TOYOHASHI UNIVERSITY OF TECHNOLOGY

IEEE UFFC Symposium Short course 2013

Where do the acoustic images come from? What are they? Where are they going?

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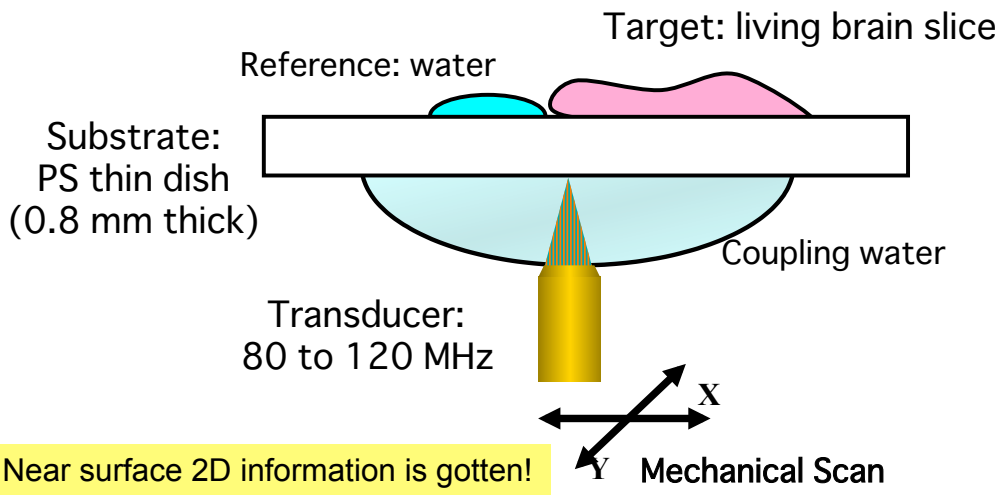
Our targets: Living organs and cells of rat cerebellum

Materials: 400μm sliced rat cerebellar cortex and cultured glial cells

Methods: Acoustic impedance microscopy



Near surface 2D information is gotten!

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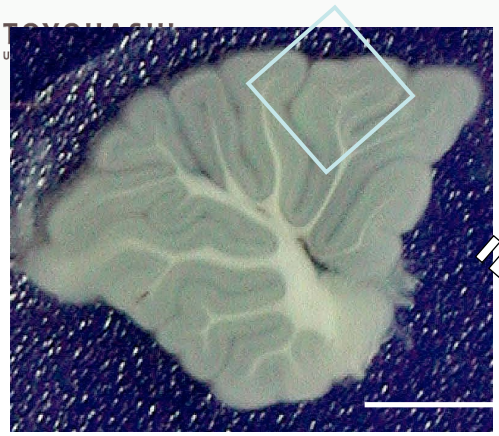
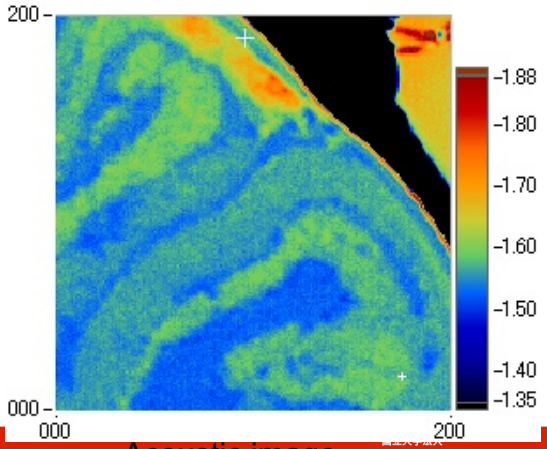
Cerebellar slice observation
using middle frequency (80 – 120 MHz)





Optical image

Acoustic image

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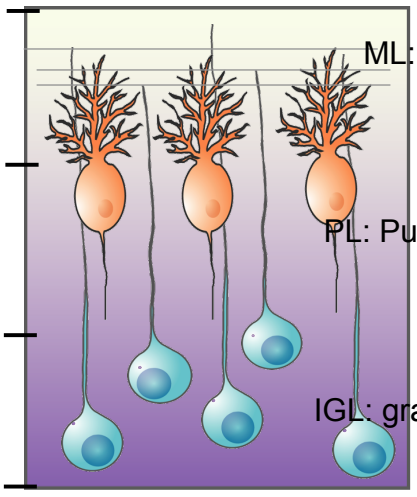
Nuclei-rich layer: high (>1.58) impedance

