HISTOKATFUSION. IMAGE REGISTRATION FOR THE ACROBAT CHALLENGE.

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Image registration is aiming to find a transformation between at least two images, so that corresponding objects appear at the same position. This document describes a registration method submitted to the ACROBAT challenge[1] and is an extension of an earlier text [1].

1 Tissue Segmentation

Our image registration method is based on a Newton-type optimization and therefore relies on a good initial guess to converge quickly. As an initial guess, a robust rough alignment of the tissue sections is required. In many image pairs in the ACROBAT data (as well as in many real-world datasets), control tissue is present in only one of the two images which makes finding the initial alignment difficult.

We extended a registration method that was previously applied in the ANHIR challenge by an additional tissue foreground segmentation step that aims at masking the images such that only tissue present in both images is taken into account.

1.1 Step 1: Foreground segmentation

The foreground segmentation is based on the implementation published by Bándi et al. in [2]. We trained the provided network (`FCNN_C` with a spacing of 8µm $\approx$ 1.25x magnification) on seven slides of the authors' public training subset, on six additional slides from the ACROBAT training data (slides `1_HE, 105_KI67, 106_HER2, 119_PGR, 156_PGR, 168_ER`), and one internal image (`SynD_3_001`) to further improve the segmentation performance.

1.2 Step 2: Choosing relevant tissues

From each of the resulting tissue masks, the largest interconnected tissue parts are selected.

We assume that there are a multitude of smaller (control) tissue parts that do not necessarily have a correspondence in the other slide of the pair. Only the few large tissue parts that are present on both images should be considered for the initial registration.

To select the relevant tissue parts, the separate masks in each image are sorted by their surface area and the differences between the areas are computed. We then identify the largest gap in area sizes that divides the tissue parts in a large and a small group. Only the tissue parts in the large-area group are kept for further processing. Additional tissue parts are included to obtain the same number of tissue parts in both images of a pair.

The resulting masked images are used for the pre-alignment step of the image registration.

[1] `https://acrobat.grand-challenge.org`

2 Image Registration

Each image pair is registered twice, once with the H&E-image as reference (fixed) image and once with the IHC image as reference image. Both results are stored and the final result is determined by comparing the overlap of the deformed masks from the foreground segmentation.

The registration method consists of 1) a robust pre-alignment, 2) an affine registration computed on coarse resolution images, and 3) a curvature-regularized deformable registration. The method is based on the variational image registration framework first described by Fischer and Modersitzki [3, 4] which has been applied to many clinical fields from histology [5] to radiology [6, 7].

Given a reference image $R : \mathbb{R}^2 \to \mathbb{R}$ and a template image $T : \mathbb{R}^2 \to \mathbb{R}$, the goal of image registration is to find a reasonable spatial transformation $y : \mathbb{R}^2 \to \mathbb{R}^2$ such that $R(\mathbf{x}) \approx T(y(\mathbf{x}))$, i.e., R and the deformed template $T \circ y$ are similar in an adequate sense.

Following [4], we formulate the image registration as the optimization problem $J(R, T, y) \xrightarrow{y} \min$ of an appropriate objective function J with respect to the desired spatial transformation. A key component of the objective function is a so-called distance or image similarity measure that quantifies the quality of the alignment. We use the Normalized Gradient Fields (NGF) distance measure [8] as it has been shown to be robust to different stains and is suitable for multi-modal image registration of histological images [9]. For the discretization, 2D images with extents n_1-by-n_2 are assumed, correspondingly consisting of $N = n_1 \cdot n_2$ pixels with uniform size $h \in \mathbb{R}$ in each dimension and pixel centers $\mathbf{x}_1, ..., \mathbf{x}_N$; $\mathbf{x_i} \in \mathbb{R}^{\mathbf{2}}$. The NGF distance measure is given by

$$\text{NGF}(R, T, y) = \frac{h^2}{2} \cdot \sum_{i=1}^{N} 1 - \left(\frac{\langle \nabla T(y(\mathbf{x}_i)), \nabla R(\mathbf{x}_i) \rangle_\varepsilon}{\|\nabla T(y(\mathbf{x}_i))\|_\varepsilon \, \|\nabla R(\mathbf{x}_i)\|_\varepsilon} \right)^2$$

with $\langle \mathbf{x}, \mathbf{y} \rangle_\varepsilon = \mathbf{x}^\top \mathbf{y} + \varepsilon^2$, $\|\mathbf{x}\|_\varepsilon := \sqrt{\langle \mathbf{x}, \mathbf{x} \rangle_\varepsilon}$, and the edge parameter ε, which controls the sensitivity to edges in contrast to noise. This image distance becomes minimal if intensity gradients and edges, respectively, are aligned and which therefore leads to the alignment of morphological structures.

