ONMD Onboard User Manual

Configure the microscope

OS installation and configuration :
- Download the OS img from openflexure website and follow their instructions

Use directly on the Raspberry Pi :
- No specific configuration needed
- You can use a touch screen, an external screen via HDMI, mouse/keyboard via USB…

Use over network connection with RDP :
- Connect the raspberry to network (if not already done)
- Activate SSH in the raspberry parameters
- Install xrdp via this script : sudo apt install -y xrdp

Install the extension

Copy the file "nanomotion_extension.py" to the following folder on the microscope :
/var/openflexure/extensions/microscope_extensions

- If the **magnification** of your optic is **different than 40x** :
  - Open the file "nanomotion_extension.py"
  - Search for "OPTIC_MAGNIFICATION = 40"
  - Change the "40" to your magnification (example : "OPTIC_MAGNIFICATION = 100")
  - Save and update the file on the microscope

Install the required libraries (if not already installed) **in the right python environment**:

- Set the **python environment** :
  - *source /var/openflexure/application/openflexure-microscope-server/.venv/bin/activate*
- **Opencv** :
  - *sudo apt install build-essential cmake git libgtk-3-dev libavcodec-dev libavformat-dev libswscale-dev*
  - *pip install opencv-python (there will be errors at the end that you can ignore)*
- **matplotlib**
  - *pip install matplotlib*
- **ffmpeg**
  - *sudo apt install -y ffmpeg*
  - *sudo chown -R $(whoami) /var/openflexure/application/openflexure-microscope-server/.venv*
  - *pip install ffmpeg-python*

Record videos

- Set the position (with the "Navigate" panel or by double-clicking on the stream, see Figure 1 "Navigate" panel)
- Do a correct focus with fast autofocus or manually (no need to be perfect, the focus will be done precisely when recording videos)
- Go to the "Base_extension" panel (see Figure 2 "Base_extension" panel)
- Set your parameters:

