Guidance to process microscopic overview images for AutoFoci

Requirements:

- Computer equipped with at least 8 GB RAM.
- Download the provided "Cellect_Installation" zip file and unpack it.
- Download the test images provided as "Test_Images_ImageJ" zip file and unpack it.
  If you want to use own images obtained from cells stained for γH2AX (green), 53BP1 (red) and DAPI (blue) in a similar magnification and image resolution, make sure that the file and subdirectory structure containing your images is exactly the same as provided in the "Test_Images_ImageJ" folder. Image titles must include the colour of the channel: blue, red, green.

Preparation:

1. Copy the provided "ImageJ" program folder to a desired destination. Make sure you have administrator permissions for the selected destination (due to the fact that a Cellect.config file is generated automatically by first use).

2. Create a folder named "AcquisitionData" directly in the root of any partition (for example D:\AcquisitionData). Create a second folder named "Templates" in the same partition (in our example D:\Templates).

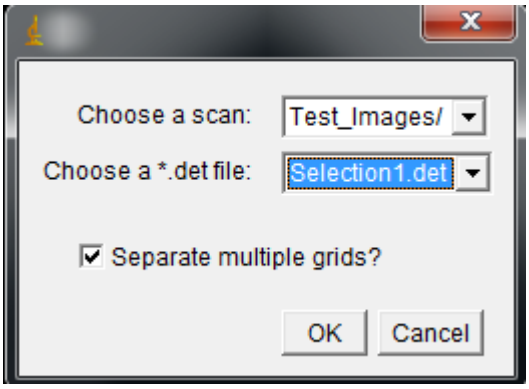
3. Copy the two files "Selection1" and "Selection2" into the "Templates" folder, and place the folder containing the images you want to analyse e.g. our folder "Test_Images_ImageJ" into "AcquisitionData".

Generation of single cell images based on overview images:

1. Open ImageJ and select "**Cellect Tools**" by clicking on 
2. Start detection of nuclei by clicking on 

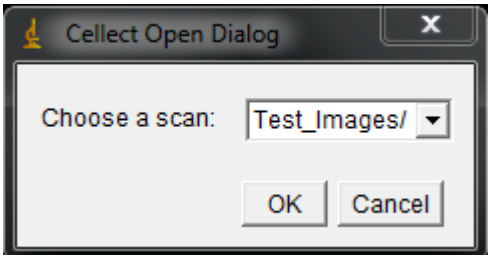
   IMPORTANT: If you use this function for the first time, the location of the folders "AcquisitionData" and "Templates" will be requested. Please direct the program to the drive you selected before (in our example D:\).

   Another window will open:
   - Select image folder "Test_Images_ImageJ" as a scan.
   - Select "**Selection1.det**" as your .det file.
   - IMPORTANT: choose the option "separate multiple grids" if you are analysing own samples and have more than one folder containing subfolders with images to analyse. This option will enable a fast image processing for multiple samples.