The NGF distance measure is used in all three steps of the registration pipeline: Pre-alignment, affine registration, and deformable (non-linear) registration. In addition, we use a multilevel optimization scheme that starts with the registration of images at low resolution levels and then refines the transformation to higher image resolutions to reduce the risk of converging too early to local minima and to speed up the optimization process [10]. The per-level optimization is performed using a Gauss-Newton type (affine registration) and L-BFGS quasi-Newton (deformable registration) method, see e.g. [4] or [11, 12] for a more detailed discussion and additional strategies.

All of the three following registration steps rely on the edge parameter ε, the number of levels N_{level} of the image pyramid, and the image resolution at the finest level. The parameters are set independently for each step and such that the registration error is minimal and the deformation grid is regular in the sense that it is not folded in the image domain. These parameters are shown in Table 1.

2.1 Step 1: Automatic rotation alignment (ARA)

Before histological images are scanned, the tissue is cut, preprocessed, and stained in a pathology lab.

After this manual process, neighboring tissue slices can end up in arbitrary positions on the object slide (such as upside down or turned in various ways). In general, no assumptions can be made on the initial tissue positioning and—in a first step—we aim to find a rigid alignment, correcting for global translation and global rotation.

The NGF distance measure is based on structural changes expressed through the image gradient and therefore, color information is of limited value. To reduce the amount of image data to be handled, all images are converted from color to gray scale and inverted to obtain a black background while loading from disk.

Automatic Rotation Alignment (ARA) first determines the center of mass [13] of both images, using the gray values of the pixels as the weights. The relevant, corresponding tissue parts are masked according to the segmentation procedure in Section 1.

Let (t_1, t_2) be the vector pointing from the center of mass of the reference image to the center of mass of the template image, and let $\phi_k = 2\pi(k-1)/(N_{\text{rotations}} - 1)$, $k = 1, \ldots, N_{\text{rotations}}$ be equidistant rotation angles sampling the interval $[0, 2\pi)$. For each angle, a rigid registration is computed with initial guess (φ, t_1, t_2). Among all $N_{\text{rotations}}$ rigid

Registration step	Parameter values
Step 1: Pre-Alignment	
No. of levels N_{level}	5
No. of rotations N_{rot}	32
image resolution ($\frac{\mu m}{\text{px}}$)	approx. 128
image size (px)	approx. 300×200
NGF ε	0.1
Step 2: Affine	
image resolution ($\frac{\mu m}{\text{px}}$)	approx. 4
image size (px)	approx. 9000×6000
No. of levels N_{level}	8
NGF ε	0.1
Step 3: Deformable	
image resolution ($\frac{\mu m}{\text{px}}$)	approx. 2
image size (px)	approx. 18000×12000
No. of levels N_{level}	8
NGF ε	0.1
regularizer weight α	0.1
control point grid m	1025x1025 nodes

Table 1: Parameters used in the registration pipeline.

registration results, the minimizer (ϕ^*, t_1^*, t_2^*) with the smallest image distance is selected as an initial guess for the subsequent affine registration.

2.2 Step 2: Affine registration

In a second step, again an NGF-based image registration is computed based on the original, unmasked images. To allow for additional degrees of freedom, the registration is optimized with respect to an affine transformation y_{affine} and based on a finer image resolution. The resulting transformation is then used as initial guess for a subsequent deformable registration.

2.3 Step 3: Deformable registration

The final step is a deformable image registration. Here, the transformation y is given by

$$y(\mathbf{x}) = \mathbf{x} + u(\mathbf{x})$$

with so-called displacement $u : \mathbb{R}^2 \to \mathbb{R}^2$, $u = (u_1, u_2)$ [4].

In contrast to an affine registration, the deformation is not restricted to a particular parameterizable deformation model and the nonlinear transformation is controlled by introducing a regularization term into the objective function that measures the deformation energy and penalizes unwanted transformations. Here we use the so-called curvature regularization, which penalizes second-order derivatives of the displacement [14] and which has been shown to work very well in combination with the NGF distance measure [6, 7]. As with the NGF distance, we evaluate the displacements in the pixel centers $\mathbf{x}_1, \ldots, \mathbf{x}_m$ with uniform grid spacing h and use finite differences to approximate the derivatives. Thus, the discretized curvature regularizer is defined as

$$\text{CURV}(y) = \frac{h^2}{2} \sum_{i=1}^{m} |\Delta^h u_1(\mathbf{x}_i)|^2 + |\Delta^h u_2(\mathbf{x}_i)|^2$$

where Δ^h is the common 5-point finite difference approximation of the 2D Laplacian $\Delta = \partial_{xx} + \partial_{yy}$ with Neumann boundary conditions. In summary, for deformable registration, we minimize the objective function

$$J(R, T, y) := \text{NGF}(R, T, y) + \alpha\text{CURV}(y) \to \min,$$

with respect to the deformation y. The parameter $\alpha > 0$, is a regularization parameter that controls the smoothness of the computed deformation. The parameter α is chosen manually to achieve a smooth deformation and avoid topological changes (lattice folds), while being flexible enough to correct for local changes that improve image similarity. The resolution of the control point grid is independent of the image resolution and is typically chosen to be coarser than the image resolution (see also Table 1). A higher number of grid points allows for a more accurate representation of local deformations. Linear interpolation is used to evaluate the deformation between its grid nodes.

