Duolink®

# 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 tips 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.

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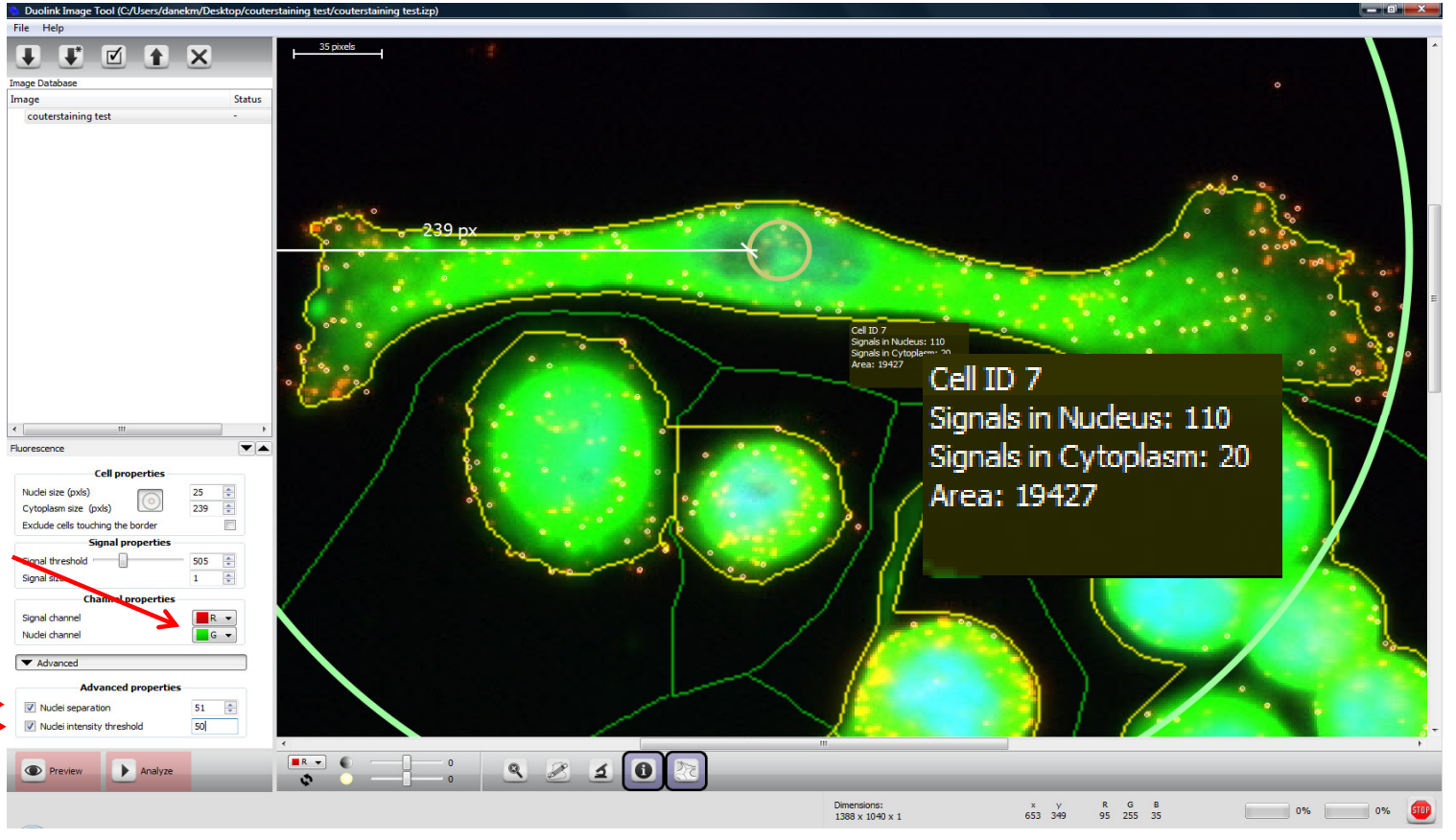

