

DeleteROI for Cleaning CiliaQ Output of Non-ciliary Contamination

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Abstract

The ImageJ plugin CiliaQ developed by Hansen and colleagues (Hansen et al., 2021) provides for sophisticated analysis of ciliary parameters in three-dimensional space. However, midbodies and other non-ciliary structures can contaminate the output and require significant effort to remove. Furthermore, the manual removal of contamination risks subjective bias as the data is not blinded to the investigator. To address these problems, we developed an ImageJ plugin that presents images of the cilia region-of-interests (ROIs) identified by CiliaQ in a clickable grid that allows for marking and automated removal of non-ciliary contaminants. To reduce subjective bias, our plugin works on a dataset of multiple images and presents the cilia ROIs randomly. If the dataset contains both control and experimental conditions, the cilia are randomly interspersed with no visible information about their experimental group, thus reducing subjective bias. After removal of contamination, the cleaned data is output maintaining the CiliaQ file formats initially used.

Installation and Operation

CiliaQ-DeleteROI is intended to be used after the third CiliaQ module, which is simply called CiliaQ. The user is expected to have used the CiliaQ Preparator or an equivalent method to segment cilia in a series of images and processed them through CiliaQ. The use of the CiliaQ Editor is optional and may be

needed to improve segmentation. There is little value to remove segmented objects with the CiliaQ Editor as this is handled more efficiently with CiliaQ-DeleteROI.

CiliaQ-DeleteROI is dependent on installations of FIJI (Schindelin et al., 2012) and the output from CiliaQ (Hansen et al., 2021). After installation of FIJI, place DeleteROI_.py in the FIJI/scripts/Plugins folder and DeleteROI-1.0.1.jar (or latest version number, ensure only the latest version is in the directory) in the FIJI/jars/Lib (you may need to create the Lib directory) After downloading the DeleteROI_.jar file from GitHub, use the FIJI **Plugins->Install...** (not **Install Plugin...**) to install this module. Windows users will need to be running FIJI in administrator mode for this operation.

Restart FIJI and run the software via **Plugins -> DeleteROI**. A popup menu will allow you to navigate to a folder containing your images and the CiliaQ output files. DeleteROI uses two files. The first is a tif image that ends with CQ_RP.tif or CQ_date_time_RP.tif containing the ciliary masks and the immunofluorescence channels. The second is a spreadsheet generated by CiliaQ that ends with CQ.txt or CQ_date_time.txt. Note that the txt file can be in either US (0.00...) or German (0,00...) format and this will be maintained. The menu allows three options to control how the upcoming grid of ROIs is displayed (Fig 2). The first option “Saturation” controls the percentage of the pixels that are saturated with higher numbers producing more saturated images. The second “Min/Max” sets minimal and maximal display values for each of the three channels. The third “Manual” allows you to view the tif image and adjust on an image-by-image basis.

Once you have selected the folder containing your images and chose the display settings, use **Update** to start the process. DeleteROI will survey your folder and will update the popup to present the number of images and ROIs to be processed from each of the tifs in the folder (Fig 3). Select **Next** to continue. If there are more than 500 ROIs, the data will be presented in sessions (Fig 4). At the end of each session, the analysis is stored so if there is an interruption, the completed sessions do not have to be reanalyzed. Advanced Options allow for scaling the ROIs and determining how big the ROI needs to be (Fig 5). 32 pixels and 3x are good options for 40x images of fibroblast cilia. Higher magnification or longer cilia would require larger ROIs and possibly smaller scales. Advanced options also allow one to enter Debug mode where the tif images remain open and the ROIs will have an identifier included to track them back to the originating tif image. This allows one to see the ROI in context of the image, which can be useful in initial efforts to understand what ROIs represent cilia but removes the blinding of the data. By default, the image name is stored in column 1 of the CQs file but this option can be changed on the advanced option screen.

Once **Next** is selected, a montage grid of ROIs will be presented (Fig 6). These ROIs are randomly chosen from the dataset to reduce subjective bias. Clicking on ROIs of contamination will put

an X over them and will mark them for removal from the dataset. Clicking a second time will remove the X and allow them to remain in the dataset. Select **Completed** to move to the next group of ROIs and continue until the session is completed. Once the session of about 500 ROIs is completed, a new set of files will be written to a subfolder called “Group_1” that will contain two new spreadsheets. One, ending CQ.txt, will be similar to the original CQ.txt CiliaQ file except that the ROIs that were selected for removal are marked with an # in column 1 effectively commenting them out. This file is duplicated in the original folder as CQ-active.txt. The second, CQ-stripped.txt, only contains the active entries from CQs.txt CiliaQ file plus the addition of the image name in column 1 (or wherever was selected in the advanced options). The CQ-stripped.txt file is intended to be directly used for secondary data processing. The original CQ.txt file remains unchanged in the original folder. A text file (README.txt) with the metadata of the session and deletion history is also present in the folder.

