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Q1 Cell monitoring with optical coherence tomography

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ABSTRACT

Background aims: We evaluated a commercially available instrument, OCTiCell (chromologic.com/octicell), for monitoring cell growth in suspended agitated bioreactors based on optical coherence tomography. OCTiCell is an in-line, completely non-invasive instrument that can operate on any suspended-cell bioreactor with a window or transparent wall. In traditional optical coherence tomography, the imaging beam is rastered over the sample to form a three-dimensional image. OCTiCell, instead uses a fixed imaging beam and takes advantage of the motion of the media to move the cells across the interrogating optical beam.

Results: We found strong correlations between the non-invasive, non-contact, reagent-free OCTiCell measurements of cell concentration and viability and those obtained from a traditional invasive sampling based automated cell counter (Innovatis CedexHiRes), and the XTT viability assay, which is a colorimetric assay for quantifying metabolic activity.

Conclusions: This novel cell monitoring method can adapt to different bioreactor form factors and could reduce the labor cost and contamination risks associated with cell growth monitoring.

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Introduction

The high cost of biologics and cell therapies is in large part a result of their increased manufacturing cost compared with small molecule drugs [1]. This cost is in part due to the time and resource-consuming process of quality control that is required for Good Laboratory Practice/Good Manufacturing Practice facilities [2]. These quality control measures are especially critical in cell therapies such as chimeric antigen receptor-T-cell therapy [3], where quality variations often determine the effectiveness of the treatment. Typical quality controls for cell growth involve daily subsampling of the culture, adding a stain such as trypan blue to the sample to distinguish live from dead cells and then imaging the cells either manually or through automated image analysis [4]. This process introduces significant labor costs and is typically performed once per day [4]. This limits the ability of scientists and technicians to correct or restart problematic cultures. Additionally, each time the culture is subsampled, some of it is lost and the rest is put at increased risk of contamination. Some cell culture parameters can be measured in-line and non-destructively, such as temperature, pH, dissolved O₂ and dissolved CO₂ [5]. These can be measured optically with the addition of an indicator [6] as well as electrochemically with ion-selective electrodes [5]. While these sensors have shown that continuous monitoring can indicate

problems with cell growth, they cannot be used to quantify the number of cells in solution. Furthermore, these sensors are not completely non-invasive. They either require media additives or the probe to be in direct contact with the media. This undercuts the industry's move to disposable bioreactors [7]. Capacitive cell-monitoring systems are capable of inline viable biomass measurements but also require the capacitive probe to be directly in contact with the culture media [8]. Optical density monitors, unlike capacitive instruments, are completely non-invasive. However, they cannot measure cell viability or count cells, but instead estimate the biomass using infrared absorbance [9]. Raman spectroscopy has shown promise as an optical method for cell quantification [10], but cell-monitoring solutions based on Raman spectroscopy are not currently available due to difficult data-interpretation issues. Both laboratory research and clinical production would benefit from a cell-monitoring method that is completely non-invasive and reduces the labor costs associated with monitoring.

The OCTiCell system (ChromoLogic, Monrovia, CA, USA; chromologic.com/octicell) is able to monitor growing cell cultures in bioreactors using a non-invasive optical probe mounted outside of the bioreactor. This enables the user to monitor the culture without opening the bioreactor to remove samples and thereby reducing the risk of contamination. Since the system's measurements require neither consumable materials nor interaction with the user, they can be run continuously in order to give a more fine-grained picture of how the cell cultures are developing.

