Interdisciplinary Technical Journal Club:
Special series on Laboratory Animal Science recognized by the Veterinary Office of the Canton of Zurich

In vivo imaging

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In accordance with 3R policies

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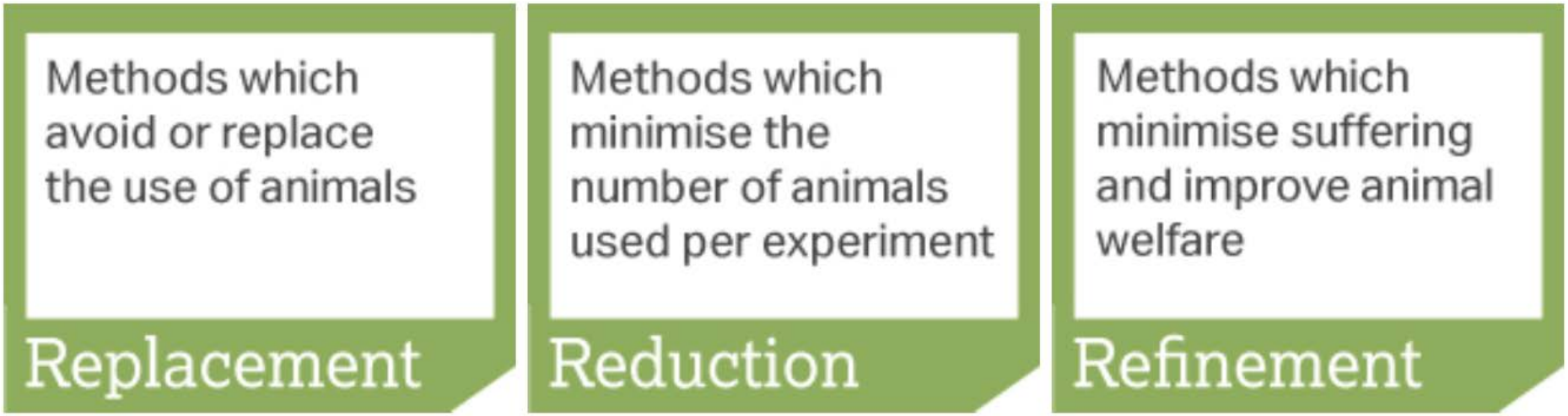
The Three R‘s

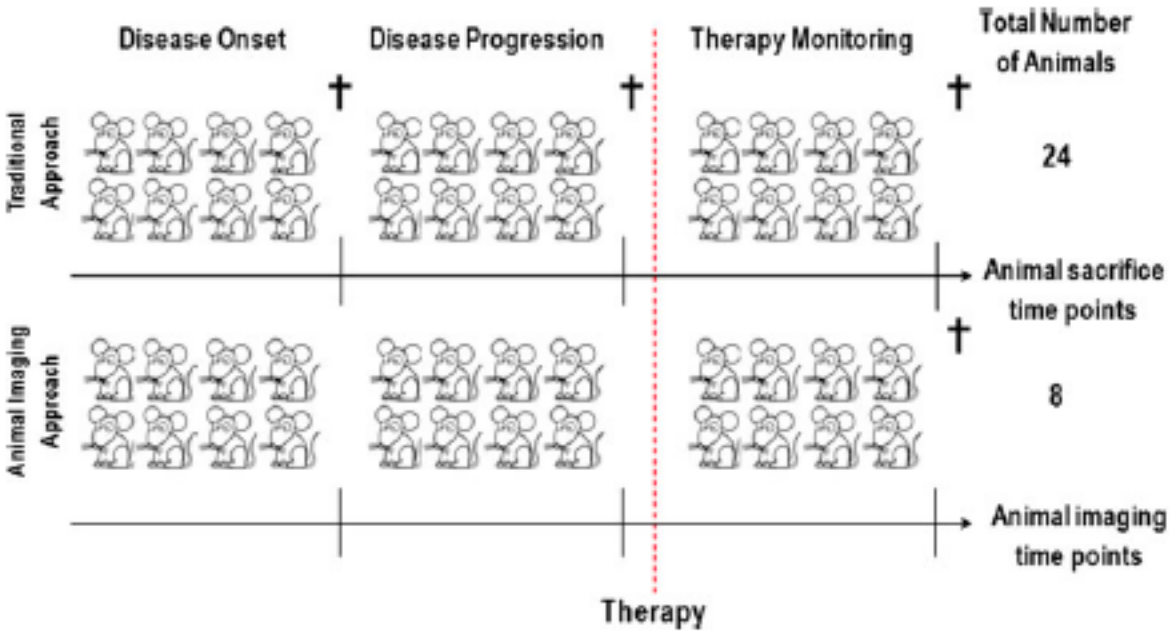
Small-animal models a bridge between basic science and clinic application

Human animal research (Russell, 1957):



https://www.nc3rs.org.uk

Reduction: Longitudinal *in vivo* studies



- Reduction of variance : each mouse is it‘s own control
- Monitoring of disease progression in the individual mouse
- Enabling a systematic collection of tissue (in further studies) :
  Time course and the anatomic hot-spots of the disease (model) are known

Refinement by the use of in vivo imaging

'It constitutes a way of assessing biological structures and function in vivo by non invasive means, allowing the collection of quantitative information, both in health and disease states' (Zanzonico, 2011)

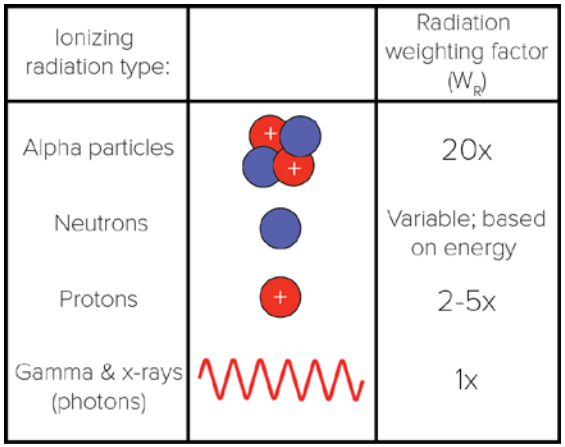
- Acquisition of an impressive amount of unique often multi-modular information (without interfering with the biological process under study)
- Real time studying of disease in a quantitative way
- Macroscopic level and molecular level
- Repeatedly and non-invasively monitor disease progression or response to treatment
- Translational aspect : nearly identical settings than used in clinic

In vivo imaging : main modalities

Table 1 Summary of general properties of diagnostic imaging modalities

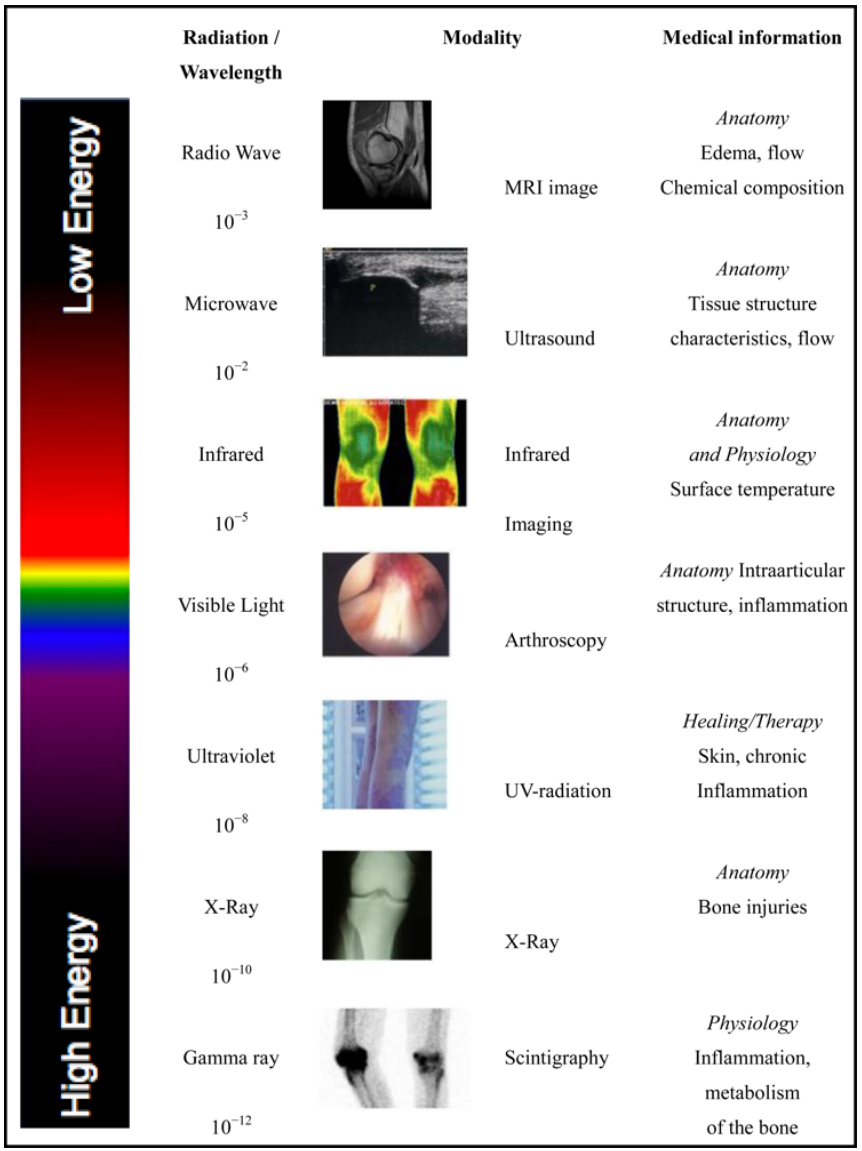
Imaging modality	Physical basis	Information supplied
Positron emission tomography (PET)	Gamma-radiation (derived from positron emission)	Tracer uptake
Single photon emission computed tomography (SPECT)	Gamma-radiation	Tracer uptake
Optical imaging (OI)	Light emissions (ex: fluorescence)	Probe uptake
Computed tomography (CT)	X-rays	Tissue density
Magnetic resonance imaging (MRI)	Magnetic properties	Tissue composition
Ultrasound (US)	Sound reflection of high-frequency sound waves	Internal movements and flows, differences of tissues

Partical radiation :



Hildebrandt, 2010

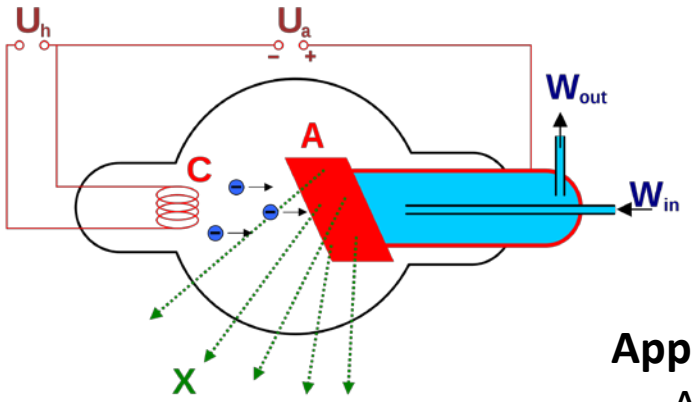
Electromagnetic radiation:



http://www.whiteripleyradsafety.com/3-3/

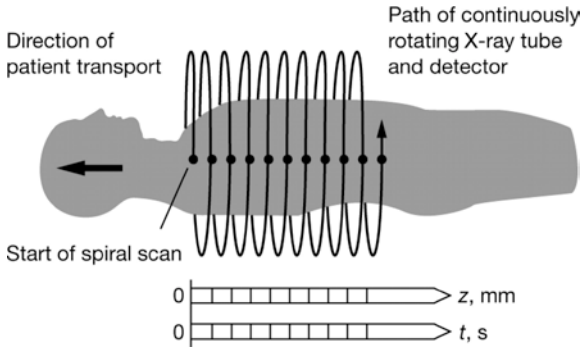
X-ray computed tomography

X-Ray tube:



https://en.wikipedia.org/wiki/X-ray

Spiral CT scanner:

