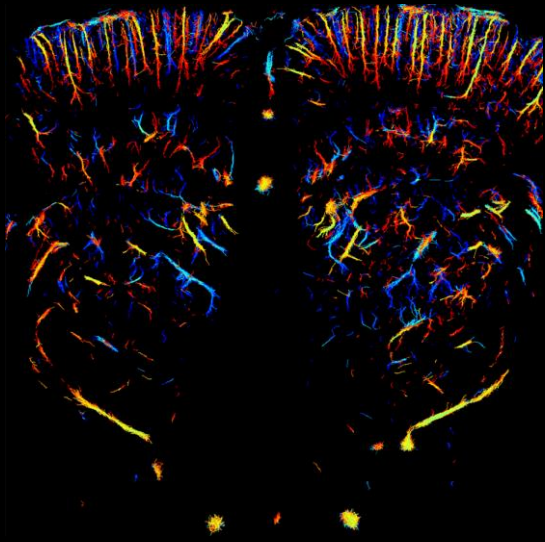
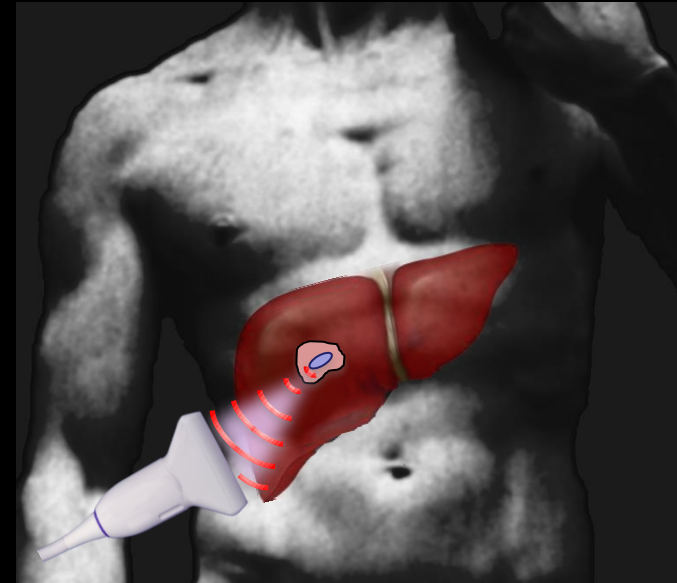


# Largage de médicaments par ultrasons et imagerie super-résolue



Olivier Couture  
Institut Langevin

4 mai 2017



**Inserm**

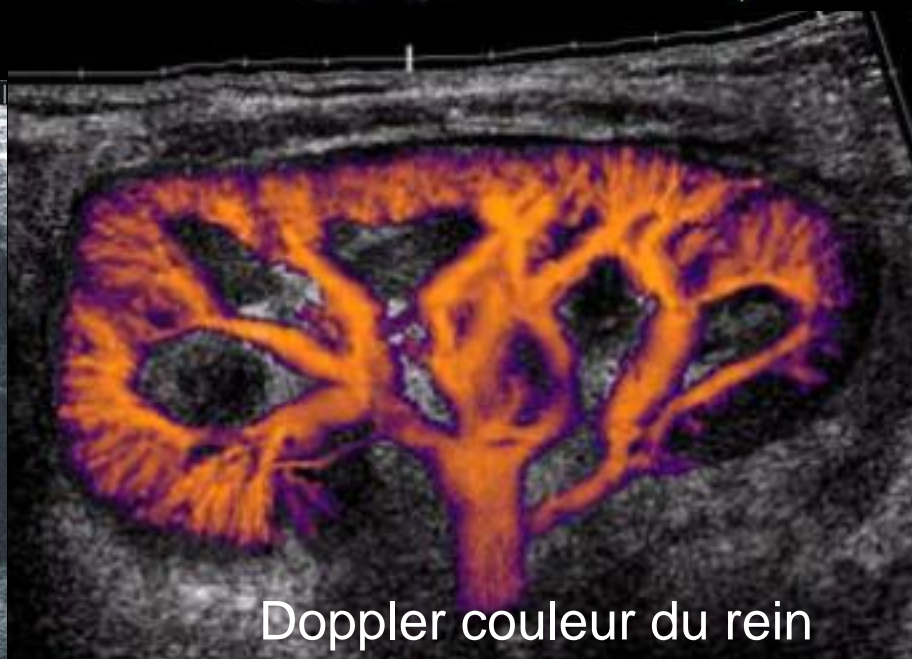
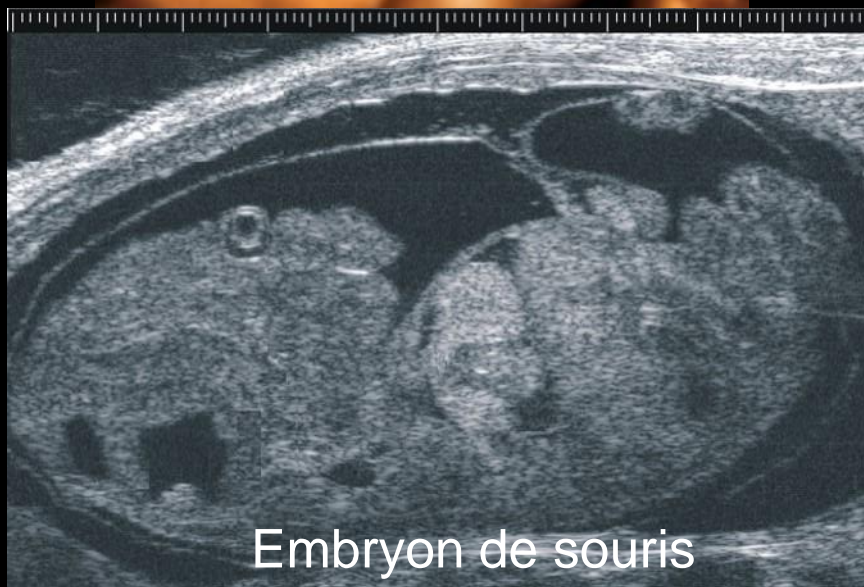
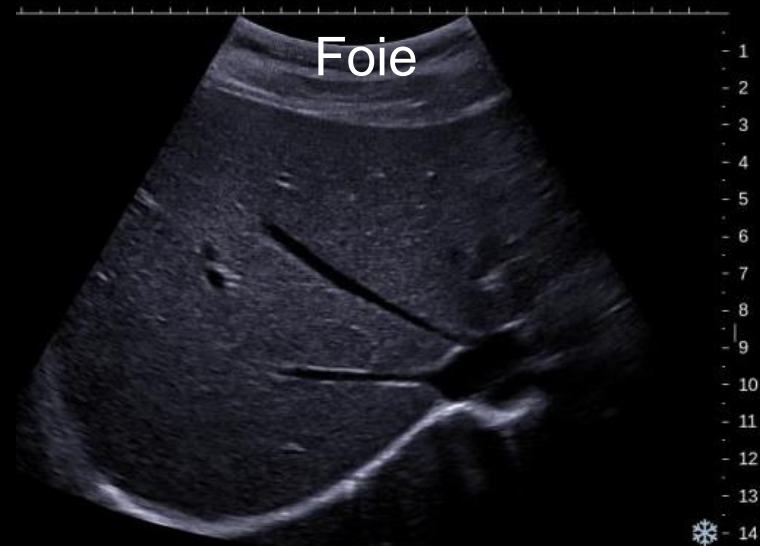


## Équipe de Physique des Ondes pour la Médecine Institut Langevin - Paris



# L'échographie est une modalité d'imagerie flexible

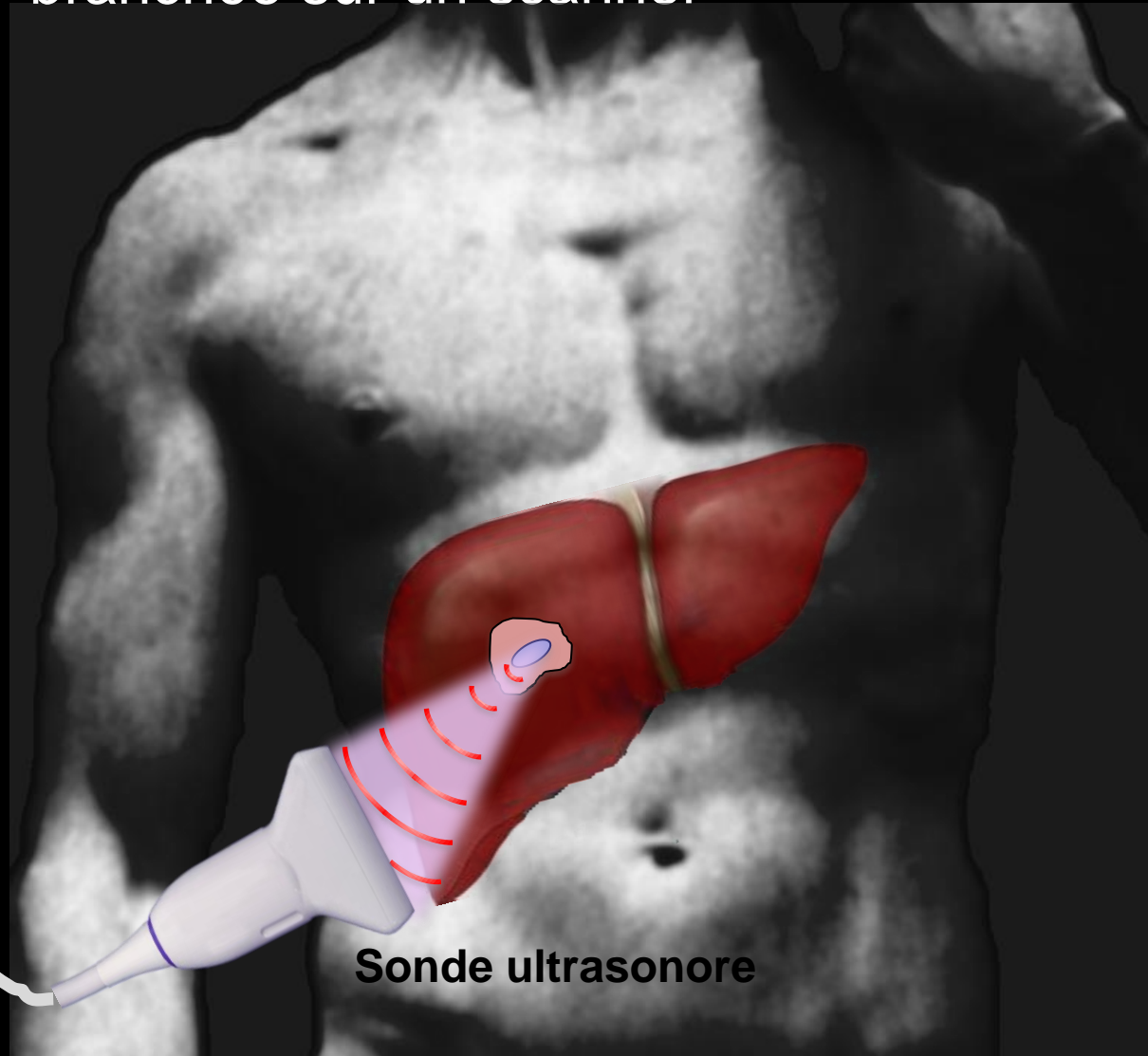
Échographie foetale 3D



L'échographie est effectuée grâce à une sonde ultrasonore branchée sur un scanner



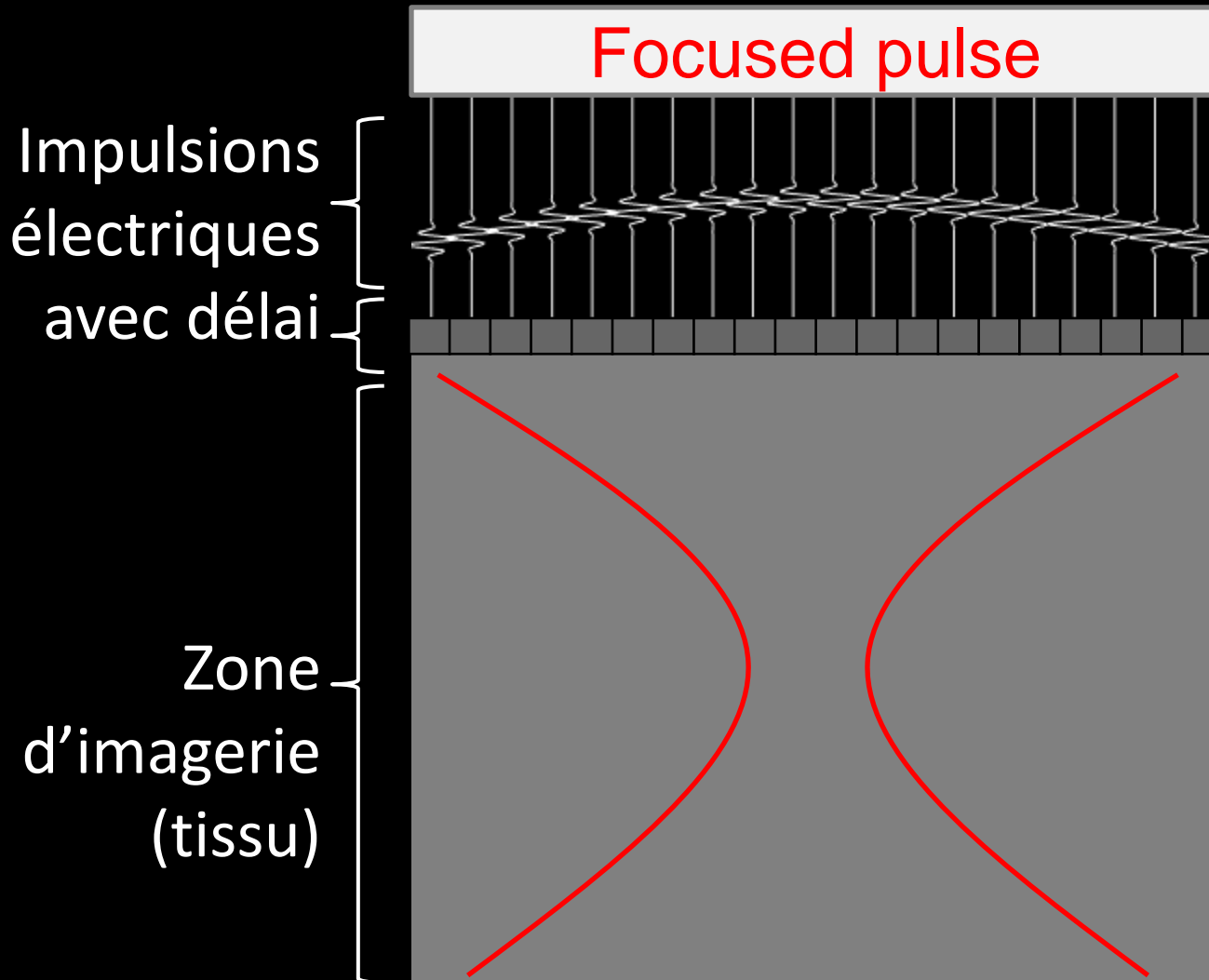
Scanner ultrasonore



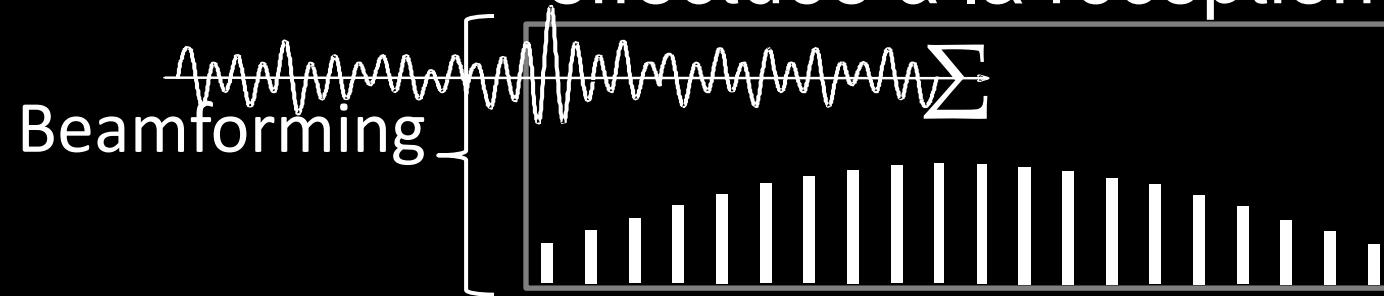
Sonde ultrasonore



# Une sonde multi-éléments permet de focaliser

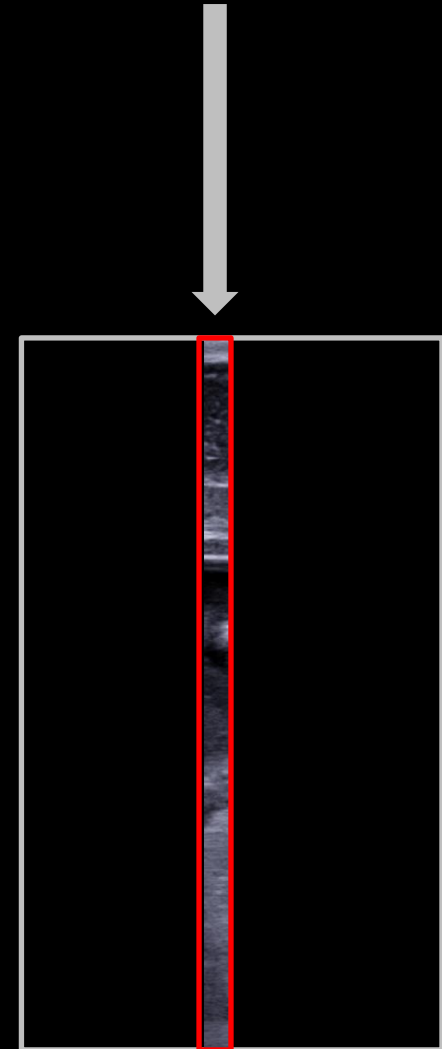
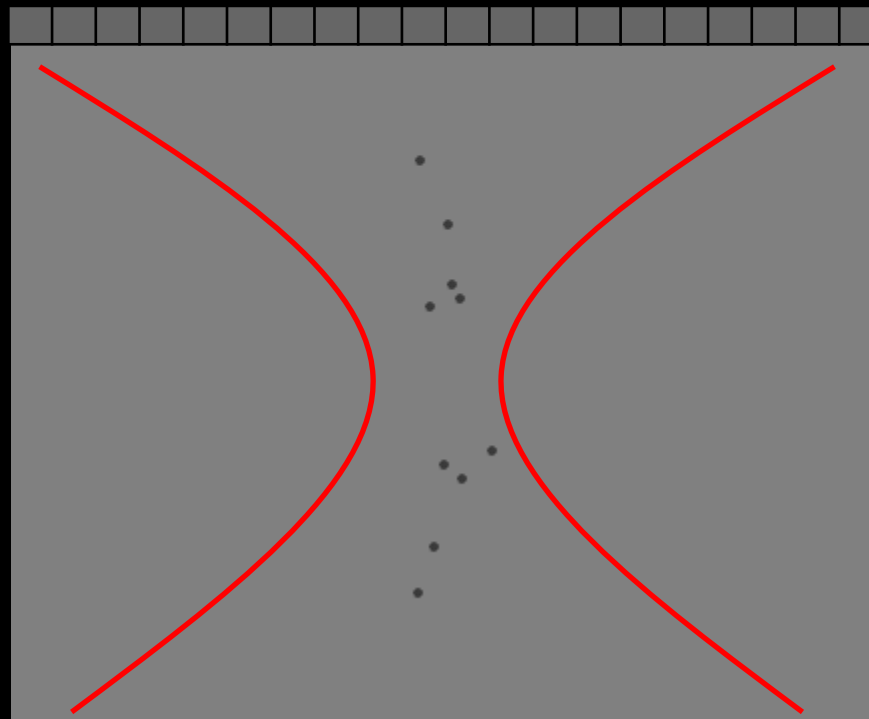


# La focalisation est également effectuée à la réception

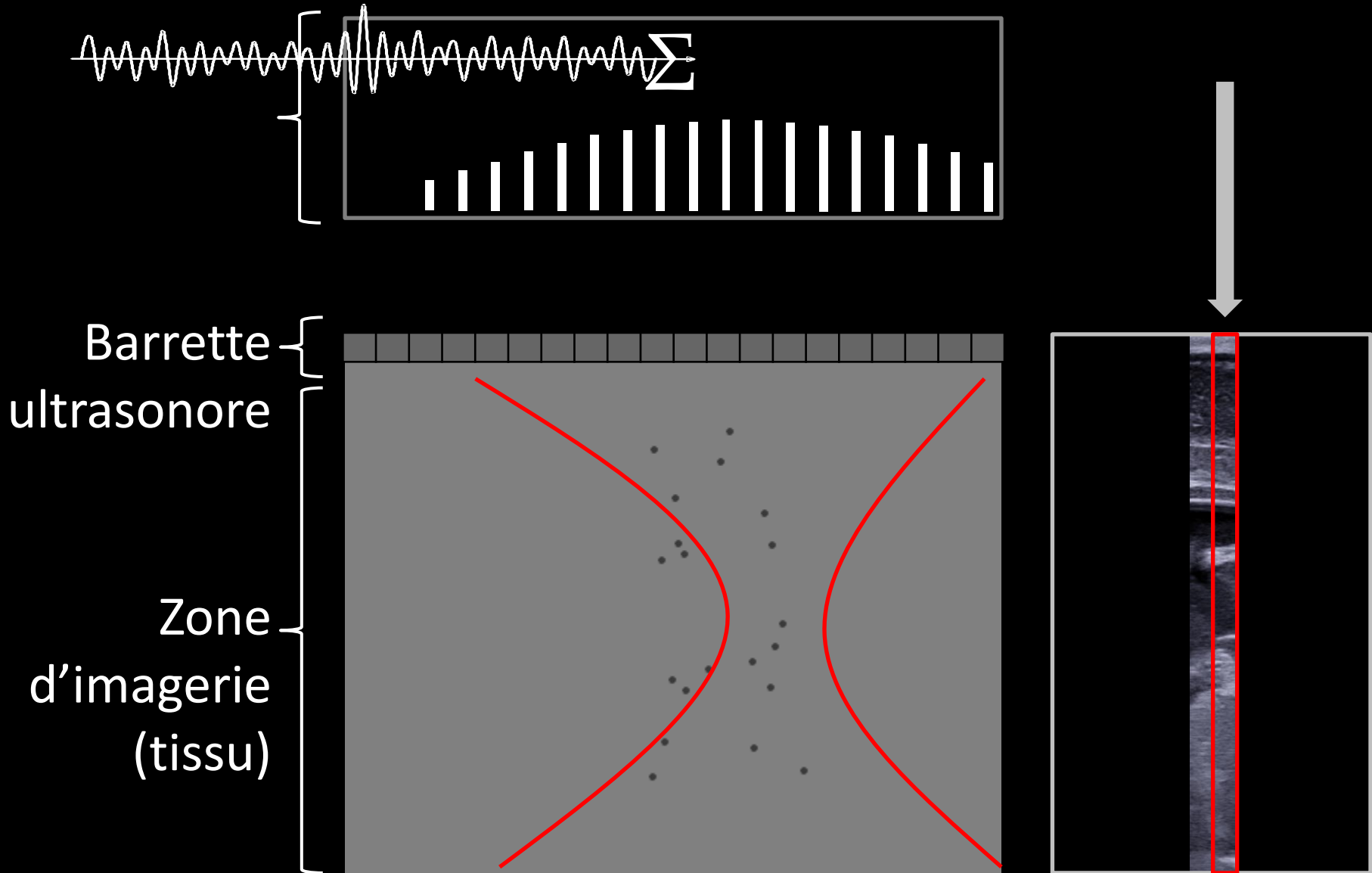


Barrette  
ultrasonore

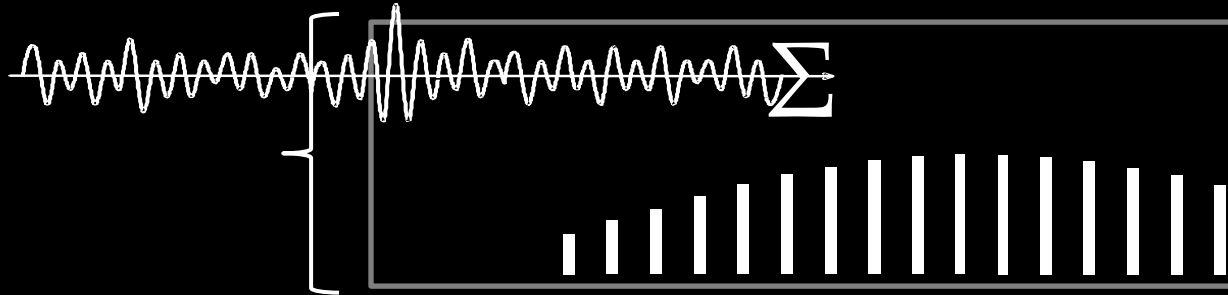
Zone  
d'imagerie  
(tissu)



# Les images sont reconstruites ligne par ligne

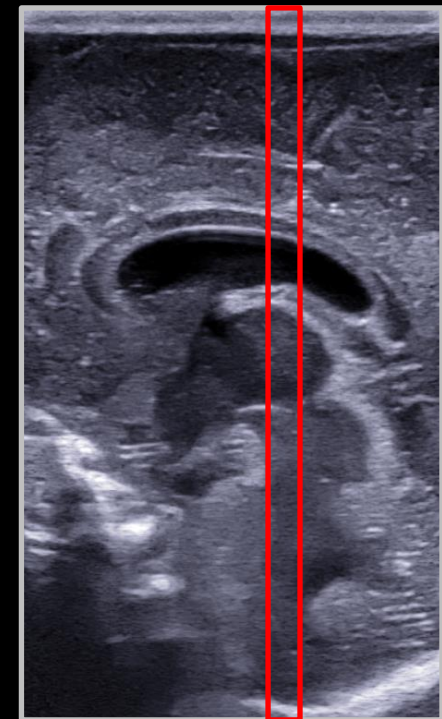
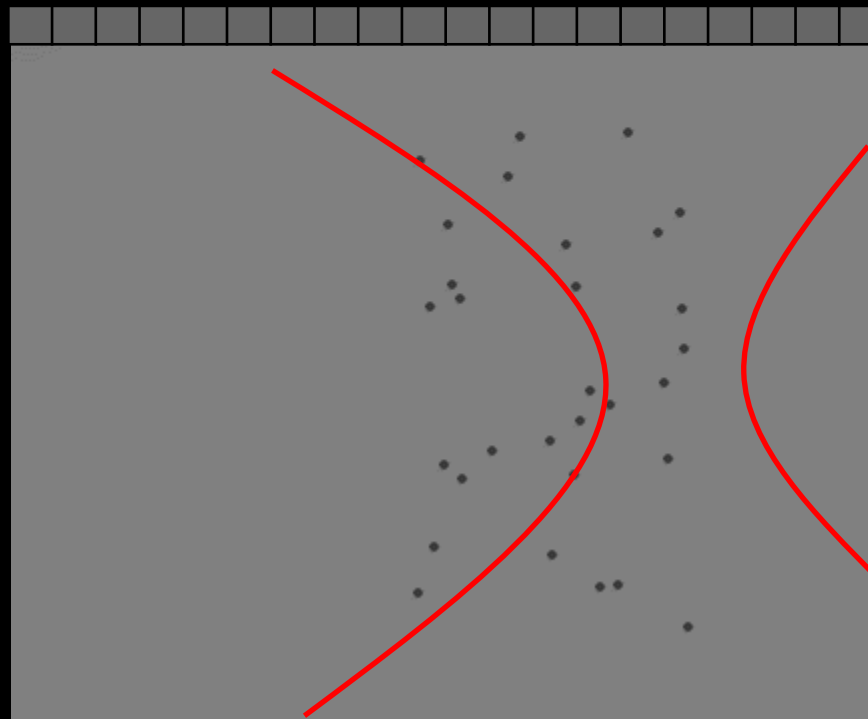


# Les images sont reconstruites ligne par ligne



Barrette  
ultrasonore

Zone  
d'imagerie  
(tissu)

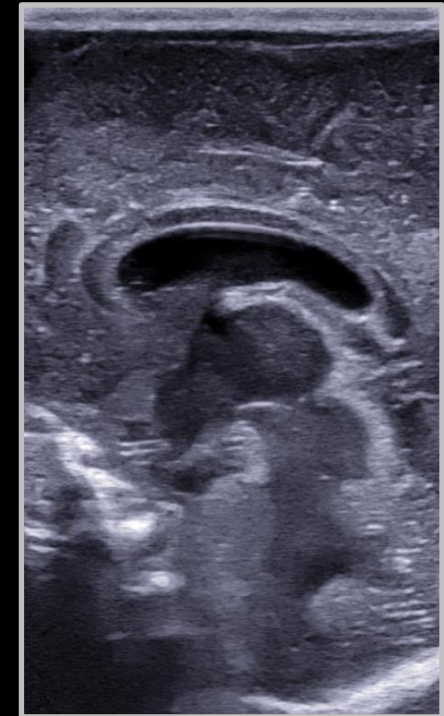
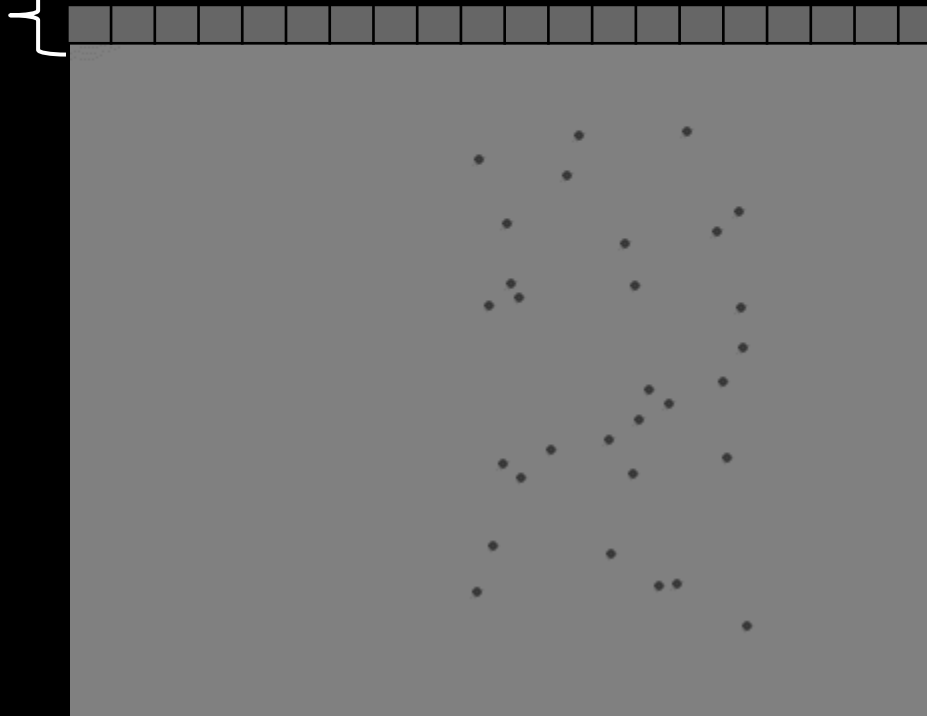




# La focalisation limite la cadence d'imagerie

 Frame Rate  $\sim 50\text{Hz}$

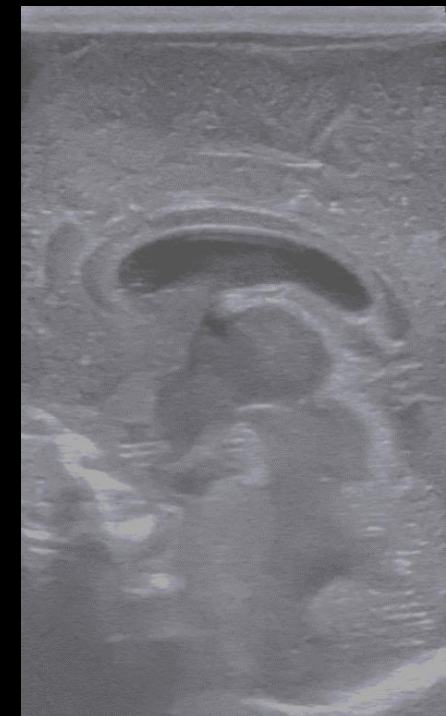
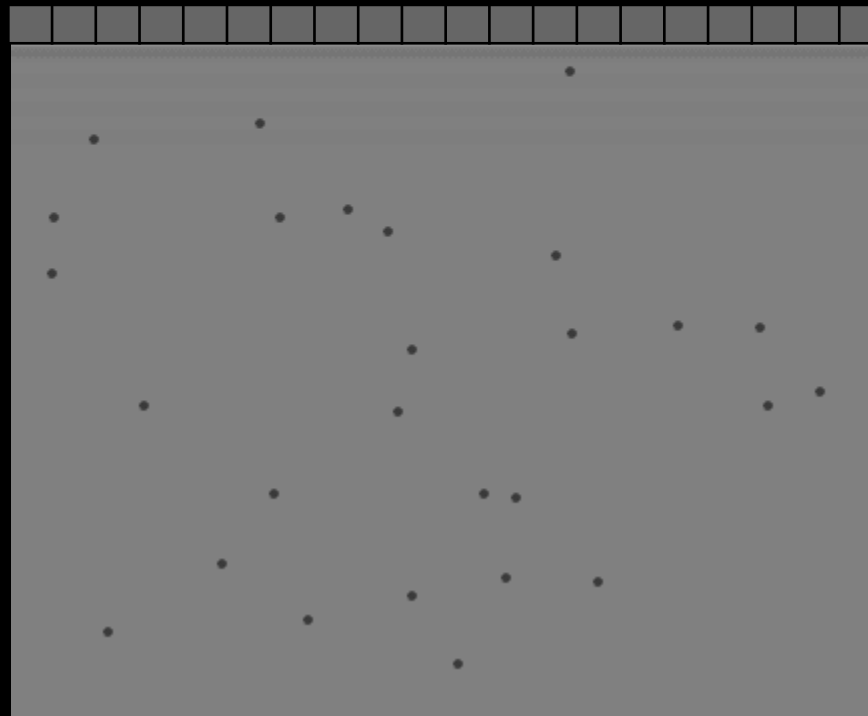
Environ 100  
éléments



L'imagerie ultrarapide est effectuée grâce à des ondes planes

Beamforming Logiciel (GPU)

RAM memory



# L'imagerie ultrarapide peut reconstruire l'élasticité des tissus en optique



Camera : 50 fps

BHW: (Belly Half Width) : 20 cm

Shear Wave speed :  $c = \text{BHW}/0.2 \text{ s} = 1 \text{ m/s}$

$c = 1 \text{ m/s}$  gives  $E = 3 \text{ kPa}$

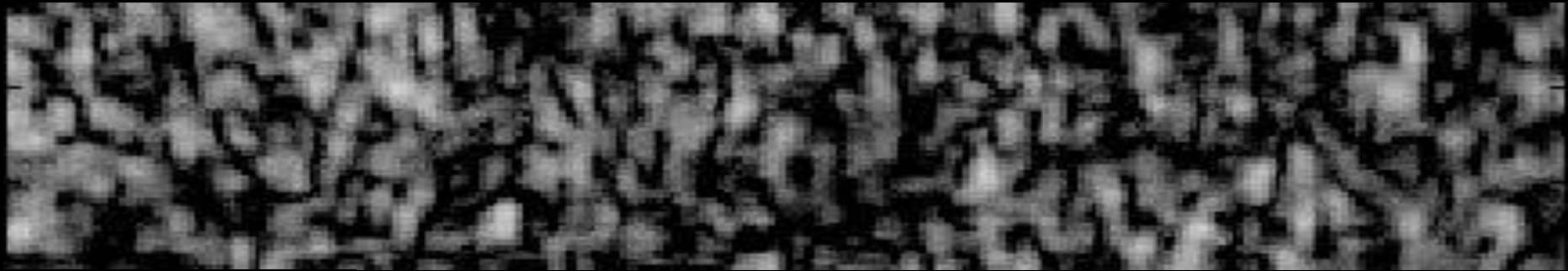
Conclusion :  $E = 3 \text{ kPa}$  correspond à l'élasticité du gras!



