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Scanning Acoustic Microscopy for Medical and Biological Applications

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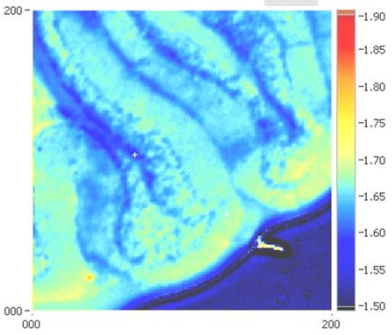
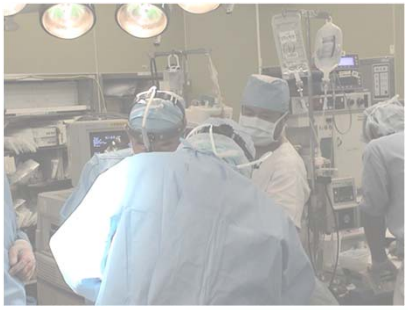
JAPAN





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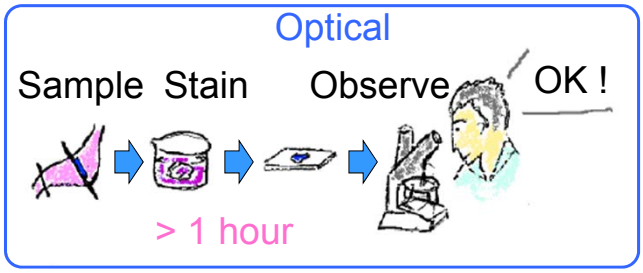
A clinical need.



3

Sound Speed Microscope

Acoustic Impedance Microscope

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Scanning Sound Speed Microscopy

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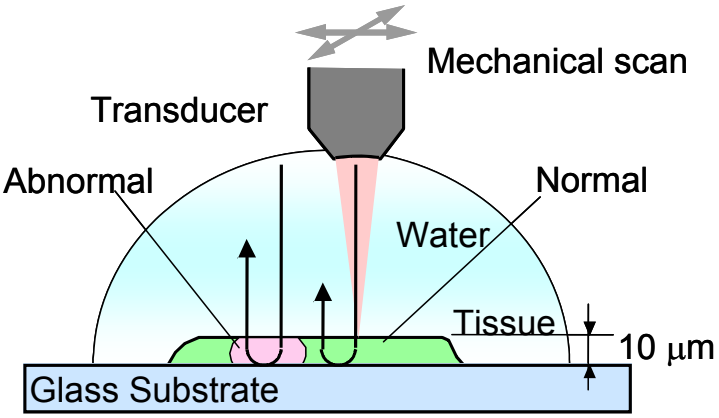
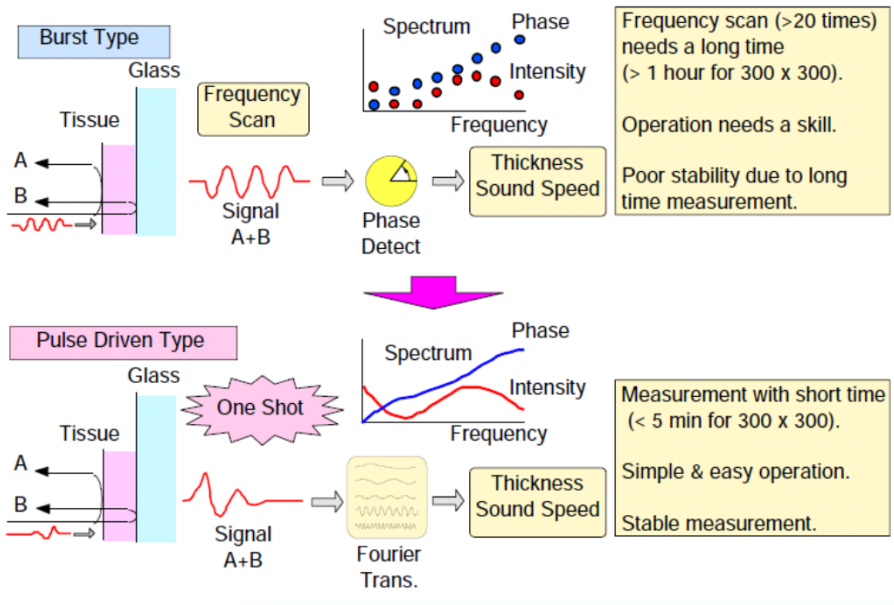
Basic idea.



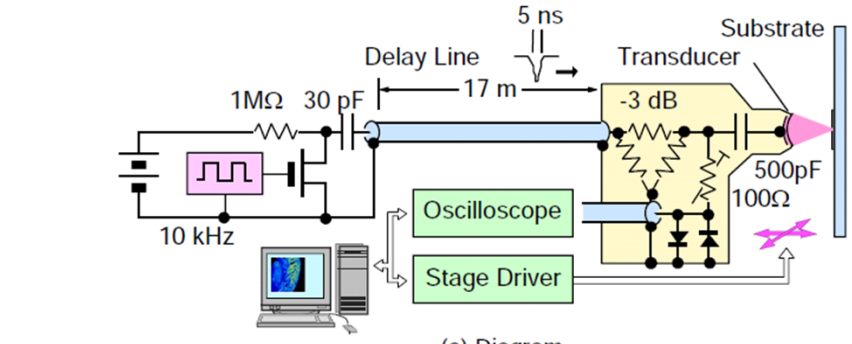
Visualize sound speed and attenuation.

6

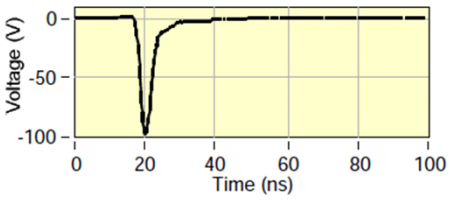
Comparison of two types of measurement.



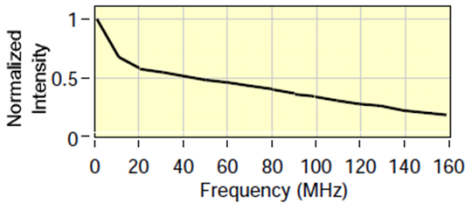
The system is simple.



(a) Diagram

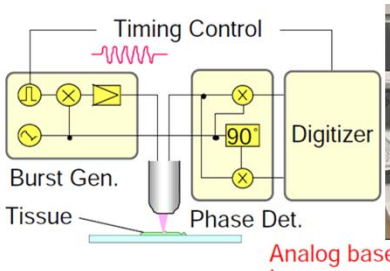


(b) Waveform of pulse voltage



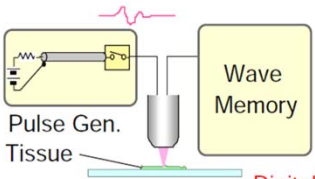
(c) Intensity spectrum of (b)

Development of proto-type.



Conventional

Analog based
Large
High cost - $500k



Proposed

Digital based
Compact
Low cost - <$100k?

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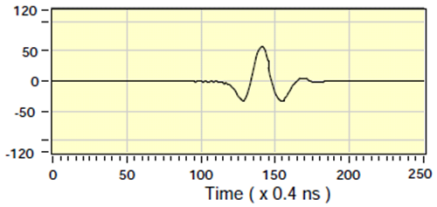
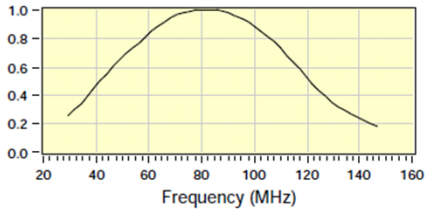
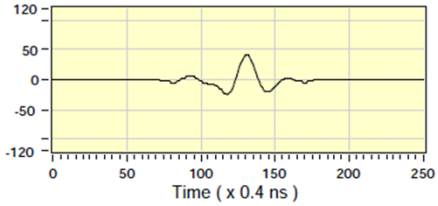
Waveforms.



(a) Reflection from glass



(b) Intensity spectrum of the reflection



(c) Reflection from tissue

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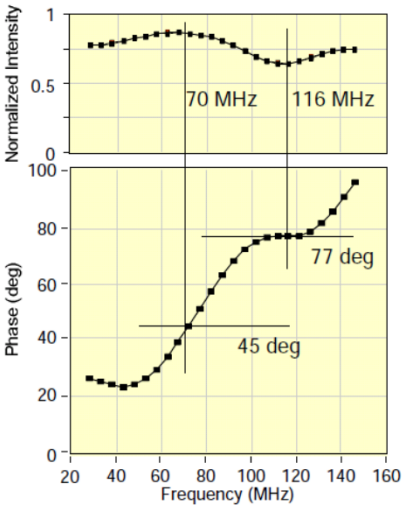
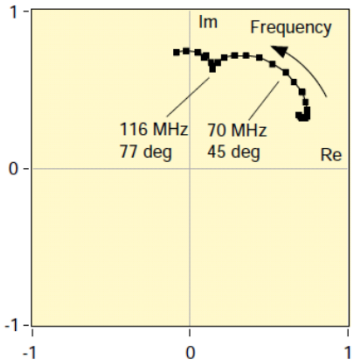
Analysis in the frequency domain.





(dip) $2\pi f_m \times \frac{2d}{c_0} = \phi_m + (2n-1)\pi$ $\quad d = \frac{c_0}{4\pi f_m}\{\phi_m + (2n-1)\pi\}$

(maxima) $2\pi f_m \times \frac{2d}{c_0} = \phi_m + 2n\pi$ $\quad d = \frac{c_0}{4\pi f_m}(\phi_m + 2n\pi)$

$2\pi f_m \times 2d(\frac{1}{c_0} - \frac{1}{c}) = \phi_m$ $\quad c = (\frac{1}{c_0} - \frac{\phi_m}{4\pi f_m d})^{-1}$

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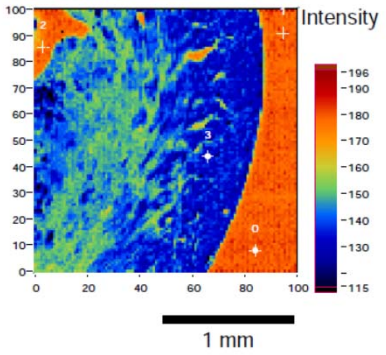
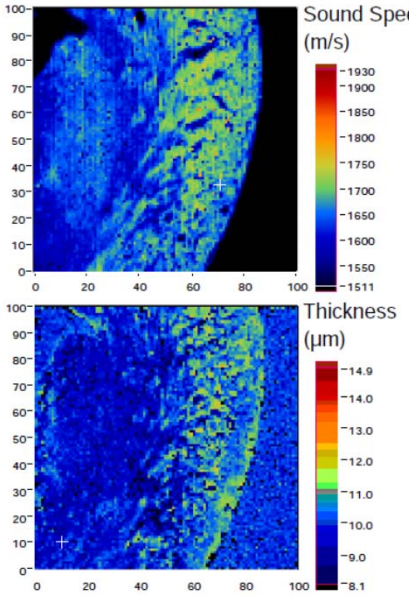
Observation of a rat cardiac allograft model.





