**Display Range:** X to Y. Display range is *purely* for the purpose of better visualization. *It is not used for segmentation*. When displaying images for membrane or antigen, any pixel with intensity value below X will be displayed as complete black and any pixel with intensity value above Y will be displayed as complete white.

**Parameters related to the Antigen Channel:**

**T antigen**: Abbreviation for ‘Threshold of antigen’. Any pixel in the antigen channel with intensity >= **T antigen** will be classified as antigen. The segmentation result will be further filtered by **Size antigen**.

**Size antigen**: X to Y. Among all the pixels classified by **T antigen** as antigen, the size (number of pixels) of a cluster of connected pixels must be in the range of [X,Y) so as to be considered as antigen. X is the lower limit and is intended to remove ‘salt pepper noise’ caused by random fluctuations of pixel values. Y is the upper limit and is intended to remove giant particles that is clearly not antigens (e.g. dust, cell aggregates).

**Background antigen**: This value is not used for segmentation of antigen. It is an estimation of the mean value of the background noise in the antigen channel. When calculating the percentage of antigen intensity on membrane, this estimated background value will be subtracted from the antigen pixel value.

When clicking the ‘Estimate’ button, the background value is estimated by: 1) segmenting the antigen channel by T antigen 2) then remove connected pixels with size below lower limit of ‘Size antigen’ 3) then calculate the mean value of all pixels that are not antigen.

**Parameters related to the Membrane Channel:**

**T membrane**: Abbreviation for ‘Threshold of membrane’. Any pixel in the membrane channel with intensity >= **T membrane** will be classified as membrane. The segmentation result will then be ‘filled’ to form ‘cell’ which will be further filtered by **Size cell**.

See here for how the ‘fill’ is done: <https://www.mathworks.com/help/images/ref/imfill.html>

**Size cell**: X to Y. Among all the pixels classified by **T membrane** as membrane and then filled to become cell, the size (number of pixels) of a cluster of connected pixels must be in the range of [X,Y) so as to be considered as cell. X is the lower limit and is intended to remove ‘salt pepper noise’/broken cells. Y is the upper limit and is intended to remove clusters of cells that are overlapping with each other.

**Parameters related to selection of Valid Cells:**

**T cytosol ratio**: Abbreviation for ‘Threshold of cytosol ratio’. For a cell to be considered as a valid cell, the ratio of cytosol to whole cell (in other words, percentage of cytosol) must be above **T cytosol ratio**. The general idea is to exclude cells with abnormally high percentage of membrane.

**T antigen pixels**: Abbreviation for ‘Threshold of number of antigen pixels’. For a cell to be considered as a valid cell, the number of antigen pixels in that cell must be above **T antigen pixels**. To make a reliable estimation of antigen on membrane ratio, it is desirable for a cell to have enough amount of antigen pixels.

**Parameters related to plot of Valid Cells:**

**N cells to plot**: Number of cells to plot. Each cell gets one image. For example, setting this value to 10 will lead to 10 images. Plotting a large number of cells (>50) might take a few minutes to finish.

**Sub image width**: Each image is composed of six sub-images whose width is specified by sub image width (unit is pixel). The three images on the first row: 1) Overlapped images of antigen and membrane channel. 2) Mask of membrane. 3) Mask of cytosol. The three images on the second row: 4) Mask of antigen. 5) Mask of antigen on membrane. 6) Mask of antigen in cytosol.

**Image width and Image height**: Width and height of the image.

Columns in results\_filtered.csv and results\_not\_filtered.csv

Each row corresponds to a cell. Cells in results\_not\_filtered.csv are not selected by **T cytosol ratio** and **T antigen pixels**. Cells in results\_filtered.csv are selected.

**Column 1:** Number of pixels classified as cytosol.

**Column 2:** Number of pixels classified as cell (cell = membrane + cytosol).

**Column 3:** Number of pixels classified as antigen in cytosol.

**Column 4:** Number of pixels classified as antigen.

**Column 5:** Sum of intensity of pixels classified as antigen in cytosol.

**Column 6:** Sum of intensity of pixels classified as antigen.

**Column 7:** Percentage of antigen intensity on membrane (1-Column 5/Column 6).

**Column 8 and 9**: x and y coordinates of the center of the cell.