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2013.7.13 バイオ超音波顕微鏡研究会

# Acoustic staining

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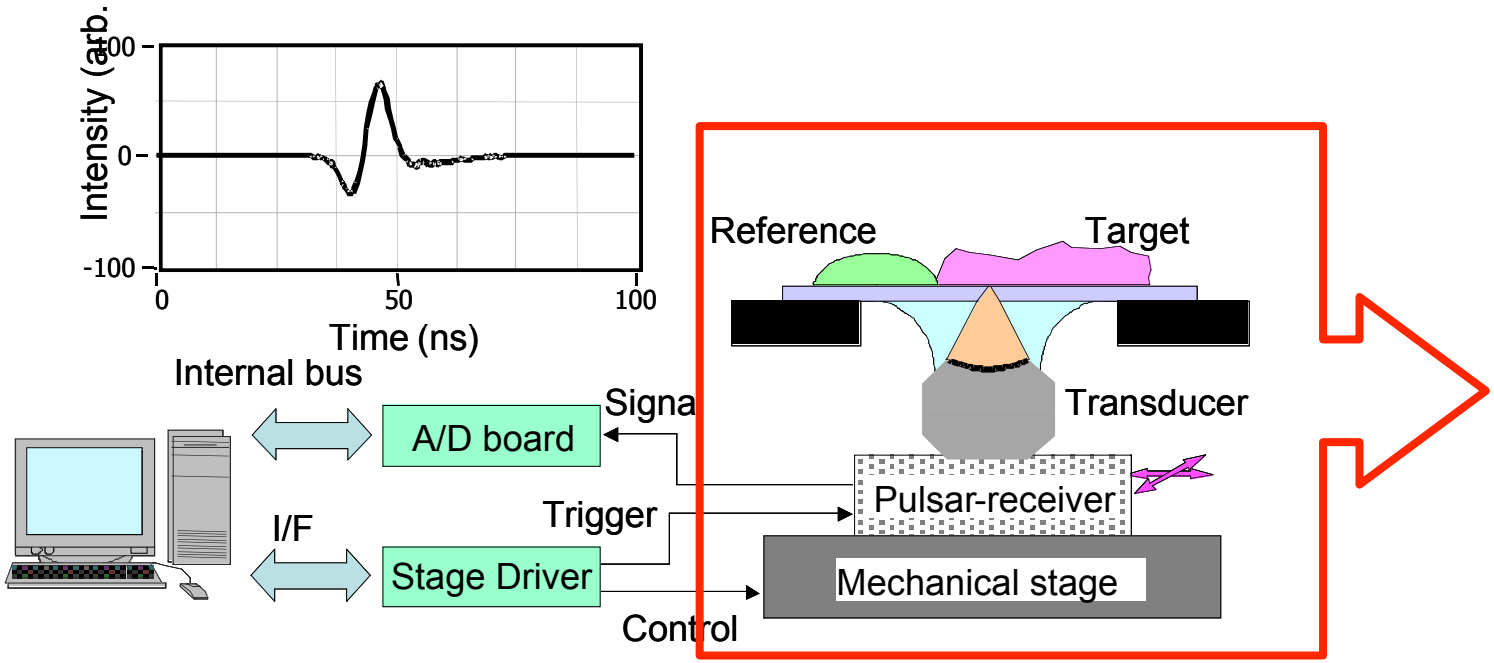
・音響インピーダンス顕微鏡とは何か

・音響インピーダンス顕微鏡で、生体を観察する

・音響染色の可能性

・音響インピーダンス顕微鏡の展開

# ・音響インピーダンス顕微鏡とは何か




・Radiation of ultrasonic wave through the polystyrene plate or film

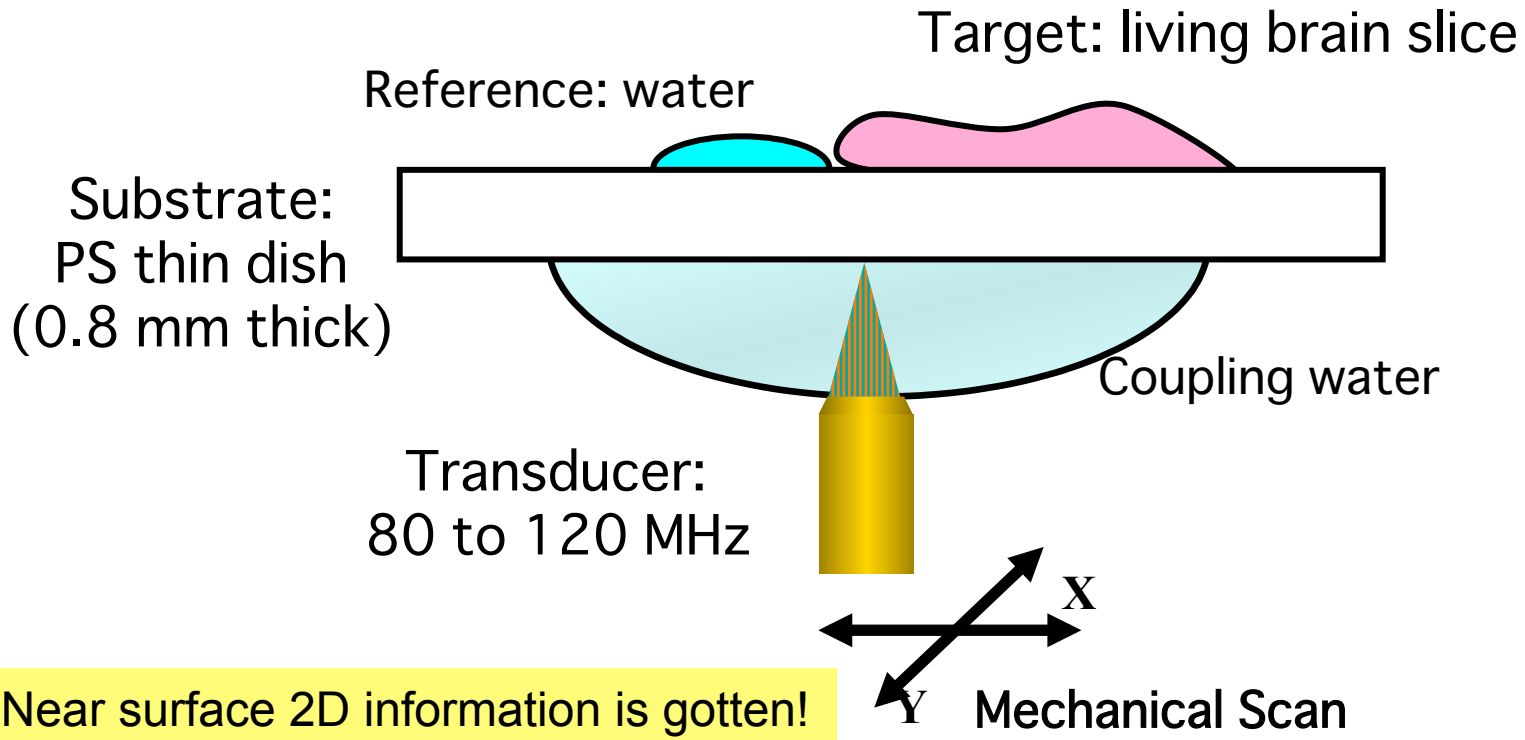
・Image processing of reflecting waves for minutes

・Reference material: water

Our targets: Living organs and cells

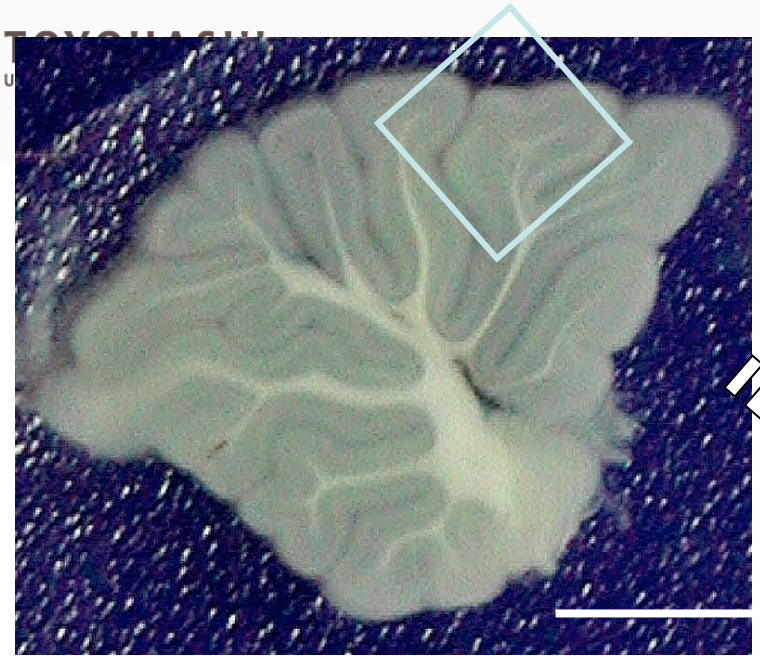
Materials: 400μm sliced rat cerebellar  cortex and cultured glial cells

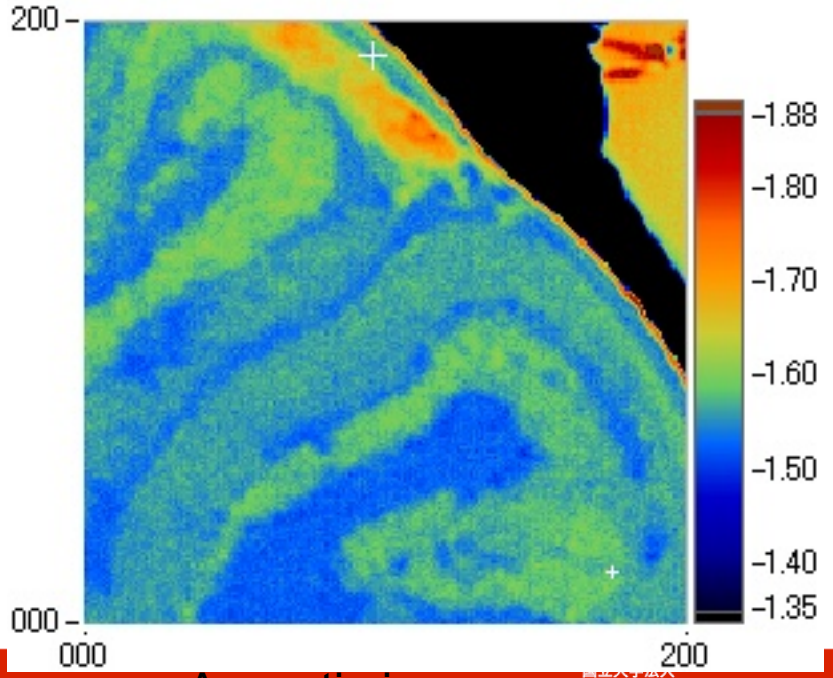
Methods: Acoustic impedance microscopy




Near surface 2D information is gotten!

