

MYOCYTER v1.2 – Quickstart

This is the **quickstart manual** of **MYOCYTER v1.2**, which sets out its **workflow as short as possible**.

MYOCYTER is a **macro** for the free image processing software „**ImageJ**“, enabling **extraction** of an **unmatched amount of parameters** from **videos of contracting cells** like (cardio-)myocytes.

For a better insight into the **technological background**, implementation of different features and the **effects of the available settings**, please take a look in the much more **detailed manual**.

The **latest version of MYOCYTER**, the **quickstart**, the **detailed manual** and several **videos for testing** purposes can be found here: www.scyrus.de

You may also visit our [YouTube-channel](#) or discuss the macro on [GitHub](#).

MYOCYTER v1.2 – Hardware

- Please be aware that **ImageJ or FIJI** require lots of **RAM (16 GB+)** for processing of large and/or high-resolution video material.
- **Video material** should be either **uncompressed or in „Motion JPEG“ (MJPEG) format** in order to be processible with ImageJ or FIJI.
- The **framerate** of the material should be at **least 120 frames per second** (fps).
- The experimental setup can be even reduced to a **common transmission light microscope** with a **middle class mobile phone with „slomo“ function** (high-speed video recording at +120 fps) **attached to the ocular** using an adapter (like *Vizzlema* “Universal”).
- MOV-, MPEG- or MPG-videos can be **converted to MJPEG** via freeware (like „FFmpeg“).

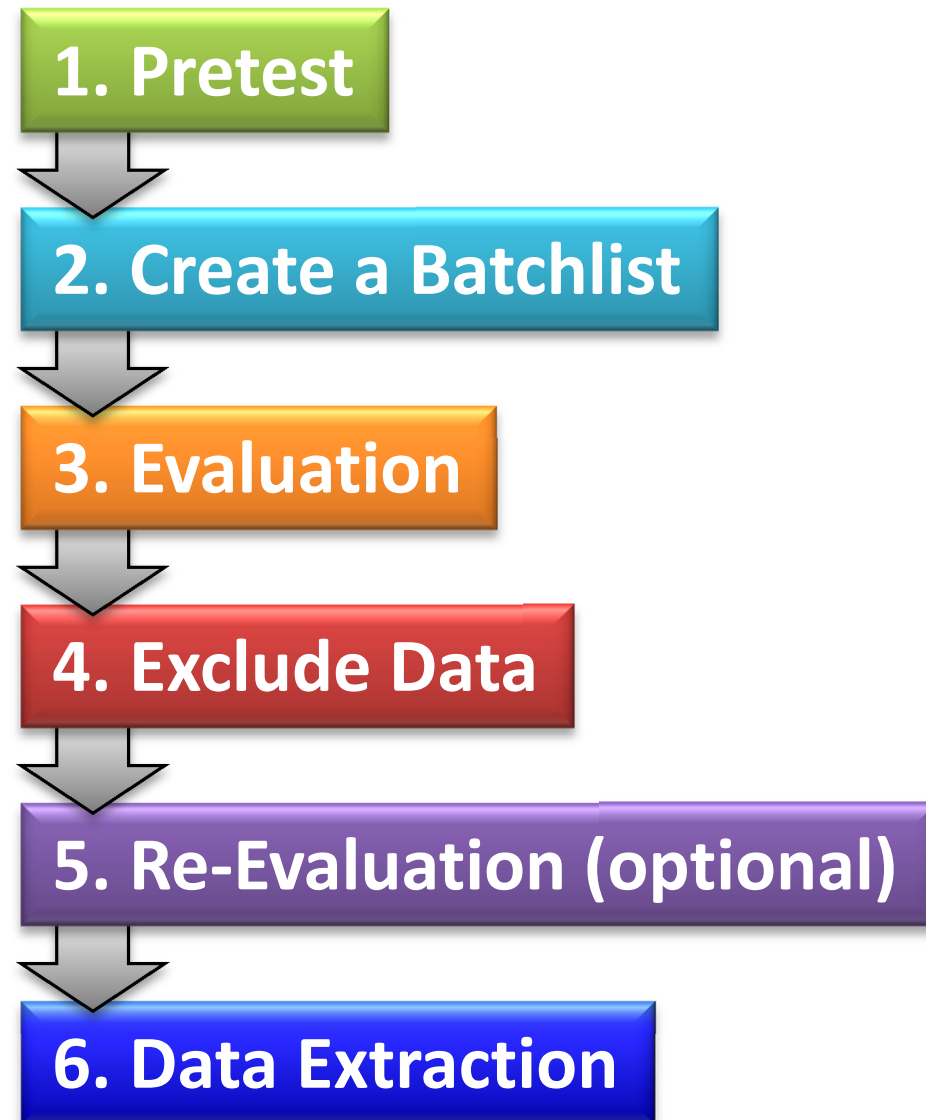
MYOCYTER v1.2 – Changes and updates

Compared to the previously published version 1.0 of MYOCYTER (*ScientificReports*), **version 1.2 provides:**

- **3 completely new features:**
 - **Use of batch lists:** Enabling multi-file evaluation with individual settings applied
 - **Exclusion of data:** Exclude recognized cells after evaluation via mouse click
 - **Data extraction:** Draws the essence from huge datasets for further statistics
- **Improved evaluation:** Enhanced detection in noisy videos with low amplitudes
- **Improved workflow:** Just a few steps from video files to statistical data.

The Workflow of MYOCYTER v1.2

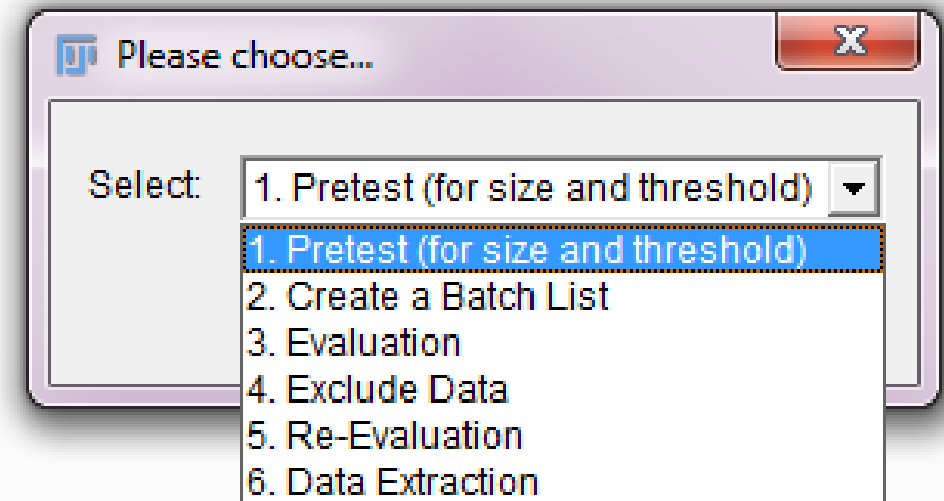
This is both the new **menu** and the **typical workflow of MYOCYTER v1.2**:



The Workflow of MYOCYTER v1.2

0. Before you start

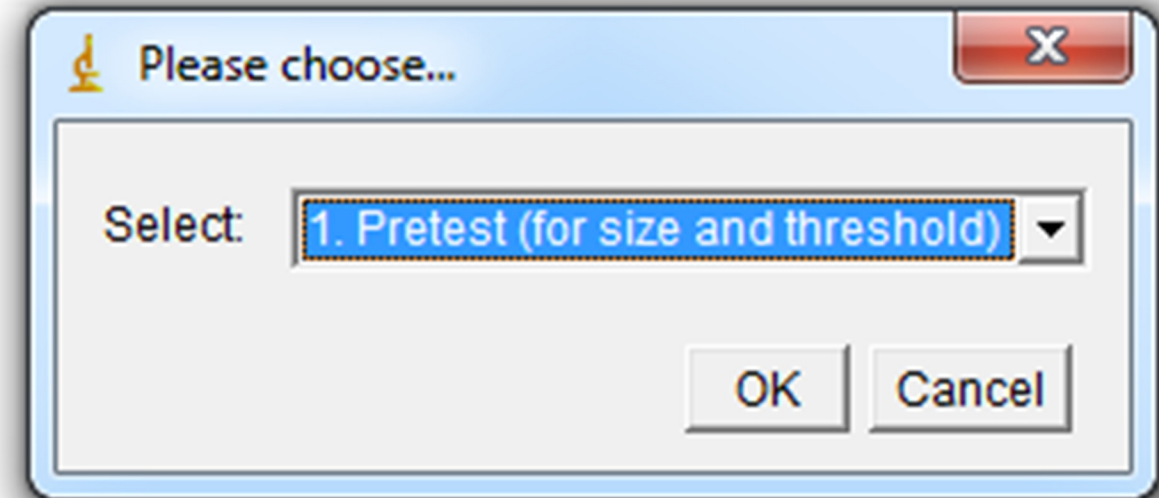
- You have all your videos in an **ImageJ-compatible format** (**MJPEG** or **uncompressed *.avi** files) in a folder.
- You need **ImageJ v. 1.52q** (or higher).
- You need the **MYOCYTER-Macro** as *.txt file.
- A computer with **≥16 GB of RAM** and a **64-bit operating system** is recommended.
- Open the file containing the macro code, copy it and **start ImageJ**.
- Select „Plugins“ → „Macros“ → „Record“.
- Paste the code into the new window („**Recorder**“), click the „**Create**“-button and close the „Recorder“-window.
- The macro code is now in a new window („**Macro.ijm**“). Select „Macros“ → „Run Macro“ (or press „Strg+R“).
- MYOCYTER starts and you should see its **user-interface**.