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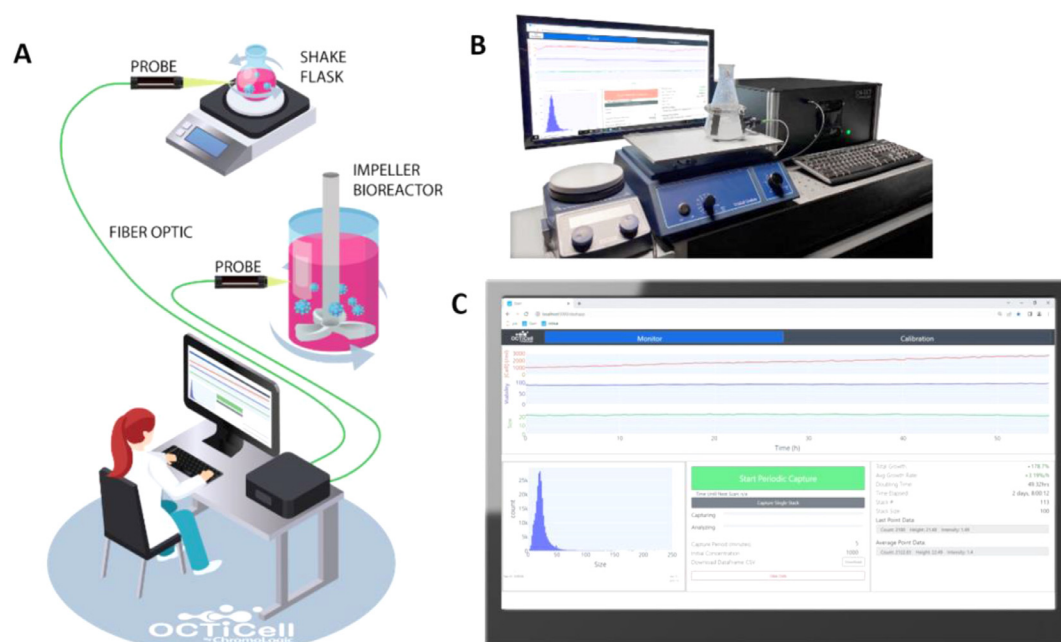


Figure 1. OCTiCell concept of operation. (A) The OCTiCell system uses a fixed OCT imaging beam that extends into a suspended cell bioreactor and detects cells as they move through the beam. The system can attach to different bioreactor types, including shake flasks, impeller bioreactors and rocking-bag bioreactors. (B) Photo of the OCTiCell system monitoring a shake flask. (C) Web-based user interface showing the changes in cell concentration, viability, size and size distribution over time.

OCTiCell is based on optical coherence tomography (OCT), an interferometric technique that can create cross-sectional images of biological samples with micron resolution [11]. The OCTiCell system is based on spectral-domain OCT, which uses a wide bandwidth light source to perform low-coherence Michelson interferometry. Traditionally, OCT systems raster the imaging beam to produce a three-dimensional (3D) image of a sample. This method has been used to monitor 3D-printed tissue growths [12], organoids [13] and scaffolded cell growths [14], and it can detect intracellular motion [15], cellular motility [16] and viability [17]. However, this method of imaging is not feasible for large-scale cell cultures in which the cells are suspended in moving media and cannot remain fixed while the image is taken. Instead, OCTiCell uses a one-dimensional OCT scan, which images cells along the direction of the imaging beam, which passes perpendicularly through the bioreactor window (Figure 1A). As the media moves, cells cross this beam and over time the system forms a distance-versus-time image that is then analyzed to determine cell concentration, size and viability (Figure S2). In this way, OCTiCell takes advantage of cell movement in order to quantify a representative sample of the cultured cells. OCTiCell uses the number of detected cells per second to calculate concentration and the cross-sectional width to determine cell size. OCTiCell also can quantify the intensity of backscattered light from each cell. We hypothesized that morphological changes such as bulging of the plasma membrane that take place during apoptosis could be detected by OCTiCell as an increase in this intensity [18]. These morphological changes make dead Chinese hamster ovary (CHO) cells identifiable in traditional light microscopy [19]. These changes give the cell more internal surfaces for the imaging beam to reflect on and therefore yield a greater OCTiCell signal intensity. Our results show that this is in fact the case.