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






Optical image

Acoustic image

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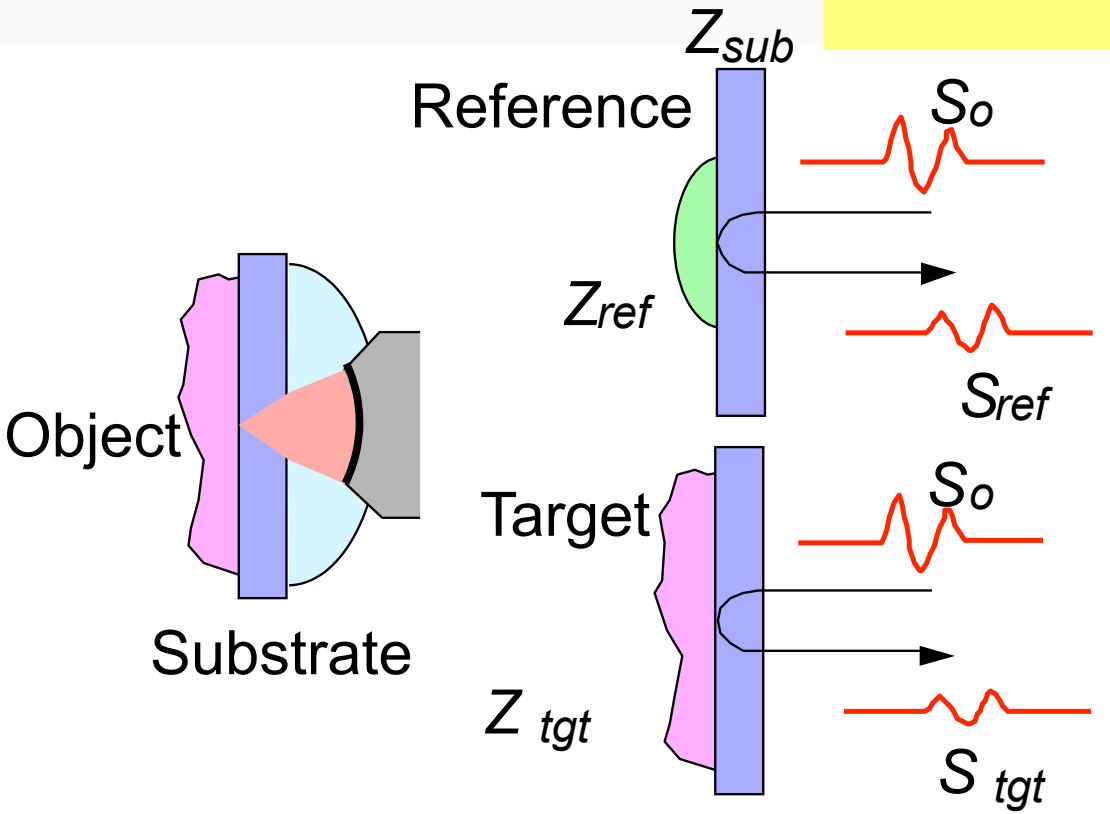
# Transformation of reflecting signal intensity into acoustic impedance



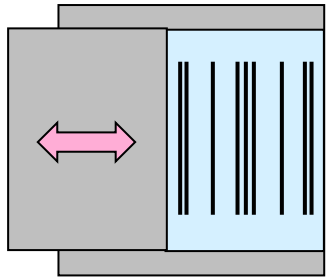

$$S_{ref} = \frac{Z_{ref} - Z_{sub}}{Z_{ref} + Z_{sub}} S_0$$

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

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

$$c_p = \sqrt{(K + \frac{4}{3}G)/\rho},$$

$$Z_p = \rho c_p \quad K >> G$$



気体・液体
・固体

*Z*: 特性音響インピーダンス
*c*: 音速　*ρ*: 密度
*K*:体積弾性率
*p*: 縦波(pressure wave)

| | |
|---|---|
| 水　（リファレンス物質） | 1.5 |
| 培養培地　（NaCl 0.8%） | 1.52 |
| 1.0M $ZnSO_4$含培養培地 | 1.78 |
| 400μM $ZnSO_4$含培養培地 | 1.53 |
| 5% NaCl水溶液 | 1.59 |

インピーダンスを0.1上昇させるには、62.5g/Lの密度上昇が必要

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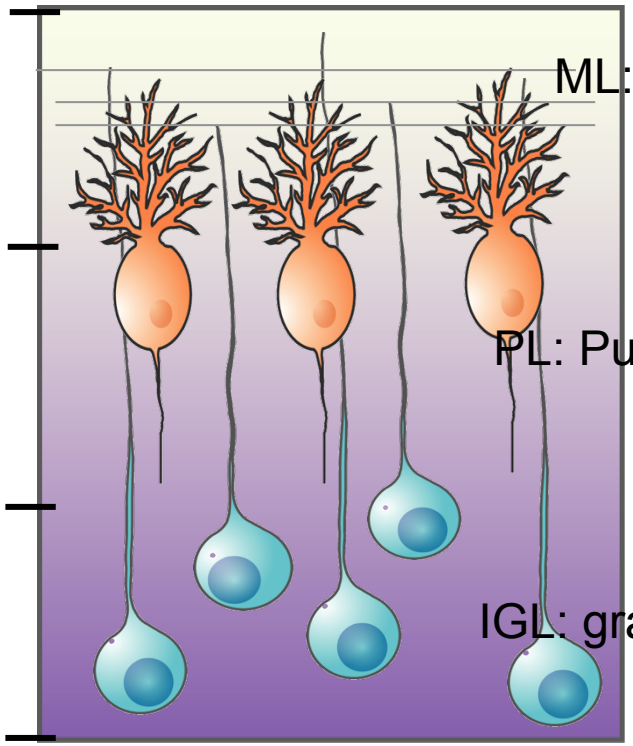
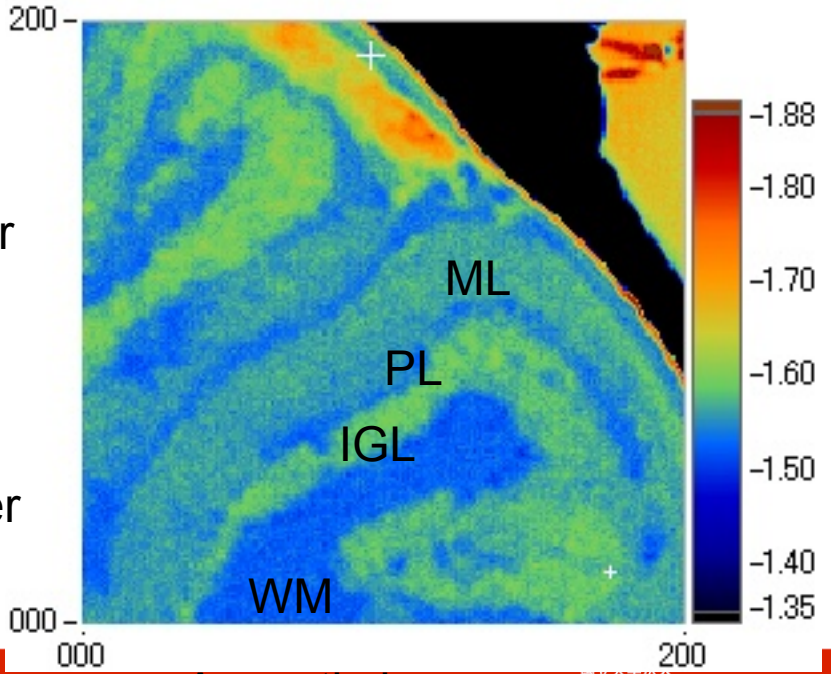
# ・音響インピーダンス顕微鏡で、生体を観察する

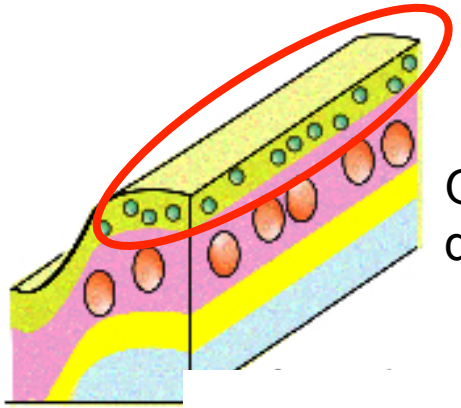
| | |
|---|---|
| Nuclei-rich layer: | high (>1.58) impedance |
| Cytoskeleton-rich layer: | middle (≅1.56) impedance |
| Lipid-rich layer: | low (<1.54) impedance |