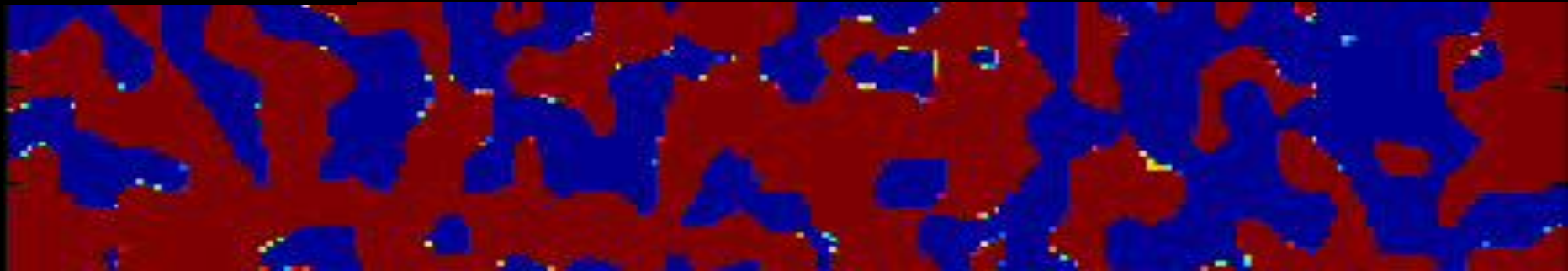
# La propagation d'une onde de cisaillement peut être filmée avec l'imagerie ultrarapide

10000 images par seconde

Speckle



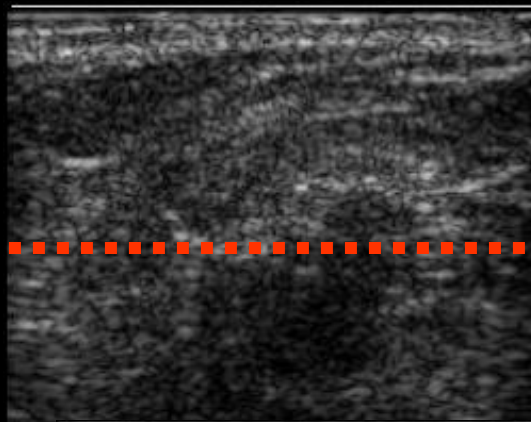
Motion tracking



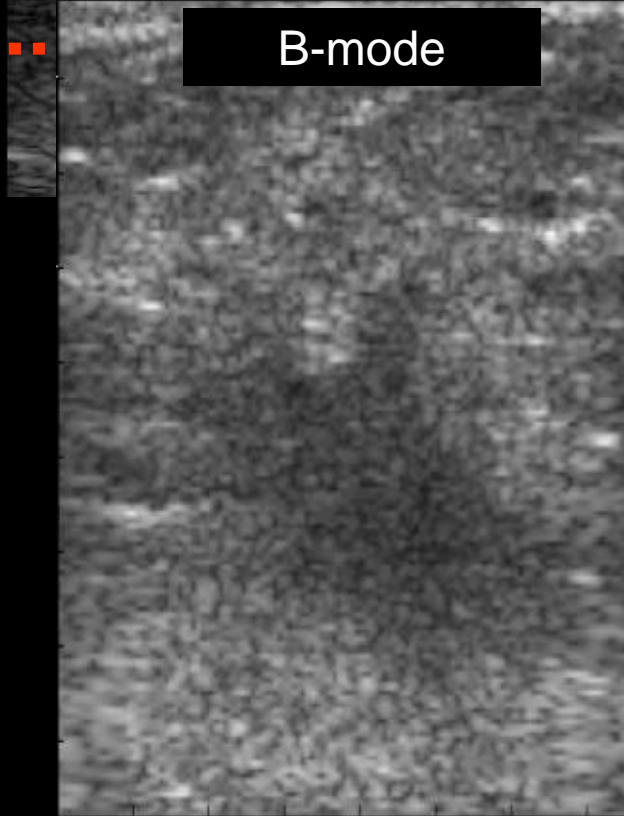
M. Fink, M. Tanter, "Multiwave Imaging and Superresolution"  
Physics Today, 63(2), 28-33, Feb. 2010

# L'élasticité des tumeurs du sein est mesurée

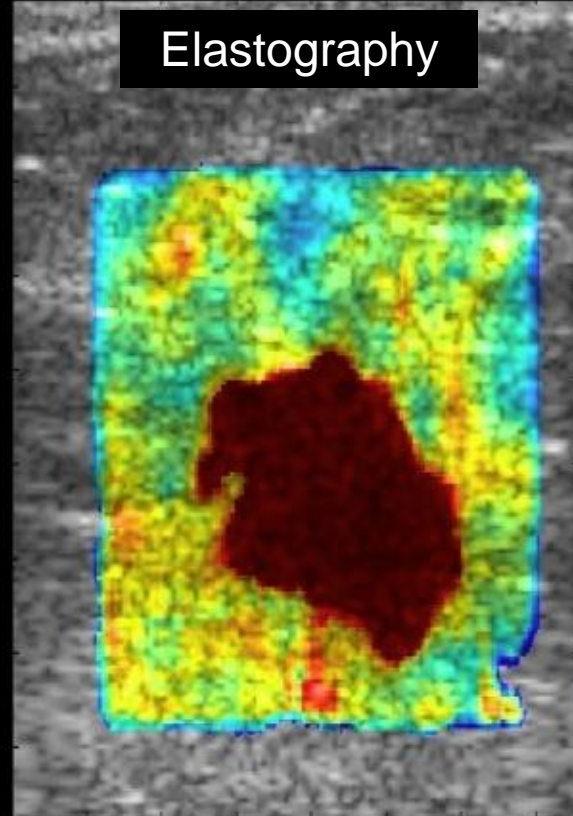
Tumeur  
du sein



Propagation  
de l'onde de  
cisaillement

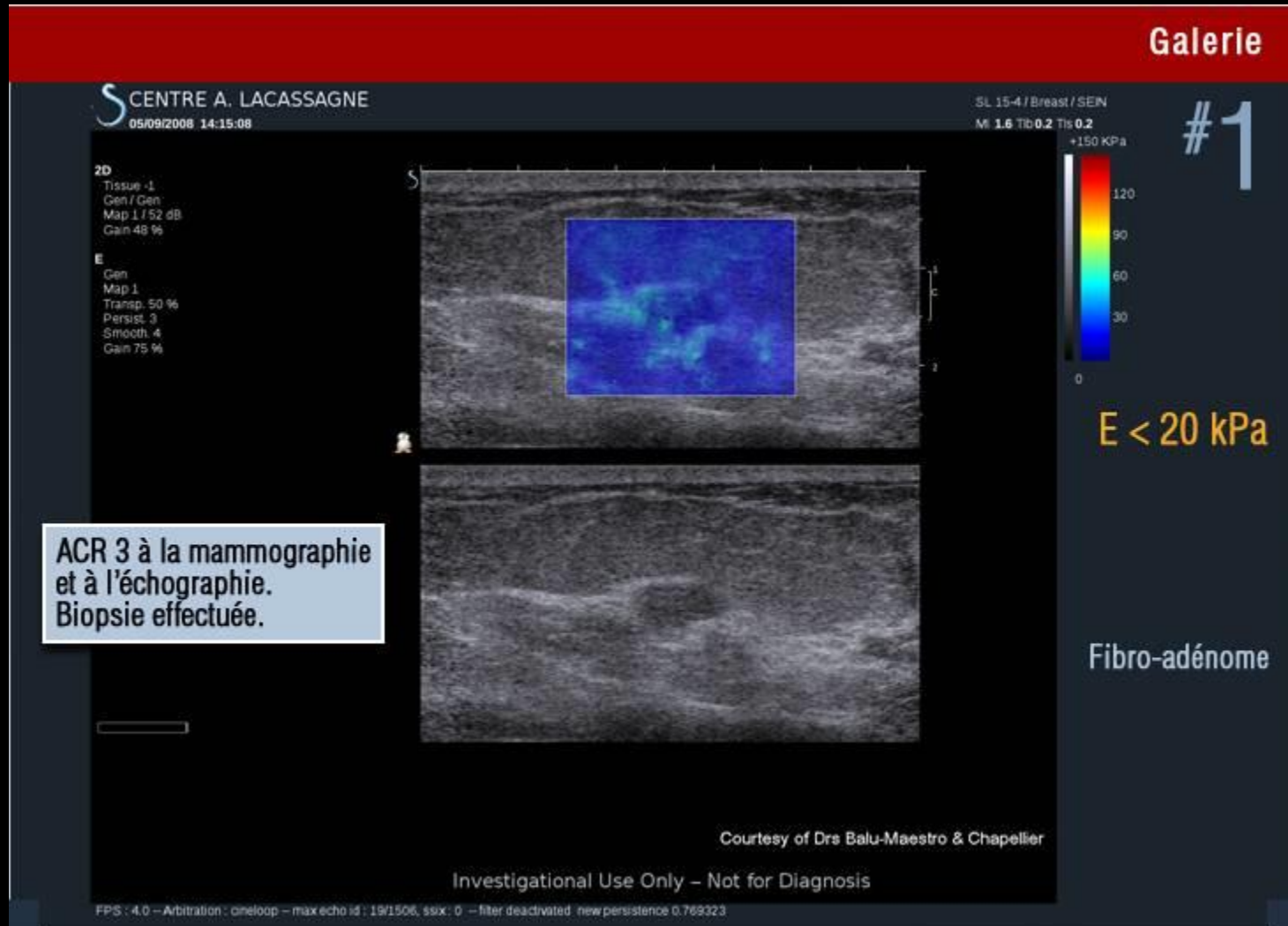


B-mode

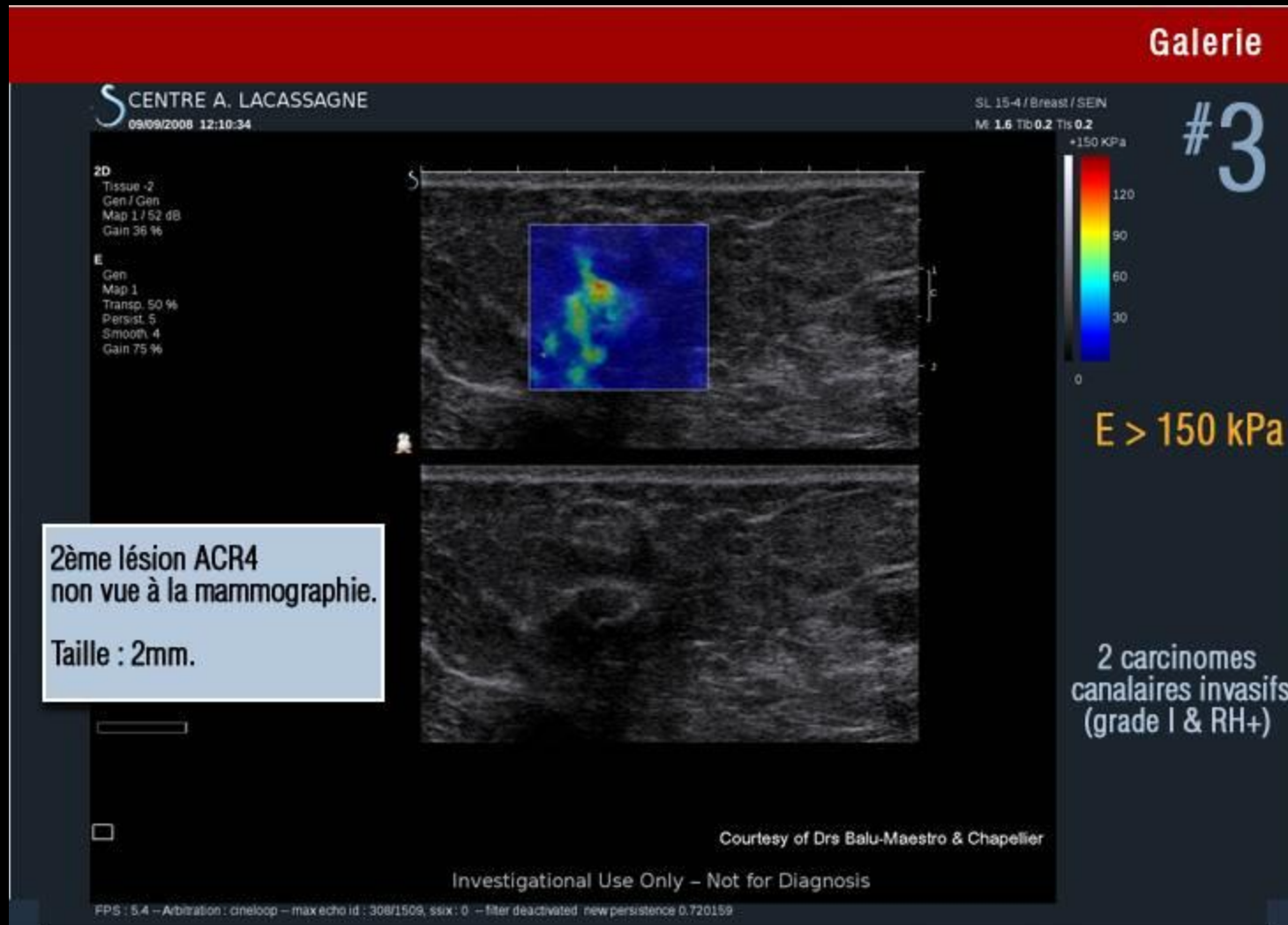


Elastography

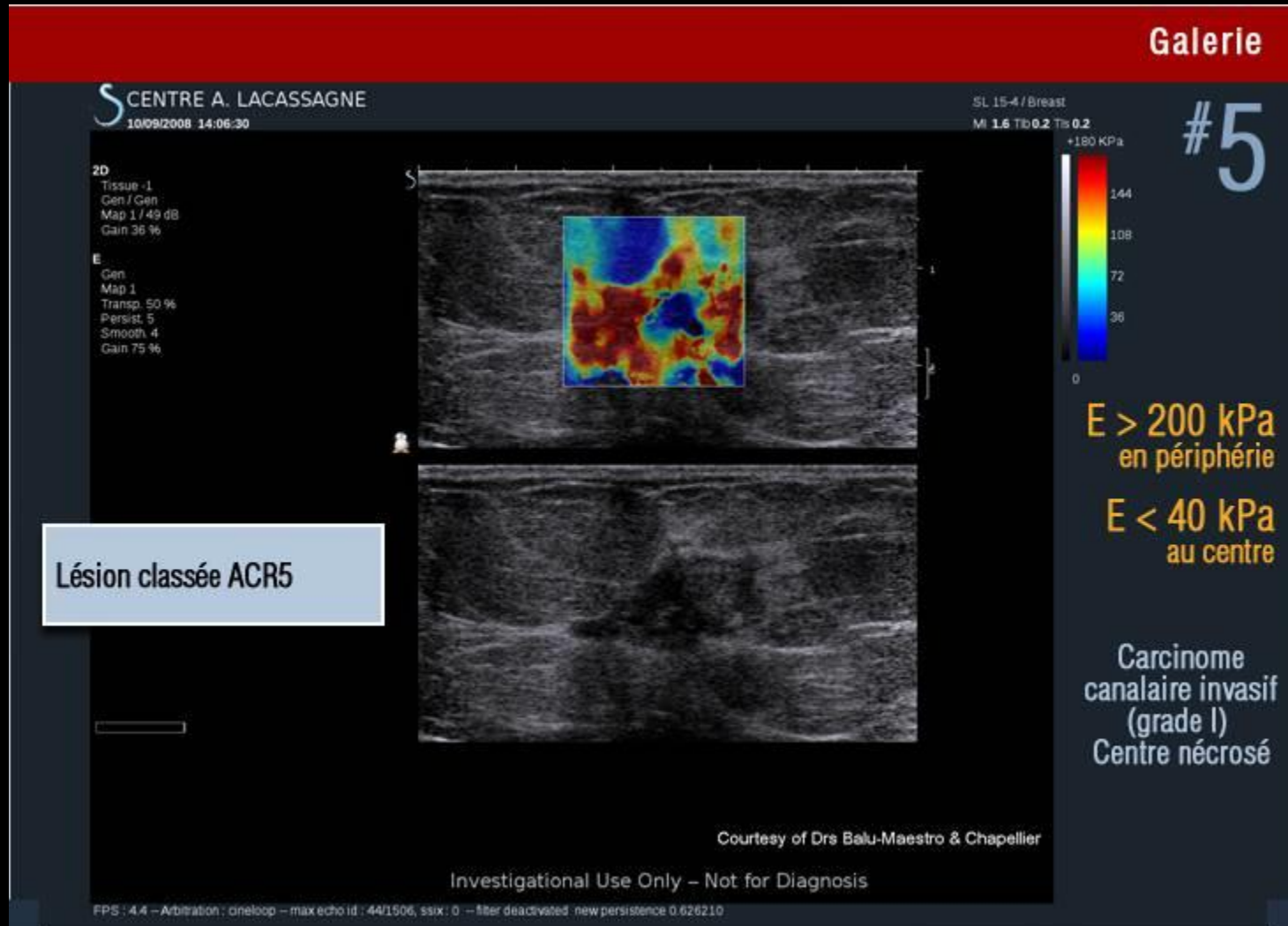
# L'élastographie peut maintenant distinguer les tumeurs bénignes et malignes



# L'élastographie peut maintenant distinguer les tumeurs bénignes et malignes



# L'élastographie peut maintenant distinguer les tumeurs bénignes et malignes



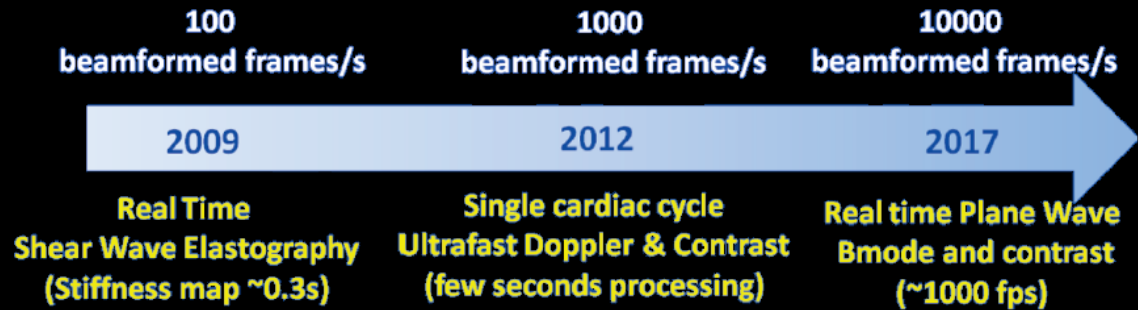
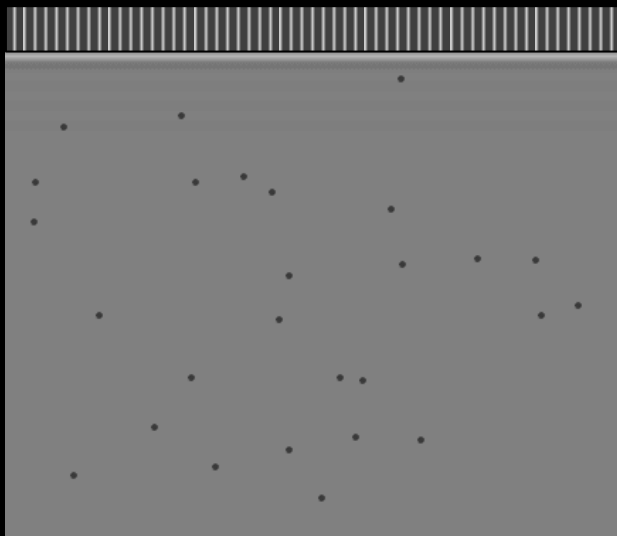
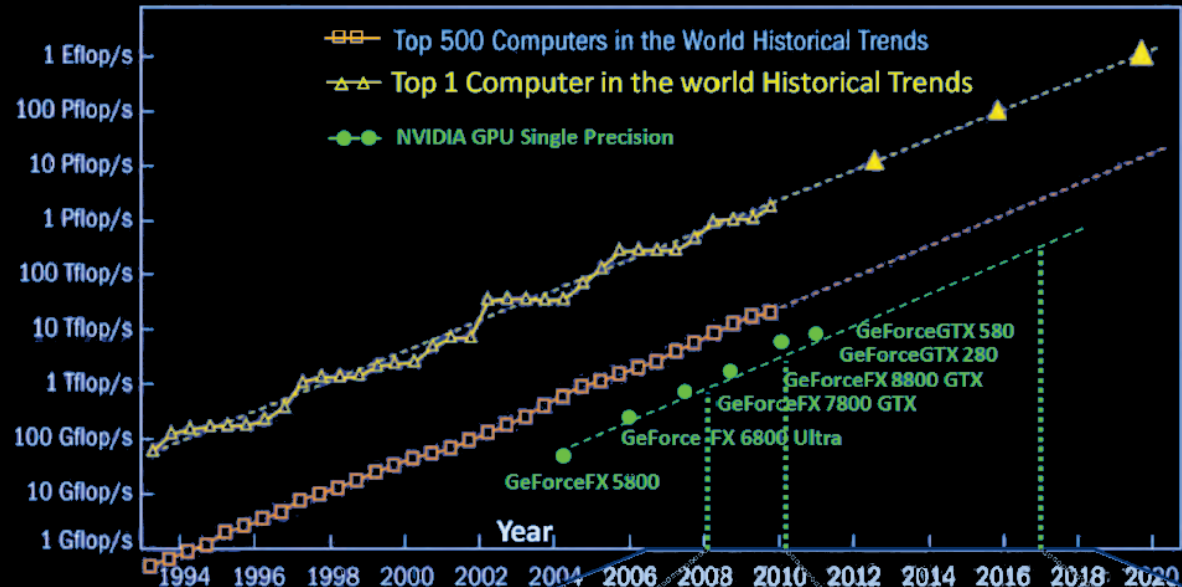


# Le prototype a permis un autre prototype et puis un scanner clinique



# Le rêve de l'imagerie ultrarapide temps réel est devenu réalité grâce aux cartes graphiques

100 lines  
 1000 time points  
 1000 frames per second  
 = 1 Gb/s



Tanter et al. IEEE UFFC 2013

O. Couture et al., Mai 2017

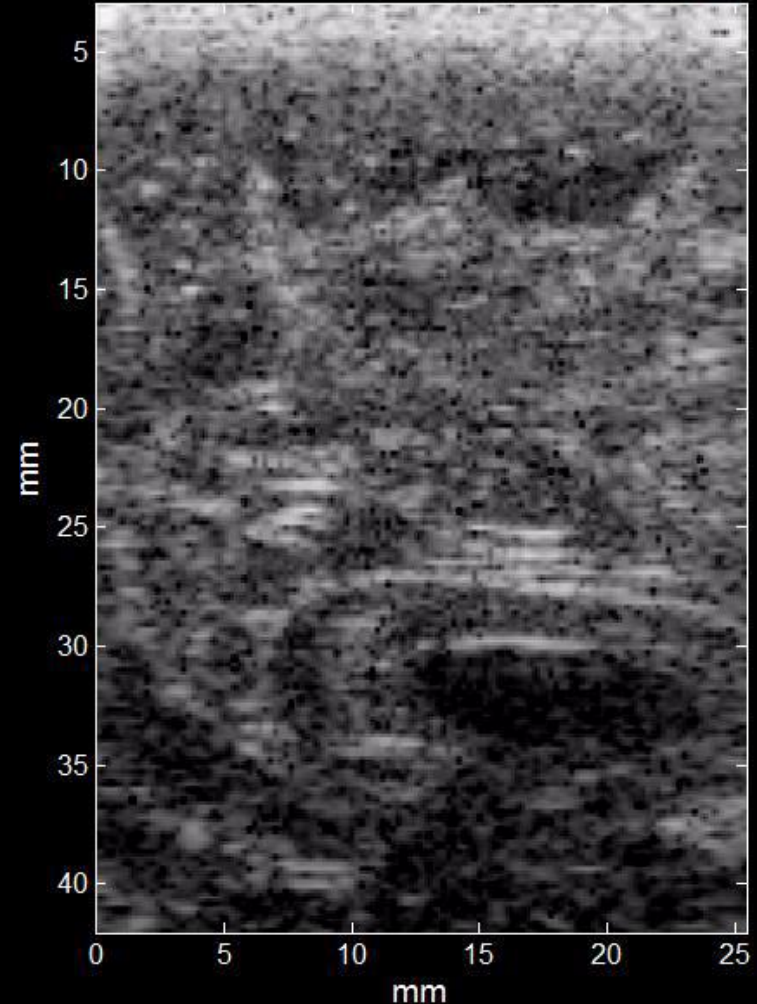
# L'imagerie ultrarapide donne accès à une nouvelle physique

## Shear wave travelling



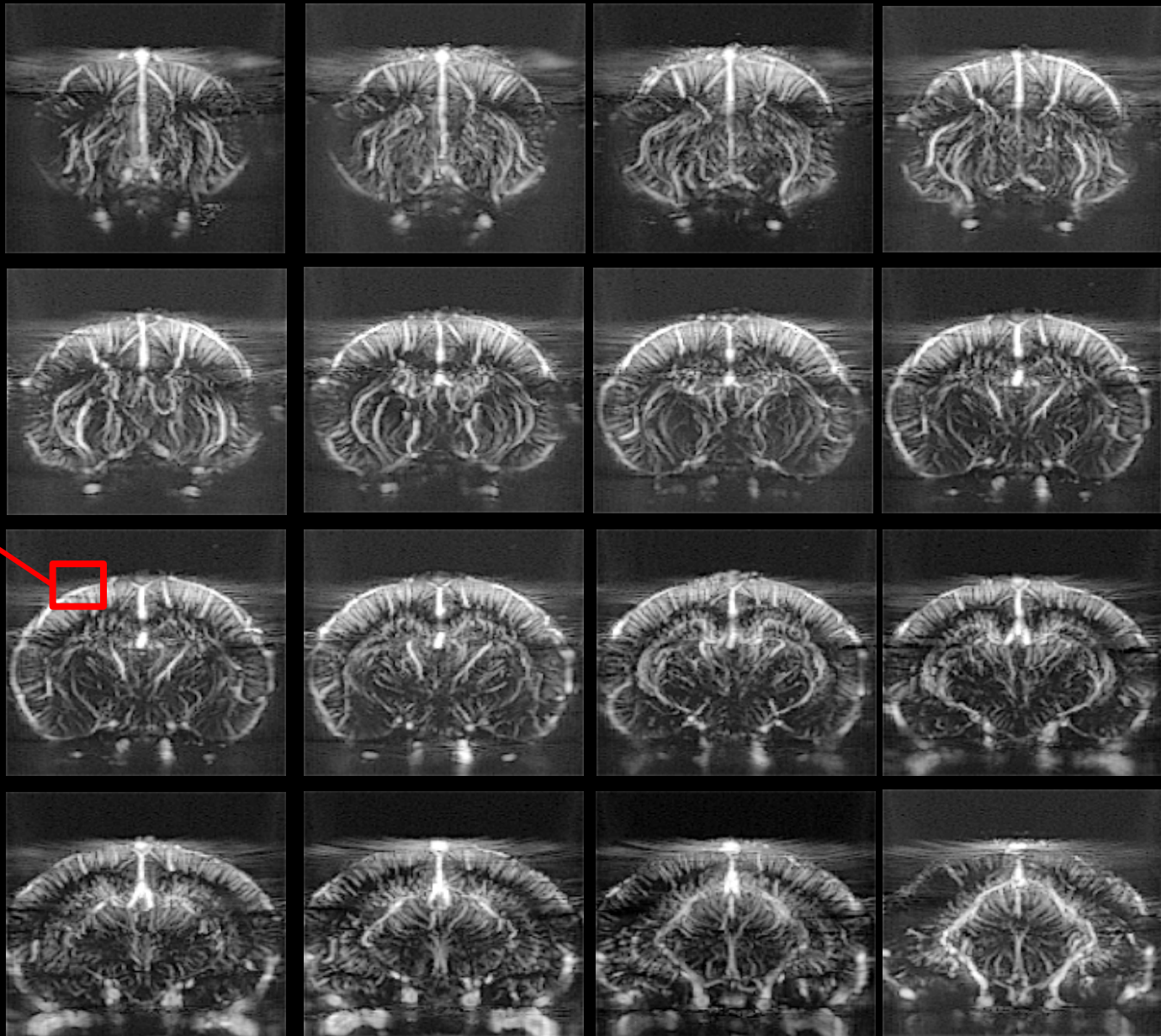
## Blood motion (Doppler)

$t = -0.20$  ms

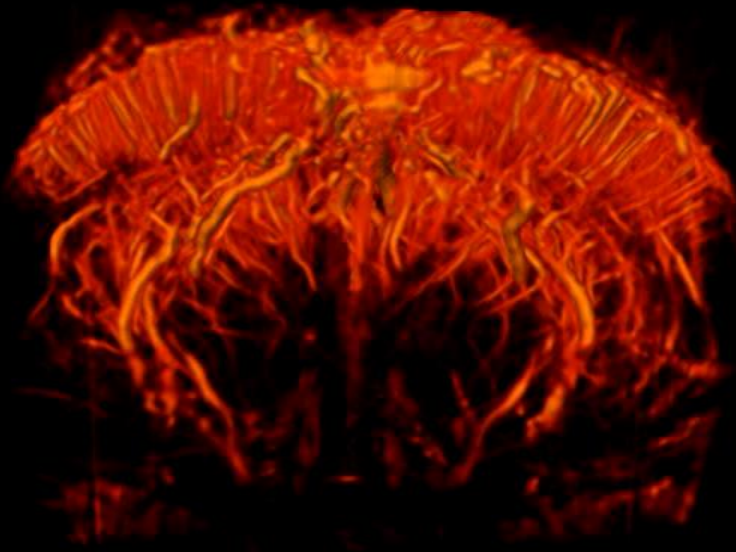


# L'imagerie ultrarapide permet un gain significatif en sensibilité. Ici sur un cerveau de rat.

Local  
blood  
flow



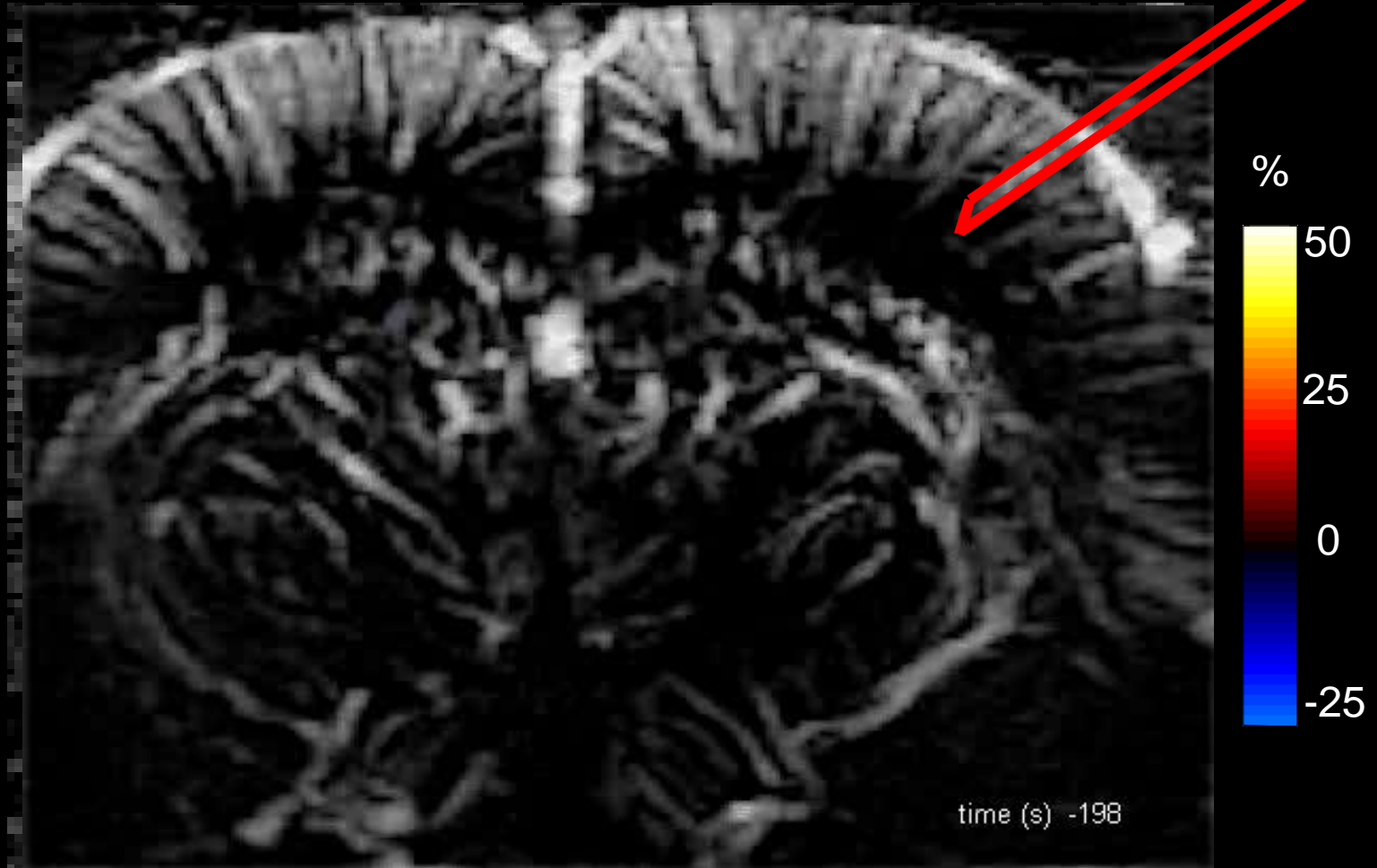
Tue Dec 09 15:34:02 2014



Amira

Demene et al. 2015

# L'activation de régions du cerveau, lors de l'épilepsie, peut être observée par échographie fonctionnelle



Macé et al., Nature Methods 2011

# Les ultrasons fonctionnels permettent d'imager les animaux vivants et éveillés



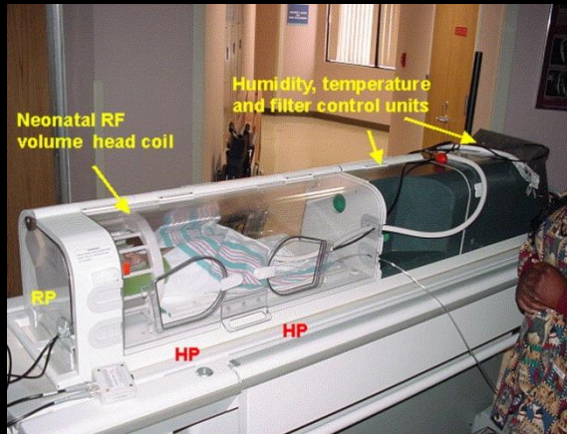
fMRI



fUltrasound

# L'échographie fonctionnelle clinique pourrait combler les lacunes de l'IRM

## Neuroimagerie des nouveaux-nés

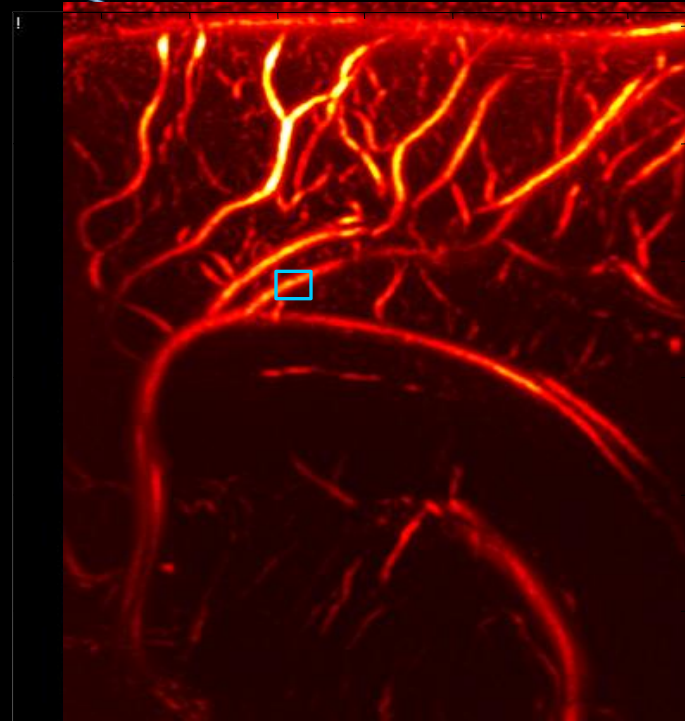
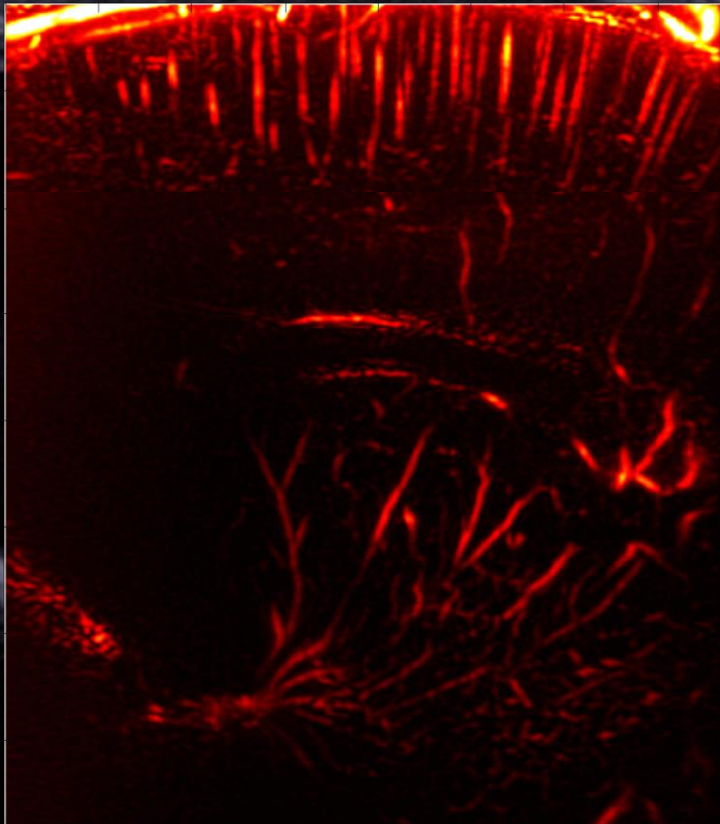


