Overview of the oCelloScope applications for real-time monitoring of microbial dynamics

Introduction

The oCelloScope is a walkaway system based on an open technology platform that can be scaled to a wide array of applications and research areas, providing a new, real-time method in microbiology, medicinal chemistry and drug development, as we as cell biology.

As a walkaway bright-field imaging system, the oCelloScope captures the early stages of microbial growth, visualises the dynamics of cell response to chemical and biotechnological compounds and provides quantification of morphological features together with real-time monitoring of cell growth/inhibition. The system consists of the small portable oCelloScope instrument (Fig. 1), which can fit inside standard laboratory incubators, and the UniExplorer software for instrument control and data analysis.

Figure 1 The oCelloScope system consists of the oCelloScope instrument and the UniExplorer software. The instrument has dimensions of 45 × 26 × 25 cm to allow portability and fit inside standard laboratory incubators for biological applications (temperature: 20 – 40 °C, humidity: 20 – 93%).

Applications

The oCelloScope is suitable for any kind of liquid sample including bacteria, yeasts and moulds. The samples do not require any additional staining and can be loaded in several types of containers spanning from microscope slides to microtiter plates up to 96 wells. The analysis can be performed on both liquid samples and solid cultures of single bacterial colonies growing on a semi-transparent support. Images can be used for (1) generation of time-lapse videos, (2) generation of growth and growth inhibition curves (including antimicrobial susceptibility testing, AST), as well as (3) quantitative analysis of morphological features.

Compared to conventional methods, the oCelloScope:

1. Provides detailed real-time information, presented as growth/growth inhibition curves and supported by high-quality image videos;
2. Monitors and analyses the microbial behavior[1], growth and morphological changes at a single cell level[2] under a range of different conditions;
3. Enables faster determination of the minimum inhibitory concentration (MIC) of antimicrobial compounds[3–5];
4. Facilitates the estimation of mould growth by considering the uneven distribution of hyphae and formation of spores[6];

5. Provides a richer data set to evaluate the efficacy of new antimicrobials and combinatorial drug treatments[7];
6. Reduces hands-on, incubation time and data processing[5];
7. Allows excluding irrelevant objects to the analysis (e.g., culture medium components and exogenous contaminants in clinical and environmental isolates), hence increasing the sensitivity;
8. Facilitates the handling of samples requiring a high biosafety level. The software communicates with the instrument via an Ethernet connection, which allows to set up the experiment, monitor the samples and process the data outside of the laboratory.

Rapid and sensitive detection of microbial growth by image-based algorithms

The oCelloScope measures microbial growth using advanced image-based algorithms (BCA, SESA and TA) developed by BioSense Solutions ApS and implemented in the UniExplorer software. Each algorithm is designed to give specific advantages depending on the type of analysis and the sample properties, such as cell concentration and translucency. All three algorithms produce reliable real-time visualisation of growth rates (Fig. 2 and 3).

BCA algorithm

The Background Corrected Absorption (BCA) algorithm 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.

SESA algorithm

The Segmentation and Extraction of Surface Area (SESA) algorithm identifies all the objects in a scan based on their contrast 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. Limitation: when more than 20% of the total image area is covered by objects, the algorithm accuracy starts to decline.

TA algorithm

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.