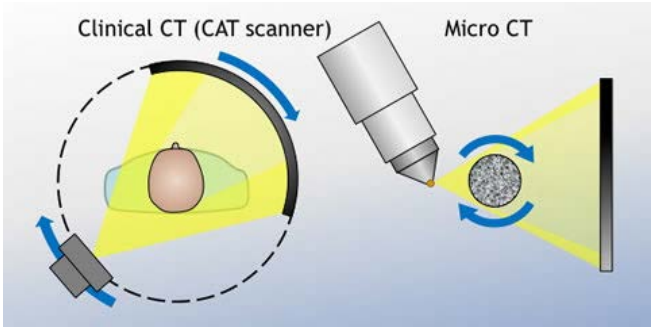


https://dta2gzjs97bag.cloudfront.net/content/ehjsupp/7/suppl_G/G4/F3.large.jpg

Application:

- Assessment of skeletal and lung abnormalities
- Heart function
- Tumor growth and angiogenesis

Micro CT:



http://www.le.ac.uk/richardiii/science/microct.html

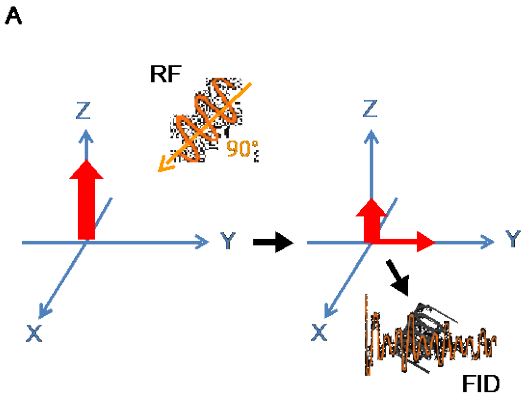
Advantages:

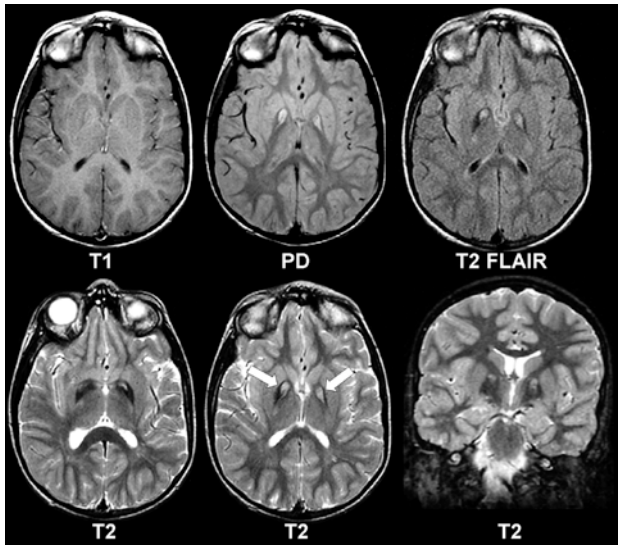
- High spatial resolution with a relative low time required for scanning

Drawbacks:

- Radiation burden
- Volume of contrast agent (animal research)

Magnetic Resonance Imaging





http://www.ajnr.org/content/27/6/1230/F1.large.jpg

Micro-MRI:

- Require stronger magnets (at least 4.7 T), specific receiver, stronger gradient sets
- 23 % of all small-animal imaging

Application:

Multiple due to the big variety of MRI techniques

Advantages:

- Non-ionizing, use of tissue properties
- Excelent contrast and spatial resolution
- Variety of MRI techniques (signal weighting, contrast agents, DWI, fMRI)

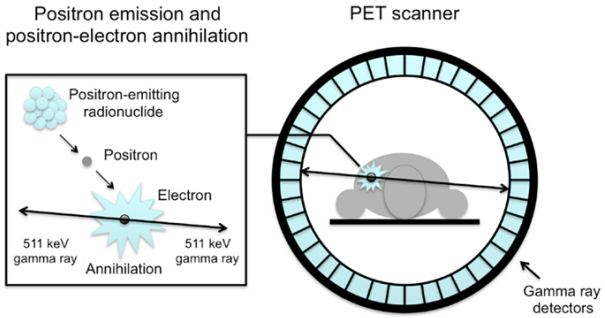
Drawbacks:

- Very expensive
- Longer aquisition time

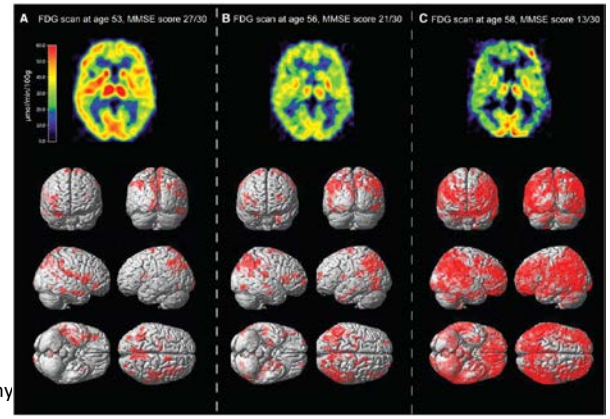
Positron emission tomography
Single-photon emission computed tomography

PET

- Emits positrons
- Higher re solution
- Costlier scanner
- Limited halflife of radiopharmaceuticals



https://www.physicsforums.com/insights/basics-positron-emission-tomography

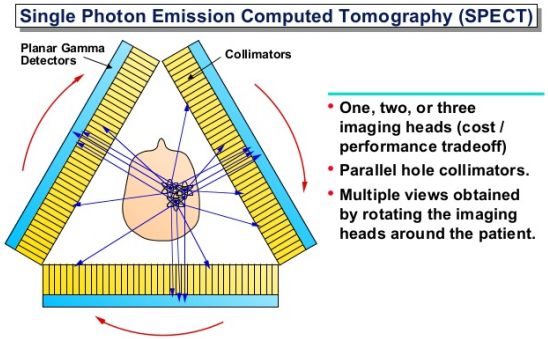


https://d1gqps90bl2jsp.cloudfront.net/content/brain/134/1/301/F3.large.jpg

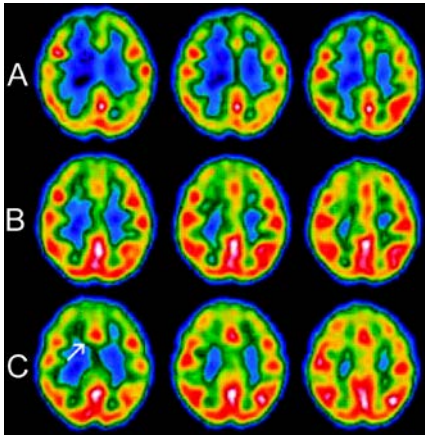
SPECT

- Emits gamma radiations
- Lower re solution
- Less capital intensive scanner
- Longer lived radioisotopes

http://www.marketsandmarkets.com



http://image.slidesharecdn.com

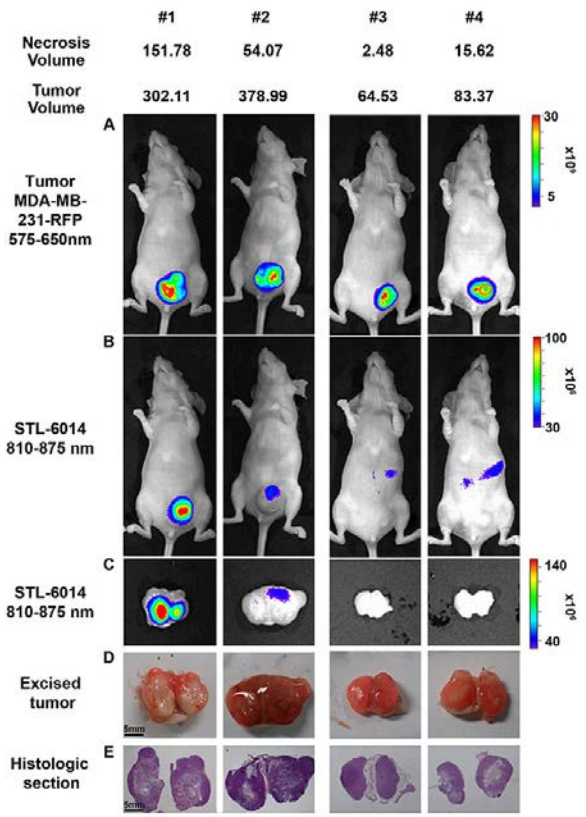


https://sites.google.com/site/dashascienceclass/spect-scan

Application:

- SPECT: cardiology, neurology (brain perfusion, neurotransmission)
- PET: metabolism, angiogenesis, hypoxia, amyloid imaging (11C-PiB PET)

Optical imaging



Modalities:

- Bioluminescence (luciferin substrate)
- Fluorescence and near-infrared fluorescence (NIR)
- Diffuse optical tomography (DOT)

Application:

- Molecular imaging of reporters
- Enzymatic imaging, tumor angiogenesis

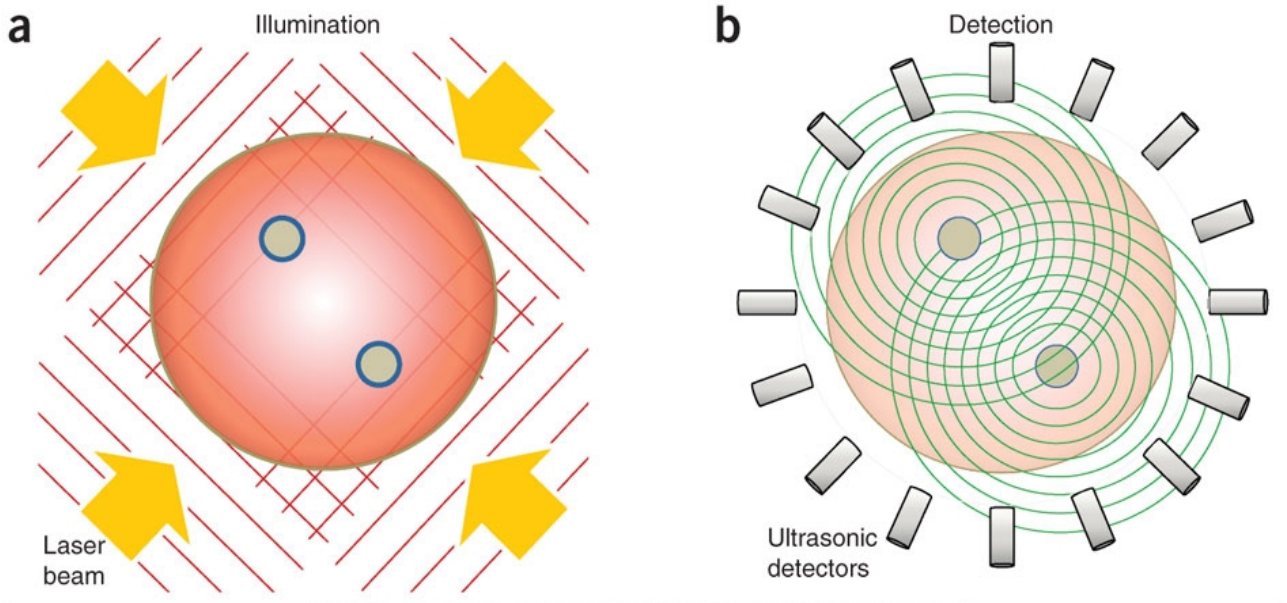
Advantages:

- High sensitivity
- Low costs
- Relatively high throughput
- Short acquisition time

Drawbacks:

- Low resolution
- Limited depth

Photoacousting imaging: Listen to absorption



Is the combination of optic imaging and US.

a, Pulsed light of time-shared multiple wavelengths illuminates the tissue of interest

b, In the tissue there are absorbing elements. In response to the fast absorption of light by this elements acoustic responses are generated. They can then be detected with acoustic detectors.

