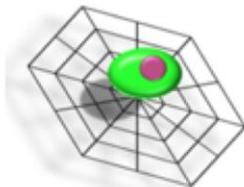


# ACCEPT MANUAL

Version 1.0.0 beta



**MIRA RESEARCH INSTITUTE**



CTCTrap



# ACCEPT Software 1.1.0 beta – Manual

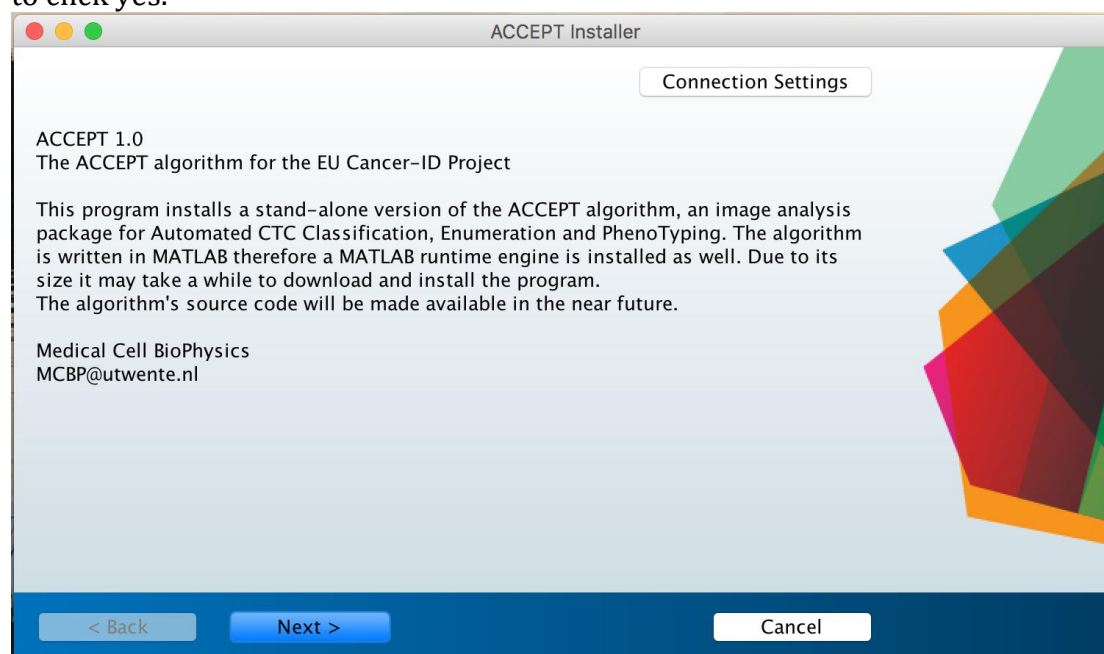
The **ACCEPT** Software developed within the EU Cancer-ID project is an open source image analysis package for the **A**utomated **CTC** Classification, **E**numeration and **P**henotyping. This version (Version 1.1.0 beta) contains the tool CTC Marker Characterization to quantify the expression of markers on pre-scored CTCs and a Full Detection tool in which all events in the images are detected and can be analyzed. Two more tools are under development; the Scoring Tool to provide a score of 1-5 to an prescored event, enabling comparisons between different operators, and a tool for automated CTC classification.

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## 1. Installation

To install the software, download the installer and double-click on it to start the installation. The start screen of the installation tool is shown in Figure 1. You can simply click “Next” on every screen and use the standard configuration and wait until the installation is finished. If you use a Windows computer, you are asked if you want to have a shortcut of the program on your desktop; it is recommended to click yes.