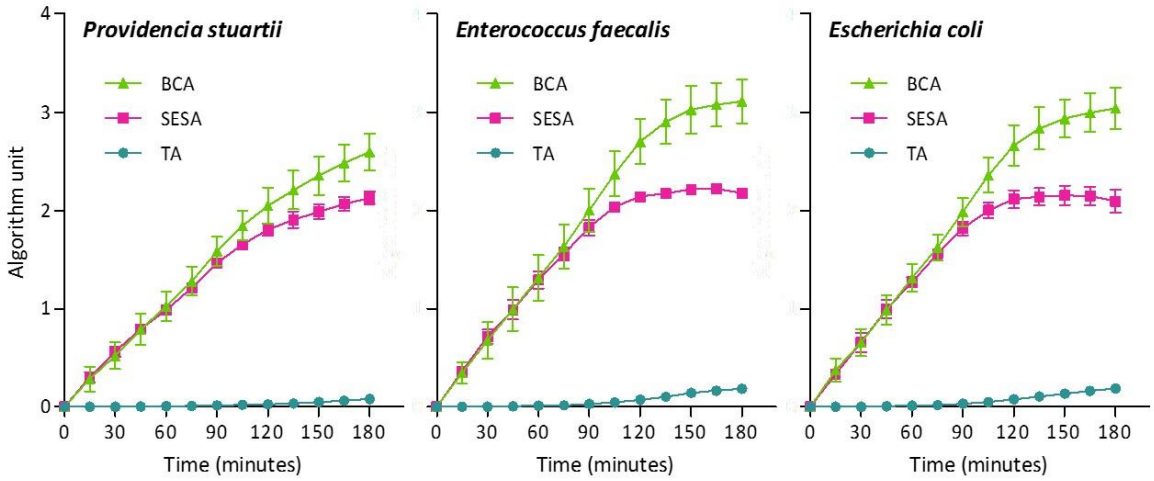

Figure 2 Growth kinetics of *Providencia stuartii*, *Enterococcus faecalis* and *Escherichia coli* incubated at 37 °C for 180 min. Bacteria inocula in exponential growth phase were diluted to 1×10^6 cells/mL and added in triplicate to a 96-well microtiter plate (100 µL/well)

before measurement of growth using the oCelloScope. Graphs show growth kinetics measured by the Normalised BCA, SESA and TA algorithms.

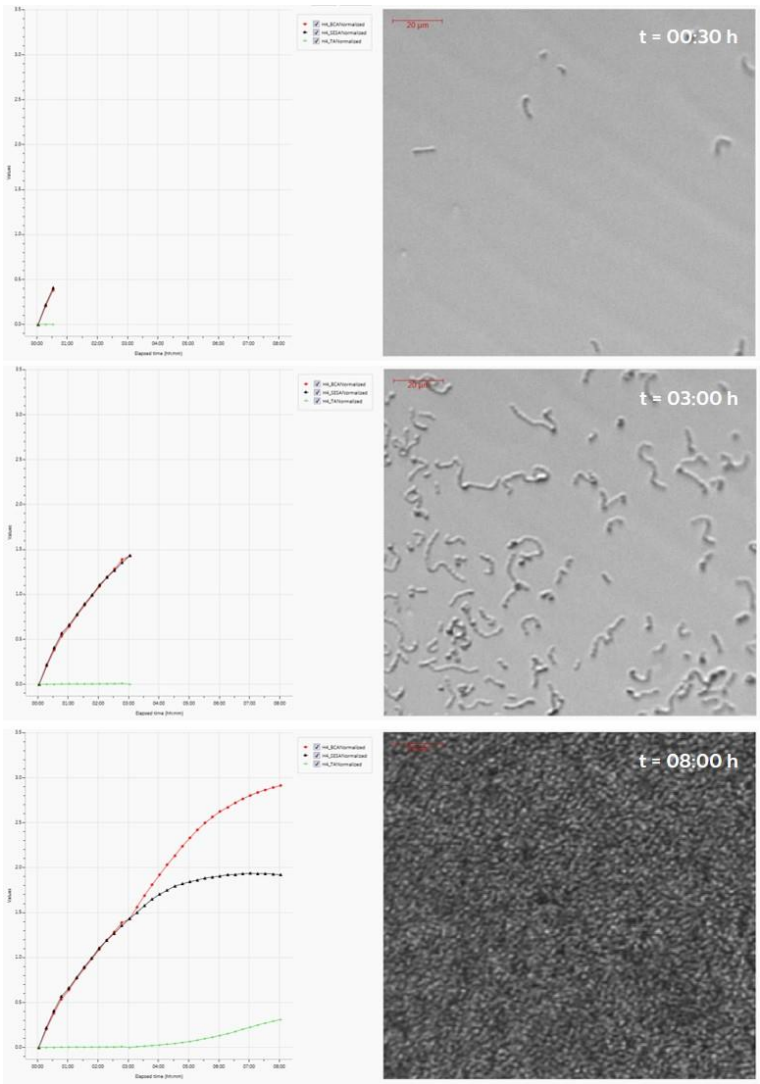

Figure 3 Growth kinetics of *Enterococcus faecalis* incubated at room temperature for 8 hours (Normalised BCA ●, SESA ●, and TA ●). Bacteria in exponential growth phase were diluted to 1×10^7 cells/mL and added in triplicate to a 96-well microtiter plate (100 µL/well). Growth was measured every 15 minutes using the oCelloScope. The BCA and SESA curves (left) clearly reflect the microbial growth shown on the corresponding image data (right).

Image-based monitoring of *Saccharomyces cerevisiae* growth using the oCelloScope

The oCelloScope facilitates in-depth microbial analysis by combing growth curves with high-quality image data of the cell population. This unique feature makes the oCelloScope an essential tool for rapid and sensitive detection of growth/growth inhibition while providing further information about growth patterns and morphology, such as in the case of budding yeast (Fig. 4).