Advantage: Combination of high optical contrast and submillimeter ultrasound resolution

V. Ntziachristos, Nature America 2010

Contrast agent versus molecular imaging probe

Anatomical imaging: = Contrast agent

CT: Iodine-based, barium
MRI: Gd, Mn, SPIO

Molecular imaging: = imaging probes

PET: ^{18}F, ^{11}C, ^{13}N, ^{68}Ga
SPECT: ^{99m}Tc, ^{123}I, ^{111}In
OI: Organic fluophores, inorganic semiconductor nanoparticles

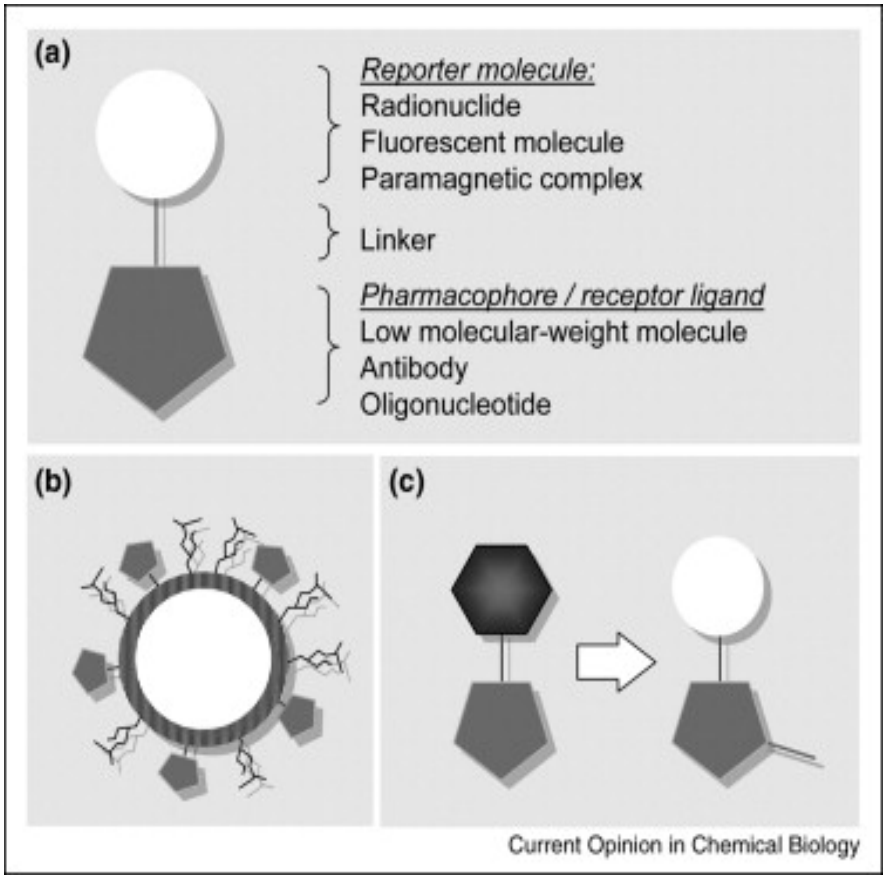
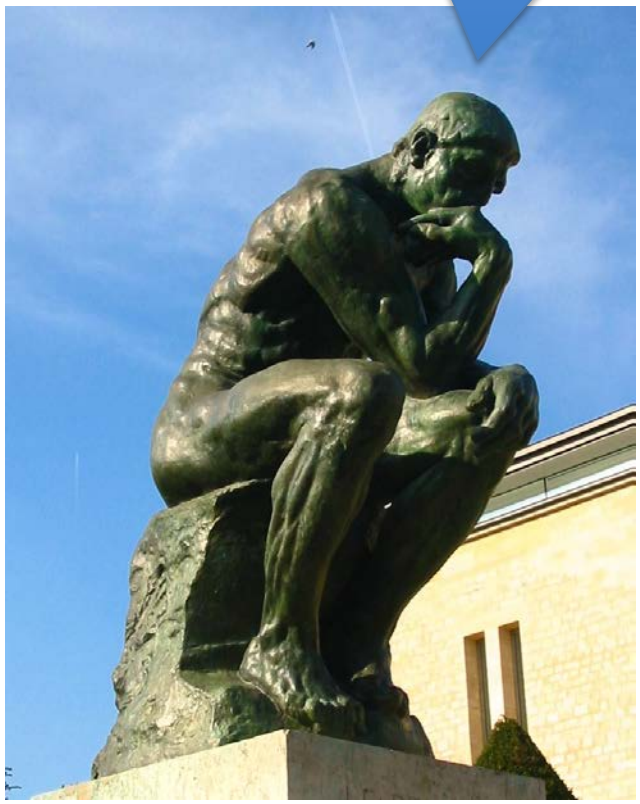


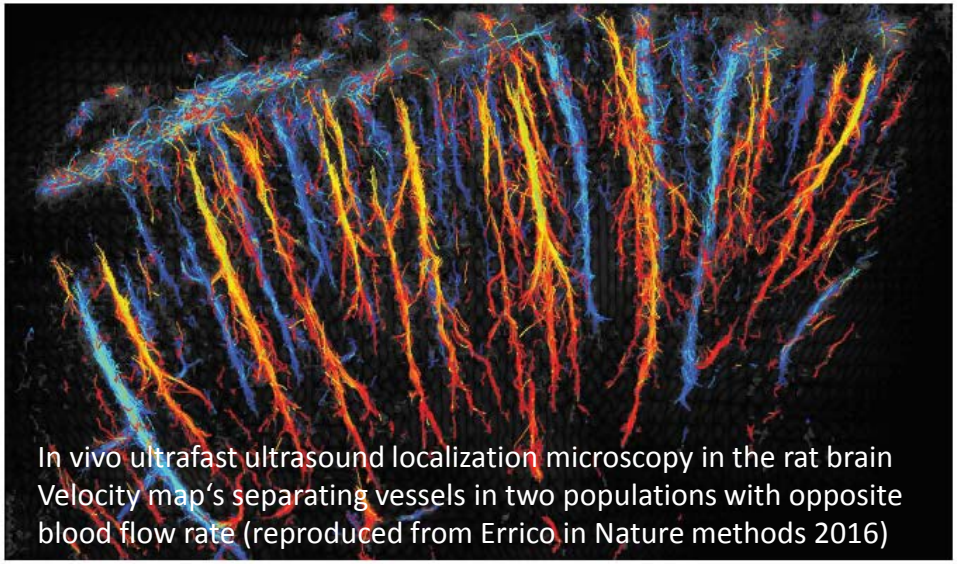
Table 6 Summary of the main characteristics of small-animal imaging modalities

Imaging modality	Spatial resolution (mm)	Sensitivity	Depth	Imaging period (min)	Radiation exposition (Gy)	Maximum number of animals/ same study	Equip. cost (M€)	Procedure relative cost	Type of probe	Major advantages	Major limitations	Major applications
SPECT	0.5–2	pM	No limit	30–90	0.1–1.0	4	0.6–0.8	$$	Variety of molecules labeled with low-energy gamma emitters	Longer physical half-lives; multiple radionuclides can be detected simultaneously; physiological and molecular information	Less sensitive than PET	Oncology; cardiology; neurology (brain perfusion and neurotransmission)
PET	1–2	pM	No limit	20–60	0.1–1.0	4	0.6–0.8	$$$	Variety of molecules labeled with positron emitters	High sensitivity; accurate quantification; diversity of biological probes available; physiological and molecular information	Short-lived radionuclides; impracticability of distribution of some of them; expensive equipment and overall procedure	Oncology (tumor metabolism/ proliferation, angiogenesis, hypoxia); neurology
CT	0.05	mM	No limit	10–15	0.1–0.6	1	0.2–0.4	$$	Radiopaque contrast agents	Spatial resolution, particularly for lung and bone imaging; morphological and physiological information	Poor soft tissue contrast; radiation exposure	Bone, lungs, vascular imaging
MRI	0.1	μM–mM	No limit	60	None	10	1.0	$$$	Paramagnetic metal chelates/ superparamagnetic iron oxide	Spatial resolution; high soft tissue contrast; morphological, physiological and molecular information	Low sensitivity and long acquisition times	Oncology (tumor metabolism and oxygenation); cardiology (heart perfusion)
OI	1–2	pM–nM	mm	1–10	None	5	0.1–0.4	$	Fluorophores or β-emitters	High sensitivity, high throughput; low cost; physiological and molecular information	Limited depth of penetration	Oncology (tumor angiogenesis, and enzymatic activity)
US	<0.1	–	cm	60	None	1	0.2–0.3	$	Microbubbles	Vascular and soft tissue imaging; morphological and physiological information	Difficult to image hollow organs and bone	Vascular imaging

CT computed tomography, *Equip* equipment, *Gy* Gray, *μm* micromolar, *mm* millimeter, *MRI* magnetic resonance imaging, *M€* million euros, *min* minutes, *nM* nanomolar, *OI* optical imaging, *PET* positron emission tomography, *pM* picomolar, *SPECT* single photon emission computed tomography, *US* ultrasound

How are these images made?
Nice pictures .... Are they biomedical useful?
And from the view of a small animal?





In vivo ultrafast ultrasound localization microscopy in the rat brain
Velocity map's separating vessels in two populations with opposite blood flow rate (reproduced from Errico in Nature methods 2016)

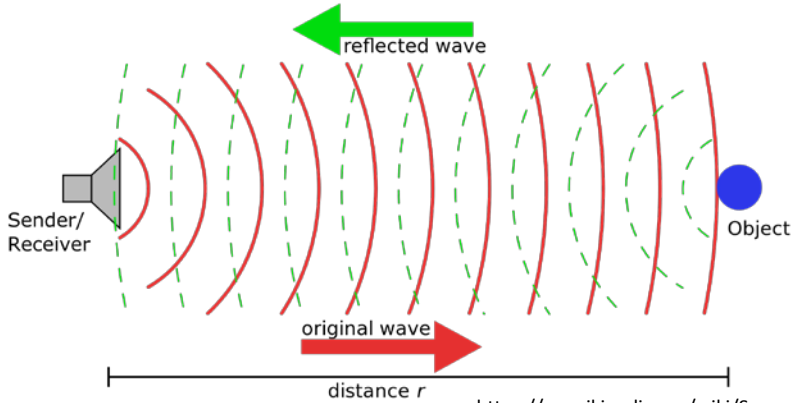
Ultrafast ultrasound localization microscopy for deep super-resolution vascular imaging

Claudia Errico, Juliette Pierre, Sophie Pezet, Yann Desailly, Zsolt Lenkei, Olivier Couture*, Mickael Tanter* Nature, November 2015

- Unprecedented spatiotemporal resolution
- Deep penetration & super-resolution
- Images are acquired in 150s !
- In-vivo, „Minimal-invasive“: Catheterized jugular vein for the administration of the contrast agent (conventional microbubbles also used in clinical applications), thinned-skull imaging window

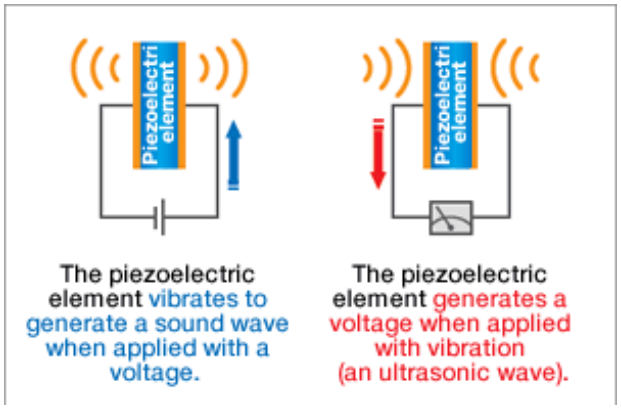
Basic principle of Ultrasound

Sonar imaging, Langevin (20th century)



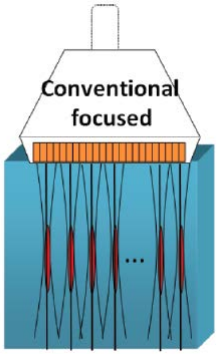
https://en.wikipedia.org/wiki/Sonar

Piezoelectric elements



http://www.ndk.com/en/sensor/ultrasonic/images/basic02/pic_01.gif

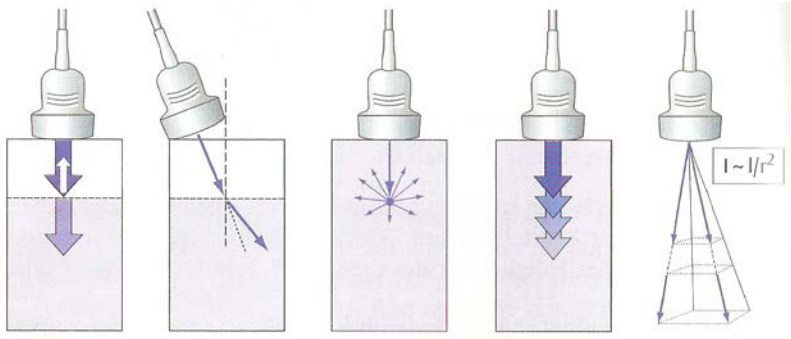
Acoustic waves : wavelength (λ), frequence (f), amplitude (A)



