

## Assignment 1: Registration of Medical Images.

### 1 Introduction

The purpose of this assignment is to perform *landmark-based image registration*. Both rigid registration, using Euclidean transformations, and Procrustes alignment, using similarity transformations, are considered. The landmarks will be generated automatically using SIFT and RANSAC, and are therefore to be considered as *mathematical landmarks*. Landmark-based methods are especially interesting when dealing with image pairs having different modalities. This is illustrated clearly in the second part of the assignment.

Each assignment in the course is given a grade. This assignment has three tasks, stated in the gray frames below. If you complete the first task you will pass and the assignment gets the grade 3. Completing the two first tasks gives grade 4. Completion of all three tasks results in the highest grade, 5. The teachers will correct and comment your solution and if you fail to pass the first time around we will ask you to improve and complement your report.

### 2 The Rules

The assignment is published on the course's Canvas page **Tuesday 4 November**. The deadline is on **Sunday 16 November** (23:59 CET). Each student should hand in his or her own individual solution and should, upon request, be able to present the details in all the steps of the used algorithm. You are, however, allowed to discuss the assignment with others. You may also ask your teachers and the course assistants for advice, if needed. It is not allowed to use generative AI to produce text and/or solutions to the assignment.

**The report.** Present your work in a report written in English or Swedish. The report should (at least) contain an introduction, a description of the theory and methods used, a section with the results, including necessary tables and a few images illustrating obtained results, and a conclusion where your results and findings are discussed. In particular, make sure to describe all essential choices made in the implementation of your algorithm, preprocessing steps included.

**Submitting your work.** Submit the report as a pdf-file with the name format `assignment_1_yourname.pdf` on the Canvas course page. Do not submit any code. Everything you wish to communicate should be written in the report. A well-written and well-structured report will have a positive influence on the grade you obtain.

**Supervision sessions.** There will be four opportunities get help with the assignment; see TimeEdit. The assistants are *Anders Heyden* and the PhD-student *Amanda Nilsson*.

### 3 The Data

Two data sets (both of them collections of image pairs) are provided for the assignment. They can be downloaded from the course webpage.

**Collection 1.** This data consists of 29 pairs of microscopy ( $20\times$ ) images of histopathological material obtained from radical prostatectomy (surgically removed prostates). There are two folders, each containing one image from each pair. The images were created in the following manner. Cylindrical cores of 1mm in diameter were punched out from the specimens, arranged in tissue microarrays (TMAs), and embedded in paraffine. Each image pair consists of two consecutive sections of thickness  $4\text{ }\mu\text{m}$  from these cores. One slice was stained with hematoxolin-eosin (H&E), which looks red or pink, and the other slice with p63AMACR, which appears blue or purple. The tissue samples in the image pair will therefore appear to have similar, but not identical, shapes. Since the images were acquired with the same magnification our assumption is that they may be aligned using a Euclidean transformation (translation+rotation).