The design of DeleteROI allows for multiple passes through the data to cull additional ROIs without having to start over. This can be useful when the first pass was too conservative and more ROIs need to be removed. Alternatively, it can be used for subsequent subclassification of cilia with a particular property e.g. positive for an experimental marker. Each pass through the data creates a subfolder “Group_x” with x incremented with each pass and the CQ-active file updated with the most recent iteration. To run subsequent passes through the data, run DeleteROI as before, pointing to the same folder or images, and select **New Session** (Fig 8) after selecting **Update** (Fig 1).

Code Availability

Code is available at Github/pazourg/DeleteROI. A practice dataset of cilia labeled with acetylated tubulin and Arl13b is available at <https://Cilia.Pro/DeleteROI/ExampleData> .

Acknowledgments

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Conflict of Interest

The authors have no conflicts of interest in this work.

Figures

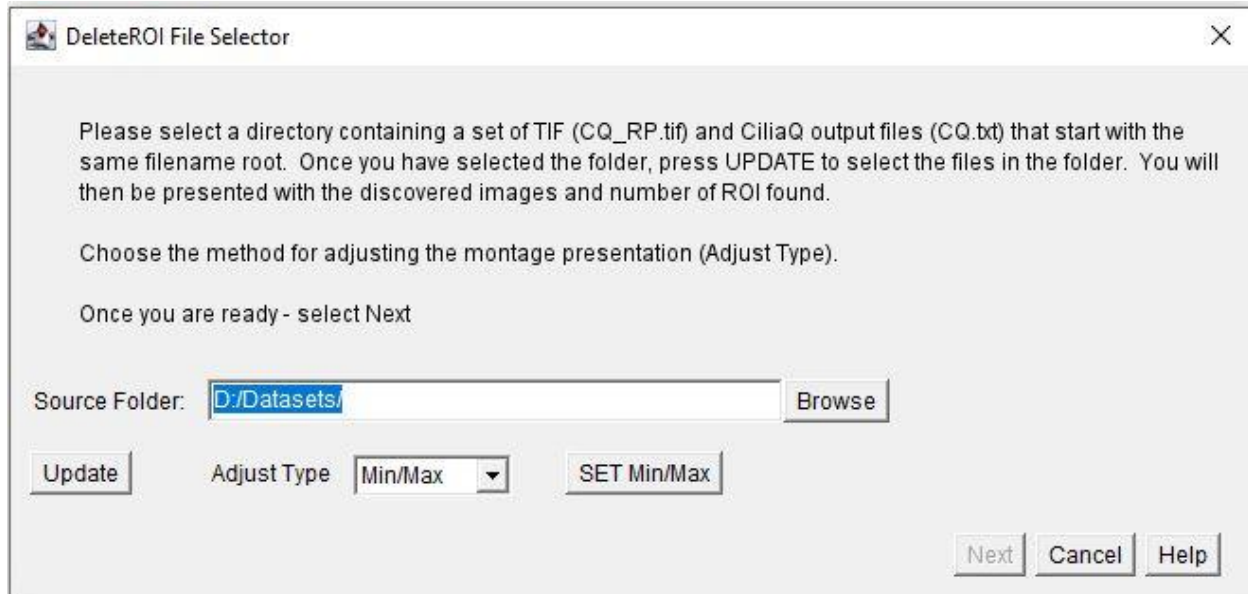


Figure 1. Initial menu

Upon starting DeleteROI you will be asked to navigate to a folder containing the dataset to be analyzed and to set the ROI display parameters. All images in this folder will be considered to be part of the overall dataset and ideally would contain images from both control and experimental conditions.