Tanter and Fink, 2015

- Array of multiple piezoelectric elements
- Line-per-line
- Transmission of a slightly defocused US beam and parallel processing of 4 US beam in the receive mode (25 fps)

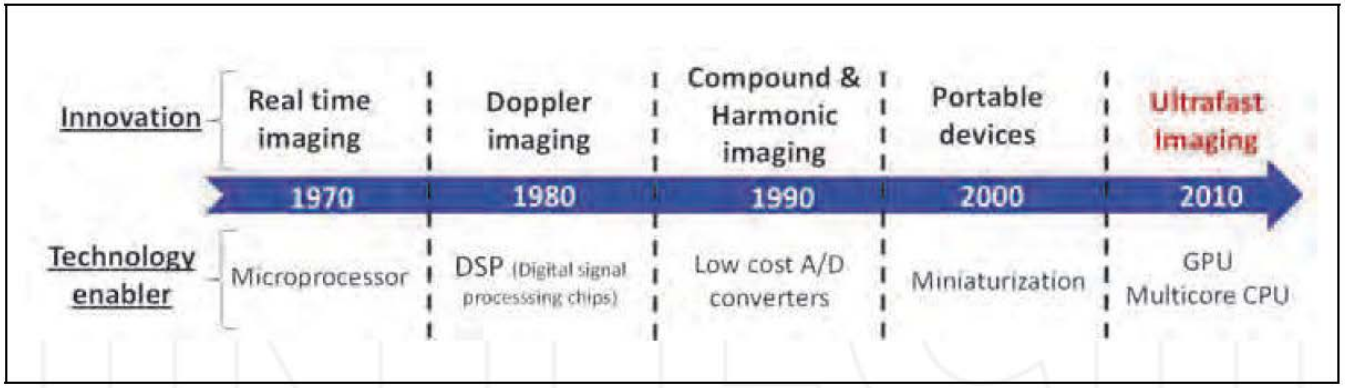
Basic for the contrast in US:



reflection scattering divergence

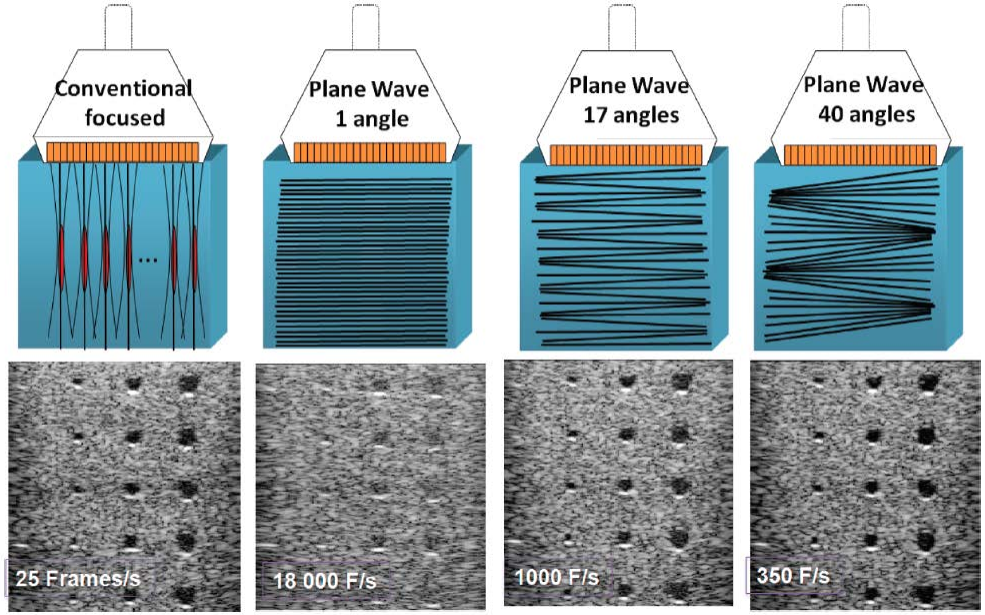
interference absorption

Ultrafast Ultrasound Imaging



Bercoff, 2011

Leaving sonar principles: Plane wave insonifying



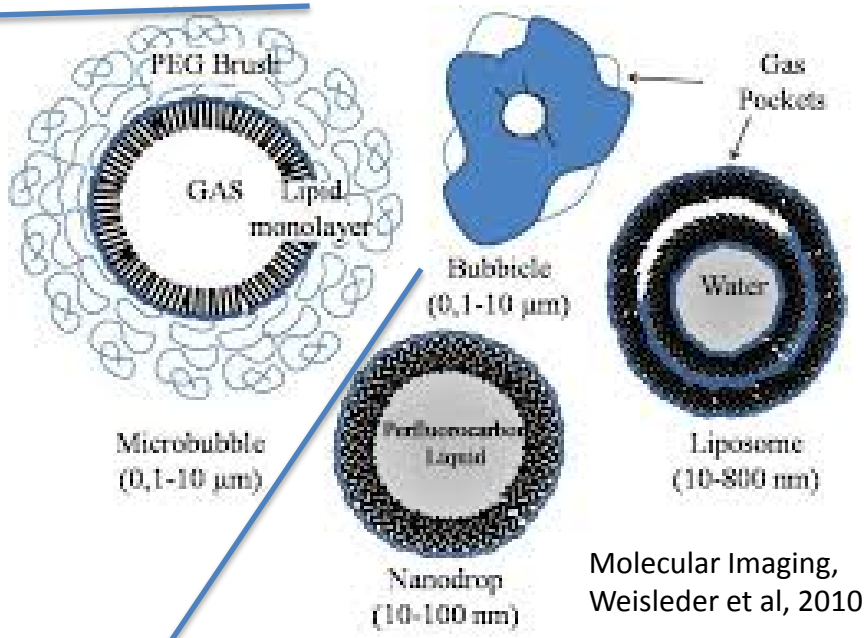
Tanter and Fink, 2015

- Transmission of plane (or unfocused) waves
- Tilted plane waves with different angles
- Full region of interest, using all array elements
- Higher amplitudes
- Short acquisition (hundreds of microseconds) : functional imaging (tissue motion, brain activity via blood flow)

Ultrasound Contrast agent

Primarily designed to detect small blood vessels
Biocolloid : Colloid particles made from biocompatible materials
Smaller than the wavelength of diagnostic ultrasound (100 – 1000 µm)

- Gas spheres (perfluocarbon)
- Gas core with a low density
  - Basic for resonation
- A clinical US system is in principle capable of detecting the signature from a single microbubble (resolution …)
- Shell : in clinical use phospholipid (older albumin, protein shelled)
- A clean microbubble is inherently unstable



Molecular Imaging, Weisleder et al, 2010

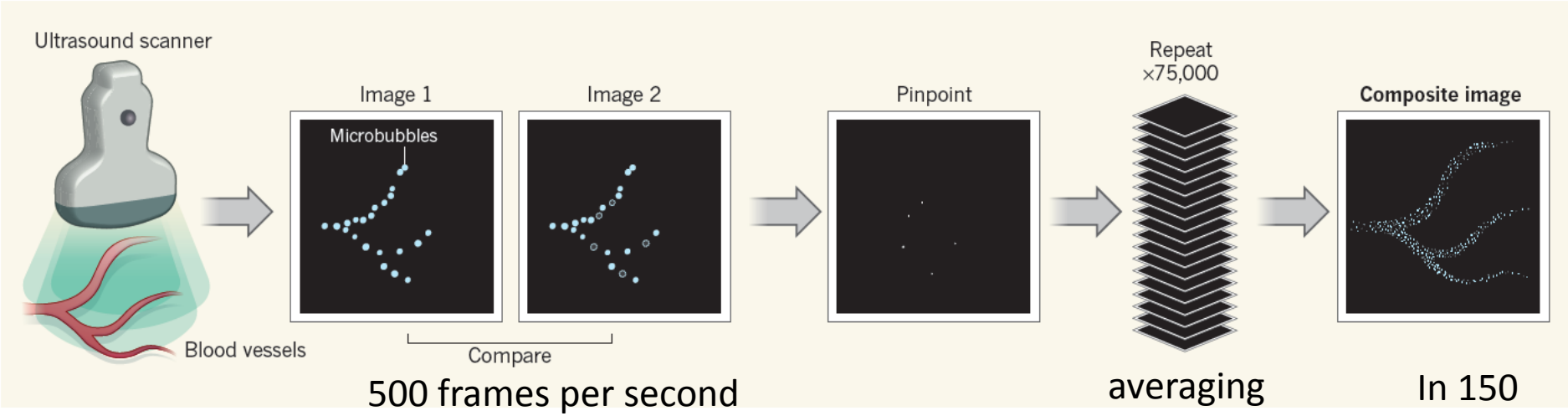
Solid and liquid nanoparticles:

- Less echogenic than gas bubbles (incompressible)
- Stable ; Advantageous pharmacokinetic properties
- Enhanced permeability and retention principle

The PALM approach applied to ultrasound

The problem to solve:

Conventional ultrasound contrast imaging is limited by the classical wave diffraction theory and corresponds roughly to the ultrasonic wavelength (200 µm – 1 mm)



500 frames per second averaging In 150

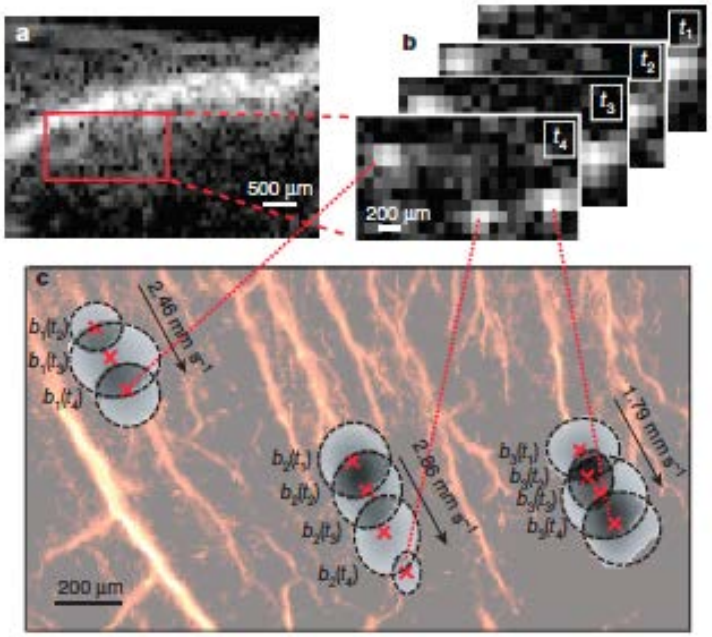
Solution:

