Submission ID #: 68941
Scriptwriter Name: Sulakshana Karkala
Project Page Link: https://review.jove.com/account/file-uploader?src=21027053

Title: Functional Cardiac Imaging in Zebrafish Embryos Using Standard Microscopy and Video Analysis: Applications in Environmental and Biomedical Research

Authors and Affiliations:

Nikola Mitovic[1], Sanjin Kovacevic[1], Jelena Nesovic Ostojic[1], Darko Puflovic[2], Marija S. Stankovic[1]

[1]Department of Pathophysiology, Medical Faculty, University of Belgrade
[2]Faculty of Electronic Engineering, University of Nis

Corresponding Authors:
Nikola Mitovic (nikola.mitovic@med.bg.ac.rs) ; (nikolamitovic@gmail.com)
Email Addresses for All Authors:
Nikola Mitovic (nikola.mitovic@med.bg.ac.rs) ; (nikolamitovic@gmail.com)
Sanjin Kovacevic (sanjin.kovacevic@med.bg.ac.rs)
Jelena Nesovic Ostojic (jelena.nesovic-ostojic@med.bg.ac.rs)
Darko Puflovic (darko.puflovic@elfak.ni.ac.rs)
Marija S. Stankovic (marija.stankovic.med@gmail.com)

Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: MM**/DD/YYYY**

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare.

Current Protocol Length

Number of Steps: 07
Number of Shots: 11

Introduction

NOTE TO VO: Please record this section

INTRODUCTION:

~~What technologies are currently used to advance research in your field?~~

1.1. ~~**Nikola Mitovic:**~~ High-resolution microscopy, fluorescent reporters, and digital image analysis are some technologies that enable detailed assessment of embryonic cardiac function in zebrafish.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.1*

~~What are the current experimental challenges?~~

1.2. ~~**Nikola Mitovic:**~~ Maintaining consistent imaging conditions and minimizing embryo movement remain major challenges for accurate cardiac function measurements.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.5*

CONCLUSION:

~~What research gap are you addressing with your protocol?~~

1.3. ~~**Nikola Mitovic:**~~ The protocol addresses the lack of standardized, accessible methods for quantifying zebrafish embryonic cardiac function with reproducible workflows.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.7*

~~What advantage does your protocol offer compared to other techniques?~~

1.4. ~~**Nikola Mitovic:**~~ This protocol uses standard microscopy and open-source analysis, making high-quality cardiac assessment feasible without specialized equipment.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.3*

~~How will your findings advance research in your field?~~

1.5. ~~**Nikola Mitovic:**~~ The findings provide a reliable protocol that improves comparability between studies and enables more precise evaluation of cardiac phenotypes.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.2*

Ethics Title Card

The study was approved by the Animal Care and Use Committee of the Faculty of Medicine, University of Belgrade

Protocol

NOTE: Scripted from author provided footage

2. Assessment of Zebrafish Embryo Heart Dynamics via Image-Based Motion Analysis

Demonstrator: Nikola Mitovic

2.1. To begin, record videos of zebrafish embryos at 96 hours post in uncompressed formats to allow accurate analysis of cardiac motion dynamics **[1-TXT]**.

2.1.1. LAB MEDIA: 1st.mp4 00:00-00:16
TXT: Frame rate: 30 fps

2.2. Sequentially click on **File**, **Open** then choose the AVI *(A-V-I)* file in ImageJ *(Image-J)* to import the videos **[1-TXT]**. Use **Analyze** then click on **Set Scale**, input the known field diameter and enable the **Global** option to apply the scale across all measurements **[2]**.

2.2.1. LAB MEDIA: 2nd.mp4 00:00-00:14
TXT: If needed, convert to image stack

2.2.2. LAB MEDIA: 2nd.mp4 00:23-00:35

2.3. Use the ROI *(R-O-I)* tools to mark the long and short ventricular axes during end-systole and end-diastole **[1]**.

2.3.1. LAB MEDIA: 2nd.mp4 00:45-00:51, 00:58-01:02, 01:13-01:16

2.4. Then determine the ventricular surface area using the ellipse approximation formula **[1]**. Estimate the ventricular volume at end-diastole and end-systole using the ellipsoid volume formula **[2]**.

