

# Recent Developments in Using Scanning Radio-Frequency Impedance Measurements on Cell Culture Processes

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The viable cell concentration is of prime importance in monitoring cell culture manufacturing processes. Of the available online biomass assays, the radio-frequency impedance (RFI) method has some clear advantages for manufacturing because it is an unambiguous reflection of viable cell biovolume rather than the total number of cells. This allows RFI to be used to control feeding rates or to maintain a constant level of biomass within the bioreactor. RFI is also suitable for measuring the live cell density in bioreactors when the cells are attached to microcarriers and to inert discs.<sup>1,2</sup>

Traditional RFI-based systems measure the capacitance at either one frequency (typically 0.5 MHz) or in a dual frequency mode. However, scanning the capacitance in a frequency range, typically between 0.1 and 20 MHz, also makes it possible to measure other important parameters such as the cell size, the cell membrane capacitance, and the cell interior conductivity online.<sup>3,4</sup> Online measurement of such properties can provide useful information regarding the physiological state of the cells,

which can assist in better understanding and controlling the fermentation or cell culture process.

In this article, we introduce the concept, theory, and challenges of applying RFI frequency scanning using the Aber Biomass Monitor (Aberystwyth, UK) to cell culture processes, and show examples of how additional process parameters can be derived.

## Principles

The principle behind RFI measurement of biomass levels is an electrical polarisation process set into motion

when a radio-frequency alternating current is applied across a suspension of cells. This results in a charge separation across the insulating cell membrane and the cell, attaining an induced dipole. The cells with their induced dipoles can be compared to tiny capacitors. If lots of tiny capacitors (*i.e.*, cells) are present in the medium, then the capacitance of the suspension will go up; and the more there are, the higher the capacitance. The presence of an insulating membrane is essential. Dead cells, which often have a leaky or broken membrane, are not polarized as much. Hence, the technique is relatively insensitive to

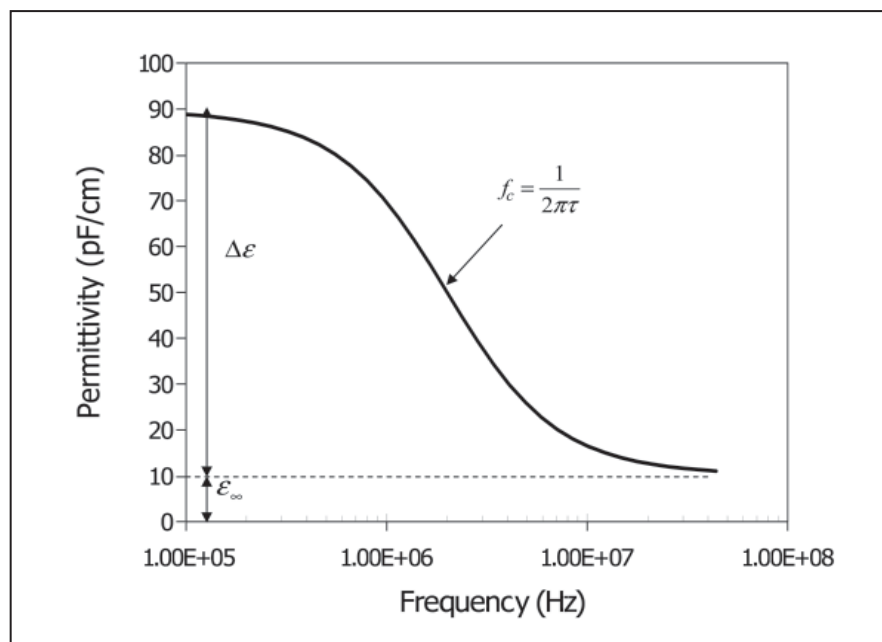


Figure 1.  $\beta$ -dispersion curve.