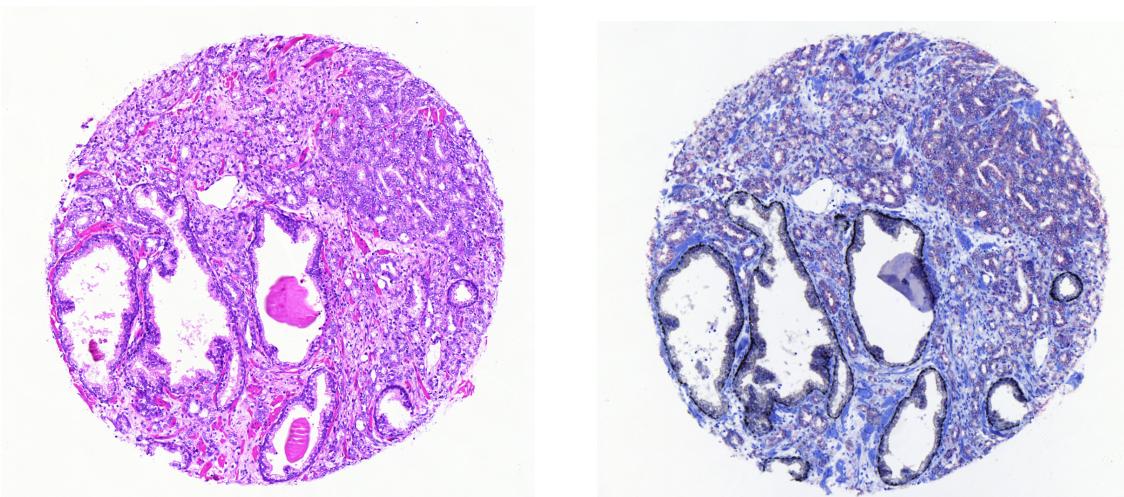


Figure 1: An image pair from Collection, prostate tissue cores. The two tissue samples are adjacent slices from the same core. The sample to the left is stained with H&E, the one to the right with p63AMACR.

**Task 1:** Perform rigid registration of the image pairs in Collection 1. For each image pair, record the *magnitude* of the translation vector in pixels and the *rotation angle* in degrees. Perform visual assessment of the performance of the method for each image pair. Present the results in a table with one row for each image pair. Include example images of both successful and less successful alignments. Describe the required theory adequately. This is the minimum requirement for the grade 3 (pass).

**Collection 2.** Here 12 pairs of images are provided. Each pair consists of an image ( $10\times$ ) stained with H&E and another ( $40\times$ ) stained with a fluorescent marker and acquired using time-resolved fluorescence microscopy (TRF), which looks as a faint nebulous signal on a dark background. Both images comes from the same slice, where the fluorescent staining was applied and the TRF image acquired first. Then the fluorescent staining was washed off, the H&E staining was applied subsequently and the corresponding image acquired. Due to the difference in magnification, the

TRF image shows only a detail of the H&E image. The alignment should therefore use a similarity transformation (translation+rotation+scale).

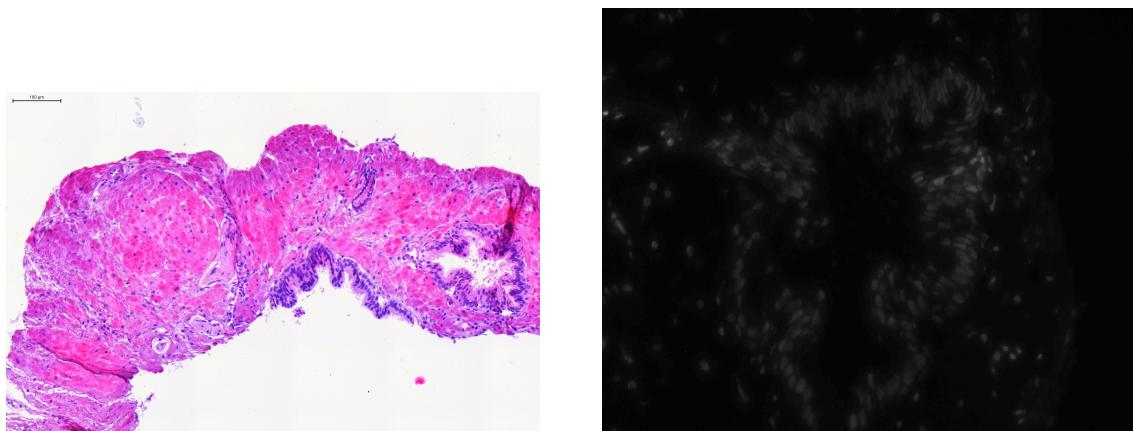


Figure 2: An image pair from Collection 2. The tissue is from a prostate biopsy. The images are obtained from the same slice. The left one with a H&E-staining and 10 $\times$  magnification, the right one with a fluorescent staining and 40 $\times$  magnification and amplified.

More information about the images can be found in Lippolis et al. [1].

**Task 2:** Perform image registration of the image pairs in Collection 2 using similarity transformations. For each image pair, record the *magnitude* of the translation vector in pixels, the *rotation angle* in degrees and the *scale factors*. Perform visual assessment of the performance of the method for each image pair. Present the results in a table with one row for each image pair. Include example images of both successful and less successful alignments. Describe the required theory adequately. This is the minimum requirement for the grade 4.

