# Development of an active-matrix biosensor array

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Thanks to: NSF XYZ-on-a-chip

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# About this file

These are the slides from an invited presentation at the *Symposium on Dielectrics and the Dielectric-Electrolyte Interface in Biological and Biomedical Applications,* Electrochemical Society Fall Meeting, Los Angeles, CA, October 19, 2005.

The full presentation used a small number of illustrations copyrighted by others. These have been removed and replaced by a reference to the original sources. A few slides included here were not actually presented due to time constraints.



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# Outline

- motivation
- some relevant prior work
- cell sensing with impedance measurements
- active matrix concepts
- results so far and future prospects

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# Motivation

- drug discovery
- medical diagnostics

(short movies showing cancer and normal cells, obtained from website below)

tumor and normal cells move and grow differently
observation of movement may lead to a prognostic test that can be used to tailor therapy

[http://www.mwri.magee.edu/latimer/livecell.htm]

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# Impedance-based cell sensor

Giaever and Keese,
*Proc. Natl. Acad. Sci.*
**88**, 7897 (1991)

(figure from Giaever reference)

(figure from Giaever Nobel prize talk and official photo)

*I. Giaever won the Nobel prize in 1973 along with B. Josephson and L. Esaki.*

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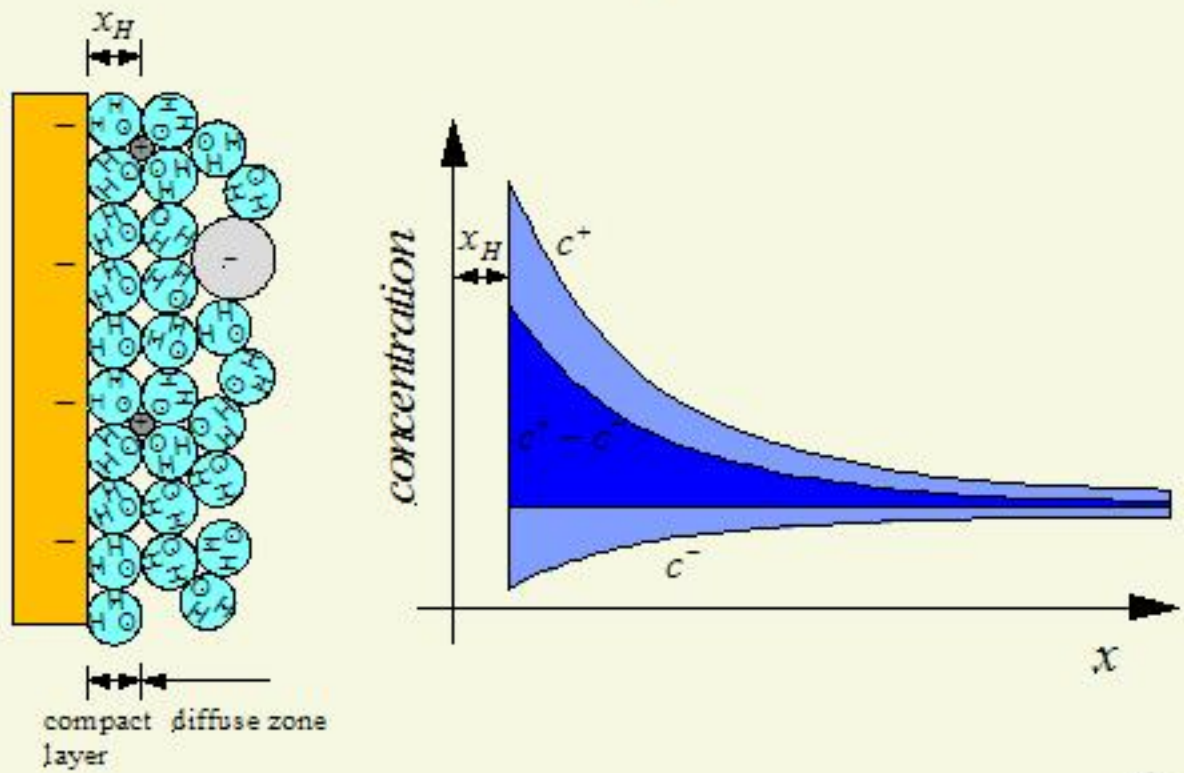
# Impedance of electrode

- cell growth medium is highly ionic ($\sigma \sim 0.015\ (\Omega\cdot\text{cm})^{-1}$)
- an *electrical double layer* forms at electrode

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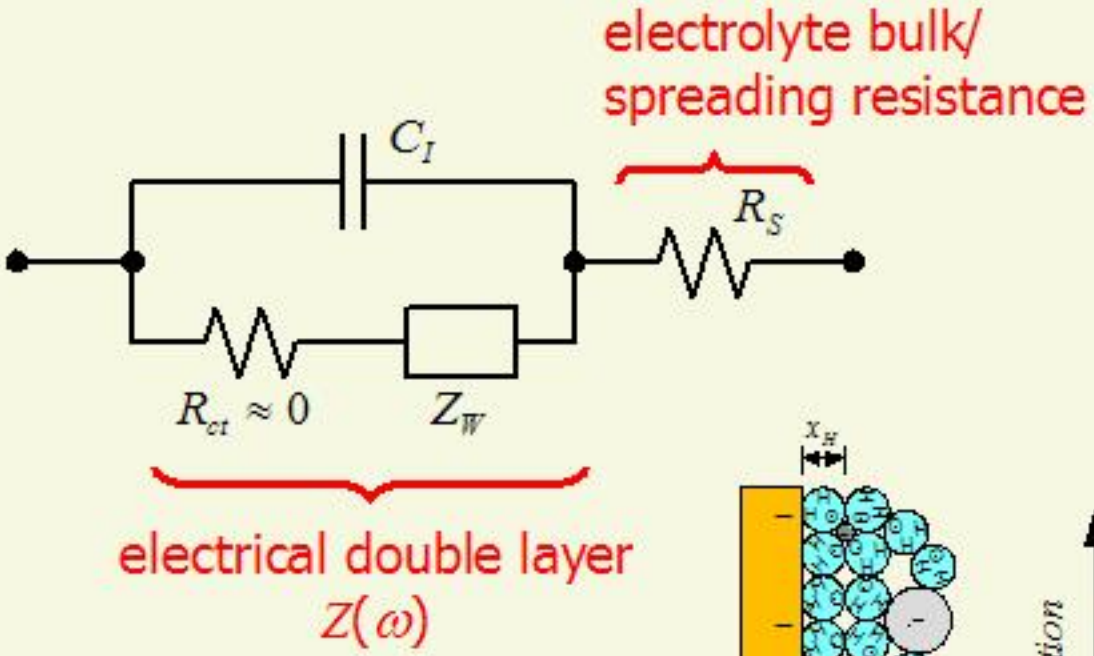
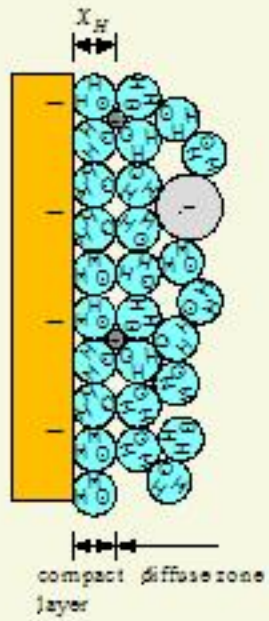
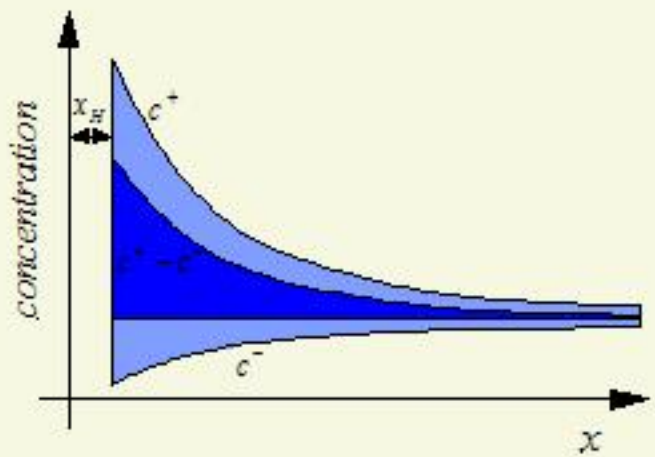
# Electrode impedance

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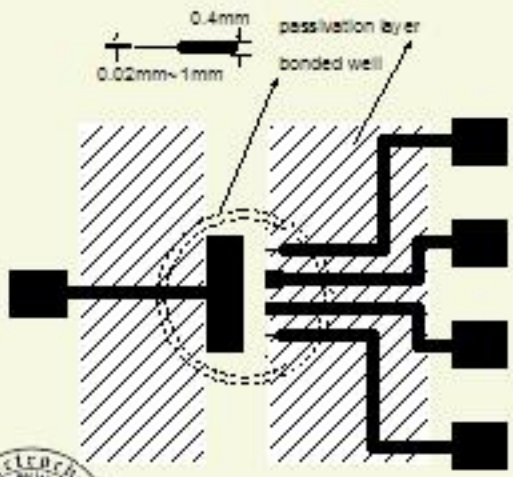
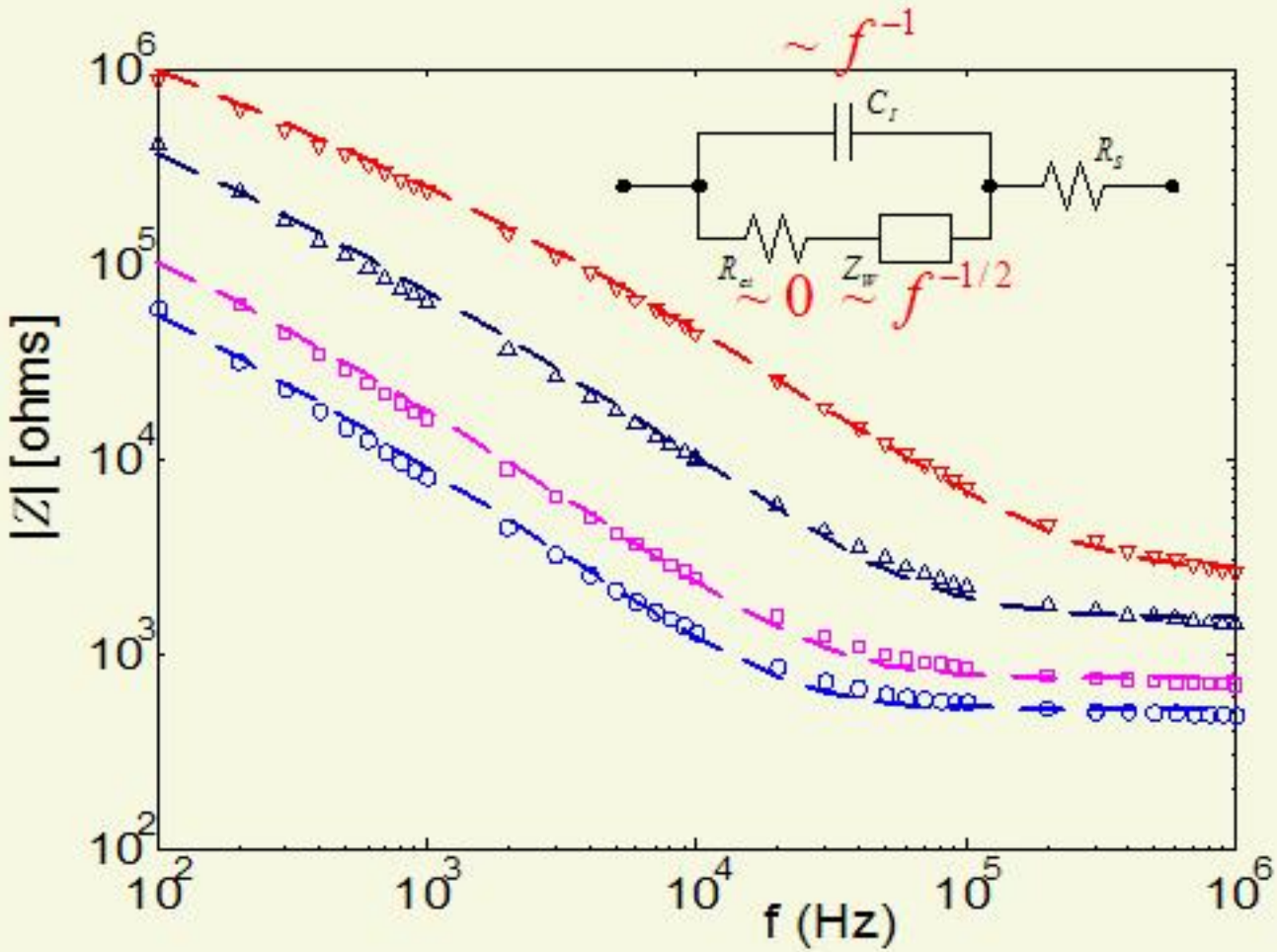
# Measured electrode impedance

○ 0.00032 cm²
□ 0.0016 cm²
△ 0.0032 cm²
▽ 0.000064 cm²

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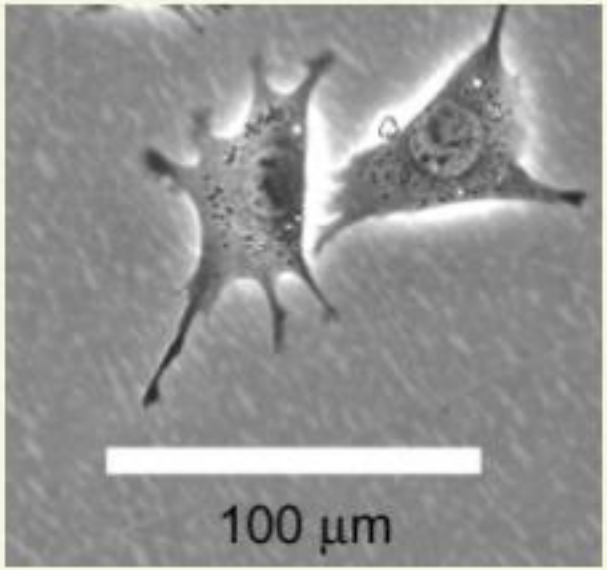
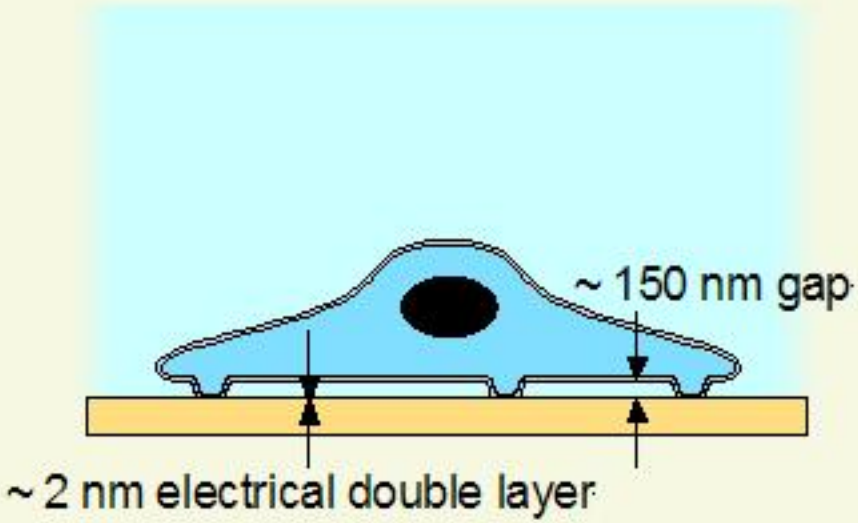
# Length scales (3T3 fibroblasts)

cell extent = 30-50 μm
focal adhesion regions 2 μm × 5-10 μm
cell-electrode gap = ~150 nm
electrical double layer ~ 2 nm

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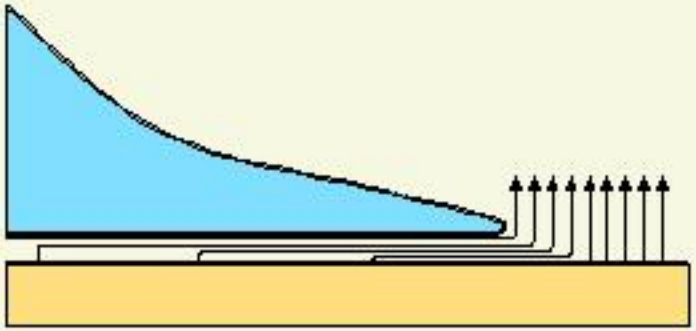
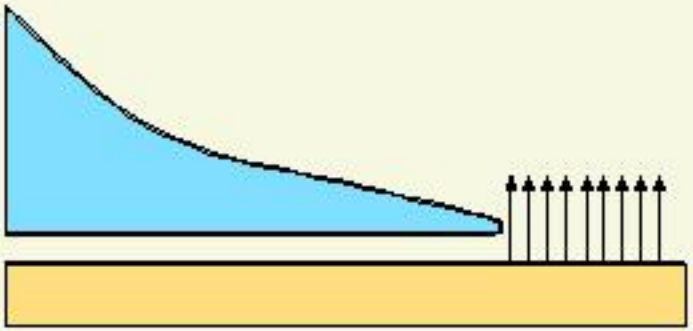
# Qualitative picture