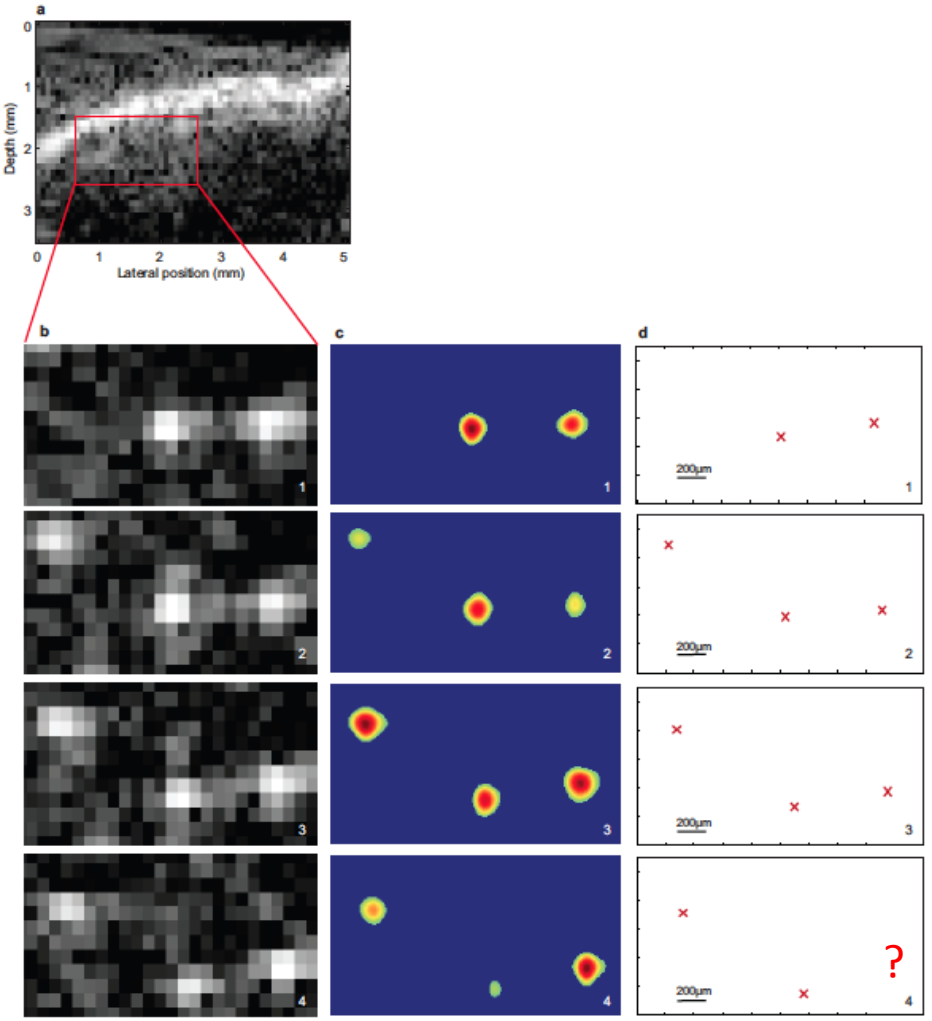
Cox and Beard, 2015 about:
Claudia Errico, (…), Olivier Couture*, Mickael Tanter* , 2015

Pinpointing the location of the few, well-separated microbubbles that degraded between each image (“blinking of bubbels”)

Viessmann et al. 2013: Achievement of the necessary separation by sufficient dilute of microbubbles. However hour-long imaging acquisition.

The principle of ultrafast ultrasound localization microscopy



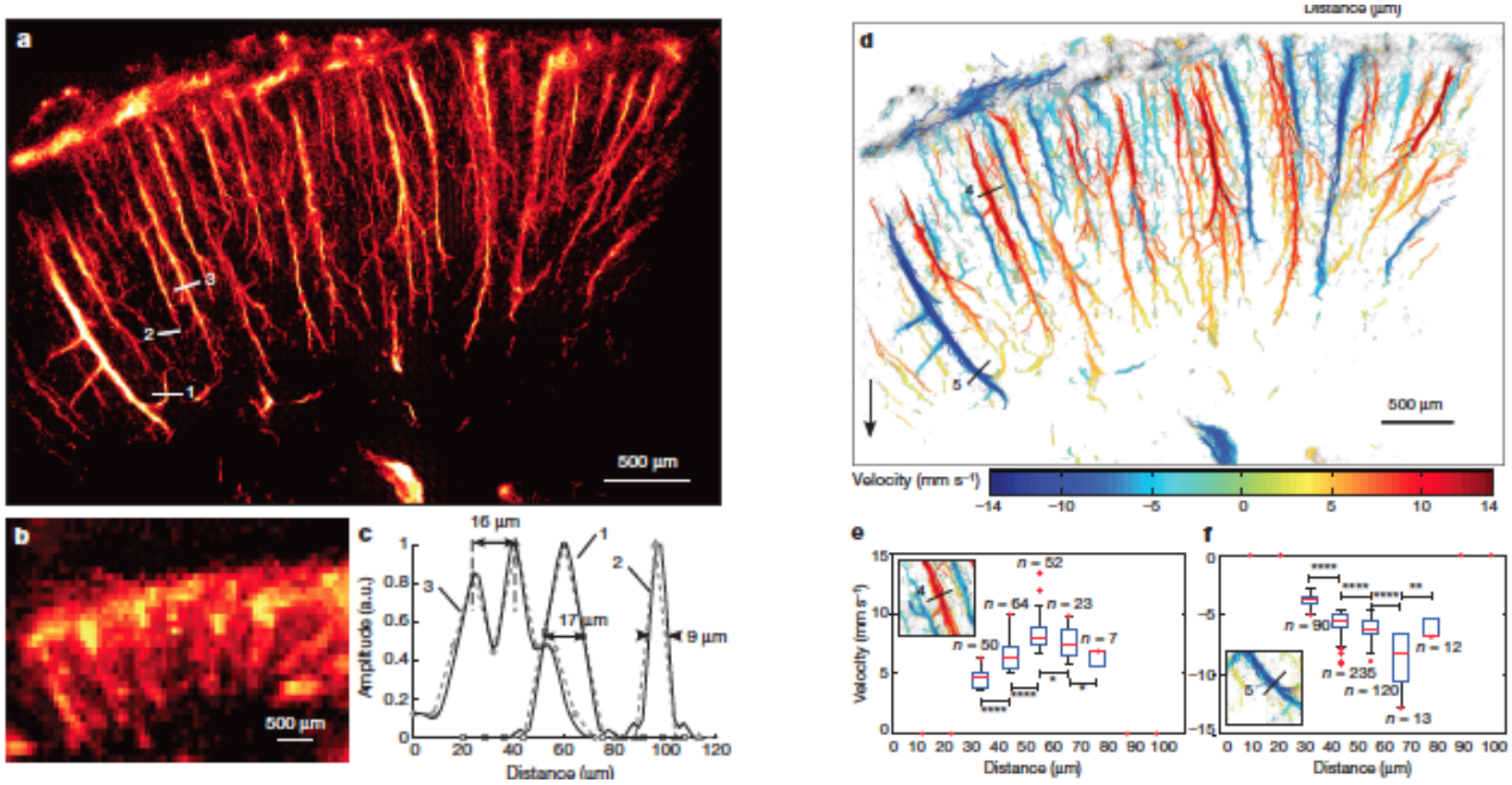


a, Average stack of 250 beamformed images
b, Frames separated by 44 ms and filters (to remove the tissue signal)
c, three independent microbubbles blinking over several miliseconds
Red cross: Exact position of centroid (deconvolution with point-spread function)

http://www.nature.com/nature/journal/v527/n7579/fig_tab/nature16066_SV1.html

Claudia Errico, 2015

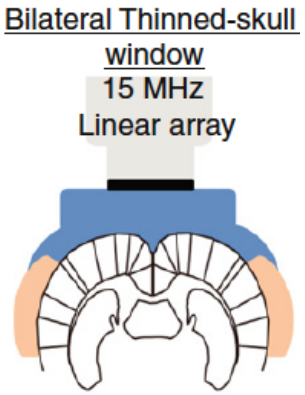
Spatial resolution and bubble velocity maps

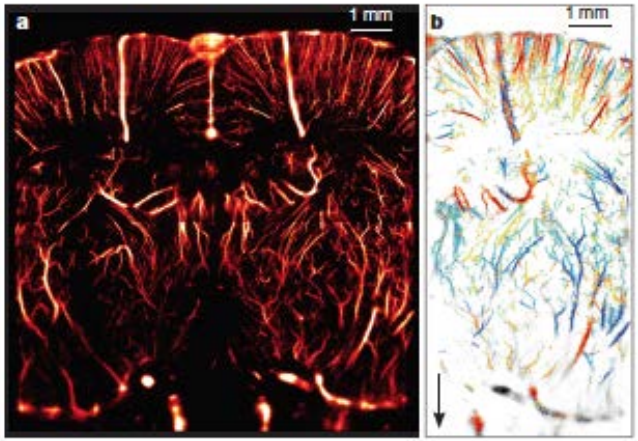


a, Microbubble density maps with a spatial resolution of λ/10
Resolution in depth and lateral direction : 8 µm x 10 µm
b, Same area in a conventional power Doppler image
c, Interpolated profiles the lines marked in a
d, dynamic tracing of bubbles separates vessels in two populations
e, f, Velocity lines associated with d (n = number of bubbles)

Claudia Errico, 2015

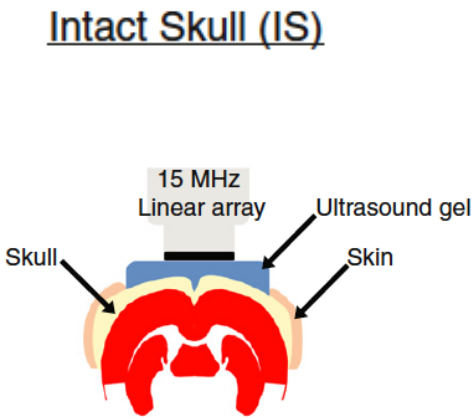
Thinned skull window versus intact skull

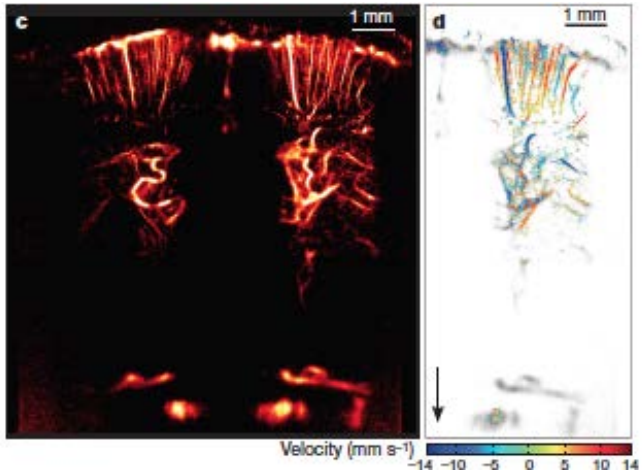




a, uULM preformed through shinned skull (8 µm x 10 µm)
b, corresponding velocity map

http://www.nature.com/nature/journal/v527/n7579/fig_tab/nature16066_SV2.html

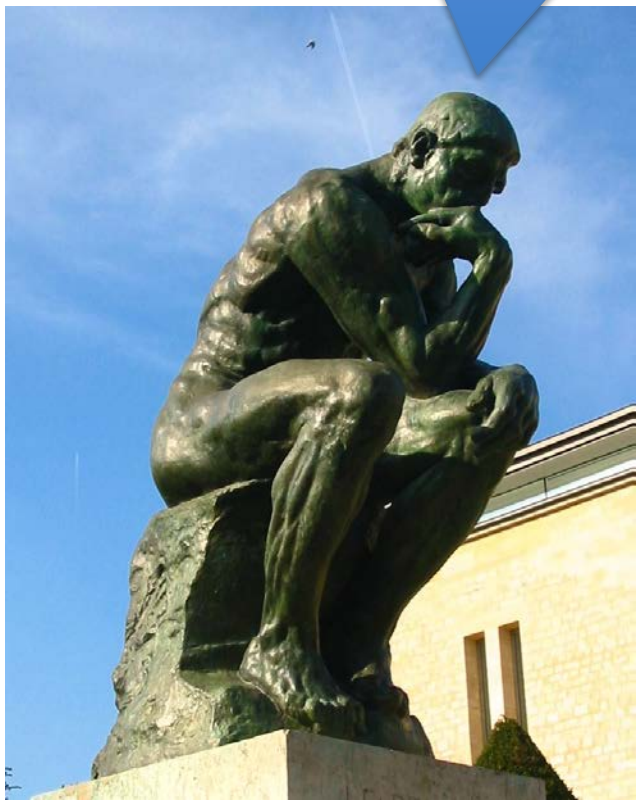




c, uULM performed through the intact skull : attenuation of the ultrasound wave in the presence of bone (12.5 µm x 10 µm)
d, corresponding velocity map

Claudia Errico, 2015

Nice pictures .... Are they biomedical useful?



