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Aditya Bhat

John Poppleton

[1]Aber Instruments Ltd

Using capacitance for measuring cell density in-line is not a new concept. The technology was first developed over 30 years ago (1) for determination of biomass in suspension and was then commercialized by Aber Instruments (Aber). In fact, since the 1990s capacitance measurement has been used extensively in biopharmaceutical companies and contract development and manufacturing organizations (CDMOs) world-wide. It is now a standard technology for monitoring and automating cell cultures in research and process development laboratories through to manufacturing scale facilities for production of biologics and vaccines.

What does capacitance measure and how is that related to cell density and health?

A cell in suspension culture has an outer membrane consisting of a bilayer that is impermeable to ions and is non-conducting. The medium is a complex of many components that includes ions in suspension and when in an electric field, cells become polarized (Figure 1). With a live cell, the cell membrane is intact, and the cell will act as a capacitor to store electrical energy.

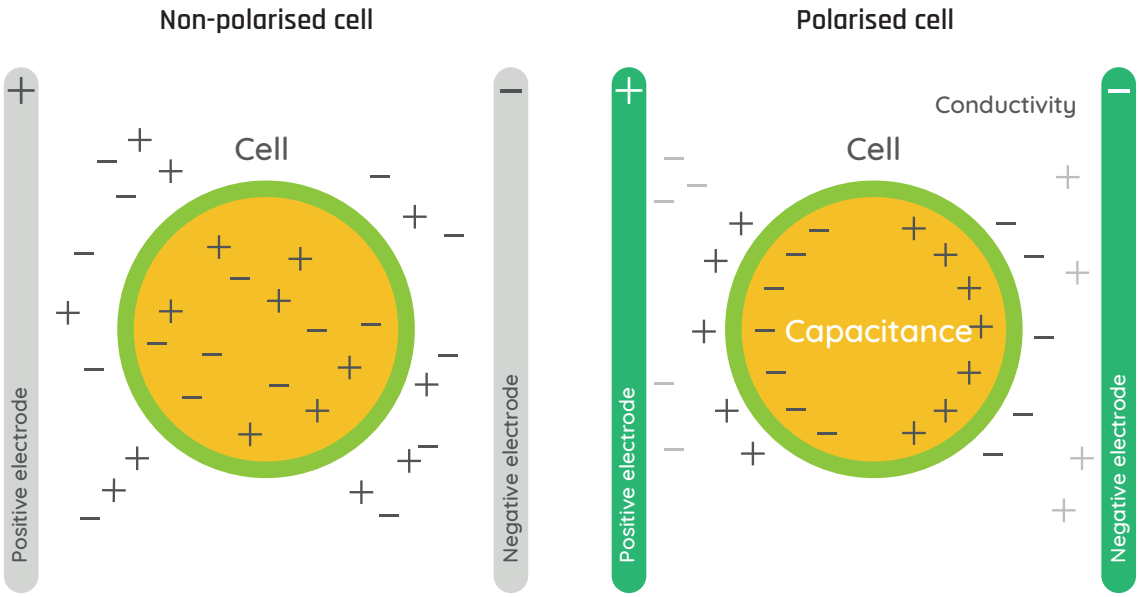

Figure 1: Live cells acting as capacitors within an electrical field.

As cell number and volume increases, so the number of polarized cell membranes increases which means capacitance increases. Thus, the capacitance of a cell suspension at one or more frequencies, is directly proportional to the total membrane bound volume of the cells. Since dead cells have leaky cell membranes and solid particles and gas bubbles in the media do not have cell membranes, they cannot store electrical charge and do not contribute to capacitance in a cell suspension. Therefore, capacitance measurement reflects cell density and cell size, (the viable biovolume), as well as the electrical properties of a cell membrane.

So how does capacitance compare with at-line and off-line measurements and is there a case for using raw capacitance data to monitor cell density and health in place of these methods?

At-line and off-line measurements of cell density

In bioprocessing, scientists use either at-line or off-line methods of measuring cell density using viable cell concentration (VCC) as a key performance indicator (KPI). The commonest methods are listed in Table 1.

Table 1: At-line and off-line methods of monitoring VCC

Method	Example of technology used for analysis
Trypan Blue Dye Exclusion – Analyses cells by color - live cells are colorless and dead cell are stained blue	Cedex Bio® Analyzer (Roche), Vi-CELL BLU Cell Viability Analyzer (Beckman-Coulter Life Sciences)
Particle Detection – Measures particles by electrical zone sensing. Does not differentiate between live or dead cells.	Multisizer 4e Coulter Counter (Beckman-Coulter Life Sciences)
Flow Cytometry - Analyses cells by fluorescence. Live cells emit low wavelength red fluorescence, dead cells emit high wavelength red fluorescence.	Guava® easyCyte ™ (Merck)

The Trypan Blue Dye Exclusion method has long been considered the gold standard in bioprocessing to measure VCC (2). This method relies on cells with extensively damaged membranes that are incapable of growth or other functions associated with normal metabolism taking up dye and turning blue. However, there is currently some debate by process experts stating that VCC values obtained by this method overestimate VCC (2).

