GeoMx® DSP: Creating Geometric Segmentation Masks using ImageJ: Custom Shapes

v2 for software
v1.4 and v1.5

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

1

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 2 is not possible. Instead, use a USB to transfer ROI and mask files to and from the instrument.

2

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**.
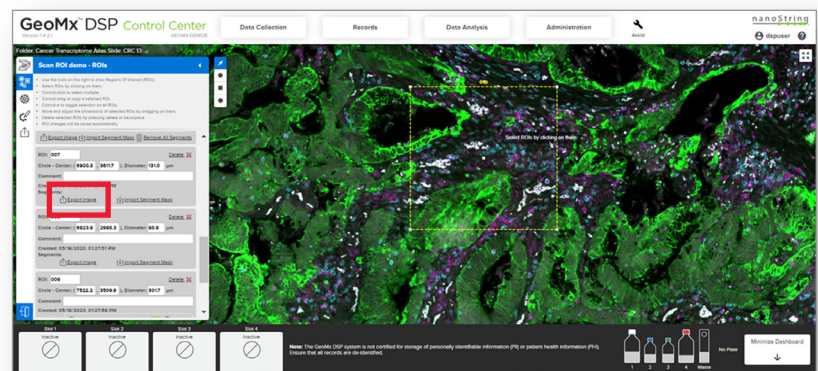
3

In the GeoMx Scan Workspace, create or select an ROI. The segmentation masks you create in subsequent steps will be within this ROI area.

4

On the left panel where the ROIs are listed, click the "**Export image**" button underneath the ROI details (Fig 1, red box). It will save the ROI as a stacked TIFF image. Note where the .tif is saved.
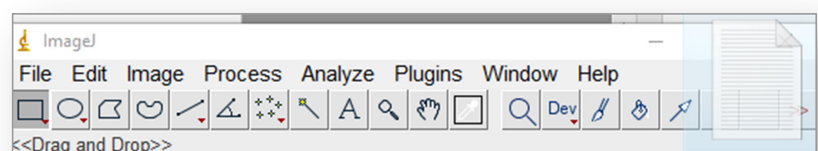
Fig. 1

5

Navigate to the saved .tif file, then drag & drop it onto the ImageJ toolbar. (Fig 2).

Fig. 2

6

Set the dimensions in ImageJ (in pixels) to the dimensions in the GeoMx DSP (in microns) as follows:
- Under the Analyze tab, click **Set Scale** to open the dialog box (Fig 3).
- **Distance in pixels:** enter the length of the longest edge of your .tif (shown at the top of the image window as in Fig 4).
- **Known distance:** enter the length of the longest edge of your ROI created in the GeoMx DSP software; this value is listed in your ROIs panel (Fig 5, red box).
- **Pixel aspect ratio:** 1.0
- **Unit of length:** um
- If you tick the **'Global'** box, this scale will be applied to all images opened during this ImageJ session (helpful if you will create custom masks from multiple, same-sized ROIs).
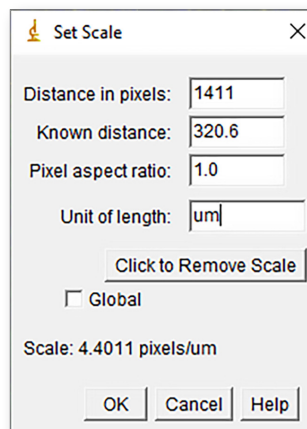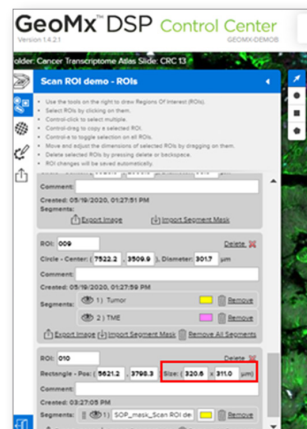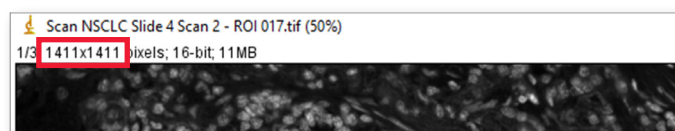
Fig. 3