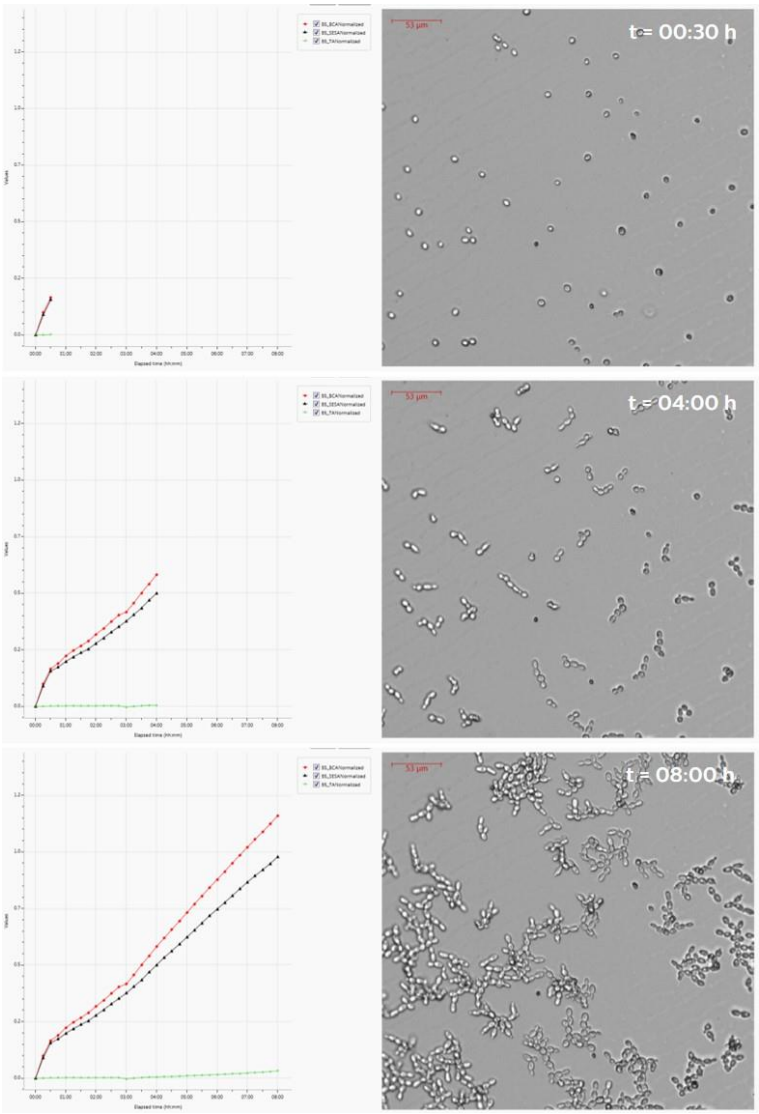

Figure 4 Growth kinetics of baker's yeast (*Saccharomyces cerevisiae*, 2.5×10^5 cells/mL) cultured in apple juice for 8 hours at room temperature. Cells were monitored every 15 minutes using the oCelloScope. For each sample, results are provided as a growth curve (Normalised SESA ●, TA ●, and BCA ●) together with image data for each time point. Image data can be shown as a video.

Germination of *Aspergillus niger* monitored using the oCelloScope

Compared to conventional methods, the oCelloScope facilitates fast real-time analysis of mould growth and growth inhibition, scanning up to 96 samples at the time[4]. Images can be used to generate growth curves and quantify morphological changes (e.g., mould perimeter, area of spores, growth and branching of hyphae). Therefore, the development of a single spore can be followed by considering (1) the germination time, (2) the increase in size of spores and hyphae fragments over time and (3) morphological differences between treated and non-treated samples with antimicrobial agents. Aunsbjerg et al.[6] showed that the image analysis performed with the oCelloScope allows detection of mould growth within 15 hours, compared with more than 30 hours when using standard OD measurements. Fig. 5 shows the morphological development of *Aspergillus niger* over time.

