

Introduction to TissueMaps & Image Analysis

BIO325: Systems dynamics in cell and developmental biology

Riccardo Murri & Joel Lüthi

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 divided into sub-groups Fly & Yeast

Learning Objectives

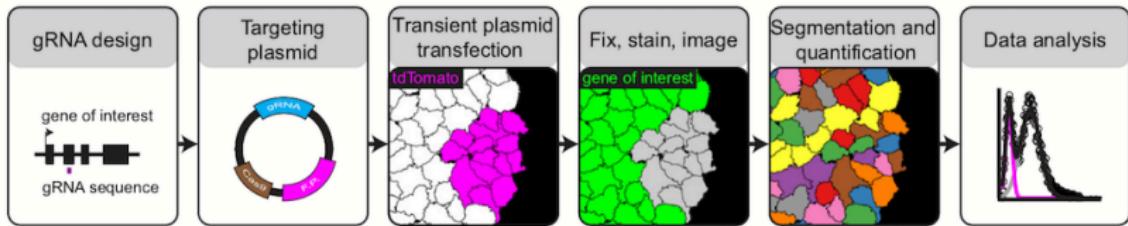
- * At the end of the day, you will be able to:
 1. Understand the use-case for TissueMaps
 2. Use TissueMaps to process your image data
 3. Process single cell measurements using python
 4. Create plots of your data using python

What Will We Do Today?

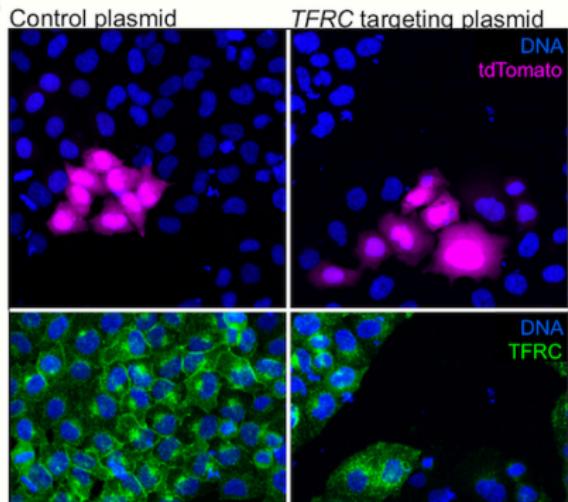
- * 9:20 - 10:00 Upload data to `TissueMaps`
 & start processing it
- * 10:20 - 11:30 Image processing &
 cell segmentation in `TissueMaps`
- * 12:30 - 13:15 Using machine learning & downloading data
- * 13:15 - 13:45 Intro to Python
- * 14:00 - 14:45 Data processing
- * 14:45 - 15:00 Discussing data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