2.4.1. LAB MEDIA: 3rd.mp4 00:00-00:15
Video Editor: Please Highlight the top formula VA = ((D_L - D_S)/2) × π and the values of VAS and VAD

2.4.2. LAB MEDIA: 3rd.mp4 00:00-00:15
Video Editor: Please Highlight the middle formula VV = (1/6) × π × D_L × D_S² and the values of VVS and VVD

2.5. Now, calculate the fractional area change and fractional shortening **[1]**. Then determine the stroke volume SV *(S-V)* and Ejection Fraction EF *(E-F)* **[2]**.

2.5.1. LAB MEDIA: 3rd.mp4 00:00-00:15
Video Editor: Please Highlight the formulae
FPP (%) = ((VAD - VAS) / VAD) × 100 and
FS (%) = ((D_L - D_S) / D_L) × 100

2.5.2. LAB MEDIA: 3rd.mp4 00:00-00:15
Video Editor: Please Highlight the formulae
SV= VVD=VVS= 367039 µL and
EF (%) = (SV / VVD) × 100

2.6. For automated analysis, launch the ZembryoAnalyzer *(Z-Embryo-Analyzer)* software and click on **File** then **Open Video** to import the desired video **[1]**. Click on the **Detect Heart** button to allow the software to automatically locate the region of interest based on brightness fluctuations across video frames **[2-TXT]**.

2.6.1. LAB MEDIA: 4th.mp4 00:00-00:02

2.6.2. LAB MEDIA: 4th.mp4 00:02-00:30
TXT: Adjust region manually if needed

2.7. Let the software analyze brightness changes within the detected region of interest to estimate heart rate then calculate cardiac output **[1-TXT]**.

2.7.1. LAB MEDIA: 4th.mp4 00:31-00:55 **TXT: Export heart rate and corresponding intensity plots**

Results

3. Results

3.1. The average ventricular area in zebrafish embryos at 96 hours post fertilization was significantly higher at end-diastole compared to end-systole **[1]**. The average ventricular volume in zebrafish embryos at 96 hours post fertilization was significantly higher at end-diastole compared to end-systole **[2]**.

3.1.1. LAB MEDIA: Figure 2A and C. *Video editor: Highlight the red violin plot labelled "EDA" in 2A*

3.1.2. LAB MEDIA: Figure 2B and B . *Video editor: Highlight the red violin plot labelled "EDA" in 2 B*

3.2. In healthy zebrafish embryos, the average stroke volume was 0.213 nanoliters **[1]**, cardiac output was 27.8 nanoliters per minute **[2]**, and ejection fraction was 41.99 percent **[3]**.

3.2.1. LAB MEDIA: Figure 3 *Video Editor: Please highlight 3D*

3.2.2. LAB MEDIA: Figure 3 *Video Editor: Please highlight 3A*

3.2.3. LAB MEDIA: Figure 3 *Video Editor: Please highlight 3C*

3.3. In healthy zebrafish embryos, the average fractional area change was 61.2 percent **[1]**, and fractional shortening was 44.5 percent **[2]**.

3.3.1. LAB MEDIA: Figure 4 *Video Editor: Please highlight A*

3.3.2. LAB MEDIA: Figure 4 *Video Editor: Please highlight B*

3.4. Cadmium-exposed embryos exhibited abnormal cardiac morphology with enlarged ventricles and pericardial edema **[1]**.

3.4.1. LAB MEDIA: Figure 5 *Video Editor: Please highlight A*

3.5. In cadmium-exposed embryos, stroke volume was significantly lower than in controls **[1]**, cardiac output was diminished **[2]**, and heart rate was reduced **[3]**.