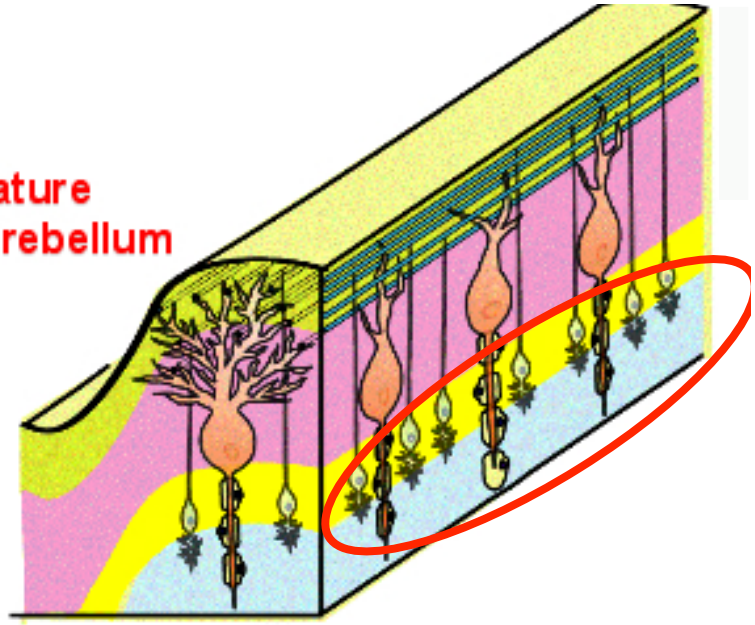

Optical image

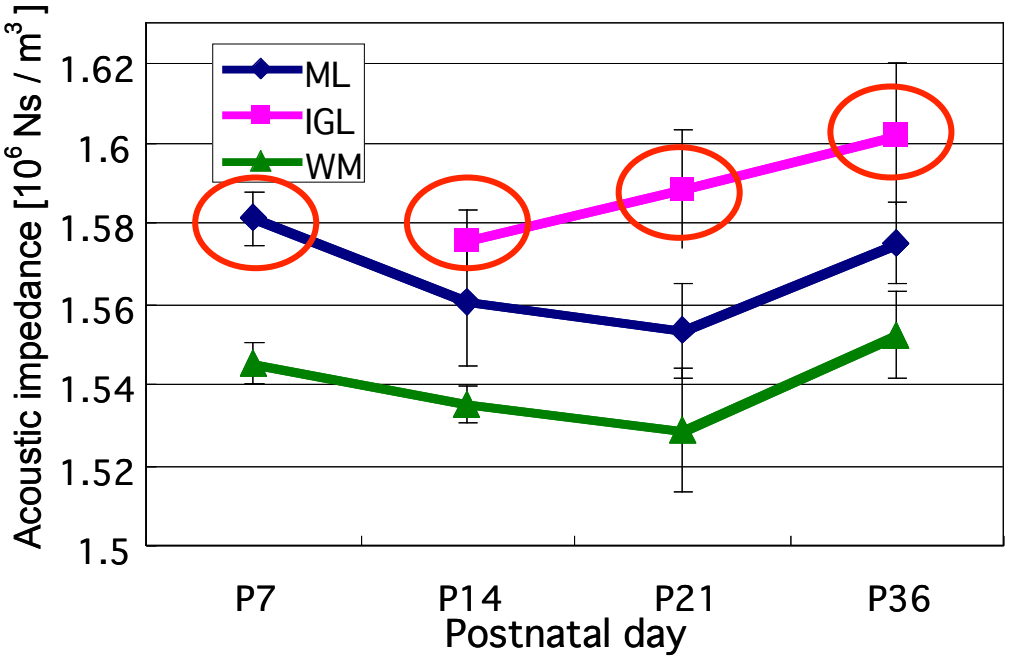
Acoustic image



Granule cells move down to deep layer in development

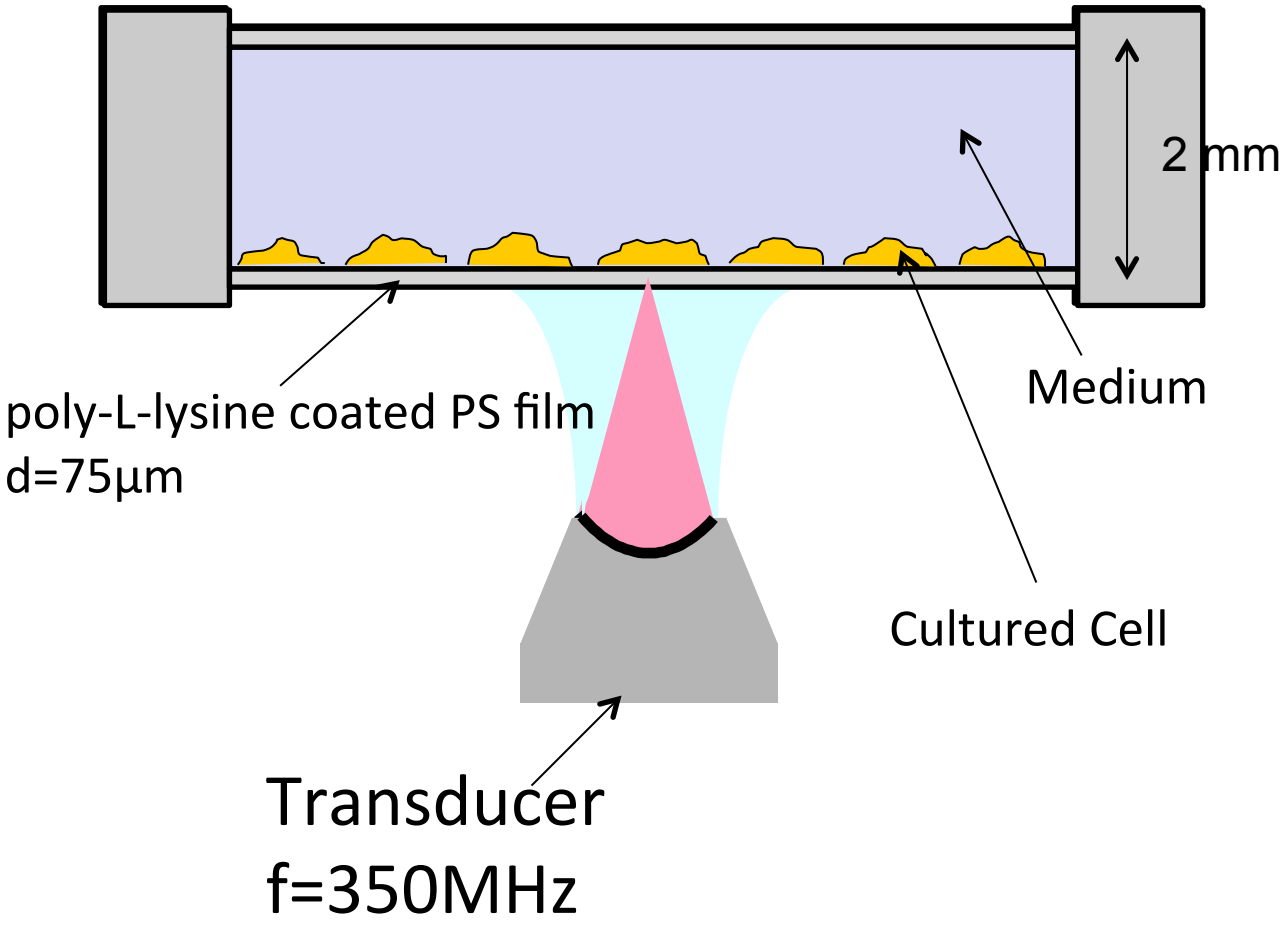
Mature cerebellum



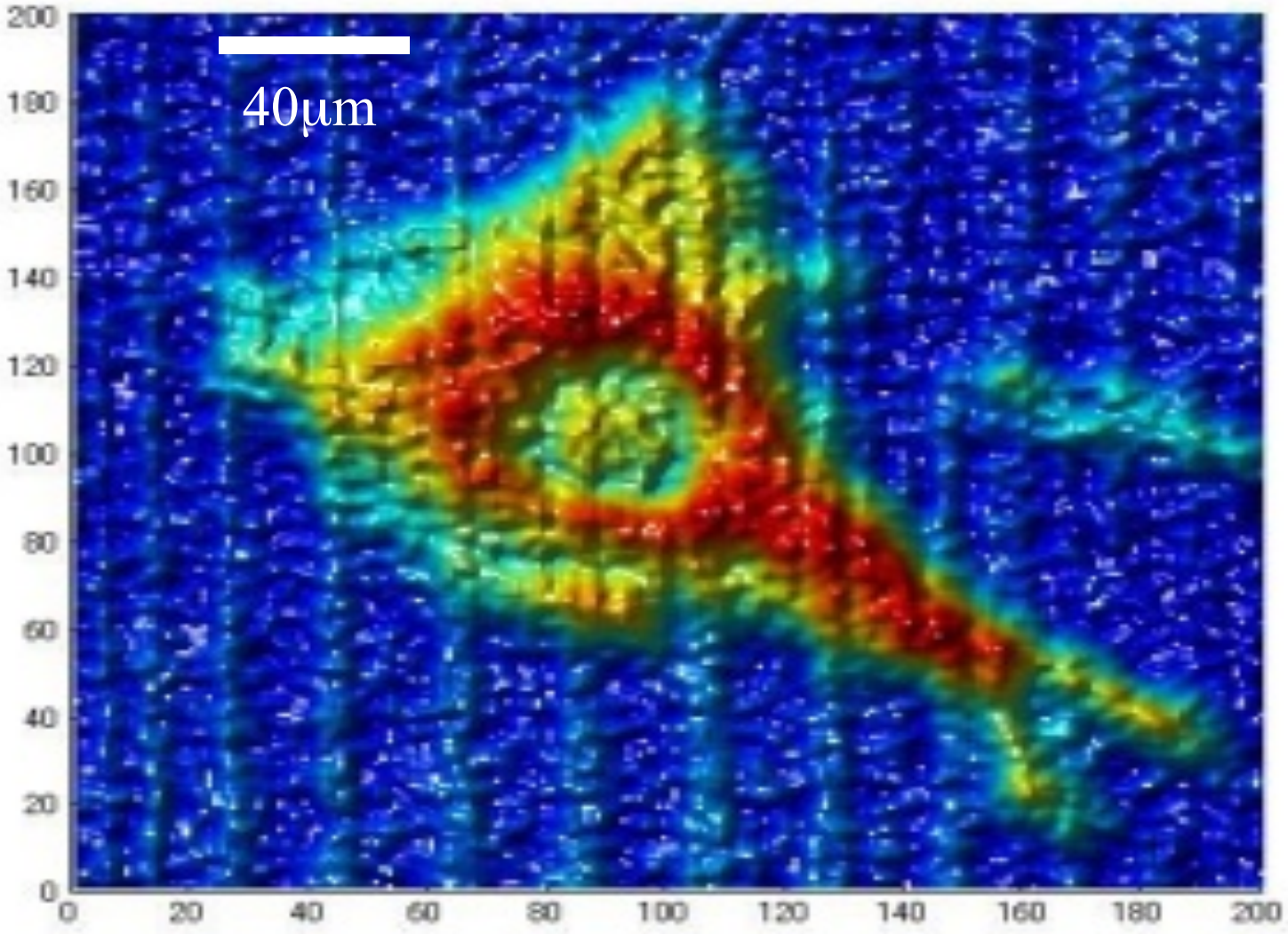



High impedance region moves to deep layer

# Subcellular observation




OptiCell




