

# Imagerie harmonique & Produits de contraste

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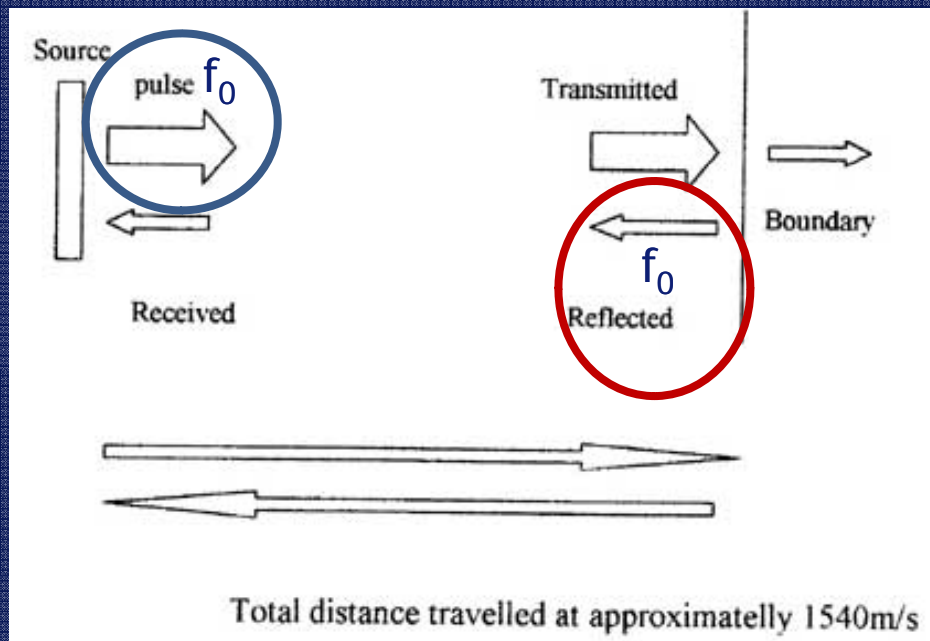
- Interaction bulles-Ultrasons
- Imagerie harmonique de contraste

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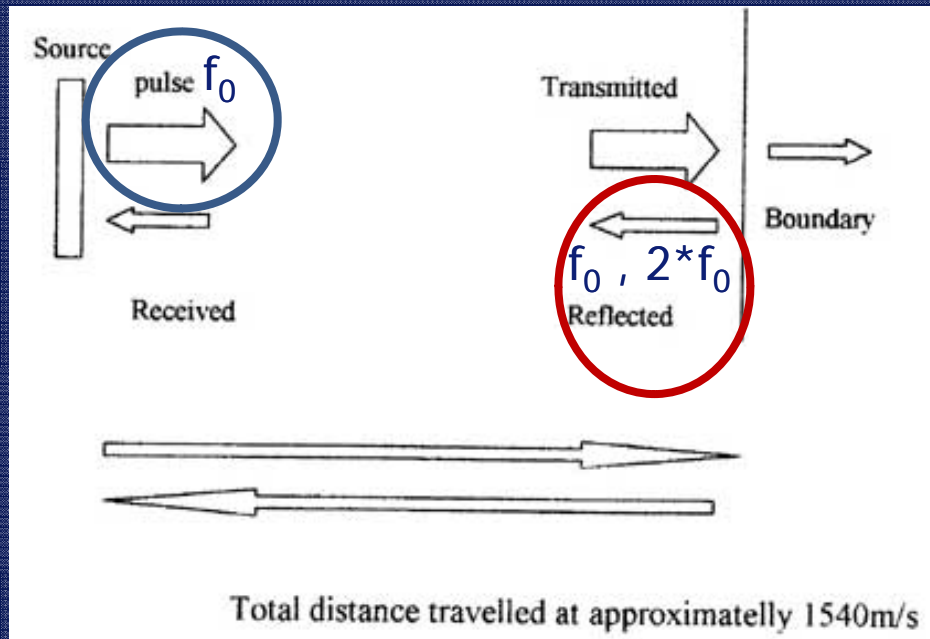
# Formation de l'image et Imagerie conventionnelle (fondamentale)

- ➡ Ondes ultrasonores émises à la fréquence de la sonde ( $f_0$ )
- ➡ Echos réfléchis à la fréquence ( $f_0$ )
- ➡ Image reconstruite à la fréquence ( $f_0$ )



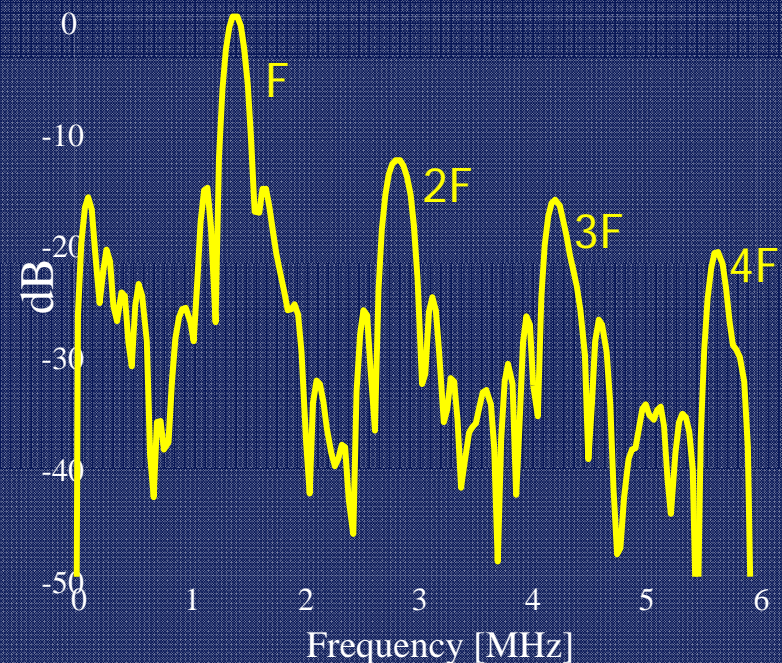
# Formation de l'image et Imagerie harmonique (non linéaire)

- ➡ Ondes ultrasonores émises à la fréquence de la sonde ( $f_0$ )
- ➡ Echos réfléchis sont composés de deux fréquences :  $f_0 + 2 * f_0$
- ➡ Image reconstruite à 2 fois la fréquence émise :  $2 * f_0$



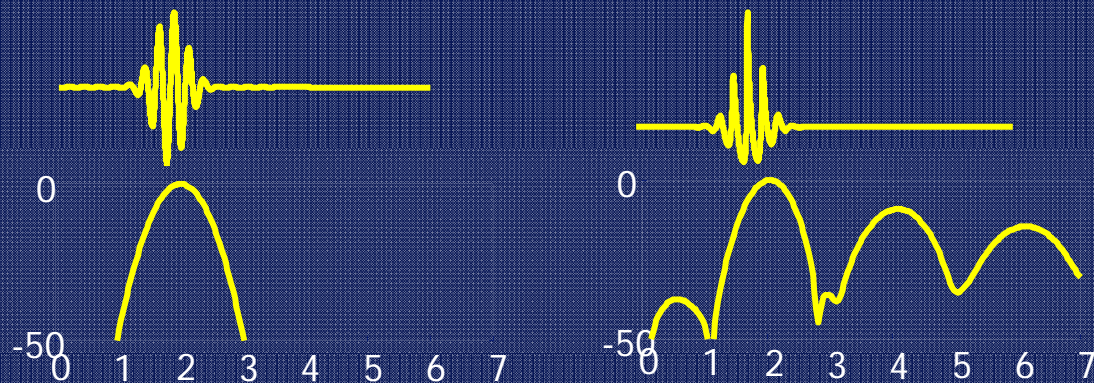
# Formes de l'imagerie harmonique

➔ **Imagerie harmonique de contraste:** Les microbulles réémettent (réflexion) de l'énergie aux fréquences harmoniques (non linéaires), à des fréquences multiples de la fréquence d'émission.



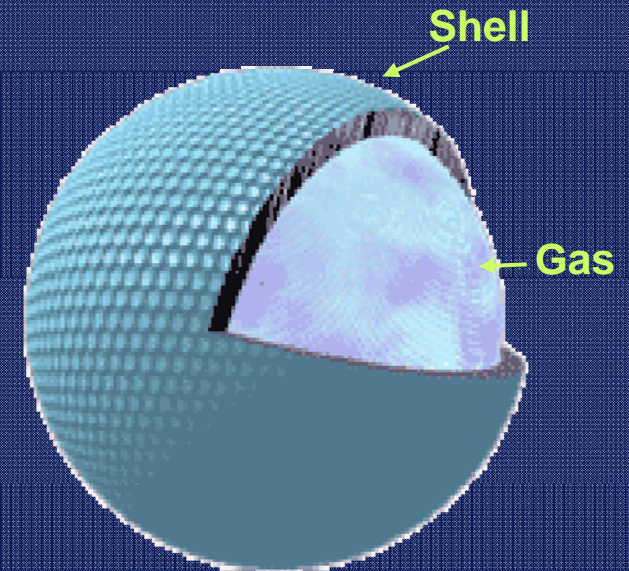
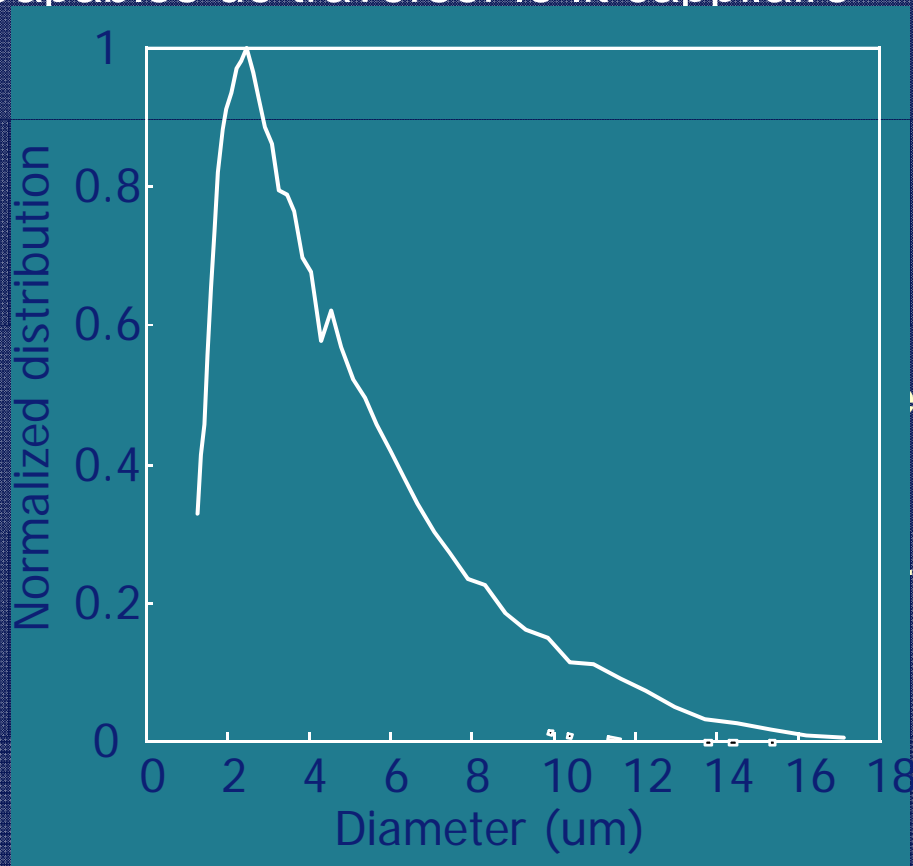
# Formes de l'imagerie harmonique

- ➡ **Imagerie harmonique tissulaire:** Des composantes fréquentielles harmoniques (non linéaires) sont générées lors de la propagation dans les tissus.



# Agents de contraste US

- Suspension de microbulles de gaz pour injection intraveineuse.
- Capables de traverser le lit capillaire

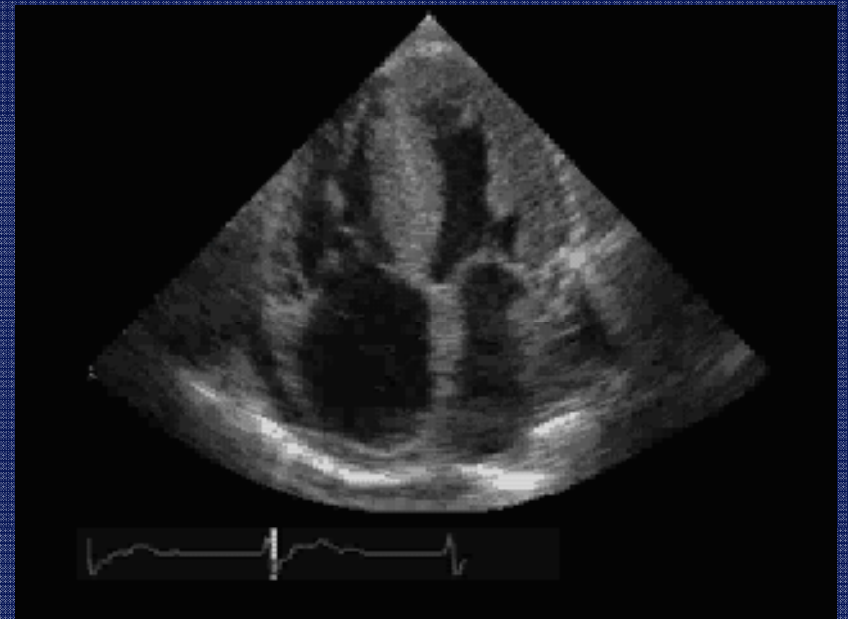


# Agents de contraste homologués

Company	Agent	Gas	Shell	Where
Bracco	<b>Sonovue</b>	<i>Sulfur hexafluoride</i> SF <sub>6</sub>	Phospholipid	Europe/China
Bracco	Lumason	<i>Sulfur hexafluoride</i> SF <sub>6</sub>	Phospholipid	USA
Dupont (BMS)	Definity	<i>Octafluoropropane</i> C <sub>3</sub> F <sub>8</sub>	Liposome	USA/CA
Mallinckrodt	Optison	<i>Octafluoropropane</i> C <sub>3</sub> F <sub>8</sub>	Albumin	USA/CA
Nycomed (GE)	Sonazoid	<i>Perfluorobutane</i> C <sub>4</sub> F <sub>10</sub>	Phospholipid	Japan

# Pourquoi un agent de contraste US?

- Le sang réfléchit très peu les ultrasons
- Les vaisseaux sanguins ont un fort coefficient de réflexion
- Aux fréquences ultrasonores, les petits vaisseaux ( $<2$  mm) ne sont pas visibles



# Pourquoi un agent de contraste US?

## Pour amplifier l'écho en provenance de la circulation sanguine

- L'écho US (image) est basé sur la différence d'impédances acoustiques
- L'amplitude de l'écho est proportionnelle à la différence d'impédances acoustiques
  - $Z = \rho c$ ;  $\rho$ =densité,  $c$ =vitesse de propagation
  - $Z_{\text{tissus}} \approx 1.55 \times 10^6$  [kg.m<sup>-2</sup> s<sup>-1</sup>]
  - $Z_{\text{air}} \approx 4 \times 10^2$  [kg.m<sup>-2</sup> s<sup>-1</sup>]
- Air-eau (tissus) est le réflecteur idéal des ultrasons

# Interaction bulle-Ultrasons

# MODELE LINEAIRE

## Réflexion passive

$$P_d = I_i \Sigma_p \quad \Sigma_p = \text{Section efficace de diffusion}$$

$$\Sigma_p = \frac{4\pi}{9} k^4 R^6 \left[ \left( \frac{\kappa_p - \kappa}{\kappa} \right)^2 + \frac{1}{3} \left( \frac{3(\rho_p - \rho)}{2\rho_p + \rho} \right)^2 \right]$$

$$\Sigma_p \propto f^4; \quad \Sigma_p \propto R^6$$

$$(R = 1.5 \mu\text{m}, f = 3 \text{ MHz})$$

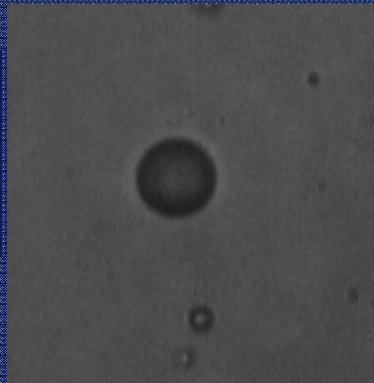
	particule air	Particule métal
$\Sigma_p (\mu\text{m}^2)$	113	$6 \cdot 10^{-7}$

# MODELE LINEAIRE

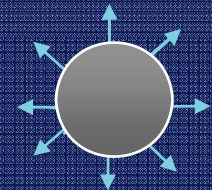
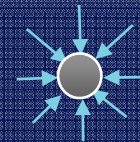
## Réflexion active: vibration & résonance

La bulle agit comme un oscillateur harmonique

US 2MHz ==> 2 millions oscillations / sec



La taille change avec la pression



Pression incidente

Positive

Négative

0.1 – 10  $\mu$ s

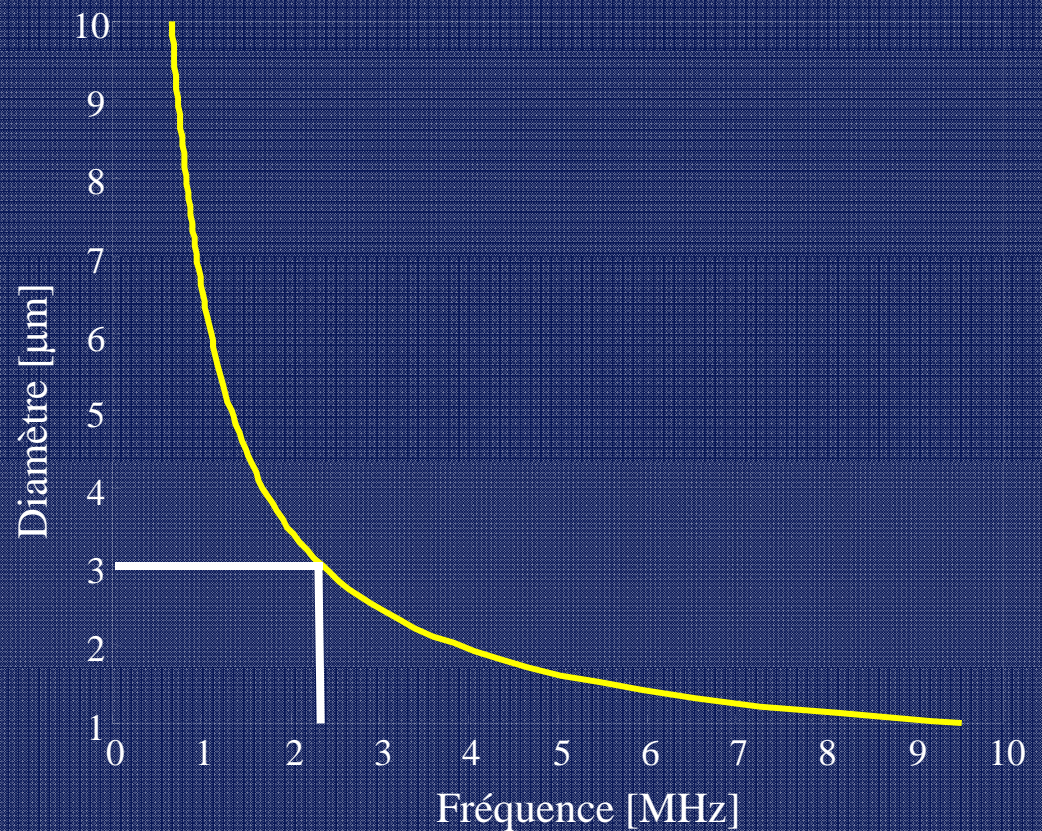


# Fréquence de Résonance

Bulle nue

$$f_o = \frac{1}{2\pi R} \sqrt{\frac{3 \kappa P_o}{\rho}}$$

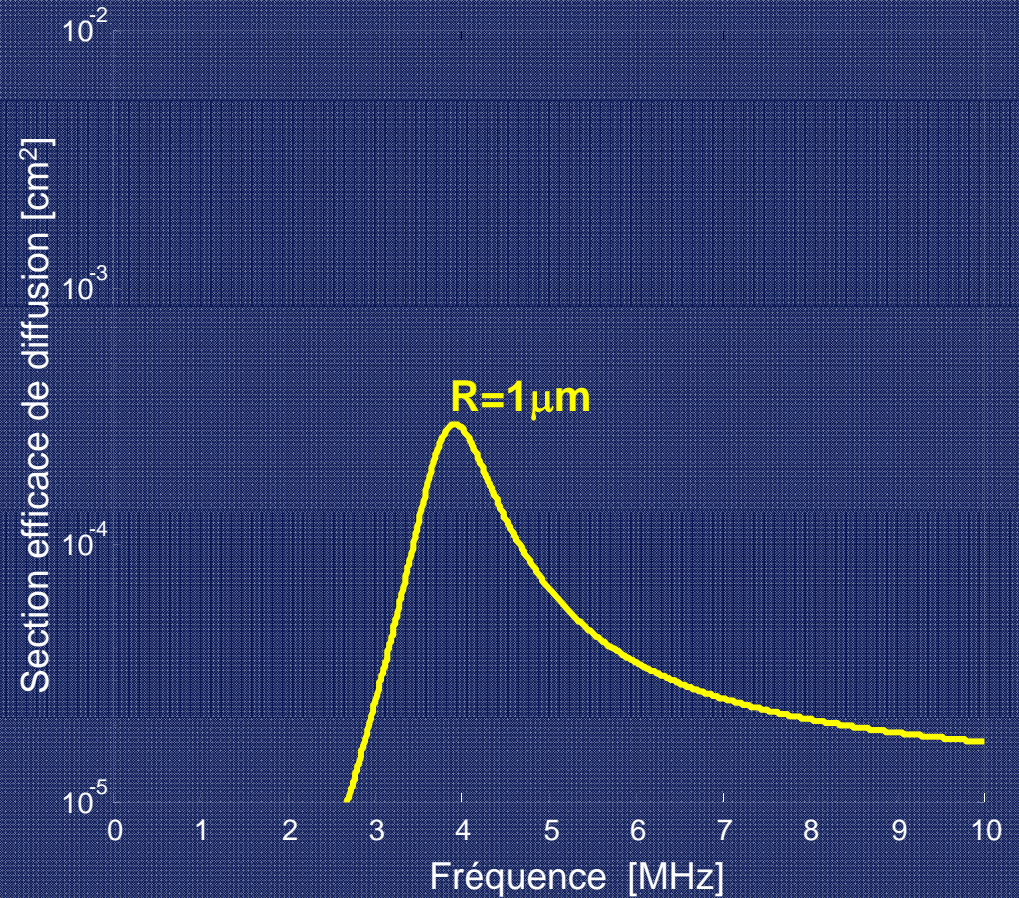
$\Phi = 3 \mu\text{m}$ ,  $f_r = 2.5\text{MHz}$