The Workflow of MYOCYTER v1.2

1. Pretest

- The **pretest** assures **optimal recognition** of contracting cells and **exclusion of the background**.
- Select the first option „**1. Pretest (for size and threshold)**“ from the user interface and click „OK“.

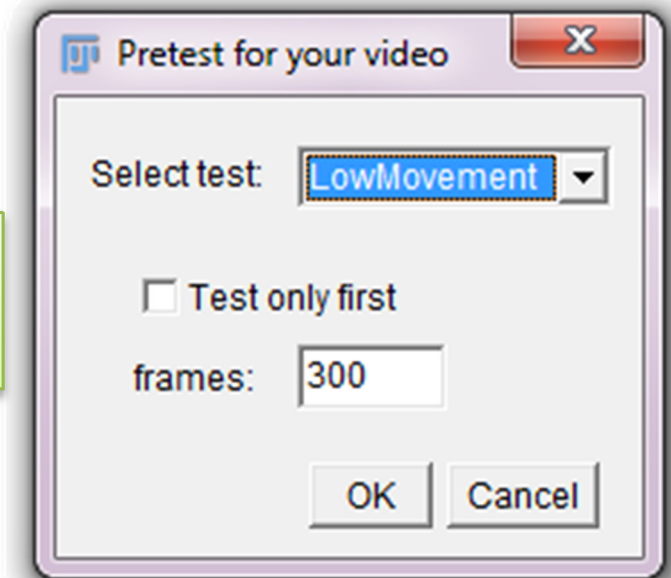


The Workflow of MYOCYTER v1.2

1. Pretest

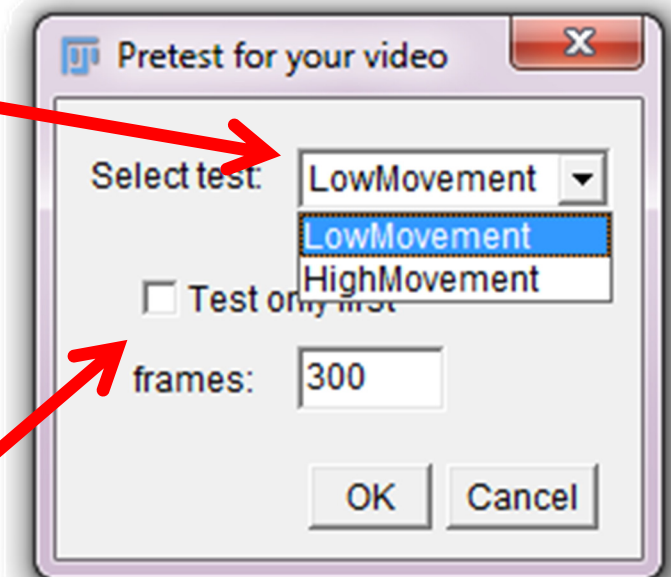
- Stick with the **default settings** (for the beginning).
- **Select** the folder containing your videos, **DO NOT open** it, click „**OK**“ and **wait** until the macro finished.
- A new folder („**pretest**“) should be found in the folder containing your videos.

Default settings



Use „LowMovement“ for cells that show not much contraction – which setting to use comes with some experience.

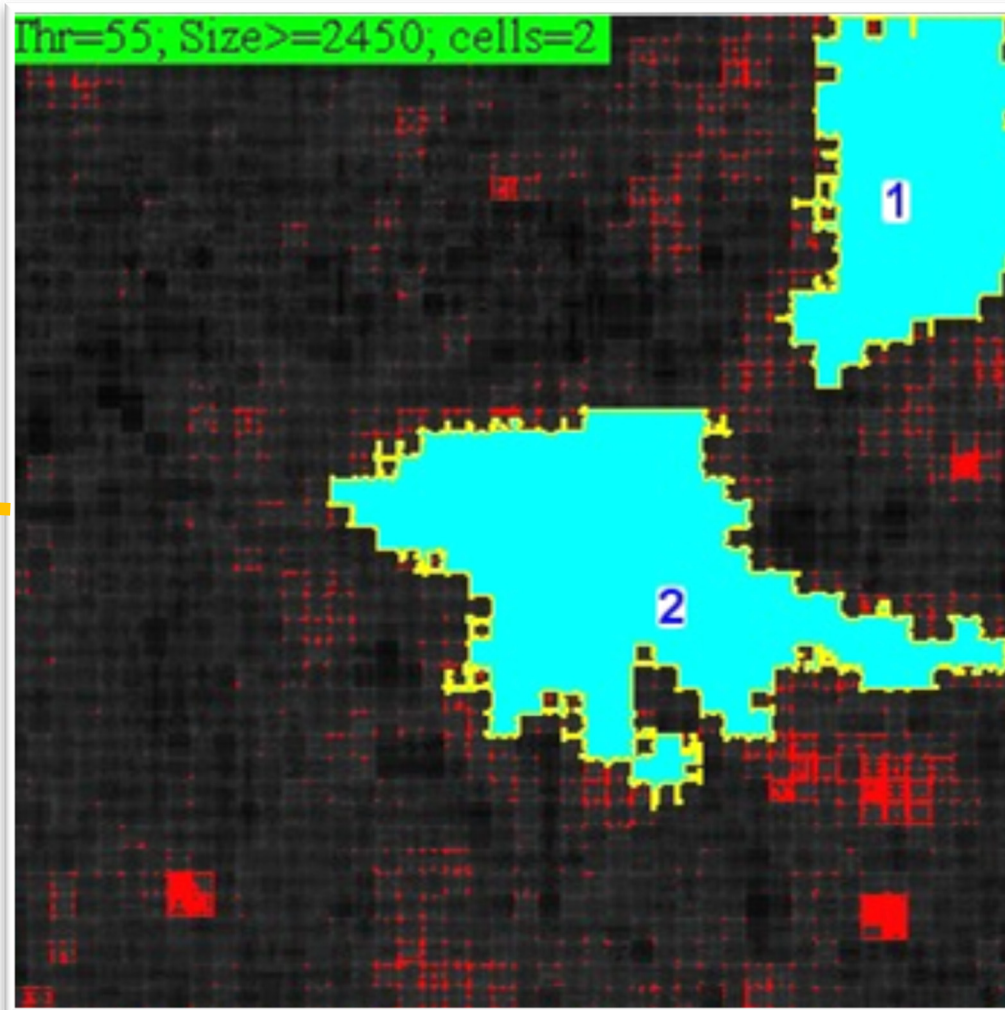
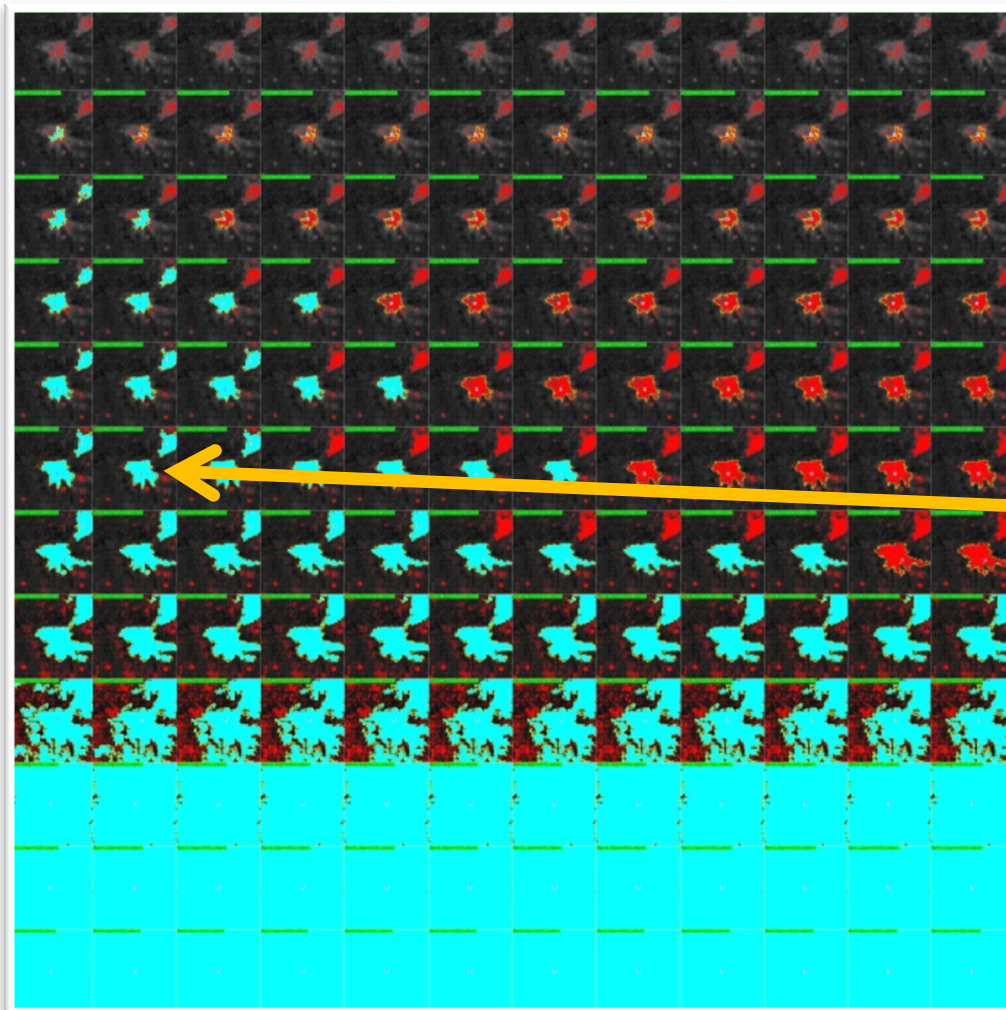
To save time, pretest only the first xxx frames of your video



