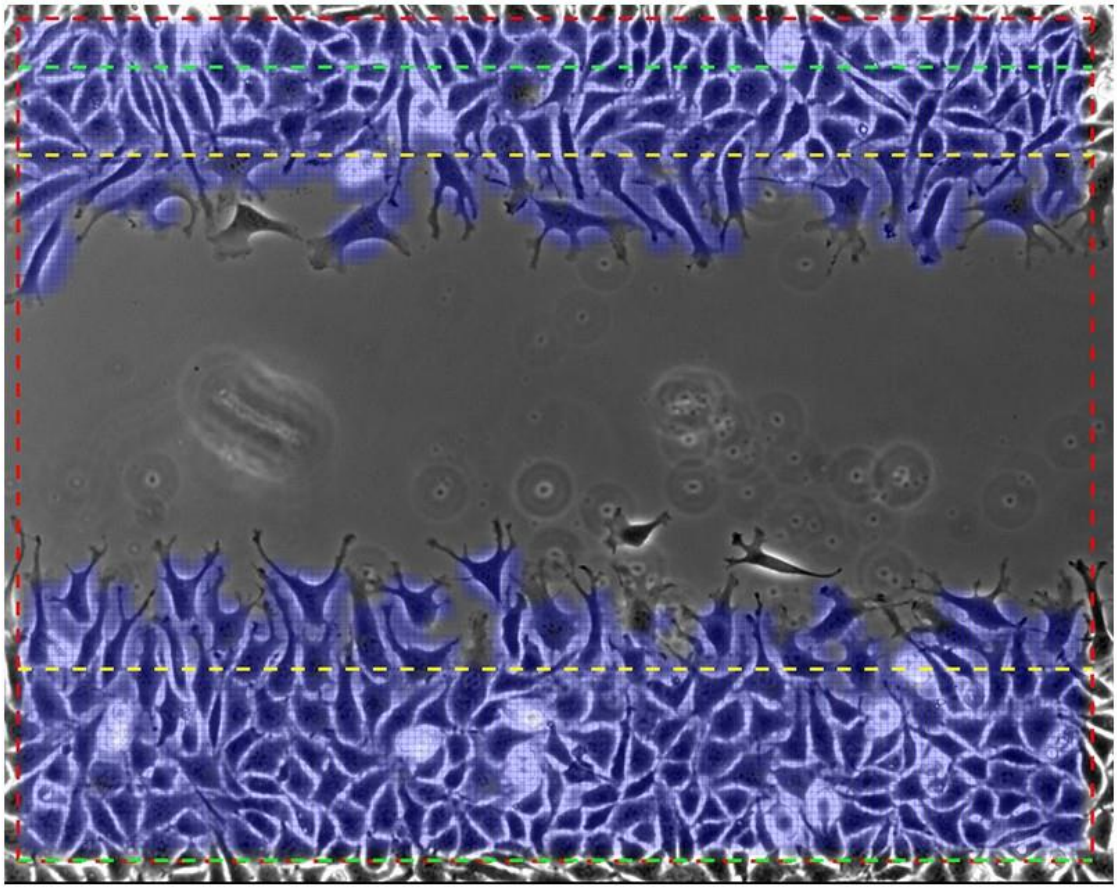
Wound Healing Analysis in the Automated Cellular Analysis System



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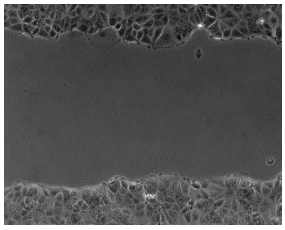
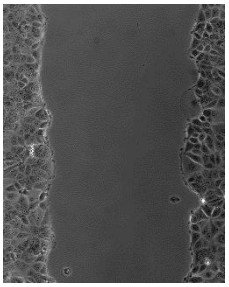
Specifications

Input

Compatible image formats	jpg, png, bmp, tif
Compatible video formats	mp4, avi, mov
Image Size	500 px x 500 px (min) 3840 px x 2160 px (max)
Image aspect ratio	Acceptable range is 4:3 to 16:9
Gap orientation	Horizontal or Vertical
Gap center	Must be clearly visible in the center of the first image
Contrast	As show in sample images below





