Two-Dimensional Gel Electrophoresis Image Registration

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Abstract

Two-dimensional gel electrophoresis (2DGE) is the leading technique to separate individual proteins in biological samples with many biological and pharmaceutical applications, e.g., drug development. The technique results in an image in which the proteins appear as dark spots on a bright background.

The analysis of these images is very time consuming and requires a large amount of manual work. So there is a great need for fast, objective, and robust methods based on image analysis techniques in order to significantly accelerate this key technology.

To enable comparison of protein patterns between different samples, it is necessary to match patterns so that homologous spots are identified. This project presents an algorithm for the purpose of aligning two different electrophoresis images.

The proposed alignment algorithm is based on three different concepts, including segmentation, point matching, and elastic registration. The segmentation technique is based on the Laplacian of the Gaussian filter, using a threshold and connected region processing. The point matching is based on the probabilistic relaxation technique that was proposed in [11]. The final registration is based on the matched points, thin plate Spline interpolation and bicubic interpolation

The proposed algorithm improves the correlation coefficient between the two images by 31% and the mutual information between the two images by 24%.

Keywords: Electrophoresis, Image Registration, Point Matching, Probabilistic Relaxation, Thin Plate Splines

1. Introduction

The field of Proteome[1] analysis (known as proteomic) has become an important part of life sciences especially after the completion of sequencing the human genome. Proteome analysis is the science of the separation, identification, and quantification of proteins from biological samples in order to reveal the function of the living cells. Applications range from prognosis of virtually all types of cancer over drug development to monitoring of environmental pollution.

Electrophoresis has become a leading technique for separation of the proteins. Any charged ion or group will migrate when placed in an electric field. The higher the ratio of charge to mass, the faster the molecule will migrate. The application of an electric field to a protein mixture in solution will therefore result in different proteins migrating at different rates towards one of the electrodes. The pattern of the separation of a sample is an indicator of whether there is any perturbation in the sample or not. Different conditions including genetical changes, the presence of a tumor and leukemia, can be diagnosed using the acquired pattern from the electrophoresis.

For years electrophoresis was just based on one-dimension. In other words the basis of separation was the molecular weight or iso-electric point. Because of the co-migration of polypeptides, there are likely to be problems in the interpretation of gel patterns which are separated on the basis of one feature. Therefore the two-dimensional electrophoresis was suggested by the scientists in order to tackle the problem. All two-dimensional methods should be designed so that the polypeptides are separated on the basis of a different molecular property in each dimension. The commonest two-dimensional electrophoresis method for analyzing mixtures of polypeptides is to separate the proteins in the first dimension on the basis of charge and then to separate the polypeptides in the second dimension on the basis of molecular mass of the polypeptides.

One of the major problems of working with two-dimensional gels is the analysis and comparison of different images. In fact matching of patterns of 2D gels obtained in experiments with previously characterized maps with identified proteins from databases, or, comparison of control and experimental gels, may allow the determination of proteins, expressed under certain environmental conditions, stress treatment, or in some pathological processes. The protein pattern differences between gel images can be very subtle and tedious to detect by eye, therefore digital image analysis is a natural part of the 2-dimensional-gel-electrophoresis process. In order to compare patterns from several experiments it is necessary to overlay the patterns. In order to adjust the coordinates so that two patterns are

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[1] The analysis of the entire Protein complement expressed by a genome, or by a cell or tissue type

superimposed, references (in image registration known as landmarks (control points)) must be selected. In conventional alignment, standard proteins are added to the sample before electrophoresis. Having done this, using the control points (standard proteins) a transformation between the coordinate system of one image to the other image can be obtained.

In order to exclude the presence of the standard proteins, different algorithms have been suggested by the scientists. Some of these algorithms are described next.

Previous Works

Several techniques have been implemented for 2D electrophoresis registration. These techniques can be divided into two main categories:

1. Algorithms based on the characteristics of the spots on the images.
2. Algorithms based on direct comparison of the distribution of the image intensities.

The algorithms of the first group are used in such programs as PDQuest [1], Phoretix 2D [2], Melanie [3]; they are used in numerous proteomic studies. The standard procedure in these kinds of algorithms is to detect the spots (using different segmentation methods) and extract different features, for example shape, area and location, from each spot. Using the extracted features, different pattern matching algorithms and transformations are designed and utilized in order to match and align the spots of the two images. The matching algorithms and the extracted features are the main differences between these algorithms. For instance in [4] different features such as distance, shape context, image context, and feature context is extracted from the spot regions. The correspondence (matching) between different extracted features is acquired using ICP (Iterative Correspondence Point) algorithm. These corresponded features are used in order to calculate the transformation between the two images. The transformation used in this algorithm is CPS (Clamped-Plate Splines). In [5] the correspondence is obtained using RPM-TPS (Robust Point Matching-Thin Plate Spline). In this scheme the point matching and the transformation between the images are optimized simultaneously. In other words the cost function in this scheme is designed so that it optimizes the transformation and the point matching simultaneously.

