# oCelloScope technology

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 technology is well suited for liquid samples such as microbial cultures of bacteria, yeasts and moulds as well as mammalian cell cultures and other kind of analytes (e.g., spores, pollen and drug crystals). The method relies on an imaging system that consists of a digital camera, an illumination unit and a lens where the optical axis is tilted 6.25° relative to the horizontal plane of the stage (Fig. 1 and 2). The tilting of the optical axis grants more freedom of operation at both high and low concentrations of the analyte.

The whole 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. 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.

### How it works

When considering, for instance, a bacterial suspension in a microwell (Fig. 1), the oCelloScope acquires a sequence of 6.25°-tilted images along the horizontal plane to form an image Z-stack. The stack contains the best-focus image, as well as the adjacent out-of-focus images (which contain progressively more out-of-focus cells, the further the distance from the best-focus position). The acquisition process is repeated over time, so that the time-lapse sequence of best-focus images is used to generate a video.

All the acquired images are saved and used for data analysis. Both the best-focus Z-stack layer and all the out-of-focus Z-stack layers are analysed by the growth kinetic algorithms based on *measurement of light absorption* (i.e., the same principle of optical density, OD, measurements), which are the background corrected absorption (BCA) and the total absorption (TA) algorithms.

- The *BCA algorithm* provides increased sensitivity and robustness than OD measurements, even at very low or high cell concentrations. To achieve this, the BCA algorithm corrects background intensities with respect to the images acquired at the first time point. 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 value is calculated as

$$BCA\ value = \log_{10}\left(\sum(object\ pixel\ intensities)\right)$$

- The *TA algorithm* is designed as an equivalent of OD measurements. During microbial growth, the increasing number of microorganisms 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 TA value is calculated as

$$TA\ value = \log_{10}\left(\sum(pixel\ intensities)\right)$$

Only the best-focus Z-stack layers are analysed by the growth kinetic algorithms based on *object-based image analysis* (OBIA), which are the segmentation and extraction of surface area (SESA) and segmentation and extraction of average length (SEAL) algorithms. In general terms, OBIA employs two main processes: segmentation and classification. OBIA groups image pixels into homogeneous ‘objects’, which can have different shapes and intensity scale. The ‘objects’ are also associated with statistics that can be used for classification of such ‘objects’ and include geometry, context and texture.

- The *SESA algorithm* uses the simplest method for image segmentation, which is called ‘linear thresholding’. The 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. Therefore, this method is not based on absorbance but uses the object covered area, so that it is not affected by background intensity changes (such as shadow effects caused

by, e.g., condensation on the microtiter plate lid and air bubbles in the culture medium) and can measure microbial growth with high accuracy at very low cell concentrations. The SESA value is calculated as

$$SESA\ value = \log_{10}\left(\sum(object\ covered\ area)\right)$$

However, when more than 20% of the total image area is covered by objects, the SESA algorithm accuracy starts to decline. The SESA algorithm gives faster results compared to conventional OD measurements. Finally, since 'linear thresholding' uses object surface area, this method cannot handle out-of-focus images.

- The *SEAL algorithm* is specifically designed to detect filamentation of rod shaped bacteria. The SEAL algorithm performs segmentation to identify all the 'objects' in a scan and determines their average length. The segmentation approach is identical to the SESA algorithm ('linear thresholding'). The SEAL value is calculated as

$$SEAL\ value = \frac{\sum(object\ length)}{number\ of\ objects}$$

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.

As SESA and SEAL algorithms, the oCelloScope *segmentation analysis* is also based on OBIA. OBIA is performed on the best-focus layers of the Z-stacks to identify and quantify up to twenty different morphological features (e.g., area, eccentricity, symmetry, …) of individual 'objects' (or group of objects), such as microbes, spores and cells. Moreover, it is also possible to monitor the development of such morphological features over time with the *segmentation kinetics analysis*.

In the following page, Fig. 1 shows the oCelloScope technology applied to a bacterial suspension.

When all the bacteria are sedimented at the bottom of the microwell (Fig. 2), all the bacteria will be in focus along the horizontal plane. Therefore, the image Z-stack will contain the all-in-focus image as well as the adjacent out-of-focus images along the vertical axis. The generation of video and data analysis are the same shown in Fig. 1.

