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Registration Decisions during 3D Medical Volume Reconstructions from Confocal Laser Scanning Microscopy

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We address the problem of optimal registration decisions during 3D medical volume reconstruction and their impact on anticipated accuracy of aligned images, uncertainty of obtained results, repeatability of alignment, and computational requirements. The registration decisions include image size used for registration, transformation model, invariant registration feature (intensity or morphology), automation level, evaluations of registration results (multiple metrics and methods for establishing ground truth), and assessment of resources (geographically local or distributed computational resources and human expertise). Our goal is to introduce data-driven mechanisms for evaluating the tradeoffs between accuracy of 3D volume reconstructions and registration variables. In this work, we present links between registration decisions and 3D reconstruction results in terms of accuracy, uncertainty, consistency and computational complexity characteristics. We also illustrate examples of optimizing image size and transformation model by using web-enabled software tools. We have built these software tools to enable geographically distributed researchers to optimize their data-driven registration decisions. The software is based on using web services and is available to the general community.

Key words: Computational Methods for Microscopy, Image Simulation and Image Processing Techniques

1. Introduction

The problems of 3D volume reconstruction and medical cross section registration have been approached by an overwhelming number of researchers over the past several decades [5][6][9][10][11][13] and remain still a very challenging problem. There are several survey papers about registration approaches that include selection of registration variables based on user decisions [2][8][14]. In this paper, we focus on optimal selections of registration variables that are inherent parts of 3D volume reconstruction process. In our work, the 3D volume reconstruction problem is defined as a registration problem without fiduciary markers [8]. The goal of 3D reconstruction is to form a high-resolution 3D volume with large spatial coverage from a set of spatial tiles (small spatial coverage and high-resolution 2D images or 3D cross section volumes. Our objective in this work is to describe the impacts of registration decisions made during 3D volume reconstruction and outline data-driven simulations that can support optimal registration decisions.

Based on our knowledge, there has been limited work on understanding accuracy, uncertainty, consistency and computational complexity characteristics of 3D volume reconstruction and their relationships to registration decisions. The past work usually addressed only certain aspects of registration decisions, for example the choice of transformation models [8], the combination of invariant registration features [18], the image data quality evaluation metrics [15], the choice of shape metrics [19], or the process of geometric (spatial registration related) and radiometric (intensity related) adjustments [12]. The past work has originated primarily from the computer vision community when tackling the problem of matcing and alignment from points and frames while modelling rigid motion of objects. For example researchers, such as Pennec and Thirion [16][17] have developed a theoretical model defining the relationship between uncertainty of a rigid transformation applied to a set of 3D points or 2D frames and the registration accuracy. However, the model is defined for only a very small subset of typical registration decisions. A researcher performing 3D volume reconstruction is usually facing registration decisions about (1) image size used for registration, (2) transformation model (e.g., rigid, affine or elastic), (3) invariant registration feature (intensity, morphology or a combination of the two), (4) automation level (manual, semi-automated, or fully-automated), (5) evaluations of registration results (multiple error metrics and methods for establishing ground truth), and (6) assessment of resources (geographically local or distributed computational resources and human expertise). Thus, there is a need to provide a mechanism for making optimal registration decisions, as well as to build a good understanding of the decision impacts on registration accuracy. Our work addresses this need by developing data-driven web-enabled analyses to support optimal registration decisions during 3D volume reconstruction. The analyses can be viewed as trade-off studies of multiple registration decisions in terms of registration accuracy.

This paper describes data-driven optimization approaches to four registration decisions including image size selection, rigid or affine transformation model, intensity or morphological invariant feature selection, and manual (pixel-based) or semi-automated (centroid-based) automation level. The reason for using data-driven approaches lies in the diverse appearance of objects/specimens of interest, and the wide variations of specimen preparation techniques, imaging modalities, and specific instrumentation characteristics just to name a few. These variations are extremely difficult to model analytically with any generality whatsoever. Thus, we describe the problem by presenting the links between registration accuracy and variables. Then, we present examples of data-driven simulations that support optimal selection of registration variables.

