Utilizing capacitance measurement to improve vaccine and viral vector production processes

from Aber Instruments

Aditya Bhat BSc, MSc, PhD
VP Technology

Housekeeping

- Length – about 30 minutes, followed by 10 minutes for questions.
- Please mute yourselves, just in case.
- Any questions during the webinar, please feel free to type them.
- We will try and address as many questions as possible. Any that are not addressed, we will respond via email.
- Sit back and enjoy the webinar!

Structure

Introduction

- Viral vector/vaccine development
- Challenges/Opportunities

Capacitance measurement

- How does it work?

Utilizing capacitance

- Benefits to the process

Take home points

- Summary

Viral vector/vaccine development and production

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Vaccine development and production

- CAGR for global vaccine market is 8%.
- 95% of the world's vaccines are developed for humans. Among these, 90% are prophylactic vaccines and 10% are therapeutic.
- Significant proportion of development/manufacturing strategies are based around *viral vector or virus platforms.*

Typical process flow

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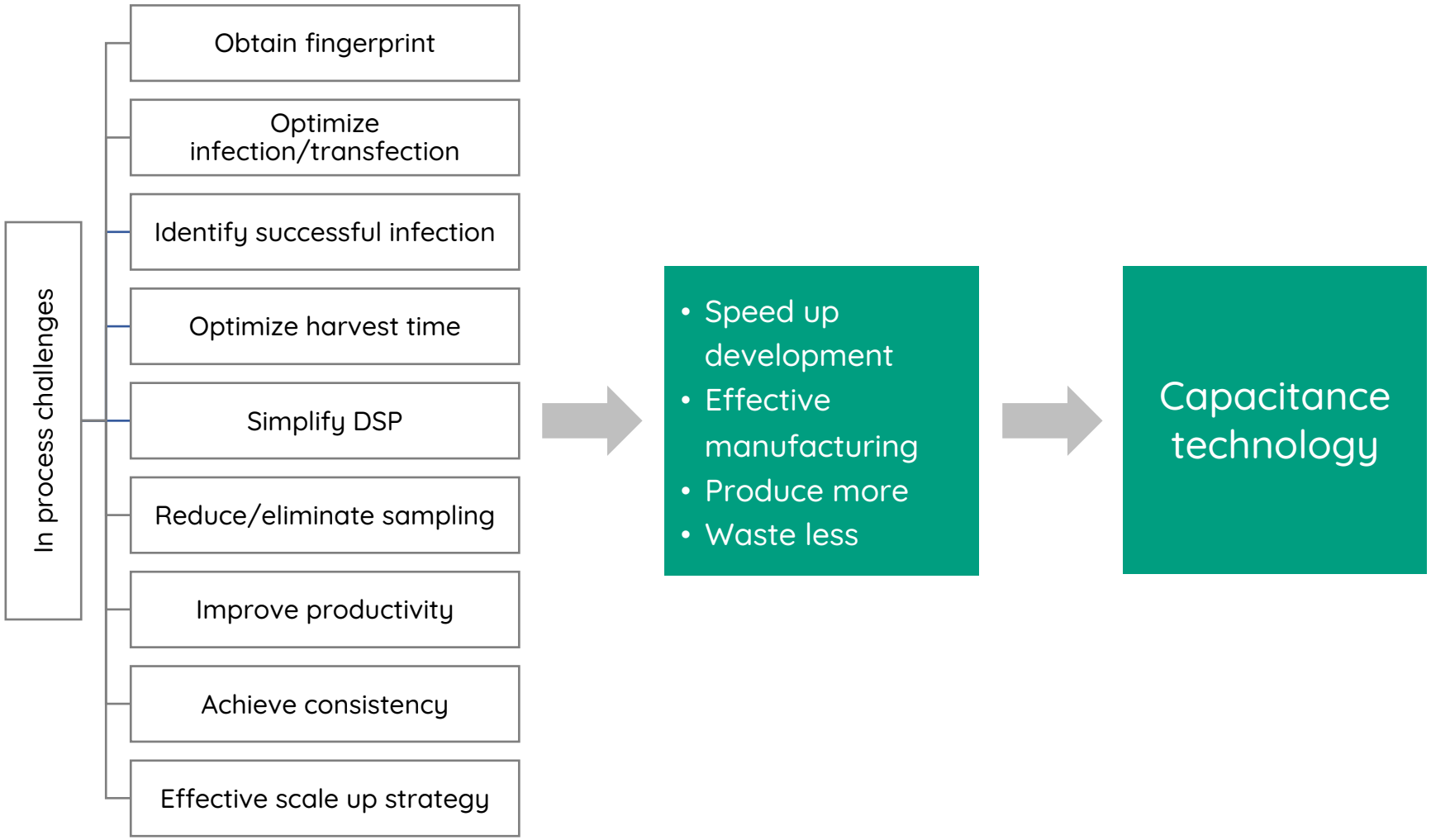
In process challenges



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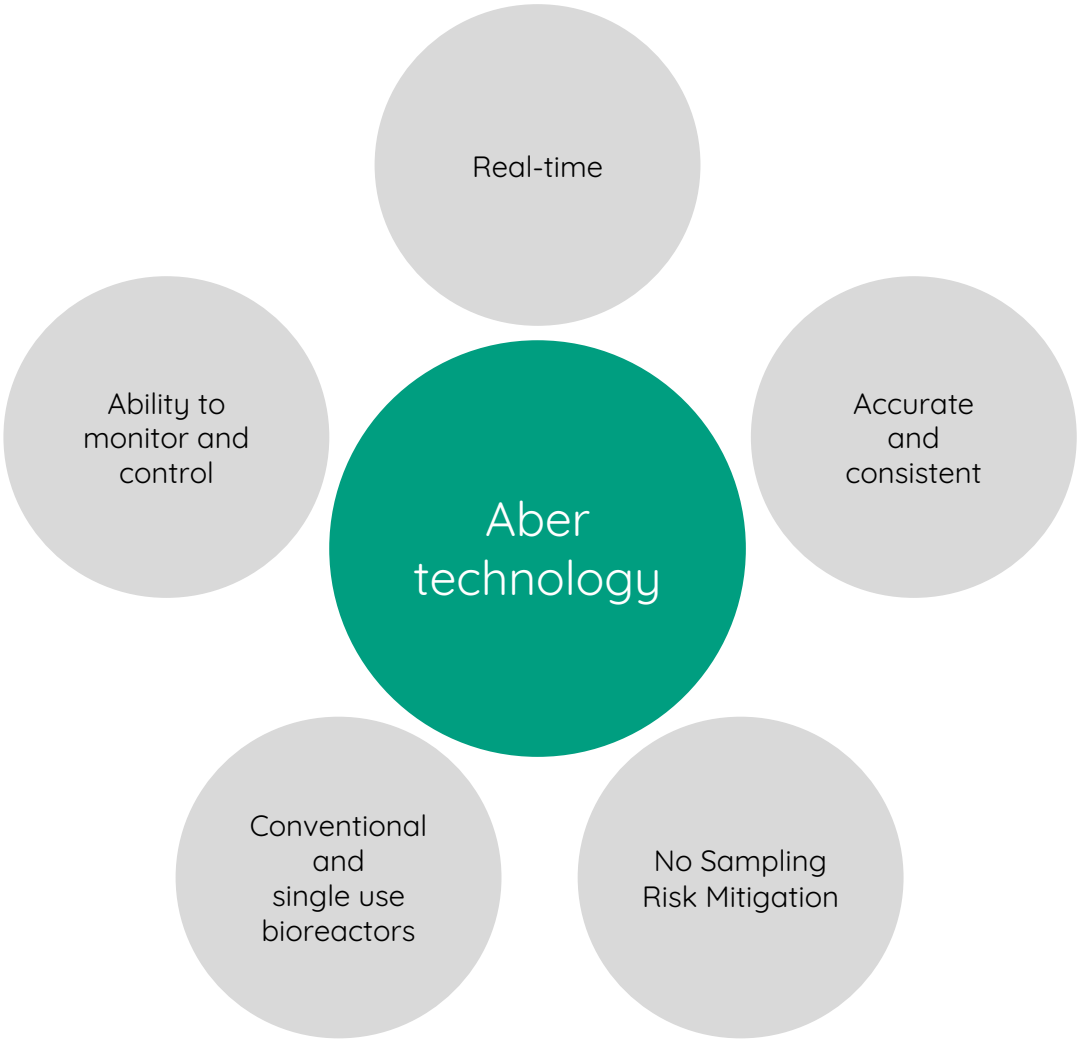
Capacitance technology (Dielectric Spectroscopy)



Complete knowledge of your parameter for PAT

Capacitance Technology by Aber

Capacitance technology

- Invented in 1988 by Aber Instruments, Aberystwyth UK
- Extensive knowledge

Experience

- Mature technology
- Over 30 years of focused & dedicated experience in biomass measurement

Worldwide customer base

- Customers in over 140 countries
- Major pharmaceutical companies

Complete portfolio

- Installations from R&D through to Manufacturing
- From mini & small glass bioreactors to Stainless Steel vessels
- Single use capacitance measurement technology

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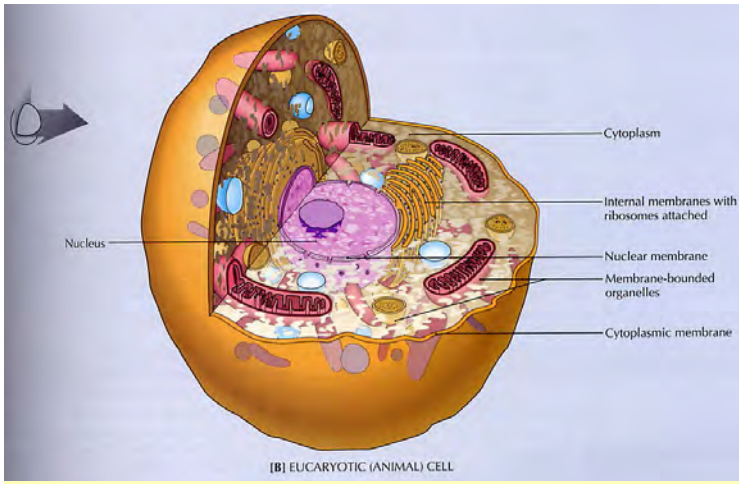
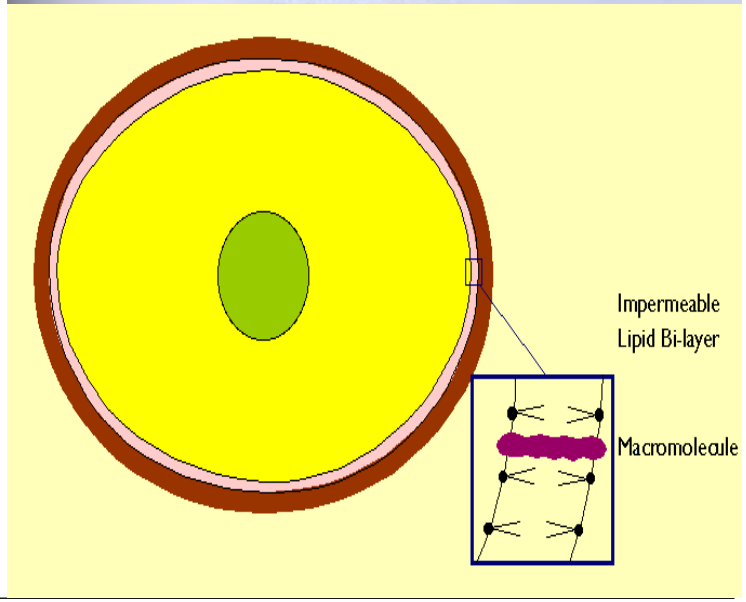
The theory
How does it work?

