

# Manual

## ***MotiQ: a toolbox to quantify the cell motility and morphology of microglia***

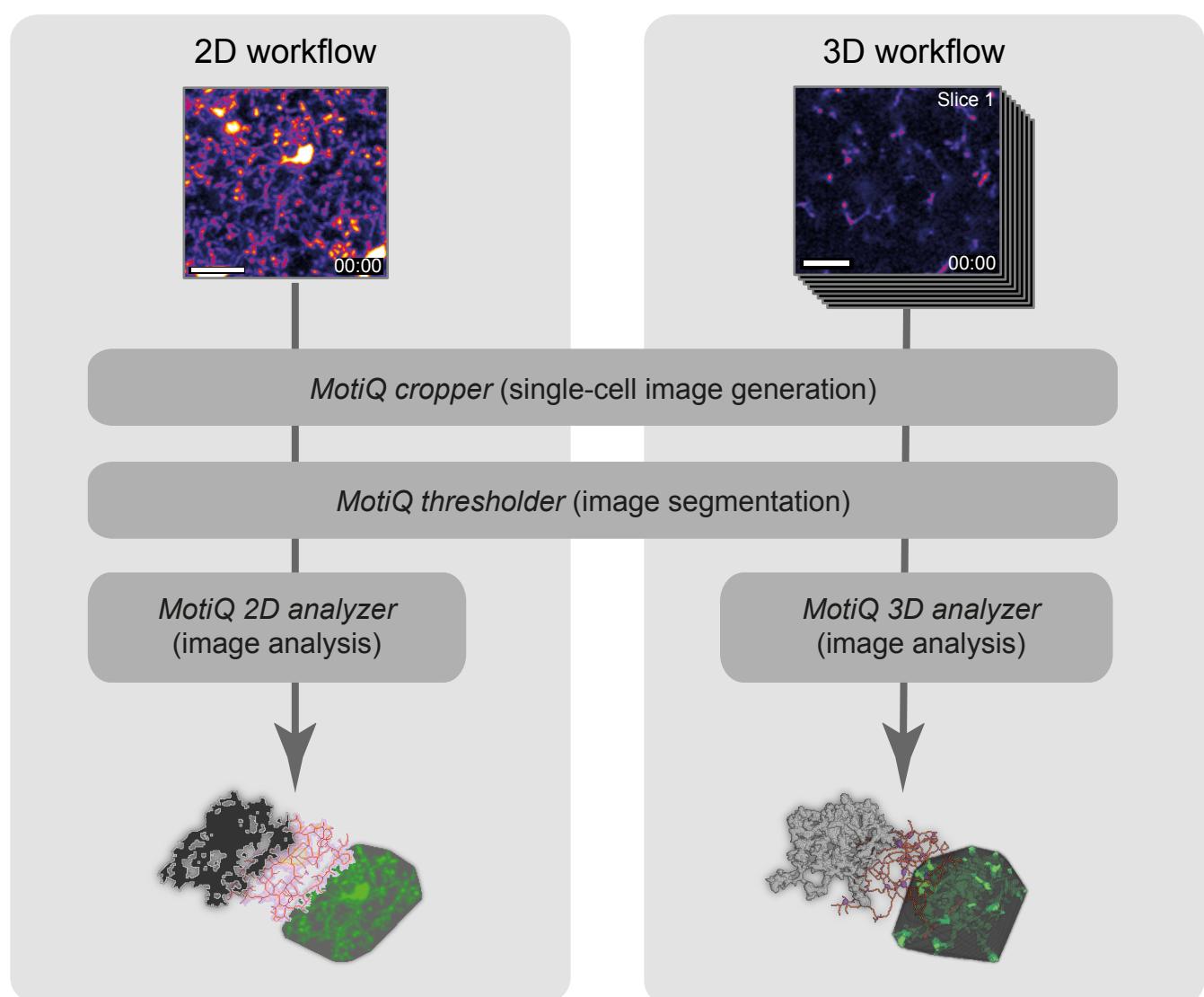
### **Introduction and Overview**

*MotiQ* is a suite of modular ImageJ plugins that allows the comprehensive analysis of microglial morphology and motility in diverse imaging data. *MotiQ* offers two workflows; one for the analysis of 2D images and time series and a second for the analysis of 3D images and time series. Both workflows consist of three steps: (A) single-cell image generation using *MotiQ cropper*, (B) image segmentation using *MotiQ thresholder*, and (C) image analysis using *MotiQ 2D analyzer* or *MotiQ 3D analyzer*. *MotiQ cropper* and *MotiQ thresholder* are used for work steps A and B of both workflows. Optionally, step A can be skipped and *MotiQ* can be used to separate particles in the image automatically; To this end, select to track all particles in the image separately in step C (see section C below, settings dialogs).

To start either workflow, download and install ImageJ (<https://imagej.net/Downloads>) or FIJI (<https://fiji.sc/>) and install into ImageJ or FIJI all four *MotiQ* plugins from the newest *MotiQ* release (<https://github.com/hansenjn/MotiQ/releases>). Each plugin is independent and can be used in other analysis workflows.

In this manual, the background and the settings for each *MotiQ* plugin are explained in detail.

