Geometric Alignment
of 2D Gel Electrophoresis Images

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Abstract. A key technique for protein analysis is the geometric alignment of 2D gel electrophoresis (2-DE) images. We introduce a new hybrid elastic registration approach for 2-DE images, which is based on analytic solutions of the Navier equation. With this approach cross-effects in elastic deformations can be handled, which is important for the registration of 2-DE images. We have successfully applied our approach to register 2-DE gel images of different levels of complexity and have performed a quantitative evaluation of the results. We have also performed a comparison with a previous hybrid elastic registration scheme.

1 Introduction

2D gel electrophoresis (2-DE) is the method of choice for analyzing protein expression in the field of proteomics. By this technique a very large number of proteins can easily and simultaneously be separated, identified, and characterized. This is important for understanding protein function. 2-DE separates proteins based on their electrical charge and molecular mass resulting in a 2D array of dark spots (proteins) on a bright background (Fig. 1). Often a reference population is compared with a test population to analyze the effect of medication.

To accurately and quantitatively compare the generated 2D protein profiles, three main image analysis steps are required, namely, (1) geometric alignment of 2-DE images, (2) detection of spots, and (3) quantification of spots (e.g., shape, contrast). For a general overview we refer to [1]. In this work, we focus on the geometric alignment of 2-DE images. The core task is to find an optimal geometric transformation between image data, which is known as image registration. Due to complex physical and chemical processes the locations of proteins generally vary in different 2-DE images and therefore nonrigid or Elastic registration schemes have to be applied (for a survey see, e.g., [2]). Severe problems in the case of 2-DE images are that the spot shapes are largely different, that certain spots do not appear in corresponding images, and that spots may smear over a larger area as well as overlap each other.

Previous work on 2-DE image registration can be classified into landmark-based and intensity-based schemes. Landmark-based schemes first extract landmarks from the images and then compute a transformation based on these features (e.g., [3]). With intensity-based schemes the image intensities are directly

exploited to compute the transformation (e.g., [4]). Moreover, hybrid approaches that combine landmark-based and intensity-based methods have been proposed, which allow to combine advantages of both types of methods (e.g., [5, 6, 7]). However, with these approaches only coarse physical deformation models have been used, for example, based on thin-plate splines [7] or clamped-plate splines [6], or using a Gaussian function for regularization of the deformation field [5].

In this contribution, we introduce an approach for the registration of 2-DE images, which is based on an improved physical deformation model using analytic solutions of the Navier equation. In contrast to previous spline-based approaches for the registration of 2-DE images (e.g., [5, 6, 7]), with this approach cross-effects in elastic deformations can be handled, which is important in the case of 2-DE images. Moreover, our approach allows to include landmark correspondences to aid the registration in regions which are difficult to register using intensity information alone. In contrast to [8] where brain images have been registered, we here use a different energy functional where landmark correspondences are directly incorporated instead of requiring an additional deformation field.

2 Materials and Methods

We have developed a new hybrid approach for elastic registration of 2-DE images. This approach is based on an energy minimizing functional J_{Hybrid}, which comprises three energy terms to incorporate the intensity and landmark information as well as to regularize the deformation field $\mathbf{u}$, and reads

$$J_{Hybrid} = \lambda_I \, J_{\text{Data},I}(g_1, g_2, \mathbf{u}) + J_{\text{Data},L}((\mathbf{p}_i, \mathbf{q}_i), \mathbf{u}) + \lambda_E \, J_{\text{Elastic}}(\mathbf{u}) \quad (1)$$

To constrain the transformation to elastic deformations we chose the elastic energy J_{Elastic} according to the (force-free) Navier equation, which represents the regularization of the deformation field. Since the approach is based on the Navier equation, cross-effects in elastic deformations can be taken into account, i.e., a contraction in one direction leads to a dilation in orthogonal directions, which allows to model local deformations caused by the fixation of the gel.

To register images with minimal user interaction, the idea is to exploit the intensity information as much as possible. Since in our application the images
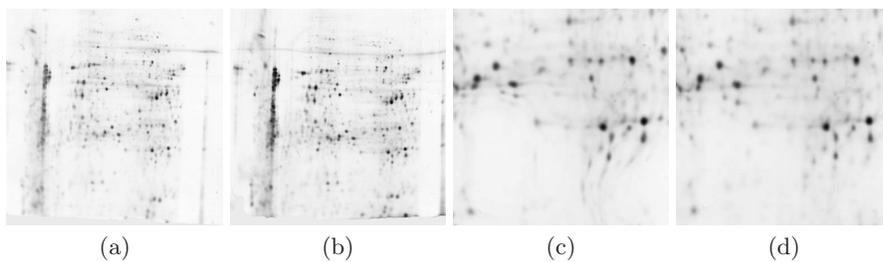

(a)	(b)	(c)	(d)

Fig. 1. Source (a) and target (b) 2-DE images as well as sections of registration results using the previous approach [5] (c) and the new approach (d)

are of the same modality, we use sum-of-squared intensity differences for the intensity similarity measure $J_{\mathrm{Data},I}$. Moreover, our approach allows to also include landmark correspondences $(\mathbf{p}_i, \mathbf{q}_i), i = 1, \ldots, n$, to aid the registration in regions which are difficult to register using intensity information alone. To include the landmarks we define $J_{\mathrm{Data},L}$ based on Gaussian elastic body splines (GEBS), which are analytic solutions of the Navier equation and which have been shown to be superior compared to previously proposed elastic body splines [9]. Note that in contrast to [8], we here directly incorporate the landmark correspondences in J_{Hybrid} instead of requiring an additional deformation field. An advantage is that the landmark and intensity information can be weighted w.r.t. each other more directly and thus the weighting is easier to control.