Measurement of cells by Particle Detection measures particles electronically by electrical zone sensing as they pass through an orifice. This method does not differentiate between live and dead cells. As well as not being able to differentiate between live and dead cells, this method can also show some data distortion if the sample has significant cell debris or cell aggregates so is less well used in bioprocessing.

The Flow Cytometry method relies on using two fluorescent binding dyes. One dye can bind to all live cells which emit low wavelength red fluorescence. The second dye only binds to DNA in dead cells and emits high wavelength red fluorescence. Like the Dye Exclusion method, this again depends on the integrity of the cell membrane as the second dye will only dye DNA after it enters cells with damaged membranes. There is also an argument by bioprocess experts that this method may also overestimate VCC (2).

Challenges of off-line and at-line measurement

At-line and off-line measurements are not always optimum for monitoring cell health and density. One reason is that they involve sampling which reduces the volume in the bioreactor and means that samples can only be taken at 12–24-hour intervals. These methods therefore cannot produce a detailed fingerprint of the process or help to provide timely data for feedback to control the process. These measurement techniques are also not ideal as automated liquid handlers or scientists physically remove a sample from the bioreactor. This not only takes time and effort but can also introduce potential errors in measurement if sampling is done manually by different operators or if there is variation due to calibration issues between different or the same equipment used for off-line analyses. Additionally, removing samples from a bioreactor can provide a contamination risk as it involves entering the bioreactor and cell culture for sampling.

Advantages of monitoring cell density and health using capacitance

The main benefit of using capacitance measurement to monitor cell density and health is that it is an in-line Process Analytical Technology (PAT) technique. The FUTURA probes from Aber, for example, can produce a signal every few seconds. Additionally, it is regarded by bioprocess experts as the most accurate and consistent in-line method to monitor live-cell density in mammalian cell culture (2,3). Capacitance is in-line and sample-less which allows scientists to produce a detailed fingerprint of critical process parameters (CPPs) and key performance indicators (KPIs) that can be used for automated feedback control without any cell culture sampling.

One advantage of developing a capacitance fingerprint for the 'golden' batch is that it can be compared with a capacitance trend for an ongoing run to more rapidly determine when CPPs and KPIs begin to behave outside of acceptable process specification. This can trigger a manual or automated response and can help to troubleshoot a production run to correct process deviations and save an expensive production batch while the run is in progress.

Another benefit is that the technology to measure capacitance is scalable (4) and is available in a range of reusable sizes and types that can be used from smaller glass bioreactors through to larger stainless-steel vessels. These sensors are available for use in pilot and manufacturing scale stainless-steel bioreactors from all major life science suppliers. Additionally, single-use sensors known as the BioPAT® Viamass (Sartorius) and Futura neotf (Thermo Scientific) which are fully integrated into single-use bioreactors up to 2000 L (Figure 2) have been available via collaboration with Aber since 2013 (5) and 2021 (6) respectively.

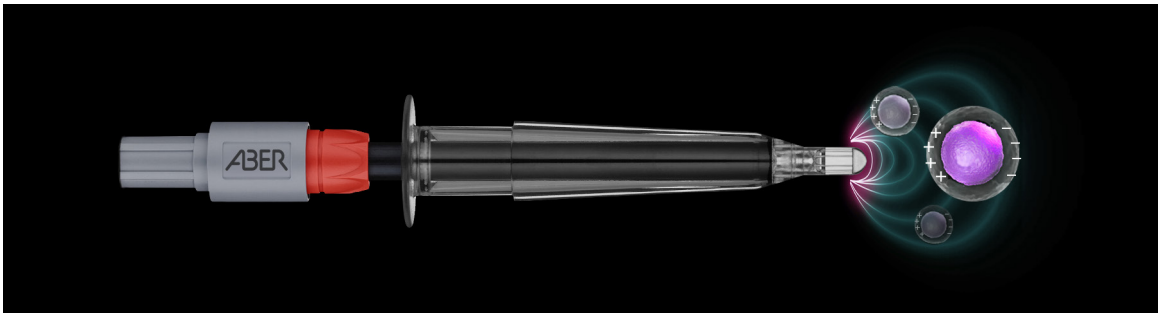

Figure 2: ABER FUTURA neotf biocapacitance sensor

How to monitor cell density and health using capacitance

In bioprocessing many scientists convert raw capacitance data to cells/mL. This is done for several reasons. Firstly, it can help validate the technology against a reference gold standard method. Also, many control strategies are based on off-line cell measurements of cells/mL so when using an in-line method of cell measurement such as capacitance many scientists are more comfortable converting back to cells/mL. Finally, scientists cannot include capacitance earlier in process development as it can only currently be used from the ~1 L scale.

