Performing cell migration experiments
using the oCelloScope and the CellDirector® 2D assay

Introduction

Performing cell migration analysis requires that cells are monitored for a period of time where images are recorded at equidistant time points. In order to track the entire dynamic process of migration at a single-cell level, it is essential to (i) define a proper time interval of analysis where adjacent time points are optimally spaced and (ii) use a reliable imaging apparatus to make sure that the scanned area is always precisely recorded at the same location at each time point.

The CellDirector® 2D assay (Gradientech AB, Uppsala, Sweden) is a cell-based assay optimised for the analysis of cell migration and cell behaviour in response to chemotactic concentration gradients. By using a dedicated sample holder, the oCelloScope can support the CellDirector® 2D assay (Fig. 1). The CellDirector® experiments require the CellDirector® Start-Kit, the oCelloScope for real-time imaging and the Tracking Tool™ PRO software (which can be downloaded for free on Gradientech AB's website). The Tracking Tool™ PRO software allows to track, analyse and plot cell migration and chemotaxis.
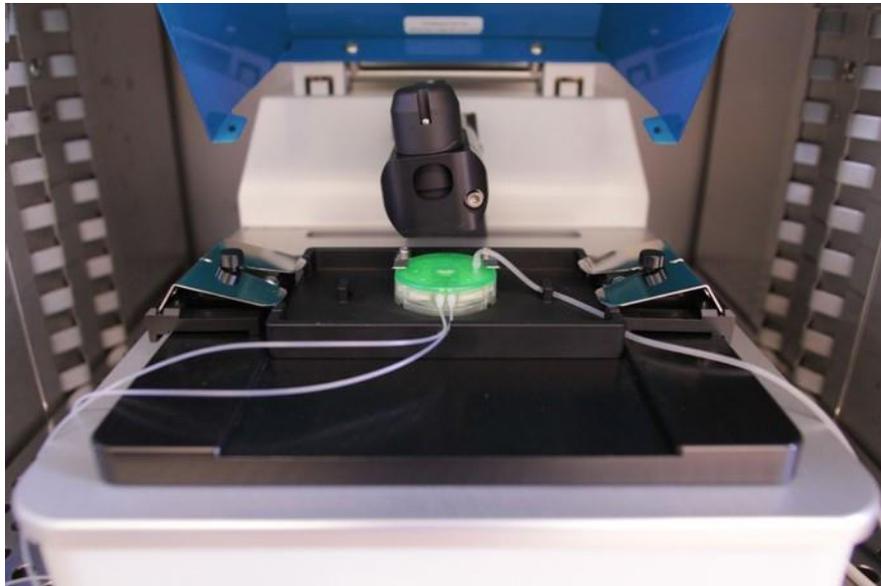

Figure 1. The oCelloScope supports the CellDirector® 2D assay for the analysis of cell migration and cell behaviour in response to chemotactic concentration gradients.

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 and mammalian cells behaviour as well as morphological features. The oCelloScope enables the scanning of volumes by recording a series of images to form an image stack where all the cells are caught in focus. The 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. The UniExplorer software generates time-lapse videos of the acquired images, growth and growth inhibition curves, as well as performs quantitative analysis of morphological features.

The present application note describes how to configure the UniExplorer software to perform cell migration analysis using the CellDirector® 2D assay and the oCelloScope. Support on preparation and seeding of cells as well as connection of pump and syringes can be found on Gradientech AB's website.

Performing cell migration experiments

1. Start a new job by clicking the 'New job' button.
2. Give the new job a name, then choose the acquisition module from the list to the left by double clicking on (or dragging) the 'Acquire' icon for image recording.

3. 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.
4. Select 'CellDirector® 2D, Gradientech 11-001' from the dropdown list at the bottom of the window.
5. Click the 'Select all' button at the top to select all the scan areas.
    <u>Note</u>: The CellDirector® 2D assay has 3 channels (one gradient and two controls). In UniExplorer, each channel has 5 scan areas named 'Control A', 'Control B' and 'Gradient' followed by a number from 1 to 5 to identify the sequence of the scan areas within each channel.
6. Set illumination level. The default value of 2 ms is usually the best solution.
7. Find the focus manually.
8. 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 cell growth and behaviour.
9. Press 'Start' to start the experiment.
10. Imaging data can be inspected and compared during the experiment in the 'Saved images' tab. The images can be zoomed by scrolling the mouse wheel and individual time points can be seen by using the slider below the image.

Exporting the imaging data for the cell migration analysis

The video and images acquired with UniExplorer can be exported to perform the cell migration analysis using the Gradientech Tracking Tool^{TM} PRO software. The Tracking Tool^{TM} PRO software handles only *.tiff* and *.jpeg* image formats.

- The recorded video (*.avi*) must be converted to individual images using a third-party software, such as ImageJ (http://imagej.nih.gov/ij/).
    1. Open the video with ImageJ.
    2. Select 'File' → 'Save as' → 'Image Sequence'.
    3. Select the image format (*.tiff* or *.jpeg*) and press OK.
- An alternative and more laborious method is to use UniExplorer to export all the single images in the time-lapse sequence. Click 'Export' → 'Save as image'. The images are saved as *.bmp* files and must be converted to *tiff* or *.jpeg* formats in order to be imported in the Tracking Tool^{TM} PRO software.
- Support on usage of the Tracking Tool^{TM} PRO software for tracking, analysing and plotting cell migration and chemotaxis can be found on Gradientech AB's website.

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

This application note is for RESEARCH USE ONLY. It is not intended for food, drug, household, or cosmetic use. Its use must be supervised by a technically qualified individual. The above information is correct to the best of our knowledge. Users should make independent decisions regarding completeness of the information based on all sources available. BioSense Solutions ApS shall not be held liable for any damage resulting from handling or contact with the above product.

Product disclaimer

BioSense Solutions ApS reserves the right to introduce changes in the product to incorporate new technology. This application note is subject to change without notice.

Copyright