- current flows beneath cell at low frequencies
- $Z(\omega)$ decreases with increasing frequency
- at high frequencies resistance of medium limits current flow beneath cell



low frequencies
$Z(\omega)$ large



high frequencies
$Z(\omega)$ small

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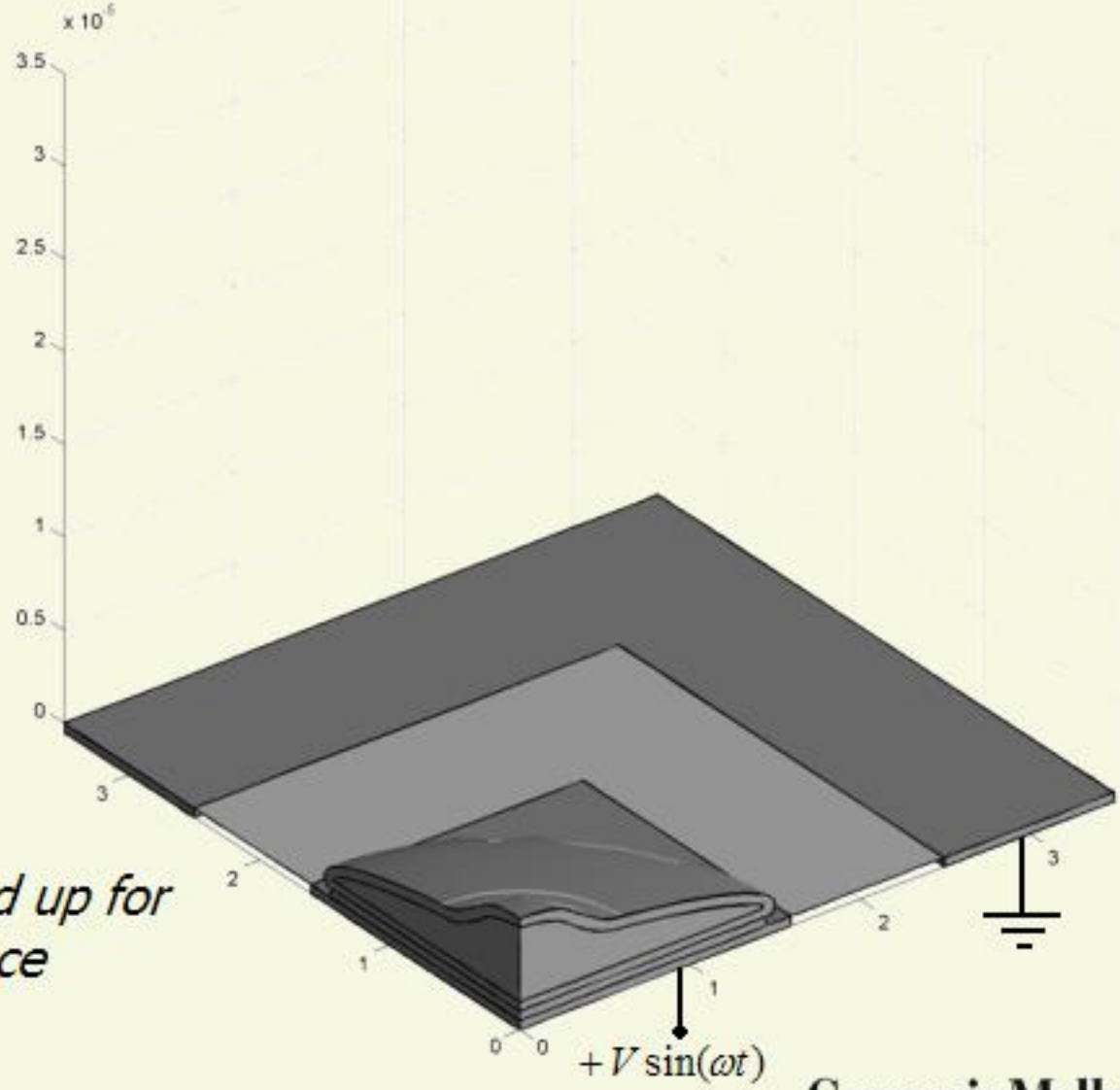
# Cell and electrode geometry

32 x 32 $\mu$m
sensing electrode

30 $\mu$m
diameter cell




*some thicknesses scaled up for visibility and convergence*

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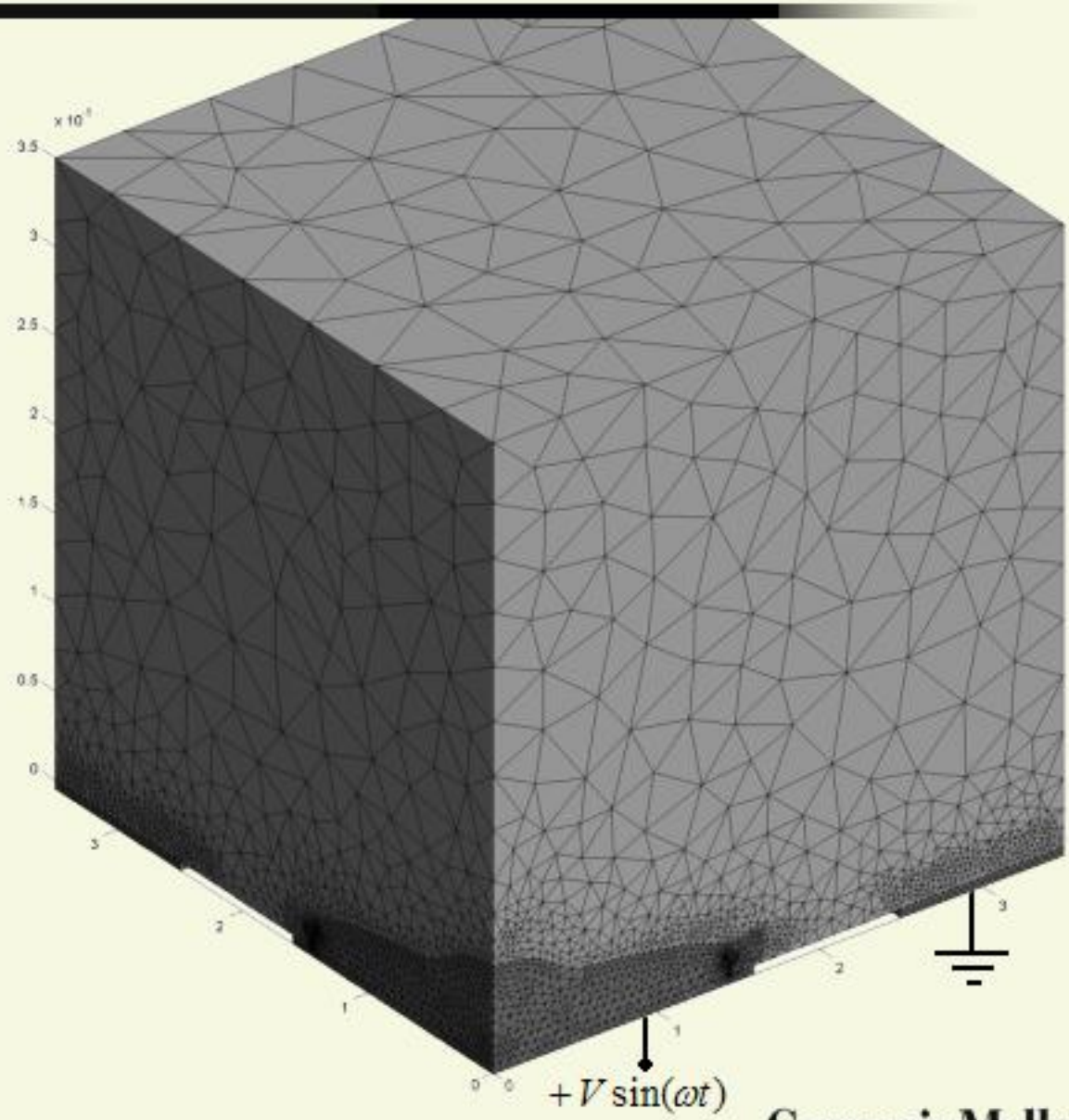
# Simulation domain and mesh

FEMLAB conductive DC media with complex $\sigma$




*148,403 elements*

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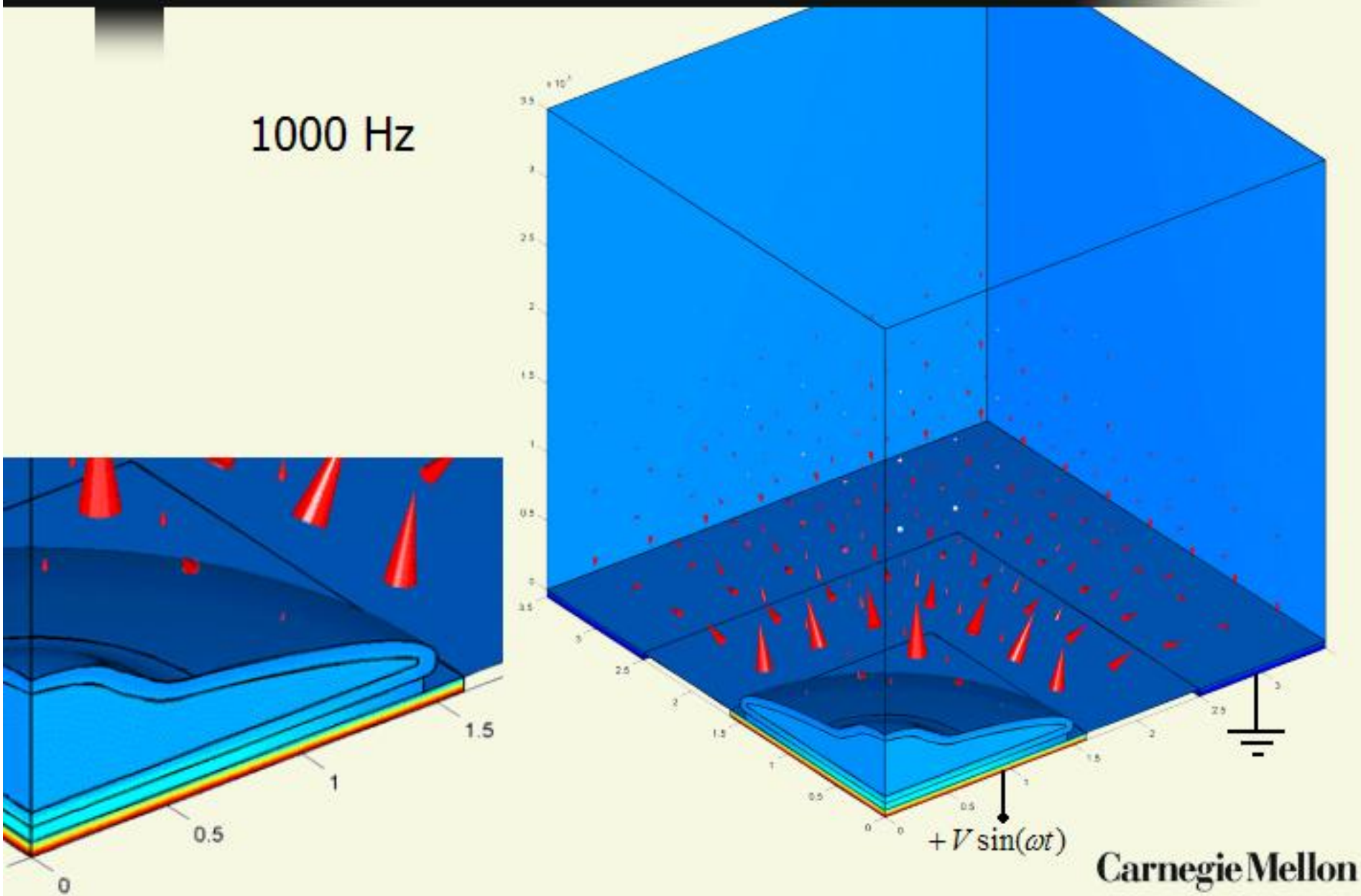
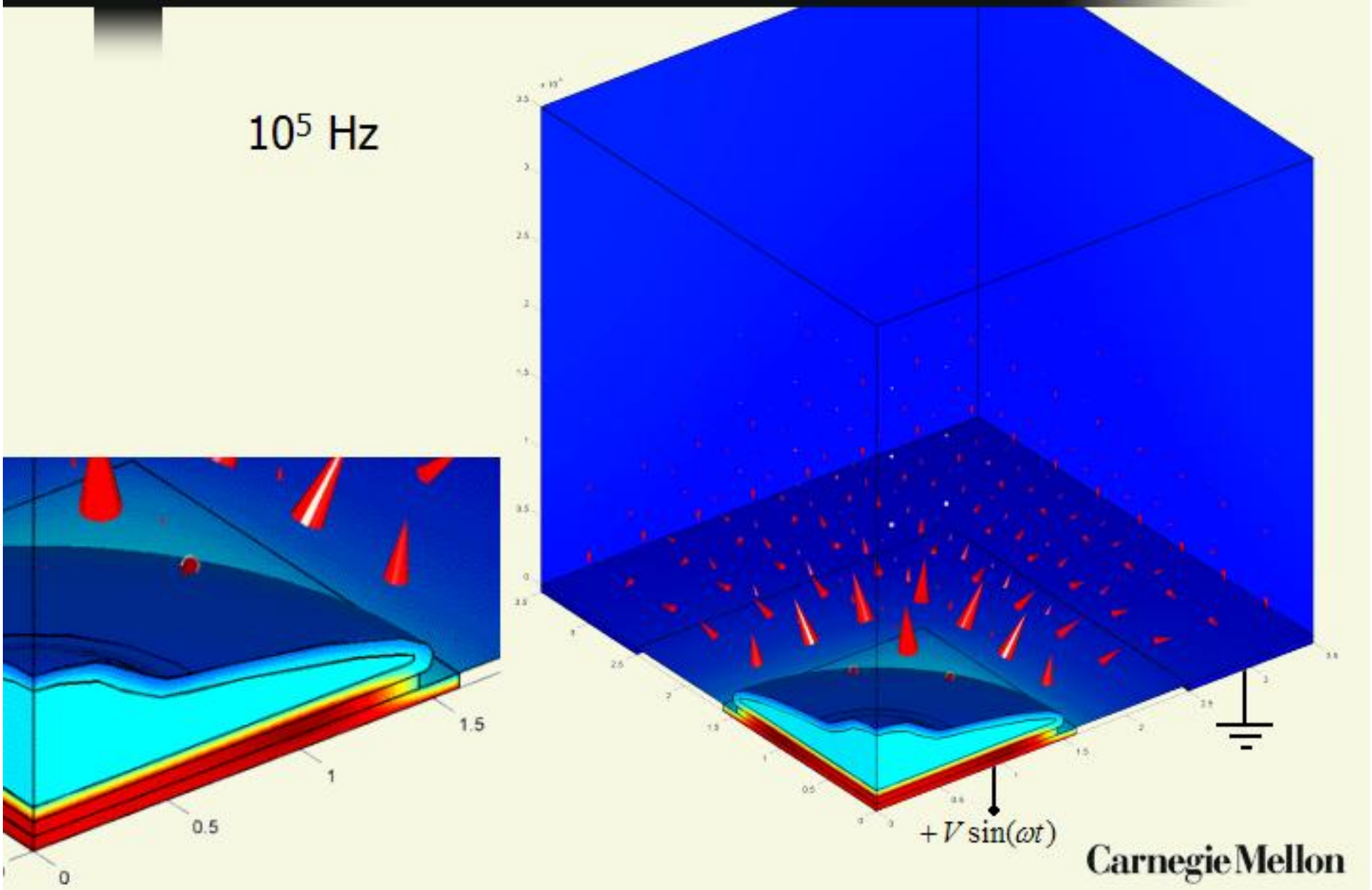
# Finite element simulation

