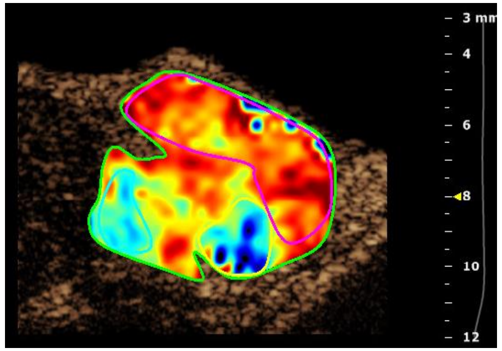
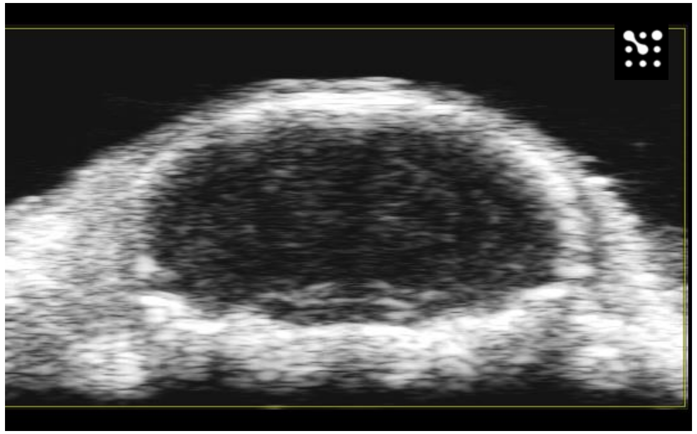
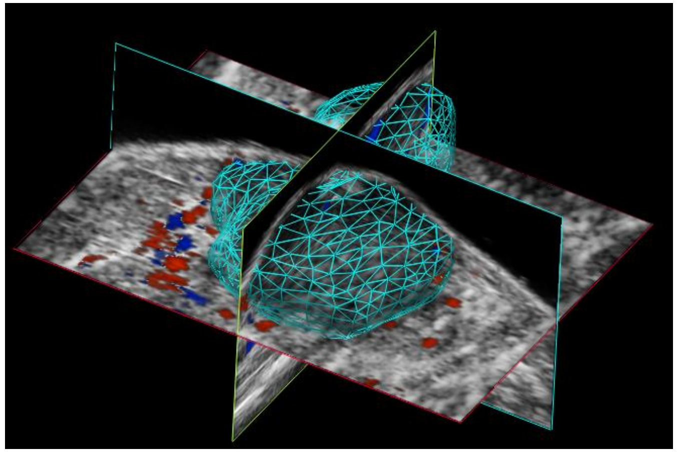
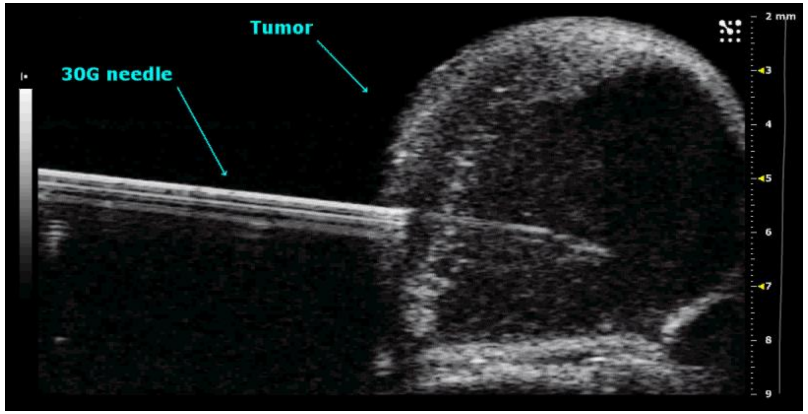
Firenze 21.1.2020

Mouse model and High-frequency ultrasound and Photoacoustic imaging in preclinical research





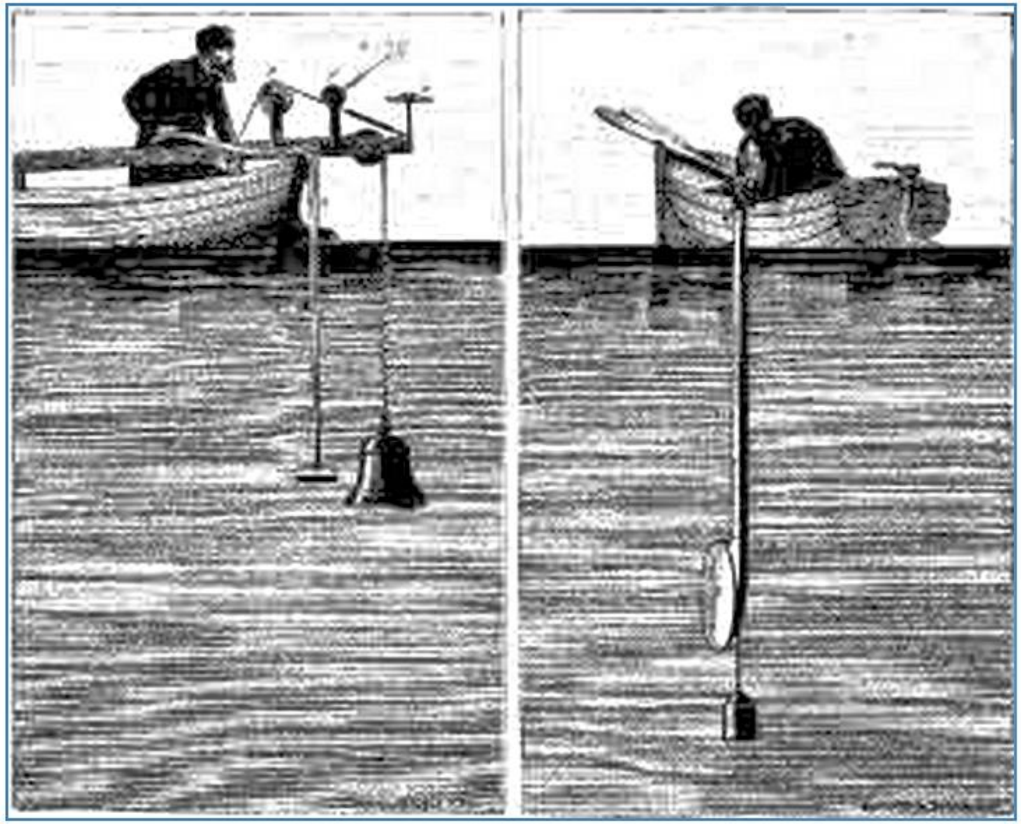




Tiziano Lottini

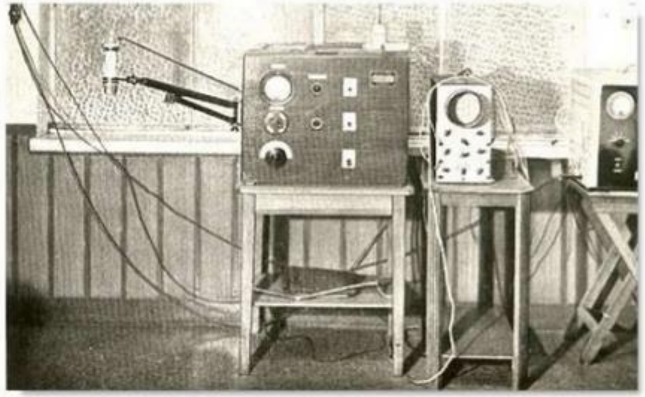
First applications of ultrasounds

In 1826 on Lake Geneva, Switzerland, Jean-Daniel Colladon and Charles-Francois Sturm made the first recorded attempt to determine the speed of sound in water.



The sonar was invented by the American Lewis Nixon in 1906 and was intended to ships to find the **iceberg** in time to avoid collisions. Later, during the First World War, it was developed a special type of sonar suited to detect **submarines.**

History of Ultrasounds



Denier's Ultrasonoscopic apparatus with ultrasound generator, emitter transducer and oscilloscope. This can be adapted for both therapeutic and diagnostic purposes

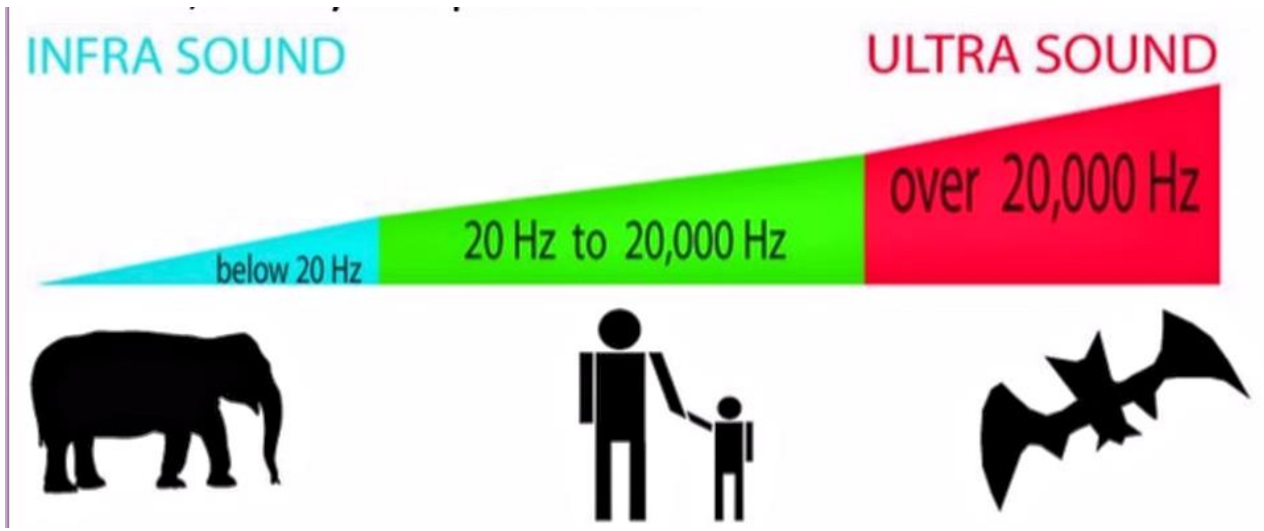
The first hand-held imaging instrument was developed by John Wild and John Reid in the early 1950's

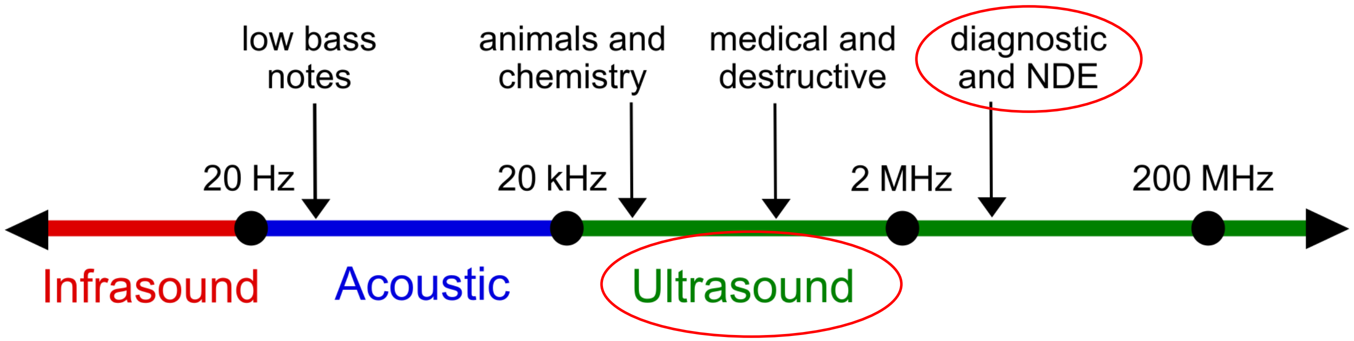
The use of ultrasounds in medicine for diagnostic purposes dates back to 1949 with the first scan by Karl Dussik.

Nowadays ultrasounds are widely used for medical diagnosis

What are Ultrasounds ?

Ultrasound is sound wave with frequencies higher than the upper audible limit of human hearing.



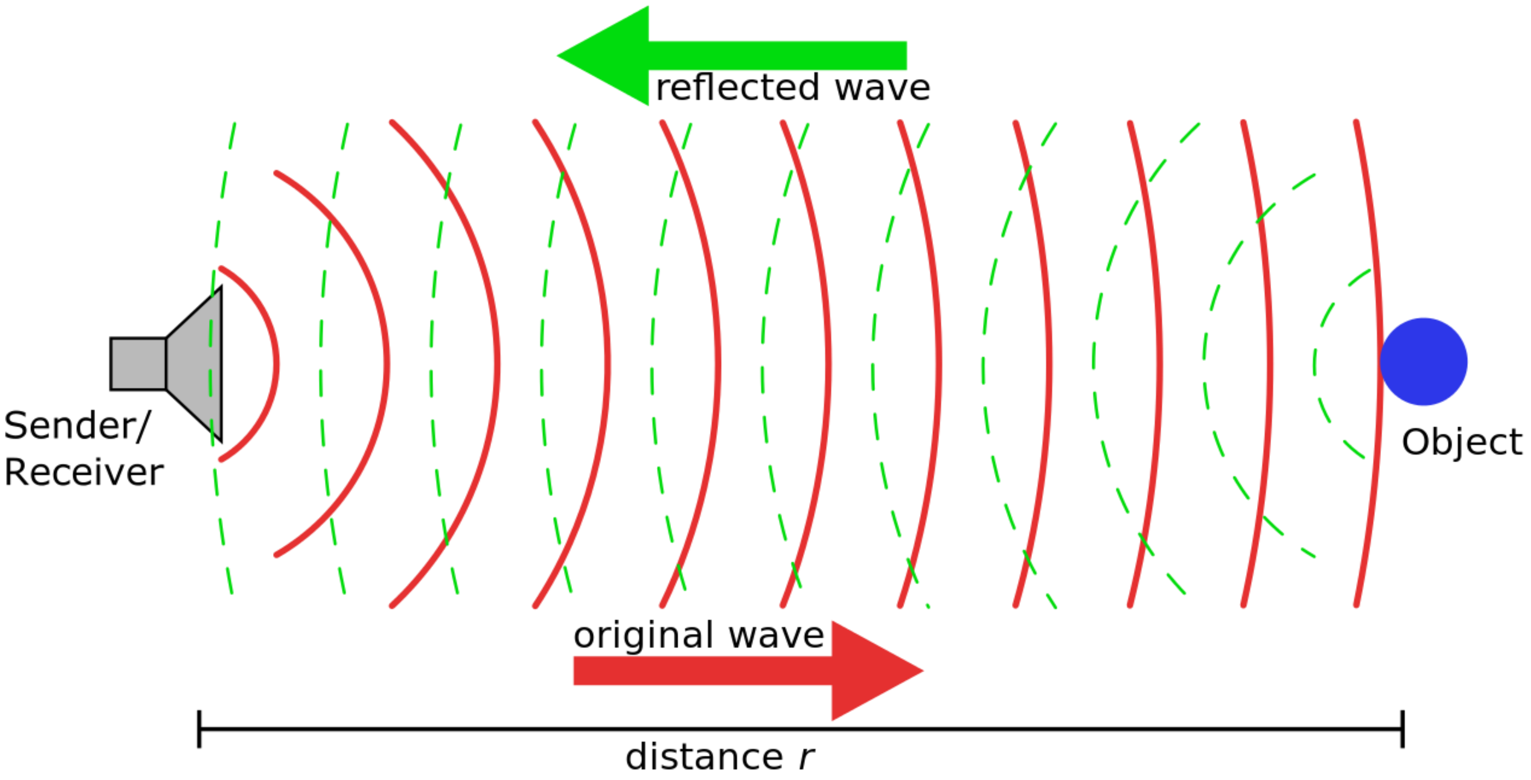


Interaction of Ultrasound with matter:

