14th International Conference & Exhibition on

Biosensors & Bioelectronics

Webinar, Monday, Sept. 28, 2020

Microwaving a Biological Cell Alive – Label-free Non-invasive Cell Characterization by Broadband Impedance Spectroscopy

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Cornell University

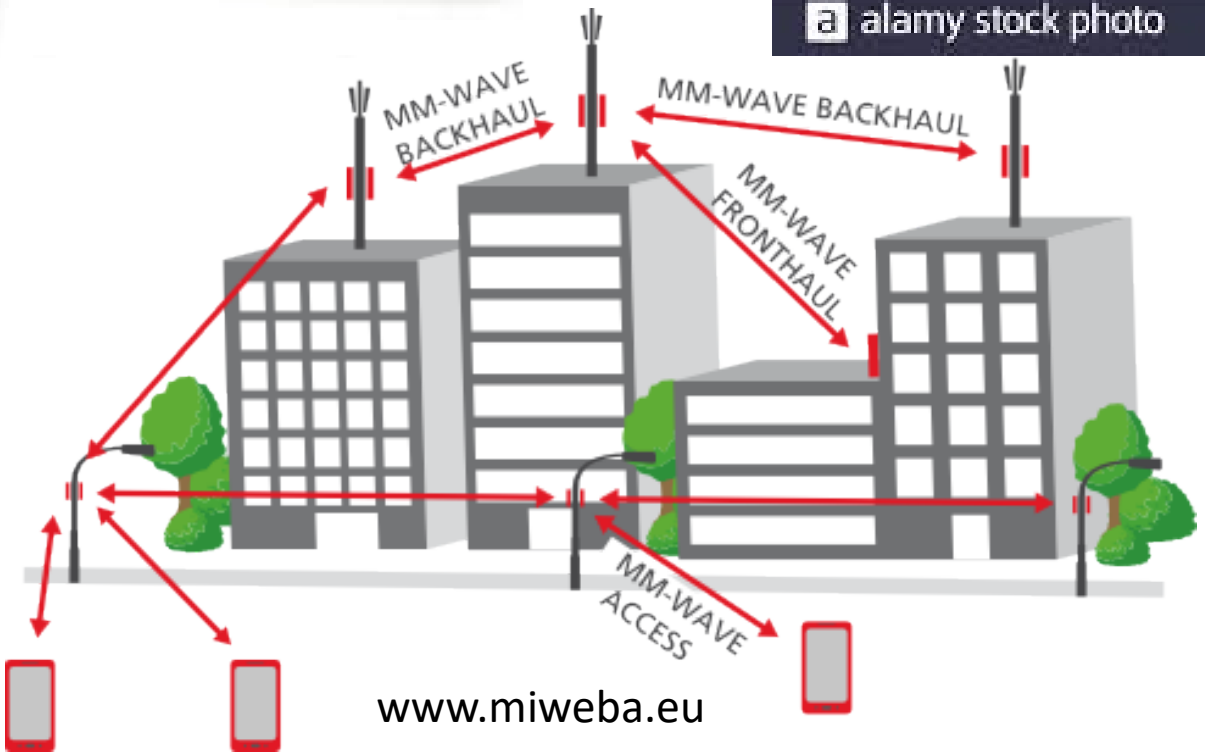
Outline

- **Introduction to microwave in bio applications**
- **Impedance spectroscopy of a biological cell**
- **Cell trapping and detrapping by dielectrophoresis**
- **Broadband microwave cell characterization**
- **Real-time monitoring of reversible electroporation**
- **Conclusion**

Familiar Applications of Microwave

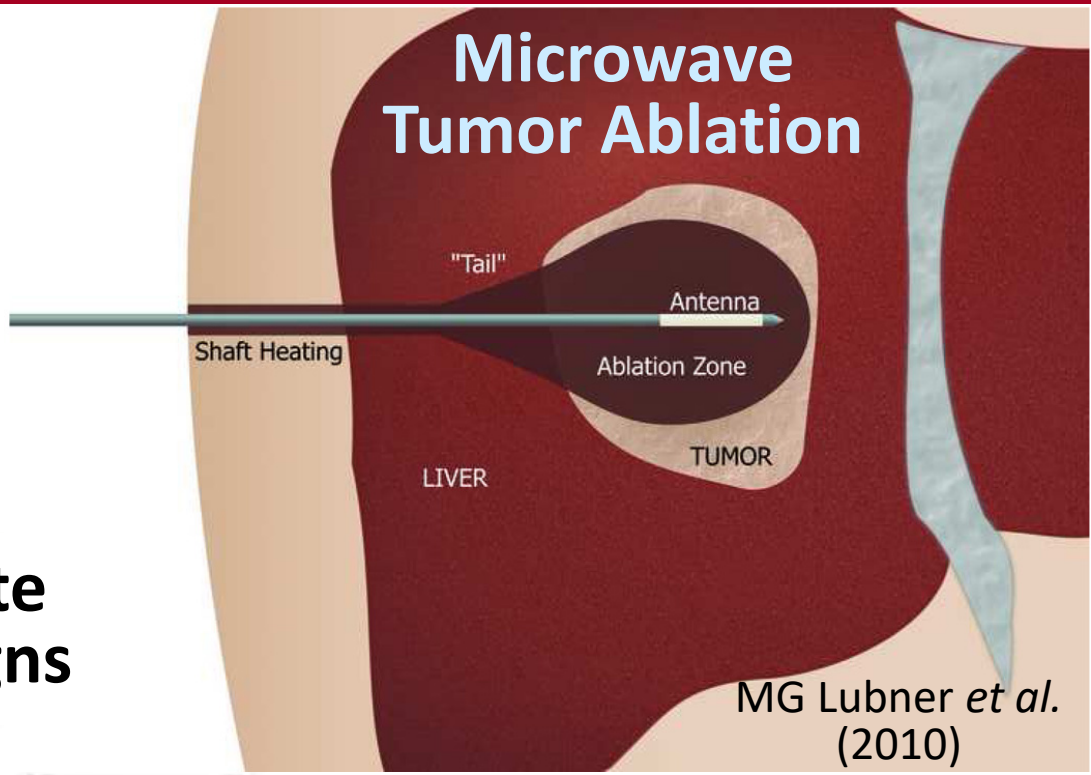






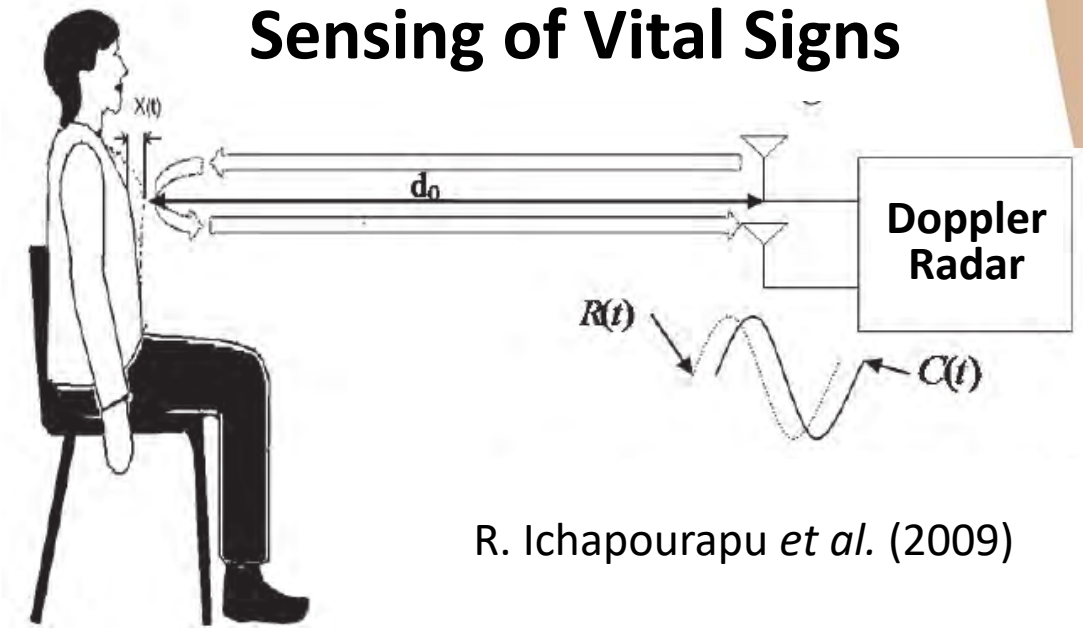
www.miweba.eu

Biomedical Applications of Microwave



MG Lubner *et al.* (2010)

Microwave Remote Sensing of Vital Signs



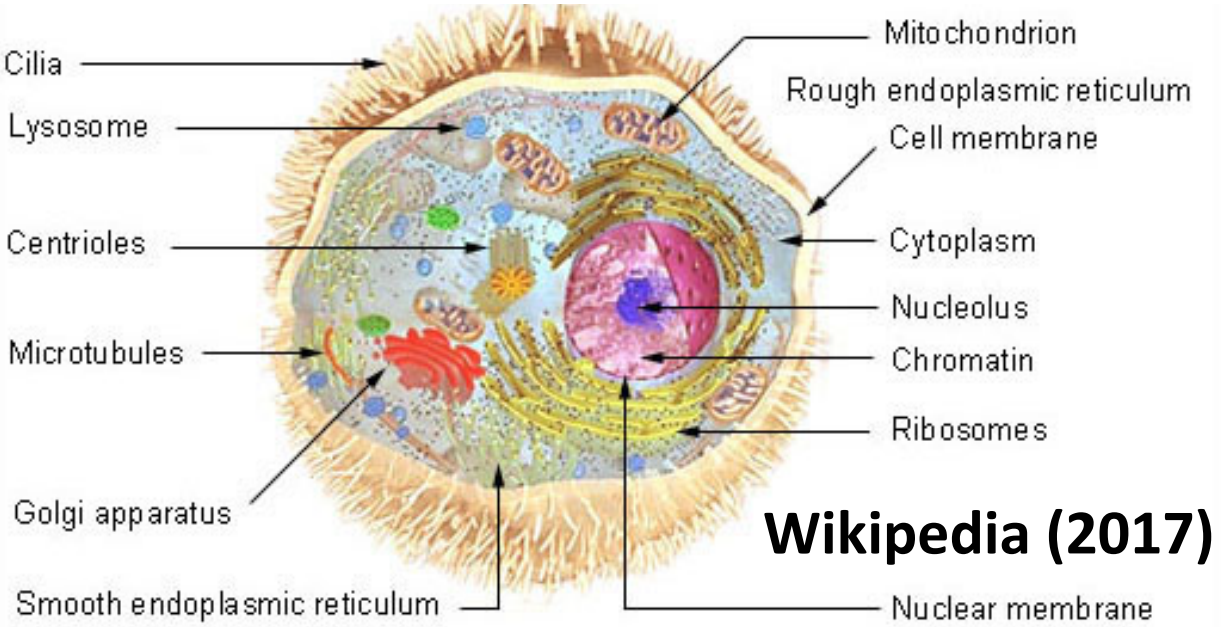
R. Ichapourapu *et al.* (2009)

Impedance Spectroscopy of a Biological Cell

J. Bernstein (1902): "A cell is made of a membrane wrapped around a cytoplasm of electrolytes. The membrane is selectively permeable to potassium so that the concentration of potassium inside is around 50 times that in the external solution."



