

# 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

## Learning Objectives

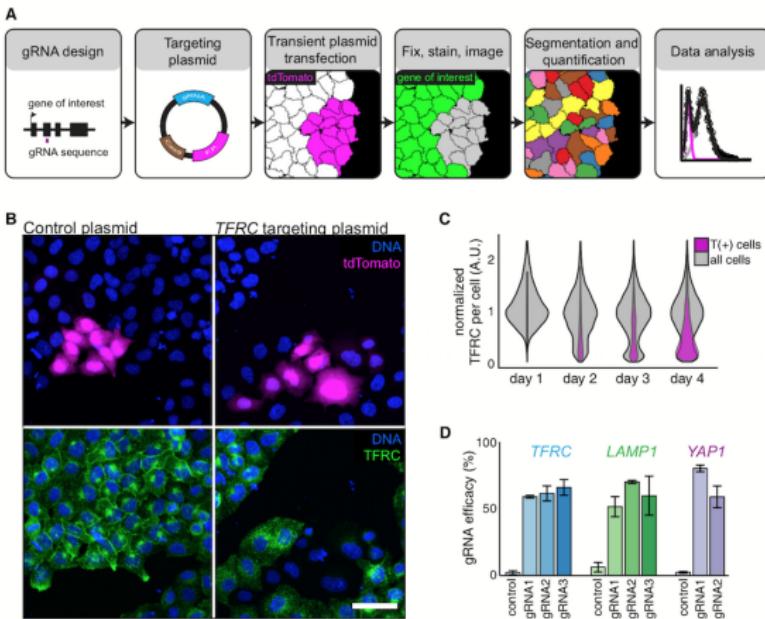
At the end of the day, you will be able to:

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.

# Connect to Jupyter Notebook

`http://172.23.X.Y:2000/`

The screenshot shows a Jupyter Notebook interface. At the top, there is a header with the Jupyter logo and navigation links for 'Logout' and 'Control Panel'. Below the header, there are tabs for 'Files', 'Running', and 'Clusters', with 'Files' being the active tab. A sub-header below the tabs says 'Select items to perform actions on them.' On the right side of this sub-header are buttons for 'Upload', 'New', and a refresh icon. The main area displays a list of files and folders in a table format. The columns are 'Name' (with a dropdown arrow) and 'Last Modified'. The table contains the following data:

Name	Last Modified
0 /	
bio325-intro-to-tissuemaps	un minuto fa
Medium_Yap	21 giorni fa
seaborn-data	11 ore fa
TissueMAPS	un giorno fa

# Start a terminal (to be used later)

The screenshot shows the Jupyter Notebook interface. At the top, there is a logo, the word "jupyter", and two buttons: "Logout" and "Control Panel". Below the header, there is a navigation bar with three tabs: "Files" (selected), "Running", and "Clusters". A message "Select items to perform actions on them." is displayed above the file tree.

The file tree on the left shows the following structure:

- 0 /
  - blo325-intro-to-tissuemaps
  - Medium\_Yap
  - seaborn-data
  - TissueMAPS

To the right of the file tree is a context menu with the following options:

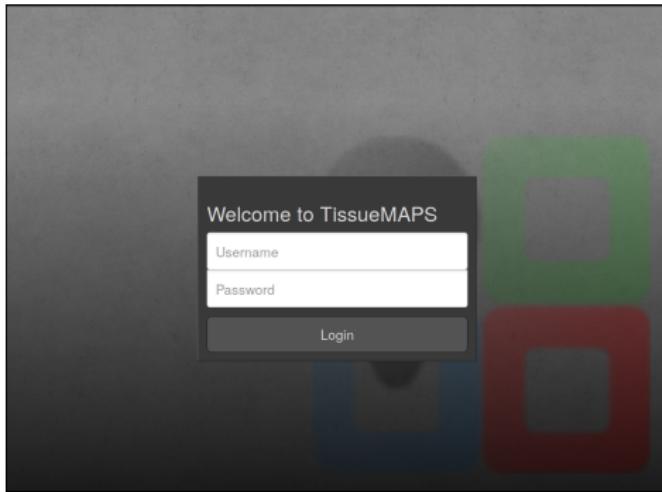
- Upload
- New ▾
- ↻
- Notebook:
  - Bash
  - Python 2
  - Python 3
  - R
- Other:
  - Text File
  - Folder
  - Terminal

At the bottom left of the interface, there is a URL: "172.23.88.49:2009/user/tissuemaps/tree#".