Red arrows indicate an explanation for a setting. Green arrows indicate the workflow.

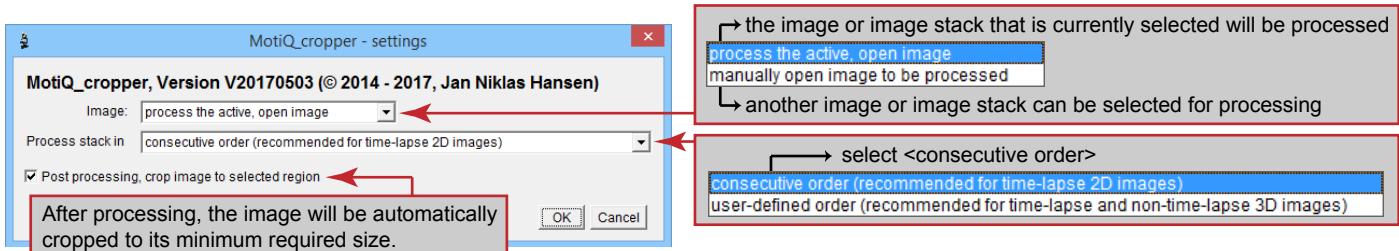


## A) Single-cell image generation (*MotiQ cropper*)

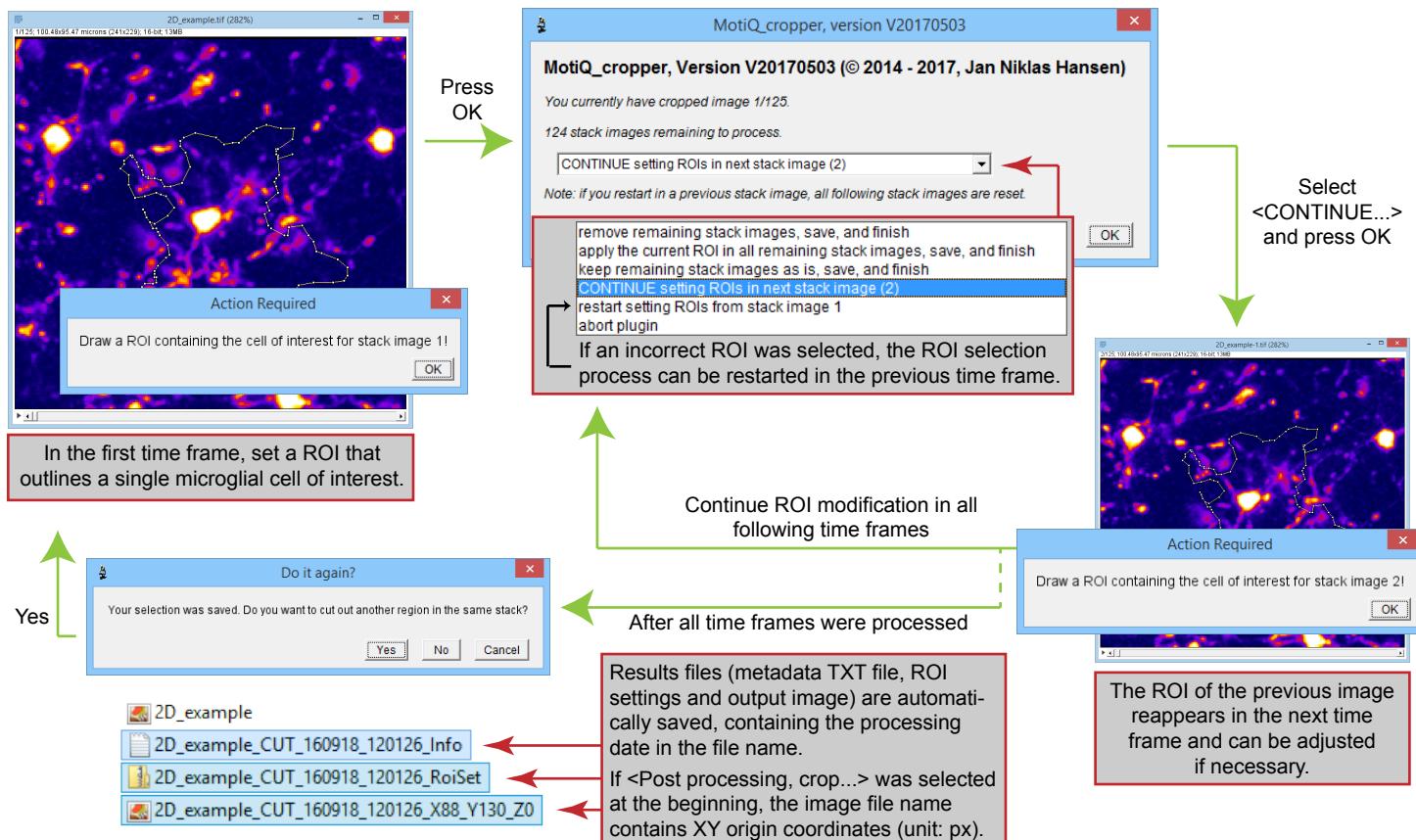
*MotiQ cropper* is a tool for generating single-cell images of microglial cells in image stacks and time series. To start the single-cell selection process, open the respective images or image stacks in ImageJ and launch the plugin *MotiQ cropper* via the ImageJ menu: Plugins > MotiQ > MotiQ cropper.

