



## CELL-IQ ANALYSER MANUAL

CELL-IQ®, FOR YOUR RESEARCH NEEDS

Version MA4.4.0.0

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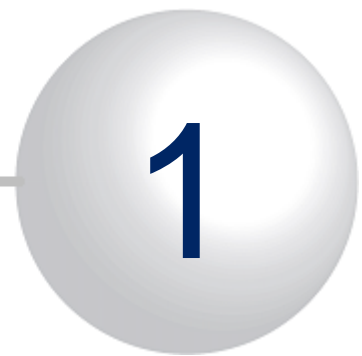
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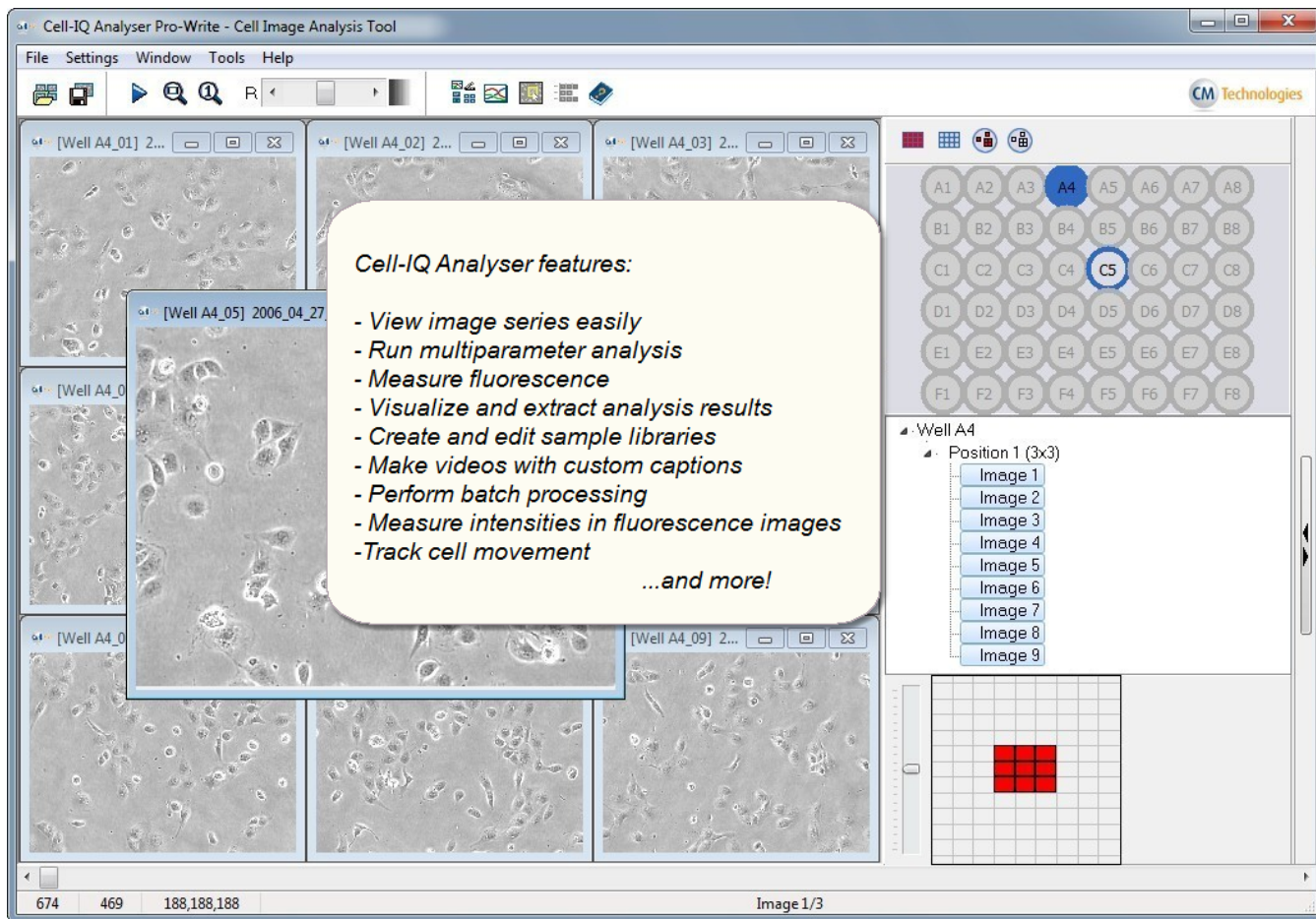
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## Analyser program overview

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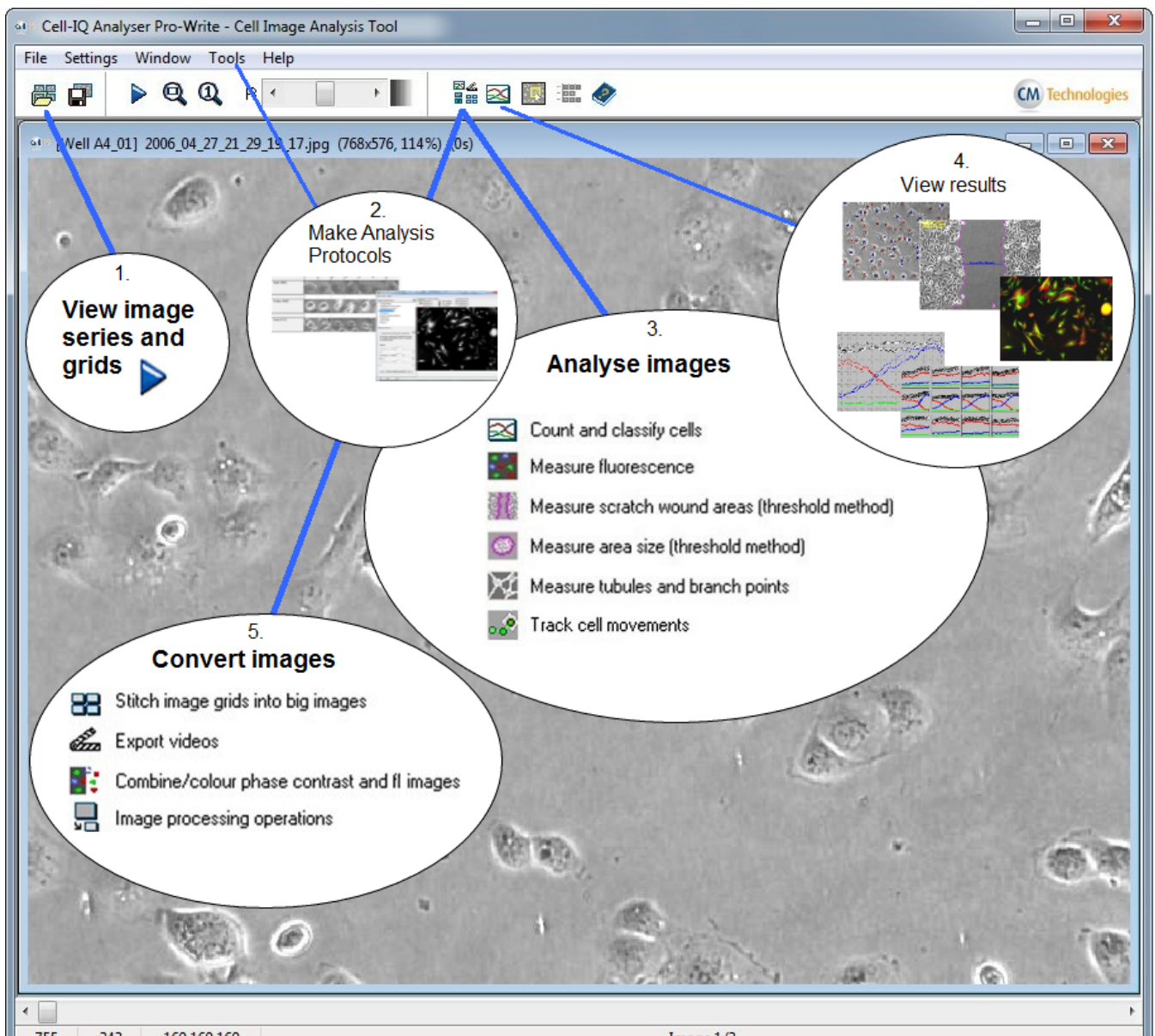


# 1 Analyser program overview



## 1.1 Analyser basic features

1. **View image series and grids**
2. **Make Analysis Protocols**
3. **Analyse images**
4. **View results**
5. **Convert images**



### Analyser basic features

#### 1. View image series and grids

- View image series and image grids captured with **Cell-IQ Imagen**.
- Open images into main window to create Protocols, i.e. to define parameters for analysis of the plate.
- Cell-IQ Analyser supports three image formats, bitmap (\*.bmp), jpeg (\*.jpg) and tiff (\*.tif).

## 2. Make Analysis Protocols

- Analysis protocol defines cell analysis for specific cell test. It includes analysis parameters, and can include cell samples.
- Batch process analysis of a well plate typically requires an analysis Protocol, which in some tools has to be available before Batch process analysis is started.

## 3. Analyse images

- Automatic Cell image analysis is made in the Batch Process tool in the Cell-IQ Analyser.
- The analysis tools in the Cell-IQ Analyser:
  - Count and classify cells: Cell, neurite and area recognition tools that use user defined cell sample library. How this is used in Batch Process:
    - a. Load the Protocol.
    - b. Select source images.
    - c. Define saving directory for the results.
    - d. Edit analysis settings (Optional).
    - e. Click Start.
  - Measure fluorescence: Single and multichannel fluorescence cell intensity measurement.
  - Measure scratch wound areas: Measure scratch wound areas and closure speed in horizontal migration test. Uses threshold method.
  - Measure area size: Measure cell population areas and growth speed. Uses threshold method.
  - Measure tubules and branch points: Analyse angiogenesis images.
  - Track cell movements: Track cells and show cell movement trajectories and statistics.

## 4. View results

- Cell-IQ Analyser results are normally presented in time curves that show changes in population over the entire cell test duration.
- After Batch Process analysis finishes, the result curve window pops up. Analyser also saves the results into text (\*.txt) files that can be re-opened later.
- Also result image series are typically saved during analysis, and they can later be for example converted into videos.

## 5. Convert images

- Batch Process tool includes also several image conversion tools:
  - Stitch image grids into big images: Combine e.g. the nine image series of 3x3 image grid into one series with large images.
  - Export videos: Make video from image series.
  - Combine/colour phase contrast and fluorescence images: Cell-IQ Imagen saves images in grey scale, and different channels into separate folders.  
This tool combines and colours them.
  - Image processing operations: Collection of image processing tools (including e.g. background correction and image series judder removal).

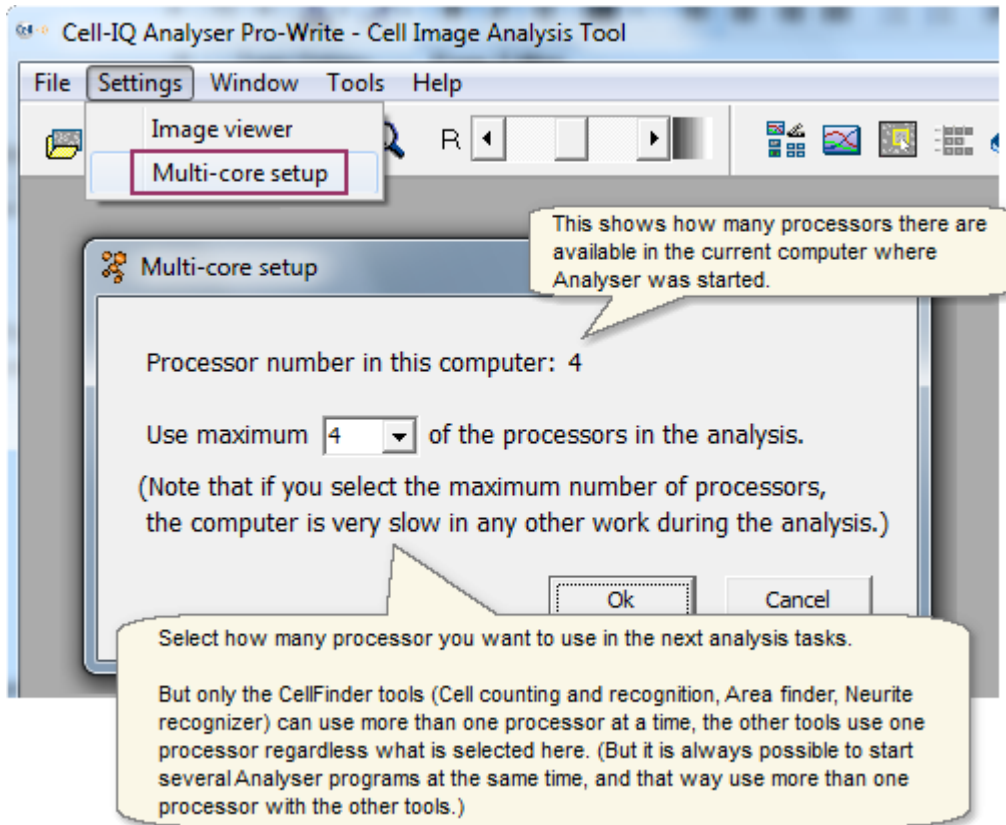
## 1.2 System requirements

### System Requirements - Cell-IQ Analyser program

- Microsoft Windows 7, Vista, XP or 8 with the latest service packs installed (32-bit or 64-bit editions)
- 1.5 GHz processor (2 GHz or higher recommended. Dual- or quad-core recommended if the computer is used also for other purposes.)
- 2 GB of RAM (3 GB or higher recommended)
- 5 GB of free hard drive space (Minimum 350 GB hard drive recommended for result saving)
- Minimum display resolution: 1024 x 768 (24-bit color)

### Multi-core computer support

By default the Cell-IQ Analyser program uses all the processor cores in the Cell Finder analysis (Cell counting and recognition, Area finder and Neurite measurement). But user has option to select that only part of the available processors will be used in analysis, see the image in below. If all cores are used in analysis, the computer can be very slow with other processes.





## 1.3 Change log

### Analyser program versions change log:

#### --- AN4.4.0 -----

- Update: Phase contrast cell classification of fluorescence emitting cells.
- Update: Cell Tracking: Several positions can be selected simultaneously for result graphs.
- Update: Cell Tracking: Axis scales for Rose plots and centered trajectory graphs can be changed.
- Update: Cell Tracking: Option to set time point and distance limits for including cell to average results.
- Update: Cell Tracking: Centered trajectories: Possible to use different color for each cell.
- Update: Manual Cell Tracking tool: Backwards tracking added.
- Update: Manual Cell Tracking tool: Cell positions can be added from different channels to same time series.
- Update: Greyscale image support added (jpeg, tiff, bmp). All tools now accept them.
- Update: 16 bit tiff image reading support added. They are converted to 8 bit when read.
- Revamp: Previewing large image grid does not reserve much memory anymore.
- Revamp: Manual Cell Tracking tool: Confirmation prompt added for time point deletion.
- Revamp: Area size tool: The Growth result curve now starts from 0 instead setting the smallest value to 0.
- Revamp: Protocol making tool -windows cannot be opened anymore without selecting images for them.
- Revamp: Area Finder result graph background class legend now uses class name instead of "Background".

#### --- AN4.3.0 -----

- Update: CellFinder image pre-processing brightness normalization level adjustment added.
- Update: CellFinder cell counting parameter slide bar selection added.
- Update: NeuriteRecognizer: Cell counting dots can be hid from result image.
- Update: Tracking tool: The data values from the Distance3D chart are saved to an xml file to result folder.
- Update: TubuleFinder: Blur (advanced user-) parameter added to grey level threshold.
- Update: Image area -curve removed from results of all area measurement tools.
- Update: Movement tracking: Cell coordinates and time points added to the result xml file.
- Update: Launch Analyser -button from Imagen 4 opens the current Imagen cycle in Analyser.
- Update: Windows 8 support added.
- Update: New manual cell tracking tool. Also lineage tool added to this version.
- Revamp: Batch Process uses resolution also without cycle.dat if resolution selected from Main Window settings.
- Revamp: Judder fixer: Small performance improvements.
- Revamp: All slide plate image positions now fit to the well map when cycle.dat file read.
- Revamp: CellFinder: Result curve \*.txt file saved already after analysis of one image series, not after all series.
- Revamp: BatchProcess: Result image saving made more secure and memory efficient.
- Revamp: Tracking tool: Backwards tracking does not change the cell positions user selects from last image.

#### --- AN4.2.0 -----

- Update: HW Analyser in Cell-IQ leaves one processor core entirely free for Imagen.
- Revamp: Plate map in main window: Image position can be selected with arrow key from the list view.

#### --- AN4.1.0 -----

- New tool: Cell tracking tool. Track cells and show cell movement trajectories and rose chart etc. movement statistics.
- New tool: Manual cell tracking tool. Shows cell movement trajectories and rose chart etc. movement statistics.
- New feature: Use different cell counting parameters for different recognition classes in Cell Finder.

- Update: Remaining analysis time estimate displayed during Batch Process.
- Update: Result curve graph default background changed from grey to white and x-axis shows hours.

#### --- AN4.0.0 -----

- Update: Well Plate map view kept visible in main window for preview image selection.
- Update: Well Plate map added to the Protocol making tools (Scratch wound tool etc.).
- Update: Manual measurement tools added to the Scratch wound and Area Threshold tools.
- Update: Result curves can be generated directly from the Protocol making tools (Scratch wound tool etc.).
- Update: Current result image can be saved to automatically generated Well folder from the Protocol making tools (Scratch wound tool etc.).
- Update: Windows 7 style buttons etc. UI components instead of Win XP style.
- Update: Use micrometers instead of pixels in all the results that tell distance or area.
- Update: Batch process: Added option to select what cycles from the image series are analysed.
- Update: Batch process: Select multiple source image wells from plate map by drawing with mouse.
- Update: Grid stitching: Stitching combines correct time points, even if some image positions have less time points than others.
- Update: Grid stitching: Image resizing integrated to the stitching tool.
- Update: Grid stitching: Background correction integrated to the stitching tool.
- Update: Grid stitching: Match image edge brightness between the grid images -feature added.
- Update: Grid stitching: Brightness adjustment bar added to the Batch process stitching tool.
- Update: Grid stitching: Stitched image preview button added to the stitching tool.
- Update: FL measurement and Combining/colouring: Do not use FL channel in combined image if it was disabled from imaging in that time point (but enabled on some other time points). Earlier the closest FL time point was taken in all cases.
- Update: Result curve graph: XY axes and legend font editing added.
- Update: Result curve graph: Show LD50% point. Also show point of N% of total cell count.
- Update: Result curve graph: Smoothen curves by polynomial curve fitting.
- Update: Result Curve grid: Show combined curve for well. Show Y axis scale. Use curve smoothing.
- Update: Scratch wound tool: New parameter added: Pre-filtering level for the threshold source.
- Update: Cell Finder: Neurite Cell diameter parameter (B) will not anymore remove neurites from areas of cells recognized to a disabled recognition class.
- Update: Analyser main window is hidden when Batch Process is started, and shown when it finishes.
- Update: Image brightening value from Main window is applied also in the cell image in the Threshold tools.
- Update: FL result curve graph sheet names changed. E.g. Area count -> Cell count.
- Update: FL tool: When cells selected manually in FL tool, they are remembered in Batch Process.
- Update: Open one image series -button removed from the main Window. Its functionality added to Open grids - button.
- Revamp: Do not allow result saving to the source image folder.
- Revamp: Confirm that data lost is ok if user changes e.g. from Cell finder to Area Finder.

#### --- AN3.3.0 -----

- New Tool: Manual Cell Lineage analysis.
- Update: FL tool: Let user select cells from any image in series when manual cell selection mode selected.
- Update: Video export: Show scalebar in the video.
- Update: Video export: Add more than one free-word text fields to video.
- Update: Image file name for every time point added to the exported XML result files.

#### --- AN3.2.0 -----

- New Tool: Automatic Cell Lineage analysis.
- Update: Protocol making guide merged to the Analyser help manual.

#### --- AN3.1.0 -----

- New feature: Wells or well groups can be combined and compared to each others in the result curve window.
- New feature: Standard deviation can be shown for combined curves in the result curve window.
- New feature: New curve graph settings window, with several graph modification options (for titles and axes etc).
- Update: FL tool: Tiff saving added to Batch process. Earlier it saved Tiff source image in bitmap format.

#### --- AN3.0.1 -----

- Update: The user interfaces of the Scratch wound finder and the other threshold finders updated with some new visualization and tools presented in the new FL tool: E.g. the batch process quick start buttons added.
- Update: FL measurement: Single cells now identified with colours and IDs in the scattergram result curve, when the manual cell selection method used. This way the same cell can be found from curve and from result image.
- Update: Neurite Classifier made faster: Only half of the neurite pixels are classified, and the result is copied to the adjacent pixels.

#### --- AN3.0.0 -----

- New tool: The multilabel Fluorescence intensity measurement.
- Update: The image colouring and combining tool replaced with the new tool. Now also possible to save the colouring and combining parameters to a protocol.
- Update: Cell tracking added to the Batch Process.
- Update: Scratch wound tool: Single cell removal using cell area size criteria added. Added feature for removal all the gaps that are not connected to the big centre wound, or keep also the gaps that has area close enough to the biggest gap. Remove cells inside the biggest gap.
- Update: Judder fixer: Colour image support and tif saving added.
- Update: Cell count etc. results are displayed in the Cell Finder protocol test image.
- Update: Images can be opened to the Analyser main window using \*.dat file.
- Update: Tiff saving added to the Image Processing operations in Batch Process
- Update: \*.dat file version 3.0 support added. (Imagen IM2.8.0 uses this.)
- Update: Grids can be stitched even if image folders for the grid corner positions do not exist.

#### --- Version AN2.3.1 -----

- Update: Result curve window: The class sum curve option added in addition to the class average curve.
- Update: Line width etc. graph options can be changed from curve window for all Analysis methods.
- Update: All the result curves now start from 0 time point in the result graph.

#### --- Version AN2.3.0 -----

- New feature in Tubule finder: Tubule recognition by classification added.
- New feature in Tubule finder: Manual measurement tools added.
- Update: Tubule finder: Tubule width, BranchP width and color in result images can be changed and saved to Protocol
- Update: Different wells have different line types in result curve graph
- Update: Grey level threshold added to tubule finder and Area Thr finder.

#### --- Version AN2.2.2 -----

- Cell coordinate export changed to work with Image-Pro Cell-IQ plugin. Also Image name saved in full length to the CellCountCurve.txt file.
- Empty CellCountCurve.txt files and their folders are not created anymore in manual export.

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--- Version AN2.2.0 -----

- New feature: Interactive parameter selection tool Tubule finder
- New feature: Mouse wheel operated zoom added
- New tool: Image series XY Judder fixer added to Batch process Image processing tool

--- Version AN2.1.0 -----

- New feature: Large cell analysis. User can define two areas, a circle and surrounding disk for cell recognition.
- New tool: Scratch wound measurement using threshold methods. Also in Batch process.
- New tool: Area size measurement using threshold methods. Also in Batch process.

--- Version AN2.0.1 -----

- Scale bar added to preview images. Units are in pixels in default, or user can write resolution and select display in micrometers
- Several samples can be moved as a batch from one class to another
- Help file changed to pdf format

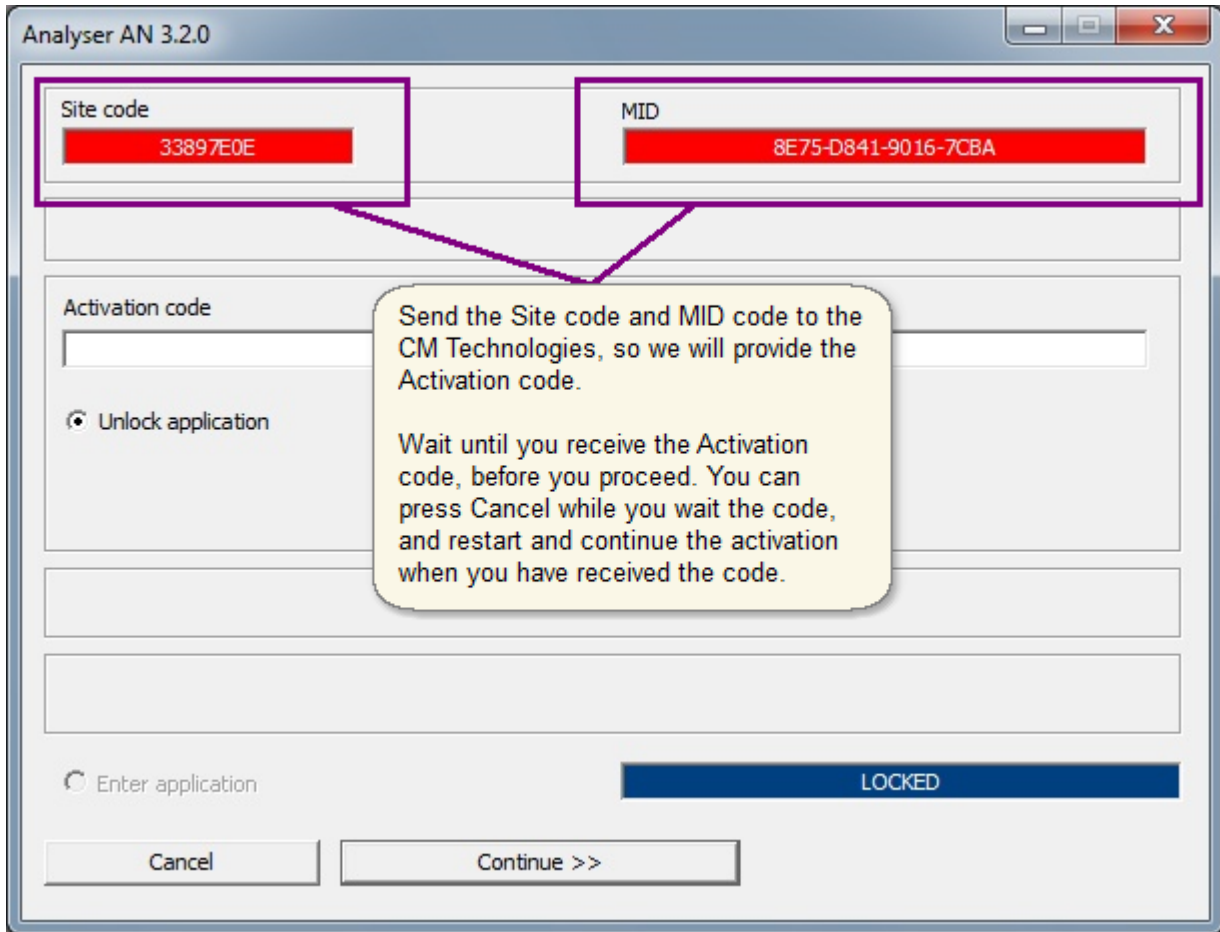
--- Version AN2.0.0 -----

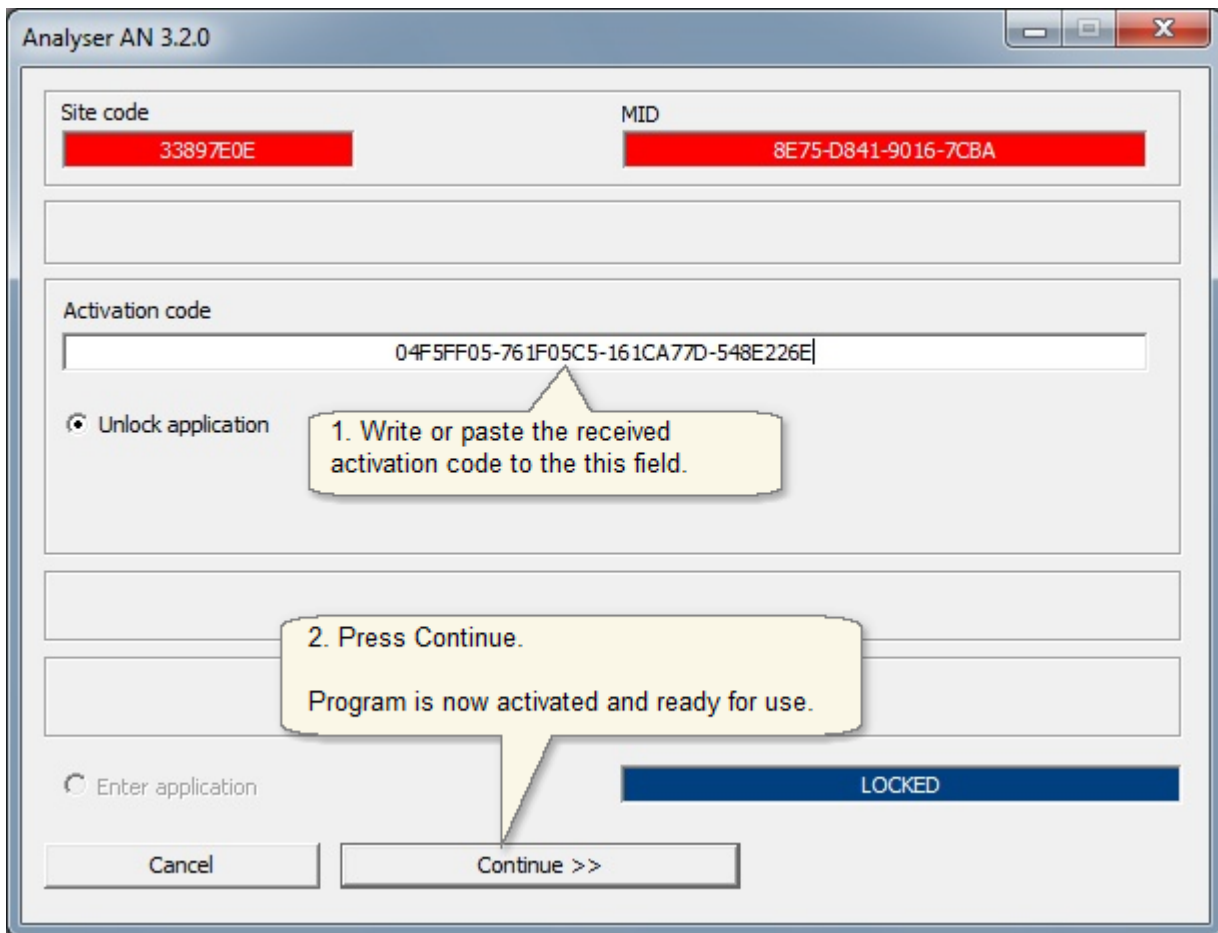
- New user interface. The controls were removed from the right hand side of the main window
- Improvements to Protocol making:
  - New window made for segmentation parameter selection
  - Graphical tools added to the segmentation parameter selection
  - Automatic sample selection: Segmentation takes the found cells to sample library
  - Classifier building moved to own thread, and hence program does not jam while building
- Updates to cell tracking: User can select if the tracking vectors are drawn to cell images or not.
- Angiogenesis analysis v1 added to batch process

## 1.4 Analyser installation

Software is supplied in an installer program exe file. Start the installer and follow the instructions in the dialogs.

After installation is complete and the program is started the first time, it asks for the activation code. Please send the site code and MID code to the CM Technologies, so we will provide the Activation code.

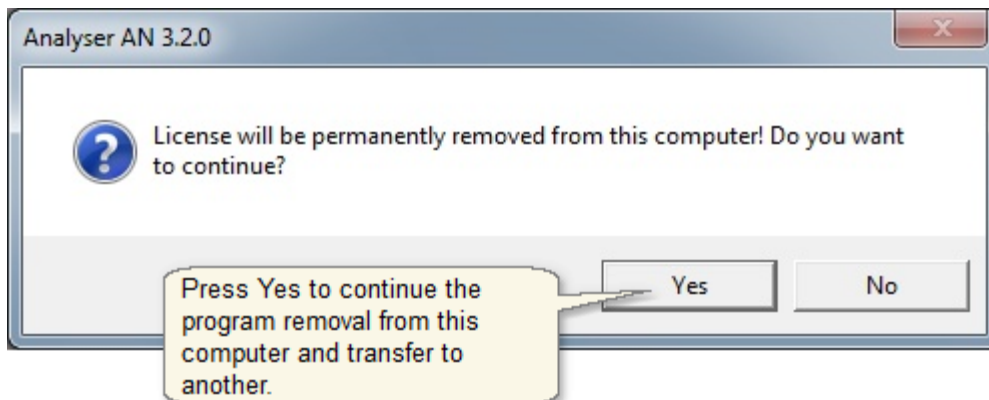
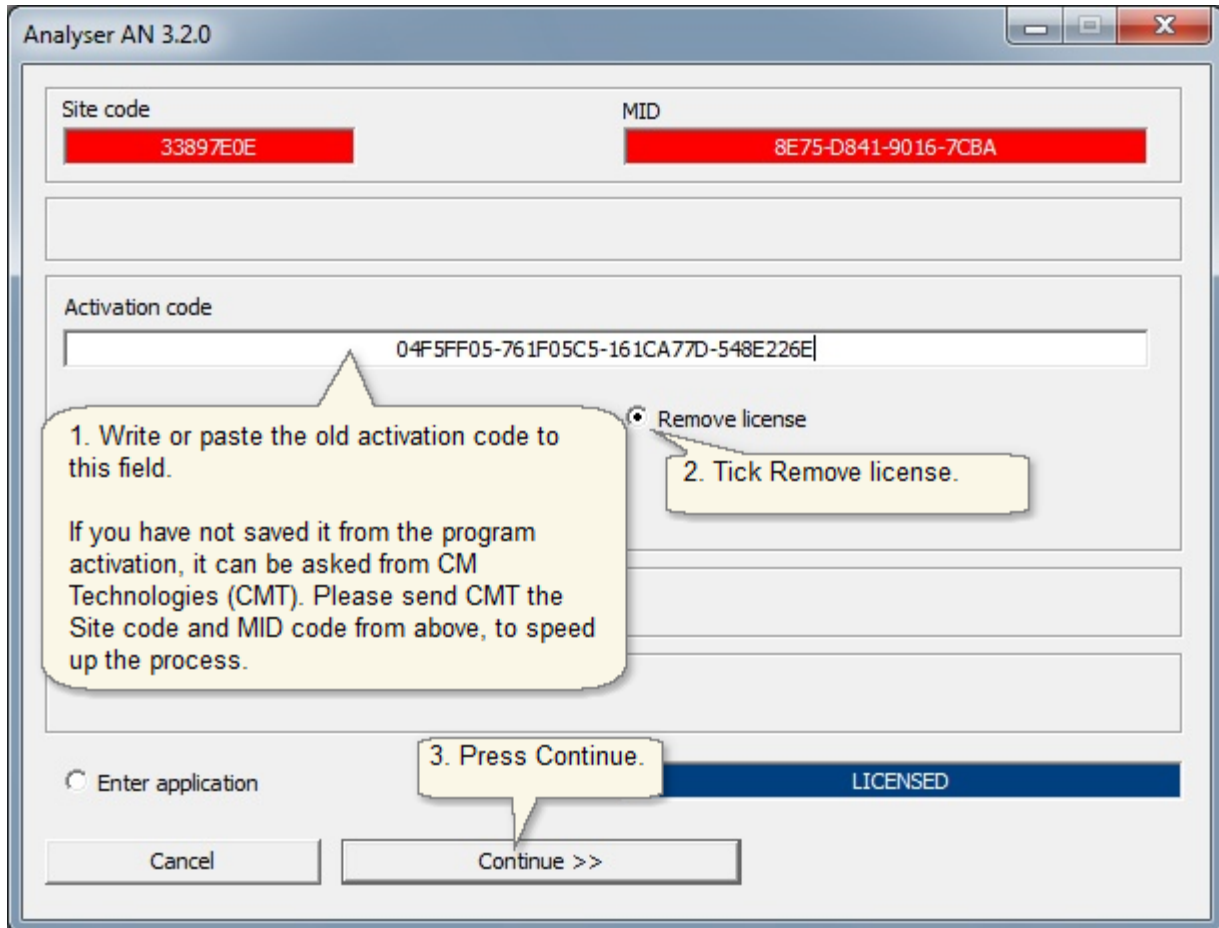


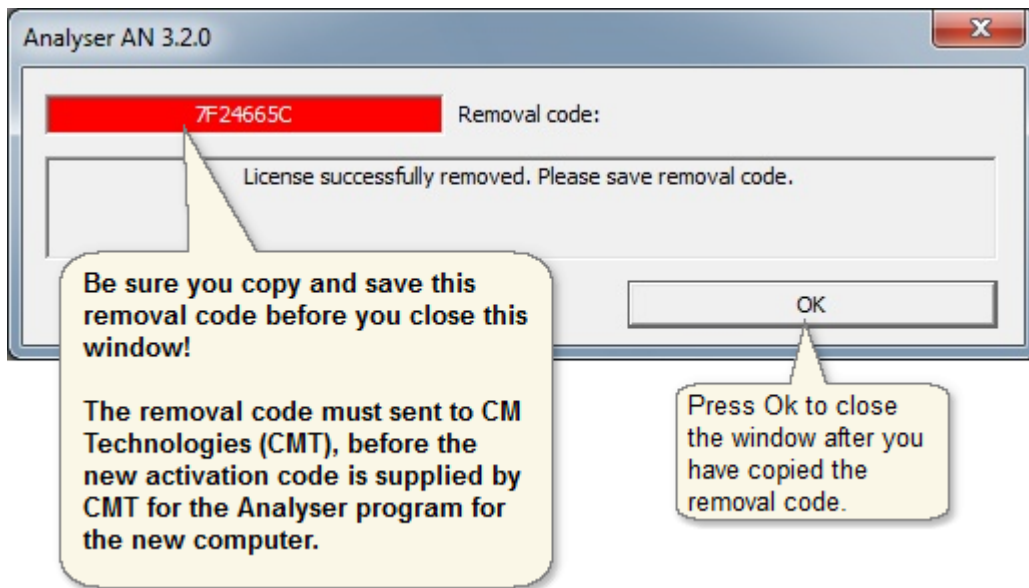


## 1.5 License transfer

The program can be moved to another computer by following the instructions below. Note that the program must be first deactivated from the old computer, and the deactivation must be **proved by supplying CM Technologies (CMT) the removal code**, before the new activation code is supplied for the new computer!

Hold the Ctrl- or Shift-key down and Start the program from the Cell-IQ Analyser program icon. The program activation dialog appears. Follow the instructions given in the images below.

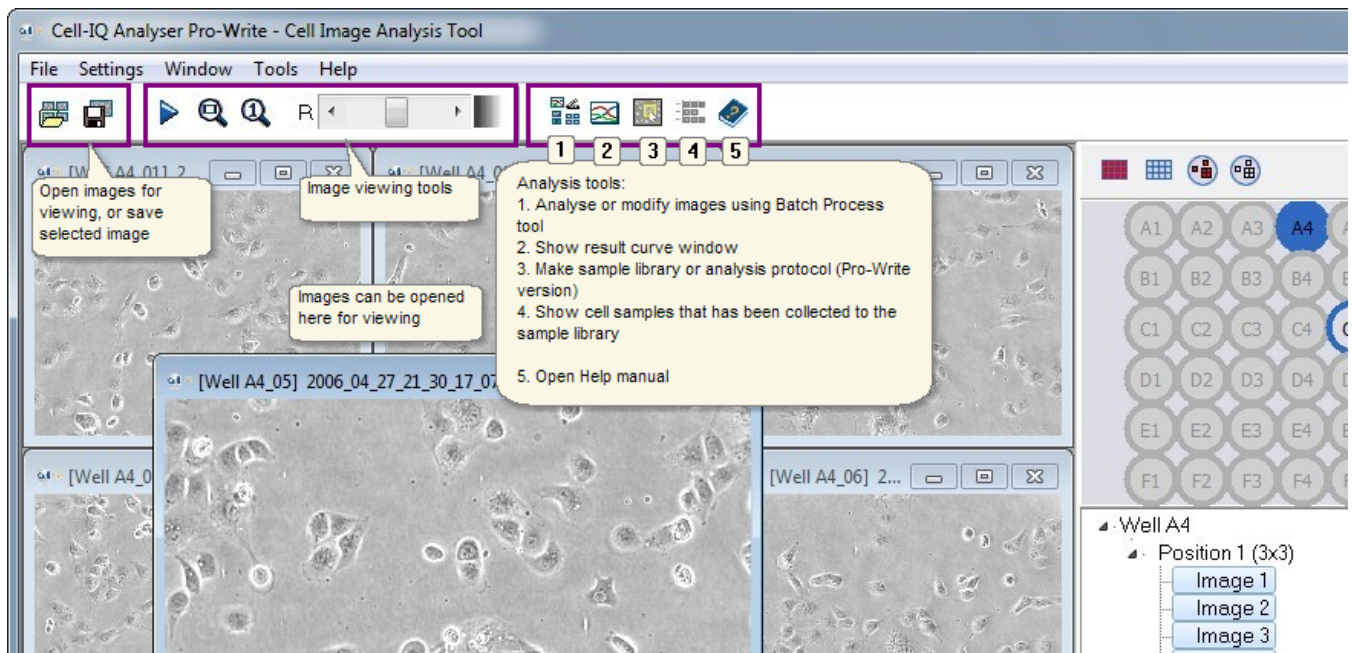




After this you can use the Analyser installer exe for [installing the Analyser](#) for the new computer. Please ask guidance from CMT for which installer version should be used. Please send the removal code (received in the deactivation) to CMT together with the new Site code and MID codes the Analyser gives in the first start after the installation, and CMT will then send you the new activation code.



## 1.6 Analyser main window



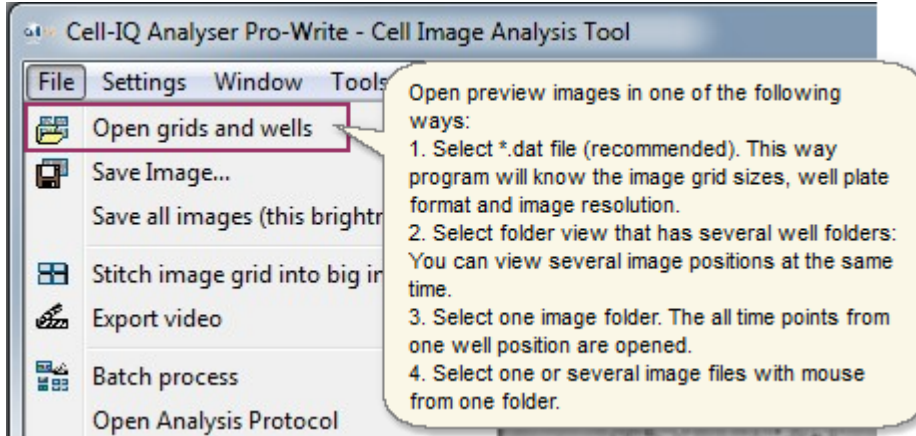
## View image series and grids

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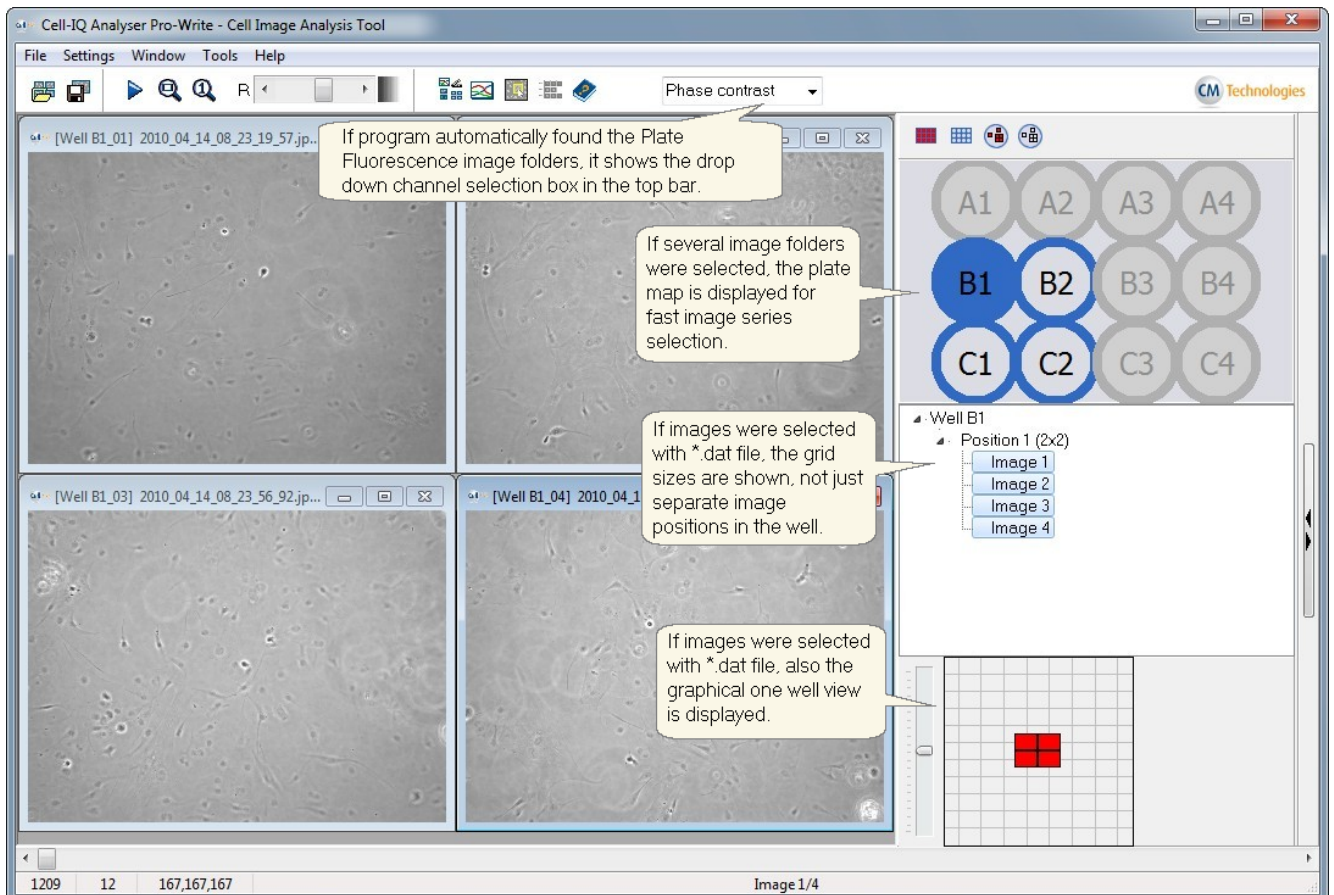


## 2 View image series and grids

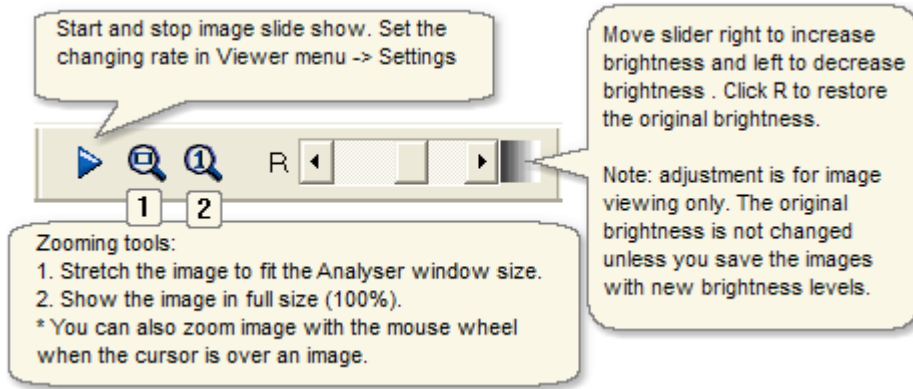
- **Open images for preview:** It is recommended to open images by selecting the \*.dat file from the Plate folder. This way program will know the image grid sizes, well plate format and image resolution.



- If several image folders were selected, the plate map is displayed on the right side of the main window. If images were selected with \*.dat file, also the graphical one well view (bottom right) is displayed. If program automatically found the Plate Fluorescence image folders, it shows the drop down channel selection box in the top bar.

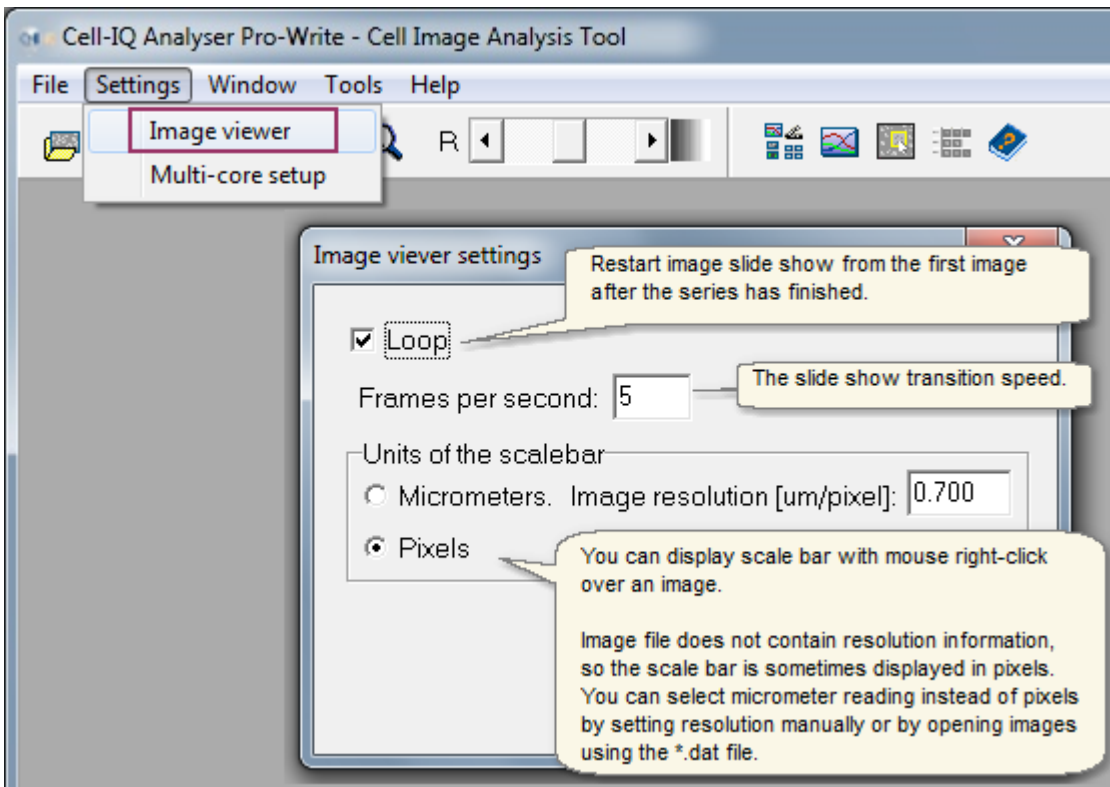


- Image viewing tools

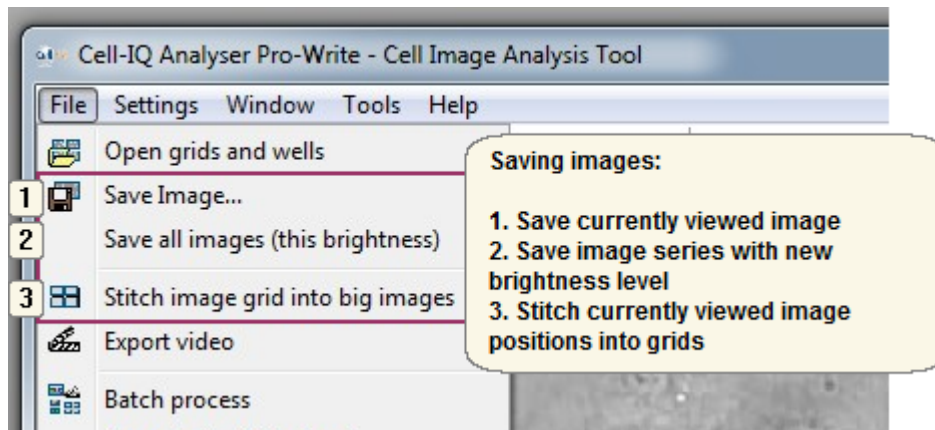


**Note:** Use mouse wheel to zoom images without resizing the image frame. This way you can zoom also when image grid is open, without changing the image positions.

- The speed of the image slide show and the scale bar settings can be changed from "Settings → Image viewer".



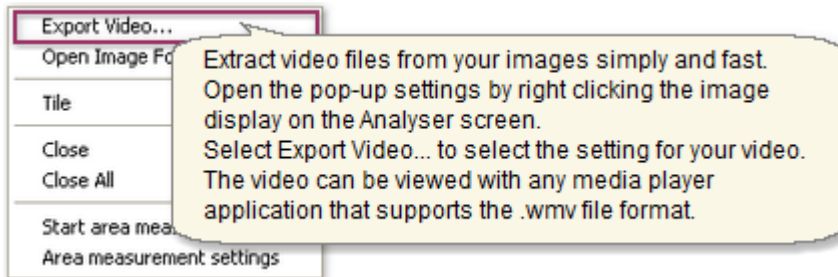
## Saving images



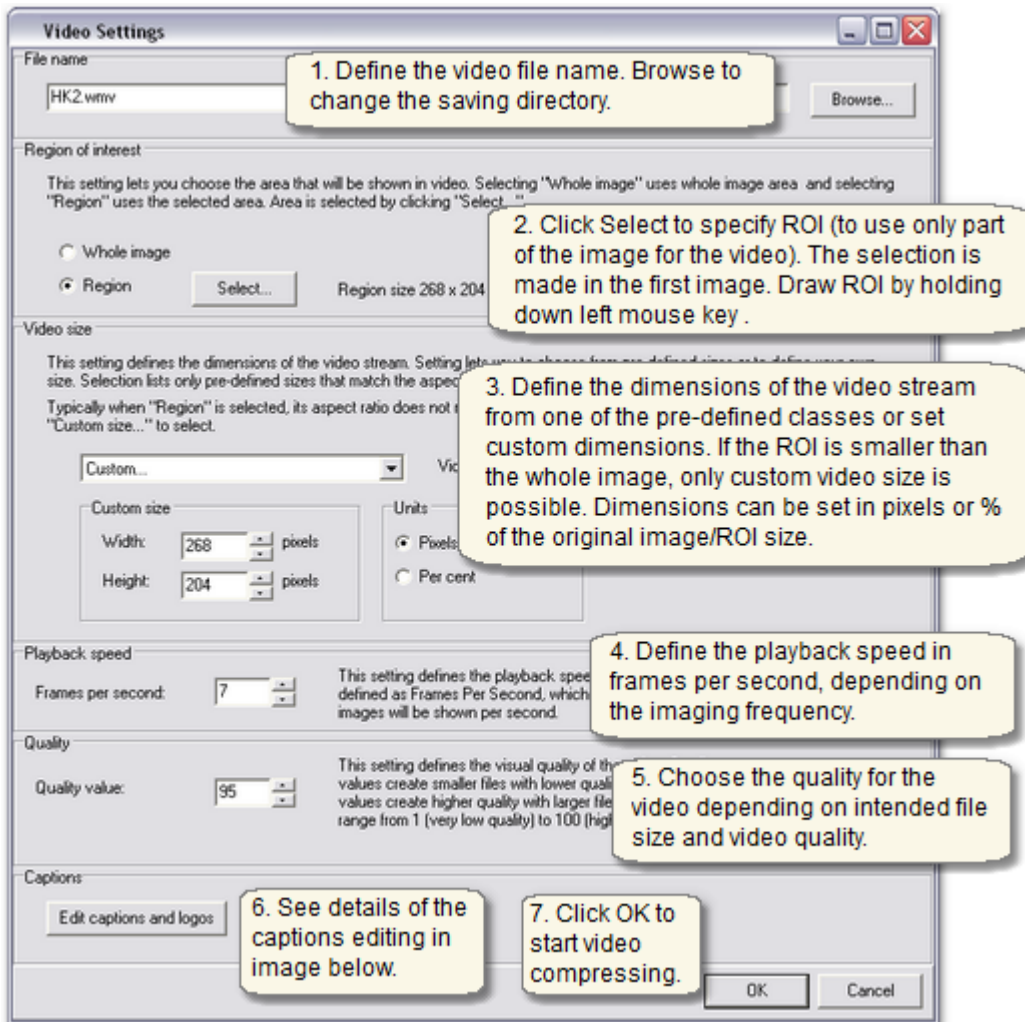
Movie maker



### 3 Movie maker



#### Video settings



### Captions and logos

The screenshot shows the 'Captions and logos' dialog box with several callouts:

- Text field 1**: Callout: "There is no limit how many caption text fields you can add."
- Edit caption font**: Callout: "Edit caption font"
- Select caption text position in image**: Callout: "Select one of the predefined positions for the caption. Different captions can be in the same or different position, and they can have different font."
- Edit caption text.**: Callout: "Edit caption text."
- Image capture time**: Callout: "Image capture time can be added to the image. Edit the type, format and position for the time and date."
- Select capture time text position in image**: Callout: "The text field distance from image border can be adjusted."
- Logo**: Callout: "Edit logo colour, size and position in image"
- Scalebar**: Callout: "Add scalebar"
- Preview image with the selected fields.**: Callout: "Preview image with the selected fields."
- Show preview image.**: Callout: "Show preview image."

The dialog box includes sections for:

- Caption**: Text field 1, Add new, Delete all, Change font, Edit caption font, Select caption text position in image, Vertical 15 pixels.
- Image capture time**: Show (Time offset from first image, Capture time, Image name), Date format, Time format (51h 25min 20s), Select capture time text position in image, Distance from border (Horizontal 15 pixels, Vertical 15 pixels), Show preview.
- Logo**: Cell-IQ Colored, Width: 120 pixels, Height: 71 pixels, Cell-IQ logo, Select logo position in image, Distance from border (Horizontal 15 pixels, Vertical 15 pixels), Show preview.
- Scalebar**: Show (Black scalebar, White scalebar), Add scalebar, Scalebar position in image, Distance from border (Horizontal 15 pixels, Vertical 15 pixels), Show preview.

Buttons: Ok, Cancel



## Image analysis steps

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## 4 Image analysis steps

The image analysis is made with the following steps:

1. View images and evaluate which Analysis method(s) give required results.
  - See the list of analysis methods from the [Analyser basic features](#) chapter.
2. Make and save Analysis Protocol that defines the analysis task and parameters.
  - See how [Cell recognition Protocol](#) and the [other Protocol types](#) are made from the corresponding chapters.
3. Run analysis in Batch Process for the entire plate or selection of wells.
  - See from the the [Batch process](#) chapter how the plate is analysed with the different tools.
4. View results in Analyser and export them to Excel if necessary. Videos can also be made from result images.
  - See from the [Analysis results](#) chapter how the results are viewed in the curve graph window.
  - See from the [Batch process](#) chapter how the result images can be post processed and e.g. exported to videos.

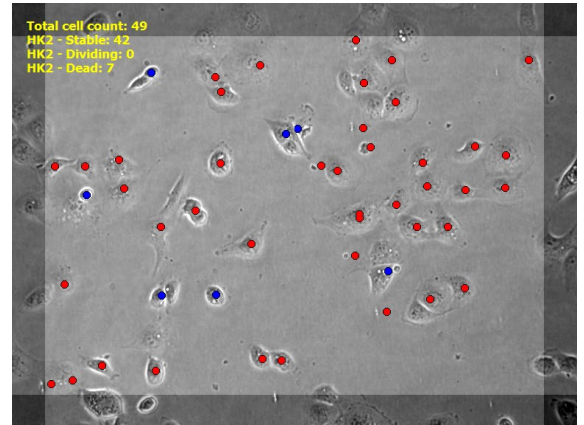
## Making cell recognition Protocol

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# 5 Making cell recognition Protocol

The Cell Recognition protocol is needed when cells are analysed in Batch Process with the Cell counting and recognition tool. The tool counts cells and classifies them into the user defined classes. The analysis protocol can define cell size, population density, neurites and areas in a specific cell test.



