Application note

Measuring cell density in HyPerforma S.U.B.s with ABER FUTURA neo$_{tf}$ single-use sensors

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Introduction

Monitoring critical process parameters (CPPs) and key performance indicators in bioreactor control systems is crucial to ensure proper cell growth and protein production. For years, the most important parameters have generally been dissolved oxygen (DO), pH, temperature, and cell concentration. Continual advancement of technology to evaluate these parameters has resulted in faster and more reliable measurements. Additionally, the move to single-use bioreactors (S.U.B.s) for bioproduction has motivated vendors to develop single-use sensors to incorporate into fully single-use workflows.

Recent advances in sensing technologies and a move toward controlling critical quality attributes of a culture have led to the development and use of more advanced sensors. Among the most highly monitored and controlled culture parameters has been cell concentration. However, to monitor cell concentration, the industry has generally relied on off-line measurement methods and analysis based on daily spot checks. The challenges to this method are well known and include contamination risks, labor-intensive work, infrequent sample points, sample variability, etc. Online measurement methods are used occasionally, mainly by development lab professionals who look to implement advanced technologies and develop higher-order control schemes.

Dielectric spectroscopy, or capacitance measurement, is a reliable and proven method of determining cell density online. This technology was invented and developed in 1988 by ABER Instruments Ltd, Aberystwyth UK. Today, most of the major biopharmaceutical companies employ capacitance measurement, in R&D and through process development to manufacturing.

Owing to the increased use of S.U.B.s and building on ABER's experience with single-use capacitance sensors, the latest FUTURA™ neo$_{tf}$™ single-use capacitance sensors (Thermo Fisher Scientific Cat. No. SV21621.01) have been specifically developed for integration into Thermo Scientific™ BioProcess Containers (BPCs) for use in Thermo Scientific™ HyPerforma™ S.U.B.s. This application note summarizes the sensor's function, integration into the S.U.B. BPC, and showcases functional cell culture data obtained both internally and with a primary customer.

Method of operation

Cells with intact plasma membranes in a bioreactor can be considered to act as tiny capacitors under the influence of an infinitesimal electric field. The nonconducting nature of the plasma membrane allows a build-up of charge, i.e., capacitance. The resulting capacitance is directly proportional to the membrane-bound volume of these viable cells (Figure 1).

