

Automated monitoring of bacterial growth/growth inhibition

Product description

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 system consists of the small portable oCelloScope instrument, which can fit inside standard laboratory incubators, and the UniExplorer software.

Application

The oCelloScope can be used for non-invasive, sensitive and rapid image-based detection of bacterial growth. It supports several types of sample containers including microscope slides and microtiter plates up to 96 wells. The oCelloScope is well suited for liquid samples, such as cultures of single or multiple strains as well as clinical isolates. Therefore, microbial growth kinetics can be examined in their source environment with no requirement for additional staining.

The oCelloScope system allows scanning of volumes by recording a series of images to form an image stack where all the microorganisms are caught in focus. 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.

Introduction

There is an increasing demand for sensitive and rapid methods to detect and monitor microbial growth/growth inhibition over time. The oCelloScope is an easy-to-use portable imaging system delivering rapid and real-time analysis of microbial growth. Compared to conventional optical density measurements, the oCelloScope allows for deeper investigation of bacterial growth/growth inhibition as it couples quantitative information to images describing changes in morphology over time.

The oCelloScope system can measure growth at a single cell level by excluding image background noise from irrelevant elements. Hence, it is possible to analyse complex and inhomogeneous samples of microorganisms in their source environment. The visualisation of low bacterial concentrations improves the ability to capture the early stages of growth that could not otherwise be detected with optical density measurements. Furthermore, since the oCelloScope supports several types of 96-well plates, it is possible to measure different samples in the same run, making the technology optimal for e.g. screening of antibiotic susceptibility.

The UniExplorer software uses advanced image-based algorithms (TA, BCA and SESA) for accurate real-time visualisation of growth kinetics. Each algorithm is designed to provide specific advantages depending on analysis type and sample properties required, such as cell concentration and translucency. Examples on growth curves obtained with the oCelloScope and the associated UniExplorer software are shown in Fig. 1. For details concerning each of the available algorithms, please refer to the user manual, Section 6.1.

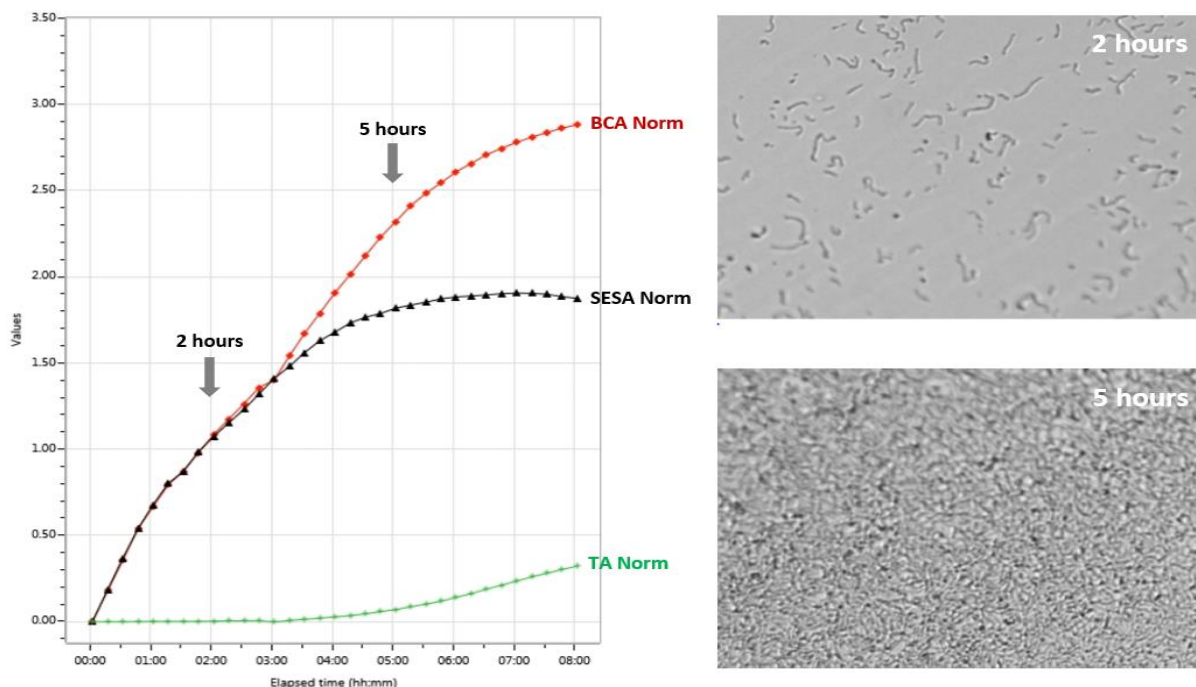


Figure 1. Growth kinetics of *Enterococcus faecalis* incubated at room temperature for 8 hours. Bacteria in exponential growth phase were diluted to 1×10^7 cells/mL and added in triplicate to a 96-well microtiter plate (100 μ L/well). Growth was measured every 15 minutes using the oCelloScope. Growth data are reported using the normalised version of BCA, SESA and TA algorithms.