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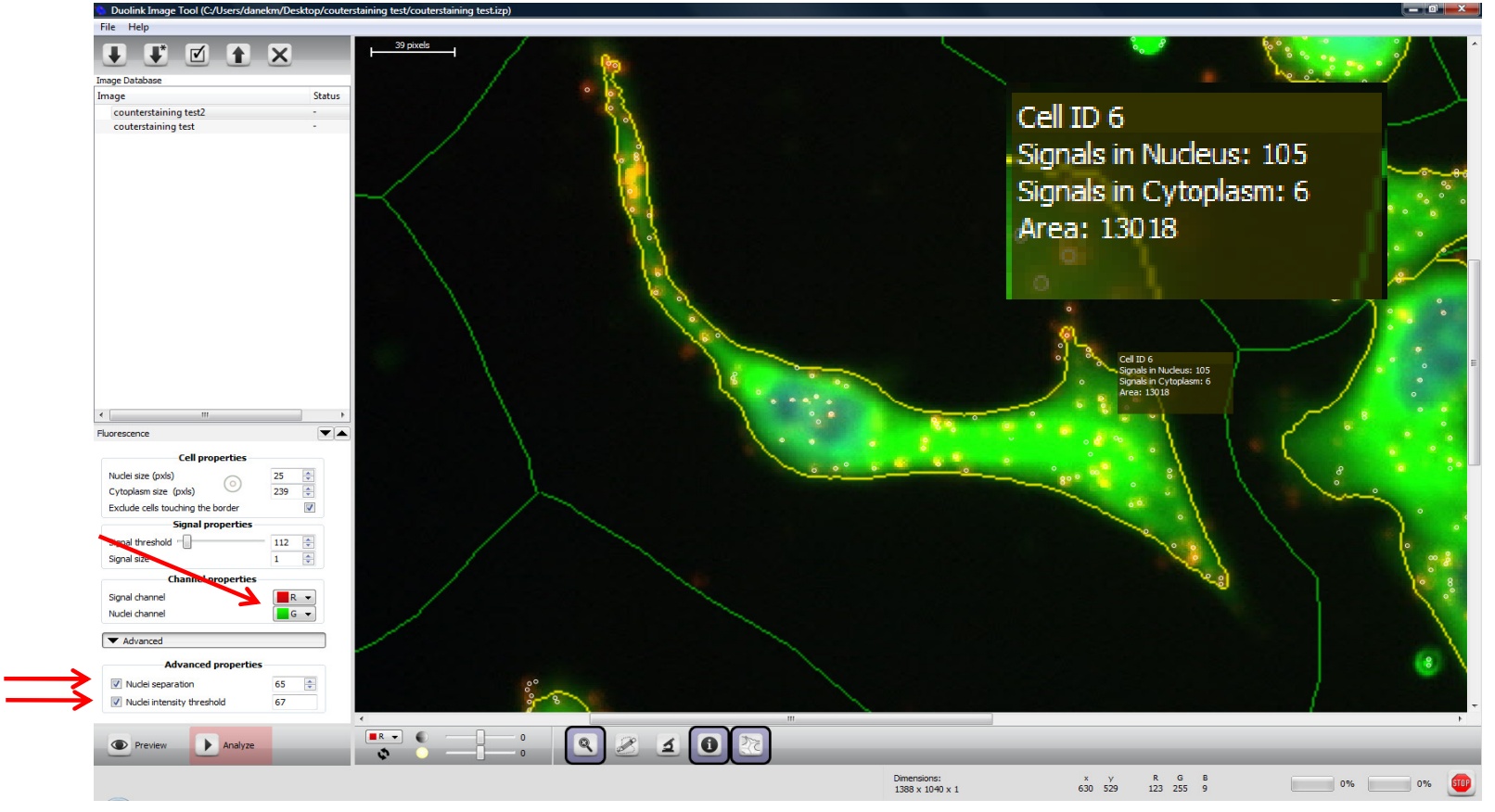

# SECOND APPROACH: REGION OF INTEREST

- 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.