### 2D Workflow:

After launching *MotiQ cropper*, a dialog will open. Select the following options and press ok:

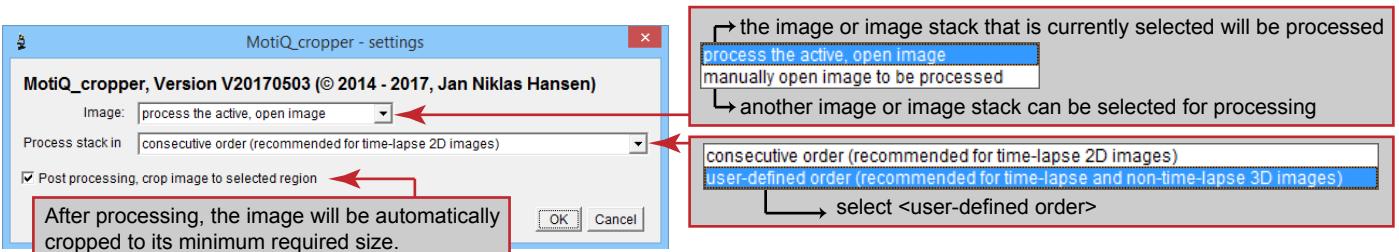


In the image display window, select and confirm a ROI that outlines a single microglial cell with its cell processes. Then proceed to potential consecutive time frames. After all time frames are processed, the processed image stack is automatically saved (together with metadata) in the same directory, where the original image or image stack was saved while opening:

