USER-GUIDE for the Junction Analyzer Program (JAnaP)

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Please refer to the following article for more information and/or cite
when using the JAnaP for published works:
Gray, K.M., Katz, D.B., Brown, E.G. et al. *Ann Biomed Eng* 47(7): 1675-1687(2019).
https://doi.org/10.1007/s10439-019-02266-5

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Image Requirements:

1) Image resolution must be 1024 x 1024 pixels or 2048 x 2048 pixels. Record the resolution of your images.

2) Record the image scale (i.e. pixels to µm conversion). This will be required in the "parameter" section for correct result calculations.

3) Images must be in tiff format.

4) When saving your images, consider naming the files in a consistent manner that includes the experimental conditions, the stain or fluorescence channel, the date, etc. These are all helpful as unique identifiers (i.e. "variants" or "dimensions") for processing and organization via the JAnaP.

5) Note that this program has been used and validated on images collected using a 60x oil objective. Ensure your images are taken with enough magnification to clearly see the edge-localized junctions.

Computer Requirements:

1) This current version of the user guide is for use on PCs.

Download Instructions:

1) Navigate to the JAnaP repository on GitHub at https://github.com/StrokaLab/JAnaP
2) Click "Clone or download" and select "Download ZIP"
3) Unzip the contents of the repository:
   - Right click on the downloaded zip file and select "Extract All."
   - Create Documents/GitHub folder on your PC and extract files to this location.

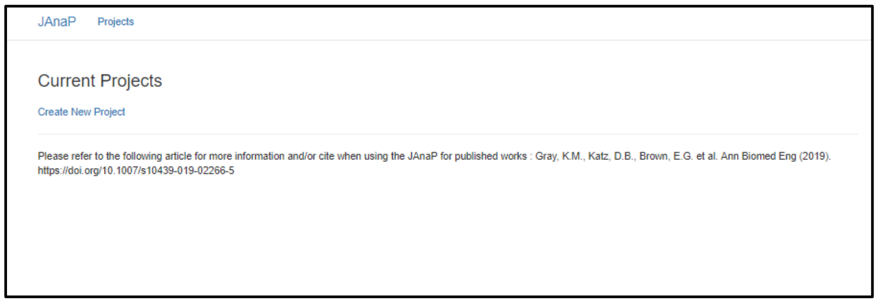
4) On Windows, run the "setup.bat" file located in the bin folder. This will install Python 2.7 and all of the required packages.
   - If you use virtualenv you can find the requirements.txt file in the bin folder
5) Run web application.py as described below

Program Instructions:

1) Open command prompt:
   - Click on windows/start icon (in bottom left corner of screen)
   - Type "cmd" and select the "Command Prompt"

2) Within command prompt, navigate to folder containing "JAnaP-master":
   - Within the command prompt type: cd Documents\GitHub\ JAnaP-master
   - Hit "Enter" then type: python web\application.py
   - Note: To stop the program, type "Ctrl+C" in the command prompt

3) Open web browser and type "127.0.0.1:5000". The following screen should load:

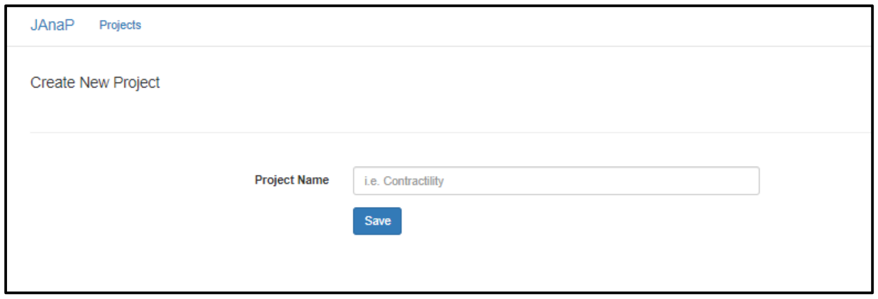


Current Projects

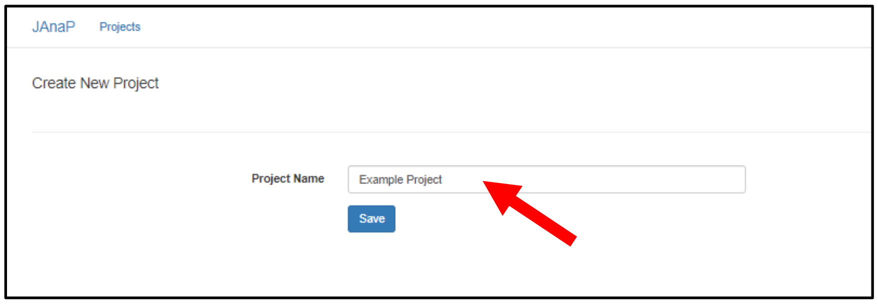
Create New Project

Please refer to the following article for more information and/or cite when using the JAnaP for published works : Gray, K.M., Katz, D.B., Brown, E.G. et al. Ann Biomed Eng (2019). https://doi.org/10.1007/s10439-019-02266-5

4) Click “Create New Project” and when the following screen appears, type in the name of the new project (e.g., Example Project) you would like to create and click “Save”

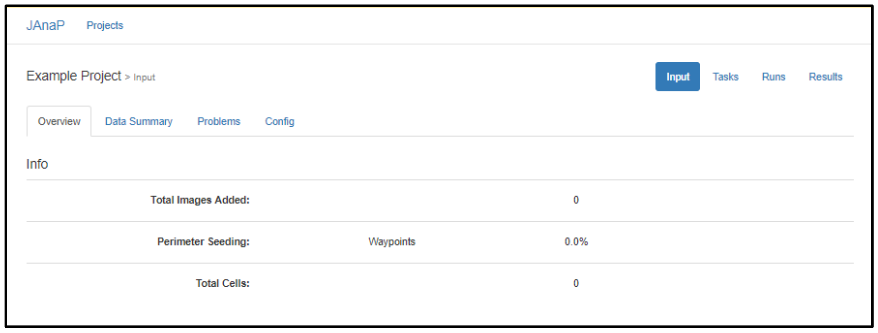


Project Name: i.e. Contractility



Project Name: Example Project

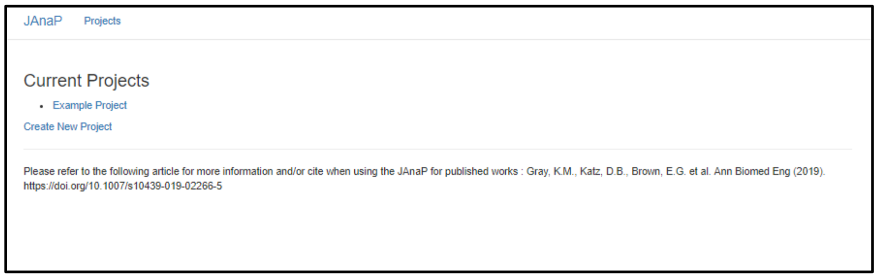
5) The following screen should load:



Info

Total Images Added:		0
Perimeter Seeding:	Waypoints	0.0%
Total Cells:		0

- NOTE: If you click on “JAnaP” the home page will re-appear, and your project will now be listed in the “Current Projects” list. Click on the link to your project to return to the screen above.


JAnaP Projects

Current Projects

- Example Project

Create New Project

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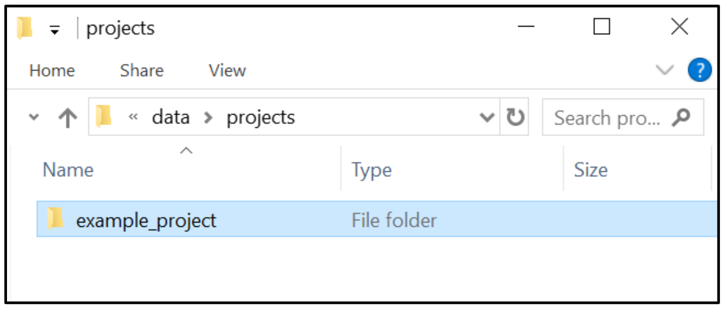
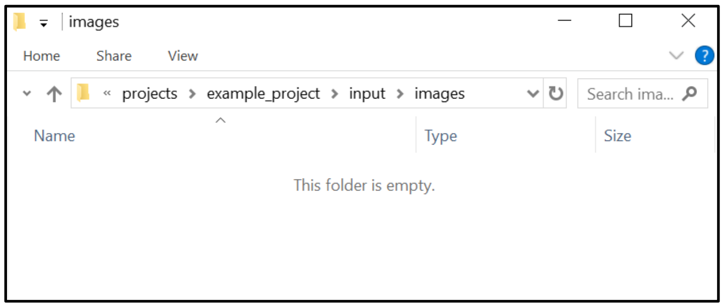
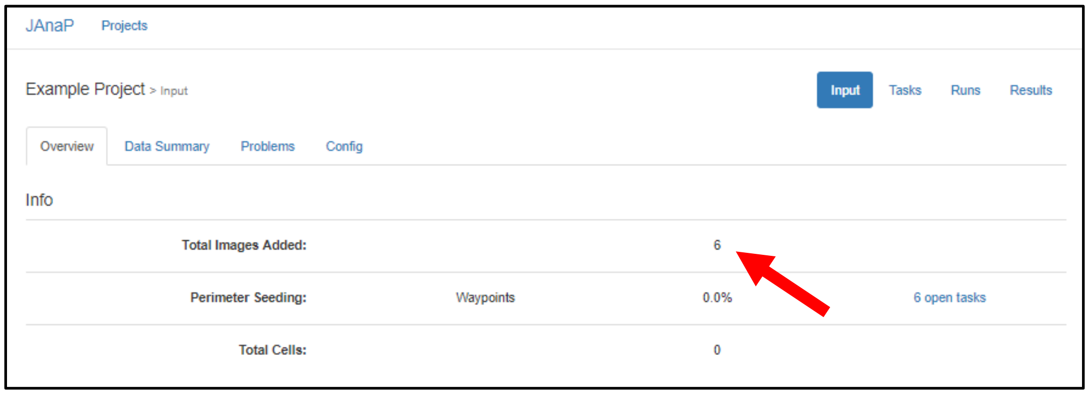
6) To load images into your project:
   - Navigate to the “projects” folder within the “JAnaP-master” folder on your computer
     - Documents > GitHub > JAnaP-master > data > projects



   - Select your project folder (e.g., “example_project”) and navigate to the “images” folder
     - example_project > input > images



   - Drag and drop the images you would like included in your analysis into this folder. When you refresh your browser, your project should then reflect the total number of images added.
     - Here, 6 images were added into the example_project>input>images folder
     - Note: only tiff files should be uploaded



JAnaP Projects

Example Project > Input

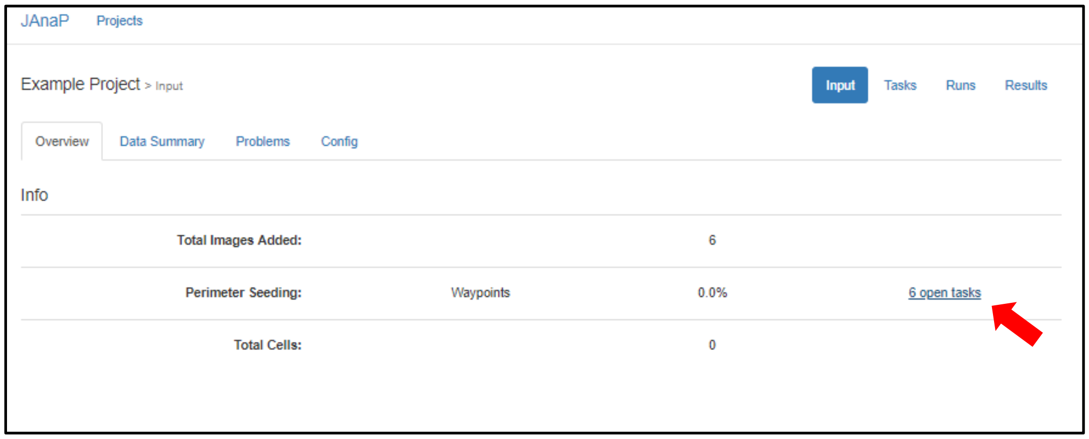
Input Tasks Runs Results

Overview Data Summary Problems Config

Info

Total Images Added:		6	
Perimeter Seeding:	Waypoints	0.0%	6 open tasks
Total Cells:		0	

7) To select the cells of interest to be "waypointed" and analyzed, click "6 open task":
   - Note: This "6" value will change depending on the number of images in the "Total Images Added" section, the total number of "open" versus "done" tasks, and the "Variants" identified (described below).



JAnaP Projects

Example Project > Input

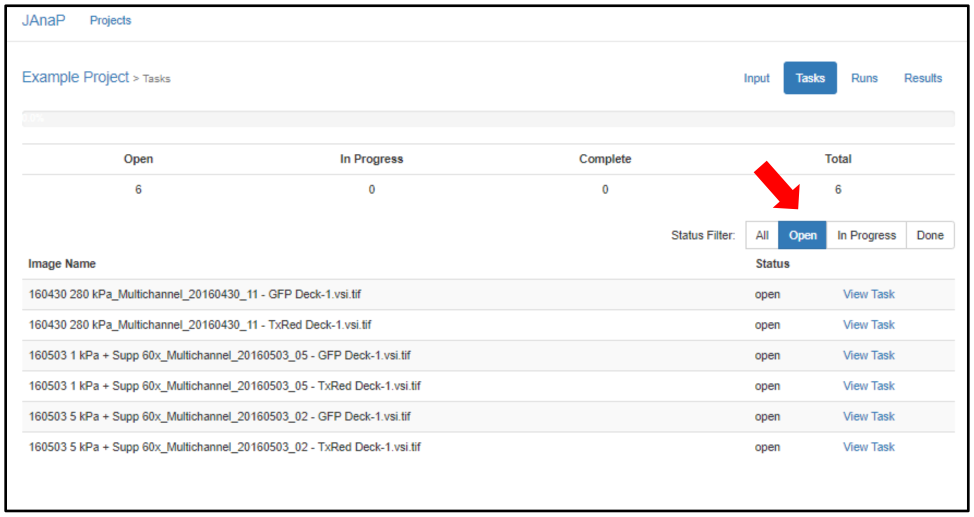
Input Tasks Runs Results

Overview Data Summary Problems Config

Info

Total Images Added:		6	
Perimeter Seeding:	Waypoints	0.0%	6 open tasks
Total Cells:		0	

8) The following screen should appear, where the tab selected is "Open" tasks and the number of images listed should reflect the "# open tasks" listed on the previous screen:



JAnaP Projects

Example Project > Tasks

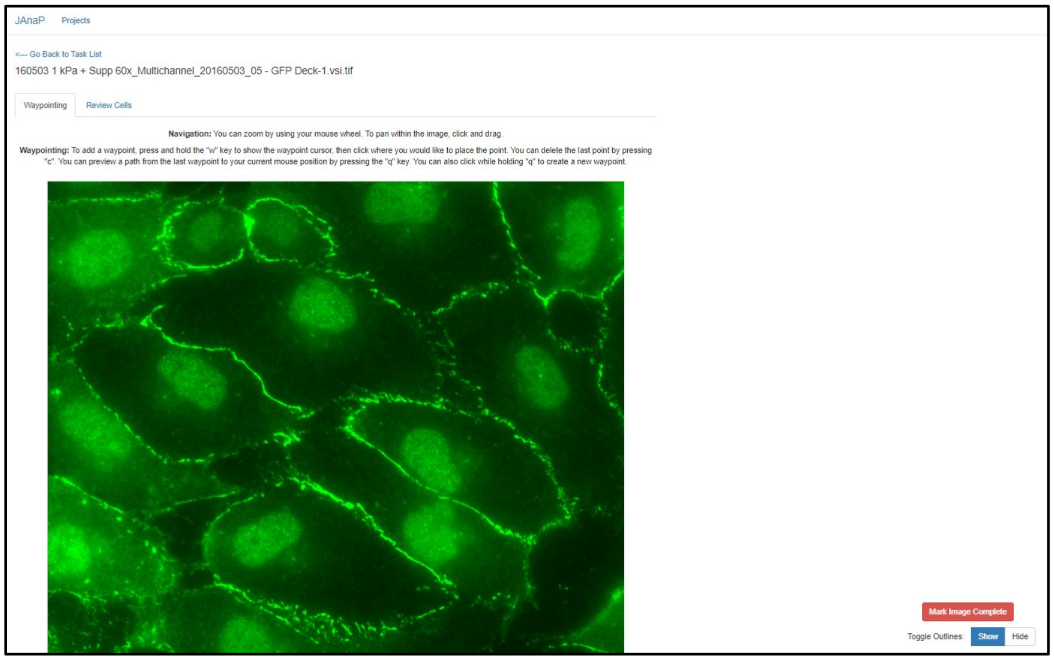
Input Tasks Runs Results

Open	In Progress	Complete	Total
6	0	0	6

Status Filter: All Open In Progress Done

Image Name	Status	
160430 280 kPa_Multichannel_20160430_11 - GFP Deck-1.vsi.tif	open	View Task
160430 280 kPa_Multichannel_20160430_11 - TxRed Deck-1.vsi.tif	open	View Task
160503 1 kPa + Supp 60x_Multichannel_20160503_05 - GFP Deck-1.vsi.tif	open	View Task
160503 1 kPa + Supp 60x_Multichannel_20160503_05 - TxRed Deck-1.vsi.tif	open	View Task
160503 5 kPa + Supp 60x_Multichannel_20160503_02 - GFP Deck-1.vsi.tif	open	View Task
160503 5 kPa + Supp 60x_Multichannel_20160503_02 - TxRed Deck-1.vsi.tif	open	View Task

9) Select "View Task" next to the image you would like to view and/or waypoint cells, and the following screen should appear presenting the image you selected:

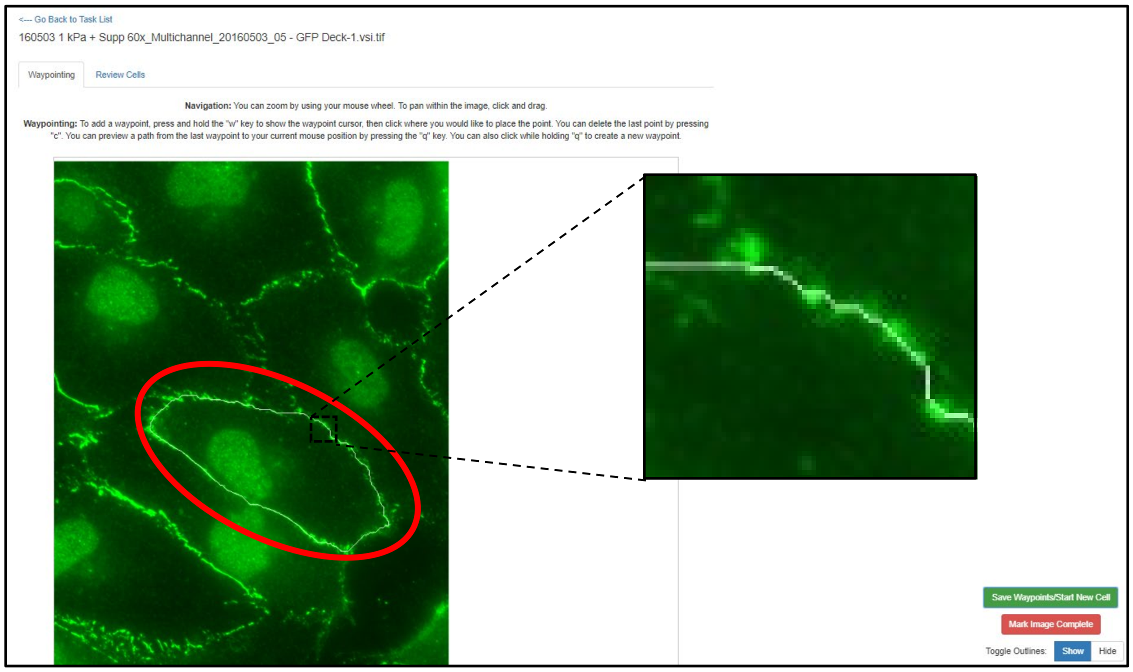

JAnaP
Projects
<--- Go Back to Task List
160503 1 kPa + Supp 60x_Multichannel_20160503_05 - GFP Deck-1.vsi.tif
Waypointing
Review Cells
Mark Image Complete
Toggle Outlines:
Show
Hide

Navigation: You can zoom by using your mouse wheel. To pan within the image, click and drag.

Waypointing: To add a waypoint, press and hold the "w" key to show the waypoint cursor, then click where you would like to place the point. You can delete the last point by pressing "c". You can preview a path from the last waypoint to your current mouse position by pressing the "q" key. You can also click while holding "q" to create a new waypoint.

10) Under the “Waypointing” tab, you can begin waypointing each cell using the instructions on the page.
- NOTE: A mouse should be used for waypointing (i.e., a laptop trackpad will likely not work)
- Hold “q” and click on a spot in the perimeter of a cell.
- Move your cursor to another spot on the perimeter while holding “q” and the connecting path should appear. If you agree with the connecting path, click on that spot to add it as a waypoint. Otherwise, move your cursor around the cell perimeter until you agree with the automated path.
- Continue seeding waypoints until the entire cell perimeter is traced.
  - Number of waypoints – This depends on the cell, the amount of junction presentation, and the quality of staining. If a cell has mostly continuous junctions and/or the edge of the cell is very distinct relative the background, less waypoints are likely required. Try seeding a waypoint and then trace the edge with your mouse holding “q” for as long as the path accurately follows the cell edge. Once it begins to deviate, move your mouse back closer to the last waypoint to seed a waypoint where the path is accurate.
  - Use your best judgement when deciding the location of the cell perimeter when little to no stain is present around the cell edge. More waypoints will likely be required in this region.
- If you misplace a waypoint, click “c” to remove the last waypoint seeded.

