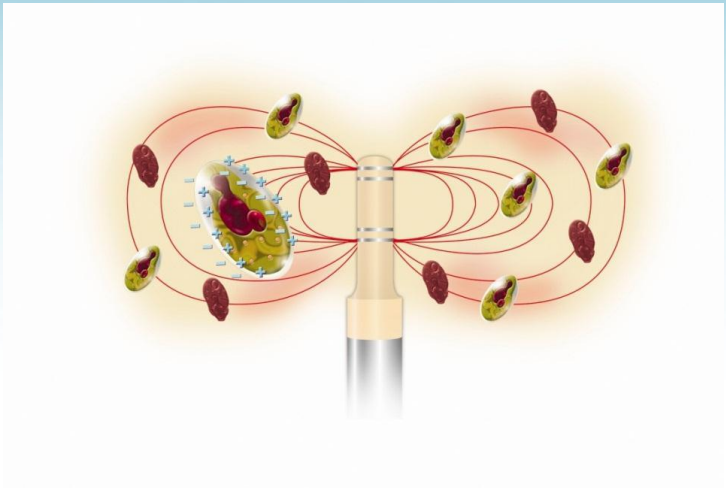
“Development application and single use probes for building Design Space of live cell concentration by dielectric spectroscopy”

BY: JOHN CARVELL

ABER INSTRUMENTS, Wales, UK



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ABER Instruments Ltd – originally a spin out company from the University of **ABER**ystwyth, Wales.

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Wales also has the longest station name in the world!

Fortunately it is shortened to Llanfair PG

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Scope of Talk

- Principle of Dielectric Spectroscopy (DS) –emphasis on scanning
- Correlation with offline readings
- Case study Gedeon Richter
- Case study GSK
- Case study Fujifilm Diosynth
- Single use probe applications
- Conclusions

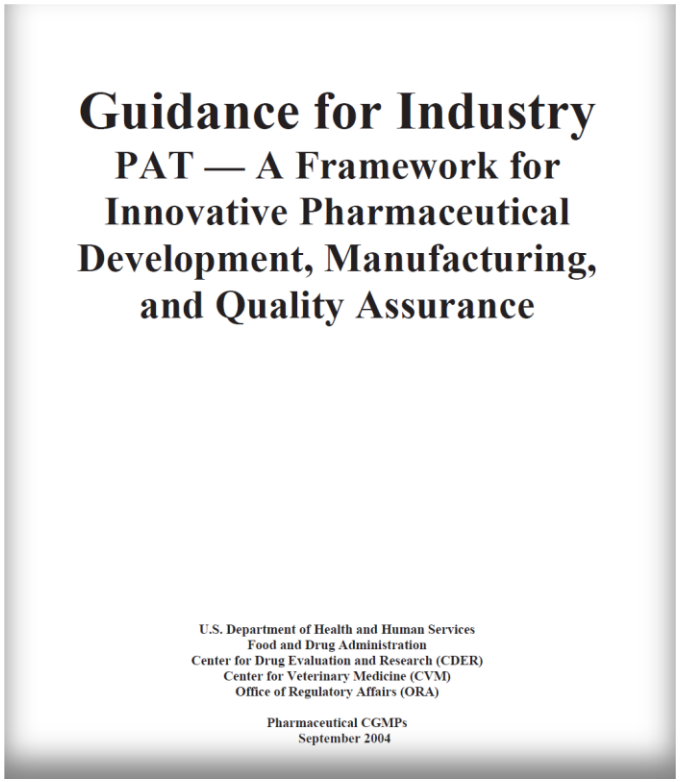
Industry Initiative

"Systems that promote greater product and process understanding can provide a high assurance of quality on every batch and provide alternative, effective mechanisms to demonstrate validation"

FDA Guidance for Industry: PAT—A Framework for Innovative Pharmaceutical Manufacturing and Quality Assurance, 2004.

Four types of tools for generation and application of process understanding:

1. **Multivariate tools for design, data acquisition and analysis**
2. **Process Analyzers**
3. **Process Control tools**
4. **Continuous improvements and knowledge management tools**



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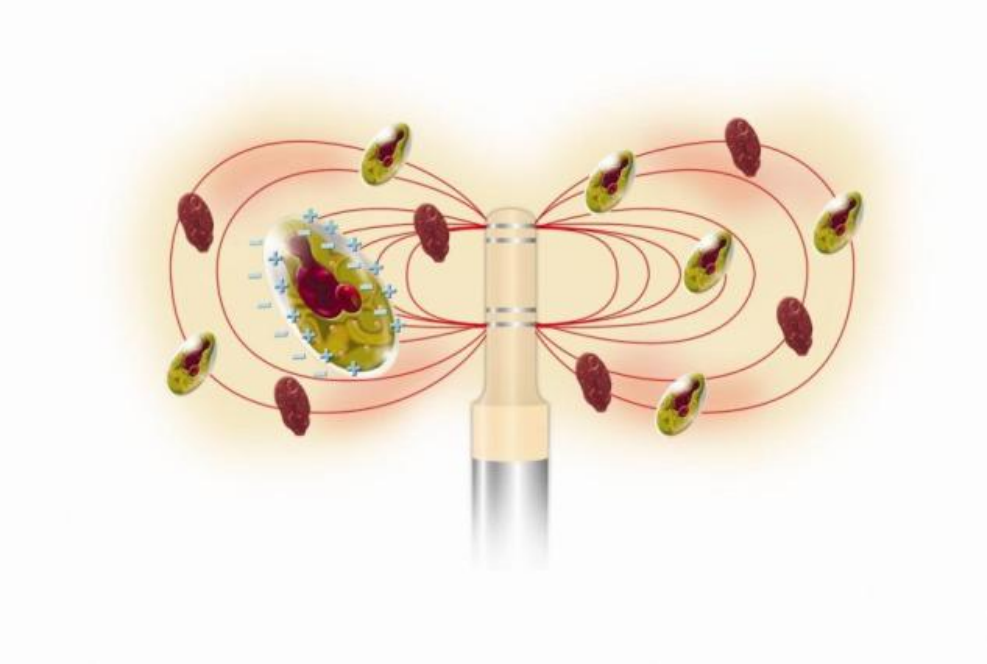
Principle of Dielectric Spectroscopy (DS)

•When an alternating electrical field is applied to an ionic solution of cells, a charge separation or polarization effect develops at the cell membrane

•Cells behave like a capacitor under an electric field

•The magnitude of this charge separation can then be measured by the capacitance of the solution at varying radio-frequencies in the range 0.1-20MHz

•The resulting capacitance is generally proportional to the total enclosed bio-volume, which itself is directly correlated with cell density or cell weight



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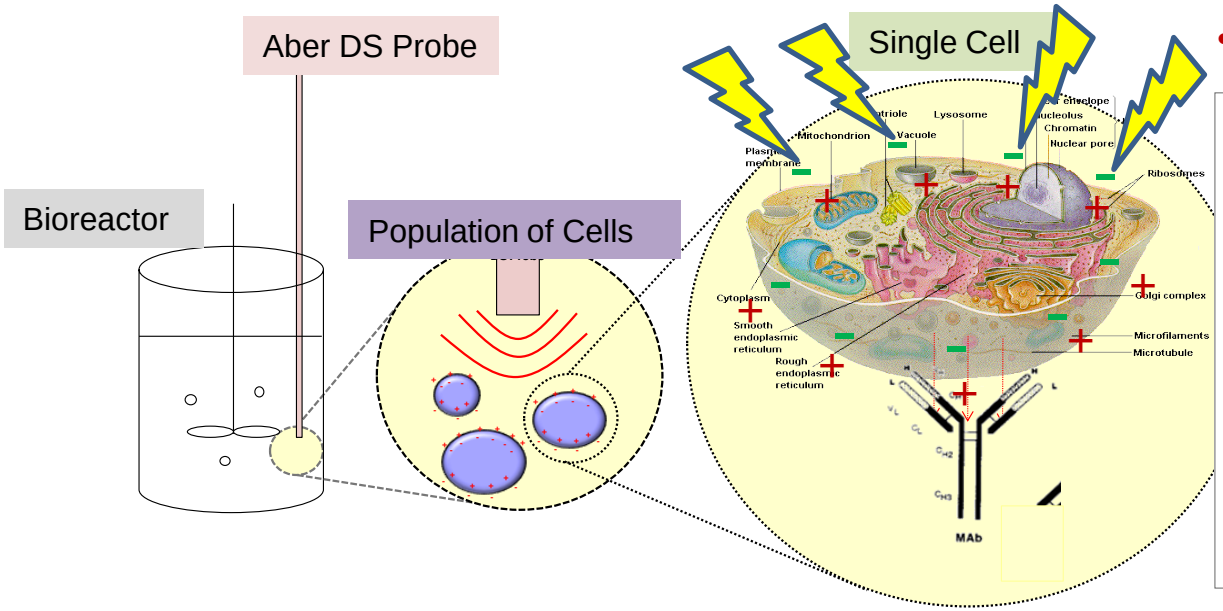
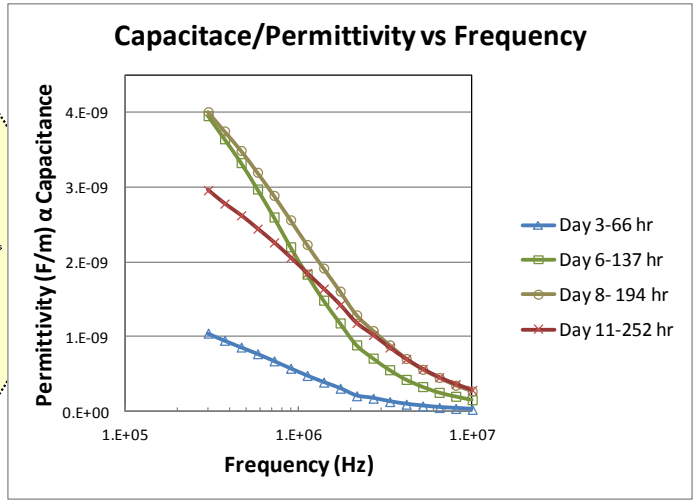
Dielectric Spectroscopy (DS) in bioreactors



Dielectric Spectroscopy Key Outputs:

- Capacitance Data Over Time
- Correlates with Viable Cell Density



Graphics courtesy of Bend Research,Oregon

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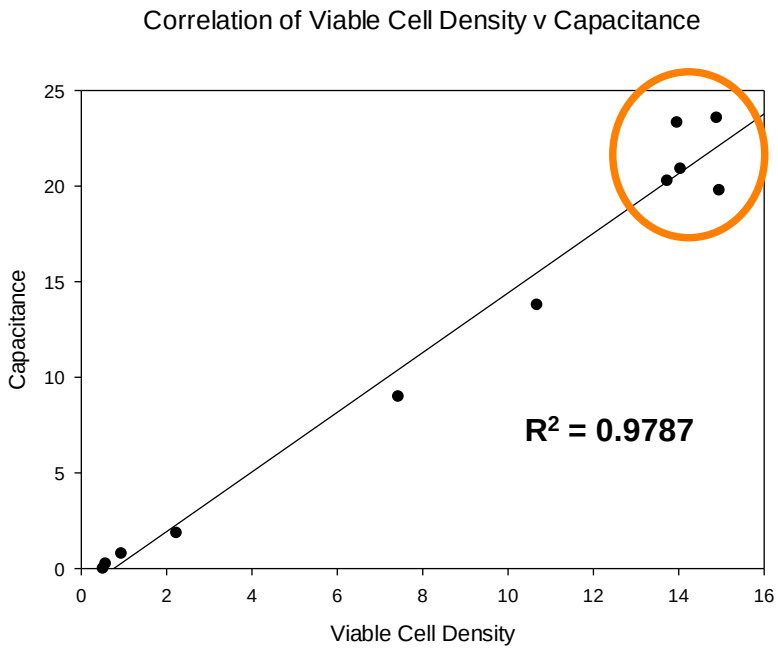
Correlation of online capacitance data with offline readings

