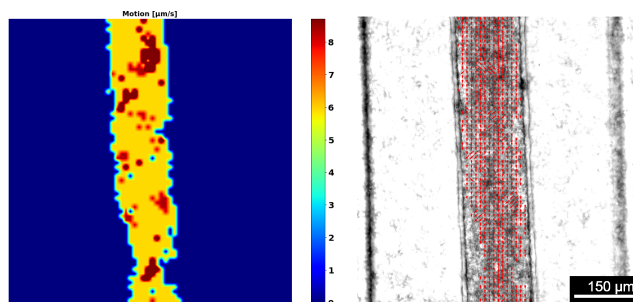# User Guide to: 'OpenHeartWare (OHW) - Software for optical determination of Cardiomyocyte contractility'

January 24, 2019

Loskill Group, Fraunhofer Institute for Interfacial Engineering and Biotechnology IGB



## Contents

# 1 Introduction and Overview

This user guide is designed to give you a guideline on how to install and use the software 'OpenHeartWare (OHW)'. OHW can be used for the automated motion detection in videos of beating cardiomyocytes.

Both the analysis of single videos as well as the batch analysis of multiple videos will be described in a step-by-step guide.

## 2 Installation and Setup

To install and use OpenHeartWare, follow this step-by-step guide. Install the following:

1. **Install Anaconda:**
   Anaconda can be downloaded from the Anaconda website. Choose the right version for Python3: `https://www.anaconda.com/download/`.

   - Install it according to the installation wizard. Install with the option "Install for all users". Do not add Anaconda to my PATH environment variable. Make sure this option is not checked. It is best if you install it in the standard folder (see Figure 1).

   - If, for any reason, you cannot install Anaconda for all users, you will need to change a variable in the setup file before moving on to Step 2. To change the variable, do the following:

     - Open the file `setup_OpenHeartWare.bat` (located in the OpenHeartWare folder) in any texteditor. The content should look like in Figure 2.

     - Search on your computer for the location of the `activate.bat` file. Use .e.g. the search term *Anaconda3* and you will find a folder located e.g. at `C:\Users\carla\Anaconda3` (see Figure 3). Copy the path or write it down.

     - You will now need to change the line
       `SET anaconda_folder=` `C:\ProgramData\Anaconda3``\Scripts\activate.bat`
       to your location, e.g.:
       `SET anaconda_folder=``C:\Users\carla\Anaconda3``\Scripts\activate.bat`

     - Save the file and close it.

     - You will need to change the very same line also also in the file `run_OpenHeartWare.bat` (also located in the OpenHeartWare folder). Open this file in a texteditor and change the same line. Save the file and close it.

2. **Run the setup file to install required packages:**
   Double-click on `setup_OpenHeartWare.bat`. This will open a command window where all required packages will be installed automatically. You do not need to do anything else. Just wait until all packages are installed. When the setup is completed, it will look similar to Figure 4.

