



How to count PLA signals in counterstained areas
using Duolink ImageTool

OLINK
BIOSCIENCE

FIRST APPROACH: SET GREEN NUCLEI



- ▶ Choose “green” as nuclei channel instead of “blue”.
- ▶ Info about how many PLA signals that lie within the area defined as nuclei is thus obtained. In this case that area is the counterstained area.
- ▶ Drawbacks when following this approach:
 - It can be difficult to set the parameters so that the program can identify the counterstained area. One tip is to have a pretty high value on nuclei separation and a low value on nuclei intensity (both parameters are located under advanced settings).
 - With these settings the program gives no value on how many “real” nuclei there are, which means that one can/will get a wrong value on how many cells there are in the image.

FIRST APPROACH: SET GREEN NUCLEI



The screenshot displays the Duolink Image Tool interface. The main window shows a fluorescence image of cells with green and red signals. A specific cell, Cell ID 7, is highlighted with a yellow outline. A tooltip for Cell ID 7 provides the following data:

- Cell ID 7
- Signals in Nucleus: 110
- Signals in Cytoplasm: 20
- Area: 19427

The left sidebar contains several control panels:

- Image Database:** Shows the current image name "couterstaining test".
- Fluorescence:** Includes "Cell properties" (Nuclei size: 25, Cytoplasm size: 239), "Signal properties" (Signal threshold: 505, Signal size: 1), "Channel properties" (Signal channel: R, Nuclei channel: G), and "Advanced properties" (Nuclei separation: 51, Nuclei intensity threshold: 50).

Red arrows point to the "Signal channel" dropdown (set to R) and the "Nuclei channel" dropdown (set to G) in the Channel properties panel. Another red arrow points to the "Nuclei separation" checkbox in the Advanced properties panel. A third red arrow points to the "Analyze" button at the bottom of the interface.

At the bottom of the window, the status bar shows dimensions: 1388 x 1040 x 1, and color calibration values for R (95), G (255), and B (35).

FIRST APPROACH: SET GREEN NUCLEI



The screenshot displays the Duolink Image Tool software interface. The main window shows a fluorescence image of cells with green nuclei and yellow cytoplasm. A tooltip for Cell ID 6 provides the following data:

- Cell ID 6
- Signals in Nucleus: 105
- Signals in Cytoplasm: 6
- Area: 13018

The left sidebar contains several property panels:

- Image Database:** Lists 'counterstaining test2' and 'counterstaining test'.
- Fluorescence:** Includes 'Cell properties' (Nuclei size: 25, Cytoplasm size: 239, Exclude cells touching the border: checked), 'Signal properties' (Signal threshold: 112, Signal size: 1), 'Channel properties' (Signal channel: R, Nuclei channel: G), and 'Advanced properties' (Nuclei separation: 65, Nuclei intensity threshold: 67).

Red arrows point to the 'Signal channel' dropdown (set to 'R') and the 'Nuclei channel' dropdown (set to 'G') in the Channel properties panel. Another red arrow points to the 'Analyze' button at the bottom of the interface.

At the bottom of the window, the status bar shows dimensions: 1388 x 1040 x 1, and color values: X: 630, Y: 529, R: 123, G: 255, B: 9. There are also percentage indicators (0%) and a 'STOP' button.

SECOND APPROACH: REGION OF INTEREST



- ▶ Keep the ordinary channel settings with “red” signals and “blue” nuclei.
- ▶ Use the tool for “Region of interest” to mark the area that has to be analysed and perform the analysis.
- ▶ If very small areas need to be selected as region of interest, it may be necessary to import images with a higher resolution.

SECOND APPROACH: REGION OF INTEREST



The screenshot displays the Duolink Image Tool interface. The main window shows a fluorescence image with a large green ROI polygon drawn over a cell. A tooltip over the ROI displays the following data:

Total Signals	113
ROI Area	22587

The interface includes several panels:

- Image Database:** Lists the current image as "couterstaining test".
- Cell properties:** Nuclei size (pxls) 25, Cytoplasm size (pxls) 239, Exclude cells touching the border (checkbox).
- Signal properties:** Signal threshold 504, Signal size 1.
- Channel properties:** Signal channel R (Red), Nuclei channel B (Blue).
- Advanced properties:** Nuclei separation 51, Nuclei intensity threshold 73.
- Single Cell Results:** A table with columns Image, ID, SN, SC, NA. It lists two rows for "couterstaining test".
- Summary results:** A table with columns Image, SN, SC, TS, NC. It lists one row for "couterstaining test".

At the bottom, a toolbar contains icons for Preview, Analyze, and ROI selection. A red arrow points to the ROI selection icon. Below the toolbar, a text instruction reads: "Draw a number of polygons to define ROI. Right click on the polygon to remove." The status bar at the bottom shows dimensions (1388 x 1040 x 1) and color channel values (x: 607, y: 159, R: 4, G: 2, B: 7).

SECOND APPROACH: REGION OF INTEREST



Draw a number of polygons to define ROI. Right click on the polygon to remove.

Dimensions: 1388 x 1040 x 1

x	y	R	G	B
482	339	8	8	6

Image	ID	SN	SC
counterstaining test2	BG	0	27
counterstaining test2	1	9	75
counterstaining test	BG	0	14
counterstaining test	1	24	114
counterstaining test	2	21	38
counterstaining test	3	11	37
counterstaining test	4	3	28
counterstaining test	5	4	35
counterstaining test	6	17	156
counterstaining test	7	6	30
counterstaining test	8	6	60
counterstaining test	9	2	29
counterstaining test	10	32	79
counterstaining test	11	4	20
counterstaining test	12	2	14

Image	SN	SC	TS
1 counterstaining test2	9	75	111
2 counterstaining test	202	1052	1268