Possible fields:

- Normal and diseased blood-vessel function,
- identification of microvessel-related disorders,
- angiogenesis in neoplasms,
- vascular dementia etc
- **functional imaging in neuroscience** (combining this technique with functional ultrasound)

Conventional clinical ultrasound:

Resolution inversely correlates to penetration (f)

In ultrafast ultrasound localization microscopy:

Resolution is related to:

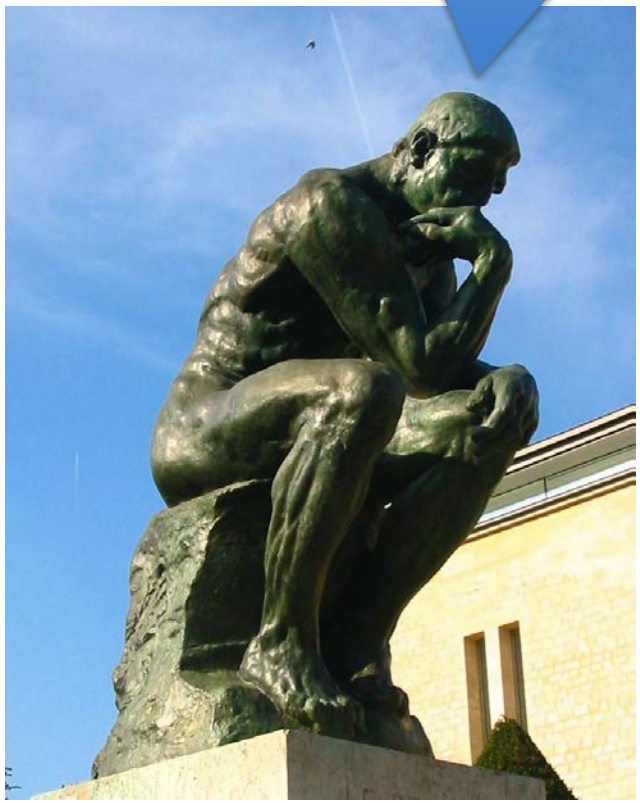
- SNR, - BW of backscattered echoes, -N of array elements

= high resolution deep into organs could be reached

= clinical application's (liver, kidney, breast)

Human brain? By the use of longer wavelength maybe the challenge of the thick human skull can be circumvented

And from the view of a small animal?

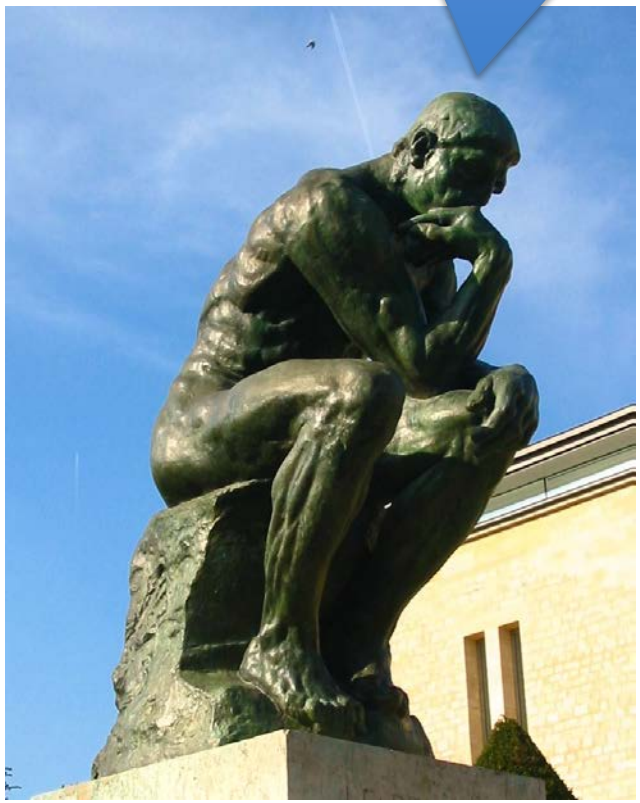


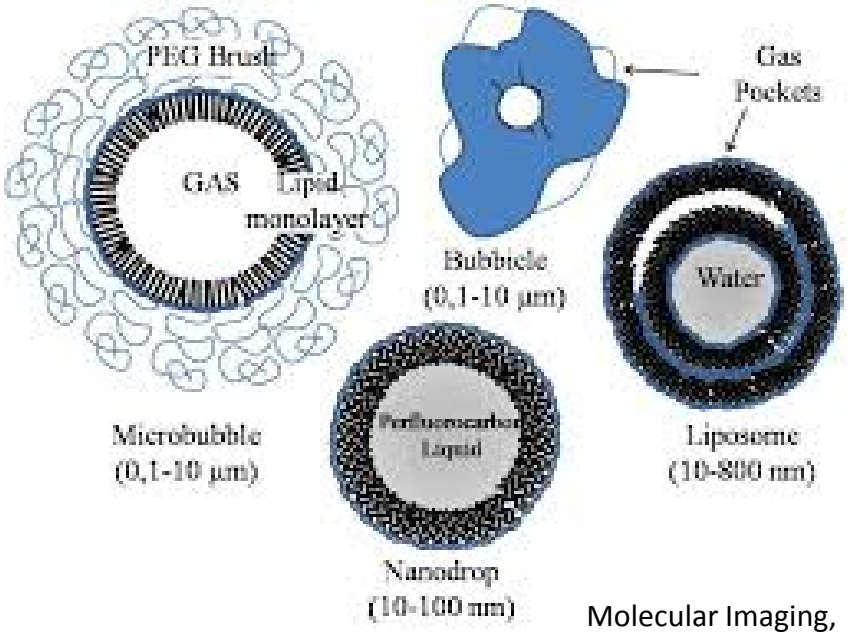
Replacement : Realistic non-invasive human application

Reduction : Longitudinal (functional) studies

Refinement : Fast acquisition, minimal invasive

Are this biccoloids of further use?



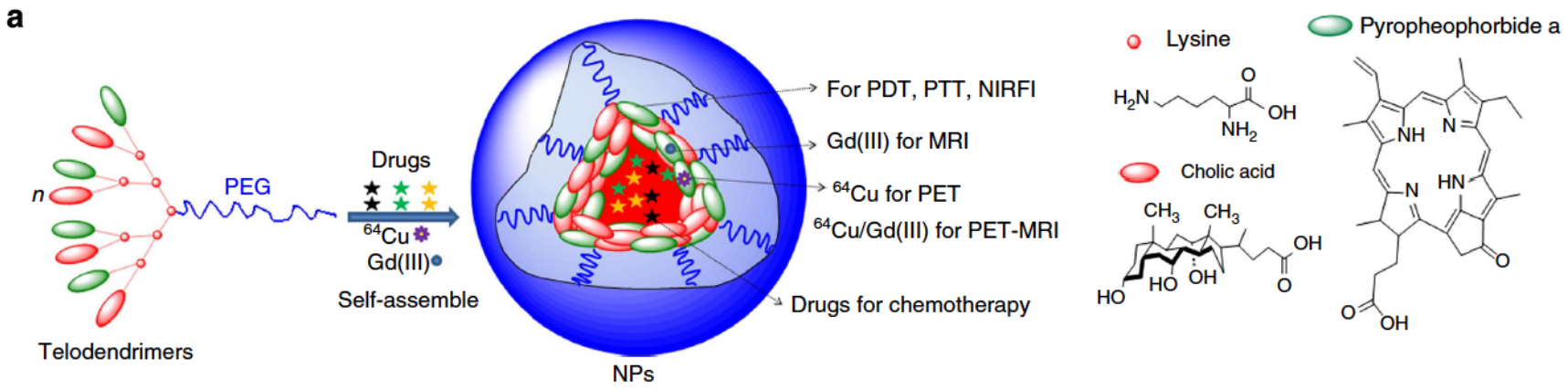


Molecular Imaging,
Weisleder et al, 2010

Theranostics: Agents for diagnosis and Therapy

Theranostic: The combination of diagnostic and therapeutic entities into one drug delivery vehicle for simultaneous diagnosis and treatment of disease

A large variety of inorganic and organic based nanoparticles

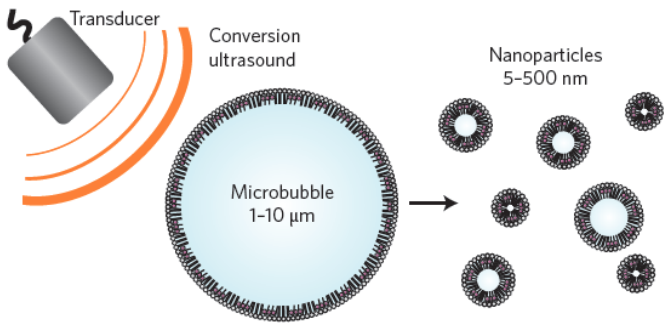


Yuanpei Li et al , 2014

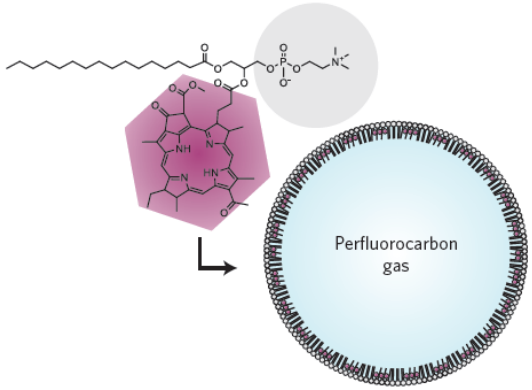
Nanoporphyrin:

- All-in-one porphyrin-based organic nanoconstruct (nanoporphyrin NP)
- NP platform which integrates a variety of imaging and therapeutic functions

From micro to nano



bacteriochlorophyll-lipid

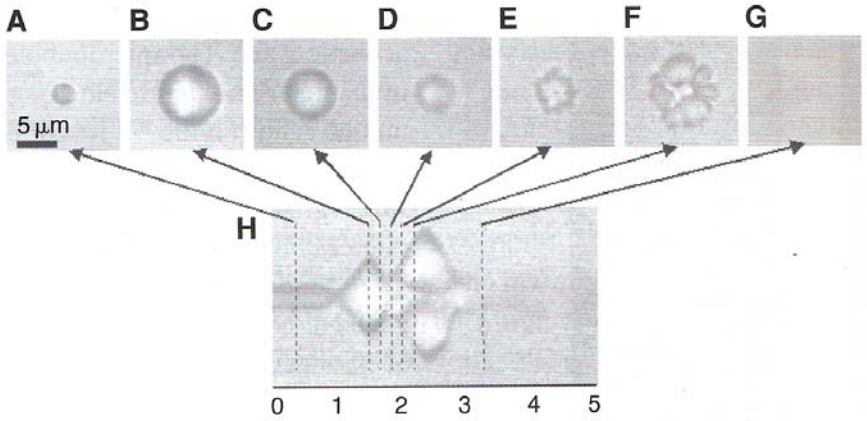


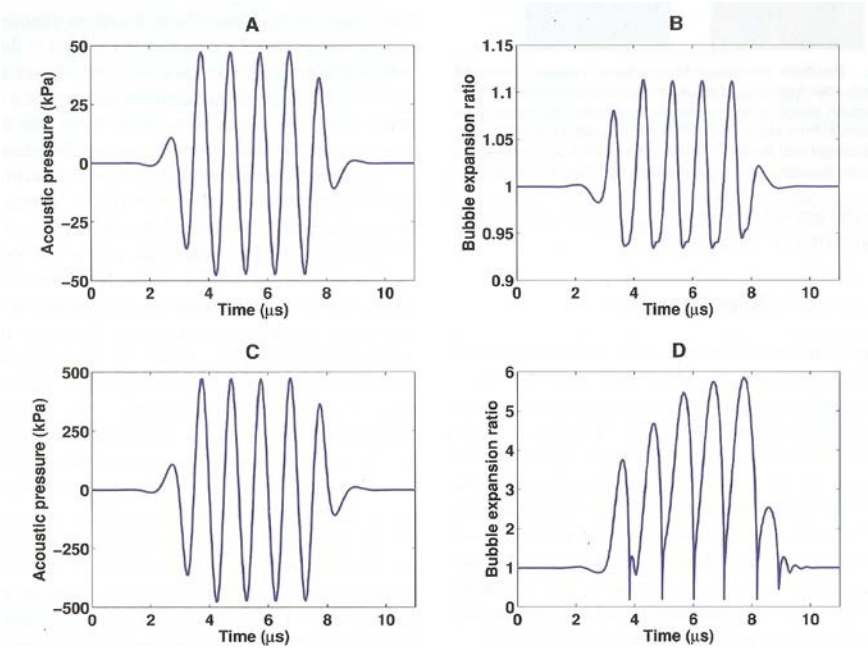
In situ conversion of porphyrin microbubbles to nanoparticles for multimodality imaging