## Segmentation and classification

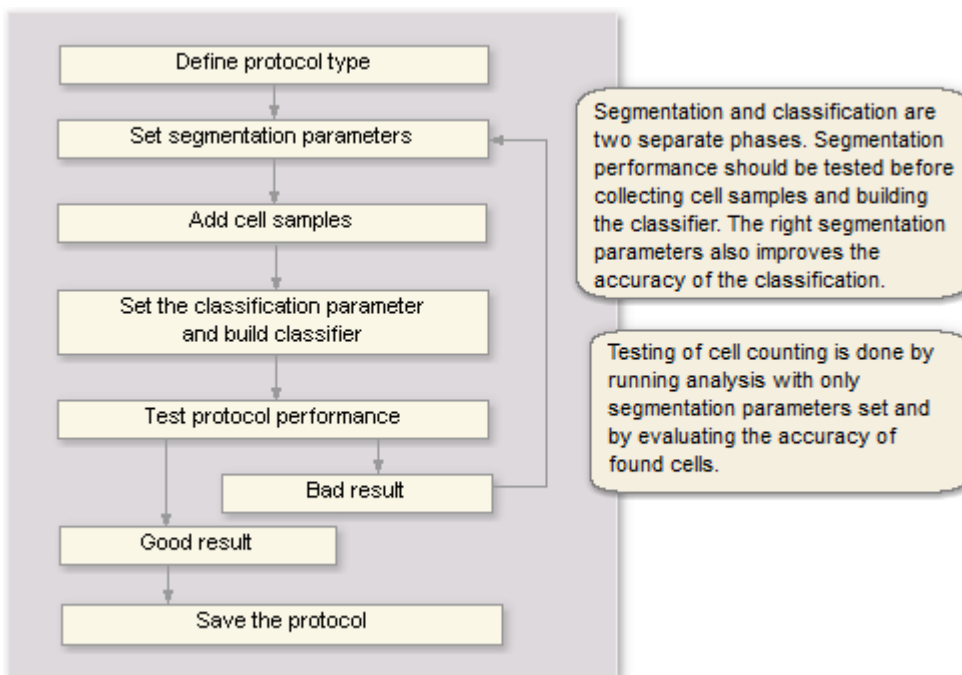
Creating an analysis protocol has two major phases:

- (1) Segmentation parameter selection and
- (2) sample library making.

Correspondingly, in the analysis run, the system first segments the images, in other words finds the interesting objects (= cells, neurite or area) in the image. Next these objects are recognized into user defined classes using the classifier that has been created based on information in the sample library.

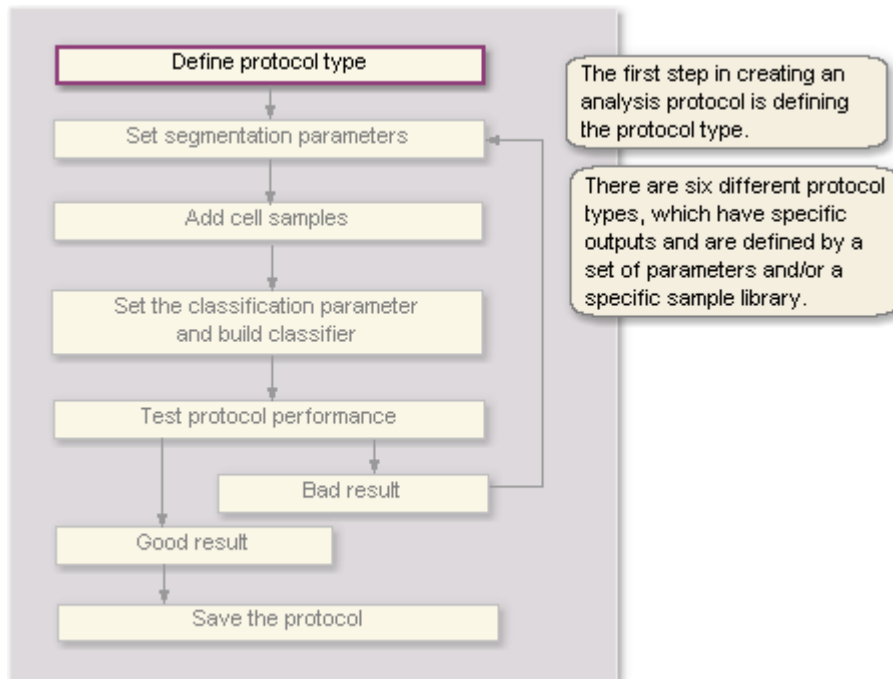
## Overview on Protocol making process

The process of creating an analysis protocol starts with defining the protocol type e.g. cell classifier + neurite finder. The following steps are setting the segmentation parameters, collecting a sample library and setting the classification parameter. The classifier is then ready to be built. Then test run is performed with the completed analysis protocol to evaluate its performance. If the result is not satisfactory, the segmentation parameters can be adjusted, the sample library can be reviewed and the classification parameter can be adjusted. This cycle is repeated until the analysis results are correct.

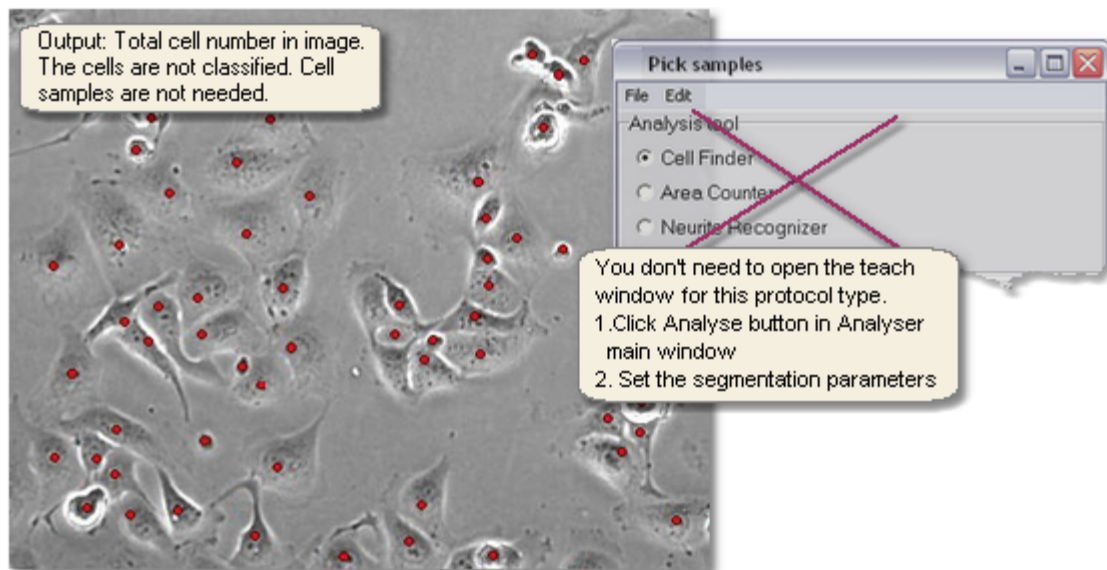


## 5.1 Protocol types

- [Cell Counter](#)
- [Cell Analyser](#)
- [Neurite Finder \(with cell counter\)](#)
- [Cell Analyser + Neurite Finder](#)
- [Neurite Recognizer](#)
- [Area Counter](#)



## 5.1.1 Cell counter



## 5.1.2 Cell Analyser

The screenshot displays the Cell-IQ Analyser software interface. The main window shows a grayscale image of cells with green and red markers indicating segmentation. A 'Pick samples' dialog box is open, showing the 'Cell Finder' tool selected under the 'Analysis tool' section. Below the main image is a grid of sample images, with the first two rows labeled 'Non stressed [H292]' and 'Stressed [H292]'. A list of sample numbers (88-97) is visible above the grid.

**Output:** Total cell number and cell number in each class in image.  
**Cell are classified and protocol includes:** cell sample library

1. Select Tools -> Pick samples in Analyser main window.
2. Select Cell Finder under Analysis tool.
3. Set segmentation parameters.
4. Create sample library.
5. Set Recognition complexity and Build Protocol.

**Pick samples**  
 File Edit  
 Analysis tool  
 Cell Finder  
 Area Counter  
 Neurite Recognizer

Example of the sample library with three classes.

### 5.1.3 Neurite finder (with cell counter)

The screenshot displays a microscopy image of neurons with blue segmentation lines. Three windows are overlaid on the image:

- Pick samples:** A window with a red 'X' over it, indicating it is not needed for this protocol. It lists 'Analysis tool' with options: 'Cell Finder', 'Area Counter', and 'Neurite Rec'.
- Cell counting parameters:** A window with the following settings:
  - Neurite segmentation parameters:
    - (A) Find Neurites
    - (B) Neurite treshold: 100
    - (C) Cell diameter: 31
  - Cell counting parameters:
    - (D) Segm grad thres.: 35
- Instructional callout:** A yellow box with the text: "You don't need to open the teach window for this protocol type. 1. Click Analyse button 2. Set the segmentation parameters 3. Check the Find neurites checkbox in run Analysis window."

In the bottom-left corner of the image, a text box states: "Output: Total cell number and total neurite length (in pixels) in image. Note! Cells are not classified. Cell sample library not needed."



### 5.1.4 Cell Analyser + Neurite finder

Output: Total cell number, cell number in each class and total neurite length (in pixels) in image. Cells are classified and protocol includes cell sample library

**Pick samples**

File Edit

Analysis tool

- Cell Finder
- Area Counter
- M...

1. Click 'Pick samples' in Analyser main window
2. Select Cell Finder under Analysis tool
3. Create cell sample library
4. Set segmentation parameter & build classifier
5. Check find neurites checkbox in Run analysis window.

**Cell counting parameters**

Neurite segmentation parameters

- (A) Find Neurites
- (B) Neurite treshold
- (C) Cell diameter

Cell counting parameters

- (D) Seam grad thres.

### 5.1.5 Neurite recognizer

Output: Total cell number and total neurite length (in pixels) in image. User creates neurite sample library. Thick or thin neurites can be excluded from the measurement. The cells are not classified.

1. Click 'Pick samples' in Analyser main window
2. Select Neurite Recognizer under Analysis tool
3. Create neurite sample library
4. Set segmentation parameter & build classifier

Pick samples  
File Edit  
Analysis tool  
 Cell Finder  
 Area Counter  
 Neurite Recognizer

All neurites [Neurites]

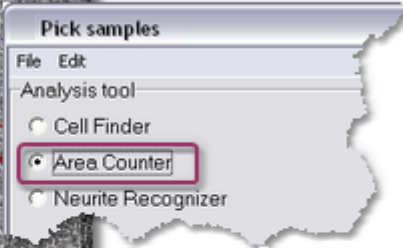
Example of the sample library, including and excluding objects that are counted.

**\*Note:** Assign just one class for the neurites and one or more for **all objects** that are excluded from the measurement, also including cells. Two different types of neurites can be classified with a combination of two separate neurite recognizer protocols.

### 5.1.6 Area finder

Output: Ratio between background area and target area (e.g. cell colony), and size of the area (in pixels) of each class. All pixels are analysed and assigned to a class. User creates an area sample library.

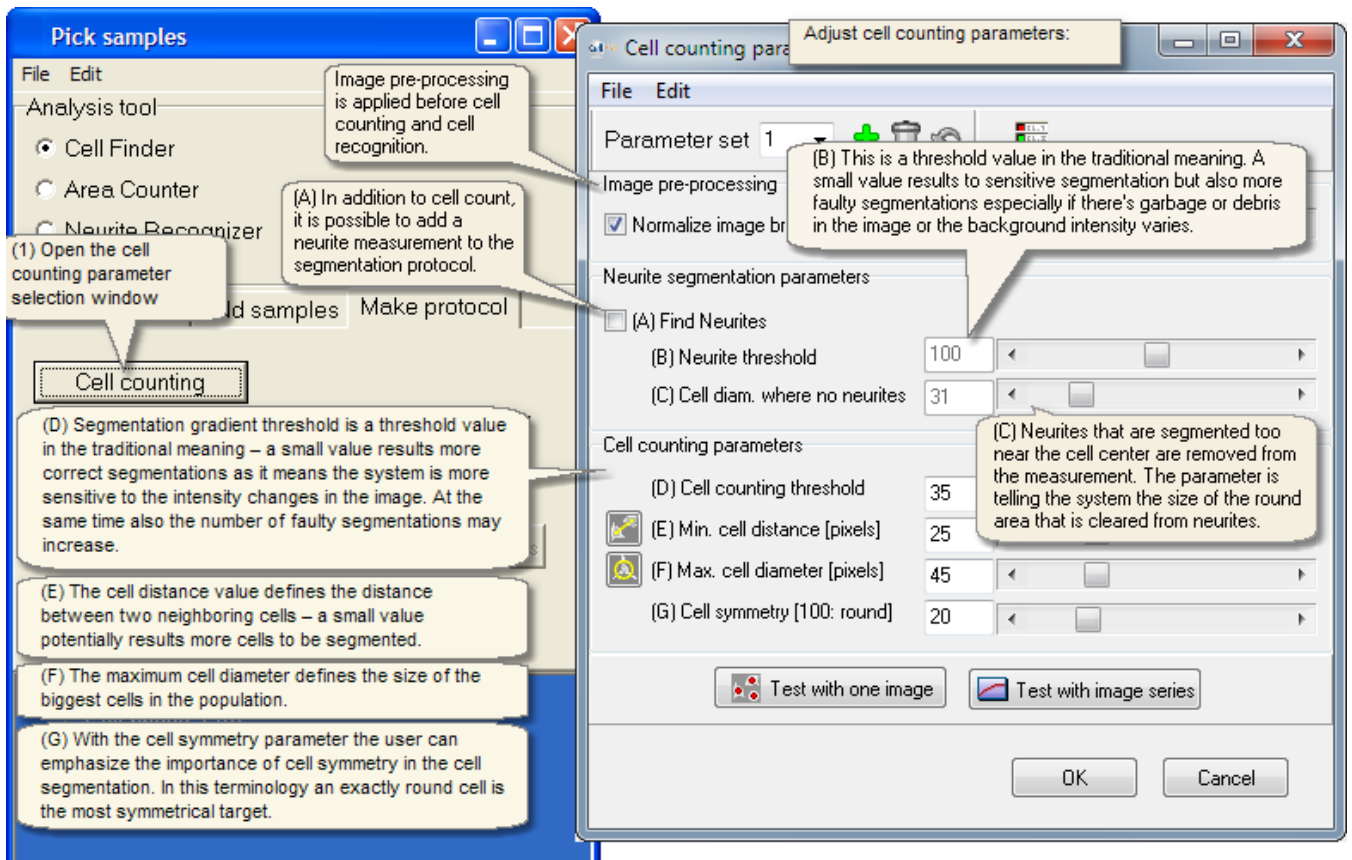
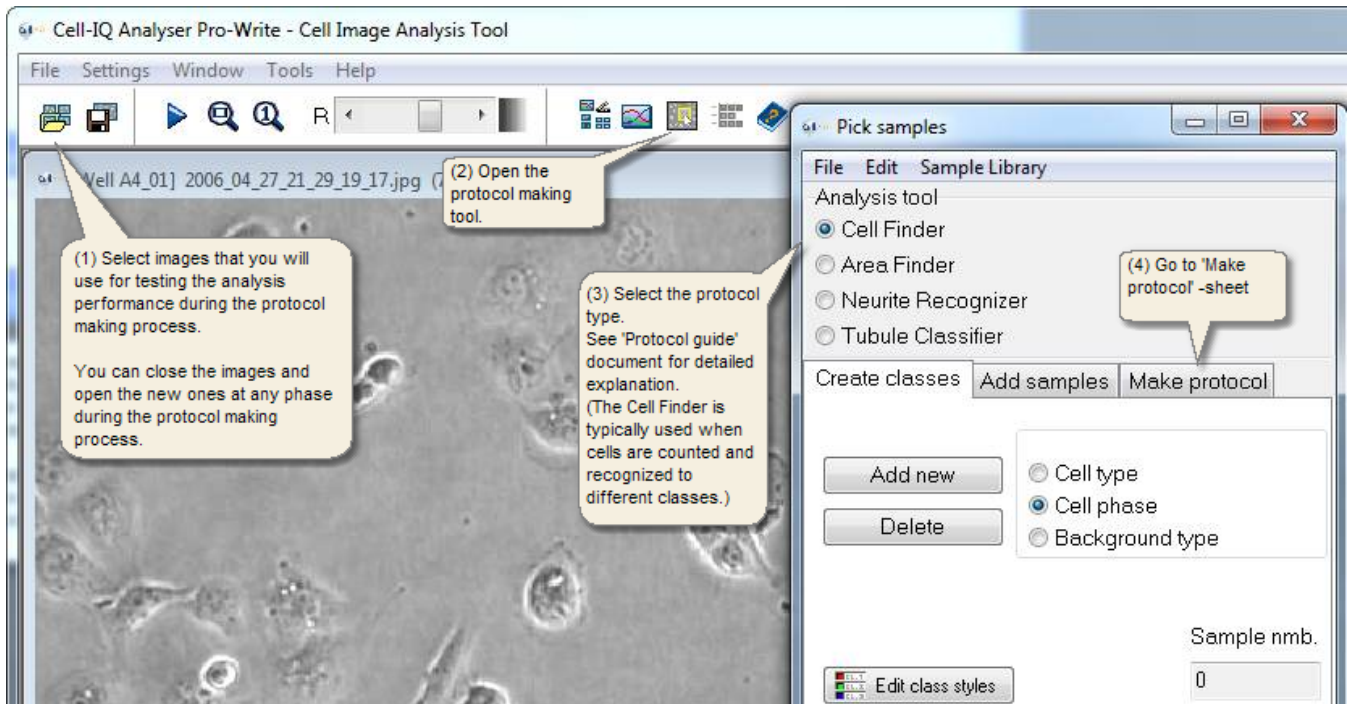
1. Click 'Pick samples' in Analyser main window
2. Select Area Counter under Analysis tool
3. Create sample library

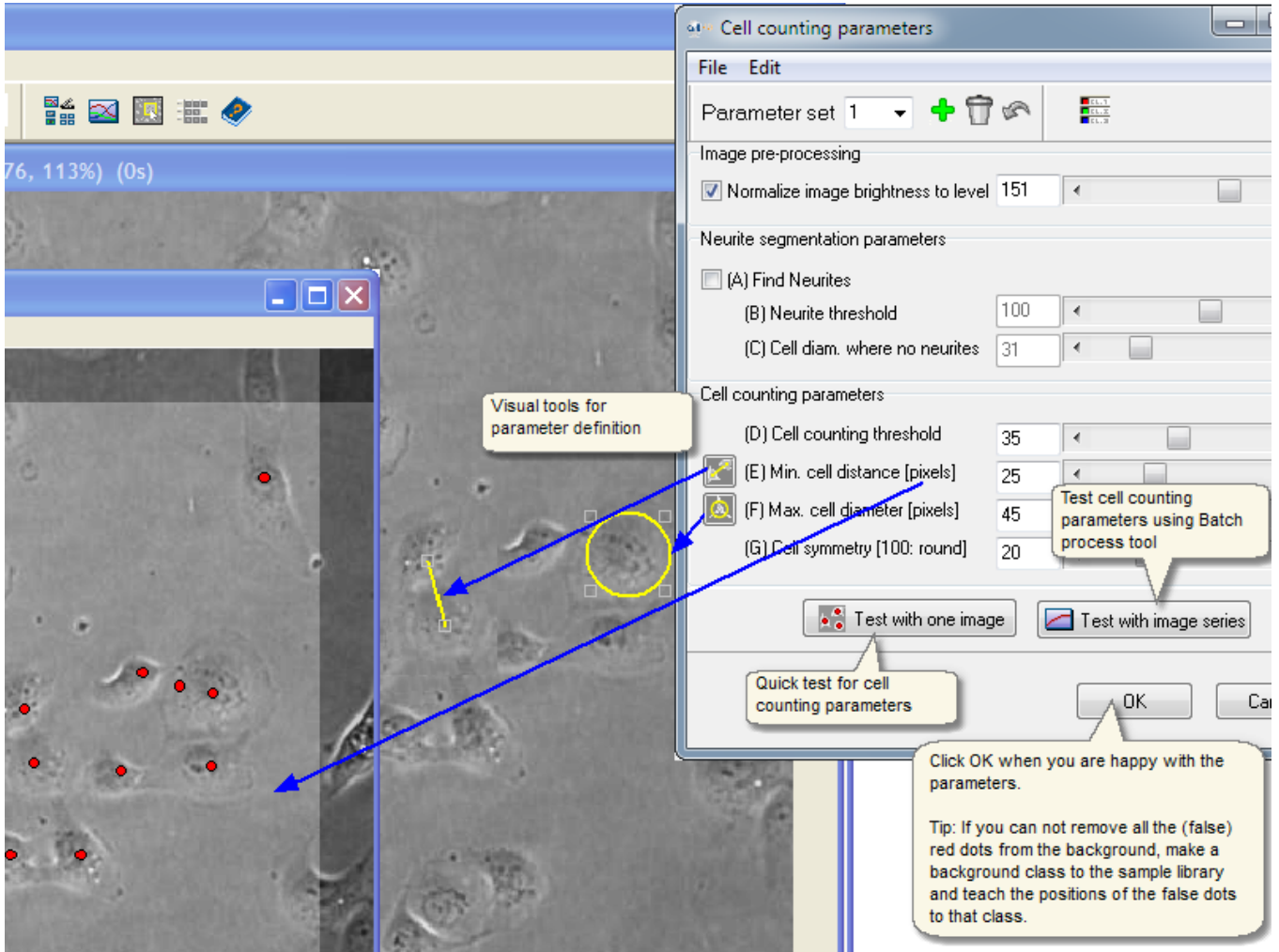


Example of a sample library in Area protocol. A background class is always included to the protocol (areas that are not of interest) and one or more classes for areas of interest.

	1	2	3	4	5	6	7	8	9	10	11	12	13
All background [Backgr													
Cell phase 1 [Cell type 1													
Cell phase 2 [Cell type 1													

## 5.2 Protocol making steps





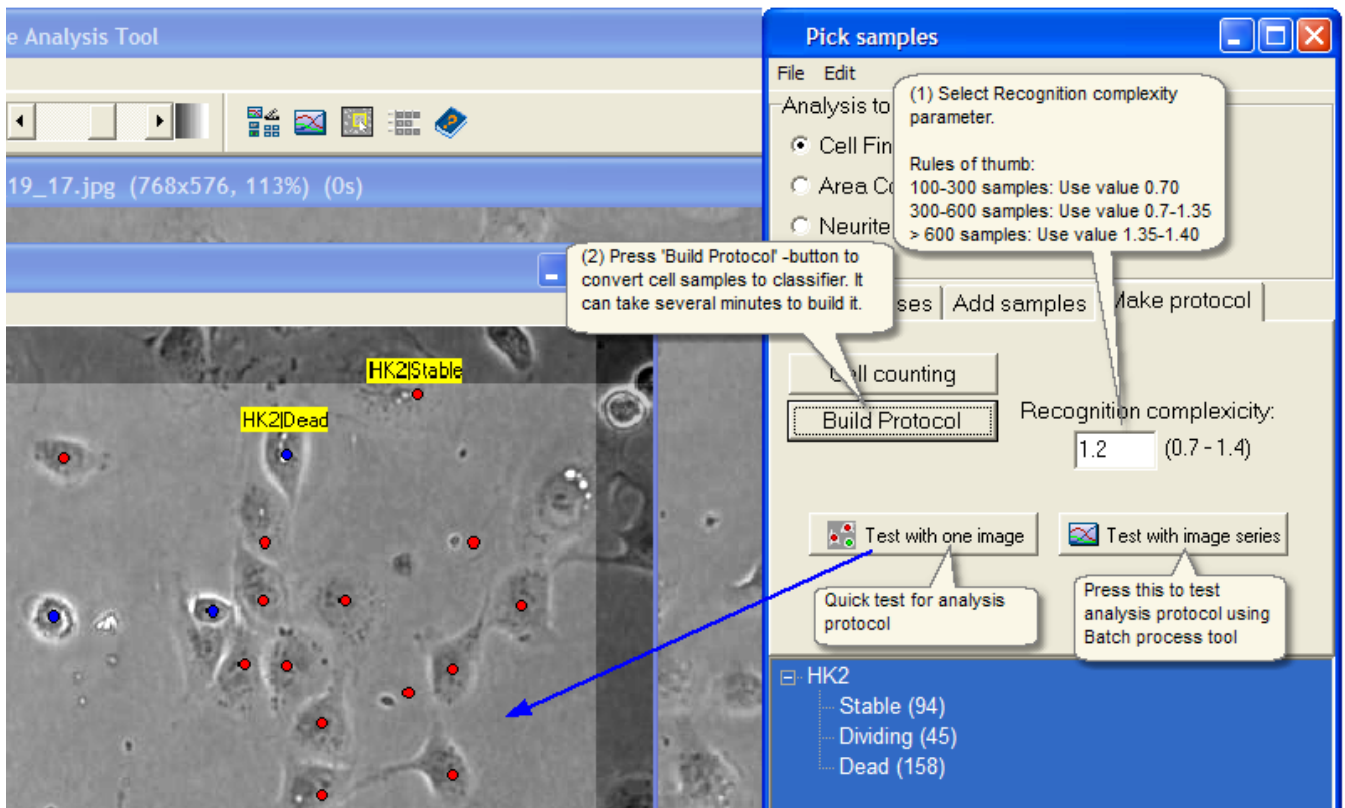
Now the cell counting part of the protocol is ready. Save the protocol from the Analyser main window: File -> Save Analysis Protocol.

## Sample library making

Next you need to make the sample library, as instructed in the following topic: [Sample library -> Creating sample file](#). It is recommended to add minimum 100 samples from the whole experiment to each class for analysis to be accurate. See more detailed explanation about the sample selection in the document **Protocol guide**.

## Build and test the protocol

After the sample library has been created, the classifier has to be built (see the image in below) and tested in case it is needed to modify the sample library for even better analysis accuracy. When Protocol building finishes, program prompts to save it to file.

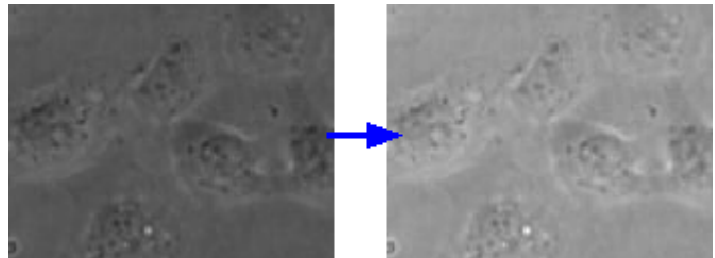


The protocol is now finished and ready to be used in the [Batch Process tool](#) for running an analysis. Save the protocol from the Analyser main window: File -> Save Analysis Protocol, if it was not yet saved.

### 5.3 Image pre-processing

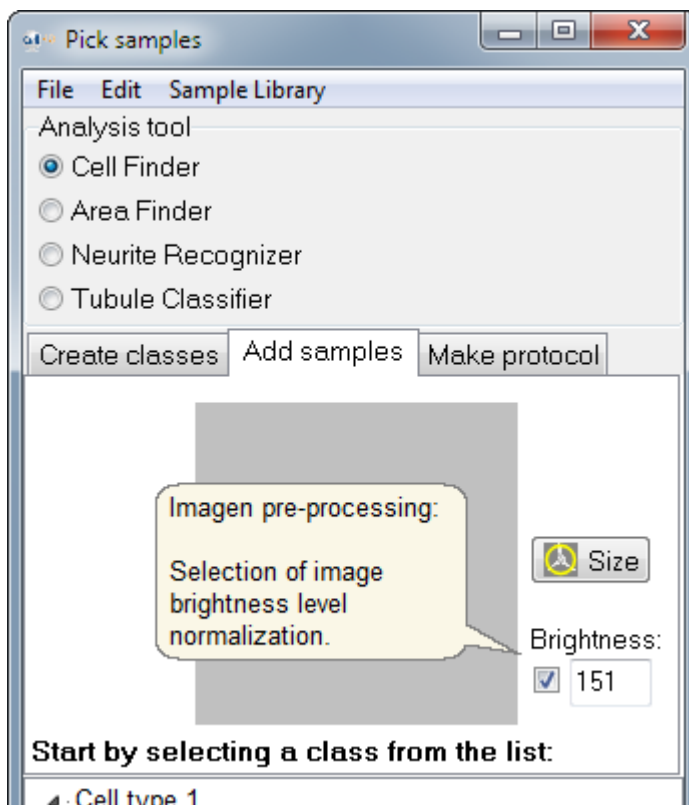
#### Image brightness level normalization

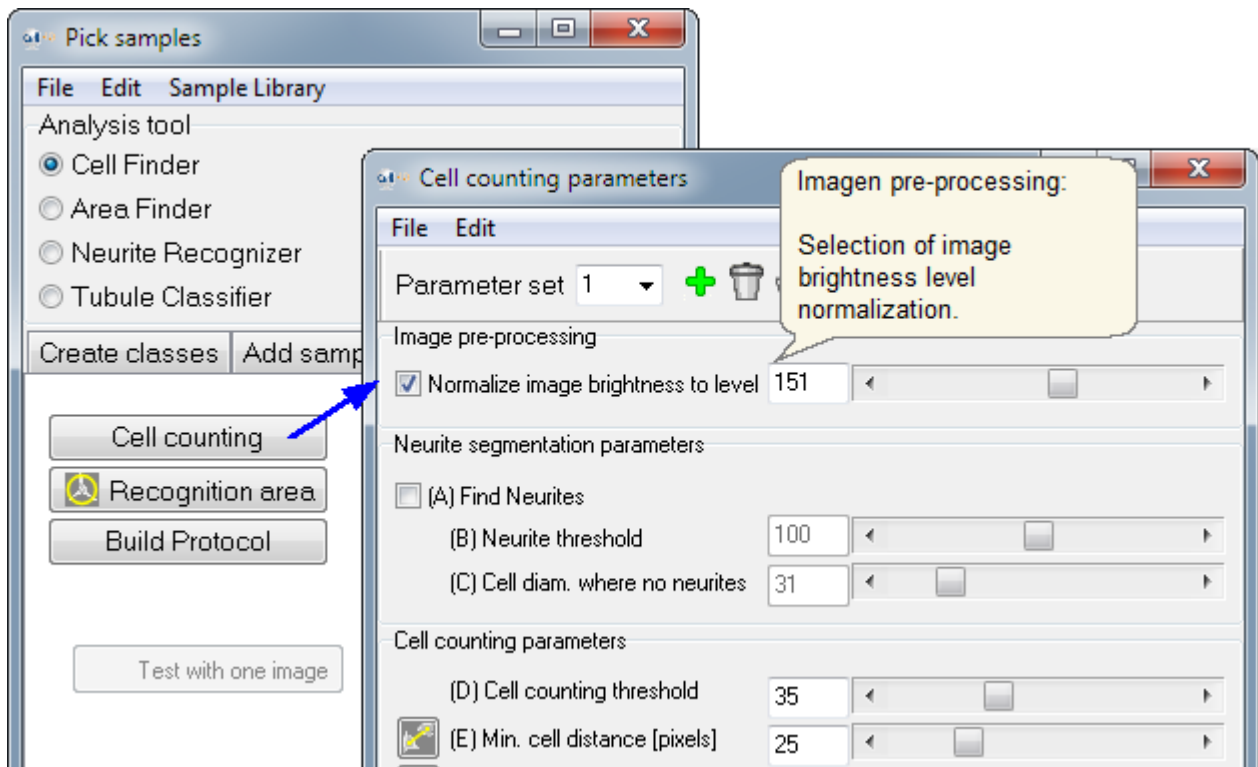
By default the Image brightness is normalized to fixed level (151) before any analysis is started, so that the same Analysis Protocol works accurately even if the source image brightness changes.



- The same brightness parameter is applied both before the cell counting and before the cell recognition.
- First adjust the brightness parameter and only after then add the samples.
- The cell sample brightness is changed only when it is added to the sample library, hence the source image brightness must stay in the same value what was selected when samples were added.
- The cell sample brightness must match to the image pre-processing brightness, for accurate cell recognition.
- > The brightness parameter is disabled in the user interface after you have added the first cell sample.

The image brightness parameter can be selected from two places, but the parameter that changes is anyway the same regardless from where it is changed:







## 5.4 Sample Library

### Sample library guide

- Defining classes
- Creating a sample library file (\*.ims or \*.cts)
- Viewing the samples in a sample library file
- Editing sample library file by moving and deleting samples, renaming and merging classes.

Sample library is the tool for designing analysis protocols for specific cell tests. Also, if an analysis protocol is requested from CM Technologies, a sample library will need to be attached corresponding for the analysis needs. A sample library is a collection of samples of cell, neurite and area types selected by the user. The sample library is essential for the creator of the analysis protocol. Samples are selected from cell images in Analyser and saved into a \*.cts sample file. Create an analysis protocol by setting the segmentation parameters, the classification parameter and by building the classifier. The detailed instructions are in the **Protocol Guide**. Contact CM Technologies to request for an analysis protocol made by a protocol specialist.

Note: If a Sample library is done and saved into a \*.ims protocol file instead, the saved file has all the same information as in the \*.cts file, and additionally the cell counting parameters. And if the protocol had been built prior to saving, also the \*\_classif.txt classifier file is automatically saved.

### Number of samples

The Sample library in a completed Protocol has normally a minimum of 100 samples in each class, and there is no upper limit to how many samples can be included; the higher number normally results in better accuracy. If the protocol is ordered from CM Technologies, normally 20 samples in a class is sufficient. But it is important to clearly demonstrate the difference between the classes. Each class can have a different number of samples.

### Cell size and position

Recognition is done from a single cell, not from the position of cell in a cell colony. One may be tempted to define cells into the classes based on their position in a colony (near center / near edge), but the cells must look different inside the sample window. Cells that are larger than the sample window can still be recognized if there are visible differences to other classes inside the sample window.

### Sample window

The recognition of cells is based on features extracted from the sample image. Those features describe the surface structure and outer shape of the cells. Features must be seen inside the sample image. But since the cells are not counted based on the sample window, the entire cell area does not need to fit inside the sample window. The size of the sample window can be changed from the Recognition area -settings.

### The classifier

The classification is defined by the classifier. For building the classifier you need to have a sample library that defines the classes in the protocol. You also need to set up the classification parameter and build the classifier.

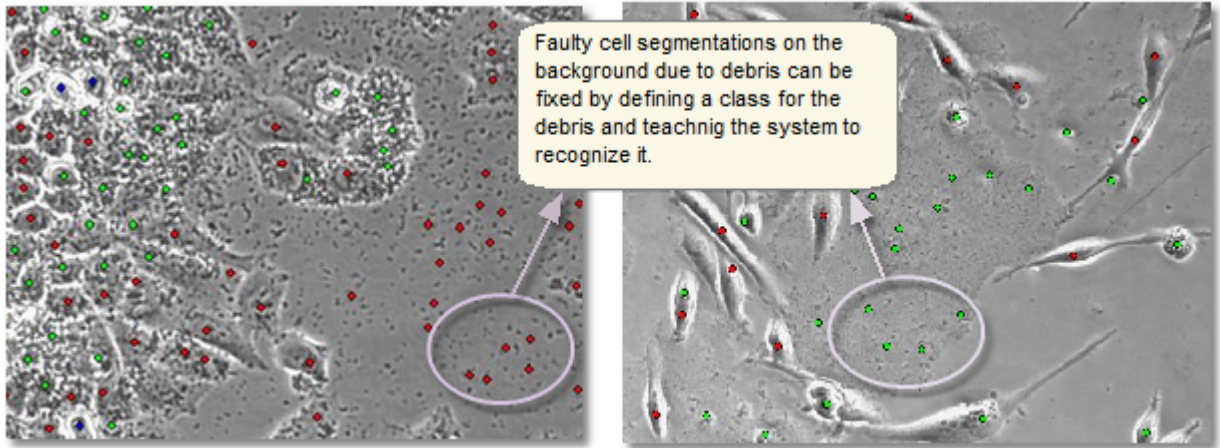
### Summary

- ? Define only classes with clear difference between them that is visible inside the sample square.
- ? You can combine uninteresting objects into one class. Different looking objects can be assigned to the same class.
- ? Objects that look too identical can't be in different classes. The recognition accuracy suffers in both of those classes.
- ? Classification is not based on cell movement or cell history.

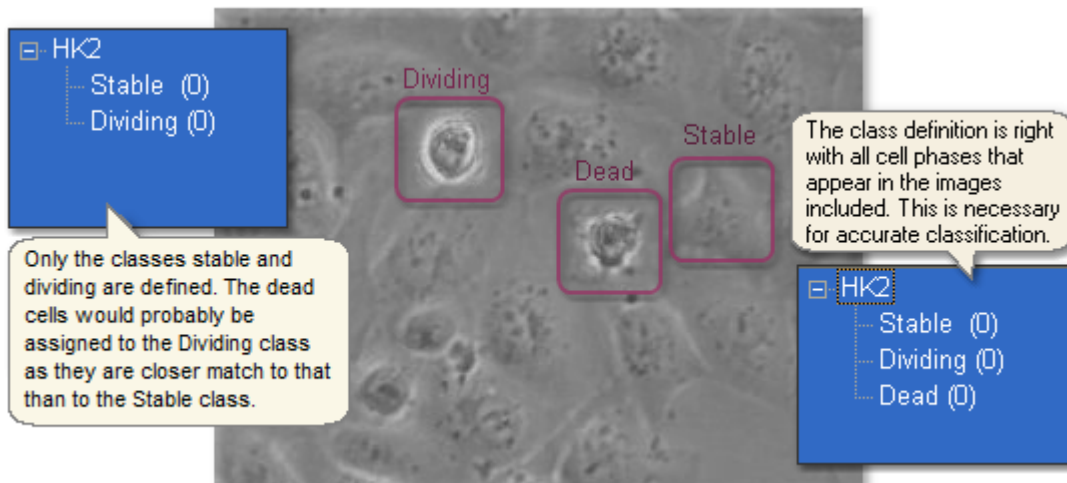
## 5.4.1 Defining classes

### About creating classes

- ? Browse the cell images throughout the cell test data and examine the morphology of different phases.
- ? Create two or more classes for the cell types and phases you want to analyse. Remember to assign all objects present in the images to one of these classes including debris, garbage or other particles. If there are a lot of uninteresting objects in respect of the cell count and recognition, you can make one class for all those items. For example, if there are garbage particles, unwanted cell type or multiform trash on the background, they can all be defined into a class "other", see image below. Everything is counted anyway in the segmentation phase.

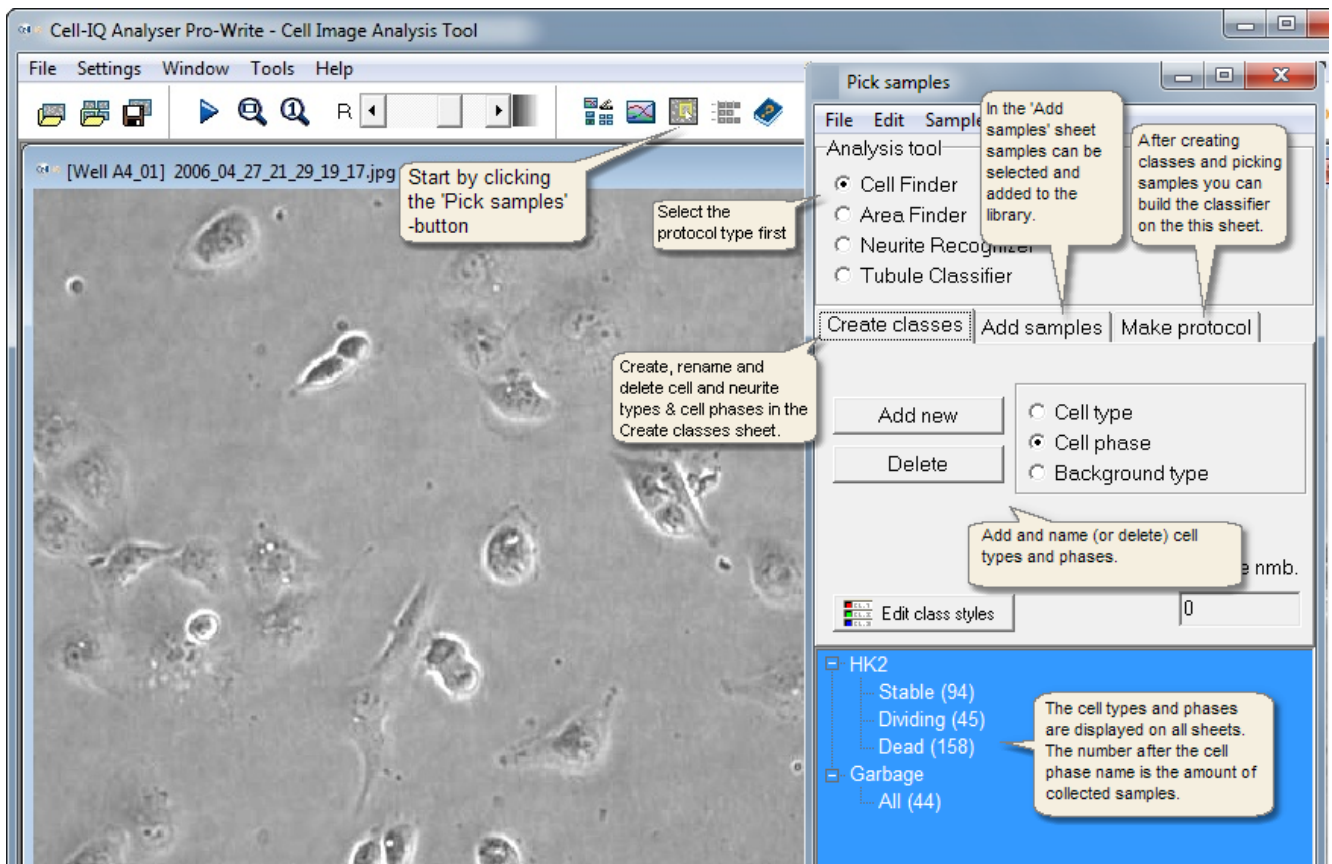


- ? Defining too many classes e.g. more than six, the classes might not have clear enough differences between them to be distinguished accurately.
- ? Cells are recognized from one image view, not based on cell movement or cell history.
- ? A clear difference in features must be seen between all the sample images of two different classes.
- ? Assign each sample into the right class based on the currently viewed image, not based on the images before or after.



## 5.4.2 Creating sample file

- Click **Pick samples** button (see image below) or go to **Tools** menu to open the **Pick samples** window.
- **Add cell types/phases:** Select Cell type or Cell phase first and click **Add new**. Each cell phase will be a class in the protocol where as each Cell Type is meant to group cell phases clearly and doesn't affect the classification.
- **Delete cell types/phases:** Click the type/phase once and then click **Delete**.
- **Rename the cell types and phases:** Click the cell type/phase name. Then click the active name a second time. Edit the name in the active box.
- Note that defining several phases e.g. more than six, the classes might not have clear enough differences between them to be distinguished accurately.

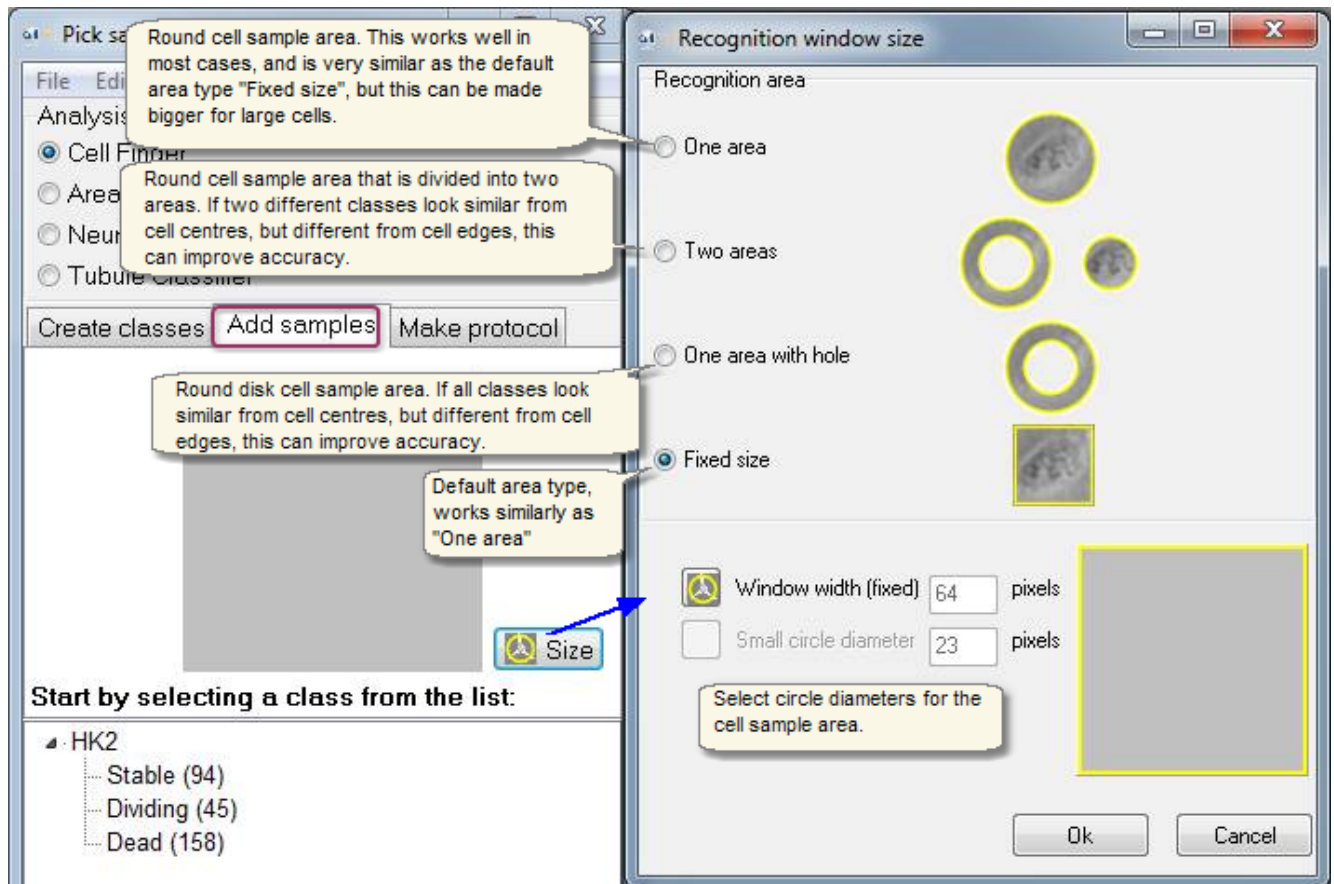


## Teaching

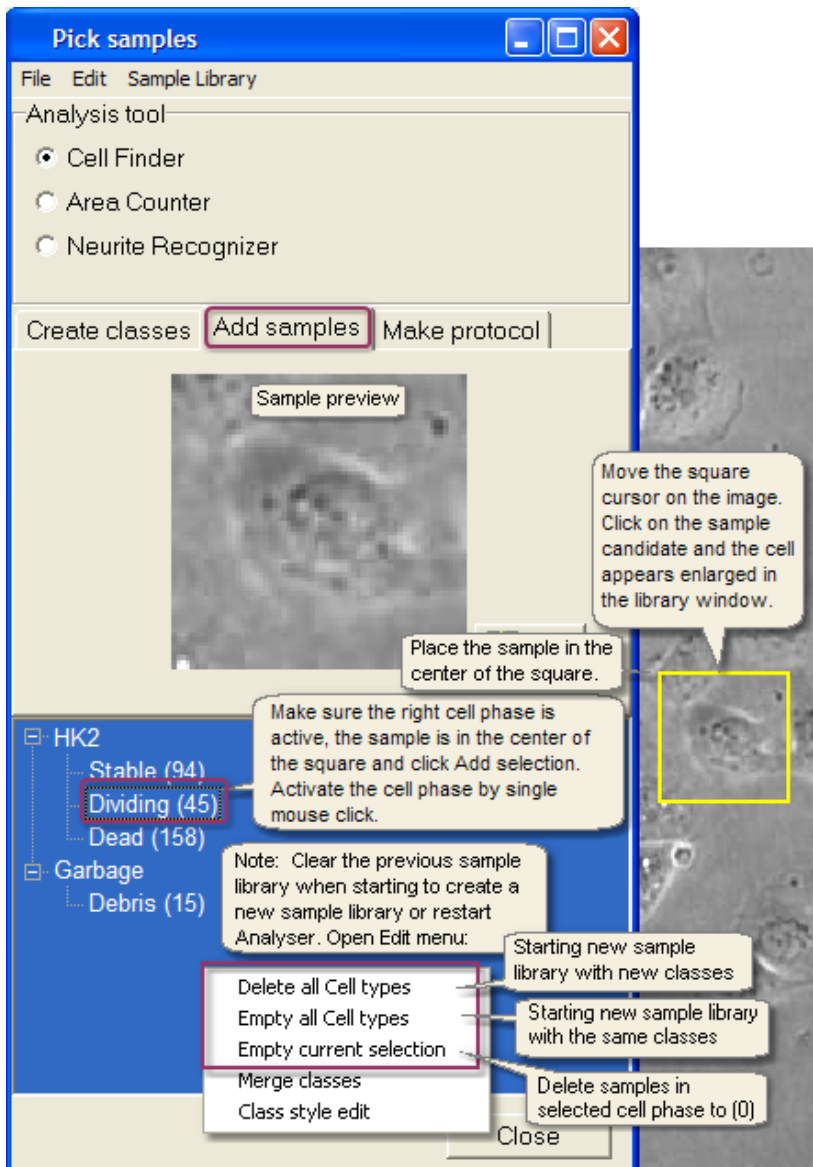
Select the **Add samples** sheet to collect samples to the classes.

- If there are no samples added yet, program offers to change the cell sample window size. Default window size is good in most situations, and you can click **No** to keep it. You can change the window size at any time later (with some constraints) also by clicking the **Size** button.

**Note:** The window size for the cell sample images in the saved protocol file depends on the selected sample window size. If the selected size is small, the saved size is small, and when you increase the window size in the program later, the sample image size will be increased artificially, and the analysis accuracy may decrease somewhat. The program never decreases the saved sample size, so if you first increase the size, add samples, and then decrease the size, the bigger save size is used for all samples, even if displayed size is smaller.



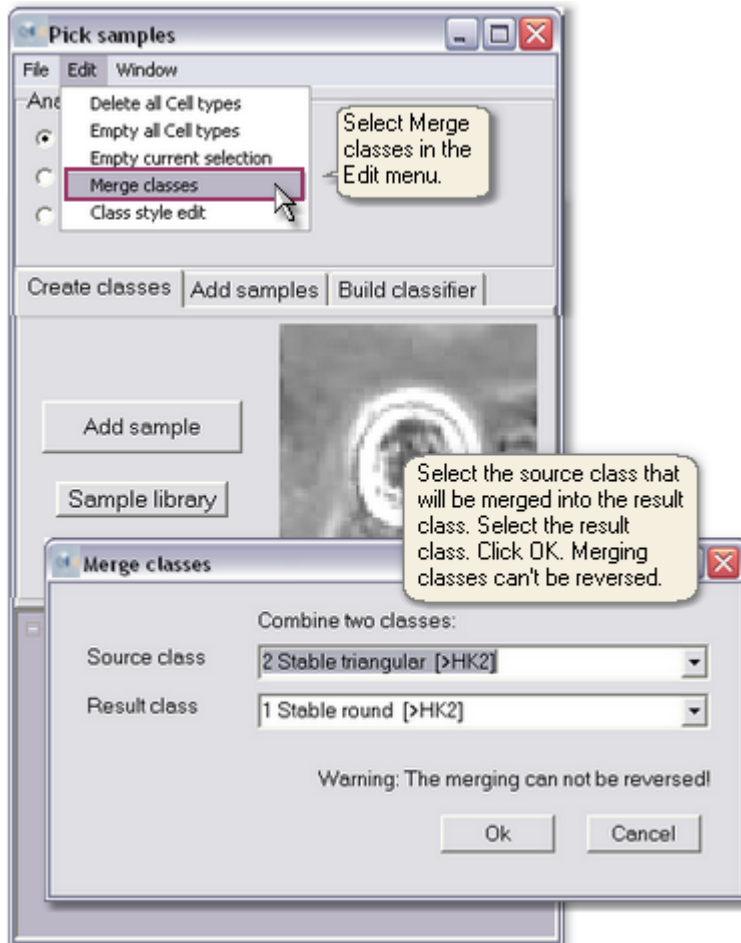
- Click the name of a cell phase to add samples. The active cell phase becomes highlighted by a dashed rectangle.
- Search samples in the image and place the square cursor on a sample candidate. Make sure that the cell is in the center of the square in the sample preview and click **Add sample**. The number after the cell phase increases by one.
- Repeat this procedure to add all samples. The ideal amount of sample is highly case specific but usually bit over 100 samples in each cell phase is sufficient for good recognition and 10 - 20 samples for demonstrating for protocol maker what classes will be needed for the protocol. Select samples throughout the cell test. Browse through images to obtain a comprehensive sample collection.
- Save the sample library into protocol file (\*.ims) from Analyser main window by clicking **Save**. Before you exit the program remember to double check that you have saved the sample library file as \*.ims. The protocol will have to be completed by building the classifier on the [Building Protocol](#) sheet.



**Note:** When you make an analysis protocol, you can [add cell samples automatically](#) after you have selected the cell counting parameters.

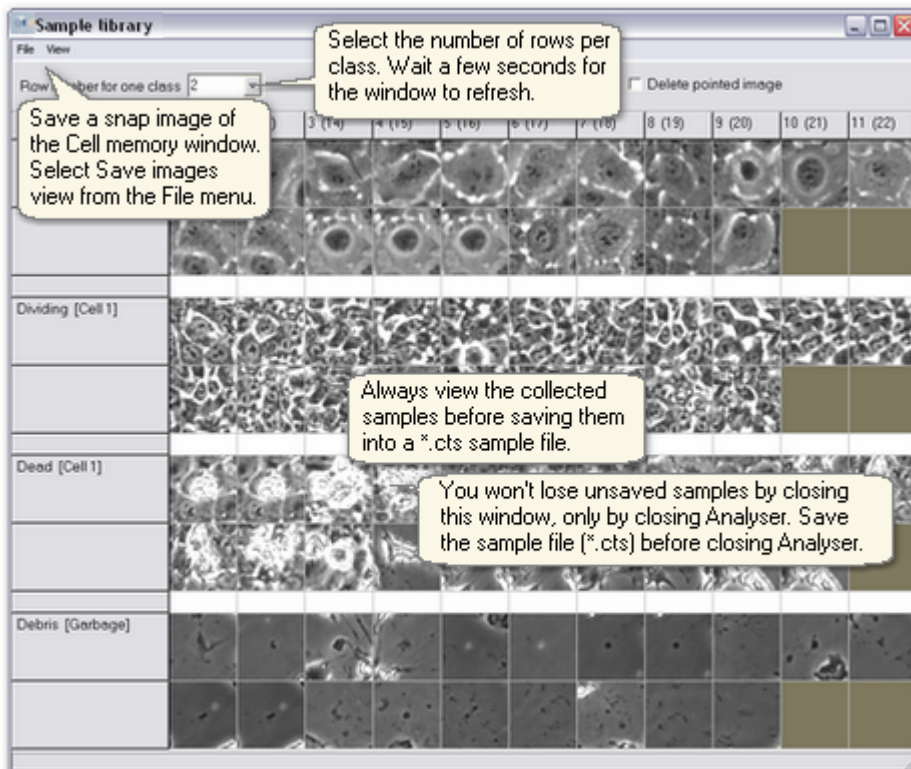
## Merging classes

Any two classes in the sample file can be merged into one. If two classes turn out to be too similar, merging them into one will save the samples unlike when deleting a class. The operation can not be reversed.



### 5.4.3 Viewing sample file

- Always review the collected samples before saving and closing the sample file. Remove any inappropriate samples and move misplaced samples to the right class. See next topic [Editing sample file](#).
- Open the sample file into Analyser and select **Tools** -> **Sample library** or click **Sample library** button on the Add samples -sheet of Pick samples -window.
- The samples can be saved only from the Analyser main window by clicking **Save**. However, you can save a jpeg snapshot of the current Cell sample window. Select **Save images view** from the **File** menu. The sample information is not included in the jpeg image but in an ims file.



### 5.4.4 Editing sample file

To have a good sample library, go through your sample selections carefully and make necessary changes. Some samples may be placed into the wrong class. Move misplaced samples into the correct class. Delete any incorrect samples, such as seen in the example of a poor sample library.

**1. MOVING SAMPLES**

- Select **Move image**.
- Select the sample by a single mouse click.
- Click any sample in the target class.
- Wait for the view to refresh.
- Save the sample file after any changes.

**2. DELETING SAMPLES**

- Select **Delete pointed image**.
- Move the cursor on a sample image and click on it once.
- Wait for the view to refresh.
- Save the sample file after any changes.

### Example: good sample library

Excellent sample library with enough samples in each class and no false or bad samples

Take a snapshot (\*.jpg)

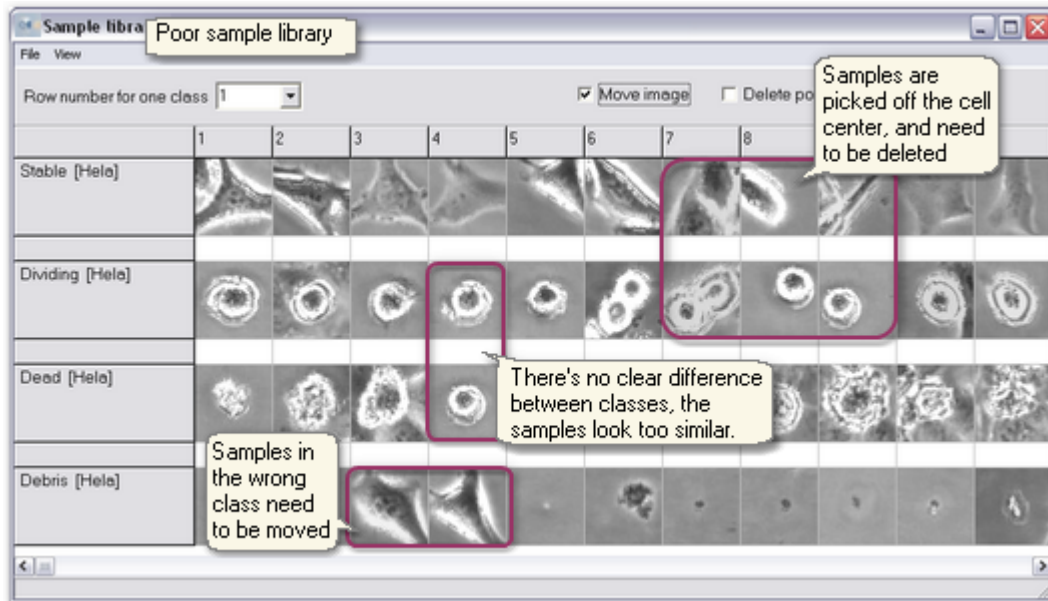
Cells are in the center of the sample square

Clear difference between classes

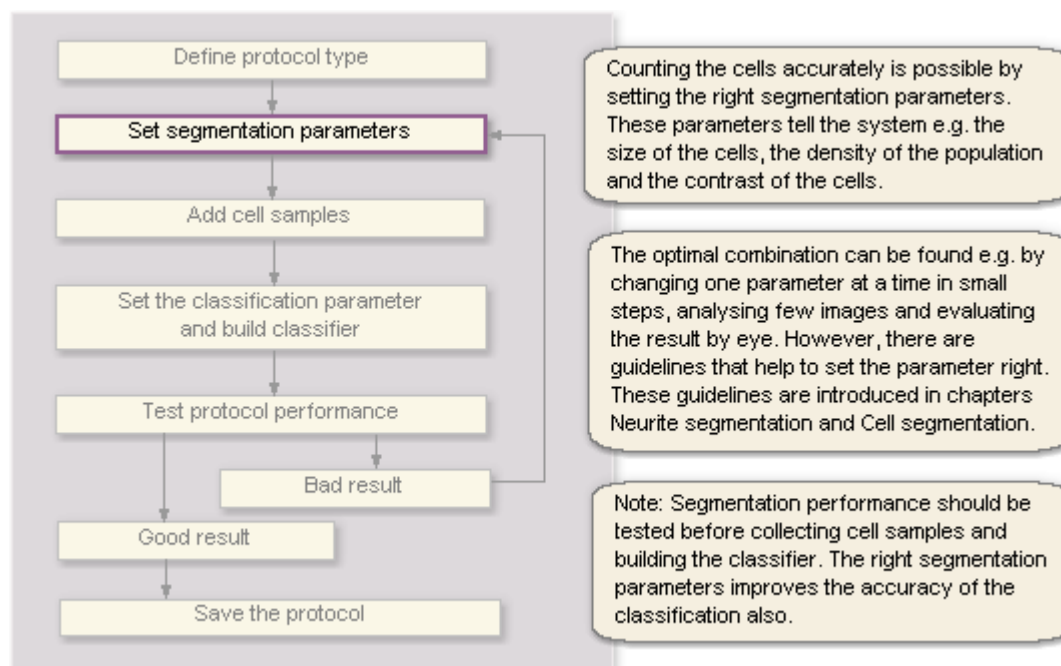
Save sample file (\*.cts) in the Analyser main window after making any changes. Click Save.



