

Sequential Injection Capillary Electrophoresis for Bioprocess monitoring

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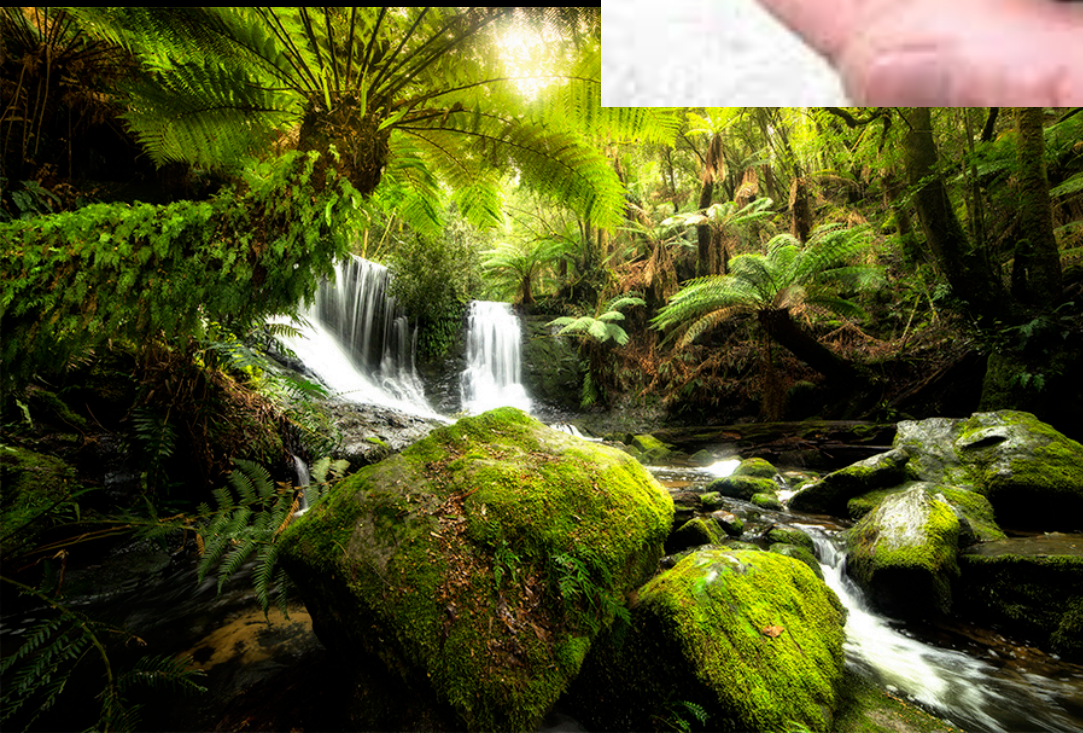
School of Medicine & School of Physical Sciences

University of Tasmania

Australian Centre for Research on Separation Science (ACROSS)

Microfluidic Chip Shop GmbH

From July: Deakin University, Geelong, Australia

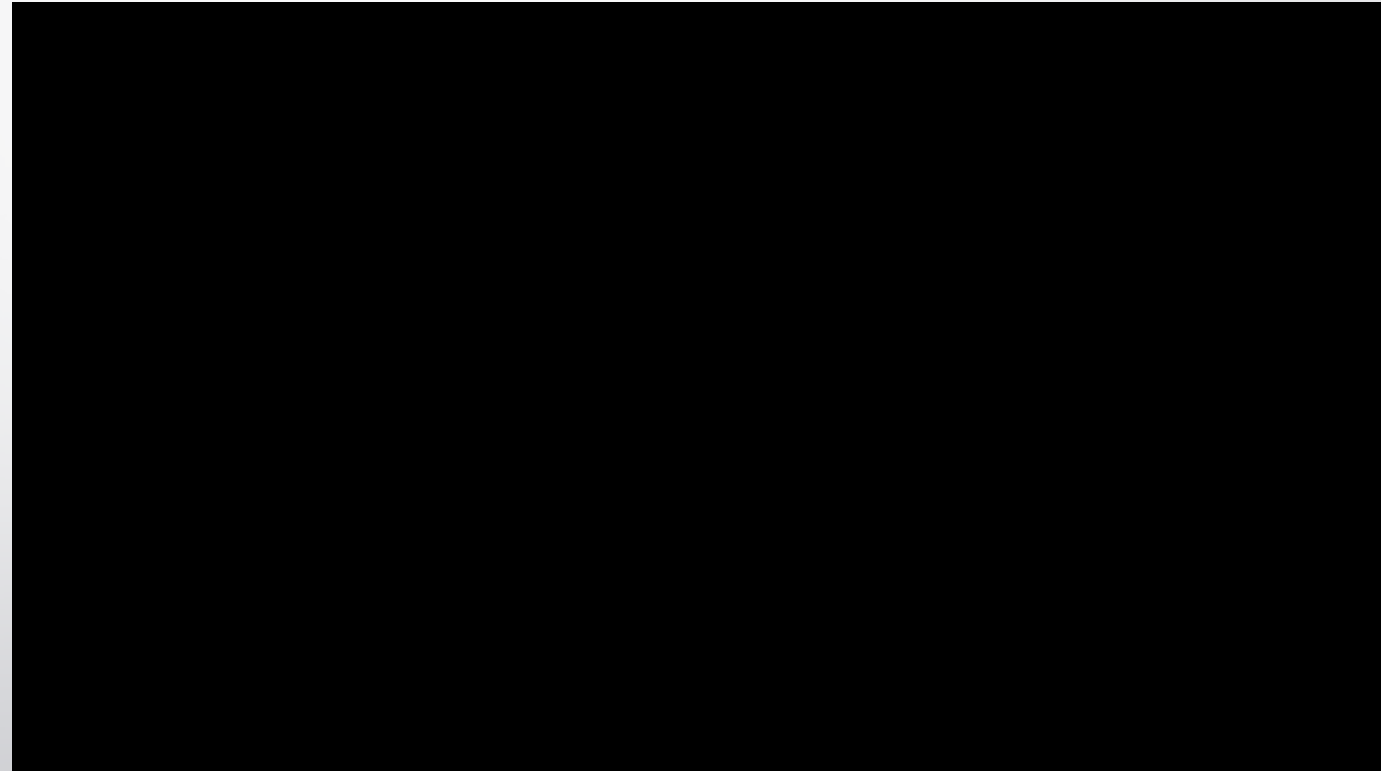


When a photograph falls short

Photograph



Movie (deskcam)



Rationale: need for more chemical information

Fast



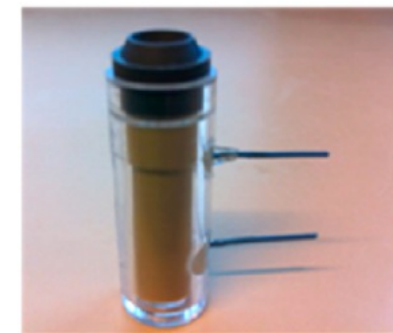
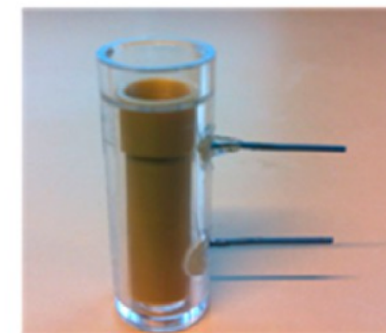
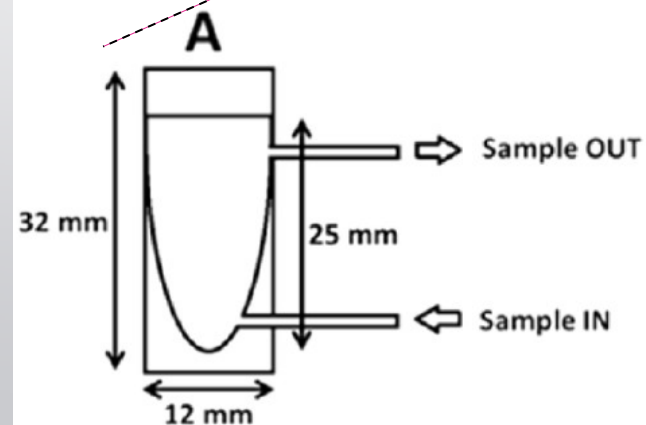
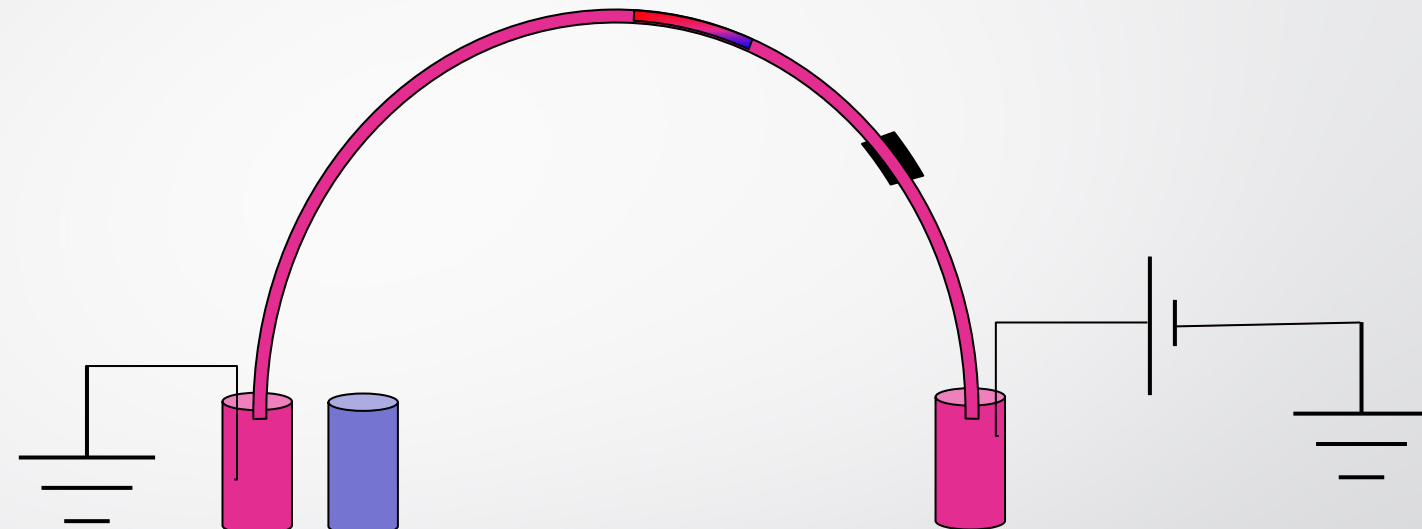
On-site