3. **Installation and Setup finished:**
   The installation and setup of OpenHeartWare is now finished. Steps 1 and 2 only need to be performed once during the installation and setup process. Whenever you want to use the software, you simple need to double-click on the file `run_OpenHeartWare.bat`.
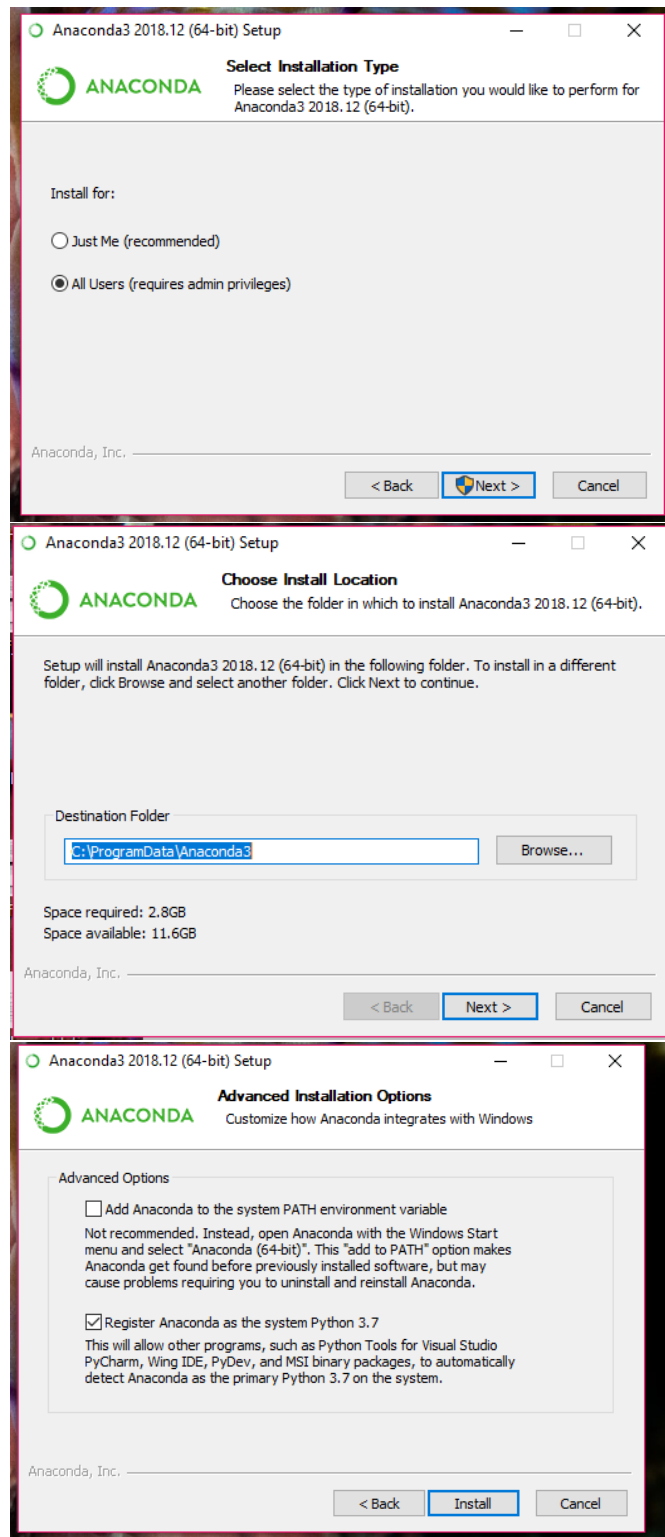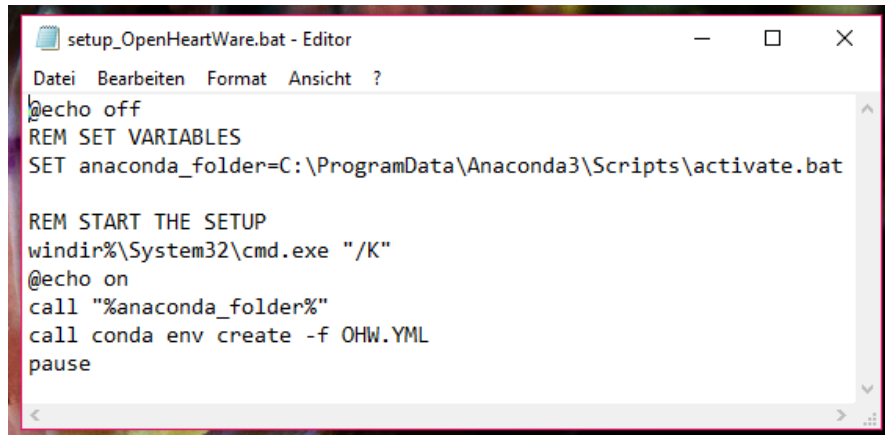
Figure 1: Anaconda installation: Install Anaconda for all users without adding it to PATH environment variable. Use the standard folder.

Figure 2: Setup file opened in a texteditor.
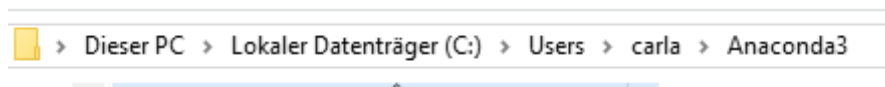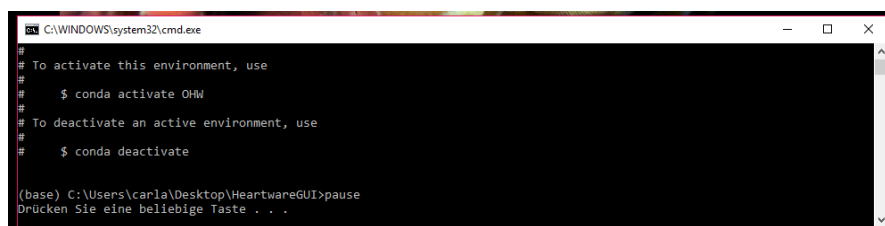


Figure 3: Path where your Anaconda is installed.



Figure 4: Successful run of setup file.

# 3 Evaluation of One Folder With OpenHeartWare

This section describes how to analyze one folder of input data with OpenHeartWare.

