oCelloScope vs. OD plate reader

Introduction

The Optical Density (OD) detection limits for standard bacterial cultures like *Escherichia coli* is extremely variable in the literature and lies between $2 \cdot 10^6$ and $8 \cdot 10^6$ cells/ml. The detection limits vary according to the optics and instruments[1]. Although most biotechnological applications still rely on OD measurements, researchers are showing an increased interest in alternative methods that offer rapid analytical capacity and reproducibility to overcome the high request of new antibacterial compounds.

The oCelloScope is an automated digital real-time bright field imaging system that enables rapid monitoring of microbial growth and morphological features. The UniExplorer software which controls the instrument and data analysis has embedded image analysis algorithms that recognise the objects and accurately tracks the growth or inhibition of bacteria, yeast, fungi or mammalian cells. The UniExplorer software can also generate time-lapse videos of the acquired images, as well as performing quantitative analysis of morphological change.

oCelloScope 250 times more sensitive than OD plate readers.

The UniExplorer offers multiple algorithms used in microbiology for monitoring growth: TA and BCA are both based on absorbance, where TA is designed to be equivalent to OD and BCA designed with much greater sensitivity. (visit www.biosensesolutions.dk for more details). A comparison study between the Thermo-Fisher Scientific VarioSkan plate reader and oCelloScope have been made, measuring the growth of *E. coli* every 15 min, in triplicates with 5 different initial inoculums, 10^7, 10^6, 10^5, 10^4 and 10^3 CFU/ml. The minimum detection limit of *E. coli* cells necessary to induce the OD signal with the plate reader is measured to $2 \cdot 10^6$ CFU/ml, which corresponds to the minimum detection limit of *E. coli* found in literature [2,3].
By contrast, the BCA algorithm detects the bacterial growth immediately for 10^4 CFU/ml (Fig.1-A). Using start inoculum of 10^3 CFU/ml (Fig.1-B), growth is detected after 45 min, corresponding to $8 \cdot 10^3$ CFU/ml, a factor of **250 times** greater sensitivity than the minimum detection limit obtained by the plate reader.

This great difference in **sensitivity** implies that using the oCelloScope in combination with the BCA algorithm results can be achieved much faster. Accordingly, *McLaughlin H. et al.* (2017), at the U.S. Center of Disease Control (CDC), concluded that with the oCelloScope they calculated the M.I.C. value for *B. anthracis* in only 4 hours, which is a **time-to-result 4 times faster** than standard methods[4].

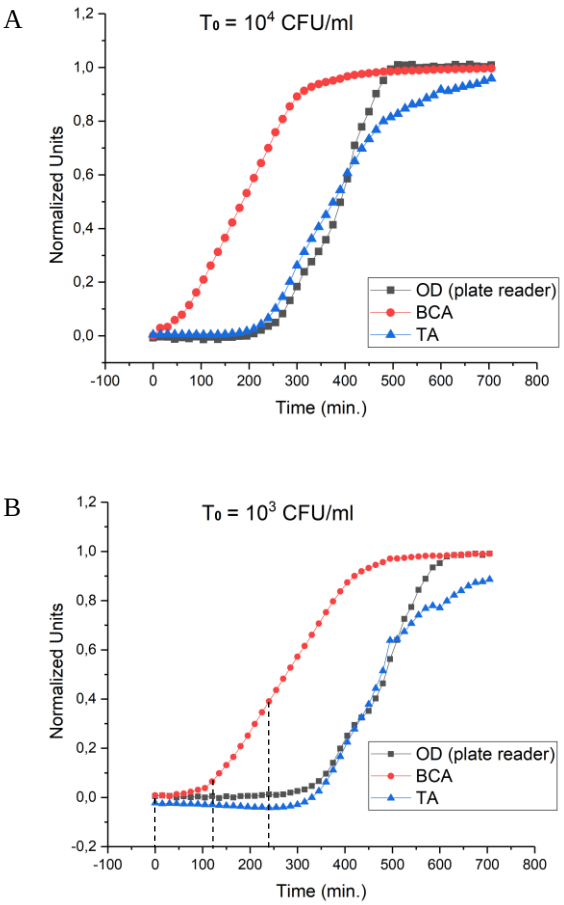

Figure 1. *E. coli* growth curve acquired with Thermo-Fisher Scientific VarioSkan (black) and the two different oCelloScope algorithms, BCA (red) and TA (blue). E. *coli* initial cell inoculum was 10^4 (A) and 10^3 (B) CFU/ml