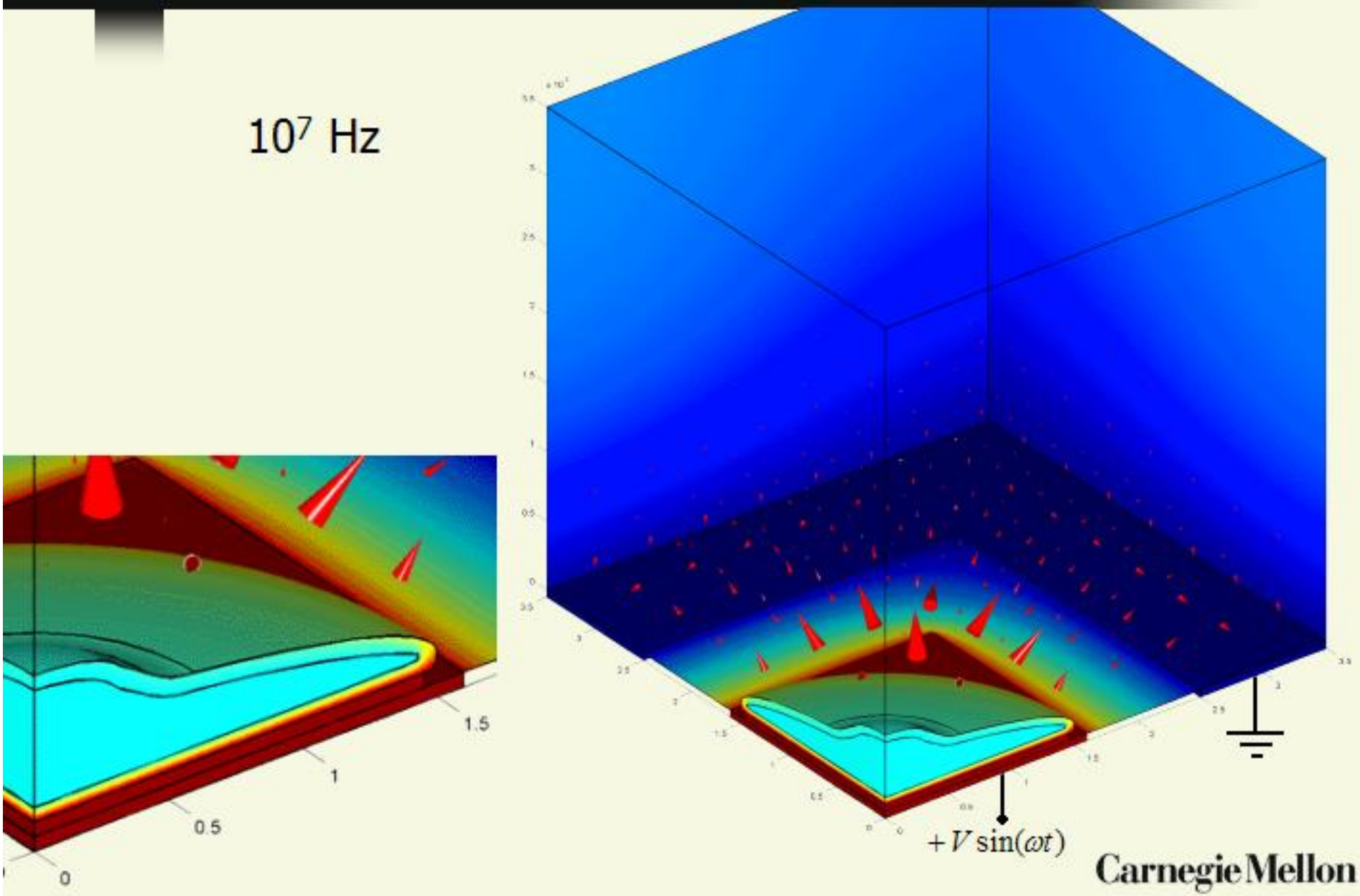


# Finite element simulation



# Finite element simulation

$10^7$ Hz

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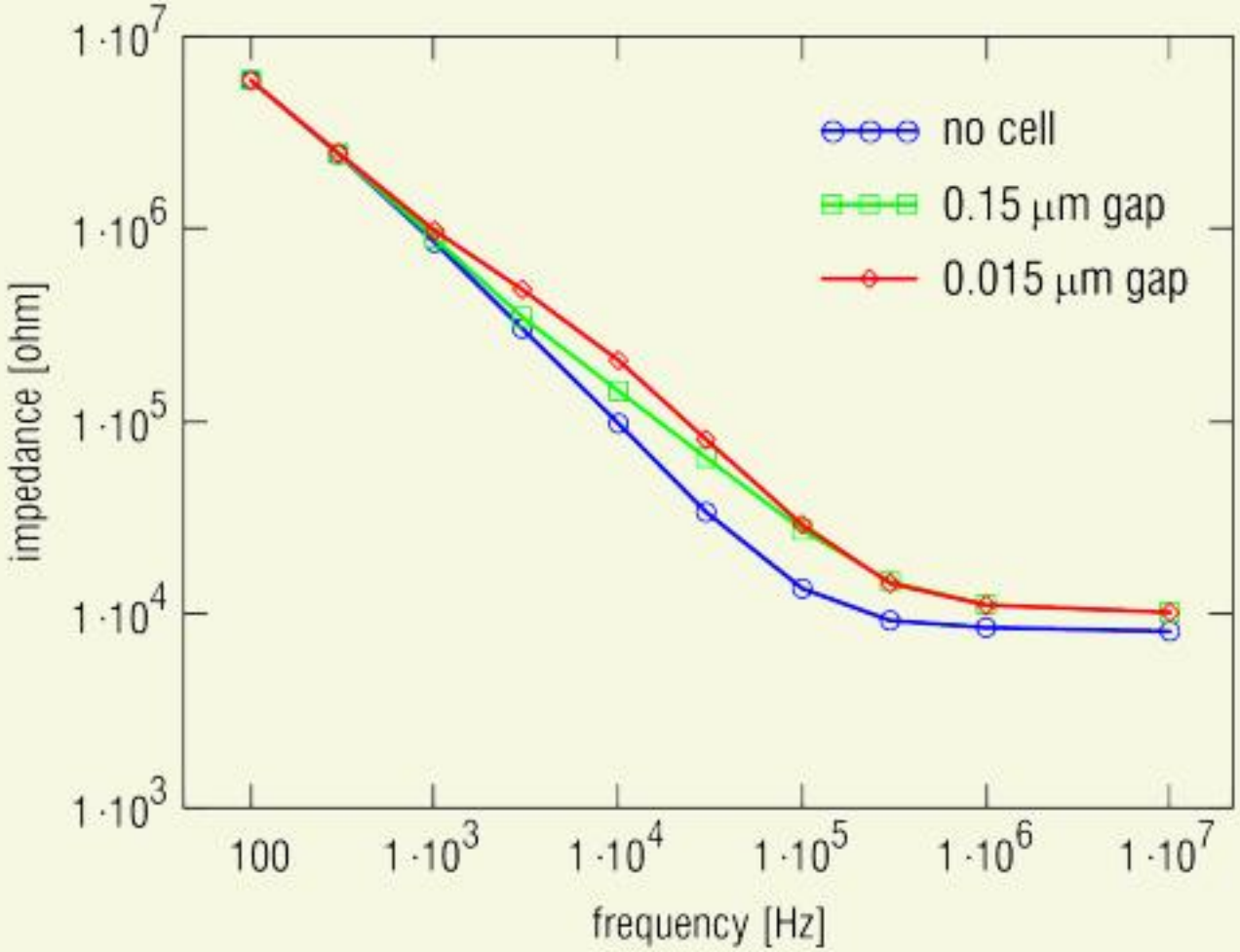
# Finite element simulation

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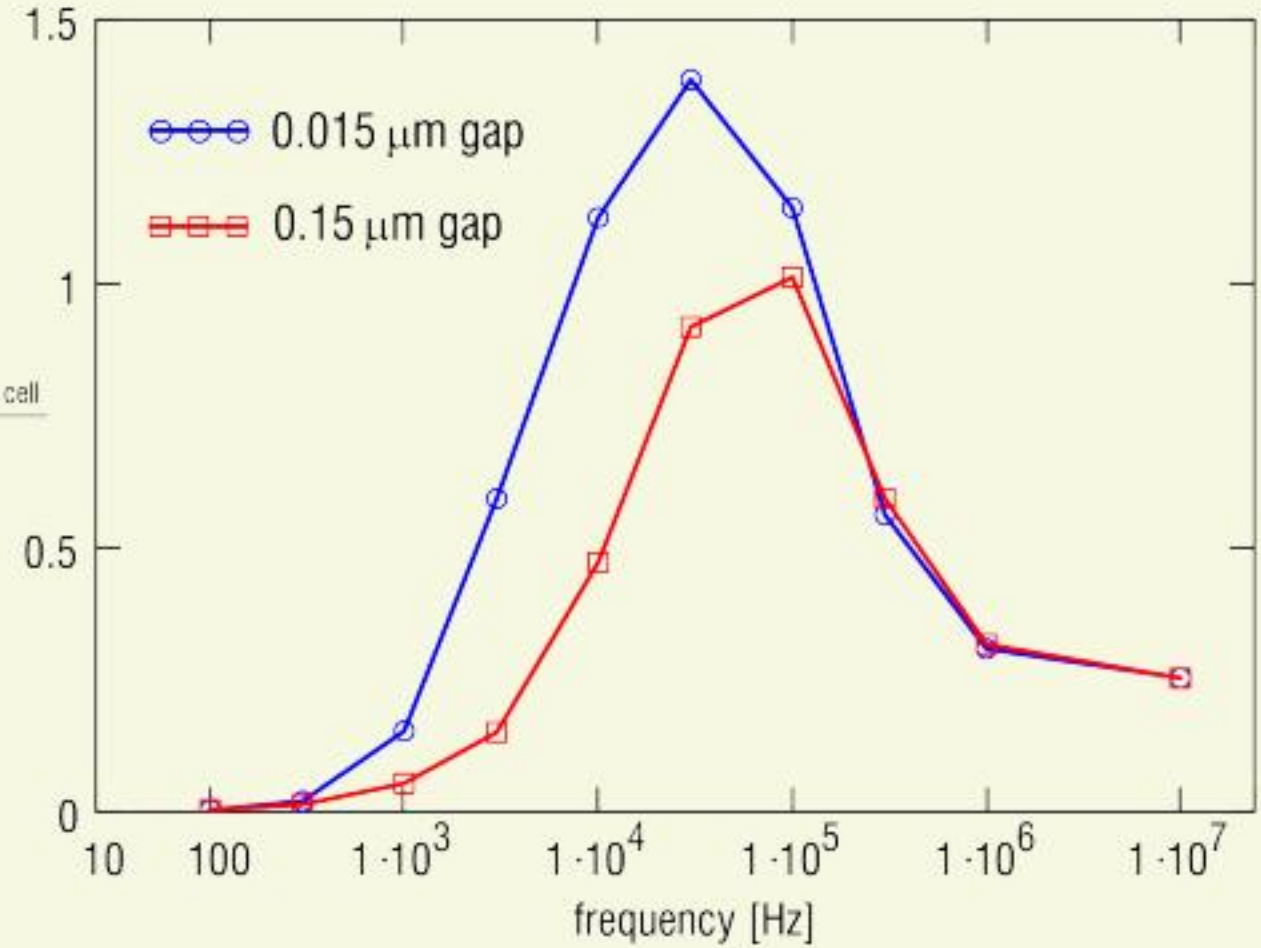
# Normalized impedance change $r$

$$r = \frac{Z_{cell} - Z_{no\ cell}}{Z_{no\ cell}}$$

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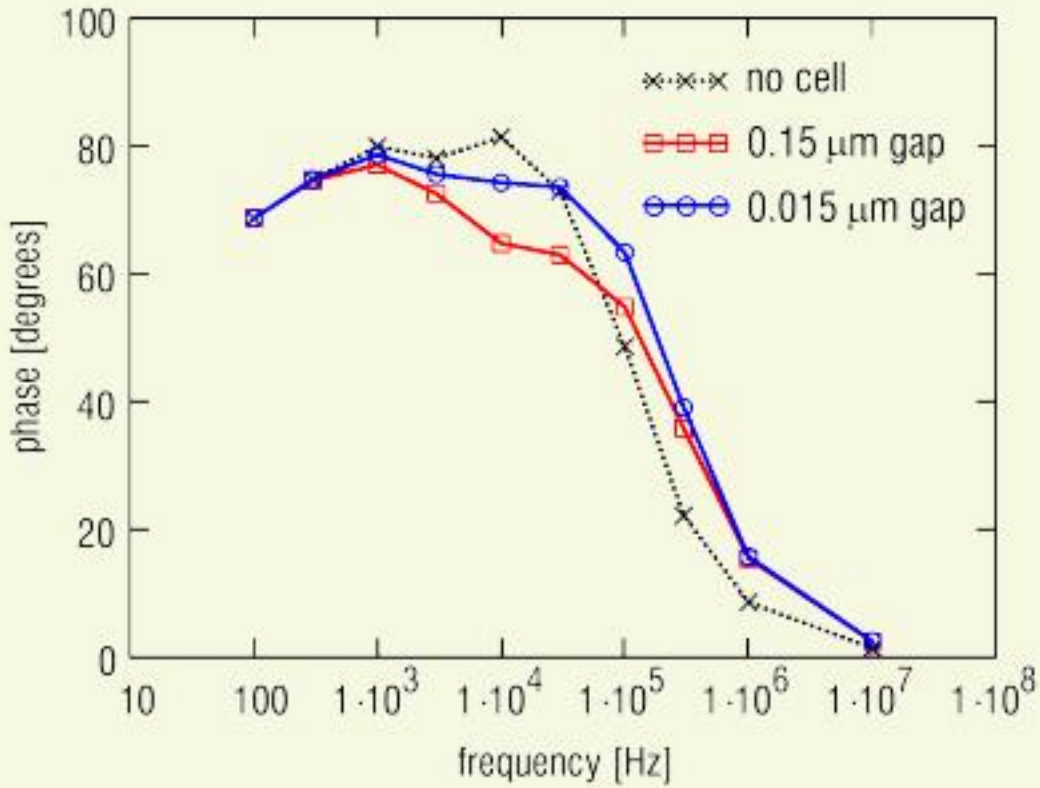
# Design lessons from simulation

- reference electrode location doesn't matter
- reference electrode must be large in area
- measure impedance magnitude, not phase

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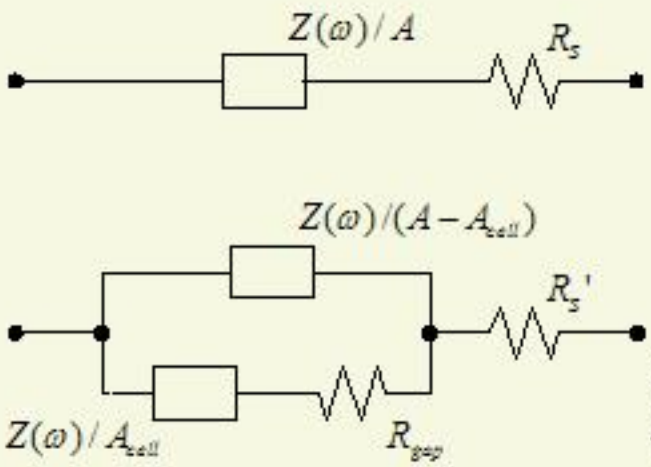
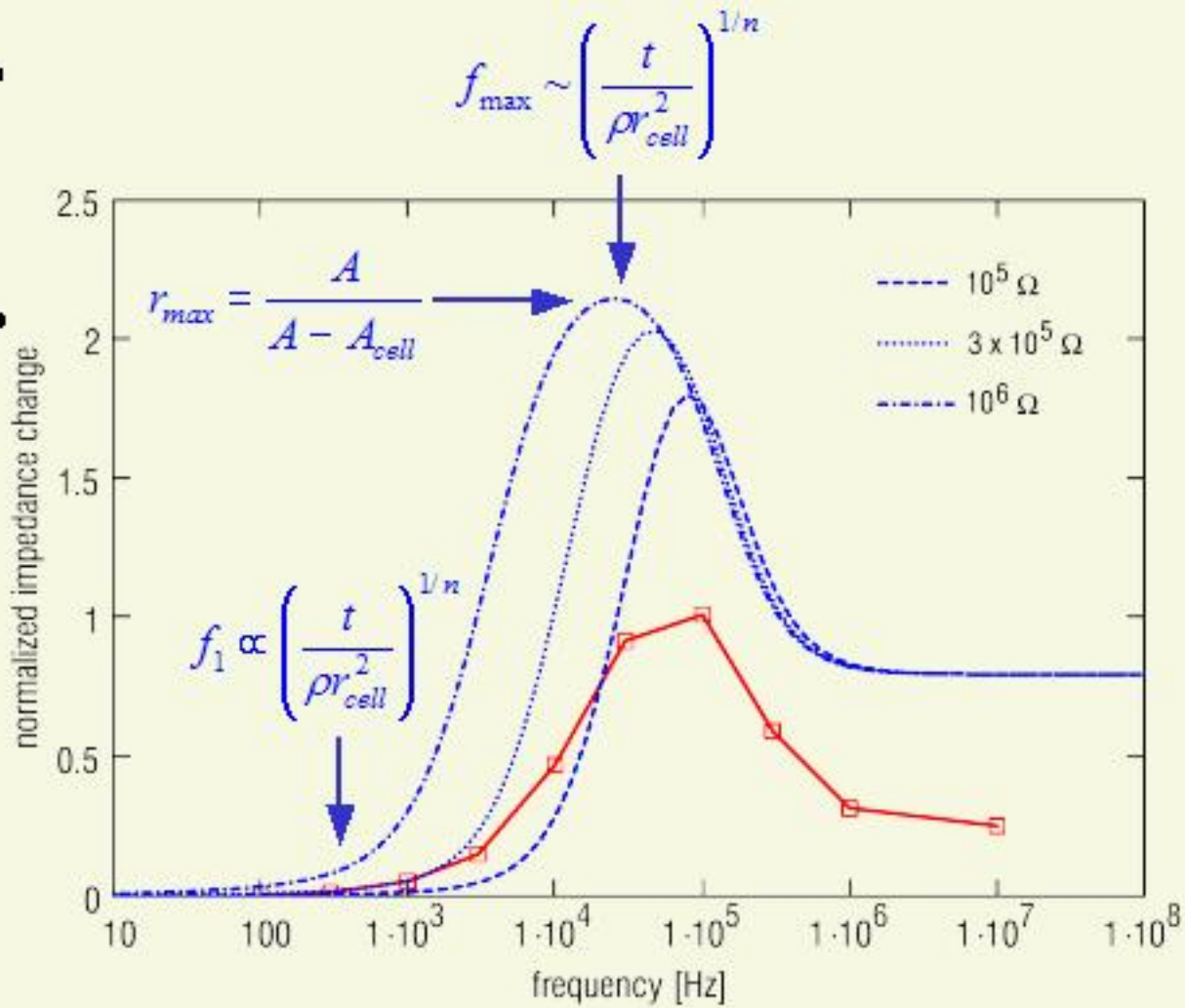
# An equivalent circuit model