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their presence. Microcarriers and similar materials used to support cell growth do not have a membrane either, making them near-invisible to the RFI field, which allows the technique to measure biomass levels in situations where optical methods cannot be used.<sup>1,2</sup>

### The $\beta$ -Dispersion

In the frequency range of 0.1–20 MHz the capacitance of a suspension of viable cells tends to decrease with an increase in the frequency of measurement. This phenomenon is associated with the polarisation process across the cell membrane (described previously), and is called the  $\beta$ -dispersion. The  $\beta$ -dispersion is useful for biomass determination since its amplitude is intimately related to the volume fraction occupied by the cells in a suspension.

Figure 1 shows the change in the capacitance or in this case, permittivity—also known as dielectric constant (a simple calibration step allows one to convert capacitance to permittivity)—as a function of frequency. The  $\beta$ -dispersion takes the form of a sigmoid curve with a high plateau permittivity region at low frequencies, and a low plateau permittivity region at high frequencies. The difference in the two plateaus,  $\Delta\epsilon$ , is called the dielectric increment. The dielectric increment is proportional to the volume fraction of biomass  $P$  in the suspension, according to the equation:<sup>5,6</sup>

$$\Delta\epsilon = \frac{9PrC_m}{4\epsilon_0} \quad (1)$$

The  $r$  is the cell radius,  $C_m$  is the membrane capacitance and  $\epsilon_0$  is the permittivity of free space.

### Frequency Scanning

As already shown in Figure 1, the change of permittivity as a function of frequency associated with the  $\beta$ -dispersion generally takes the form of a sigmoid curve with high and low frequency plateaus. Using this whole curve rather than just the dielectric increment provides an opportunity to improve the accuracy of the measurement of the biomass. In addition, frequency scanning also makes it possible to measure

other important parameters such as the cell size, the cell membrane capacitance, and the cell interior conductivity online. Conventional methods to measure such properties online are still very challenging, but can provide useful information regarding the physiological state of the cells. This will help in understanding and controlling the fermentation, and provide guideposts to the major transition points in the process.

The change in the permittivity as a function of the frequency  $f$  can be described by the Cole-Cole equation:<sup>7</sup>

$$\epsilon' - \epsilon_\infty = \frac{(\epsilon_s - \epsilon_\infty) \left[ 1 + \left( \frac{f}{f_c} \right)^{1-\alpha} \sin \frac{\alpha\pi}{2} \right]}{1 + 2 \left( \frac{f}{f_c} \right)^{1-\alpha} \sin \frac{\alpha\pi}{2} + \left( \frac{f}{f_c} \right)^{2(1-\alpha)}} \quad (2)$$

The  $\alpha$  represents the Cole-Cole alpha, and the value of  $\alpha$  is dependent on the cell size distribution. However, it is also dependent on the volume fraction, average cell size, medium conductivity, and other factors. Hence, it is not possible to directly correlate the value of  $\alpha$  to the cell size distribution.

The critical or characteristic frequency is  $f_c$ ; the frequency at which the change in permittivity is half-complete. The value of  $f_c$  depends on the internal cytoplasmic ( $\sigma_i'$ ) and exter-

nal ( $\sigma_o'$ ) conductivities, the (mean) cell radius  $r$  (taken across the cytoplasmic membrane, excluding the cell wall), and the magnitude of the specific membrane capacitance ( $C_m$ ) according to the relation:<sup>5</sup>

$$\frac{1}{2\pi f_c} = rC_m \left( \frac{1}{\sigma_i'} + \frac{1}{2\sigma_o'} \right) \quad (3)$$

The value of  $f_c$  can be obtained from fitting the Cole-Cole equation to the measured frequency scan. The  $\sigma_o'$  can be estimated from the suspension conductivity. Unfortunately, it is often very difficult to separate the simultaneously occurring changes in  $r$ ,  $C_m$ ,  $\sigma_i'$ , and  $\sigma_o'$ . However, assuming a constant value of  $\sigma_i'$  can sometimes be used to gain insight into what happens during fermentation.

As an example, we will look at a Human Embryonic Kidney (HEK293) cell culture in a fed-batch process. Using an Aber Biomass Monitor (see Figure 2), capacitance values were continuously recorded at 25 different frequencies between 100 kHz and 20 MHz. In addition, samples were obtained and analysed offline for the viable cell count, total cell count, and cell size.

