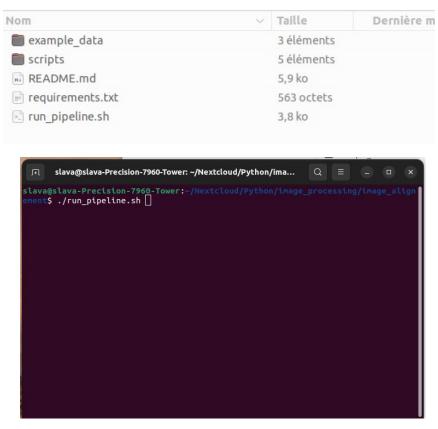
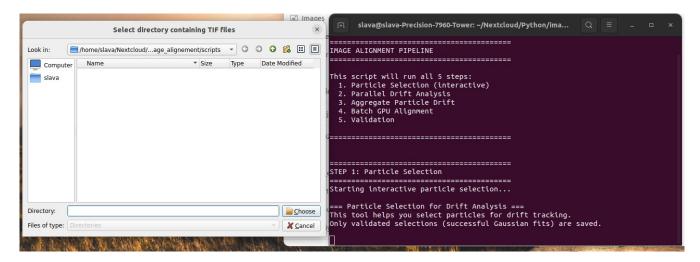
## Short example of script usage on the example data

Run run\_pipeline.sh in the terminal window, making sure to install all requirements beforehand.

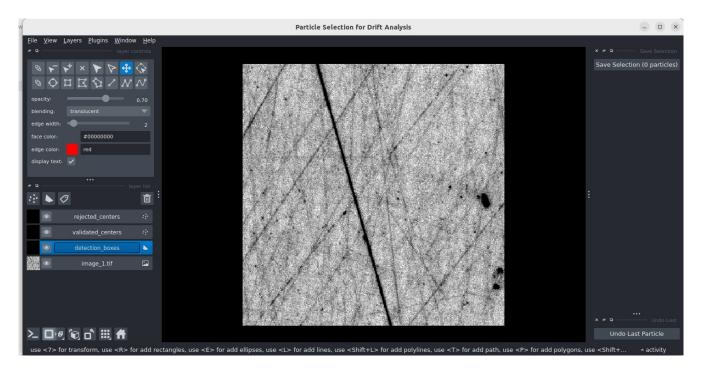


Follow the instruction appearing and guiding you through 5 steps of image alignment.

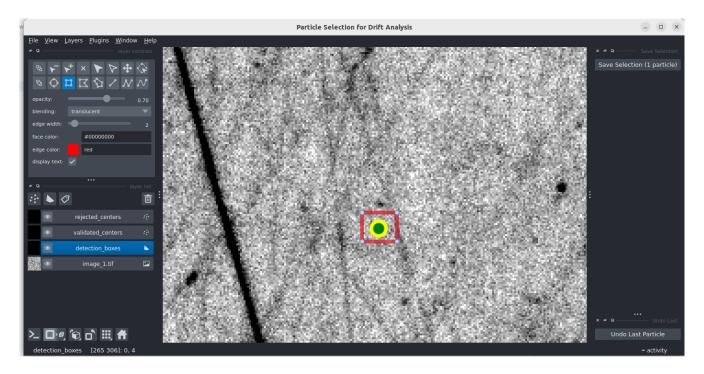


In the first step, select a folder containing TIFF files. The script will then prompt you to choose multiple sets of folders with corresponding data, if necessary, as it can process several data sets in parallel.

Choose 'detection boxes':



Then use the rectangle tool from the toolbox to manually locate the particles. Each particle will be fitted to a Gaussian distribution and, if successful, marked with a dot; if not, it will be indicated with a red cross. To remove a particle, click on "Undo last particle" (bottom right). Once you've made your selections, click on "Save selection" (top right) and proceed to another dataset if prompted by the interface. We recommend selecting at least 4 particles in each corner of the image. Ideally, aim for 4–6 particles per corner. Keep in mind that selecting more particles increases the chances of successful fits, especially if some image areas are not fitted correctly.



After you finish, observe the terminal as the script executes all necessary image processing steps, providing information about the results and the output folder containing all data, along with the before and after drift values.

```
.==============
 STEP 5: Validation
    == Validation of Aligned Images ===
Using user-specified JSON file: ../scripts_output/particle_selections_20251016_133257.json
Loading configuration from: ../scripts_output/particle_selections_20251016_133257.json
Found 1 aligned image set(s) to validate
   et 0: example_data1
Aligned folder: /home/slava/Nextcloud/Python/image_processing/image_alignement/example_data/example_data1_aligned
    Particles: 5
 Step 1: Validating aligned images (Gaussian fitting)...
 Using 1 parallel workers
                                                                                                                                                      | 0/Set 0: Validating example_data1: 24%|
| 25/25 [00:00<00:00, 32.60fit/s]
 Set 0: Validating example_data1: 0%|
| 6/25 [00:0Set 0: Validating example_data1: 100%|
 Step 1 complete: Gaussian fitting on aligned images
 [Set 0] Validation CSV: example data1 z6z.csv
   -----
 Step 2: Aggregating validation drift data...
| 0/
| Set 0: Aggregating example_data1_z62.csv: 0%| | 0/
| [Set 0] Loaded 25 records, 5 images
| Set 0: Aggregating example_data1_z62.csv: 0%| | 0/
| [Set 0] Successful fits: 24/25 (96.0%)
| Set 0: Aggregating example_data1_z62.csv: 0%| | 0/
| [Set 0] / Aggregated CSV: drift_example_data1_z62.csv
| Set 0: Aggregating example_data1_z62.csv: 0%| | 0/
| [Set 0] Images: 5, Avg particles/image: 4.8
| Set 0: Aggregating example_data1_z62.csv: 0%| | 0/
| [Set 0] Residual Drift X: 0.0000 to 0.1862 px
| Set 0: Aggregating example_data1_z62.csv: 0%| | 0/
| [Set 0] Residual Drift Y: 0.0000 to 0.4052 px
| Set 0: Aggregating example_data1_z62.csv: 0%| | 0/
| Set 0: Aggregating example_data1_z62.csv: 0%| | 0/Set
                                                                                                                                                       | 0/Set 0: Aggregating example_data1_z6z.csv: 100%|
 Step 2 complete: Aggregation finished
 Step 3: Generating validation comparison plots...
 [Set 0] ✓ Validation plot: validation plot example data1 z6z.png
 VALIDATION SUMMARY
 Total processing time: 0.79 seconds
Image sets validated: 1
Successful validations: 1
 Set 0: example_data1
Total fits attempted: 25
Successful fits: 24
Success rate: 96.0%
 Updating JSON file with validation file paths...
✓ JSON file updated: particle_selections_20251016_133257.json
Added validation file paths to each image set
 Validation files saved to: ../scripts_output/validation
   === VALIDATION COMPLETE ===
 If residual drift is near zero (< 0.1 px) and rotation is near zero (< 0.01^{\circ}), then the alignment was successful!
 PIPELINE COMPLETE!
 All steps completed successfully!
    esults:
- JSON file: scripts_output/particle_selections_20251016_133257.json
- Particle tracking CSV: scripts_output/particles_tracking/
- Drift analysis CSV: scripts_output/particle_drift/
- Aligned images: (sibling to input folders with _aligned suffix)
- Validation results: scripts_output/validation/
```

## Validation: example\_data1\_z6z Before vs. After Alignment

