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Matlab coding: Willy Oesterheld

Matlab EmbryoMotion GUI for PMR and STC analysis

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1. Aim of the program

The program is designed to analyse videos obtained with the Viewpoint zebrabox in order to calculate the motion index for a selected time bin (for PMR) or the number of contractions (for STC). Subsequent calculations/data arrangements, particularly concentration-response analysis and calculation of effect concentration need to be processed in different software, e.g. using KNIME workflow or R-scripts.

The same program is applied to PMR and STC videos but different settings have to be used and different data have to be selected from the results of the video analysis.

KNIME workflows for subsequent data analysis are available from the UFZ.

The software particularly allows to analyse videos from microplate wells with rectangular shapes (96 wells) or round wells (24 wells). Other formats may be detected as well but have not been tested. Videos with too low (weak sensitivity) or too high (computationally demanding) could be difficult to analyse.

Example videos that will work: 30-60 sec videos recorded with the Viewpoint Zebrabox system. AVI format with no compression (approx.. 6 GB size).

A detailed manual and a license is provided.

Please note that the software should be considered as under development.

Known issues and required improvements:

- More flexible identification of well plate and dish formats is needed.
- Embryo tracking: Contraction of embryos can result in moving them out of the area that is used for quantification. A combination with a track of the embryo region could help in the future. You can also increase the area around the embryo to compensate for slight movements.
- Association with layout file: Only layouts with one test chemical in different concentrations are accepted. Workaround for screen different chemicals and numbers: Assign an arbitrary number to each sample.

1.1. STC

For the the STC the number of spontaneous contraction is calculated based on the selected threshold.

1.2. PMR

Specify the lag, excitation and refractory phase for the analysis (modify presets if needed). The activity of embryos in these three phases is calculated.

2. Description

The program is identifying the area in the video covered by the embryo. For this area subtraction images will be calculated and used to quantify the movement.

Potential confounding factors: Moving embryos can lead to false responses. You can check whether embryos have been moved by using the video slider. It is recommended to use a region for the slighter bigger than the embryo (e.g. 20-40 pixels radius if embryo region is 10 pixels radius).

The software was coded and compiled by Willy Oesterheld.

3. Installation and prerequisites

Either use the code in matlab (additional toolboxes may be required) or install the Windows version (required matlab runtime installation). The latter does not require a matlab license.

Prerequisites:

- Works on desktop computers but GPU computers provide faster performance
- The software was developed and tested with videos obtained with a ViewPoint Zebrabox System, but videos from other devices may work as well.
- For 96square well plates the video resolution used for development of the software was 5 Mio pixels. Lower resolutions may provide too low sensitivity for movement detection. Higher resolution may increase the duration of analysis.

- It is recommend to avoid compression of videos (e.g. record avi-files without any compression option). Compression introduces artificial differences between subsequent frames.
- Automatically detects square wells on videos of different numbers. Circular wells may also detected and square grid will be overlaid. There is also an option to detect circular wells but this has not yet been tested.