1. **Start OpenHeartWare**. Start the application OpenHeartWare by navigating to the OpenHeartWare folder and by double-clicking on the file `run_OpenHeartWare.bat`.
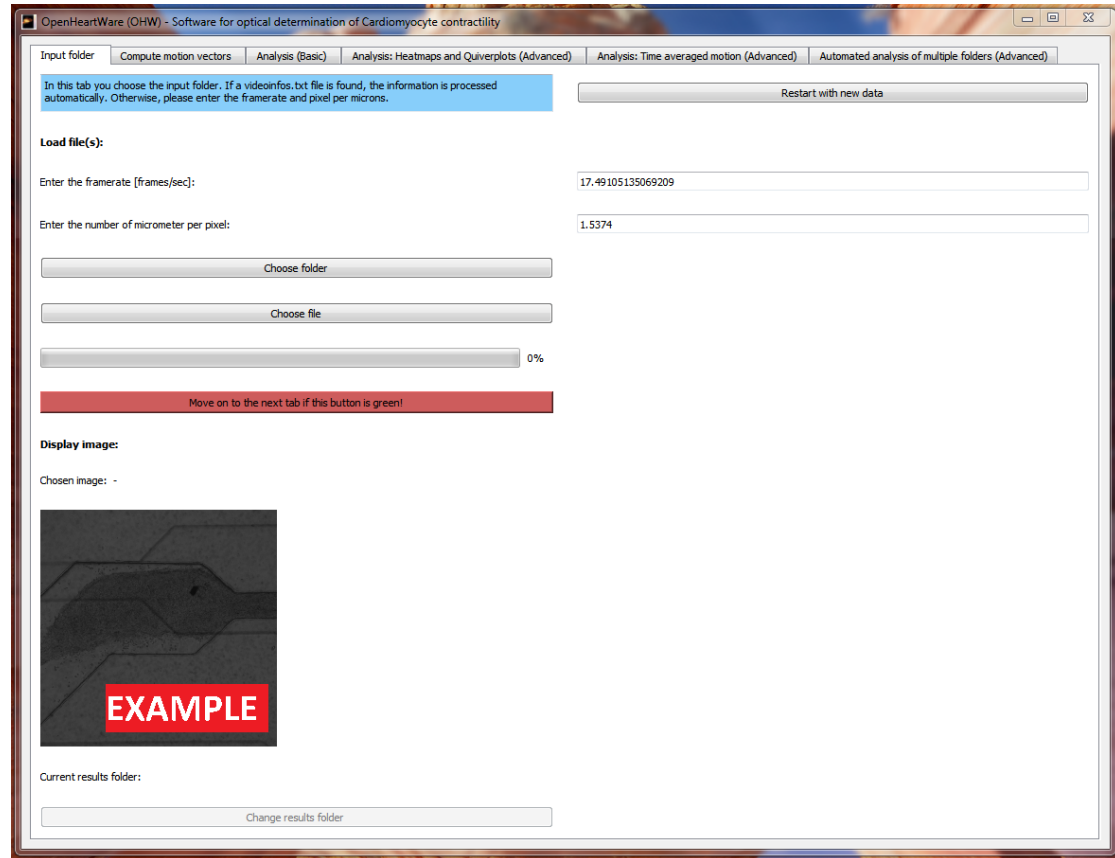


Figure 5: *Initial view of the first tab where you choose your input folder.*

If you want to restart the application, do so by clicking on the button 'Restart with new data'. However, keep in mind that all of your settings will go back to default and all chosen folders etc. will be deleted. It is advised to restart the application every time you analyse a new folder.

If you want to close the application, use the cross in the top right corner or press the 'Esc' key on your keyboard.

2. **Choose a folder:** The software opens and you see different tabs: 'Input folder', 'Compute motion vectors', 'Analysis (Basic)', 'Analysis: Heatmaps and Quiverplots (Advanced)', 'Analysis: Time averaged motion' and 'Automated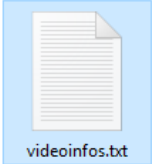 analysis of multiple folders (Advanced)'. If you cannot see all of the tabs use the little arrows on the top right corner or enlarge the window. Switch to a new tab by clicking on it.

   a) **Choose a folder (images) as input:** You can choose a folder containing a sequence of `.tiff` images as input to OpenHeartWare. To choose a folder, stay on the first tab 'Input folder' and click on the button 'Choose folder'. A new window appears. Navigate to your input data and choose this folder as input folder.

   b) **Choose a file (video) as input:** Alternatively, you can choose an input file of type `.mp4`, `.avi` or `.mov`. To choose a file, stay on the first tab 'Input folder' and click on the button 'Choose file'. A new window appears. Navigate to your videofile and choose this file as input file.

   c) **Monitor the loading:** Wait for the loading to be completed. You can monitor the progress of the loading process via a progress bar. The loading is finished when the progress bar shows 100% and the button below the progress bar changes its color from red to green. As soon as the loading is completed, one image of your input frames will be displayed in the lower part of the application.

   d) **Framerate and number of micrometer per pixel** Remember to think about the framerate in frames per second you used while acquiring the images. Without a correct framerate and number of micrometer per pixel, calculations performed in the subsequent steps will not be correct. Enter the framerate and the number of micrometer per pixel manually by editing the corresponding fields in the application. Another possibility is to provide a **videoinfos.txt** file in your input data. This text file then contains the required information. In the demo data folder, a videoinfos.txt file is provided. Make sure, your file only differs in the values but is otherwise exactly the same to the demo file.



Figure 6: *Manually set the framerate and the number of micrometer per pixels or provide a 'videoinfos.txt' file in the same setup as the provided demo file.*

e) **Check the results folder:** After you loaded the images, the default results folder will be displayed at the bottom of the first tab. It is a new subfolder within your inputfolder called `results`. If you wish to change the folder into which all results will be saved click on the button 'Change results folder'. A new window will open. Navigate to your new desired results folder and choose it. The new folder will be displayed.