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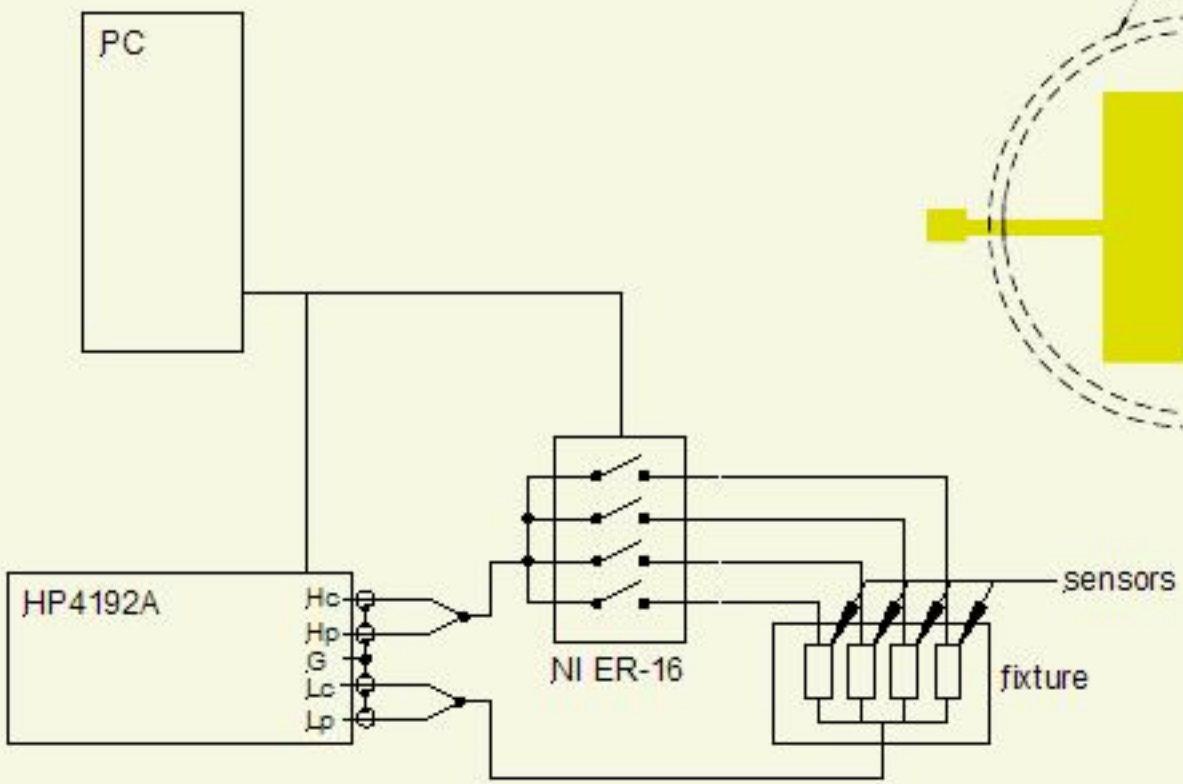
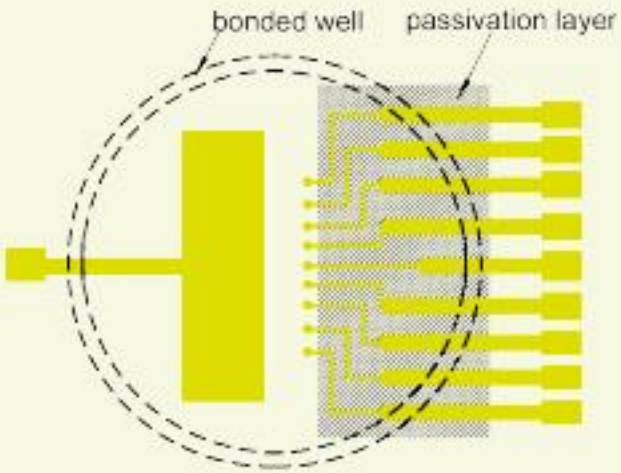
# Experiments-individual electrodes

- gold/chromium electrodes, 0.015-0.02 $mm^2$
- 50 mV p-p, measure $|Z(\omega)|$
- relay addressing

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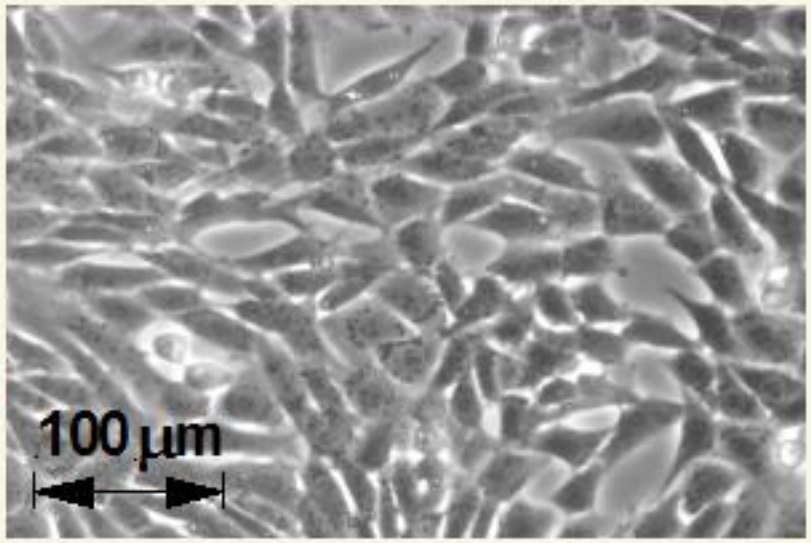
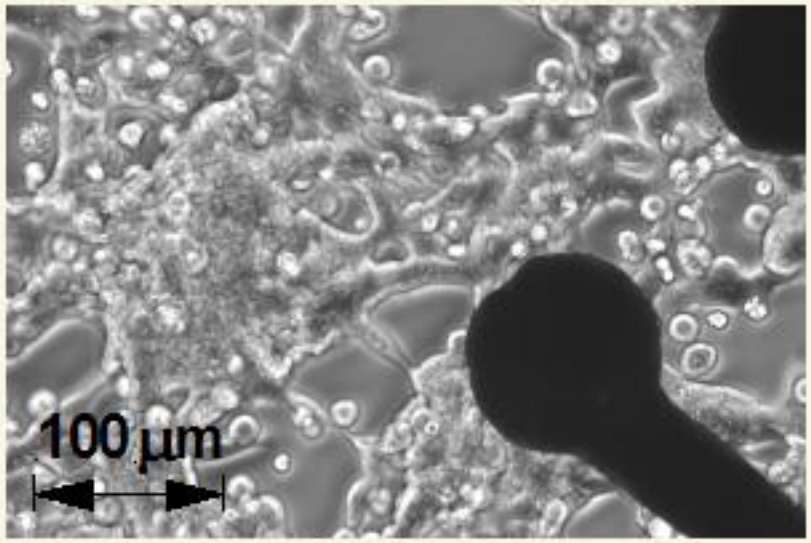
# Experiment-cell proliferation

3T3 mouse fibroblasts
mobile, involved in wound healing
grow to form a flat, nearly
confluent monolayer




HCT116 human cancer cells
smaller (~20 μm), round
do not form continuous sheet

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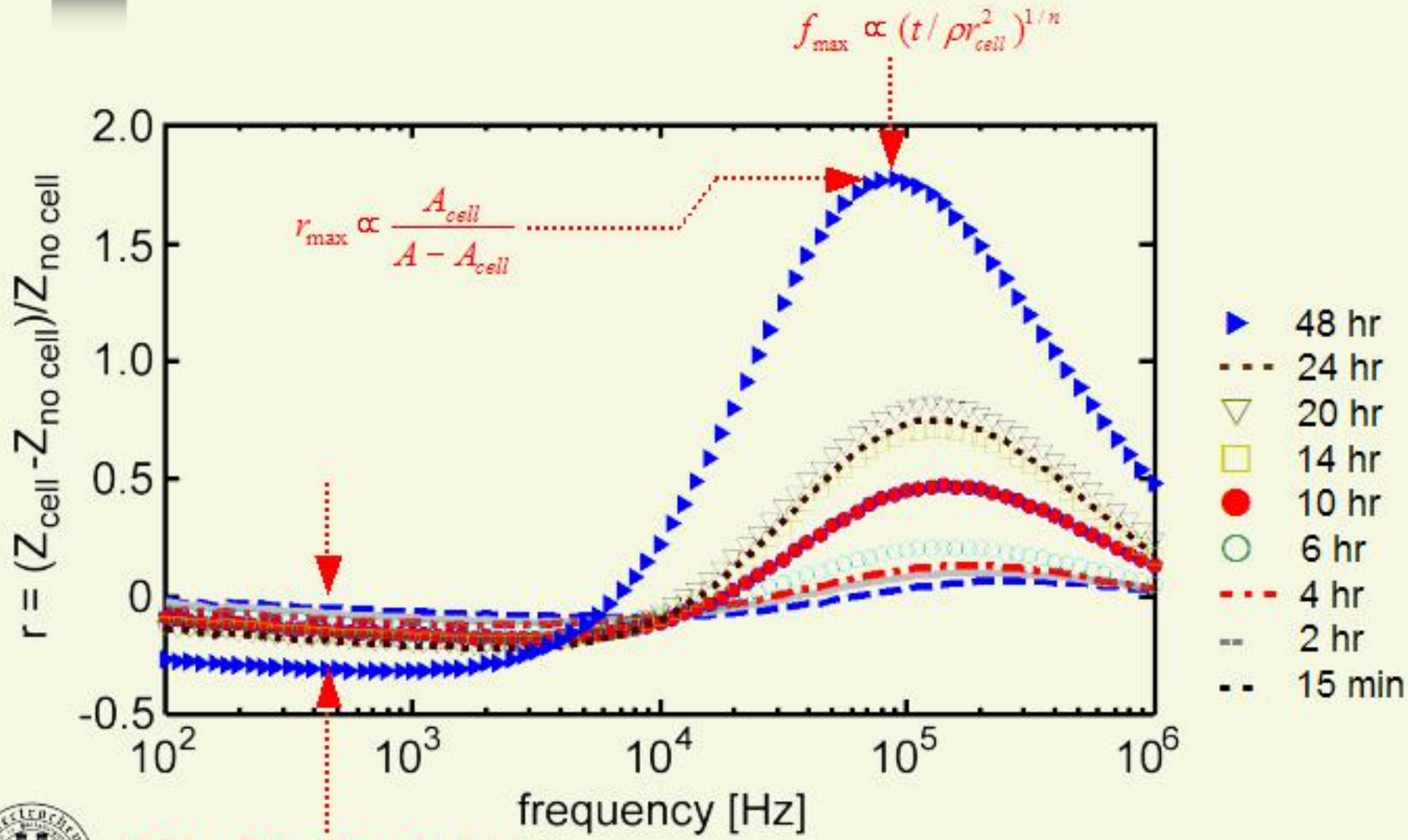
# HCT-116 (cancer cells)




ECM = Extra-Cellular Matrix
(proteins secreted by the cell)

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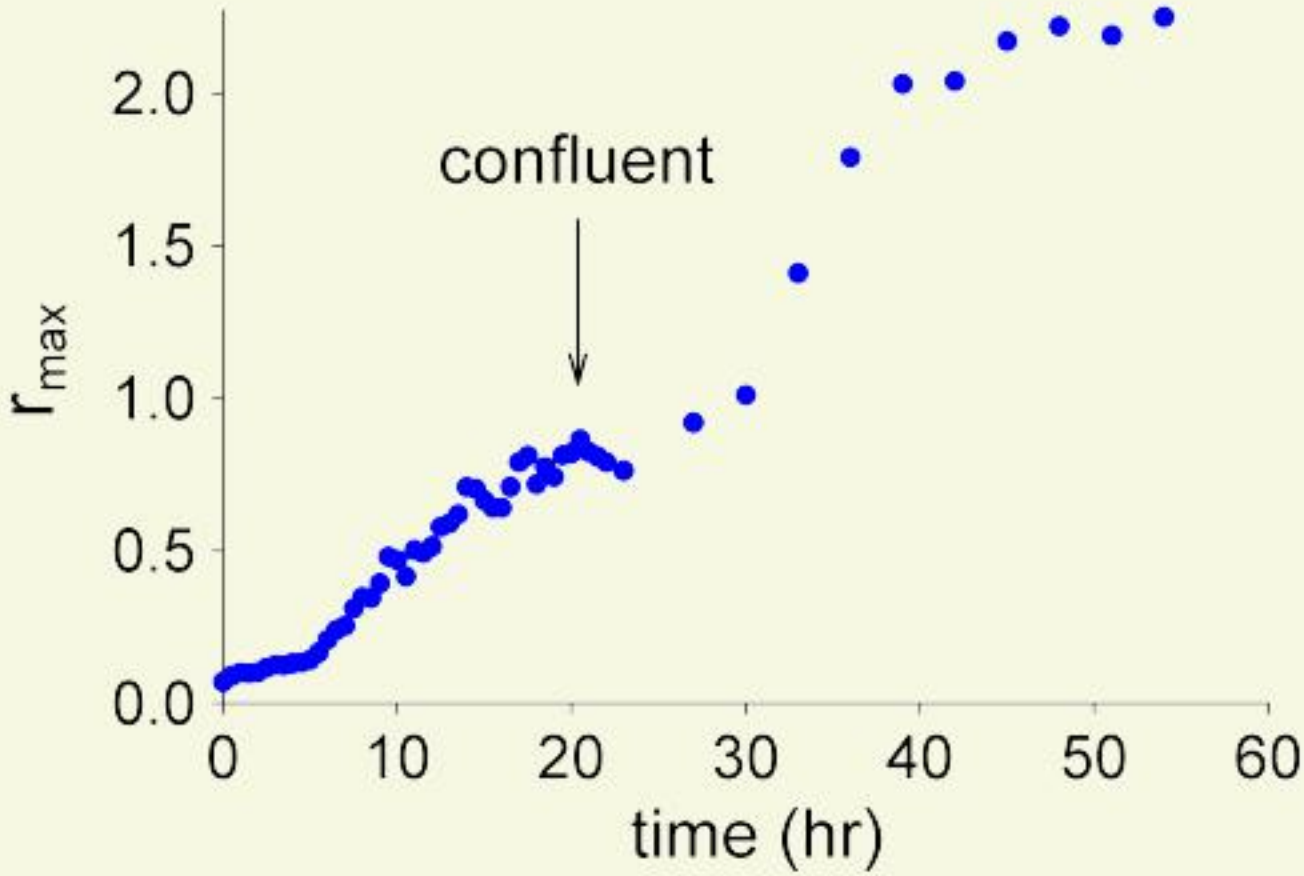
# HCT-116 (cancer cells)

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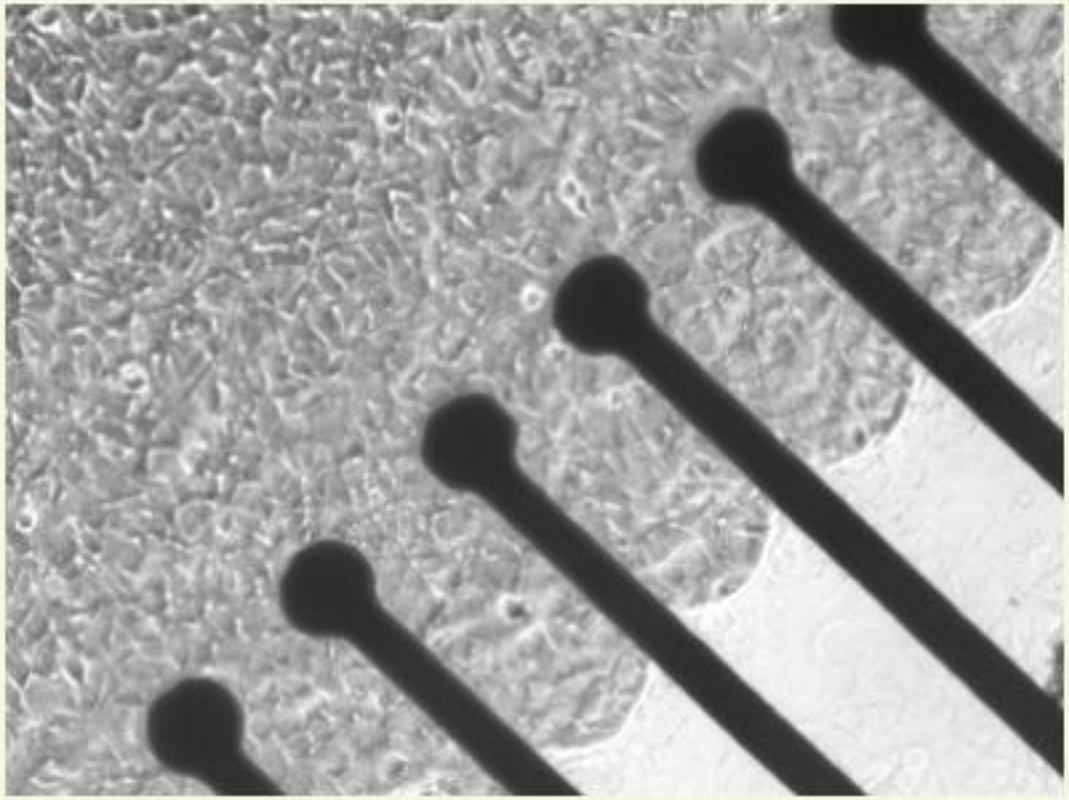
# Experiment-fibroblast proliferation

transmission image (48 hrs growth)



top view (reflective)

