DESIGNING A PYTHON-BASED PLATFORM TO EXAMINE AND ANALYZE ZEBRAFISH

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Thesis for the Master's Program in Electrical and Electronics Engineering

Graduate School
Izmir University of Economics
Izmir
2025

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A Master’s Thesis
Submitted to
the Graduate School of Izmir University of Economics
the Department of Electrical and Electronics Engineering

Izmir
2025

Approval of the Graduate School

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I hereby declare that I am the sole author of this thesis and that I have conducted my work in accordance with academic rules and ethical behaviour at every stage from the planning of the thesis to its defence. I confirm that I have cited all ideas, information and findings that are not specific to my study, as required by the code of ethical behaviour, and that all statements not cited are my own.

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ABSTRACT

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Master’s Program in Electrical and Electronics Engineering

Advisor: Asst. Prof. Ebru SAYILGAN

June, 2025

This study focuses on the development of a platform designed to facilitate the visual examination and analysis of zebrafish. The platform is particularly aimed at researchers, biologists, toxicologists, and pharmacologists working in biomedical and toxicological research. It addresses common challenges encountered in zebrafish analysis, such as accurately tracking movement, effectively monitoring heart rate, obtaining clear microscopic images, and generating comprehensive visual data. The platform will consist of four main modules. These modules are as follows: “Tracking Free-Moving Zebrafish in a Container Module” it measures the speed variation and movement data of zebrafish in relation to a fixed point. By utilizing this module, changes in the nervous and muscular systems of zebrafish can be analyzed. “Examination of the Heart Rate/Frequency of a Stationary Zebrafish Module” facilitates the analysis of the zebrafish's cardiac response to pharmaceutical and toxicology tests. By employing this module, researchers can effectively monitor the heart’s reaction to various substances. “Zebrafish Focus Stacking Module” that the microscopes used for zebrafish studies are primarily designed for two-dimensional imaging. This module has been developed to enhance the visualization of three-dimensional zebrafish tissues with greater clarity. “Zebrafish Image Stitching Module” The microscopes used for zebrafish imaging have a limited field of view, making it challenging to capture the entire zebrafish in a single frame. Instead of using expensive microscopes with a wider field of view, this module digitally merges with multiple images to produce a composite image with an expanded field of view.

Keywords: Zebrafish, Zebrafish Analysis Software, Image Processing, Zebrafish Heart Rate Measurement, Zebrafish Tracking.

ÖZET

ZEBRABALIKLARINI İNCELEMEK VE ANALİZ ETMEK İÇİN PYTHON TABANLI BİR PLATFORM TASARIMI

ÇOLAK, Mehmet Furkan

Elektrik ve Elektronik Mühendisliği Yüksek Lisans Programı

Tez Danışmanı: Dr. Öğr. Üyesi Ebru SAYILGAN

Haziran, 2025

Bu çalışma, zebra balıklarının görsel olarak incelenmesi ve analizini kolaylaştırmak amacıyla tasarlanmış bir platformun geliştirilmesine odaklanmaktadır. Platform, özellikle biyomedikal ve toksikolojik araştırmalarda çalışan araştırmacılar, biyologlar, toksikologlar ve farmakologları hedeflemektedir. Zebra balığı analizinde karşılaşılan yaygın zorluklara çözüm üretmeyi amaçlamaktadır; bu zorluklar arasında hareketin doğru bir şekilde takip edilmesi, kalp atış hızının etkin biçimde izlenmesi, net mikroskobik görüntüler elde edilmesi ve kapsamlı görsel verilerin oluşturulması bulunmaktadır. Platform dört ana modülden oluşacaktır. Bu modüller şunlardır: "Kap İçinde Serbest Hareket Eden Zebra Balıklarının Takip Modülü": Zebra balıklarının sabit bir referans noktasına göre hız değişimi ve hareket verilerini ölçer. Bu modül sayesinde zebra balıklarının sinir ve kas sistemlerindeki değişiklikler analiz edilebilir. "Hareketsiz Zebra Balığının Kalp Atış Hızı/Frekansı İnceleme Modülü": Zebra balığının farmasötik ve toksikolojik testlere verdiği kalp tepkisinin analizini mümkün kılar. Araştırmacılar bu modül ile kalbin çeşitli maddelere karşı tepkisini etkin bir şekilde izleyebilir. "Zebra Balığı Focus Stacking (Odak Yığma) Modülü": Zebra balıkları için kullanılan mikroskoplar genellikle iki boyutlu görüntüleme için tasarlanmıştır. Bu modül, üç boyutlu zebra balığı dokularının daha net bir şekilde görselleştirilmesini sağlamak üzere geliştirilmiştir. "Zebra Balığı Görüntü Birleştirme (Image Stitching) Modülü": Zebra balığı görüntülemelerinde kullanılan mikroskoplar dar bir görüş alanına sahiptir ve zebra balığının tamamını tek bir karede yakalamak zordur. Geniş görüş alanına sahip pahalı mikroskoplar yerine, bu modül birden fazla görüntüyü dijital olarak birleştirerek daha geniş görüş alanına sahip bileşik bir görüntü oluşturur.

Anahtar Kelimeler: Zebra Balığı, Zebra Balığı Analiz Programı, Görüntü İşleme, Zebra Balığı Kalp Ritmi Ölçme, Zebra Balığı Takibi.

Dedicated to all the loved ones…

ACKNOWLEDGEMENTS

I would like to express my heartfelt gratitude to my supervisor, Dr. Ebru Sayılgan, for her continuous support, insightful guidance, and genuine encouragement throughout my academic journey. Her thoughtful feedback and deep understanding have had a lasting impact on both my undergraduate and postgraduate studies. It has been a privilege to be her student. I would also like to express my sincere thanks to the ÇETİN Lab at IBG for their valuable guidance and for generously providing the zebrafish data that formed the foundation of this research.

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CHAPTER 1: INTRODUCTION

Due to the critical role of animal testing in ensuring human health and safety, zebrafish have been increasingly utilized as experimental models, offering notable advantages such as genetic similarity to humans, optical transparency, rapid development, and the ability to monitor immune and toxicological responses in real time (Choi et al., 2021). Approximately (Orthologous total/ Coding-gene total) 71.4% of human protein-coding genes have at least one orthologue in zebrafish. This underscores the suitability of zebrafish as a highly effective model organism for human genetic research and highlights the necessity of specialized zebrafish analysis programs (Howe et al., 2013).

Table 1. A comparative analysis of human and zebrafish protein-coding genes and their orthologous relationships. (Source: Howe et al., 2013)

Relationship type	Human	Core Relationship	Zebrafish	Ratio
One to one	-	9,528	-	-
One to many	3,105	-	7,078	1:2.28
Many to one	1,247	-	489	2.55:1
Many to many	743	233	934	1:1.26
Orthologous total	14,623	13,355	18,029	1:1.28
Unique	5,856	-	8,177	-
Coding-gene total	20,479	-	26,206	-

1.1 Importance of Animal Testing Tools

Building these advantages on Zebrafish, recent advancements in high-throughput screening (HTS) technologies have underscored the significance of integrated imaging systems, automated analysis tools, and model-specific software platforms in animal testing. As conculuded, small animal models such as Caenorhabditis elegans, Drosophila melanogaster, and Danio rerio not only offer genetic and physiological relevance but also support scalable drug discovery pipelines through the use of automated devices like the COPAS Biosort and image-based phenotypic tracking systems. These tools allow for real-time, in vivo observation of disease models, improved throughput, and reproducibility, thereby enhancing the predictive value and efficiency of preclinical research (Giacomotto & Ségalat, 2010).

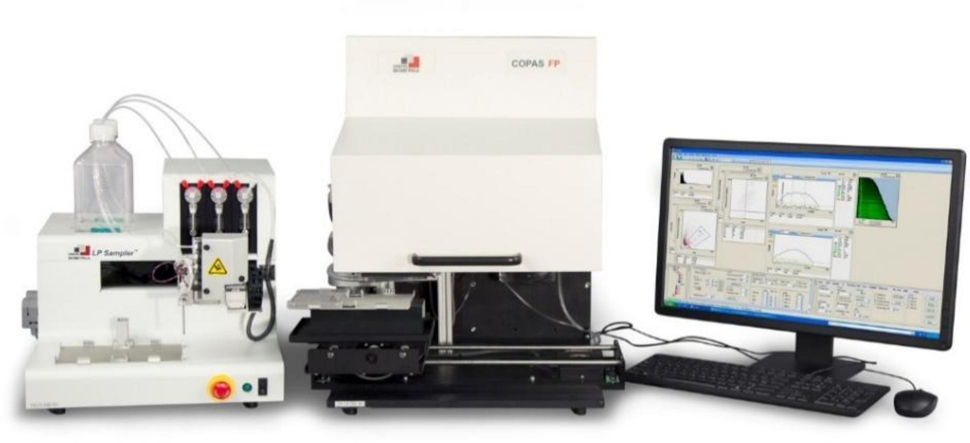


Figure 1: COPAS Biosort device (url1: https://www.unionbio.com/copas/software.aspx)

1.2 Brief History of Animal Testing

Human beings have always been in pursuit of a more comfortable and convenient life. This relentless quest for comfort has often driven them to minimize the difficulties and challenges they encounter. In the early stages of human civilization, efforts to overcome various diseases led to numerous discoveries, many of which involved seeking subjects for potentially dangerous experimental methods. These early experiments were primarily aimed at finding cures and treatments for illnesses that plagued human populations (Kinter et al., 2021). As time progressed, the focus of these experimental endeavors expanded. The quest for test subjects was no longer confined solely to the realm of disease treatment. Instead, it began to encompass aesthetic concerns as well. The desire to enhance physical appearance and achieve societal standards of beauty prompted further experimentation and innovation in medical science. This shift reflected a broader trend in human behavior: the pursuit of not only physical well-being but also aesthetic improvement (Sreedhar et al., 2020).

Throughout history, this dual pursuit of health and beauty has led to significant advancements in medical and cosmetic procedures. However, it has also raised ethical questions regarding the use of human subjects in experimental research. The balance between achieving scientific progress and ensuring the safety and dignity of individuals has been a topic of ongoing debate and regulation. This ongoing debate has also shifted the focus toward the ethical implications of animal use in scientific experiments, a practice with a long and complex history. Humans have observed animals for at least 17,000 years to gain critical knowledge for survival. The systematic use of animals as human surrogates in scientific research began in earnest in the late 19th century, driven by the need to characterize new compounds resulting from synthetic chemistry for their potential therapeutic utility and safety (Kinter et al., 2021).

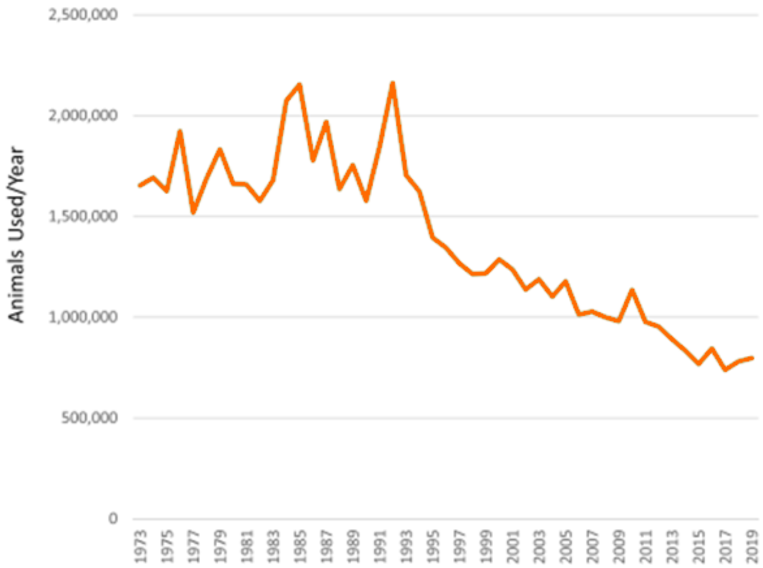


Figure 2: Annual data on animal testing reported by USDA/APHIS from 1973 to 2019 are presented. (Source : Kinter et al., 2021).

