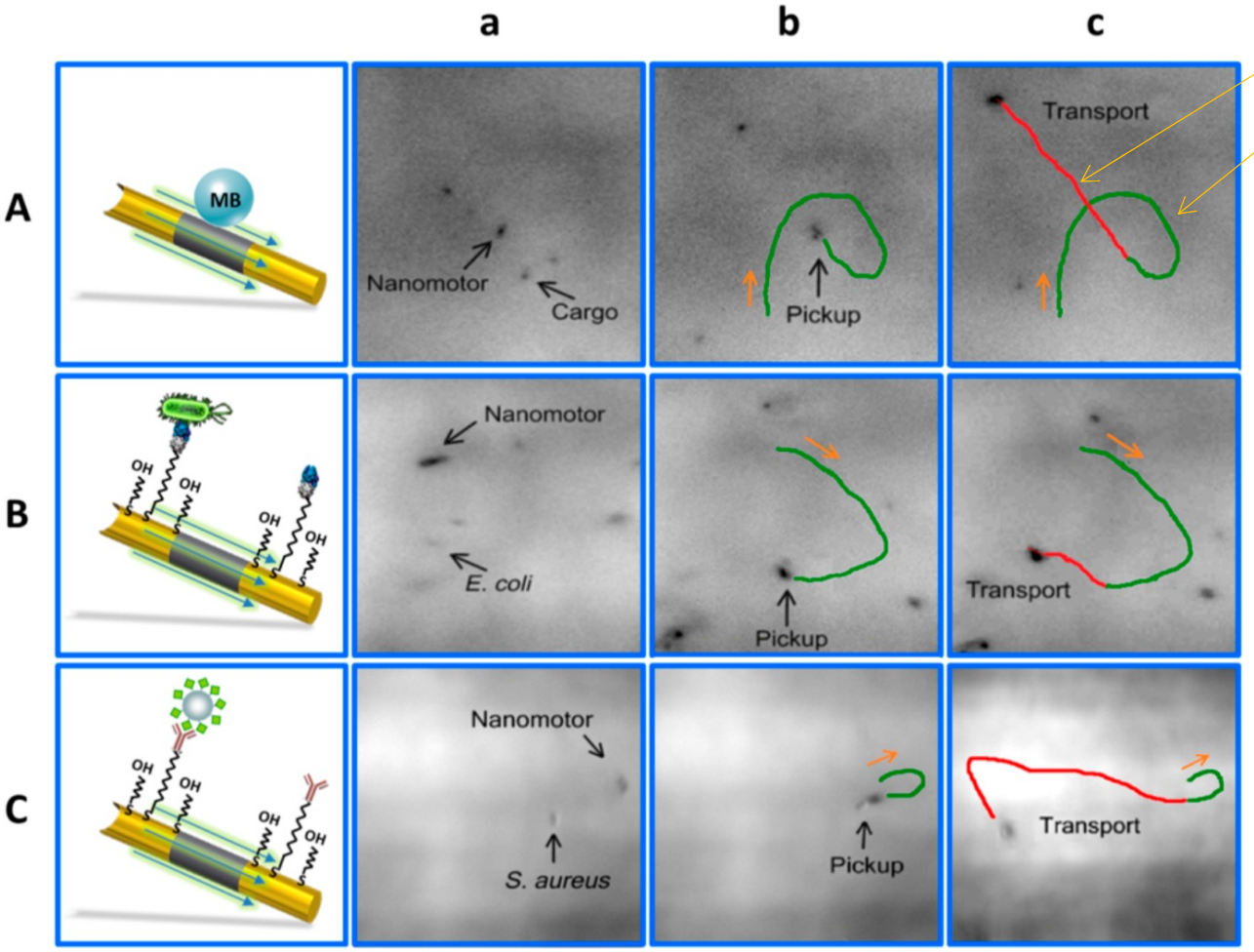




Speed modulation by ultrasound

Garcia-Gradilla 2013 et al. http://pubs.acs.org/doi/abs/10.1021/nn403851v

Deterministic functionalized transportation



After pickup

Before pickup

Collecting a magnetic particle

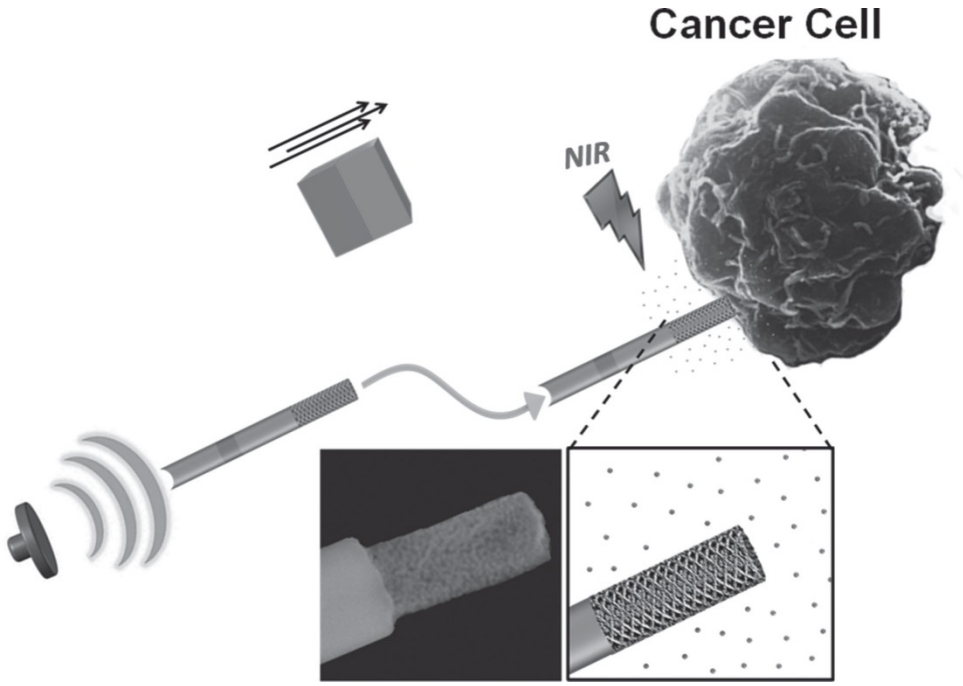
Collecting *E. Coli*

Collecting *S. aureus*

Figure 5. (A) Capture and transport of a 0.89 μm magnetic bead by an ultrasound-propelled Au/Ni/Au unmodified nanomotor approaching (a), capturing (b), and transporting (c) the magnetic particle. (B) Capture and transport of *E. coli* bacteria by a lectin-modified ultrasound-propelled nanomotor: (a) approaching, (b) pickup, and (c) transport the *E. coli* bacteria. (C) Capture and transport of *S. Aureus* bacteria by a Con