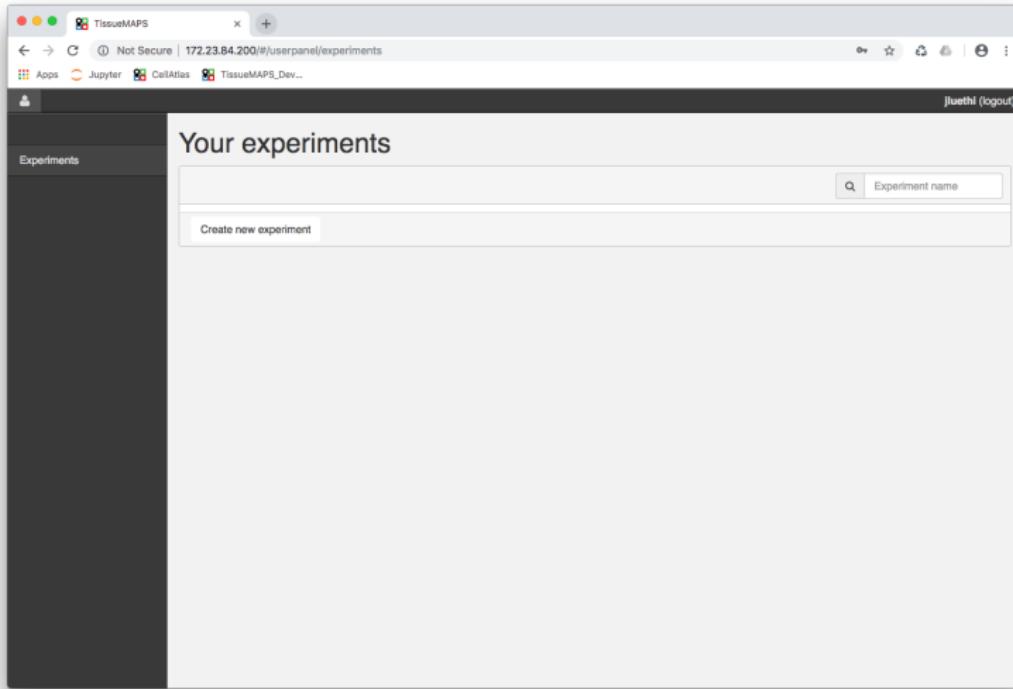
## Connect to TissueMAPS

`http://172.23.X.Y:1000/`

(Same address as Jupyter, but with :1000 at the end.)



# 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 user is logged in as "Juethi". The main content area is titled "Create experiment". It contains several input fields:

- 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 blue "Create experiment" button.

# You Made a TissueMaps Experiment!

The screenshot shows a web browser window titled "TissueMAPS" with the URL "Not Secure | 172.23.84.200/userpanel/experiments". The browser's address bar also lists "Apps", "Jupyter", "CellAtlas", and "TissueMAPS\_Dev...". The top right corner shows the user "Juethi (logout)". The main content area is titled "Your experiments" and contains a search bar with "Experiment name" and a magnifying glass icon. Below the search bar is a table with one row, "BIO325\_CRISPR\_Yap", which has three buttons to its right: "Delete" (red), "View" (green), and "Modify" (blue). At the bottom of the table is a link "Create new experiment".