In the following image, the circled cell has been fully waypointed. The cell path has been traced in white, and a zoomed-in cropped image has been added to help show the tracing (Note: this is for the user guide only, it will not appear this way in the GUI):



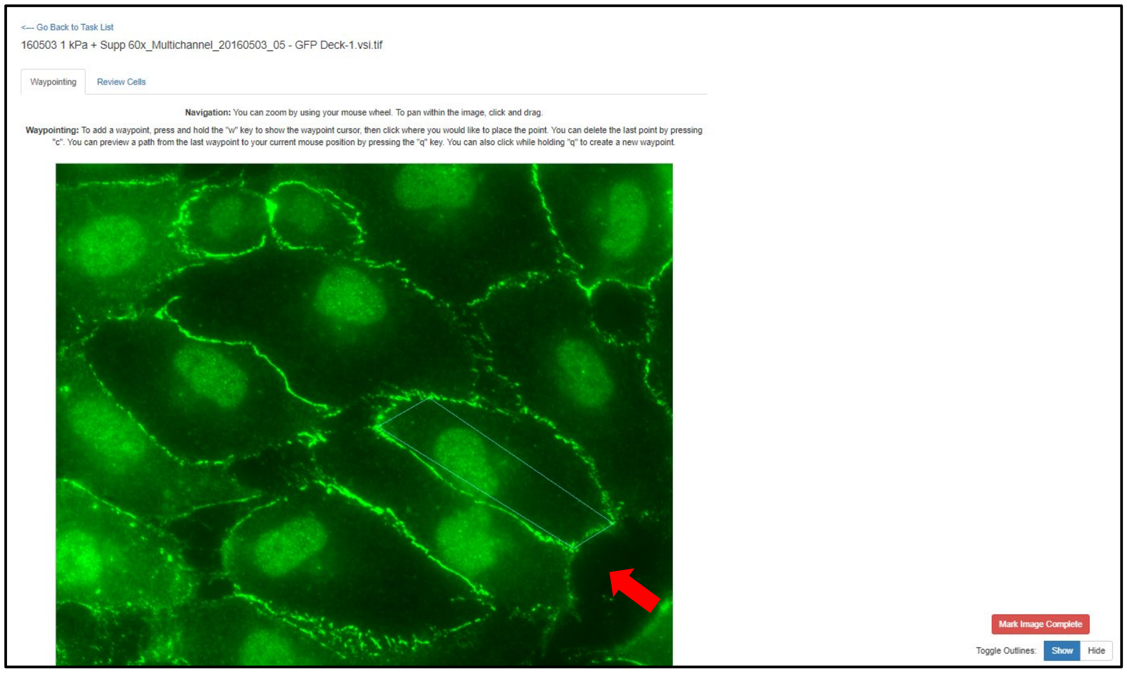
160503 1 kPa + Supp 60x_Multichannel_20160503_05 - GFP Deck-1.vsi.tif

Navigation: You can zoom by using your mouse wheel. To pan within the image, click and drag.

Waypointing: To add a waypoint, press and hold the "w" key to show the waypoint cursor, then click where you would like to place the point. You can delete the last point by pressing "c". You can preview a path from the last waypoint to your current mouse position by pressing the "q" key. You can also click while holding "q" to create a new waypoint.

11) Click "Save Waypoints/Start New Cell" once the cell perimeter has been fully traced.

12) The image will reappear with a rough tracing (i.e. lines connecting the waypoints) of the waypointed cells in blue to indicate which have already been traced within that image.

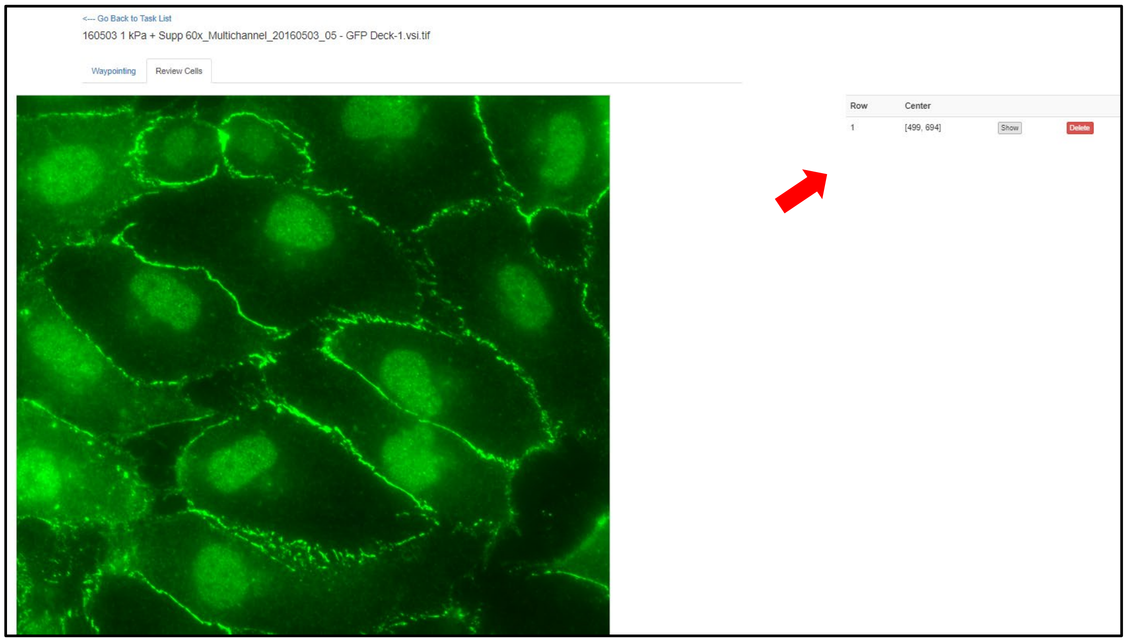


160503 1 kPa + Supp 60x_Multichannel_20160503_05 - GFP Deck-1.vsi.tif

Navigation: You can zoom by using your mouse wheel. To pan within the image, click and drag.

Waypointing: To add a waypoint, press and hold the "w" key to show the waypoint cursor, then click where you would like to place the point. You can delete the last point by pressing "c". You can preview a path from the last waypoint to your current mouse position by pressing the "q" key. You can also click while holding "q" to create a new waypoint.

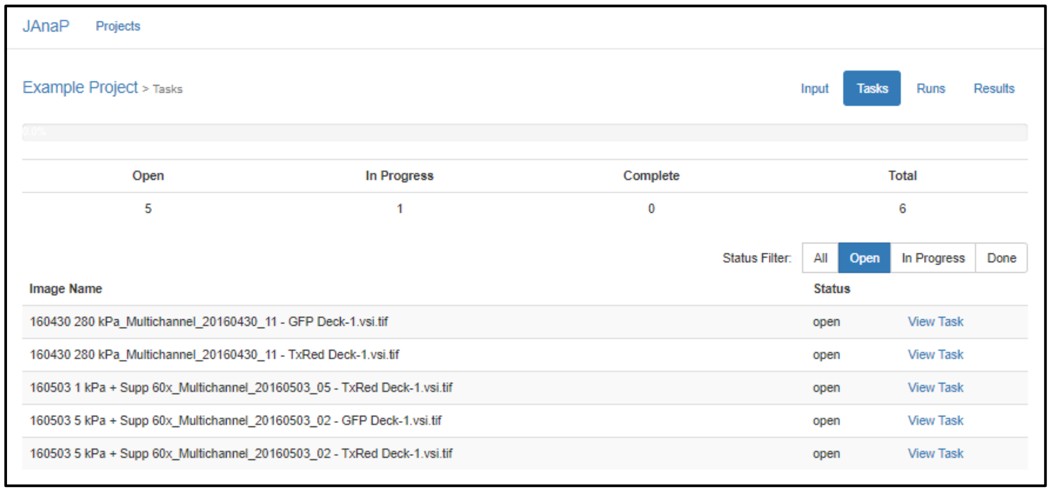
13) To view or remove previously waypointed cells, click the "Review Cells" tab. The list of traced cells will appear on the right side, designated by their center waypoints. Here, you can click "show" to visualize the traced perimeter of each cell or click "Delete" to remove the waypoints associated with that cell.



160503 1 kPa + Supp 60x_Multichannel_20160503_05 - GFP Deck-1.vsi.tif

Row	Center
1	[499, 694]

14) To navigate back to the "Tasks", click "← Go Back to Task List" in the upper left corner. This will bring you to following screen:

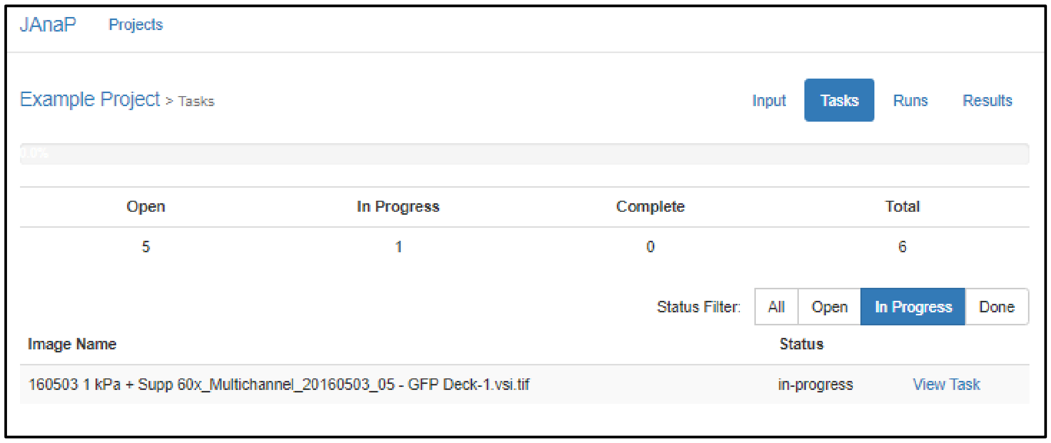


Open	In Progress	Complete	Total
5	1	0	6

Image Name	Status	
160430 280 kPa_Multichannel_20160430_11 - GFP Deck-1.vsi.tif	open	View Task
160430 280 kPa_Multichannel_20160430_11 - TxRed Deck-1.vsi.tif	open	View Task
160503 1 kPa + Supp 60x_Multichannel_20160503_05 - TxRed Deck-1.vsi.tif	open	View Task
160503 5 kPa + Supp 60x_Multichannel_20160503_02 - GFP Deck-1.vsi.tif	open	View Task
160503 5 kPa + Supp 60x_Multichannel_20160503_02 - TxRed Deck-1.vsi.tif	open	View Task

The remainder of the open tasks will be listed when the "Open" tab is selected, and the total tally should be reflected across the top of the page. Here, 5 images are still listed as open, but the one image that we have started is now listed as "In Progress".

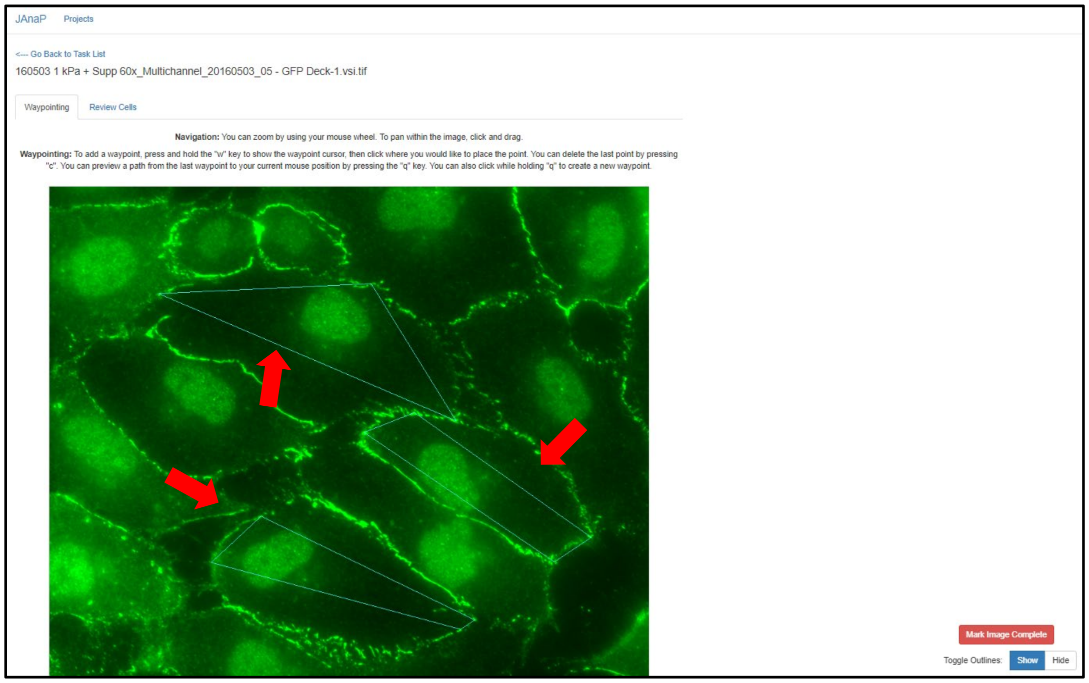
When you click on the "In Progress" tab, all images that have been started will be listed:



Open	In Progress	Complete	Total
5	1	0	6

Image Name	Status	
160503 1 kPa + Supp 60x_Multichannel_20160503_05 - GFP Deck-1.vsi.tif	in-progress	View Task

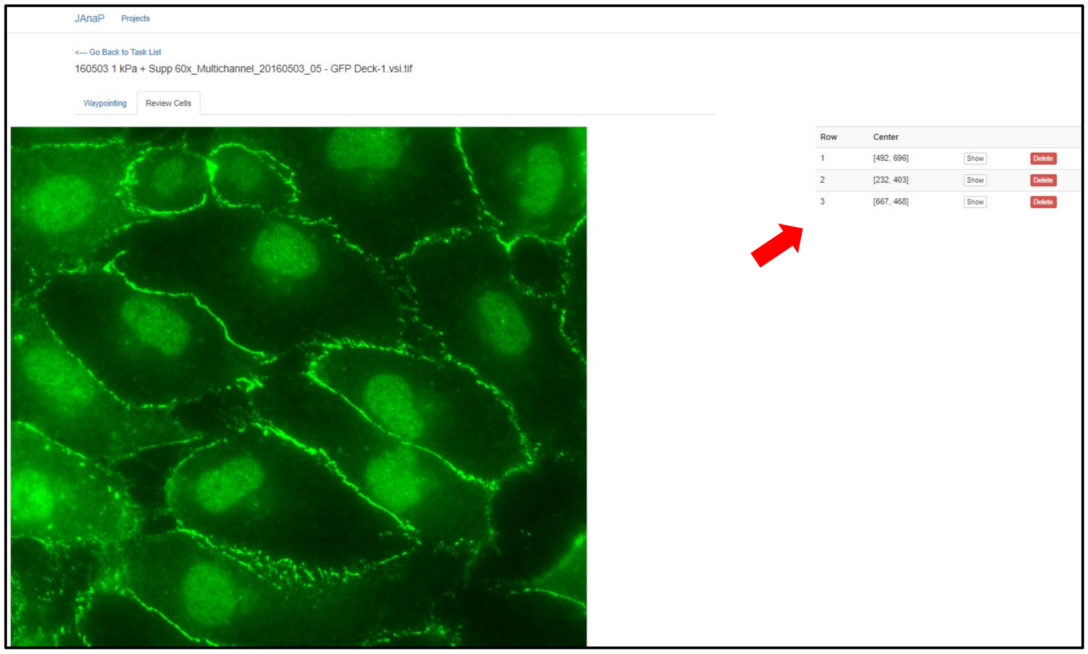
15) To continue waypointing the cells in any image in the “Open” or “In Progress” tab, click “View Task” and repeat steps 10-12.
   - As an example, 3 cells have been traced here, each indicated by the blue outlines in the image below and listed in the “Review Cells” tab in the second image.



160503 1 kPa + Supp 60x_Multichannel_20160503_05 - GFP Deck-1.vsi.tif

Navigation: You can zoom by using your mouse wheel. To pan within the image, click and drag.

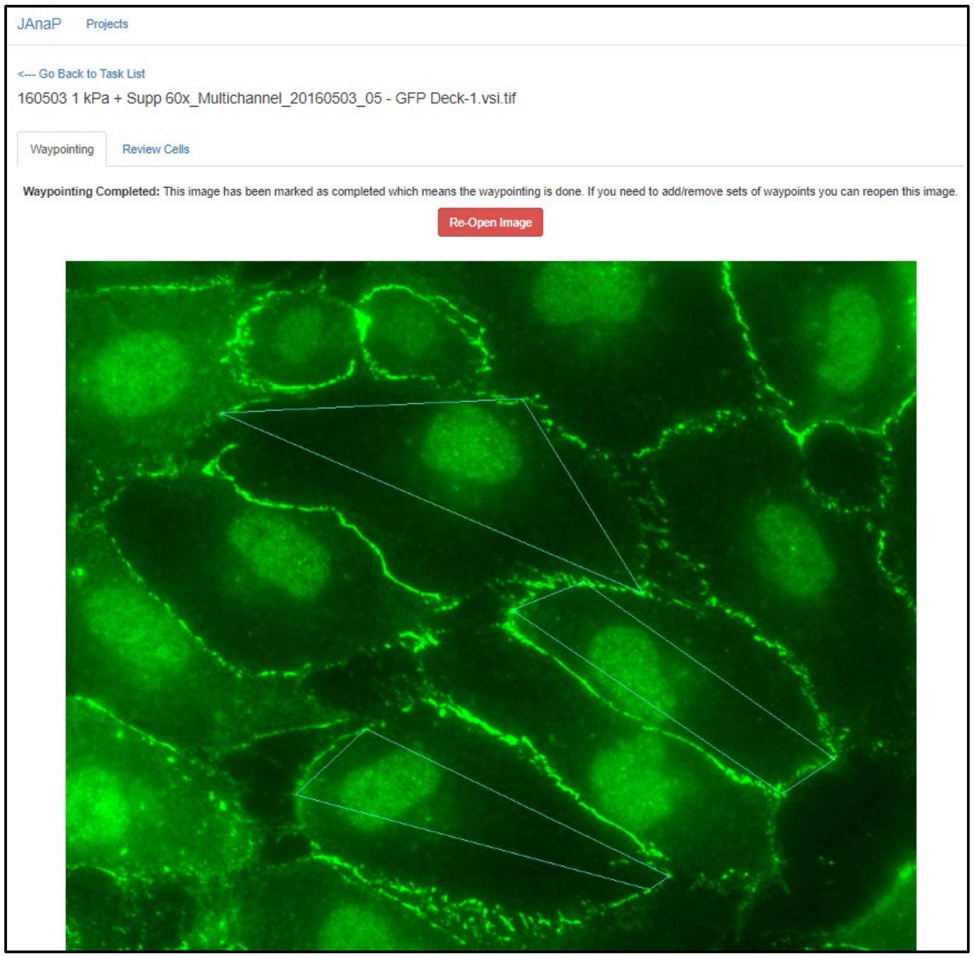
Waypointing: To add a waypoint, press and hold the "w" key to show the waypoint cursor, then click where you would like to place the point. You can delete the last point by pressing "c". You can preview a path from the last waypoint to your current mouse position by pressing the "q" key. You can also click while holding "q" to create a new waypoint.



160503 1 kPa + Supp 60x_Multichannel_20160503_05 - GFP Deck-1.vsi.tif

Row	Center		
1	[492, 696]	Show	Delete
2	[232, 403]	Show	Delete
3	[667, 468]	Show	Delete

16) Once all cells of interest in the image have been traced, navigate back to the “Waypointing” tab and click “Mark Image Complete” in the bottom right corner. The following screen will appear:

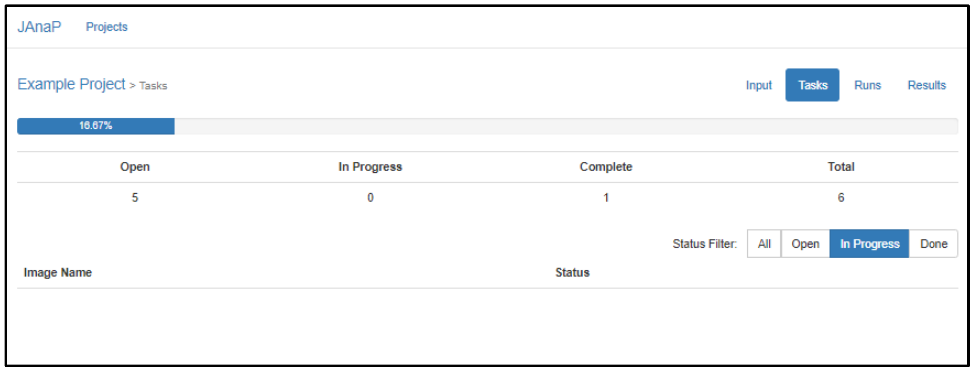


160503 1 kPa + Supp 60x_Multichannel_20160503_05 - GFP Deck-1.vsi.tif

Waypointing Completed: This image has been marked as completed which means the waypointing is done. If you need to add/remove sets of waypoints you can reopen this image.