A-modified ultrasound-propelled nanomotor: (a) approaching, (b) pickup, and (c) transport the *S. Aureus* bacteria. Conditions: media, deionized water (A) and PBS (pH 7.0) solutions (B and C); ultrasound field, 6 V and 2.51 MHz.

Garcia-Gradilla et al. http://pubs.acs.org/doi/abs/10.1021/nn403851v

Deterministic drug delivery to a cancer cell



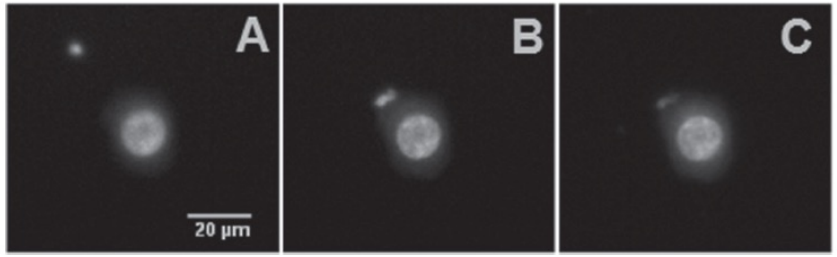
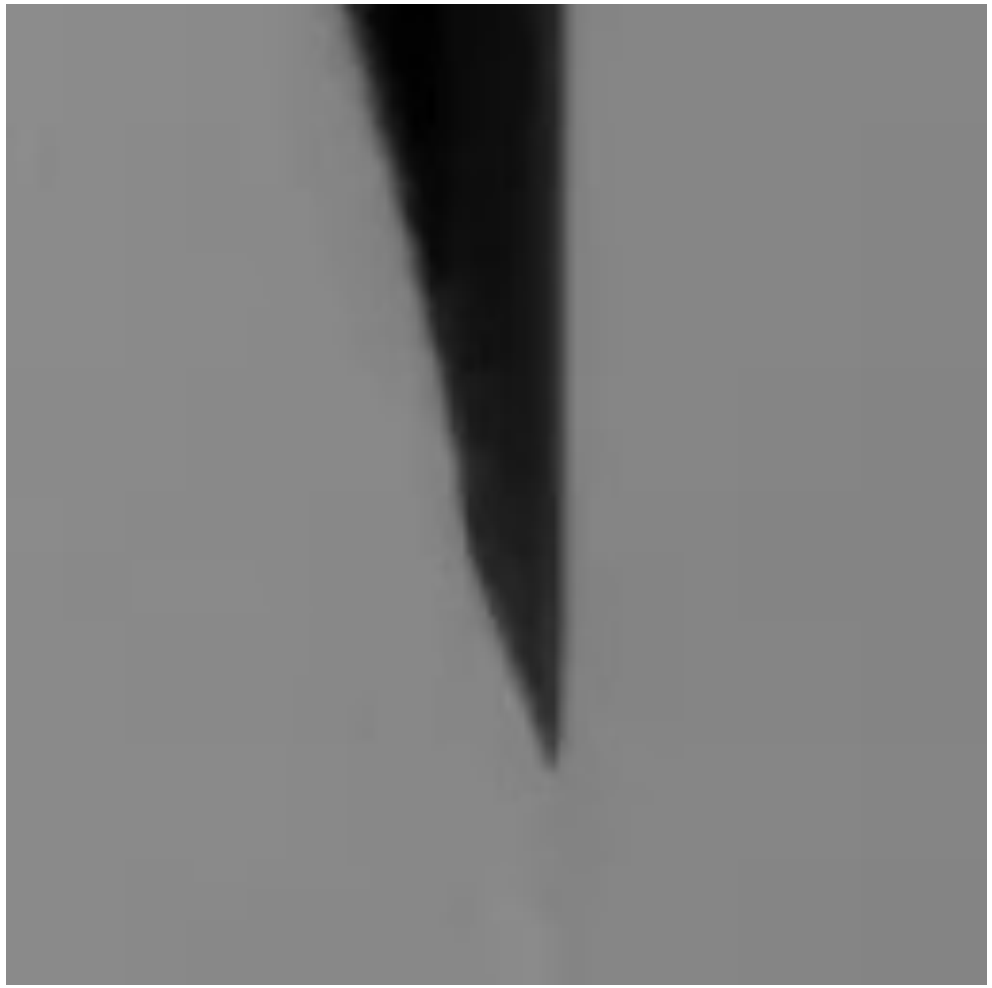


Figure 6. Time-lapse fluorescent images of the DOX-loaded NPAu nanomotor A) travelling towards a HeLa cell, B) approaching the HeLa cell, and of C) the nanomotor and the HeLa cell after 15 min of NIR irradiation. DOX release conditions, as in Figure 4. Motion of NPAu nanomotors was achieved under ultrasound propulsion at 6 V and 2.01 MHz.