## Example: Poor sample library

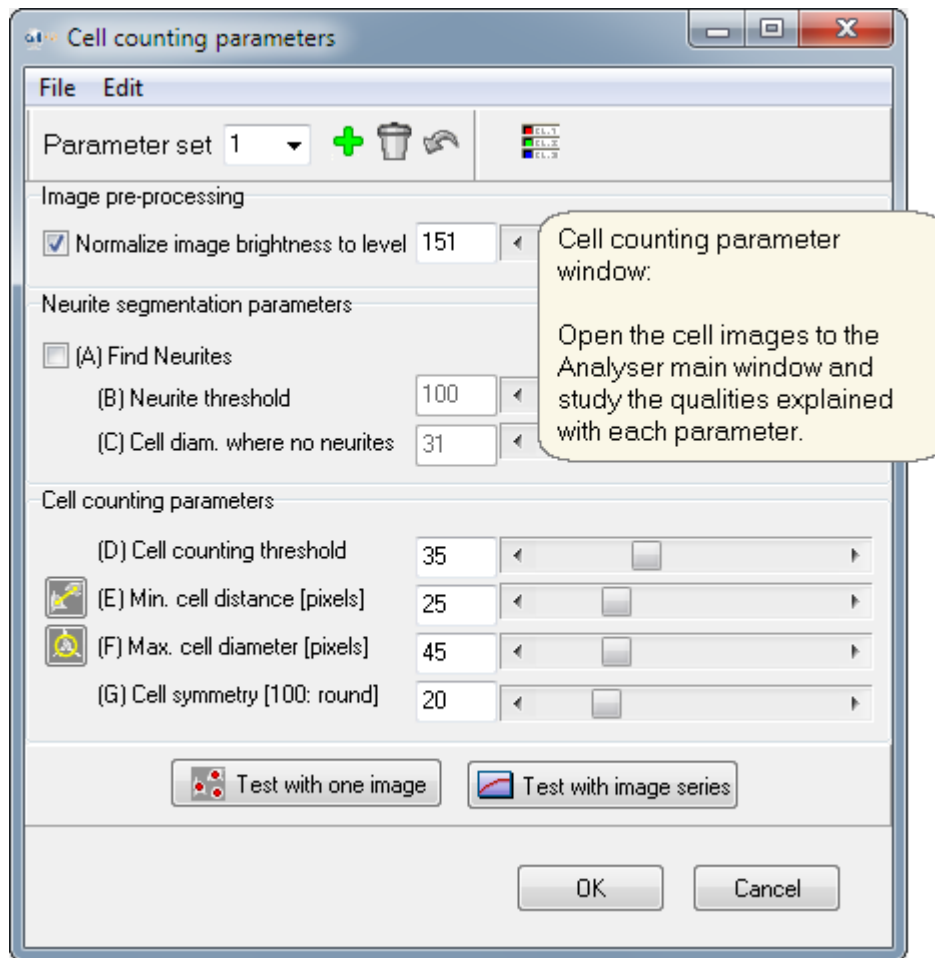


## 5.5 Cell counting parameters

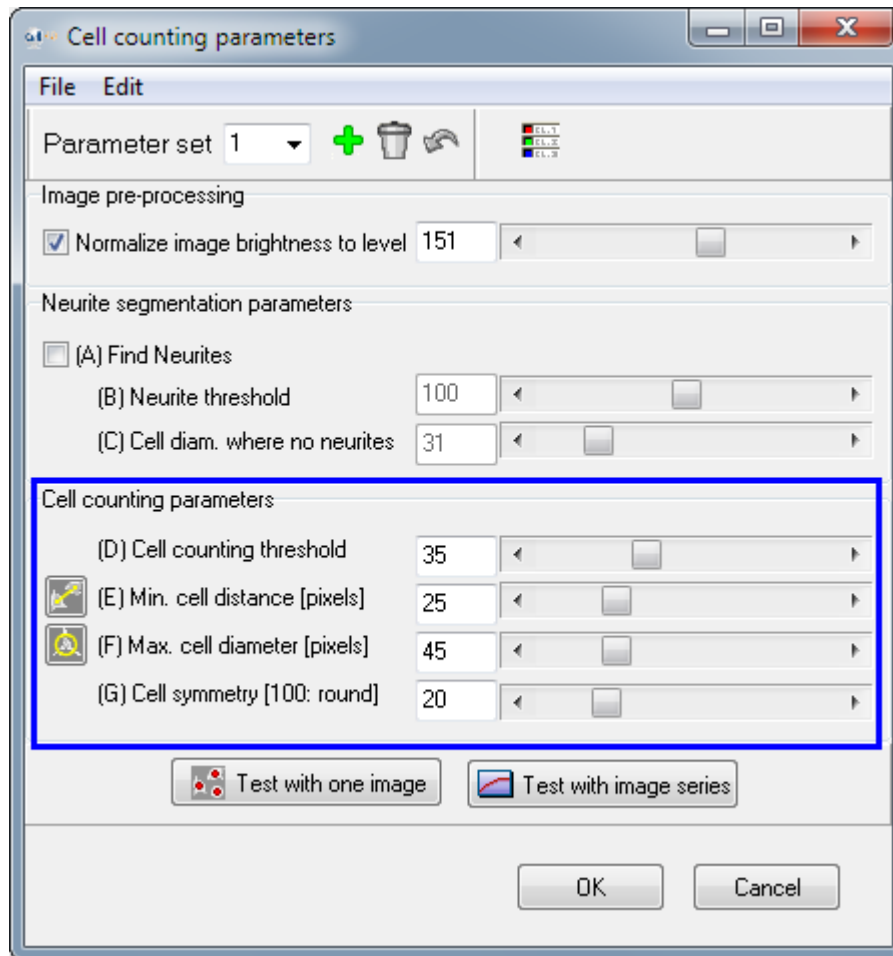


Note: The instructions in the following chapters give suggestions and usable ranges for the cell counting parameters, but the user can freely try any such parameters that exceed the given limits, though it is unlikely that they will give the best result.

### SEGMENTATION PARAMETER WINDOW



## 5.5.1 Cell counting



### (D) SEGMENTATION GRADIENT THRESHOLD

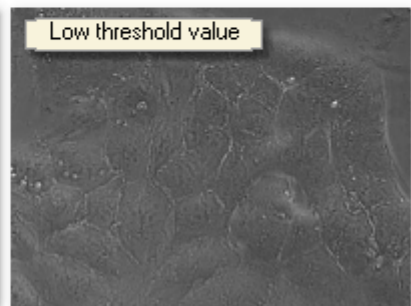
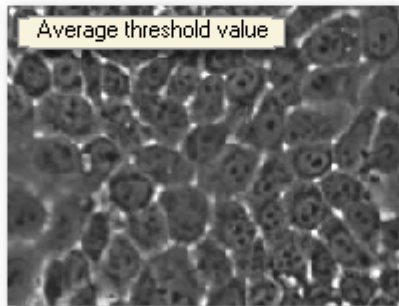
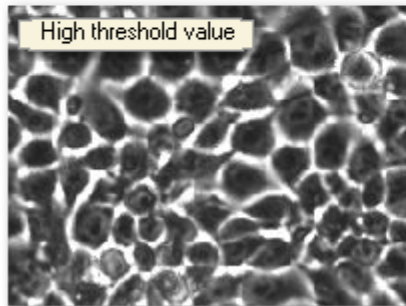
Segmentation gradient threshold is a threshold value in the traditional meaning – a small value results more correct segmentations as it means the system is more sensitive to the intensity changes in the image. At the same time also the number of faulty segmentations increases because some intensity changes are not cells.

High contrast in the cell edges and structures suggests the use of high threshold value.

Dim cells with low contrast suggest using low threshold value.

Default value: 35

Range: 5...55



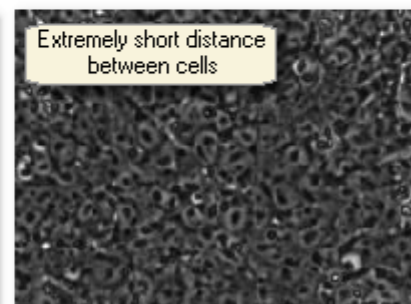
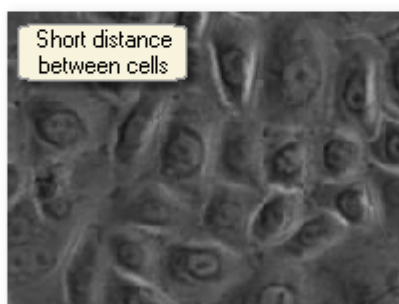
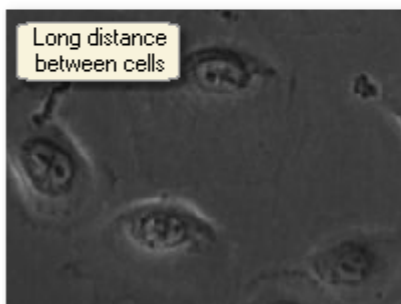
## (E) CELL DISTANCE

The cell distance defines the distance between two neighboring cells – a small value potentially results more cells to be segmented.

If the value is set to e.g. 25 -> the minimum distance between the segmented cells is 25 pixels.

Default value: 25

Range: 13...33, odd value



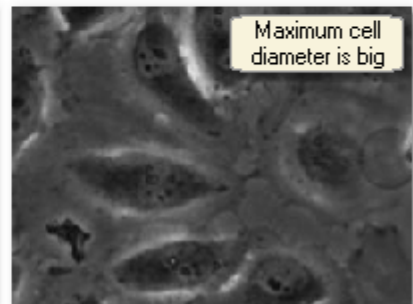
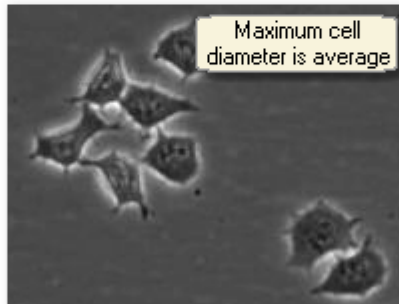
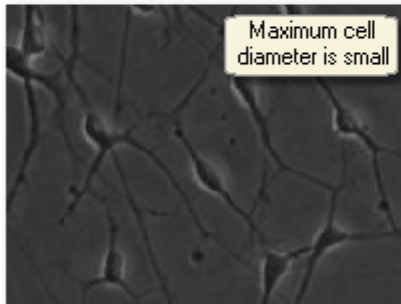
## (F) MAXIMUM CELL DIAMETER

The maximum cell diameter defines the size of the biggest cells in the population.

If the value is set to 37, the cell edges should fit inside a 37x37 pixel window.

Default value: 37

Range: 21...75, odd value



### (G) CELL SYMMETRY PARAMETER

With the cell symmetry parameter the user can emphasize the importance of cell symmetry in the cell segmentation. In this terminology an exactly round cell is the most symmetrical target.

The system always first identifies the cell centers in the image. Sometimes small and dim cells coexist with big and bright cells. In such case the system can find cells in the background right next to the bright cells even though there's really nothing.

However, the symmetry parameter implies that there are cell edges found in every direction (360°) around the cell center position.

Default value: 0

Range: 0...100

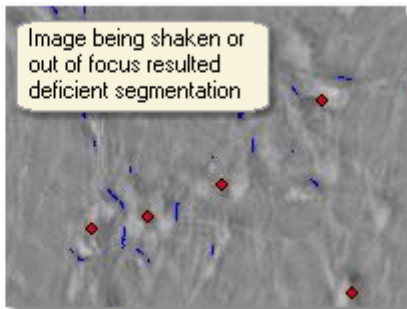
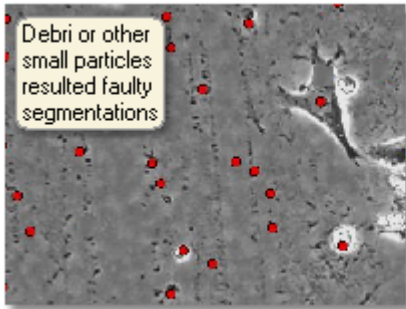
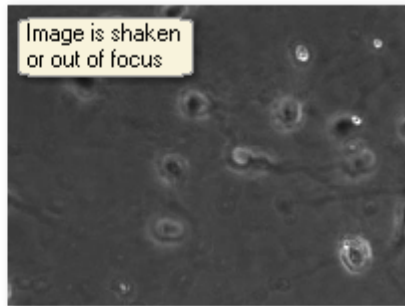
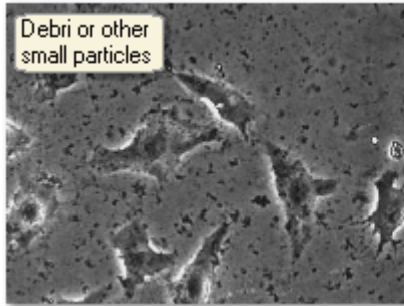
No influence: 0, and big influence: 100

Cells are often nonsymmetrical. Define the other segmentation parameters first, and only after that see if using the symmetry parameter makes the segmentation more accurate.

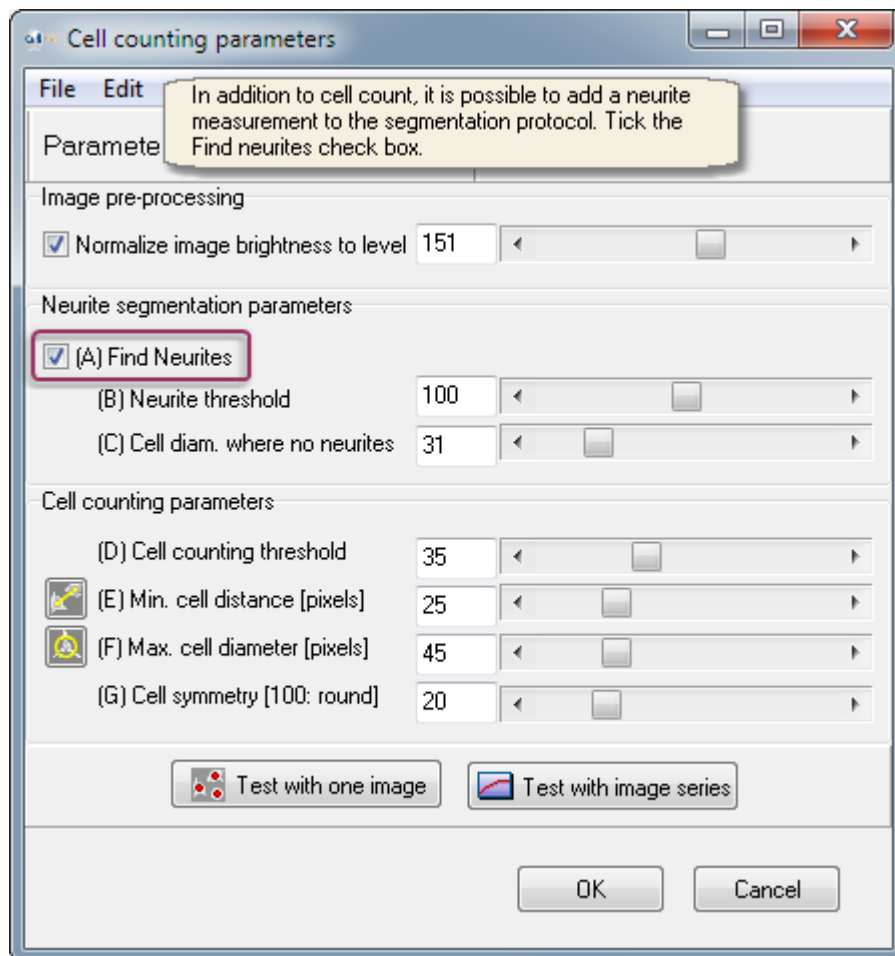
### SEGMENTATION PROTOCOL PERFORMANCE

Objects and patterns in the images that affect the accuracy of the segmentation and can cause faulty or deficient segmentation:

- ? Garbage and debris on the background
- ? Image is out of focus or shaken

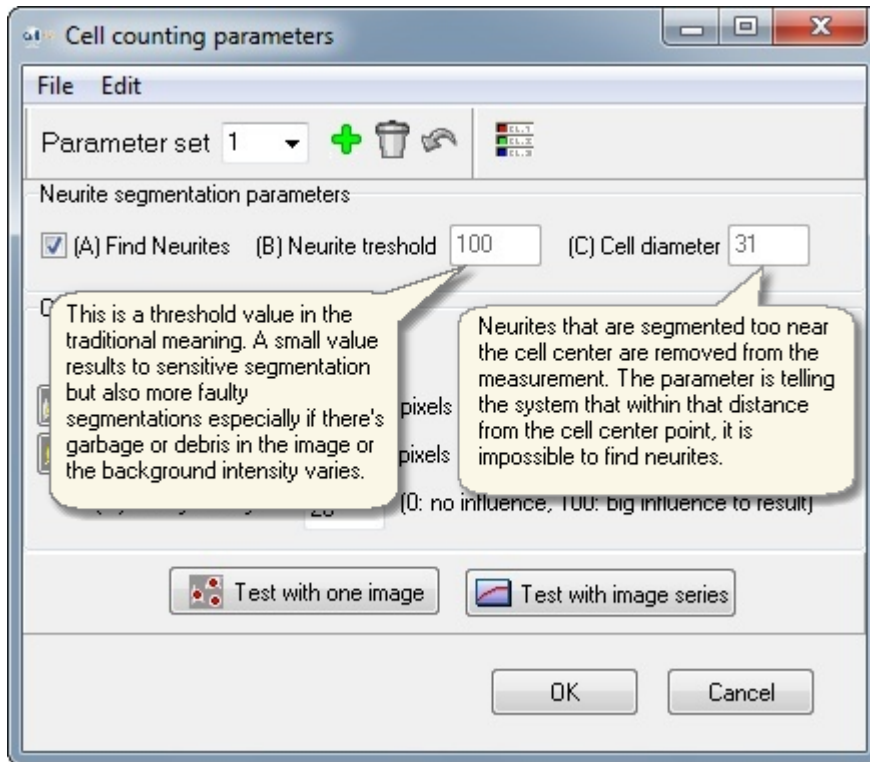


## 5.5.2 Neurite segmentation



### SETTING NEURITE PARAMETERS





**1. Neurite threshold value guidelines**

Thick neurites with high contrast suggests the use of high threshold value.

Thin neurites with low contrast suggests the use of low threshold value.

Default value: 100

Range: 30...130

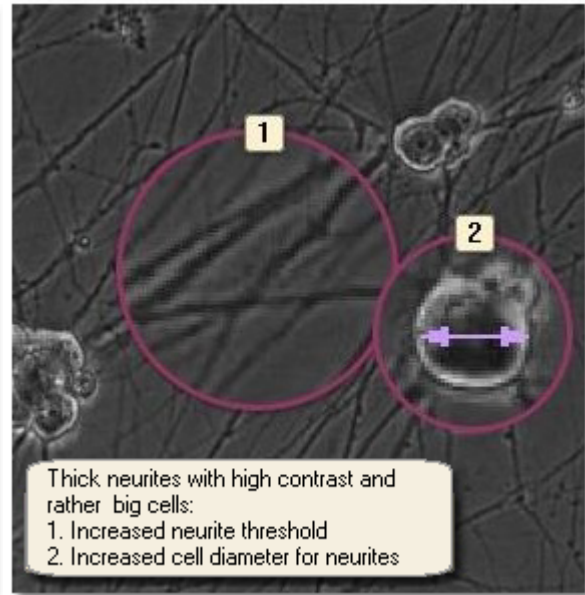
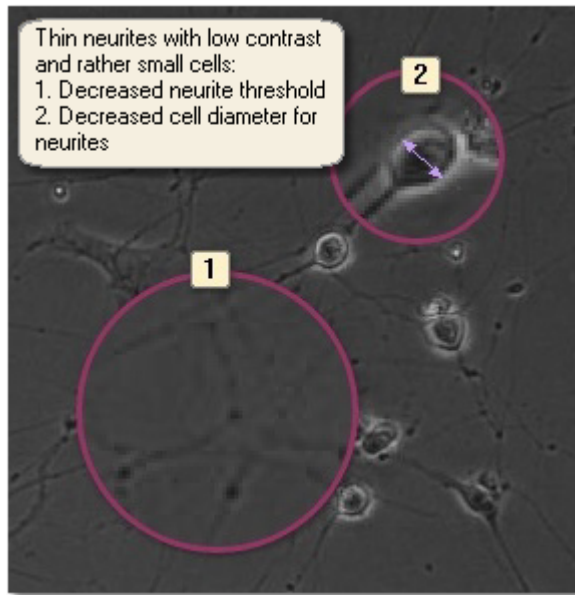
**2. Cell diameter for neurites guidelines**

If the value is set to 31, the found neurites are removed from the 31/2 pixel radius from the cell center point.

Default: 31

Range: 17...41

**EXAMPLE: TWO NEURITE SEGMENTATION CASES**



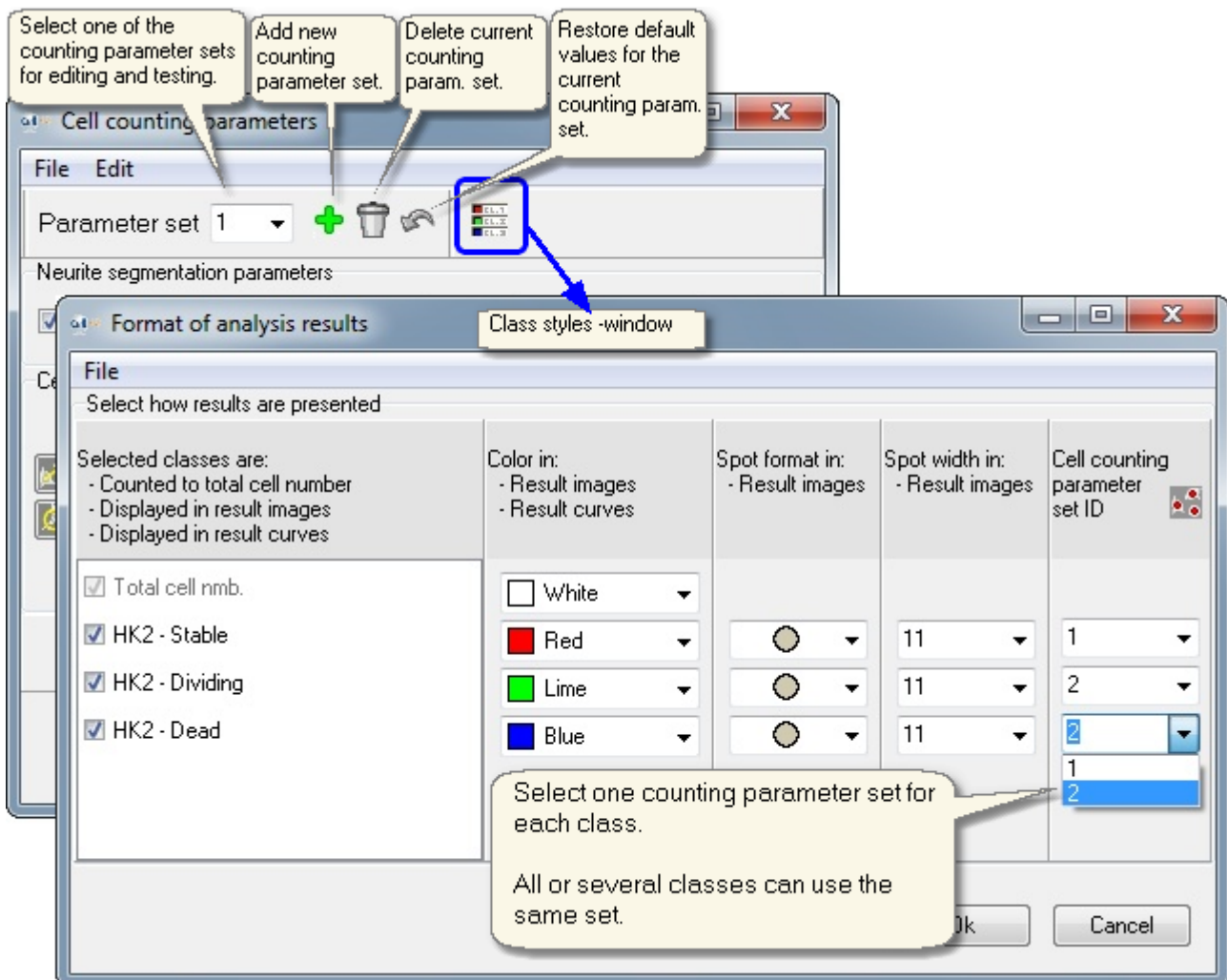
### 5.5.3 Class specific counting parameters

Different counting parameters can be selected to find small cells and different parameters for the large cells.

- Often one counting parameter set is enough for all cells, but sometimes it can be difficult to find one set of counting parameters that work for all the cells in image, image series or well in the plate.
- One set of counting parameters can be selected to find small cells and different counting parameters for the large cells. The same sample library is always used both for small and large cells.
- The number of the counting parameter sets is not limited to two, or to any other number. Different counting parameters can be selected for every recognition classes if needed.
- Neurite segmentation can be enabled in one or more counting parameters sets. Program will take the found neurites from them all to the results.

Steps:

1. Make CellFinder protocol the same way as with only one counting parameter set. Optimize the counting parameters for the first cell type. If counting is now accurate for all cells in image, and for all recognition classes, you can ignore the next steps.
2. Add new counting parameter set from the "+" -button in the counting parameter window. Optimize these parameters for the other cell type.
3. Go to the Class styles -window, and select the counting parameter set 2 for the recognition classes that work best with these counting parameters.



## 5.5.4 Tips

### GETTING STARTED

Even though the images can never be perfectly segmented with any device the result can be optimized by improving the parameters according to the cell test qualities.

It is recommended to collect a set of images which represent the cell test as comprehensively as possible. It might be a few images from the start, the middle and the end of the cell test and depending on the well plate size. Select positions from different conditions.

### ADJUSTING PARAMETERS

Once this set of images is selected, the analysis can be run with the default parameters. If the result is not correct, adjust one parameter at a time in small steps and run the analysis through the test set. Evaluate the result images by eye. Repeat the iteration until the result is acceptable.

<p><b>Segmentation gradient threshold</b></p> <ul style="list-style-type: none"> <li>? If the cells have low contrast try decreasing this parameter in small steps from the default value</li> <li>? If the cells have high contrast try increasing this parameter in small steps from the default value</li> </ul>
<p><b>Cell distance</b></p> <ul style="list-style-type: none"> <li>? If the population is dense try decreasing this parameter in small steps from the default value</li> <li>? If the population is sparse try increasing this parameter in small steps from the default value</li> </ul>
<p><b>Maximum cell diameter</b></p> <ul style="list-style-type: none"> <li>? If there are relatively small cells try decreasing this parameter in small steps from the default value</li> <li>? If there are relatively big cells try increasing this parameter in small steps from the default value</li> </ul>
<p><b>Cell symmetry</b></p> <ul style="list-style-type: none"> <li>? If the cells are symmetrical and there are faulty segmentations on the background near cells, try increasing this parameter in small steps from the default value. You might have to increase the segmentation gradient threshold a step too.</li> </ul>

### ACTION TO TAKE IN CASE...

#### Cell segmentation

- ? There are too many spots -> increase the segmentation threshold value
- ? All the cells are not counted -> decrease the segmentation threshold value
- ? Individual cells have several spots -> increase cell distance and/or the maximum cell diameter
- ? There are a lot of spots on the background near cell edges -> increase the cell symmetry parameter
- ? There are some spots on the background and no cell edges near -> increase the segmentation threshold value until they disappear.

#### Neurite segmentation

- ? There are neurites found inside cells -> increase the neurite threshold value and/or cell diameter for neurites
- ? All the neurites are not found -> decrease the neurite threshold value
- ? There is garbage or debris in the background which are counted as neurites -> increase the neurite threshold value

### DETERMINING THE MAXIMUM CELL DIAMETER

Slide the cursor over the biggest cells on the image and see the pixel dimensions on the bottom. This gives you an idea of the maximum cell diameter. However, if the majority of the cells are smaller, then decreasing the MCD moderately to ensure correct segmentation.

### PARAMETER INTERRELATION

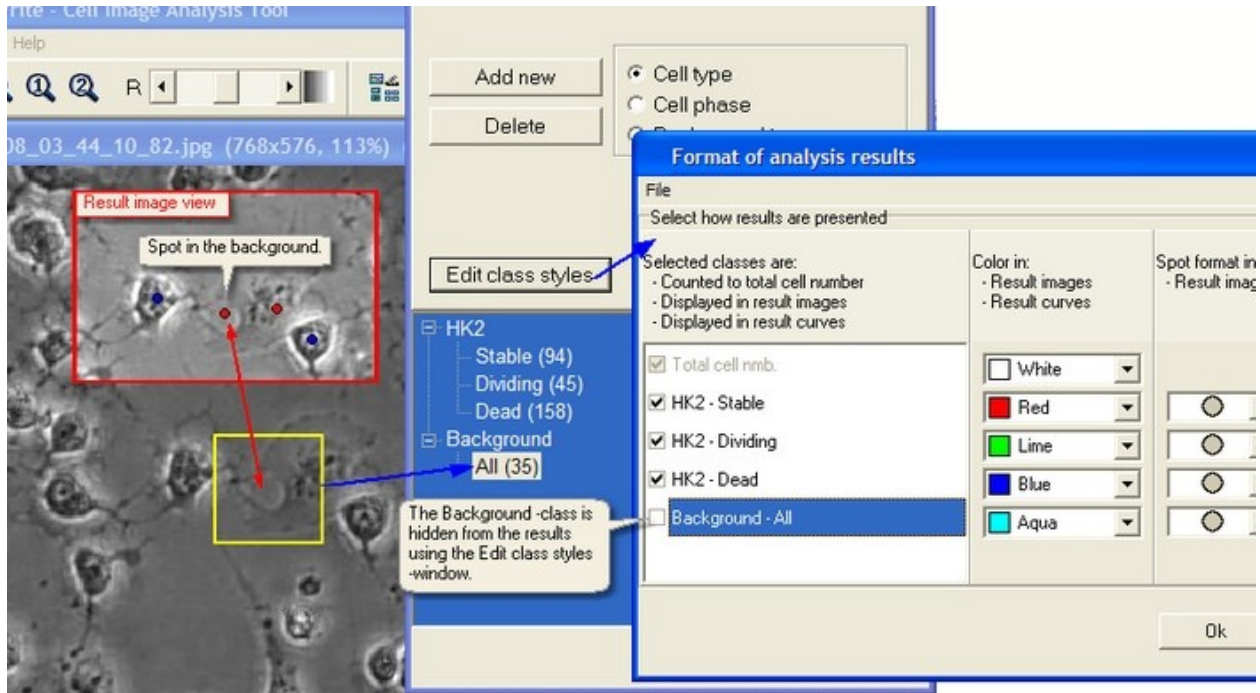
Sometimes changing one parameter might require to change another one. For example, increasing the symmetry parameter can require decreasing the segmentation gradient threshold, and increasing the maximum cell diameter might require increasing the segmentation gradient threshold. There's no universal rule as this is case dependent and can be detected only by testing. For instance when changing the maximum cell diameter the

threshold value can be adjusted in case of it still improves the analysis result.

**WHAT IF THERE ISN'T A PARAMETER COMBINATION THAT GIVES GOOD ENOUGH CELL COUNTING ACCURACY?**

In many cases the cell counting accuracy can be improved significantly using the cell recognition feature (that is explained later in this guide):

- If there are spots in the background between the cells, and it is not possible to delete them, the following can be performed: Make a 'Background' -class and teach the false background spots to that class. Use the tools in the 'Edit class styles' -window in the Analyser to make the background spots invisible in the result images and remove them from the result curves at the same time.



- If you have spots in the edges of the cells, in the membrane, you can for example teach them also to the 'Background' -class.

### 5.5.5 Examples

The selected set of cell counting parameters often apply for the same cell type if the objective magnification (4X, 10X, 20X or 40X) is kept constant in the imaging system. If it is changed, the new parameters need to be found.

#### EXAMPLE 1: RAT NEURAL CELLS, 10X OBJECTIVE

The selected parameters are:

- (A) Find neurites Enabled
- (B) Neurite threshold 55
- (C) Cell diameter (for neurites) 29
- (D) Segment grad. thresh. 37
- (E) Cell distance 23 pixels
- (F) Max cell distance 31 pixels
- (G) Cell symmetry 75

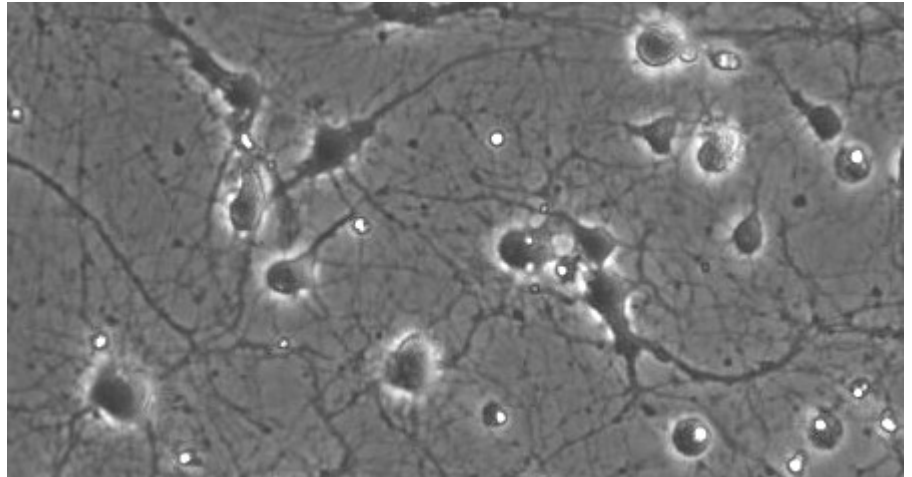


Image from Institute of Biotechnology, University of Helsinki

#### EXAMPLE 2: FLUORESCENCE CELLS, 10X OBJECTIVE

The fluorescence images are counted with the same tools as phase contrast images, and the parameter selection follows the same principles.

Below is a step by stem case example for the parameter selection for fluorescence image. We start with the parameters (D) Segment grad. thresh. 35, (E) Cell distance 27 pixels, (F) Max cell distance 37 pixels, (G) Cell symmetry 0.

1. Segmentation gradient threshold 27 is too low and there are faulty segmentations. Increase the threshold to remove them.

2. Segmentation gradient threshold was increased too much (37) and the segmentation is deficient. Decrease the threshold a step or two.

3. Segmentation seems ok with threshold 33, except the spot on the background near bright cell edges.

4. The optimal result is finally achieved with threshold 33 but increasing the symmetry parameter to 35. [E = 33, F = 39]

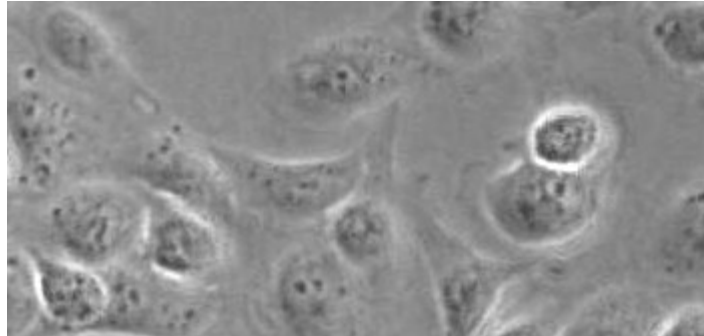
The selected parameters are:

- (D) Segment grad. thresh. 33
- (E) Cell distance 33 pixels
- (F) Max cell distance 39 pixels
- (G) Cell symmetry 35

### EXAMPLE 3: HK2 CELLS, 10X OBJECTIVE, JAI CAMERA

The selected parameters are:

- (D) Segment grad. thresh. 35
- (E) Cell distance 27 pixels
- (F) Max cell distance 37 pixels
- (G) Cell symmetry 0



*Image from Cell Research Center, University of Tampere*

## 5.6 Sample teaching tips

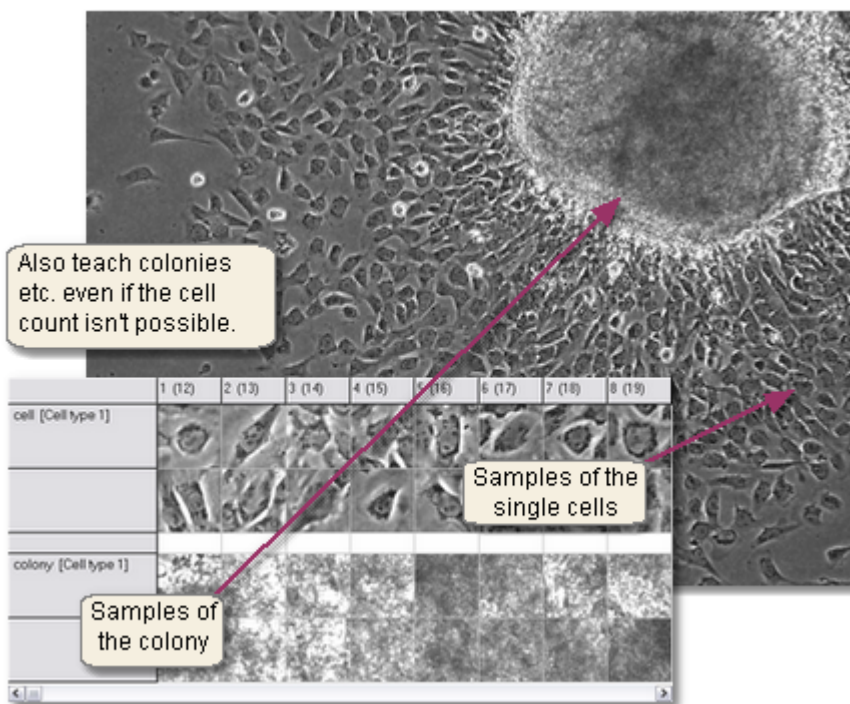
### Cell counting and recognition

A good analysis accuracy is usually achieved by

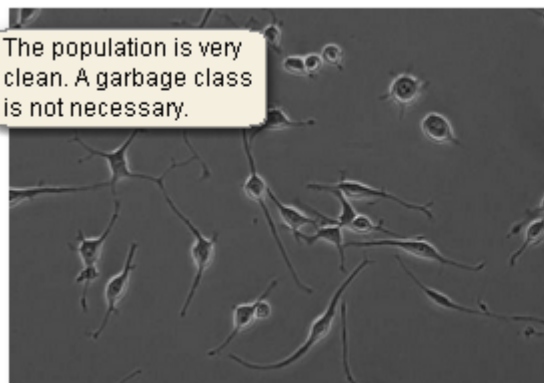
- Clear representation of classes in the sample library
- Picking samples throughout the cell test
- Picking samples by clicking in the geometric cell center supposing the cell edges are visible.

However, some conditions may require picking samples of areas where single cells are not distinguishable, e.g. a cell colony. In that case a separate colony class should be created and taught to avoid any error in other classes. The cell count for that colony class is not accurate, but the other classes will be more accurate.

#### CELL COLONY

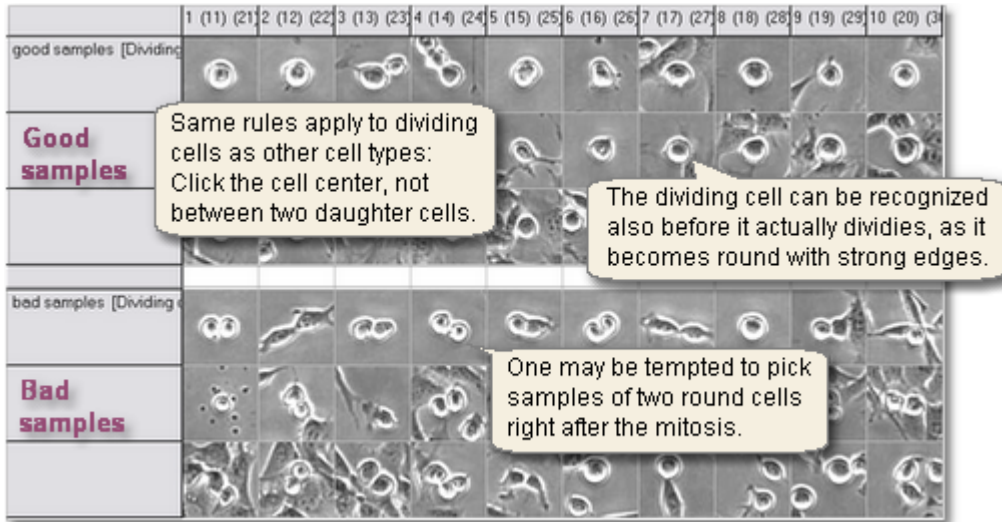


#### DEBRIS AND BACKGROUND



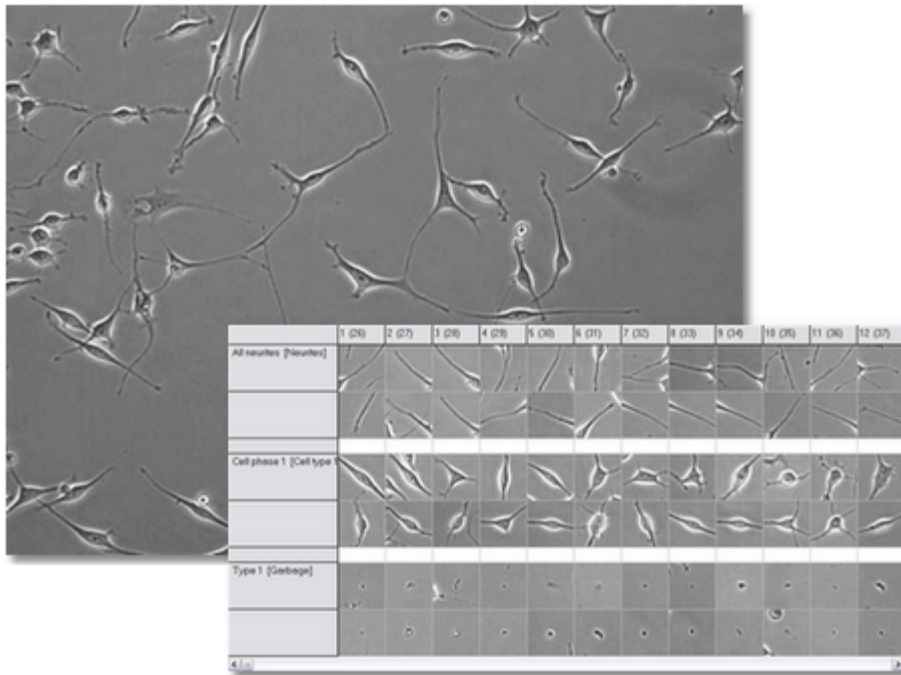
#### DIVIDING CELLS





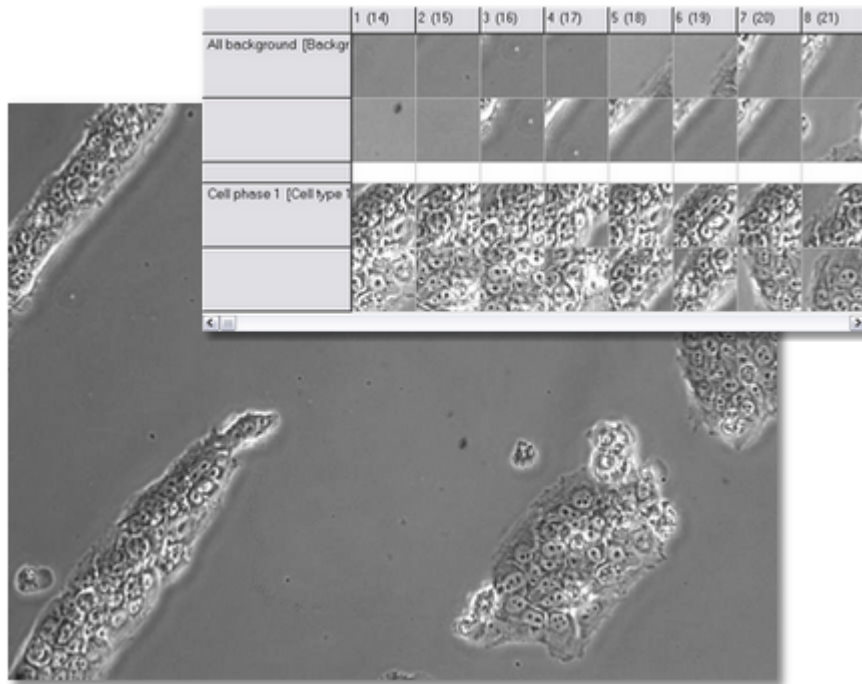
### Neurite recognizer

One class for neurites is to be included into the measurement and everything else in other class(es), also cells. Click neurites so that they go through the center point of the sample window. You can for example include thick neurites into the measurement by selecting thick neurite samples into the "All neurites" class and thin neurite samples into another.



### Area finder

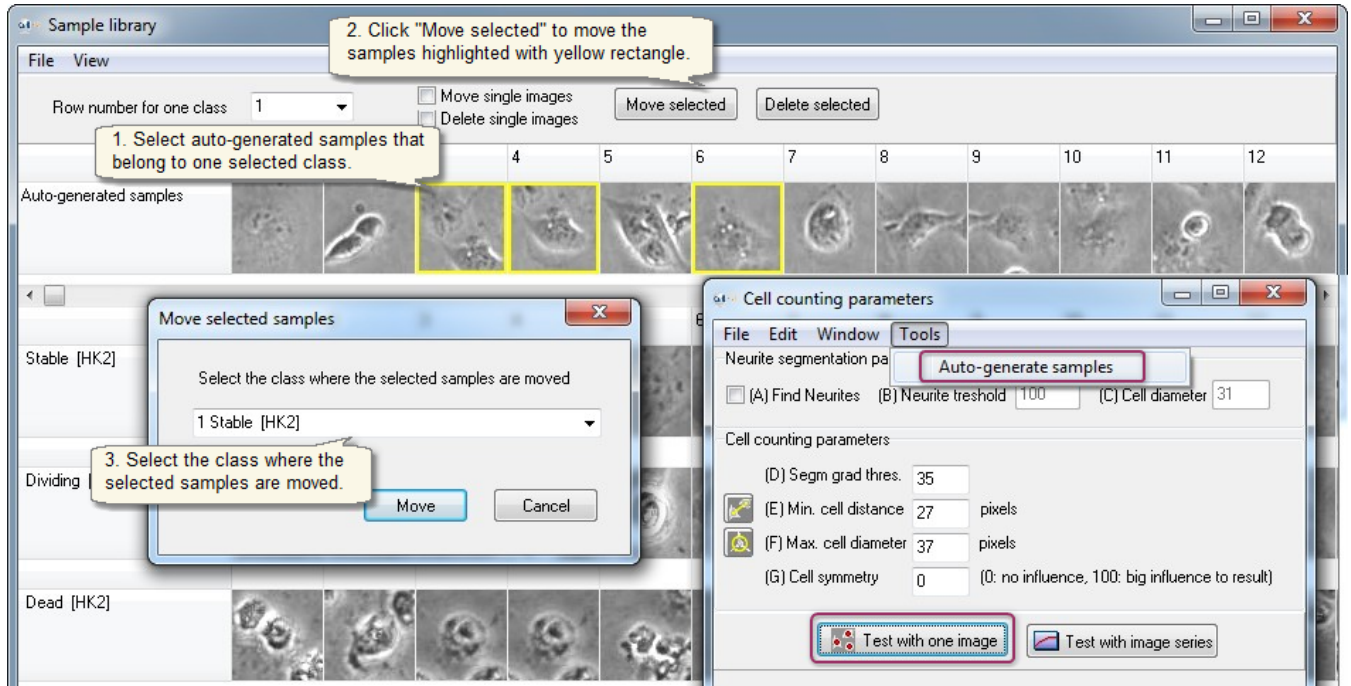
When picking samples of the different areas, make sure to also pick samples of the borders of each area type, just few pixels away from their borders. It doesn't matter if almost half of the sample is background or other area type, as long as in the center of the sample window is in the right class. Always teach the background.



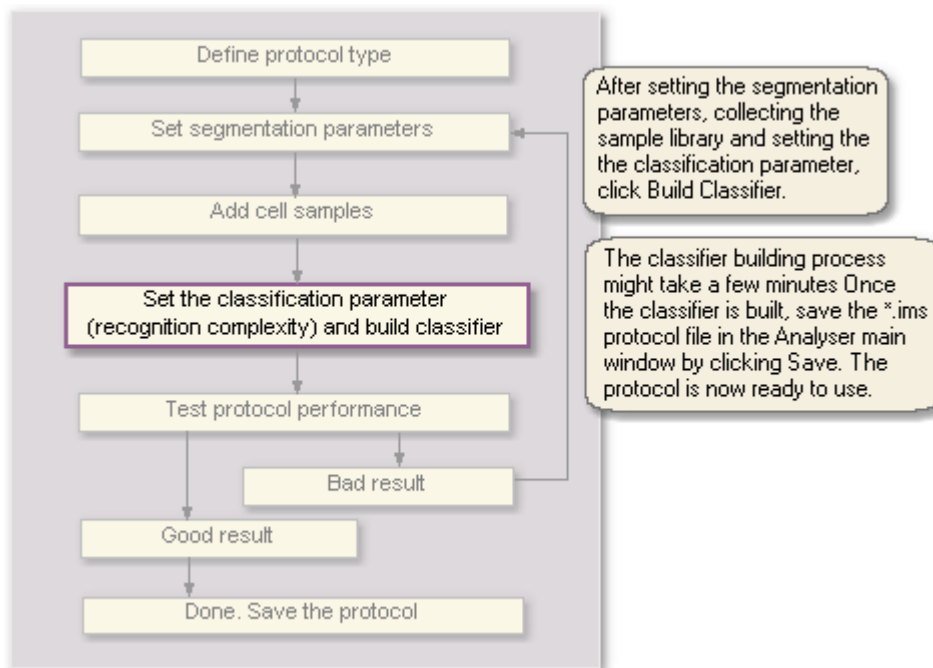
## 5.7 Auto-generate cell samples

After satisfactory cell counting parameters have been selected, the cell samples for sample library can be generated in a more automatic way to speed up the process of building the sample library:

- After you have clicked "Test one image" button, you can use the found cell coordinates to generate cell samples automatically, by clicking "Tools -> Auto-generate samples".
- Move auto-generated samples to the user defined classes using the sample library window.



## 5.8 Building Protocol

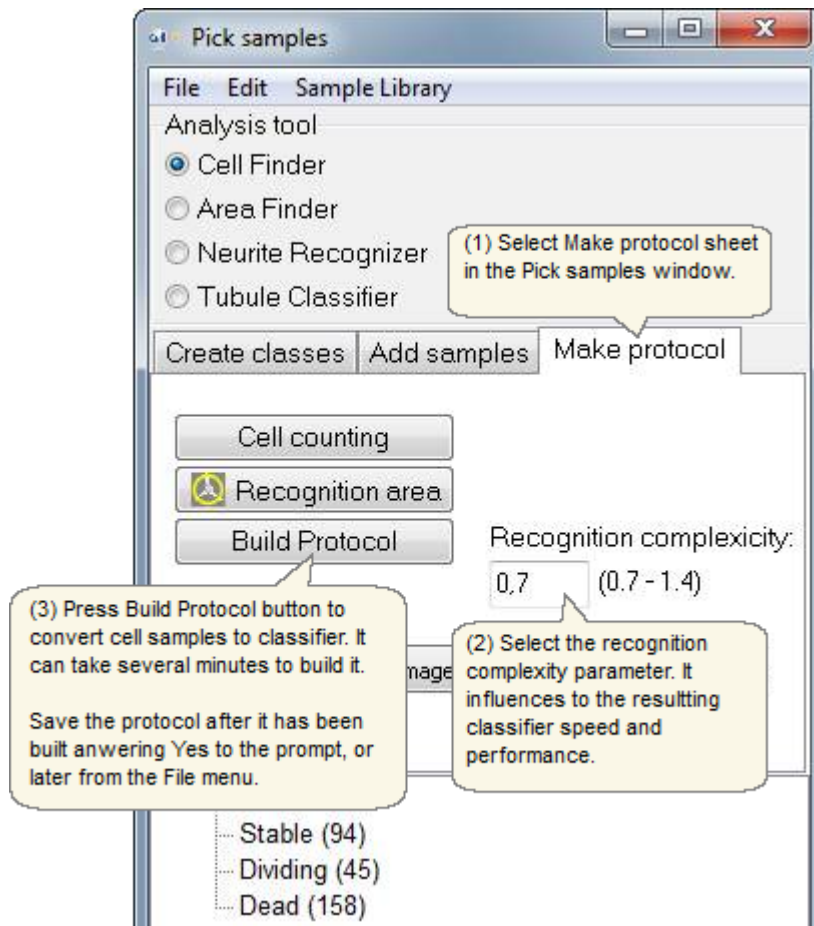


### BUILD PROTOCOL

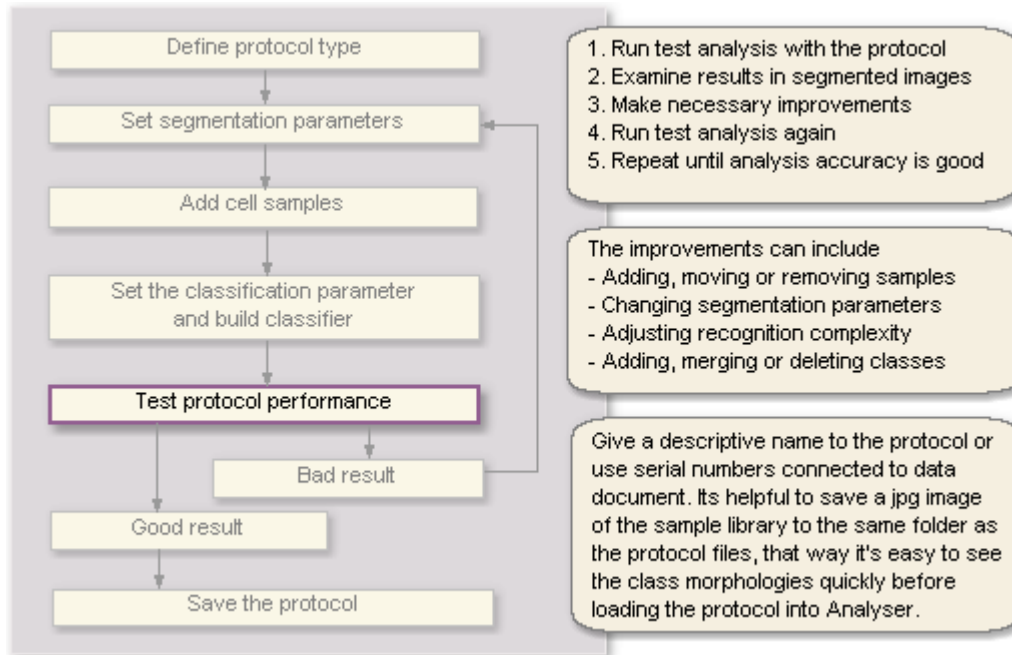
The protocol has to be completed by converting the Sample library into classifier, with the Build Protocol button, as explained in the image below.

### THE CLASSIFICATION PARAMETER (=RECOGNITION COMPLEXITY) GUIDELINES

- Small total cell sample number (e.g. 300 samples) -> g is small (e.g. 0.7)
- Large total cell sample number (e.g. 600 samples) -> g is big (e.g. 1.35)
- Too small value results inaccurate analysis, since a large part of the cell samples are deleted in learning process regarded as unnecessary.
- Too big value results inaccurate analysis as well since the single cell samples have smaller visibility.
- Usable range: 0.7 - 1.4



## 5.9 Testing protocol performance



### REVIEW, ADD OR REMOVE SAMPLES

- Examine the misclassified cells in the segmented result images. If you can see a pattern there you can teach certain type of appearance by adding 10-15 such samples and try again.
- Make sure every class is clearly recognizable by eye. Also make sure that all samples are selected in the center of the cell. If not, remove such samples.
- If the color coded spots are not at all in the center of the cells, try fixing the segmentation parameters.
- If the spots are in a right place but the class is wrong in many cases, try teaching the more comprehensively by adding samples to all classes with poor accuracy.

### DELETE, MERGE OR REORGANIZE CLASSES

- If two classes *x* and *y* are both perfectly round and it is impossible to distinguish these two morphologies at some stage, merge them into one round class.
- The dividing cells can sometimes look very similar to dead cells, round and non-adherent. However, the amount of cell division can be obtained directly by the derivative of the cell count curve. Therefore, teaching the dividing class might not be necessary as not all divisions can be captured due to the cycle time being greater than mitosis time. If creating a class for dividing cells is relevant, only assign samples to the *dead* class that are clearly granular, and the perfectly smooth ones into the *dividing* class.
- Sometimes distinguishing between *early differentiation* and *late differentiation* is not accurate and those classes can be merged into one *differentiating* class.
- Multinuclear cells and cells with single nucleus might not look different enough in the sample window. Distinguishing between the two might not be accurate enough so they can be merged.

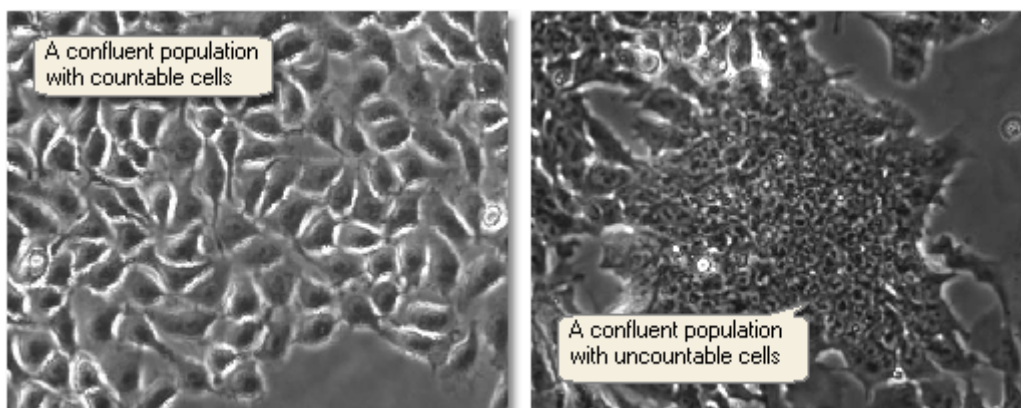
### LIMIT TIME LINE & MAKE MULTIPLE PROTOCOLS

If the population changes so dramatically that towards the end there are completely different cell morphologies and cell sizes and they get confused with some cells in the beginning, you could divide the analysis in to different phases and create a protocol for each phase to obtain more accurate analysis.

