

TissueMAPS

BIO325: Systems dynamics in cell and developmental biology

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Administration

Divide into two groups for the course:

- ▶ Group 1 goes to the Pelkmans Lab first
- ▶ Group 2 goes to the Brunner Lab first:
- ▶ Group 2 is further divided into sub-groups Fly & Yeast

Learning Objectives

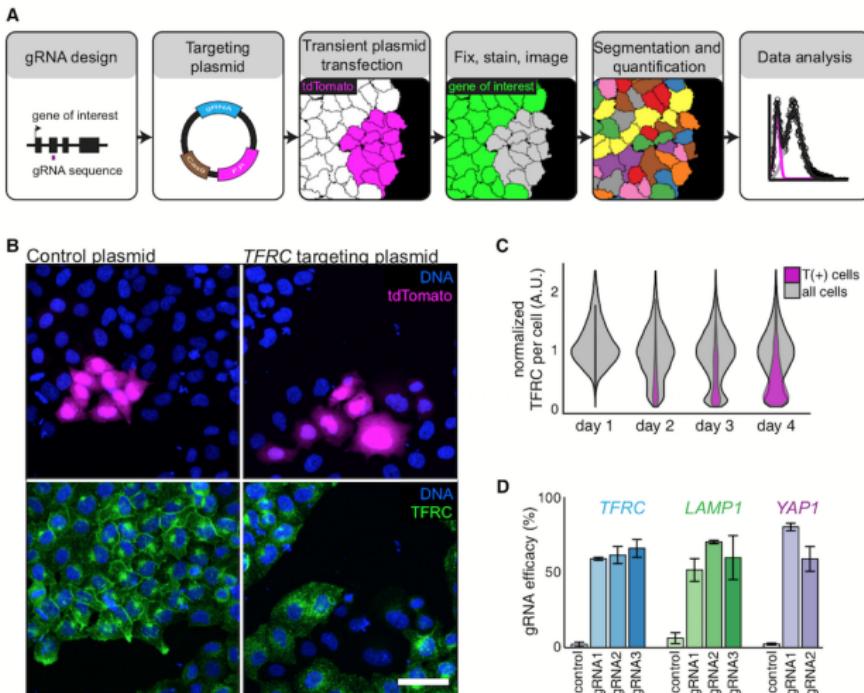
At the end of the day, you will be able to:

1. Understand the use-case for TissueMaps
2. Use TissueMaps to process your image data
3. Process single cell measurements using python
4. Create plots of your data using python

What Will We Do Today?

- ▶ 9:20 - 10:00 Upload data to TissueMaps & start processing it
- ▶ 10:20 - 11:30 Image processing & cell segmentation in TissueMaps
- ▶ 12:30 - 13:15 Using machine learning & downloading data
- ▶ 13:15 - 13:45 Intro to Python
- ▶ 14:00 - 14:45 Data processing
- ▶ 14:45 - 15:00 Discussing data processing
- ▶ 15:15 - 16:00 Plotting data
- ▶ 16:00 - 16:30 Discussing plotting & wrap up

