A Pipeline for Constructing a 3D Coordinate Map of PMCs In Developing Embryos

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Abstract

For years, developmental biologists have relied upon spatial expression patterns of key genes to understand regulatory mechanisms behind embryogenesis. However, due to the difficulty of making quantitative comparisons of small morphological differences across embryos, analysis is often necessarily qualitative and imprecise. Excitingly, by first aligning embryonic images to a common coordinate system, we can more accurately and robustly analyze spatial patterns in developing embryos. Here we present a pipeline that constructs a coordinate system onto which we can map 3D images of primary mesenchyme cells (PMCs) from developing sea urchins, which secrete the larval skeleton during skeletogenesis. To generate a coordinate system, we first orient images along structural landmarks and subsequently perform multi-image alignment to produce a template embryo. New embryonic images can be transformed onto this template and compared across the common coordinate system. We optimized parameters by aligning stereotypic PMC images and measuring their mutual information scores, which reflect the efficacy of alignment. Preliminary results showed that learning transformations from binary images (images that delineate background and foreground elements) and using nonlinear image transformations produced higher mutual information scores than other image alignment techniques.

Background

- Understanding mechanisms for embryonic development is imperative to studying biological problems involving disease, regenerative medicine, and tissue engineering

- Sea urchins are a model organism for development because they have a simple structure and are conducive to induction of synchronous, rapid embryogenesis

- Spatial expression patterns of key genes provide insight into regulatory mechanisms behind embryonic development, however, without a common coordinate system as reference, quantitative comparisons are difficult

- Primary mesenchyme cells (PMCs) form the sea urchin larval skeleton and produce a stereotypical structure. Thus, sea urchin embryos share structural landmark points along the PMCs that are helpful for orienting and aligning images of their PMC stains.

Objective

- Apply image-registration techniques to create a pipeline that constructs a template coordinate system onto which we can map 3D images of primary mesenchyme cells (PMCs) from sea urchin embryos

Pipeline Overview
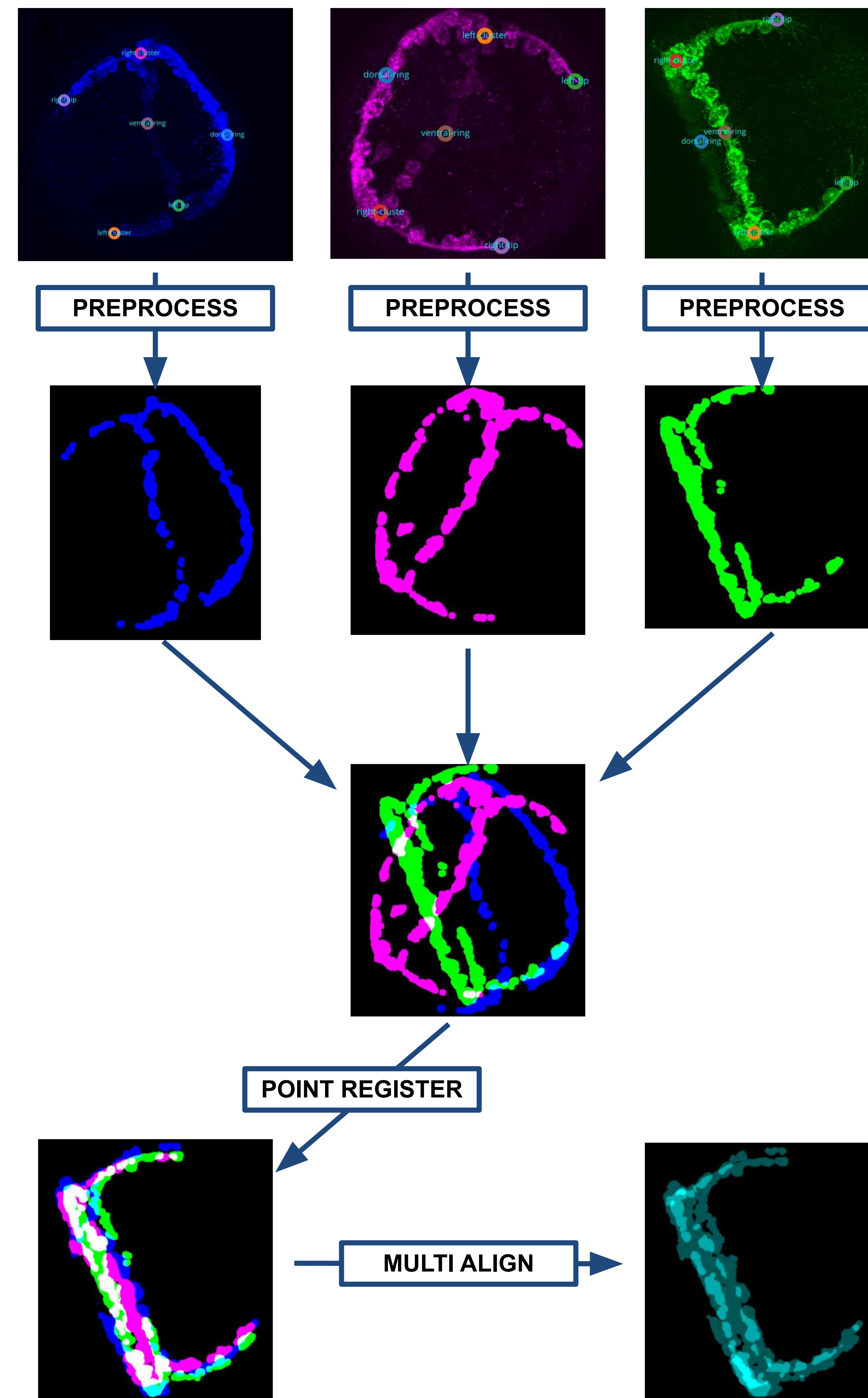
Figure 1. Schematic depicts approach for producing a template 3D coordinate system onto which we can map embryonic images for spatial comparisons. Images of PMC stains are preprocessed, producing cleaned and normalized binary images. The images are aligned using their structural landmark points (point-registration). A template image is generated by averaging the intensity values of the point-registered images. The point-registered images are aligned to the template via symmetric normalization, a transform that optimizes mutual information scores. Algebraic transformations learned from the registration process are applied to the image landmark points, which are then averaged to produce the template landmark points. Images of other embryos can be passed into the pipeline and mapped onto the template coordinate system via the registration process.

