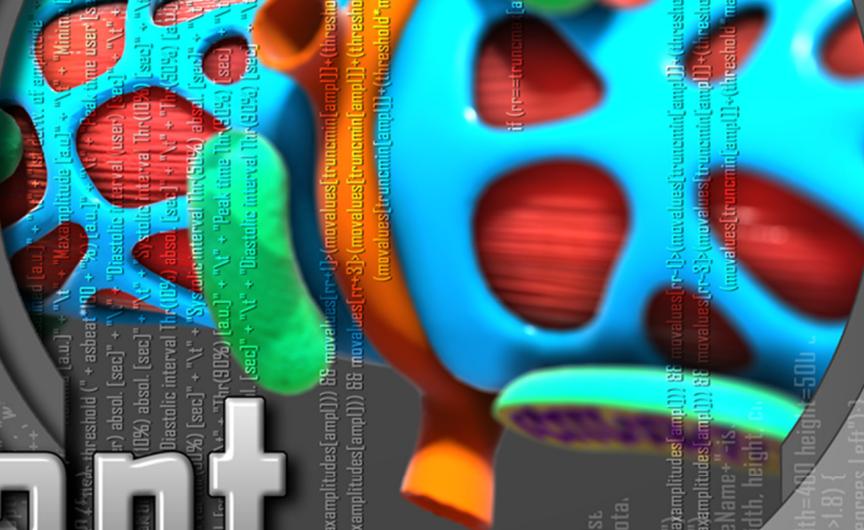


# MyoCytex V1.5

# Turning cellular and cardiac contractions into numbers via ImageJ

# wicket



# MYOCYTER v1.5 – Hard- and software

Please be aware that **ImageJ** and **FIJI** require lots of **RAM (16 GB+)** for processing of **large and/or high-resolution video material.**

MYOCYTER requires a **Windows-based operating system** like Win7 or Win10.  
Use of „**MacOS**“ or „**OS X**“ may cause dysfunction of our software.

# MYOCYTER v1.5 – Quickstart

This is the **quickstart manual** of **MYOCYTER v1.5**, 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.

Please read the **complete quickstart first** to familiarize yourself with the possibilities of the software and to be able to decide **which settings are best suited** for your samples/data.

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](http://www.scyrus.de)

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

# MYOCYTER v1.5 – New features

**New features** of MOCYTER v1.5 (optimizations of the workflow):

- When **batch-labeling ROIs** of videos in a folder, a **counter indicates** the **remaining files** to be marked.
- The **extracted data** of all single cells are stored both in **one large “Results”-file** and in **individual files** (named by file name and identified cell); the **same** applies **for the according amplitudes (only after “5. Exclude Data” is applied!)**.
- At the **end of** the **“Results”-file** is now a **summary of the statistics** for each individual cell.
- These **summary statistics** are also **saved as a separate file** (“Results (final summary)”).

# The Workflow of MYOCYTER v1.5

1. Pretest

2. Create a Batchlist

3. Manual ROI selection

4. Evaluation

5. Exclude Data

6. Re-Evaluation

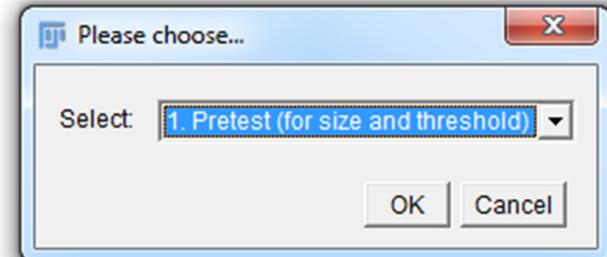
7. Data Extraction

This is both the **menu** and the **typical workflow of MYOCYTER v1.5** during data extraction from videos and further processing.

# The Workflow of MYOCYTER v1.5

## 0. Before you start

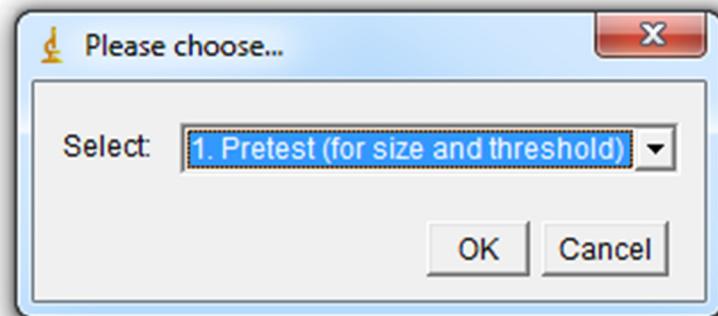
- You have all your videos in an **ImageJ-compatible format (MJEPG or uncompressed \*.avi files)** in a folder.
- You need **ImageJ v. 1.52q** (or higher). [Download a functional version here](#) (do **NOT** update)!
- You need the **MYOCYTER-Macro** as \*.txt file.
- A computer with **≥16 GB of RAM** and a **64-bit Windows-OS** 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 v1.5

## 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“.

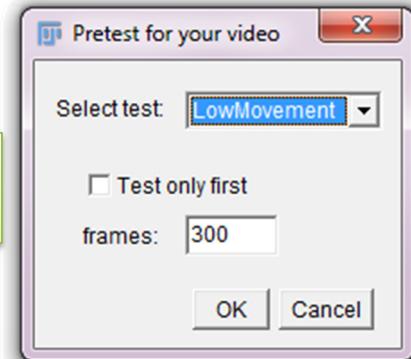


# The Workflow of MYOCYTER v1.5

## 1. Pretest

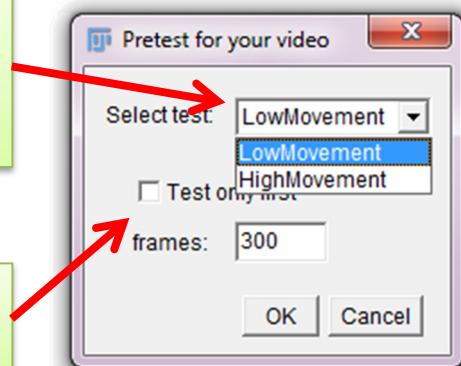
- Stick with the **default settings** (for the beginning).
- Select the folder containing your videos, **DO NOT open** it, click „**OK**“ and **wait** until the macro finished.
- A new folder („**pretest**“) should be found in the folder containing your videos.