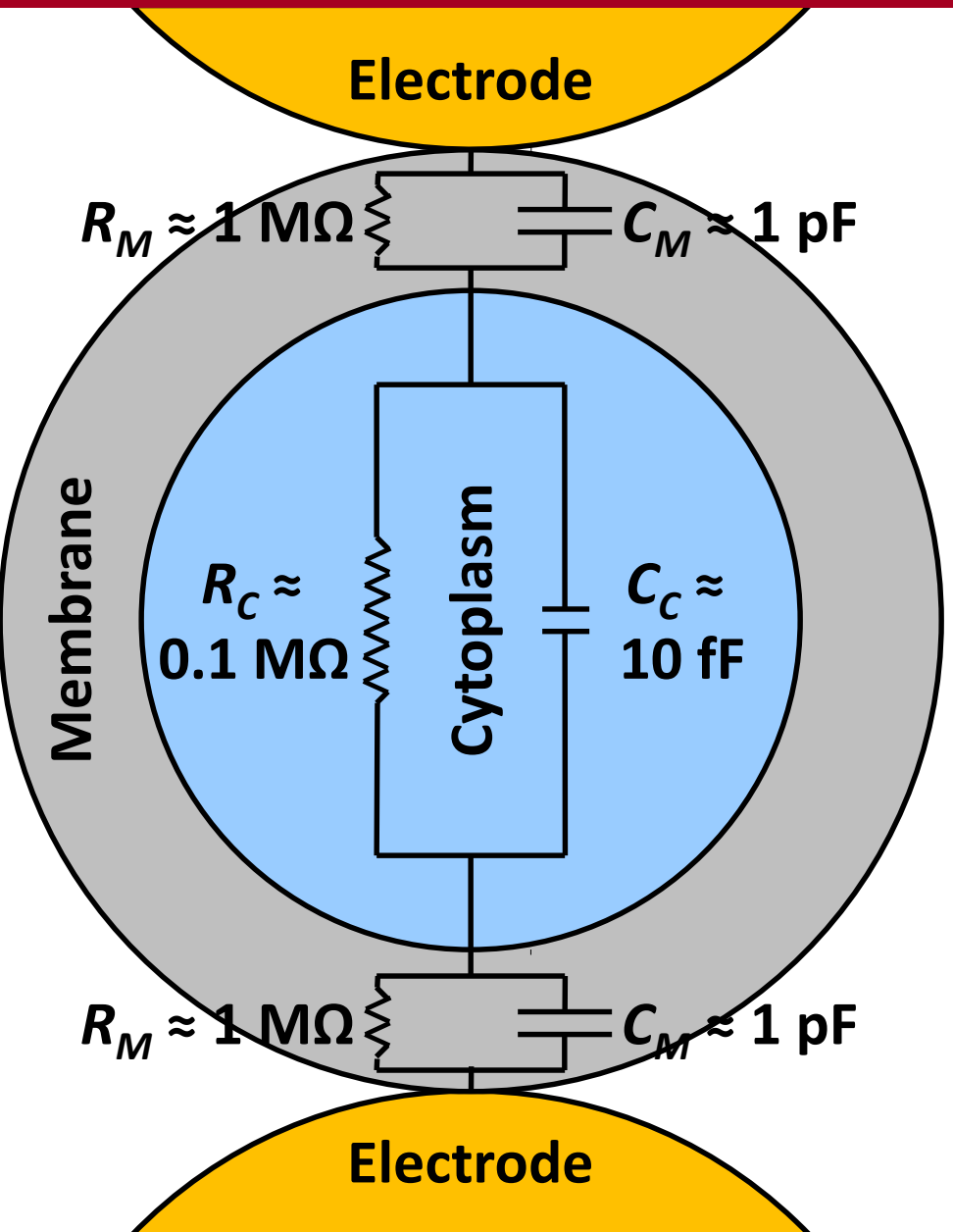
Harrytoystore.com



Wikipedia (2017)

A cell is nothing but a bag of salty water?

Equivalent Circuit of a Cell



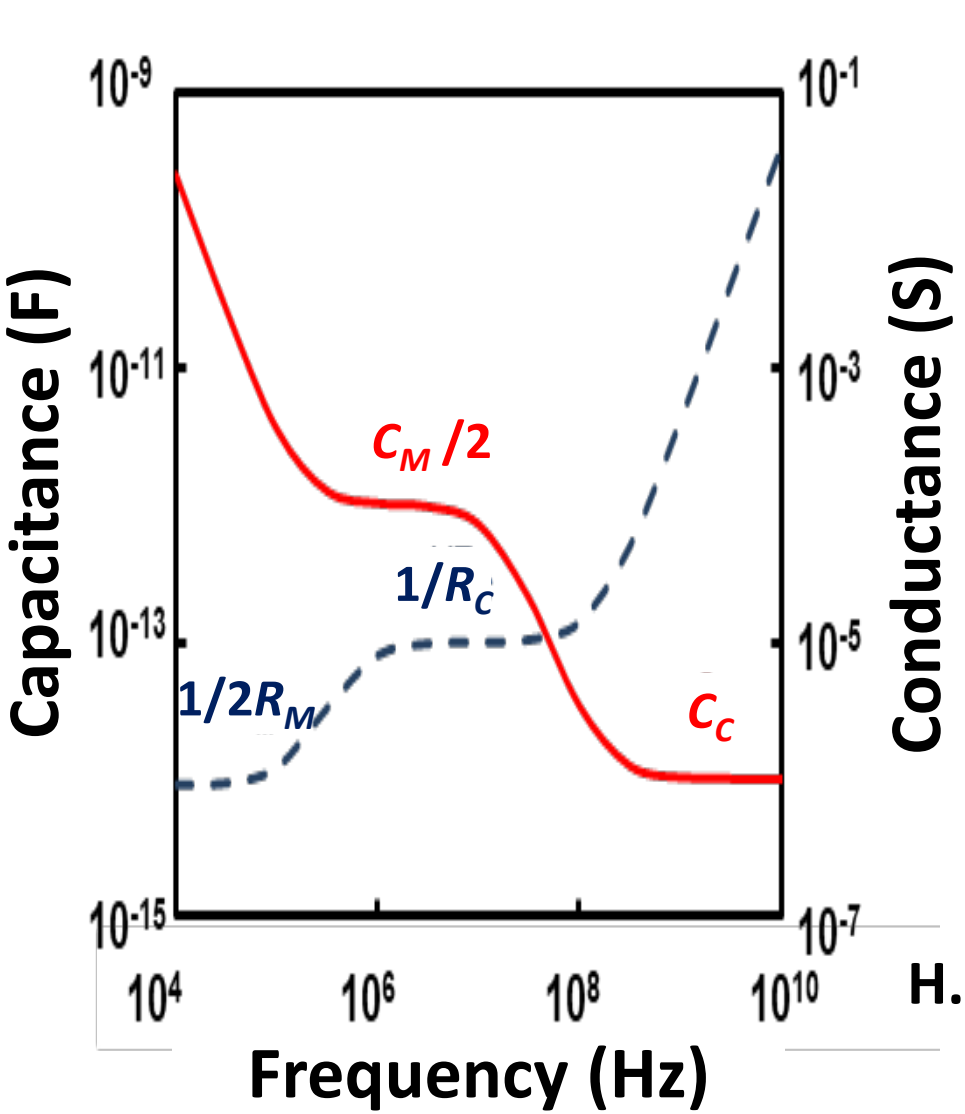
$r_C \approx 1\ \Omega\cdot m$
R. Hoeber (1910)

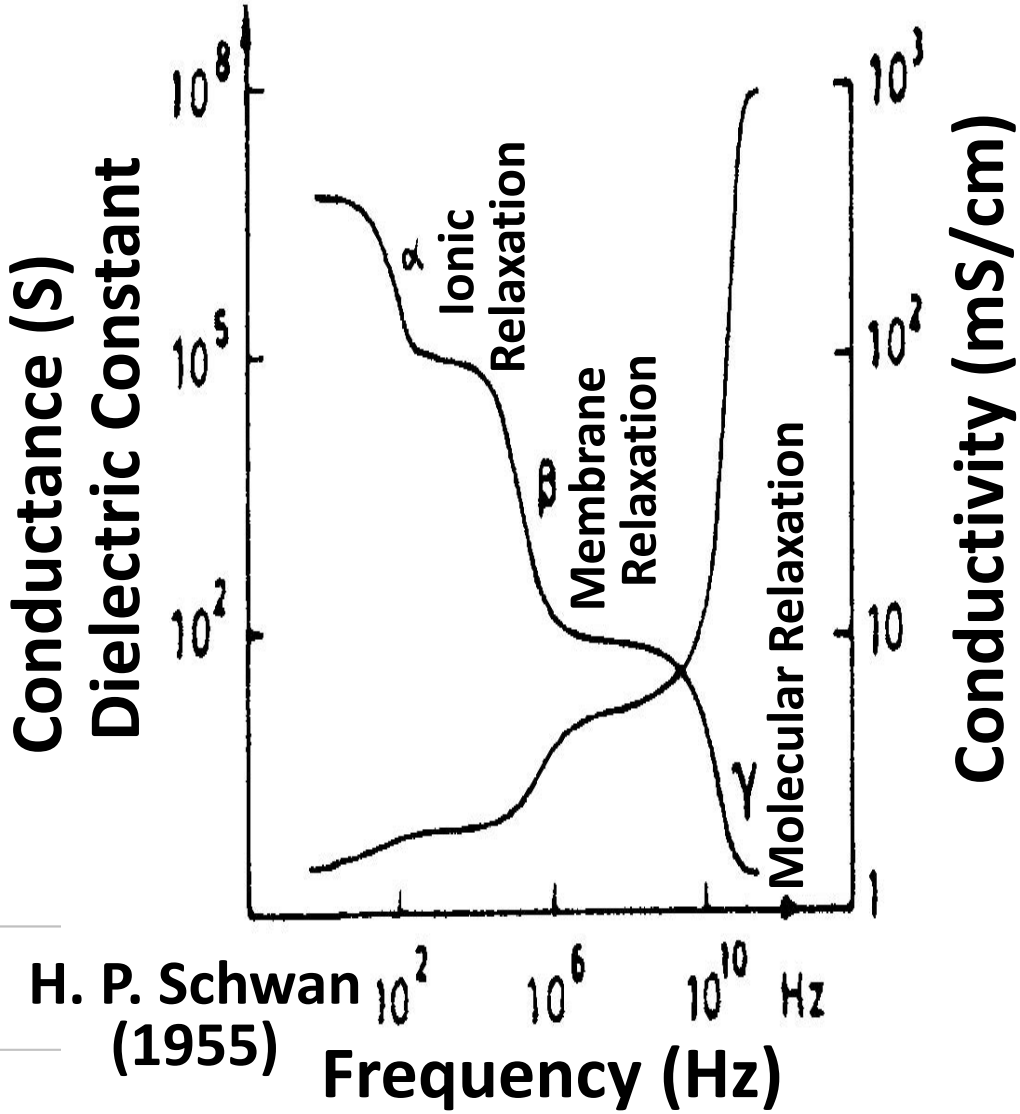
$\varepsilon_C \approx \varepsilon_{WATER} \approx 80$

$r_M \approx 2\ k\Omega/cm^3$
M. Phillipson (1921)

$c_M \approx 0.01\ F/m^2$
H. Fricke (1924)