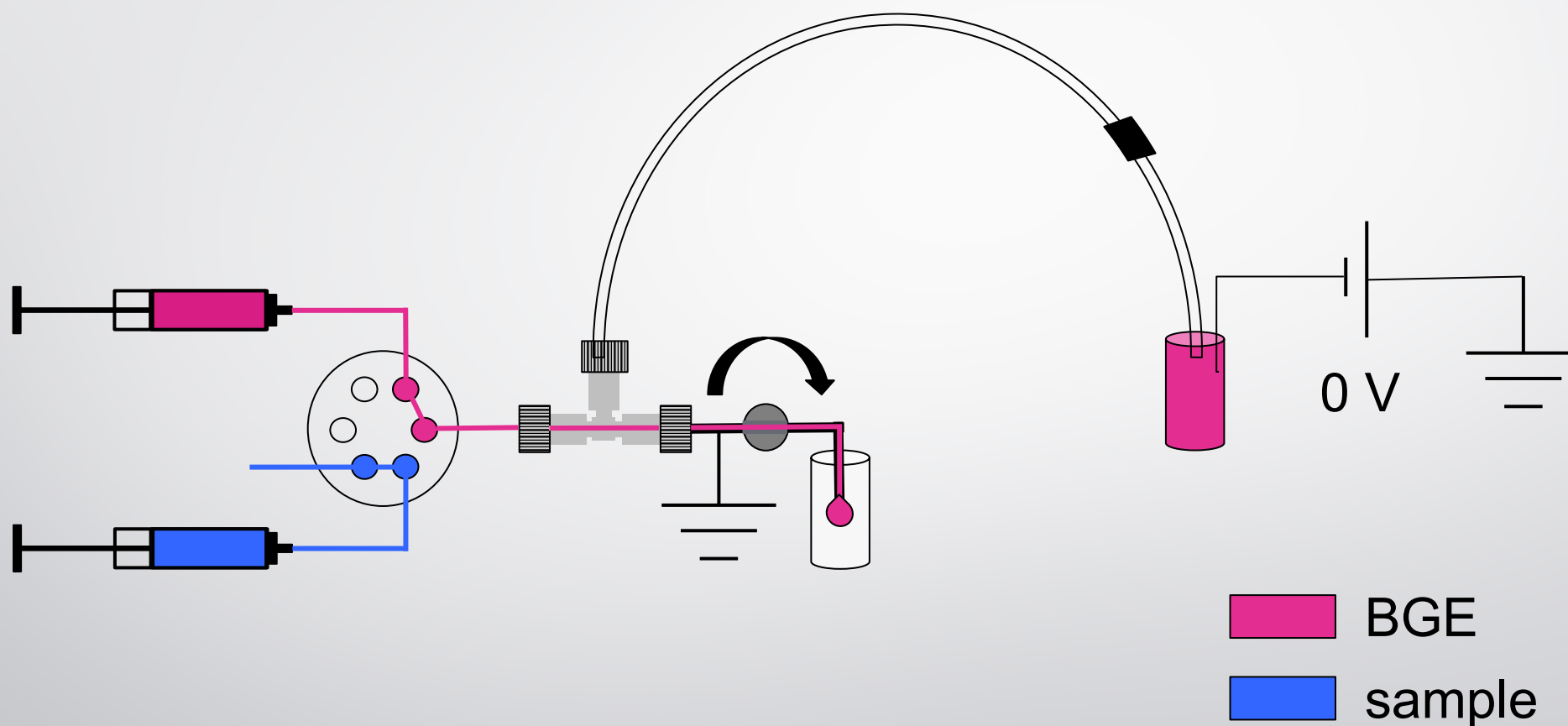
Often

- High resolution analytical technique
- Instrumentally simple (unlike HPLC no need for high pressure pumps)
- Fast
- Compatible with miniaturisation
 - Forward compatible with autonomous onsite monitoring

Turkia et al. [dx.doi.org/10.1021/ac402113x](https://doi.org/10.1021/ac402113x) |
Anal.Chem. 2013, 85, 9705–9712

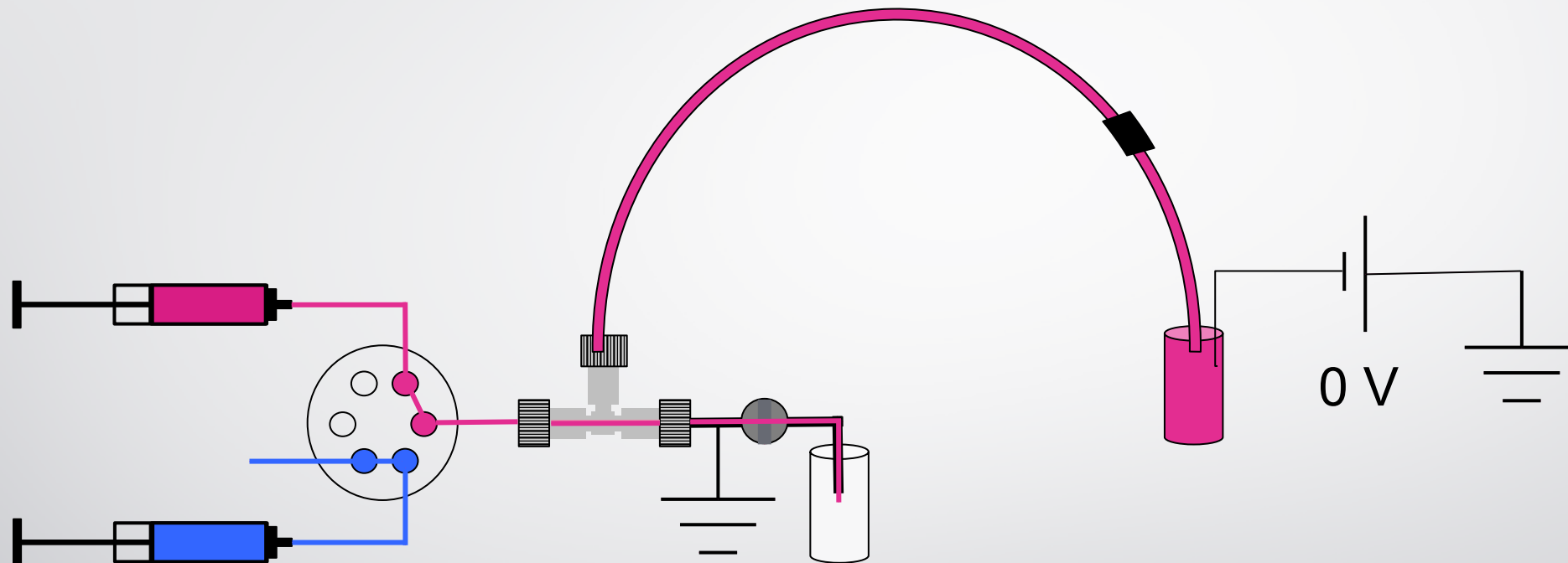
- Flow-through vial
- 9 mL per analysis



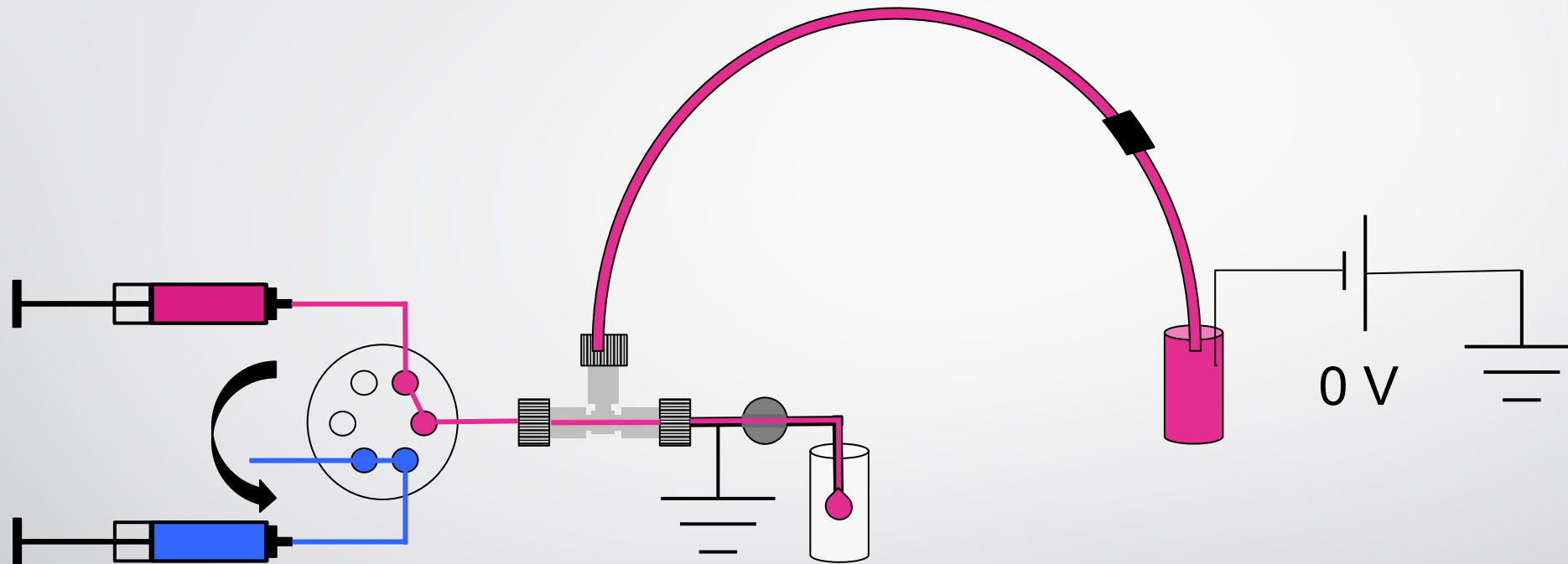


Blanco, Nai, Hilder, Shellie, Dicinoski, Haddad and Breadmore,
Anal Chem. 2011 83(23):9068-75. doi: 10.1021/ac2020195