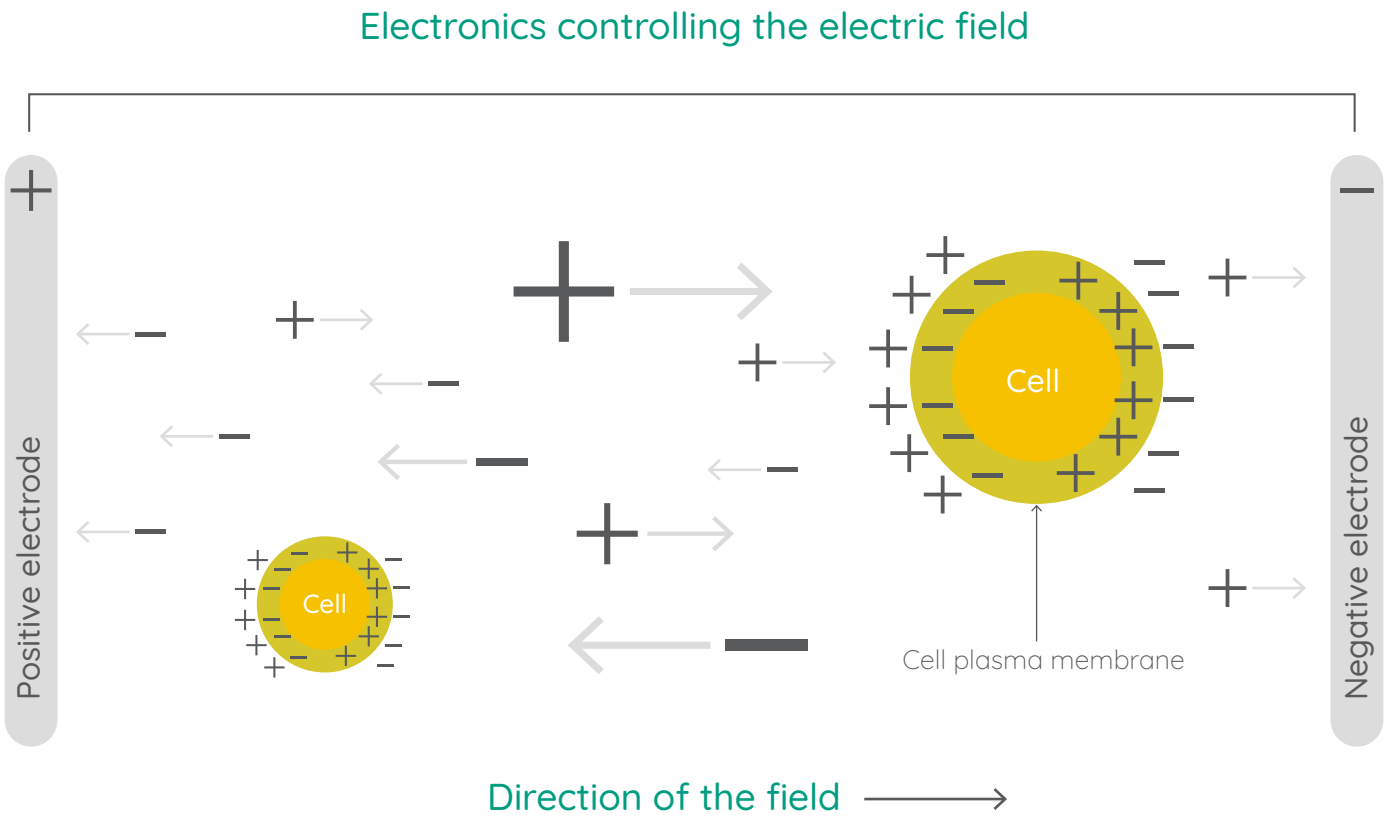
Principle of measurement

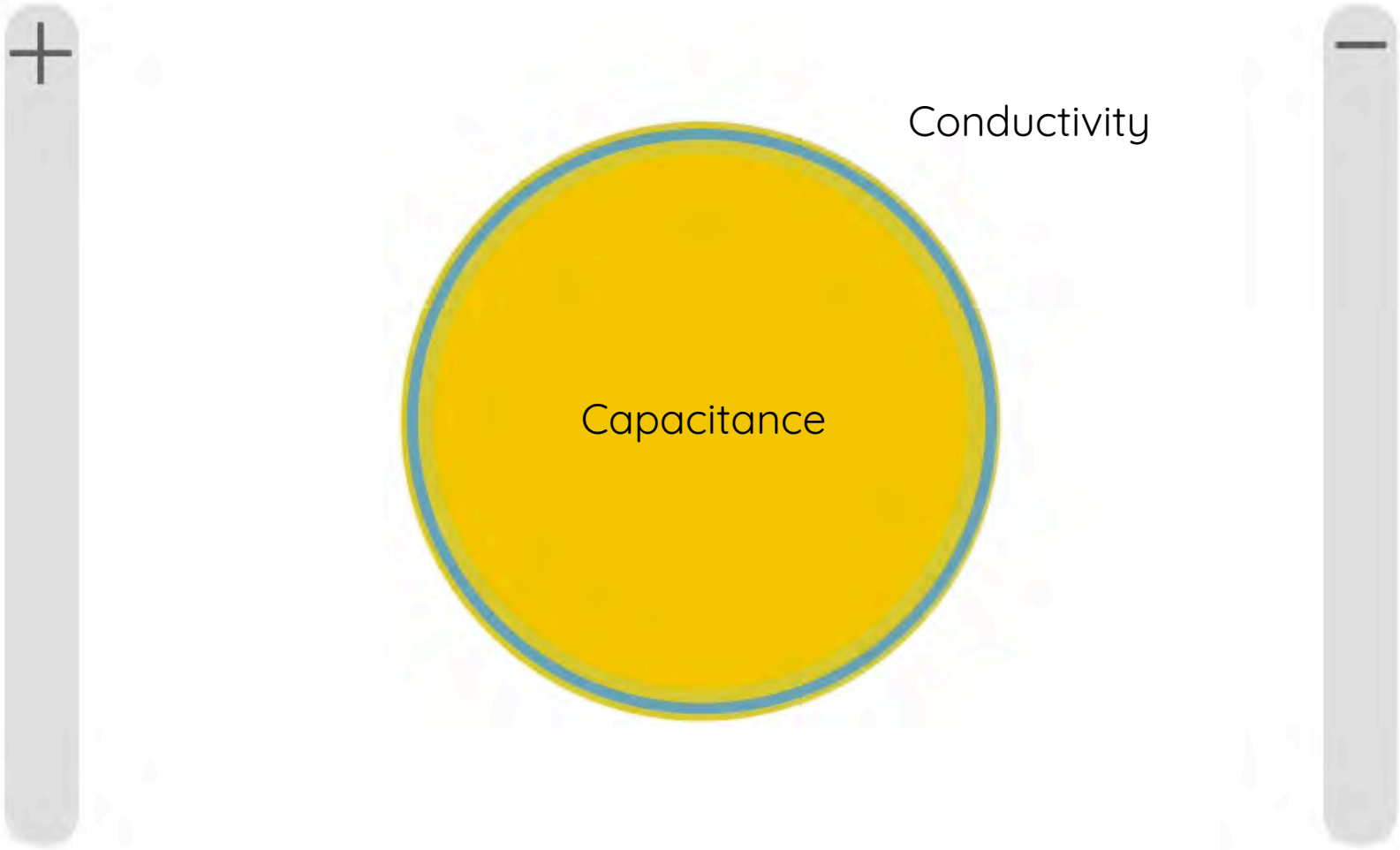
- Capacitance and Conductivity
- The Cell Membrane
  - Bilayer
  - Is impermeable to ions (insulator)
  - Under the influence of an electric field, gets polarised
- When membrane is undamaged, each live cells acts as a capacitor





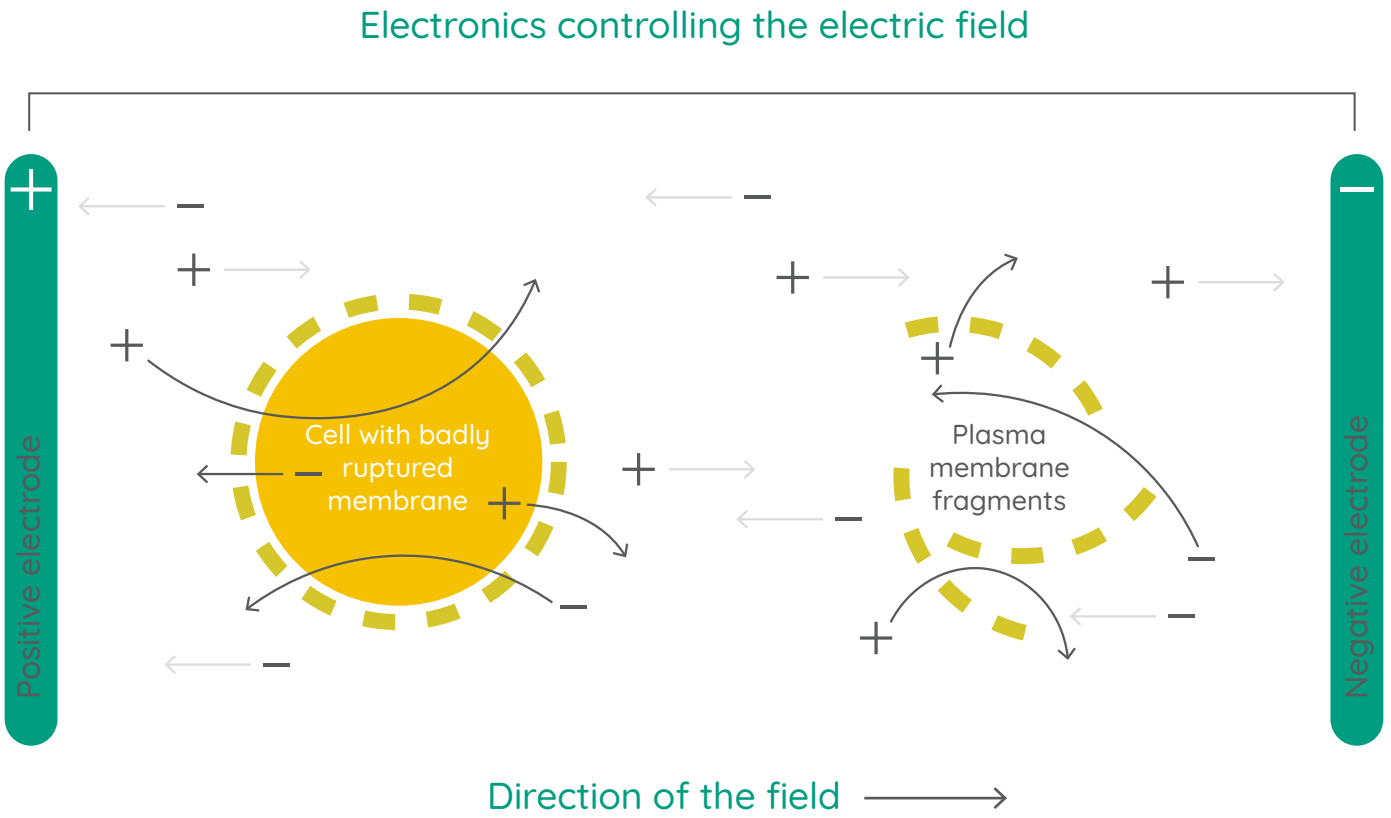
Influence of electric field to 'polarise' viable cells





- Measure the build up of charge around live cell membranes
- Capacitance reflection of cell density AND cell size + electrical properties of cell membrane
- Aber capacitance is a measure of the viable biovolume

Failure of electric field to ‘polarise’ dead and ruptured cells



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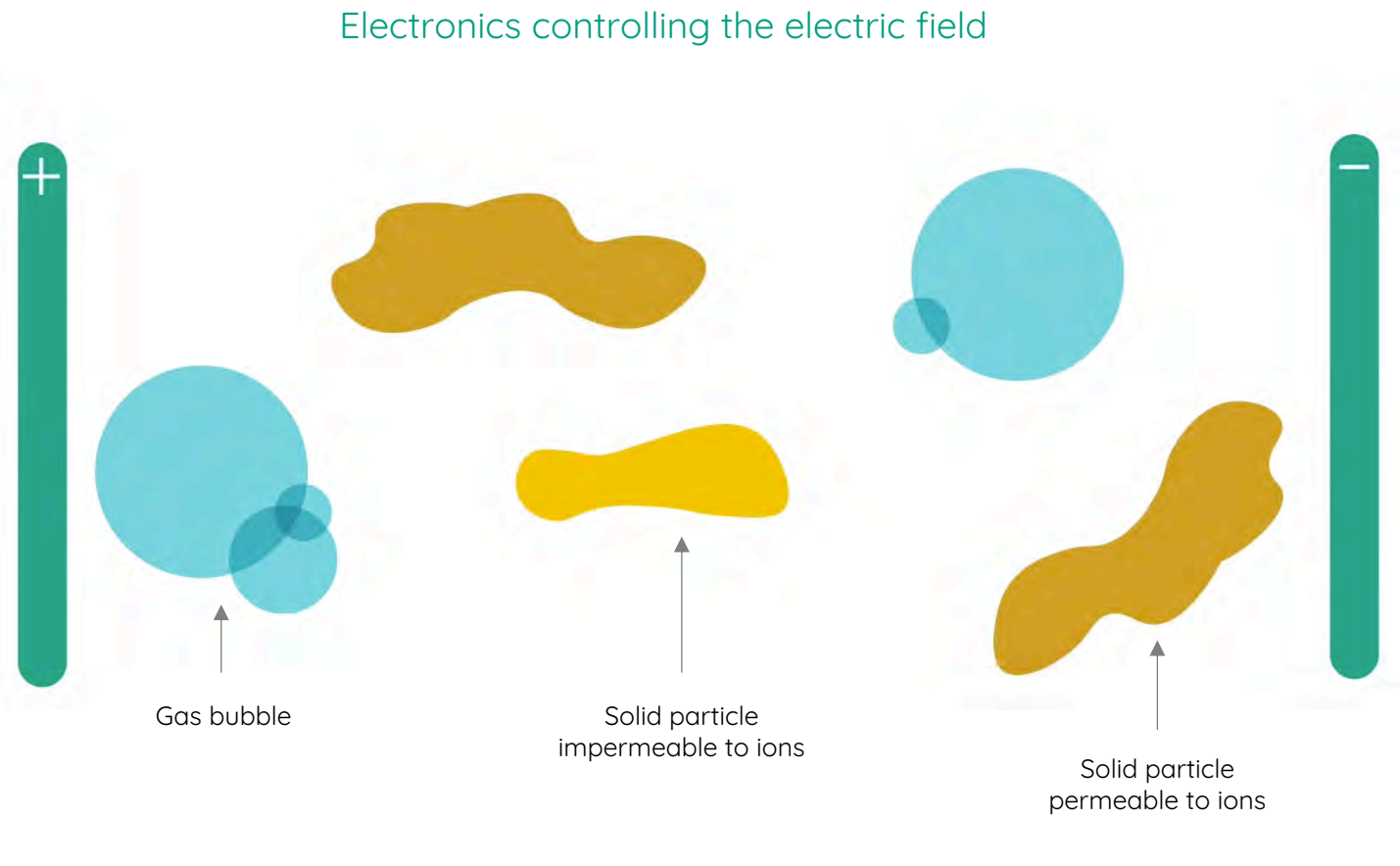
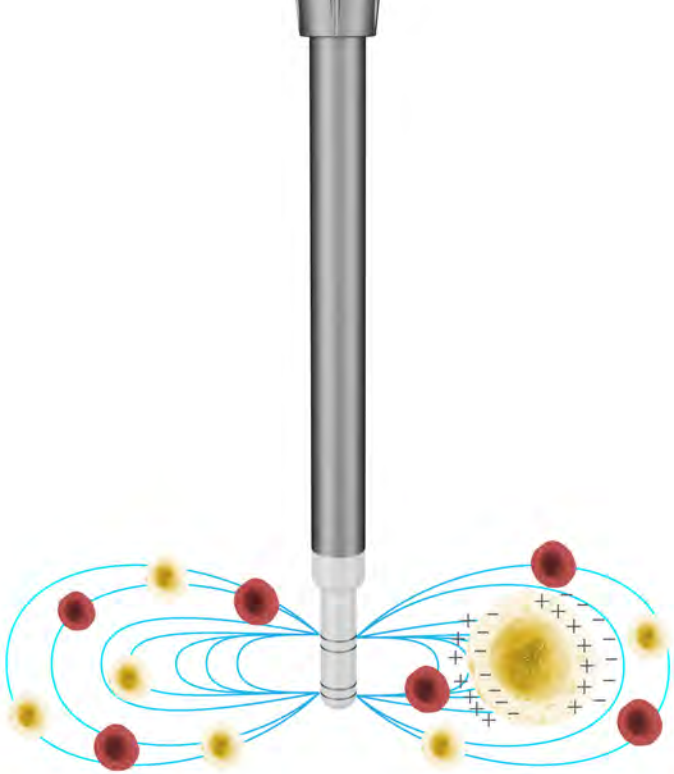
Influence of gas bubbles and solid media particles on capacitance measurements.