- Plot of capacitance at specific single frequency versus VCD should produce straight line correlation with an R^2 value close to 1
- Correlation during log phase of cell growth is well maintained
- Divergence observed as cells enter stationary/death phase at which point cell viability decreases and LDH levels increase proportionally
- Published data on models for correcting this divergence available *eg* Bend, UMASS, University of Manitoba



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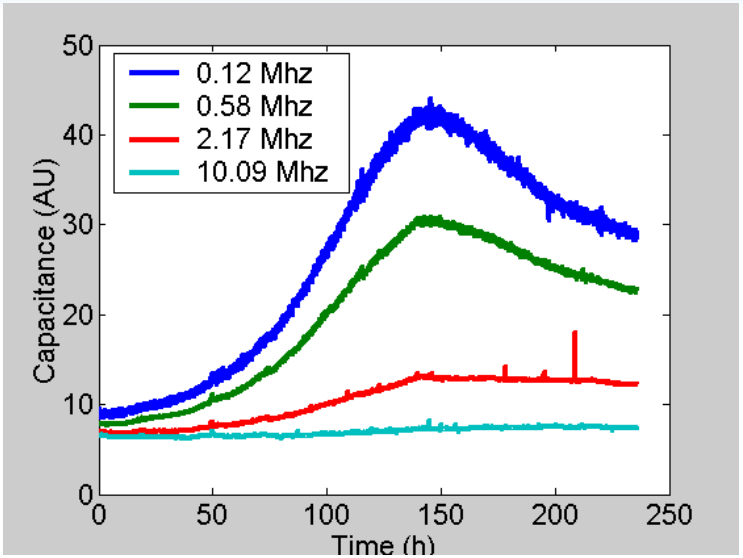
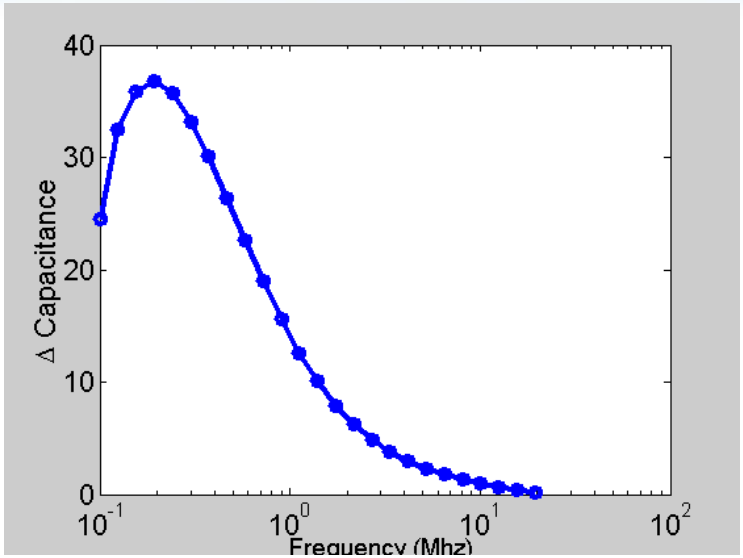
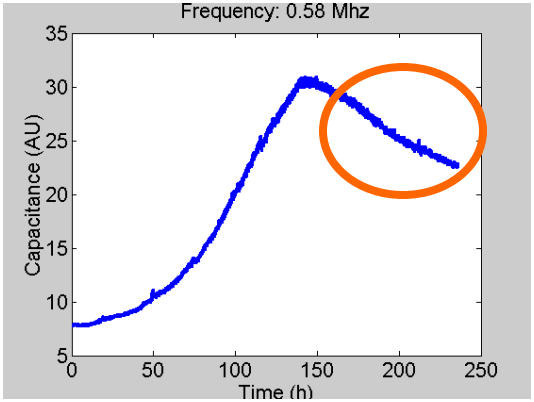
CHO Cell Density Monitoring in Bioreactor Culture – Comparison of four methods (data courtesy) University of Manitoba)

Fed-Batch HEK cells (data courtesy of CNRC, Canada)

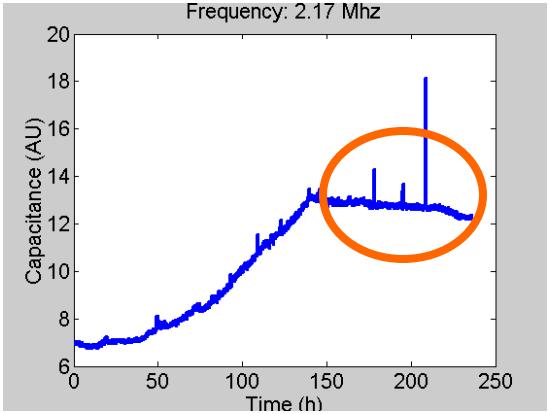




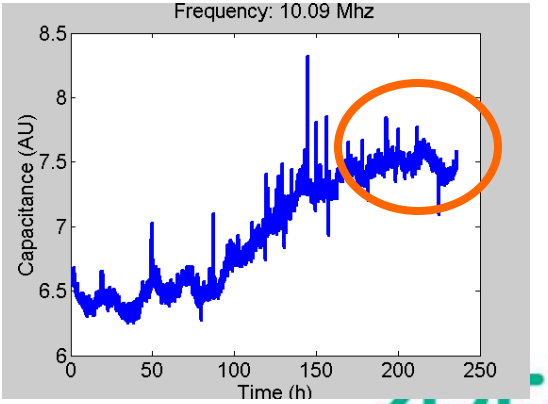
0.6 MHz



2.2 MHz



10 MHz



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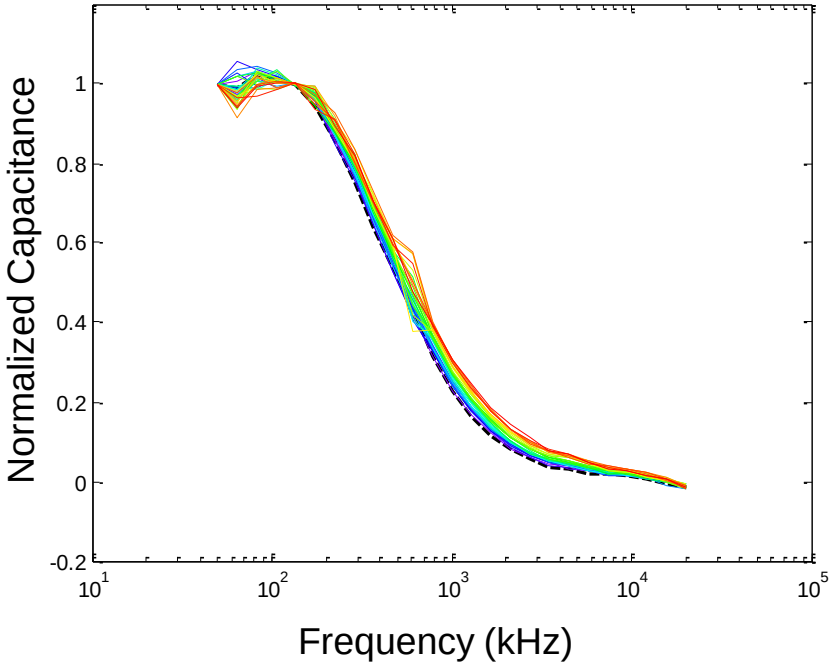
Change in shape of Spectrum- fundamentals of frequency scanning

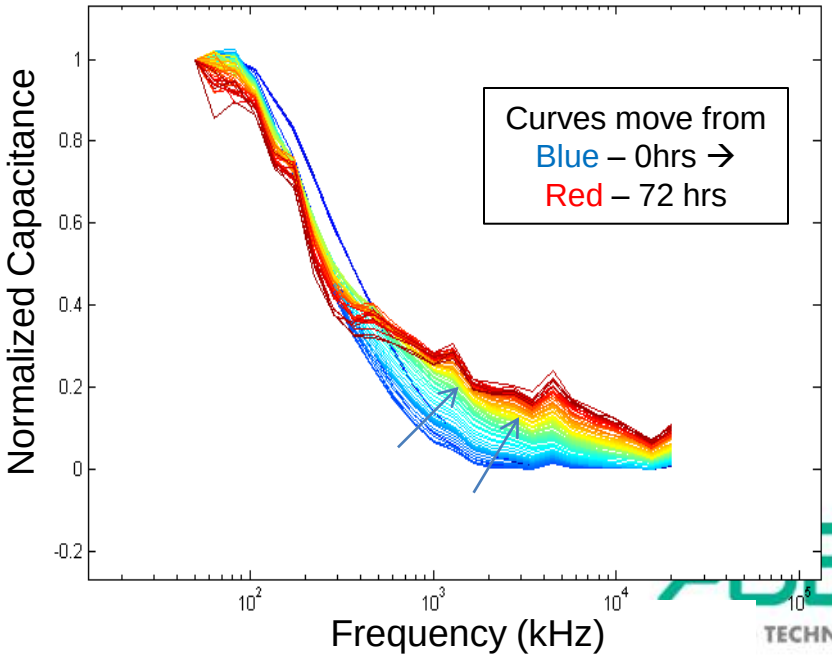
Control

Timeseries of Normalized Capacitance vs. Frequency



CHO cell undergoing induced apoptosis

Timeseries of Normalized Capacitance vs. Frequency



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Bioprocess Biosyst Eng
DOI 10.1007/s00449-013-0998-z

ORIGINAL PAPER

Application of dielectric spectroscopy for monitoring high cell density in monoclonal antibody producing CHO cell cultivations

László Párta · Dénes Zalai · Sándor Borbély · Ákos Putics

Case Study 1

Gedeon Richter, Hungary

Contact Dénes Zalai
zalaid@richter.hu

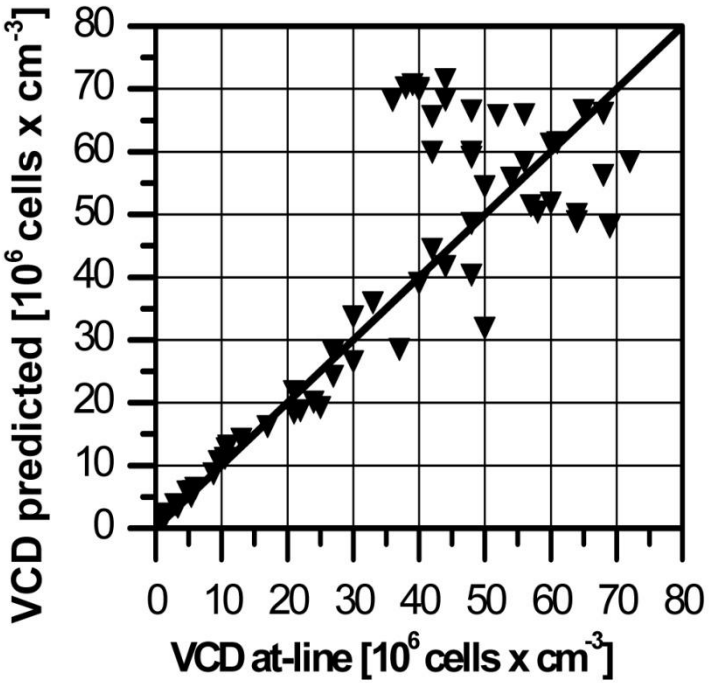
Details of study at Gedeon Richter

- 6 runs with 1L bioreactors and ATF2 technology (Refine,USA) with CHO expressing Mab
- High cell densities up to 75 x10E6 cells/ml
- Utilized Aber Biomass Monitor 220 with AberScan software
- Scans at 25 pre-defined frequencies every 8 minutes
- Spectral data exported for linear regression or multivariate data analysis

