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Developing workflow tools for quantifying kidney regeneration post laser injury

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Abstract

High content screening microscopy enables the automated imaging of various biological events in cellular and whole-organism model systems**. Zebrafish embryos** and larvae are an ideal model system for screening microscopy allowing large scale phenotypic scoring within the complexity of a live vertebrate. While several protocols for automated zebrafish screening experiments have been established, the development of an **automated image analysis workflows** remains a major challenge, especially when dynamic phenotypes are evaluated.

This study introduces an automated analysis workflow to **quantify kidney regeneration over time** in the developing zebrafish upon **automated laser-induced injury** on a screening microscope equipped with a photomanipulation module. The screening dataset consists of multiple time-lapse images of injured and fluorescently labelled distal pronephric tubules of the transgenic Tg(*cdh17:egfp*) zebrafish line.

To overcome the lack of image analysis tools for whole-organism screening data and to automate the quantification of kidney regeneration post photodamage, we developed a workflow combining image-processing and data-analysis using the **open-source software tools FIJI, Ilastik and KNIME**. To restrict the analysis to the wounding site, image data was automatically cropped after stack alignment and registration. Fluorescently labelled tissue was segmented, and the regenerating tubule tip was tracked over time. The established workflow provides a **semi-automated solution for the tracking, quantification and intuitive graphical visualization of the regeneration process.** Hence, the workflow enables the analysis of regeneration dynamics and facilitates the identification of specimen with impaired or enhanced regeneration.

Ultimately, we expect that workflow components can be directly applied for the analysis of other complex timelapse datasets, and can be adapted for the quantification of growth or migration of other fluorescently labelled tissues.

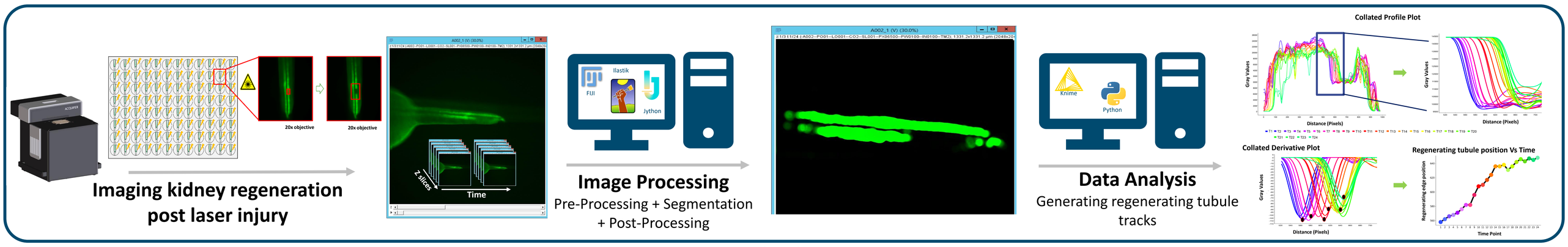
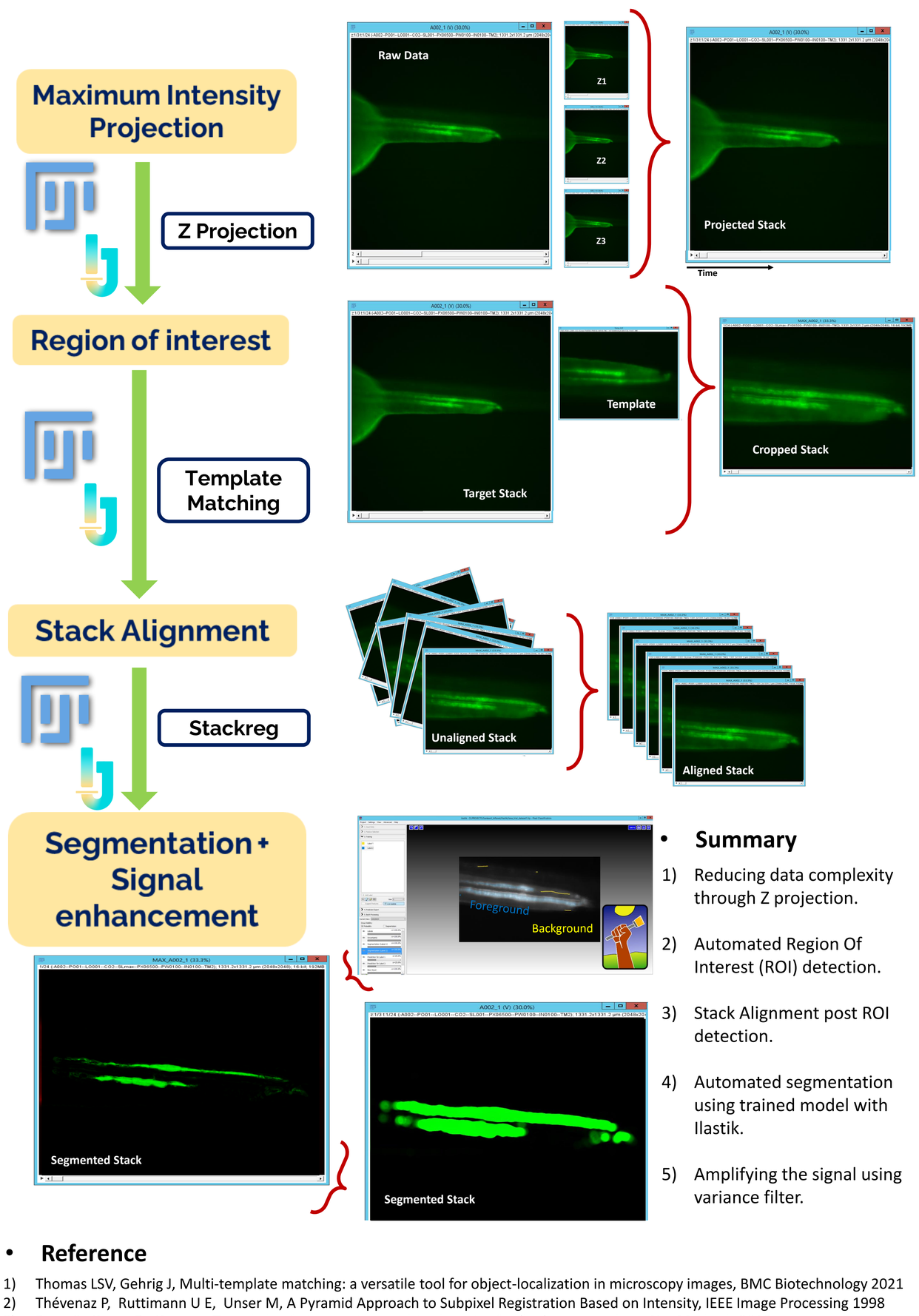