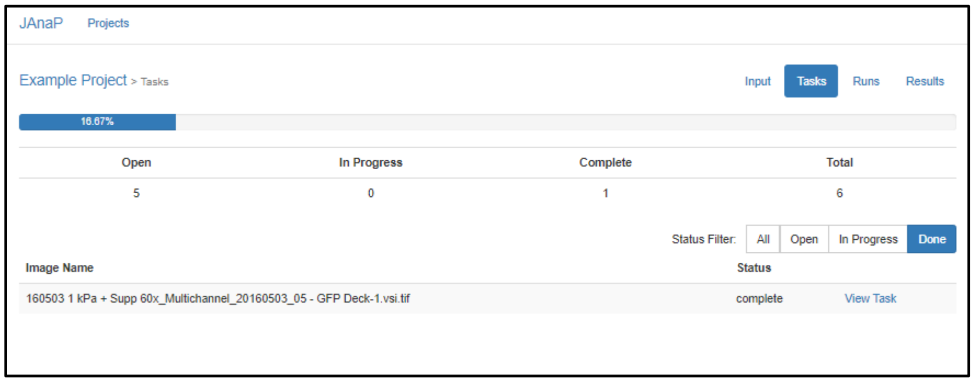
- The “Re-Open Image” button can be clicked if any adjustments are needed after the image has been marked as “complete”. “Re-opening” the image will allow you to continue waypointing new cells, or review or delete previously waypointed cells as described in step 14.
  - Note: If you re-open the image, be sure to “Mark Image Complete” once all changes have been made. “Open” images will not be processed, even if cells have been waypointed within that image.

17) Click on the “← Go Back to Task List” link in the top left of the screen to bring you back to the previous “Tasks” tab, where the “Status Filter” will be updated accordingly:



Open	In Progress	Complete	Total
5	0	1	6

- In this example, the one image we have marked as complete is now registered as “Complete” and nothing is listed as “In Progress”. Select the “Done” tab to view the list of images marked as complete:

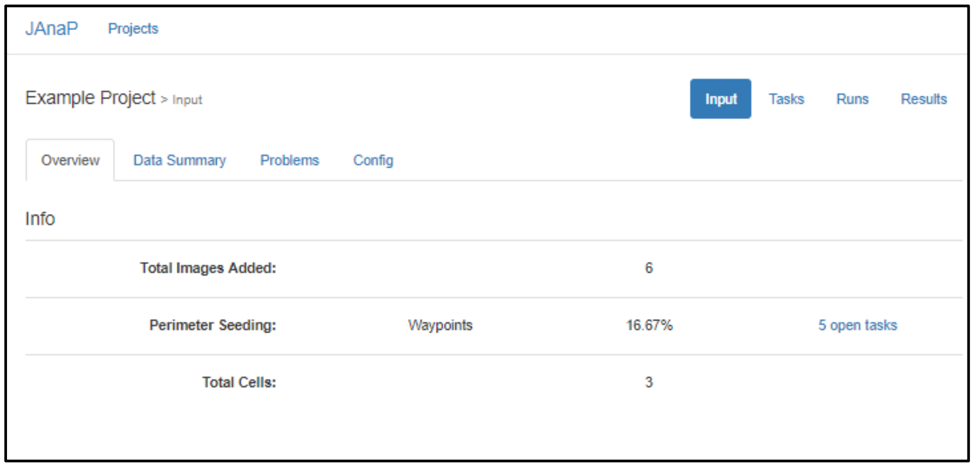


Open	In Progress	Complete	Total
5	0	1	6

Image Name	Status
160503 1 kPa + Supp 60x_Multichannel_20160503_05 - GFP Deck-1.vsi.tif	complete

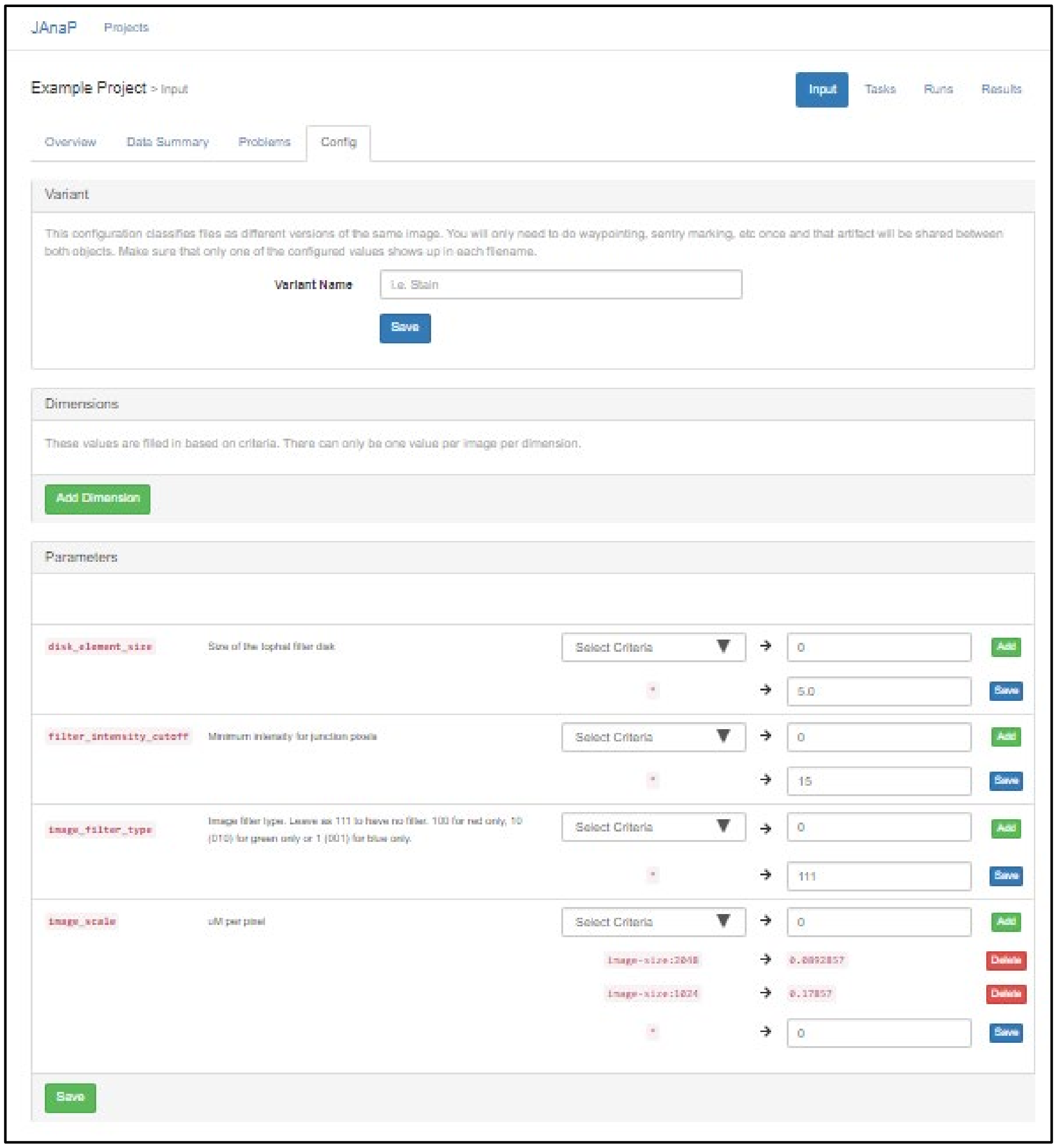
- NOTE: Clicking “View Task” next to the image in the “Done” tab will bring you back to the screen in step 17 and allow you to re-open the image if needed.

18) To navigate back to the project overview, click the “Input” button next to “Tasks” in the upper right corner. The “Overview” tab will appear and provide an updated count of the total images added, the percentage of the images marked as “Done”, and the total number of waypointed cells at that time:



Total Images Added:		6	
Perimeter Seeding:	Waypoints	16.67%	5 open tasks
Total Cells:		3	

19) Click the “Config” tab to add in the user-designated and project-specific input. The follow screen will appear:


JAnaP Projects

Example Project > Input

Input Tasks Runs Results

Overview Data Summary Problems Config

Variant

This configuration classifies files as different versions of the same image. You will only need to do waypointing, sentry marking, etc once and that artifact will be shared between both objects. Make sure that only one of the configured values shows up in each filename.

Variant Name: i.e. Stain

Save

Dimensions

These values are filled in based on criteria. There can only be one value per image per dimension.

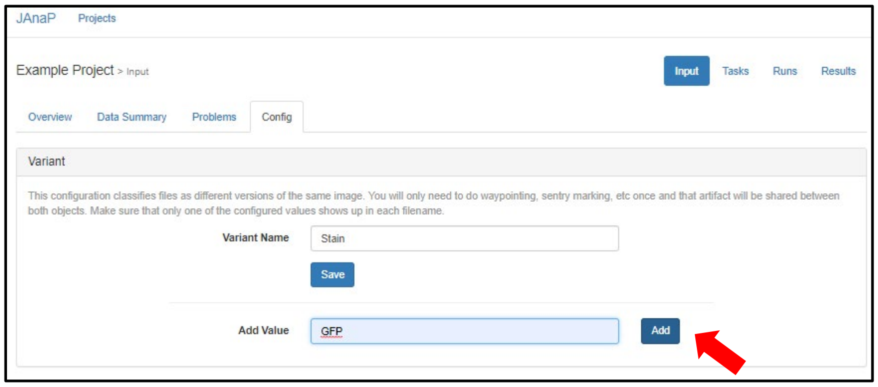
Add Dimension

Parameters

`disk_element_size`	Size of the tophat filter disk	Select Criteria	→	0	Add
		*	→	5.0	Save
`filter_intensity_cutoff`	Minimum intensity for junction pixels	Select Criteria	→	0	Add
		*	→	15	Save
`image_filter_type`	Image filter type. Leave as 111 to have no filter. 100 for red only, 10 (010) for green only or 1 (001) for blue only.	Select Criteria	→	0	Add
		*	→	111	Save
`image_scale`	uM per pixel	Select Criteria	→	0	Add
		`image-size:2048`	→	`0.0892857`	Delete
		`image-size:1024`	→	`0.17857`	Delete
		*	→	0	Save

Save

- **Variant -** The first option is to designate a “Variant Name”. Typically, this is used if you include more than one stain/channel of the same image. This allows you to waypoint a cell one time but analyze the junctions immunostained for different proteins using different secondary fluorophores. It requires that the same image name is used for the different filters/channels except for the specific designation of the different filter/channel. Note that the variant values must be written out within the image name.
  - In this case, the “Variant Name” could be something like “Stain” or “Filter”.
  - Each value of the variant (e.g., type of stain or filter, like GFP or TxRed) would then be typed in the “Add Value” field and “Add” would be clicked. These must be the specific designations that differentiate between the same images that otherwise have the same name. Be sure to check how it is written within the image filenames (e.g., TxRed versus TexasRed) and include each value.

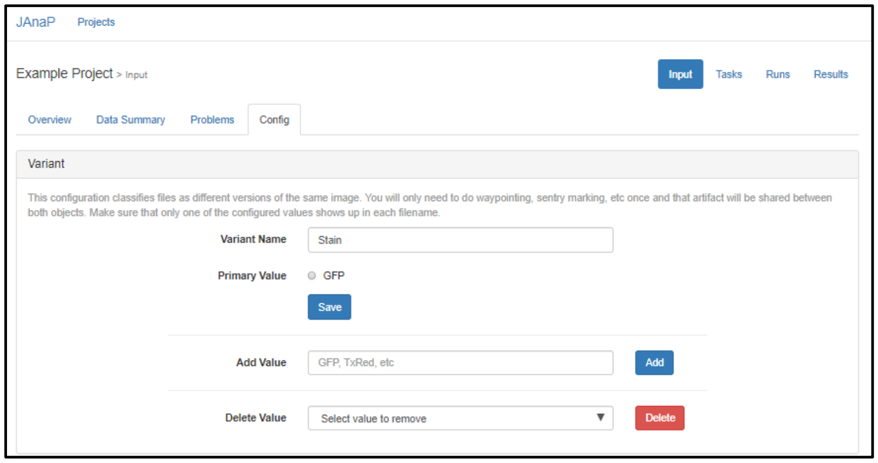


This configuration classifies files as different versions of the same image. You will only need to do waypointing, sentry marking, etc once and that artifact will be shared between both objects. Make sure that only one of the configured values shows up in each filename.

Variant Name: Stain

Add Value: GFP

- The added values will appear under the “Primary Value” field and can be deleted via the “Delete Value” selection field:



This configuration classifies files as different versions of the same image. You will only need to do waypointing, sentry marking, etc once and that artifact will be shared between both objects. Make sure that only one of the configured values shows up in each filename.

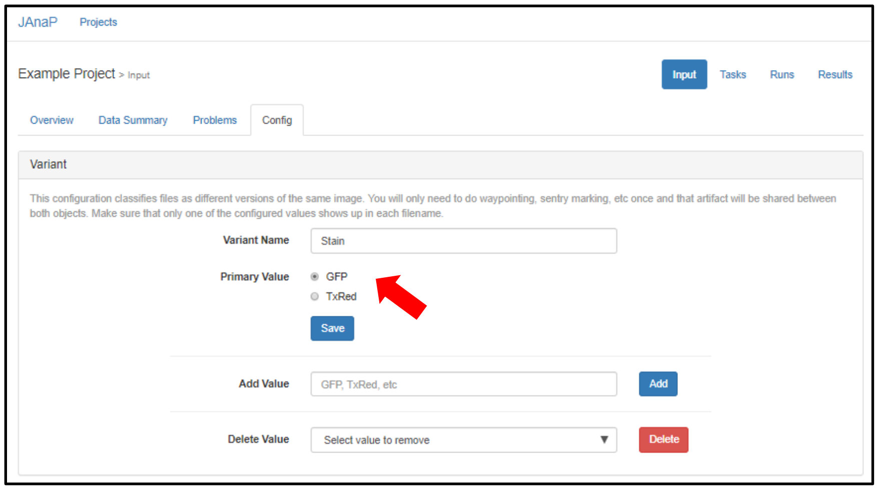
Variant Name: Stain

Primary Value: ○ GFP

Add Value: GFP, TxRed, etc

Delete Value: Select value to remove

- Once you’ve added all variant values, you can then select a “Primary Value” which designates the channel you will perform the waypointing on. Then click “Save.” This will remove the other channels/stains/variant values from the “Tasks” lists, since the waypoints are only identified on the “Primary Value” images and will be applied to the other channels/values automatically for processing.
    I. Here, GFP has been selected as the “Primary Value”:



This configuration classifies files as different versions of the same image. You will only need to do waypointing, sentry marking, etc once and that artifact will be shared between both objects. Make sure that only one of the configured values shows up in each filename.

Variant Name: Stain

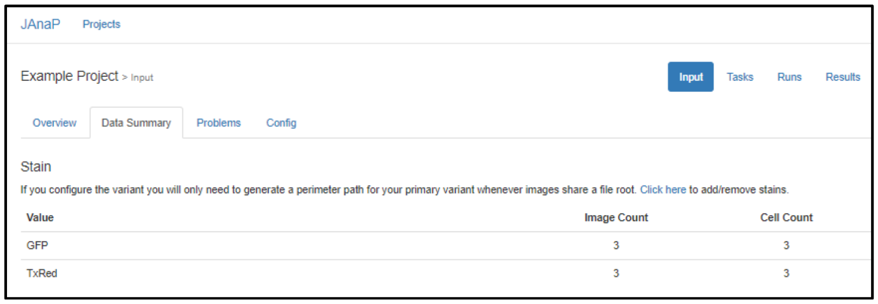
Primary Value: ● GFP ○ TxRed

Add Value: GFP, TxRed, etc

Delete Value: Select value to remove

- Once variants have been added, they will be included in the “Data Summary” tab along with the breakdown of image and cell counts within each variant value.

  In this example we have included 3 GFP images and 3 TxRed images, which is reflected in the “Image Count”. At this point, we have waypointed 3 cells in one GFP image. Those 3 cells are reflected in the “Cell Count” column. Since we added GFP and TxRed as variants, and selected GFP as the primary value, however, those 3 waypoints were also transferred to the TxRed counterparts of those GFP images, thus 3 cells are listed in the “Cell Count” column for that variant as well:

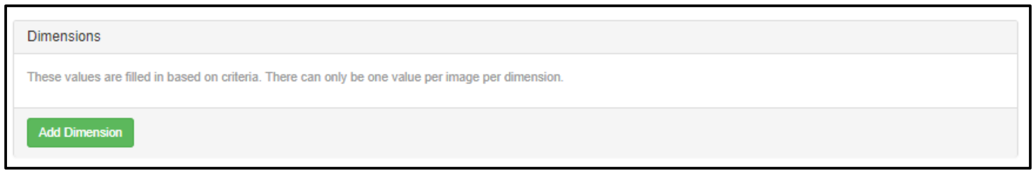


Stain

If you configure the variant you will only need to generate a perimeter path for your primary variant whenever images share a file root. Click here to add/remove stains.

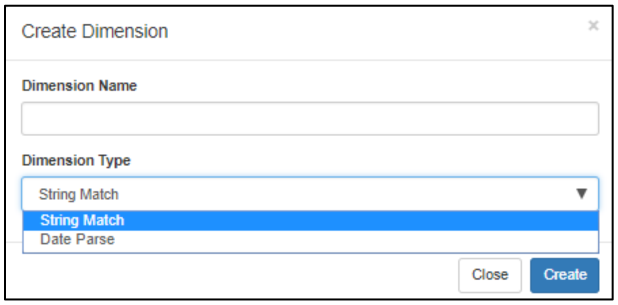
Value	Image Count	Cell Count
GFP	3	3
TxRed	3	3

- **Dimension -** The next option is to designate “Dimensions” located in the “Config” tab under the “Variant” section. This allows you to designate different conditions, experiments, trial dates, etc. within this one project based on the image filenames, that will help ease data filtering/sorting for post-processing data analysis. To do this, click the green “Add Dimension” button:

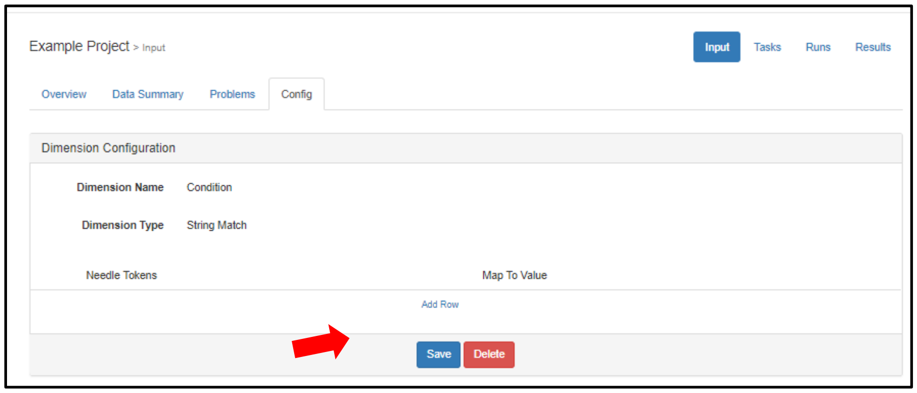


These values are filled in based on criteria. There can only be one value per image per dimension.

- The following screen will appear and allow you to identify the name and type of dimension. Then click “Create”.
  I. The **Dimension Name** should be a descriptive title for the category you are sorting, for example, the name could be “Treatment Group” or “Date”, etc.
  II. The two options for the **Dimension Type** are:



  - “Date Parse” - uses date within the filename or associated with the file to sort cells/files by date. If this is selected, click “create” and then select “Config” tab (clicking “create” auto-saves the Dimension)
  - “String Match” - uses character strings within the filename to sort cells/files into the specific dimension category. If this is selected, click “create”, and the following screen will appear.
    - This is where the different groups within the specified category can be defined. Click “Add Row” to input each category sub-type.