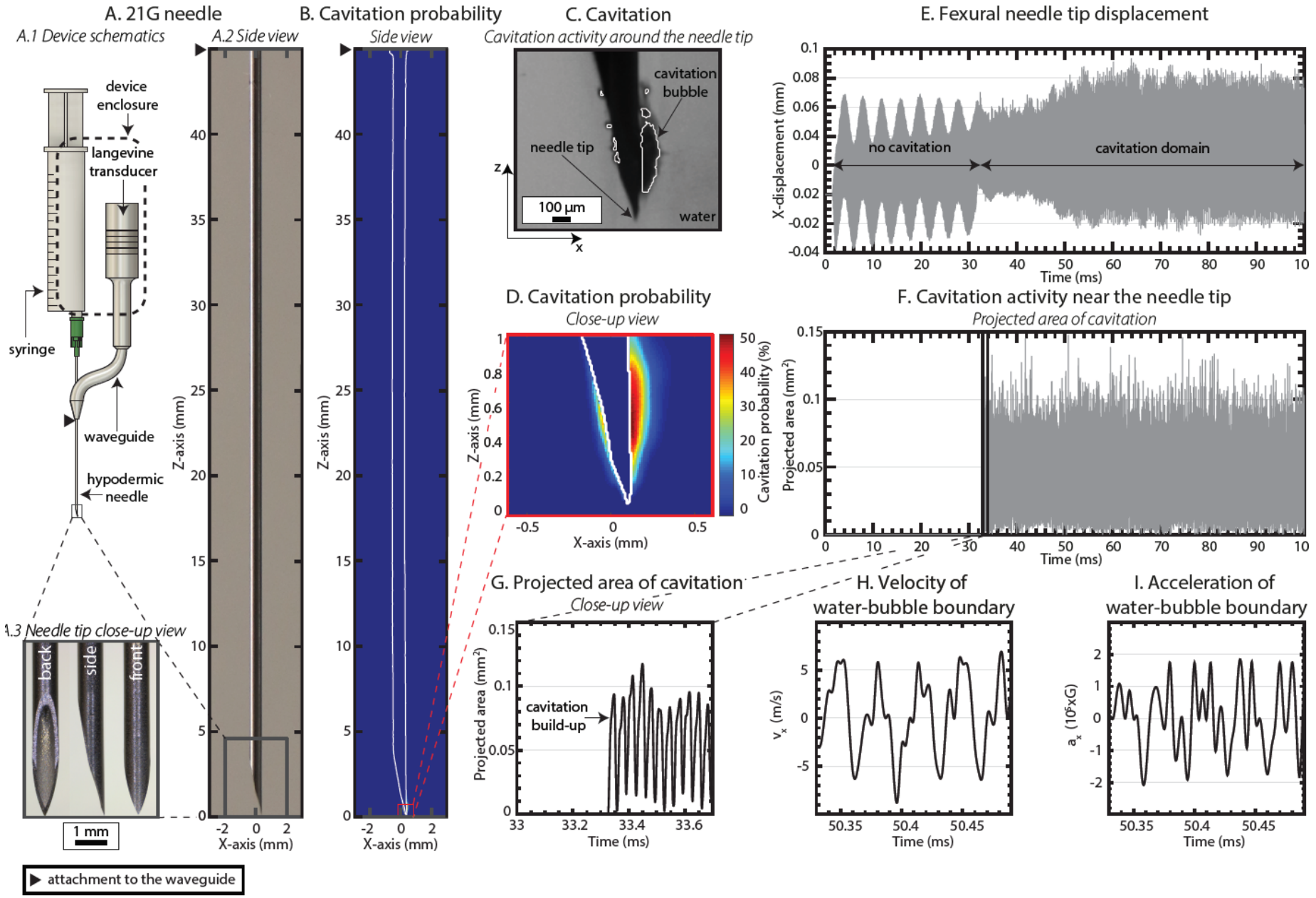
Garcia-Gradilla et al. 2014: http://onlinelibrary.wiley.com/doi/10.1002/smll.201401013/pdf

Ultrasonic needle

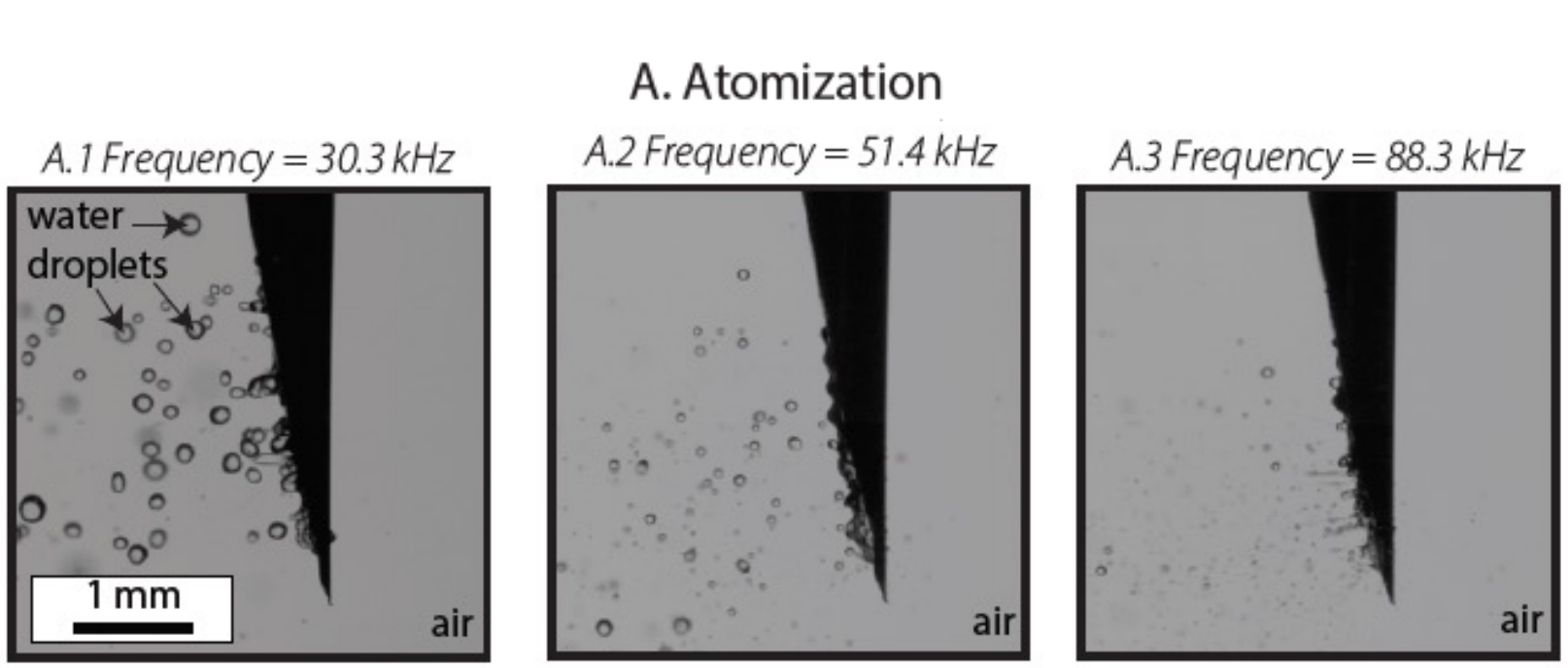
Ultrasonic needle (Video)