Elizabeth Huynh, Ben Y. C. Leung, Brandon L. Helfield, Mojdeh Shakiba, Julie-Anne Gandier, Cheng S. Jin, Emma R. Master, Brian C. Wilson, David E. Goertz and Gang Zheng* Nature Nanotechnology, March 2015

- Drug delivery by using ultrasound to implode microbubbles into nanoparticles
- Bacteriochlorophyll-lipid (BChl-lipid) shell; porphyrins confer **photoacoustic** and fluorescent properties
- In-vivo anatomical guidance by the microbubble US contrast
- Nanodroplets are better able to penetrate fenestrated or compromised vessel
- Interaction of ultrasound with drug-loaded microbubbles causing non-lethal transient pores in blood vessels

The echogenity of microbubbles





Microbubble passage of an acoustic wave
A) Bubble with an initial radius of 1.5 μm
B-E) Expanding and Contraction
E-G) Collaps

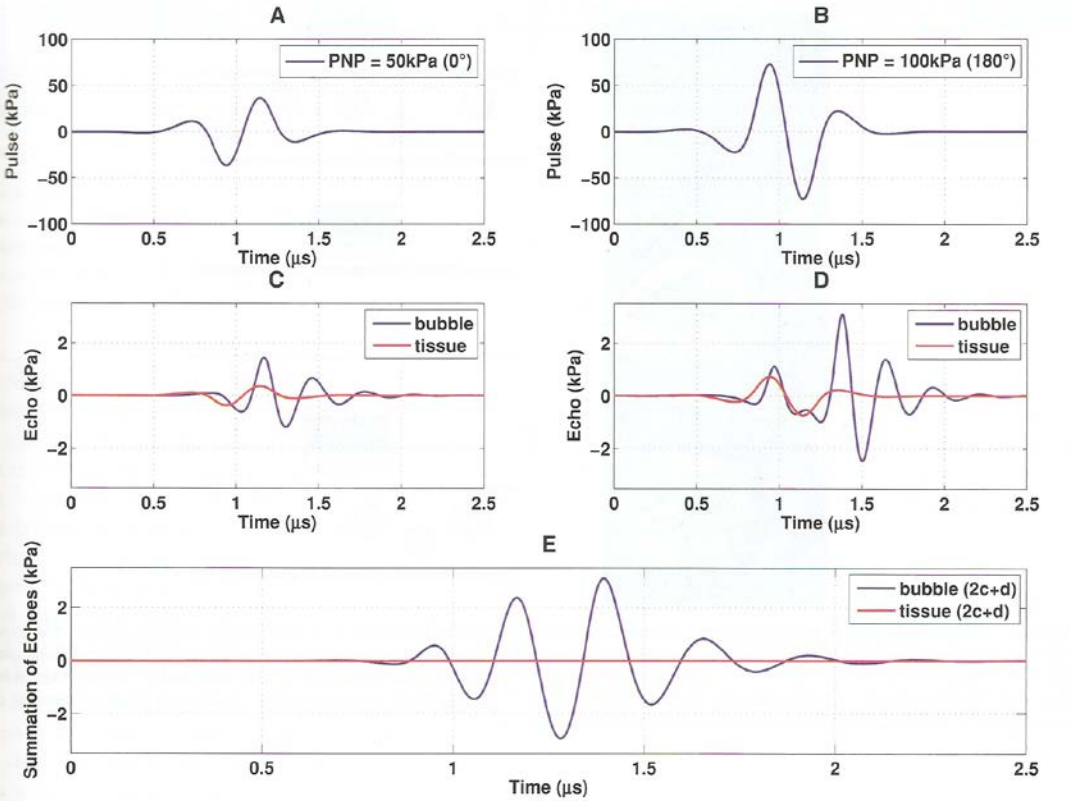
- Basis for a strong and unique echo, microbubble resonate at frequencies typical used in US imaging

Effect of pulse driving pressure on bubble radial oscillation
A and C) Driving (acoustic) pressure;
A = 50 kPa and C = 500 kPa
B and D) Expansion ratio in response to either A or C

- B represents a linear dynamic
- D represents a nonlinear dynamic

Molecular Imaging, Weisleder et al, 2010

Pulse-inversion imaging : Contrast between bubbles and tissue

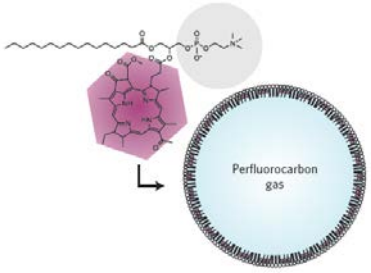


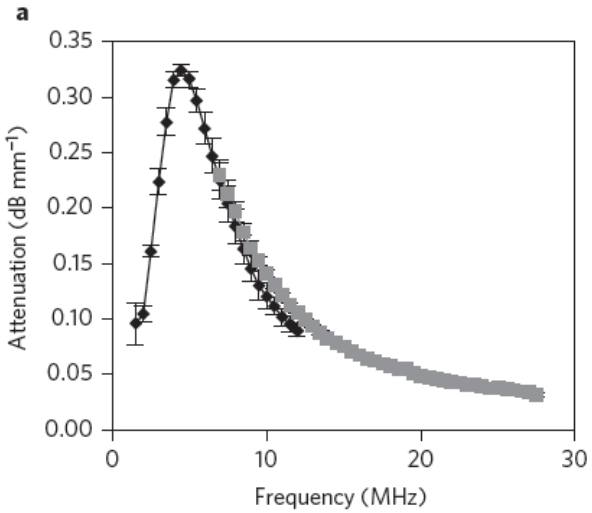
A and B) Transmitted pulses (A: 50kPa, B: 100 kPa); B is inverted (180°)
C and D) Corresponding echoes from 1-micron bubble and tissue
- Bubble echoes: With the higher pressure non-linear dynamic

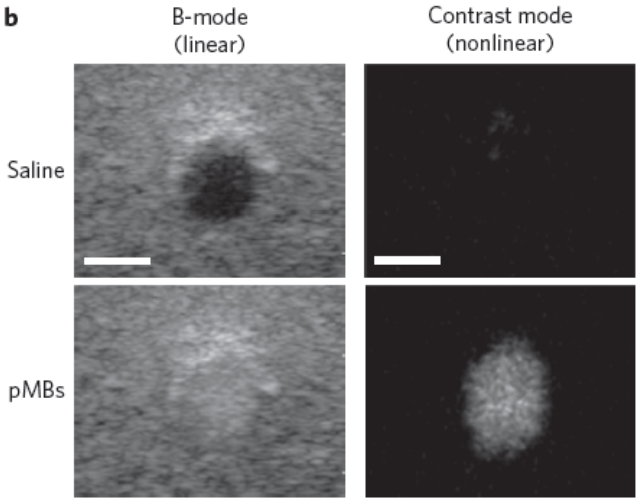
E) Summation of 2 times echo in C plus echo in D
- The linear echoes from tissue are cancelled while nonlinear echoes from the bubble are acquired

Molecular Imaging, Weisleder et al, 2010

Basic properties of BChl-lipid microbubbles





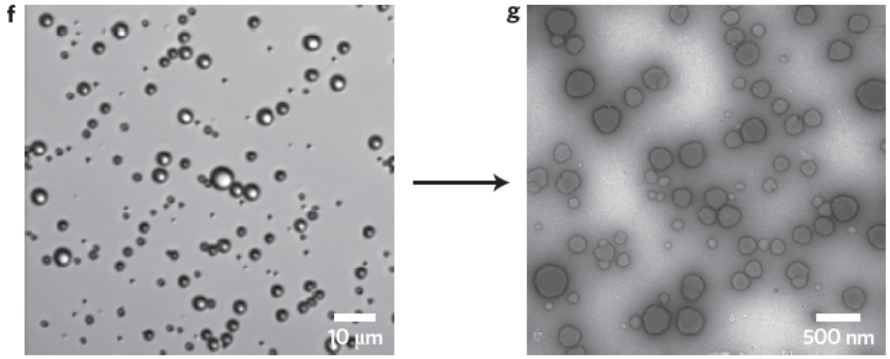


a) Acoustic attenuation measurement of pMBs, Ressonance attenuation peak at 4.5 MHz
b) Linear and nonlinear ultrasound properties of pMBs
   Tissue-mimicking flow phantom (agar and graphite)
   Microbubbles generate both linear and non-linear ultrasound signals

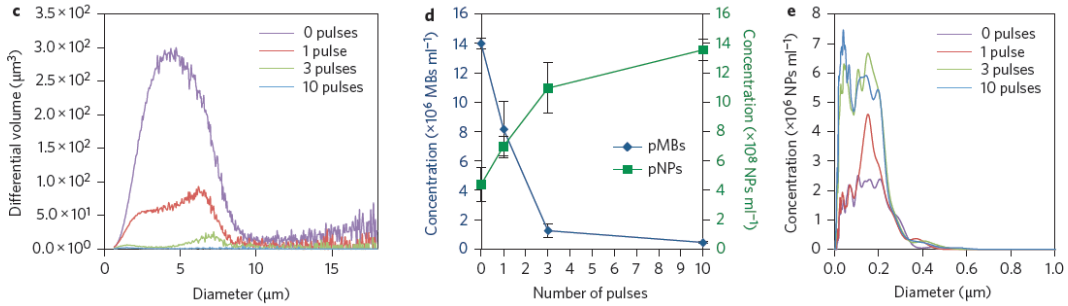
Elizabeth Huynh, 2015

“Conversion ultrasound”

“Conversion ultrasound”: 1 MHz, high-duty-cycle (50 %), ultrasound (2 W cm^{-2})



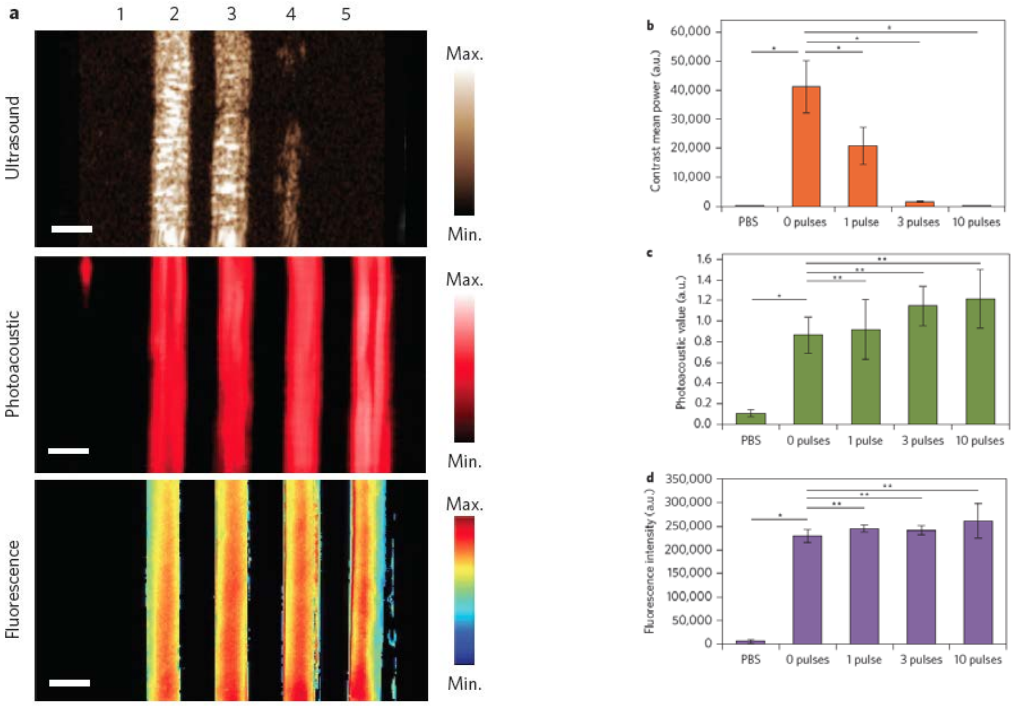
f, light microscopy image of microbubbles before conversion ultrasound
g, Electron microscopy of porphyrin nanoparticles after ten ultrasound pulses



c, Size distribution of microbubbles before and after conversion ultrasound
d, Concentration of microbubbles and nanoparticles before and after conversion ultrasound
e, Size distribution of nanoparticles before and after conversion ultrasound

