NON-RIGID REGISTRATION FOR STAINED HISTOLOGICAL SECTIONS OF ATHEROSCLEROTIC ARTERIES

M. Auer
Computational Biomechanics
Graz University of Technology
Schiesstattgasse 14-B, A-8010 Graz
e-mail: ma@biomech.tugraz.at

P. Regitnig
Institute of Pathology
Medical University Graz
Auenbruggerplatz 25, A-8036 Graz
e-mail: peter.regitnig@uni-graz.at

G. A. Holzapfel*
Computational Biomechanics
Graz University of Technology
Schiesstattgasse 14-B, A-8010 Graz
e-mail: gh@biomech.tugraz.at

ABSTRACT
An automatic computer-based analysis of histological sections, which are differently stained, requires that they are related to each other. Histological images are, in general, accompanied with several artifacts and different contrasts, which require non-rigid registration. In this proceedings we present a novel hierarchical non-rigid registration algorithm able to align images, which contain small image artifacts. The proposed algorithm is decomposed into a fast coarse rigid registration step and a slower, but finer non-rigid step using the elastic thin-plate spline interpolation. Accuracy tests, performed for 20 histological images obtained from human arteries, have shown that the error measure is acceptable, and that the image noise does not cause any problem. The algorithm can be applied to various multi-contrast elastic registration problems in medical imaging.

KEYWORDS
Medical Imaging, Registration, Histological Image, Artery, Atherosclerosis.

1 Introduction
In several clinical diagnostic procedures, it is important, to compare images of the same topological location, in order to detect certain changes during a certain time period. The documentation of, for example, tumor growth during therapy over time is of crucial clinical importance. The images may be obtained from different acquisition methods and include often artifacts. Manual analyses of such sections would be time consuming, prone to errors and tedious if many samples are to be investigated. Therefore, more and more image diagnostic procedures are performed by machine and complex computer algorithms, which are sensitive to artifacts and which require that the images are related to each other. For a meaningful and objective comparison of images these artifacts must be removed and the images be related to each other. The process which tries to align the images is called registration in digital image processing.

The great majority of methods consider rigid geometric transformations, because for many applications it can be assumed that only rigid-body motions appear. A variety of applications of registration methods may be found in medical imaging, see, for example, [1], [2], [3]. Typical image acquisition methods are, for example, MR (Magnetic Resonance), CT (Computer Tomography), PET (Positron Emission Tomography), US (Ultra Sound) and IVUS (Intra Vascular Ultra Sound). These methods provide digital images, which can be used directly for subsequent computer-based analyses. However, this is not the case for images which are obtained from stained histological sections. For example, for computerized methods, which are developed to segment the vessel wall and the plaque architecture by means of high-resolution MRI, histological analyses and registration methods are necessary to identify the underlying tissue type and the morphological structure of each individual tissue component [4]. The three-dimensional geometries of the different tissue components of diseased arterial walls are then used as input data to simulate, for example, the process of angioplasty on a computational basis [5].

Digital image acquisition of histological sections requires a specified microscope and, in most of the cases, this is performed by manual interaction. Hence, the quality of the digital images depends on several parameters, which vary from one recording to another. Therefore, the images are not well-suited for comparison purposes, and many general registration methods fail. Since elastic deformations in the specimens will occur during the preparation, rigid registration methods cannot be applied anymore and a non-rigid method is required. In this proceedings we present a methodology for an automatic and robust non-rigid registration process of histological sections that enables a subsequent machine-driven analysis of the images.

2 Materials and Methods

2.1 Specimen and Image Acquisition
Stenotic iliac arteries with macroscopic visible high grade atherosclerotic changes including stiff plaque components were harvested during autopsy. After alcohol fixation the
and the observed section. The slides were stained with a scope at a resolution of B correction [6]. It is important to note that there are still ar-

tifacts present in the intensity-corrected images due to the proces,

preparation, staining and cutting procedures (Fig. 1 (a), (b),

together with the following section.

2.2 Registration Methods

Many different registration methods exist in the literature, see, for example, [7], [1]. The registration process is an optimization process, where one image I, is transformed by a geometric transformation T into an image I’ in such a way that some ‘similarity measure’ SIM between some reference image I_ref and the transformed image I’ is optimized. As a result we obtain appropriate parameters T_opt by means of a numerical optimizer OPT. A registration algorithm can then be classified based on the used geometric transformation and the used similarity measure.