Dielectric Relaxations in a Cell

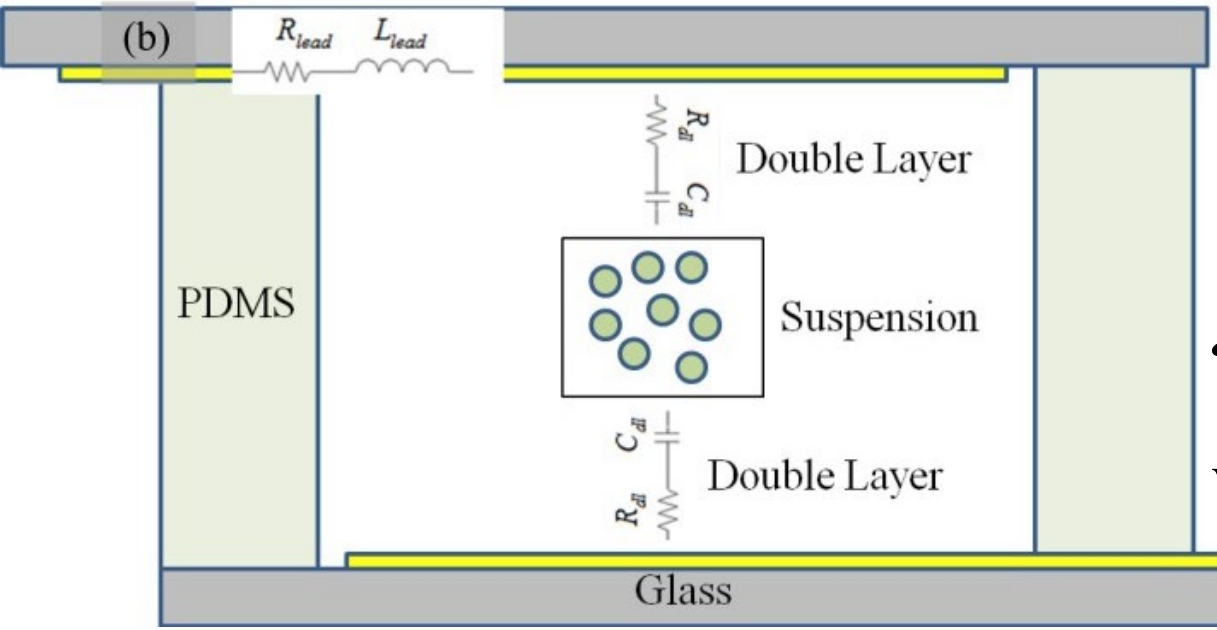




H. P. Schwan (1955)

Lumped equivalent circuit can represent cell dispersion

Solution Effects



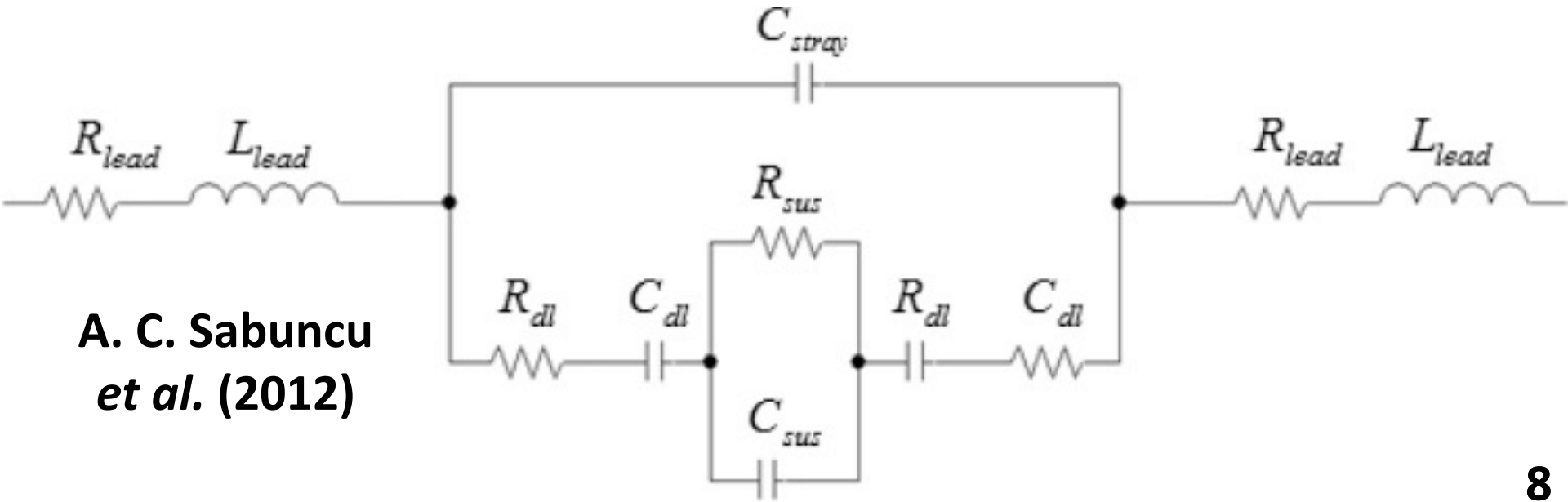
Mixture Model

J. C. Maxwell (1891)

K. W. Wager (1914)

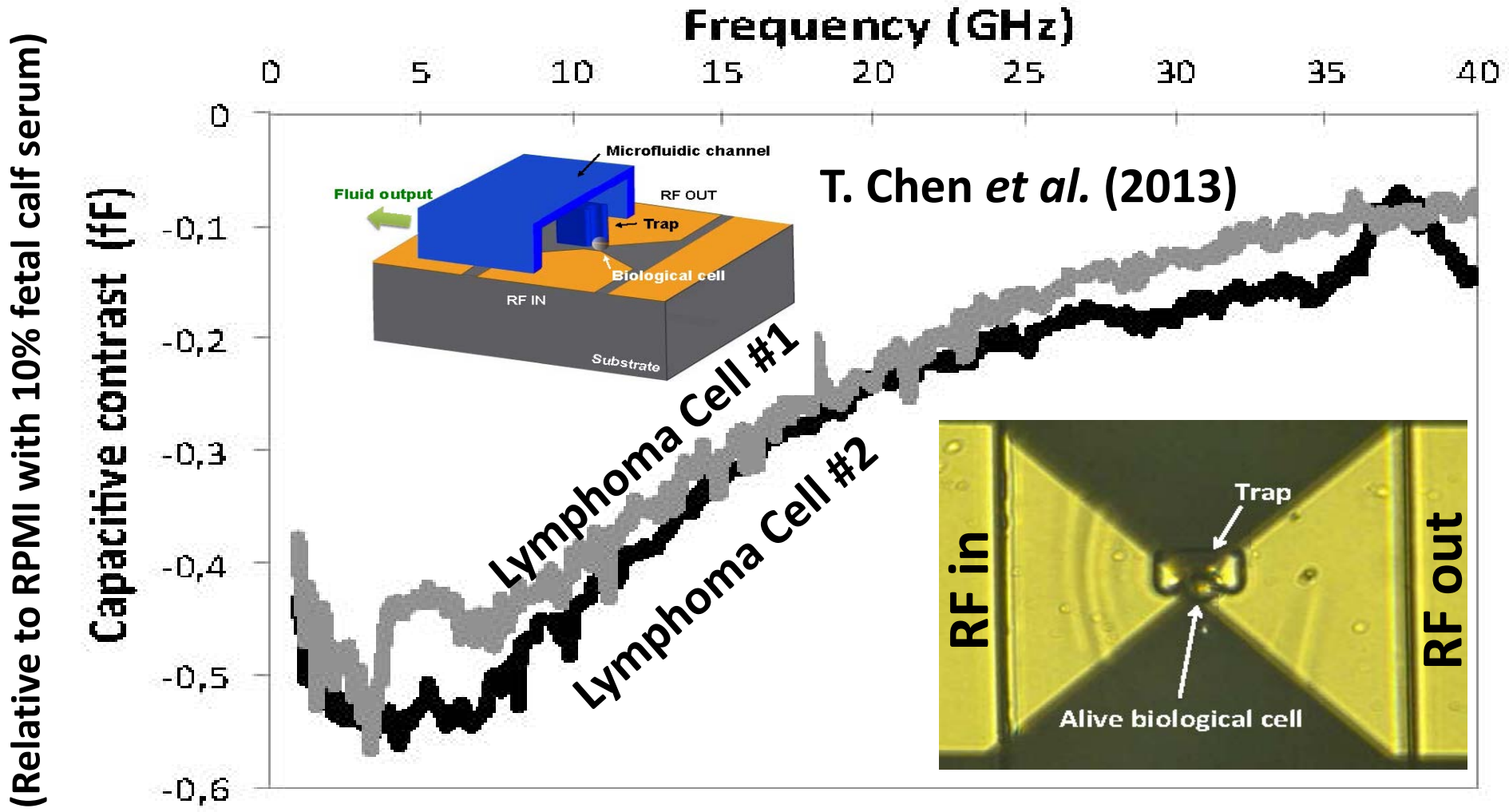
$$\varepsilon = \varepsilon_1 \frac{(2\varepsilon_1 + \varepsilon_2) - 2p(\varepsilon_1 - \varepsilon_2)}{(2\varepsilon_1 + \varepsilon_2) + p(\varepsilon_1 - \varepsilon_2)}$$

volume fraction $p << 1$



A. C. Sabuncu *et al.* (2012)

Broadband Single-Cell Sensing



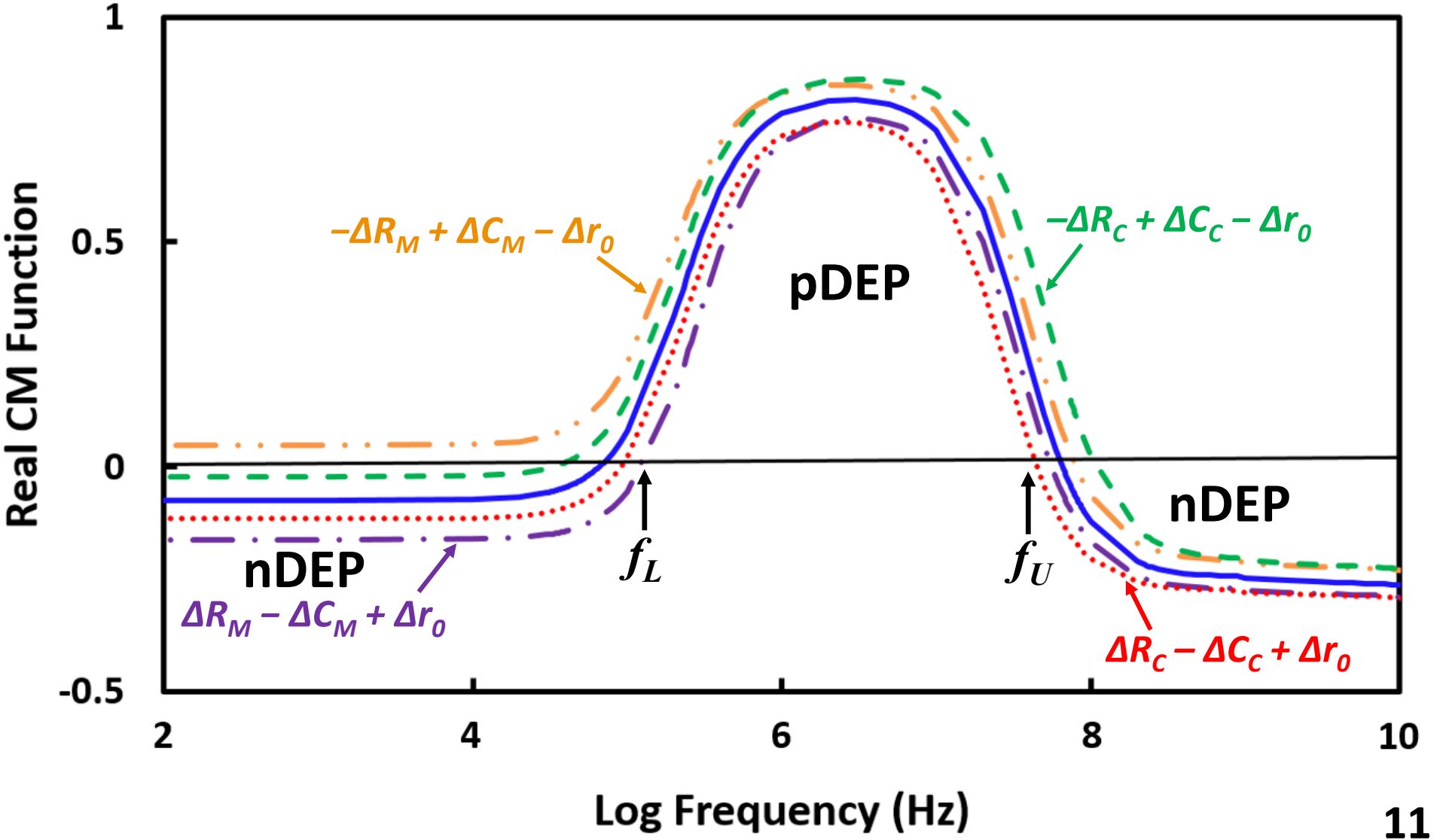
Bypassed solution effects at microwave frequencies.

Our Innovative Approaches

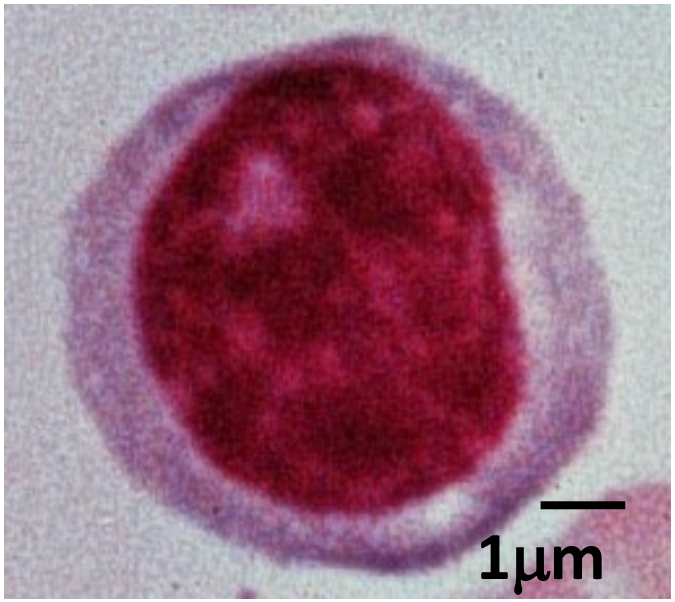
- **Cells resuspended in isotonic sucrose solution to minimize solution effects while keeping them alive**
- **AC dielectrophoresis (DEP) to trap cells between narrowly spaced electrodes for maximum cell-to-solution volume ratio and for quick trap/release**
- **2-port *S*-parameter measurement instead of 1-port impedance measurement for wide dynamic range**
- **Rapidly successive measurements with and without a cell to compensate for background drift**
- **Ultra-wideband trapping/detrapping, characterization & electroporation between 9 kHz and 9 GHz**

Clausius-Mossotti Function

$$F = 2\pi\varepsilon_S r_0^3 \,\mathrm{Re}\left[f_{CM}(\omega)\right]\nabla\left|E\right|^2 \qquad f_{CM}(\omega) = \left(\varepsilon_P^* - \varepsilon_S^*\right)\Big/\left(\varepsilon_P^* + 2\varepsilon_S^*\right)$$