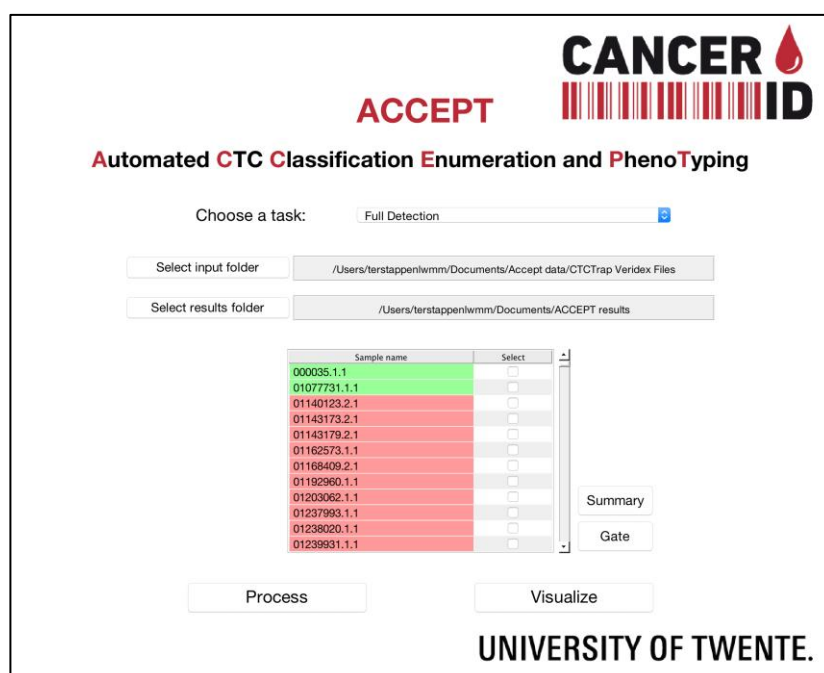
**Figure 1:** Start screen of the installation tool.

## 2. ACCEPT requirements for image files

The general image requirements are that the images contain an automated scan of the full scanning area (no thumbnails of cells) and should not be stitched together. Moreover, they should be raw .tiff files, that means no compressed files. The folder structure is flexible, but once we programmed a script to load your images, the folder structure from all exported scans should remain the same. If you can export an xml file that contains some sample/microscope information, please provide that as well. The current version of ACCEPT can read already read images acquired by CellSearch, DEPArray, the VyCAP puncher and the microscope systems used in the MCBP UT laboratories. For all other systems, a specific loader needs to be programmed before ACCEPT can handle these images.

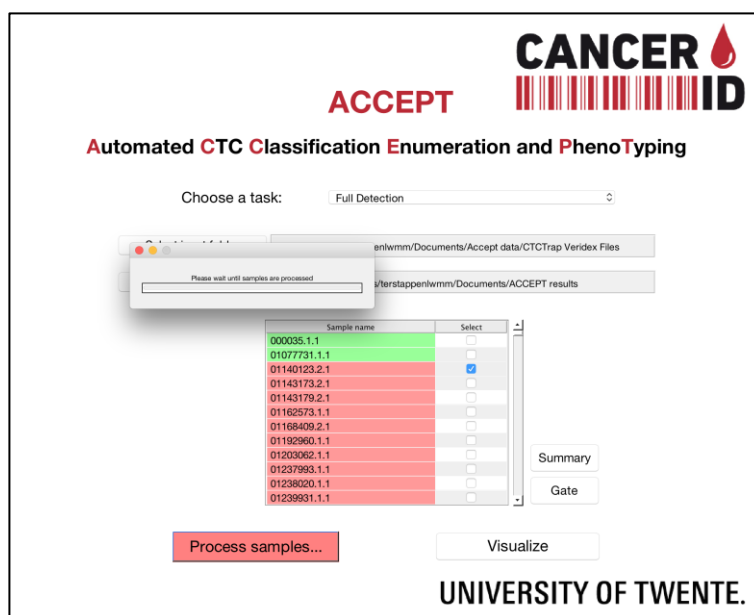
## 3. Processing your samples

After opening the ACCEPT program the display illustrated in Figure 2 appears. First one has to choose one of the four tasks 1. CTC Marker Characterization, 2. Scoring Tool (not active in this version), 3. Full Detection, or 4. Automated CTC Classification (not active in this version). In Figure 2, Full Detection is chosen as task. To process your samples, you first have to specify an input and results folder. First click on “Select input folder” and choose the folder where the samples you want to process are located. This should not be the sample folder itself! Next, choose a folder where you want to save the results. After specifying both links a list is updated below where all sample folders that were found in your input folder are listed (see Figure 2). Green colored sample names indicate that the samples are already processed with the chosen task, red colored sample names indicate that the samples still need to be processed. Note that this is always relative to the results folder that you have specified. If you already processed a sample but saved it in a different results folder, it will not be marked as processed. You can select the little box on the right side of every sample to indicate that you want to Process, Visualize, Gate or obtain a Summary of the performed analysis of the selected samples.