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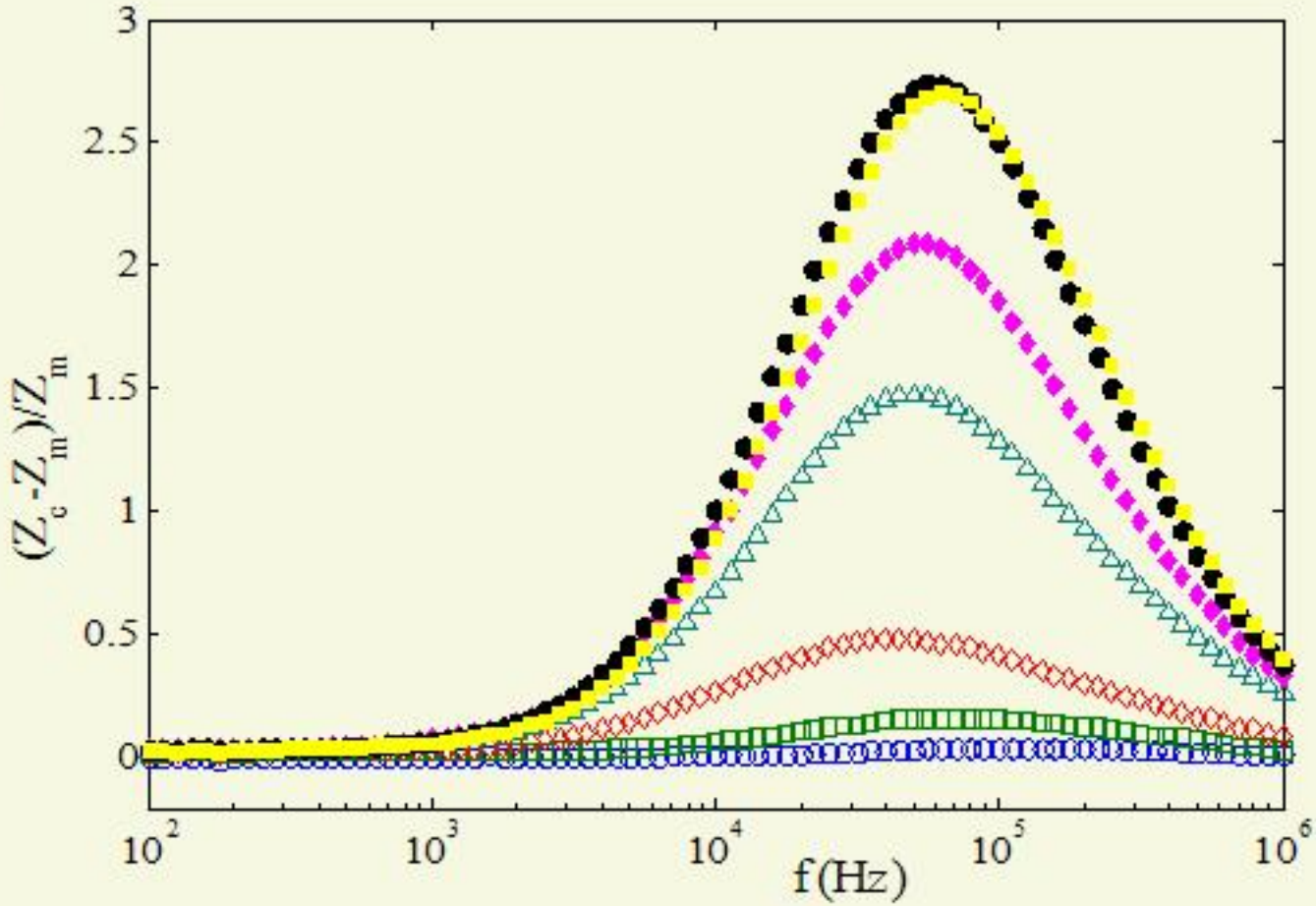
# 3T3 (fibroblast) proliferation

37 °C
area = 0.4 mm²

- 0 hr
- 0.5 hr
- 1 hr
- 2 hr
- 3 hr
- 4 hr
- 6 hr

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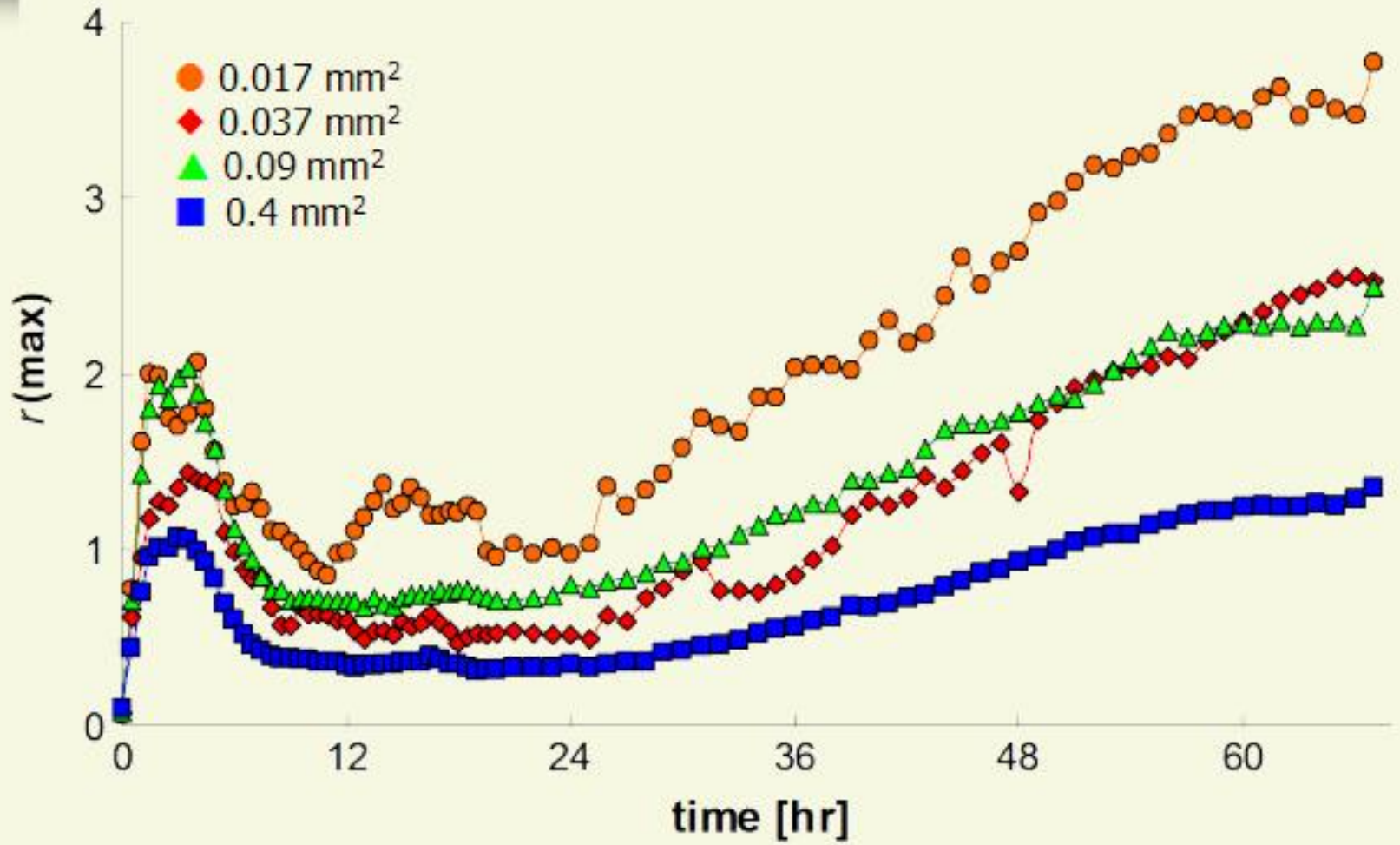
# 3T3 mouse fibroblasts




attachment and spreading

motion, relief from surface

migration and proliferation

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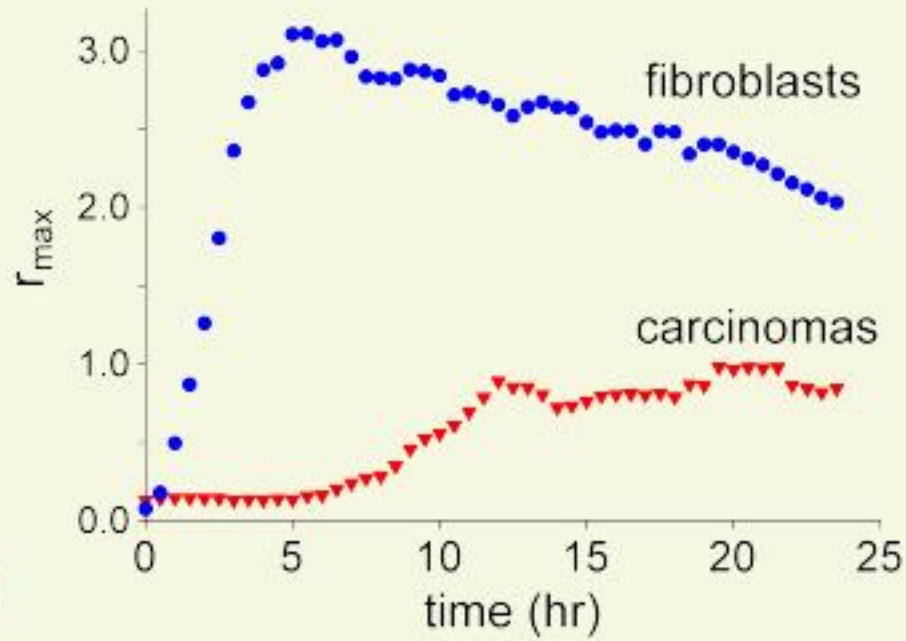
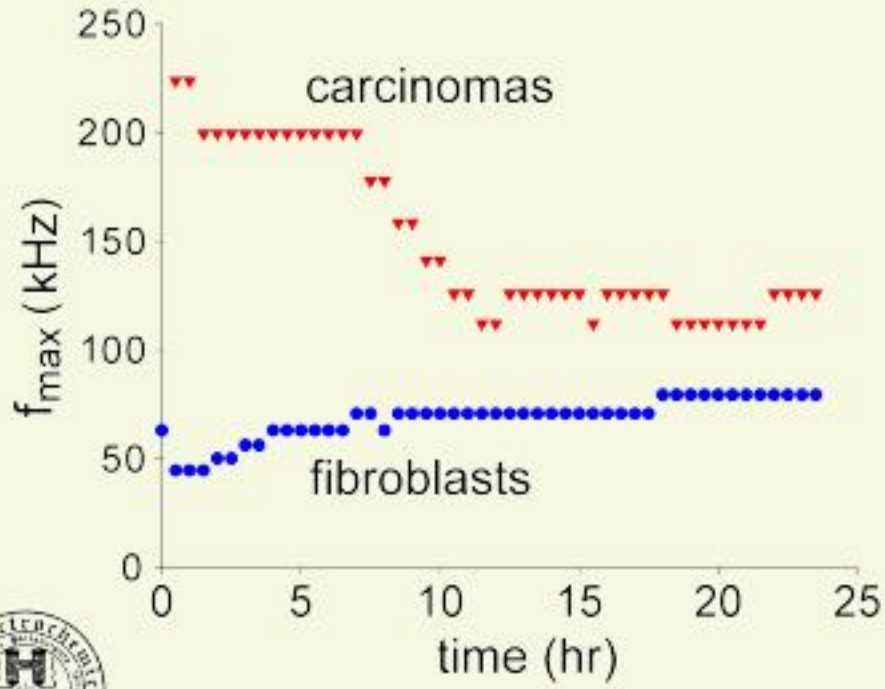
# Comparison-24 hr

$$r_{max} \propto \frac{A_{cell}}{A - A_{cell}}$$




$$f_{max} \propto (t / \rho r_{cell}^2)^{1/n}$$

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# Comparison- longer duration

| cell line | spreading time | $f_{max}$ (value) | $f_{max}$ (change) | $r_{max}$ (change) |
|---|---|---|---|---|
| 3T3 | ~5 hr | $< 10^5$ Hz | ↑ | rapid increase, lag, then slow increase |
| HCT-116 | ~10 hr | 1.3- 2.0 $\times 10^5$ Hz | ↓ | slow increase, plateau, then slow increase |

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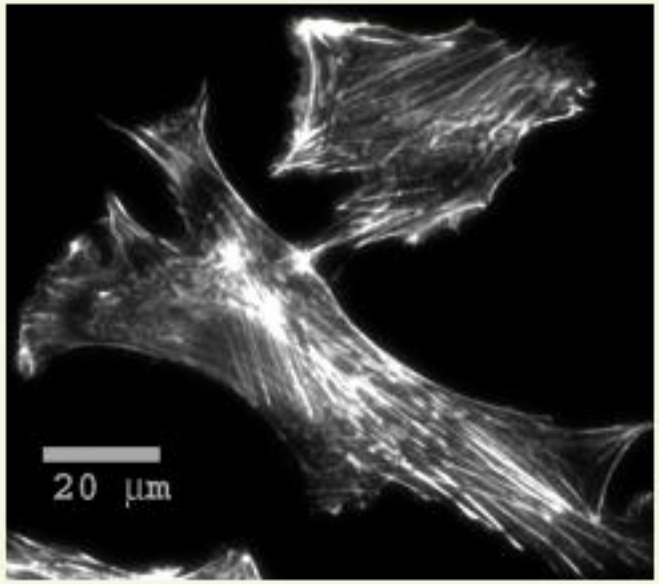
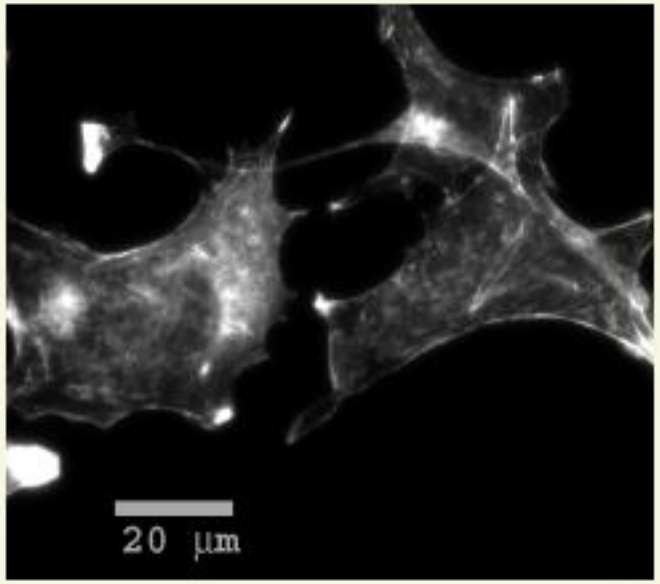
# Experiment-H-7 effect

protein kinase inhibitor

- influences cell adhesion (focal contacts)
- small influence on cell-cell junctions