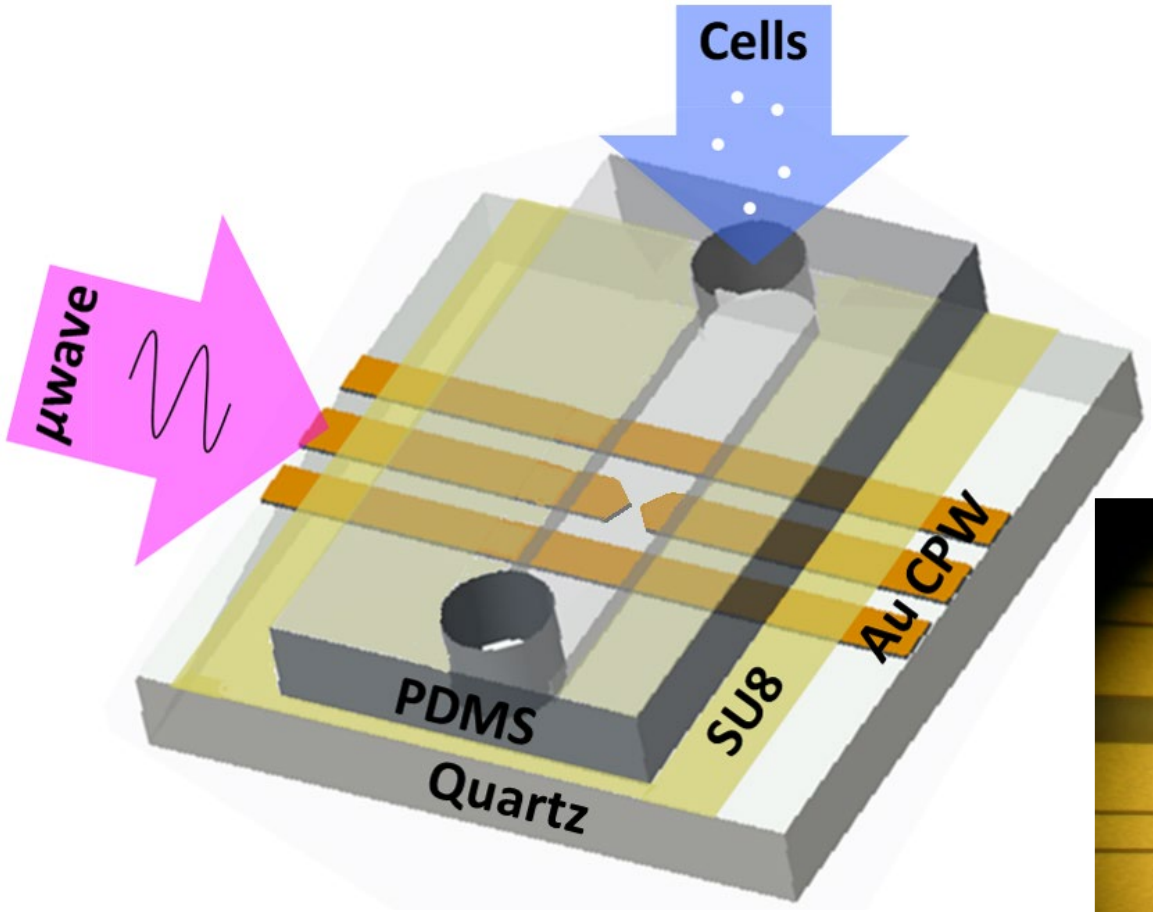


Cells Chosen for Proof of Concept

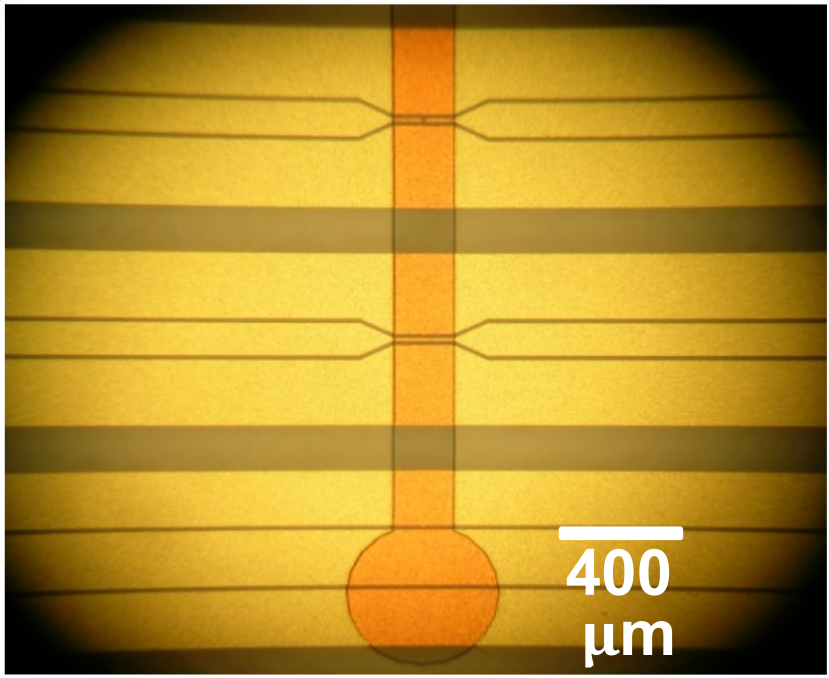


- Jurkat T-lymphocyte human cell
- Large diameter ~ 10 µm
- Simple Structure
- Non-adherent
- Well studied biologically

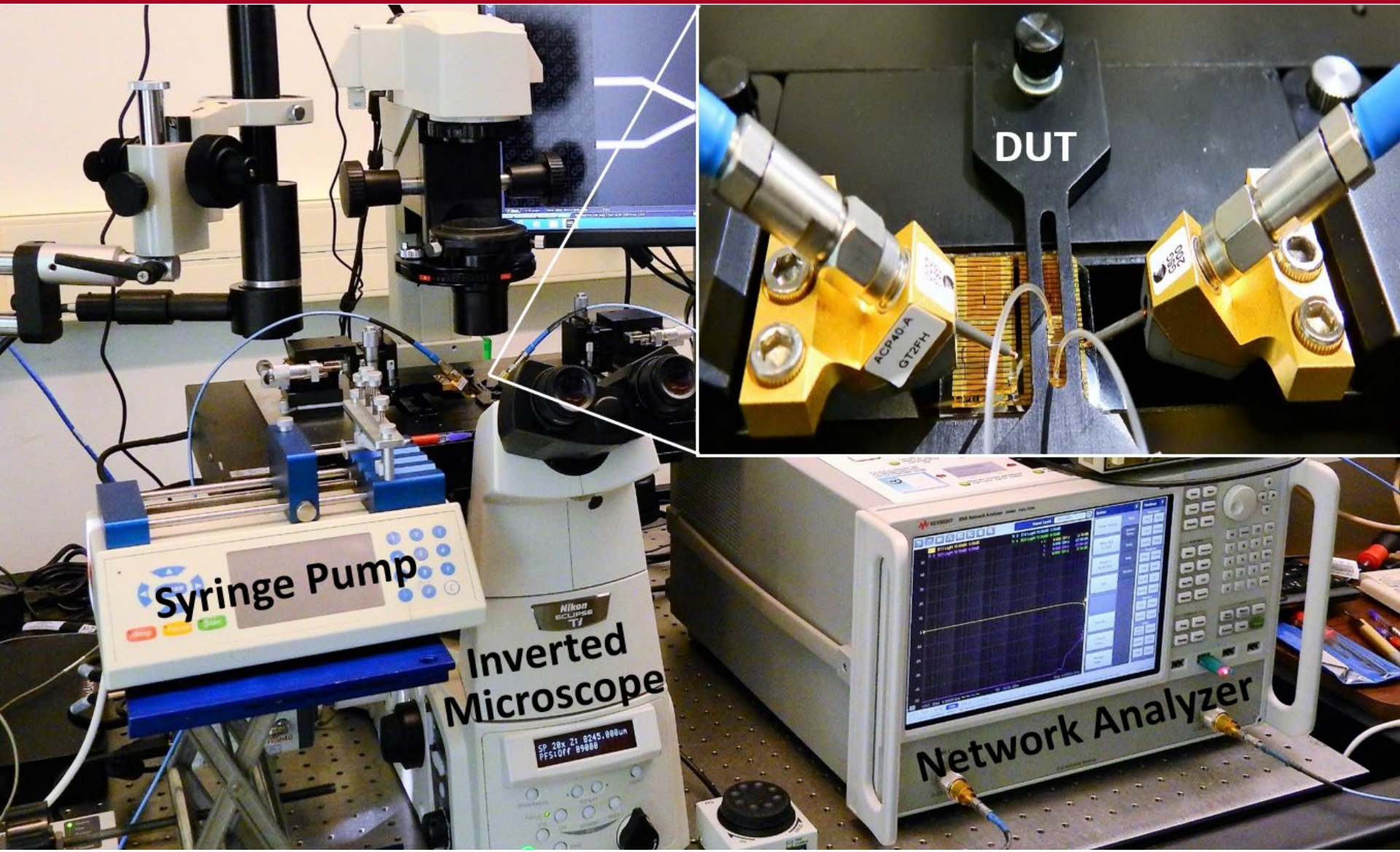
Device Under Test



Microfluidic Channel

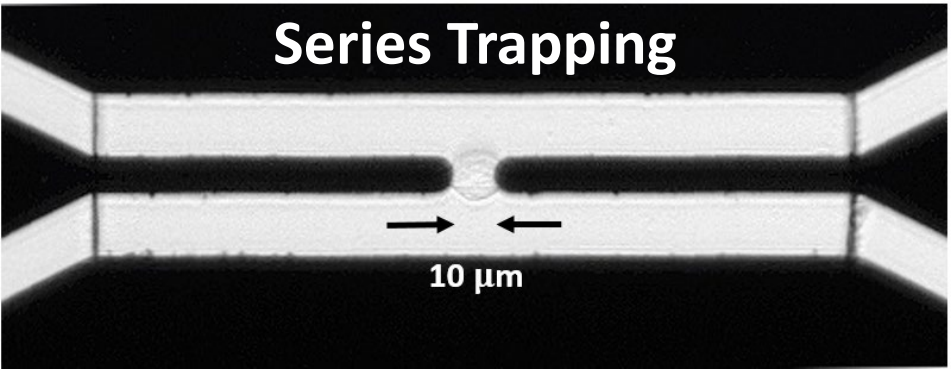


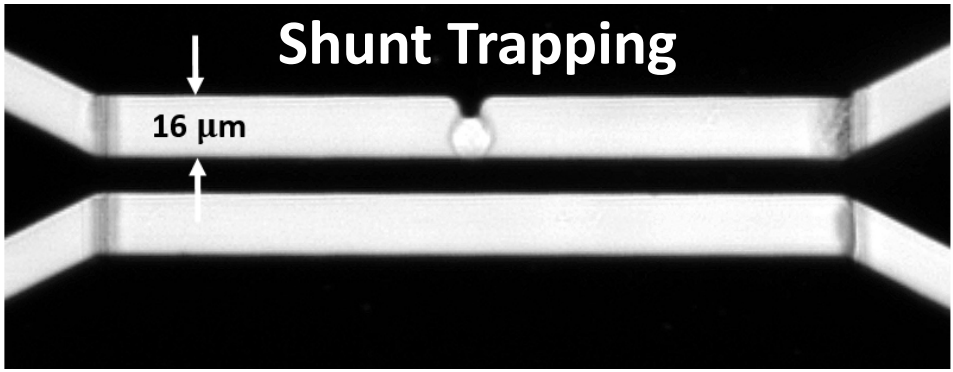
UWB Characterization Setup



Same network analyzer for DEP, characterization, & electroporation

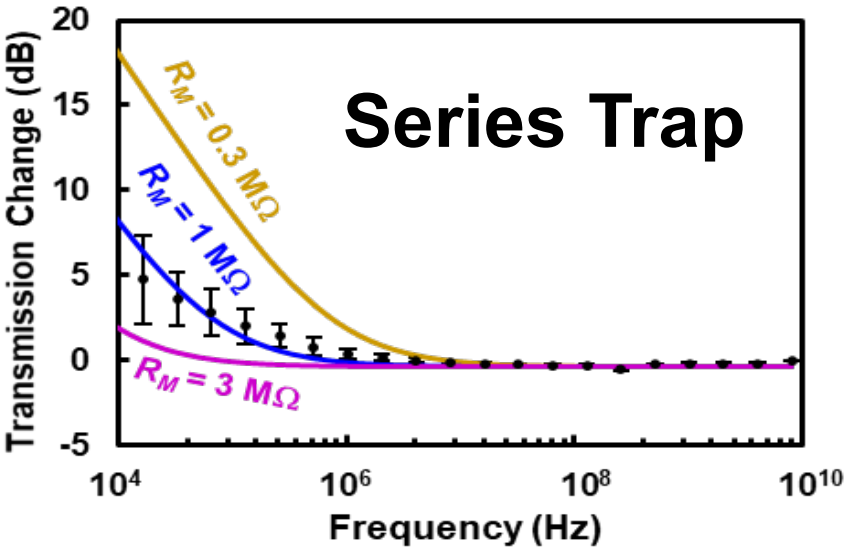
UWB Trapping/Detrapping, Characterization, & Electro-poration