The use of animals in biomedical research and education reached its peak in the latter half of the 20th century but has since undergone significant changes, influenced by technological advancements and evolving scientific methodologies. Official data from the United States Department of Agriculture and the United Kingdom Home Office reveal a marked shift in research practices, with a decline in the use of larger, more publicly sensitive species in favor of smaller animals such as mice, rats, and fish. This shift reflects the complex impact of emerging technologies, which can lead to both reductions and increases in overall animal use, while redistributing the experimental burden across different species (Kinter et al., 2021).

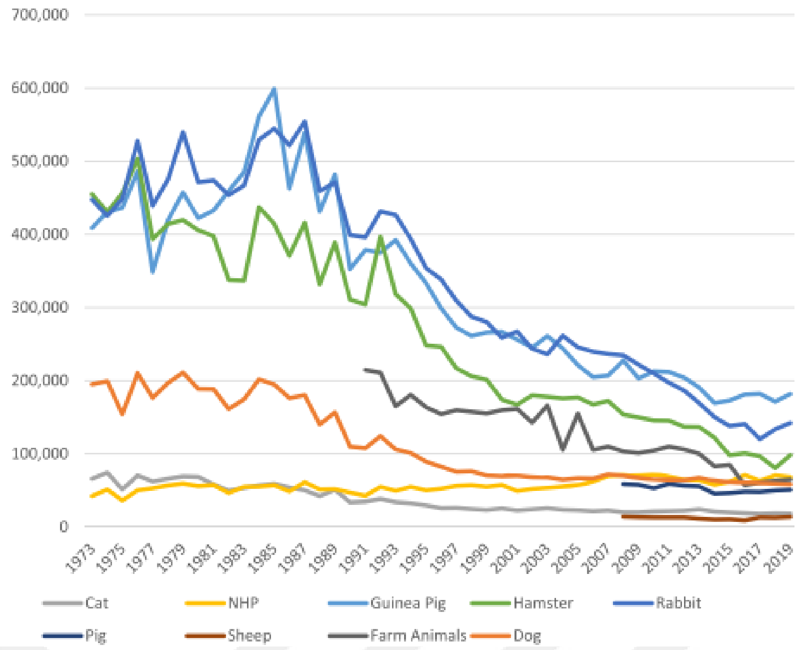
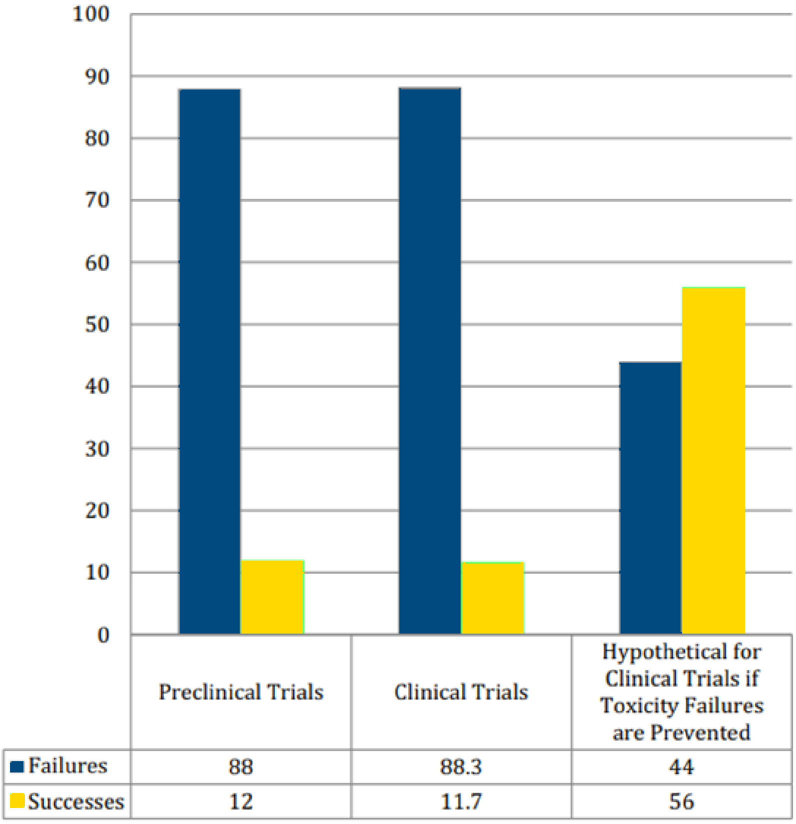


Figure 3: Annual data on animal testing reported by USDA/APHIS from 1973 to 2019 are presented. (Source : Kinter et al., 2021).

Furthermore, animal testing remains a widely employed method in pharmaceutical and industrial research for assessing potential human toxicity. Nonetheless, a growing body of evidence suggests that animal models frequently fall short in accurately forecasting drug safety in humans. The reliance on animal-based studies carries significant costs, not only in terms of financial expenditure and delayed regulatory approval but also through the possible rejection of therapeutically valuable compounds. Notably, there have been instances where drugs considered safe in animal trials have caused harm during human clinical testing. As a result of these concerns, there is growing scrutiny within the scientific community regarding the validity and translational relevance of animal-based research methodologies (Van Norman, 2019).



	Preclinical Trials	Clinical Trials	Hypothetical for Clinical Trials if Toxicity Failures are Prevented
Failures	88	88.3	44
Successes	12	11.7	56

Figure 4: The percentage of drug failures occurring after progression beyond preclinical and clinical trials (Source : Van Norman, 2019)

1.3 The Significance of Zebrafish Research

While traditional animal models remain fundamental to many aspects of biomedical research, the growing interest in more ethical and cost-effective alternatives has led to the increased use of zebrafish (Danio rerio) in recent years, particularly in vaccine development. Due to its genetic, anatomical, and physiological similarities with mammals, zebrafish offers significant advantages in both human and veterinary medicine. The zebrafish model allows for real-time observation of immune responses, embryonic development, and organ-specific effects of vaccines, while also adhering to the 3Rs principle, Replacement, Reduction, and Refinement (Bailone et al., 2020). Zebrafish possess both innate and adaptive immune systems, and their use in evaluating vaccine efficacy and toxicity has demonstrated results comparable to traditional mammalian models. Moreover, their optical transparency, rapid development, and the availability of transgenic lines enhance their utility in high-throughput screening and immune system visualization. Studies have also shown zebrafish to be effective in modeling numerous infectious diseases such as tuberculosis, salmonellosis, and shigellosis, allowing for preclinical testing of vaccine candidates. Thus, integrating zebrafish into research pipelines complements existing animal models and contributes to more humane and efficient scientific practices (Bailone et al., 2020).

In conclusion, the human drive for a more comfortable life has fueled centuries of medical and aesthetic innovation, shaping the trajectory of scientific advancement. From the earliest disease treatments to modern-day cosmetic procedures, this pursuit has necessitated experimentation, often involving animal models. While animal testing has contributed immensely to our understanding of biology and drug development, it has also sparked enduring ethical, scientific, and economic debates. In response, alternative approaches such as in vitro methods, computational models, and more recently, the zebrafish model have gained momentum, offering promising results in areas like vaccine development and toxicology. The zebrafish, in particular, presents a viable and humane complement to traditional animal models due to its genetic similarity to humans, rapid development, and suitability for high-throughput and visual analysis. As research increasingly embraces zebrafish for modeling human diseases and evaluating treatments, there is a growing need for accessible, efficient, and data-driven technological tools that can streamline experimentation and analysis processes (Bailone et al., 2020).

In light of this need, the development of a Python-based platform designed to observe and analyze zebrafish represents a significant step forward. Unlike conventional zebrafish research methods, this project aims to develop an integrated software platform that supports motion tracking, heart rate analysis, image enhancement, and panoramic stitching, all within a single, modular system. This approach is expected to significantly reduce manual labor and subjectivity in zebrafish analysis.

CHAPTER 2: LITERATURE REVIEW

While the introduction has provided the historical, ethical, and scientific context of animal experimentation, this chapter focuses on exploring the current state-of-the-art tools and research specifically related to zebrafish (Danio rerio) analysis. Zebrafish have gained increasing prominence in biomedical and toxicological studies due to their genetic similarity to humans, transparency during early development, and compatibility with high-throughput screening techniques (Bailone et al., 2020). In response to this growing interest, several software platforms have been developed to facilitate the visual analysis, behavioral tracking, and physiological monitoring of zebrafish. This chapter begins by reviewing prominent zebrafish analysis tools, including Athena, IN Carta® SINAP, ZebrafishMiner, Zebrabeat, BonZen and ZeBraInspector. It then critically examines the four main modules of the proposed platform, namely locomotion tracking, heart rate analysis, focus stacking, and image stitching, supported by relevant academic literature, emphasizing the need for an integrated, modular, and accessible solution in the field.

2.1 Comparative Overview of Zebrafish Analysis Platforms

- **ZebraBeat**, Zebrafish imaging software programs have become pivotal tools in biomedical research due to the zebrafish's transparency, rapid embryonic development, and genetic similarity to humans, making it an excellent model organism for cardiovascular studies. Among these advanced platforms is ZebraBeat, a specialized software designed to non-invasively quantify heart rate and cardiac function in zebrafish embryos. ZebraBeat processes high-resolution images obtained through confocal microscopy using two distinct analytical approaches: the Area Variation Mode, which tracks dynamic heart chamber movements through fluorescently labeled cardiac tissues, and the Blood Pool Variation Mode, which monitors circulating fluorescently labeled blood cells. This dual-mode functionality offers flexibility in experimental design, allowing the evaluation of heart rate variations caused by genetic modifications, pharmacological treatments, or environmental factors (De Luca et al., 2014). Furthermore, ZebraBeat's automated, user-friendly interface and high-throughput analysis capability ensure precise and reproducible measurements, supporting extensive applications ranging from genetic screenings to pharmacological studies. Ultimately, this powerful combination of advanced imaging techniques and robust computational analysis positions ZebraBeat as an indispensable tool for investigating cardiac physiology, pathology, and drug toxicity in developmental biology research (De Luca et al., 2014).

- **BonZeb**, Behavioral analysis software designed for zebrafish has significantly advanced biomedical research by enabling precise, automated tracking and quantification of zebrafish behavior in various experimental contexts. Among these innovative tools is BonZeb, an open-source, modular software platform specifically created for high-resolution tracking and analysis of larval zebrafish behaviors. It leverages Bonsai, a flexible, visual programming environment known for rapid data stream processing, allowing users to effortlessly perform high-speed, online and offline behavioral tracking (Guilbeault et al., 2021). This software uniquely supports dynamic visual feedback through closed-loop and open-loop stimulation paradigms, including predator avoidance, prey capture, and optomotor responses, as well as multi-animal tracking, optogenetic stimulation, and simultaneous calcium imaging. BonZeb's architecture is optimized for precise kinematic analysis, featuring specialized modules for background subtraction, centroid calculation, and detailed tail and eye tracking, making it highly versatile for complex experimental setups. The combination of ease-of-use, adaptability, and powerful computational capabilities provided by BonZeb significantly enhances the ability of researchers to investigate neural mechanisms underlying zebrafish behavior, thereby facilitating high-throughput behavioral and neuroscientific studies (De Luca et al., 2014).

- **ZeBraInspector (ZBI)** is a specialized platform designed for comprehensive three-dimensional imaging of fixed zebrafish larvae. By using confocal scanning and tissue clearing techniques, it captures detailed volumetric datasets that highlight anatomical structures, such as brain regions and overall morphology. Researchers can automatically segment these structures with high precision, enabling quantitative analyses of features like volume, shape, or fluorescent marker distribution. Built on a Python-based environment, the system offers both user-friendliness and considerable flexibility; however, processing large 3D image files necessitates a relatively powerful computing infrastructure. While ZBI excels at revealing subtle developmental changes in fixed samples, it remains less suited for studies that require real-time assessment of live fish behaviors (Lempereur et al., 2020).

- **ZebrafishMiner** is a MATLAB-based tool aimed at quantifying fluorescence in distinct anatomical domains of zebrafish embryos or larvae. It uses reference-based segmentation to map labeled tissues onto a standard template, ensuring reliable comparisons of gene expression or other fluorescent signals across multiple specimens. The software's graphical user interface leads investigators through a pipeline that includes image alignment, landmark-based warping, and automated intensity measurements. While ZebrafishMiner is particularly adept at analyzing reporter gene expression, it is not designed for broader morphological assessments or live imaging. Its strong emphasis on standardization and reproducibility, however, makes it highly valuable for labs focusing on genetic or toxicological screenings (Reischl et al., 2017).

