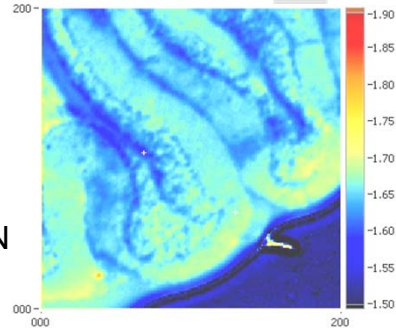


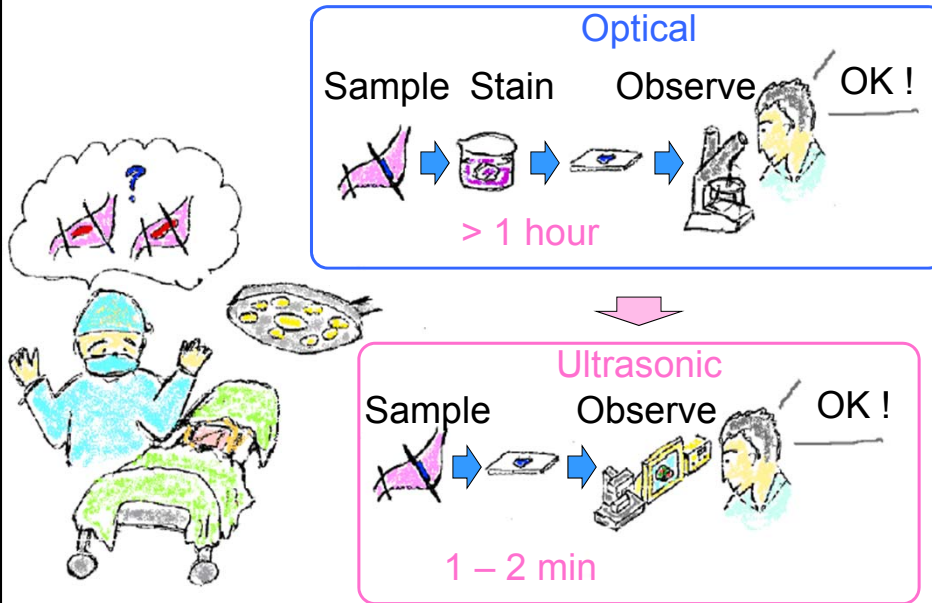
# Scanning Acoustic Microscopy for Medical and Biological Applications

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Honda Electronics Co., Ltd.

JAPAN



## A clinical need.

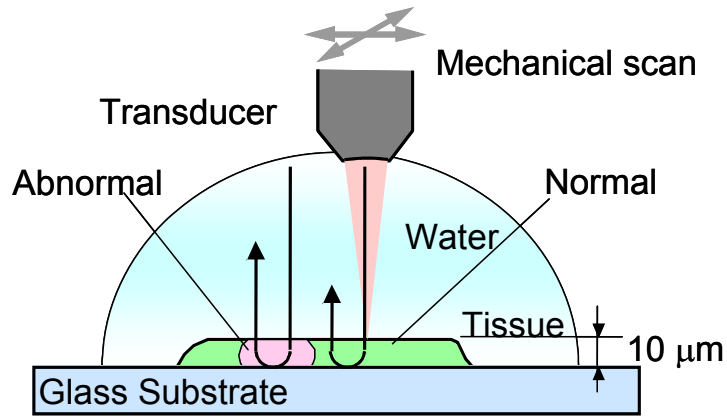


Sound Speed Microscope  
Acoustic Impedance Microscope

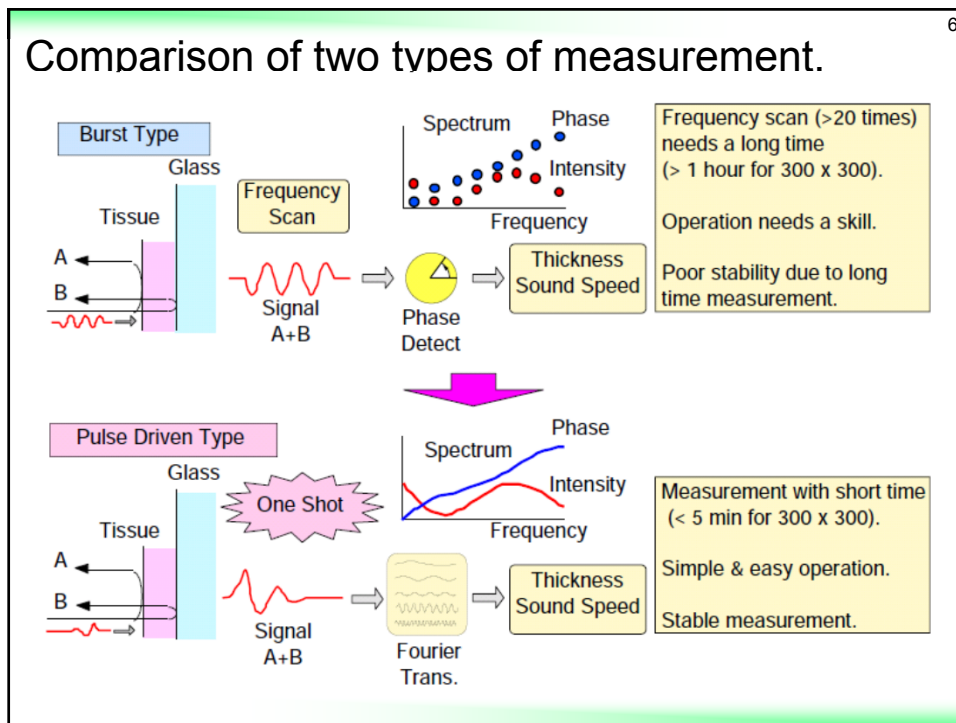
Scanning Sound  
Speed Microscopy

# Basic idea.

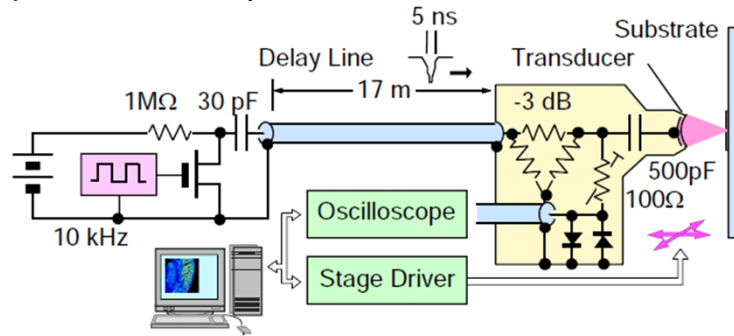
5



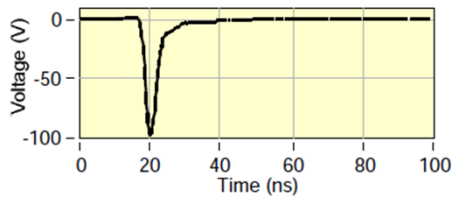
Visualize sound speed and attenuation.



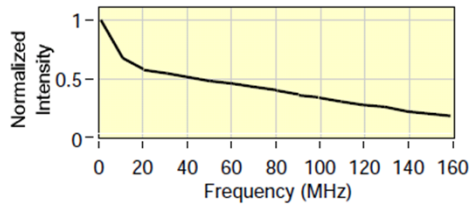
The system is simple.



(a) Diagram

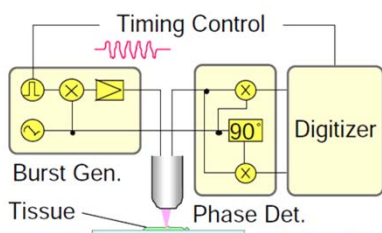


(b) Waveform of pulse voltage



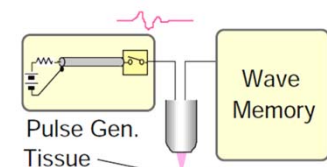
(c) Intensity spectrum of (b)

Development of proto-type.



Conventional

Analog based  
Large  
High cost - \$500k

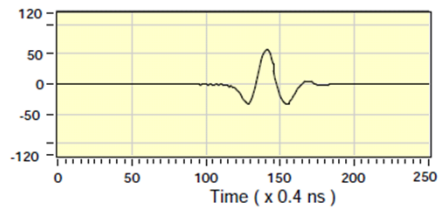


Proposed

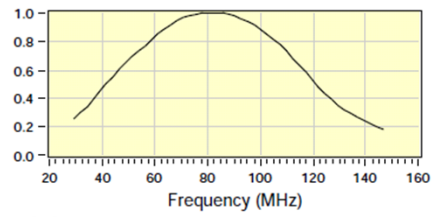
Digital based  
Compact  
Low cost - <\$100k?



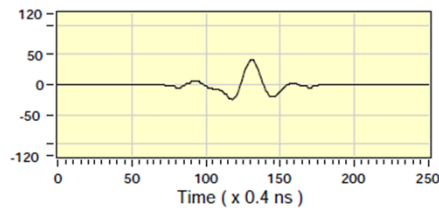
# Waveforms.



(a) Reflection from glass

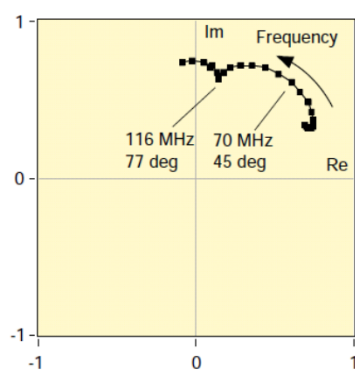
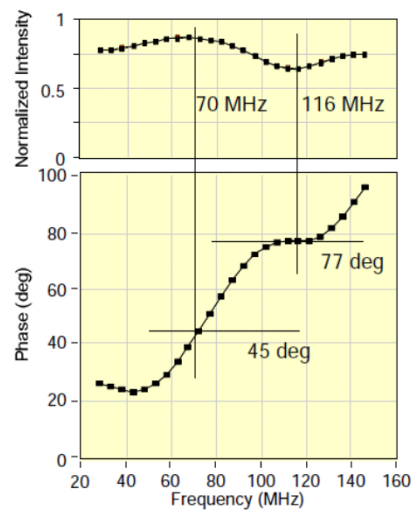


(b) Intensity spectrum of the reflection



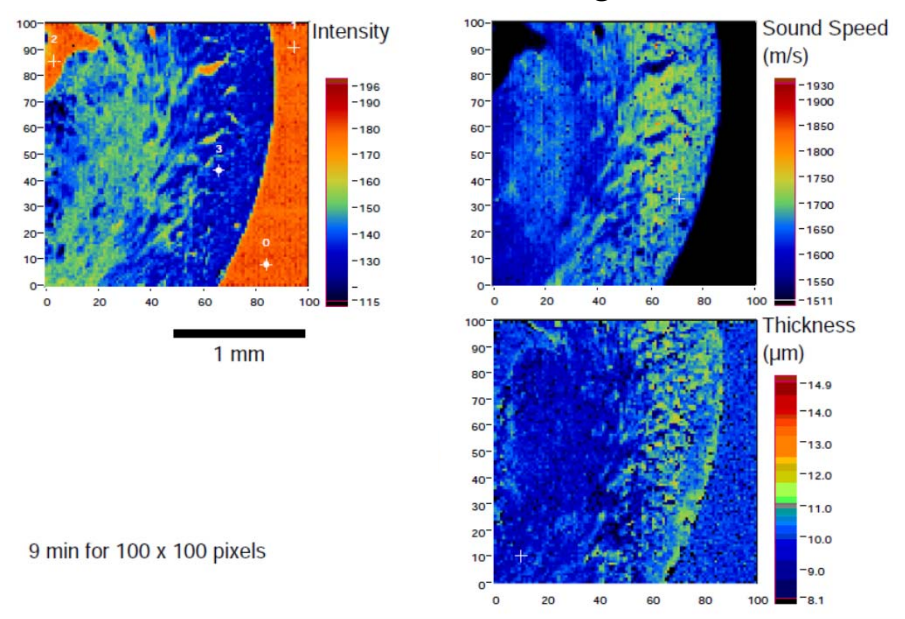
(c) Reflection from tissue

# Analysis in the frequency domain.

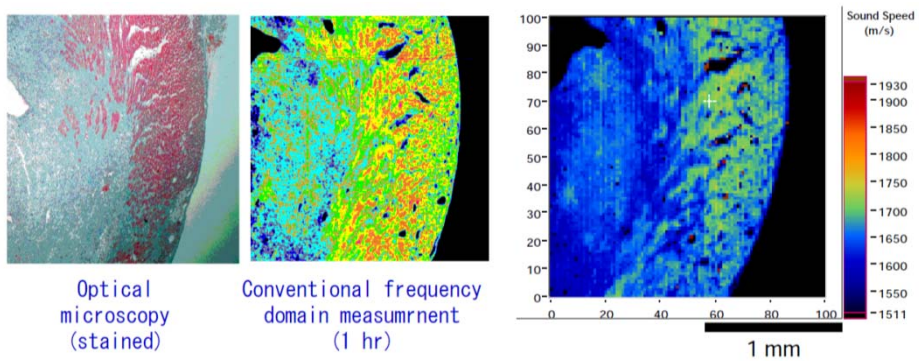


$$\begin{aligned}
 \text{(dip)} \quad 2\pi f_m \times \frac{2d}{c_0} &= \phi_m + (2n-1)\pi & d &= \frac{c_0}{4\pi f_m} \{ \phi_m + (2n-1)\pi \} \\
 \text{(maxima)} \quad 2\pi f_m \times \frac{2d}{c_0} &= \phi_m + 2n\pi & d &= \frac{c_0}{4\pi f_m} (\phi_m + 2n\pi) \\
 2\pi f_m \times 2d \left( \frac{1}{c_0} - \frac{1}{c} \right) &= \phi_m & c &= \left( \frac{1}{c_0} - \frac{\phi_m}{4\pi f_m d} \right)^{-1}
 \end{aligned}$$

### Observation of a rat cardiac allograft model.

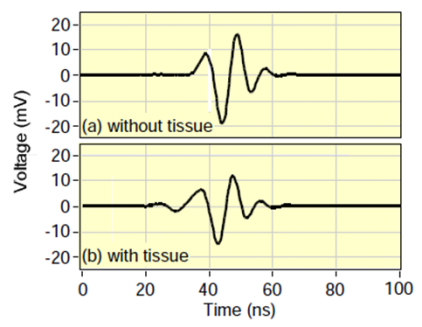


### Comparison with optical microscopy.

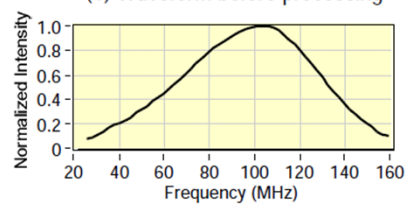


Massive hyalinization in the endocardial side (left side of the figure) classified as severe allograft rejection.