# Efficacité en réflexion

## Section efficace de diffusion

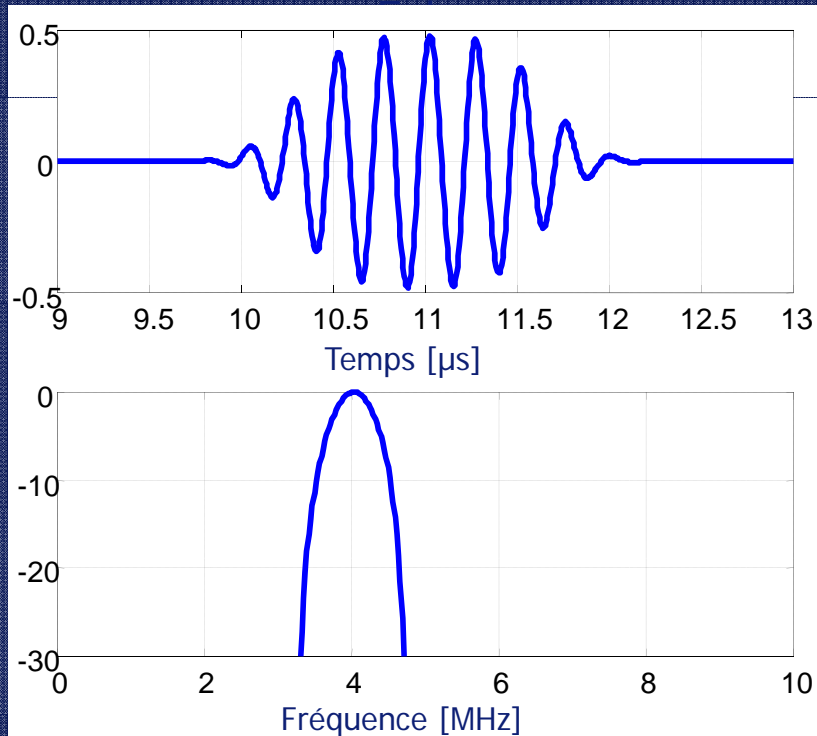
$$\Sigma_p = \frac{P_s}{I_i} = \frac{4\pi R^2}{\left(\frac{f_r^2}{f^2} - 1\right)^2 + \delta^2}$$



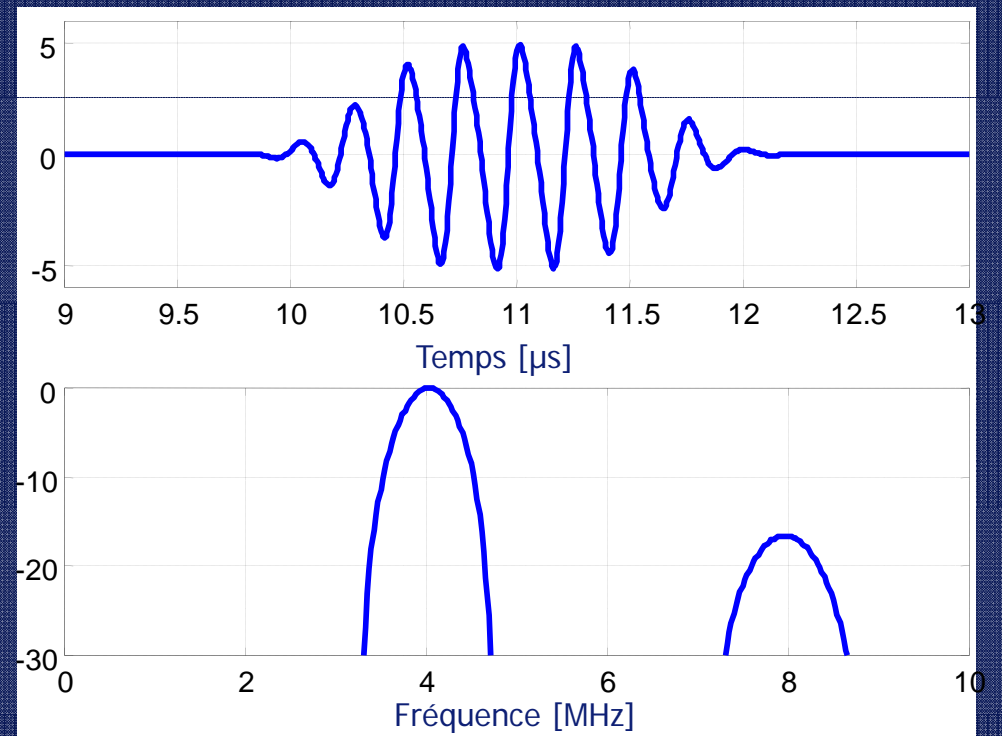
# Modèle non linéaire et Harmoniques

$r=1\mu\text{m}$ ;  $f=f_0$  [4MHz]

Amp=5kPa

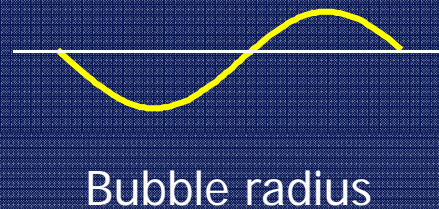
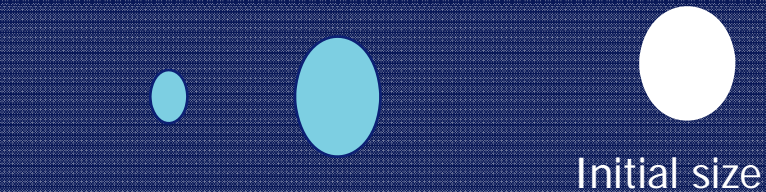
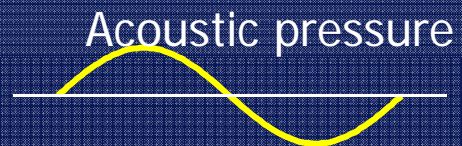


Amp=50kPa

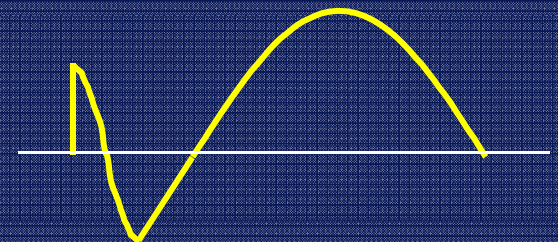
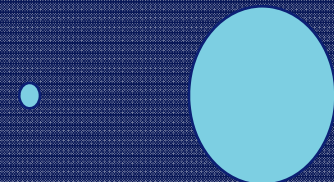
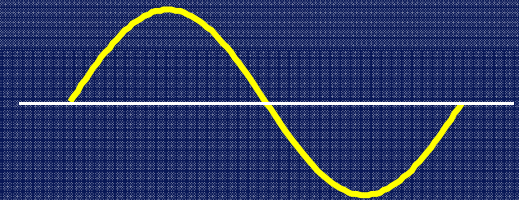


# Harmoniques

Linear oscillation:  
Fundamental imaging



Nonlinear oscillation  
Harmonic imaging



# Caractérisation optique: régime linéaire

US:  $f=1.8\text{MHz}$ ,  $50\text{kPa}$  ( $\text{IM}<0.1$ )

Optic: Frame rate  $16\text{MHz}$

Soft-shelled agent (Sonovue Bracco, Italy)

Soft-shelled agent

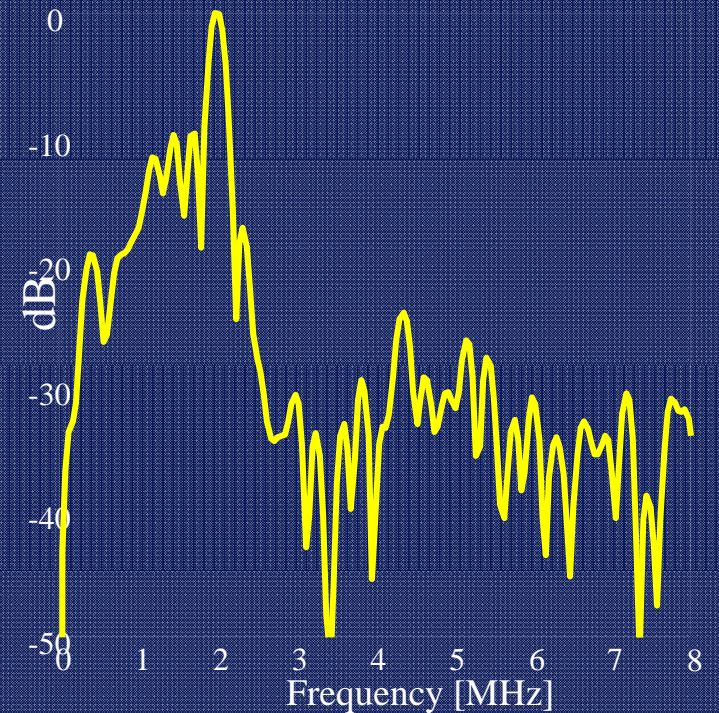
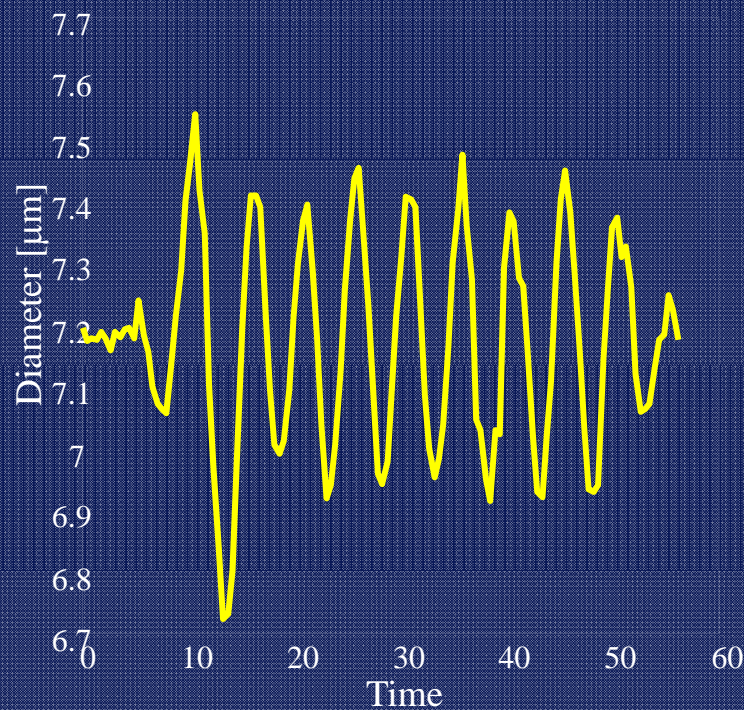


# Caractérisation optique: régime linéaire

US:  $f=1.8\text{MHz}$ ,  $50\text{kPa}$  ( $\text{IM}<0.1$ )

Optic: Frame rate  $16\text{MHz}$

Soft-shelled agent (Sonovue Bracco, Italy)

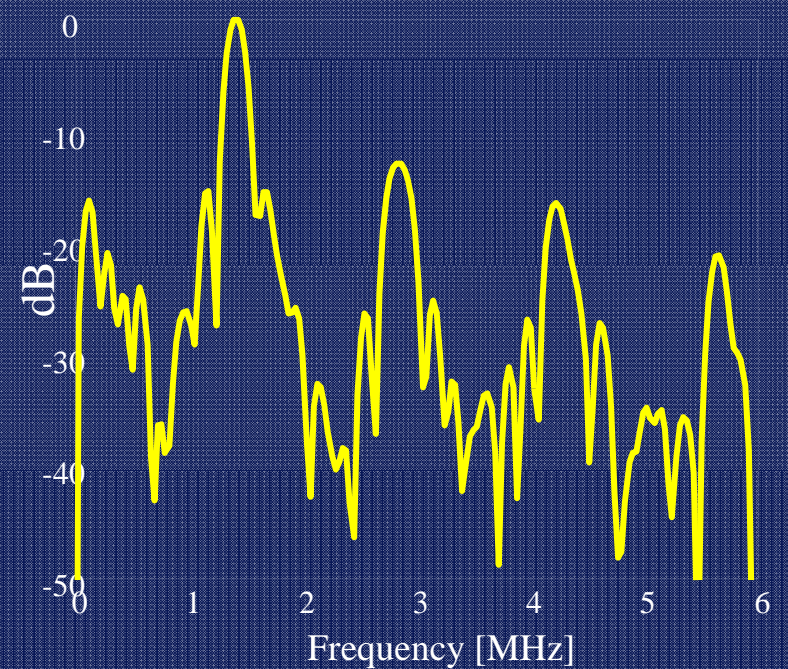
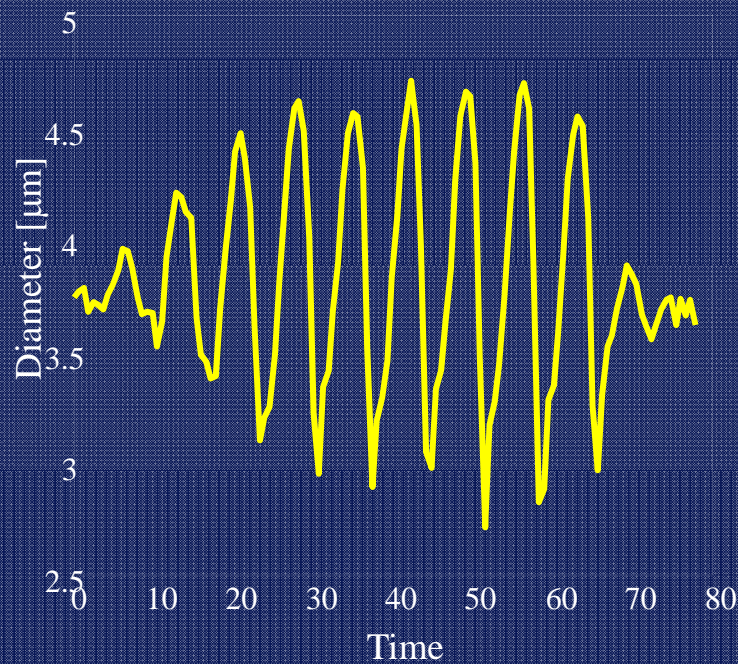
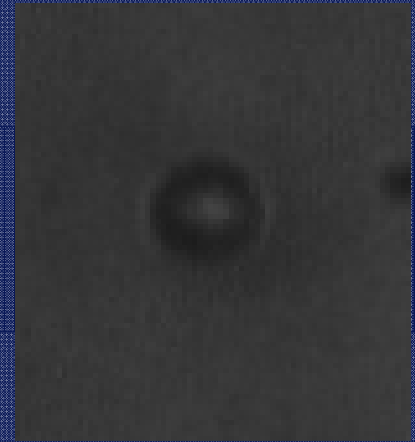


# Caractérisation optique: régime non linéaire

US:  $f=1\text{MHz}$ ,  $200\text{kPa}$  ( $\text{IM}=0.2$ )

Optic: Frame rate  $16\text{MHz}$

Soft-shelled agent (Sonovue Bracco, Italy)



# Caractérisation optique: destruction

US:  $f=1.7\text{MHz}$ ,  $1.7\text{MPa}$  ( $MI=1.3$ )

Optic: Frame rate  $14.69\text{MHz}$

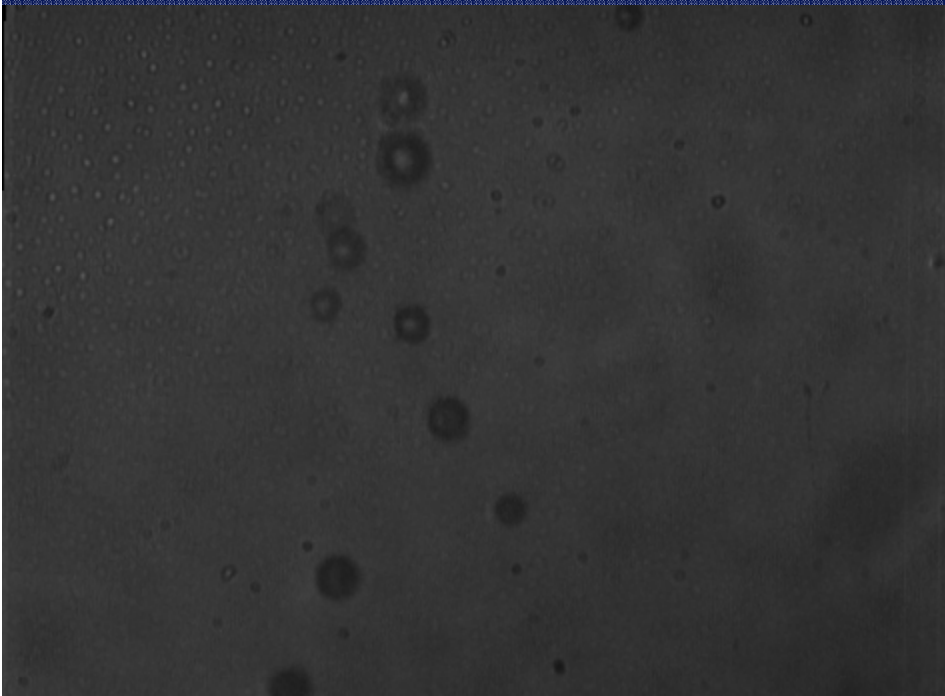


# M1212

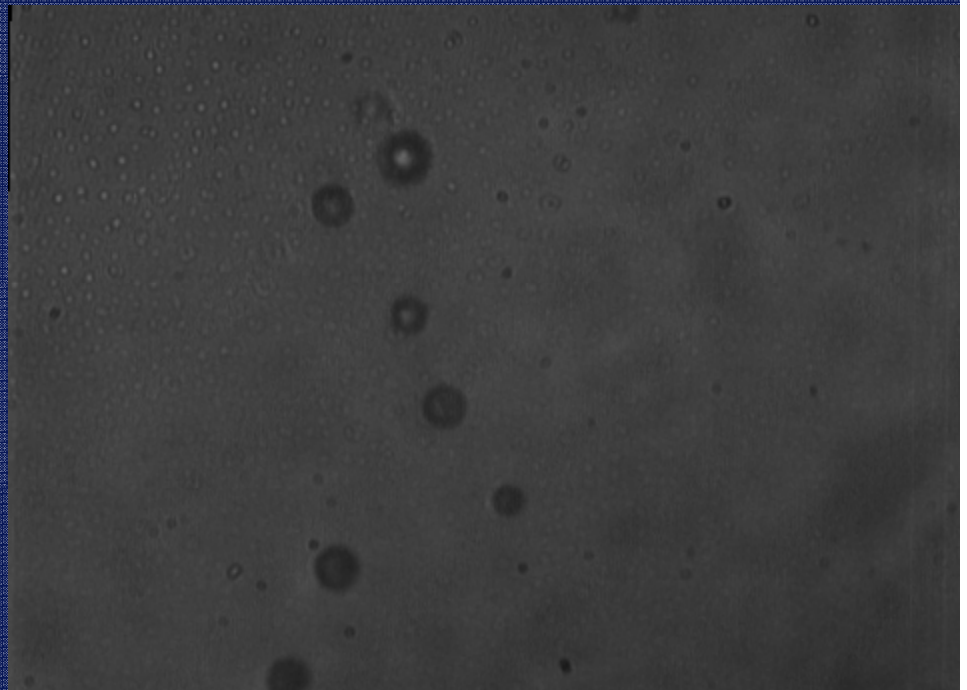
M1212 Hard shell bubble

1MPa (MI=0.8),  $f=1.7\text{MHz}$ , frame rate $\approx 15\text{Mfps}$

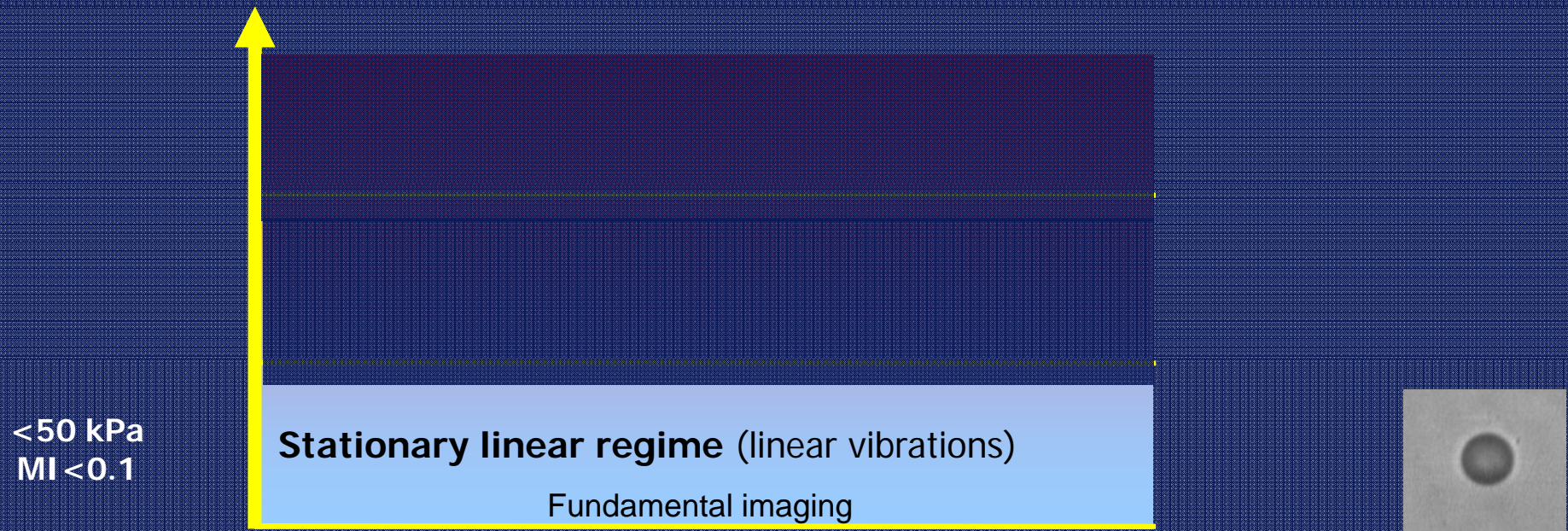
1<sup>st</sup> US pulse



2<sup>nd</sup> US pulse (100ms later)



