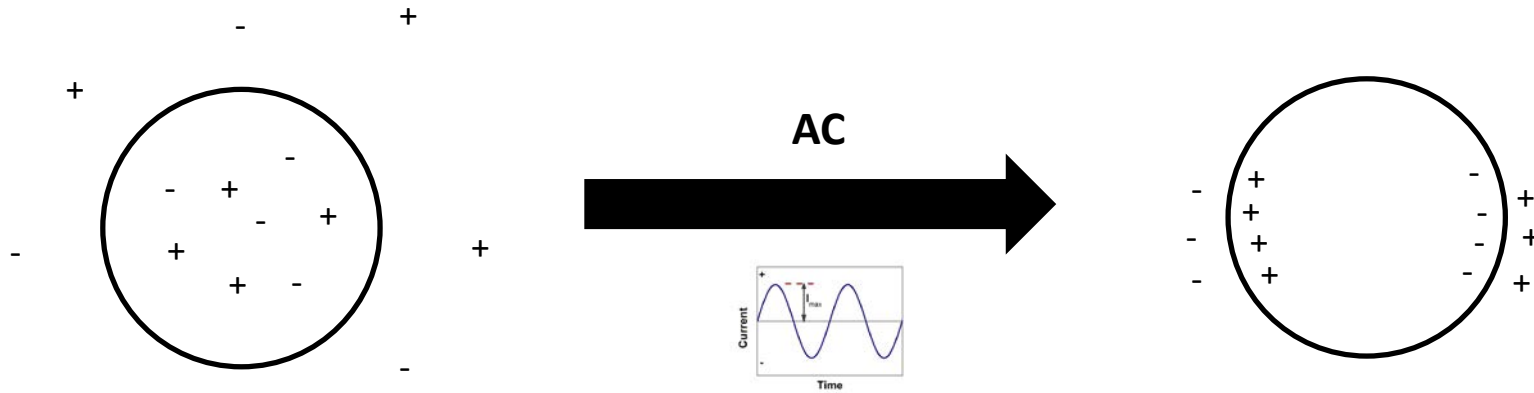


Bioproduction / Citywest October 19-20th 2016

Dielectric Monitoring of Mammalian cells in a Bioreactor

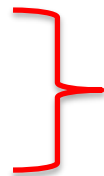
Michael Butler
NIBRT,
Dublin, Ireland

Dielectric properties of cells



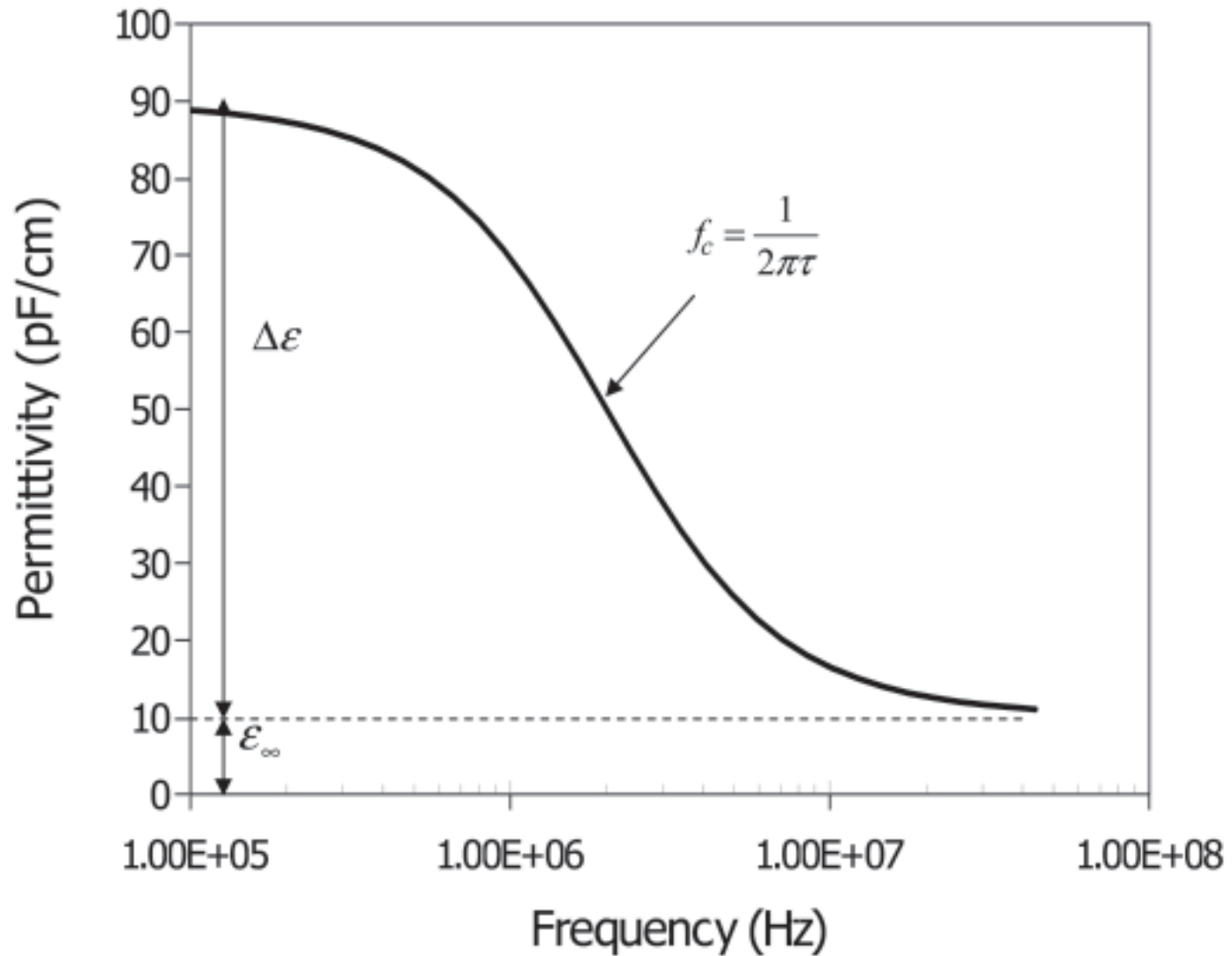
- Formation of dipole
 - Dependent on frequency applied