Default settings



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

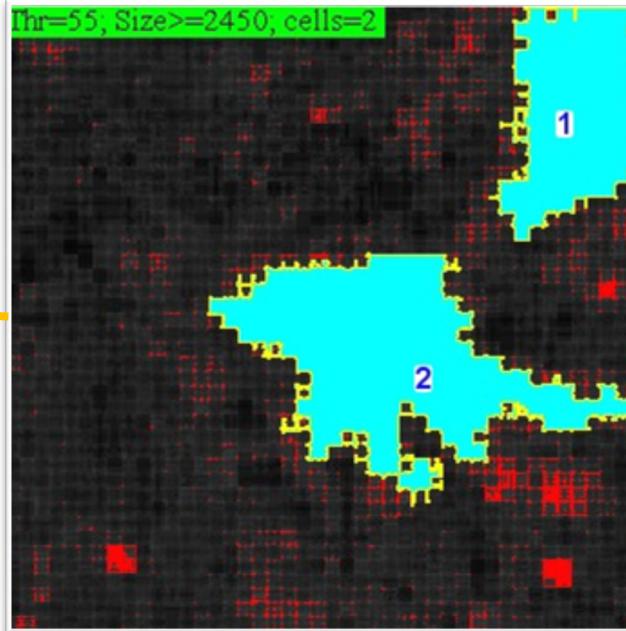
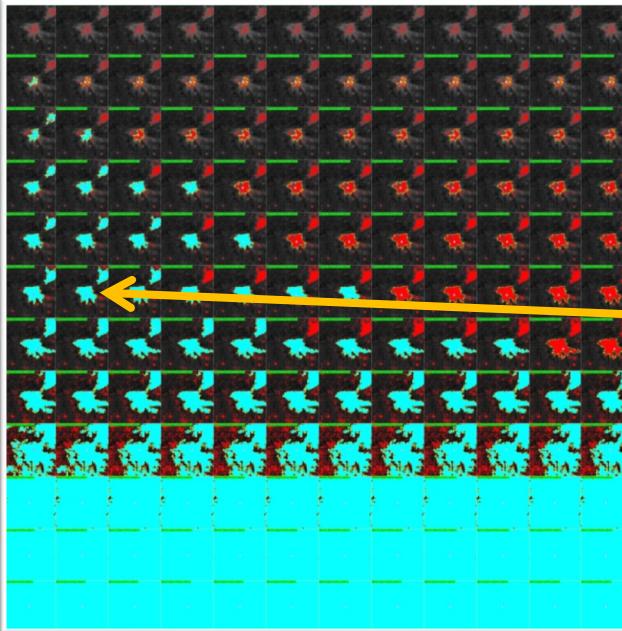
To save time, pretest only the first xxx frames of your video



# The Workflow of MYOCYTER v1.5

## 1. Pretest

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



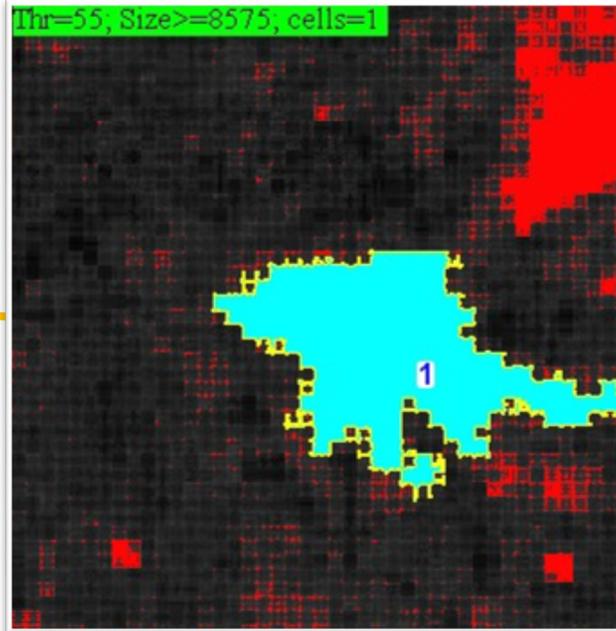
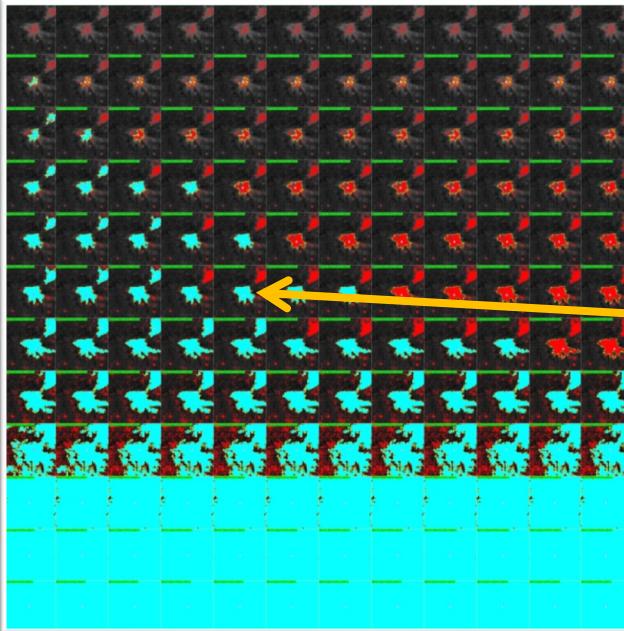
**Two cells** have been recognized. To restrict evaluation to the moving cells only, we should apply (in this case) a **Threshold of 55** and a **Size of 2450**.

Only the **turquoise areas** will be evaluated with the according settings, while the **background is completely excluded**.

# The Workflow of MYOCYTER v1.5

## 1. Pretest

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

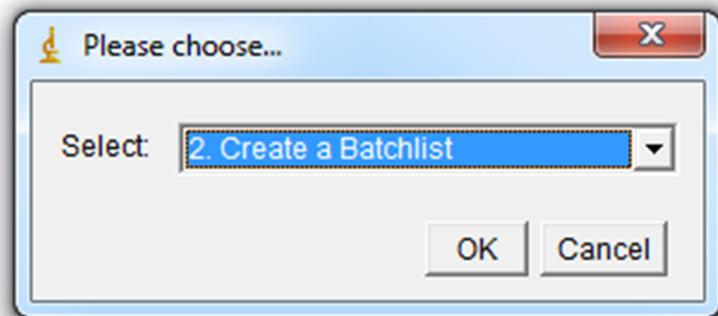


**Two cells** have been recognized.  
To exclude the one cropped through the picture frame, apply a **Threshold of 55** and a **Size of 8575**.  
**Only the turquoise areas** will be evaluated with the according settings, while the **background is completely excluded**.