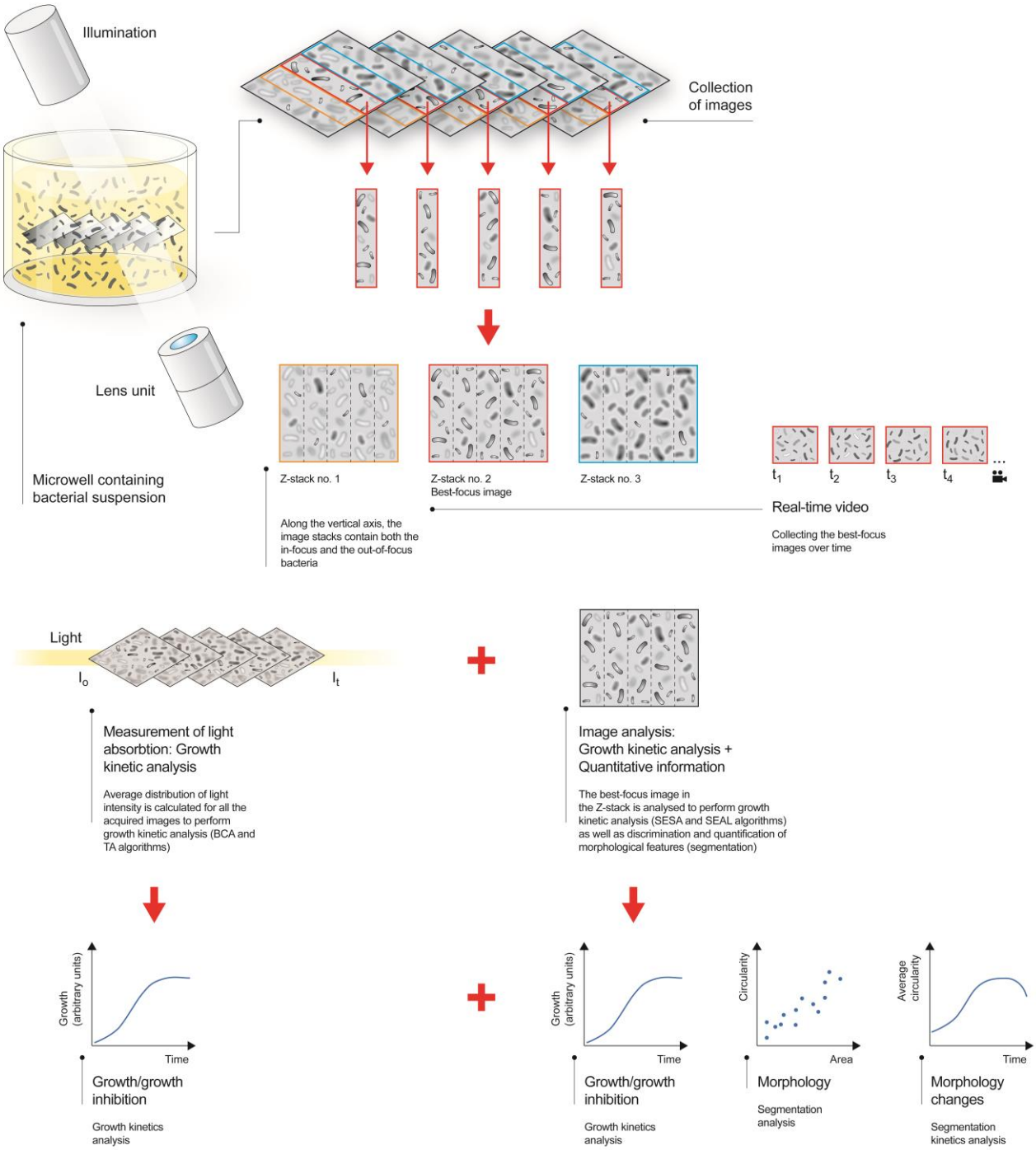


**Figure 1.** Schematic showing the oCelloScope optical scanning technology and image analysis applied to a microwell containing a bacterial suspension.

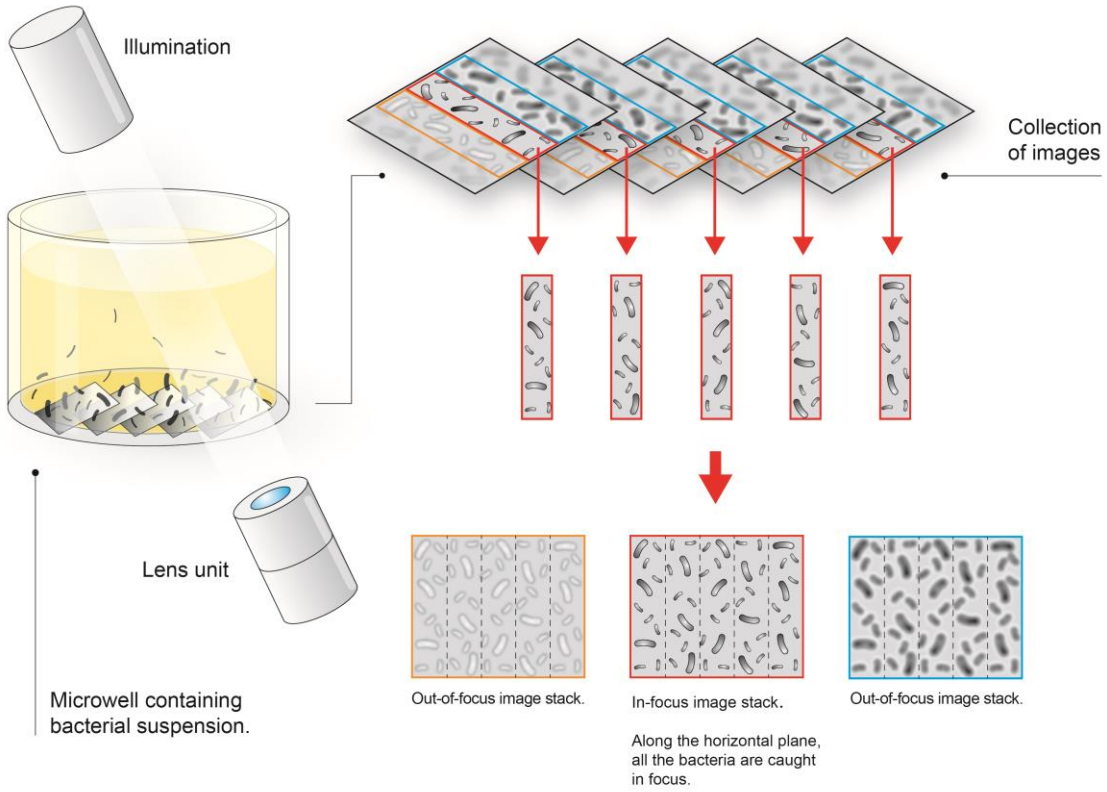


**Figure 2.** Schematic showing the oCelloScope optical scanning technology and image analysis applied to a microwell containing bacteria sedimented at the bottom.