Volume
Membrane capacitance
Cytoplasm conductivity

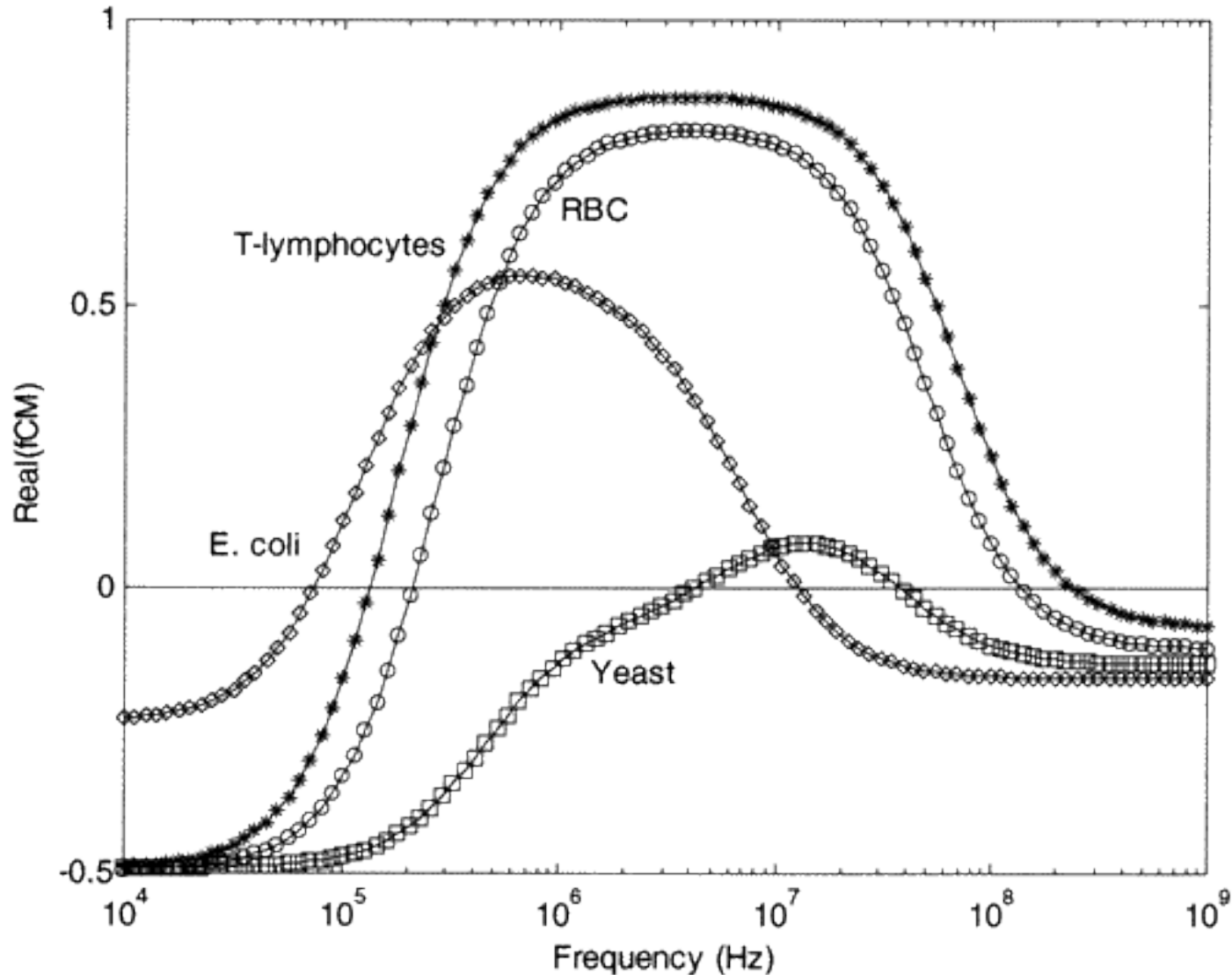


Indicators of physiological cell conditions

Beta dispersion curve



Frequency spectra for the polarization of various cell types



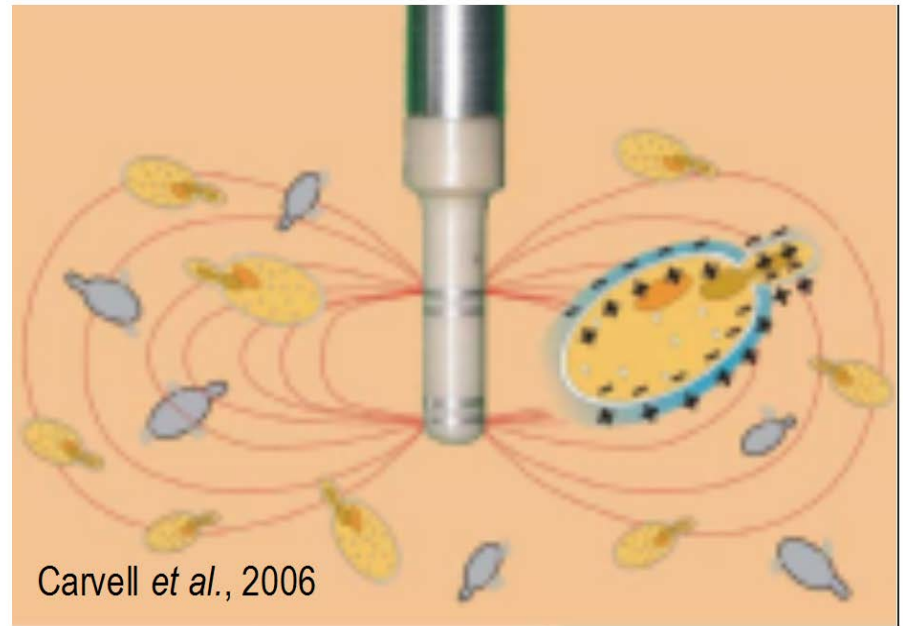
Dielectric Measurement

Biomass Monitor: Aber Instruments

On-line measurement

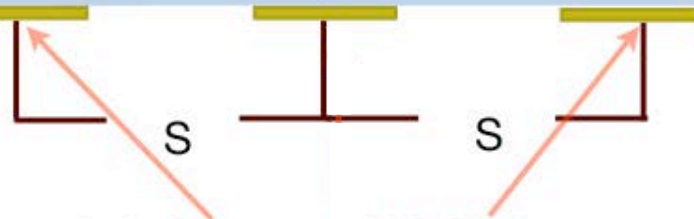
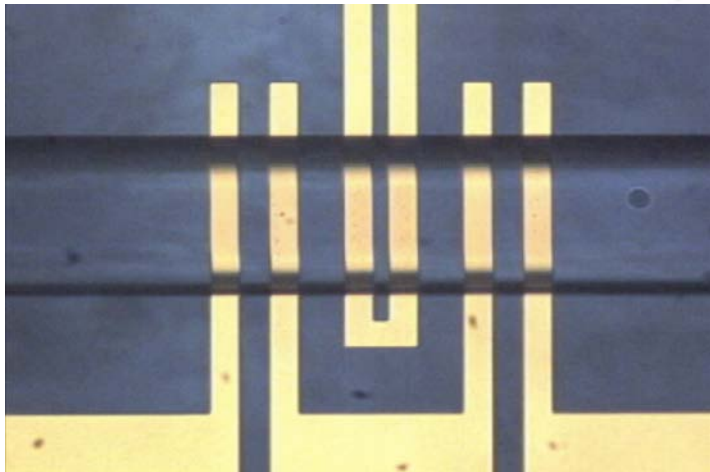


The Standard Remote FUTURA



DEP Cytometer



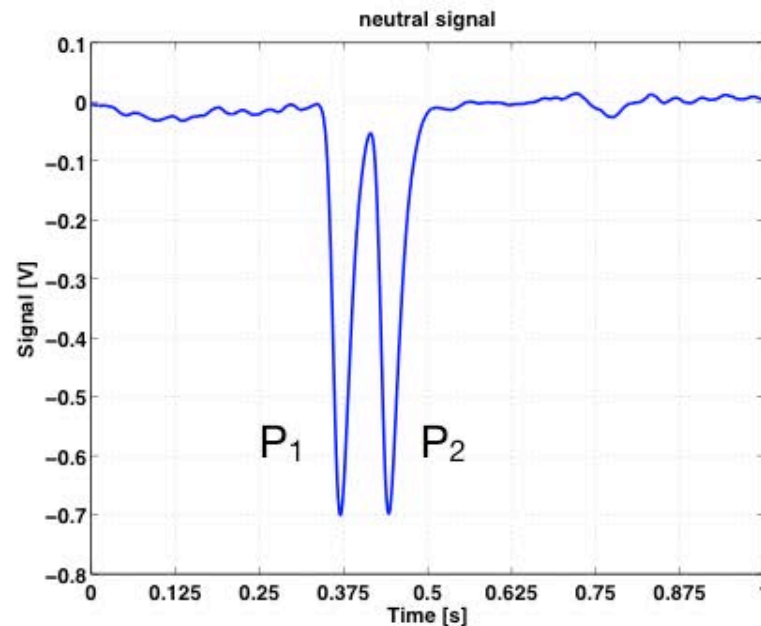


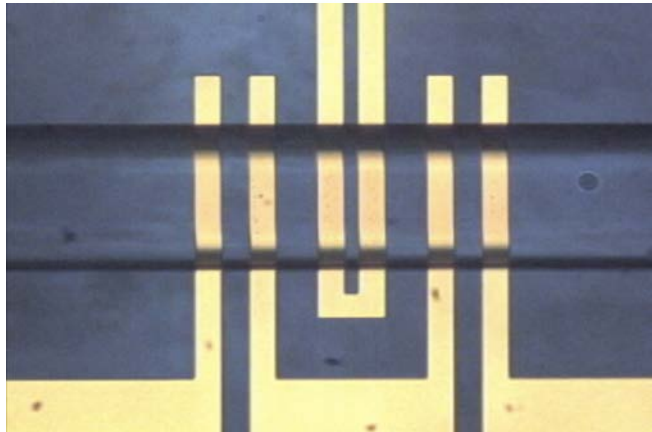
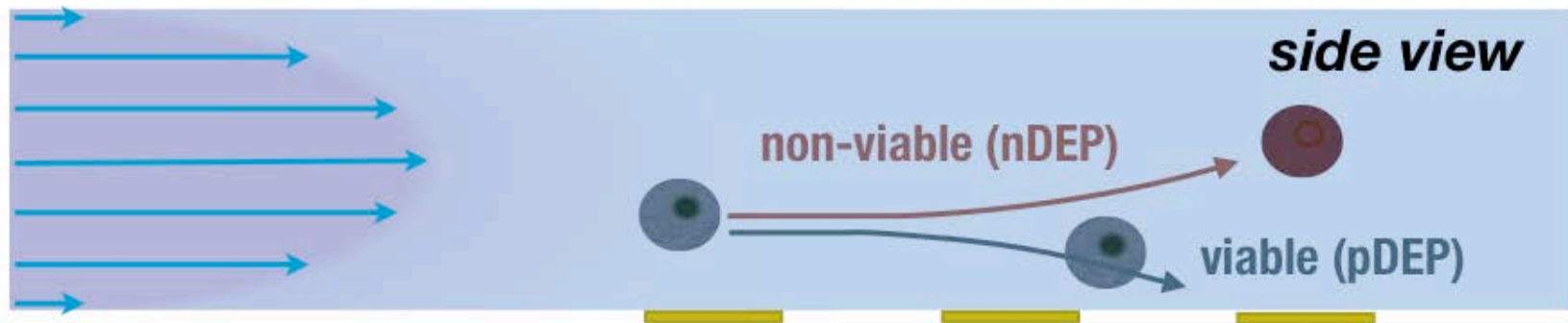
high frequency (1.5 GHz) waves
used for **detection**

$$S \sim C (E^2)$$

Without low frequency wave
there is **no actuation**.