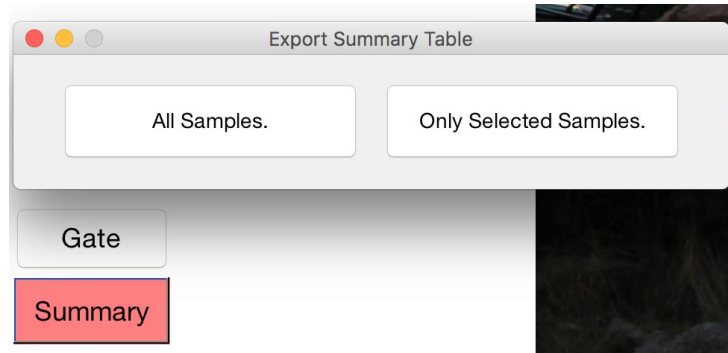
**Figure 2:** Opening display ACCEPT

Choose the samples you want to process by clicking on the little box (they will be marked in blue when they are selected). Click on Process and ACCEPT will start processing the selected samples. In Figure 3 the sample 1140123.2.1 was selected and the processing was started. As long as the selected samples are processed, the “Process” button turns red and shows “Process samples...” indicating that ACCEPT is busy and a pop-up “Please wait until samples are processed” appears. The time it takes to process the sample depends on the chose task, the capacity of the processor of your computer and the number of objects in the chosen sample. After the sample is processed, the sample name will turn green and one can visualize the data in a particular file by selection of the click box next to the file and select “Visualize”.



**Figure 3: Processing samples with ACCEPT**

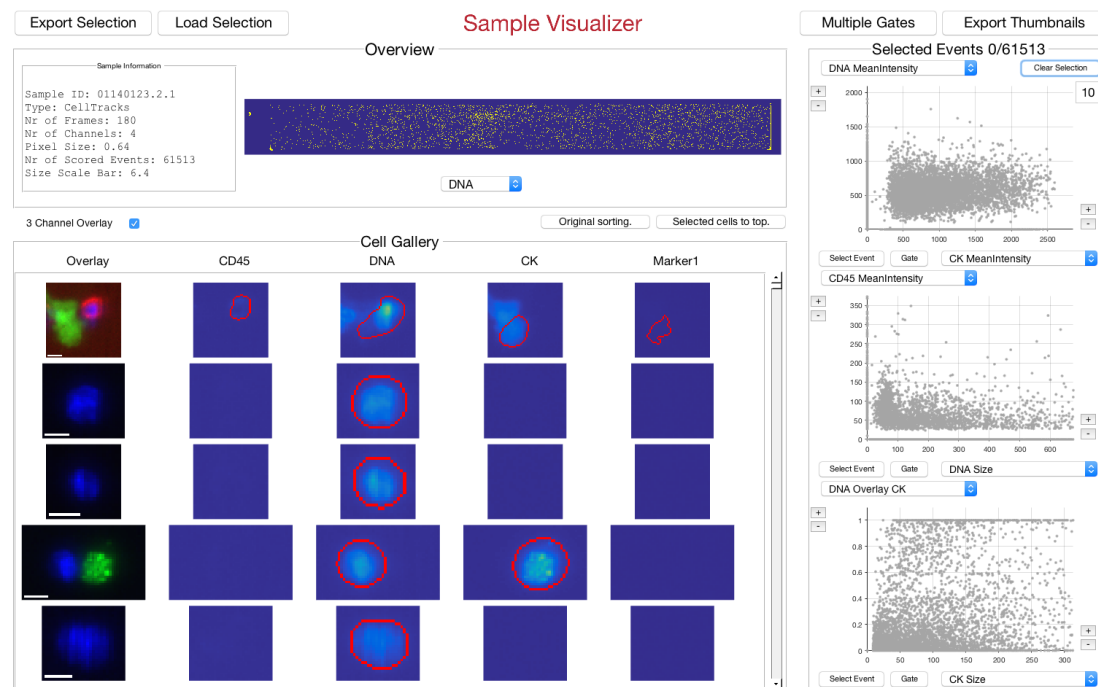
Apart from processing and visualizing the selected samples, you can also apply a gate or create a summary table of the results. These two functions require, that you processed the selected samples beforehand, that means they have to be marked green. A detailed description of the “Gate” button can be found in the next section; the only difference is if you use the button on the start screen (Figure 3) instead of the sample visualizer (Figure 5) the gates are applied to all selected samples and the results are directly saved and can be used later. If you click on the “Summary” button, you can first select, if you want a summary of all samples in the list or only the selected ones (Figure 4). You can then specify where you want to save the summary excel file. This excel file will contain for every listed sample the number of events that fall into a certain gate and will list all gates found in one of the listed samples.