Gap orientation examples: horizontal or vertical

User Input

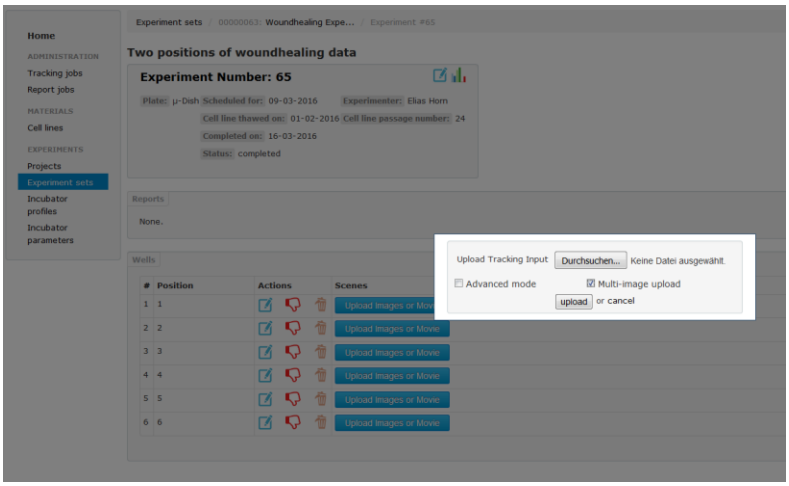
Pixel size	User must input the pixel size in px/µm
Time lapse frame rate	User must input the time between frames
Field of view	0.5 mm to 2 mm

Output

Scratch open area over time	µm²
Covered area over time	µm²
Gap closure speed	µm²/min
Data table	.csv format