Acoustic image of a migrating glial cell

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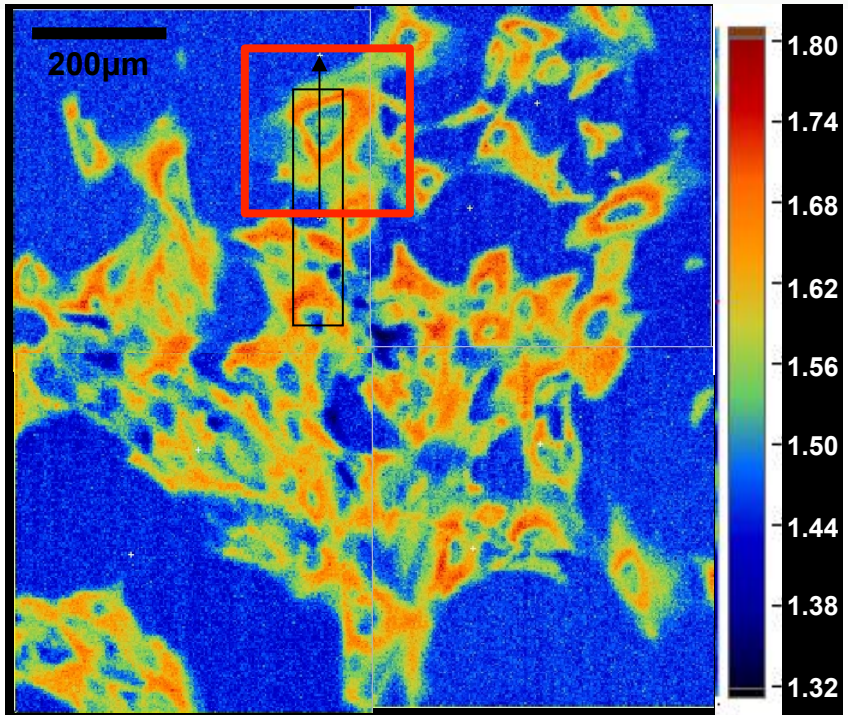
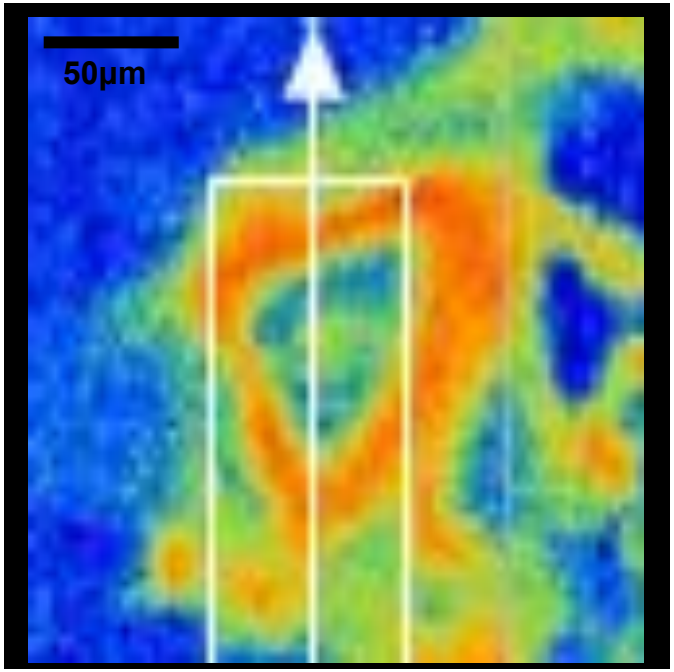
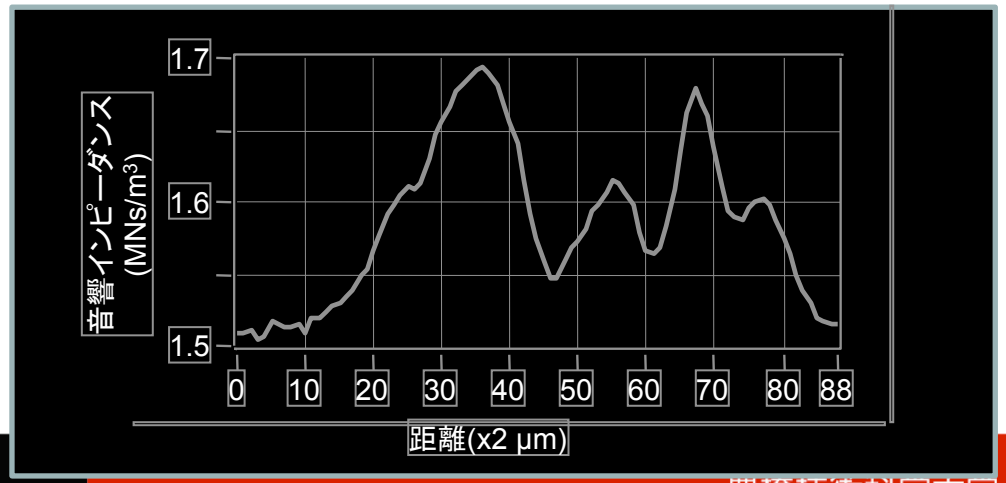
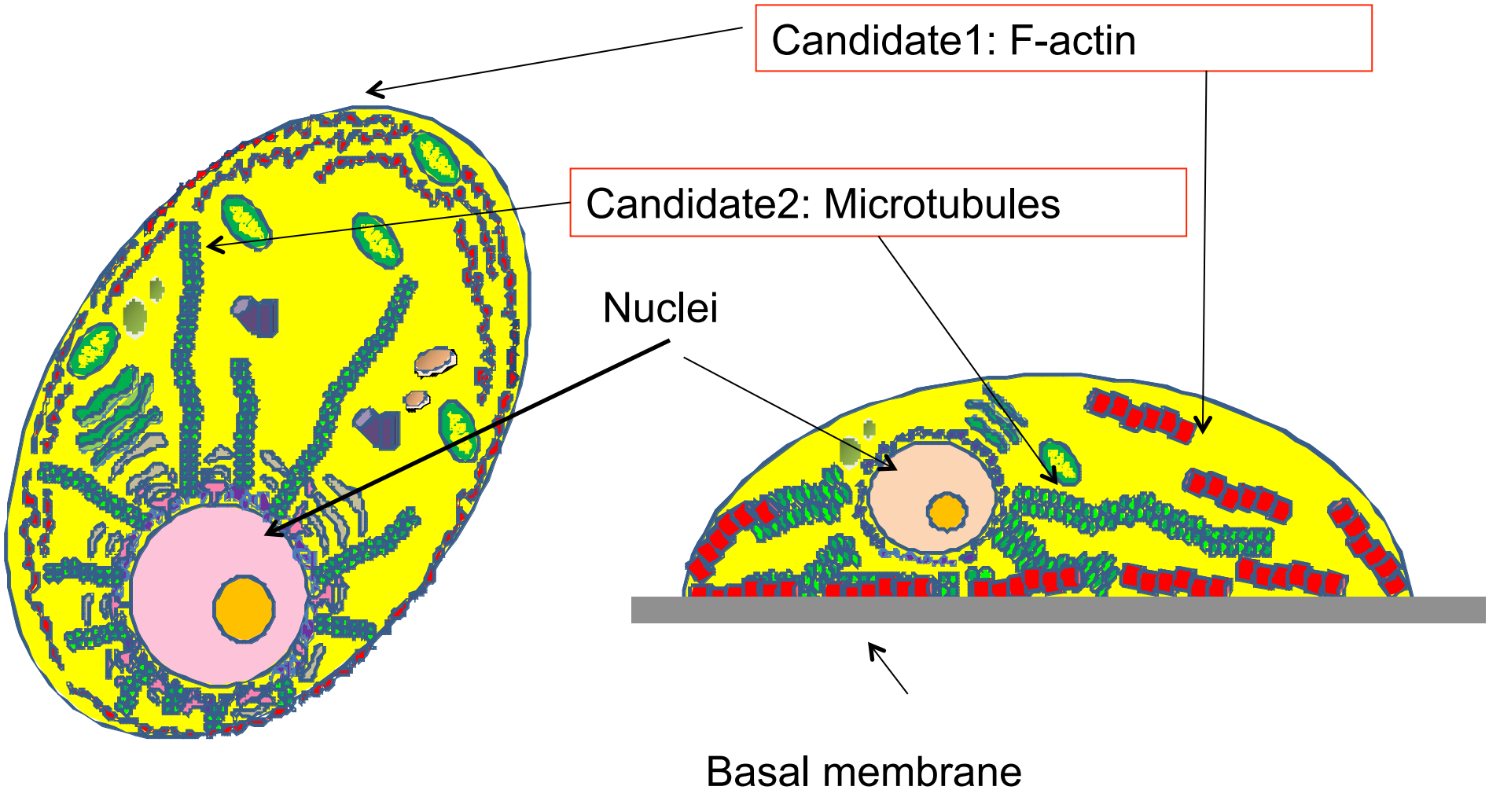
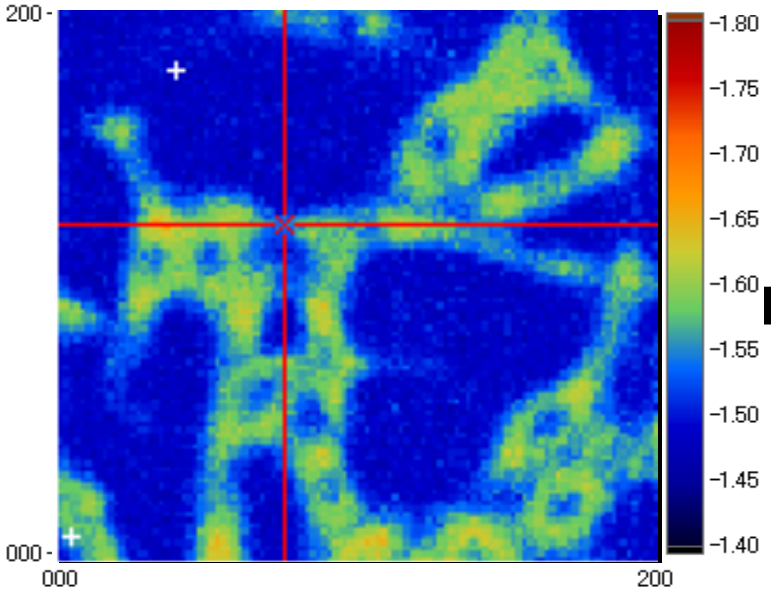


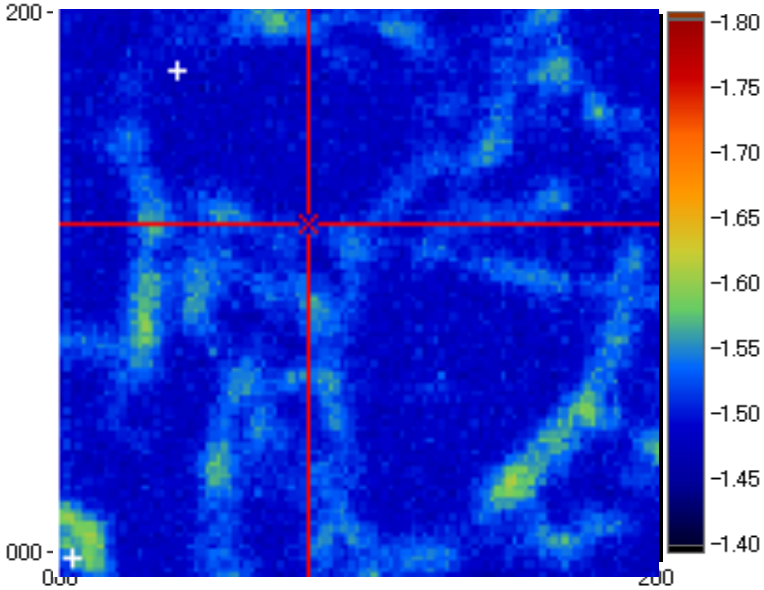

What is this organelle?