Results
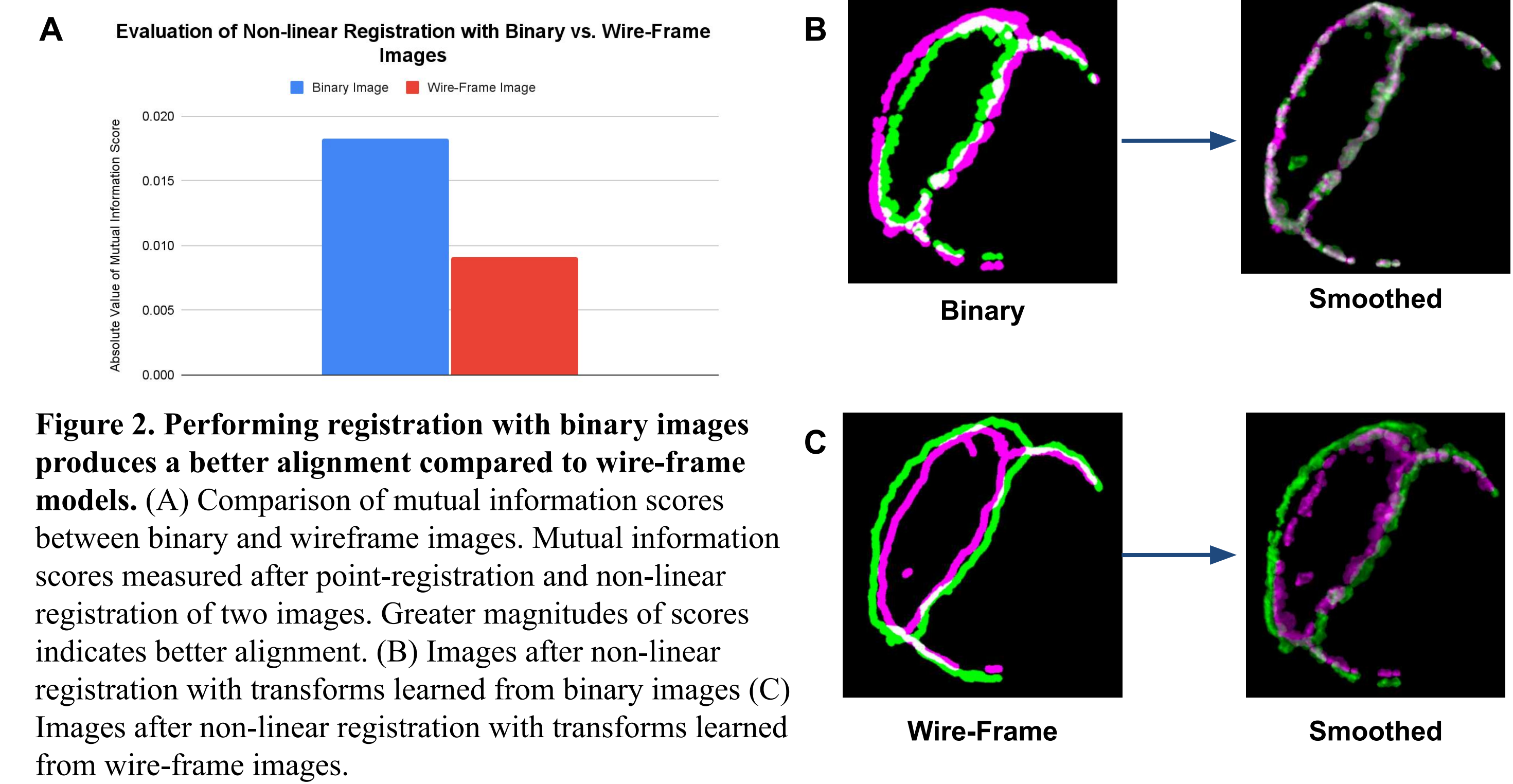
Figure 2. Performing registration with binary images produces a better alignment compared to wire-frame models. (A) Comparison of mutual information scores between binary and wireframe images. Mutual information scores measured after point-registration and non-linear registration of two images. Greater magnitudes of scores indicates better alignment. (B) Images after non-linear registration with transforms learned from binary images (C) Images after non-linear registration with transforms learned from wire-frame images.
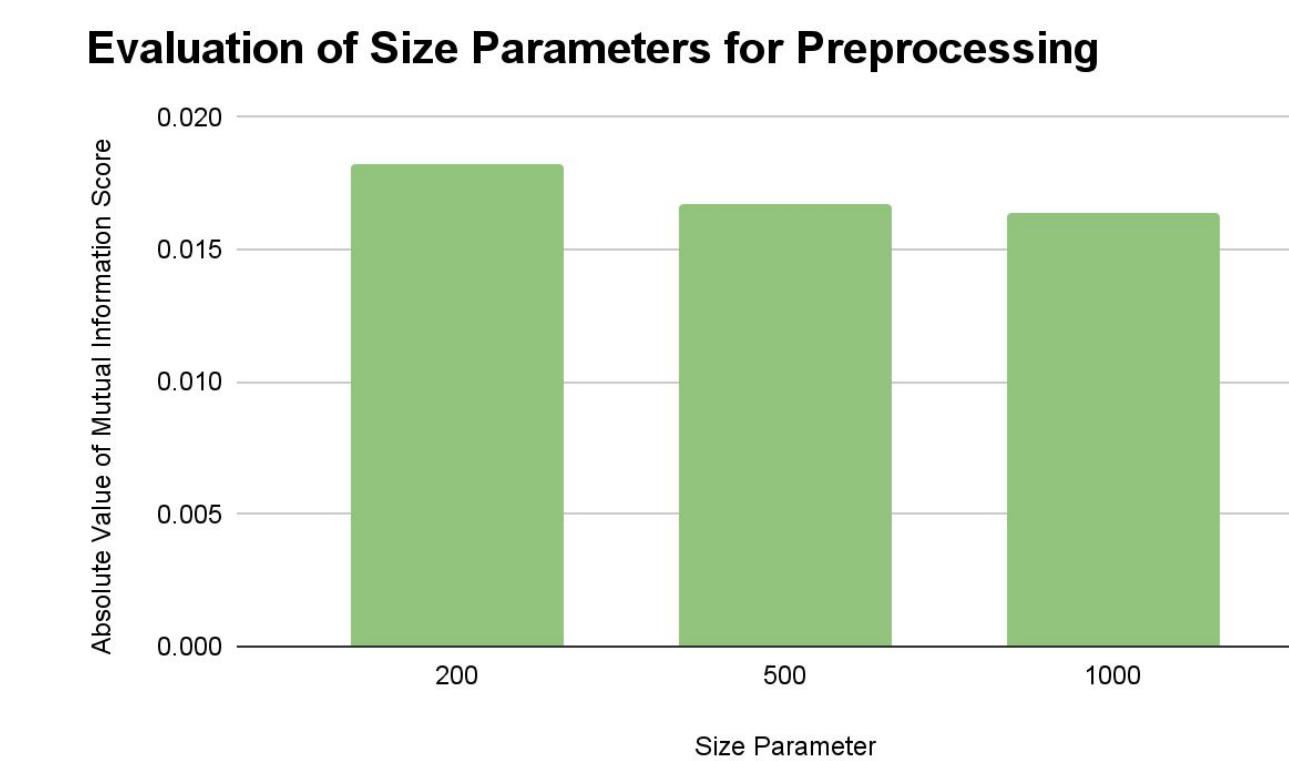
Figure 3. Applying a smaller size threshold allows for better alignment. Comparison of mutual information scores