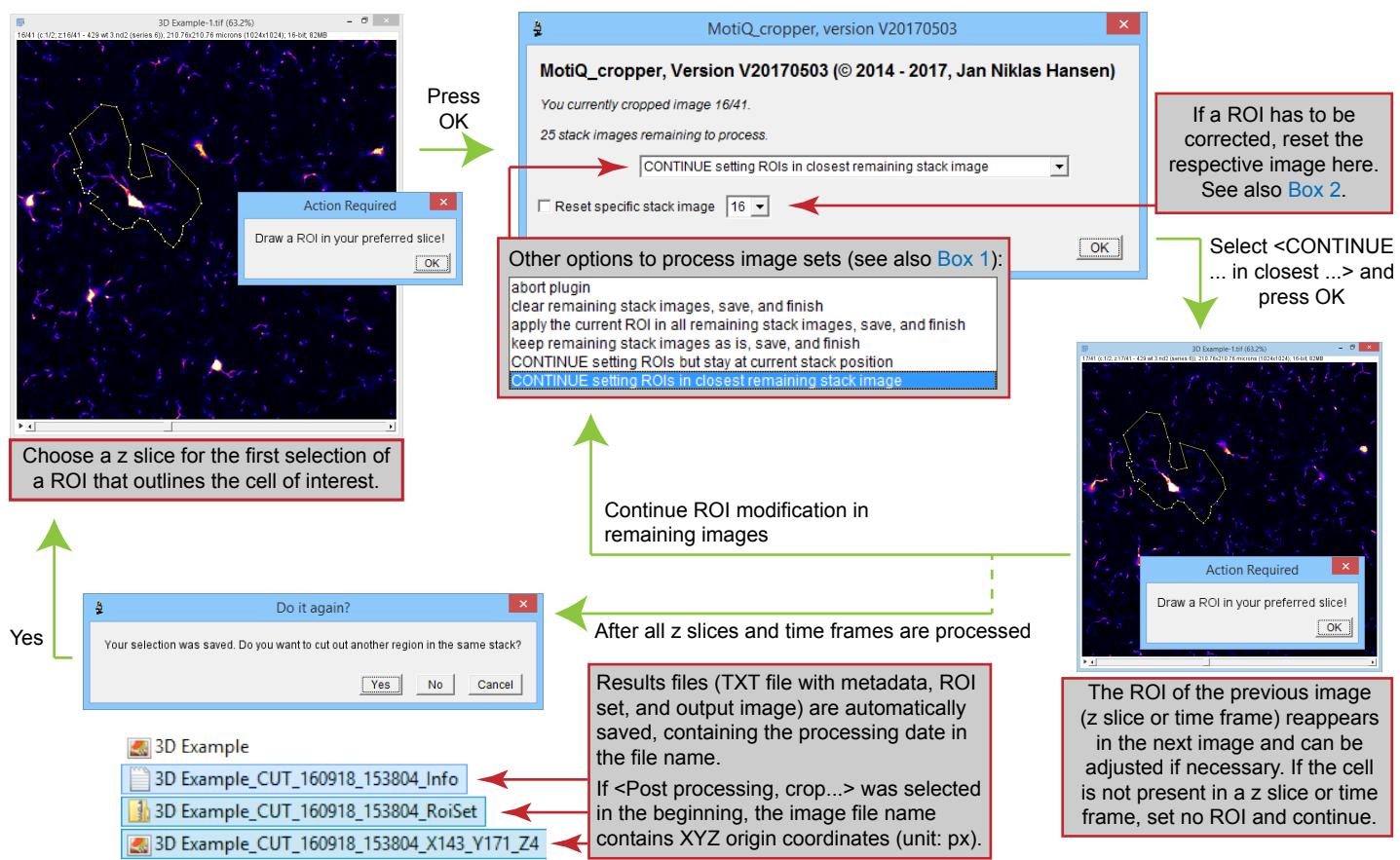


### 3D Workflow:

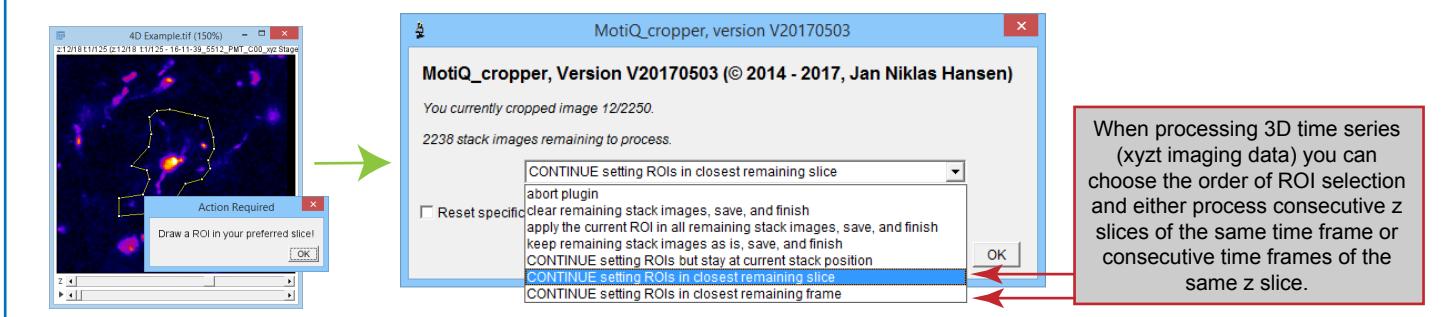
After launching *MotiQ cropper*, a dialog will open. Select the following options and press ok:



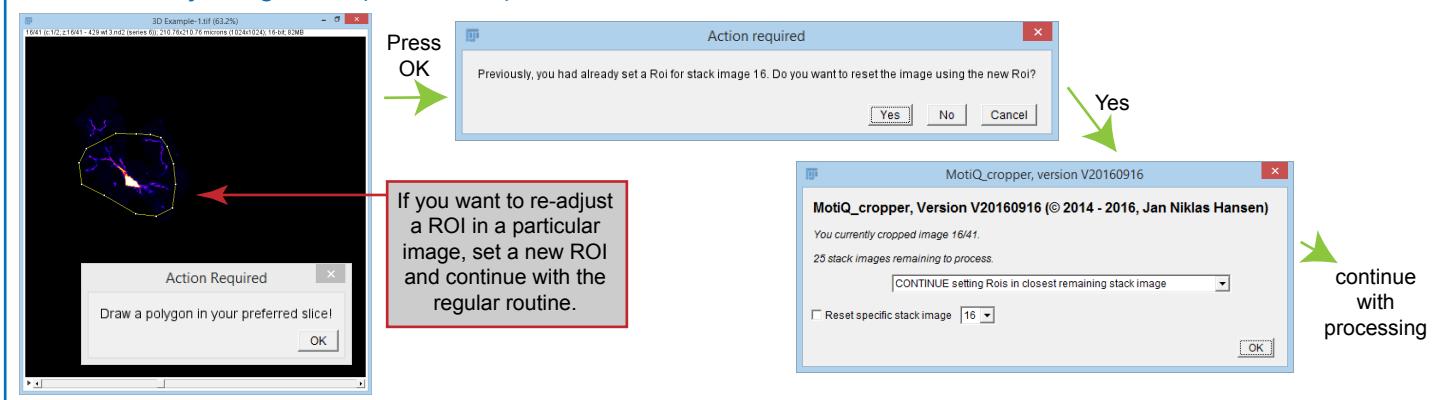
In the image display window, select and confirm a ROI that outlines a single microglial cell and its cell processes. Then proceed to consecutive time frames and z slices. After processing of all images is completed, processed images and metadata are automatically saved in the same directory, where the original image stack was saved while opening:



### Box 1: Processing 3D time series



## Box 2: Re-adjusting ROIs (alternative)



## B) Image segmentation (*MotiQ threshold*er)

*MotiQ threshold*er is a tool for automated and standardized background removal and image segmentation of 2D and 3D images and time series. It offers a variety of thresholding strategies. A list of recommended settings for different imaging data is provided in Supplementary Table 1 of the original publication. *MotiQ threshold*er processes all 8-, 16-, and 32-bit grayscale images and contains a multi-task manager to automatically process large data sets (Manual, page 9).

For optimized image segmentation, *MotiQ threshold*er creates a copy of the input image that serves as a reference image for threshold calculation (section **Reference image** in the settings dialog). The reference image is then automatically pre-processed in order to optimize the image histogram for threshold calculation (section **Pre-processing** and, partly, section **Threshold determination**). Next, an intensity threshold is automatically calculated in the reference image (section **Threshold determination**) and applied to the original input image, generating either a background-depleted or a binary image (section **Image segmentation and output**).