The Workflow of MYOCYTER v1.2

1. Pretest

The **images of the pretest** should look like this:

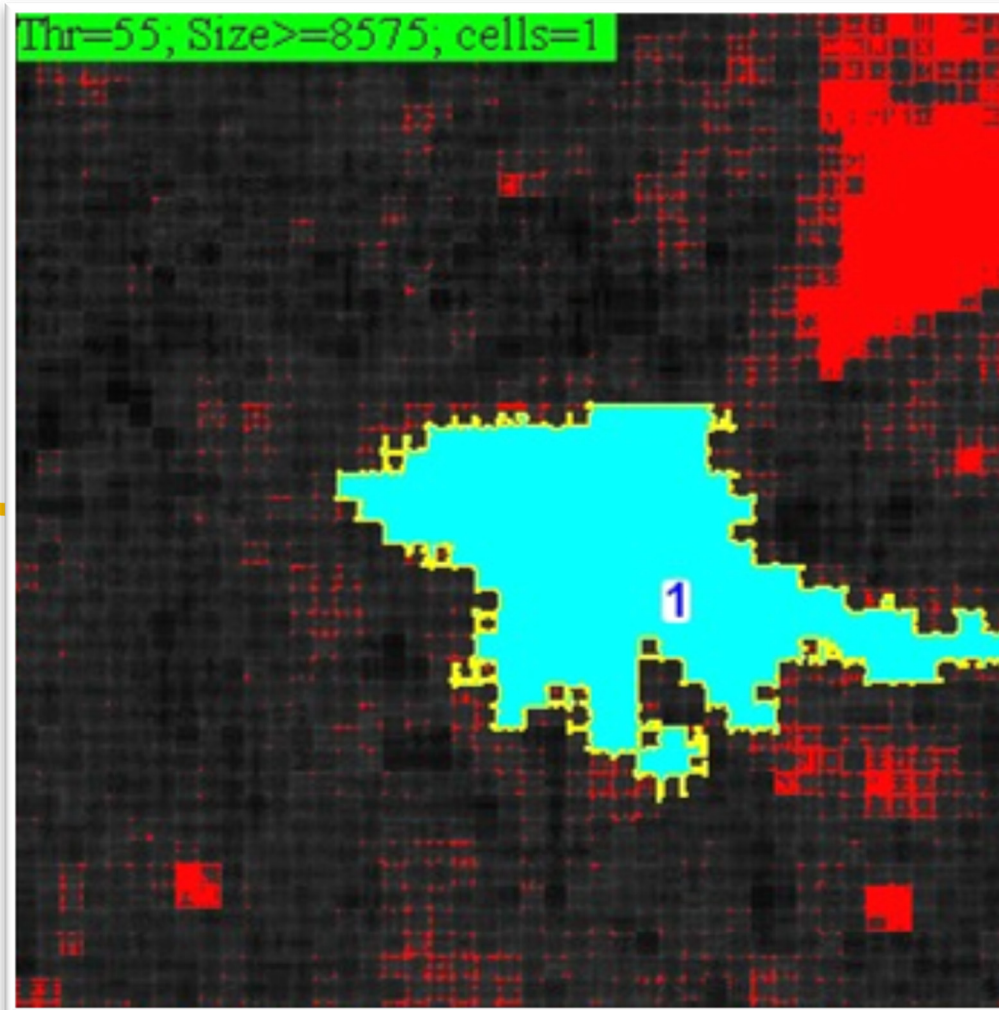
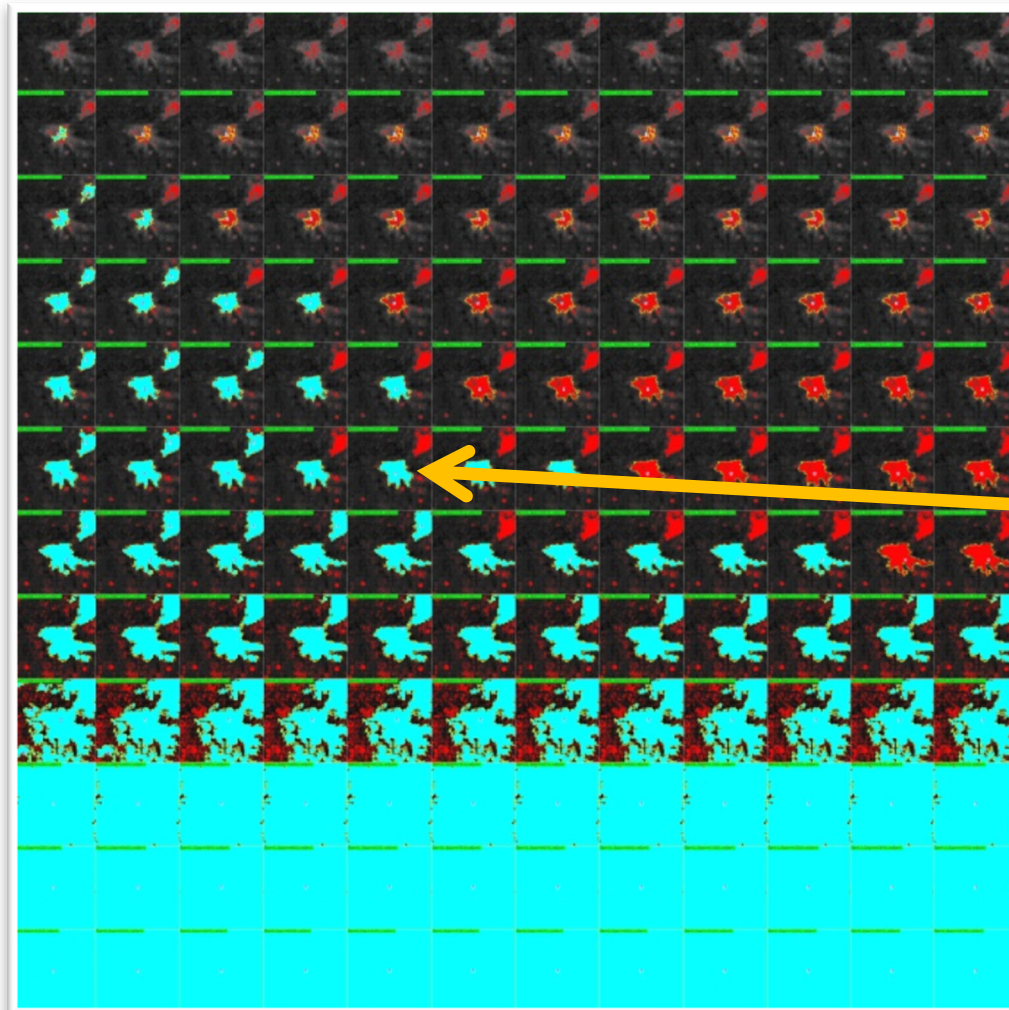


Two cells have been recognized. To restrict evaluation to the moving cells only, we could apply (in this case) a **Threshold of 55** and a **Size of 2450** (right image). **Only the turquoise areas** will be evaluated with the according settings, while the **background is completely excluded**.

