Integrating AC-Electric Fields into the Cell Microenvironments

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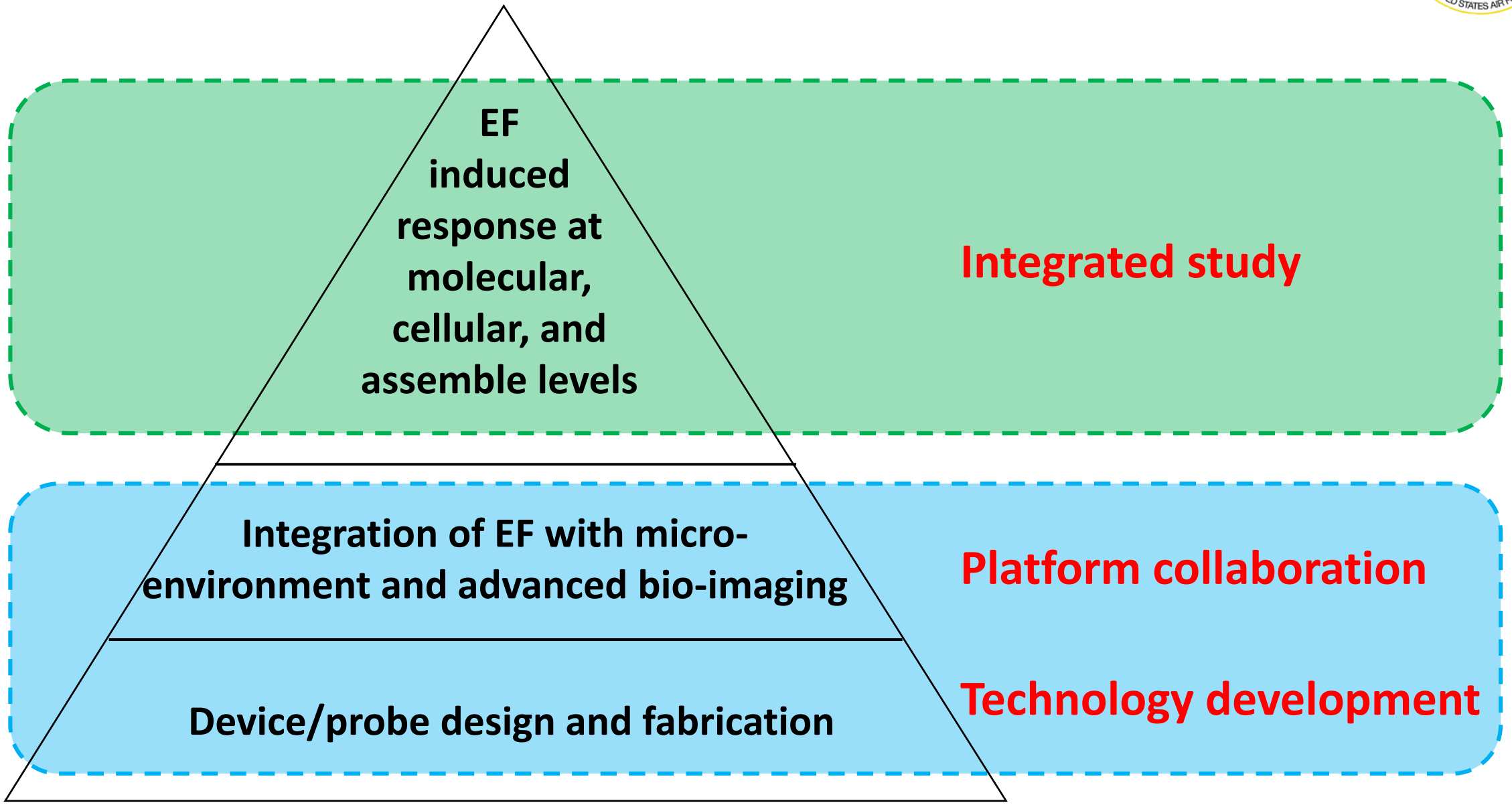
Overview: Our role in this MURI project