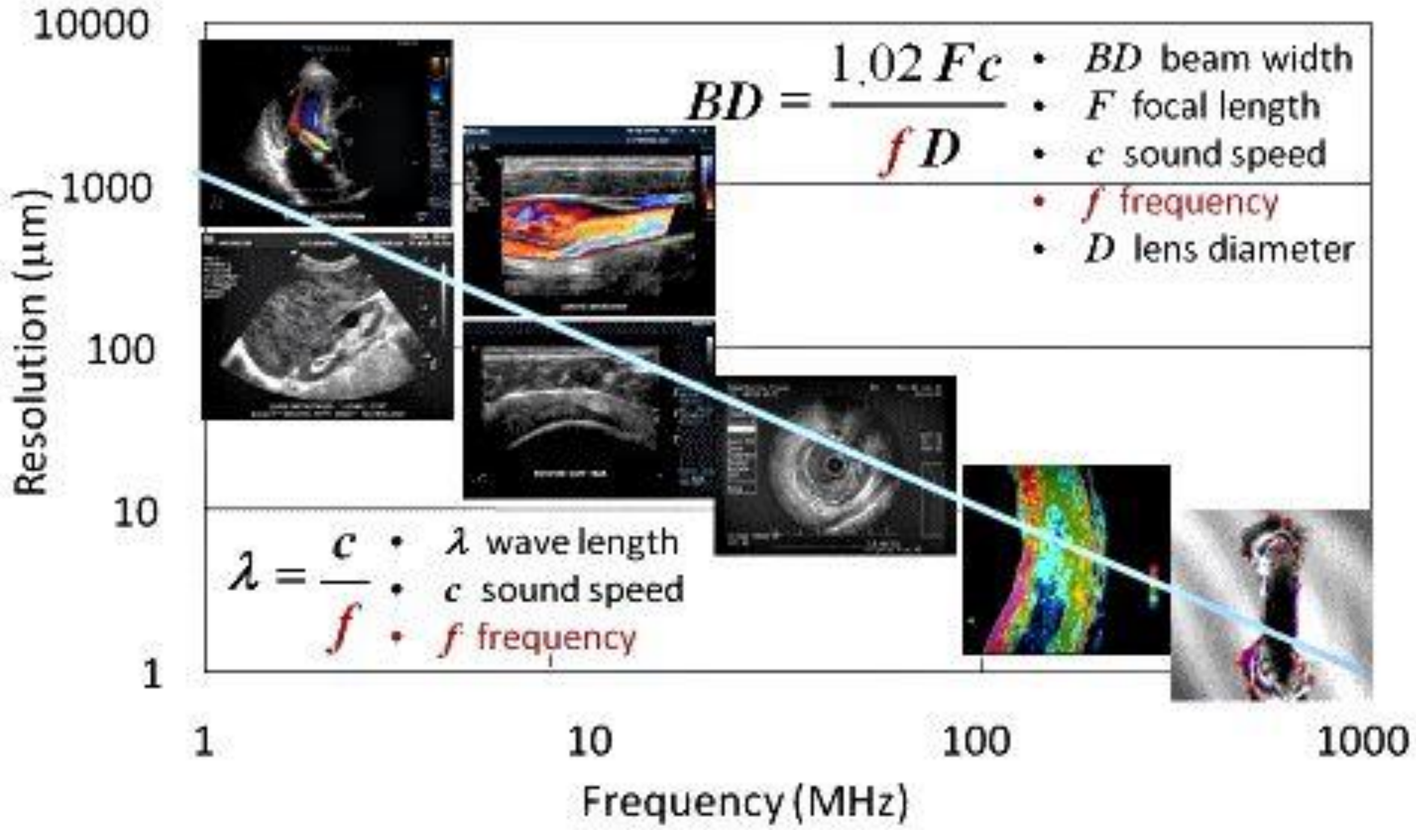
- **Reflection**
- Transmission
- Attenuation
- Scattering

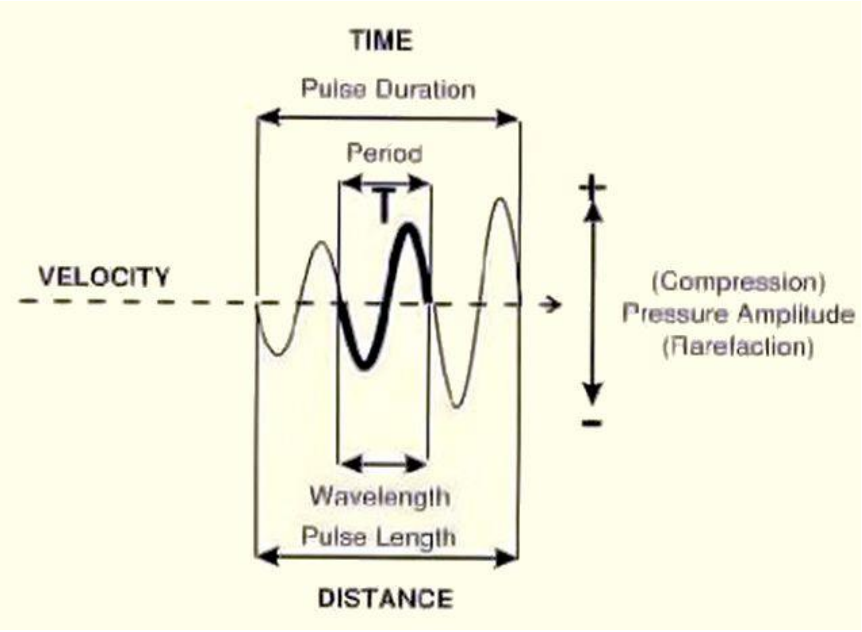
Piezoelectric effect

Deformation of certain materials that result from the application of electric field



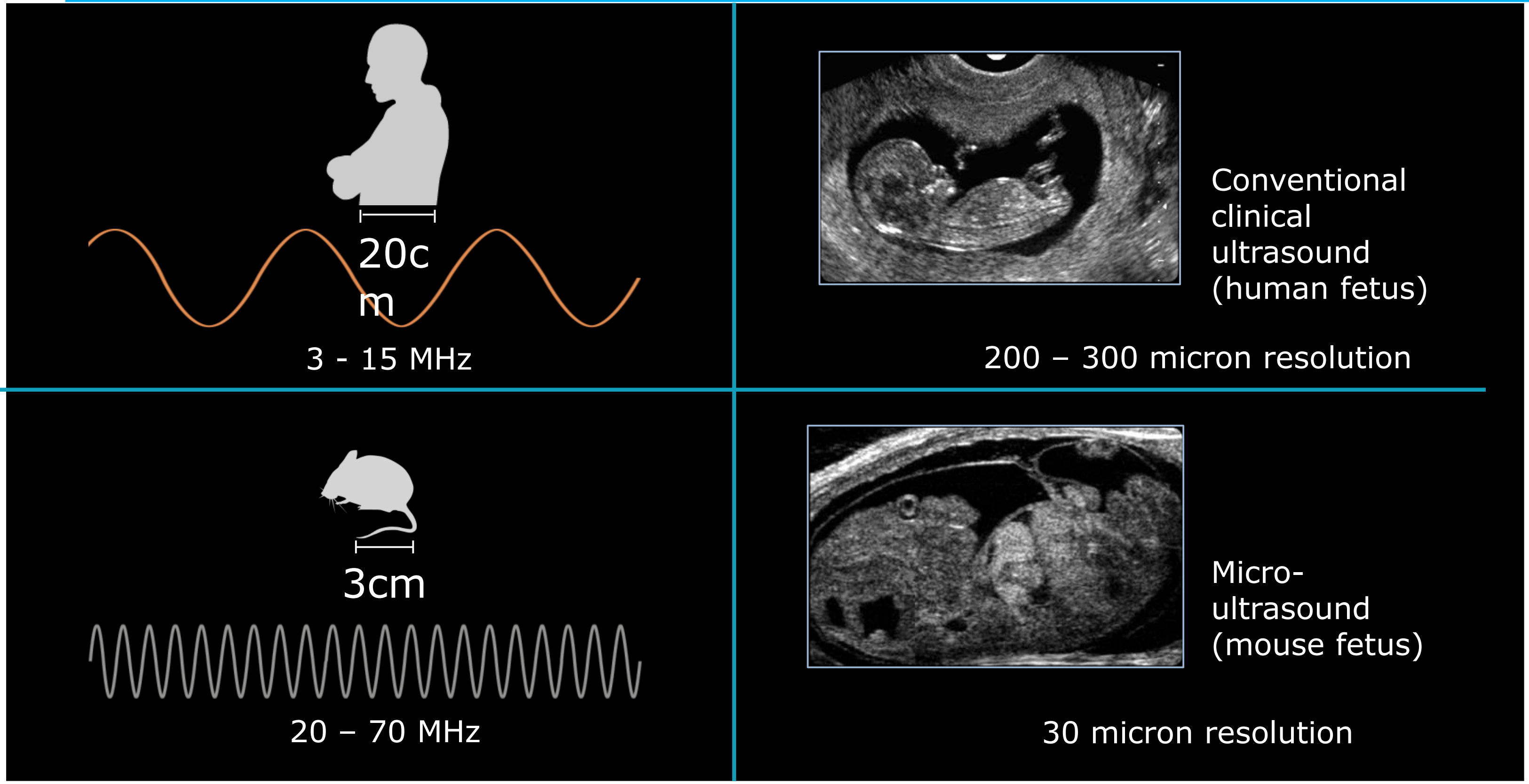
Ultrasound/Micro-Ultrasound

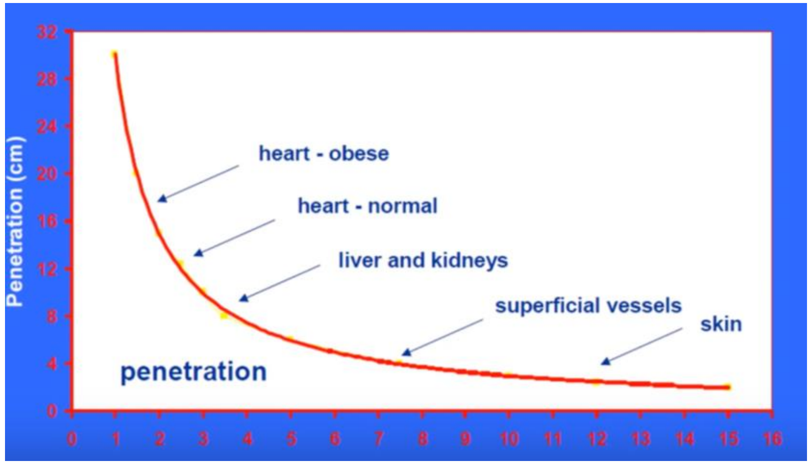




Ultrasound is a wave defined by **frequency**, **wavelenght** and **amplitude**

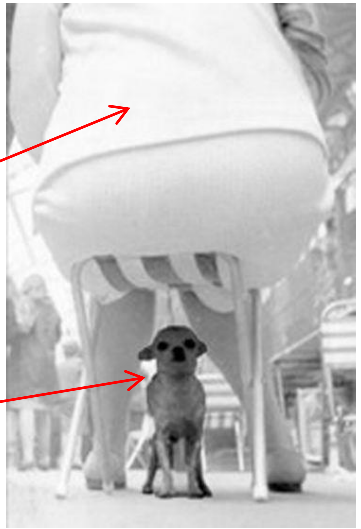
Micro-Ultrasound



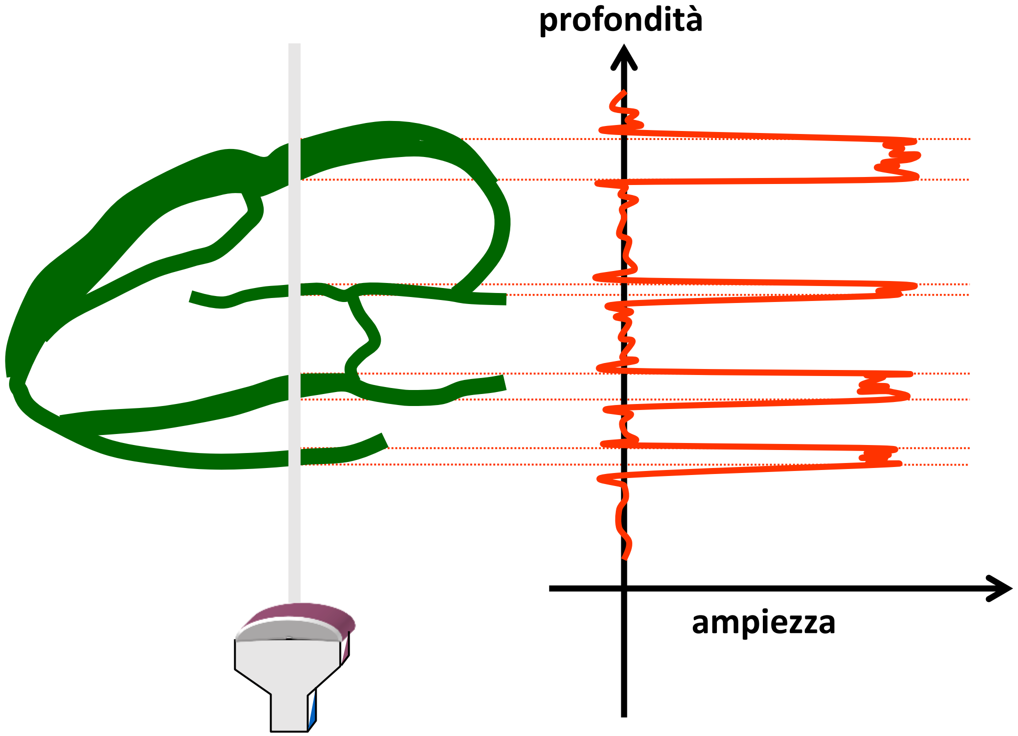


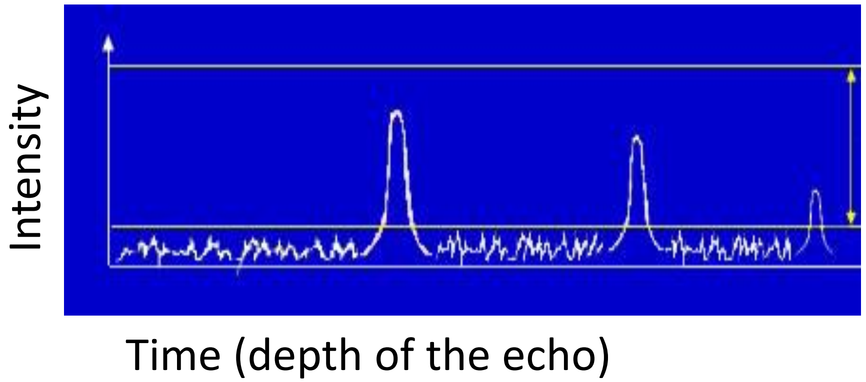
Patient that need low-frequencies transducers