The paper is organized as follows. First, we present the CLSM imaging and data in Section 2. Next, we describe registration decisions in Section 3.1 and their impacts on (a) anticipated accuracy of aligned images, (b) uncertainty of obtained results, (c) repeatability of alignment, and (d) computational requirements in Section 3.2. We use illustrations of data-driven analyses in order to demonstrate approaches to gaining better understanding of the relationships between registration variables and the quality of 3D reconstruction results. Section 4 summarizes our work.

2. Confocal Laser Scanning Microscopy Data

In order to demonstrate the issues related to selecting registration variables, we consider 3D volume reconstruction of blood vessels in histological sections of uveal melanoma [3] from paraffin-embedded serial sections labeled with antibodies to CD34 and laminin and studied by confocal laser scanning microscopy (CLSM) imagery [1][7]. The set of spatial tiles is acquired by CLSM and consists of images that came from one cross section (same axial coordinate) in different lateral coordinates or multiple cross sections of a 3D volume (different axial coordinates). The 3D volume reconstruction objectives are to register (stitch together) spatial tiles that came from the same cross section, i.e., image mosaic, and align spatial tiles from multiple cross sections with the end use for visual inspection or quantitative analysis [1]. An overview of processing steps to create a visual display of a specimen in-silico is illustrated in **Figure 1**.

When the image tiles are stitched (mosaicked), one typically assumes that:

- Identical frame indices in multiple image tiles would have the same physical axial depth if they came from the same physical cross section.
- Structural deformation is negligible in overlapping regions of spatially adjacent image tiles when compared at the same depth (e.g., frame index).
- Image tiles are acquired by only translational motions of the microscope stage to capture high resolution images across entire specimen.

Similarly, when images are aligned, several properties of acquired images could be assumed:

- All physical sections are assumed to be parallel to the same two-dimensional plane.
- During slide preparation, tissue slide could be only rotated, translated, and slightly sheared.

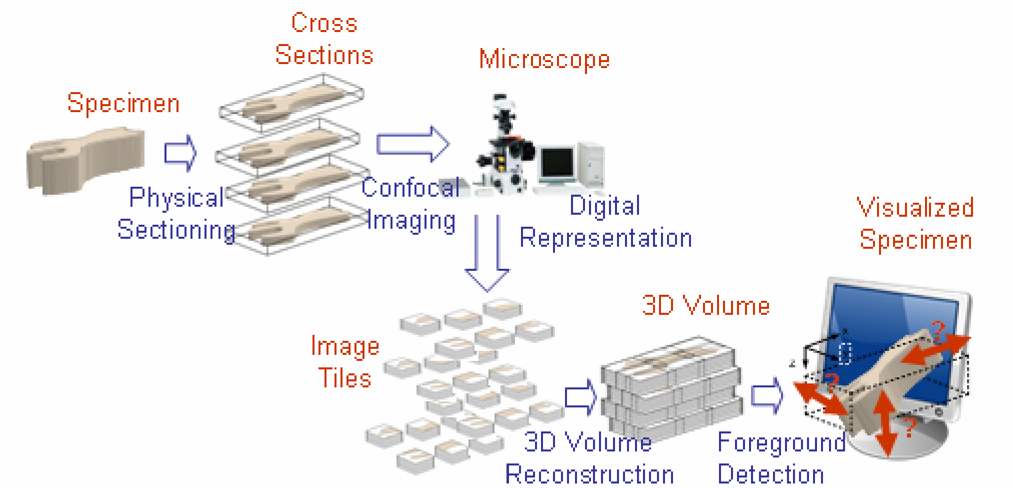

Figure 1. An overview of processing steps to create a visual display of a specimen in-silico that includes 3D volume reconstruction. The red arrows denote possible errors and uncertainties due to the sequence of processing steps.

3. Registration Decisions

The overview of most common registration decisions is provided in Figure 2. The list of registration decisions was introduced in Section 1 (image size, transformation model, invariant feature, automation level, evaluations of registration results, and assessment of resources). In Figure 2, one should view black arrows as possible decision outcomes during the registration process and hence any possible combination of user decisions would characterize the obtained registration result. Some of the decisions could be easily expanded, e.g., other transformation models. Other decisions could be elaborated, such as methods for establishing ground truth could be classified into visual inspection, comparison with ground truth data, or measuring the degree of deviations from assumed data model. The purpose of Figure 2 is to present basic registration decisions rather than an exhaustive list of possible decision selections.

