Cell-ACDC

A **GUI**-based framework for **segmentation**, **tracking** and **cell cycle annotations** of microscopy imaging data. It includes two of the latest deep learning methods, Cellpose and YeaZ.

Cell-ACDC can load **2D**, **3D** (either single z-stacks or 2D images over time) and **4D** (3D z-stacks over time) images.

Written in Python 3 by Francesco Padovani and Benedikt Mairhoermann.

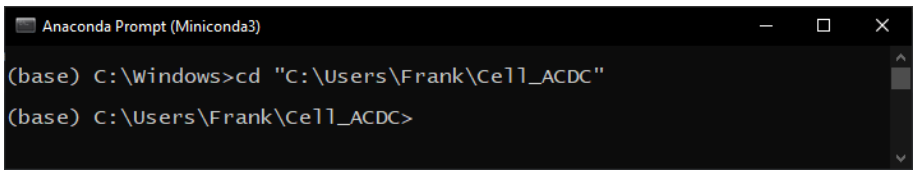
*Tested on Windows 10 64 bit, macOS , and Linux Mint 20.1

Installation

1. Download the latest release from here.
2. If you don't already have Python or Anaconda, download, and install Miniconda for Python 3.8 here. We recommend using Anaconda even if you already have Python.
3. Follow the instructions below specific to your OS

Installing on Windows using conda

1. Unzip the latest release you downloaded before. For this example, I will assume it was unzipped into `C:\Users\Frank`
2. Open the Anaconda Prompt (you should be able to find it from the search bar)
3. Navigate to the folder where you unzipped Cell_ACDC, (in this example it is `C:\Users\Frank\Cell-ACDC`) by typing `cd "C:\Users\Frank\Cell_ACDC"` Press "Enter" to confirm. Note that if you unzipped into a drive different from `C:\` you first need to change the drive letter in your terminal. To do so, type the letter first (e.g., `G:`) and then you can navigate with the `cd` command.



4. Now type the following commands **one at the time** (press "Enter" after each command and type "Y" when requested):

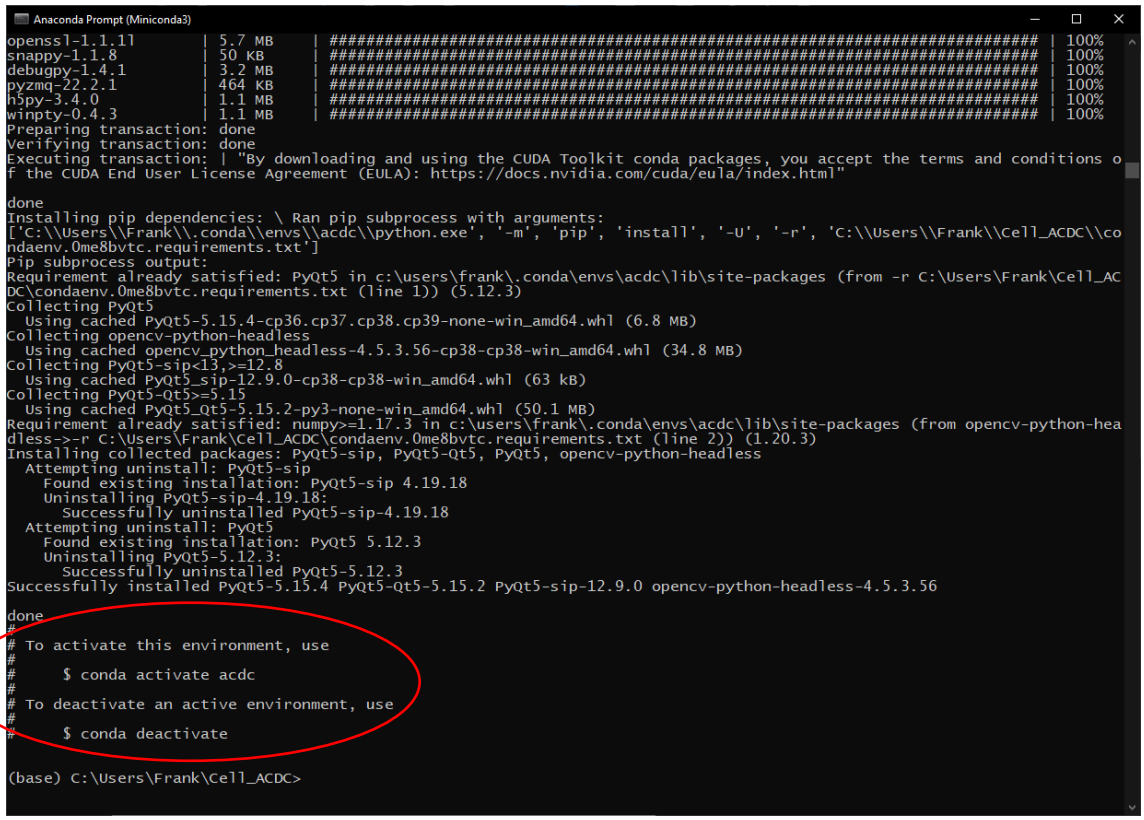
```
conda update -n base -c defaults conda
```

```
conda clean --all
```

```
conda env create --file acdc.yml
```

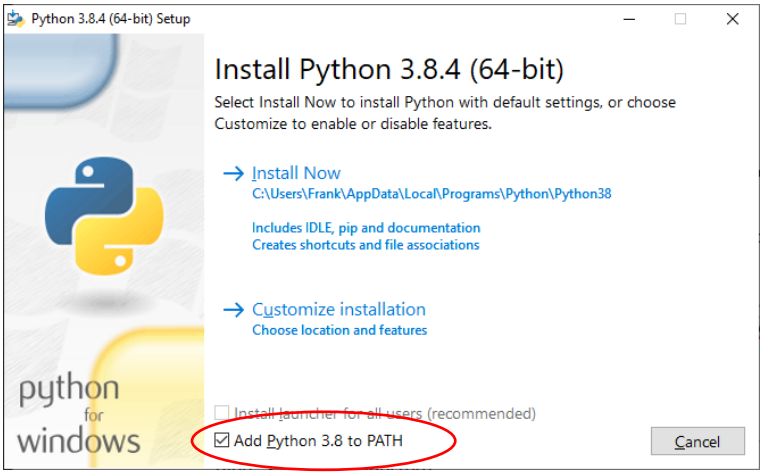
Anaconda will create the environment with Python 3.8 and all the packages required. This step can take several minutes (about 20 minutes if I have to guess, but it depends on your internet connection speed).

If successful, your terminal should now look like the screenshot below (red circle around the part that will tell you that the installation was successful). If you had an error, you could try installing using pip (see instructions below) or open an issue here.