Patient that need high-frequencies transducers

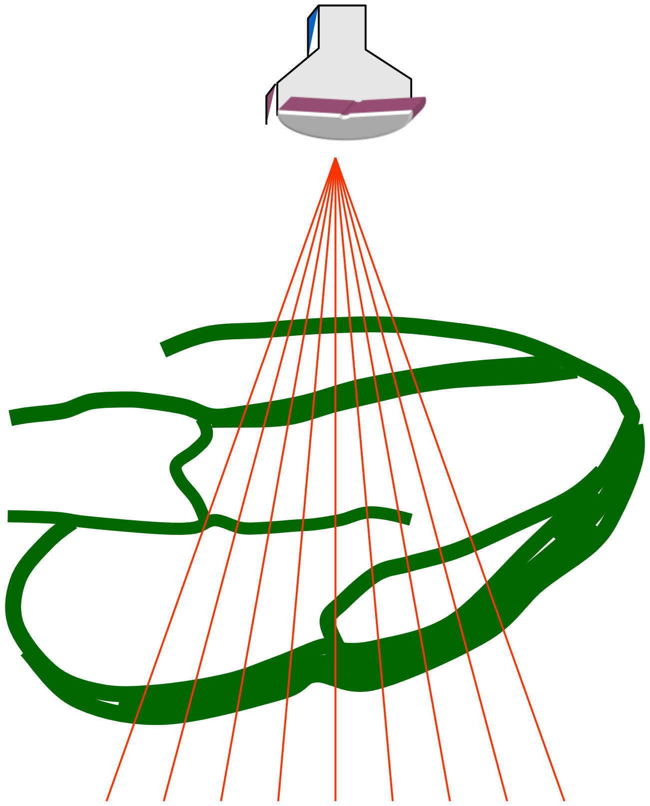


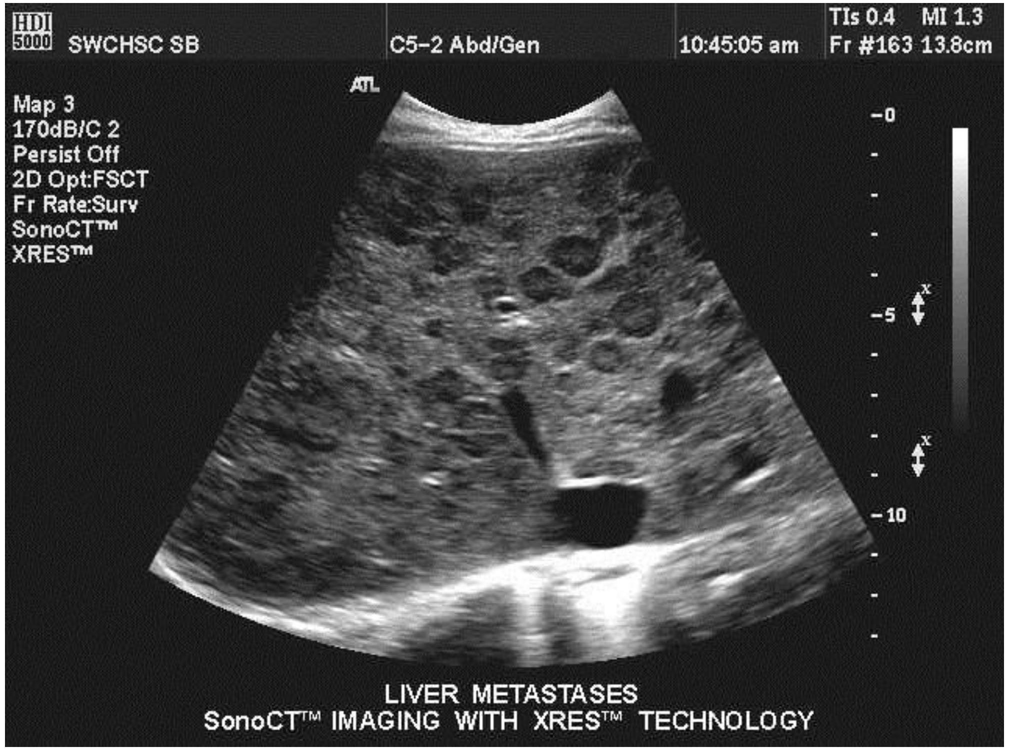
- **A-Mode** (Amplitude Mode). First developed, is the display of amplitude spikes of different heights. A-Mode consists of a x and y axis, where x represents the depth of the echo and y represents the Amplitude. Used in ophtalmology



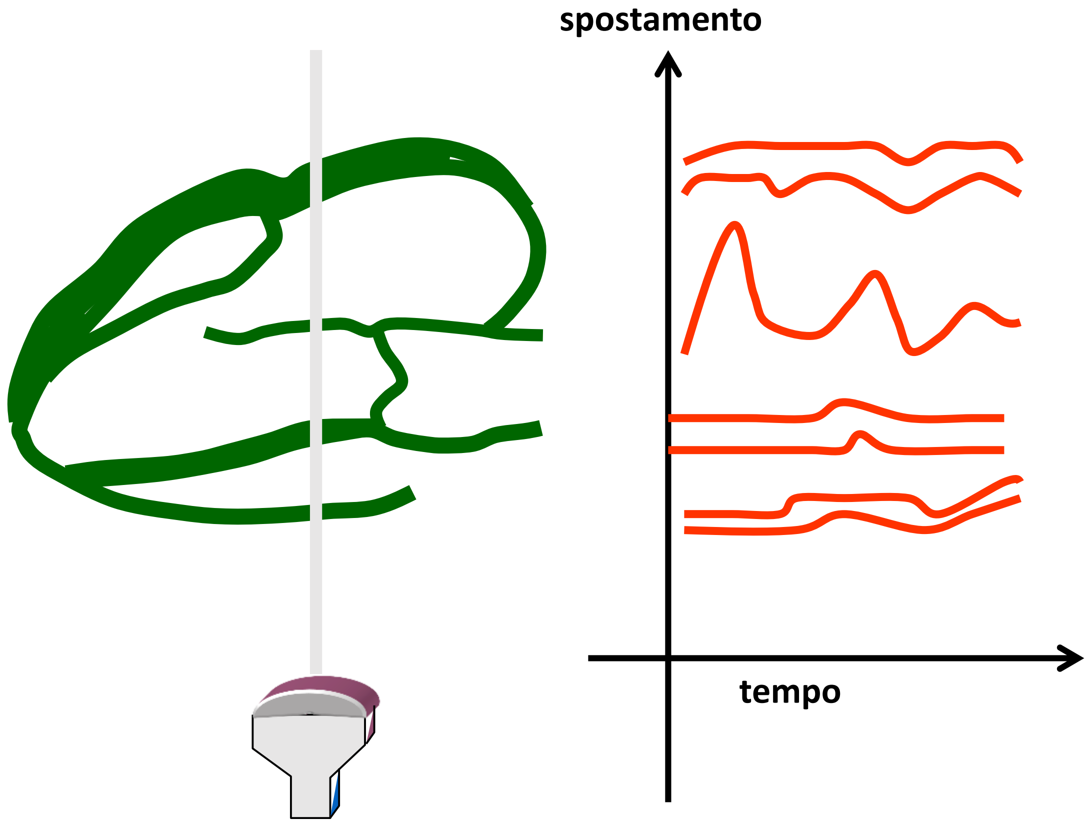


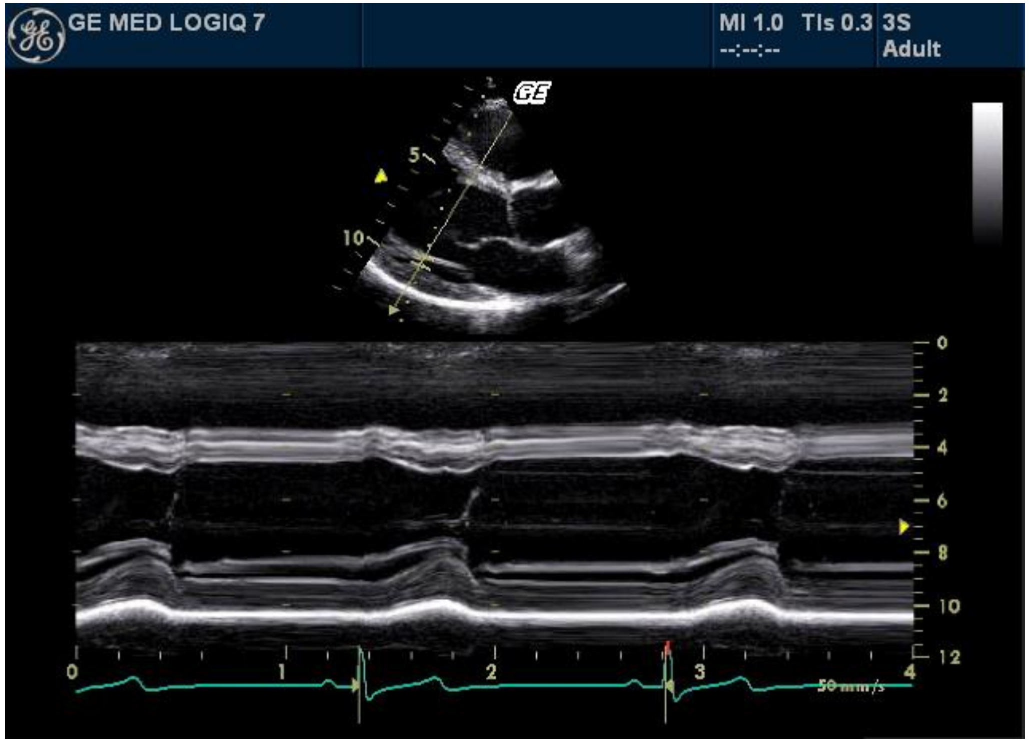
•**B-Mode** (Brightness Mode) a linear array of transducers simultaneously scans a plane through the body that can be viewed as a two-dimensional image on screen. The brightness depends upon the amplitude or intensity of the echo. Commonly known as 2D mode.



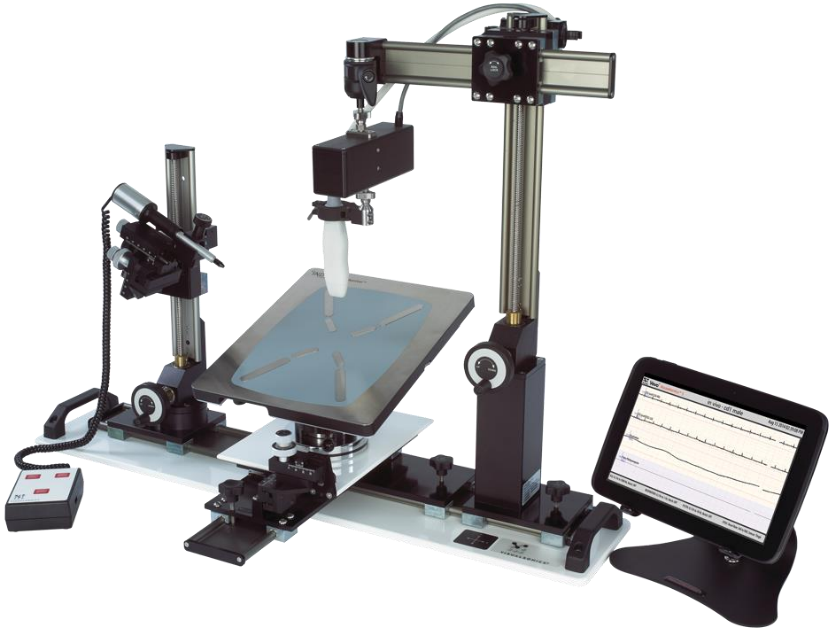


•**M-Mode** (Motion Mode) is the display of a one-dimensional image that is used for analyzing moving body parts commonly in cardiac and fetal cardiac imaging (e.g. cardiac valves).





Ultrasound with VevoLAZR-X



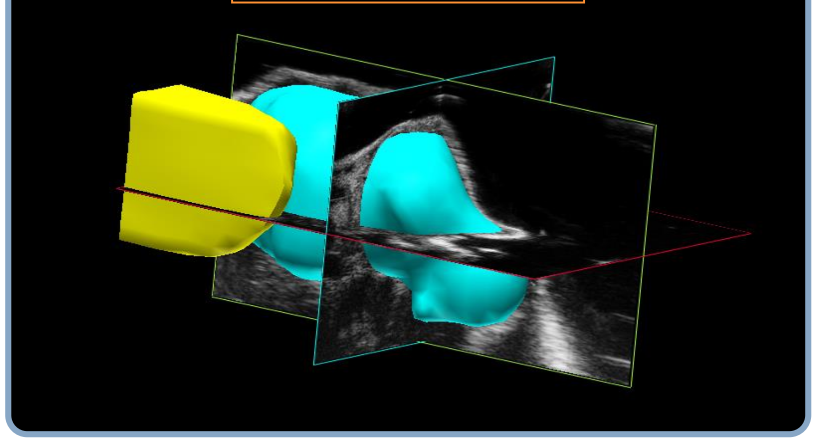
Multimodal imaging sistem

Vevo-LAZR-X

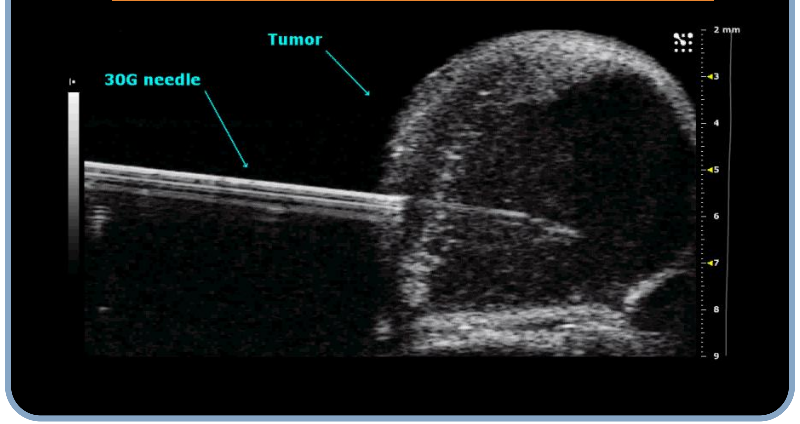
(Visualsonics Fujifilm)