# The Workflow of MYOCYTER v1.5

## 2. Create a Batchlist

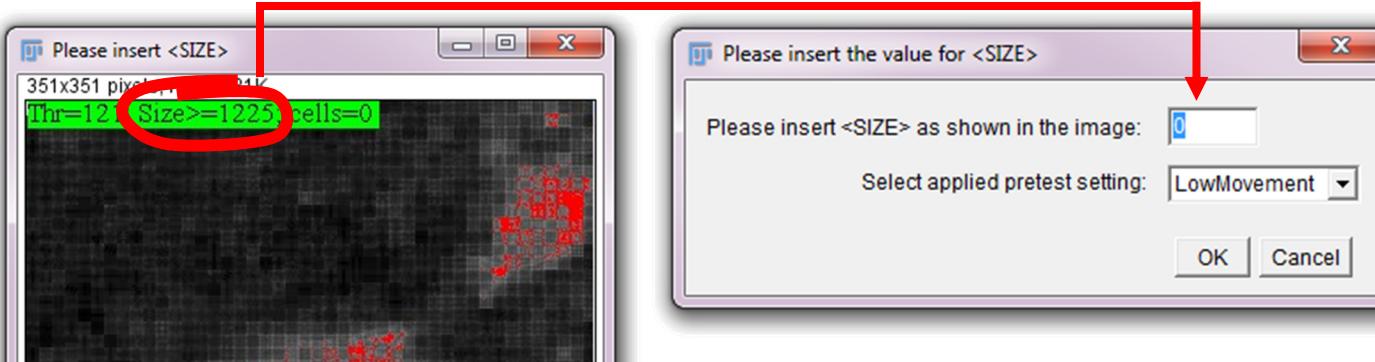
- Application of **individual settings** for **Threshold** and **Size** from „1. Pretest“ **to every single video** can be completely automatized.
- Select option „**2. Create a Batchlist**“.
- **Just select** the folder containing your pretest images, **DO NOT** open it.
- Click „**Okay**“.



# The Workflow of MYOCYTER v1.5

## 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.

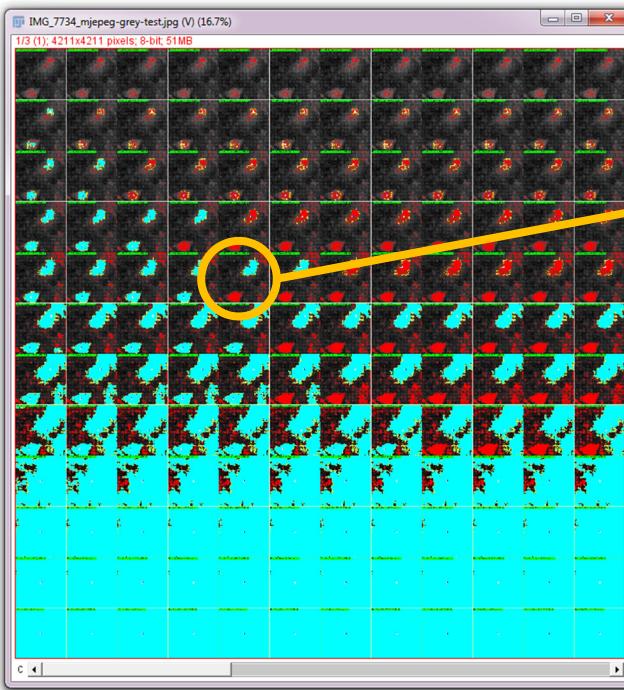
**NOTE:** All videos in the folder should have same dimensions!

- 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 v1.5

## 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.

# The Workflow of MYOCYTER v1.5

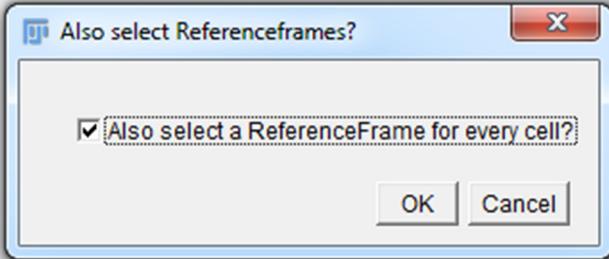
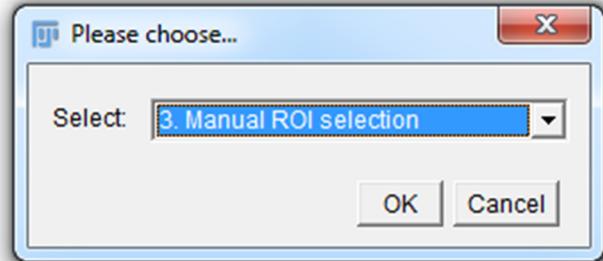
## 2. Create a Batchlist

- 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 v1.5

## 3. Manual ROI selection

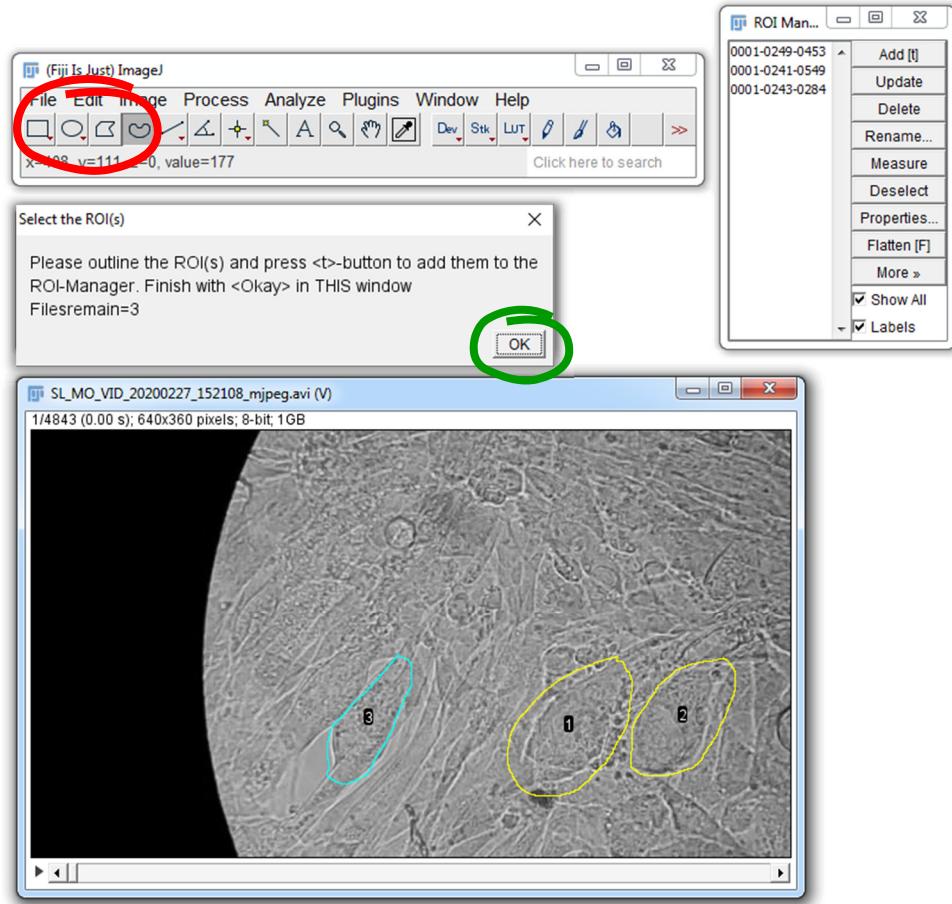
- Select „3. Manual ROI selection“ and click „OK“.
- This feature enables **manual outlining** and **labelling of cells without any pretest**.
- Just select (**do not open**) the folder containing your videos, click „OK“.
- In the next step you will be **asked about (manual) selection of a reference frame** for every single defined cell. In most cases, you can uncheck this feature, because **automatic recognition is quite reliable and robust**.