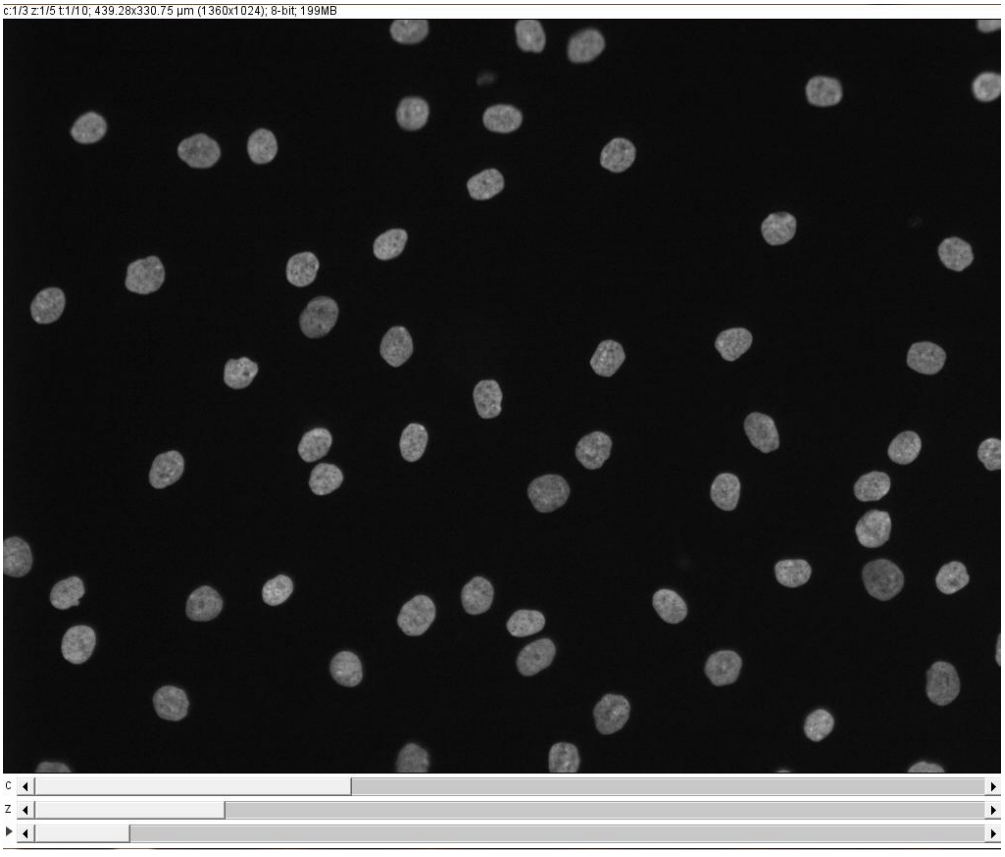


Dependent on the number of images, the object detection takes some minutes. A "Log" and "Result" window will open during the calculation process. If the calculation is done, an "Exception" window will open. Close all windows without saving any results.

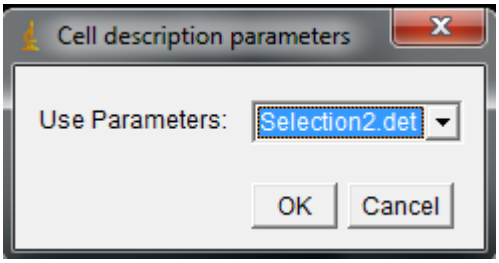
3. Click on  and choose the desired file and click "OK"



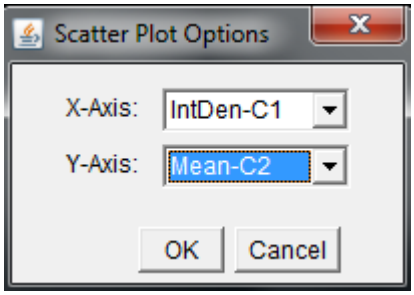
An additional window called "v_Stack" will open showing all images as stack.



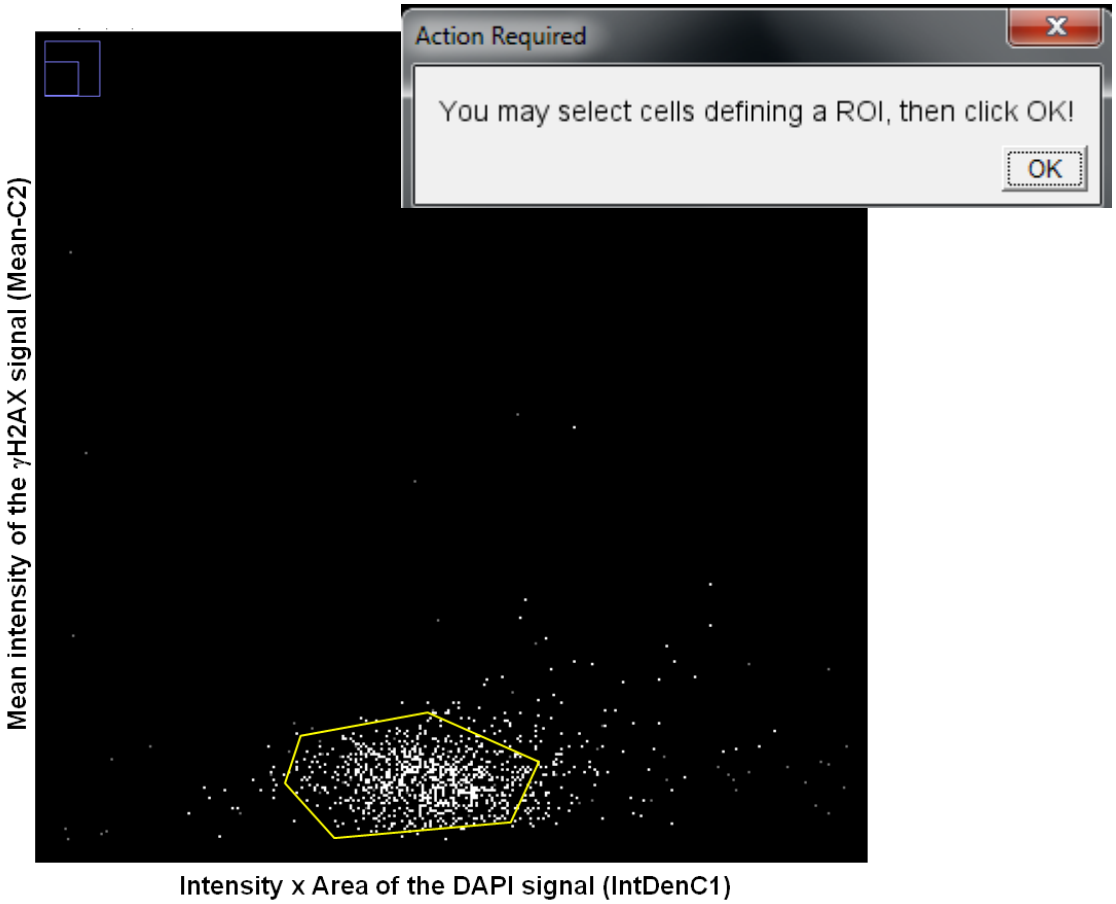
4. Click on  and select "**Apply_CellDescriptor**", then choose "**Selection2.det**" as parameter.