Visualization with micro-ultrasound

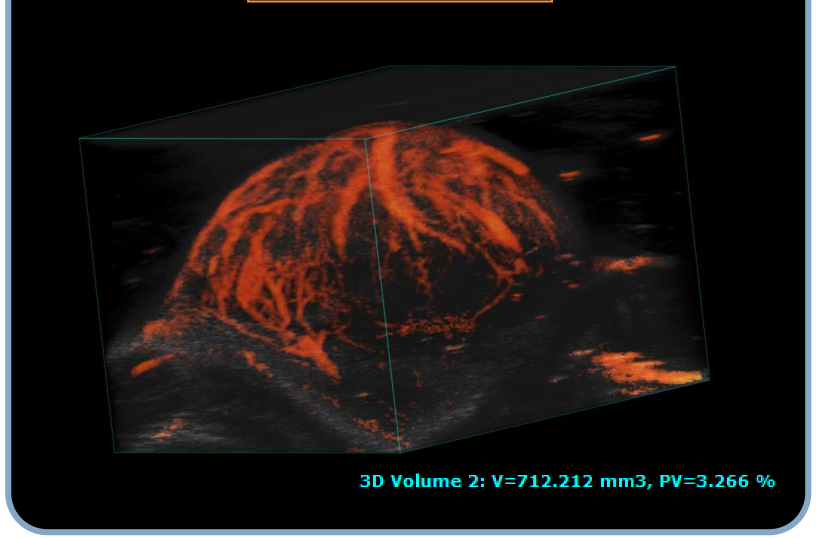
Volumetrics



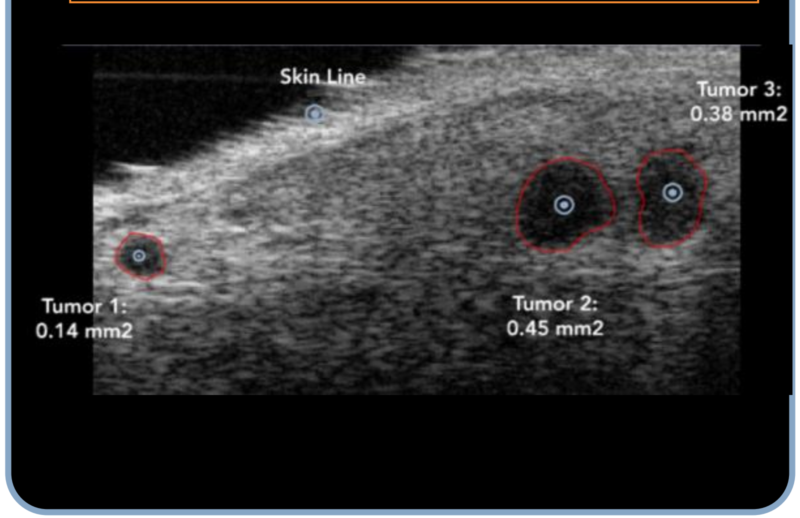
Echo-guided injection



Vascularity



Early detection/screening



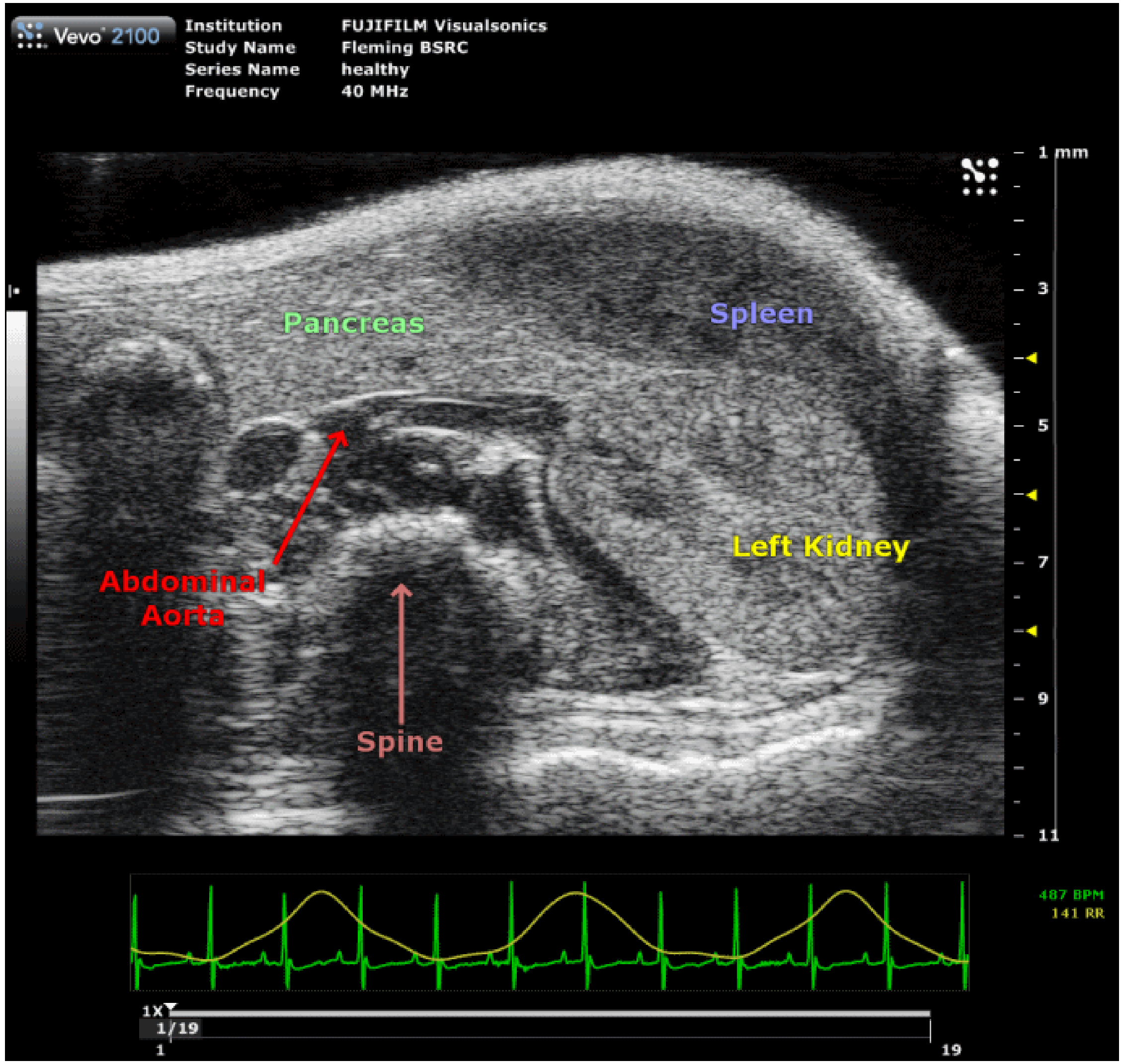
Ultrasound in B-Mode

Mouse abdominal echografy

An-echogenic tissue: absence of reflection

Hyperechogenic tissue: High acoustic impedance (the wave is reflected but not trasmissed)

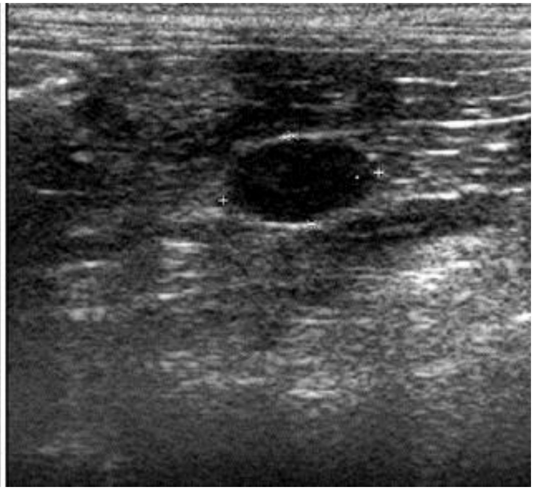
Hypoechogenic tissue



Artefacts…

Images or part of images that do not rapresent the real anatomy of the tissue

Ovarian cysts: Liquid inside, **Back enhancement**, lateral shadow

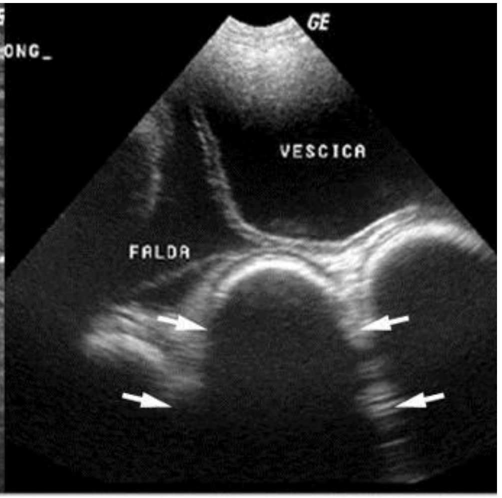


Lymphomatous lymph node: No Back enhancement

Back shadows

calculus

calcolo vescicale



Air bubble

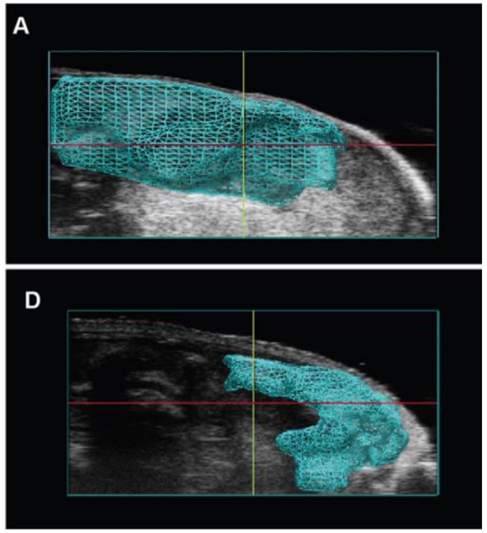
gas intestinale

Rebound «ringing»