3 Selection of the superior registration result

After registering each image pair twice, once with each image as reference image, the better of the two registrations is determined by comparing the alignment of the masks generated by the foreground segmentation.

The alignment quality is computed by the sum of squared differences (SSD) of the reference and the deformed template mask. Deformations with foldings determined by negative parts in the jacobi determinant are rejected beforehand.

References

[1] J. Lotz, N. Weiss, and S. Heldmann, "Robust, fast and accurate: A 3-step method for automatic histological image registration," arXiv:1903.12063 [cs], Mar. 28, 2019.

[2] P. Bándi, M. Balkenhol, B. van Ginneken, J. van der Laak, and G. Litjens, "Resolution-agnostic tissue segmentation in whole-slide histopathology images with convolutional neural networks," *PeerJ*, vol. 7, e8242, Dec. 17, 2019, ISSN: 2167-8359. DOI: 10.7717/peerj.8242.

[3] B. Fischer and J. Modersitzki, "Fast Image Registration - A Variational Approach," in *Numerical Analysis and Computational Mathematics*, G. Psihoyios, Ed., 2003, pp. 69–74.

[4] J. Modersitzki, *FAIR: Flexible Algorithms for Image Registration* (Fundamentals of Algorithms FA06). Philadelphia: Society for Industrial and Applied Mathematics, 2009, 189 pp., ISBN: 978-0-89871-690-0.

[5] O. Schmitt, J. Modersitzki, S. Heldmann, S. Wirtz, and B. Fischer, "Image Registration of Sectioned Brains," *International Journal of Computer Vision*, vol. 73, no. 1, pp. 5–39, Jun. 2007, ISSN: 0920-5691, 1573-1405. DOI: 10.1007/s11263-006-9780-x.

[6] J. Rühaak, S. Heldmann, T. Kipshagen, and B. Fischer, "Highly Accurate Fast Lung CT Registration," *Proceedings of SPIE 8669, Medical Imaging 2013: Image Processing*, Mar. 2013.

[7] L. König, J. Rühaak, A. Derksen, and J. Lellmann, "A Matrix-Free Approach to Parallel and Memory-Efficient Deformable Image Registration," *SIAM Journal on Scientific Computing*, vol. 40, no. 3, B858–B888, Jan. 2018, ISSN: 1064-8275, 1095-7197. DOI: 10.1137/17M1125522.

[8] E. Haber and J. Modersitzki, "Intensity Gradient Based Registration and Fusion of Multi-modal Images," *Methods of Information in Medicine*, vol. 46, no. 03, pp. 292–299, 2007, ISSN: 0026-1270, 2511-705X. DOI: 10.1160/ME9046.

[9] W. Bulten, P. Bándi, J. Hoven, *et al.*, "Epithelium segmentation using deep learning in H&E-stained prostate specimens with immunohistochemistry as reference standard," *Scientific Reports*, vol. 9, no. 1, Dec. 2019, ISSN: 2045-2322. DOI: 10.1038/s41598-018-37257-4.

[10] E. Haber and J. Modersitzki, "A Multilevel Method for Image Registration," *SIAM Journal on Scientific Computing*, vol. 27, no. 5, pp. 1594–1607, Jan. 2006, ISSN: 1064-8275, 1095-7197. DOI: 10.1137/040608106.

[11] G. Song, J. Han, Y. Zhao, Z. Wang, and H. Du, "A Review on Medical Image Registration as an Optimization Problem," *Current Medical Imaging Reviews*, vol. 13, no. 3, Jul. 20, 2017, ISSN: 15734056. DOI: 10.2174/1573405612666160920123955.

[12] J. Nocedal and S. J. Wright, *Numerical Optimization*. New York: Springer, 2006, ISBN: 978-0-387-30303-1.

[13] M. F. Beatty, *Principles of Engineering Mechanics* (Mathematical Concepts and Methods in Science and Engineering 32-33). New York: Plenum Press, 1986, 2 pp., ISBN: 978-0-387-23704-6.

[14] B. Fischer and J. Modersitzki, "Curvature based image registration," *Journal of Mathematical Imaging and Vision*, pp. 81–85, 2003. DOI: 10.1023/A:1021897212261.