Example Project > Input

Input Tasks Runs Results

Overview Data Summary Problems Config

Dimension Configuration

Dimension Name Condition

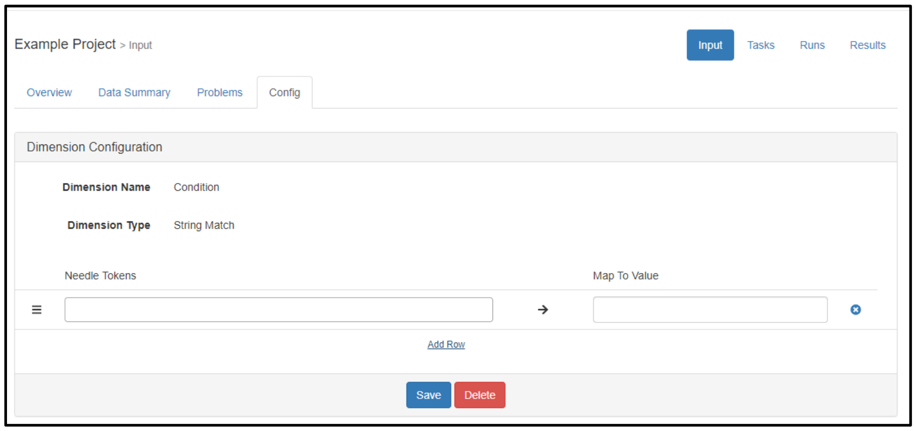
Dimension Type String Match

Needle Tokens Map To Value

Add Row

Save Delete

- The follow screen will appear:


Example Project > Input

Input Tasks Runs Results

Overview Data Summary Problems Config

Dimension Configuration

Dimension Name Condition

Dimension Type String Match

Needle Tokens Map To Value

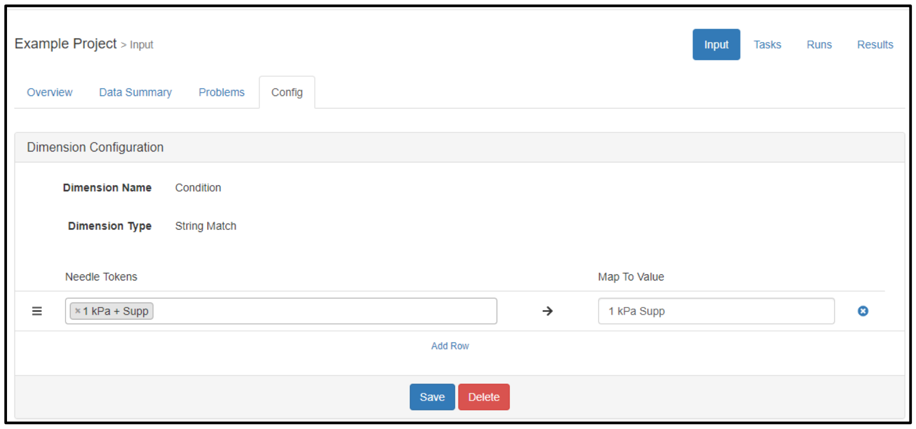
Add Row

Save Delete

- Here, a "Needle Token" is a phrase within the image title that will be used to sub-categorize the image and the "Map to Value" is the sub-category to which you would like it assigned.
  - Needle Tokens:
    - Type in the phrase and then hit "Enter" or "Shift + Enter" to register it in the needle token field
    - Several needle tokens can be associated with one "Map to Value". This is particularly helpful if there are differences in naming schemes between different dates or trials.
    - Note that the Needle Tokens are case and space sensitive so they must exactly match the phrase within the image title.
    - Importantly, the algorithm cycles through the list of needle tokens and assigns the filename to the "Map to Value" associated with the first "Needle Token" found within the filename. Therefore, start with the most specific "Needle Tokens" first on the list.
  - Map to Value:
    - This will be the sub-category listed next to the cells/images in the output, so use something that allows for easy "filtering" or "sorting" in the spreadsheet
    - This phrase must be at least 5 characters long, so if needed, add spaces between each letter to increase the total phrase character length
  - Click "Save"
  - To add more sub-categories, click "Add Row" and repeat the above steps to enter in each sub-category needed

- As an example, here the filename is: 160503 1 kPa + Supp 60x_Multichannel_20160503_05 - GFP Deck-1.vsi.tif

- We want to group this image into a sub-category called “1 kPa Supp” so we assign the “Needle Token” as “1 kPa + Supp” and the “Map to Value” as “1 kPa Supp” as below:



Example Project > Input

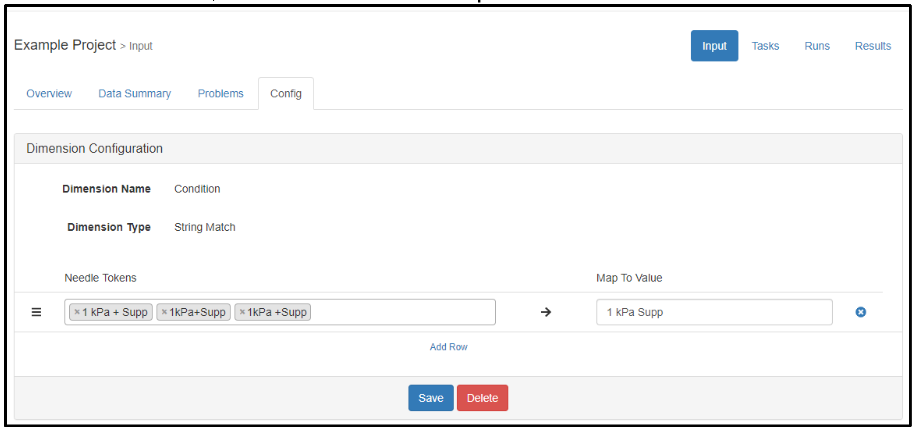
Dimension Configuration

Dimension Name: Condition

Dimension Type: String Match

Needle Tokens	Map To Value
1 kPa + Supp	1 kPa Supp

- Now, all images with “1 kPa + Supp” in the filename will be sub-categorized to “1 kPa Supp” in the output excel spreadsheet.
- Say, though, that for some of the images, the naming scheme was altered such that some of the files were instead missing a space between a few or all the phrases in the needle token (e.g., 160503 1kPa+Supp 60x_Multichannel_20160503_05 – GFP Deck-1.vsi.tif, or 160503 1kPa +Supp 60x_Multichannel_20160503_05 – GFP Deck-1.vsi.tif). You still want them to be sub-categorized as “1 kPa Supp” since they are the same condition, but the needle tokens don’t exactly match the phrases in the filename to be accurately sub-categorized. Therefore, you would add each different naming structure in as a needle token, all to the same “Map to Value” as below:



Example Project > Input

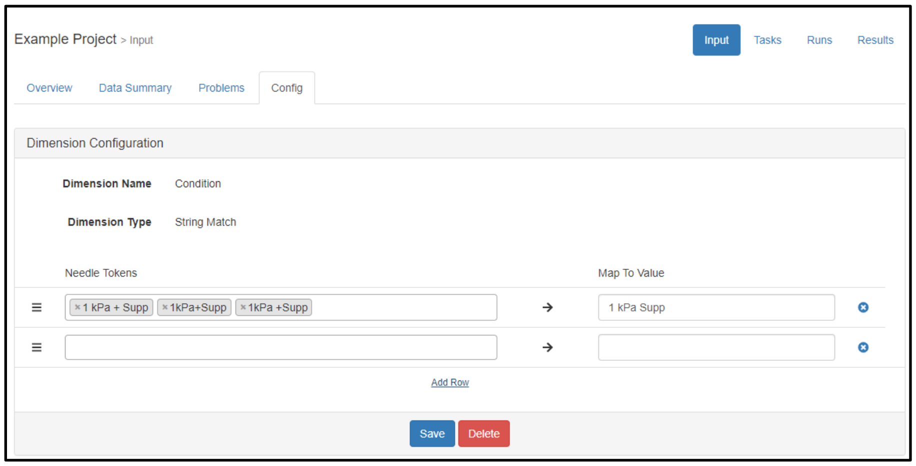
Dimension Configuration

Dimension Name: Condition

Dimension Type: String Match

Needle Tokens	Map To Value
1 kPa + Supp, 1kPa+Supp, 1kPa +Supp	1 kPa Supp

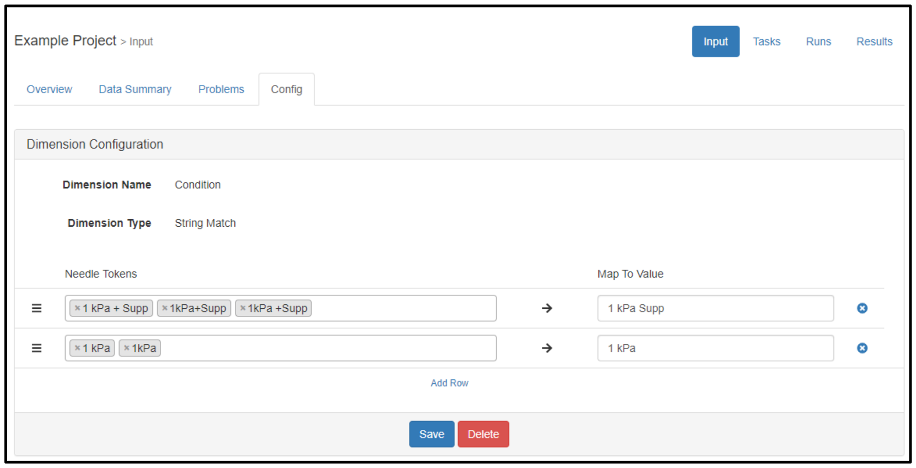
- Now, in this example, we also have a condition on 1 kPa without supplement. So, we click “Add Row” so another box appears as below:


Example Project > Input

Dimension Name	Condition
Dimension Type	String Match

Needle Tokens	Map To Value
1 kPa + Supp, 1kPa+Supp, 1kPa +Supp	1 kPa Supp

- We type in “1 kPa” as a “Needle Token” and as a “Map to Value”. Since perhaps the naming scheme was also an issue here, we also include “1kPa” as an additional “Needle Token” as below, and hit “save”:


Example Project > Input

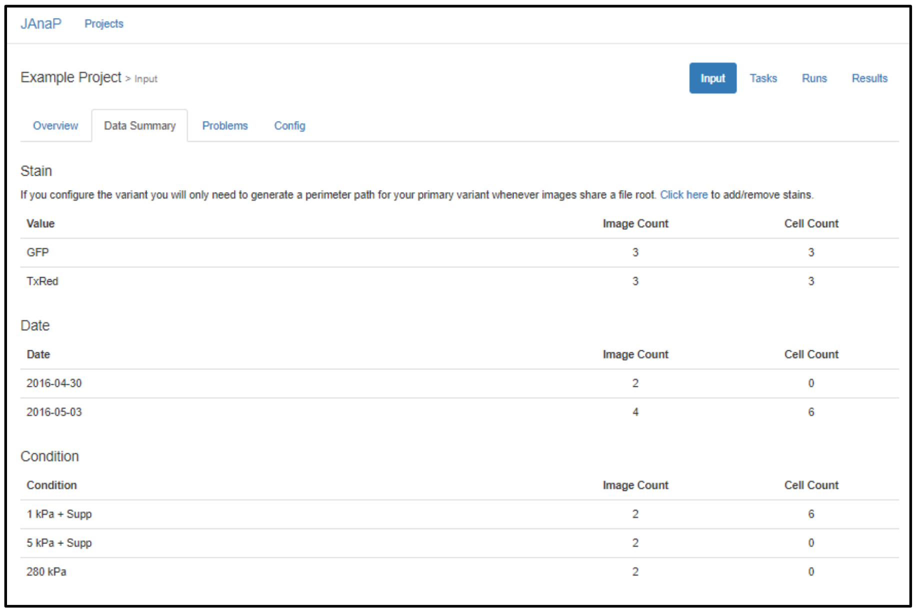
Dimension Name	Condition
Dimension Type	String Match

Needle Tokens	Map To Value
1 kPa + Supp, 1kPa+Supp, 1kPa +Supp	1 kPa Supp
1 kPa, 1kPa	1 kPa

- Importantly: We added 1 kPa + Supp first before 1 kPa since the program cycles through the list of needle tokens in order and assigned the image to the first sub-category to which it fits. Since “1 kPa” is included in the image names containing “1 kPa + Supp”, all images would have been assigned to the “1 kPa” Map to Value if it were listed first. As written, the image name must meet the first criteria (e.g., include 1 kPa + Supp) otherwise it will be grouped a “1 kPa”.

---------- End Example

- Once all Dimensions have been put in and saved, you can navigate to the “Data Summary” tab. This section should now be populated to reflect the “Variants” and the “Dimensions” and the respective Image and Cell Counts associated with each categorization:



JAnaP Projects

Example Project > Input

Stain

If you configure the variant you will only need to generate a perimeter path for your primary variant whenever images share a file root. Click here to add/remove stains.

Value	Image Count	Cell Count
GFP	3	3
TxRed	3	3

Date

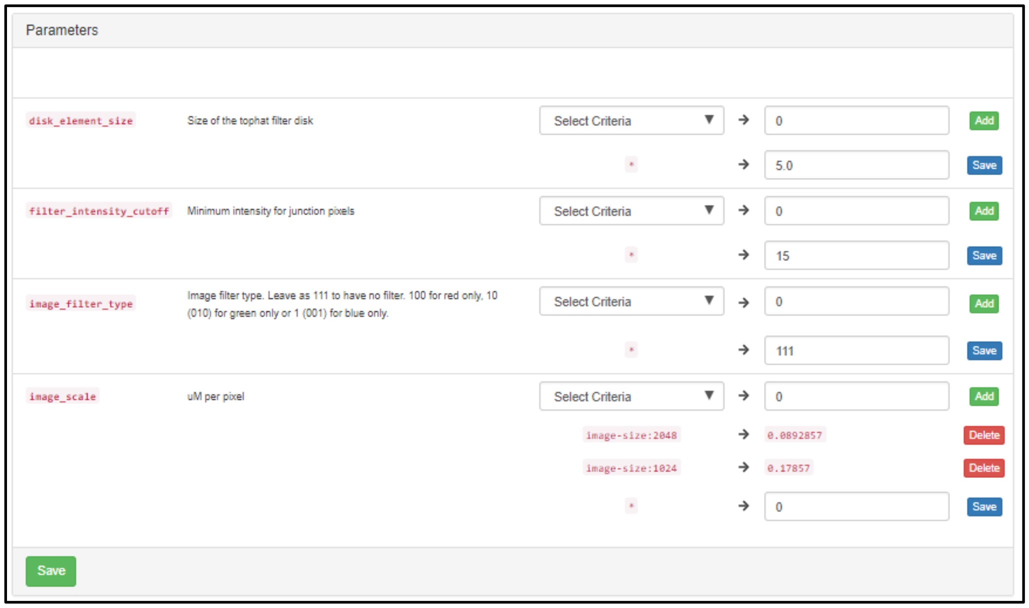
Date	Image Count	Cell Count
2016-04-30	2	0
2016-05-03	4	6

Condition

Condition	Image Count	Cell Count
1 kPa + Supp	2	6
5 kPa + Supp	2	0
280 kPa	2	0

Here, the counts still reflect the 3 cells in the 1 image that has been marked complete plus the TxRed counterpart based on the variants identified.

- **Parameters -** The next option on the "Config" tab (below "Variant" and "Dimension") is to designate "Parameters":



Parameters

disk_element_size	Size of the tophat filter disk	Select Criteria	→	0	Add
		*	→	5.0	Save
filter_intensity_cutoff	Minimum intensity for junction pixels	Select Criteria	→	0	Add
		*	→	15	Save
image_filter_type	Image filter type. Leave as 111 to have no filter. 100 for red only, 10 (010) for green only or 1 (001) for blue only.	Select Criteria	→	0	Add
		*	→	111	Save
image_scale	uM per pixel	Select Criteria	→	0	Add
		image-size:2048	→	0.0892857	Delete
		image-size:1024	→	0.17857	Delete
		*	→	0	Save

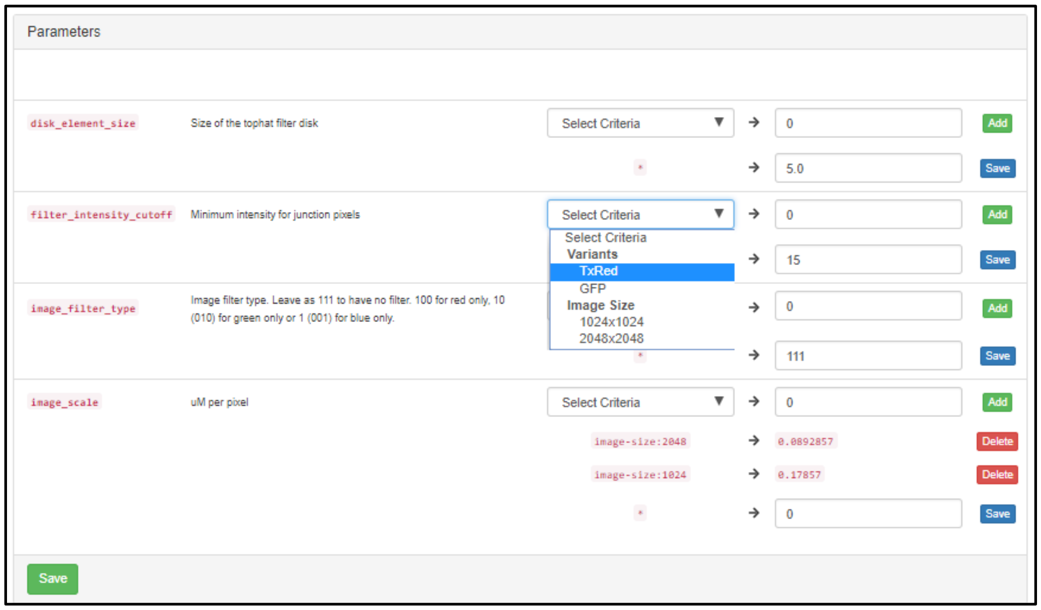
- This section allows you to adjust the following:
  - **I.** disk_element_size – this allows the user to define the size of the disk used for the tophat filter. The default is a disk size of 5 and should only be changed in specific circumstances.
  - **II.** filter_intensity_cutoff – this defines the intensity threshold value used to differentiate between regions of junction and no junction. This value should be determined for each project (using the python notebook described below) but a good starting point is 15 for GFP images and 5 for TxRed images with low levels of background. See the bottom section "Jupyter Notebook" for how to check the appropriate threshold values for your project.

III. <u>image_filter_type</u> – this defines which color channels are included in the analysis where the three numbers represent RGB. The default is 111 which keeps full RGB images in the analysis and should only be changed in specific circumstances.

IV. <u>image_scale</u> – this defines the conversion from pixel to microns so that the results are reported in terms of microns. The input values are based on image size and put in units of micron per pixel. This value should be changed for each project depending on the objective and camera used for image collection and the resolution of the images.

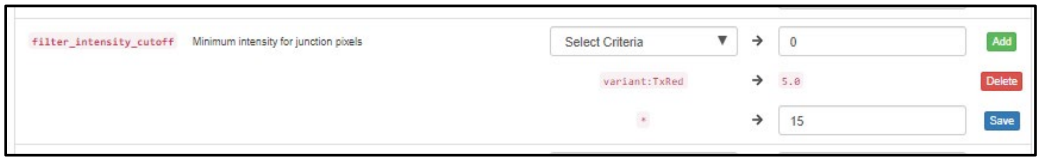
- NOTE: The default values reflect the Stroka Lab IX83 microscope using the 60x oil objective for 1024 x 1024 and 2048 x 2048 pixel images

Note that a * indicates the default value applied to all images. There is a dropdown menu next to each variable that allows the user the designate a specific variable for each type/category of image. The "Delete", "Add", "Save" buttons can be used to adjust all inputs as necessary.



Parameters

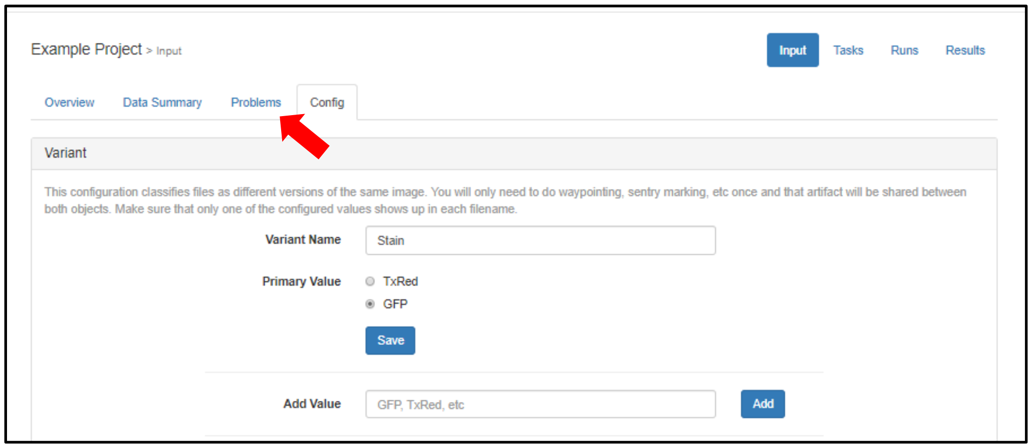
disk_element_size	Size of the tophat filter disk	Select Criteria	→	0	Add
		*	→	5.0	Save
filter_intensity_cutoff	Minimum intensity for junction pixels	Select Criteria	→	0	Add
		*	→	15	Save
image_filter_type	Image filter type. Leave as 111 to have no filter. 100 for red only, 10 (010) for green only or 1 (001) for blue only.	Select Criteria	→	0	Add
		*	→	111	Save
image_scale	uM per pixel	Select Criteria	→	0	Add
		image-size:2048	→	0.0892857	Delete
		image-size:1024	→	0.17857	Delete
		*	→	0	Save



filter_intensity_cutoff	Minimum intensity for junction pixels	Select Criteria	→	0	Add
		variant:TxRed	→	5.0	Delete
		*	→	15	Save

20) Click save in the bottom left hand corner of the Config tab to save any changes

21) To check if there are any issues with the uploaded images based on the configurations set up in the Config tab, scroll up to the top of the "Input" screen and click on the "Problems Tab" indicated below



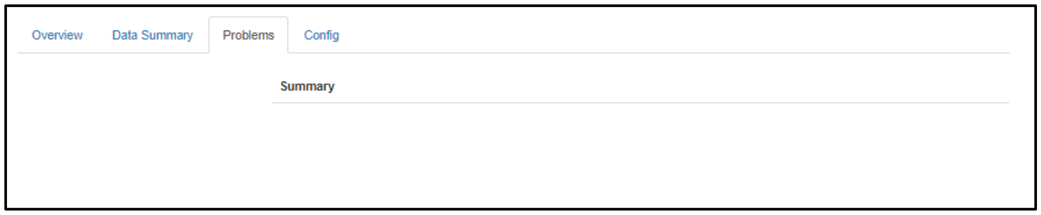
Variant

This configuration classifies files as different versions of the same image. You will only need to do waypointing, sentry marking, etc once and that artifact will be shared between both objects. Make sure that only one of the configured values shows up in each filename.