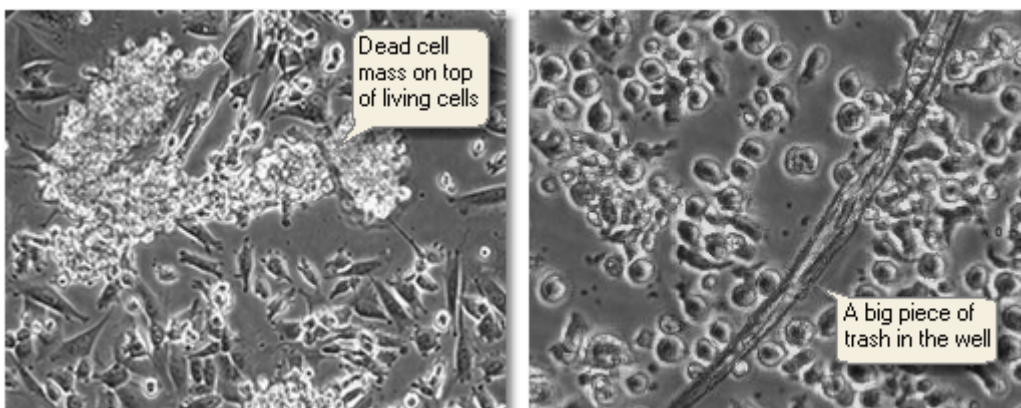
## 5.10 About analysis performance

### CELL POPULATION

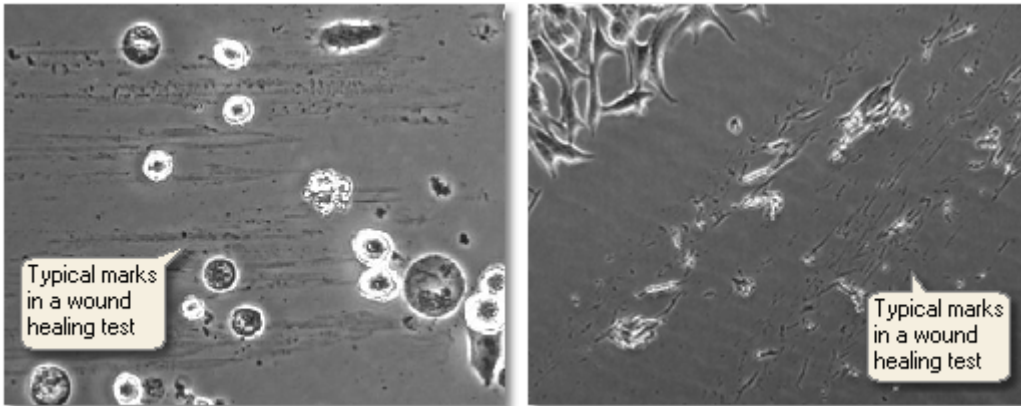
- The confluency of the population is best kept at low to medium for the best results. If the cells in the population cannot be distinguished by eye, it is likely that the cell counting accuracy will decrease. If the images contain areas of high confluency, it is best to include them to avoid false classification of other classes.
- If there is a cluster of dead cells or debris together with live cells, the clustered population often cannot be counted. However this rarely interferes with the healthy population, as long as the clusters are included into a background class. The same principle applies for any large objects in the field of view, they can be added to the background and thus do not affect the counting of the target cells



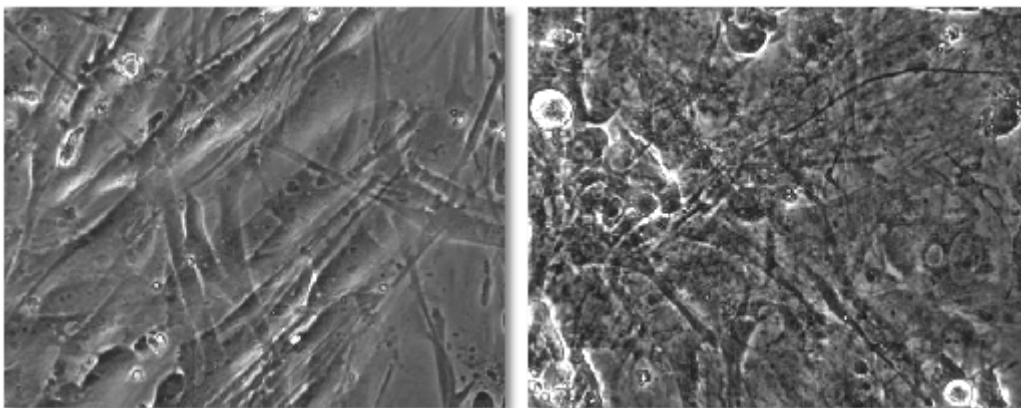
- If there's a lot of dead cells tangled together over the healthy population, the dead cells can't be distinguished and counted. However, the stable cells can usually be counted accurately. In this case include samples of the dead cell mass also into its own class or combine it with other such as the dead cell class etc. If for some reason there is a big piece of trash in the image you can include samples of that into e.g. a garbage class to avoid its interference with the cell count.



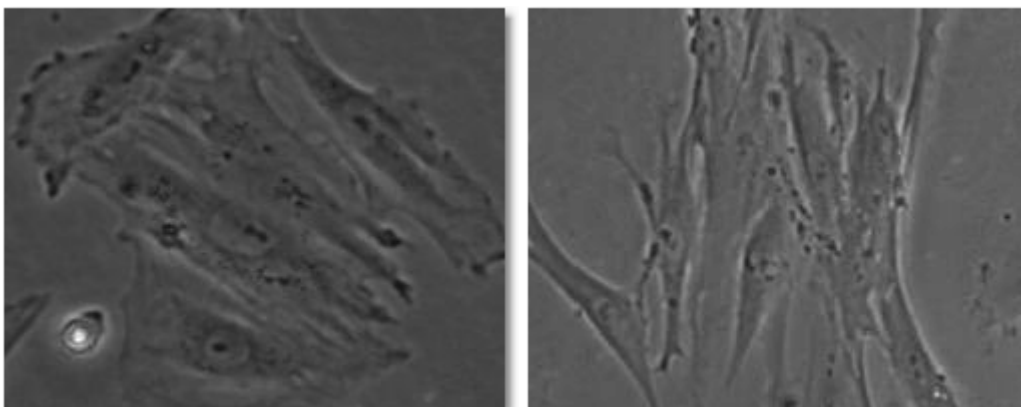
- If there's a lot of garbage in wells, define a garbage class to sample library to avoid the garbage being faultily counted as cells. Small particles can interfere the neurite measurement in Neurite finder tool, but by using the Neurite Recognizer protocol you can teach the system to recognize and discard the small garbage.
- Small particles can interfere also the neurite measurement in Neurite finder tool, but by using the Neurite Recognizer protocol the system can be taught to recognize and discard the debris and scratches on the plastic.



- Two-layered cell population: Cells are counted and recognized from a single image, so if the two cells are on top of each other, at least one of them is not counted and the recognition accuracy can decrease.



- Cell colonies, tissue samples and embryos: Cell-IQ can take images that show features in the selected Z-range from a thick target. This slice can be analysed using the Area Analysis method, where the system divides the target into the different areas based on the texture. In this case it analyses more the structure, rather than the individual cells.
- Long, low contrast cells: The cell analyser finds the cell centers (segmentation) in analysis before they are recognized, and the segmentation is based on the features inside the cells and the cell edges. Hence the foil-like cell types that have a body that is very flat and long (tens of micrometers) and has no clear edges, may not be segmented and recognized accurately.

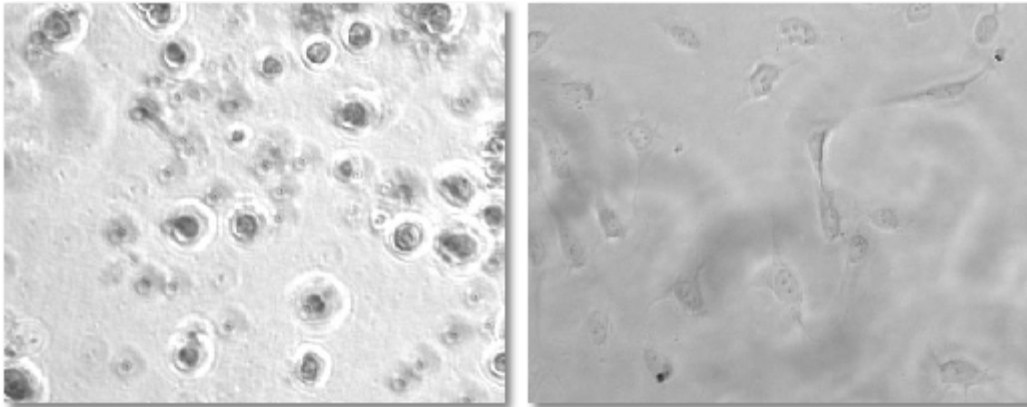


**IMAGE QUALITY**

- Poor image quality due to e.g. images being out of focus can decrease the accuracy of the analysis. Images can be out of focus because:
  - Cells are not adherent.
  - There are particles floating in the medium.
  - Imagen parameters are not optimal for the cell population type, or the cell test is not started according to the given guidelines.



- Poor image quality due to images being taken too close to the well edge, where the phase contrast illumination field is not optimal, can decrease the accuracy of the analysis.



### **CLASS DEFINITIONS**

- If the cells look similar even if they are biologically in different phases or cell types the system might not be able to distinguish them.
- If the cells in two classes look similar only inside the cell sample window, even if they move differently or their processes have different number of branches, the system might not be able to distinguish them.
- Cells actively change shape and look similar from time to time, even if they are different phases and look different most of the time. In this case it is possible to define an additional class for the uncertain cell shape. This way it is easier to quantify the analysis error.

### **HARDWARE CHANGES**

- If the image resolution changes due to changing the objective or the camera, a new analysis protocol must be created for the new resolution.

## Making the other Protocol types

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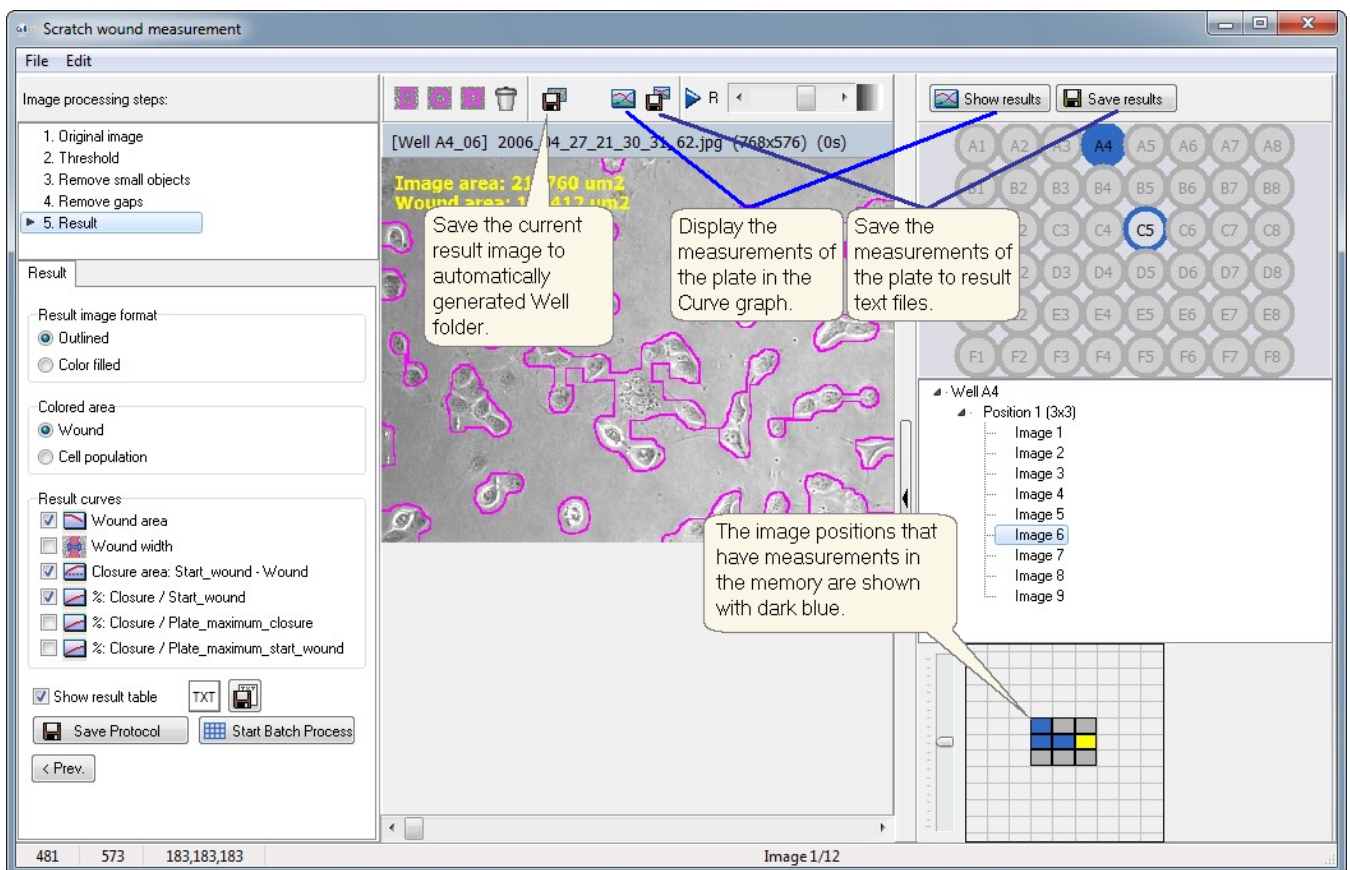
## 6 Making the other Protocol types

The following analysis protocols are made with similar parameter selection interface:

- [Fluorescence intensity measurement](#)
- [Fluorescence image combining and colouring](#)
- [Scratch wound measurement](#)
- [Area size measurement](#)
- [Tubule and branch point measurement](#)

Optionally the result curves can be generated directly from the protocol making tools, without the Batch Process:

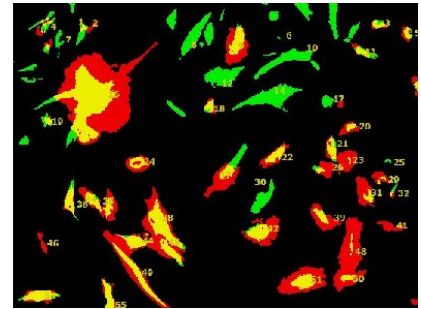
- Each measurement that is made, with automatic or manual tools, is automatically saved to memory for the plate for that image positions and time point.
- The measurements of the plate can be displayed in the Curve graph with the Show results -button.
- The measurements of the plate can be saved to result text files with the Save results -button.



## 6.1 Fluorescence intensity measurement

Analyse images from single and multichannel fluorescence cell tests with the Fluorescence intensity measurement tool.

- Measurement is based on thresholds and selected parameters, and no cell sample image teaching is necessarily needed, but it can be utilized, as later is shown.
- The fluorescence cell count, area and intensity are measured.
- The tool can utilize both the phase contrast and fluorescence images at the same time.



### Fluorescence intensity measurement procedure

There are two optional ways to do the analysis:

Option 1:

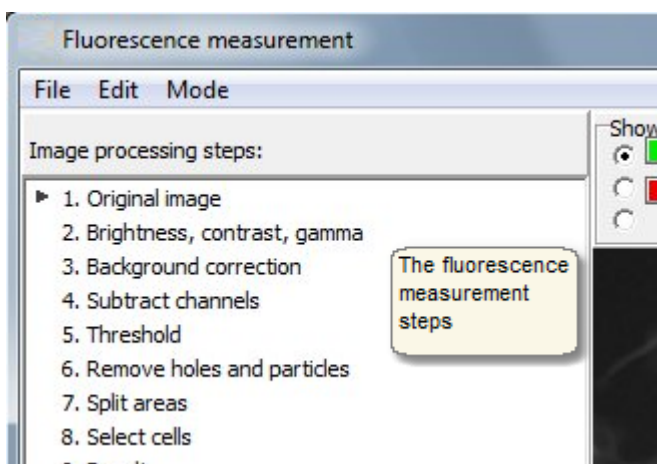
Step 1: Select the analysis parameters and save them to a Protocol file.

Step 2: Go to Batch process, open the Protocol from file when asked, and fine tune the parameters if needed.

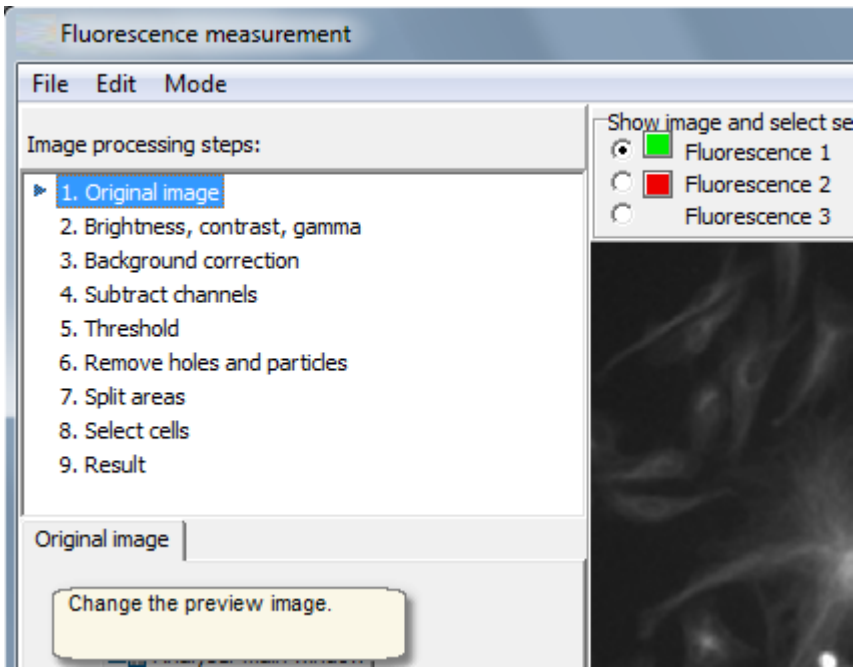
Option 2: Go directly to Batch process, and select the analysis parameters on the go. No protocol file is needed.

### Making a fluorescence analysis Protocol

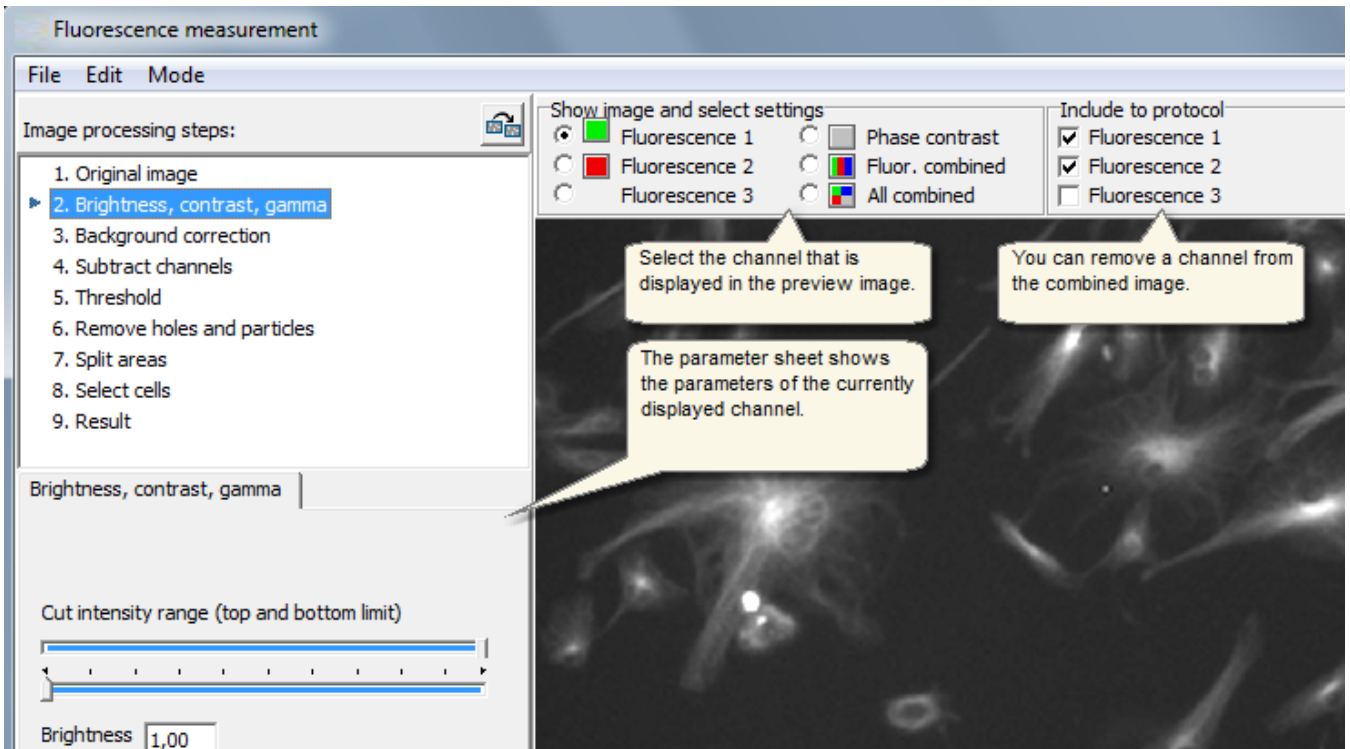
- [Open images](#) to the analyser main window **using the \*.dat file**, and open the Fluorescence intensity measurement tool from "Tools -> Fluorescence - intensity measurement".
- The preview image that was in the main window is automatically opened to the Fluorescence measurement tool preview window.
- Select the measurement parameters step by step. The intermediate results image is always displayed on the right hand side of the window.



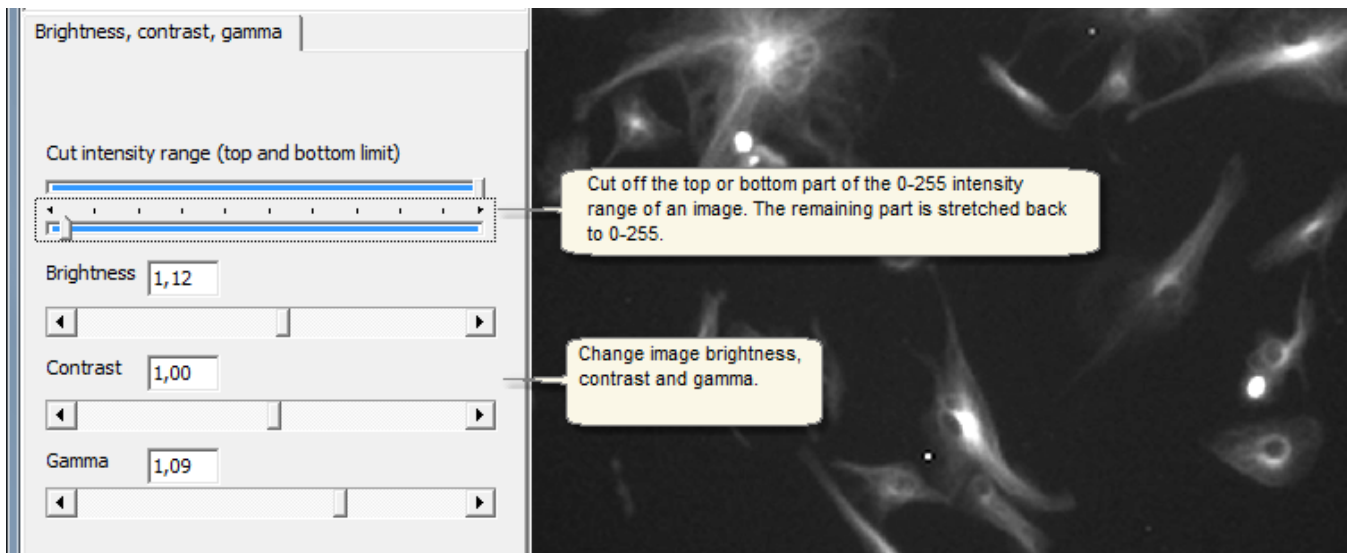
- You can change the preview image at any time.



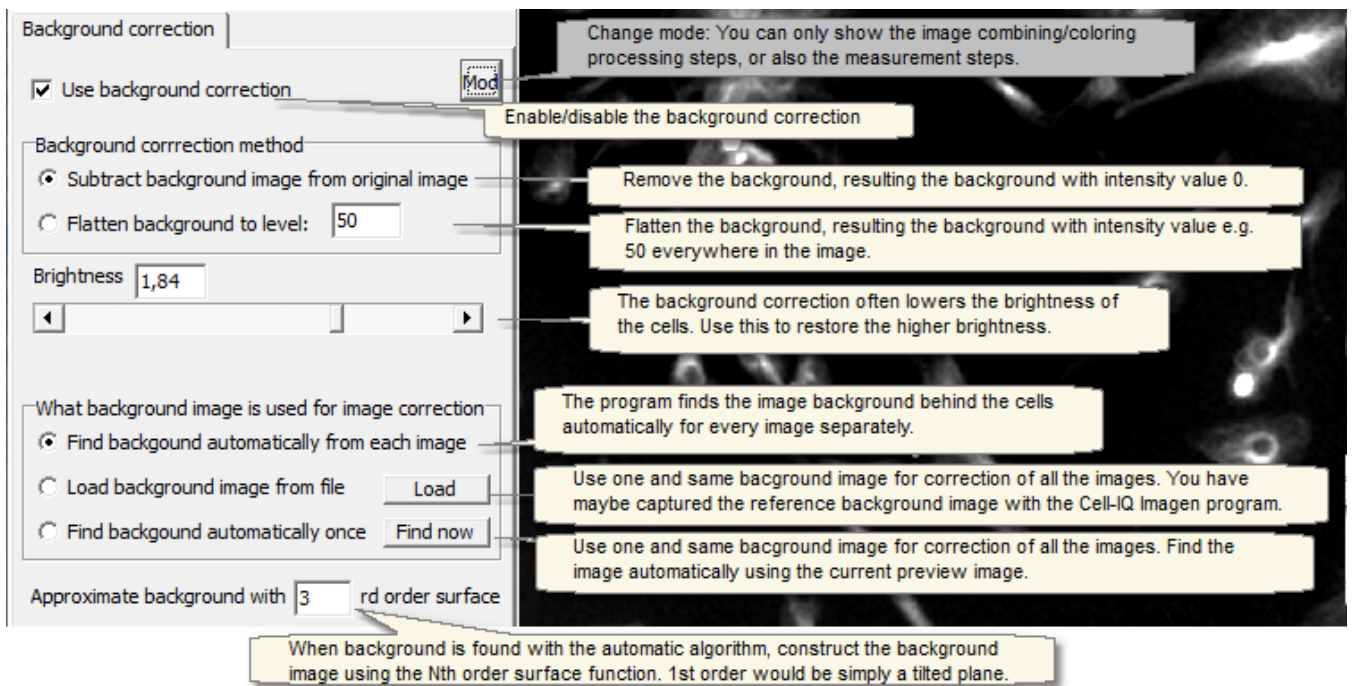
- When the images were opened using \*.dat file, images from all the channels were loaded at once also to the Fluorescence tool. You can preview any channel at any time by clicking the radio buttons above the image. You can also preview all the channels at the same time as a combined image, with or without the phase contrast channel.
    - The result parameter selection sheet on the left side of the image shows the parameters of the currently displayed channel. Each channel has its own set of parameters.
- Hint: If you want to remove some of the fluorescence channels from the combined image, disable it from the "Include to protocol" selections.



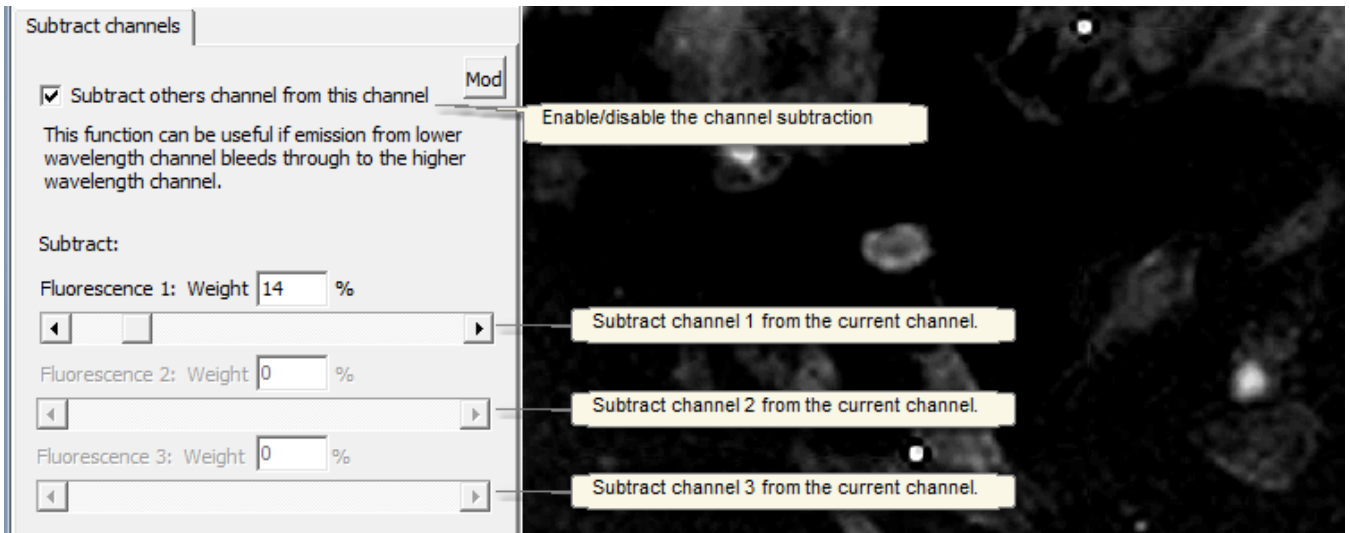
- Image brightness, contrast and gamma can be adjusted, so that it is later easier to select the threshold etc. parameters, and that the result images will look better.
  - These parameters will be set for each channel separately. Each channel can be set up individually through steps 1-9 or completing each step for all channels.



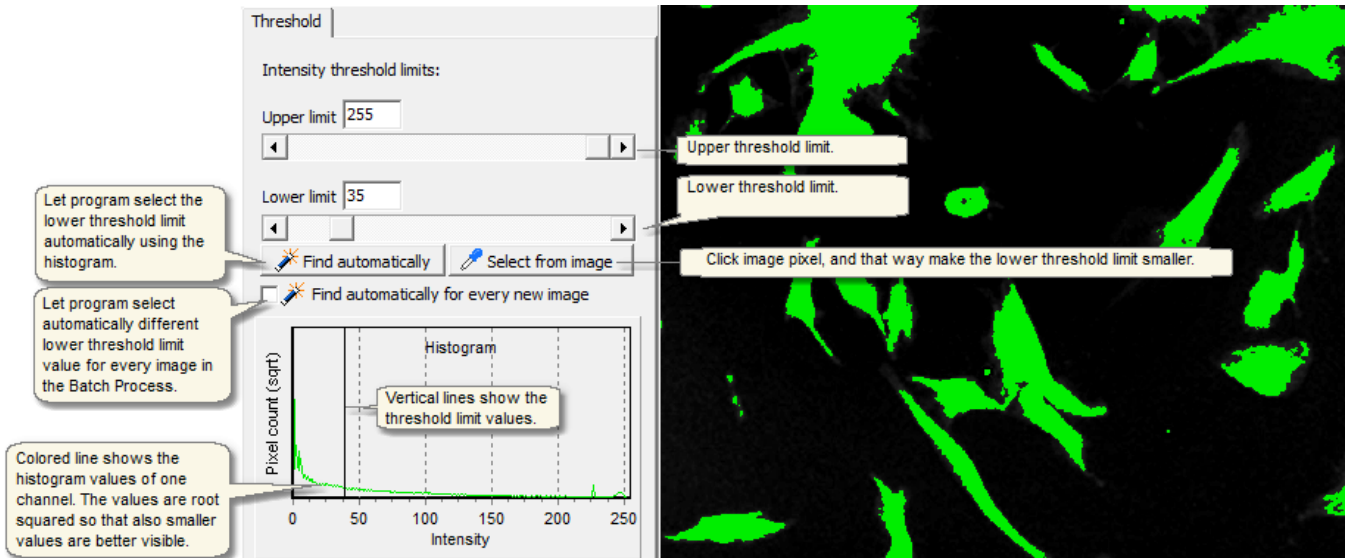
- Make background correction. Typically the image background gradient is removed, resulting background with an intensity value of 0. This will make it easier to select the threshold value later.



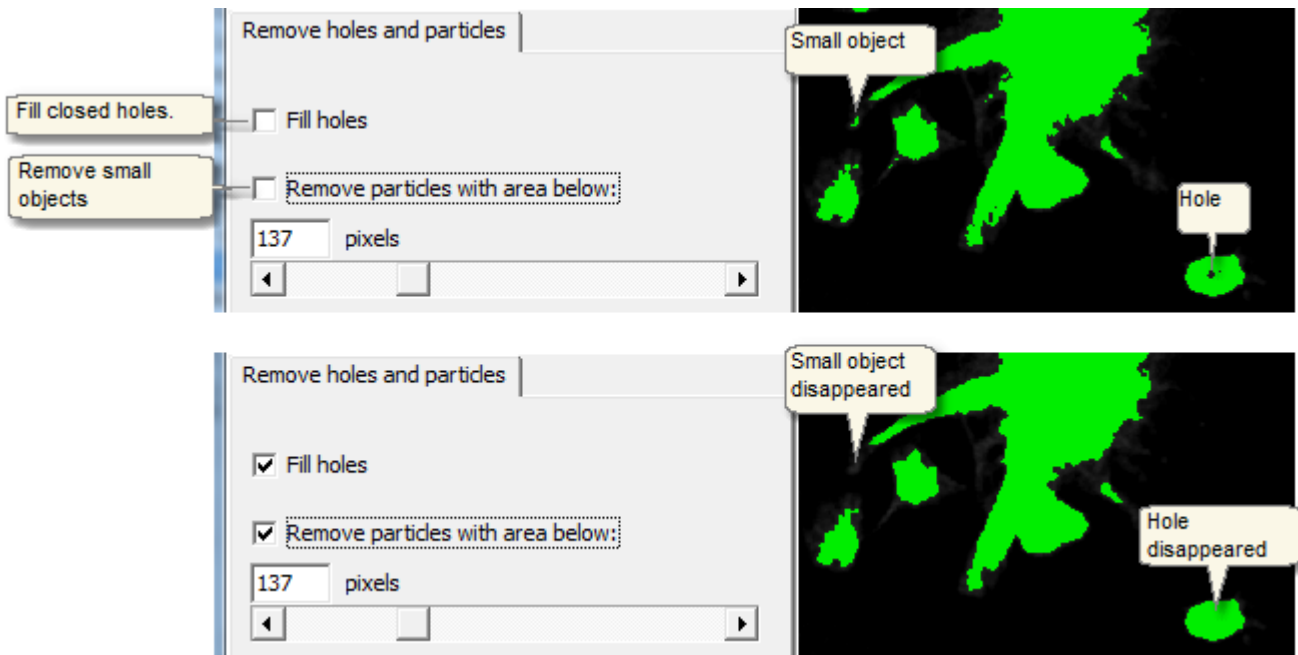
- Subtract one channel from an other. The signal from a channel sometimes bleeds through to another channel, especially from the lower wavelength emission channel to the higher wavelength channel. In the image below the 14% of the pixel intensities of the GFP channel (FL1) is subtracted from the pixels intensities of the Red channel (FL2). The calculation would be:  $Red\_result = Red\_original - 0.14 \times GFP\_original$ .



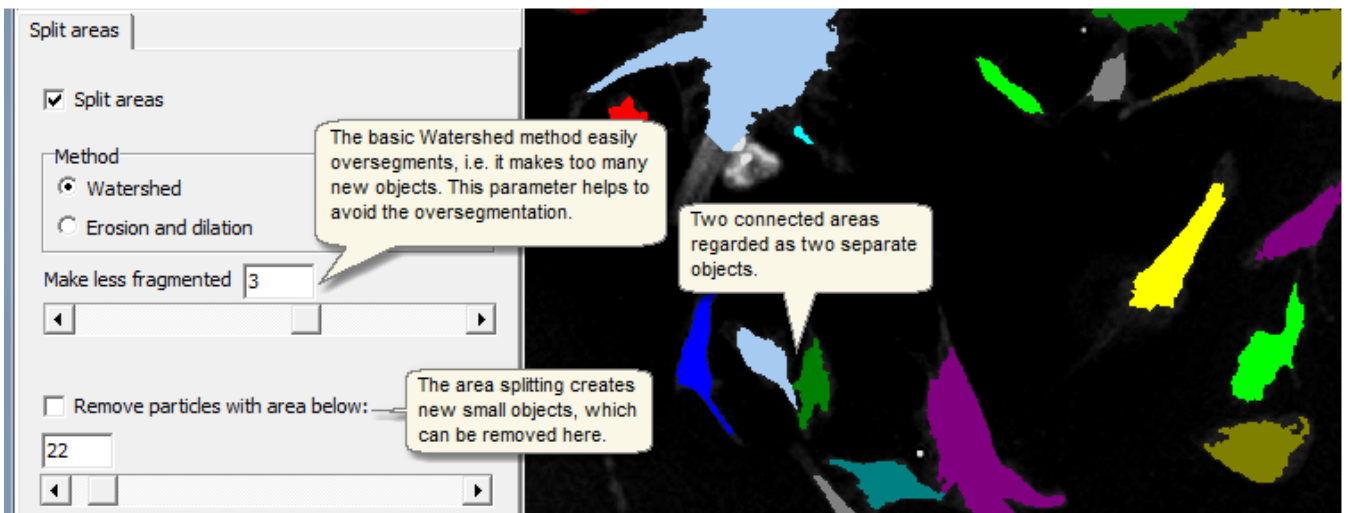
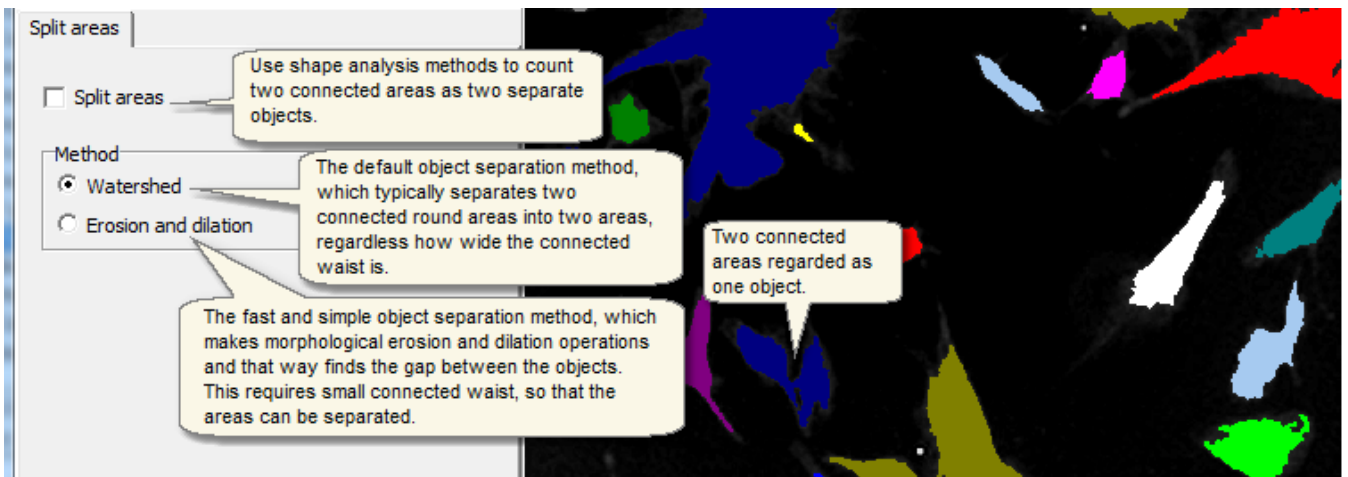
- Apply intensity threshold. In the resulting binary image the objects of interest are separated from the background and hence can later be measured for intensity, area and count. Select the upper and lower threshold limits - the intensity values between the limit values are segmented as the objects of interest.



- Fill closed holes from the segmented areas and remove objects that have small area size.
  - The number of fluorescent objects is counted from the image in the later steps, and it is often more illustrative to get the count of only the large size objects.



- Split areas: Use shape analysis methods to count two **connected** areas as two separate objects.
  - This step also automatically counts all the non-connected objects, using the threshold result image as a source image. The area color shows which areas are connected and which are non-connected and hence counted as several objects.





- Select how the cells are counted. There are the three basic options available: (1) Count from fluorescence image, (2) count from phase contrast image with Cell Finder protocol, (3) or user can manually select the cells from the first image in the series, and the cells are automatically followed through the series.
  - Unique IDs for cells: If the default settings are used, the same cell has different ID number in the different fluorescence channels. But if the Cell Finder is used or if one of the fluorescence channels is used as a mask (see image below), each cell has the same unique ID in all the fluorescence channels.

The screenshot displays the 'Select cells' panel in the Cell-IQ software. The 'FindCells' section has three radio button options: 'From fluorescence images using threshold', 'From phase contrast images using Cell Finder R', and 'Select manually, and track using Cell Finder'. The 'From phase contrast images using Cell Finder R' option is selected and highlighted with a red box. Below this, there are options for 'Use one channel as template for measurement' (Disabled, Fluorescence 1 is template, Fluorescence 2 is template, Fluorescence 3 is template) and an 'Open Analysis Protocol' button. At the bottom, there are input fields for 'Measurement area diameter' (95 pixels) and 'Cell tracking area diameter' (110 pixels). The right side of the image shows a grayscale microscopy image with several cells highlighted in different colors (green, red, blue, cyan) and green circles around them. Callout boxes provide detailed explanations for various features and settings.

Callout boxes include the following text:

- The cell count comes from the number of the non-connected fluorescence areas.
- The cell count and positions come from the phase contrast image analysis.
- If the fluorescence area of the cell is 0 in any of the enabled channels, the cell is removed from Cell Finder results entirely.
- The cell positions are manually selected from each image series, from the first image in the series, and then cells are automatically tracked through the series using the phase contrast image analysis.
- If you select that Fluorescence 2 (FL2) is template, for the current, say FL1, then the FL1 areas that are outside the FL2 areas are removed from the measurement.
- When a template is used, each cell has the same ID number in all of the channels!
- The dots show where the cell was found with the phase contrast image analysis.
- The circle shows where the fluorescence area is searched so that it can be assigned to the cell/dot.
- If the centre of a fluorescence area is inside the circle, the area is assigned to that cell.
- Select Cell Finder protocol, so that cells will be counted accurately from the phase contrast image. Otherwise the default cell counting parameters are used.
- The green circle diameter.
- This parameter is used only when the cell positions are selected manually from the image, and tracked using Cell Finder.
- This shows how far one cell can move between the two time points and the tracking algorithm can still find it.
- But if the tracking algorithm does not find any new position for a cell from the next image, the old position is simply copied to the new image.

- Select how the results are displayed and saved in Batch Process.

**Image processing steps:**

1. Original image
2. Brightness, contrast, gamma
3. Background correction
4. Subtract channels
5. Threshold
6. Remove holes and particles
7. Split areas
8. Select cells
9. Result

**Result**

Result image format

- Outlined with channel color
- Outlined with label color
- Filled with label color

Show result data TXT PDF

Show area IDs TXT

Show result table PDF  Show scattergram

Save result table for every image in Batch Process

Batch Process result images:

- Combined final result - only fuorescence
- Combined final result - phase and fuorescence
- Single channel(s) final result
- Combined image from step 4. - only fuorescence
- Combined image from step 4. - phase and fuoresc.

Save Protocol Start Batch Process

**Image area: 1447680**  
**Result area: 186294**  
**Intensity: 17143145**  
**Area count: 78**

Callout boxes:

- Select how the result areas are displayed in the preview image.
- Select what and how the result values are displayed in the preview image.
- This will also apply to Batch Process result images.
- Show single cell areas and intensities in a scattergram. Click the dots in it to highlight the corresponding cell in the image.
- Show single cell areas and intensities in a table. Click the rows in it to highlight the corresponding cell in the image.
- Select what result images will be saved in the Batch Process for each time point.
- Click these items to see the image!
- Go directly to the file selection phase of the Batch Process.

Save the fluorescence measurement parameters to protocol file.

The default Protocol name is Protocol01.ims

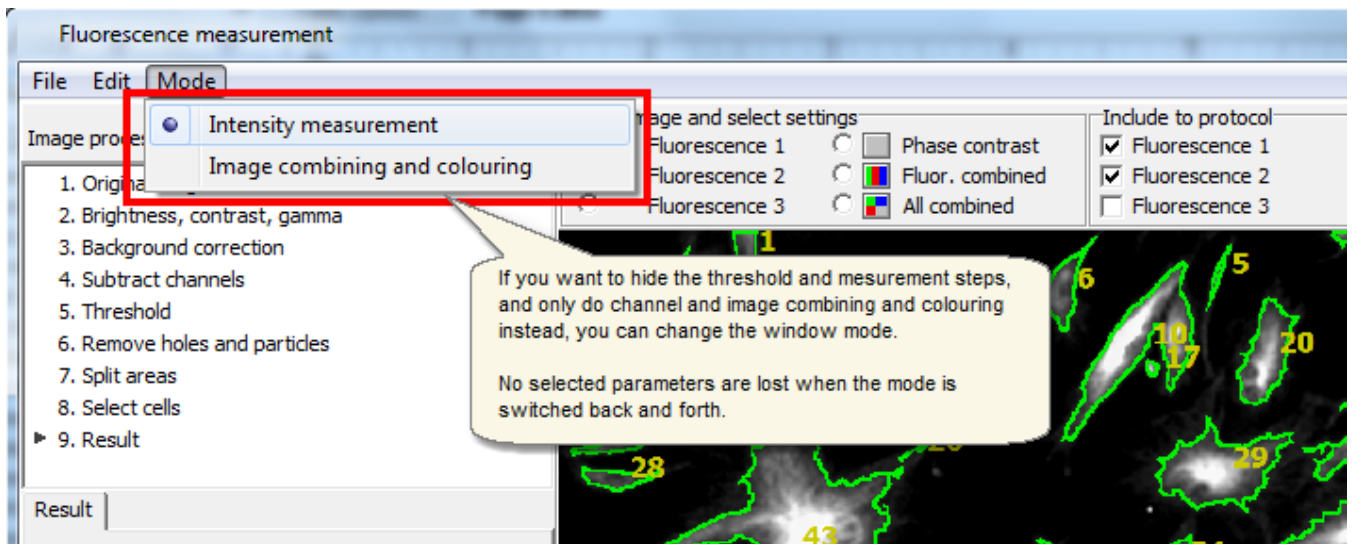
If you use Cell Finder for cell selection, and you select the default name for the Protocol, the following three files are automatically saved:

```

Protocol01.ims           //FL parameters
Protocol01_SampleLib.ims //Sample library and cell counting parameters
Protocol01_SampleLib_classif.txt //Cell classification parameters, if the sample library protocol has been built
    
```

In the Batch Process you only need to select the Protocol01.ims, and the other two files are opened automatically when named correctly.

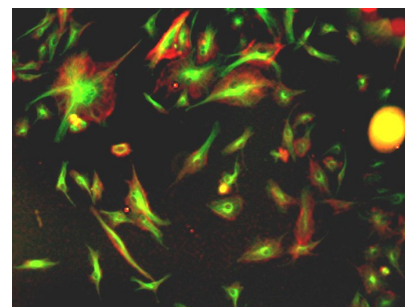
- Change mode if you simply want to combine and colour images.



## 6.2 Fluorescence image combining and colouring

Combine and colour the images from single and multichannel fluorescence cell tests with the Fluorescence image combining and colouring tool.

- No measurement values are produced, just the combined and coloured result images.
- The tool uses both the phase contrast and fluorescence images at the same time.



### Fluorescence image combining/colouring procedure

There are two optional ways to do the analysis:

Option 1:

Step 1: Select the analysis parameters and save them to a Protocol file.

Step 2: Go to Batch process, open the Protocol from file when asked, and fine tune the parameters if needed.

Option 2: Go directly to Batch process, and select the parameters on the go. No protocol file is needed.

### Make fluorescence image combining/colouring Protocol

- [Open images](#) to the analyser main window **using the \*.dat file**, and open the Fluorescence image combining and colouring tool from "Tools -> Fluorescence - image combining and colouring".
- The preview image that was in the main window is automatically opened to the Fluorescence measurement tool preview window.
- Select the measurement parameters step by step. The intermediate results image is always displayed on the right hand side of the window.

**The first four parameter selection steps are identical to the parameter selection in the Fluorescence intensity measurement tool.** See from the [Fluorescence intensity measurement topic](#) how the parameters are selected.

- Select in the last step which type of result images will be saved in the Batch Process, and save the protocol.

Combine/colour phase contrast and fluorescence images

File Edit Mode

Image processing steps:

1. Original image
2. Brightness, contrast, gamma
3. Background correction
4. Subtract channels
- ▶ 5. Result

Result

Batch Process result images:

- Combined image - only fuorescence
- Combined image - phase and fuorescence

Select what result images will be saved in the Batch Process for each time point. Click these items to see the image!

Save the fluoreescense combining/colouring parameters to protocol file.  
The default Protocol name is Protocol01.ims

Go directly to the file selection phase of the Batch Process.

Save Protocol Start Batch Process

< Prev.

1372 872 6,2,0

## 6.3 Scratch wound measurement

Measure scratch wound areas and closure speed with the scratch wound measurement tool. Measurement is based on thresholds and selected parameters, and no cell sample image teaching is needed.

### Scratch wound measurement procedure

There are two optional ways to do the scratch wound analysis:

Option 1:

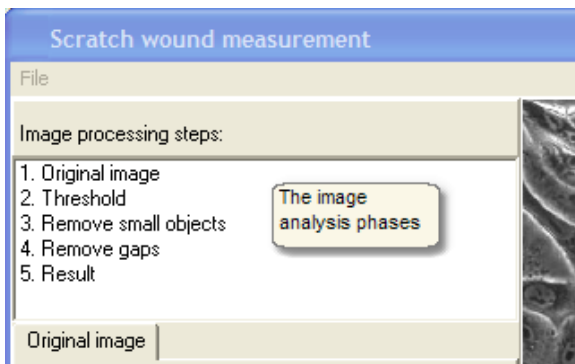
Step 1: Select scratch wound analysis parameters and save them to a Protocol file.

Step 2: Go to Batch process, open the Protocol from file when asked, and fine tune the parameters if needed.

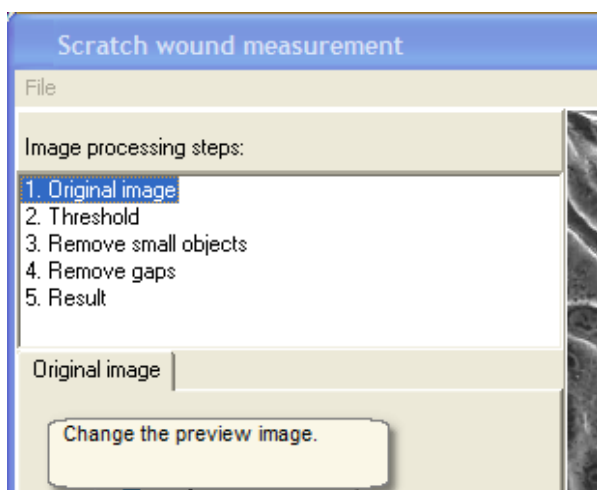
Option 2: Go directly to Batch process, and select the scratch wound analysis parameters on the go. No protocol file is needed.

### Make scratch wound analysis Protocol

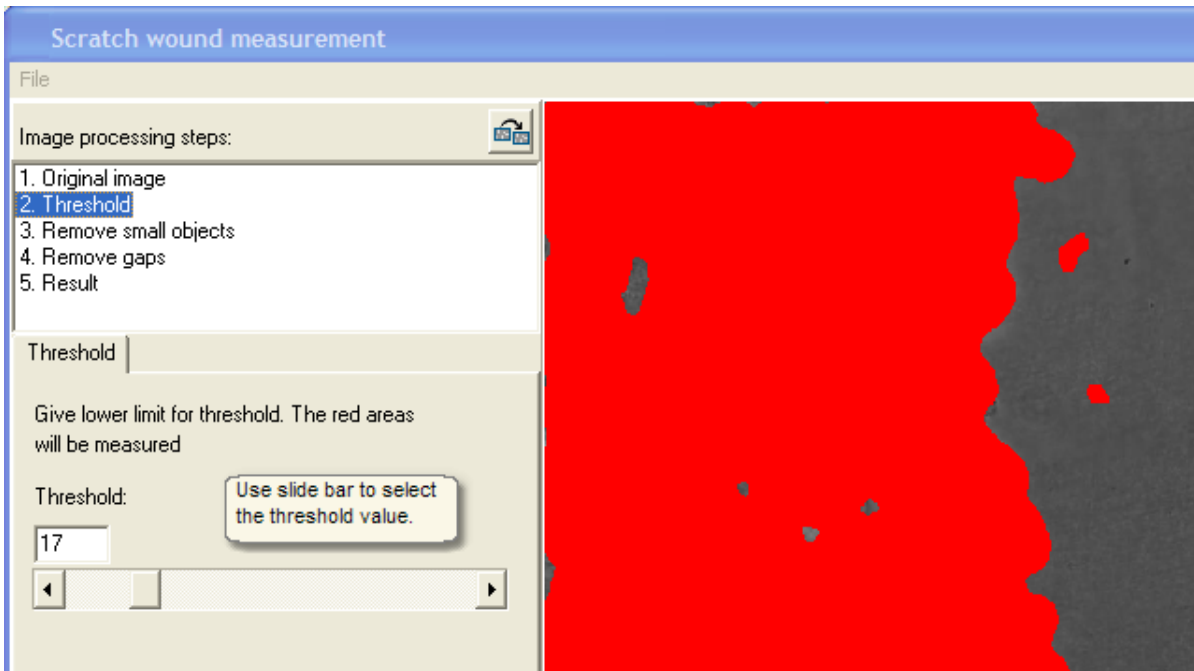
- [Open images](#) to the analyser main window, and open Scratch wound tool from "Tools -> Scratch wound measurement".
- Main window image is automatically opened to Scratch wound analysis preview window.
- Select analysis parameters. The intermediate image analysis results are displayed, when you click the image analysis phases.



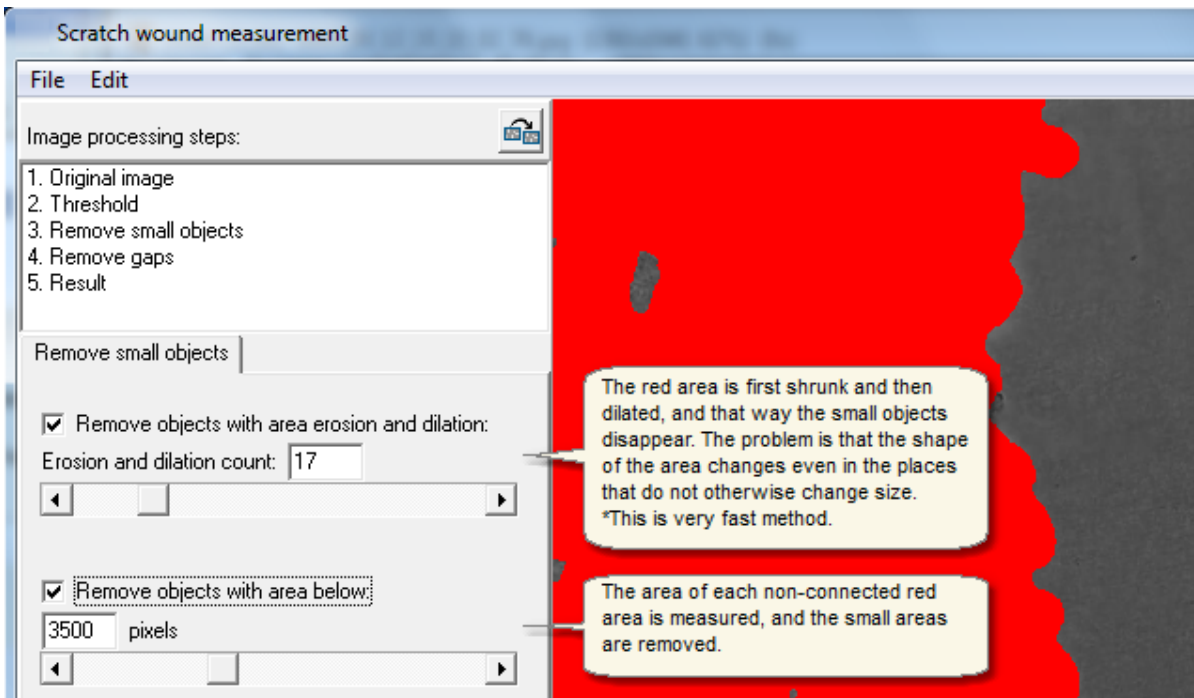
- You can change the preview image at any time.



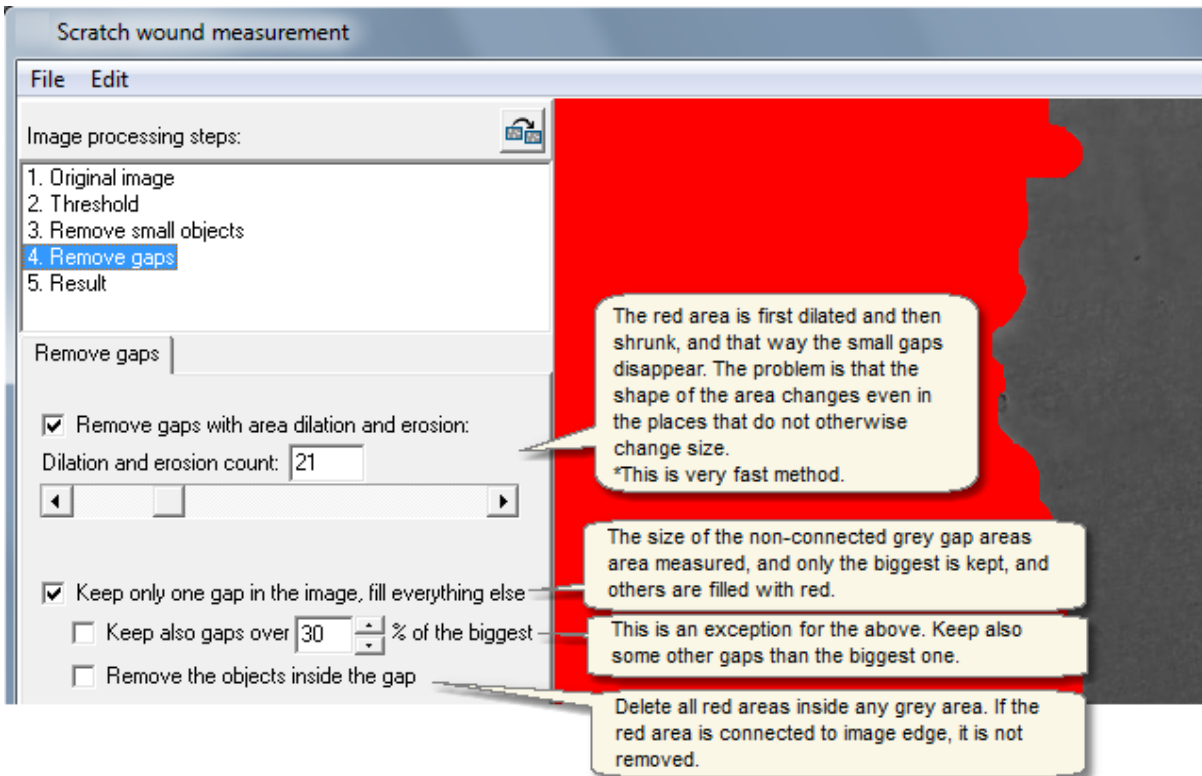
- Select threshold so that the all the cells and none of the background is thresholded in, and hence colored with red. Even if cells are now segmented, the background area is finally measured!