before H-7




4 hours after H-7

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# Experiment-H-7 effect

cells incubated for 16 hours

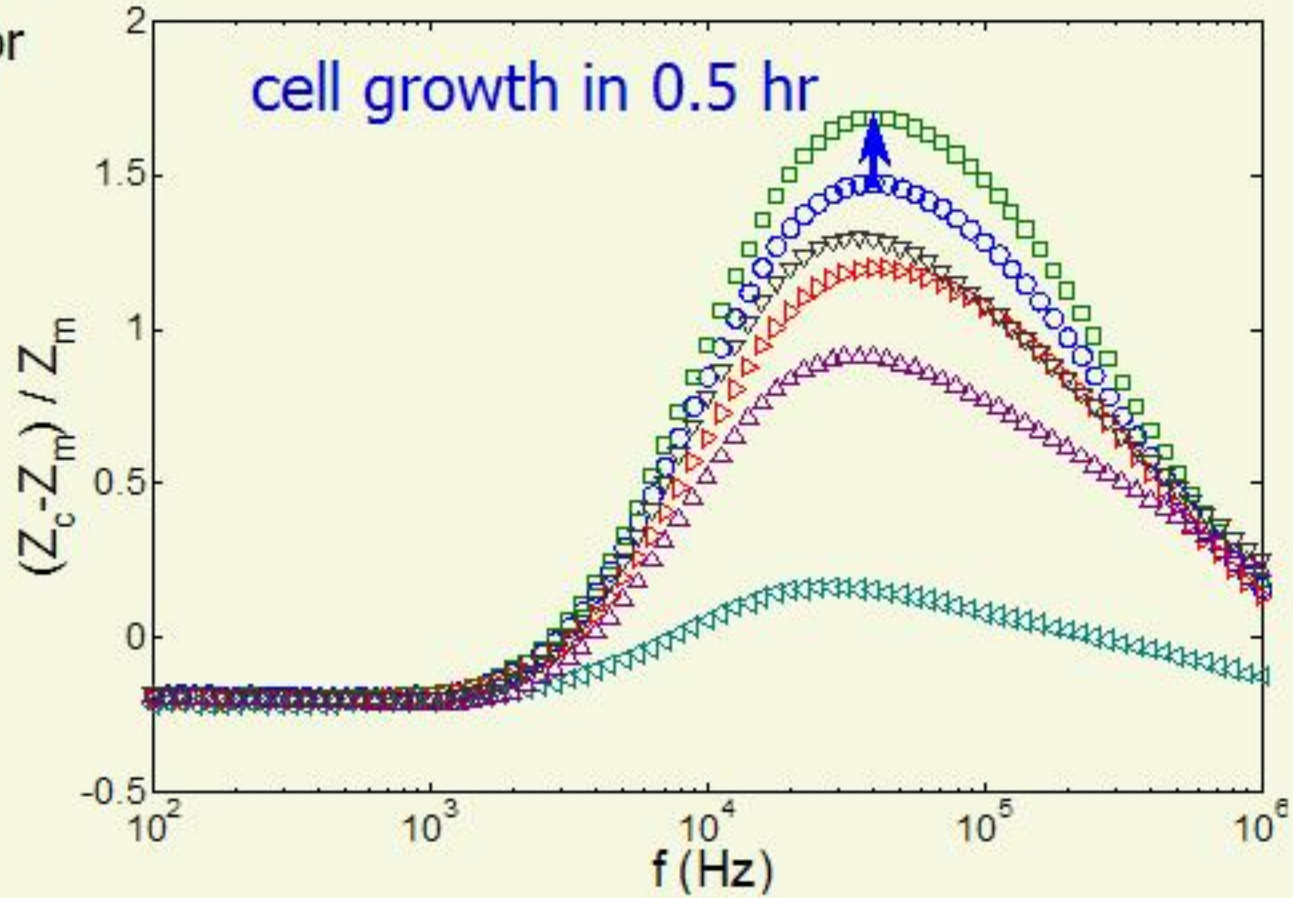
- ○ +0 hr
- □ +0.5 hr

H-7 added

- ▷ +15 min
- ◁ +4 hr

fresh medium

- △ +1 hr
- ▽ +2 hr

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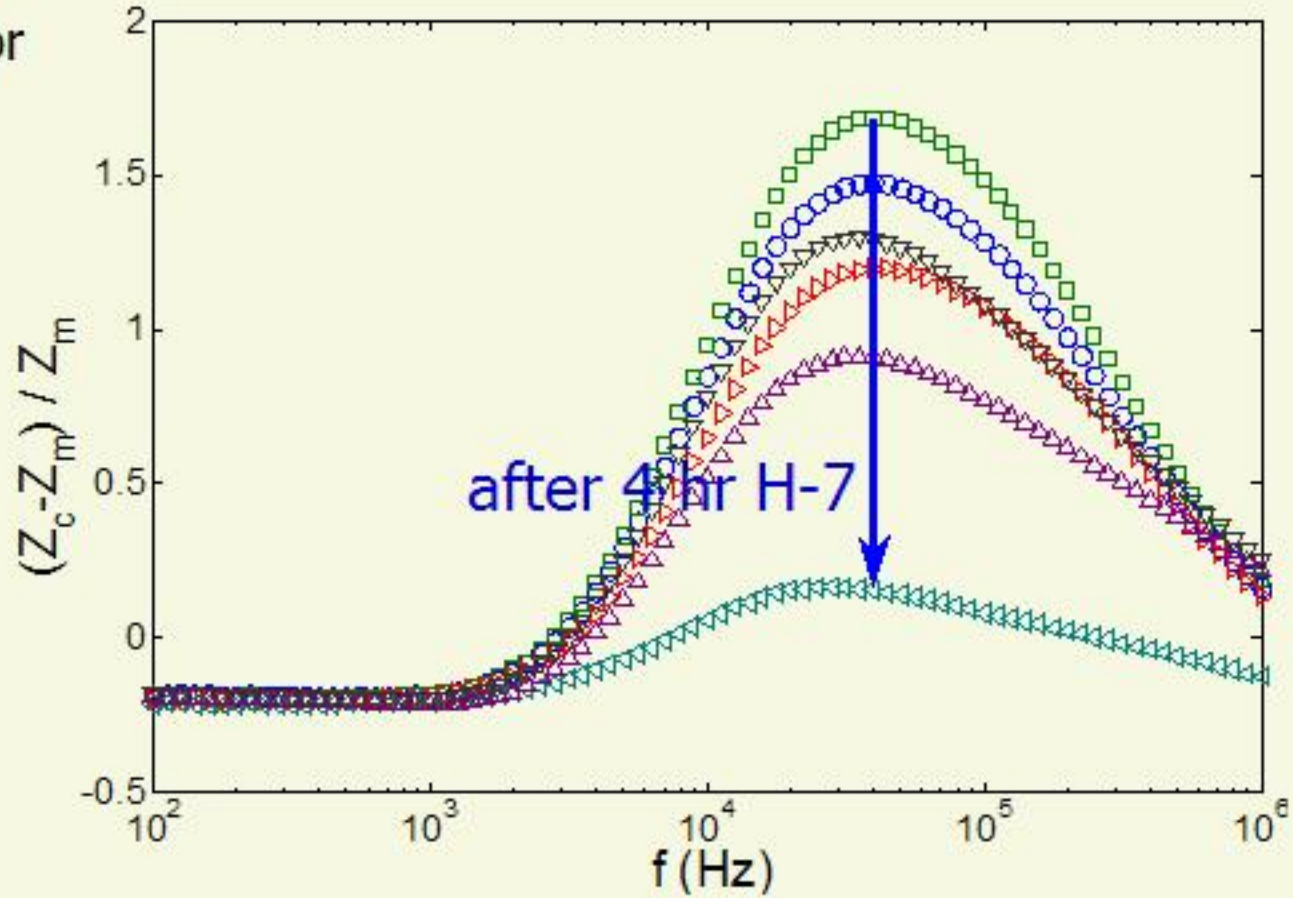


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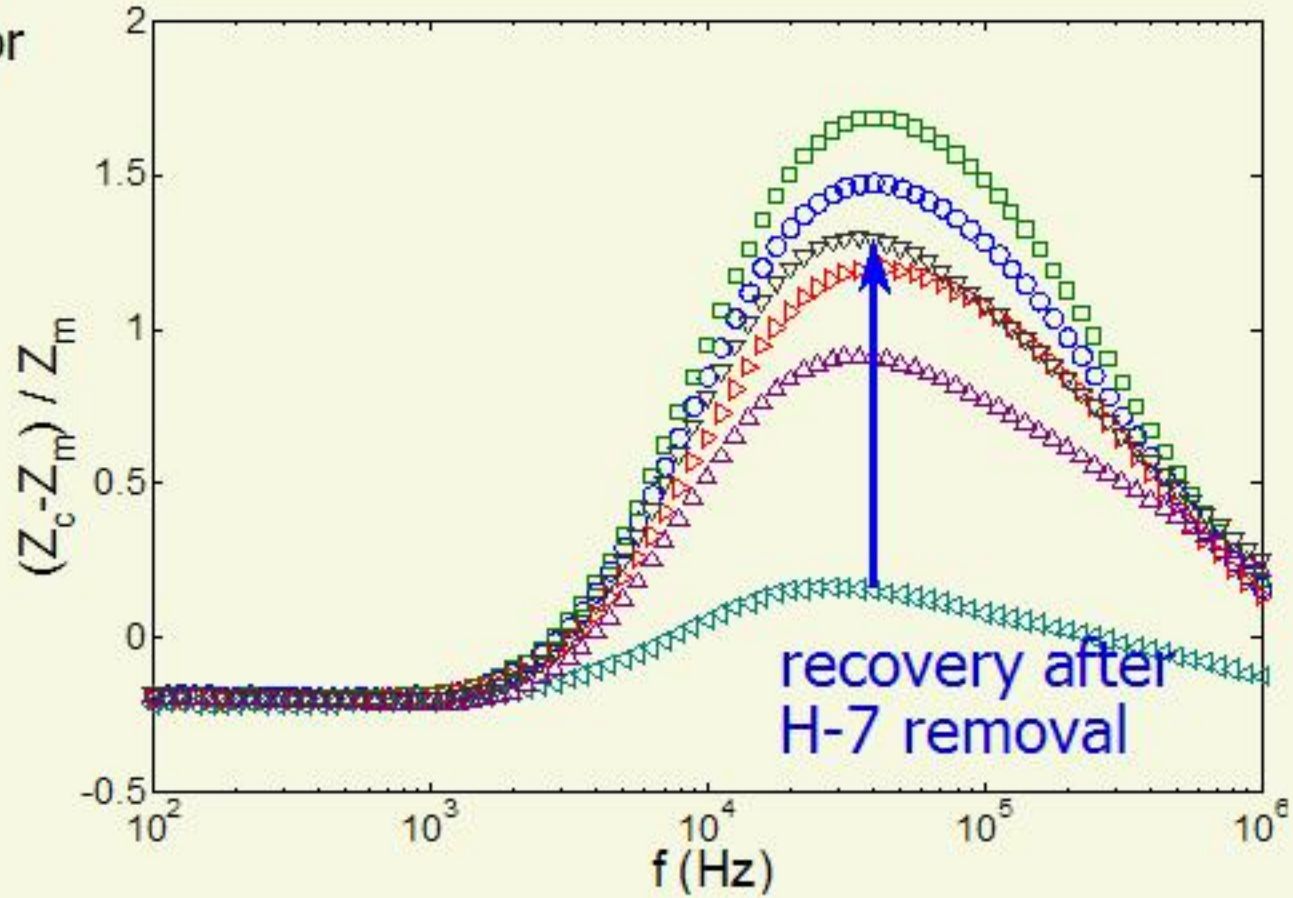


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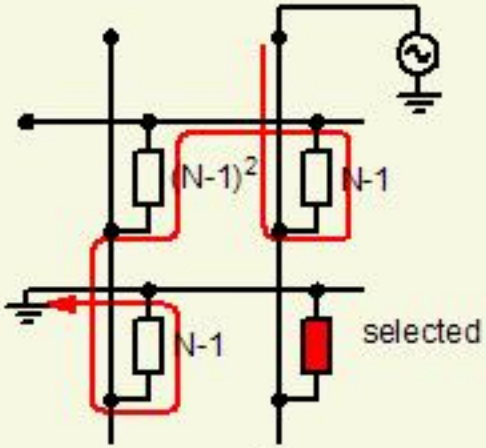
# Biosensor arrays

Goal: to collect data on many cells, using cell-sized electrodes

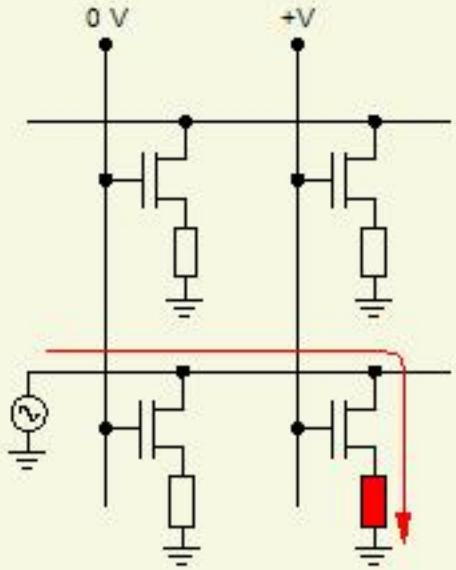
(photo from website below)

60 electrodes 40 μm × 40 μm
200 μm spacing
[http://www.ayanda-biosys.com]

(figure from US patent)




Giaever et al.,
US patent 5187096




Active matrix addressing-
most scalable

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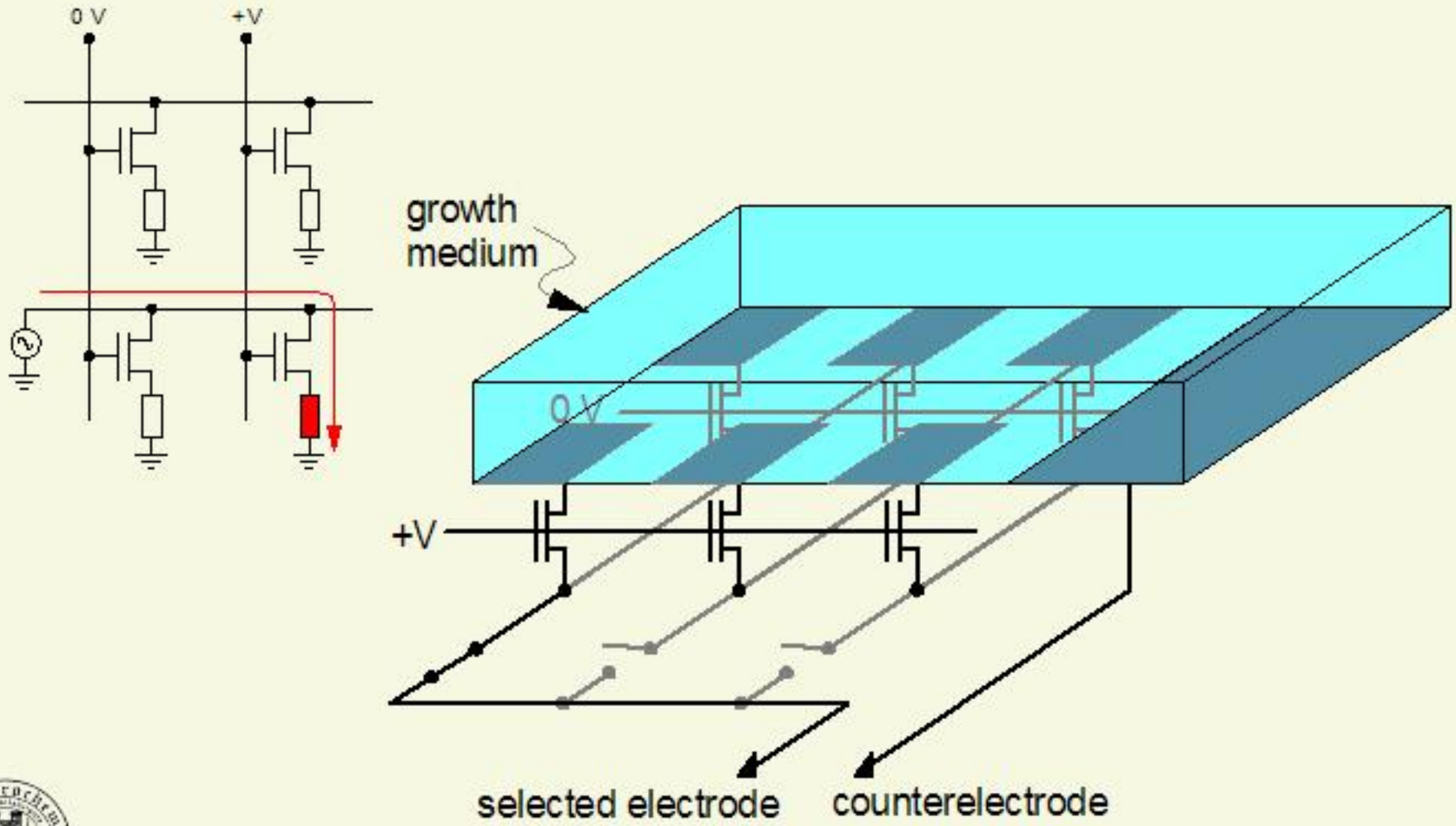
# Biosensor array

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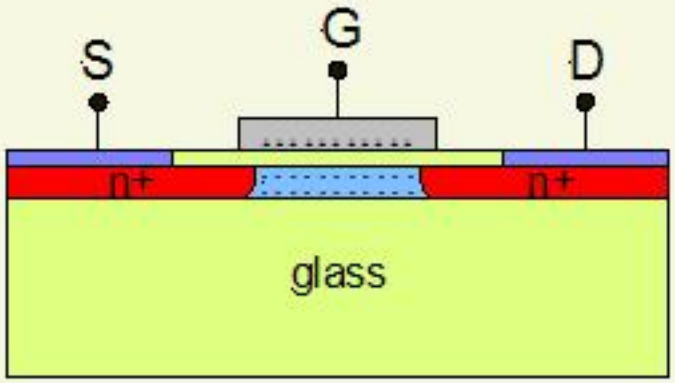
# Thin film FETs

can be made on glass or plastic

commonly used in flat panel displays




(figure showing TFT in AMLCD)

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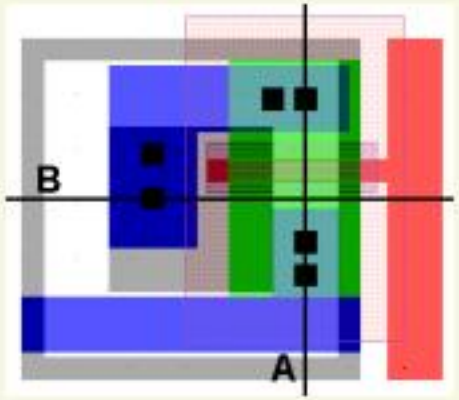
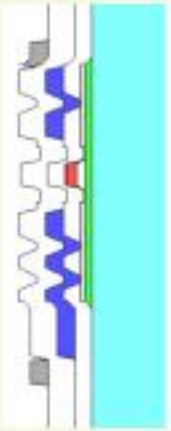
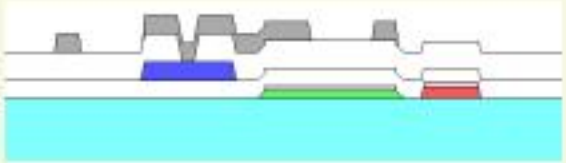
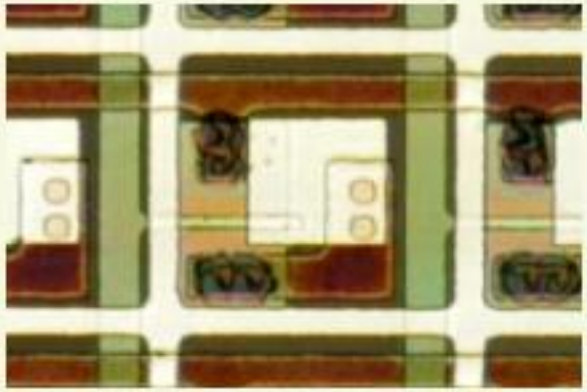
# Fabricated arrays

polysilicon TFTs, gold metallization, 10 x 10 array







[D. Nguyen, H. Xiaoqiu, T. Afentakis, M. Hatalis, M. Domach, and D. Greve, 225th ACS National Meeting, New Orleans, LA, March 23-27, 2003, New Orleans]