4. Instructions and settings for using the program

Main window

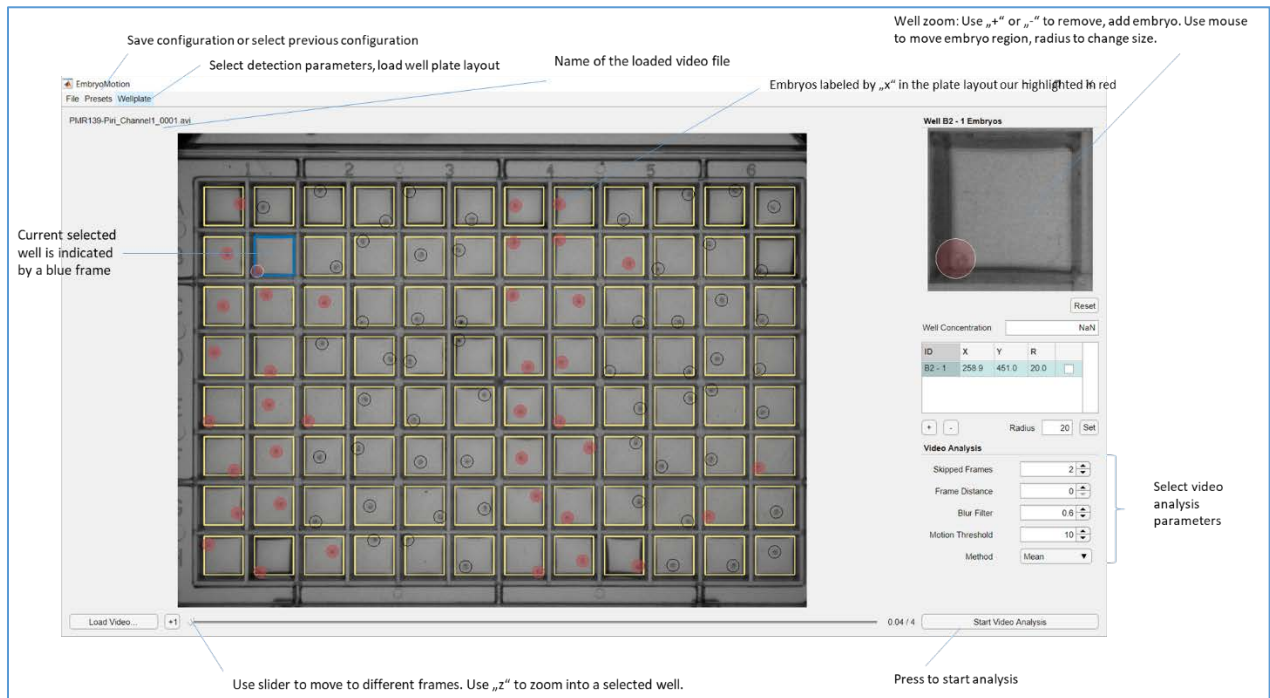


Fig. 1: Main window description

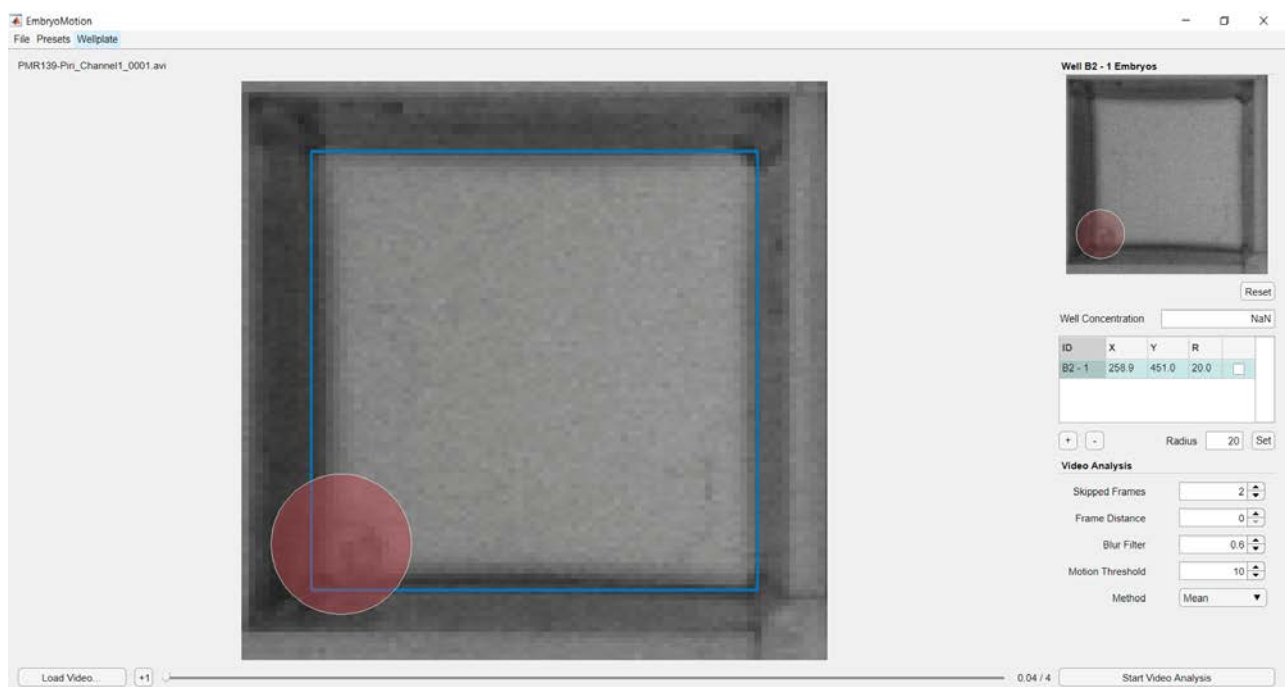


Fig. 2: Zoom into well by using the key for “z”. To leave zoom mode, press “z” again. The zoom mode is also available from the result window and can be used to visually inspect the movement of an individual embryo using the slider.

Video loading options:

Recommended settings for PMR and STC:

PMR	STC
Video Analysis Skipped Frames <input type="text" value="0"/> Frame Distance <input type="text" value="0"/> Blur Filter <input type="text" value="0.0"/> Motion Threshold <input type="text" value="10"/> Method <input type="text" value="Mean"/> Blur filter change to 0.6	Video Analysis Skipped Frames <input type="text" value="2"/> Frame Distance <input type="text" value="0"/> Blur Filter <input type="text" value="0.6"/> Motion Threshold <input type="text" value="10"/> Method <input type="text" value="Max"/>

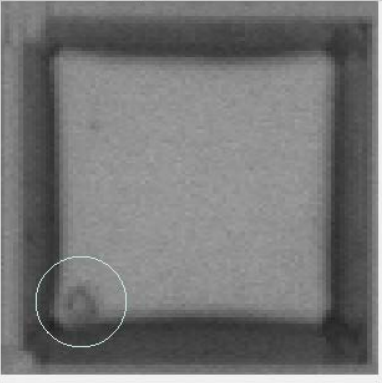
Fig. 3. Recommended settings for PMR and STC: Blur filter 0.5 or 0.6