9 min for 100 x 100 pixels

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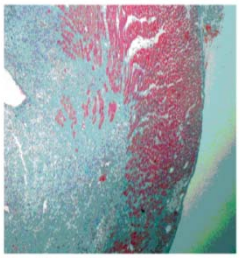
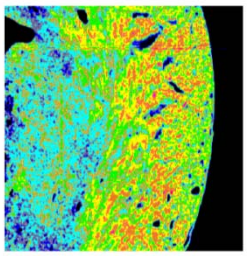
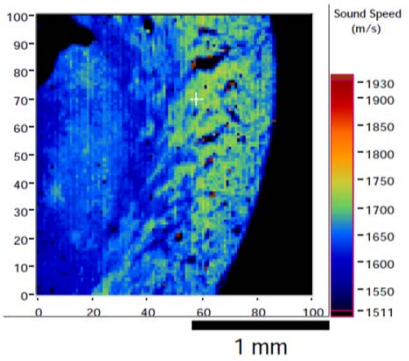
Comparison with optical microscopy.



Optical microscopy (stained)



Conventional frequency domain measumrnent (1 hr)



Pulse driven type (10 min)

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

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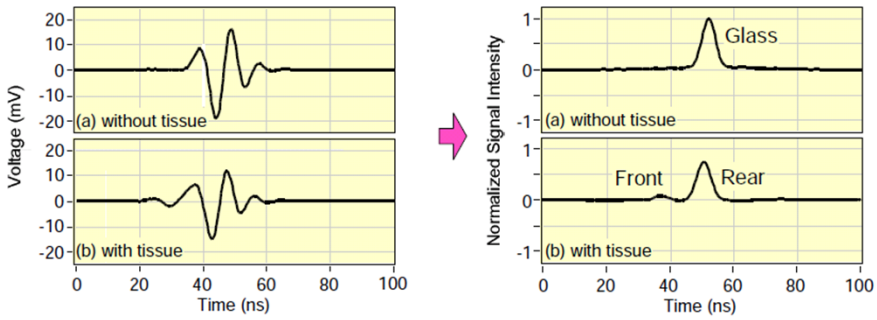
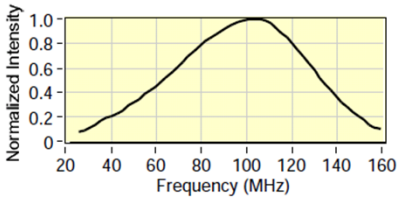
Waveforms before and after the processing.



(1) Waveform before processing

(2) Waveform after processing



Intensity spectrum of (1-a)

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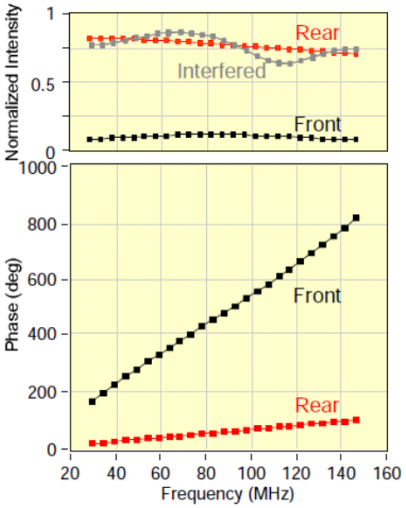
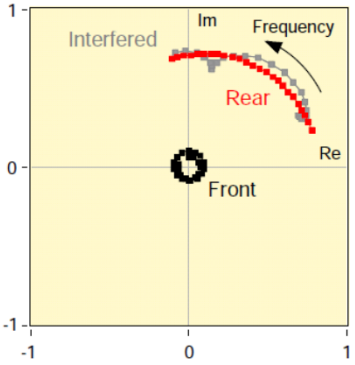
Analysis for separated waveforms.





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

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

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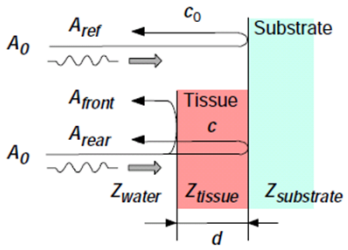
Calculation for specific parameters.

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



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:

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

Attenuation:

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

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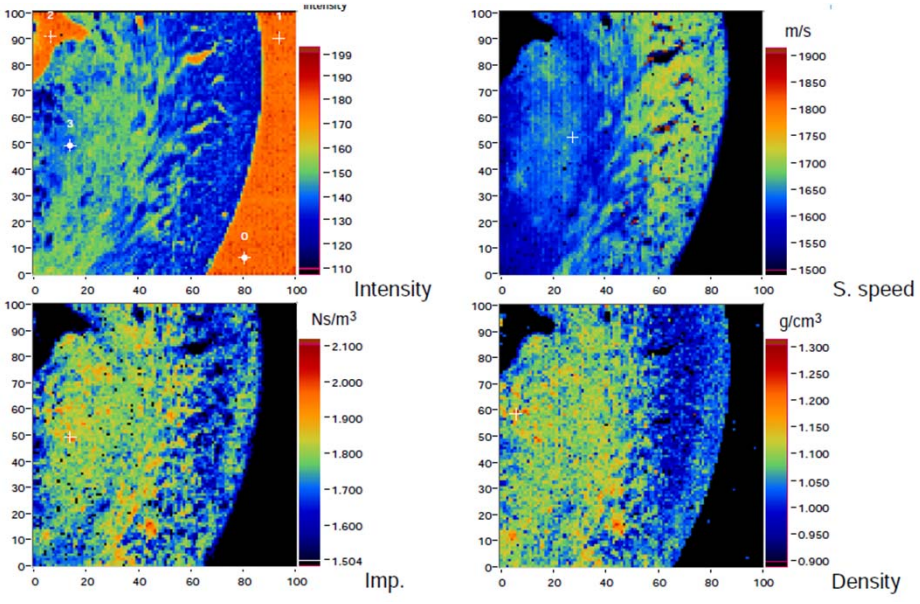
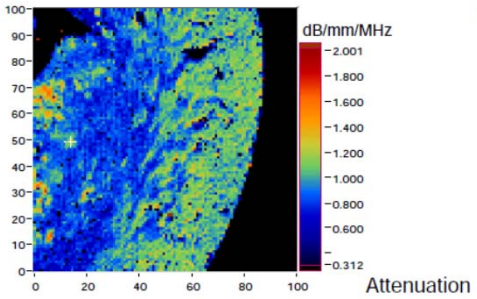
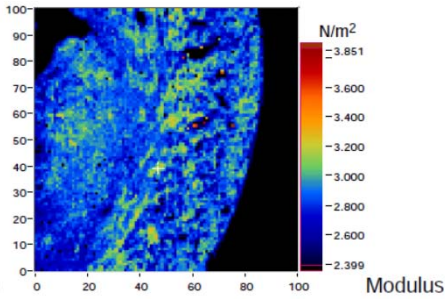
Some other parameters I (at 120 MHz).



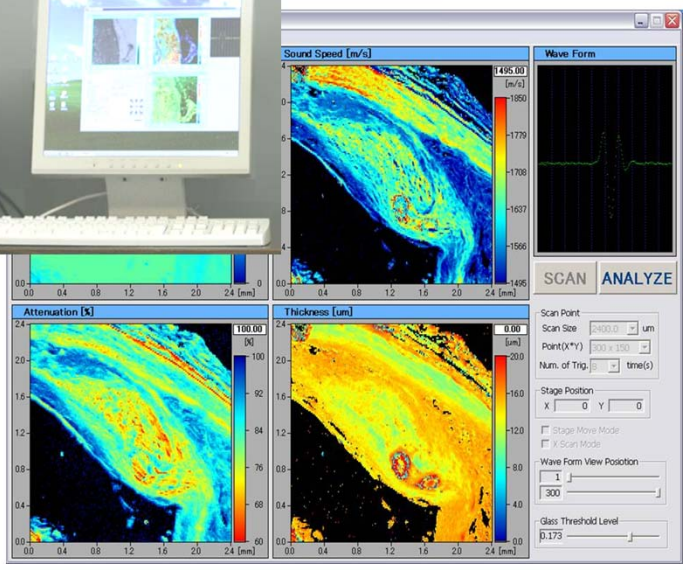
Some other parameters II (at 120 MHz).





Commercialized system.

Honda Electronics
HMS-1000
300 × 300
one minute



vascular wall with calcification

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Summary

- Pulse driven ultrasonic microscope.
- Quick, Precise, Easy, Compact.
- Prototype: 100 x 100 resolution within 9 min.
- Commercial type: 300 x 300 resolution within1.5 min.
- Rat miocadium was successfully observed.

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Scanning Acoustic Impedance Microscopy

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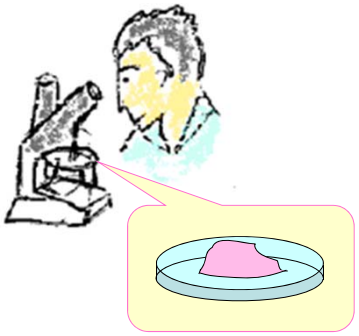
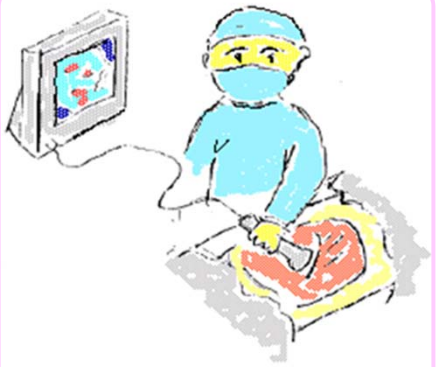
Additional needs.



Non-sliced specimen



Direct probing

This presentation

Future ?

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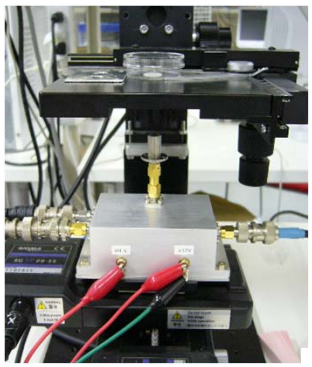
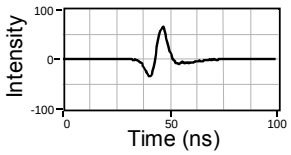
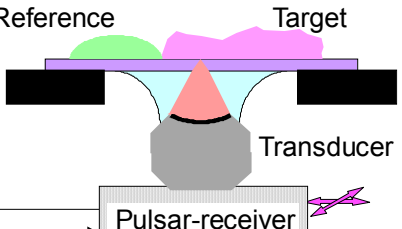
System setup.







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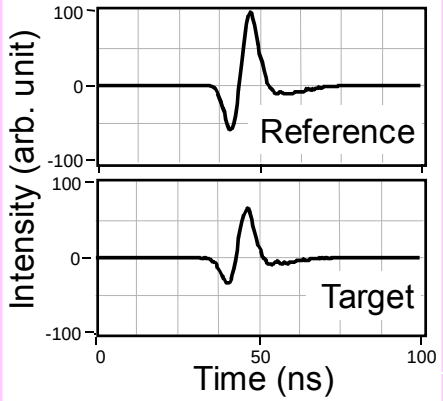
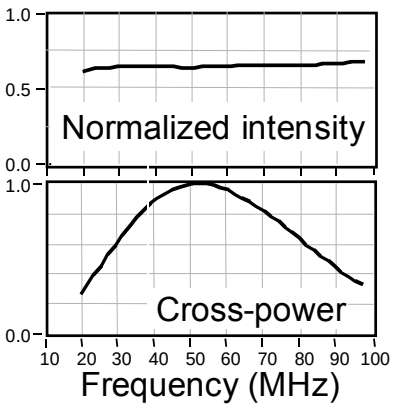
Waveforms.