**Figure 4:** Creating a summary table.

#### 4. Full detection

After the task “Full Detection” is performed, all objects in the images of the samples that are processed will be identified using the algorithms described in detail elsewhere (L. Zeune et al., Multiscale Segmentation via Bregman Distances and Nonlinear Spectral Analysis, SIAM Journal on Imaging Sciences 2017 10:1, 111-146). After the “Visualize” button is clicked it will turn red until the display of the Sample Visualizer as shown in Figure 5 opens. In the box Overview the sample information retrieved from the data files is contained and a display of all the

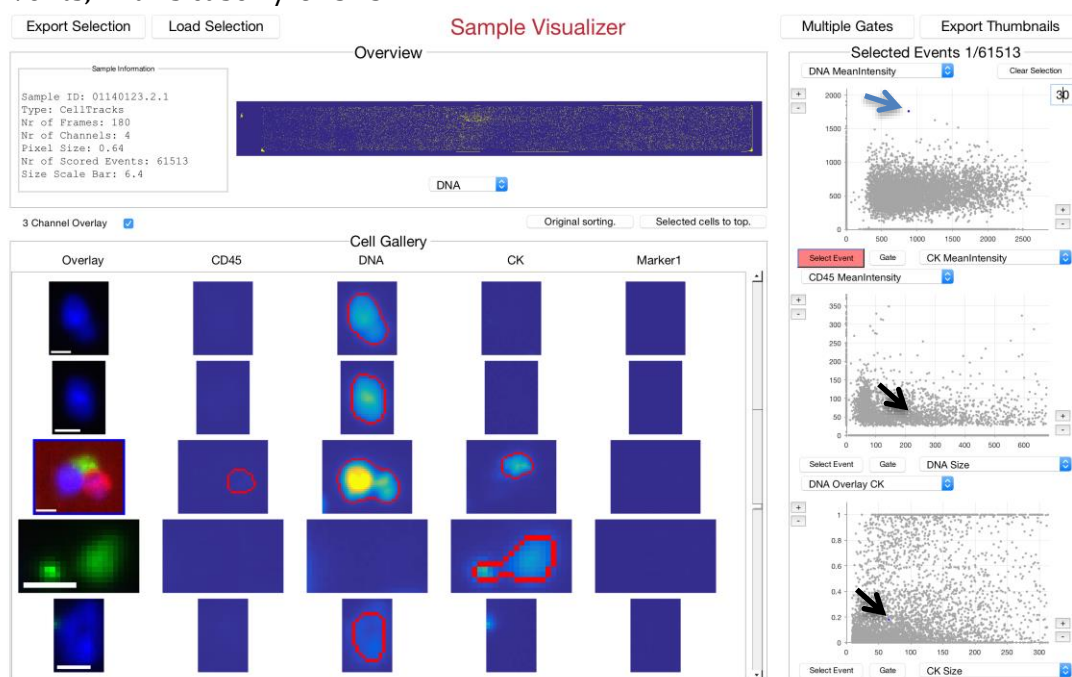


**Figure 5:** Visualize the results.

images in the sample. Below the display is a box indicating the channel that is illustrated. By clicking on the box one can alter the parameter.