Motivation for Doing Image Analysis



de Groot et al., 2018

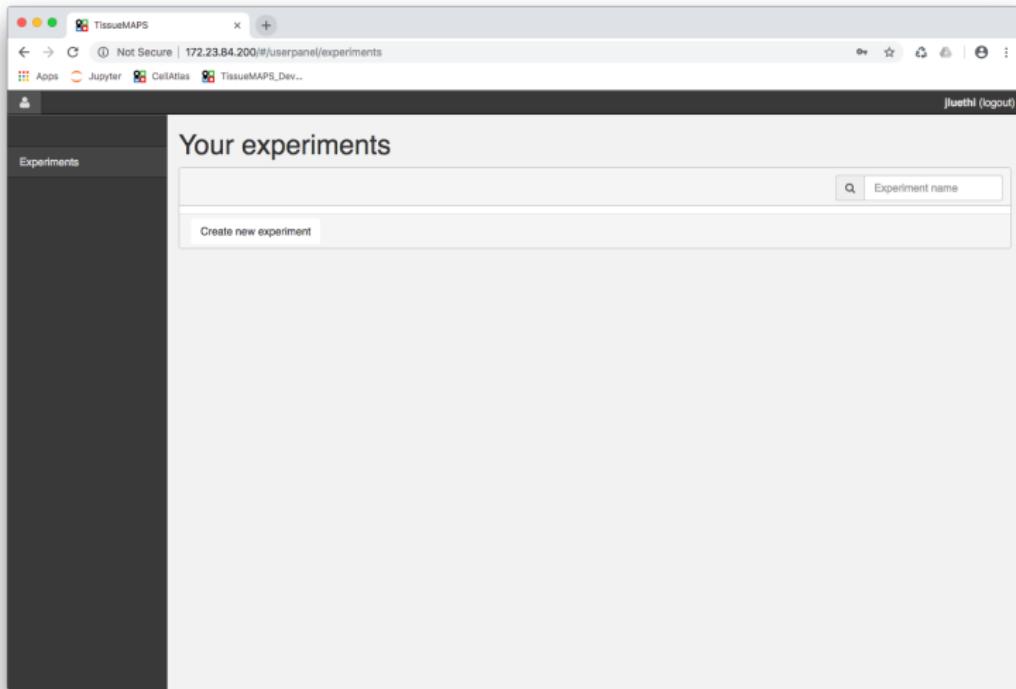
What is TissueMaps

- ▶ Cloud platform for image viewing & analysis
- ▶ Scalable & interactive
- ▶ Accessible by web browser

Your Jupyter Notebook & TissueMaps Server

Get one sheet with instructions & passwords per 2 people

Creating a New Experiment in TissueMaps



Name Your Experiment

The screenshot shows a web browser window titled "TissueMAPS" with the URL "Not Secure | 172.23.84.200#/userpanel/experiments/create". The page is titled "Create experiment". It contains several input fields:

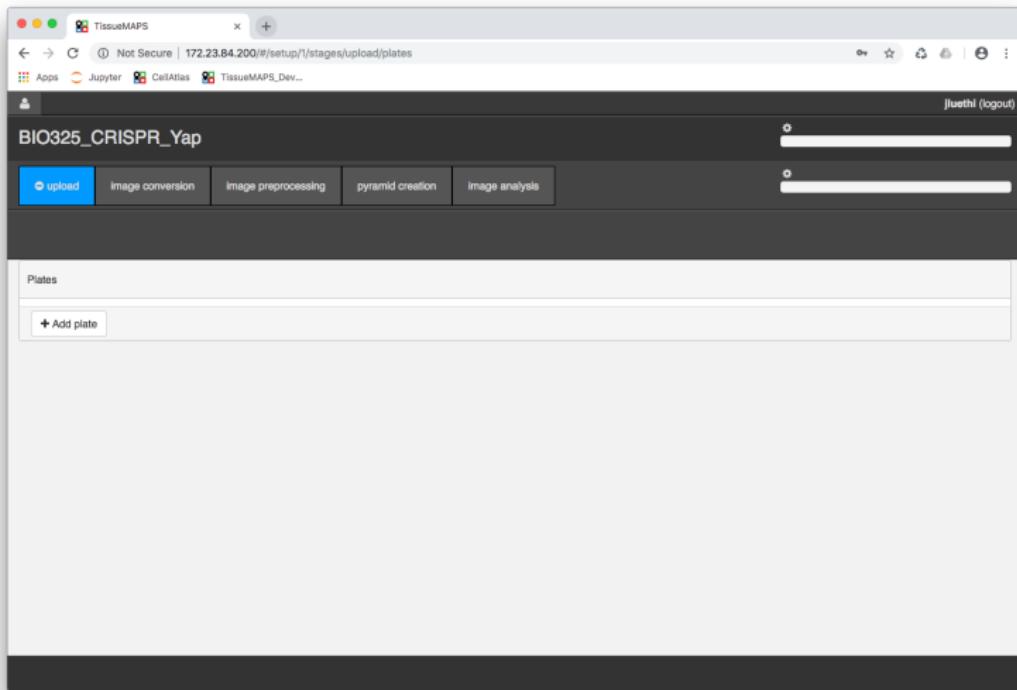
- Experiment name:** BIO325_CRISPR_Yap
- Description:** (Empty text area)
- Workflow type:** canonical
Please specify which workflow type should be used for processing the experiment.
- Plate format:** 384
Please specify the number of wells per plate. A plate represents a container with multiple reservoirs for different samples (wells) that might be stained independently, but imaged under the same conditions. For consistency, a slide is considered a plate with one well.
- Plate acquisition mode:** basic
Please specify how images were acquired by the microscope. When "multiplexing", sequential acquisitions are interpreted as different channels, while when "basic" they are treated as time points.
- Microscope type:** celvoyager
Please specify which microscope was used to acquire the images for this experiment.