The PMR settings reveal results very close to those obtained previously using the KNIME analysis. However, using appropriate combinations of threshold and blur the assessment could be optimised.

Blur = Reduces sharpness and hence background signalling. However, it will also decrease signals from moving embryos and hence, optimal combinations of threshold and blur need to be identified.

Adding and removing embryo or moving an annotation

Well D9 - 1 Embryos



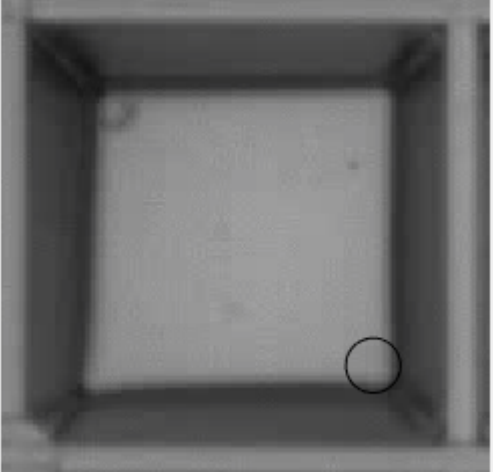
Well Concentration

ID	X	Y	R	
D9 - 1	1406.3	777.3	20.0	<input checked="" type="checkbox"/>

Radius

Add/remove:
 Click "+" or "-" on the selected well. Then click "Add Embryo"
 If a well contains several embryos and only individual embryos shall be removed from analysis use the tick box.

Well B5 - 1 Embryos



Move:
 Click on the circle with the mouse. While pressing the mouse button move the circle to the embryo.

Select the same size for all embryos

- Make sure that NO well is selected (by e.g. clicking outside the well to border of the well plate)
- Label all well in the well list. Enter the desired radius (e.g. 12 pixels) and confirm by "Set".


