

Anchor: Registration by Alignment

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Spatial organization within tissues plays a central role in regulating cellular interactions and functions.¹ Advances in microscopy have enabled increasingly detailed measurements of this organization, but multiplexed phenotyping remains challenging. Single tissue sections are limited in the number of markers that can be detected simultaneously. This limitation can be addressed by repeated cycles of staining on the same section or by imaging serial slices for different marker subsets, using modalities such as Iterative Bleaching Extends Multiplexity (IBEX)² or Cyclic Immunofluorescence (CycIF).³ These approaches are substantially easier and more cost-effective to implement than specialized mass spectrometry equipment.

However, combining information across multiple rounds or slices introduces a new challenge: the images often do not align automatically. Variations in tissue placement, stretching, tearing, folding, or structural changes between slices can create spatial offsets that must be corrected before meaningful analysis.⁴ In electron microscopy workflows, these effects can be substantial due to stage instability, thermal drift, and sparse axial sampling.⁵ Artifacts introduced during imaging are often corrected through manual hardware adjustments, but these can fail when overlap between rounds is partial or when deformation varies locally, such as in tissue tears. Software-based alignment using global metrics, such as intersection-over-union or cross-correlation, can also obscure misregistration in specific regions, producing alignments that appear reasonable but are incorrect at the level of individual features.⁶

To overcome the inconsistency and limitations of manual or global alignment, automated methods should follow the logic of a careful human: use reliable landmarks to fix lateral alignment, restrict distortions to realistic transformations, and indicate where alignment is reliable, while remaining fully automated and reproducible.

Proposed Solution

We propose a landmark-centric alignment pipeline designed for multi-round microscopy data with drift and incomplete axial coverage. The goal is to align a moving imaging round to a fixed reference with subpixel lateral accuracy and the best achievable axial consistency under partial overlap.

First, a coarse global XY translation is estimated using phase correlation on down sampled two-dimensional projections. This step provides a robust initial alignment without introducing non-rigid deformation. Next, segmented landmarks are matched across rounds using identity embeddings learned from local image structure and intensity patterns. Each nucleus is represented by an embedding vector, and candidate matches are restricted using physical distance constraints. Nuclei are encoded using a 2D-CNN, using two separate branches. Nucleus level features are encoded by a direct convolution, while neighborhood level features are encoded by down sampling the image prior to convolution. One-to-one assignment is enforced to prevent duplicate matches. High-confidence correspondences are identified based on embedding similarity margins and geometric consistency. These correspondences are used as anchors to fit a

single smooth two-dimensional deformation field across the field of view. Low-confidence matches are excluded from deformation of fitting. After lateral alignment is stabilized, axial mismatch is estimated separately. Because axial sampling is sparse, axial alignment is performed using overlap-tolerant matching that scores only overlapping slices. Local axial shifts are estimated on spatial tiles and interpolated into a smooth axial displacement field.

Results

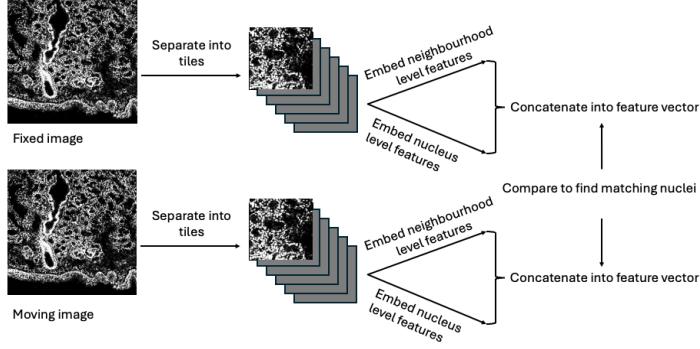


Figure 1. Workflow of developed pipeline.

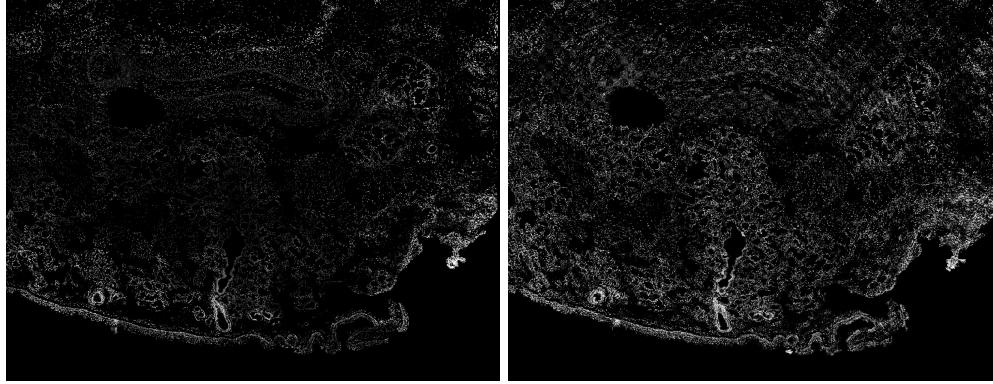


Figure 2. Alignment using manual registration (left) in comparison to Deep Learning (right).

The pipeline produces an aligned moving volume, the associated transformation fields, and a correspondence table with linking features across rounds. In addition, each feature match is assigned to a confidence label based on independent criteria, including similarity margin and geometric residual. Confidence labeling serves two purposes. First, it prevents unreliable matches from influencing deformation estimation. Second, it provides a diagnostic output indicating regions where alignment is uncertain and where downstream analysis should be interpreted cautiously. Axial alignment confidence is assessed independently using the sharpness and separation of overlap-matching scores across candidate shifts. This approach addresses alignment in multi-round microscopy experiments where drift, deformation, and partial overlap are present. By separating lateral and axial alignment, restricting deformation to smooth fields, and explicitly tracking confidence, the method avoids common failure modes of global or unconstrained registration techniques. Future work will focus on validation across additional datasets and refinement of confidence thresholds.

References

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