3.5.1. LAB MEDIA: Figure 5B. *Video editor: Highlight the yellow violin plot labelled "Cd^{2+}"*

3.5.2. LAB MEDIA: Figure 5C. *Video editor: Highlight the yellow violin plot labelled "Cd^{2+}"*

3.5.3. LAB MEDIA: Figure 5D. *Video editor: Highlight the yellow violin plot labelled "Cd^{2+}"*

Pronunciation Guide:

1. Zebrafish
   Pronunciation link: https://www.merriam-webster.com/dictionary/zebrafish
   IPA: /ˈzɛbrəˌfɪʃ/
   Phonetic Spelling: ZEB·ruh·fish
2. Embryos
   Pronunciation link: https://www.merriam-webster.com/dictionary/embryo
   IPA: /ˈɛmbrioʊ/
   Phonetic Spelling: EM·bree·oh
3. Functional
   Pronunciation link: https://www.merriam-webster.com/dictionary/functional
   IPA: /ˈfʌŋkʃənəl/
   Phonetic Spelling: FUNK·shuh·nuhl
4. Cardiac
   Pronunciation link: https://www.merriam-webster.com/dictionary/cardiac
   IPA: /ˈkɑːrdiːæk/
   Phonetic Spelling: KAR·dee·ak
5. Microscopy
   Pronunciation link: https://www.merriam-webster.com/dictionary/microscopy
   IPA: /maɪˈkrɑːskəpi/
   Phonetic Spelling: my·KRAH·skuh·pee
6. Fluorescent
   Pronunciation link: https://www.merriam-webster.com/dictionary/fluorescent
   IPA: /flʊˈrɛsənt/
   Phonetic Spelling: floor·EH·suhnt
7. Phenotypes
   Pronunciation link: https://www.merriam-webster.com/dictionary/phenotype
   IPA: /ˈfiːnəˌtaɪp/
   Phonetic Spelling: FEE·nuh·type
8. Ventricular
   Pronunciation link: https://www.merriam-webster.com/dictionary/ventricular
   IPA: /vɛnˈtrɪkjələr/
   Phonetic Spelling: ven·TRIK·yuh·ler
9. End-diastole
   Pronunciation link: https://www.merriam-webster.com/dictionary/diastole
   IPA: /daɪˈæstəli/
   Phonetic Spelling: dye·AS·tuh·lee
10. End-systole
    Pronunciation link: https://www.merriam-webster.com/dictionary/systole
    IPA: /ˈsɪstəli/
    Phonetic Spelling: SIS·tuh·lee

11. Ellipsoid
    Pronunciation link: https://www.merriam-webster.com/dictionary/ellipsoid
    IPA: /ɪˈlɪpsɔɪd/
    Phonetic Spelling: ih·LIP·soyd
12. Fractional
    Pronunciation link: https://www.merriam-webster.com/dictionary/fractional
    IPA: /ˈfrækʃənəl/
    Phonetic Spelling: FRAK·shuh·nuhl
13. Stroke Volume
    Pronunciation link: https://www.merriam-webster.com/dictionary/stroke%20volume
    IPA: /ˈstroʊk ˌvɑːljuːm/
    Phonetic Spelling: strohk VOL·yoom
14. Ejection Fraction
    Pronunciation link: https://www.merriam-webster.com/dictionary/ejection%20fraction
    IPA: /ɪˈdʒɛkʃən ˈfrækʃən/
    Phonetic Spelling: ih·JEK·shuhn FRAK·shuhn
15. Cardiac Output
    Pronunciation link: https://www.merriam-webster.com/dictionary/cardiac%20output
    IPA: /ˈkɑːrdiːæk ˈaʊtpʊt/
    Phonetic Spelling: KAR·dee·ak OUT·poot
16. ImageJ
    Pronunciation link: https://www.howtopronounce.com/imagej
    IPA: /ˈɪmɪdʒ dʒeɪ/
    Phonetic Spelling: IM·ij jay
17. Region of Interest
    Pronunciation link: https://www.merriam-webster.com/dictionary/region%20of%20interest
    IPA: /ˈriːdʒən əv ˈɪntrəst/
    Phonetic Spelling: REE·juhn uhv IN·truhst
18. Pericardial
    Pronunciation link: https://www.merriam-webster.com/dictionary/pericardial
    IPA: /ˌpɛrɪˈkɑːrdiəl/
    Phonetic Spelling: pair·ih·KAR·dee·uhl
19. Edema
    Pronunciation link: https://www.merriam-webster.com/dictionary/edema
    IPA: /ɪˈdiːmə/
    Phonetic Spelling: ih·DEE·muh
20. Cadmium
    Pronunciation link: https://www.merriam-webster.com/dictionary/cadmium
    IPA: /ˈkædmiəm/
    Phonetic Spelling: KAD·mee·uhm