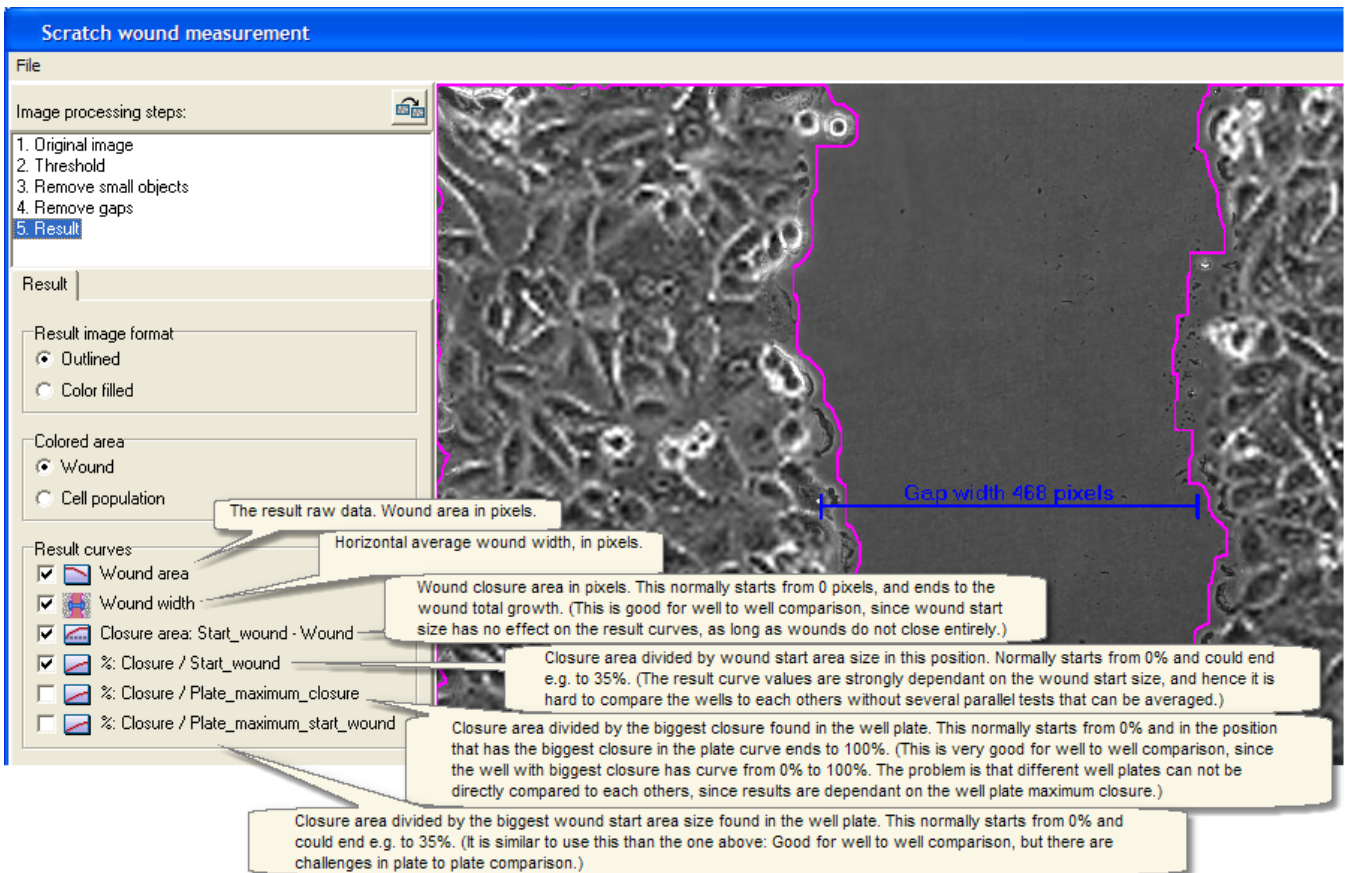
- Select settings for removing the small unwanted red particles from the measured cell area. You can see that the two small red areas have disappeared.



- Select settings for removing the small unwanted grey background gaps from the cell area. You can see that several gaps have disappeared.

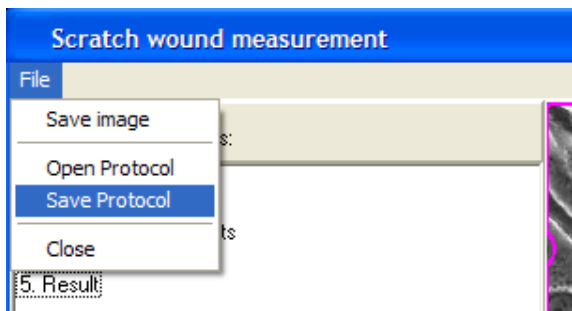


- Select how the result images are drawn and which type of result curves will be displayed.



- Save the scratch wound analysis parameters and result settings to protocol file.

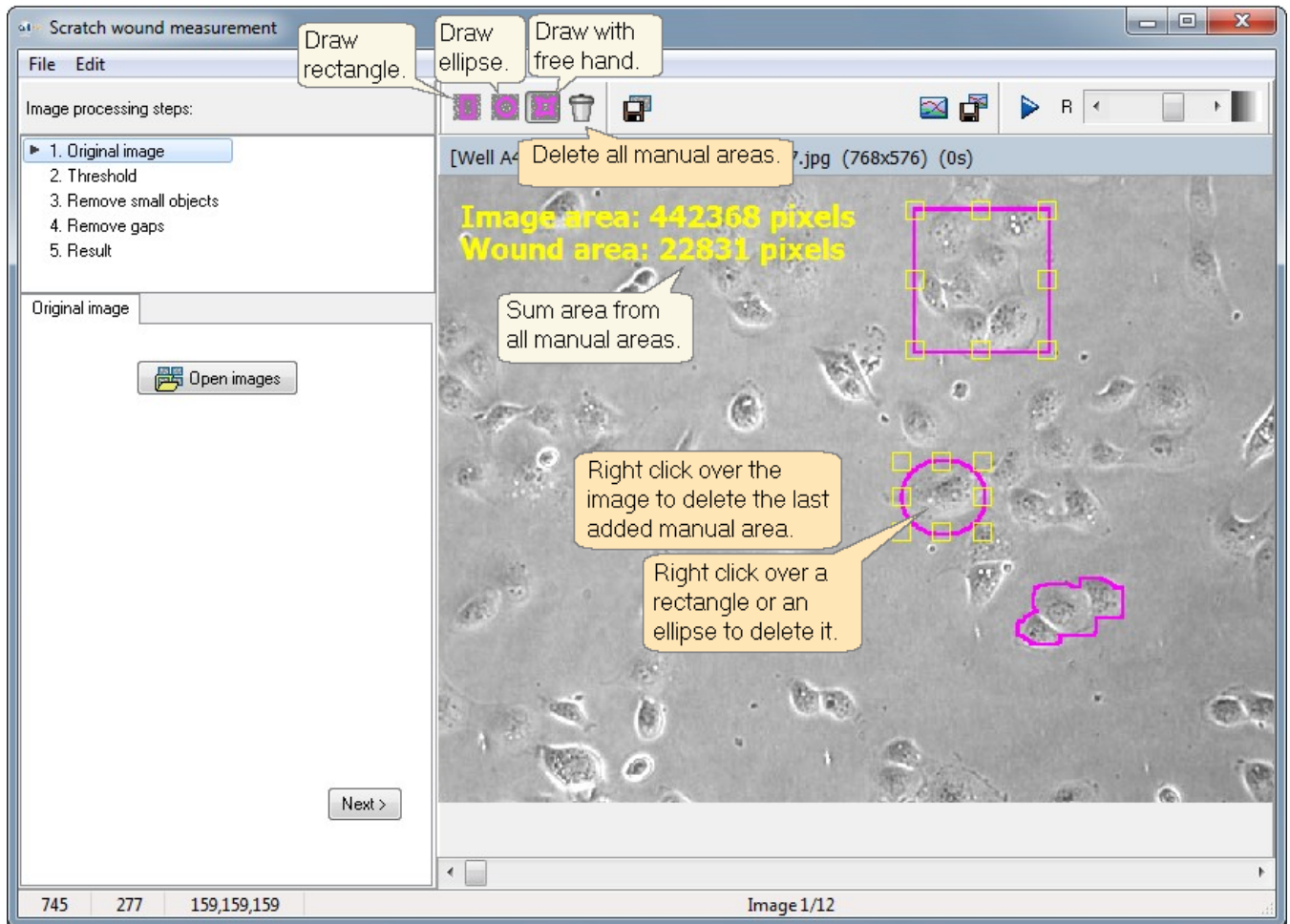




### 6.3.1 Manual tools

The measurement area can be defined manually with the manual tools found from the center top panel.

- There is no limit on how many manual areas are added.
- The result area displayed in picture is a sum of the areas.
- Area can be measured either manually or automatically, not with both for one time point.



## 6.4 Area size measurement

Measure cell population areas and growth speed with the area size measurement tool. Measurement is based on thresholds and selected parameters, and no cell sample image teaching is needed.

### Area size measurement procedure

There are two optional ways to do the area size analysis:

Option 1:

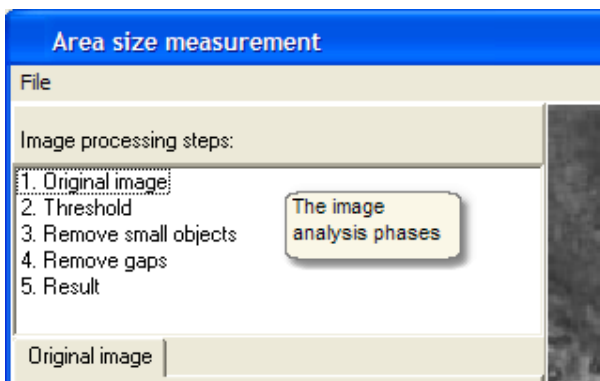
Step 1: Select area size analysis parameters and save them to a Protocol file.

Step 2: Go to Batch process, open the Protocol from file when asked, and fine tune the parameters if needed.

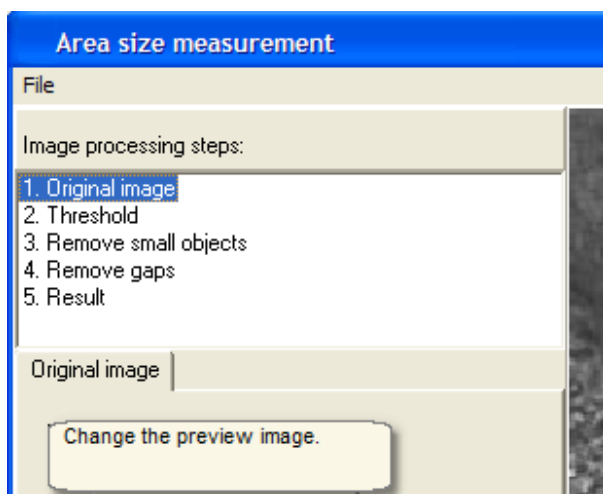
Option 2: Go directly to Batch process, and select the area size analysis parameters on the go. No protocol file is needed.

### Make Area size analysis Protocol

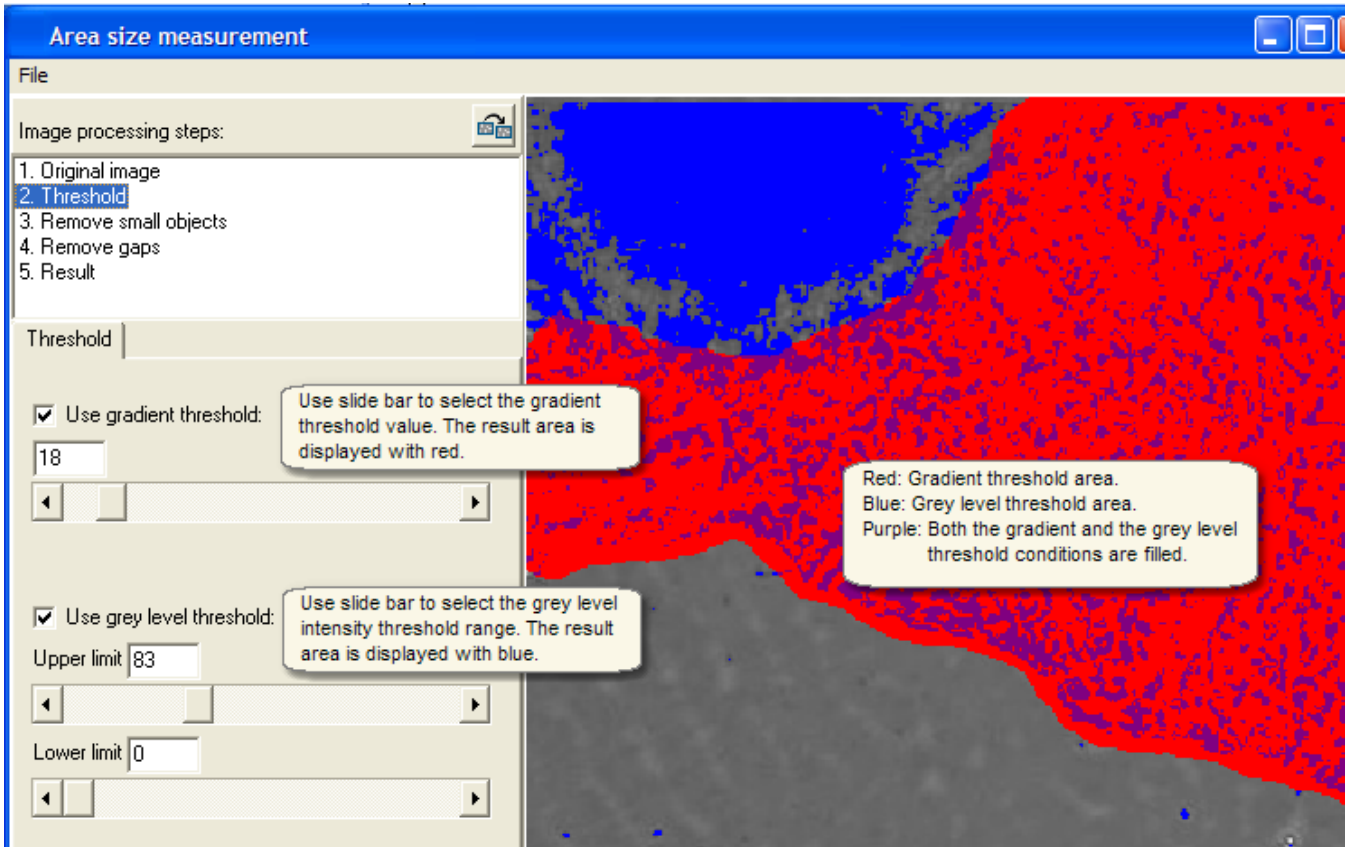
- [Open images](#) to the analyser main window, and open area size tool from "Tools -> area size measurement".
- Main window image is automatically opened to area size analysis preview window.
- Select analysis parameters. The intermediate image analysis results are displayed, when you click the image analysis phases.



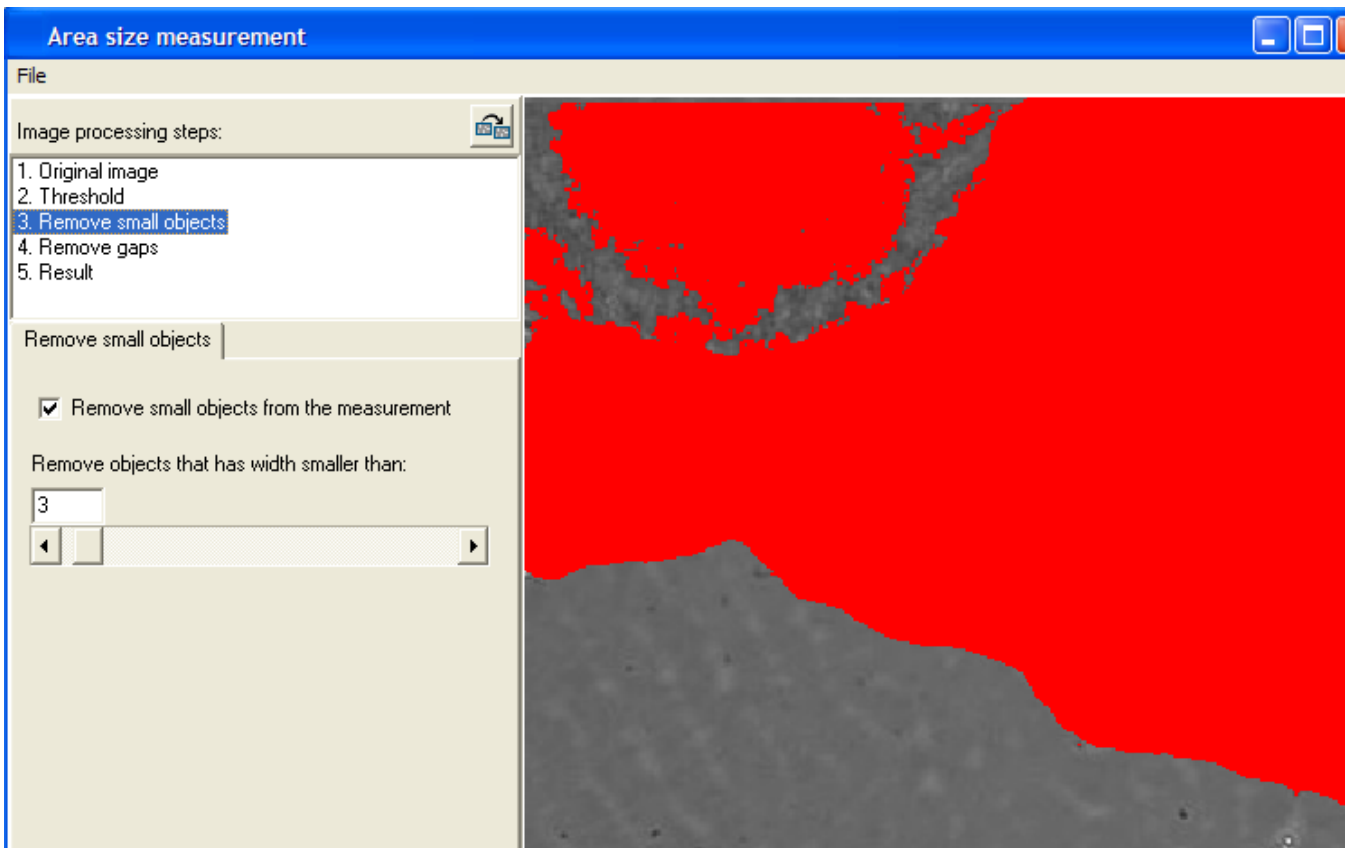
- You can change the preview image at any time.



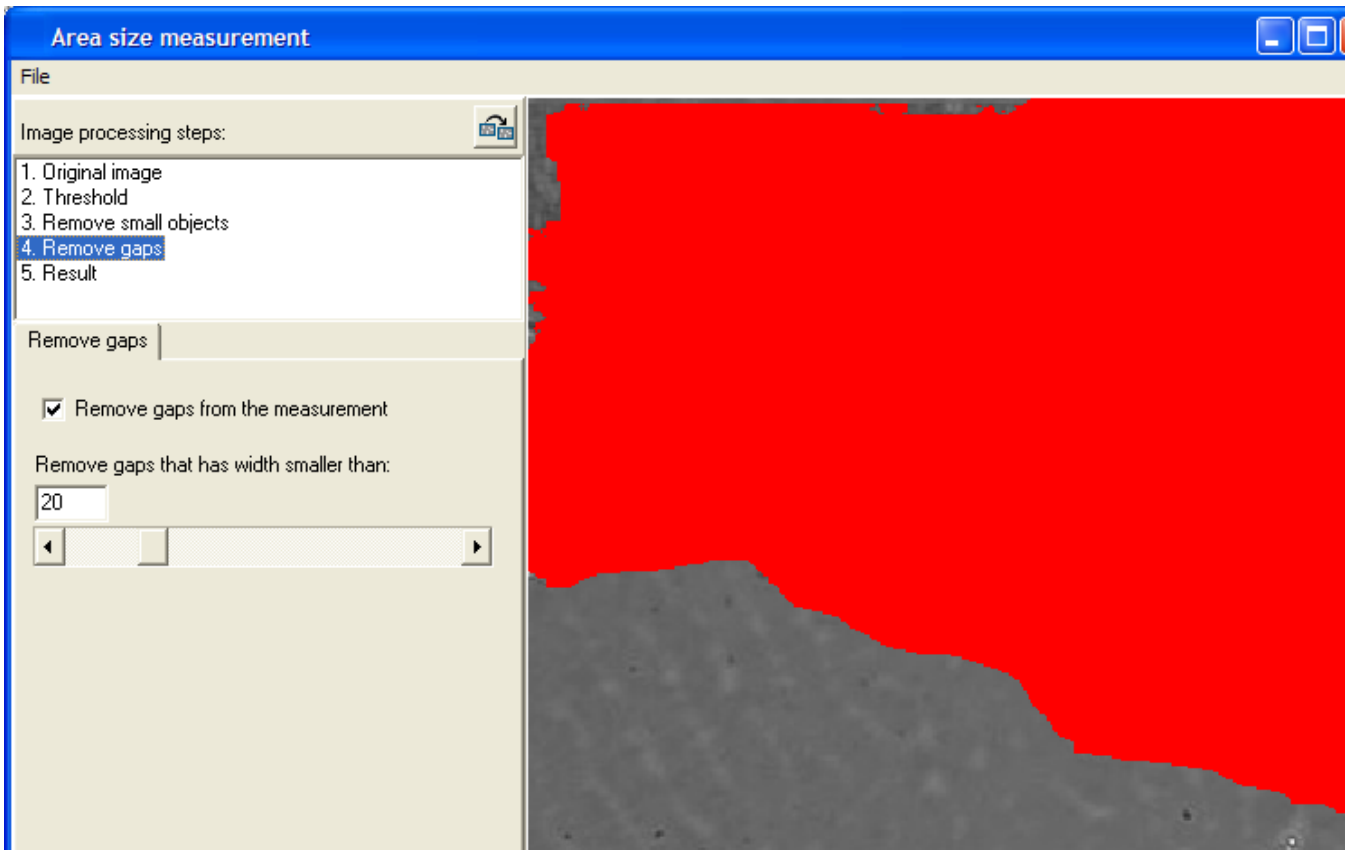
- Select thresholds so that the all the cells and none of the background is thresholded in. The red, blue and purple areas are combined after this analysis step.



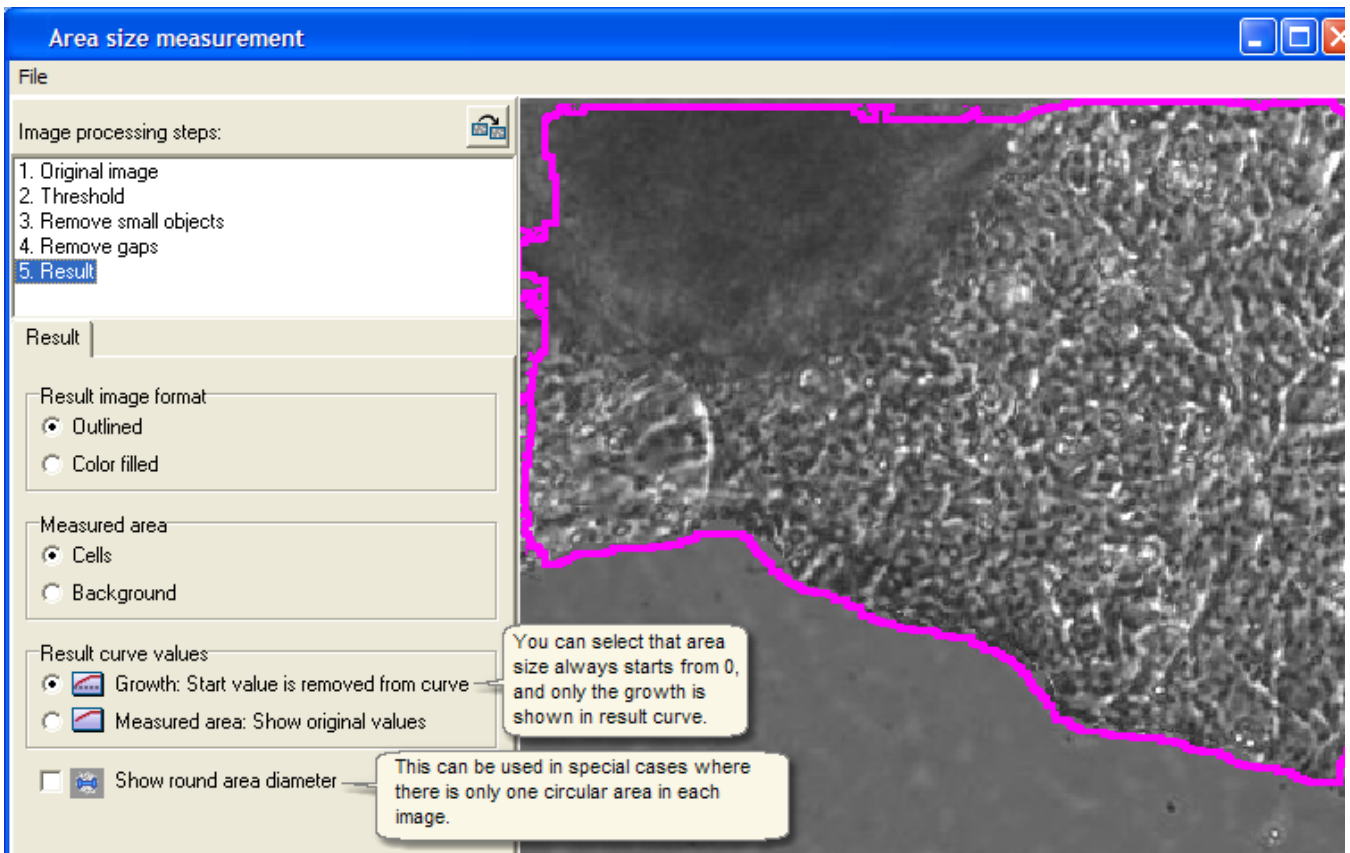
- Select particle size for removing small unwanted particles from the measured cell area. You can see that some small blue areas from the bottom have disappeared (are not displayed in red in below).



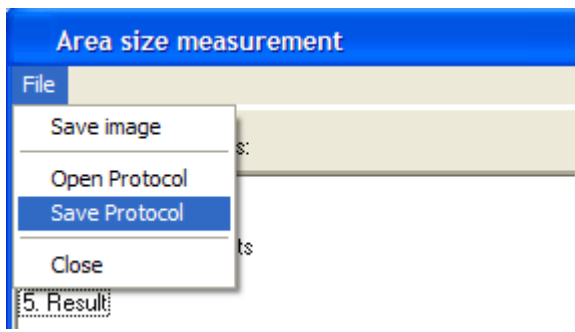
- Select size of gaps for removing the small unwanted background gaps from the cell area. You can see that several gaps have disappeared.



- Select how the result images are drawn and which result curves will be displayed.



- Save the area size analysis parameters and result settings to protocol file.



## 6.4.1 Manual tools

The measurement area can be defined manually with the manual tools found from the center top panel. The area size measurement manual tools are similar to the manual tools for the Scratch wound measurement, see the topic: [Making the other Protocol types -> Scratch wound measurement -> Manual tools](#).

## 6.5 Tubule and branch point measurement

Analyse angiogenesis images with the tubule and branch point measurement tool. Measurement is based on thresholds and selected parameters, and no cell sample image teaching is necessarily needed, but it can be utilized, as described later. The tubule length and the number of different type branch points (different branch count) are measured. The images should be normal phase contrast images.

### Tubule and branch point measurement procedure

There are two optional ways to do the analysis:

Option 1:

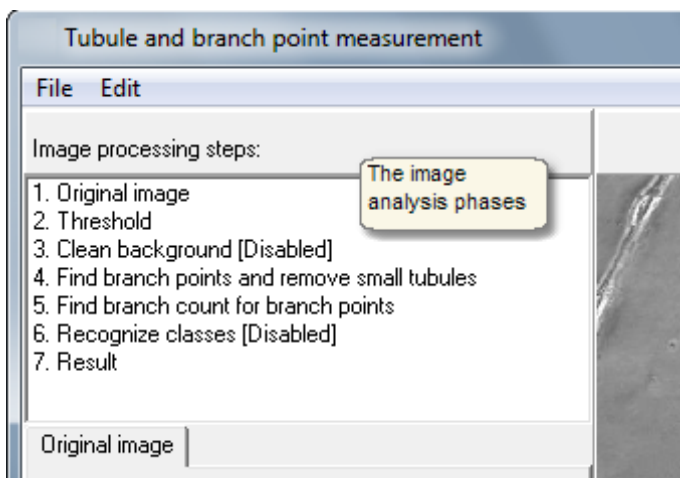
Step 1: Select the analysis parameters and save them to a Protocol file.

Step 2: Go to Batch process, open the Protocol from file when asked, and fine tune the parameters if needed.

Option 2: Go directly to Batch process, and select the analysis parameters on the go. No protocol file is needed.

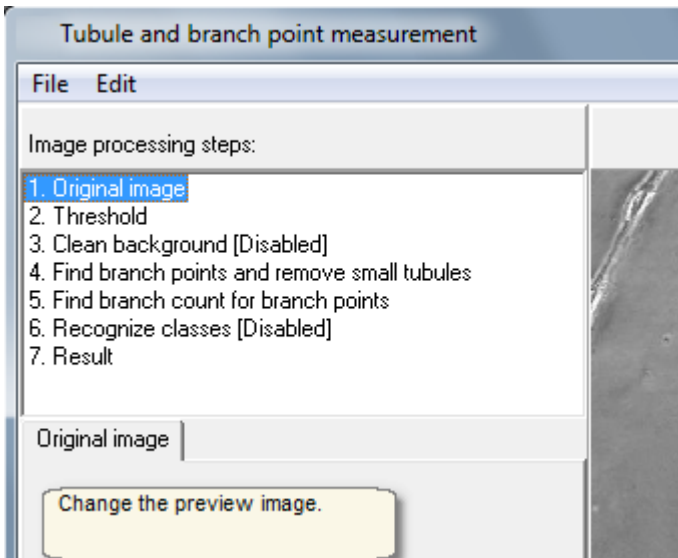
### Make analysis Protocol

- [Open images](#) to the analyser main window, and open tubule and branch point measurement tool from "Tools -> Tubule and branch point measurement".
- Main window image is automatically opened to area size analysis preview window.
- Select analysis parameters. The intermediate image analysis results are displayed when you click the image analysis phases.

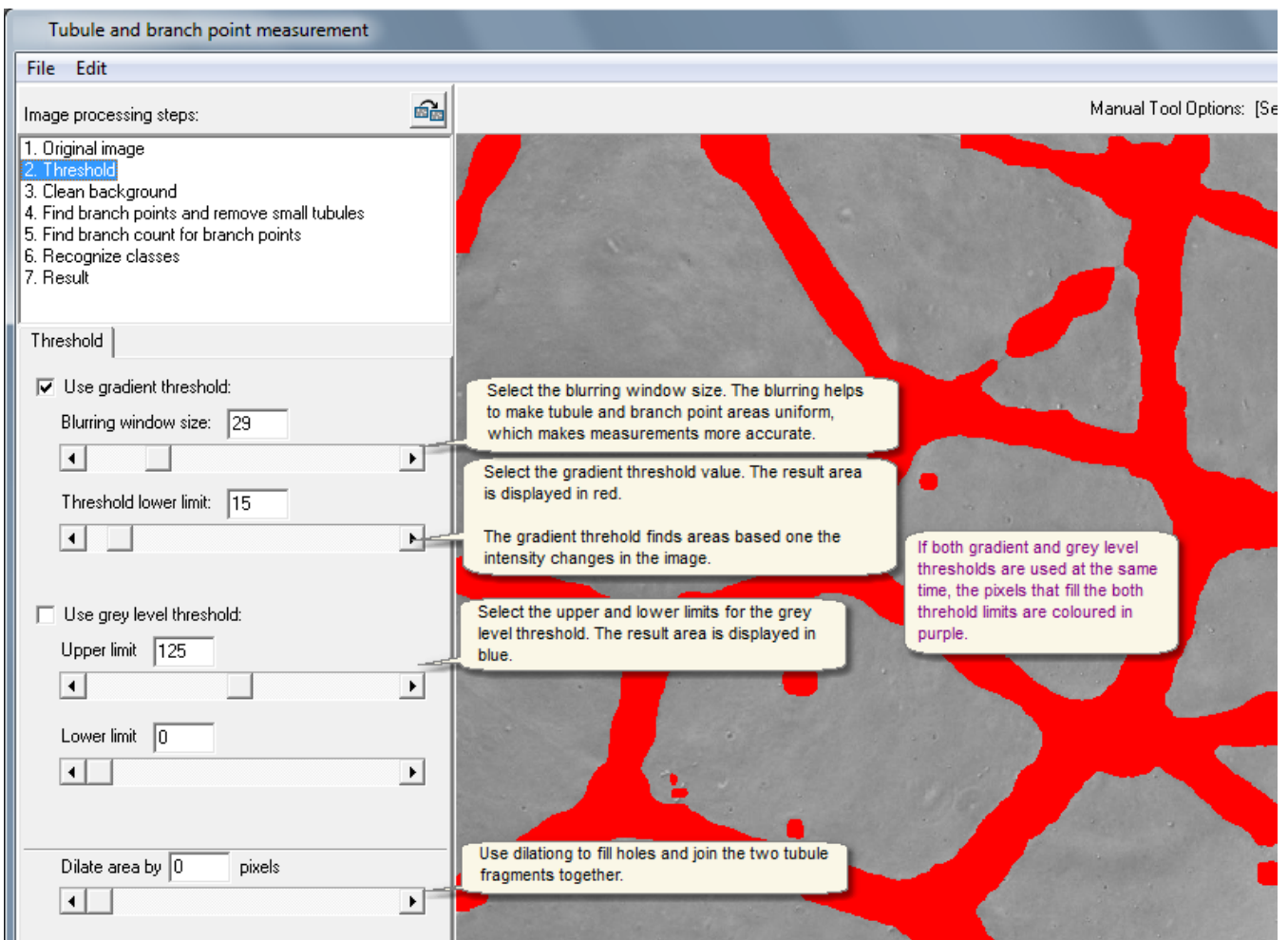


- You can change the preview image at any time.

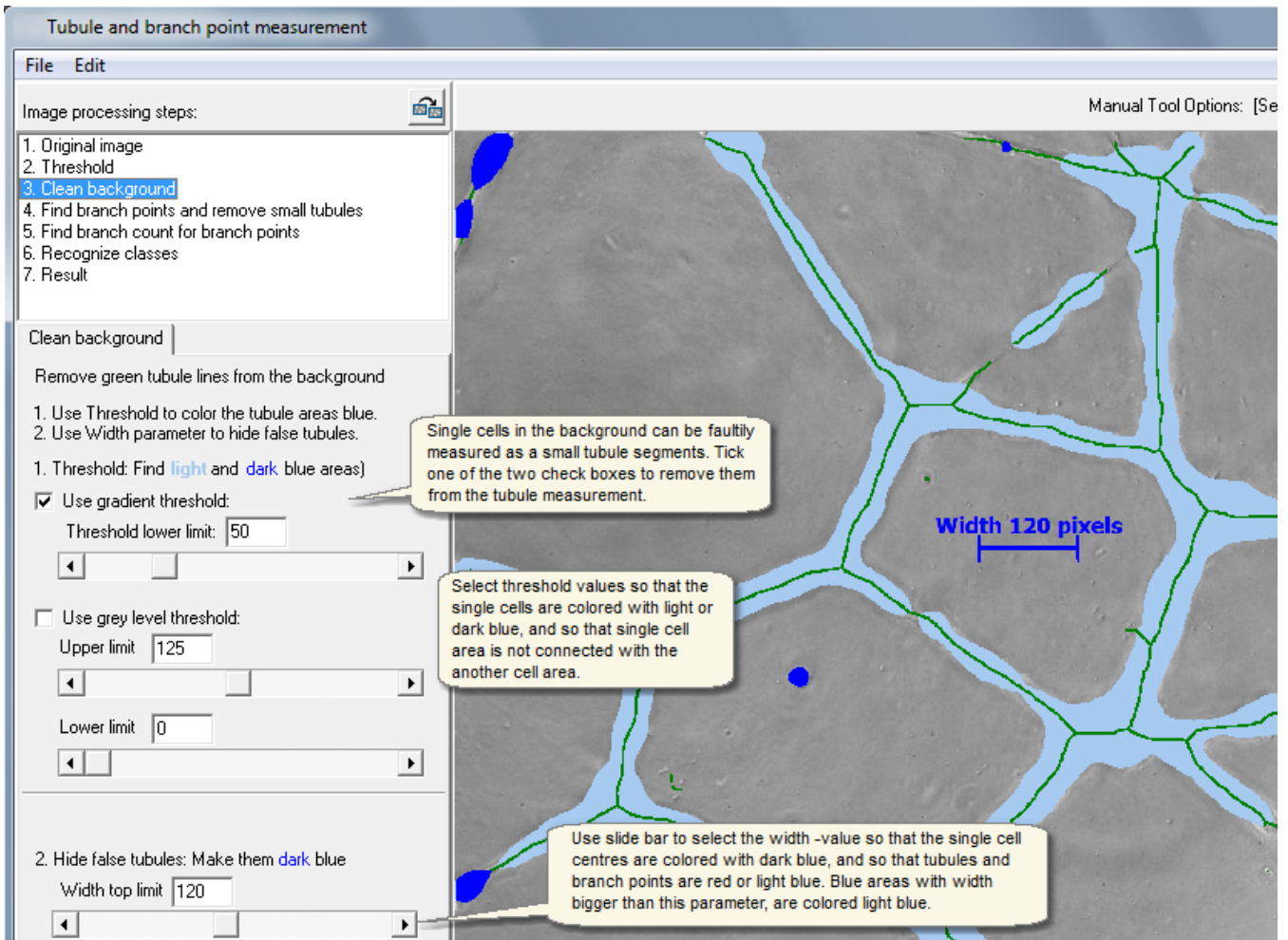




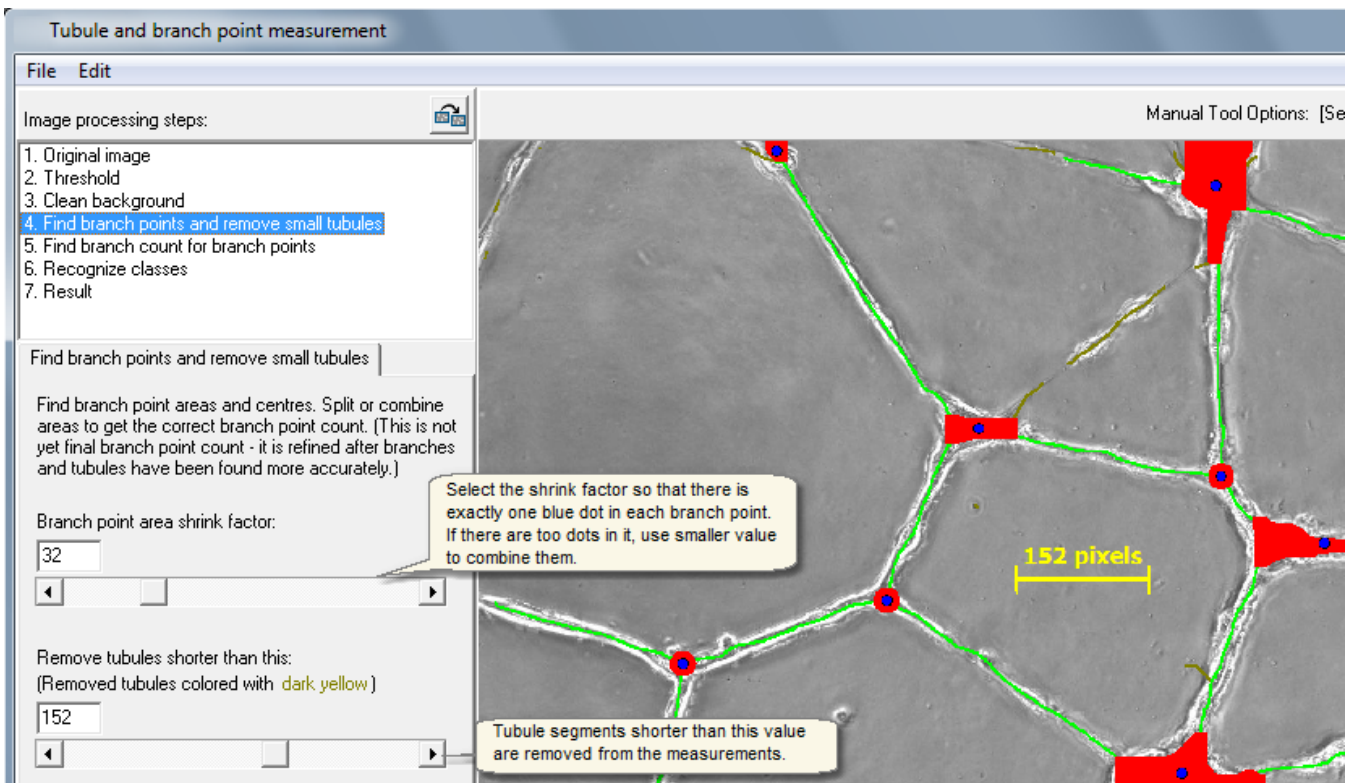
- Select threshold parameters so that the all the tubules and branch point areas are thresholded in. The following is targeted: Branch point areas have no holes, tubules are continuous with no cuts, and no single cells or garbage from the background is colored (but this is secondary target, since they can be fixed also during later steps). The width of the colored tubule area is not relevant.



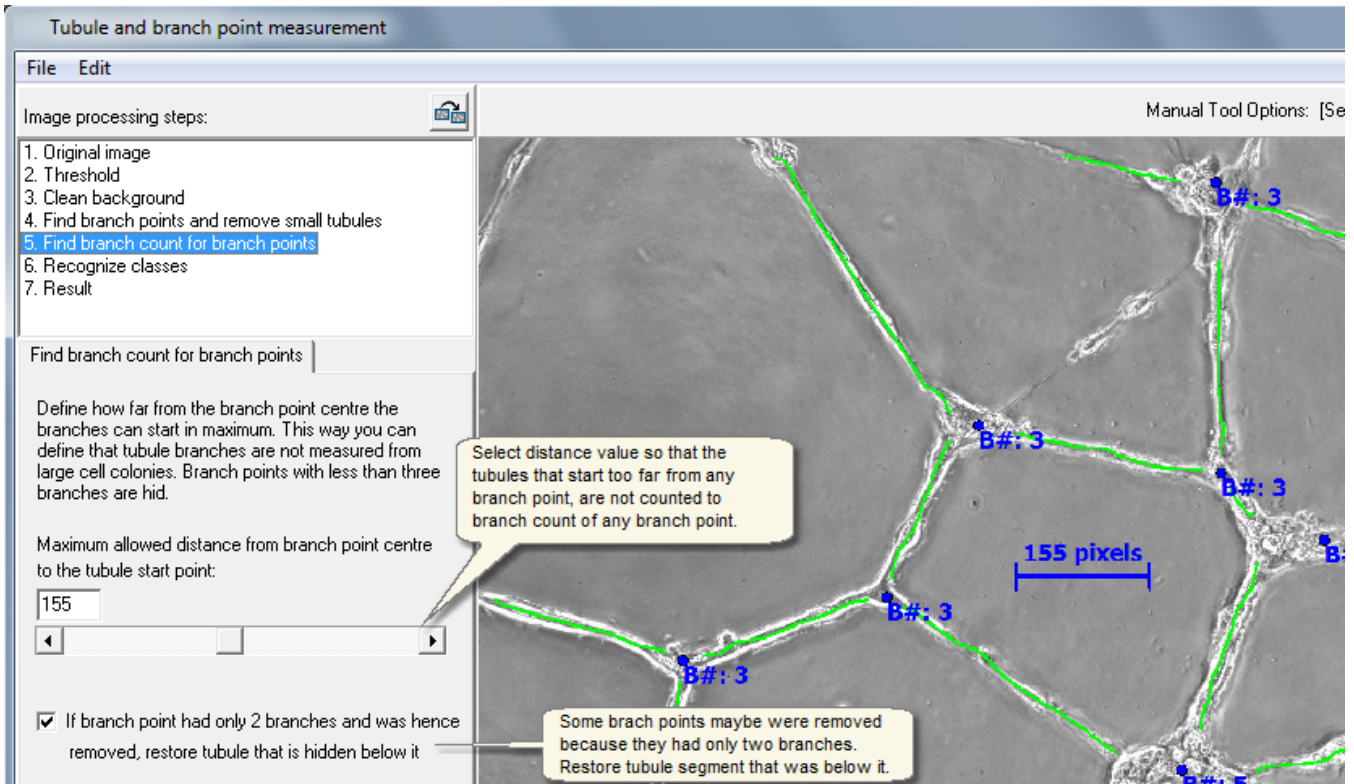
- Single cells in the background can be faultily measured as a small tubule segments. Use these parameters to remove them from the tubule measurement.



- Find the center of each branch point. Branch point is assigned to the center of every disconnected area. Adjust the red area size so that there is exactly one blue dot in each branch point. If there are too many single branch points found, some of them are removed automatically in the next analysis stage.



- Count how many branches each branch point has. Remove some tubules from the branch count of the branch point, by defining the minimum distance value, so that the tubules that start too far from center of the branch point, are not included in the branch count. Branch points that have less than three branches are hidden and removed from the results.



- Classify tubules using Tubule Classifier protocol. If it is disabled, the total tubule length is measured in any case, but the tubules will not be classified to different classes.
  - Tubules can be classified to different classes such as thin and thick tubule classes, and false tubules can be removed from the measurement with the classifier protocol.

**Tubule and branch point measurement**

File Edit

Image processing steps:

1. Original image
2. Threshold
3. Clean background
4. Find branch points and remove small tubules
5. Find branch count for branch points
6. Recognize classes
7. Result

Recognize classes

Classify tubules using Analysis Protocol

Open Analysis Protocol **Select Tubule Classifier protocol**

Edit class styles **Modify Tubule Classifier protocol.**

Protocol ready!

Unify the class of a tubule

All the pixels in one continuous tubule segment will be assigned to the same recognition class.

**Format of analysis results**

File

Select how results are presented

Selected classes are:

- Counted to total cell number
- Displayed in result images
- Displayed in result curves

Total tubule length

Tubules - Thin

Tubules - Thick

Color in:

- Result images
- Result curves

White  Red  Lime

Spot format in:

- Result images

Spot width in:

- Result images

Ok Cancel

Thin tubule recognized and colored with red.

Thick tubule recognized and colored with green.

An example Tubule Classifier protocol

**Sample library**

File View

Row number for one class: 1

Move single images  Delete single images

Move selected Delete

	1	2	3	4	5	6	7
Thin [Tubules]							
Thick [Tubules]							

**Pick samples**

File Edit Sample Library

Analysis tool

- Cell Finder
- Area Finder
- Neurite Recognizer
- Tubule Classifier

Create classes | Add samples | Make protocol

Add new Delete

Cell type  Cell phase  Background type

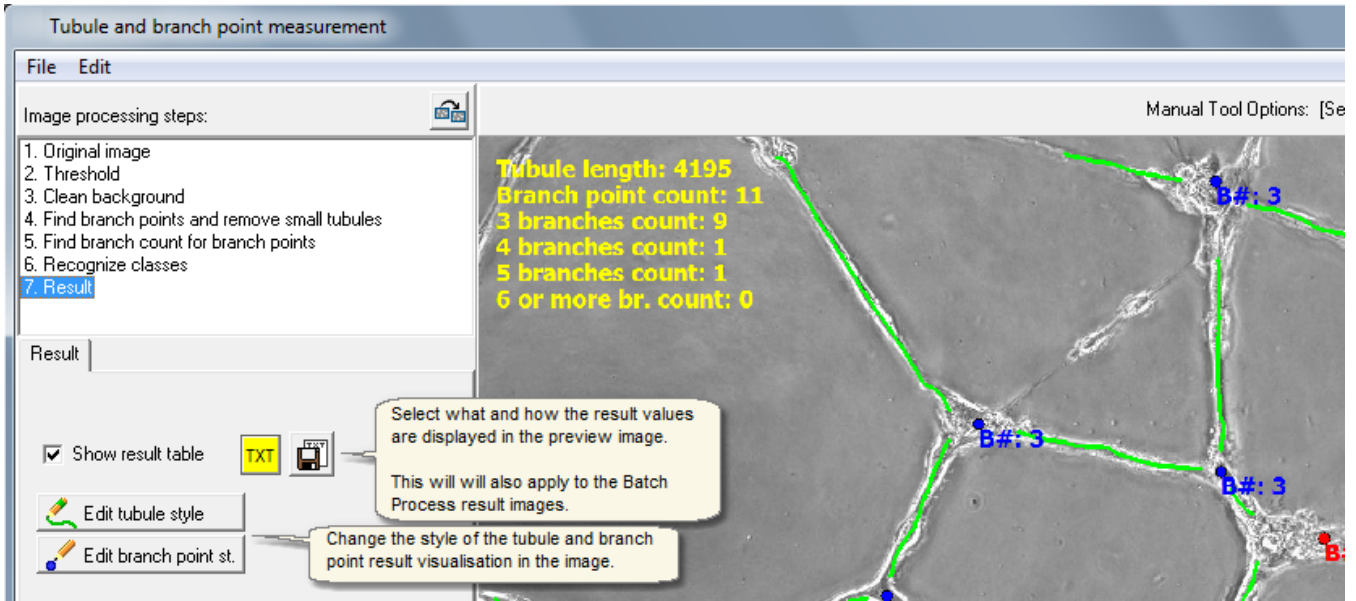
Sample numb. 157

Edit class styles

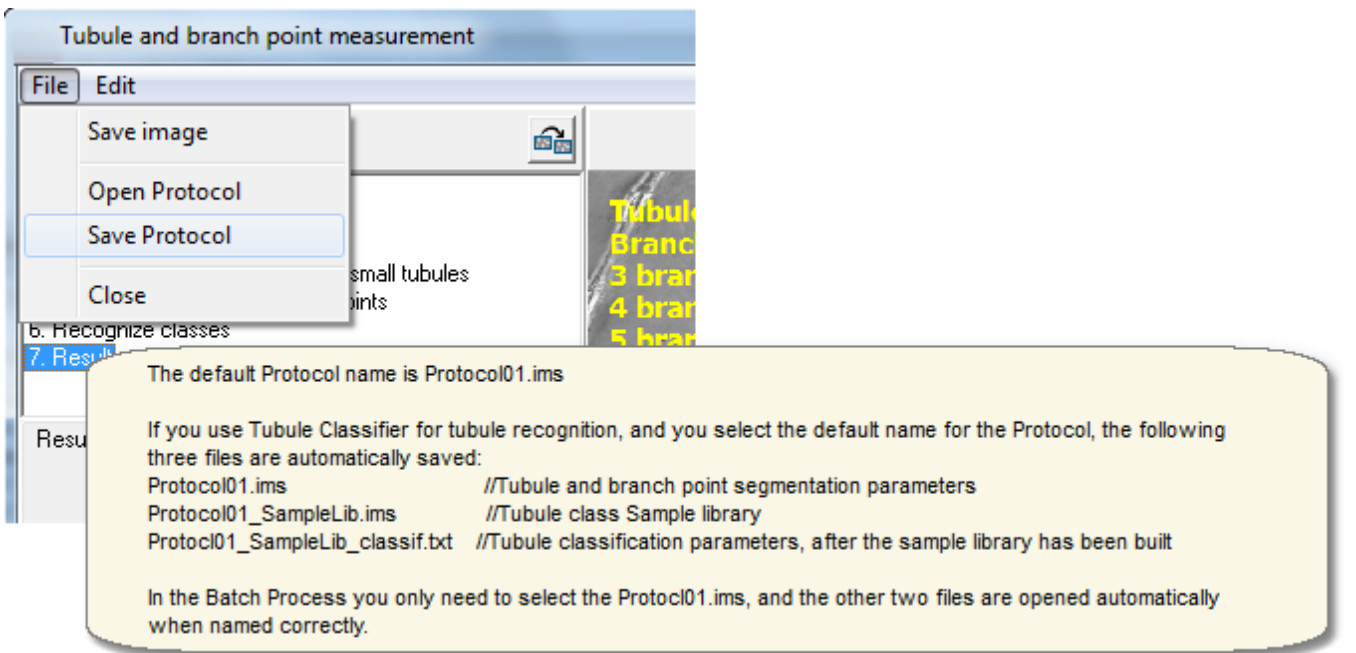
- Tubules
  - Thin (73)
  - Thick (84)

Close

- Display the final result image.



- Save the tubule and branch point analysis parameters to protocol file.



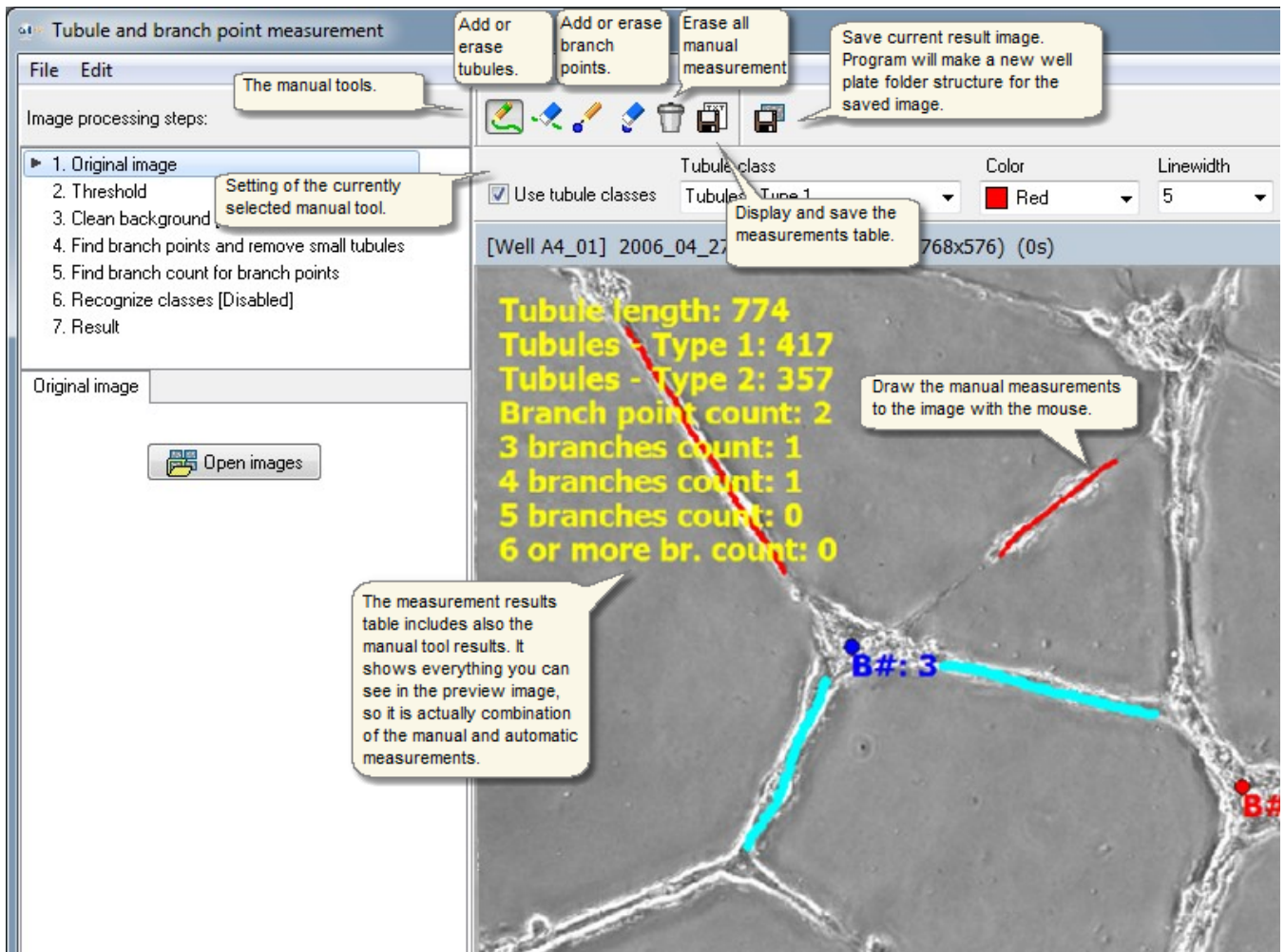
### 6.5.1 Manual tools

The tubule and branch point results can be manually edited with these tools in the protocol parameter selection window, for single images, but they do not change the measurement results when the Batch Process is used!

The image below shows the step 1 (see the left top corner of the image) of the tubule parameter selection, so all the results displayed in the preview image have been created with the manual tools. The manual tools can be used in the step 1 and in the final result step 7, where also the automatically created measurement results results can be edited (erased) with them.

With the manual tools you can:

- Add tubules. You can define the classes, e.g. thin and thick tubules.
- Add branch points. You can change the branch point display style.
- Erase tubules. You can erase the automatically created tubules in the step 7.
- Erase branch points. You can erase the automatically created branch points in the step 7.

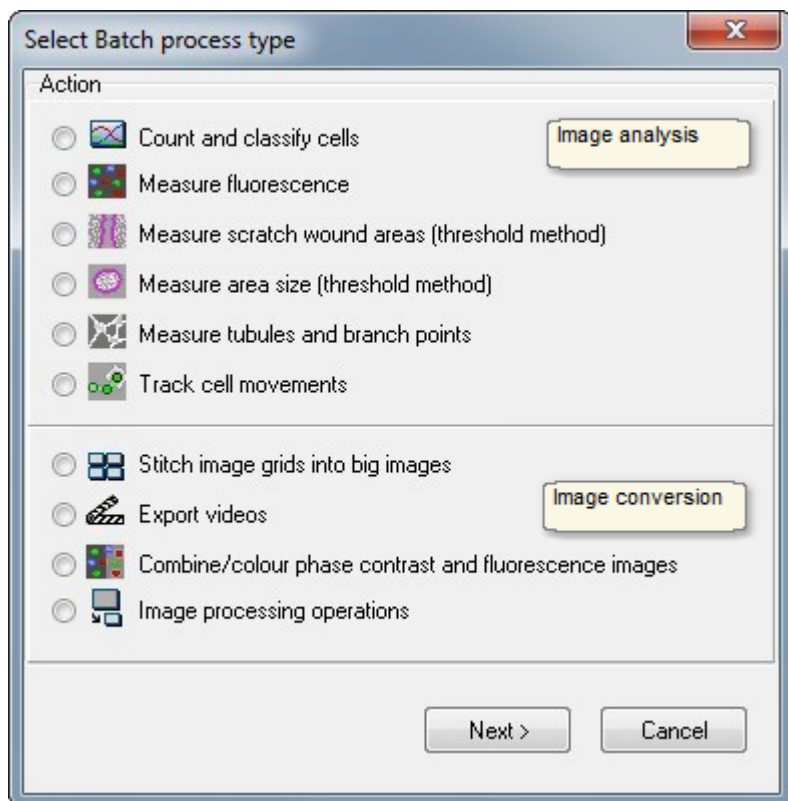


Batch process



## 7 Batch process

Use Batch process for applying the selected image analysis or conversion routines easily for all images from an entire well plate, with a single run.