# Modify Your Experiment

The screenshot shows a web-based application window titled "TissueMAPS". The URL in the address bar is "Not Secure | 172.23.84.200/l/setup/l/stages/l/upload/plates". 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)". The main content area is titled "BIO325\_CRISPR\_Yap". Below the title is a horizontal navigation bar with five tabs: "upload" (highlighted in blue), "Image conversion", "Image preprocessing", "pyramid creation", and "Image analysis". The "upload" tab has a progress bar below it. The main workspace is labeled "Plates" and contains a button labeled "+ Add plate".

# 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...". On the right side of the header, there is a user profile icon and the name "Juethi (logout)". Below the header, a dark navigation bar contains five tabs: "upload" (highlighted in blue), "Image conversion", "Image preprocessing", "pyramid creation", and "Image analysis". The main content area is titled "Create new plate". It has two input fields: "Name" with the value "p1" and "Description" which is empty. A "Create" button is located at the bottom left of the form.

# Create an Acquisition

The screenshot shows a web browser window titled "TissueMAPS" with the URL "Not Secure | 172.23.84.200/setup/1/stages/upload/plates/1/acquisitions/create". The page header includes a user profile for "Juethi (logout)" and navigation links for "Apps", "Jupyter", "CellAtlas", and "TissueMAPS\_Dev...". Below the header, the main content area is titled "BIO325\_CRISPR\_Yap". A horizontal menu bar contains five items: "upload" (highlighted in blue), "Image conversion", "Image preprocessing", "pyramid creation", and "Image analysis". The main content area displays a message: "Plate: p1 (NOT READY FOR PROCESSING)". It includes fields for "Name" (set to "a1") and "Description" (empty). A "Create" button is located at the bottom left of the form.

# 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,f
eature-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,s
egmentations,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  
pe-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 -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" with the URL "Not Secure | 172.23.84.200/i/setup/1/stages/upload/plates/1". The top navigation bar includes links for "Apps", "Jupyter", "CellAtlas", and "TissueMAPS\_Dev...". A user icon and "Juethi (logout)" are also present. The main content area is titled "BIO325\_CRISPR\_Yap". Below the title is a horizontal bar with five tabs: "upload" (highlighted in blue), "Image conversion", "Image preprocessing", "pyramid creation", and "Image analysis". To the right of this bar is a progress bar labeled "TERMINATED". The main content area is titled "Plate: p1 (READY FOR PROCESSING)". Under the "Acquisitions" section, there is a list item "a1 (COMPLETE) - 400 files" with a red "Delete" button to its right. A "Add acquisition" button is located below the list.

# 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 'Not Secure' warning, and the URL '172.23.84.200/l/stages/l/image\_conversion/steps/metaconfig'. Below the header is a navigation menu with links for 'Apps', 'Jupyter', 'CellAtlas', and 'TissueMAPS\_Dev...'. On the right side of the header, there's a user profile for 'Juethi' with a 'logout' link.

The main content area has a title 'BIO325\_CRISPR\_Yap'. Below it is a horizontal navigation bar with several tabs: 'upload' (disabled), 'image conversion' (selected), 'image preprocessing', 'pyramid creation', 'image analysis', 'metaextract' (disabled), 'metaconfig' (selected), and 'imextract' (disabled). The 'metaconfig' tab is highlighted with a blue background.

Below the tabs, the section title 'Configuration of image metadata' is displayed. A sub-instruction reads: 'Configuration of extracted OMEXML metadata and integration with additional microscope-specific information about the image acquisition process.'

The configuration is divided into two main sections:

- Batch arguments**:
  - mip**:  (disabled)
  - description: 'perform maximum intensity projection along z axis'
  - n\_horizontal**:
    - description: 'number of images along the horizontal axis of the stitched well overview mosaic image (optional)'
    - input field: '5'
  - n\_vertical**:
    - description: 'number of images along the vertical axis of the stitched well overview mosaic image (optional)'
    - input field: '5'
  - regex**: (disabled)
- Submission arguments**:
  - cores**:
    - 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)'
    - input field: '1'
  - duration**:
    - description: 'waittime 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)'
    - input field: '06:00:00'
  - memory**:
    - description: 'amount of memory in megabytes that should be allocated to each "run" job'
    - input field: '3133'

At the bottom of the configuration area, there are several buttons: 'Submit' (blue), 'Resume', 'Resubmit', 'Kill' (disabled), and 'Save'.