## 4 Evaluation of Results

Discuss how the registration results should be presented and the performance of the proposed algorithm can be adequately evaluated. In your report, only the simplest kind of evaluation is required. This could be simply a subjective visual assessment of the obtained results. However, this will not be enough in a scientific paper, and we therefore need to be aware of how we could proceed in order to get more objective assessments.

**Task 3:** This is the minimum requirement for the highest grade (5): Make an objective assessment of the performance of the algorithm used above on Collection 1. Use manually recorded landmarks (5 to 10 correspondences for each image pair) and follow the evaluation method outlined in the paper by Lippolis et al. [1]. Perform the evaluation on at least four images, preferably on both images where your algorithm works well and where it fails.

## 5 Landmark Generation and Alignment

In both task 1 and task 2, the given images should be converted to gray scale images. It may also be advisable to downscale the images to e.g. half the size (a quarter of

the number of pixels) to reduce runtimes. In the case of the TRF images, it may be an advantage to make an inversion of the image and a histogram equalization as well.

## 5.1 Keypoints and descriptors – SIFT

The first step in the landmark-generation is to find salient points in the images (keypoints) which are tagged with “fingerprints” (descriptors) which will allow us to find corresponding keypoints in pairs of images showing the same scene. To do this we use D. Lowe’s SIFT-algorithm, [2], using the function `detectSIFTFeatures` available in Matlab (introduced in R2021b).

## 5.2 Generating preliminar matchings from the keypoints – Lowe’s criterion

We first generate a set of preliminary matchings. This can be done either using Lowe’s criterion as outlined below, or the Matlab function `matchFeatures` with suitable parameters.

Let  $(\mathbf{x}_i, \mathbf{f}_i)_{i=1}^N$  denote the  $N$  pairs of keypoints  $\mathbf{x}_i \in \mathbf{R}^2$  and corresponding descriptors  $\mathbf{f}_i \in \mathbf{R}^{128}$  found by SIFT in the first image. Let similarly  $(\mathbf{x}'_j, \mathbf{f}'_j)_{j=1}^M$  denote keypoint-descriptor pairs detected in the second image. Then we first compute the  $N \times M$ -matrix  $\mathbf{D} = [d_{ij}]$  where the entries  $d_{ij} = \|\mathbf{f}_i - \mathbf{f}'_j\|$  are the euclidean distances between all pairs of descriptors, one from each image. Next, for each index  $i$  we find the index  $j^*$  such that

$$d_{ij^*} = \min_{1 \leq j \leq M} d_{ij}.$$

The keypoint  $\mathbf{x}'_{j^*}$  in image two is preliminarily matched to the keypoint  $\mathbf{x}_i$  in image one if Lowe’s criterion is fulfilled:

$$\frac{\min_j d_{ij}}{\min_{j:j \neq j^*} d_{ij}} < 0.77.$$

This criterion states that  $(\mathbf{x}_i, \mathbf{x}'_{j^*})$  is a preliminary match only if the corresponding descriptors  $\mathbf{f}_i$  and  $\mathbf{f}_{j^*}$  have a mutual distance  $d_{ij^*}$  which is *significantly* shorter than that from  $\mathbf{f}_i$  to any other descriptor  $\mathbf{f}'_j$  from the second image. The number 0.77 is heuristically chosen. The parameter may be chosen to be any number between zero and one.

## 5.3 Generating landmarks from the matchings – RANSAC

Among these matches we find real pairs of matching keypoints using RANSAC, [3]. When RANSAC is used, it is necessary to compute the registration models (rigid or similarity referred to below) on minimal or near-minimal sets of preliminary keypoint correspondences. The result of this step is to obtain a maximal set of inliers, i.e. a maximal set of keypoint-pairs which are consistent with the registration model used. This set of inliers will be our landmarks. The final registration is estimated from these landmarks.

## 5.4 Alignment using rigid transformation

The landmarks should be aligned using a transformation  $T : \mathbf{R}^2 \rightarrow \mathbf{R}^2$  of the following type,

$$T(\mathbf{x}) = \mathbf{t} + \mathbf{R}\mathbf{x} \quad (1)$$

where  $\mathbf{t} \in \mathbf{R}^2$  is a translation vector and  $\mathbf{R}$  is a  $2 \times 2$  orthogonal matrix, i.e. satisfying  $\mathbf{R}^T \mathbf{R} = I$ . An algorithm for computing  $\mathbf{t}$  and  $\mathbf{R}$ , based on singular value decomposition of a correlation matrix for the data, is presented in the lectures. This algorithm is formulated for the three-dimensional case, but is easily modified to suit our purpose.