Installing on Windows using pip

1. Download and install Python 3.8.4 from here. **Make sure to check the option** Add Python 3.8 to PATH and then install with default options.



2. Unzip the latest release you downloaded before. For this example, I will assume it was unzipped into `C:\Users\Frank`
3. Open a terminal (either a Command Prompt or PowerShell, you can find both from the search bar)
4. Navigate to the folder where you unzipped Cell_ACDC, (in this example it is `C:\Users\Frank\Cell_ACDC`) by typing `cd "C:\Users\Frank\Cell_ACDC"` Press *Enter* to confirm. Note that if you unzipped into a drive different from `C:\` tou first need to change the drive letter in your terminal. To do so type the letter first (e.g., `G:`) and then you can navigate with the `cd` command.
5. Now type the following commands **one at the time** (press "Enter" after each command and type "Y" when requested):

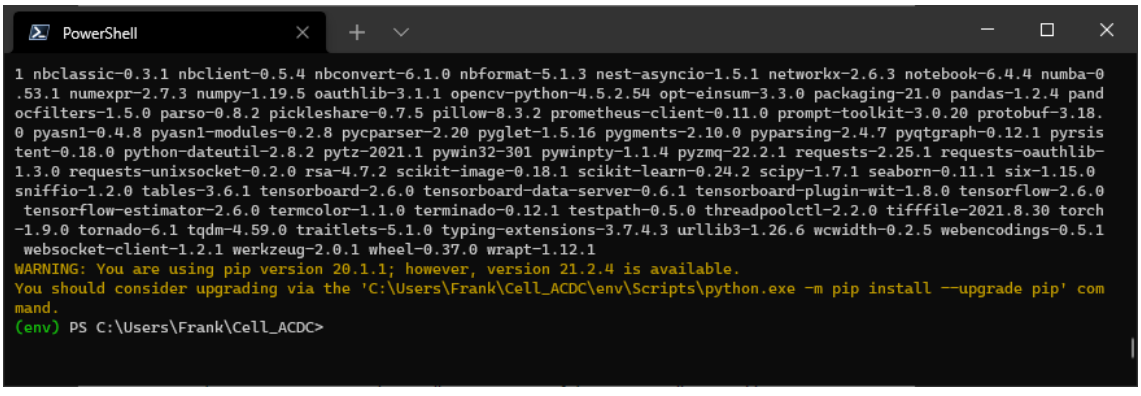
```
py -m pip install --upgrade pip
```

```
py -m venv env
```

```
.\env\Scripts\activate
```

```
py -m pip install -r requirements.txt
```

You will now see all the required packages being installed. If successful, your terminal should look like the screenshot below (you can ignore that warning). If you had an error, you could try installing using Anaconda (see instructions above) or open an issue here.



Installing on macOS using conda

1. Unzip the latest release you downloaded before. For this example, I will assume it was unzipped into `SCREENSHOTPATH`
2. Open a **Terminal** (Click the Launchpad icon in the Dock, type “Terminal” in the search field, then click Terminal)
3. Navigate to the folder where you unzipped Cell_ACDC, (in this example it is `SCREENSHOTPATH/Cell_ACDC`) by typing `cd “SCREENSHOTPATH/Cell_ACDC”` Press “Enter” to confirm.

SCREENSHOT

4. Now type the following commands **one at the time** (press “Enter” after each command and type “Y” when requested):

   ```
   conda update -n base -c defaults conda
   ```

   ```
   conda clean --all
   ```

   ```
   conda env create --file acdc.yml
   ```

   Anaconda will create the environment with Python 3.8 and all the packages required. This step can take several minutes (about 20 minutes if I have to guess, but it depends on your internet connection speed).

   If successful, your terminal should now look like the screenshot below (red circle around the part that will tell you that the installation was successful). If you had an error, you could try installing using pip (see instructions below) or open an issue here.

SCREENSHOT

Installing on macOS using pip

1. Download and install Python 3.8.5 from here. Install with default options.
2. Unzip the latest release you downloaded before. For this example, I will assume it was unzipped into `/Users/anikavanessaseel/Documents/GitHub/Cell_ACDC`
3. Open a **Terminal** (Click the Launchpad icon in the Dock, type "Terminal" in the search field, then click Terminal)
4. Navigate to the folder where you unzipped Cell_ACDC, (in this example it is `/Users/anikavanessaseel/Documents/GitHub`) by typing `cd "/Users/anikavanessaseel/Documents/GitHub/Cell_ACDC"` Press *Enter* to confirm.
5. Now type the following commands **one at the time** (press "Enter" after each command and type "Y" when requested):

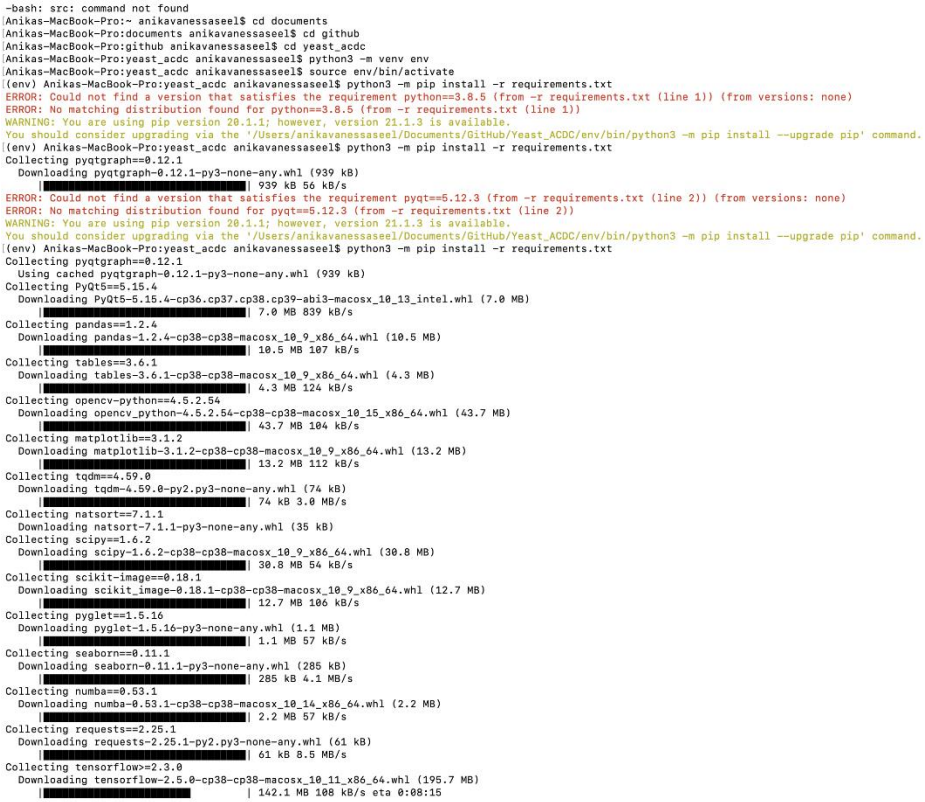
   ```
   python3 -m pip install --user --upgrade pip
   ```

   ```
   python3 -m venv env
   ```

   ```
   source env/bin/activate
   ```

   ```
   python3 -m pip install -r requirements.txt
   ```

   You will now see all the required packages being installed. If successful, your terminal should look like the screenshot below. If you had an error, you could try installing using Anaconda (see instructions above) or open an issue here.