The Workflow of MYOCYTER v1.2

1. Pretest

The **images of the pretest** should look like this:

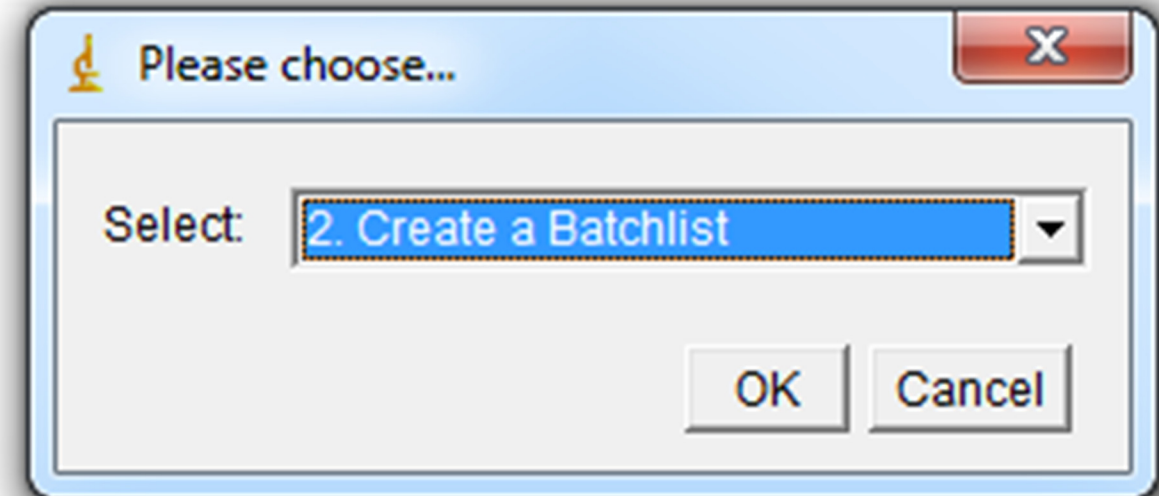


Two cells have been recognized.
To exclude the one cropped through the picture frame, apply a **Threshold of 55** and a **Size of 8575**.
Only the turquoise areas will be evaluated with the according settings, while the **background is completely excluded**.

The Workflow of MYOCYTER v1.2

2. Create a Batchlist

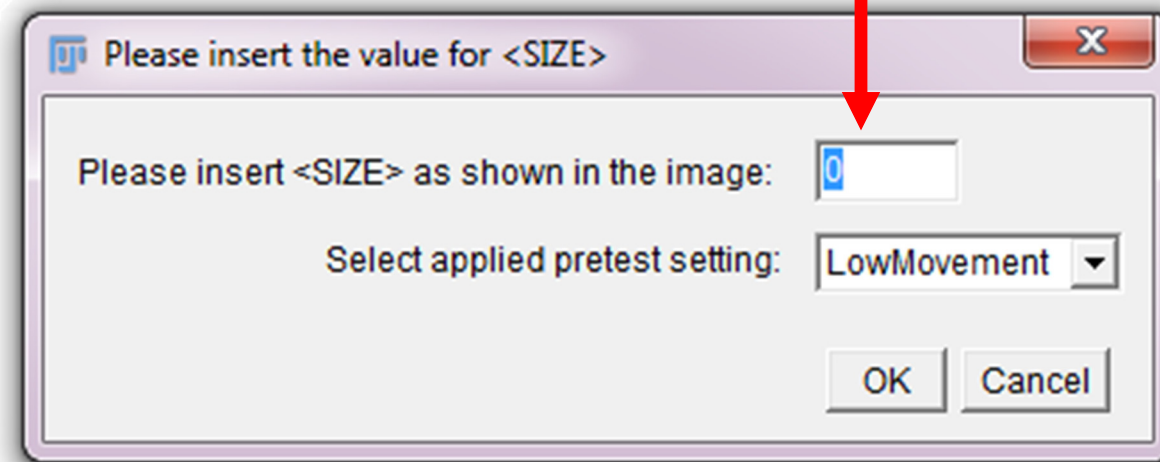
- Application of **individual settings** for **Threshold** and **Size** from „1. Pretest“ **to every single video** can be completely automatized.
- Select option „**2. Create a Batchlist**“.
- **Just select** the folder containing your pretest images, **DO NOT** open it.
- Click „**Okay**“.



The Workflow of MYOCYTER v1.2

2. Create a Batchlist

- An image opens and you will be asked to insert the „**Size**“-value (left image) into the **according field** (right image).



Please type the value for „**Size**“ in this field (in this case 1225). This has to be done **only once**.

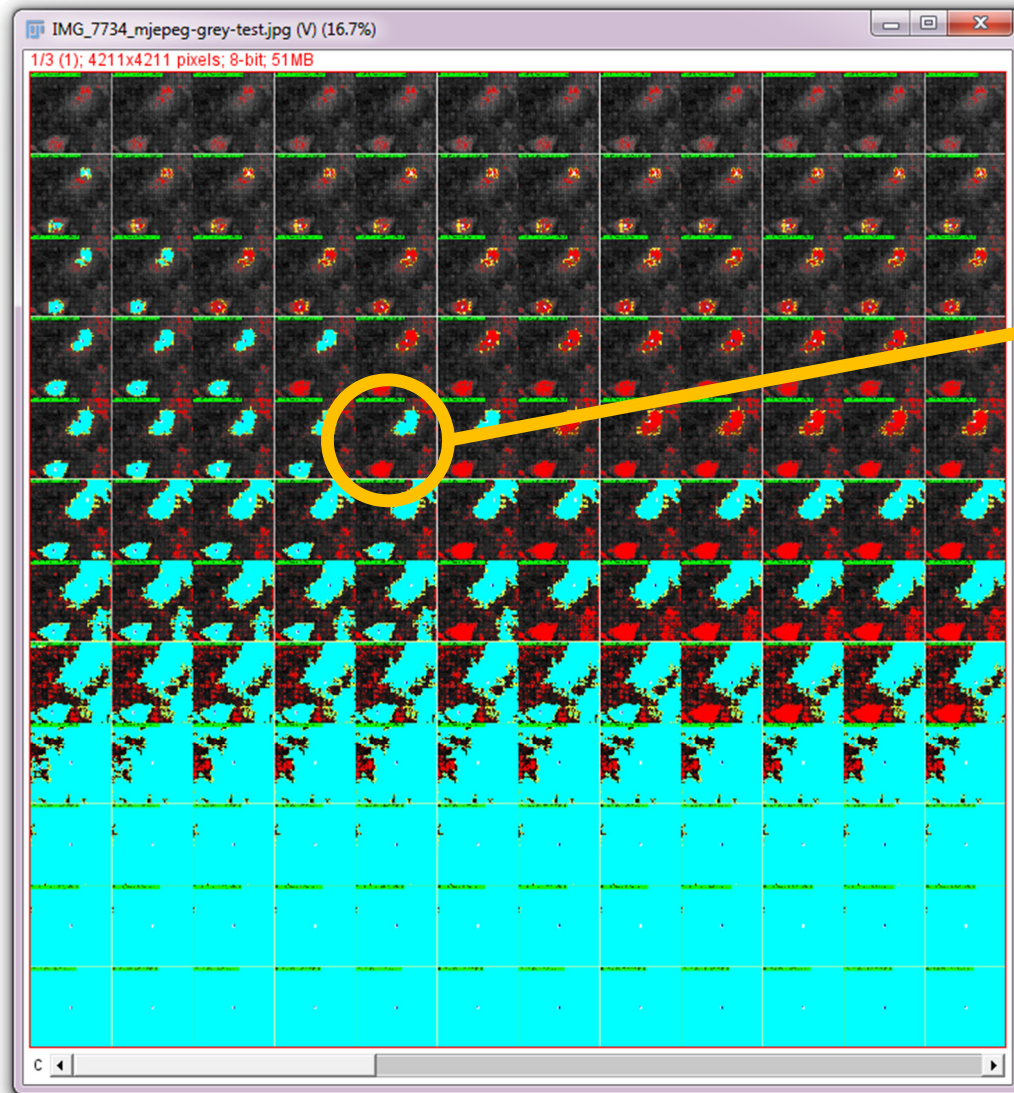
NOTE: All videos in the folder should have same dimensions!

- After this, please select „**LowMovement**“ or „**HighMovement**“ – depending on your **pretest settings**. This information is also given in the **name of the folder** containing the pretest images. For example: „pretest (**LowMovement**) 2019-Sep-21 22h09“.
- Click „**OK**“ to start selection of the individual settings.

The Workflow of MYOCYTER v1.2

2. Create a Batchlist

- Now, **just left click the panel** of the pretest images **matching the settings you want to apply**.