## Neuroimagerie per-opératoire









Medulla

5 10 15 20 25 30 35  
mm

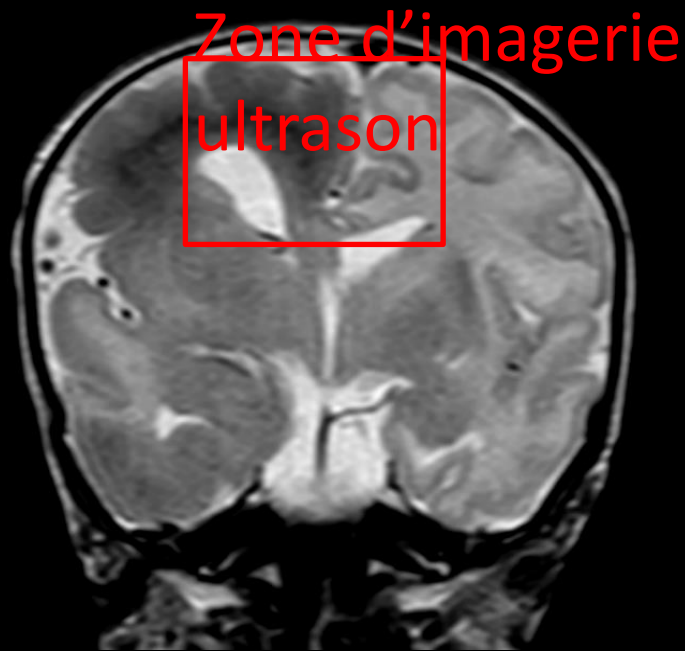
Cerebellum

Collaboration  
Institut Langevin & Hopital Robert Debré, Paris

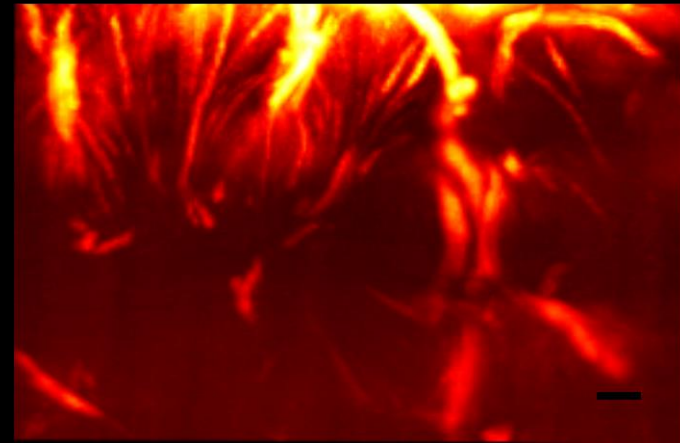
C. Demené, O. Baud, V. Biran, M. Alison  
M. Pernot and M. Tanter



# Imagerie temps-réel d'une pathologie d'un grand prématuré

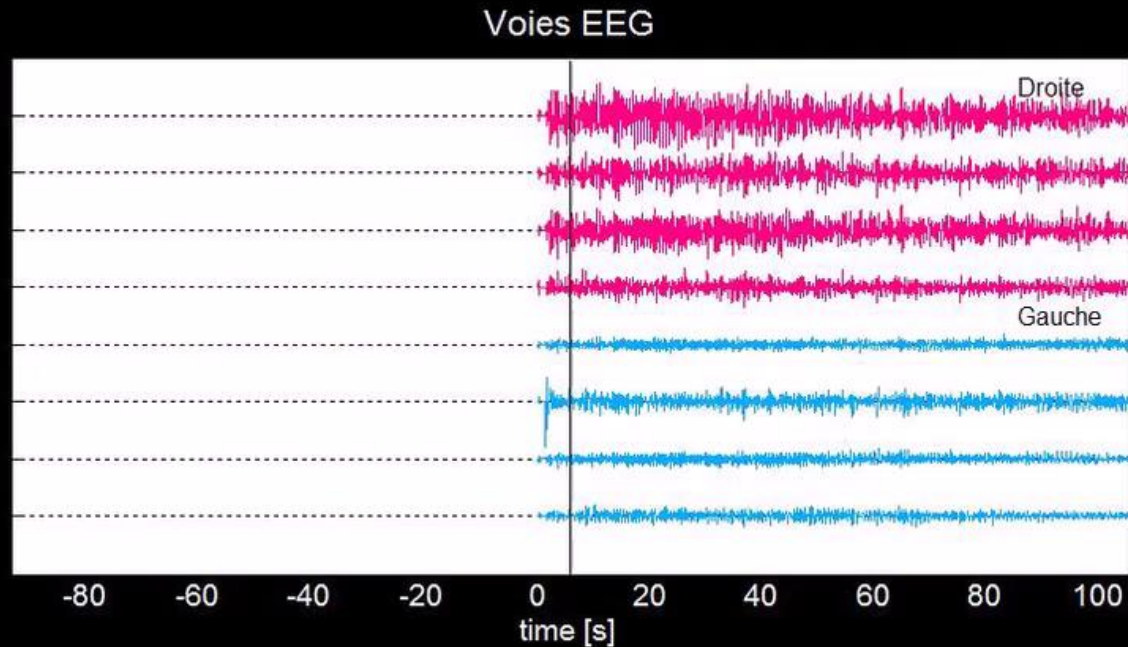
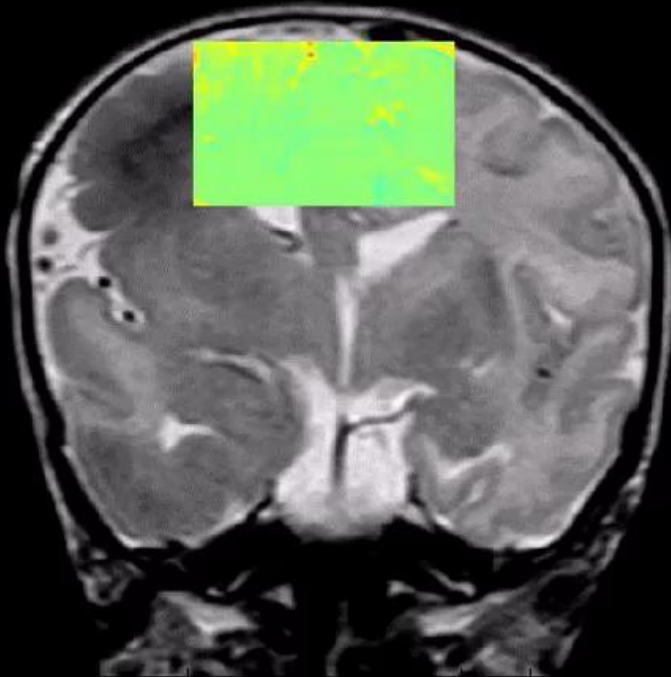


IRM T2  
Coronal



C.Demené et al, under review, 2017

# Le Doppler ultrasensible peut détecter les crises d'épilepsie chez le nouveau-né

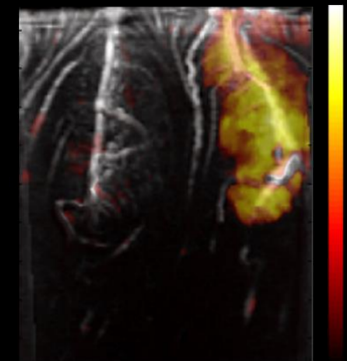
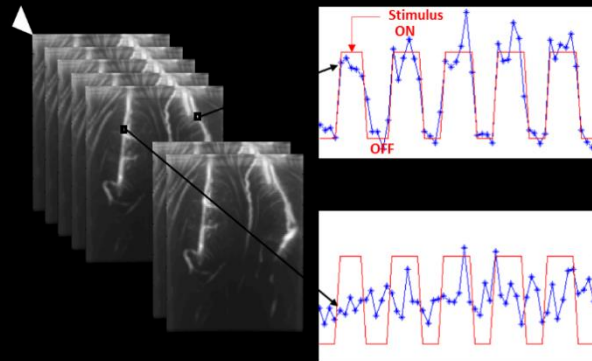
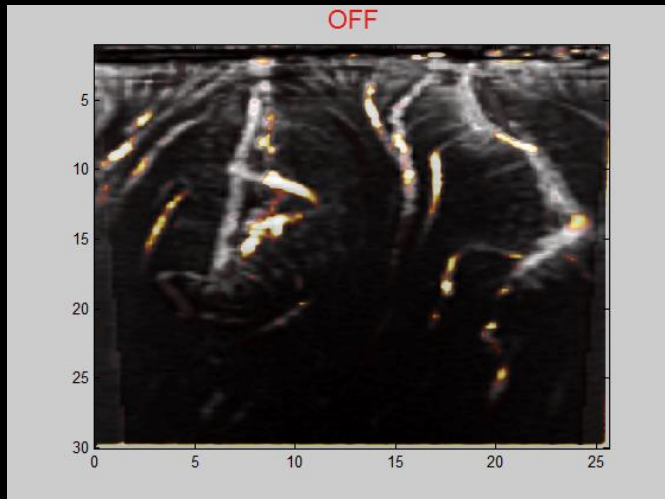
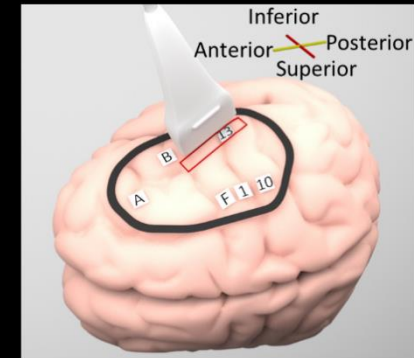
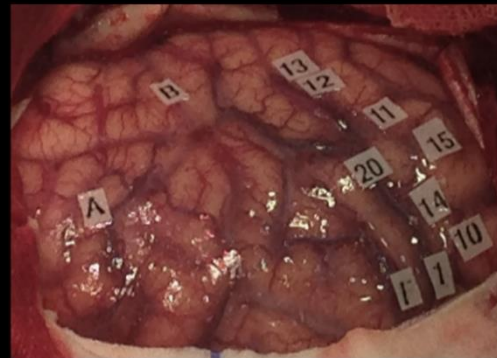


C.Demené et al, under review, 2017

# L'échographie fonctionnelle peut être réalisée pendant une chirurgie

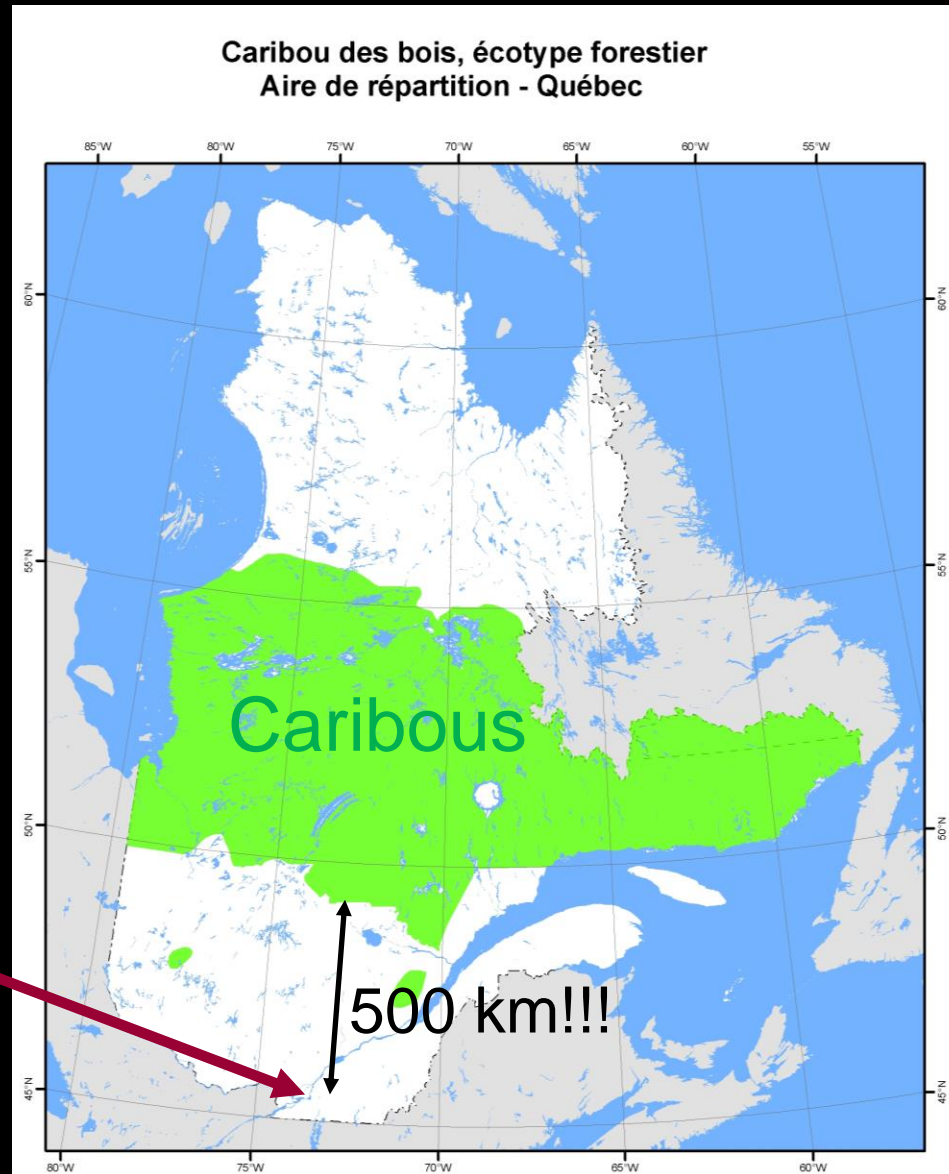


Collaboration D. Chauvet, L. Capelle  
Service de Neurochirurgie, Pitié Salpêtrière  
PhD Marion Imbault



M.Imbault et al, under review, 2017

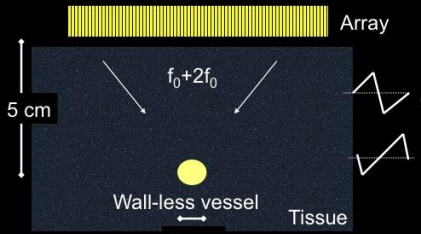
# Il n'y a pas de caribou à Montréal



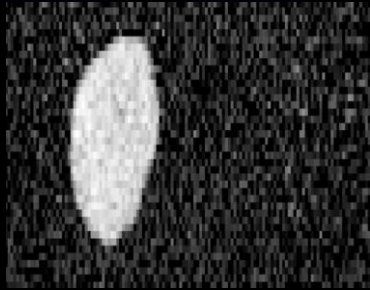
Montréal

# Mes intérêts: la synergie ultrasons + agents injectables

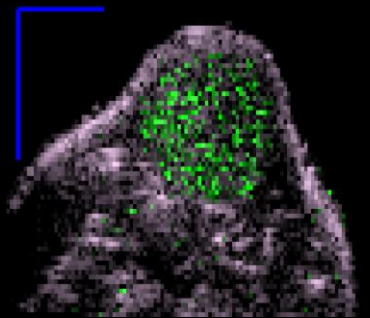
## Imagerie



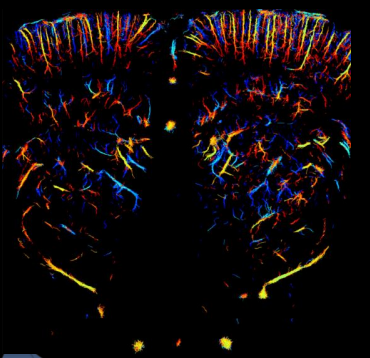
Nonlinear propagation correction



Ultrafast contrast imaging



Molecular imaging and functional imaging



Ultrasound localization microscopy

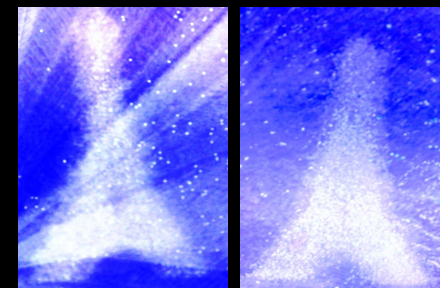
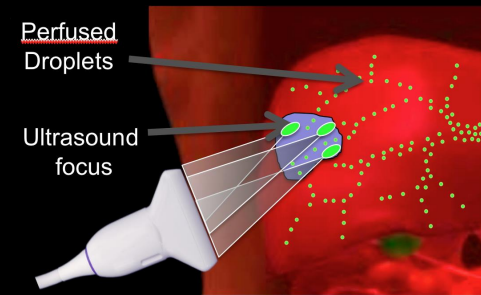
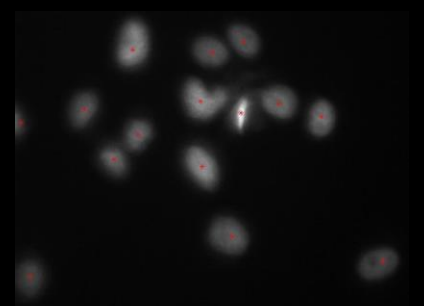
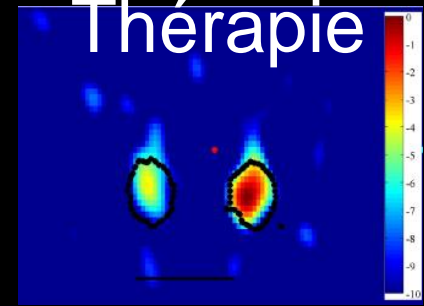
Molecular focusing

Sonoporation

Targeted drug-delivery

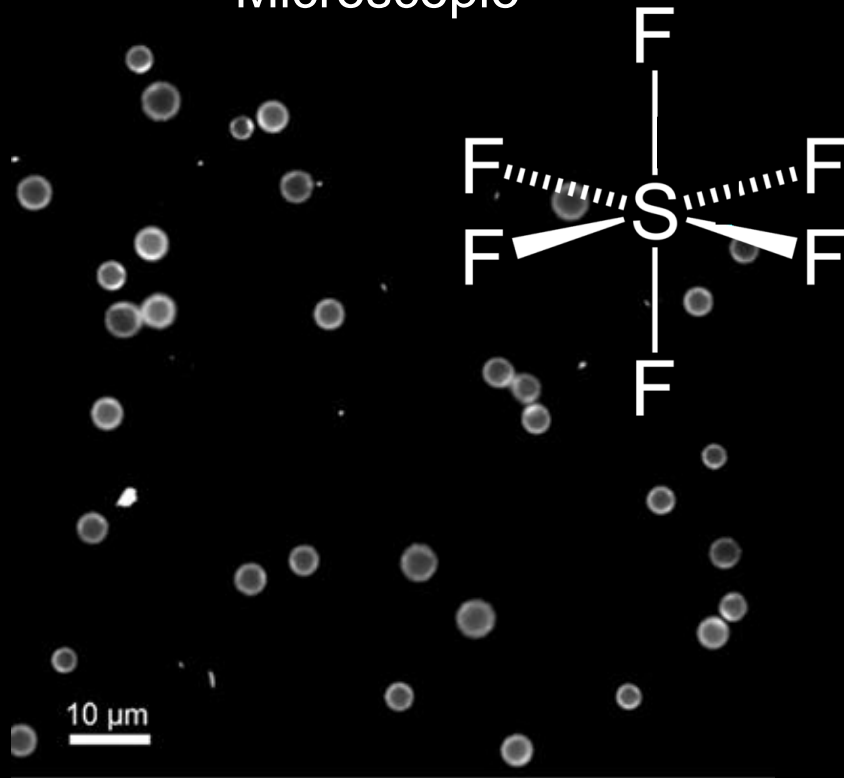
Chemistry in-situ

## Thérapie



# En échographie, les microbulles sont utilisées pour révéler les vaisseaux sanguins

Microscopie



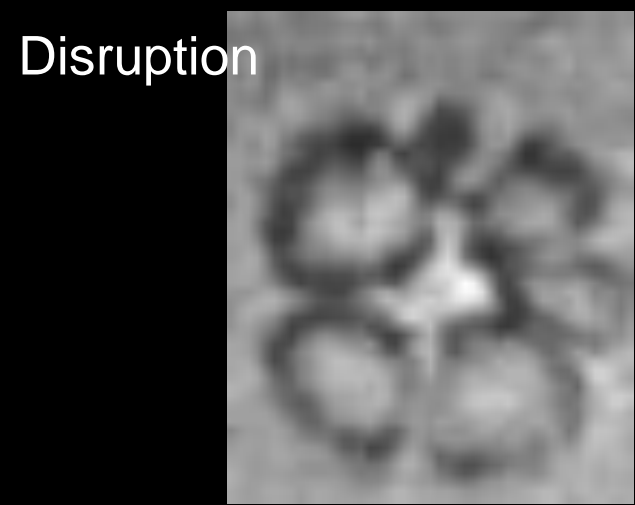
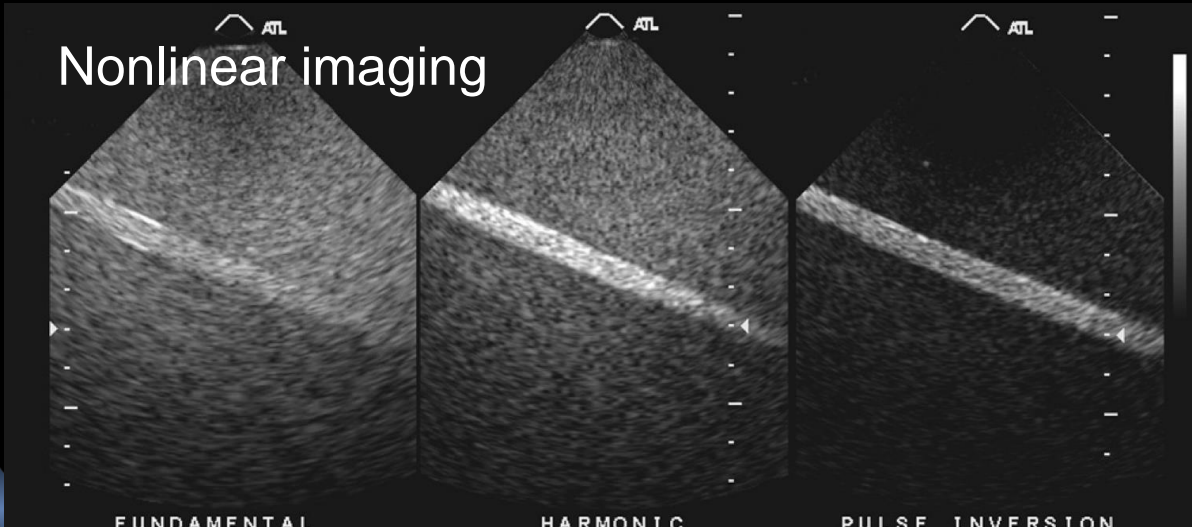
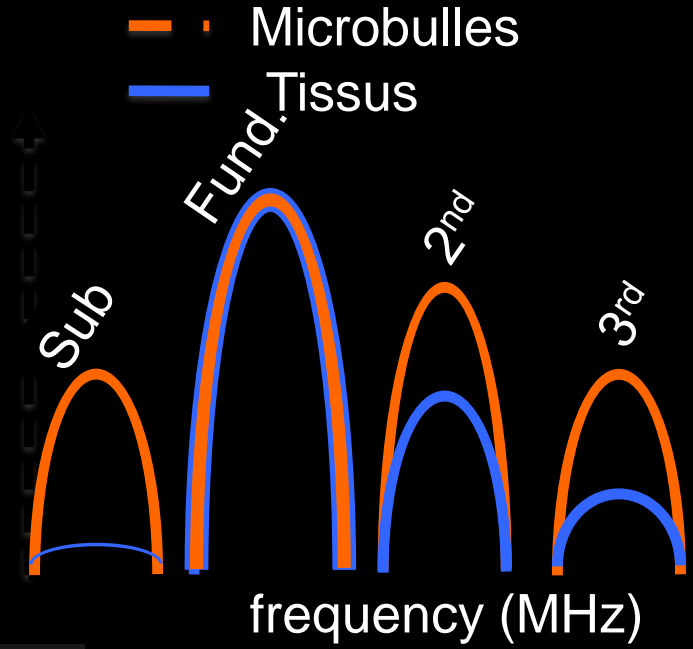
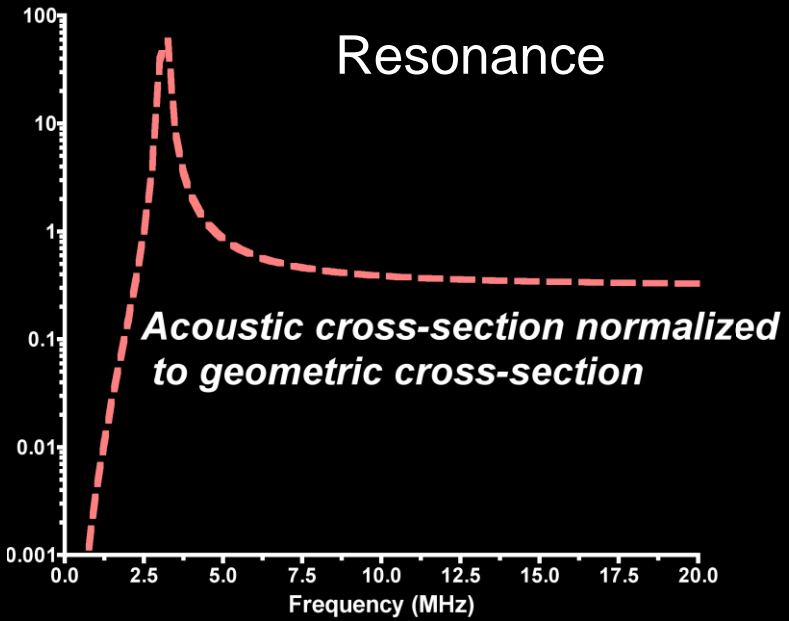
Agents de contraste ultrasonore



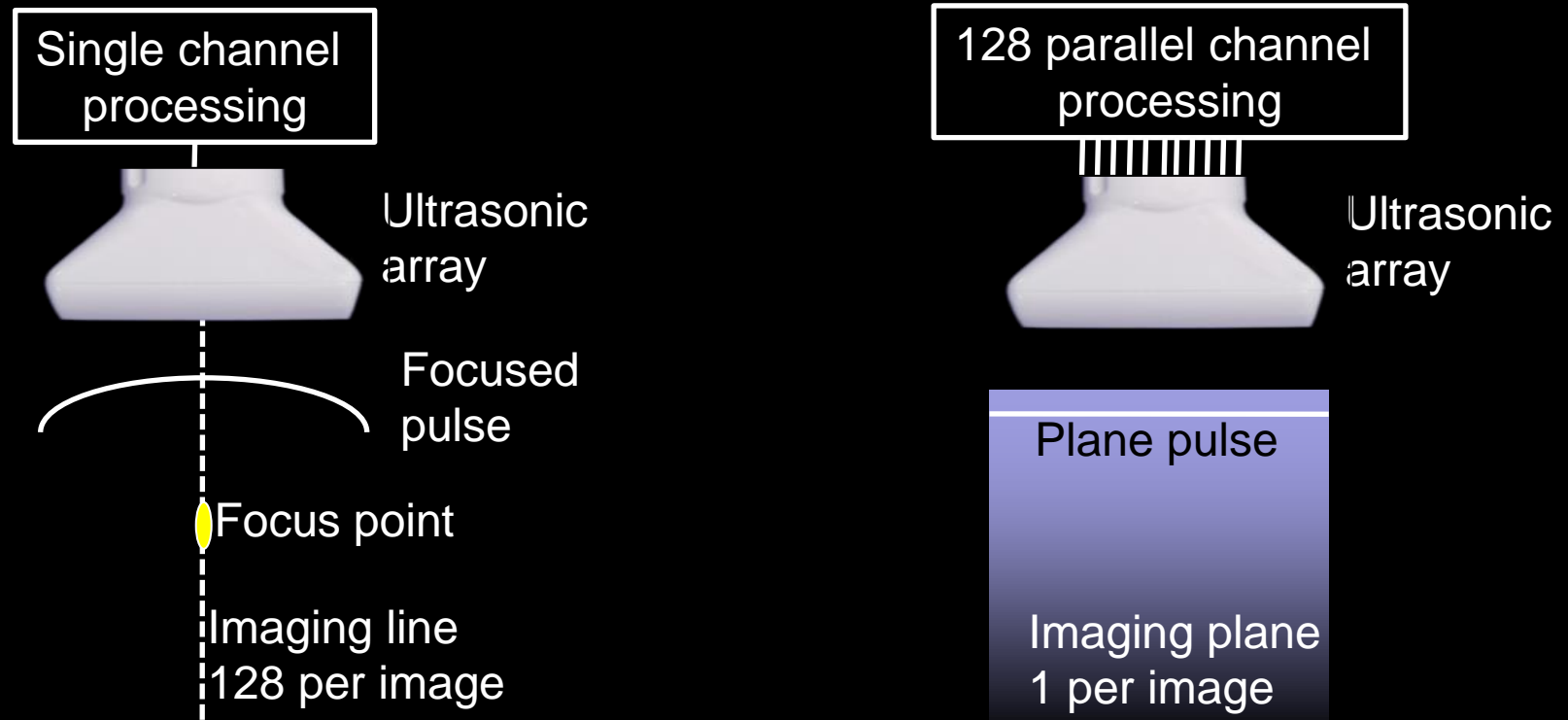
See Burns et al., 2006



# Les bulles cliniques sont résonantes et non-linéaires



# L'imagerie ultrarapide est adaptée à l'imagerie des agents de contraste



Couture et al. UMB 2009

Couture et al. Molecular imaging 20122

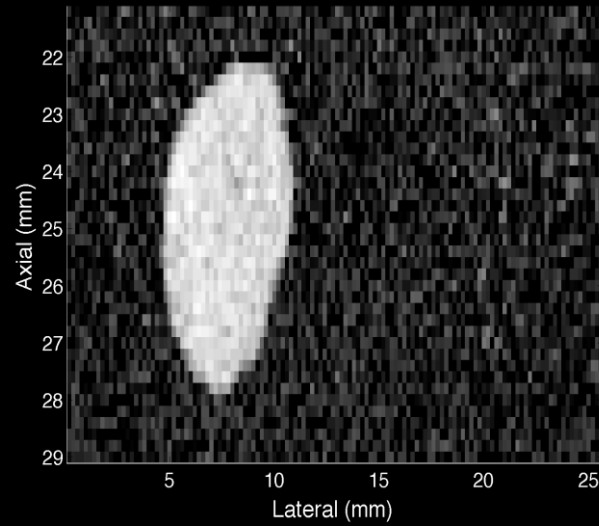
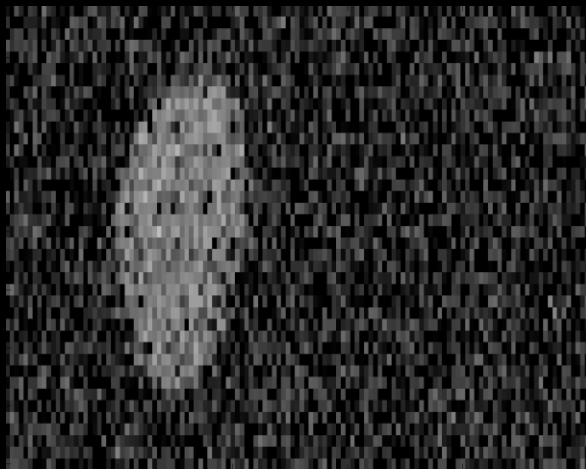
Couture et al. IEEE UFFC 2012

# Les agents de contraste sont plus facilement détectés en imagerie ultrarapide

## Conventionnel

## Ultrarapide

PIAM



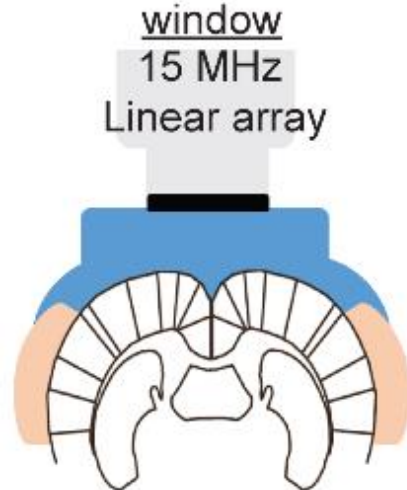
12.5 dB  
Gain



# Les agents de contraste ultrasonore permettent une imagerie transcrânienne



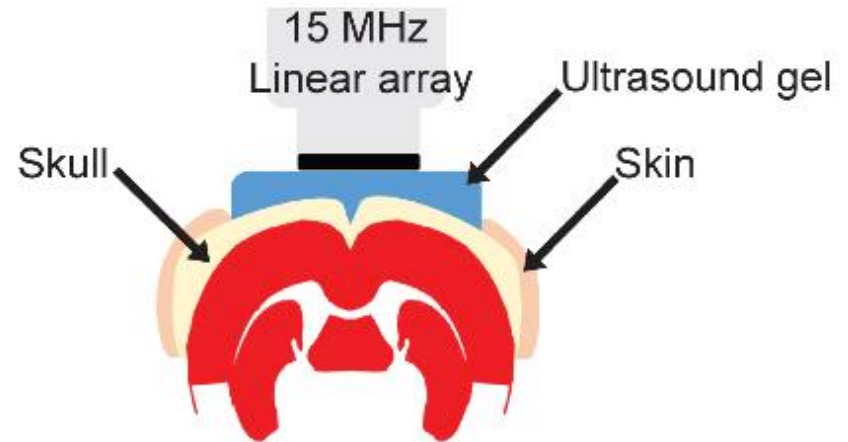
Bilateral Thinned-skull



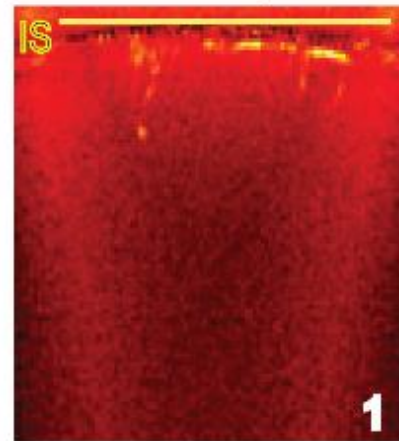
Without Microbubbles



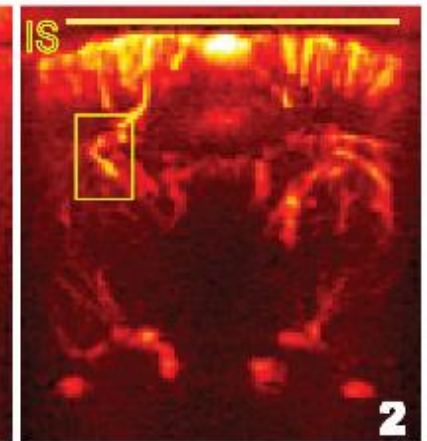
**B** Intact Skull (IS)



