A BioMEMS Device for On-chip Single-Cell, Flow-based Diagnostic Assays

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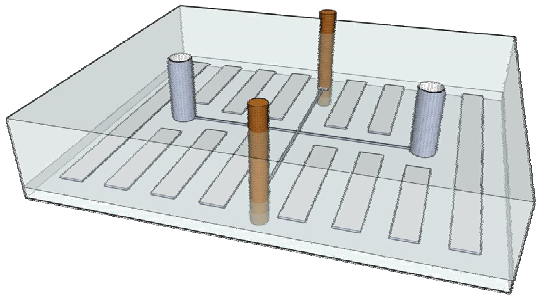
Overview

- Background
  - Cell Viability and Apoptosis
  - Apoptosis detection
  - Motivation
- Materials and Methods
  - Cell Assays
  - Microfluidic chip design and fabrication
- Results
  - Conductivity cell detection
  - Modeling of electrodes
- Summary & Future Works

Overall Objective

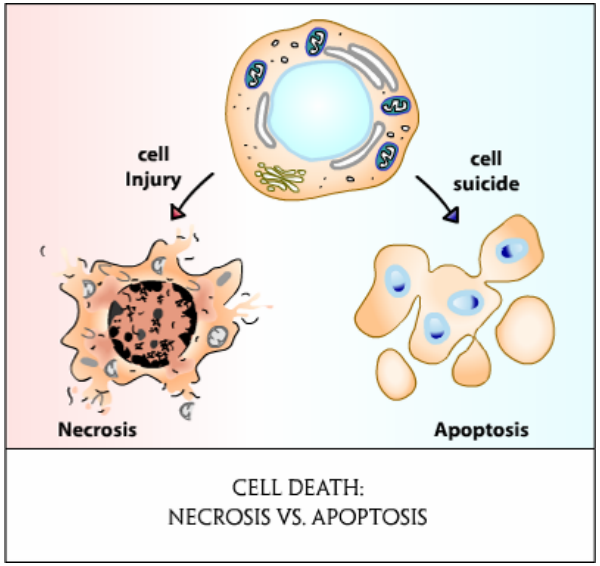
- Develop a microfluidic platform for sensitive detection and discrimination of single-cell live, necrotic, and early apoptotic cells using electrochemical techniques

- Proposed BioMEMS device
  - Label-free, Non-optical
  - Fluorescence for optical validation
  - Measure Impedance or Conductivity
  - Real-time capability



What is Apoptosis?

- Apoptosis- programmed cell death
  - Membrane, DNA, mitochondria, caspase alterations
- Maintains balance in organisms
  - Tissue homeostasis
  - Defense mechanism
  - Aging process
- Inappropriate regulation of apoptosis leads to disease



Necrosis:

Pathological Cell Death

- Ruptured membrane
- Random DNA fragments
- Inflammation

Apoptosis:

Programmed Cell Death

- Intact membrane
- DNA ladder fragmentation
- No inflammation

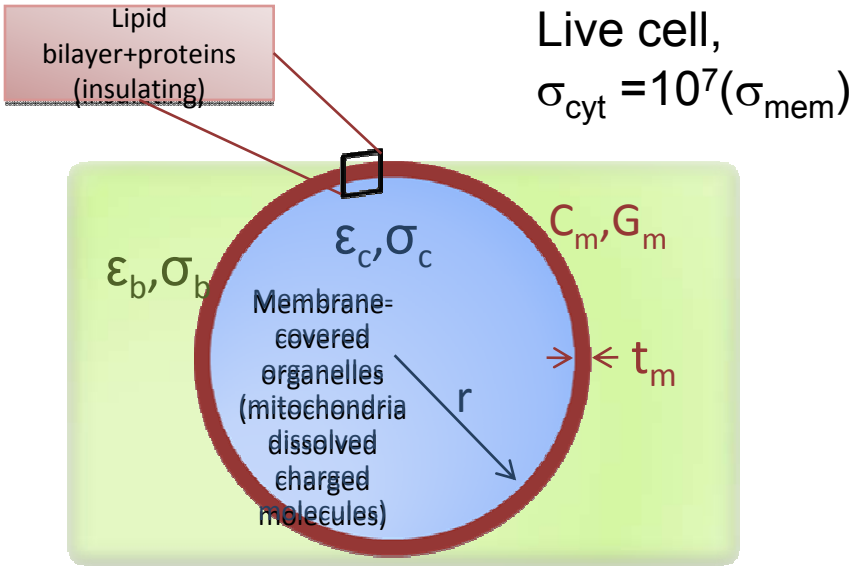
Applications
Apoptosis Detection-on-a-chip