Just **left click** the **desired panel** (small images).
To **magnify the image**, you can use the **mouse wheel** while pressing „**Strg**“.
After selection, the current pretest image will be closed and the next one opens automatically until every single one has been processed.

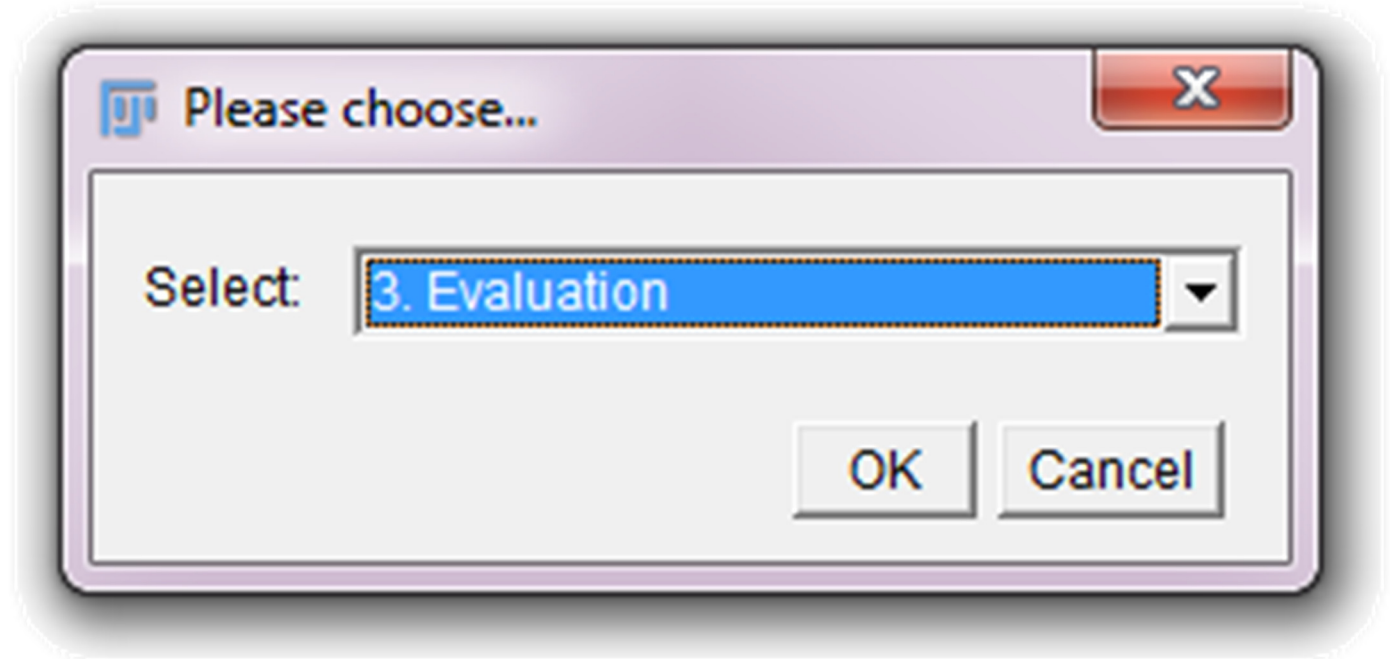
2. Create a Batchlist

- After processing the last pretest image, the **individual settings** will be found as table (**batch.txt**) in the pretest folder.
- **Copy this file (batch.txt)** into the **folder containing your videos**.
- Using the file **batch.txt**, MYOCYTER applies individual settings for every single video during evaluation. **ONLY** the videos **actually listed in this file** will be processed.

The Workflow of MYOCYTER v1.2

3. Evaluation

- Select „3. Evaluation“ and click „OK“.



The Workflow of MYOCYTER v1.2

3. Evaluation

Creates an animated plot of every single recognized cell synchronized with its amplitude. This takes time, so you may apply this feature only to selected videos.

Higher values will recognize smaller amplitudes as contractions. **Can be changed even after evaluation.**

If checked, you will be asked to define the **resting state** of every single cell manually. Usually, **automatic recognition** of the **reference-frame** works very fine, so this field can be left unchecked.

Activate „**Batching**“ if you want to use a **Batch List**; the values for „Lower Threshold“ and „Size“ can be ignored and are over-written by the individual ones of the list.

Please insert values for lower t...

Lower Threshold: 22

Size: 1600

☐ Video output

[%] of max recognized as beat: 20

Detection: 4

☐ Smoother

☐ Force reference-frame

☒ Replace zero from RefFrame

☒ Batching

OK Cancel

If you have **only a single video** or videos with **very similar cells**, you can apply pretest-values for **Threshold** and **Size** directly here without creating a batch-list and **deselect „Batching“**.

If you want to **evaluate the whole frame** without restriction to the moving cells only, apply a **Threshold of 0** (Sizes becomes irrelevant in this case) and **deselect „Batching“**.

If the **amplitude** exceeds this value (here: 20%) of its **lokal peak**, the times for **systole** and **diastole** are measured. The values **10, 50** and **90%** are applied anyway. This value **can also be changed even after evaluation.**

The Workflow of MYOCYTER v1.2

3. Evaluation

- After applying the desired **settings for evaluation**, click „**OK**“.
- **Just select** the folder **containing your videos** (and also the file „**batch.txt**“, if you activated „**Batching**“), **DO NOT** open it.
- Evaluation **may take some time**, depending on filesize and number of your videos. After finishing evaluation ALL windows will be closed automatically.



Please, **DO NOT** use the computer for **any other task during evaluation** – this **may lead to premature termination** of MYOCYTER.

The screenshot shows a dialog box titled "Please insert values for lower t...". It contains several input fields and checkboxes. The "Lower Threshold" is set to 22, and the "Size" is set to 1600. There are checkboxes for "Video output", "Smoother", "Force reference-frame", "Replace zero from RefFrame", and "Batching". The "Detection" field is set to 4. The "[%] of max recogized as beat" field is set to 20. The "OK" and "Cancel" buttons are at the bottom right.

Lower Threshold:	22
Size:	1600
<input type="checkbox"/> Video output	
[%] of max recogized as beat	20
Detection	4
<input type="checkbox"/> Smoother	
<input type="checkbox"/> Force reference-frame	
<input checked="" type="checkbox"/> Replace zero from RefFrame	
<input checked="" type="checkbox"/> Batching	
OK	Cancel

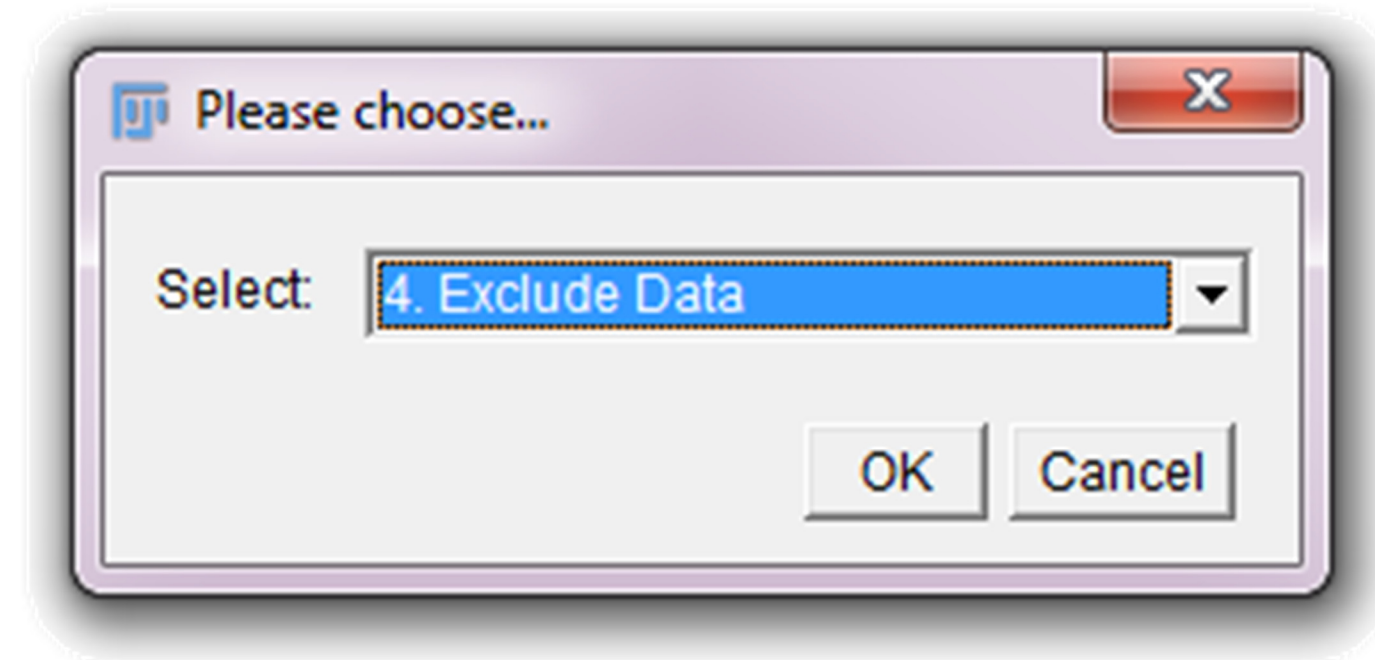
4. Exclude Data

- After evaluation, new **folders are created** in the one containing your videos: This folder should have the **subfolders „diffMov“ → „dataplots“**.
- The **folder „dataplots“** contains (amongst other things) **plots of the amplitude of every single recognized cell** as well as a **text-file named „Results“**.
- Possibly also cells were evaluated the **user wants to exclude from further analytics** - nobody wants to do this manually in a table that contains several tens of thousands lines.
- This process (**excluding data**) is largely automatized, the **original data remain untouched**.