Candidate1: F-actin
Candidate2: Microtubules
Nuclei
Basal membrane

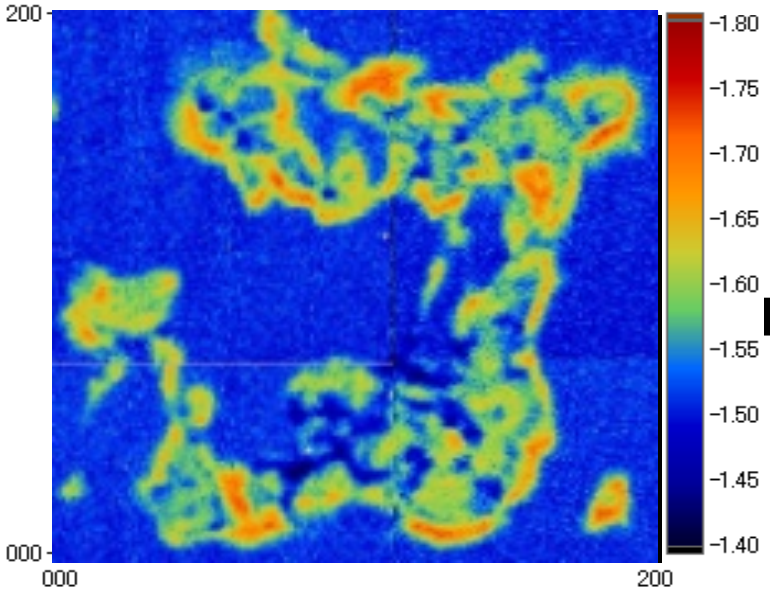



Living glioma

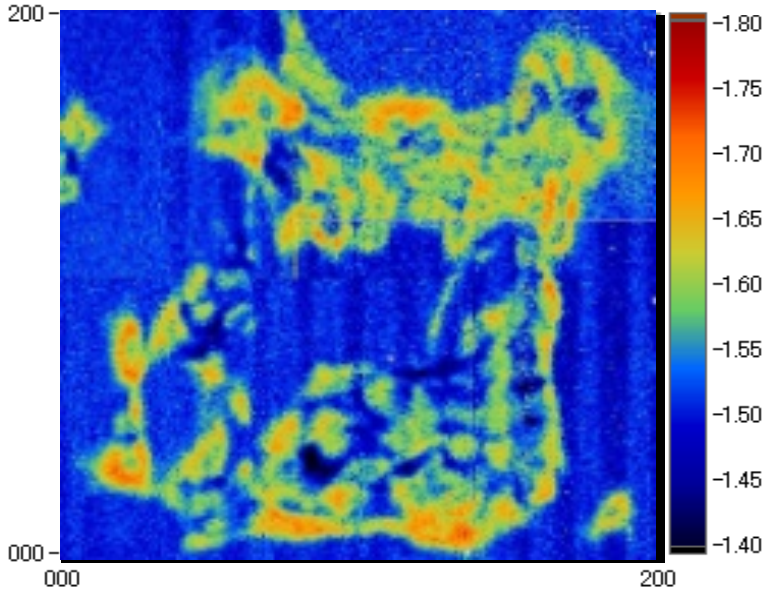



1 hr after Cytocharacin B application

Depolymerization of F-actin decreased intracellular impedance



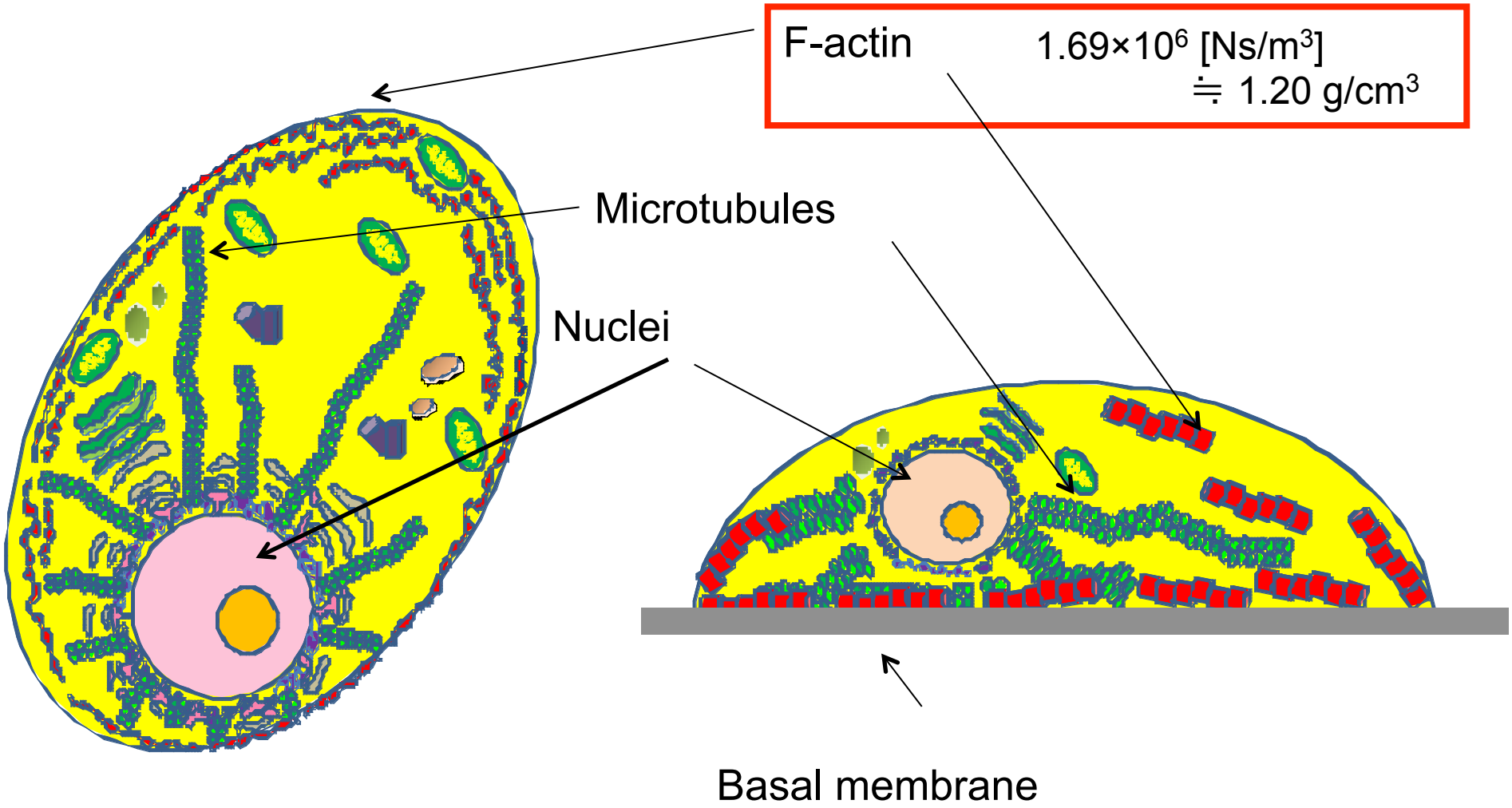

Living astrocyte



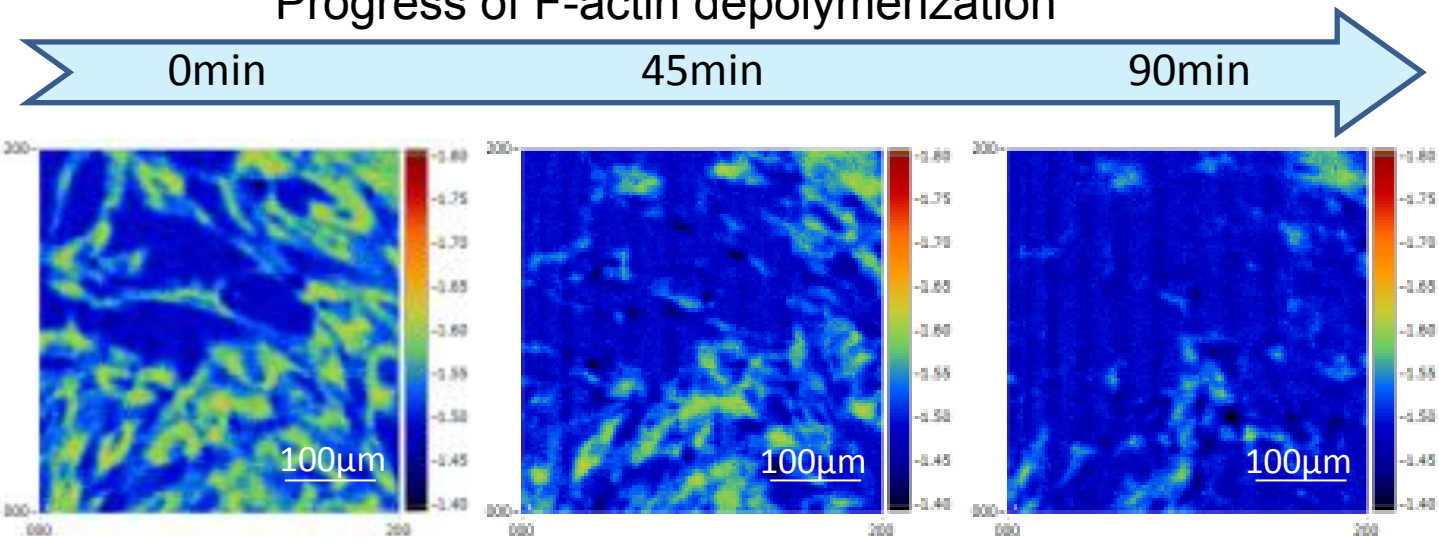

1 hr after Demecolcin application

Depolymerization of microtubules had little effect to intracellular impedance

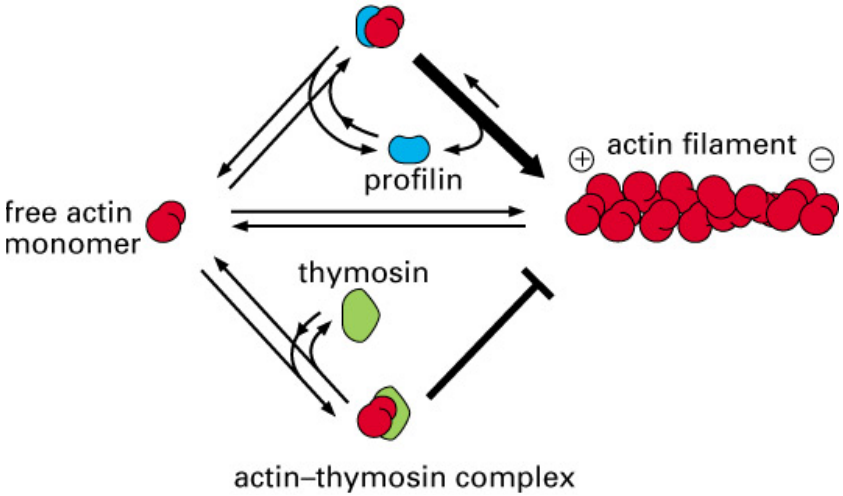
# result: Subcellular F-actin bundle is observed



## Progress of F-actin depolymerization




Cytoplasm: 1.63 → 1.58




Current densities could be estimated on the impedance

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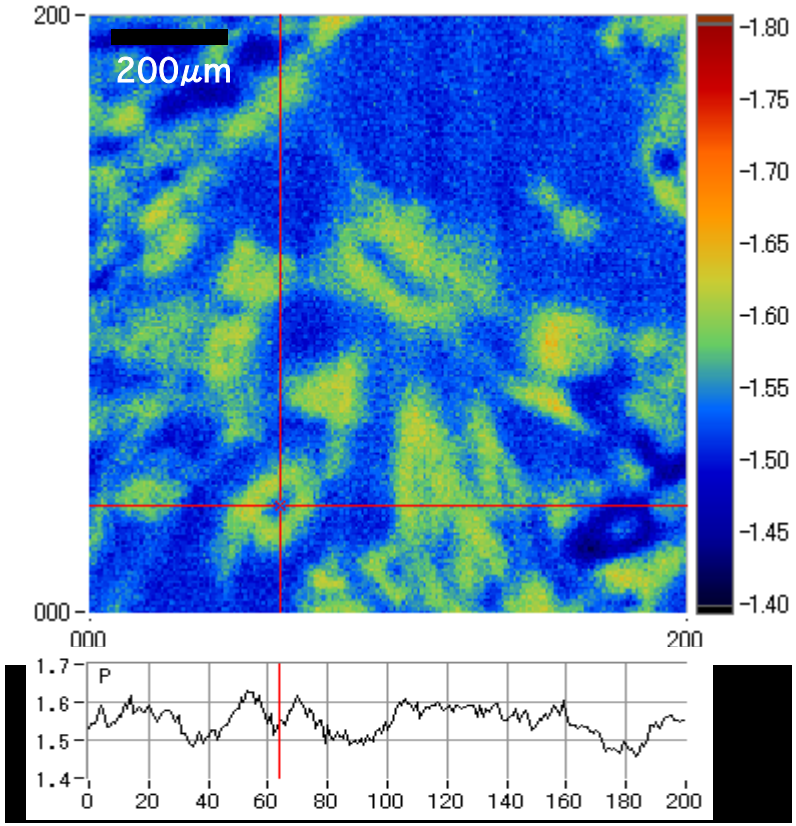
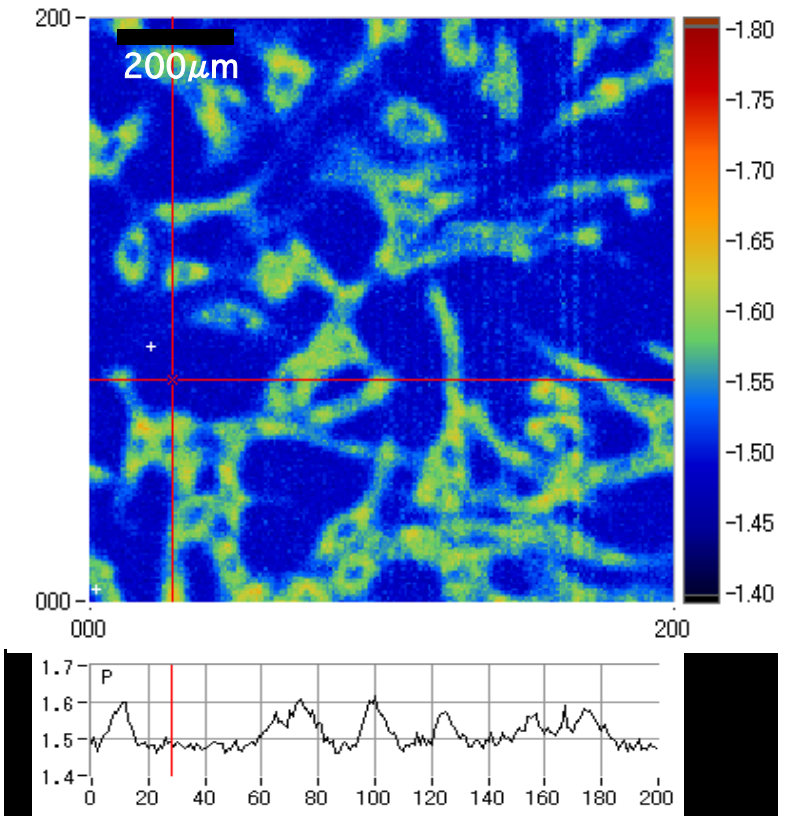
# Identification of a specific type of cells is difficult with no staining