The user-driven registration decisions define the complexity of (a) registration model, (b) model parameter estimation, (c) registration computations to be performed and (d) evaluation strategy. For example, the case of a manual registration (alignment) of two image sub-areas containing a few features (visually salient pixel arrangements) using rigid transformation (rotation and translation) by overlaying two sub-areas and visually assessing the quality of alignment would be considered as a low complexity registration. It would use the simplest transformation model (rigid), subjective parameter estimation (visual), no computation (manual), and visual method for evaluating registration quality. In contrast, the case of a fully-automated alignment of two large images containing several millions of features using affine transformation (rotation, translation, scale and shear) by exhaustively evaluating the range of affine transformation parameters based on invariance of intensity (e.g., using normalized cross correlation or normalized mutual information) would be considered as a high complexity registration. Although there exist fast implementations of the intensity-based cross correlation methods, e,g., pyramid-based techniques, these methods cannot be applied in the case of CLSM due to large intensity heterogeneity. Registration uses consistent parameter estimation by evaluating invariance of intensity, consuming significant computational resources and performing registration quality evaluations using mathematically defined metrics and based on a set of assumptions about data.

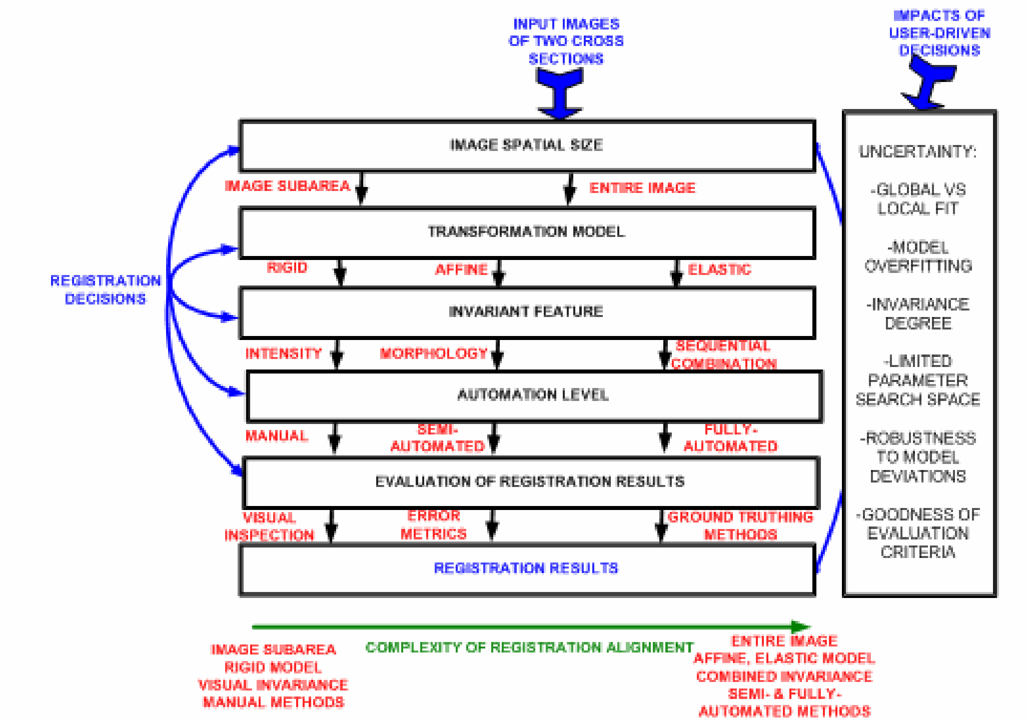

Figure 2: Registration decisions and their impact on registration results.