- **Diagnostics**
  - Early detection
  - Portable point-of-care device
  - Monitoring cancer treatment efficacy
- **High-throughput drug screening**
  - Apoptosis & toxicity testing in drug screening
  - Monitoring cells in recombinant protein production
- **Novel tool**
  - Better understand signaling pathways
  - Potentially help characterize cell phenotype

- **Label-free detection Advantages**
  - Non-invasive method to quantify cell properties
  - Able to be integrated in microfluidics
  - Cheaper peripheral equipment (compared to optical and MS methods)

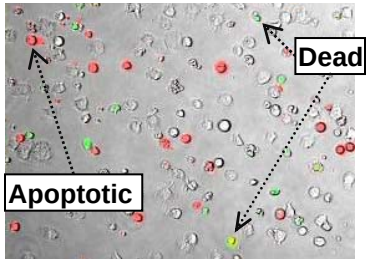
Dielectric Summary of Cells

- Useful to compare relevant parameters which are a function of cell viability:
  - C_m (cross-over frequency)
  - Cell size (r)
  - Membrane complexity
- Still no published account of conductive, label-free, single-cell apoptosis detection on-chip

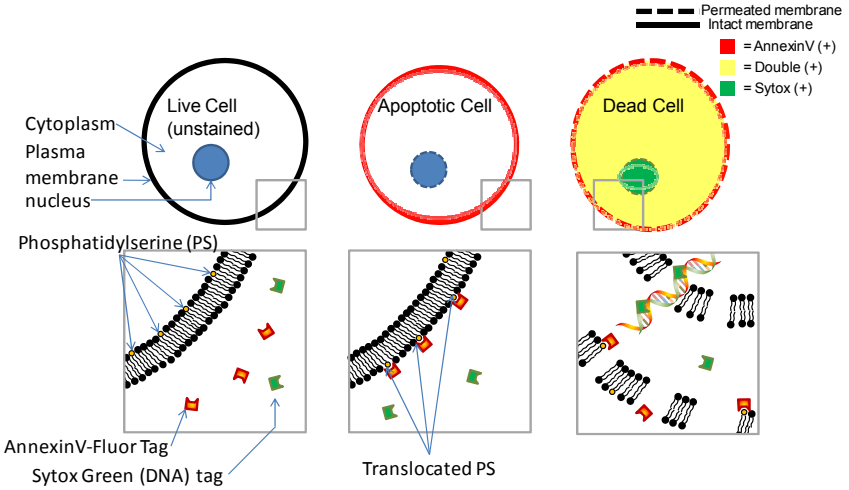


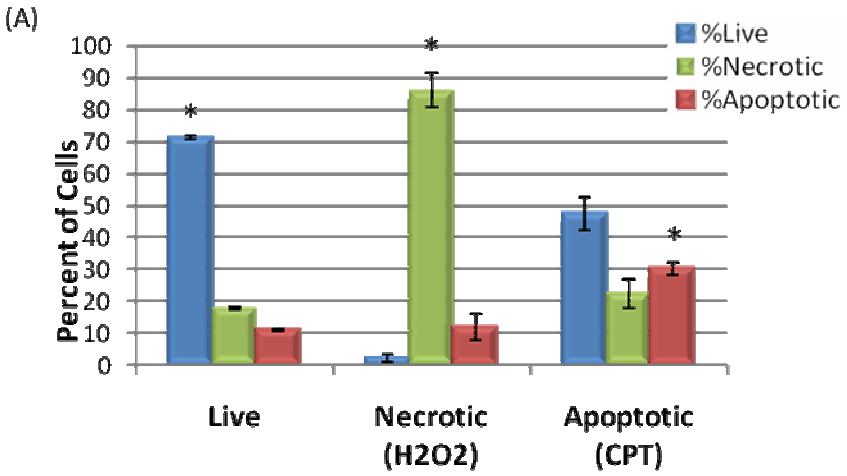
Cell Population	Membrane Capacitance (mF/m^2) HL-60 cells (Genistein) (Wang et al. 2002)	Membrane Capacitance (mF/m^2) Jurkat cells (Etoposide) (Pethig et al. 2007)
Live	17.6 ± 0.9	13.34 ± 2.88
Apoptotic	9.1 ± 0.5	10.49 ± 4.00
	ΔC_m ~ 8.5 mF/m^2 (48%)	**ΔC_m~ 2.85 mF/m^2 (21%)**