# Microbubble – Ultrasound interaction

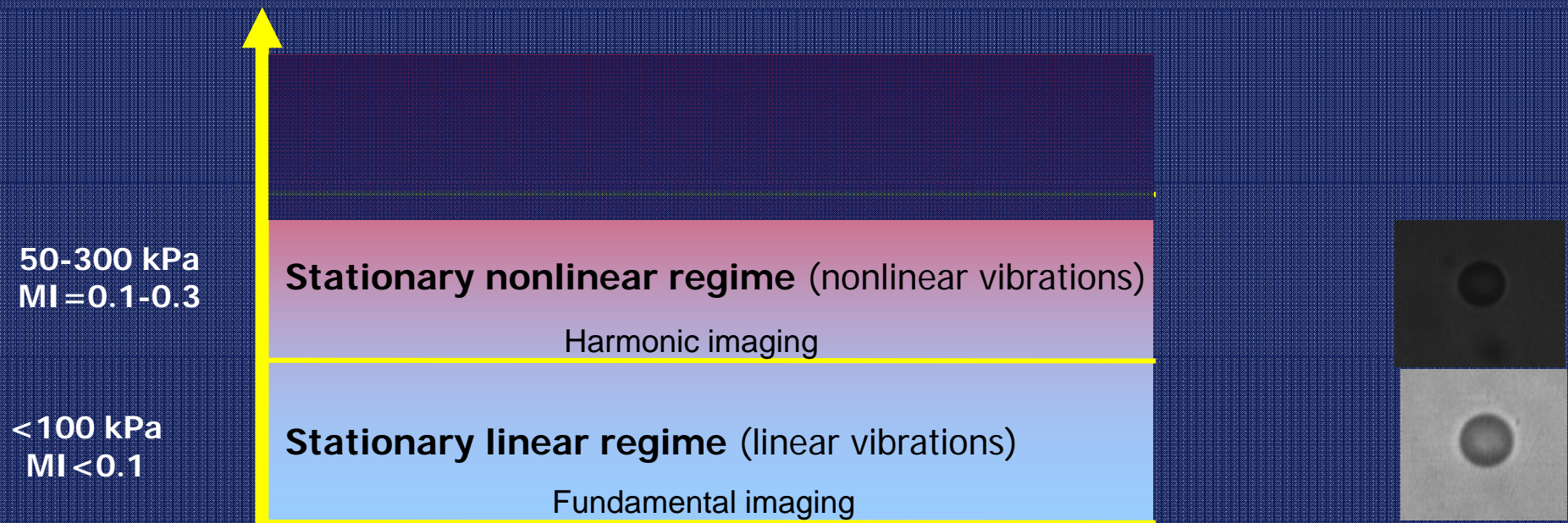


$$MI = \frac{P_r \text{ (MPa)}}{\sqrt{f \text{ (MHz)}}}$$

**Mechanical index**

$$MI = 0.1 - 1.9$$

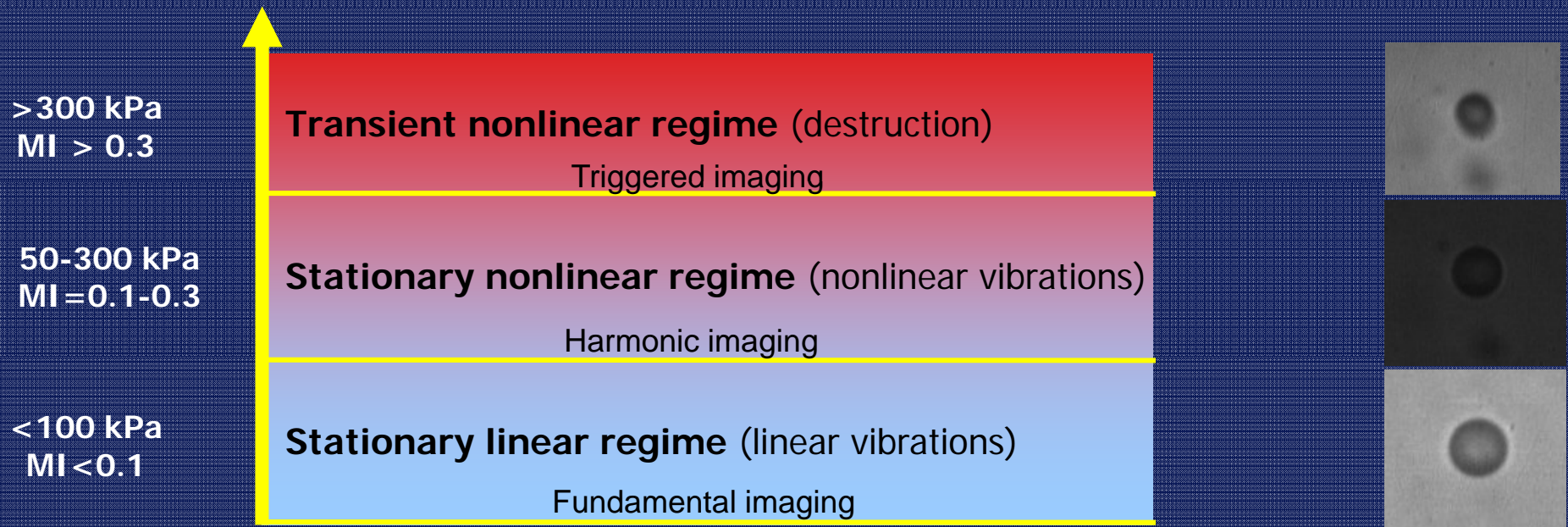
# Microbubble – Ultrasound interaction



$$MI = \frac{P_- (MPa)}{\sqrt{f (MHz)}}$$

$$MI = 0.1 - 1.9$$

# Microbubble – Ultrasound interaction



$$MI = \frac{P_- (MPa)}{\sqrt{f (MHz)}}$$

$$MI = 0.1 - 1.9$$

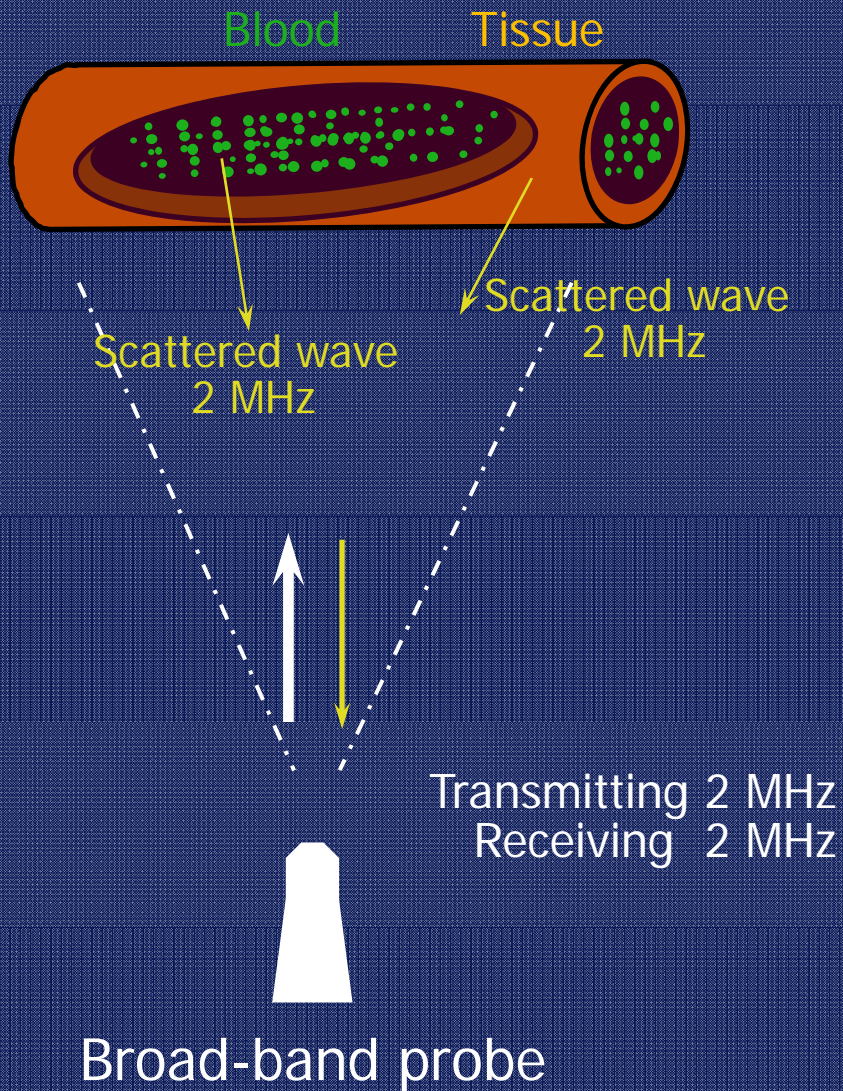
# Contrast Imaging Methods

# Méthodes d'imagerie de contraste

- Second harmonic imaging (filtering)
- Pulse inversion imaging (Pulse inversion Doppler)  $\Rightarrow$  ATL (Philips)
- Power modulation imaging  $\Rightarrow$  Agilent (Philips)
- Pulse inversion/power modulation
- Contrast Pulse Sequence (CPS)  $\Rightarrow$  Acuson (Siemens)

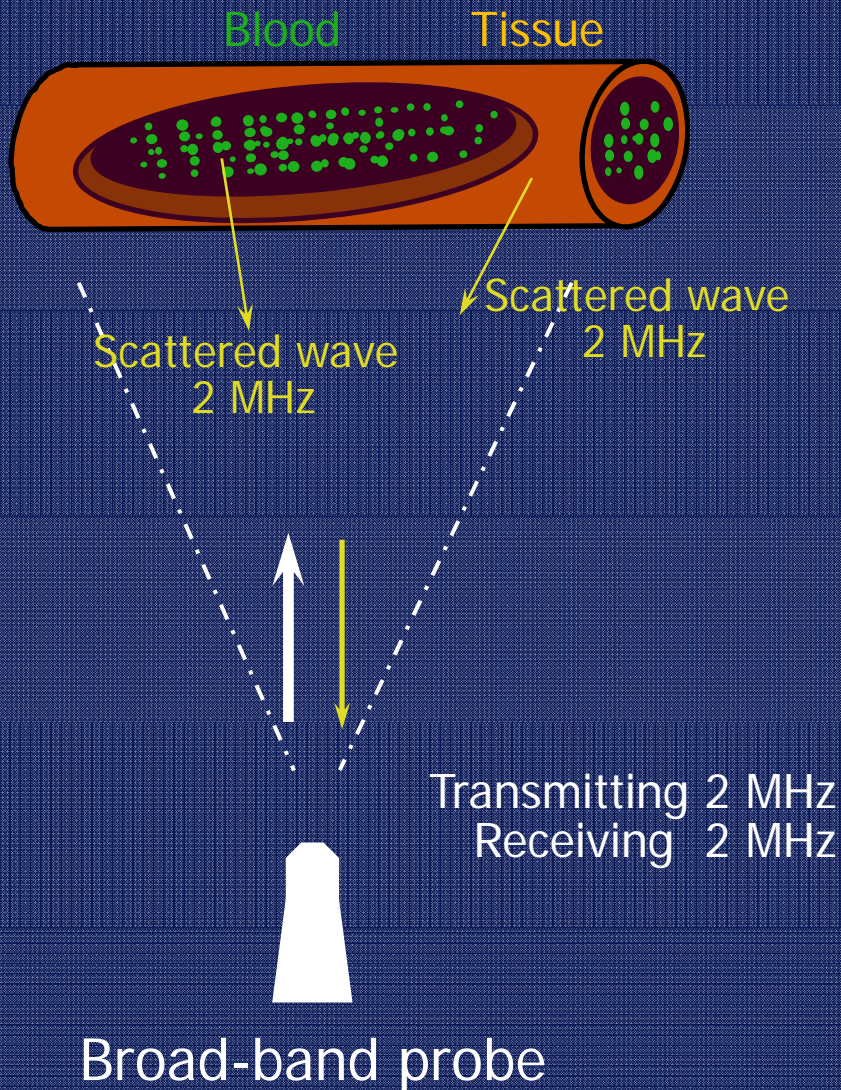
# Principe de l'imagerie harmonique de contraste

## Fundamental Imaging

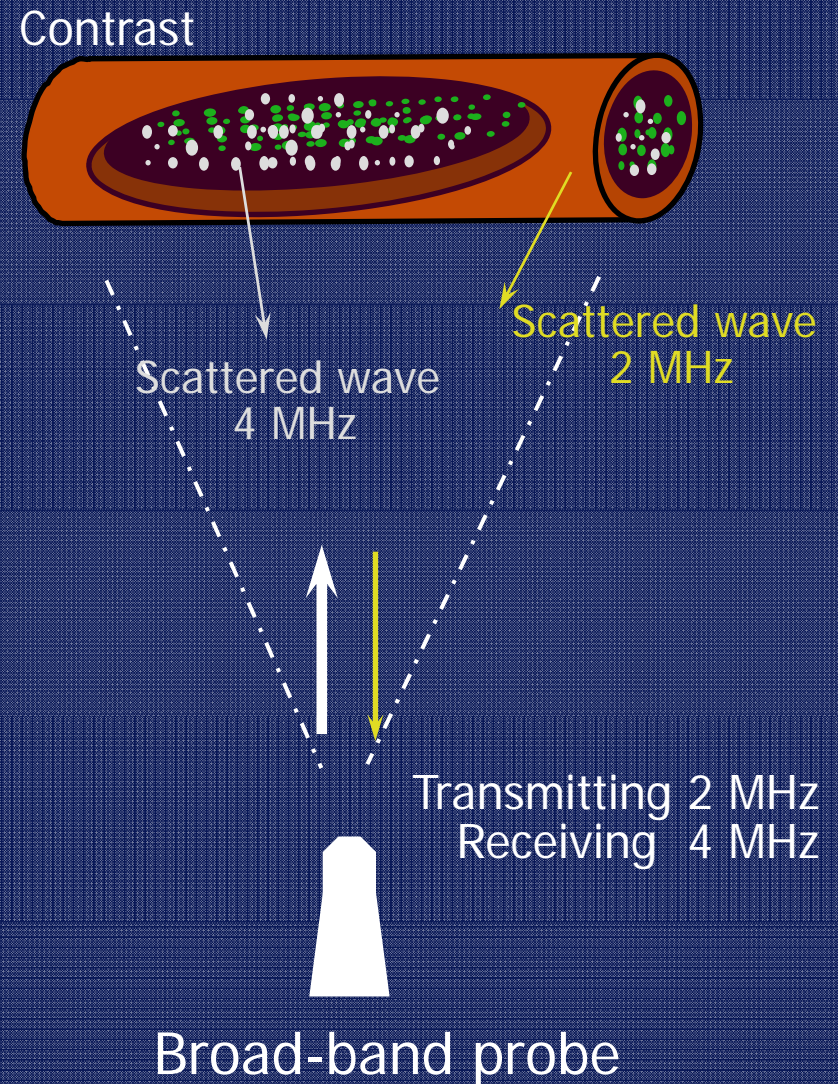


# Principe de l'imagerie harmonique de contraste

## Fundamental Imaging

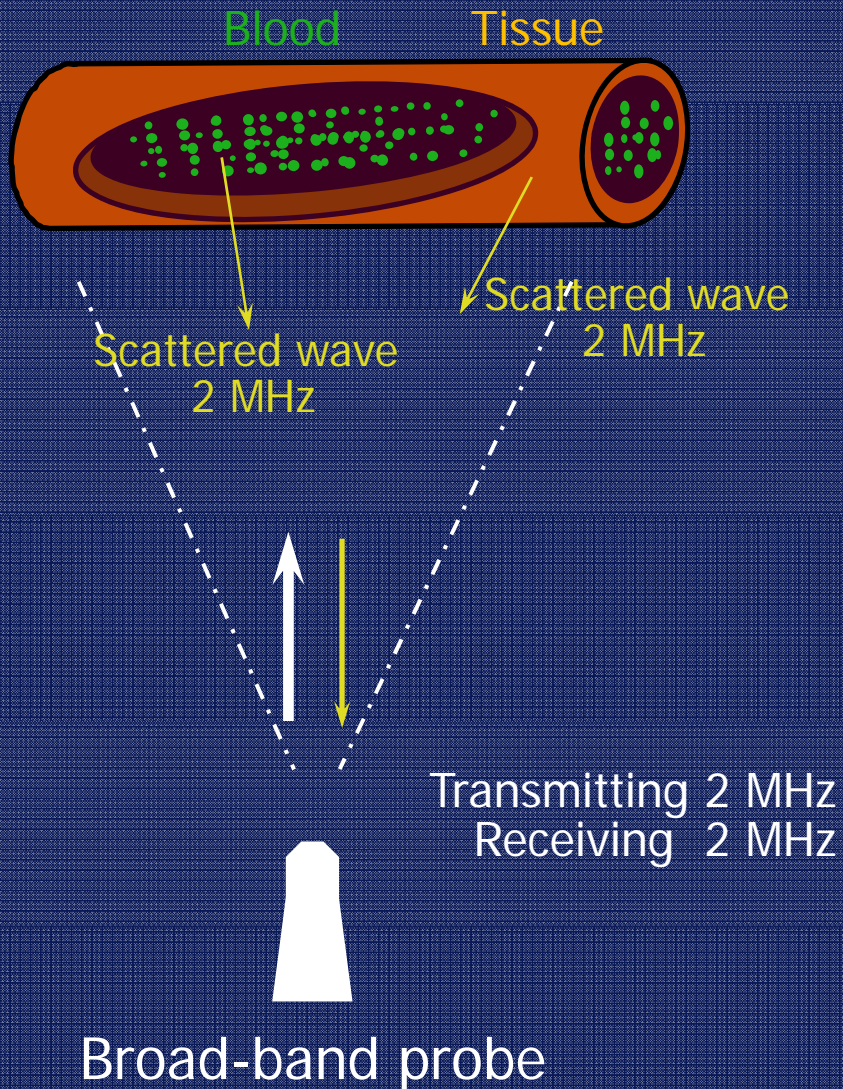


## Contrast Harmonic Imaging



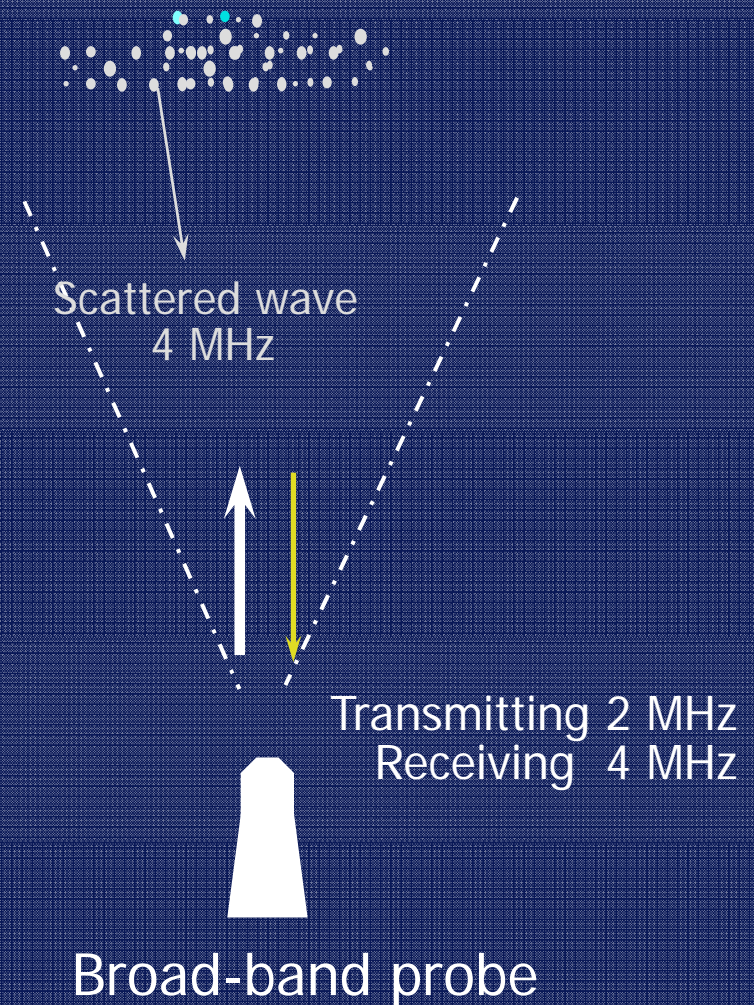
# Principe de l'imagerie harmonique de contraste