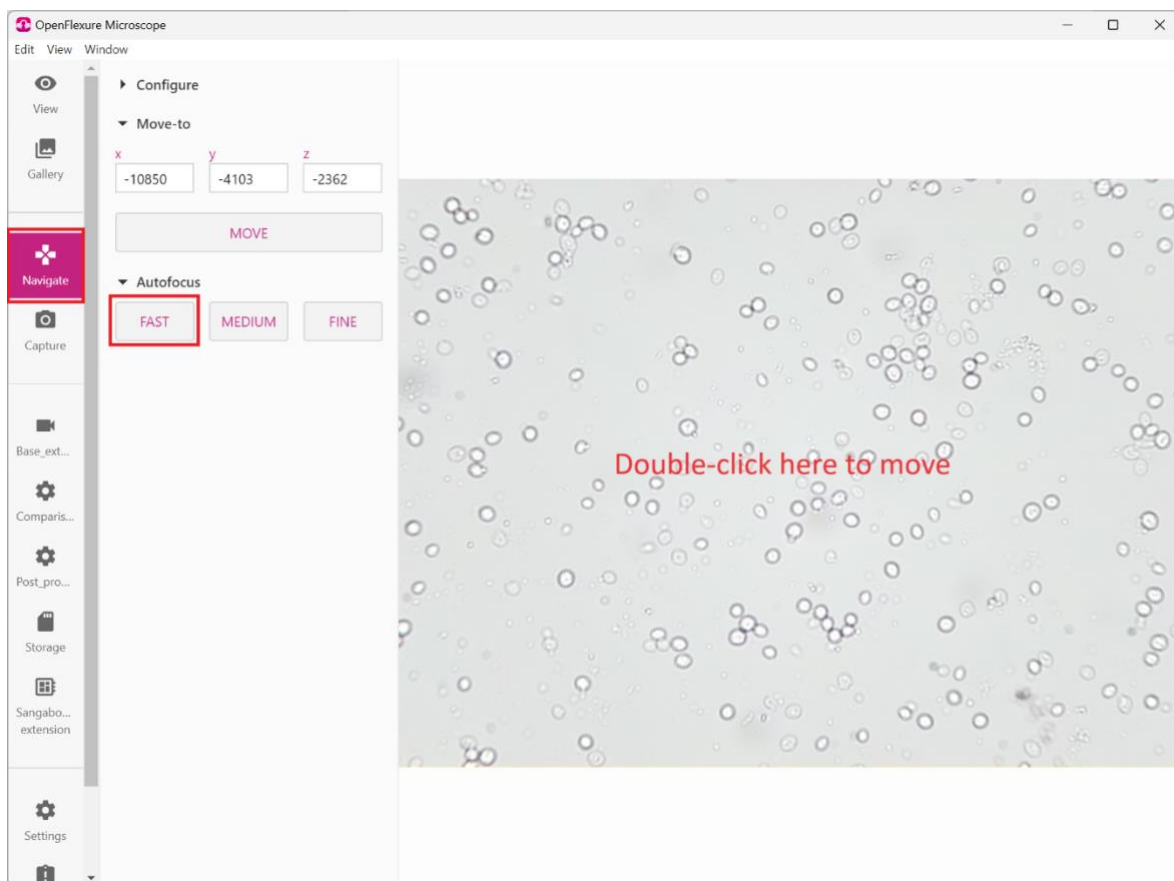| Parameter	Comments
Length (seconds) of video	
Displacement in x/y axis	In pixels.
Number of displacements in x/y axis	- For only one video, set both to 0 - Example: if x = 1 and y = 2 => 6 videos will be recorded
Folder name	It will be created in the defaut openflexure directory for records: **/var/openflexure/data/micrographs**
Video definition	For 480p, only video recording works, not the analysis
Cell type	Use "Universal" by default. "Bakers yeast" can be use only with bakers yeast, and should be used if you want a deep video processing (explained after).
Minimum number of cells	If less cells are detected, the stage will move further until enough cells are detected.
Video processing	Check if you want to directly process the video after recording. Do not check if you want to process it later or with another program. Warning: processing videos can that several minutes depending on the parameters you set. Count about 2mn for one 10s video.
Deep video processing	**Works only with Baker's yeast (and cell type defined accordingly)**. In addition of standard processing with the diff picture, it will analyze individual cells movements. Warning: deep video processing takes a lot of time. It may be easier to first record all videos and then process them.
MP4 video	By default, videos are only saved in the H264 format. Check if you want to save it in MP4 too.

- Click on "START RECORDING"
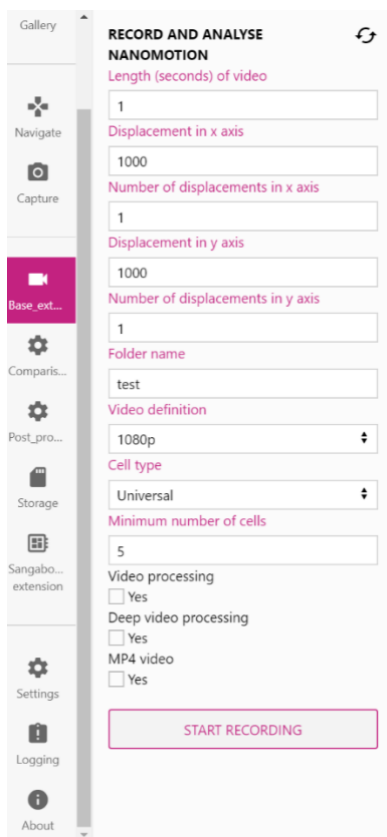
Figure 1 "Navigate" panel

Figure 2 "Base_extension"
panel

Analyse videos

- Go to the "post_process_extension" panel

- Set your parameters:

Parameter	Comments
Path	If you have only one folder: path to the folder (the folder that contains subfolders 1_1, 1_2…)
	If you have multiple folders: path to the parent folder (the folder that contains subfolders which contains 1_1, 1_2…)
	See below for an example
Analyse subfolders	If you have only one folder: do not check
	If you have multiple folders: check, it will analyze every subfolders one by one
	See below for an example
Cell type	Use "Universal" by default. "Bakers yeast" can be use **only with bakers yeast**, and should be used if you want a deep video processing (explained after).
Deep video processing	**Works only with Baker's yeast (and cell type defined accordingly)**. In addition of standard processing with the diff picture, it will analyze individual cells movements.