Figure 3 shows the changes in the parameters obtained from the frequency scans. The  $\beta$ -dispersion curve obtained



Figure 2. The Aber Biomass Monitor model 220; suitable for frequency scanning.

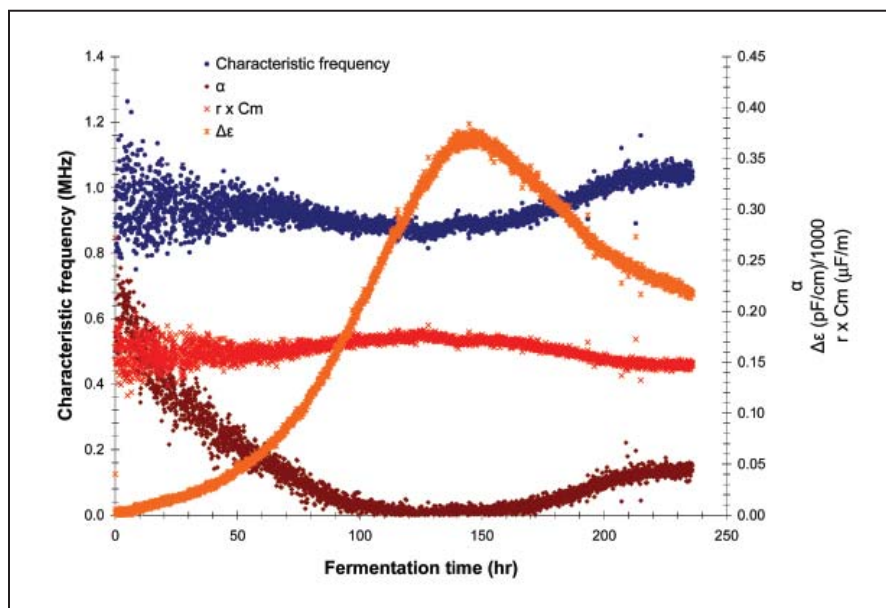


Figure 3. Change in the values of parameters derived from permittivity scans over time during the fermentation of HEK293 cells.

online for the HEK fermentation was fitted to the Cole-Cole equation (equation 1) to calculate the values of  $f_c$  and  $\alpha$ . The value of  $f_c$  obtained from the Cole-Cole equation was then substituted in equation 3 to obtain  $rC_m$ , assuming  $\sigma_i'$  to be constant (15 mS/cm). The value of the conductivity of the medium  $\sigma_o'$  was calculated from the suspension conductivity using the Bruggeman equation:<sup>8</sup>

$$\sigma = \sigma_o' (1 - P)^{3/2} \quad (4)$$

Figure 4 plots the results of offline counts using the Aber Biomass Monitor Model 220, all in the same graph: 1) the total number of cells; 2) the number of viable cells; and 3) the volume fraction obtained. In this case, there was a good correlation between the volume fraction and the offline viable cell count. Changes in the cell radius, the cell membrane capacitance, the cell membrane conductance, and the viability of the HEK293 cells will contribute to the difference between the two viable biomass estimation methods towards the end of the culture period.

If the value of  $rC_m$  is known, it is possible to estimate the value of cell radius online during the fermentation if  $C_m$  does not change significantly. To determine whether  $C_m$  changes during fermentation, offline samples were taken at regular intervals to measure

the cell size. The measured value of cell radius was then used to calculate  $C_m$  during the fermentation. Figure 5 shows how the measured cell diameter and the calculated value of  $C_m$  changes during fermentation.