## Fundamental Imaging



## Contrast Harmonic Imaging

Contrast

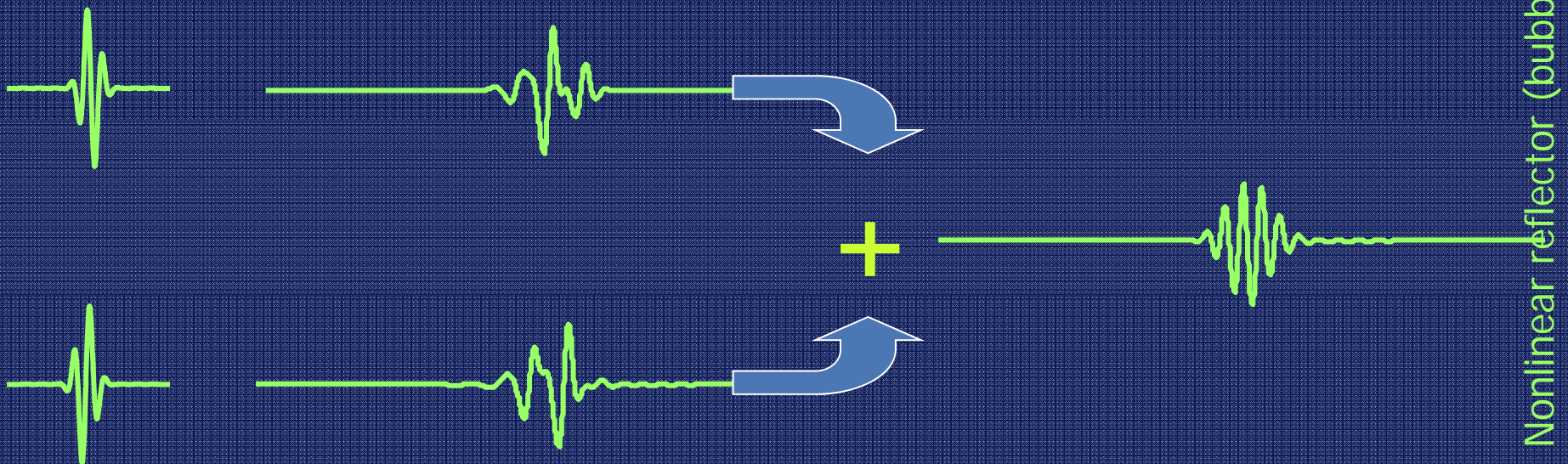
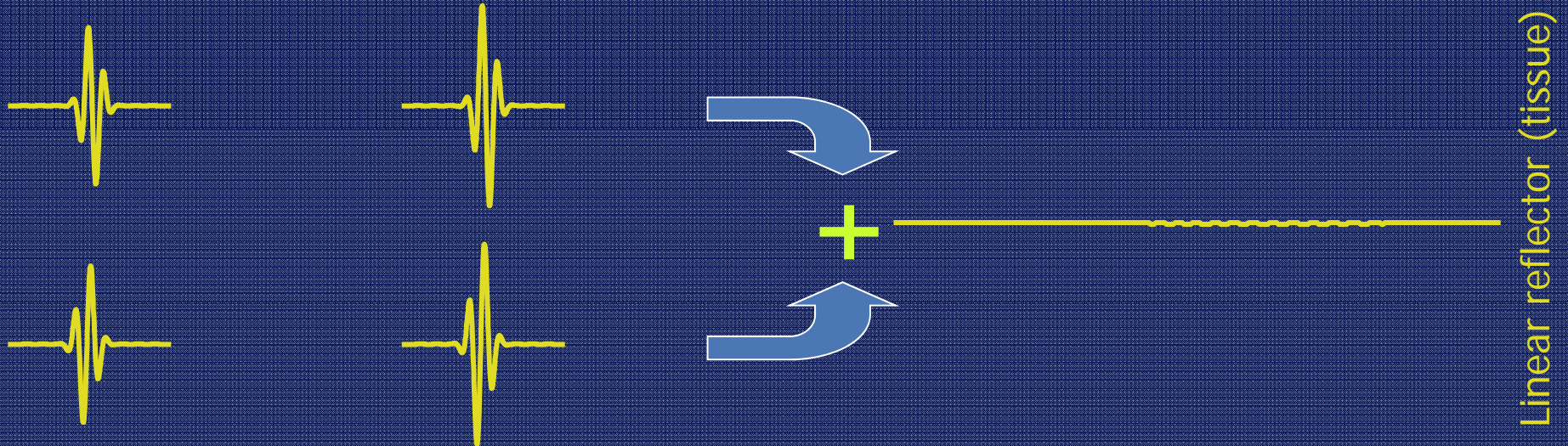


# PULSE INVERSION

transmit

receive

Display



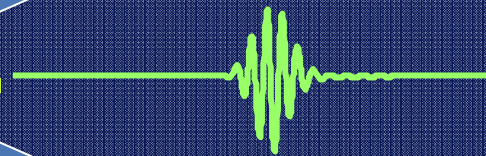
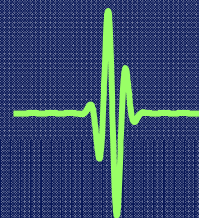
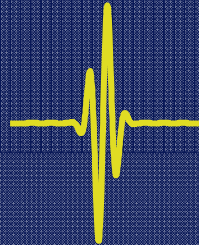
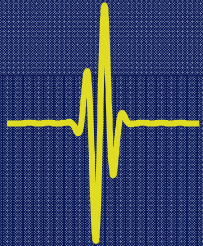
# POWER MODULATION

**transmit**

**receive**

**Scale x2**

**Display**



Linear reflector (tissue)

Nonlinear reflector (bubble)

# Microbulles en imagerie

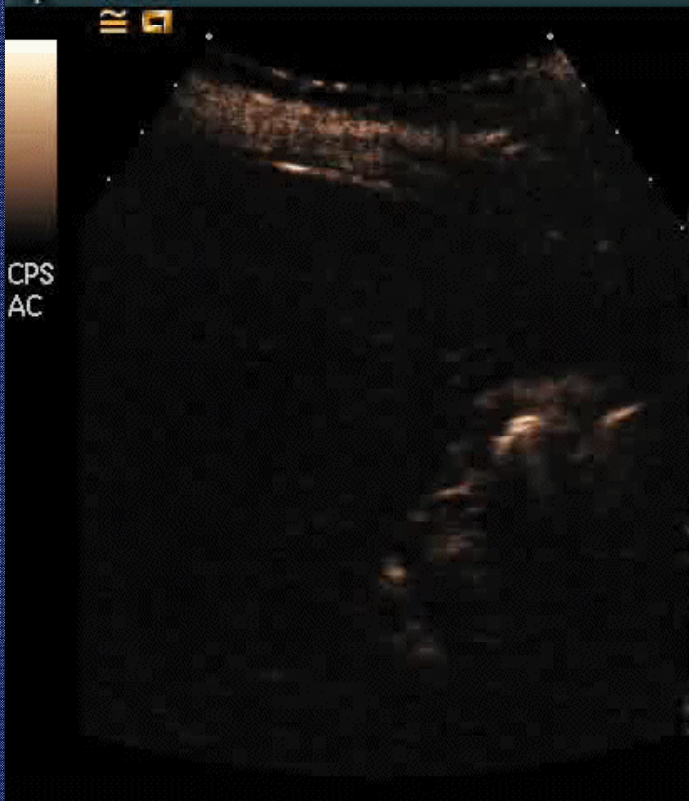
## What do we need contrast agent for?

- “Boost” image quality
- Enhance diagnostic confidence
- Clinical applications
  - Estimation of myocardial perfusion
  - Detection and characterization of tumors
  - Amplification of Doppler signal

# Exemples cliniques

## Caractérisation des lésions focales hépatiques

### Contraste Pulse Sequence (CPS)

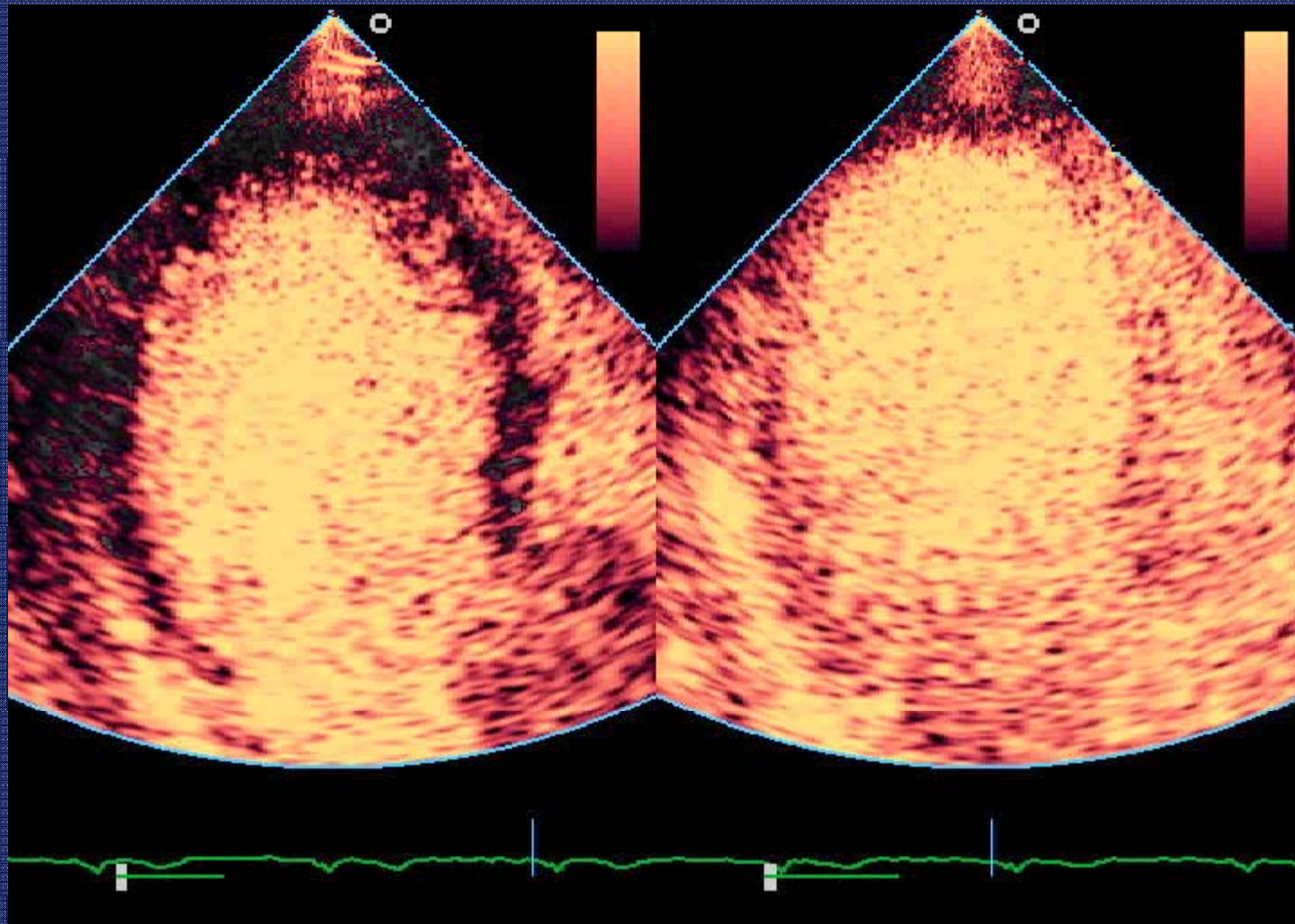


Tranquart, CHU Tours, France

- Chez tout patient avec une lésion hépatique de nature incertaine et particulièrement:
  - La découverte fortuite d'un nodule lors d'une échographie
  - La suspicion ou la découverte d'une lésion en cas d'hépatite chronique ou de cirrhose
  - La suspicion ou la découverte d'une lésion chez un patient porteur de cancer
  - L'absence de certitude diagnostique en TDM/IRM ou histol.

## Exemples cliniques

Cardiologie: estimation de la perfusion myocardique



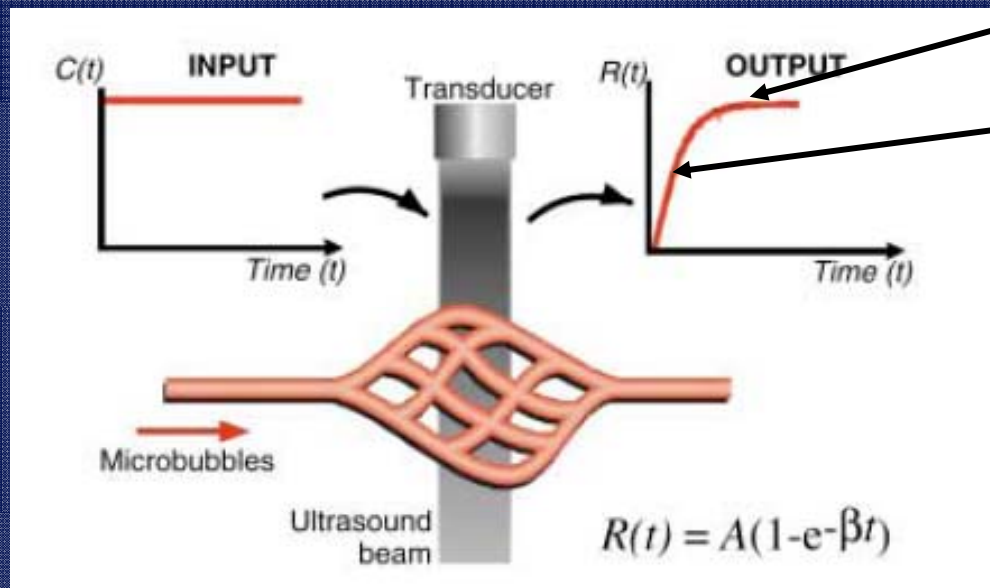
*Courtesy: F. ten Cate, Thorax Center Rotterdam, NL*

# Exemples cliniques

## Cardiologie: estimation de la perfusion myocardique

### Destruction-reperfusion

- Destroying the vast majority of microbubbles and measuring their tissue replenishment in real time to assess blood flow velocity.
- Assessment of myocardial perfusion

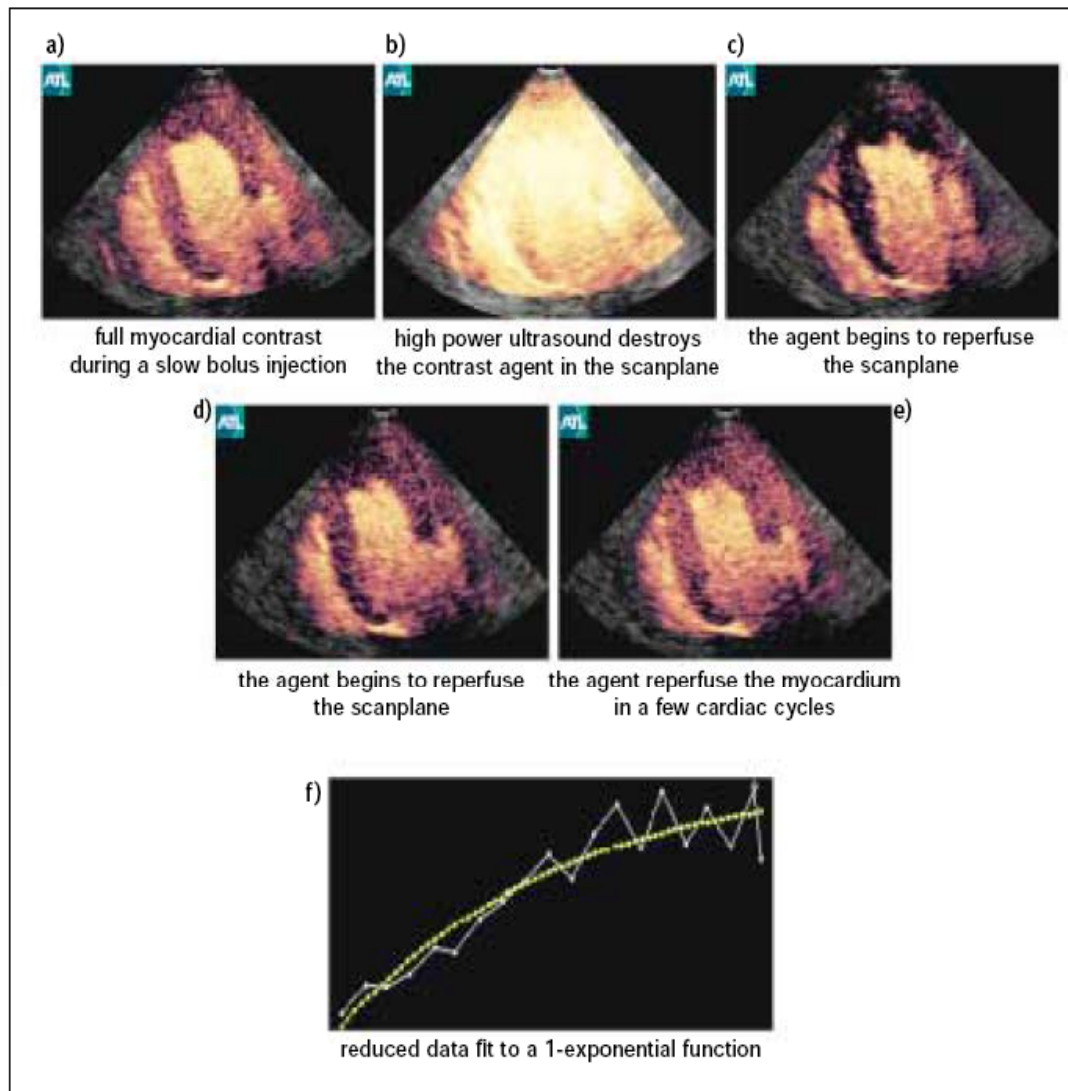


Total number of bubbles present in ROI (vascular volume)  $A$

Rate at which the level is attained reflects the speed (mean velocity)  $\beta$

$A \times \beta = \text{flow (perfusion) rate}$

# Destruction-reperfusion



**Fig. 13** Real-time myocardial contrast during a slow bolus injection at low MI using Power Pulse Inversion imaging (a). High power frames destroy all of the contrast agent in the scanplane (b). The agent begins to reperfuse the scanplane (c-d), and fully reperfuses the myocardium in a few cardiac cycles (e). Real-time data collection begins just after the high power frames. The data is reduced to include only end diastolic frames, and a monoexponential curve is fitted (f).

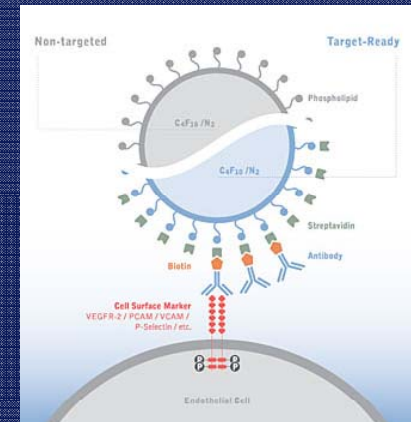
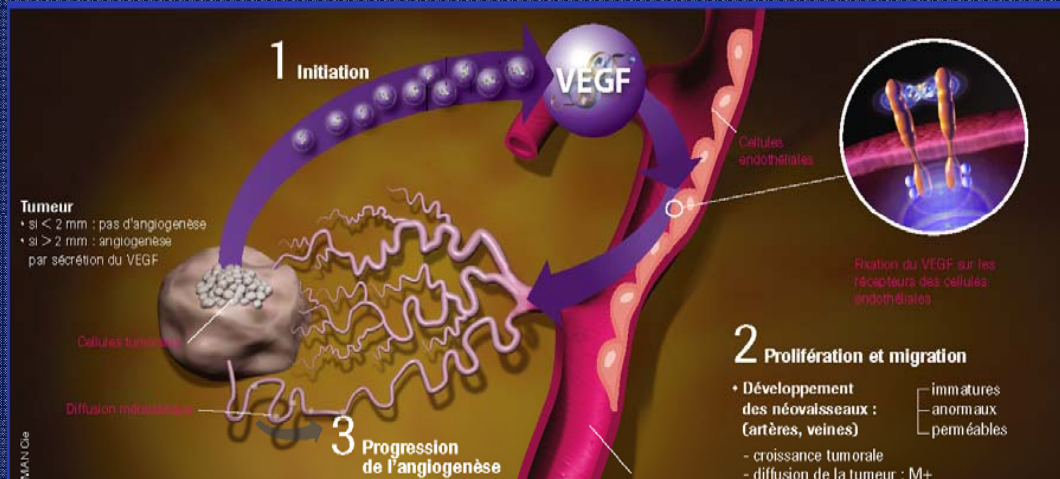
REAL TIME

## Agents de contraste US: Contre-indications

- Allergie à un composant
- Sonovue:
  - Insuffisance cardiaque sévère
  - Cardiopathie ischémique instable
  - Troubles du rythme sévères
- Par précaution: femme enceinte, allaitement, nourrisson et enfant

# Ultrasound molecular contrast imaging

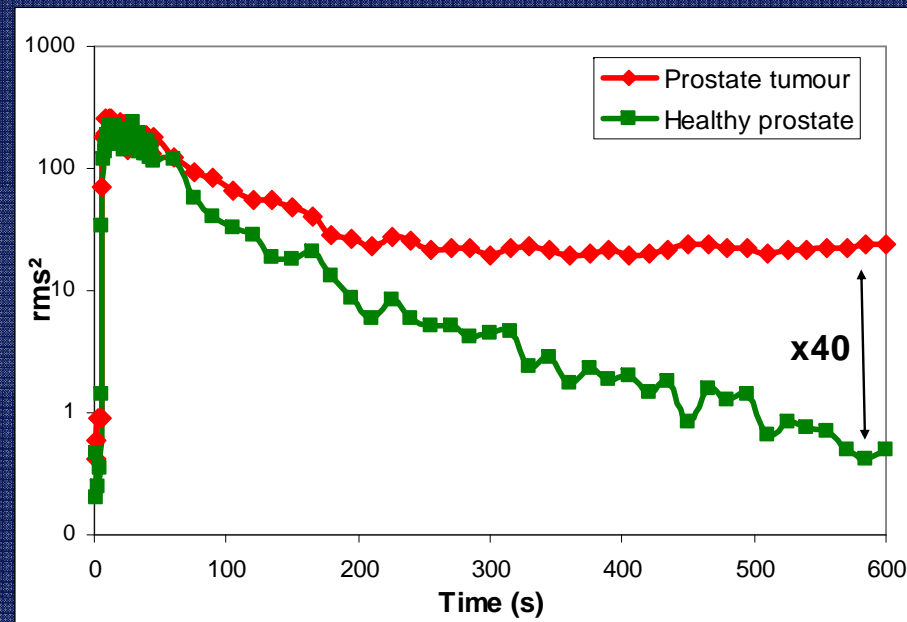
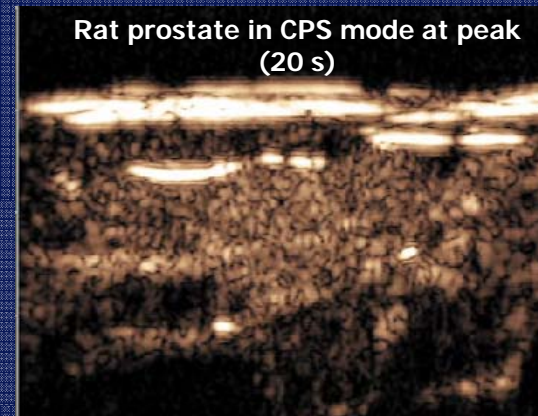
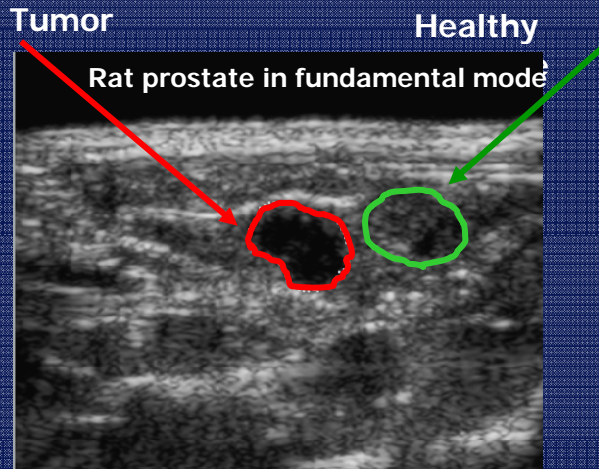
## BR55: Targeted US contrast agent



- Angiogenesis is an important process involved in tumor growth: the angiogenic switch occurs when the tumour reaches 1-2 mm size.
- Among the various markers of angiogenesis, **Vascular Endothelial Growth Factor Receptor 2 (VEGFR2)** is recognized as a major player.
- **BR55 microbubbles are designed to target specifically VEGFR2, thanks to a specific lipopeptide.**

<sup>3</sup>Pillai R, Fan H, Marinelli E et al. .Phospholipid Linked Peptide for Ultrasound Imaging of Angiogenesis Amino acids., vol. 37, supplement 1, July 2009, 11<sup>th</sup> International congress on Amino Acids Peptides and Proteins, Vienna , Austria , Aug 3<sup>rd</sup> -7 , 2009.

