Quick Reference

## GeoMx® DSP: Creating Geometric Segmentation Masks using ImageJ: Contouring

This workflow describes geometric segmentation, as opposed to fluorescent segmentation based on morphology marker signal.

1

Please request copies of the custom ImageJ scripts from **GeoMxSupport@nanostring.com**. Two types of contouring scripts are available: i) Concentric or ii) Non-concentric.
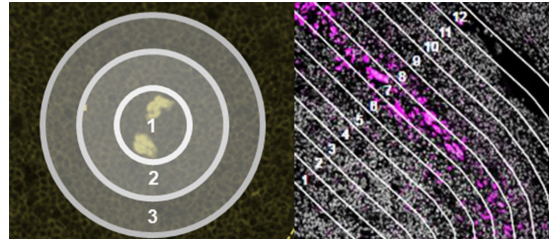
Concentric   Non-Concentric

2

To perform these steps, you must be working on a computer that has ImageJ installed. If the GeoMx DSP is not networked, remote access as described in Step 3 is not possible. Instead, use a USB to transfer ROI and mask files to and from the instrument.

3

From the external computer, **open ImageJ**. Then, open Chrome and access your GeoMx workspace remotely:
- Browse to **https://geomx-####** where #### is the GeoMx instrument number; find this under **Administration tab >> Network >> Machine Name**.

4

In the GeoMx Scan Workspace, create or select a geometric ROI. The segmentation masks you create in subsequent steps will be within this ROI border.
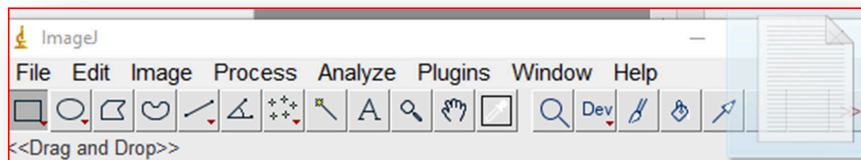
5

On the left panel where the ROIs are listed, click the "**Export image**" button (red box, right) underneath the ROI details. It will save the ROI as a stacked TIFF image. Note where the .tif is saved.
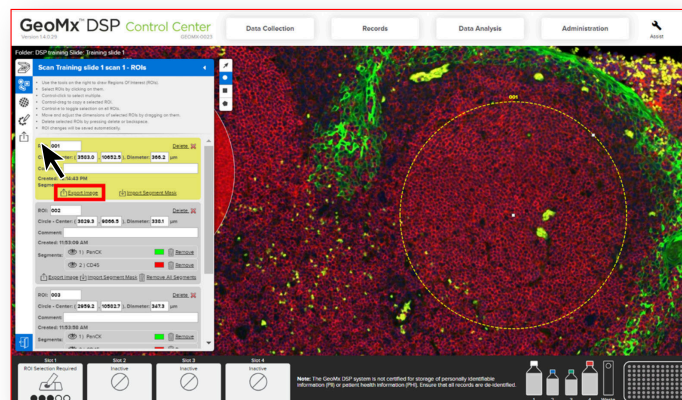
6

Navigate to the saved .tif file, then drag & drop it onto the ImageJ toolbar.

7

Next, drag & drop the ImageJ script (.txt file) into the ImageJ workspace.
- Note: it is important that the file is dragged & dropped rather than loaded from the Plugins > Macros menu. Drag & drop will enable you to set the number of contours and their spacing in the next step.

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After the drag & drop of the script file, the script window will open, within which you can set the parameters for your segmentation masks:
- '**ringWidth**' defines in micrometers the width of each contour.
- '**ringNumber**' defines the number of contours.

After setting your parameters, click **Macros > Run Macro** from the script toolbar.

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Next, a dialog box will pop up instructing you to draw your first segment. **Don't click OK yet!**
- For concentric rings script: draw one circle – this will be the first segmentation mask (To draw a perfect circle, hold Shift while drawing). When you **click OK** in the dialog box, the software will auto-draw the other circles based on your parameters.
- For contouring script: draw a line by clicking where the line should start, clicking at each vertex along your desired line, then clicking back at the first dot to finish the contour line. That last line segment should disappear, leaving you with a line following the path you designated. Then, you must click the first point in your line and drag it off the ImageJ window; do the same for the last point, effectively dividing the ROI image in two parts. When you **click OK** in the dialog box, the software will then auto-draw contouring lines following the same shape of your drawn contour line, on either side of it.

