

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

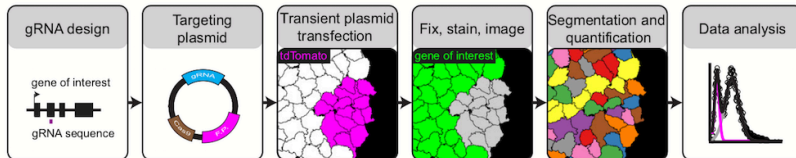
- * At the end of the day, you will be able to:
 1. Understand the use-case for TissueMaps
 2. Use TissueMaps to process your image data
 3. Process single cell measurements using python
 4. Create plots of your data using python

What Will We Do Today?

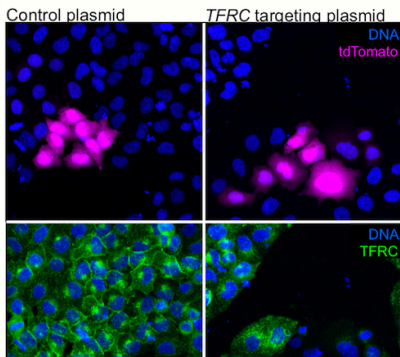
- * 9:20 - 10:00 Upload data to TissueMaps
& start processing it
- * 10:20 - 11:30 Image processing &
cell segmentation in TissueMaps
- * 12:30 - 13:15 Using machine learning & downloading data
- * 13:15 - 13:45 Intro to Python
- * 14:00 - 14:45 Data processing
- * 14:45 - 15:00 Discussing data processing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