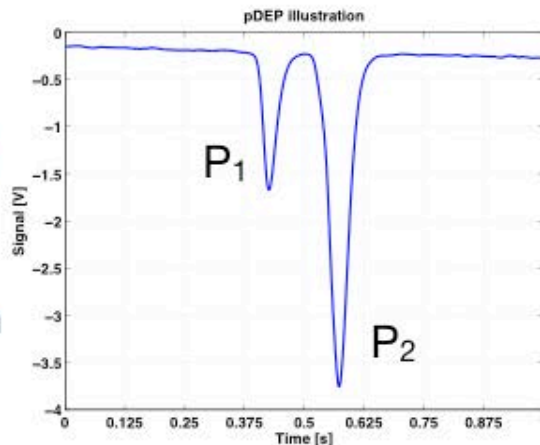
==> Signal peaks P_1 and P_2 have
the **same amplitude**
and **width**.





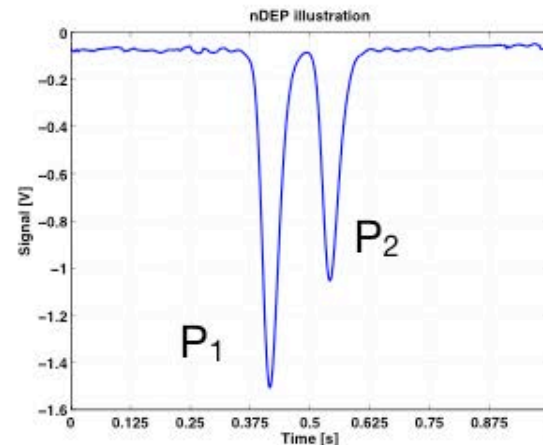
low frequency wave
(100 kHz - 10 MHz)
 $F \sim \nabla E^2$

$$\text{viability} = \frac{\text{number of pDEP signals}}{\text{total number of signals}}$$



viable cells
attracted
(pDEP),
slowed down

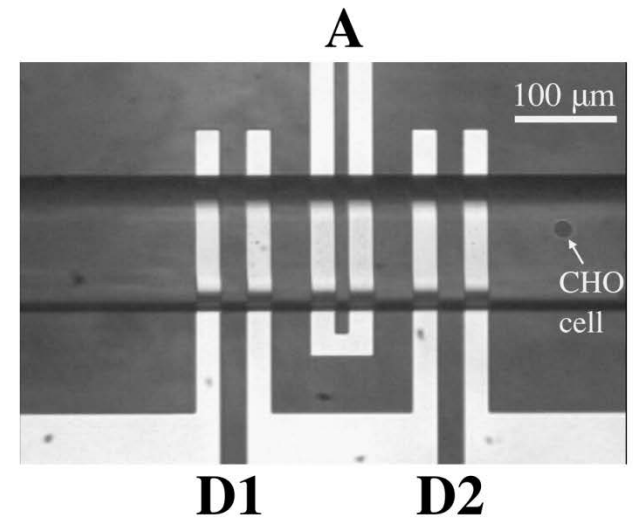
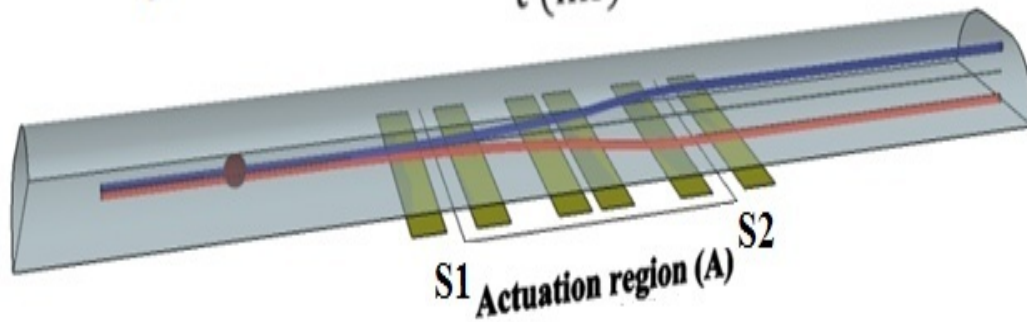
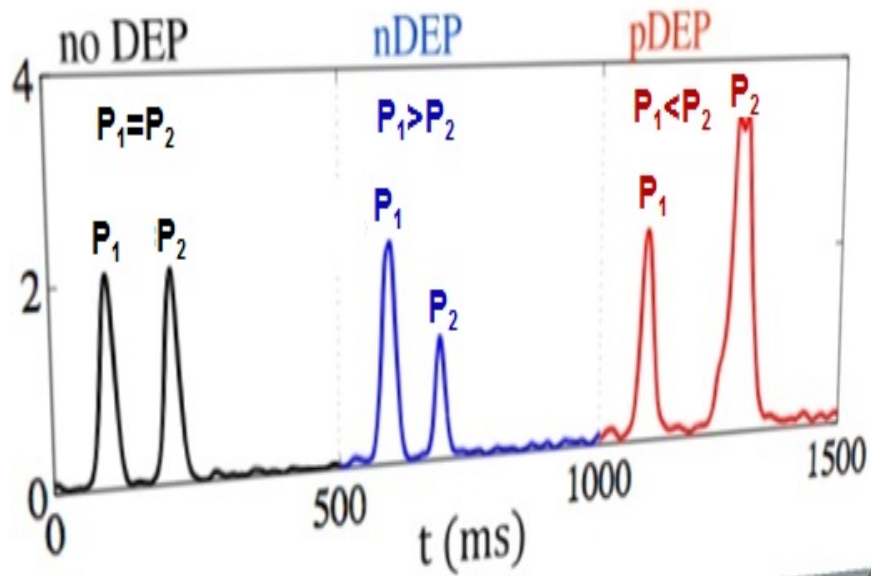
P₂ is stronger and wider than P₁.



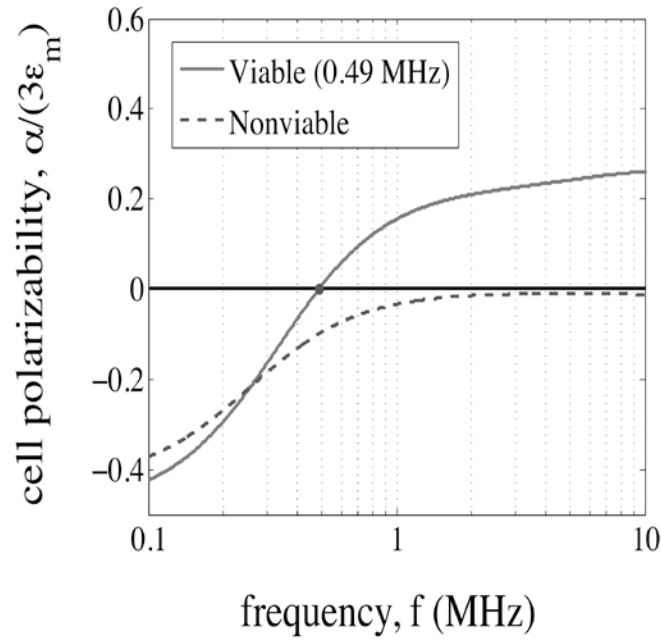
non-viable cells
repelled
(nDEP),
sped up

P₁ is stronger and wider than P₂.

$$\text{Force Index (FI)} = \frac{2(P_2 - P_1)}{(P_1 + P_2)}$$

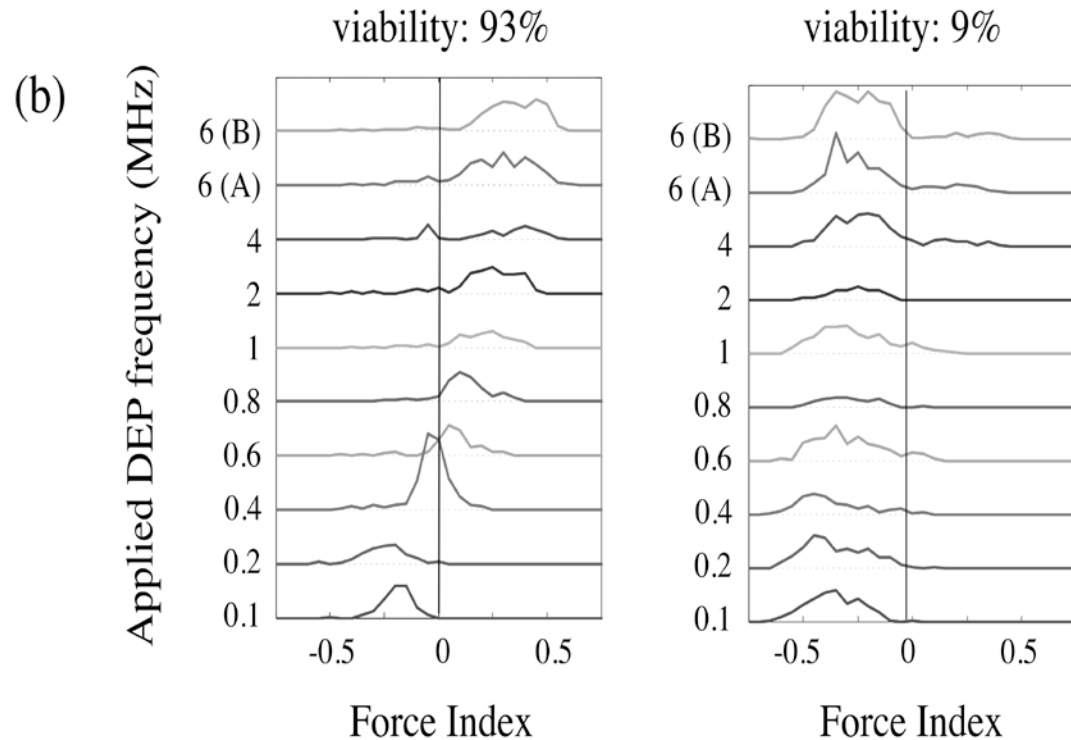


The effect of AC frequency of the actuating electrode on response



(a)