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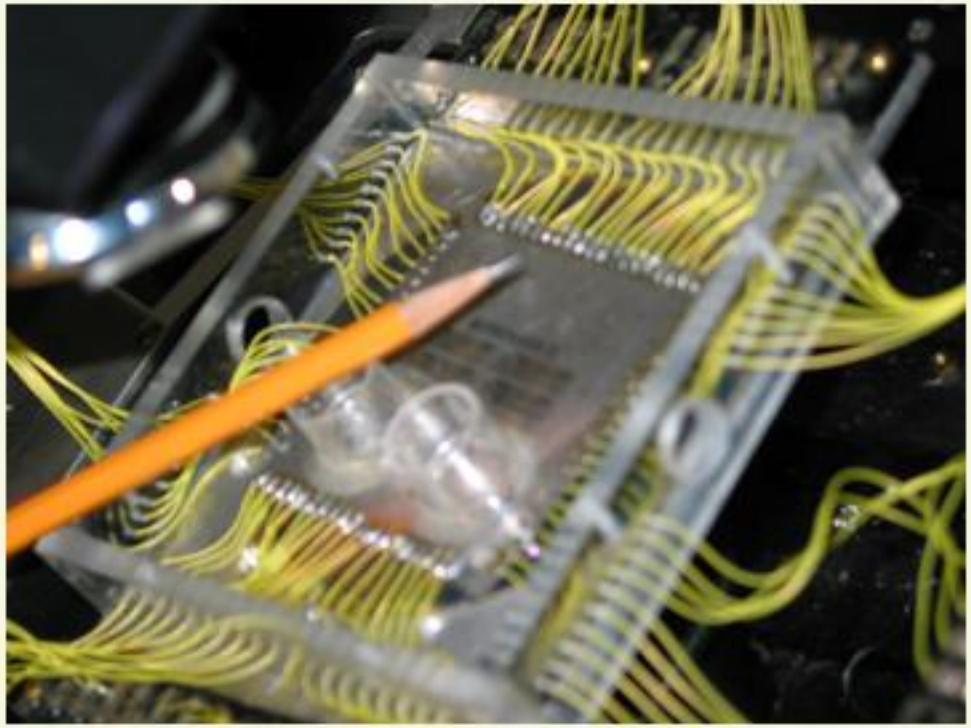
# Completed array



*there were some problems...*

*passivation pinholes*

*transistor gate leakage...*

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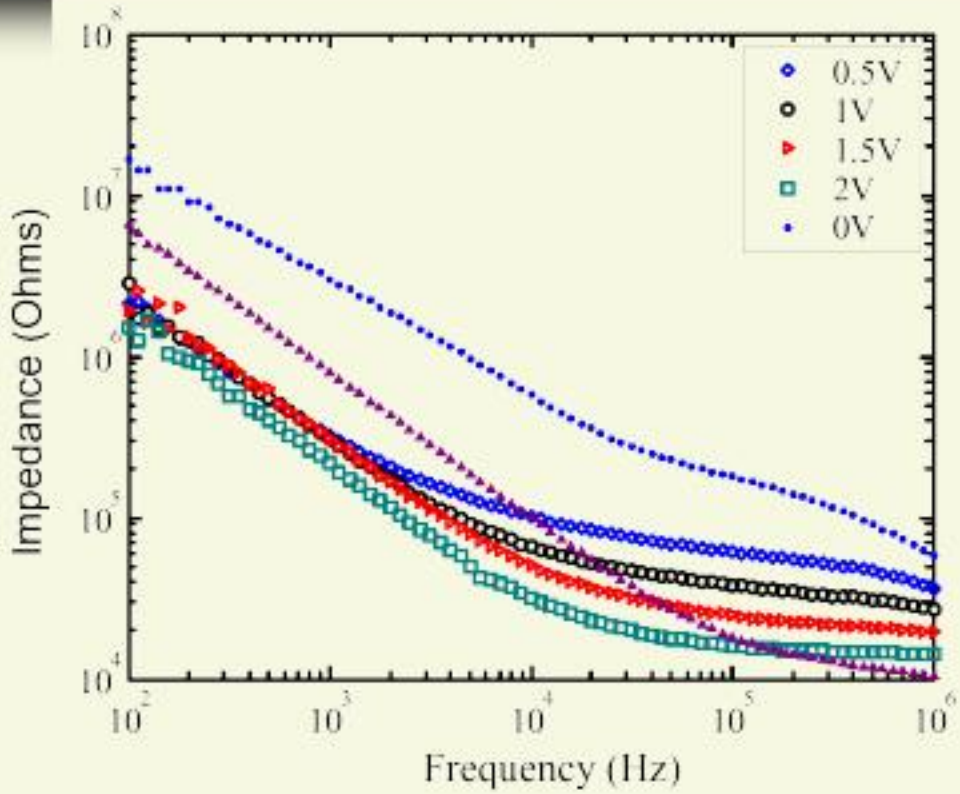
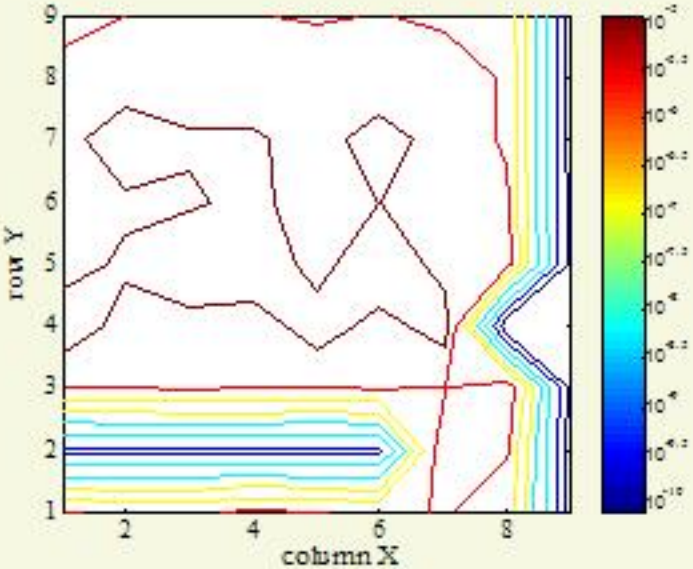
# TFT array problems




electrode addressing test
$Z$ < electrode impedance with access transistor on

measured gate current leakage (no medium)

Also: passivation pinholes caused rapid destruction of metal lines

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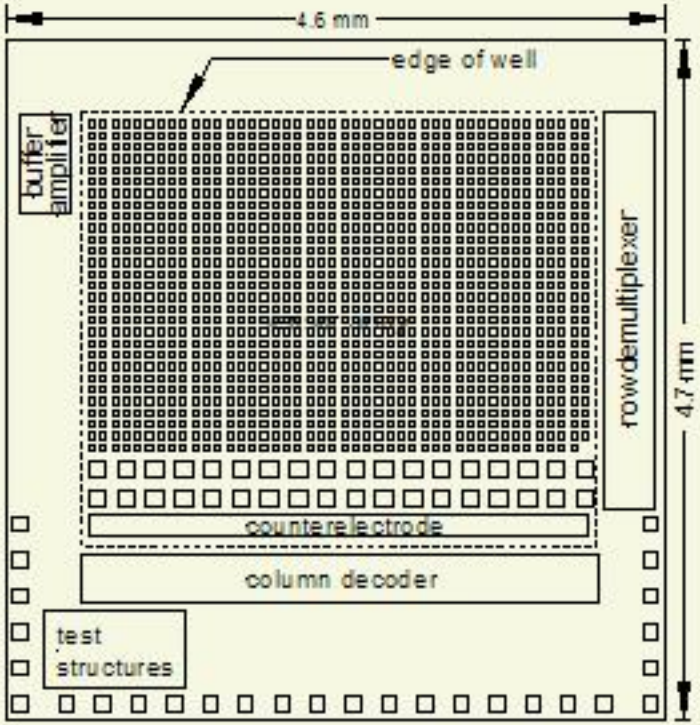
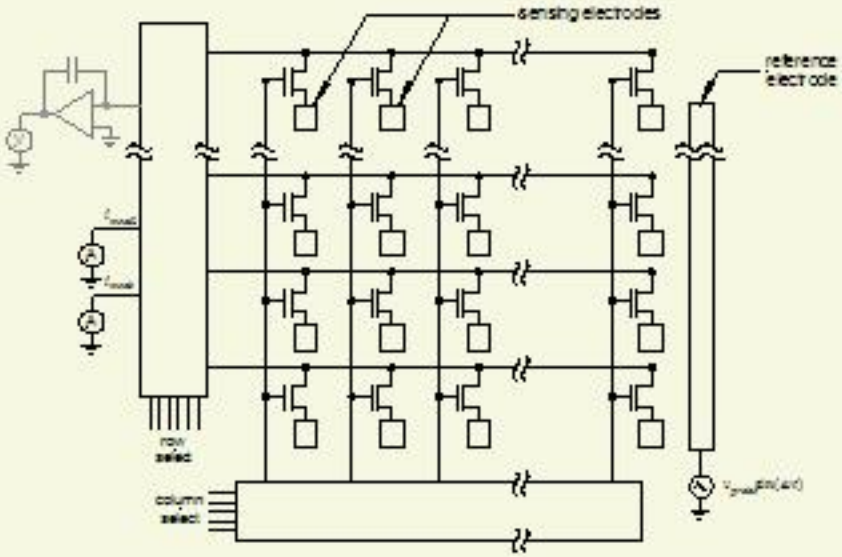
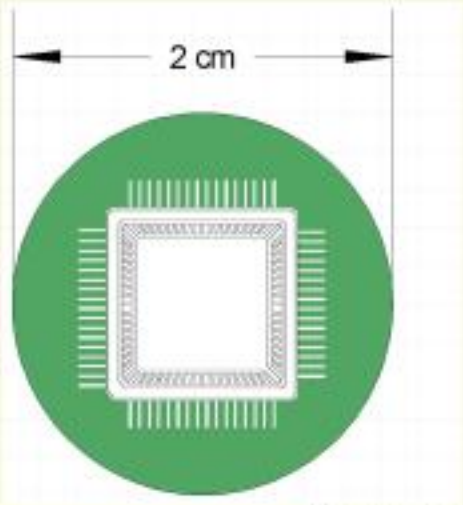
# Do we *need* TFTs?




chip floor plan based on CMOS process
40 x 40 $\mu$m "probe pads" for electrodes
1480 sensing sites *plus* space for
- column decoder
- row multiplexer
- buffer amplifier

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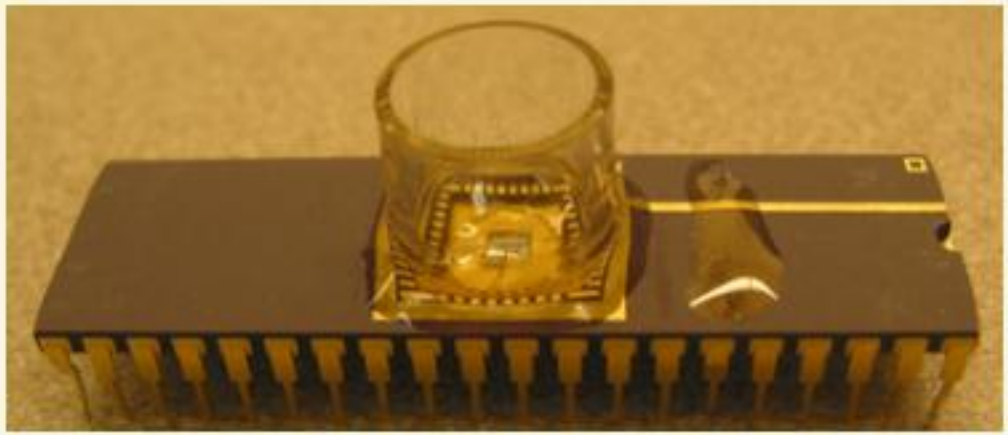
# Problems with CMOS

aluminum electrodes- not bio-compatible

⇒ unmasked, electroless gold plating

bond wires (medium containment)

⇒ low-resolution photolithography



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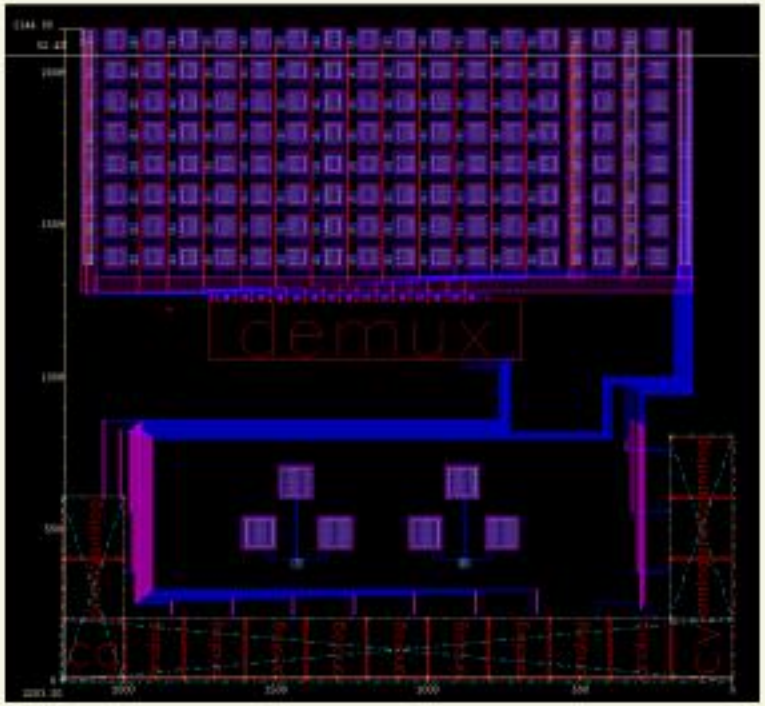
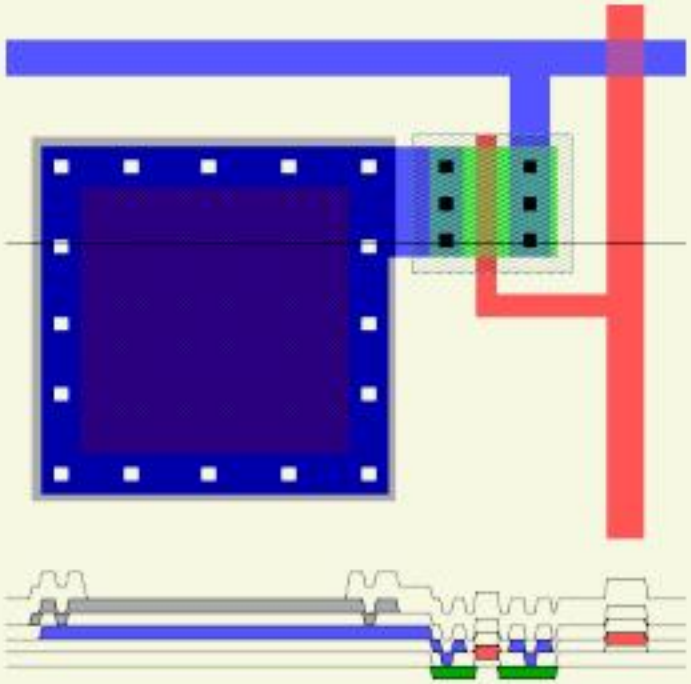
# CMOS array (2 mm x 2 mm)

8 x 15 electrodes + access transistors
column decoder



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# Electroless gold plating

1. surface oxide removal
2. zincation
3. desmut
4. nickel plating
5. gold plating

*deposits metal only on exposed aluminum*

[see Datta et al., ," IEEE Trans. Components and Packaging Technology **22**, 299-306 (1999)]

