



Quick guide to:

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/growth inhibition. 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 setting of the experiment, image analysis and generation of time-lapse videos can be performed through a dedicated software (UniExplorer, BioSense Solutions ApS). The UniExplorer software communicates with the instrument via an Ethernet connection, which can favourably reduce the time required in the laboratory when working with hazardous microorganisms. The acquired images can be processed for online and offline quantification of several morphological parameters, microbial growth and inhibition over time. The output data can also be exported to Microsoft Excel or in the CSV format for further processing.

How to get started – UniExplorer installation

For PC requirements and details about UniExplorer installation, refer to Chapter 2.2 in the user manual.

To install the UniExplorer software:

1. Insert the USB stick that came with UniExplorer.
2. Locate the file “Install.UniExplorer-x.x.x.xxxx-RL9.exe” using Windows Explorer.
3. Double-click the file to start the installation.
4. Follow the instructions on the screen.
5. Accept the terms of use.
6. Select location to install UniExplorer.
7. Select location for Datastore.

Note: All data will be saved in Datastore. A minimum 1Tb of free disk space is needed.

- 7.1 To increase data speed, save Datastore on a high-speed access drive with no operating system installed. USB drives are not recommended.
8. Terminate installation. No user interaction is needed.

How to perform growth kinetic analysis to monitor bacterial growth/growth inhibition

General guidelines for sample preparation prior to oCelloScope analysis are stated 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 μL . 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 (Fig. 1).
 - b) Select the object size to $\leq 20 \mu\text{m}$ 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.

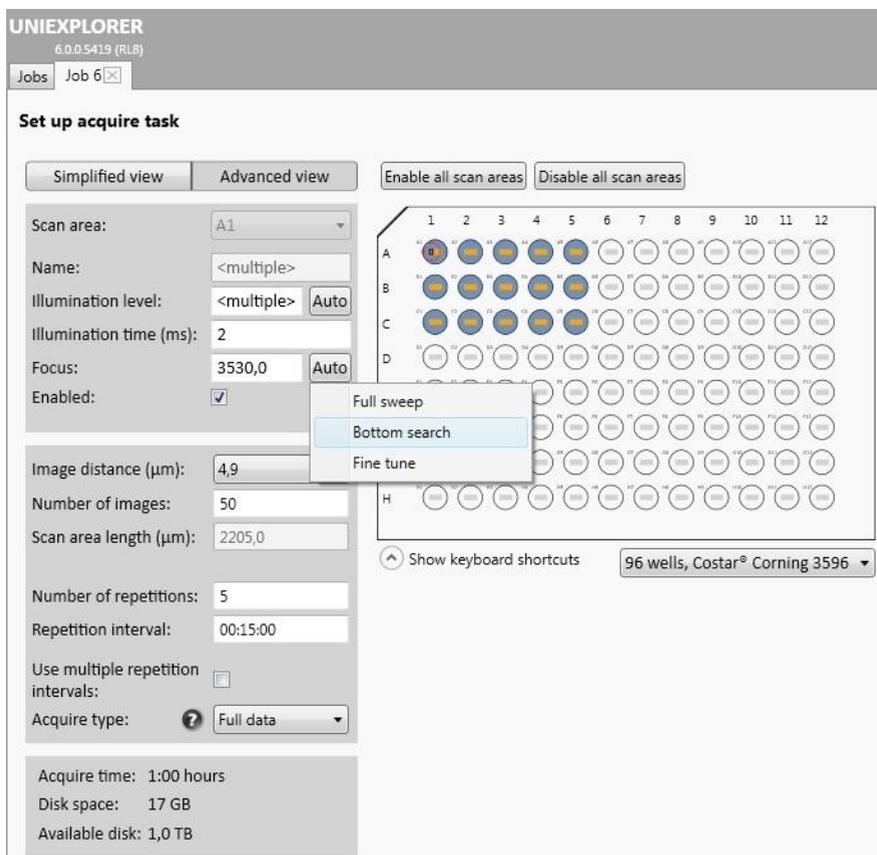


Figure 1. In order to start the analysis, the sample container type as well as the wells of interest should be chosen. Then, set the illumination level and find the focus (‘Bottom search’ is recommended when working with microtiter plates among the suggested ones in the available sample containers list).

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.

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

How to perform data analysis

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 give specific advantages depending on analysis type and sample properties, such as cell concentration and translucency. A normalised version of each algorithm (Norm) is also available. The normalised version is computed in the same way as the respective algorithm but the value of the first image is subtracted from the subsequent ones to build the growth/growth inhibition curve (Fig. 2).