Fig. 5

Fig. 4

7

In this example, we show a freehand geometric mask based on cell/tissue morphology. Use the freehand selection tool to click and drag to form the first shape. To draw subsequent shapes as part of the same mask, simply hold the 'Shift' key and continue to draw.
- Other geometric objects such as rectangles, circles, etc. can be created too.
- To rotate a single object such as a rectangle, click **Edit > Selection > Rotate**. (Leave the "rotate around image center" box empty). Move the rectangle into place using the keyboard arrow keys. Make adjustments to the size and position of the shape to match the area of the sample you wish to mask.
- From the Edit menu, click **Selection > Specify**... and set the dimensions of the rectangle as you wish. Click the '**scaled units**' box to use micron units.

8

To make the mask, click **Edit > Fill**. Answer '**yes**' to "**Process all two images?**". Then, click **Edit > Clear outside**. This yields a black background with the mask in white. This series of steps is shown in Fig 6.

Fig. 6
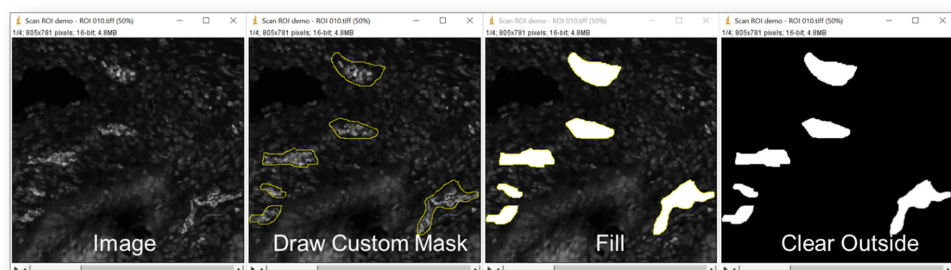
9

Ensure the mask is in the right format by clicking **Image > Type > 8-bit**, then **File > Save as > PNG** file format. Save the file where you will be able to find it in the next step.

10

Return to the GeoMx DSP interface. Click "**Import segment masks**" under the ROI. Locate the PNG file just saved, highlight, and click **Open**. The custom mask will be imported to the scan workspace, overlaying the custom mask on your ROI (Fig 7).

Fig. 7
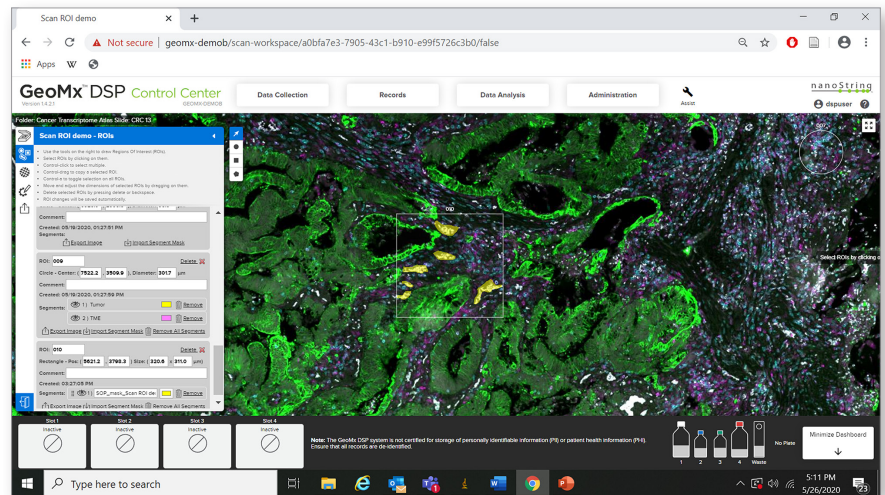
11

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

12

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

13

To design more than 2 masks/collections per ROI, simply draw separate masks in ImageJ and upload them individually to the ROI. Note that masks cannot overlap.

14

Remember that every custom mask you import to the GeoMx DSP will be illuminated, collected and counted separately. Keep this in mind when planning your collection plate and downstream reagent usage.

For more information, please visit nanostring.com/GeoMx

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