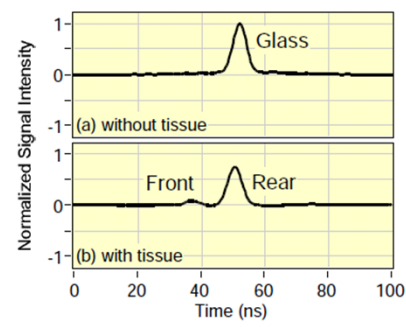
### Waveforms before and after the processing.



(1) Waveform before processing

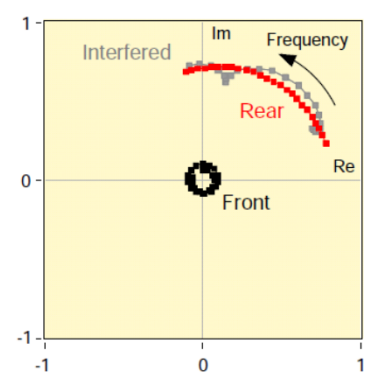
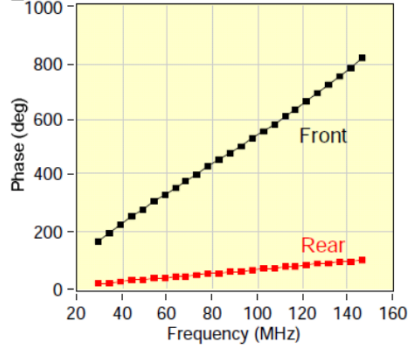
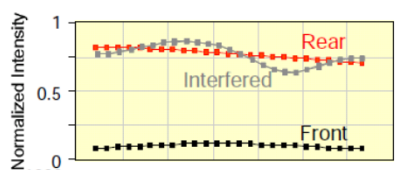


Intensity spectrum of (1-a)



(2) Waveform after processing

### Analysis for separated waveforms.



$$2\pi f \times \frac{2d}{c_0} = \phi_{front} \quad \rightarrow \quad d = \frac{c_0}{4\pi f} \phi_{front}$$

$$2\pi f \times 2d \left( \frac{1}{c_0} - \frac{1}{c} \right) = \phi_{rear} \quad \rightarrow \quad c = \left( \frac{1}{c_0} - \frac{\phi_{rear}}{4\pi f d} \right)^{-1}$$

## Calculation for specific parameters.

$$S_0(f) = \frac{S_{ref}(f) \exp\{2\alpha_{water}(f)d\}}{\frac{Z_{substrate} - Z_{water}}{Z_{substrate} + Z_{water}}} \approx \frac{S_{ref}(f)}{\frac{Z_{substrate} - Z_{water}}{Z_{substrate} + Z_{water}}}$$

Acoustic impedance:

$$Z_{tissue}(f) = Z_{water} \times \frac{S_0 - S_{front}}{S_0 + S_{front}}$$

Density:

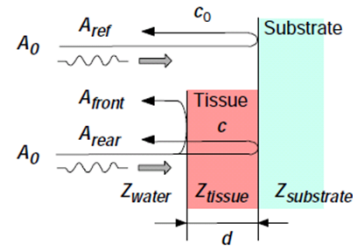
$$\rho(f) = \frac{Z_{tissue}}{c}$$

Bulk modulus:

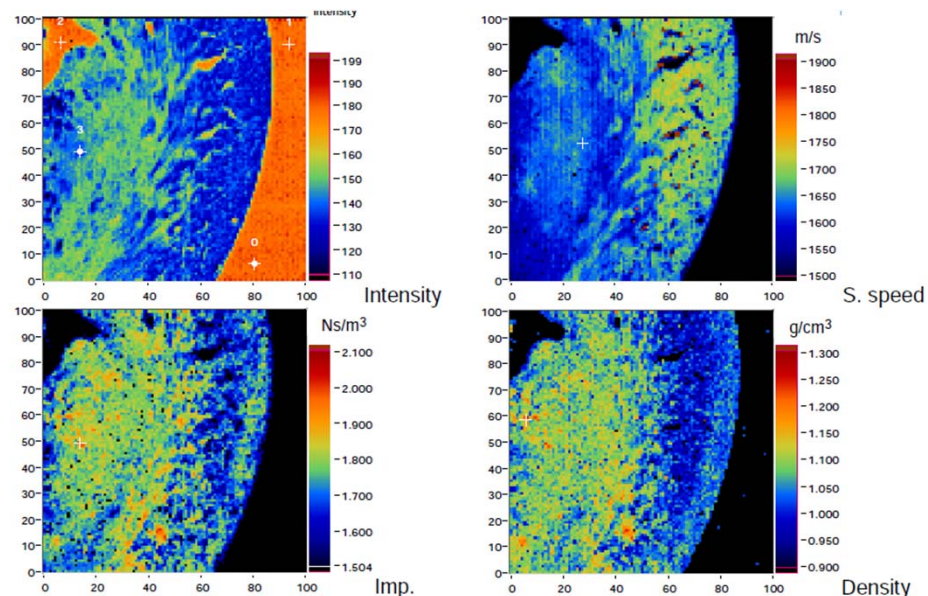
$$K(f) = c^2 \rho = c Z_{tissue}$$

Attenuation:

$$\alpha_{tissue}(f) = \frac{1}{2d} \times \ln \left( \frac{S_0 \times \frac{2Z_{tissue}}{Z_{tissue} + Z_{water}} \times \frac{Z_{substrate} - Z_{tissue}}{Z_{substrate} + Z_{tissue}} \times \frac{2Z_{water}}{Z_{water} + Z_{tissue}}}{A_{rear}} \right) \approx \frac{1}{2d} \times \ln \left( \frac{S_{ref}}{S_{rear}} \right)$$

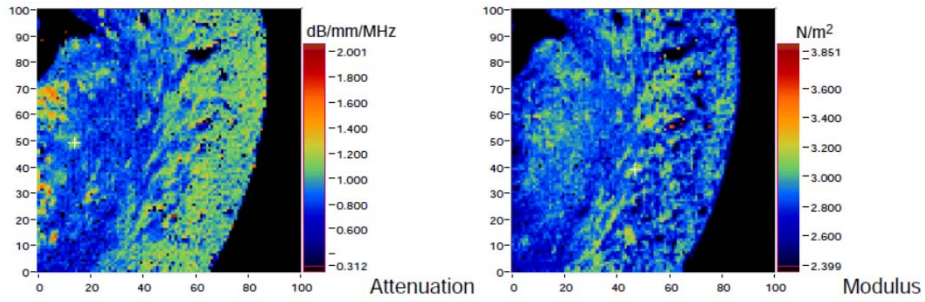


## Some other parameters I (at 120 MHz).





# Some other parameters II (at 120 MHz).

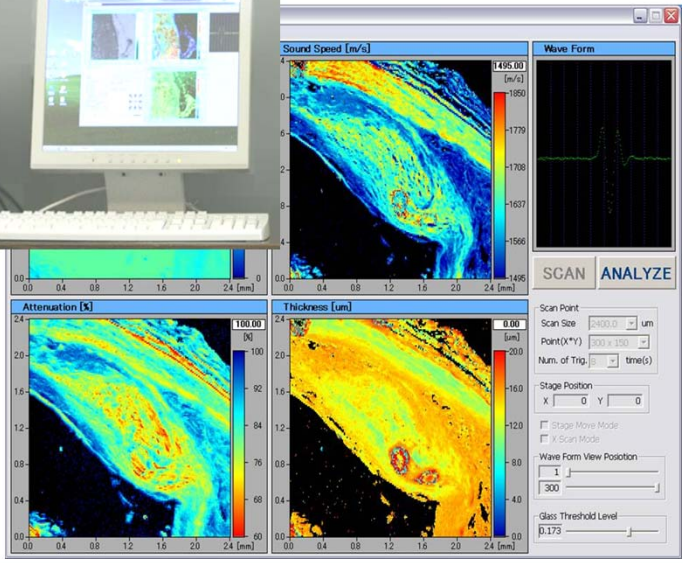


# Commercialized system.



**Honda Electronics  
HMS-1000  
300 × 300  
one minute**

vascular wall with calcification

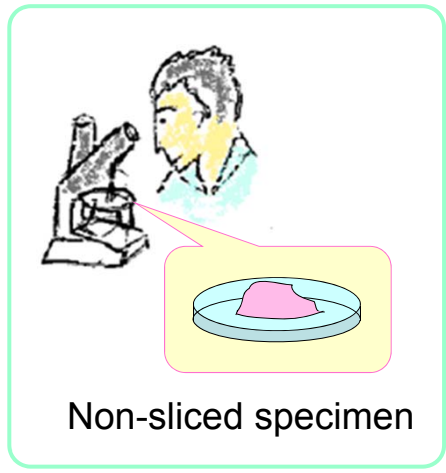


## Summary

- **Pulse driven** ultrasonic microscope.
- **Quick, Precise, Easy, Compact.**
- Prototype: 100 x 100 resolution within 9 min.
- Commercial type: 300 x 300 resolution within 1.5 min.
- Rat myocardium was successfully observed.

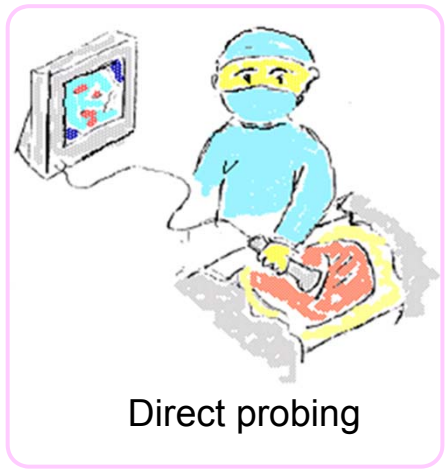
## Scanning Acoustic Impedance Microscopy

### Additional needs.



Non-sliced specimen

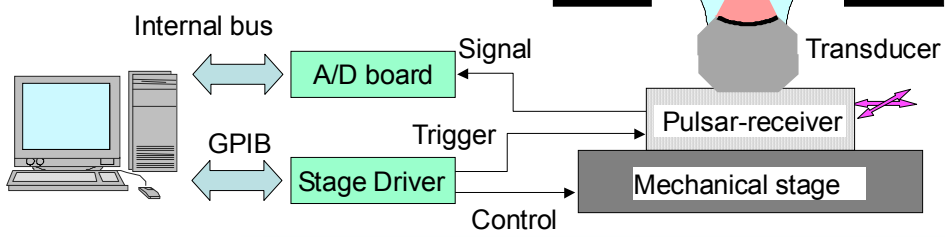
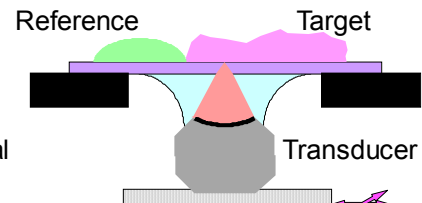
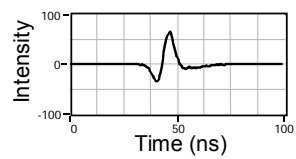
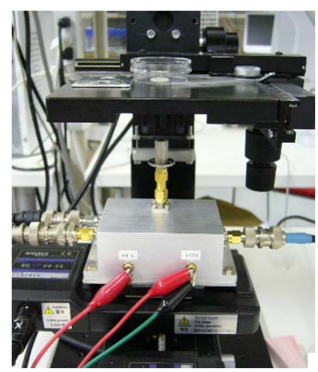
↓  
This presentation