Cytoskeleton-rich layer: middle (≅1.56) impedance

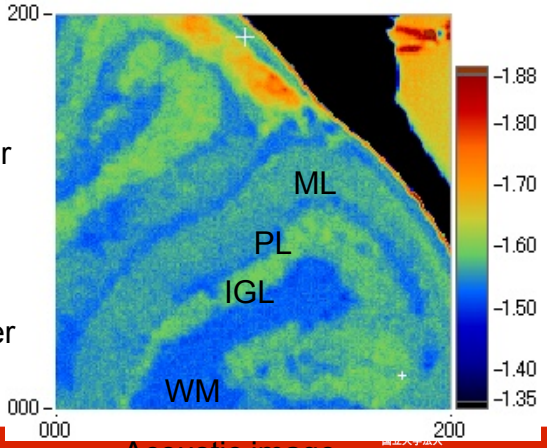
Lipid-rich layer: low (<1.54) impedance



ML: network layer

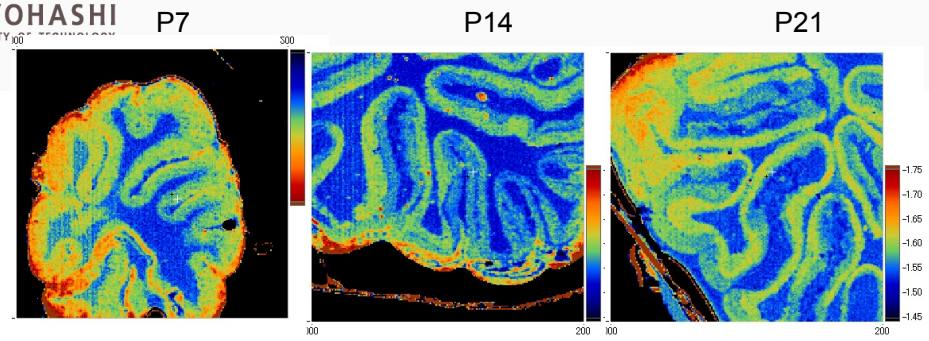
PL: Purkinje cell layer

IGL: granular cell layer

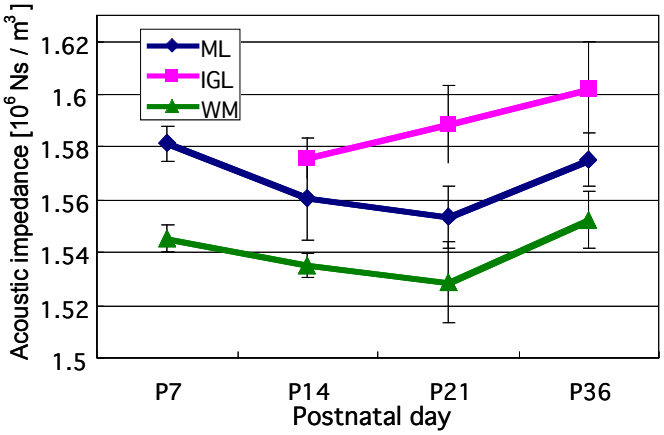


Optical image

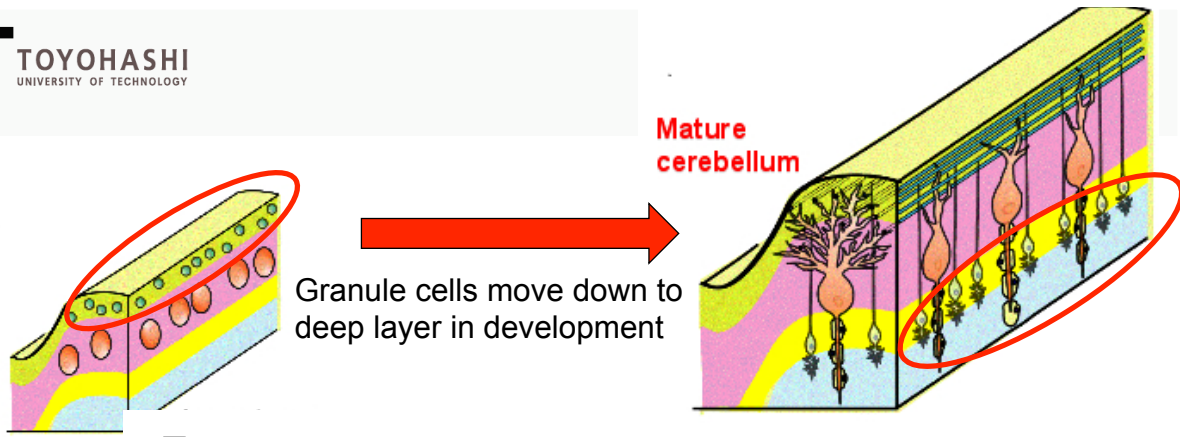