Motivation for Doing Image Analysis

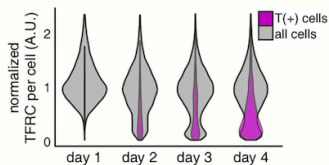
A



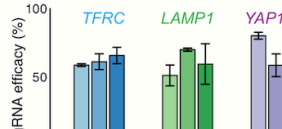
B



C



D



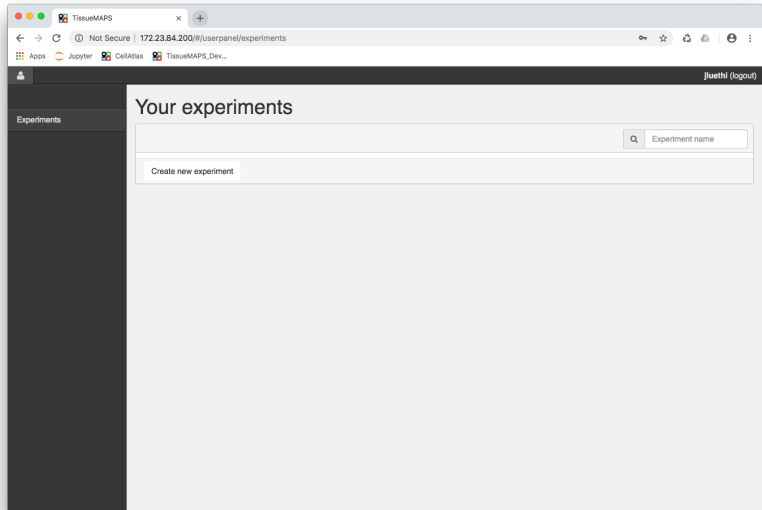
What is TissueMaps

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

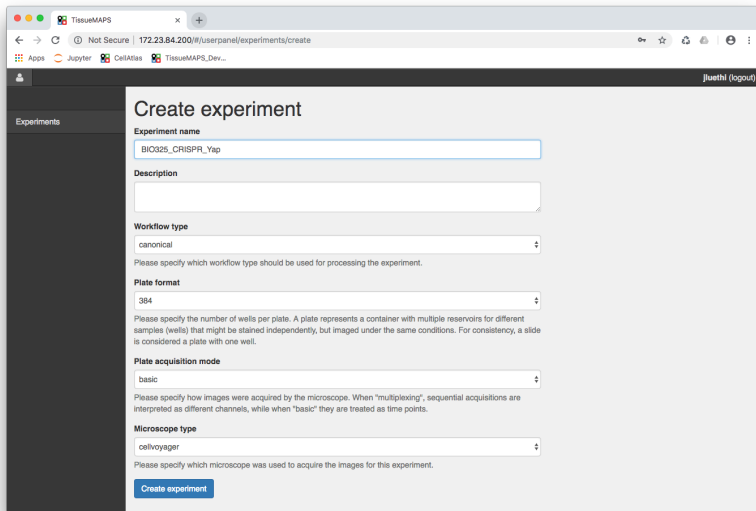
Your Jupyter Notebook & TissueMaps Server

- * 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 with the TissueMAPS application. The address bar shows the URL `172.23.84.200/#userpanel/experiments/create`. The page title is "Create experiment". On the left, there is a dark sidebar with a "Experiments" menu item. The main content area contains several form fields: "Experiment name" (text input with value "BIO325_CRISPR_Yap"), "Description" (text area), "Workflow type" (dropdown menu with value "canonical"), "Plate format" (dropdown menu with value "384"), "Plate acquisition mode" (dropdown menu with value "basic"), and "Microscope type" (dropdown menu with value "cellvoyager"). Each dropdown menu has a small upward and downward arrow icon. Below the "Plate acquisition mode" dropdown, there is a paragraph of text explaining the difference between "multiplexing" and "basic" acquisition modes. At the bottom of the form, there is a blue button labeled "Create experiment". The top right corner of the page shows the user "juethi (logout)".

Not Secure | 172.23.84.200/#userpanel/experiments/create

Apps Jupyter CellAtlas TissueMAPS_Dev...

juethi (logout)

Create experiment

Experiment name

BIO325_CRISPR_Yap

Description

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.

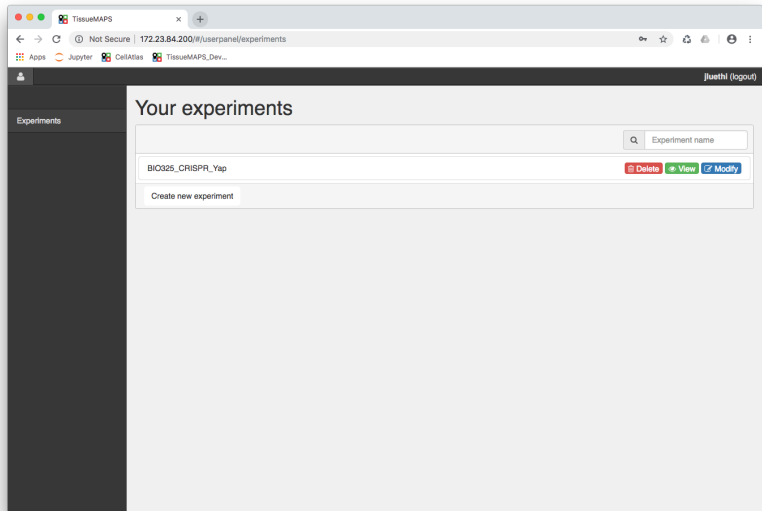
Microscope type

cellvoyager

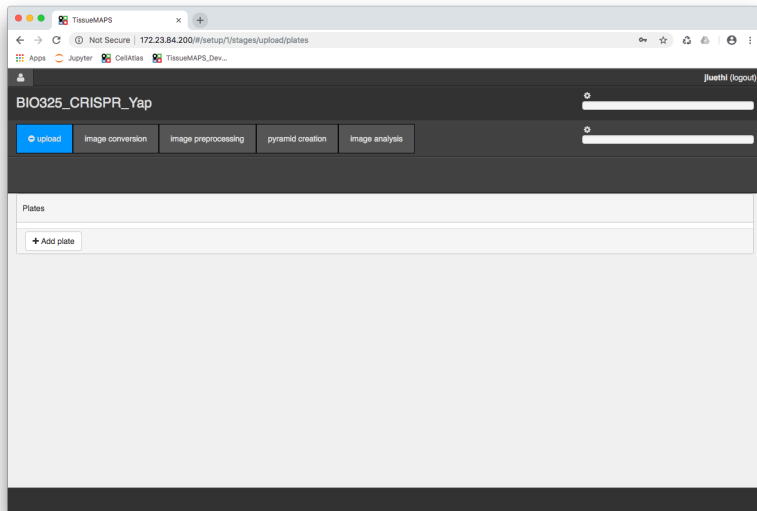
Please specify which microscope was used to acquire the images for this experiment.