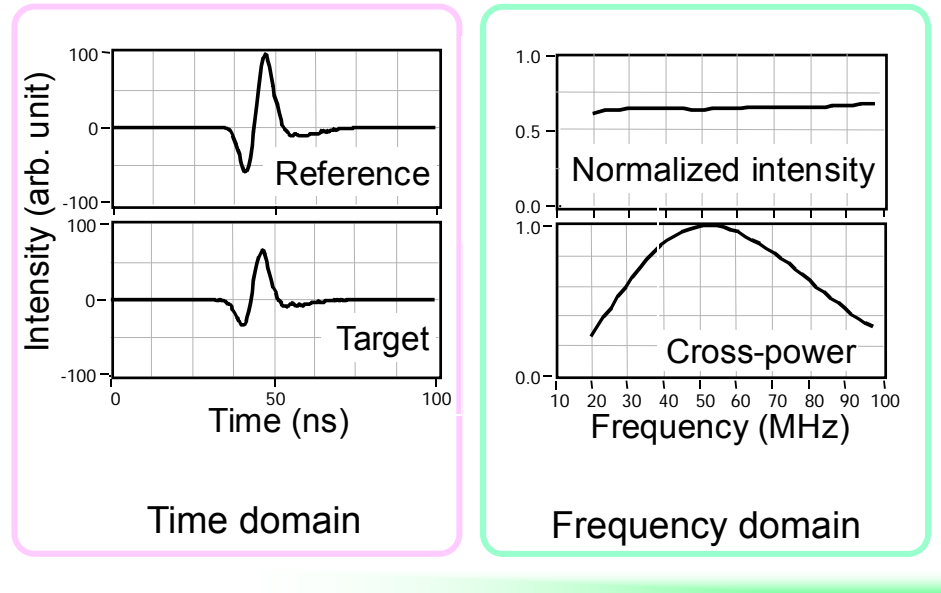
Direct probing

↓  
Future ?

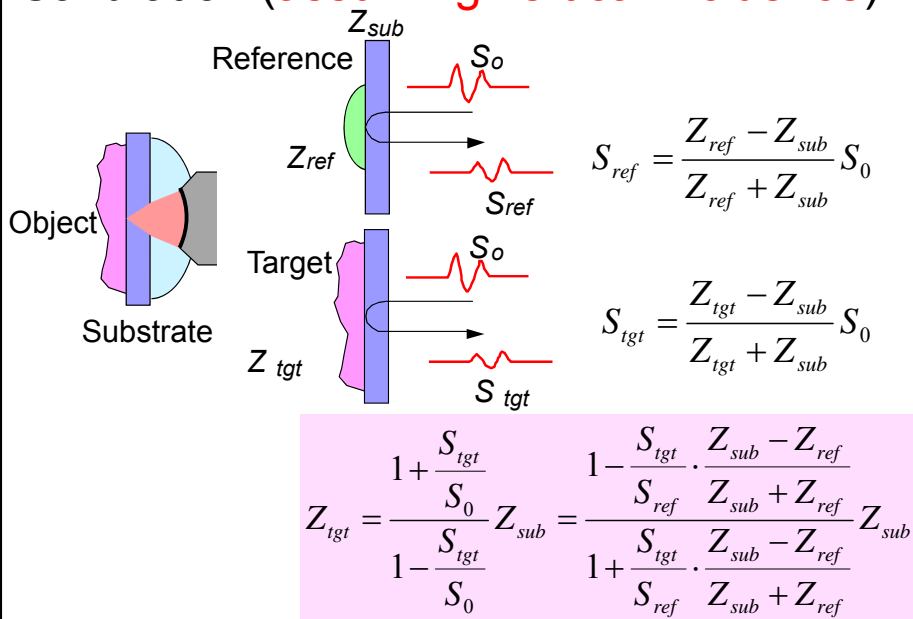
### System setup.



## Waveforms.



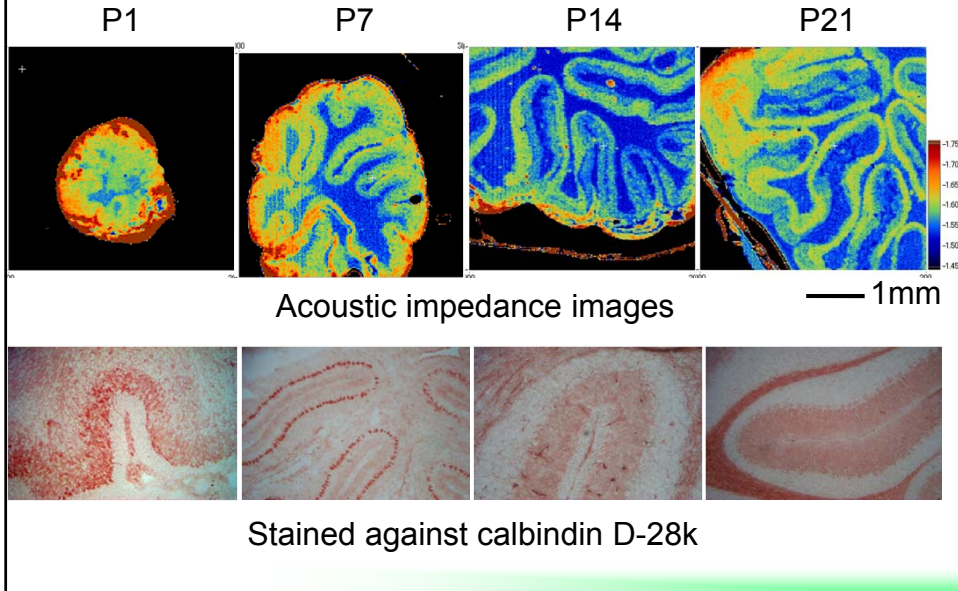
## Calibration (assuming vertical incidence).



# Cerebellum, rat.

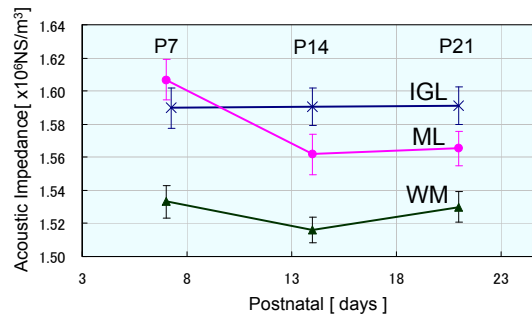
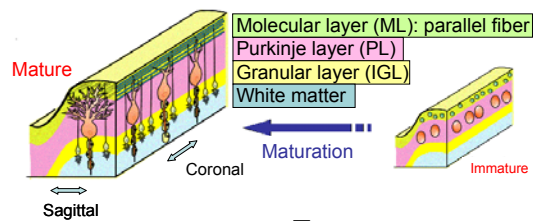
25

60-100 MHz  
not fixed, postnatal 1 to 21 days



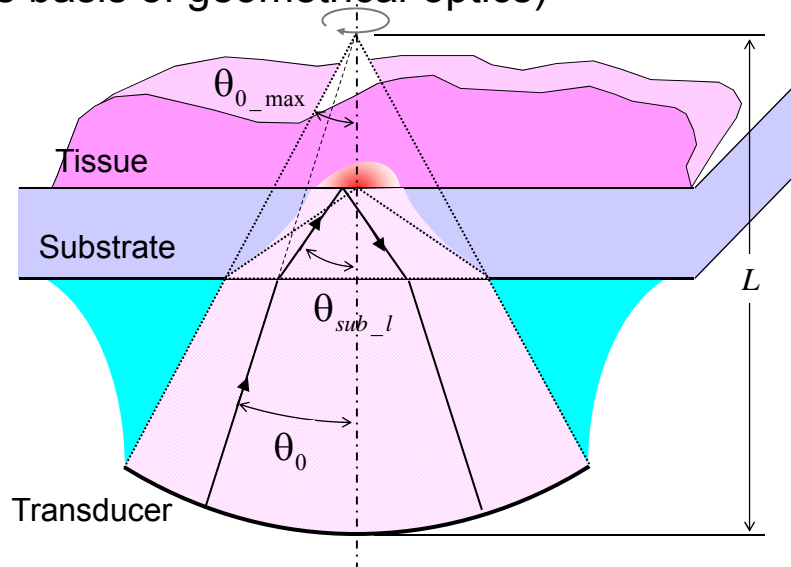
# Quantitative assessment

26

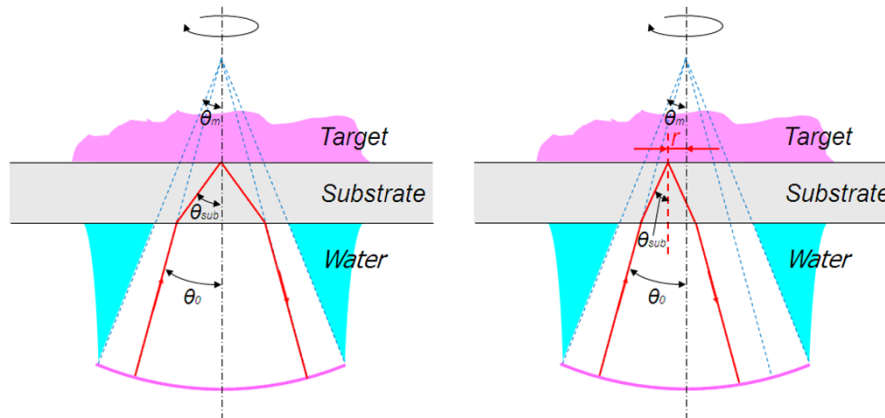


# Improvement of spatial resolution

Aberration produced by the substrate  
(on the basis of geometrical-optics)



## Propagation of beam component

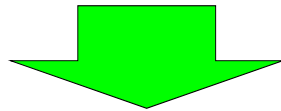


This is not true if the substrate is thick and its sound speed is not the same as water.

Each beam component forms ring-like focus. Its radius depends on the incident angle.

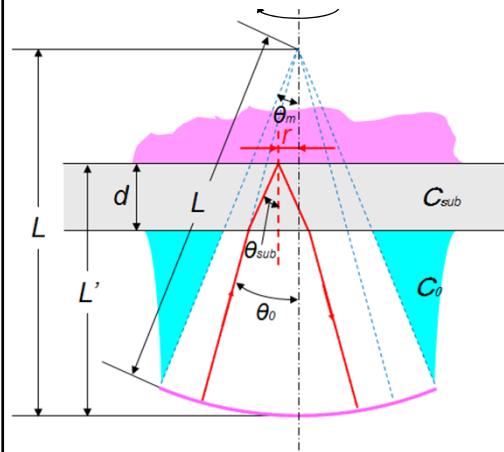
## Signal processing using 3D deconvolution

Calculate impulse response  
of the acoustic system



Improvement of spatial resolution.  
Quantitative observation.