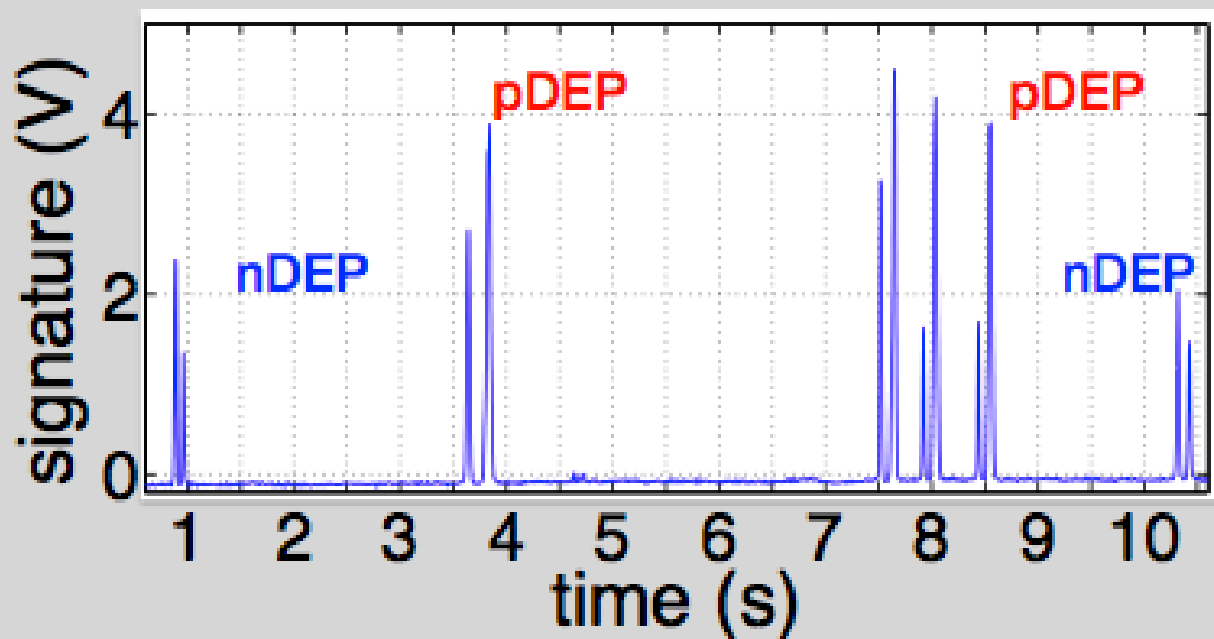
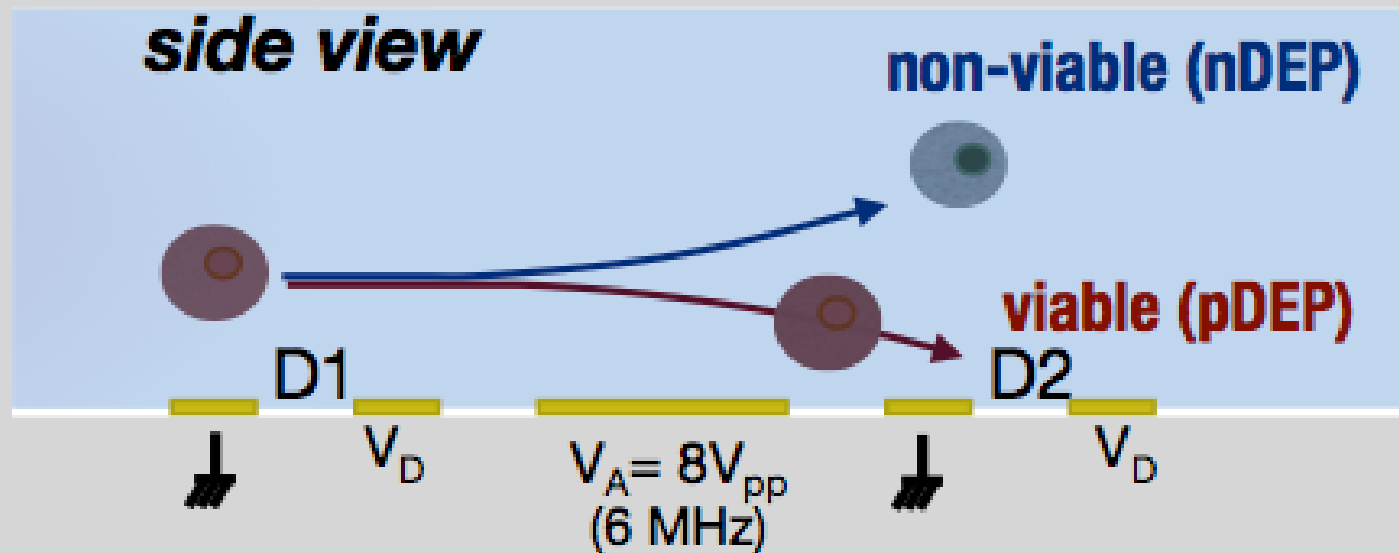
Numerical simulation of the polarizability, produced for a double-shell model (Jones, 1995)



(b)

Signatures were collected at nine discrete steps in frequency. Two collections were made at 6 MHz frequency at the beginning and at the end

Actuation (DEP on)

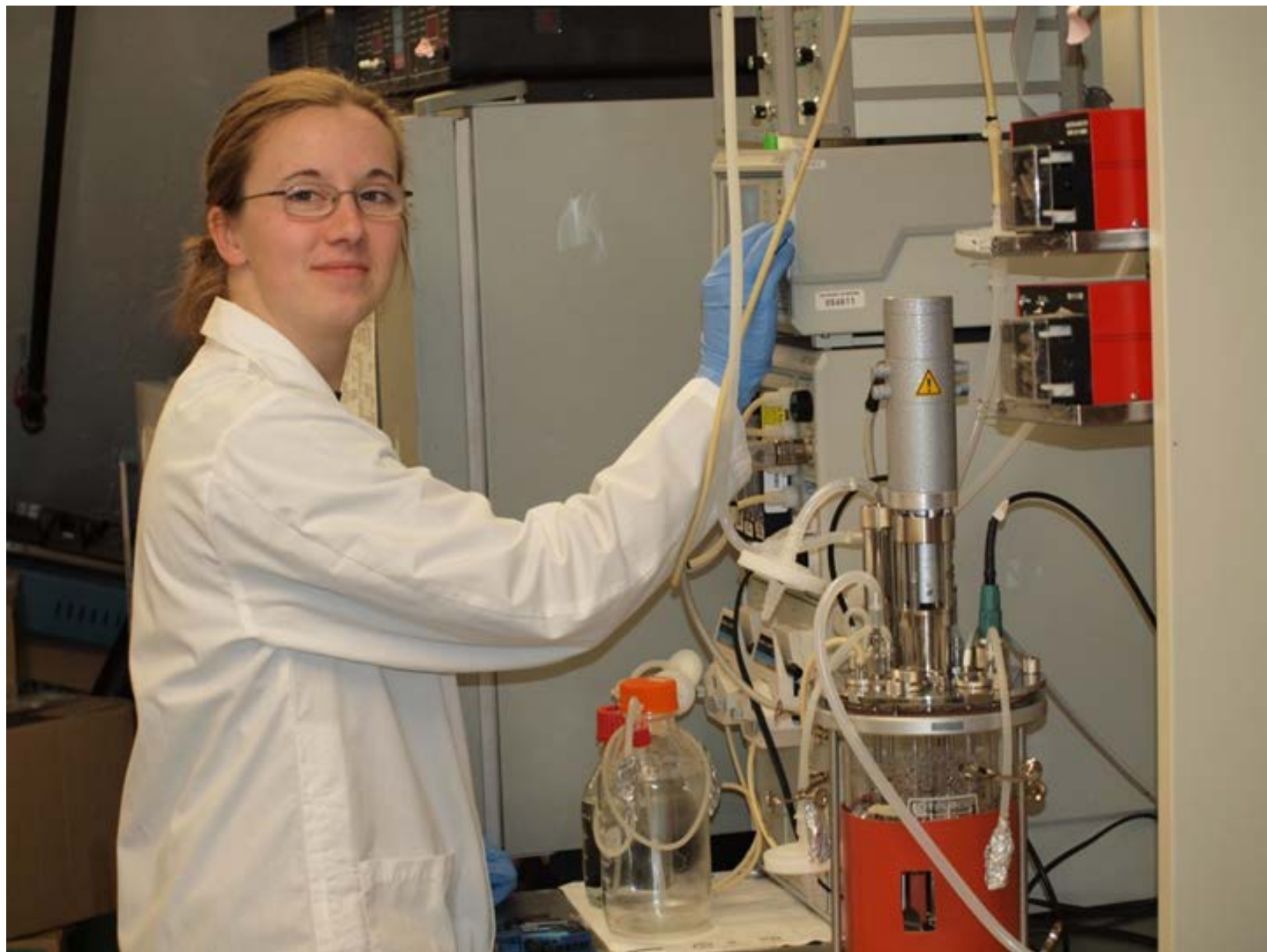


RESULTS

Application of counting methods to a bioprocess for monoclonal antibody production