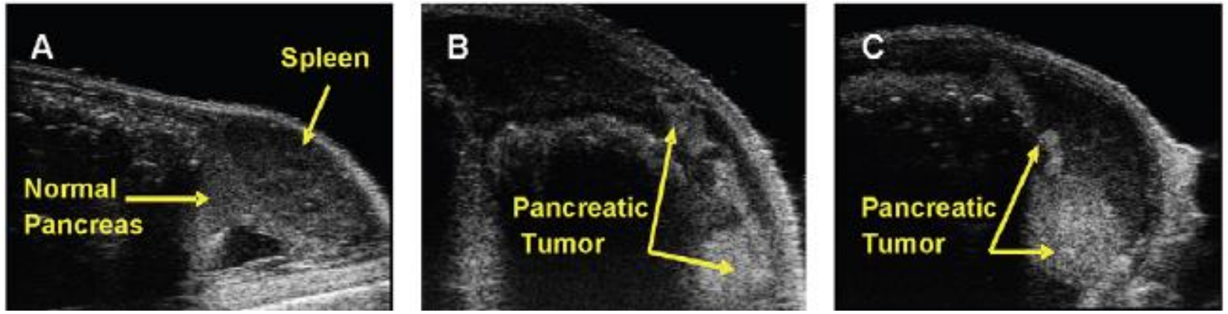
Ultrasound in B-Mode

Echo-guided intervention into the heart

3D volume reconstruction



Monitoring tumor development



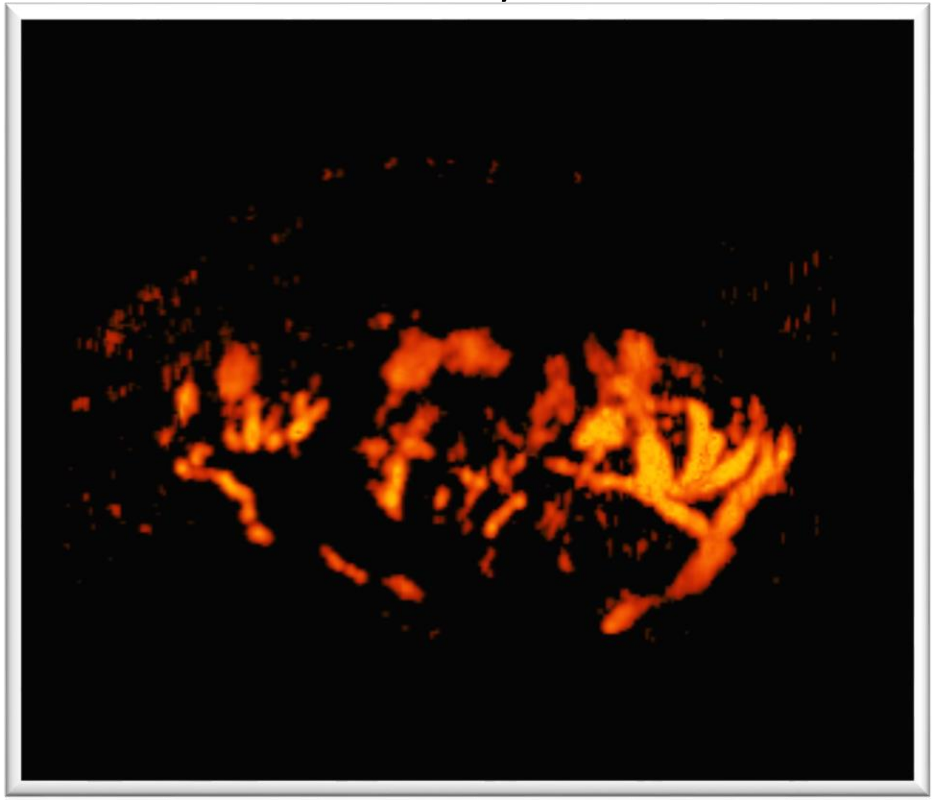
Ultrasound in B-Mode

How to study angiogenesis

Ultrasound Power Doppler Imaging

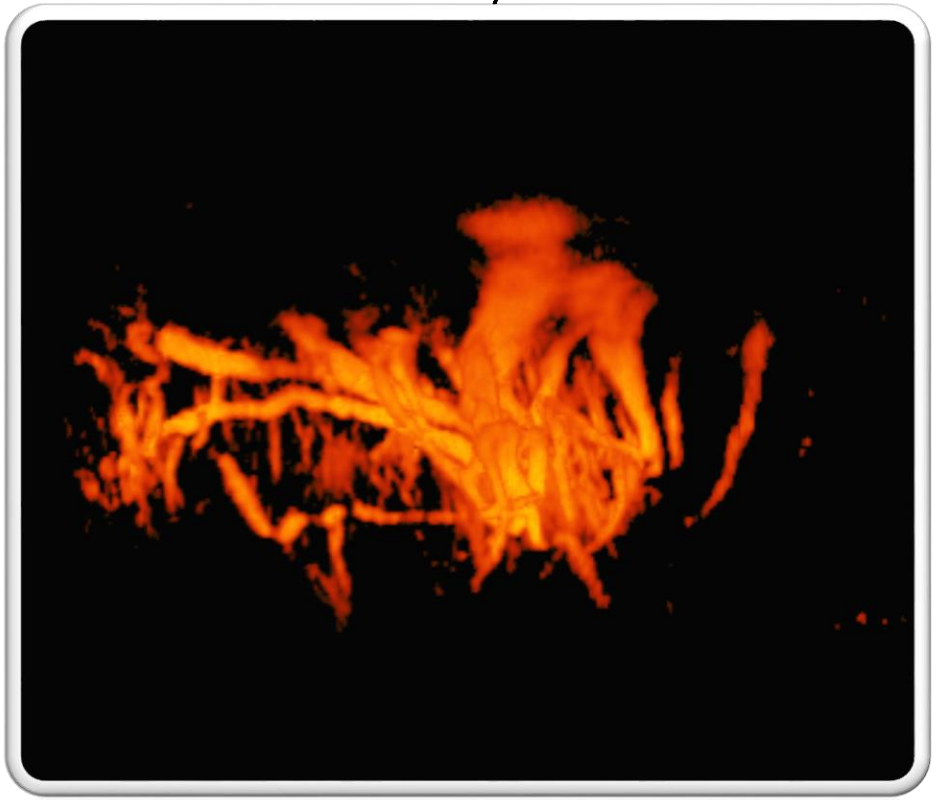
Power Doppler Mode Imaging

Day 2



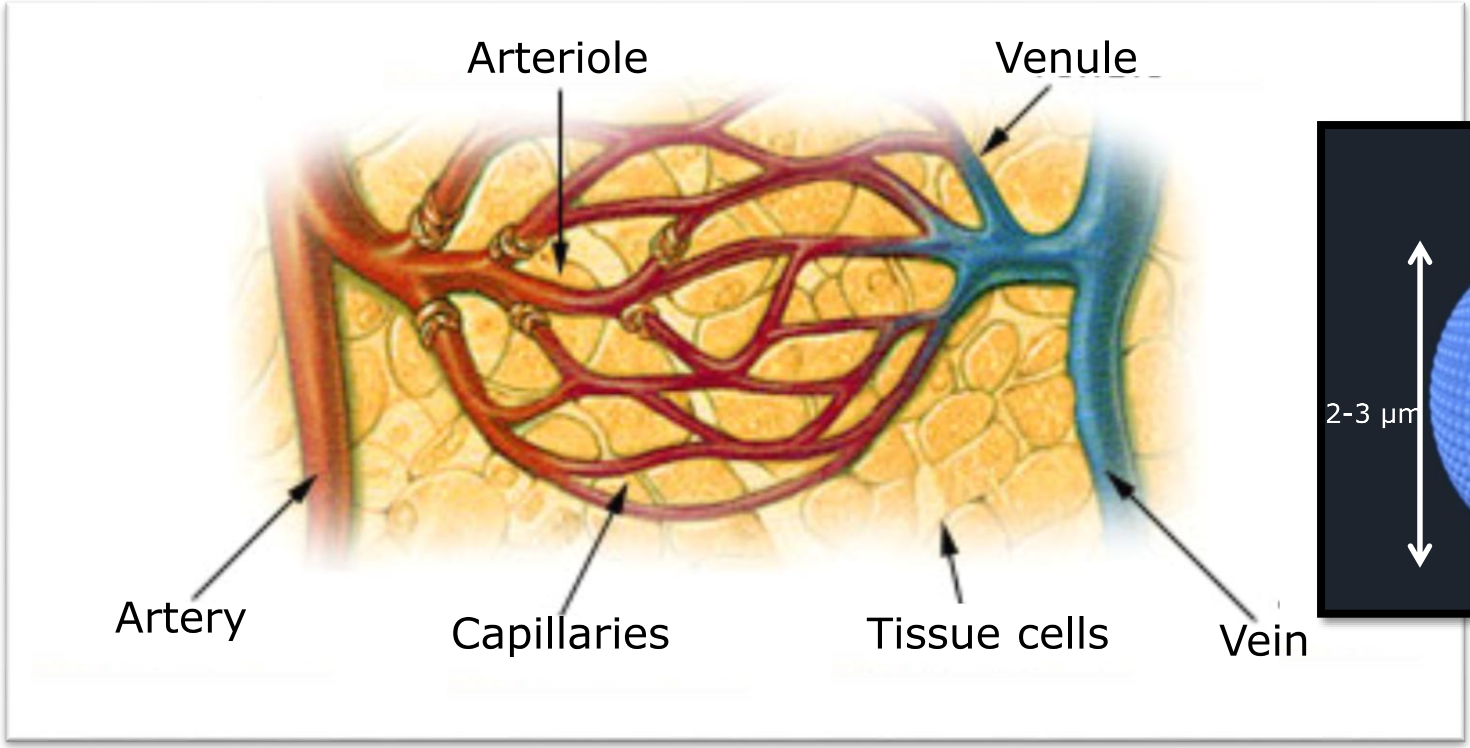
PV = 3.18%

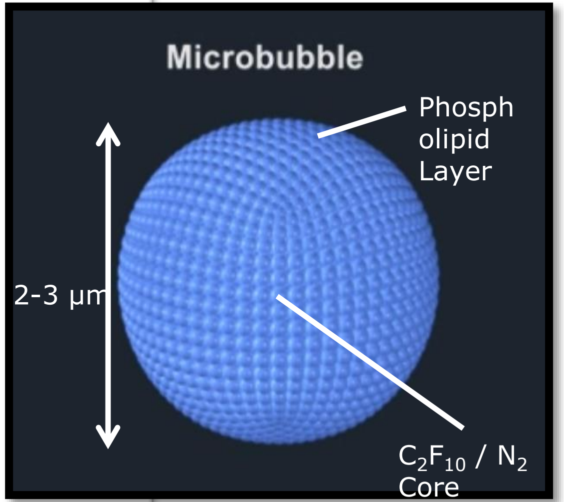
Day 10



PV = 11.22%

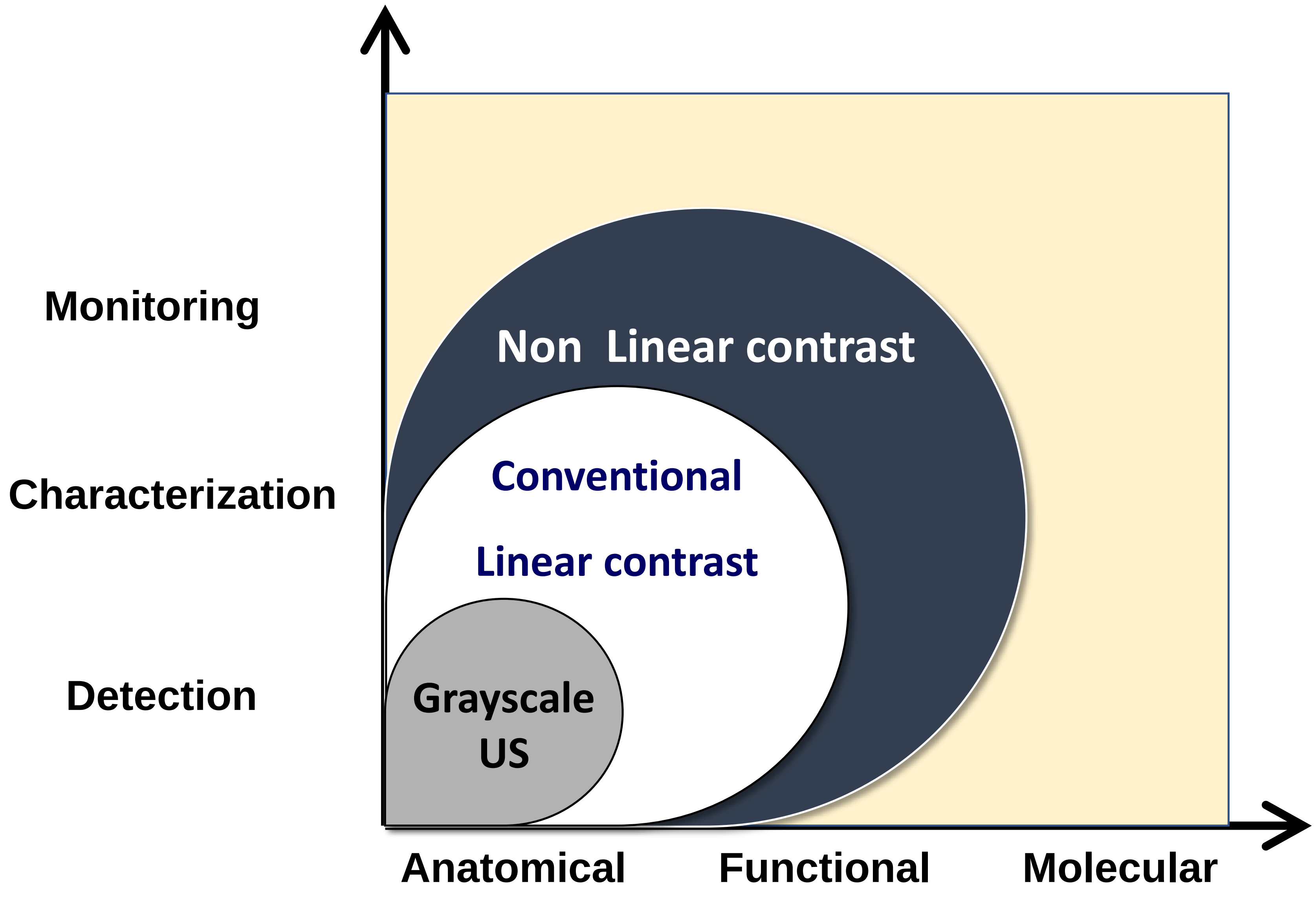
Ultrasound in B-Mode



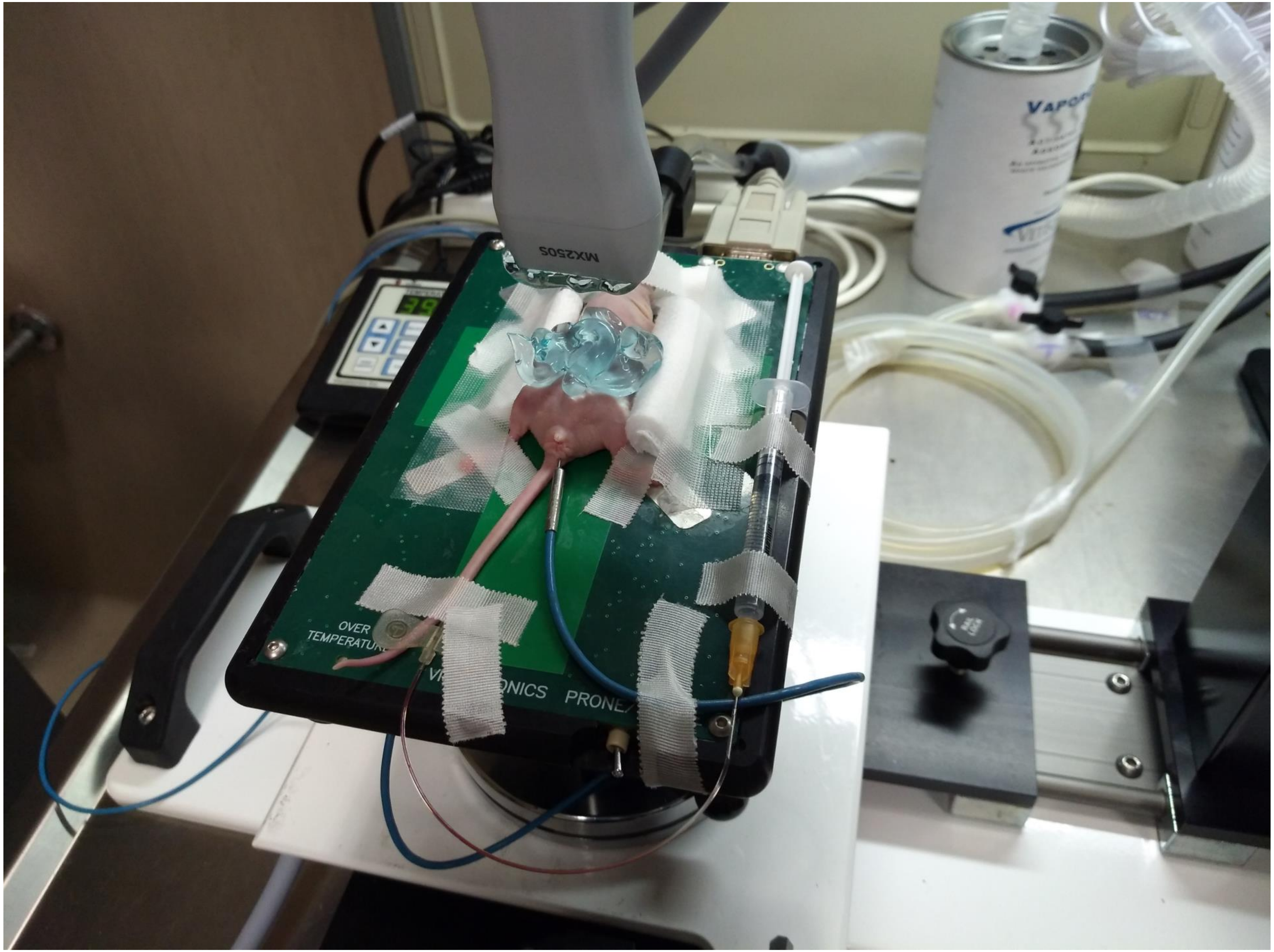


Arteries & Veins (500-1000 μM) → Arterioles & Venules (20-500 μM) → Capillaries (5-10 μM)