3. **Compute motion vectors:** Move to the second tab 'Compute motion vectors'.
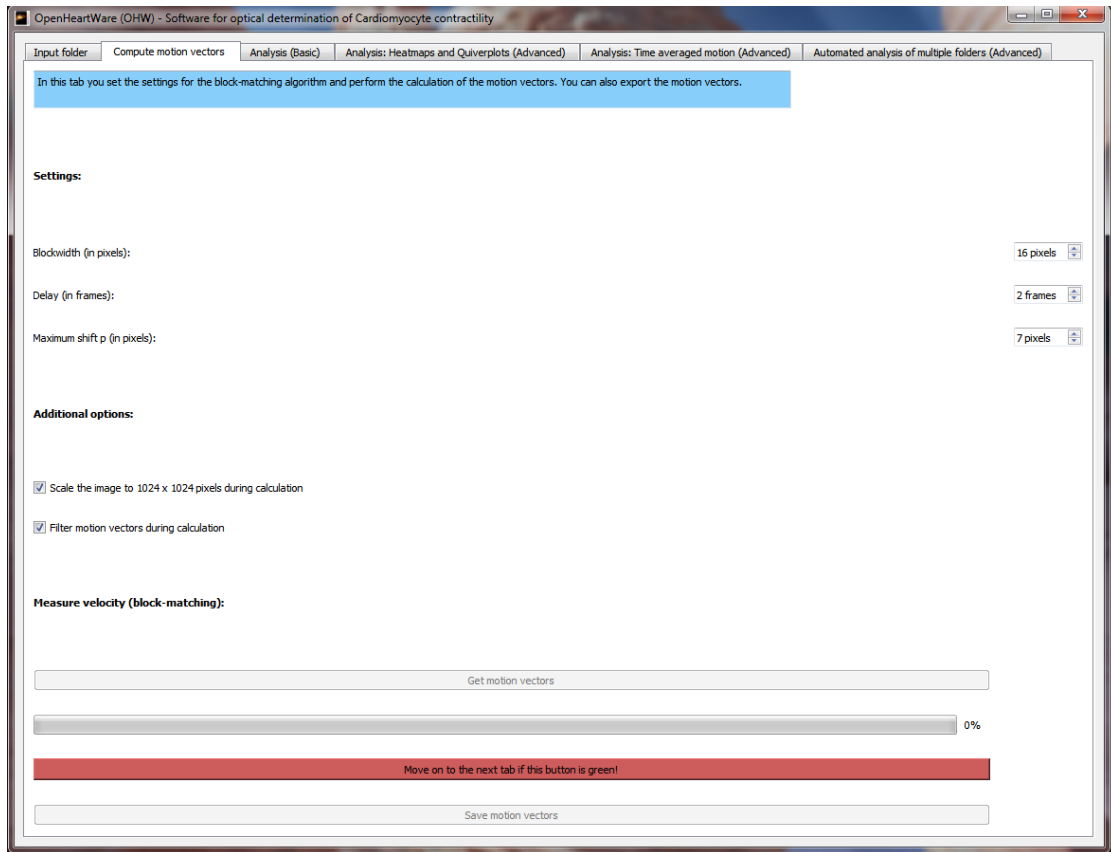


Figure 7: Second tab to calculate the motion vectors.

The calculation of the motion vectors is performed using a block-matching algorithm.

a) Choose the settings for performing the calculation. If you are not sure about the correct settings, leave the default values as they are: a **blockwidth** of 16 pixels, a **delay** of 2 frames, a **maximum shift p** of 7 pixels. You will need to find your own optimum settings.

b) Choose between different additional options. **Scaling** the image to 1024x1024

pixels during the calculation speeds the algorithm up and has little influence of the quality of the result. It is therefore advised to scale the image.
It is also advised to **filter** the motion vectors during calculation.
You can remove the checked options by clicking on the tick.

c) If all settings and options are how you want them to be, click on the button 'Get motion vectors'. The calculation of the motion vectors starts. Again, you can monitor the progress of the calculations in a progress bar. The process is finished when the progress bar shows 100% and the button below the progress bar changes its color from red to green.



> 10% Calculation of motion vectors...
>
> Move on to the next tab if this button is green!
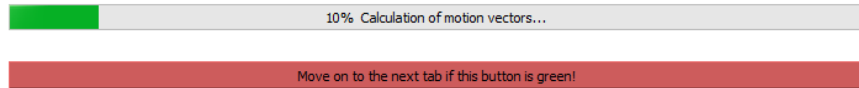
Figure 8: Progress bar during calculation of motion vectors.

d) **Export motion vectors:** If you wish to export the calculated motion vectors, you can do so by clicking on the button 'Save motion vectors'. The motion vectors will be saved as 'MotionVectors.npy' in your previously chosen results folder, a NumPy Array file that can be loaded into a Python script for further use. This export option is only available after a successful calculation of the motion vectors.

4. **Beating kinetics and statistical analysis:** Move to the third tab 'Analysis (Basic)'.
   After the successful calculation of the motion vectors, an EKG-like graph will be displayed as mean absolute motion in $\mu$m per second versus time in seconds.