Acoustic image



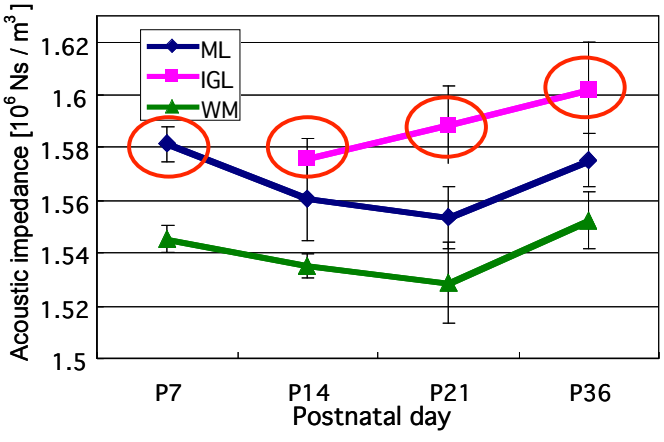
Acoustic impedance images　　1mm



High impedance region moves to deep layer

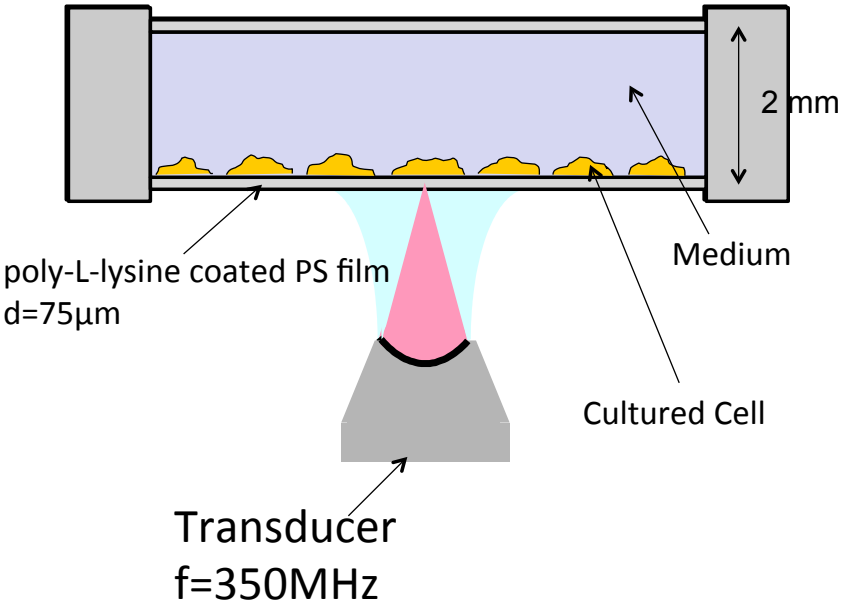


Granule cells move down to deep layer in development

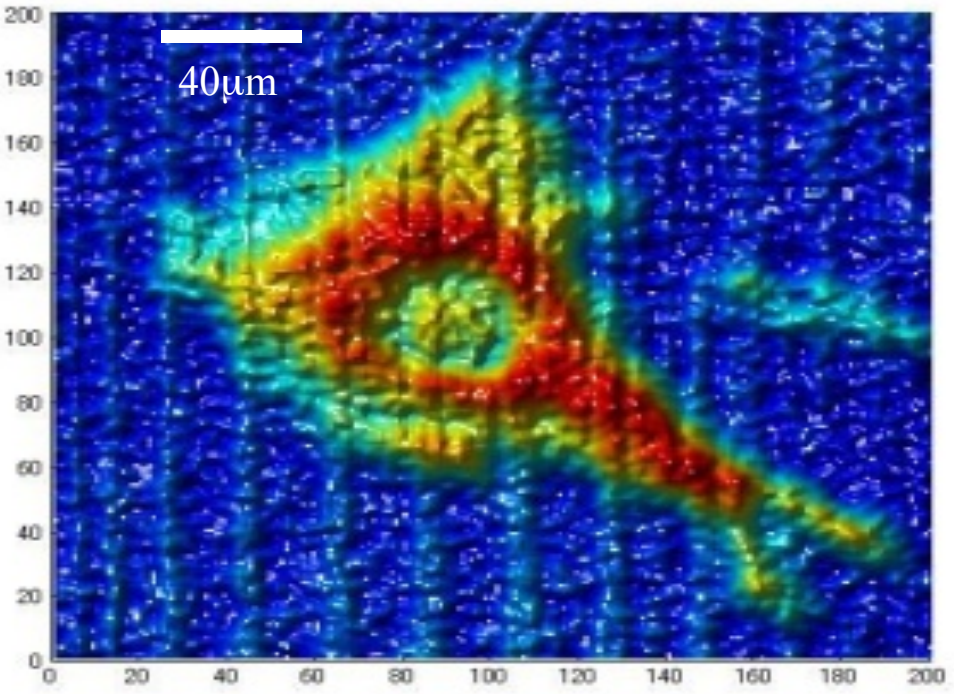


High impedance region moves to deep layer

