Evaluating the differences between optical density measurements and the oCelloScope to monitor microbial growth

Introduction

Optical density (OD) measurements of microbial liquid cultures are an established technique used in biotechnology for a wide range of applications[1,2] including antimicrobial susceptibility testing (AST) and minimum inhibitory concentration (MIC) studies, as well as in the production of recombinant proteins and fermentation processes. Since such measurements rely on the amount of light scattered by the culture, the OD value depends on the light wavelength reaching the culture. In theory, any wavelength could be chosen, as long as it is kept for all the following measurements. In practice, OD measurements of microorganism cultures are generally performed at 600 nm. This allows avoiding any wavelength which corresponds to absorption of molecules in the medium or inside bacteria (e.g. 230 nm and 260 – 280 nm for proteins and nucleic acids, respectively). Furthermore, 600 nm is a good compromise between easy availability of filters on the market and easy detection of light scattering, since longer wavelengths generate lesser scattering. OD measurements are highly dependent upon the optical system used and its geometry (e.g., area and sensitivity of the detector, distance between sample and detector, etc.)[3]. Accordingly, it is shown that spectrophotometers with different optical configurations give different OD values for the same culture[4]. Although most biotechnological applications still rely on OD measurements, researchers are showing an increased interest in alternative methods that offer analytical reproducibility and repeatability for monitoring bacterial growth and response to chemicals.

The oCelloScope is a robust, automated digital time-lapse bright field imaging system that enables rapid higher throughput, non-invasive, real-time monitoring of microbial growth and morphological features. The oCelloScope enables the scanning of volumes by recording a series of images to form an image stack where all the microorganisms are caught in focus. The system consists of the small portable oCelloScope instrument, which can fit inside standard laboratory incubators, and the UniExplorer software for instrument control and data analysis. The oCelloScope supports several types of sample containers including microscope slides and microtiter plates up to 96 wells. It is well suited for liquid samples, such as cultures of single or multiple strains as well as clinical isolates. Microbial growth kinetics can, therefore, be examined in their source environment with no requirement for additional staining. The UniExplorer software generates time-lapse videos of the acquired images, growth and growth inhibition curves, as well as performing quantitative analysis of morphological features. Up to 7 images/sec can be acquired.

Results

Fredborg et al. showed that OD measurements have a lower limit of detection of 8×10^6 cells/mL for *Escherichia coli*, while with the oCelloScope, the Segmentation and Extraction of Surface Area (SESA) algorithm allows to detect concentrations < 8×10^6 cells/mL. The SESA algorithm also allowed the detection of the statistically significant antibiotic effect on *Escherichia coli* within 6 min[5]. Additionally, the oCelloScope can exclude contaminants, dormant microbial cells and host cell from the analysis of complex cultures by using optimised algorithms. This would not be possible with conventional OD measurements. The oCelloScope can provide (i) real-time estimation of microbial growth and growth inhibition (Fig. 1), (ii) differentiation between normal growth patterns[5] and bacterial filamentation[6] (Fig. 1), and (iii) quantitative morphological analysis over time, such as cell elongation (Fig. 2), shrinking and sporulation. In fact, the oCelloScope correlates each growth curve to the respective video and, hence, it provides high specificity of analysis. The videos facilitate curves interpretation in relation to the biological events occurring in the sample. This would otherwise be impossible using OD measurements and is particularly relevant when testing β-lactam antibiotics such as penicillins, cephalosporins, carbapenems and monobactams, which typically induce morphological changes in bacteria such as filamentation and spheroplast formation.

For real-time estimation of microbial growth and growth inhibition using the oCelloScope, three different algorithms may be used. Each algorithm is designed to give specific advantages depending on analysis type and sample properties, such as cell concentration and translucency.

1. The **Background Corrected Absorption** (BCA) algorithm is based on the same principle of OD measurements but with increased sensitivity and robustness even at very low or high cell concentrations. To achieve this, the BCA algorithm corrects background intensities with respect to the first image acquired. This allows obtaining images with an even light distribution, which are used for calculating an intensity threshold. The threshold

divides pixels into 'background' and 'objects'. Growth curves are generated based on changes in 'objects' so that the effect of background intensities are significantly reduced.