   a) **Peak Detection:** To further analyze the beating kinetics, the peaks need to be detected. This happens in a semi-automated process. Start with the default values for 'Ratio between peaks' and 'Number of neighbouring values for evaluation' and click 'Start peak detection'. The peaks will be shown. Maximum peaks will be marked by a red circle and minimum peaks will be marked by a red triangle. If not all of the peaks have been detected correctly, change the peak ratio or the number of neighbours and repeat the peak detection until the result is satisfactory.
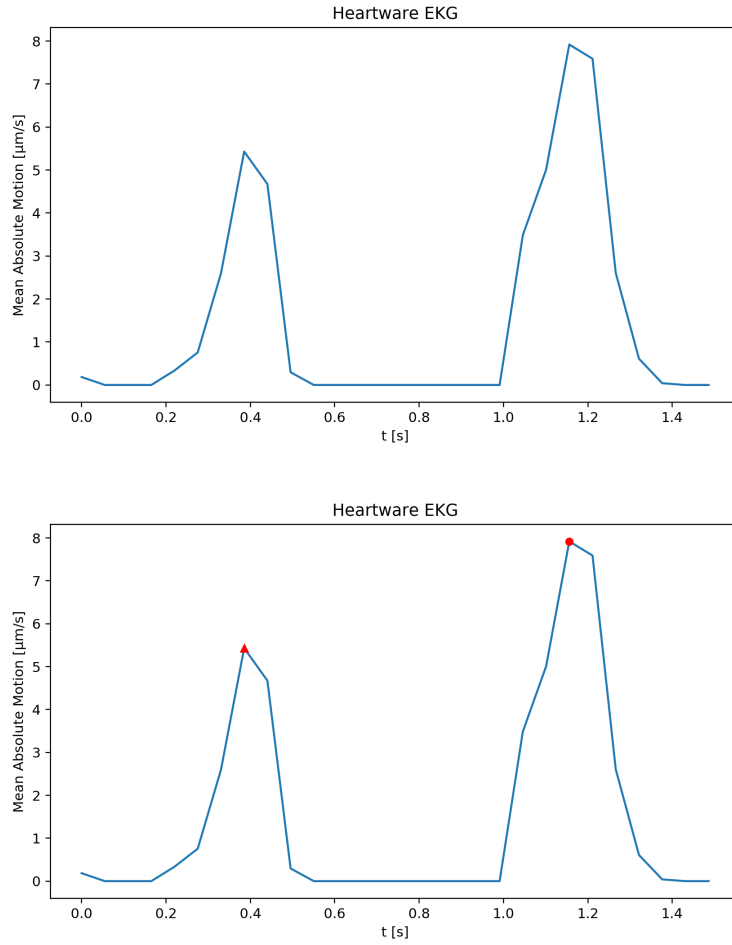
Figure 9: *Beating kinetics after the successful calculation of the motion vectors and after peak detection.*

10

b) **Save beating kinetics:** If you wish to save the beating kinetics either before or after the peak detection, click on 'Save current EKG graph as ...'. A new window will open. You can choose a filename and a file extension (PNG, JPEG, SVG, BMP, TIFF). The graph as you see it in the application will be saved under the chosen filename, e.g. 'ekg_raw.png'.

c) **Statistics:** If the peak detection was successful, the following statistical values will be calculated automatically:

- **detected maximum contraction ($\mu$m/sec):**
  amplitude of mean absolute motion corresponding to the high peaks (contraction) averaged over the number of these peaks

- **detected maximum relaxation ($\mu$m/sec):**
  amplitude of mean absolute motion corresponding to the less high peaks (relaxation) averaged over the number of these peaks

- **mean contraction interval (sec):**
  average time in seconds between two contraction peaks (high peaks)

- **mean relaxation interval (sec):**
  average time in seconds between two relaxation peaks (less high peaks)

- **mean interval between contraction and relaxation (sec):**
  average time in seconds between a contraction peak and the next relaxation peak

- **heart rate (beats/min):**
  number of contraction-relaxation pairs for a given time interval corresponding to a heartbeat

d) **Save peaks:** If you wish to save the peaks, click on the button 'Save raw and analyzed peaks'. The peaks will then be exported to a Excel file and saved as 'Peaks_raw.xlsx' and 'Peaks_analyzed.xlsx' in your previously chosen results folder.

e) **Save EKG as .xlsx file:** It is also possible to export the x and y values of the beating kinetics to an Excel file. Click on 'Save EKG as excel file (.xlsx)' and the beating kinetics will be saved in your results folder as 'EKG.xlsx'.

| | A | B | C |
|---|---|---|---|
| 1 | time | mean absolute motion | high/low |
| 2 | 0,385283 | 5,430711056 | low |
| 3 | 1,15585 | 7,919819969 | high |

Figure 10: *Example of exported peaks in the Excel file.*

f) **Save statistical analysis:** Export the calculated statistics to an Excel file by clicking on 'Save statistical analysis'. The values will be saved in a file called 'Statistics.xlsx' in your previously chosen results folder.

| Evaluated folder: | | C:/Users/carla/twSyncAndShare/B - UNI FÜR MICH/N | | |
|---|---|---|---|---|
| Used parameters: | | | unit | |
| image width (scaling factor!) during calculation: | | 1024 | pixels | |
| width of macroblocks | | 16 | pixels | |
| delay between images | | 2 | frames | |
| framerate | | 18,16845323 | frames/sec | |
| maximum allowed movement | | 7 | pixels | |
| | | | | |
| Results of statistical analysis: | | | | |
| | mean | standard deviation | unit | |
| maximum contraction | 7,9198 | 0 | µm/sec | |
| maximum relaxation | 5,4307 | 0 | µm/sec | |
| mean contraction interval | | | sec | |
| mean relaxation interval | | | sec | |
| mean interval between contraction and relaxation | | | sec | |
| heart rate | | | beats/min | |

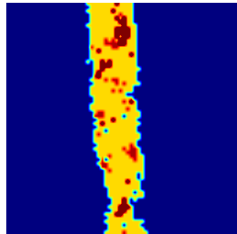Figure 11: *Example of exported statistics in the Excel file.*

5. **Heatmaps and Quiverplots:** Move to the fourth tab 'Analysis: Heatmaps and Quiverplots (Advanced)'.
   In this tab, you can have a look at and save heatmaps and quiverplots that were calculated based on the detected motion.