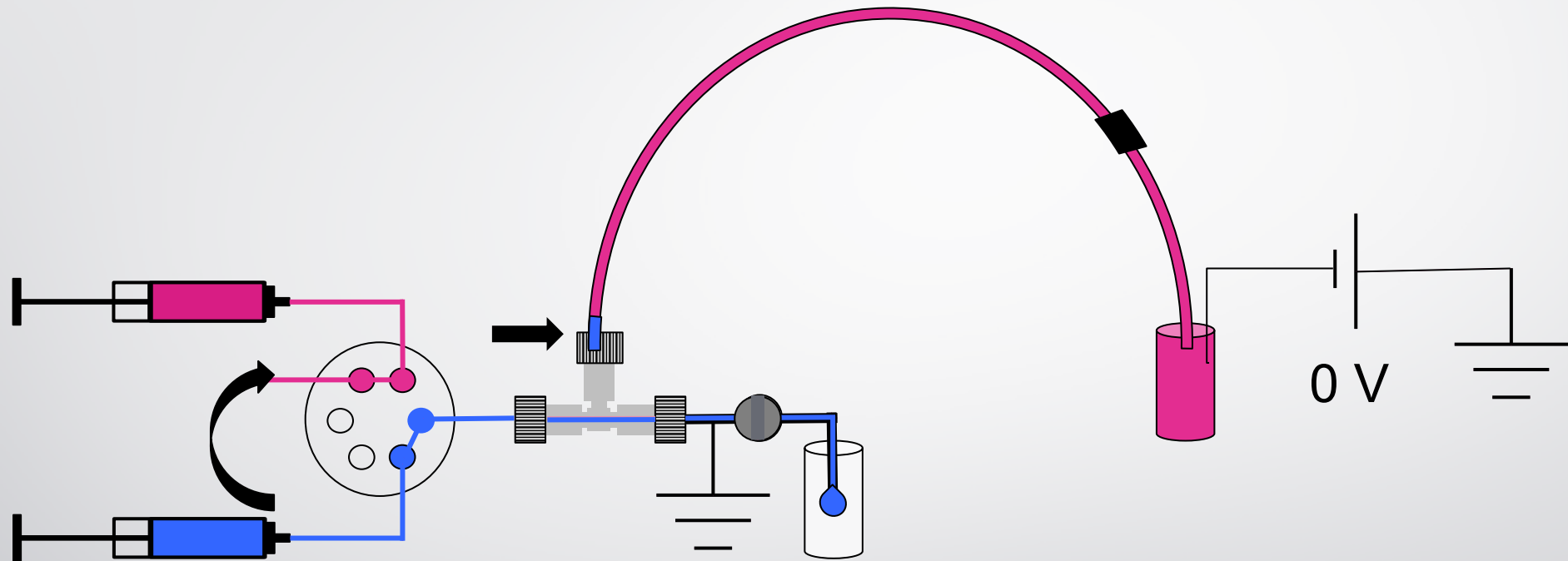
Fill capillary with BGE



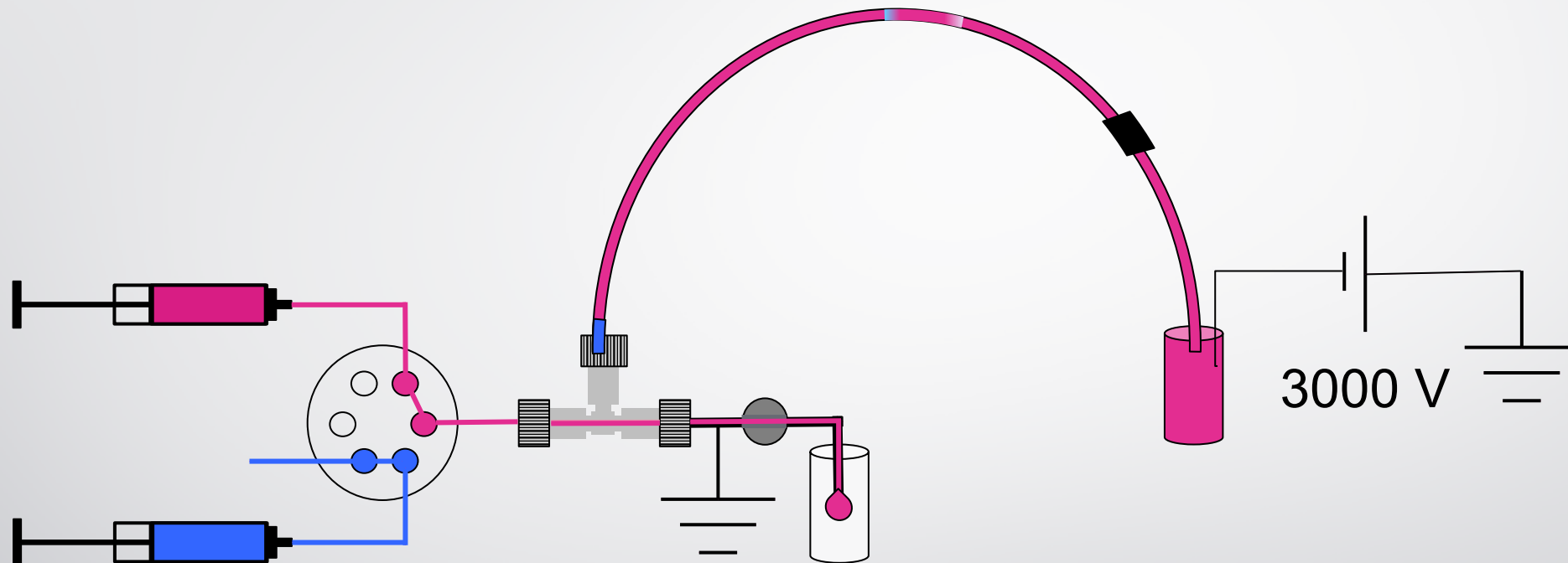
Switch 6 port valve and fill interface with sample



Close 2 way valve to inject sample in capillary, change 6 way valve to BGE

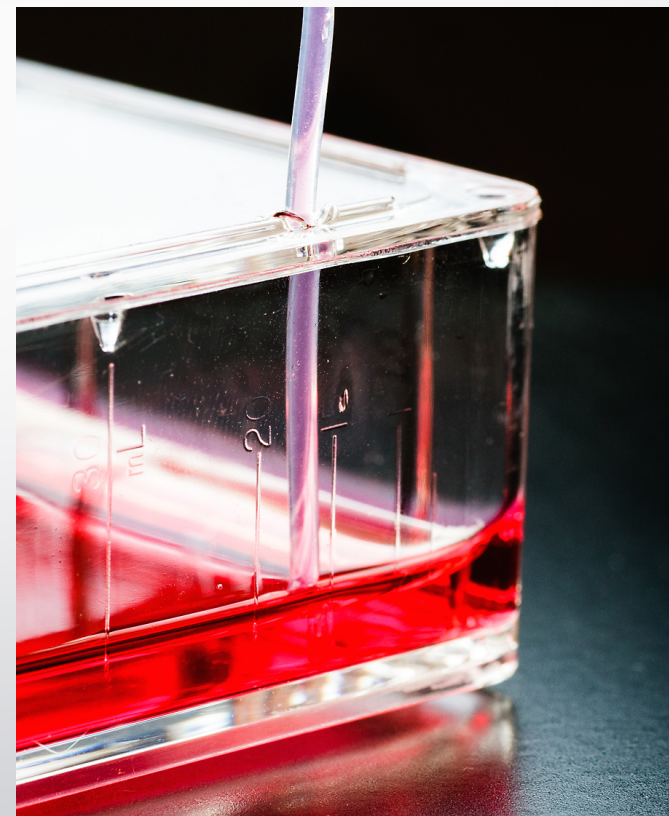
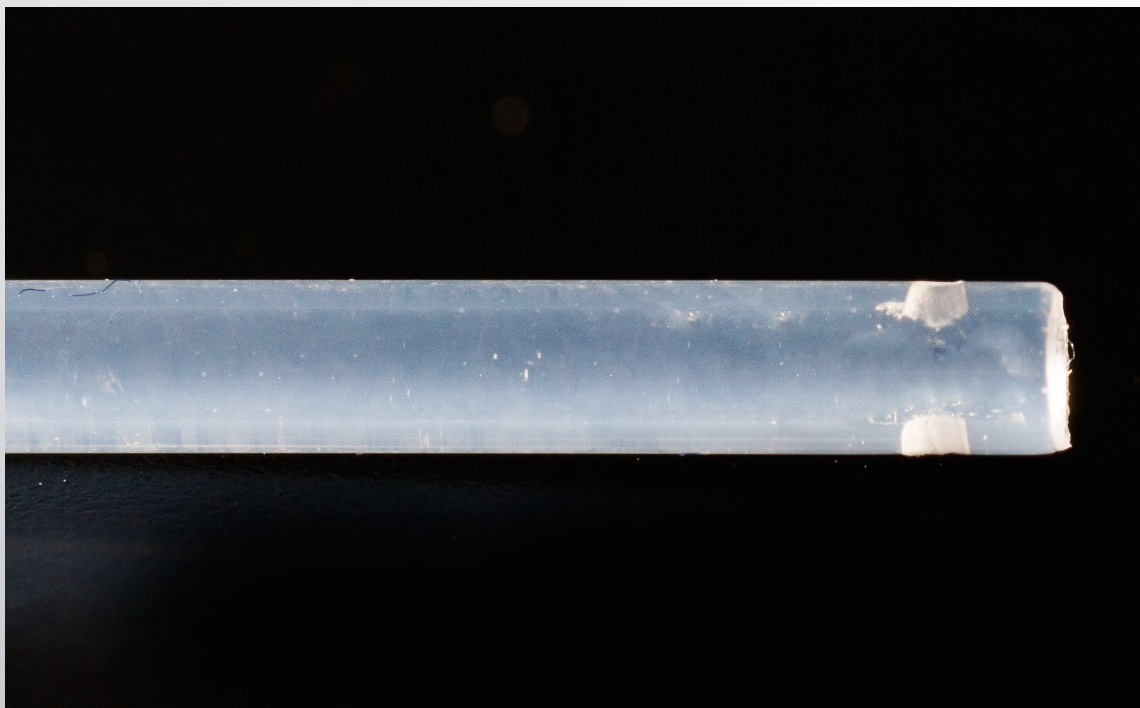


