Scientific paper

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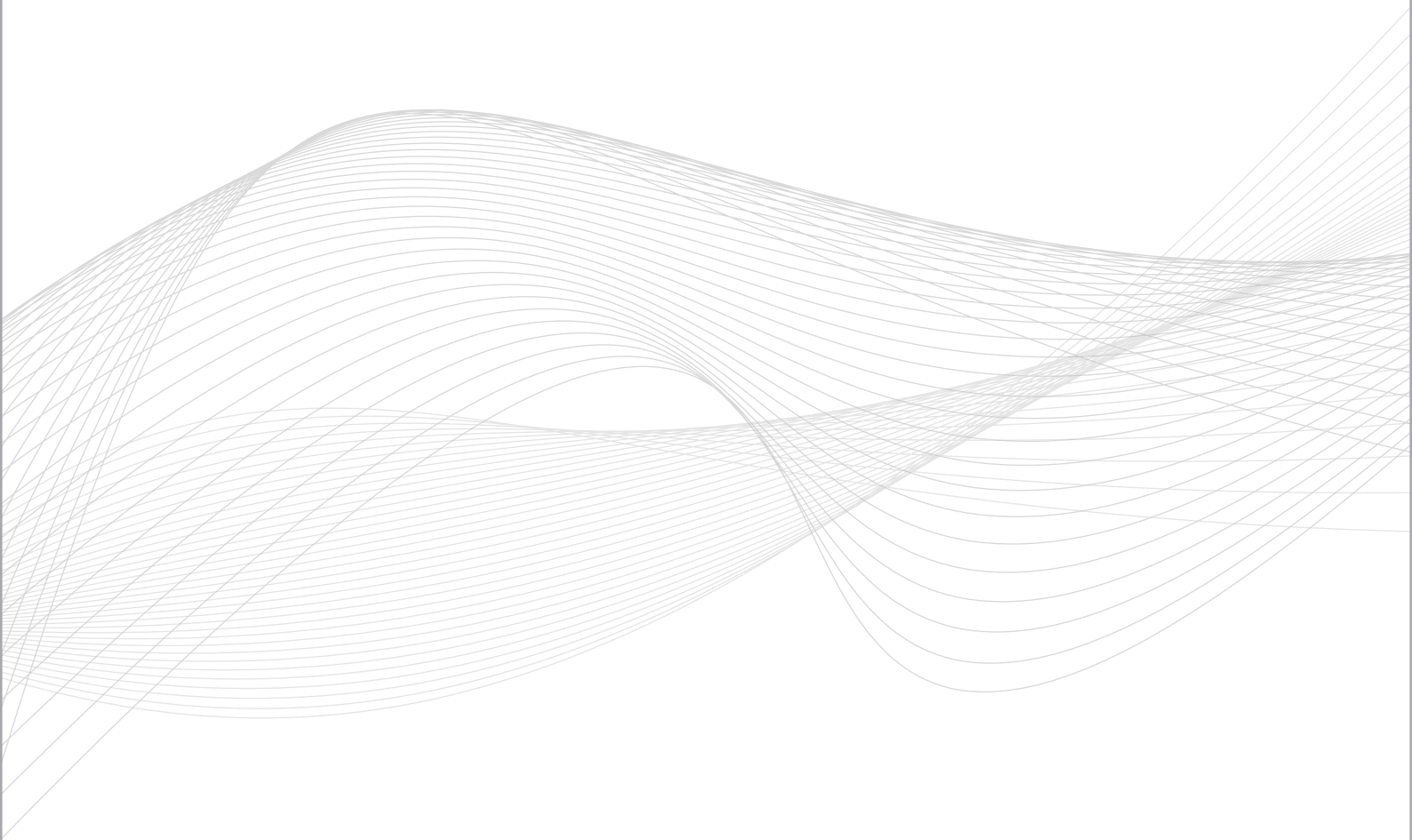

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The Introduction of the Futura Biomass Monitor for monitoring conventional and disposable bioreactors.

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Abstract

Of the available on-line biomass assays, the radio-frequency impedance method (often simply referred to as capacitance) is generally regarded as the most robust and reliable method to monitor the viable biomass during fermentation and cell culture. The capacitance method has been used to estimate microbial biomass for more than 20 years (1) and today the technology is used routinely for monitoring and controlling mammalian cell culture processes and high density yeast and bacterial fermentations in research, process development and manufacturing applications. The capacitance method has shown to be robust, scalable, and insensitive to gas bubbles or debris when measuring cells in suspension or attached to inert carriers (2).