- Cells/ml derived from Aber using 3 different methods:
- Simple measurement at 580KHz (linear modelling)
- Cole- Cole
- PLS using Umetrics SIMCA software

GEDEON RICHTER

Linear Modelling at 580KHz



- at-line measured VCD values throughout Run 1-4 were plotted against predicted VCD values and depicted as triangles

- linear equation for prediction:
  $VCD = 414492 \times C_{580} + 1.187 \times 10^6$

- linear model over-estimated VCD at the later phases of the cultivation

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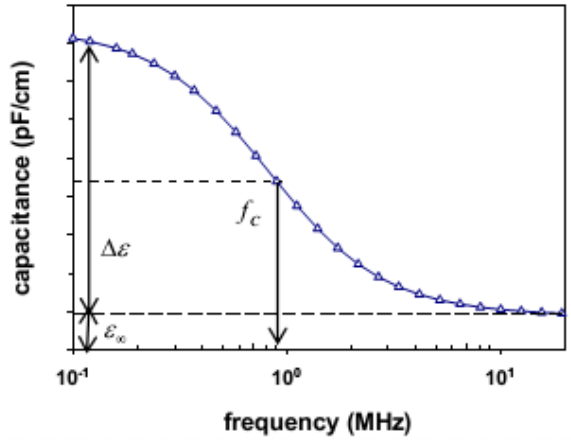
Deriving VCD using Cole-Cole using frequency scanning between 100 and 20,000KHz

- **Theoretical equations for β-dispersion curve (Cole-Cole equation)**



$$\Delta\varepsilon = \frac{9pRC_m}{4(1+RG_m(1/\sigma_i + 1/2\sigma_a))^2} \approx \frac{9pRC_m}{4} = 3\pi R^4 C_m \cdot VCD$$

$$f_C = \frac{2\sigma_i\sigma_a + RG_m(\sigma_i + \sigma_a)}{2\pi RC_m(\sigma_i + 2\sigma_a)} \approx \frac{1}{2\pi RC_m(1/\sigma_i + 1/2\sigma_a)}$$

- The known values of C_m, σ_i, and σ_a together with $\Delta\varepsilon$ and f_c → *VCD* and *R* (cell radius) can be calculated from the above two equations
- The known values of C_m, σ_i, and σ_a together with $\Delta\varepsilon$ and f_c → Assume that cell radius, R is constant during culture →

$$\Delta\varepsilon \propto pR \propto VCD \cdot R^4 \propto VCD$$

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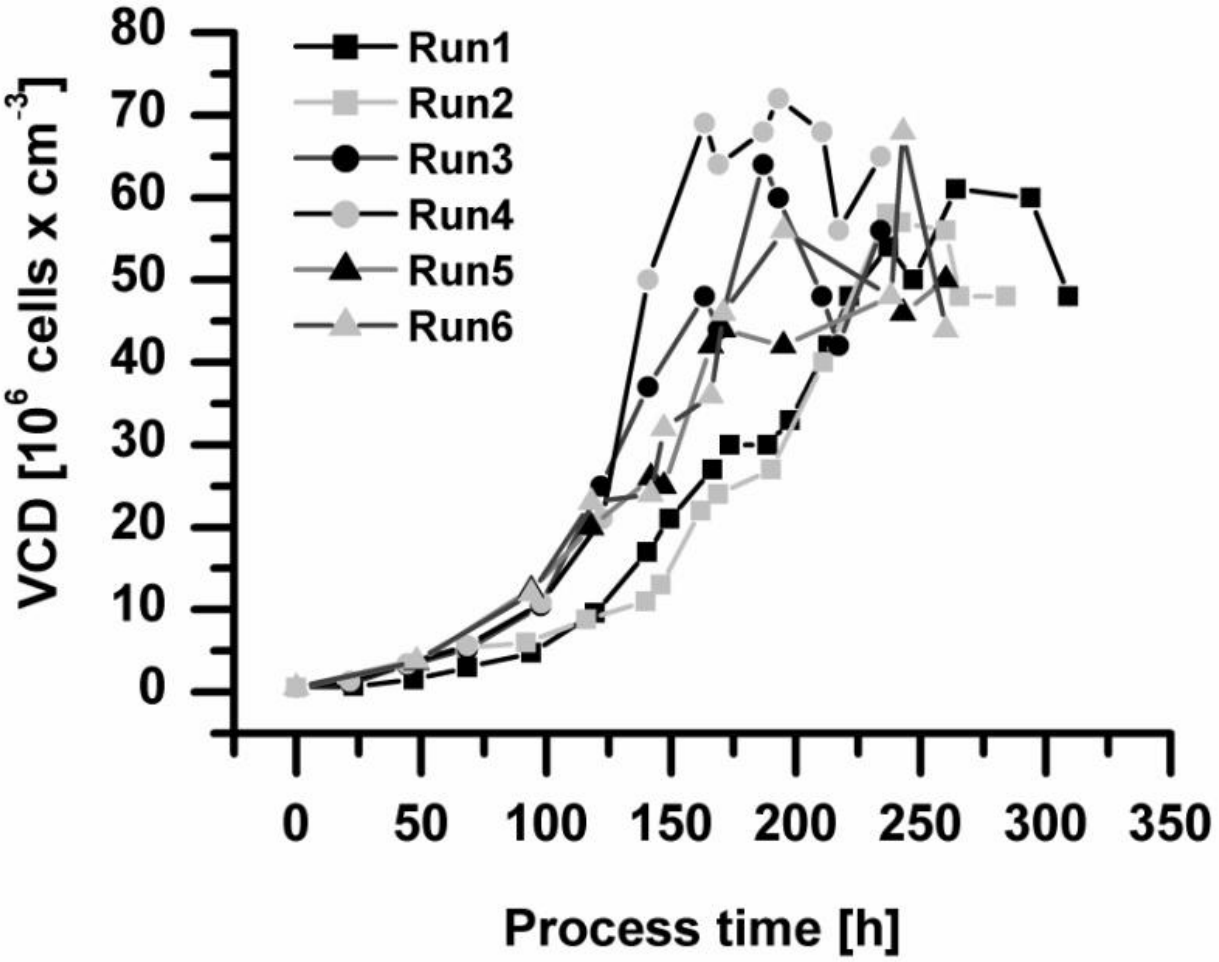
Off-line cell counts (VCD) for 6 runs with REFINE system



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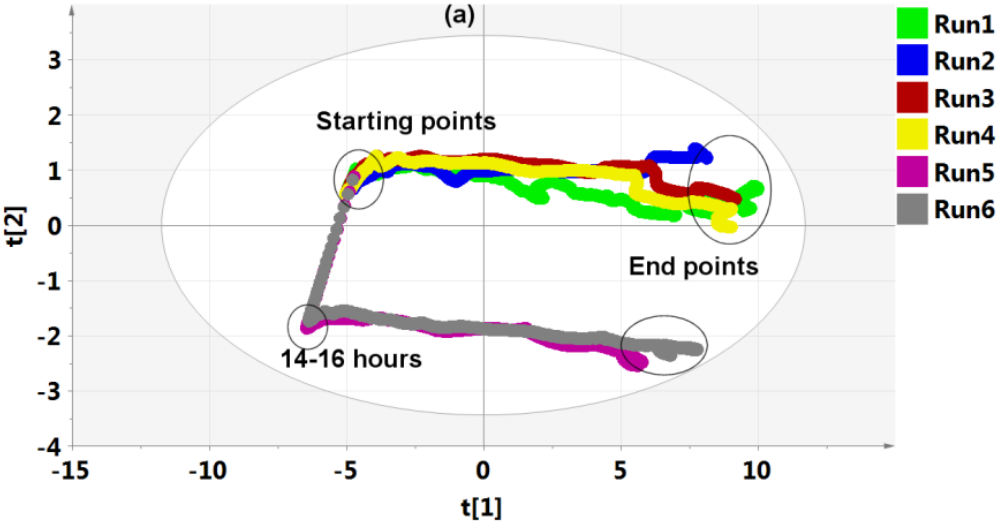
Score plots for 6 runs



- Multivariate analysis (PCA) of capacitance spectra resulted in detection of measurement disturbances
- Principle component t(1) captured 92% of variance in spectra and showed same trend for all 6 runs
- **DIELECTRIC SPECTRA NOT INFLUENCED BY BATCH TO BATCH DIFFERENCES OF CRITICAL CULTIVATION PARAMETERS INDICATING ROBUSTNESS OF probe**
- VCD prediction with multivariate PLS model developed

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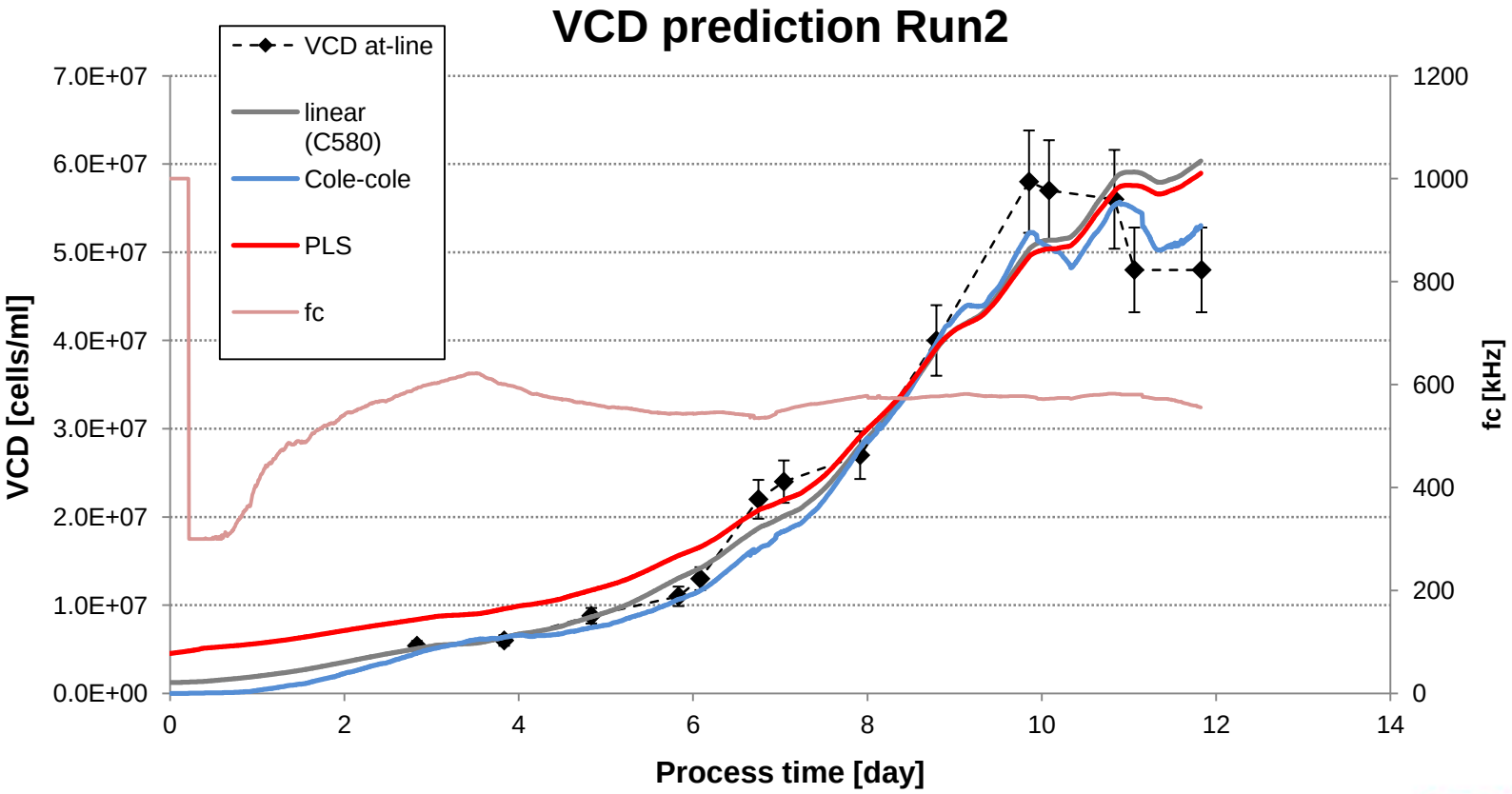
CHO with continuous feed-Comparison of 3 methods to predict VCD



