Guidance to process microscopic overview images using Cellect

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. (The provided data set shows many staining artifacts/cell depris outside the cell nuclei to demonstrate the ability of Cellect to sort these out).

  If you want to use own images obtained from cells stained for γH2AX (green), 53BP1 (red) and DAPI (blue) in the same 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 names must start with "img" and should contain the colour of the channel "blue", "green" or "red".

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). Please note that the exact spelling of the folder names is important.

3. Copy the four files "Selection1", "Selection1MIP", "Selection1_1plane" and "Selection2" into the "Templates" folder, and place the folder containing the images you want to analyze e.g. our folder "Test_Images_ImageJ" into "AcquisitionData".

Generation of single cell images based on overview images:

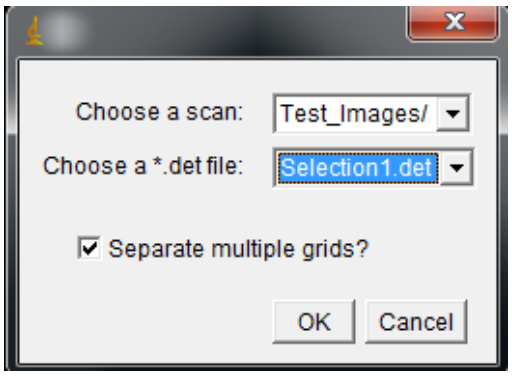
1. Open ImageJ and select "**Cellect Tools**" by clicking on 
2. Start detection of nuclei by clicking on 

   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:\). If the wrong path was accidentally entered, an error message is displayed. Before you can repeat the step 2 to enter a new file path, delete the configuration file "Cellect.conf" created in the ImageJ folder.

   Another window will open:
   - Select image folder "Test_Images_ImageJ" as a scan.
   - To analyze later in autofoci single cell images with the best plane as presented in the paper (starting from 1 DAPI image and 5 images for each

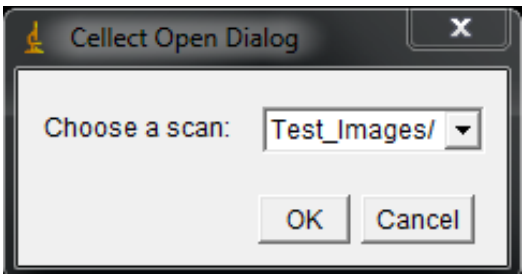
damage marker), select "**Selection1.det**" as .det file. Select "**Selection1_MIP.det**" to create maximum intensity projection (MIP) images suitable for the analysis in AutoFoci. If you need to start with 3 images in total (1 DAPI image and 1 image for each damage marker) select **"Selection1_1plane"**. The further procedure remains identical regardless of whether "Selection1.det" or "Selection1_MIP.det" or "Selection1_1plane.det" was selected in this step. Please note that we just provide "Selection1_MIP.det" and "Selection1_1plane.det" as additional feature but never used it for our analysis and thus didn't test them thoroughly.

IMPORTANT: choose the option "separate multiple grids" if you are analysing own samples with "Selection1.det" and have more than one folder containing subfolders with images to analyze. This option will enable a fast image processing for multiple samples.