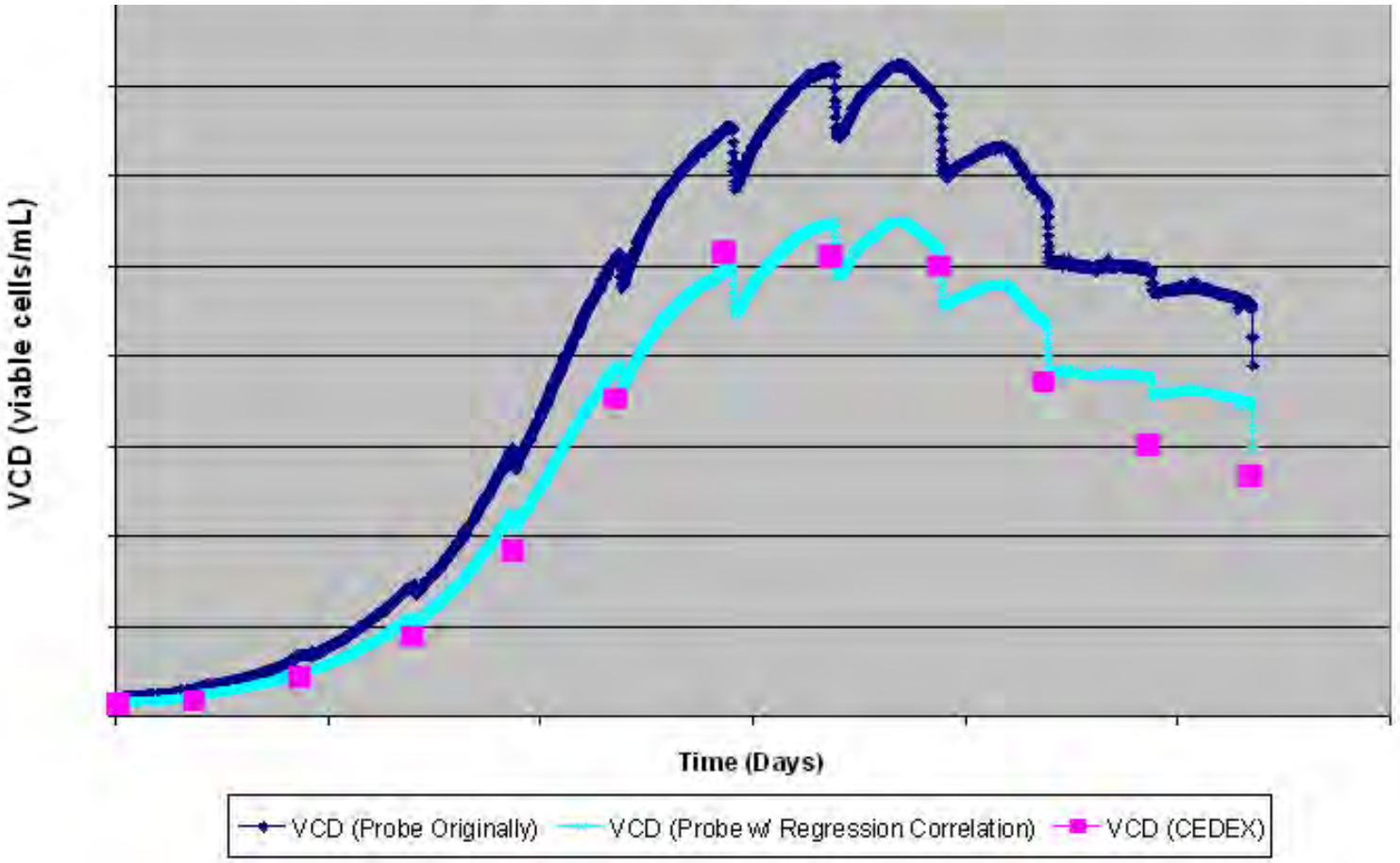
How does Aber detect this capacitance?

- The Aber probe and electronics creates and monitors the electric field
- The probe's
  - Outer electrodes create the field
  - Inner electrodes measure the voltage
- The electric field is in the Radio Frequency spectrum, typically between 50kHz and 20MHz



NB: the field projects approximately 30 – 40 mm

Monitoring a Fed-Batch Culture



- Notice how cells have consumed critical nutrients before feed occurs!

Key points

- Live cells with intact membranes become polarised and thus measured.
- Dead cells with leaky membranes with respect to charge are not measured.
- The Resulting capacitance is directly proportional to the total membrane bound volume of the cells
  - The number of cells, and the size of the cells.

Applications

Where has the technology been utilized?

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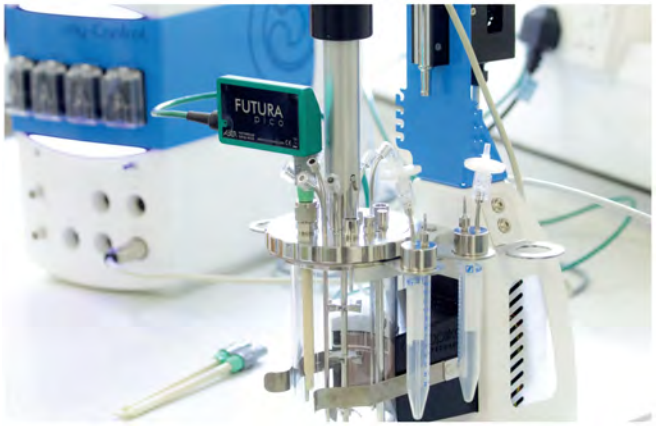
Applications - Platforms



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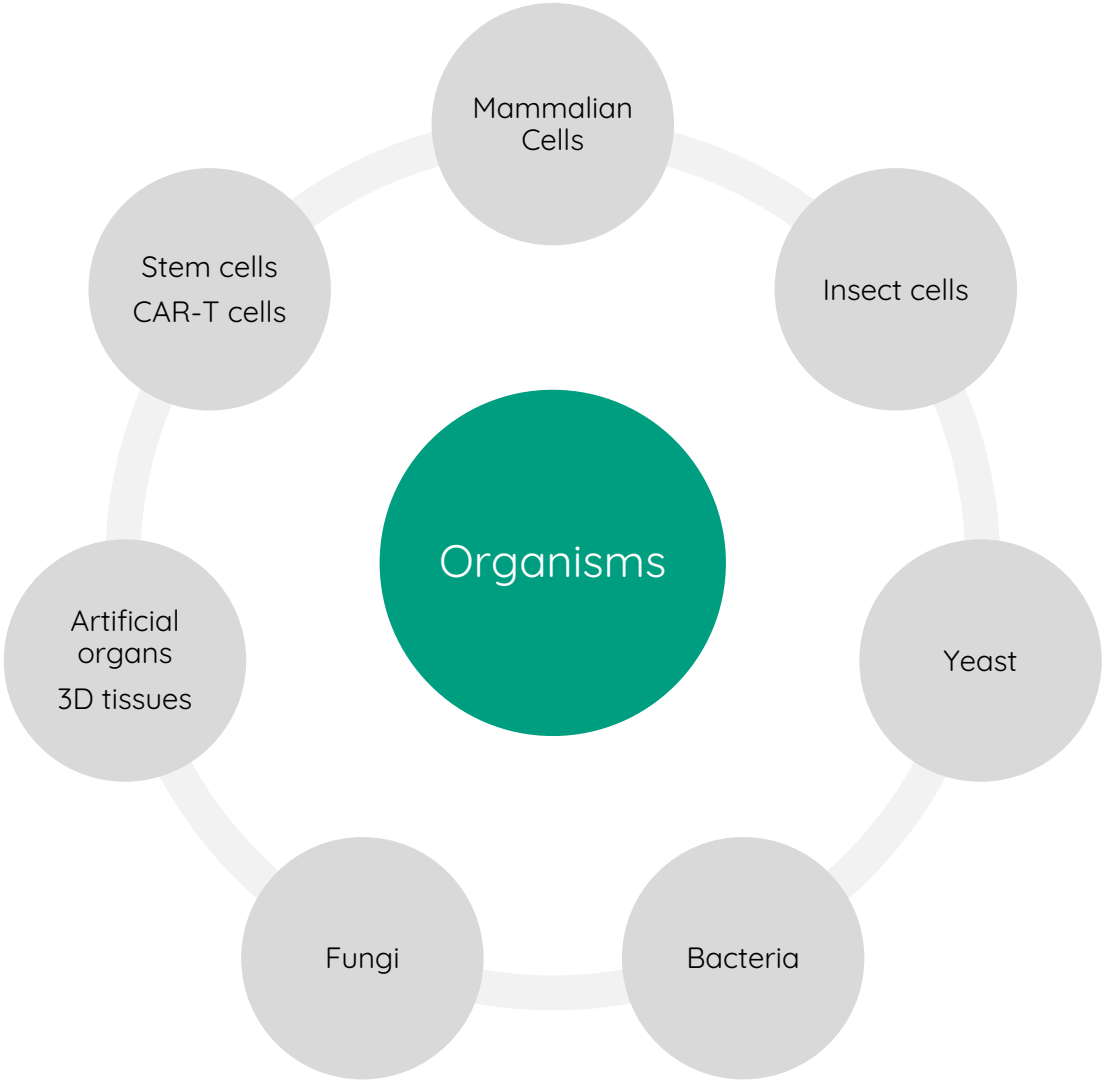
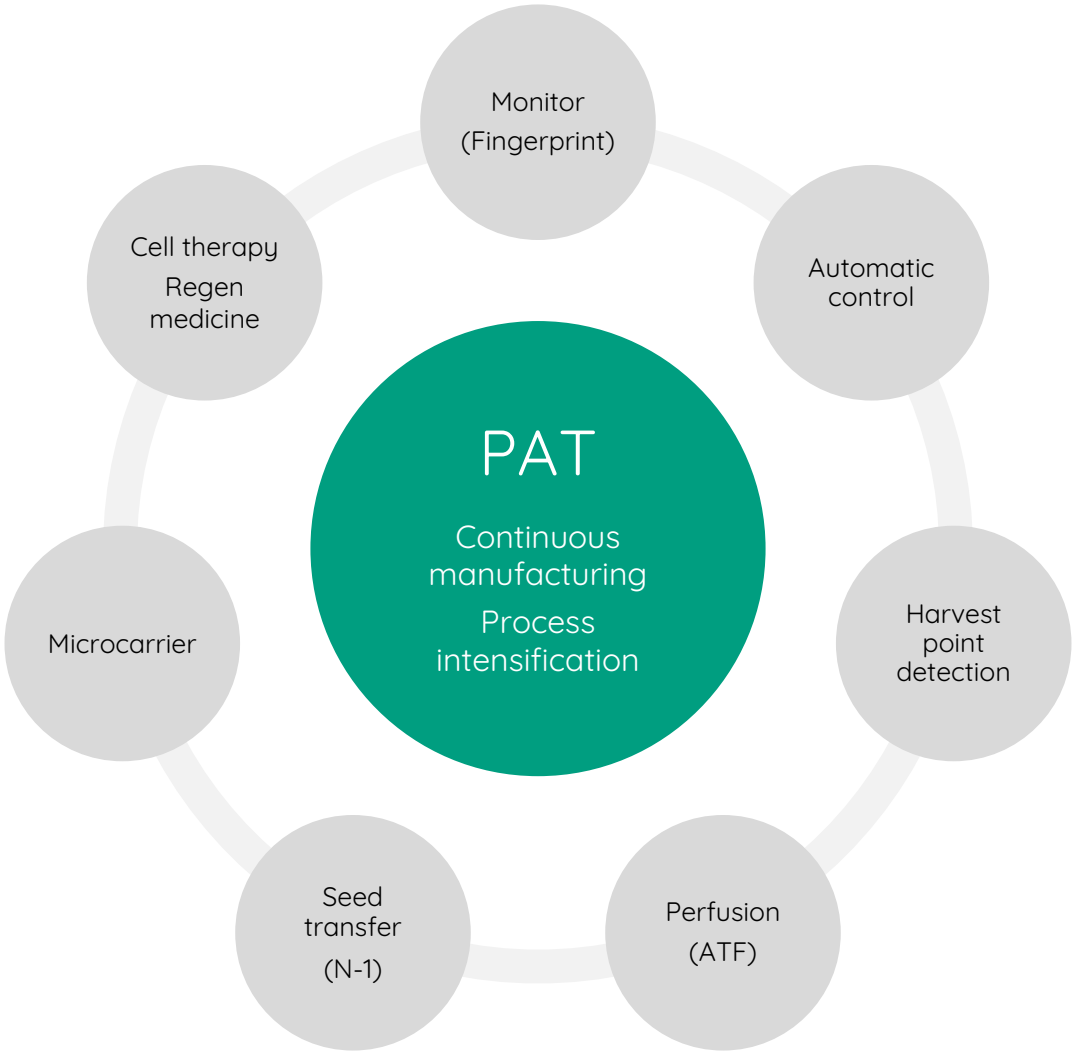
Organisms