Motivation for Doing Image Analysis

A

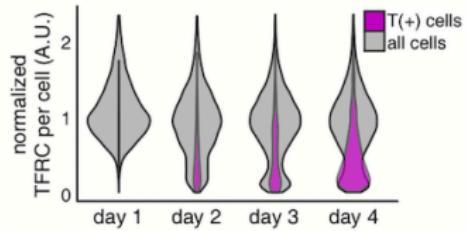


B

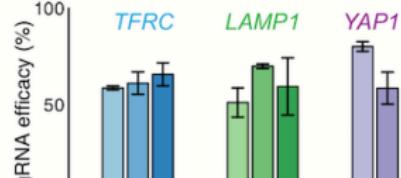


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C



D



Introduction to TissueMaps & Image Analysis

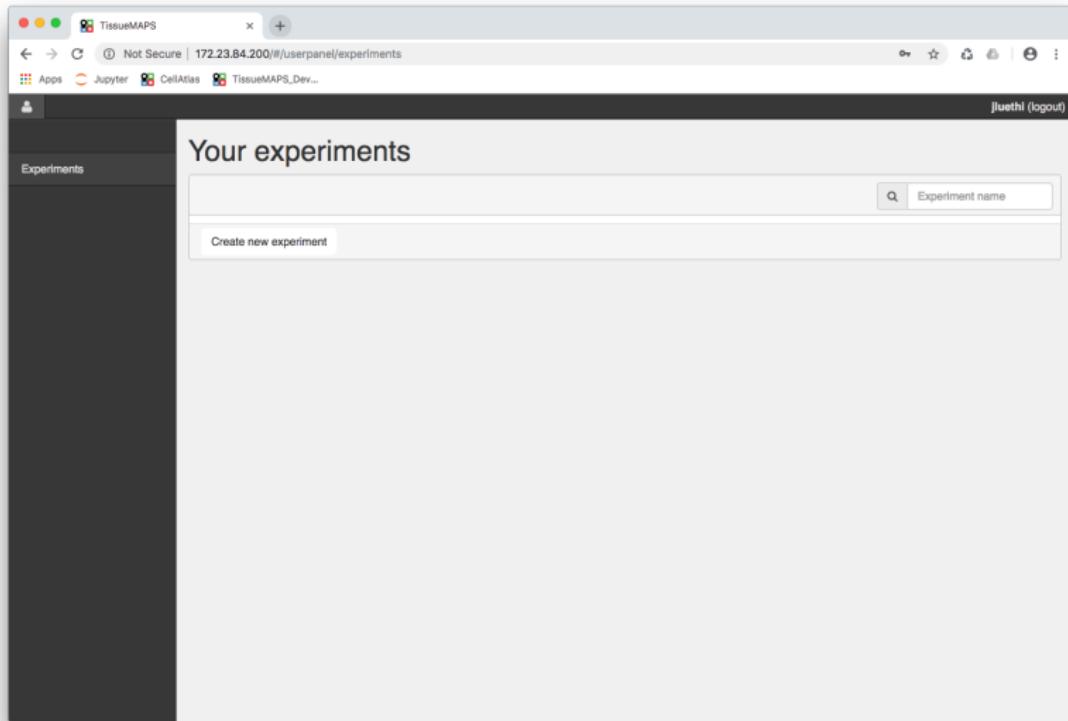
What is TissueMaps

- * 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



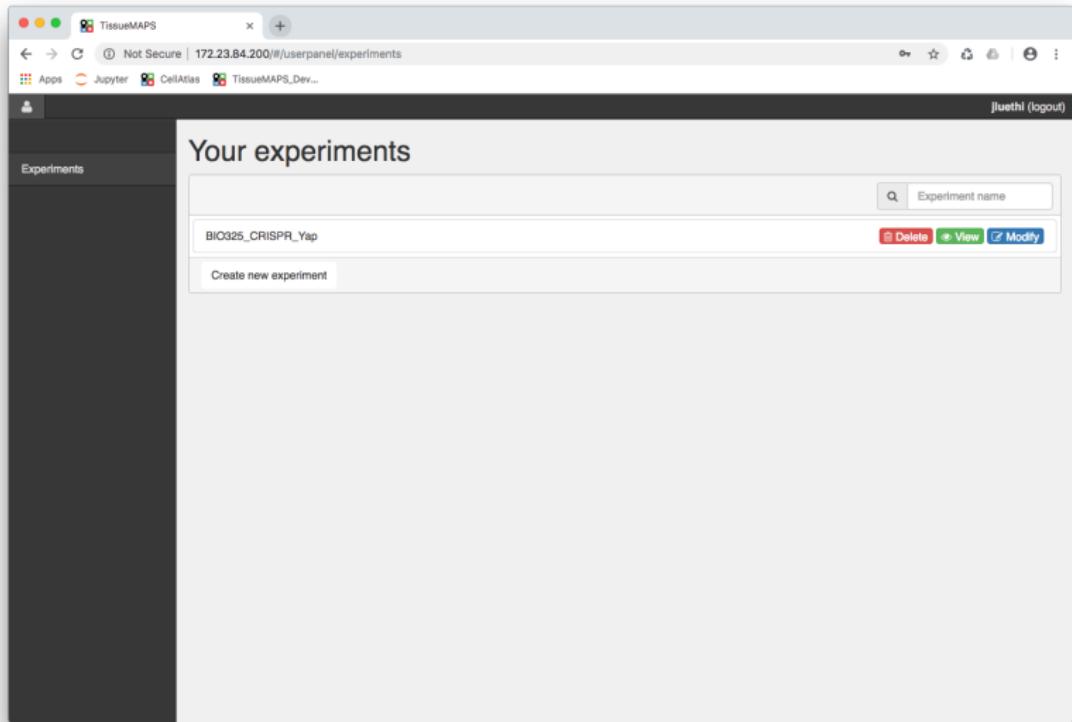
Name Your Experiment

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 and dropdown menus:

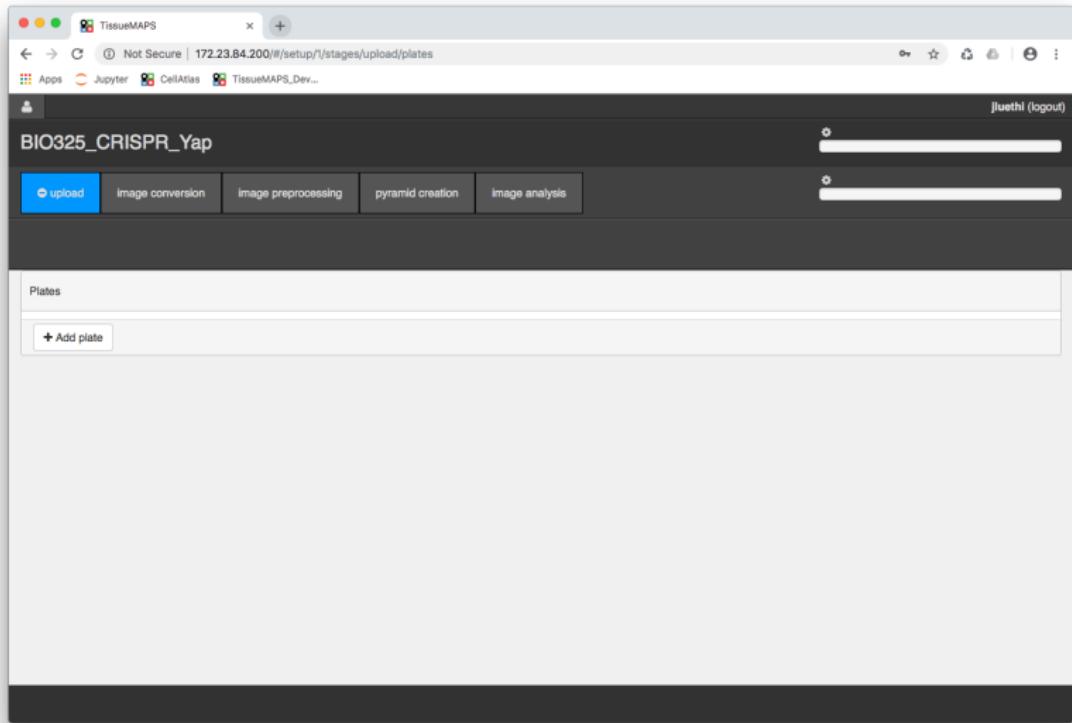
- Experiment name:** BIO325_CRISPR_Yap
- Description:** (Empty text area)
- Workflow type:** canonical
- Plate format:** 384
- Plate acquisition mode:** basic
- Microscope type:** celvoyager

Below the input fields is a large blue button labeled "Create experiment".

You Made a TissueMaps Experiment!