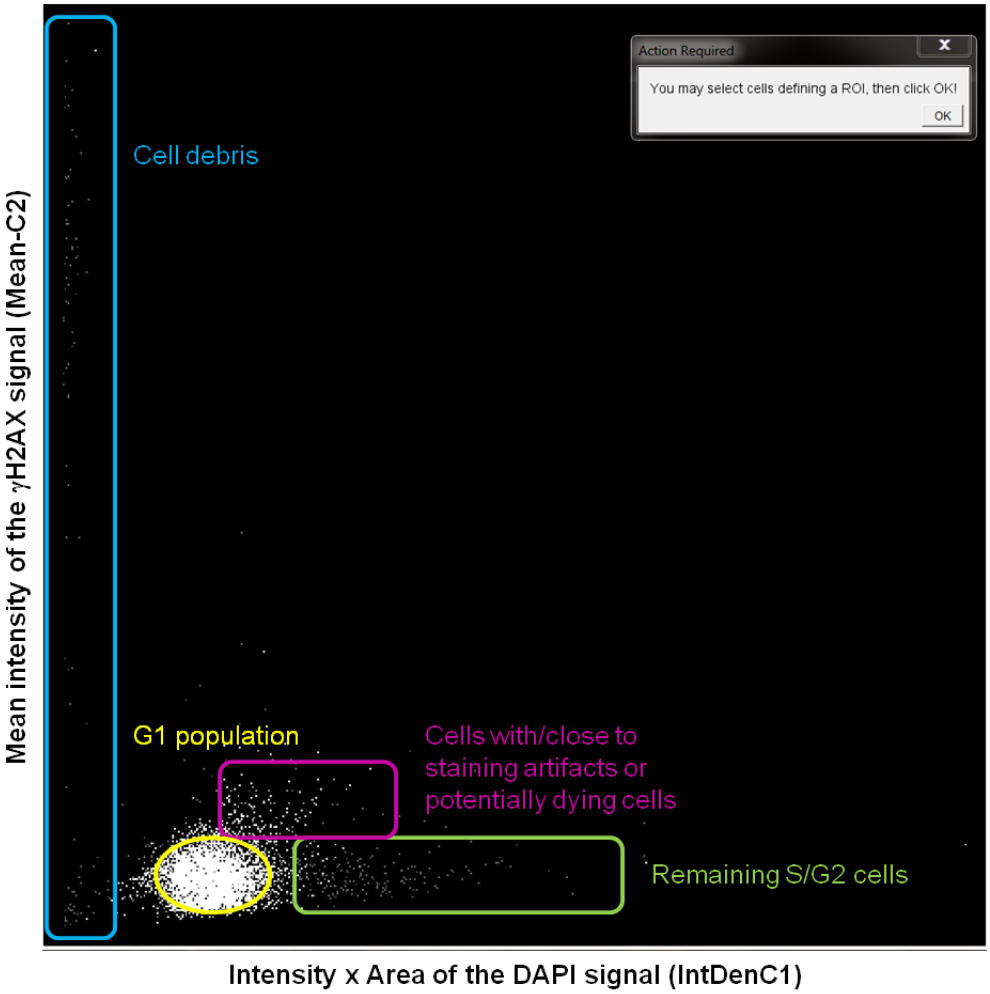
5. Click on  to create a scatter plot. Plot **IntDen-C1** (blue channel, DAPI) against **Mean-C2** (green channel, γH2AX).



Select the G1 population of cells (as region of interest, ROI) in the lower left corner of the created scatter plot. Select "OK" and close the "v_Stack".



If you are analysing own samples containing more cells, your scatter plot might look like this example created by an extended data set with more than 5 thousand cells (from which the provided test images originate).



IMPORTANT: If you are analysing own samples with more than one folder containing subfolders with images to analyse, please repeat the steps 3. to 5. for each file.

6. Click on  and select "**Steffens_Gallery**" and define a file path for saving the created single cell images to your desired folder. Single cell images can be found in the selected file path under the name you picked before and copied into "AcquisitionData", in our example "Test_Images_ImageJ". These images are ready to be analysed in AutoFoci (https://github.com/nleng/AutoFoci/tree/master/AutoFoci).

   Please try not to load (single cell) images saved in "AquisitionData" into AutoFoci. The "_Gallery" folder which is created there (in our example "D:/AcquisitionData/ Test_Images_ImageJ/_Gallery") is an intermediate product and does not represent the final selection of appropriate G1 cells you can analyse further in AutoFoci.