## Aberration along lateral direction



### Transducer

film type with PVDF-TrFE  
focal length ( $L$ ) 3.2 mm  
angle of focusing ( $\theta_m$ ) 21.5deg

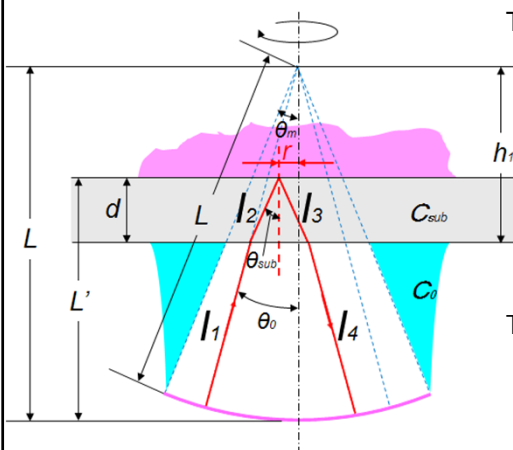
### Substrate

polystyrene 0.8 mm thick  
sound speed ( $c_{sub}$ ) 2.3 km/s

$$\theta_{sub}(\theta_0) = \sin^{-1}\left(\frac{c_{sub}}{c_0} \sin \theta_0\right)$$

$$r = d \tan \theta_{sub} - (L - L' + d) \tan \theta_0$$

## Aberration along time direction



### Traveling distance

$$l_1 = L - \frac{1}{\cos \theta_0} (L - L' + d)$$

$$l_2 = l_3 = \frac{d}{\cos \theta_{sub}}$$

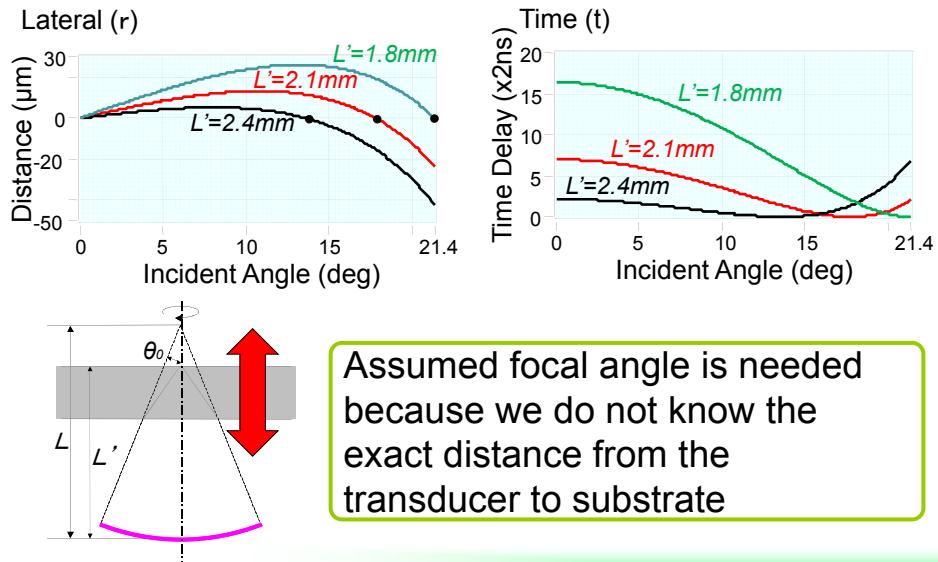
$$l_4 = \sqrt{L'^2 - (2r \cos \theta_0)^2} + 2r \sin \theta_0 - \frac{h_1}{\cos \theta_0}$$

### Traveling time

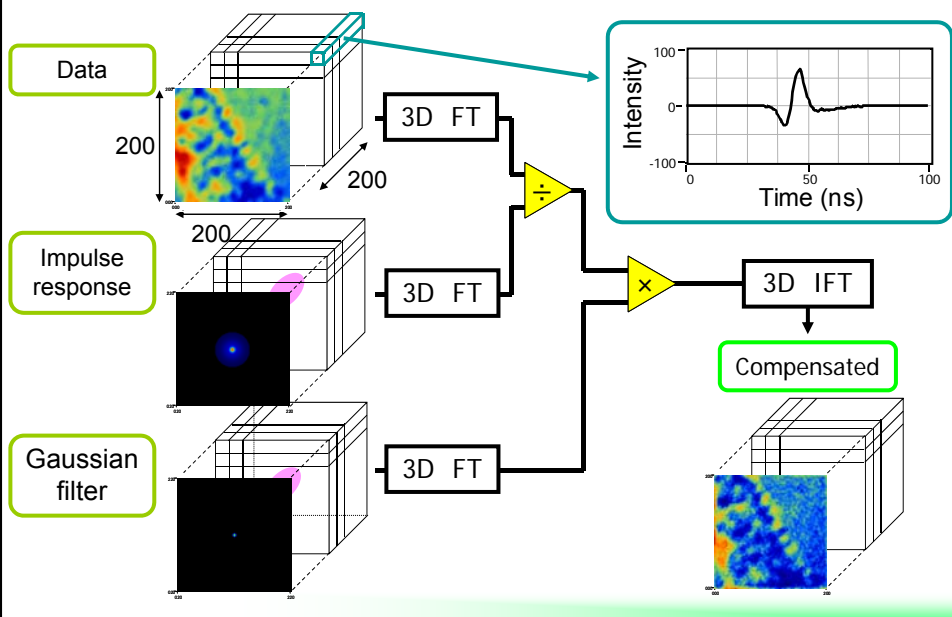
$$t = \frac{l_1}{c_0} + \frac{l_2}{c_{sub}} + \frac{l_3}{c_{sub}} + \frac{l_4}{c_0}$$

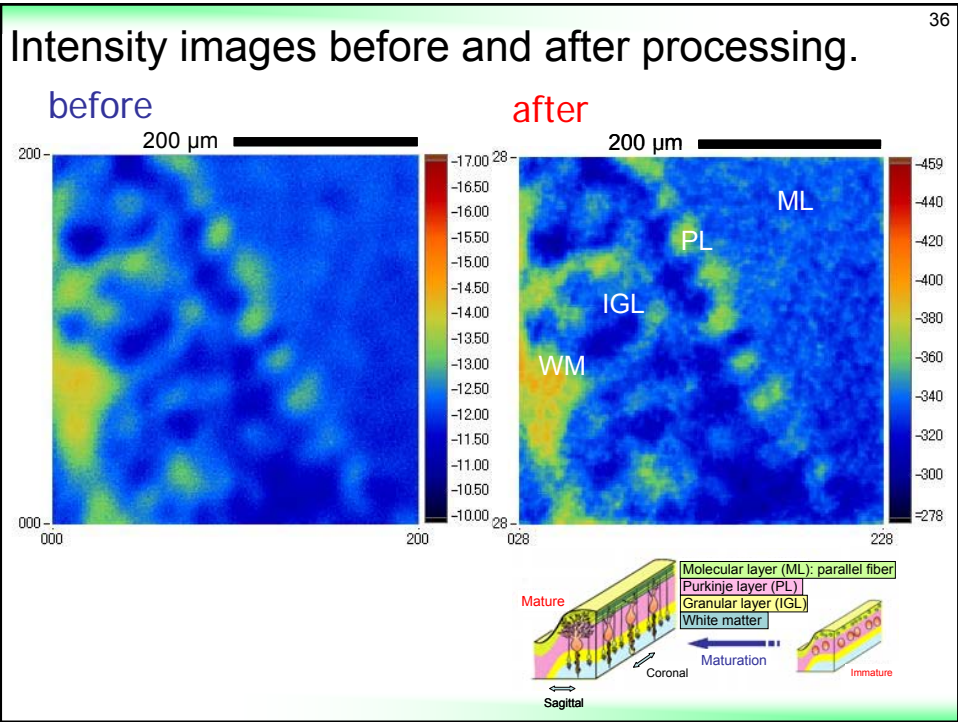
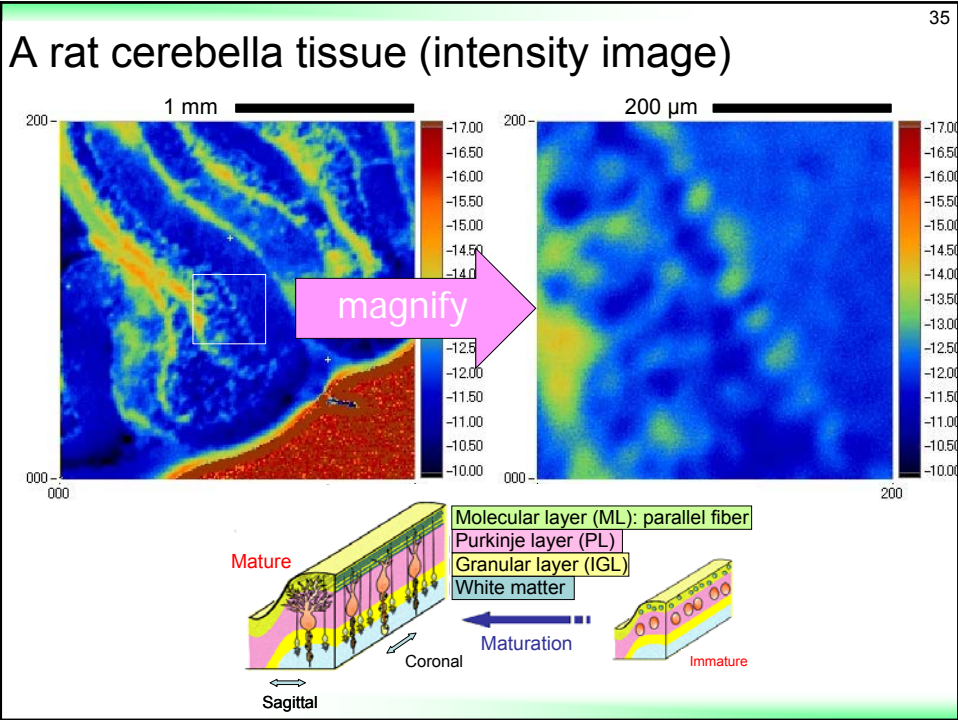


## The impulse response

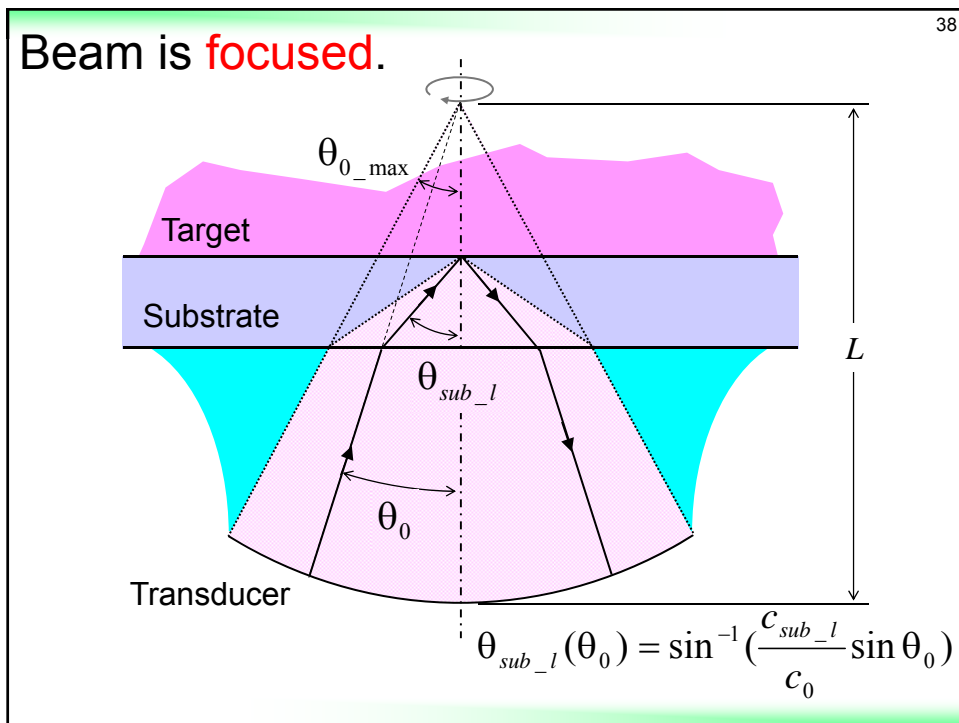


## 3D deconvolution processing

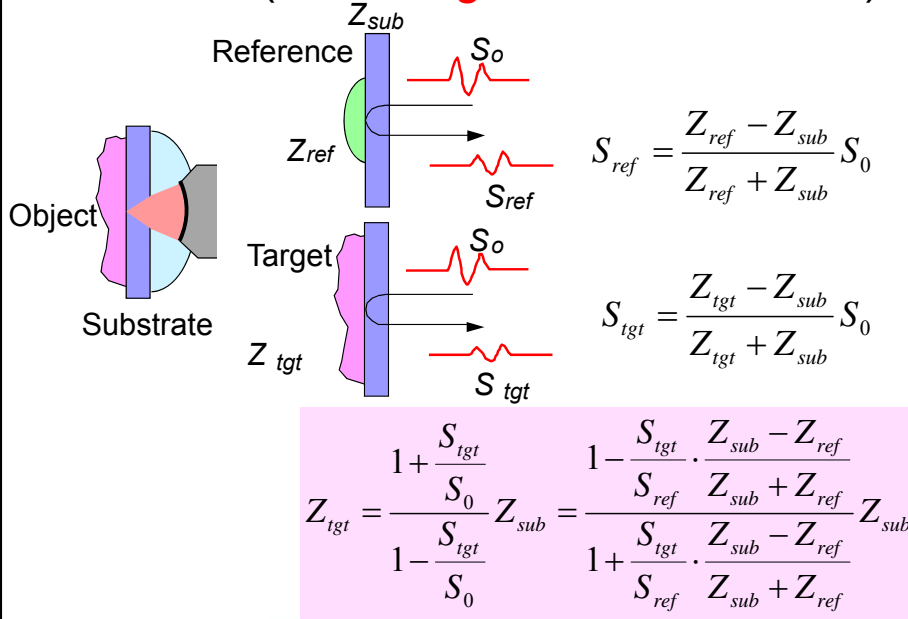




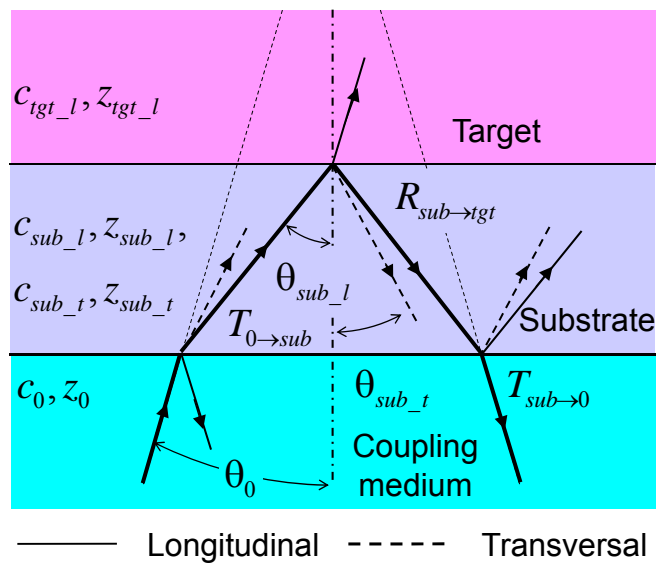
# Improvement of accuracy



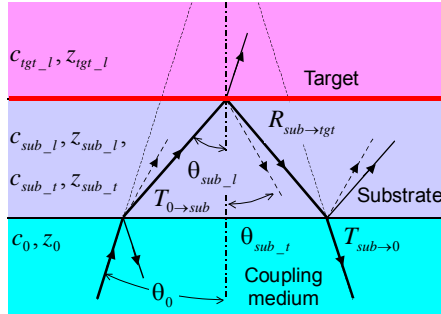
# Calibration (assuming vertical incidence).