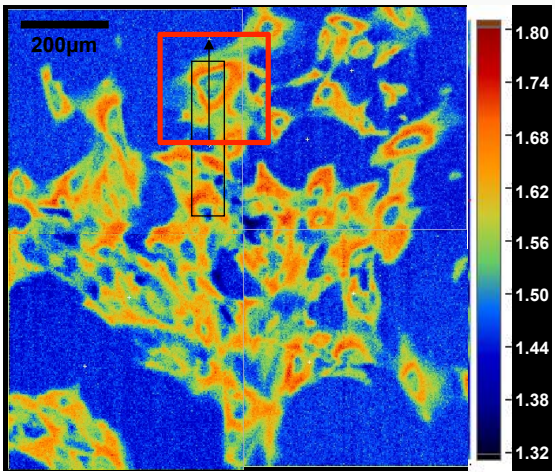
Subcellular observation

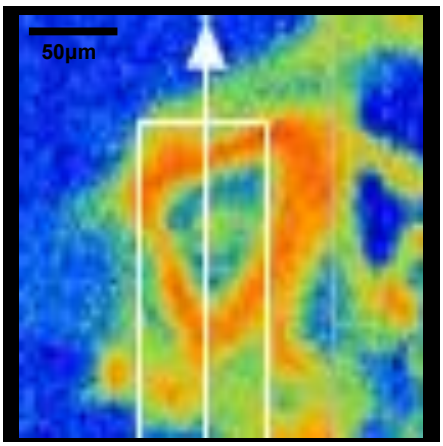


OptiCell

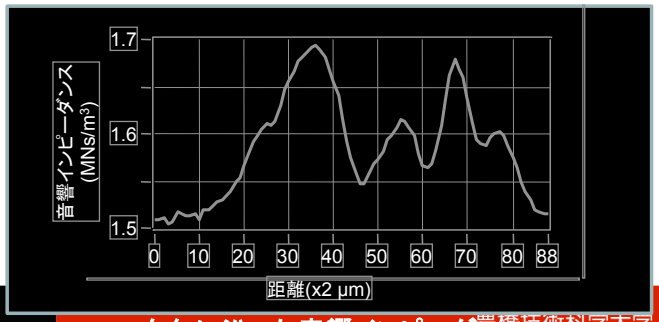


Acoustic image of a migrating glial cell

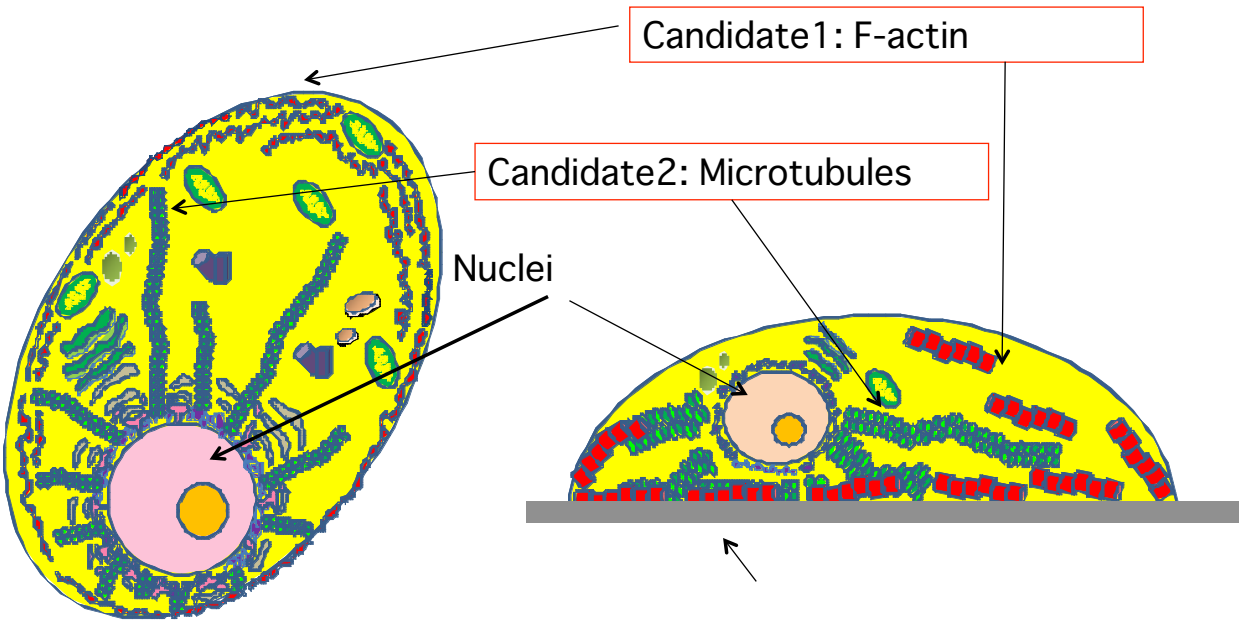


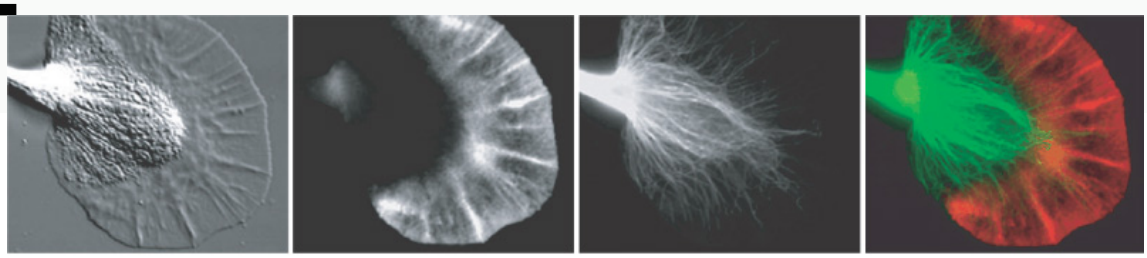


What is this organelle?