obtained using different size parameters. In the preprocessing step, objects with a voxel size smaller than the size parameter are removed from the image.
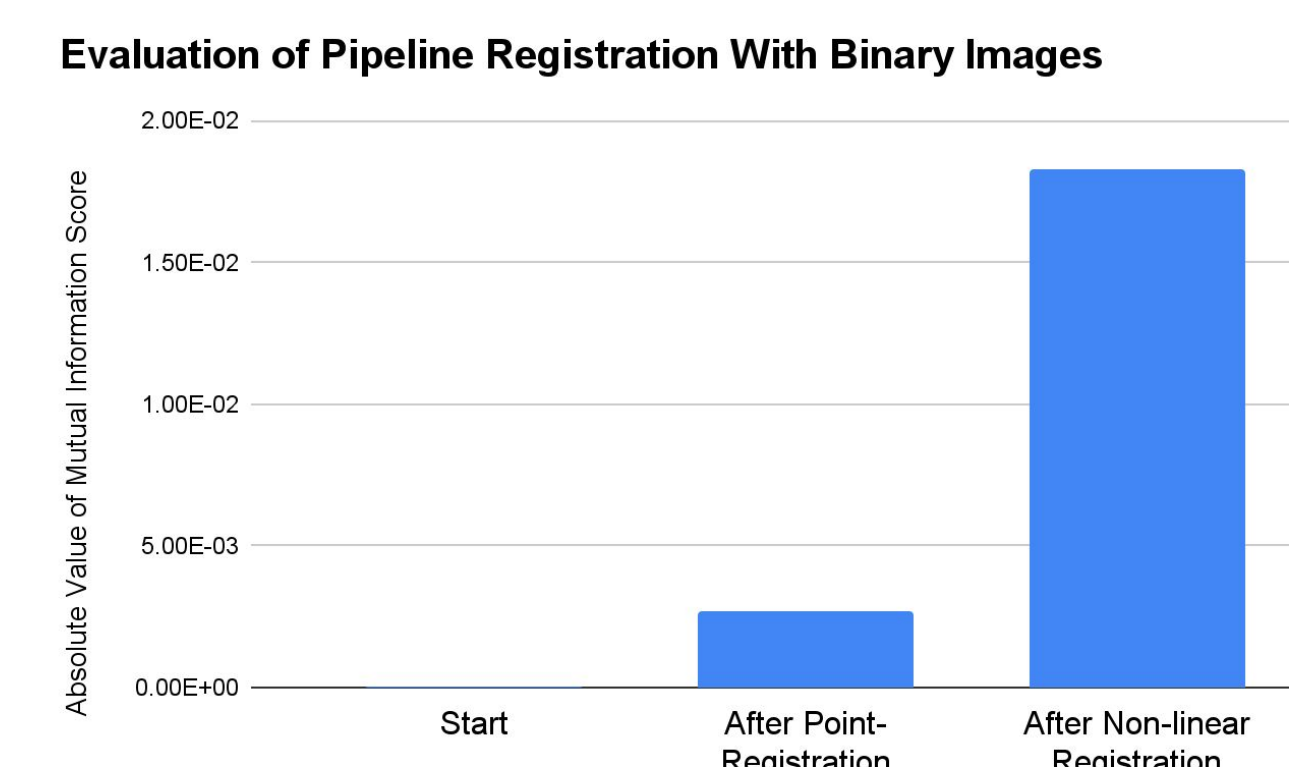
Figure 4. Point registration followed by non-linear registration of images produces an effective alignment. Comparison of mutual information scores obtained after point-registration and non-linear registration of two images. Point registration uniformly orients images and marginally increases the score. Non-linear registration utilizes affine and deformable transformations, and significantly increases the score.

Conclusions

- Figure 1 shows that the pipeline was successful at generating a template 3D coordinate system onto which we can map other images for more accurate spatial comparison

- According to Figure 2, the pipeline was effective at aligning images of PMC stains, and learning transformations from the binarized images produced the best result

- As displayed in Figure 3, applying a smaller voxel size threshold for image noise removal produces a better alignment outcome

- Figure 4 suggests that embryonic images can be effectively aligned using point-registration followed by non-linear registration techniques.

- While the pipeline currently only supports stereotypic control embryos, future work may entail expanding support to irregular, perturbed embryos.

References & Acknowledgments

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