Methods of monitoring cell growth

- **Particle counting:** total cell count
- **Haemocytometer:** cells with intact membrane
- **Image analysis:** cells with intact membrane
- **Capacitance probe:** polarizable cells on-line (population)
- **Flow cytometer:** stages of apoptosis; fluorescence
- **DEP cytometer:** polarizable cells (individually); markerless

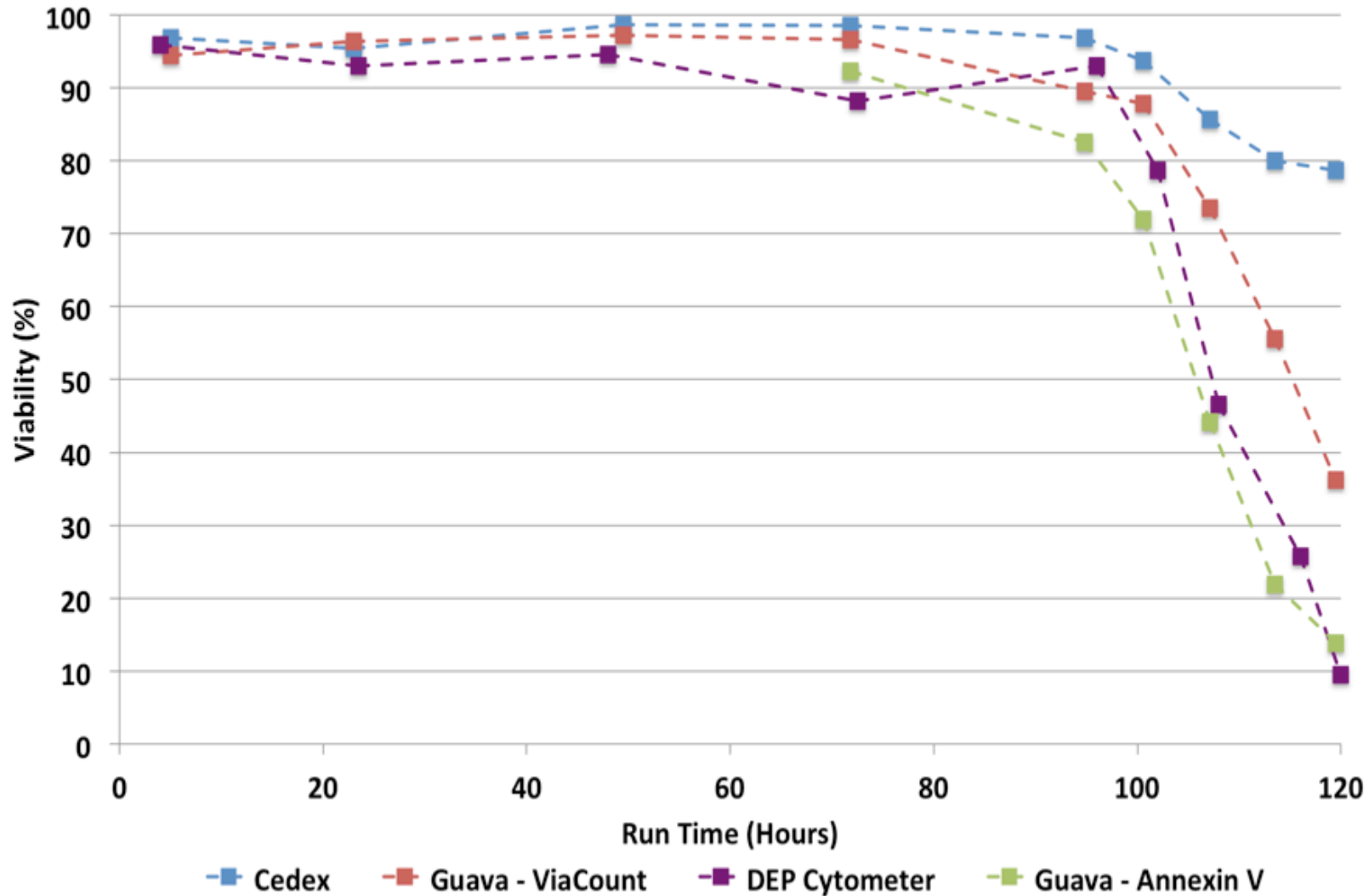


Growth Curve

Cell density and capacitance determined for CHO EG2 cells in batch culture



Cell Viability Monitoring (Run A)



Adenylate Energy Charge

interconversion of the three adenylate nucleotides in the cell:



$$\text{energy charge} = \frac{[ATP] + 1/2 [ADP]}{[ATP] + [ADP] + [AMP]}$$

decrease in the value gives an early indication of loss of viability in a cell population (< 0.7-0.9)

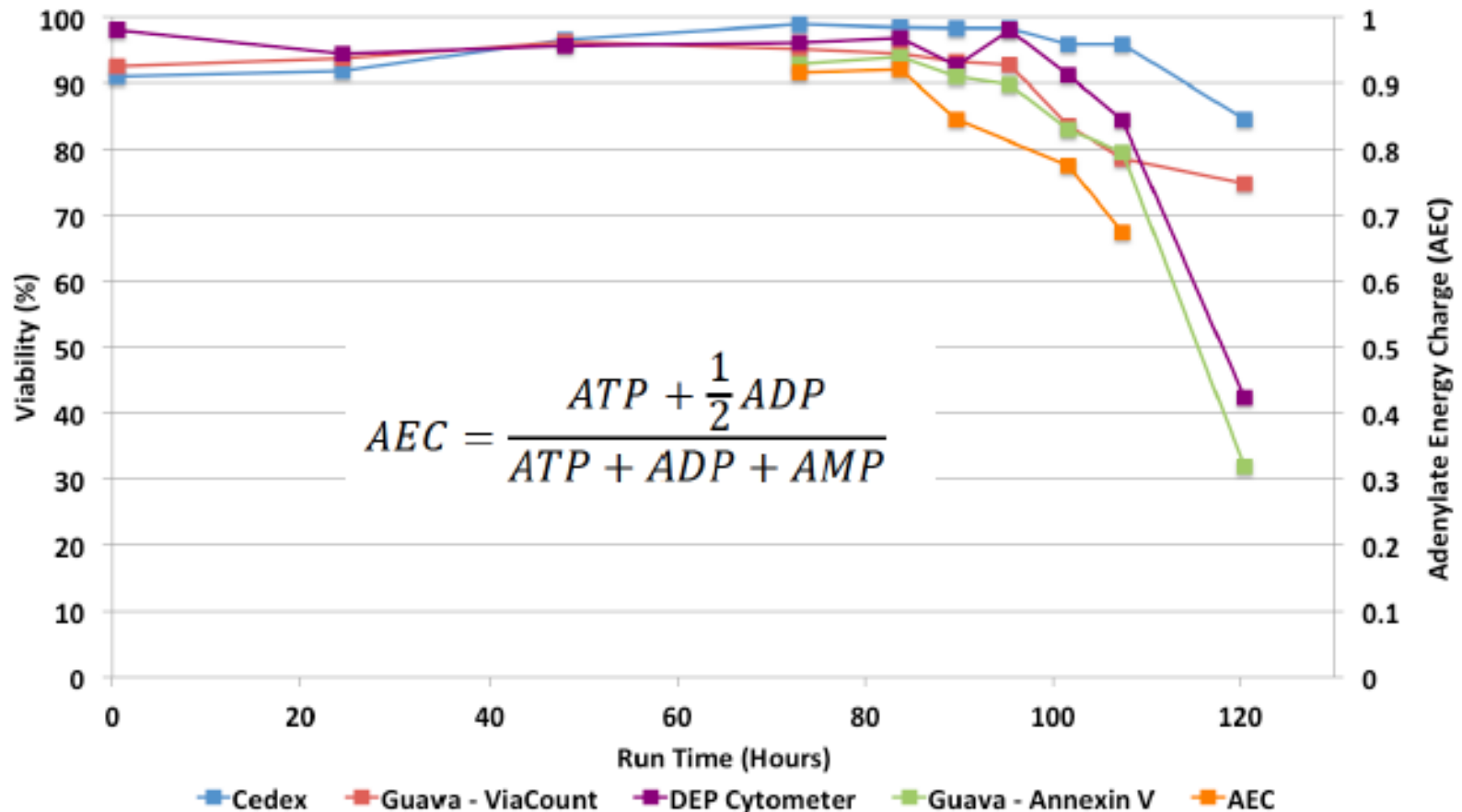
measured by HPLC or luciferase-luciferin enzyme system