10

The individual masks will pop up in separate windows. ImageJ will prompt you to save them - **don't click Cancel** here or you'll have to save them one-by-one. Save all the masks (anywhere, as long as you can find them).

11

Return to the GeoMx DSP interface. Click "Import segment masks" under the ROI.

12

Import masks by navigating to the individual mask files, highlighting all of them, and clicking Open. (It does not work to import the entire folder they are in). You'll see all the masks imported to your scan and ROI details.

13

(Optional) To change the collection order, drag individual masks up and down the "Segment list" (red box, below).
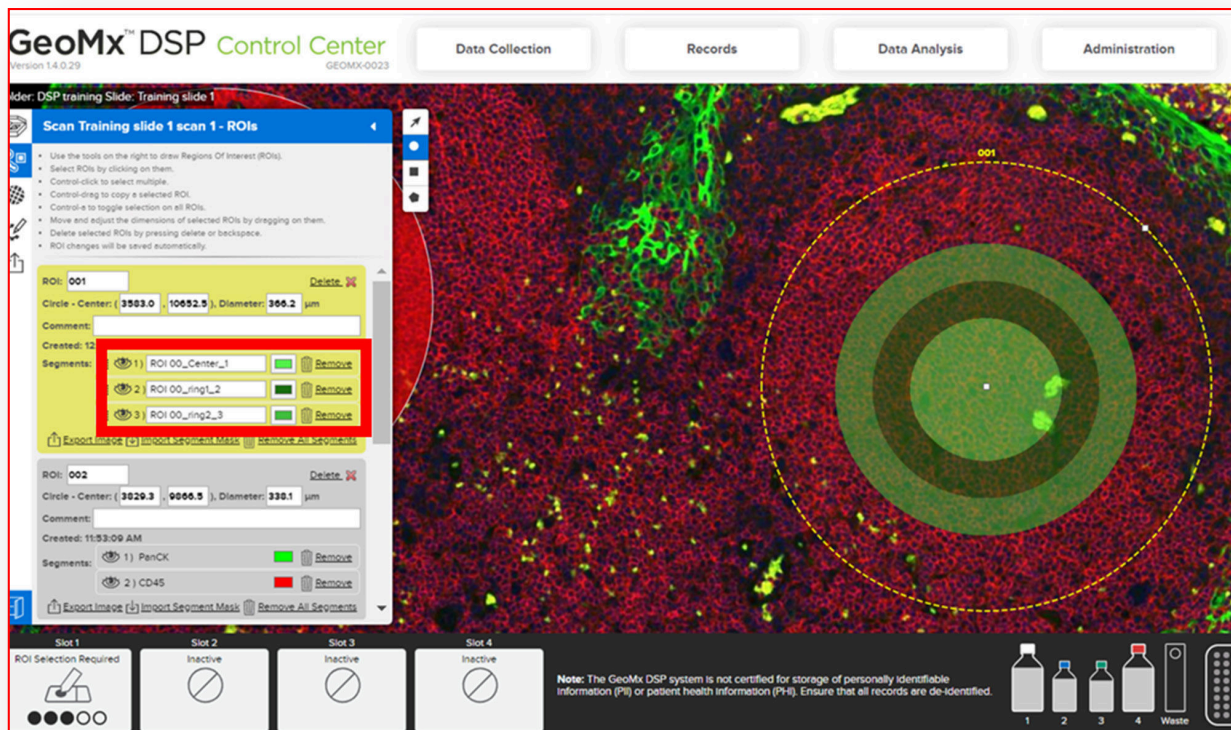
14

Proceed with other ROIs, then finalize ROIs and proceed to Collection.

For more information, please visit nanostring.com/GeoMx

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Additional Customer Resources

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Support > Product Support to view manuals and other technical product literature.

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