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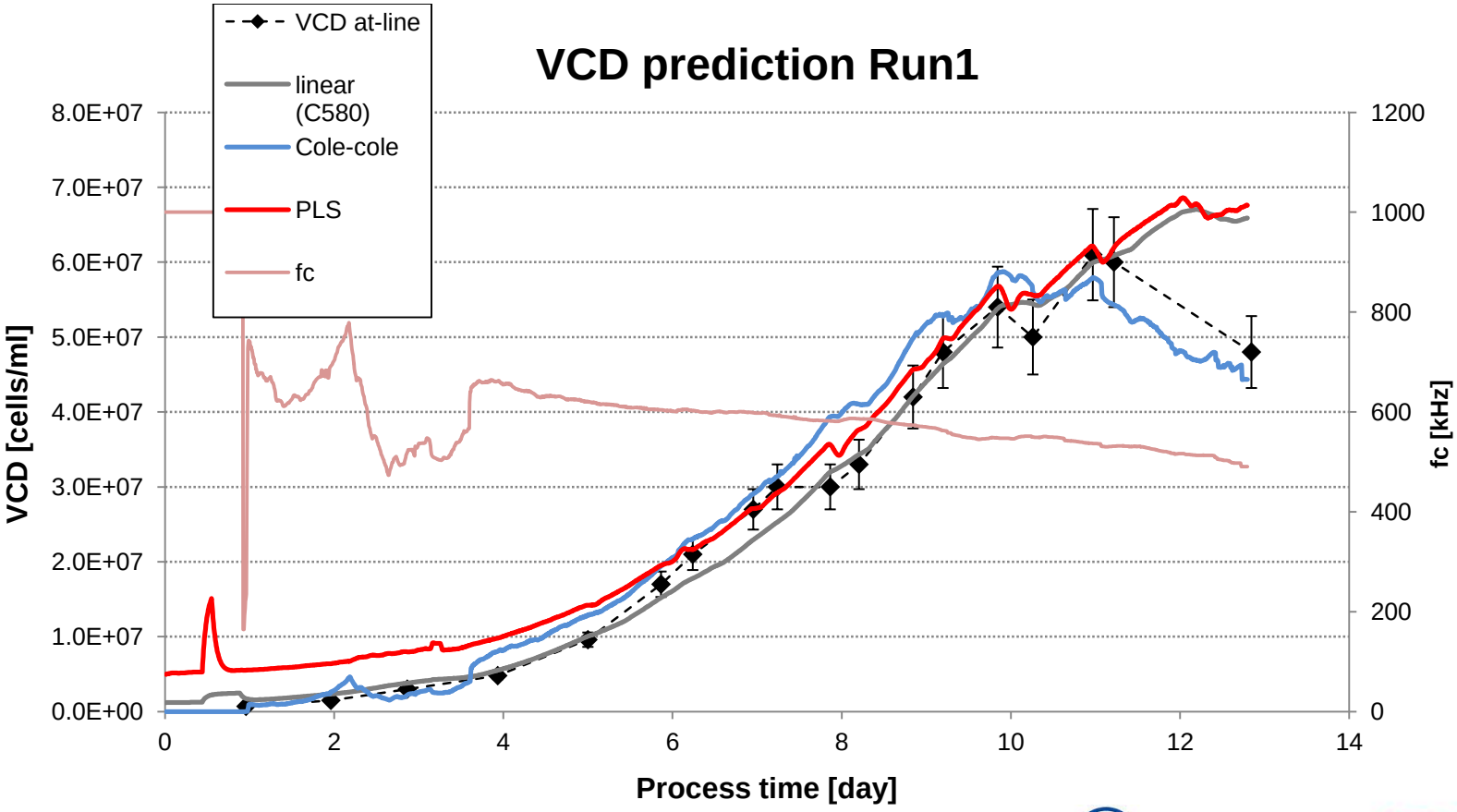
CHO with continuous feed-Comparison of 3 methods to predict VCD



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Case study 2
GSK, Stevenage, UK

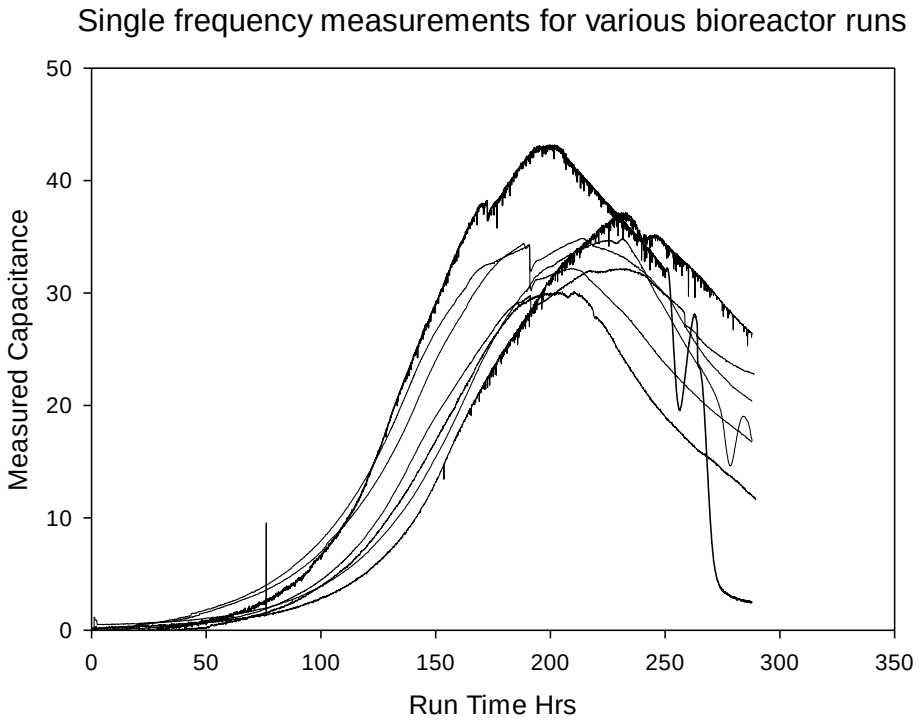
Dielectric Spectroscopy and its application in process development

Andrew Heinrich
Biopharm R&D, GSK Stevenage

PAT & QbD Forum, Feb 18th
Goettingen, Germany

Use of capacitance in scale-up and technology transfer

- Background
  - Cytokine produced in modified CHO cells
  - Initial cell line selection carried out in shake flask studies
  - Process scoping in 2L bioreactor system
- In-situ Aber FUTURA Probe used in 2L glass bioreactors with multiple frequency scanning
- What information can we glean from this data?
  - Relatively similar process profiles
  - Perturbations in capacitance profiles appear to correspond with feed points in process
  - Can this data be used to help build a 'process model' for scale up (and scale-down)?



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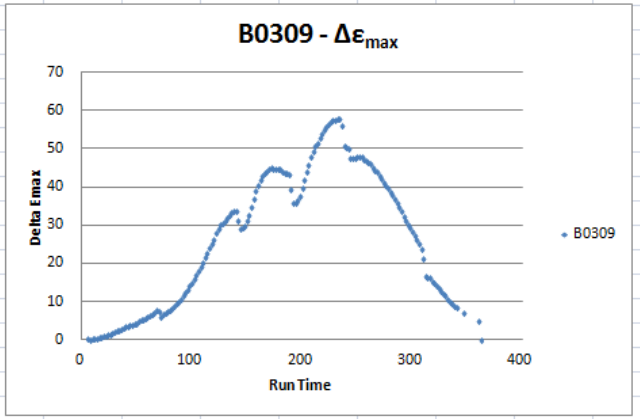
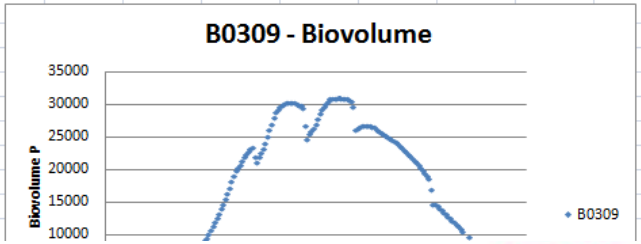
Curve fitting the data

Scanning frequency analysis

- GSK developed an XL-fit macro for rapid and reproducible automatic analysis of scanning data sets

				XLFIT	Emax	Slope	EC50	E0		Delta Emax	Biovolume P
12139	15582	20000									
-0.05384	-0.02003	-0.02867019		#Ok	-0.02213	67.9377	50	0		-0.02212655	-1.10632767
-0.05609	-0.02926	-0.02333686		#Ok	0.15623	-84.3199	50	0		0.1562311	7.81155525
-0.06892	-0.04473	-0.04530769		#Ok	0.12267	-10.0714	1096.66	0		0.12266904	134.526142
-0.05755	-0.0459	-0.03802019		#Ok	0.24021	-6.3871	1017.35	0		0.24021494	244.383568
-0.06545	-0.03558	-0.03590353		#Ok	0.30406	-6.23227	1057.29	0		0.30405766	321.476758
-0.07727	-0.03394	-0.03028269		#Ok	0.40628	-5.25257	1053.34	0		0.40628334	427.955626
-0.07307	-0.04495	-0.02983686		#Ok	0.52413	-4.33303	973.588	0		0.52412527	510.281922
-0.0644	-0.04436	-0.03675353		#Ok	0.65062	-4.10737	954.47	0		0.65062089	620.99819
-0.06359	-0.04909	-0.03325353		#Ok	0.85102	-3.63535	910.199	0		0.85102195	774.599067
-0.03963	-0.01646	0.005313141		#Ok	1.09639	-3.23762	869.539	0		1.09638907	953.352867
-0.04287	0.00657	0.029250641		#Ok	1.30887	-3.082	848.211	0		1.30886902	1110.1977
-0.04326	-0.00259	0.018208974		#Ok	1.52516	-2.88532	825.095	0		1.52515804	1258.40068
-0.04691	-0.01544	0.013429808		#Ok	1.73297	-2.77729	813.643	0		1.73296655	1410.01534
-0.0404	-0.01336	0.022763141		#Ok	1.99487	-2.64025	781.499	0		1.99486599	1558.98574
-0.0071	0.02674	0.055717204		#Ok	2.31913	-2.41333	760.452	0		2.31912931	1763.58753
0.01036	0.05772	0.096129808		#Ok	2.60404	-2.33824	759.638	0.01012		2.59391923	1970.44014
0.00974	0.05008	0.087875641		#Ok	2.81761	-2.33608	762.594	0.01426		2.80334183	2137.81106
0.02664	0.06881	0.095208974		#Ok	3.07825	-2.28322	756.015	0.01675		3.06149186	2314.53463
0.02434	0.07905	0.105646474		#Ok	3.40322	-2.16663	730.877	0.02845		3.37476971	2466.54145
0.01963	0.06479	0.091971474		#Ok	3.57071	-2.21813	747.204	0.02232		3.54839176	2651.37382
0.00873	0.05317	0.080313141		#Ok	3.8613	-2.16156	738.955	0.01006		3.85123908	2845.89176
-0.023	0.00977	0.026117308		#Ok	3.90199	-2.20813	731.644	0		3.90198695	2854.8648
-0.03276	0.00119	0.016242308		#Ok	4.27703	-2.12814	707.708	0		4.27703031	3026.88694
-0.07777	-0.06187	-0.05085769		#Ok	4.35397	-2.24076	712.702	0		4.3539736	3103.08611