It can be seen from Figure 5 that the cell diameter significantly decreases during fermentation, and that the calculated value of  $C_m$  increases (from 1.9–2.7  $\mu\text{F cm}^{-2}$ ). Whether the actual change

in  $C_m$  is as large as the one calculated here is uncertain. We had to make some large assumptions during the calculations, including the assumption that the membrane conductance and internal conductivity didn't change. Cell viability decreases during the latter stages of the fermentation, most likely through apoptosis. Apoptosis is accompanied by large changes in cell properties including cell shrinkage, which may be associated with folding of the cell membrane. Such folding could explain the increase in  $C_m$ . Cell shrinkage accompanying apoptosis can also lead to an increase in cell interior conductivity.<sup>9</sup> An increase in internal conductivity could well be the cause of the increase in  $f_c$  seen during the later stages of fermentation. However, although there is some uncertainty about the actual value of  $C_m$ , clearly it would be incorrect to assume  $C_m$  to remain constant.

Finally, Figure 6 shows the calculation of the specific growth rates of the HEK293 cells online during fermentation using data obtained with the Aber Biomass Monitor. At the start of the culture, biomass levels are very small, and it is therefore difficult to obtain a good estimate of the specific growth rate. However, this becomes easier as time progresses and the biomass level increases. It can be observed in Figure 6

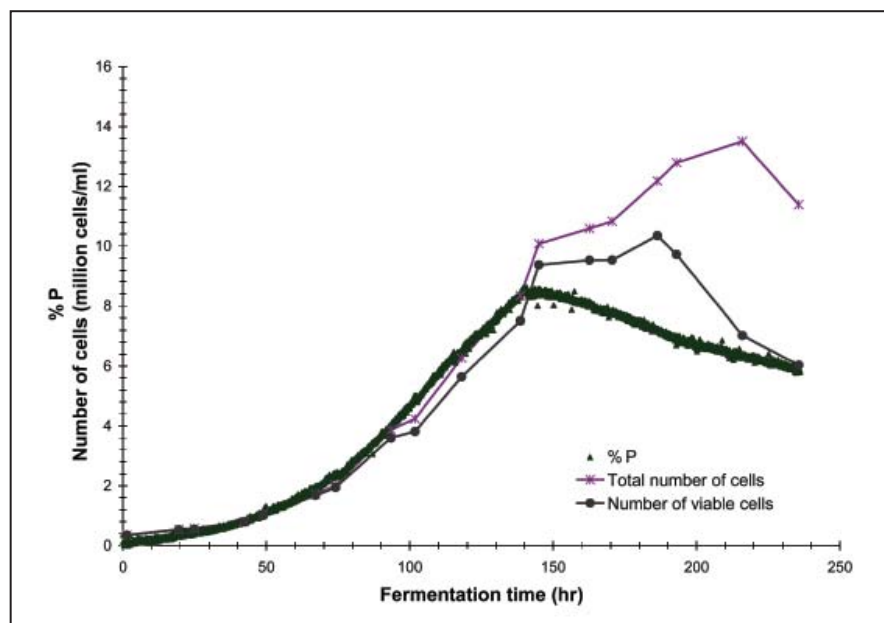


Figure 4. Changes in the % volume fraction of the HEK293 cell culture biomass as measured using the Aber Biomass Monitor. Also shown are the cell counts obtained offline.