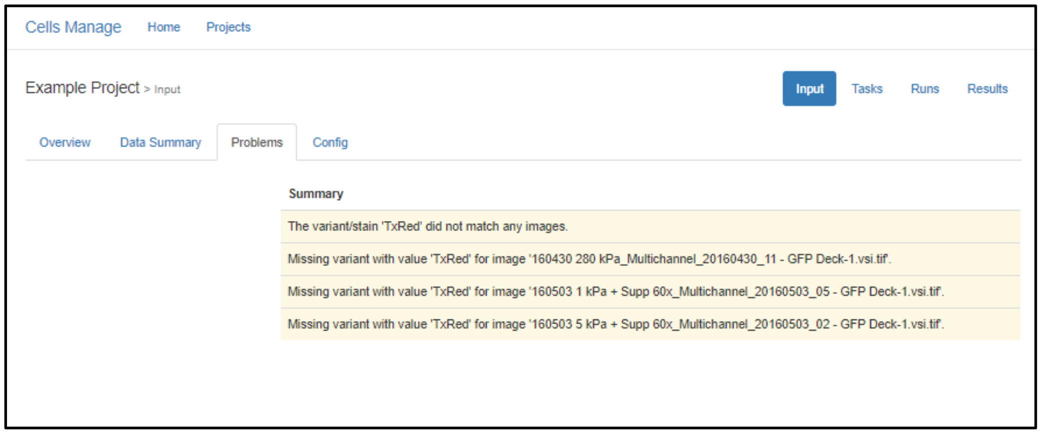
Variant Name: Stain

Primary Value: ○ TxRed ● GFP

If no problems exist, the “Problems” tab will be blank as below:



If a problem is present, it will be listed under “Summary” with an explanation of the problem. Below is an example of this situation:



Summary

The variant/stain 'TxRed' did not match any images.

Missing variant with value 'TxRed' for image '160430 280 kPa_Multichannel_20160430_11 - GFP Deck-1.vsi.tif'.

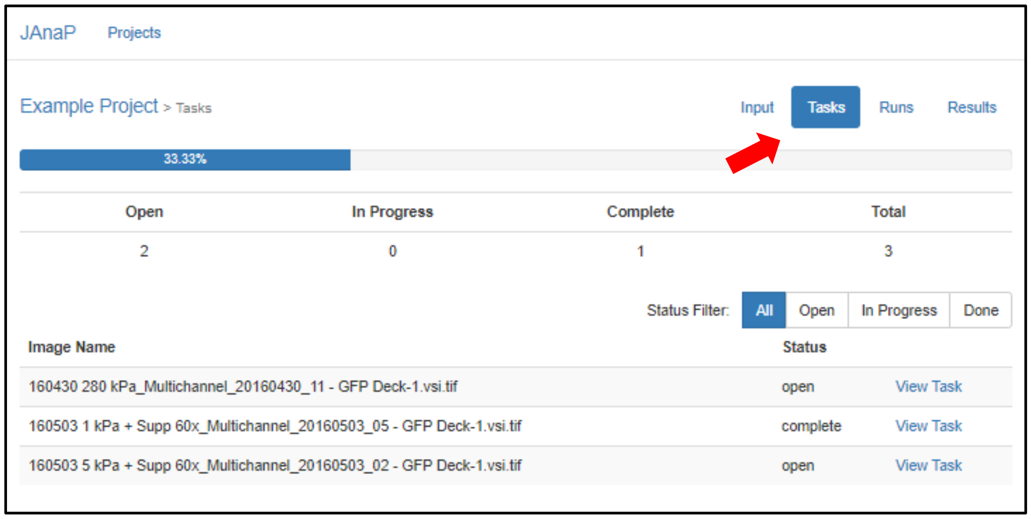
Missing variant with value 'TxRed' for image '160503 1 kPa + Supp 60x_Multichannel_20160503_05 - GFP Deck-1.vsi.tif'.

Missing variant with value 'TxRed' for image '160503 5 kPa + Supp 60x_Multichannel_20160503_02 - GFP Deck-1.vsi.tif'.

Here, two variants were added to the configuration (i.e., GFP and TxRed) but no images with TxRed in the filename were uploaded into the program. This could be because those images were accidentally skipped when uploading the images or because the files do not have TxRed in the filenames. Note: variants must be an exact match to the filename (e.g., Texas Red in the filename will not be included with the TxRed variant).

As problems are fixed, they will be removed from this list on the Problems tab.

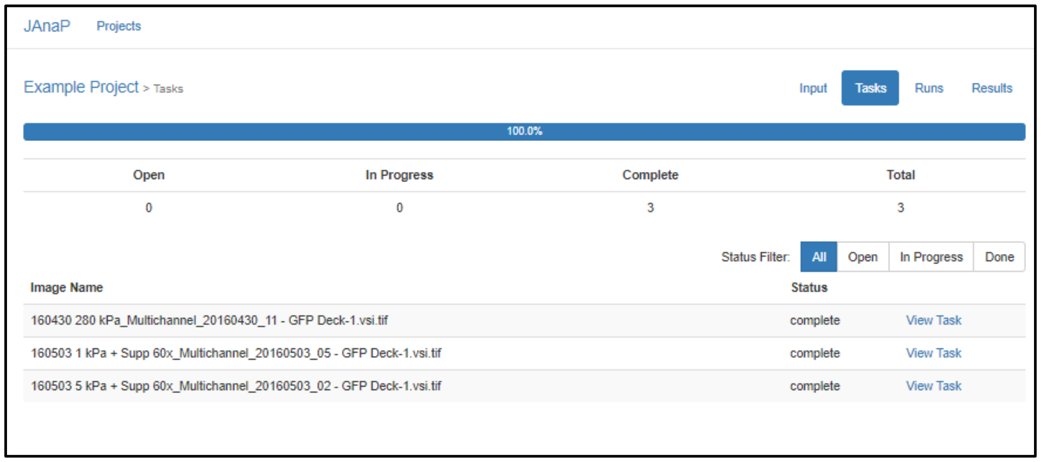
22) Click on the Tasks tab button in the top right of the project to check your waypointing progress on based on any changes to your configuration. Note that the number of images requiring waypoints will decrease depending on the number of variants you include (e.g., only half of the images will be listed, if two variants are included).



Open	In Progress	Complete	Total
2	0	1	3

Image Name	Status	
160430 280 kPa_Multichannel_20160430_11 - GFP Deck-1.vsi.tif	open	View Task
160503 1 kPa + Supp 60x_Multichannel_20160503_05 - GFP Deck-1.vsi.tif	complete	View Task
160503 5 kPa + Supp 60x_Multichannel_20160503_02 - GFP Deck-1.vsi.tif	open	View Task

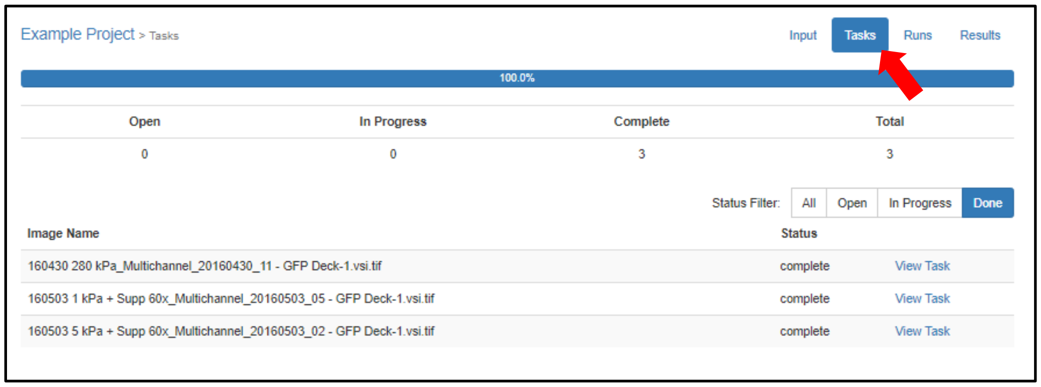
Complete the waypointing process for all images in the “Open” and “In Progress” tabs until the counters read “0” for each and the “Complete” count matches the “Total” count as below:



Open	In Progress	Complete	Total
0	0	3	3

Image Name	Status	
160430 280 kPa_Multichannel_20160430_11 - GFP Deck-1.vsi.tif	complete	View Task
160503 1 kPa + Supp 60x_Multichannel_20160503_05 - GFP Deck-1.vsi.tif	complete	View Task
160503 5 kPa + Supp 60x_Multichannel_20160503_02 - GFP Deck-1.vsi.tif	complete	View Task

23) The program is now ready to be run. To do this, click on the “Runs” tab in the top right corner:



Open	In Progress	Complete	Total
0	0	3	3

Image Name	Status	
160430 280 kPa_Multichannel_20160430_11 - GFP Deck-1.vsi.tif	complete	View Task
160503 1 kPa + Supp 60x_Multichannel_20160503_05 - GFP Deck-1.vsi.tif	complete	View Task
160503 5 kPa + Supp 60x_Multichannel_20160503_02 - GFP Deck-1.vsi.tif	complete	View Task

24) The “Overview” page will load as below:

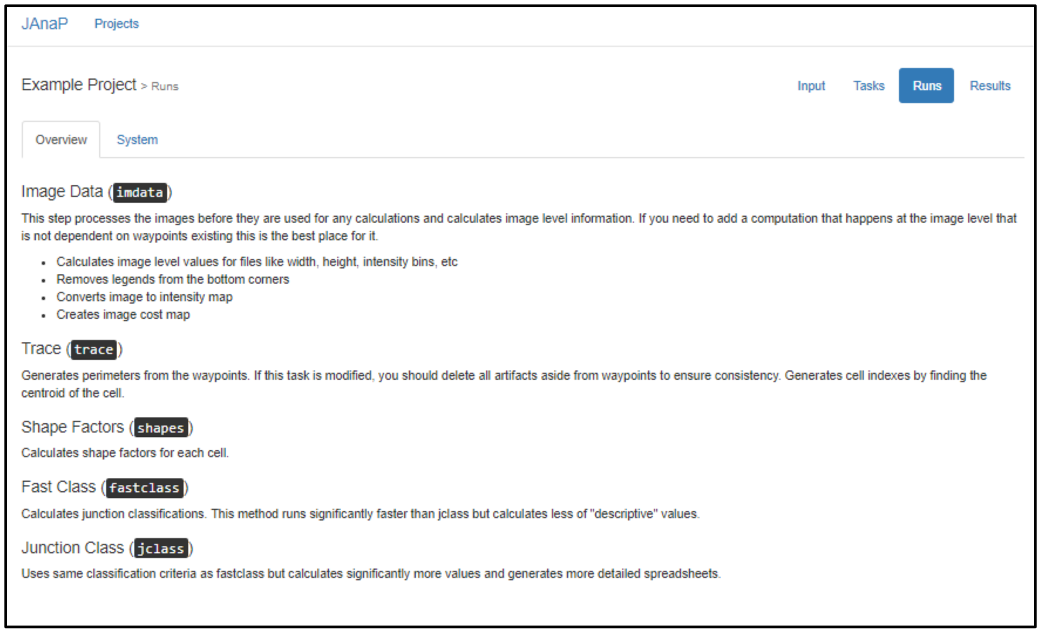


Image Data (`imdata`)

This step processes the images before they are used for any calculations and calculates image level information. If you need to add a computation that happens at the image level that is not dependent on waypoints existing this is the best place for it.

- Calculates image level values for files like width, height, intensity bins, etc
- Removes legends from the bottom corners
- Converts image to intensity map
- Creates image cost map

Trace (`trace`)

Generates perimeters from the waypoints. If this task is modified, you should delete all artifacts aside from waypoints to ensure consistency. Generates cell indexes by finding the centroid of the cell.

Shape Factors (`shapes`)

Calculates shape factors for each cell.

Fast Class (`fastclass`)

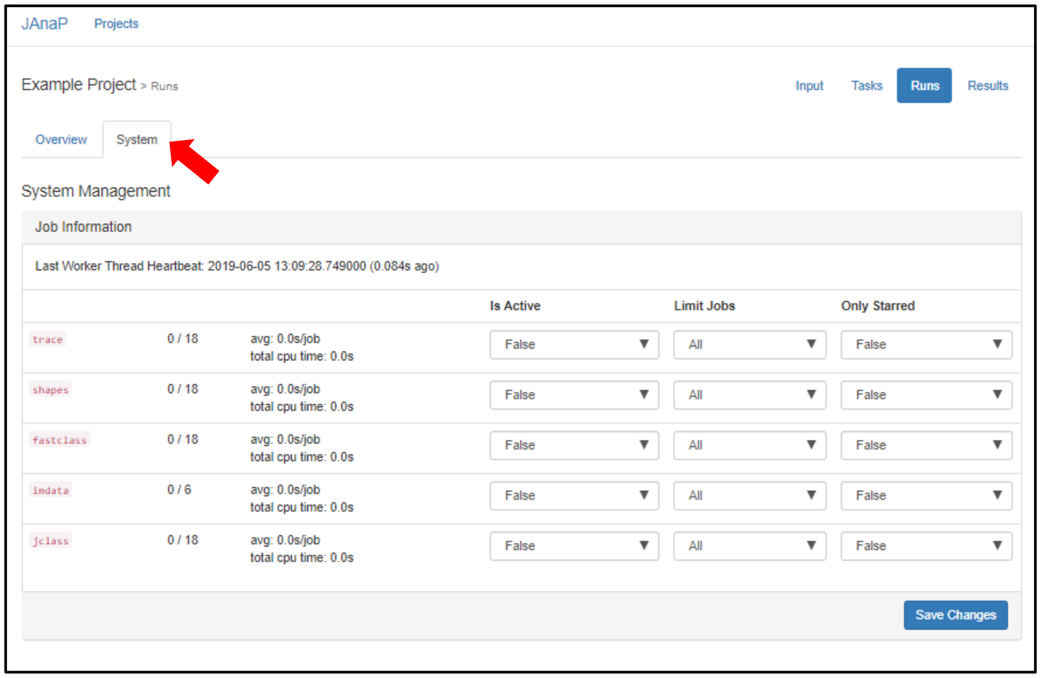
Calculates junction classifications. This method runs significantly faster than jclass but calculates less of "descriptive" values.

Junction Class (`jclass`)

Uses same classification criteria as fastclass but calculates significantly more values and generates more detailed spreadsheets.

- The categories listed on this tab indicate each group of calculations to be processed by the program:
  - Image Data: processes the images as described
  - Trace: traces the outline of each cell to generate the cell path & perimeter
  - Shape Factor: uses trace data to calculate shape factors of each cell (e.g., area, circularity, solidity)
    - Note: a variant must be included for the shape factors to process. If a variant is not needed for your specific application, simply add something that is included in every image title as a placeholder variant and select it as the primary value.
  - Fast Class: categorizes junctions into continuous, punctate and perpendicular
  - Junction Class: generates more detailed calculations for the junctions in Fast Class, such as the start and end index, length, aspect ratio, tip-to-tip distance, etc. for each junction feature coinciding with the cell path.

25) To process the images, navigate to the "System" page by clicking on the "System" tab:



System Management

Job Information

Last Worker Thread Heartbeat: 2019-06-05 13:09:28.749000 (0.084s ago)

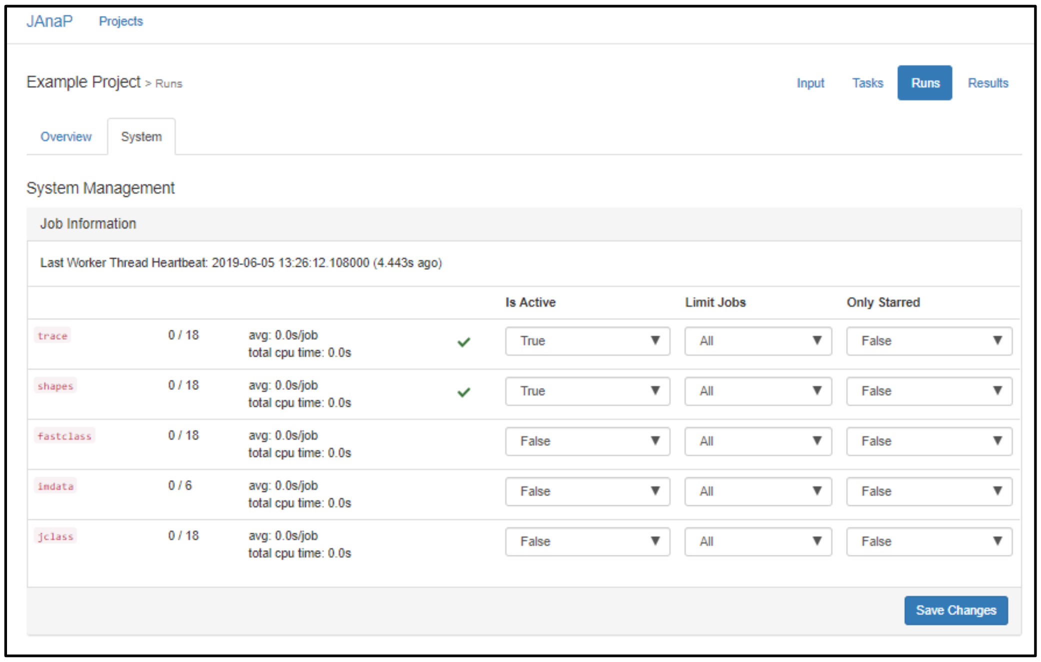
			Is Active	Limit Jobs	Only Starred
trace	0 / 18	avg: 0.0s/job total cpu time: 0.0s	False	All	False
shapes	0 / 18	avg: 0.0s/job total cpu time: 0.0s	False	All	False
fastclass	0 / 18	avg: 0.0s/job total cpu time: 0.0s	False	All	False
imdata	0 / 6	avg: 0.0s/job total cpu time: 0.0s	False	All	False
jclass	0 / 18	avg: 0.0s/job total cpu time: 0.0s	False	All	False

Save Changes

- Here, you will find the categories described on the "Overview" page listed to control which groups are being processed at any time.

- Note: The denominator of the counters (e.g., 0/18) indicates the total number of cells to be analyzed except for "imdata" which indicates the total number of images to be analyzed. Here, we have 6 total images (3 GFP and 3 TxRed) which included 18 total cells (9 from GFP and 9 from TxRed).

