

### 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. Shuffle colors:

- Change spot and centroid colors in main axes

## 9. Save and export:

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

# 10. 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

### 11. Add/delete masks and nuclei:

- Mask spots in current channel or mask nuclei for all channels
- Delete erroneous nuclei and add missing nuclei
- Press **Enter** to complete operation
- If nucleus is masked/added/deleted, spots in view will be reassigned

### 12. Threshold axes:

- Drag vertical blue line to set spot intensity threshold
- Or use text box in upper right corner