**SegmentationTool**

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**Section 1 - Description:**

This tool was created to develop a ground truth for AI or a neural net approach to cell segmentation of the nuclei. This tool reads in the image segmentation output from Seyoun’s machine learning ‘superpixel’ algorithm, finds the corresponding inform segmentation output for that image, computes the overlapping cells and displays the overlapped cells one cell at a time. The user selects the best case segmentation for each cell, if neither cell is acceptable then user can draw their own segmentation before moving to the next cell. Cells are considered overlapping if the joint overlap is 10%. Cells with a joint overlap of 80% are considered the same cell object and are accepted as true cells without user input.

**Section 2 - Installation:**

Download the installer located here:

[\\halo1\Taubelab\Ben\Code\SegmentationTool\dist\SegmentationTool\_installer.exe](file:///\\halo1\Taubelab\Ben\Code\SegmentationTool\dist\SegmentationTool_installer.exe)

To run the installer after downloading, simply double click the installer icon. When the installation window opens, click through the installation dialog. Be sure accept the prompt to download ‘Runtime’ if you do not already have this version of runtime installed on your machine. It is best to allow the installer to create a desktop shortcut. Otherwise the program can be run by double clicking on the executable which, unless the default installation path is changed during installation, should be located at:

“C:\Program Files\Johns Hopkins University\SegmentationTool\application\SegmentationTool.exe”.

**Section 3 - File Structure:**

The program relies that the images files are set up in a unique file structure which is modeled after the Clinical Specimen directory structure, in order to find the corresponding inform images.

In summary, there should be two adjacent directories for a given slide as follows:



Each folder should contain their own respective ‘Component\_Tiffs’ folder, the images for each algorithm should reside in their respective folder.

As a more distinct definition for this structure we first split the directory tree into 4 main parts which will be labeled “root”, “slideID”,”superpixel\_tree”, and ”IF\_tree”.

<root>\<slideID>\< superpixel\_tree>\< superpixel\_Image> **OR** <root>\<slideID>\<IF\_tree>\<IF\_Image>

EX. “\\bki04\Segmentation\TMAs\Liver\_TMA\_145\_23\_01.30.2020\superpixel\Component\_Tiffs\Liver\_TMA\_145\_23\_01.30.2020\_[6435,55763]\_component\_data\_seg.tif”

<root>: “\\bki04\Segmentation\TMAs”

<slideID>: “Liver\_TMA\_145\_23\_01.30.2020”

<superpixel \_tree>: “superpixel\Component\_Tiffs”

<superpixel \_Image>: “Liver\_TMA\_145\_23\_01.30.2020\_[6435,55763]\_component\_data\_seg.tif”

For the corresponding inForm image, replace the < superpixel\_tree> with the <IF\_tree> as follows:

EX. “\\bki04\Segmentation\TMAs\Liver\_TMA\_145\_23\_01.30.2020\IF\_data\Component\_Tiffs\Liver\_TMA\_145\_23\_01.30.2020\_[6435,55763]\_component\_data\_w\_seg.tif”

<root>: “\\bki04\Segmentation\TMAs”

<slideID>: “Liver\_TMA\_145\_23\_01.30.2020”

<IF\_tree>: “IF\_data\Component\_Tiffs”

<IF\_Image>: “Liver\_TMA\_145\_23\_01.30.2020\_[6435,55763]\_component\_data\_w\_seg.tif”

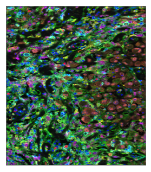
**Section 4 - Usage:**

***4.1 Launching and getting started***

1. Launch the program either by double clicking on the icon: or by locating the SegmentationTool.exe (see above in *Installation*).



* 1. An icon will appear on the windows tool bar:



* 1. a loading visual will appear on the desktop:
  2. The visual and icon may disappear then the app will open. This may take a few minutes and is normal.