# Define settings for the Pyramid / Image Browser

BIO325\_CRISPR\_Yap

pyramid creation

illumini

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

The screenshot shows a web-based application window titled "TissueMAPS". The URL in the address bar is "Not Secure | 172.23.84.200/l/stages/pyramid\_creation/steps/illumina". The top navigation bar includes links for "Apps", "Jupyter", "CellAtlas", and "TissueMAPS\_Dev...". A user profile icon for "Juethi" with "(logout)" is also present.

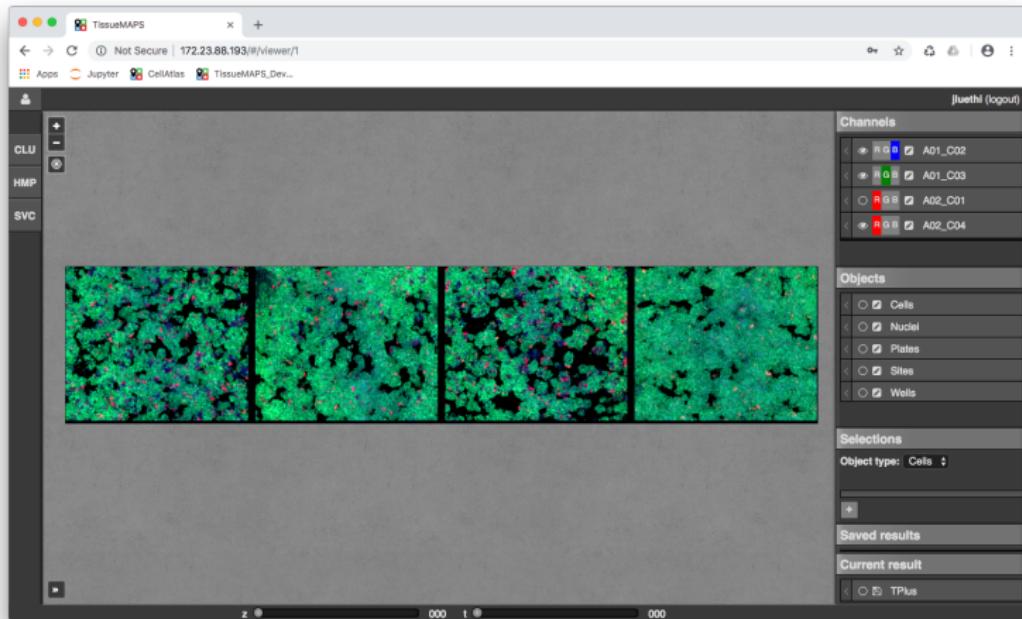
The main content area displays a workflow titled "BIO325\_CRISPR\_Yap". The workflow consists of several steps: "upload", "image conversion", "image preprocessing", "pyramid creation", and "illumina". The "illumina" step is currently selected, indicated by a blue background.

A modal dialog box titled "Warning" is displayed, asking "Do you really want to submit the workflow?". It contains two buttons: "Cancel" and "Ok".

The left side of the interface contains a "Pyramid image builder" section with the sub-section "Batch arguments". It includes configuration for "align" (unchecked), "batch\_size" (set to 100), "clip" (checked), and "clip\_percent" (set to 99.9). Below these are buttons for "Submit", "Resume", "Resubmit", "Kill", and "Save".

The right side contains a "Submission arguments" section with fields for "cores" (set to 1), "duration" (set to 06:00:00), and "memory" (set to 3133). There is also a note: "Arguments that control how computational jobs are submitted to the cluster".