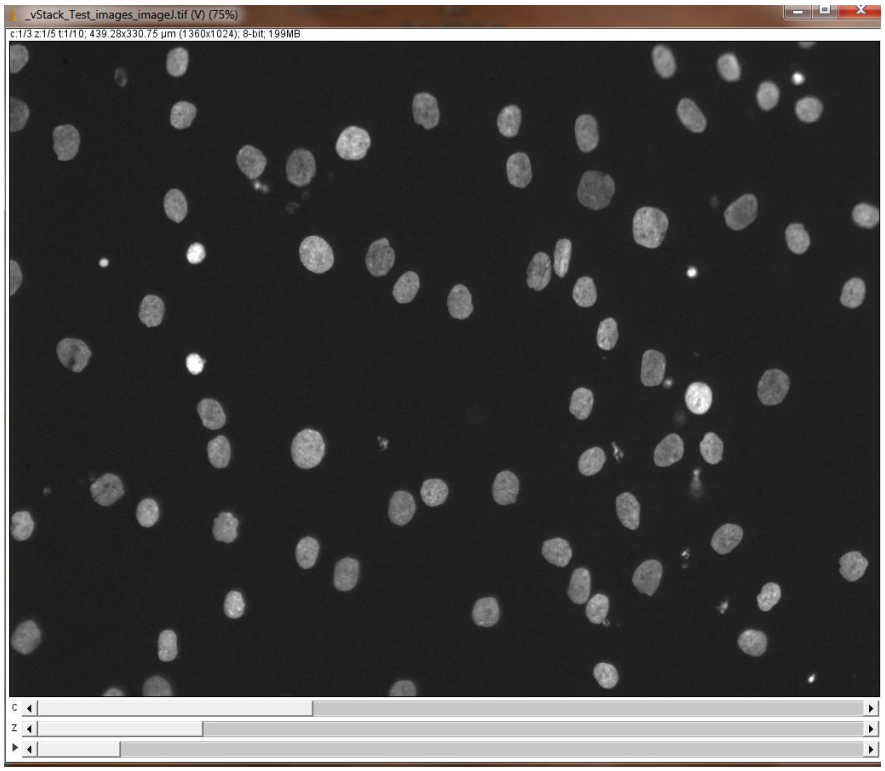


Depending on the number of images, the object detection takes some minutes. A "Log" and "Result" window will open during the calculation process. If the calculation is done, an "Exception" window will open. Close all windows without saving any results.

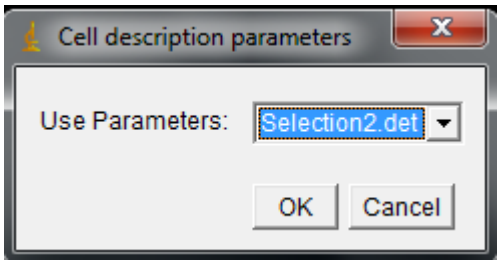
3. Click on  and choose the desired file and click "OK"



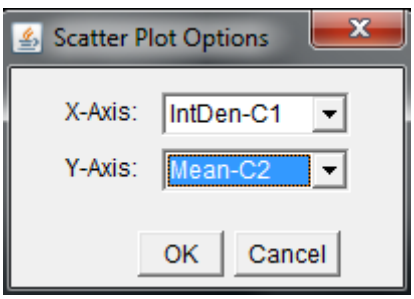
An additional window called "v_Stack" will open showing all images as stack.



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

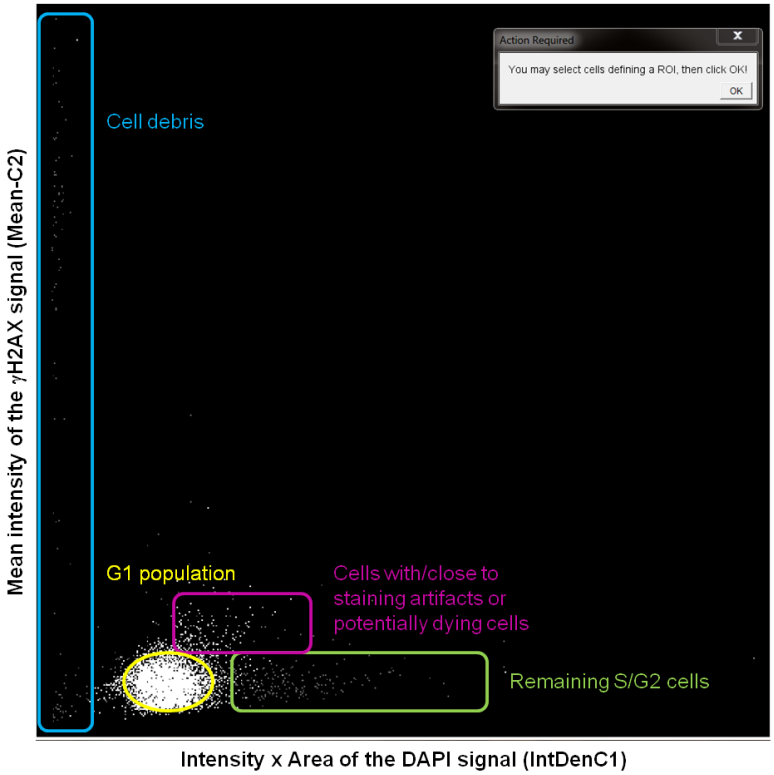


5. Click on  to create a scatter plot. Plot **IntDen-C1** (blue channel, DAPI) against **Mean-C2** (green channel, γH2AX).



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 samples containing more cells, your scatter plot might look like this example created by an extended data set with more than 5 thousand cells.



IMPORTANT: If you are analysing own samples with more than one folder containing subfolders with images to analyze, 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 are stored in a subdirectory of the path you selected. The subdirectory has the name of the original folder, in our example "Test_Images_ImageJ" These images are ready to be analyzed 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 analyze further in AutoFoci.