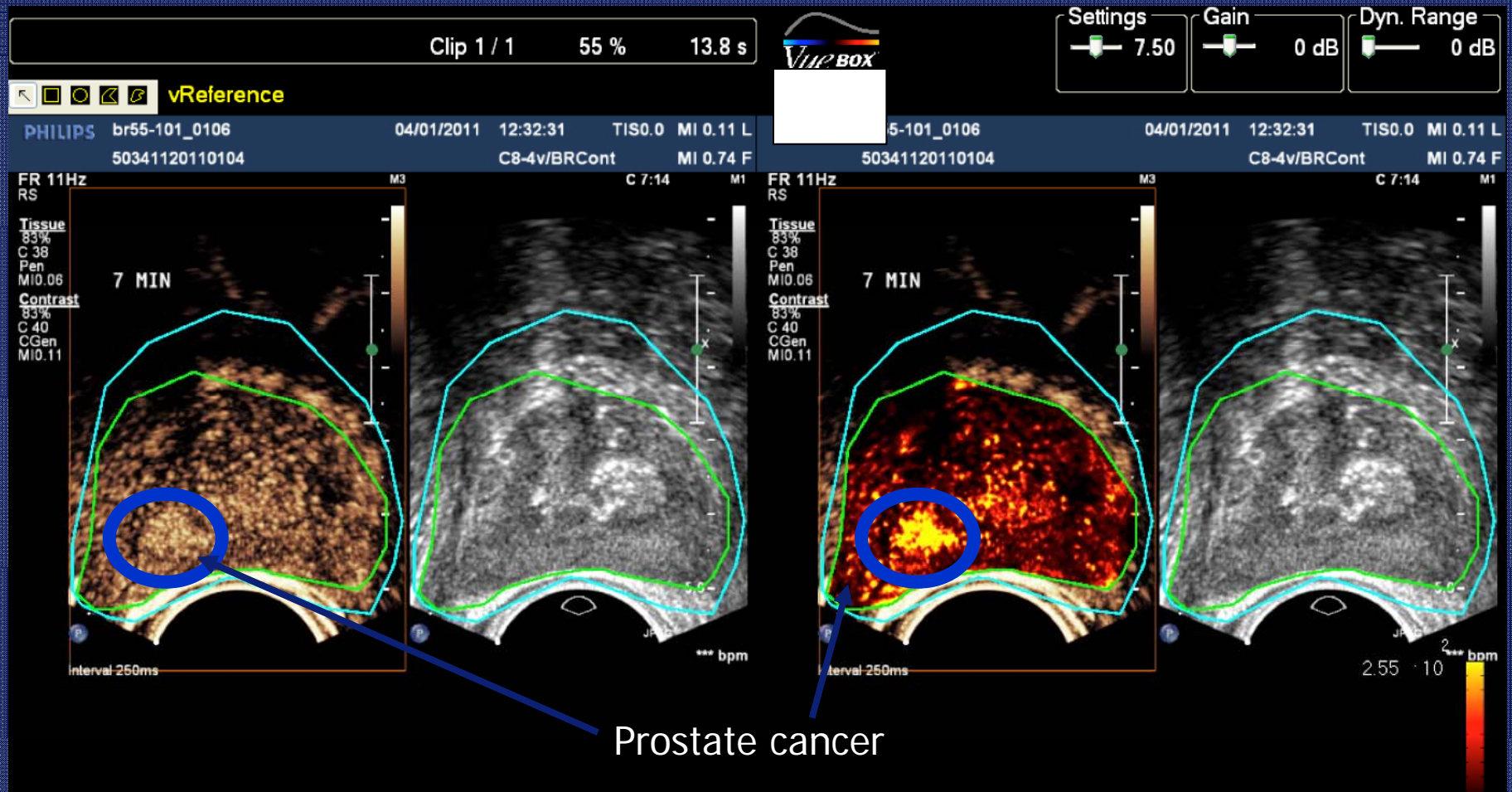
# Molecular imaging of prostate cancer lesion in a rat Dunning model with BR55



BR55 can differentiate tumor from normal prostate based on angiogenesis

Tardy I, Pochon S, Theraulaz M, Emmel P, Passantino L, Tranquart F, and Schneider M, Investigative Radiology (2010), 45, 573

# Proof of concept: human studies

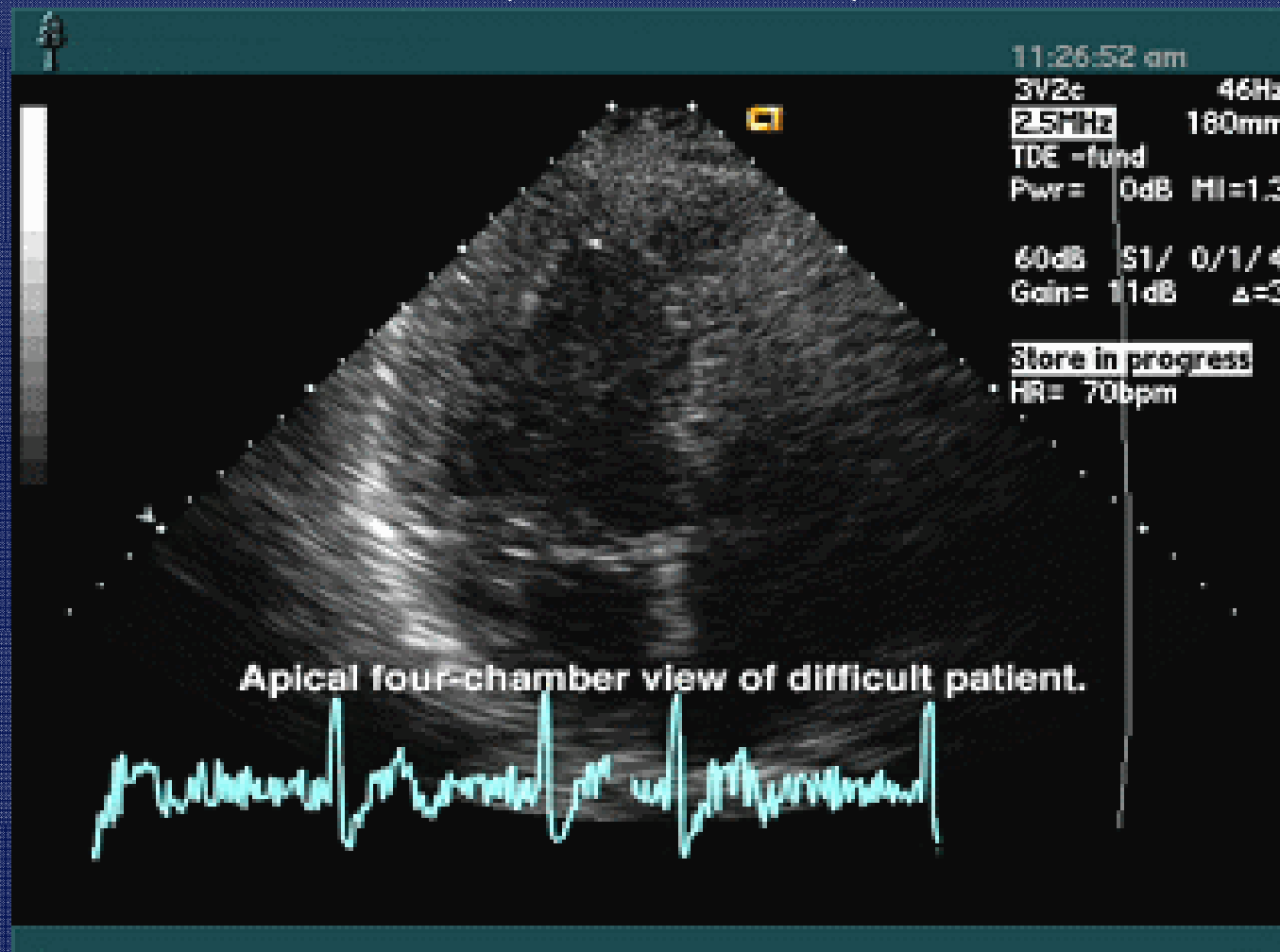


Immunostaining demonstrates moderate VEGFR2 expression in that PCa lesion

BR55 is able to bind to VEGFR2 in humans and is safe and well-tolerated

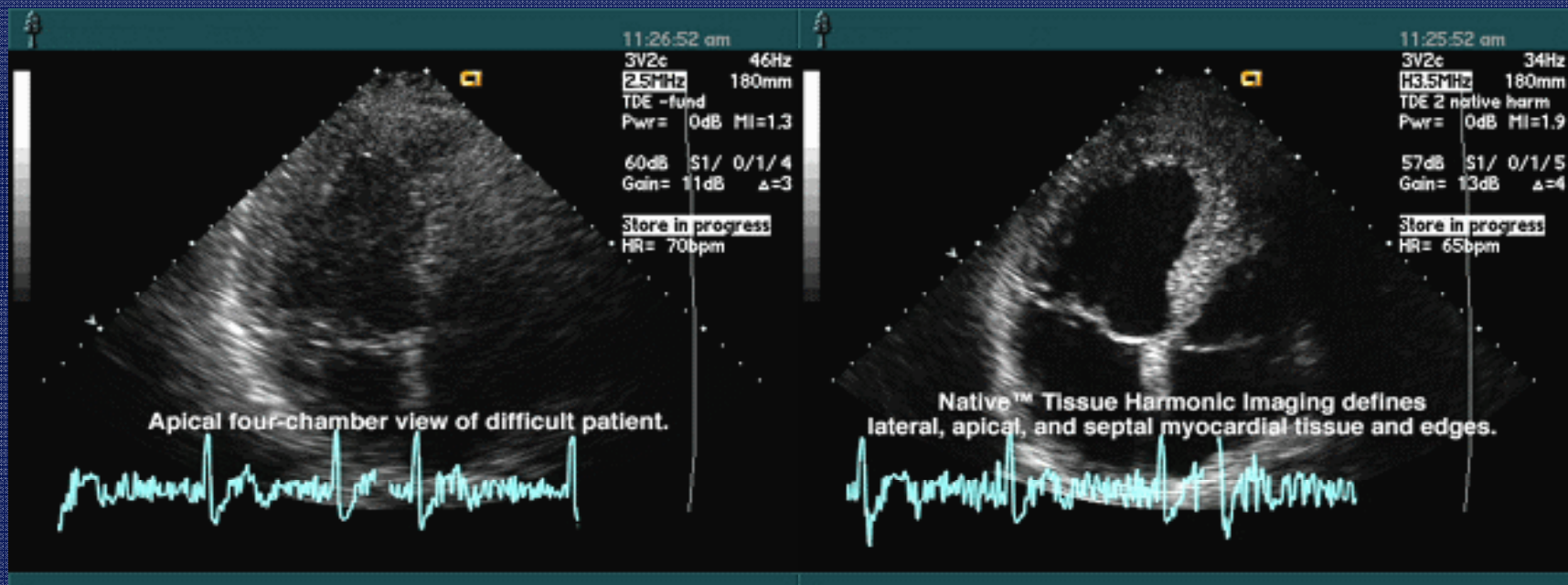
# Imagerie harmonique tissulaire

# Conventional B-mode image (AP4CH)

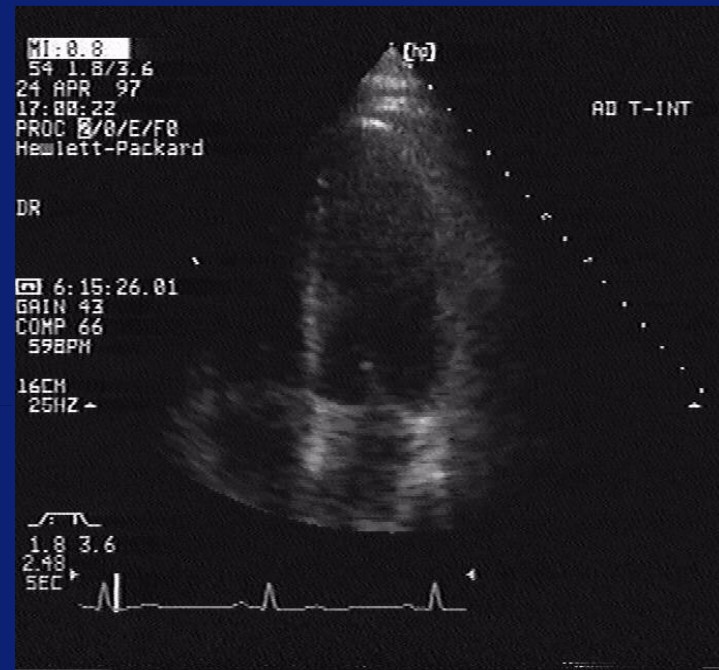
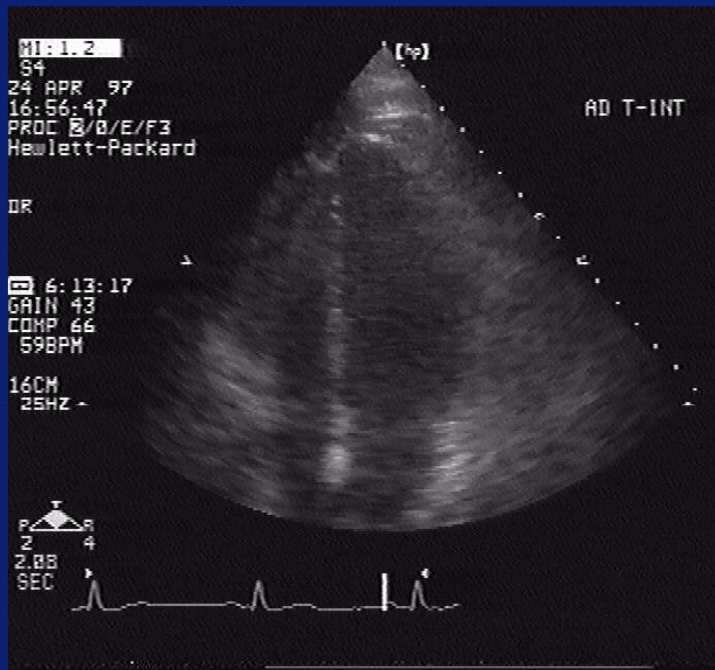


# Fundamental

# THI



# Exemples cliniques



4 chamber view

# Génération de fréquences harmoniques

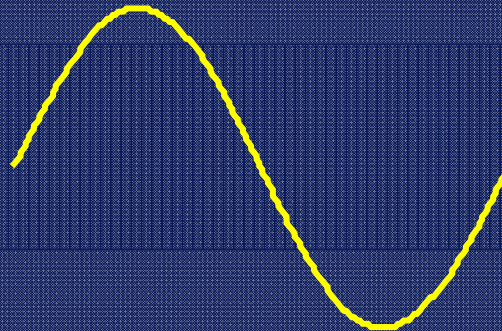
## ⇒ Non linéarité:

- Apparition des effets d'acoustique non linéaire
- Création de composantes fréquentielles absentes dans le faisceau émis

→ Harmonique tissulaire (native) → propagation

# Origine physique de la distorsion de l'onde

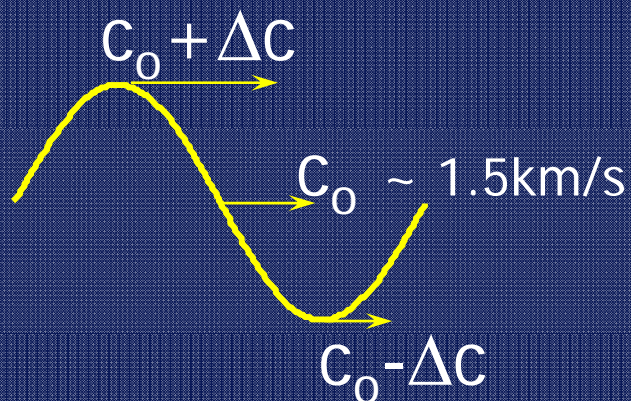
Pression positive  $\Rightarrow$  Tissu comprimé (forte densité)  $\Rightarrow$  Vitesse



Pression négative  $\Rightarrow$  Expansion du tissu (faible densité)  $\Rightarrow$  Vitesse



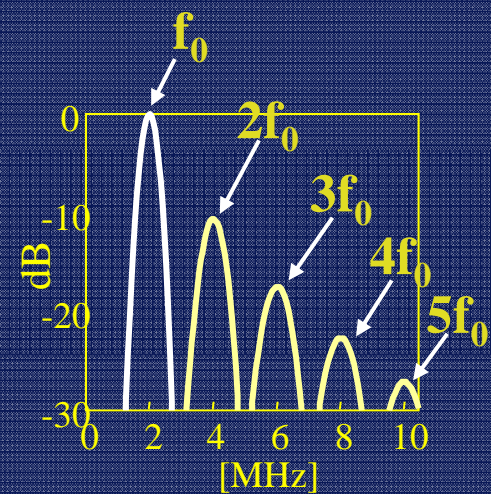
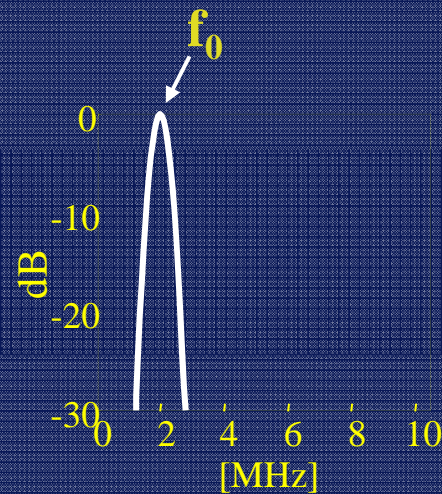
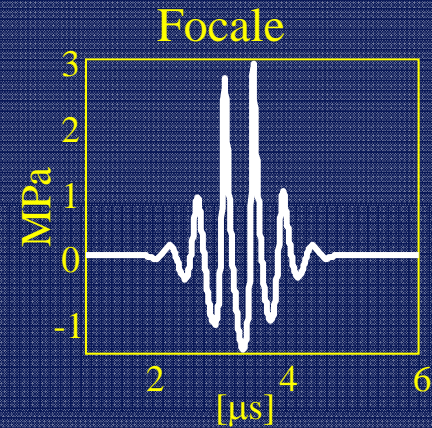
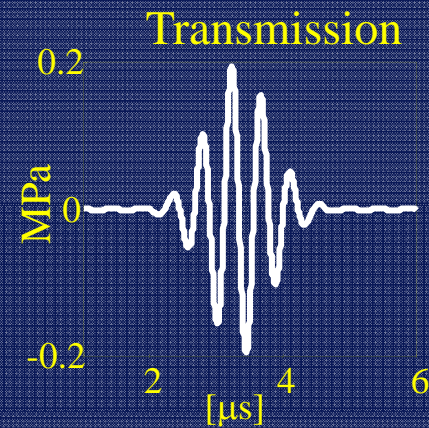
## Accumulation de la distorsion



direction de propagation

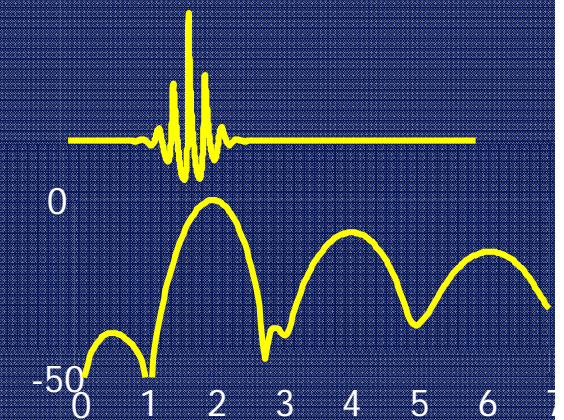
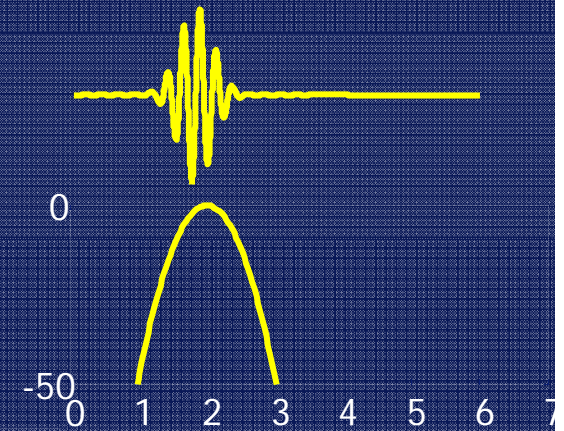
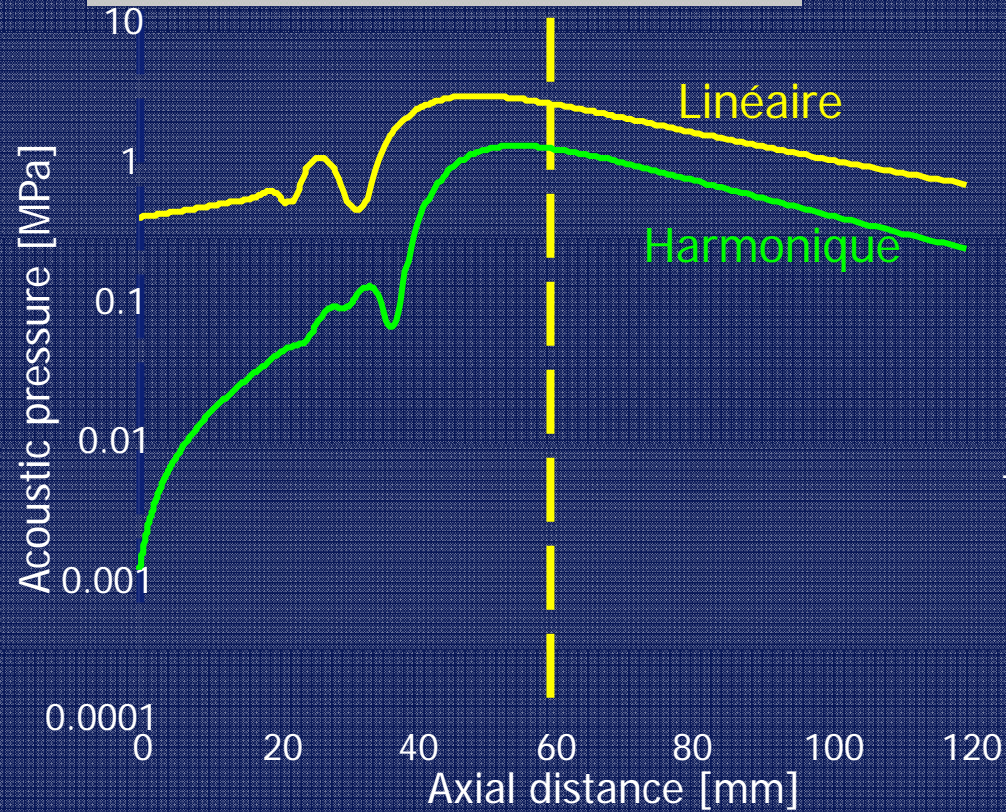
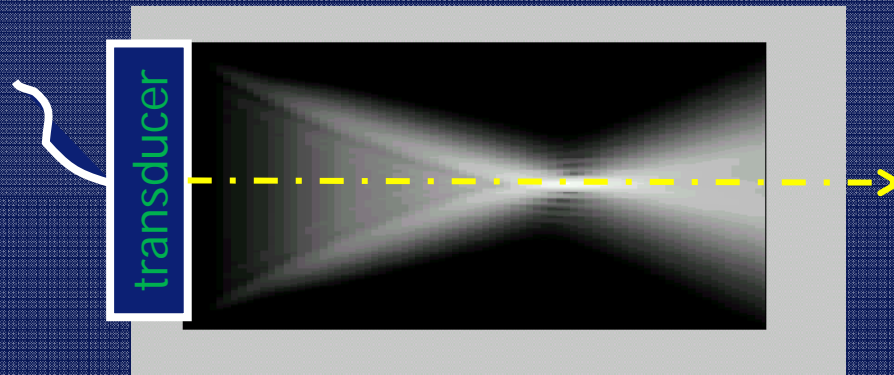
# Génération des fréquences harmoniques