Astrocyte (normal cell)




Ave. of Impedance 1.63

C6 Glioma (cancer cell)




Ave. of Impedance 1.57

・音響染色の可能性

$$c_p = \sqrt{(K + \frac{4}{3}G)/\rho},$$

$$Z_p = \rho c_p$$

密度の特異的増大による染色

Acoustic staining

1. Physiological heavy metal absorption

2. Immunohisitological metal binding

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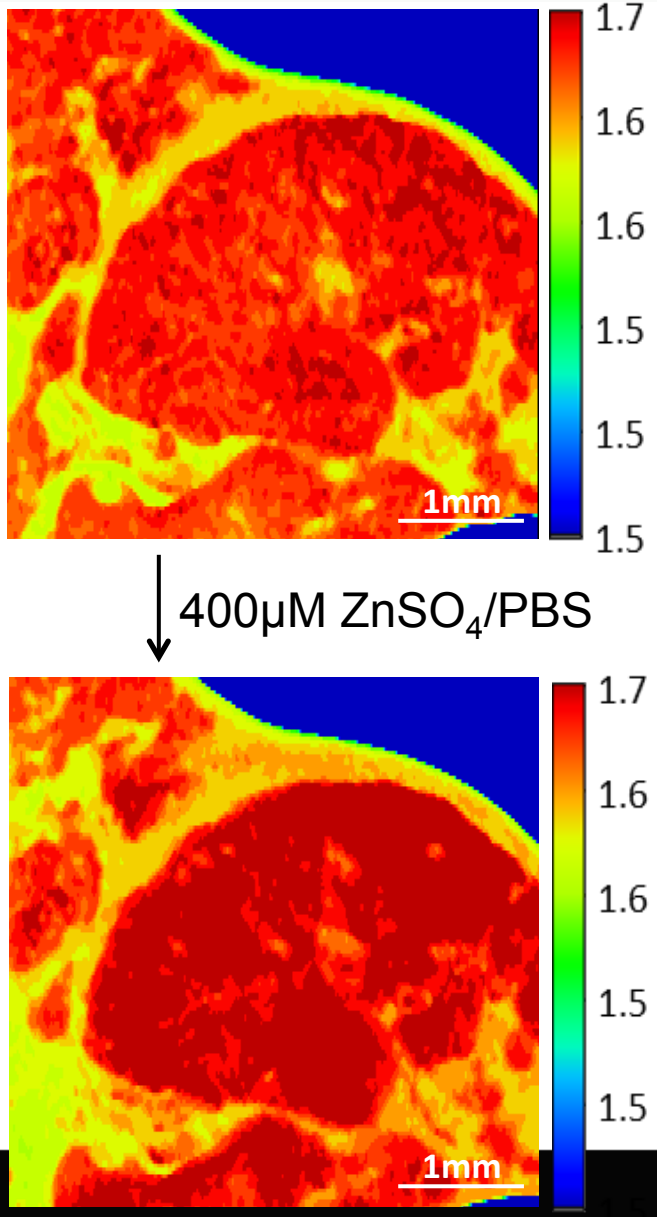
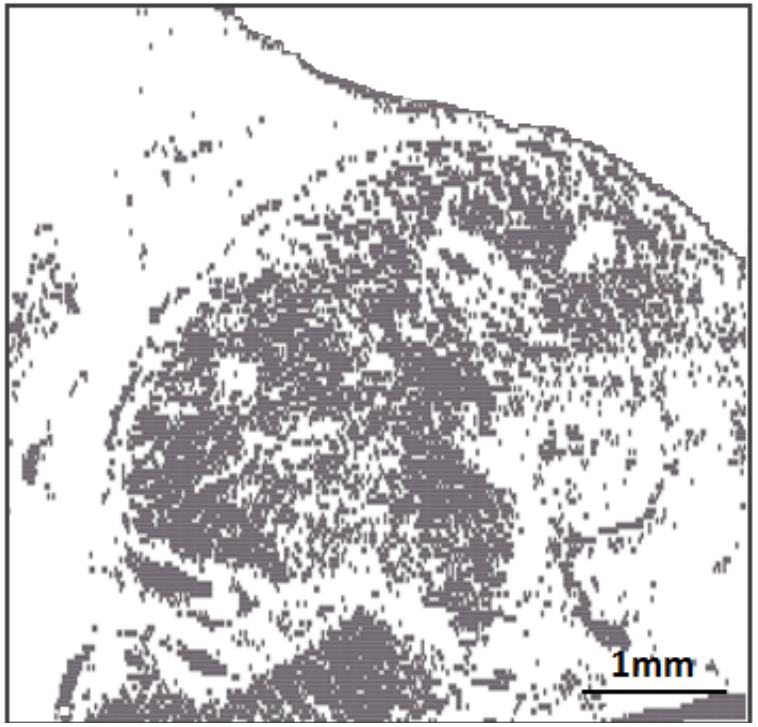
# Zn application to mouse hepatoma




The impedance elevated points
(0.01 -0.5)

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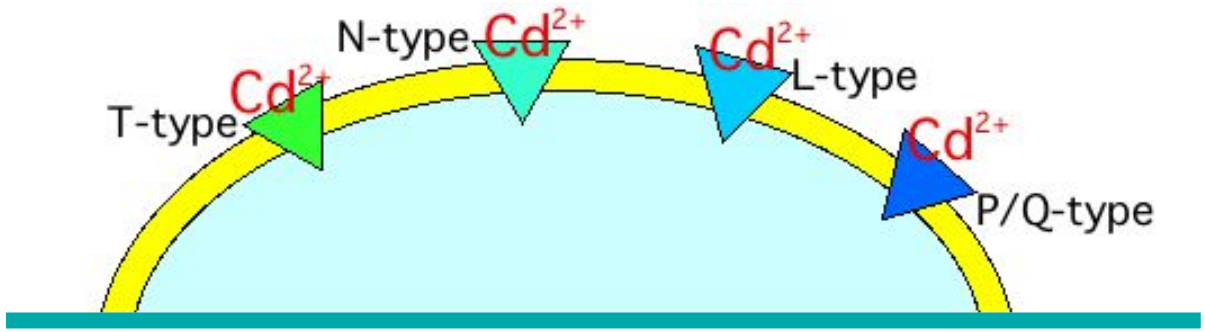
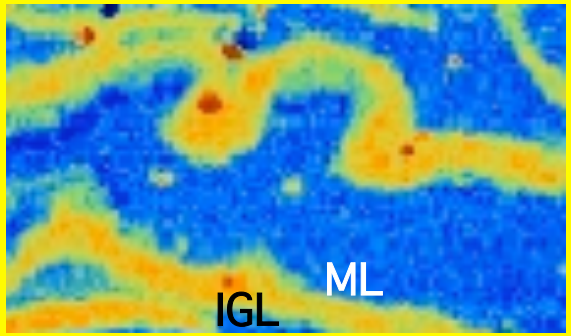
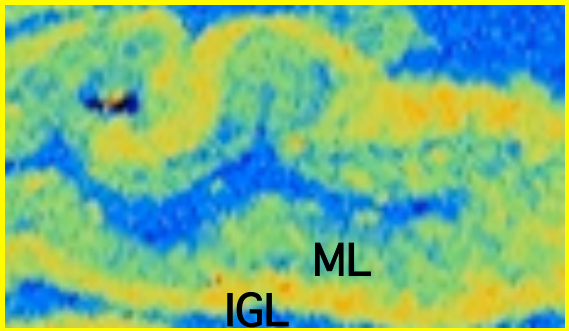
## **Heavy metal absorption** to $Ca^{2+}$ channel



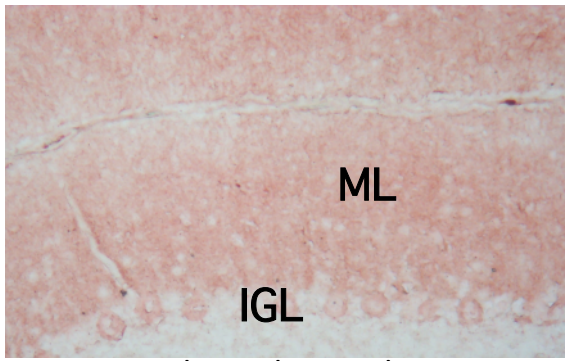




HEPES condition at P21



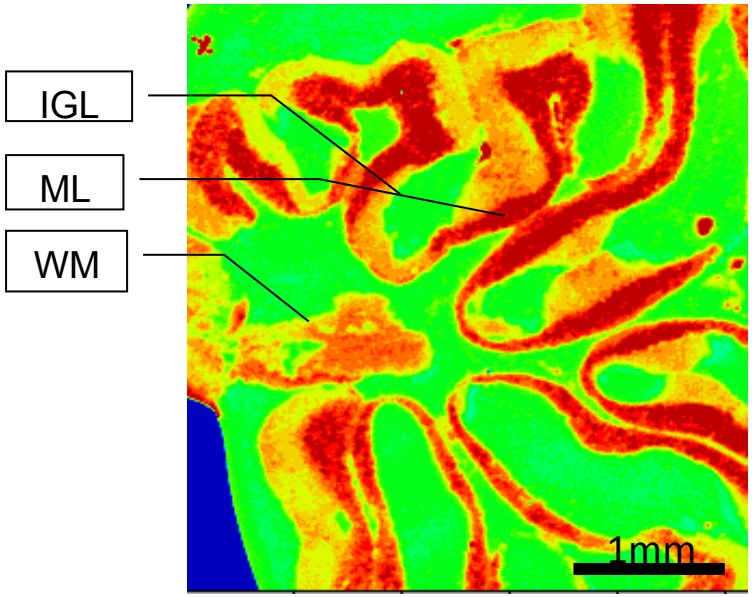

$Cd^{2+}$ condition at P21

Calcium channel proteins of Cerebellar neurons were treated by 100 μm $Cd^{2+}$, or 50 μm $Ni^{2+}$