26) To begin processing the cells, change the "Is Active" column to "True" using the drop-down menu for each category you would like to process and click "Save Changes". See below as an example:



System Management

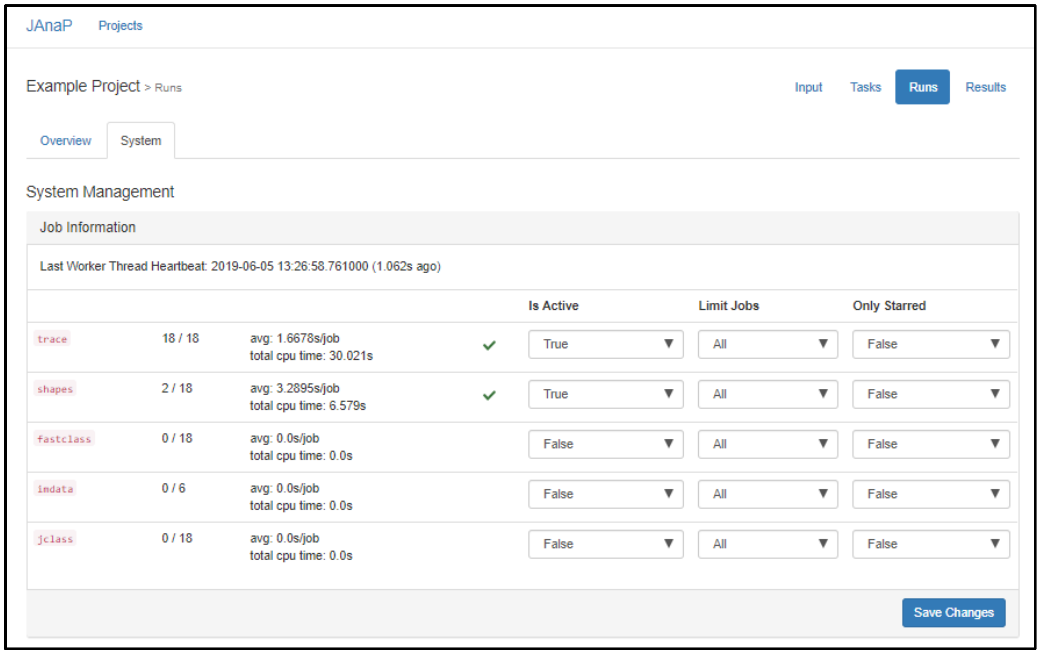
Job Information

Last Worker Thread Heartbeat: 2019-06-05 13:26:12.108000 (4.443s ago)

				Is Active	Limit Jobs	Only Starred
trace	0 / 18	avg: 0.0s/job total cpu time: 0.0s	✓	True	All	False
shapes	0 / 18	avg: 0.0s/job total cpu time: 0.0s	✓	True	All	False
fastclass	0 / 18	avg: 0.0s/job total cpu time: 0.0s		False	All	False
imdata	0 / 6	avg: 0.0s/job total cpu time: 0.0s		False	All	False
jclass	0 / 18	avg: 0.0s/job total cpu time: 0.0s		False	All	False

- To speed up the processing time, open a new command prompt and navigate to the project folder (as in step 1).
  - Within the command prompt, type cd Documents\GitHub\JAnaP-master
  - Hit "Enter" then type: python web\fast-worker.py
  - Note: To stop the fast worker application, type "Ctrl+C" in the command prompt

- Reload the window in your browser at any time to check the progress of the run. The counter will update to show how many images/cells have been processed out of the total. Below is an example where all the cells have been processed for the first category:



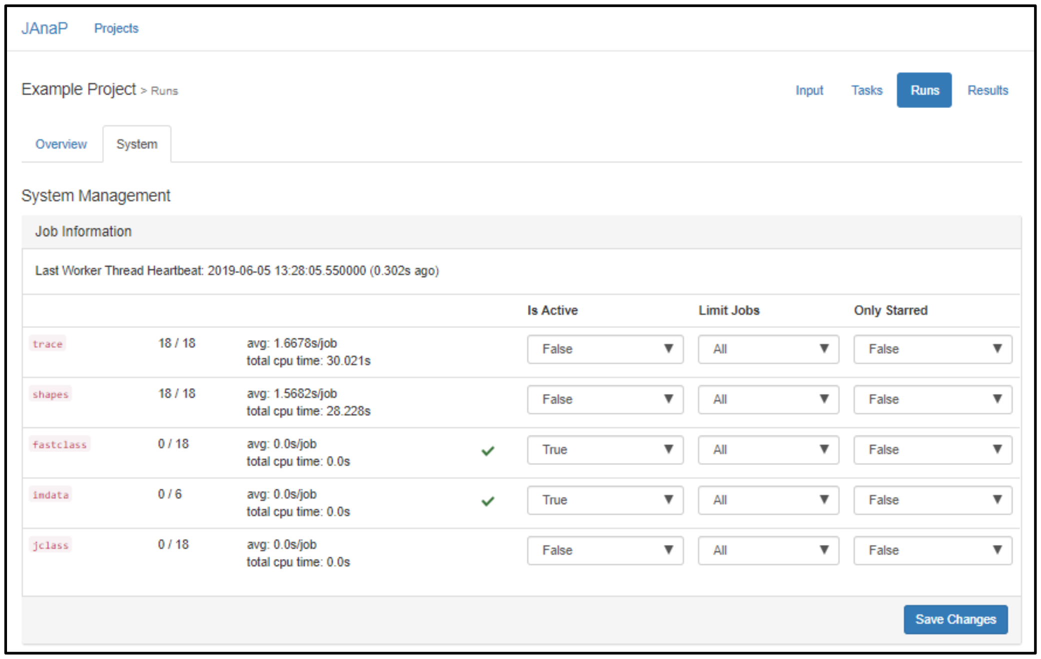
System Management

Job Information

Last Worker Thread Heartbeat: 2019-06-05 13:26:58.761000 (1.062s ago)

				Is Active	Limit Jobs	Only Starred
trace	18 / 18	avg: 1.6678s/job total cpu time: 30.021s	✓	True	All	False
shapes	2 / 18	avg: 3.2895s/job total cpu time: 6.579s	✓	True	All	False
fastclass	0 / 18	avg: 0.0s/job total cpu time: 0.0s		False	All	False
imdata	0 / 6	avg: 0.0s/job total cpu time: 0.0s		False	All	False
jclass	0 / 18	avg: 0.0s/job total cpu time: 0.0s		False	All	False

- To continue processing the other categories, change the "Is Active" status to "True" for the other categories. Since the run is complete for the "trace" and "shape" categories, the status can now be switched to "False".


JAnaP Projects

Example Project > Runs

Input Tasks Runs Results

Overview System

System Management

Job Information

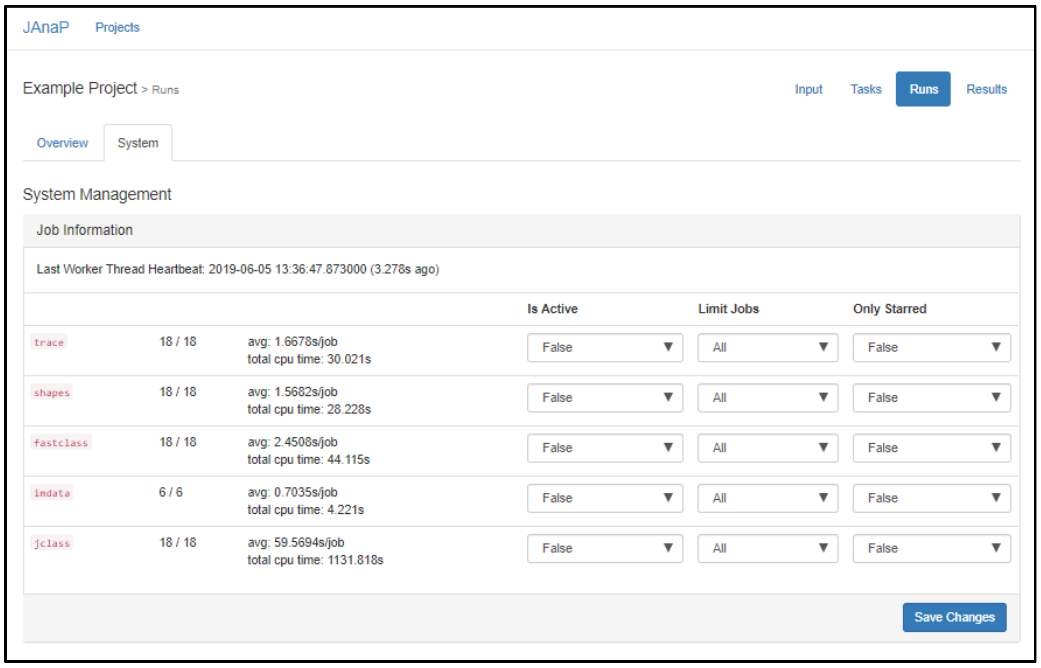
Last Worker Thread Heartbeat: 2019-06-05 13:28:05.550000 (0.302s ago)

				Is Active	Limit Jobs	Only Starred
trace	18 / 18	avg: 1.6678s/job total cpu time: 30.021s		False	All	False
shapes	18 / 18	avg: 1.5682s/job total cpu time: 28.228s		False	All	False
fastclass	0 / 18	avg: 0.0s/job total cpu time: 0.0s	✓	True	All	False
imdata	0 / 6	avg: 0.0s/job total cpu time: 0.0s	✓	True	All	False
jclass	0 / 18	avg: 0.0s/job total cpu time: 0.0s		False	All	False

Save Changes

- Note that you can switch all columns to “True” if you would like to leave the program alone to run for a long period of time (e.g., overnight). The first four categories are all required to generate the categorization spreadsheet which includes the shape factors and the junction analysis. <u>The jclass takes much longer to process</u>, so only switch this to “True” if you specifically need the data generated in that separate spreadsheet.

Once everything is finished, make sure all “Is Active” columns are set to “False”:


JAnaP Projects

Example Project > Runs

Input Tasks Runs Results

Overview System

System Management

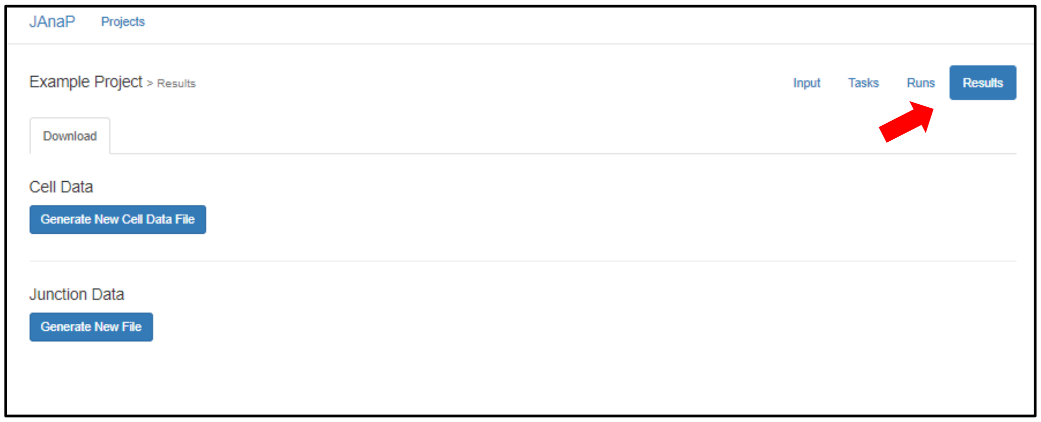
Job Information

Last Worker Thread Heartbeat: 2019-06-05 13:36:47.873000 (3.278s ago)

			Is Active	Limit Jobs	Only Starred
trace	18 / 18	avg: 1.6678s/job total cpu time: 30.021s	False	All	False
shapes	18 / 18	avg: 1.5682s/job total cpu time: 28.228s	False	All	False
fastclass	18 / 18	avg: 2.4508s/job total cpu time: 44.115s	False	All	False
imdata	6 / 6	avg: 0.7035s/job total cpu time: 4.221s	False	All	False
jclass	18 / 18	avg: 59.5694s/job total cpu time: 1131.818s	False	All	False

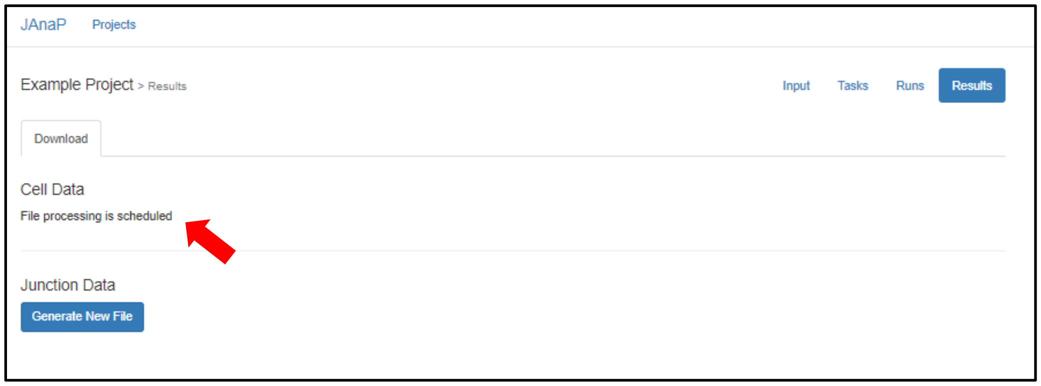
Save Changes

27) Click on the “Results” button in the top right corner of the project page to generate the excel files for export. The following screen will load:

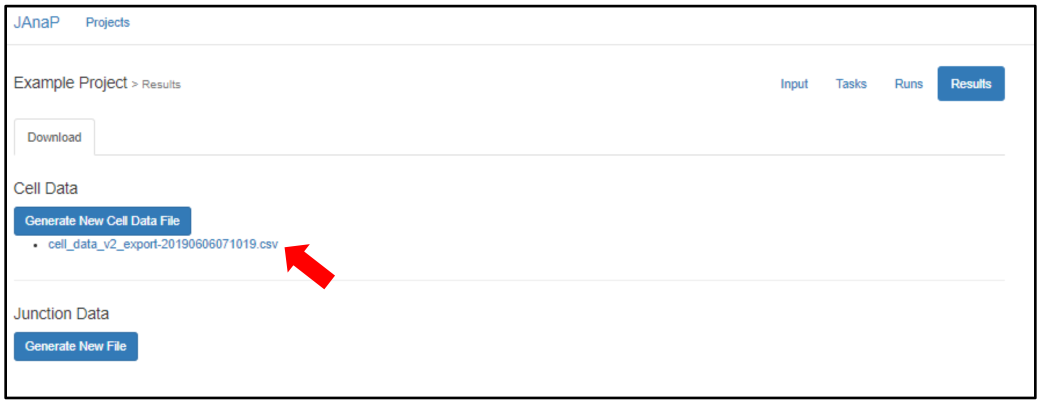


28) Click on “Generate New File” for any of the files types to generate that spreadsheet at any point in the processing period. The details of each category are discussed in the next step.

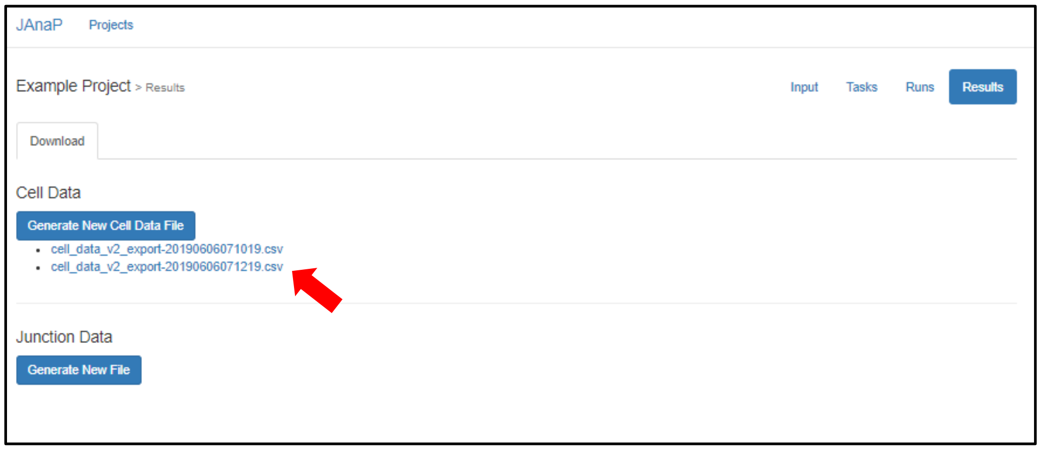
- Once the button has been pushed, the page will read “File processing is scheduled” under that category, as below under “Cell Data”:



- After a few minutes, the file link will appear under the category header or the page can be re-loaded to load the file link.
  - Clicking on the link will download the file which will be opened as an excel spreadsheet.
  - Note that the name indicates the category of the file (e.g., “cell_data_v2), and the numbers indicated the date and time when the file was generated (e.g., 20190606071019 means the file was generated on June 06, 2019 at 07:10.19).


JAnaP
Projects
Example Project > Results
Input
Tasks
Runs
Results
Download
Cell Data
Generate New Cell Data File
cell_data_v2_export-20190606071019.csv
Junction Data
Generate New File

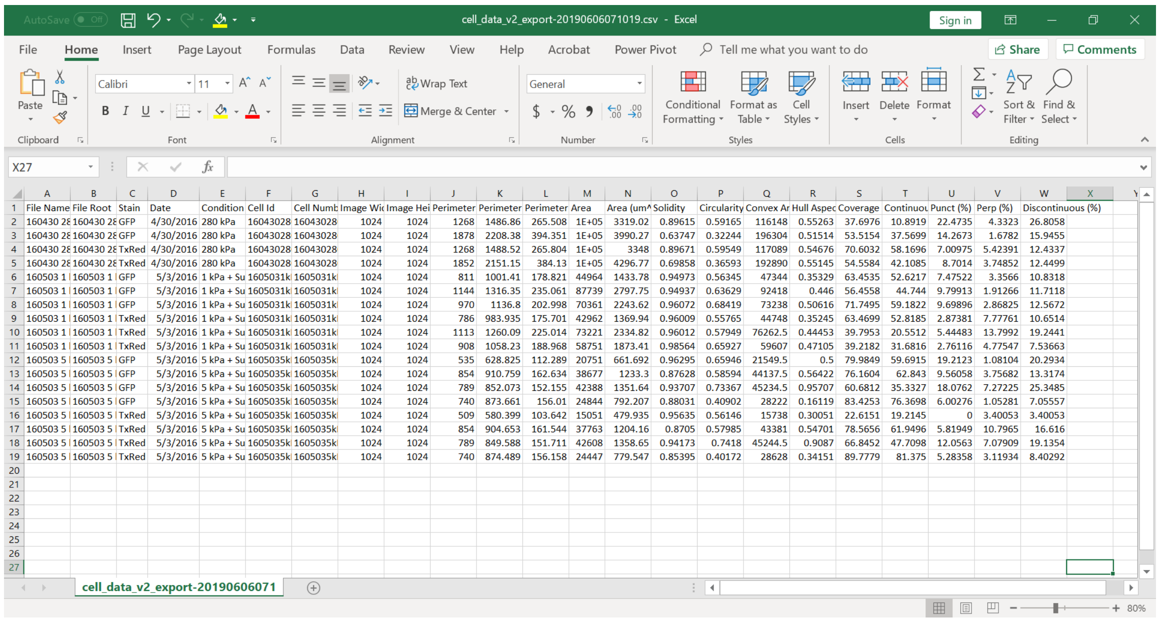
- A new file can be generated at any point by re-clicking on the blue “Generate New File” button. If so, a new file will appear with the appropriate date/time reflected in the filename:


JAnaP
Projects
Example Project > Results
Input
Tasks
Runs
Results
Download
Cell Data
Generate New Cell Data File
cell_data_v2_export-20190606071019.csv
cell_data_v2_export-20190606071219.csv
Junction Data
Generate New File

29) The spreadsheet categories are as follows:

- **Cell data:** This provides the shape factors and junction presentation percentages for every cell waypointed within the project
    - File Name: The name of the image in which the specific cell is located in
    - File Root: The base name of the image (e.g., TxRed or GFP identifier removed)
    - Variant Columns: A column will be generated for each variant and dimension added in the configurations tab. Here, stain: GFP or TxRed, was added as the variant and date and Condition: 1 kPa + Supp, 5 kPa + Supp, and 280 kPa, were added as dimensions. This allows for easy filtering within the spreadsheet to groups results by the various sub-dimensions.
    - Cell ID & Cell Number: This is the unique identifier assigned to each cell. It consists of the file/image name and the center coordinates of the waypoints. The center coordinates will be the same for every cell sharing the same waypoints (i.e., the same cell with more than one variant or stain) such that the difference in the Cell ID only lies in the image name differentiating it between variants.
    - Image Height: The height of the image in pixels calculated by the "Image Data"
    - Image Width : The width of the image in pixels calculated by the "Image Data"
    - Perimeter Count: The perimeter of the cell based on counted pixels
    - Perimeter Calc: The perimeter of the cell that accounts for pixel diagonals since cells are round objects not solely composed of linear edges
    - Perimeter (μm): The perimeter calc value converted to μm based on the conversion designated in the configuration tab for that image size
    - Area: The cell area (pixels^2) calculated using the same correction for pixel diagonals as in perimeter calc
    - Area (μm^2): The cell area converted to μm based on the conversion designated in the configuration tab for that image size
    - Solidity: Calculated using the formula: $(cell\ area)/(convex\ hull\ area)$ as a measure of how solid the cell is. A value of 1 indicates a completely solid shape while a lower value indicates more protrusive features.
    - Circularity: Calculated using the formula: $4\pi(area)/perimeter^2$ as a measure of how close the cell is to a perfect circle. A value of 1 indicates a perfect circle while a lower value indicates a more oblong or protrusive shape.
    - Convex Area: Calculated using the Shoelace Method through the Scipy library and used to calculate solidity.
    - Hull Aspect Ratio: The inverse aspect ratio of the cell calculated by taking the minor axis of the cell divided by the major axis of the cell. The axes are determined based on an ellipse fit to the convex hull.
    - Coverage (%): The percent of the cell perimeter presenting junction.
    - Continuous (%): The percent of the cell perimeter presenting continuous junction.

