Virtual Double Staining Using Elastic Image Registration

Multiple Stainings in the Age of Digital Pathology

Double staining techniques are often used to analyze the colocalization of different antibodies on a tissue sample. In this process the first stain is applied to the tissue which is then washed before the second stain is applied likewise. However, **multiple staining of tissue has disadvantages**. Different stains can interfere and crossreact making the analysis more difficult. Even worse, the multiple chemical treatment of the tissue can lead to tissue loss.
With the increasing availability of whole slide scanners, high resolution scans of histological samples can be obtained and analyzed digitally. **Adjacent, differently stained slides can be combined in an analysis, which is often referred to as virtual double staining**.
However, **spatial correspondence between adjacent slides is lost during the sectioning process** and various distortions are introduced to the tissue, such that a naïve overlay of the slides is not possible.

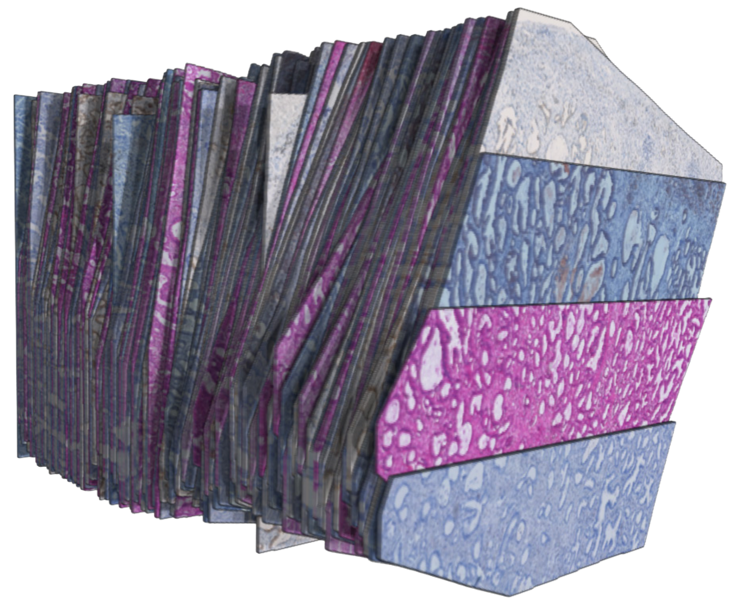

Figure 2: Reconstructed volume around a user-chosen point of interest using elastic registration. First four slides are cropped to better see the four different stains and the well aligned structures.

References:

1. Haber, Eldad, and Jan Modersitzki. 2006. "Intensity Gradient Based Registration and Fusion of Multi-modal Images." Ed. Rasmus Larsen, Mads Nielsen, and Jon Sporring. *Methods of Information in Medicine 46 (3): 292–299.*
2. Papenberg, N., J. Modersitzki, and B. Fischer. 2008. "Registrierung Im Fokus." *Bildverarbeitung für die Medizin 2008: 138–142.*

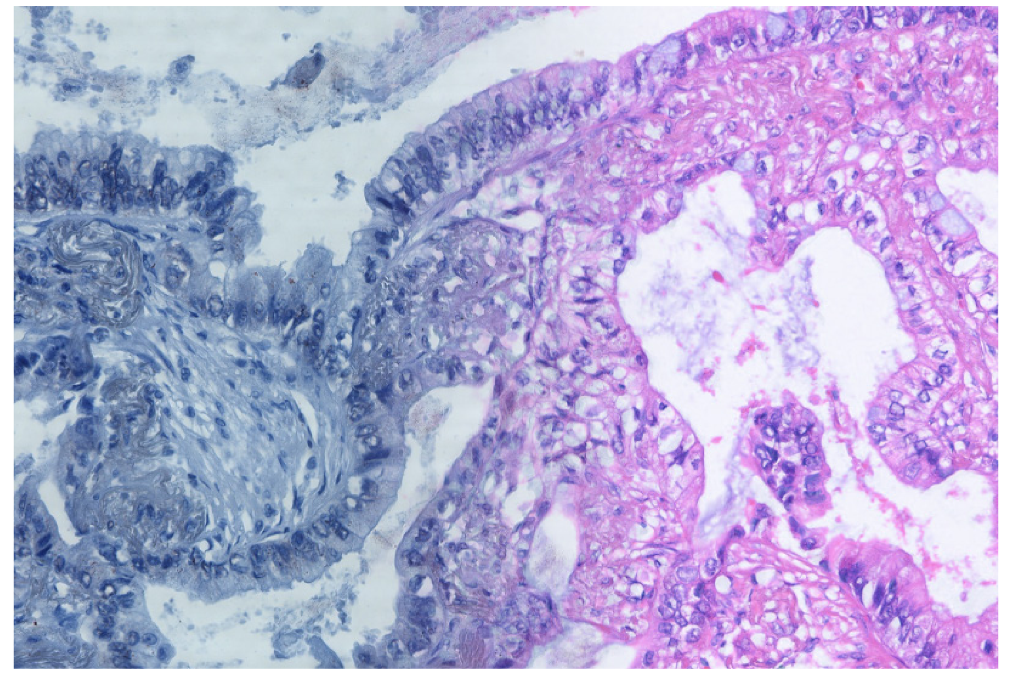

Figure 1: Virtual double staining of a KL-1 and the neighboring H&E stained slide using a fade over. The H&E slide is registered to the KL-1 and deformed accordingly.