- Click on "START ANALYSIS"

- USEFUL TIP: If, for any reason, you want to reanalyze a video, delete only the file "result.txt" (and/or "deep_processing_result.txt") in the folder. If this file isn't present, the software will consider that the analysis isn't already done, and it will do it (and overwrite every old files)

ANALYSE VIDEOS

Path

/var/openflexure/data/micrographs/

Analyse subfolders

☐ Yes

Cell type

| Universal | ⬍ |

Deep video processing

☐ Yes

START ANALYSIS

View
Gallery
Navigate
Capture
Base_ext...
Comparis...
Post_pro...
Storage
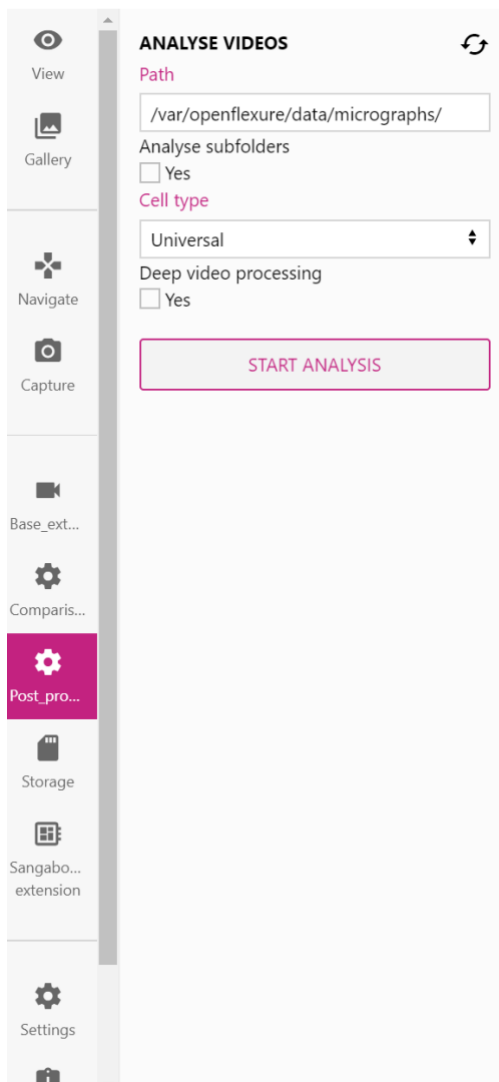Sangabo...
extension
Settings

Figure 3 "Post_process_extension" panel

Example for path parameter :

If we have the following hierarchy :
- Saccharomyces Cerevisiae
-- After10mn
--- 1_1
--- 1_2
-- After20mn
--- 1_1
--- 1_2
-- After30mn
--- 1_1
--- 1_2

If we want to analyse only "After10mn" :
- Path : "Saccharomyces Cerevisiae/After10mn"
- Analyse subfolders : No

If we want to analyse every subfolders in "Saccharomyces Cerevisiae", in this example "After10mn", "After20mn" and "After30mn" :
- Path : "Saccharomyces Cerevisiae"
- Analyse subfolders : Yes

Compare videos

How it works:
- This will always create a comparative plot of all video processing results available in the subfolders of the path folder (see Figure 5 Default plot)
- If at least one subfolder name contains "[…]alive[…]" or "[…]dead[…]", it will additionally create another comparative plot with only those subfolders, and only "Alive" or "Dead" labels (see Figure 4 Dead versus alive plot)

How to use it :
- Go to the "comparison_extension" panel
- Set your parameters:

Parameter	Comments
Path	

- Click on "COMPARE"