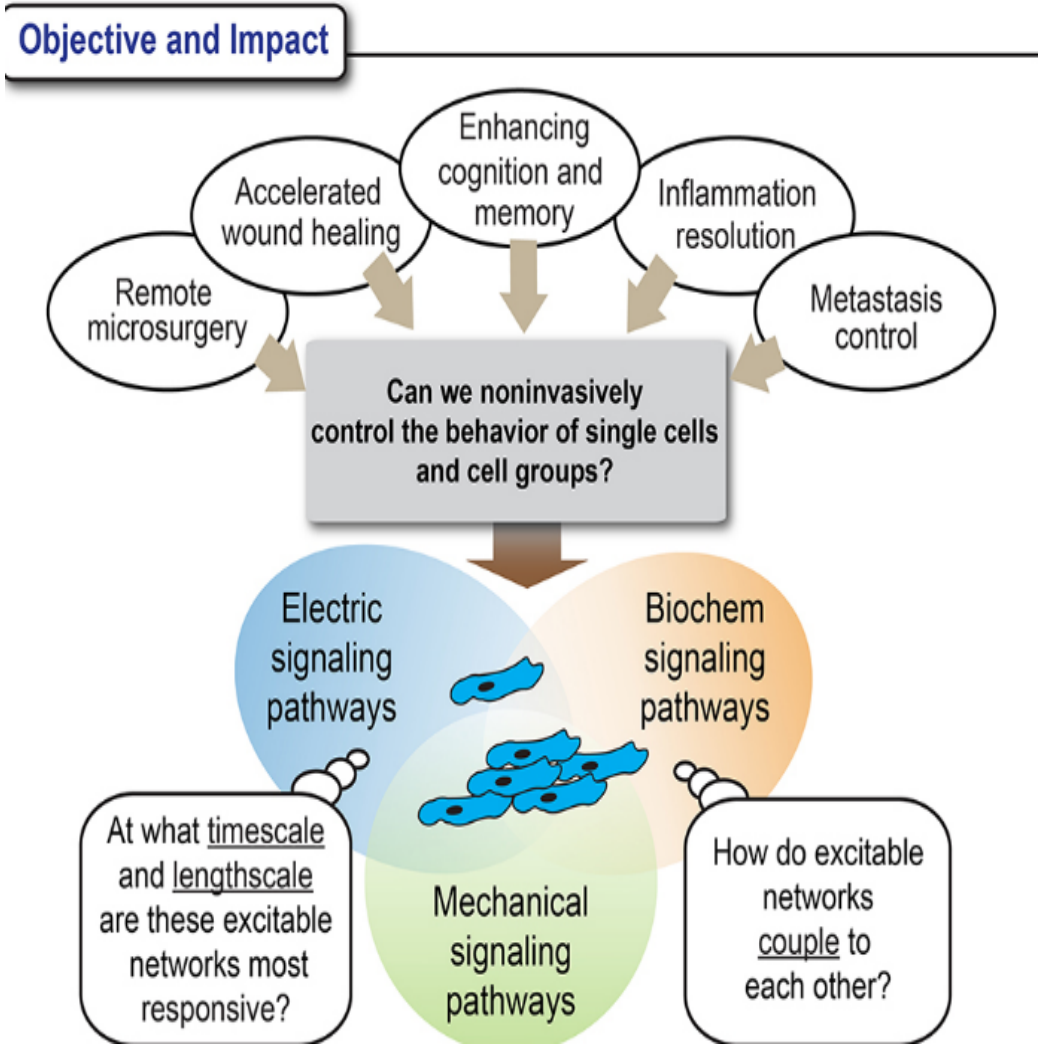


Synergetic activities:

- Visits to UCDavis (4 times). Each visit took one week with fully scheduled experiments
  - Prof. Min Zhao, Dr. Liang Guo, Prof. John G. Albeck at UC Davis
- Visits to UMD (3 times). Each visit took one week with fully scheduled experiments, and joint discussions with UMD and John Hopkins (1 day).
- Extensive preparation through online meetings and emails among all groups before each visit
  - Identify key scientific questions
  - Design experiment plans
  - Prepare samples and protocols
- Customized chips and electronic controllers shared among MURI groups with quick turn around time to meet specific requirements in collaborative studies
- Introduced new cell lines from UCDavis and UMD to ASU
- Built bioimaging platform with environmental control systems at ASU with help from UCDavis and UMD.
- Biweekly web conference discussion between ASU and UCDavis
  - Tracking progress
  - Discussion on data analysis
  - Preparation of manuscript
- Regular discussion with all MURI team members through web conference to receive additional comments and suggestion

Our interest

Combining spatiotemporally modulated electric field stimuli with biochemical and mechanical signals to study intra-/inter-cellular signaling network couplings



Electric Field:

- Polarized field – direct interaction on charges and dipoles
- Localized – high spatial resolution
- Accurate timing – high temporal resolution

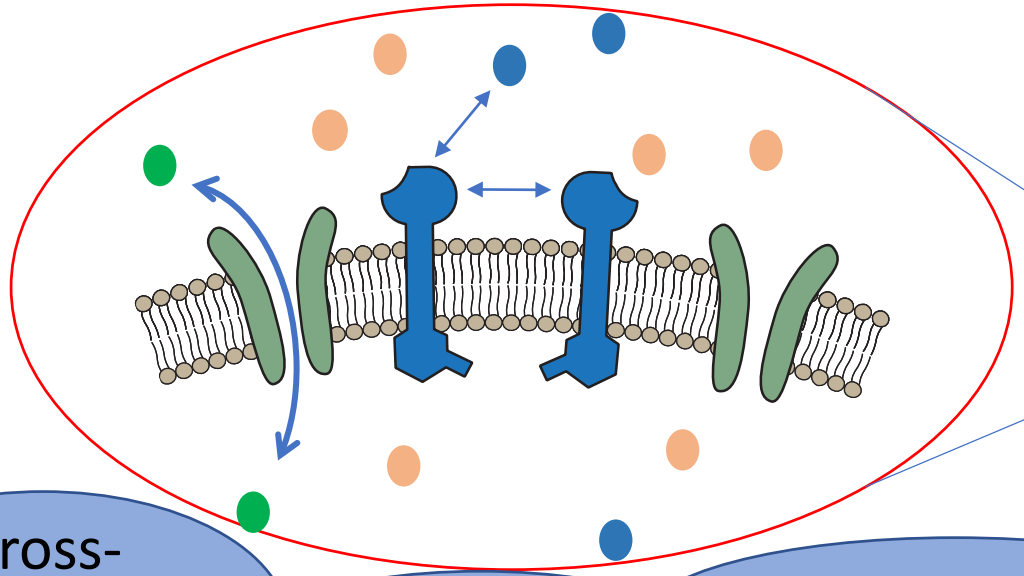
Biochemical stimuli:

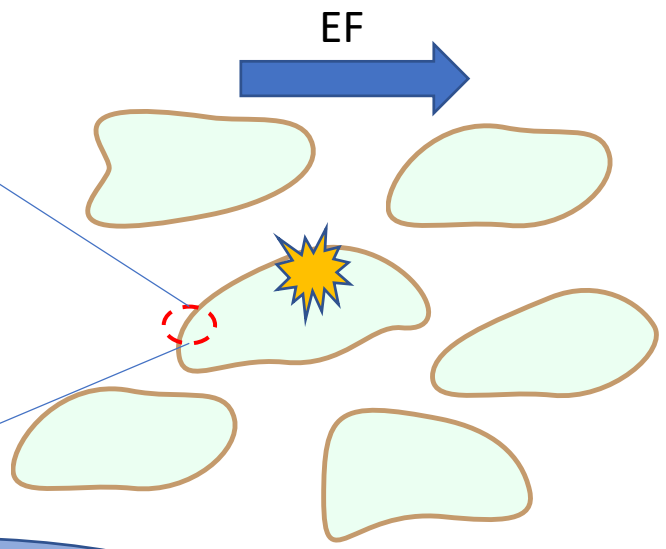
- Rich toolbox
- Highly specific
- Molecular level information
- Typically can only apply to a group of cells – low spatial resolution
- Gradient change is usually slow – low temporal resolution

Mechanical stimuli:

- Typically static patterns, passive stimulation only
- Can achieve subcellular spatial resolution
- Distinct behavioral consequence

A lot of things can happen when EF is applied to a cell





- Heterogeneity
- Intercellular communication
- Coordinated behaviors

Cross-membrane potential

Non-Faradaic ion flux

Electroporation

Heat stress

Membrane permeability

Faradaic/Redox process

- Global environment change
- Multiple signaling pathways involved
- Long time scale
- Large length scale (diffusion and inter-cellular process could be involved)

- Changes of binding energy/kinetics at the membrane
- EF-dipole, EF-charge interactions of membrane protein/lipid domains
- Others?