In this article we show how the recent launch of the Futura Biomass Monitor from Aber Instruments (Aberystwyth,UK) has increased the range of applications for measuring the live cell concentration in various bioreactors including single use and mini-bioreactors.

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New Futura launch in 2010

Since 1988 when Aber Instruments first invented the technology for measuring live biomass on-line, the company has released a series of five different instruments with each generation providing an improvement in terms of performance and advances in electronics.

The latest Futura Biomass Monitor, launched by Aber in 2010, is already acknowledged as the most compact, cost effective, on-line instrument in the market for use in conventional, miniature and disposable bioreactors.
The Futura is available in three configurations with each version optimized for the type or size of bioreactor.

The “SF” Standard Futura (Figure 1) has all the biomass monitoring processing power contained within a single lightweight, compact housing. It is suitable for most conventional bioreactors above 1Litre.

Figure 1 Standard Futura

Figure 2 Standard Remote Futura

The “SRF” Standard Remote Futura (Figure 2) incorporates a slim, light-weight pre-amplifier making it ideal for small bioreactors as low as 100ml working volume. The main Futura housing can be mounted away from the bioreactor vessel. The SRF has been designed to be used on any small bioreactor where there is restricted head plate space including Das Gip bioreactors.

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Futura Biomass Monitors are already monitoring fermentations in research environments. The growth of *E. coli* in a 1L, highly aerated and agitated Das Gip bioreactor has been measured with an "SRF" type biomass monitor **(Figure 3)**. The improved noise performance over previous biomass monitors makes possible an interrogation of secondary features found in biomass growth curves.

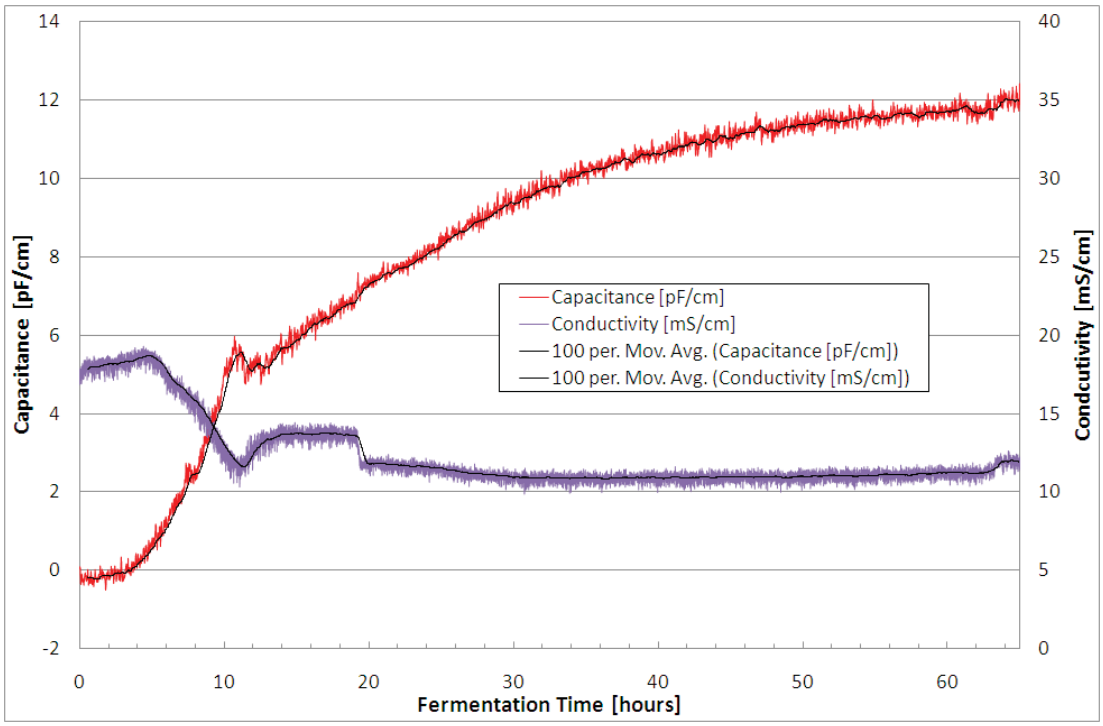

Figure 3 Plots of capacitance (pF/cm) and conductivity (mS/cm) for a fermentation in a 1litre Das Gip bioreactor using the Standard Remote Futura.

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The “MRF” Mini-Remote Futura **(Figure 4)** is a brand new instrument design for disposable bioreactors. It incorporates an ultra lightweight pre-amplifier for connecting to single use, disposable probes. Details of the range of disposable probes are provided in the following section.