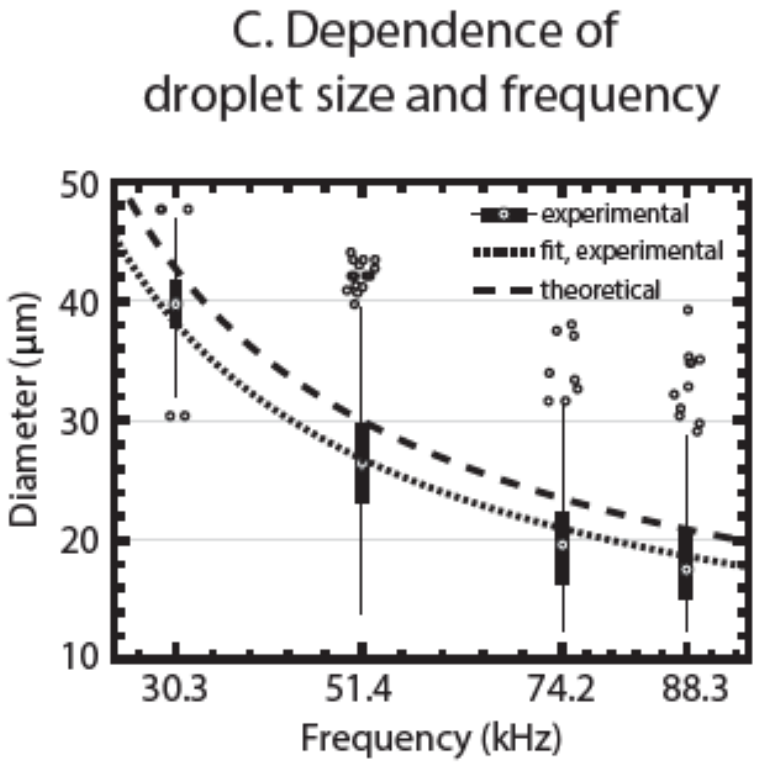


Ultrasonic needle

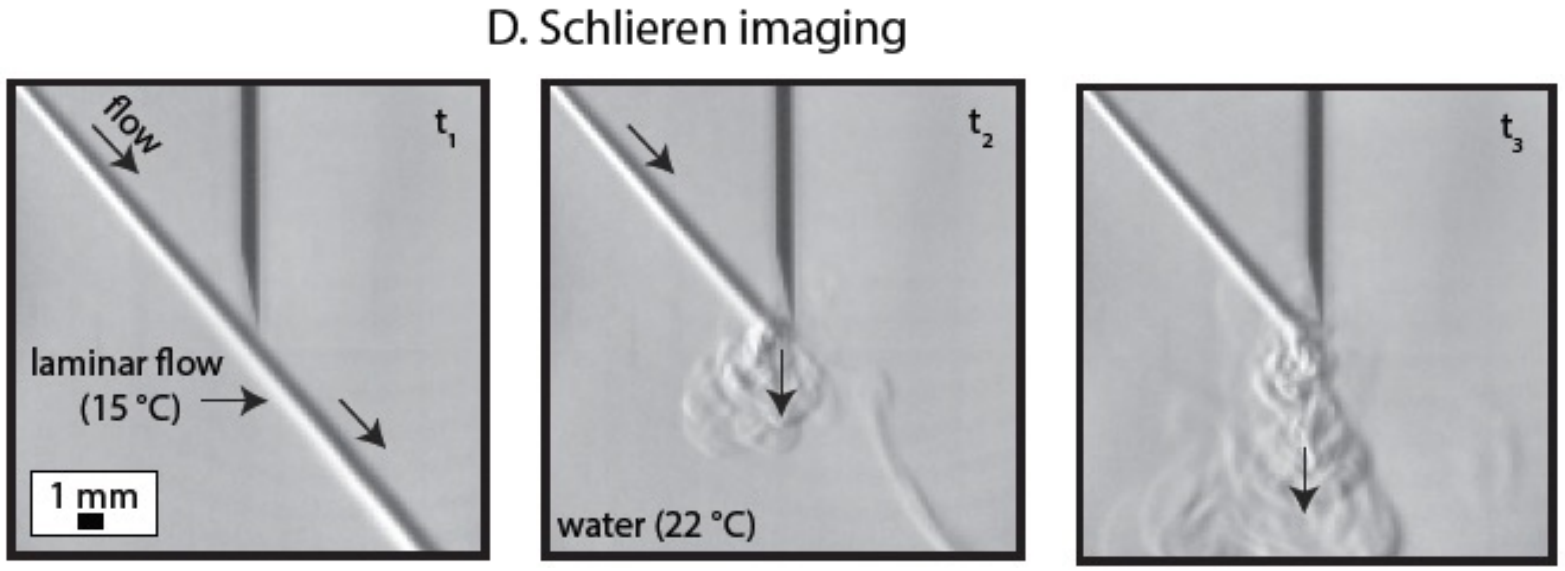


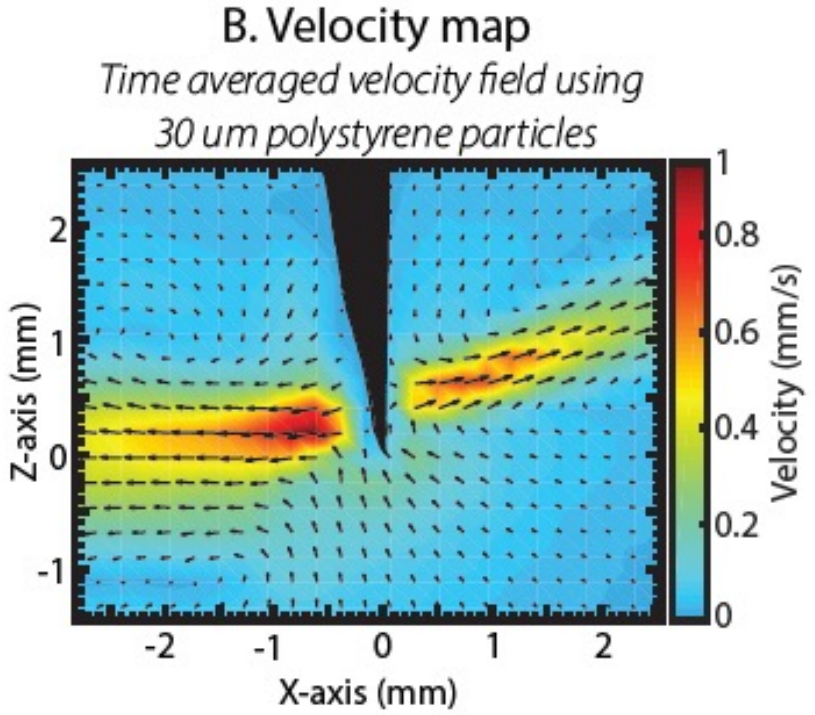