Installing on Linux using conda

1. Open a **Terminal**
2. Unzip the latest release you downloaded before. For this example, I will assume it was unzipped into `/home/elpado/GitHub/`
3. Navigate to the folder where you unzipped Cell_ACDC, (in this example it is `/home/elpado/GitHub/`) by typing `cd "/home/elpado/GitHub/Cell_ACDC"`
4. Now type the following commands **one at the time** (press "Enter" after each command and type "Y" when requested):

   ```
   conda update -n base -c defaults conda
   ```

   ```
   conda clean --all
   ```

   ```
   conda env create --file acdc.yml
   ```

   Anaconda will create the environment with Python 3.8 and all the packages required. This step can take several minutes (about 20 minutes if I have to guess, but it depends on your internet connection speed).

   If successful, your terminal should now look like the screenshot below (red circle around the part that will tell you that the installation was successful). If you had an error, you could try installing using pip (see instructions below) or open an issue here.

Installing on Linux using pip

1. Open a Terminal
2. Make sure you have Python 3.8 and pip installed. Check if you have Python with `python –version` command and check if you have pip with `pip help` command. If you don't have them install with the following commands:

   ```
   sudo apt-get update
   ```

   ```
   sudo apt-get install python3.8
   ```

   ```
   sudo apt-get install python3-pip
   ```

3. Unzip the latest release you downloaded before. For this example, I will assume it was unzipped into `/home/elpado/GitHub/`
4. Navigate to the folder where you unzipped Cell_ACDC, (in this example it is `/home/elpado/GitHub/`) by typing `cd "/home/elpado/GitHub/Cell_ACDC"`
5. Now type the following commands **one at the time** (press "Enter" after each command and type "Y" when requested):

   ```
   python3 -m pip install --user --upgrade pip
   ```

   ```
   python3 -m venv env
   ```

   ```
   source env/bin/activate
   ```

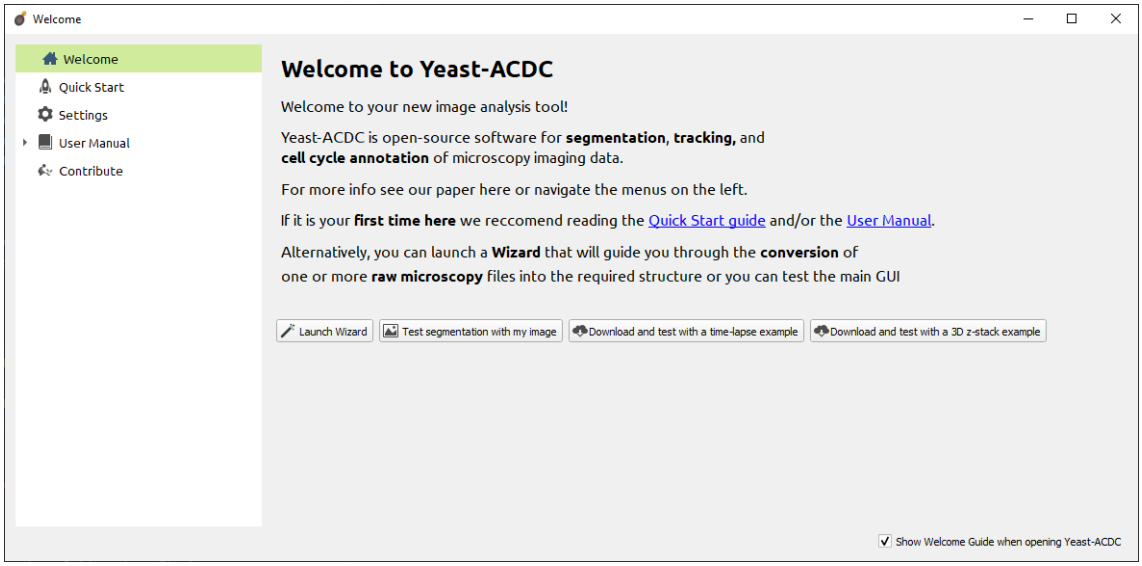
   ```
   python3 -m pip install -r requirements.txt
   ```

   You will now see all the required packages being installed. If you had an error, you could try installing using Anaconda (see instructions above) or open an issue here.

First steps

Starting the main launcher

1. Open a terminal:
    - Windows: **Anaconda Prompt** if you installed with conda
    - Windows: Command Prompt or **PowerShell** if you installed with pip
    - Unix/maxOS: **Terminal**
2. Navigate to the Cell-ACDC folder with the command `cd` like you did when you installed it.
3. **Activate** the environment:
    - Conda: `conda activate acdc`
    - pip on Windows: `.\env\Scripts\activate`
    - pip on Unix/macOS: `source env/bin/activate`
4. Run the main launcher:
    - Windows: `python main.py`
    - Unix/macOS: `python3 main.py`
5. If you get the error ImportError: No module named 'Tkinter' you need to install tkinter with the command `sudo apt-get install python3-tk`
6. The first time, it will take 1 or 2 minutes to launch. The next times it will be faster. Once launched, you should get the following Welcome Guide window.

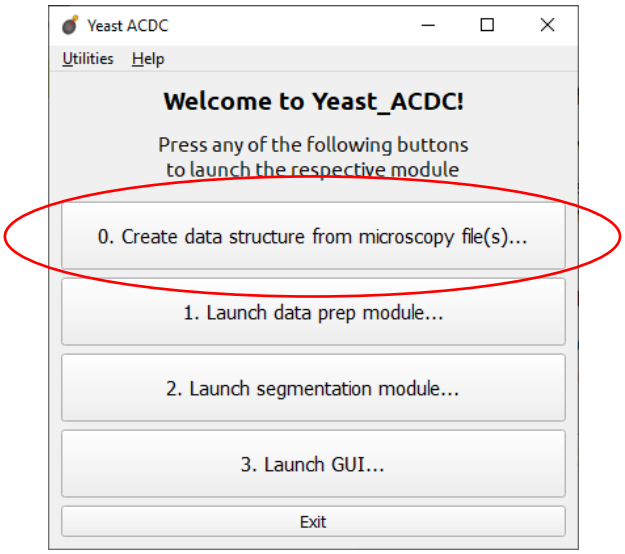


Load microscopy file

To load a microscopy file, Cell-ACDC uses the java library Bio-Formats and the Python library python-bioformats. The python-bioformats library was developed for CellProfiler and it is **embedded** into Cell-ACDC. It essentially allows you to run the java code from Bio-Formats from Python. Have a look here for a list of supported file formats.