   - Heatmap: The heatmap of this application displays the absolute motion in micrometers per second. If you save one of the heatmaps to a file, a colorbar will be provided indicate which color corresponds to which amount of motion. Red pixels correspond to very strong motion whereas blue pixels correspond to little or no motion in the respective pixel.

   - Quiverplot: In the quiverplot, the original image is overlayed by arrows indicating the direction and strength of motion corresponding to the pixel. Additionally, a scale is provided. The scale is calculated based on the provided number of pixels per micrometer in the first tab 'Input folder'.

   a) **Slide through the heatmaps and quiverplots:** To get an overview, you might want to slide through the different frames and visually inspect the heatmaps and quiverplots. After the successful calculation of the motion vectors, both the heatmap and quiverplot of the initial frame will be displayed automatically in the middle of the window. To change between the heatmaps of the different frames, use the slider on the left side below the heatmap image. To change between the quiverplots of the different frames, use the slider on the right side below the quiver plot image. Keep it pressed, drag it either to the left or right and the corresponding image will be displayed.
   The number of the frame as well as the time in seconds will be displayed above the heatmap or quiverplot image.
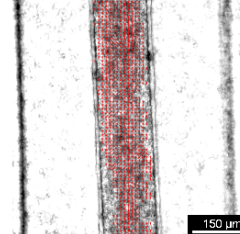


Figure 12: *Example preview of heatmap and quiver plot after the successful calculation of the motion vectors.*

   b) **Save a single heatmap or quiverplot:** If you wish to save a single heatmap or quiverplot, use the slider to navigate to the desired frame. Click on the

button 'Save this heatmap frame to image file...' or on the corresponding button 'Save this quiver frame to image file...'. The heatmap or quiverplot will be saved as a PNG file with the filename 'heatmap_frameX.png' or 'quivers_frameX.png' to your previously chosen results folder.

c) **Create a heatmap or quiverplot video:** If you wish to create a video file of all the heatmaps or quiverplots, click on the button 'Create Heatmap Video' or 'Create quiver video' respectively. The video will be saved as 'heatmapvideo.mp4' or 'quivervideo.mp4' to your previously chosen result folder.
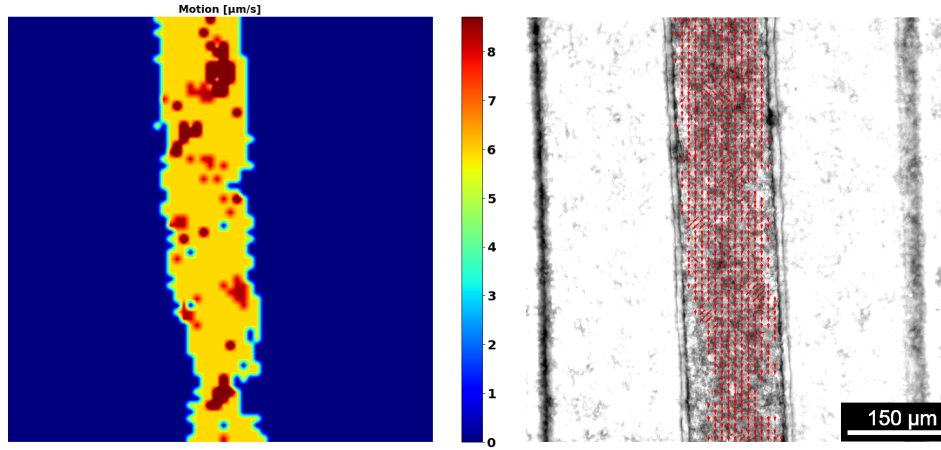


Figure 13: *Example of manually exported heatmap and quiver plot.*

14

6. **Time Averaged Motion:** Move to the fifth tab 'Analysis: Time averaged motion (Advanced)'.
   In this tab, you can have a look at and save heatmaps where the contractility has been averaged over all frames. After the successful calculation of the motion vectors, three heatmaps are displayed where the motion has been averaged over time. The first one shows the time averaged motion averaged over both x and y directions: the absolute contractility. The second one visualizes the time averaged motion in the direction along the x-axis. The third one displays the time averaged motion in the direction along the y-axis.
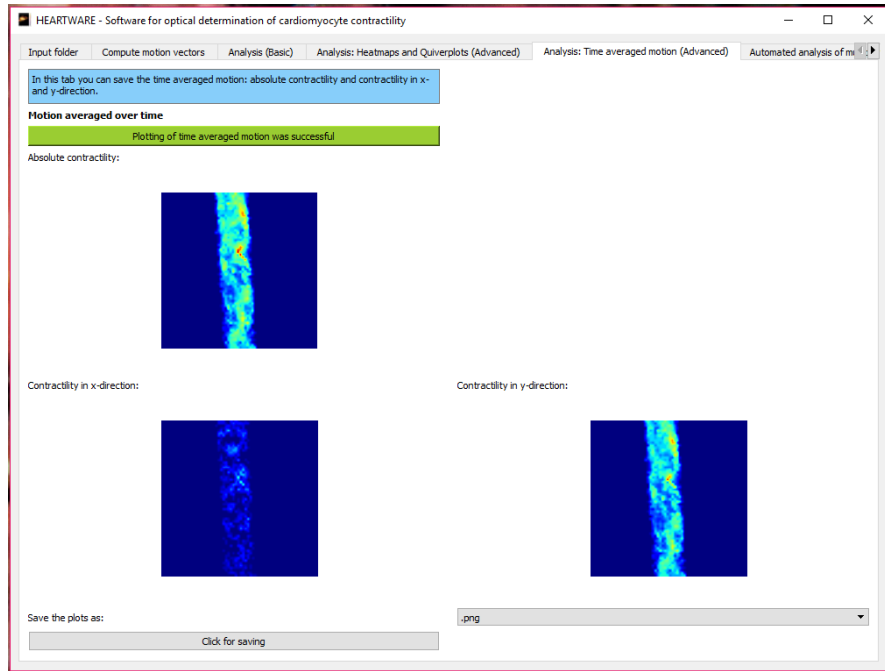