Well Concentration 0.000 - 0.000

ID	X	Y	R	
G11 - 1	1812.1	1197.8	11.9	✓
G12 - 1	1972.5	1131.1	10.0	✓
H1 - 1	70.1	1306.3	12.4	✓
H2 - 1	301.3	1361.2	12.1	✓
H3 - 1	397.4	1291.8	10.7	✓
H4 - 1	560.3	1393.3	10.2	✓
H5 - 1	725.0	1339.0	11.7	✓
H6 - 1	931.5	1393.9	11.9	✓
H7 - 1	1155.3	1291.1	9.6	✓
H8 - 1	1213.9	1402.1	6.5	✓
H9 - 1	1377.8	1393.4	10.0	✓
H10 - 1	1641.9	1396.6	12.0	✓
H11 - 1	1725.6	1305.7	11.3	✓
H12 - 1	1867.4	1296.3	11.2	✓

+ -

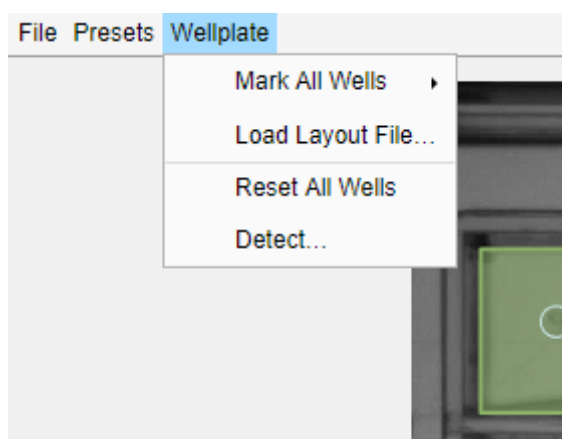
Radius 12
Set

No well should be labelled in order to obtain a table for all wells (click outside a well to clear a label)



Load well plate layout

The well plate layout can be loaded via the Menu “Wellplate” and the option “Load Layout File”. It is recommended to load the layout prior to analysis to see wells that are labelled with an “x” in plate layout (invalid, dead, empty or to be excluded from analysis for other reasons).



Example of plate layout (use template, edit green area, rest will be adjusted automatically))

Note: Load the video before loading the layout file prior to starting the analysis if you have a layout file.

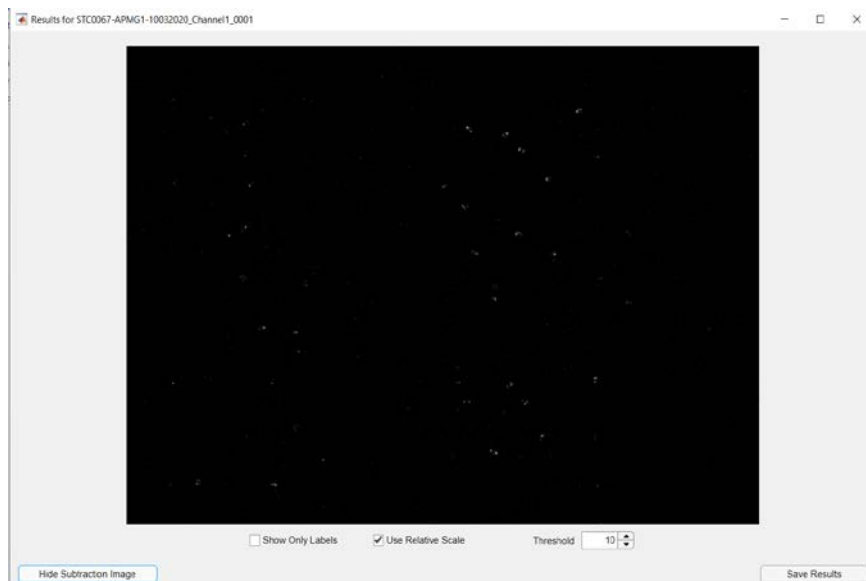


Fig. 6: Result window with subtraction image. Tick “Relative scale” to show embryos or regions with movement, respectively