# Wave propagation.



## Reflection constant.



$$R_{sub \to tgt}(Z_{sub\_l}, c_{sub\_l}, c_{sub\_t}, Z_{tgt}, c_{tgt}, \theta_0)$$

$$= \frac{M_{sub} - \cos^2(2\theta_{sub\_t}) + N_{sub \to tgt}}{M_{sub} + \cos^2(2\theta_{sub\_t}) + N_{sub \to tgt}}$$

$$\theta_{sub\_l} = \sin^{-1}\left(\frac{c_{sub\_l}}{c_0} \sin \theta_0\right)$$

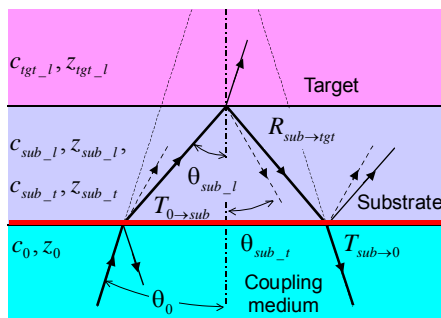
$$\theta_{sub\_t} = \sin^{-1}\left(\frac{c_{sub\_t}}{c_{sub\_l}} \sin \theta_{sub\_l}\right)$$

$$M_{sub} = \left(\frac{c_{sub\_t}}{c_{sub\_l}}\right)^2 \sin \theta_{sub\_t} \sin \theta_{sub\_l}$$

$$N_{sub \to tgt} = \frac{Z_{tgt} \cos \theta_{sub\_l}}{Z_{sub\_l} \cos \theta_{tgt}}$$

$$\theta_{tgt} = \sin^{-1}\left(\frac{c_{tgt}}{c_{sub\_l}} \sin \theta_{sub\_l}\right)$$

## Transmission constant.



$$T_{0 \to sub} \cdot T_{sub \to 0}$$

$$= T'_{sub \to tgt}(Z_0, c_0, Z_{sub\_l}, c_{sub\_l}, c_{sub\_t}, \theta_0)$$

$$= \frac{4N_{0 \to sub} \cos^2(2\theta_{sub\_t})}{\{M_{sub} + \cos^2(2\theta_{sub\_t}) + N_{0 \to sub}\}^2}$$

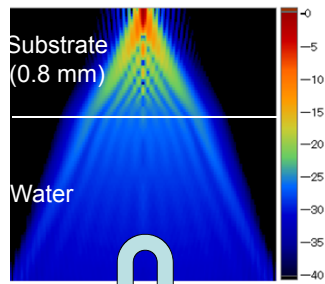
$$\theta_{sub\_l} = \sin^{-1}\left(\frac{c_{sub\_l}}{c_0} \sin \theta_0\right)$$

$$\theta_{sub\_t} = \sin^{-1}\left(\frac{c_{sub\_t}}{c_{sub\_l}} \sin \theta_{sub\_l}\right)$$

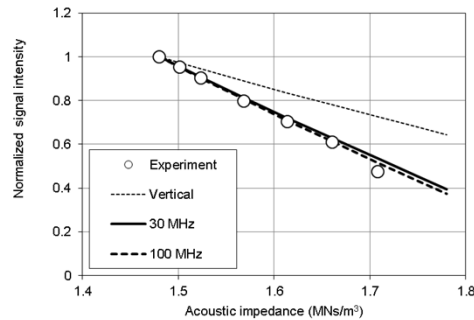
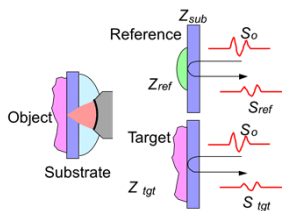
$$M_{sub} = \left(\frac{c_{sub\_t}}{c_{sub\_l}}\right)^2 \sin \theta_{sub\_t} \sin \theta_{sub\_l}$$

$$N_{0 \to sub} = \frac{Z_0 \cos \theta_{sub\_l}}{Z_{sub\_l} \cos \theta_0}$$

## Normalized reflection intensity.

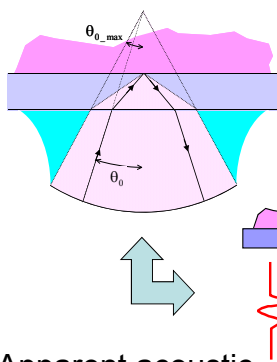


Reflection Intensity



## Apparent acoustic impedance of the target assuming vertical incidence.

44



Apparent reflection constant target:

$$S_{tgt}(Z_{tgt}, c_{tgt}, \theta_{0\_max}) / S_0 = \int_0^{\theta_{0\_max}} 2\pi L^2 \sin \theta_0 R_{sub \rightarrow tgt}(\theta_0) T'_{0 \rightarrow sub}(\theta_0) d\theta_0$$

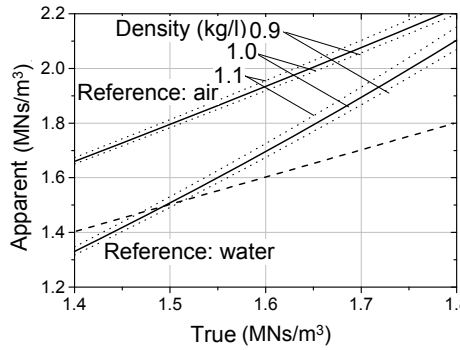
reference:

$$S_{ref}(Z_{ref}, c_{ref}, \theta_{0\_max}) / S_0 = \int_0^{\theta_{0\_max}} 2\pi L^2 \sin \theta_0 R_{sub \rightarrow ref}(\theta_0) T'_{0 \rightarrow sub}(\theta_0) d\theta_0$$

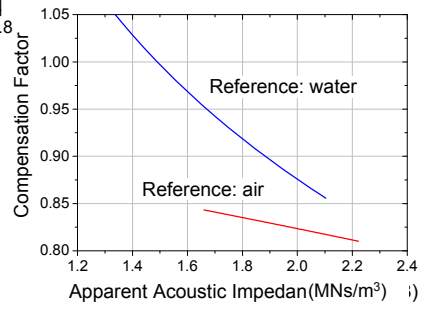
Apparent acoustic impedance of the target:

$$Z_{tgt\_app} = \frac{1 + \frac{S_{tgt}(Z_{tgt}, c_{tgt}, \theta_{0\_max})}{S_{ref}(Z_{ref}, c_{ref}, \theta_{0\_max})} \cdot \frac{Z_{ref} - Z_{sub\_l}}{Z_{ref} + Z_{sub\_l}}}{1 - \frac{S_{tgt}(Z_{tgt}, c_{tgt}, \theta_{0\_max})}{S_{ref}(Z_{ref}, c_{ref}, \theta_{0\_max})} \cdot \frac{Z_{ref} - Z_{sub\_l}}{Z_{ref} + Z_{sub\_l}}} \cdot Z_{sub\_l}$$

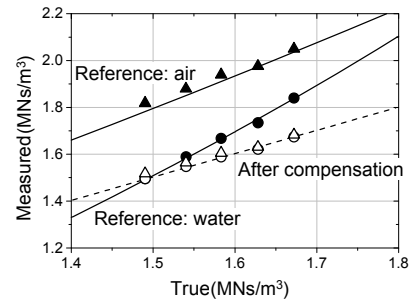
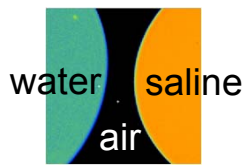
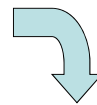
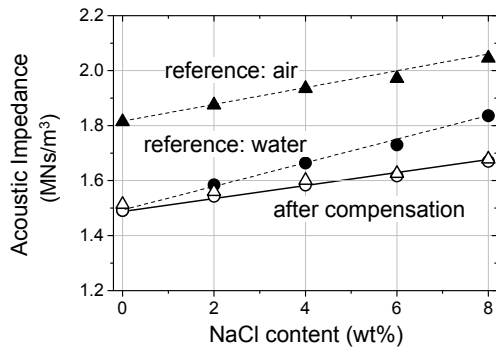
### Compensation curves.



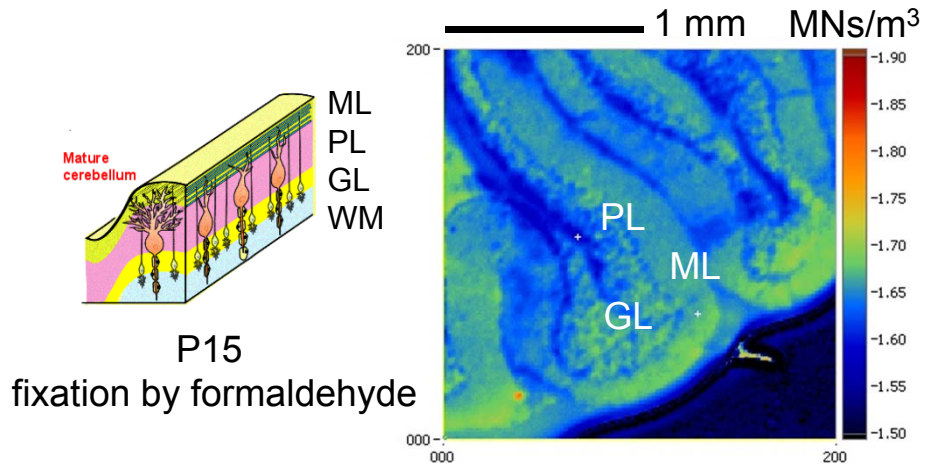
Angle of focusing: 22 deg



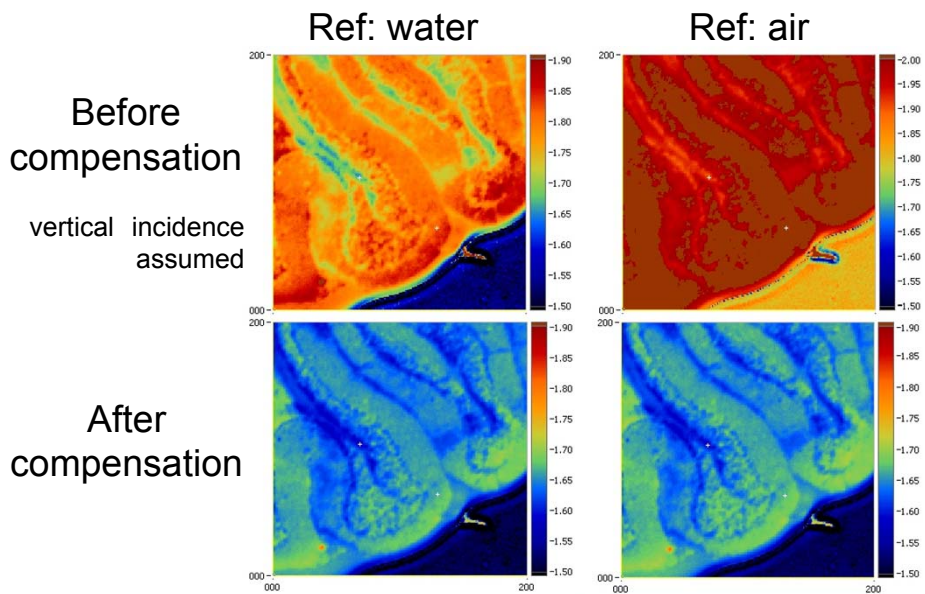
### Measurement for saline solution.