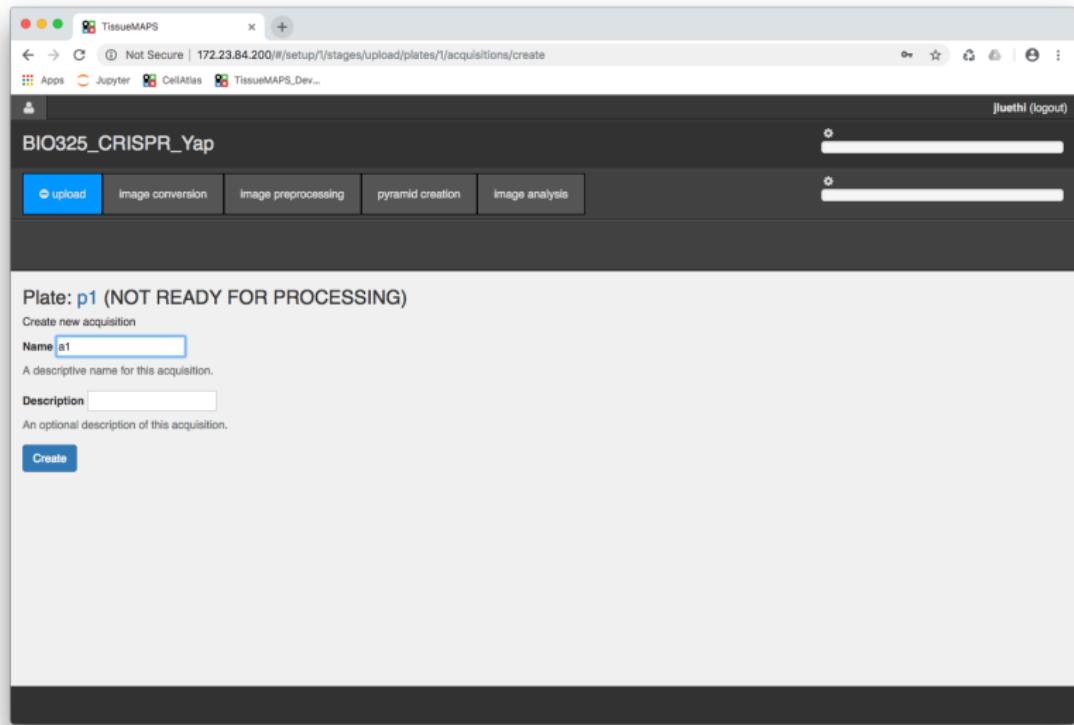
Modify Your Experiment



Create a Plate

The screenshot shows a web-based application window titled "TissueMAPS". The URL in the address bar is "Not Secure | 172.23.84.200//setup/l/stages/upload/plates/create". The top navigation bar includes links for "Apps", "Jupyter", "CellAtlas", and "TissueMAPS_Dev...". A user profile icon and the name "jluethi (logout)" are visible on the right. Below the header, a navigation bar contains five tabs: "upload" (which is highlighted in blue), "image conversion", "image preprocessing", "pyramid creation", and "image analysis". The main content area is titled "Create new plate". It features two input fields: "Name" with the value "pt" and "Description" which is empty. A "Create" button is located at the bottom left of the form.

Create an Acquisition



Getting Data into TissueMaps

```
jluethi@pelkmanslab-slurm-master-001:~$ tm_client -h
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).

positional arguments:
  {tools,workflow,jtproject,experiment,plate,well,site,acquisition,microscope-file,channel,object-type,feature,feature-values,
segmentation,channel-image}
      resources
      tools          tools resources
      workflow       workflow resources
      jtproject      iterator 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] -e 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 BI0325_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

```
joel — ssh cluster — 106x28
jluethi@pelkmanslab-slurm-master-001:~$ tm_client -H 172.23.XX.YY -u clever_student -p myPassword microscope-file -e BI0325_CRISPR_Yap upload -p pl -a a1 --directory ~/BI0325_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 URL in the address bar is "Not Secure | 172.23.84.200//setup/l/stages/upload/plates/l". 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 "jluethi (logout)". Below the header, the main content area has a title "BIO325_CRISPR_Yap". A horizontal progress bar at the top of the content area is labeled "TERMINATED". Below this, a section titled "Plate: p1 (READY FOR PROCESSING)" contains a table under the heading "Acquisitions". The table lists one entry: "a1 (COMPLETE) - 400 files", which is accompanied by a red "Delete" button. At the bottom of the acquisition table is a button labeled "+ Add acquisition".

Add Metadata about the Experiment

The screenshot shows a web-based interface for the TissueMAPS software. At the top, there's a header bar with the TissueMAPS logo, a search bar, and a user dropdown for "jluethi (logout)". Below the header, a navigation bar has tabs for "upload", "Image conversion", "Image preprocessing", "pyramid creation", "Image analysis", "metaextract", "metaconfig" (which is highlighted in blue), and "Imextract".

The main content area is titled "BIO325_CRISPR_Yap". It displays a "Configuration of image metadata" section. This section is divided into two main sections: "Batch arguments" and "Submission arguments".