Create experiment

You Made a TissueMaps Experiment!



Modify Your Experiment



Create a Plate

The screenshot shows a web browser window with the TissueMAPS application. The browser's address bar shows the URL `172.23.84.200/#/setup/1/stages/upload/plates/create`. The application header includes a user profile icon, the text "BIO325_CRISPR_Yap", and a "logout" link. Below the header is a navigation bar with buttons for "upload", "image conversion", "image preprocessing", "pyramid creation", and "image analysis". The main content area is titled "Create new plate" and contains a form with the following fields:

- Name:** A text input field containing the value "p1". Below it is a placeholder text: "A descriptive name for this plate."
- Description:** A text input field. Below it is a placeholder text: "An optional description of this plate."
- Create:** A blue button to submit the form.

Create an Acquisition

The screenshot shows a web browser window with the TissueMAPS application. The address bar shows the URL: `172.23.84.200/#/setup/!/stages/upload/plates/!/acquisitions/create`. The page title is "TissueMAPS". The user is logged in as "juethi (logout)".

The main heading is "BIO325_CRISPR_Yap". Below this is a navigation bar with five buttons: "upload" (highlighted in blue), "image conversion", "image preprocessing", "pyramid creation", and "image analysis".

The main content area is titled "Plate: p1 (NOT READY FOR PROCESSING)". Below this is the section "Create new acquisition".

The "Name" field contains the text "a1". Below it is a descriptive text: "A descriptive name for this acquisition."

The "Description" field is empty. Below it is a descriptive text: "An optional description of this acquisition."

At the bottom left is a blue "Create" button.

Getting Data into TissueMaps

```
Joel — ssh cluster — 169x38
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                resources
  workflow             tools resources
  jtproject            workflow resources
  experiment           jtproject resources
  plate               experiment resources
  well                plate resources
  site                well resources
  acquisition          site resources
  microscope-file      acquisition resources
  channel             microscope file resources
  object-type         channel resources
  feature             object type resources
  feature-values      feature resources
  segmentation        feature values resources
  channel-image       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
jluethi@pelkmanslab-slurm-master-001:~$
```

Tell TmClient Who You are

```
Joel — ssh cluster — 122x16
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