A blue "Create experiment" button is located at the bottom left of the form.

You Made a TissueMaps Experiment!

The screenshot shows a web browser window titled "TissueMAPS". The address bar indicates the URL is "Not Secure | 172.23.84.200/#/userpanel/experiments". The top navigation bar includes links for "Apps", "Jupyter", "CellAtlas", and "TissueMAPS_Dev...". On the right side of the header, there is a user profile icon and the text "Juethl (logout)". The main content area is titled "Your experiments". It features a search bar with the placeholder "Experiment name" and a magnifying glass icon. Below the search bar is a list containing one item: "BIO325_CRISPR_Yap". To the right of this item are three buttons: "Delete" (red), "View" (green), and "Modify" (blue). At the bottom of the list, there is a link "Create new experiment".

Modify Your Experiment



Create a Plate

The screenshot shows a web-based application window titled "TissueMAPS" with the URL "Not Secure | 172.23.84.200/#/setup/1/stages/upload/plates/create". The top navigation bar includes links for "Apps", "Jupyter", "CellAtlas", and "TissueMAPS_Dev...". On the right, there is a user profile icon and the name "Juethi (logout)". Below the header, the project name "BIO325_CRISPR_Yap" is displayed. A horizontal menu bar contains five items: "uploaded" (highlighted in blue), "Image conversion", "Image preprocessing", "pyramid creation", and "Image analysis". The main content area is titled "Create new plate". It contains two input fields: "Name" with the value "b1" and "Description" with the placeholder "An optional description of this plate.". A "Create" button is located at the bottom left of this section.

Create an Acquisition

The screenshot shows a web browser window for the TissueMAPS application. The title bar reads "TissueMAPS". The address bar shows the URL "Not Secure | 172.23.84.200/#/setup/1/stages/upload/plates/1/acquisitions/create". The navigation bar includes links for "Apps", "Jupyter", "CellAtlas", and "TissueMAPS_Dev...". On the right side of the header, there is a user icon and the name "Juethl (logout)". Below the header, the page title is "BIO325_CRISPR_Yap". A horizontal menu bar contains five items: "uploaded" (highlighted in blue), "Image conversion", "Image preprocessing", "pyramid creation", and "Image analysis". The main content area displays the following message: "Plate: p1 (NOT READY FOR PROCESSING)". Below this, there is a section titled "Create new acquisition" with two input fields: "Name" containing "a1" and "Description" containing an empty field. A "Create" button is located at the bottom left of this section.

Getting Data into TissueMaps

```
jluethi@pelkmanslab-slurm-master-001:~$ tm_client -h
Joel — ssh cluster — 127x39
usage: tm_client [-h] [-H HOST] [-P PORT] [-u USERNAME] [-p PASSWORD] [-v]
                  {tools,workflow,jtproject,experiment,plate,well,site,acquisition,microscope-file,channel,object-type,feature,feature-values,segmentation,channel-image}
                  ...
TissueMAPS REST API client (version: 0.3.3).

optional arguments:
  --resources
    tools          tools resources
    workflow       workflow resources
    jtproject     jterator project resources
    experiment    experiment resources
    plate         plate resources
    well          well resources
    site          site resources
    acquisition   acquisition resources
    microscope-file microscope file resources
    channel        channel resources
    object-type   object type resources
    feature       feature resources
    feature-values feature values resources
    segmentation  segmentation resources
    channel-image channel image resources

optional arguments:
  -h, --help            show this help message and exit
  -H HOST, --host HOST  TissueMAPS server host (default: localhost)
  -P PORT, --port PORT  TCP port number on which the server listens (default: 80)
  -u USERNAME, --user USERNAME
                        name of TissueMAPS user (default: jluethi)
  -p PASSWORD, --password PASSWORD
                        password of TissueMAPS user (default is the value of
                        environmental variable TM_PASSWORD)
  -v, --verbosity      increase logging verbosity
```