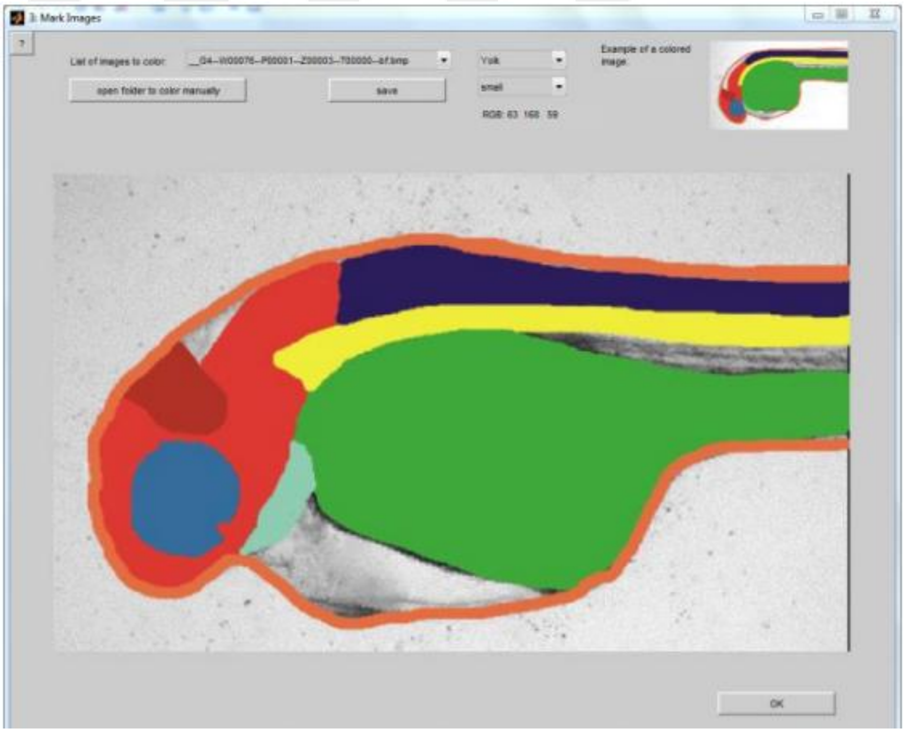
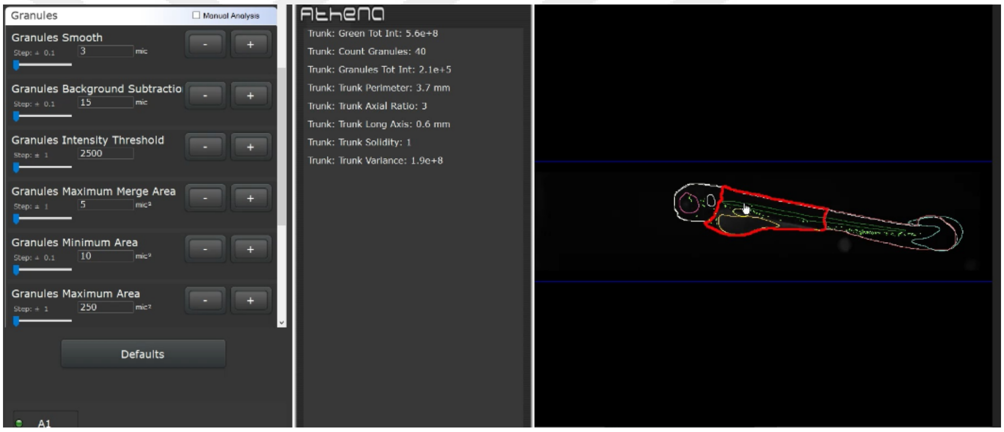


Figure 5: GUI of ZebrafishMiner Tissue manager (Source : Reischl et al., 2017)

- WiSoft Athena, used alongside the WiScan Hermes high-content imaging system, provides a streamlined approach to automated zebrafish analysis. The system utilizes artificial intelligence algorithms to detect larvae in brightfield images, identify essential anatomical structures, and quantify fluorescent signals with minimal user intervention. This integration of hardware and software allows for rapid throughput, making it appealing for large-scale screening where speed and consistency are top priorities. The platform can handle both brightfield and fluorescence images, generating data on body shape, organ size, and signal intensity. Although it offers an impressive range of automated features, its proprietary nature may restrict customization options, and it is optimized primarily for early larval stages.