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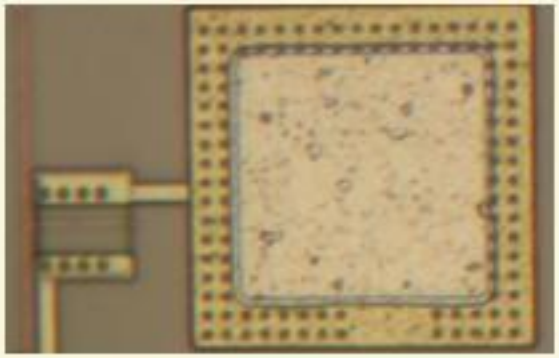
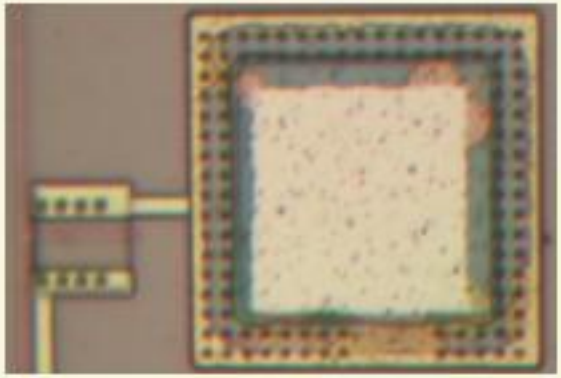
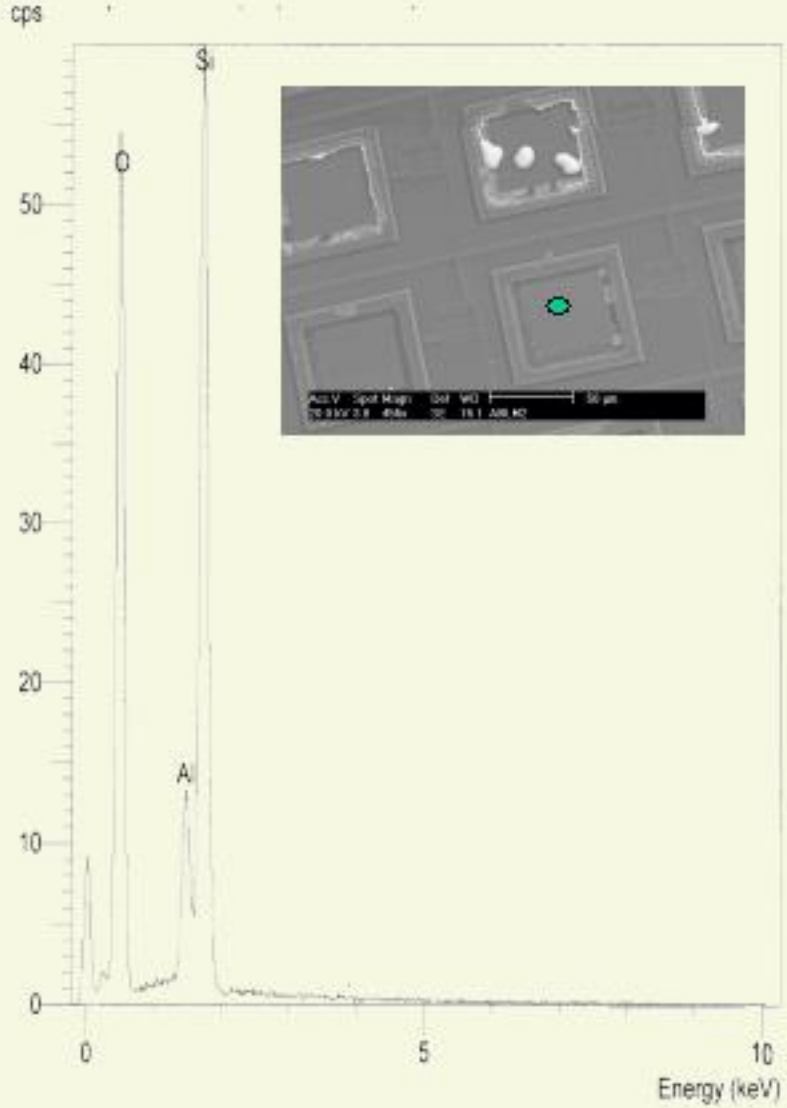
# Plating of packaged chip



before



after plating process

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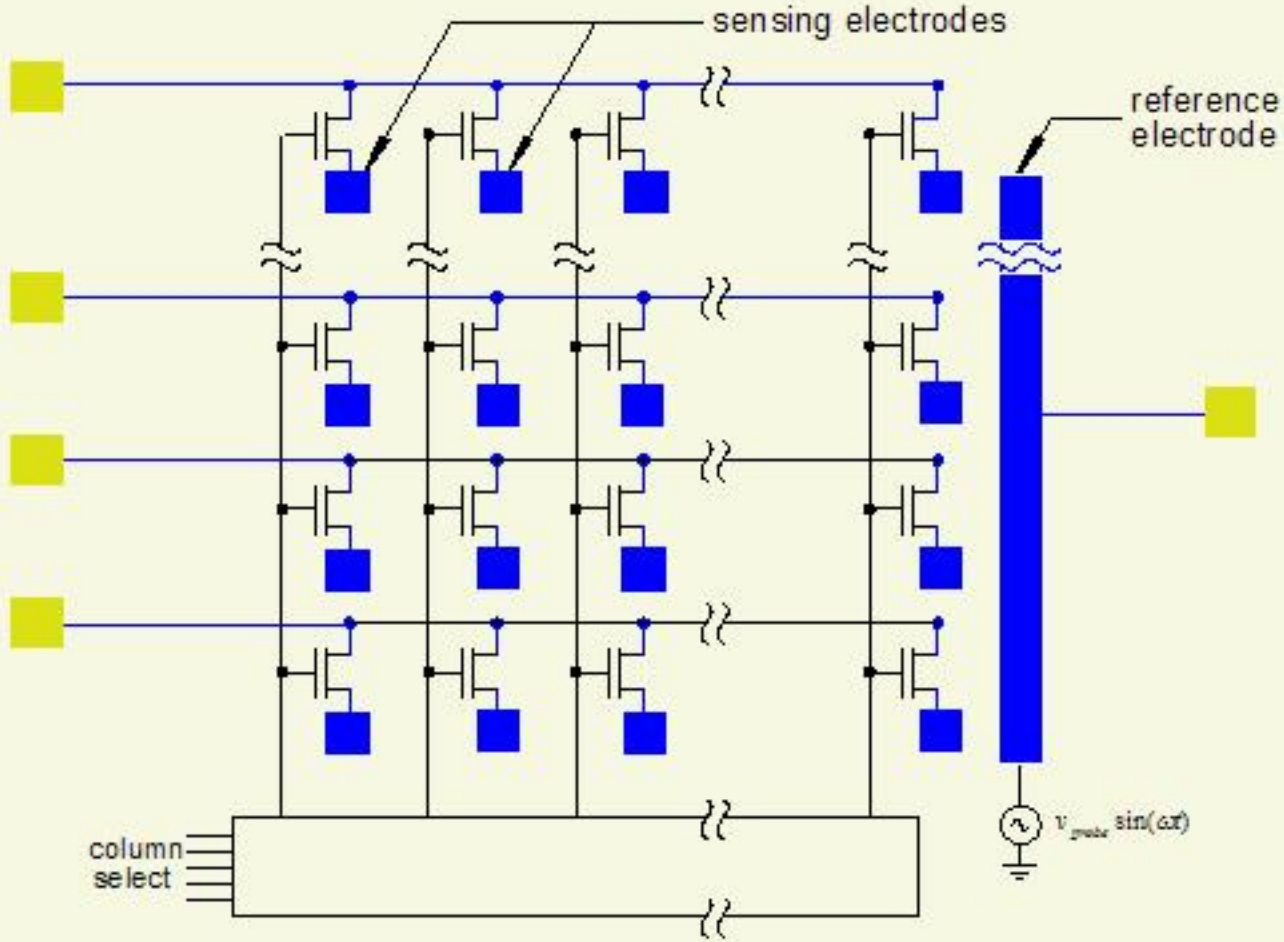
# What went wrong

standard electrode potentials

Au +0.8 V

Al -1.6 V

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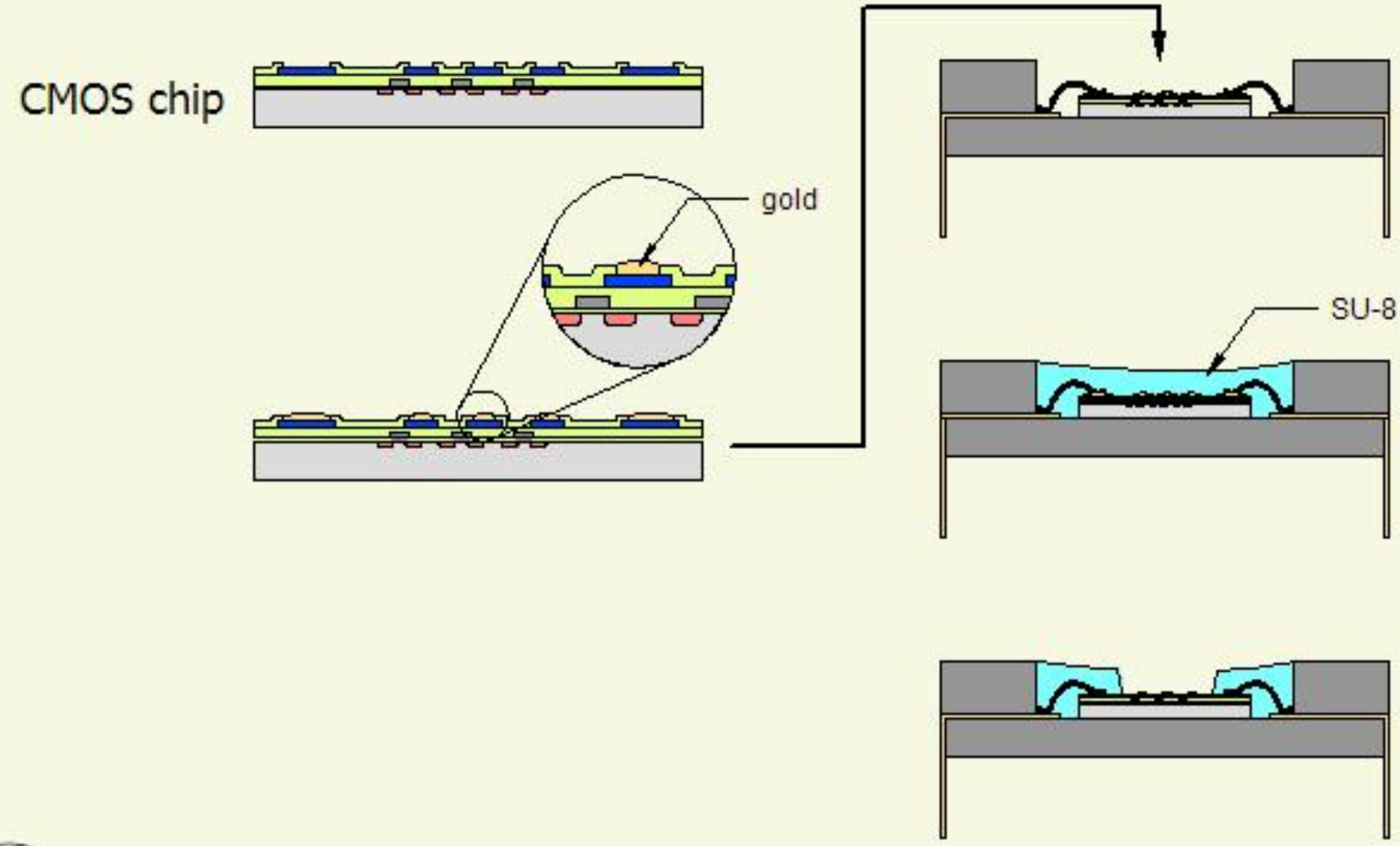
# Plating-first process

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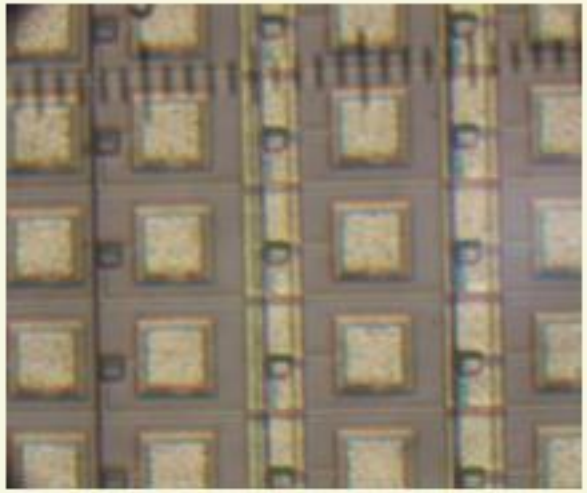
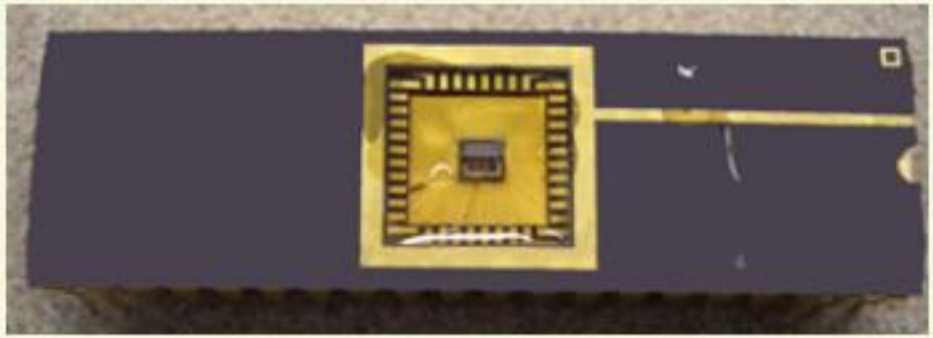
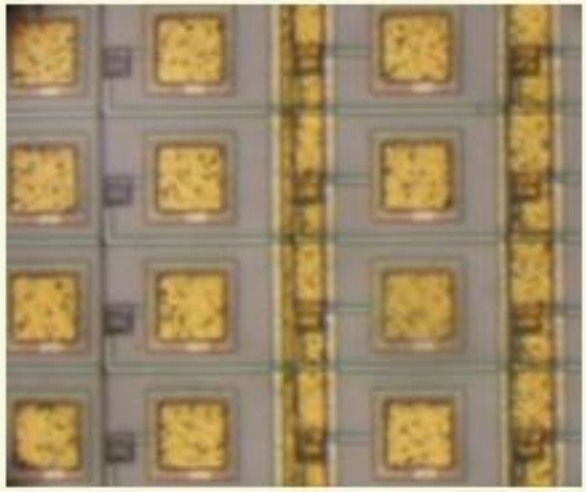
# Gold plating before packaging



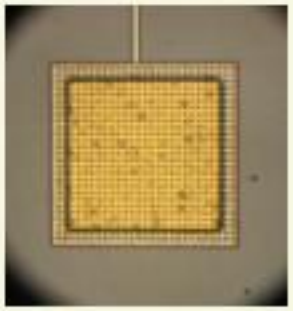
before



after mounting, wirebonding, and SU-8 photolithography



after plating



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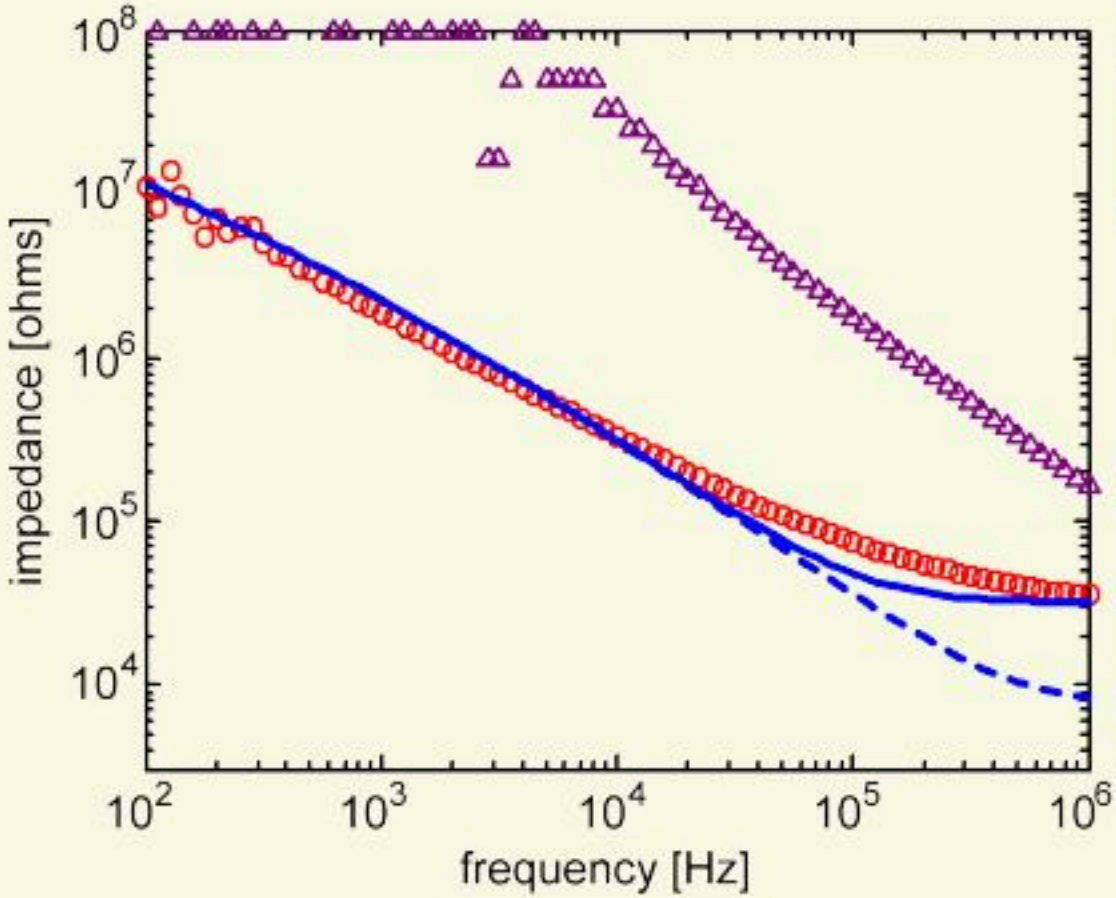
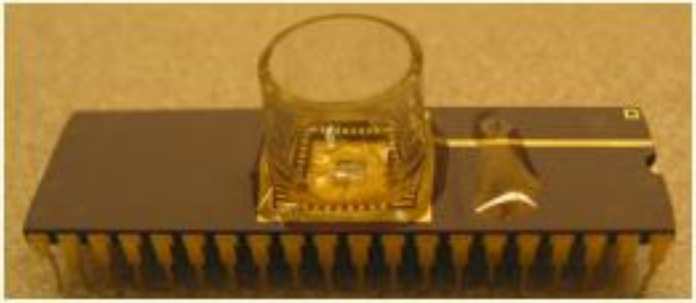
# Gated impedance measurements



50 $\mu$m × 50 $\mu$m electrode

△ $V_g$ = -1 V
○ $V_g$ = 5 V
-- predicted impedance
— simulated impedance

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# Some other applications

- pH, ion sensing
- other biosensors

(some figures from reference below)

[R. Thewes et al., "Sensor arrays for fully-electronic DNA Detection on CMOS," ISSCC Proceedings 2002, paper 21.1, (2002)]

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# Summary

- further development of cell impedance sensing
  - detection of cells, cell growth
  - detection of proteins
  - sensitive to cell-electrode separation (H-7)
- active matrix-addressed, cell-sized impedance sensors have been developed
- other potential applications of similar technology

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