Non Linear contrast imaging



Non-Linear
Contrast
Mode

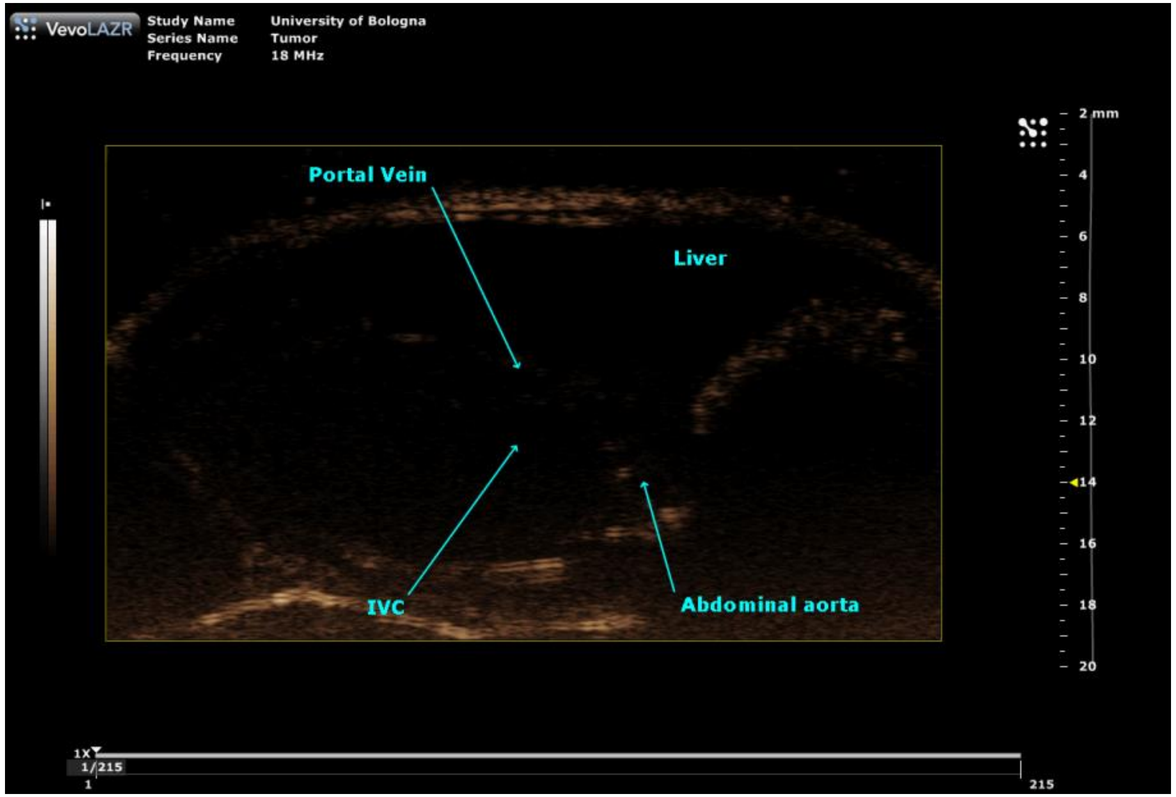


Microbubble applications

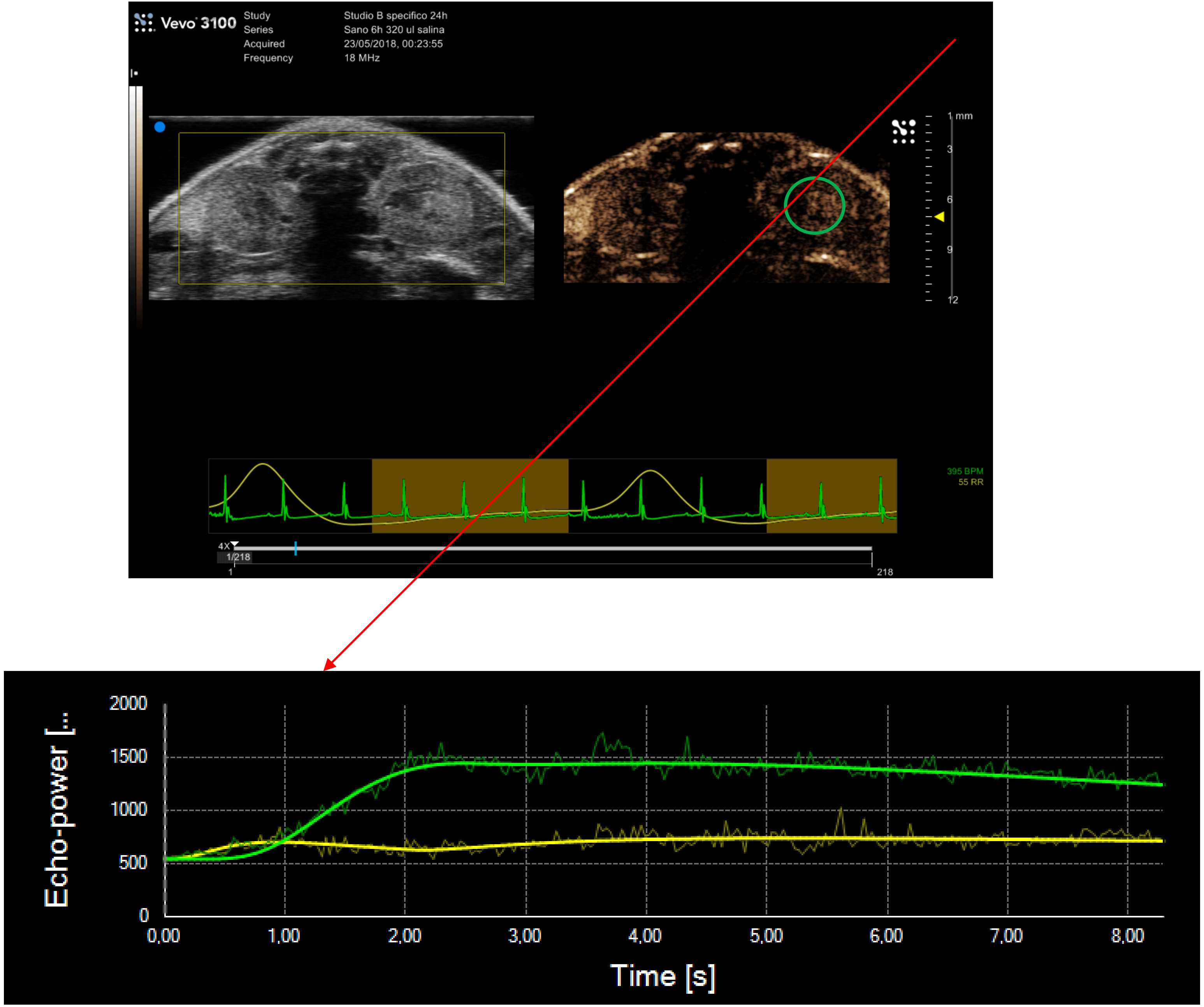
Contrast Enhanced Ultrasound

❖ The non linear contrast Mode exploit the **nonlinear response of the microbubbles** to ultrasound pulses

❖ the goal is to **suppress the tissue signal** while **increasing the detection of the contrast agents**, providing a much more sensitive imaging technique

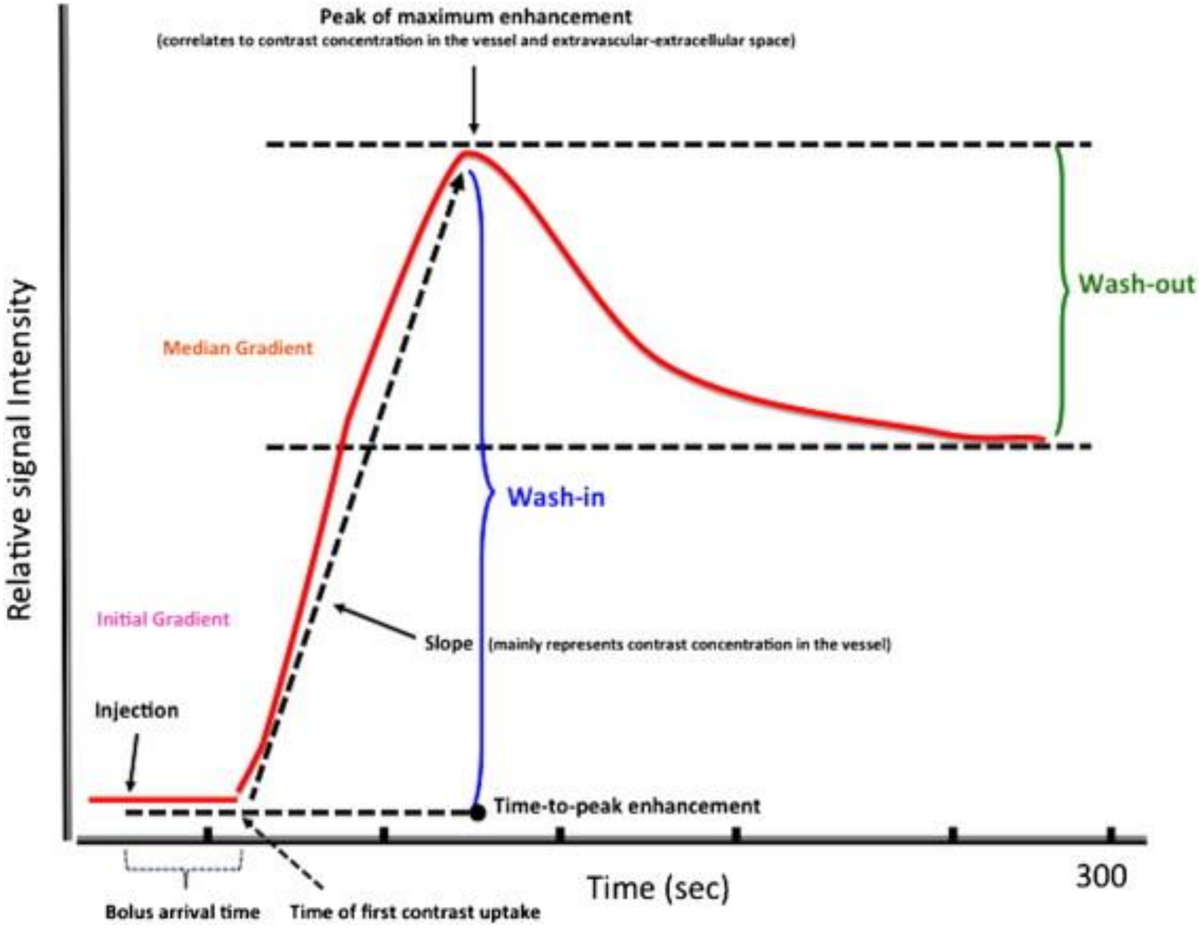


- Contrast-enhanced ultrasound show any selective enhancement in the venous and arterial phases
- Contrast agent uptake may be fast or slow depending on circulation speed



Vevo 3100
Study
Series
Acquired
Frequency
Studio B specifico 24h
Sano 6h 320 ul salina
23/05/2018, 00:23:55
18 MHz
1 mm
3
6
9
12
395 BPM
55 RR
4X
1/218
1
218
Echo-power [...
2000
1500
1000
500
0
0,00
1,00
2,00
3,00
4,00
5,00
6,00
7,00
8,00
Time [s]

Perfusion curve



PE	**Peak Enhancement**
WiAUC	Area Under the Curve (Wash-in)
RT	Rise Time
TTP	**Time To Peak**
WiR	**Wash-in Rate**
WiPI	Wash-in Perfusion Index (WiAUC / RT)
Area	ROI area

---------- BASED ON EXTRAPOLATED DATA ----------

AUC	Area Under the Curve
mTT	mean Transit Time
PI	**Perfusion Index (AUC / mTT)**

PE = relative blood volume

TTP = relative velocity of blood

What is Photoacoustic

The PA effect is a combination of both light and sound

1) Laser pulse illumination (reach the object)

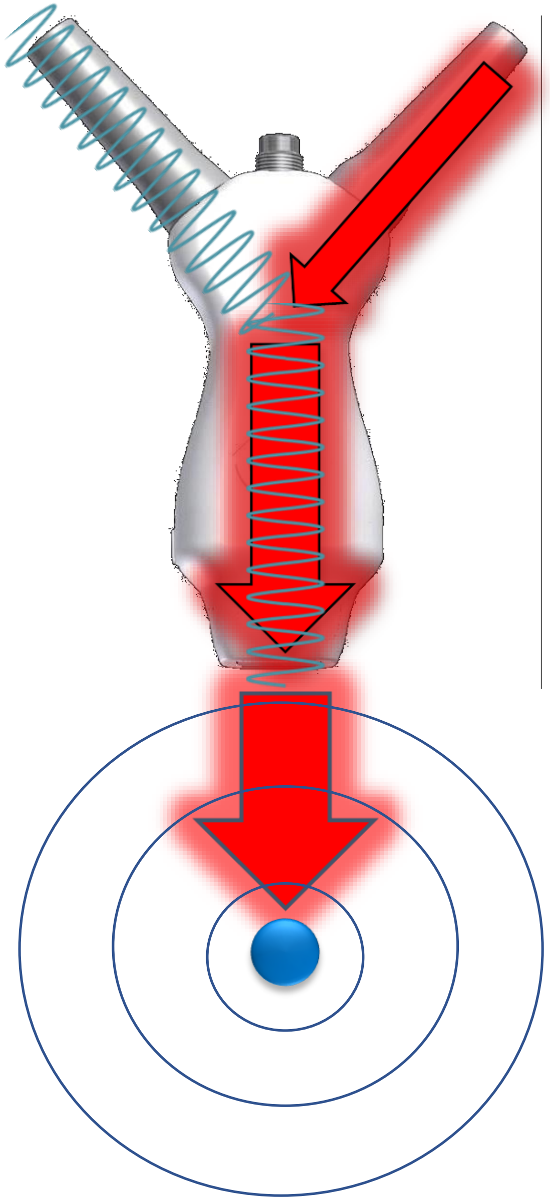
2) Optical absorption, heating and thermoelastic expansion