Figure 4 Mini-Remote Futura

Unlike previous biomass models from Aber, that have always incorporated a transmitter, Futura’s flexible bus connectivity allows multiple units to be easily connected to a PC, bioreactor controller or SCADA via a simple hub. This connectivity reduces the overall costs of complex systems and requires a small footprint **(Figure 5a)**. The hub can be provided with 1, 4 or 8 inputs from local fermenters. If a smaller local display than the previous biomass systems is required, the Futura can be connected to a colour, touch screen PLC that can be panel or table mounted **(See Figure5b)**.

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Figure 5 Options for connecting the Futura to control systems, PC, SCADA or local transmitter.

Figure 5a Futura Connect 8 Way Hub. Provides USB, Modbus and 4 - 20 mA Outputs.

Figure 5b V-350 touch screen display connected to the Standard Remote Futura.

The Futura range of probes is designed for maximum sensitivity, reduced fouling and easy cleaning. The probe sensitivity is maximized by the generation of large, even electric fields from annular electrodes. The smooth surface reduces potential areas of entrapment and the durable construction allows sterilisation in situ or autoclaving. For cGMP cell culture systems the Futura range also includes a robust, 25mm probe with flush electrodes that provide an optimum surface to reduce a build-up of cells during fermentations of up to 3 months duration. Each connected probe is uniquely calibrated allowing the Futura to provide highly accurate measurements. Information on the probe life cycle is provided by intelligent sensor management software.

Like some of the earlier instruments from Aber (*e.g.* Model 200 Biomass Monitor) the Futura is configured to run in four different configuration modes. These modes allow the end user to quickly pre-set the measuring frequency and filtering appropriate for the type of cells being monitored (termed bacterial, microbial, and cell culture modes).

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A fourth custom “mode” allows the end user to change the frequencies and filtering allowing further optimization of the conditions or matching settings from previous experience with other Aber products.

The Futura has been designed to provide full IOQ documentation making it the ideal choice for cGMP and cell culture manufacturing processes

Biomass Probes For Disposable Bioreactors

Many biopharmaceutical companies are switching from conventional glass and stainless steel vessels to disposable, single use bioreactors. On-line probes are provided in a small number of disposable bioreactors but these options are limited with usually just pH, temperature, and pO2 available. New processes requiring cGMP production are now being developed using disposable bioreactors and, consequently, there will be increasing demand for the same range of sensors (including viable biomass) used with conventional bioreactors.

Figure 6 Aber disposable coupon suitable for welding into single use bioreactors.

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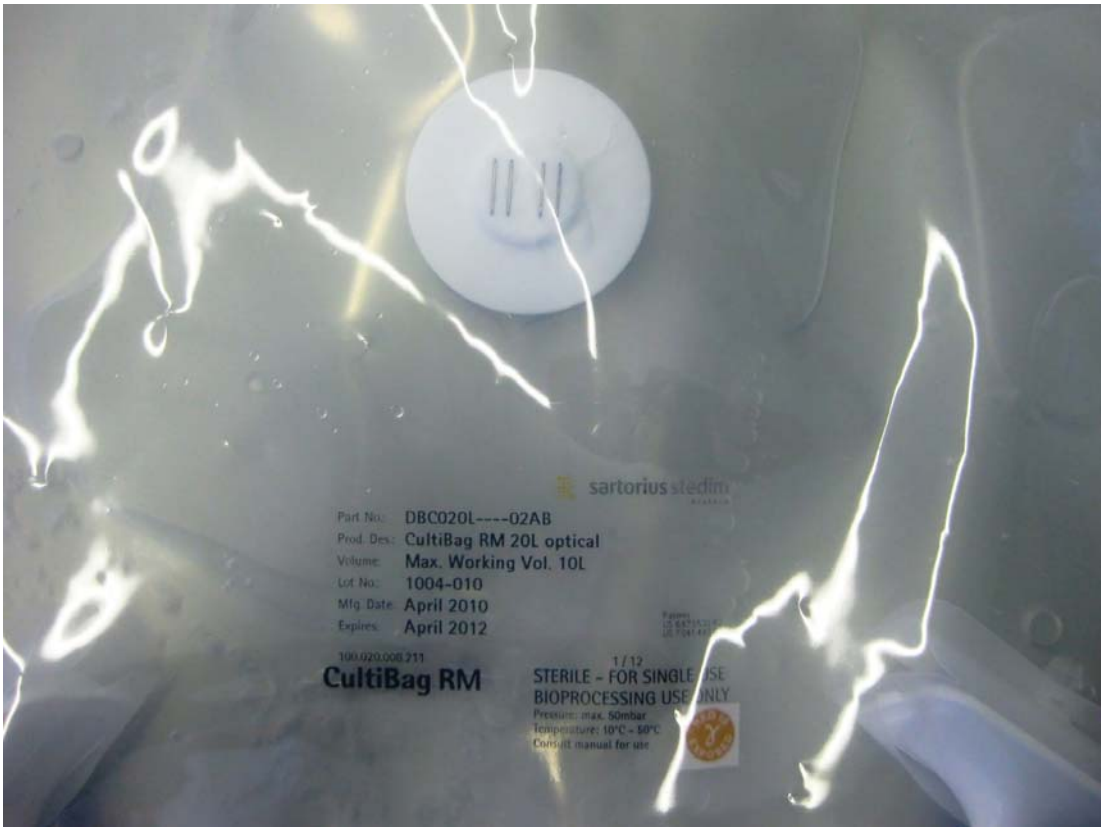
A truly disposable biomass probe has been carefully designed to be welded into most single-use bioreactor bags including those with agitators (*e.g.*Hyclone SUB, XCellerex XDR, Sartorius Stedim Biotech CultiBag STR and Millipore Mobius CellReady) or those using a rocking platform (e.g. Sartorius Stedim Biotech CultiBag RM and Wave Bioreactor). The disposable probe is easily connected to a mini-lightweight pre-amplifier (see Figure 6) so that the weight load or torque on the bag is minimal and the bulk of the electronics is located away from the bag. The disposable probe has already been welded into various bags, an example of a disposable probe welded into a prototype Sartorius Stedim Biotech CultiBag RM is pictured in **Figure 7.**