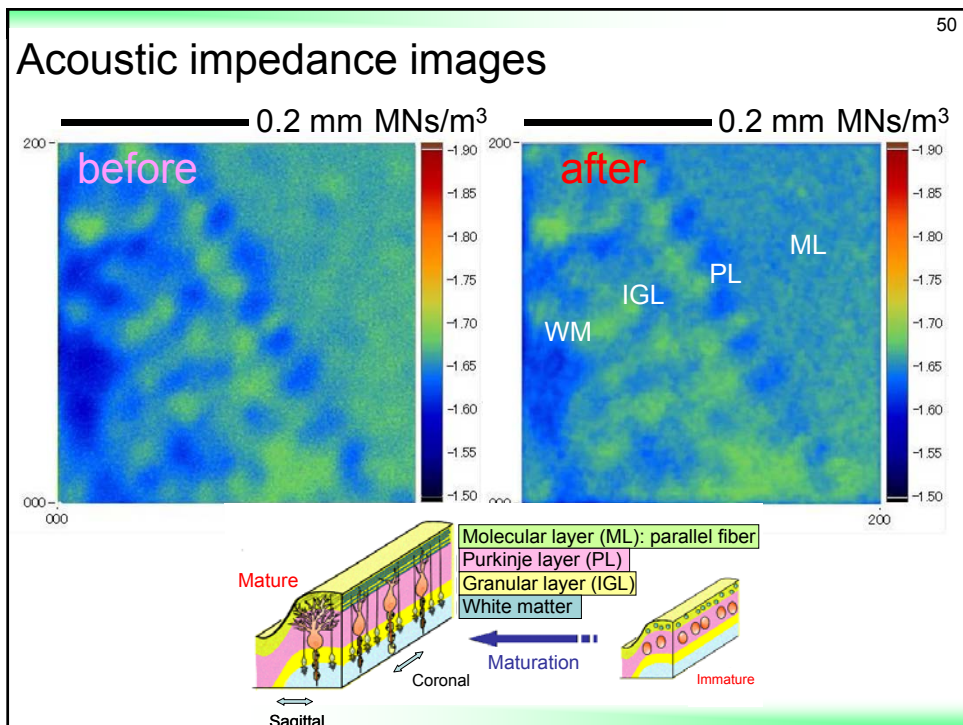
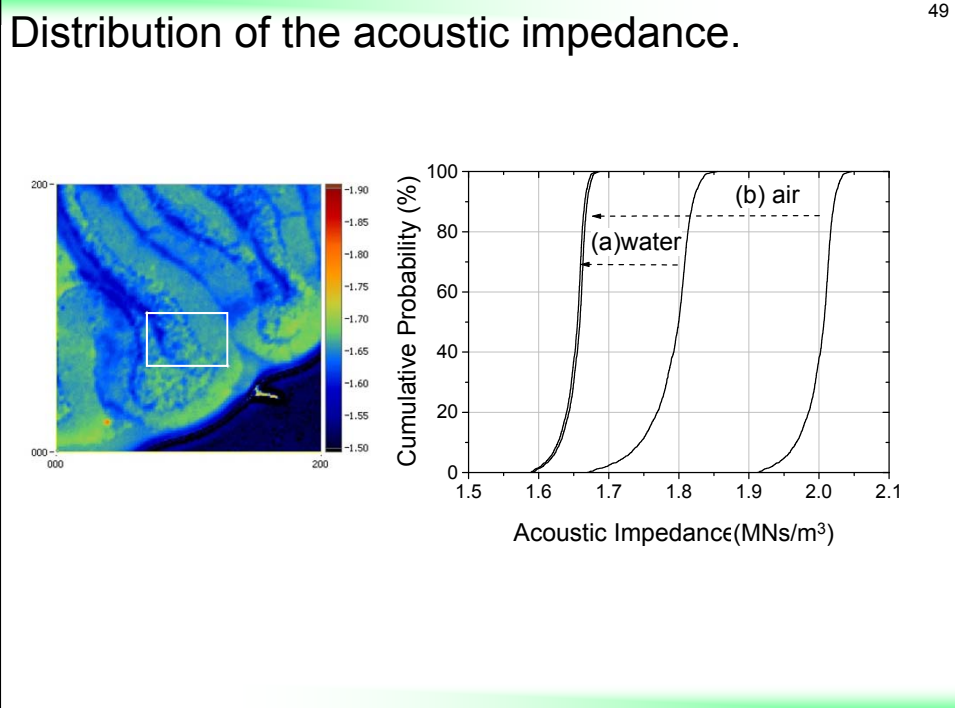
Cerebellum tissue of a rat.



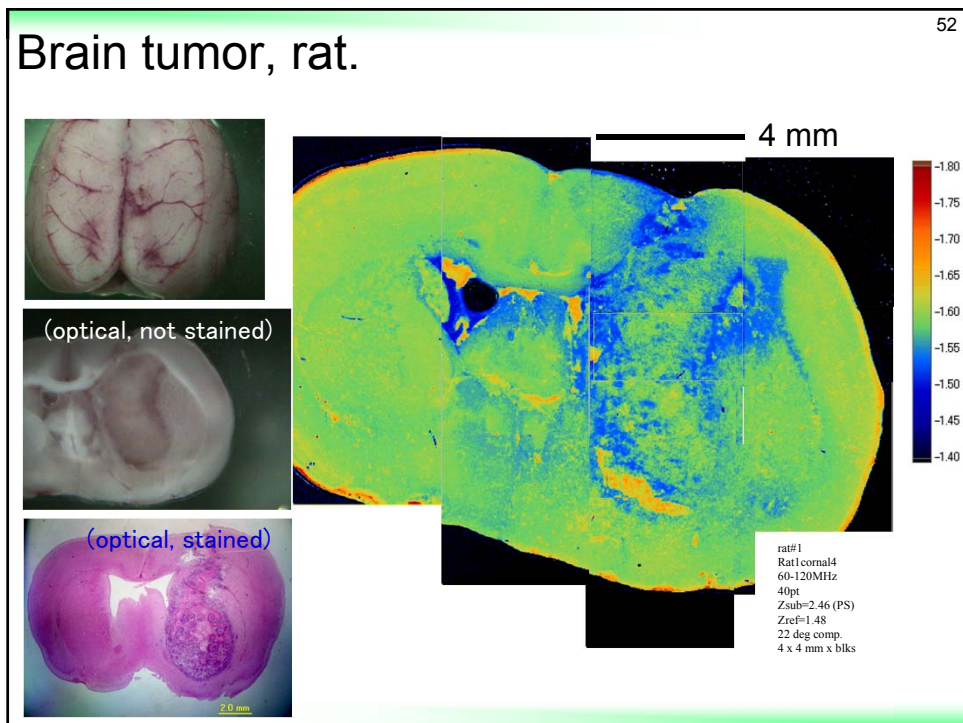
Effect of compensation.





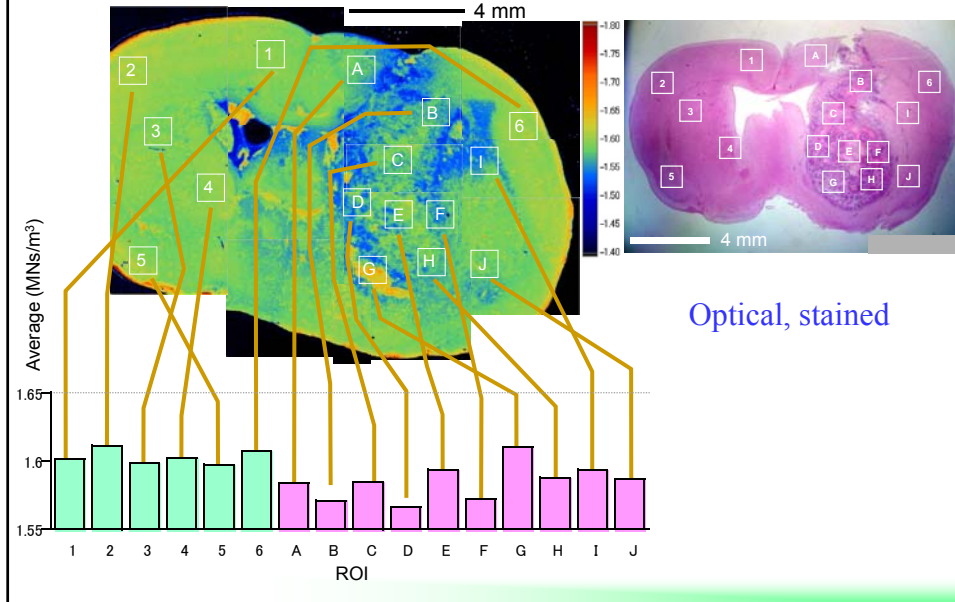


# Observation of brain tumor model of rat



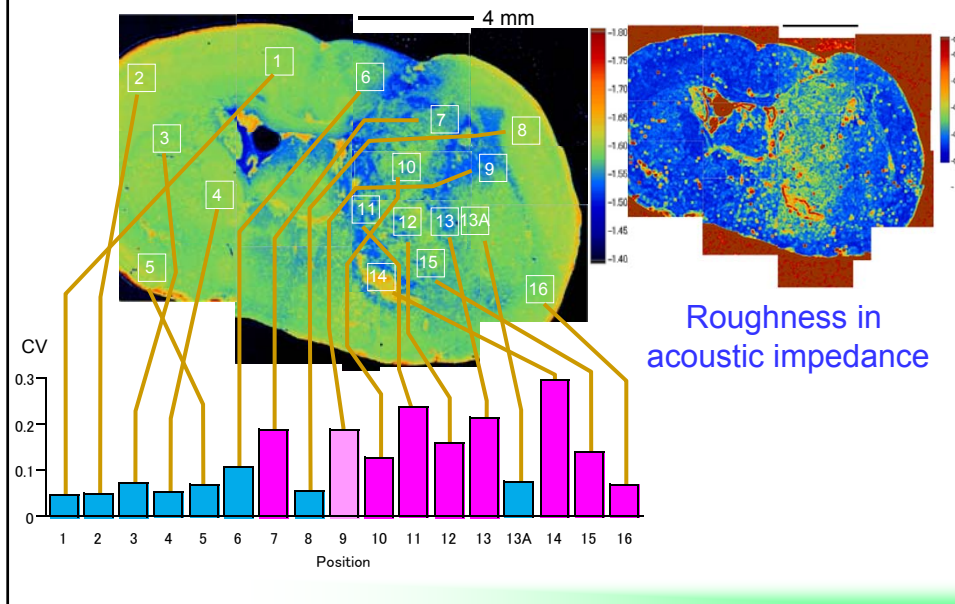
## Quantitative analysis (average)

53



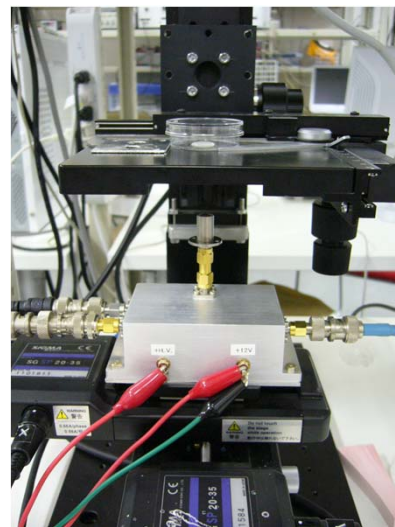
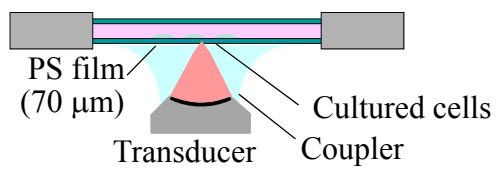
## Quantitative analysis (standard deviation)

54

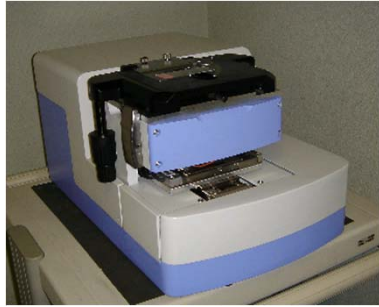


# Cell size observation

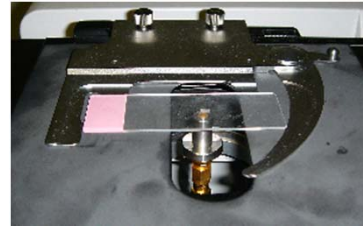
## Observation system for cultured cells.