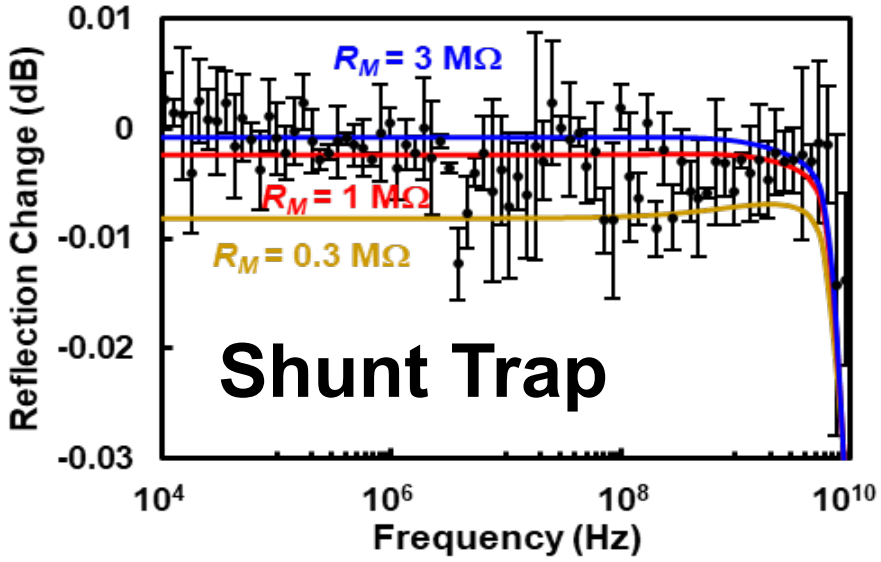


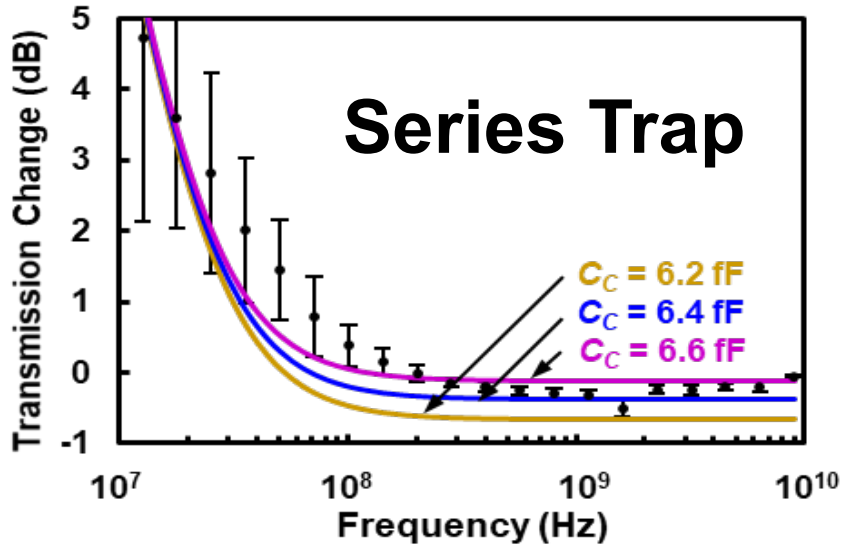


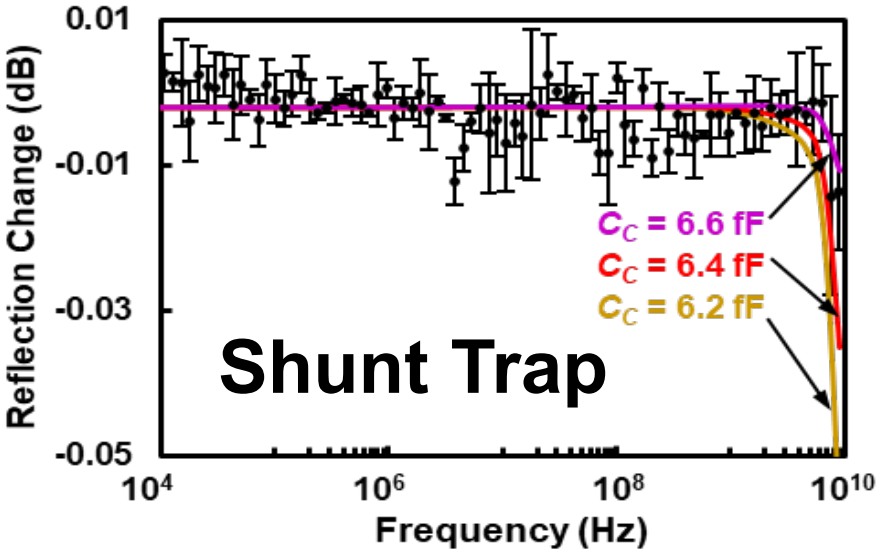
Function	Frequency	Power
Trapping	10 MHz	0 dBm
Characterization	9 kHz–9 GHz	–18 dBm
Electroporation	100 kHz	9 dBm
De-trapping	10 kHz	3 dBm

Single Jurkat Cell Characteristics



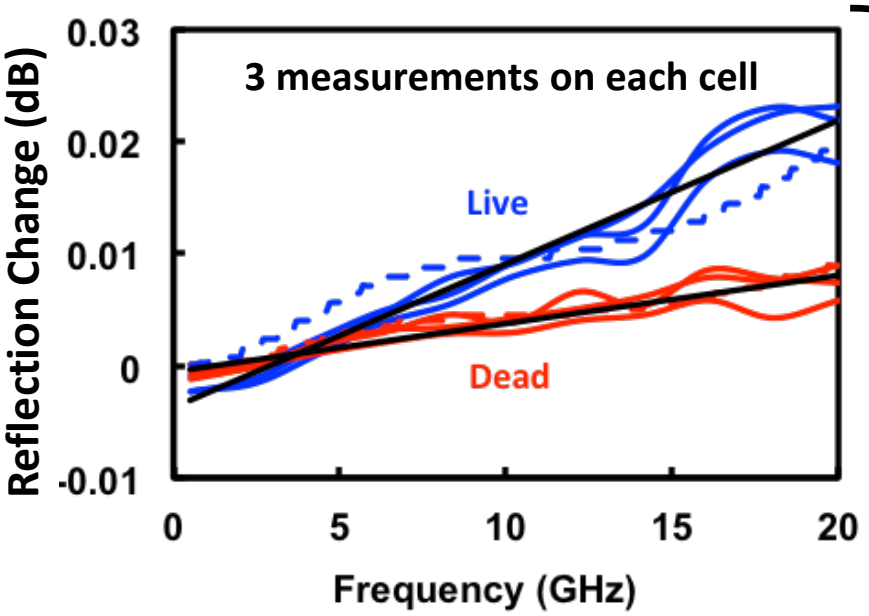


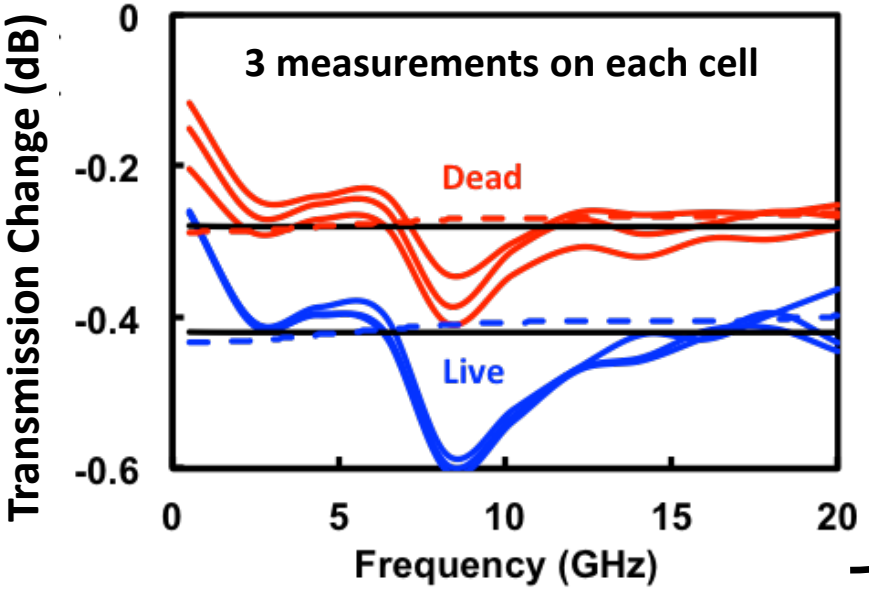


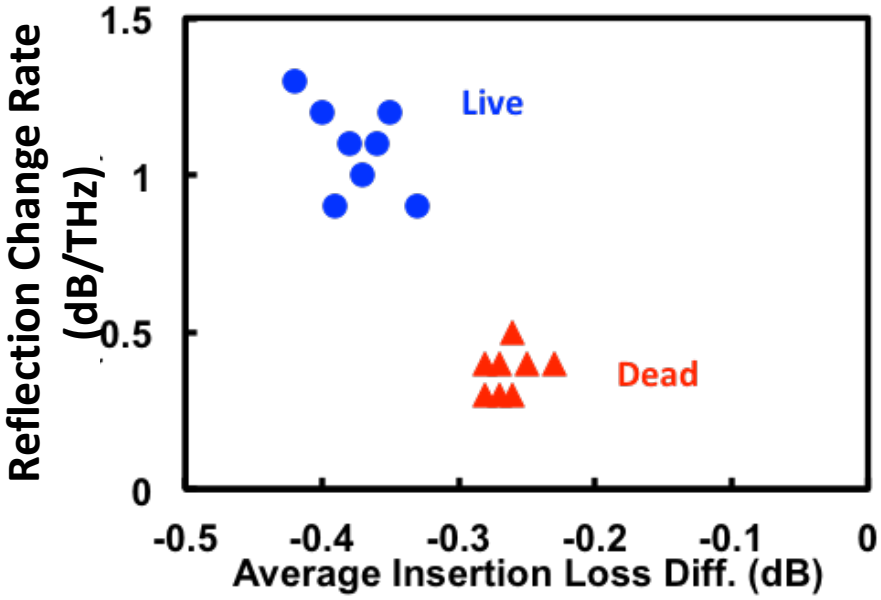


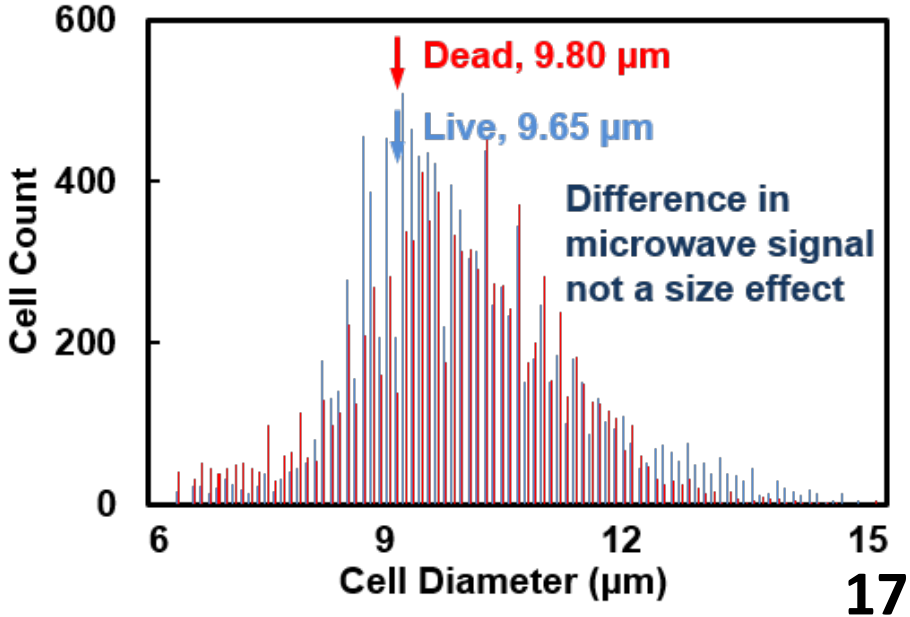
Accurate extraction of cell parameters despite weak & noisy signals