Mammalian Cells
Insect cells
Yeast
Bacteria
Fungi
Artificial organs
3D tissues
Stem cells
CAR-T cells
Organisms

Diverse application tree



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Viral vector/ vaccine development or production

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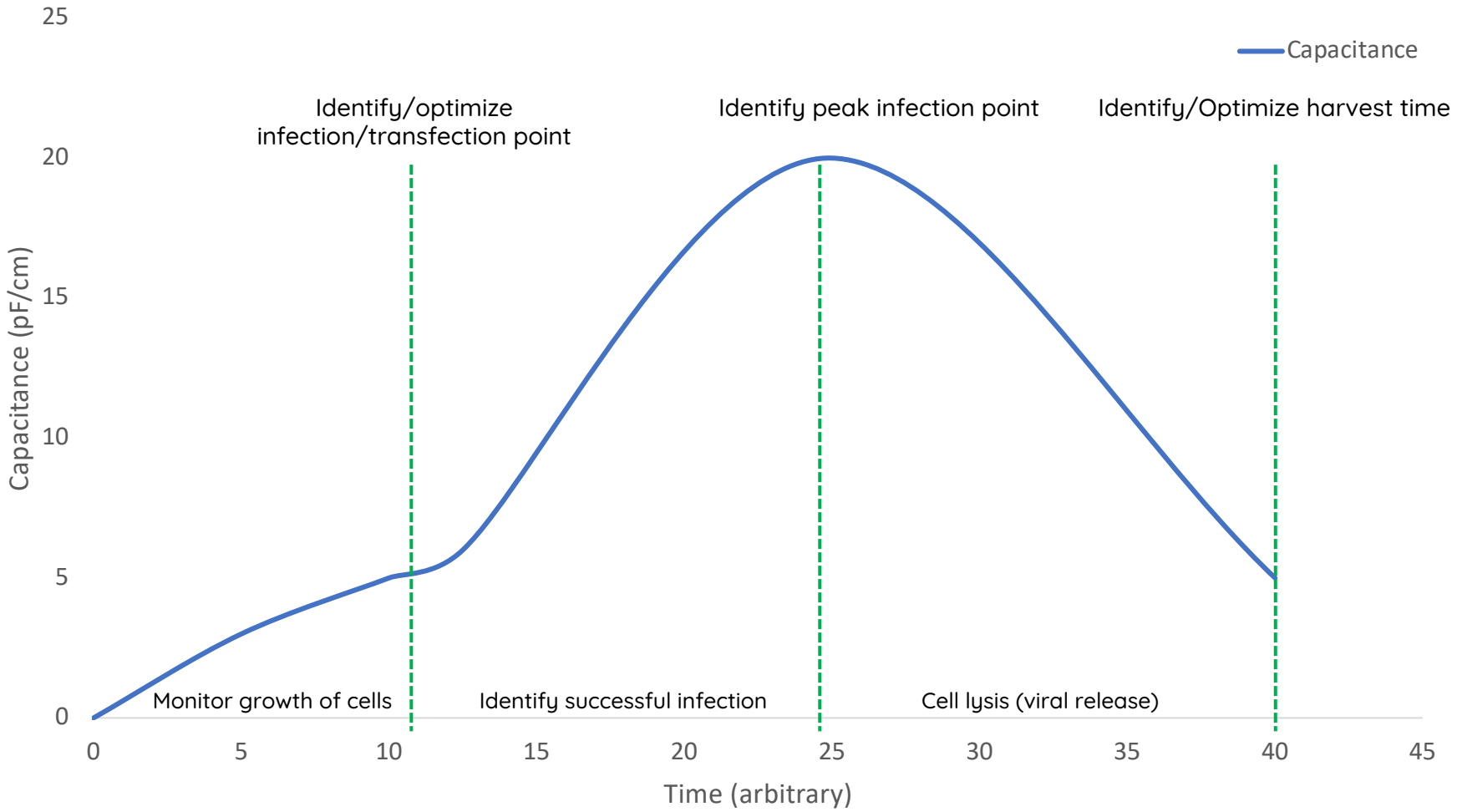
Publications utilizing capacitance measurement

Cells	Viral vector	Publication using capacitance measurement
Sf-9	Baculovirus	Zeiser, A., Elias, C.B., Voyer, R., Jardin, B. and Kamen, A.A., 2000. On-line monitoring of physiological parameters of insect cell cultures during the growth and infection process. Biotechnology progress, 16(5), pp.803-808.
HEK-293	Lentiviral	Ansorge, S., Lanthier, S., Transfiguracion, J., Henry, O. and Kamen, A., 2011. Monitoring lentiviral vector production kinetics using online permittivity measurements. Biochemical engineering journal, 54(1), pp.16-25.
HEK-293	AAV	Lesch, H.P., Heikkilä, K.M., Lipponen, E.M., Valonen, P., Müller, A., Räsänen, E., Tuunanen, T., Hassinen, M.M., Parker, N., Karhinen, M. and Shaw, R., 2015. Process development of adenoviral vector production in fixed bed bioreactor: from bench to commercial scale. Human gene therapy, 26(8), pp.560-571. Kamen, A. and Henry, O., 2004. Development and optimization of an adenovirus production process. The Journal of Gene Medicine: A cross-disciplinary journal for research on the science of gene transfer and its clinical applications, 6(S1), pp.S184-S192. Henry, O., Dormond, E., Perrier, M. and Kamen, A., 2004. Insights into adenoviral vector production kinetics in acoustic filter-based perfusion cultures. Biotechnology and bioengineering, 86(7), pp.765-774.
HEK293 and Sf-9	Reovirus, influenza, lentivirus and baculovirus	Petiot, E., Ansorge, S., Rosa-Calatrava, M. and Kamen, A., 2017. Critical phases of viral production processes monitored by capacitance. Journal of biotechnology, 242, pp.19-29.
HEK293	Influenza and lentivirus	Emma, P. and Kamen, A., 2013. Real-time monitoring of influenza virus production kinetics in HEK293 cell cultures. Biotechnology progress, 29(1), pp.275-284.
SF9	Recombinant adeno associated vectors	Negrete, A., Esteban, G. and Kotin, R.M., 2007. Process optimization of large-scale production of recombinant adeno-associated vectors using dielectric spectroscopy. Applied microbiology and biotechnology, 76(4), pp.761-772.
Proprietary Human Cell Line	Adenovirus	Monica, T.J., Montgomery, T., Ayala, J.L., Schoofs, G.M., Whiteley, E.M., Roth, G., Garbutt, J.J., Harvey, S. and Castillo, F.J., 2000. Monitoring adenovirus infections with on-line and off-line methods. Biotechnology progress, 16(5), pp.866-871.
Vero	Measles virus	Grein, T.A., Loewe, D., Dieken, H., Salzig, D., Weidner, T. and Czermak, P., 2018. High titer oncolytic measles virus production process by integration of dielectric spectroscopy as online monitoring system. Biotechnology and bioengineering, 115(5), pp.1186-1194.

Typical real-time fingerprint - viral vector or vaccine production process



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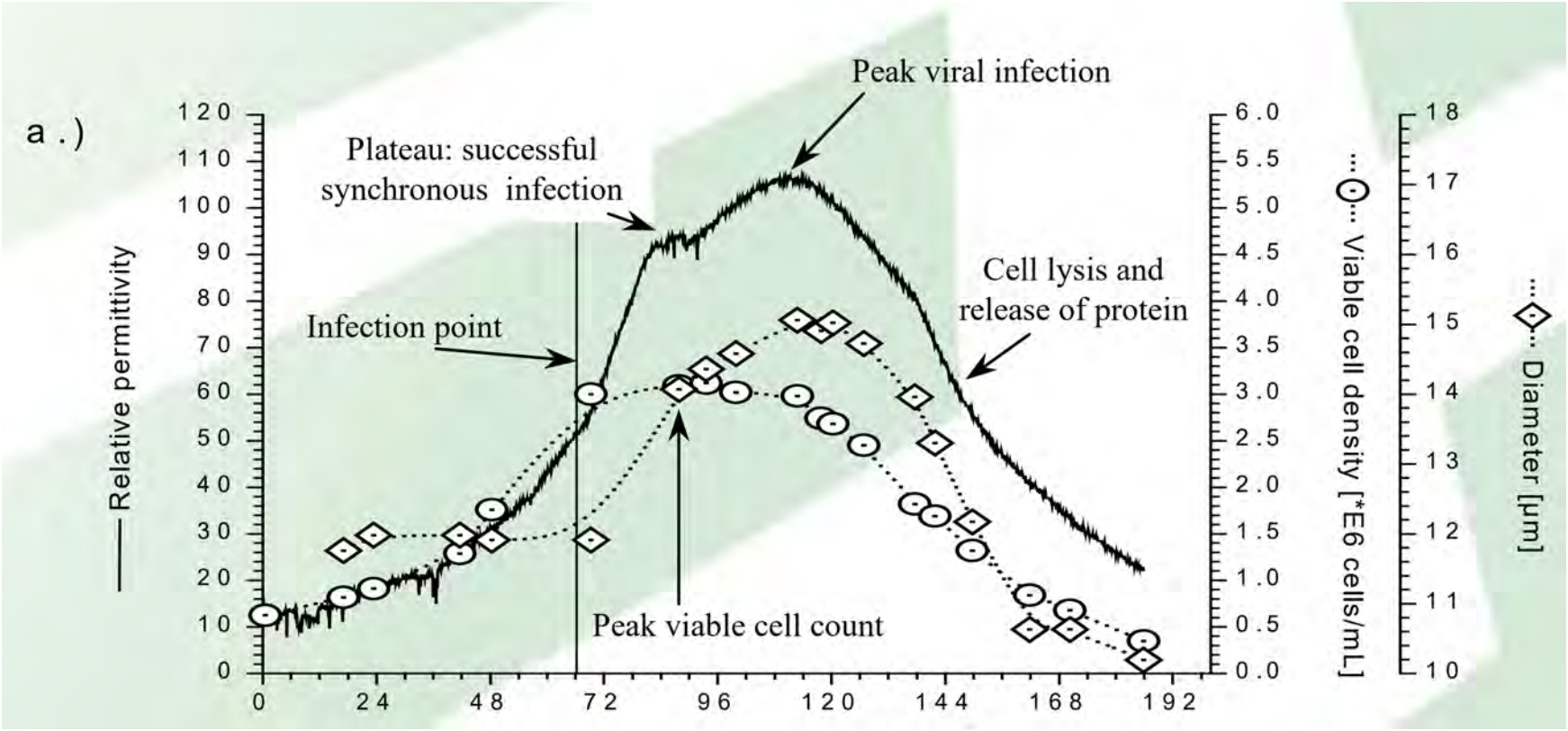
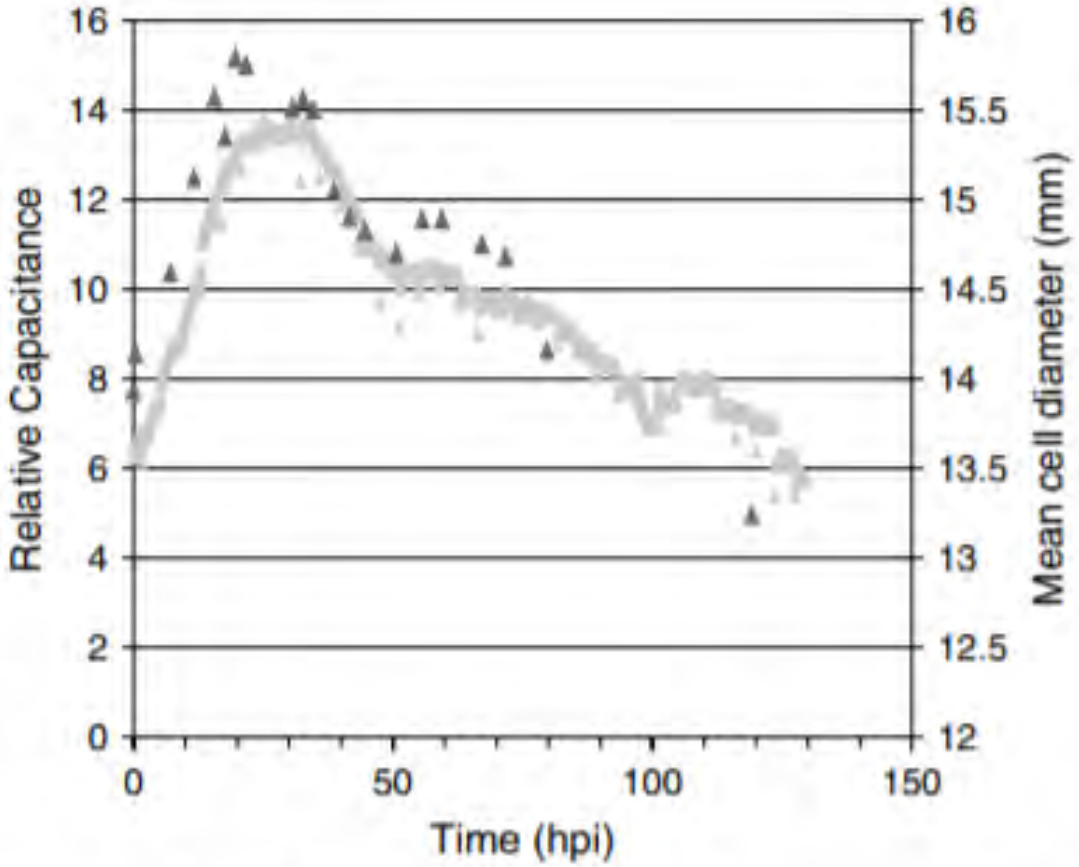
Monitoring Sf-9 cells infected with a virus