To load a microscopy file into the Cell-ACDC pipeline, we first have to **convert it into a specific data structure**. We included a module that allows you to **automatically create the required data structure**. However, if it fails, you can create it manually with ImageJ/Fiji. Read the section of this manual called "Manually create data structure from microscopy file(s)".

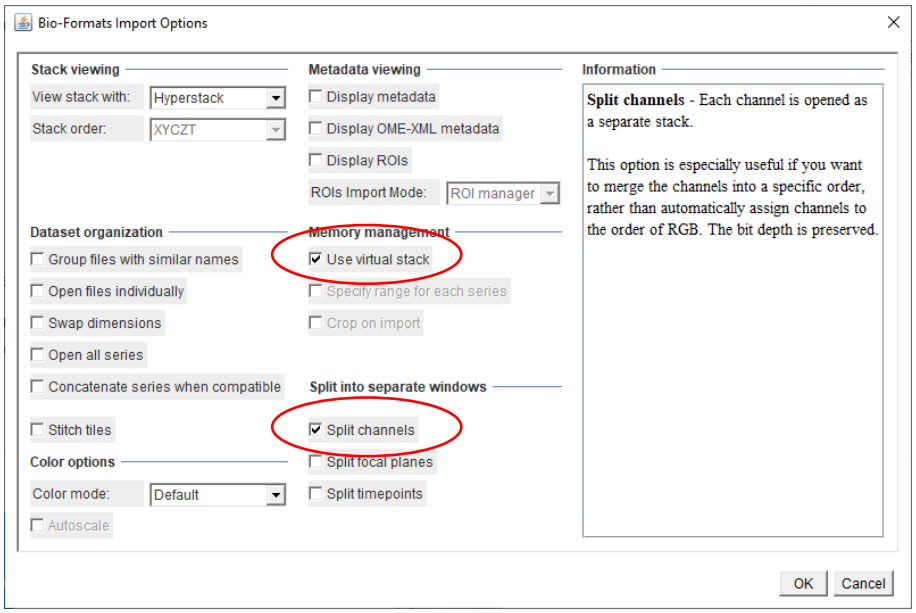
1. From the main launcher (could be behind Welcome Guide window) click on the "Create data structure from microscopy file(s)..." button and follow the instructions of the Wizard.



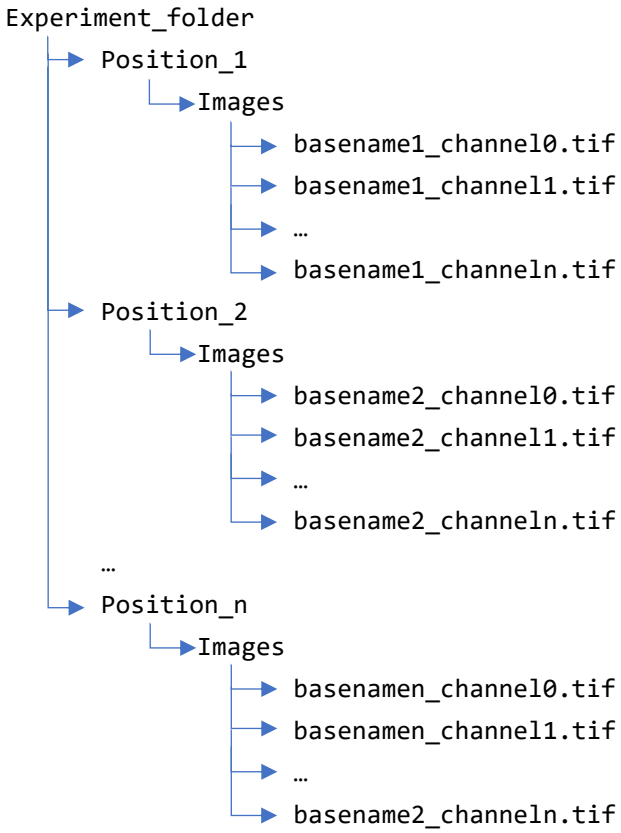
2. Once the creation of the data structure is finished, you are ready to start using your new labelling tool! The easiest way to start is from the Quick Start section of this User Manual.

Manually create data structure from microscopy file(s)

1. If you don't have it, download and install Fiji from here
2. Open the Fiji app and launch the Bio-Formats importer from the menu "Plugins → Bio-Formats → Bio-Formats Importer" and select your microscopy file (one at the time).
3. Check the options "Use virtual stack" and "Split channels" as in the screenshot below



4. If you have multiple positions (series) in the file you opened, you will be asked to select a position. We recommend opening one position at the time, to avoid memory issues.
5. You should now have one image window for each channel you had in the file. Select the window with the image data from the first channel (the window name should be something like "filename … C=0"), then "File → Save as… → Tiff"
6. As a filename we recommend calling it with the same name of the original microscopy file (if it is not too long) **WITHOUT the extension** plus something like "_channel0.tif", where instead of "channel0" you can write whatever you like (e.g., DAPI or GFP etc.). So for example the phase contrast channel of a .czi (Zeiss microscope) file called `ASY15-1_15nM-01.czi` can be save as `ASY15-1_15nM-01_phase_contr.tif`
7. Save the .tif file to a path called "/Position_1/Images".
8. Repeat 2-7 for all the other positions.
9. In the end you should have the following folder structure:



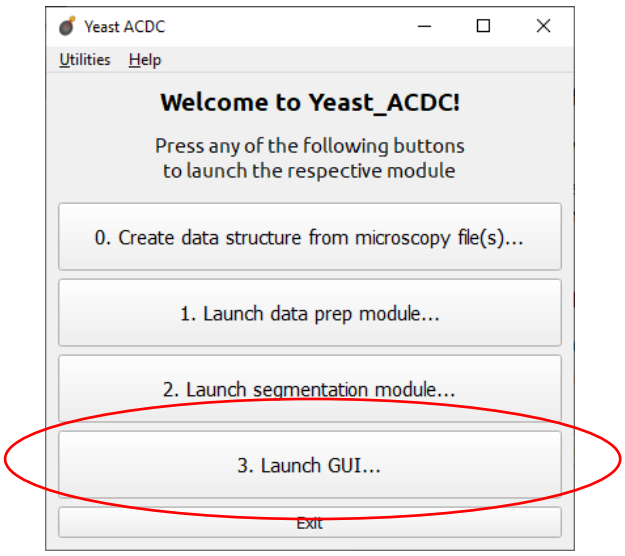
10. Once the creation of the data structure is finished, you are ready to start using your new labelling tool! The easiest way to start is from the Quick Start section of this User Manual.