Reproducible Measurements on 16 Cells

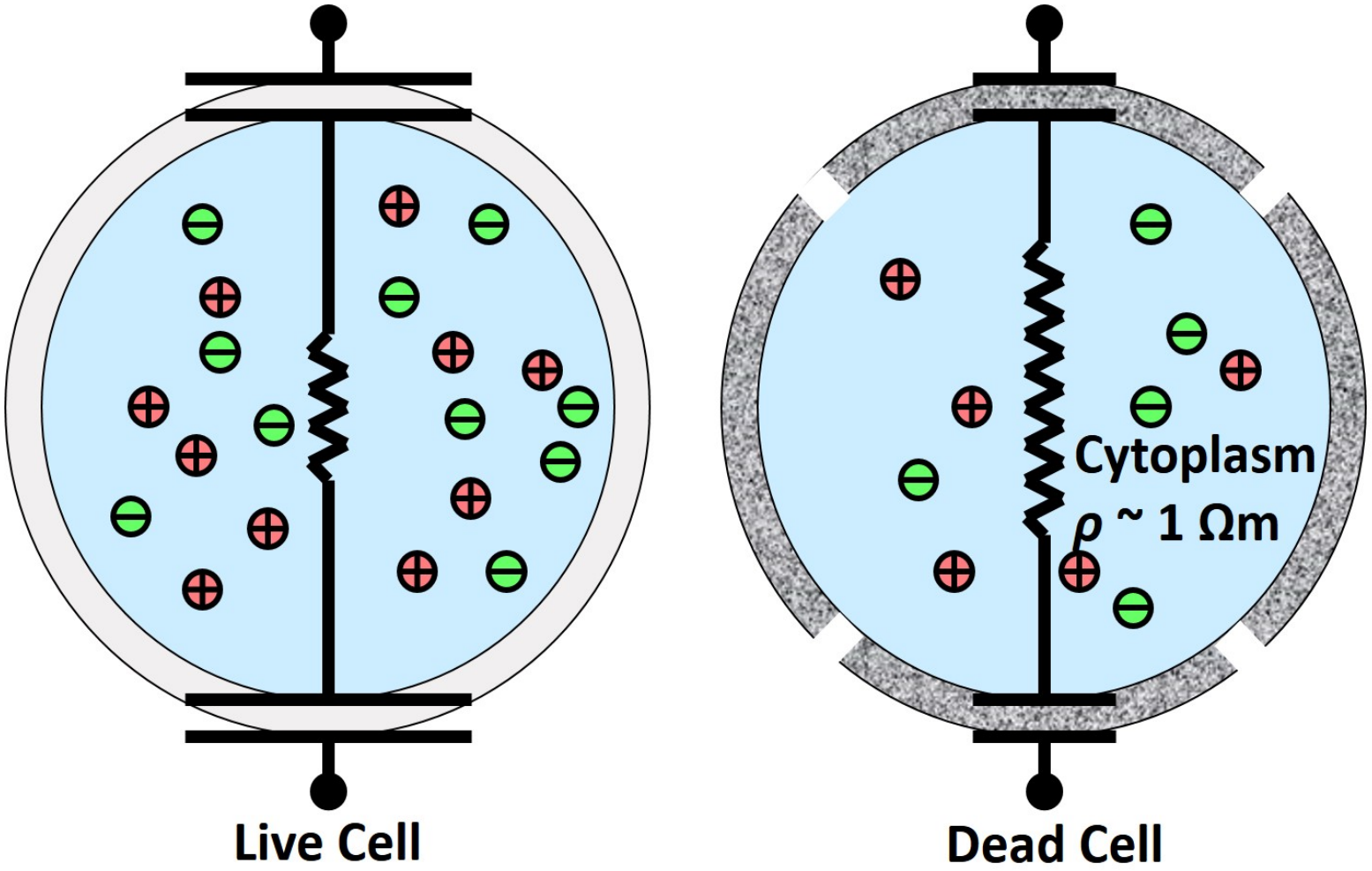








Live vs. Dead Cells



As a cell dies, membrane becomes more porous allowing more ions to leak out

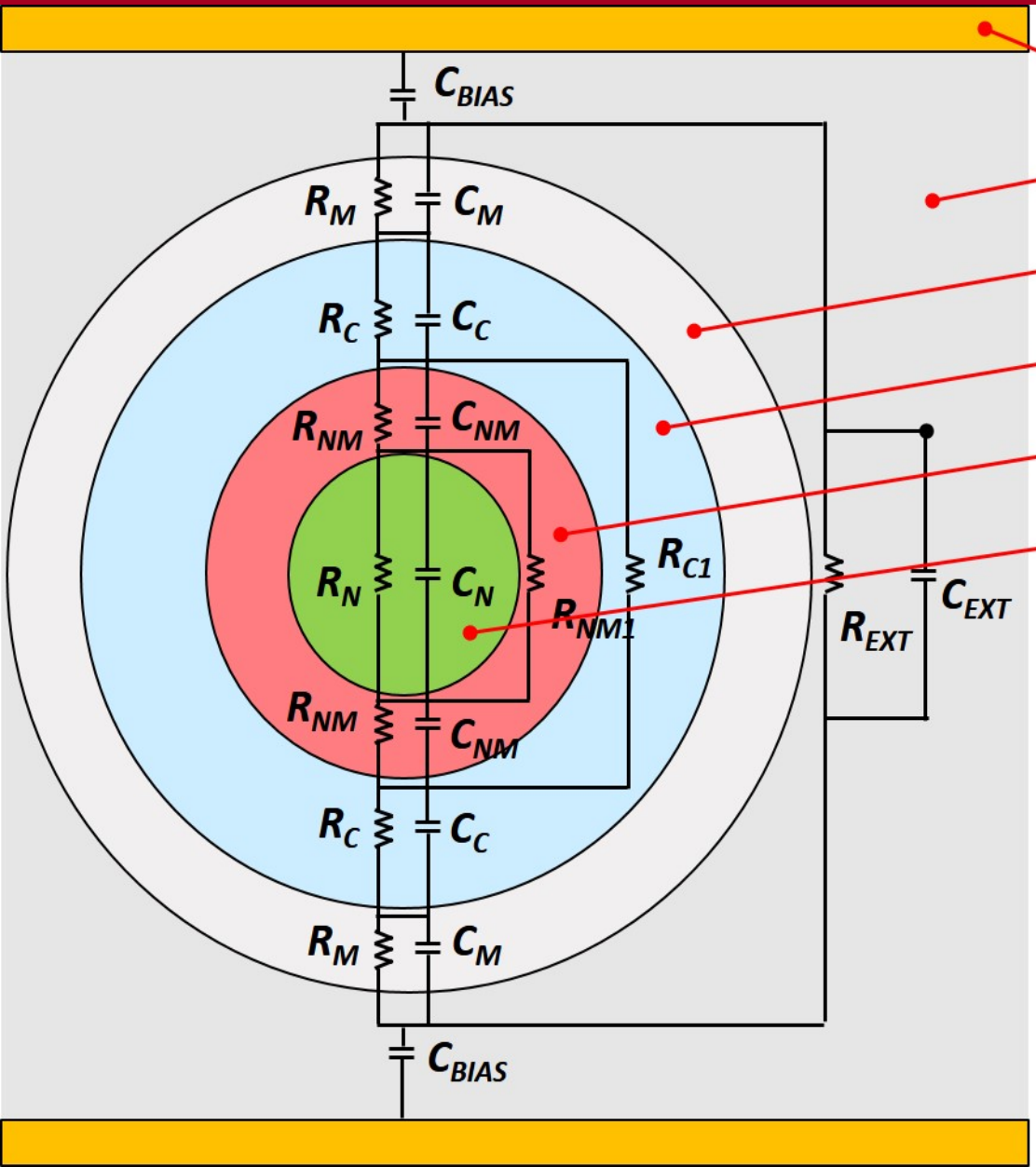
Electrical Characteristics of a Jurkat Cell

Year	Organization	Bandwidth	R_M (MΩ)	C_M (pF)	R_C (MΩ)	C_C (fF)
1995	Wales	10 kHz-100 MHz		1.1	0.13	6
2001	Wurzburg	10 kHz-100 MHz	3.6	1.4	0.15	6
2006	Aura BioSystems	10 kHz-100 MHz	80	1.1	0.2	12
2007	Old Dominion	ns Pulse	20	0.9	0.5	
2012	Old Dominion	10 kHz-10 MHz	2	1.2	0.3	6
2013	Inje	1 kHz-100 MHz		0.7	0.2	10
2017	Lehigh	9 kHz-9 GHz	1.5 ±0.3	1.5 ±0.3	0.4 ±0.1	6.4 ±0.1

Conclusion

- **Lumped equivalent circuit with nondispersive resistance/capacitance can model kHz-GHz impedance spectrum without Debye formula of dispersive permittivity.**
- **UWB cell characterization compares well with traditional impedance spectroscopy, but more accurate.**
- **Approach may be extended to include more resistors/capacitors over an even wider bandwidth for better representation of subcellular structures.**
- **Microwave signal can be a fast, compact and label-free technique to distinguish live and dead cells, as well as reversible and irreversible electroporation of cells.**