Time domain



Frequency domain

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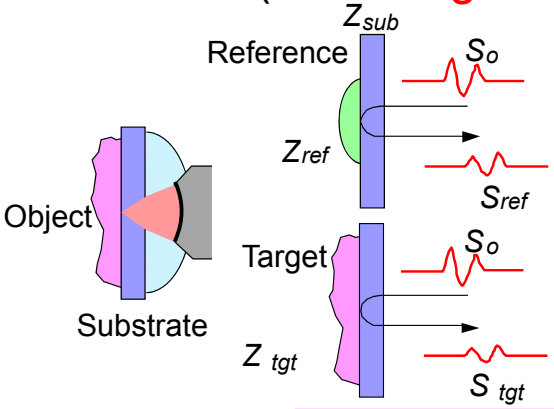
Calibration (assuming vertical incidence).



$$S_{ref} = \frac{Z_{ref} - Z_{sub}}{Z_{ref} + Z_{sub}} S_0$$

$$S_{tgt} = \frac{Z_{tgt} - Z_{sub}}{Z_{tgt} + Z_{sub}} S_0$$

$$Z_{tgt} = \frac{1 + \frac{S_{tgt}}{S_0}}{1 - \frac{S_{tgt}}{S_0}} Z_{sub} = \frac{1 - \frac{S_{tgt}}{S_{ref}} \cdot \frac{Z_{sub} - Z_{ref}}{Z_{sub} + Z_{ref}}}{1 + \frac{S_{tgt}}{S_{ref}} \cdot \frac{Z_{sub} - Z_{ref}}{Z_{sub} + Z_{ref}}} Z_{sub}$$

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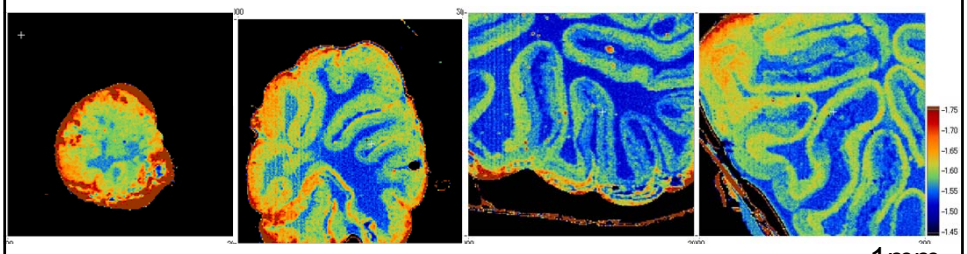
Cerebellum, rat.

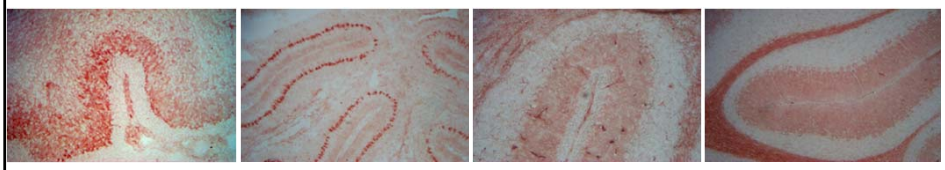
60-100 MHz
not fixed, postnatal 1 to 21 days

P1 P7 P14 P21



Acoustic impedance images

1mm



Stained against calbindin D-28k

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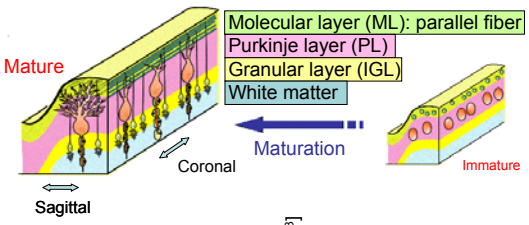
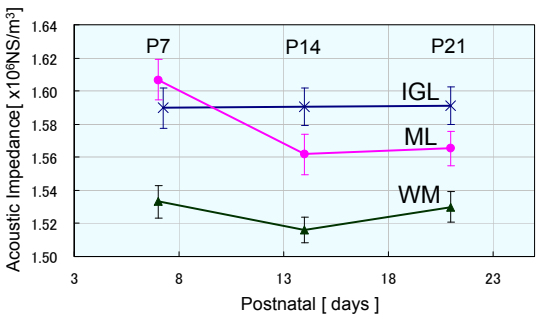
Quantitative assessment





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Improvement of spatial resolution

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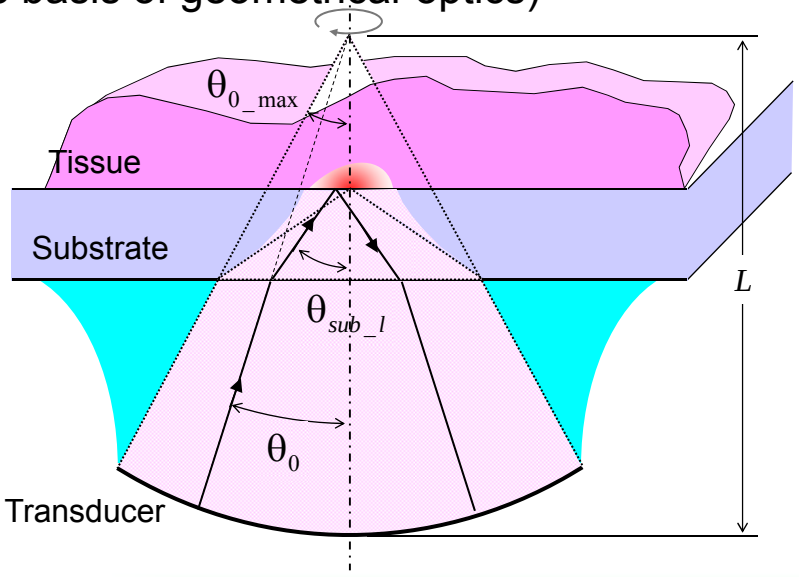
Aberration produced by the substrate (on the basis of geometrical-optics)



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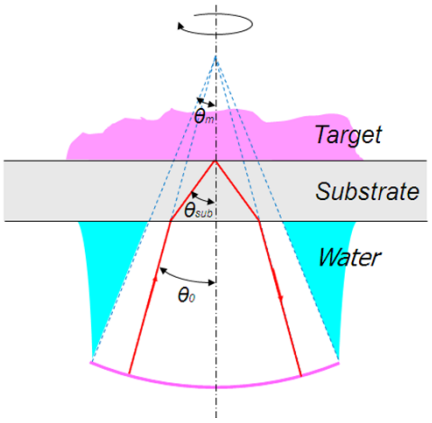
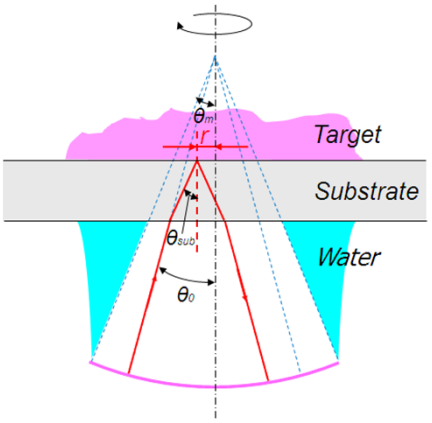
Propagation of beam component



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.

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Signal processing using 3D deconvolution

Calculate impulse response of the acoustic system

Improvement of spatial resolution.
Quantitative observation.

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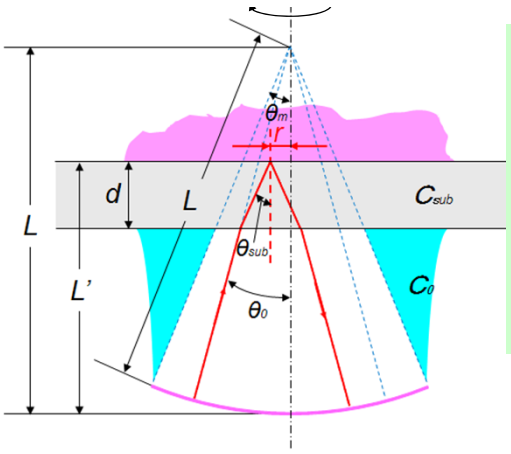
Aberration along lateral direction



Transducer

film type with PVDF-TrFE

focal length（L） 3.2 mm

angle of focusing（θ_m） 21.5deg

Substrate

polystyrene 0.8 mm thick

sound speed(c_{sub}) 2.3 km/s

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

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

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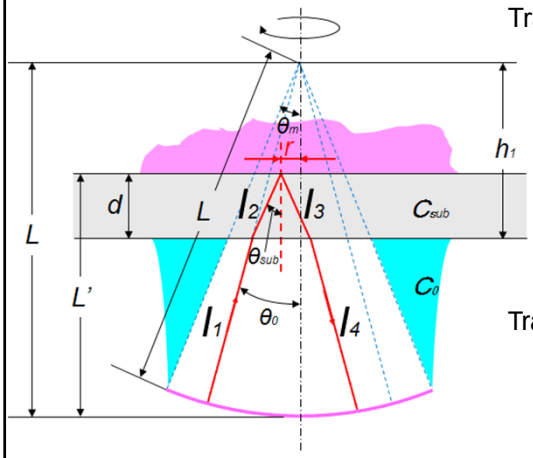
Aberration along time direction



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

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

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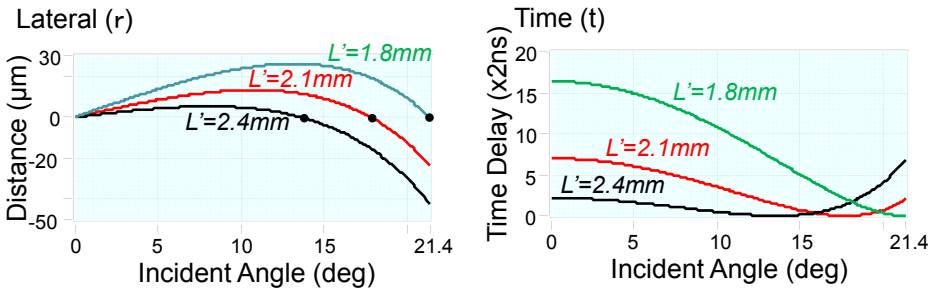
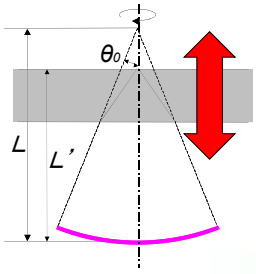
The impulse response