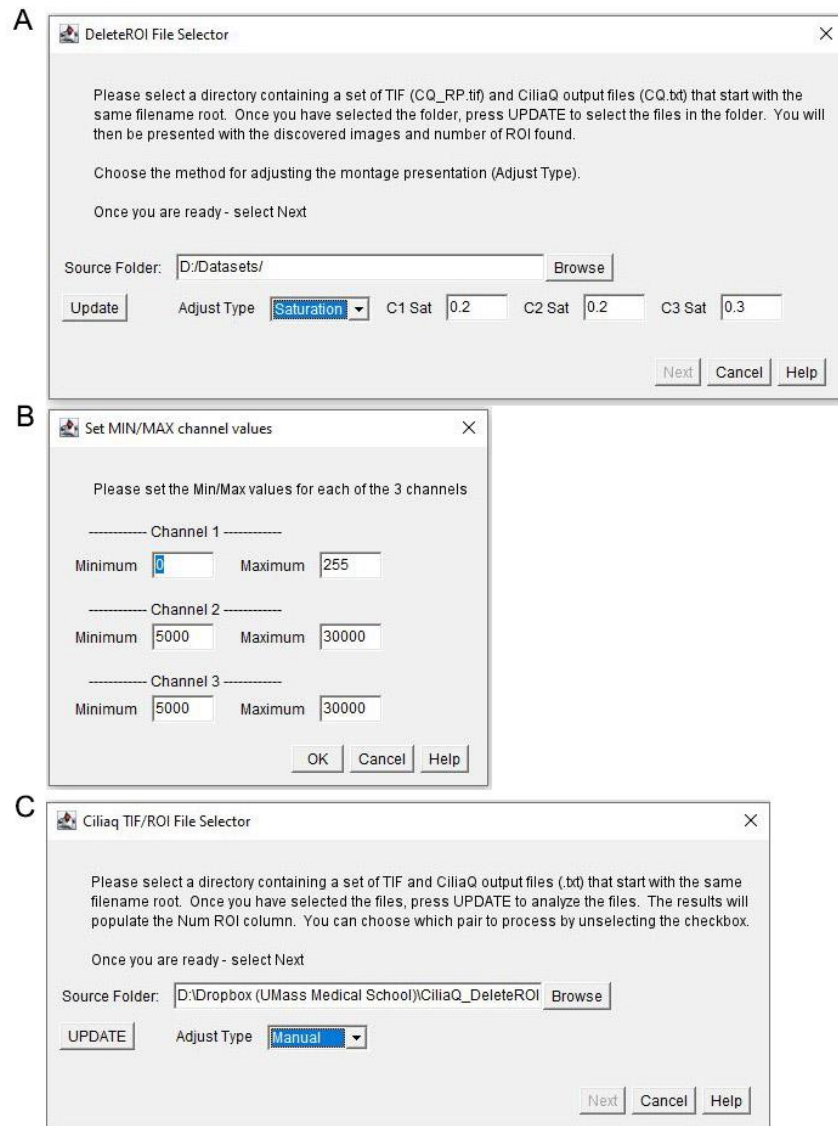


Figure 2. Setting ROI montage display parameters

DeleteROI allows three options for setting brightness and contrast on displayed ROIs. Saturation can be changed on this popup (A). Min/Max will call a new popup (B). Manual (C) will ask you to set values for each image as they are processed.