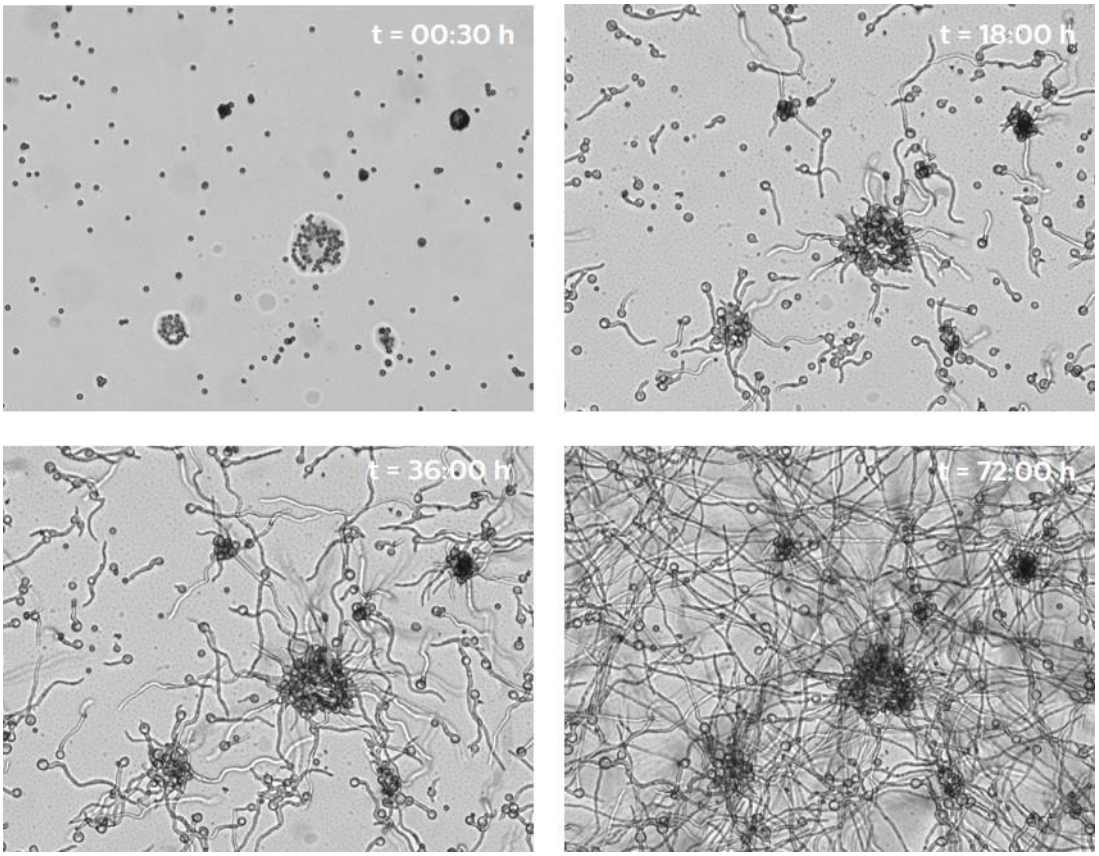

Figure 5 Morphological development of *Aspergillus niger* grown on Sabouraud liquid medium. The medium was inoculated with conidiospores and incubated at 30 °C for 72 hours.

Real-time monitoring of morphological features using the oCelloScope

In addition to growth curves and image data, the UniExplorer software allows real-time monitoring of up to 20 different morphological features at the same time. These include: area, circularity, elongation, branch points, granularity and skeleton length and find application in real-time monitoring of, e.g, cell elongation, spore germination, yeast budding and hyphae branching (Fig. 6). During the segmentation analysis, the single morphological features, or combinations of them, can be used to achieve fine identification and differentiation within the cell population based on morphological characteristics. Hence, the oCelloScope allows combining real-time growth kinetics analysis and segmentation analysis to obtain unique and comprehensive information of the dynamics associated to cell response to chemical and biotechnological compounds.