Granules Smooth
Step: ± 0.1 3 mic

Granules Background Subtractio
Step: ± 0.1 15 mic

Granules Intensity Threshold
Step: ± 1 2500

Granules Maximum Merge Area
Step: ± 1 5 mic²

Granules Minimum Area
Step: ± 0.1 10 mic²

Granules Maximum Area
Step: ± 1 250 mic²

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Trunk: Green Tot Int: 5.6e+8
Trunk: Count Granules: 40
Trunk: Granules Tot Int: 2.1e+5
Trunk: Trunk Perimeter: 3.7 mm
Trunk: Trunk Axial Ratio: 3
Trunk: Trunk Long Axis: 0.6 mm
Trunk: Trunk Solidity: 1
Trunk: Trunk Variance: 1.9e+8
```

Figure 6: Athena Zebrafish Image Analysis Software (url2: https://idea-bio.com/products/zebrafish-image-analysis/)

Table 2. Comparative Analysis of Zebrafish Imaging and Analysis Platforms

Platform	**Key Features**	**Main Advantages**	**Main Disadvantages**
ZeBraInspector	- 3D imaging via confocal scanning and tissue clearing - Automated segmentation and volumetric analysis	- Excellent for detailed morphological studies - Python-based, can be extended or customized - Robust for quantifying subtle developmental changes	- Primarily for fixed, cleared samples - Large image files require strong computing resources - Not suited for real-time behavioral analysis
ZebrafishMiner	- Reference-based segmentation for fluorescence - MATLAB-based GUI pipeline - Focuses on domain-specific quantification	- Open-source and free to use - High reproducibility using standard reference models - Automated batch-processing of fluorescence data	- Requires MATLAB (licensing and installation) - Limited to analyzing gene expression/tissue-specific fluorescence - Not designed for live imaging or broad morphology
WiSoft Athena (WiScan Hermes)	- AI-driven fish and organ detection - Brightfield + fluorescence imaging - Automated morphological and fluorescence measurements	- Rapid, high-throughput processing - Minimal user input required - Integrates well with automated imaging systems	- Proprietary and potentially expensive - Optimized mainly for early-stage larvae - Less customizable for specialized experiments
Proposed Multi-Module Platform	- Movement tracking (free-swimming zebrafish) - Heart rate analysis (stationary fish) - Focus stacking and image stitching	- Covers both functional (behavior/heart rate) and structural (imaging) aspects - Potentially unifies multiple research needs in one toolkit - Flexible, modular design	- Complex development and validation needed - Potentially higher hardware and software demands - May require advanced user expertise to handle multiple modules

2.2 The Importance of Tracking Free-Moving Zebrafish

While existing zebrafish analysis platforms such as ZeBraInspector, Athena, and ZebrafishMiner offer powerful imaging and quantification tools, they primarily focus on fixed-position embryos or static fluorescence data. None of them incorporate real-time behavioral tracking capabilities essential for evaluating neuromuscular function and dynamic physiological responses. To address this gap, the proposed platform includes a dedicated module for tracking free-moving zebrafish in a container, enabling motion-based behavioral analysis. Recent studies have demonstrated the importance of capturing locomotor activity at various developmental stages to detect subtle neurofunctional abnormalities. For example, a fluidic platform that simulates natural aquatic flow using mechanical vibration, allowing researchers to measure motor capacity based on the zebrafish's ability to swim against induced currents. Wild-type zebrafish exhibited active resistance and maintained stability in high-flow zones, while those with lipin-1 gene deficiency or exposed to anesthetics were passively displaced, thereby offering a quantitative behavioral readout (Jia et al., 2023).

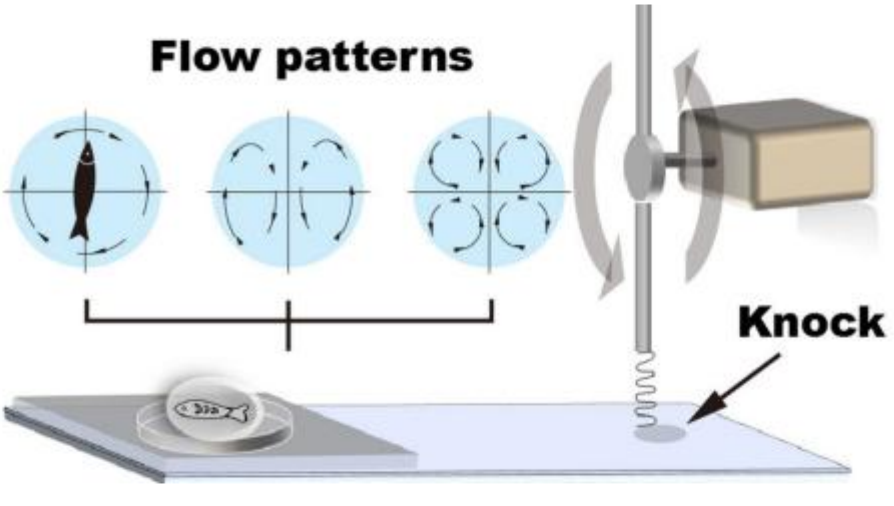

Figure 7: The schematic illustrates that the vibration frequency is controlled by the repeated tapping of the glass substrate (Source : Jia et al., 2023).

Similarly, developed a high-sensitivity behavioral assay that tracks spontaneous tail coilings in embryos and free-swimming patterns in larvae, identifying dose-dependent behavioral disruptions following chlorpyrifos exposure, even in the absence of morphological defects. Their use of distribution overlap (OA) as a statistical measure offered increased sensitivity compared to conventional t-tests (Selderslaghs et al., 2010). Together, these findings highlight the scientific and practical value of locomotor tracking as a non-invasive, early-stage biomarker for developmental neurotoxicity, thereby validating the need for this module within the proposed system.

2.3 Significance of Heart Rate Analysis in Stationary Zebrafish

In addition to behavioral analysis, physiological monitoring of internal organ function is essential for comprehensive zebrafish evaluation, particularly cardiac rhythm, which serves as a sensitive indicator of both developmental integrity and pharmacological effects (Romano et al., 2017). To complement the locomotor tracking module, the proposed platform integrates a second module focused on the non-invasive examination of heart rate and frequency in stationary zebrafish embryos and larvae. Several optical methods have been developed to detect cardiac rhythm without the need for surgical procedures. Among the most effective is video microscopy, which involves recording high-frame-rate brightfield or fluorescent footage of the heart and analyzing frame-to-frame pixel intensity fluctuations to identify systolic and diastolic contractions. This approach enables accurate measurement of heart rate, even at early developmental stages (e.g., 2–5 dpf), when the zebrafish remains semi-transparent and the heart is visible under basic microscopy setups. The use of automated software for heartbeat detection allows for quantitative assessment of cardiac arrhythmias, such as bradycardia or tachycardia, which may arise due to genetic mutations, toxic chemical exposure, or drug interactions (Santoso et al., 2020).

Furthermore, the study highlights the applicability of these techniques in high-throughput screening scenarios, where embryo arrays are immobilized in agarose and analyzed in parallel using multi-well imaging platforms. Such strategies reduce human error, increase reproducibility, and support dose-response assessments in pharmaceutical research. By embedding this functionality into a modular software environment, the proposed platform expands zebrafish analysis capabilities beyond behavior into core physiological domains, filling a major gap left by existing tools that often neglect real-time cardiac monitoring (Santoso et al., 2020).

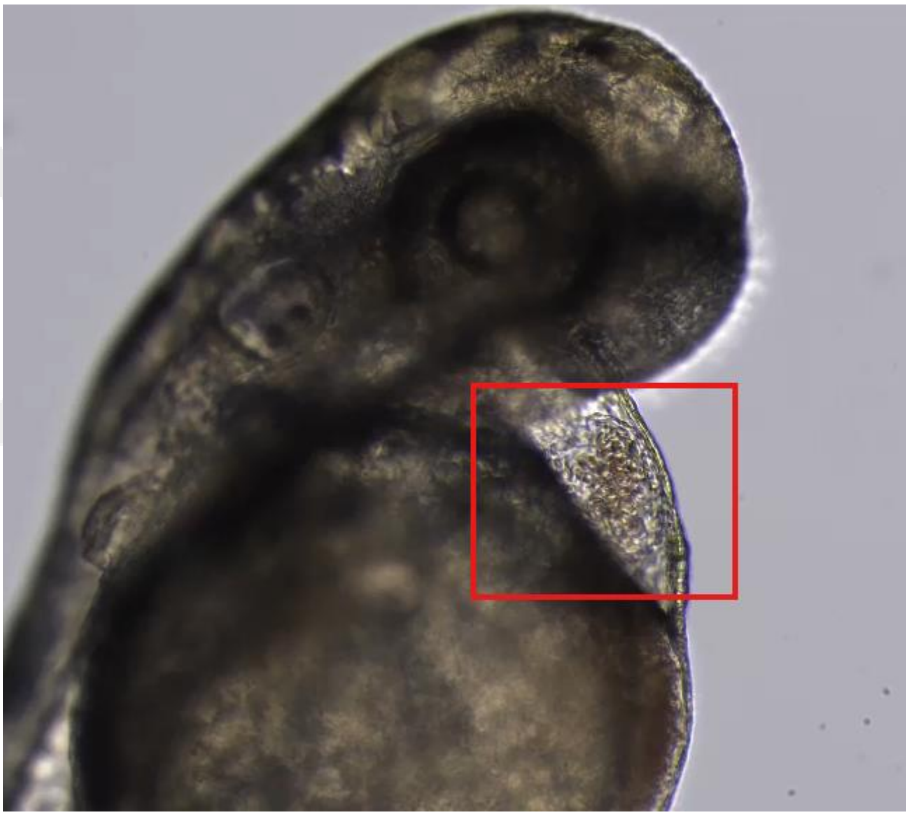

Figure 8: The image illustrates the anatomical position of the heart in a larval zebrafish.

Beyond general methods of cardiac rhythm detection, recent research has underscored the importance of hormonal signaling pathways in modulating heart function during zebrafish development, which investigated the role of the G protein-coupled estrogen receptor (GPER) in regulating embryonic heart rate. Through a combination of pharmacological activation, genetic overexpression, and gene knockout experiments, the authors demonstrated that GPER plays a pivotal role in controlling heart rate in zebrafish embryos. Activation of GPER using selective agonists resulted in a dose-dependent increase in heart rate, whereas GPER knockdown or treatment with antagonists led to bradycardia, indicating a direct and functional relationship between estrogen signaling and cardiac pacing mechanisms (Romano et al., 2017). These effects were independent of classical nuclear estrogen receptors, emphasizing the distinct and non-genomic role of membrane-bound GPER in cardiovascular physiology. The findings also suggest that environmental estrogens and endocrine-disrupting chemicals could potentially influence zebrafish cardiac function through this pathway, making heart rate a valuable and sensitive bio-indicator of both genetic and environmental modulation. Incorporating such hormonal regulation insights into heart rate monitoring modules enhances the diagnostic and screening capabilities of zebrafish-based assays. By integrating GPER-mediated signaling into analytical considerations, the proposed platform not only tracks cardiac rhythms but also supports deeper mechanistic exploration of endocrine and toxicological effects, contributing to a more holistic understanding of cardiovascular development and dysfunction in zebrafish (Romano et al., 2017).