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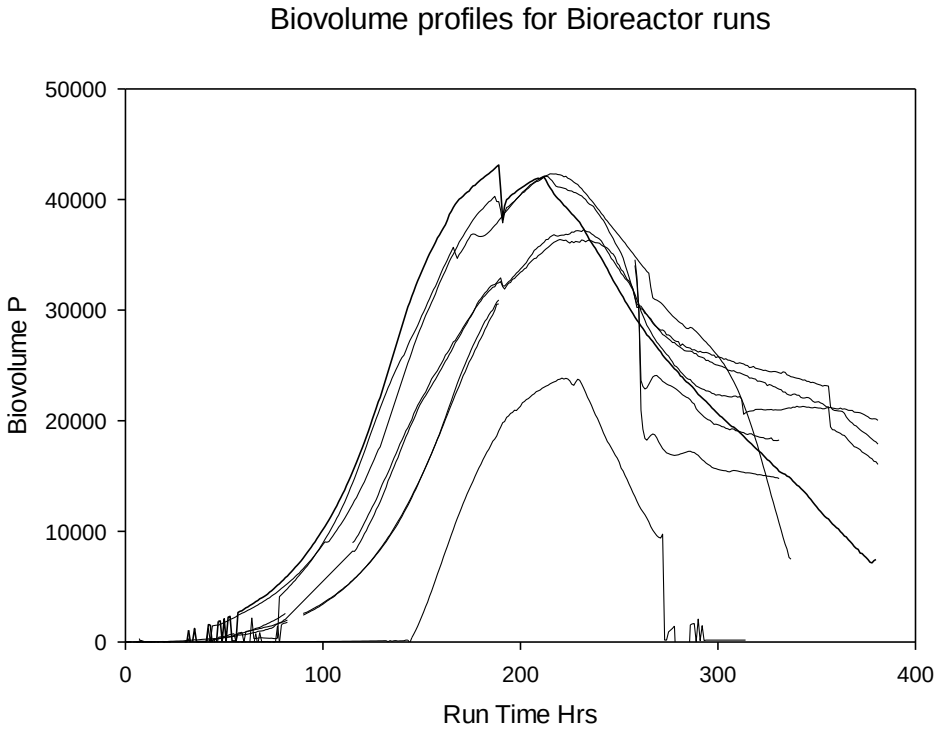
Use of capacitance in scale-up and tech transfer

- Analysis of various scanning data parameters highlights clearer differences between some of the processes
- Using this information one can eliminate runs that appeared previously to be consistent and therefore make more informed conclusions of the 'predictability' of the process conditions
- Further processing of data is still required to fully analyse these results and gain a greater confidence in the 'process model'



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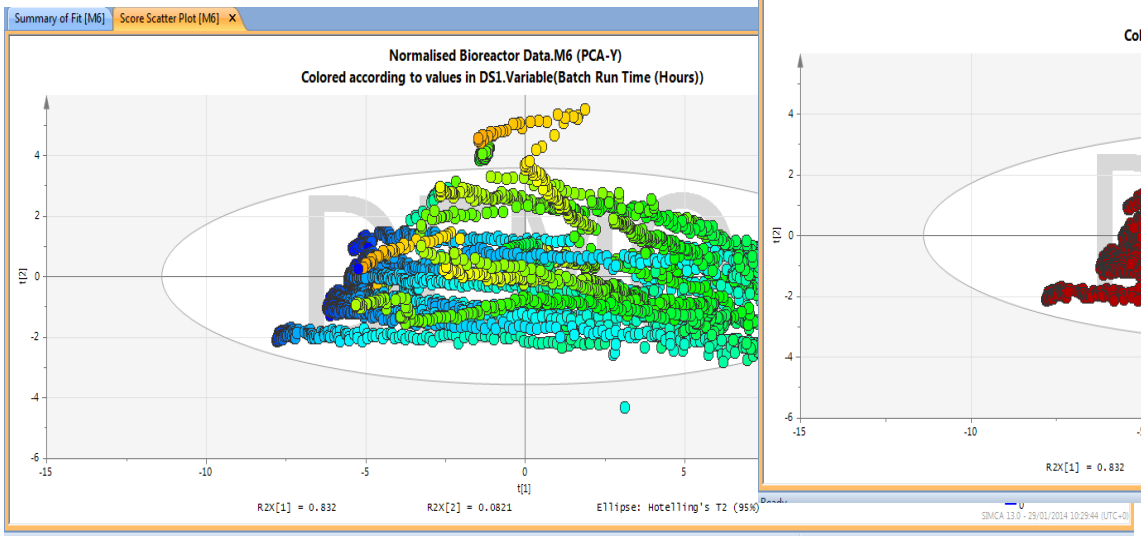
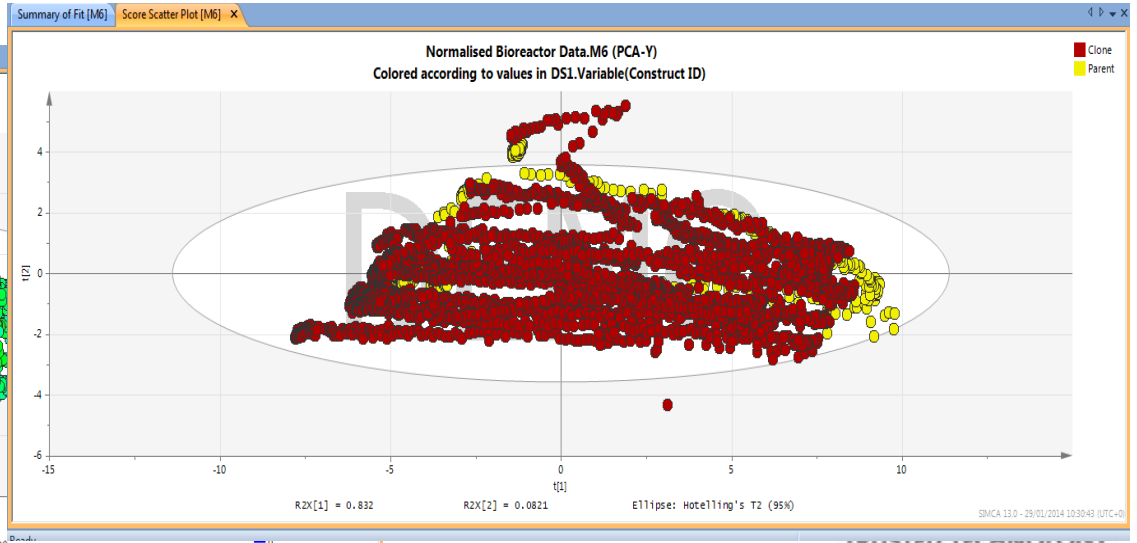
Multivariate analysis

- PCA modelling can then be used to further interpret the key components and specific characteristics of individual batches
- Pre-processing of the raw data is critical in this sort of analysis in order to ensure that inferences from the statistical tools are ‘genuine’
- This analysis then offers a method for investigating design space and process ‘limits’ suitable for development and scale up





Using DS in scale up/scale down experiments

Using conventional probes volumes down to 100ml can be monitored

Optimization of human pluripotent stem cell suspension cultures in controlled, stirred bioreactors

Ruth Olmer[1], Ulrich Martin[1] Robert Zweigerdt[1]

Leibniz Research Laboratories for Biotechnology and Artificial Organs (LEBAO), REBIRTH Cluster of Excellence, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany

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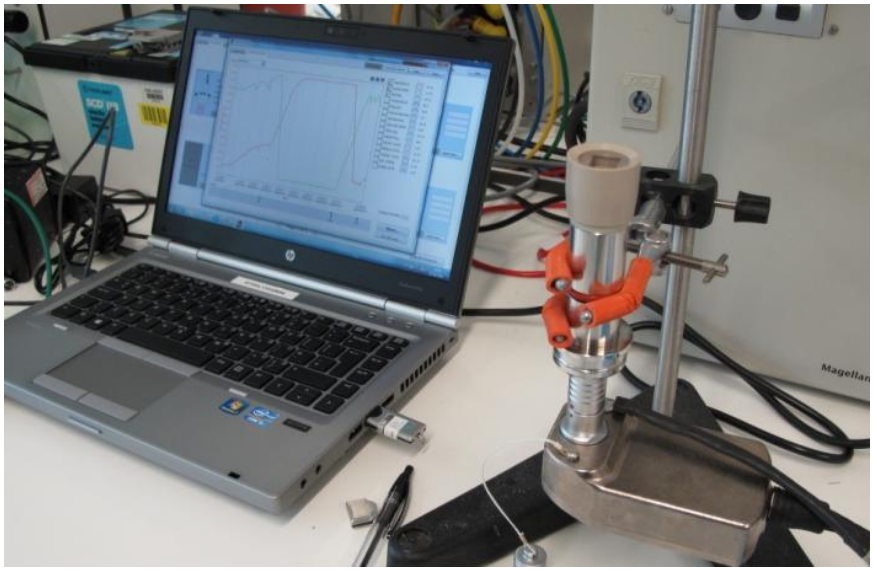
Samples down to 1ml can be taken from shake flasks for DS using an inverted probe