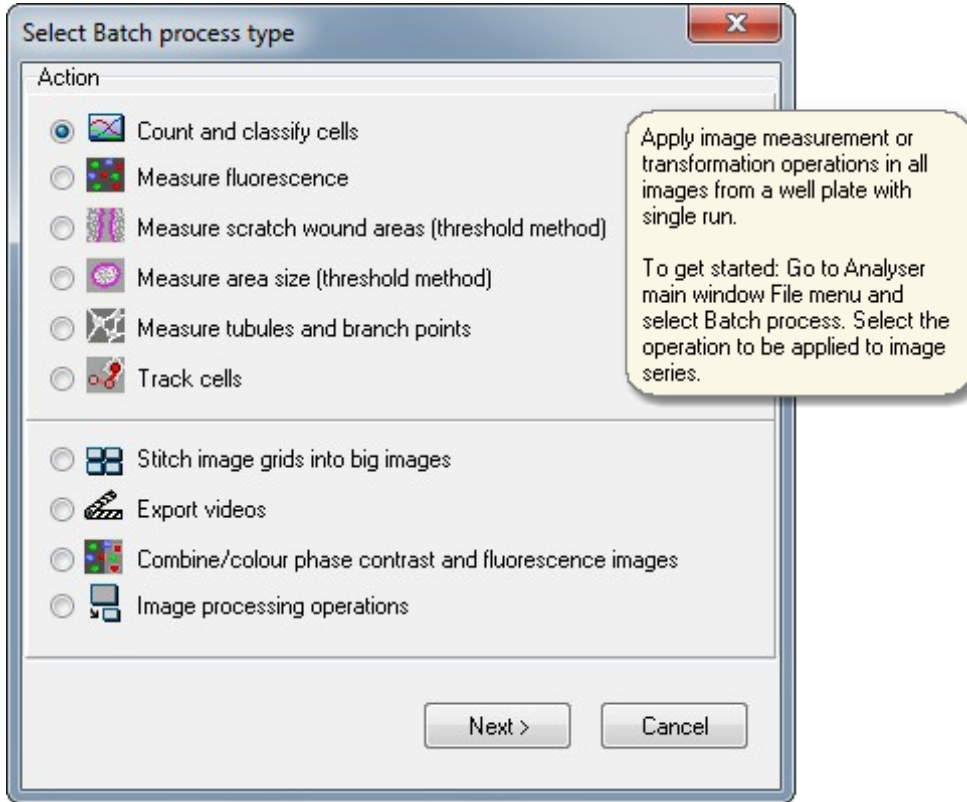




## 7.1 Select method and images

### Selecting batch process type

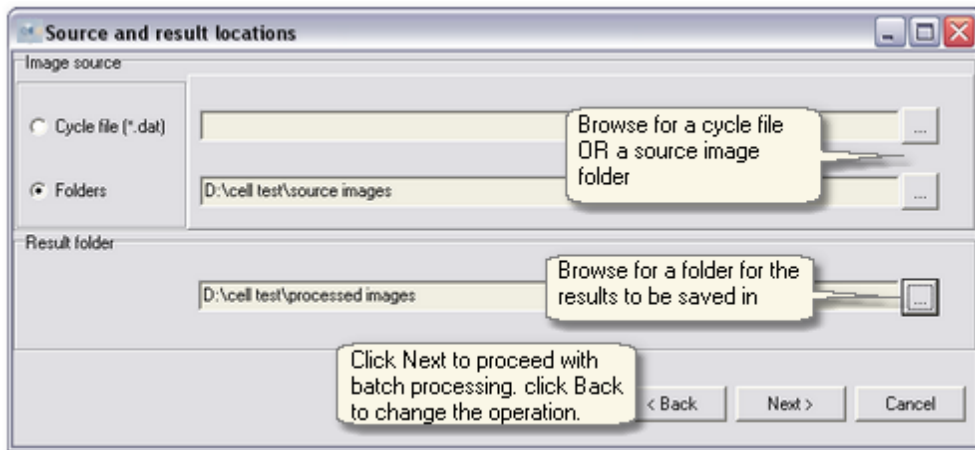
Select **Batch process** in the Analyser main window File menu. Next select batch process type:



### Defining the source and result files and folders

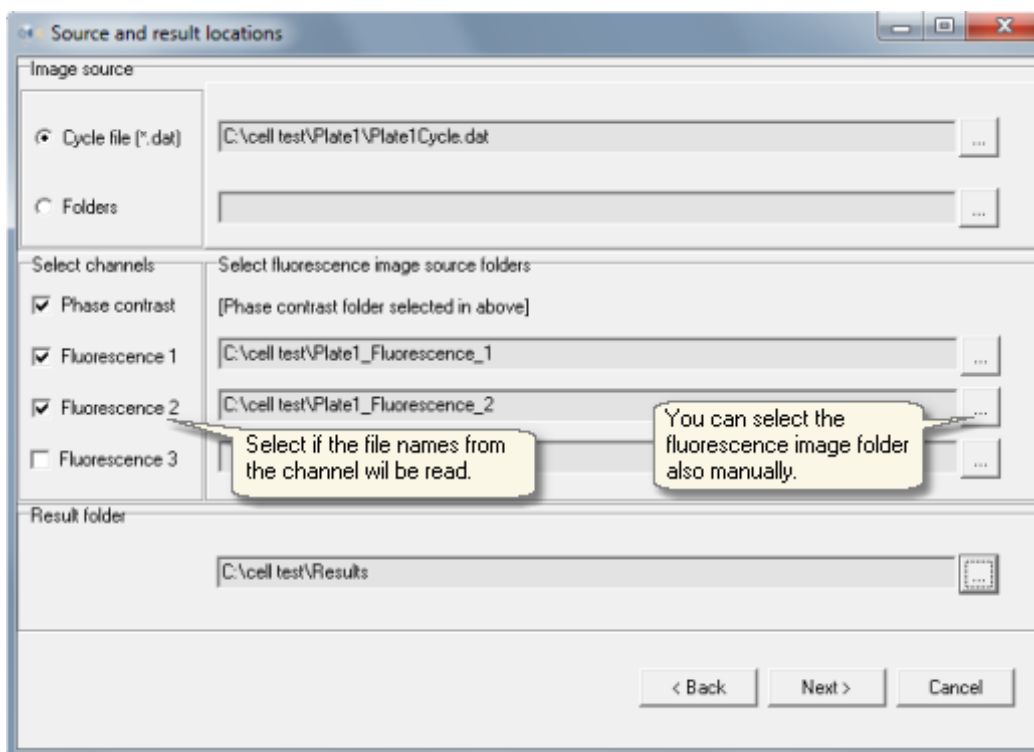
You need to define the source images for the processing, either by a cycle file or by the folder, and a folder for the results to be saved in. Click Next to continue or Back to change the processing type.

- The result curves are saved in ACSII format in a \*.txt file.
- File names and subdirectories for the results are automatically created.
- The result images are normally saved as jpeg images that can also be viewed with other programs.
- The Analyser converts the curve text files (\*.txt) into visible graphs, see more in topic [Analysis results](#).

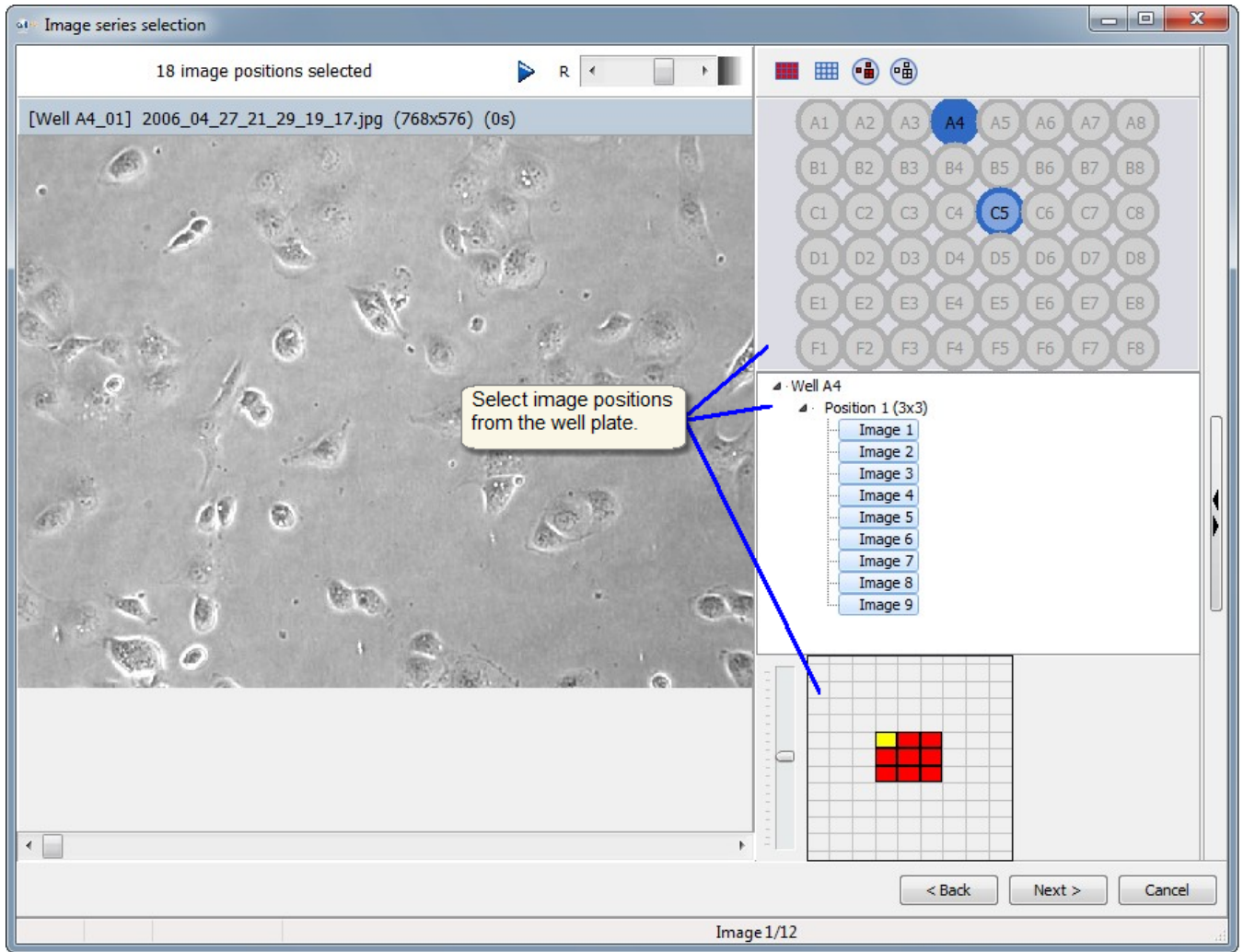


With the fluorescence intensity measurement and combining/colouring tools you will also need the images from the fluorescence channels. But the file selection method is still the same as above, because the program will find

the fluorescence image folders automatically after you have selected the phase contrast image folder. The fluorescence image folders are displayed as shown below, and you can disable the program from reading the file names for some channels using the check boxes.



**Select the image positions from the well plate**



**Previewing and refining image selection**

Preview the number of images selected, and refine the amount of processed data e.g. by selecting only every second image from series if preferred. Click Next to continue to method special settings, and further to start analysis run or image conversion.

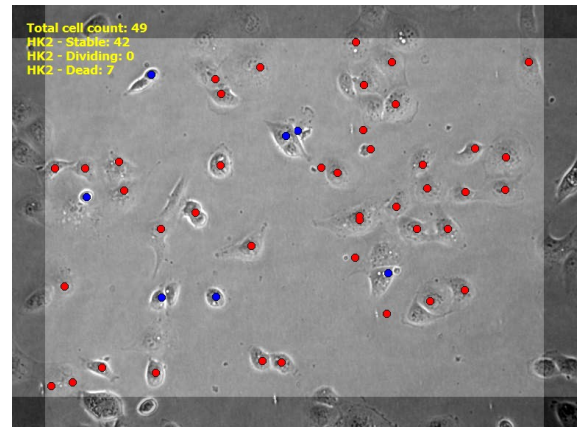
The screenshot shows a software dialog box titled "Image file selection". It contains a "Selections" section with a list of items: "Positions and images:", "- 2 wells, with", "- 2 grids, with", "- 18 image series, with", and "- 207 image files". A callout points to this list, stating "Summary of selected images." Below this is a control for "Use every 1 image" with a spin box and a checked "Save result images" option. A callout explains: "Reduce the data by using every Nth image of image series." The "Image series time range:" section has two radio buttons: "All images" and "Time". The "Time" option is selected, showing "Start time" and "End time" fields with spinners and units (0 d 2 h and 0 d 7 h). A callout points to these fields: "Select time range from image series that will be processed." Below "Time" is the "Cycles" option with "First cycle" and "Last cycle" fields (1 and 12). A callout points to these fields: "Select imaging cycle range from image series that will be processed." At the bottom are buttons for "< Back", "Next >", and "Cancel". A separate callout on the left states: "The source data can be reduced by removing start or end of the image series."

## 7.2 Count and classify cells

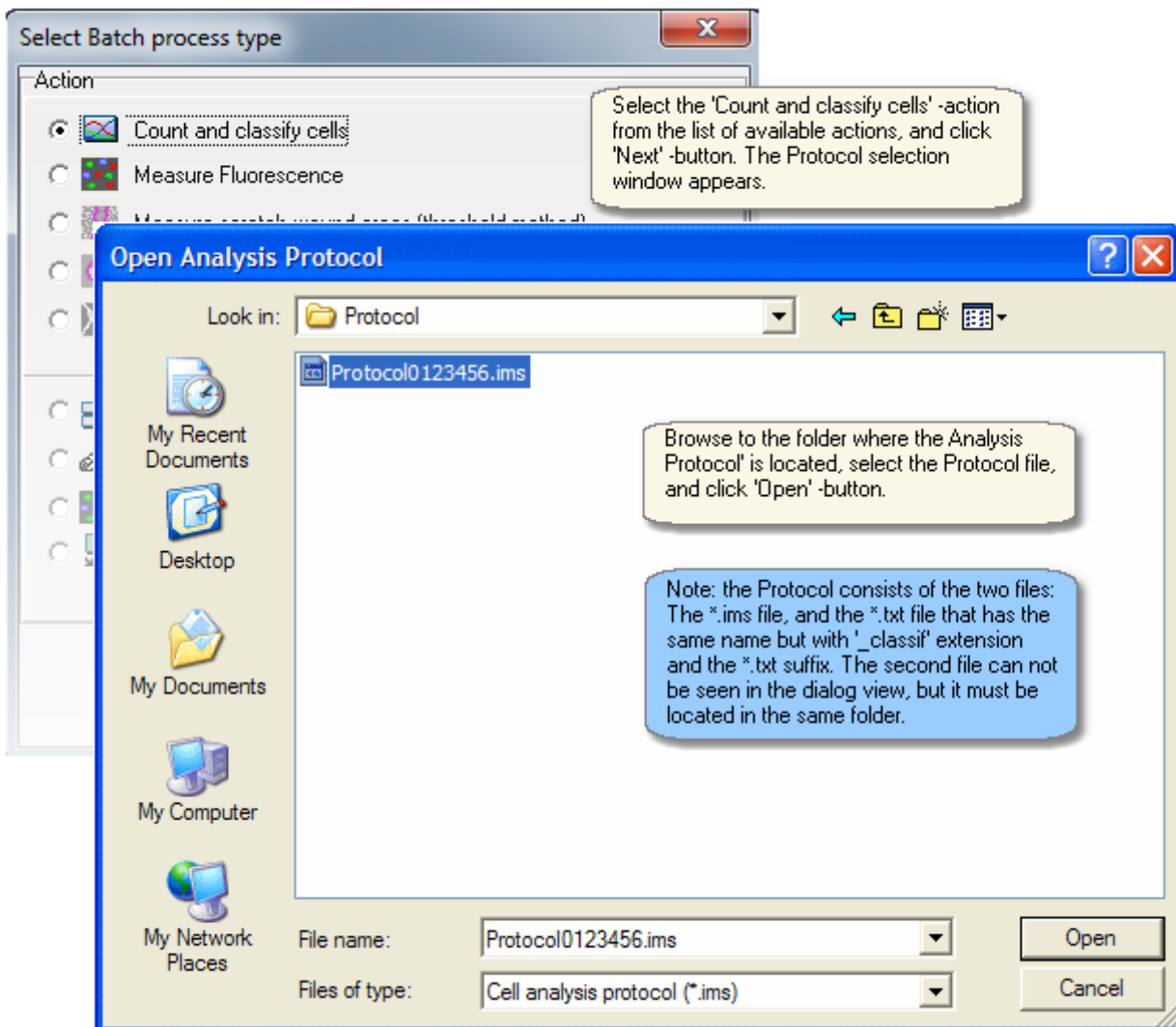
The cell counting and recognition tool counts cells and classifies them into the user defined classes.

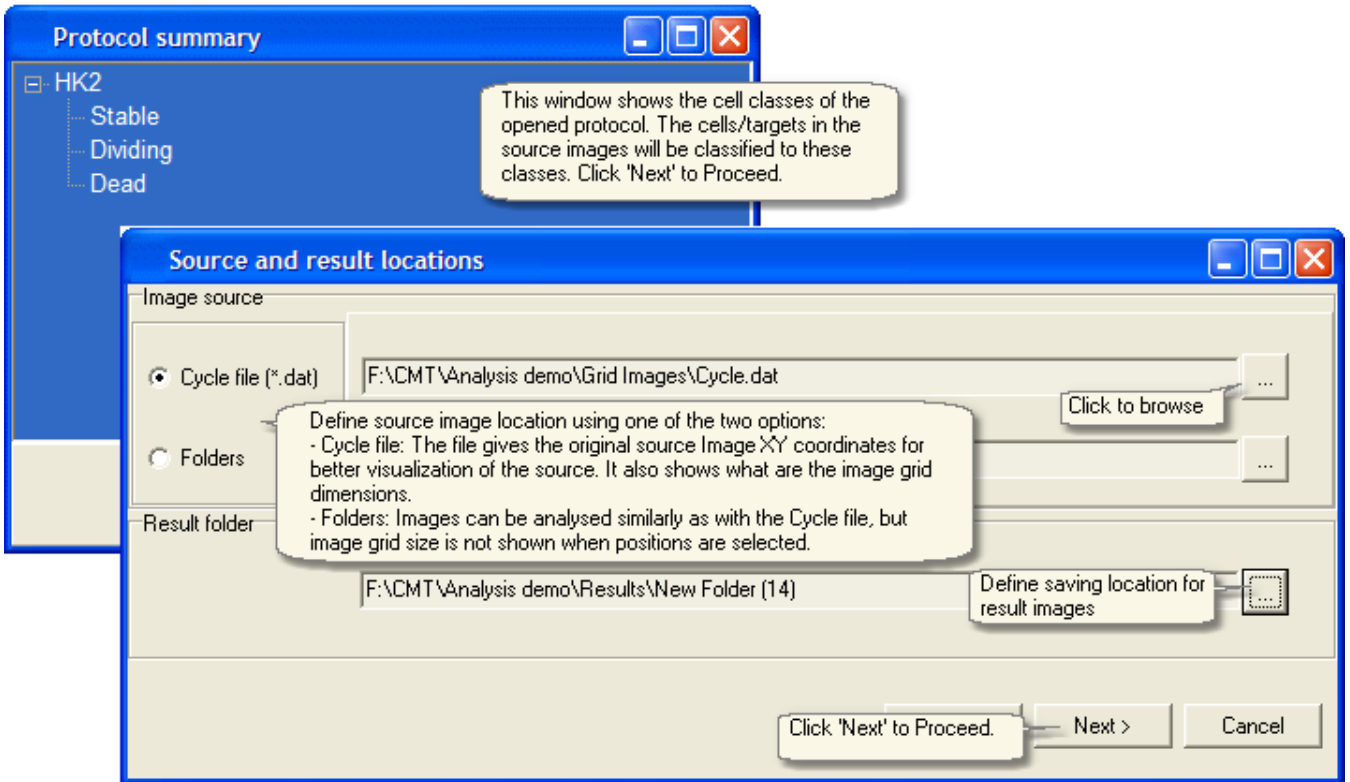
You will need to have the analysis protocol file before you start the Cell counting and recognition in the Batch Process tool. The analysis protocol defines the particular analysis task. See from the other topic [how the measurement parameters are selected and saved to protocol](#).

The Batch Process tool can be found from the menu of the Analyser main window: File -> Batch Process.

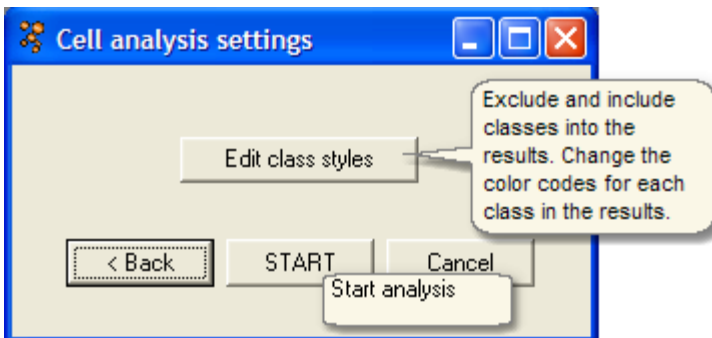


- In the start the analysis Protocol file must be selected, and then program continues to the source and result file selection.

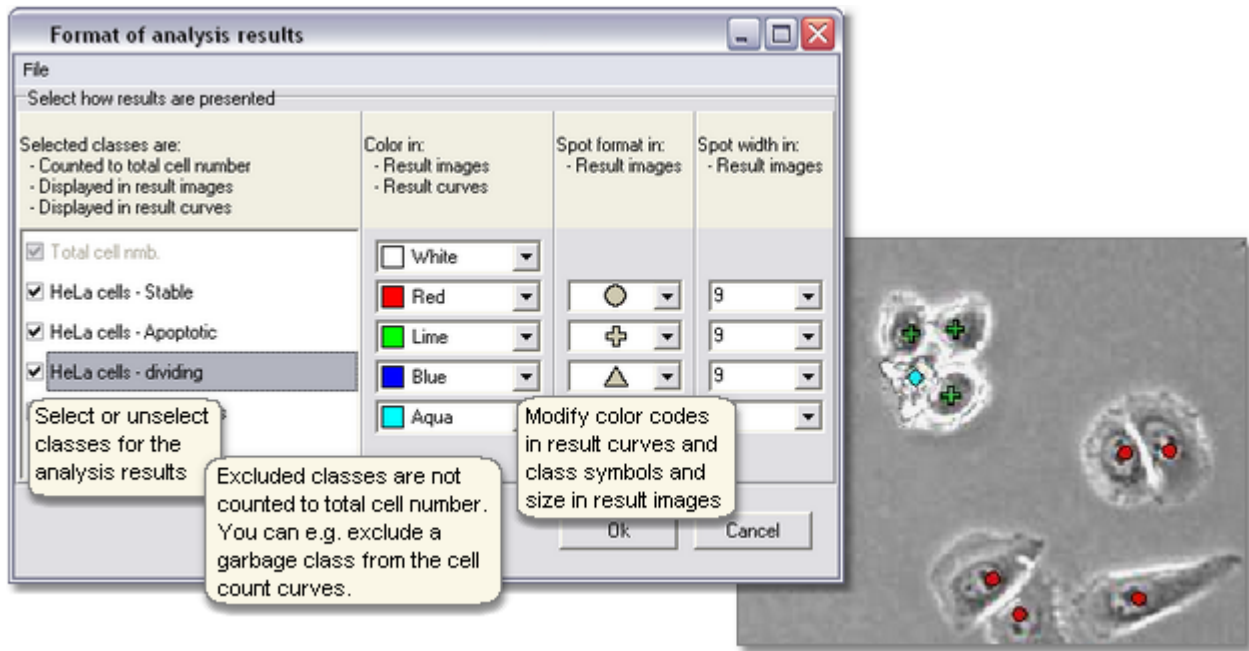




- Adjust class styles and start the analysis run.



Editing class styles

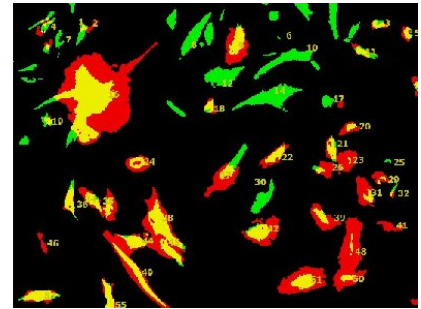


When image analysis finishes, the results are automatically displayed. Click this to go to topic '[Viewing results](#)'.

### 7.3 Measure fluorescence

Analyse images from single and multichannel fluorescence cell tests with the Fluorescence intensity measurement tool.

- Measurement is based on thresholds and selected parameters, and no cell sample image teaching is necessarily needed, but it can be utilized, as later is show.
- The fluorescence cell count, area and intensity are measured.
- The tool uses both the phase contrast and fluorescence images at the same time.



See from another topic [how the measurement parameters are selected and saved to protocol.](#)

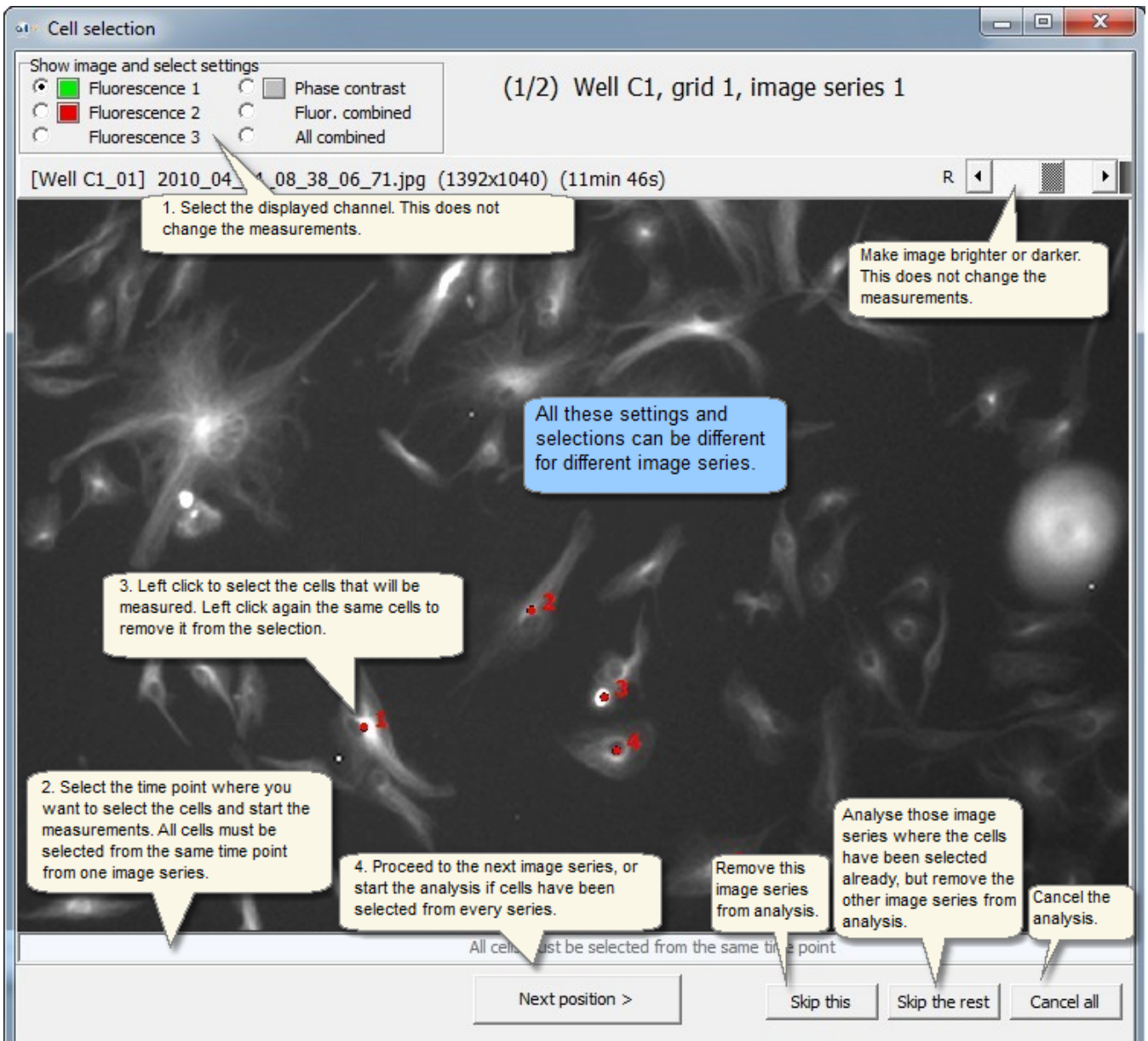
The Batch Process tool can be found from the menu of the Analyser main window: File -> Batch Process.

- Measurement parameters can be opened from Protocol file or selected on the go. After parameters are selected, click Start from the bottom of the window to run the analysis for the selected images.

The screenshot displays the 'Select Batch process type' dialog box with 'Measure Fluorescence' selected. The 'Fluorescence measurement' window is open, showing a list of image processing steps (1-9) and a 'Result' section with various output options. A 'Confirm' dialog box is overlaid on the main image, asking 'Read measurement parameters from Protocol file?' with 'Yes' and 'No' buttons. The background image shows cells with green outlines and yellow numbers, representing the measurement results.



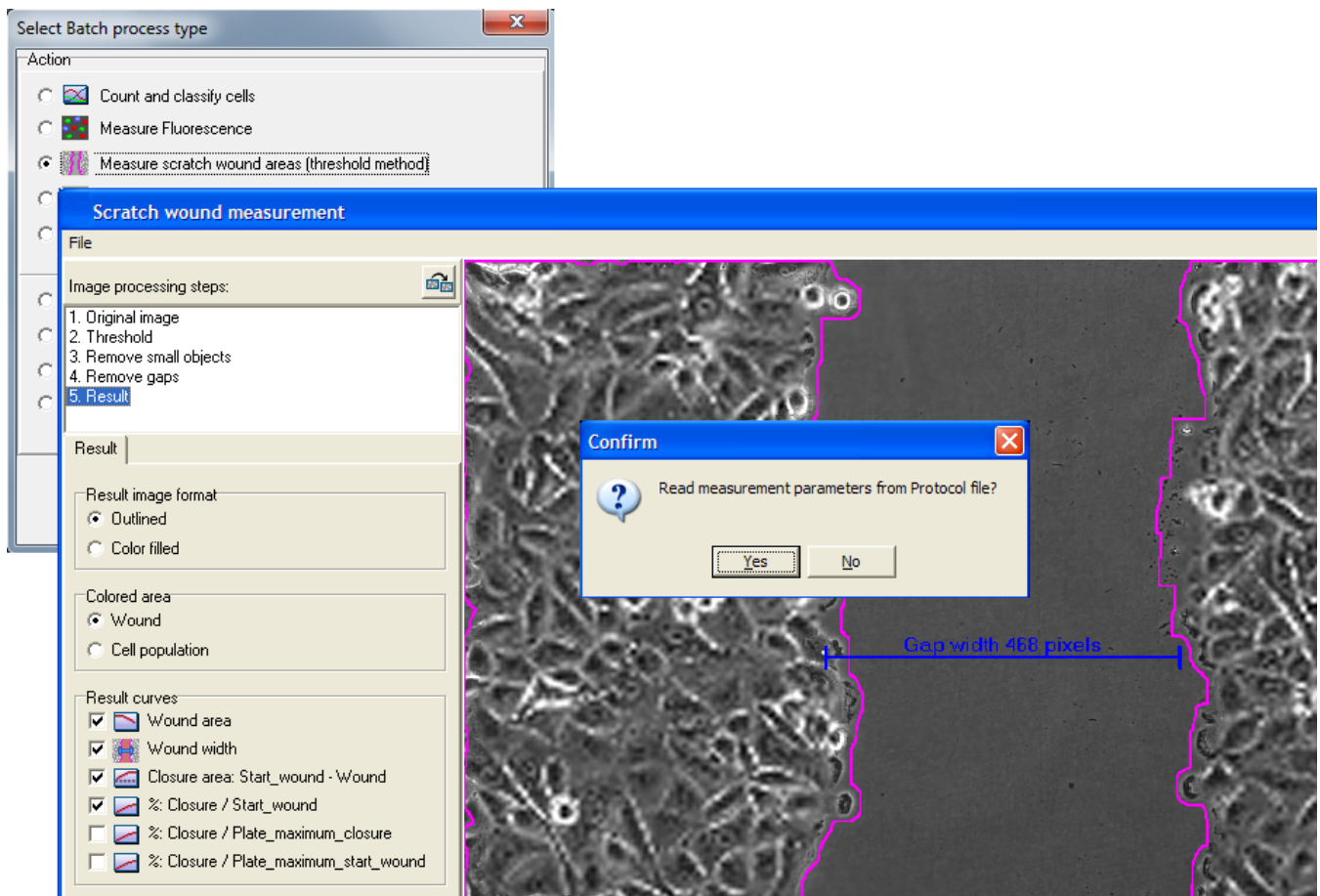
- If the manual cell selection was selected instead of the automatic, select the cells for measurement from each image series.



## 7.4 Measuring scratch wound areas

Measure scratch wound areas and closure speed. Measurement is based on thresholds and selected parameters, and no cell sample image teaching is needed. See from another topic [how Scratch wound parameters are selected](#) and [saved to protocol](#).

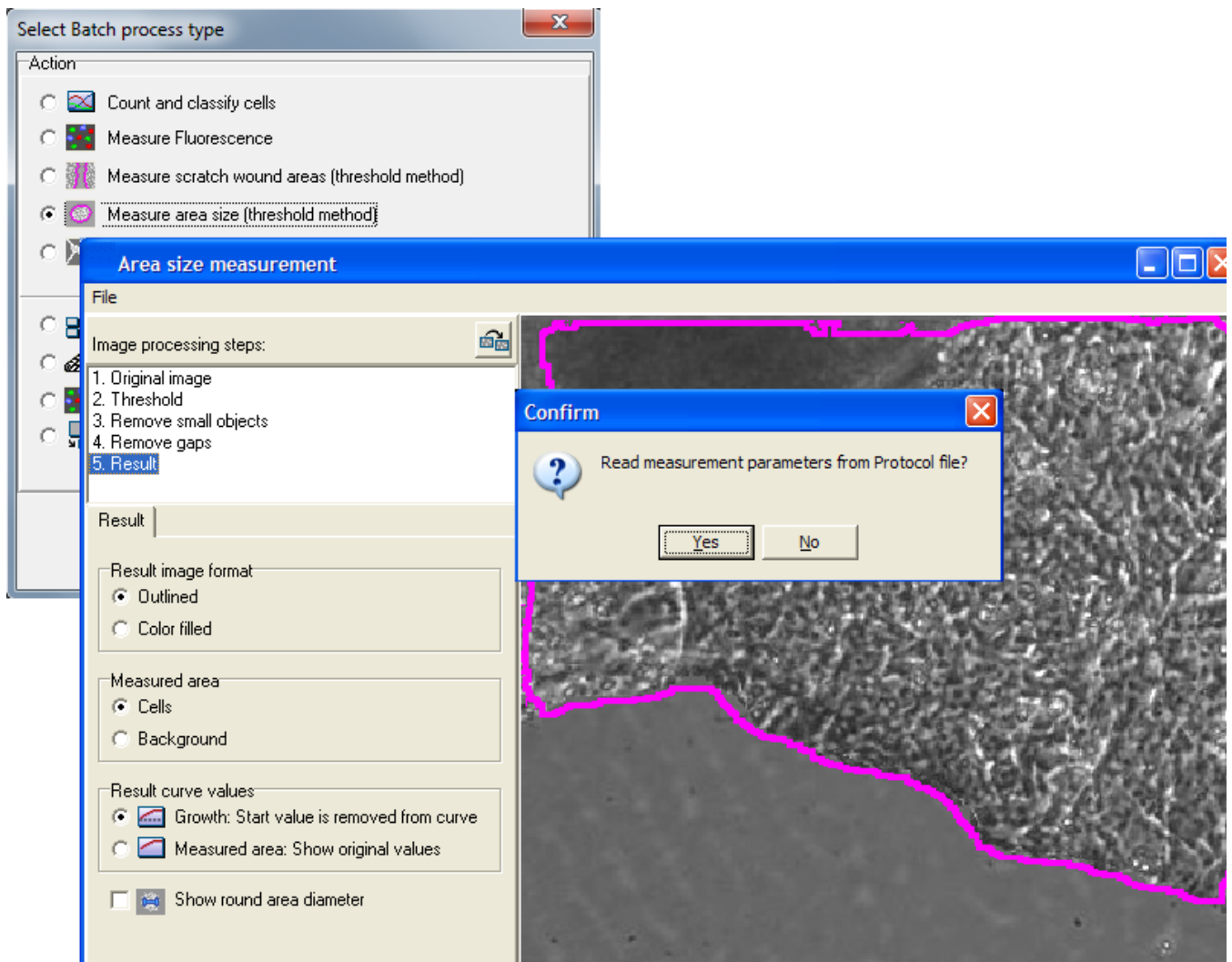
- The Batch Process tool can be found from the menu of the Analyser main window: File -> Batch Process.
- Measurement parameters can be opened from Protocol file or selected on the go. After parameters are selected, click Start from the bottom of the window to run the analysis for the selected images.



## 7.5 Measuring area sizes with threshold tool

Measure cell population areas and growth speed. Measurement is based on thresholds and selected parameters, and no cell sample image teaching is needed. See from another topic [how Area size measurement parameters are selected](#) and [saved to protocol](#).

- The Batch Process tool can be found from the menu of the Analyser main window: File -> Batch Process.
- Measurement parameters can be opened from Protocol file or selected on the go. After parameters are selected, click Start from the bottom of the window to run the analysis for the selected images.

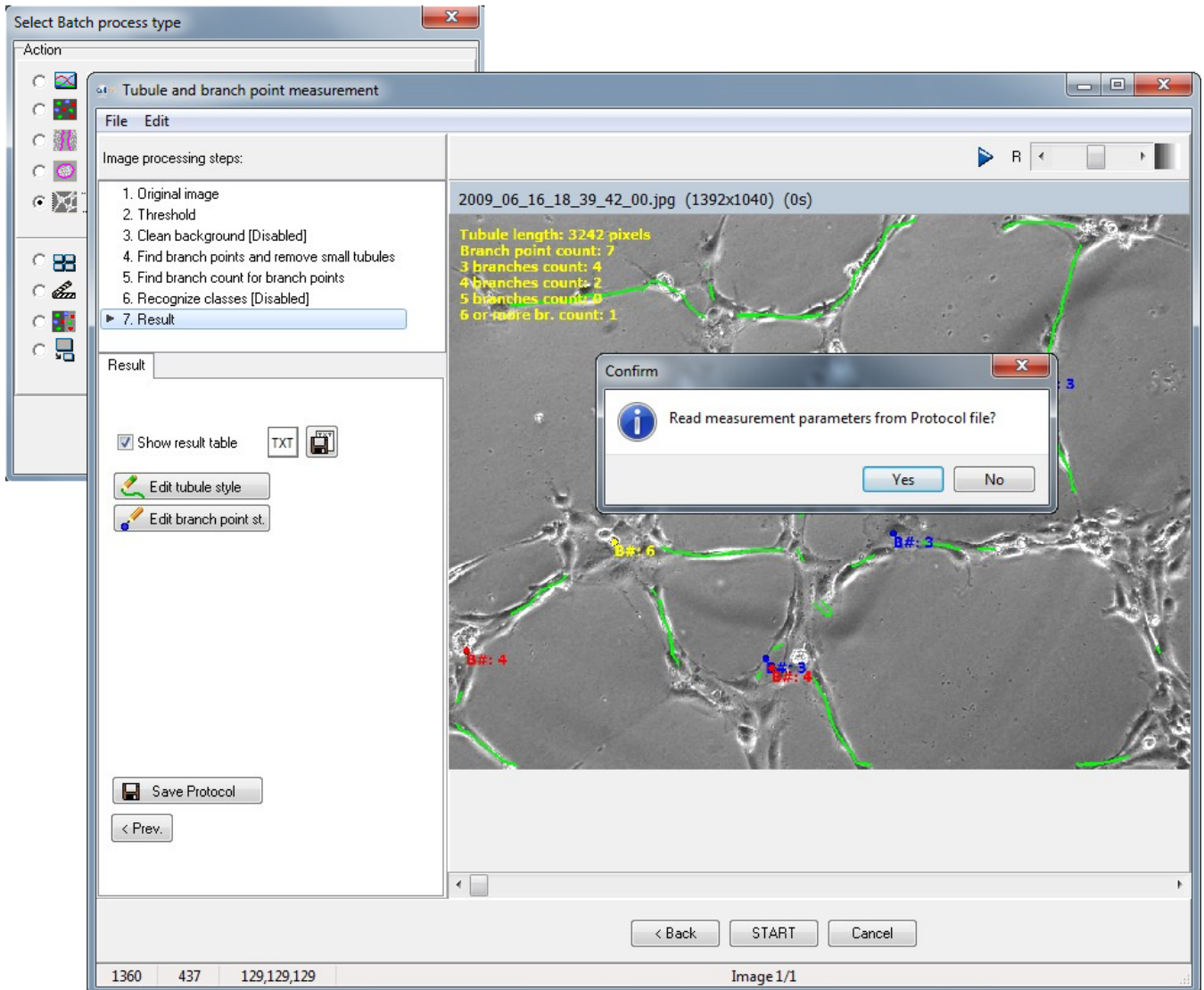


## 7.6 Measure tubules and branch points

Analyse angiogenesis images. The images should be normal phase contrast images. The tubule length and the number of different type branch points (different branch count) are measured. Measurement is based on thresholds and selected parameters, and no cell sample image teaching is necessarily needed, but it can be utilized. See from another topic [how the measurement parameters are selected](#) and [saved to protocol](#).

The Batch Process tool can be found from the menu of the Analyser main window: File -> Batch Process.

- Measurement parameters can be opened from Protocol file or selected on the go. After parameters are selected, click Start from the bottom of the window to run the analysis for the selected images.



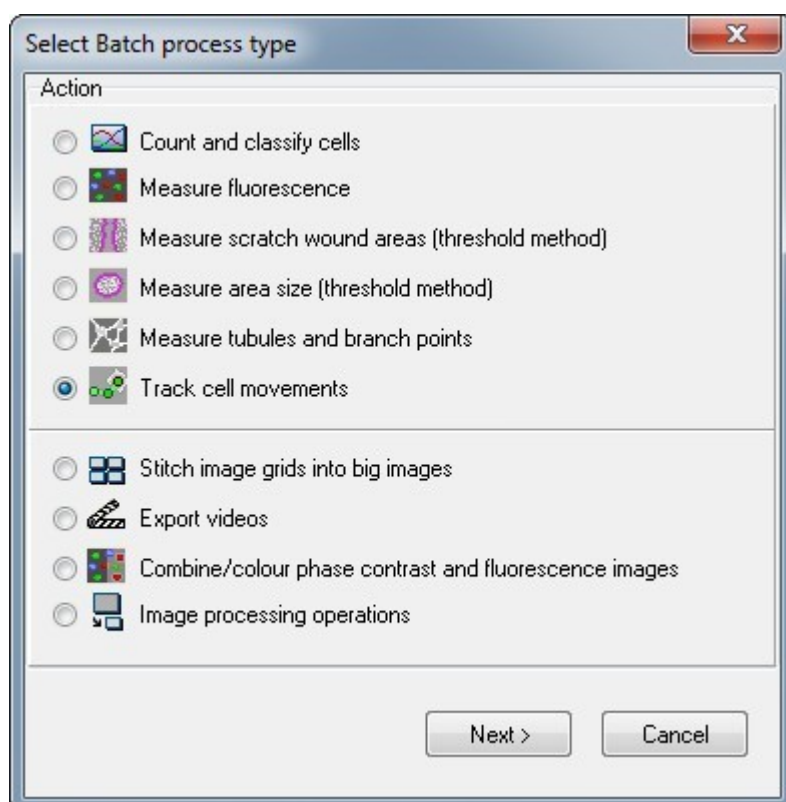
## 7.7 Cell movement tracking

Track cell movements automatically for the image series with this tool. The images should be normal phase contrast images. Movement trajectory images and rose charts etc. statistics are generated. Cell Finder cell recognition or cell counting Protocol is required before this tool is started.

The Batch Process tool can be found from the menu of the Analyser main window: File -> Batch Process.

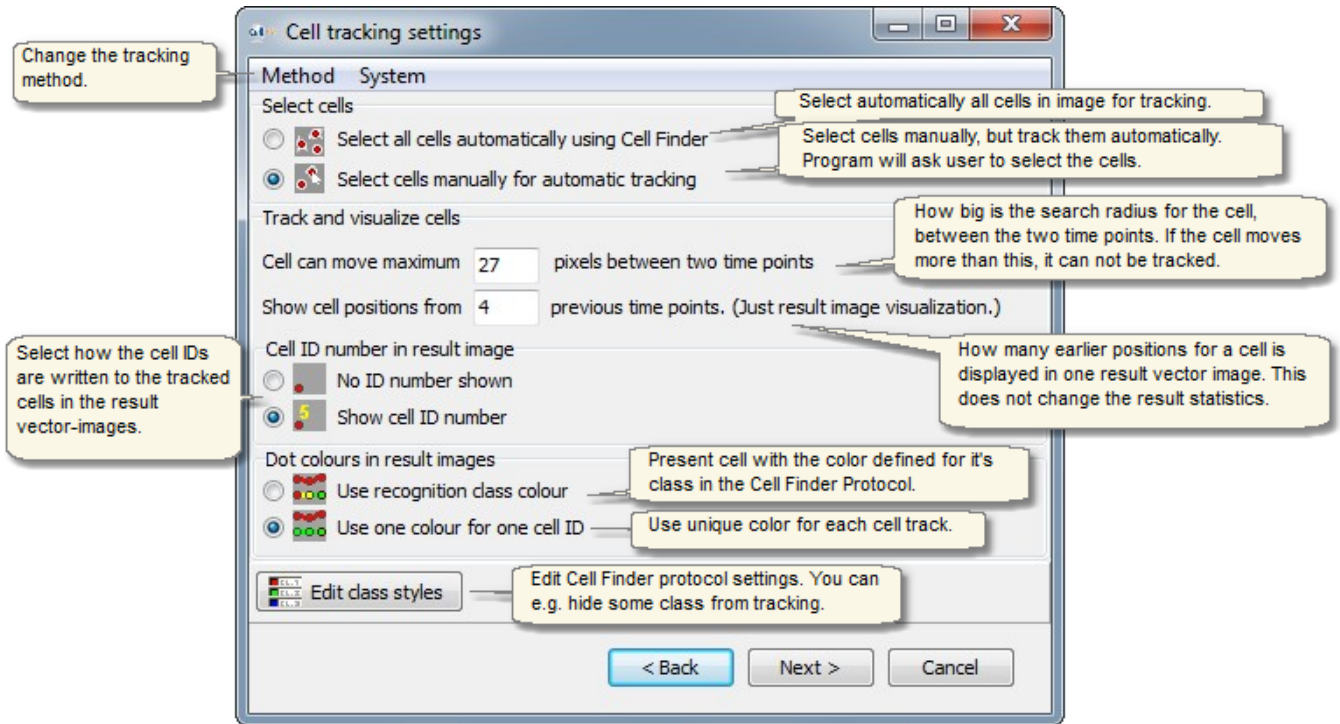
### Run Cell movement tracking

- Select Track cell movements and click Next button.

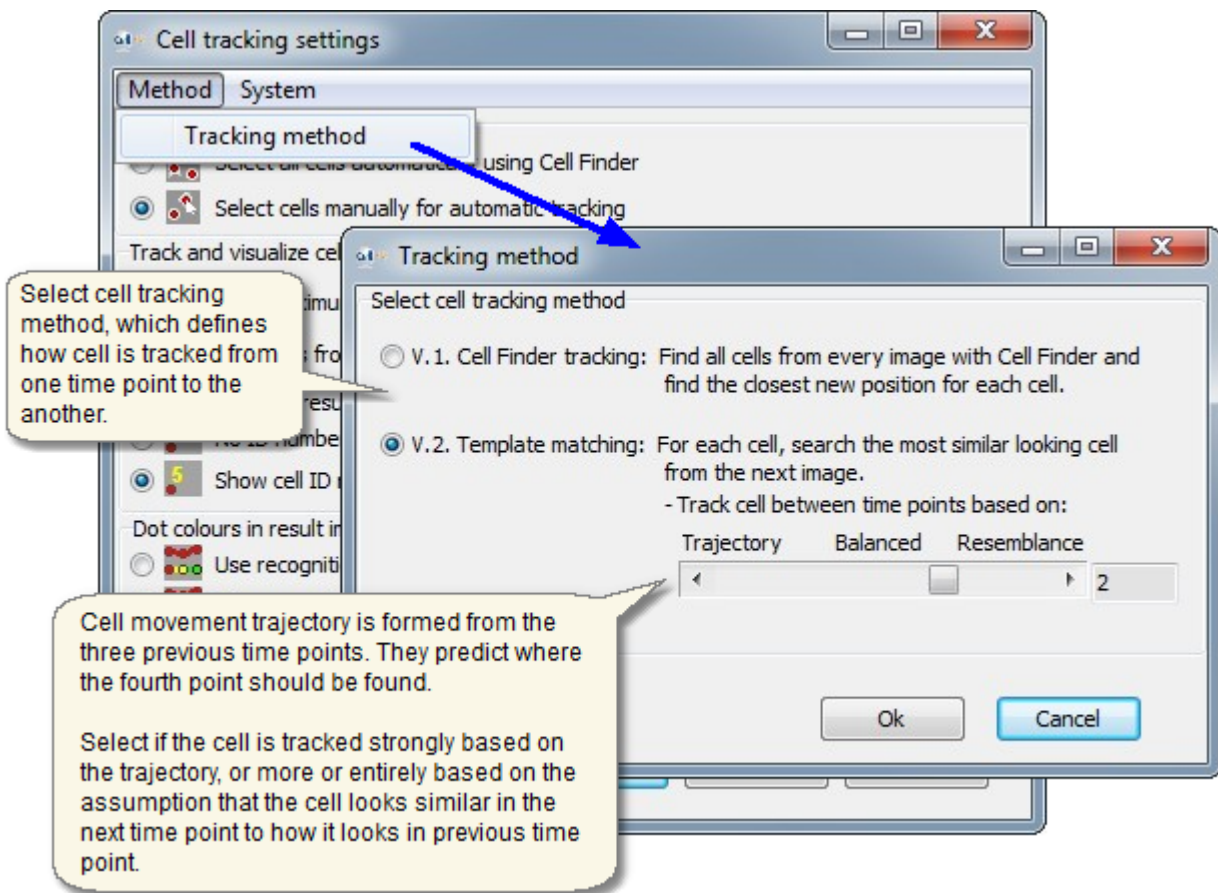


- Select pre-saved Cell Finder Protocol, and go through the source image selection steps.
- In the source file selection, select the **\*.dat file** instead of just the source folder, so that cell travel in the results will be presented **in micrometers instead of pixels**. Select also the folder where the result data will be saved.

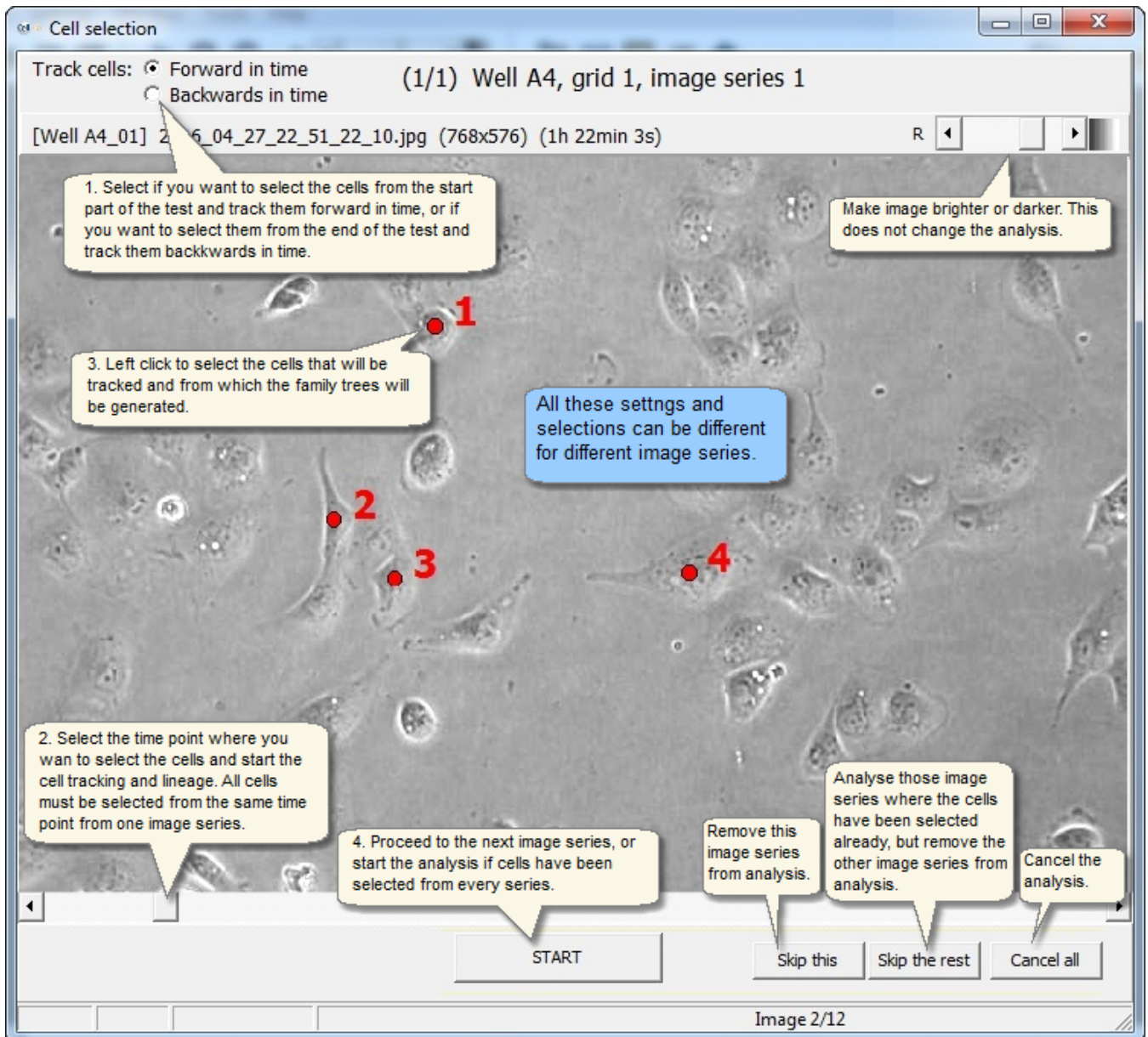
- Select the cell tracking and tracking and result settings.



Tracking method. There are two methods available. The v.2. is more recently developed and has better performance in most cases.



- If the manual cell selection was selected instead of the automatic, select the cells for measurement from each image series.



## Cell movement tracking Results

When tracking finishes, the [Cell Finder result curve window](#) and the tracking result window (see image below) open.

- Select results from different image series to display from the plate map and position list in the right hand side of the image.
- The "Trajectory" sheet shows the entire journey the selected cells have traveled in the entire time series.

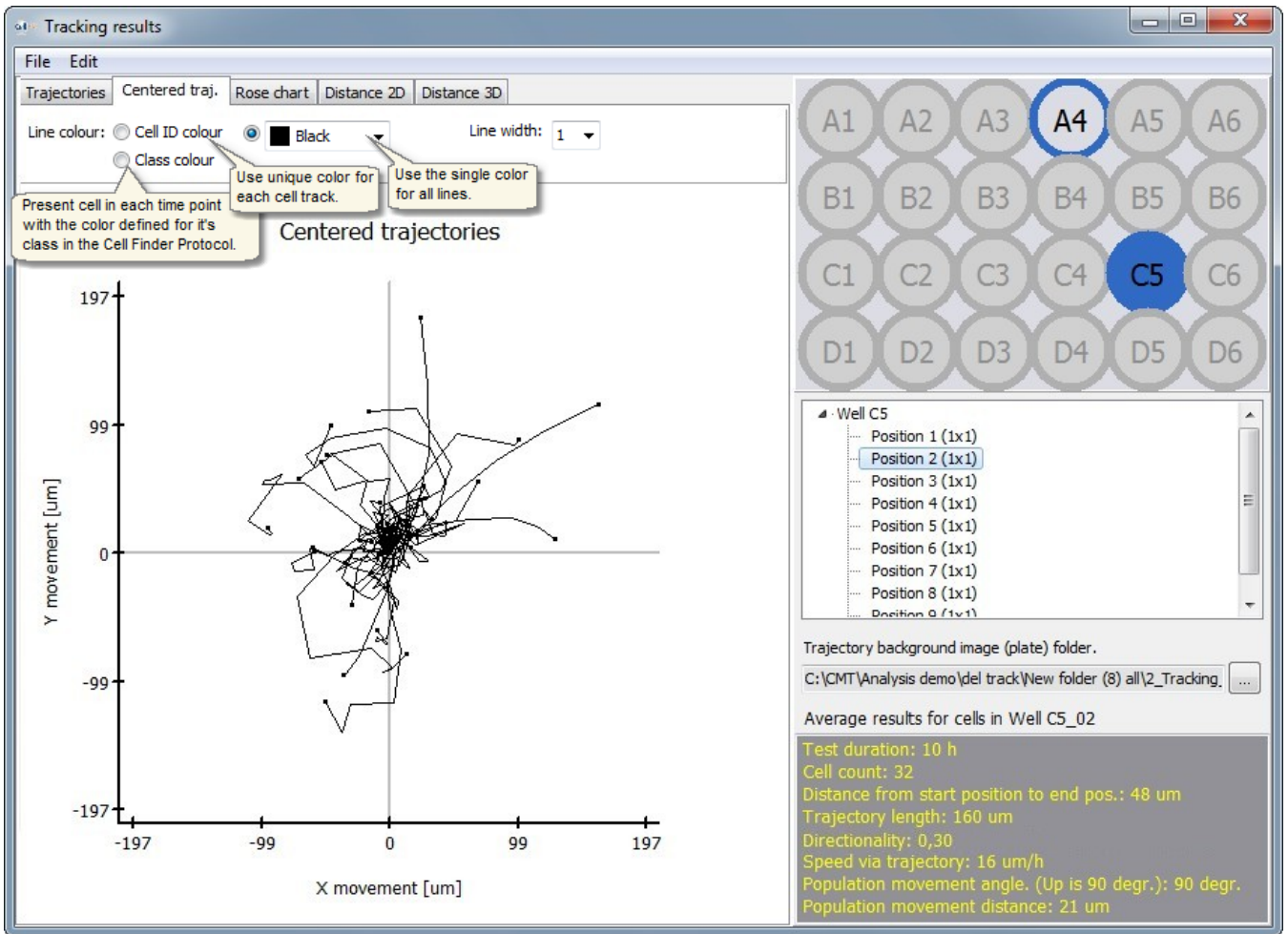
The screenshot displays the 'Tracking results' window. At the top, there are tabs for 'Centered traj.', 'Rose chart', 'Distance 2D', and 'Distance 3D'. Below these are settings for 'Line colour' (Cell ID colour selected) and 'Line width' (2). A grid of wells (A1-A6, B1-B6, C1-C6, D1-D6) is shown on the right, with A4 and C5 highlighted. A summary table for 'Well A4' provides the following data:

Average results for cells in Well A4_01	
Test duration:	11 h
Cell count:	4
Distance from start position to end pos.:	54 um
Trajectory length:	179 um
Directionality:	0,30
Speed via trajectory:	16 um/h
Population movement angle. (Up is 90 degr.):	153 degr.
Population movement distance:	13 um

Below the table are options to 'Exclude cell from average results if it...' with checkboxes for 'has < 5 time points' and 'moved < 30 um distance'.