On the right are three scatter plots. One can change the displayed features of all parameters by clicking on the box next to the x or y-axis. You can select the Area, Eccentricity, Perimeter, Mean Intensity, Max Intensity, Standard Deviation of the Intensity, Mass (Sum of the Intensity) and Perimeter2Area (a circularity measurement) for each channel. Also the possibility to use overlay between the

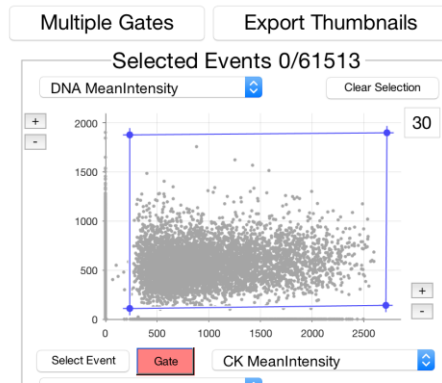
different contours can be chosen. The dots in the scatter plot represent all objects identified in the sample. By clicking on the + and – buttons next to the x or y-axis one can increase or decrease the scale at which the objects are displayed. By changing the number 10 in the top right corner of the top scatter plot one can alter the size of the dots. In this example 61513 objects are identified. Thumbnails of the objects are displayed for each parameter. The left thumbnail is an overlay of the first three parameters. Red is used for the first parameter in this case CD45, blue for the second parameter here DNA and green for the third parameter here CK (cytokeratin). The white scale bar in the overlay represents 10 pixels. In this example the pixel size is 0.64  $\mu\text{m}$  (retrieved from the sample information) and thus the bar has a size of 6.4  $\mu\text{m}$ . The red lines in the thumbnails show the contour of the object as identified by the image analysis algorithm. One can visualize all objects by using the scroll function at the right of the thumbnails. By pushing the select button under each scatter plot one can click on an object, the thumbnail associated with this object appears in the thumbnails and is highlighted by a blue contour drawn around the overlay thumbnail. Figure 6 shows an example in which an object is identified in the top scatter plot (position indicated with a blue arrow). The object also appears in the other scatter plots and is identified by the black arrows. The number of objects selected is indicated by the number after Selected Events, in this case 1/ 61513.



**Figure 6:** Selection of an object

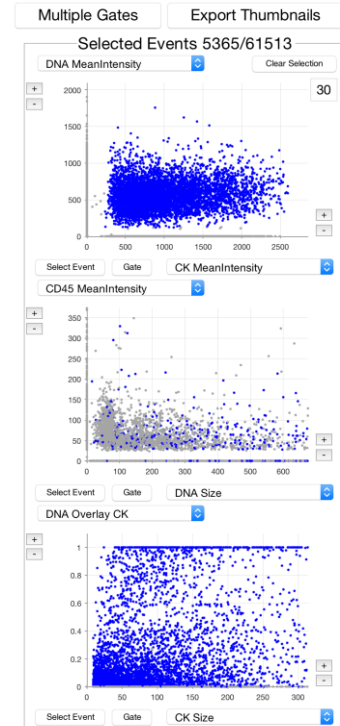
Next to the Select Event button is the Gate button. Pushing this button allows one to indicate a region in the scatter plot by selection the corners of the desired region and closing the region by returning to the starting point (a blue open circle appears when reaching the starting point). Figure 7 shows a gate set on the scatter plot. Figure 8 shows the results of the gate in this case 5365 of the 61513 objects fall within the gates and are depicted blue.





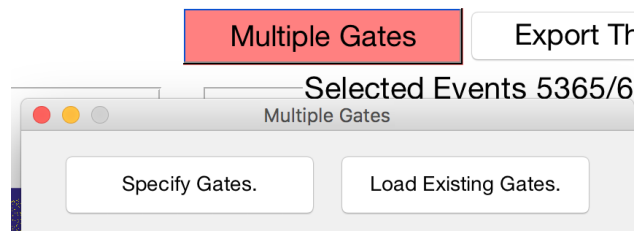
**Figure 7: Gating of objects**

To visualize the thumbnails associated with the gated events one can push the button “Selected cells to top”. For the simultaneous use of more features from the measured parameters one can use the “Multiple Gates” feature.