# The Image Viewer Gives You an Overview of Your Experiment

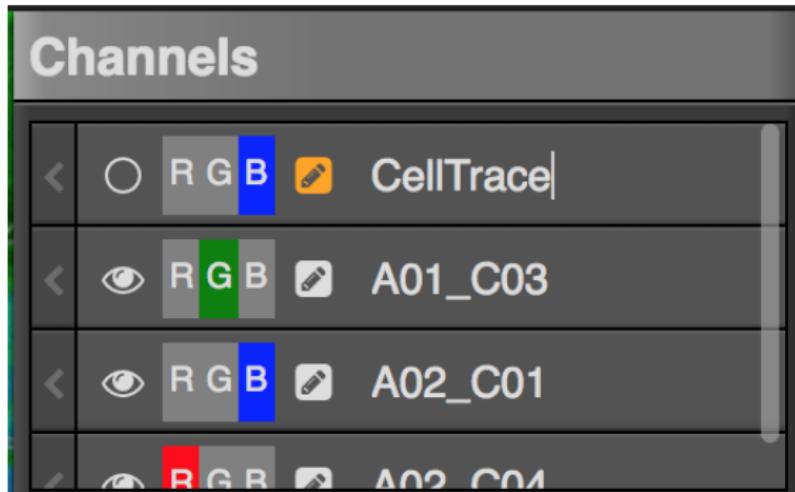


# Rename Your Channels

---

A01_C02 → CellTrace	A01_C03 → Yap
A02_C01 → DAPI	A02_C04 → Plasmid

---



# Go into the Image Processing Pipeline

The screenshot shows a web-based application window titled "TissueMAPS". The URL in the address bar is "Not Secure | 172.23.88.193/#/setup/1/stages/image\_analysis/steps/iterator". The top navigation bar includes links for "Apps", "Jupyter", "CellAtlas", and "TissueMAPS\_Dev...". On the right, a user profile indicates "jluethi (logout)". Below the header, a progress bar for the pipeline step "BIO325\_CRISPR\_Yap" shows four completed steps: "upload", "image conversion", "image preprocessing", and "pyramid creation", followed by the currently selected step, "image analysis". A final step, "iterator", is shown as pending. The main content area is titled "Image analysis pipeline engine" and describes the application of a sequence of algorithms to images. It contains two sections: "Batch arguments" and "Submission arguments". Under "Batch arguments", there is a field for "batch\_size" set to "10" with an "Edit Pipeline" button. An arrow points to this button. Under "Submission arguments", there are fields for "cores" (set to "1") and "duration" (set to "06:00:00"). At the bottom, there 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
- CellTrace
- Correct illumination
- Yap
- Correct illumination
- Transfection
- Correct illumination

**+ Add**   **- Remove**   **List jobs**

**Pipeline**

Define the **modules** and the order in which they should be processed:

- smooth\_DAPI
- threshold\_otsu
- filter\_nuclei
- separate\_clumps
- label\_nuclei
- register\_objects
- segment\_secondary
- register\_objects\_Cells
- measure\_intensity\_DAPI
- measure\_intensity\_CellTrace
- measure\_intensity\_2\_Yap
- measure\_intensity\_3\_Transfection
- measure\_morphology\_Cells
- measure\_morphology\_1\_Nuclei
- measure\_texture\_Transfection

**Submit**   **Kill**   **Save**

**Output**

Select **objects** that should be saved:

- Nuclei  
Represent as polygons
- Cells  
Represent as polygons

# Resubmission

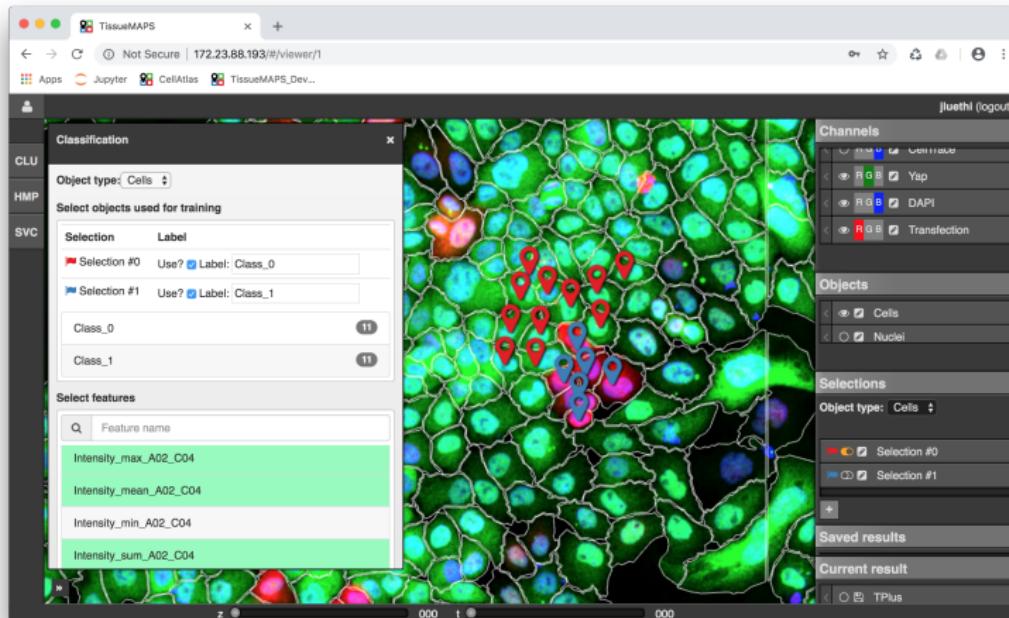
- ▶ Batch Size 15
- ▶ Resubmit from image\_analysis

The screenshot shows the TissueMAPS software interface. At the top, there's a navigation bar with tabs like 'TissueMAPS', 'Not Secure | 172.23.88.193/#/setup/1/stages/image\_analysis/steps/iterator', and user information 'Jluethi (logout)'. Below the navigation bar, a modal dialog titled 'Resubmit' is open, with a dropdown menu set to 'image\_analysis'. A red arrow points from the 'batch\_size' input field in the pipeline configuration area to this dropdown. The pipeline configuration area shows a step named 'iterator' with a value of '15' in the 'batch\_size' field. Another red arrow points to the 'iterator' step. The pipeline has four stages: 'upload', 'Image conversion', 'Image segmentation', and 'iterator'. The 'iterator' stage is highlighted with a blue background. The pipeline is described as an 'Image analysis pipeline engine' for segmenting images and extracting features. The bottom of the screen features a toolbar with buttons for 'Submit', 'Resume', 'Resubmit' (which is highlighted in blue), 'Kill', and 'Save'.

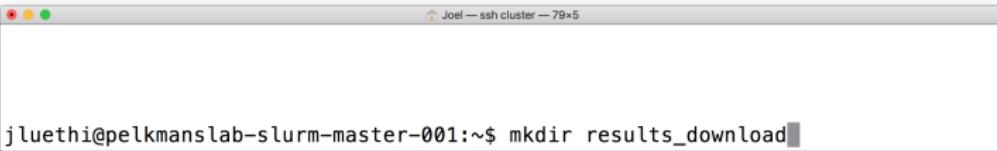
## What Will We Do Today?

- ▶ 9:20 - 10:00 Upload data to TissueMaps & start processing it
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- ▶ 14:45 - 15:00 Discussing data processing
- ▶ 15:15 - 16:00 Plotting data
- ▶ 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 titled "Joel — ssh cluster — 79x5". The window has three colored window control buttons (red, yellow, green) at the top left. The title bar also shows the window's name and size. The main area of the terminal contains the command:

```
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:~$
```