Non-invasive tool to assess heart rhythm in Zebrafish embryos

A NON INVASIVE TOOL TO ASSESS HEART RHYTHM IN ZEBRAFISH EMBRYOS

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Introduction

Background

In the last years the zebrafish (Danio Rerio) has emerged as animal model for cardiac research. In consequence of the similarity to humans in the early function, the zebrafish embryo (Fig.1) has been suggested as an ideal model i) to study the molecular mechanism of cardiac development, and ii) to identify genes related to congenital cardiac defects in human [1]. Moreover the overall similarity of zebrafish and human, in responses to cardiotoxic drugs, was demonstrated [2]. For these reasons, the non-invasive monitoring of the cardiac function in zebrafish has become a challenging issue, in very recent years.

The development in digital imaging has recently made easier the assessment of the cardiac functions in zebrafish embryos [3,4]. This could allow to monitor non-invasively heart rate in zebrafish embryos from videos of beating heart.

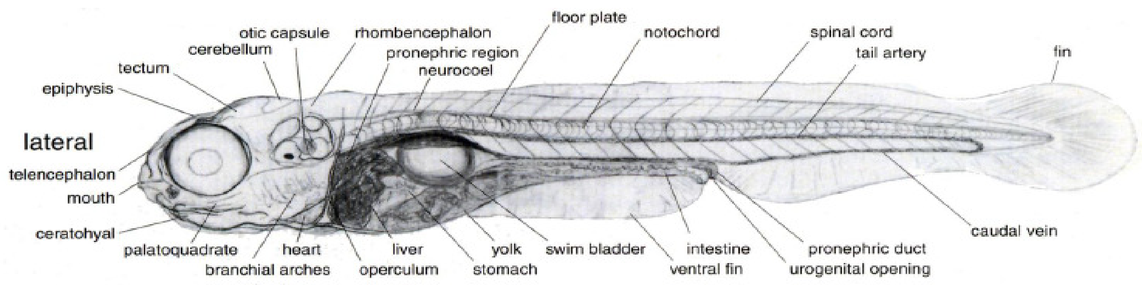

Figure 1. a lateral representation of a 5 days post fertilization embryo of zebrafish [5]

Aim of the study

We present a non-invasive method that, by video-recording zebrafish embryo images of the heart chambers (atrium, ventricle, bulb) (Fig. 2) using confocal microscopy, and integrating image processing and power spectral analysis, allows to measure the heartbeat rhythm.