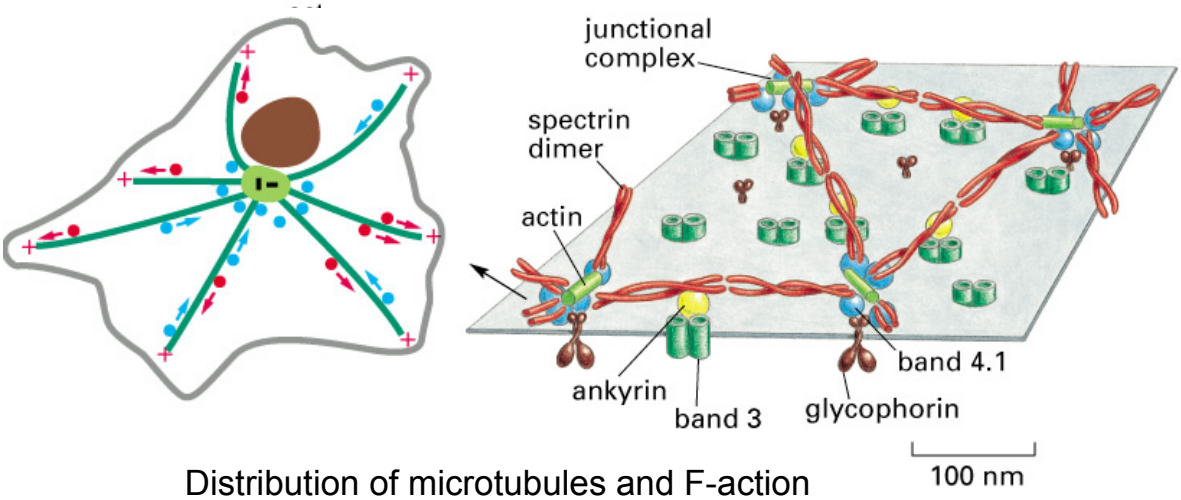


白矢に沿った音響インピーダンス





actin filament (From Paul Forscher) microtubules MERGED



Distribution of microtubules and F-action

Excerpt from Molecular biology of the Cell

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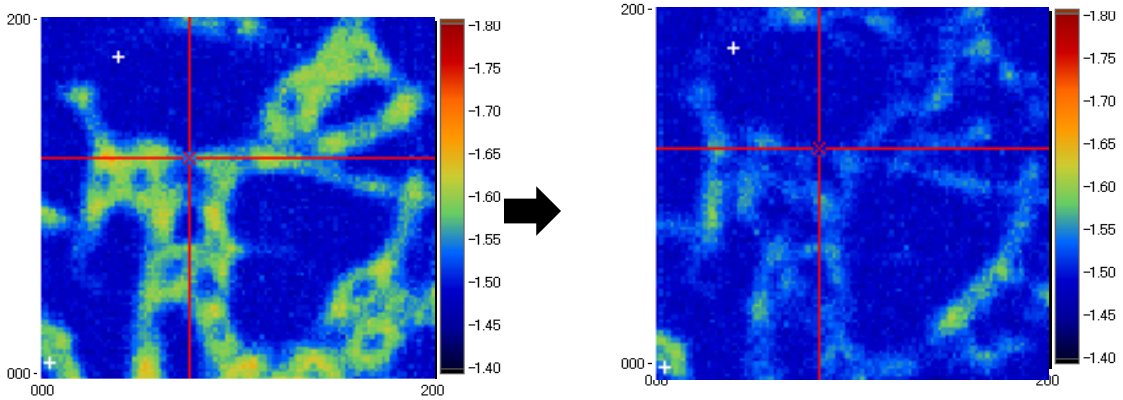
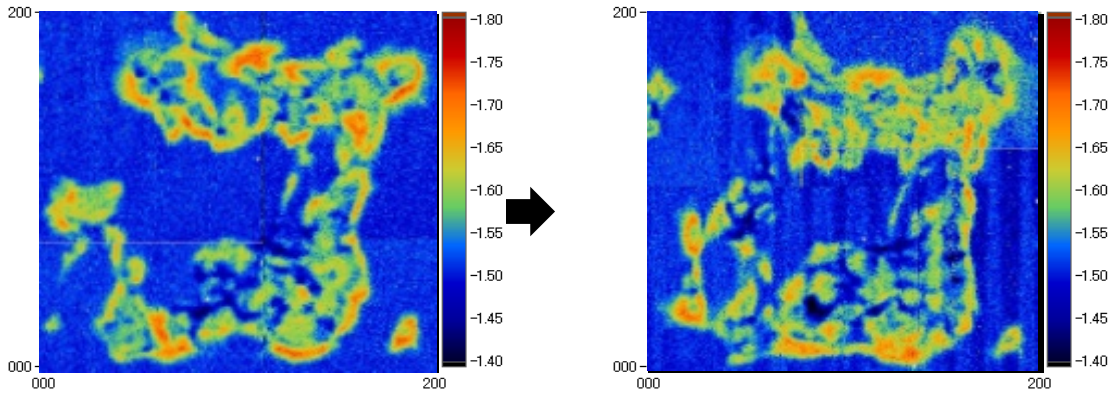