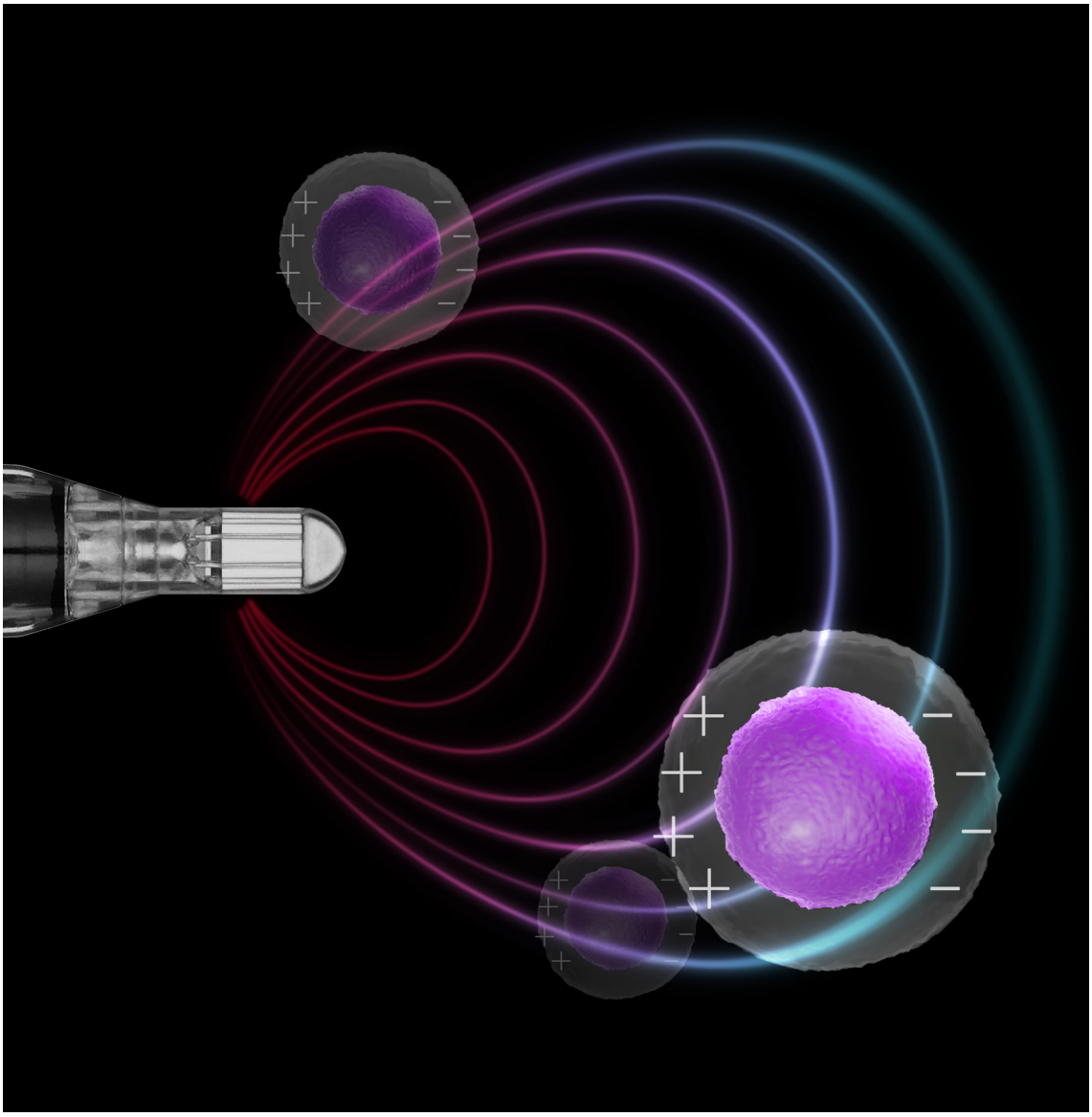

Figure 1 ABER FUTURA neo$_{tf}$ biocapacitance sensor, and representation of measurement field.

Correlations between total capacitance and viable cell density or volume can easily be determined to monitor cell growth and health, and can be incorporated into control pathways as a basis for feeds, gas flow rates, or cell bleed in the case of perfusion cultures.

Integration into the S.U.B. BPC

The FUTURA neo$_{tf}$ single-use sensor is integrated into the S.U.B. BPC by way of the standard 1.27 cm (1/2 in.) bioreactor tube port (Thermo Fisher Scientific Cat. No. SV20716.01). Care was taken during the design to establish proper penetration depth into the BPC while ensuring that film damage due to packaging, shipping, irradiation, and deployment was avoided. Additionally, guidance is provided to BPC design engineers and customers to ensure proper spacing of sensors in the case where multiple pieces are used to monitor the bioprocess. As a rule, a minimum of 6 cm (2.36 in.) of separation in the sensor port of the S.U.B. should be maintained to avoid signal interference. Ideally, these sensors should be placed on opposite ends of the sensor belt.

The FUTURA neo$_{tf}$ single-use sensor can be connected to the FUTURA neo$_{tf}$ electronics, which in turn are connected to a laptop with FUTURA software via the Connect Hub. For direct integration options, please contact your local Thermo Fisher Scientific or ABER representative. Figure 2 represents the connections required for connecting the FUTURA neo$_{tf}$ sensor to the FUTURA neo$_{tf}$ transmitter, the Connect Hub, and the communication to the local computer running the FUTURA software.