Methods

OCTiCell system

In this study, ChromoLogic evaluated the OCTiCell system, an interferometry system for monitoring cell growths. It uses two

super-luminal diodes, which give the system a center wavelength of 890 nm and a total bandwidth of 185 nm, which results in a theoretical axial resolution of 1.85 μm . It also uses a 2048-pixel spectrometer that is sensitive to 774- to 991-nm light. The OCT system acquired axial scans (A-scans) at a rate of 6.5 kHz, which results in 12.7 z-versus-time images per second. In order to estimate cell concentration, it uses an initial concentration measurement supplied by the user and calculates subsequent concentrations based on the number of objects detected per second. For each detected cell, OCTiCell measures the cell size along the direction of the imaging beam and the intensity of backscattered light from the cell. For cell count and viability calculations, OCTiCell only considers objects that are larger than 10 μm in diameter in order to remove cell debris. It uses an initial cell concentration measurement supplied by the user to calculate the concentration of subsequent measurements based on the number of objects detected per second. Diagrams, photos and raw data from the system are shown in Figures S1 and S2.

Impeller bioreactor study

In order to test OCTiCell's ability to measure cell concentration, viability and size, we performed a preliminary study in a small impeller-bioreactor study (Wheaton Celstir Double Sidearm Spinner Flask, 38 mm, 25 mL). The bioreactor was powered by a magnetic stir plate running at 600 rpm as recommended by the manufacturer. We used 20 mL of either UT7-erythropoietin erythroleukemic cells (UT7) or CHO cells at concentrations between 2×10^4 and $80 \times 10^4/\text{mL}$. Before imaging, cells were grown as adherent cultures to 90% confluency in 75-cm² flasks in Iscove's Modified Dulbecco's Medium + 2 U/mL erythropoietin for UT7 cells or Ham's Nutrient Mixture F12 for CHO cells. Once sufficient cells had been grown, they were trypsinized and pelleted at 1500 rpm for 5 minutes before being resuspended and diluted in their respective media to the target concentration. The cells were then imaged for 40 seconds with OCTiCell. Immediately after imaging, the cells were analyzed by hemocytometer to determine cell concentration. The experiment was repeated three times to