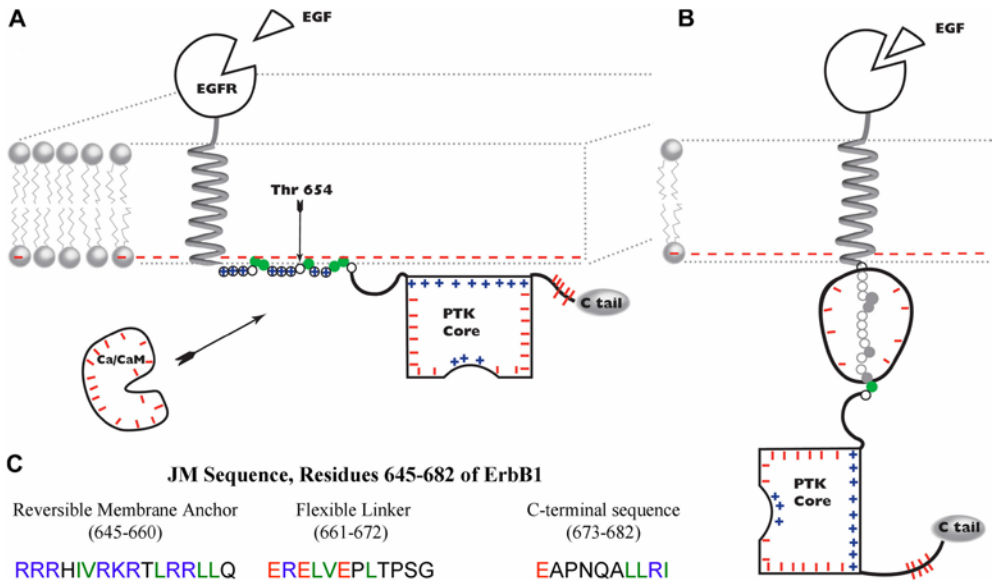
- **Localized?**
- **Time scale?**
- **Frequency dependency?**
- **Specificity?**

Hypothesis and predictions

- Direct, specific EF modulation at molecule level without chemical complication
  - We hypothesize that AC EF could modulate the electrostatic interactions of proteins or other biomolecules at the cell membrane interface, leading to functional conformational changes

- Predictions:
  - Highly specific to membrane protein
  - Threshold behavior
  - Time scale (0.1 μs ~ 1 ms?)
  - Frequency dependent

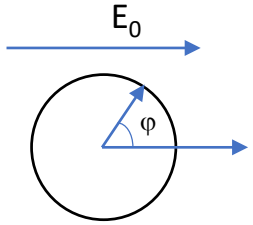
How do we separate/distinguish direct EF interaction from other effects?



McLaughlin S, et. al, *J. Gen. Physiol.* 126(1):41–53.

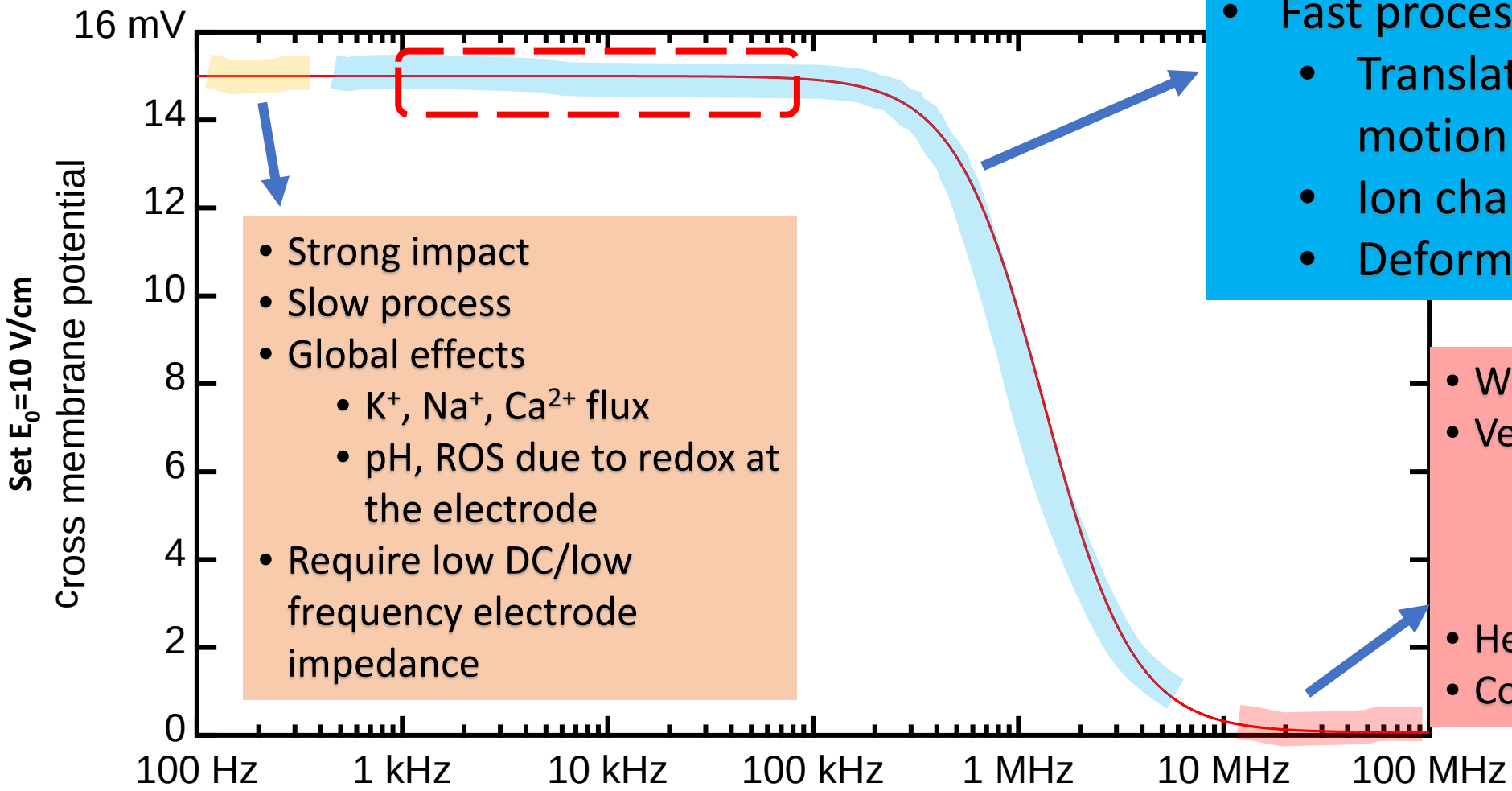
Charge/dipole interactions ⟺ Conformational changes