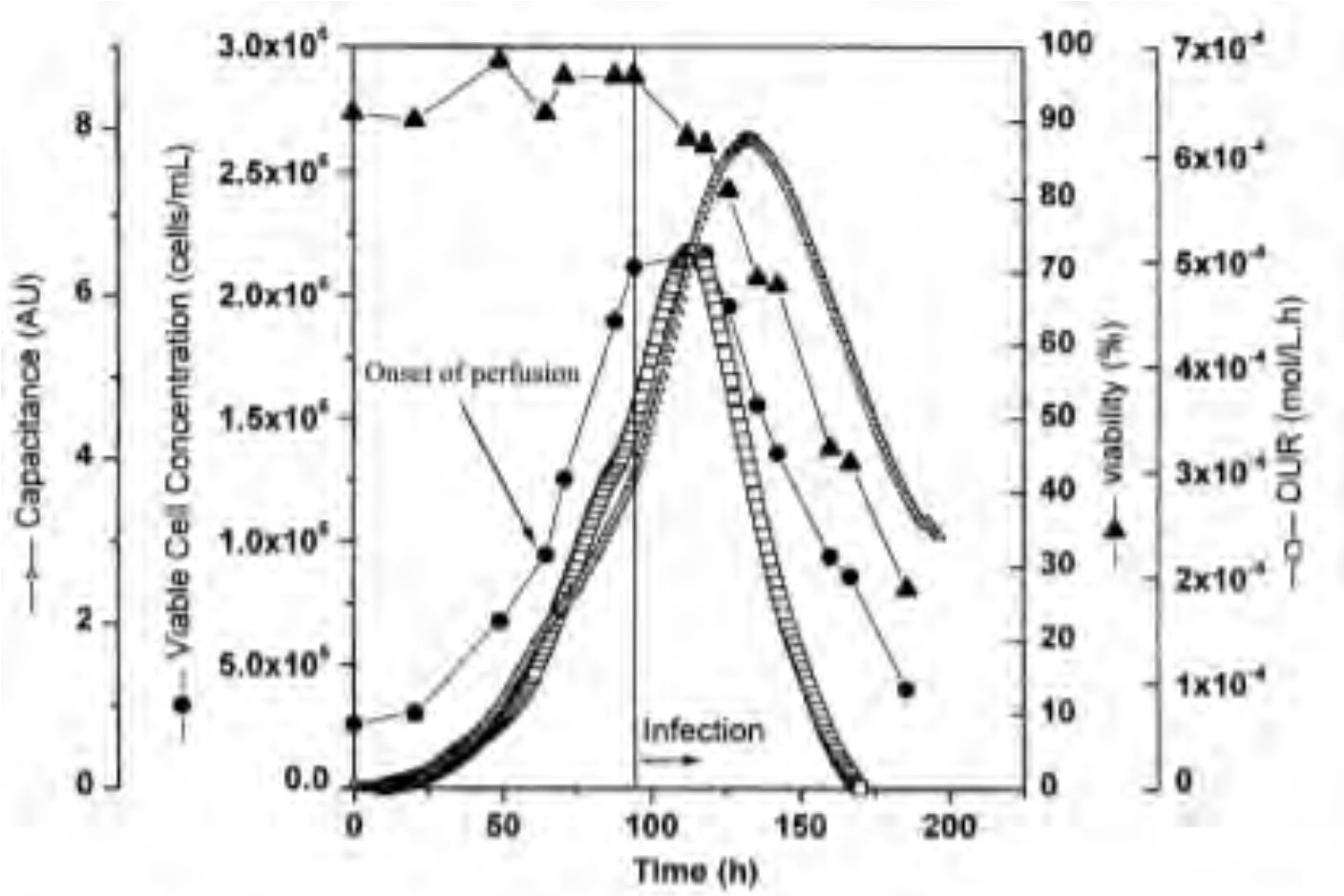
Zeiser, A., Elias, C.B., Voyer, R., Jardin, B. and Kamen, A.A., 2000. On-line monitoring of physiological parameters of insect cell cultures during the growth and infection process. Biotechnology progress, 16(5), pp.803-808.

Tracking adenovirus production process (HEK293)



Kamen, A. and Henry, O., 2004. Development and optimization of an adenovirus production process. *The Journal of Gene Medicine: A cross-disciplinary journal for research on the science of gene transfer and its clinical applications*, *6*(S1), pp.S184-S192.

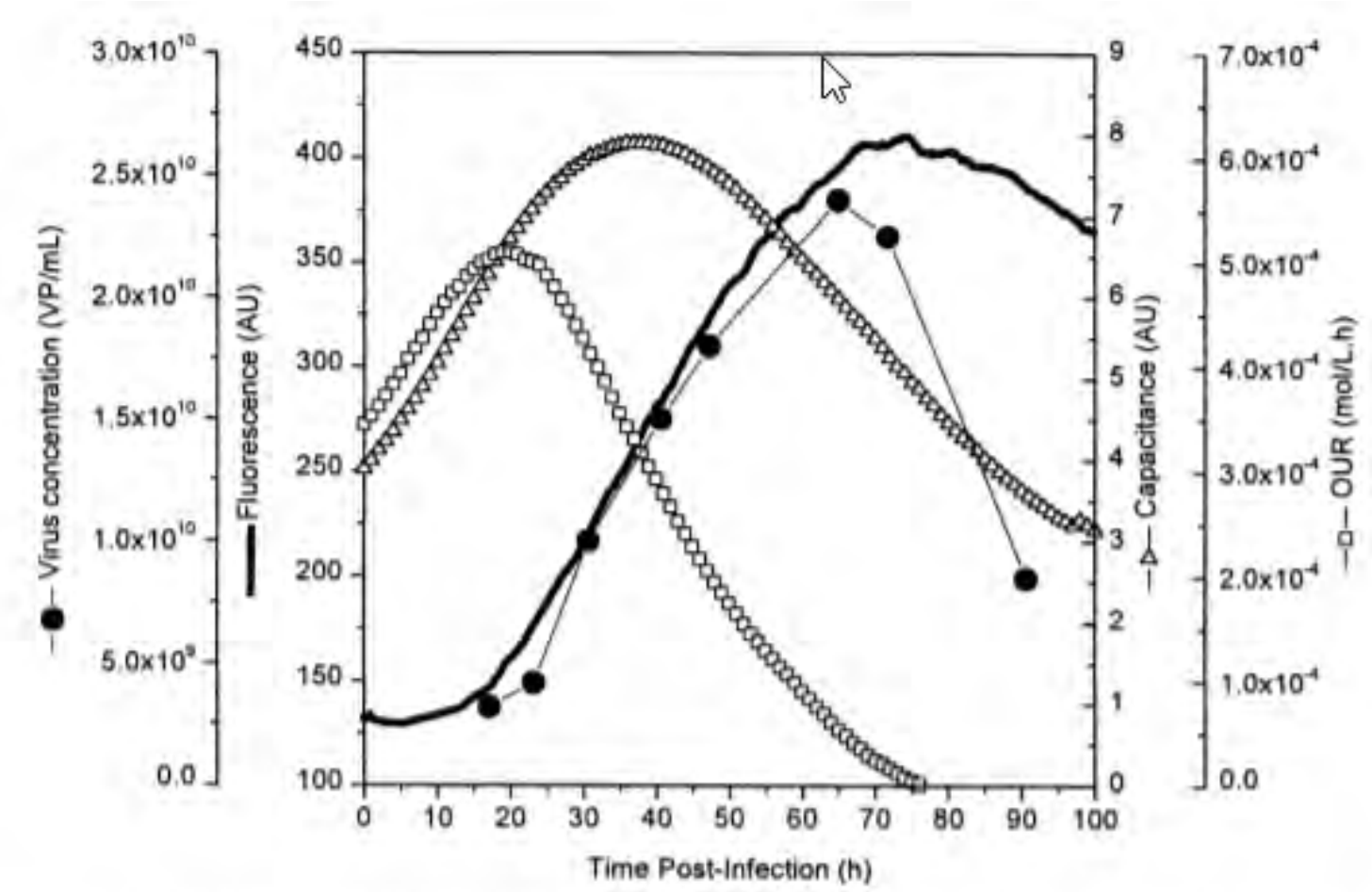
Tracking adenoviral vector production kinetics – HEK293



Viral protein synthesis phase: Mean cell radius of HEK293 – 14 micron to 16 micron – 50% increase in cell volume

Henry, O., Dormond, E., Perrier, M. and Kamen, A., 2004. Insights into adenoviral vector production kinetics in acoustic filter-based perfusion cultures. *Biotechnology and bioengineering*, *86*(7), pp.765-774.

Tracking adenoviral vector production kinetics – HEK293



Cap peaks 18 h after OUR and offline cell concentration peaks. Can be closely linked to virus concentration

Henry, O., Dormond, E., Perrier, M. and Kamen, A., 2004. Insights into adenoviral vector production kinetics in acoustic filter-based perfusion cultures. *Biotechnology and bioengineering*, *86*(7), pp.765-774.

Perfusion
Cell concentration control

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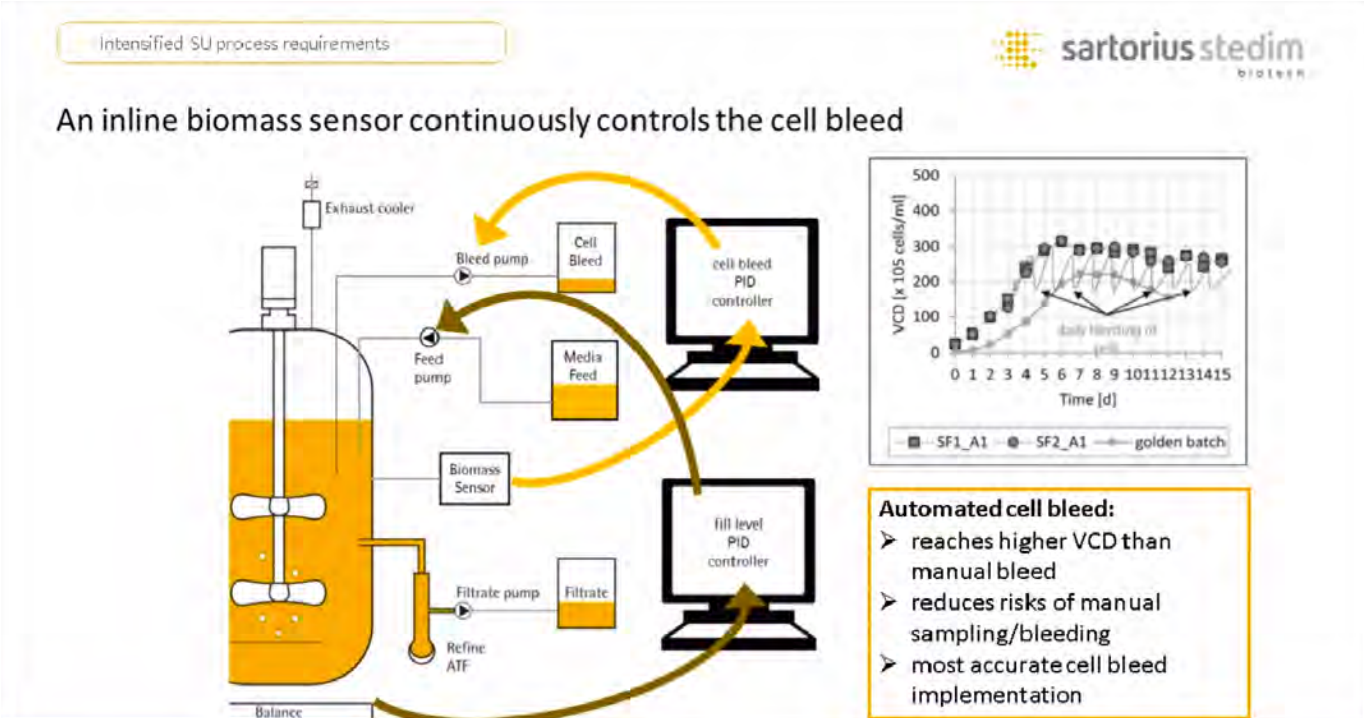
Cell Concentration Control Using Capacitance



Cell bleed: You need:

- Biomass sensor
- PID controller
- Bioreactor scale

Advances in Single-Use Platforms for Commercial Manufacturing, *Bioprocess International*, Dec 2017

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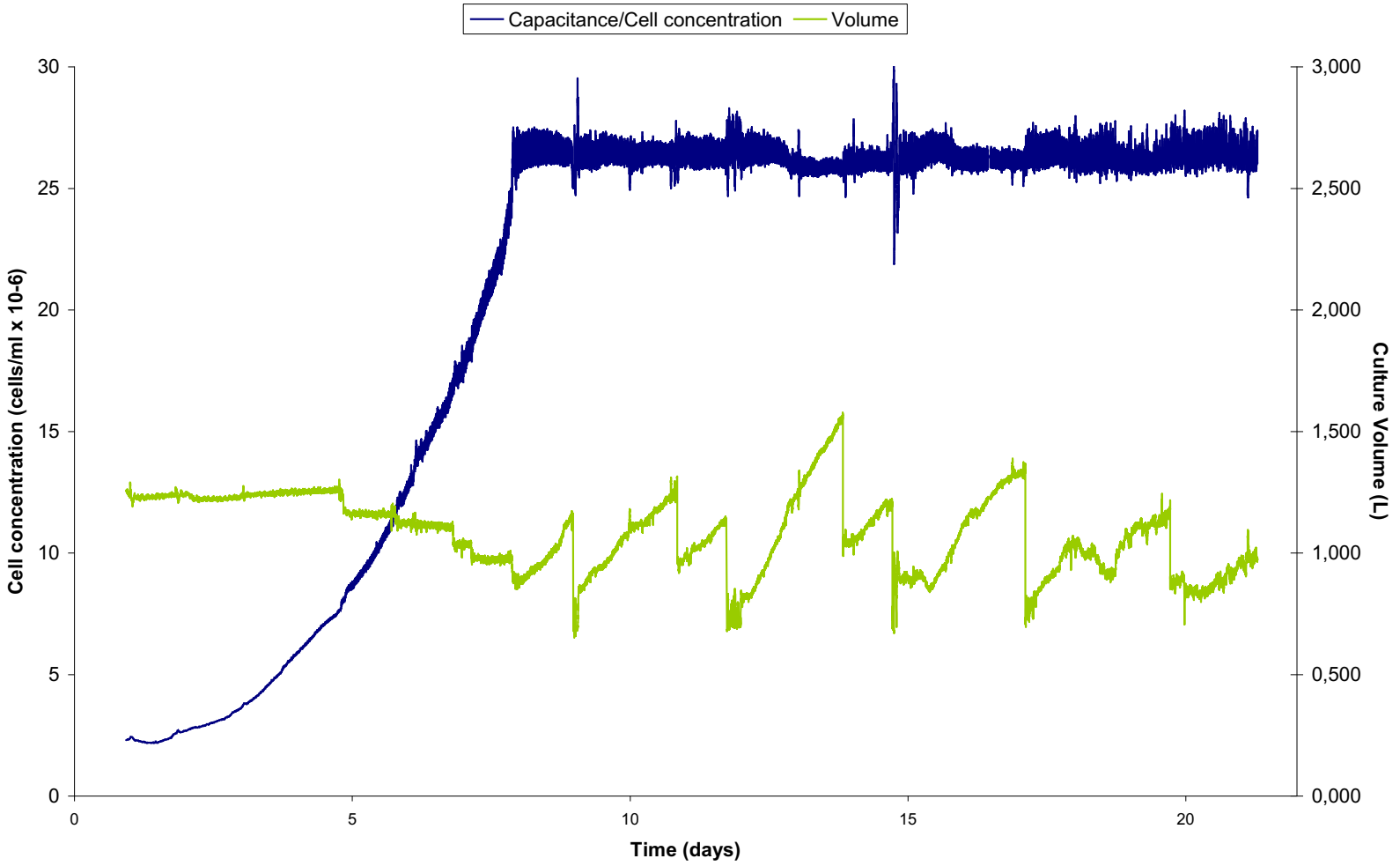
Cell concentration control using Aber