Quick Start

Cell-ACDC is composed of **three main modules**:

- **Data prep**: align time-lapse data, crop, and select a z-slice or z-projection and a ROI for segmentation. More details…
- **Segmentation**: to automatically segment multiple experiments and multiple positions with the embedded deep learning models (YeaZ for yeast cells and Cellpose for various model organisms). More details…
- **Main GUI**: to visualize segmentation masks, correct segmentation and tracking errors, and cell cycle annotations. More details…

The easiest way to start is to **open the main GUI**. Next, if you already created the data structure (see Load microscopy file section) you can click on the "Open Folder" button on the toolbar, otherwise you can go to "File →Open image/video file…". To start the main GUI, click the "Launch GUI…" button on the main launcher.



Data Prep module

To use the data prep module, you need to **first create the required data structure.** See this section.

Use the **Data Prep** module if you need to do one of the following **tasks**:

a) Select a **z-slice** or **z-projection** for segmentation of 3D z-stacks.

b) **Align frames** of time-lapse microscopy data (RECOMMENDED, it is revertible).

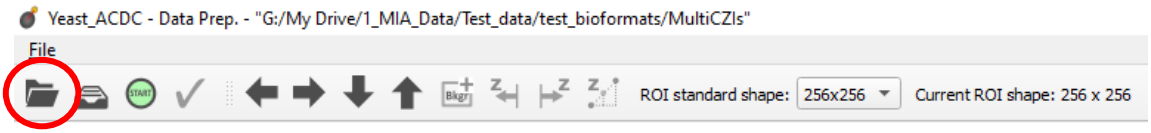
c) Calculate **background metrics** (median, mean etc.) from one or more **rectangular areas**. The median will be used later for background subtraction. The areas are movable and resizable.

d) Select a region of interest (**ROI**) for segmentation.

e) **Crop** images to reduce memory usage (RECOMMENDED, if possible).

Loading data

1. **Launch** the data prep module, click on the "1. Launch data prep module…" button on the main launcher.
2. Click on the "**Open Folder**" button on the toolbar.



3. Select a specific Position folder or the entire experiment folder.
4. Follow the instructions in the pop-up windows. Make sure to enter the **correct metadata.**

   NOTE: For time-lapse microscopy you can load only one position at a time. Select multiple positions only if you have single 3D z-stacks or single 2D images.

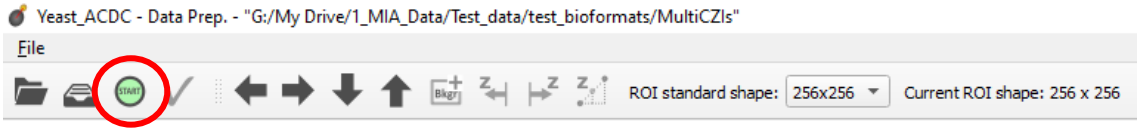
Usage

1. If your data does not contain 3D z-stacks go to point 2. Otherwise, you can visualize the **z-slices** with the **scrollbar** below the image or choose a **z-projection** method with the selector on the right side of the scrollbar.

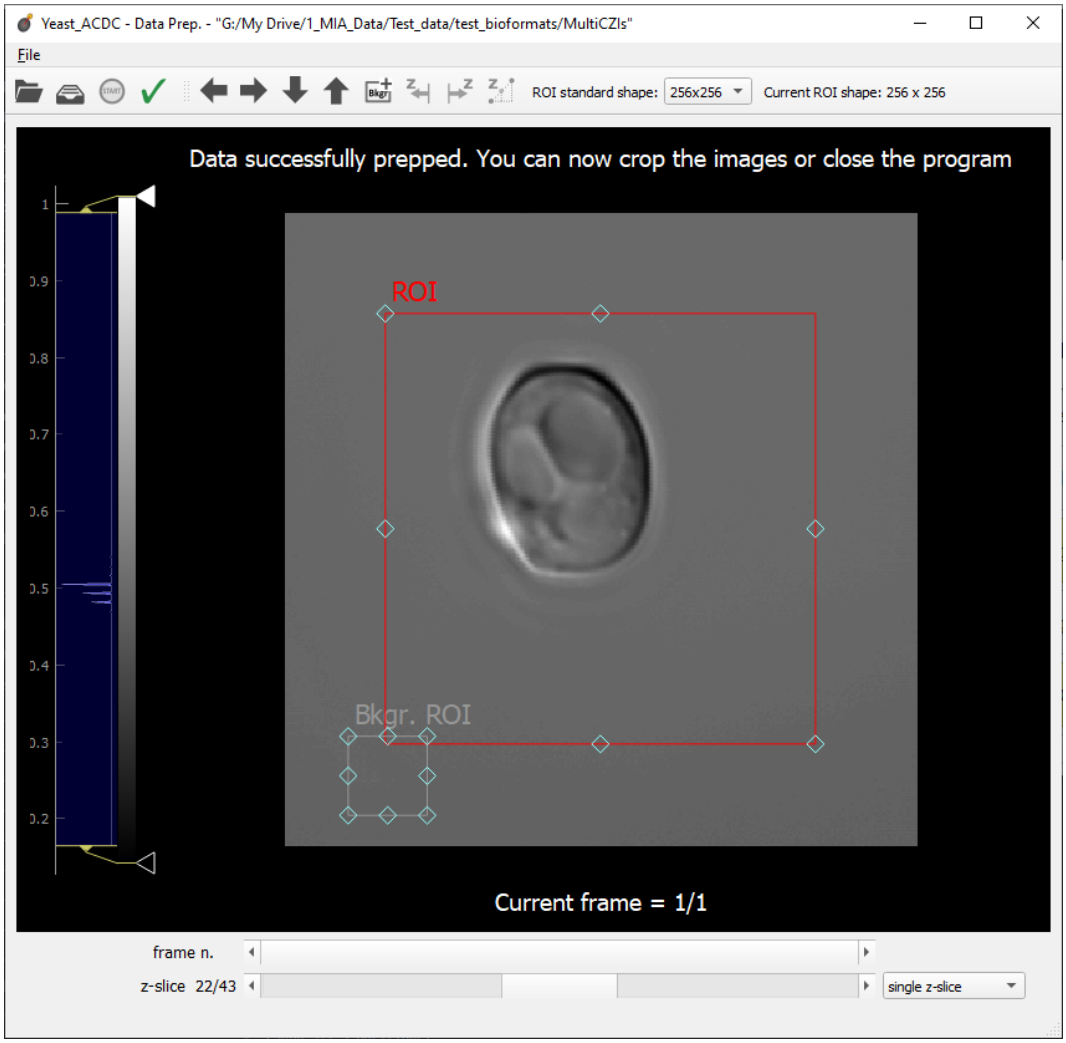
   Every time you change the visualization method, the system will save it. It will then assume that the **last visualization is the preferred one** and it will be used for **segmentation**.