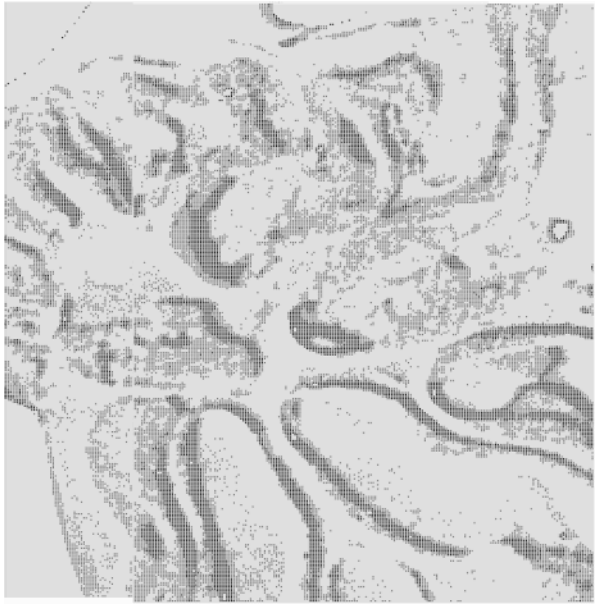



Immunohistochemical staining

# **Heavy metal absorption** to $Ca^{2+}$ channel




音響インピーダンス像



音響インピーダンス増加エリア

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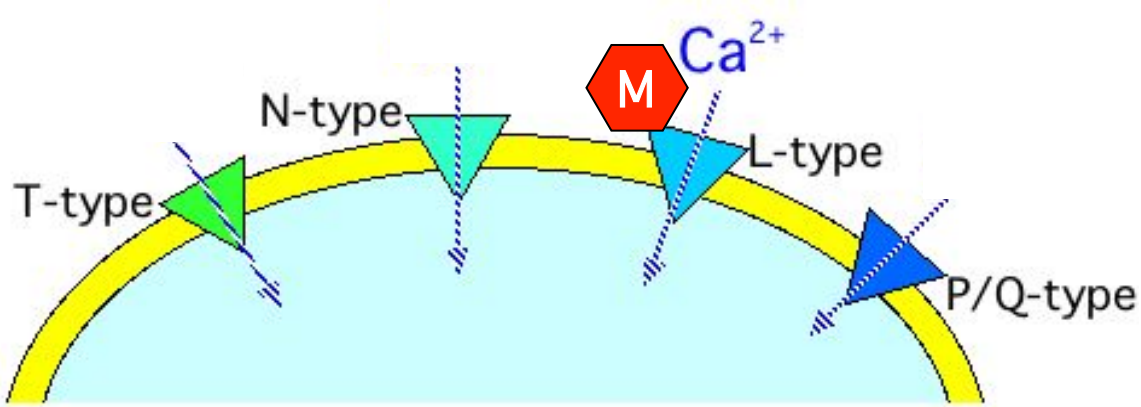
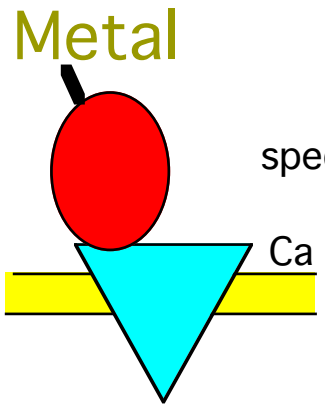
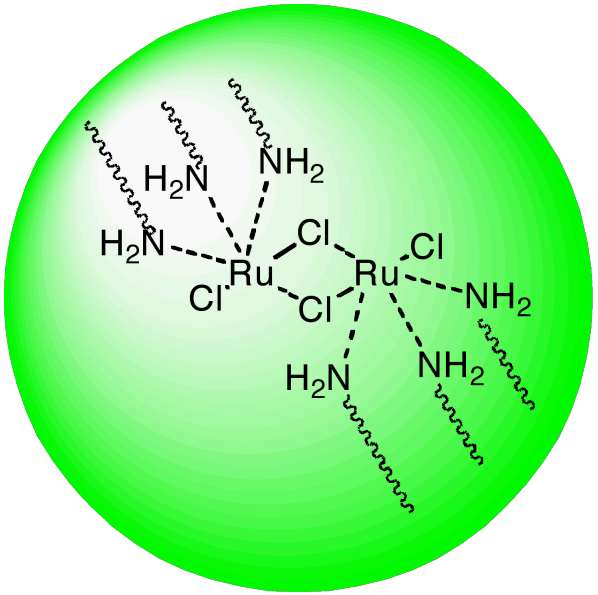
# Construction of specific **acoustic 'dye'** for $Ca^{2+}$ channel using heavy metal complex




Ca channel-binding polypeptide

*p*-cymene Ru (II) chloride

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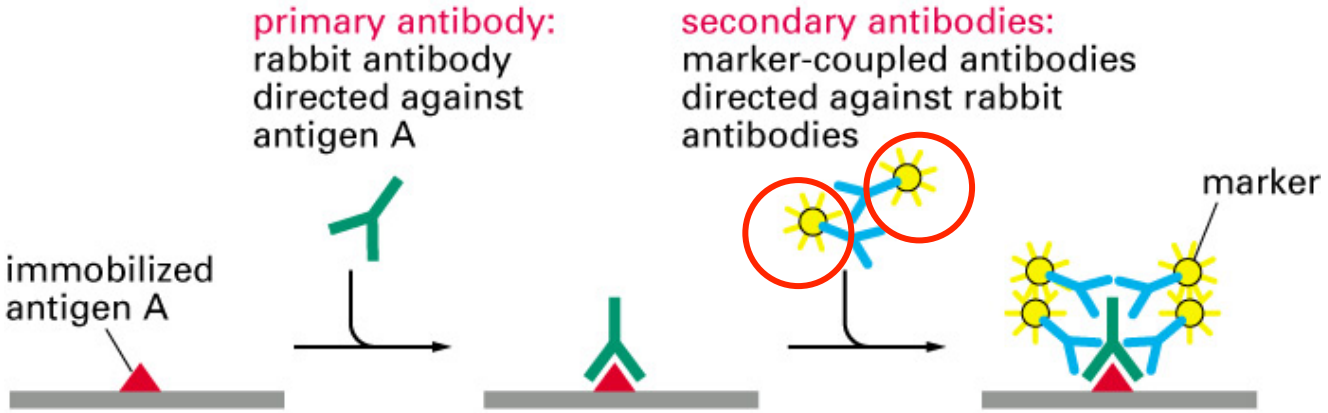
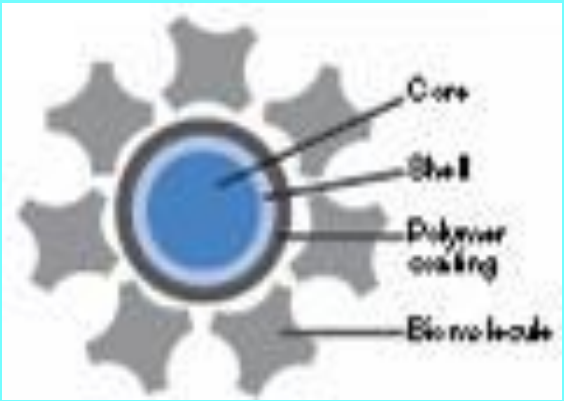
# **Acoustic immunohistology** using Heavy metal crystals




## Qdot : Fluorescent heavy metal semiconductor nano particles




Φ:10~20nm
Core：Cd
shell：Zinc sulfide
1 particle : 500 atoms

4th

## Immunogold : Gold colloid with antibodies.

Φ:10~40nm
Core：Au
1 particle : 5000-200000 atoms

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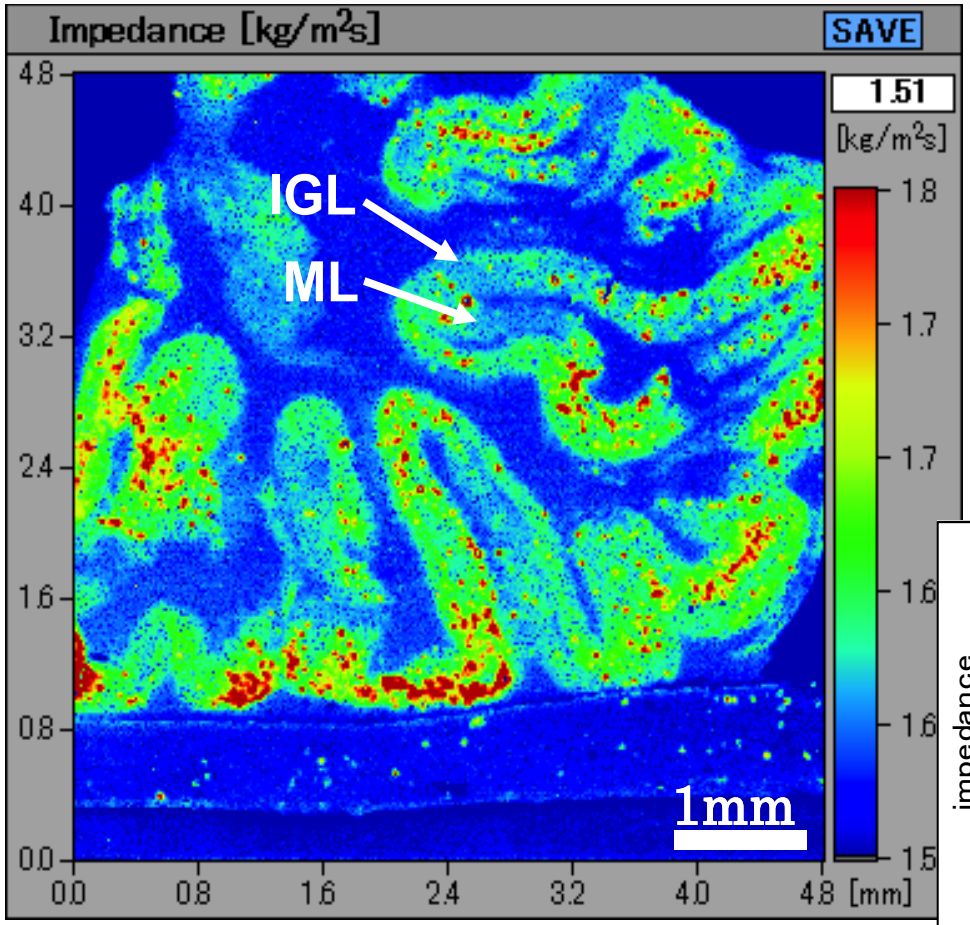
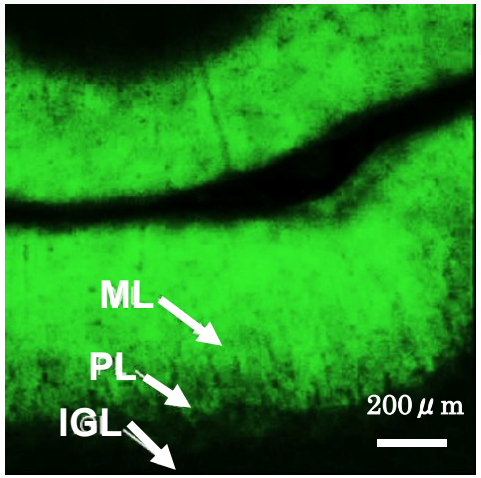
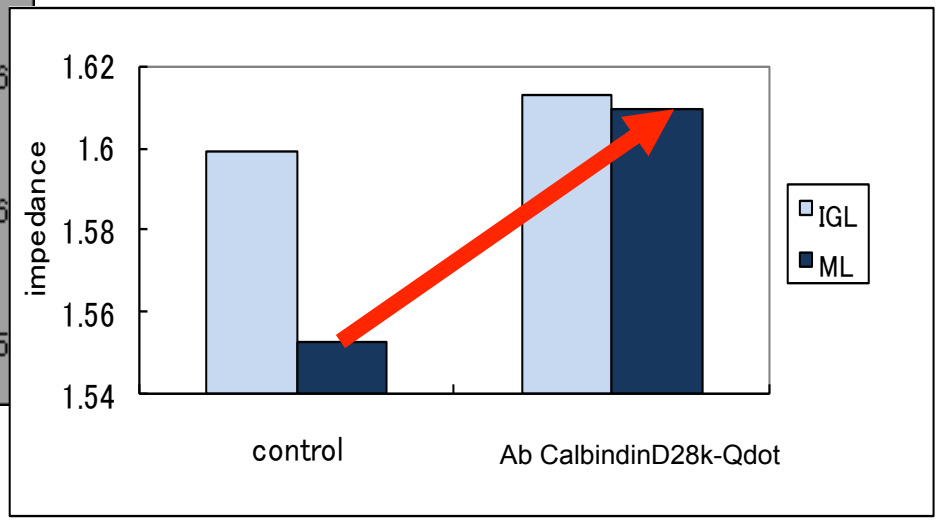
# Primary antibody: Calbindin D28k




Acoustic staining image




Fluorescent image




Impedance was increased by acoustic immunostaining to much proteins.