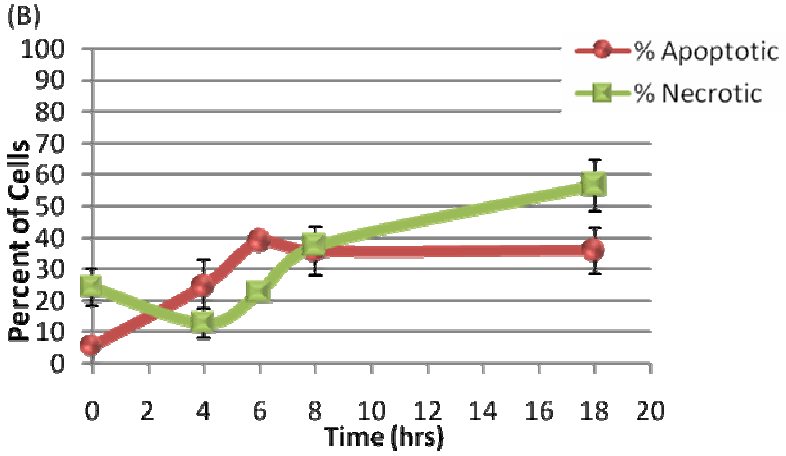
Cellular Annexin V Assay



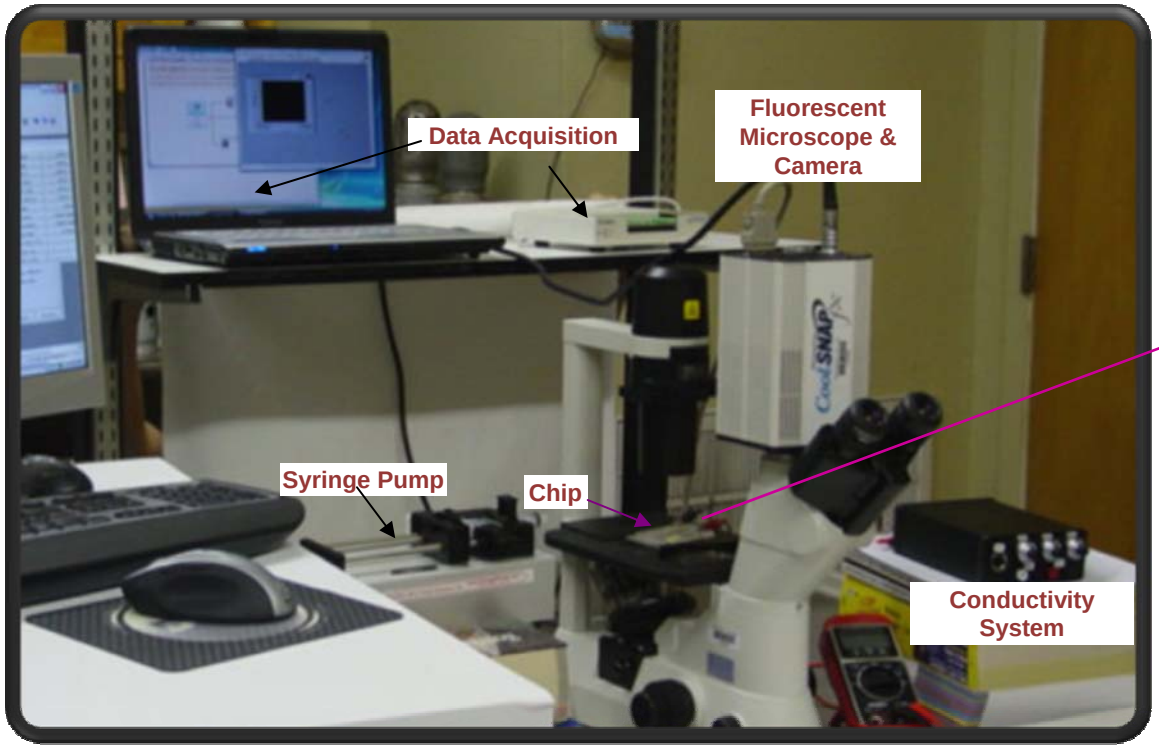
- Jurkat cells
- Camptothecin (CPT)- apoptotic control
  - Inhibitor of topoisomerase I- which is required for DNA synthesis
  - 10uM CPT @ 4-6 hrs
- Annexin V/SYTOX assay
  - Phosphatidylserine (PS) translocation- AnnexinV
  - Membrane integrity- SYTOX Green
- Fluorescence microscopy optimization