   For **time-lapse data** you have **additional buttons** to help with the selection. Go to the section "Additional functions" for details about their functionality.

2. If you do not need to select a ROI, crop, align or calculate background metrics you can close the window. Otherwise press the "**Start**" button on the toolbar and follow the instructions on the pop-up windows.



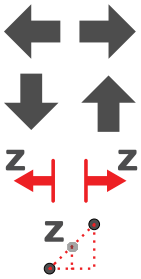
3. The GUI now will be **unresponsive** until the process terminates, so do not close it. You can follow **progress** in the **terminal**. Once it finishes, a **red rectangle** will appear, along with a grey rectangle (see screenshot below). If you do not need to select a **ROI,** calculate **background metrics,** or crop you can close the window now, otherwise go to the next point.



4. The **red ROI** is used for either **cropping** or saving the coordinates where to compute **segmentation**. The **grey ROI** (Bkgr. ROI) is used to calculate **background metrics** from that area (median, mean, quantiles etc.). You can add more background ROIs with the "Add ROI where to calculate background intensity" (Bkgr) button on the toolbar.

5. **Resize** and **move** the ROIs until you are happy with their position and size, click on the **green tick** button on the toolbar, then follow the instructions in the pop-up windows. The GUI will be **unresponsive** until the process terminates, so **do not close it**. You can check progress in the terminal.

Additional functions

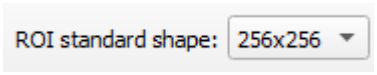


Go to **previous/next** position or frame (time-point).

Go **10** positions or frames **backward/forward.**

Use the same **z-slice** from current frame to all past/future frames.

Use **linearly interpolated z-slices** from first frame to current frame.



Select one of the **standard shapes** for the red ROI.

Segmentation module

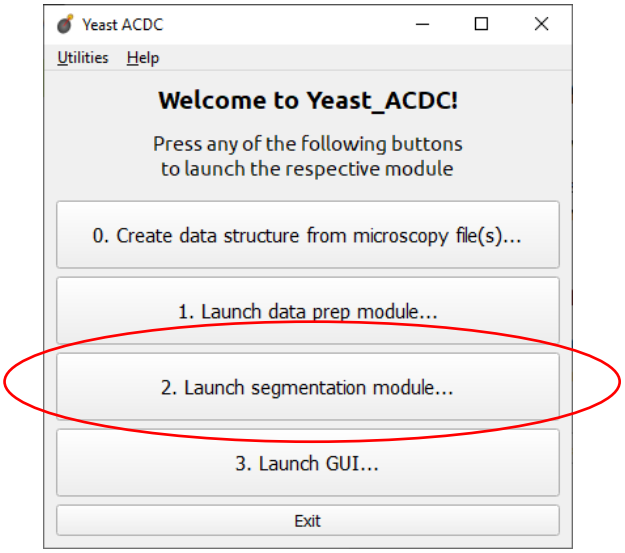
The segmentation module is used for **automatically segmenting multiple experiments and multiple positions** in one session.

To use the segmentation module, you need to **first create the required data structure.** See this section.

NOTE: if you are just testing, you can also segment in the main GUI. Use this module when you need to segment many experiments and/or many positions.

Usage

To use this module, simply follow the **instructions** in the **pop-up windows**. To **launch** the module, click on the "2. Launch segmentation module…" button on the main launcher.



Main GUI

The main GUI is the actual **core of Cell-ACDC**. It serves multiple purposes:

a) **Test** which **segmentation method** works best for your dataset.

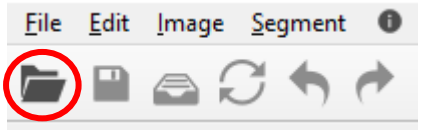
b) **Correct** segmentation and tracking errors.

c) Cell cycle **annotations.**

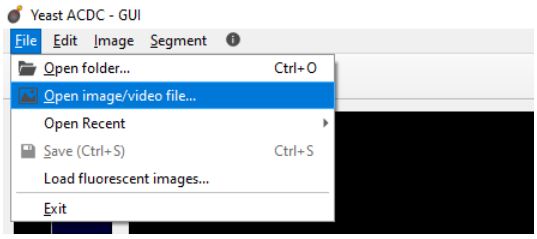
As for all the other modules, you can load **2D**, **3D** (either single z-stacks or 2D images over time) and **4D** (3D z-stacks over time) images or videos.

Loading data

1. **Launch** the GUI module by clicking on the `3. Launch GUI...` button on the main launcher.
2. Depending on the data structure, do one of the following actions:
    - If you already created the **data structure** following the instructions in this section (recommended) then click on the Open Folder button.



    - If you have a single image (.tif, .png, .jpg, etc.) or video (.mov, .avi) go to `File → Open image/video file...`



3. Select a specific file, a Position folder, or the entire experiment folder.
4. Follow the instructions in the pop-up windows. Make sure to enter the **correct metadata.**

*NOTE: if you load a **single image** or **video** file **without the required data structure,** the Cell-ACDC output will be saved in a sub-folder called* `<timestamp>_acdc_output`

Usage with time-lapse data

For **time-lapse data**, you can load one position (one video) at a time. With this data, the GUI has **three modes** that can be toggled from the selector on the toolbar:

a) `Viewer` mode (default mode, used only for visualisation).
b) `Cell cycle analysis` mode.
c) `Segmentation and tracking` mode.

The **main idea** is that when you visit a frame for the first time, some automatic functions are triggered: **tracking** in `Segmentation and tracking` mode, **mother-bud pairing** in `Cell cycle analysis` mode.

These functions are **not triggered** when you visualize a frame that you **already visited before**. You can always call any function manually (see this, this, or this section of the manual).