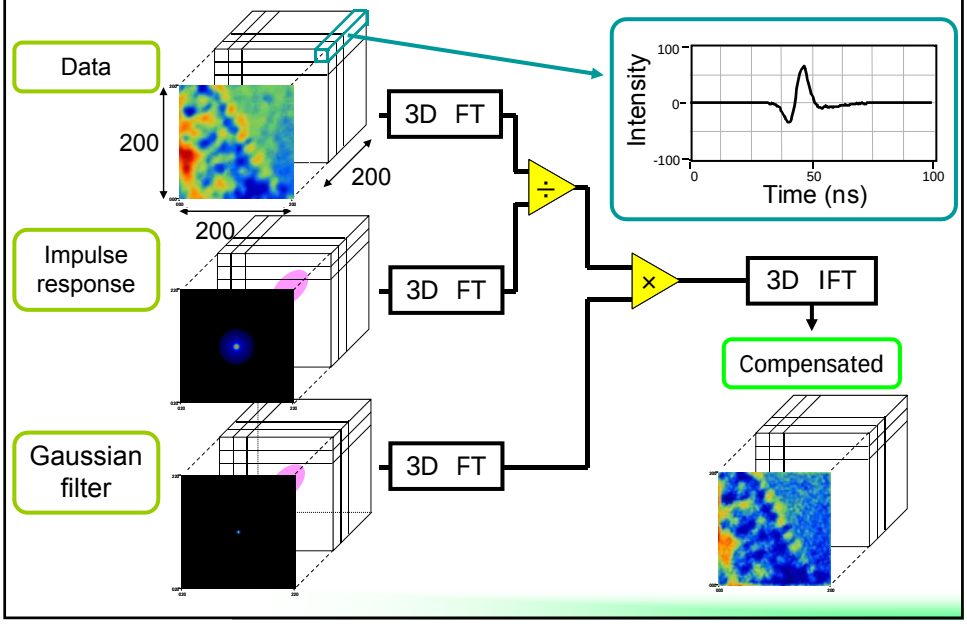




Assumed focal angle is needed because we do not know the exact distance from the transducer to substrate

34

3D deconvolution processing



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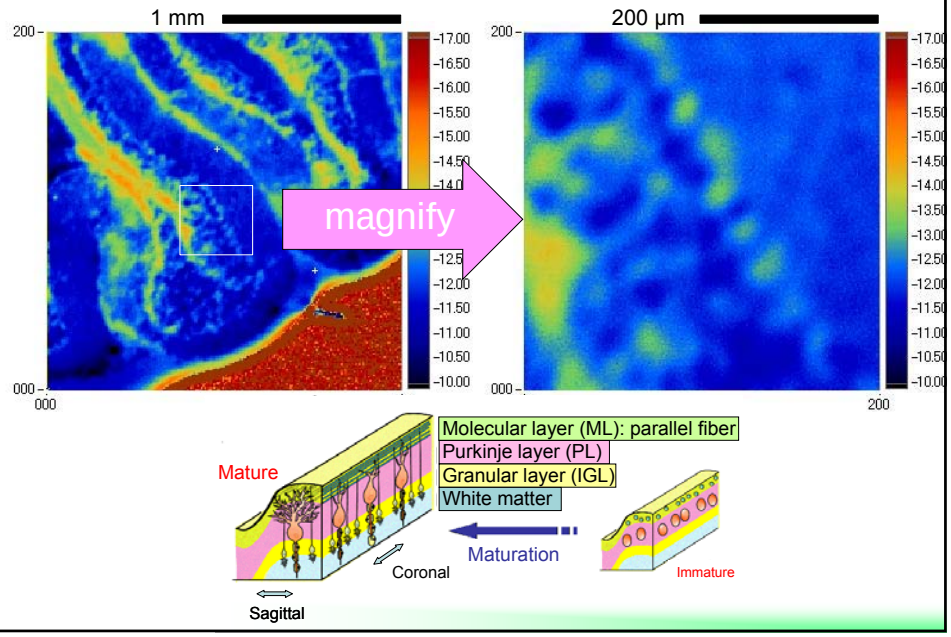
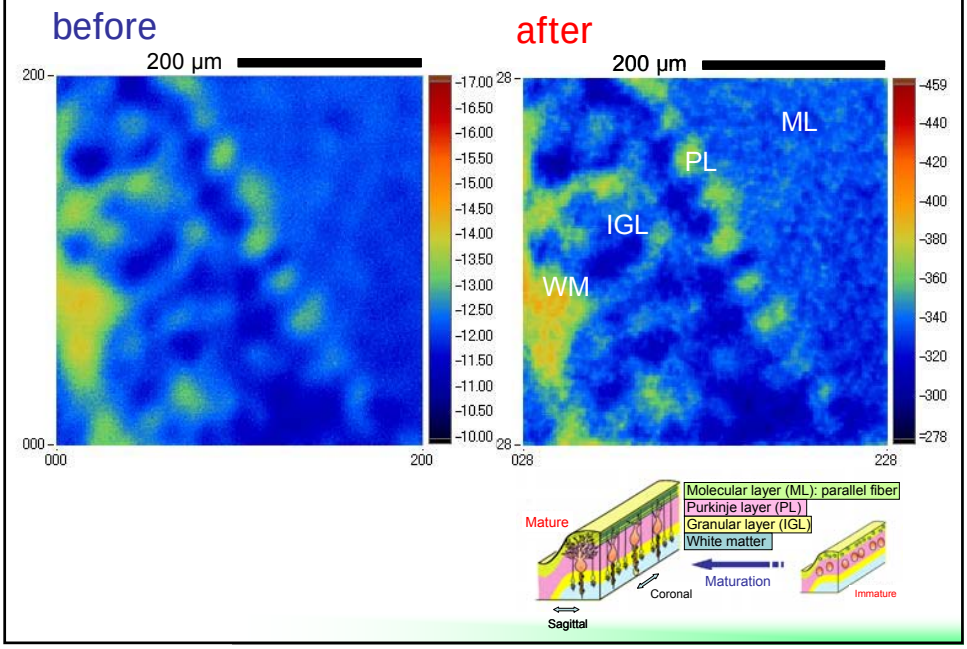
A rat cerebella tissue (intensity image)



36

Intensity images before and after processing.



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Improvement of accuracy

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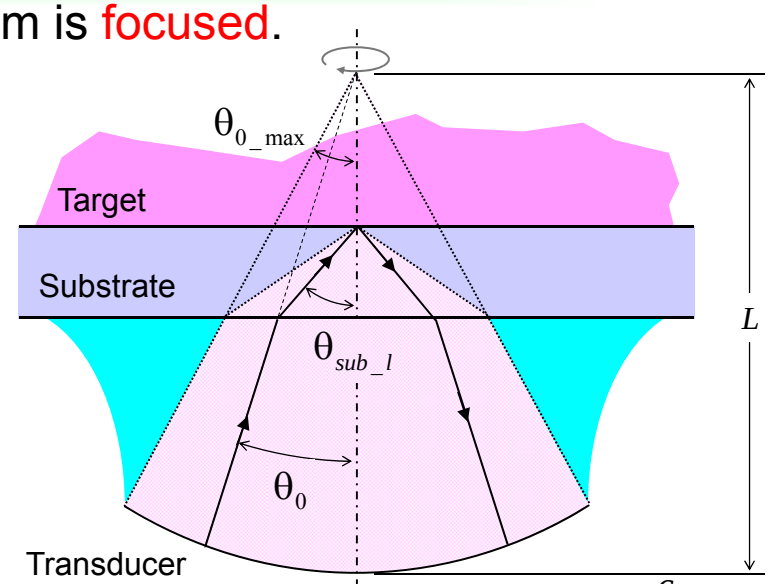
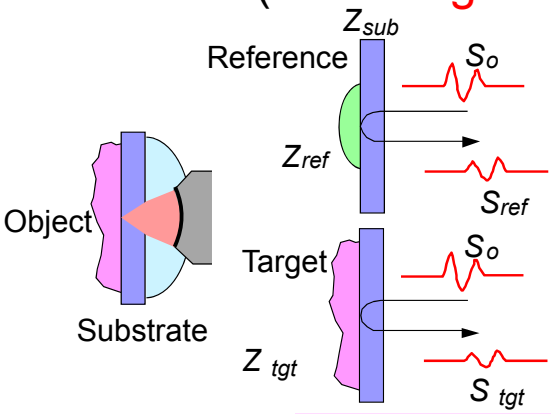
Beam is focused.



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

Calibration (assuming vertical incidence).

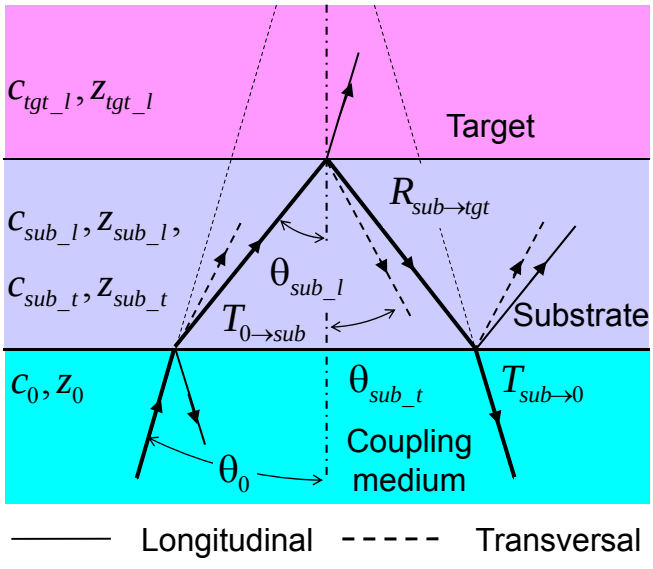


$$S_{ref} = \frac{Z_{ref} - Z_{sub}}{Z_{ref} + Z_{sub}} S_0$$

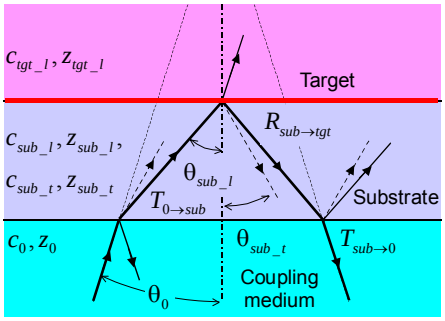
$$S_{tgt} = \frac{Z_{tgt} - Z_{sub}}{Z_{tgt} + Z_{sub}} S_0$$

$$Z_{tgt} = \frac{1 + \frac{S_{tgt}}{S_0}}{1 - \frac{S_{tgt}}{S_0}} Z_{sub} = \frac{1 - \frac{S_{tgt}}{S_{ref}} \cdot \frac{Z_{sub} - Z_{ref}}{Z_{sub} + Z_{ref}}}{1 + \frac{S_{tgt}}{S_{ref}} \cdot \frac{Z_{sub} - Z_{ref}}{Z_{sub} + Z_{ref}}} Z_{sub}$$

Wave propagation.