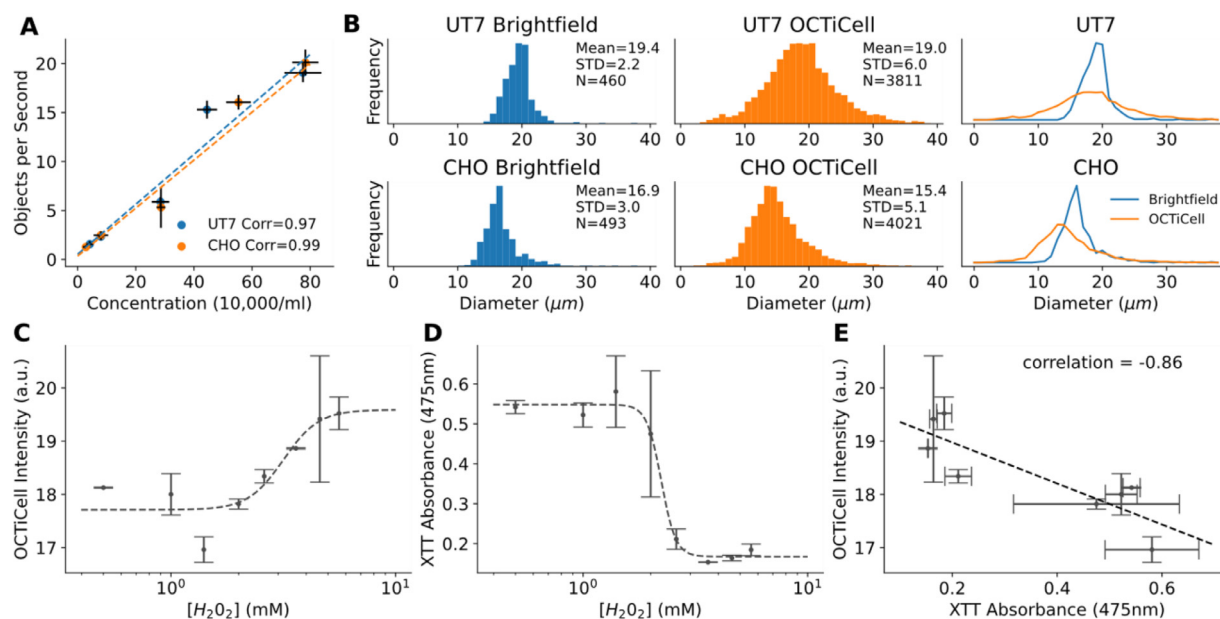


Figure 2. Analysis of suspended UT7 and CHO cells. (A) Objects detected per second in the OCTiCell system versus cell concentration as measured by hemocytometer for both UT7 and CHO cells. Dashed lines show linear fit to the data. (B) Distribution in detected sizes of UT7 and CHO cells as measured by brightfield image analysis and OCTiCell. The mean cell sizes match well with OCTiCell showing slightly lower sizes (0.4–1.5 μm lower for UT7 and CHO, respectively). The standard deviations were greater for OCTiCell (4.21 and 5.58 μm greater for UT7 and CHO, respectively). The distributions were normalized before plotting so that the y-axes are in arbitrary frequency units. (C–E) H_2O_2 was added to CHO cell cultures at varying concentrations. (C) Average cell backscatter intensity as measured by OCTiCell at each H_2O_2 concentration. (D) XTT assay absorbance for each H_2O_2 concentration. Error bars show standard deviation over two separate trials. (E) Viability measures from OCTiCell and XTT plotted against each other.

determine the experimental error, and the results are shown for varying cell concentrations in Figure 2A,B.

In order to examine the effects of cell death on the OCT signal, we added hydrogen peroxide (H_2O_2) into CHO cell cultures at concentrations between 1 and 5 mmol/L for 2 hours before measuring their viability by XTT and OCTiCell backscatter intensity. The XTT assay (Biotium 30007) uses a tetrazolium dye, which changes color when it is reduced as a result of cell metabolism [20]. The assay is a standard viability test for growing cell cultures and measures the change in absorbance of 475-nm light, with greater absorbance indicating greater viability. The results are shown in Figure 2C–E.

Shake flask bioreactor study

In order to directly compare OCTiCell measurements with conventional measurements on a growing 3D cell culture over time, we performed a growth using Human Embryonic Kidney Cells (Expi293F) growing in Expi293 Expression Medium (#A1435101; Thermo Fisher Scientific, Waltham, MA, USA) on an orbital shaker (130 rpm) in a 500-mL Erlenmeyer flask. The probe was attached to the clamp arm with an aluminum mount (Figure S1) and image distortion from probe movement was determined to be negligible ($<1 \mu\text{m}$). For comparison, the culture was split into two flasks of 250 mL, one of which was monitored with OCTiCell while the other was used to take daily 300- μL samples for analysis on an Innovatis Cedex HiRes (Cedex). The Cedex instrument uses trypan blue staining and automated brightfield imaging to analyze cells and other objects such as aggregates or cell debris. OCTiCell measurements were taken every 30 minutes over the course of the growth. The cells are typically grown for 72 hours before splitting, but we extended the growth to 160 hours in order to observe a decrease in cell viability due to starvation. The experiment was repeated three times with fresh cells. Each reading measured between 135 and 6456 cells depending on concentration (Figure 3).

Results

Impeller bioreactor study

Titration of the cell concentration showed strong correlation between the number of objects detected per second by OCTiCell and the concentration as measured by hemocytometer for both CHO cells and UT7 cells (Figure 2A, $\rho = 0.97$ UT7, 0.99 CHO). This shows the potential for objects detected per second to be used as a measure of cell concentration. We investigated these data further by comparing the distribution in cell size measured by OCTiCell with the distribution in cell size measured by brightfield microscopy using a $10\times$ objective. The brightfield images of the cells were taken immediately after imaging in our bioreactor, and the cells were not adhered to the surface. Cell sizes in the images were measured manually using ImageJ software (National Institutes of Health, Bethesda, MD, USA), and the mean cell sizes found in the brightfield images and OCTiCell differed by $0.4 \mu\text{m}$ (UT7) and $1.5 \mu\text{m}$ (CHO), which indicates that OCTiCell measurement of size is consistent with brightfield measurements of cell size (Figure 2B). However, we did note that the wider distribution (higher standard deviation) in size measured by OCTiCell indicates that there is 4.21 – $5.58 \mu\text{m}$ of non-systematic error in the measurement.