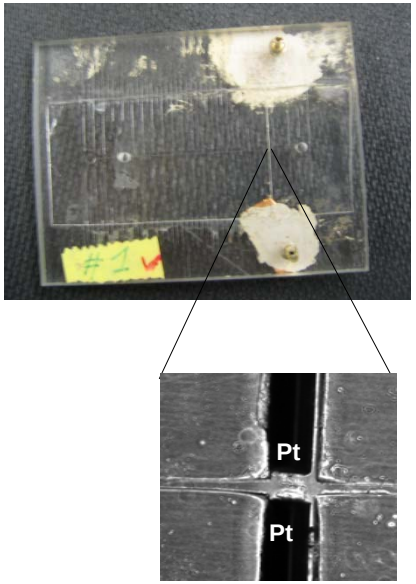






Experimental Setup



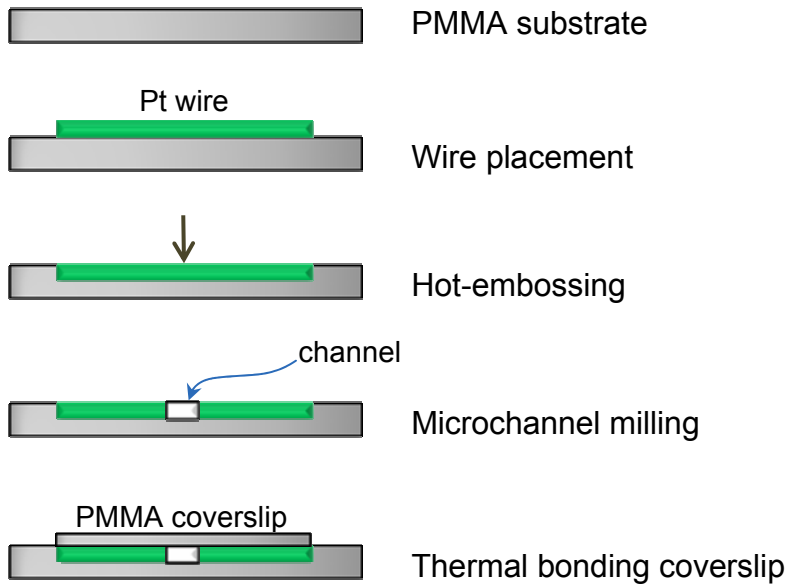


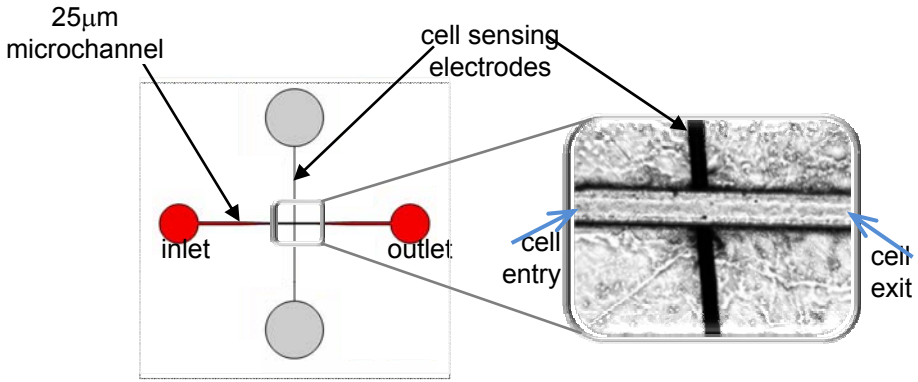
Microchip showing the 25um-wide microchannel and electrode detection area

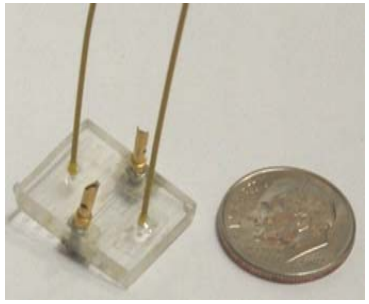
- Microchip visualized via Microscope
  - BF and Fluorescence microscopy
- Conductivity measurements acquired
  - Conductivity system
  - Data Acquisition via DAQ-Pad and LabVIEW