Optical Pat

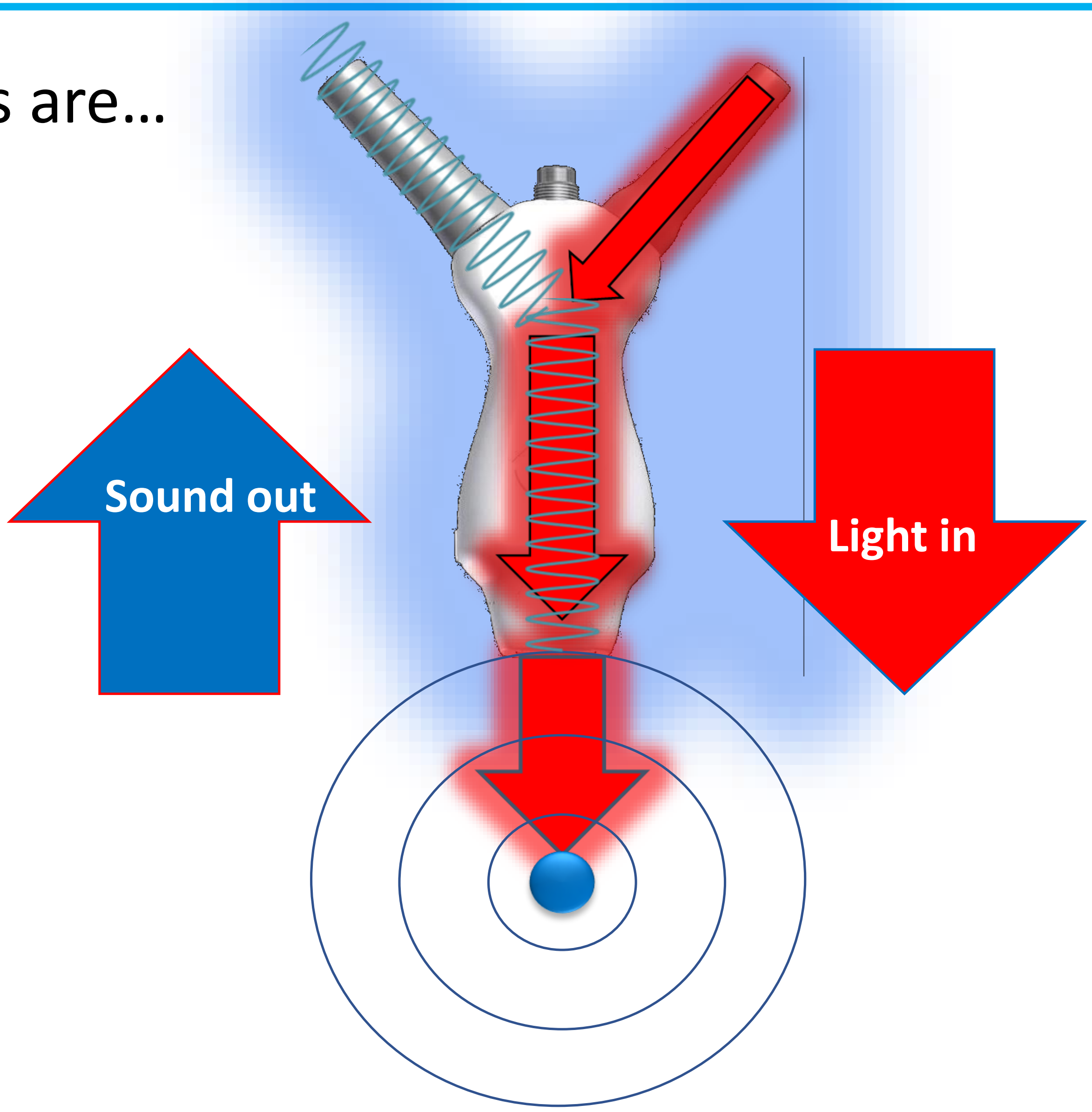
3) Emitted pressure (sound) wave

4) Detection of ultrasound and creation of image

Sound Pat

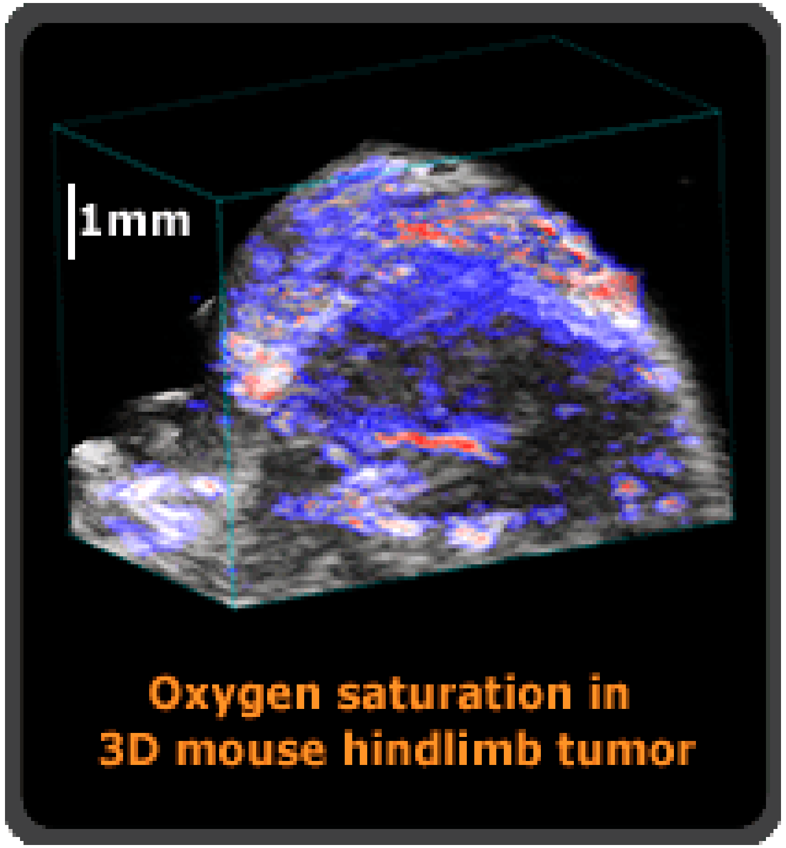


The bases are…



What Photoacoustic can do

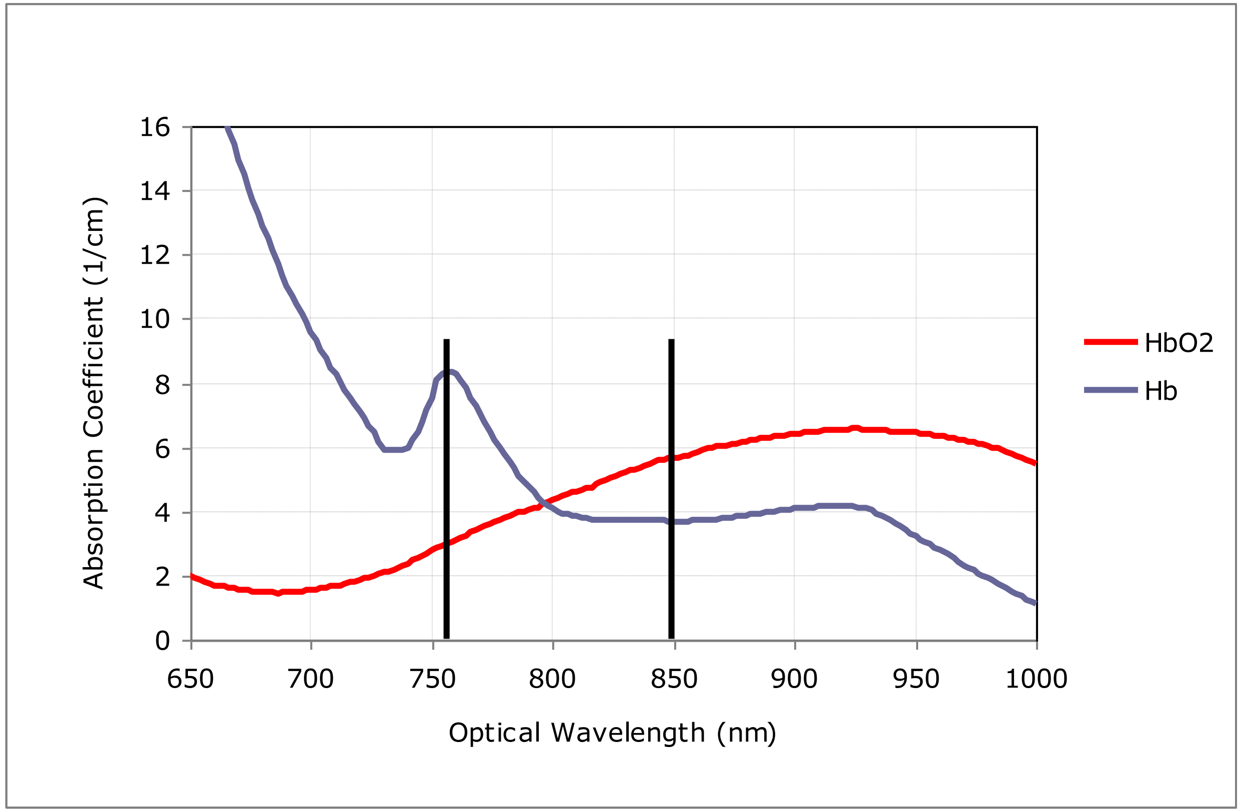
Endogenous PA contrast agents



Oxygen Saturation

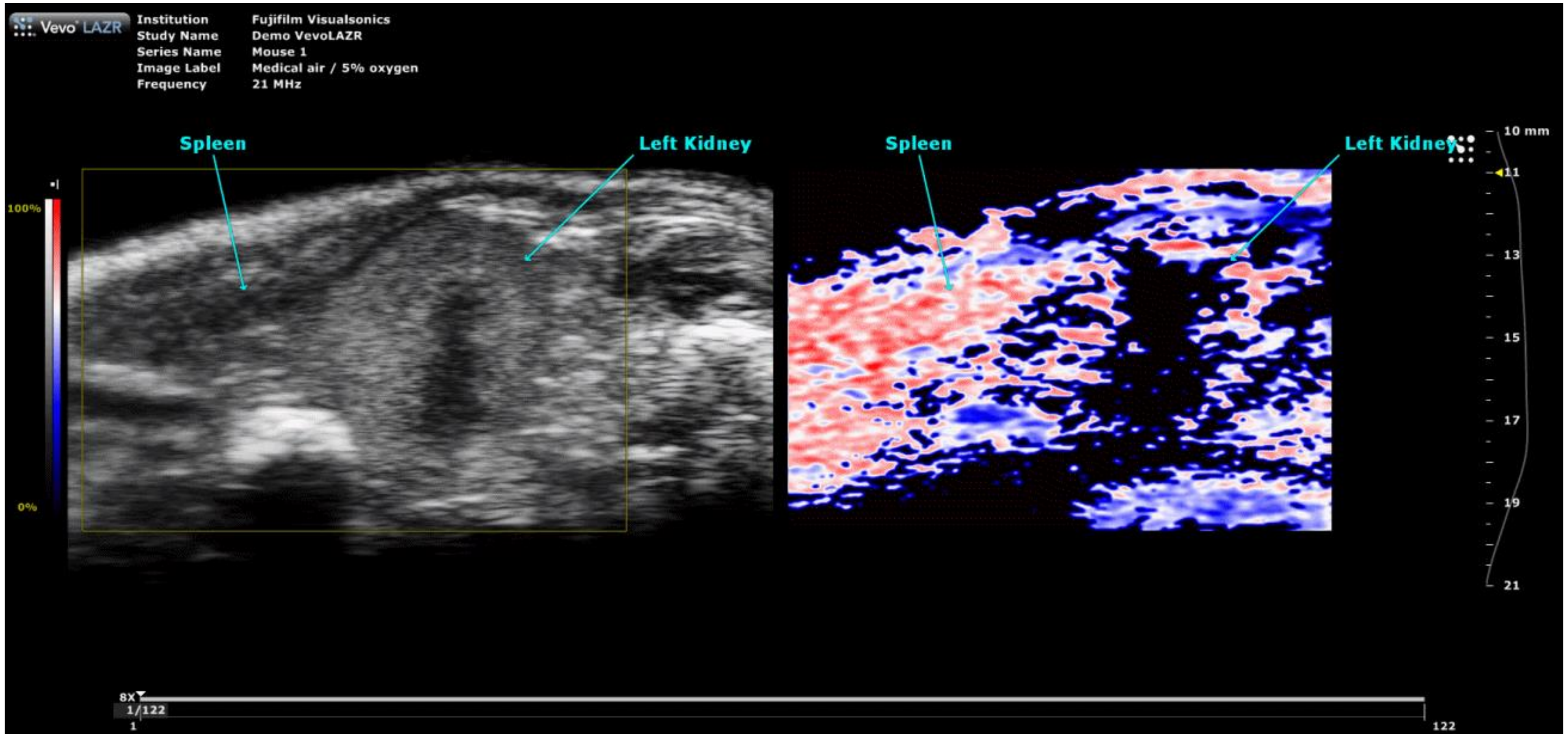
3D Quantification

Oxygen saturation → oxygenated and de-oxygenated hemoglobin discrimination



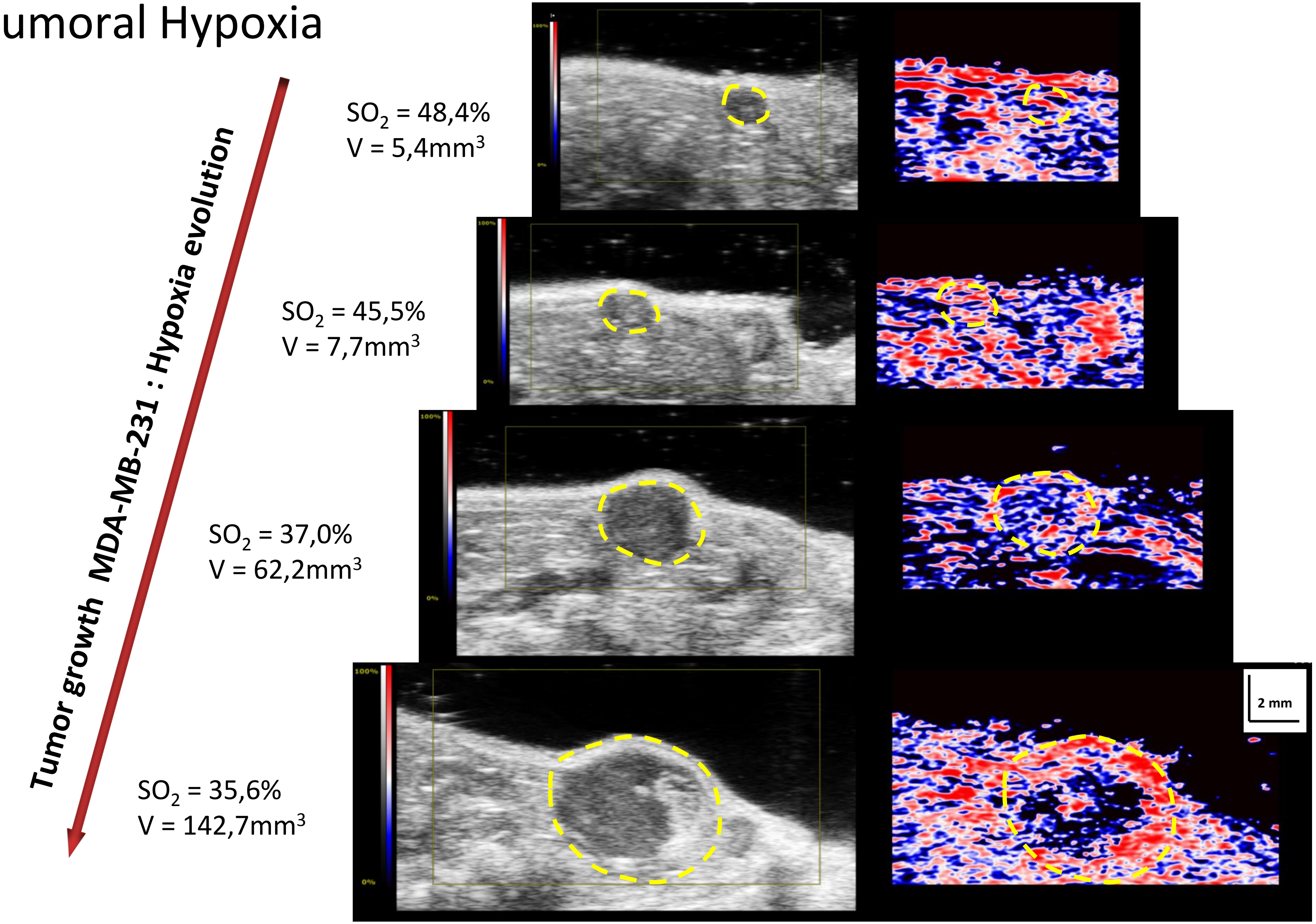
Images are acquired at both **750**nm and **850**nm, an algorithm is applied to create a spectroscopic image of oxygen saturation in real time

What Photoacoustic can do



Medical Air | 5% Oxy. | Med. Air

Evolution of the Intratumoral Hypoxia



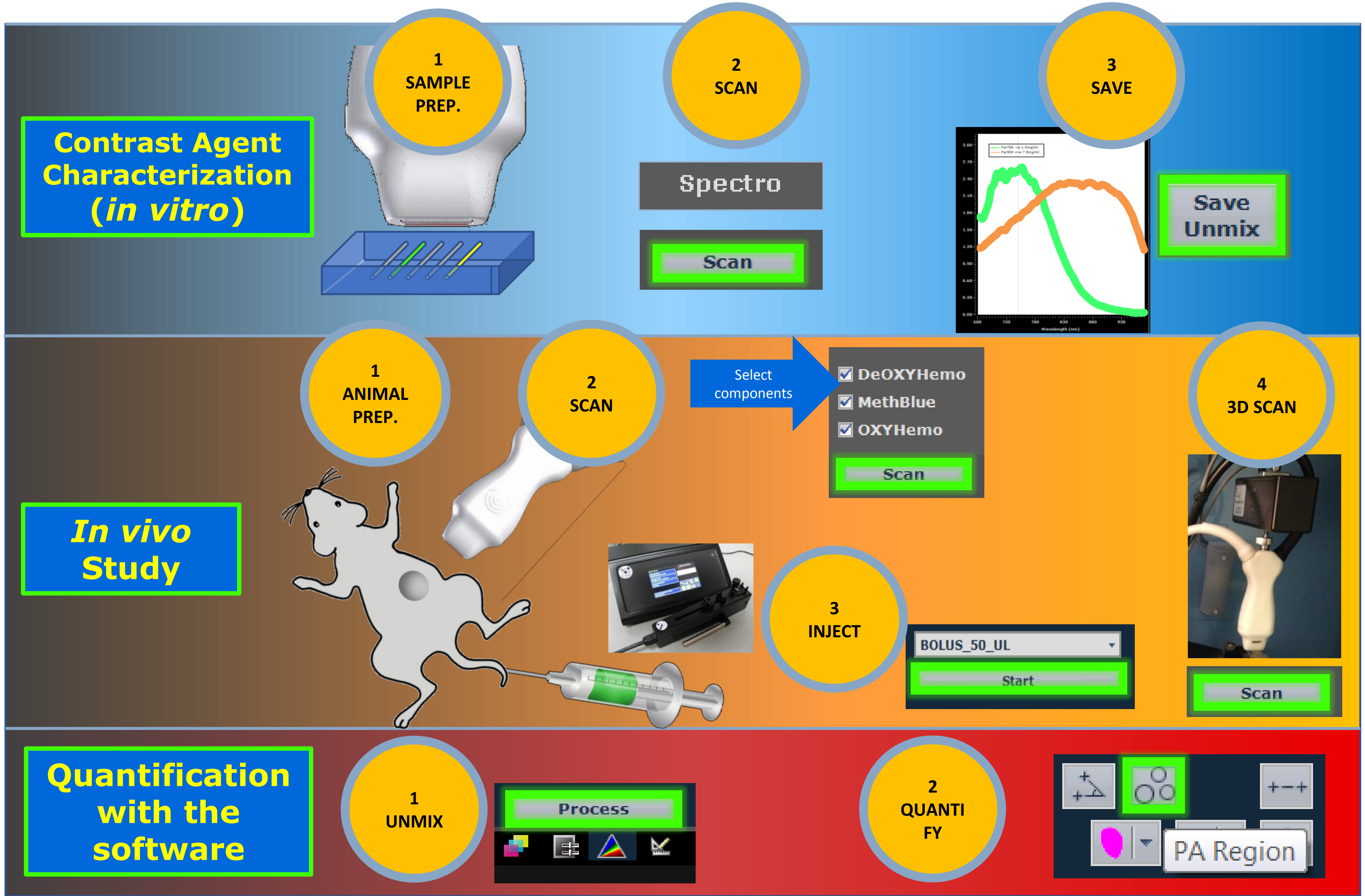
Nanoparticle Tracking with PA analysis

Exogenous PA Contrast Agents

Table 1. Contrast agents for photoacoustic imaging.