More Complicated Equivalent Circuit



Electrode

Solution

Plasma Membrane

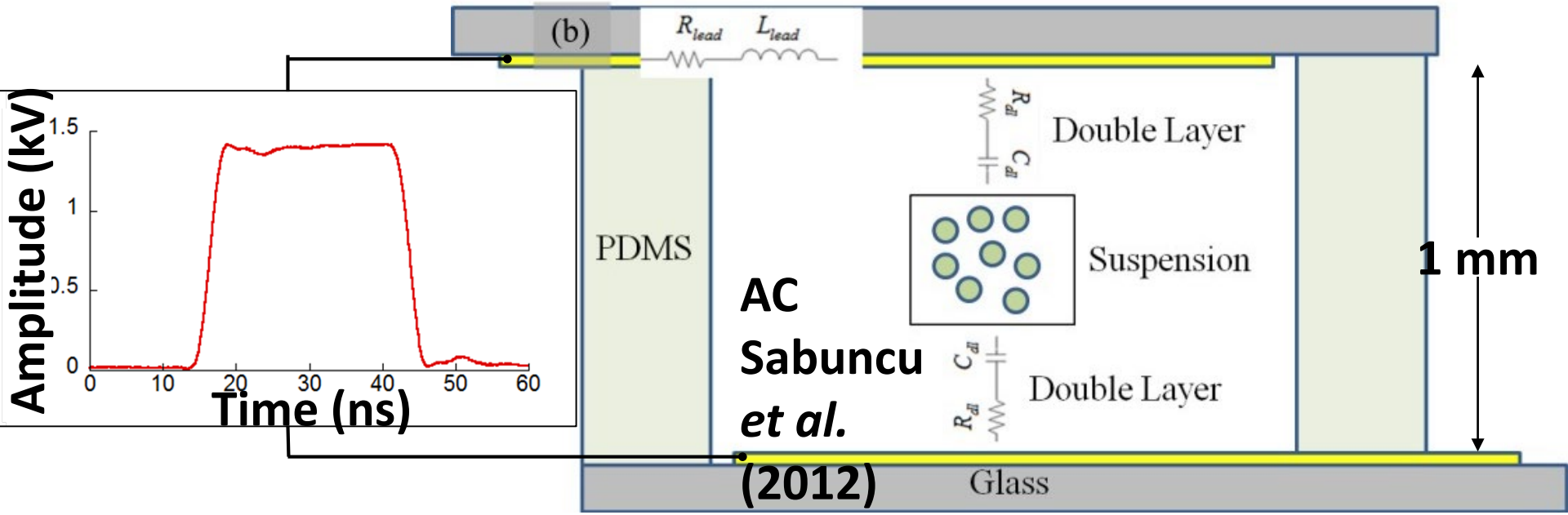
Cytoplasma

Organelle/Nucleus Membrane

Organelle/Nucleus



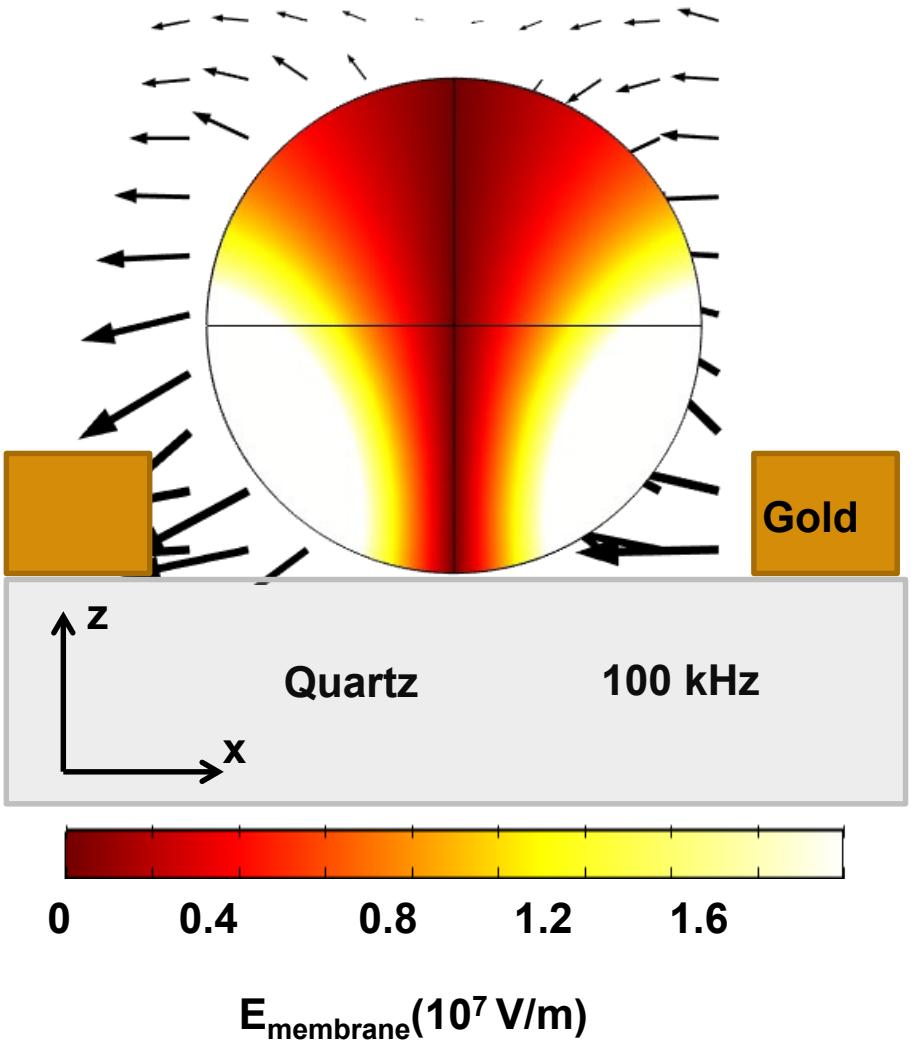
Traditional Electroporation



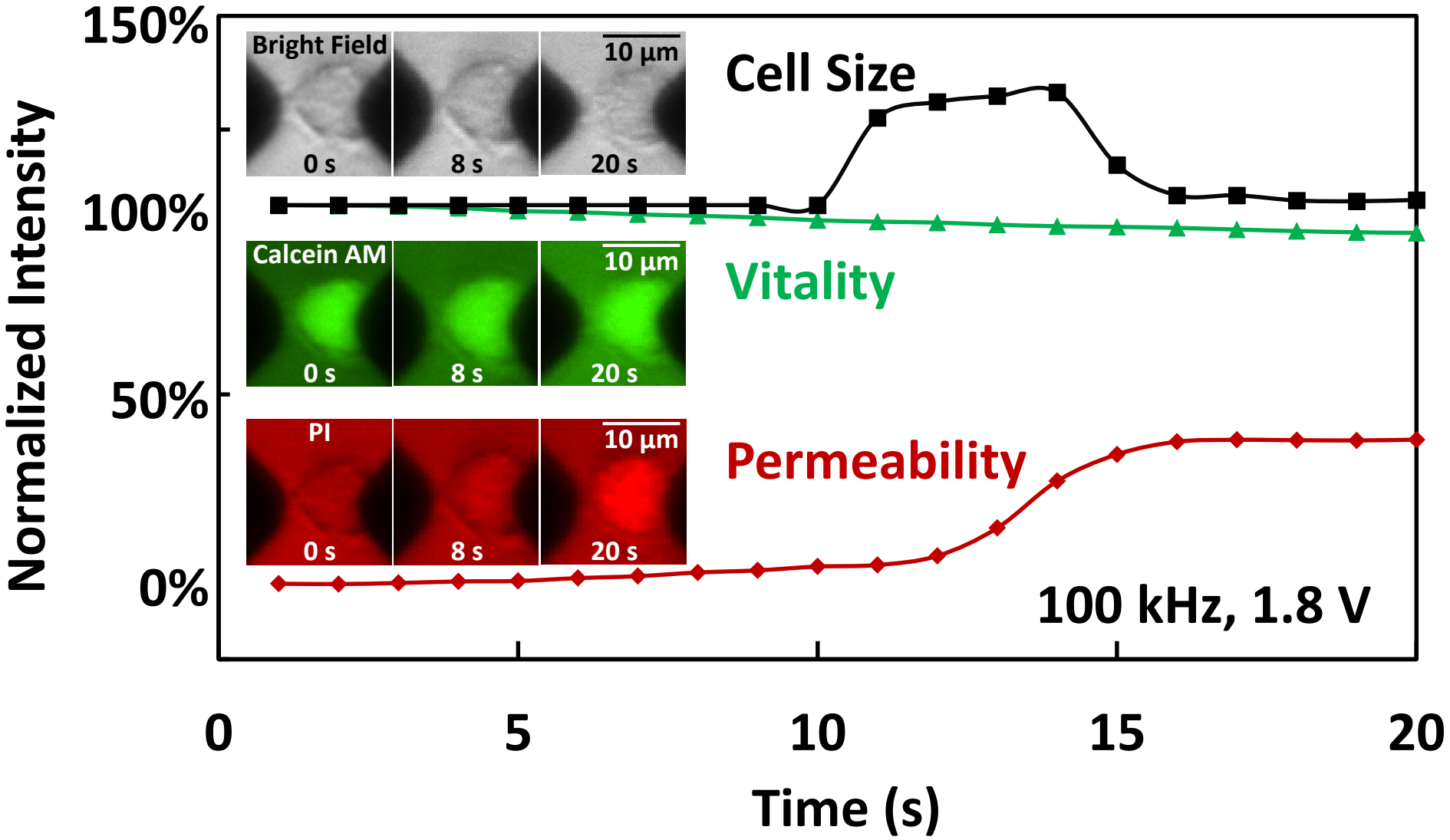
- **Commonly used to create temporary nanopores on cell membrane for drug delivery, DNA extraction, etc.**
- **Nanosecond, kilovolt pulses applied to cell suspensions**
- **Pulse waveform distorted and actual field on cell unknown**
- **Poration efficiency < 50 %, despite high voltage that can cause side effects such as heating and electrolysis**
- **Cell poration/healing dynamics masked by statistics**

Single-cell Electroporation

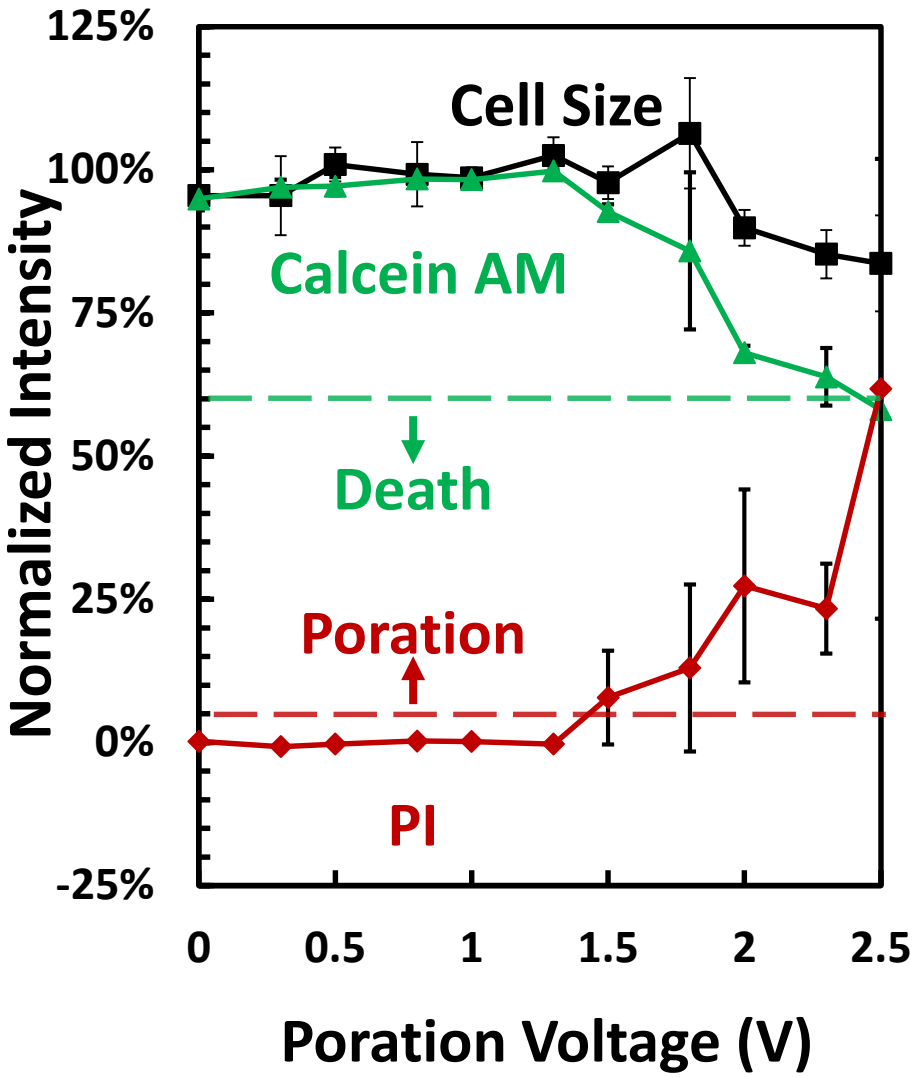
- Poration and healing dynamic observed individually instead of statistically
- Applied voltage fine-tuned around 1 V for improved poration efficiency and slow poration/healing dynamics for real-time monitoring
- Highly nonuniform distribution of electric field greatly disturbed by cell presence