Living glioma

1 hr after Cytocharacin B application

Depolymerization of F-actin decreased intracellular impedance



Living astrocyte

1 hr after Demecolcin application

Depolymerization of microtubules had little effect to intracellular impedance

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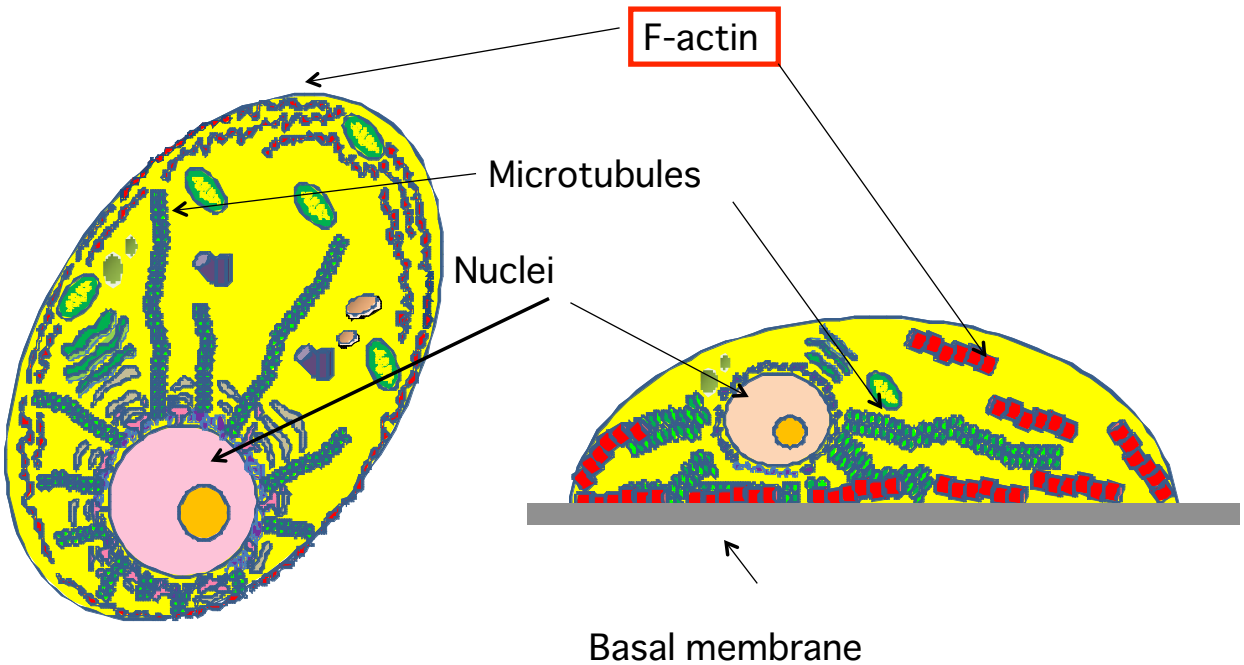
Result: Subcellular F-actin bundle is observed



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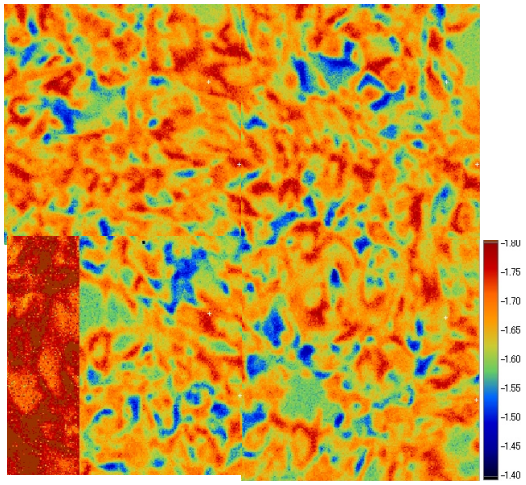
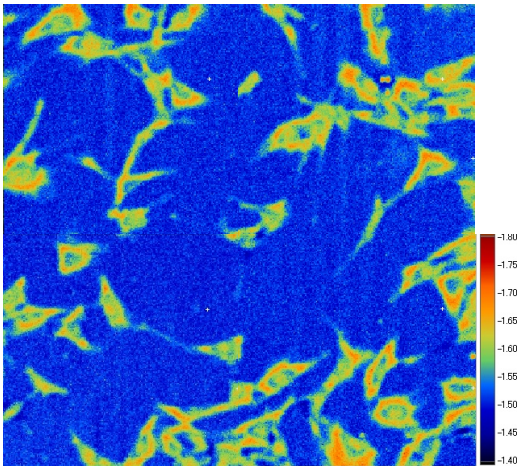
Identification of a specific type of cells