The Workflow of MYOCYTER v1.2

4. Exclude Data

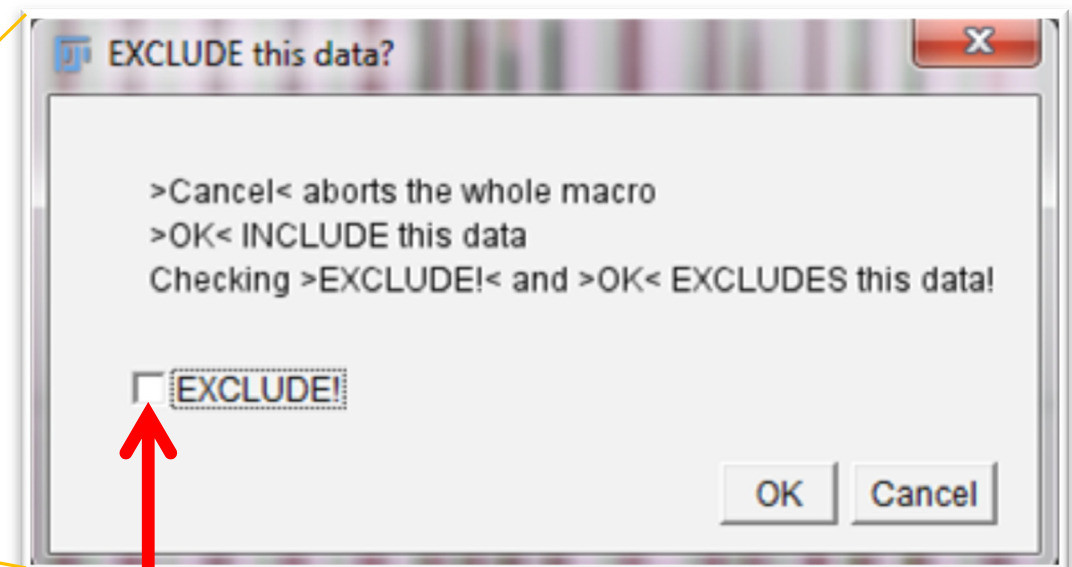
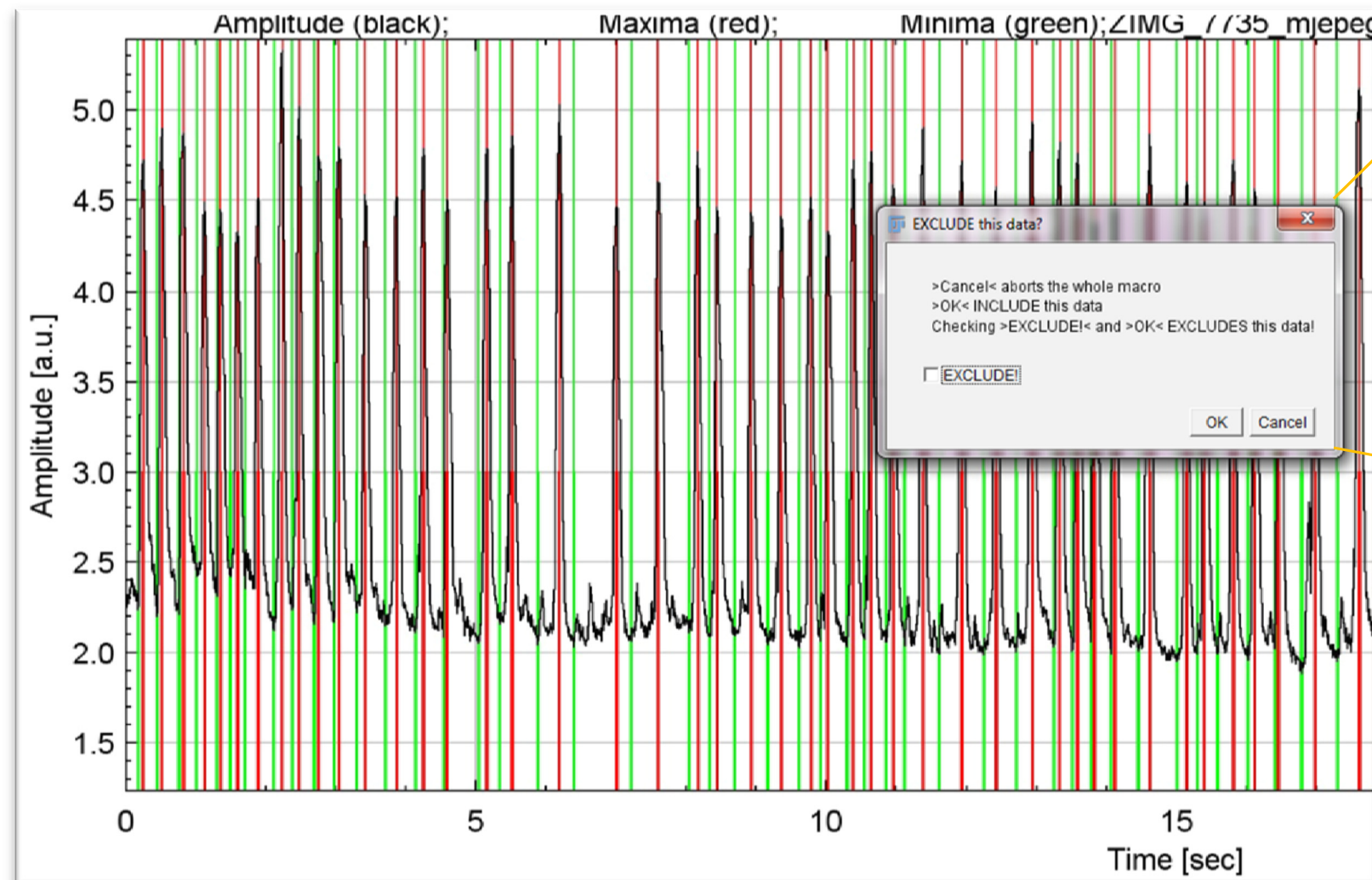
- Select „4. Exclude Data“ and click „OK“.
- **Just select** the „dataplots“ folder created after evaluation (**DO NOT open** it), containing the **amplitude plots** and the „Results“ (as text file).
- After selecting your data, you will be asked again to choose **another** „dataplots“ folder until you skip the macro.



The Workflow of MYOCYTER v1.2

4. Exclude Data

- Now, you will be asked to **decide for every single recognized cell** if the according **data should be EXCLUDED**.



Click „OK“ to **INCLUDE** the shown dataset.
Check „EXCLUDE“ and click „OK“ to **EXCLUDE** it.