Viability (Run B)

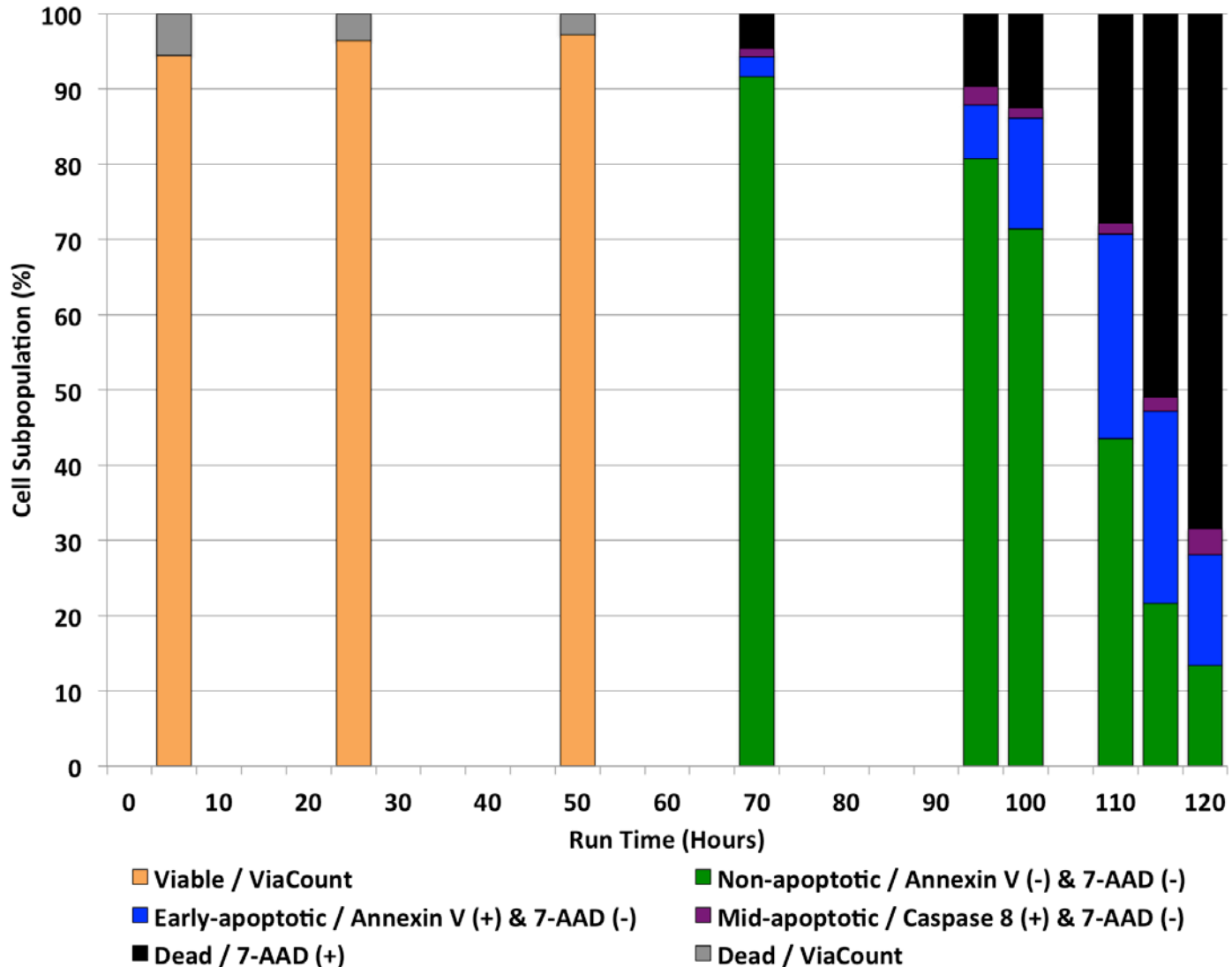
Viability determined for CHO EG2 cells in batch culture

Adenylate energy charge indication for cell health

- normal or mid-exponential phase AEC = 0.8 - 0.9

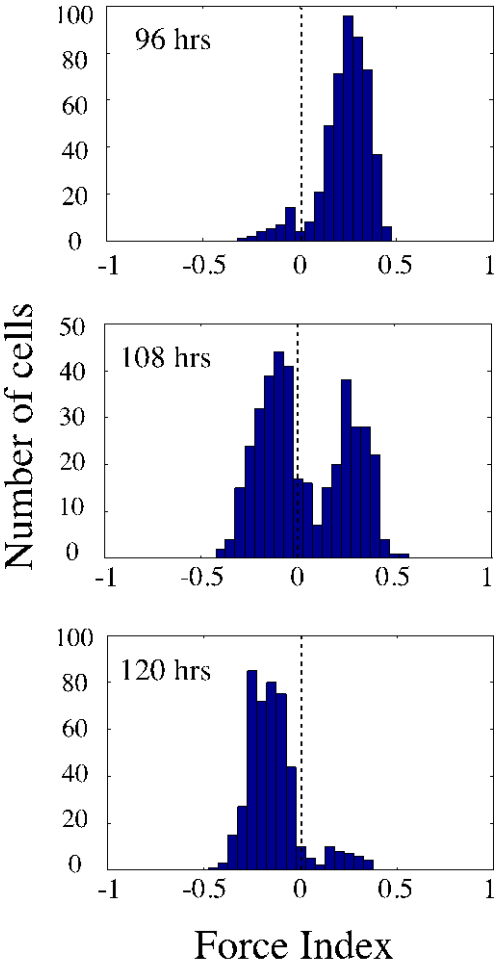


Cell Subpopulation Monitoring in Bioreactor Culture - Comparison

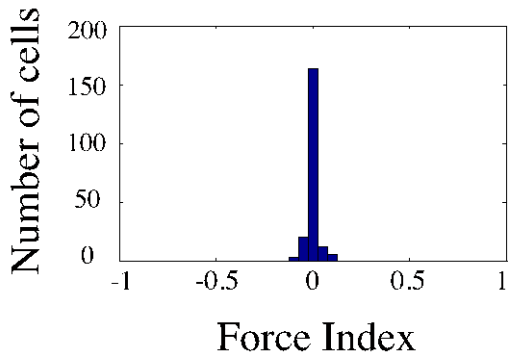


Time-lapse histograms showing binary population of cells

Actuated with 8 Vpp, 6 MHz



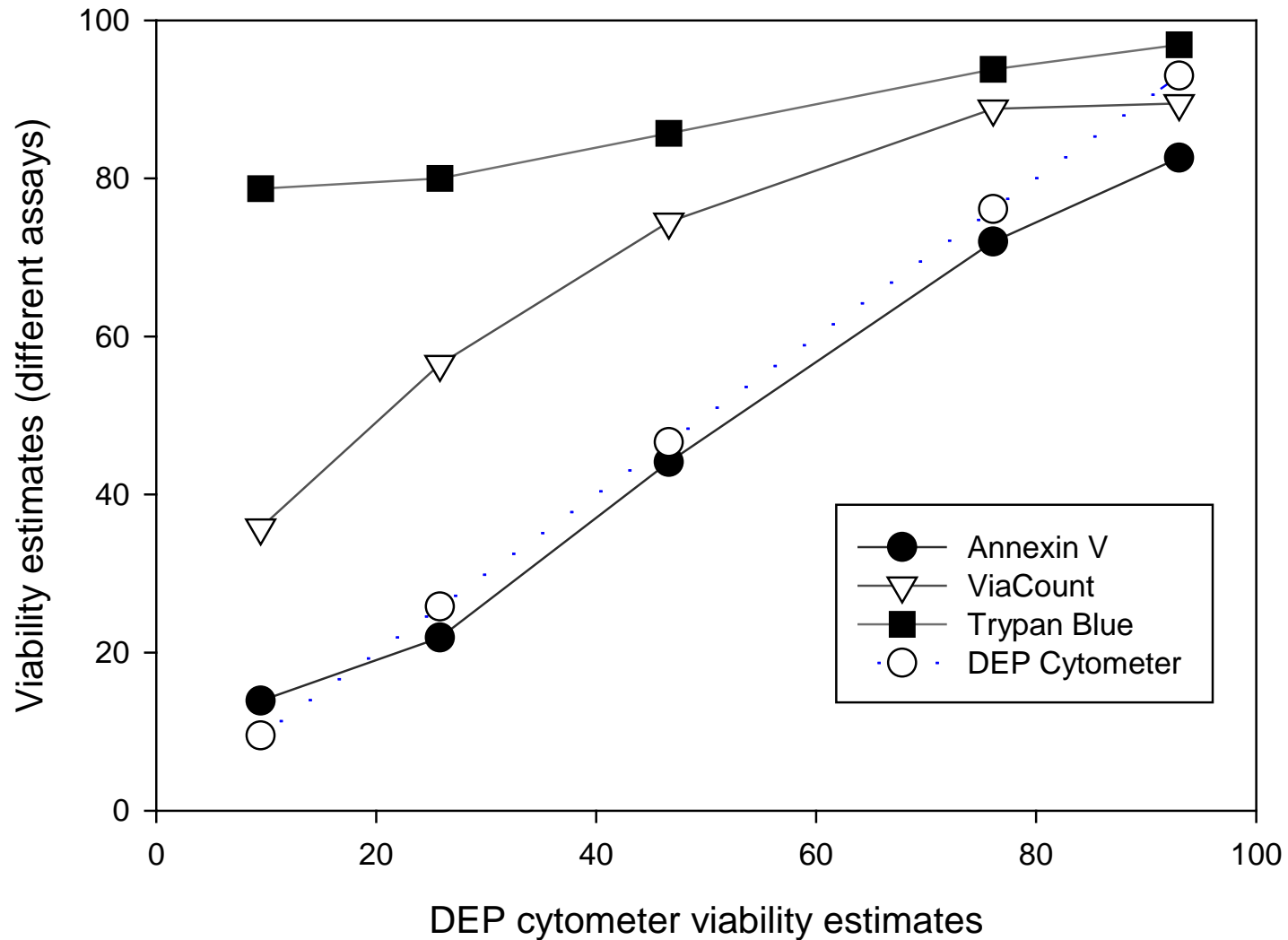
Control set (no DEP applied)