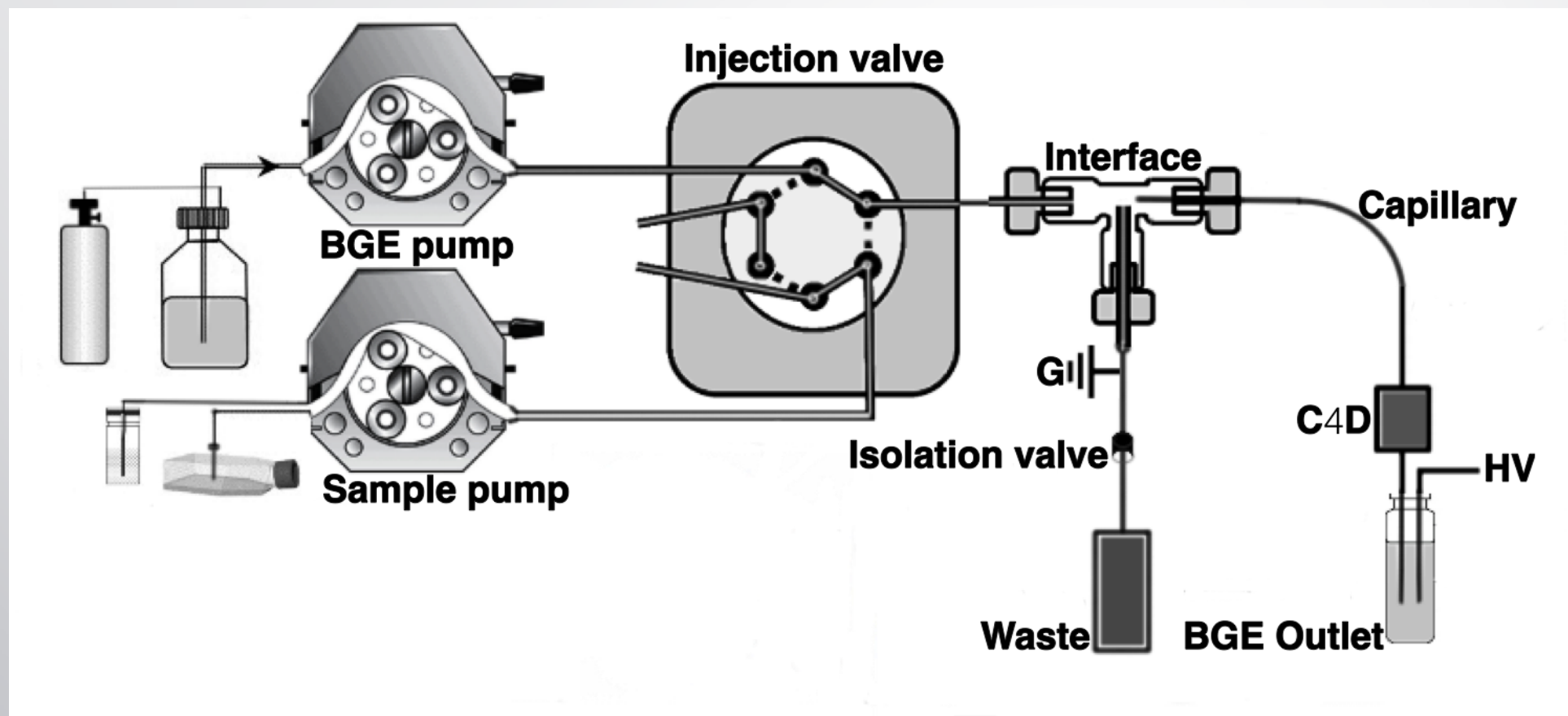
Clean interface with BGE and start HV for separation



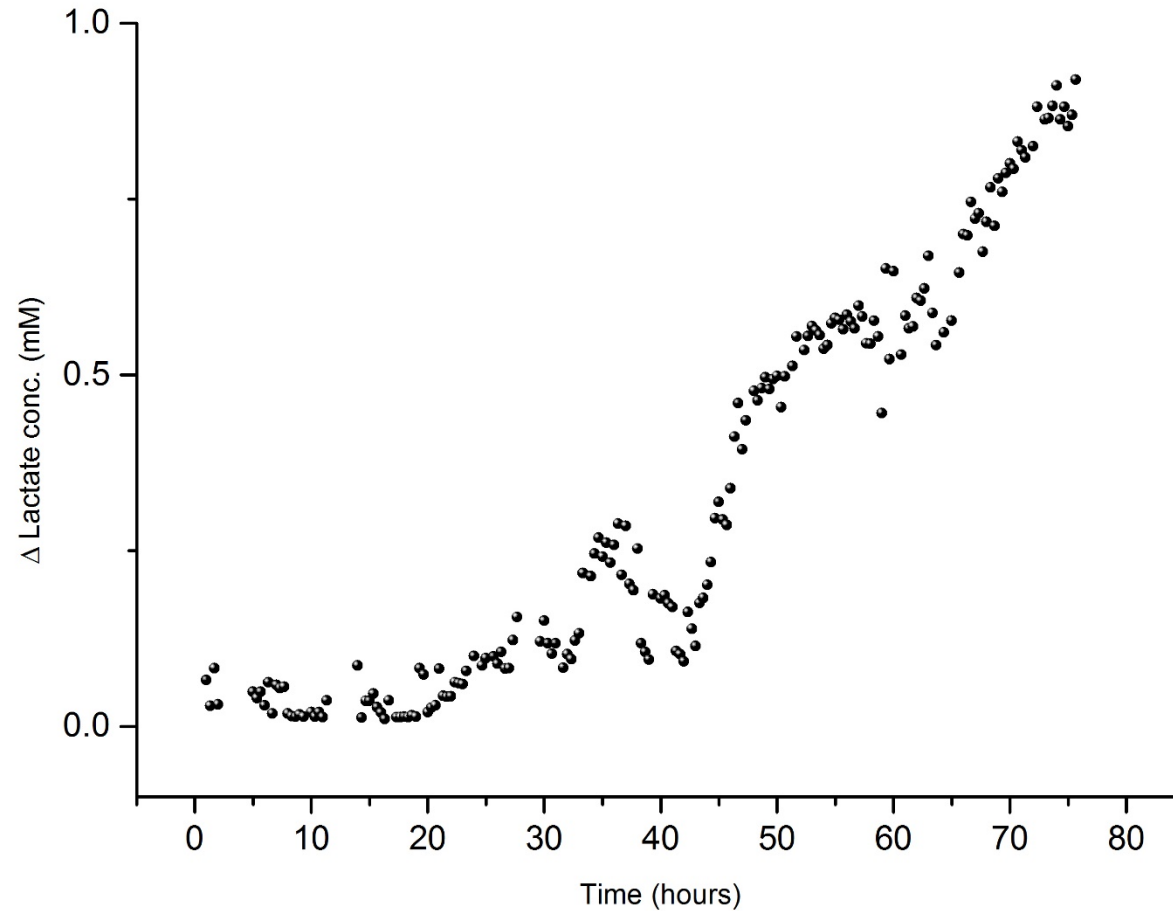
- Requirements
 - CE method
 - Separate lactate from components in medium
 - Stable over >3 days
 - Sampling interface for sampling cell-free medium
 - Sampling volume should not exceed 10% of media volume
 - 20 mL media in standard flask with a surface area of 75 cm²
 - Cannot sample more than 2 mL

Sampling interface for adhesion culture





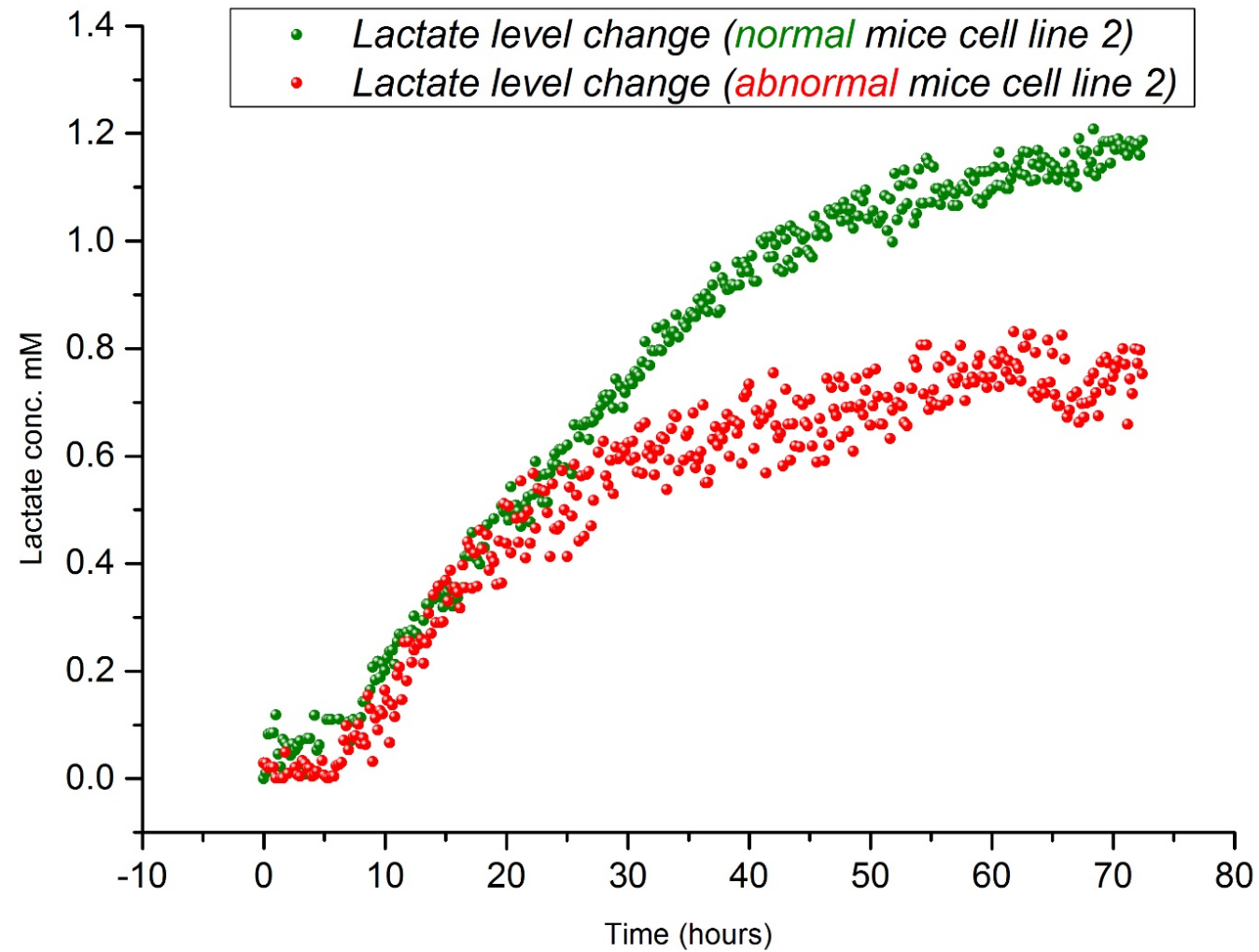
Continuous monitoring lactate human embryonic kidney cell line HEK293



