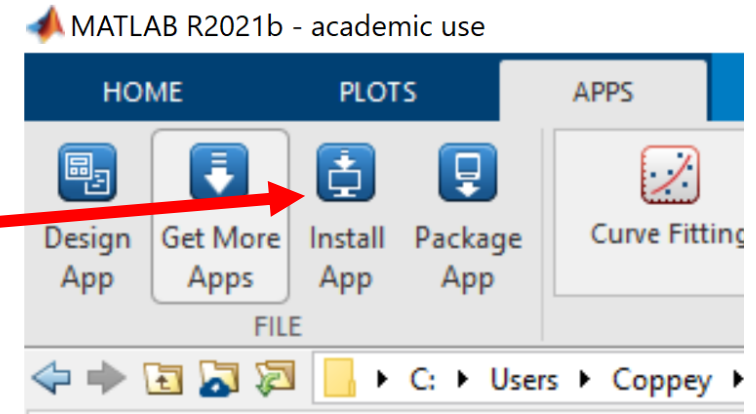# Foci analysis

For the software:

➢ If you have matlab (R2021b), just install the app

➢ If you don't have matlab, install the .exe file and the matlab runtime:
https://fr.mathworks.com/products/compiler/matlab-runtime.html

For the images:

➢ The soft works for two single TIFF images, which corresponds to the two channels to be quantified

➢ The two images need to be of the same size, and with no shift (perfect registration, no chromatic aberrations). There should be only one nucleus per image to be analyzed (crop images in imageJ if needed)

➢ It is better if images are background subtracted (in imageJ, measure the background using a rectangle in a black zone and substract the value to the image)

➢ The images can be taken as single plane of a multistack, or max intensity projection of the whole stack (or part of it)

Load here the first image that will be used to find peaks

Load here the second image that will be used to see if peaks colocalize

Move the cursor to change the value for segmenting the nucleus

Crop the image to fit full screen

Move the cursor to fit peaks on the image, and modify here the mean distance between peaks
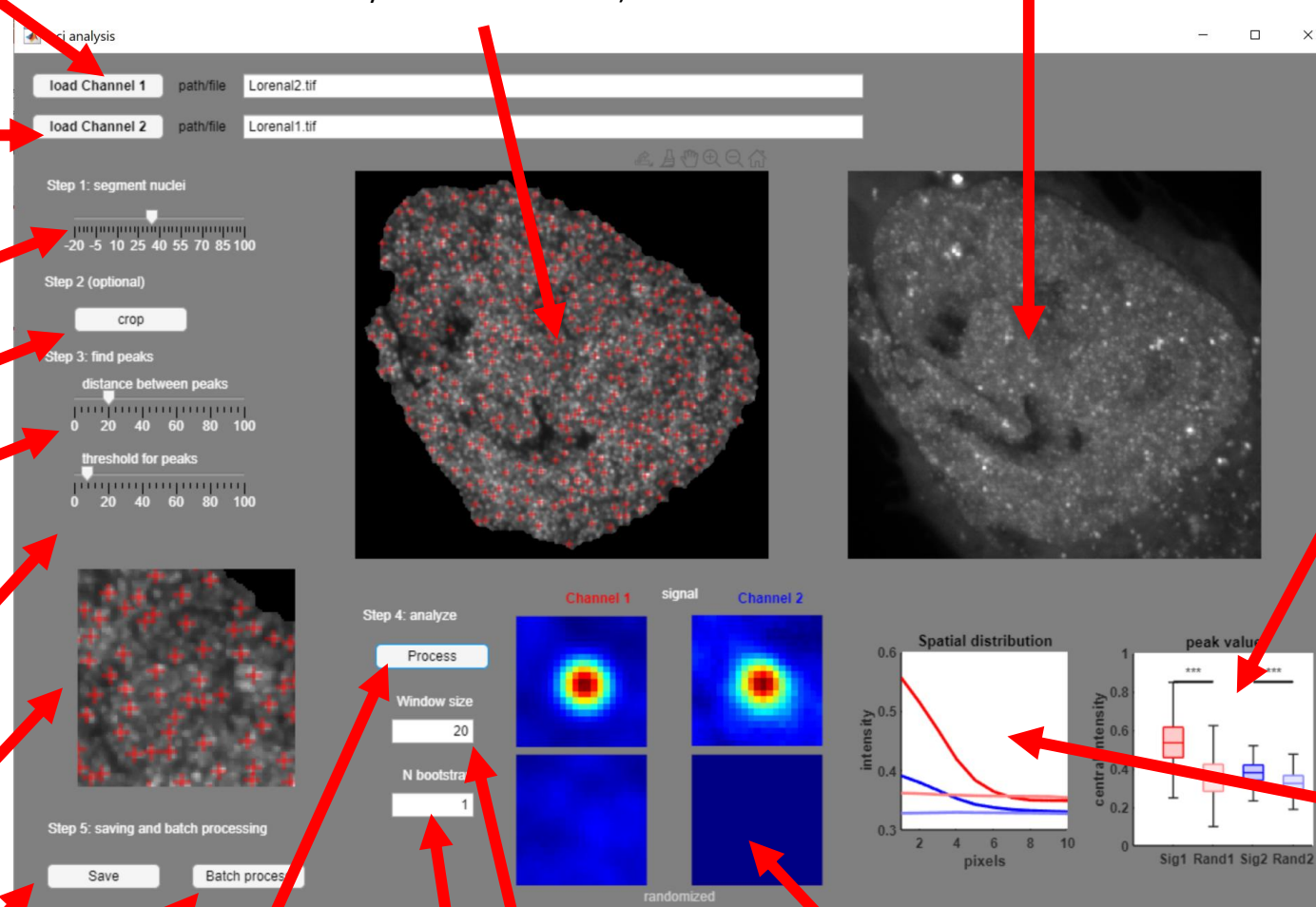
modify here the minimal value accepted for a peak