Ultrasonic needle



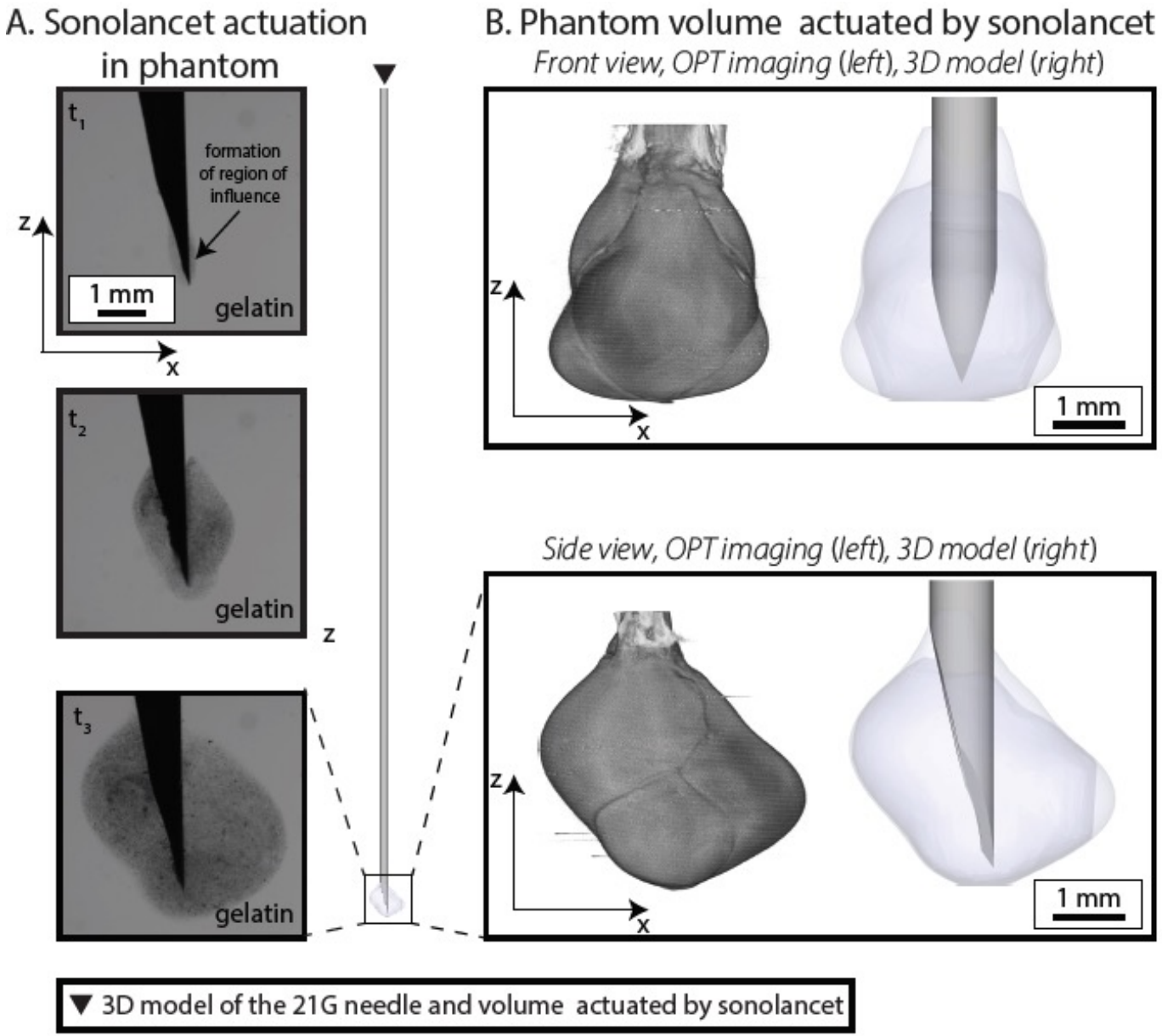


Ultrasonic needle

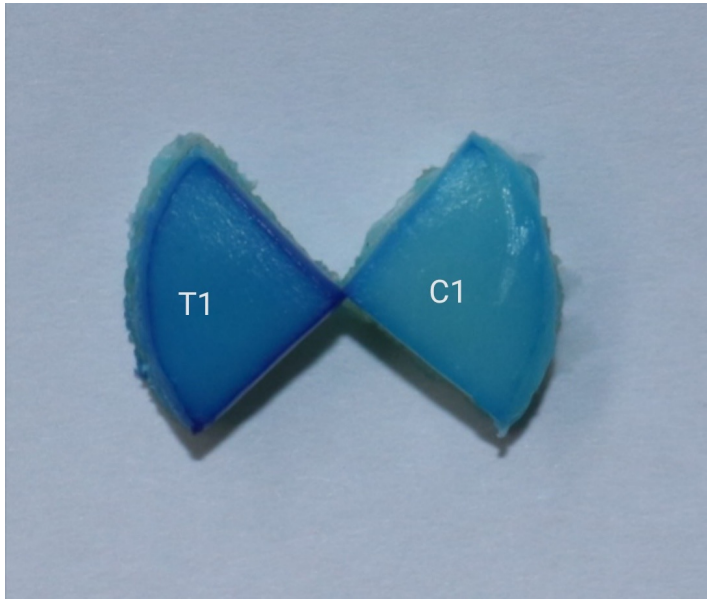