EF at the cell membrane depends on frequency



$$\psi(j\omega) = \frac{3}{2} E_0 R\cos\varphi \frac{1}{1 + j\omega\tau_m} \quad \text{Where } \tau_m = RC_m\left(\frac{1}{\sigma_1} + \frac{1}{2\sigma_e}\right)$$

$$\psi(j\omega) = \frac{3}{2} E_0 R\cos\varphi \frac{1 + j\omega\tau_{m2}}{1 + j\omega\tau_{m1}} \quad \text{Where } \tau_{m1} = \frac{\varepsilon_m}{\frac{d\ 2\sigma_e\sigma_1}{R\sigma_1 + 2\sigma_e} + \sigma_m},\ \tau_{m2} = \frac{\varepsilon_1 + 2\varepsilon_e}{\sigma_1 + 2\varepsilon_e}$$

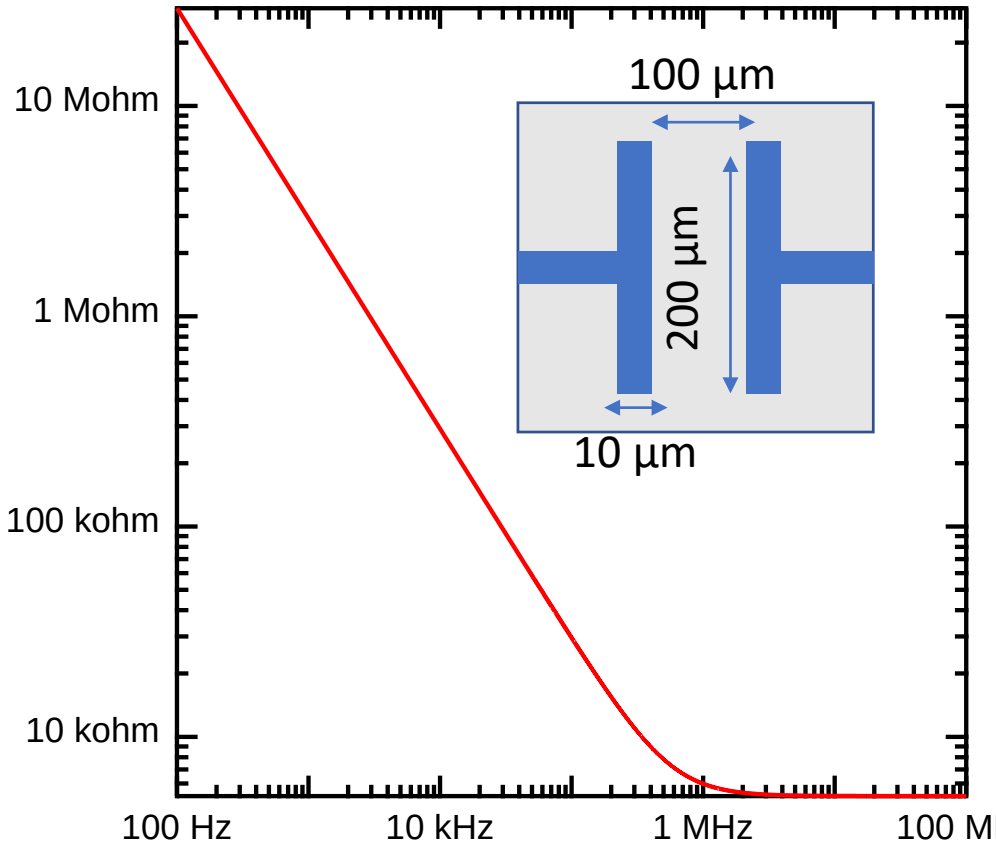


- Strong impact
- Slow process
- Global effects
  - K^+, Na^+, Ca^{2+} flux
  - pH, ROS due to redox at the electrode
- Require low DC/low frequency electrode impedance

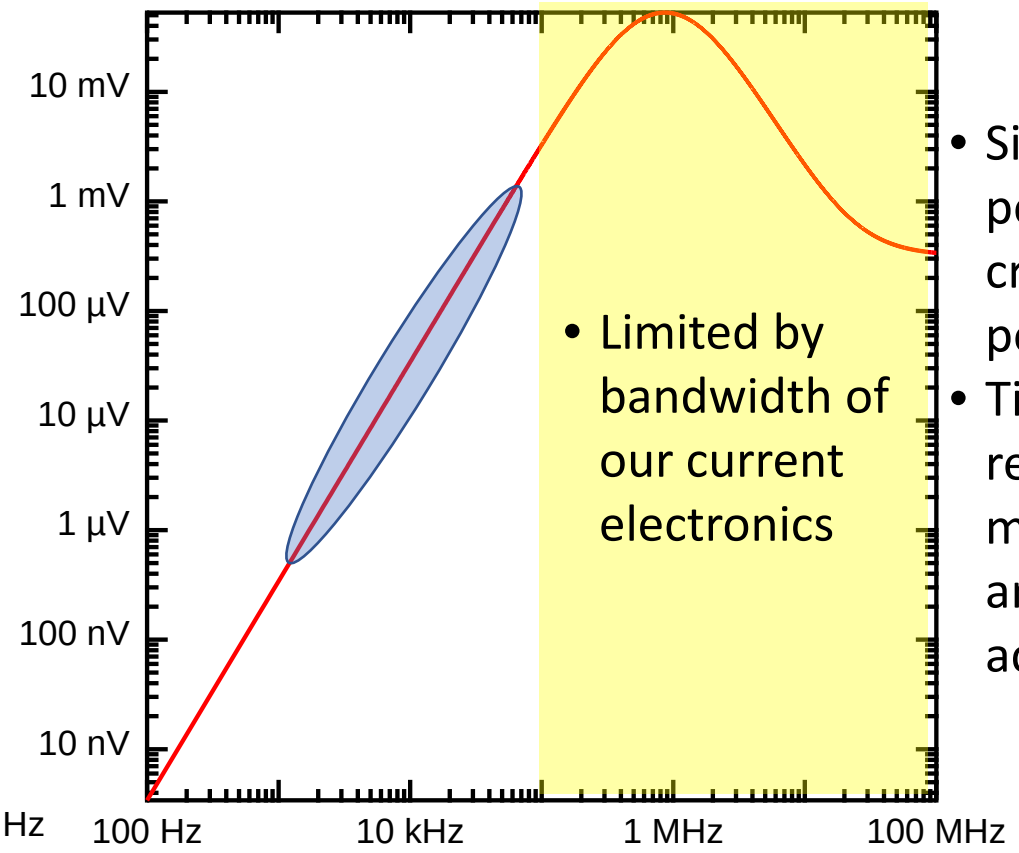
- Strong ~ medium impact
- Fast process
  - Translational/rotational motion of proteins/domains
  - Ion channel polarization
  - Deformation of membrane