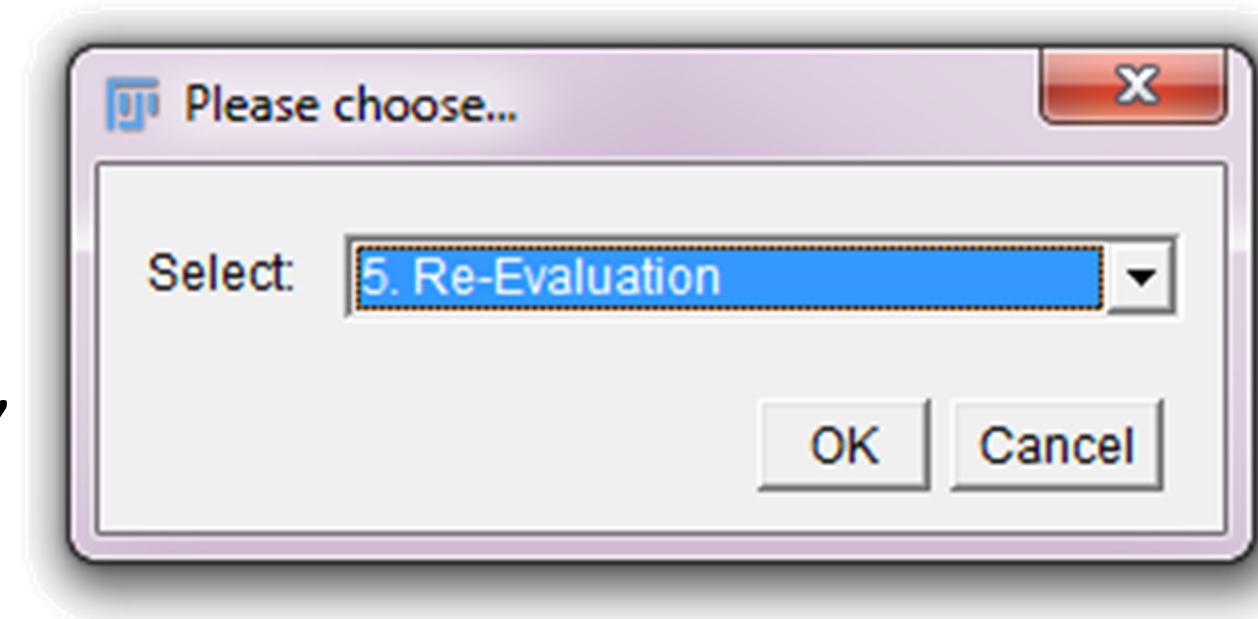
4. Exclude Data

- A new file is generated in the „**dataplots**“ folder:
„**Results (selected)**“.
This file contains **only the data you included** as well as a **list of the excluded data**.
- You can now process/compare this data table in a stats software.

The Workflow of MYOCYTER v1.2

5. Re-Evaluation

- Using „**5. Re-Evaluation**“, you can apply changed settings to your data, even after evaluation.
- Select „**5. Re-Evaluation**“ and click „OK“.
- Go to the „**dataplots**“ subfolder of your evaluation, **open it** and select the „**Amplitudes only**“ text file.



5. Re-Evaluation

- Now, you are asked to **apply other settings** to your **evaluated data**.

Please apply your...

Detection	20
Threshold 1 [%]	10
Threshold 2 [%]	20
Threshold 3 [%]	50
Threshold 4 [%]	90
Framerate [fps]	120

OK Cancel

Higher values for „**Detection**“ include smaller local **maxima** as detected peaks/contractions.

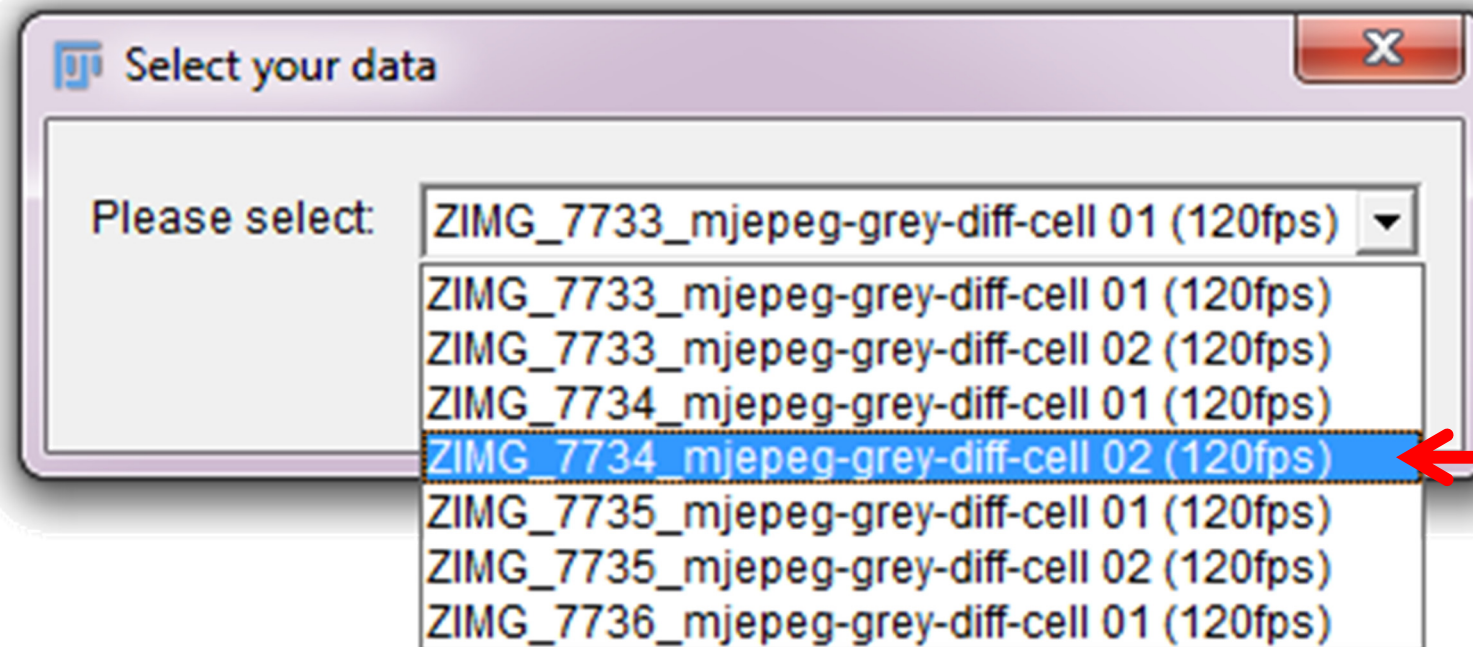
Apply **four different thresholds** to the extracted **amplitude**. Please see the manual for details.

Change the detected framerate of the video (if necessary).

The Workflow of MYOCYTER v1.2

5. Re-Evaluation

- And to **select the dataset** you want to re-evaluate.