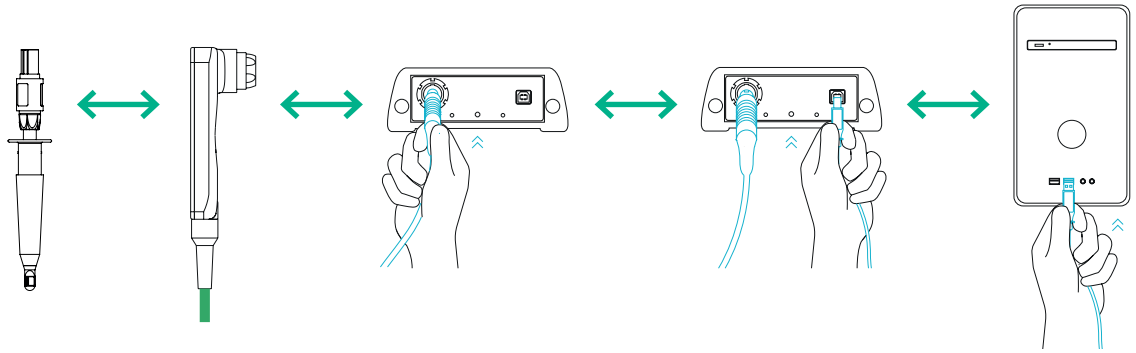

Figure 2 Connections between the FUTURA neo$_{tf}$ sensor, FUTURA neo$_{tf}$ transmitter, Connect Hub, and computer

Case study 1—Fed-batch culture

Cell runs were performed to test the function of the FUTURA neo$_{tf}$ sensor using a reusable FUTURA annular probe (12 mm sensor) as control (installed via the standard probe insertion kit) and comparing the data against traditional off-line Trypan Blue staining (Beckman Coulter). Three single-use FUTURA neo$_{tf}$ sensors were built into a 50 L S.U.B. BPC and irradiated between 25–40 kGy; a total of three BPCs were prepared. The BPCs were installed in a 50 L S.U.B., filled with growth medium (Gibco™ ExpiCHO™ Stable Production Medium (SPM)), and inoculated with CHO cells at a density of 0.3 x 10^5 cells/mL. Cells were grown in a standard 14-day fed-batch process with a continuous flow of Gibco™ EfficientFeed™ C+ Advanced Granulation Technology™ (AGT™) feed and 45% glucose from days 3 to 14. Daily samples were taken and analyzed for cell density and viability using a Beckman Coulter Vi-Cell™ XR analyzer using trypan blue exclusion staining.

For each run, three FUTURA neo$_{tf}$ sensors were tested and compared with a FUTURA annular probe (the reusable sensor was disconnected in run 2). The software (FUTURA Tool v3.0.4.0) was placed in cell culture mode to log all sensors. The sensors were fully immersed in liquid medium, allowed to equilibrate at operating temperature (noting the correct conductivity of 12–14 mS/cm), zeroed in cell-free medium, and the sensor signals were logged for the duration for each run. Note: Grounding of the FUTURA neo$_{tf}$ sensor is possible, if required. For the case study presented here, prototype FUTURA neo$_{tf}$ transmitters without grounding were used.

A correlation model was built between the off-line high-viability viable cell volume (VCV) and the online capacitance signals. VCV was calculated as the total number of cells multiplied by the average diameter of the viable cells as measured by the Vi-Cell XR analyzer. Separate correlations were determined for either the reusable FUTURA annular probe or the single-use FUTURA neo$_{tf}$ sensors. Based on these correlations, an online VCV was determined for the individual sensors, and plotted in Figures 3–5. The data show consistent performance among the online sensors for each run, while the off-line VCV varied near peak cell density and as viability dropped to below 90%. This is especially noted in the data from run 3, where off-line counts differed significantly from the online measurements. Variability in off-line counts at lower viability led to differences between off-line and online measurements. However, online capacitance measurements were very consistent among all sensors.

Variance among the single-use sensors was within 5% for the duration of the cultures and was consistent throughout each run, i.e., the variance from the average value didn't drift over the course of the culture. This demonstrates that the sensors exhibit a reliable signal to determine cell mass despite changing bioreactor conditions, and that no sensor drifted from expected results through the cell runs. This is key to linking feed volumes based on cell mass and nutrient demands during fed-batch or perfusion culture processes, allowing for optimal performance of culture conditions to maximize cell production.