Tell TmClient Who You are

```
jluethi@pelkmanslab-slurm-master-001:~$ tm_client -H 172.23.XX.YY -u clever_student -p myPassword
microscope-file -h
usage: tm_client microscope-file [-h] --EXPERIMENT {ls,upload,register} ...

Access microscope file resources.

positional arguments:
  {ls,upload,register}  access methods
    ls                list microscope files
    upload            upload microscope files
    register         register microscope files

optional arguments:
  -h, --help          show this help message and exit
  -e EXPERIMENT, --experiment EXPERIMENT
                      name of the experiment
jluethi@pelkmanslab-slurm-master-001:~$
```

Tell TmClient What You Want to Do

```
jluethi@pelkmanslab-slurm-master-001:~$ tm_client -H 172.23.XX.YY -u clever_student -p myPassword microscope-file -e BIO325_CRISPR_Yap upload -h
usage: tm_client microscope-file upload [-h] -p PLATE -a ACQUISITION
                                         [--convert [FMT]] [--delete]
                                         [--directory] [--parallel [NUM]]
                                         [--retries NUM] [--no-retry]
                                         path

Upload microscope image and metadata files.

positional arguments:
  path                  path to file or directory to upload

optional arguments:
  -h, --help            show this help message and exit
  -p PLATE, --plate PLATE
                        name of the plate
  -a ACQUISITION, --acquisition ACQUISITION
                        name of the acquisition
  --convert [FMT]        Convert files to the specified format during upload.
                        The format is specified by giving the converted file's
                        extension, e.g., `png`, `jpeg`, etc. If `--convert` is
                        given without specifying a format, images files will
                        be converted to PNG. The conversion process depends on
                        the availability of ImageMagick's `convert` program --
                        if it cannot be executed, file upload will fail.
  --delete              Delete files after uploading. USE WITH CAUTION!
  --directory           Unused. Retained for backwards-compatibility only.
  --parallel [NUM]       Use NUM parallel processes for upload (default: 1). If
                        NUM is omitted or 0, the degree of parallelism is
```

Tell TmClient Where the Images Are



```
jluethi@pelkmanslab-slurm-master-001:~$ tm_client -H 172.23.XX.YY -u clever_student -p myPassword microscope-file -e BIO325_CRISPR_Yap upload -p p1 -a a1 --directory ~/BIO325_Yap_Data -h
usage: tm_client microscope-file upload [-h] -p PLATE -a ACQUISITION
                                         [--convert [FMT]] [--delete]
                                         [--directory] [--parallel [NUM]]
                                         [--retries NUM] [--no-retry]
                                         path

Upload microscope image and metadata files.

positional arguments:
  path                  path to file or directory to upload

optional arguments:
  -h, --help            show this help message and exit
  -p PLATE, --plate PLATE
                        name of the plate
  -a ACQUISITION, --acquisition ACQUISITION
                        name of the acquisition
  --convert [FMT]        Convert files to the specified format during upload.
                        The format is specified by giving the converted file's
                        extension, e.g., 'png', 'jpeg', etc. If '--convert' is
                        given without specifying a format, images files will
                        be converted to PNG. The conversion process depends on
                        the availability of ImageMagick's 'convert' program --
                        if it cannot be executed, file upload will fail.
  --delete              Delete files after uploading. USE WITH CAUTION!
  --directory           Unused. Retained for backwards-compatibility only.
```