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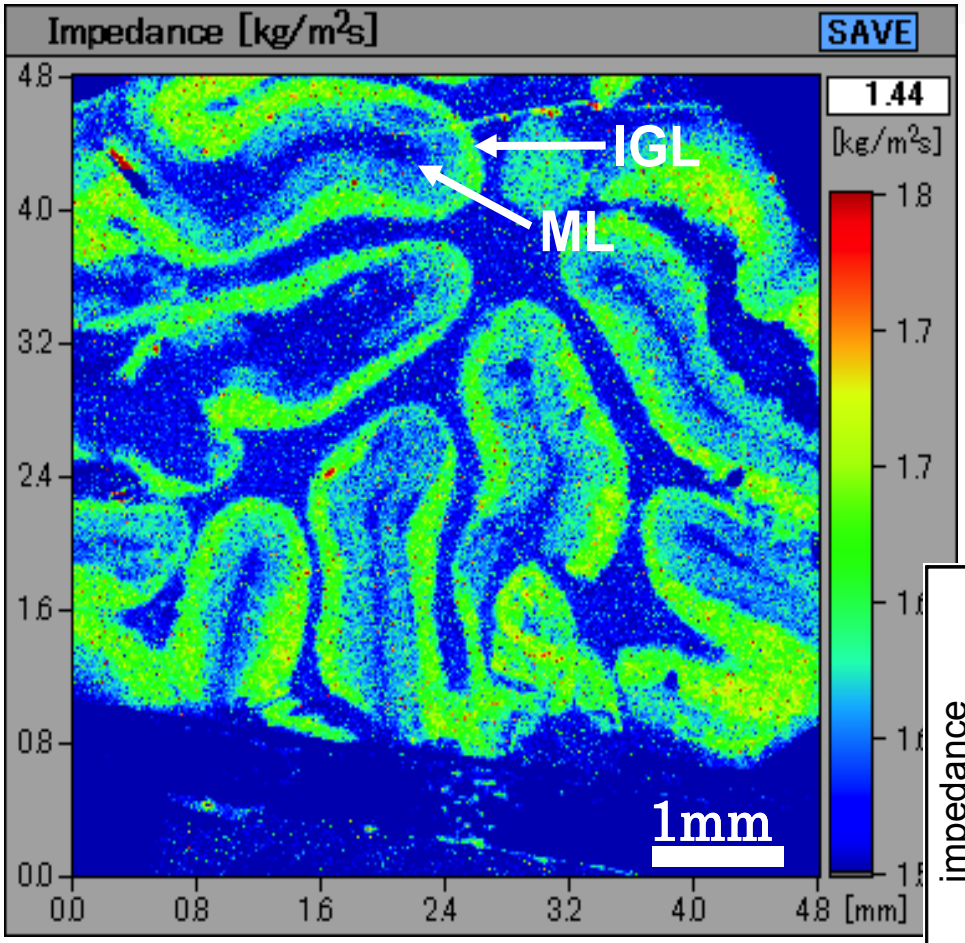
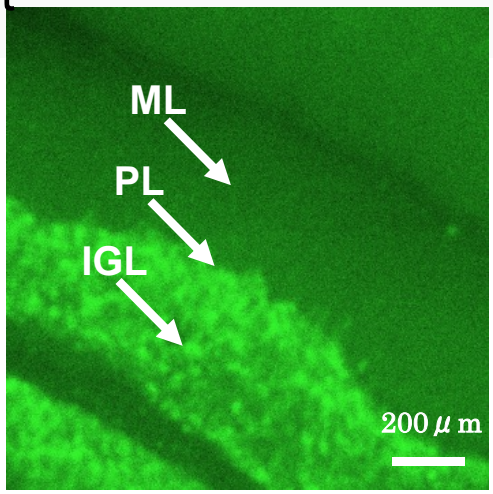
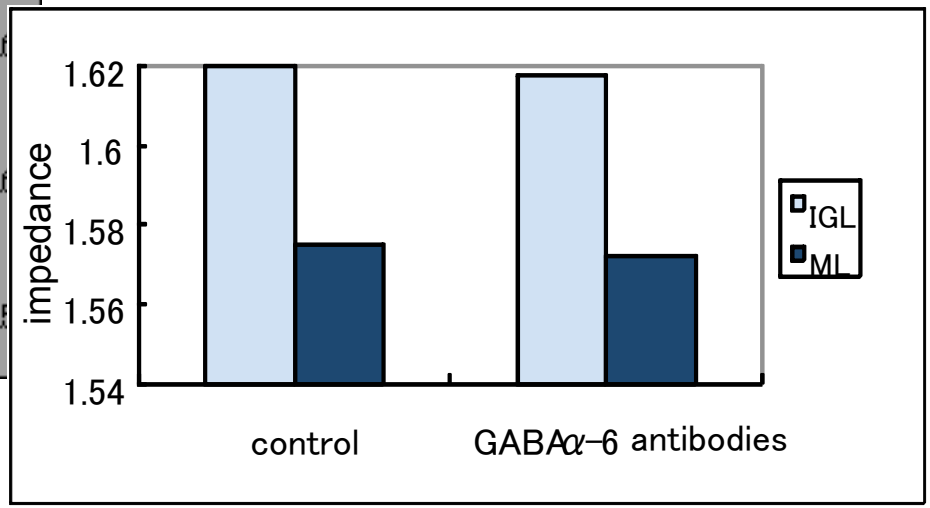
# Primary antibody: GABARα6 subunit




Acoustic staining image




Fluorescent image




Impedance was not increased by acoustic immunostaining to less proteins.

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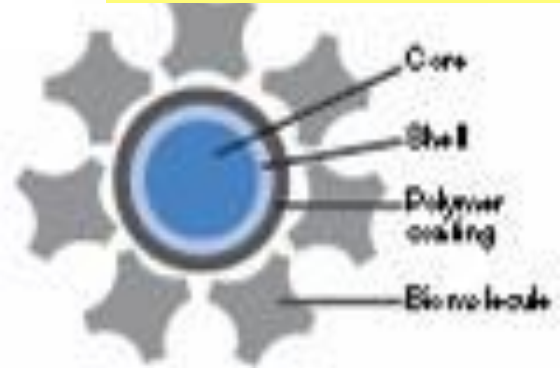
# **Acoustic immunohistology** using Heavy metal crystals


Immunostainingの技術的・時間制約的問題

Qdot : Fluorescent heavy metal ser

23.0 mM の分子に対し、
インピーダンスを0.1上昇可能




Φ:10~20nm
Core：Cd
shell：Zinc sulfide
1 particle :
500 atoms

4th

Immunogold : Gold colloid with ant

16.0 μM の分子に対し、
インピーダンスを0.1上昇可能

Φ:10~40nm
Core：Au
1 particle :
5000-200000
atoms

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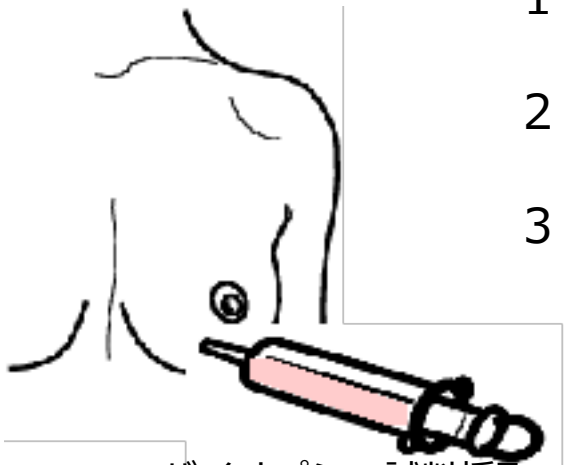
# ・音響インピーダンス顕微鏡の展開

術中迅速診断の問題

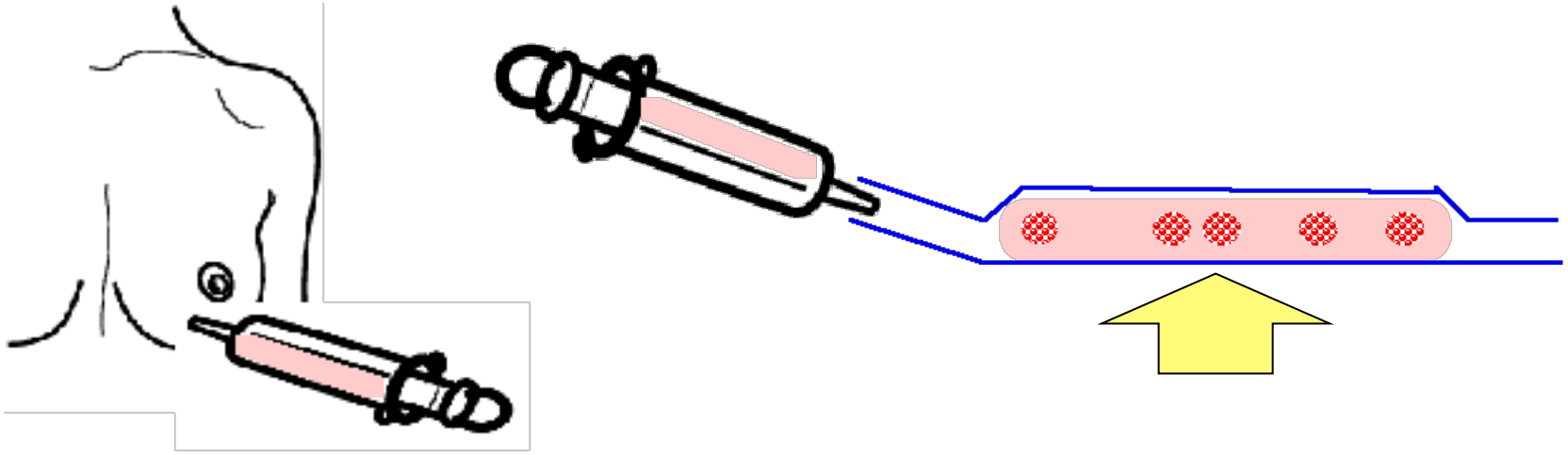


バイオプシー試料採取

1　薄切と固定に、時間と習熟した技術者が必要
　　←　非固定、非薄切試料の観察
2　非特異的な染色像を判断する経験が不可欠
　　←　特異的で、閾値設定できる病理診断技術
3　医療技術者の感染を完全には予防できない
　　←　採取試料を閉鎖系で観察

# ・音響インピーダンス顕微鏡の展開

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 .

# 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.
- Cell specificity and surface proteins in living cells are marked with heavy metal absorption or Qdot.

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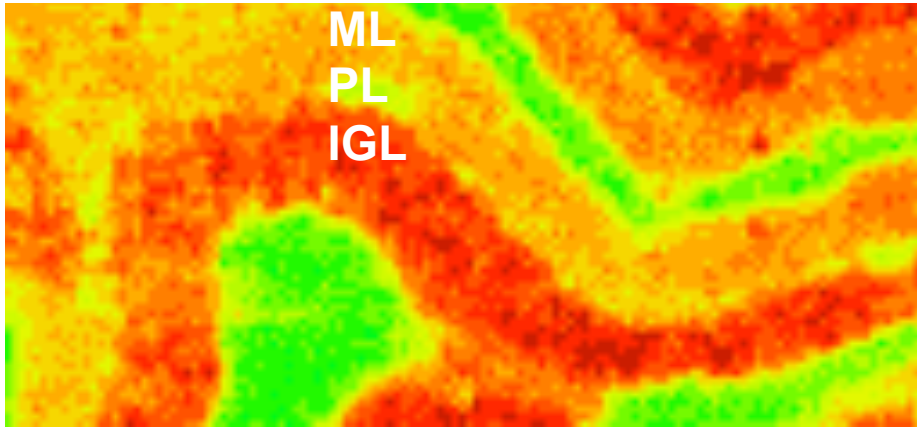
# Thank you for your attention

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