The solution: Digital Processing

We present a method that **recaptures the spatial correspondence of neighboring tissue slides and corrects locally varying deformations using nonlinear image registration**. Limitations of globally linear transformations like rotation, translation, shearing, and scaling or combinations thereof are exceeded. A virtual double staining can be displayed as seen in Figure 1.

The obtained alignment enables us to **transfer deduced tissue properties** gained in one slide to regions of interest in a neighboring, differently stained slide. This process can substitute physical double staining.

Furthermore we use our method to **reconstruct the 3D structure** of a serially sectioned sample by consecutively aligning all slices, such as seen in Figure 2.

Elastic Image Registration

We present an image registration based method to **recapture spatial correspondence** between neighboring and differently stained slides. Our method consists of two main components

- a **preliminary coarse alignment** based on an affine registration
- a **nonlinear registration** to correct local, non-uniform deformations.

For both components, image registration can be formulated as an **optimization problem** with respect to a deformation y. The objective function

$$\mathcal{J}[y] = \mathcal{NGF}[y] + \mathcal{S}_{\text{elastic}}[y] \xrightarrow[y]{} \min$$

to be minimized consists of a **distance term** ($\mathcal{NGF}$) to measure image similarity and a **regularizing term** ($\mathcal{S}_{\text{elastic}}$).

The regularizer is only needed in nonlinear registration where the deformation model as such has a high number of degrees of freedom. In this case the **regularizer models elasticity**

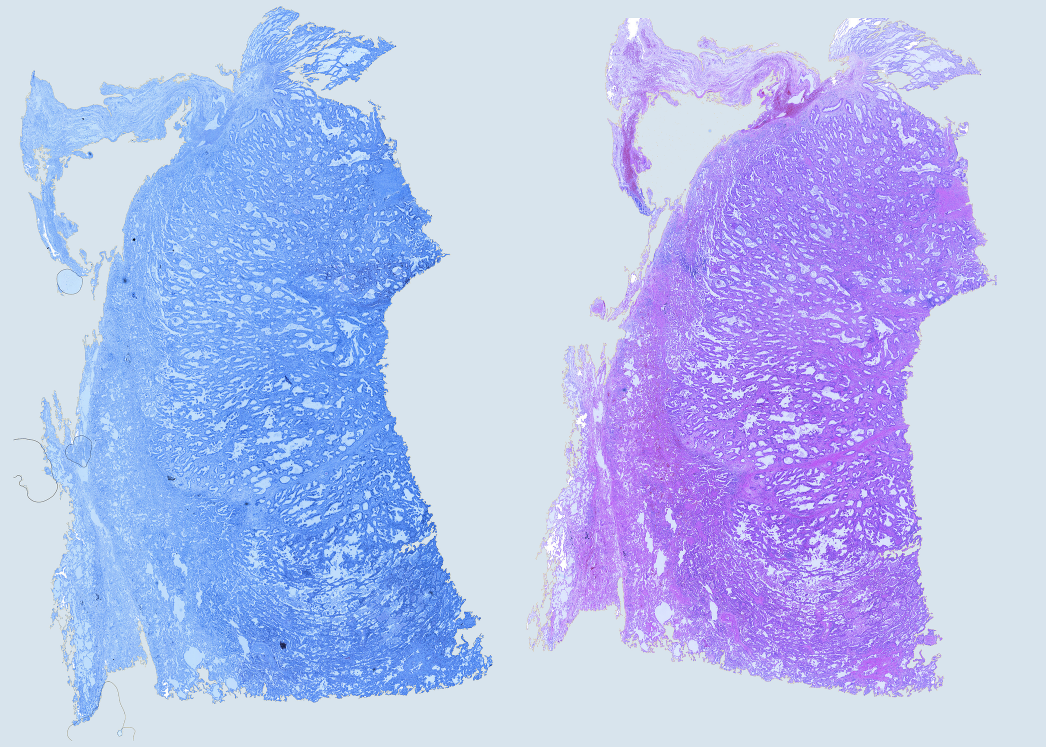

Figure 3: Whole slide images of a human lung tumor (NSCLC).

and controls the plausibility of the deformation. Minimizing the **Normalized Gradient Field (NGF)** [1] functional, we focus on aligning edges in the images and can thus cope with the multi modality of different stains.