# The Workflow of MYOCYTER v1.5

## 3. Manual ROI selection

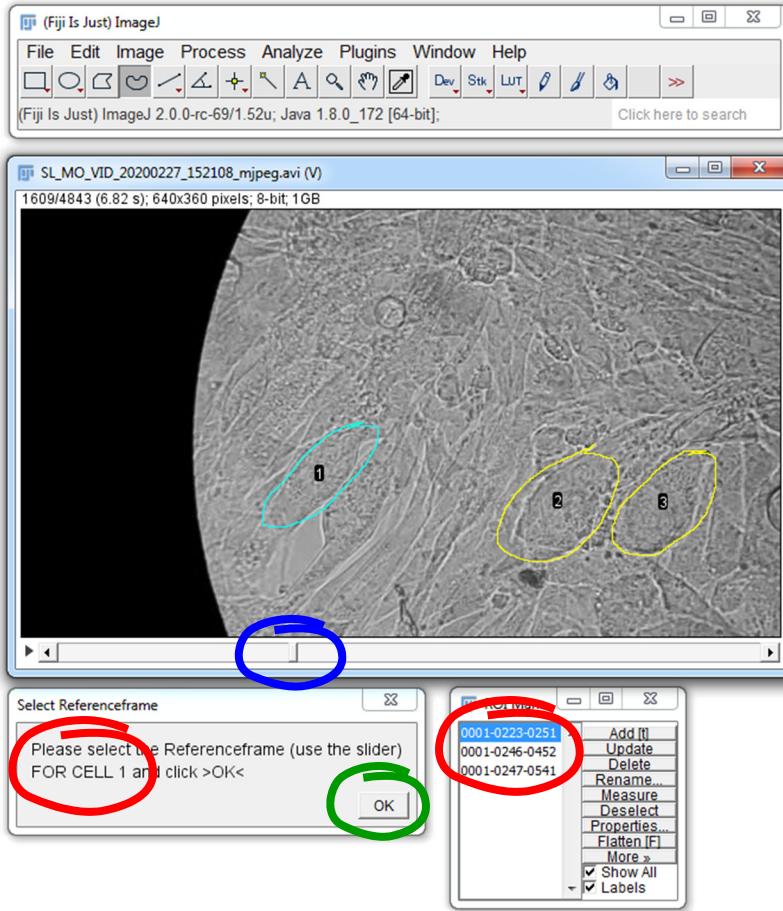
- Now, please select (**do not open**) the folder containing your video-files.
- Every single file will be opened successively and the user is asked to **outline single cells**.
- Pick one of the **selection tools** (**red circle**).
- Label the first cell** (see video-panel) and press the „**t**“-key to assign the selection to the „**ROI Manager**“ (top right panel). Repeat for **every single cell** to be analyzed.
- To **finish the current video**, click „**OK**“ in the „**Select the ROI(s)**“-window (**green circle**).



# The Workflow of MYOCYTER v1.5

## 3. Manual ROI selection

- IF you checked „Also select a ReferenceFrame for every cell“ at the beginning, you are asked to adjust the according reference frame for every single selection using the slider of the video (blue circle) after defining the individual ROIs.
- Define the appropriate reference frame for every single ROI (red circles) using the slider of the video-frame, then click „OK“ (green circle) to proceed to the next ROI.



# The Workflow of MYOCYTER v1.5

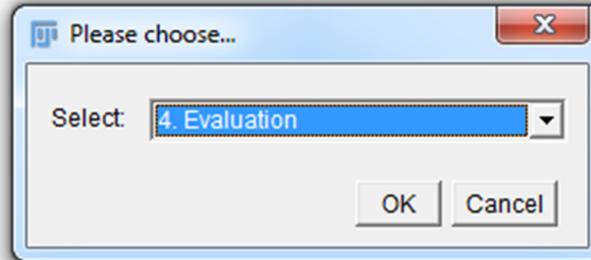
## 3. Manual ROI selection

- Repeat this selection of ROIs and also reference frames (if you activated this option) for **every single video**.
- MYOCYTER will **terminate after the last file**, and a „**manrois**“-subfolder is created in the folder containing your videos.
- How this manual ROI selection is used during evaluation is explained in the next section (4. Evaluation).

# The Workflow of MYOCYTER v1.5

## 4. Evaluation

- Select „4. Evaluation“ and click „OK“.



# The Workflow of MYOCYTER v1.5

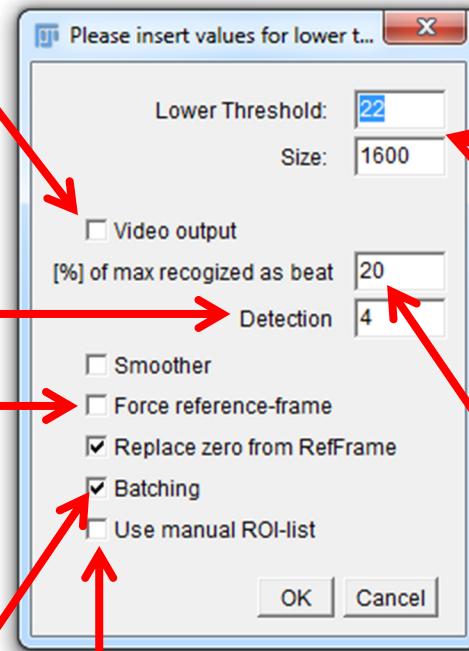
## 4. Evaluation

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 overwritten by the individual ones of the list.



Check this option to use the **manual ROI-selection** created in section „3. Manual ROI selection“.

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 **lateral 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**.

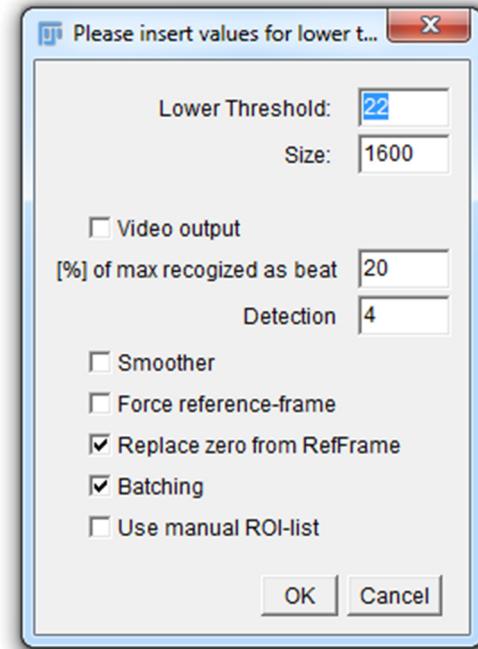
# The Workflow of MYOCYTER v1.5

## 4. 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 selected „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.