Batch arguments:

- mip**: A checkbox labeled "perform maximum intensity projection along z axis".
- n_horizontal**: A text input field containing the value "5". Description: "number of images along the horizontal axis of the stitched well overview mosaic image (optional)".
- n_vertical**: A text input field containing the value "5". Description: "number of images along the vertical axis of the stitched well overview mosaic image (optional)".
- regex**: A text input field containing the value "3133".

Submission arguments:

- cores**: A text input field containing the value "1". Description: "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**: A text input field containing the value "06:00:00". Description: "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)".
- memory**: A text input field containing the value "3133". Description: "amount of memory in megabytes that should be allocated to each "run" job".

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

Define settings for the Pyramid / Image Browser

BIO325_CRISPR_Yap

pyramid creation

Illuminati

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

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

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

Submit Resume Resubmit Kill Save

Submit the Pipeline & Enjoy Your Break

The screenshot shows a web-based application for creating pyramid images. At the top, there's a navigation bar with links for 'upload', 'image conversion', 'image preprocessing', 'pyramids', and 'Illuminati'. A 'Warning' dialog box is centered over the page, asking 'Do you really want to submit the workflow?' with 'Cancel' and 'Ok' buttons.

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

clip whether images intensities should be clipped

clip_percent
threshold percentile at which image intensities should be clipped

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)

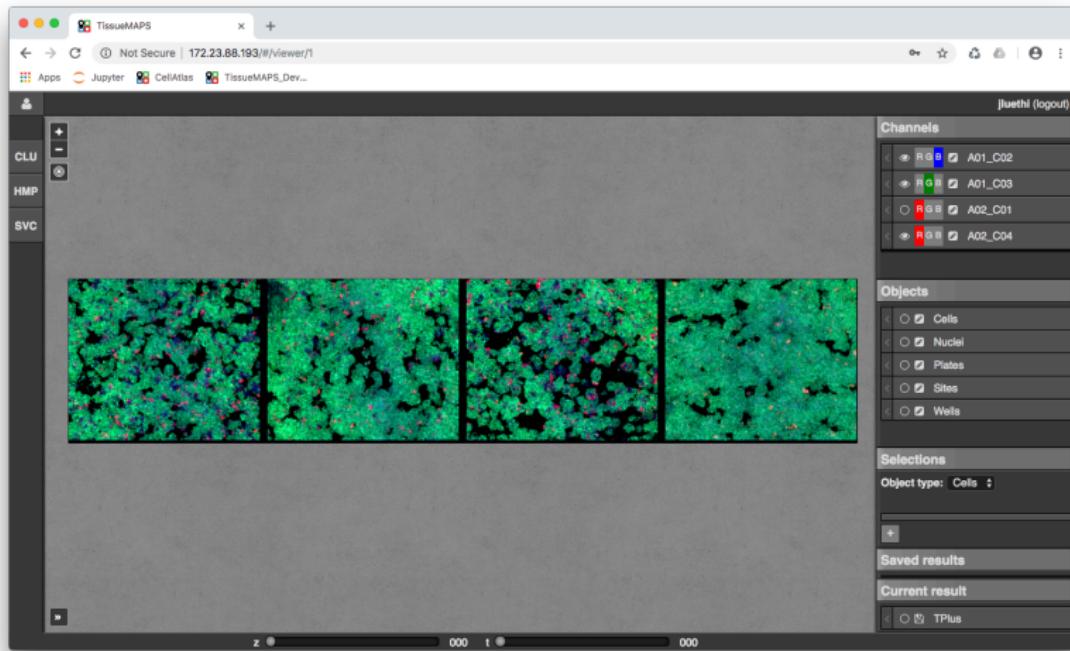
duration
walltime that should be allocated to each "run" job in the format "HH:MM:SS" (may need to be adapted depending on the choice of batch size)