**E** Without Microbubbles

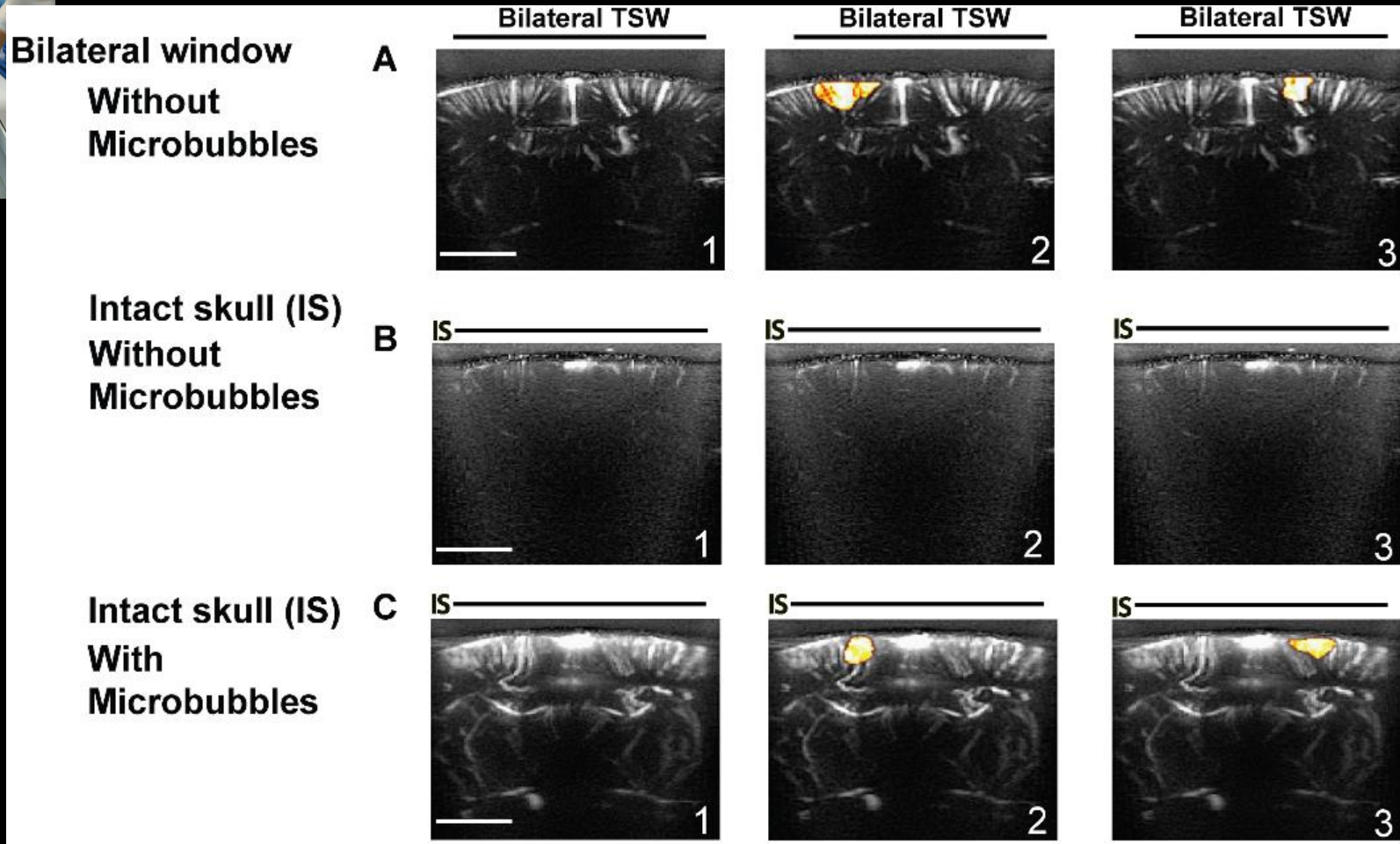


With Microbubbles



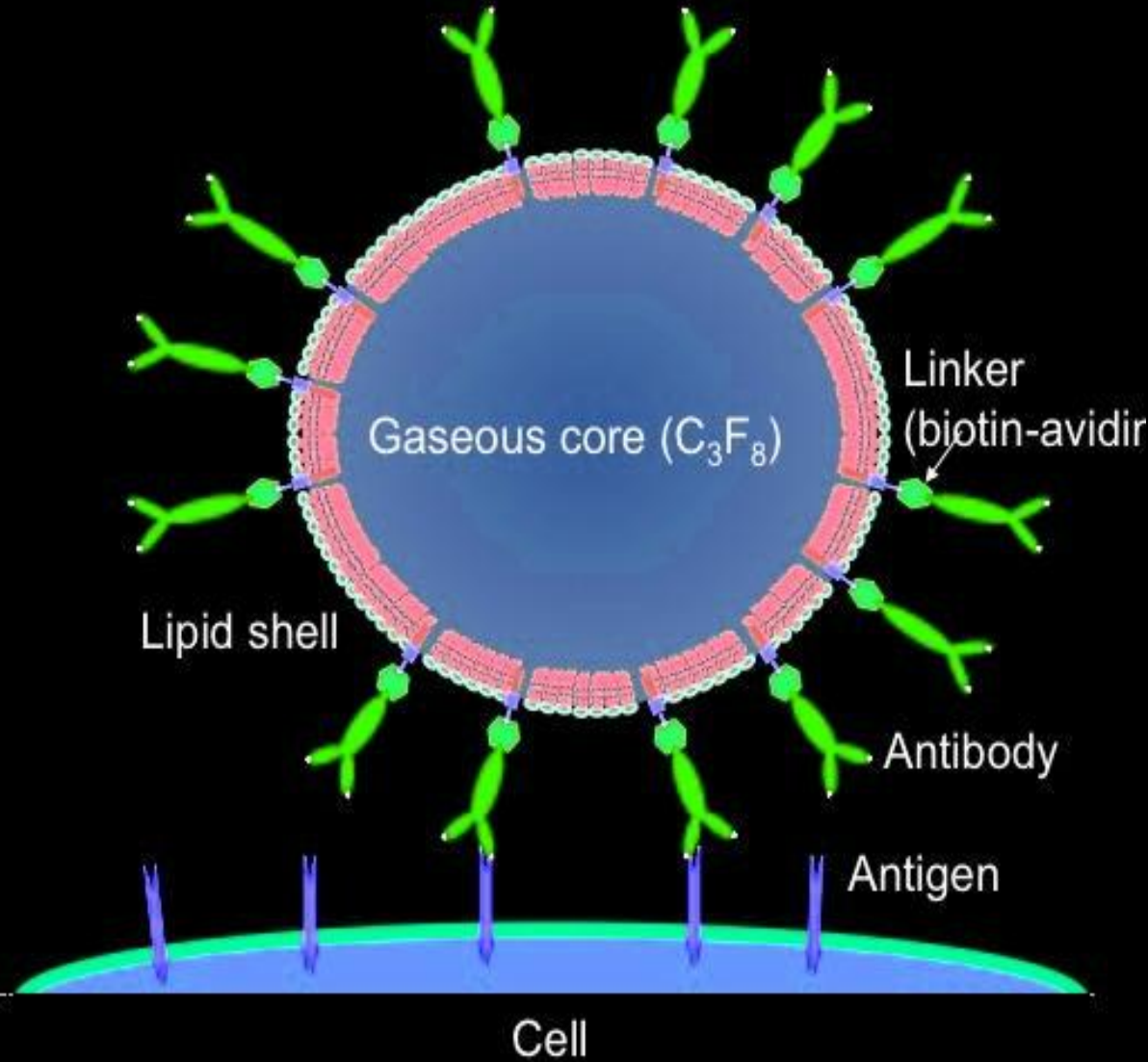
**F**

# L'imagerie ultrarapide des agents de contraste est sensible à l'activité cérébrale à travers le crâne

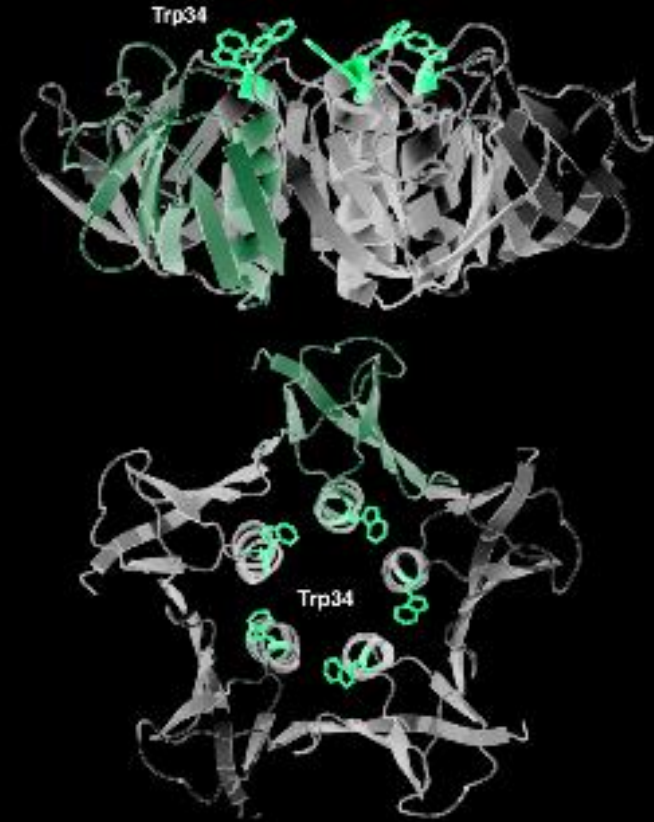


Errico et al. Neuroimage 2015

# Les processus moléculaires sont détectables à l'aide d'agents de contraste ciblés



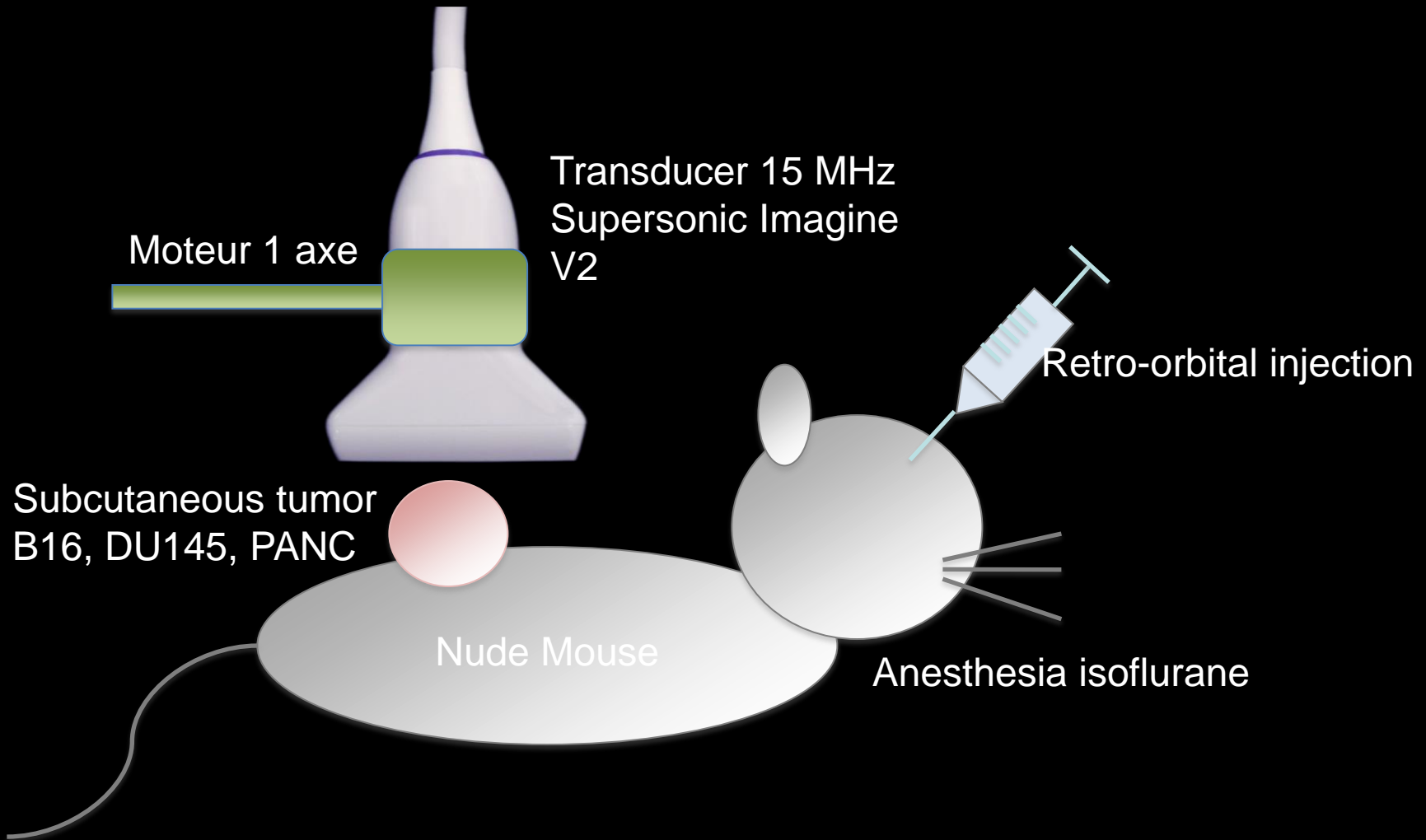
## Shiga toxin



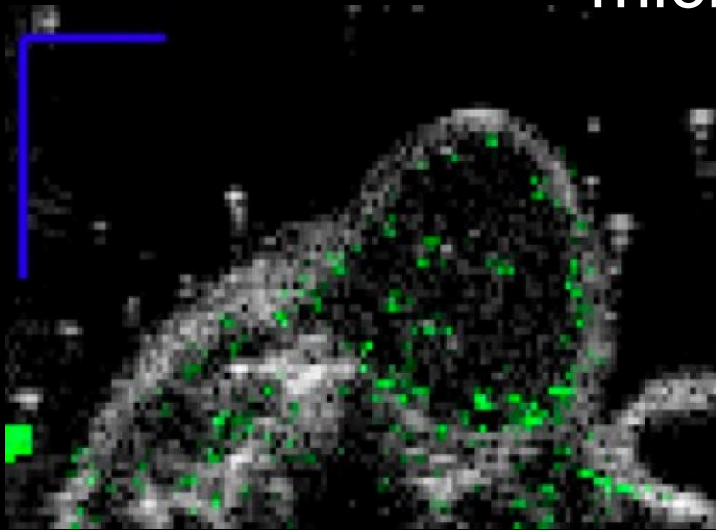
Couture et al. 2007

O. Couture et al., Mai 2017

# Les expériences ont été réalisées sur des tumeurs sous-cutanées



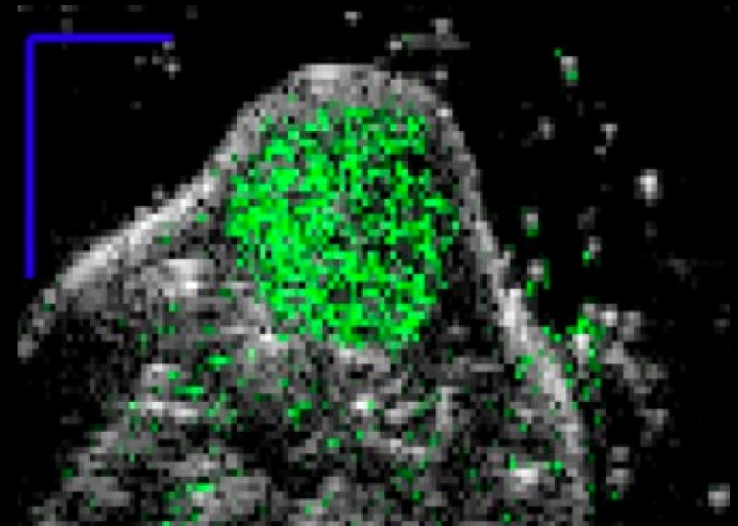
# L'angiogénèse tumorale est détectée grâce aux microbulles ciblées



Non-targeted bubbles

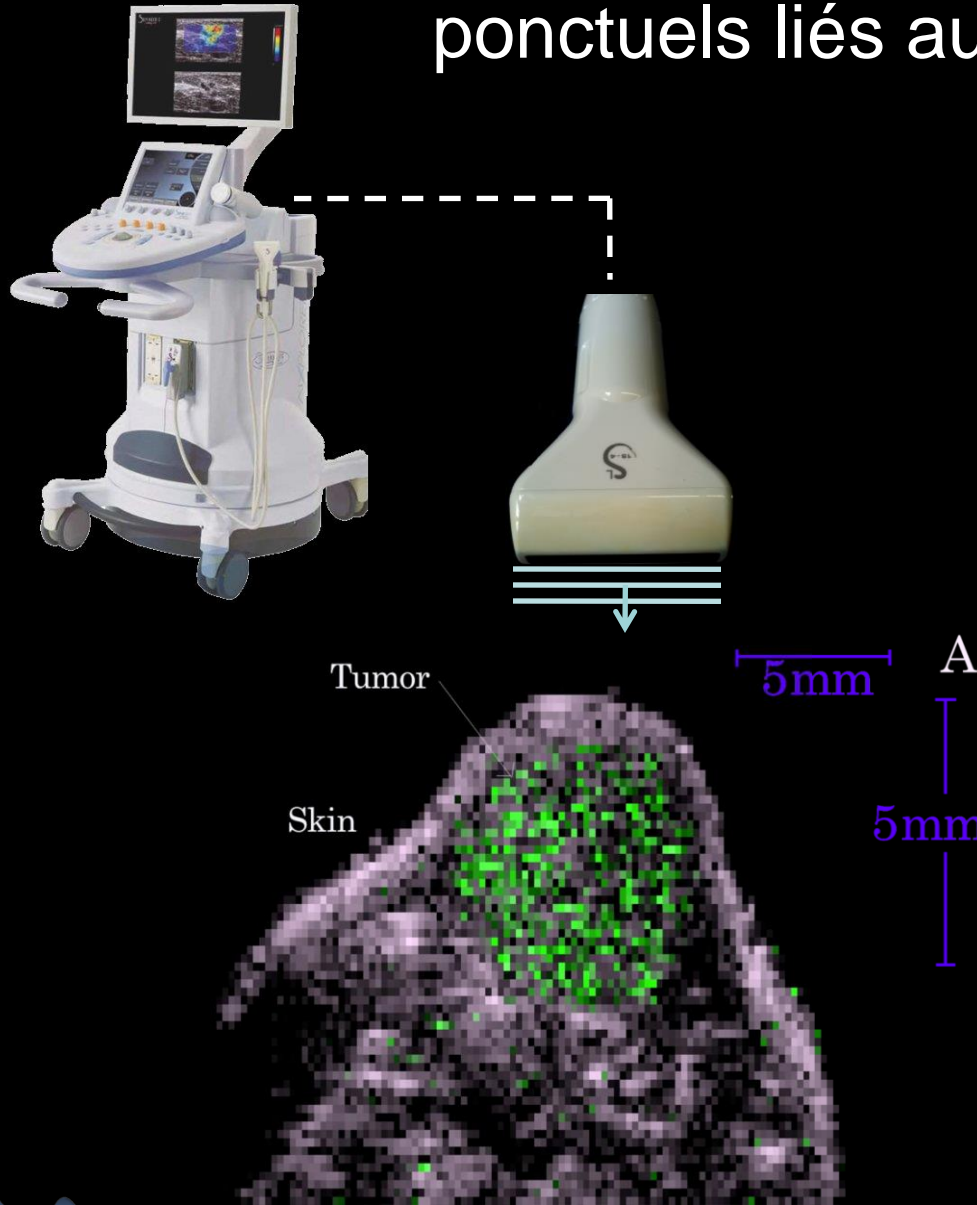
Green: Plane-wave  
Amplitude Modulation

Tumor targeted with  
Shiga





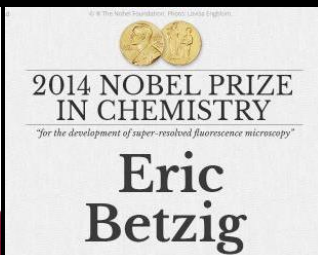
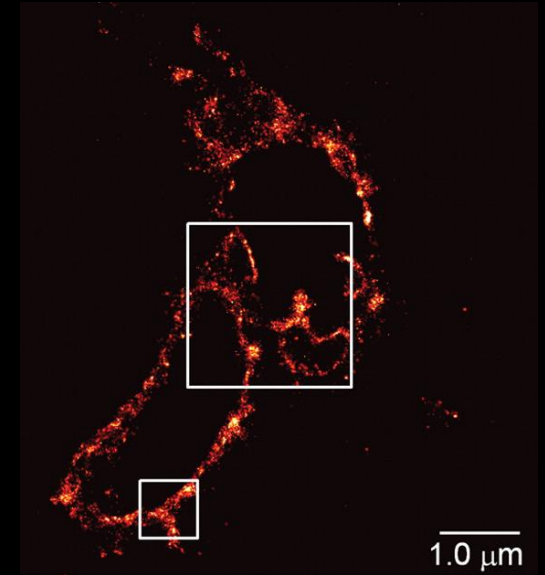
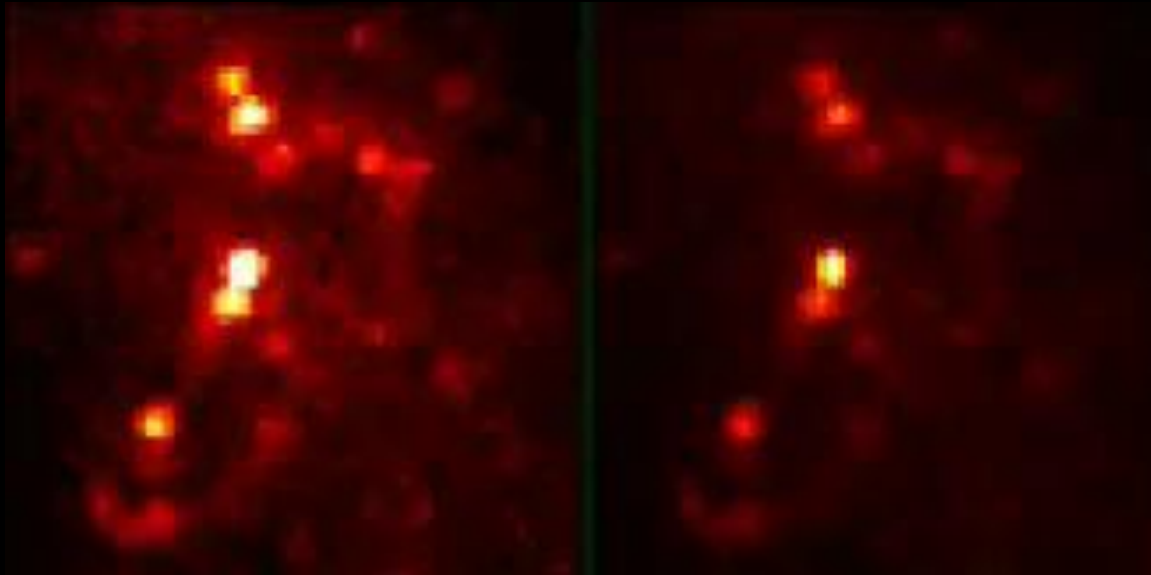
# L'imagerie ultrarapide révèle des événements ponctuels liés aux microbulles



Time=2 ms

Frame rate:  
5000 kHz  
Differential imaging

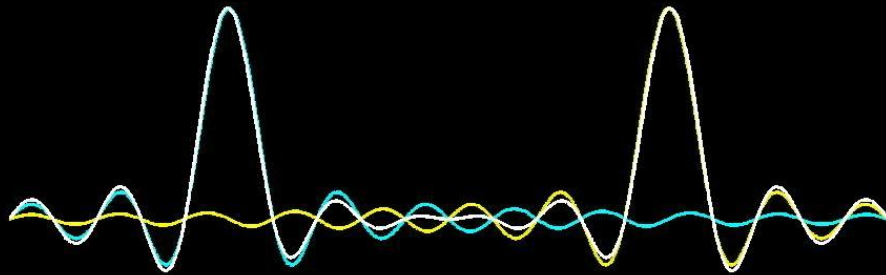
# FPALM localise des sources fluorescentes avec une résolution du nanomètre



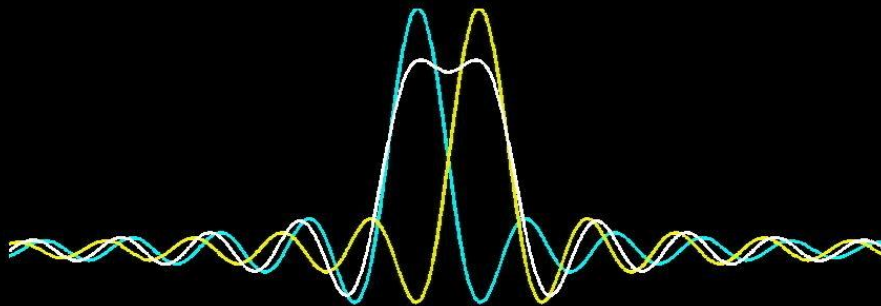
*Betzig et al, 2006, Science*

Des millions d'évènements individuels sont requis pour reconstruire une image

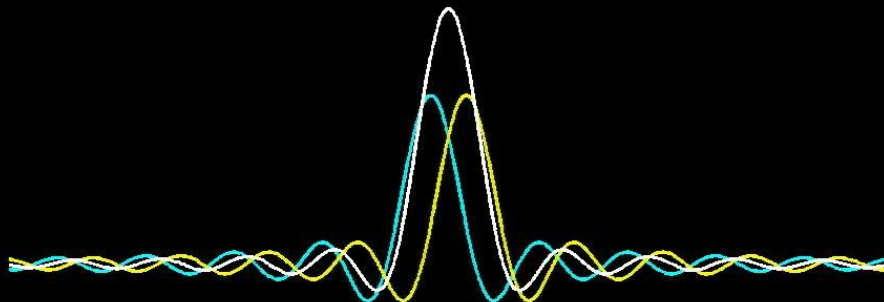
# La resolution d'une imagerie est limitée par la longueur d'onde



Deux sources distinctes

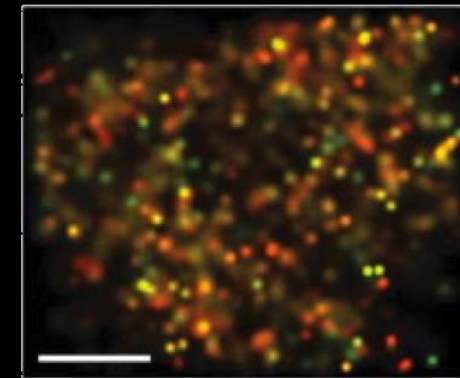
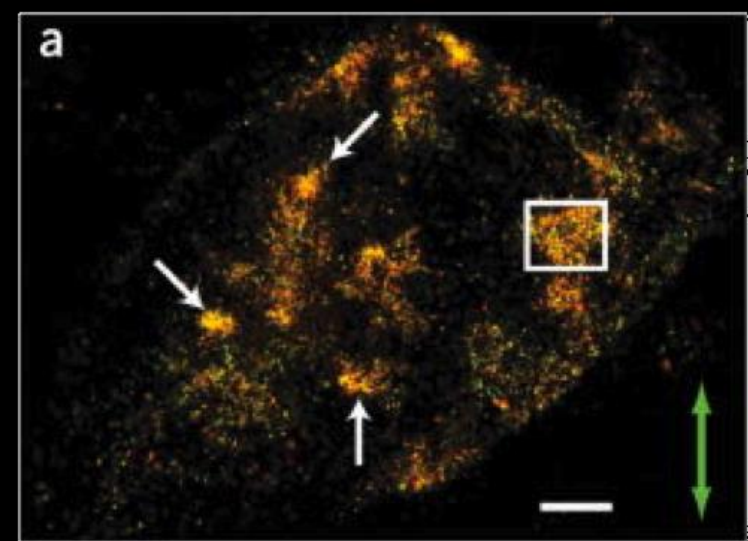
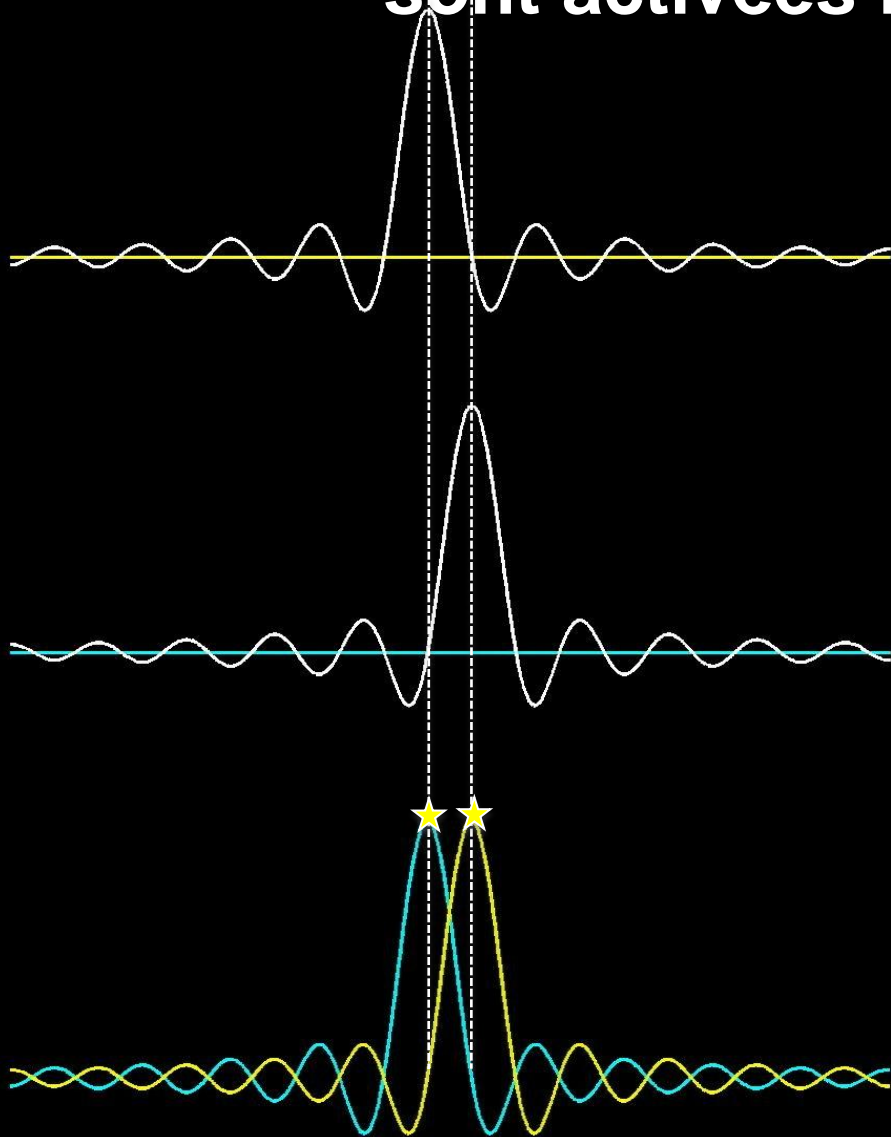


Critère de Rayleigh



Deux sources indistinctes

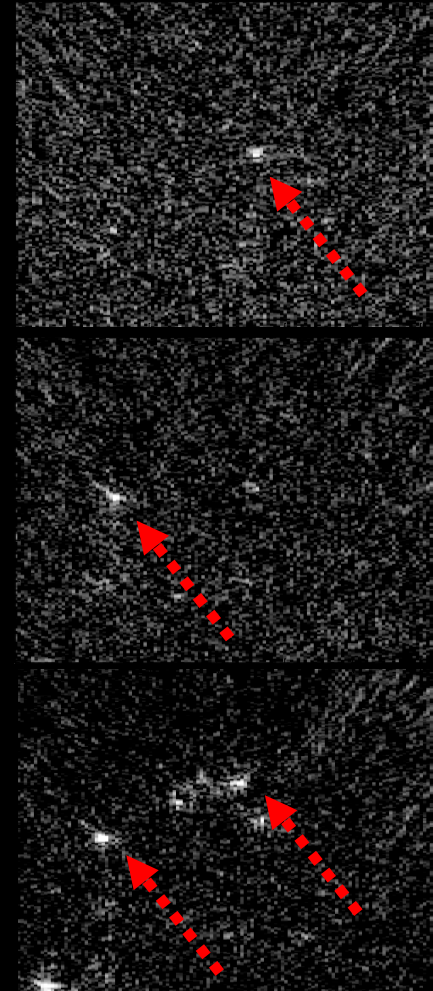
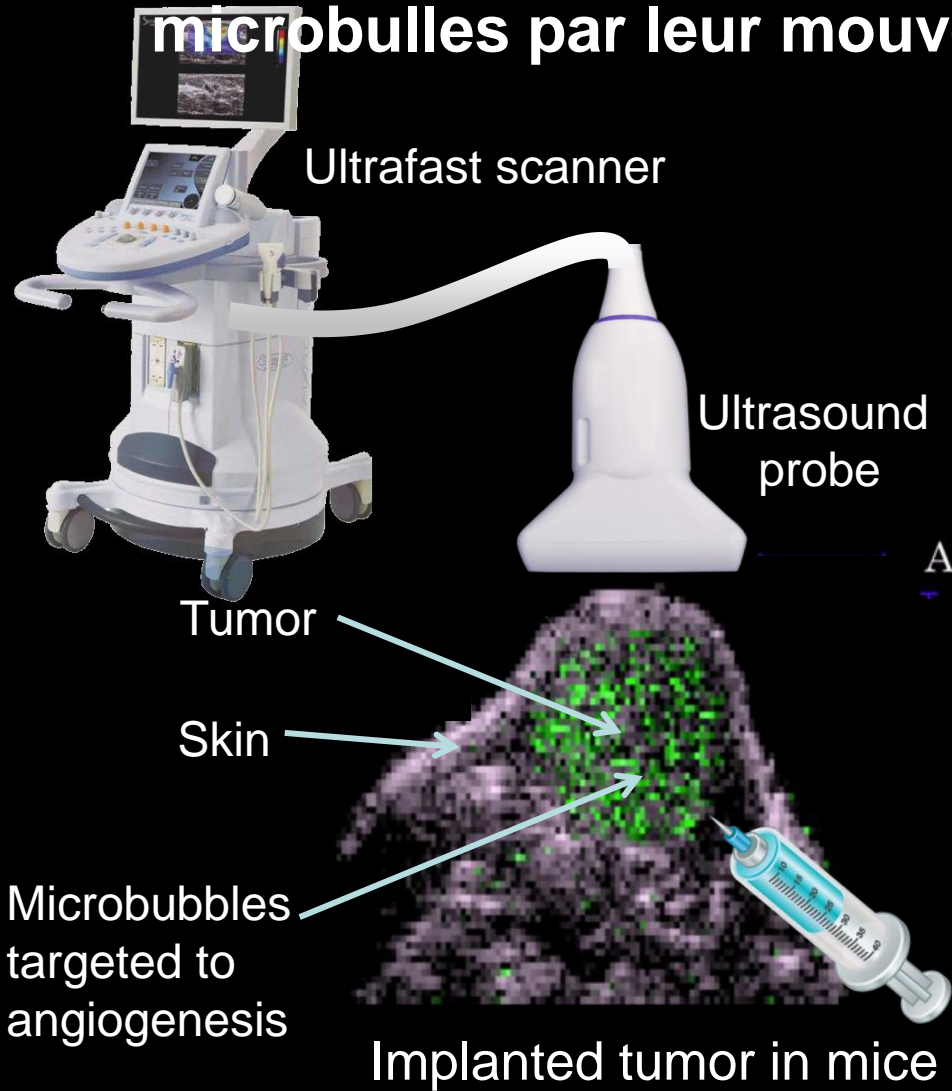
# Les sources deviennent distinctes lorsqu'elles sont activées individuellement



P-FPALM image of a fixed fibroblast (scale bar = 1  $\mu\text{m}$ ).