Assay	% viability at approx. hours				
	96	102	108	114	120
Trypan blue	96.9	93.8	85.7	80.0	78.7
ViaCount*	89.5	88.8	74.5	56.5	35.8
Nexin (annexinV)	82.6	72.0	44.1	21.9	13.9
DEP cytometer	93.0	76.1	46.6	25.8	9.5

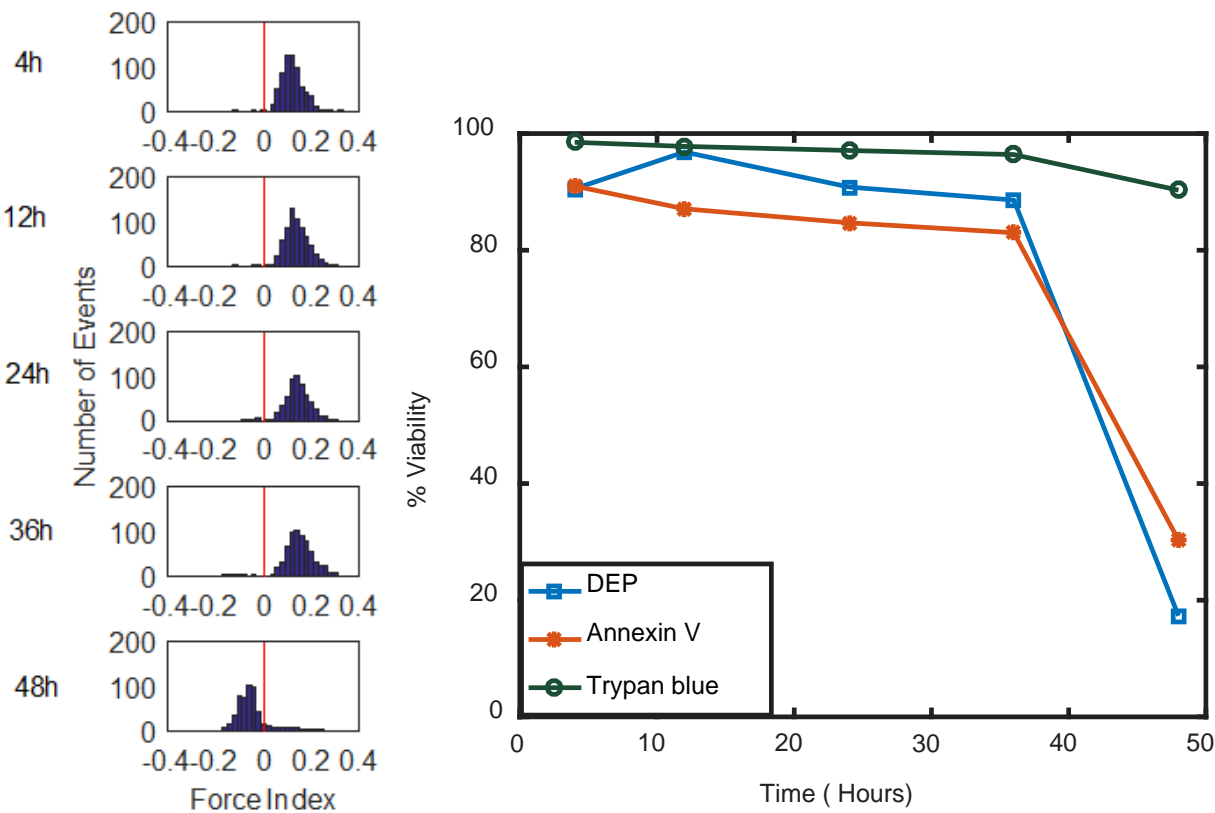
*uses a proprietary mix of two DNA-binding dyes

Correlation of estimates of viability by different methods



Induction of Apoptosis by Starvation

Results over 48 hours of Starvation



CHO cells seeded at
25 mM glucose
1 mM glutamine
4 mM GlutaMax
Density= 2×10^5 cells/mL
37°C
10% CO₂
120-160 rpm
Passage number:
31, 22, 7



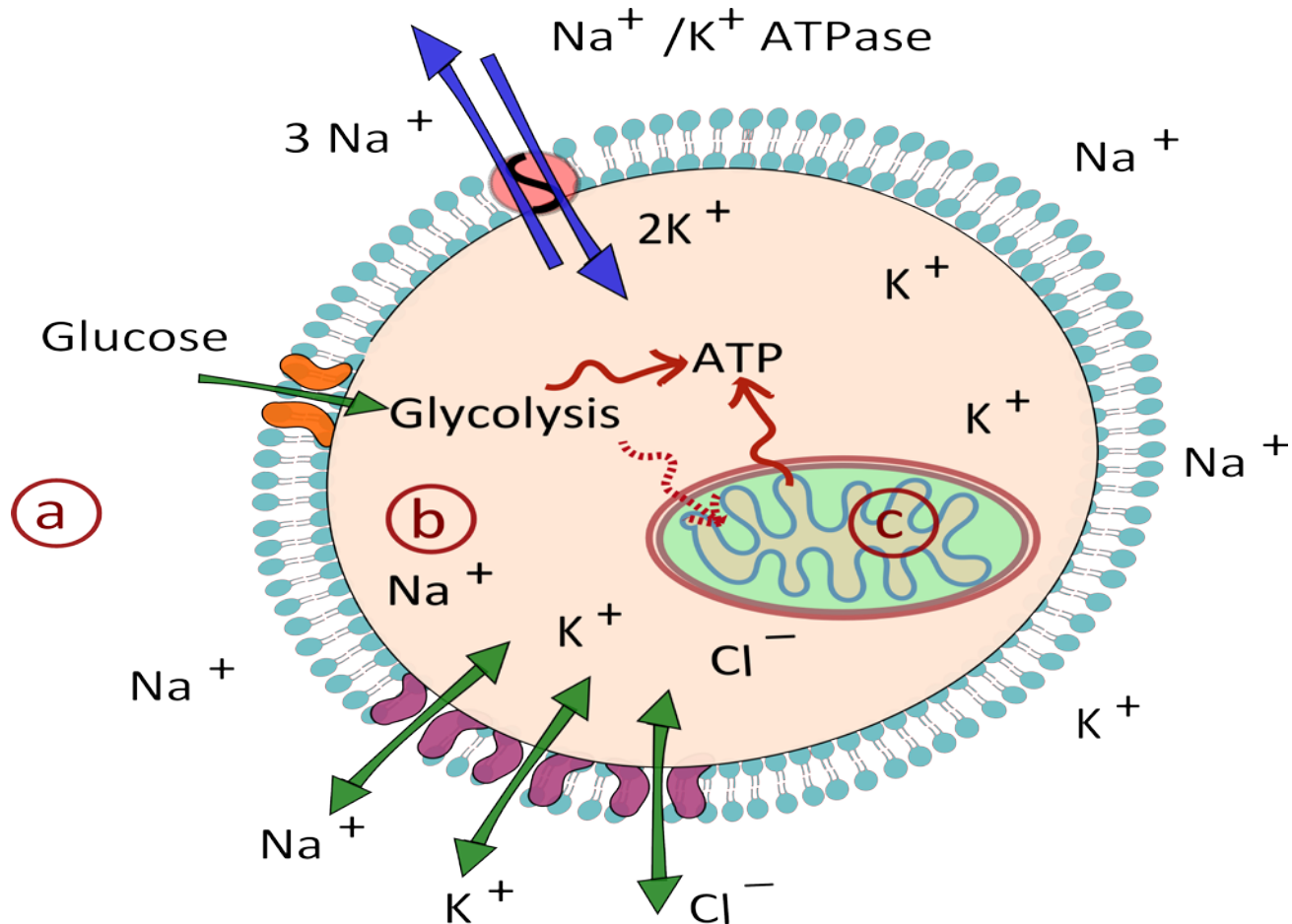
Day 3 cells extracted with
centrifuge and resuspended into
medium without glucose,
glutamine and GlutaMax
Density= $2-5 \times 10^5$ cells/mL