- Weak impact on membrane
- Very fast process
  - small ions? electronic states? polarization states?
- Heating?
- Coupled wirelessly

Impedance of microelectrodes and the cell membrane together provide a band-pass of AC EF



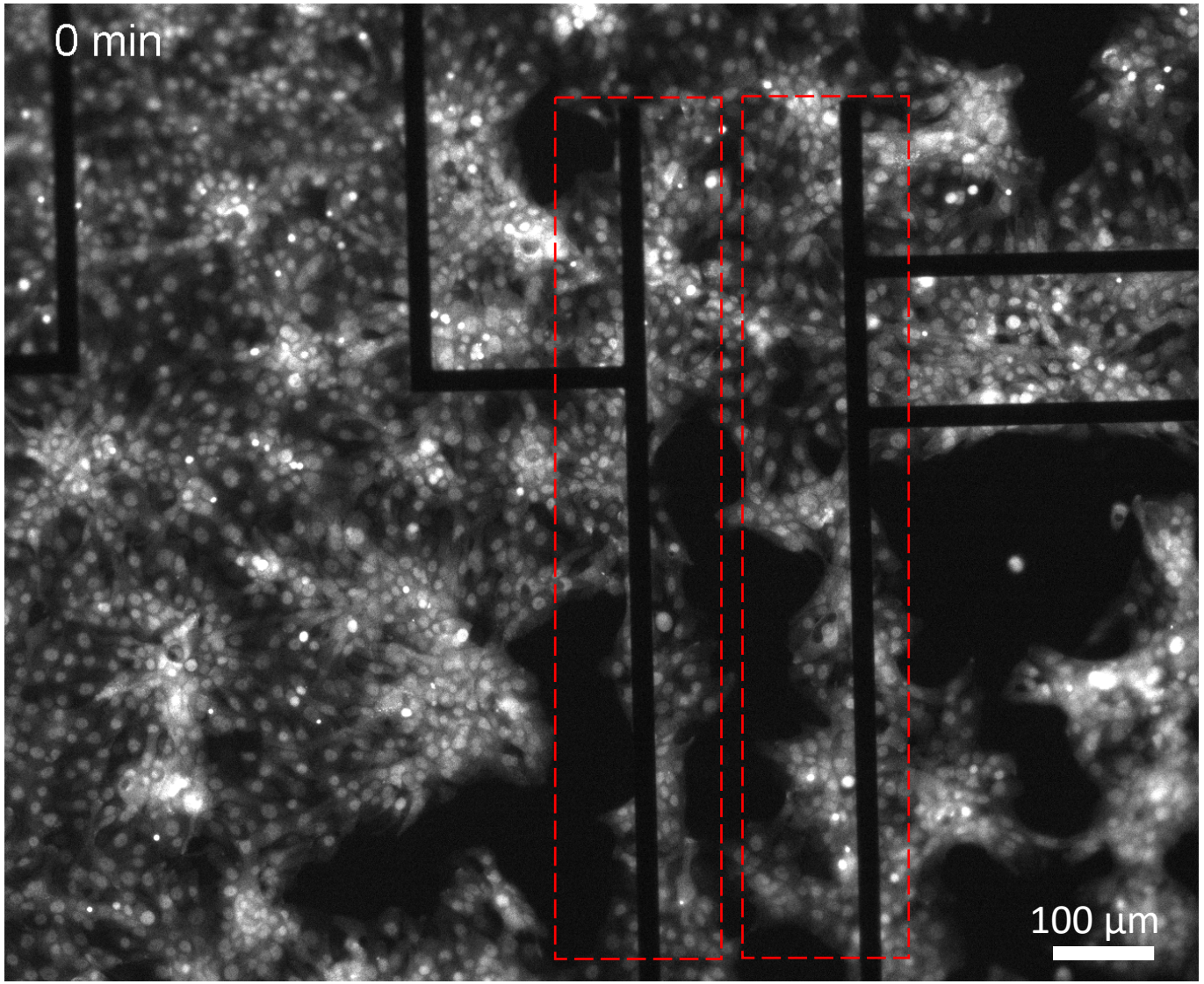
Impedance of microelectrodes – high pass of signals

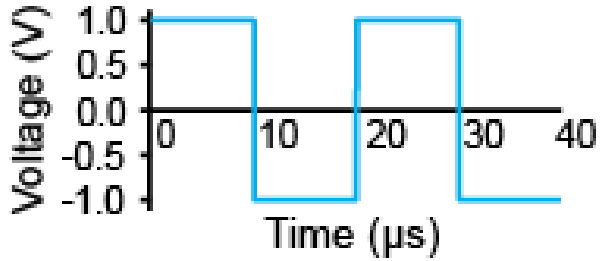


Simulation of cross-membrane potential when applying ± 1 V AC bias at the microelectrode pairs

- Significant perturbation of cross-membrane potential
- Time scale (µs~ms) relevant to membrane protein and ionic channel activities

A showcase:
Surprising observation of localized AC EF activation of ERK in MCF10A cells





- Reproducible, non-invasive, and highly localized ERK activation by bipolar AC EF pulses
- Threshold of activation: nominal EF strength 10~100V/cm
- Faradaic process excluded at the electrode/cell interface