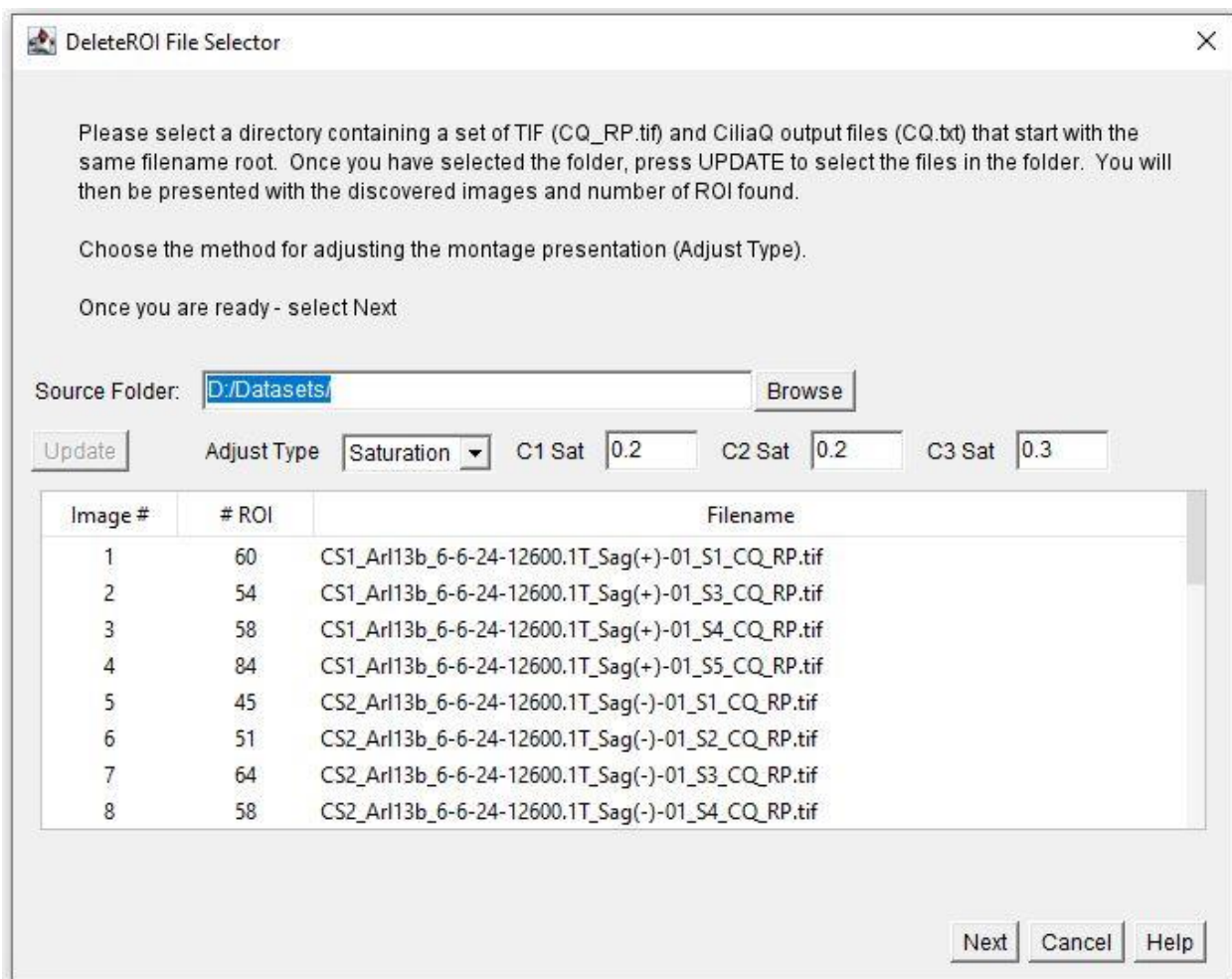


Figure 3. List of images and the number of ROIs that will be processed.

After pointing the plugin to the folder and selecting **Update**, the popup will refresh showing the images and number of ROIs in the dataset. Select **Next** to continue the process

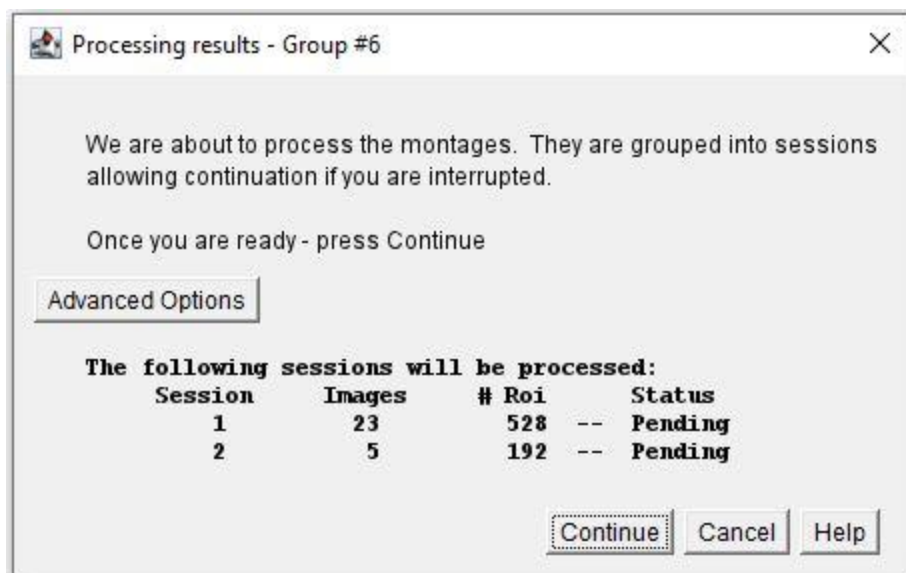


Figure 4. Information about the number of sessions that the data has been allotted to and the number of ROIs in each.