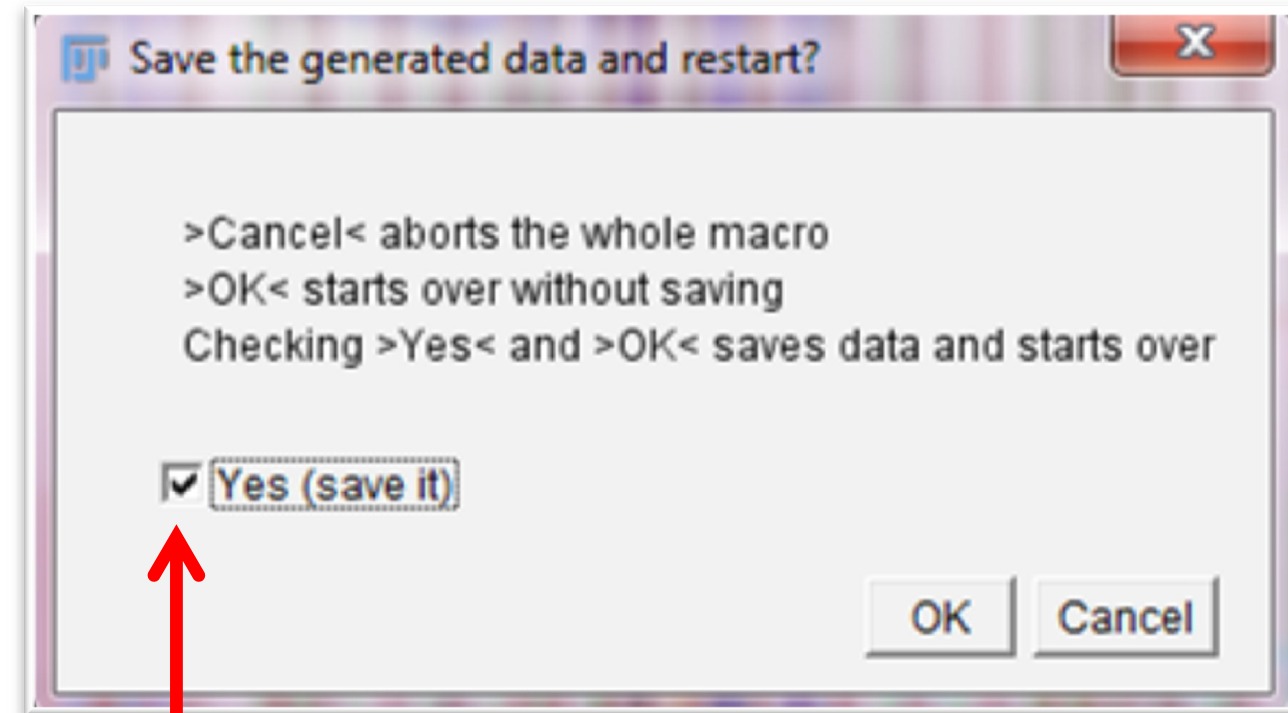
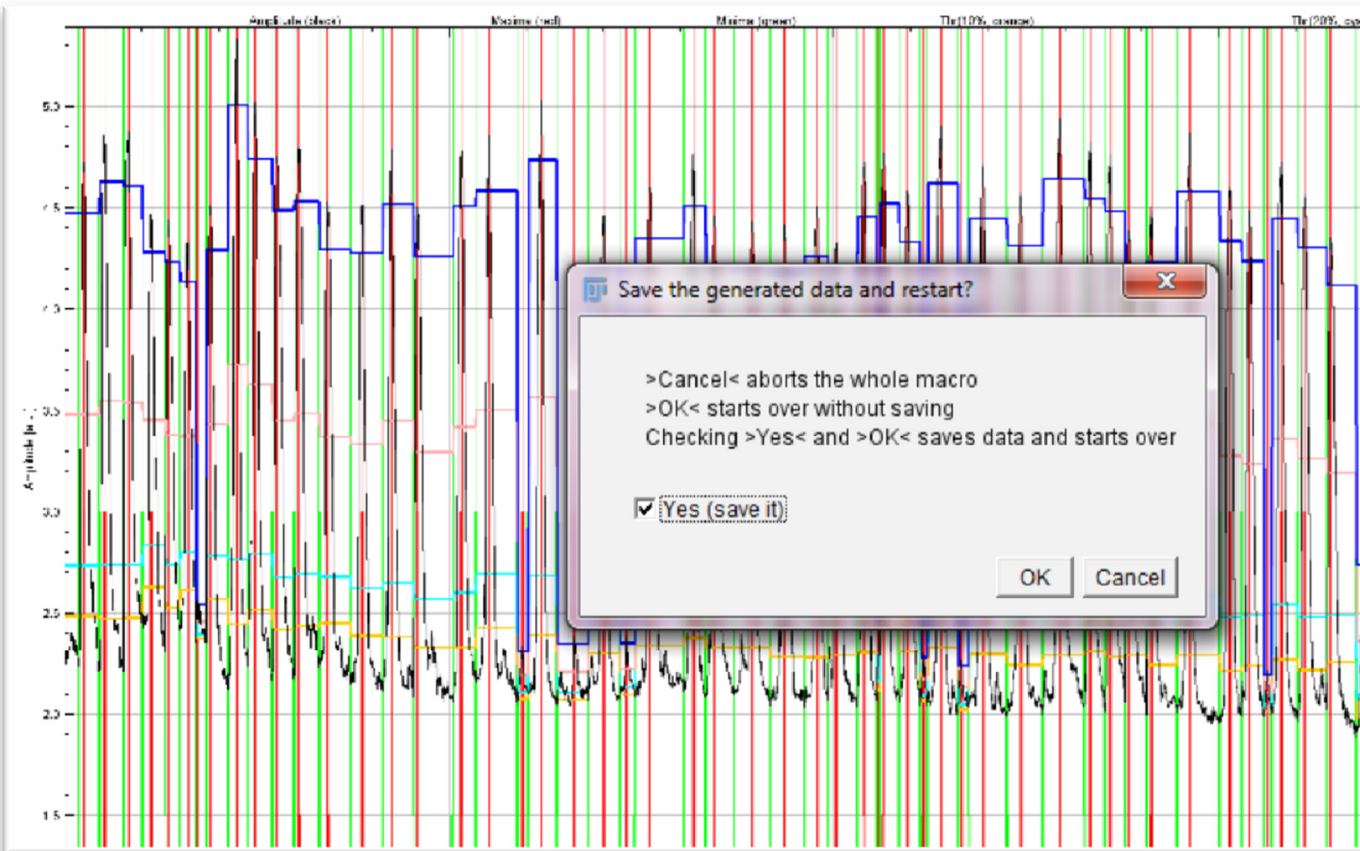


Every single cell from your evaluation is listed here – just pick one.

The Workflow of MYOCYTER v1.2

5. Re-Evaluation

- And to **select the dataset** you want to re-evaluate.



Check „Yes“ and click „OK“ to save the new plot as well as the new results table.
Just click „OK“ to restart re-evaluation with other settings and data (without saving).

5. Re-Evaluation

- All **re-evaluated data** will be **stored** both **as plots**:

old filename + „re-evaluated“ and the current date and time (JPEG-format)

and **as a single data table**:

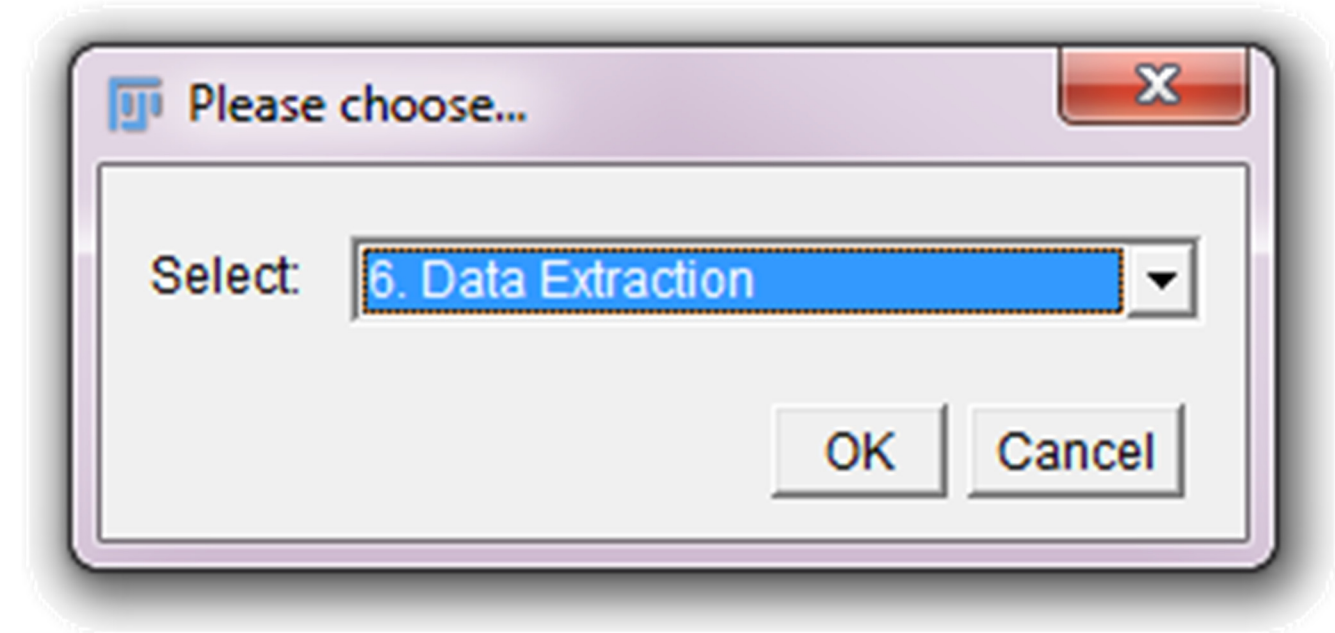
„Amplitudes only“ + „re-evaluated“ and the current date and time (text file).

Nevertheless, this „Amplitudes only re-evaluated“ file contains all information available also in a „Results“ file.

The Workflow of MYOCYTER v1.2

6. Data Extraction

- Evaluation provides both „continuous“ data for plotting (like the amplitude over time) or „discrete“ data for statistics (like only the maximum amplitude for every single contraction).
- This feature extracts all „discrete“ data from a „Results“ file for further data processing in statistical software.
- Select „6. Data Extraction“ and choose a „Results“ file.



6. Data Extraction

- **14 different discrete parameters** from the whole file will be extracted completely, **without any information about** the according **files and recognized cells**.
- This **feature is only useful** if **ALL cells** in the according „Results“ file are **from the same group** and should be compared to another group (like „Control“ and „Treatment“).
- **Two different files** are generated and stored in the folder of the „Results“ file:
„**Extracted results (eng)**“ (**points as decimal separator**)
and
„**Extracted results (ger)**“ (**commas as decimal separator**)
- The data are arranged for **direct copy/paste transfer into a statistics software**.
- You will be asked **immediately** for the **next „Results“ file** to extract until you skip the macro.



Please cite us when you use MYOCYTER

If you use our macro, **please cite the according peer-reviewed publication:**

**“The MYOCYTER – Convert cellular and cardiac contractions into numbers with ImageJ”,
Grune, T.; Ott, C.; Häseli, S.; Höhn, A.; Jung, T., *ScientificReports*, October 2019.**

If you have **any questions about MYOCYTER**, please contact me:

Tobias Jung, **e-mail:** tobias.jung@dife.de

Phone: +49 (0)33200 88-2490

German Institute of Human Nutrition Potsdam-Rehbruecke (DIfE)

Arthur-Scheunert-Allee 114-116

14558 Nuthetal, Germany