## 5.5 Alignment using similarity transformation

The landmarks should be aligned using a transformation  $T : \mathbf{R}^2 \rightarrow \mathbf{R}^2$  of the following type,

$$T(\mathbf{x}) = \mathbf{t} + s\mathbf{R}\mathbf{x} \quad (2)$$

where  $\mathbf{t} \in \mathbf{R}^2$  is a translation vector and  $\mathbf{R}$  is a  $2 \times 2$  orthogonal matrix, and  $s > 0$  is a scale factor. Again,  $\mathbf{t}$ ,  $\mathbf{R}$  and  $s$  may be computed using an algorithm presented in the lectures. Alternatively one may use the method employed in Lippolis et al. [1].

# 6 Suggested Pipeline

This short section provides a brief outline of the steps needed in landmark-based registration using landmarks provided by distinctive image features from scale-invariant keypoints (SIFT), see [2].

1. Load the images in corresponding pairs and rescale (possibly). Convert to grayvalue. In the case of the TRF images, it may be an advantage to make an inversion of the image and an histogram equalization as well. The Matlab commands `dir`, `imread`, `imresize`, `rgb2gray` and `histeq` may be used, but the step can be completed in different ways.
2. Compute SIFT keypoints and descriptors in both images using the commands

```
points = detectSIFTFeatures(im);  
[features, valid_points] = extractFeatures(im, points);
```
3. Find preliminary matchings of pairs of keypoints using the descriptors. Either you make your own implementation of Lowe's criterion, as outline briefly above, or you employ the commands

```
indexPairs = matchFeatures(features1,features2);  
matchedPoints1 = valid_points1(indexPairs(:,1),:);  
matchedPoints2 = valid_points2(indexPairs(:,2),:);  
matchedLocations1 = matchedPoints1.Location';  
matchedLocations2 = matchedPoints2.Location';
```

The function `matchFeatures` has parameters `MatchThreshold` and `MaxRatio` which might require some tuning.

4. Find the “true” matches among all preliminary matchings using RANSAC. This step requires the implementation of the alignment model - orthogonal Procrustes for the rigid registration and Procrustes for the similarity transformations [4]. **You have to implement these yourself.** Since the alignment model will be used multiple times it should be implemented as a function in Matlab, taking coordinates of matched points as inputs and giving the computed variables ( $\mathbf{R}, \mathbf{t}, s$ ) as output. The “true” matches are defined to be the maximal inlier set found from the RANSAC procedure. Useful commands are `svd`, `diag`, `sum`, `mean` and `randperm`.
5. Align the images using the alignment model (rigid or similarity) using the maximal inlier set as landmarks. An image may be transformed and overlayed on top of another image using the Matlab script similar to the following:

```
T = [s*R, t ;
      0, 0, 1];
tform = affine2d(T');
im1_warp = imwarp(im1, tform, 'OutputView', imref2d(size(im2)));
figure;
imshow(imfuse(im2, im1_warp, 'blend'));
```

6. Evaluate the result on both image collections and write the report. To make the objective assessment of the performance (requirement for grade 5), the Matlab function `ginput` could be useful.

## References

- [1] G. Lippolis, A. Edsjö, L. Helczynski and N. C. Overgaard: *Automatic registration of multi-modal microscopy images for integrative analysis of prostate tissue sections*, BMC Cancer, **13**:408, 2013 (Open Access).
- [2] D. G. Lowe: *Distinctive Image Features form Scale-Invariant Keypoints*, International J. Comp. Vis. **60**:2, 91–110, 2004.
- [3] M.A. Fischler and R.C. Bolles: *Random sample consensus - a paradigm for model-fitting with applications to image-analysis and automated cartography*, Comm. of the AMC **24**(6), 381–395, 1981.
- [4] Horn, B. K. P., Hilden, H. M., & Negahdaripour, S. *Closed-form solution of absolute orientation using orthonormal matrices*. Journal of the Optical Society of America A: Optics and Image Science, and Vision, **5**(7), 1127-1135, 1988.