Figure 14: *Example preview of heatmap and quiver plot after the successful calculation of the motion vectors.*

   a) **Save the time averaged motion heatmaps:** If you want to save the displayed time averaged motion heatmaps, choose a file extension for your image files in the drop-down menu next to 'Save the plots as:' and click on the button 'Click for saving'. You can choose between .png, .jpeg, .tiff, .svg, .eps. After you clicked for saving, the files will be saved as 'TimeAveraged_x', 'TimeAveraged_y', 'TimeAveraged_totalMotion'.
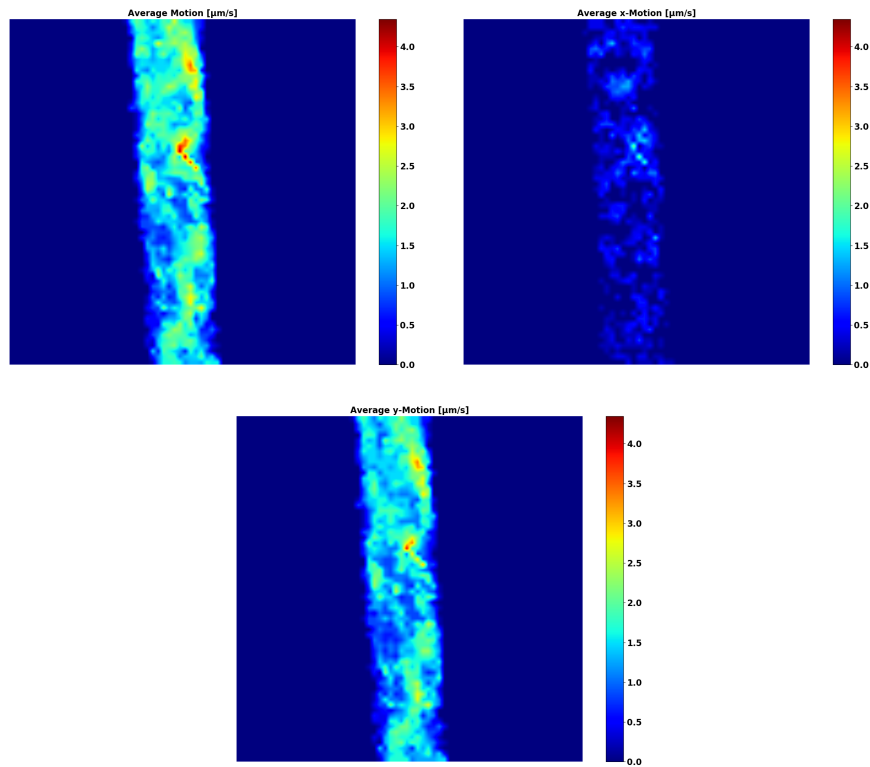
15

Figure 15: *Example of manually exported time averaged motion images for total motion, motion in x- and motion in y-direction.*

If you wish to evaluate another folder, please use the 'Restart with new data' button in the first tab 'Input folder'.
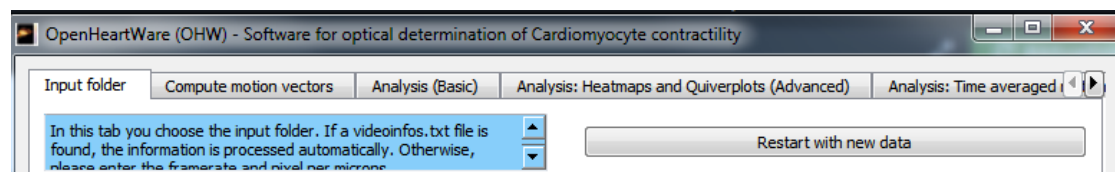


Figure 16: *Button to restart the application, e.g. for analysis of a second folder.*

# 4 Evaluation of Multiple Folders at Once With OpenHeartWare

This section describes how to analyze multiple folders of input data at once with Open-Heartware.