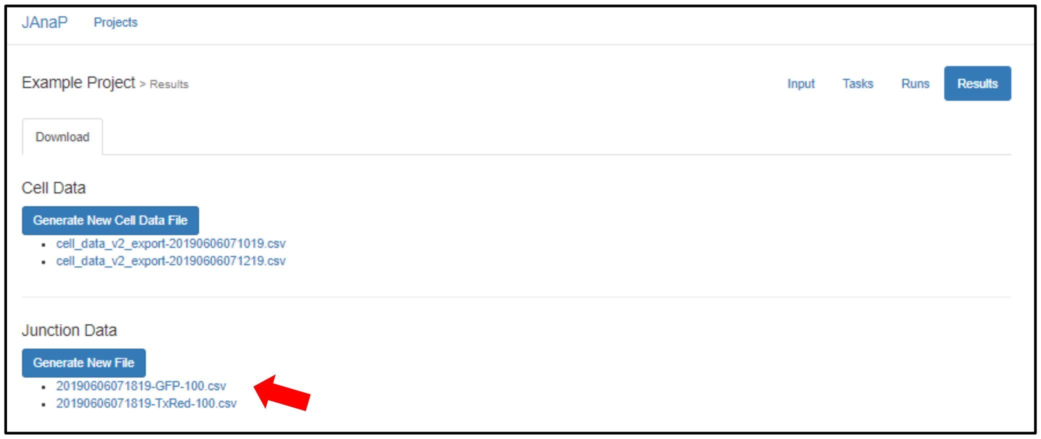
- Punct (%): The percent of the cell perimeter presenting punctate junction.
- Perp (%): The percent of the cell perimeter presenting perpendicular junction.
- Discontinuous (%): The percent of the cell perimeter presenting punctate and perpendicular junction.


cell_data_v2_export-20190606071019.csv - Excel
File
Home
Insert
Page Layout
Formulas
Data
Review
View
Help
Acrobat
Power Pivot
Tell me what you want to do
Share
Comments
X27
cell_data_v2_export-20190606071

	A	B	C	D	E	F	G	H	I	J
1	File Name	File Root	Stain	Date	Condition	Cell Id	Cell Numb	Image Wic	Image Hei	Perimeter
2	160430 28	160430 28	GFP	4/30/2016	280 kPa	16043028	16043028	1024	1024	1268
3	160430 28	160430 28	GFP	4/30/2016	280 kPa	16043028	16043028	1024	1024	1878
4	160430 28	160430 28	TxRed	4/30/2016	280 kPa	16043028	16043028	1024	1024	1268
5	160430 28	160430 28	TxRed	4/30/2016	280 kPa	16043028	16043028	1024	1024	1852
6	160503 1	160503 1	GFP	5/3/2016	1 kPa + Su	1605031k	1605031k	1024	1024	811
7	160503 1	160503 1	GFP	5/3/2016	1 kPa + Su	1605031k	1605031k	1024	1024	1144
8	160503 1	160503 1	GFP	5/3/2016	1 kPa + Su	1605031k	1605031k	1024	1024	970
9	160503 1	160503 1	TxRed	5/3/2016	1 kPa + Su	1605031k	1605031k	1024	1024	786
10	160503 1	160503 1	TxRed	5/3/2016	1 kPa + Su	1605031k	1605031k	1024	1024	1113
11	160503 1	160503 1	TxRed	5/3/2016	1 kPa + Su	1605031k	1605031k	1024	1024	908
12	160503 5	160503 5	GFP	5/3/2016	5 kPa + Su	1605035k	1605035k	1024	1024	535
13	160503 5	160503 5	GFP	5/3/2016	5 kPa + Su	1605035k	1605035k	1024	1024	854
14	160503 5	160503 5	GFP	5/3/2016	5 kPa + Su	1605035k	1605035k	1024	1024	789
15	160503 5	160503 5	GFP	5/3/2016	5 kPa + Su	1605035k	1605035k	1024	1024	740
16	160503 5	160503 5	TxRed	5/3/2016	5 kPa + Su	1605035k	1605035k	1024	1024	509
17	160503 5	160503 5	TxRed	5/3/2016	5 kPa + Su	1605035k	1605035k	1024	1024	854
18	160503 5	160503 5	TxRed	5/3/2016	5 kPa + Su	1605035k	1605035k	1024	1024	789
19	160503 5	160503 5	TxRed	5/3/2016	5 kPa + Su	1605035k	1605035k	1024	1024	740

	K	L	M	N	O	P	Q	R	S	T	U	V	W
1	Perimeter	Perimeter	Area	Area (um^	Solidity	Circularity	Convex Ar	Hull Aspec	Coverage	Continuou	Punct (%)	Perp (%)	Discontinuous (%)
2	1486.86	265.508	1E+05	3319.02	0.89615	0.59165	116148	0.55263	37.6976	10.8919	22.4735	4.3323	26.8058
3	2208.38	394.351	1E+05	3990.27	0.63747	0.32244	196304	0.51514	53.5154	37.5699	14.2673	1.6782	15.9455
4	1488.52	265.804	1E+05	3348	0.89671	0.59549	117089	0.54676	70.6032	58.1696	7.00975	5.42391	12.4337
5	2151.15	384.13	1E+05	4296.77	0.69858	0.36593	192890	0.55145	54.5584	42.1085	8.7014	3.74852	12.4499
6	1001.41	178.821	44964	1433.78	0.94973	0.56345	47344	0.35329	63.4535	52.6217	7.47522	3.3566	10.8318
7	1316.35	235.061	87739	2797.75	0.94937	0.63629	92418	0.446	56.4558	44.744	9.79913	1.91266	11.7118
8	1136.8	202.998	70361	2243.62	0.96072	0.68419	73238	0.50616	71.7495	59.1822	9.69896	2.86825	12.5672
9	983.935	175.701	42962	1369.94	0.96009	0.55765	44748	0.35245	63.4699	52.8185	2.87381	7.77761	10.6514
10	1260.09	225.014	73221	2334.82	0.96012	0.57949	76262.5	0.44453	39.7953	20.5512	5.44483	13.7992	19.2441
11	1058.23	188.968	58751	1873.41	0.98564	0.65927	59607	0.47105	39.2182	31.6816	2.76116	4.77547	7.53663
12	628.825	112.289	20751	661.692	0.96295	0.65946	21549.5	0.5	79.9849	59.6915	19.2123	1.08104	20.2934
13	910.759	162.634	38677	1233.3	0.87628	0.58594	44137.5	0.56422	76.1604	62.843	9.56058	3.75682	13.3174
14	852.073	152.155	42388	1351.64	0.93707	0.73367	45234.5	0.95707	60.6812	35.3327	18.0762	7.27225	25.3485
15	873.661	156.01	24844	792.207	0.88031	0.40902	28222	0.16119	83.4253	76.3698	6.00276	1.05281	7.05557
16	580.399	103.642	15051	479.935	0.95635	0.56146	15738	0.30051	22.6151	19.2145	0	3.40053	3.40053
17	904.653	161.544	37763	1204.16	0.8705	0.57985	43381	0.54701	78.5656	61.9496	5.81949	10.7965	16.616
18	849.588	151.711	42608	1358.65	0.94173	0.7418	45244.5	0.9087	66.8452	47.7098	12.0563	7.07909	19.1354
19	874.489	156.158	24447	779.547	0.85395	0.40172	28628	0.34151	89.7779	81.375	5.28358	3.11934	8.40292

- **Junction Data:** This provides additional junction detail for that calculated in “Cell Data” generated in spreadsheets by variant (Here, one for GFP and one for TxRed).
  - Note that if you do not “run” the “jclass” job, the spreadsheets will either not generate, or will still be generated but will not contain any information.



Example Project > Results

Cell Data

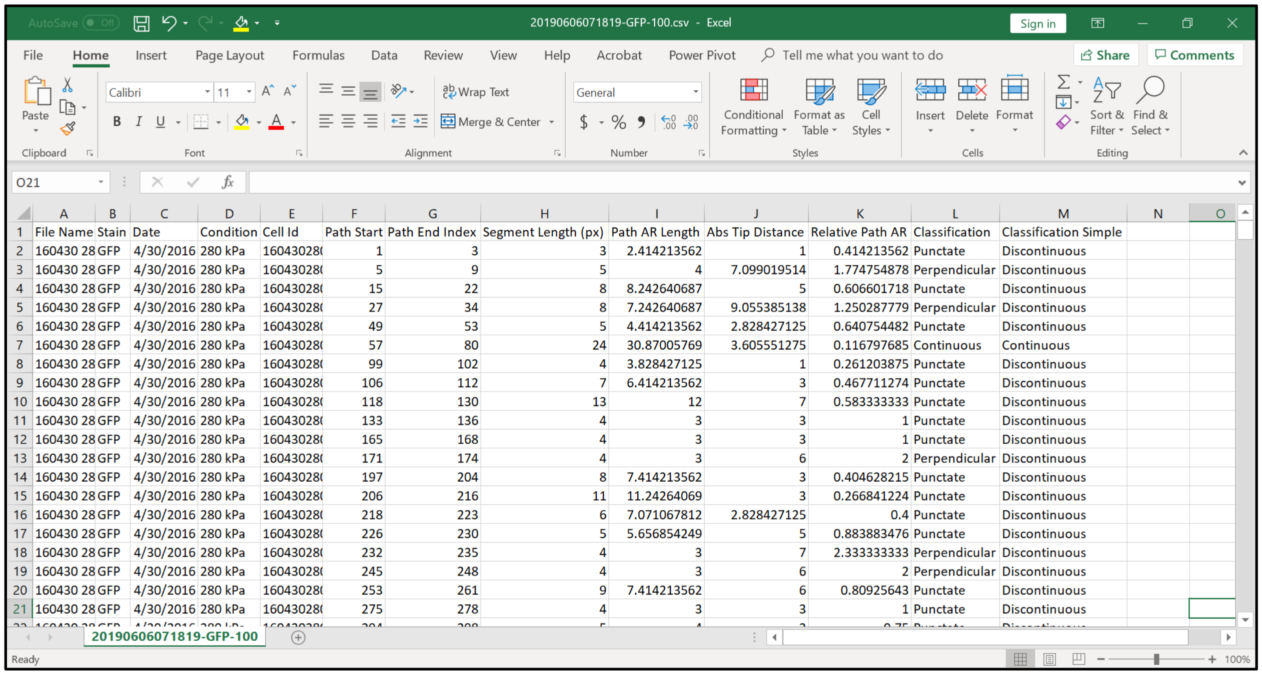
- cell_data_v2_export-20190606071019.csv
- cell_data_v2_export-20190606071219.csv

Junction Data

- 20190606071819-GFP-100.csv
- 20190606071819-TxRed-100.csv

- File Name, Variant Columns, Cell ID: All the same as in “Cell Data” spreadsheet
- Path Start: Counter around the cell path indicating where junction piece begins, determined by the first waypoint seeded and continues in the direction of waypoint seeding around the cell
- Path End: Counter around the cell path indicating where junction piece ends
- Segment Length: Length (in pixels) that the junction piece coincides with the cell path
- Path AR Length: Length of the segment taking into account square-roots for pixel diagonals since the segments are not solely composed of linear edges (similar to perimeter count versus perimeter calc as described in the above section).
- Abs Tip Dist: Measure of junction thickness or maximum protrusion radially from cell path.

- Relative Path AR: Calculated by dividing "Abs Tip Dist" by "Path AR Length", used to determine whether a discontinuous junction is perpendicular or punctate
- Classification: Category to which junction piece is designated – Continuous, Perpendicular or Punctate
- Classification Simple: Simplified category to which junction piece is designated – Continuous or Discontinuous



	File Name	Stain	Date	Condition	Cell Id	Path Start	Path End Index	Segment Length (px)	Path AR Length	Abs Tip Distance	Relative Path AR	Classification	Classification Simple
2	160430 28	GFP	4/30/2016	280 kPa	16043028(	1	3	3	2.414213562	1	0.414213562	Punctate	Discontinuous
3	160430 28	GFP	4/30/2016	280 kPa	16043028(	5	9	5	4	7.099019514	1.774754878	Perpendicular	Discontinuous
4	160430 28	GFP	4/30/2016	280 kPa	16043028(	15	22	8	8.242640687	5	0.606601718	Punctate	Discontinuous
5	160430 28	GFP	4/30/2016	280 kPa	16043028(	27	34	8	7.242640687	9.055385138	1.250287779	Perpendicular	Discontinuous
6	160430 28	GFP	4/30/2016	280 kPa	16043028(	49	53	5	4.414213562	2.828427125	0.640754482	Punctate	Discontinuous
7	160430 28	GFP	4/30/2016	280 kPa	16043028(	57	80	24	30.87005769	3.605551275	0.116797685	Continuous	Continuous
8	160430 28	GFP	4/30/2016	280 kPa	16043028(	99	102	4	3.828427125	1	0.261203875	Punctate	Discontinuous
9	160430 28	GFP	4/30/2016	280 kPa	16043028(	106	112	7	6.414213562	3	0.467711274	Punctate	Discontinuous
10	160430 28	GFP	4/30/2016	280 kPa	16043028(	118	130	13	12	7	0.583333333	Punctate	Discontinuous
11	160430 28	GFP	4/30/2016	280 kPa	16043028(	133	136	4	3	3	1	Punctate	Discontinuous
12	160430 28	GFP	4/30/2016	280 kPa	16043028(	165	168	4	3	3	1	Punctate	Discontinuous
13	160430 28	GFP	4/30/2016	280 kPa	16043028(	171	174	4	3	6	2	Perpendicular	Discontinuous
14	160430 28	GFP	4/30/2016	280 kPa	16043028(	197	204	8	7.414213562	3	0.404628215	Punctate	Discontinuous
15	160430 28	GFP	4/30/2016	280 kPa	16043028(	206	216	11	11.24264069	3	0.266841224	Punctate	Discontinuous
16	160430 28	GFP	4/30/2016	280 kPa	16043028(	218	223	6	7.071067812	2.828427125	0.4	Punctate	Discontinuous
17	160430 28	GFP	4/30/2016	280 kPa	16043028(	226	230	5	5.656854249	5	0.883883476	Punctate	Discontinuous
18	160430 28	GFP	4/30/2016	280 kPa	16043028(	232	235	4	3	7	2.333333333	Perpendicular	Discontinuous
19	160430 28	GFP	4/30/2016	280 kPa	16043028(	245	248	4	3	6	2	Perpendicular	Discontinuous
20	160430 28	GFP	4/30/2016	280 kPa	16043028(	253	261	9	7.414213562	6	0.80925643	Punctate	Discontinuous
21	160430 28	GFP	4/30/2016	280 kPa	16043028(	275	278	4	3	3	1	Punctate	Discontinuous

30) All the data generated using the GUI is saved in the GitHub project folder:
- Documents > GitHub > JAnaP-master > data > projects > *your project*:
  - Artifacts – This includes all the raw files generated for each cell during the waypointing process and the information calculated during the run process.
  - Input – input images of the cells you placed here in step 6
  - Output – this is where all the excel spreadsheets that you generate in step 28 & 29 are stored
  - System – this stores the system information and logs of any problems during processing

31) The program can be re-run (e.g., with different parameters) at any time:
- To do this, the artifacts need to be deleted from the individual folders within the artifacts folder described above. This will register in the GUI and reset the counters in step 26 to "0".
  - *Important* DO NOT DELETE THE WAYPOINTS FOLDER (unless the waypoints specifically need to be re-done for the project). This will remove the saved waypoint information and require the entire waypoint process to be performed again. If you leave the waypoints, the cell IDs will remain, and the calculations will be re-calculated based on the parameters in the configurations tab at that time.
  - Be sure you save the original artifacts folders and/or excel spreadsheets of the original data in case you need to reference back, as well as the configuration data used to generate them (saved in the _project.config.json file within the project folder). The results will be saved over and the excel files generated do not save the configuration data within them.

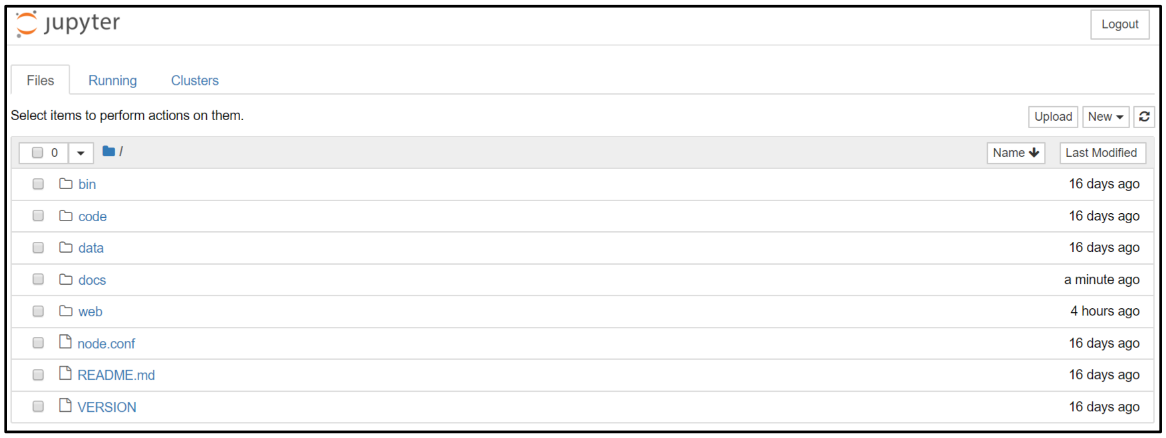
Jupyter Notebook:

This notebook allows you to identify threshold values for use in JAnaP processing and generate figures based on the calculated data. The following instructions provide guidance on how to work through the notebook as it is currently written. Note that at any time, the notebook can be copied and re-saved using a different name for future reference or to make changes to the graphing or calculations themselves.

A. First time use/install:
   1. Open a command prompt
   2. Right click and select "Run as Admin."
   3. Type in "Pip install jupyter"

B. Subsequent Usage:
   1. In a new command prompt, navigate to folder containing "JAnaP-master" then type jupyter notebook. For example:
      - cmd > cd Documents\GitHub\ JAnaP-master\
      - cmd > jupyter notebook

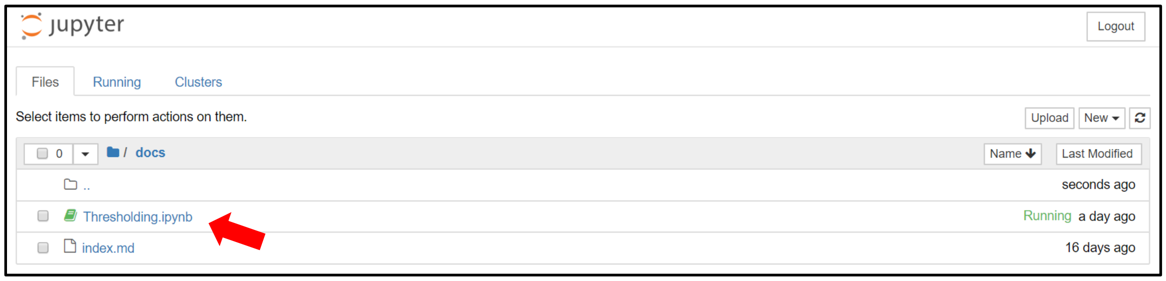
   2. The web page will open and look like the following:



Select items to perform actions on them.

bin	16 days ago
code	16 days ago
data	16 days ago
docs	a minute ago
web	4 hours ago
node.conf	16 days ago
README.md	16 days ago
VERSION	16 days ago

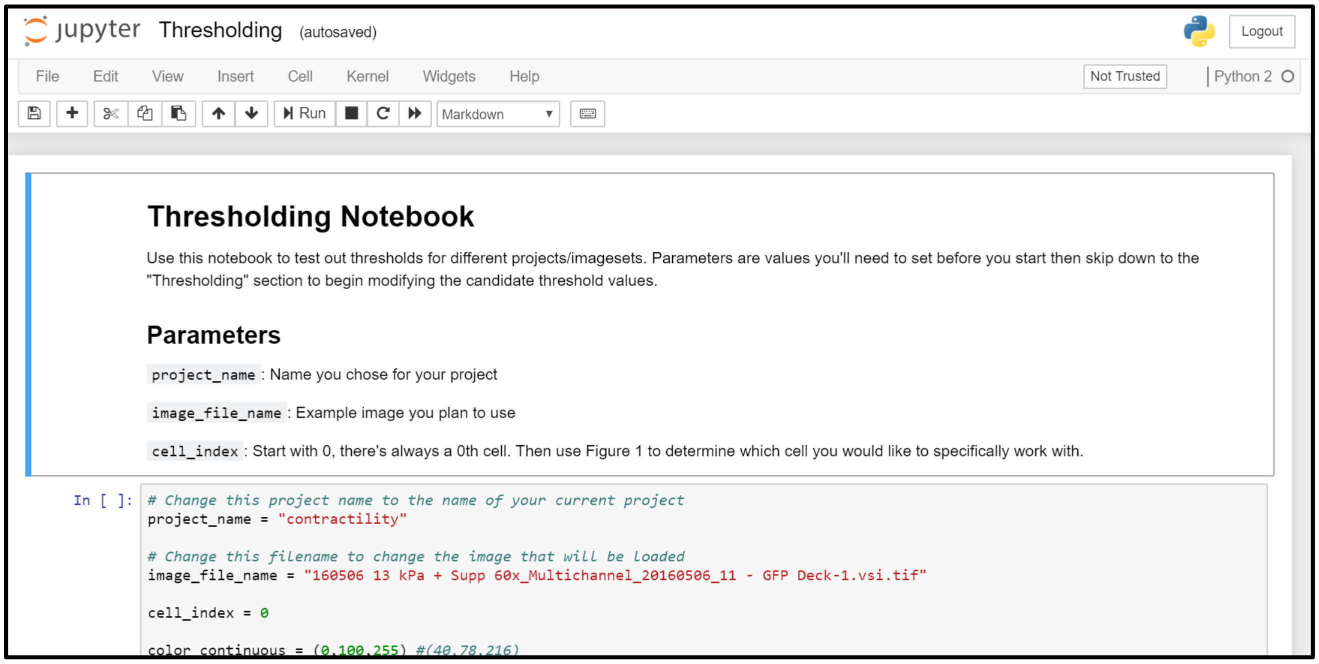
   3. Click on the "docs" folder then select the link to the "Thresholding":



Select items to perform actions on them.

..		seconds ago
Thresholding.ipynb	Running	a day ago
index.md		16 days ago

   4. The following page will appear in a new window:


jupyter Thresholding (autosaved)
Logout
File Edit View Insert Cell Kernel Widgets Help
Not Trusted
Python 2
Run
Markdown

Thresholding Notebook

Use this notebook to test out thresholds for different projects/imagesets. Parameters are values you'll need to set before you start then skip down to the "Thresholding" section to begin modifying the candidate threshold values.