Upload Completed

The screenshot shows a web-based application window titled "TissueMAPS". The address bar indicates the URL is "Not Secure | 172.23.84.200/#/setup/1/stages/upload/plates/1". The top navigation bar includes links for "Apps", "Jupyter", "CellAtlas", and "TissueMAPS_Dev...". On the right side, there is a user profile icon and the text "Juethil (logout)". Below the header, the project name "BIO325_CRISPR_Yap" is displayed. A progress bar at the top right shows the status as "TERMINATED". A horizontal menu bar contains five items: "uploaded" (highlighted in blue), "Image conversion", "Image preprocessing", "pyramid creation", and "Image analysis". The main content area is titled "Plate: p1 (READY FOR PROCESSING)". It displays a table under the heading "Acquisitions" with one entry: "a1 (COMPLETE) - 400 files". To the right of this entry is a red "Delete" button. At the bottom left of the acquisition table is a button labeled "+ Add acquisition".

Add Metadata about the Experiment

The screenshot shows a web-based application window titled "TissueMAPS" at the URL "Not Secure | 172.23.84.200/#/setup/1/stages/image_conversion/steps/metaconfig". The top navigation bar includes links for "Apps", "Jupyter", "CellAtlas", and "TissueMAPS_Dev...". A user icon and "Juethil (logout)" are also present. Below the header, the experiment name "BIO325_CRISPR_Yap" is displayed. A horizontal menu bar contains several tabs: "upload", "image conversion" (which is highlighted in blue), "image preprocessing", "pyramid creation", "image analysis", "metaextract" (disabled), "metaconfig" (disabled), and "imextract" (disabled). The main content area is titled "Configuration of image metadata" and describes the integration of OME XML metadata with additional microscope-specific information. It is divided into two sections: "Batch arguments" and "Submission arguments".

Batch arguments
Arguments that control how data is partitioned into computational jobs.

mip perform maximum intensity projection along z axis

n_horizontal
number of images along the horizontal axis of the stitched well overview mosaic image
(optional)

n_vertical
number of images along the vertical axis of the stitched well overview mosaic image
(optional)

regex

Submission arguments
Arguments that control how computational jobs are submitted to the cluster.

cores
number of cores that should be allocated to each "run" job (may be increased in case memory requirements of a job exceed resources of a single core)

duration
walltime that should be allocated to a each "run" job in the format "HH:MM:SS" (may need to be adapted depending on the choice of batch size)

memory
amount of memory in megabytes that should be allocated to each "run" job

At the bottom of the page are buttons for "Submit", "Resume", "Resubmit", "Kill", and "Save".

Define settings for the Pyramid / Image Browser

BIO325_CRISPR_Yap

pyramid creation

illuminate

batch_size
number of image files that should be processed per job
100

clip
whether images intensities should be clipped

clip_percent
threshold percentile at which image intensities should be clipped
99.9

clip_value
threshold value at which image intensities should be clipped (defaults to 99.99th percentile; the set value overwrites calculated percentile)
(optional)

illumcorr
whether images should be corrected for illumination artifacts

illumcorr_exceptions

Submit Resume Resubmit Kill Save

Submit the Pipeline & Enjoy Your Break

BIO325_CRISPR_Yap

upload image conversion image preprocessing pyramid

Pyramid image builder

Creation of pyramids for interactive, web-based visualization of images.

Batch arguments

Arguments that control how data is partitioned into computational jobs.

align whether images should be aligned between multiplexing cycles

batch_size number of image files that should be processed per job
100

clip whether images intensities should be clipped

clip_percent threshold percentile at which image intensities should be clipped
99.9

Submission arguments

Arguments that control how computational jobs are submitted to the cluster.

cores number of cores that should be allocated to each "run" job (may be increased in case memory requirements of a job exceed resources of a single core)
1

duration walltime that should be allocated to a each "run" job in the format "HH:MM:SS" (may need to be adapted depending on the choice of batch size)
06:00:00

memory amount of memory in megabytes that should be allocated to each "run" job
3133