## Commercialized system by Honda Electronics.



body (new type available)



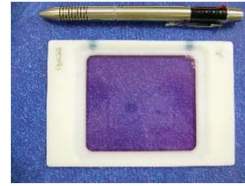
stage



dish

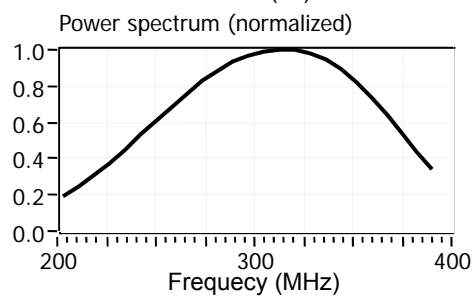
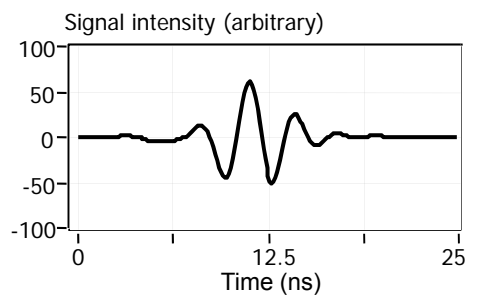


dish with film



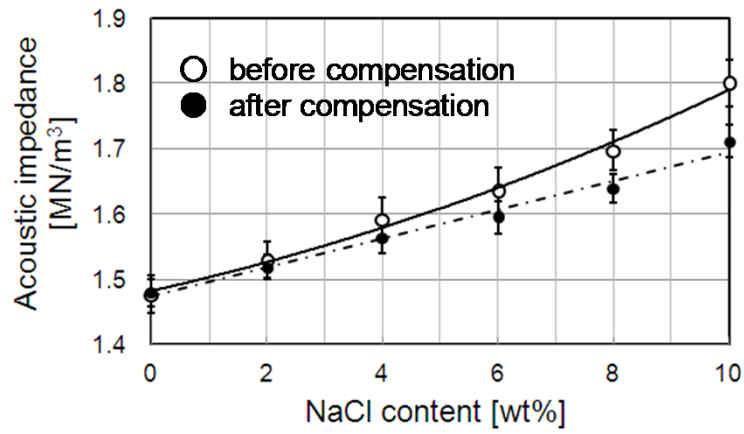
incubation cell

## Waveform and spectrum.

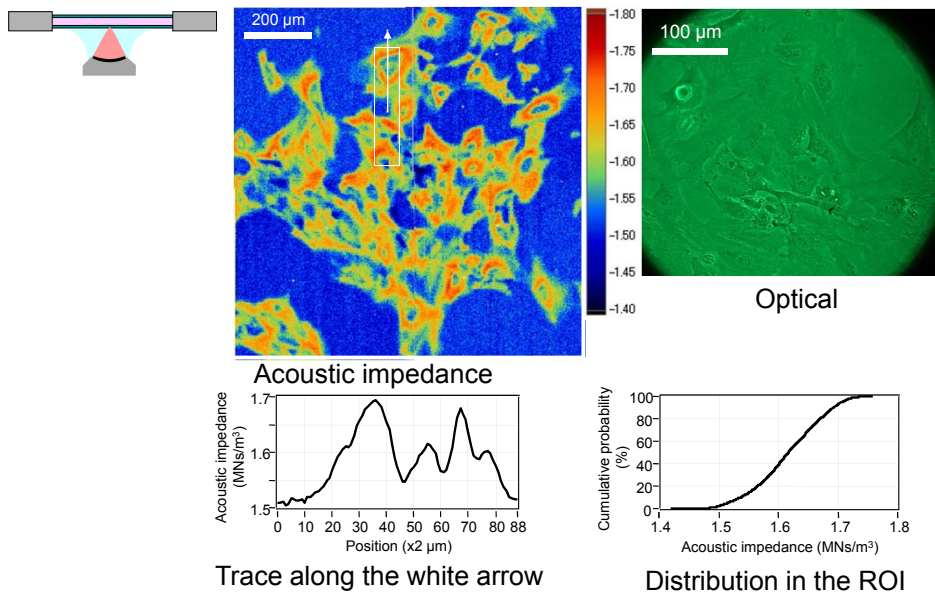


200~400 MHz  
range was used.

## Calibration by saline solution.



## Cultured glial cells, rat.



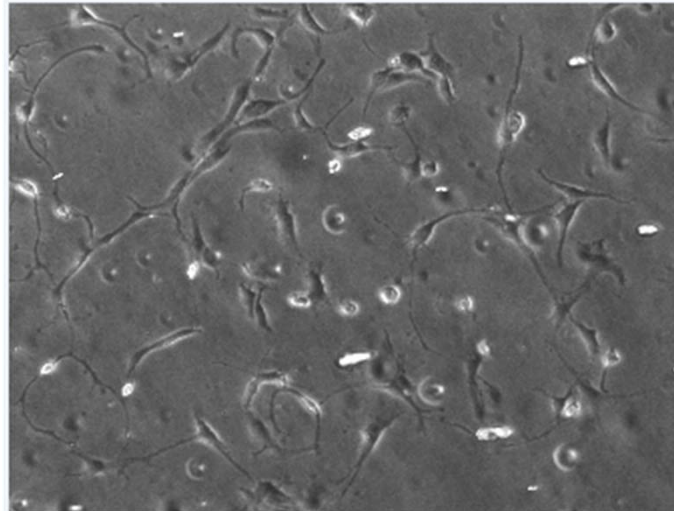
# *In situ* observation after dosage

Glioma DIV14, Cytochalasin B 25 $\mu$ g/ml

0min	30min	60min
90min	120min	150min
180min	210min	240min

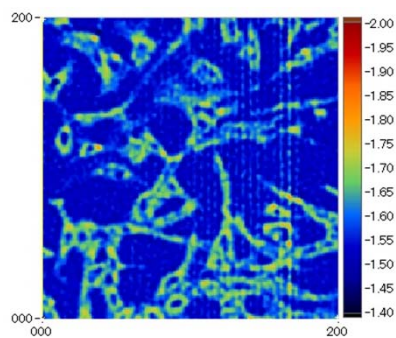
投薬後30分から1時間の間では大きな変化は確認  
できないですが、4時間後には全体的に細胞の萎縮  
や浮いてくるような様子が見られる。

Glioma DIV14, Cytochalasin B 25 $\mu$ g/ml



0 min after injection. Optical Image

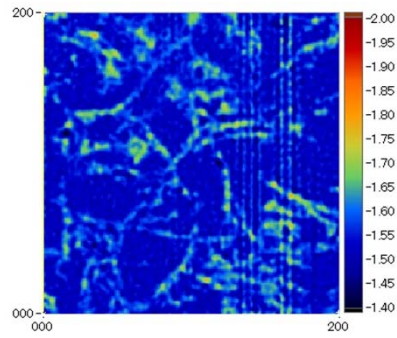
Glioma DIV14, Cytochalasin B 25 $\mu$ g/ml



Before injection.  
Acoustic impedance

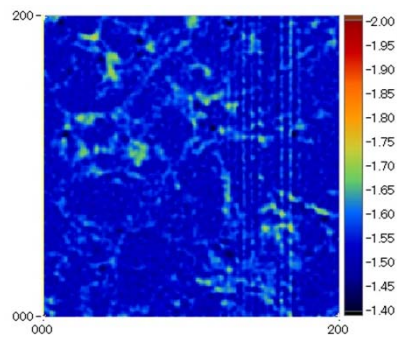


Glioma DIV14, Cytochalasin B 25 $\mu$ g/ml



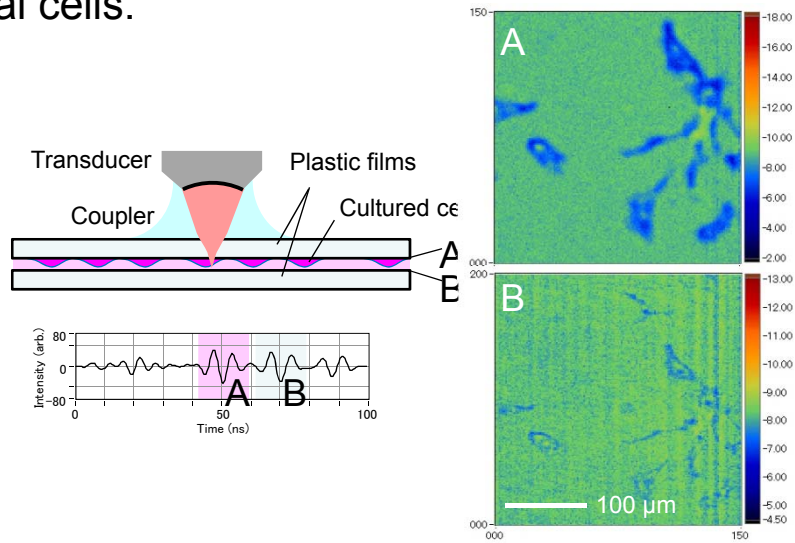
60 min after injection.  
Acoustic impedance

Glioma DIV14, Cytochalasin B 25 $\mu$ g/ml

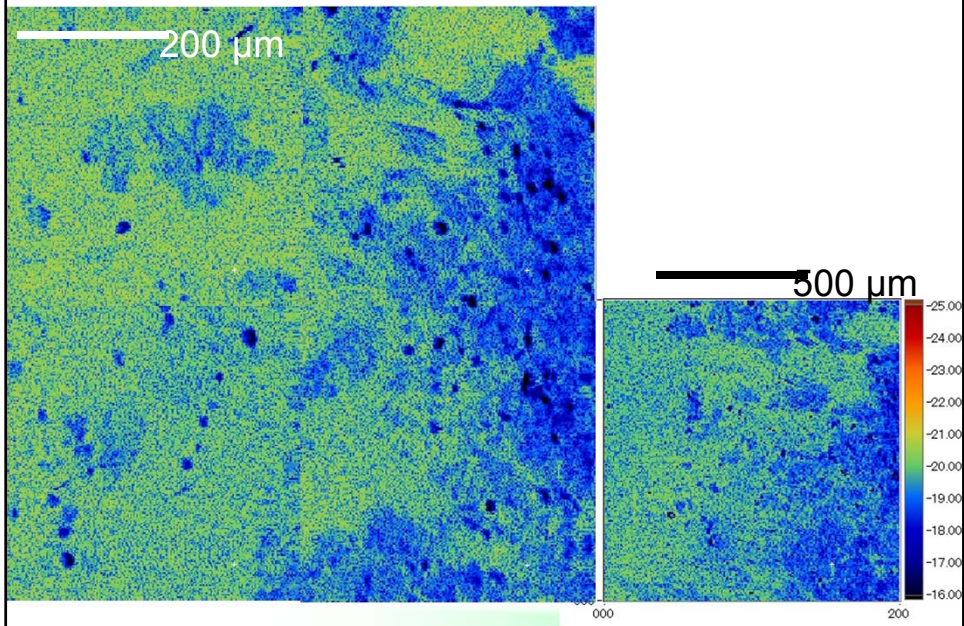


150 min after injection.  
Acoustic impedance

## Reflection mode and projection mode, Glial cells.



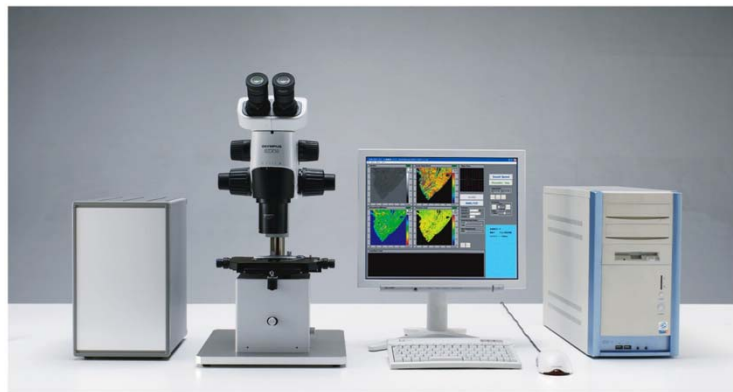
## Projection mode, cancerous liver, rat.