Sergeant D., Moser M., Carvell JP., Measurement and control of viable cell density in a mammalian cell bioprocessing facility: Validation of the software (2007), European Society for Animal Cell Technology

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Scale up – key performance indicator

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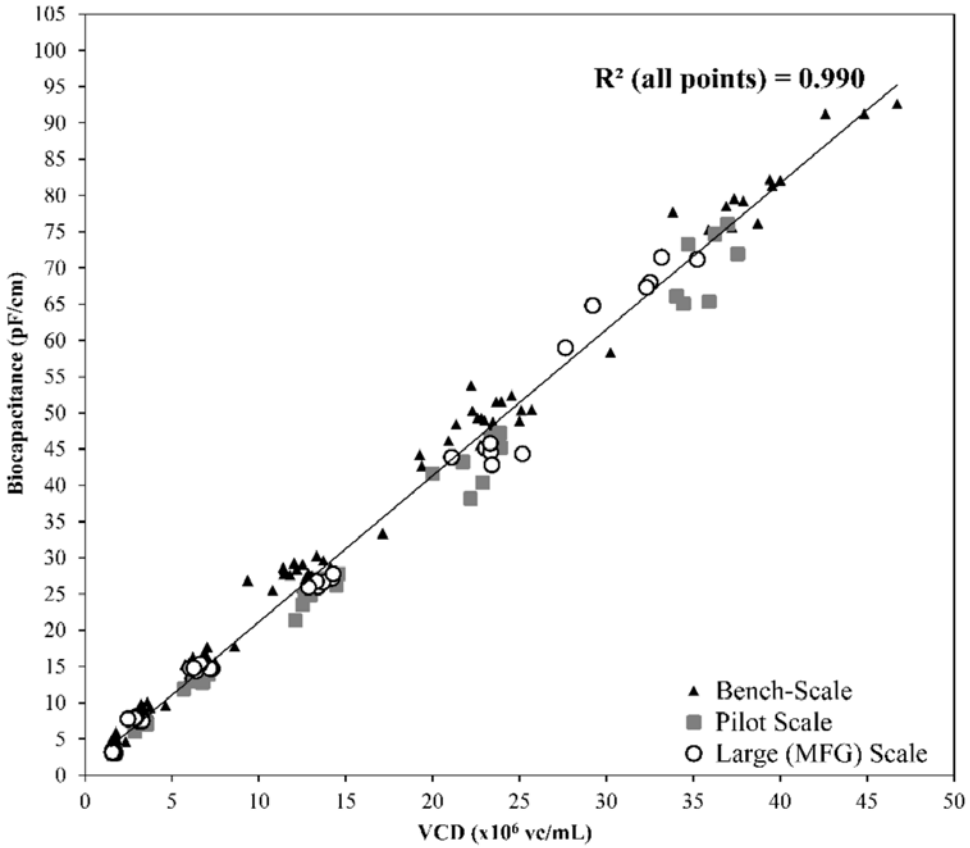
Scalability evaluation

Scalability determined over different scales:

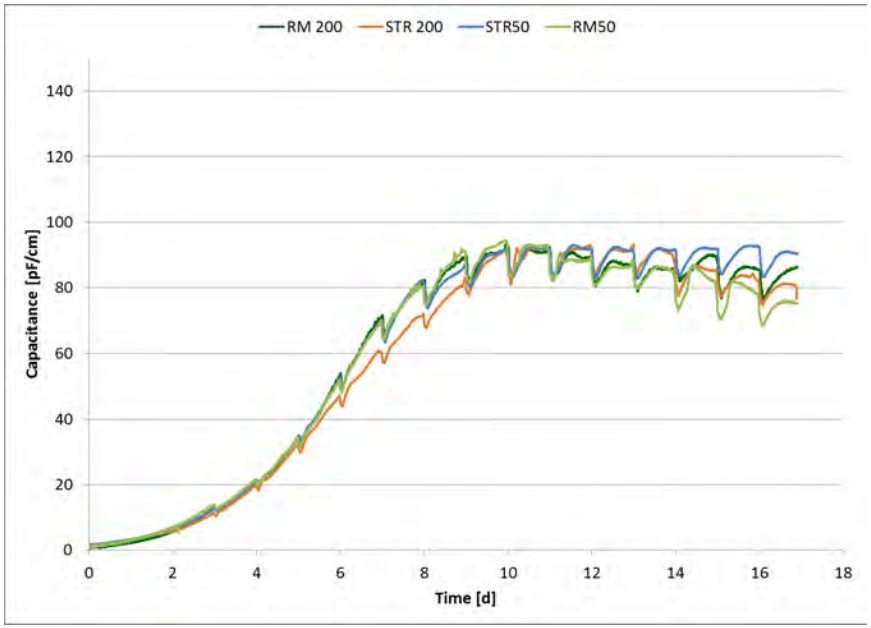
5L (bench scale), 200 & 315 L (pilot scale) and 15000 L (manufacturing scale)

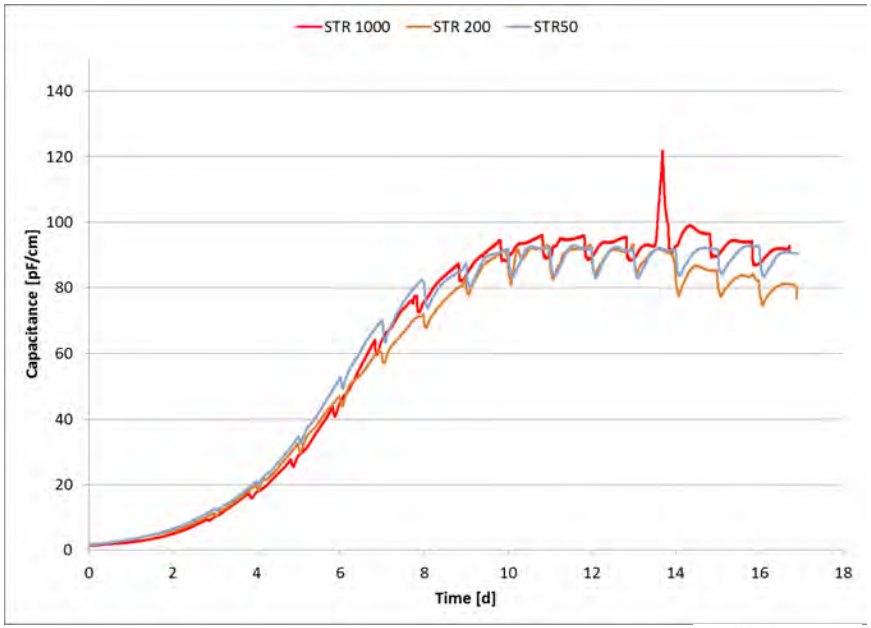


Moore, B. *et al.*, 2019; *Case study: The characterization and implementation of dielectric spectroscopy (biocapacitance) for process control in a commercial GMP CHO manufacturing process*, **Biotech Prog**

Scale up (single use systems)

- Cultivations in RM 50 (2x), RM 200, STR 50 (2x), STR (2x) and STR 1000 performed
- Scale Up was successful
  - ✓ Good agreement n capacitance trend Flexsafe® RM to STR and throughout scales





Measuring cells on microcarriers

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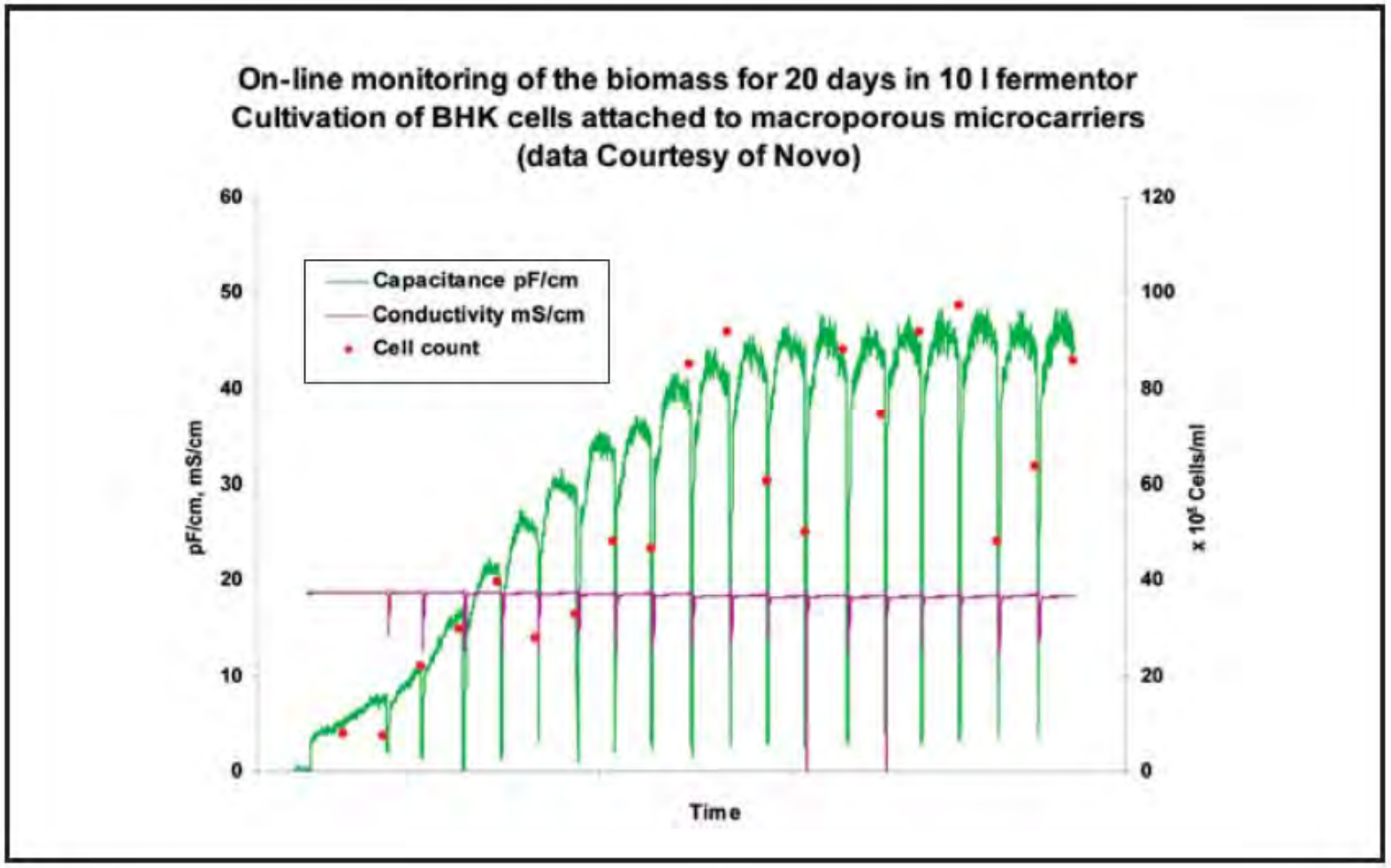
Monitoring Microcarrier Culture Using Aber



Data courtesy of Novo (Denmark)

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Monitoring Microcarrier Culture Using Aber

Cell adhesion

Both conductivity and capacitance were used to determine the adhesion time. Typically, it was seen that once the Vero cells were fully spread, as determined by microscopy, the conductivity remained constant whereas the capacitance correlated well with the offline cell concentration. Even with constant experimental parameters, the time needed for the Vero cells to adhere to the microcarrier varied from 3.8 to 7.3 h post inoculation, thus making an online real time tool to determine adhesion time even more useful.

Grein, T.A., Loewe, D., Dieken, H., Salzig, D., Weidner, T. and Czermak, P., 2018. High titer oncolytic measles virus production process by integration of dielectric spectroscopy as online monitoring system. Biotechnology and bioengineering, 115(5), pp.1186-1194.