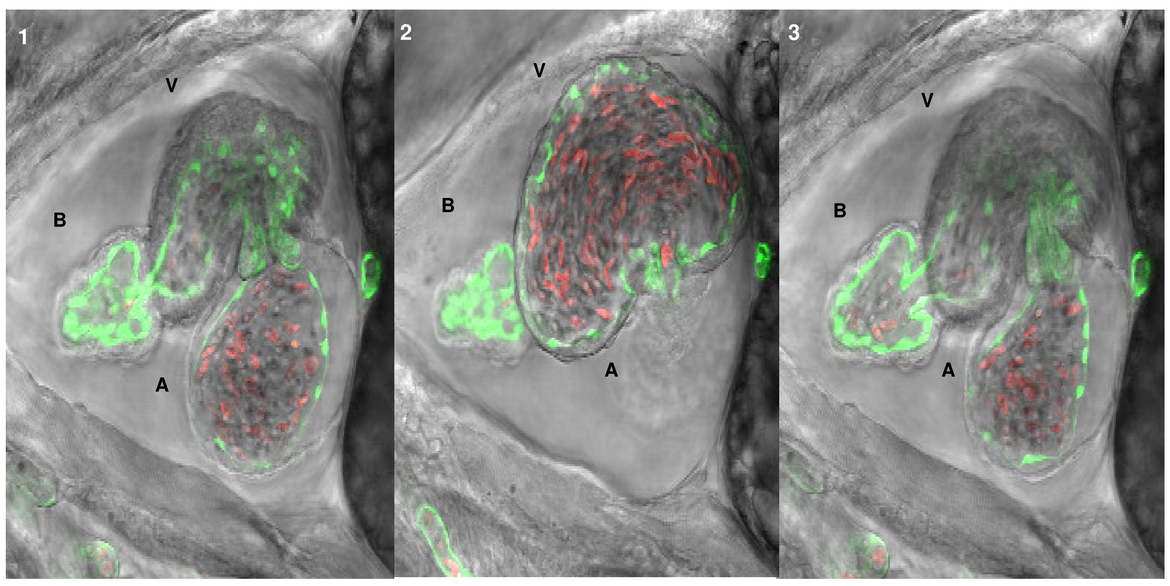

Figure 2. A 3D acquisition obtained through the Leica confocal microscope (256 x 256 pixels); the images represent the 3 channel together: the 3 channel are respectively the green channel that shows the endothelial cells (Tg(flk1:GFP)s843); the red channel that shows the erythrocytes (Tg(gata1:dsRed)sd2); the phase channel represented in the gray scale; the letters are referred to the specific chambers: A for atrium; B for Bulb; V for Ventricle.

Materials and Methods

• Zebrafish embryos at 96 hpf developmental stage were mounted in 0.5% agarose solution inside of a viewing chamber and analyzed with a Leica confocal laser-scanning microscope (model TCS SP5).

•We used Tg(flk1:GFP)s843 and Tg(gata1:dsRed)sd2 transgenic lines, which express green fluorescent protein in the vasculature [6], and red fluorescent protein in blood cells, respectively.

• Images (20× magnification) including atrium, ventricle, and aortic bulb (Fig. 3) were captured in mono-directional mode at a 36 fps sampling frequency. Recordings were grabbed and stored in TIFF format.

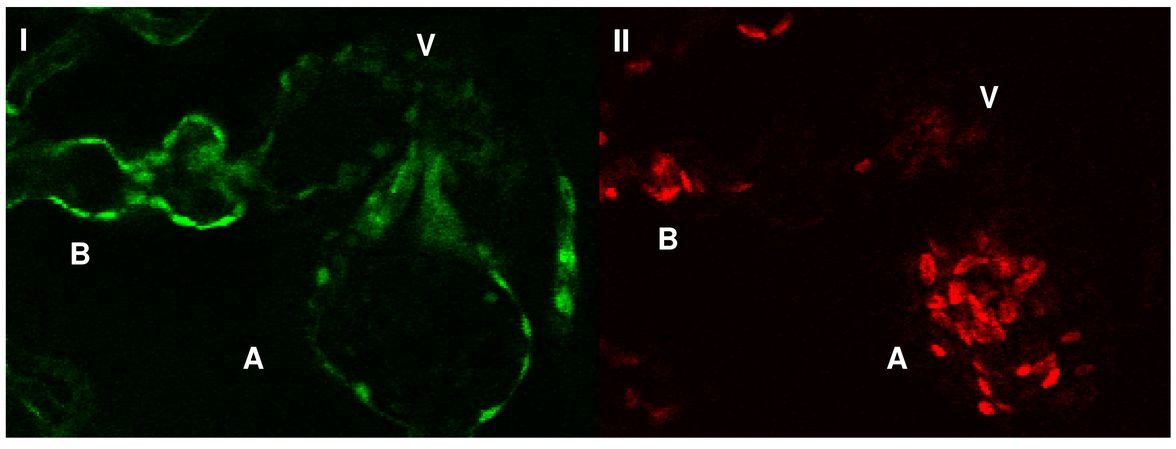

Figure 3. a typical 2D acquisition obtained through the microscope (512 x 200 pixels) from a 96 hpf embryo; images are bidimensional slices of the entire heart muscle (Figure 2); 3I shows the green channel (endothelial cells); 3II shows the red channel, (erythrocytes). Letters: A for atrium; B for bulb; V for ventricle.

