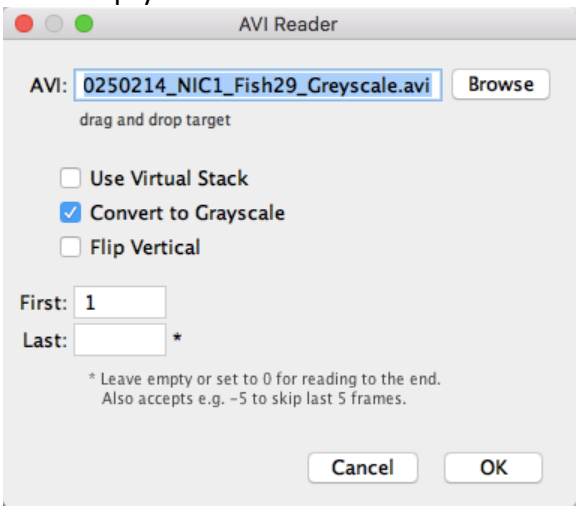
Heart Rate Macro Protocol

HR_Macro

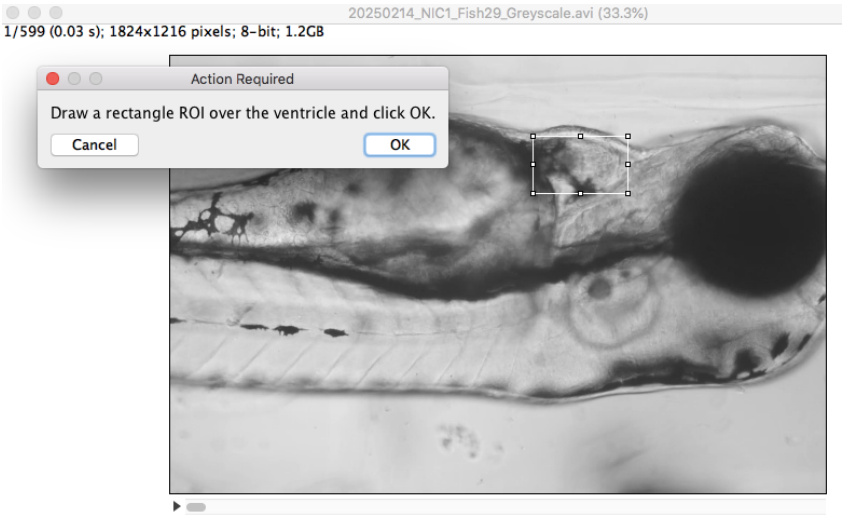
This ImageJ macro code is developed for processing of zebrafish videos to calculate the average heart rate, plot the heart brightness curve with detected beats and detect possible arrhythmia.

Using HR_Macro (Protocol)

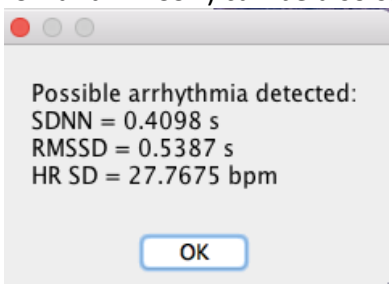
1. Copy the .txt file titled “HR_Macro” to your desktop.
2. Open ImageJ and install the macro:
    a. Plugins > Macros > Install > Choose HR_Macro
3. Open the video (.avi format):
    a. Select ‘*Convert to Grayscale’* and **deselect ‘***Use Virtual Stack’.* Leave the ‘Last’ frame number empty or set to 0 to make sure all frames are loaded.



4. Run the macro:
    a. Installed macro is ready to run: Plugins > Macros > HR_Macro
    OR go to Plugins > Macros > Run… > Choose HR_Macro
5. Draw a rectangle around the ventricle, move between the frames to see if the ventricle exceeds the rectangle. (*Make sure the heart is within the rectangle, a rectangle too large or too small can lead to inaccuracy*).



6. Save the measurements on your Excel sheet (HR average & average interval time at the bottom of the results table). You can save the plot if needed.
    a. The macro will give a warning only if arrhythmia is detected, arrhythmia parameters (SDNN, HR SD and RMSSD) can be also saved if needed.



About the Plot: The plot is good to check the beat detection visually and can be useful to compare zebrafish lines. If you do not need it, you can delete the last part of the code after 'Step 8' so a plot does not show up each time (make sure you make the changes in a copy txt file and not the original script).

Parameter Guideline

The macro has adjustable parameters to ensure accuracy and optimize detection. Default values are tested and optimized according to existing data. Besides fps (frames per second, depends on the camera used for filming), no prior adjustment is required. If you suspect any results (especially for mutants which may have smaller ventricles or undetectable heart beats), please refer to the guideline.

The best is to analyze every dataset using the same parameters if possible, to avoid incorrect comparison. Adjust when there is an issue for multiple videos or a whole dataset, make sure they are not just outliers to be discarded.

When you change a threshold, save the changes, reinstall the macro or use the 'Run…' option to test. Note down new threshold values for further reference.

Detection Parameters

- fps: Frames per second, set to your video's frame rate
- minSpacing: Minimum frame distance between beats
    - Prevents false detection of multiple beats, evaluating each beat
    - In case of false peaks (multiple detections per beat) > increase minSpacing (to 7-9)
    - If beats are missed (likely in high HR recordings) > decrease minSpacing (to 3-5)
- prominenceThreshold: Sensitivity for dip detection
    - Noisy signal, too many false beats > increase prominenceThreshold (try small changes first, 0.07 etc.)
    - Weak intensity changes, beats missed (likely in some mutants) > decrease prominenceThreshold (try small changes first, 0.03 etc.)
- minInterval: Minimum frames between beats, filters out false peaks
    - For very fast zebrafish hearts, decrease slightly (minimum 2) to avoid discarding valid beats.
    - For very low fps, may need to increase slightly.

Arrhythmia Parameters

- SDNN (s): Standard deviation of beat intervals (overall HR variability)
- RMSSD (s): Root mean square of successive interval differences (sensitive to irregular rhythm)
- HR SD (bpm): Standard deviation of HR values (beat-to-beat stability)
- For high sensitivity (detection of minor irregularities) lower the thresholds.
- For high specificity (avoid false arrhythmia and detect major irregularities only) raise thresholds.
- Refer to literature for adjustments.

Default Thresholds

fps	minSpacing	prominenceThreshold	minInterval	SDNN	RMSSD	HR SD
30	6	0.05	4	>0.1	>0.2	>15