Note that in this study, capacitance:VCV ratio correlations between the FUTURA neo$_{tf}$ sensors were slightly different (approximately 8%). This could possibly be due to the way the reusable FUTURA annular sensor is installed in the S.U.B. via the probe kit.

The FUTURA neo$_{tf}$ sensors have been specifically designed for the S.U.B.s to measure comparably with the reusable FUTURA annular sensors in reusable vessels, making ABER capacitance technology truly scalable across various platforms.

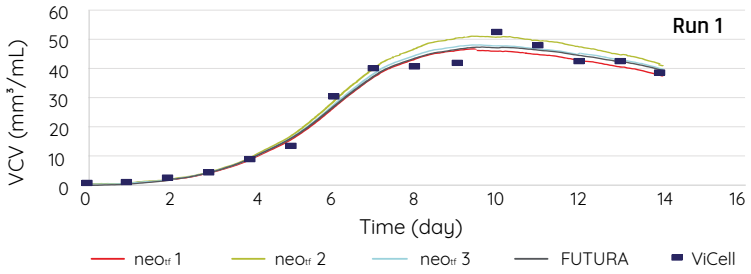

Figure 3. Sensor and off-line data for cell run 1. VCV for the capacitance sensors was calculated based on raw capacitance readings and off-line VCV.

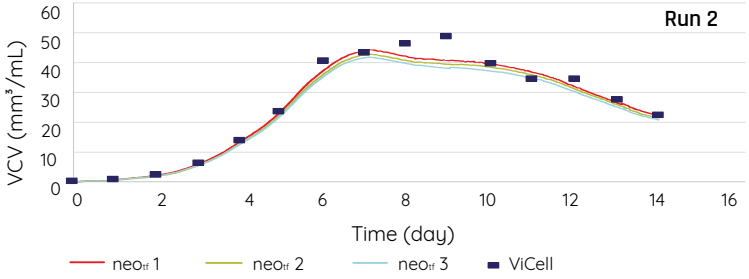

Figure 4. Sensor and off-line data for cell run 2. Note: The FUTURA reusable annular sensor was disconnected in this run. VCV for the capacitance sensors was calculated based on raw capacitance readings and off-line VCV.

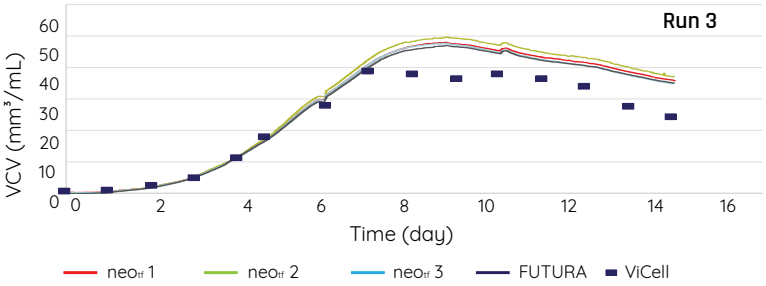

Figure 5. Sensor and off-line data for cell run 3. VCV for the capacitance sensors was calculated based on raw capacitance readings and off-line VCV.

Case study 2—Biogen fed-batch culture

A 50 L S.U.B. containing both a single-use FUTURA neotf sensor and a reusable FUTURA annular sensor was used to perform a fed-batch culture using a CHO clone. For 15 days, nutrient was dynamically added using a biocapacitance-based feeding strategy, with the reusable FUTURAl annular sensor used for feed control while the FUTURA neotf sensor was monitored for comparison. Sensors were placed at an appropriate distance from each other to minimize potential sensor-to-sensor signal interference. ABER's standard cell culture mode was used to monitor and collect the data.

Results in Figure 6 show near-perfect alignment of the signals for the duration of the culture with minimal variance of up to 2% of signal reading. Both sensors showed the daily culture dilutions due to the bolus feeds with similar timing and magnitude. It can be noted that the capacitance signal from the FUTURA neotf sensor was slightly noisier than the reusable FUTURA annular sensor. The signal could easily be smoothed using a higher filter value in the ABER software and ensuring proper grounding. Conductivity was also nearly identical between the sensors.

