

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 where the code is and find Full_GUI.m.

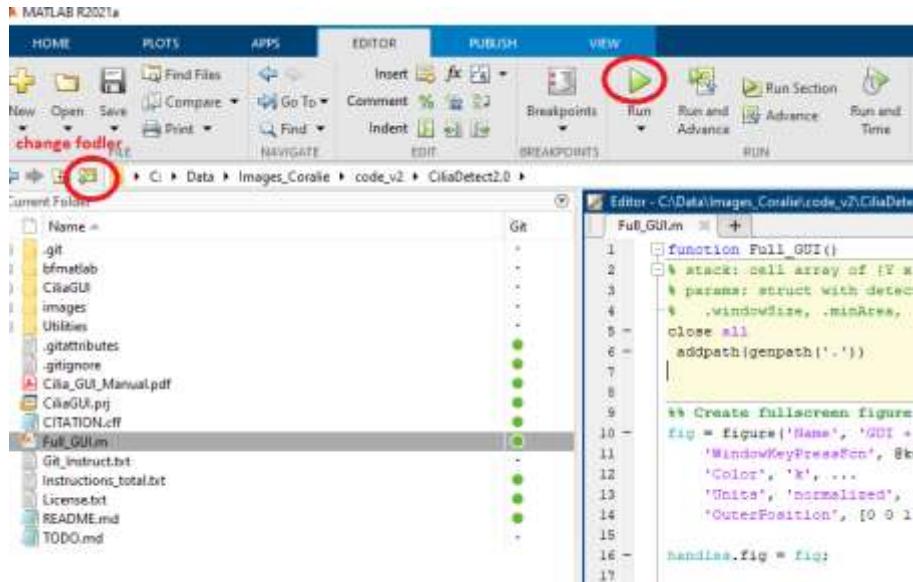
- Double-click it. If MATLAB was not open, this will automatically launch MATLAB and set the current folder correctly.

⚠ The correct current folder in MATLAB (shown in the top bar) is the one that contains Full_GUI.m. If it doesn't, choose the folder with this button it manually. (see pic below)

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.

Should look like this :



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 - Click "Tune parameters" for loading pre-set parameters or set your own custom parameters. A mini-GUI open to interactively optimize detection on a small set before running it broadly.

→ **Use pre-set for cilia detection** (cilia_defaults.mat used as default or cilia_final.mat) or axon initial segments (AIS_final.mat), that can be loaded in the mini-GUI. Click "apply" set these parameters active in the main GUI and as new defaults.

→ **To generate your own custom parameters**, • First pick a representative Z-plane and channel in the main GUI. • Click on a few typical cilia to be detected and press Space to detect. Open the mini-GUI (click tune parameter) • Adjust sliders/toggles:, save the set parameters and click "apply" set these parameters active in the main GUI and as new defaults.

→ **Recompute ROIs** for any detection made prior to the parameter tuning this is done by clicking "Recompute ROIs" in the main GUI after applying the parameters

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.
 - o Provide an approximate number of cilia expected in the current Z-plane.
 - o  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.
 - o 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:
 - o They are not saved.
 - o 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.
 - o Additional points will be tested.
- After auto-detection, you may:
 - o Manually suppress unwanted auto-detected ROIs with **S**.
 - o 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'.
- this 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, either using the miniGUI (only detection related parameters, see section 10 of this guide) or with the button “edit any parameters” where parameters for detection and quantification can be modified.

→ The main parameters for detection are:

a. Window Size (region considered for segmentation)

- Defines the square region (in pixels) around the clicked seed used to isolate the cilium for detection. Recommended range: **50–400 px** depending on image resolution and cilia length.

b. Sensitivity Threshold

- Adjusts how permissive the adaptive threshold is. Recommended range: **0.3–0.7** (use **0.4–0.5** in most cases), or 0.6-1 if using Otsu global threshold

c. Detection Prefilter (used only for detection, not quantification)

- Applies a light edge-preserving **spatial smoothing** to simplify punctated or noisy images before segmentation. This setting **Does not** affect quantitative measurements (intensity, length, etc.), only the initial mask detection. **Enable prefilter** only if cilia appear fragmented or speckled. **Spatial scale: ~2 px** (try between **1–4 px**).

d. Line bridging

- Connects nearby fluorescent fragments that likely belong to the same elongated structure. This operation helps recover continuity in partially broken or dim cilia/AIS. It affects only the segmentation mask, not the quantitative measurements. Enable bridging if cilia appear discontinuous or interrupted. Use typically StrengthBridge: **0.1–0.5** and strengthShrink: **0.02–0.1**.

e. Inclusion/Exclusion criteria:

- Applies shape-based filters (area, thinness, elongation, eccentricity) to keep only ROIs consistent with typical cilia geometry. Used mainly during automated detection. If no ROI meets these criteria, the algorithm returns an empty placeholder ROI, which should be deleted.

→ The parameters for quantification are:

Define how background fluorescence is estimated around each ROI. The **background spread** sets the width of the surrounding ring used for background sampling (in pixels), while the **background padding** defines the gap between the ROI edge and that ring. B