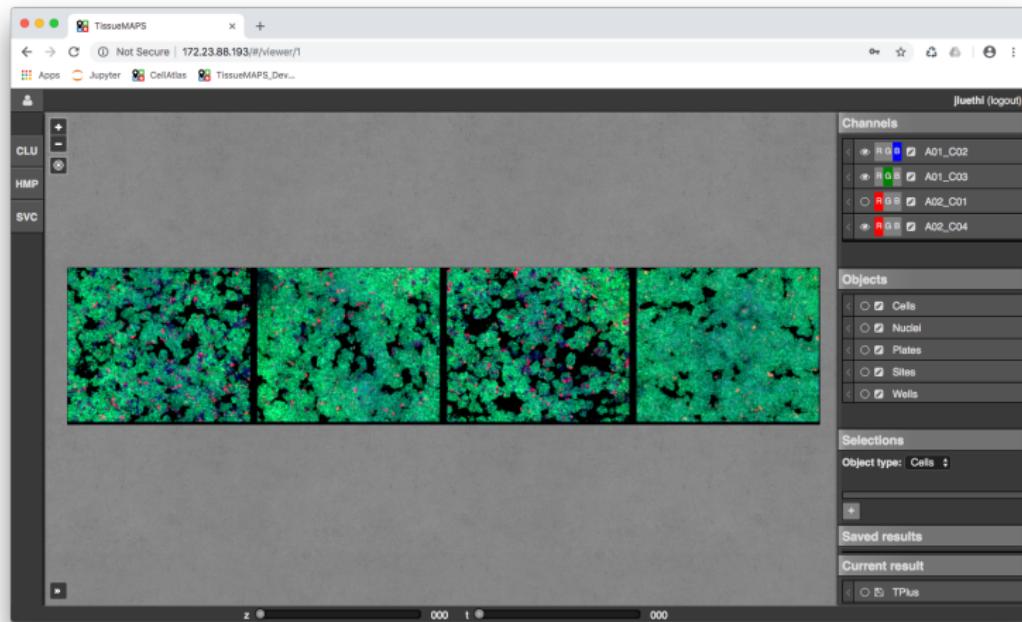
Submit Resume Resubmit Kill Save

Warning

Do you really want to submit the workflow?