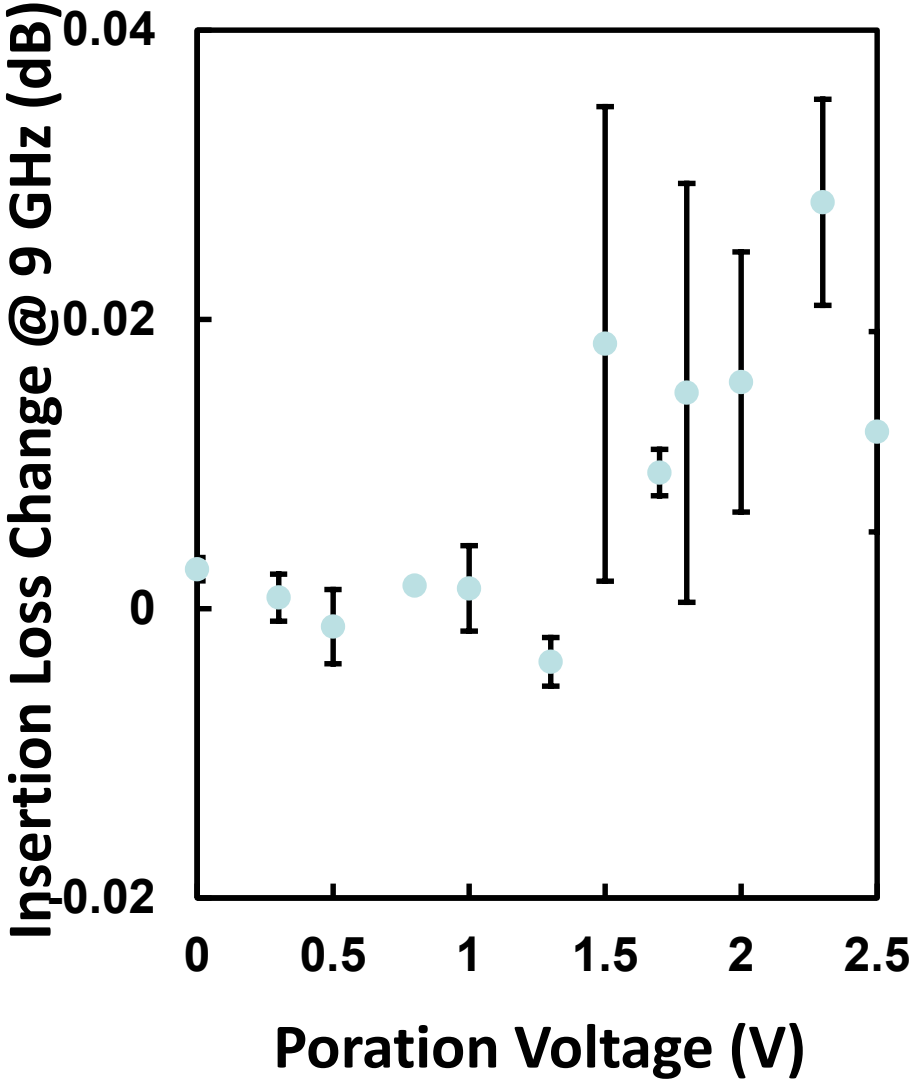


Poration Dynamics

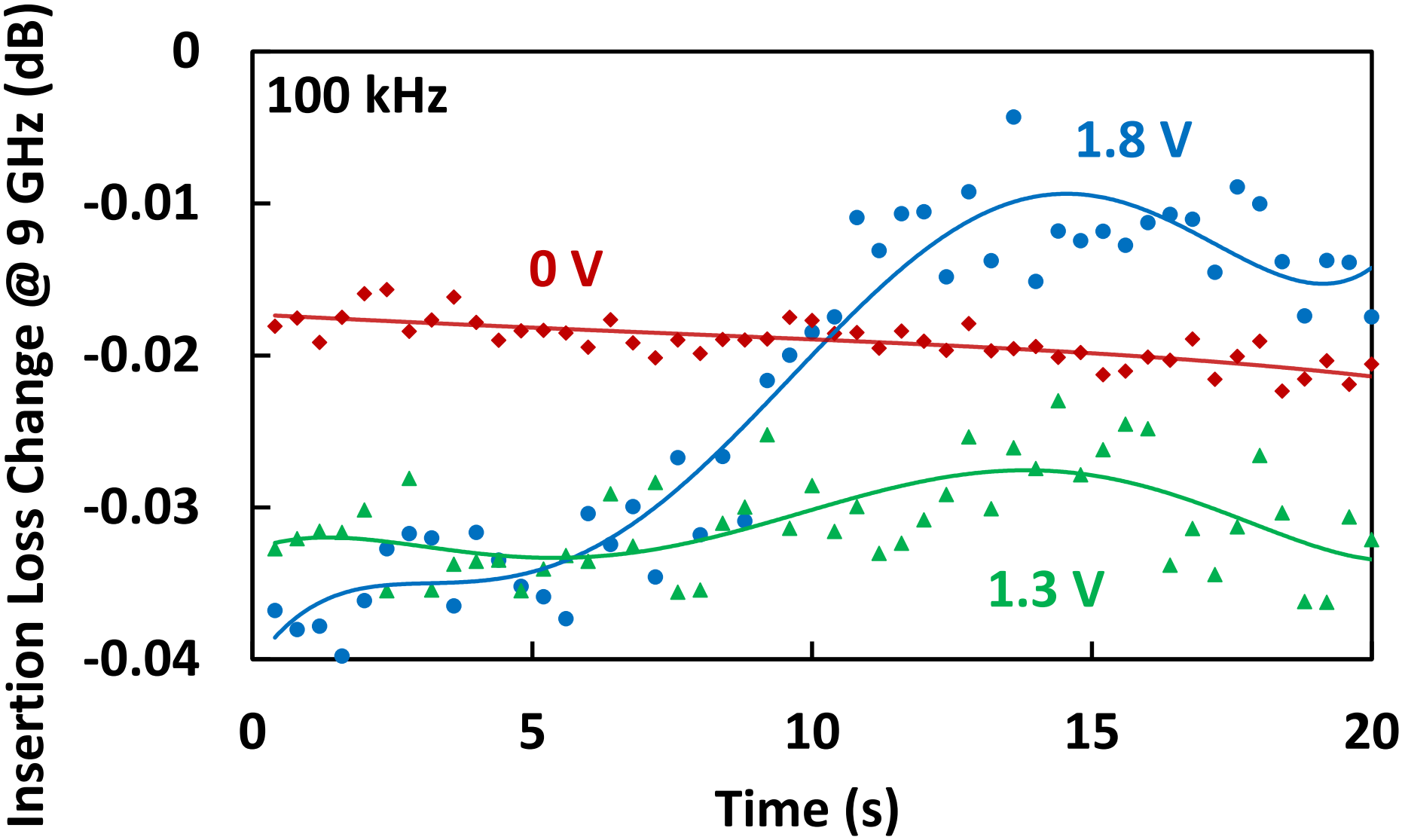


Poration Threshold

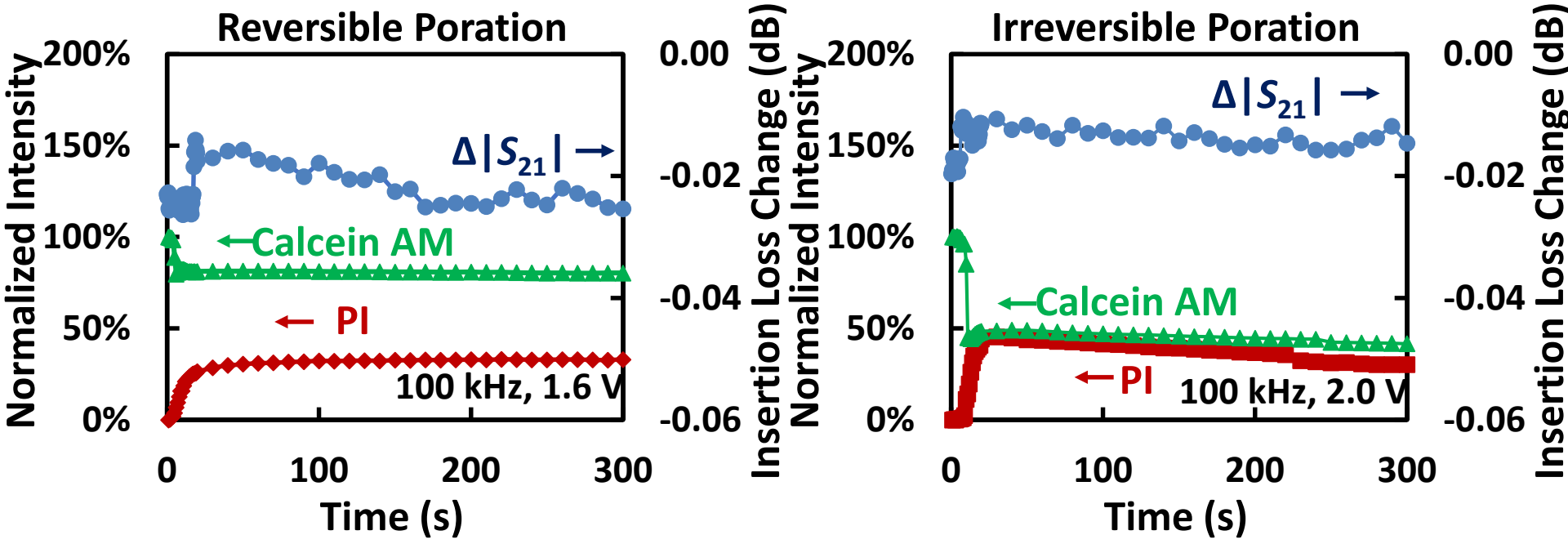




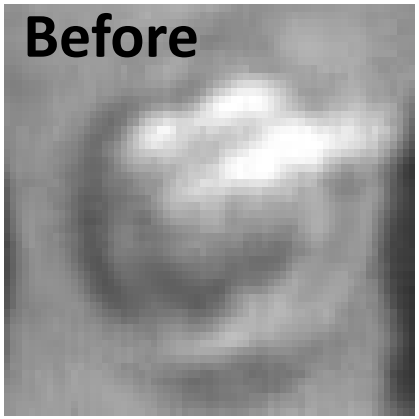
Microwave Poration Indicator

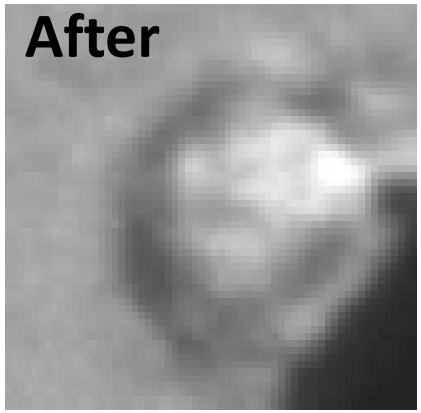


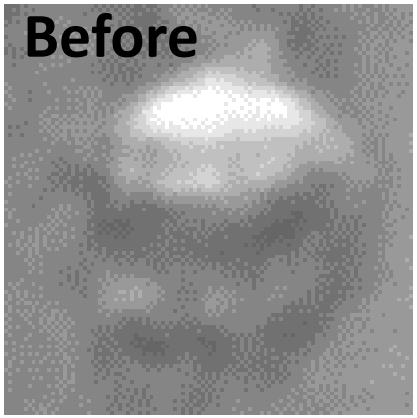
Healing Dynamics

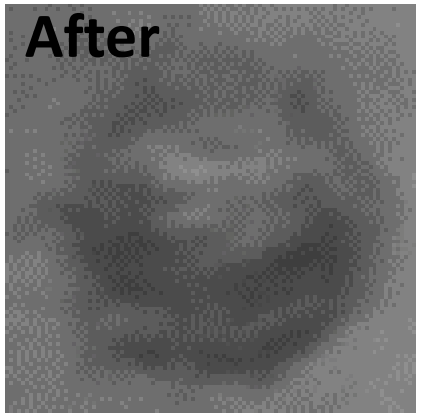


Trypan Blue Staining

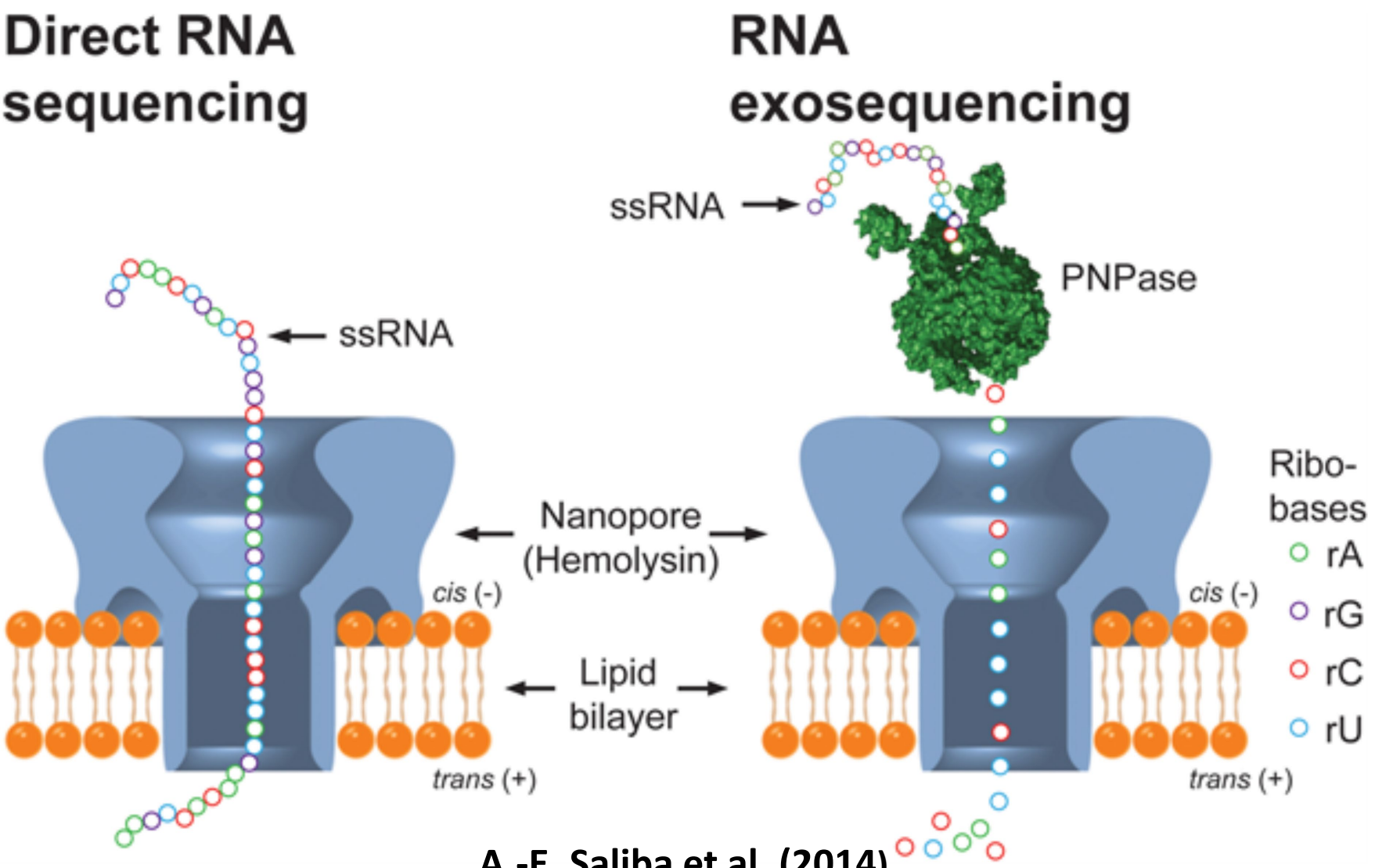








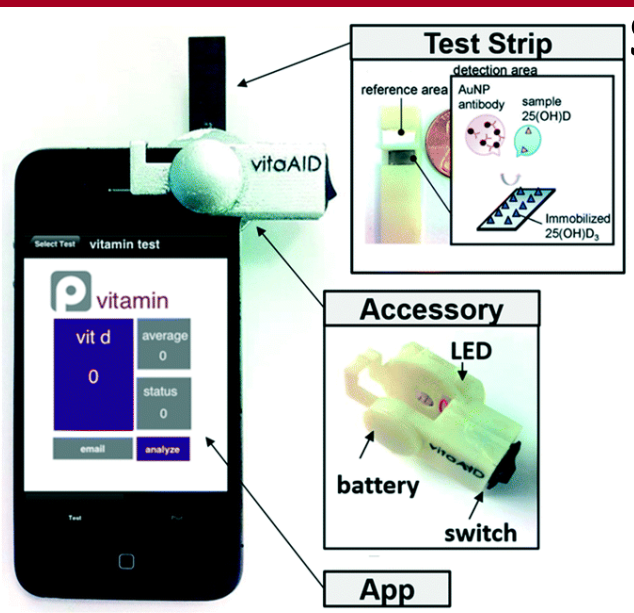
Nanopore RNA Sequencing

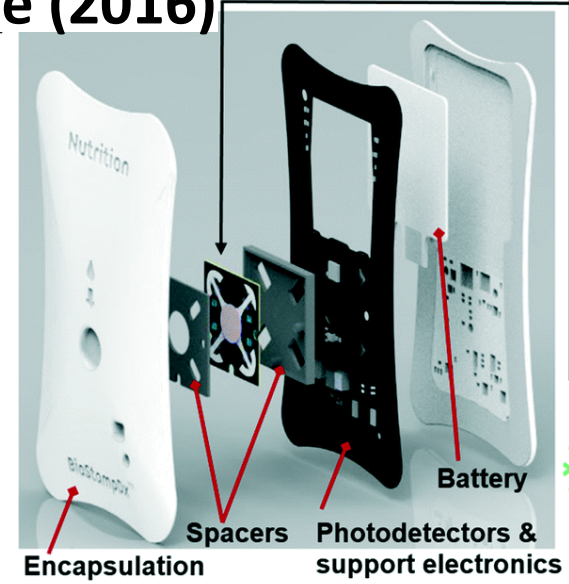


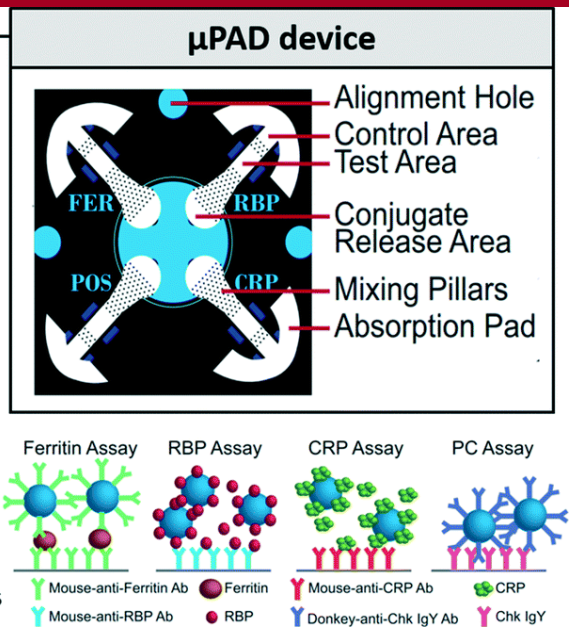
A.-E. Saliba et al. (2014)

Long-Range Plan

S Lee (2016)







MS Draz (2017)





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