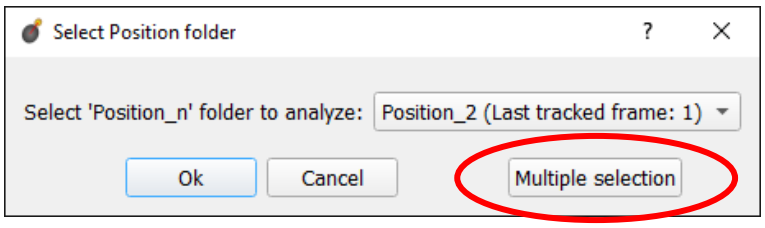
Give a **quick read** to

Tips and tricks and then **start using the GUI**.

If you are unsure about the function of any item in the GUI, see this, this, or this section of the manual.

Usage with snapshot data (no time-lapse)

For **snapshot data**, you can load **multiple positions** at the same time. When prompted, simply click on multiple selection button, and then select the positions with `Ctrl+click` for selecting specific positions, or `Shift+click` to select a range, or `Ctrl+A` to select all.



Once loaded, you can **navigate** through positions with **left and right arrow** or with the Position **scrollbar** below the left image.

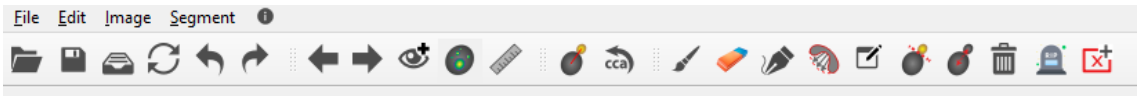
Give a **quick read** to

Tips and tricks and then **start using the GUI**.

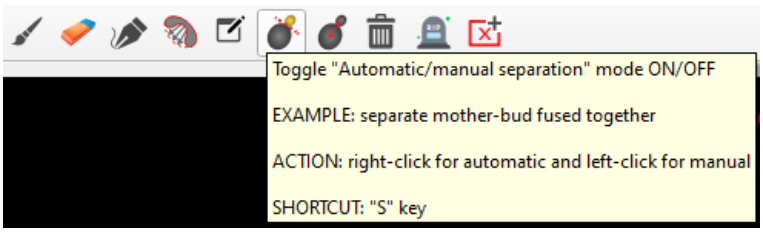
If you are unsure about the function of any item in the GUI, see this, this, or this section of the manual.

Tips and tricks

- Most of the **functions** are available from the **toolbar** on the top of the window:



- Activate the function with a ***SINGLE-click*** on the button
- When you **hover a button** with the mouse cursor you get a **tool tip** on how to use that function:



- The **tool tip** will tell you whether you need ***RIGHT-click*** or ***LEFT-click*** for that function
- Functions **NOT** present on the **toolbar**:
  - *Middle-click* (scrolling wheel) → delete the segmented object you click on
  - `H` key → automatic zoom on the segmented objects
  - `Ctrl+P` → visualize cell cycle annotations in a table
  - `L` key → relabel object IDs sequentially (1,2,3...etc)
- To **test** the available **segmentation models,** use the `Segment` menu.
- To **navigate frames** (time-lapse data) or **positions** (snapshots data), use the `←→` **arrows** on the keyboard.
- To visualize the frames of time-lapse data in a second window click on the Slideshow button on the toolbar: 
- **Personalize** settings such as **Font Size**, **overlay colour** and **text's colour** from the `Edit` menu.

Functions activated from the toolbar

File toolbar

Open folder: used to **automatically load** single or multiple positions from the standard data structure. See this section for details on how to create the required data structure**.**

Save: used to save **all data** (segmentation mask, cell cycle annotations and metrics calculated from the loaded fluorescent images).

Show in Explorer/Finder: open the loaded folder into your Explorer/Finder.

Reload segmentation file: used to reload the segmentation labels from the hard drive. Use it if you want to go back to the saved state.

Undo (*Ctrl+Z*): almost all the performed actions are undoable. Currently you can undo up to 5 actions in the past.

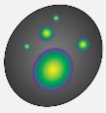
Redo (*Ctrl+Y*): repeat an undone action.

Visualize toolbar

Go to previous frame (time-lapse data) or Position (snapshot data).
SHORTCUT: Left arrow on the keyboard

Go to next frame (time-lapse data) or Position (snapshot data).
SHORTCUT: Right arrow on the keyboard

Open the images in a **second window.** This window will have no annotations, which means it is the fastest way possible to visualize the frames. Handy in many situations.



Overlay a second signal: when you press this button for the first time, you will be asked to choose which signal to overlay. The next times it is used to toggle **overlay on/off**.

Ruler: draw a line with the left button to **measure the distance** between two points (*pixels* and *μm*). Distance will be displayed in the bottom-left corner of the window.

Edit toolbar

Brush (*left-click motion*)**:** used to modify a segmented object or paint a new object with a circular brush. Increase/decrease the size of the brush with up/down arrows on the keyboard. *SHORTCUT: B key*

Two **modes**:

- To **draw a new object** start painting on the background (brush cursor is white).
- **Modify an object** by starting to paint from the object (brush cursors takes the color of the object you are about to modify)

NOTE: The brush will add the new object UNDER existing objects unless you first press the B key twice. The brush button will then turn red and new objects will be added ABOVE existing objects. Restore default behaviour by pressing B twice again.

Eraser (*right-click motion*): used to erase parts of a segmented object with a circular cursor. *SHORTCUT: X key*

Two **modes**:

- Start erasing from the background to erase **all the objects** you will pass over (eraser cursor is red).
- Start erasing from a specific object to erase ONLY that object (eraser cursor keeps the colour of the object you start erasing from).

NOTE: To enforce erasing any object you pass over, even if you start from a specific one, press the X key twice. The eraser button will then turn RED. Restore default behaviour by pressing X twice again.

Curvature tool: used to draw new objects by drawing a spline with multiple anchor points. *SHORTCUT: C key*

Two **modes**:

- Consecutive **left-clicks** to **manually** draw an object with a spline.
- **Right-click** with a drawing motion to **automatically** follow an intensity line.

Hull contour: replace object with its hull contour image. Useful to **fill holes** and **cracks**. *SHORTCUT: F key*

Usage: first *activate the button* (left-click or shortcut) and then *RIGHT-click* on the requested object.

Edit ID: replace the ID of an object with a manually inserted one. *SHORTCUT: N key*

Usage: first *activate the button* (left-click or shortcut) and then *RIGHT-click* on the requested object.