Depending on the number of ROIs in the dataset, the workflow will be broken into sessions of about 500 ROIs. Once processing of a session is completed, the data is written to the disk. If there are interruptions during analysis, the process can restart at the first unfinished session without needing to redo all sessions. **Advanced Options** is described in Fig 5. **Continue** will start the process of displaying the ROI montages.

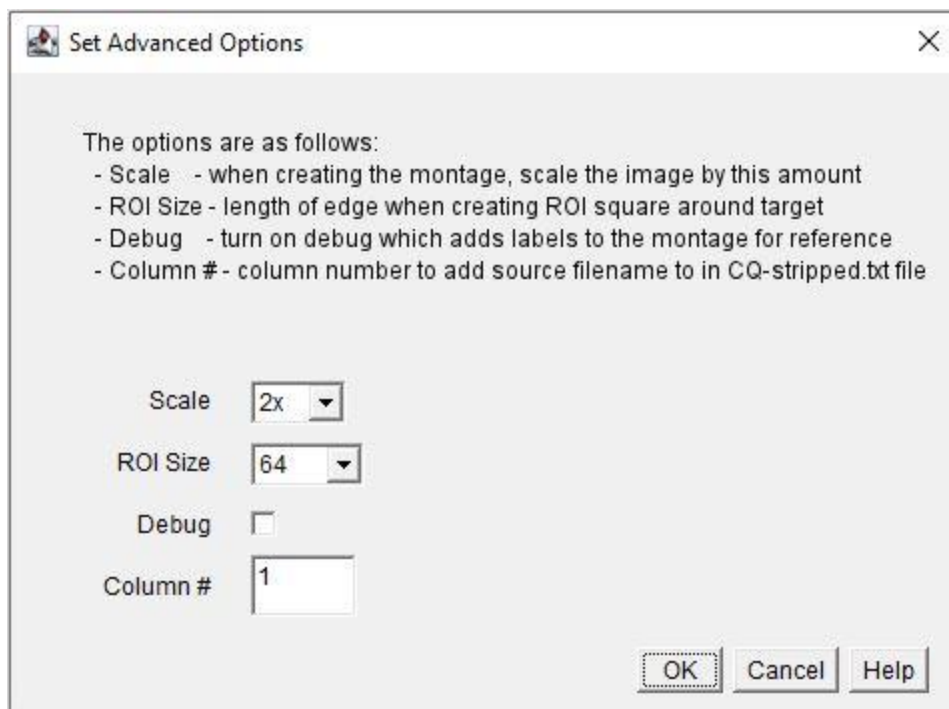


Figure 5. Advanced options.

Scale and ROI Size should be adjusted to the magnification of your image and the length of the cilia. Debug allows the images with ROIs marked to remain open. Column # allows for the insertion of the source filename into the CQ-stripped.txt spreadsheet of data.