Gould et al. Nat Methods 2008. (Hess group)

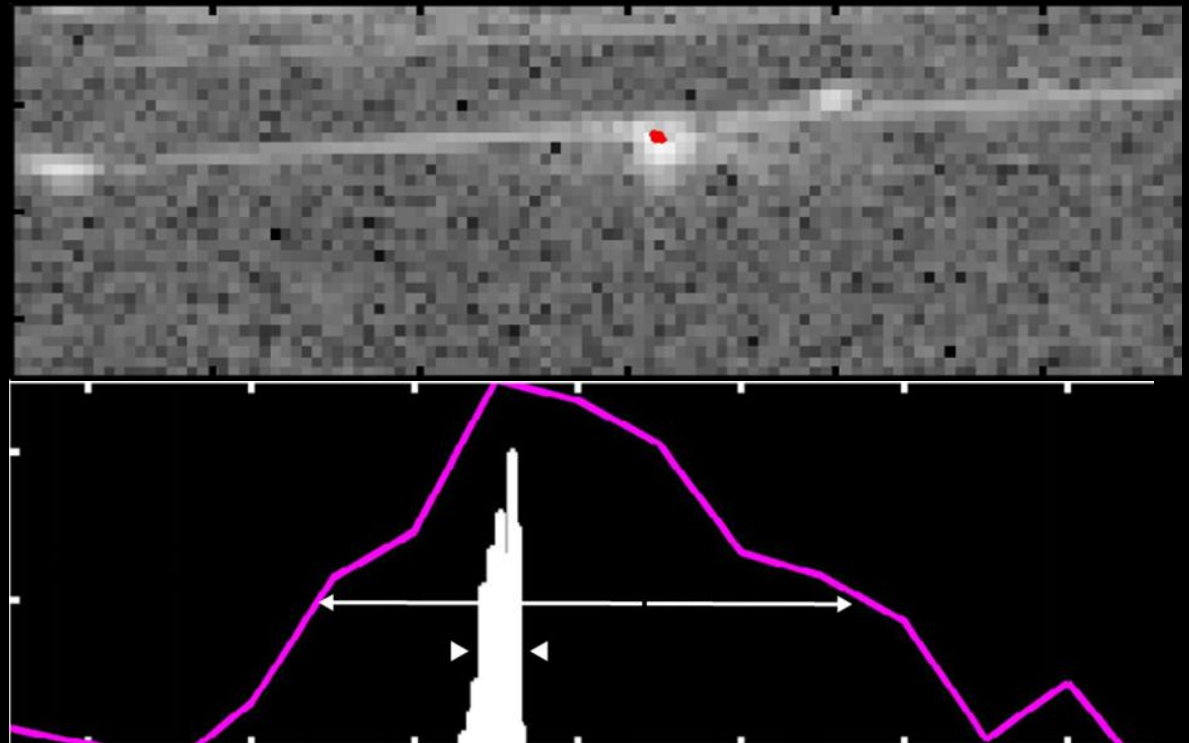
# L'imagerie ultrarapide permet la décorrélation des microbulles par leur mouvement et leur destruction



# La super-résolution ultrasonore a été introduite en 2011 *Ultrafast Ultrasound Localization Microscopy (uULM)*



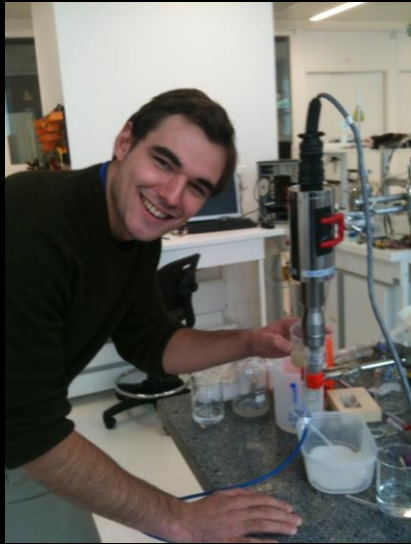
Cross-section of a channel with flowing microbubbles, imaged with ultrafast ultrasound



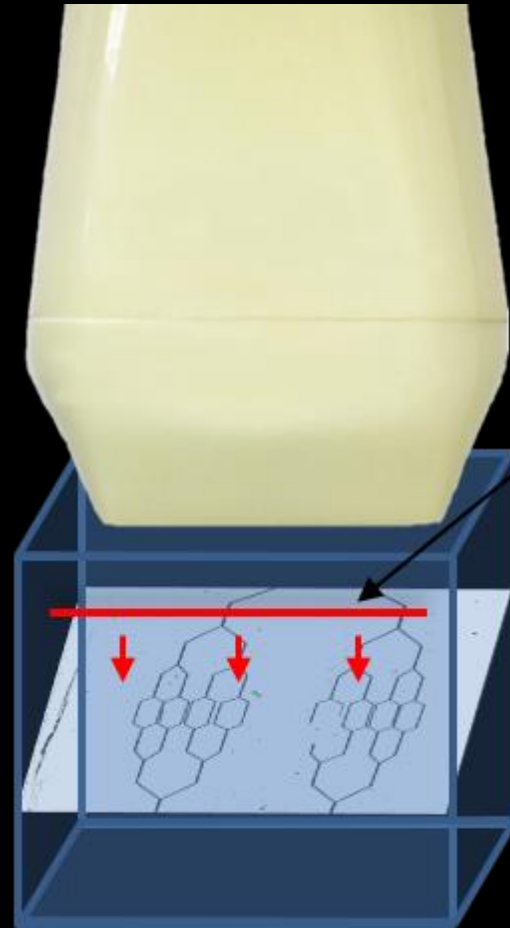
*"Microbubble ultrasound super-localization imaging (MUSLI)", O. Couture et al., IEEE-IUS Symposium 2011*

**Patent:** Couture O, Fink M, Tanter M (2010), "Method and Device for Ultrasound Imaging", PCT/FR2011/052810

Les bulles sont détectables dans des canaux microfluidiques plus fins que la longueur d'onde ultrasonore



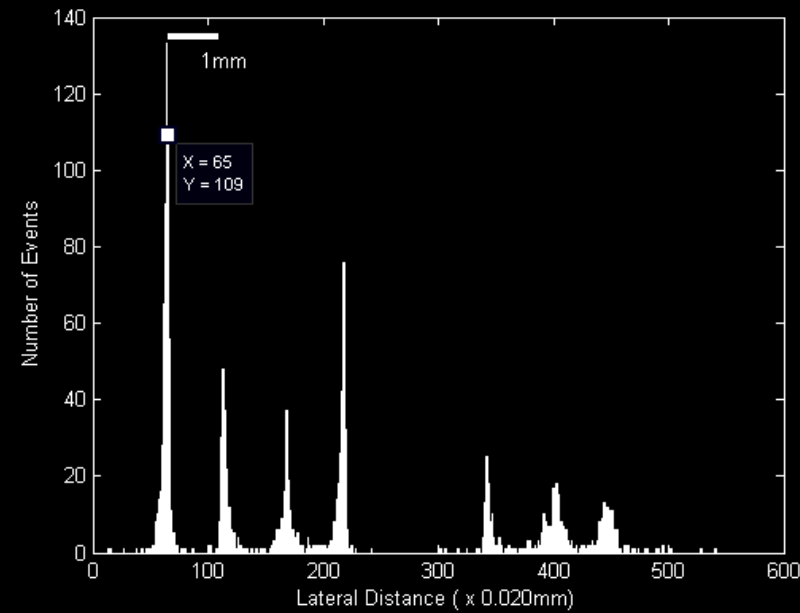
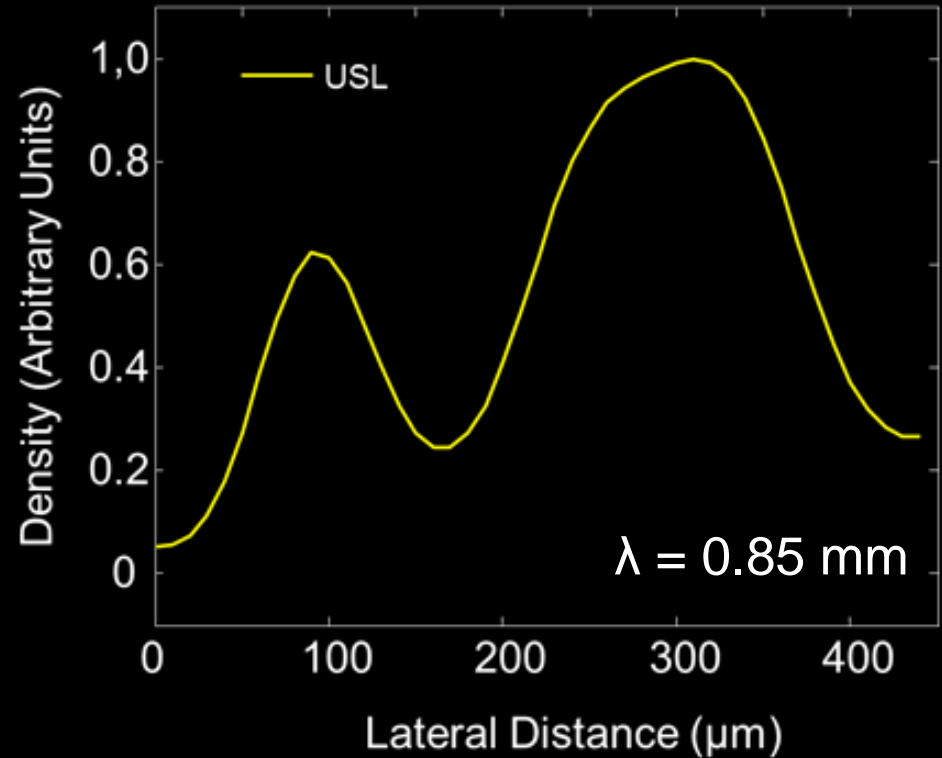
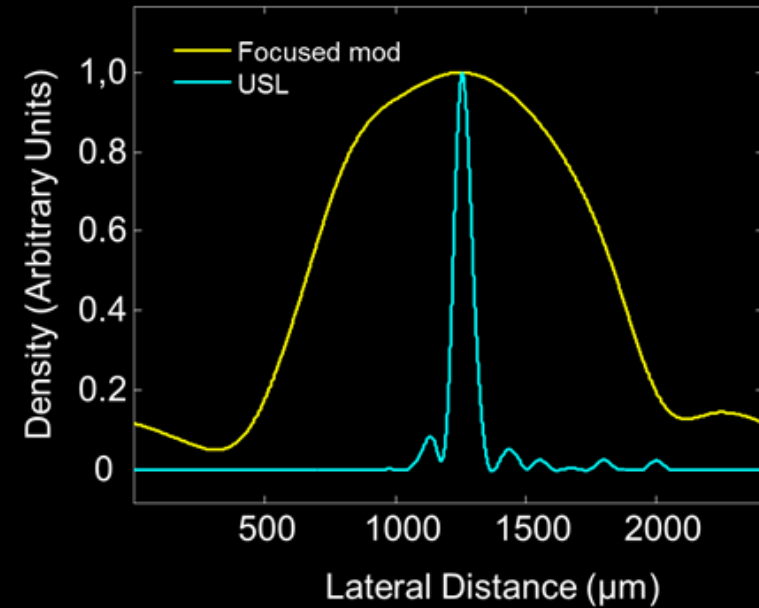
*Desailly et al.,  
APL, 2013*



2D Matrix

Vascular model  
in PDMS

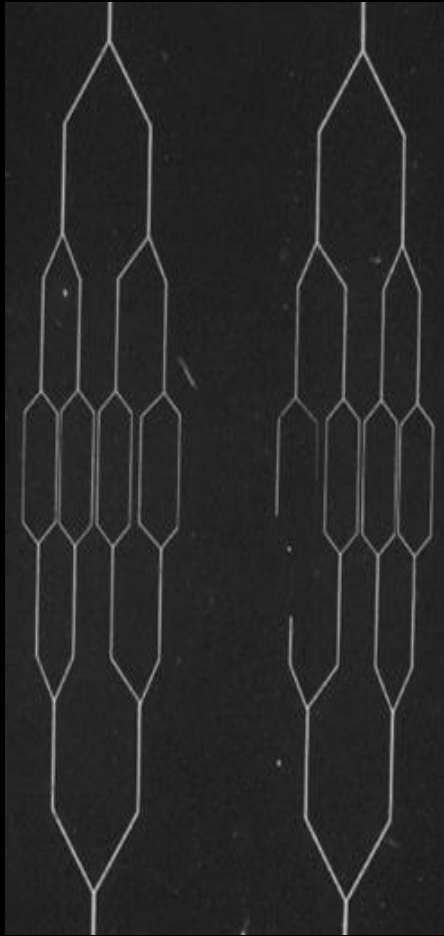
# Le critère de Rayleigh est dépassé



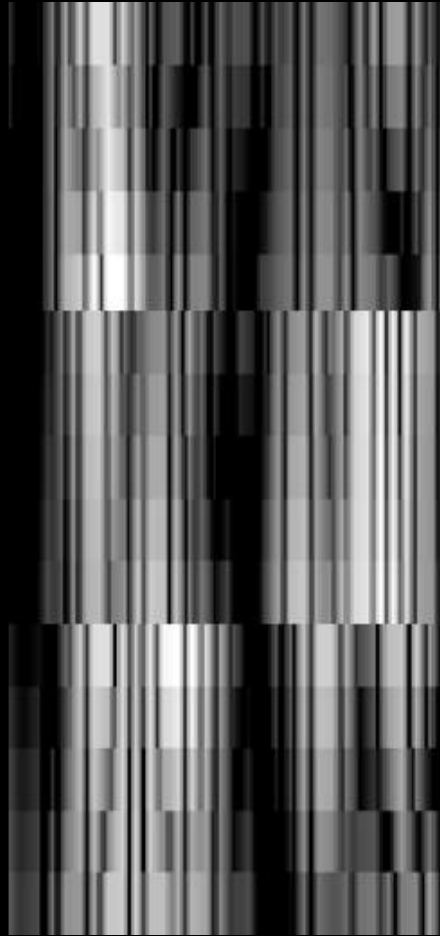
*Desailly et al., APL, 2013*



# Un modèle de microvasculature peut être reconstruit grâce à l'imagerie par localisation ultrasonore



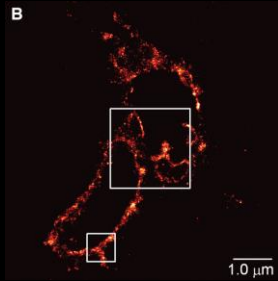
Optical  
microscopy



Conventional  
US

*Desailly et al., APL, 2013*

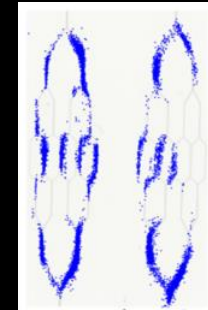
# Jusqu'où peut aller la resolution?



Resolution limit in FPALM

$$\sigma_{(x,y)} \approx \frac{s}{\sqrt{N}}$$

S: Standard deviation



Resolution limits in  
ultrasound localization microscopy

$$\sigma_x \approx A \frac{c \cdot \sigma_\tau}{\sqrt{n}} \frac{z_0}{L_x}$$

A: Constant

c: Speed of sound

$\sigma_\tau$ : Timing resolution  
of the system

$z_0$ : Target depth

“Resolution limits in ultrafast ultrasound localization microscopy”, Y. Desailly et al., *PMB*, 2015

# La microscopie par localisation ultrasonore devrait atteindre des résolutions micrométriques

<b>Biomedical application</b>	<b>US Frequency</b>	<b>Imaging depth (mm)</b>	<b>Number of elements</b>	<b>Predicted Lateral Resolution</b>
Breast	7 MHz	30	128	10 $\mu\text{m}$
Liver, Heart	3 MHz	70	192	20 $\mu\text{m}$
Brain (rodents)	15 MHz	10	128	5 $\mu\text{m}$

*“Resolution limits in ultrafast ultrasound localization microscopy”, Y. Desailly et al., **PMB**, 2015*

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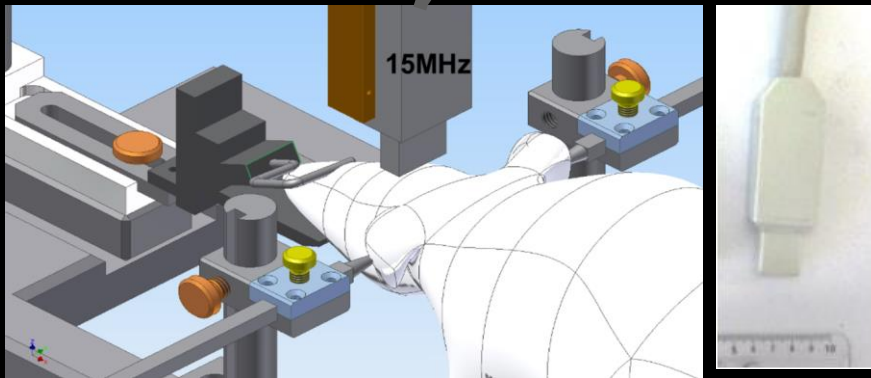
*“Resolution limits in ultrafast ultrasound localization microscopy”, Y. Desailly et al., **PMB**, 2015*

# Setup experimental in-vivo



## Ultrasound imaging parameters

- Imaging Frequency=15MHz
- 128 emitting elements
- Pitch=0,08 mm
- Frame Rate=500Hz



## Ultrasound contrast agents

Initial concentration :  $2 \cdot 10^8$   
 $\mu\text{bubbles/mL}$

Boluses of 150  $\mu\text{L}$  were injected  
every 15 minutes

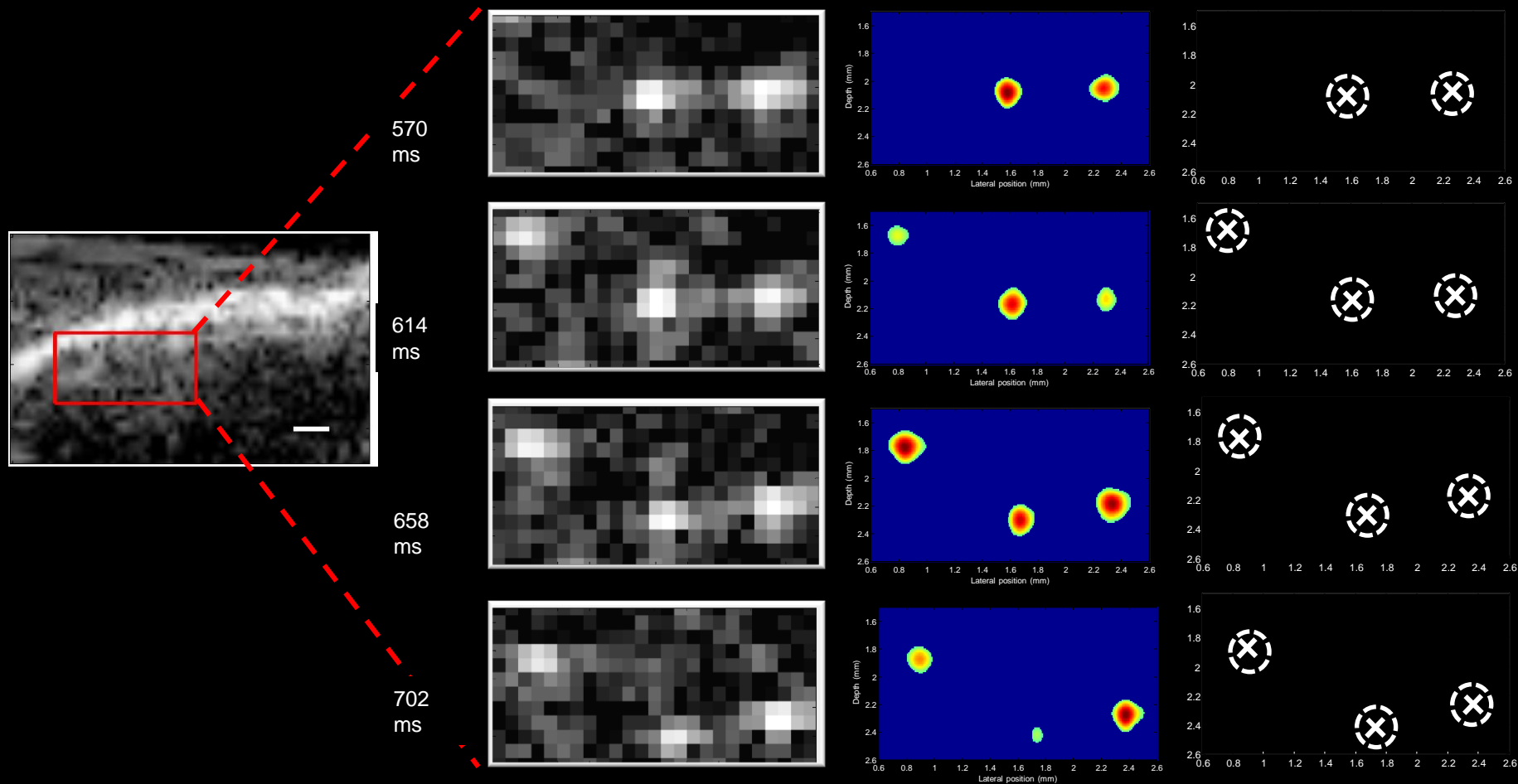


Claudia Errico

# L'imagerie ultrarapide distingue des sources ponctuelles dans le cerveau du rat



# L'imagerie ultrarapide distingue des sources ponctuelles dans le cerveau du rat

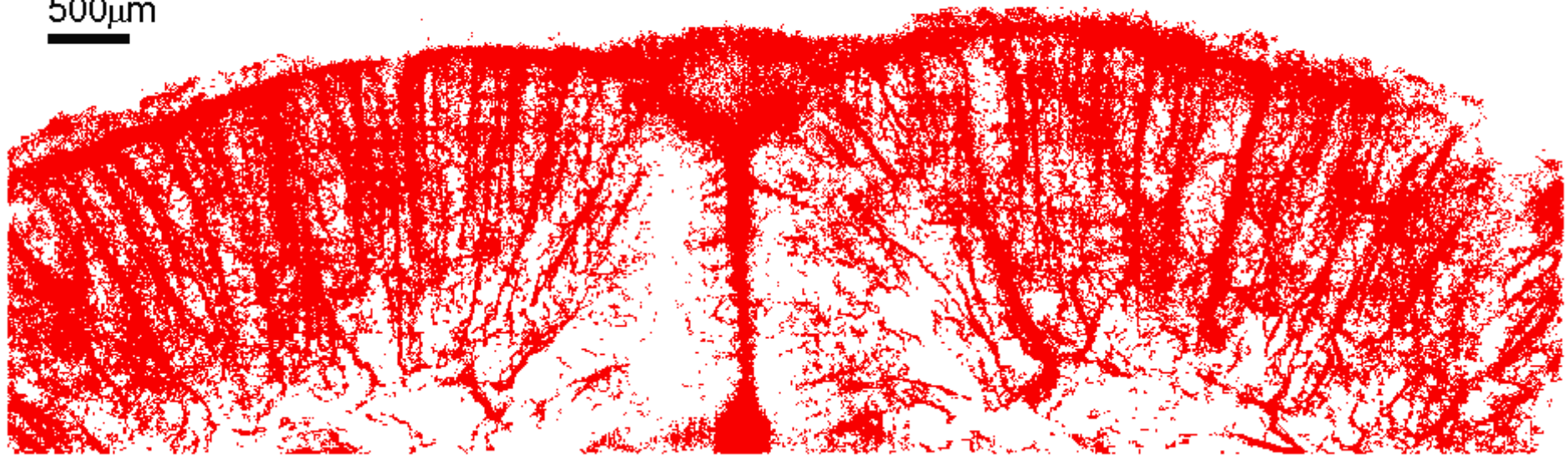


"Ultrafast ultrasound localization microscopy for deep super-resolution vascular imaging", *C. Errico et al., Nature*

En quelques dizaines de secondes, des millions de microbulles ont été localisées

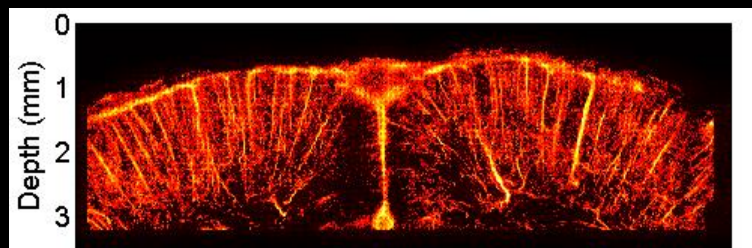
500 $\mu\text{m}$

149 s



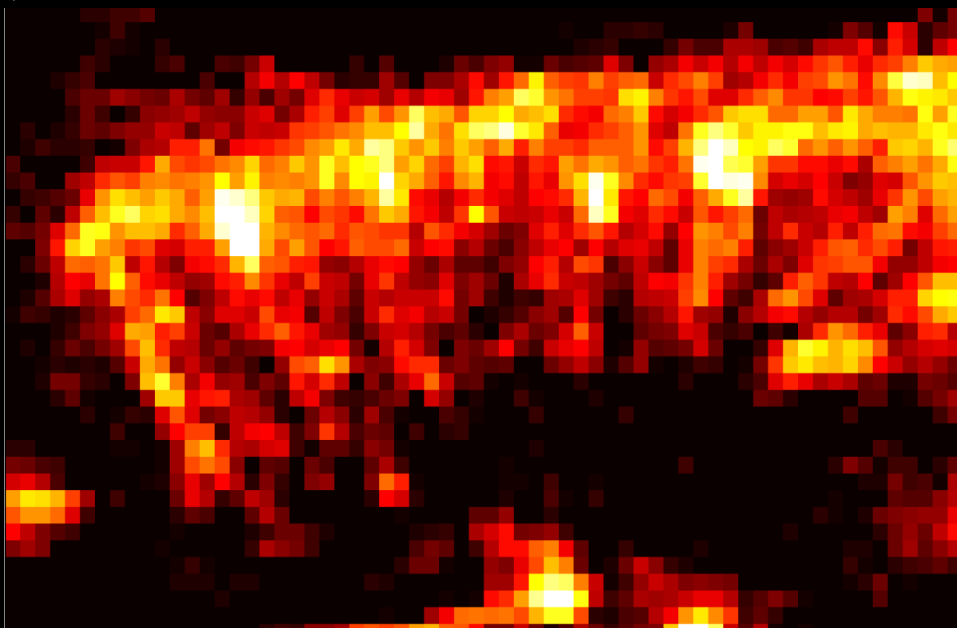
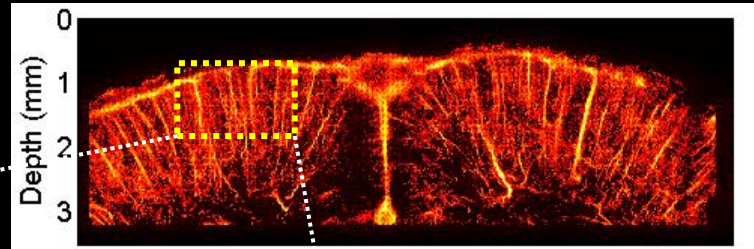


# La résolution des ultrasons est améliorée d'un facteur 10



"Ultrafast ultrasound localization microscopy for deep super-resolution vascular imaging", C. Errico et al., *Nature*, 2015

# La résolution des ultrasons est améliorée d'un facteur 10

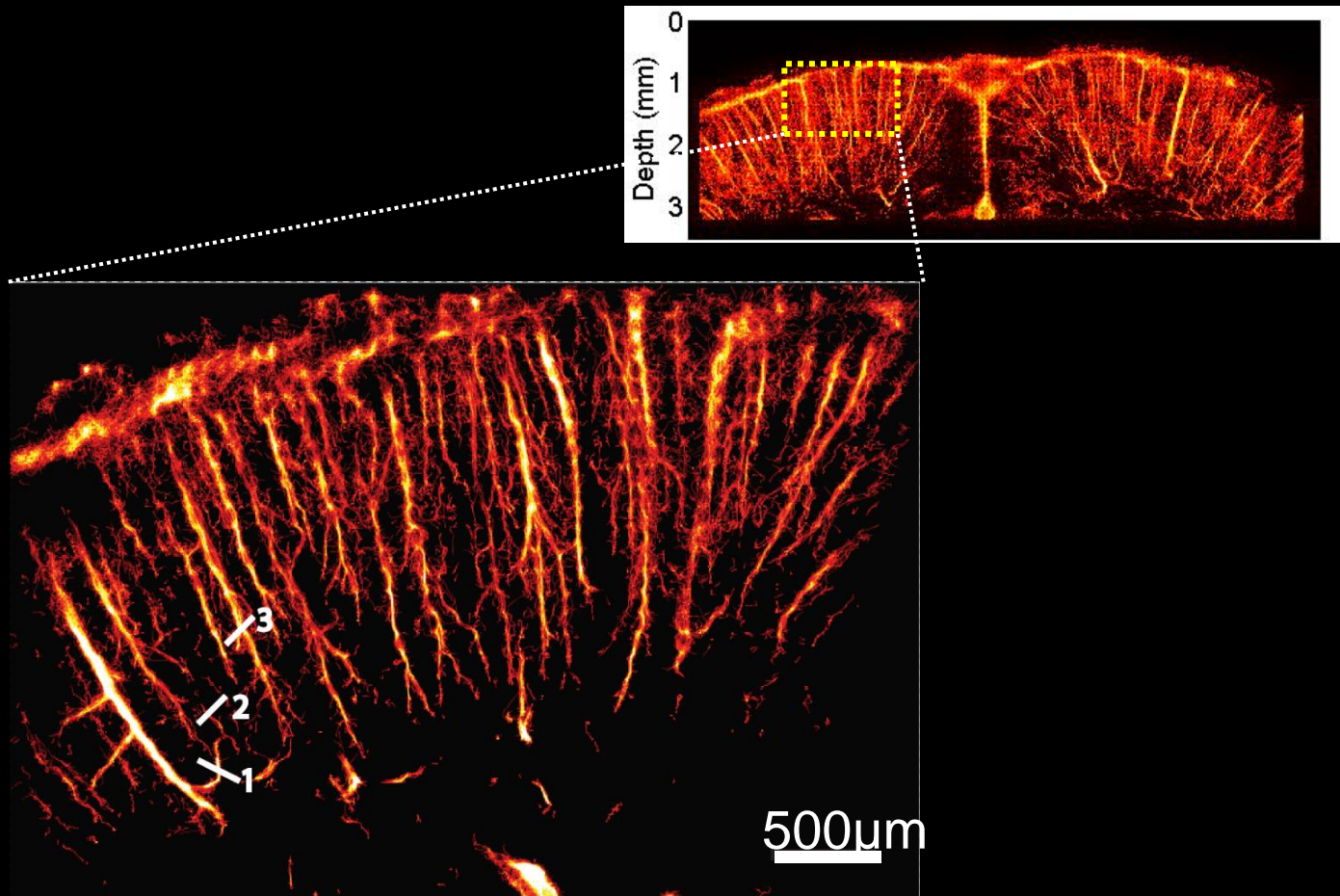


500 $\mu$ m

Imagerie Doppler conventionnelle

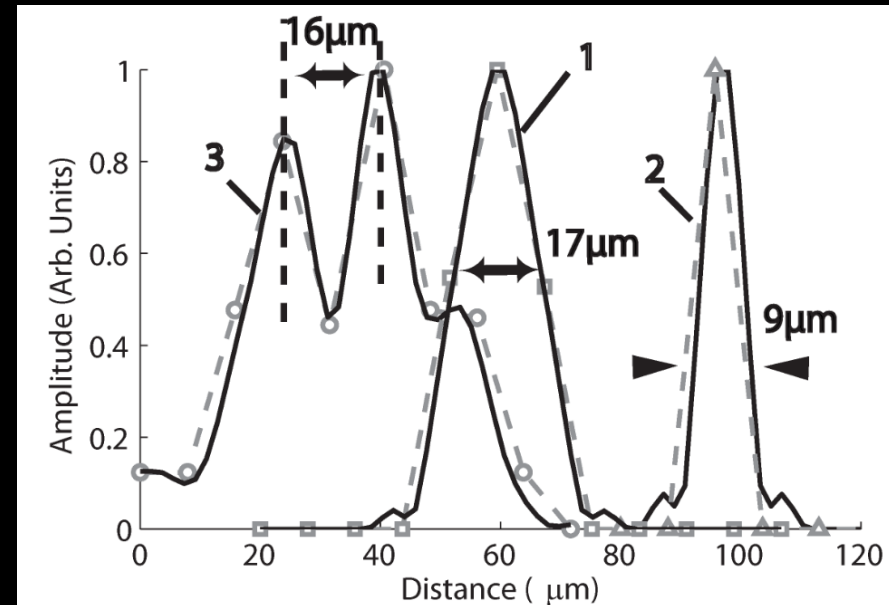
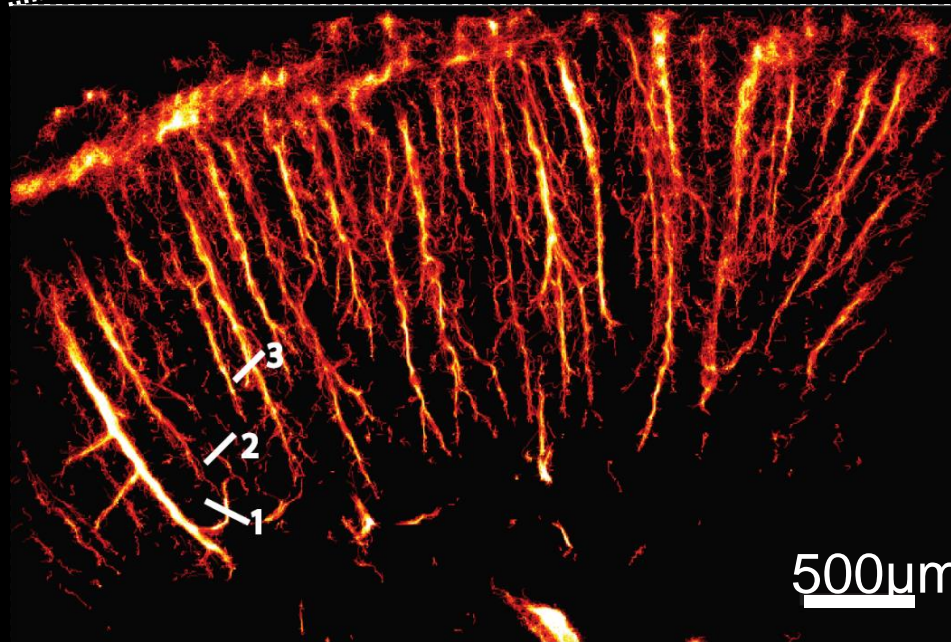
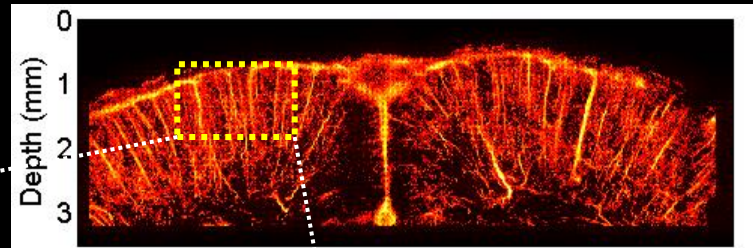
C. Errico et al., *Nature*, 2015

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"Ultrafast ultrasound localization microscopy for deep super-resolution vascular imaging", C. Errico et al., *Nature*, 2015

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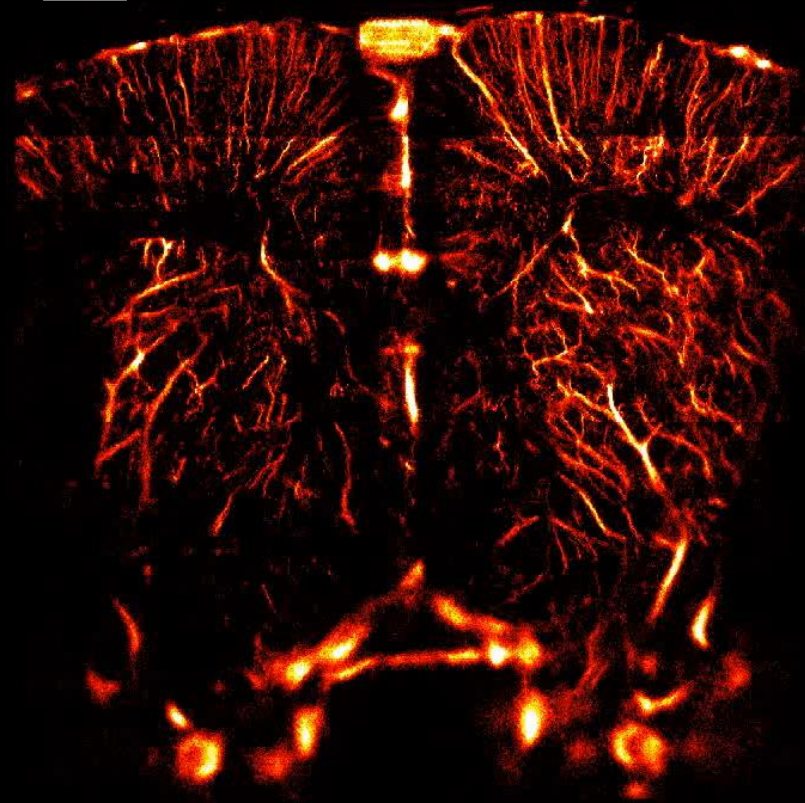


"Ultrafast ultrasound localization microscopy for deep super-resolution vascular imaging", C. Errico et al., *Nature*, 2015

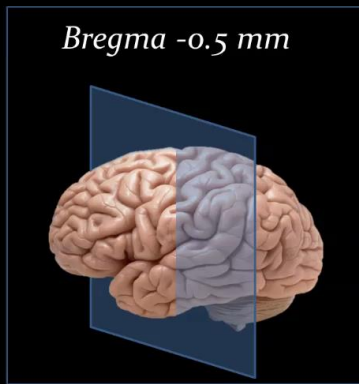
# La microvasculature du cerveau peut être reconstruite plan par plan à une résolution micrométrique