## Propagation non linéaire (native)



# Profile axial du champs acoustique

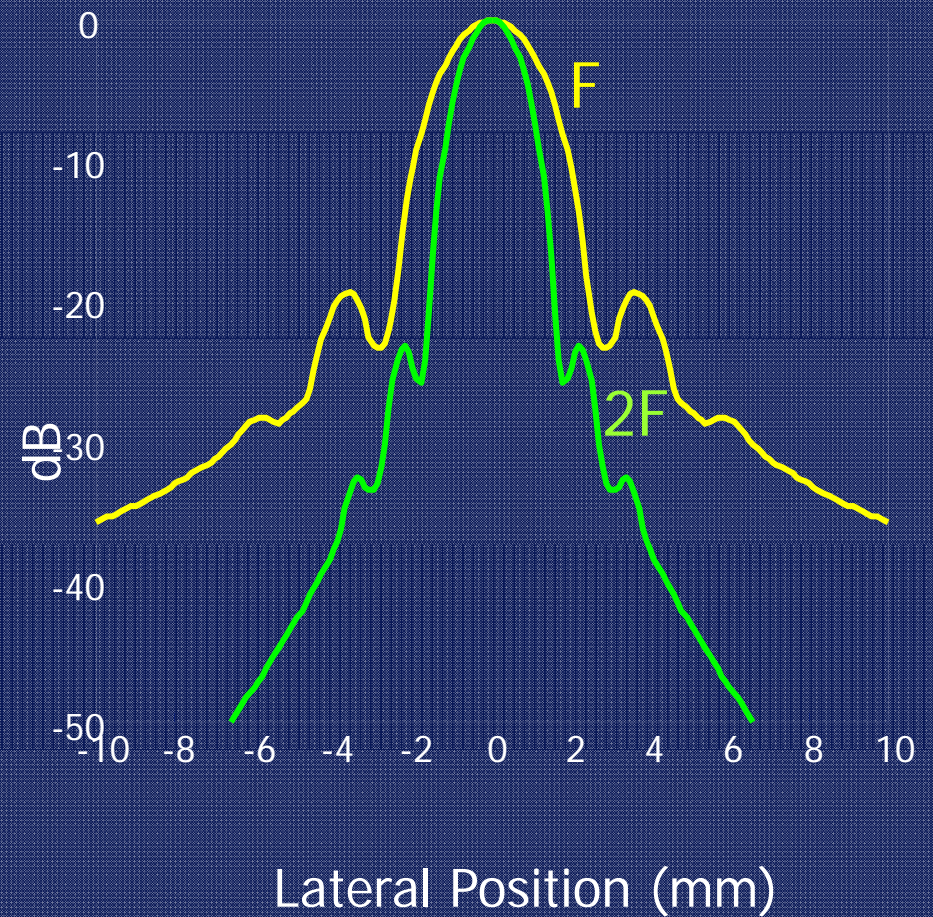
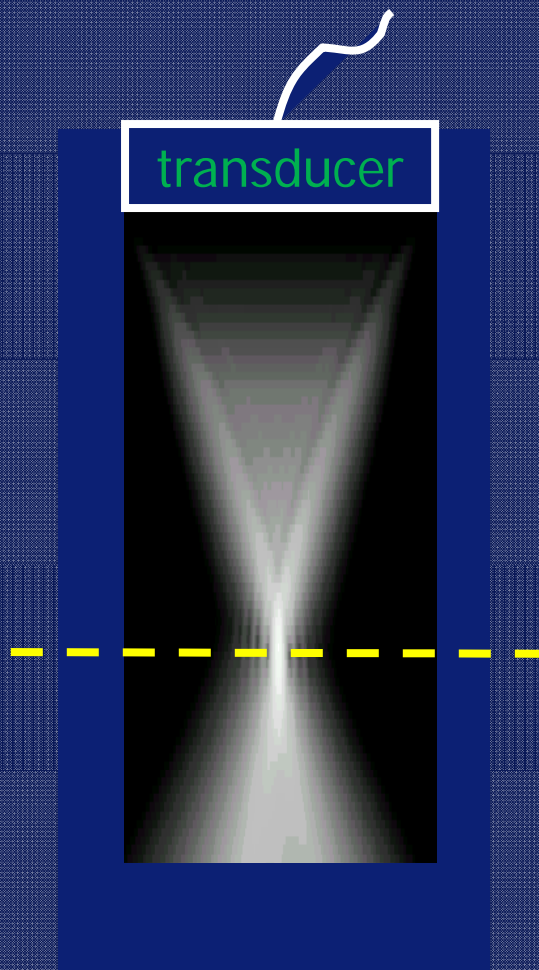
500 kPa (IM=0.3), 2.25 MHz,  $\phi=12$  mm



# Profile latéral du champs acoustique

500 kPa (IM=0.3), 2.25 MHz,  $\phi=12$  mm

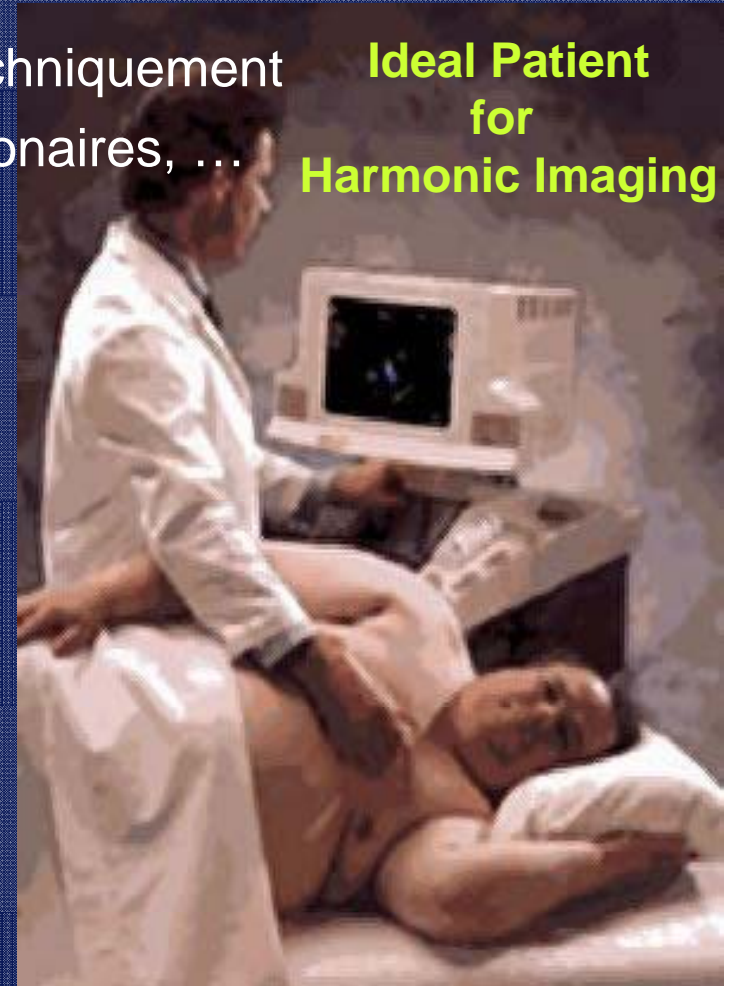
Beam profile at the focus



# Intérêt de l'imagerie harmonique

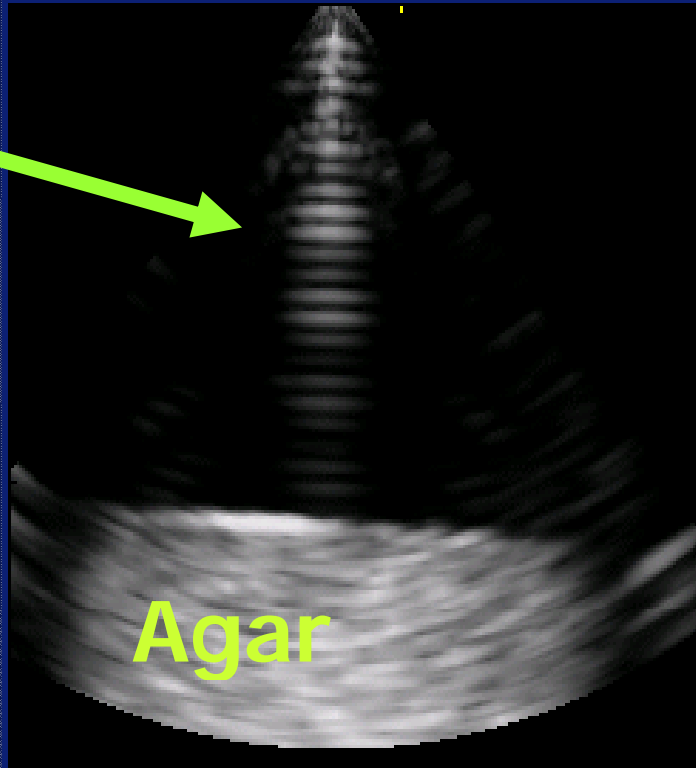
En cardiologie, plus de 40% des patients sont "techniquement difficiles en raison de: obésité, pathologies pulmonaires, ...

**Ideal Patient  
for  
Harmonic Imaging**

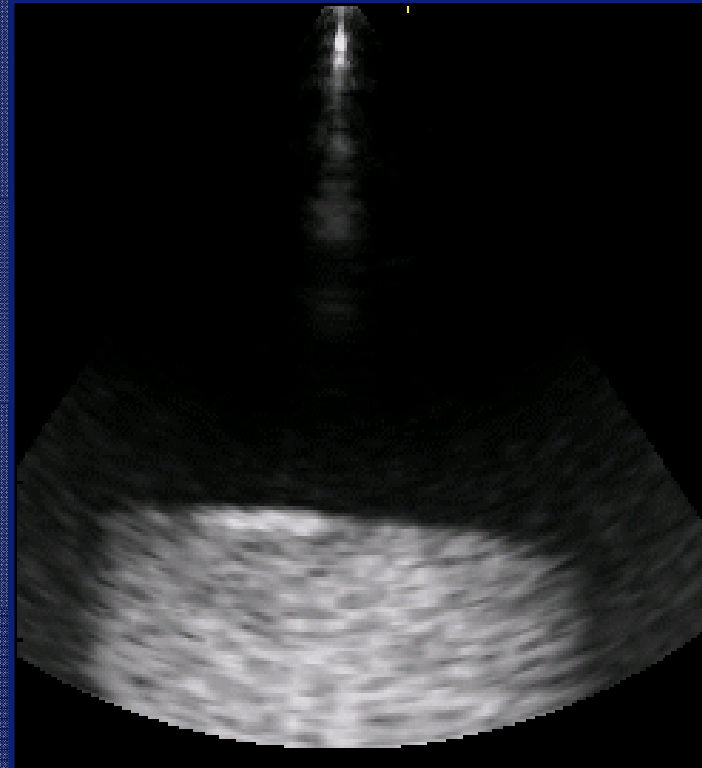


# *In vitro data*

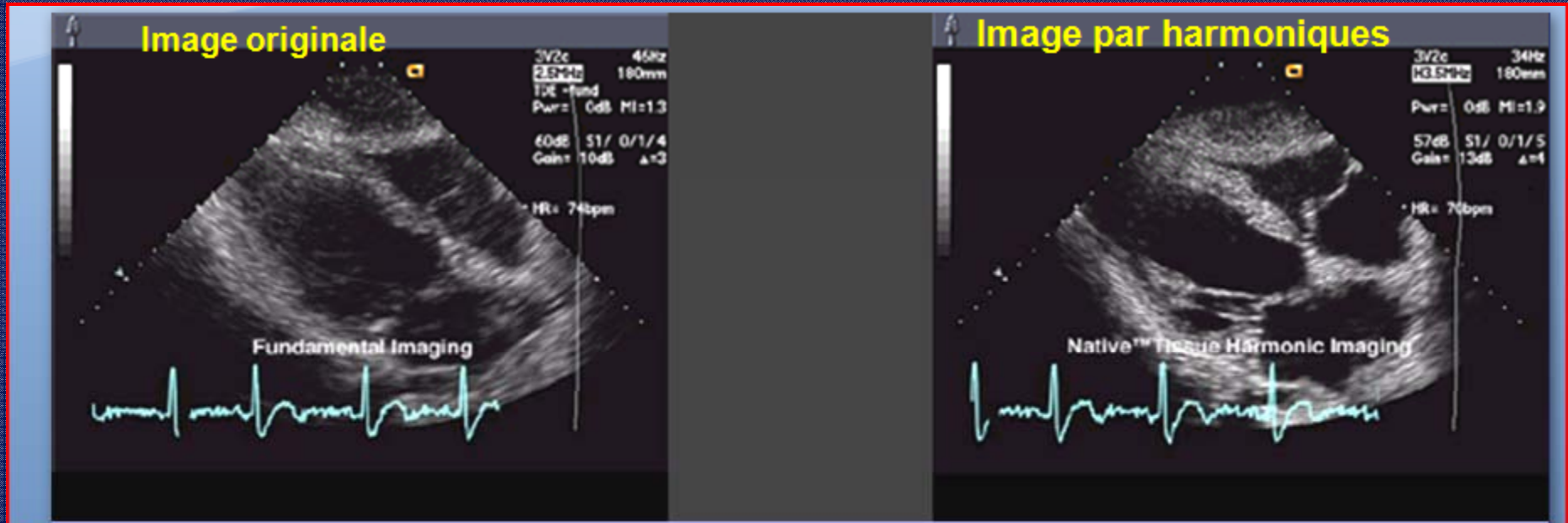
Fundamental 3.3 MHz



Harmonic 1.7 MHz

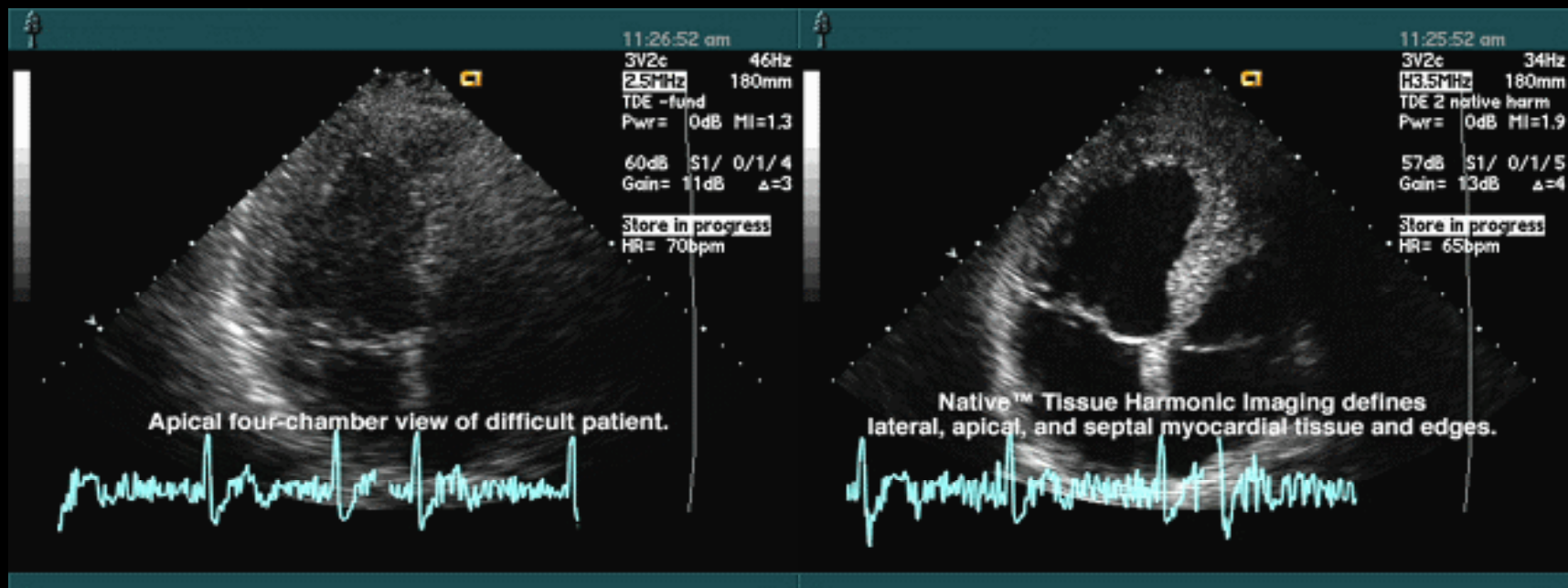


# Exemples cliniques



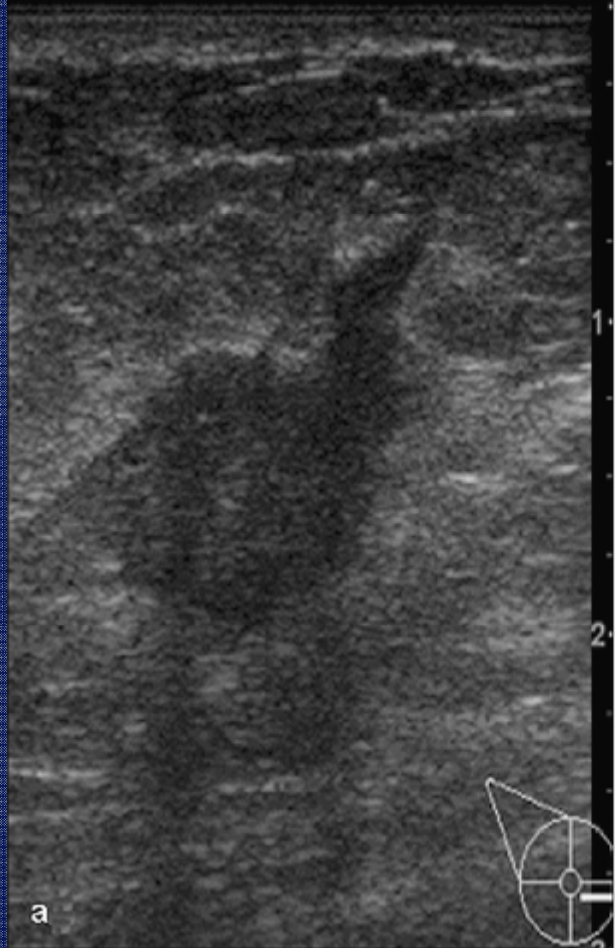
# Fundamental

# THI

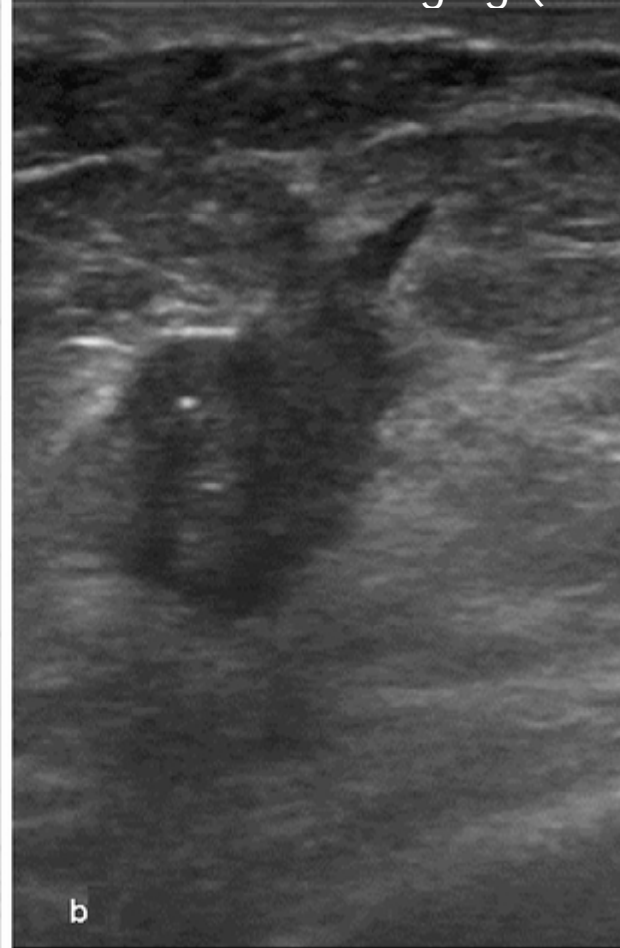


# Exemples cliniques

Fundamental B mode



Tissue harmonic imaging (THI)



Effect on visibility of calcifications and delineation of tumour margin

# **Bubbles And Ultrasound As A Therapeutic Modality**

# Therapy

- **Potential therapeutic applications of microbubbles:**

- **Sonothrombolysis**

- *Accelerate clot lysis*

- **Drug delivery to brain**

- *Reversible opening of blood brain barrier*

- **Drug delivery**

- *Acoustic active carriers*

- **Gene delivery**

- *Co-administration of genes with microbubbles*

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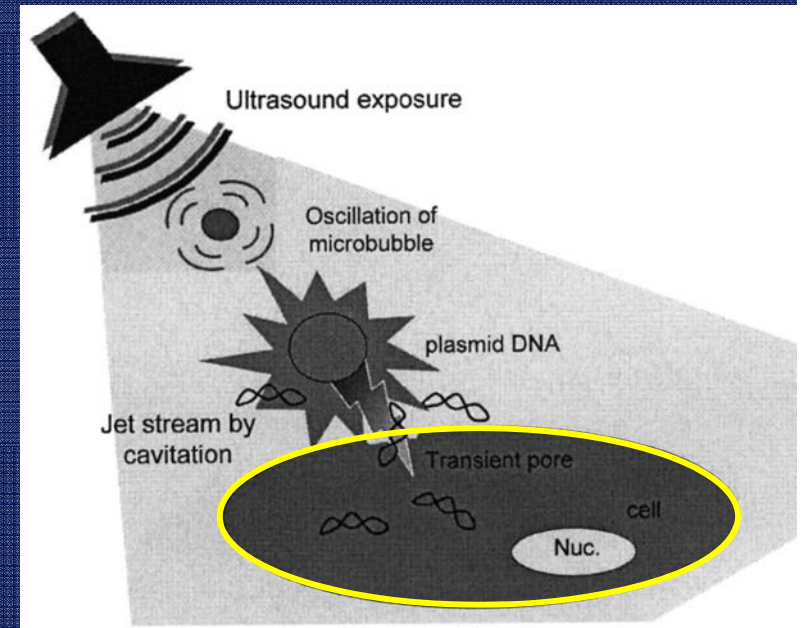
- *Co-administration of genes with microbubbles*

**Sonoporation**

# Sonoporation

The use of ultrasound and microbubbles for modifying the permeability of the cell plasma membrane.

