

# Stitching Misoriented and Misaligned Non-Overlapping Images

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## ABSTRACT

Image stitching is a fundamental task in biomedical imaging, enabling reconstruction of large specimens that exceed the field of view of a single acquisition. Conventional stitching methods rely on overlap between neighboring tiles and known acquisition geometry. These methods typically use feature matching registration to estimate spatial transformations and alignment [1–3]. Although effective under controlled conditions, these assumptions do not hold when tiles do not overlap or when their orientations are unknown. This situation often occurs during the physical handling of samples [4]. We address this challenge by proposing a framework for stitching non-overlapping biomedical image tiles with unknown orientations.

We conduct the study using a single biomedical dataset that is divided into two subsets: (1) a translation-only (alignment) subset, and (2) a translation-and-orientation subset. In the first subset, orientations are known, but both horizontal and vertical offsets between tiles are unknown. In the second subset, tile orientations are unknown, and only horizontal offsets between tiles are considered. The proposed framework follows a two-stage strategy that decouples orientation determination from alignment. First, tile orientation is inferred using a compatibility measure (CM). Next, tile translation is estimated using phase correlation applied to tile boundaries, rather than relying on shared pixel content. This approach achieves approximately 91% correct reconstructions (182 out of 200) on the translation-and-orientation subset.

This result demonstrates that accurate reconstruction is possible even without overlap or

orientation metadata. However, this result is obtained on small grid sizes (single rows), where exhaustive brute-force assembly strategies are still computationally feasible. However, scaling to larger grid sizes makes brute-force assembly computationally infeasible. This motivates the use of derivative-free optimization (DFO)-based assembly strategies, including Bayesian optimization and Tabu search, as scalable alternatives.

**Table 1.** Comparison of pairwise compatibility measures on the small-grid translation-and-orientation subset (Subset 2).

| Compatibility Measure | Assembly Strategy          |                          |
|-----------------------|----------------------------|--------------------------|
|                       | Bayesian Optimization      | Tabu Search              |
| New CM                | 121 out of 166<br>(72.89%) | 182 out of 200<br>(91%)  |
| MGC [5]               | –                          | 200 out of 200<br>(100%) |

A key focus of this study is the comparison of pairwise compatibility measures used in determining the orientation of tiles. Specifically, we compare the compatibility measure we developed with the Mahalanobis Gradient Compatibility (MGC) measure from the image puzzle-solving literature [5,6]. Table 1 presents preliminary results for this comparison using the single row, translation-and-orientation subset (Subset 2). Both measures perform strongly in this setting. The developed CM achieves up to 91% accuracy, while MGC achieves 100% accuracy. These results motivate

the integration of both measures into scalable, optimization-based assembly frameworks for large grids. Overall, this work demonstrates the progression of non-overlapping stitching frameworks from small, controlled experiments to scalable and computationally efficient solutions for large-scale biomedical image reconstruction.

## REFERENCES

1. Brown M, Lowe DG. Automatic panoramic image stitching using invariant features. *Int J Comput Vision* 74:59–73 (2007).  
<https://doi.org/10.1007/s11263-006-0002-3>
2. Preibisch S, Saalfeld S, Tomancak P. Globally optimal stitching of tiled 3D microscopic image acquisitions. *Bioinformatics* 25: 1463–1465 (2009).  
<https://doi.org/10.1093/bioinformatics/btp184>
3. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez JY. Fiji: an open-source platform for biological-image analysis. *Nature Methods* 9:676–682 (2012).  
<https://doi.org/10.1038/nmeth.2019>
4. Chalfoun J, Majurski M, Blattner T, Bhadriraju K, Keyrouz W, Bajcsy P, Brady M. MIST: accurate and scalable microscopy image stitching tool with stage modeling and error minimization. *Scientific Reports* 7:4988 (2017).  
<https://doi.org/10.1038/s41598-017-04567-y>
5. Gallagher AC. Jigsaw puzzles with pieces of unknown orientation. In 2012 IEEE Conference on computer vision and pattern recognition (pp. 382–389). (2012)  
<https://doi.org/10.1109/CVPR.2012.6247699>
6. Cho TS, Avidan S, Freeman WT. A probabilistic image jigsaw puzzle solver. In 2010 IEEE Computer society conference on computer vision and pattern recognition (pp. 183–190) (2012).  
<https://doi.org/10.1109/CVPR.2010.5540212>

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