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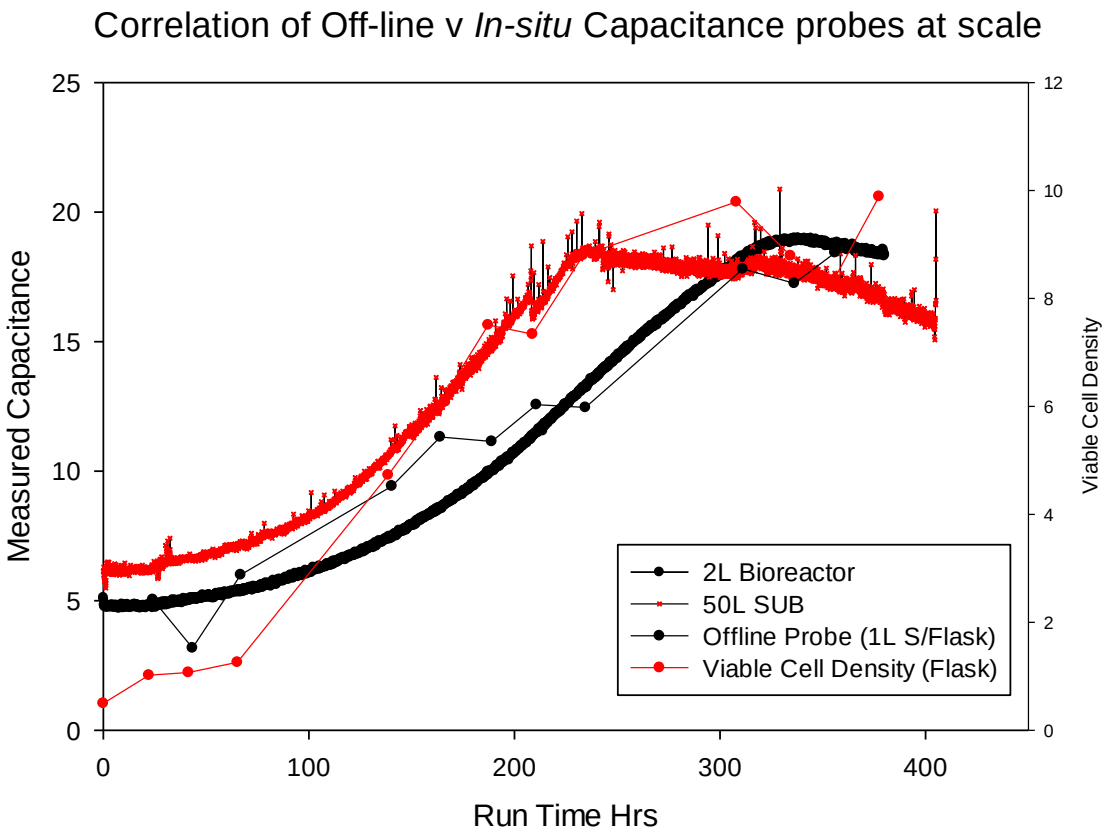
Scale up data from GSK

- Capacitance as a scale parameter from shake flask to 50L single use bioreactor
- Inverted probe technology from Aber Instruments provides real-time offline capacitance readings that correlate well with online data



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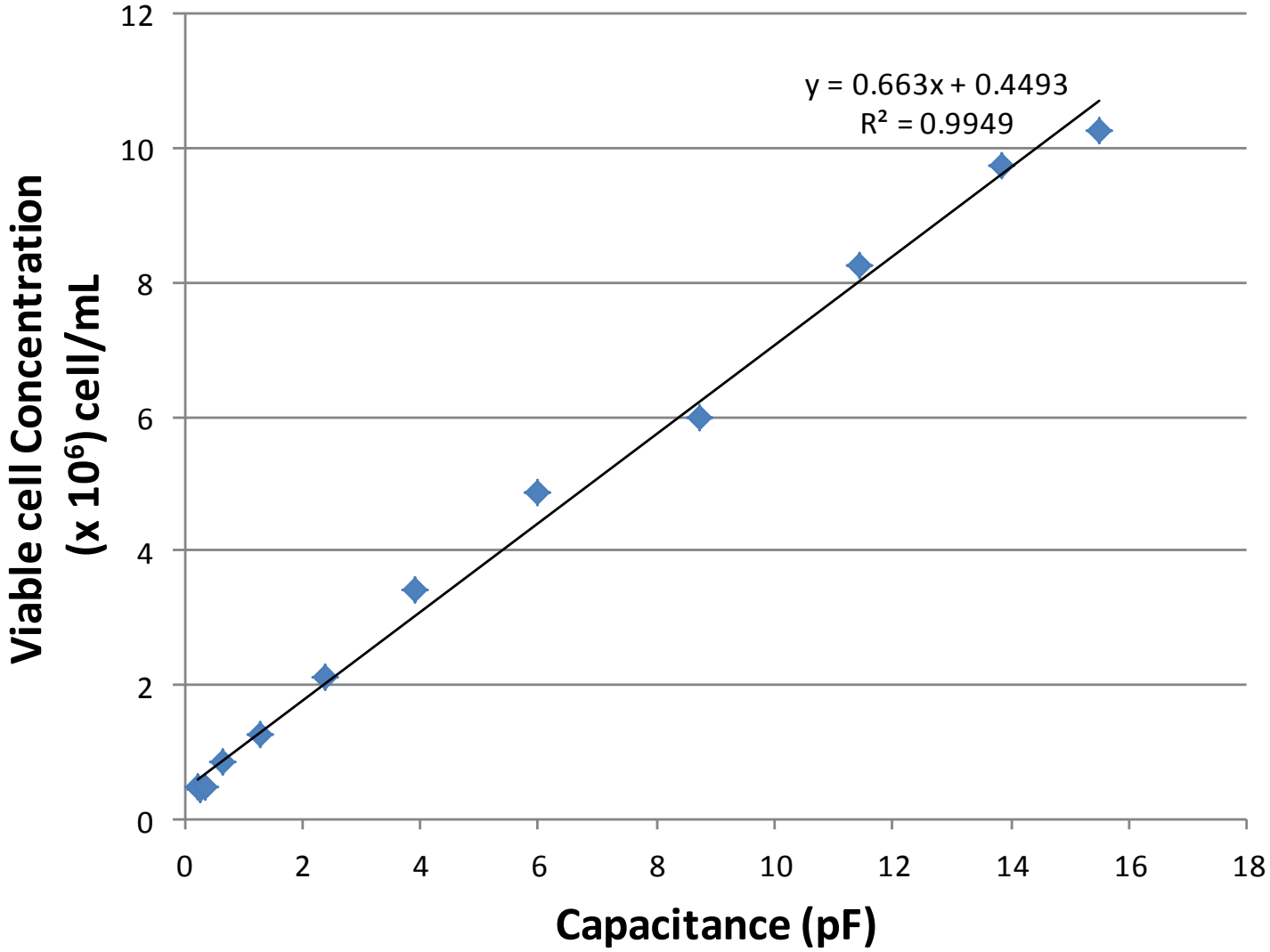
Aber evaluation

FUJIFILM
Diosynth
biotechnologies

- Aims : Acquire capacitance spectra data & compare conventional calibration to PLS calibration

  - Glass 3L bioreactor
  - CHO DG44 clone
  - Fed batch process
    - Cell count & Viability data from Vicell
    - Conventional calibration carried out after run and applied to historical capacitance data
    - PLS calibration carried out after run and applied to historical capacitance data

Case study 3
Fujifilm Diosynth, UK



FUJIFILM Diosynth biotechnologies

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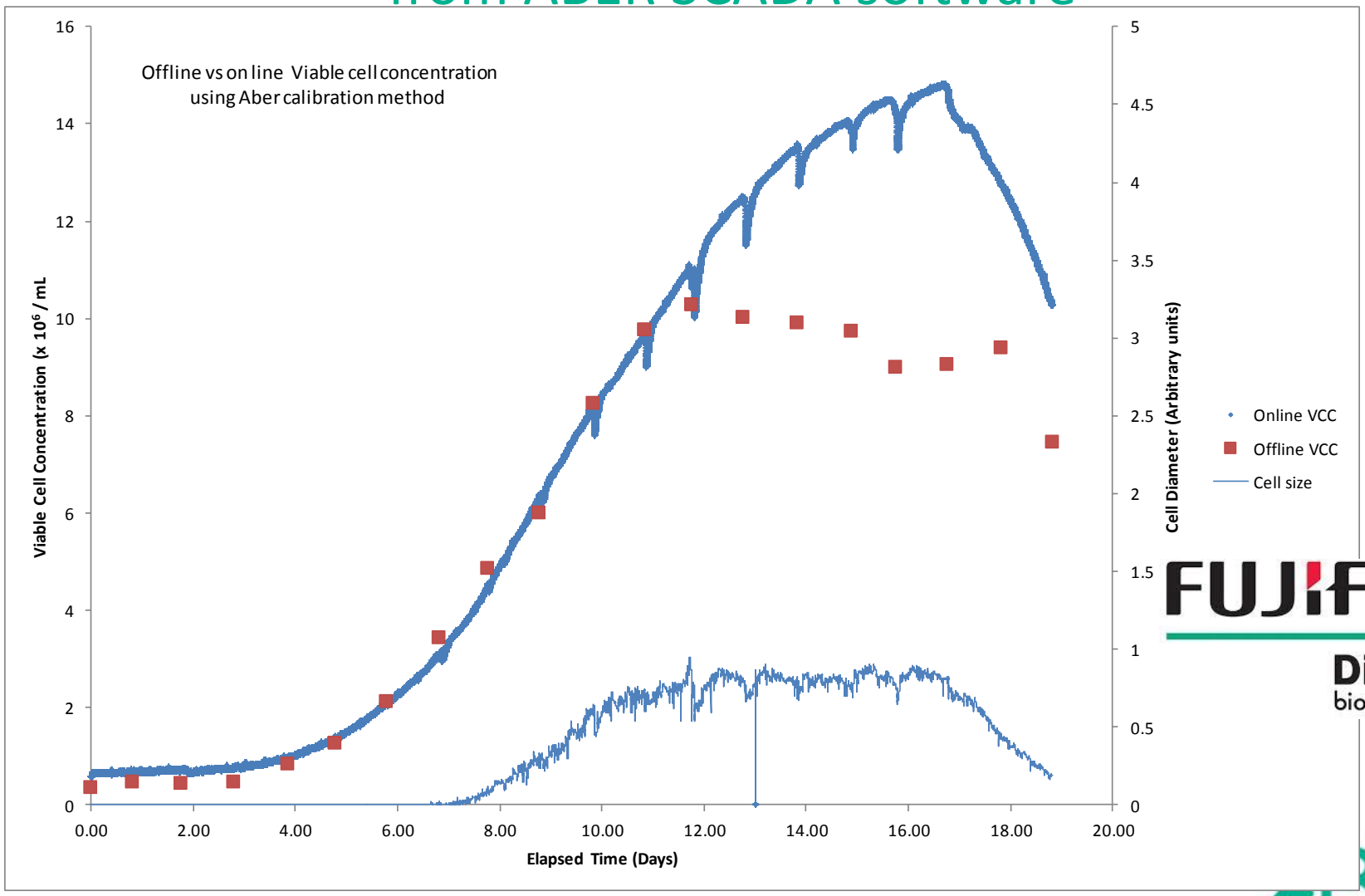
Conventional Calibration showing online cell diameter from ABER SCADA software



