WimScratch

Application note

Cell migration plays a central role in many complex physiological and pathological events. One approach is to isolate characteristics from the multi-step events happening in vivo through the help of the in vitro "Scratch Assay".

This assay is based on the assumption that cell migration into a blank area is a pivotal event. It is a commonly used method for studying migration. The major advantage of this assay is that it offers the opportunity to monitor cell migration over time and therefore enabling the estimation of migratory response.

The general idea of this assay is to prepare two cell patches with defined gap, e.g. by inflicting an artificial "wound" on a cell layer. Then, the speed of wound closure can be measured by monitoring the cell migration over the gap area.

The objective, reliable, and precise quantification of images of Scratch assays is ensured by the Wimasis WimScratch Solution.

Components required:

- Wimasis' myWim account (you can get it **here**).
- Adherent cells like Fibroblasts, Keratinocytes, Epithelial cells, Cancer cells, or other experimental cell lines.
- Cell culture medium.
- Sterile Tweezers.
- Inverted microscope, preferably with automated image acquisition system and stage top incubator for live cell imaging

Experimental endpoints:

- Inhibition of wound healing and migration.
- Substance mediated enhancement of wound healing and cell migration.

Key benefits of WimScratch:

⚙ Automated assessment of image data.

🕐 Quick results and nearly live analysis.

📝 Objective and reliable measurements.

🌐 Worldwide accessibility via Wimasis myWim image analysis platform.

◔ Optimized assay-specific algorithms to enhance data quality.

Step Process of the Assay:

1. Cell seeding and gap formation.

2. Acquisition of microscopy images.

3. Quantitative image analysis

4. Data analysis and evaluation.

Step 1: Cell Seeding And Gap Formation

- Prepare cell suspension as usual. Depending on your specific cell type, the application of a 3 to 7 x 10 5 cells/ml should result in a confluent layer within 24 hours.

- Apply cell suspension into each well. Avoid shaking because this will result in inhomogeneous cell distribution.

- Incubate at 37 °C and 5 % CO_2 as usual.

- Optionally, it is possible to fill the outer area with cell suspension or cell medium. Use the recommended volume of the dish.

- After appropriate cell attachment (24 hours) gently generate the scratch by using a sterile tip and generate a straight line "wound" on the cell monolayer.

Step 2: Acquisition Of Microscopy Images

- Set up your inverted microscope and measure the µm-per-pixel metric used for the imaging process. If your microscope doesn't provide this data, please use a length reference standard. One practical way is the use of a Neubauer Slide.

- Move the dish until you have the gap and both cell fronts captured on image.
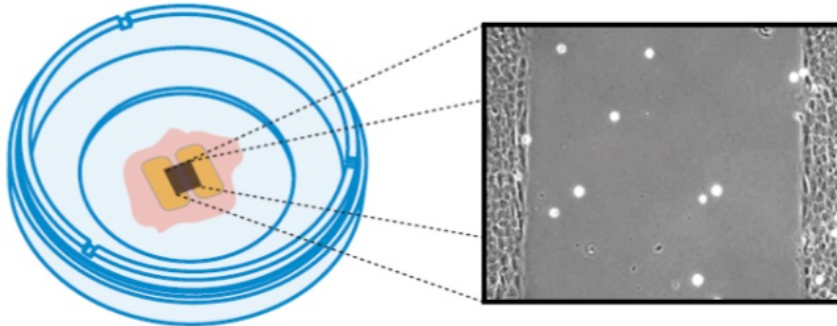
- Start the observation process by taking images several times throughout the following hours. The time between images largely depends on the type of cells and the migration speed.

- Optional: if you include a timestamp in your image filenames, WimScratch can include that time information in the output measurements and calculate time-dervied parameters like wound closing velocity and acceleration. In order for the analysis to be able to extract the timestamp, your files must meet these requirements:

    1. Images from each time series must be saved in its own folder. For instance, if you have two time series, they must be organized into two different folders.

    2. All images inside each folder must share the same filename, apart from the timestamp part, which must follow one of these templates:

    - FilenamePrefix**X.Y**FilenameSufix
    - FilenamePrefix**XhY**FilenameSufix

    In which FilenamePrefix and FilenameSufix can be any text in your filenames but must be the same for all your files. X and Y are any number of digits representing your timestamp.

    For instance, the following files would be a valid time series:
    Experiment1_00h00_Good.jpg, Experiment1_08h30_Good.jpg,
    Experiment1_12h00_Good.jpg, Experiment1_24h00_Good.jpg

Step 3: Quantitative Image Analysis

- Register and log in on the Wimasis myWim image analysis platform.

- Upload your images to WimScratch.

- After successful transfer of the data, you will be notified via e-mail about the status of your order. Detailed results will become available in the results section of Wimasis myWim.

Step 4: Data Analysis And Evaluation

- After having been processed, you will be able to download the overlay images and CSV data files with the migration metrics of your image set.

- In addition, the downloadable results will include a plotted chart of your experiment. Based on your preferences, the chart is either .png or .svg file format.

- The analysis readout (CSV files) will include the data outlined in the "WimScratch: Analysis results in detail" document that you can download here.