Tutorial for cell nucleus detection and counting on histochemically stained tissue microarray (TMA) images with Color Deconvolution.

<http://www.nexus.ethz.ch/> -> Software -> TMARKER

# Prerequisites:

* Java 1.7 (Runtime Environment JRE).
* Tissue images of IHC stained tissue (see DemonstrationData.zip)

# WorkFlow

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| 1. Open TMARKER. |  |
| 2. Drag and Drop the images (one image per TMA spot) from the demonstration dataset (Folder “RCC”) into TMARKER.  (Or alternatively click *“File -> Open…”* and select the images). |  |
| 3. Set the nucleus radius to 5. |  |
| 4. Select all images that you want to process with nucleus detection. |  |
| 5. Open the “**Color Deconvolution**” plugin in the tools and use following parameters in the upcoming window:  *Staining Protocol* = H DAB.  *Tolerance* = 5  *Blur* = 2  *T\_hema* = 55  *T\_dab* = 110  Click on „Estimate“.  This is the ***Color-Deconvolution*** method. The two color channel images Hema and DAB are separated. They can be seen by selecting “Display Channel 1” or “2” in the plugin after click on “Estimate”.    ***How are nuclei detected?***  The two channels are blurred and local intensity minima are detected in the image. A local minimum is accepted if it is below the threshold and if it has a minimum difference to the neighboring local minimum of *tolerance*.  Accepted nuclei are filtered not to overlap to each other according to their radius. |  |
| ***Repeat step 5 with different parameters until you are satisfied with the result. TMARKER gives immediate feedback in the preview images and recalculates fast the detected nuclei.*** You might want to select the optimal parameters on one image and then process all images. | |
| 6. The nuclei are displayed in the main window as “***Unknown***” nuclei (since they do not have a known cancer/benign state – they are just detected).  As the nuclei arise from the *Hema* (unstained) channel and from the *DAB* (stained) channel, they appear in the “***Clear***” and “***3+***” column of the TMA List.  *Example here: 2.2k blue nuclei (unstained) and 2.6k brown nuclei (stained) have been found (54% stained).* |  |
| ***TIP:*** You might want to adjust the shape and the color of the nuclei for better visualization:  **Shape:** Click on *Tools -> Options -> Layout* and select “Circle”.  **Color:** Double click on the *Unknown* Label and select the color. |  |
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| 7. Save the analysis output.   1. As a HTML Report. Go to “*File -> Save As…*” and select a new HTML file.   A HTML report with a sub-folder for the needed image files is created. |  |
| 1. Save the analysis as XML which can be reloaded in future TMARKER sessions.   The stored XML file contains all program options and all TMA spots and their nuclei. The file can be opened (or drag’n’dropped) in the next TMARKER session.  **The raw images have to co-exist on the hard disk (they are not included in the XML file itself).** |  |