Converting Capacitance to VCC

To convert capacitance to an at-line or off-line KPI measurement such as VCC a calibration must be performed for every cell line. This is because studies have shown that with two cell lines tested an off-line method generates values that can differ from in-line capacitance measurements (7). The commonly used method for calibration requires scientists to take a sample every 24 hours for VCC measurement while simultaneously recording the capacitance measurement in pF/cm using an in-line sensor, for example the FUTURA sensor (Aber). The sample is analyzed using an off-line system such as the Cedex Bio® Analyzer (Roche) to generate cells/ml value for each data point. The capacitance value is then plotted against cells/ml and the slope of the equation of this curve produces a calibration factor. Using this method, several groups have successfully converted capacitance to their reference off-line method. In a few cases, there is a deviation seen between capacitance and the off-line method, especially towards the end of a culture run.

What is the Reason Behind this Deviation?

One reason for the differences seen between capacitance and other off-line and at-line measurement of VCC is thought to be that each of the methods define live and dead cells differently.

For example, a study at the University of Manitoba (8) compared off-line and at-line particle detection, dye exclusion and fluorescent flow cytometry with in-line capacitance to monitor the VCC of Chinese hamster ovary (CHO) cells grown in a batch culture. The study showed that these techniques produced similar results during the exponential growth phase (80-hours in culture), but the dye exclusion and particle detection methods produced higher estimates of VCC after 80 hours to 120 hours (Figure 3). The fluorescent flow cytometry gave comparable results to capacitance and additional assays confirmed that these two methods are detecting apoptotic cells which become non-viable earlier than the other methods. This study shows that the different methods of measuring VCC are monitoring different points of cell death.

Figure 3: Comparison of off-line, at-line and in-line methods of measuring VCC, (published with kind permission from the University of Manitoba)

Another reason for the differences in VCC seen with capacitance and off-line and at-line measurements is also because cell size changes can affect VCC measurements. In a study by scientists at Sartorius (5) of CHO cells cultured for 15-days in rocking motion single-use bioreactor, capacitance using a BioPAT® Viamass sensor (Sartorius) was compared with VCC measurement using the trypan blue dye exclusion method (Cedex Bio® Analyzer) and wet cell weight. The results (Figure 4) show that during the exponential phase (6 days) capacitance measurements correlate well with both VCC and viable cell volume (VCV). However, when the average cell diameter increases, VCC measurements differ but the correlation with calculated biomass volume is maintained to the end of the run. This demonstrates that capacitance is proportional to the volume of viable cell membranes, and shows every biomass change during the culture run. These results indicate that capacitance is an accurate method of monitoring viable biomass.

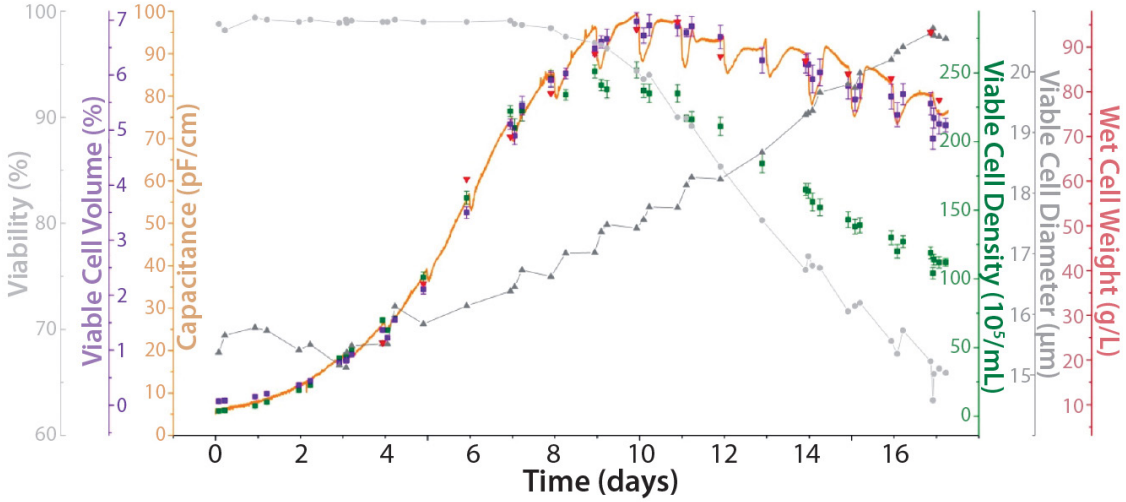

Figure 4: Comparison of at-line and in-line methods of measuring VCC with viable cell volume, (published with kind permission from Sartorius)