```
Joel — ssh cluster — 154x35
jluethi@pelkmanslab-slurm-master-001:~$ tm_client -H 172.23.XX.YY -u clever_student -p nyPassword microscope-file -e B10325_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
                        proportional to the number of available CPUs.
  --retries NUM         Retry failed uploads up to NUM times. If this option
                        is omitted, 'tm_client' will retry failed uploads up
                        to 5 times.
  --no-retry            Do not retry failed uploads.
jluethi@pelkmanslab-slurm-master-001:~$
```

Tell TmClient Where the Images Are

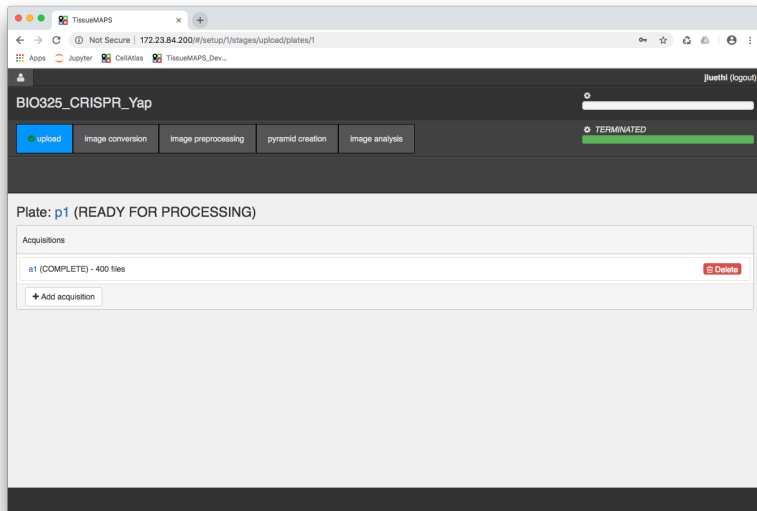
```
Joel — ssh cluster — 187x35
jluethig@pelkmanlab-slurm-master-001:~$ tm_client -h 172.23.XX.YY -o clever_student -p myPassword microscope-file -e B10325_CRISPR_Yap upload -p p1 -s a1 --directory ~/B10325_Yap_Data -h
usage: tm_client microscope-file upload [-h] -p PLATE --acquisition ACQUISITION
                                         [--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
                        proportional to the number of available CPUs.
  --retries NUM         Retry failed uploads up to NUM times. If this option
                        is omitted, 'tm_client' will retry failed uploads up
                        to 5 times.
  --no-retry            Do not retry failed uploads.
jluethig@pelkmanlab-slurm-master-001:~$
```


Upload Completed



Add Metadata about the Experiment

TissueMAPS

Not Secure | 172.23.84.200/#/setup/1/stages/image_conversion/steps/metaconfig

AppsJupyterCellAtlasTissueMAPS_Dev...

juethi (logout)

BIO325_CRISPR_Yap

uploadimage conversionimage preprocessingpyramid creationimage analysis

metaextractmetaconfigimextract

Configuration of image metadata

Configuration of extracted OMEXML metadata and integration with additional microscope-specific information about the image acquisition process.

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

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)

memory

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

Submit

Resume

Resubmit

Kill

Save

Define settings for the Pyramid / Image Browser

The screenshot shows a web browser window with the URL `172.23.84.200/#/setup/1/stages/pyramid_creation/steps/illuminate`. The page title is "BIO325_CRISPR_Yap" and the user is logged in as "juethi". The interface has a dark theme and a navigation bar with buttons for "upload", "image conversion", "image preprocessing", "pyramid creation" (highlighted in blue), and "image analysis". Below the navigation bar, there are three tabs: "illuminate" (highlighted in blue), "pyramid creation", and "image analysis". The "illuminate" tab is active, showing a form with the following settings:

- batch_size**: number of image files that should be processed per job. Value: 100.
- clip**: ☒ whether images intensities should be clipped.
- clip_percent**: threshold percentile at which image intensities should be clipped. Value: 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). Value: (empty).
- illumcorr**: ☒ whether images should be corrected for illumination artifacts.