Cancel Ok

The Image Viewer Gives You an Overview of Your Experiment



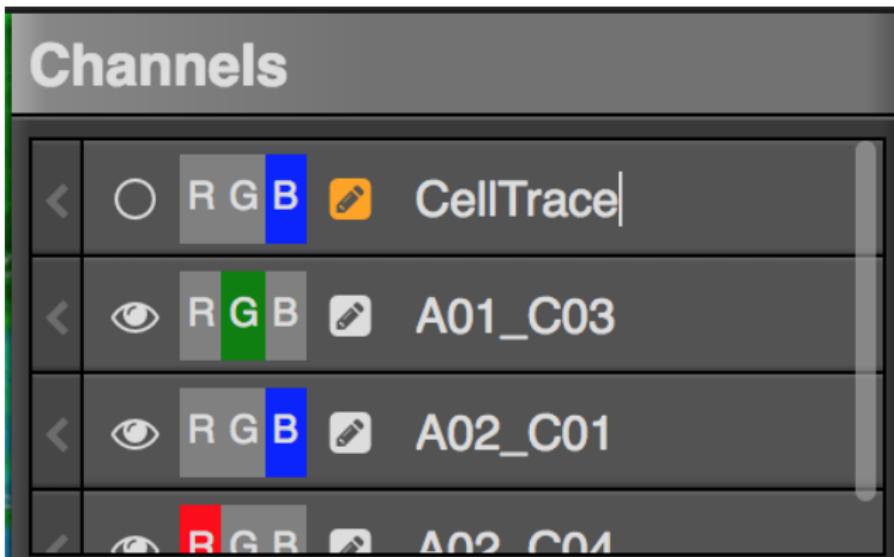
Rename Your Channels

A01_C02 → CellTrace

A02_C01 → DAPI

A01_C03 → Yap

A02_C04 → Plasmid



Go into the Image Processing Pipeline

The screenshot shows a web browser window titled "TissueMAPS" with the URL "Not Secure | 172.23.88.193/#/setup/l/stages/image_analysis/steps/iterator". The page displays a pipeline engine interface for a project named "BIO325_CRISPR_Yap". The pipeline consists of several steps: upload, image conversion, image preprocessing, pyramid creation, and image analysis. The "image analysis" step is currently selected. The status of each step is shown as "TERMINATED" with a green progress bar. Below the pipeline, there are two sections: "Batch arguments" and "Submission arguments". The "Batch arguments" section contains a "batch_size" field set to "10" with an "Edit Pipeline" button. A red arrow points to this button. The "Submission arguments" section contains a "cores" field set to "1" and a "duration" field set to "06:00:00". At the bottom of the interface are buttons for "Submit", "Resume", "Resubmit", "Kill", and "Save".

A Good Pipeline for Today

Input	
Select channels that should be available in the pipeline:	
DAPI	
Correct illumination <input checked="" type="checkbox"/>	
CellTrace	
Correct illumination <input checked="" type="checkbox"/>	
Yap	
Correct illumination <input checked="" type="checkbox"/>	
Transfection	
Correct illumination <input checked="" type="checkbox"/>	
<input type="button" value="+ Add"/>	<input type="button" value="Remove"/>
<input type="button" value="List jobs"/>	

Pipeline	
Define the modules and the order in which they should be processed:	
<input checked="" type="checkbox"/>	smooth_DAPI
<input checked="" type="checkbox"/>	threshold_otsu
<input checked="" type="checkbox"/>	filter_nuclei
<input checked="" type="checkbox"/>	separate_clumps
<input checked="" type="checkbox"/>	label_nuclei
<input checked="" type="checkbox"/>	register_objects
<input checked="" type="checkbox"/>	segment_secondary
<input checked="" type="checkbox"/>	register_objects_Cells
<input checked="" type="checkbox"/>	measure_intensity_DAPI
<input checked="" type="checkbox"/>	measure_intensity_CellTrace
<input checked="" type="checkbox"/>	measure_intensity_2_Yap
<input checked="" type="checkbox"/>	measure_intensity_3_Transfection
<input checked="" type="checkbox"/>	measure_morphology_Cells
<input checked="" type="checkbox"/>	measure_morphology_1_Nuclei
<input checked="" type="checkbox"/>	measure_texture_Transfection

Output	
Select objects that should be saved:	
Nuclei	
Represent as polygons <input checked="" type="checkbox"/>	
Cells	
Represent as polygons <input checked="" type="checkbox"/>	

Resubmission

- ▶ Batch Size 15
- ▶ Resubmit from image_analysis

The screenshot shows the TissueMAPS software interface. At the top, there's a navigation bar with tabs like 'upload', 'Image conversion', 'Image analysis', and 'Iterator'. The 'Iterator' tab is currently selected, indicated by a blue background. A red arrow points to the 'batch_size' input field in the 'Batch arguments' section, which contains the value '15'. In the center, a modal dialog titled 'Resubmit' is open. It has a dropdown menu labeled 'Stage from which workflow should be resubmitted:' with 'image_analysis' selected. Another red arrow points to this dropdown. Below the dropdown are 'Cancel' and 'OK' buttons. In the bottom right corner of the main window, there's a status bar showing 'Jluethi (logout)'.

BIO325_CRISPR_Yap

Resubmit

Stage from which workflow should be resubmitted:
image_analysis

Cancel OK

Batch arguments

Arguments that control how data is partitioned into computational jobs.

batch_size
number of sites that should be processed per job
15

Submission arguments

Arguments that control how computational jobs are submitted to the cluster.

cores
number of cores that should be allocated to each "run" job (may be increased in case memory requirements of a job exceed resources of a single core)
1