An efficient way of minimizing J_{Hybrid} is to minimize it alternatingly w.r.t. the intensity information and w.r.t. the remaining functional. For the minimization w.r.t. the intensity information we use the method of Levenberg/Marquardt, which typically converges within few iterations. For the minimization of the overall functional we have derived an analytic solution, which uses matrix-vector convolutions and which is given by

$$\mathbf{u}(\mathbf{x}) = \mathbf{x} + \phi_I(\mathbf{x}) * \left(\mathbf{u}^I(\mathbf{x}) - \mathbf{x}\right) + \phi_L(\mathbf{x}) * \sum_{i=1}^{n} \mathbf{G}(\mathbf{x} - \mathbf{p}_i)\, \mathbf{c}_i \qquad (2)$$

where "$*$" denotes the convolution and $\mathbf{G}$ is the matrix-valued GEBS basis function. Furthermore, $\mathbf{u}^I$ is the result of Levenberg/Marquardt minimization and the coefficients $\mathbf{c}_i$ are obtained from the landmark correspondences using GEBS. The matrix-valued functions ϕ_I and ϕ_L are computed based on the landmark correspondences, the GEBS basis function, as well as the scalar weights λ_I and λ_E. To cope with affine differences in the images, we apply a pure intensity-based affine registration scheme [10] prior to elastic registration.

3 Results

We have applied the new hybrid registration approach to register 2D polyacrylamide gel electrophoresis images of three different levels of complexity (easy, medium, and difficult). The images are courtesy of Prof. G-Z. Yang, Royal Society/Wolfson MIC Laboratory, Dept. of Computing, Imperial College of Science, Technology, and Medicine, London/UK. In each case, gel images are compared from a reference group with those from a test group [4]. All images represent human protein expressions and have a size of 1024×1024 pixels.

To analyze the performance of our approach, we have carried out a quantitative evaluation of the registration results. An obvious measure for the registration accuracy is the number of correctly matched spots. However, note that counting the spots is a non-trivial task in the case of 2-DE images, and also tedious and time consuming. Based on spot counting we obtain the overall number of spots that should ideally match as well as the correctly and incorrectly matched spots. The quantitative evaluation has been performed for images of easy and medium complexity. Tables 1 and 2 show the resulting number of correctly matched spots, the number of incorrectly matched spots, and the fraction of correct matches.

Table 1. Quantitative evaluation results for images of easy complexity (208 ideally matching spots) using affine registration, a previous elastic registration approach [5], and the new approach: Number of correctly (n_{correct}) and incorrectly ($n_{\text{incorrect}}$) matched spots as well as fraction of correct matches

Approach	Information	n_{correct}	$n_{\text{incorrect}}$	Correct
Affine [10]	intensity	128	80	61.5%
Gaussian [5]	intensity	187	21	89.9%
	hybrid	201	7	96.6%
GEBS	intensity	200	8	96.2%
	hybrid	203	5	97.6%

Table 2. Same as Table 1 but for images of medium complexity (158 spots)

Approach	Information	n_{correct}	$n_{\text{incorrect}}$	Correct
Affine [10]	intensity	130	28	82.3%
Gaussian [5]	intensity	137	21	86.7%
	hybrid	149	9	94.3%
GEBS	intensity	150	8	94.9%
	hybrid	153	5	96.8%

4 Discussion

Applying our elastic registration approach without using landmarks, we achieved good overall registration results for all considered 2-DE images. Only in some regions, in particular, regions with a large number of tiny spots or regions located at the image border, the results are worse compared to other regions. However, by inclusion of a relatively small number of landmarks the registration accuracy could be significantly improved in the respective regions. Moreover, we have quantitatively evaluated the registration result. For the images of easy complexity (Table 1) 208 spots should ideally match. Applying the new registration scheme and using only intensity information, we obtain 200 correctly matched spots (8 incorrectly matched) which calculates to 96.2% correct matches. In contrast, the previous approach in [5] yields only 187 correctly matched spots (21 incorrectly matched), and a pure affine approach is much worse (80 incorrectly matched). By additionally incorporating landmarks, the performance of the new approach improves, i.e., only 5 spots are incorrectly matched. Applying the approach in [5] using additional landmarks yields 7 incorrectly matched, i.e., the result is improved but worse compared to the new approach. For the images of medium complexity we obtained comparable results (Table 2). From Tables 1 and 2 it can be seen that both elastic approaches yield quite good results. However, it also turned out that for the previous approach [5] the use of additional landmark information is crucial to achieve satisfactory matching results of 96.6% and 94.3%. In contrast, the new approach achieves this level of accuracy without including landmarks, and yields even better results when landmark information is used (97.6% and 96.8%). Therefore, the new approach is well suited in a fully

automatic setting and the performance can further be improved when landmark correspondences are available.

Moreover, by comparing the resulting deformation grids of both elastic approaches we found that the new approach yields more realistic deformations, in particular, distortions leading to spot smearing as in the case of using [5] are reduced. As an example, Fig. 1 shows a section (384×384 pixels) of the registered images for both elastic approaches for images of easy complexity. It can be seen that the new approach (d) yields a registration result without significant distortions. In contrast, the previous approach [5] yields distortions leading to significant smearing, for example, see the bottom right in (c).

In summary, we introduced a new hybrid elastic registration approach for the geometric alignment of 2-DE gel images. With this approach cross-effects in elastic deformations can be handled, which is important for the registration of 2-DE images. We have successfully applied our approach to register 2-DE gel images of different levels of complexity and we found that the approach is superior to a previous hybrid scheme.

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