Bregma 0.5mm

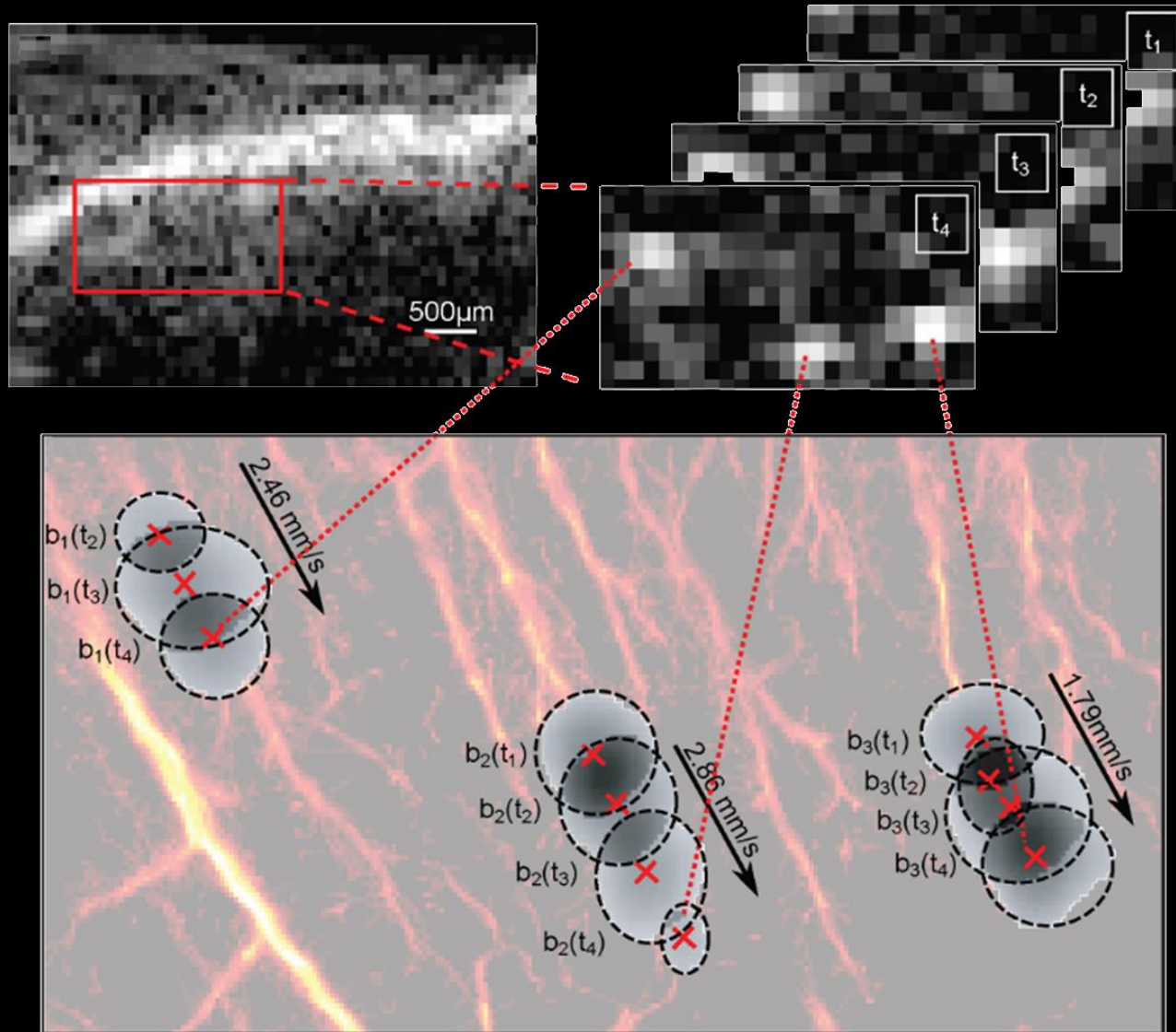
1 mm



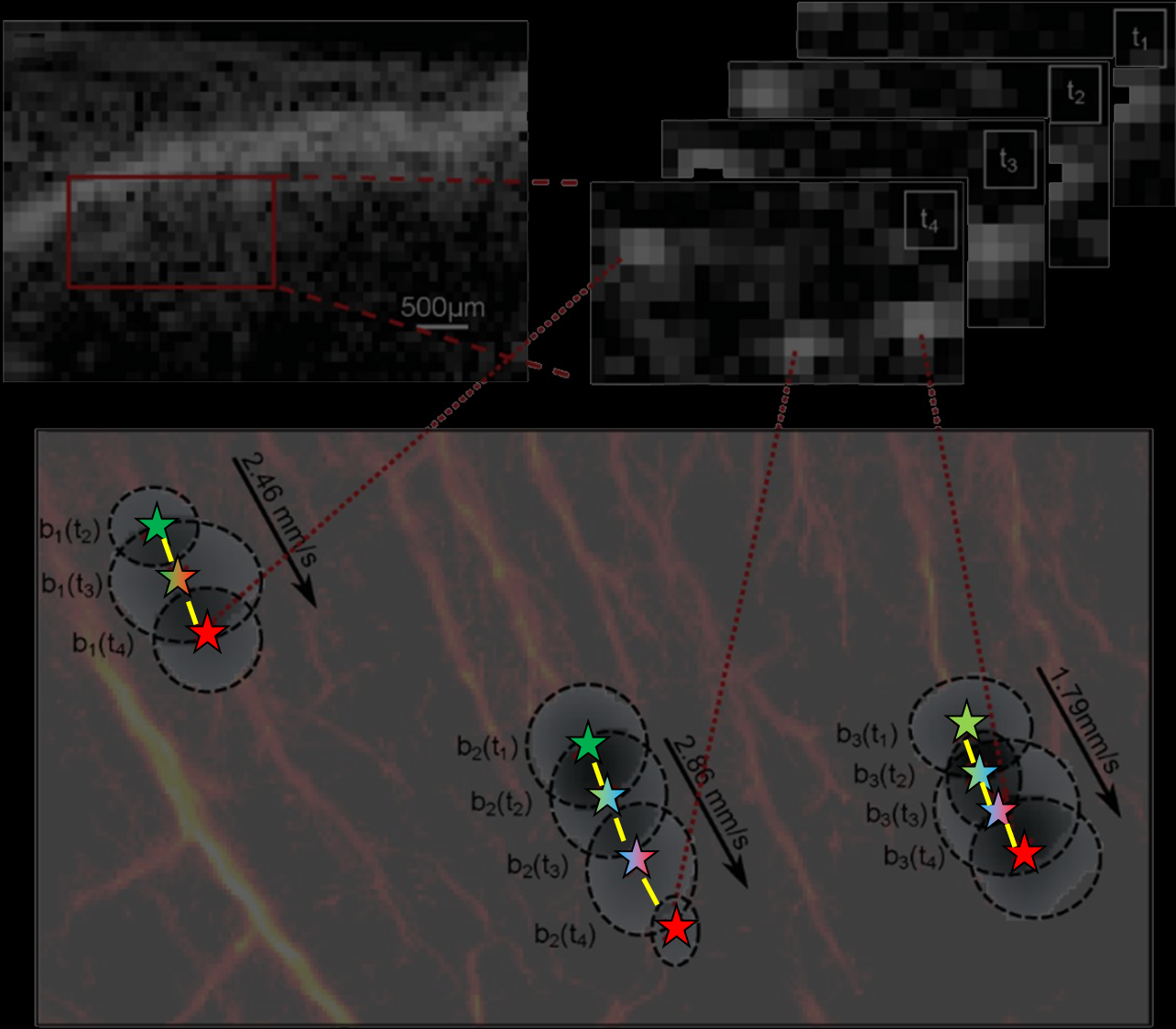
Bregma -0.5 mm



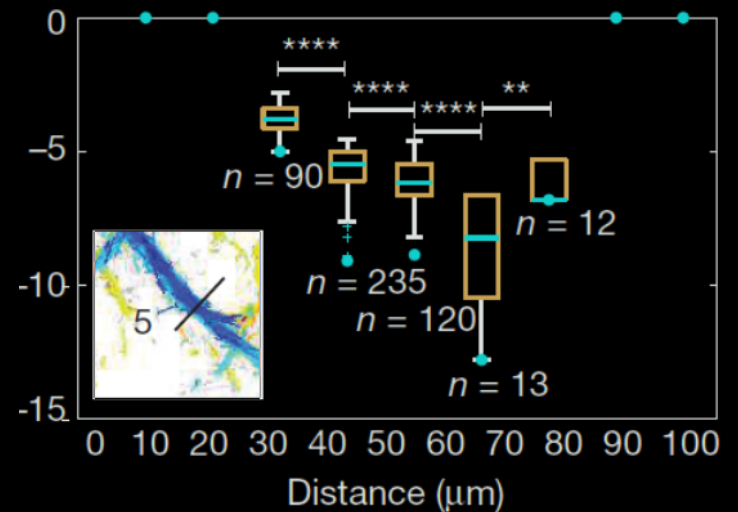
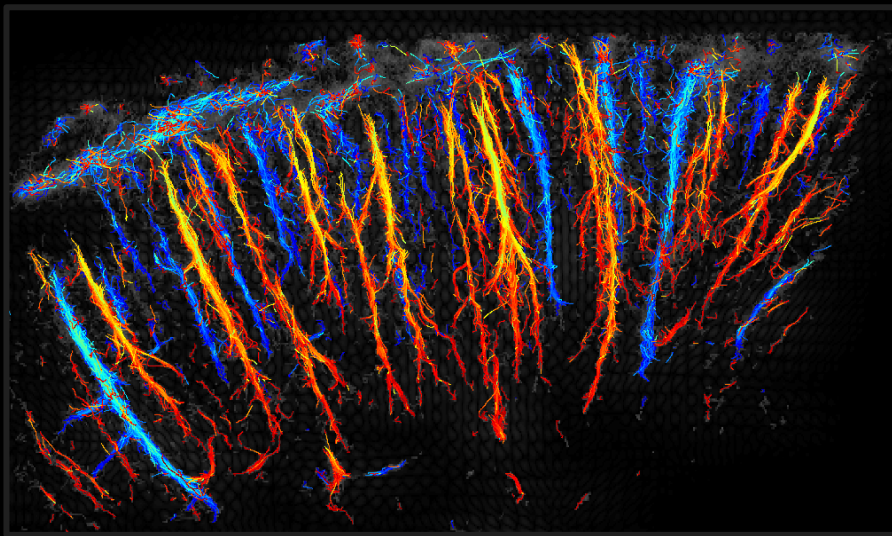
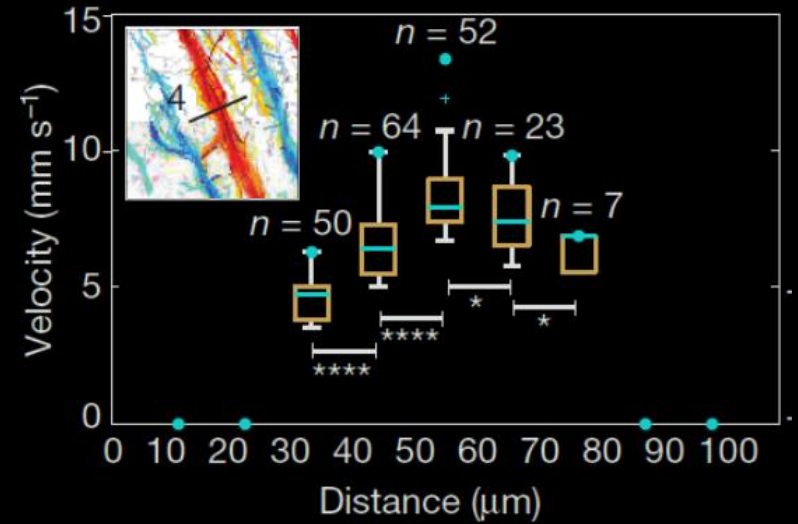
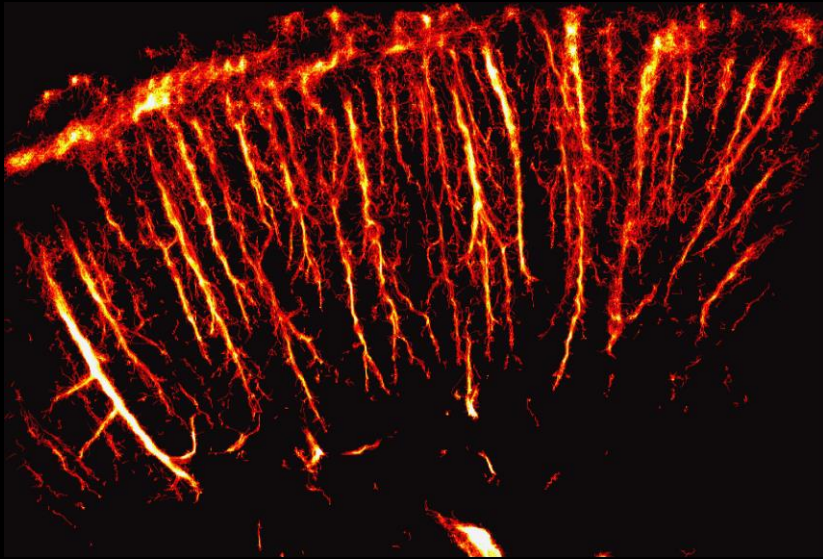
Le suivi des microbulles à l'échelle de la milliseconde permet de créer un vecteur vitesse pour chacune d'elle



# Le suivi des microbulles à l'échelle de la milliseconde permet de créer un vecteur vitesse pour chacune d'elle



# L'imagerie ultrarapide permet une grande gamme dynamique de vitesse

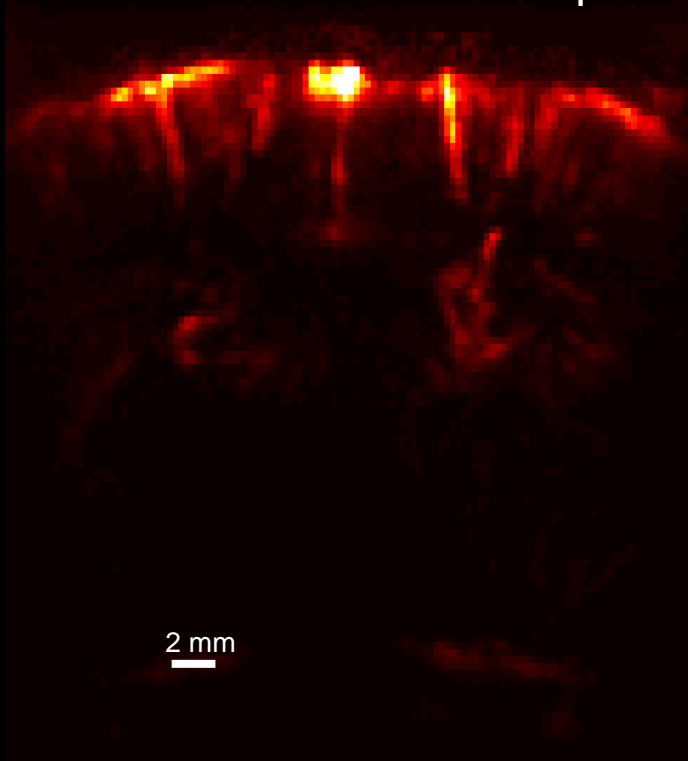


C. Errico et al., *Nature*, 2015

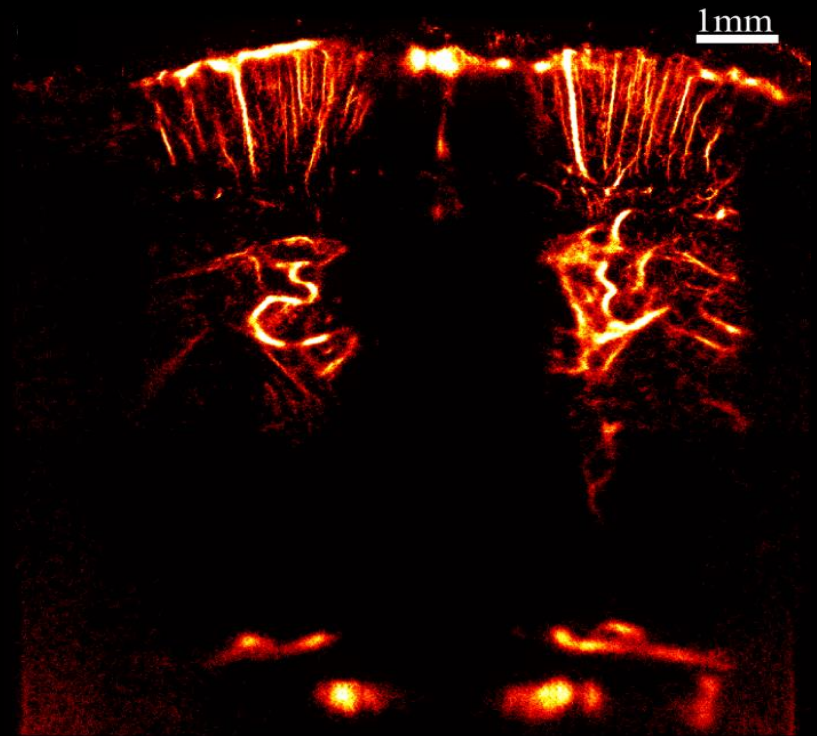


# L'imagerie super-résolue a été exploitée en transcranien

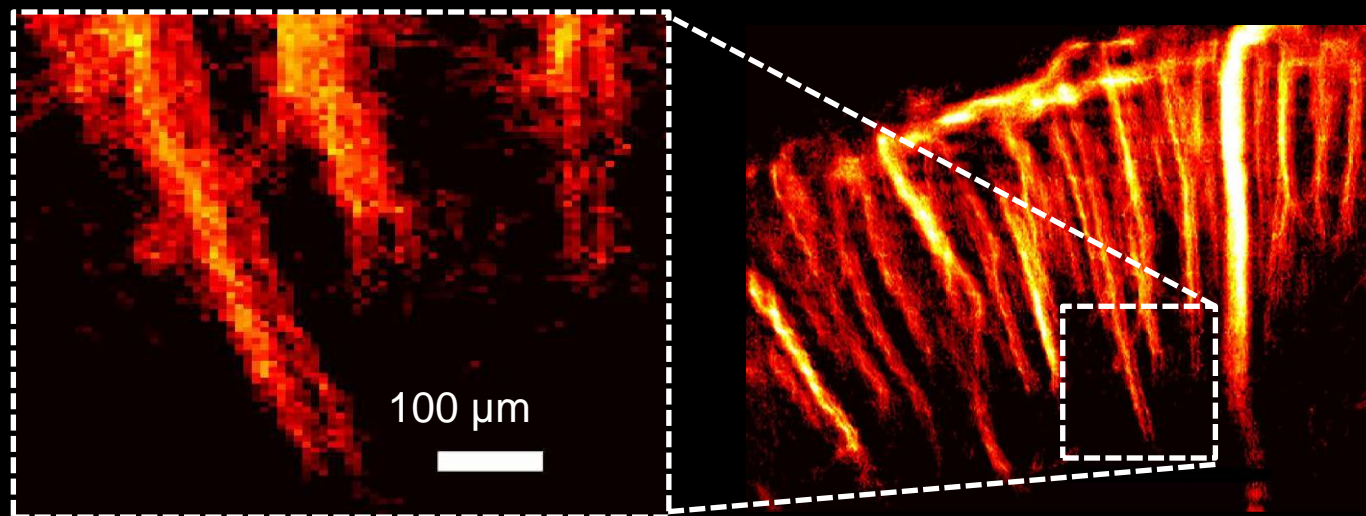
Transcranial ultrafast Doppler  
vascularization map



Transcranial uULM

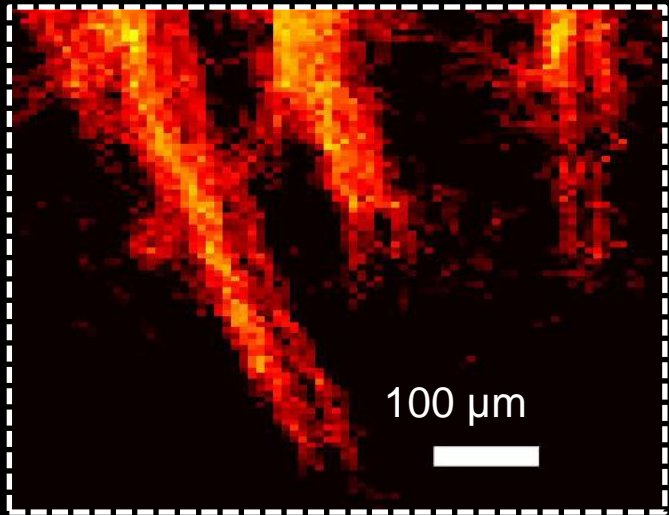


# Le mouvement affecte fortement les images super-résolues

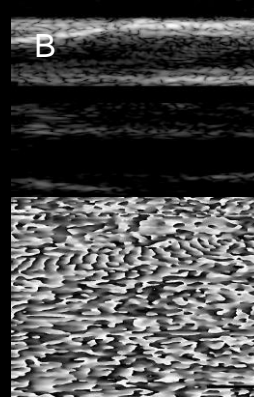


*Hingot et al. 2016a, in preparation*

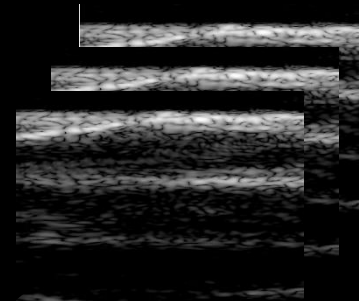
# La corrélation de phase permet de corriger ces mouvements



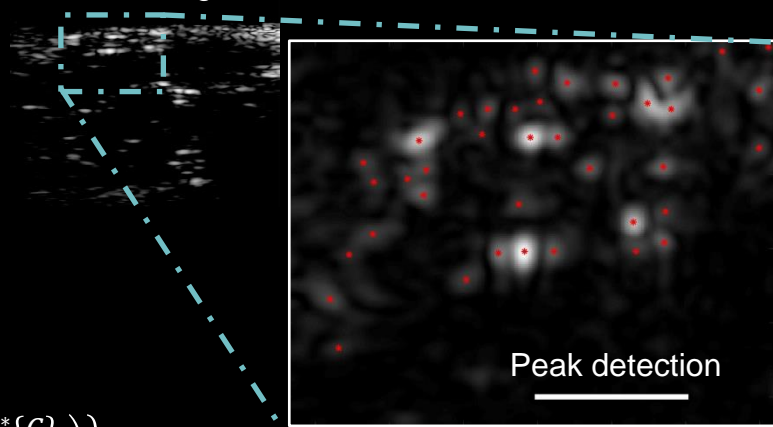
Tissue Singular vectors



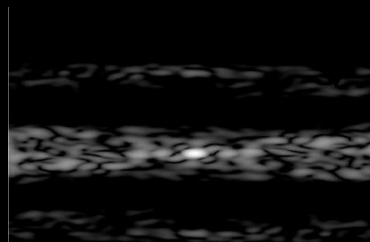
Bubbles singular vectors



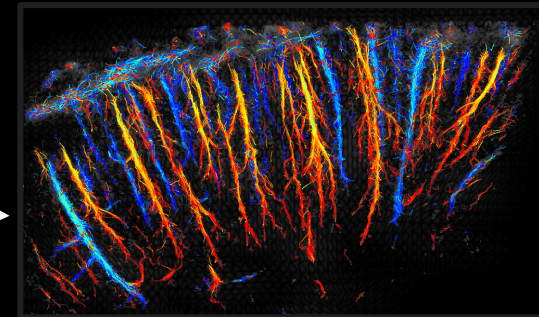
Original dataset  
74 000 images  
10x interpolation



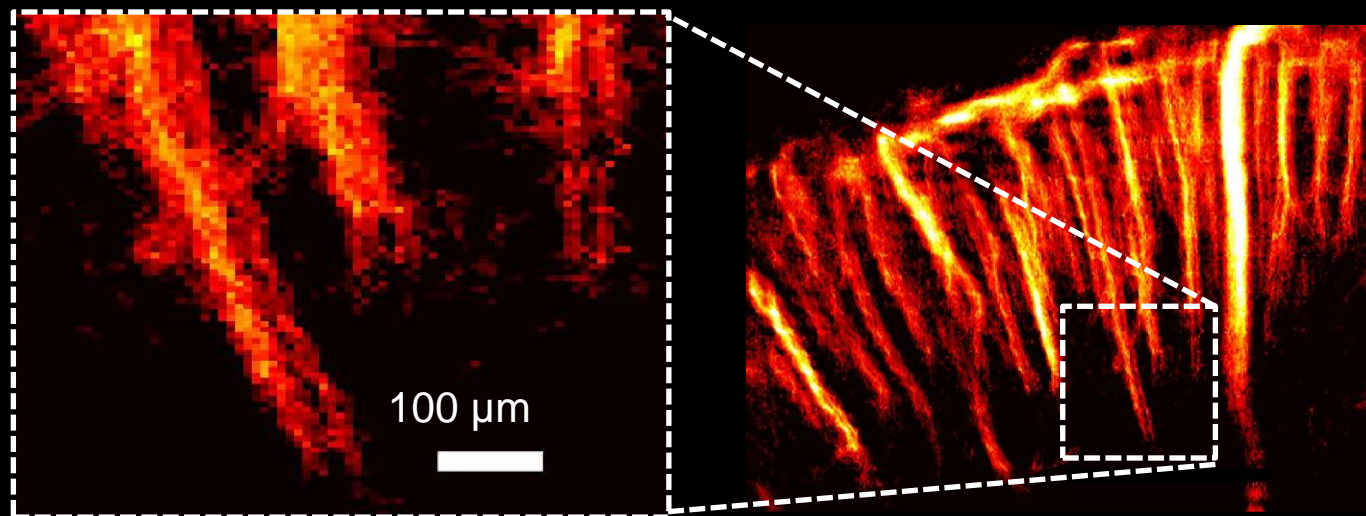
$$(\Delta x, \Delta z) = \underset{(x, z)}{\operatorname{argmax}} \left\{ \mathcal{F}^{-1} \left( \frac{\mathcal{F}\{B\} \circ \mathcal{F}^*\{C\}}{|\mathcal{F}\{B\} \circ \mathcal{F}^*\{C\}|} \right) \right\}$$



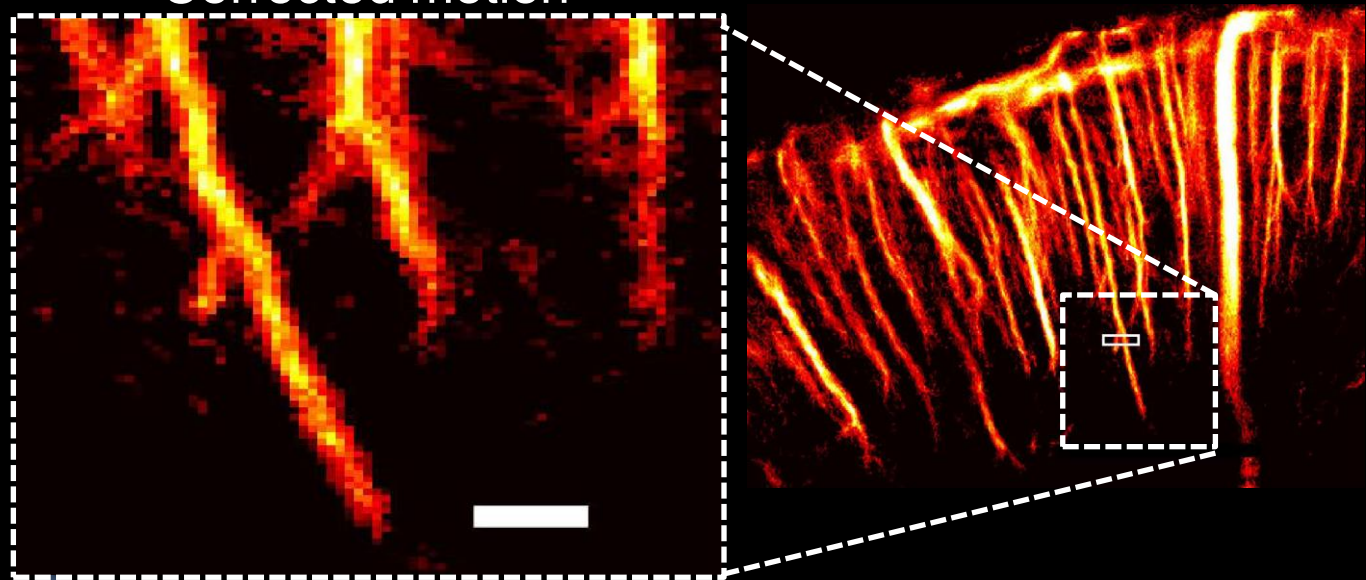
→ Δx, Δz →



# La correction de mouvement améliore les images floutées

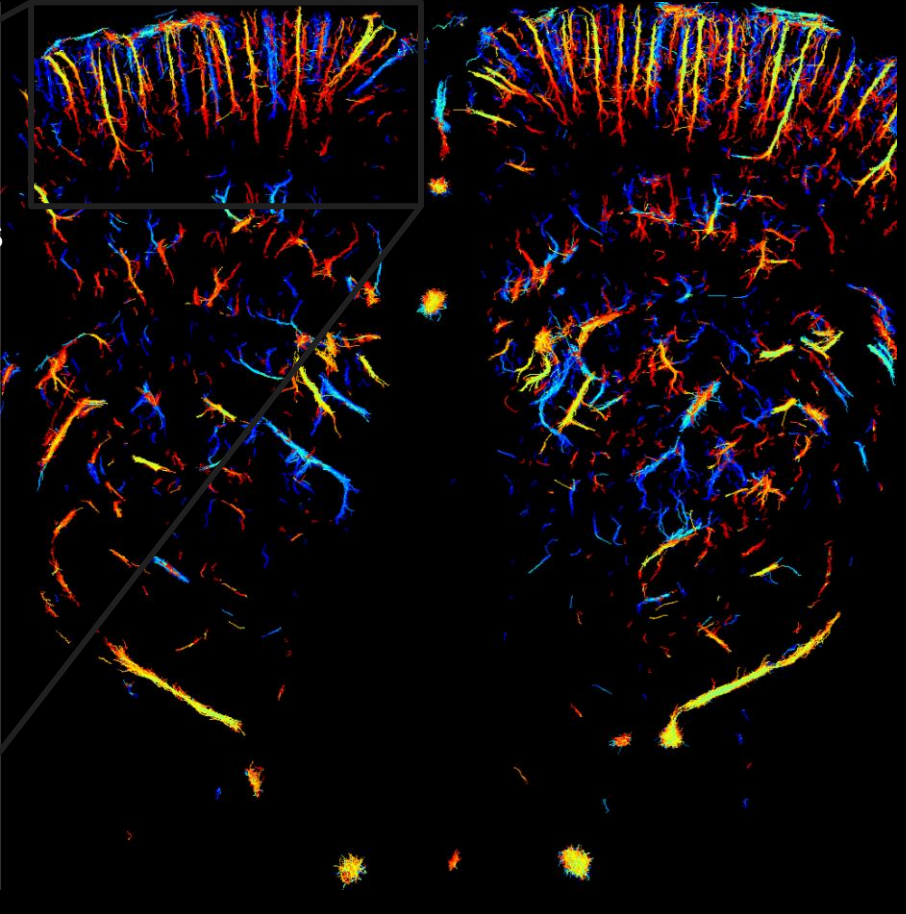
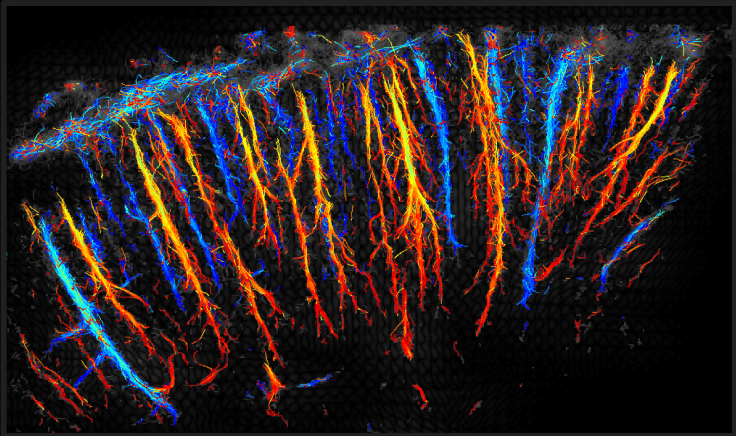


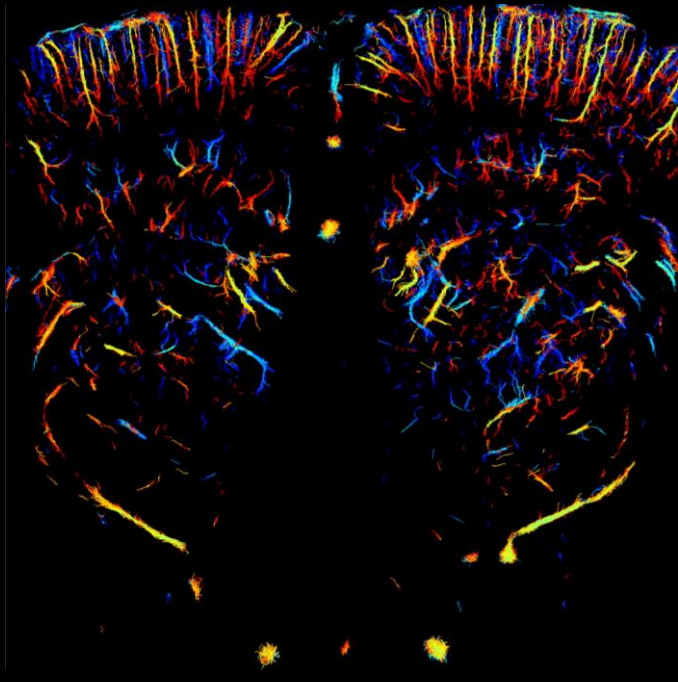
Corrected motion



# L'imagerie ultrarapide permet de dépasser la limite de résolution ultrasonore

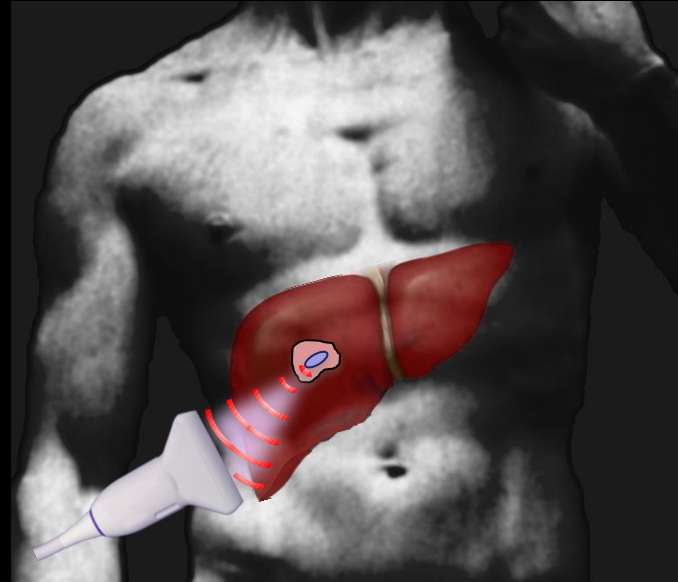
- Des millions de microbulles sont localisées
- La vitesse du sang est facilement mesurable
- Les doses de microbulles sont déjà utilisées en clinique
- La correction de mouvement est nécessaire





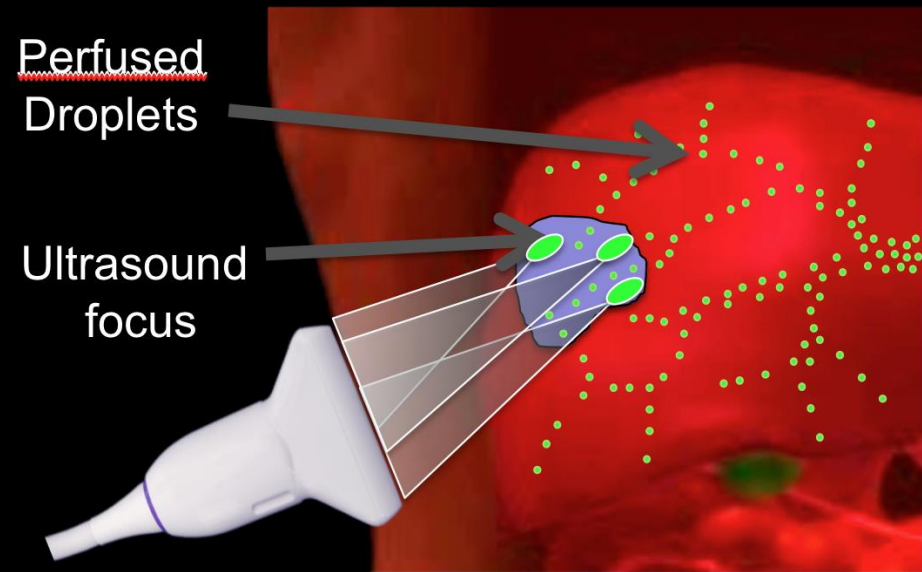
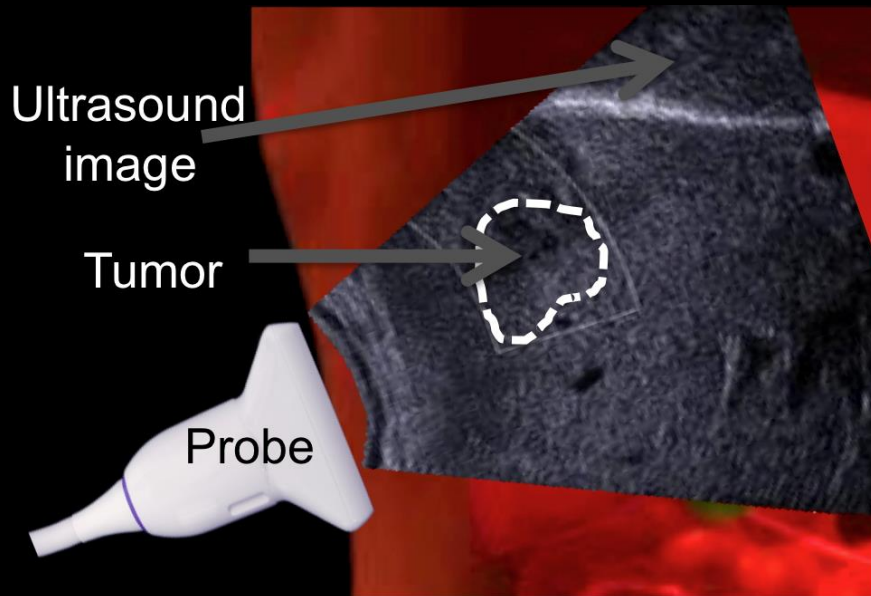
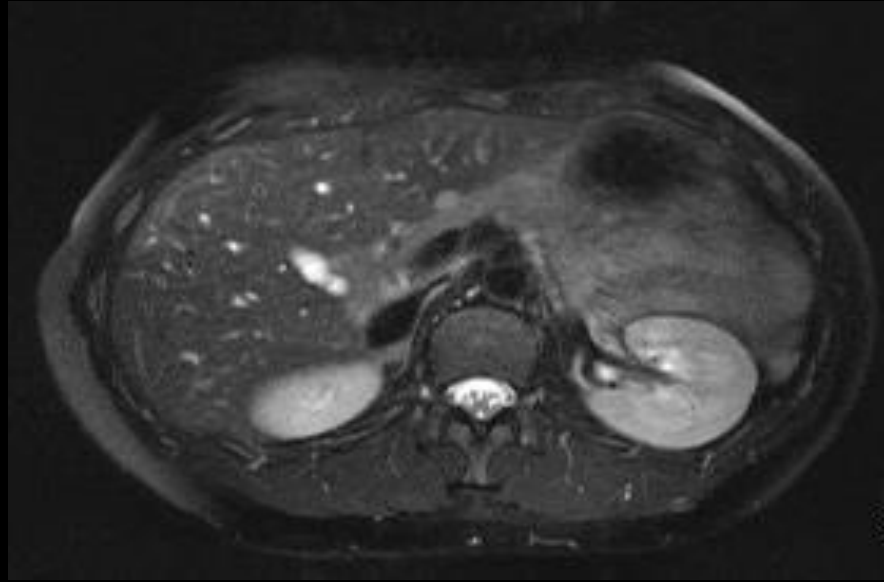
Microscopie par localisation  
ultrasonore