3.1. Variables for Registration Decisions

Image Spatial Size: From a medical user view point, one would like to obtain 3D volume reconstructions over a large spatial area at a high spatial resolution, and with the best possible visual alignment of all salient image features. Let us assume that the visual alignment of salient image features is measured by a normalized correlation coefficient. Then, our goal is to provide data-driven analysis of input data to understand the tradeoffs between these two conflicting requirements, such as to maximize aligned image area and its measure of alignment goodness (e.g., normalized correlation coefficient) with other images. In order to compute a data-driven dependency between normalized correlation metric and image sub-area size, at least two frames have to be aligned otherwise other registration unknowns would be inseparable from the variables under our scrutiny. In the case of CLSM, we can establish the data driven dependency by using any two frames from one stack of images. The assumptions are that these two selected frames are representatives of the two frames from two adjacent cross sections without any distortion due to specimen slicing, and the frame-to-frame morphology and intensity changes in the selected frames from one sub-volume are similar to those in the adjacent sub-volumes.

Figure 3 (a) shows a method supporting registration decisions by uploading two images from a 3D volume, selecting a sub-area of interest, and then it reports a visualization of a correlation coefficient as a function of incrementally increasing sub-area size, as shown in **Figure 3**(b) The correlation coefficient is used as registration accuracy metric in this case. If two identical frames would be compared then the correlation metric value equals to one. The frames that would be completely dissimilar would lead to the value of zero.

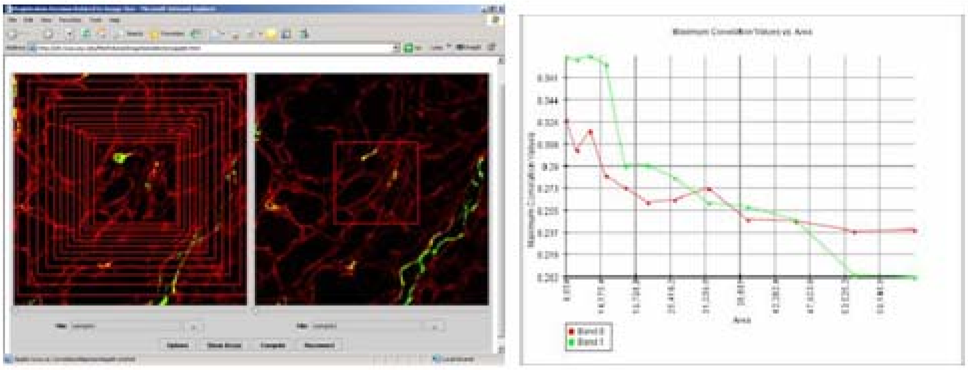

Figure 3 : Supporting registration decisions about image sub-area size. Left – two image frames from the same CLSM stack with the overlay of all considered sub-areas. Right: A graph of the normalized correlation as a function of sub-area size (two curves refer to two correlations of green and red bands in the original image frames).

Transformation Model: Let us assume that a class of CLSM images acquired from similar tissues can be perfectly registered by an unknown transformation with N parameters. The N parameters define the order of transformation model. It is well known that a higher order transformation model than N would lead to data overfitting while a lower order transformation model than N would lead to large registration errors. In our case, overfitting would lead to distortions in the transformed image that could never happen in the original cross sections although the registration error would be small and indicate a good alignment. Using lower order transformation model might likely never satisfy the transformation error defined as user requirements.

The tradeoffs between transformation model complexity and registration error are usually resolved by practitioners based on a good understanding of medical specimen preparation. For example, for imaging cross sections of solid and hard specimens, rigid transformation might be appropriate (rotation and translation). If cutting the specimen introduces additional shear and scale changes, then affine transformation would be appropriate (rotation, translation, scale and shear). Our goal is to provide data-driven analysis of input data to understand the tradeoffs between these two conflicting requirements, such as to minimize transformation model complexity and registration error.

Figure 4 shows a method allowing uploading two images to be registered, manually placing disks of variable size over visually matching features, and selecting rigid or affine transformation to compute the registered image pair. The reported registration transformation error is shown in **Table 1**, and the level of distortion in the overlaid registered images with registration disks are shown in **Figure 4** for aiding the choice of registration model.