Wild type epidermal cells



Immortalized epidermal cells

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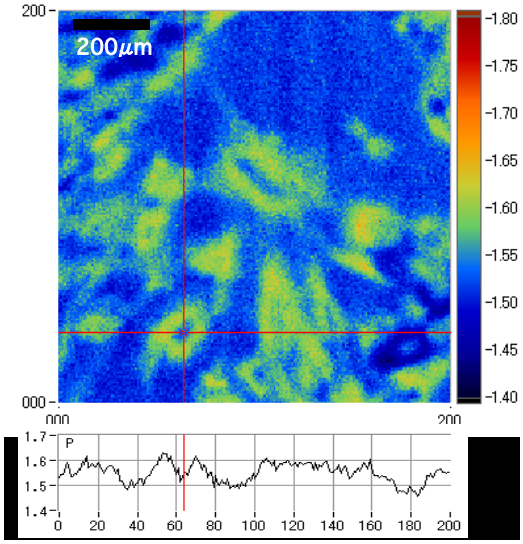
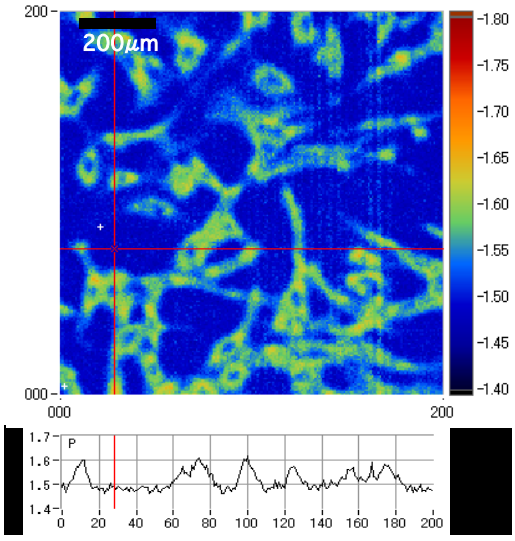
Astrocyte (normal cell)



Ave. of Impedance 1.63

C6 Glioma (cancer cell)



Ave. of Impedance 1.57

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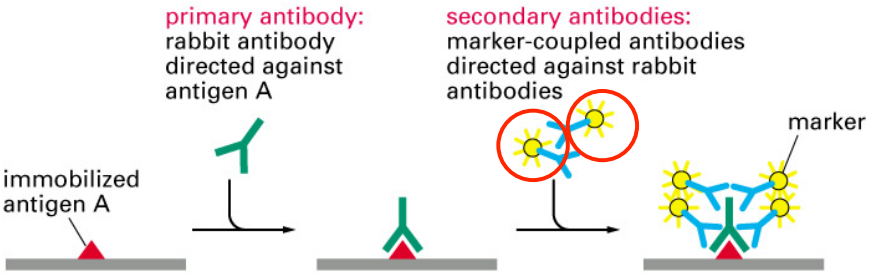
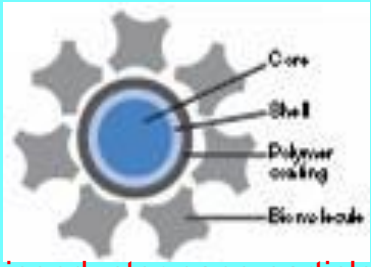
Experiment: **Acoustic immunohistochemistory** using Metal crystal



10~20nm diameter
core：semiconductor Cd
shell：Zinc sulfide
1 particle : 500 atoms



Q-Dot : Fluorescent heavy metal semiconductor nano particles

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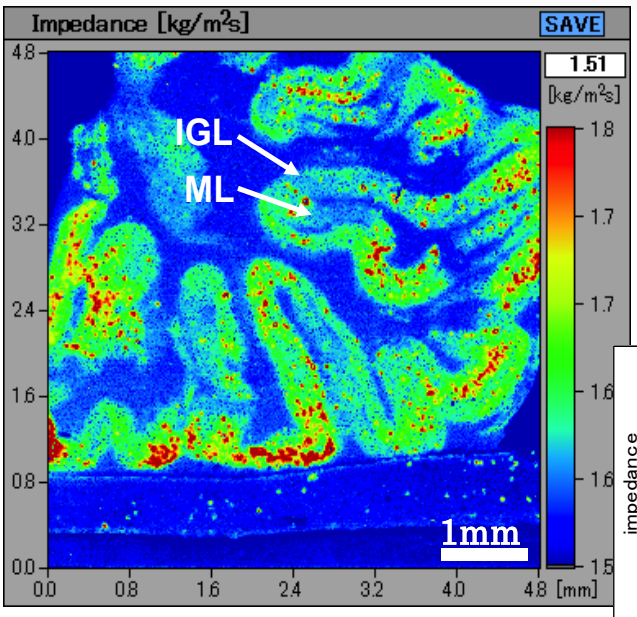
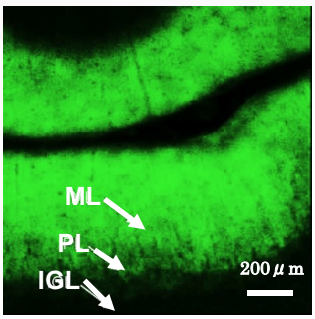
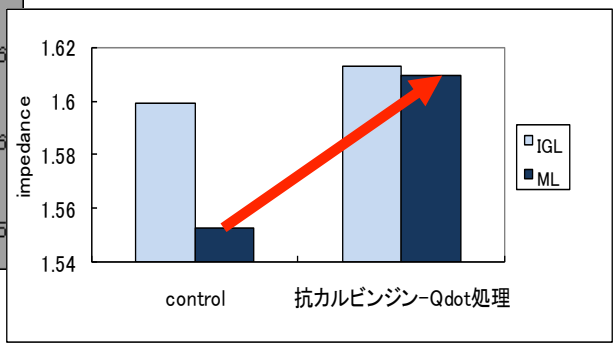
Primary antibody: Calbindin D28k



Acoustic staining image



Fluorescent image



Impedance was increased by acoustic immunostaining to much proteins.