To launch *MotiQ threshold*er go to the menu of ImageJ: Plugins > MotiQ > MotiQ threshold.

Different options for selecting input images, including a multi-task manager, are offered:  
the active, open image  
multiple images (use multi-task manager to open images)  
all open images

Check box if single-cell images derived by *MotiQ cropper* are processed. The threshold of single-cell images should best be calculated in the original image that was processed with *MotiQ cropper* (i.e., alternate reference image) in order to include enough background and to avoid that blank areas (cleared by *MotiQ cropper*) hamper threshold calcuation.

Do not check box if other images (i.e., not derived by *MotiQ cropper*) are processed. In this case, a copy of the input image is automatically generated as a reference image for pre-processing and threshold calulation. All \*-marked settings are then obsolete and do not need to be selected.

Scaling down image resolution in the reference image reduces noise for improved threshold calculation.

When processing time series, thresholds can be calculated for each time step individually - recommended when image intensities largely vary among time steps.

A threshold can be calculated locally for every pixel within a circular ROI of a specified radius around the pixel.

Holes within foreground structures can be filled and thereby added to the foreground.

Check box if single-cell images derived by *MotiQ cropper* are processed. With this function the alternate reference image (i.e. the original image that was processed with *MotiQ cropper* and saved in the same directory) is automatically identified and loaded via the file name and suffix. For example, for an input image <3D Example\_CUT\_160918\_153804\_X143\_Y171\_Z4.tif>, the reference image <3D Example.tif> is opened and used as the reference image for pre-processing and threshold calculation. If not checked, a dialog will be offered to manually select a reference image.

Check box if the input image is derived by *MotiQ cropper* and local intensity levels vary in the reference image. If checked, theshold calculation is restricted to the region of interest.

Converting input and reference image to 8 bit results in histogram binning to improve threshold calculation.

All current ImageJ threshold algorithms are implemented in *MotiQ threshold*er and can be freely selected.

Choose between different options for calculating and applying the threshold in image stacks:

- apply average threshold of independent stack-image thresholds
- apply threshold determined in the stack histogram
- threshold every stack image independently
- apply threshold determined in a maximum-intensity-projection**
- no stack processing

If checked, the image background is removed, while intensity information of foreground pixels (pixels above the determined threshold) is fully preserved. Note that intensity information is required to calculate signaling and intensity parameters in *MotiQ 2D* or *3D analyzer*. If this box is not checked, a binary image (with intensity of foreground pixels set to maximum intensity) is generated that lacks intensity information.

Check box to process only selected time frames of a time series.

Images are automatically saved in the original folder under the input image name followed by the suffix ("\_pBIN", or "\_BIN") together with a TXT file containing the *MotiQ threshold*er settings. If not checked, the output image is kept open in ImageJ and no metadata file is saved.

Avoids overwriting of already existing output files by adding date and time of processing in the output file name.

MotiQ\_thresholder - Settings

MotiQ\_thresholder, version 20170503 (© 2015 - 2017, Jan Niklas Hansen)

Process: the active, open image

Reference image

Use alternate reference image

Automatically find alternate reference image in origin directory (via file name)\*

→ Begin of suffix in file name of input image: CUT\*

→ Additional suffix in file name of alternate reference image: .tif\*

Restrict threshold calculation to size and position of input image\*

Pre-processing

Scale down reference image for calculation - factor: 0.5

Convert input and reference image into 8-bit before processing.

Threshold determination

Threshold algorithm: MinError

Stack handling: apply threshold determined in a maximum-intensity-projection

Threshold every time-step separately.

Threshold only distinct time series. Start / end time-step: 1 10

Calculate an individual threshold for each pixel - local threshold radius [px]: 50

Image segmentation and output

Fill holes in mask (independent for every stack image)