1. **Start OpenHeartWare**. Start the application OpenHeartWare by navigating to the OpenHeartWare folder and by double-clicking on the file `run_OpenHeartWare.bat`

2. **'Automated analysis of multiple folders (Advanced):** Move to the tab 'Automated analysis of multiple folders (Advanced).
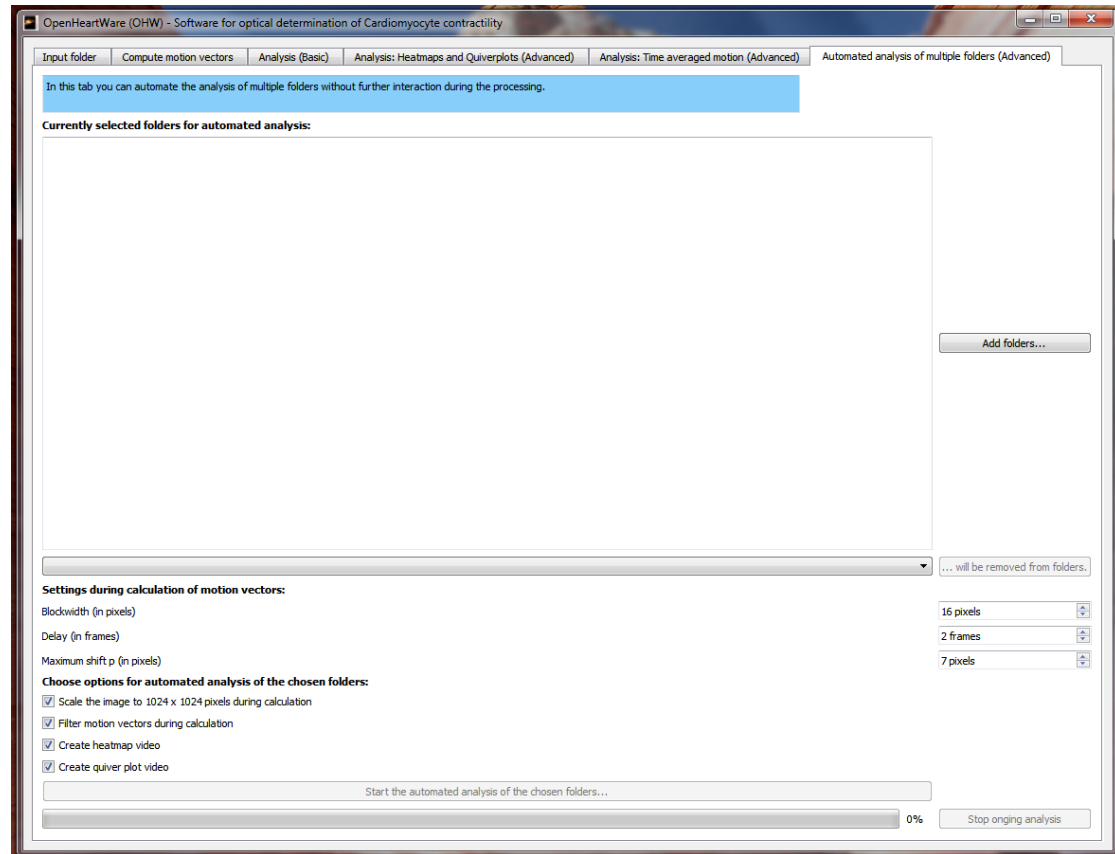


Figure 17: *View of the tab for the analysis of multiple folders after choosing two folders for analysis.*

3. **Choose input folders:** Click on the button 'Add folders...'. A new windows opens and you can choose your input folders. You can either select one folder, click on 'Choose', open the folder navigation again by clicking on 'Add folders...', choose another folder and so on. Alternatively, you can choose multiple folders at one by pressing the CTRL-key (Windows) on your keyboard and clicking on the desired folders one after another. Click 'Choose'. All chosen folders will be

17

displayed under the title 'Currently selected folders for automated analysis.' If you chose a folder but do not want to run the analysis on it, you can remove it by selecting the specific folder in the drop-down menu below the display of the chosen folders. Make sure the folder to be removed is selected in the drop-down menu and click on '... will be removed from folders.'.



Figure 18: *Removing of a folder.*

4. **Choose the settings:** Before starting the analysis, check the settings for the calculation of the motion vectors. Similarly to the analysis of one folder, set the desired blockwidth in pixels, delay (in frames) and maximum shift p (in pixels). You can also leave the default values of blockwidth = 16 px, delay = 2 frames and maximum shift p = 7 px in place.

5. **Choose further options:** Check or uncheck options for the automated analysis.

   - Scaling: First, decide whether you wish to scale the image to 1024x1024 pixels during the calculation. Checking this is advised as it accelerates the calculation without having a strong impact on the quality of the results.

   - Filtering: Secondly, decide whether you wish the calculated motion vectors to be filtered after calculation. Checking this box is also strongly advised.

   - Create heatmap video: See Chapter 3 Section 5 for further information on heatmaps. You can decide whether you want that a video of the heatmaps is saved for each folder.

   - Create quiverplot video: See Chapter 3 Section 5 for further information on quiverplots. You can decide whether you want that a video of the quiverplots is saved for each folder.

6. **Start the analysis:** After choosing the settings and further options, you can start the analysis of the chosen folders by clicking on the button 'Start the automated analysis of the chosen folders...'. A new window opens. Choose a folder to save the results in by navigating to the desired folder. You can monitor the progress of the analysis with a progress bar below this button showing the process in percent.

7. **Stop ongoing analysis:** If you wish to stop the current analysis, e.g. because you forgot some settings or options, click on the button 'Stop ongoing analysis'. You can then change your folders, settings and options. Then start the analysis again.
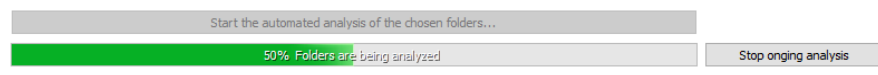
Figure 19: *Progress bar and button for stopping the ongoing analysis after starting.*