CiliaGUI – Installation and User Guide

Installation

To run the toolbox, you need to:

1. Install MATLAB (2021 or later).

- MATLAB is not free; check with your institution for access.

- If you cannot get a license, contact me — we may try a standalone version of the program.

- Installation instructions: https://www.mathworks.com/help/install/ug/install-products-with-internet-connection.html

- During installation, make sure to install the following toolboxes:

  • **Statistics and Machine Learning Toolbox**

  • **Image Processing Toolbox**

2. Download the code from GitHub:

- Repository: https://github.com/pmgarderes/CiliaDetect2.0

- Click 'Download as ZIP file'.

3. Unzip and place the folder on your hard drive.

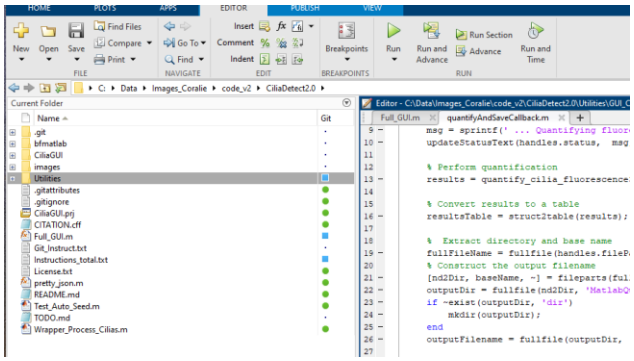
- Example: on Windows, I use WinRAR and put the files into a folder named ciliaGUI_demo at the root of C:/.

Launching a Session

4. Navigate to the folder and find Full_GUI.m.

- Double-click it. If MATLAB is not open, this will automatically launch MATLAB and set the current folder correctly.

 Make sure the current folder in MATLAB (shown in the top bar) contains Full_GUI.m. If it doesn't, adjust it manually. → it should like like this (roughly)



5. Start a session:

- Go to the Editor panel.

- With Full_GUI.m active (double click Full_GUI in matlab to make sure it's active

- click Run.

Preprocessing – Z-Planes

6. Prepare your microscopy images before using the GUI.

- The program only reads *_reduced.mat , i.e. files formatted for matlab. Thus we need to pre-process microscopy image and format them.

- Strategy: reduce the number of frames in a to a few averaged Z-planes (down to a single Z-plane), so multiple Z-planes can be separated from the same Z-stack.

To preprocess:

1. Click the 'Pre-process' button.

2. Enter the downsampling factor (number of frames to average together).

- Example: enter '1000' to collapse the full stack into a single Z-plane.

3. A file browser window will open. Navigate to the folder containing your microscopy files.

 You will not see the individual files — just select the directory where the files are located.

4. The GUI text box will display messages ('WAIT') and begin downsampling.

Notes:

- Preprocessing large stacks may take a long time. Tip: you can batch-process many files overnight.

- Output: a new folder 'Matlab_quantif' is created. Each original file generates a reduced version with the suffix '_reduced.mat'.

Loading a File

7. Load files into the GUI:

- Only files ending in '_reduced.mat' can be loaded.

- Click 'Select File', navigate to the directory, and choose the file.

GUI Display

8. The central panel displays one Z-plane in one channel.

- Adjust fluorescence intensity with numeric keypad: /, *, -, +.

  • These settings apply across all Z-planes in the same channel but not across channels.

- Navigate between channels: left/right arrow keys.

- Navigate between Z-planes: up/down arrow keys.

Detecting ROIs

9. To add a new ROI:

- Left-click directly on the cilium (not beside it).

- Press Space.

- The program segments the cilium around your click.

- If detection fails (e.g., low contrast), it will appear as a single point.

  • Delete failed detections by pressing U once (undo).

 Do not leave empty ROIs.

Parameters to Adjust Detection

10. Sensitivity is the key parameter.

- Recommended range: 0.3–0.7 (use 0.4–0.5 in most cases).

- Higher sensitivity: easier detection, less accurate ROIs.

- Lower sensitivity: stricter detection, fewer but more accurate ROIs.

Merging ROIs

11. If a cilium is split across Z-planes or incompletely detected:

- Click near the junction point.

- Press M to merge ROIs (works if they are close enough).

Deleting ROIs

12. To delete ROIs:

- Single ROI: click near it and press S.

- All ROIs: click 'Clear Detections'.

Automated ROI detection

Automatic detection of cilia ROIs (for strong signals).
If your images contain strong cilia signals, you can use the automatic detection feature instead of manually clicking each ROI.

To run auto-detection:

1. Click the **Auto-detect** button.
2. A pop-up window will ask how many ROIs you want to detect.
    - Provide an approximate number of cilia expected in the current Z-plane.
    - □ Tip: start with a small number (e.g., `10`).
3. The program will search the brightest `N` points in the current Z-plane and channel.
    - For each candidate point:
      • If a cilium-shaped structure is found, the ROI is added to the detection list.
      • If not, the point is marked as tested and excluded, shown with a **red dot**.

Notes:

- Newly detected ROIs can be merged (`M`) or deleted (`S`) the same way as manually detected ROIs.
- Excluded points are temporary:

    - They are not saved.
    - They disappear if you close the file or select a new file.
- You can re-run **Auto-detect** in the same Z-plane or in new Z-planes.
    - Additional points will be tested.
- After auto-detection, you may:
    - Manually suppress unwanted auto-detected ROIs with **S**.
    - Add new ROIs near excluded points manually with **Space**.

Other Useful Buttons

13. Refresh ROI display: press R.

Saving Detections

14. Save your work:

- Click 'Save Detections' at any time.

- This creates a new file in 'Matlab_quantif' inside 'reduced_stack/'.

- File name format: originalName_reduced_cilia_detections.mat

- Next time you open the reduced file, saved detections load automatically.

 Only load '_reduced.mat' files. Do not load detection files directly.

Visualizing Masks

15. To view ROI and background masks:

- Click 'Visualize Masks'.

- Two images will appear:

  • Left: cilia mask overlay.

  • Right: background mask.

- Images are saved automatically.

- Adjustable parameters:

  • Padding: gap between ROI and background (pixels).

  • Background mask width (pixels).

Quantify and Save Fluorescence

16. To export quantified data:

- Click 'Quantify and Save Fluorescence'.
- Creates one Excel file (.xls) per image in 'Matlab_quantif/'.
- Each row = one cilium.

Fluorescence outputs (per channel):

- F_StackSum_ch# – sum across the Z-stack.
- F_plan_ch# – mean in the Z-plane.

Morphological measures (if metadata available):

- Length (µm)
- Width (µm)
- Curviness (length/chord length)
- Length/Width ratio
- Area (μm^2)

Notes:

- Both methods correct for background.
- Both are valid for comparisons if exposure and Z-range are similar.
- Recommendation:
  - Use stack sum if Z-range > cilium span.
  - Use mean method otherwise.

Editing Parameters

17. Parameters are user-editable:

- Any parameter can be modified.
- Settings are automatically saved and reloaded in the next session.