2. The **Segmentation and Extraction of Surface Area** (SESA) algorithm identifies all the objects in a scan based on their contrast against the background and then calculates the total surface area covered by such objects. It is not sensitive to background intensity changes (caused by, e.g., condensation on the microtiter plate lid) and can measure microbial growth with high accuracy at very low cell concentrations. However, when more than 20% of the total image area is covered by objects, the SESA algorithm accuracy starts to decline.
3. The **Total Absorption** (TA) algorithm is designed as an equivalent of OD measurements. During microbial growth, the increasing number of objects will reduce light transmission through the sample and the image will get progressively darker. A darker image is equivalent to a higher TA value. Sensitivity is limited if compared to the BCA algorithm as growth and cell concentration need to be quite considerable before affecting light transmission.

The Normalised version of each algorithm (BCA Norm, SESA Norm and TA Norm) is also available, which subtracts the value of the first image acquired from the following images to generate the growth curve.

Fig. 1 shows the effect of cefotaxime, a third-generation cephalosporin antibiotic, on growth and length of *Escherichia coli* monitored with the oCelloScope. Growth inhibition due to the presence of the antibiotic was assessed using the SESA algorithm. In the same figure, the Segmentation Extracted Average Length (SEAL) algorithm allows early detection of antibiotic-induced filamentation. The SEAL algorithm is specifically designed to detect filamentation of rod shaped bacteria[6]. The SEAL algorithm determines the average bacterial length along the major axis and performs segmentation. This is done by (i) calculating the image contrast against the background and (ii) performing a morphological filtering on the acquired images. The SEAL algorithm is limited when bacterial cells or filaments are overlapping and may lead to inaccurate determination of bacterial length at high cell concentrations.

Fig. 2 shows an example of morphological analysis performed with the oCelloScope. Up to twenty quantitative parameters, including cell area, elongation, symmetry and optical intensity, can be used for sample characterisation.

Table 1 shows the advantages that the oCelloScope system has over conventional OD measurements.

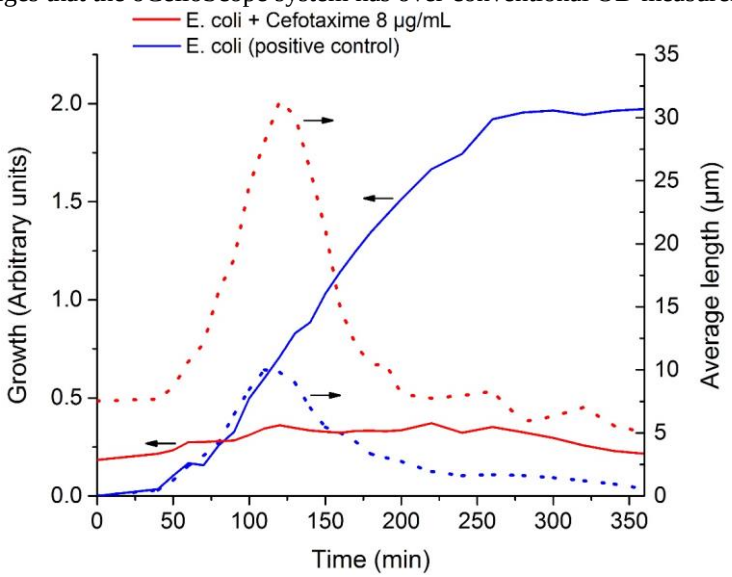

Figure 1. Effect of cefotaxime on growth and length of *Escherichia coli* monitored with the oCelloScope. Discrimination between bacterial growth kinetics (solid lines) and filamentation (dotted lines) was performed for bacteria treated with a concentration of 8 µg/mL (red lines) and for a positive control grown in plain medium (blue lines). Growth kinetics were monitored using the SESA algorithm, and variation in bacterial length was measured using the segmentation extracted average length (SEAL) algorithm.