1. Click on the ‘Load new image’ button:
2. A windows file explorer should open, navigate to the < superpixel\_tree> folder and open the < superpixel\_Image> of interest.
3. If the file structure was set up appropriately, the program will find the corresponding inForm segmentation output image, otherwise it will throw an error.
4. The segmentation overlap on the images will be computed or, if it exists, the corresponding overlap image and .csv file comparison will be loaded for the image.
5. The first cell for comparison will appear in the UI as a set of four images.
   1. The top images will contain only the DAPI signal, the bottom images will contain the DAPI and the Membrane signal
   2. On the left will be used to display the machine learning or superpixel segmentation and the right will be used to display the inform segmentation

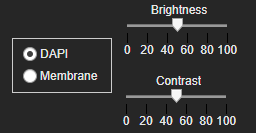
***4.2 Selecting a segmentation approach***

Once the first cell appears, the figure title will be populated with the image name and the cell count.

1. Select one of the 5 options in the first box on the right panel:
   1. ‘Select Superpixel Segmentation’: this option saves the superpixel image segmentation shown on the left panels
   2. ‘Select InForm Segmentation’: this option saves the inform image segmentation on the right panels
   3. ‘Draw Segmentation on DAPI’: this option allows the user to draw their own segmentation on the InForm DAPI only image. (Drawing explained below)
   4. ‘Draw Segmentation on Membrane’: this option allows the user to draw their own segmentation on the InForm DAPI + Membrane image. (Drawing explained below)
   5. ‘Reject Cell’: This cell is thrown out for some reason, ie. The segmentation is a result of image artifact
2. Select ‘Go to next cell’
   1. This jumps to the next cell that has not yet been segmented
      1. Sometimes this means skipping cells

***4.3 Additional buttons***

* ‘Go back one cell’: this goes back one cell in the numeric ordering (shown in the figure header at the top of the page)
* ‘Go forward one cell’: this moves forward a single cell and a single cell only in the numeric ordering. This differs from the ‘Go to next cell’ button by ignoring which cells have already been checked off.
* ‘Jump to cell’: this button jumps to the cell entered in the input box under it. This can be used to ask for confirmation or review the segmentation of a given cell.
* The brightness and the contrast of the DAPI and Membrane are toggled separately. Select the color of interest in the button selection, then vary the appropriate parameter.



* ‘Toggle Segmentation’: Toggles the segmentation on and off for all four image stamps.

***4.4 Drawing feature***

When you select one of the drawing options:

1. Move the cursor over the corresponding image (DAPI or Membrane + DAPI).
2. There are two options for drawing the segmentation.
   1. Click and drag
      1. Left click and hold
      2. then drag around the cell to draw (while holding the left mouse button)
      3. Release the left click ***and*** right click to end the segmentation
   2. Create waypoints
      1. Left click once on the segmentation
      2. release
      3. Then move the mouse and left click again
      4. Do this all the way around the cell, creating ‘waypoints’
      5. right click on the mouse to end the segmentation

Notes:

* To clear a segmentation and draw a new segmentation
  1. Right click to end the segmentation
  2. Click the ‘Reject cell’ button
  3. Then click the corresponding ‘Draw’ button and attempt to draw again

- Sometimes the drawing tool gets hung up when selecting between Draw on ‘DAPI’ or ‘Membrane’. You may have to select reject cell to clear the drawing tool, then select back into the ‘Draw’ button desired.

***4.5 Additional notes for segmentation selection***

- We are segmenting the nuclei only at this point

- If one of the two segmentations are correct select that segmentation before rejecting or drawing a new segmentation.

- Since the cells are usually order by location, segmentation on adjacent cells may show up in sequential ordering. Often this means that over-segmented or under-segmented examples are directly next to each other. If one of the approaches correctly defines the over-segmented cell, *it is safe to reject the second cell that appears or select the correct version again.*

EX.

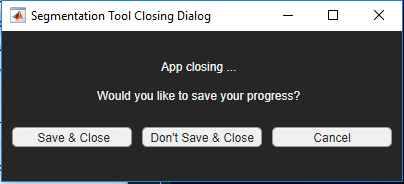
Cell1: Cell2:

**Section 5 - Saving and Output:**

***5.1 Saving and closing***

There are two ways to save progress.