Theranostique ultrasonore  
super-résolu



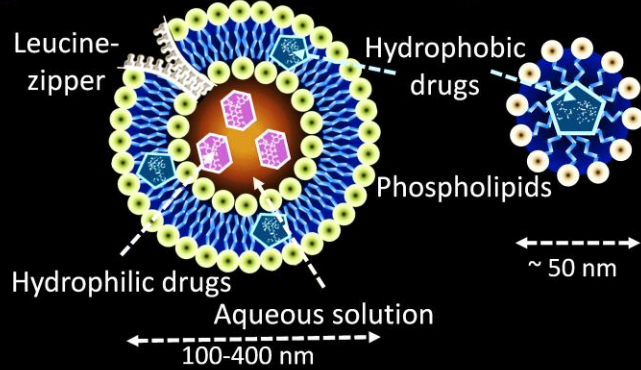
Délivrance ultrasonore

# Le tatouage in-vivo pourrait améliorer la chirurgie

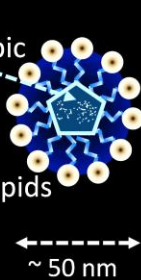


# Il y a plusieurs stratégies d'encapsulation sensible aux ultrasons

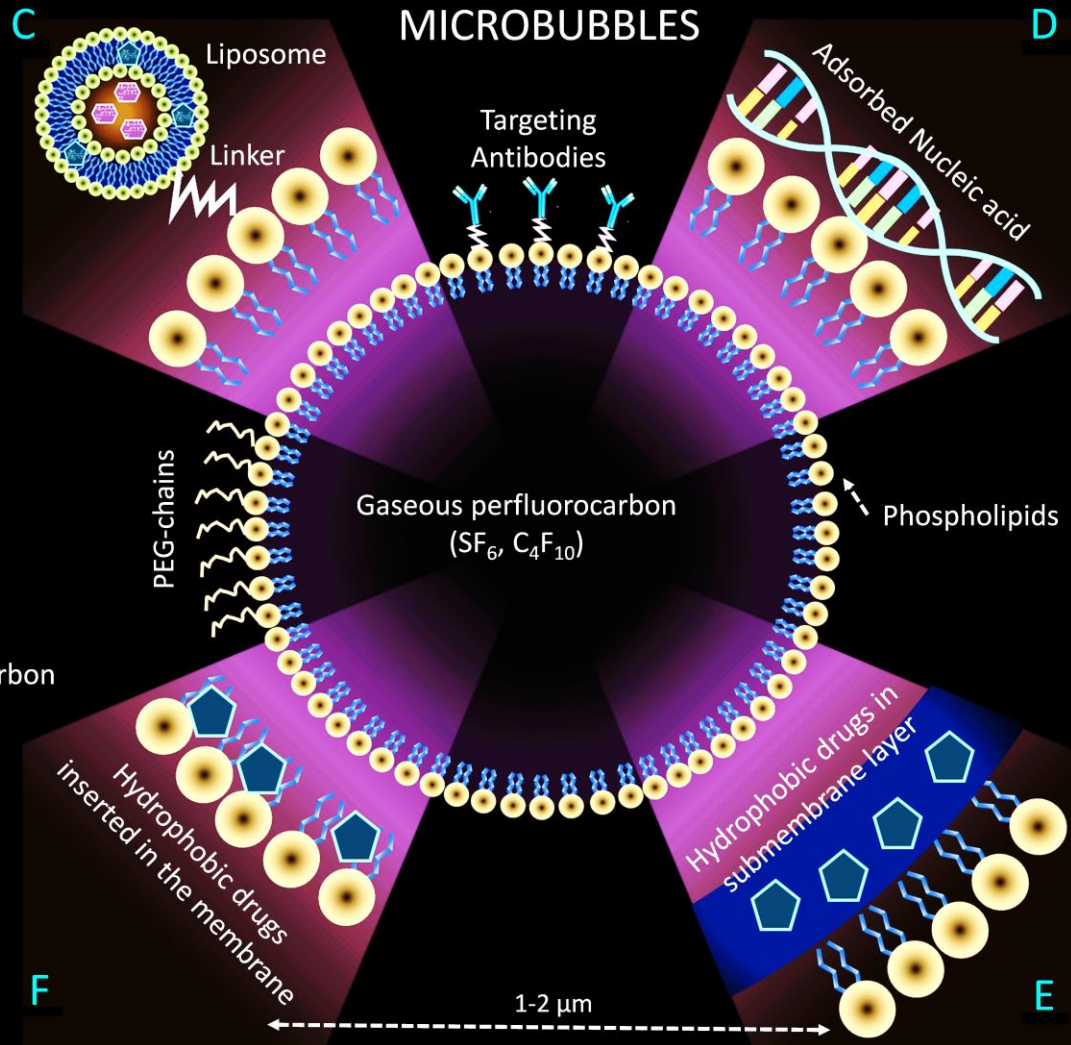
## A LIPOSOMES



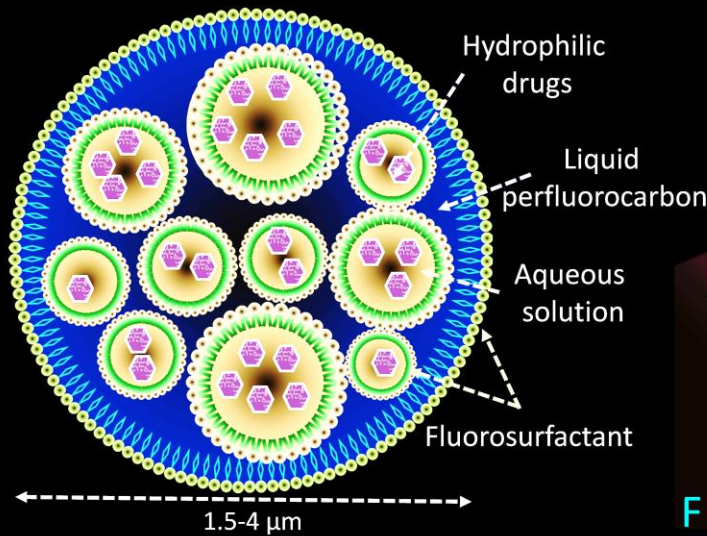
## B MICELLES



## C



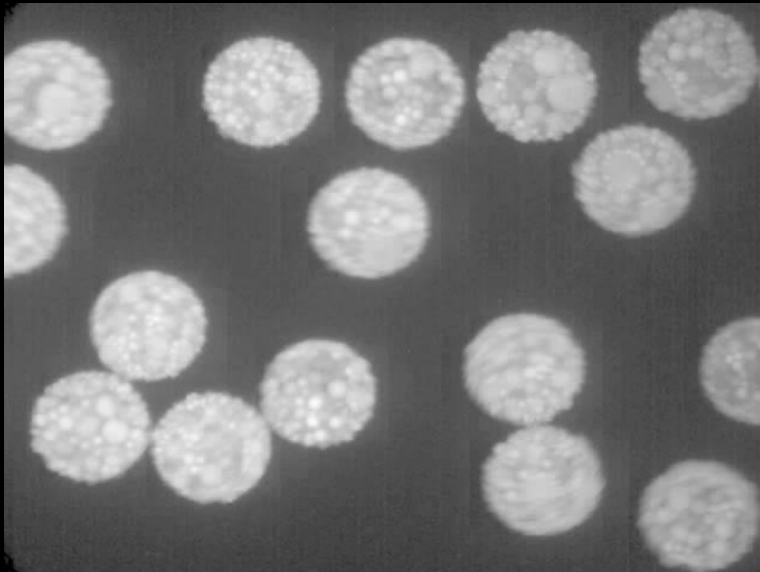
## G COMPOSITE DROPLETS



(Couture et al., 2014)

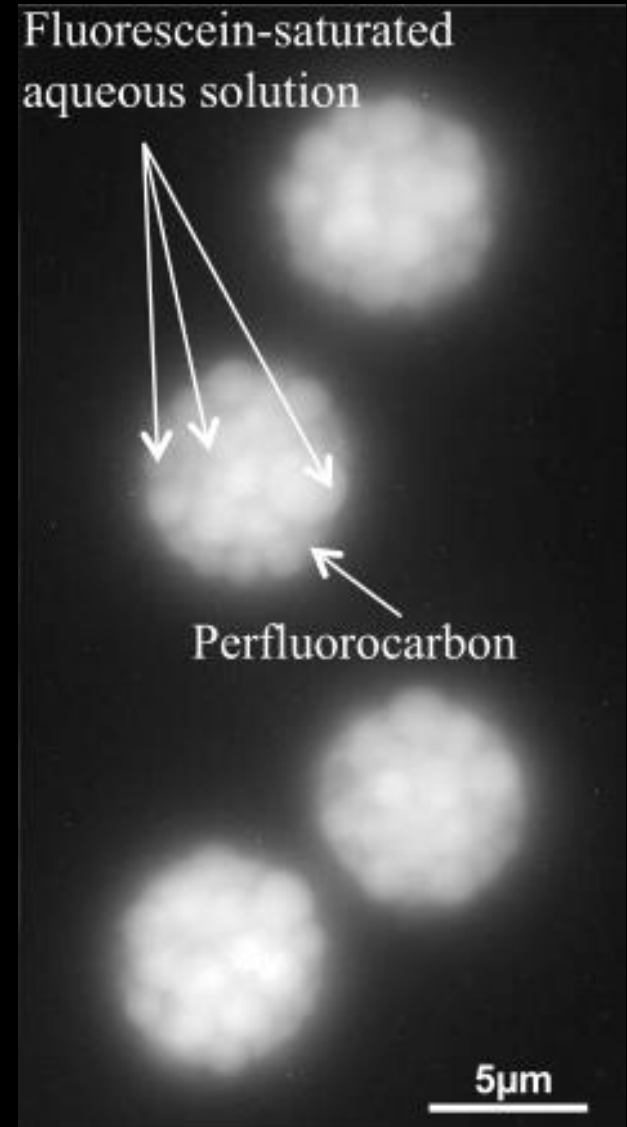


# Des grandes quantités de fluoresceine peuvent être encapsulées dans les gouttes composites

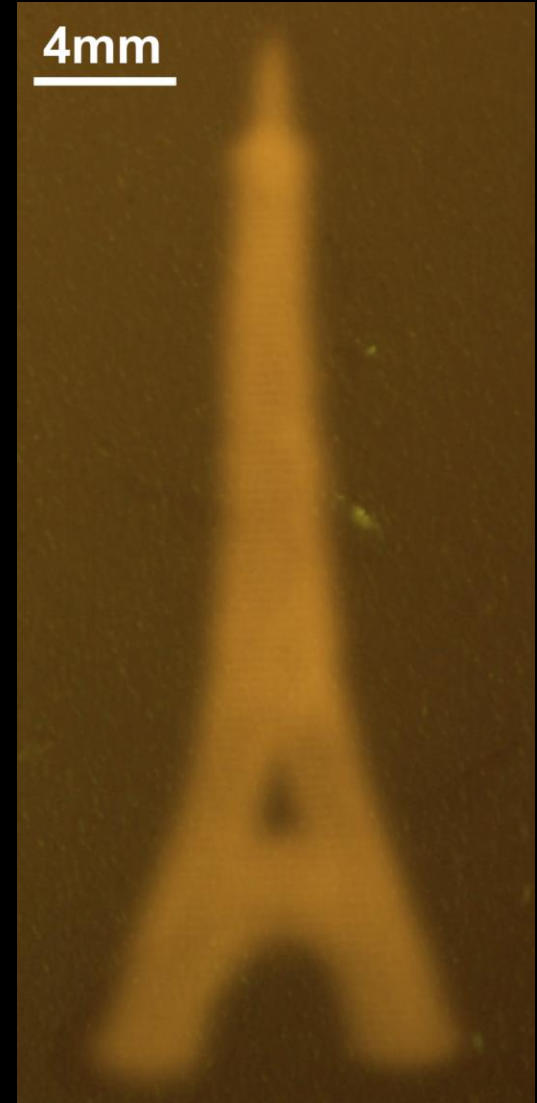
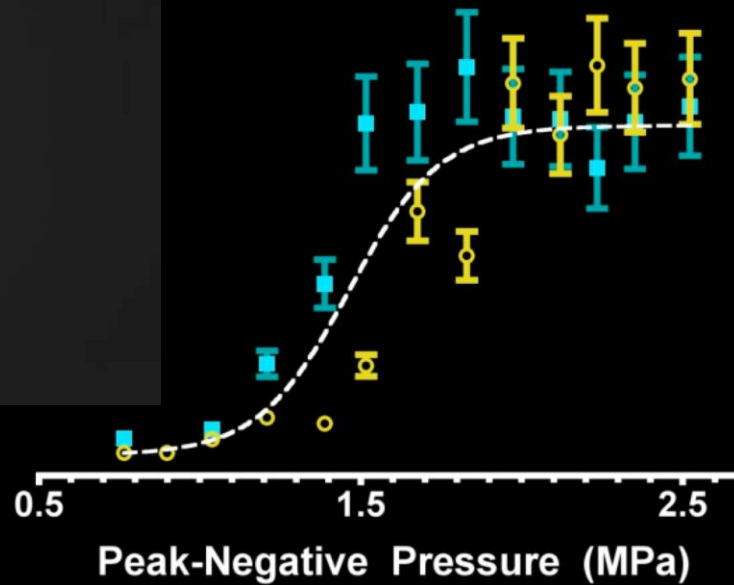
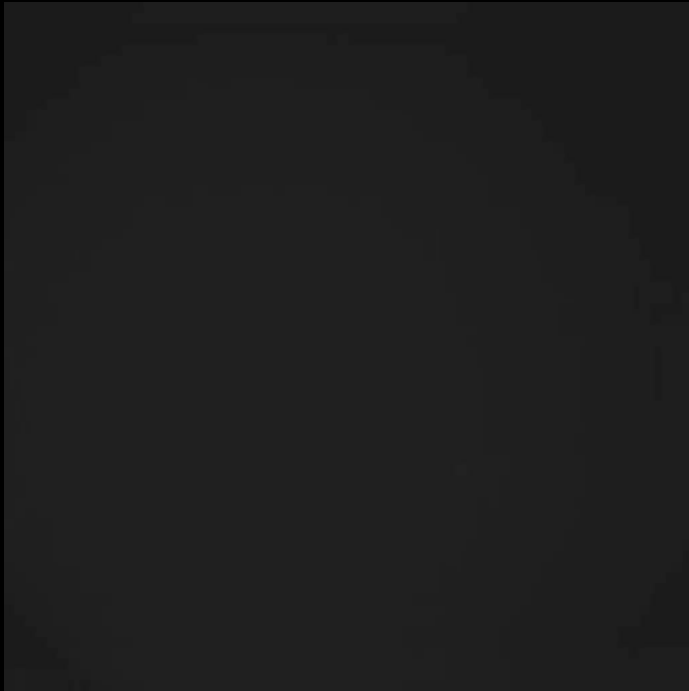


Monodispersity

Parallelized production



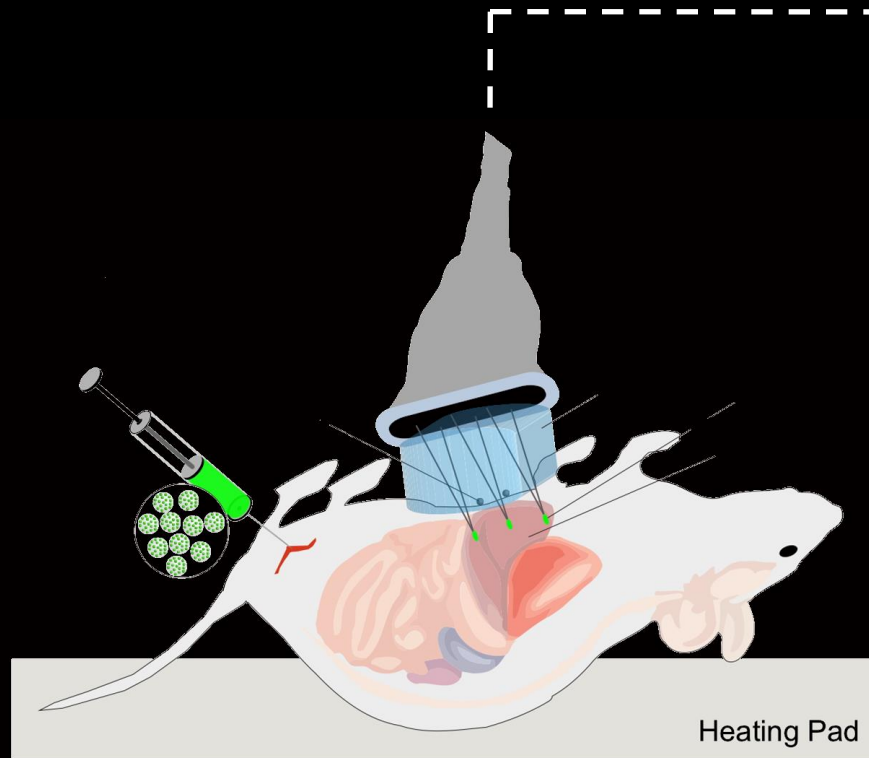
# Le largage est induit par ultrasons à un seuil très précis



Couture et al. 2009, 2011, 2012

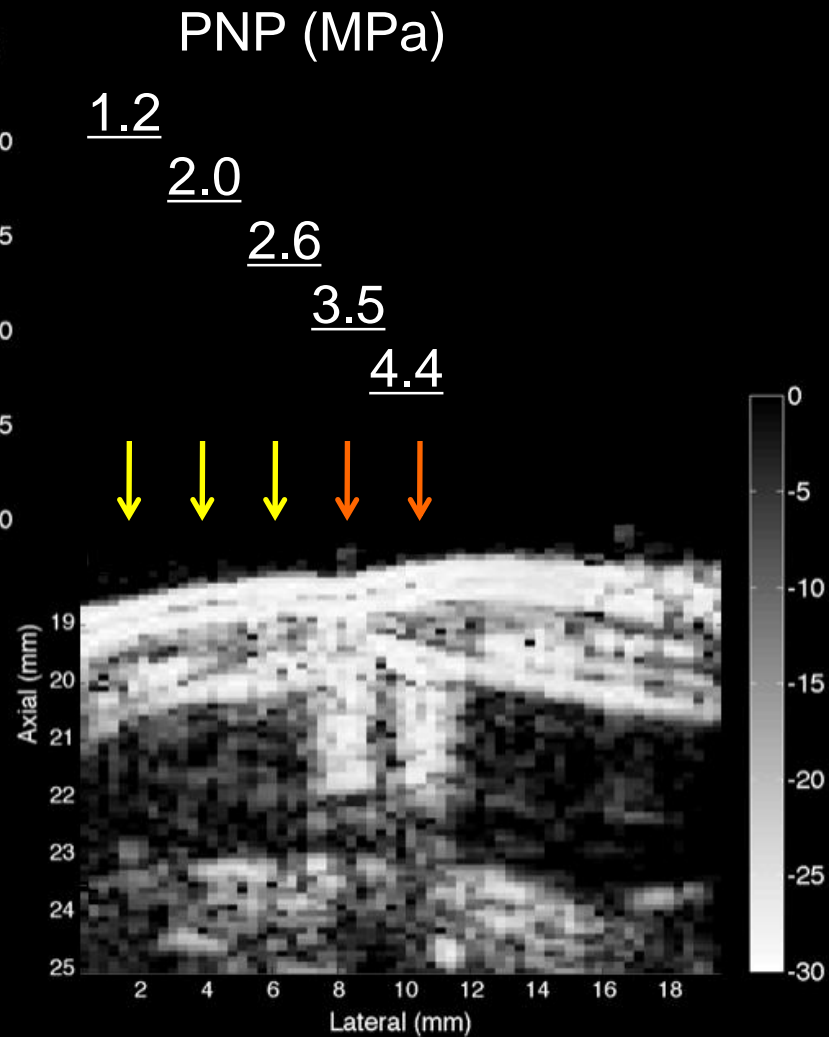
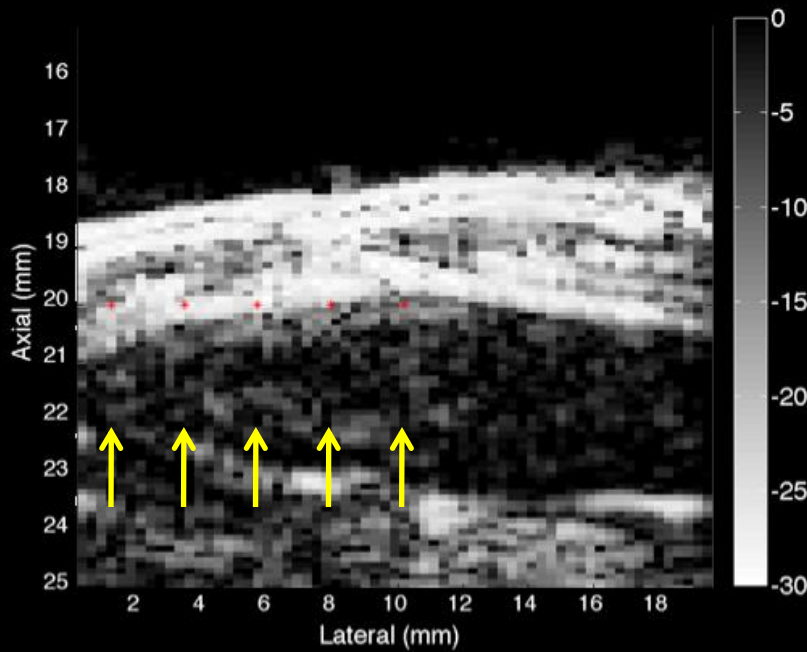


# Les microbulles sont injectées en intra-veineuse dans un rat

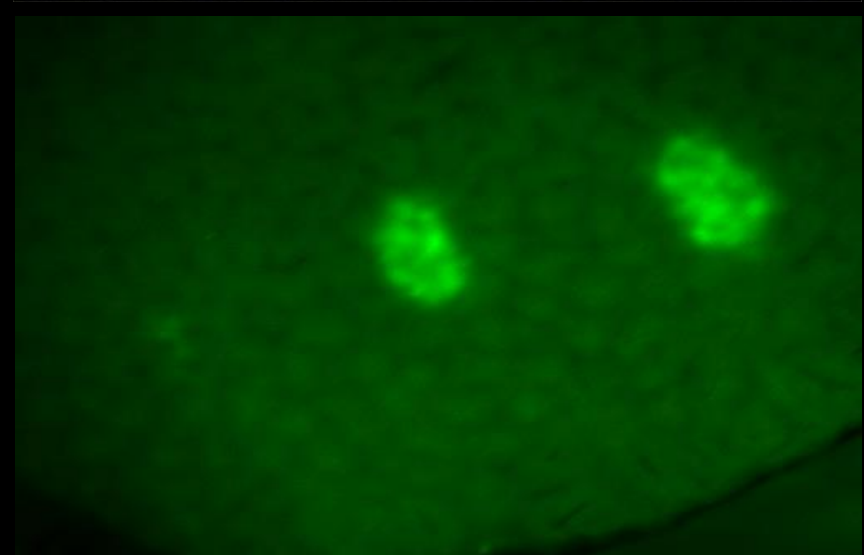
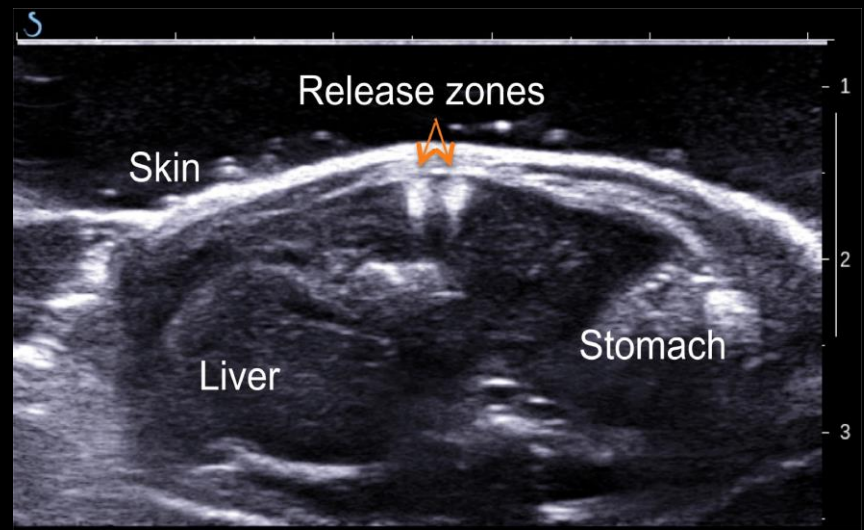
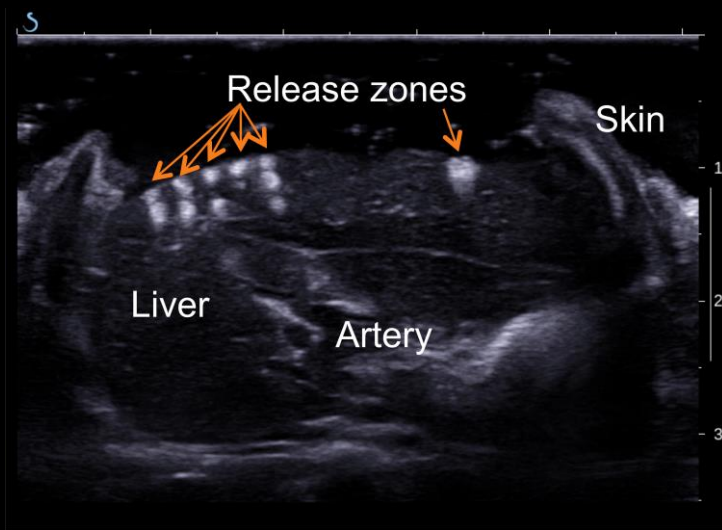


Supersonic Imagine

# Le suivi ultrasonore montre des zones plus échogènes

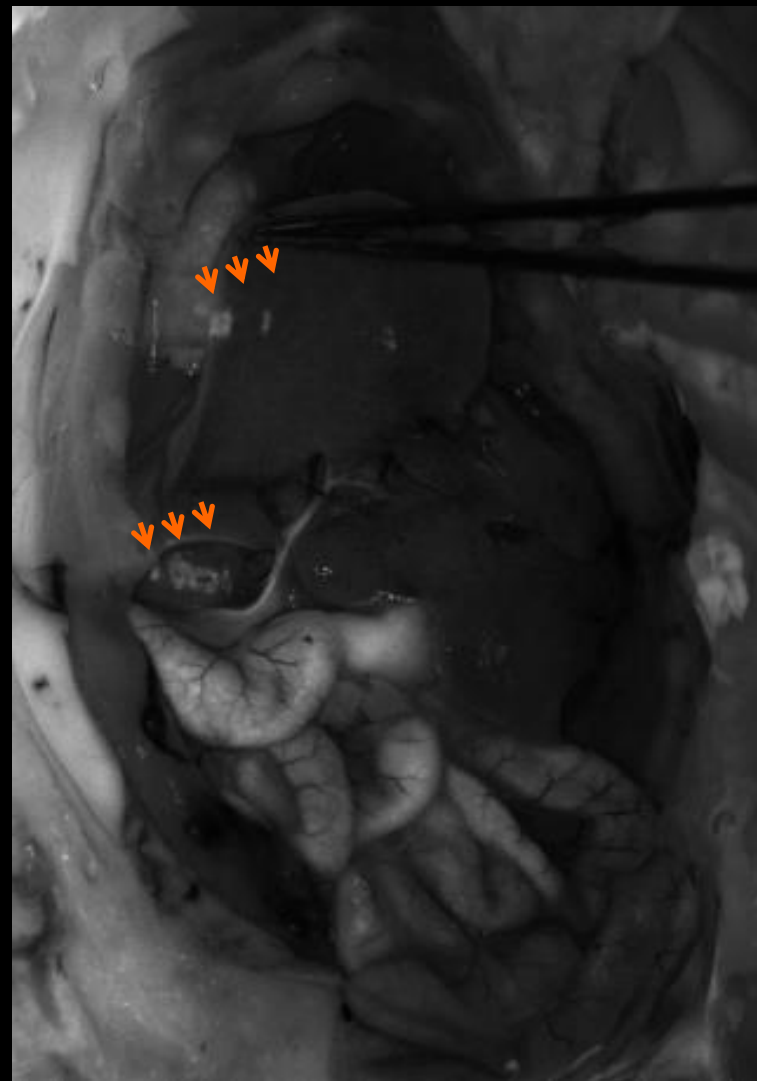
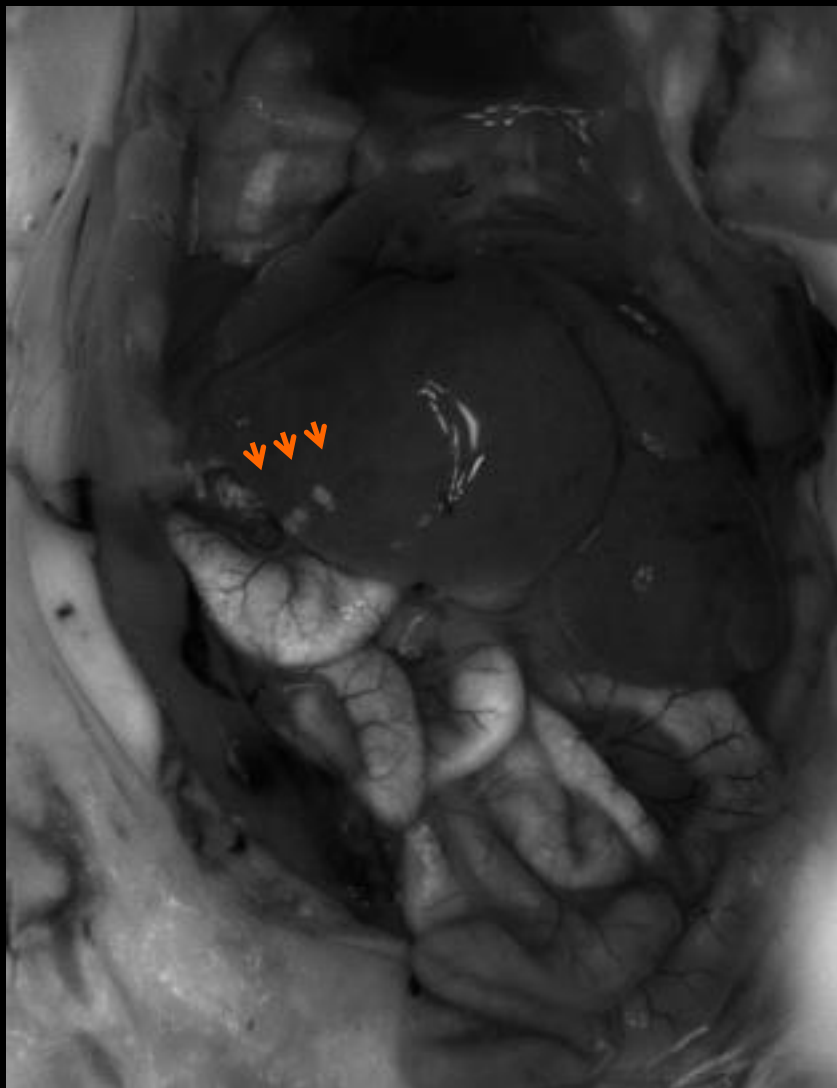


# Les cibles de focalisation sont observables par imagerie de fluorescence



Couture et al. 2012

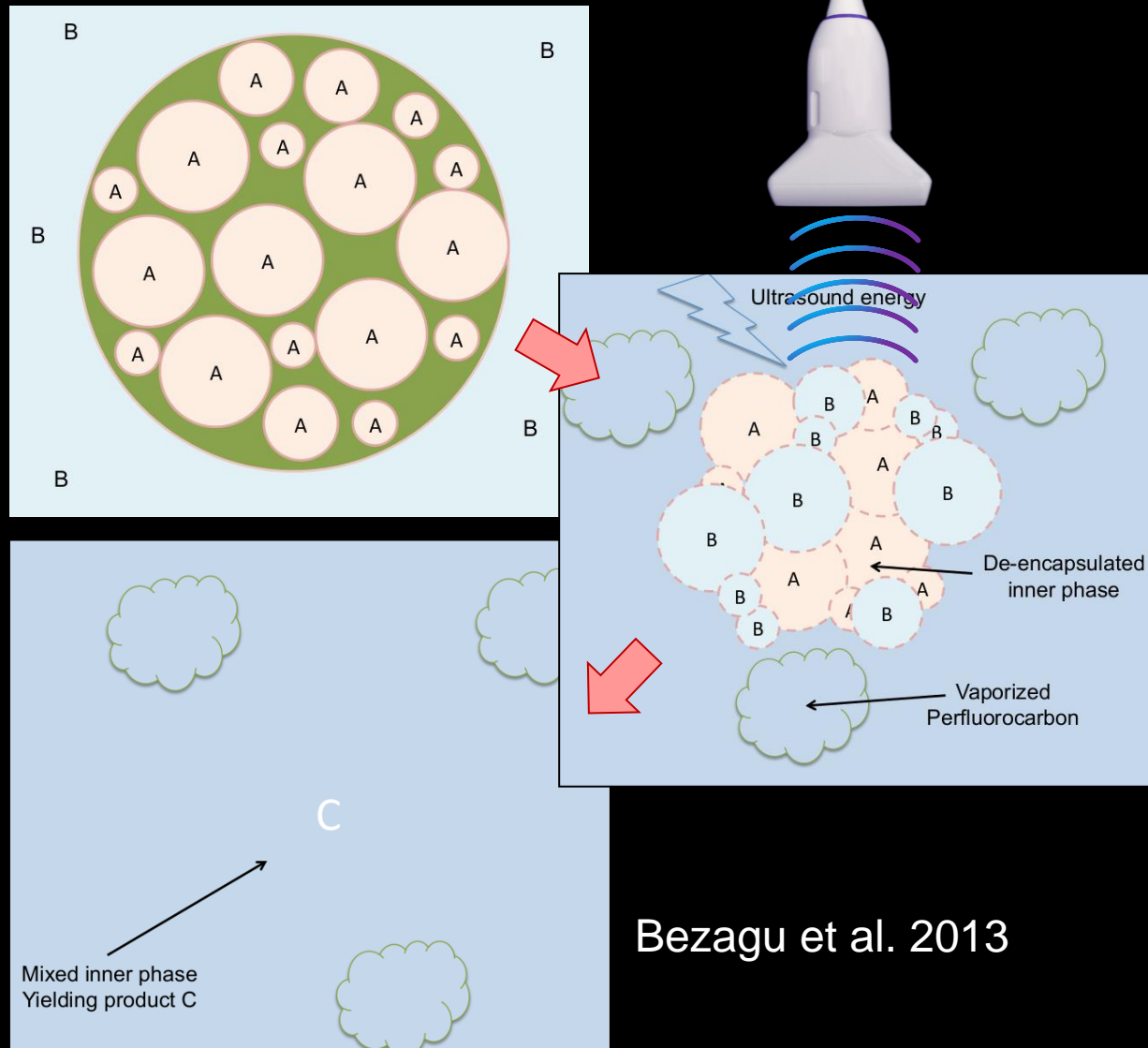
# Une caméra intra-opératoire détecte les zones de largage



Fluobeam (Fluoptics, Grenoble)

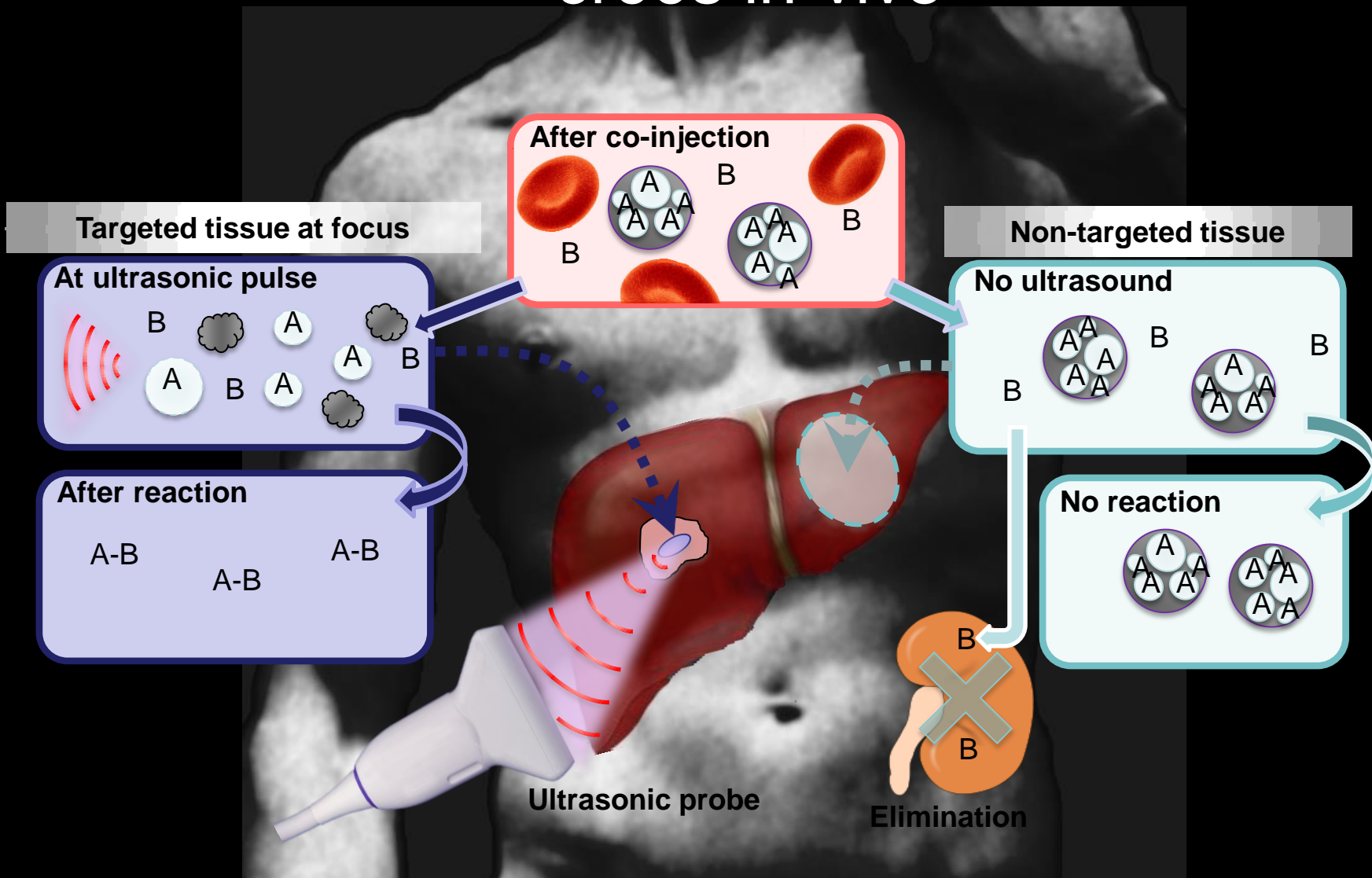


# Les gouttes pourraient être utilisées pour créer des réactions chimiques



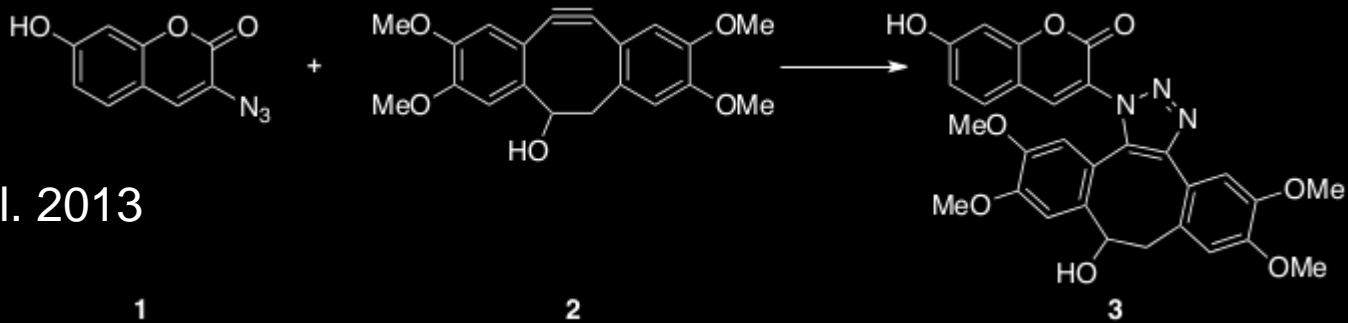
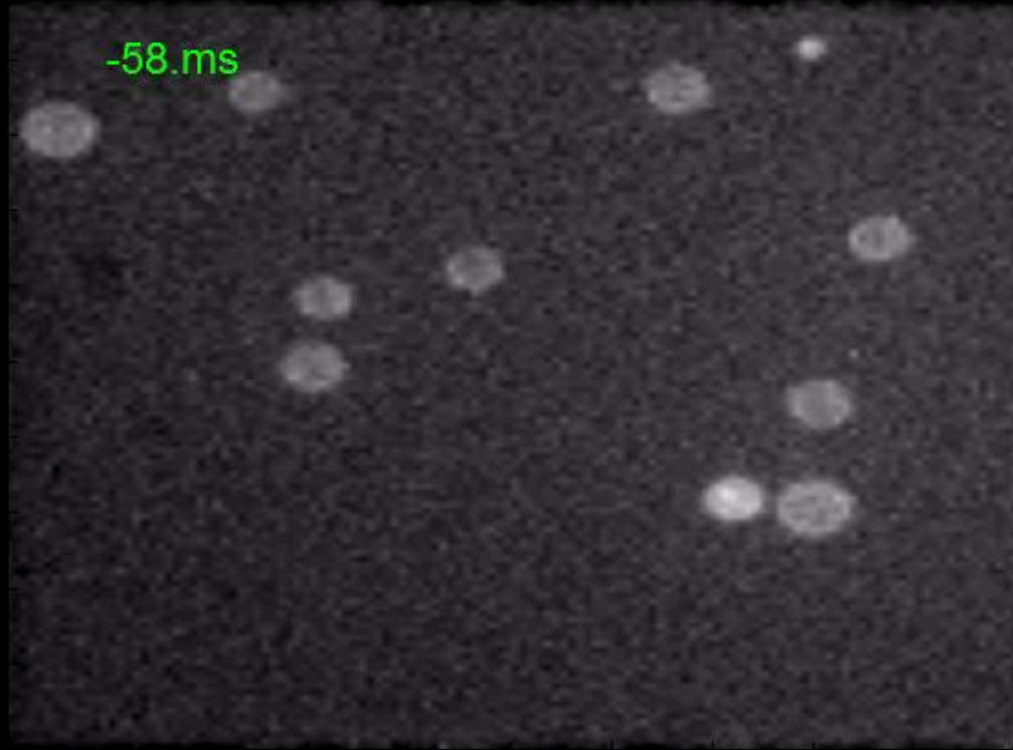
Bezagu et al. 2013