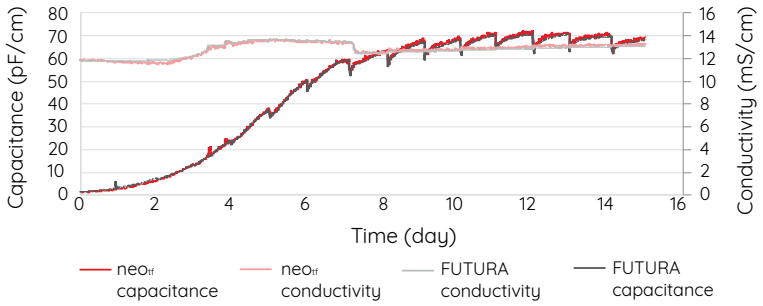

Figure 6. Sensor data for the fed-batch culture from a customer.

Case study 3—Biogen perfusion culture

An S.U.B. containing both a FUTURA neotf sensor and a reusable FUTURA annular sensor was used in a continuous perfusion culture of CHO cells. The reusable FUTURA annular sensor was used to control cell mass in the process while the FUTURA neotf sensor was used for monitoring only. For 6 days, the cells underwent an exponential growth phase, followed by approximately 2–3 days of continued growth as the cells transitioned into steady state. Once the steady state cell mass was achieved, the reusable FUTURA annular sensor was linked to a pump to remove culture automatically as required in order to maintain cell mass at the target value. Similar to case study 2, the sensors were spaced sufficiently to minimize potential sensor-to-sensor signal interference. ABER software in cell culture mode was used to monitor and control the process.

Results for this study (Figure 7) show very good alignment between the sensor signals, with less than 5% variance at the steady-state cell density. As in the other studies, it is important to note that neither sensor exhibited drift compared to the other sensor for the culture duration. This becomes increasingly important when utilizing these sensors for either feed-based or cell-density controls, where consistent measurement is critical to ensuring that cells have sufficient nutrients for growth or protein production. Notably in this case, the signal remained consistent between the sensors even after high cell densities were reached.

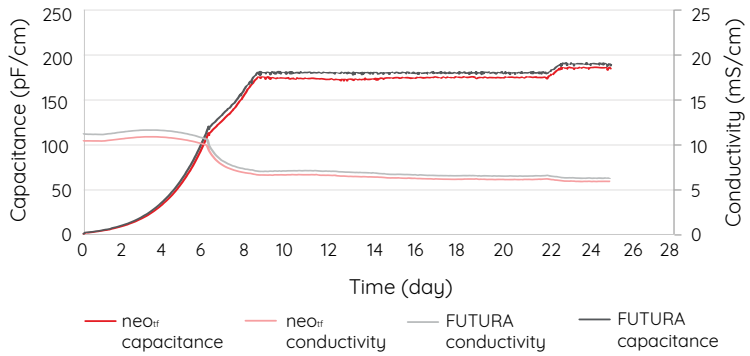

Figure 7. Sensor data for perfusion culture from a customer.

Conclusion

The data presented in the case studies clearly show accurate and consistent performance among the tested sensors in various cell culture conditions and across a wide range of cell densities. Good correlation was observed between reusable and single-use capacitance sensors with off-line measurements. Consistency between FUTURA neo$_{tf}$ single-use sensors across runs was also demonstrated (less than 5% variance). The data presented support simple integration and ultimate replacement of traditional reusable capacitance sensors with this latest single-use capacitance sensor. This custom-designed FUTURA neo$_{tf}$ single-use sensor, for use solely in HyPerforma S.U.B.s, is a plug-and-play, single-use alternative to move toward higher-order control of cell densities and feed strategies with real-time, online measurements.

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Note: The views and opinions presented in this article were made independently of Biogen and should not be considered to imply an affiliation with or an endorsement by Biogen.

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For over three decades, ABER has pioneered the development and use of dielectric instrumentation to measure cell membrane capacitance and media conductivity. Since its inception, ABER has provided the biotech industry with three generations of biomass monitors. Now with the recent introduction of the FUTURA neo$_{tf}$ system, ABER technology is available for single-use bioreactors.

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