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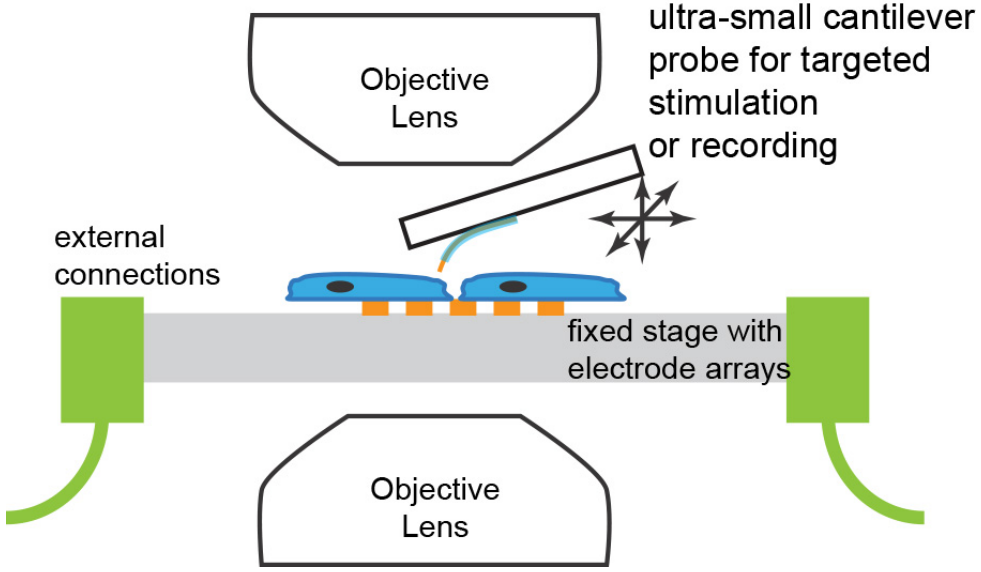
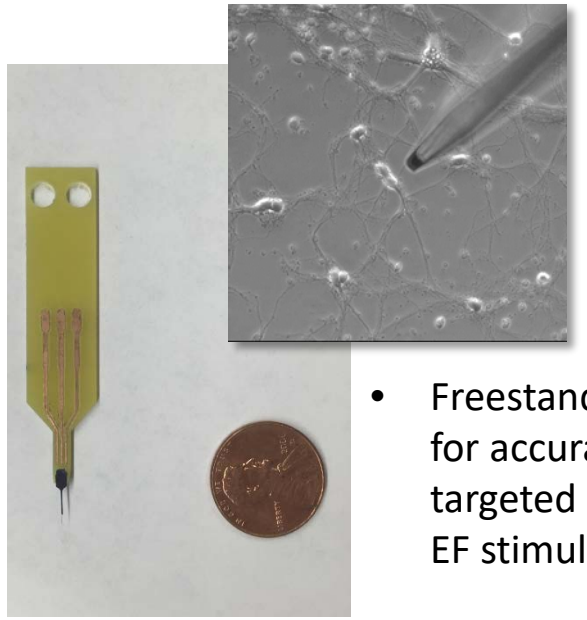
ASU

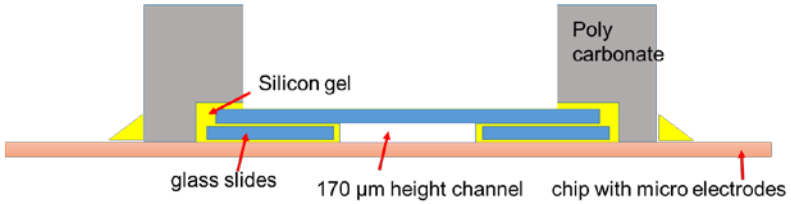
Microelectrode Arrays and Freestanding probes provide highly localized EF delivery to cells, allowing sub-cellular level spatial resolution

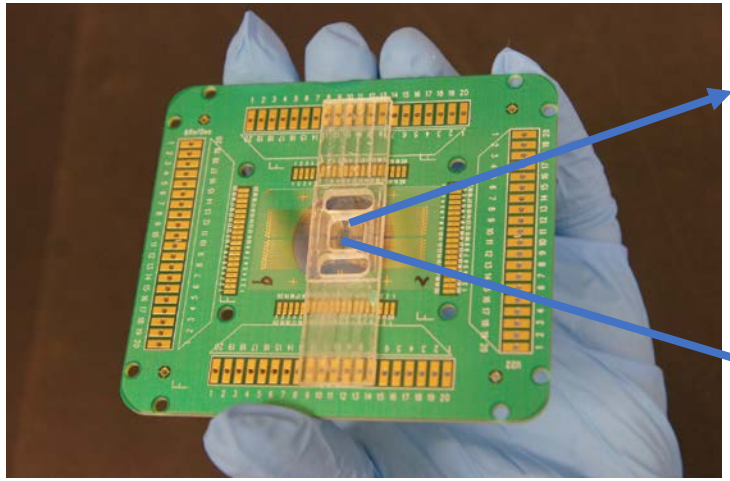


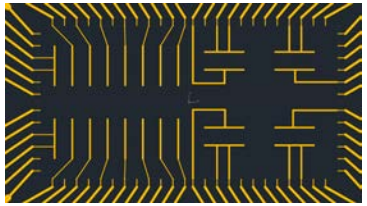


- Freestanding probe for accurately targeted sub-cellular EF stimulation

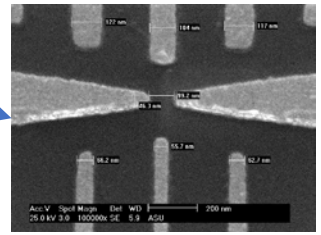
We have utilized UC Davis experience in their petridish-based system, and designed a chamber that can be assembled around our chips to give optimal delivery of global EF, and imaging capability.