Zoomed snap of the image of channel 1 to see if peaks are localized where you want

Click here to save data (at the end, after processing data)

No working yet

Click here to perform the analysis when you're happy about peaks

Specify the size (in pixel) of subimages used to quantify intensities locally

Specify the number repetitions of the random generation of peaks

Image of channel 1 (peaks are overlayed with red crosses)

Image of channel 2

Boxplot showing the values of the peaks (central point) for all peaks. In red, peaks of channel 1 (light red is for randomized position of the peaks in the image). In blue, peaks of the channel 2 taken at the position of the peaks found in channel 1 or randomly (light blue). If channel 2 colocalize with channel 1 on peaks, there will be a statistical difference between signal and randomized.
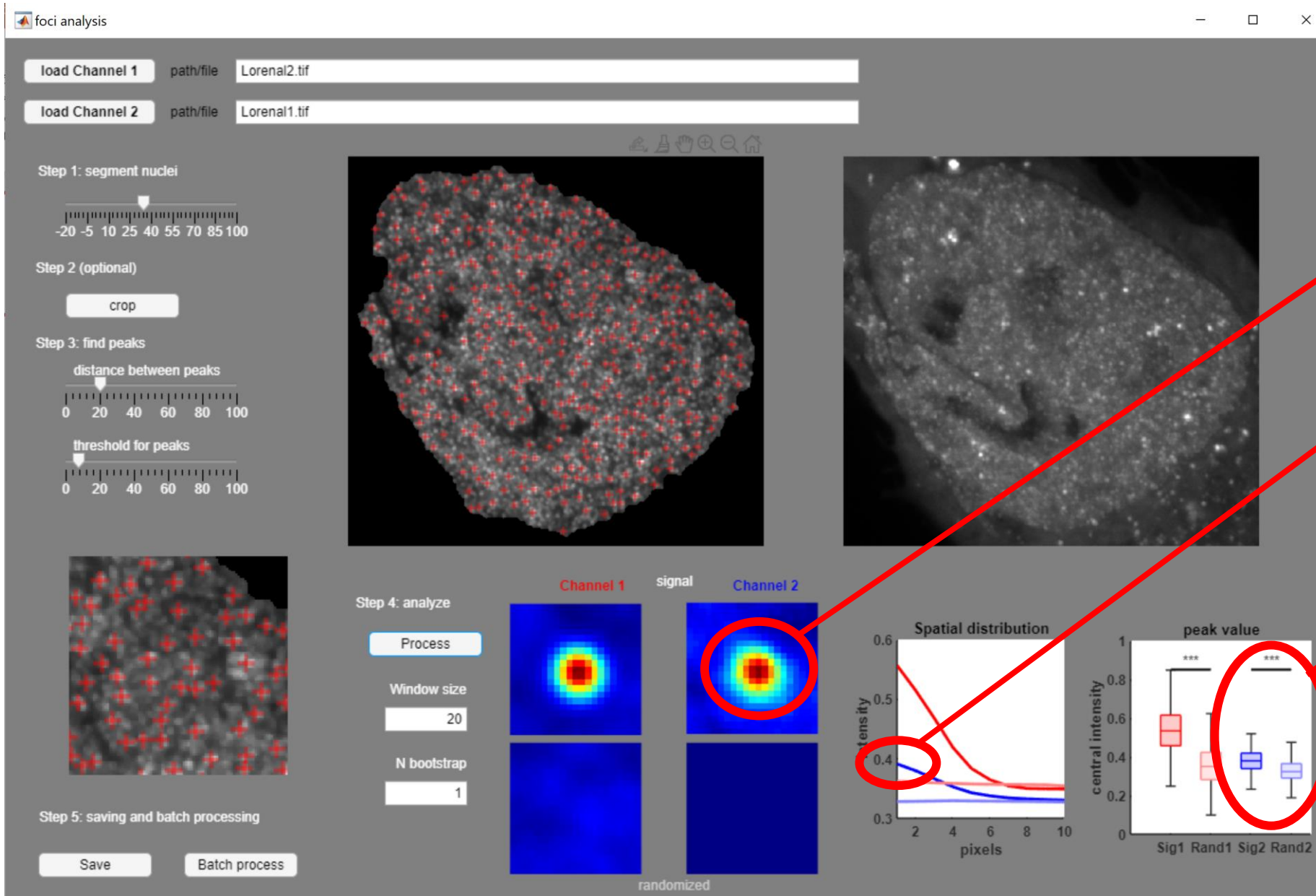
Radial plot of the intensities in the averaged images of peaks. Light color are for randomized peaks.