Procedure

General guidelines for sample preparation prior to oCelloScope analysis are reported in Chapter 3 of the user manual. Procedures can be optimised depending on the sample source and the bacterial strains.

To perform bacterial growth/growth inhibition studies using the oCelloScope:

1. Place the oCelloScope in a standard laboratory incubator for precise regulation of temperature and humidity. – This step is optional depending on the requirements of the sample under investigation.
2. Introduce the sample container loaded with the samples in the sample holder, tighten firmly and close the instrument lid. The recommended total volume of bacterial suspension to add to each well is 50 – 200 μ L. Smaller volumes can be used as long as the sample fully covers the bottom of the well during the entire period of analysis.
3. Configure the UniExplorer software for time-lapse analysis by clicking the ‘New job’ button in the top left corner of the main window.
4. Give the new job a name, then choose the acquisition modules from the list to the left by double clicking (or dragging) the icons in the following order: (i) ‘Acquire’ (for recording new image data) and (ii) ‘Growth Kinetics Analysis’ (for monitoring bacterial growth/growth inhibition over time). Press ‘Next’.
5. Let the UniExplorer software recognise the oCelloScope instrument by selecting the instrument to use from the instrument list with a left click. Press ‘Next’ to continue with experimental setup.
6. Select the sample container type (e.g., ‘96 wells, Costar® Corning 3596’) from the list of available sample containers.
7. a) Select and enable the wells that should be included in the analysis with the cursor. Enabled wells are shown as blue: bacterial growth/growth inhibition is only monitored for enabled wells.
b) Select the object size to ≤ 20 μ m for measurement of bacteria.
8. a) Set illumination level: the default value of 2 ms is usually the best solution.

- b) Find the focus using one of the three algorithms. ‘Bottom search’ is suitable when working with well plate types included in the UniExplorer list. ‘Full sweep’ searches the bacteria vertically through the full focus range to find the best focus. ‘Fine tune’ is commonly used after manual focus adjustments to automatically refine the focus around the one that has been manually set.
9. Set the scan area for each well. The scan area is shown as an orange rectangle and placed in the centre of each well by default. Multiple scan areas can be added in different positions in each well and labelled with customised names.
 10. Adjust the time of analysis by selecting the number of acquired images (‘Number of repetitions’) and the time interval between two sequential images (‘Repetition interval’). The first image is taken at $t = 0$. By choosing ‘Multiple repetition intervals’, two separate phases of analysis can be set, each one having a specific ‘Repetition interval’. The two repetition intervals can be adjusted according to the expected phases of bacterial growth, which depend on microorganism type and growth conditions.
 11. Select or deselect the algorithms to use for image analysis by ticking the boxes. For detailed description, refer to Chapter 6 in the user manual. Press ‘Start’ to start the experiment.
 12. Curves for real-time growth/growth inhibition as well as image data and time-lapse videos can be inspected and compared during the experiment in the ‘Current scan’ tab.

Refer to the user manual for further details on how to set up a new experiment (Chapters 4 and 5) and perform image analysis (Chapter 6).

Troubleshooting

Your oCelloScope is not displayed on the instrument list

Check cables and power. If the instrument is still not displayed, press ‘Refresh instrument list’. Otherwise, IP address of the instrument (e.g., 012.34.56.789) can be manually typed.

Imprecision of the autofocus

Check that the sample container is not scratched or contaminated and the right type of container has been selected when setting the analysis. When analysing samples with few and/or small bacteria, it may be beneficial to add polystyrene beads of an appropriate size to facilitate the focusing process. During the analysis, the segmentation algorithm will be able to ignore the beads.

Poor image quality

Image quality is strongly affected by condensation. The sample container should optimally be conditioned at the same temperature and humidity of the incubator or environment where the experiment will be performed. Therefore, it is recommended to let the instrument and sample container equilibrate inside the incubator for at least 2 hours before performing any measurements to avoid condensation.

The equipment is sensitive to vibrations and tremours. Place the oCelloScope on a stable level surface to avoid disturbance of samples during measurements.

Keep the top and bottom surfaces of the sample container as clean as possible. Protect the oCelloScope from exposure to dust and other fine particles that may interfere with the optical system.

For further details on trouble shooting of technical issues, please refer to the user manual, chapter 8.6

Maintenance

The instrument surface should be frequently cleaned to avoid cross-contamination between samples and to avoid bio hazard. An alcohol based solution, e.g. 70% ethanol, and a smooth lint free wipe should be used. Do not use any other organic solvent.

The optical components and the illumination lens do not usually need to be cleaned.

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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