• The red scale of each RGB frame was considered. Images of embryo's ventricle, atrium, and bulb, respectively were acquired. Considering that i) a 8 bits image intensity colour range is [0-255], and that ii) the value of static pixels located in non-moving areas was zero, to reduce noisy artefacts we fixed a threshold value of 50 in pixel intensity over each frame, and the amount of static over-threshold pixels was counted, and expressed as percentage to the number of the pixels of each frame which are contained inside the edges of the chamber investigated, following the relation:

$$PercentageofPixelsOverThreshold(i) = \frac{NumberofPixelsOverThreshold(i)}{NumberofPixelsInsideTheChamberAnalyzed} \bullet 100$$

Where i = i - esime image

• The automated evaluation of the heart rhythm of zebrafish embryos was performed by means of a spectral analysis: the Fast Fourier Transform (FFT) algorithm was applied for the calculation of the power spectral density (PSD).

•Treated and no-treated embryos were investigated: treated embryos were anesthetized with a 4mg/ml tricaine solution, in order to depress the cardiac rhythm.

•The validation was executed on video acquired on 14 embryos at 96 hpf. A pool of healthy embryos were measured and then undergone treatment by tricaine, a cardiac anaesthetizing drug, in consequence of which a decrease of the heart rate is expected, were analyzed. The software results were compared to the optometric count of the heart beats from the video histories as concerning the no-treated embryos as the larvae treated.

Results

The embryos flow is pulsatile, with a rhythm of fast and slow movement. Our image analysis allowed to record this oscillatory movement of blood cells in any heart chamber and to map it into a time history. It can be noticed from Fig. 4 that i) a rhythm of fast and slow movement was present within the extracted time histories, and ii) a phase shift among waveforms extracted from atrium, ventricle and bulb was present, as physiologically expected.

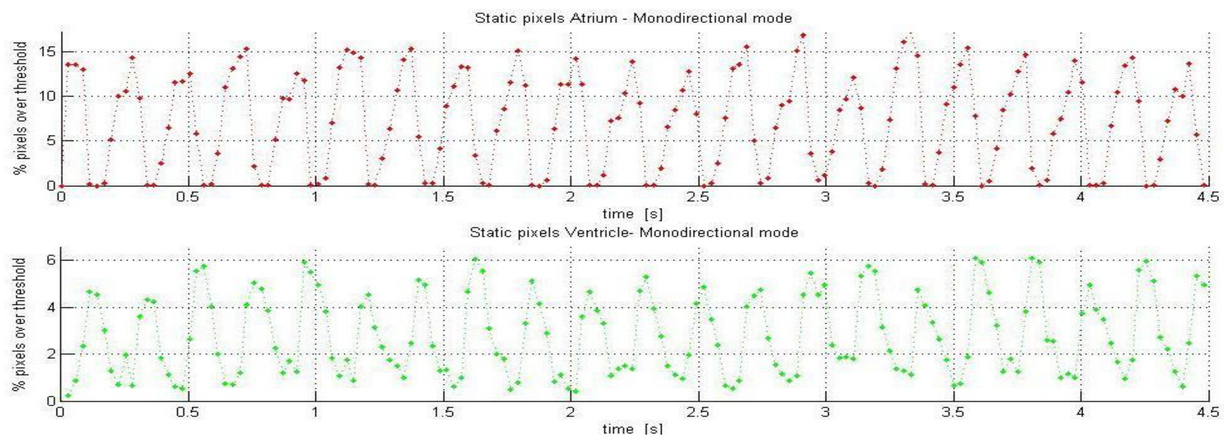

Figure 4. A typical example of the static pixels signal: the curves are relative to the same acquisition; we observed that atrium peaks (upper panel) anticipated, in time, the ventricle peaks (lower panel).