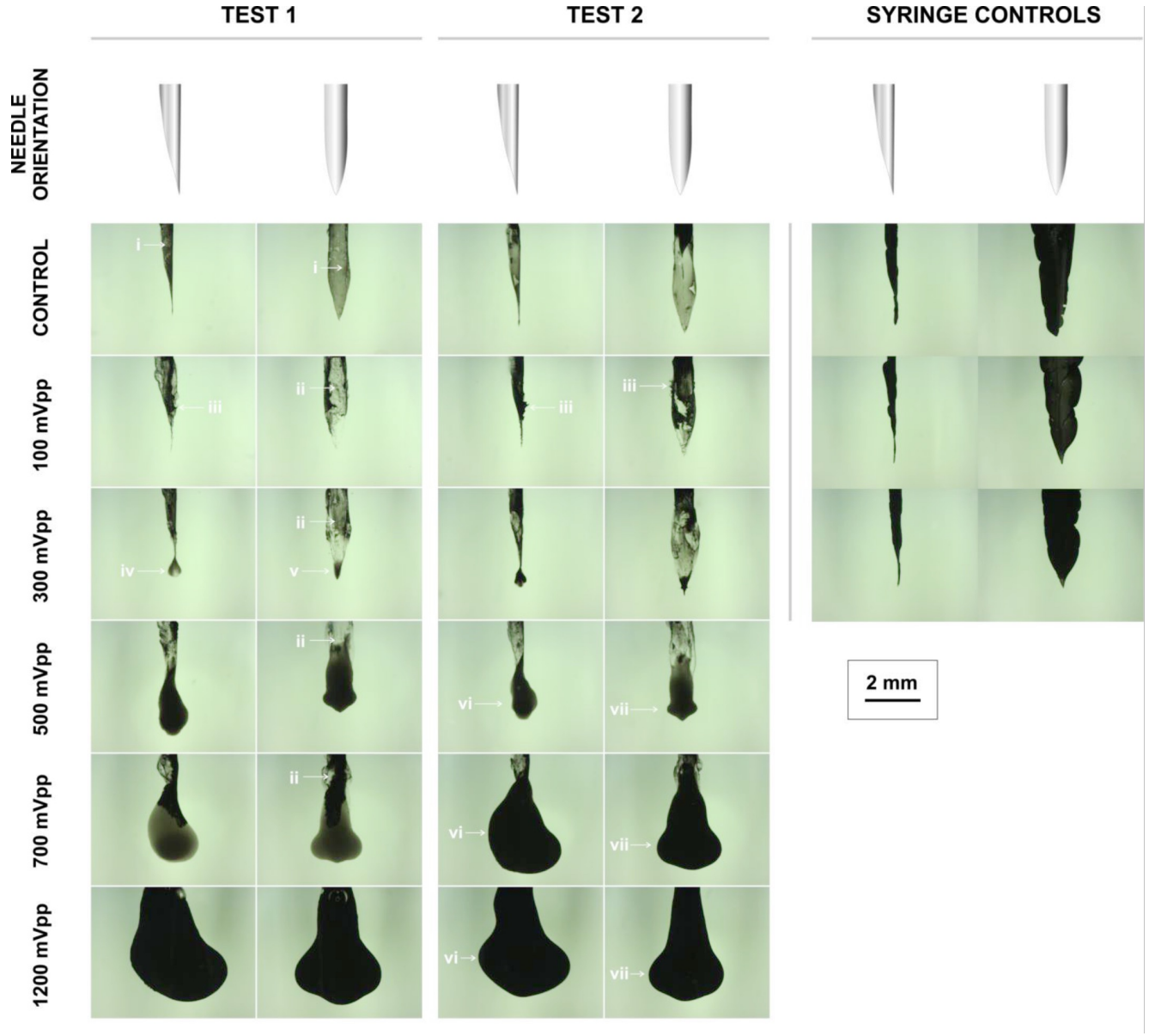
Ultrasonic needle



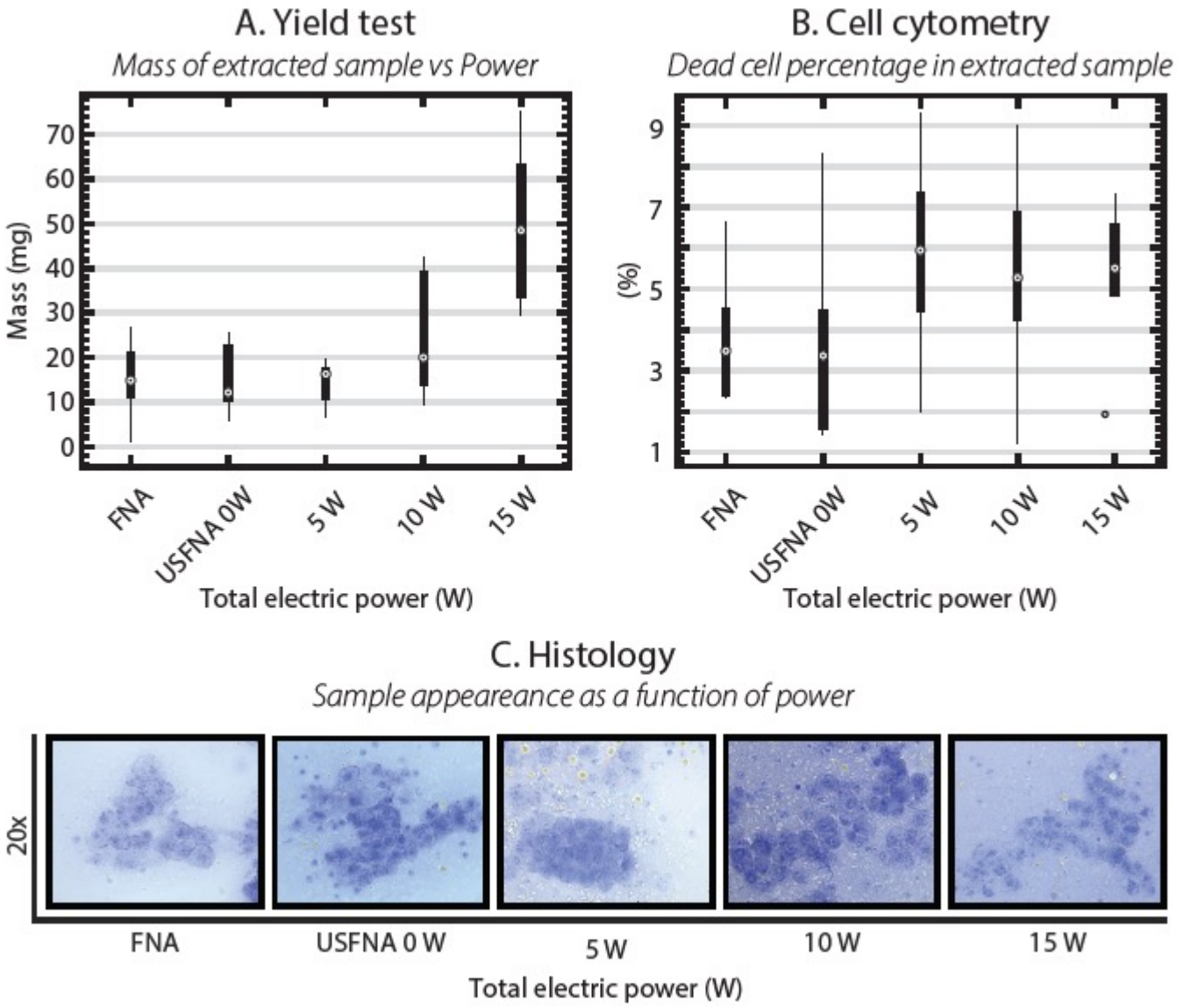
Delivery into articular cartilage