Keep original intensities in pixels above threshold

Automatically save image/metadata into origin directory and close image

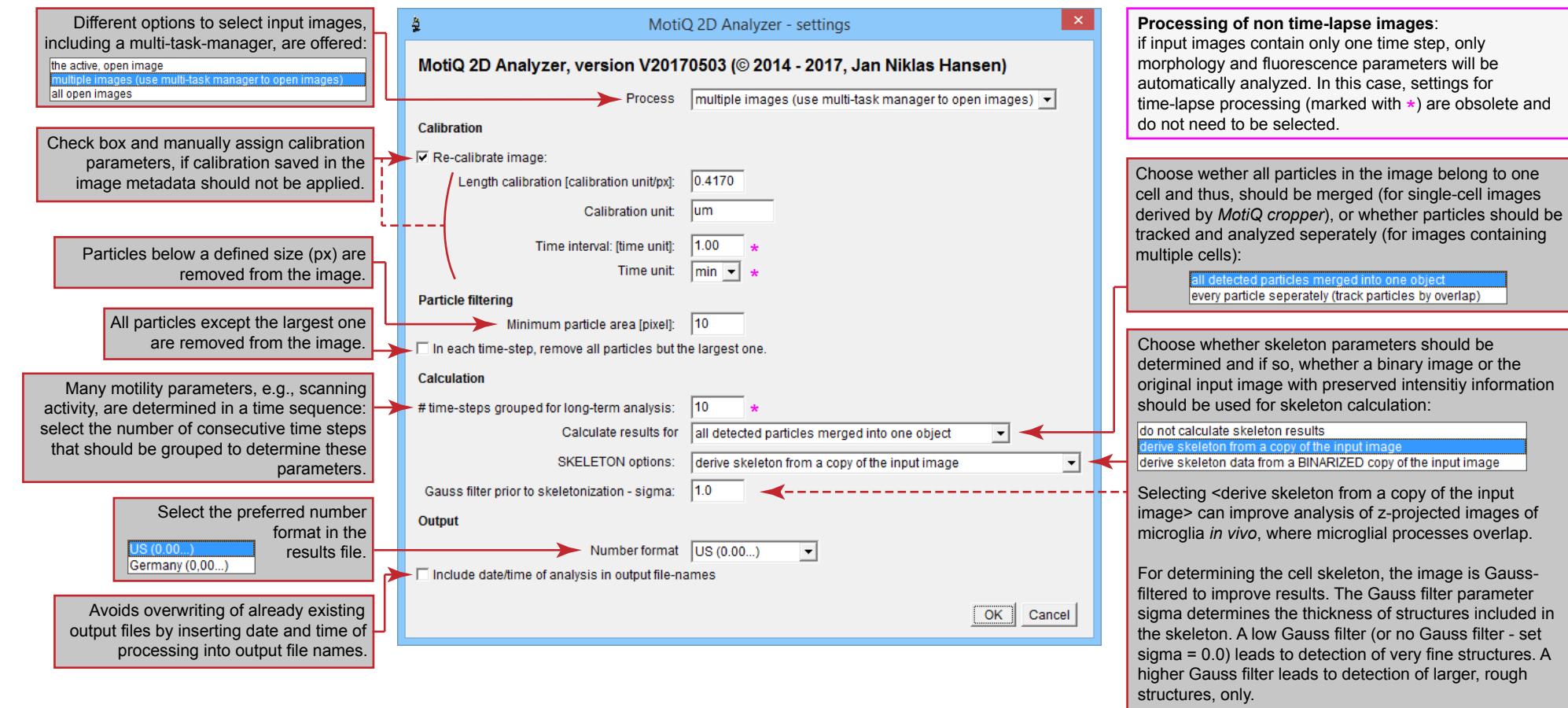
Include date in output file names

OK Cancel

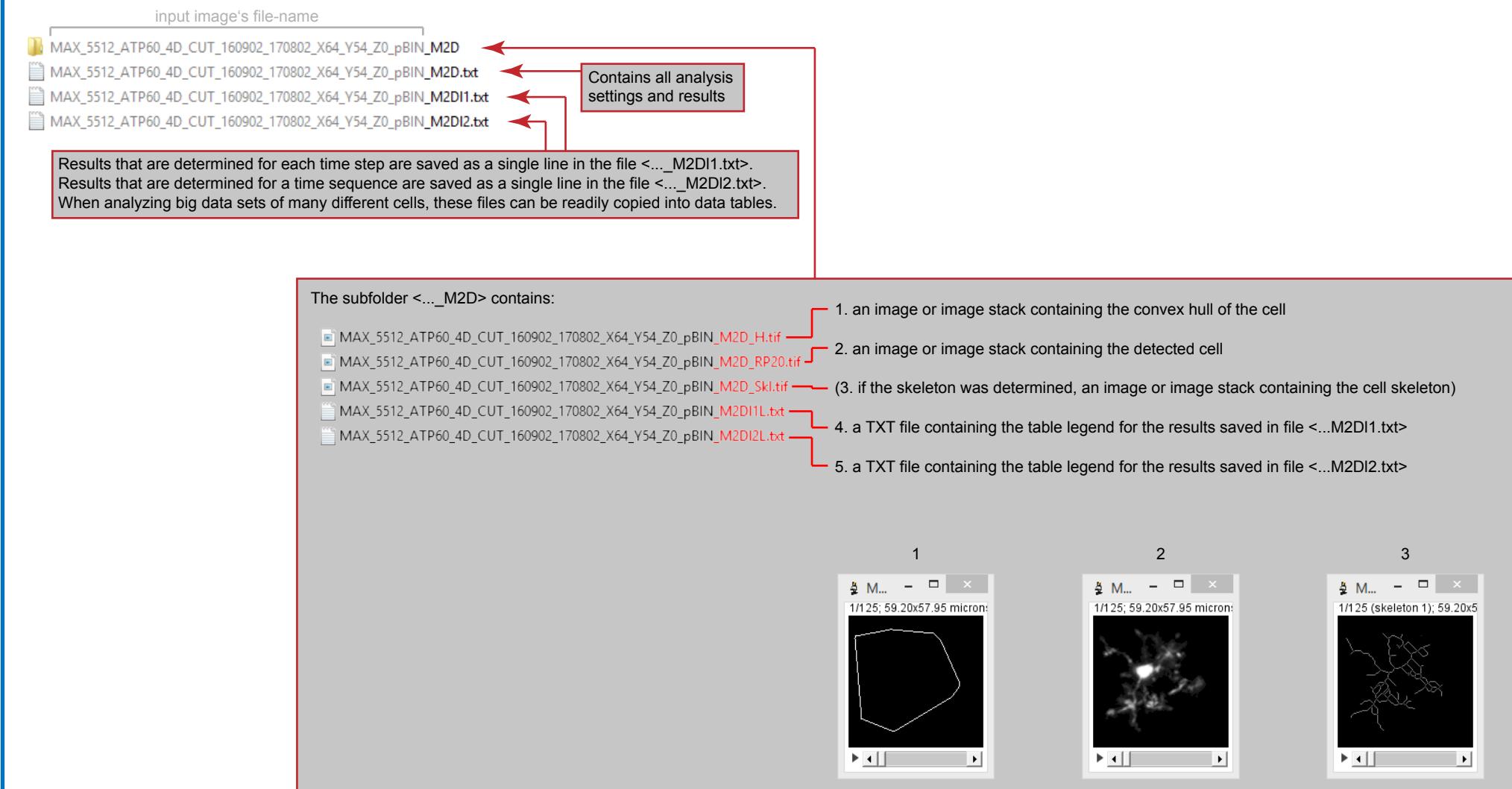
## C) Image analysis

### 2D workflow (*MotiQ 2D analyzer*)

To analyze 2D images or 2D time series, launch the plugin *MotiQ 2D analyzer* via the menu of ImageJ: Plugins > MotiQ > MotiQ 2D analyzer. Several analysis options can be selected in the dialog. *MotiQ 2D analyzer* contains a multi-task manager to automatically process large data sets (Manual, page 9). The output of *MotiQ 2D analyzer* is explained in [Box 3](#) (Manual, page 6).