2.3 Geometric Transformations

Let us consider an image I as a finite two-dimensional do-

mains, where each point (x, y) is associated with a gray level. Then a geometric transformation T of an image can be described by I’(x’, y’) = T[I(x, y)], where (x’, y’) and I’ denote the transformed point and the image, respectively.

In the present proceedings, we use a transformation, which is based on elastic Thin-Plate Splines (TPS) [8]. Such elastic transformations are more general in contrast to rigid, affine, projective or curved transformations. The TPS transformation is defined as

\[ x' = Ax + t + \sum_{i=1}^{n} t_i U(||x_i - x||), \]

where the points, characterized by the position vectors x_i, i = 1, \ldots, n, can be considered as control points. These points can, for example, be a set of registered points. The parameter values summarized in A and t represent the linear affine part of the transformation, and the parameters [t_i] = [t_{x_i}, t_{y_i}]^T are the weights of the non-linear radial interpolation function, U say, which is defined for any scalar r by U(r) = r^2 \log(r^2). The parameters t_i, t and A can be obtained by means of eq. (1) using the known transformation of n sample points x_j, j = 1, \ldots, n to their corresponding points \hat{x}_j, j = 1, \ldots, n. For a detailed algebraic treatment see [8]. The purpose of the registration algorithm is now to find the appropriate parameters. To do this we compare the reference image I_ref with the transformed image I’ and calculate a so-called measure of similarity, which defines the quality of matching, as discussed in the following section.

2.4 Similarity Measures

In the present communication we prefer to use the mutual information [9] as a similarity measure, which is appropriate to compare images with different contrasts, and allows an accurate and robust registration of images infected with artifacts.

The normalized mutual information Y, introduced by STUDHOLME et al. [3], is defined by \[ Y(A, B') = \left( H(A) + H(B') \right) / H(A, B'), \]

where A denotes the reference image and B’ is obtained from the image B to be registered by a geometric transformation T determined by maximizing Y. \[ H(A, B) \]

1 denotes the joint entropy, while H(A) and H(B) are the Shannon entropies, which are measures for the amount of information. The entropies can be estimated from the normalized intensity histograms [3] of the images A and B’. To avoid difficulties in finding a maxima in the parameter-space for small sample data, we propose to use the modified joint probability [10], which combines the probability of the transformed image B’ and the probability of B before transforming to compute H(A, B’). This leads to a modified normalized mutual information \[ \hat{Y}(A, B’). \]

2.5 Numerical Implementation of the Hierarchical Approach

To ensure high robustness and efficiency of the registration algorithm a hierarchical approach is the method of
choice. A hierarchical approach is accurate and accelerates the registration process by reducing the computational costs. In particular, we develop a hierarchical registration algorithm by basically using a fast coarse rigid registration step, which is followed by a slower, but finer non-rigid registration step.

We begin our description with the coarse registration step. For that we use an image pyramid, which is a multi-resolution representation of an image. We start with the registration by using a rigid transformation, which allows only translation and rotation, of the image with the smallest image resolution. The optimal parameters \( T_{\text{opt}} \) are then to be found during the registration by maximizing \( \mathcal{Y} \) and by using Powell’s multi-dimensional directional set method, which are used for the starting parameters for the registration of the next pyramid level.

Now we continue our description with the non-rigid registration step. During this second step the achieved resulting image is split sequentially into four sub-images. The sub-images are registered from top to down. Before proceeding it is necessary to determine an image mask from the images which are used for the starting parameters for the registration of the sub-images of the background, and, therefore, not used during the registration by maximizing \( \mathcal{Y} \) and by using Powell’s multi-dimensional directional set method, which are used for the starting parameters for the registration of the next pyramid level.

In the following section we present a meaningful performance test, which works without using ground truth data. We analyze a set of digital-acquired images selected from different histological stains and different specimens. The computed mean squared error of the pixel disparities between aligned images is used as a measure of accuracy. The influence of Gaussian noise with different standard deviations is investigated. Finally, certain limitations of the used mutual information measure, the TPS-interpolation and the investigated sample material are demonstrated.