Average image of channel 1 at peaks found in channel 1 (top left). Average image of channel 2 at peaks found in channel 1 (top right). If a peak is seen in this image, it means that channel 2 tends to form foci at channel 1 foci. Bottom, average images when peak positions are randomized in the nucleus.

Example: Lorena's data (EWS-FLI1 and EWS-SSPB-GFP)

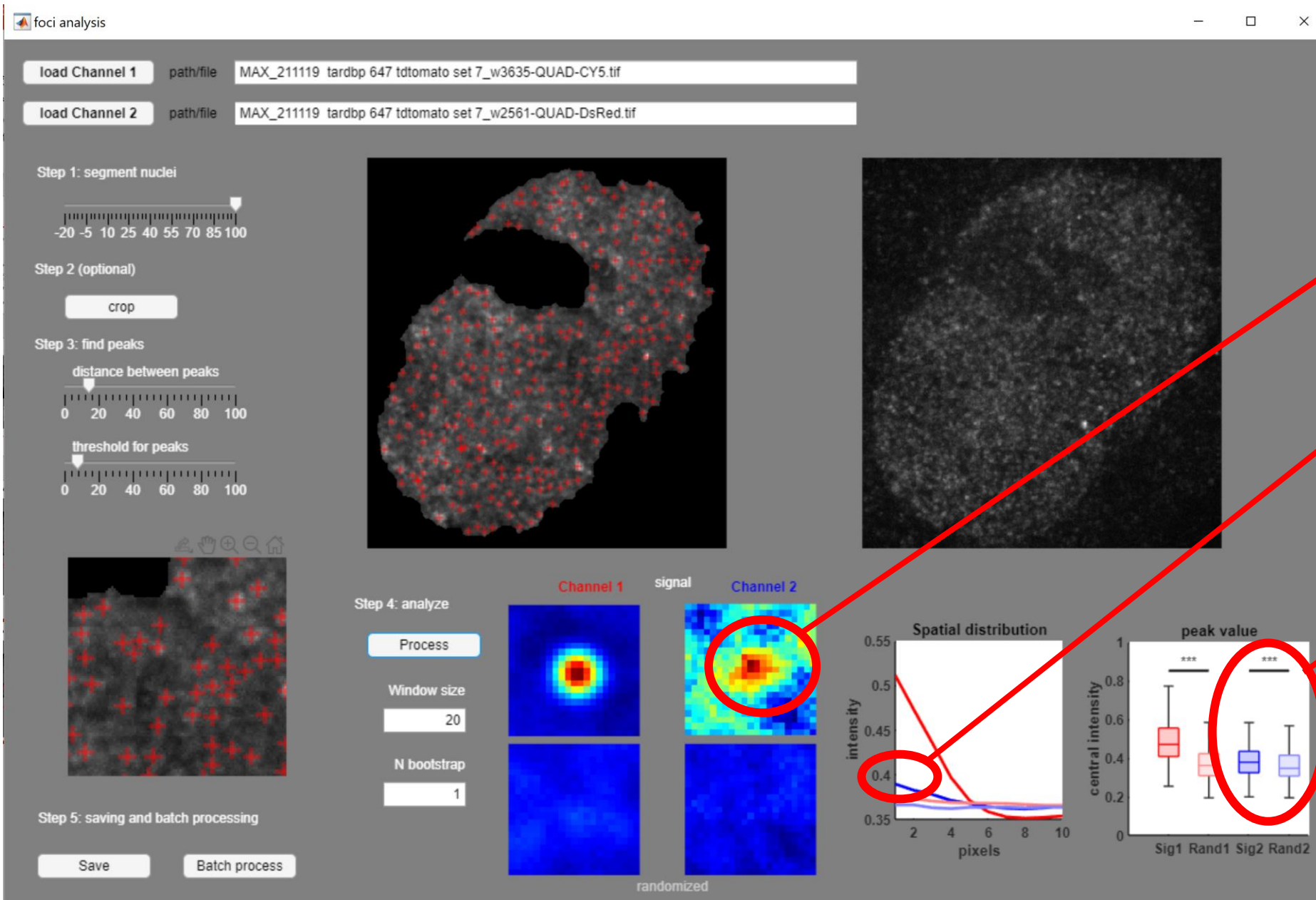# Example: Calvin's data (EWS-FLI1 and TARDBP)

There is a peak seen in channel 2

The peak in channel 2 is quantified here

There is a significant increase of intensity at peaks in channel 2 compared to randomized data

→ TARDBP colocalizes with EWS-FLI1