Elizabeth Huynh, 2015

Multimodal imaging of Microbubbles (pMBs) and resulting nanoparticles (pNPs) following ultrasound-induced conversion

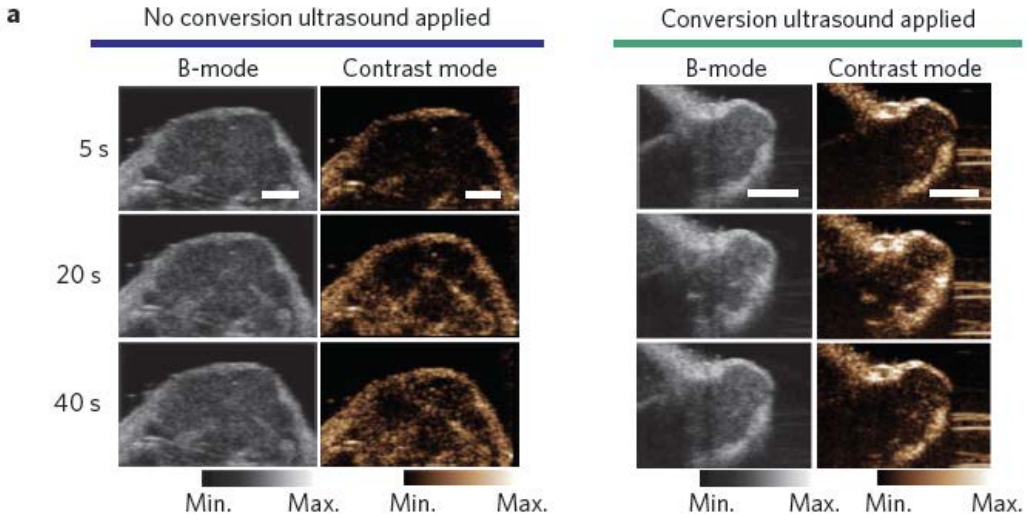


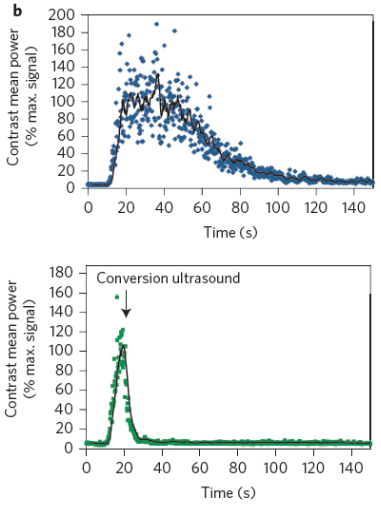
a, Acrylamide gel phantom imaged with ultrasound, photoacoustic and fluorescence
1) PBS 2) without conversion US 3) one pulse of conversion US 4) three pulses 5) ten pulses
b-d, Quantified signals (b: ultrasound, c: photoacoustic, d: fluorescence

Elizabeth Huynh, 2015

Conversion of pMBs to pNPs in vivo in mice

Mouse model: Subcutaneous inoculation of 2x106 KB cells (HeLa derivative) into the right flank of athymic nude mice. Experiments were performed when tumors reached a surface of 5-7 diameter.

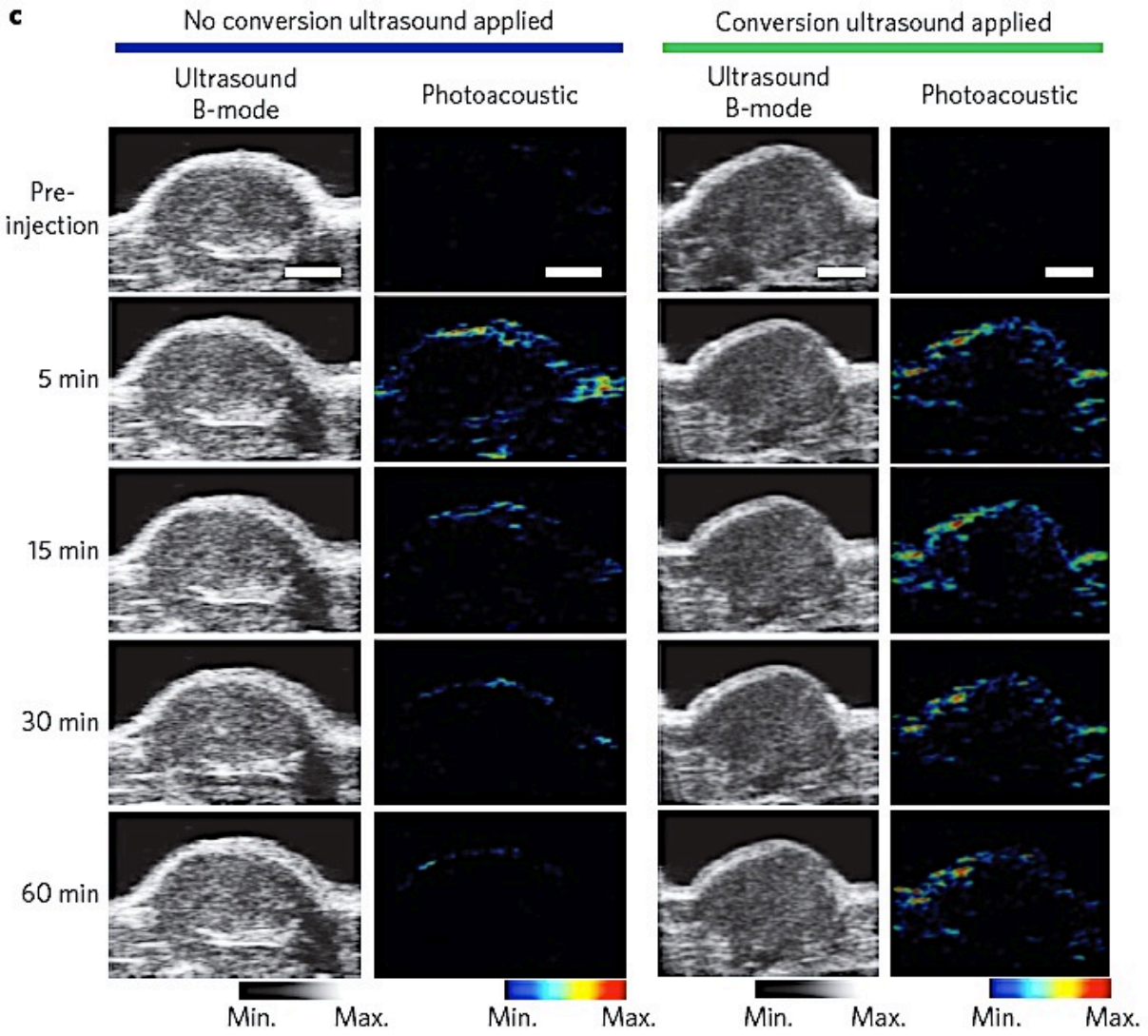


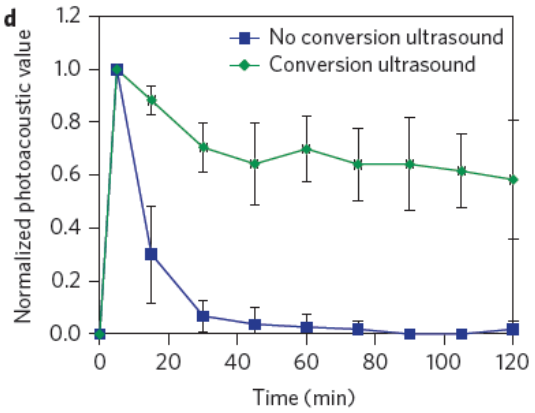


a, Cross section of the tumor (linear and non-linear imaging)
Non con. US: After injection, the pMBs circulate into the tumor, reaching a peak in circulation at 20 s and could be observed continuously in the circulation beyond 40 s
With con. US: A decrease of non-linear signal after the 20 s time point
b, ROI analysis without conversion ultrasound (top) and with conversion ultrasound (bottom)

Elizabeth Huynh, 2015

Successful delivery of pNPs into the tumor xenograft

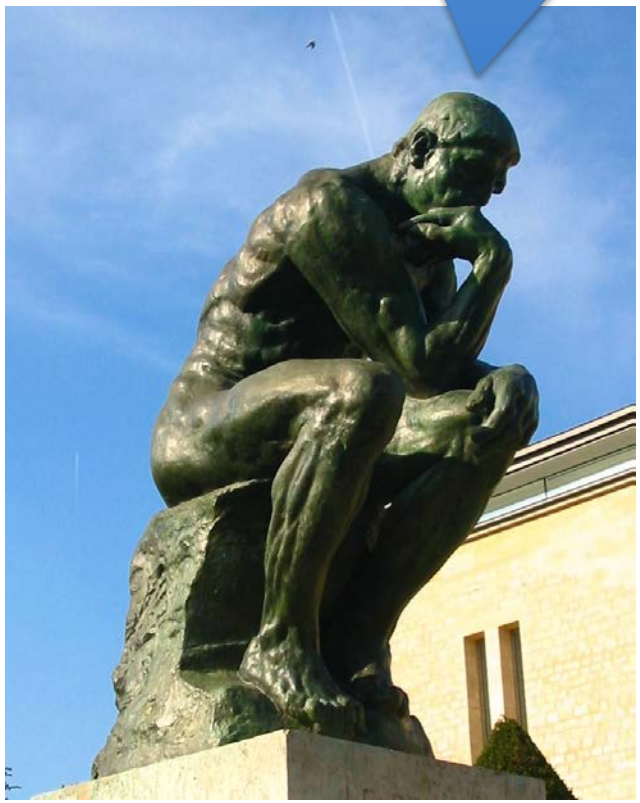




c, Retention of pNPs in the tumor xenograft enabled by conversion from pMBs to pNPs
d, Normalized photoacoustic signal over time in the tumor, indicative for a successful delivery

Elizabeth Huynh, 2015

Nice pictures .... Are they biomedical useful?



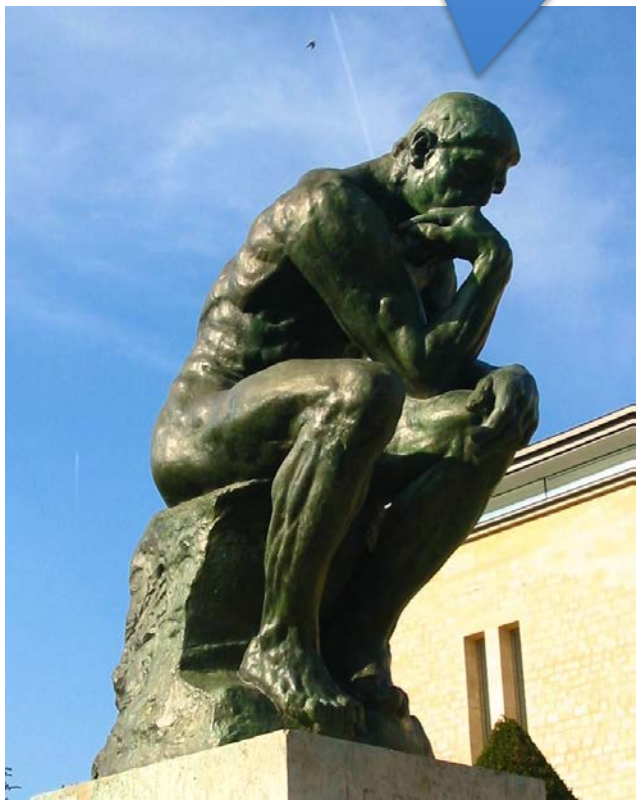
Not adressed:

- Penetration of the conversion ultrasound?
  (1 MHz = high penetration)
- Limitations for the drug delivery?
  (size, chemical properties etc)

Possible fields:

- Translational medicine (drug delivery)
- The use of nanoporphyrin offer an expansion to other imaging modalities

And from the view of a small animal?



Replacement : (further investigation needed, before application in humans are possible)

Reduction : Longitudinal studies are possible (Reaches the drug the target tissue?)

Refinement : Multimodality imaging (multiple information)

Drawbasks: Setup “experimental”, photoacoustic longitudinal studies performed over 2 h

Thank you for your attention!

-

Questions?

-

Get your forms signed!