2.4 Importance of Focus Stacking and Image Stitching in Zebrafish

As zebrafish models gain widespread acceptance in preclinical research, the demand for more sophisticated image acquisition and analysis techniques has grown correspondingly, particularly in studies focused on developmental biology, toxicology, and vaccine efficacy (Tavares & Lopes, 2013). The third and fourth modules proposed in this platform, namely the Focus Stacking Module and the Image Stitching Module, address fundamental imaging limitations encountered in traditional zebrafish microscopy. Due to the curved anatomy and three-dimensional structure of zebrafish larvae, standard two-dimensional microscopy often fails to maintain consistent focus across the depth of field, resulting in incomplete or blurred representations of key anatomical regions. The Focus Stacking Module addresses this challenge by acquiring multiple images along the z-axis and algorithmically combining them into a single, fully focused composite image, thereby enhancing the visualization of internal structures such as the brain, heart, and developing organs. This technique is especially valuable in the context of vaccine research, where tissue-level responses and cellular localization of immunological markers must be precisely analyzed (Bailone et al., 2020). Complementarily, the Image Stitching Module extends the field of view by digitally merging multiple adjacent image tiles, allowing for whole-organism imaging without the need for high-cost, large-field microscopes. This is particularly beneficial when screening multiple embryos in parallel, as it ensures consistent documentation of systemic effects, including inflammation, vascular responses, or developmental anomalies throughout the entire body axis.

The importance of these imaging emphasized that zebrafish offer a highly tractable model for vaccine evaluation due to their transparent embryos, genetic similarity to mammals, and well-characterized innate and adaptive immune systems (Bailone et al., 2020). In their review, they underscore that high-resolution imaging techniques greatly improve the detection of immunological effects, such as lymphocyte migration and antigen-specific tissue responses, especially when using transgenic lines with fluorescent reporters. Integrating focus stacking and stitching workflows not only elevates the quality of image-based endpoints but also facilitates high-throughput screening by automating complex visual analyses. Moreover, these imaging strategies support the 3Rs principle (Replacement, Reduction, and Refinement) by reducing the number of required experimental animals and enhancing the scientific validity of non-mammalian models. By embedding these modules within the proposed platform, researchers are empowered to conduct detailed, organism-level evaluations with minimal manual intervention, lower cost, and high reproducibility, thereby closing a critical gap in zebrafish image analysis for vaccine and drug development workflows (Bailone et al., 2020).

2.5 Advantages of Python Programming

Python has emerged as a highly effective programming language, particularly advantageous in biomedical research and graphical user interface (GUI) development due to its ease of use, flexibility, and extensive capabilities. Its readability and straightforward syntax reduce the learning curve, making it accessible to researchers who may not possess extensive programming expertise, significantly shortening the development time compared to more complex languages such as Java or C++ (Cutting & Stephen, 2021). Additionally, Python's dynamically typed nature facilitates automatic data type management, minimizing potential errors and further simplifying code maintenance (Cutting & Stephen, 2021). The language's suitability for biomedical applications is notably enhanced by a comprehensive suite of scientific libraries. Libraries such as NumPy and SciPy enable efficient numerical computations and statistical analyses, while TensorFlow facilitates the implementation of complex machine learning and artificial intelligence algorithms, crucial for handling biomedical datasets and predictive modeling. Moreover, Pandas simplifies data manipulation, making the preprocessing of large datasets more manageable (Saabith et al., 2021). Python also excels in GUI development, an essential feature in biomedical software, allowing researchers to interact intuitively with the software environment. Robust GUI frameworks including Tkinter, PyQt, and PySimpleGUI enable developers to craft responsive, user-friendly interfaces, significantly improving user engagement and data visualization capabilities within biomedical platforms (Cutting & Stephen, 2021). Moreover, Python supports interoperability with other programming languages like C, C++, and Java, offering biomedical researchers additional flexibility in optimizing performance-critical components through integration with existing specialized libraries. Additionally, Python’s Integrated Development Environments (IDEs), such as PyCharm, Spyder, and JupyterLab, provide comprehensive development support with advanced debugging, interactive testing, and integrated visualization tools that enhance productivity and code quality, essential for rigorous biomedical software development (Saabith et al., 2021).

Due to these strengths, Python has gained widespread acceptance in the biomedical community, establishing itself as a preferred solution for diverse scientific computing and GUI-intensive applications, thereby ensuring reliability, scalability, and efficiency in complex biomedical research scenarios

CHAPTER 3: METHODOLOGY

This chapter presents the methodological framework for the development and validation of the proposed zebrafish analysis platform. Section 3.1 addresses the free-swimming zebrafish tracking module, with particular attention to container setup and the computer vision algorithms employed for movement tracking. Section 3.2 focuses on the heart rate analysis module for stationary zebrafish, outlining optical detection methods, immobilization protocols, and automated measurement strategies. In Sections 3.3 and 3.4, the focus stacking and image stitching techniques are introduced, followed by an explanation of how these functionalities are integrated into a modular Python environment. By providing a step-by-step account of each module's design, this chapter demonstrates the platform's capacity to support both behavioral and physiological studies of zebrafish in a scalable, reproducible, and user-friendly manner.

3.1 Tracking Free-Moving Zebrafish in a Plate

Accurately tracking the movement of free-swimming zebrafish within a controlled environment is essential for evaluating their locomotor responses and physiological condition under dynamic stimuli. Recent advancements underscore the significance of assessing zebrafish mobility as a critical indicator of neurological and developmental health, particularly in response to genetic alterations or exposure to neurotoxic compounds. Changes in locomotor patterns, such as altered frequency and duration of spontaneous tail coiling in embryos and swimming behavior in larvae, provide sensitive measures of developmental neurotoxicity, highlighting the potential for early-stage locomotor activity to predict toxicological impacts (Selderslaghs et al., 2010). Furthermore, there are fluidic platforms, which simulate natural aquatic flow through controlled mechanical vibration, enable researchers to quantitatively distinguish zebrafish with compromised motor function from healthy controls based on their ability to actively resist and stabilize against induced currents (Jia et al., 2023). These methodologies allow researchers to perform precise behavioral analyses under near-natural flow conditions, facilitating a deeper understanding of neuromuscular function and environmental interactions. Thus, tracking free-moving zebrafish in a container provides valuable data critical for high-throughput screenings, elucidating subtle developmental anomalies, and assessing the physiological and behavioral implications of genetic or toxicological perturbations.