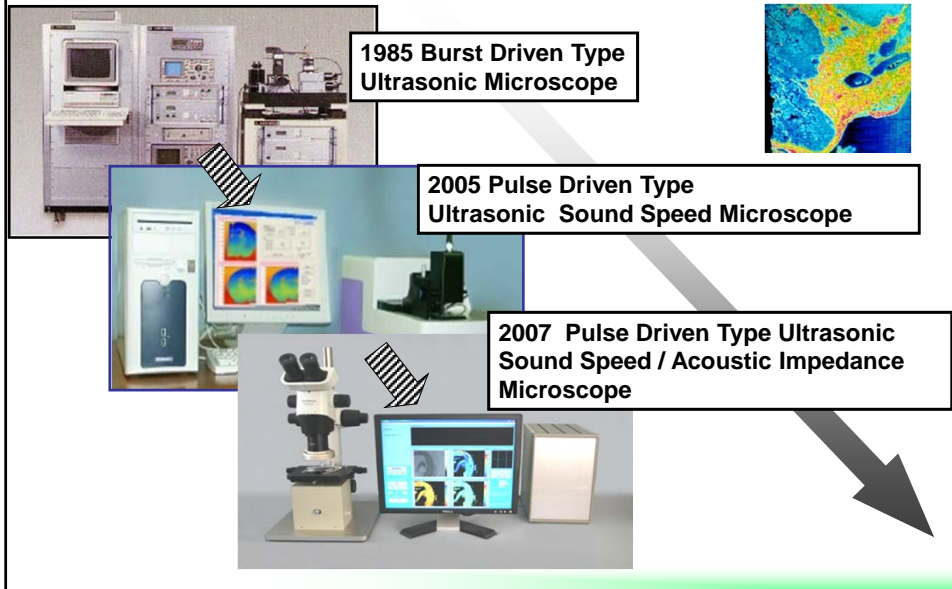
Commercialized by  
HONDA Electronics

HONDA Electronics

**AMS-50SI**



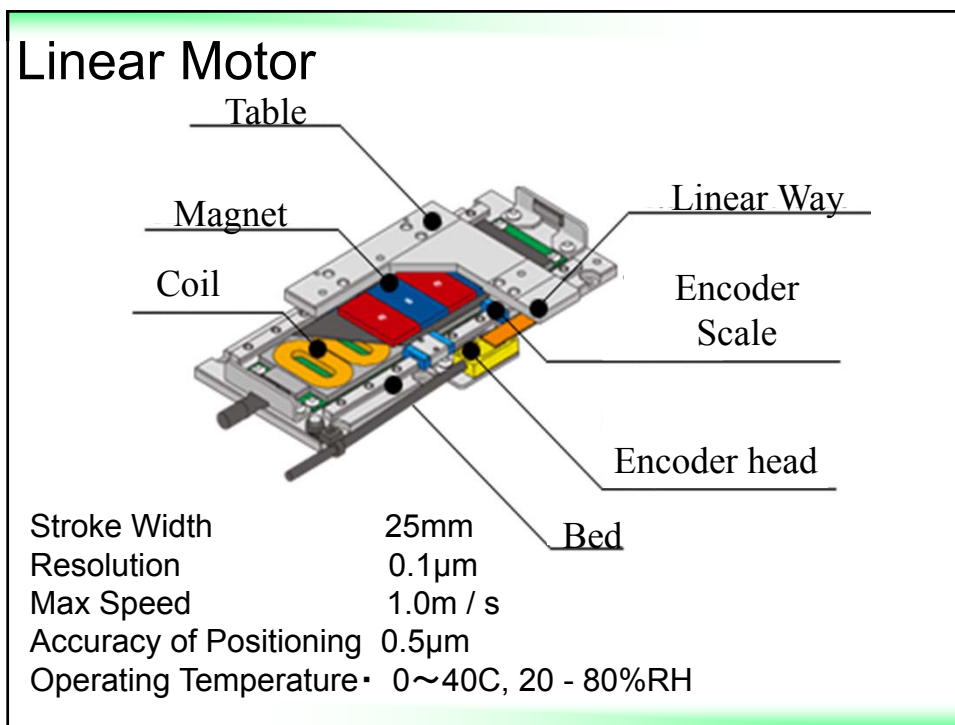
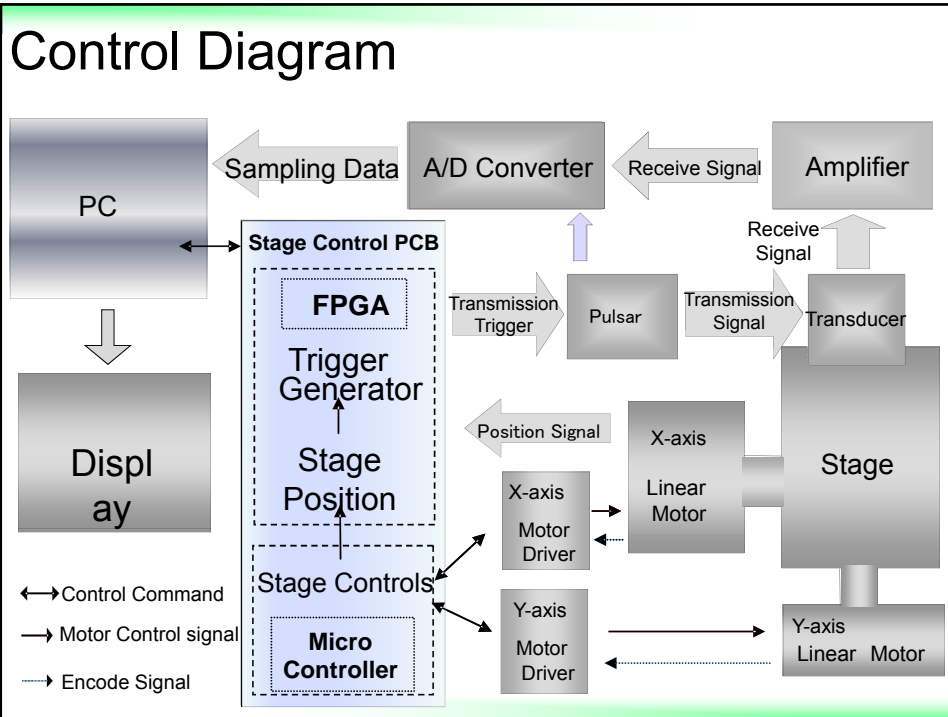
## Development in Honda Electronics



## Digitizer Unit DP1400 (ACQIRIS)



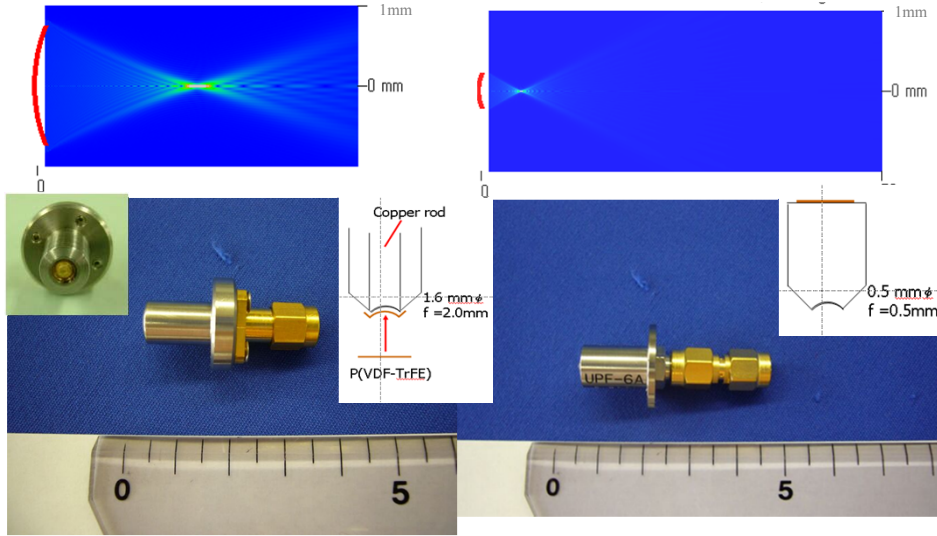
- 2 GS/s Sampling Rate
- 500 MHz Bandwidth
- 256 k points Acquisition Memory
- 50  $\Omega$  and 1 M $\Omega$  Input Impedance
- Complete Pre and Post Triggering
- 2 ppm Clock Accuracy
- for Accurate Timing Measurements
- Very high Data Transfer Rate
- Sampling Jitter 2psec
- Transfer Speed 220MB/sec



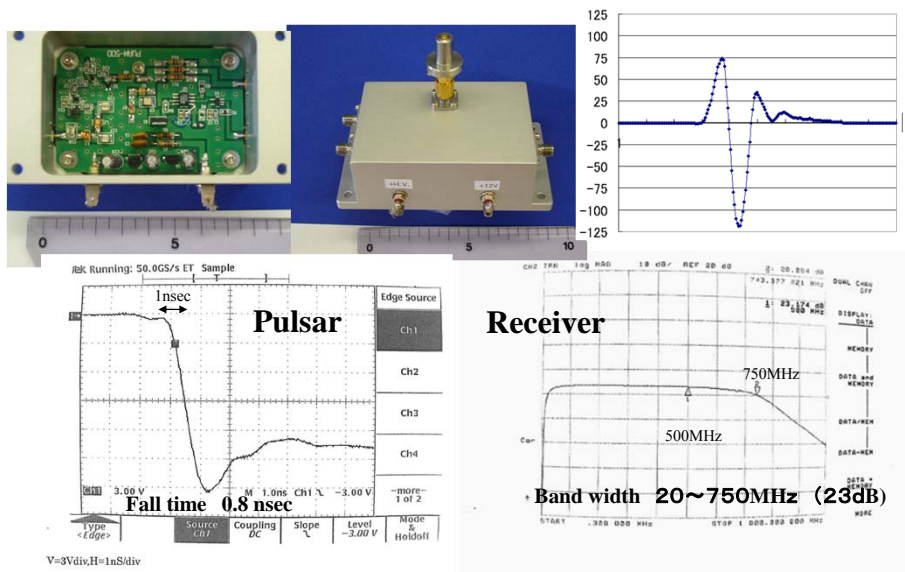
# Transducers

**P(VDF-TrFE) 80MHz D=1.6 F=2.0**

**ZnO 320MHz D=0.5 F=0.5**



# Pulsar Receiver (20MHz - 500MHz)



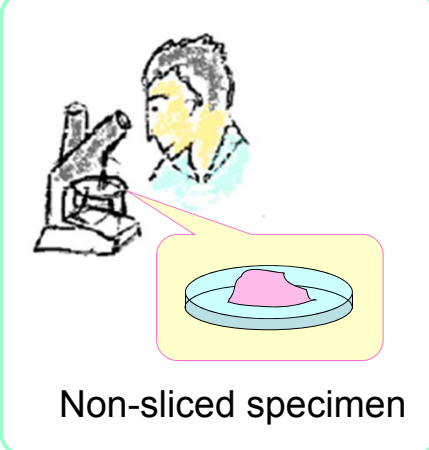
## Summary

1. **Acoustic impedance microscope** was proposed.
2. No need to slice, stain. Non-contact.
3. Estimation error **by assuming vertical incidence** is significant if;  
(1) angle of focusing is large  
or  
(2) reference material is far different from target.
4. The error may be **compensated**.
5. Calibration by using **saline solution**.
6. **Cerebellar tissue** was observed.
7. **Cultured cells** were observed with a sufficient resolution.

What can we do next ?

79

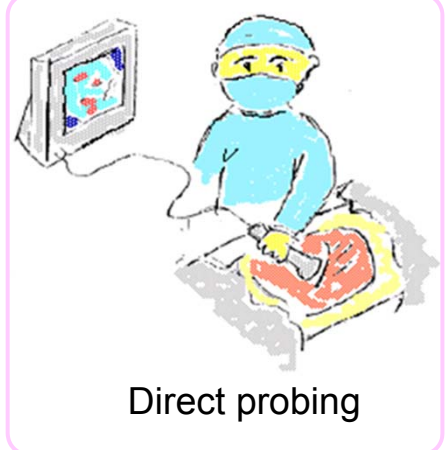
## Direct probing



Non-sliced specimen

↓

This presentation



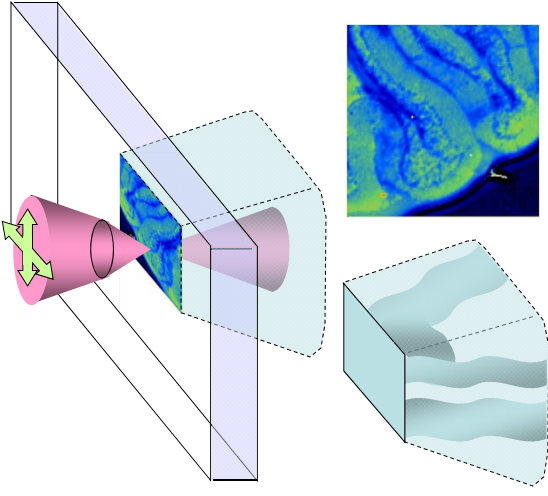
Direct probing

↓

Future ?

80

## A hybrid 2D 3D microscope



2D Profile of Acoustic Impedance

**Quantitative**

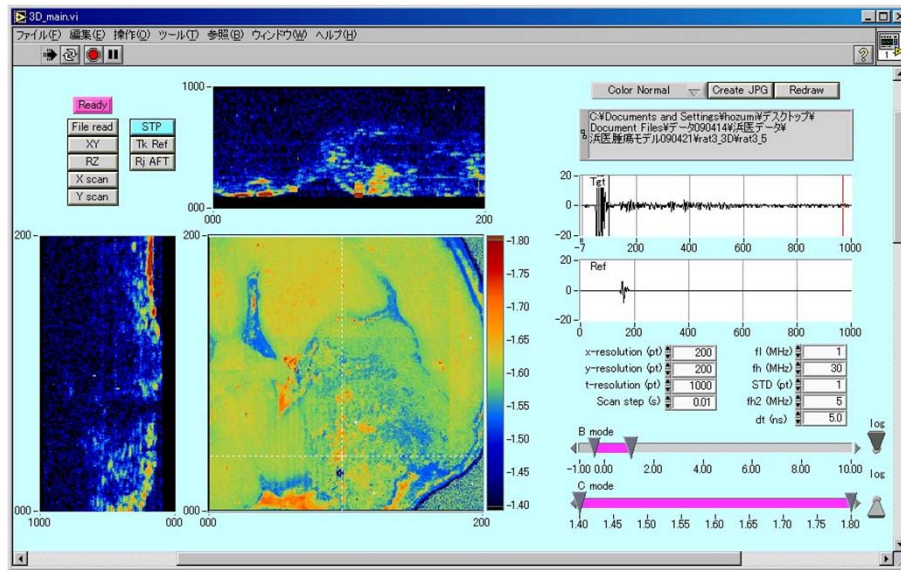
  

3D Profile by Synthetic Aperture

**Morphological**



## A hybrid 2D 3D microscope



Thank you for your attention.