# Des médicaments pourraient être créés in-vivo





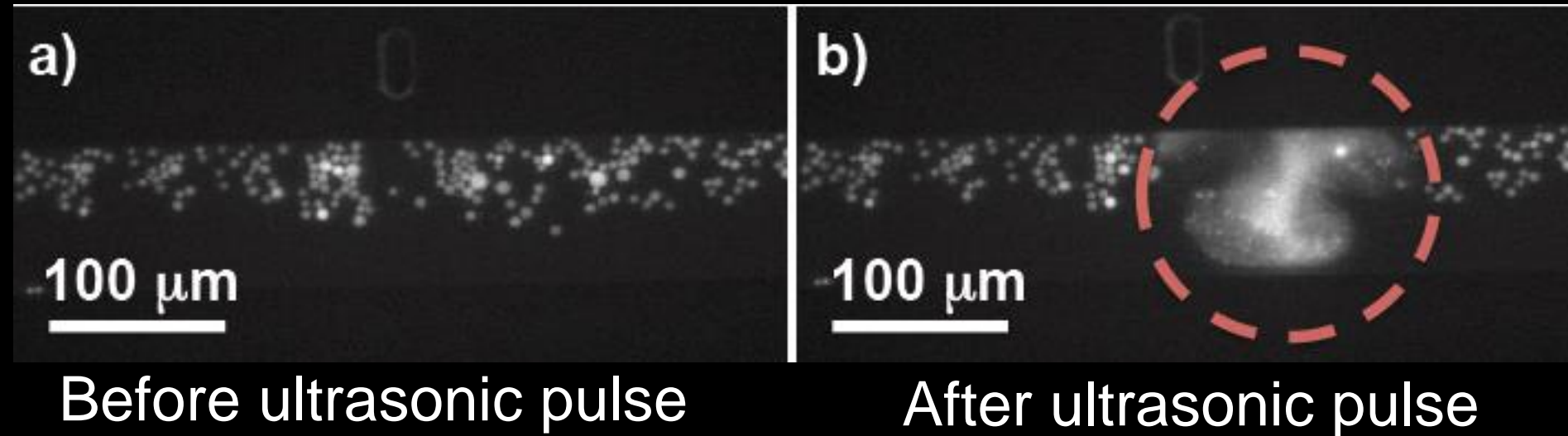
# La chimie click peut être induite localement par ultrasons



Bezagu et al. 2013



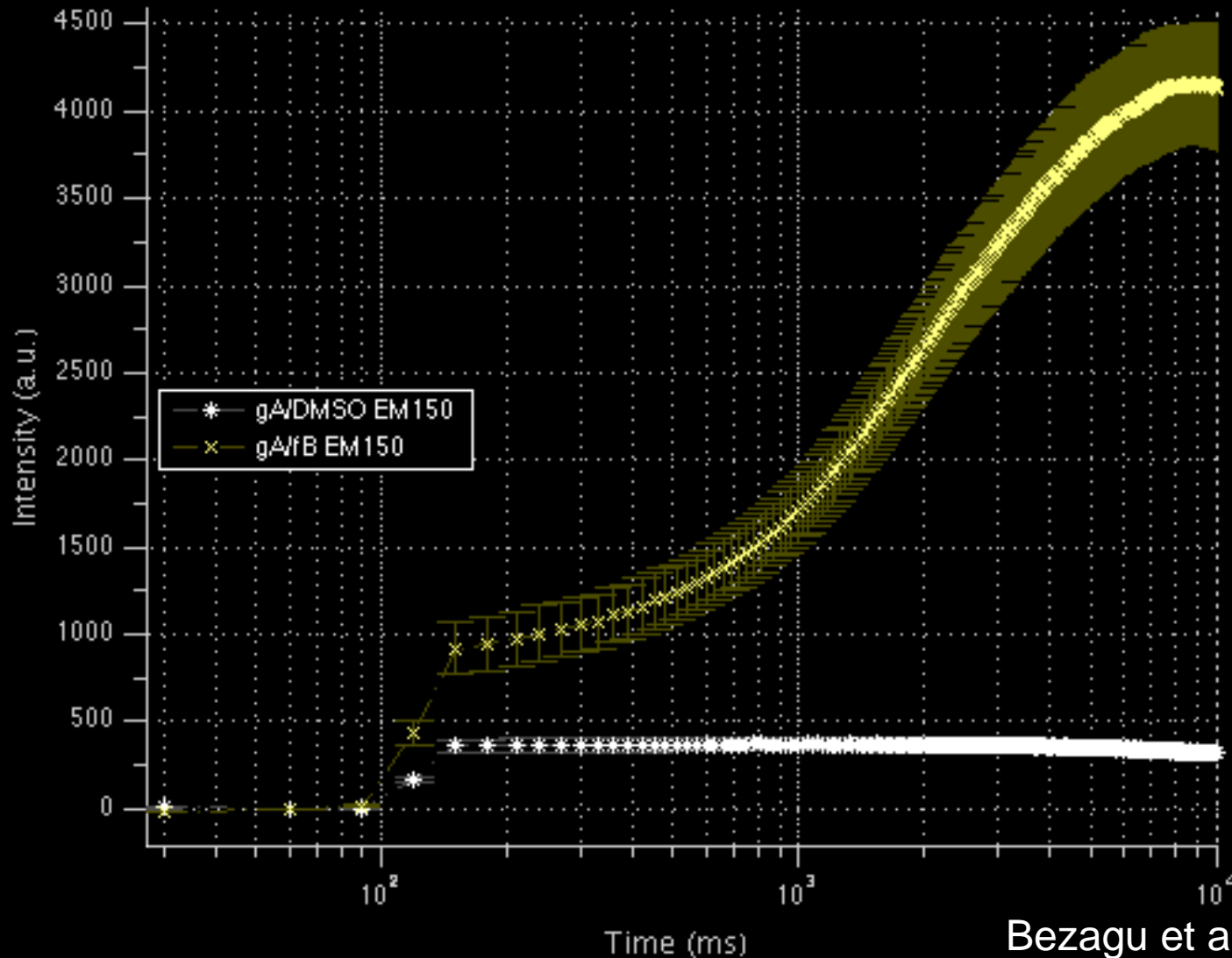
# La réaction chimique est contrôlée dans l'espace et dans le temps



Bezagu et al. 2013

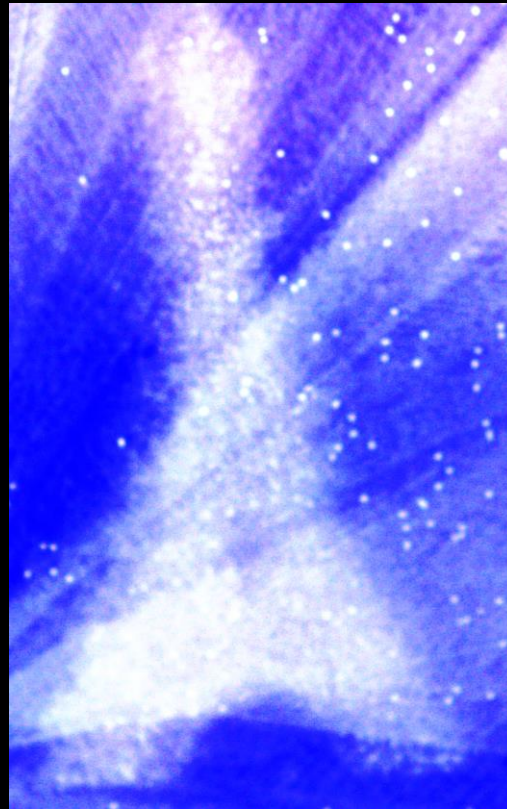


# La fluorescence, le marqueur de la réaction, est détectable après induction ultrasonore



Bezagu et al. 2013

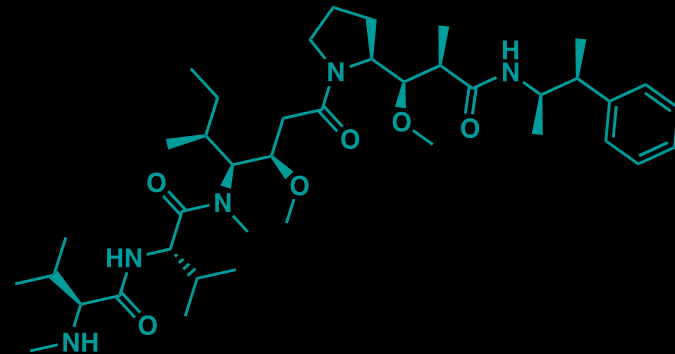
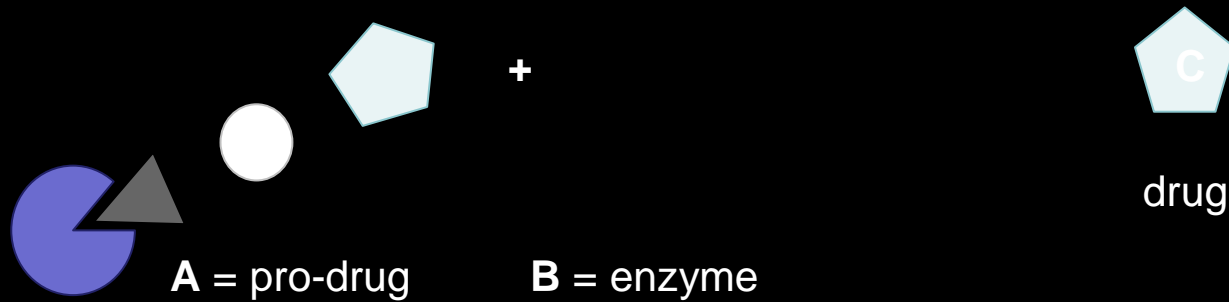
# La réaction chimique peut être guidée grâce à l'échographe



Bezagu et al. 2013



# Des médicaments anti-cancéreux pourraient être induits localement



## Monométhylauristatine E (MMAE)

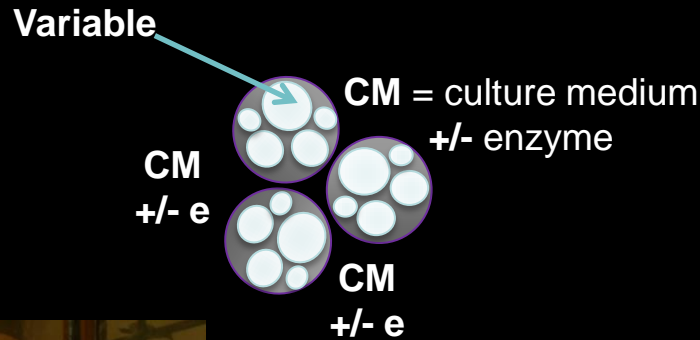
IC<sub>50</sub>: 0.19 nM (KB, human mouth carcinoma)

*Non commercial*

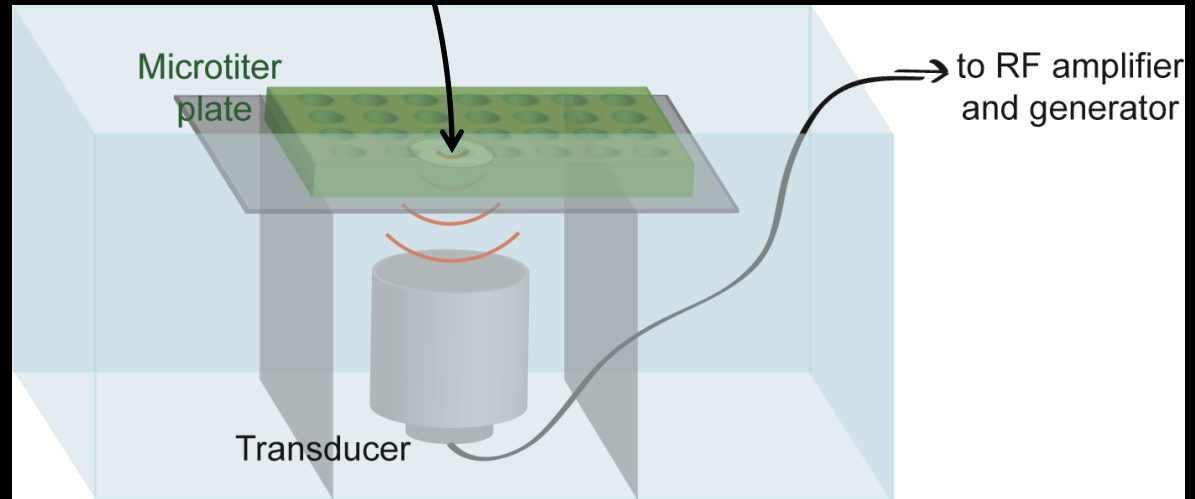
## Prodrug: MMAE-glu

IC<sub>50</sub>: 20.8 nM (KB, human mouth carcinoma)

# Les gouttes ont été larguées dans des plaques 96 puits avec des cellules



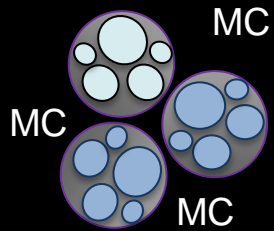
Marine Bézagu



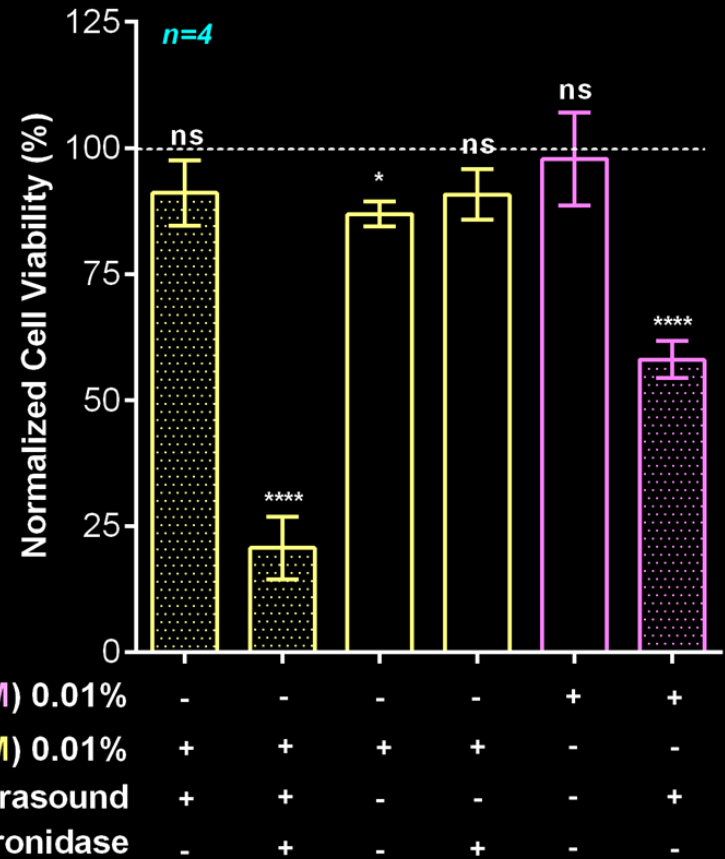
4 x 1 pulse of 5 cycles,  $\approx 11$  MPa PNP, 1.5 MHz, 37 ° C  
KB cancer cells

XTT cell viability assay, normalization by non-treated cells

# Les prodrogues sont bien encapsulées dans les gouttes et une mortalité spécifique est observable sur les cellules cancéreuses

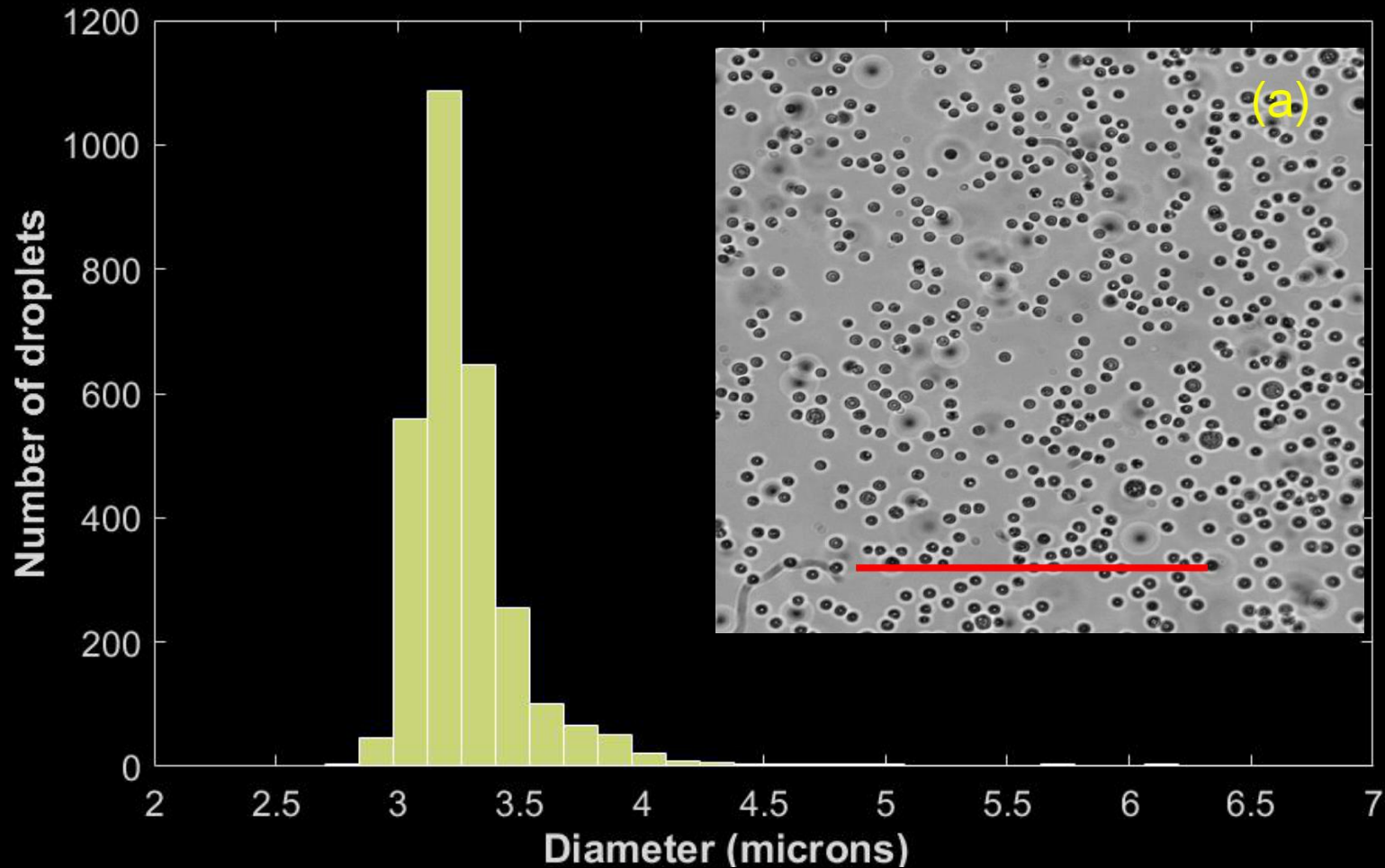


● = MMAE-glu 0.1 mM  
in cell culture medium



Droplets (MMAE 0.1mM) 0.01%	-	-	-	-	+	+
Droplets (MMAE-glu 0.1mM) 0.01%	+	+	+	+	-	-
Ultrasound	+	+	-	-	-	+
$\beta$ -glucuronidase	-	+	-	+	-	-

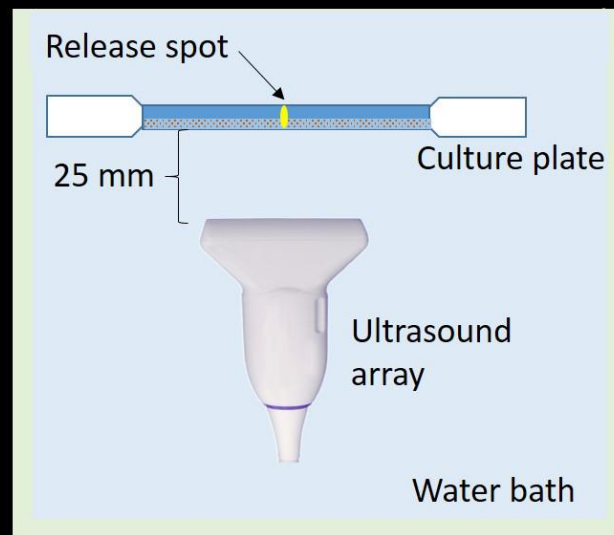
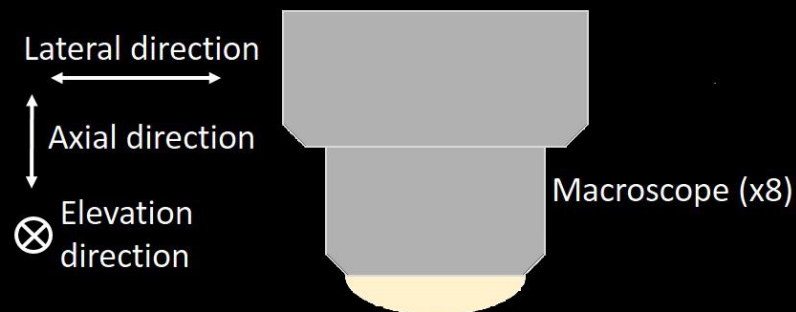
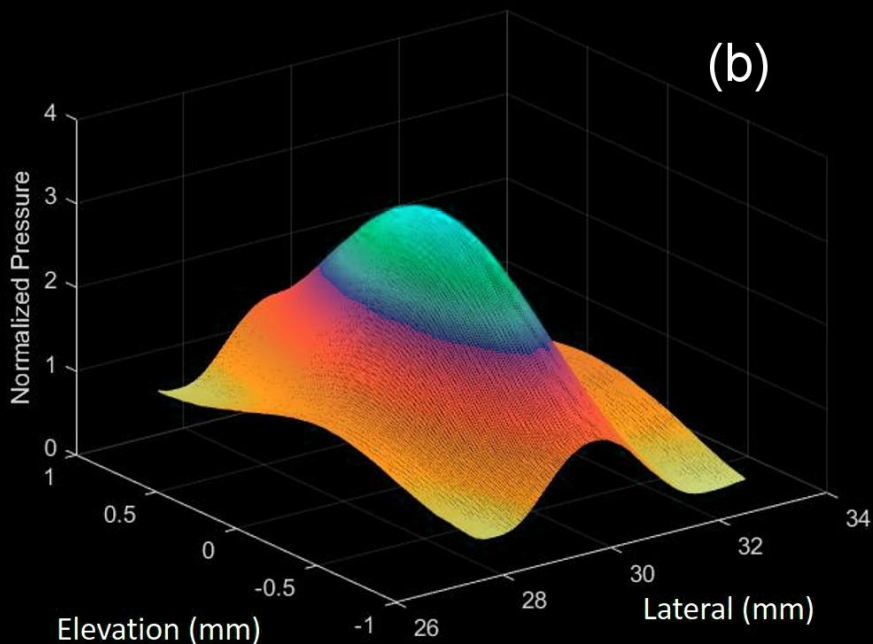
# Peut-on faire de la thérapie ultrasonore ultrarésolue? Le cas des gouttes composites



*Hingot et al. 2016b, accepted*

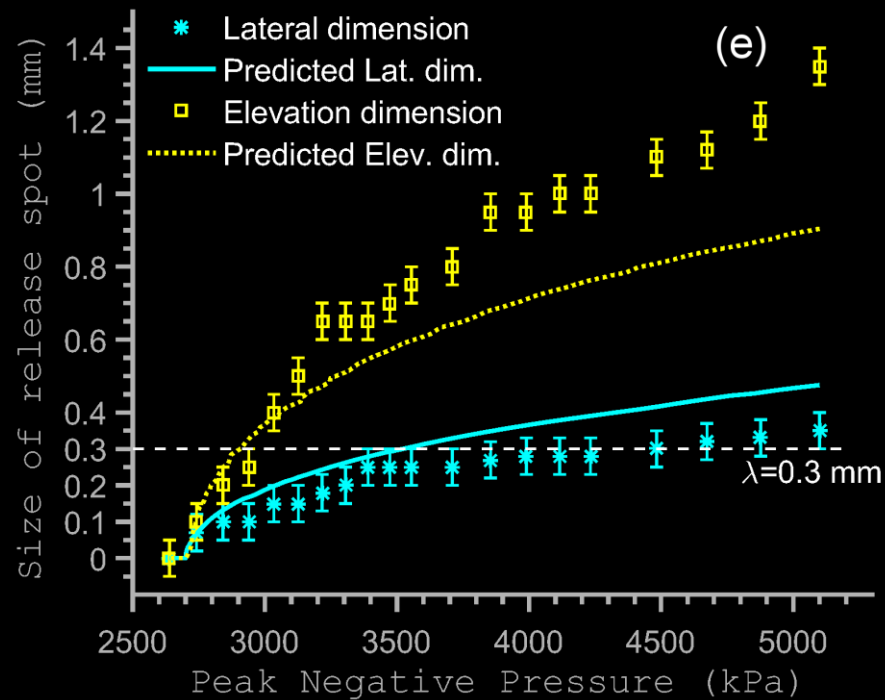
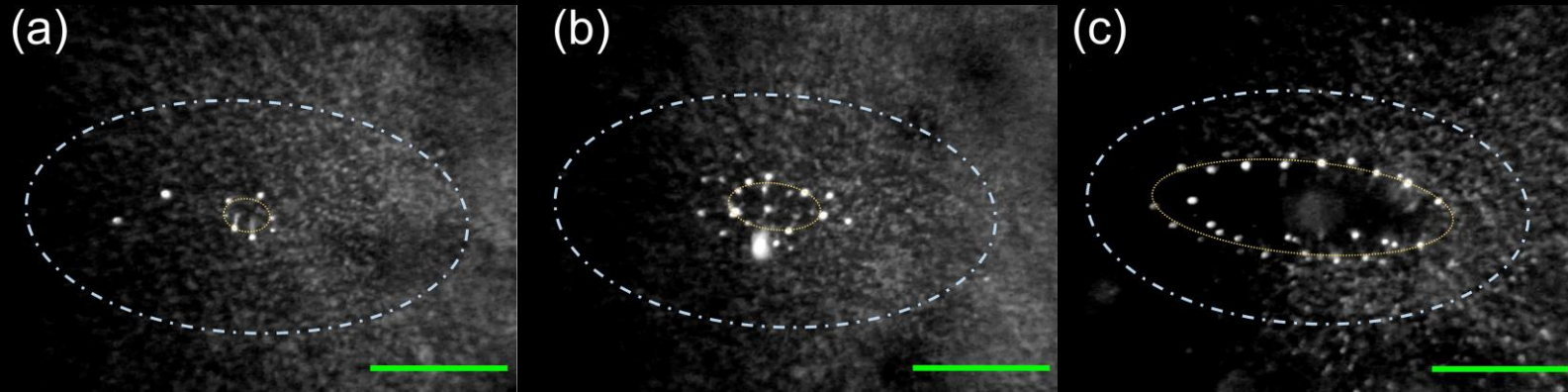


Un suivi constant et une augmentation graduelle des pressions permet de limiter le largage dans une zone plus fine que la longueur d'onde

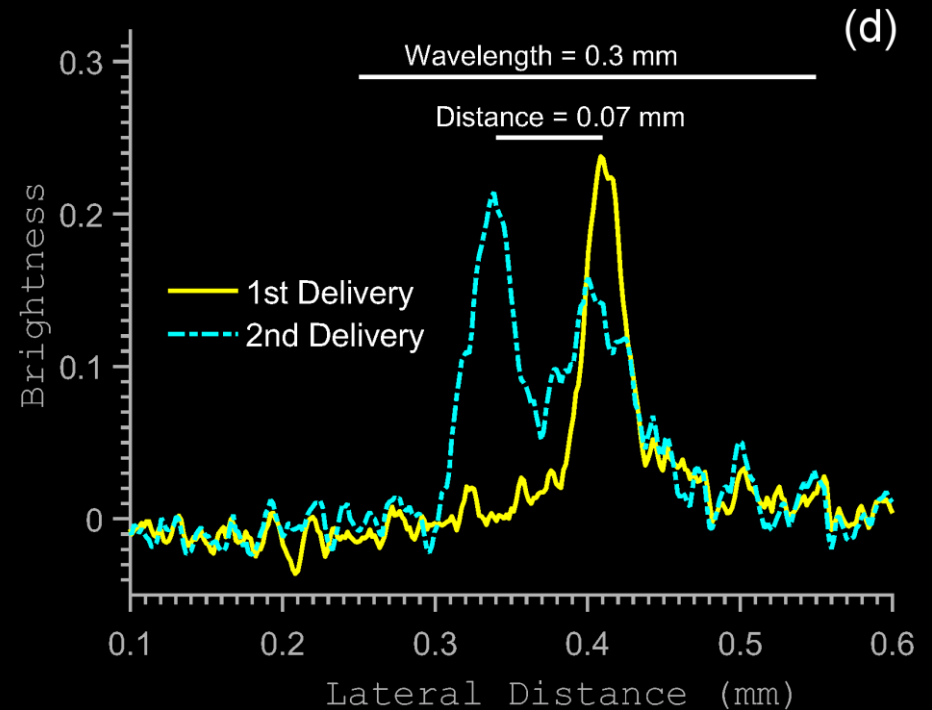
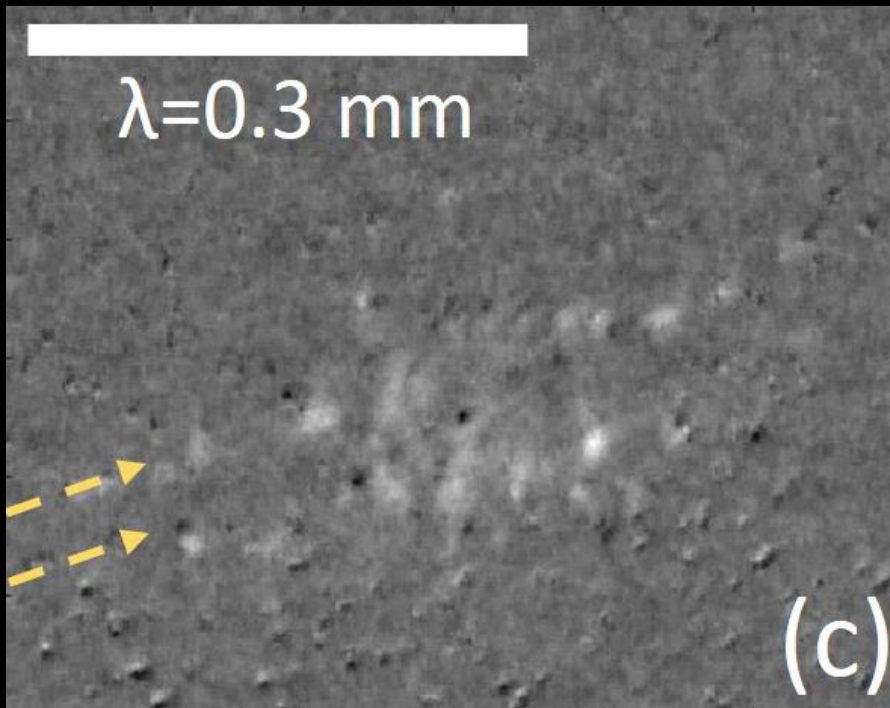


Hingot et al. 2016b, accepted

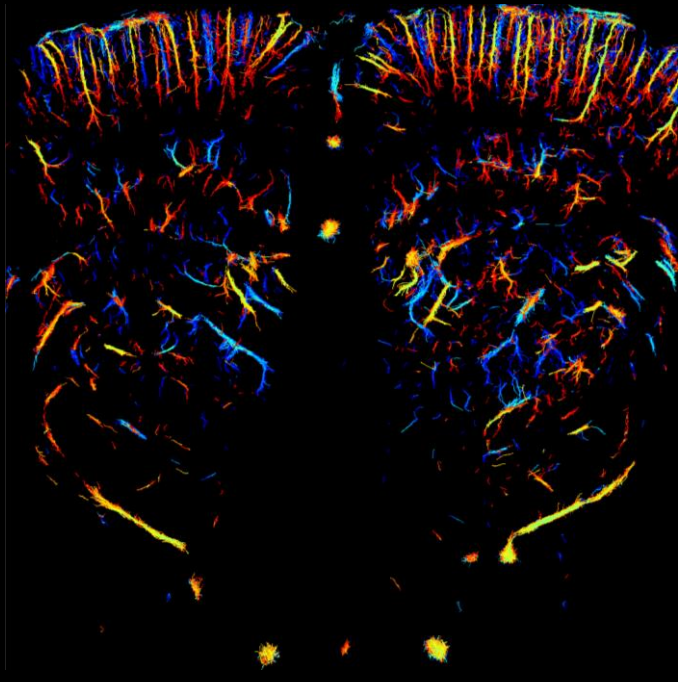
# Une résolution d'un quart de longueur d'onde est atteignable



Deux points de largage peuvent être induits à une distance d'un quart de longueur d'onde

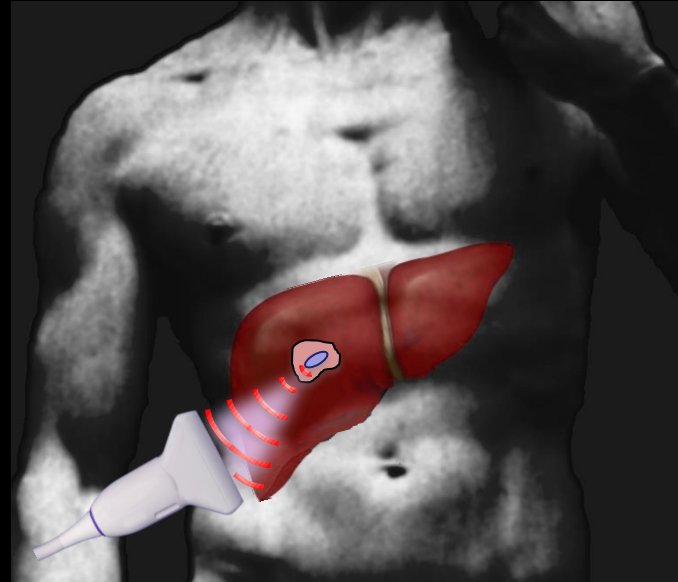


Hingot et al. 2016b, accepted



Microscopie par localisation  
ultrasonore

Theranostique ultrasonore  
super-résolu



Délivrance ultrasonore