In case you are not satisfied by the results and need to adjust threshold and blur options, close the window, change the settings in the main window and repeat analysis. Changing of threshold in the subtraction image window has no effect on analysis”

5. Calculation, saving and exporting results

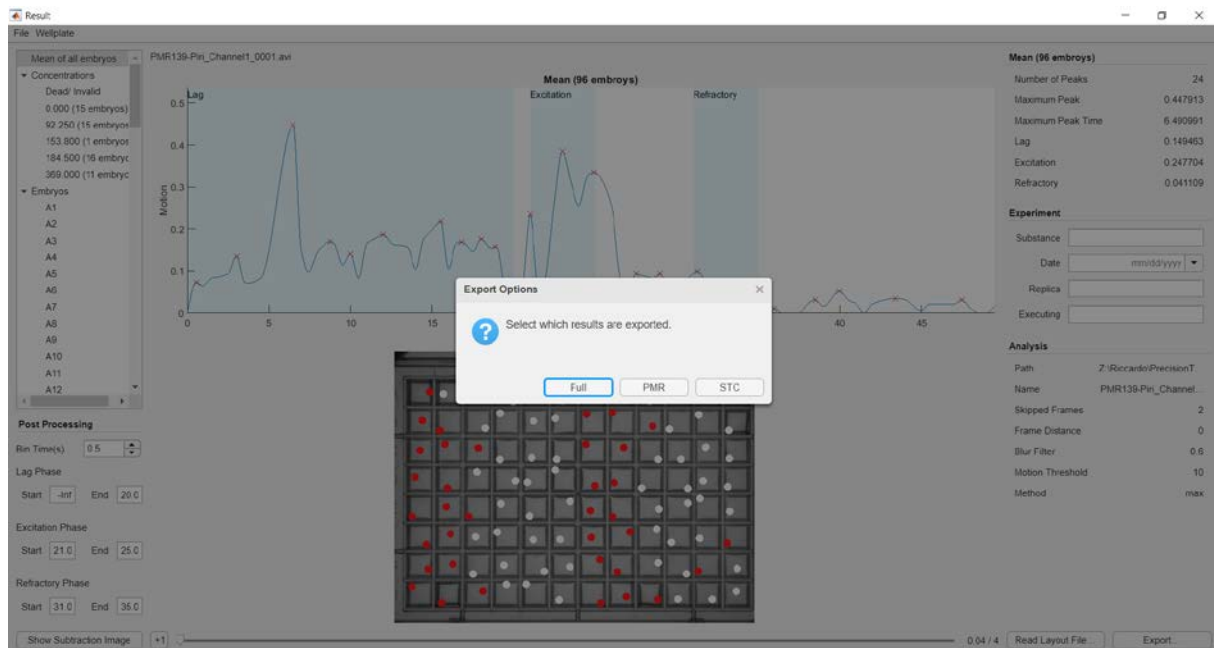
<p>Experiment Info</p> <p>Test Substance <input type="text"/></p> <p>Concentration Range <input type="text"/></p> <p>Date <input type="text" value="03.04.2020"/> ▼</p> <p>Replica <input type="text"/></p> <p>Executing <input type="text"/></p>	<p>Fig. 7 Information on the experiment can be added in the menu “Experiment Info”</p>
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NOTE: A working Excel installation on the same PC that is running Embryo Motion is required to write and export data into an excel file!!!!

Save results by clicking on “Export” Button. Data (data.xlsx) and various images (labels, subtraction, first video frame) are saved into the same excel file.

Please note that the same evaluations and calculations are conducted regardless whether you load videos for STC or PMR assessment. However, some of the calculations make only sense for either STC and PMR. Therefore, when exporting results you can restrict the export to STC or PMR relevant data.