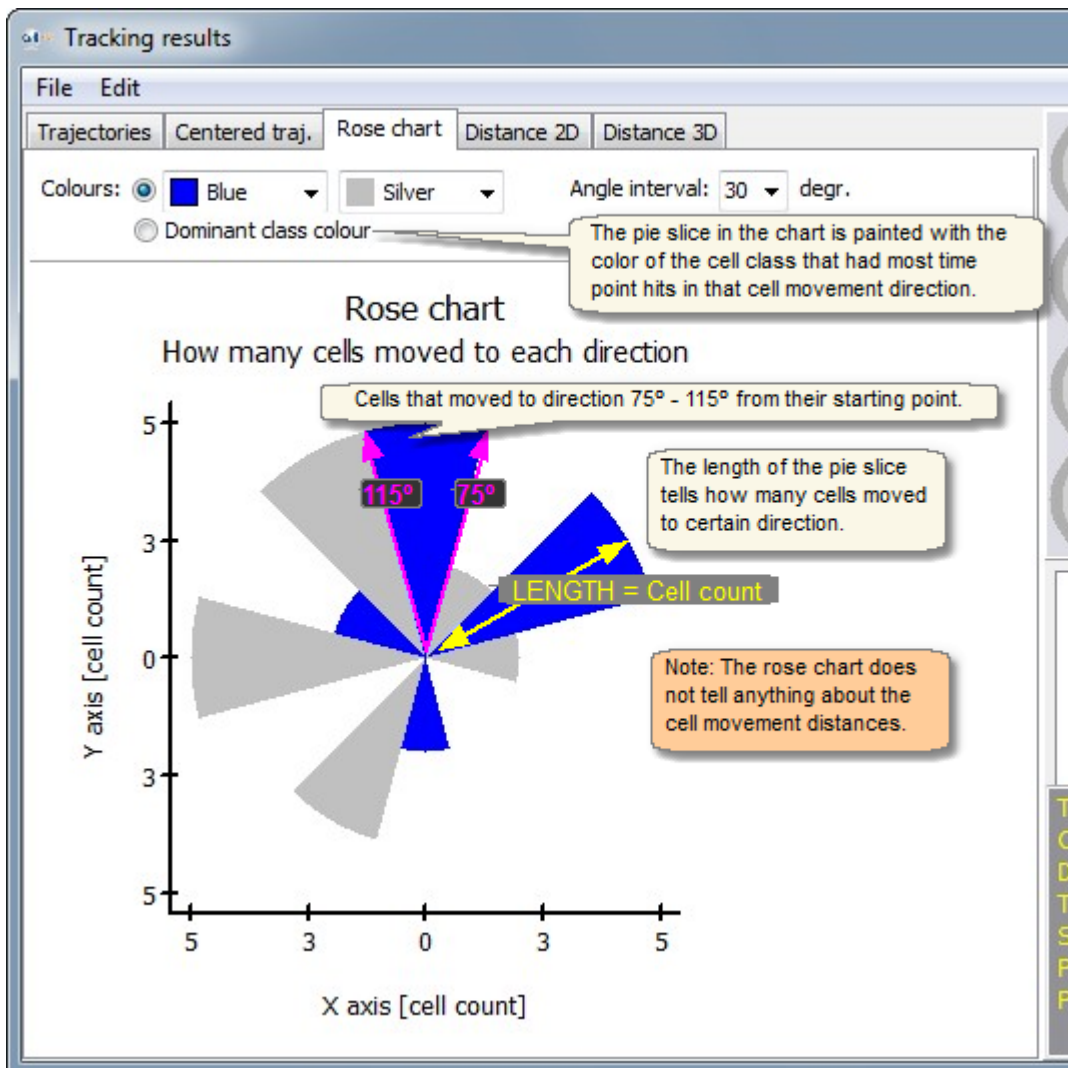
- Also the "Centered trajectory" sheet shows the entire journey the selected cells have traveled in the entire time series, but here the starting point of each cell is shifted to the center of the image.



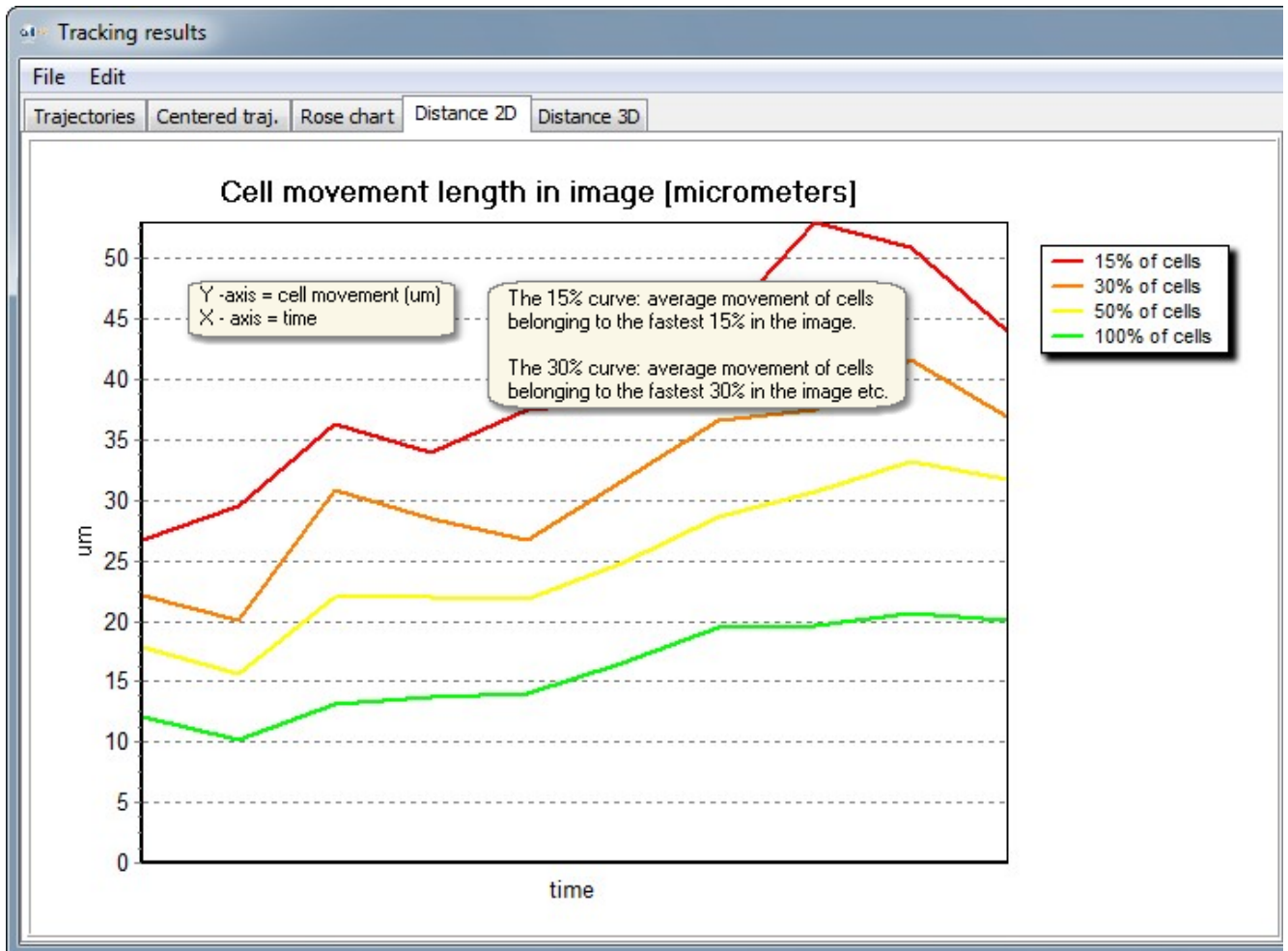


- The Rose chart sheet shows the direction where the cells moved from start point to the end point in the entire time series.

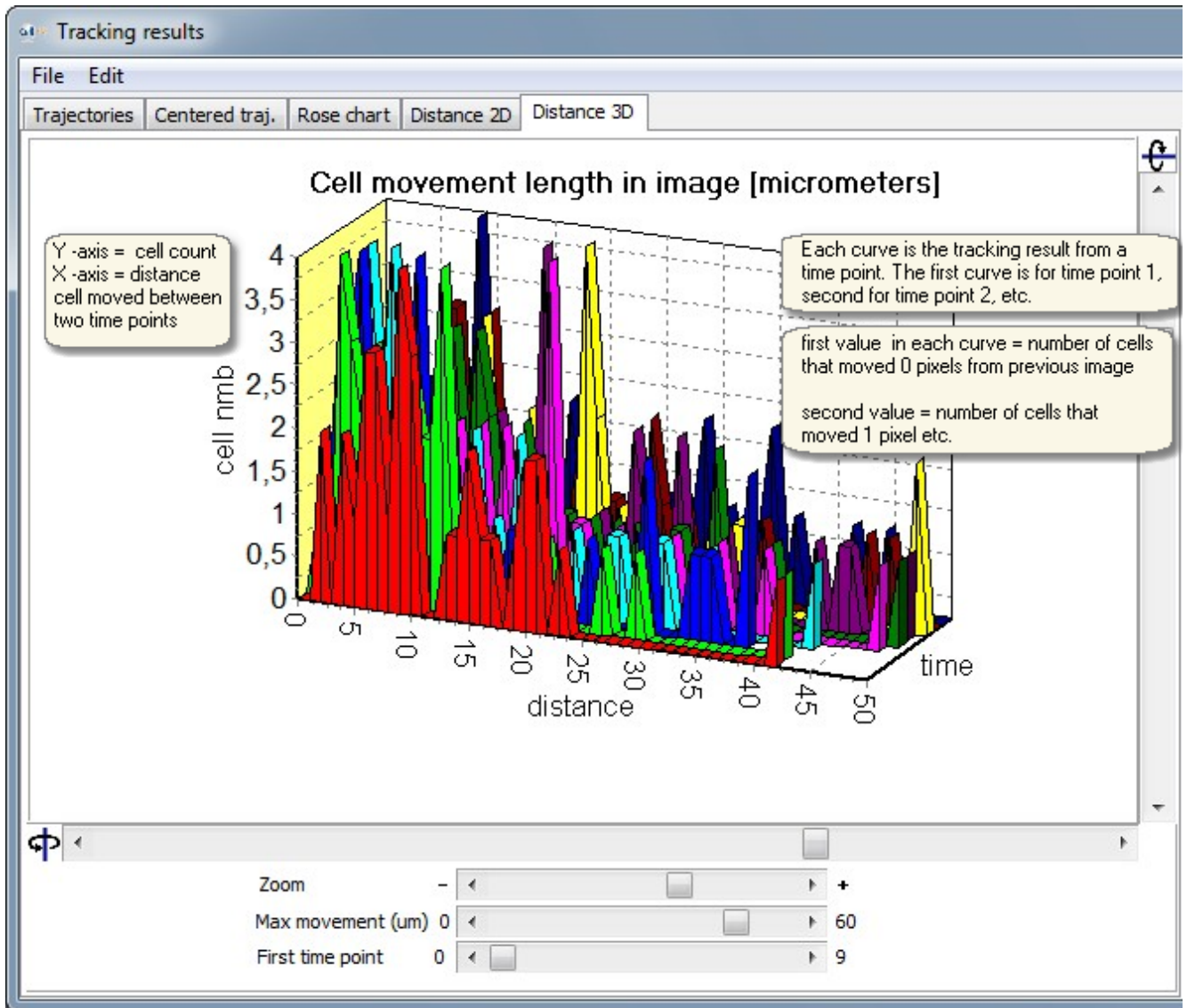
\*Note: The rose chart does not tell anything about the cell movement distances, only about the movement directions.



- The 2D distance graph shows the average movement distance of the fastest cells at each time point



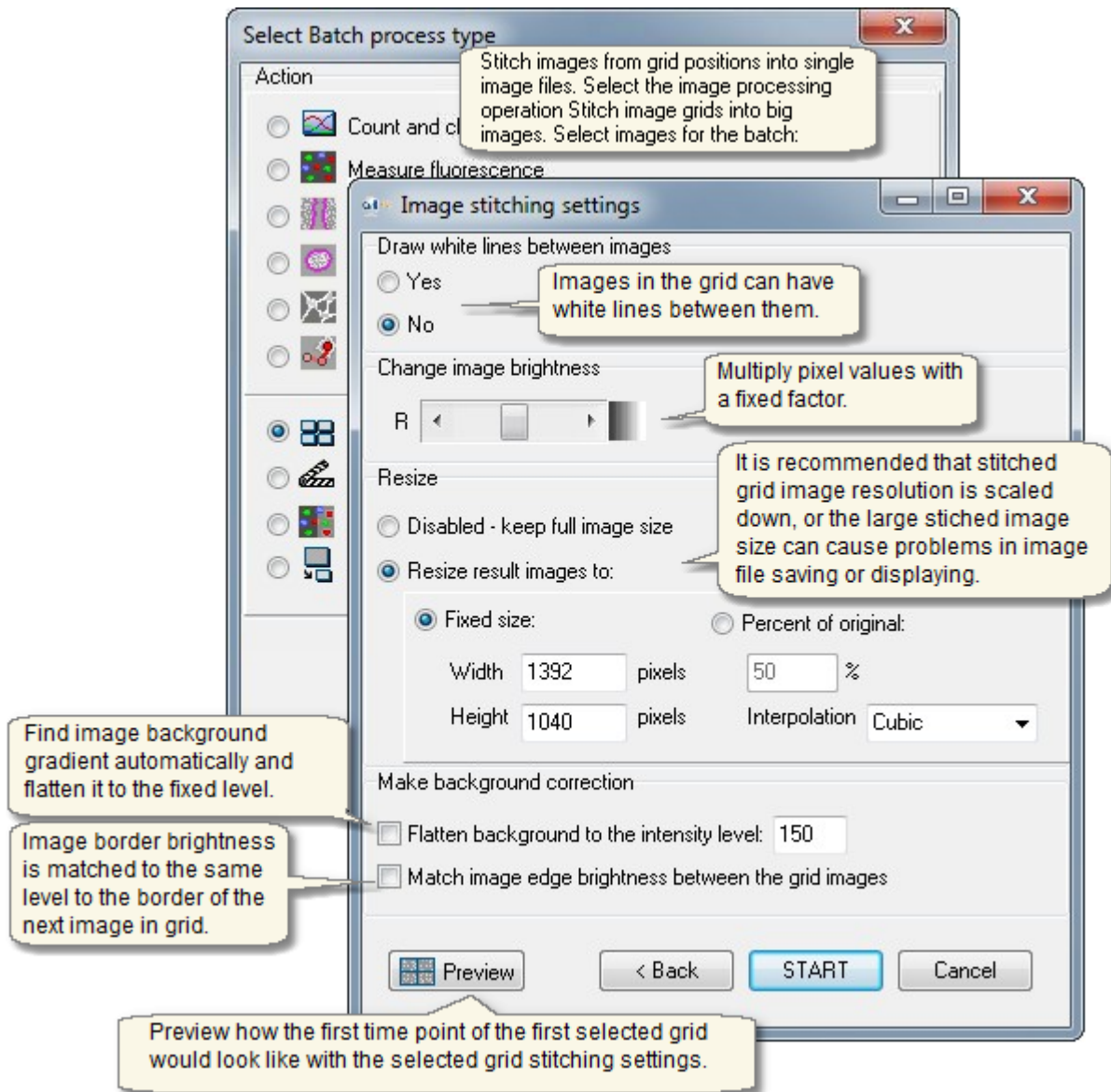
- The 3D distance graph: Each entire curve is the tracking result from one time point.



- The result \*.xml file is saved automatically for each image series to the "2\_tracking\_results" folder. The first three rows present average result for the image series, and the latter rows have detailed data of each cell.

Average results for image series:							
Cell count	Test duration [h]	Distance from start position to end pos. [um]	Trajectory length [um]	Directionality	Speed via trajectory [um/h]	Population movement angle [degr.]. (To right is 0, up is 90 degr.)	Population movement distance [um]
49	11	54	179	0.3	16.1	153	13
Results for each cell:							
Cell ID	Time from start position to end pos. [h]	Distance from start position to end pos. [um]	Trajectory length [um]	Directionality	Speed via trajectory [um/h]	Movement angle [degr.]. (To right is 0, up is 90 degr.)	
1	11	13	149	0.09	13.4	221	
2	11	93	210	0.44	18.9	25	
3	11	81	266	0.3	23.9	140	
4	11	9	149	0.06	13.4	104	
5	11	82	200	0.41	18	274	
6	11	95	255	0.37	22.9	207	
7	11	20	52	0.38	17	86	

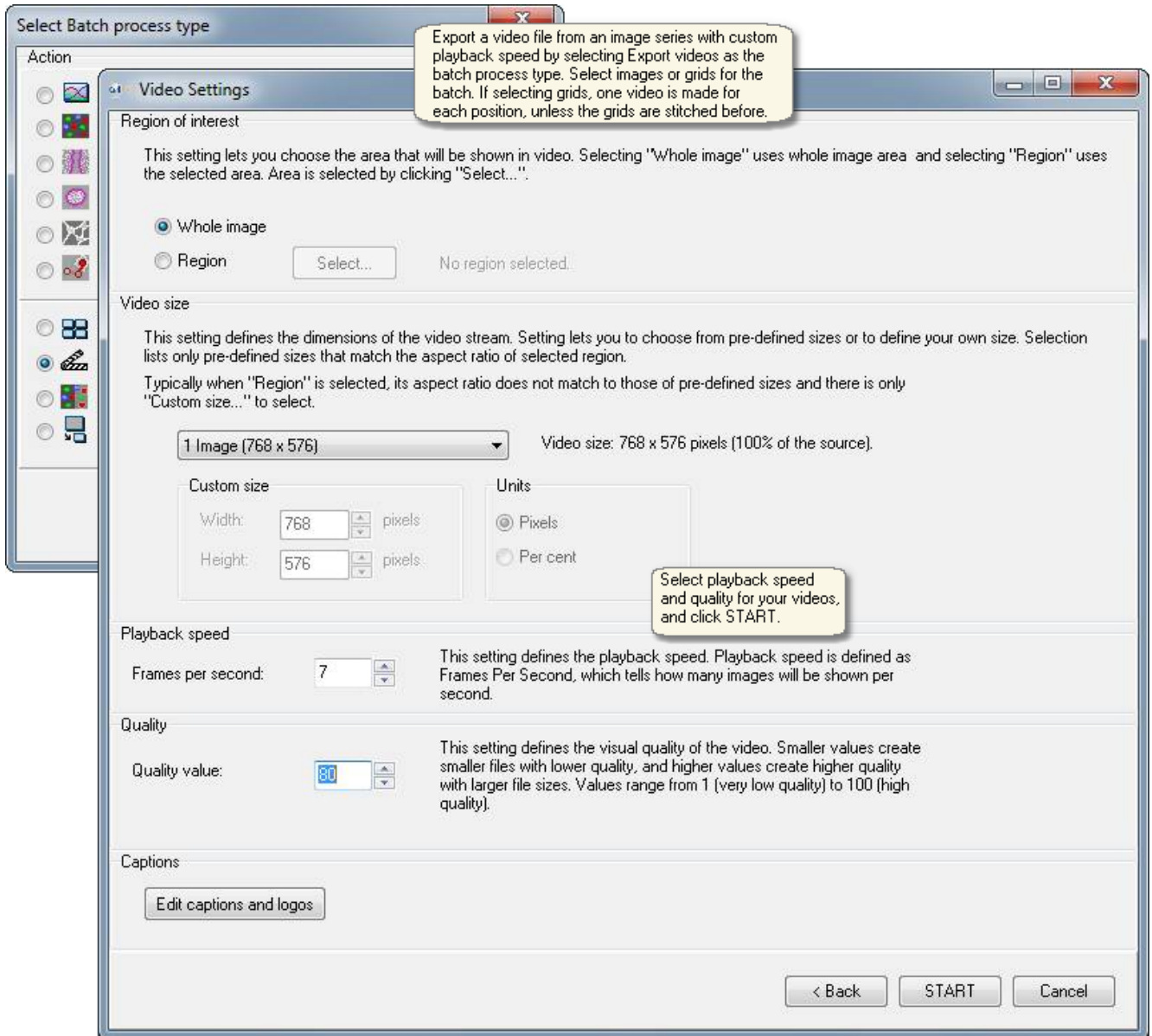
## 7.8 Stitching grids



## 7.9 Exporting videos

Select the playback speed and the quality for the videos. To add the caption texts and time stamps in the videos click the **Captions and logos** button, see more in [Movie Maker](#).

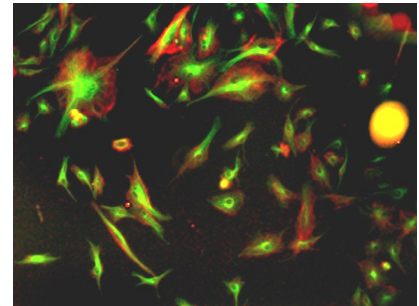
- View cell videos with any media player application that supports the .wmv file format.



## 7.10 Combine and colour images

Combine and colour the images from single and multichannel fluorescence cell tests with the Fluorescence image combining and colouring tool.

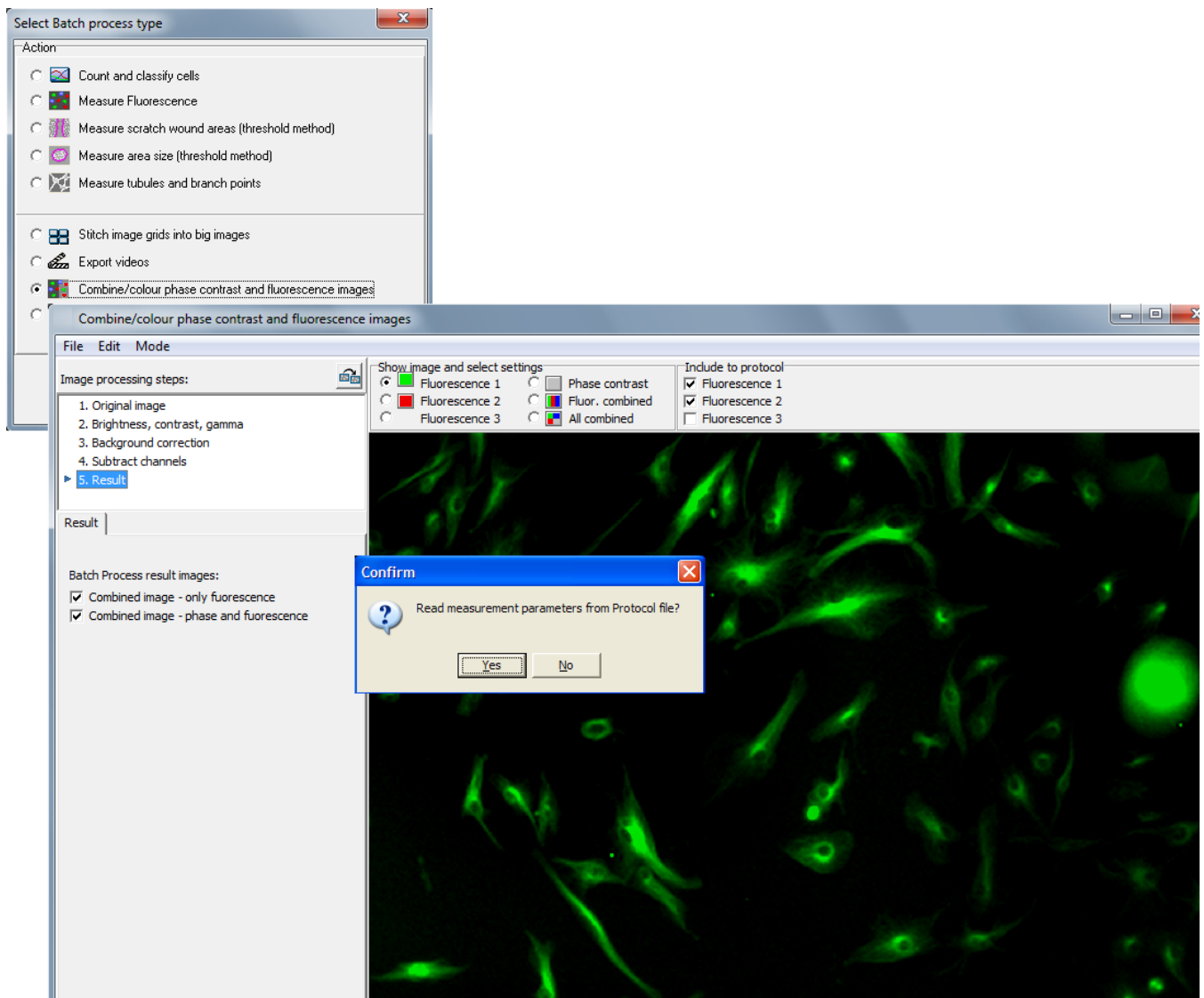
- No measurement result values are produced, just the combined and coloured result images.
- The tool uses both the phase contrast and fluorescence images at the same time.



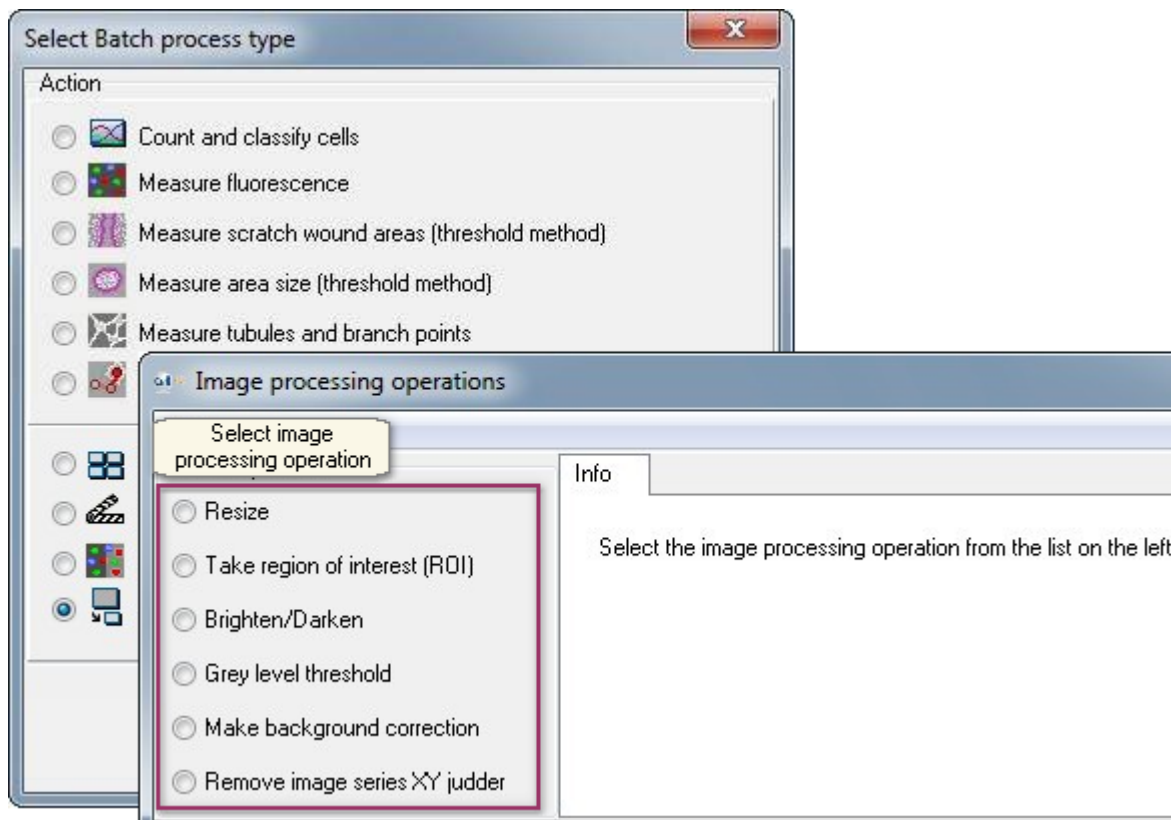
See from another topic [how the measurement parameters are selected and saved to protocol](#).

The Batch Process tool can be found from the menu of the Analyser main window: File -> Batch Process.

- Measurement parameters can be opened from Protocol file or selected on the go. After parameters are selected, click Start from the bottom of the window to run the analysis for the selected images.



## 7.11 Image processing operations

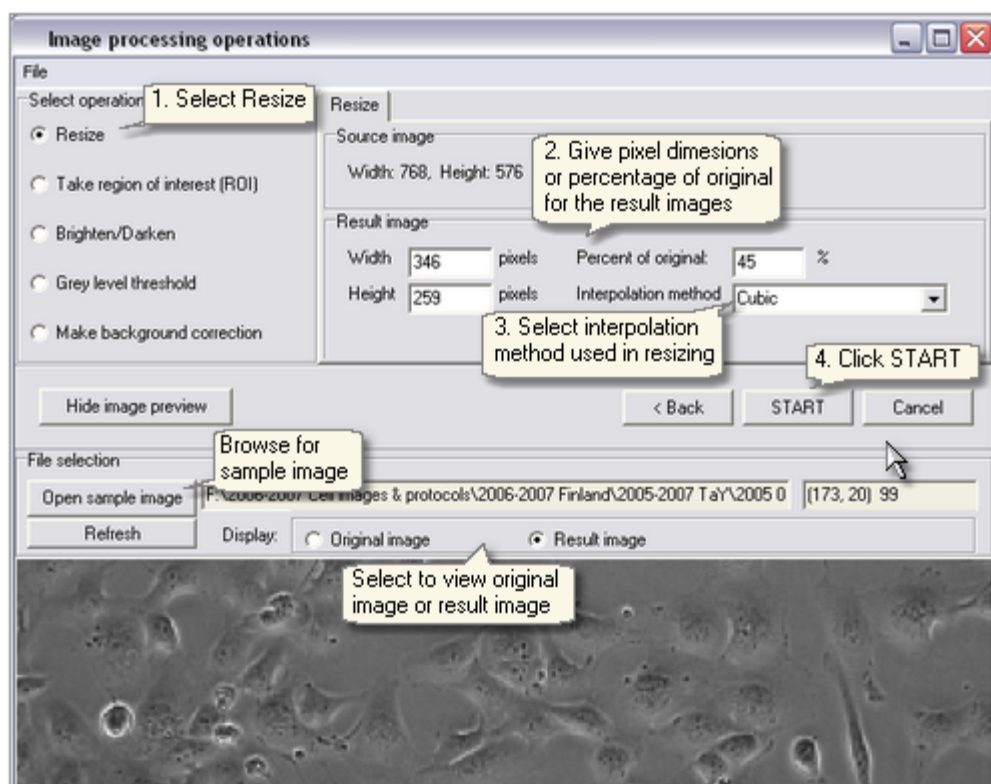




## 7.11.1 Resize

### Change the pixel dimensions of images

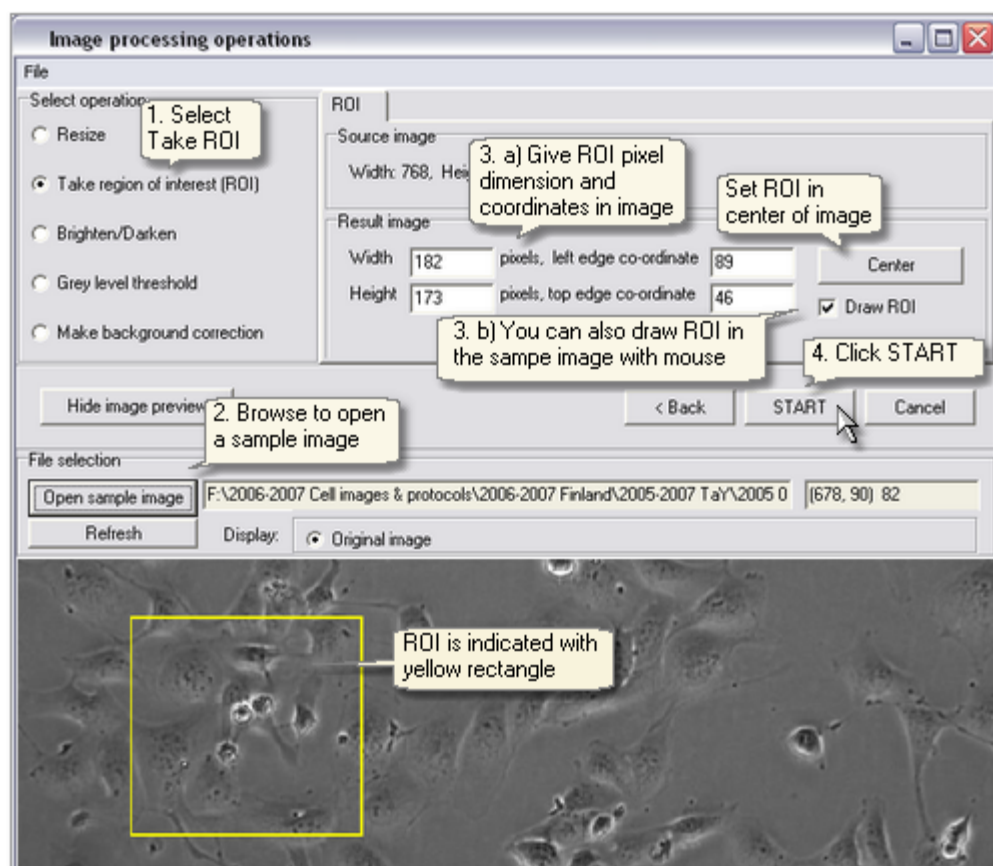
1. Select **Resize**
2. Define the dimensions of the resized images. Fill in the dimensions in pixels or give a percentage of the original. Click **Show image preview** to preview a resized image.
3. Select the interpolation method from the drop menu. You can compare the original image and the result with different interpolation method in the preview window by switching from **Original image** to **Result image**.
4. Click **Next**.



## 7.11.2 Region of interest

### Take a custom size region from images

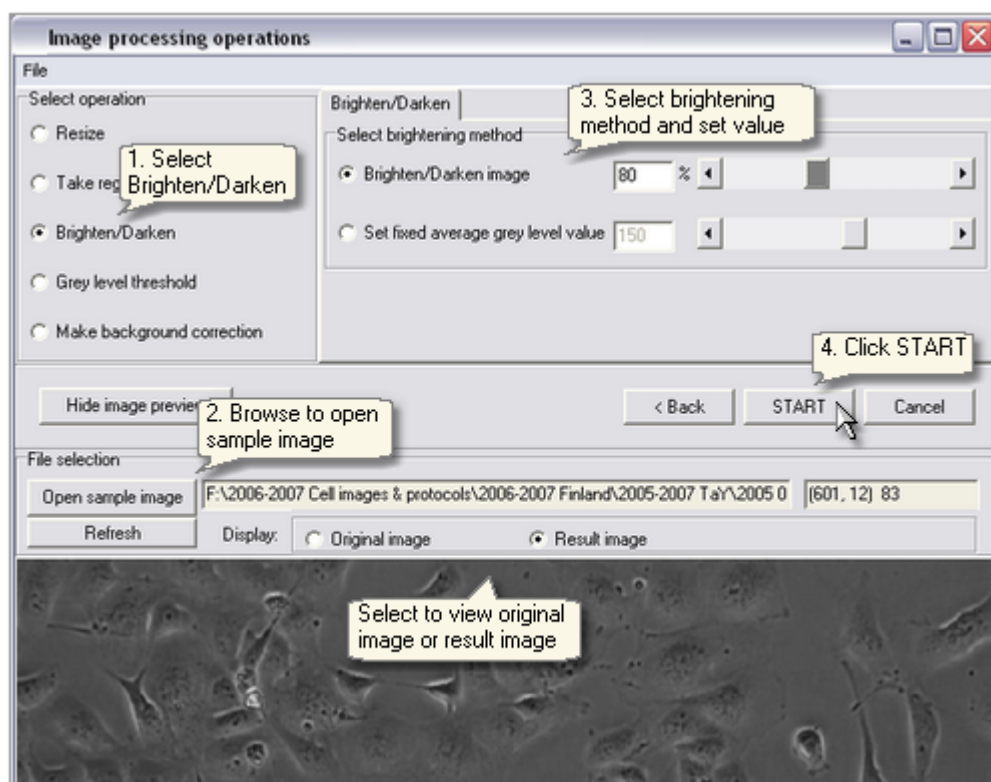
1. Select **Take region of interest (ROI)**
2. Click **Show image preview** and open sample image to preview ROI, which is indicated with a white dotted rectangle in the preview image.
3. Define the dimensions of ROI and give the coordinates in the image, or select **Draw ROI** and draw it with mouse on the preview image. Click the **Center** button to set the ROI to the center of the image.
4. Click **Next**.



### 7.11.3 Brighten/Darken

#### Brighten/darken: Change the brightness of images

1. Select **Brighten/Darken**
2. Click **Show image preview** and open a sample image to preview image brightness. You can compare the original and the result image in the preview window by switching from **Original image** to **Result image**.
3. Select brightening method. Adjust brightness by percentage by selecting **Brighten/Darken image** image or select **Fixed average gray level value** and fill in the desired value.
4. Click **Next**.



## 7.11.4 Grey level threshold

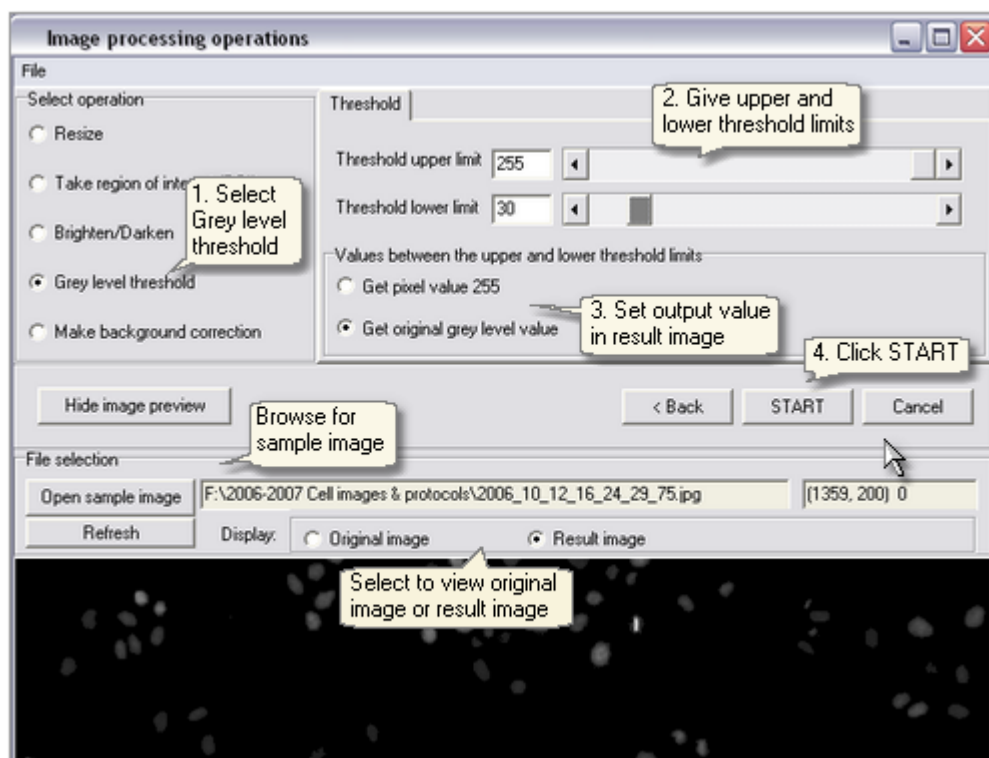
### Limit the grey level range or produce a binary image

1. Select **Grey level threshold**

2. The upper and lower threshold limits are set between 0-255 and pixels that have values outside these limits are set to zero. Click **Show image preview** and browse for a sample image to compare the original image with the result image.

3. The grey value between these limits in the output image can be set to 255 (binary image) or the original grey level can be maintained.

4. Click **Next**.



## 7.11.5 Background correction

### Background correction: Even out background trend in image

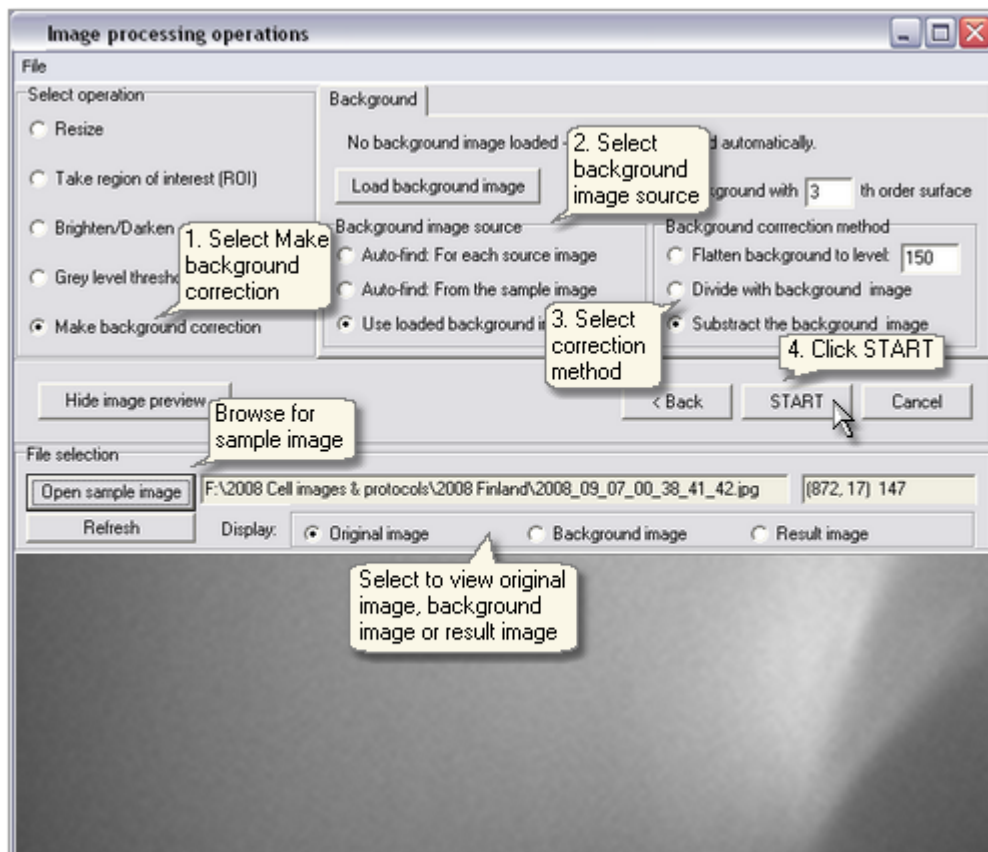
1. Select **Make background correction**
2. Select background image source
3. Select the correction method.
4. Click **Next**.

### Selecting background image source:

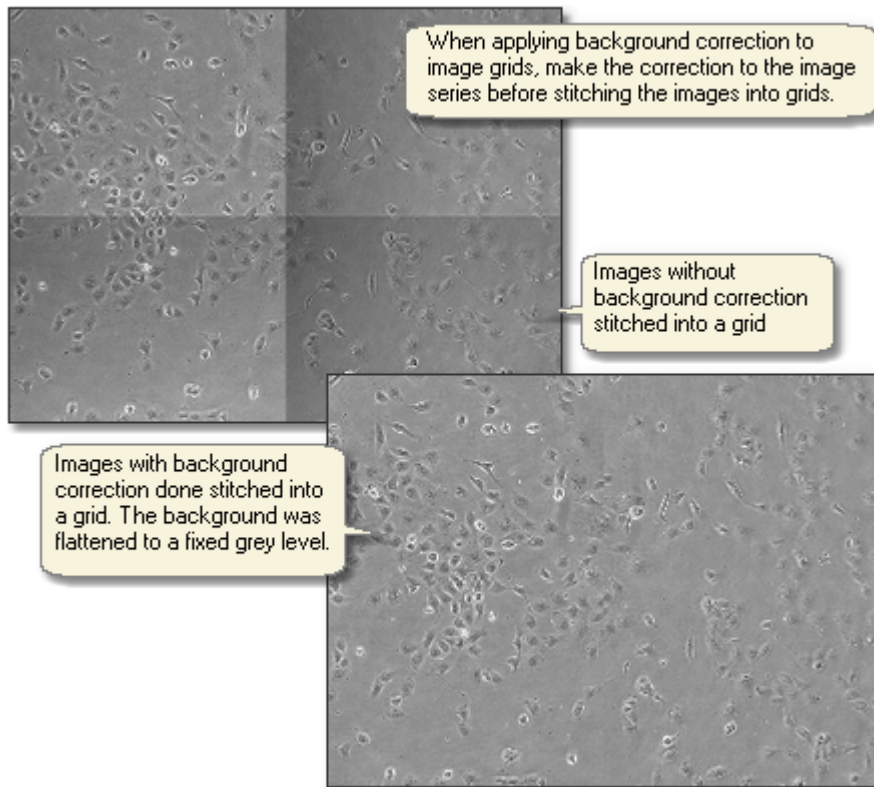
- Auto-find for each source image
- Auto-find from the sample image to be used to correct all images. Click **Show image preview** and **Open sample image** to define the sample image. You can choose to view the sample image, the background image or the result image in the preview window.
- Use loaded background image. Click **Load background image** and open the file.

### Select one of the background correction methods:

- Flatten to a certain gray level. The image is first divided with background image and then multiplied to a fixed grey level.
- Divide with background image
- Subtract the background image



## Example: Background corrected images stitched to a grid



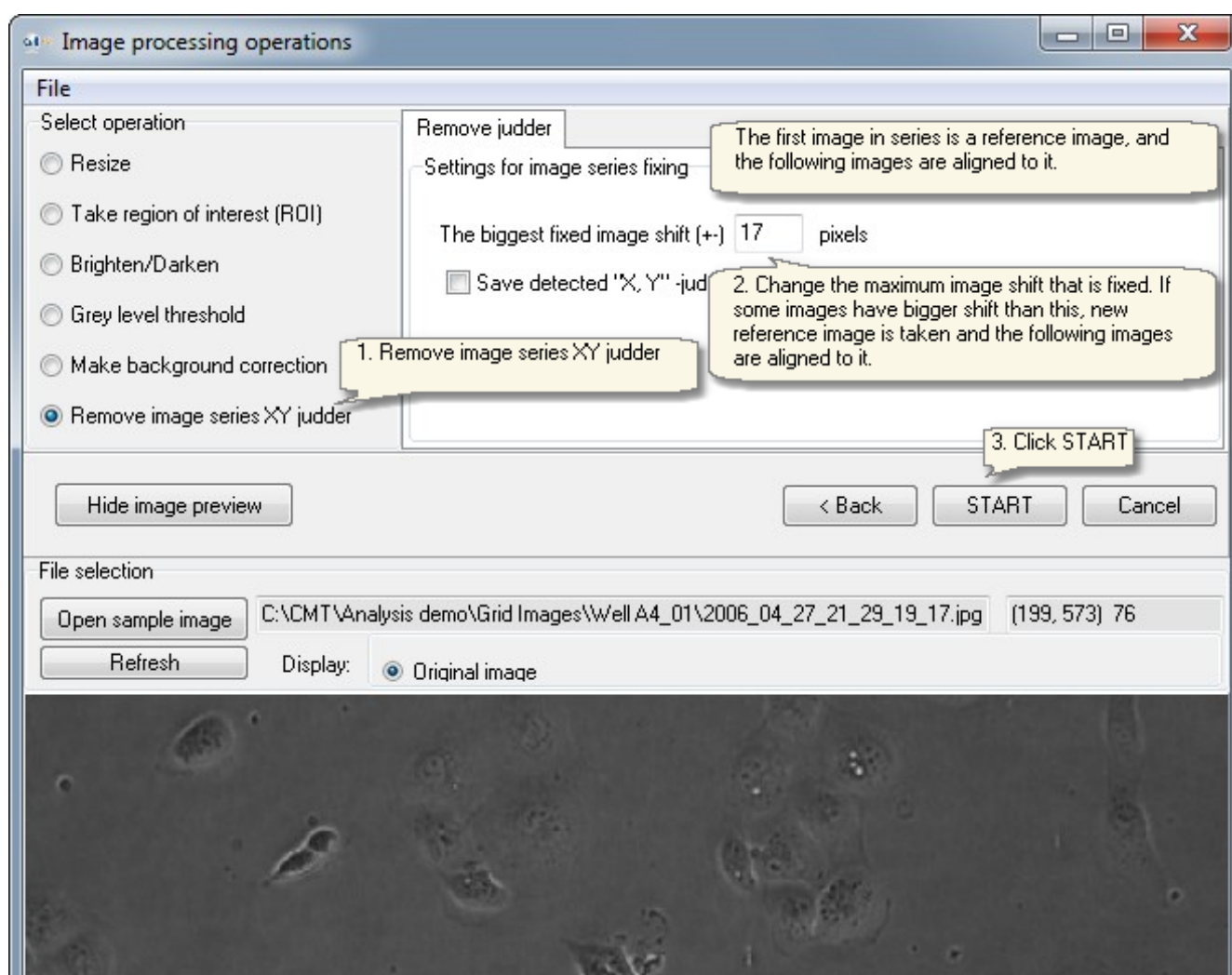
## 7.11.6 Remove image series XY judder

**Remove image series XY judder: Align images from series so that images do not appear to jump when video is played**

1. Select **Remove image series XY judder**

2. Select judder fixing parameter that defines the maximum image shift that is fixed. Smaller parameter makes the fixing faster and bigger correspondingly slower. The result images have the same size as the source images, and when the image is shifted, the blank space is filled by mirroring the image edges. Preserving the original image size is beneficial if image grid is later stitched together.

4. Click **Next**.



## Analysis results

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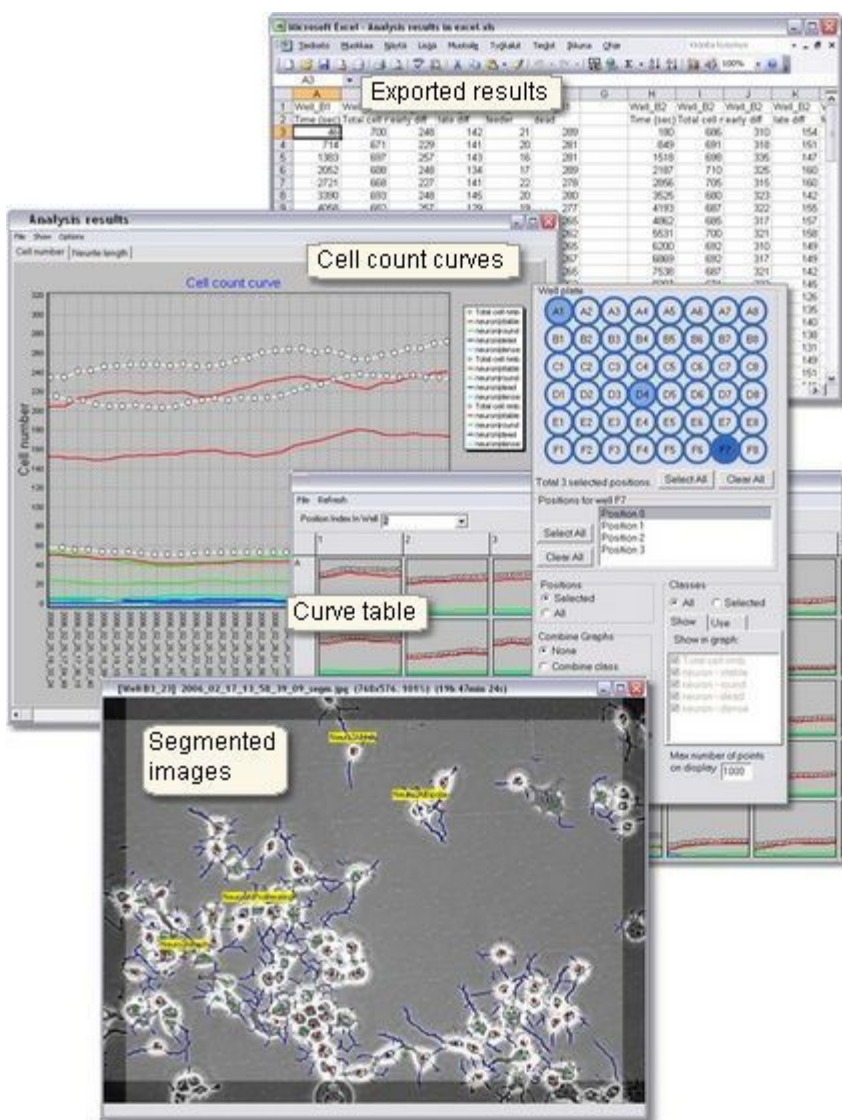




# 8 Analysis results


## View, present and extract analysis results

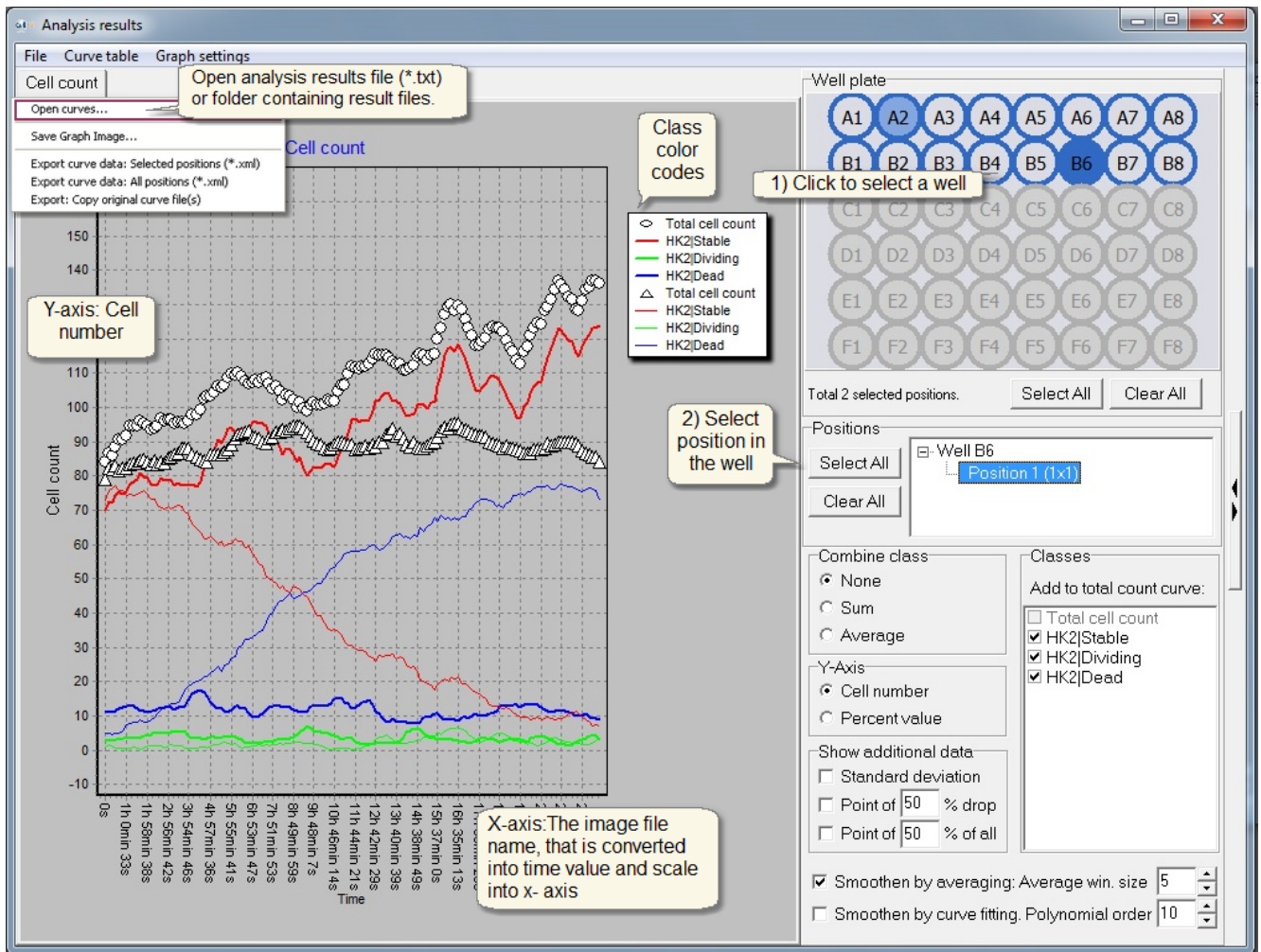
- Result curves
- Graph functions
- Saving graphs
- Curve table
- Segmented result images
- Export results into Excel
- Extract cell count curves



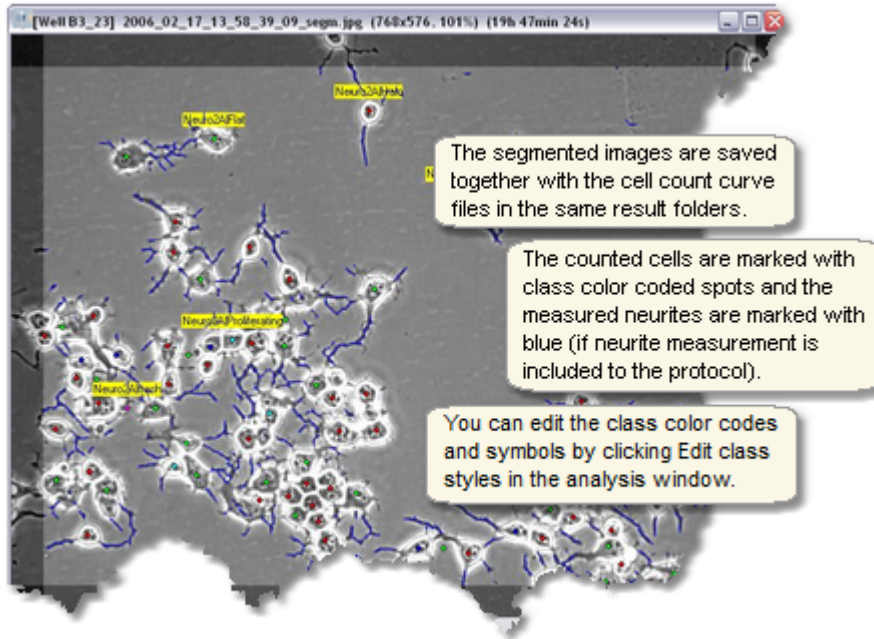
## 8.1 Viewing results

### Result curves

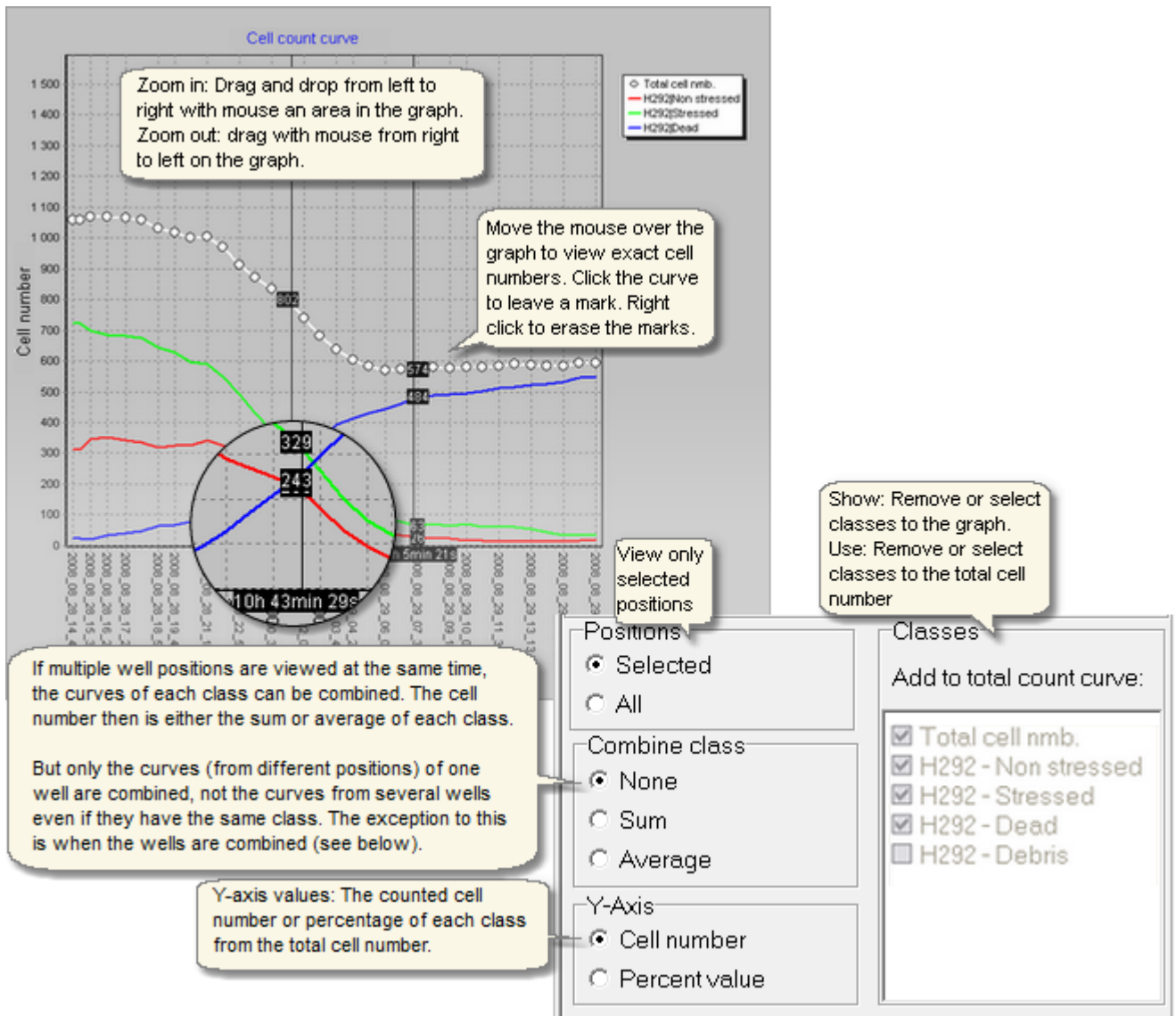
- Click the **Show result curve window**- button  in the Analyser main window to view the cell count curves of analysed data. The results window also opens automatically when analysis is finished.
- When the Cell Finder tool is used, the white dotted curve is the total cell number and the colored curves are cell numbers in each class of the protocol. The color codes are displayed next to the graph.
- The line colors in graphs can be customized, click **Options** menu to select custom line colors and graph background style with or without color gradient.