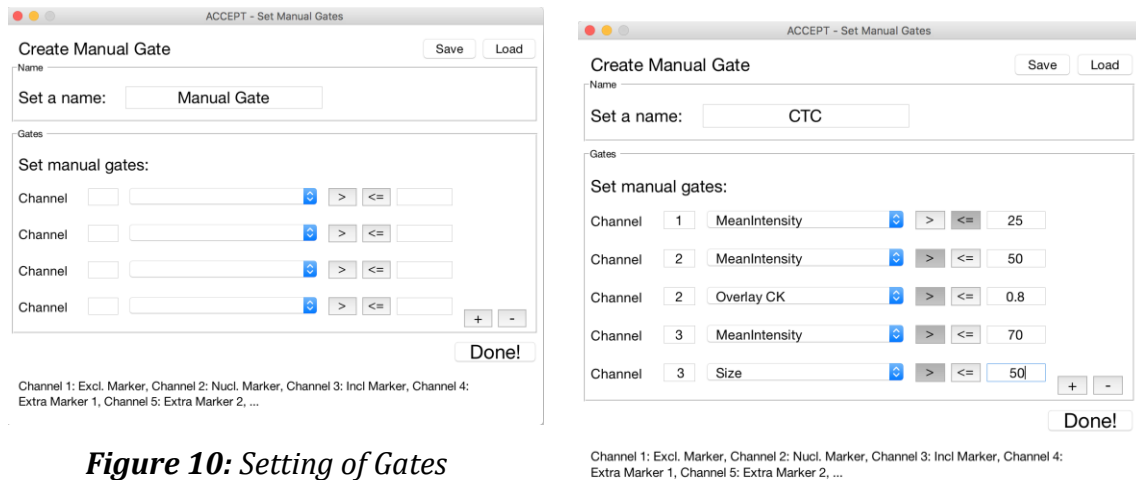
**Figure 8: Gated objects**

After a click on the button the display in Figure 9 shows up and one can select a new gate (“Specify Gates.”) or use an already existing gate (“Load Existing Gates.”). After choosing the Specify Gates button, the display on the left of Figure 10 appears. In the box “Set a name” the “Manual Gate” will have to be replaced by the



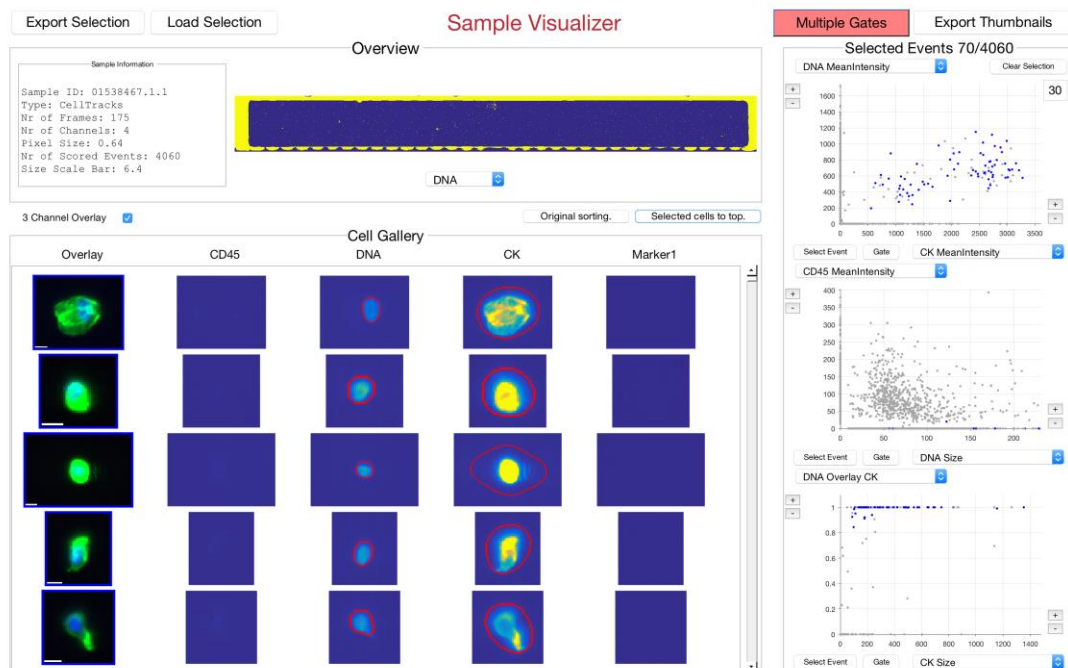
**Figure 9: Multiple Gates**

name one would like to assign to the gate. Under “Set manual gates:” the channel number has to be indicated and next to it the feature that one would like to use and next to it the value either > or <=. In case more features are needed one can click the “+” button and more gates can be added. In the display on the right of Figure 10 the name of the Manual Gate is set to CTC and five features are used to arrive at the definition of the CTC gate. The “Save” button is needed to save the gate settings, the “Load” button can be used when one would like to load an existing gate and by pressing the “Done!” button the display will disappear and the results of the chosen gate combination will be visualized as shown in Figure 11. If you already saved a gate combination before, you can directly load these gates by clicking “Load existing Gates.” in Figure 9.