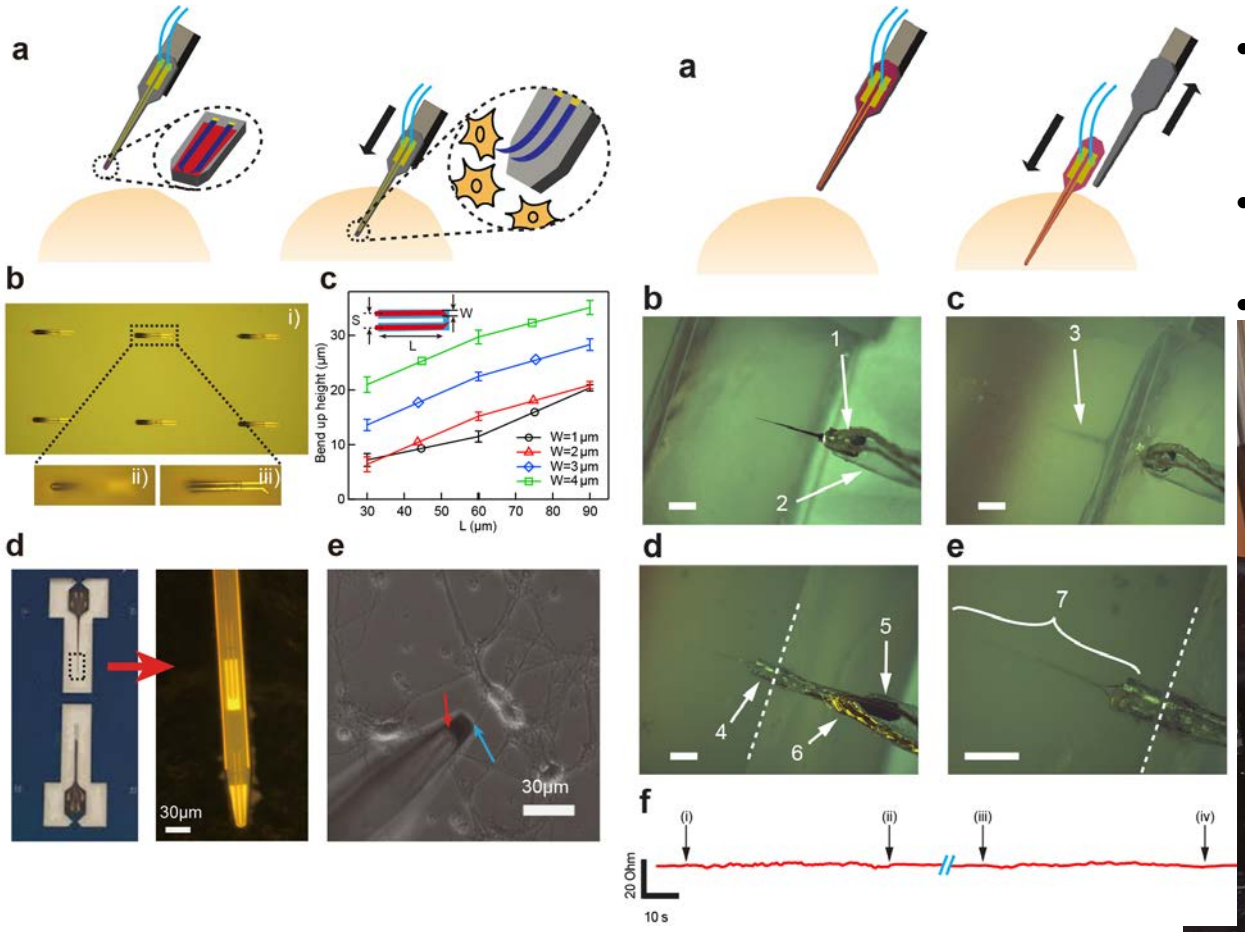


- Surface microelectrode arrays for cellular level EF stimulation

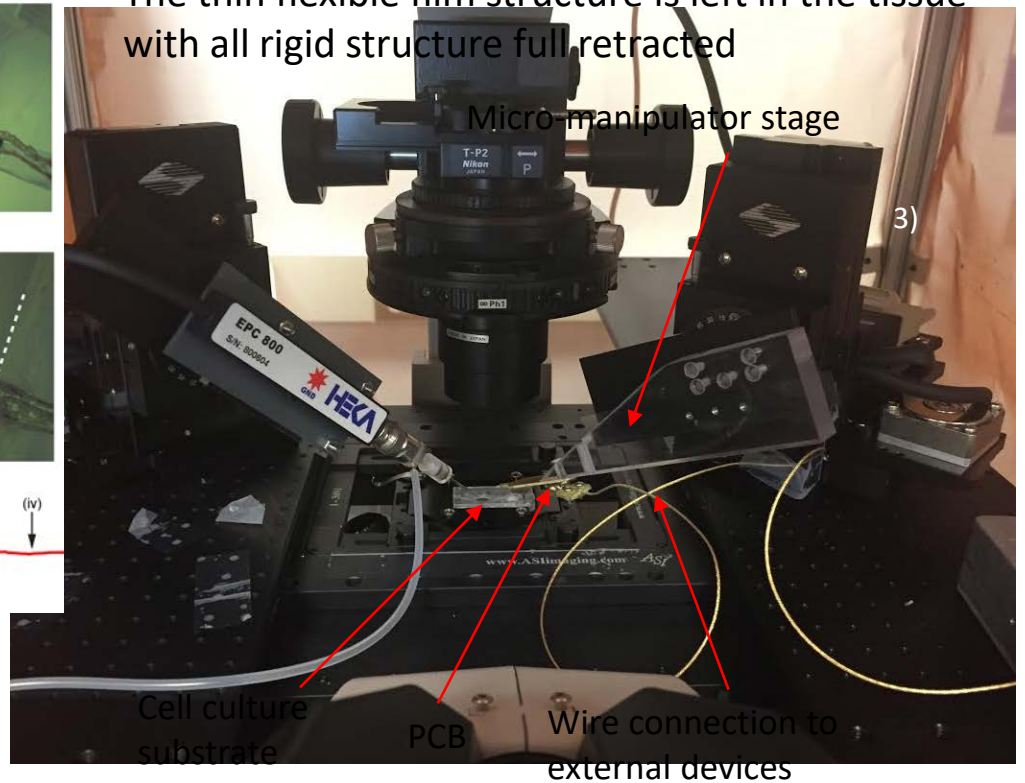


- Surface nanoelectrodes for subcellular level EF stimulation

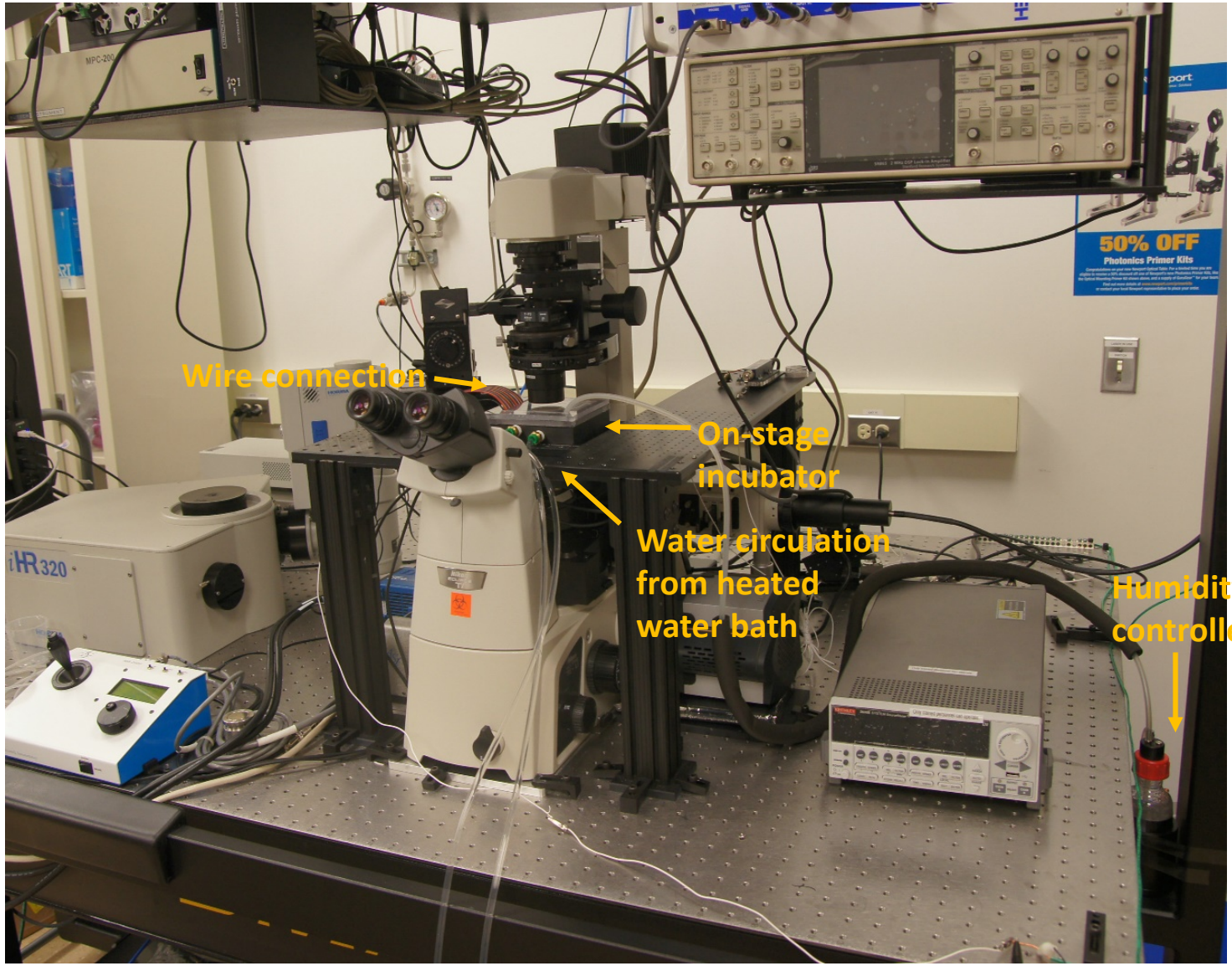
Ultra-small freestanding probe: accurate targeting individual cells



- The whole top device layer is build within flexible polymer
- The backbone silicon structure is used only for surgically deliver the probe accurately into deep tissue
- The biologically degradable sacrificial layer dissolves within 20-30 minutes after the insertion