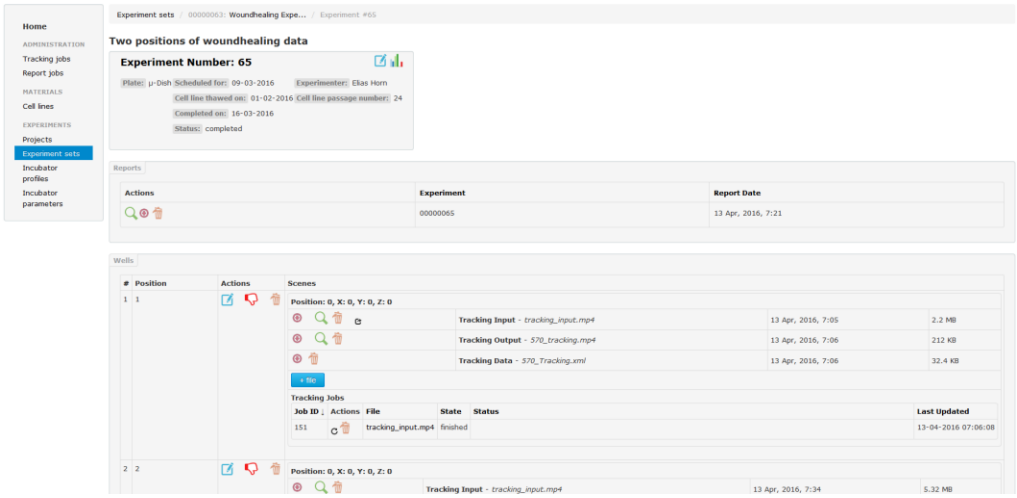
Step-by-Step Guide

1. Upload video/images to ACAS (either quick analysis or standard).



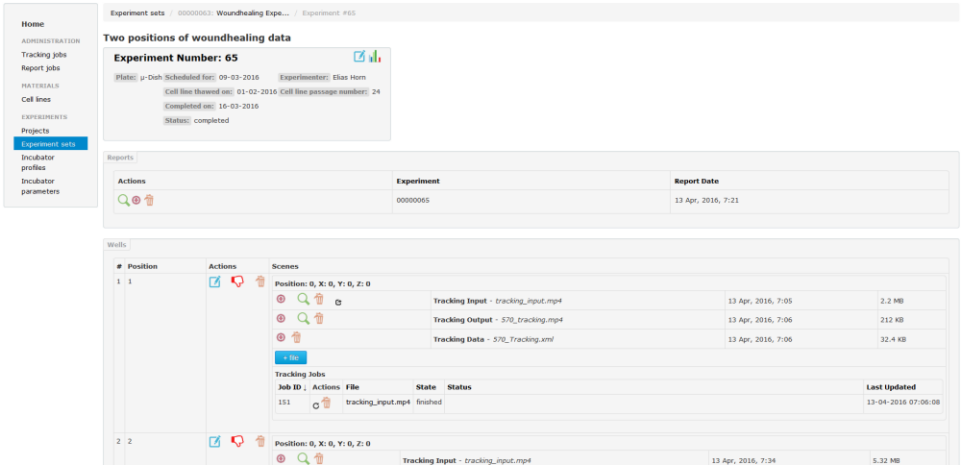
Checking Multi-image upload enables selection of more than one picture by using the “ctrl” or “shift” key. On Windows systems, all pictures in a directory can be selected with the key combination “ctrl + A”.

   a. The system will automatically create and run an analysis job after the image files are uploaded.

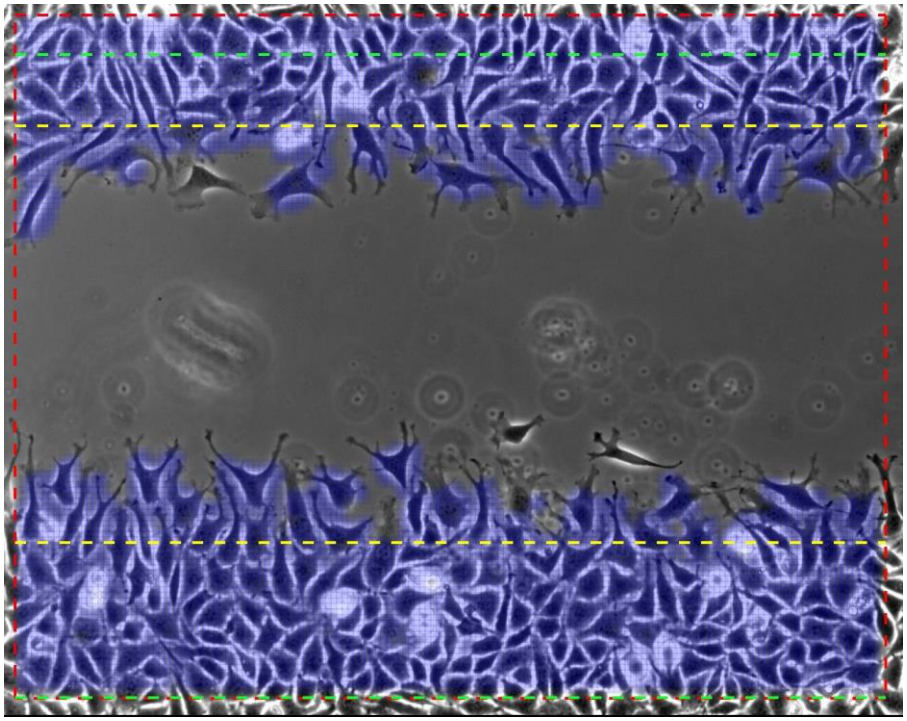


   b. Once the analysis jobs are completed for all wells, a report job will be automatically created.

2. You will find a report in the experiment page (and also in the experiment-set page) typically 5 minutes after the final well has been uploaded. Each well will also contain a tracked movie (or tif file).



The ACAS system generates many types of output images. Output image collections of less than 31 frames will be stored in a multi-frame tif file. 31 or more images will be stored in an mp4 file.