Zooming In

The high amount of data in whole slide images is challenging for numerical image processing algorithms. To cope with this challenge, we propose a **zooming approach** that consecutively refines the resolution around a user-chosen point of interest.
After an initial registration of two whole slide images (see Figure 3), correspondence is established on a coarse level. We then continue to zoom in around a specified point of interest similarly to [2]. While zooming in in terms of increasing the magnification level, we keep the number of pixels of the chosen image section constant. **In each zooming step, an affine registration is performed**, refining the result obtained at the previous step.
The zooming step is repeated until the desired image magnification is reached, **resulting in two affinely registered high resolution tiles from two adjacent whole slide images**. In order to correct local, non-uniform deformations in these image tiles, a **nonlinear, elastic registration** is performed based on the preliminary affine result. The full registration scheme is visualized in Figure 4.

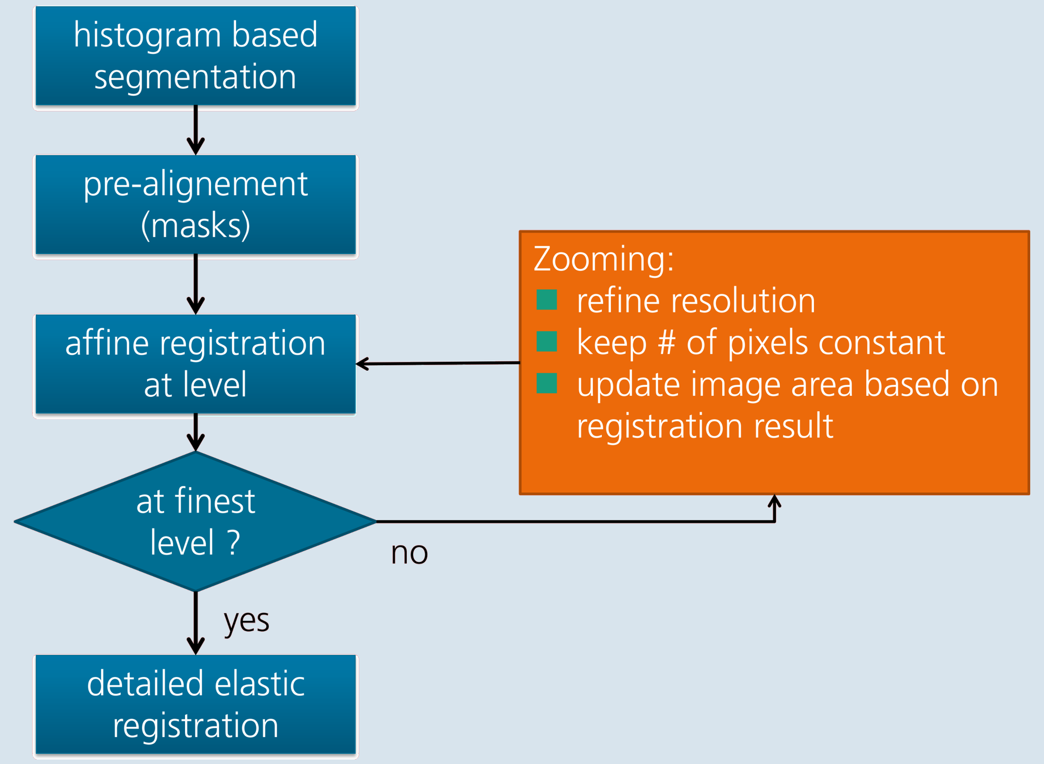

Figure 4: Flow chart displaying the zooming strategy.

Rapid Registration in High Resolution: Virtual Double Staining

We applied our registration method to adjacent slides of a human lung tumor (non-small cell lung cancer, NSCLC). In total **180 slides, stained alternatingly with CD31, H&E, Factor VIII, and KL-1, have been registered**.
Our method needs **less than 30 seconds per image pair** to perform the registration process and display the results, both in a magnification of **up to 40x**.
Thorough visual inspection of the deformed images at maximum magnification shows **accurate alignment of vessels and other morphological structures in all processed image pairs** (see Figure 5, left and Figure 6, right).
Visual inspection of the actual output of the elastic registration, the deformation, visualized by a grid plot (deformation grid shown in Figure 5, right) indicates two things: Firstly, the image is deformed a lot, but in a local environment only, which means that there are **local distortions, which are corrected by the registration.** Secondly, **the registration results in a smooth deformation** that does not show any foldings or implausible stretching or shrinking of the image.