The available algorithms to use for image analysis are described briefly below:

- The Total Absorption (TA) algorithm is an equivalent of optical density measurements at 600 nm (OD_{600}). During microbial growth, the increasing number of bacteria will reduce light transmission through the sample and the image will get progressively darker. Any darker image corresponds to a higher TA value. TA sensitivity is limited if compared to the BCA algorithm (described below) as growth and cell concentration need to be quite considerable before affecting light transmission.
- The Background Corrected Absorption (BCA) algorithm is an equivalent of OD_{600} but with increased sensitivity even at very low or high cell concentrations. To achieve such performance, the BCA algorithm considers any variation in the background intensity relative to the first acquired image. Hence, an even light distribution in the images can be obtained, which is used for calculating a threshold pixel value. Such threshold value divides image pixels into pixels belonging to the background and pixels belonging to the microorganisms. Growth curves are generated based on changes in the pixels belonging to the microorganisms. Therefore, the BCA algorithm is able to determine microbial growth/growth inhibition with

high sensitivity as the influence of the background intensity is significantly reduced compared to the same analysis performed with the TA algorithm.

- The Segmentation and Extraction of Surface Area (SESA) algorithm identifies all the objects in a scan based on their contrast with the background and then it calculates the total surface area covered by such objects. It is insensitive to variations in the background intensity (caused by, e.g., condensation on microtiter plate lid) and it is able to measure microbial growth with high accuracy at very low cell concentrations. However, when more than 20% of the total image area is covered with bacteria, the algorithm accuracy starts to decline.
- The Normalised version of each algorithm (Norm) is computed in the same way as the respective algorithm but the value of the first image is subtracted from the subsequent ones to build the growth/growth inhibition curve.

Examples of growth curves obtained with the oCelloScope and the associated UniExplorer software are shown in Fig. 2.

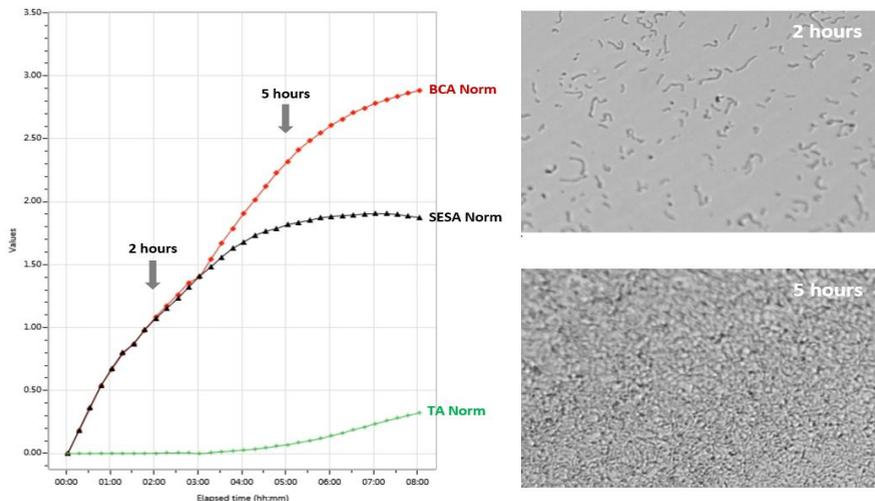


Figure 2. Growth kinetics of *Enterococcus faecalis* incubated at room temperature for 8 hours. Bacteria in exponential growth phase were diluted to 1×10^7 cells/mL and added in triplicate to a 96-well microtiter plate (100 μ L/well). Growth was measured every 15 minutes using the oCelloScope. Growth data are reported using the normalised version of BCA, SESA and TA algorithms.

For further details concerning each of the available algorithms, refer to the user manual, Chapter 6.1.

How to export data for presentations

After analysis, the recorded growth/growth inhibition curves as well as image data and videos can be exported for further processing and presentation (Fig. 3).

1. Press the ‘Export’.
- 1.1 Select ‘Export to Excel’ or ‘Export to CSV’ to export growth/growth inhibition values as Excel files or in the CSV format respectively.
- 1.2 Select ‘Export chart’ to export graphs as BMP files.
- 1.3 Select ‘Export’ and ‘Export video’ to export time-lapse videos in the AVI format.
2. Specify the file destination and save.

For further details concerning how to explore and export data, refer to Chapter 7.2 in the user manual.

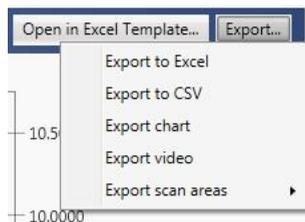


Figure 3. The UniExplorer software allows to export the recorded growth/growth inhibition curves as well as image data and videos for further processing and presentation.

Trouble shooting

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 the IP address of the instrument (e.g., 012.34.56.789) can be typed manually.

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 tremors. 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.

Interruption of data analysis

To avoid interruption of data analysis, make sure to disable automatic restart after downloading Windows updates to prevent the hard disk from going to sleep. Exclude the DataStore folder from the antivirus protection to allow fast execution. To allow communication with the oCelloScope instrument, allow firewall reconfigurations automatically created by UniExplorer.

For further details on trouble shooting of technical issues, 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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