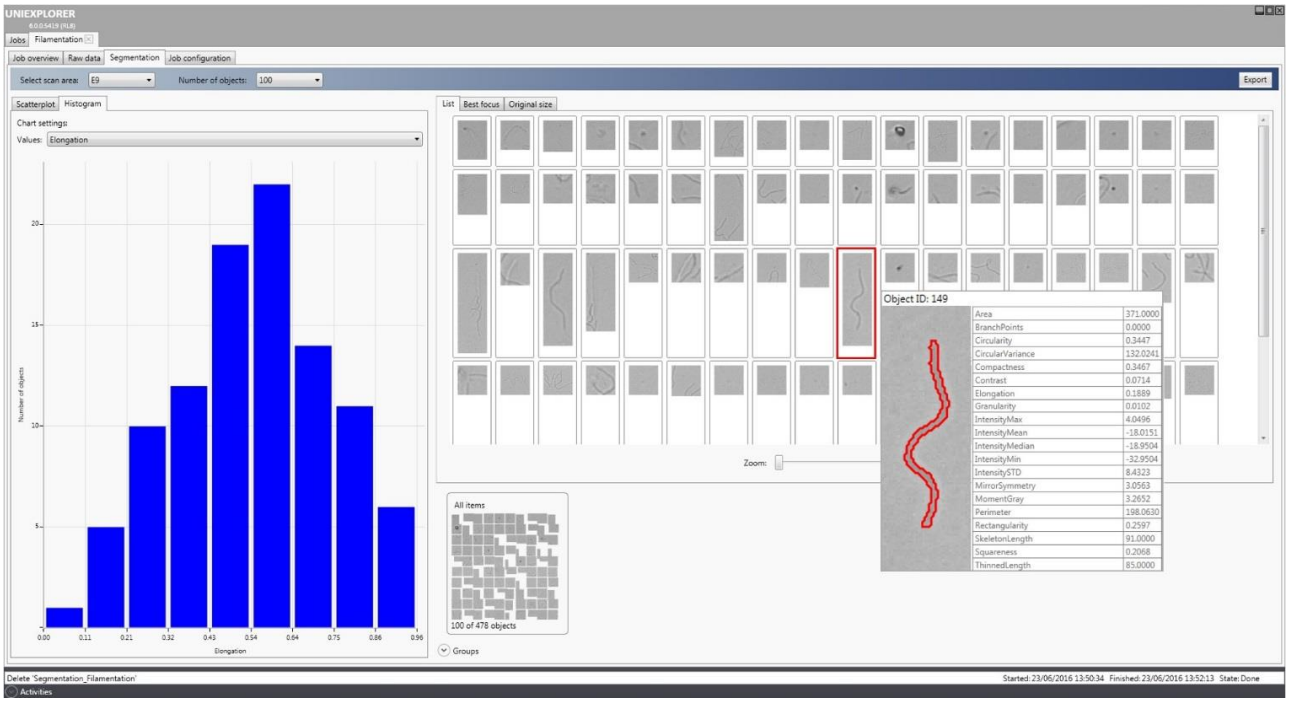

Figure 2. Morphological analysis using the oCelloScope. The histogram shows the distribution of cell elongation within the cell population for filamenting *Pseudomonas aeruginosa* incubated with 1 µM ceftazidime. Single cells and subpopulations of cells are listed to right and labeled with an ID number. For each of them, all the quantitative parameters are listed.

Table 1. Differences between oCelloScope and OD measurements for monitoring bacterial growth.

Feature	oCelloScope	Manual OD measurements	Plate Reader (OD)
Sample volume	50 – 200 µL*/entire analysis	2 – 5 mL/measurement	100 µL*/entire analysis
Sample container	1 microtiter plate/entire analysis	1 cuvette/measurement	1 microtiter plate/entire analysis
Growth/Growth inhibition curves	✓	✓	✓
Quantification of cell concentration	Relative to negative control	Relative to a blank control	Relative to a blank control
Bright field images	✓	✗	✗
Time-lapse videos	✓	✗	✗
Morphological analysis	✓	✗	✗
Analysis of mixed cultures	✓	✗	✗
Automated analysis	✓	✗	✓
On-line and off-line analysis	✓	✗	✗

*for a 96-well plate

References

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Warnings and precautions

During sample preparation, biosafety guidelines for handling biological specimens and waste should be followed. For other reagents, refer to the material data sheet from the pertaining manufacturer.

Limitations

The oCelloScope has not been validated for use in diagnostic procedures, including IVD studies. The system is for research use only.

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