Microfluidic Chip Design & Fabrication

- PMMA substrate
  - Biocompatible, optically clear, non-fluorescent
- Pt wire electrodes
  - Inert, highly conductive
- Fluidic connections
  - Inlet and outlet
- Detection volume
  - Rectangular cross-section (25umx76umx76um)
- Cell Experiments
  - Cells suspended in buffer
  - Syringe pump control laminar flow of cells
  - Electrical field applied by conductivity system
  - Simultaneous video acquisition



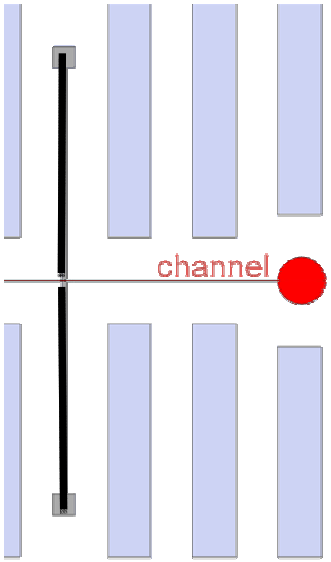




2 Primary Chip Designs

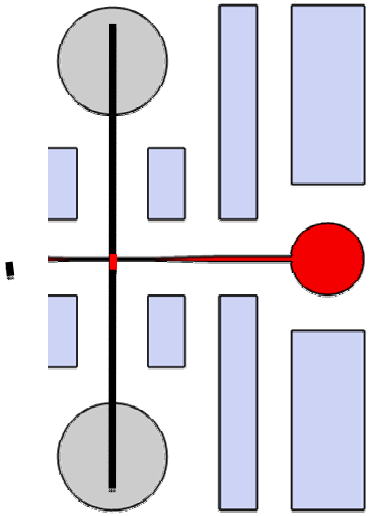
“Guide Channel”

- Chip channels micromilled
- Pt wire
  - Cut in 5mm segments
  - Placed in guide channels
  - Aligned along microchannel



“Milled-wire”

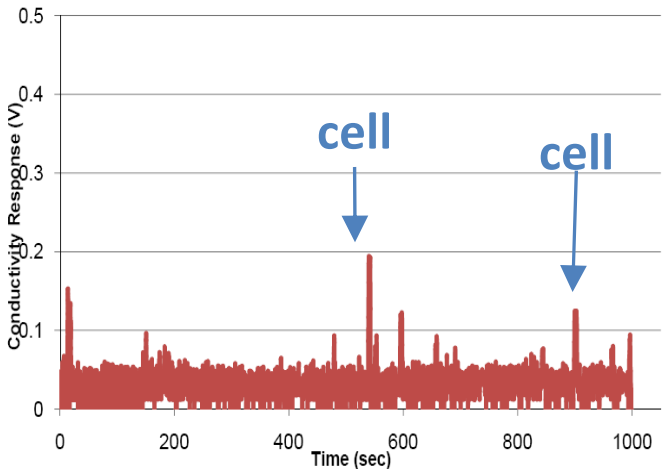
- Pt wire
  - Threaded through holes
  - Hot-embossed into PMMA
  - Cut during micromilling of channel

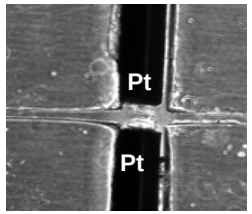


Conductivity Detection

- Conductivity System
  - Bi-polar voltage (±0.5 V)
  - 40kHz
- Resistance in channel
  - Electrode gap and size (area)
  - Conductivity of buffer
- Experiment
  - Flow of dilute suspension of Jurkat cells
  - Peaks in Voltage indicate cell present
  - Conductivity measurements will be acquired simultaneously with video microscopy for optical validation

Conductivity of biological cell buffers (Omega CDH-7X conductivity meter)	
0.1 mM PBS	0.5 mS/cm (±0.04)
1 mM PBS	1.2 mS/cm (±0.04)
10 mM PBS	10.2 mS/cm (±0.04)
Tris-Glycine	40 μS/cm (±0.04)