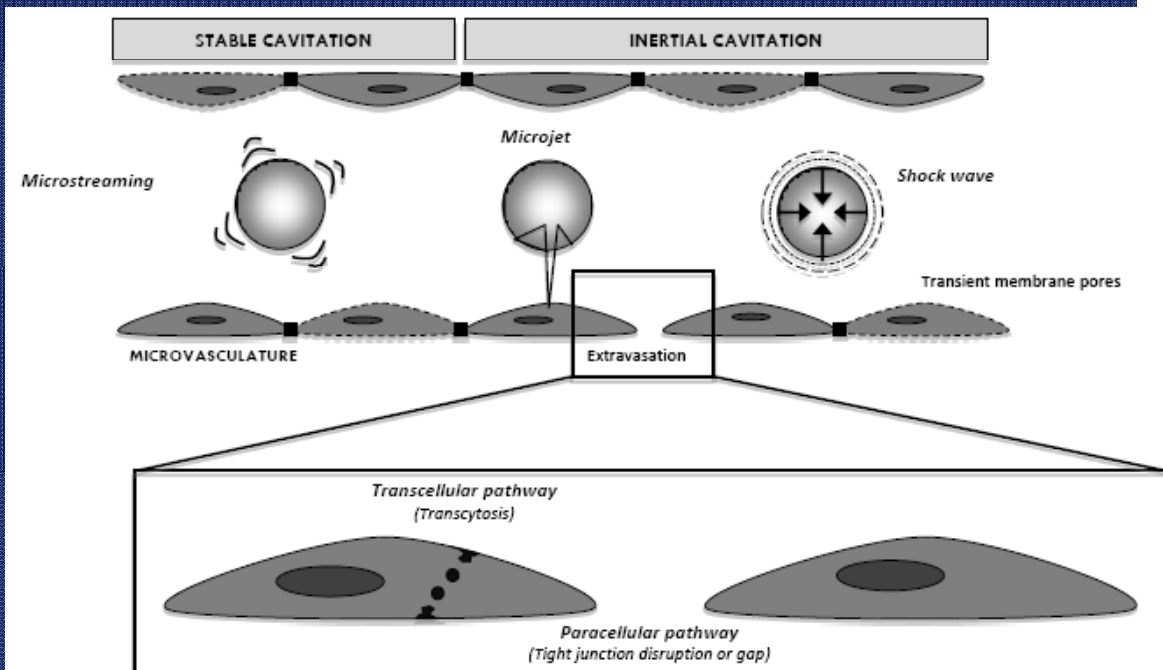
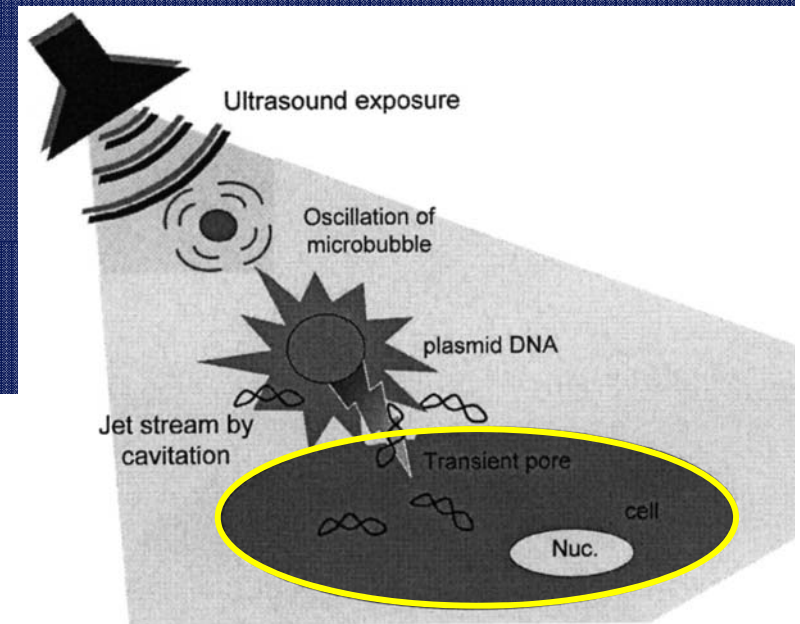
Enhance delivery of large molecules.



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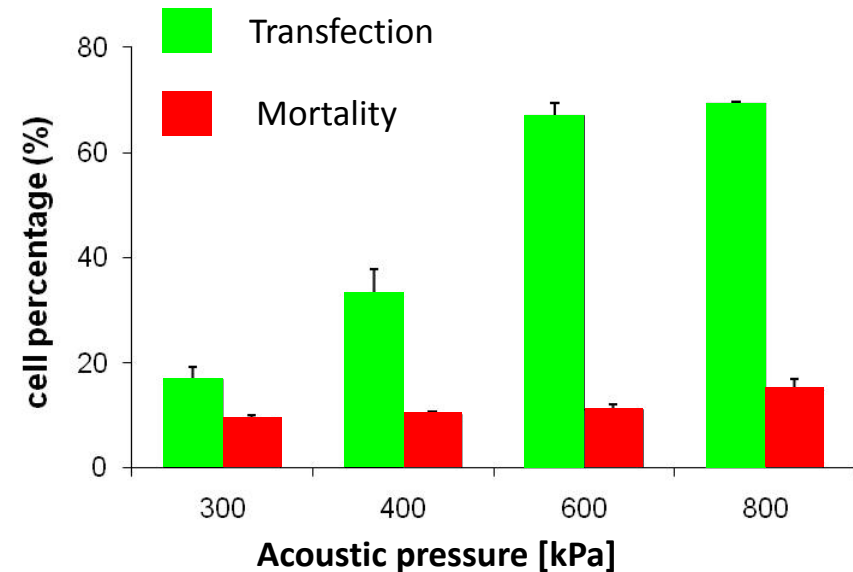
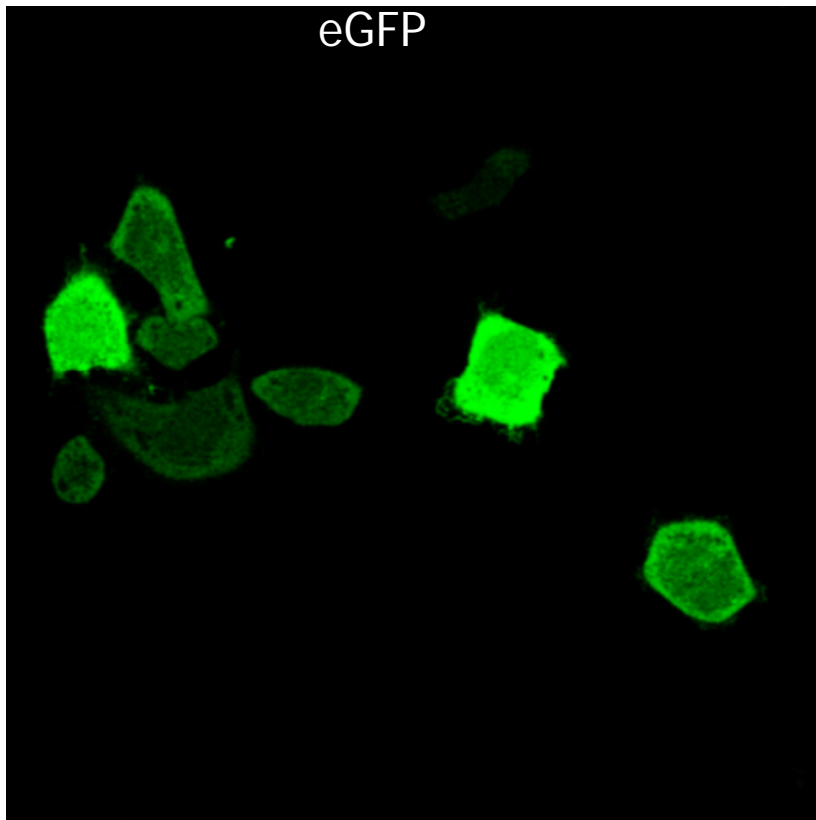


# Permeabilization of the cell membrane

Transfection: DNA encoding for **eGFP**  
Observation T6H  
U87 Cells in-vitro

*The integrity of the cell membrane is Compromised*

*High drug concentration at the site of interest while minimizing Systemic drug exposure*



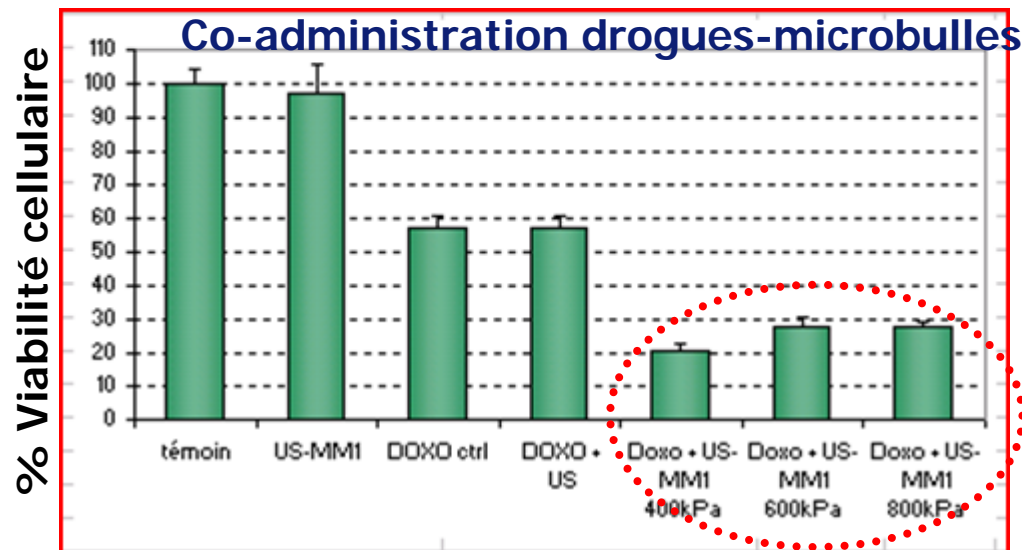
*+MM1 bubbles, 1min exposure*

# *Permeabilization of the cell membrane:*

## *Co-administration of drugs and microbubbles*

U87MG – US 30sec - 1MHz

MM1 microbulles, Doxorubicin: 3 $\mu$ g/ml

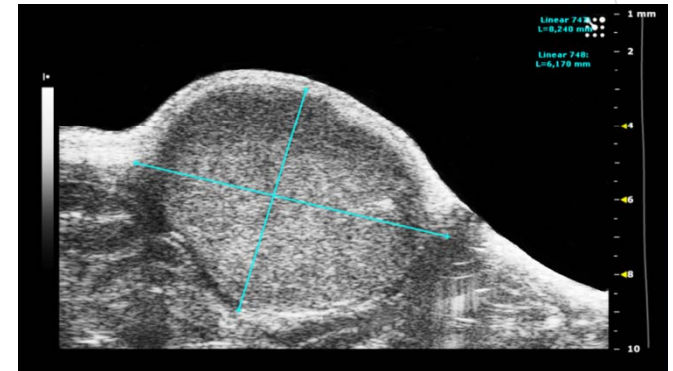
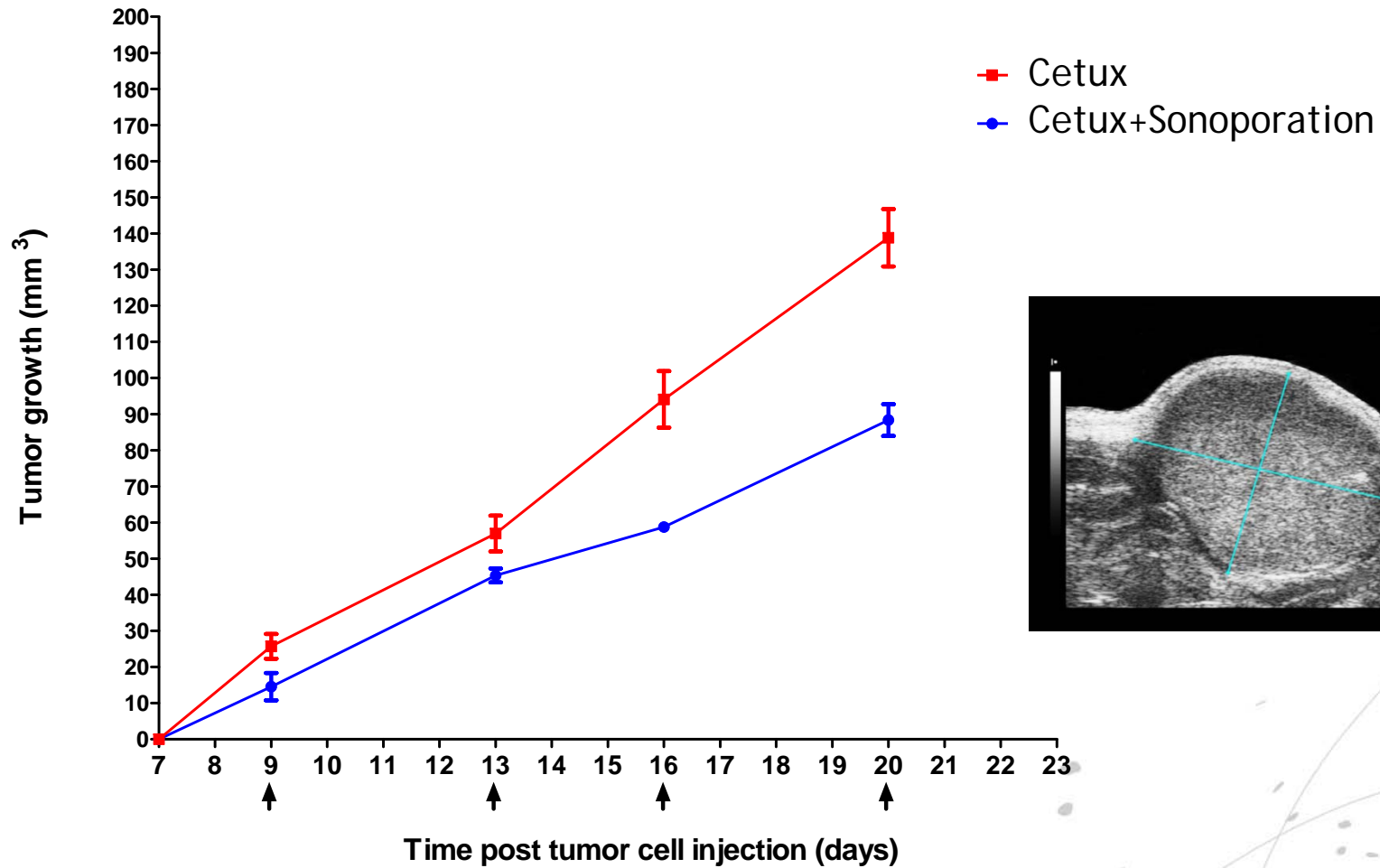


*Enhancement of doxorubicin-induced U87MG cell death with US+MM1 microbubbles*

# Subcutaneous tumor model U87 (glioblastoma)

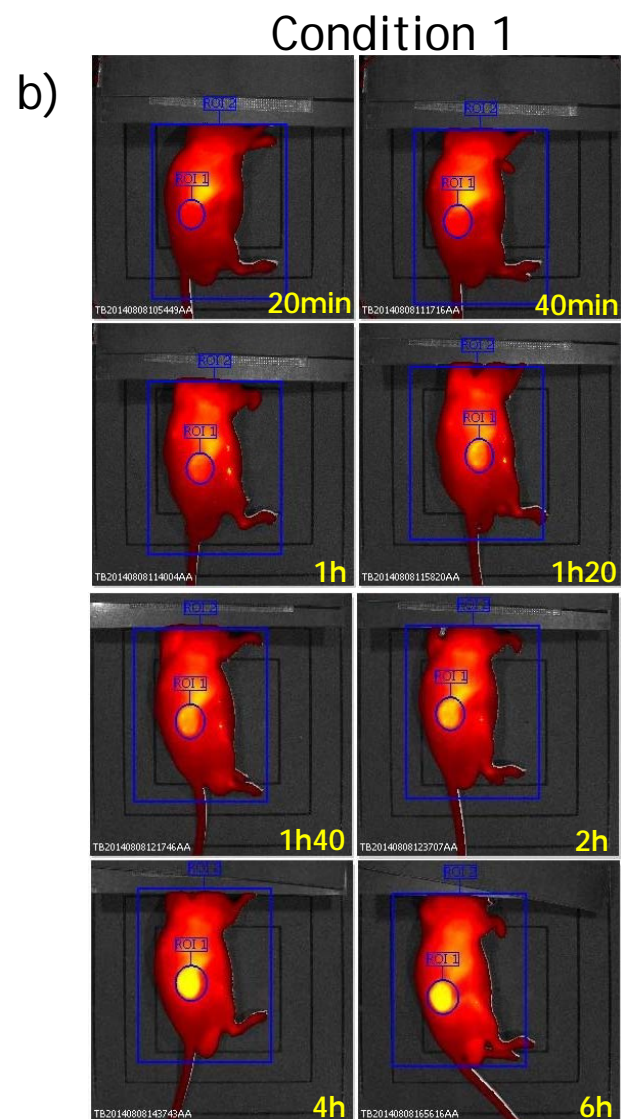
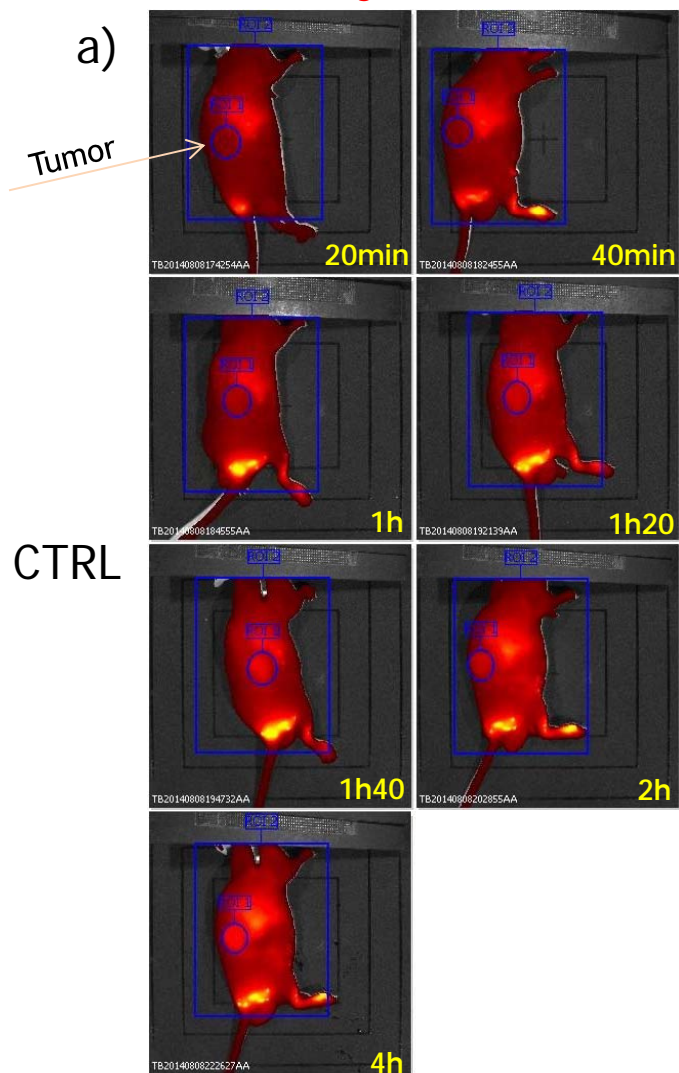
Tumor volume

Evolution of tumor volume measured by ultrasound imaging



# Results: ectopic model

## Cetux Bioavailability

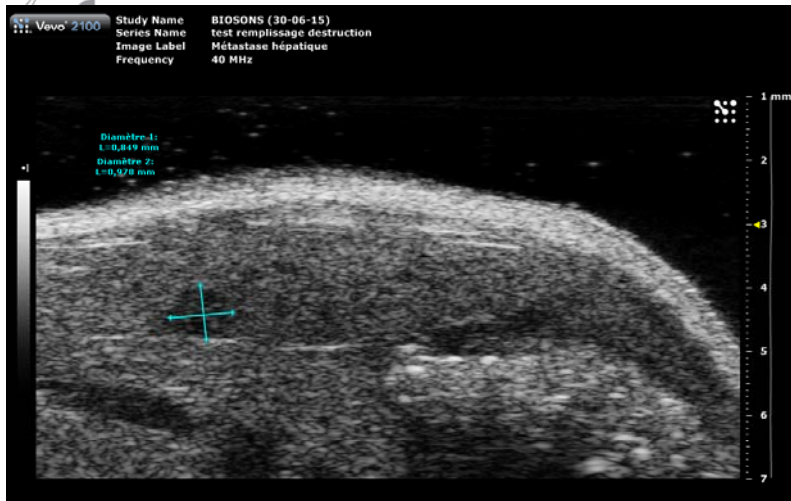


Comparison of bioavailability kinetics of cetuximab-AlexaFluor® 750 between a control tumor (a) and sonoporated tumor (b) by fluorescence imaging