In the other group of algorithms the necessity of feature extraction and pattern matching is excluded. In this scheme the complete raw gel images are matched according to their intensity distributions. One of the first systems that adopted this approach for electrophoresis is a software environment called Z3 developed by Compugen [6]. In [7], the authors use a multi-resolution representation of the gel profiles and exploit the fact that coarse approximations to the optimal matching can be extracted efficiently from low-resolution images. The optimization procedure in this method is to transform the source image to the target image so that it maximizes the similarity function (correlation) between the two images. The optimal transformation is obtained in different resolutions of the images.

The algorithm that has been designed in this project can be categorized in the first group of algorithms. In other words it tries to align the images using the characteristics of the spots in the images. The three main steps of this algorithm are shown in Figure 1.

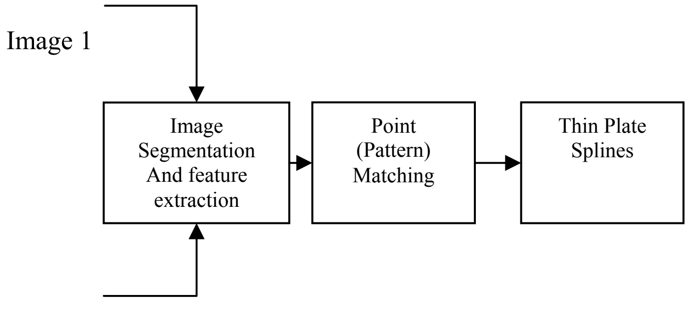

Figure 1: Different steps of the algorithm

In the first step the dark spots are discriminated from the background using a segmentation algorithm and subsequently the features are extracted from the segmented images. In the second step a feature matching scheme is used in order to find the correspondence between the extracted features. In the last step a transformation from one image to the other image is obtained using the correspondence between the extracted features. Detailed description of each of these steps is given in the following sections.

2.1 Segmentation and Feature Extraction

A segmentation method is of necessity in order to discriminate the proteins from the background and noise. The two-dimensional electrophoresis gel images show the expression levels of several hundreds of proteins where each protein is represented as a blob shaped spot of grey level values. Therefore the segmentation process can be confined into detection of blobs or spots in the image. It is crucial that the segmentation is correct in order to obtain a correct matching and alignment in the whole procedure of this algorithm. The matching becomes meaningless if the input is an erroneous segmentation.

Some blob detection (segmentation) algorithms have been suggested in previous works. These include the methods based on mathematical morphology [8] and [9], and scale space blob analysis [10]. In this project a 15×15 LoG (Laplacian of Gaussian) filter is used in order to enhance the blob structures in the image. In fact in [10] has been shown that LoG filter is an appropriate choice for detecting blob structures into an image. The 3-D plot of this filter (mask) is shown in figure 2. This mask is convolved with the original image, in order to enhance spots.

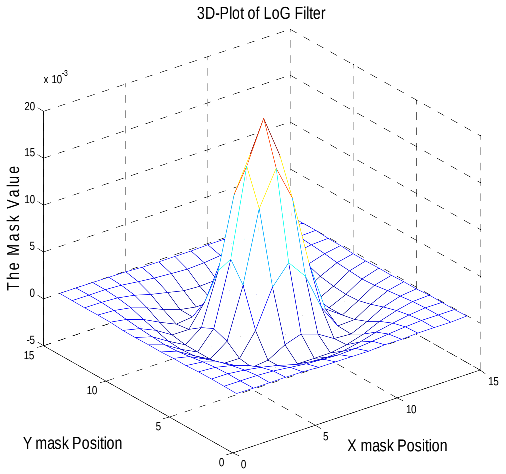

Figure 2: The 3D plot of a LoG mask

Using the histogram of the filtered image, an appropriate threshold can be obtained. This threshold is used to construct a binary image from the filtered image. Having constructed the binary image, the 8-connectivity regions are labeled. The properties of the labeled regions including the second moments in the x and y directions are extracted. According to a blob structure, a region is assumed to be a blob, if its moments in both the x and y directions are within a limited range of values. The regions that their moments are out of this pre-defined range are eliminated from the binary image. The centers of mass of the remaining regions are assumed to be the extracted features.