ONLY 1.99 mL
of sample for
228 analyses!
(8.3 μ L/run)

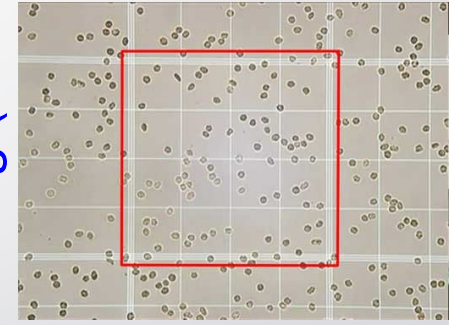
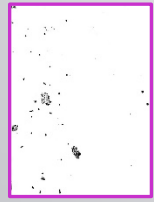
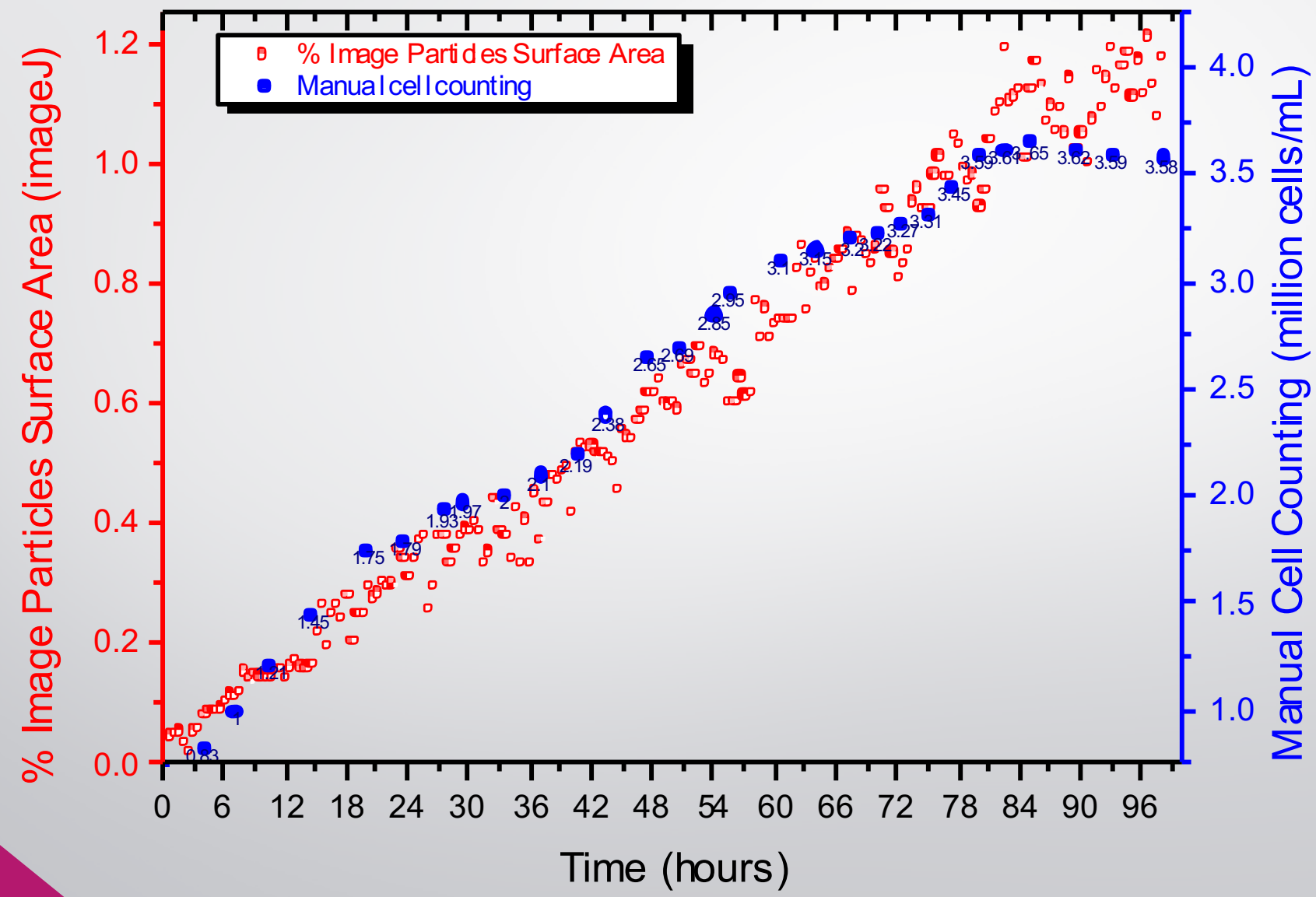
Conditions:

85 cm x 50 μ m i.d. fused silica capillary coated with HDMB/PSS/HDMB; BGE: 25 mM Tris/35 mM CHES, pH 8.65 with 0.02% PEI; +30 kV applied to outlet vial while interface was grounded. Signal was obtained using a TraceDEC conductivity detector positioned 10 cm from the outlet.

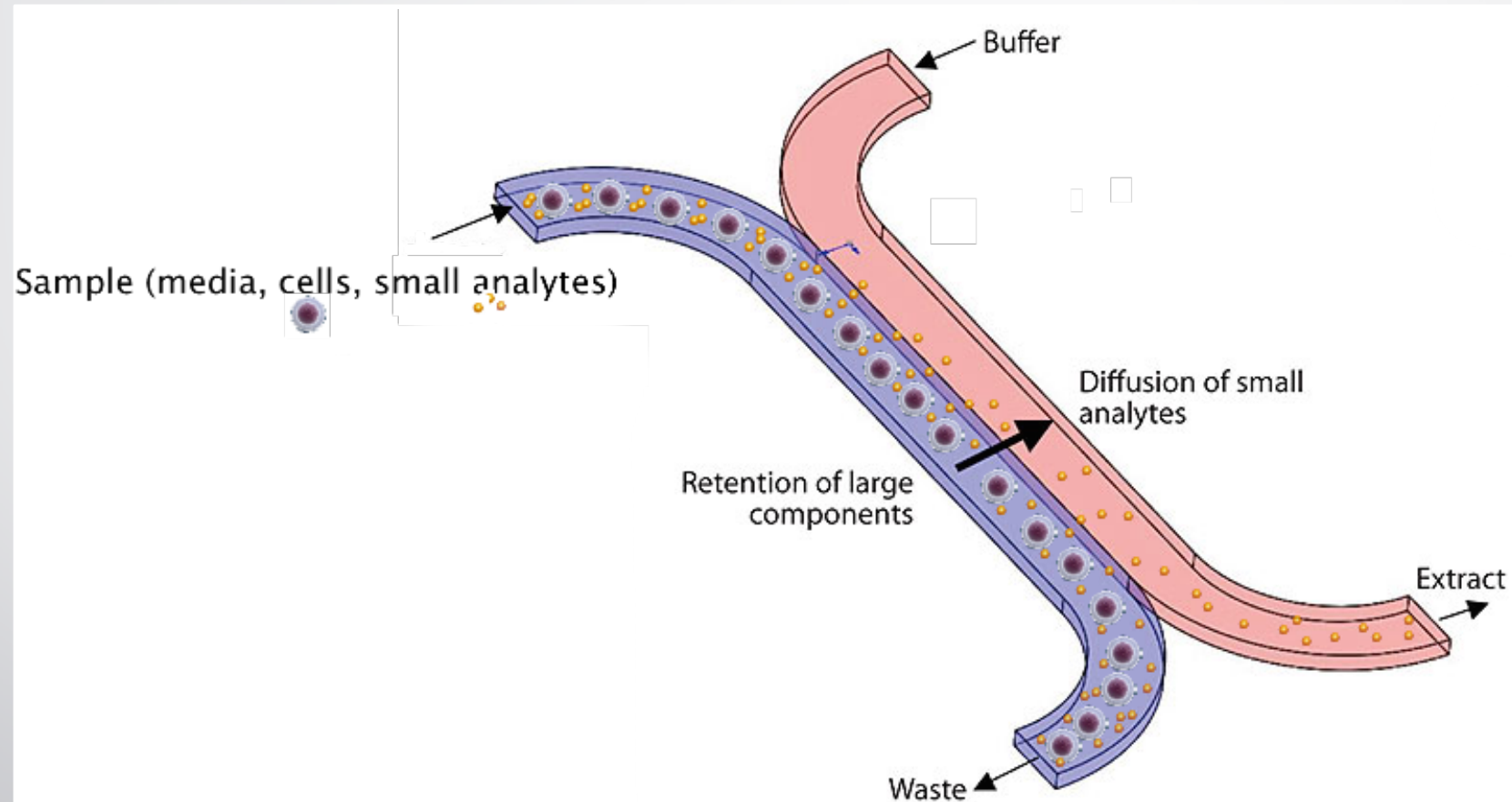


- Jurkat Human T lymphocyte cells
 - Include cell density measurement: digital microscopy
 - Different sampling approach
- Expand analyte set to include other acids, amino acids and sugars