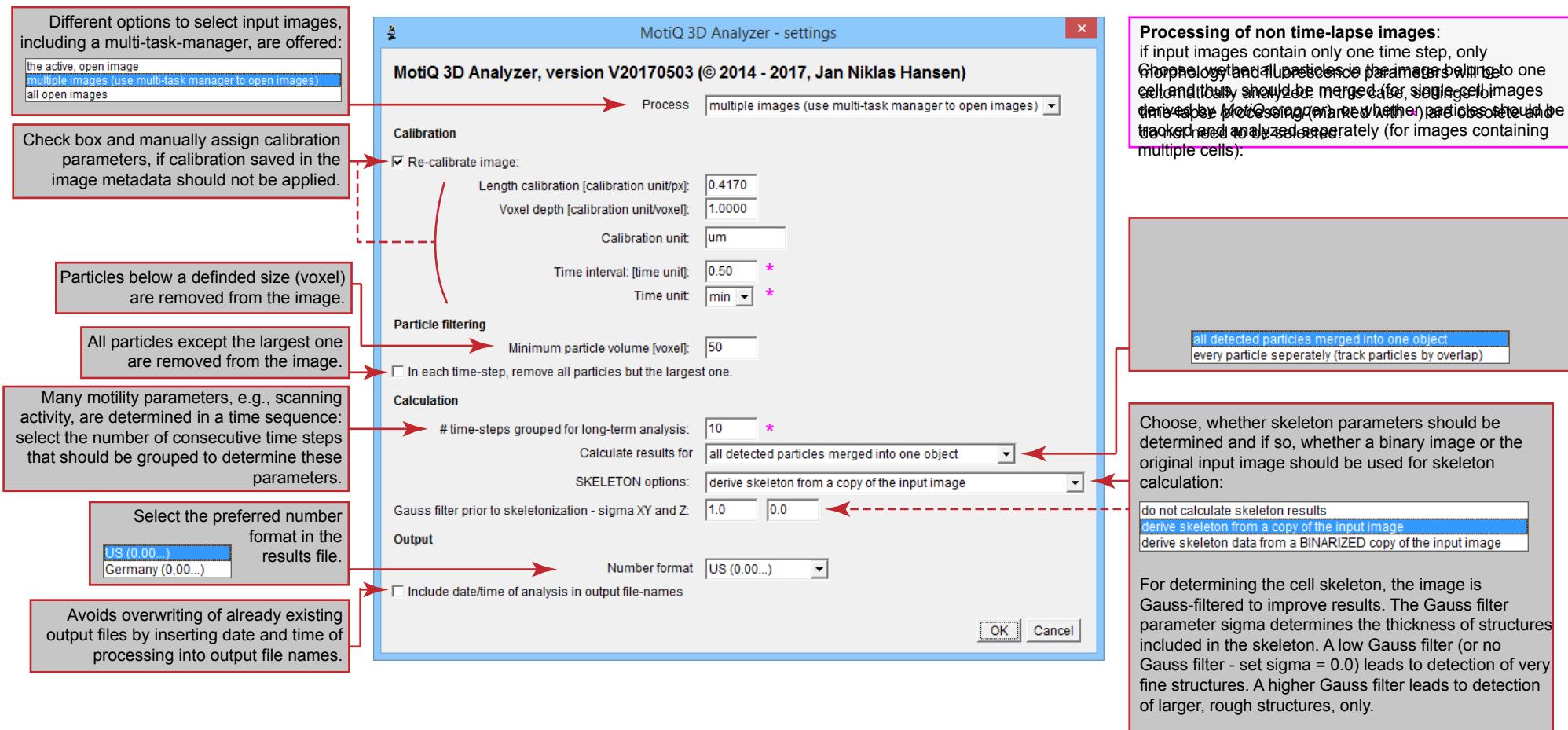


### Box 3: Output of MotiQ 2D analyzer

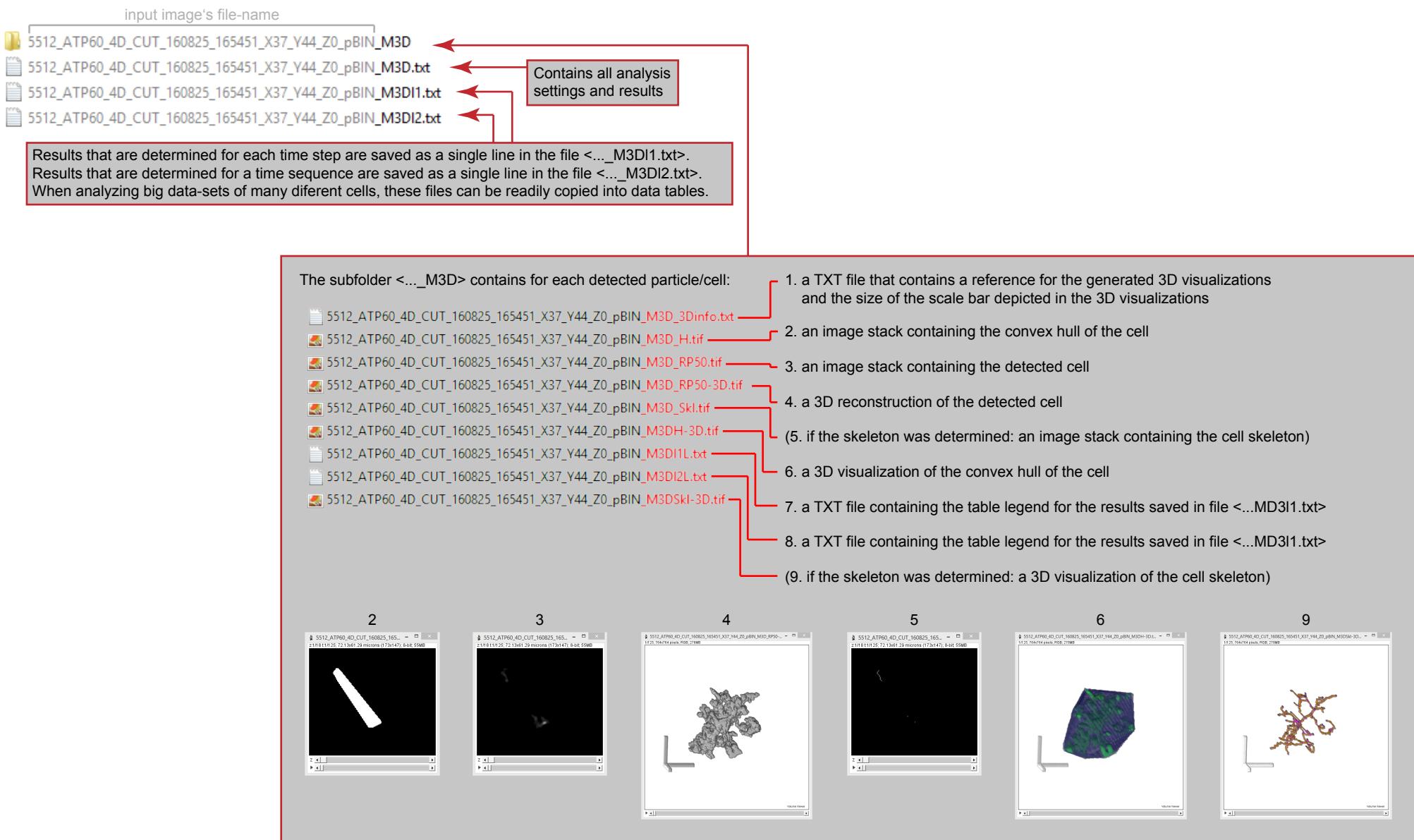


## 3D workflow (*MotiQ 3D analyzer*)

To analyze 3D images or 3D time series, launch the plugin *MotiQ 3D analyzer* via the menu of ImageJ: Plugins > MotiQ > MotiQ 3D analyzer. Several analysis options can be selected in the dialog. *MotiQ 3D analyzer* contains a multi-task manager to automatically process large data sets (Manual, page 9). The output of *MotiQ 3D analyzer* is explained in [Box 4](#) (Manual, page 8).



#### Box 4: Output of *MotiQ 3D analyzer*



## D) Multi-task management

*MotiQ thresholder*, *MotiQ 2D analyzer*, and *MotiQ 3D analyzer* contain a multi-task management (i.e., queue processing) option to process large data sets. If selected in the analysis settings, the multi-task manager dialog opens and data sets can be selected and added for processing.