Figure 5 Default plot
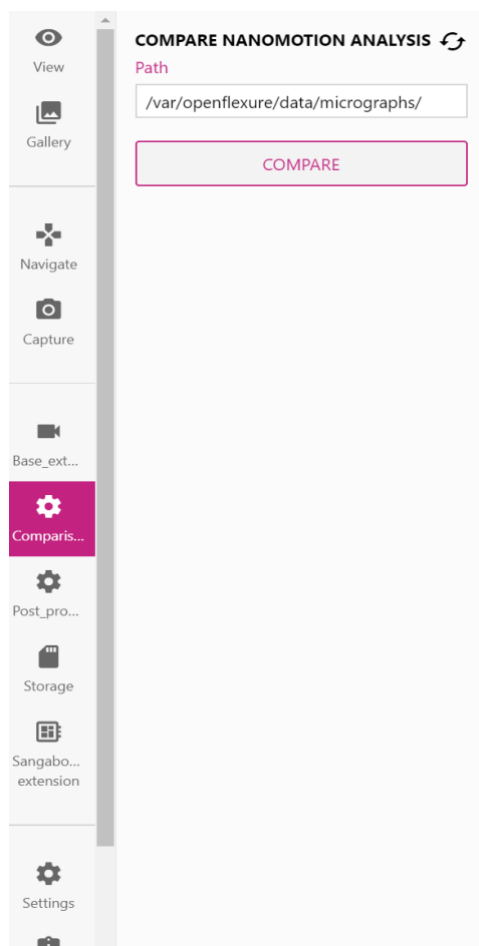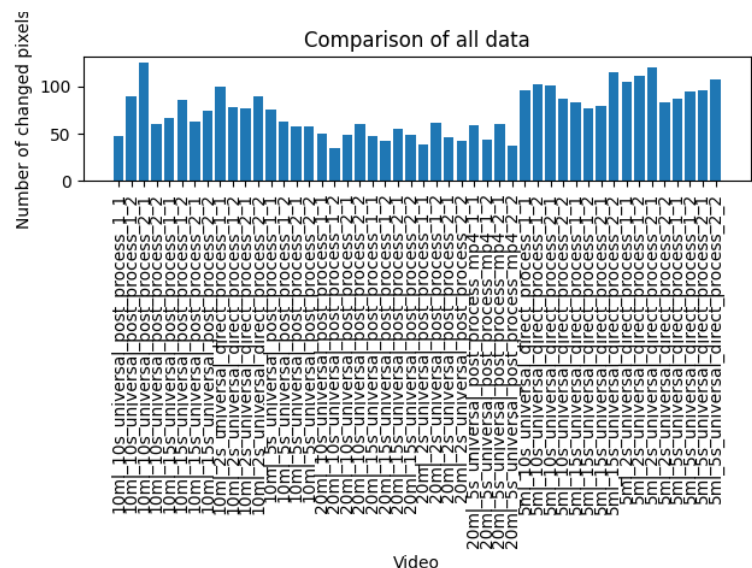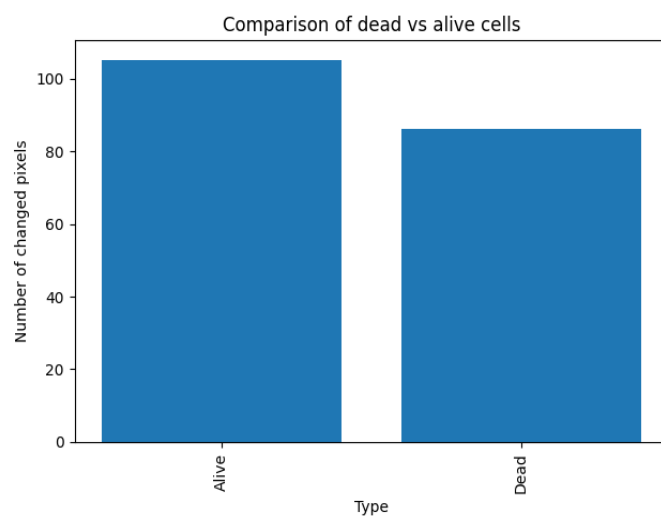

Figure 6 "Comparison_extension" panel

Figure 4 Dead versus alive plot

Check the logs

Useful thing to do when an error occurred is to check the logs :
- Go to the "Logging" panel
- Set "Show" to "All"

Debug the software

Debugging some parts of the software (like the processing parts) is easier to do locally on your computer. To do that, open the file "nanomotion_extension.py" in a code editor, and set the global variable DEBUG to True. Detailed information are written directly in the file.