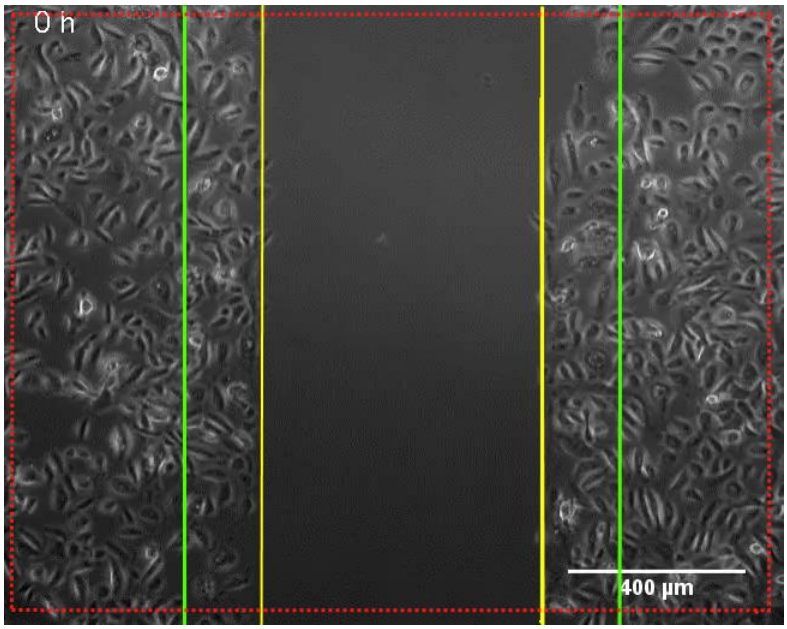
See ACAS User Guide for detailed information.

Analysis

The red outside line indicates the margin. Outside the margin the algorithms cannot reliably detect structures so this region is omitted from the analysis. The width of the margin is dependent on the resolution. The higher the resolution the smaller the margin.

On the first frame of the time sequence, the scratch is detected. A left scratch region border and right border (or top and bottom) are estimated and marked with a yellow line. From these marks, 10% of the image width (or height) are subtracted to increase the scratch analysis area, indicated by green lines.

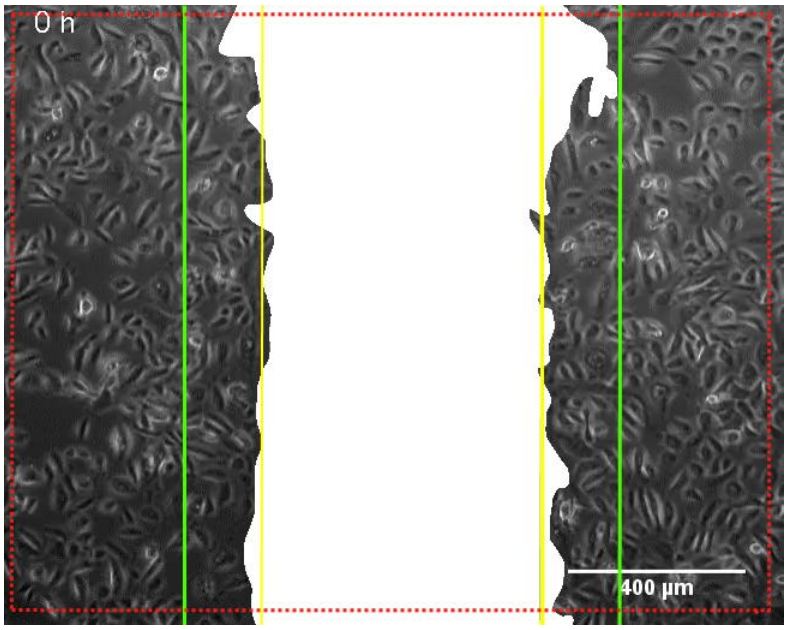
Within these green lines the scratch open area and covered areas are measured. Each pixel is considered in this gap area to be either covered by cells or not covered.