Parameters

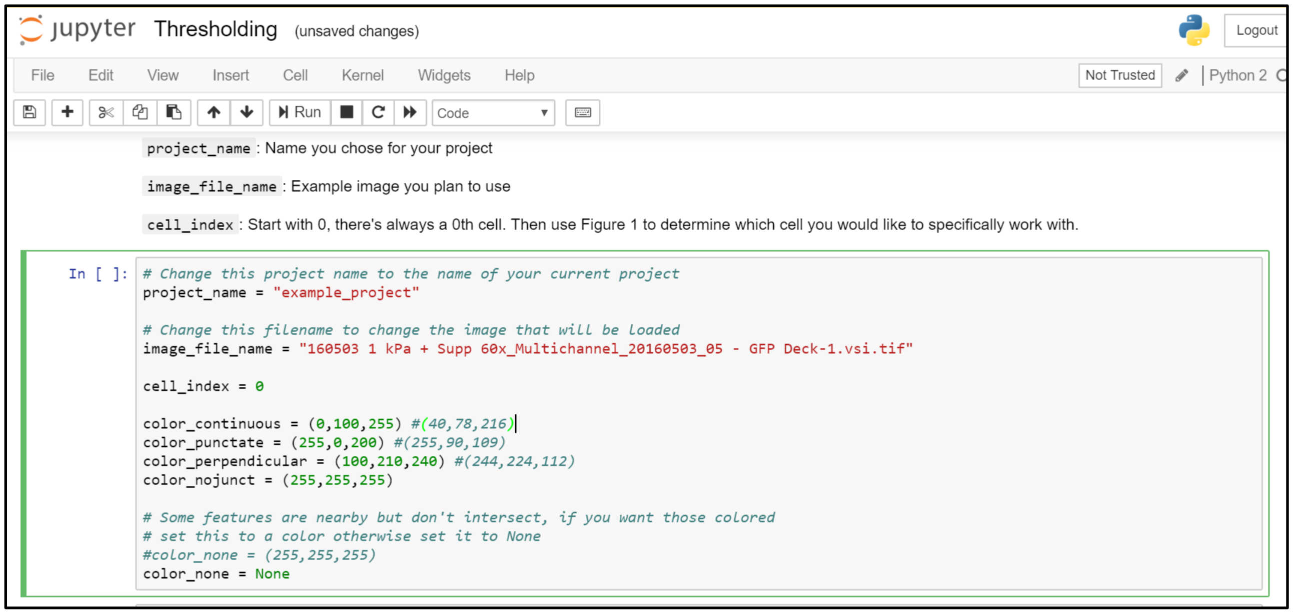
`project_name` : Name you chose for your project

`image_file_name` : Example image you plan to use

`cell_index` : Start with 0, there's always a 0th cell. Then use Figure 1 to determine which cell you would like to specifically work with.

```
In [ ]: # Change this project name to the name of your current project
        project_name = "contractility"

        # Change this filename to change the image that will be loaded
        image_file_name = "160506 13 kPa + Supp 60x_Multichannel_20160506_11 - GFP Deck-1.vsi.tif"

        cell_index = 0

        color_continuous = (0,100,255) #(40,78,216)
```

5. Click on the first box and type in the specified parameters (e.g., project name, image name, and cell_index) as follows, then hit Ctrl+Enter to run:


jupyter Thresholding (unsaved changes)
Logout
File Edit View Insert Cell Kernel Widgets Help
Not Trusted
Python 2
Run
Code

`project_name` : Name you chose for your project

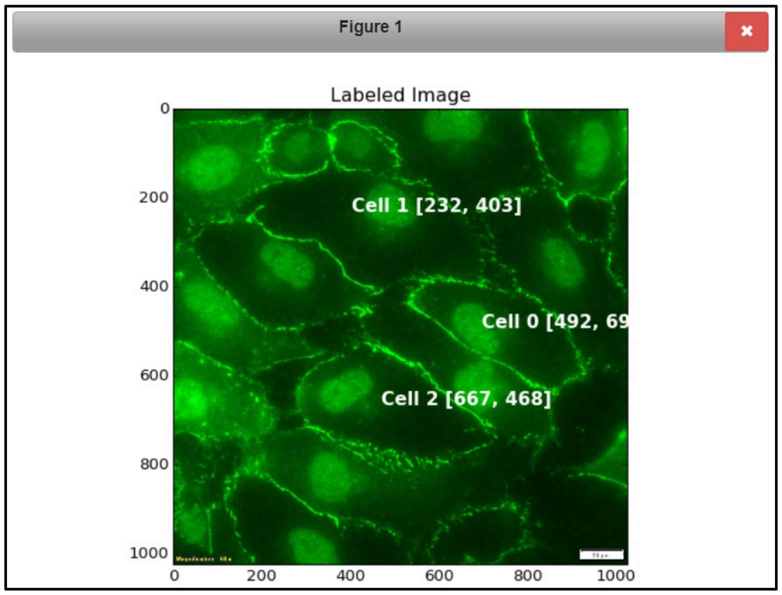
`image_file_name` : Example image you plan to use

`cell_index` : Start with 0, there's always a 0th cell. Then use Figure 1 to determine which cell you would like to specifically work with.

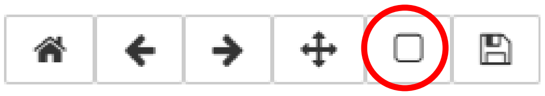
```
In [ ]: # Change this project name to the name of your current project
        project_name = "example_project"

        # Change this filename to change the image that will be loaded
        image_file_name = "160503 1 kPa + Supp 60x_Multichannel_20160503_05 - GFP Deck-1.vsi.tif"

        cell_index = 0

        color_continuous = (0,100,255) #(40,78,216)
        color_punctate = (255,0,200) #(255,90,109)
        color_perpendicular = (100,210,240) #(244,224,112)
        color_nojunct = (255,255,255)

        # Some features are nearby but don't intersect, if you want those colored
        # set this to a color otherwise set it to None
        #color_none = (255,255,255)
        color_none = None
```

- Note: If you don't know the cell index, you can continue through step 6 to view an annotated image of the waypointed cells to help identify which cell you would like to view moving forward. If you decide to change the cell index in box 1, simply rerun that box and subsequent boxes by selecting them and hitting Ctrl+Enter.
- Note: If you change any parameters in any box after Ctrl+Enter has been hit, you must re-run box 2 prior to re-running the box of interest for the modified image to appear.
- Note: What you type for "project_name" in the Thresholding notebook must exactly match the project name created in the JAnaP web interface.

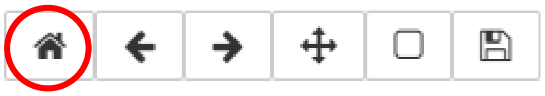
6. Click on the next two boxes and hit Ctrl+Enter to run each. After the 3rd box, your annotated image will appear as follows:



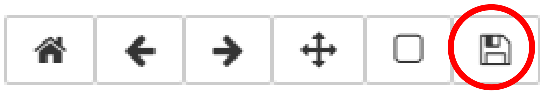
- Note: the following tool bar will appear below each image.
  - Selecting the square button will allow you to select an area within the image to zoom-in on



  - Click the "home" button to return to the original image at any point



  - The save button will **NOT** save the image.

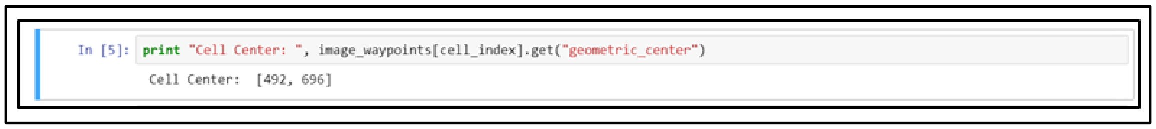


  - To save the image, you must insert the following line at the bottom of the cell that generated the image:

    cv2.imwrite(output_prefix + 'TitleYouWantImageSavedAs.png', input_image)

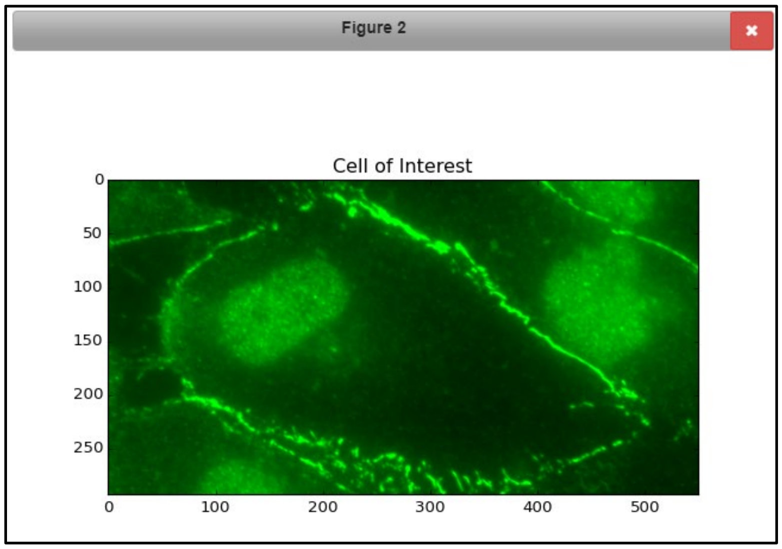
    - input_image is the plot generated in this box. This can be substituted in subsequent cells for the plot generated within that cell (e.g., in the 6th box it would be "plotter").
    - Adding a # symbol in front of this line will comment out the command if you would like to run the cell without saving the image but keeping the command there for future use.

7. Click Ctrl+Enter in the 4th box for the center coordinates of the specified cell index to appear. Here, this is for Cell 2:

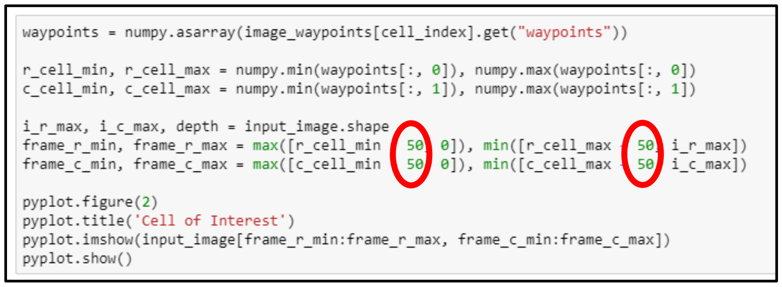


```
In [5]: print "Cell Center: ", image_waypoints[cell_index].get("geometric_center")
        Cell Center:  [492, 696]
```

8. Click Ctrl+Enter in the 5th box for a cropped image of the cell of interest to be generated:

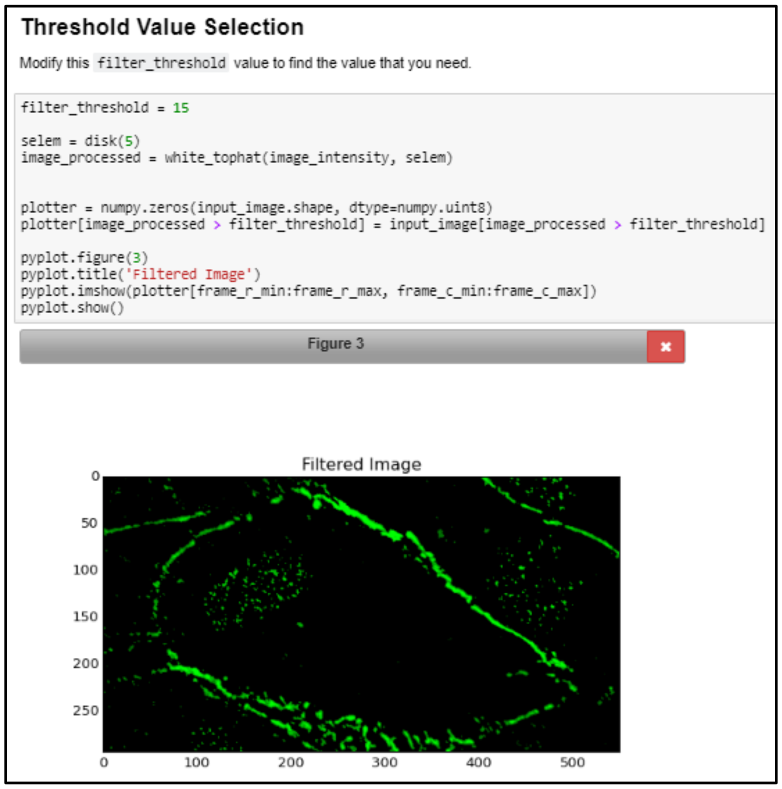


- The crop level of the image can be adjusted by changing the following values (decreasing the number provides a “tighter” crop). Note that this can be changed throughout the notebook for any of the cropped images.



```
waypoints = numpy.asarray(image_waypoints[cell_index].get("waypoints"))

r_cell_min, r_cell_max = numpy.min(waypoints[:, 0]), numpy.max(waypoints[:, 0])
c_cell_min, c_cell_max = numpy.min(waypoints[:, 1]), numpy.max(waypoints[:, 1])

i_r_max, i_c_max, depth = input_image.shape
frame_r_min, frame_r_max = max([r_cell_min  50, 0]), min([r_cell_max  50, i_r_max])
frame_c_min, frame_c_max = max([c_cell_min  50, 0]), min([c_cell_max  50, i_c_max])

pyplot.figure(2)
pyplot.title('Cell of Interest')
pyplot.imshow(input_image[frame_r_min:frame_r_max, frame_c_min:frame_c_max])
pyplot.show()
```

9. To identify the optimal threshold value, enter a value next to “Threshold” and click Ctrl+Enter in the 6th box. Repeat this process, adjusting the threshold as necessary to reflect the isolated junctions as you would expect. Note that increasing the threshold isolates less junction while decreasing the threshold isolates more junction.



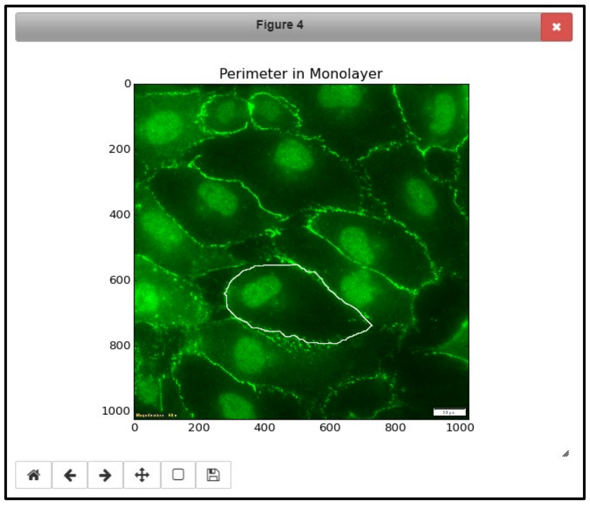
Threshold Value Selection

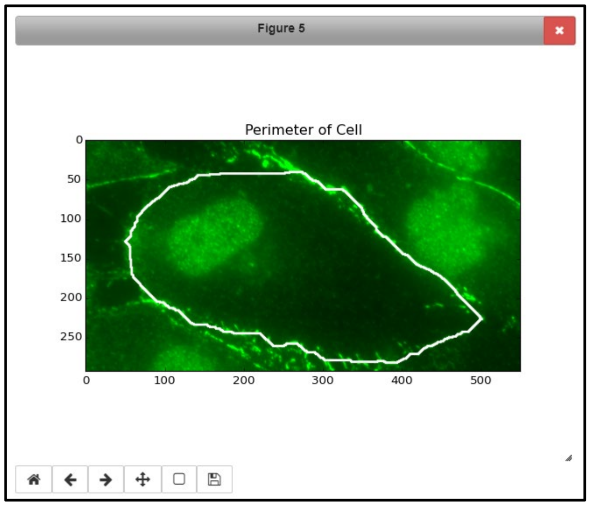
Modify this `filter_threshold` value to find the value that you need.

```
filter_threshold = 15

selem = disk(5)
image_processed = white_tophat(image_intensity, selem)


plotter = numpy.zeros(input_image.shape, dtype=numpy.uint8)
plotter[image_processed > filter_threshold] = input_image[image_processed > filter_threshold]

pyplot.figure(3)
pyplot.title('Filtered Image')
pyplot.imshow(plotter[frame_r_min:frame_r_max, frame_c_min:frame_c_max])
pyplot.show()
```

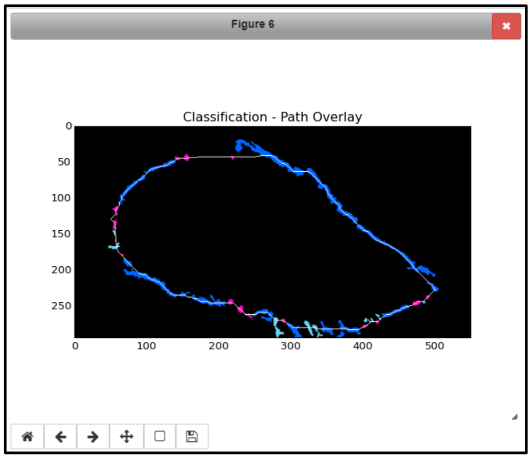
- NOTE: Repeat this step for several cells to determine the optimal threshold value for the images within your experiment. Be sure to determine the optimal threshold level for each variant (e.g., GFP and TxRed stain).
- The optimal threshold value(s) can now be used as parameter inputs in the “Config” tab of the GUI prior to processing the code in step 25.

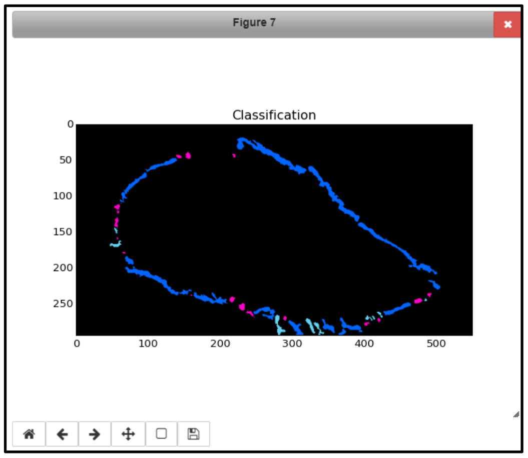
10. Click Ctrl+Enter in Box 7 to generate images of the cell perimeter:

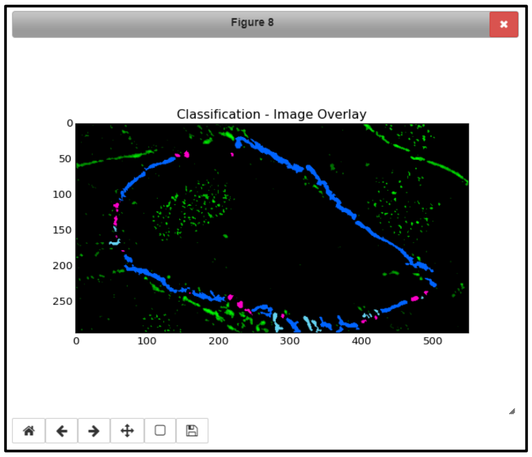


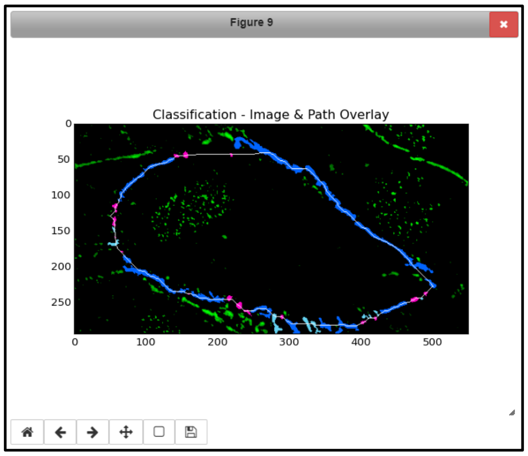


11. Clicking Ctrl+Enter in the next two boxes will generate the following images presenting the junction detail:

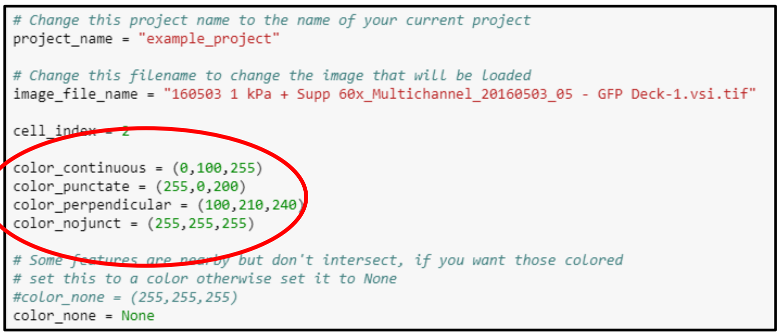






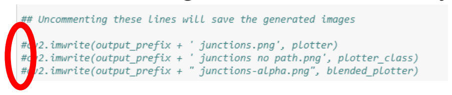


- Here, the continuous junctions are colored in blue, the punctate junctions are colored in magenta, and the perpendicular junctions are colored in light blue. These colors can be changed by modifying the RBG values specified in following section in the 1st box:

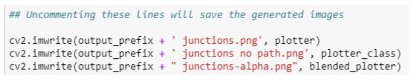


```
# Change this project name to the name of your current project
project_name = "example_project"

# Change this filename to change the image that will be loaded
image_file_name = "160503 1 kPa + Supp 60x_Multichannel_20160503_05 - GFP Deck-1.vsi.tif"

cell_index = 2

color_continuous = (0,100,255)
color_punctate = (255,0,200)
color_perpendicular = (100,210,240)
color_nojunct = (255,255,255)

# Some features are nearby but don't intersect, if you want those colored
# set this to a color otherwise set it to None
#color_none = (255,255,255)
color_none = None
```

- To save these images, remove the # symbol from the code above and re-run:



```
## Uncommenting these lines will save the generated images

#cv2.imwrite(output_prefix + ' junctions.png', plotter)
#cv2.imwrite(output_prefix + ' junctions no path.png', plotter_class)
#cv2.imwrite(output_prefix + " junctions-alpha.png", blended_plotter)
```



```
## Uncommenting these lines will save the generated images

cv2.imwrite(output_prefix + ' junctions.png', plotter)
cv2.imwrite(output_prefix + ' junctions no path.png', plotter_class)
cv2.imwrite(output_prefix + " junctions-alpha.png", blended_plotter)
```

  - The images will be saved in the project docs folder: Documents\GitHub\JAnaP-master\docs
  - Adding the # back in as shown on the left will prevent further images from being saved. This allows you to save only the images you are interested in.