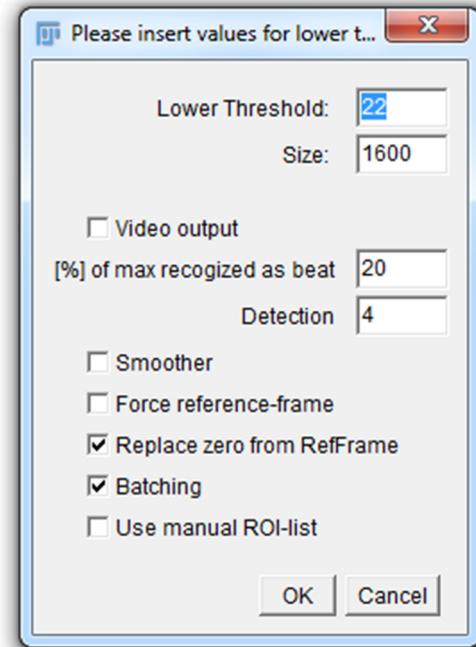
Please, **DO NOT** use the computer for **any other task during evaluation** – this **may lead to premature termination** of MYOCYTER.



# The Workflow of MYOCYTER v1.5

## 4. Evaluation

- If you want to **apply manual ROIs** (see section 3.), please check the option „**Use manual ROI-list**“.
- First, you may see a „warning“ that „batching“ was deactivated. This is just a hint that **batch-lists will be ignored**, click „OK“ to go on.
- First, you are asked to select the folder containing your videos (**just select, do not open it**).
- Second, you are asked to select (**not open**) the „**mainrois**“-folder containing the ROI-data (please see section 3 for details).



# The Workflow of MYOCYTER v1.5

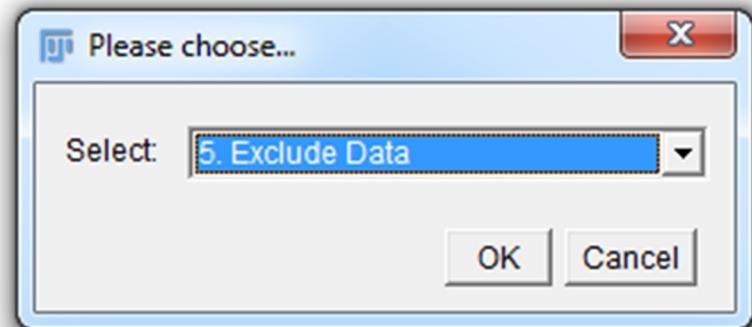
## 5. Exclude Data

- 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 many thousands of lines.
- This process (**excluding data**) is largely automatized, the **original data remain untouched**.

# The Workflow of MYOCYTER v1.5

## 5. Exclude Data

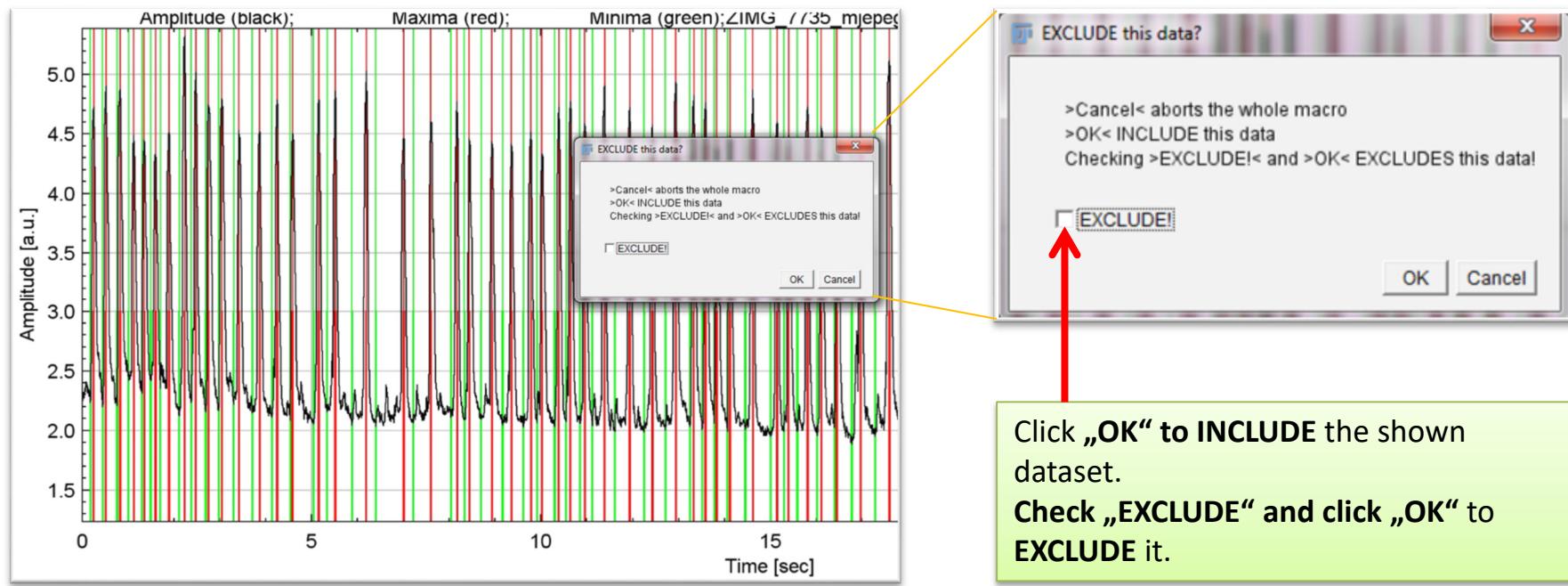
- Select „5. Exclude Data“ and click „OK“.
- Just select the „**dataplots**“ folder created after evaluation (**DO NOT open** it), containing the **amplitude plots** and the „**Results**“ (as text file).
- After selecting/excluding your data, you will be asked again to choose **another „dataplots“** folder to continue excluding until you terminate the macro.



# The Workflow of MYOCYTER v1.5

## 5. Exclude Data

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



# The Workflow of MYOCYTER v1.5

## 5. Exclude Data

- New files are generated in the „**dataplots**“ folder:  
„**Results (selected)**“ - This file contains **only the data you included** as well as a **list of the excluded data**.  
These **results are also saved** as **individual files** for **each cell**.  
Furthermore, a file with **summary statistics** is created: “**Results (final summary)**”.  
The **included amplitudes** are saved in a **large file** (“**Amplitudes (selected)**”),  
as well as in **separate files** for **every single amplitude**.
- You can now process/compare this data table in a stats software.
- The **original data remain unchanged**.

# The Workflow of MYOCYTER v1.5

## 5. Exclude Data

- The **results table** can be **quite long** (several megabytes).