FUJIFILM Diosynth biotechnologies

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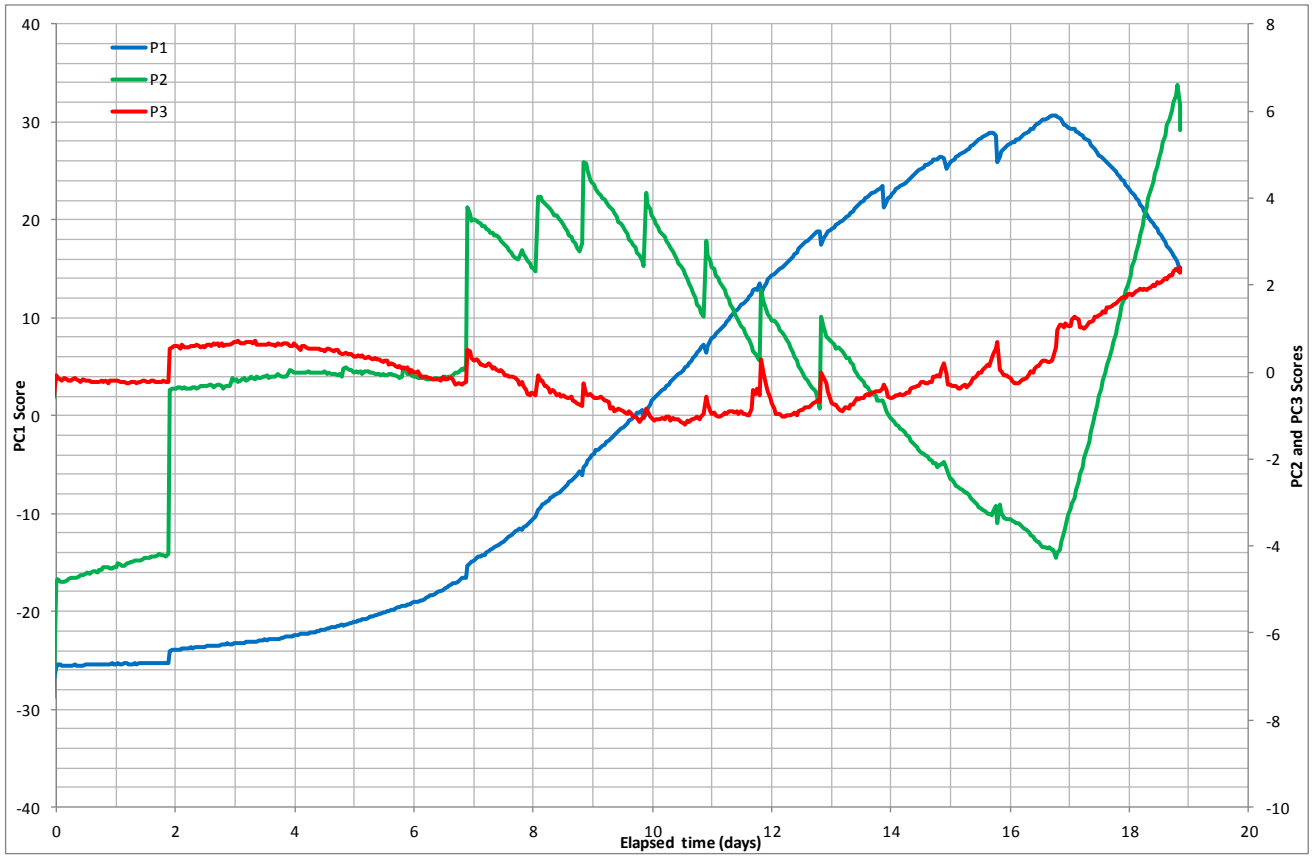
Principal Component Analysis
Raw scores plot of B-Dispersion Spectra



FUJIFILM Diosynth biotechnologies

- PCA analysis of Raw B-dispersion Spectra from CHO Mammalian cell culture
- P1 – Strong Biomass concentration signal
- PC2 and 3 Remaining Orthogonal variance in signal

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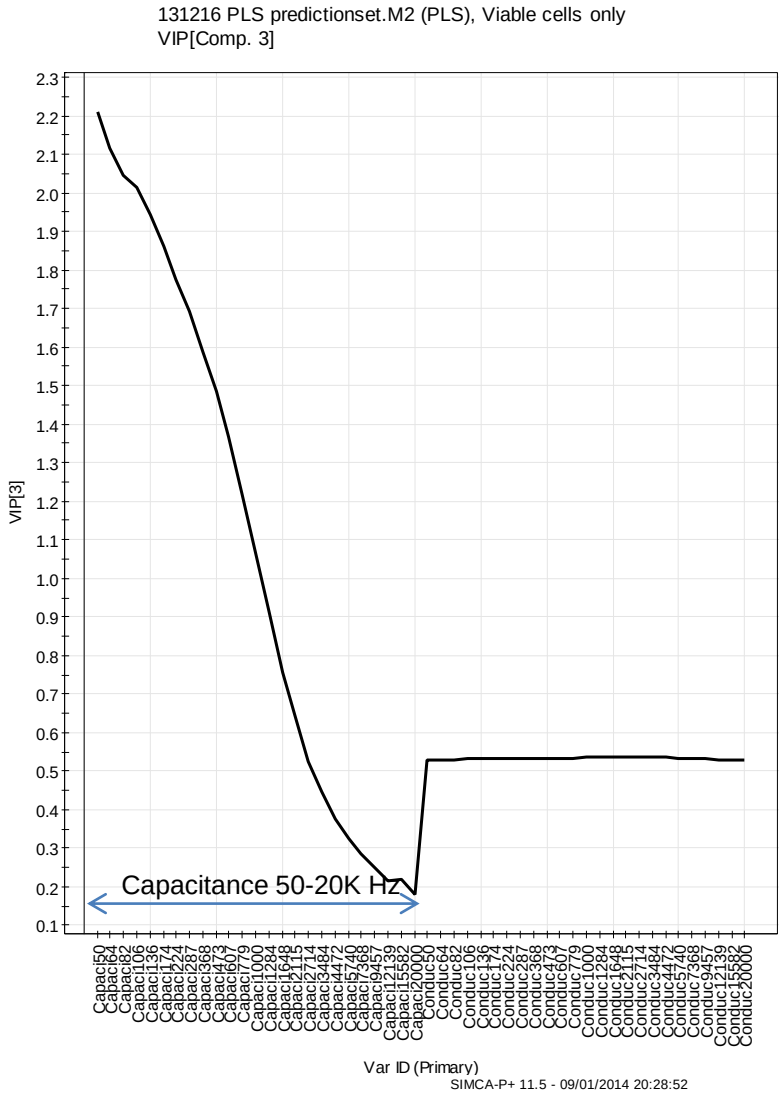
VCD- Variable importance plots vs Frequency

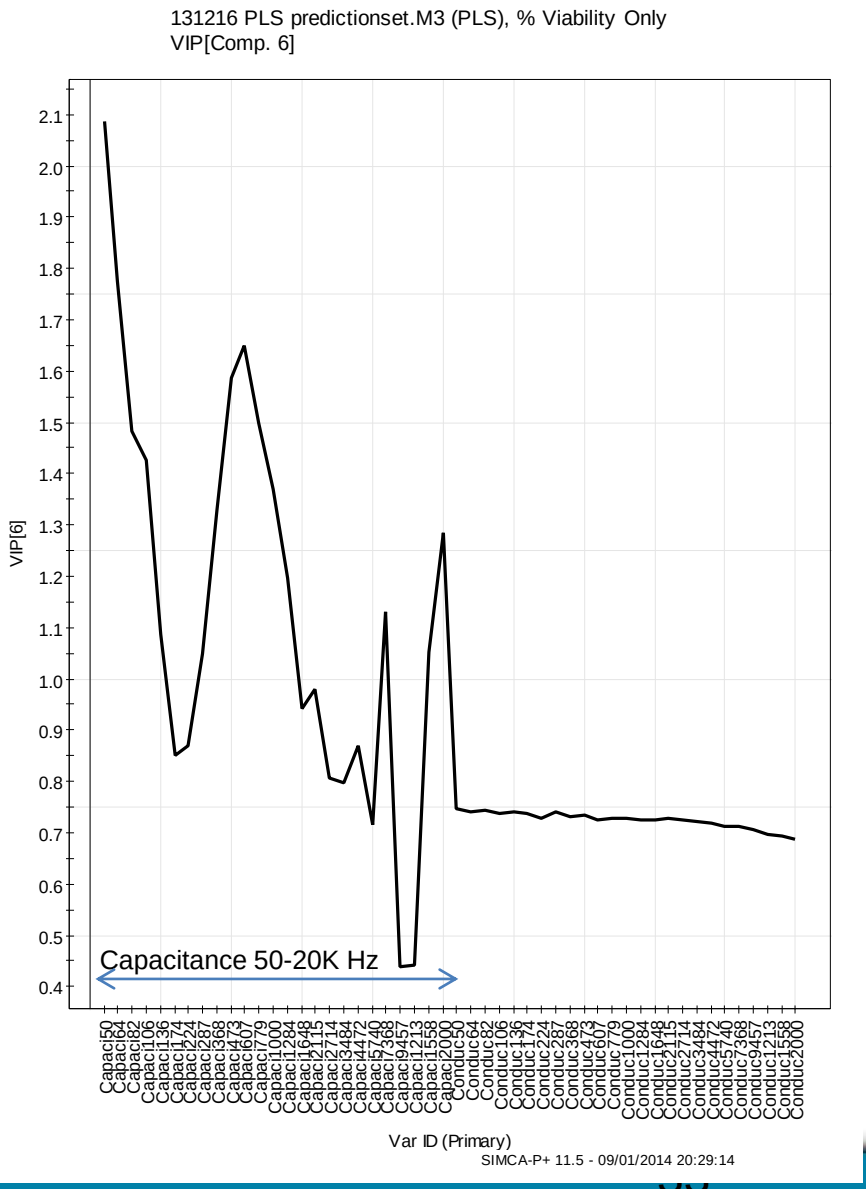


For VCD the lower frequencies correlate more strongly than the higher frequencies as expected

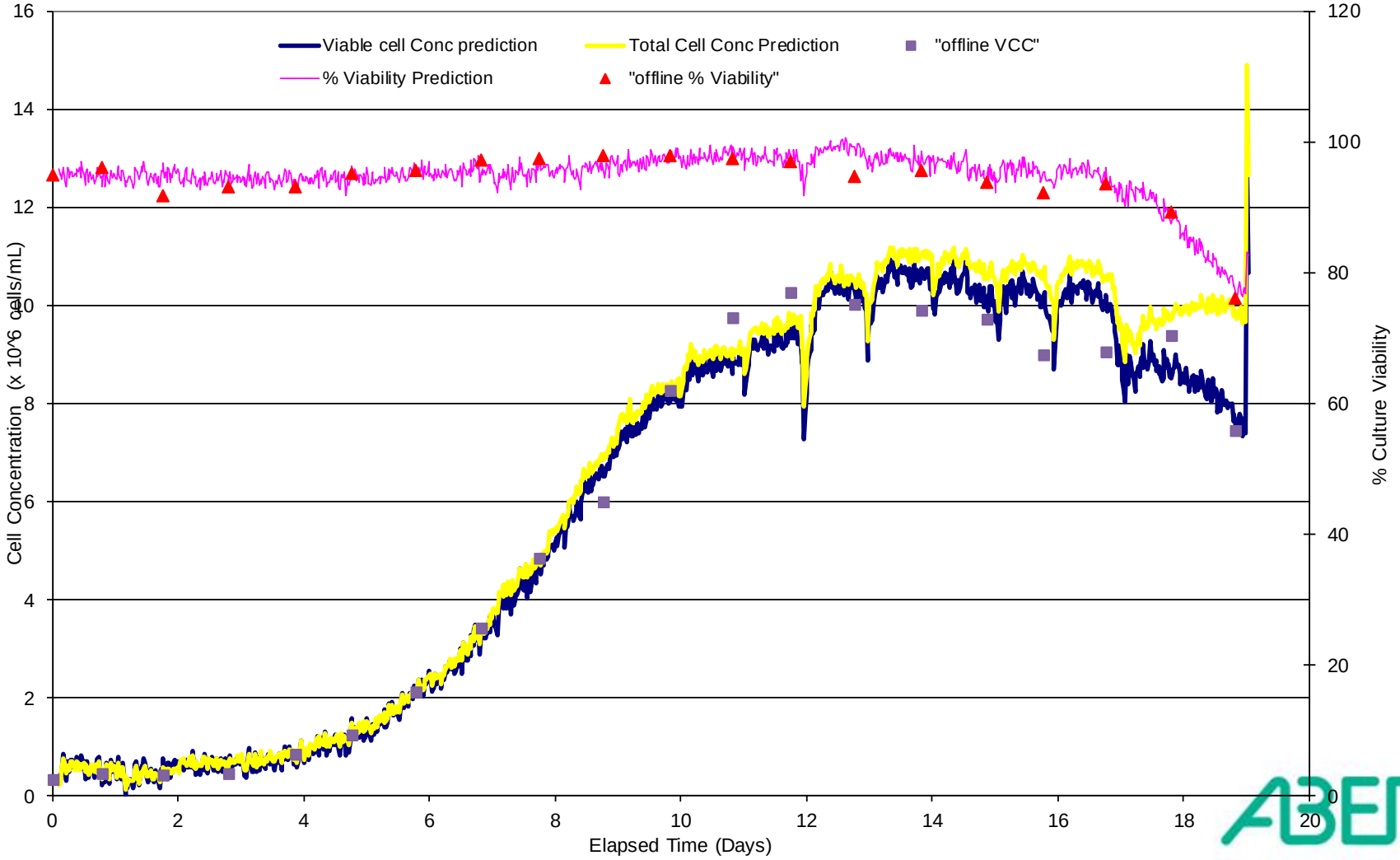
Viability-Variable importance plots vs Frequency

-Two peaks in the VIP plot vs frequency with a peak in the mid range.