Separate objects: used to separate merged objects. *SHORTCUT: S key*

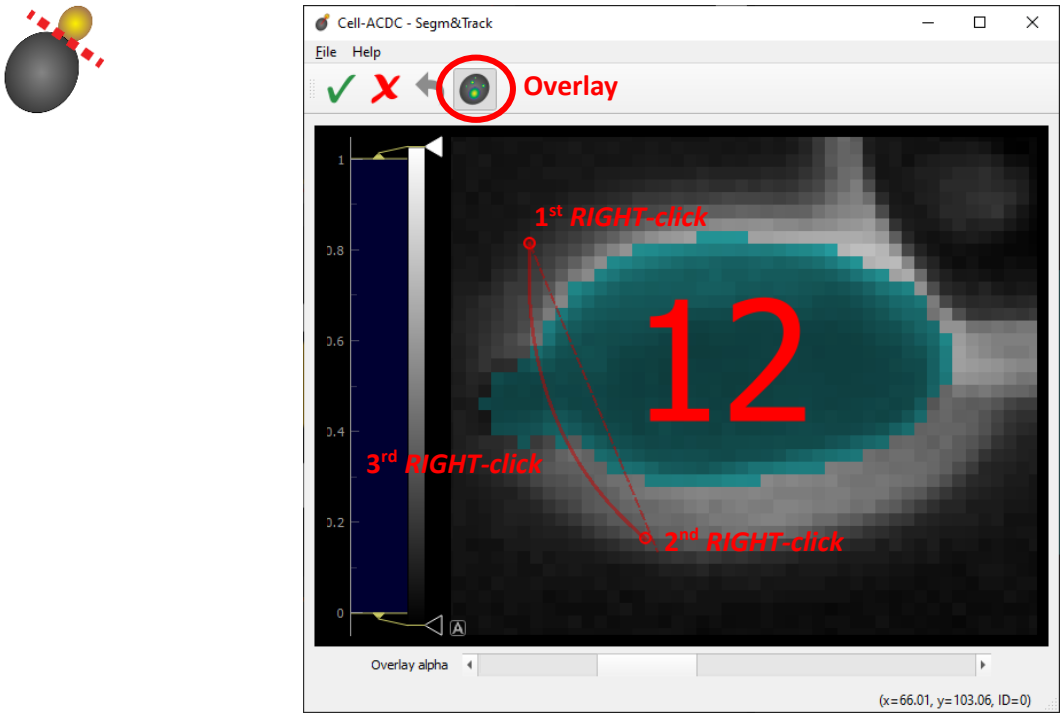
Usage: first *activate the button* (left-click or shortcut) and then *RIGHT-click* on the requested object to first attempt **automatic separation** (works well with two objects separated by a constriction) or *LEFT-click* to go straight to **manual separation**.

When **manual separation** is triggered, a window with only the object you clicked one will appear (see screenshot below). To separate the object along a curved line, you need **three *RIGHT clicks*:**

1. First *RIGHT-click* where to **start** a straight **line**.
2. Second *RIGHT-click* where to **end** the straight **line**.
3. Third *RIGHT-click* to **set the curvature** of the separating curve.

The object will then be **separated** along the drawn line.

*NOTE: to **help** with deciding where to draw the line you can **overlay** the intensity signal with the overlay button*



Merge objects. *SHORTCUT: M key*

Usage: first *activate the button* (left-click or shortcut) and then *drag-and-drop* motion with *RIGHT-button* between the two objects.

Annotate cell as "Excluded from the analysis": the column called `is_cell_excluded` (on the saved data, see this section) will have a TRUE for the clicked object from current frame to the end. *SHORTCUT: R key*

Usage: first *activate the button* (left-click or shortcut) and then *RIGHT-click* on the requested object. Repeat to undo from that frame onwards.

Annotate cell as "Dead": the column called `is_cell_dead` (on the saved data, see this section) will have a TRUE for the clicked object from current frame to the end. *SHORTCUT: R key*

Usage: first *activate the button* (left-click or shortcut) and then *RIGHT-click* on the requested object. Repeat to undo from that frame onwards.

Add a delROI: used to add a rectangular area to automatically delete all objects contained in or touched by it.

Usage: click on the button to add a ROI. **Resize and move** the ROI to either **delete** objects or **restore** objects when they are not contained/touched anymore. Delete the ROI with right-click on it → Remove ROI.

*NOTE: Objects are **permanently deleted** only when you save AND close the GUI.*

Repeat tracking: used to repeat tracking on the current frame.

*Note: The tracking in the GUI is **different** from the tracking of the segmentation module. In the segmentation module we kept the algorithm proposed by the YeaZ model.*

Cell cycle annotations toolbar

Assign bud/sister to **mother/sister**. *SHORTCUT: A key*

Usage: first *activate the button* (left-click or shortcut) and then *drag-and-drop* motion with *RIGHT-button.* Right-click on bud and release on mother, or **right-click on bud** then **right-click on mother**.

Annotate that a cell does **not** have a **fully known history.** *SHORTCUT: U key*

Usage: first *activate the button* (left-click or shortcut) and then *RIGHT-click* on the requested object.

***Tip**: two examples of cells with uknown history are cells already present at the first frame and cells appearing from outside the field of view*

Reinitialize cell cycle annotations to default. Default is all cells in **G1** without a relative assigned to it.

Functions activated from the menus

File → Load fluorescent images…

Used to **load** as many **additional images** (e.g., fluorescence signal) as you want.

Loaded images will be used to calculate metrics such as mean, median, total amount etc. See this section for more details.

Edit → Smart handling of enabling/disabling tracking

The GUI has built-in **automatic tracking** for **time-lapse data** with the following behaviour:

- If tracking is active (Disable tracking checkbox on the toolbar is UNCHECKED) when you visit a frame that you have **never visited before**, objects will be **automatically tracked** compared to previous frame.
- When you visit a frame **already visited** before, it will **not** be **tracked**.

You can disable this automatic behaviour by unchecking Smart handling of enabling/disabling tracking

When you **disable** the **smart** handling, you can **enforce tracking** on all visited frames no matter if they were previously visited or not. To enforce, use the Disable tracking checkbox on the toolbar.

***Tip**: useful when you know you have to repeat tracking on already visited frames.*

Image → Normalise intensities → …

You can choose to **normalise the intensities** of the displayed images (saved data will not be modified) with the following methods:

- Do not normalise. Display **raw image**.
- Convert to **floating point** format with values [0, 1] → simply convert to floating point, no normalisation involved.
- **Rescale** to [0,1] → intensities are first converted to floating point if needed and then STRETCHED to convert the entire [0,1] range.
- **Normalize by max** value: divide by the max of the intensities.

Additional functions

- *Middle-click* (scrolling wheel) → delete the segmented object you click on.
- `H` key → automatic zoom on the segmented objects.
- `Ctrl+P` → visualize cell cycle annotations in a table.
- `L` key → relabel object IDs sequentially (1,2,3...etc).

Cell-ACDC output data