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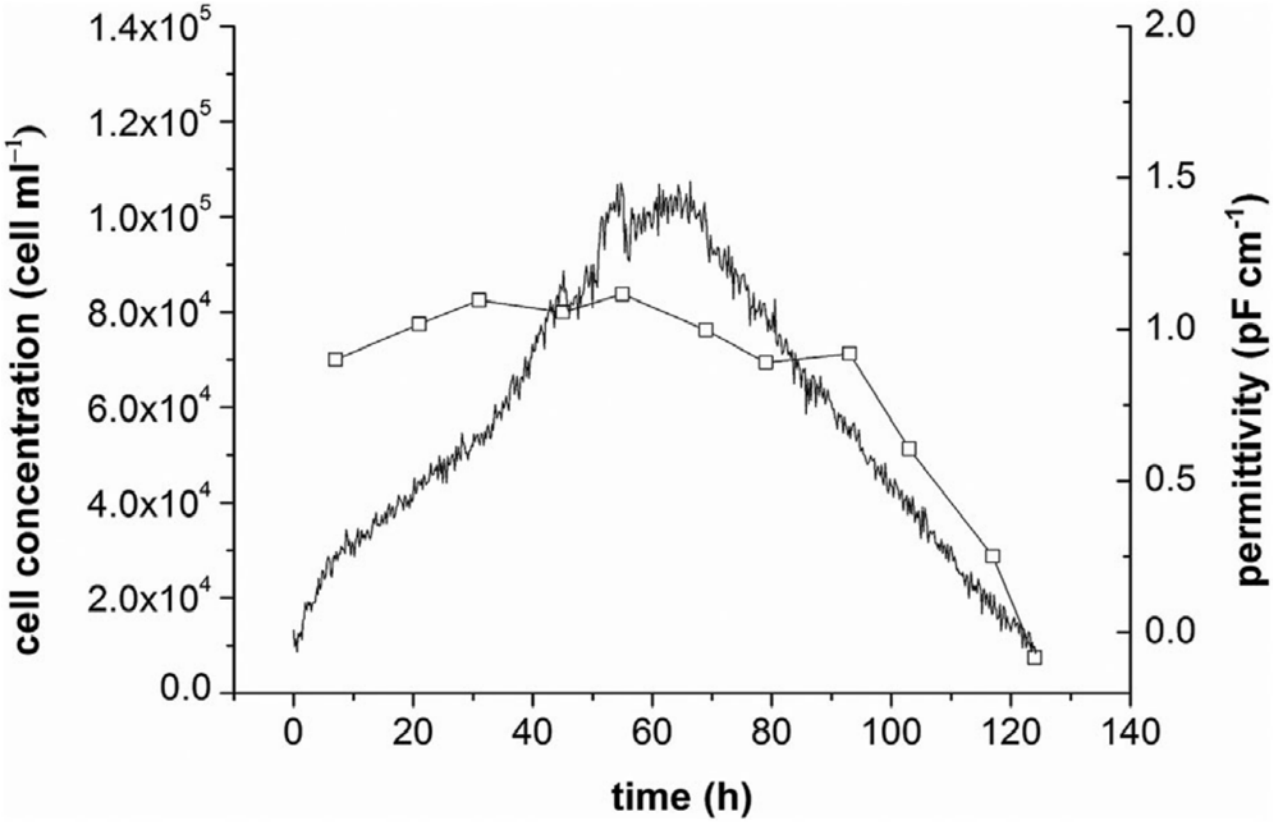
Quantification of Vero cells during Measles virus infection using a Cytodex 1 microcarriers



Grein, T.A., Loewe, D., Dieken, H., Salzig, D., Weidner, T. and Czermak, P., 2018. High titer oncolytic measles virus production process by integration of dielectric spectroscopy as online monitoring system. Biotechnology and bioengineering, 115(5), pp.1186-1194.

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Benefits and Summary

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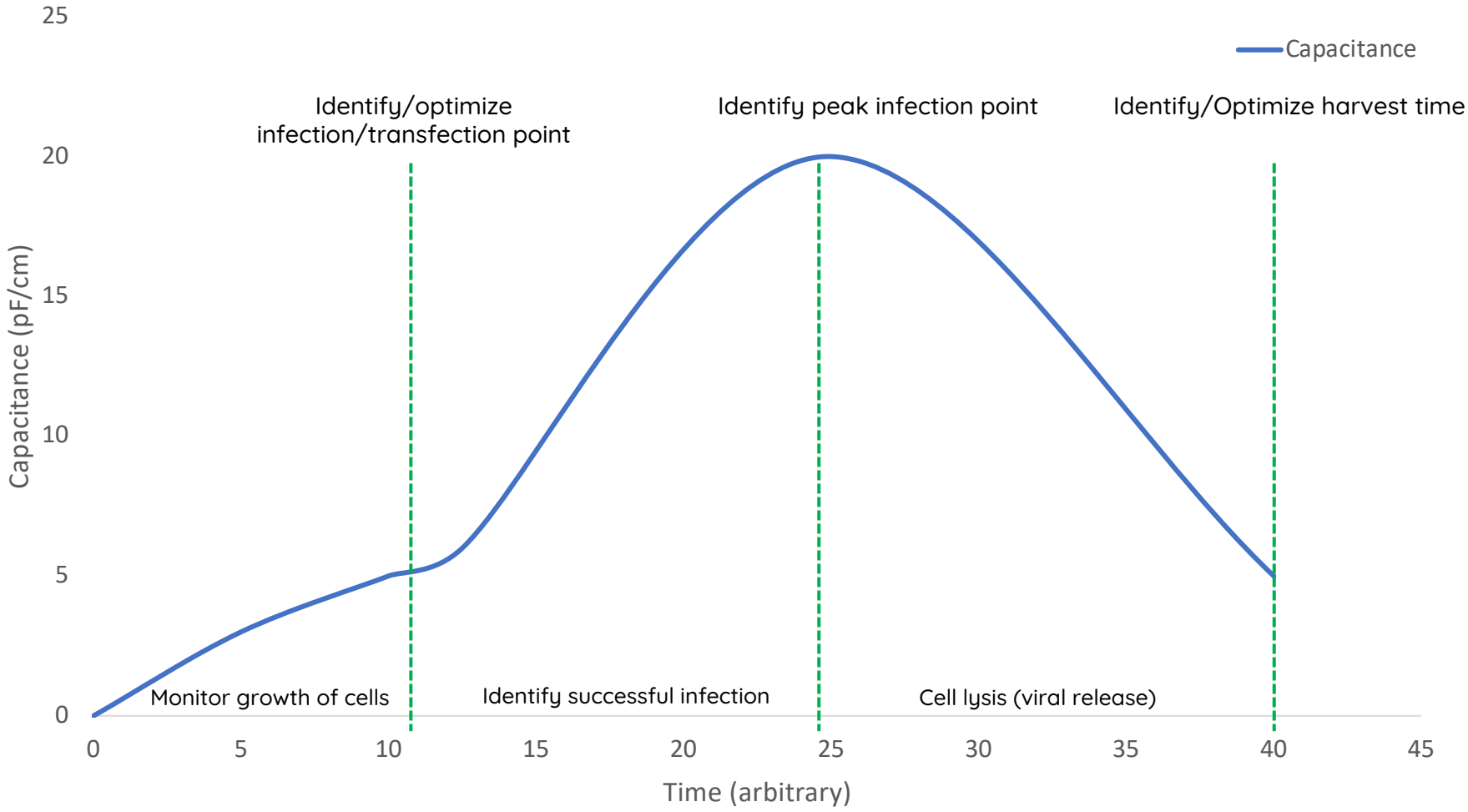
Typical real-time fingerprint - viral vector or vaccine production process



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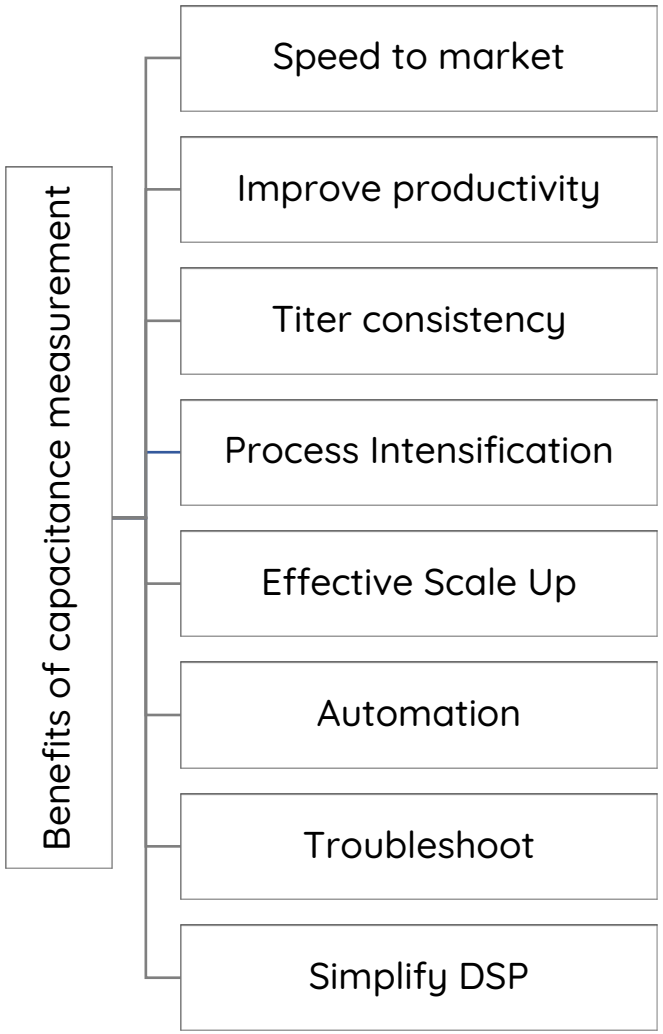
Benefits



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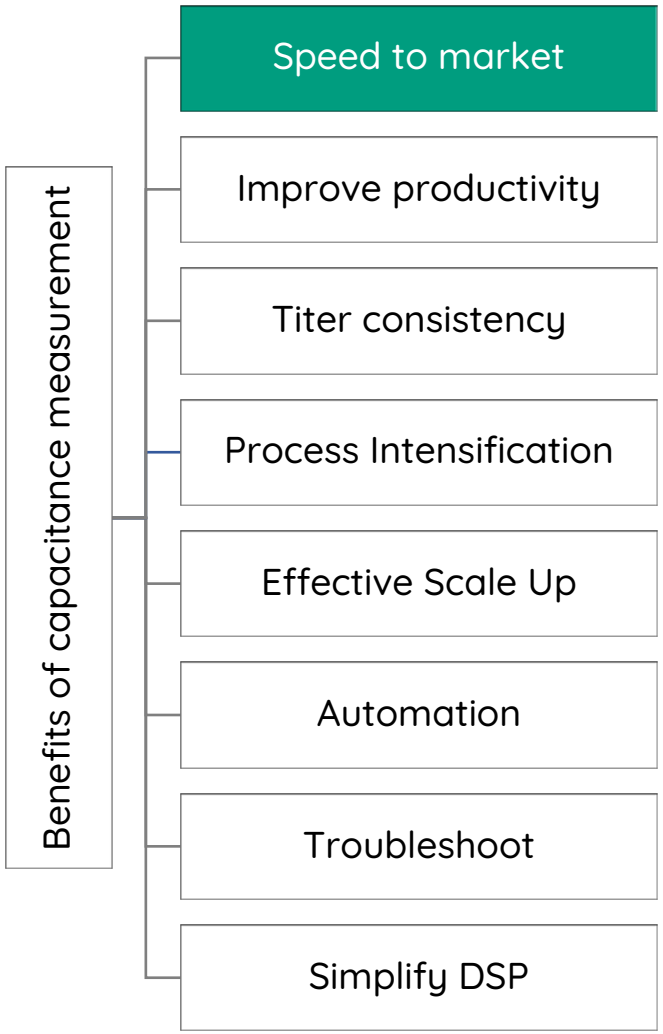

Speed to market

- Faster & detailed information
- Quicker development

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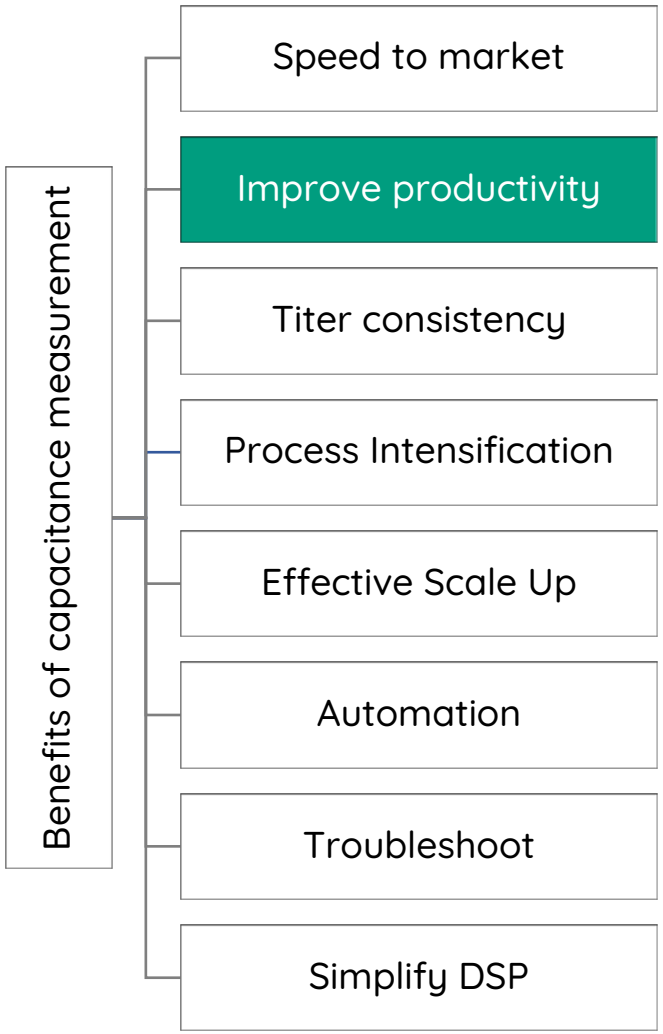

Improve productivity

- Harvest time optimization
- Viral titer closely linked to max capacitance
- Grein *et. al.* (2017): Capacitance based method increased max virus concentration by more than 3 orders of magnitude
- Can produce one dose/patient in 1 x 500 ml bioreactor as opposed to 20 L bioreactor