Reflection constant.



$$R_{sub\to tgt}(Z_{sub\_l}, c_{sub\_l}, c_{sub\_t}, Z_{tgt}, c_{tgt}, \theta_0)$$
$$= \frac{M_{sub} - \cos^2(2\theta_{sub\_t}) + N_{sub\to tgt}}{M_{sub} + \cos^2(2\theta_{sub\_t}) + N_{sub\to tgt}}$$

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

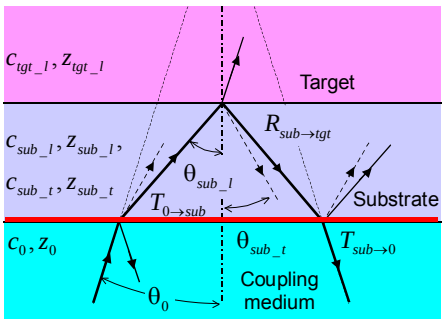
$$\theta_{sub\_t} = \sin^{-1}\left(\frac{c_{sub\_t}}{c_{sub\_l}}\sin\theta_{sub\_l}\right)$$

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

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

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

Transmission constant.



$$T_{0\to sub}\cdot T_{sub\to 0}$$
$$= T'_{sub\to tgt}(Z_0, c_0, Z_{sub\_l}, c_{sub\_l}, c_{sub\_t}, \theta_0)$$
$$= \frac{4N_{0\to sub}\cos^2(2\theta_{sub\_t})}{\left\{M_{sub} + \cos^2(2\theta_{sub\_t}) + N_{0\to sub}\right\}^2}$$

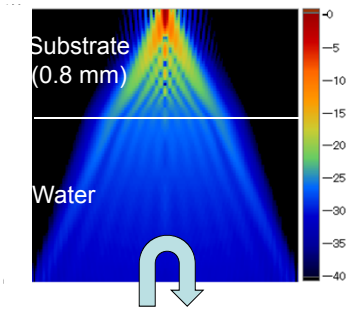
$$\theta_{sub\_l} = \sin^{-1}\left(\frac{c_{sub\_l}}{c_0}\sin\theta_0\right)$$

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

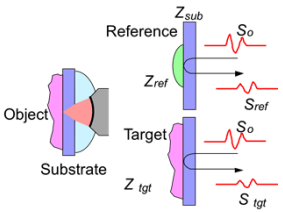
$$M_{sub} = \left(\frac{c_{sub\_t}}{c_{sub\_l}}\right)^2 \sin\theta_{sub\_t}\sin\theta_{sub\_l}$$

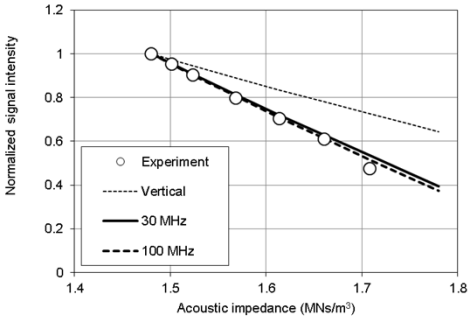
$$N_{0\to sub} = \frac{Z_0\cos\theta_{sub\_l}}{Z_{sub\_l}\cos\theta_0}$$

Normalized reflection intensity.

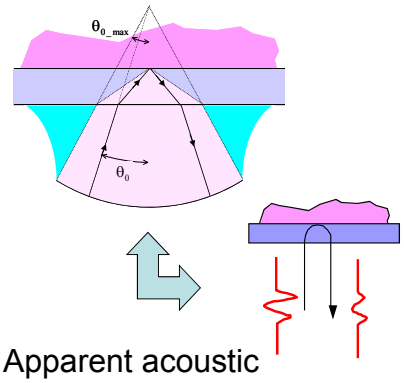


Reflection Intensity





Apparent acoustic impedance of the target assuming vertical incidence.



Apparent reflection constant

target:

$$S_{tgt}(Z_{tgt}, c_{tgt}, \theta_{0\_\max}) / S_0 =$$

$$\int_0^{\theta_{0\_\max}} 2\pi L^2 \sin\theta_0 R_{sub\to tgt}(\theta_0) T'_{0\to sub}(\theta_0) d\theta_0$$

reference:

$$S_{ref}(Z_{ref}, c_{ref}, \theta_{0\_\max}) / S_0 =$$

$$\int_0^{\theta_{0\_\max}} 2\pi L^2 \sin\theta_0 R_{sub\to ref}(\theta_0) T'_{0\to sub}(\theta_0) d\theta_0$$

Apparent acoustic impedance of the target:

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

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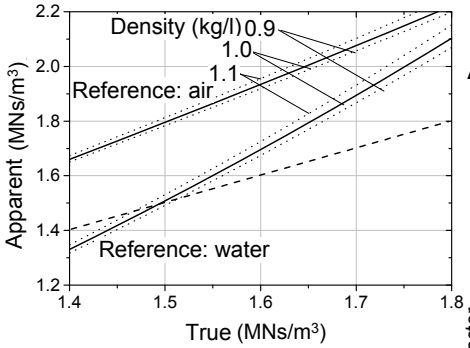
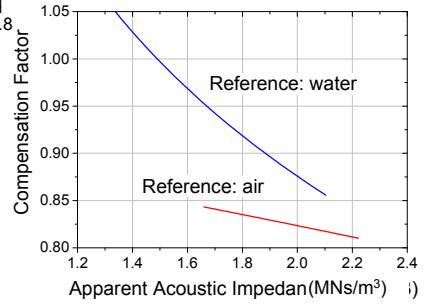
Compensation curves.



Angle of focusing: 22 deg



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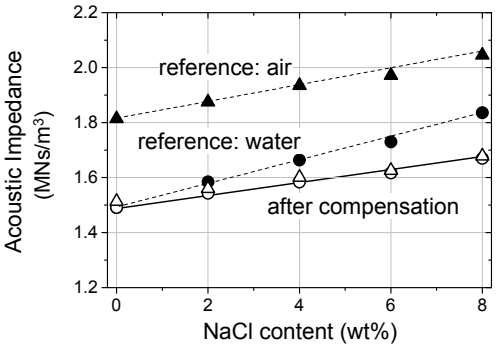
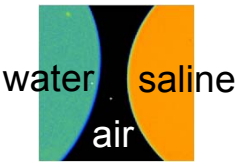
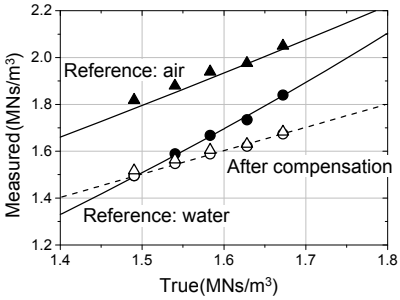
Measurement for saline solution.







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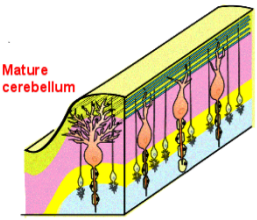
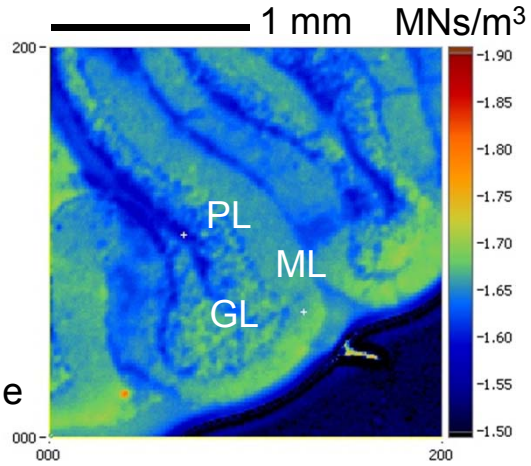
Cerebellum tissue of a rat.



ML
PL
GL
WM

P15
fixation by formaldehyde



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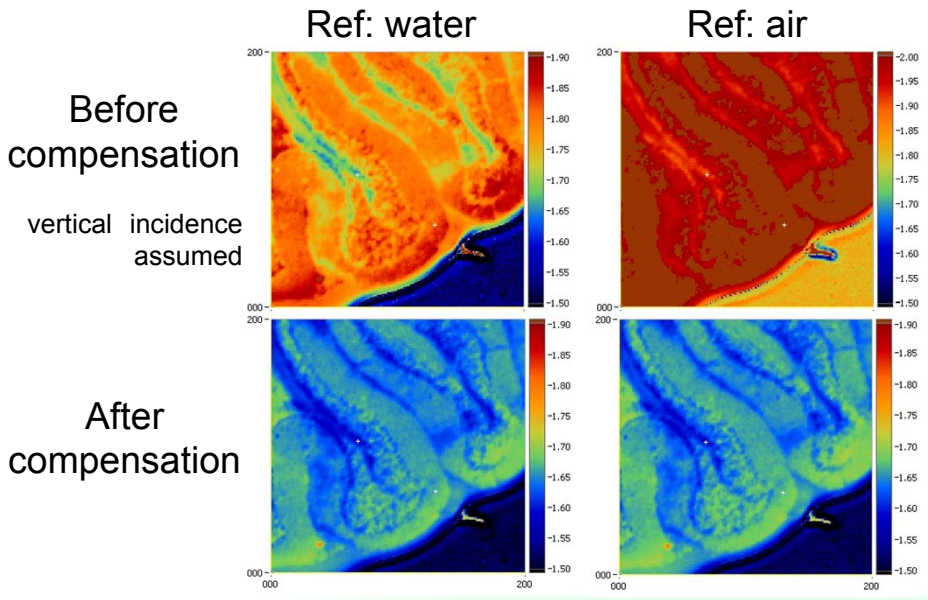
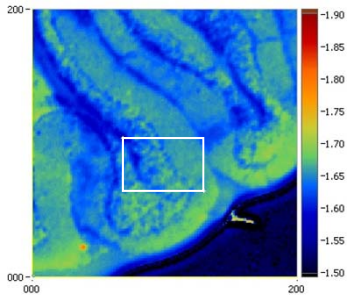
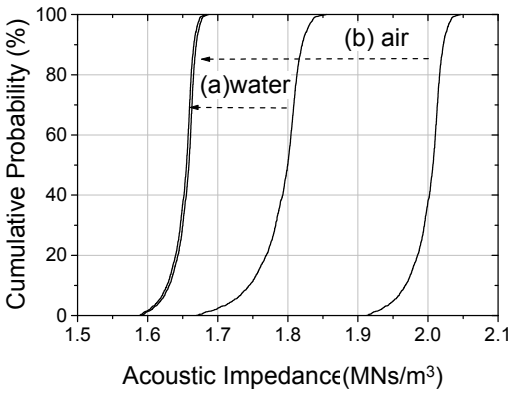
Effect of compensation.