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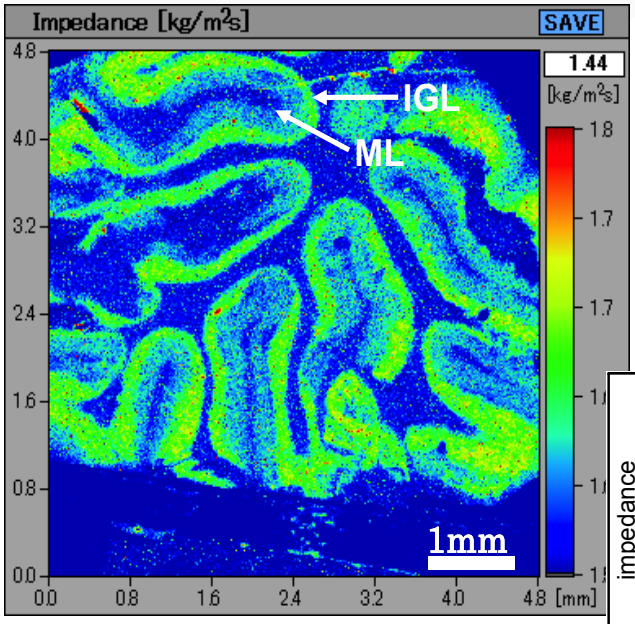
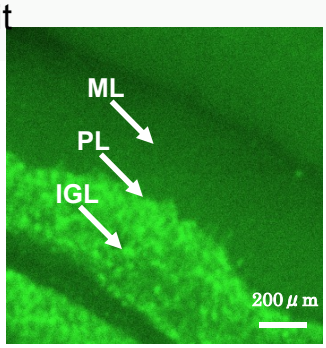
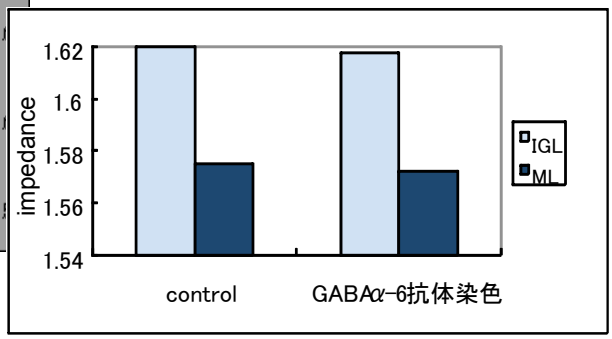
Primary antibody: GABARα6 subunit



Acoustic staining image



Fluorescent image



Impedance was not increased by acoustic immunostaining to less proteins.

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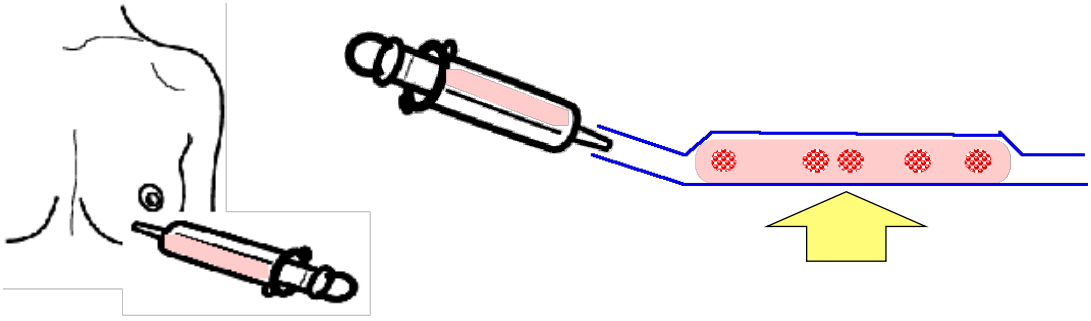
In the future

Identification of biopsied tumor organ with attenuation observation



Biopsied sample is inserted to film cartridge and observed the density of nuclei, or distribution of specific markers (model)

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Conclusions

- Middle level-reforming of substrate plate or film is effective to good acoustic impedance imaging.
- High freq. transducer makes cytoskeletal structure (F-action) visualized.
- Surface protein in living cells are marked with Qdot or some heavy metal.

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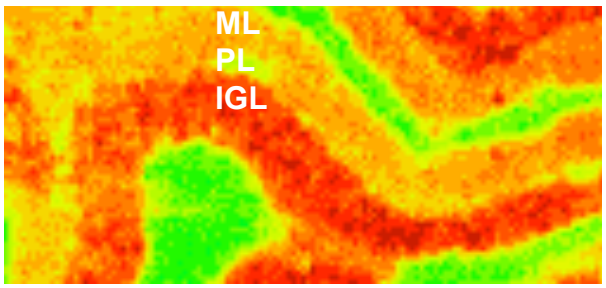
Thank you for your attention



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Hamamatsu University School of Medicine
Seiji Yamamoto

Toyohashi University of Technology
Many students

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