### Segmented result images

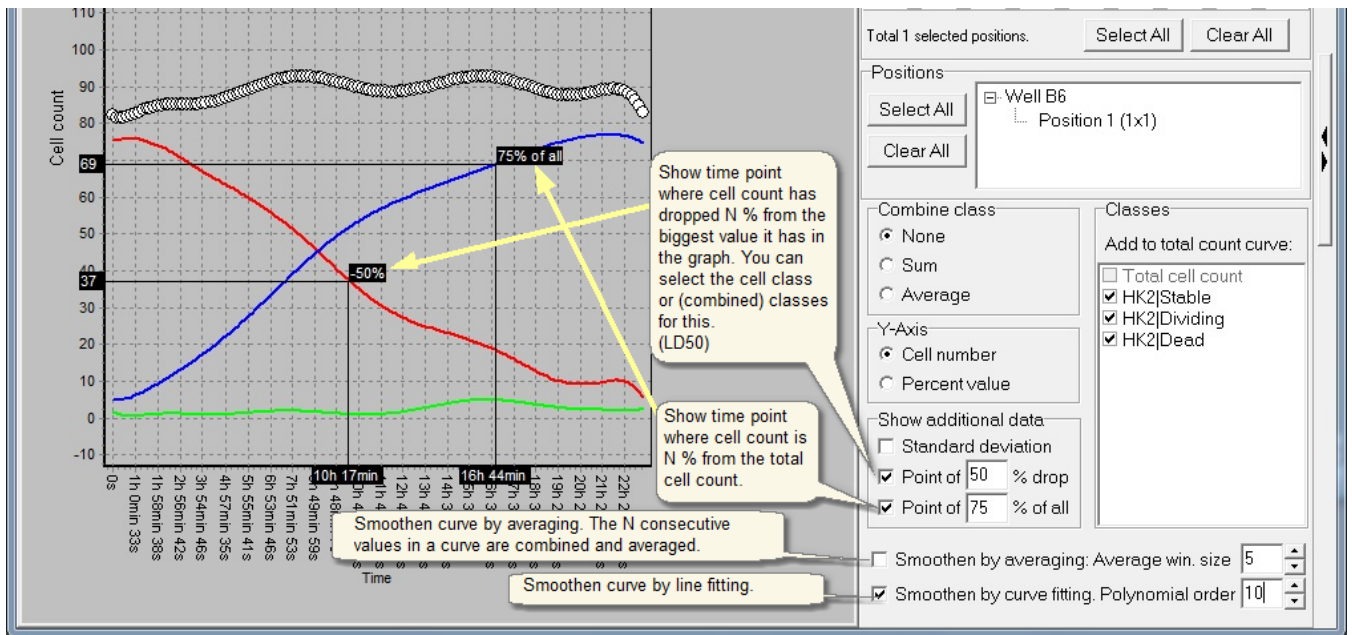


## Graph functions



Highlight time points where cell count has increased or decreased a given number, and smoothen curves.

- You can use these controls for showing the LD50 point in curve.



Compare different wells or well groups to each others, by grouping the wells from the well plate image.

The screenshot displays the Cell-IQ software interface. On the right, a 'Well plate' grid is shown with wells A1-A8 and B1-B8. Wells A4, A5, and A6 are highlighted in purple, and wells B5 and B6 are highlighted in blue. A callout box explains: '1. Paint the wells you want to be combined with left mouse button down.' Below the grid, a 'Well B6' section shows 'Position 1 (1x1)' selected. On the left, a graph shows data points for 'Total cell count' (purple dots) and 'HK2|Stable' (red line) for the selected wells. A callout box states: 'The dots are coloured with the group colour.' and 'One Total cell count curve for the three combined wells (A4, A5 and A6)'. A legend lists groups: Grp1: Total cell count (purple dot), Grp1: HK2|Stable (red line), Grp1: HK2|Dividing (green line), Grp1: HK2|Dead (blue line), Grp2: Total cell count (cyan triangle), Grp2: HK2|Stable (red line), Grp2: HK2|Dividing (green line), Grp2: HK2|Dead (blue line). A 'Confirm' dialog asks 'Combine the selected wells? (No: Select without combining.)' with 'Yes' and 'No' buttons. A callout box explains: '2. Click Yes, so that the wells will be combined together. Otherwise they will be just selected to the curve graph view without combining.' A 'Select group' dialog shows 'Add the selected wells to the group number' set to 2 and 'The group name in the graph legend' set to Grp2. A callout box explains: '3. Make a new group for the selected wells, or combine them to one of the previously added well groups.' Another callout box notes: 'You can change the name how this group is displayed in the curve graph legend.'

Edit e.g. curve graph titles and axes scales from the Graph settings window.

\* Note: This window also includes the **Standard Deviation** display option for the combined curves.

**Graph settings**

File Curve table **Graph settings**

Cell count

**SETTINGS FOR THE CURRENT GRAPH**

**Titles**

Graph main title: Cell count

Change font

**Y axis scale**

Automatic scale

Fixed scale: Min -13,08 Max 170,08

**SHARED SETTINGS FOR ALL GRAPHS**

**Panel**

Colour: Silver Use colour gradient

**Legend**

Show well name in legend

**X axis scale**

Automatic scale

Fixed scale: Min 0,00 h Max 23,22 h

**X axis labels**

Time labels in data points (1) Image name (2) Automatic scale - In hours (3) Automatic scale - In minutes (4)

Text angle: 5

**Curves**

Curve line width: 2 pixels

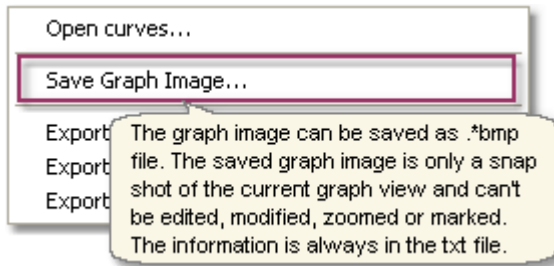
Class colour: Total cell count Black

Show Standard Deviation for the combined curves

**Callouts:**

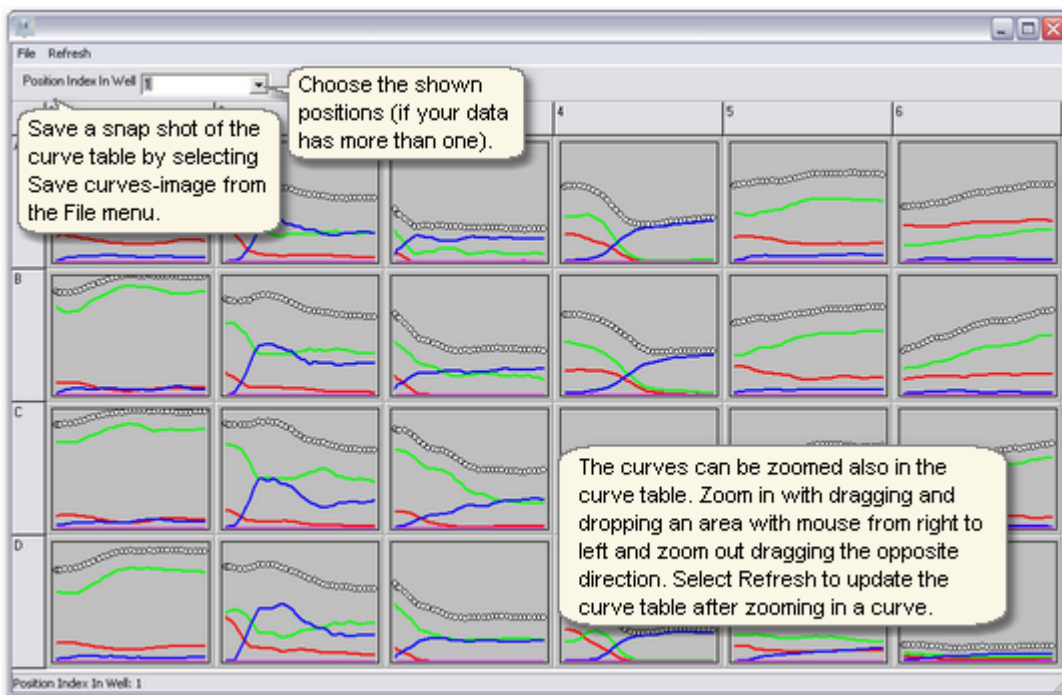
- Settings in this section is for the currently visible graph only.
- Select the title from the drop-down for editing. The title in graph is changed real-time.
- The Y-axis scale is the same for all wells, but it is selected automatically so that all the curve values are below the Y-axis top value.
- User selected scale.
- Settings in this section are the same for all result curve graphs.
- The graph background.
- All the curves are listed in the legend box in the right from the curve graph. Select if the curve name in the legend starts with the well name (or with well group name if it exists).
- Show all existing time points.
- Show only limited time range.
- X axis labels:
  - Show in every time point: "hh mm ss"
  - Show original image name in every time point.
  - Show evenly spaced labels: "hh"
  - Show evenly spaced labels: "mm"
  - Change the angle of the label text.
- The width of the thickest curve in graph.
- The class names and colours.
- Show the Standard Deviation for every time point for the combined curves.

## Saving graph image



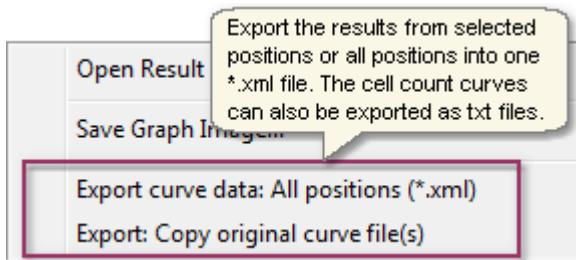
## Curve table

Click **Curve table** in the **Show** menu above the graph to view all result curves of the well plate. The curve table is a good tool in comparing the result curves in the wellplate. Select the position in the well or select all positions from the drop menu. You can save a jpg snap shot of the curve table.





## 8.2 Exporting results



### Exporting results into Excel

Select **Export curve data: All positions**. Note that there's a limitation of 256 columns on one sheet in Excel. The results might have to be saved in many separate xml files, which can be very inconvenient. That's why it's recommended to use the selected positions option to avoid exceeding the limit.

### Extracting curve files

Extract the \*.txt files by selecting **Export: Copy original curve file(s)**. The curve files are extracted from the result folders leaving out the result images. The subfolders and file names are created automatically.

### STRUCTURE OF THE EXPORTED XML FILE

Results exported in Excel

1	Well_A2_02	Well_A2_02	Well_A2_02	Well_A2_02	Well_A2_02	Well_A2_03	Well_A2_03
2	Time (sec)	Total cell nmb	Class 1	Class 2	Class 3	Time (sec)	Total cell nmb
3	245	4	1	91	254	143	
4	5946	105	0	79	5957	129	
5	11657	98	1	84	11666	116	
6	17365	104	0	81	17374	122	
7	23081				23091	123	
8	28794				28804	117	
9	34503				34512	134	
10	40211				40221	123	
11	45920	108	4	76	45931	116	
12	51622	103	2	81	51632	119	
13	57331	114	1	70	57341	123	
14	63050	103	2	78	63059	143	
15	68779	110	2	76	68789	125	
16	74495	110	3	71	74505	115	
17	80211	104	3	74	80220	121	
18	85931	97	2	85	85941	125	
19	91642	99	1	78	91651	115	
20	97357	93	1	84	97370	132	
21	103073	100	2	78	103087	122	
22	108806	103	1	76	108806	125	
23	114535	110	2	73	114535	117	

Callouts in the image:

- First row: Position index
- Second row: class name
- The columns for each position: 1. time, 2. total cell number, and rest of the columns are the cell number in each class.
- Each row is in its own sheet

## Manual measurement tools

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## 9 Manual measurement tools

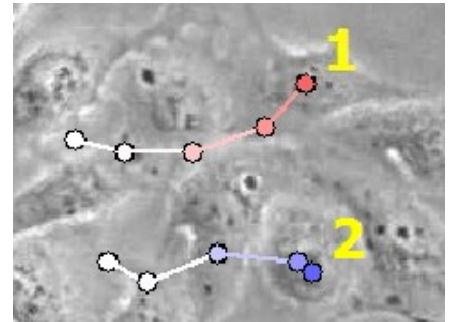
In addition to the automatic measurement tools mentioned earlier in this manual, there are some manual measurement tools available:

- [Manual cell tracking](#): Produce manually the similar results as in the [automatic cell movement tracking](#)
- [Manual cell lineage](#): Track cells through cell divisions, and generate family trees and movement vector images
- [Manual area measurement](#): Measure cell brightness and the area based on the threshold

## 9.1 Manual cell tracking

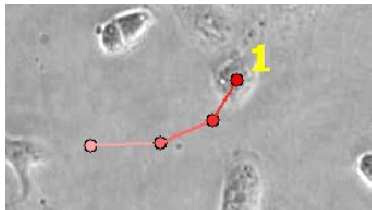
Track cells and generate movement vector images and rose charts etc. statistics. The tool is completely manual - the mouse left click adds the new cell positions to the new time points.

The results are displayed and saved in the same format as in the [automatic cell tracking measurement](#).



### Manual cell tracking:

- [Open images](#) to the Analyser main window. Using \*.dat file for image opening is recommended.
- Open Manual cell tracking tool from "Tools -> Manual cell tracking". The Manual cell tracking tool window opens.
- Select well position from the right side (if plate map visible) and scroll to interesting time point in image series from the scroll bar in the bottom.
- Track cell: Select the cell of interest from the image and track it by mouse left click. The tracking tool adds new time points to cell tree automatically.



- Track different cell: Press Add cell family–button (see image below), and left click any cell to start tracking.

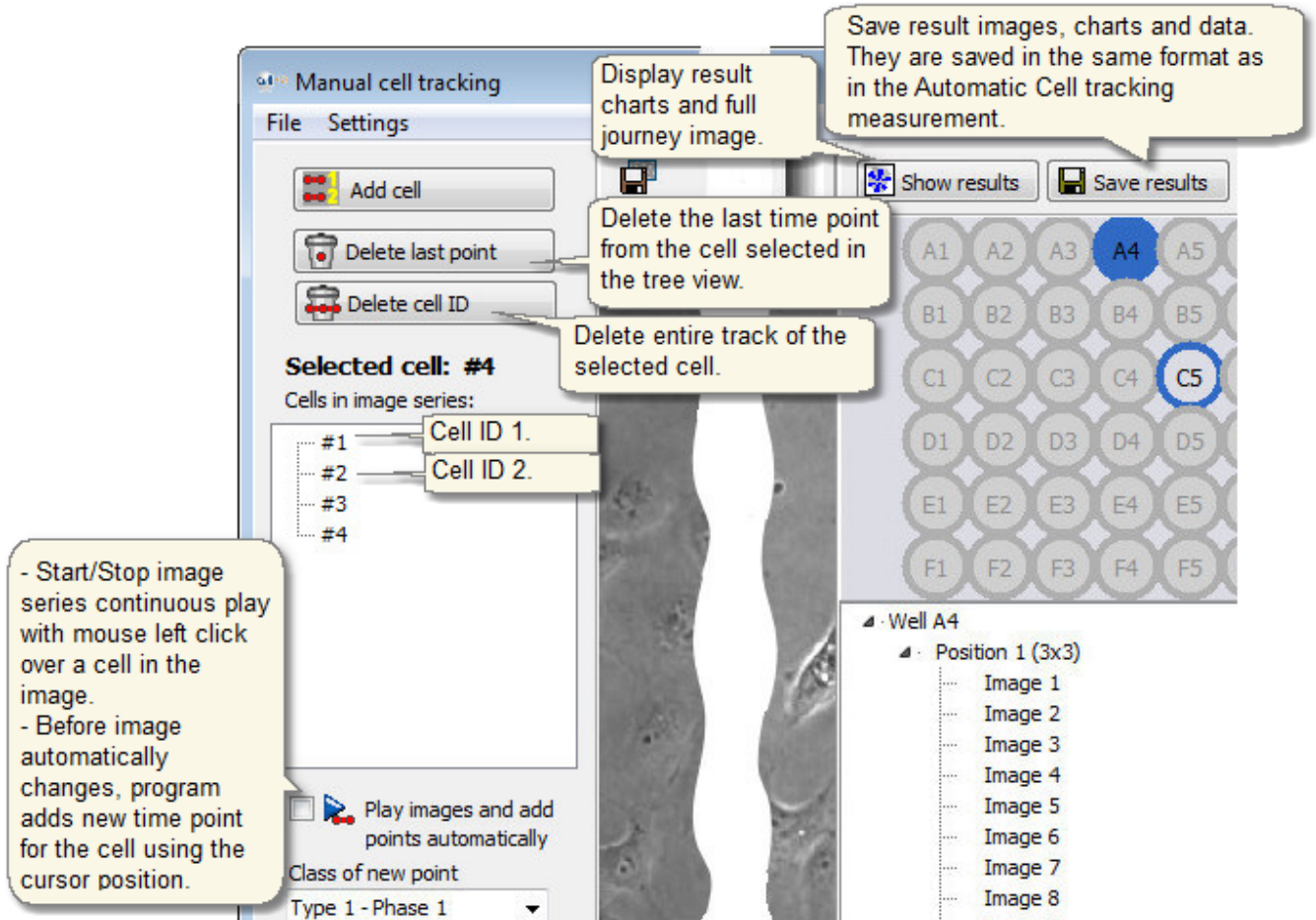
The screenshot shows the 'Tracking result editing' window with a menu bar (File, Edit, Settings) and a toolbar. The 'Track cells' section has radio buttons for 'Forward in time' (selected) and 'Backwards in time'. Below are buttons for 'Add cell', 'Delete last point', and 'Delete cell ID'. A 'Selected cell: #1' section shows a tree view with cells #1, #2, #3, and #4. The main image area shows a cell track with points #1, #2, and #3. Callout boxes provide the following instructions:

- New time points can be added to the end or to the start of the track. This selection can be changed back and forth any time.** (points to the 'Add cell' button)
- With multichannel image data you can jump between channels while adding new points to the track.** (points to the channel selection dropdown)
- Start tracking new cell.** (points to the 'Add cell' button)
- Left click to add NEW time point for the current cell.** (points to a blue dot on the track)
- Left click EXISTING time point to delete this and the following time points for the current cell.** (points to a red dot on the track)
- When you select a cell ID from tree view, the program moves to correct time point in image series, where the next position should be added.** (points to the tree view)

- Continue tracking third cell, after tracking some other cell in between: Click #3 -label from the family tree

diagram. The program drives to the correct time point where you should add the next new position for the cell.

- Display result graphics and full journey image from the Show results –button. The results are displayed and saved in the same format as in the automatic cell tracking.
- Save results from the Save results –button. You can open the saved results later from the Open result images –button (Analyser main window File-menu) the same way as the results from the Automatic Tracking.
- You can edit existing cell tracks from the different buttons in the Manual cell lineage tool, as shown in the image below.



### Visualization settings

- The manual cell tracking has similar visualization settings (image below) as the automatic Cell tracking tool.
- Cell recognition classes: You can give cell a different recognition class in each time point, even if there is not automatic cell recognition in this tool, see image in below.

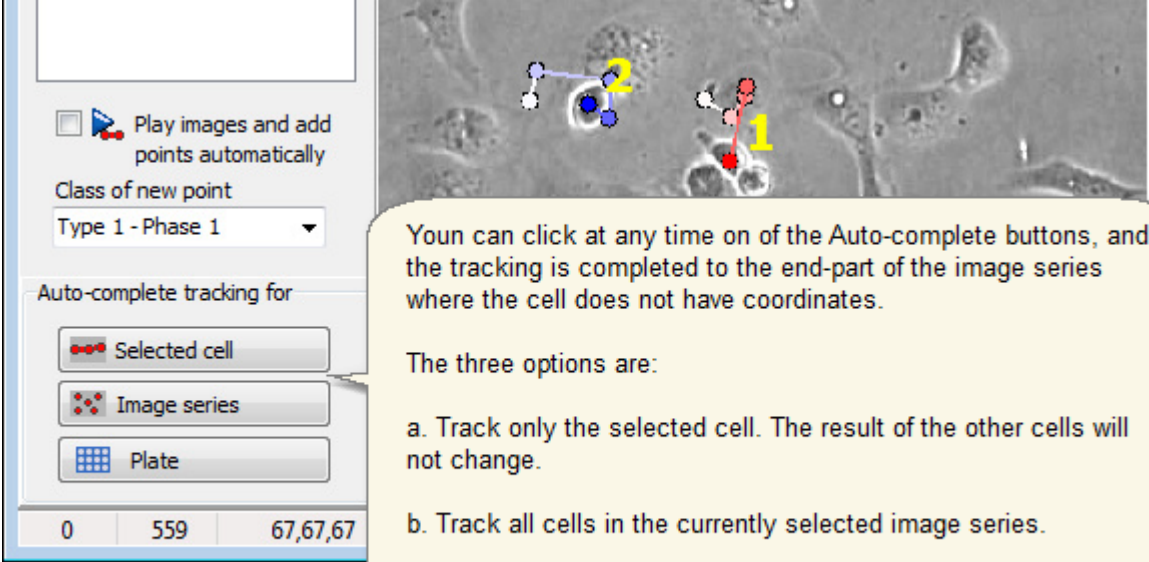
The screenshot shows the 'Lineage settings' dialog box with several callouts explaining the options:

- Visualize cells**
  - Show cell positions from** 4 previous time points. (Callout: How many earlier positions for a cell are displayed in one image. This does not change any numerical results.)
  - Cell ID number in result image**
    - No ID number shown
    - 5 Show cell ID number (Callout: Cell IDs are written to the tracked cells in the vector-images. There are no ancestors in cell tracking (as there are in Lineage) so the last two options are identical in this case.)
    - 5,2,1 Show cell ID and cell's ancestor's IDs
  - Dot colours in result images**
    - Use recognition class colour (Callout: Use class colours in the dots in the images.)
    - Use one colour for one cell ID (Callout: Use unique colour for each cell track. Class colours are not shown in images.)
- Recognition classes**
  - Buttons: Add new, Delete, Edit class styles
  - Radio buttons:  Cell type,  Cell phase
  - Tree view: Type 1
    - Phase 1
    - Phase 2 (Callout: The recognition classes that are shown in the result tree view and can be shown in the vector images.)
- Distance Units**
  - Micrometers. Image resolution [um/pixel]: 0,700
  - Pixels
- Exclude cell from average results if it...**
  - has < 5 time points
  - moved < 30 um distance
- Close button

## 9.1.1 Autocomplete Tracking

Auto complete tracking automatically in the end part of the image series where the cell does not yet have coordinates.

- The coordinates can be missing if user has not finished manual tracking till the end of the series.
- The coordinates can be missing if user has deleted time points after finding them faulty, and continued manual tracking over the time points that maybe caused problems for automatic tracking.



The screenshot shows the software interface. On the left is a control panel with the following elements:

- A checkbox labeled "Play images and add points automatically" which is currently unchecked.
- A dropdown menu labeled "Class of new point" set to "Type 1 - Phase 1".
- A section titled "Auto-complete tracking for" containing three buttons: "Selected cell" (with a red dot icon), "Image series" (with a red dot icon), and "Plate" (with a grid icon).
- At the bottom of the panel, a status bar shows the numbers "0", "559", and "67,67,67".

On the right is a microscopy image showing several cells. Some cells are marked with colored dots (blue, red, yellow) and lines, indicating they are being tracked. A yellow number "2" is next to a blue dot, and a yellow number "1" is next to a red dot.

A yellow callout box contains the following text:

You can click at any time on of the Auto-complete buttons, and the tracking is completed to the end-part of the image series where the cell does not have coordinates.

The three options are:

- Track only the selected cell. The result of the other cells will not change.
- Track all cells in the currently selected image series.
- Complete tracking for all the cells in the well plate.

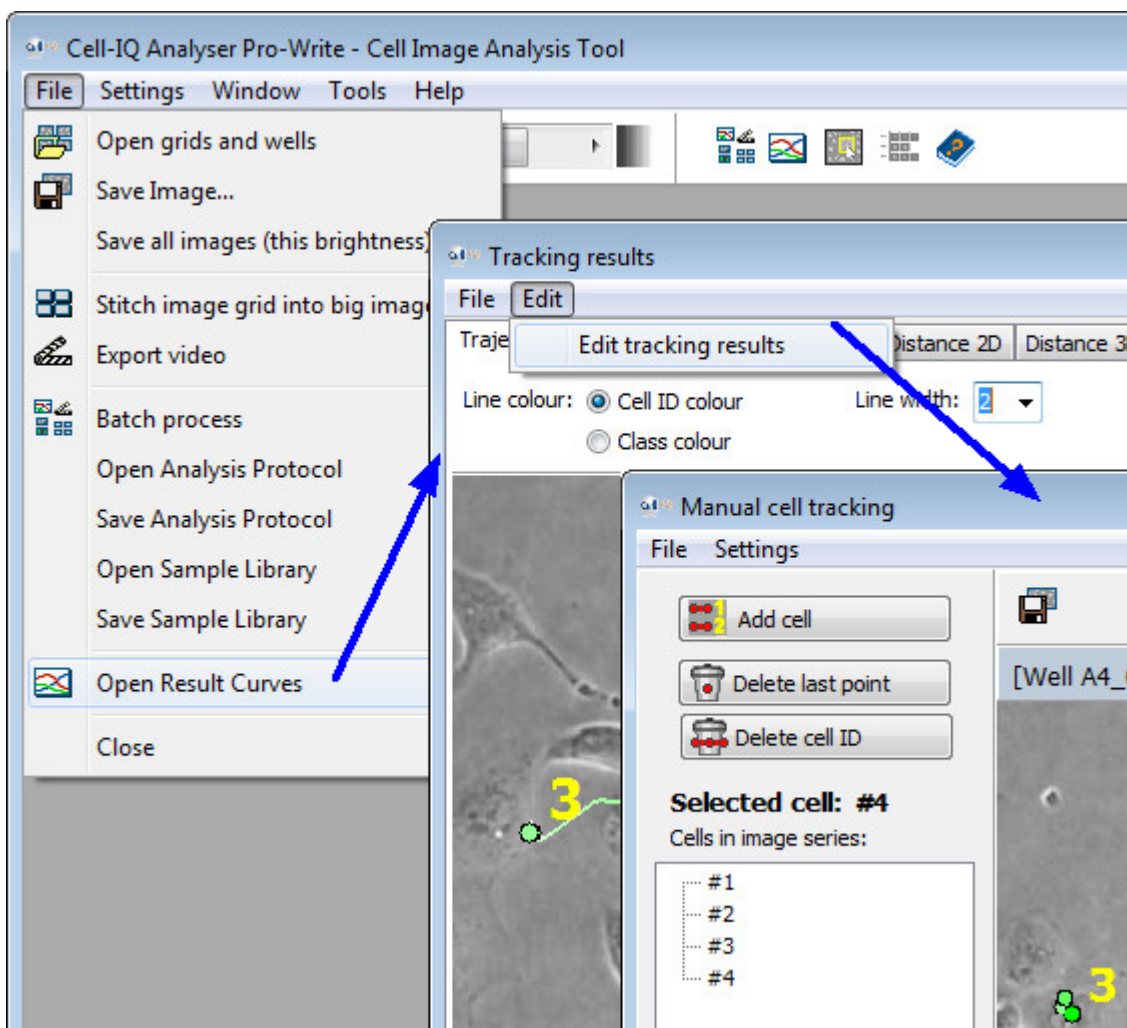
## 9.1.2 Edit Tracking results

You can complete or edit the old tracking results in the tracking Results Editing -window. Then you can save the fixed results to the new result folder.

### Edit tracking Results:

Open the tracking Results Editing -window, as shown in the image below.

- Start by opening the tracking result curves of the entire well plate.
- Results Editing -window will get a copy of the tracking results for editing - the tracking result window will keep the old values unmodified until they are later actively refreshed by the user.
- Tracking Results Editing -window will request the location of the original, unmodified source images. They are needed so that the dots and trajectories of the modified cell tracking result can be drawn to them, and also if the automatic cell tracking is utilized in the Results Editing.



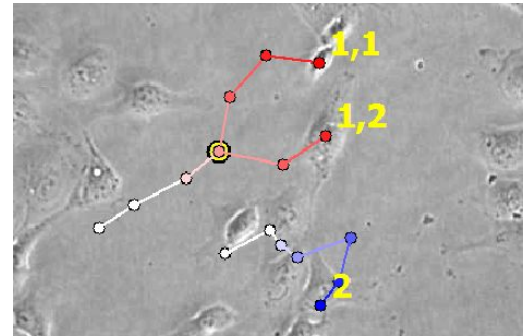
- The tracking results Editing window is identical to the [Manual cell tracking tool](#).



## 9.2 Manual cell lineage

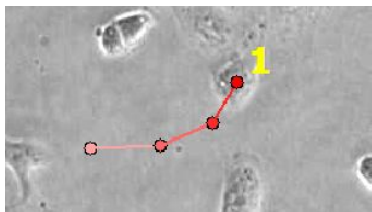
Track cells through cell divisions, and generate family trees and movement vector images. The tool is completely manual - the mouse left click adds the new cell positions to the new time points.

The results are displayed and saved in the same format as in the automatic cell lineage measurement.

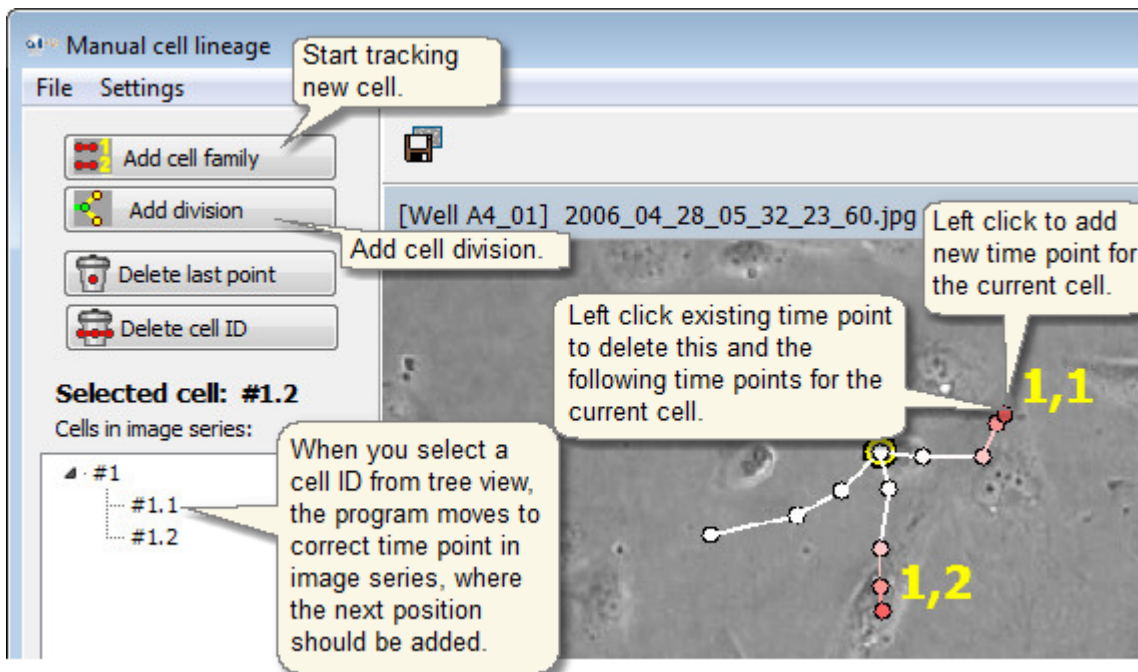


### Make manual cell lineage measurements:

- [Open images](#) to the Analyser main window. Using \*.dat file for image opening is recommended.
- Open Manual cell lineage tool from "Tools -> Manual cell lineage". The Manual cell lineage tool window opens.
- Select well position from the right side (if plate map visible) and scroll to interesting time point in image series from the scroll bar in the bottom.
- Track cell: Select the cell of interest from the image and track it by mouse left click. The lineage tool adds new time points to family tree automatically.



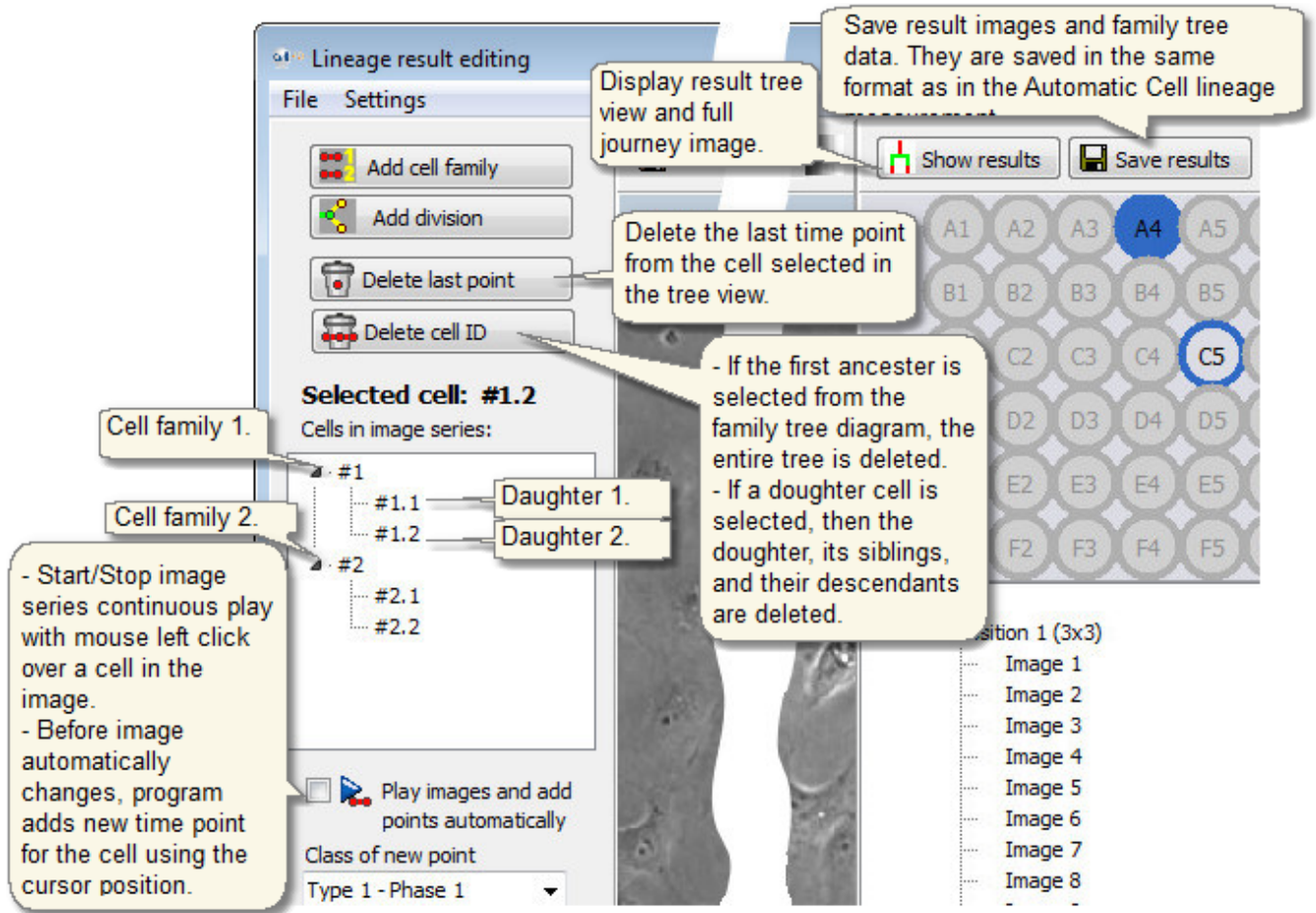
- Track different cell: Press Add cell family–button (see image below), and left click any cell to start tracking.



- Add cell division: Press Add division –button (see image above), and left click the first daughter cell in image. Then you can continue to click and add more time points for the first daughter.
- Track the second daughter: Press #1.2 -label from the family tree diagram. The program drives to the correct time point where you should add the position for the second daughter. Left click image and continue to track the

second daughter. You can jump back and forth between the two daughters, by clicking the cell IDs in the tree view.

- **Display result** tree view and full journey image from the Show results –button. The results are displayed and saved in the same format as in the automatic cell lineage measurement.
- **Save results** from the Save results –button. You can open the saved results later from the Open result images –button (Analyser main window File-menu) the same way as the results from the Automatic Lineage measurement.
- You can edit existing cell families from the different buttons in the Manual cell lineage tool, as shown in the image below.

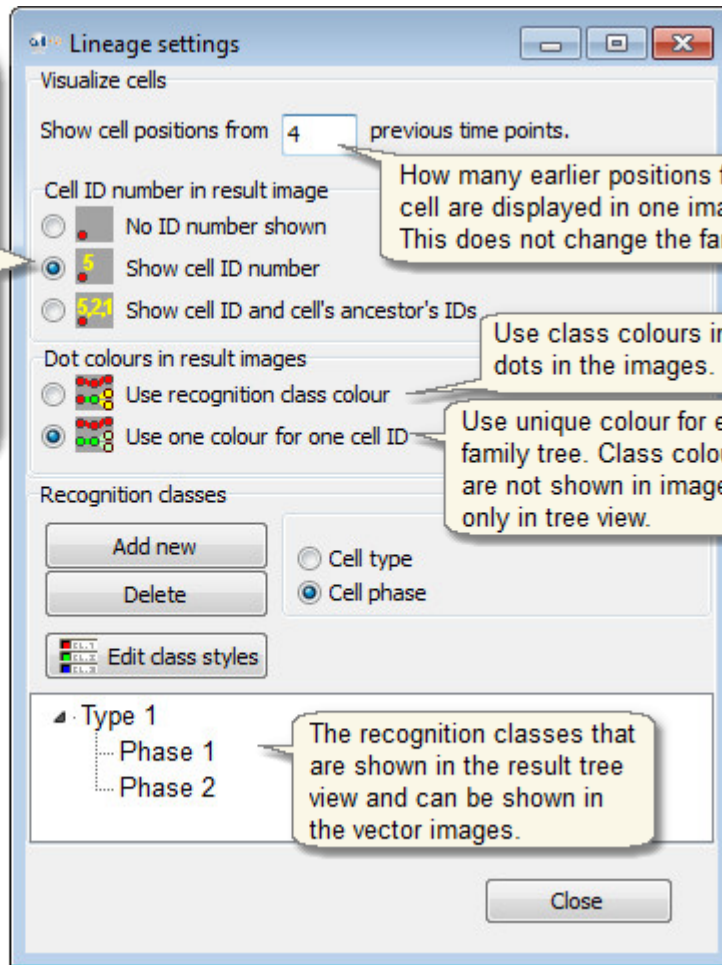


**Visualization settings**

- The manual cell lineage has similar visualization settings (image below) as the automatic Cell lineage tool.
- **Cell recognition classes:** You can give cell a different recognition class in each time point, even if there is not automatic cell recognition in this tool, see image in below.

Cell IDs are written to the tracked cells in the vector-images.

First ID number in ID label of cell comes from the first ancestor's ID of that cell, and more ID numbers in the label come if cell divides - the first daughter gets value 1, and the second daughter value 2.



The recognition classes that are shown in the result tree view and can be shown in the vector images.

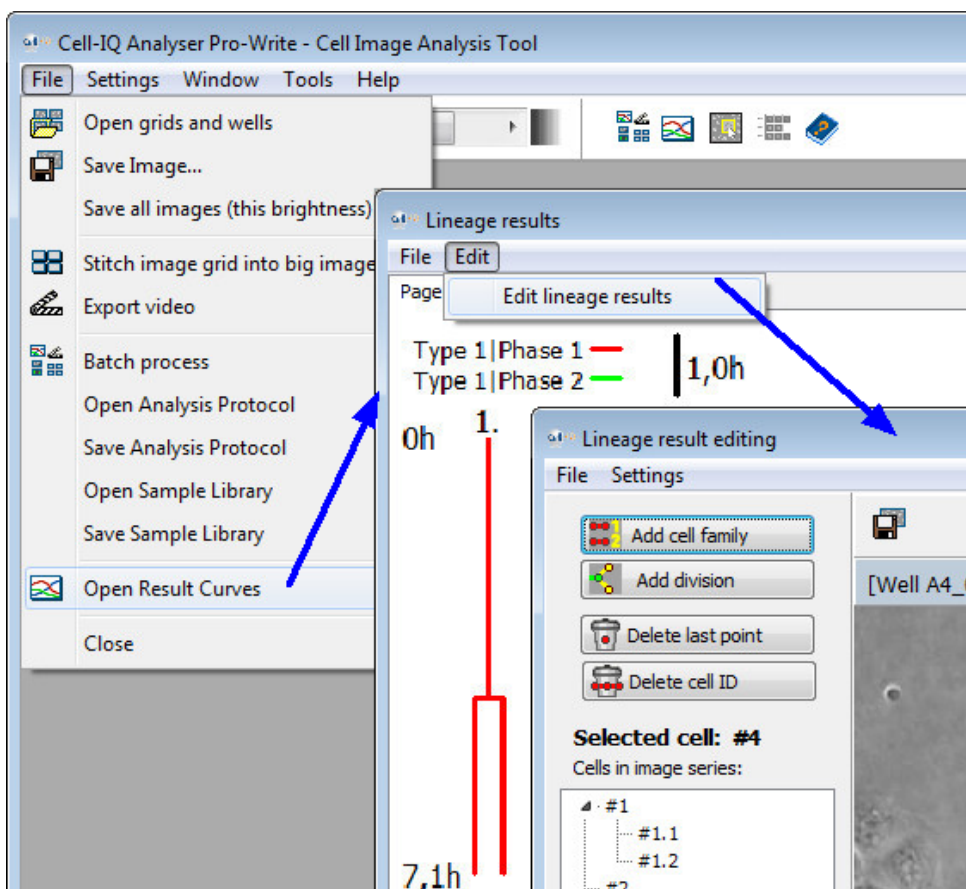
## 9.2.1 Edit Lineage results

You can fix the possible errors in the lineage results in the Lineage Results Editing -window. Then you can save the fixed results to the new result folder.

### Edit Lineage Results:

Open the Lineage Results Editing -window, as shown in the image below.

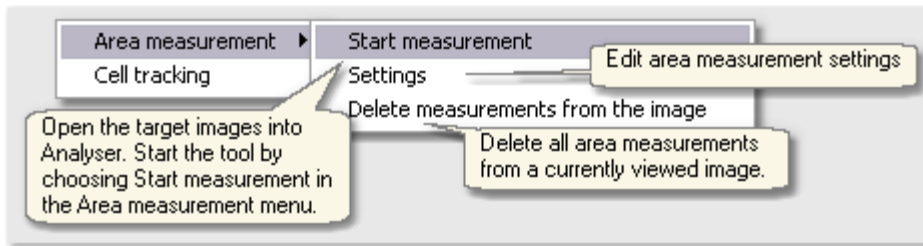
- Start by opening the Lineage result curves of the entire well plate.
- Results Editing -window will get a copy of the Lineage results for editing - the Lineage result (Tree/Trajectory) window will keep the old values unmodified until they are later actively refreshed by the user.
- Lineage Results Editing -window will request the location of the original, unmodified source images. They are needed so that the dots and trajectories of the modified cell tracking result can be drawn to them, and also if the automatic cell tracking is utilized in the Results Editing.



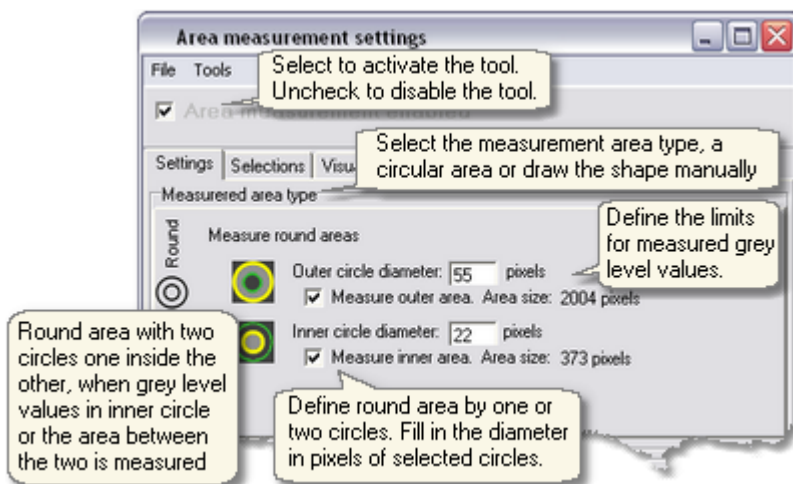
- The Lineage results Editing window is identical to the [Manual cell lineage tool](#).

### 9.3 Manual area measurement

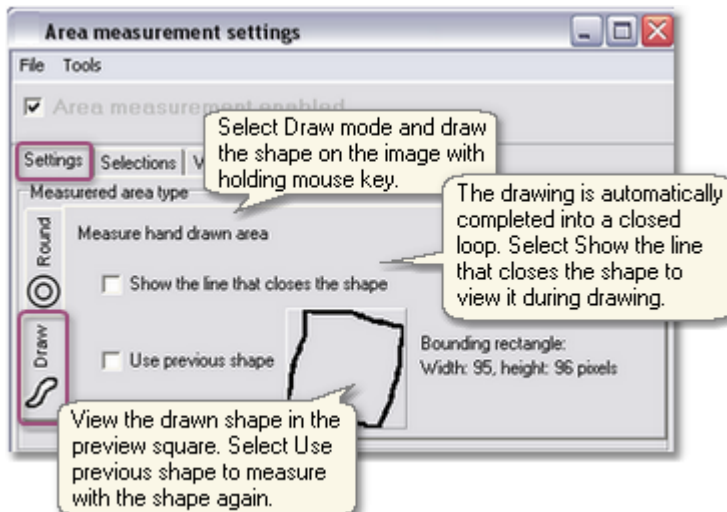
#### Measure intensities in e.g. fluorescence images



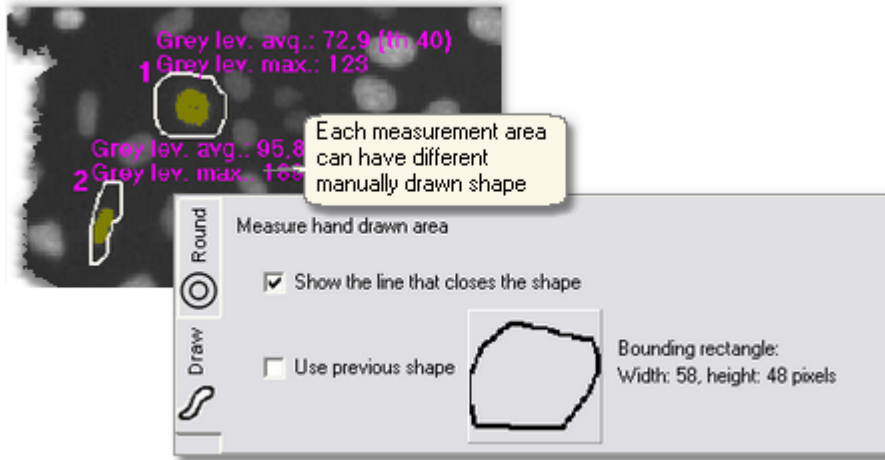
#### Area measurement settings: Round area



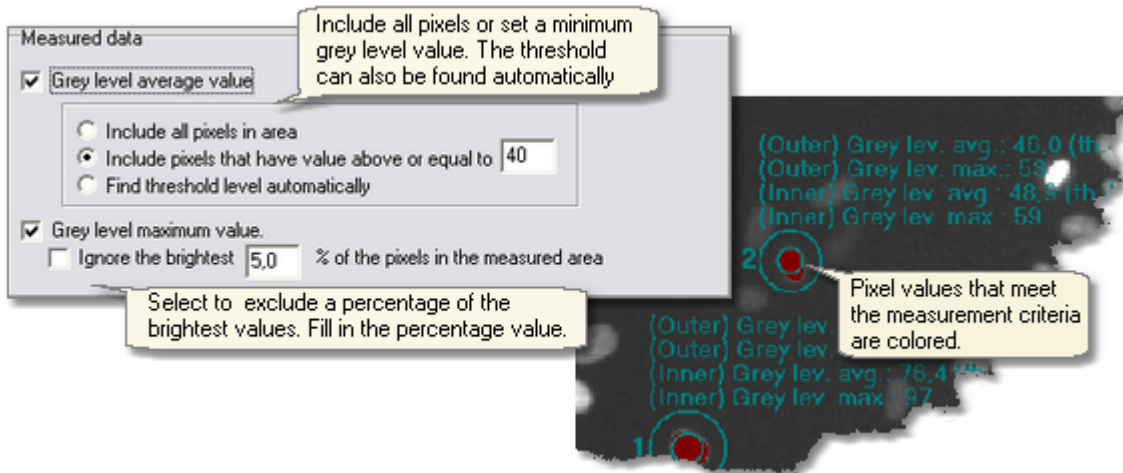
#### Area measurement settings: custom area



### Custom shape for each measurement



### Grey level values



### Display settings

Select the parameters to be displayed on the image for each area measurement

Display

Selected grey level measurements

Index of area

Colorize the measured area ■

Outer circle diameter

Inner circle diameter

Manually drawn area

Round area size

Outer area (pixel number)

Outer area (pixel number) - segmented

Inner area (pixel number)

Inner area (pixel number) - segmented

Manually drawn area size

Drawn area (pixel number)

Segmented area (pixel number)

Click to edit the color for measured area

Parameters are displayed next to each measurement area according to the selected

You can make measurements in multiple target images. The measurements are stored while browsing the target images opened in Analyser. Save the measurements before closing the images.

(Outer) Grey lev. avg.: 42.3 (th.40)  
 (Outer) Grey lev. max.: 45  
 (Outer) Area: 1404  
 (Outer) Segmented area: 6  
 (Inner) Grey lev. avg.: 54.4 (th.40)  
 (Inner) Grey lev. max.: 69  
 (Inner) Area: 973  
 (Inner) Segmented area: 709  
 Big circle diam.: 55  
 Small circle diam.: 35

### Viewing measurements

Area measurement settings

File Tools

Area measurement

Settings: **Selections** Visualization

Areas in the image:

Round area 3

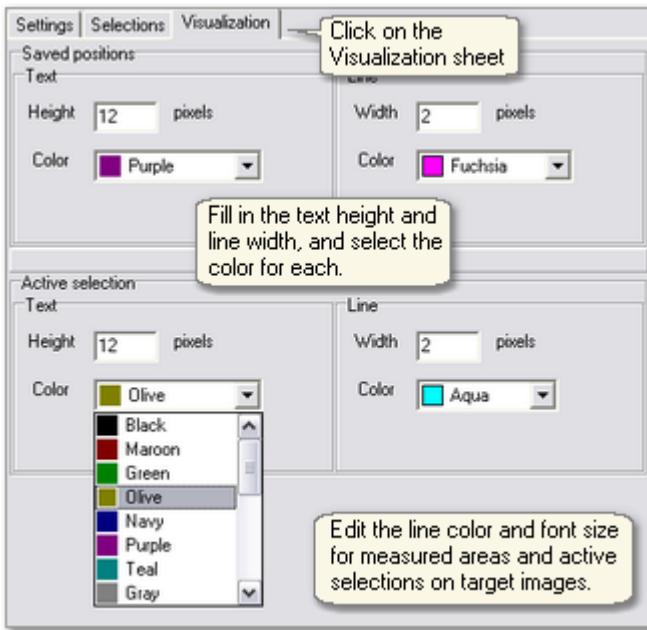
(Inner) Grey lev. avg.: 72.5 (th.40)  
 (Inner) Grey lev. max.: 105  
 (Inner) Area: 973  
 (Inner) Segmented area: 558  
 Big circle diam.: 45  
 Small circle diam.: 35

Click on the Selections sheet

View all the parameters of each measurement by choosing the index in the drop menu

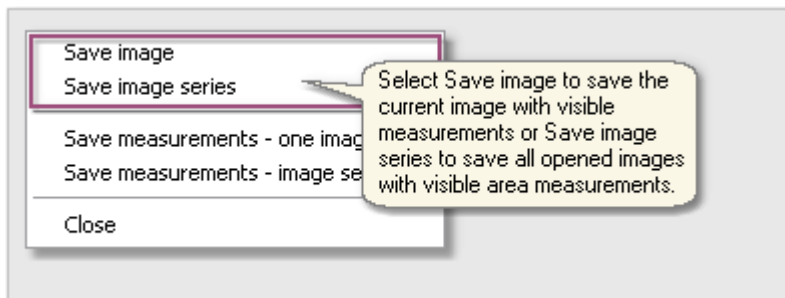
Delete the selected measurement (Delete) or all the measurements in target images (Delete all).

### Editing line color and width

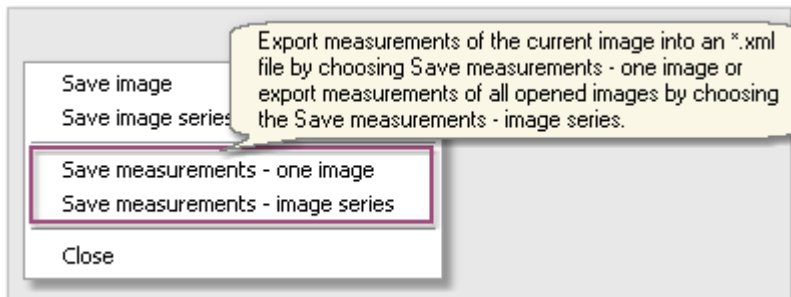


### Saving area measurements

#### Saving image(s)



#### Export measurement(s)





### Area measurement result file

The measurements are exported into an \*.xml file that can be opened with Excel.

	A	B	C	D	E	F	G	H
	Image	Area	(Outer) Grey lev. avg.	(Outer) Grey lev. max.	(Outer) Segmented area	(Inner) Grey lev. avg.	(Inner) Grey lev. max.	(Inner) Segmented area
2	2006_10_13_02_24_40_35	1	423	45	6	544	69	351
3		3	581	80	61	696	90	351
4		4	590	86	112	783	114	311
5		5	586	75	63	701	101	461
6		7	631	90	100	757	113	581
8	2006_10_13_04_42_52_46	1	420	42	1	929	135	363
9		2	559	75	82	758	118	483
10		3	588	85	33	934	128	629
11		4	445	48	4	840	180	302
12		5	618	104	206	864	117	723
14	2006_10_13_07_01_03_71	1	710	115	150	918	135	704
15		2	455	53	13	758	108	604
16		3	521	69	231	952	255	525
17		4	567	85	65	706	95	415
18		5	400	40	1	844	122	427
20	2006_10_13_10_05_56_34	1	817	129	164	1087	159	576
21		2	562	82	97	829	113	551
22		3						595
23		4						345
24		5						310

### Histogram tool

**Area measurement settings**

File Tools

Histogram

Select Histogram in the Tools menu

(Outer) Grey lev. avg.: 80.2 (th.50)  
 (Outer) Grey lev. max.: 255  
 (Inner) Grey lev. avg.: 145.8 (th.50)  
 (Inner) Grey lev. max.: 255

The histogram can be displayed from gray scale values or each individual channel separately.

You can choose the measurement area (the inner, outer or both areas in case of round area) or the entire image in the histogram.

**Histogram**

Displayed channel(s)

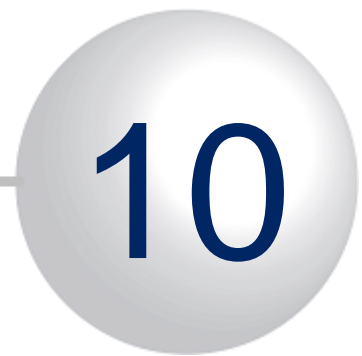
Grey scale  
 Red  
 Green  
 Blue

Measured area

Outer ROI area  
 Inner ROI area  
 Outer + Inner area  
 Entire image

Grey scale: Mean: 64 Median: 46

Contact



## 10 Contact

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