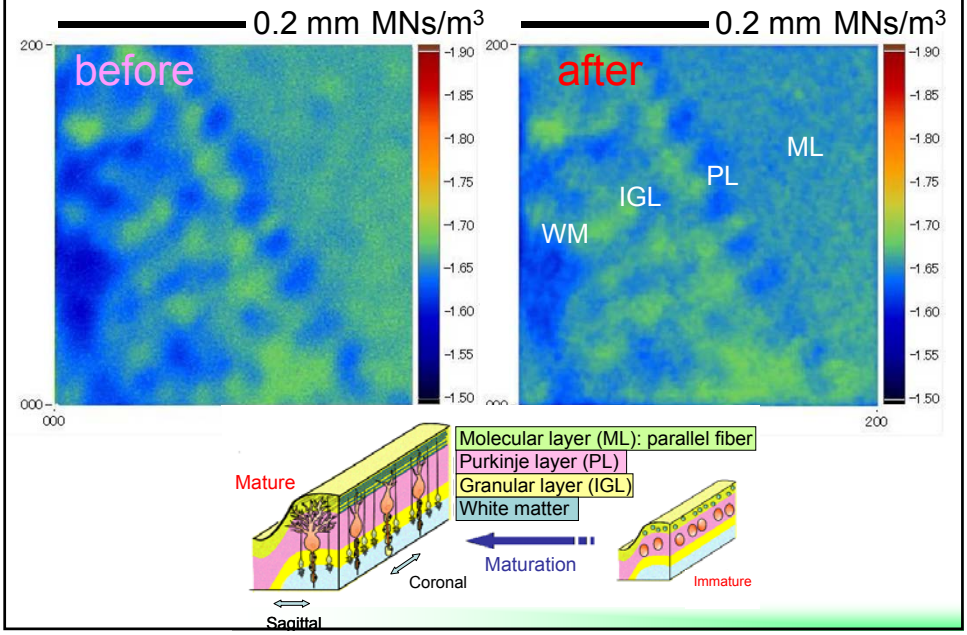


Distribution of the acoustic impedance.





Acoustic impedance images



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Observation of brain tumor model of rat

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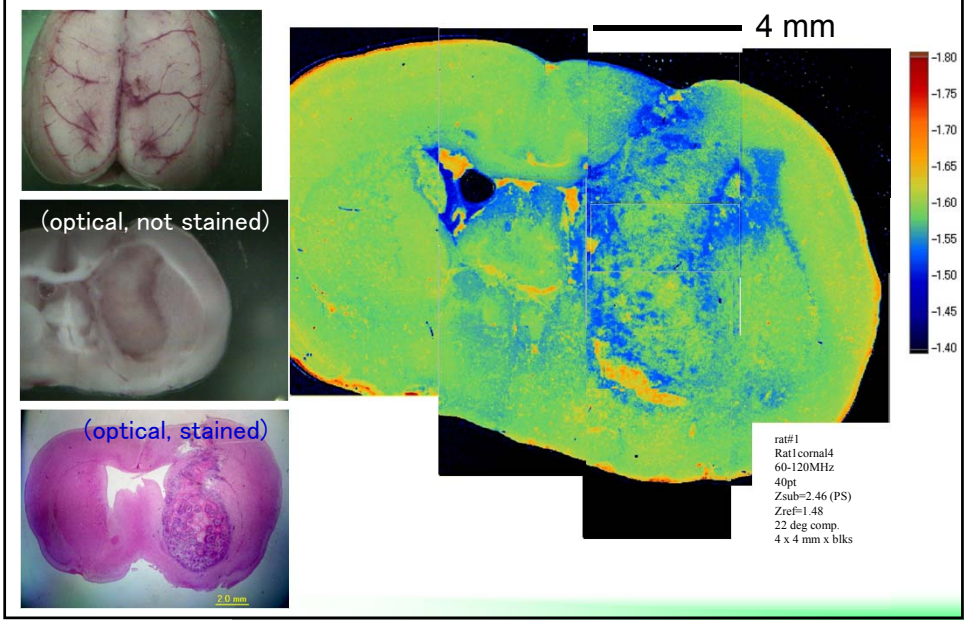
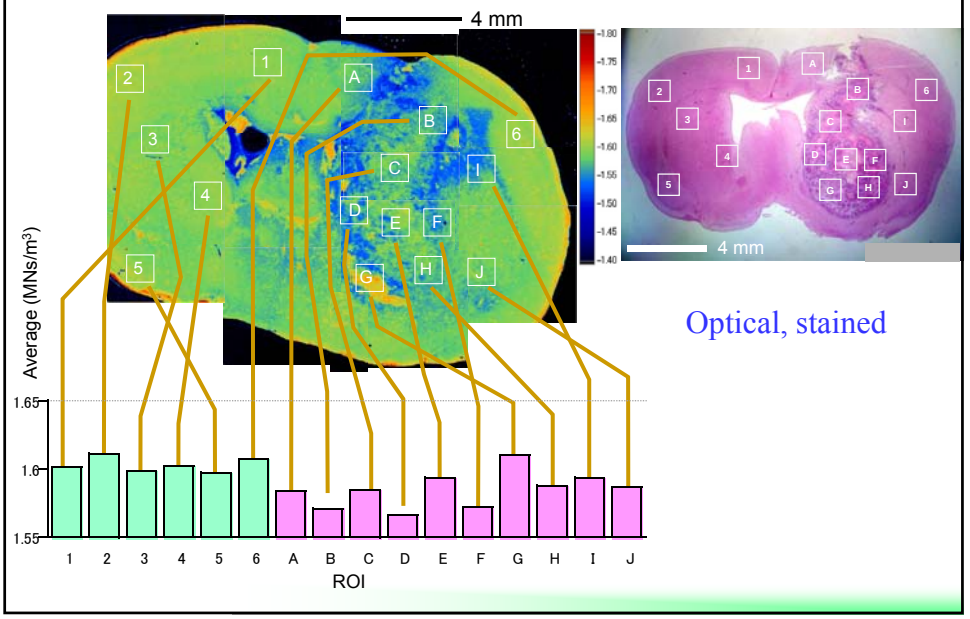
Brain tumor, rat.

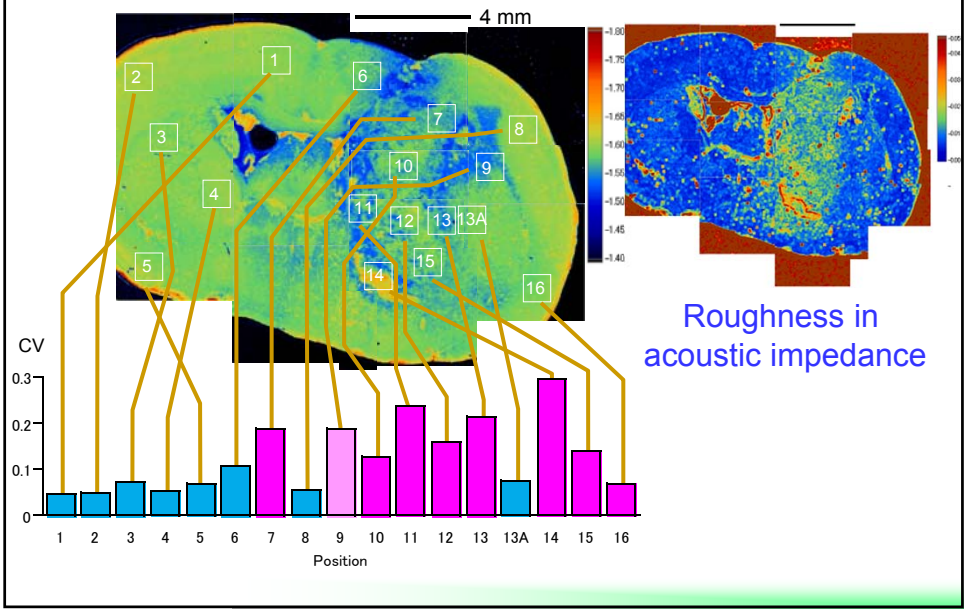


Quantitative analysis (average)



Optical, stained

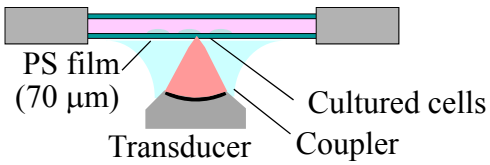
Quantitative analysis (standard deviation)

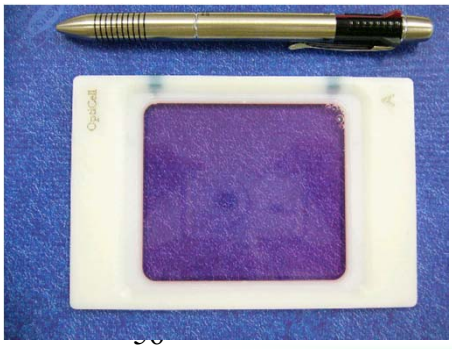


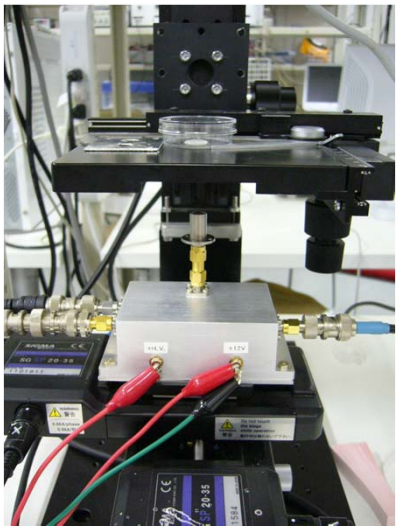
Roughness in acoustic impedance

Cell size observation

Observation system for cultured cells.







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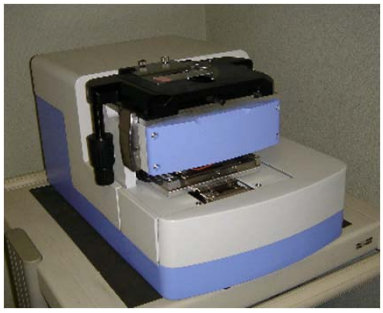
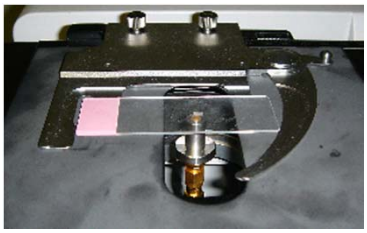
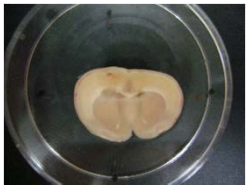
Commercialized system by Honda Electronics.



body (new type available)

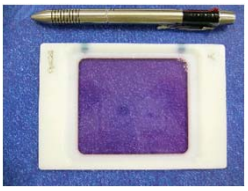


stage



dish

dish with film



incubation cell

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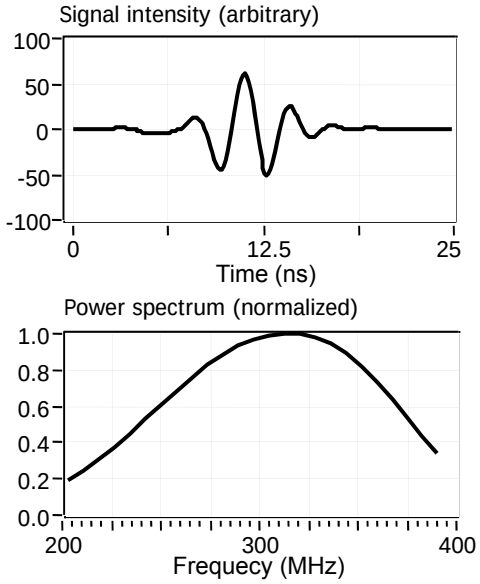
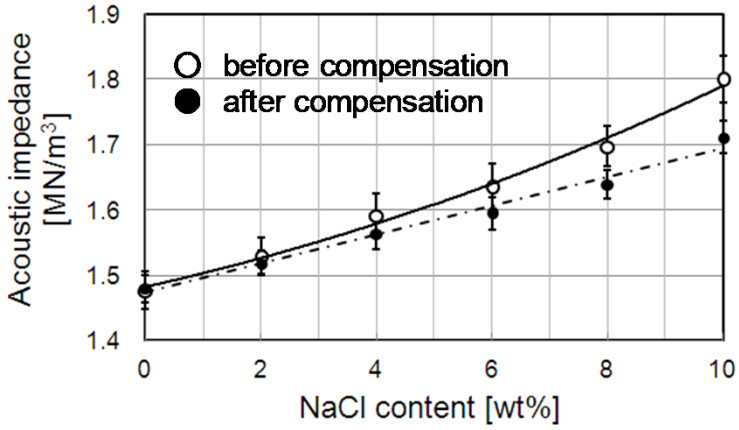
Waveform and spectrum.