Monitored every 12 hours

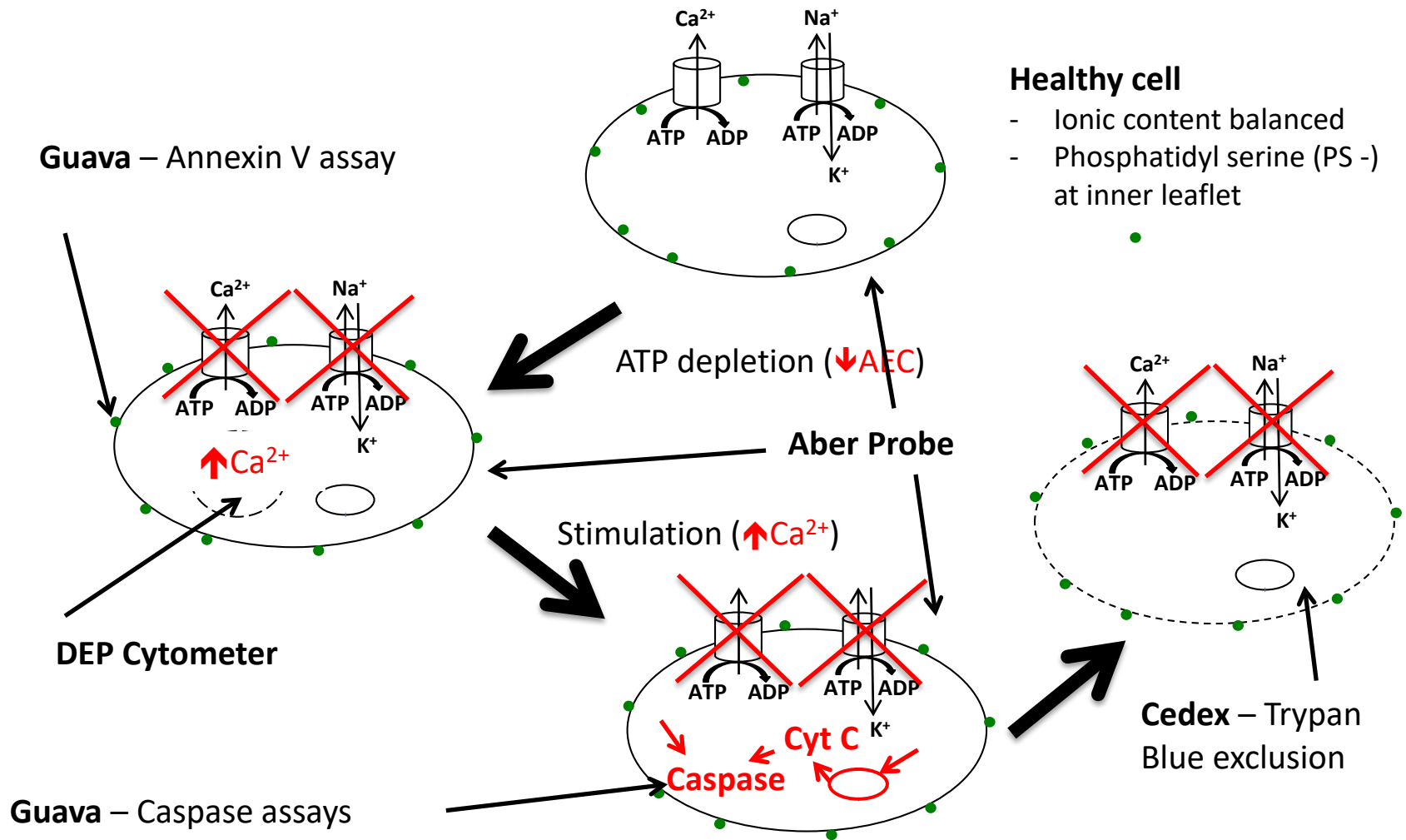
- Annexin V
- DEP
- Trypan blue

DISCUSSION and SUMMARY

Mechanisms of cellular energetics



Proposed Model for Detection of Changes during Apoptosis



Summary of findings

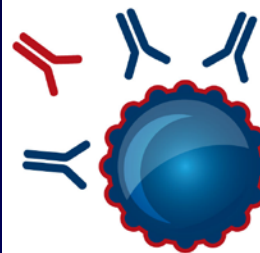
- Various methods used to monitor cells correlated during growth
- Deviations occurred during cell death
- Early apoptosis is detected and correlated with Annexin V assay
- Late stages involve membrane damage and trypan blue detection
- The capacitance probe (Aber) measures early stage apoptosis in a cell population
- DEP cytometry is a markerless electronic single cell detector
 - Identifies sub-populations of cells during apoptosis
 - Drug discovery through metabolic change detection



**NSERC
CRSNG**



UNIVERSITY
OF MANITOBA



MabNet
monoclonal antibody



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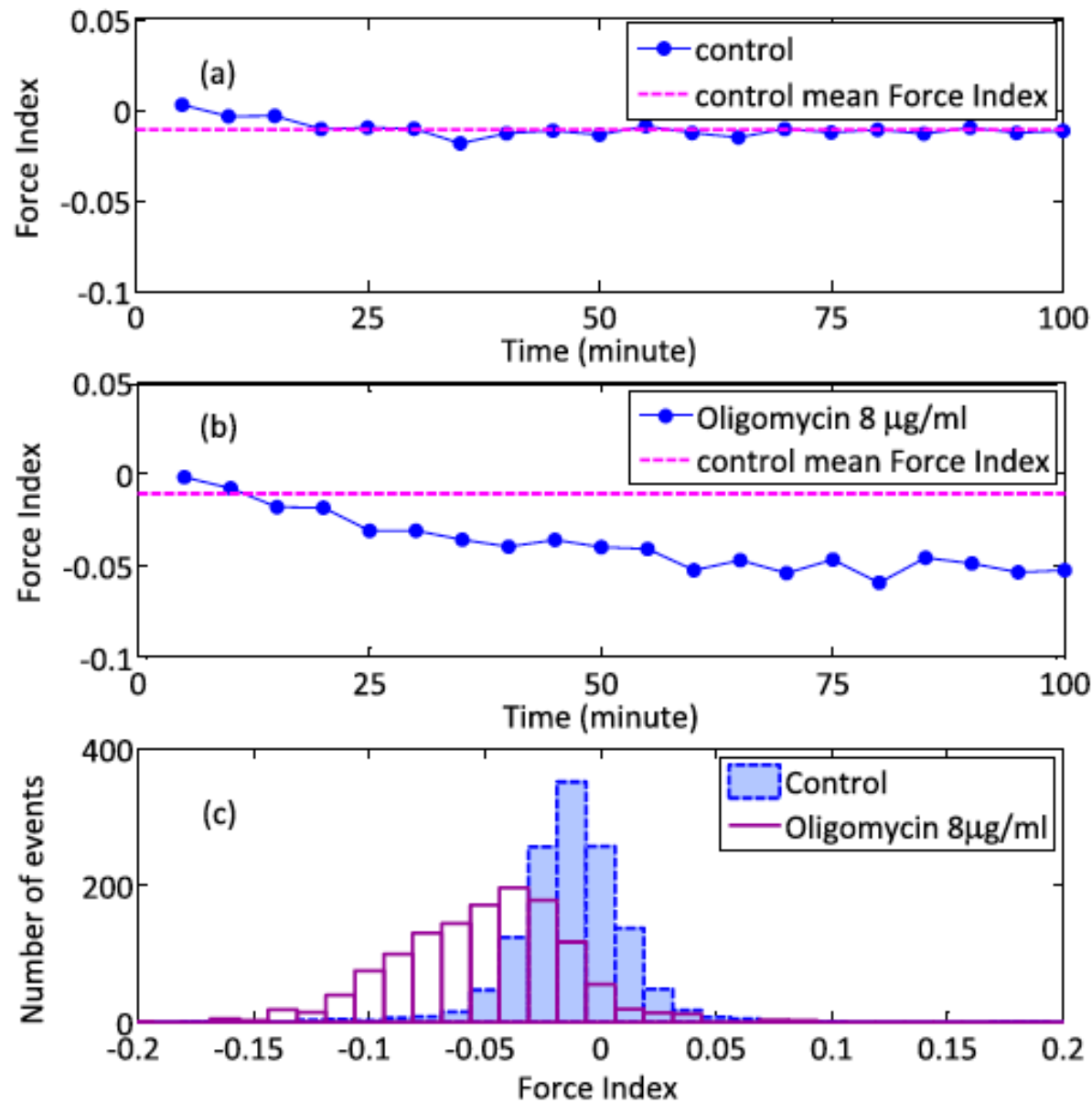


National Institute for Bioprocessing Research & Training

NIBRT's vision

- » Become a global leader in biopharmaceutical manufacturing research, education and training
- » Build out our research and development scale, capability and critical mass to establish NIBRT as a globally recognised centre for industry applied research and process development
- » Be the hub for bioprocessing manufacturing research in Ireland and internationally
- » Continue to support the growth and development of the biopharmaceutical industry in Ireland





By using medium conductivity close to cytoplasm conductivity for DEP measurements early detection of changes during oligomycin apoptosis induction