We found that cell viability drops dramatically at approximately 2 mmol/L H_2O_2 over 2 hours, as shown by the decrease in XTT absorbance and an increase in OCTiCell intensity. The experiment was performed twice using the impeller bioreactor and the two measures of viability correlated with a correlation coefficient of -0.86 (Figure 2C–E).

These results serve as a proof-of-concept for OCT quantification of moving cells. However, the experiments used cells recently transferred from two-dimensional culture and imaged at a single time point, which does not accurately represent the target environment of 3D-expanded culture.

Shake flask bioreactor study

We found that the concentration measured by OCTiCell correlated well with cell concentrations as measured by Cedex over the course

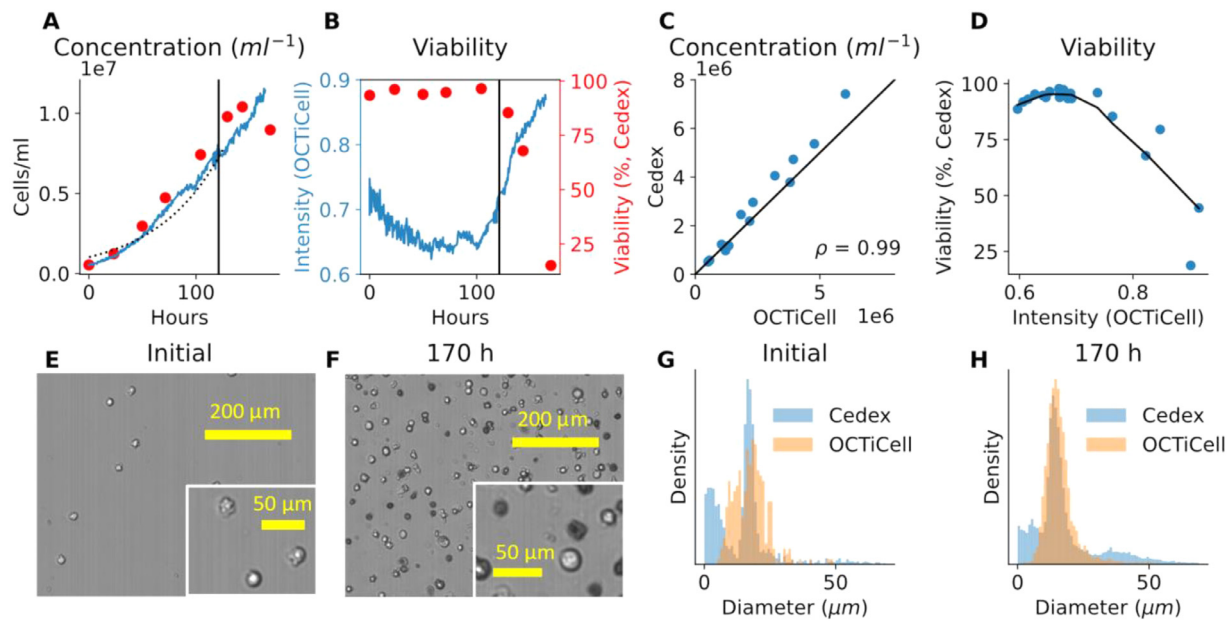


Figure 3. OCTiCell measurements correlate with Cedex standard. (A-B) Results from a 160-hour Expi293 cell growth showing OCTiCell (blue line) and Cedex measurements (red dot). Vertical lines mark the time at which the cell culture viability falls below 90% as determined by backscatter intensity. (A) Estimates of cell concentration by OCTiCell and Cedex. (B) Viability and intensity of backscattered light as measured by the OCTiCell. (C) OCTiCell and Cedex measurements of cell concentration taken with cultures with >90% viability in each of the three independent growths. Solid line indicates $y=x$. (D) Viability data from three independent growths as measured by Cedex versus intensity measured by OCTiCell. Solid line indicates LOWESS fit to the data. (E-F) Example images taken from the Cedex cell counter and used to measure the object size distribution. Cells were stained with Trypan blue, causing dead cells to appear dark. (G-H) The distribution of detected object sizes as determined by Cedex and OCTiCell at the beginning of a growth when cell viability is high (G) and after 170 hours when cell viability is low (H). Peaks for both Cedex and OCTiCell align for both time points to within $\pm 2 \mu\text{m}$. (G) $N = 1555$ (Cedex) and 345 (OCTiCell). (H) $N = 18\,693$ (Cedex) and 5156 (OCTiCell).