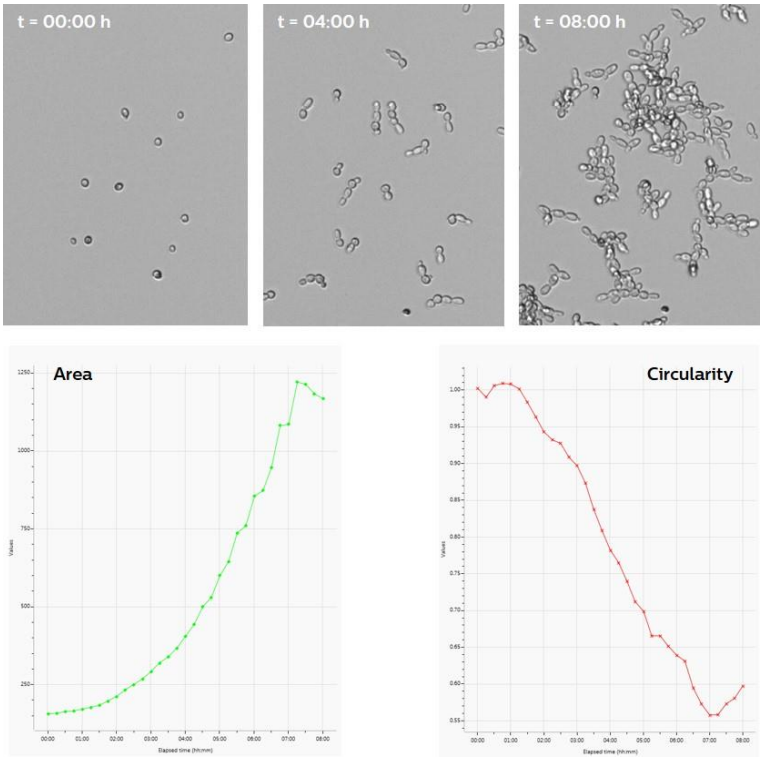

Figure 6 Cell development of baker's yeast (*Saccharomyces cerevisiae*, 2.5×10^5 cell/mL) cultured in apple juice for 8 hours was monitored every 15 minutes using the oCelloScope. In addition to growth curves, cell development was measured as change in cell area (●) and circularity (●) over time. Results show that cell area increased while circularity decreased during culturing. This is in accordance with the recorded images showing single circular cells developing into larger groups of budding yeast cells. The oCelloScope provides real-time monitoring of up to 20 different morphological parameters at the same time.

Real-time monitoring of bacterial filamentation using the oCelloScope and implications in AST

Early determination of antimicrobial susceptibility is complicated in the case of filamentous bacteria such as *Escherichia coli*. In fact, when using conventional optical density (OD) measurements, their elongation in shape due to the presence of antibiotics may be wrongly interpreted as growth. Fredborg et al. showed that the oCelloScope is able to provide early and clear detection of piperacillin/tazobactam-induced filamentation within only 30 min[2].

To allow bacterial filamentation analysis, the UniExplorer software includes the Segmentation and Extraction of Average Length (SEAL) algorithm which (1) determines the mean bacterial length in micrometers and (2) uses contrast-based segmentation followed by morphological filtering on a projected Z-stack image to measure the major axis length of each bacterium. However, an inaccurate determination of bacterial length may be obtained if (i) cells are overlapping, (ii) the cell concentration is too high or (iii) bacterial filaments are crossing each other.

A second version of the SEAL algorithm is the Normalised SEAL (SEAL Norm) algorithm which is computed in the same way as SEAL, except that the value of the first image is subtracted from the following images on the curve (Fig. 7). SEAL Norm curves are calculated for each individual scan area and always start at point 0, independently of the initial average length value.

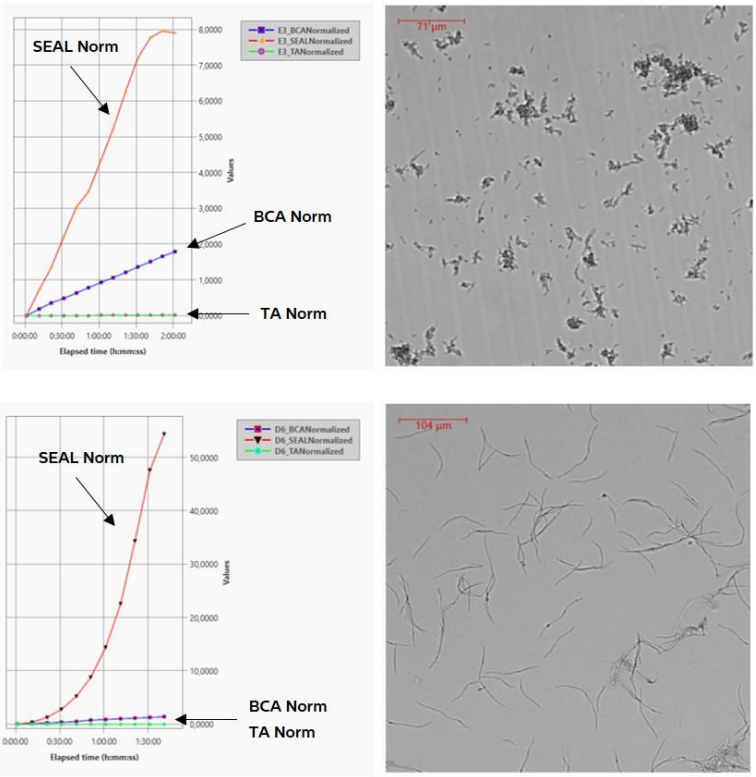

Figure 7 Effects of β-lactam–β-lactamase inhibitor combinations on bacterial length of *Escherichia coli*. The Normalised SEAL algorithm allows to specifically monitor variations in the average bacterial length over time. On the other hand, the Normalised BCA and TA algorithms allows the real-time monitoring of bacterial growth.

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.

Liability disclaimer

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