Figure 7 Aber Disposable Probe welded into a Sartorius Stedim Biotech CultiBag RM 20L optical (10 L working volume)



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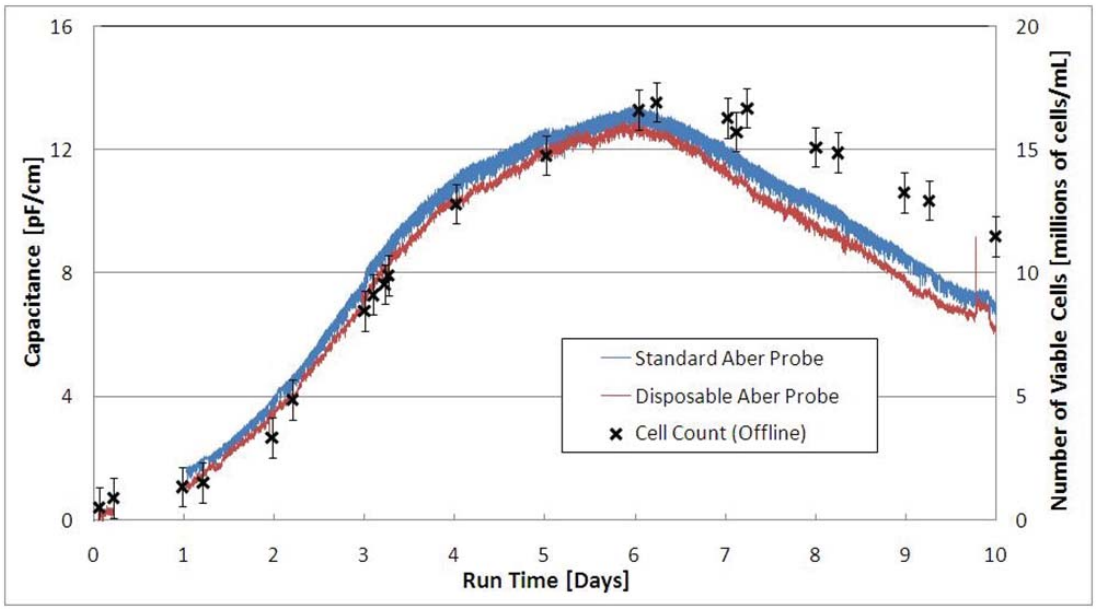
The performance of the disposable probe has been assessed in the Single-Use Mobius CellReadyTM 3L rigid body bioreactor (Millipore, USA) . In a series of experiments with the bioreactor, the viable cell density of CHO cells was monitored over a two week period using a conventional reusable annular biomass probe, a disposable probe connected to a Mini-Remote "Futura" and by off-line measurements with a Vi-cell instrument (Beckman Coulter, USA). The results shown in Figure 8 demonstrate that the capacitance measurements from both probes trend very closely to one another and to the off-line measurements(4).