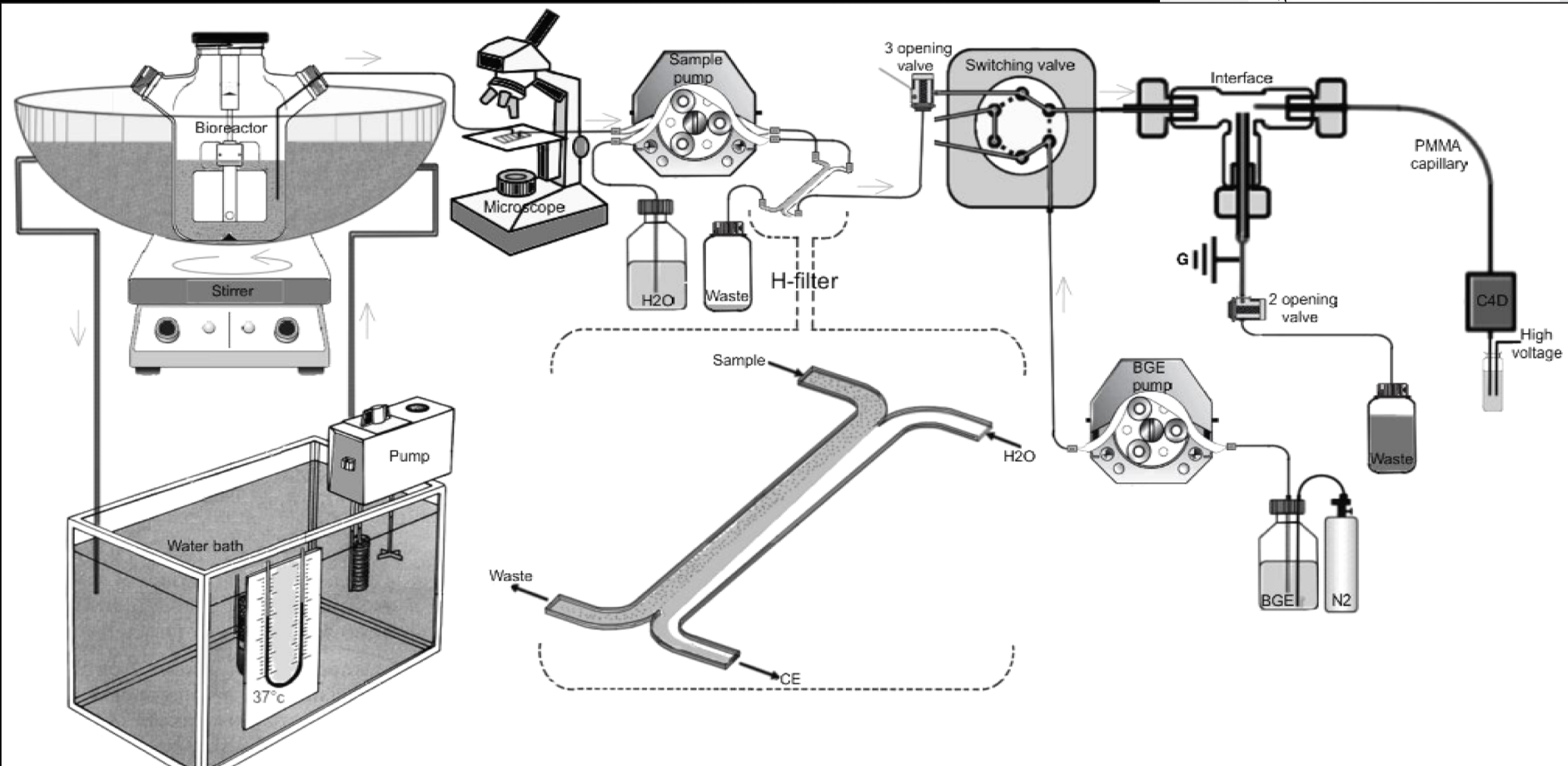
Cell count: digital microscopy vs haemocytometry