of the growth, as long as the detected viability was at least 90% ($\rho = 0.99$; Figure 3 A, C). Cedex estimates of cell viability decreased dramatically as expected after approximately 100 hours of growth, which coincided with the increase in backscatter intensity measured by OCTiCell (Figure 3B). The OCTiCell intensity measure was normalized using the average intensity at the start of the growth. The relationship between backscatter intensity and viability allowed us to estimate the viability by LOWESS interpolation (i.e., Locally Weighted Scatterplot Smoothing), which is a non-parametric interpolation approach chosen to create a model-free way to estimate viability from measured intensity [21] (Figure 3D).

The proprietary image analysis software used by the Cedex can calculate cell concentration, viability and distinguish living cells, dead cells, debris and aggregates. OCTiCell can only distinguish cells from other objects by size. Therefore, while we compared Cedex cell concentration measurements with OCTiCell cell concentration, we compared OCTiCell size measurements with the entire population of objects detected by Cedex. The distribution in detected objects size for OCTiCell gives a wider distribution of sizes for objects in the 10- to 20- μm range and is less accurate at detecting small (<10 μm) and large (>40 μm) objects (Figure 3 G–H).

Discussion

Application of OCT to monitor cells growing in agitated bioreactors is novel. By using the cell's motion for imaging, this method offers direct in-line quantification of cell parameters and can be adapted to different kinds of bioreactors. Continuous monitoring of suspension cell cultures offers a significant advantage over traditional invasive sampling methods as it avoids the risk of contamination and allows for the estimation of cell concentration and viability. OCTiCell can operate while the sample arm fiber is in constant motion, which would be required for an optical monitoring method to image cells in moving bioreactors such as the shake-flasks used in this work or WAVE

bioreactors [22], which grow cells in moving bags. Because the optical probe is small and doesn't contain electrical components, the technology was applied to medium-scale bioreactors such as the shake flasks demonstrated here, which operate in humidified CO_2 -filled incubators and for which there are currently no in-line monitoring solutions. The system has a relatively low power output (750 μW), and each measurement exposes only 0.0003% of the total cells to the full intensity of the beam so we don't expect significant effects from IR exposure of the cells. Since the system does not require user-interaction after the probe is aligned, the cells can be monitored continuously and remotely. It would also be possible to develop multi-probe systems that are able to track multiple cultures simultaneously by using an optical switch in the sample arm. The system's performance on Expi293 cells motivates further validation on clinically relevant cell lines and autologous cell cultures in order to establish the system's accuracy and operational limitations for each cell type and growth environment.

The current system is not without limitations, including high variation in the measured size and intensity of individual objects. We hypothesize that this variation is caused by objects passing the imaging beam at different distances from the beam center and which results in varying illumination between objects and non-uniform illumination of the objects. This makes the system able to determine the average size of objects with higher accuracy than individual objects. It also means that the sensitivity of the system may depend on the size of the object (Figure 3). It is still possible to accurately measure cell concentration despite this variation because the system is calibrated at the beginning of each growth and proportional increases in the concentration of objects of any size will result in a proportional increase in detected signal. However, if the relative proportions of objects in the media (single cells, aggregates and debris) change during the growth, this could lead to larger errors in cell concentration, as cell concentration would not be strictly proportional to measured signal. We saw this effect when cells began to die of starvation after 100 hours and the proportion of cell debris increased relative to cells.

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Q11 Declaration of Competing Interest

The authors have no commercial, proprietary or financial interest in the products or companies described in this article.

Q12 Author Contributions

Conception and design of the study: XXX. Acquisition of data: XXX. Analysis and interpretation of data: XXX. Drafting or revising the manuscript: XXX. All authors have approved the final article.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.jcyt.2022.09.008](https://doi.org/10.1016/j.jcyt.2022.09.008).

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