- illumcorr_exceptions**: (empty).

On the right side of the form, there are three input fields for "requirements of a job exceed resources of a single core":

- duration**: 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). Value: 06:00:00.
- memory**: amount of memory in megabytes that should be allocated to each "run" job. Value: 3133.

At the bottom of the form, there are four buttons: "Submit", "Resume", "Resubmit", and "Kill". A "Save" button is also present at the bottom right.

Submit the Pipeline & Enjoy Your Break

The screenshot shows a web browser window with the URL `172.23.84.200/#/setup/1/stages/pyramid_creation/steps/illuminate`. The page title is "BIO325_CRISPR_Yap". A "Warning" dialog box is centered on the screen, asking "Do you really want to submit the workflow?" with "Cancel" and "Ok" buttons. The background interface includes a top navigation bar with "upload", "image conversion", "image preprocessing", and "pyramid" tabs. Below these is a "Pyramid image builder" section with two columns: "Batch arguments" and "Submission arguments".

BIO325_CRISPR_Yap

upload image conversion image preprocessing **pyramid**

Warning

Do you really want to submit the workflow?

Cancel Ok

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

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)

06:00:00

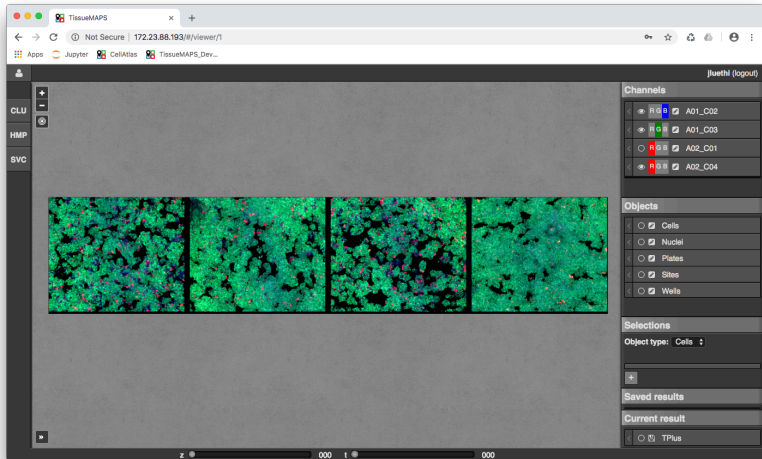
memory

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

3133

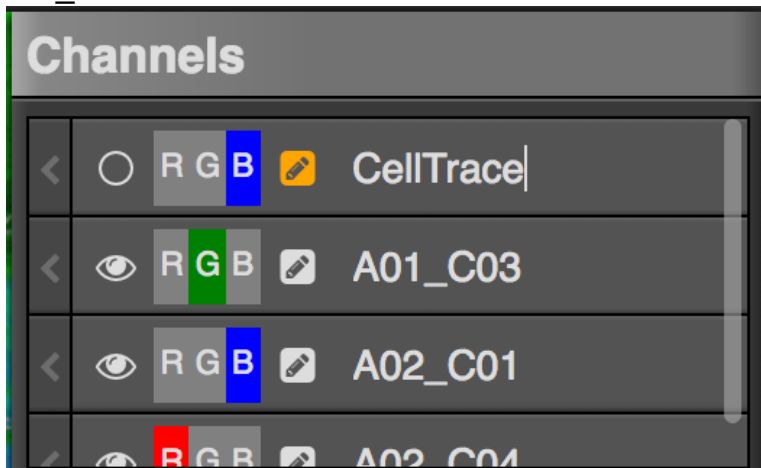
Submit Resume Resubmit Kill Save

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 with the TissueMAPS application. The address bar shows a local IP address: 172.23.88.193. The page title is "BIO325_CRISPR_Yap". The top right corner shows a user login: "jluethi (logout)".

The main interface displays a progress bar for the "Image analysis pipeline engine". The progress bar is divided into five steps: "upload", "image conversion", "image preprocessing", "pyramid creation", and "image analysis". The "image analysis" step is currently selected and highlighted in blue. To the right of the progress bar, the status "TERMINATED" is displayed with a green progress bar.

Below the progress bar, the "Image analysis pipeline engine" section is visible. It contains two main argument sections:

- Batch arguments**: Arguments that control how data is partitioned into computational jobs. The "batch_size" field is set to 10.
- Submission arguments**: Arguments that control how computational jobs are submitted to the cluster. The "cores" field is set to 1, and the "duration" field is set to 06:00:00.

A red arrow points to the "Edit Pipeline" button, which is located below the "Batch arguments" section.

At the bottom of the interface, there are several buttons: "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 ☒


Pipeline

Define the **modules** and be processed:

 smooth_DAPI

 threshold_otsu

 filter_nuclei

 separate_clumps

 label_nuclei

 register_objects

 segment_secondar

 register_objects_C

Resubmission

- ```
* Batch Size 15
* Resubmit from image_analysis
```