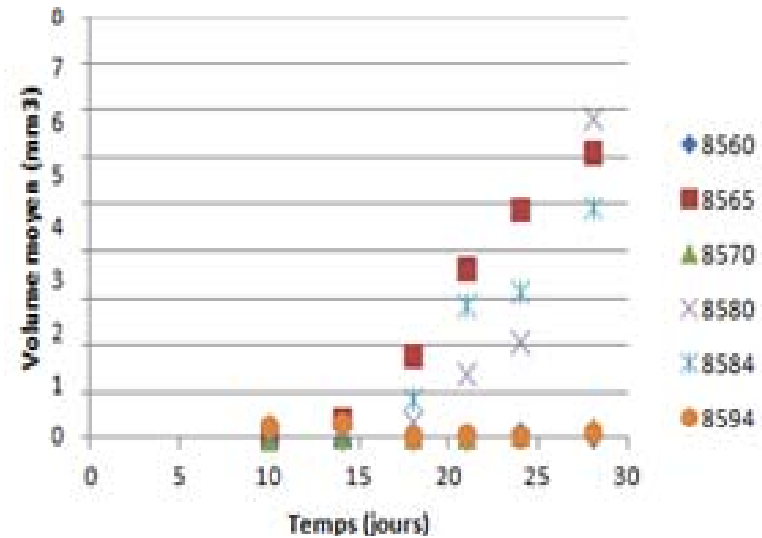


# Hepatic metastases of CCR

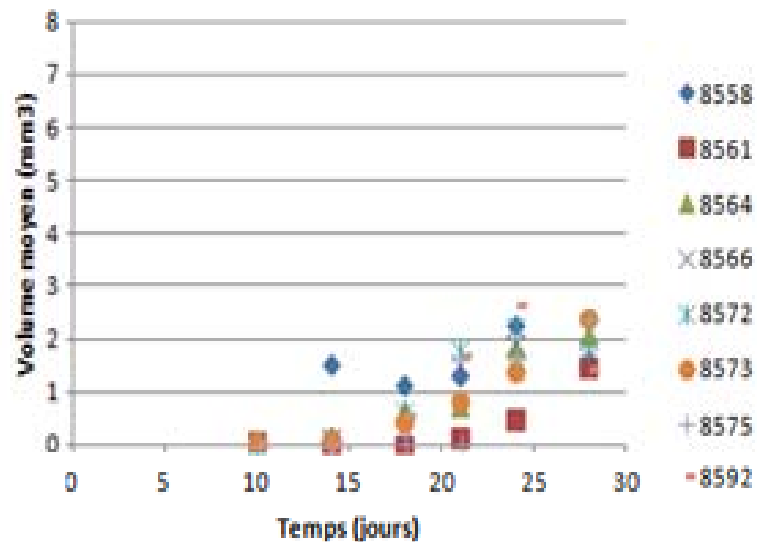
Tumor volume



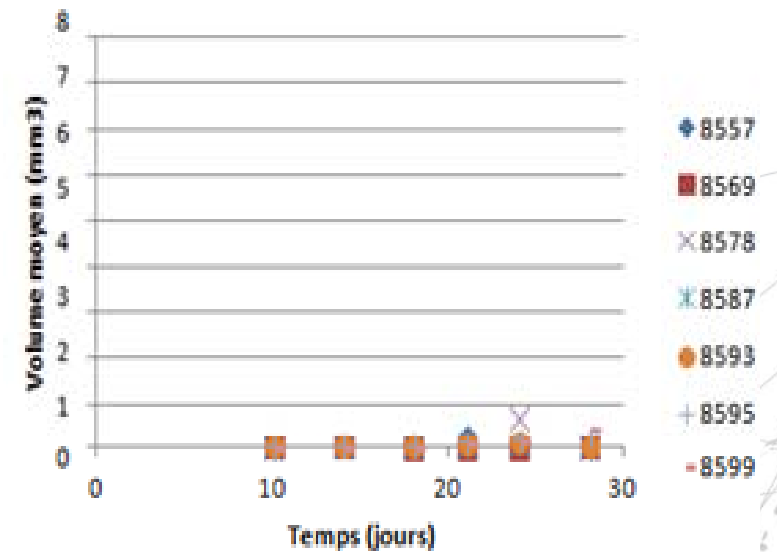
Contrôle



Chimio



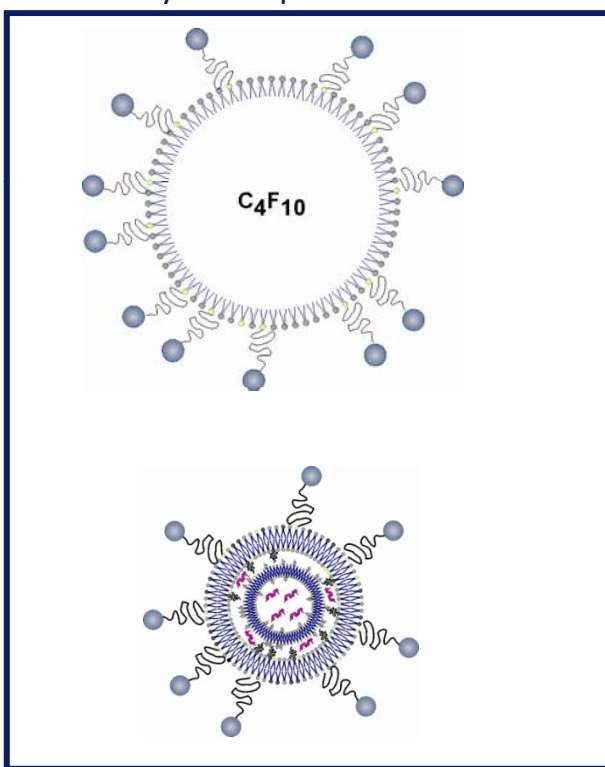
Chimio + SONO



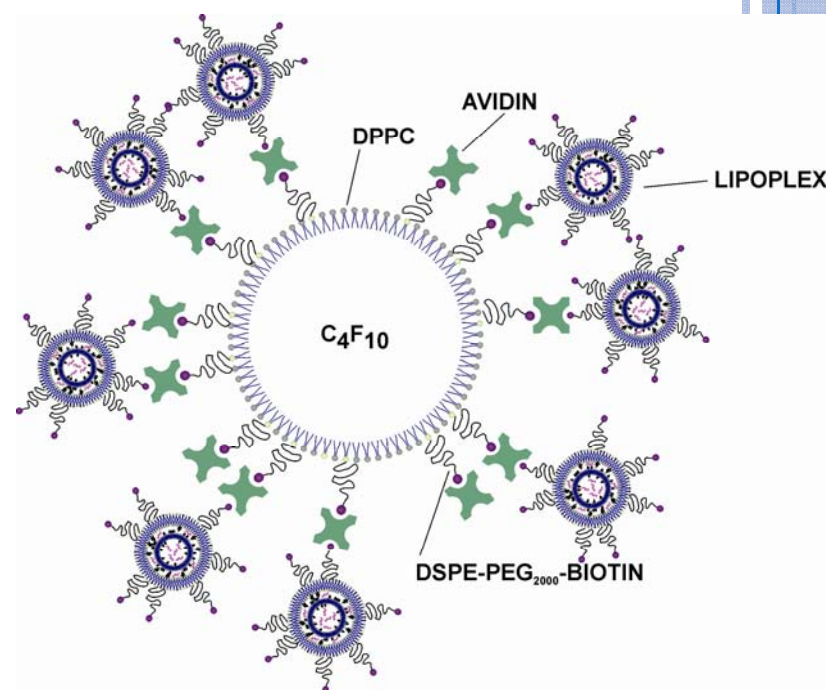
Metastases volume of various animals for 3 groups

## DRUG LOADED MICROBUBBLES

Biotinylated lipid microbubbles



Coupling  
with  
avidin

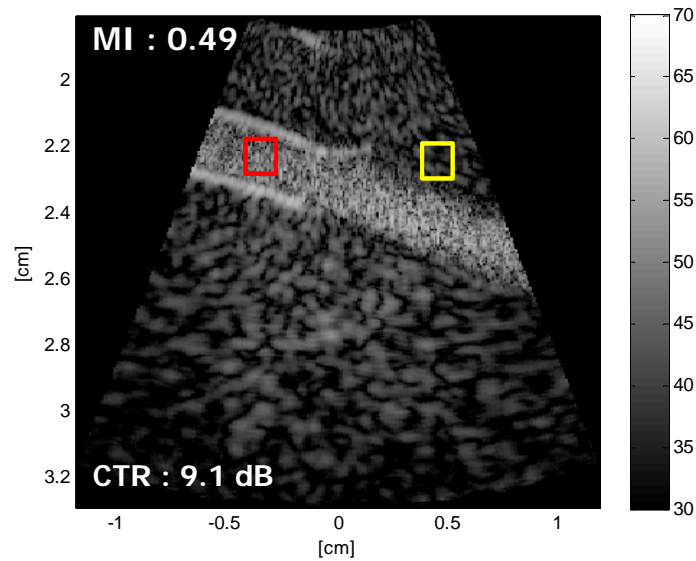


Biotinylated pDNA or siRNA containing complexes

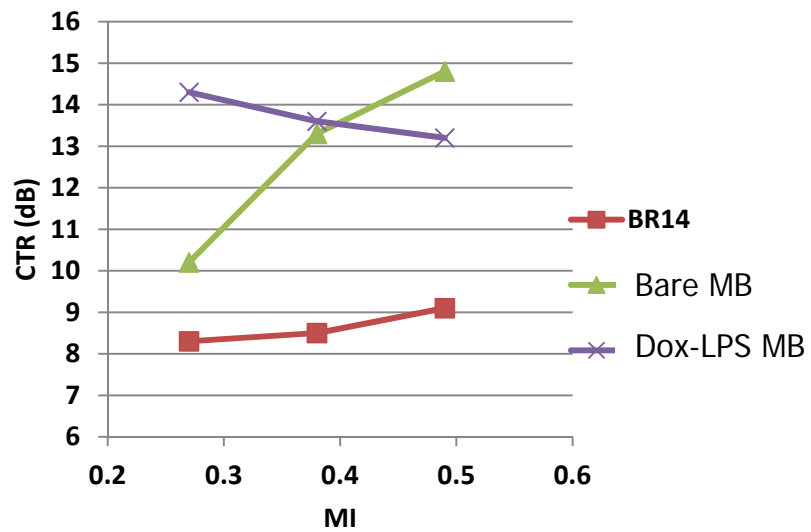
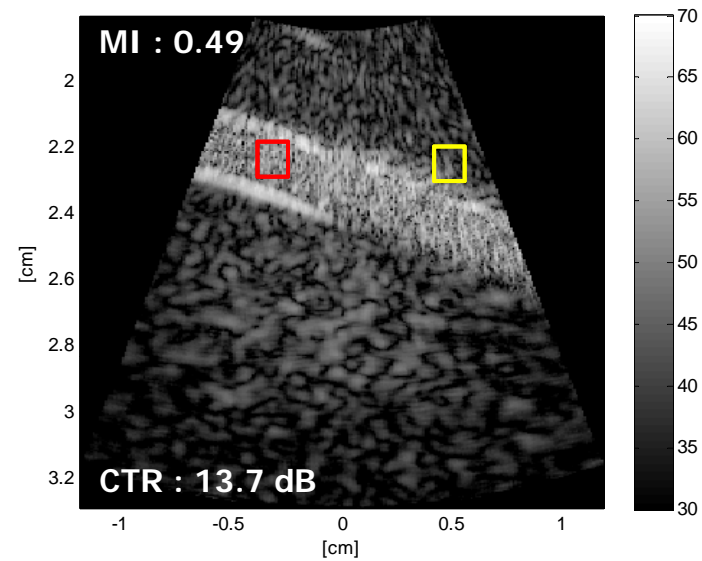
Nanoparticle loaded microbubbles

# IMAGING

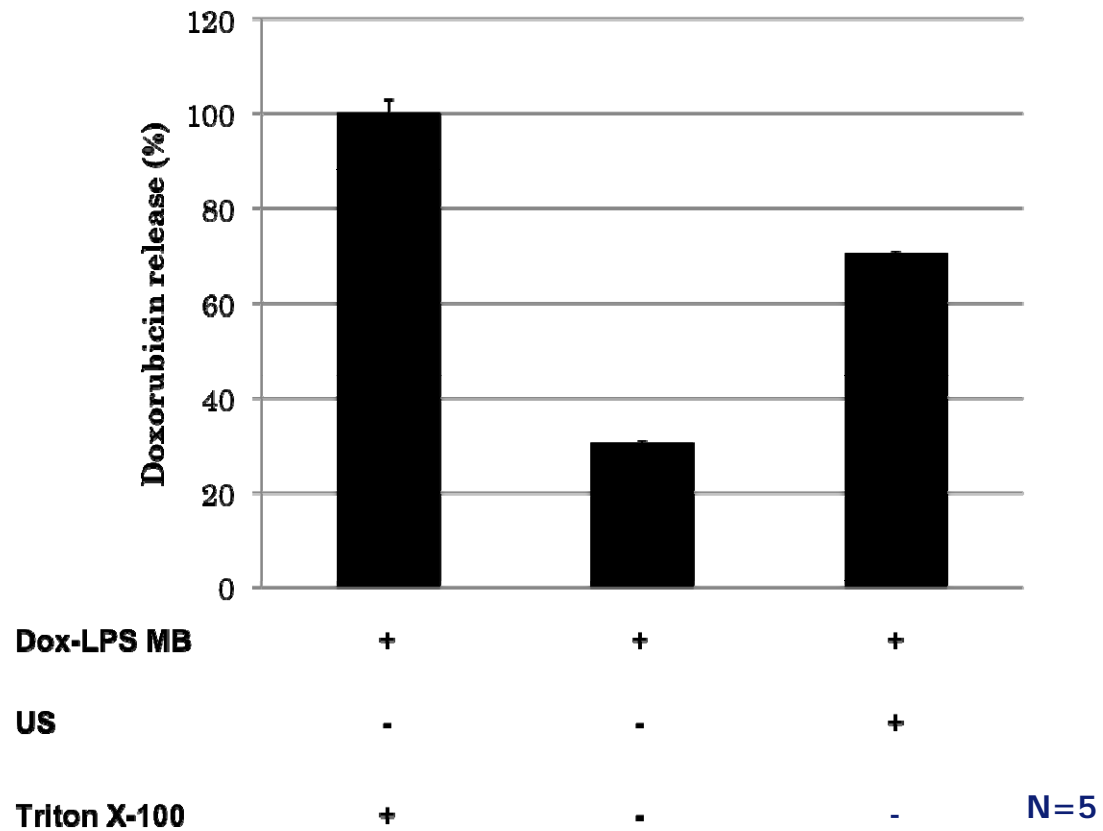
BR14 (Bracco Research)



Dox-LPS-MB



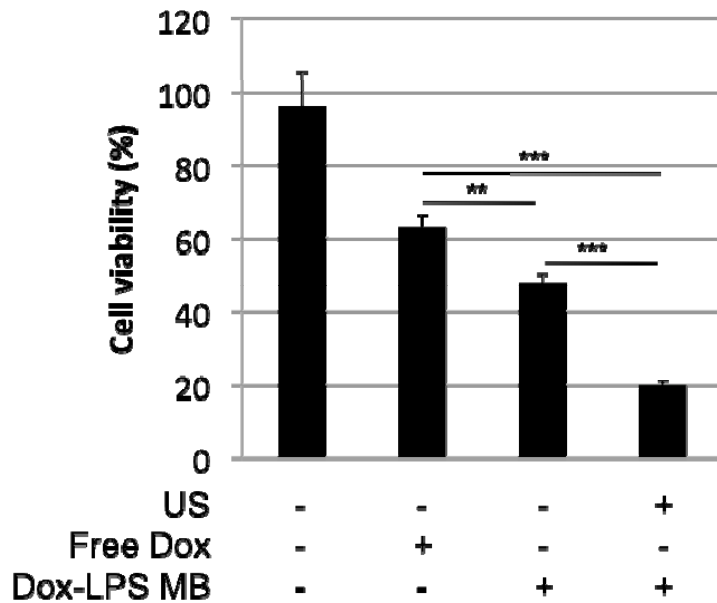
## DOXORUBICIN RELEASE FROM DOX-LPS LOADED MB



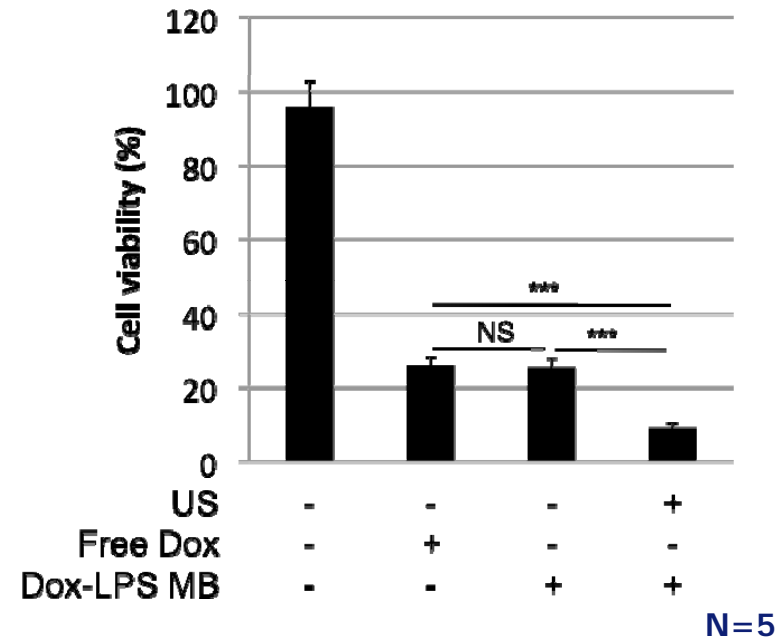
The US exposure induces the release of Dox from Dox-LPS MB

# CELL VIABILITY

24h



48h



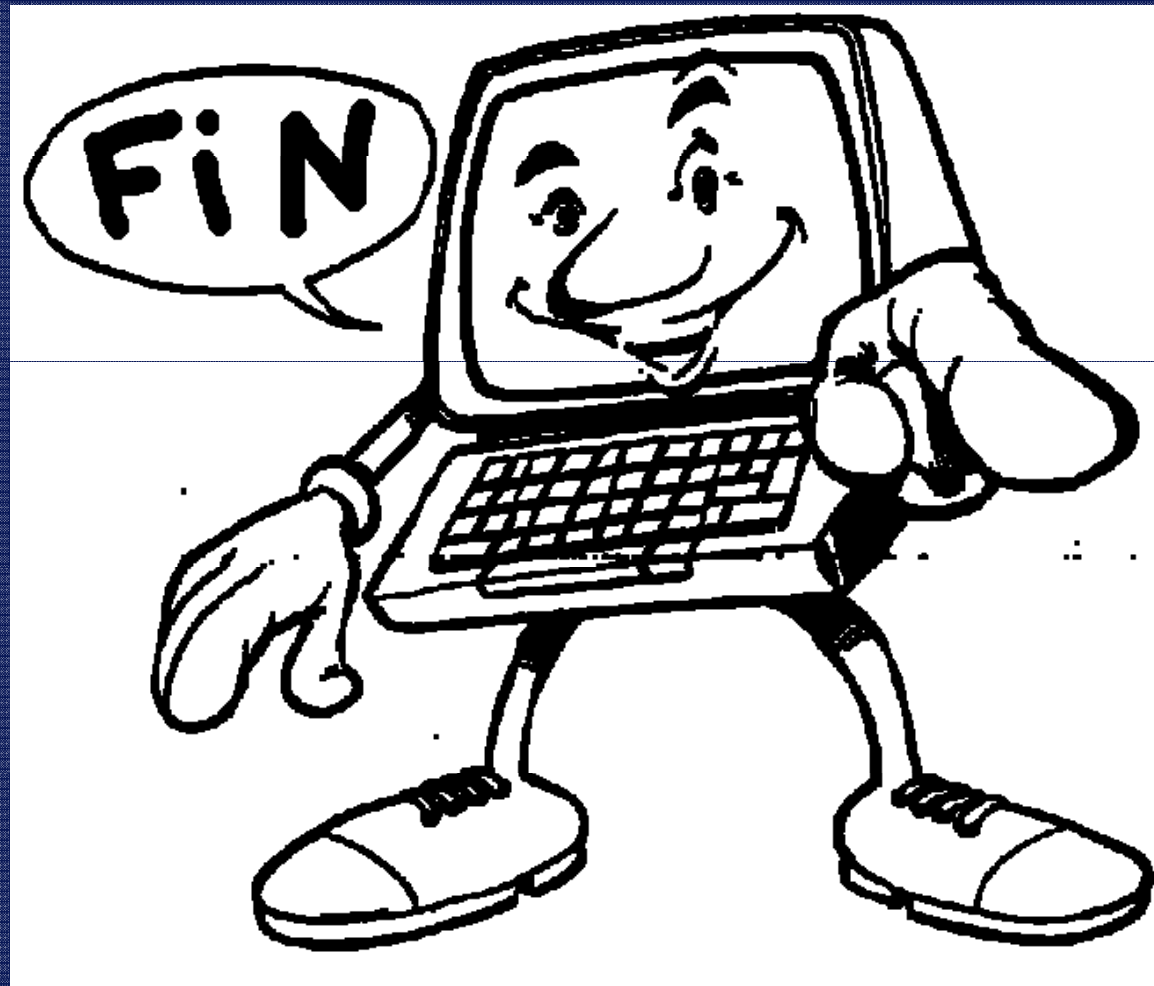
The combination of US and Dox-LPS MB are more efficient than Dox and Dox-LPS MB treatment alone

# Conclusions

**Imagerie harmonique**  $\Rightarrow$  Emettre à  $f_0$  & Recevoir à  $2f_0$

**Origine physique**  $\Rightarrow$  Contraste: réflexion des microbulles  
Tissulaire: propagation des US dans les tissus

- Imagerie non linéaire = nouvelle modalité d'imagerie US d'intérêt majeur
- Nouvelle séquences d'imagerie permettant d'identifier la signature des microbulles
- Les agents de contraste ont un rôle établi en diagnostic clinique
- Les microbulles représentent une nouvelle classe de délivrance locale de médicaments. Elles sont activées par l'énergie ultrasonore



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