3.1.1. Zebrafish Tracking Algorithms

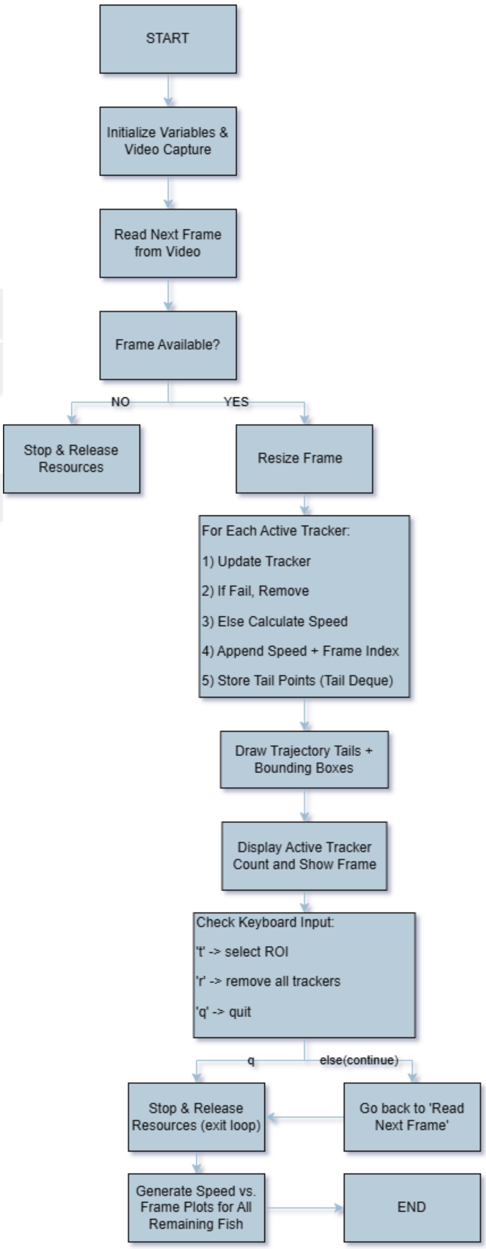

Figure 9: Zebrafish Tracking Algorithms Flowchart Scheme.

This Python script implements a multi-object tracking system specifically tailored for zebrafish analysis. At its core, the program uses MOSSE, OpenCV's legacy tracking algorithms to detect and continuously update the positions of multiple zebrafish in a video feed. Initially, users can select a new region of interest (ROI) by pressing t, which triggers OpenCV's selectROI method to define a bounding box around a single zebrafish. Each zebrafish tracker is stored in a dedicated dictionary that maintains the following information: the current tracker instance, the most recent centroid coordinates, speed values, a tail buffer (implemented via a deque) for trajectory visualization, and a unique color/label.

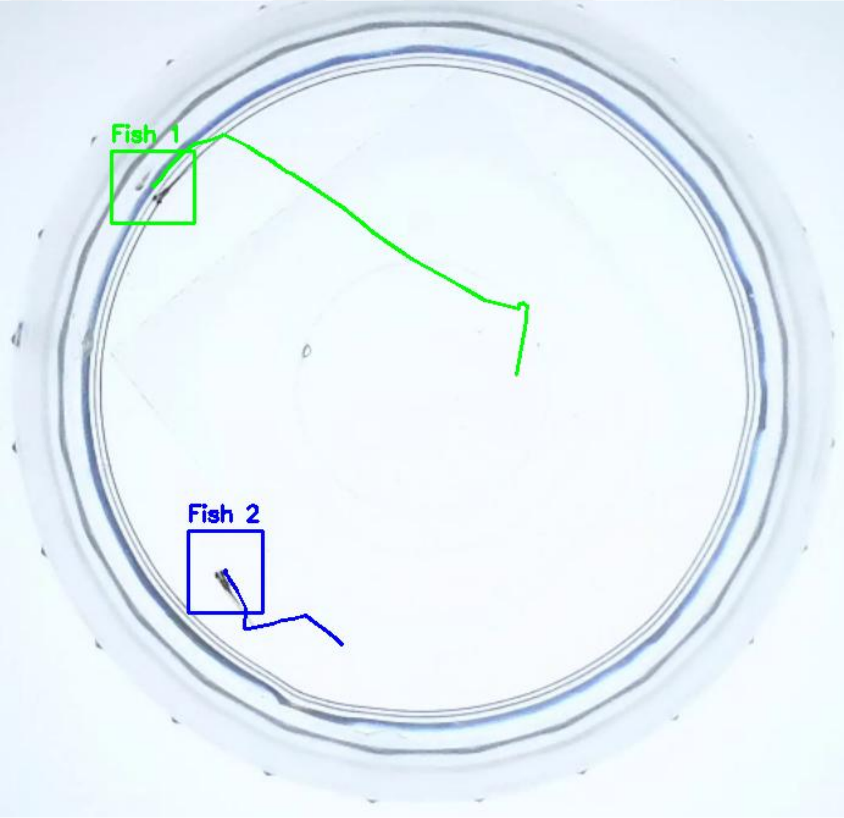

Figure 10: From Proposed Platform Tracking 2 Free-Moving Zebrafish in a Plate.

Within the main loop, frames are repeatedly captured from the input video, resized, and processed. Each tracker is then updated to extract the current bounding box of its assigned zebrafish. From there, the centroid is recalculated, and an instantaneous speed is computed as the Euclidean distance from the previous centroid to the new one. This speed, along with the current frame index, is stored for subsequent plotting. Meanwhile, each tracker's tail buffer stores a limited history of centroid positions, which is drawn frame by frame using simple line segments, thus revealing the zebrafish's trajectory in real time. If a tracker fails to update (e.g., the fish leaves the frame or the algorithm loses the object), it is removed from the active list.

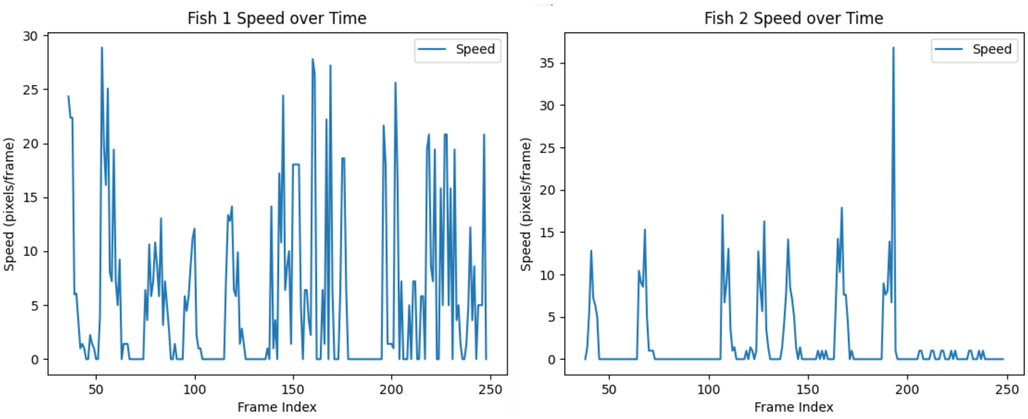

Figure 11: Graphs from proposed platform, Depicting Acceleration of Two Freely Moving Zebrafish Within a Container.

After all frames have been processed or the user presses q to quit, the script generates a speed-versus-frame plot for every tracked zebrafish. This final visualization allows researchers to assess variations in swimming velocity across the entire recording. By combining robust OpenCV trackers, real-time trajectory visualization, and post-processing speed analyses, this system provides an efficient tool for automated zebrafish locomotion studies.

3.2 Examination of the Heart Rate/Frequency of a Stationary Zebrafish

The examination of heart rate and frequency in stationary zebrafish represents a critical methodological approach for understanding cardiovascular physiology and toxicology. Zebrafish embryos, due to their optical transparency and genetic similarity to mammals, serve as effective models for observing real-time cardiac function without invasive procedures. the utility of zebrafish in cardiac rhythm detection methods, highlighting various optical techniques such as dynamic pixel change analysis, laser confocal microscopy, and artificial intelligence-based image processing (Santoso et al., 2020). These methods provide precise measurements of heart rate variations, facilitating high-throughput toxicological screenings and detailed physiological assessments. Furthermore, the pivotal role of the G protein-coupled estrogen receptor (GPER) in regulating heart rate during zebrafish embryogenesis. Their findings indicate that hormonal signaling pathways significantly influence cardiac rhythm, providing essential insights into the developmental impacts of environmental estrogens and endocrine-disrupting chemicals (Romano et al., 2017). Together, these studies underscore the importance of robust and non-invasive heart rate analysis in zebrafish models to elucidate cardiovascular function and to screen the cardiac effects of genetic, pharmacological, or environmental perturbations.

3.2.1 Zebrafish Cardiac Rate Analysis Algorithm

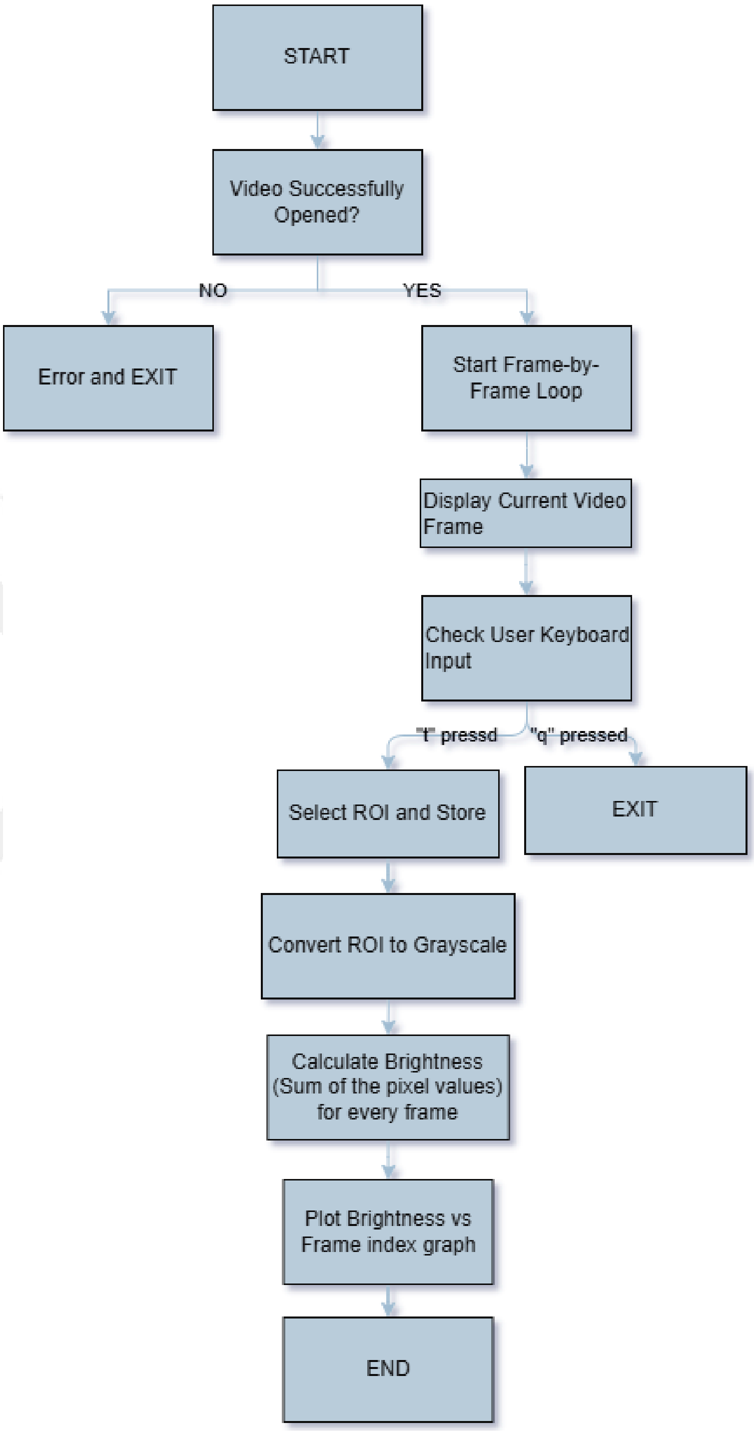

Figure 12: Flowchart of Algorithms for Zebrafish Heart Rate Analysis.

The provided Python code implements a non-invasive optical method for analyzing the heart rate of stationary zebrafish. The approach relies on fluctuations in pixel intensity within a region of interest (ROI) around the heart area, captured from video footage.Initially, the script imports essential Python libraries: OpenCV (cv2) for video processing and ROI selection, NumPy (numpy) for numerical computations, and Matplotlib (matplotlib.pyplot) for visualizing results. The program is encapsulated in the main() function, which takes a video file as input. Within the main processing loop, the video frames are displayed continuously, allowing interactive selection of an ROI. Pressing the key 't' activates OpenCV's built-in selectROI() function, enabling the user to manually specify a bounding box precisely around the zebrafish's heart area on the current video frame. Once selected, the ROI coordinates (x, y, width, height) are stored and used in subsequent computations. The defined ROI is visualized on-screen as a green rectangle to assist in verifying proper placement throughout the video playback. For each frame with a defined ROI, the algorithm extracts that specific region from the frame, converts it into a grayscale image, and computes the total brightness (pixel intensity sum). Specifically, the pixel intensities within this grayscale ROI (gray) are summed using NumPy's np.sum() function, resulting in a numerical value (bval) that correlates directly to heart contractions. These brightness values fluctuate systematically due to the rhythmic motion of the heart tissue, enabling the estimation of heart rate (Chilvers & O'Callaghan, 2000), (Santoso et al., 2020). All calculated brightness values are stored sequentially in a list as the frames are processed, effectively generating a time-series dataset that reflects cardiac rhythmicity. The main loop continues until the end of the video or until the user presses 'q'. After termination, the script closes all OpenCV video windows and releases the video capture resource.

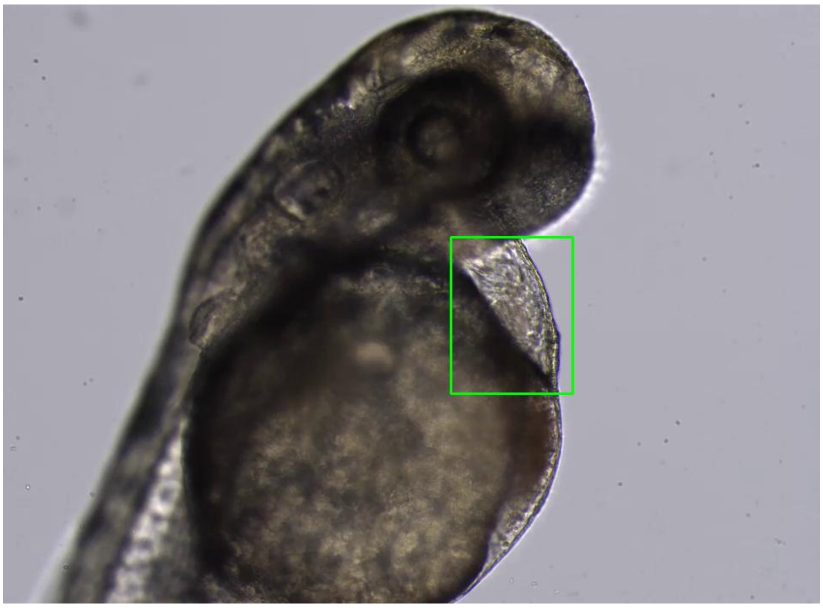

Figure 13: User Selection of Zebrafish Heart Region From Proposed Platform Indicated by Green Rectangle

In the final step, if brightness data has been successfully collected, a plot is generated displaying brightness values (bvalues) against the corresponding frame indices. This graph, produced via Matplotlib, visually represents the zebrafish's heartbeat pattern over the captured video duration, facilitating the analysis of heart rate, rhythm regularity, and potential cardiac anomalies or reactions to experimental treatments.

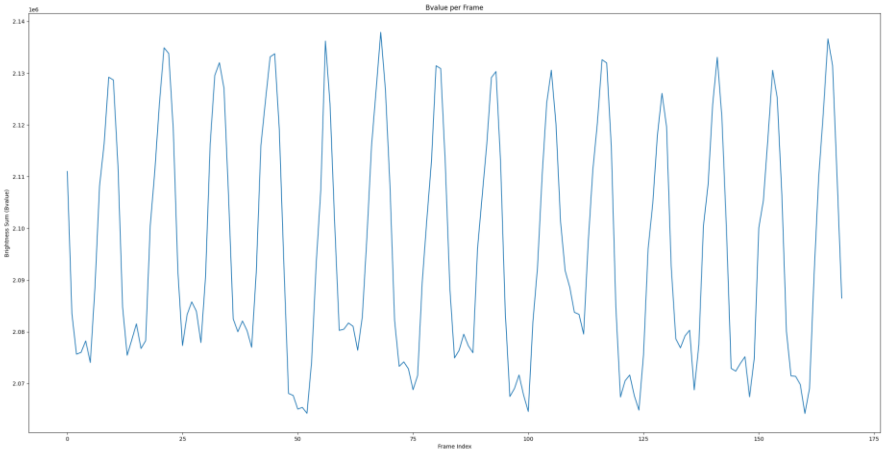

Figure 14: Plot of Analyzed Zebrafish Heart Rate Data.

3.3 Zebrafish Focus Stacking and Image Stitching Module

Zebrafish, a prominent model organism in biomedical research, possesses complex anatomical structures that require advanced imaging methods for thorough examination. Due to their three-dimensional tissue organization, capturing detailed and clear microscopic images can be particularly challenging (Choi et al., 2021). Focus stacking plays a crucial role in overcoming these limitations by amalgamating multiple images captured at varying focal depths to generate a fully focused composite image. This method is essential for visualizing intricate 3D anatomical structures such as the brain, heart, and developing organs, which otherwise appear blurred in conventional single-depth microscopic images (Sigdel et al., 2016). Concurrently, image stitching addresses the limitations posed by high-resolution microscopy, which typically has a restricted field of view. Stitching seamlessly combines multiple overlapping images, significantly expanding the field of view while preserving resolution, thus facilitating the visualization of larger tissues like the developing musculature or extensive vascular networks(Mohammadi et al., 2024). Selecting appropriate pairwise registration methods such as SURF has been shown to optimize accuracy and efficiency in image stitching, particularly benefiting high-resolution bright-field and fluorescence microscopy modalities (Mohammadi et al., 2024). Collectively, the integration of focus stacking and image stitching is indispensable for accurately analyzing zebrafish anatomical and developmental dynamics at high resolution and large scales.