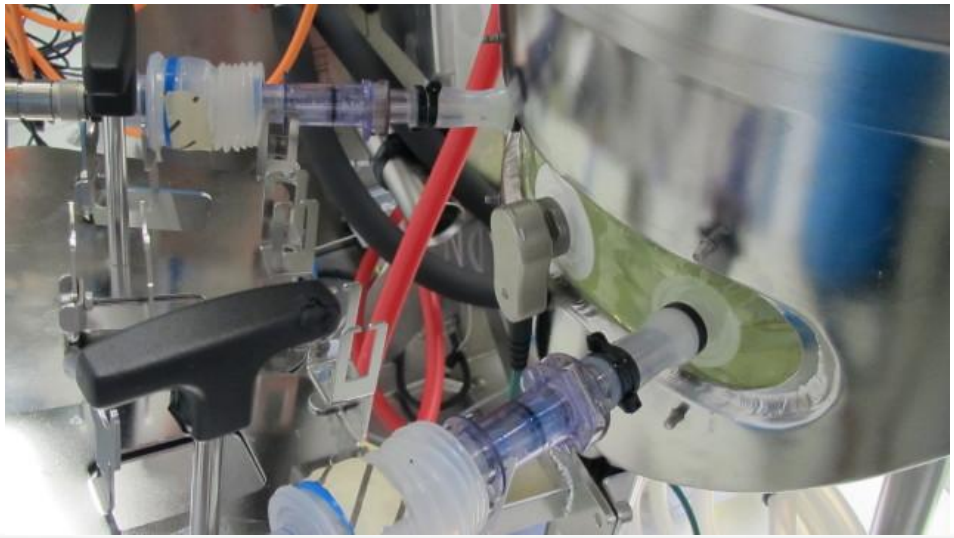
-change in the shape of the peak in the mid range over time probably due to a shift in the cell size distribution.



PLS Calibration



Single use applications



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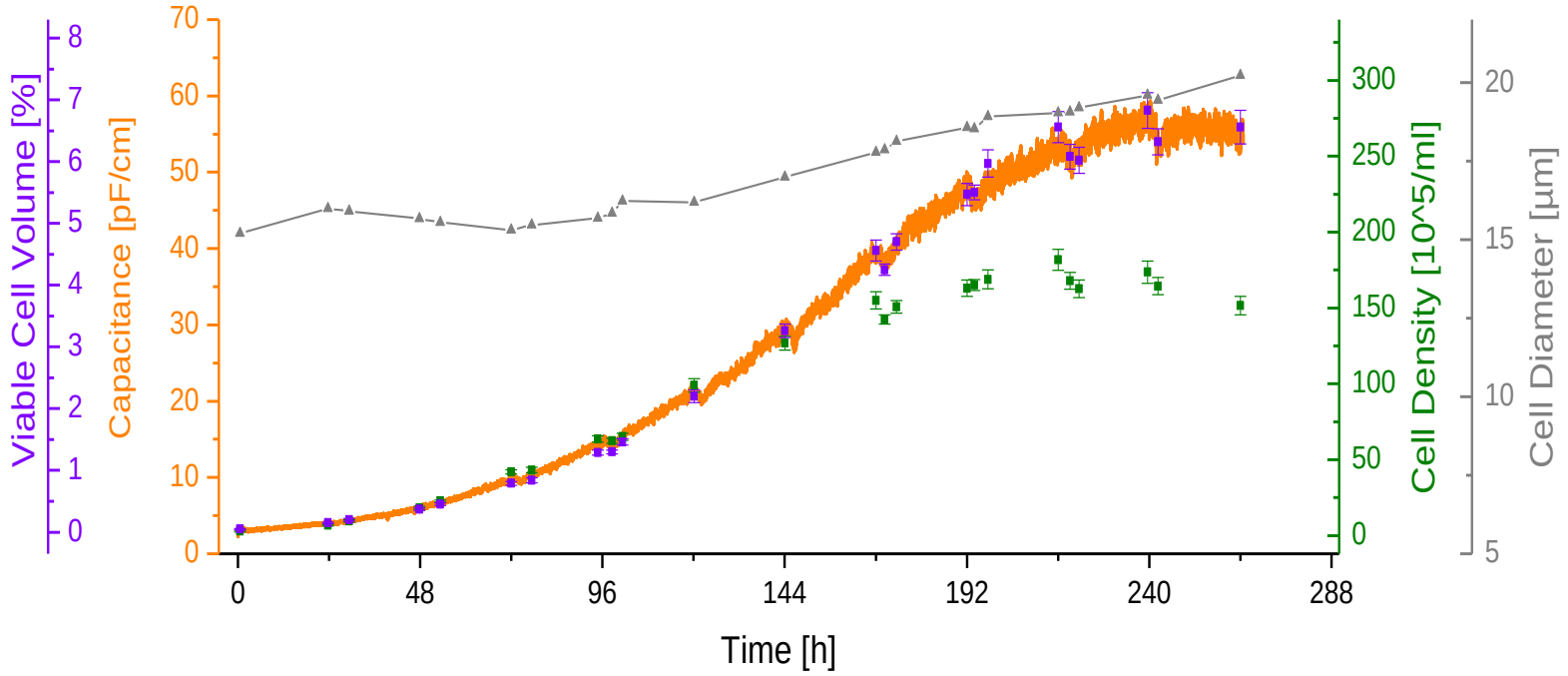
Results from Aber single use probe in a Sartorius rocking bag



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Flow past cells for use on external loops with silicone tubing

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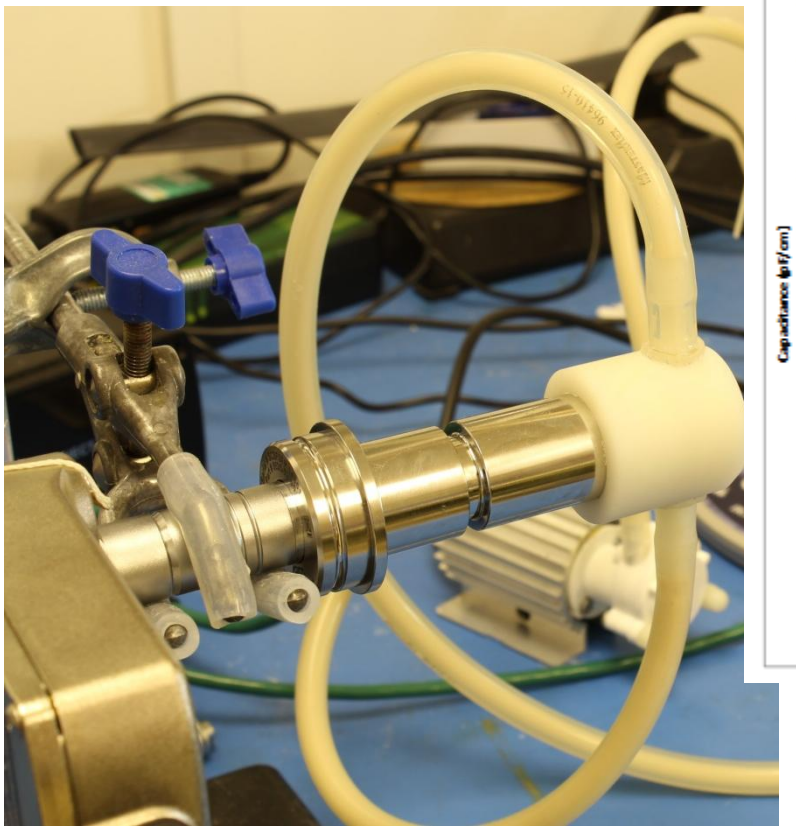
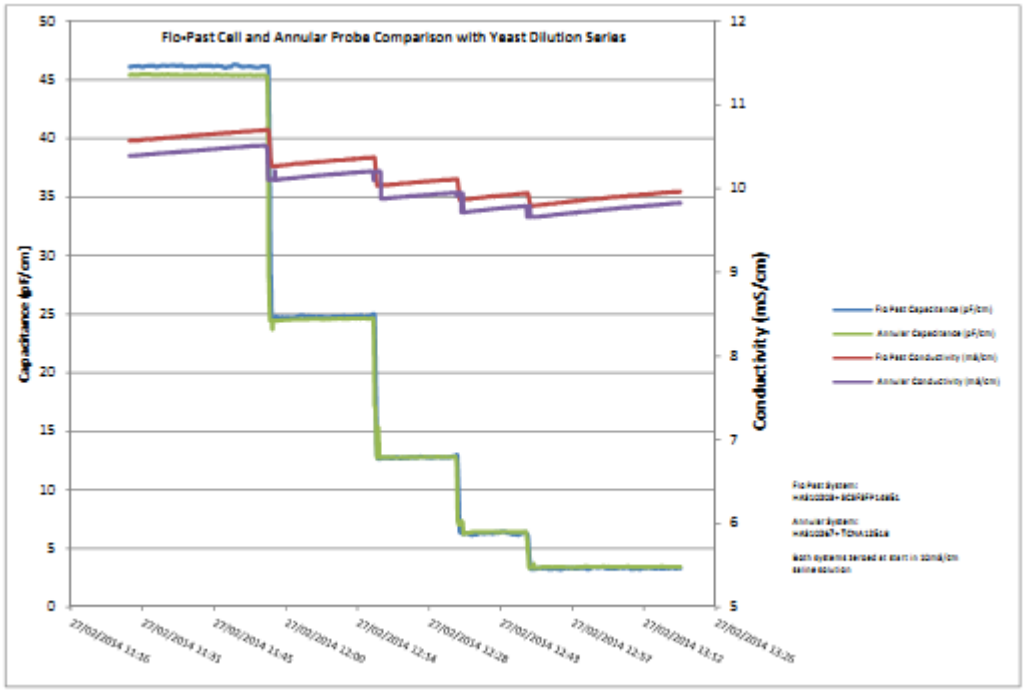
First version of Flow past cell will be tested at Shire this month





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Conclusions

- Spectral scanning of capacitance becoming increasingly used in PAT
- Software based on Cole-Cole or PLS can be used to correct VCD during death phase ,derive viability and in scale up studies
- Promising technology for studies on apoptosis on certain cell lines
- Development of single use probes has enabled technology to be applied to single use bioreactors

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