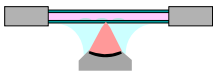


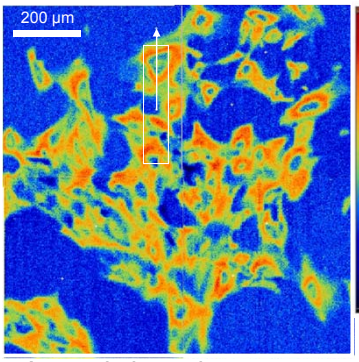
200～400 MHz range was used.

Calibration by saline solution.

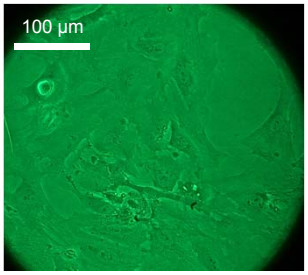


Cultured glial cells, rat.

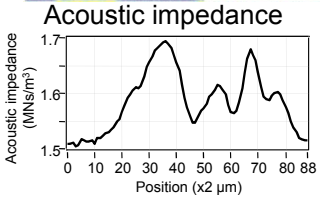




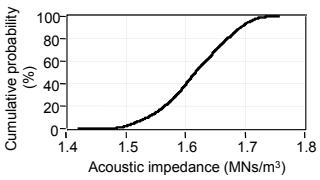
Acoustic impedance



Optical



Trace along the white arrow



Distribution in the ROI

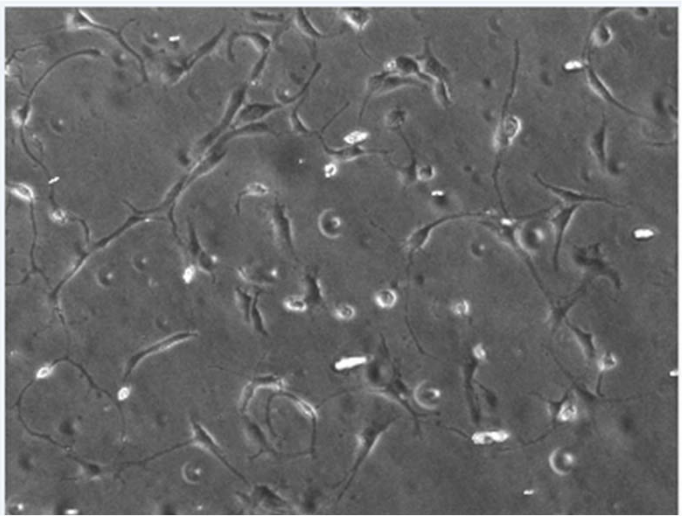
In situ observation after dosage

Glioma DIV14, Cytochalasin B 25μg/ml

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

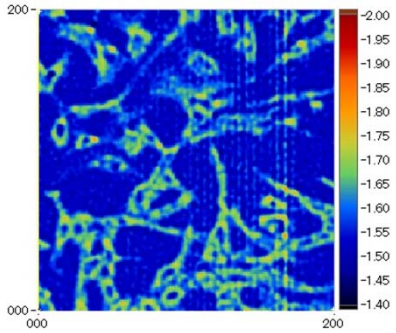
投薬後30分から1時間の間では大きな変化は確認できないですが、4時間後には全体的に細胞の萎縮や浮いてくるような様子が見られる。

Glioma DIV14, Cytochalasin B 25µg/ml



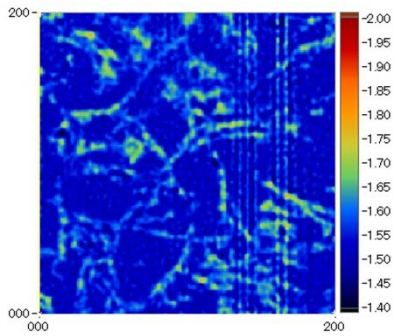
0 min after injection. Optical Image

Glioma DIV14, Cytochalasin B 25µg/ml



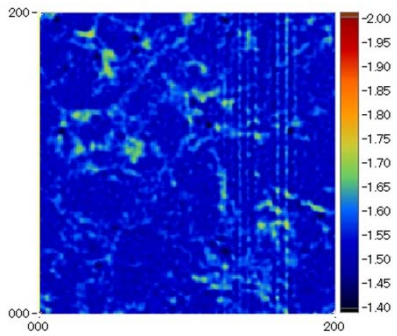
Before injection.
Acoustic impedance

Glioma DIV14, Cytochalasin B 25µg/ml



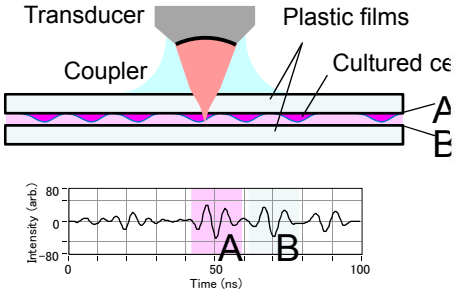
60 min after injection.
Acoustic impedance

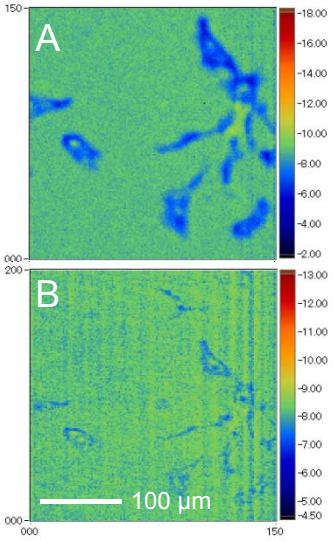
Glioma DIV14, Cytochalasin B 25µg/ml



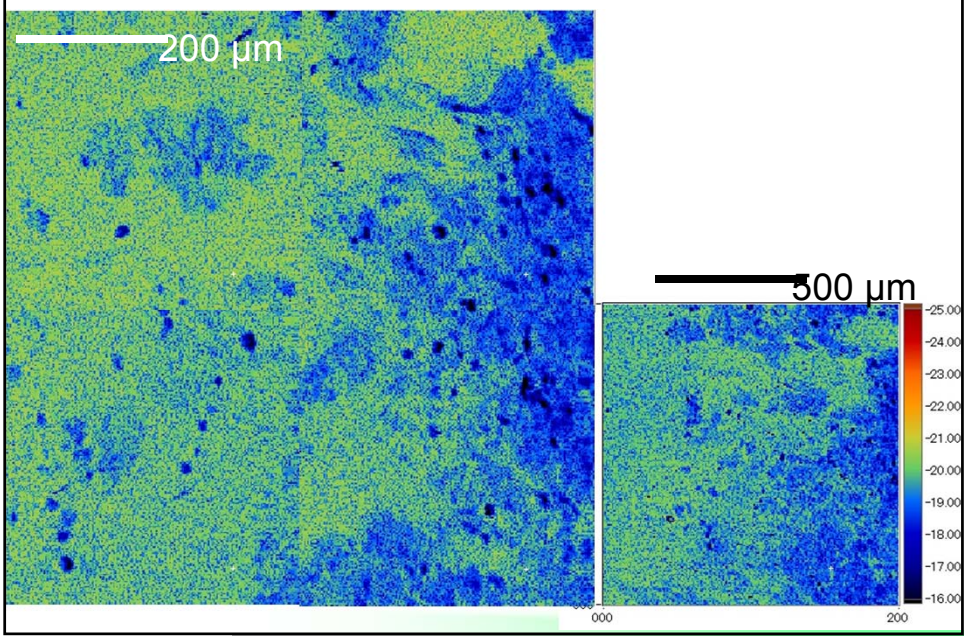
150 min after injection.
Acoustic impedance

Reflection mode and projection mode, Glial cells.





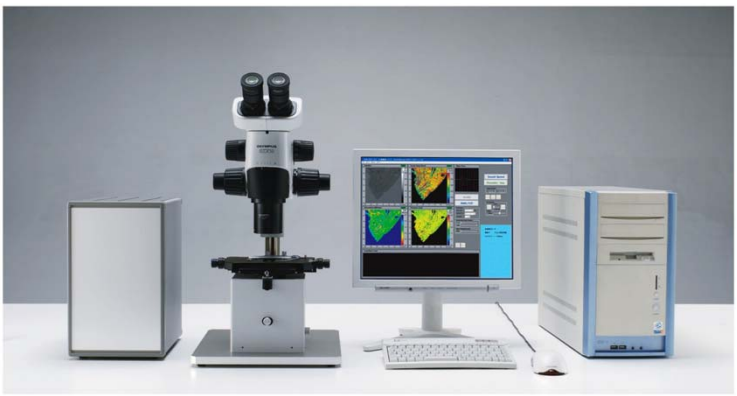
Projection mode, cancerous liver, rat.