**Figure 10: Setting of Gates**

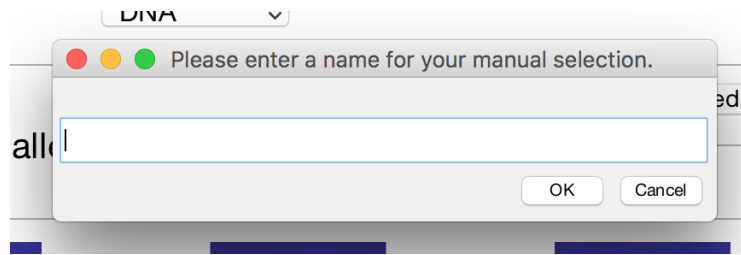
From the 4060 objects in this sample 70 fall within the settings used for the CTC gate and are being displayed blue whereas all other objects remain grey. In this case the Mean Intensity of Channel 1 representing CD45 staining was set at smaller or equal to 25. The Mean Intensity of Channel 2 representing staining of the nucleus was set as larger than 50. The overlay of the contours between DNA and Cytokeratin was set as larger than 0.8. The Mean Intensity of CK was set at larger than 70 and the size of the CK was set at larger than 50.



**Figure 11: Visualization of Multiple Gates**

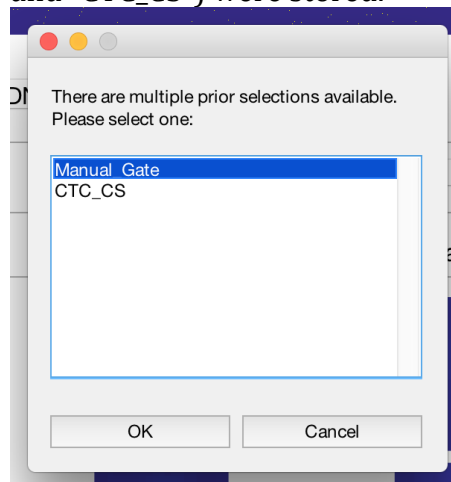
If you want to save a made selection, you can click on the “Export Selection button” and specify a name for your selection (see Figure 12).





**Figure 12:** *Saving your current selection.*

Previously stored selections or gates can be loaded by the “Load Selection” button. If multiple selections are stored for the sample that is shown, you can select which one you want to visualize. Figure 13 shows an example; in this case two selections (named “Manual Gates” and “CTC\_CS”) were stored.



**Figure 13:** *Loading a saved gate/selection.*

In case you want to store all or only the selected thumbnails as .tiff files on your hard drive (for example if you want to use them for a publication), click “Export Thumbnails”. The saved thumbnails can be found in the results folder specified in Figure 2.

## **5. CTC Marker Characterization**

The aim of the Marker Characterization tool is to evaluate the expression of certain markers on prescored CTCs. That means, instead of loading all images only small thumbnails of cells are loaded and evaluated. These cells can be either selected by the software of the machine used to record the images (like the CellSearch or DepArray system) or using the ICY plugin in case no such software is provided. The visualization is similar as described before but only these prescored cells are shown in the scatter plots and thumbnails.

## **6. Scoring Tool**

Under development.

## **7. Automated CTC Classification**

Under development.

#### **4. Contact details**

If you have any comments, suggestions or questions concerning this tool please do not hesitate to contact Leonie Zeune ([l.l.zeune@utwente.nl](mailto:l.l.zeune@utwente.nl)) or Guus van Dalum ([g.vandalum@utwente.nl](mailto:g.vandalum@utwente.nl)), MCBP group, University of Twente. As this work is still in the stage of development we will constantly provide updates of the software and are glad for every feedback.