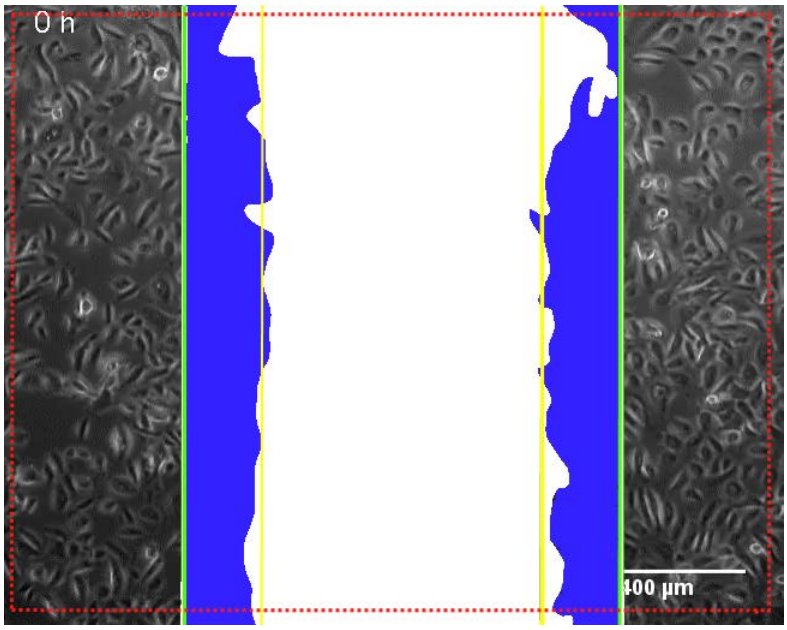
Because the scratch border is not a perfectly straight edge, open areas are considered within the analysis region of the estimated gap (that is to the green lines). But in cases of low cell density the openings or areas not covered by cells may extend far into the non-scratch area. In some cases, open areas can be traced to the outside of the frame. To limit this case, the scratch analysis area is limited to the region around the detected gap.

In the following illustration, the detected open area is marked in white. As you can see at the upper right corner of the scratch, uncovered area extends beyond the green line but the white area stops at the green line.

The white area in the image below represents the starting Scratch Open Area.

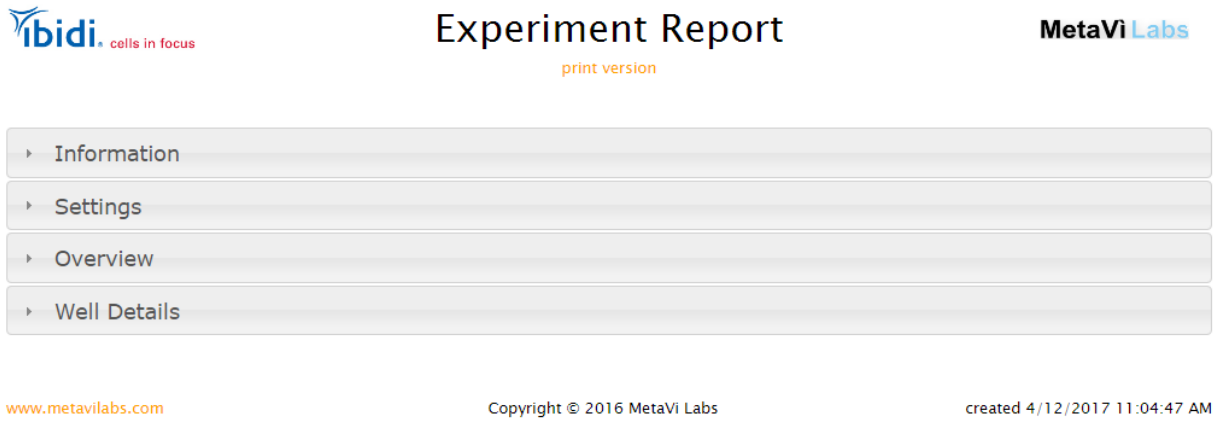


In the following illustration, the blue area marks the Starting Scratch Covered area.