### 3 Results

In the following section we present a meaningful performance test, which works without using ground truth data. We analyze a set of digital-acquired images selected from different histological stains and different specimens. The computed mean squared error of the pixel disparities between aligned images is used as a measure of accuracy. The influence of Gaussian noise with different standard deviations is investigated. Finally, certain limitations of the used mutual information measure, the TPS-interpolation and the investigated sample material are demonstrated.

#### 3.1 Performance Tests

Testing the accuracy of registration algorithms is not an easy and straightforward task since ground truth data are not available in most of the cases, or they are even impossible to get. In the present communication we tested the implemented algorithm independently with respect to (i) accuracy, and (ii) resistance to noise and missing data.

As far as the accuracy test is concerned, we split a specific shading-corrected reference image \( A \) into a red, green, and blue image, denoted as \( A_r, A_g, A_b \), and the associated shading-corrected image \( B \) to be registered into \( B_r, B_g, B_b \). Then, we register each image pair \((A_r, B_r), (A_g, B_g), (A_b, B_b)\) independently. We assume that the resulting images, denoted as \( \hat{B}_r, \hat{B}_g, \hat{B}_b \), are registered correctly. They are considered to be the ground truth data.

<table>
<thead>
<tr>
<th>( \sigma )</th>
<th>( e_{\text{pre}}, \text{eq.(2)} )</th>
<th>( e_{\text{post}}, \text{eq.(3)} )</th>
<th>( e_{\text{pre/post}}, \text{eq.(4)} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.768</td>
<td>0.771</td>
<td>0.798</td>
</tr>
<tr>
<td>0.5</td>
<td>0.881</td>
<td>0.893</td>
<td>0.881</td>
</tr>
<tr>
<td>1.0</td>
<td>0.952</td>
<td>0.961</td>
<td>0.993</td>
</tr>
<tr>
<td>2.0</td>
<td>1.053</td>
<td>1.139</td>
<td>1.376</td>
</tr>
</tbody>
</table>

Table 1. Measures of error \( e_{\text{pre}}, e_{\text{post}}, e_{\text{pre/post}} \) according to eqs. (2)-(4) with different amount of noise \( \sigma \).

To test the resistance to noise we add Gaussian noise with the standard deviations \( \sigma = 0.0, \sigma = 0.5, \sigma = 1.0, \sigma = 2.0 \), and a mean value of zero, to all the images \( A_r, A_g, A_b, B_r, B_g, B_b \). In order to test the resistance to missing data, in addition, we cut out a number of randomly distributed patches from the digital-acquired image (about 25% of the image area). With these artificially acquired images the accuracy test, as described above, is performed again. For the accuracy tests we have used 20 acquired images.

These results demonstrate the accuracy of the algorithm also to noisy images. The SNR (Signal to Noise
Ratio) in the images acquired by a CCD-camera (σ < 0.5) is much better than the critical SNR tested in the performance tests and, therefore, does not cause a problem in our registration.

3.2 Limitations

A disadvantage of the similarity measure, when based on mutual information, is that the similarity measure does not work in a satisfying manner for image areas, which contain low information, as, for example, for homogeneous areas. In order to avoid outliers, induced by low image information or image artifacts, which may result into undesirable local deformations (during the TPS transformation) we have to make use of constraints. If the obtained parameter values (\( t_x, t_y \)) of one sub-image deviate too much from the parameter values of the neighbor sub-images, then these values may be considered as outliers, this parameter values can then be estimated by interpolating the values of their neighbor sub-images.

Problems in registration may also arise when sample materials are used with artifacts. For example, tissue segments can be overlapped or missing. This type of artifact is produced during the cutting process of the specimen and cannot be registered by using a continuous transformation function.

4 Conclusion

In this proceedings we proposed an accurate and powerful non-rigid method to register histological sections. We employed a hierarchical approach, which accelerates the registration process by reducing the computational costs. The final goal of the proposed registration method is to analyze a certain section of a specimen by using the information such as the heart muscle or the breast. It is straightforward to extend the proposed algorithm to 3D.

Acknowledgement. Financial support for this research was provided by the Austrian Science Foundation under START-Award Y74-TEC.

References