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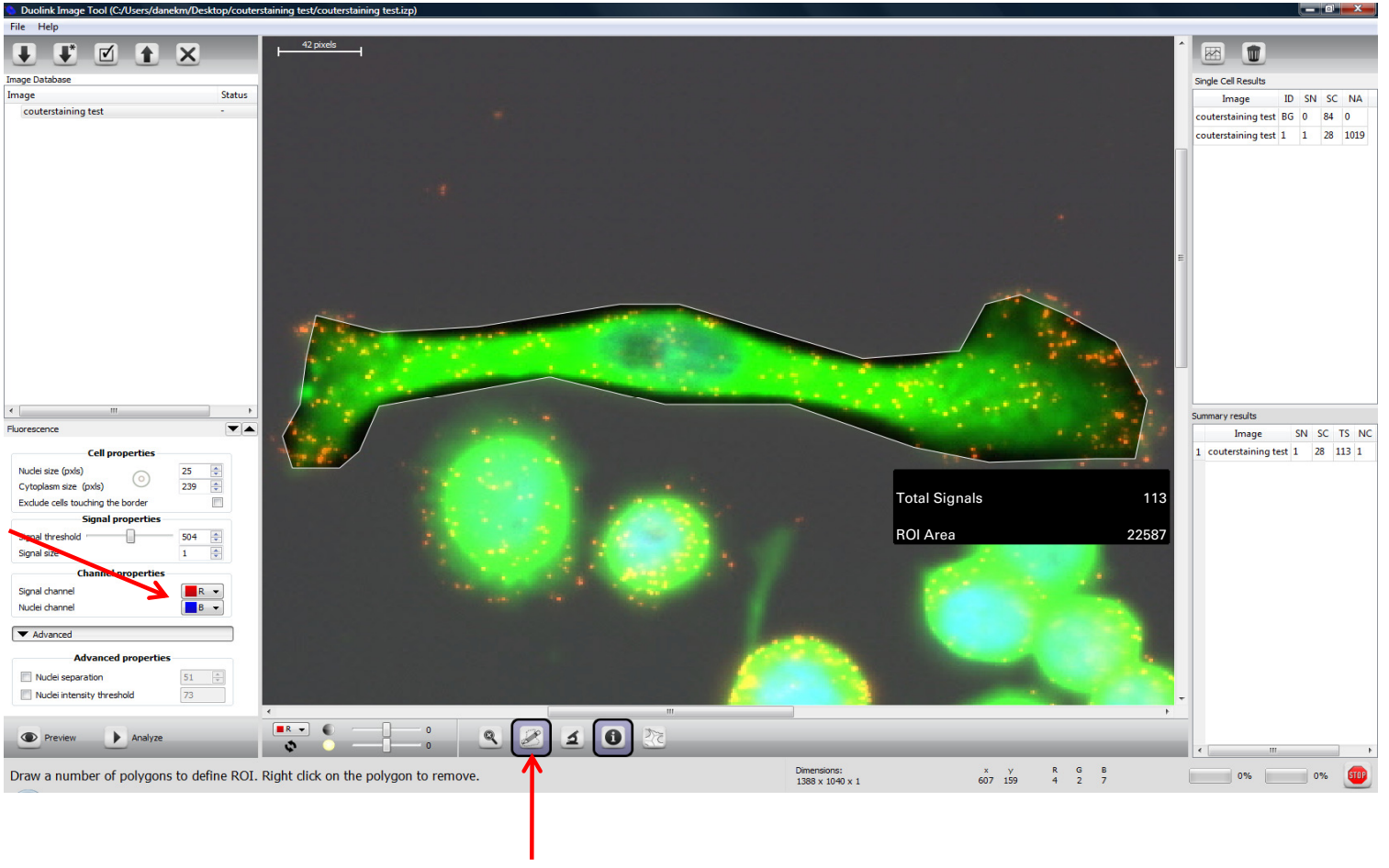

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