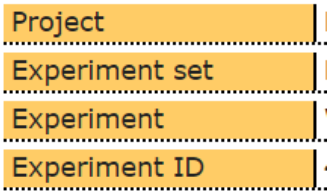
Example Report Job

The wound healing report job is contained in an HTML file, with the option to download the tracking data in a .csv format. The report job screen is subdivided in four sections that can be viewed by a click on the section respectively.



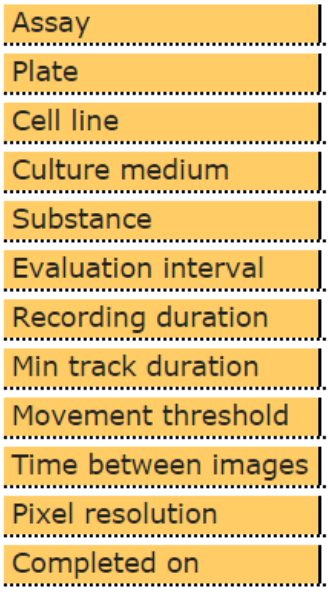
Information:

This section contains overall information of the experiment, such as the name of the project, the title of the experiment set and experiment, as well as the experiment ID number.



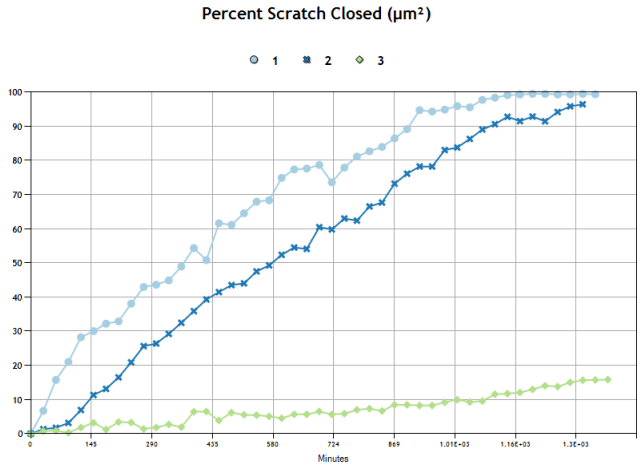
Settings:

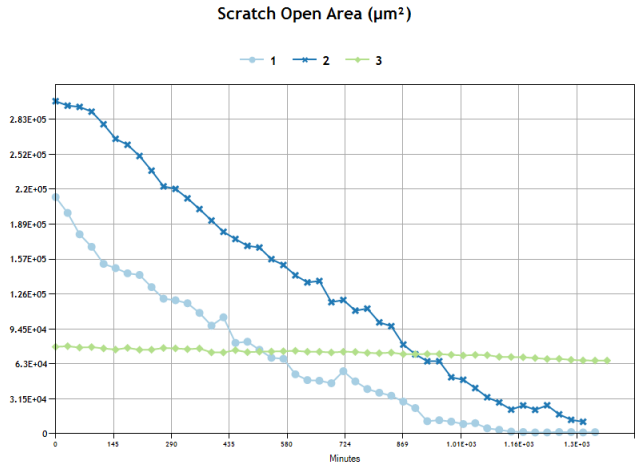
This section shows the settings that were defined in the experiment sets in order to summarize the setup and conditions for documentation purposes.