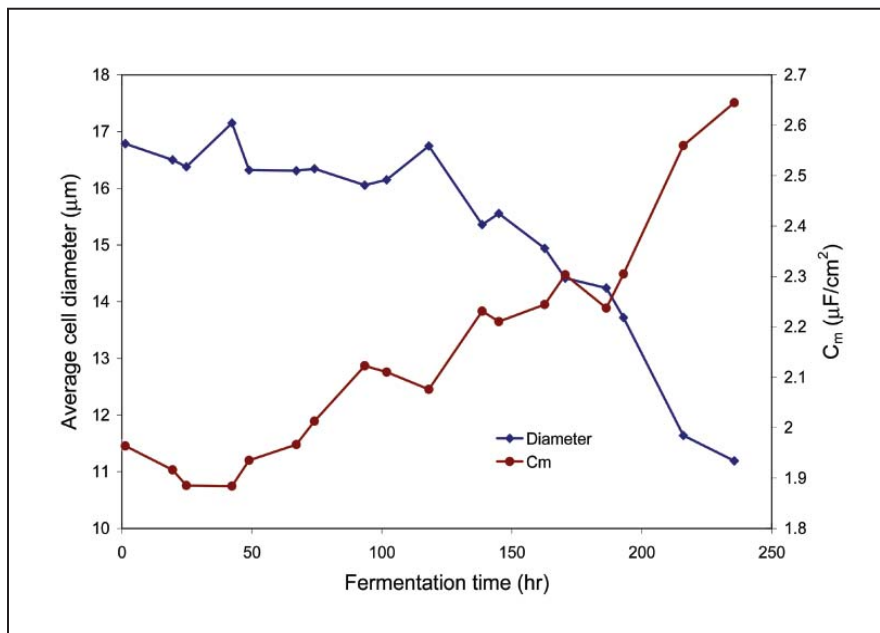


Figure 5. Changes in the average cell diameter (measured offline using a Coulter Counter Z2, Beckman-Coulter, Fullerton, CA) and calculated values of  $C_m$  during HEK293 fermentation.

that the specific growth rate decreases as the fermentation approaches stationary phase, and then turns negative as cell death overtakes cell growth. Having knowledge of such parameters online can assist the user in changing specific process variables such as the feed rate and harvesting time.

## Conclusions

Capacitance measurements are an established method for measuring viable biomass concentrations in cell culture which can be applied in process development and cGMP production. Most online measurements are based on the measurement of the capacitance at either: a) one single frequency; or b) the difference between two frequencies. In most cases, there is a good match between the capacitance signal and the live cell count. Scanning the capacitance (and conductance) over a frequency range has a lot of promise, and could prove to be a useful tool in improving the accuracy of the biomass measurement. Thus, the control and optimization of a fermentation process can be enhanced. The true value of such frequency scanning, however, will be the data made available in additional cell culture parameters. These parameters

include the critical frequency ( $f_c$ ),  $r$ ,  $C_m$  and the specific growth rate. The data can then be linked to critical events during the cell culture cycle.

## ACKNOWLEDGEMENTS

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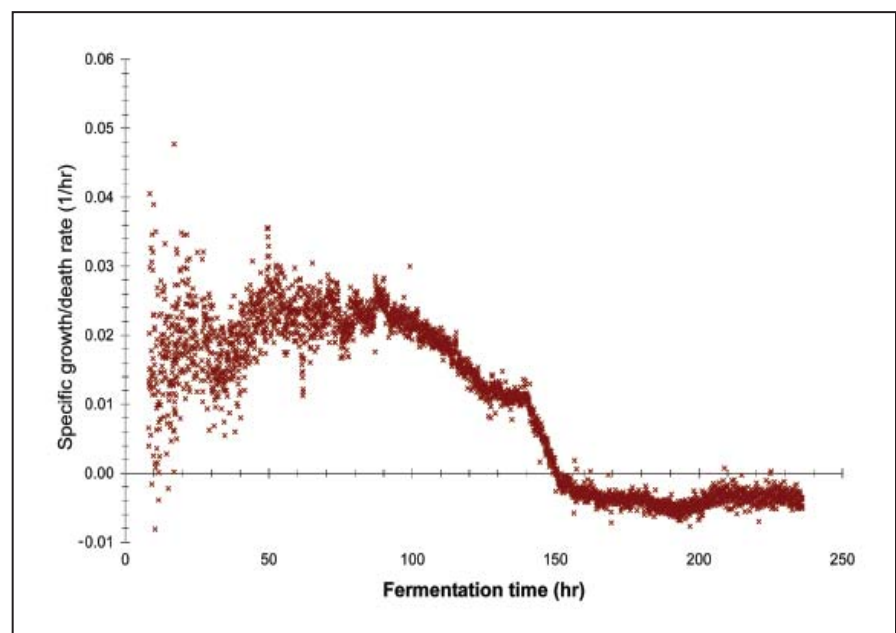


Figure 6. Online measurement of specific growth rate during HEK293 cell culture.

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