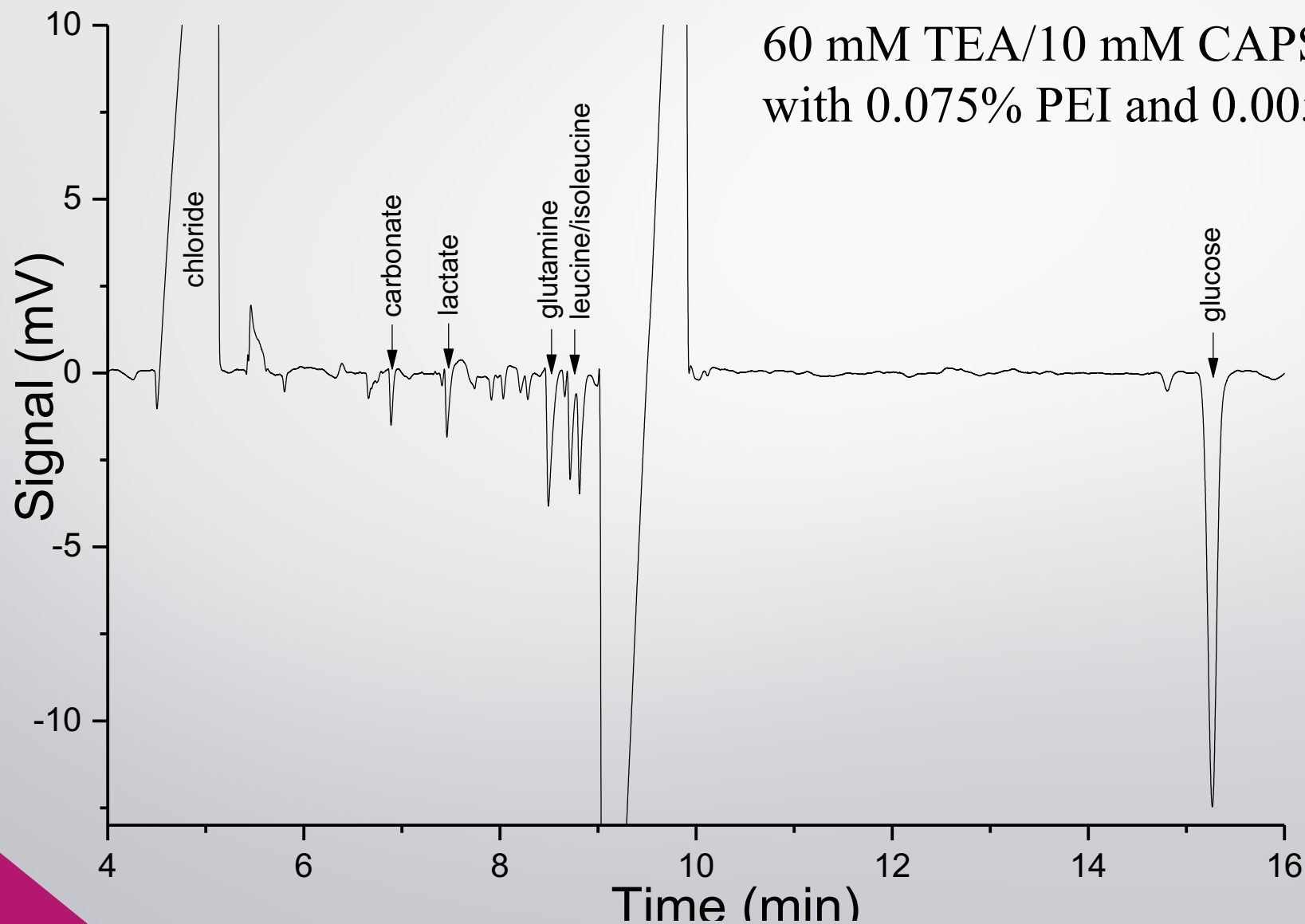


Obtain cell-free media: H filter

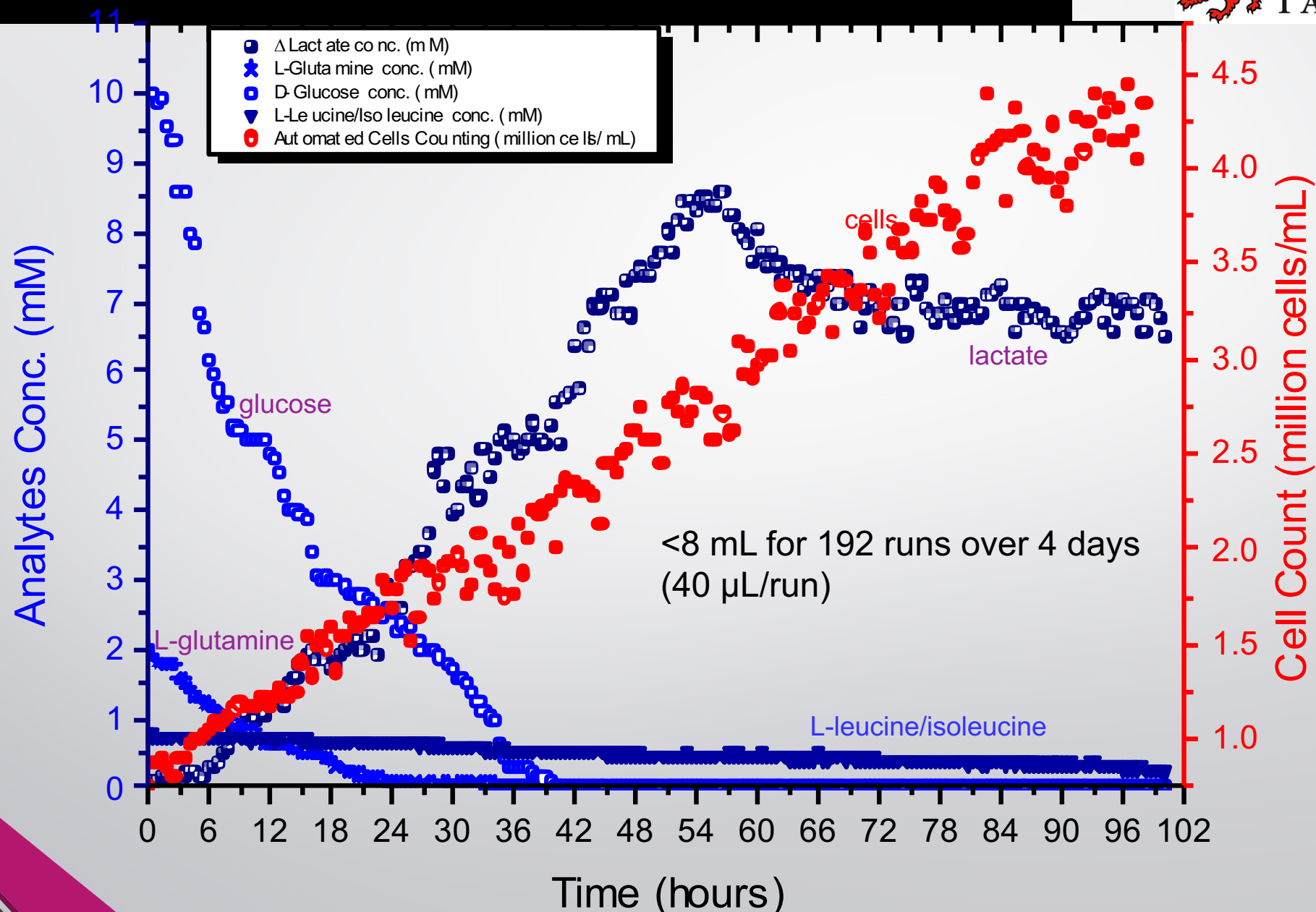


Si-CE for bioprocess monitoring

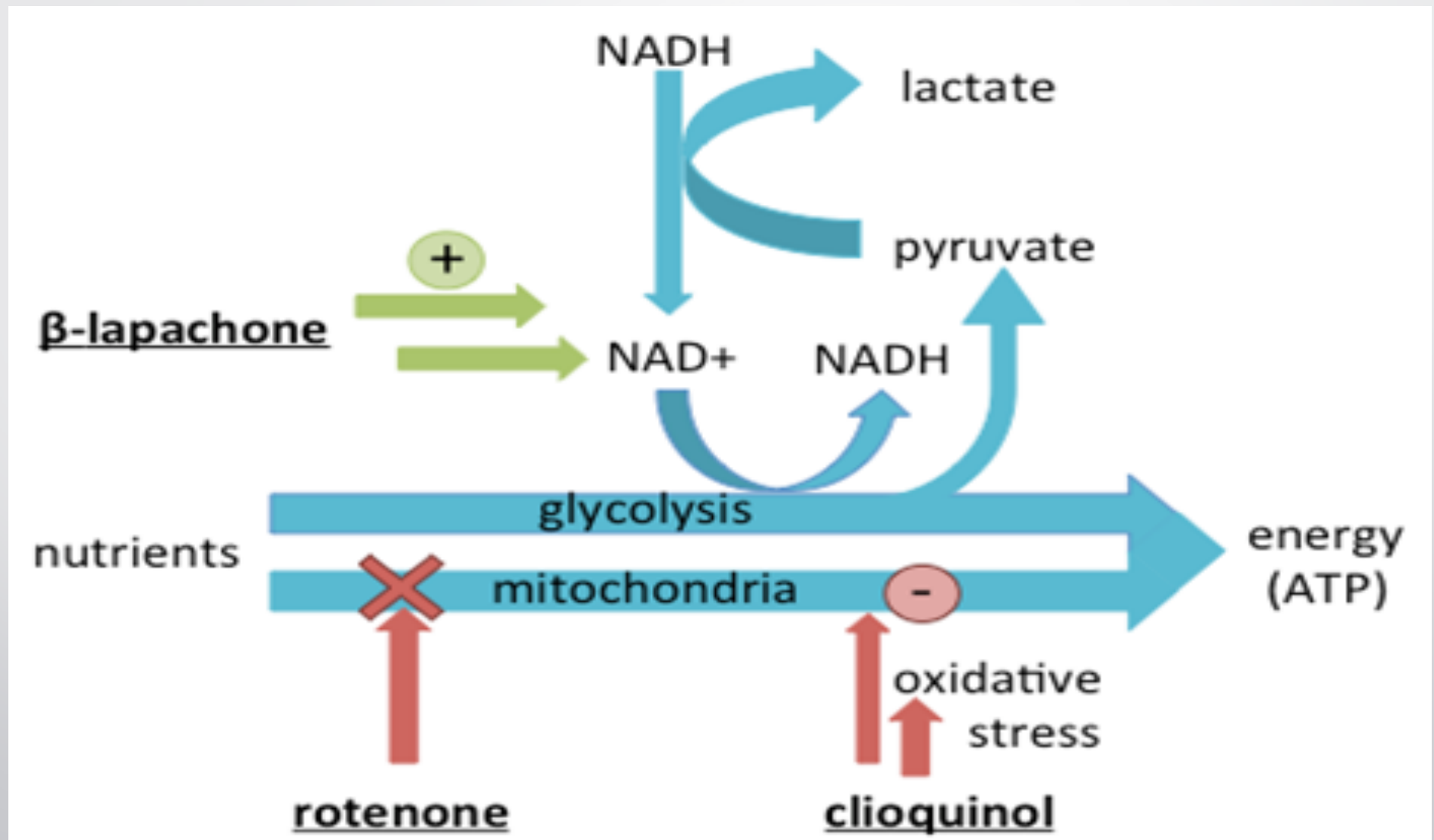




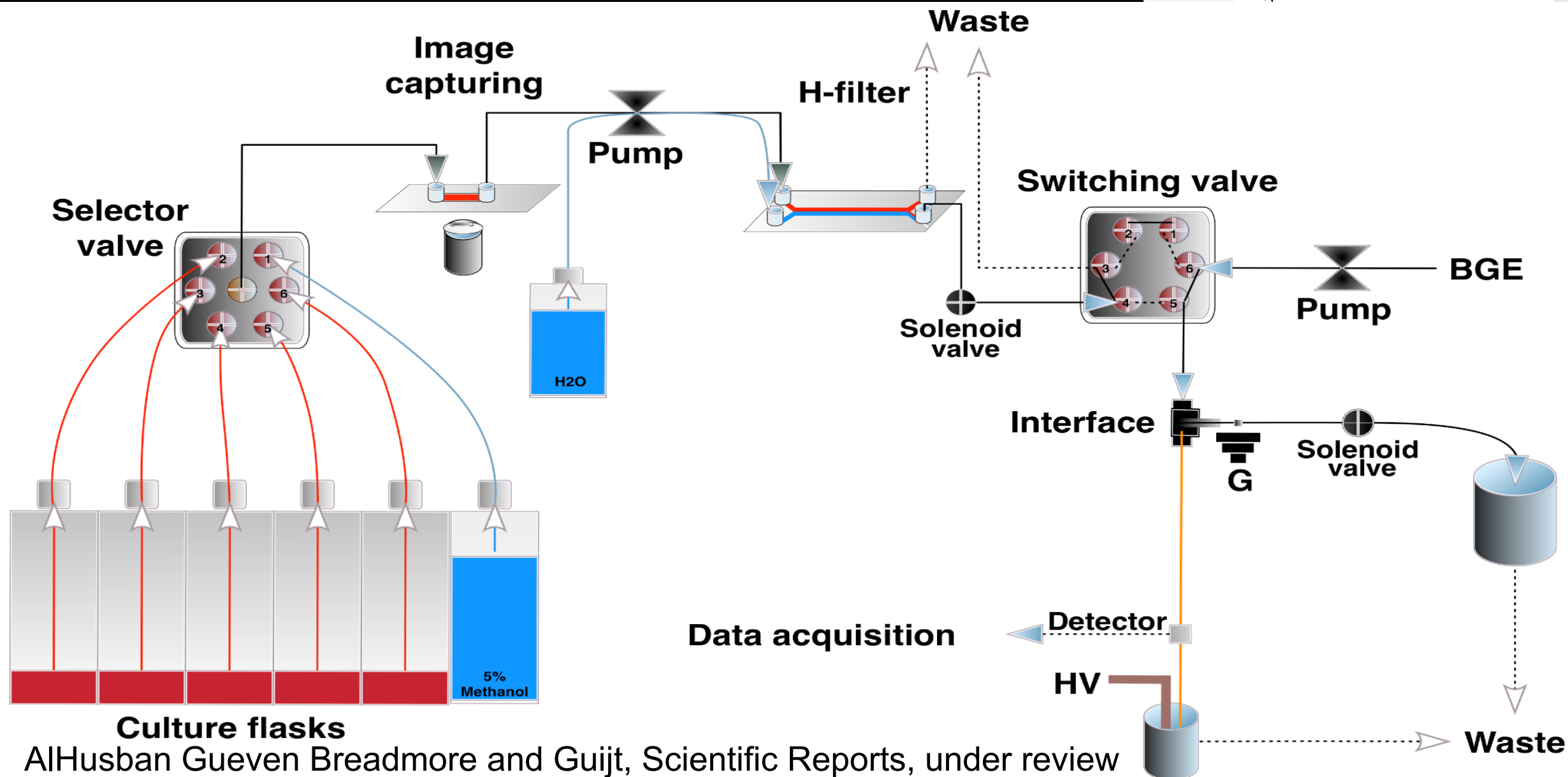
The BIG data

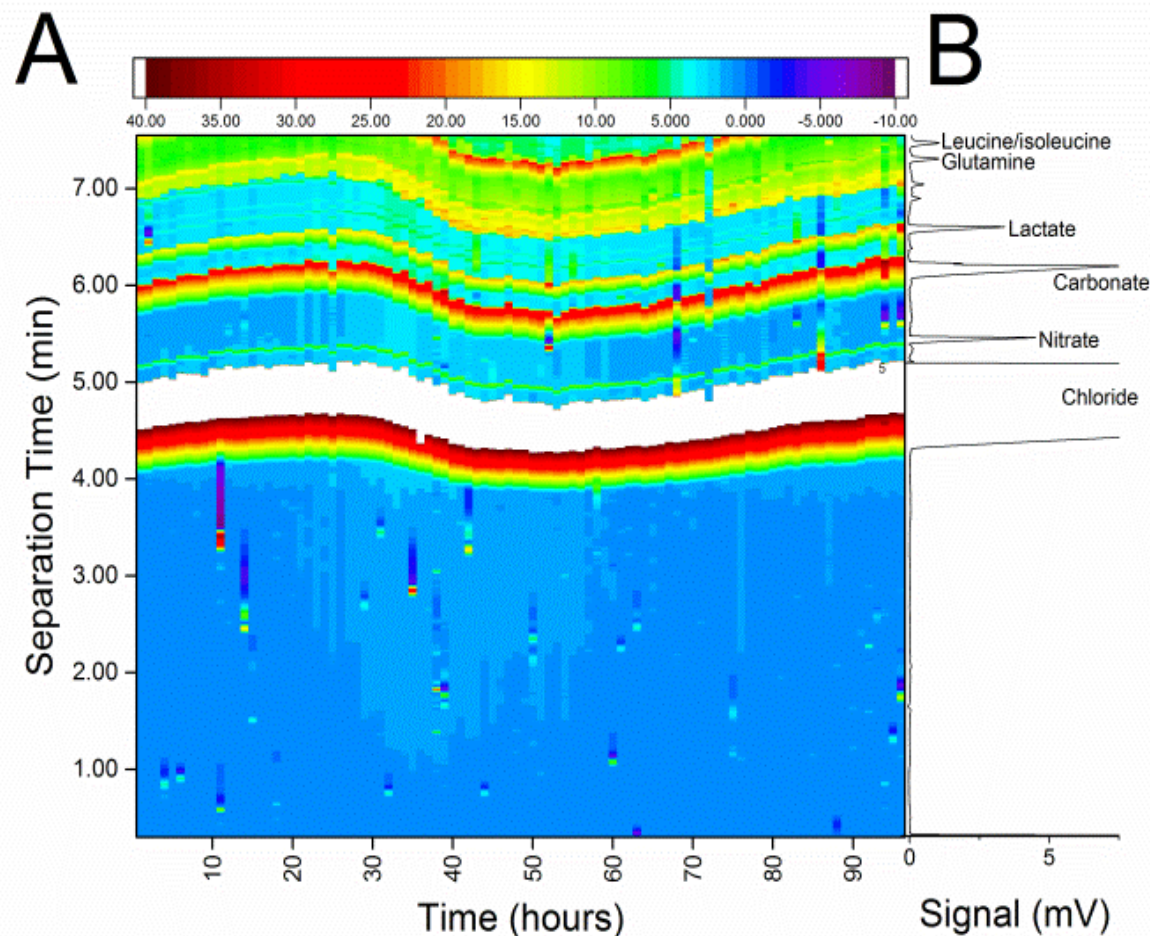


Pharmacological studies: drugs affecting lactate production



Multiplexing: sampling from 5 parallel cultures

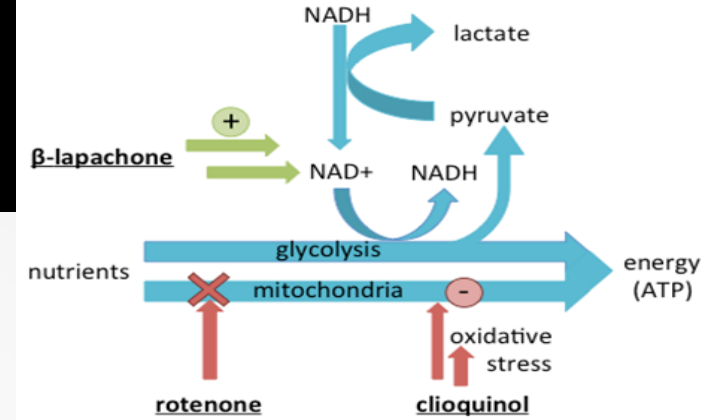
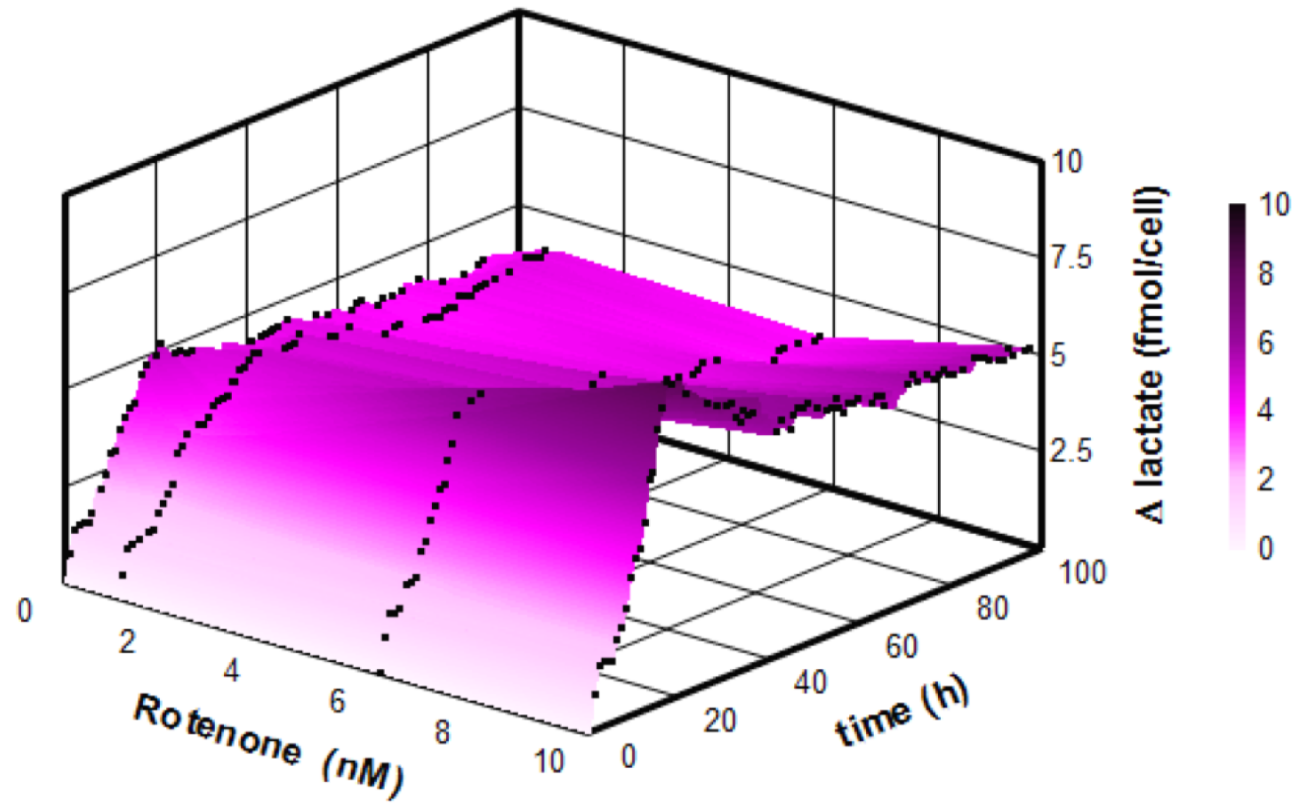




BGE: 35 mM Tris/35 mM CHES, pH 8.9 with
0.025% PEI

Dose response surface over time

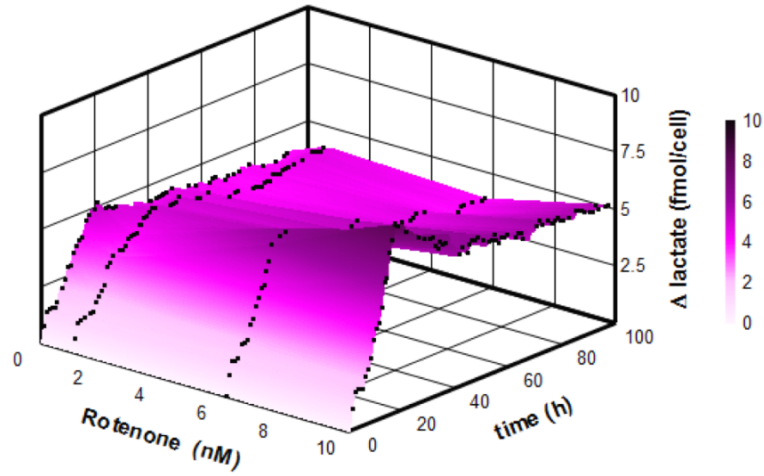
Rotenone



3D pharmacology

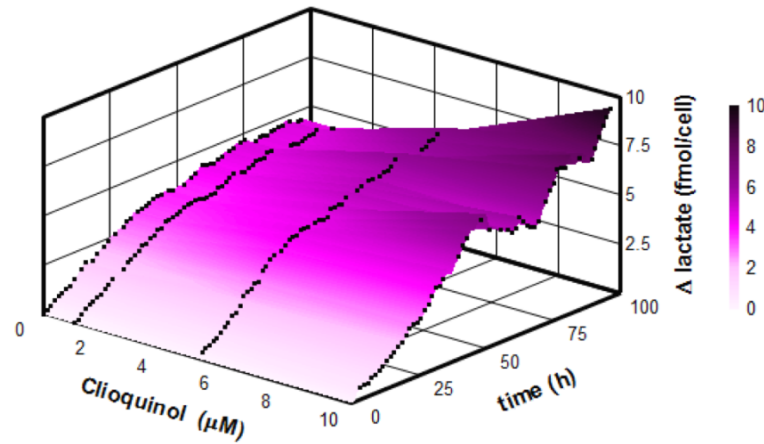
Dose response surface over time

Rotenone



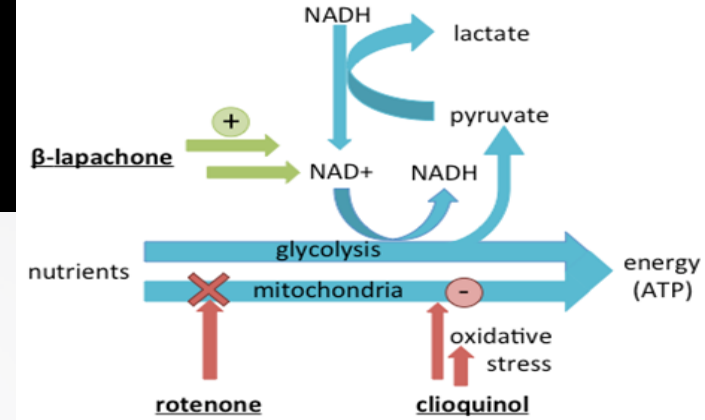
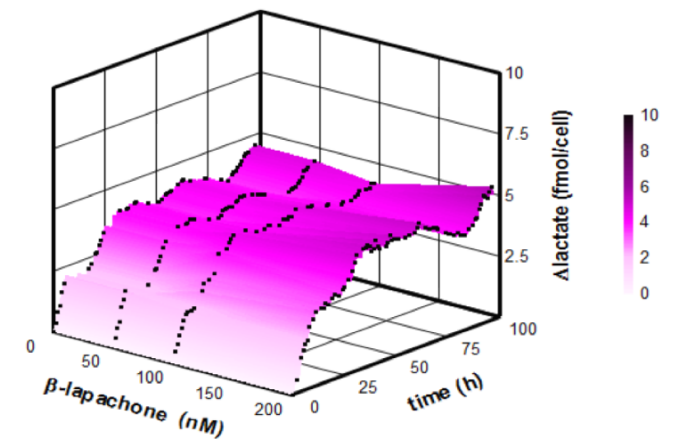
Dose Response surface over time

Clioquinol



Dose response surface over time

β -lapachone



- SI-CE successfully adapted for bioprocess monitoring
 - Adhesion culture
 - Demonstrated to distinguish between normal and mitochondrial knockout cell lines
 - Suspension culture
 - Simultaneous monitoring of cell density, glucose, lactate, leucine and glutamate
 - Correction for cell density essential for correct interpretation of results
 - Study pharmacological effect of drugs known to affect lactate metabolism over time by monitoring 5 parallel cultures
- Hardware is there, needs to be packaged and integrated

My thanks to ...

Chippers and CE-ers



- Support
 - ARC: QEII Fellowship, Future Fellowship (Breadmore),
 - Al Zaytoonah University Jordan (Al Husban)
 - Alexander von Humboldt Foundation (Guijt)