Table 1: Summary of registration errors obtained using rigid and affine transformation models for the same set of four matching pairs of points shown in Figure 4. The residual errors X, Y and (X, Y) are computed as a sum of squared differences between the transformed coordinates of image 1 and the user chosen coordinates in image 2. The original residual error (X, Y) before any transformation was equal to 12.782.

Transformation Model\Residual Error	Error X	Error Y	Error (X,Y)
Rigid	1.528	1.802	1.225
Affine	0.927	1.581	0.935

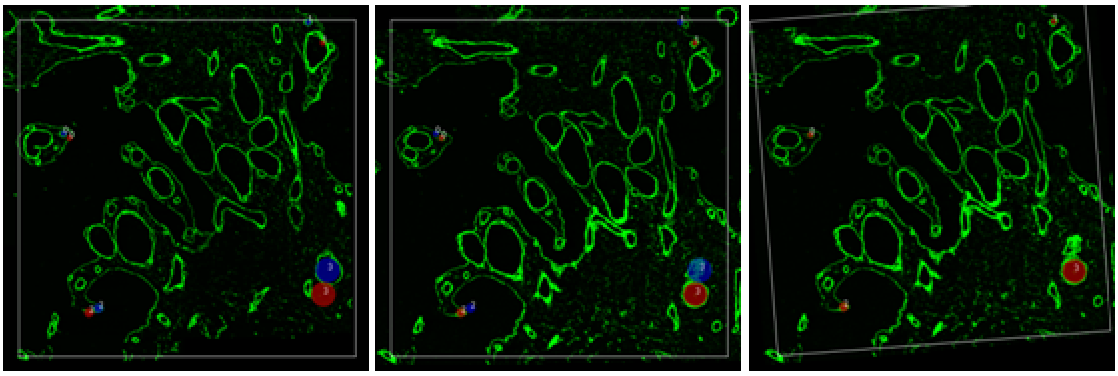

Figure 4: Transformation model selection. Target image with blue discs aligned with image features (left), source images (to be transformed) with red discs aligned with image features (middle) and transformed source image using an affine transformation model estimated based on the pairs of blue and red disc centroids (right).

Invariant Registration Feature: Registration features can span a wide range of image and volume attributes. For instance, image attributes would include pixels, edges, contours, image segments and their characteristics (centroids, sizes, etc.), and homogeneous or texture intensity profiles. Volume attributes would characterize 3D structures in each CLSM sub-volume, such as centroid trajectories of cylindrical structures, surface and volume descriptors, or intensity profiles of structures' surfaces and volumes. In general, these registration features can be divided into morphological (related to 2D or 3D shape) and intensity (related to fluorescent magnitude) attributes. One would like to understand which category of registration features is more invariant for a particle set of images and the degree of uncertainty introduced by making an assumption about feature invariance.

The challenge lies in defining and comparing metrics evaluating the degrees of change. One could choose the normalized correlation metric as a statistical measure of intensity similarity for two images of the same spatial size. In the case of morphological features, the degree of morphological distortion is related to shape, and traditionally shape characteristics have been obtained (a) from local geometry called landmarks, (b) from a set of sampled boundary points, (c) by boundary-representing basis function coefficients, or (d) by hybrids of boundary and other curve loci with landmarks, and other sampling schemes, as overviewed in [11]. Given the most appropriate choice of one morphological and one intensity feature metrics, it is not obvious how to compare the metric values. The current approaches usually select one or the other metric, or a sequential application of the two metrics.

Automation Level: The goal of this step is to decide the level of automation based on the registration accuracy, computational resources, and geographic locations of needed 3D volume reconstruction expertise. We approached this problem as follows: The evaluations of registration accuracy at multiple levels of automation require involvement of human subjects and careful preparation of ground truth (baseline) data. We have conducted a user study in the past using manual or semi-automated registration techniques [20] and decided to use the semi-automated (segment centroid based) automation level. By web-enabling the developed software, other researchers can perform similar studies to the study published in [20] and support their data-specific decision about the level of automation.

The problem of accessing necessary computational resources and combining geographically distributed expertise was solved by developing a web service-based mechanism for registration that provides access to image data from the location with 3D volume reconstruction expertise and performs computation at the location with computational resources. Other researchers would be able to use the same prototype developed for our 3D volume reconstruction and evaluate their data-driven decision about an appropriate automation level.