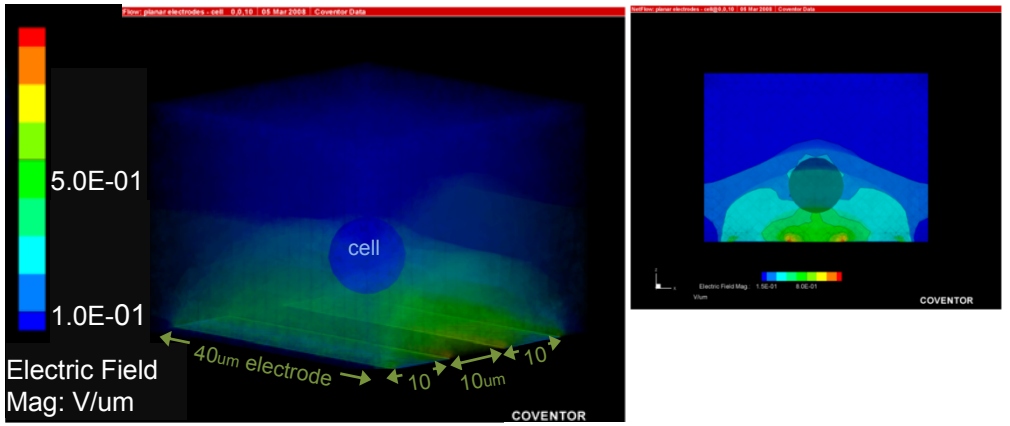




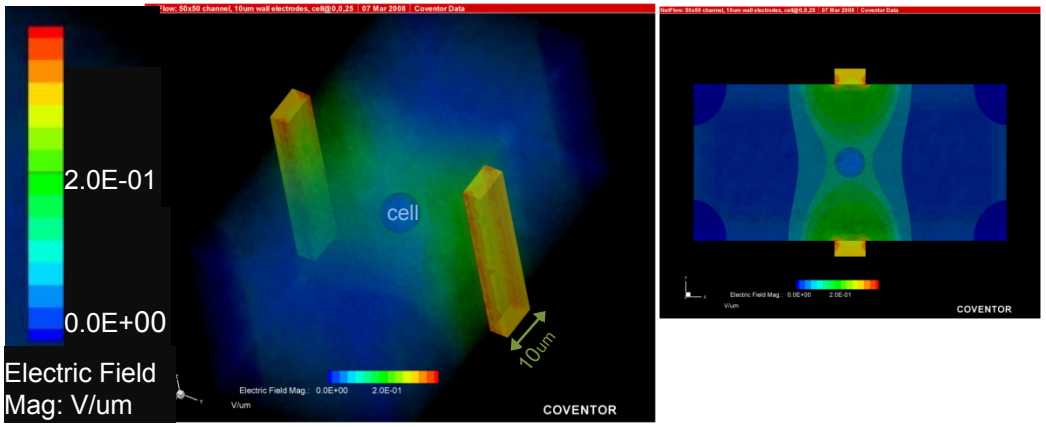
Microchip Electrode Detection Area

Modeling of Electrodes

- Simulations
  - Optimize electrode geometry
  - Help understand parameters
    - Cell size, buffer, electrodes, micro channel geometry
  - Evaluate planar vs. side-wall electrodes
- 3D Modeling of Electric Field Magnitude
  - Coventor NetFlow analyzer
  - Pt Electrodes: 10um wide, 10um spacing
  - Buffer completely filling channel (σ_{1mMPBS}=1.2mS/cm)
  - Applied Electric Potential =10V DC
  - Steady-state
- Dielectric biological cell disturbs electric field



Planar electrodes



Side wall electrodes

Summary & Future Work

- Overall Goal:
  - Establish proof-of-concept for electrical, label-free BioMEMS device for quantifying cell properties
- Early detection of apoptosis is important for drug-screening and cancer treatment
- Apoptotic-induced dielectric alterations (membrane capacitance, cytoplasm conductivity, cell size) have been described
- A PMMA microchip device with embedded Pt electrodes is proposed for evaluating live, necrotic, and apoptotic Jurkat cells
- Future generations of microfabricated chips will be evaluated
  - Consider electrode alternatives
  - Conduct modeling simulations to estimate optimal geometry and expected results

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"Matt" Hupert (Chem)	Embossing and materials expertise
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Thank You

- Any Questions?