3.3.1 Overview of Focus Stacking Technique

The implemented focus stacking technique addresses the inherent limitations of depth-of-field in zebrafish microscopic imaging by combining multiple images captured at varying focal planes into a single, sharply-focused composite. Initially, the program loads and sorts a set of input images from a designated directory, verifying image integrity. Subsequently, it applies an ORB (Oriented FAST and Rotated BRIEF) feature detector to filter out insufficiently sharp images based on the quantity of keypoints detected, thus ensuring image quality for the stacking process. Each retained image undergoes alignment relative to a chosen reference image using phase cross-correlation to correct spatial misalignments across individual color channels. To further enhance consistency, brightness normalization matches the average pixel intensity across images to that of the reference image. The core of the focus stacking operation involves calculating Laplacian responses for each image, which serve as indicators of local sharpness. The algorithm then constructs the final composite image by selecting pixels from individual images where the Laplacian response—indicating the degree of focus is maximal. This process culminates in an optimized, fully-focused image suitable for detailed anatomical and physiological analyses.

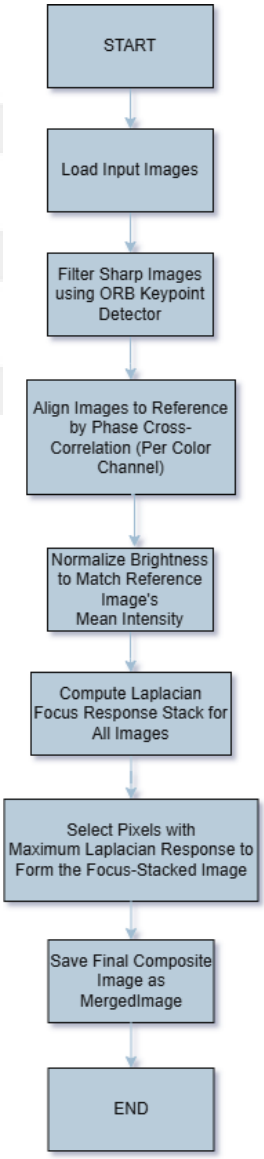

Figure 15: Flowchart of Algorithms for Zebrafish focus stacking module.

3.3.2 Technical Explanation of the Image Stitching Module

The implemented image stitching module effectively combines multiple overlapping images into a cohesive panoramic image by systematically performing several key steps (Preibisch et al., n.d.). Initially, it employs the ORB (Oriented FAST and Rotated BRIEF) algorithm to detect distinctive keypoints within each pair of consecutive images and computes their associated descriptors. These descriptors facilitate matching corresponding features between image pairs through a Brute-Force matcher using the Hamming distance metric. To enhance match reliability, the code applies Lowe's ratio test, retaining only high-quality matches that surpass a defined threshold. Subsequently, matched feature coordinates are utilized to estimate a homography matrix via the RANSAC algorithm, robustly accounting for potential outliers (Derpanis, 2010). This homography delineates the transformation required to align the new image accurately onto the existing panorama. After calculating the appropriate canvas size to accommodate the transformed images, the code applies a perspective warp and strategically translates both images into a unified coordinate space. The final composite is produced through a straightforward overlay blending, wherein non-zero pixel regions of the newly warped image supersede corresponding regions in the existing panorama. Ultimately, the resultant panoramic image is saved for further analysis or visualization, thus providing a precise and seamless integration of individual image segments.

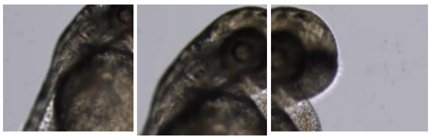

Figure 16: Part of Zebrafish images before Stitching module.

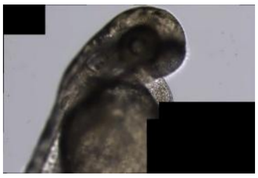

Figure 17: Zebrafish image after Stitching module.

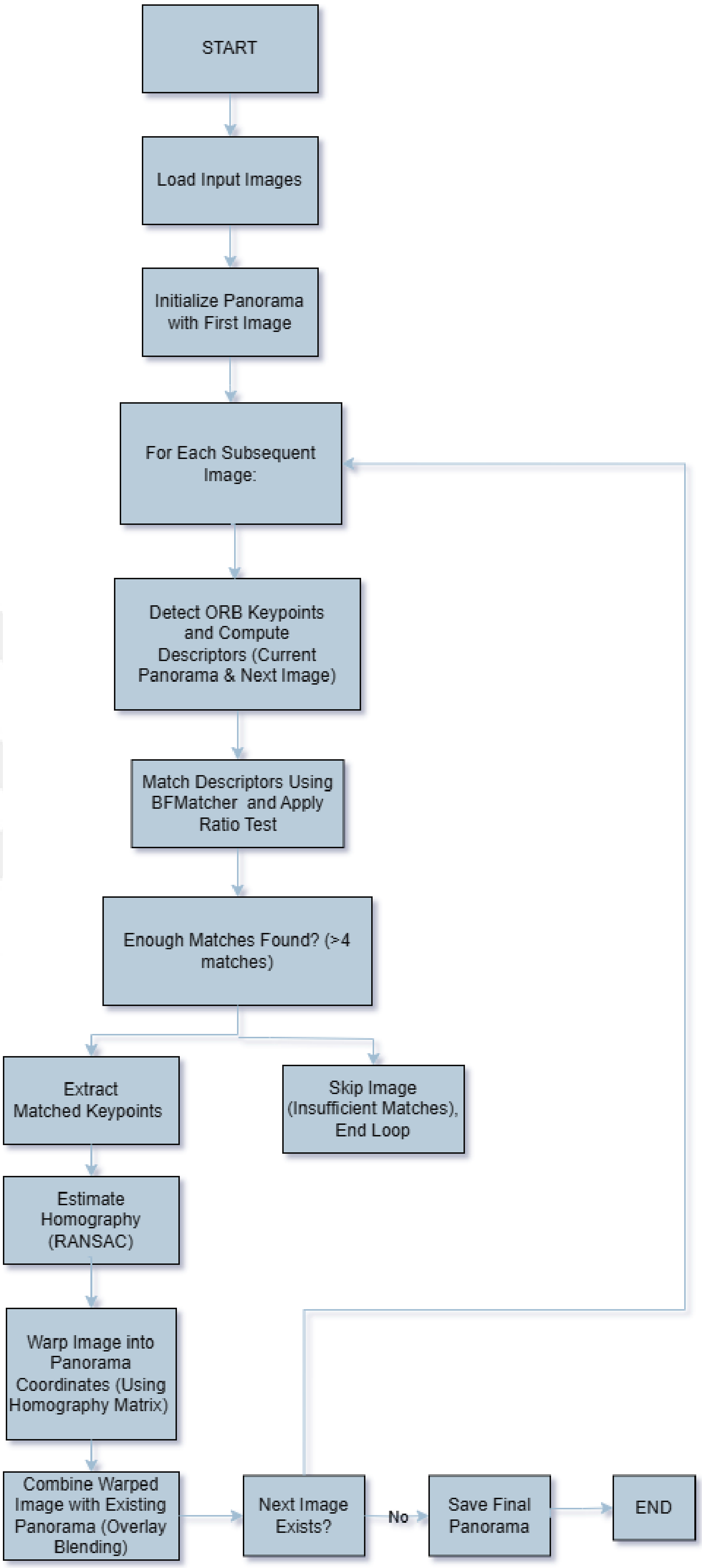

Figure 18: Flowchart of Algorithms for Zebrafish image stitching module.

CHAPTER 4: RESULT AND DISCUSSION

In this study, the primary aim was to design and rigorously test a versatile Python-based analytical tool tailored specifically to zebrafish research. This platform integrates four critical modules: tracking the movement of freely swimming zebrafish, assessing the heart rate of stationary zebrafish, enhancing image clarity via focus stacking, and producing comprehensive visuals through image stitching. This chapter outlines the

outcomes of each of these modules, discussing their practical performance and reliability in detail. Additionally, the results are contextualized within existing biological research methodologies, highlighting the benefits and limitations experienced during practical application. Through examining the strengths and potential areas of improvement within these computational methods, the discussion provides clear insights into their overall impact on facilitating zebrafish studies and opens avenues for future enhancements.

4.1 Comparative Analysis of Tracking Algorithms for Free-Moving Zebrafish

Algorithms such as MOSSE, KCF, and CSRT have been extensively employed in tracking free-moving zebrafish, with each method exhibiting distinct strengths and inherent limitations.

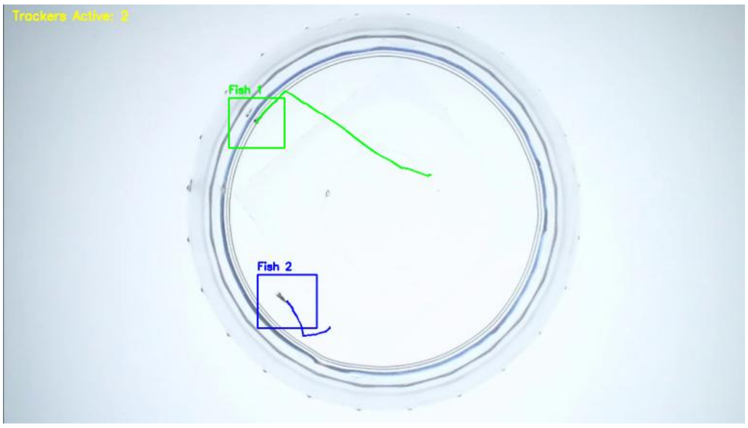

Figure 19: Experiment-1 of Free-Moving Zebrafishes

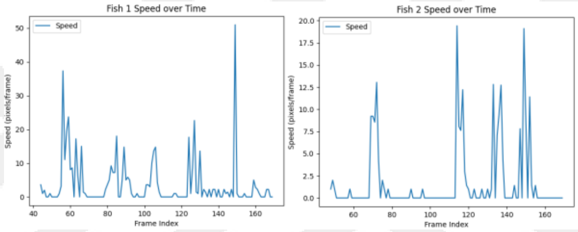

Figure 20: Result of MOSSE Tracking Algorithm for Experiment-1

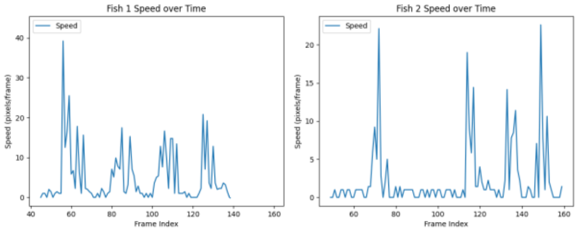

Figure 21: Result of KCF Tracking Algorithm for Experiment-1

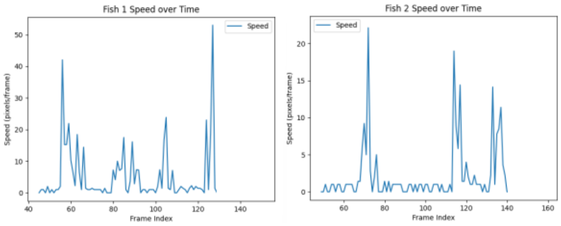

Figure 22: Result of CSRT Tracking Algorithm for Experiment-1

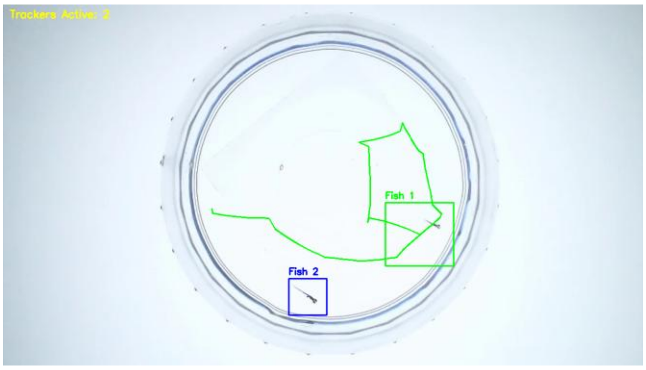

Figure 23: Experiment-2 of Free-Moving Zebrafishes

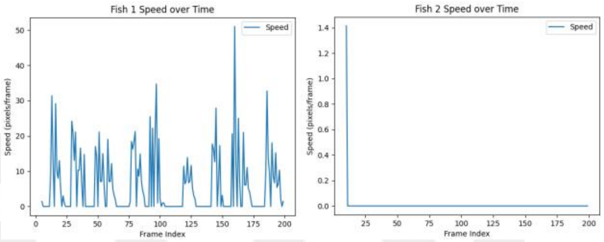

Figure 24: Result of MOSSE Tracking Algorithm for Experiment-2

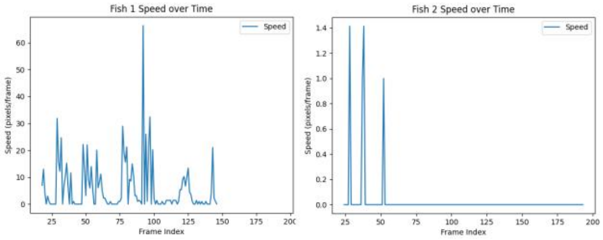

Figure 25: Result of KCF Tracking Algorithm for Experiment-2

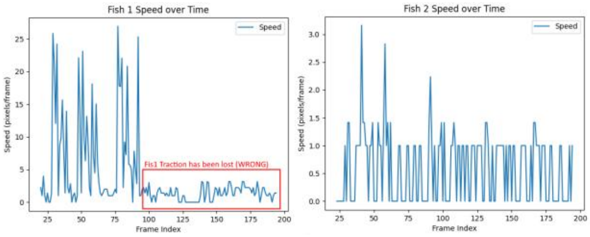

Figure 26: Result of CSRT Tracking Algorithm for Experiment-2

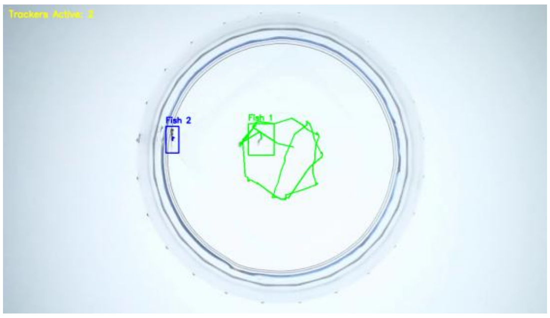

Figure 27: Experiment-3 of Free-Moving Zebrafishes

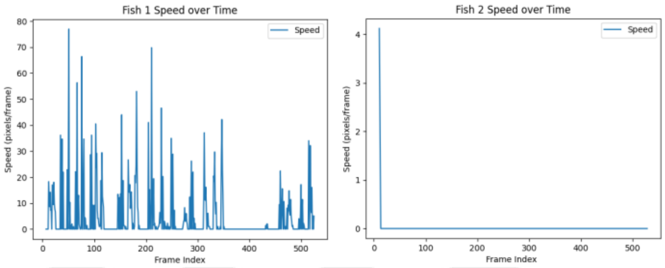

Figure 28: Result of MOSSE Tracking Algorithm for Experiment-3

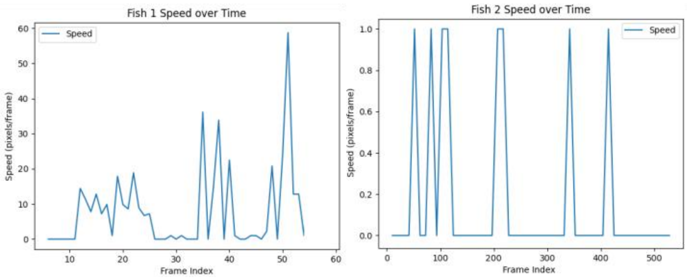

Figure 29: Result of KCF Tracking Algorithm for Experiment-3

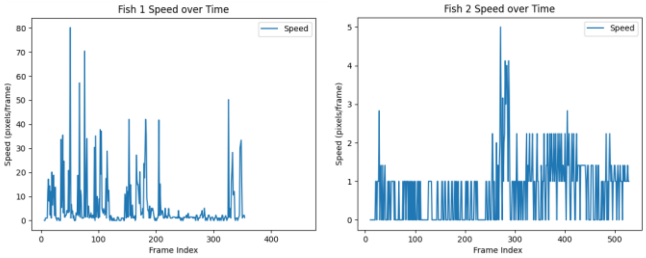

Figure 30: Result of CSRT Tracking Algorithm for Experiment-3

MOSSE (Minimum Output Sum of Squared Error), introduced by Bolme et al., is characterized by its exceptional computational efficiency and high frame rate capability, enabling robust performance even at lower computational resources. This algorithm quickly adapts to changes in lighting, scale, and pose, making it highly effective for rapid movements, such as those observed in zebrafish swimming behavior (Sidhu et al., 2016). However, MOSSE's robustness significantly depends on consistent tracking conditions, requiring careful mask filtering and region-specific preprocessing to mitigate tracking losses under challenging scenarios, including abrupt appearance changes and occlusions (Sidhu et al., 2016). Kernelized Correlation Filter (KCF) significantly builds upon the MOSSE approach by incorporating kernel methods that enhance discriminative ability without heavily compromising computational speed. Specifically, KCF leverages circulant matrices in Fourier space to expedite feature matching, substantially improving robustness against slight appearance changes and minor occlusions (Tang et al., 2018). Despite these enhancements, KCF faces limitations when dealing with large positional shifts or significant visual disturbances, as it inherently assumes moderate object displacement between frames. Such limitations become pronounced when tracking highly dynamic subjects like fast-moving zebrafish, where frequent and rapid changes occur between frames (Tang et al., 2018).The CSRT (Channel and Spatial Reliability Tracking) algorithm further improves upon earlier correlation-filter methods by introducing a spatially regularized component and reliability measures across different channels. This design choice makes CSRT particularly effective at maintaining robust tracking even in challenging environments, providing superior accuracy and stability when objects experience rapid movements, scale changes, or partial occlusions. However, these enhancements come at the expense of processing speed, which makes CSRT less suitable for scenarios where maintaining a high frame rate is critical, such as real-time zebrafish tracking at 30 frames per second or above (Farkhodov et al., 2020).