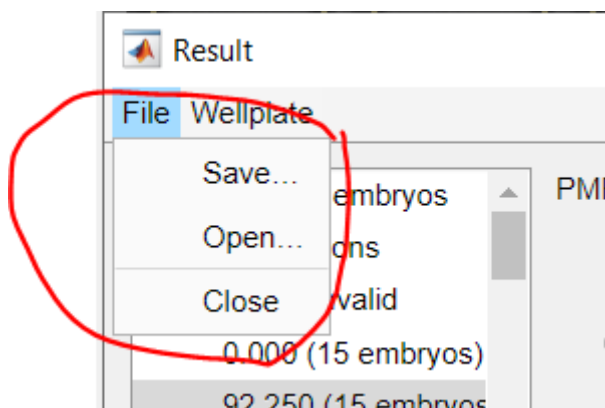
We may change this in the future by making this switch prior to the calculation and to avoid confusion. However, this requires more elaboration and hence, as in intermediate solution the selection can be made only prior to exporting data.



The following data are calculated for export to an excel file:

- MotionIndex for specific phases of the PMR (for individual wells and per concentration)
- Peak frequency for STC (for individual wells and per concentration)

Saving results in an internal format (.emr files)*



It is also possible to save the results in an internal format. This may be relevant in cases where you want to inspect the results later in the EmbryoMotion program without rerunning the analysis. However, typically the export as Excel file should be sufficient since this enables subsequent data processing. The saving option is available from the results window. Opening of saved data is possible from the start as well as the results window.

6. Optimise STC assessment

The STC analysis needs optimisation to identify parameters that allow to reliably determine the number of spontaneous contractions. Therefore, change threshold and blur settings and compare the results with manual counting of contraction in individual wells.

To facilitate the manual counting the slider can be used to browse through the video and check whether contraction aligns with the peaks in the analysis window.

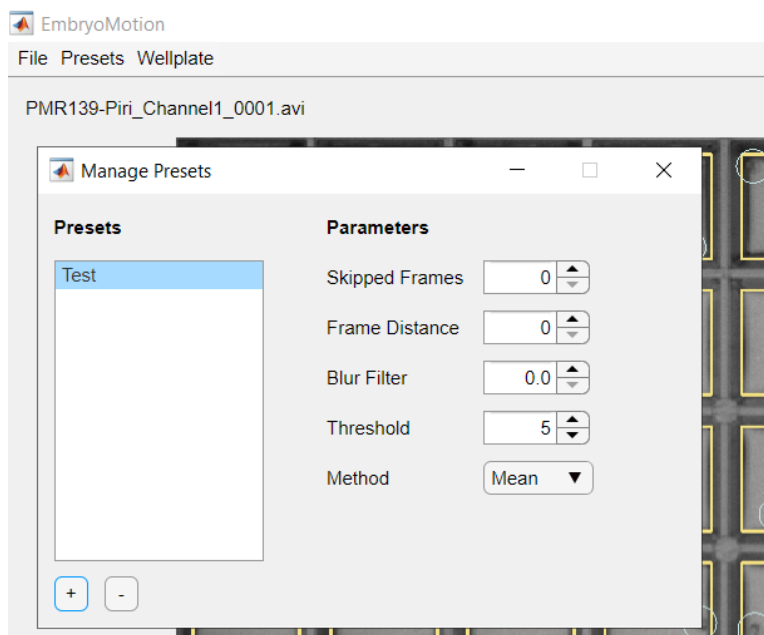
7. Creating and storing presets and projects

Presets

Using the Presets menu you can specify presets for repeated use, avoiding that you have to manually enter all parameters and securing that you are using the same set of parameters for each analysis. For instance, you could store specific presets for STC or PMR analysis.

Projects

From the start window file menu you can save your projects. A project file contains all information on embryo position and size of the embryo. It is recommended to save a project for each analysis. The advantage is that in case of reanalysis you do not need to specify embryo positions and size again and can just upload a project file. EmbryoMotion searches for a corresponding file that has been saved previously for the uploaded video in the same folder. If no file is available in the same folder or you have moved the video to a different folder since the last analysis you can select a project for upload.



8. Feedback and suggestions

....are of course welcome.