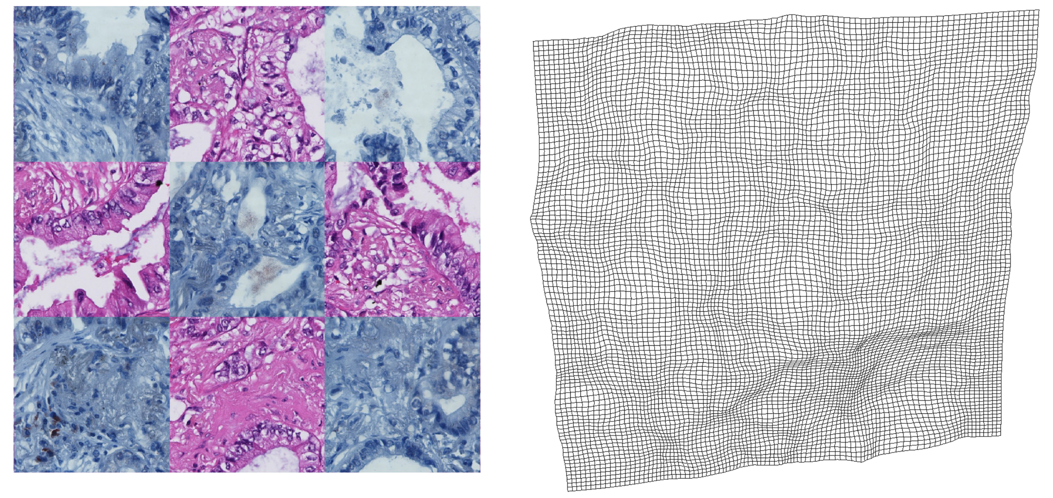

Figure 5: Left: Checkerboard visualization of a nonlinear registration result: KL-1 and deformed H&E stained slide. Right: Resulting deformation grid of the nonlinear registration showing a) the local, nonlinear distortions and b) the smoothness of the result.

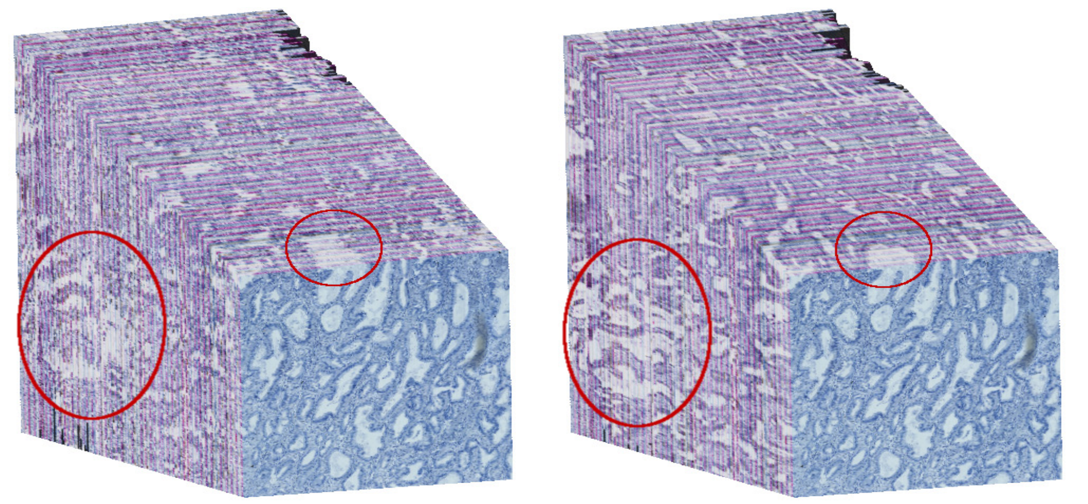

Figure 6: Reconstructed volume around a user-chosen point of interest using affine (left) and elastic (right) registration. Virtual clipping planes allow for examination of morphological structures across several slides.

Outlook: 3D Reconstruction

Our method can also be used to **analyze three dimensional structures** in a tissue block by sequentially registering pairs of serial sections.

A visual comparison of a purely affine and an elastically reconstructed volume, shown in Figure 6, indicates clearly the **benefit of the elastic registration:** Examining virtual cutting planes, morphological structures are much better visible after elastic registration.

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Contact the authors
judith.berger@mevis.fhg.de
johannes.lotz@mevis.fhg.de

Partners:

EUROPEAN UNION:
Investing in your future
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Judith Berger, Johannes Lotz, Janine Olesch, Mark Schenk
Kai Breuhahn, Benedikt Müller, Arne Warth
Niels Grabe, Bernd Lahrmann
Oliver Sedlaczek

Fraunhofer MEVIS Project Group Image Registration, Lübeck
Institute of Pathology, Heidelberg University Hospital
Tissue Imaging and Analysis Center, University of Heidelberg
Department of Radiology, University Hospital Heidelberg