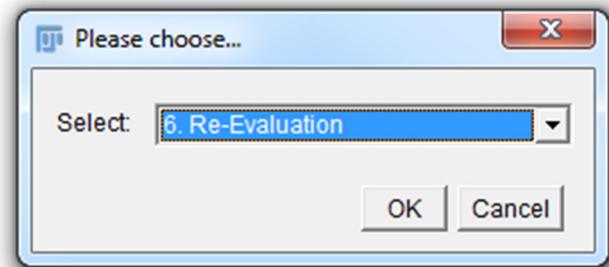
**Please note:** The **final statistics** are found **BOTH** under every single video evaluation (for every single cell) **AND at the very end of the table** also for every single video above, **AND as separate file “Results (final summary)”**.

⇒ The user who only needs the statistics no longer has to laboriously gather the individual summary statistics from the whole table.

# The Workflow of MYOCYTER v1.5

## 6. Re-Evaluation

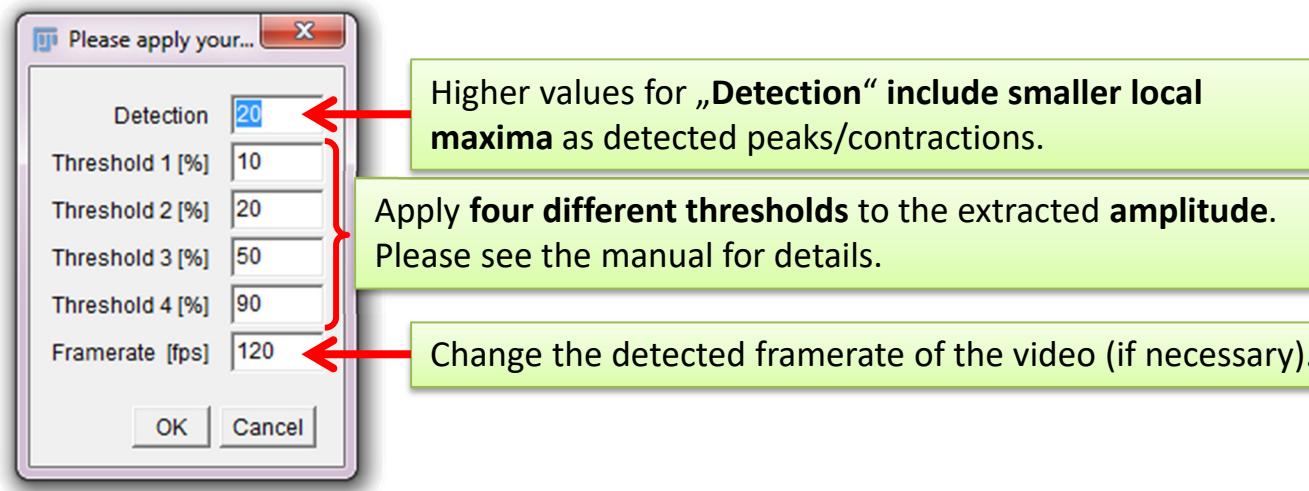
- Using „**6. Re-Evaluation**“, you can apply changed settings to your data, **even after evaluation**.
- Select „**6. Re-Evaluation**“ and click „OK“.
- Go to the „**dataplots**“ **subfolder** of your evaluation, **open it**, select the „**Amplitudes only**“ or the „**Amplitudes only (selected)**“ text file, click „OK“ to open it.



# The Workflow of MYOCYTER v1.5

## 6. Re-Evaluation

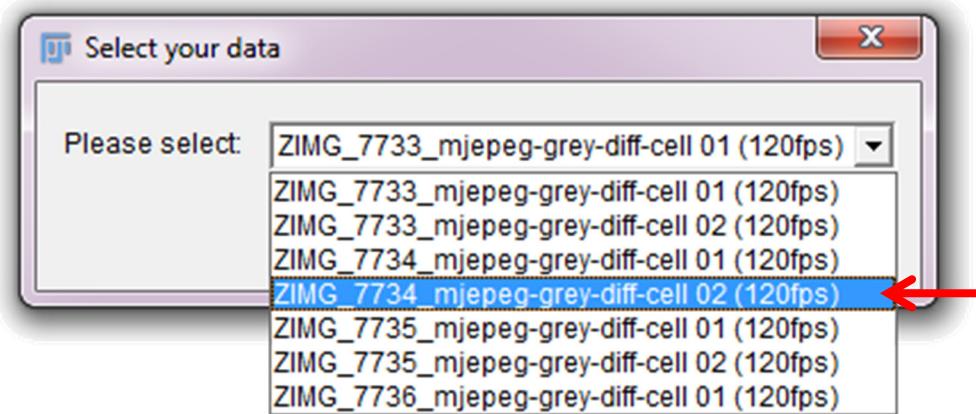
- Now, you are asked to **apply other settings** to your **evaluated data**.



# The Workflow of MYOCYTER v1.5

## 6. Re-Evaluation

- And to **select the dataset (a single cell, in this case)** you want **to re-evaluate**.

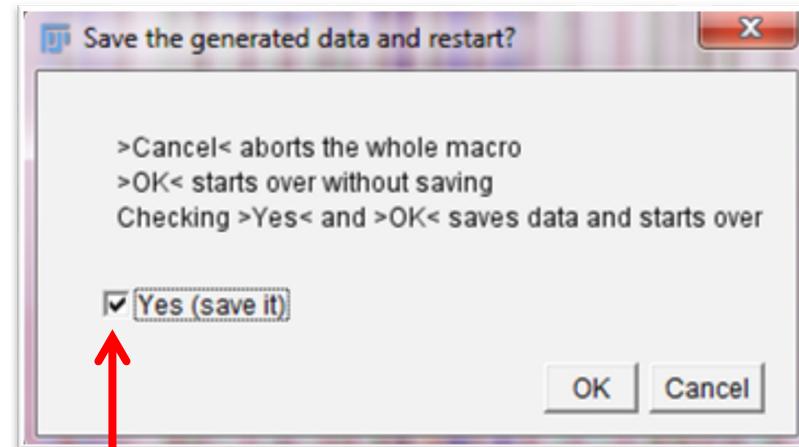


Every single cell from your **evaluation** is listed here – just pick one.

# The Workflow of MYOCYTER v1.5

## 6. Re-Evaluation

- Save or discard the (new) **dataset after re-evaluation**:



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**).