- The thin flexible film structure is left in the tissue with all rigid structure full retracted



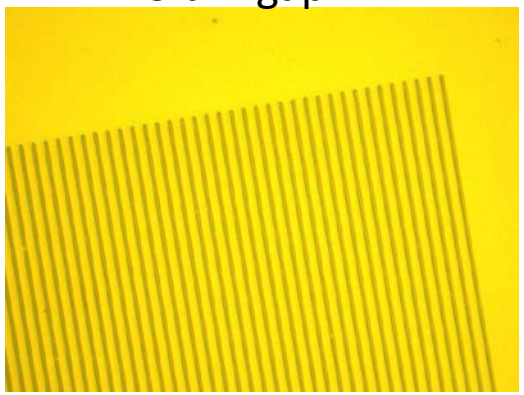
EF stimulation and imaging system built at ASU



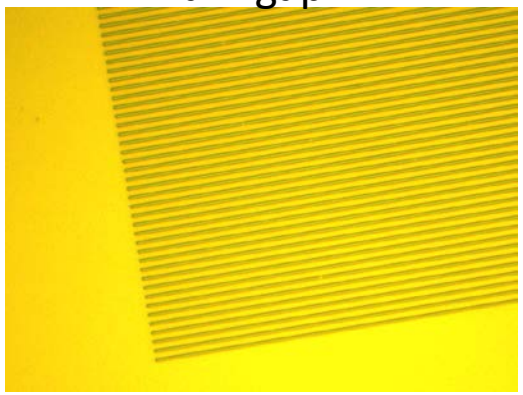
Integrating local EF with mechanical stimuli

We need to define topological features with high spatial resolution and accurate alignment with electrode edges.

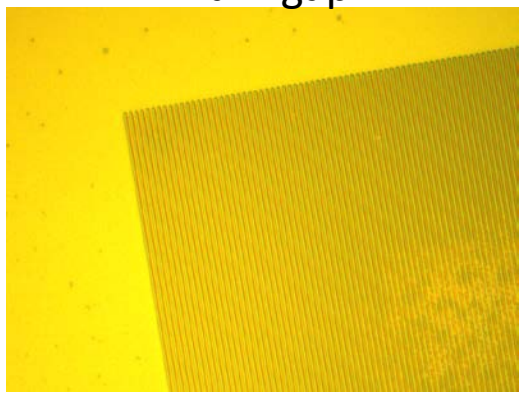
3 um gap



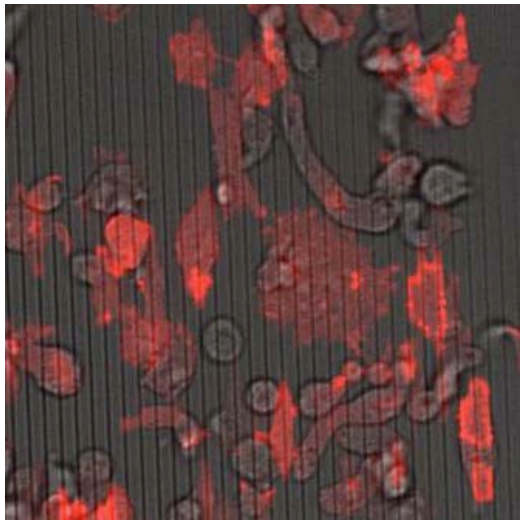
2 um gap

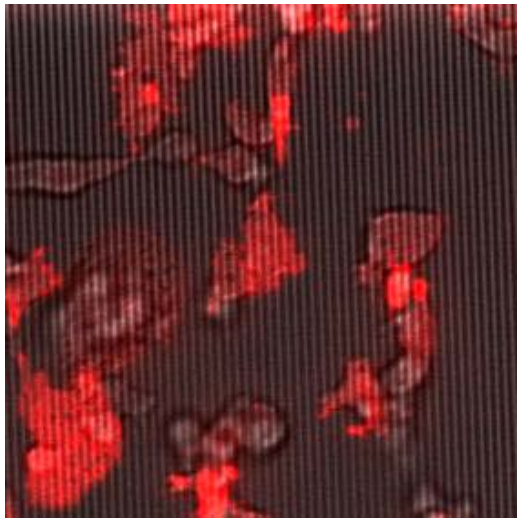


1 um gap



0.5 um thick SU8 stripes with 0.7 um width and different gap sizes





These SU8 stripes can provide good guidance to dicty cell migrations.