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

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

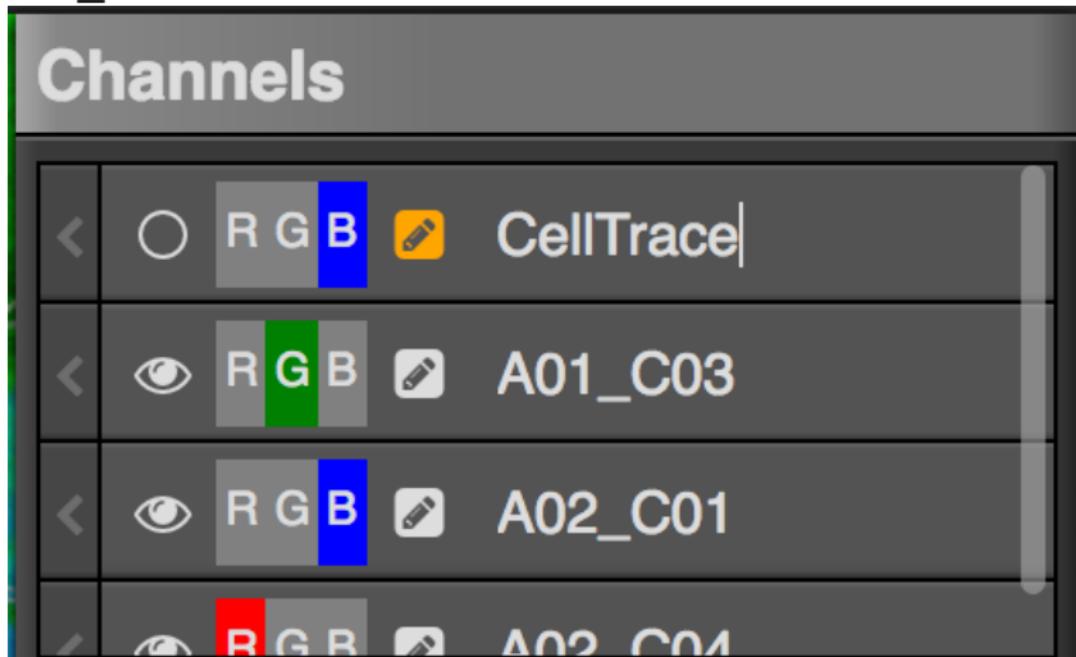
Submit **Resume** **Resubmit** **Kill** **Save**

The Image Viewer Gives You an Overview of Your Experiment



Rename Your Channels

- ▶ A01_C02: CellTrace
- ▶ A01_C03: Yap
- ▶ A02_C01: DAPI
- ▶ 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/1/stages/image_analysis/steps/iterator". The page displays a pipeline status bar at the top with several steps: upload, image conversion, image preprocessing, pyramid creation, image analysis, and iterator. The "image analysis" step is highlighted in blue, indicating it is currently active or selected. Below this, a section titled "Image analysis pipeline engine" provides details about the application of a sequence of algorithms to images. It includes two main argument sections: "Batch arguments" and "Submission arguments". The "Batch arguments" section contains a "batch_size" input field set to "10" with an "Edit Pipeline" button below it, which has a red arrow pointing to it. The "Submission arguments" section contains "cores" set to "1" and "duration" set to "06:00:00". At the bottom, there are buttons for "Submit", "Resume", "Resubmit", "Kill", and "Save".

BIO325_CRISPR_Yap

TERMINATED
TERMINATED
TERMINATED

upload image conversion image preprocessing pyramid creation image analysis iterator

Image analysis pipeline engine

Application of a sequence of algorithms to a set of images to segment the images and extract features for the identified objects.

Batch arguments

Arguments that control how data is partitioned into computational jobs.

batch_size

number of sites that should be processed per job

10

←

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

A Good Pipeline for Today

Input

Select **channels** that should be available in the pipeline:

DAPI

Correct illumination

CellTrace

Correct illumination

Yap

Correct illumination

Transfection

Correct illumination

- Pipeline**
- Define the **modules** and
be processed:
- eye smooth_DAPI
 - eye threshold_otsu
 - eye filter_nuclei
 - eye separate_clumps
 - eye label_nuclei
 - eye register_objects
 - eye segment_secondary
 - eye register_objects_C

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 'Not Secure | 172.23.88.193 // setup / stages / image_analysis / steps / iterator'. Below the navigation is a user menu with 'jiuethi (logout)'.