Commercialized by HONDA Electronics

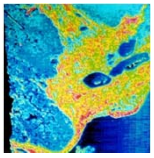
HONDA Electronics

AMS-50SI

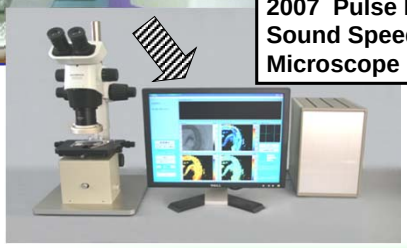


Development in Honda Electronics

1985 Burst Driven Type
Ultrasonic Microscope

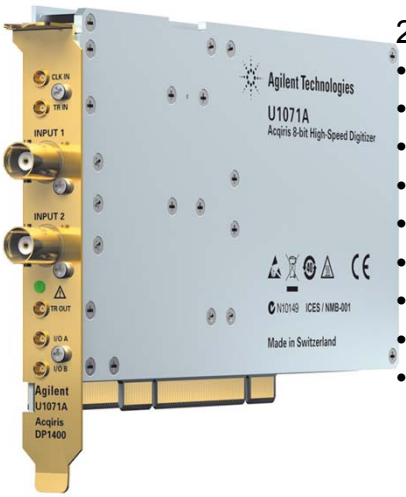


2005 Pulse Driven Type
Ultrasonic Sound Speed Microscope



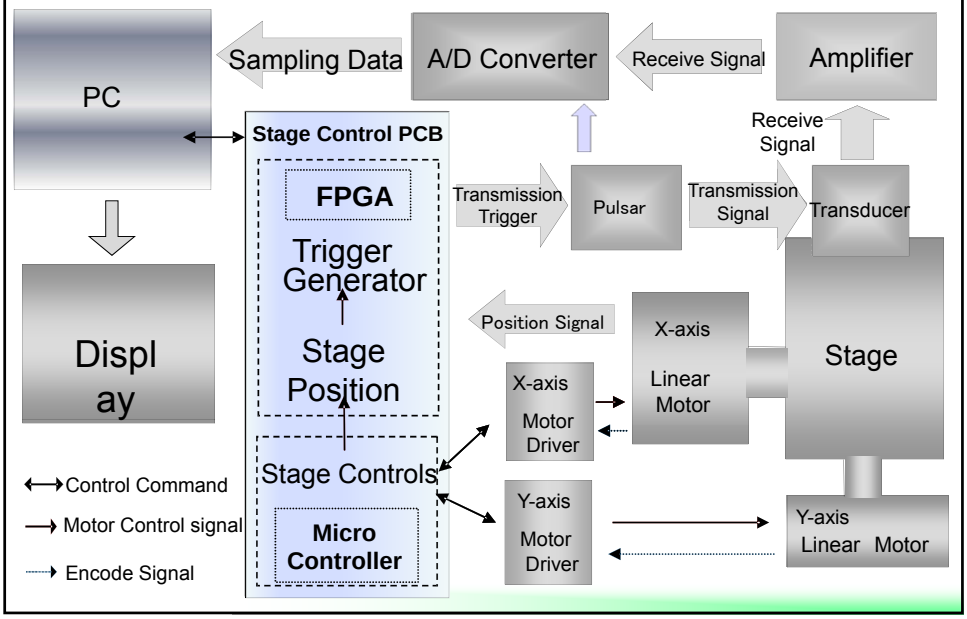
2007 Pulse Driven Type Ultrasonic
Sound Speed / Acoustic Impedance
Microscope

Digitizer Unit DP1400（ACQIRIS)

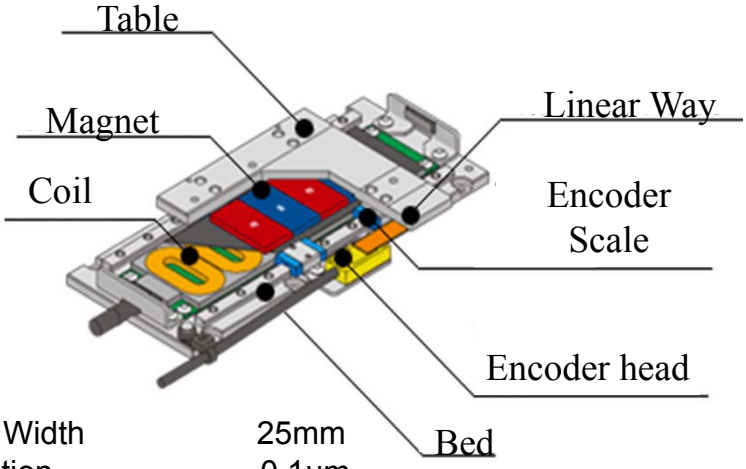


2 GS/s Sampling Rate
- 500 MHz Bandwidth
- 256 k points Acquisition Memory
- 50 Ω and 1 MΩ Input Impedance
- Complete Pre and Post Triggering
- 2 ppm Clock Accuracy
- for Accurate Timing Measurements
- Very high Data Transfer Rate
- Sampling Jitter 2psec
- Transfer Speed 220MB/sec

Control Diagram



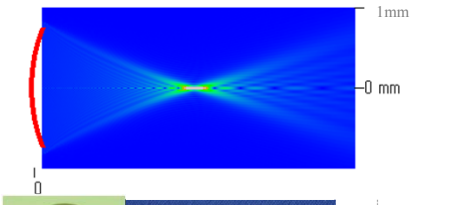
Linear Motor

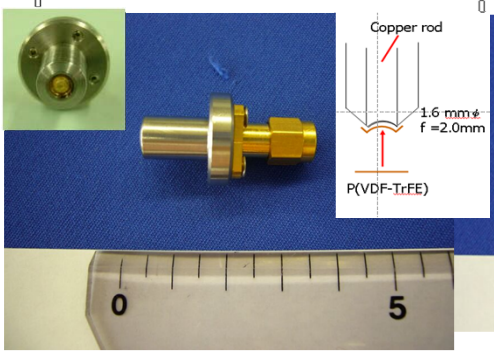


Stroke Width	25mm
Resolution	0.1μm
Max Speed	1.0m / s
Accuracy of Positioning	0.5μm
Operating Temperature・	0～40C, 20 - 80%RH

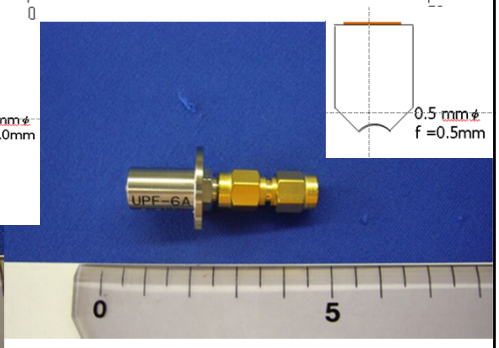
Transducers

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

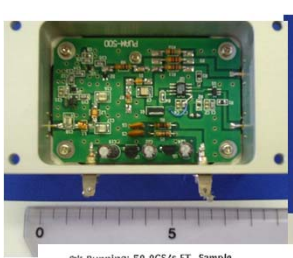


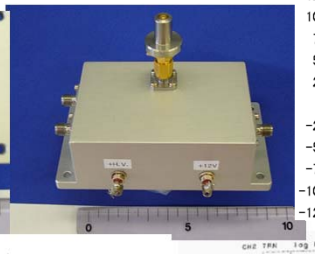


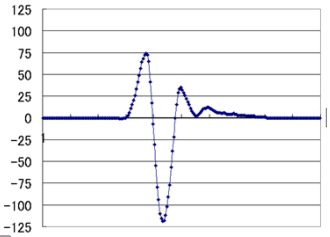
ZnO 320MHz D=0.5 F=0.5

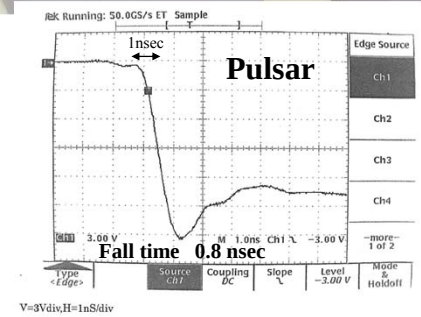


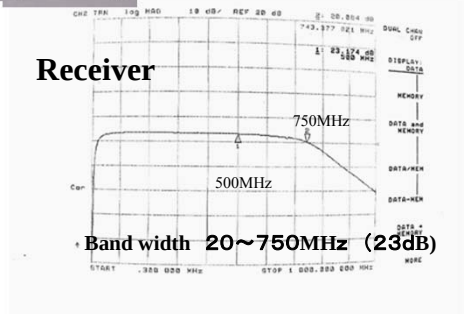
Pulsar Receiver (20MHz - 500MHz)











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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.

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What can we do next ?

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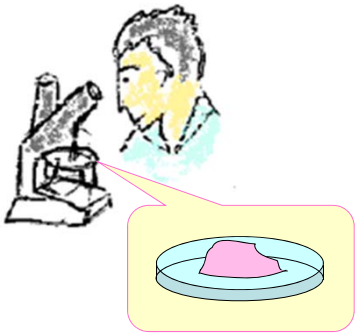
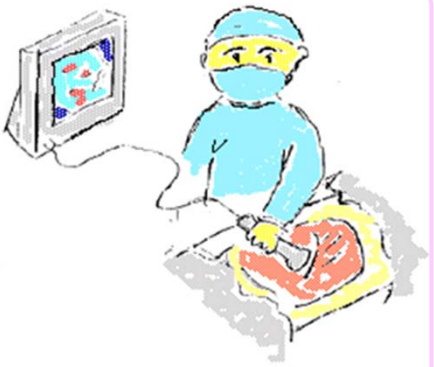
Direct probing



Non-sliced specimen



Direct probing

This presentation

Future ?

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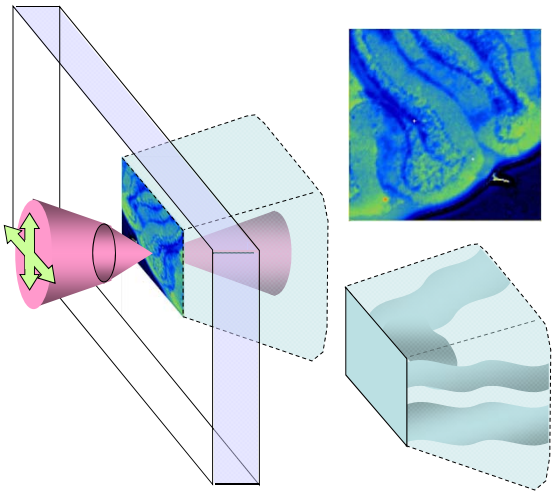
A hybrid 2D 3D microscope



2D Profile of
Acoustic Impedance

Quantitative

3D Profile by
Synthetic Aperture

Morphological

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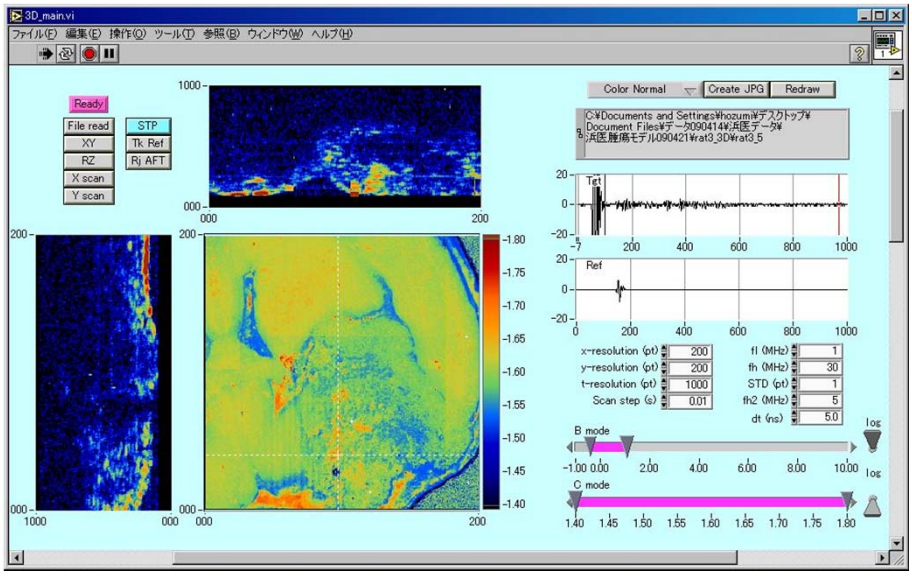
A hybrid 2D 3D microscope



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Thank you for your attention.