# The Workflow of MYOCYTER v1.5

## 6. 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 also **as a single data table**:

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

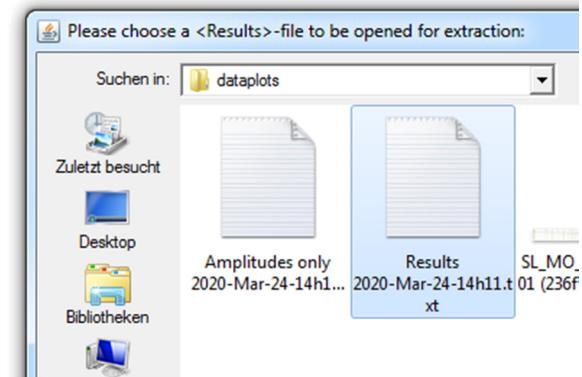
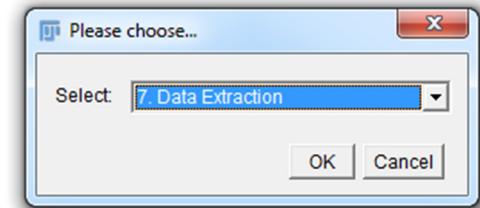
# The Workflow of MYOCYTER v1.5

## 7. Data Extraction

Evaluation provides both „continuous“ data for plotting (like the amplitude over time) or „discrete“ data (like **only the maximum amplitudes** for every single contraction) for statistics.

This feature enables **extraction of ALL „discrete“ data from a „Results“-file** for further data processing in statistical software.

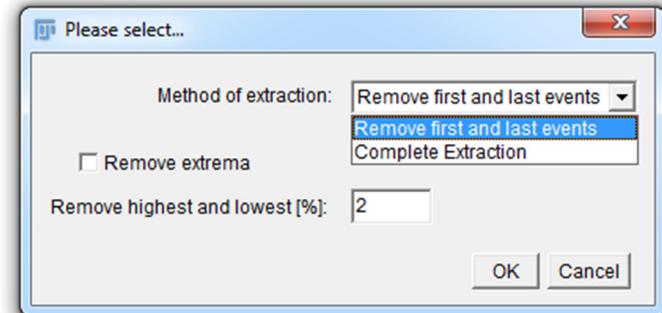
- First, you are asked to select the according „Results“- or „Results (selected)“-file (found in the „dataplots“-folder).
- Klick „OK“ to open it.



# The Workflow of MYOCYTER v1.5

## 7. Data Extraction

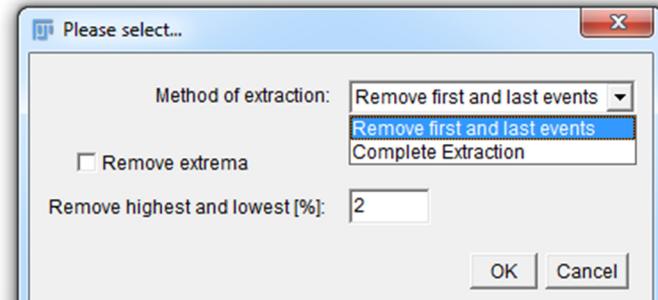
- In the next step, you determine the **method of data extraction**.
- „**Remove first and last events**“: The discrete data are completely extracted, **both first and last „events“ (contractions)** of every single recognized cell/structure are removed.
- „**Complete extraction**“: The discrete data are completely extracted (**including** first and last „events“).
- „**Remove highest and lowest [%]**“: Here, you can define the percentage of maxima that is removed. In this case, „**2**“ will result in **remove of both the 2% highest and lowest values**. This option also sorts all values by size.



# The Workflow of MYOCYTER v1.5

## 7. Data Extraction

- The **extracted data** („**Extracted results**“) are stored in the same folder as the according „**Results**“-file.
- After this, MYOCYTER will immediately ask for the **next „Results“-file to process** until you terminate the macro.





# The Workflow of MYOCYTER v1.5

## 7. Data Extraction

Please note:

- **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 separators**)  
and  
„**Extracted results (ger)**“ (**commas as decimal separators**)
- The data are arranged for **direct copy/paste transfer into a statistics software**.



## Troubleshooting – Too little RAM

A **typical problem** is the premature abort of video analysis due to a too **small working memory**, caused by **too high resolution and/or too long videos**.

To analyze such videos nevertheless, their **spatial resolution can be reduced** (details on the next page).

However, the **temporal resolution** of the videos to be analyzed **should never(!) be reduced**.



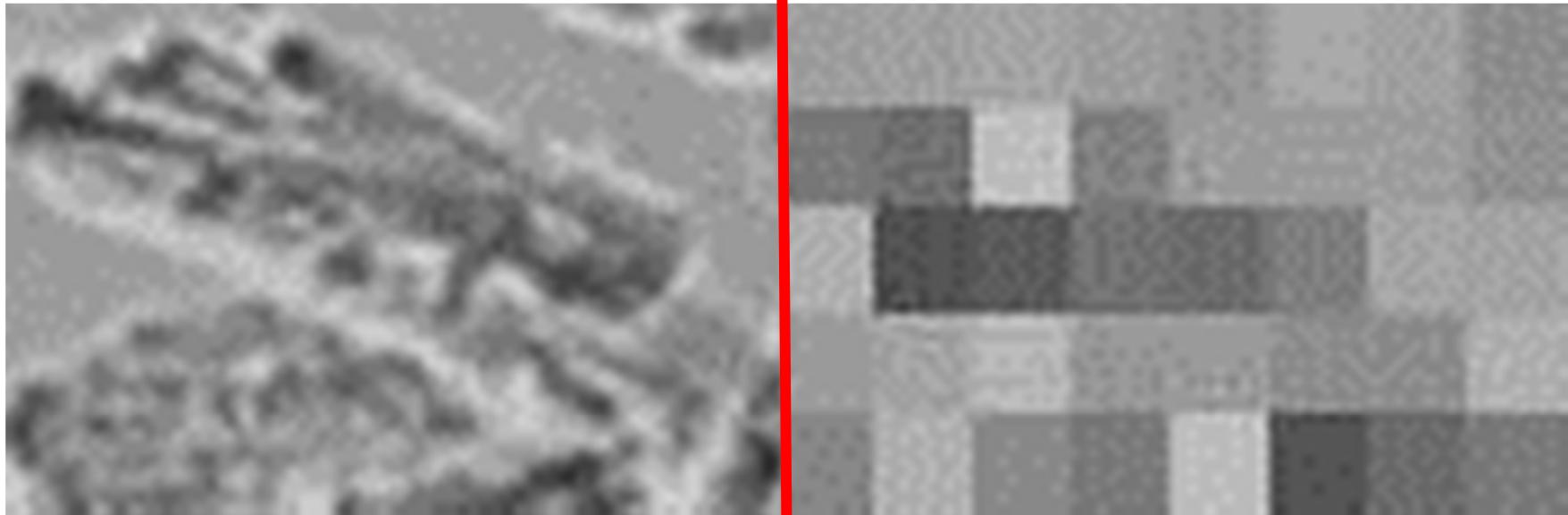
# Troubleshooting – Too little RAM

Data reduction of **spatial resolution**:

