



1. Main axes:

- Click and drag to zoom/pan view
- Scatter shows all nuclei colored by spot count
- Use DAPI+FISH overlay to mask spots & create/delete cells

2. Thumbnail Axes:

- Shows nuclei density across the entire scan
- Rectangle corresponds to the position displayed in the main axes
- Drag rectangle or double click to change positions

3. Centroid List:

- Spot counts for all nuclei (ranked high to low)
- Double click on value to move to corresponding cell
- Use up/down arrow keys to move through list

4. Sliders:

- Adjust contrast of the FISH image in main axes

5. Checkbox:

- Toggle plot settings for main axes

6. Channel drop-down:

- Change current FISH channels

7. Colormap drop-down:

- Change colormap for scatter plot in main axes

8. Zoom and pan view:

- While in zoom mode, click and drag on main axes to zoom-in
- While in pan view mode, click and drag to move through scan
- Right click to zoom-out by 2X
- Double click to return to home view

9. Shuffle colors:

- Change spot and centroid colors in main axes

10. Mask all channels checkbox

- Toggle to mask spots in current channel or all channels

11. Add/delete masks:

- Mask spots or mask nuclei by drawing on main axes.
- Delete mask(s) by selecting points inside mask(s); press **Enter** when finished.
- If nucleus is masked/added/deleted, nearby spots (all channels) will be reassigned to nearest nuclei.

12. Add/delete nuclei

- Delete erroneous nuclei and add missing nuclei.
- Press **Enter** when finished.
- Nearby spots (all channels) will be reassigned to nearest nuclei.

13. Save and export:

- Save spot, nuclei, and mask tables
- Export spot summary table (spots per nucleus)

14. Threshold axes:

- Shows the distribution of spot intensities in current channel.
- Drag vertical blue line to set spot intensity threshold
- Or use text box in upper right corner
- With zoom, click and drag on plot to zoom-in on X-axis.