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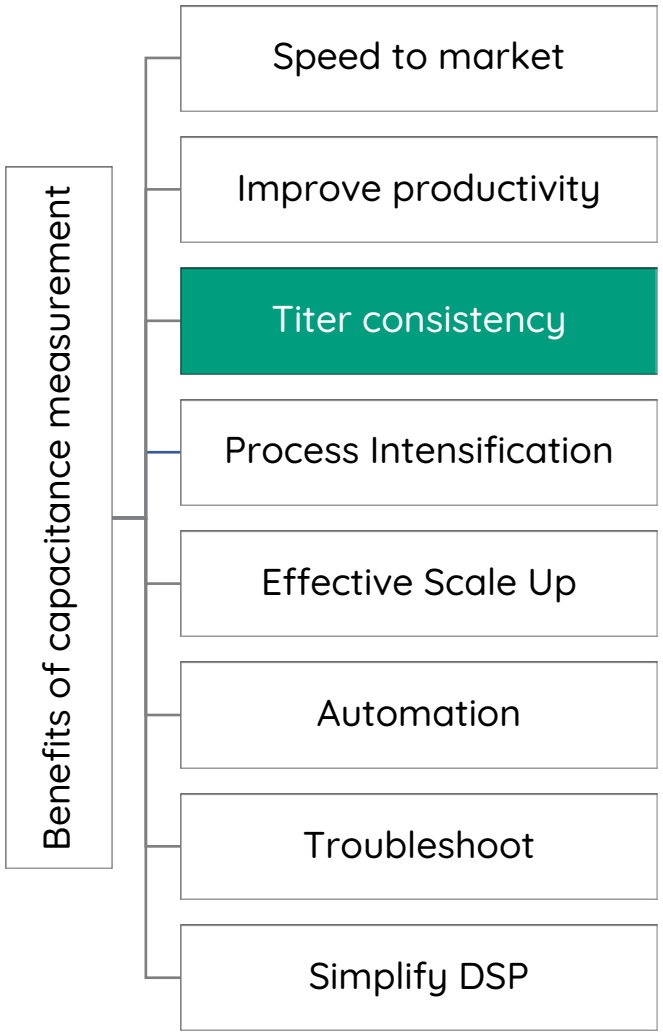

Titer consistency

- Optimal time of harvest can drift significantly in identical bioreactors and similarly run processes.
- Real time capacitance-based control can allow for consistent virus titer to be obtained, despite process changes.

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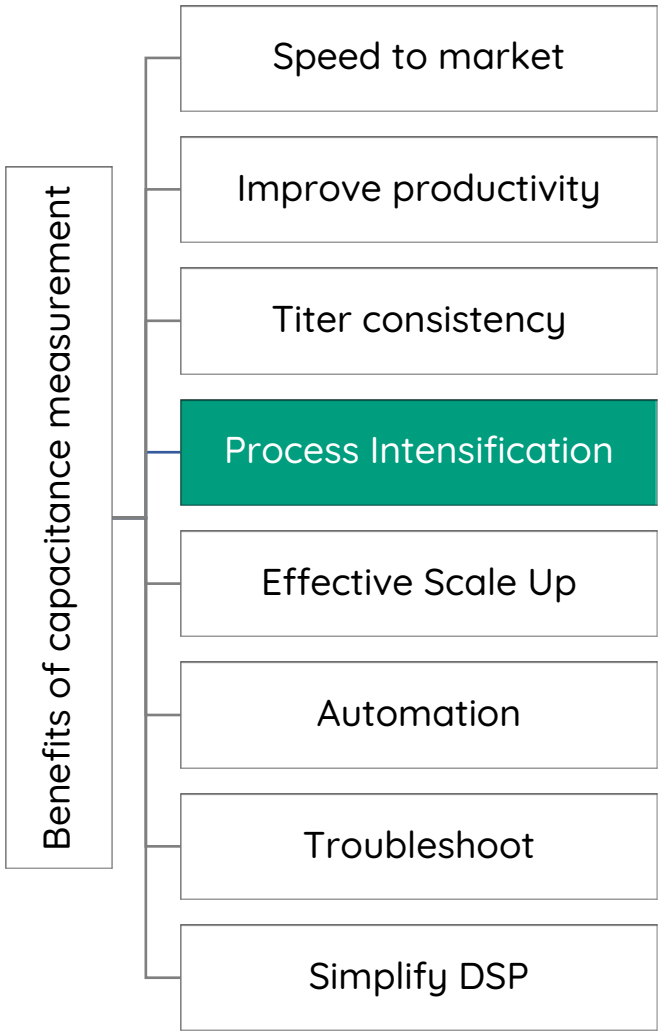

Process Intensification

- Continuous manufacturing
- Automated perfusion process

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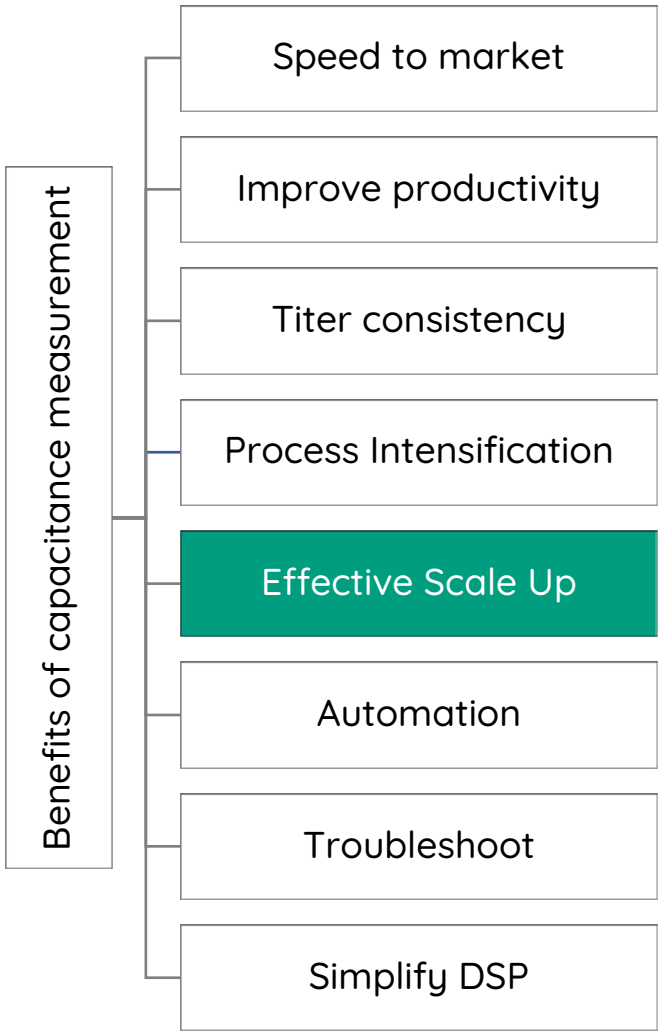

Effective Scale Up

- Capacitance can be used across different scales
- Can be used as key performance indicator to determine successful scale up

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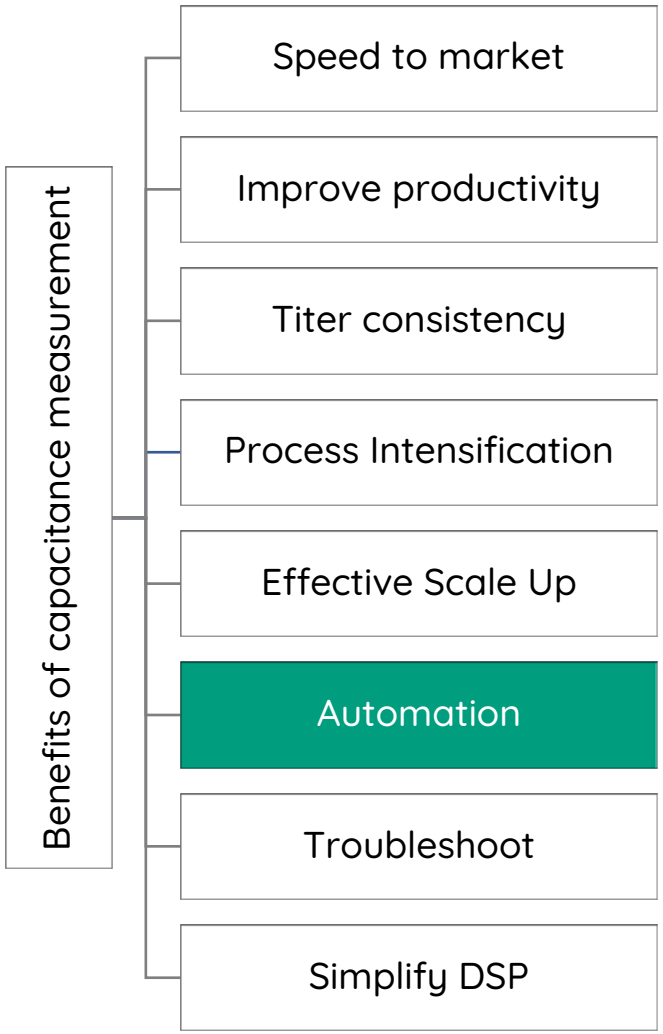

Automation

- Automate
  - ✓ Seed Transfer
  - ✓ Infection
  - ✓ Harvest

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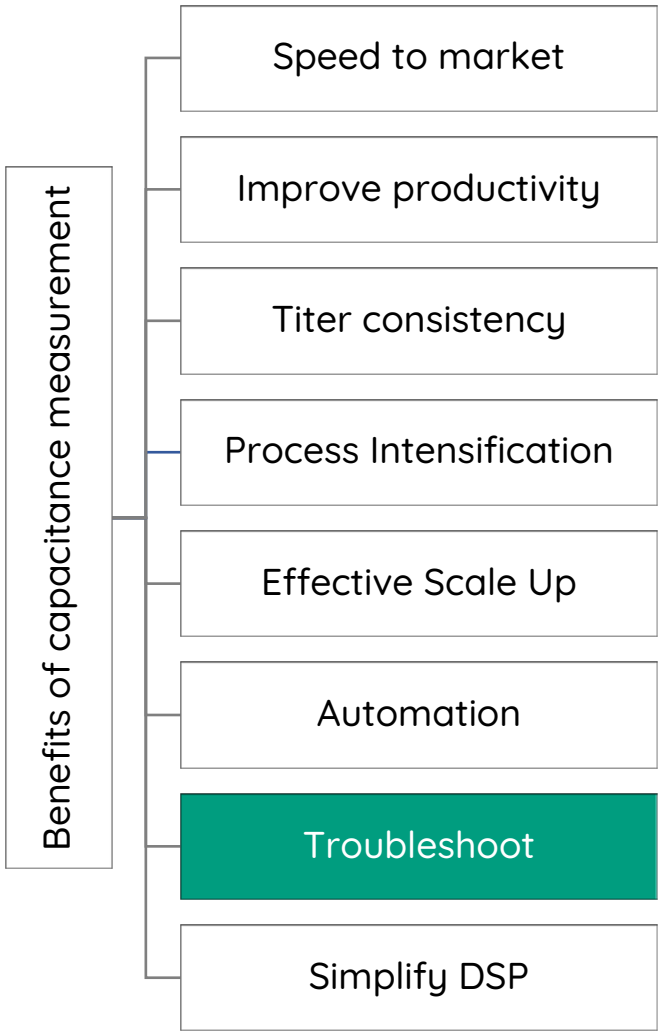
Benefits



Troubleshoot

- Real time, robust and detailed fingerprint
- Compare with historical trend
- Troubleshoot much quicker to save or abandon the process in time
- Catch contamination sooner

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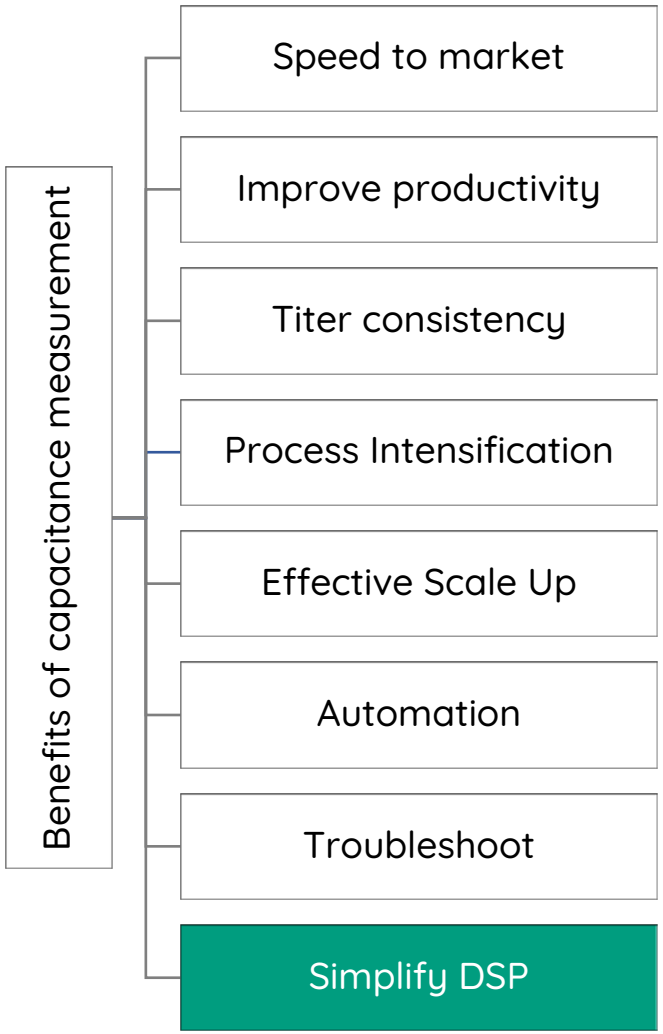
Benefits



Simplify DSP

- DSP closely linked to time of harvest
- If harvest time is optimized, purification can become less complicated

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Thank you for listening

Questions?