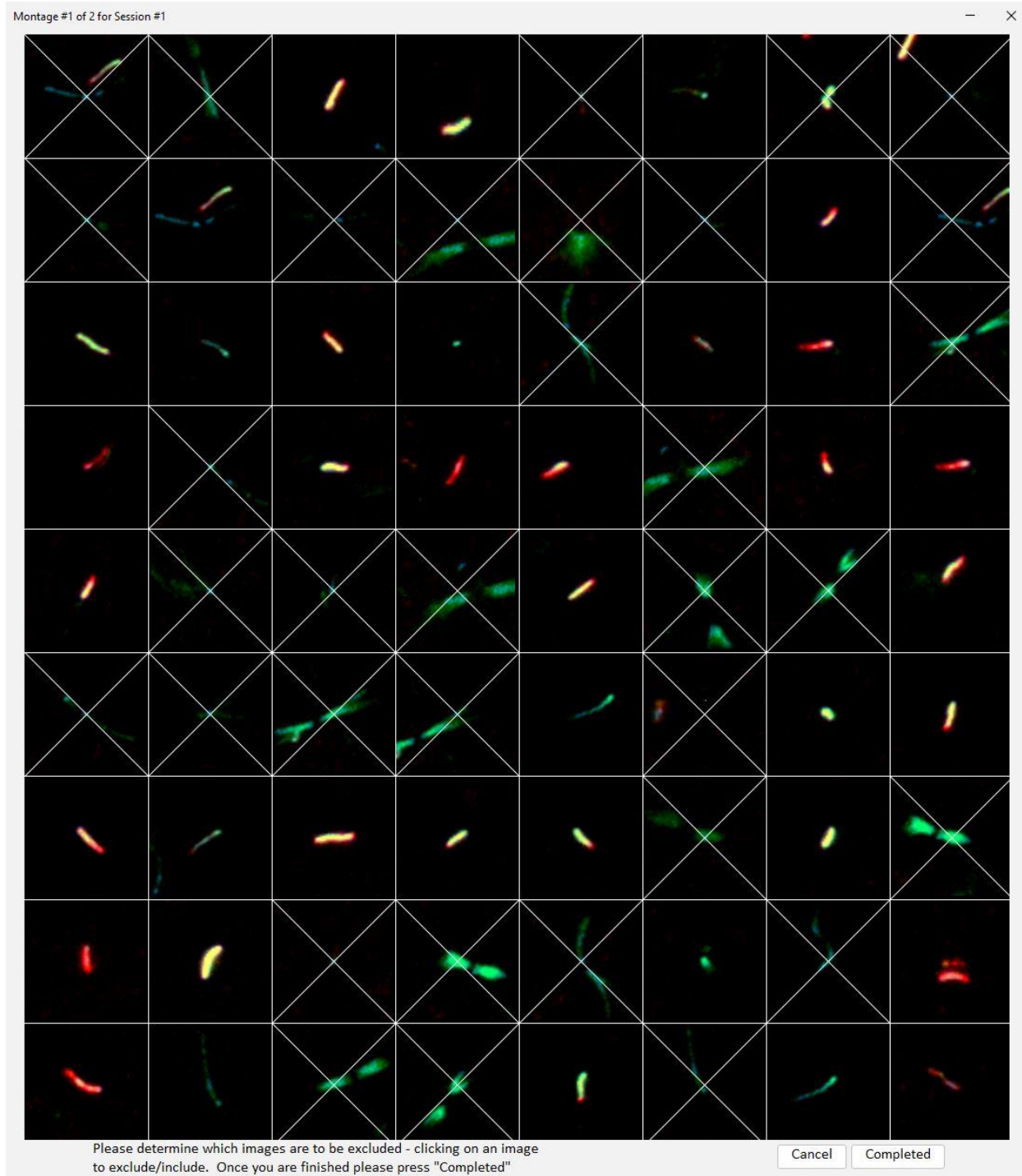


Figure 6. Example of an ROI grid

Clicking on ROIs marks them for removal from the dataset. A second click will restore them to the dataset. **Cancel** will terminate the process and exit DeleteROI. Once all ROIs have been examined,

Selecting **Completed** will move to the next montage in the group. Text at the top lists the number of montages in this group and the number of groups in the dataset.