Photoacoustic Contrast Agent	Type	Absorption Peak (nm)	Size (nm)	Modification Application	Application	Ref.
Indocyanine-green	NIR Fluorescent Dye	810	<2	CarbonNanotube, PEG, PEBBLEs	PAT, in tissue phantoms and *in vivo*	[7,16–19]
Methylene blue	NIR Fluorescent Dye	650–700	<2		PAT, in tissue phantoms	[15]
Alexa Fluor 750	NIR Fluorescent Dye	750	<2		Multispectral PAI, *in vivo*	[8,9]
IRDye800CW	NIR Fluorescent Dye	750–800	<2	NPR-1	PAS, *in vivo*	[13]
IRDye800-c(KRGDf)	NIR Fluorescent Dye	750–790	<2	Integral proteinαvβ3	PAS, *in vivo*	[20]
Evans Blue	NIR Fluorescent Dye	550	<2		PAT, *in vivo*	[10]
PPCy-C8	NIR Fluorescent Dye	754–789	<2	Perfluorocarbon	*In vivo*, dual-modality PAI-FI	[21]
Cypate-C18	NIR Fluorescent Dye	754–790	<2	Perfluorocarbon	*In vivo*, dual-modality PAI-FI	[21]
Caspase-9 Probe	NIR Fluorescent Dye	640	<2		PAI, *in vivo*	[11]
MMPSence™ 680	NIR Fluorescent Dye	620, 680	<2		PAI, in tissue phantoms	[14]
BHQ3	Quencher	672	<2		PAI, *in vitro*	[12]
QXL680	Quencher	680	<2		PAI, *in vitro*	[12]
Au Nanospheres	Plasmonic Noble Metal Nanoparticle	520–550	20–80	PEG	PAT, *in vivo*	[22,23]
Au Nanoshells	Plasmonic Noble Metal Nanoparticle	700–1100	50–500	PEG	PAT, *in vivo*	[24,25]

Molecular Imaging Workflow (PA)

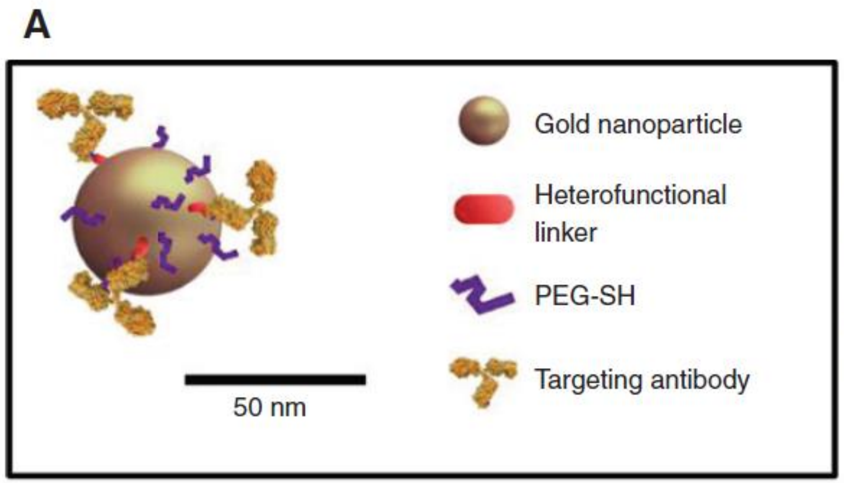


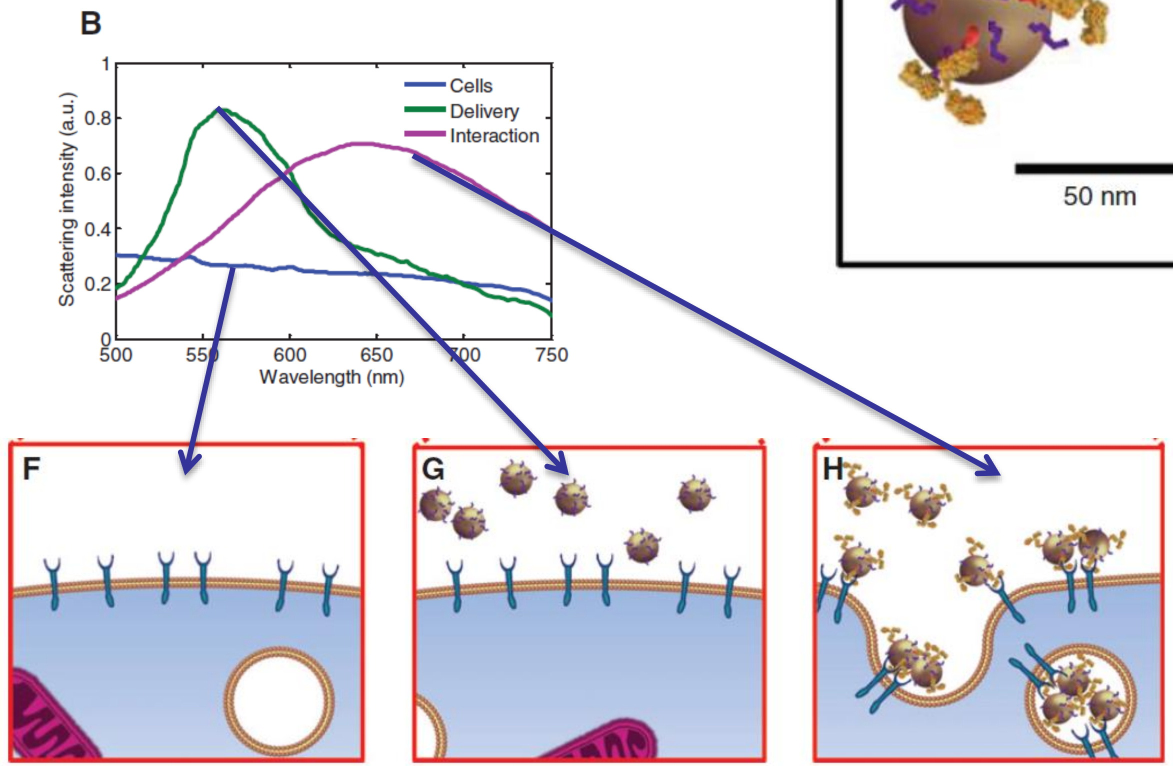
Molecular targeting

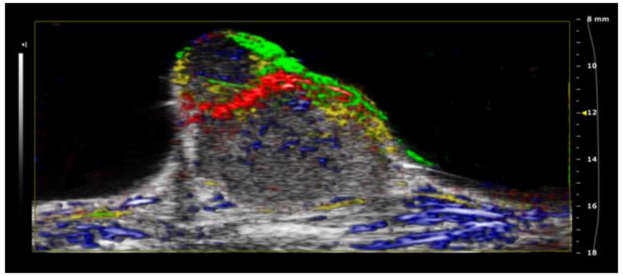
Biodistribution

Half-life

Targeted gold nanospheres





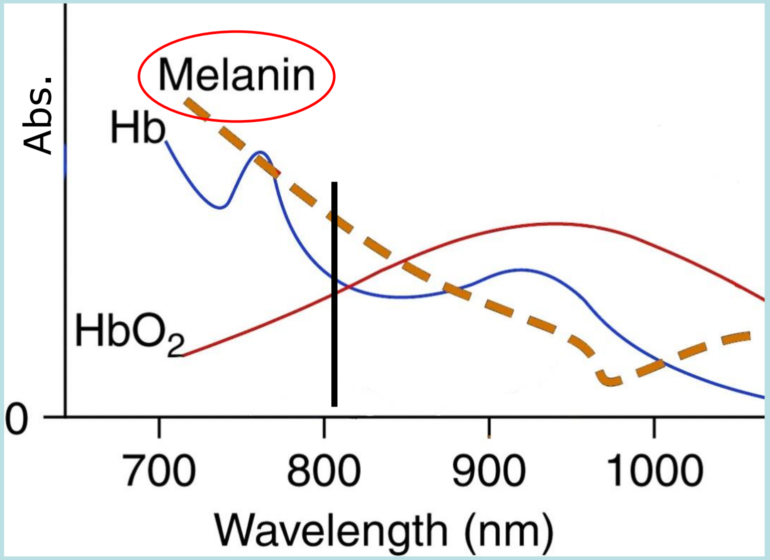



THANKS FOR YOUR ATTENTION
ANY QUESTIONS?... NO?...
GREAT!

Reporter gene imaging with Photoacoustics

- **Tyrosinase**, the enzyme responsible for **melanin** production was used as an inducible reporter
- Animals with TYR- and TYR+ tumors on each flank were imaged before and after induction of tyrosinase expression

Absorption spectra for hemoglobin and melanin



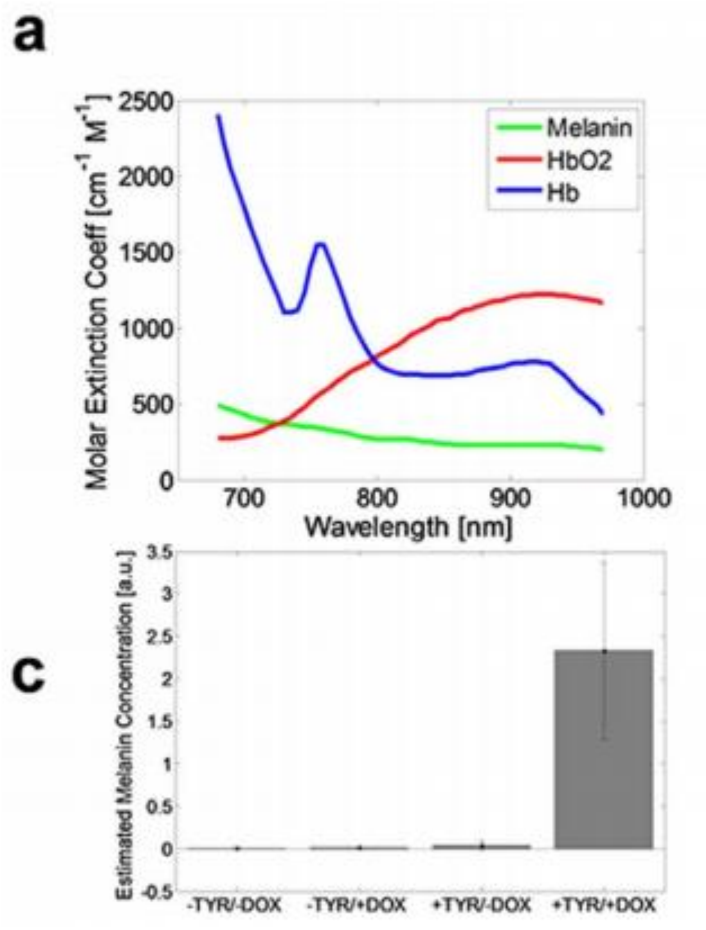
What Photoacoustic can do

SCIENTIFIC REPORTS

Multi-wavelength photoacoustic imaging of inducible tyrosinase reporter gene expression in xenograft tumors

Robert J. Paproski[1], Andrew Heinmiller[2], Keith Wachowicz[3] & Roger J. Zemp[1]

[1]Department of Electrical and Computer Engineering, University of Alberta, Edmonton, Alberta T6G 2V4, Canada, [2]FUJIFILM VisualSonics, Inc., Toronto, Ontario M4N 3N1, Canada, [3]Department of Oncology, University of Alberta, Edmonton, Alberta T6G 1Z2, Canada.



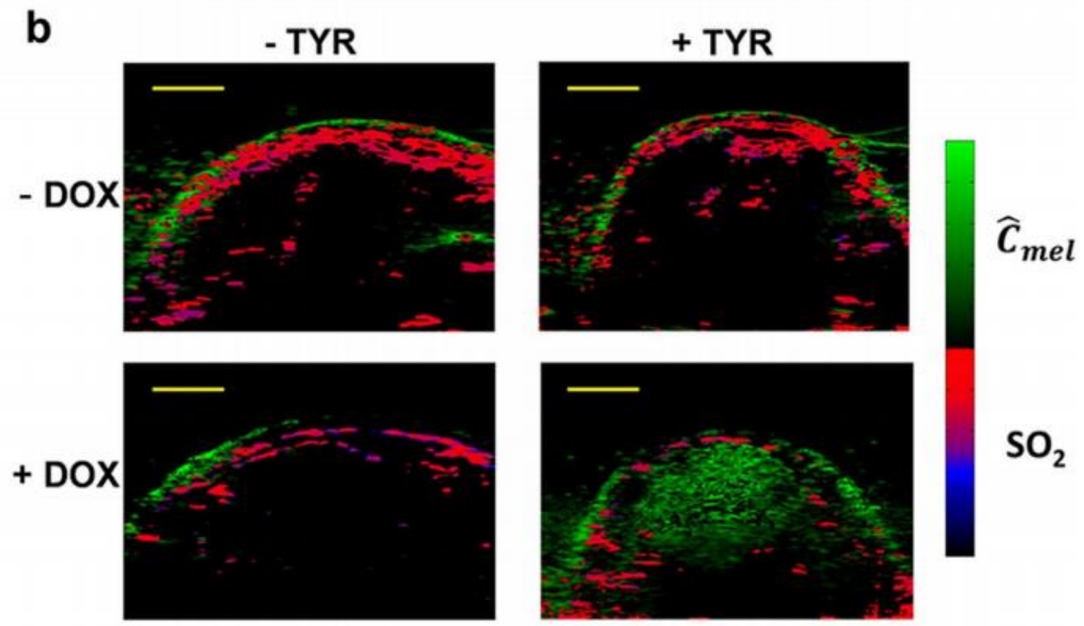


Figure 5 | Multispectral photoacoustic imaging of -TYR and +TYR tumors. (a) Molar extinction spectra of eumelanin monomers, oxy-hemoglobin (HbO_2) and deoxy-hemoglobin (Hb). (b) Multispectral photoacoustic imaging of -TYR and +TYR tumors. The green colormap represents estimated melanin concentration while the red-to-blue colormap is hemoglobin oxygen saturation. Scale bars represent 2 mm. (c) Quantitation of estimated relative melanin concentration levels using multispectral photoacoustic imaging.