Image Processing

Automated Detection and Segmentation of Region Of Interest (ROI)



- **Summary**

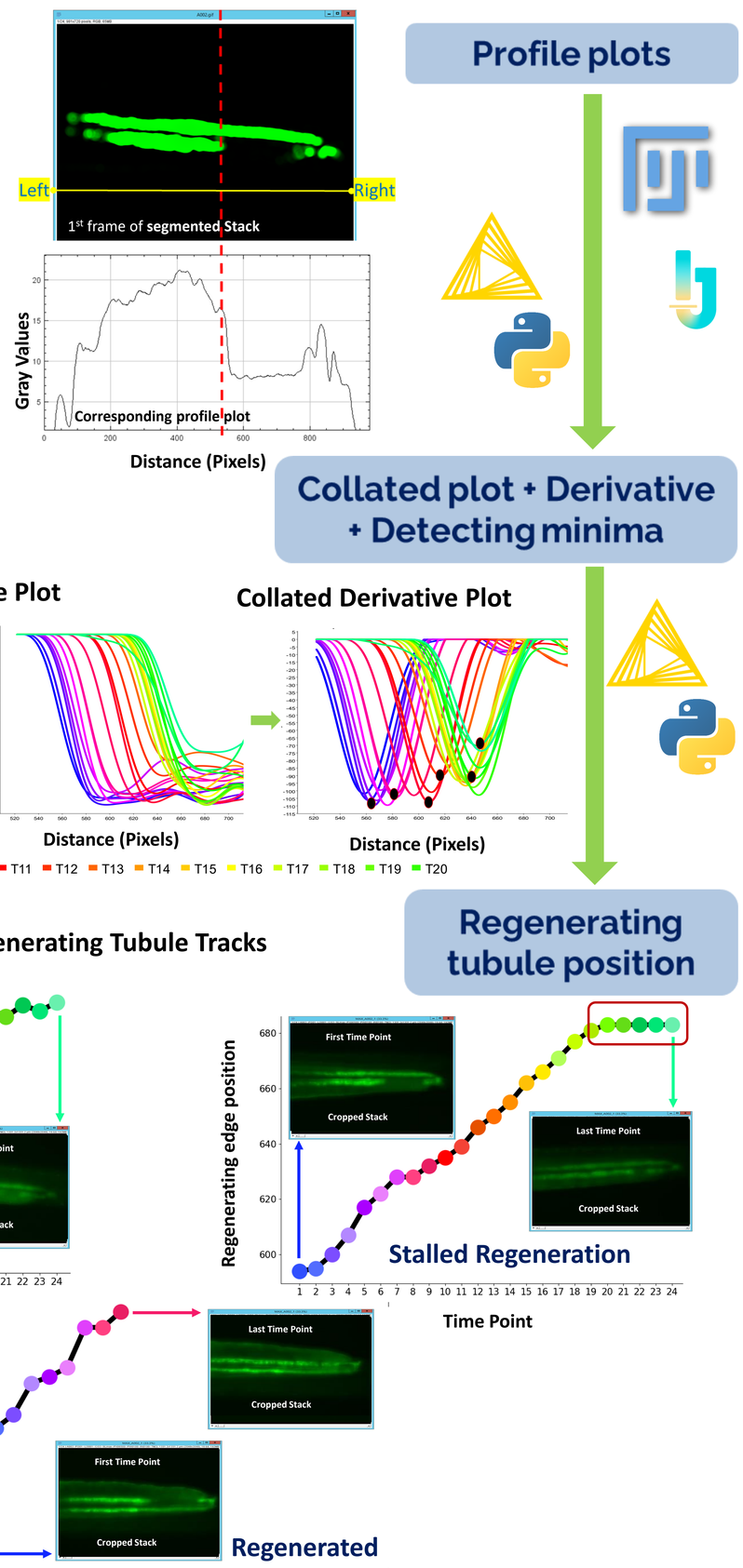
1) Reducing data complexity through Z projection.
2) Automated Region Of Interest (ROI) detection.
3) Stack Alignment post ROI detection.
4) Automated segmentation using trained model with Ilastik.
5) Amplifying the signal using variance filter.

Data Analysis

Automated Detection and Tracking Regenerating Tubule

- **Summary**

1) Spotting the injury from graph of pixel intensities along the horizontal axis. (Intensity profile)
2) Collecting all profiles and plotting as a single plot. (Collated Plot)
3) Selecting a region for analysis. (Tracking left edge of the regenerating tubule)
4) Smoothening the data and convolving using derivative filter.
5) Peak detection and creating tracks for regenerating tubule.
6) Finding different regenerating class.



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- **Reference**

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