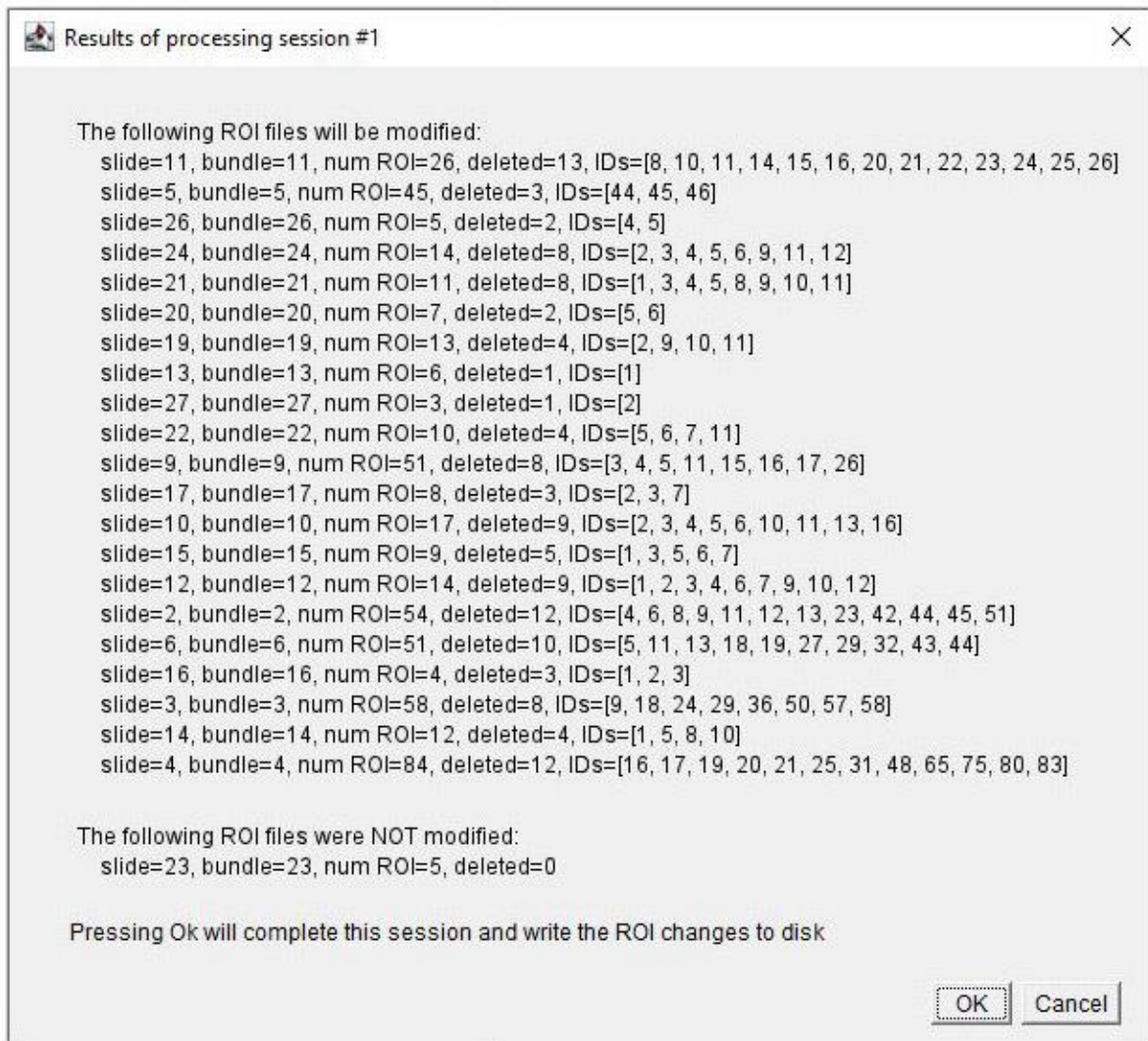
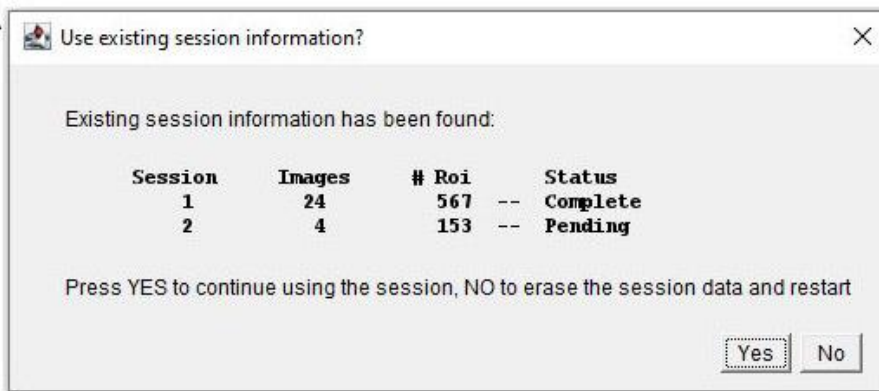
A**B**

Figure 7. Processing Complete

When you have worked through all montages in a session, a popup (A) will provide the stats on what was marked for removal. This data is also written in the README.txt file in the Group subfolder of your data folder. Selecting OK will move to the next session in the dataset or if it is the last session, to the completion popup (B).

A



B



Figure 8. Restarting a prematurely terminated session or starting a new iteration

A) If DeleteROI is run again on folder of images that were previously partially processed, this popup will appear. **Yes** will allow you to continue processing session 2, **No** will delete the work done on session 1 and restart at the beginning.

B) If DeleteROI is run again on folder of images that were fully processed, this popup will appear. **Cancel** leaves DeleteROI, **New Session** starts a new pass through the previously cleaned data. The previously removed ROIs will not be displayed. This provides an opportunity to remove additional ROIs and also can be used to classify cilia into subcategories without knowing the experimental conditions of the ROI (i.e. blinding).

References

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