Considering the specific context of tracking zebrafish in the proposed platform, MOSSE emerges as a particularly suitable choice. Its remarkable computational speed aligns well with the rapid movements typical of zebrafish, provided careful preprocessing measures, such as masking and filtering, are employed to maintain tracking stability. While KCF and CSRT offer increased robustness under more general conditions, their relatively higher computational demands may hinder real-time tracking applications. Thus, given the balance of speed, accuracy, and real-time applicability, MOSSE remains optimal for efficiently tracking fast-moving zebrafish in the proposed platform.

4.2 How the Selection of the ROI Influences Heartbeat Signal Quality

The selection of the Region of Interest (ROI) significantly impacts the quality and clarity of heartbeat signals acquired from zebrafish imaging. When the ROI size is expanded beyond the immediate vicinity of the heart, it inevitably incorporates additional anatomical structures such as adjacent organs, tissues, and muscular movements. These non-cardiac regions often introduce lower-frequency noise into the recorded signal, masking or diluting the higher-frequency cardiac activity essential for precise heart rate analysis. For instance, subtle movements originating from respiratory or muscular contractions in the zebrafish's body can generate substantial signal artifacts that degrade the reliability of the heartbeat measurement. Consequently, optimal ROI selection should narrowly encompass the heart region, effectively minimizing extraneous noise from surrounding tissues and enhancing the overall signal-to-noise ratio, thus ensuring more accurate and dependable heart rate assessments.

Figure 21: 500px ² ROI of Heartbeat Detection module.

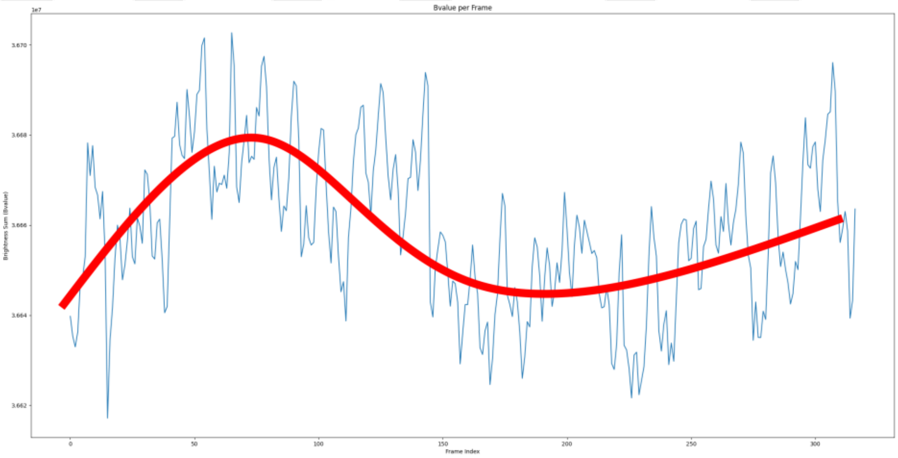

Figure 32: Result of 500px ² ROI of Heartbeat Detection module.

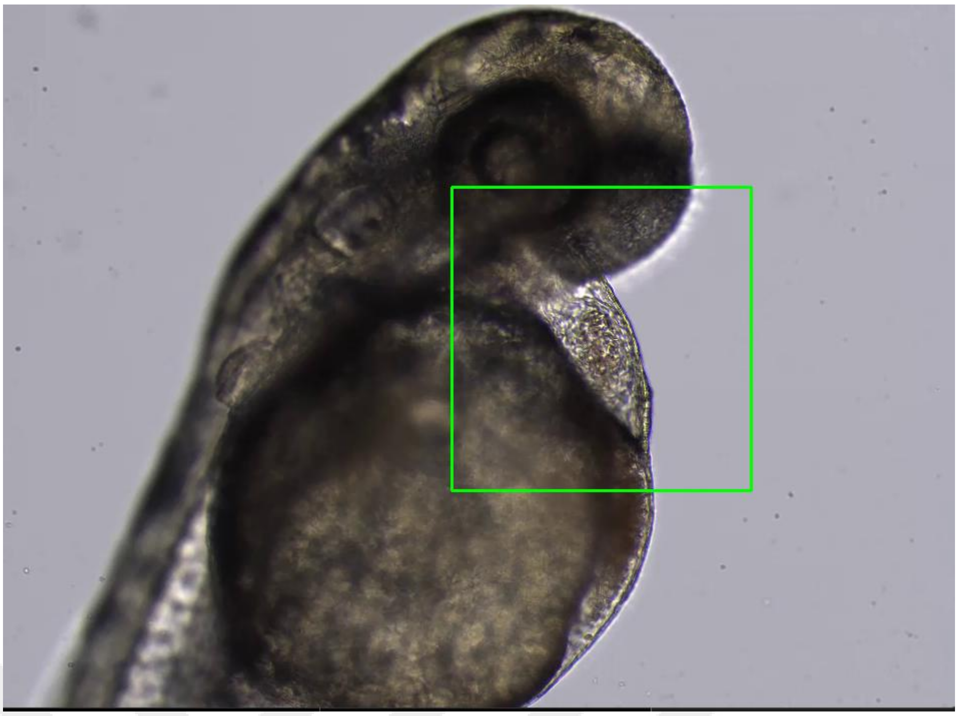

Figure 33: 280px ² ROI for Heartbeat Detection

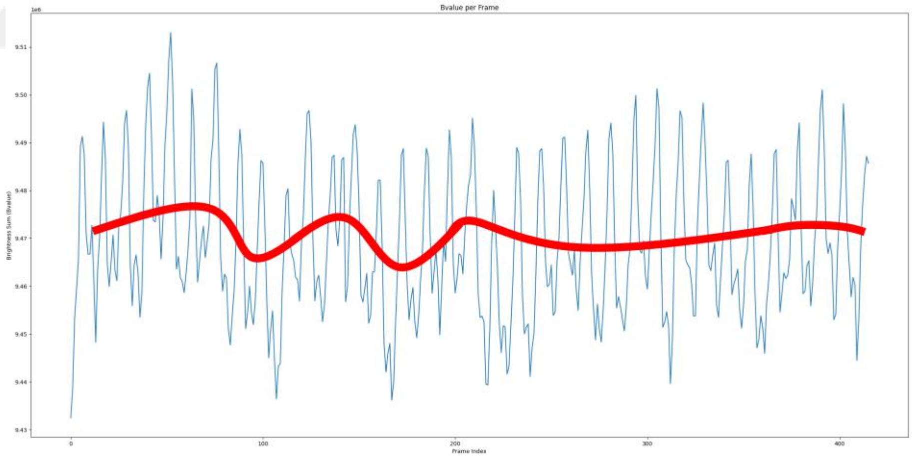

Figure 34: Result of 280px ² ROI of Heartbeat Detection

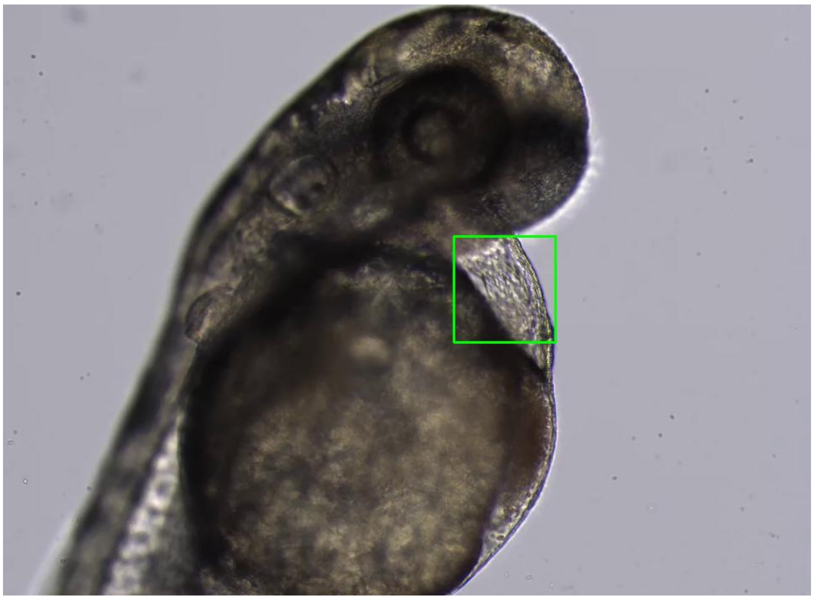

Figure 35: 90px ^{2} ROI of Heartbeat Detection

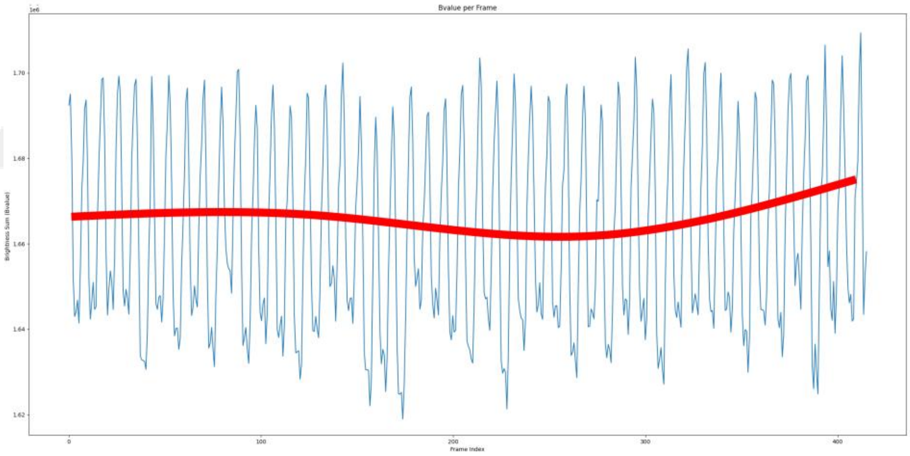

Figure 36: Result of 90px ^{2} ROI of Heartbeat Detection

Figure 37: 18px ^{2} ROI of Heartbeat Detection

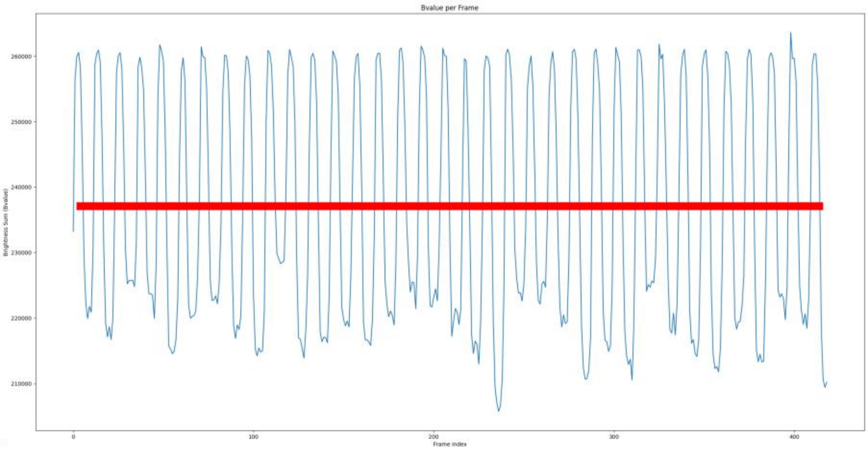

Figure 38: Result of 18px ^{2} ROI of Heartbeat Detection

4.3 Image Stitching Techniques vs. Wide-Field Objective Lenses

Wide-field microscope objective lenses have been traditionally used to acquire large-scale biological images efficiently. Such objectives are specifically designed to provide extensive fields of view coupled with high spatial resolutions and long working distances, facilitating the imaging of sizeable samples such as whole mouse brains without the need for additional processing. However, the design and fabrication of these wide-field lenses present considerable challenges, including significant costs, complex optical designs, and sophisticated correction methods for optical aberrations and chromatic shifts (Peng et al., 2022). In contrast, image stitching algorithms offer an attractive alternative by digitally combining multiple high-resolution, overlapping image segments captured through conventional microscope objectives into a unified, comprehensive view. This computational approach reduces dependency on expensive, specialized optics and significantly lowers overall costs. Particularly in zebrafish imaging, image stitching holds distinct advantages by enabling researchers to achieve a wide viewing area without compromising resolution or detail. By using standard microscope lenses combined with advanced software algorithms, stitching effectively mitigates optical distortions and aberrations that might otherwise appear prominently in wide-field lenses, providing seamless, artifact-free imaging suitable for precise anatomical and developmental analyses of zebrafish. Consequently, image stitching emerges as a cost-effective and flexible method, especially valuable for laboratories conducting high-resolution zebrafish imaging with limited optical resources (Peng et al., 2022).

4.4 Effect of Types of Focus Stacking Algorithms

Focus stacking is a pivotal technique in biomedical imaging, particularly when working with highly detailed organisms such as zebrafish (Danio rerio). Among the various approaches, pixel-based algorithms (often using local measures of sharpness or contrast) excel in balancing computational efficiency and image quality. While frequency-domain (Fourier/wavelet) and multi-scale (pyramid-based) methods can yield smoother transitions between in-focus planes, they often demand higher computational overhead and intricate parameter tuning, which may be excessive for many routine zebrafish imaging tasks. In contrast, pixel-based methods directly select the sharpest pixel across each image in the stack, effectively capturing the fine morphological details characteristic of zebrafish tissues without introducing significant blending artifacts (Kornilova et al., 2021). This streamlined approach is particularly advantageous given the complex interplay of reflective and translucent structures found in zebrafish, where maintaining clear delineation of rapidly changing features at different depths is crucial. Furthermore, pixel-based stacking is relatively straightforward to implement and adapt for high-throughput or automated pipelines commonly used in modern zebrafish research. Consequently, for researchers seeking an efficient and robust workflow that preserves the anatomical integrity of small model organisms, pixel-based focus stacking algorithms stand out as the most pragmatic solution.

CHAPTER 5: CONCLUSION

This study presents a comprehensive Python-based platform specifically designed to enhance zebrafish research through advanced computational tools. The developed platform effectively integrates several analytical modules, including real-time tracking of free-swimming zebrafish, precise cardiac rate monitoring in stationary specimens, enhanced three-dimensional visualization via focus stacking, and extended field-of-view imaging through image stitching. Through rigorous evaluation, the MOSSE algorithm emerged as the most effective tracking solution, providing a balanced combination of speed and accuracy, ideal for accurately capturing rapid zebrafish movements at high frame rates. Furthermore, the study emphasized the crucial impact of region-of-interest selection on heartbeat signal quality, underscoring the importance of precise anatomical targeting to minimize interference from surrounding tissues. Comparative analyses further highlighted the advantages of image stitching algorithms over expensive wide-field objective lenses, establishing a cost-effective yet high-quality imaging alternative suitable for detailed zebrafish anatomical assessments.

Collectively, these results validate the robustness, practicality, and versatility of the proposed analytical platform, significantly enhancing the automation, accuracy, and reproducibility of zebrafish studies within biomedical and toxicological research domains. Future investigations could further refine these computational modules by incorporating advanced machine learning methodologies, thereby broadening their applicability across diverse biological and pharmacological research contexts.

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