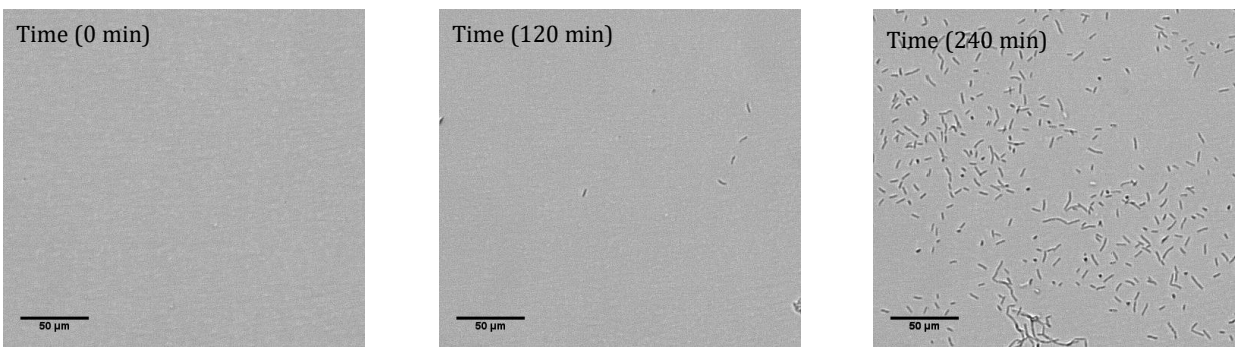

Figure 2. Images of the culture from **Fig. 1-B** at time 0, 120 and 240 minutes. The oCelloScope can detect the growth while the OD plate reader still showed no signal.

How can BCA obtain 250 times greater sensitivity than OD

In a measurement like the OD, photometers quantify the optical density of a liquid sample by comparing the intensity of light that has passed through with the intensity of the light before it enters the sample. Therefore, it needs a considerable amount of cells before the absorbance is large enough to be detected.

The oCelloScope is an imaging system and the BCA an image algorithm that measure the intensity (absorbance) of every single pixel and categorize them as objects or not, based on a background analysis conducted on the images from the first time point. This enables the BCA to detect small changes when new cells appear in the images.

The algorithm TA (Total Absorption) is comparable to OD

Among the image-analysis algorithms available in the UniExplorer software, the TA (Total Absorption) algorithm has been designed to be equivalent with OD, in this way the user can compare the growth curve as it would have been recorded with a standard plate reader.

In Figure 3 the growth curves of *E. coli* growth in LB media starting from different initial inoculum, 10^7, 10^6, 10^5, 10^4 and 10^3 CFU/ml marked in black, blue, red, magenta and green respectively. These data highlight that image-based measurements using oCelloScope are comparable with standard methods.

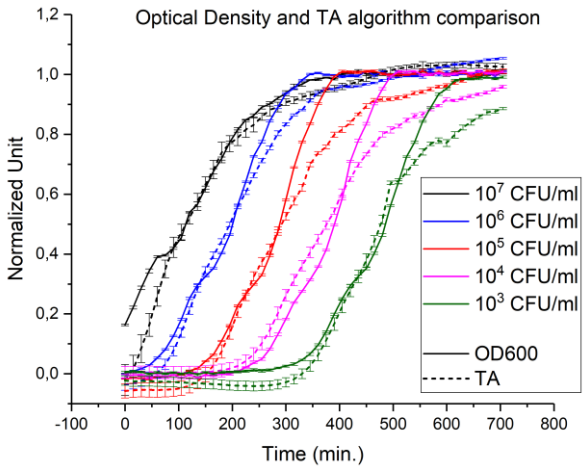

Figure 3. *E. coli* growth curves acquired with Thermo-Fisher Scientific VarioSkan (solid line) and with the oCelloScope TA algorithm (dashed line).

oCelloScope vs. Plate reader

Table 1 oCelloScope vs. plate reader

Feature	**oCelloScope**	Plate reader
Microscopy images	✓	✗
Time-lapse videos	✓	✗
Morphological analysis	✓	✗
Analysis of mixed cultures	✓	✗
Automated analysis	✓	✓
Detection limit (*E. Coli*)	$8{\cdot}10^3$ /ml	$2{\cdot}10^6$ /ml

References

1 A. L. Koch, *Anal. Biochem.*, 1970, **38**, 1970.
2 M. Fredborg, K. R. Andersen, E. Jorgensen, A. Droce, T. Olesen, B. B. Jensen, F. S. Rosenvinge and T. E. Sondergaard, *J. Clin. Microbiol.*, 2013, **51**, 2047–2053.
3 M. Fredborg, F. S. Rosenvinge, E. Spillum, S. Kroghsbo, M. Wang and T. E. Sondergaard, *BMC Microbiol.*, 2015, **15**, 1–8.
4 McLaughlin HP, Gargis AS, Michel P, Sue D, Weigel LM. 2017. J Clin Microbiol 55:959–970.

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