1. The first way it to select the ‘Save Table’ button at the bottom of the right panel: 
2. The second way is during the closing of the app. Close the app by clicking the ‘X’ in the upper right corner. A new closing dialog will appear:



* 1. ‘Save & Close’: saves your progress and closes the app
  2. ‘Don’t Save & Close’: will not save your progress and closes the app
  3. ‘Cancel’ or clicking the ‘X’ from this box will cancel the closing dialog and return to the app for segmentation

***5.2 Image Output***

The tool saves two files, both files are labeled with the file indication ‘comparison\_seg\_data’ after the image name. The first file is a csv file with 18 column headers, the second is a tiff file with 6 image layers.

1. Csv file:
   1. This file contains information on each cell object in the image
   2. Column description:
      1. IF\_cellid
         1. Description: Numeric cellid from the IF\_cellid label matrix
         2. Data type: Uint16
      2. IF\_X\_centroid
         1. Description: X value for the centroid of the IF cell
         2. Data type: Float32
      3. IF\_Y\_centroid
         1. Description: Y value for the centroid of the IF cell
         2. Data type: Float32
      4. IF\_n\_pixels
         1. Description: the number of pixels in the InForm cell object
         2. Data type: Uint16
      5. IF\_overlap
         1. Description: The pixel overlap of a given IF\_cell to a ML\_cell
         2. Data type: Uint16
      6. IF\_pct
         1. Description: the percent overlap, IF\_overlap / IF\_n\_pixels
         2. Data type: Float 32
      7. IF\_paired
         1. Description: the corresponding ML cellid that is paired to this IF cell
         2. Data type: Uint16
      8. Cellid
         1. Description: the new unique cellid given to cell pairs in the table
         2. Data type: Uint16
      9. ML\_cellid
         1. Description: Numeric cellid from the ML\_cellid label matrix
         2. Data type: Uint16
      10. ML\_X\_centroid
          1. Description: X value for the centroid of the ML cell
          2. Data type: Float32
      11. ML\_Y\_centroid
          1. Description: Y value for the centroid of the ML cell
          2. Data type: Float32
      12. ML\_n\_pixels
          1. Description: the number of pixels in the superpixel cell object
          2. Data type: Uint16
      13. ML\_overlap
          1. Description: The pixel overlap of a given ML\_cell to a ML\_cell
          2. Data type: Uint16
      14. ML\_pct
          1. Description: the percent overlap, ML\_overlap / ML\_n\_pixels
          2. Data type: Float 32
      15. ML\_paired
          1. Description: the corresponding IF cellid that is paired to this ML cell
          2. Data type: Uint16
      16. IF\_level
          1. Description: the corresponding image segmentation type for the InForm segmentation a cell comes from.
          2. Data type: Uint8
          3. Opts:
             1. 1: immune cell segmentation layer
             2. 2: tumor cell segmentation layer
      17. jp
          1. Description: the joint probability of two cells being the same object or IF\_pct \* ML\_pct
          2. Data type: Float32
      18. include\_cell
          1. Description: which segmentation is saved in the final result
          2. Data type: Uint8
          3. Opts:
             1. 0: cell has not yet been annotated
             2. 1: keep the superpixel segmentation
             3. 2: keep the InForm segmentation
             4. 3: draw a new segmentation
             5. 4: these cells have a jp over 80% and are considered the same object, keep the superpixel segmentation approach
             6. -1: reject the cell

Note: Cells can be repeated for each corresponding cell they overlap over 10% with, so all overlapped cells are included.

1. TIFF file
   1. This file contains 6 label matrices to be used in conjunction with the csv file to produce the final segmentation result. The objects are labeled with the ‘cellid’ value
   2. The 6 layers correspond to the following
      1. Cell objects accepted from the InForm approach that correspond to cells in the InForm immune segmentation
      2. Cell objects accepted from the InForm approach that correspond to cells in the InForm tumor segmentation
      3. Cell objects accepted from the superpixel approach that correspond to cells in the InForm immune segmentation
      4. Cell objects accepted from the superpixel approach that correspond to cells in the InForm tumor segmentation
      5. Cell objects that were draw in the UI which correspond to cells in the InForm immune segmentation
      6. Cell objects that were draw in the UI which correspond to cells in the InForm tumor segmentation

***5.3 Rebuilding the label matrix***

To rebuild the label matrix one must read in each layer of the Tiff file and match the cell objects in the image layers to the corresponding cellid. Overlapping regions are taken from layer 1 down, so that overlapping pixels in layer 1 are rewritten to layer 2 and so on.