Evaluating the differences between optical density measurements and the oCelloScope to monitor yeast 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 which enables rapid higher throughput, non-invasive, real-time monitoring of microbial growth and morphological features. The oCelloScope allows 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.

Facts

The oCelloScope microscope system has found a large application area in monitoring bacterial and fungal growth and growth inhibition[5–7]. The oCelloScope can acquire up to 7 images/sec and allows on-line monitoring of the microbial sample over time. With OD measurements, dormant microbial cells, host cells, complex cultures and contaminants are impossible to identify, causing biasing of the results. On the other hand, the oCelloScope can exclude contaminants and host cells by using optimised algorithms and therefore providing (i) real-time estimation of microbial growth (Fig. 1) and growth inhibition and (ii) quantitative morphological analysis over time[8,9] (Fig. 2). Morphological analysis is particularly relevant when testing morphological changes induced by antimicrobial compounds and would otherwise be impossible using conventional OD measurements.

Fig. 1 shows growth kinetics analysis of *Saccharomyces cerevisiae* using the oCelloScope. Data are shown using the background corrected absorption (BCA) algorithm, which is based on the same principle of OD measurements but with increased sensitivity even at very low or high cell concentrations. To achieve this, the BCA algorithm corrects background intensities with respect to the first acquired image. 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. The BCA algorithm may lead to inaccurate determination of growth curves when condensation (due to medium evaporation from the wells) obscures light transmission. Accordingly, condensation may result in darker images and consequently in false ‘objects’. In such case, the Segmentation and Extraction of Surface Area (SESA) algorithm is a valuable solution. In fact, the algorithms for image analysis are designed to detect cell growth with high specificity, depending on analysis type and sample properties, such as cell concentration and translucency.

In growth kinetics analysis, the oCelloScope data for growth are reported as arbitrary units and, therefore, positive and negative controls are always required for quantification. The possibility of correlating each growth curve to the respective video provides high specificity of analysis. In fact, the videos facilitate curves interpretation in relation to the biological events occurring in the sample (e.g. cell elongation, shrinking, sporulation,…). This is a concrete advantage that is not achievable when measuring OD. Furthermore, Fig. 1 shows that the oCelloScope has a wide measurable concentration range, between 1×10^3 and 1×10^6 cells/mL. However, for long term growth kinetic analysis, preliminarily experiments should be performed to determine the best starting concentration, which depends on cell properties and size. For *Saccharomyces cerevisiae* a starting concentration of 1×10^4 cells/mL may be considered.

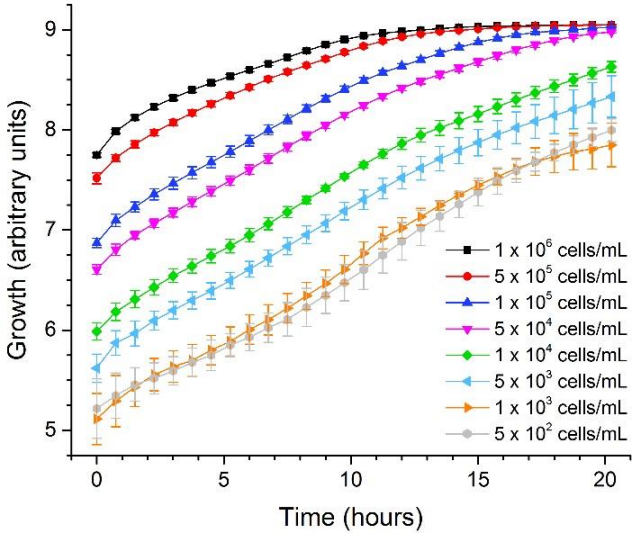

Figure 1. Growth kinetics analysis of *Saccharomyces cerevisiae* using the oCelloScope. Eight different starting concentrations ($5 \times 10^2 – 1 \times 10^6$ cells/mL) were grown in 0.22 µm filtered apple juice at 30 °C for 20 hours. Data are shown as mean ± S.D. (n = 4) using the background corrected absorption (BCA) algorithm.

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

Figure 2. Morphological analysis using the oCelloScope. The histogram shows the distribution of cell elongation within the cell population. 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.

The table below shows the advantages that the oCelloScope system has over conventional OD measurements.

Table 1. Differences between oCelloScope and OD measurements for monitoring yeast 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

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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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