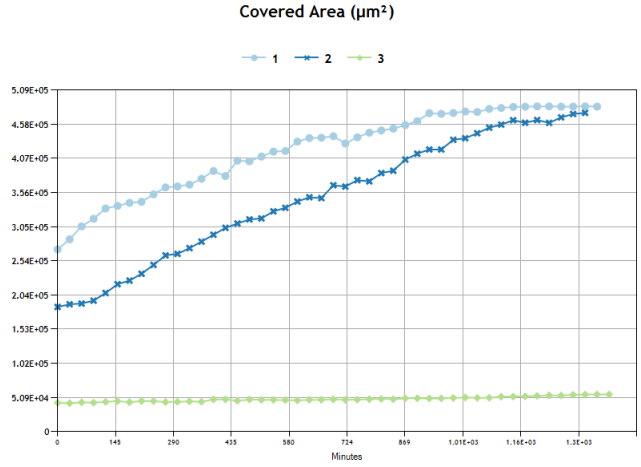


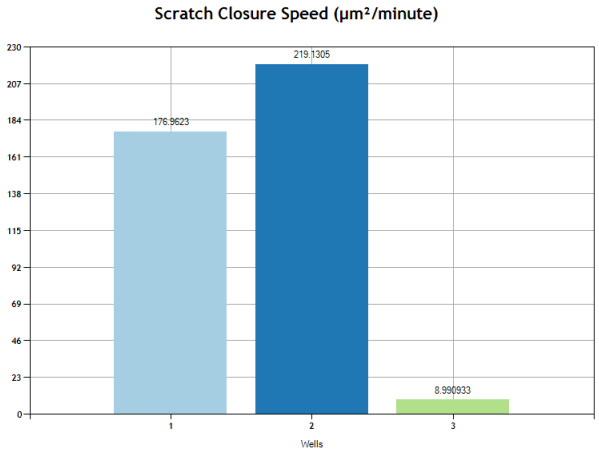
Overview:

This section contains the main results of the report shown in four different diagrams. The charts in this section are overlays of all positions (wells) of the experiment.





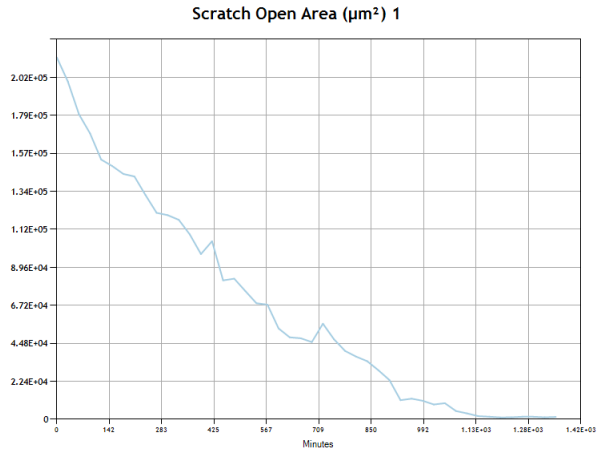


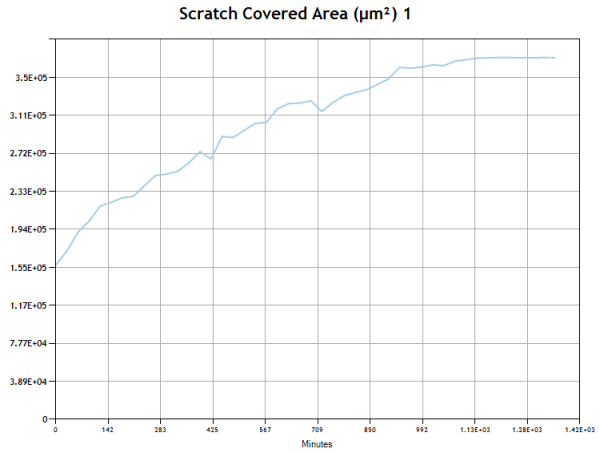


The corresponding data can be obtained by a click on the download icon ( ). The data is formatted in a .csv for use in your favorite analysis software.

Well Details:

The last section lists the data of every position (well) separately. Choose a Well under if you want to study individual detailed reports for the corresponding well. Among other things the tracking video is shown. The view will change to metrics, curves and graphs for the selected Well only.





The data that is previewed at the end of this section can be obtained as well by a click on the download icon (CSV)

Well across time:

Time point (hh:mm)	Scratch Open Area (%)	Scratch Covered Area (%)	Average Speed (%/min)
00:00	100.00	0.00	0.00
00:30	93.31	6.69	0.13
01:00	84.27	15.73	0.17
01:30	78.99	21.01	0.10
02:00	71.77	28.23	0.14
02:30	69.97	30.03	0.03
03:00	67.80	32.20	0.04
03:30	67.09	32.91	0.01