Evaluation of Registration Results: Ideally, one would like to perform data-driven evaluations with all possible registration accuracy metrics, all registration methods and approaches, and with unlimited computational resources. Each researcher would assess his/her data variability and quality with respect to the 3D reconstruction task under multiple registration assumptions and without computational constraints. While this is currently not feasible, a limited understanding might be obtained by following the data-driven evaluations presented above.

3.2. Impacts of Registration Decisions

Registration decisions during medical cross section alignment have a great impact on (1) anticipated accuracy of aligned images, (2) uncertainty of obtained results, (3) repeatability of alignment, and (4) computational requirements. As illustrated in Figure 2, the registration decisions affect (a) spatial distribution of registration error (global vs. local registration), (b) registration error composition (model over-fitting, mosaicking x-y error, and alignment z error), (c) computational requirements on registration (invariance assumption and its degree of freedom, model complexity, search space to optimize parameters) and (d) validity of the obtained results (robustness of a registration model with respect to data deviations, quality of evaluation criteria).

Alignment Accuracy: The accuracy of aligned images can be measured either by visually inspecting anticipated structures or by defining quantitative metrics to evaluate accuracy with respect to ground truth data (or a data model defined a priori).

Uncertainty of Alignment Results: Multiple registration decisions impact alignment uncertainty due to the following discrepancies between registration models and actual data distortions being compensated by the models: (1) the tradeoffs between global or local registration fit (image spatial size decision), (2) the issues of transformation model over-fitting (transformation model decision), (3) the degree of assumed intensity and morphological invariance (feature invariance decision), (4) the size of parameter search space and the algorithmic robustness to model deviations (automation level decision), and (5) the goodness of evaluation criteria for a registration problem. These uncertainties are very difficult to evaluate analytically and are very much data specific. Data-driven evaluation software tools might provide computer assisted approach to gain an insight about the anticipated result quality. The future approaches to uncertainty modelling will likely combine theoretical work on defining appropriate metrics (e.g., normalized cross correlation or normalized mutual information [4] for intensity) and the experimental work on designing data-driven methods.

Repeatability of Alignment: Repeatability can be viewed as the consistency of alignment results obtained using multiple methods and processes [21]. The alignment processes usually include humans and computer algorithms. In general, alignment repeatability varies depending on the level of automation, registration methods and the complexity of human input. The higher automation level leads to better convergence of registration methods to global extreme [18]. In the semi-automated case, the less complex human input will lead to higher alignment accuracy.

The repeatability issue is usually addressed by performing studies using human subjects [20]. The studies are based on either a class of images acquired by the same imaging techniques and from similar specimens or a class of synthetic images that simulate different degrees of deviations from a registration model. Algorithmic repeatability is often evaluated together with its robustness to test that the algorithm avoids getting trapped in local minima, and can reliably find the best global minimum in complex landscapes defined by objective functions. Similarly, any measured repeatability due to automation is based on (a) making assumptions about acquired data, for instance, assuming feature invariance, (b) allowing only a subset of possible registration transformations (model constraints), or (c) searching only a subspace of possible transformation parameters. Thus, usually the robustness and accuracy of cross section alignment is decreasing with an increasing level of automation for data sets deviating from the automation model and violating the registration assumptions.

Computational requirements: The computational requirements of alignment are directly proportional to the level of automation, the complexity of transformation model, and to the search space of transformation parameters. Furthermore, computational requirements for accommodating all researchers interested in using the same software with multiple registration tasks also have to be addressed.

4. Summary

In this paper, we addressed the problem of optimal registration decisions during 3D medical volume reconstruction. The registration decisions of interest included (1) image spatial size, (2) transformation model, (3) invariant registration feature, and (4) automation level. We demonstrated two examples how to analyze the decisions (1) and (2) using data-driven analyses. We have also built software tools for geographically distributed researchers to optimize their data-driven registration decisions by using web services and supercomputing resources. The software tools are available to the general community at http://isda.ncsa.uiuc.edu/MedVolume/.

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