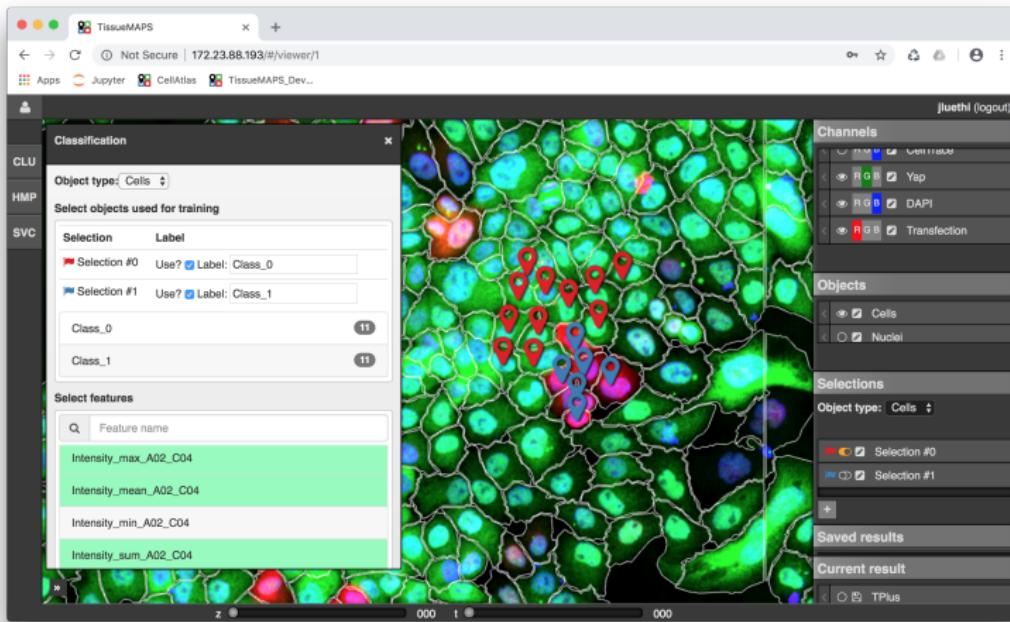
duration
walltime that should be allocated to each "run" job in the format "HH:MM:SS" (may need to be adapted depending on the choice of batch size)
06:00:00

Submit Resume Resubmit Kill Save

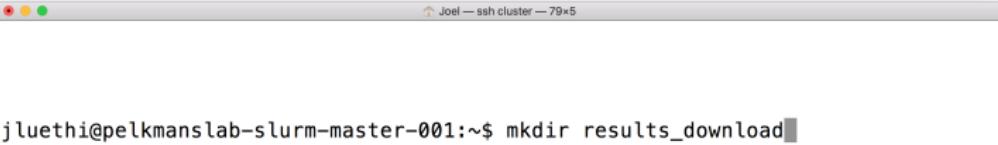
What Will We Do Today?

- * 9:20 – 10:00 Upload data to **TissueMaps**
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- * 16:00 – 16:30 Discussing plotting & wrap up

Training a Classifier to Separate Transfected from Non-Transfected Cells



Make a Directory for Your Results on Your Jupyter Machine



A screenshot of a terminal window with a light gray header bar. The title bar reads "Joel — ssh cluster — 79x5". The main window is white and contains the following text:

```
jluethi@pelkmanslab-slurm-master-001:~$ mkdir results_download
```

Download Results on Your Jupyter Machine

```
jluethi@pelkmanslab-slurm-master-001:~$ tm_client -H 172.23.XX.YY -u clever_student -p myPassword  
feature-values -e BIO325_CRISPR_Yap download -o Cells --directory ~/results_download -h  
usage: tm_client feature-values download [-h] -o OBJECT-TYPE  
                                         [--directory DIRECTORY]  
                                         [--parallel [NUM]]  
  
Download feature values for segmented objects as well as the corresponding  
metadata.  
  
optional arguments:  
  -h, --help            show this help message and exit  
  -o OBJECT-TYPE, --object-type OBJECT-TYPE  
                        name of the objects type  
  --directory DIRECTORY  
                        directory where download should be stored (defaults to  
                        temporary directory)  
  --parallel [NUM]      Use NUM parallel processes for download (default: 1).  
                        If NUM is omitted or 0, the degree of parallelism is  
                        proportional to the number of available CPUs.  
jluethi@pelkmanslab-slurm-master-001:~$
```

Intro to Python