**Original video (representative material):**

$87 \times 57 = 4959$  pixel (**100%**),

21,2 MB; 4300 single frames



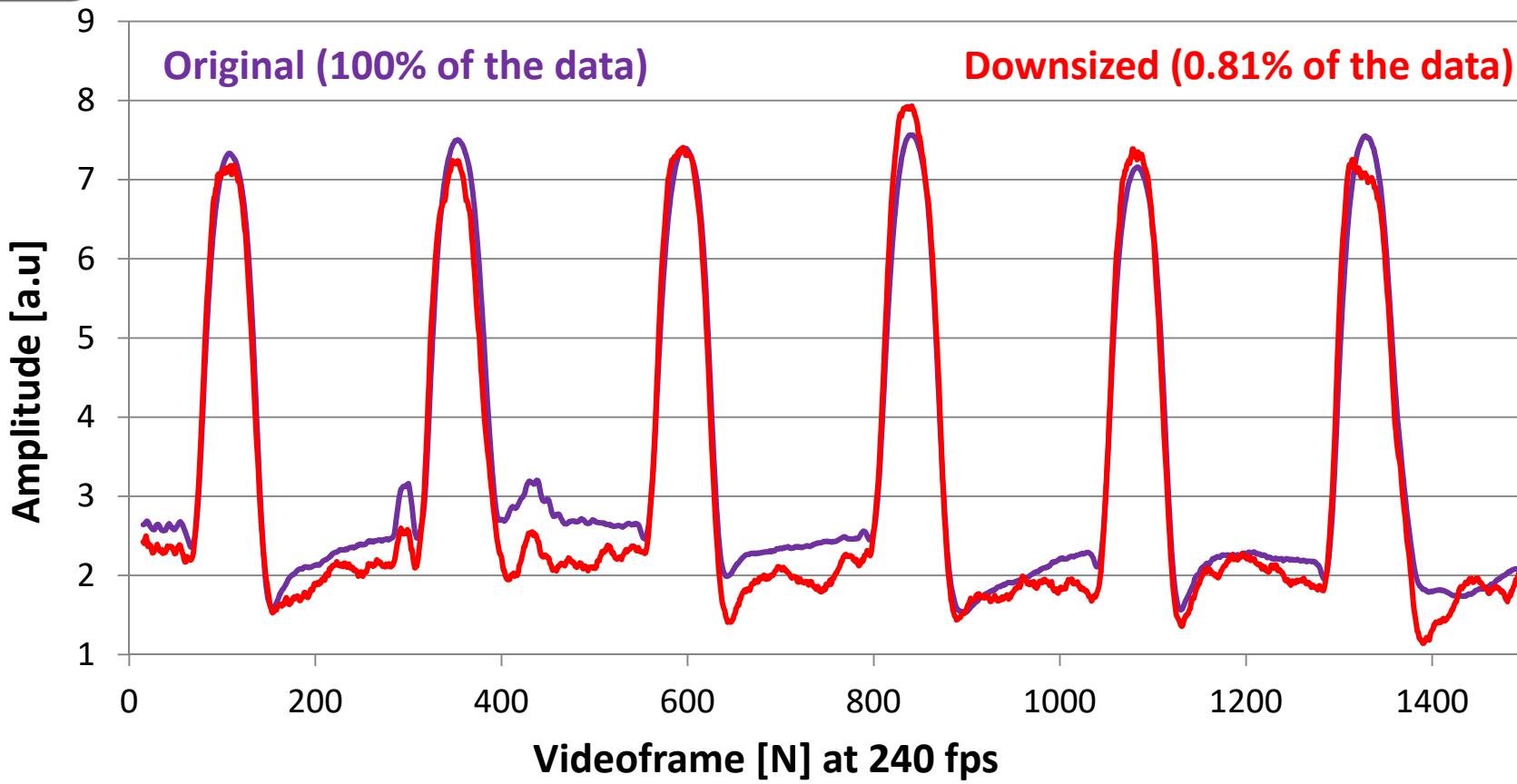
**Downsized version (hardly recognizable):**

$8 \times 5 = 40$  pixel ( **$\approx 0.81\%$** ),

0,273 MB; 4300 single frames



# Troubleshooting – Too little RAM

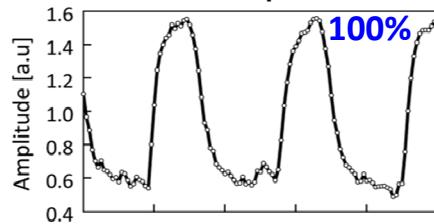




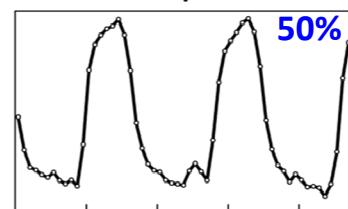
# Troubleshooting – Too little RAM

## Data reduction of temporal resolution:

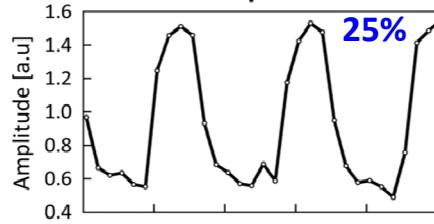
A. 120 Frames per second



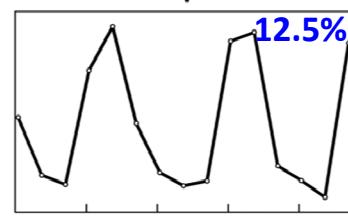
B. 60 Frames per second



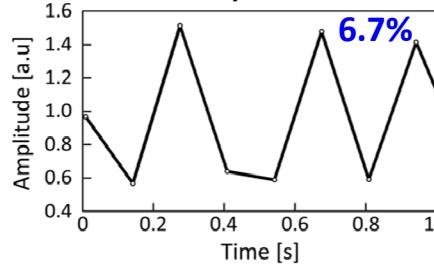
C. 30 Frames per second



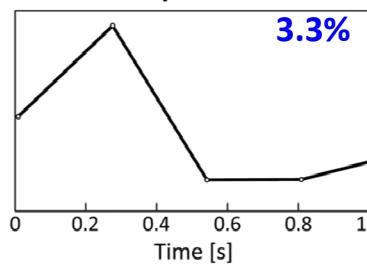
D. 15 Frames per second



E. 8 Frames per second



F. 4 Frames per second



The importance of a high frame rate for successful evaluation of cardiomyocyte videos has already been discussed in the “MYOCYTER”- publication\*. Recommended minimum: 120fps, we are already using 240fps.

**Below 25% / 30fps the videos are practically no longer evaluable**

A too low frame rate of the videos to be evaluated is a devastating problem that is insolvable afterwards.

\* The „MYOCYTER“ - Convert cellular and cardiac contractions into numbers with ImageJ. Grune, T.; Ott, C.; Häseli, S.; Höhn, A.; Jung, T. *Sci Rep.*, **9(1):15112**, 2019.



## Troubleshooting – Too little RAM

In sum:

While **temporal compression quickly results in a disaster** (below 30 fps for cardiomyocytes, 25% of the recommended minimum frame rate of 120 fps),

**spatial compression to  $\approx$ 1% of the original resolution is actually acceptable.**



# 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](mailto: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