The main area displays a pipeline named 'BIO325_CRISPR_Yap'. The pipeline steps are listed as cards: 'upload', 'image conversion', 'image', and 'iterator' (highlighted in blue). A red arrow points to the 'iterator' step.

A modal dialog box titled 'Resubmit' is open, asking 'Stage from which workflow should be resubmitted?'. The dropdown menu shows 'image_analysis' (highlighted in blue) and has a red arrow pointing to it.

The pipeline engine section below contains two main sections: 'Batch arguments' and 'Submission arguments'.

Batch arguments:
Arguments that control how data is partitioned into computational jobs.
batch_size: number of sites that should be processed per job (input field: 15, highlighted with a red arrow).

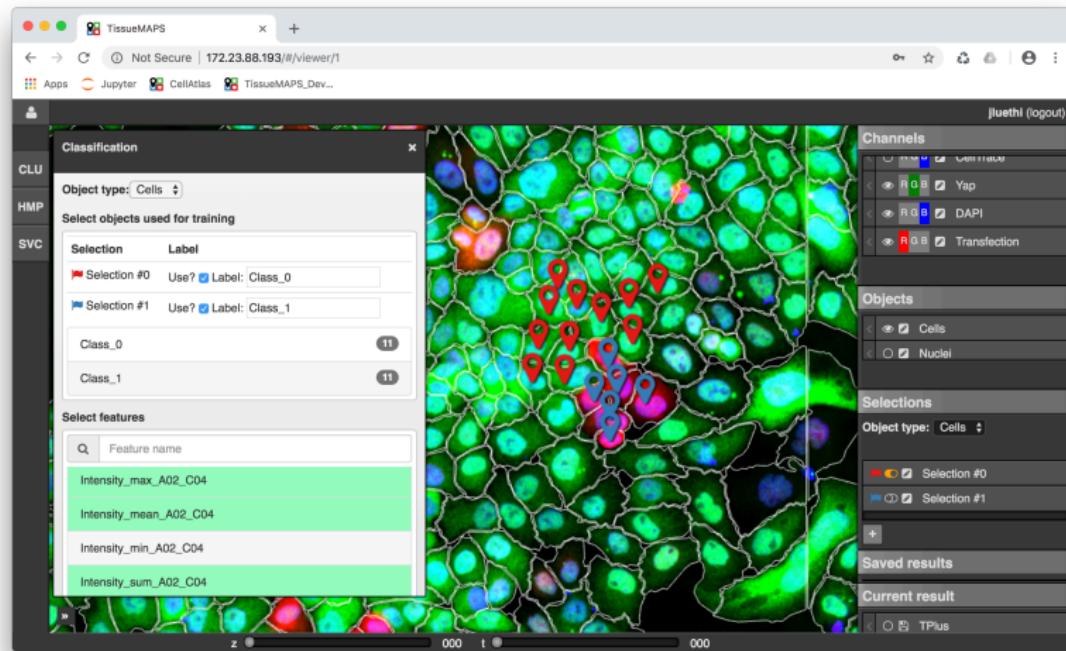
Submission arguments:
Arguments that control how computational jobs are submitted to the cluster.
cores: number of cores that should be allocated to each "run" job (may be increased in case memory requirements of a job exceed resources of a single core) (input field: 1).
duration: walltime that should be allocated to each "run" job in the format "HH:MM:SS" (may need to be adapted depending on the choice of batch size) (input field: 06:00:00).

At the bottom, there are buttons for 'Submit', 'Resume', 'Resubmit' (highlighted in blue), 'Kill', and 'Save'.

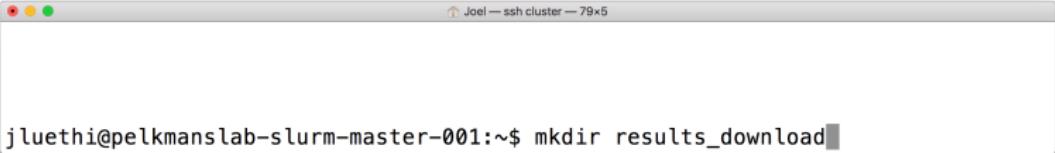
What Will We Do Today?

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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



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 BI0325_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