Fig. 5A shows the histogram obtained by 14 embryos before and after treatment (tricaine). The graph compares the optometric count with the software inspection application. Fig. 5B illustrates the PSD of waveforms extracted from ventricle image (figure 5R) analysis for larva number three of the 14 embryos used for validation: the PSD clearly shows that the embryo exhibited peaks in the spectrum at distinct frequencies (the sedated embryo at 3.941 Hz, the no-treated one at 5.649 Hz), in consequence of the anaesthetic administration (tricaine).

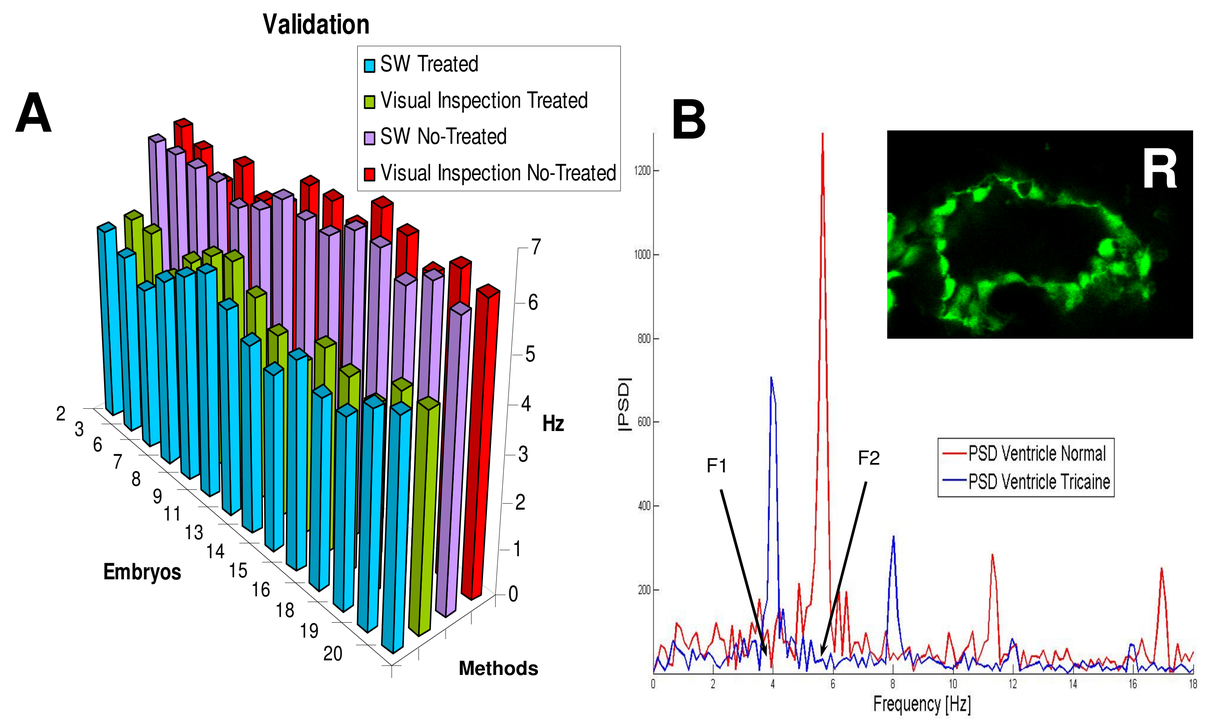

Figure 5. A: histogram obtained by 14 embryos before and after treatment; light blue bars indicate the HR frequencies of the embryos treated (tricaine) calculated by our software; green bars refer to the HR frequencies of the same embryos obtained by optometric count; violet bars indicate the HR frequencies of the larvae no treated calculated by software; red bars refer to the HR frequencies of the same embryos obtained by optometric count; **B:** example of power spectrum obtained from the ventricle analysis (R) of one embryo: arrows indicate the main frequency components F1 end F2.

Conclusions

The study demonstrates that the non-invasive estimation of the cardiac rate of zebrafish embryos is feasible by processing digital images. The main advantage of using this method is that it is rapid and fully automated, thus avoiding genetists and biologists of measuring zebrafish embryos cardiac rhythm by visual inspection.

References

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