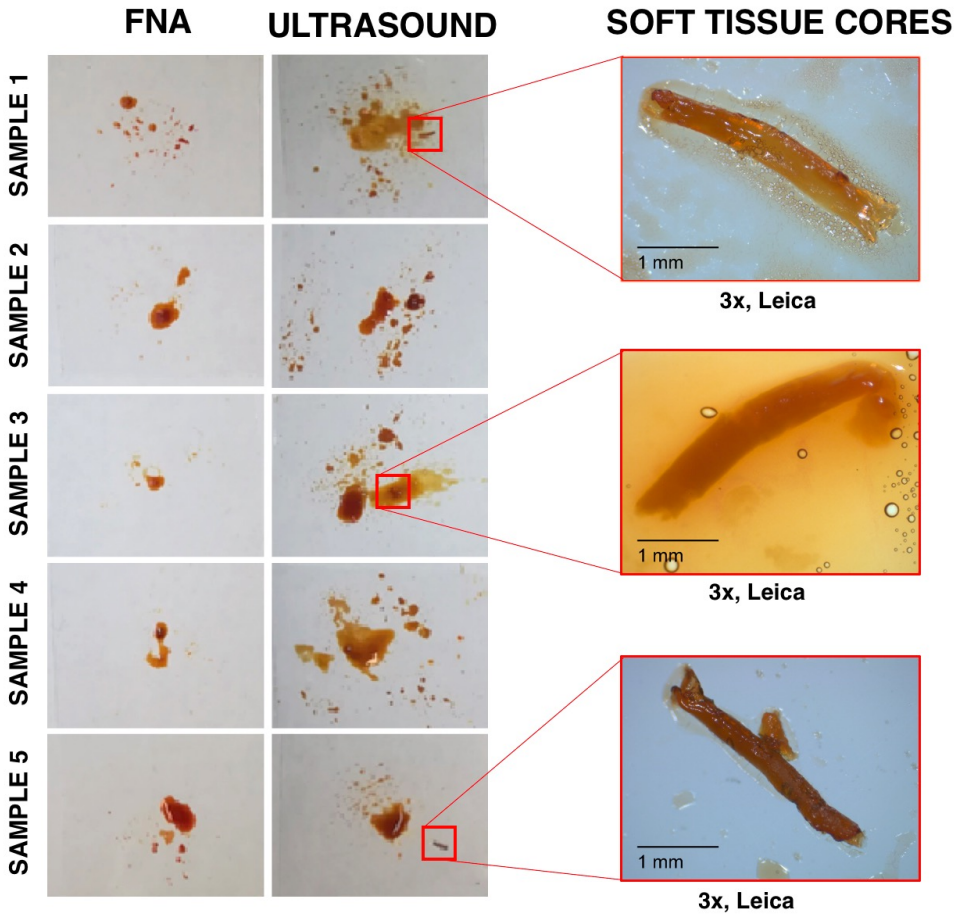


Delivery of nanoparticles inside gelatin

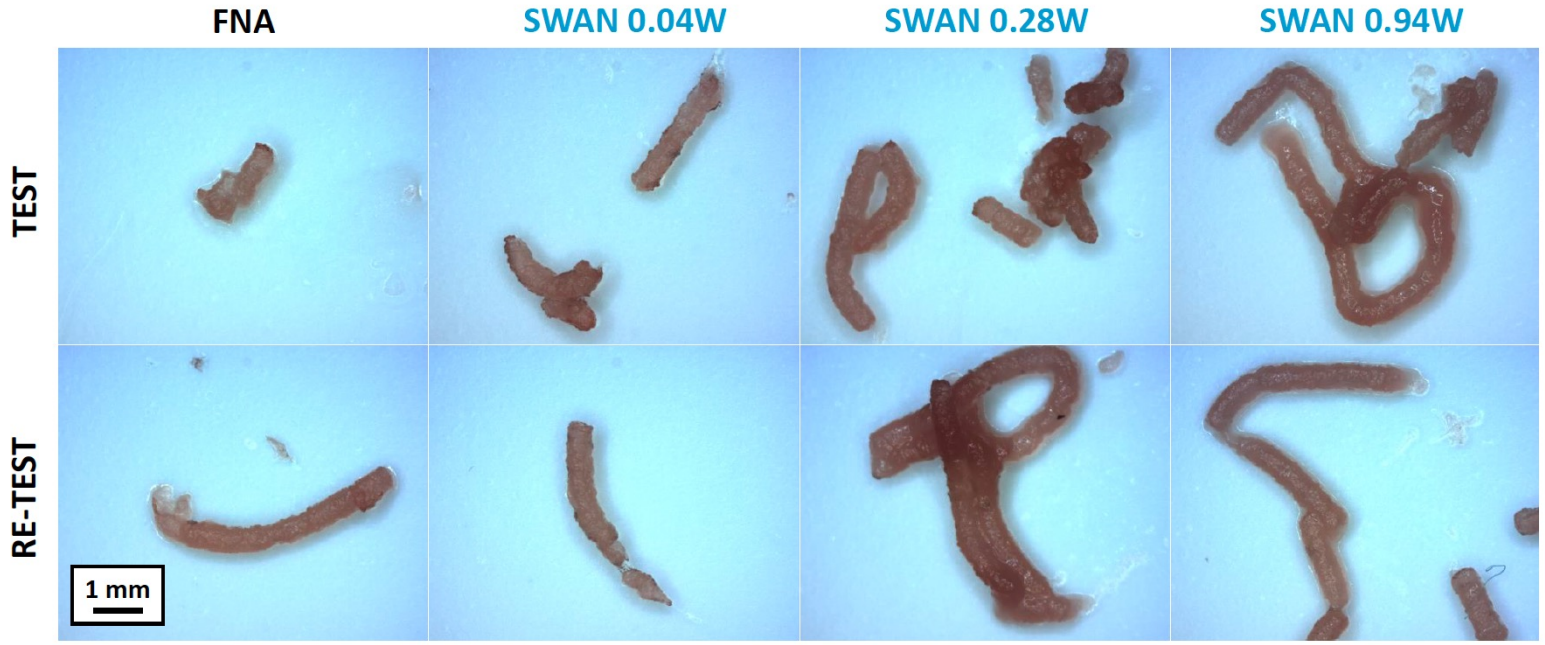


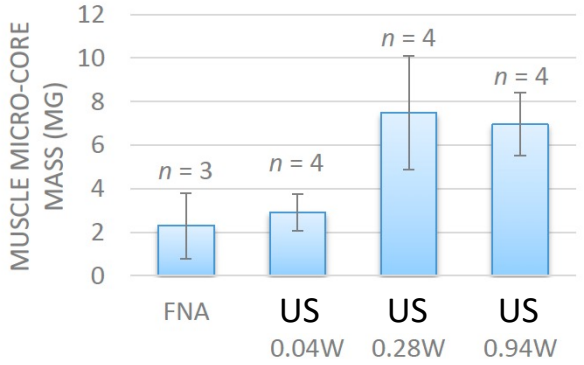
Ultrasonic needle





Bovine thigh muscle





Questions?

Feedback on this session

https://presemo.aalto.fi/bmus