Figure 8 Comparison of live biomass using on-line reusable and disposable probes and off-line viable cell concentration measurements.



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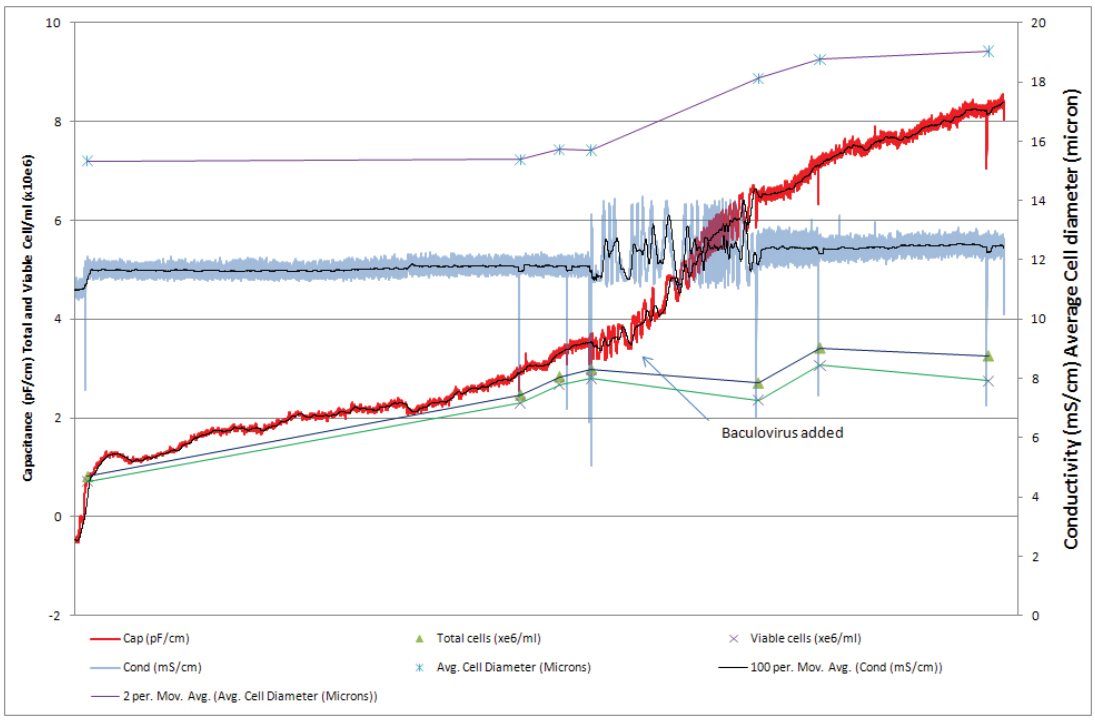
The Aber disposable probe also has been assessed in a prototype Sartorius Stedim Biotech CultiBag RM 50L optical (25 L working volume) by monitoring the growth of SF9 cells **(Figure 9).** The probe tracks the viable cell density before the addition of a Baculouvirus. The Futura biomass probe successfully detected the valid infection by the rapid increase in signal.

Figure 9 Capacitance measurement in a 50L (25L - Working volume) Sartorius Rocking Motion CultiBag RM with Aber Disposable Probe for Baculovirus Infected SF9 Cells



Data courtesy of R. Tanner, GSK, UK.

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A two part disposable probe (patent pending) also been developed by Aber Instruments (3). The design is particularly suited to the Hyclone SUB or other disposable bioreactors that have a series of available tube-ports on the side of the bag. The disposable portion consists of a plastic assembly containing the measuring electrodes that can be pre-inserted into a disposable bioreactor bag port (Figure 10 a-d) and irradiated with the bag. For compliance, all wetted materials of these disposable probes are USP class VI compliant. The Biomass Monitor system is completed by using a dedicated reusable probe adaptor designed to electrically connect the disposable section to a Futura Biomass Monitor at the other end.

Figure 10 a-d. Two part disposable biomass probe for pre-inserting in disposable bioreactors prior to gamma irradiation.

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Conclusions

The use of RF Impedance to monitor and control microbial and cell culture processes is well established in biopharmaceutical applications but the introduction of the Futura Biomass Monitor allows this technology to be used with confidence on most types of bioreactors from process development through to cGMP production. With a new calibration method and faster electronics the Futura range is Aber's most accurate biomass monitor to date. Designed with modular components, the Futura range offers customers the most connectivity options to their PC, PLC, and SCADA systems. The Futura has expanded the range of applications and reduced the complexity of live biomass measurements.

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