Using raw capacitance to monitor cultures

These studies by scientists at the University of Manitoba and Sartorius show that at-line and off-line methods depend on measuring cell death differently, which is especially true in the death phase. Therefore, it is incorrect to say one method of determining cell health and density is more accurate than another, they are just different and provides a rationale to use raw capacitance measurements to track cell growth and viability. One reason is that capacitance is a more realistic representation of how a culture is progressing because it can detect cell death sooner. Using a capacitance strategy, scientists can adjust culture parameters in real-time based on how the culture is behaving and can potentially save a batch. For example, scientists at Biogen (9) have shown that apoptosis can be reversed based on using real-time capacitance information, whereas they could not detect apoptosis early enough to reverse it using off-line VCC measurements.

Therefore, it makes sense to base feed strategies, on raw capacitance data as this means scientists can feed cells based on viable biovolume rather than cell number because larger cells demand more nutrients, which is not accounted for by using VCC as a KPI.

Biogen for example, has worked with capacitance measurements since 2016 comparing it to at-line and off-line measurements and in 2019 switched to using capacitance measurement as their gold standard method for process control in their current good manufacturing practices (cGMP) facility. They are also exploring the use of capacitance data to automate dilution of seed train cultures during scale-up and as a method of predicting glucose demand (10). They are using raw capacitance as a KPI because their studies (10,11) using capacitance to monitor CHO cultures in bench scale (5L), pilot scale (200 L and 315 L) and manufacturing scale (15000 L) bioreactors demonstrate that capacitance provides consistent data from sensor to

sensor across all bioreactor types and scales. With a R2 coefficient of variance close to 1 during the exponential growth phase, capacitance has good correlation when compared to off-line VCC measurements during this phase.

Additionally, using raw capacitance data can offer a flexible feed strategy, as cells are fed correctly irrespective of unintentional process errors such as under-inoculation. Evidence for this was shown in two studies at Biogen, one with an automated feed strategy based on in-line capacitance measurements (12) and a second where feed frequency was increased to every 4 hours (as opposed to 24 hours) using real-time continuous capacitance data (10). The second study, resulted in an increase in product titer of 21%, reduced glutamate depletion in the culture and improved cell growth. As a result of their work using capacitance measurements Biogen is currently using capacitance as a KPI instead of converting it to VCC for monitoring their cGMP processes.

Using raw conductivity to detect contamination earlier

The Aber capacitance probe also measures conductivity in mS/cm. Conductivity correlates with the concentration of charged ions in a cell suspension. An interesting application of using conductivity is in quality assurance, with a recent study (13) by researchers at the University of Massachusetts and the U.S. Food and Drug Administration (FDA), showing that unusual increases in conductivity measured with a capacitance probe in CHO cell cultures is linked to bacterial contamination. This finding indicates that in-line conductivity measurements could be used for early detection of bacterial contamination and could allow scientists to perform real-time troubleshooting to either save their batch by adding antibiotics to control the contaminant or to stop the process. The ability to detect bacterial contamination early could prevent it from impacting downstream purification processes, as well as sterilization/cleaning procedures and may save time and resources which can be costly especially in manufacturing environments.

Future perspective

Using capacitance may not replace off-line and at-line VCC measurements completely for several years, if ever. For example, in process development where bioreactor volumes of less than 1 L are used, although there are multi-use capacitance sensors which can be used, there are not yet single-use sensors available. This means there is justification to develop single-use capacitance sensors which perform well in 250 mL volumes and is an area where Aber Instruments is actively working. In cases where raw capacitance cannot be utilized, there is still significant benefit in converting real-time capacitance measurement to off-line methods and using this continuous measurement for monitoring and controlling the process.

However, this article has detailed expert opinion and evidence from several studies, which should encourage scientists, especially those developing and scaling-up new bioprocesses, to use capacitance as a KPI because it offers a more accurate approach to measure their cell health and is especially useful for controlling feed strategies. In summary, using capacitance can reduce or eliminate sampling challenges and biomass analysis variations associated with off-line and at-line measurements, as well as enable real-time monitoring of process performance to help save a costly production batch for example. All these features of using capacitance as a KPI could contribute to lower costs and shorter production timelines, which has the potential to rapidly deliver more affordable biologics and vaccines.

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Author details

*Aditya Bhat B.Sc., M.Sc., Ph.D., is Vice President – Technology and
John Poppleton is Director of Sales and Marketing, both are at Aber Instruments Inc, USA.

Web Address: www.aberinstruments.com
*Aditya Bhat is the corresponding author
Phone: +1 540 676 8113
E-mail: aditya@aberinstruments.com

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aberinstruments.com

Europe & Rest of the World:
+44 (0)1970 636 300
US:
+1 925 683 3673

sales@aberinstruments.com

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