2.2 Point Matching Using Probabilistic Relaxation

Matching is one of the still open problems in computer vision. It arises in 2D and 3D object recognition from 2D and 3D image descriptors. In fact matching is the pre-requisite of different applications in image processing and computer vision including image fusion and registration. In essence the matching problem involves a collection of object primitives or features extracted from the image.

The aim is to relate the set of primitives to a similar collection of features from another image. Each object primitive assumes a label defined from a label set. The label identifies and establishes a correspondence between the object primitives of an image to object primitives of the other image. The assignment of labels to object primitives is based on the two main sources of information:

1. Object identity, which is defined by the properties of the object itself.
2. Relations, which are defined by the relationships that the objects have with each other.

Mathematically, the primitives can be represented by the nodes of a graph and the relations which are established between the primitives can be represented by the connecting arcs of the graph. Therefore the matching problem can be formulated as a graph-matching problem. The graph matching problem is categorized between the NP-complete[2] problems in complexity theory. Some of the attempts in this field were based on graph searching techniques with some heuristic ideas in order to reduce the complexity of the problem to a manageable process. Some of the others were based on energy minimization using different algorithms including simulated annealing, deterministic annealing and relaxation labeling.

The matching algorithm which has been implemented in this project is based on a work of Christmas, Kittler and Petrou [11]. The basis of this work is probabilistic relaxation. Next the notation of probabilistic relaxation method is presented.

Notation

The nodes of the graph of the scene to be matched as a set A of N objects:

$$A=\{a_1,a_2,\ldots,a_N\}$$

In the matching problem we wish to match the scene to another similar scene or to a model. We therefore assign to each object a_i a label θ_i which may take as its value any of the $M+1$ model labels that form the set Ω:

$$\Omega=\{\omega_0,\omega_1,\omega_2,\ldots,\omega_M\}$$

where, ω_0 is the null label used to label objects for which no label is appropriate. In other words, if there does not exist any node in the model object that can be matched to the scene object then that node of the scene object will be labeled by ω_0. The notation $\omega_{\theta i}$ is used to indicate that we wish to associate a model label with a particular scene label θ_i. At the end of the labeling process it is expected that all objects in the scene have one

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[2] In complexity theory, the NP-complete problems are the most difficult problems in NP (Non deterministic time Polynomial). The reason is that if you could find a way to solve an NP-complete problem quickly, then you could use that algorithm to solve all NP complete problems quickly.

unambiguous label value. In this algorithm the matching of many-to-one[3] is allowed.

Two sets of indices are defined:

$$N_0 \equiv \{1,2,\dots,N\}$$

$$N_i \equiv \{1,\dots,i-1,i+1,\dots,N\}$$

For each object a_i a set of m_i measurements $\mathbf{x}_i$ corresponding to the unary attributes of the object are assumed:

$$x_i = \{x_i^{(1)}, x_i^{(2)}, \dots, x_i^{(m_i)}\}$$

Examples of unary attributes are the length, color, or orientation of an object. The attribute which is used in this problem is the **shape context** of the geometrical coordinate of the points extracted from the image. The abbreviation $x_{i,i\in N_0}$ denotes the set of all unary measurement vectors $\mathbf{x}_i$ made on the set A of the objects:

$$x_{i,i\in N_0} = \{x_1,\dots,x_N\}$$

For each pair of object a_i and a_j we have a set of m_2 binary measurements $\boldsymbol{A_{ij}}$:

$$A_{ij} = \{A_{ij}^{(1)}, A_{ij}^{(2)}, \dots, A_{ij}^{(m2)}\}$$

Examples of binary relations are the relative position of one object with respect to another, relative size, or orientation. In the problem of this paper the **relative Euclidian distance** between points is used as the binary relation between objects.

These measurements (binary relations and unary attributes) are computed for both images (in [11] known as scene and model). In order to discriminate between the measurements of the two images all of the 'i' and 'j' indices are replaced respectively with 'α' and 'β'.

The upper-case P is used to represent the probability of an event. For example $P(\theta_i = \omega_\alpha)$ denotes the probability that scene label θ_i is matched to model label ω_α. In other words the probability of matching object i from the scene set objects to object α from the model set objects. The lower-case p is used to represent the probability density function of a random variable.

2.2.1 Probabilistic Relaxation Theory

In [11] the general problem of matching is formulated in the Bayesian framework, from which an appropriate formula for labeling the scene objects has been derived. At the end of the algorithm it is expected that the label θ_i of an object a_i will be given the value $\omega_{\theta i}$ provided that it is the most probable label given all the information of the system. This information includes attributes of the object itself and also the relations of the object with the other objects in the scene. This can be written in the following mathematical manner:

$$P(\theta_i = \omega_{\theta i} \mid x_{j,j\in N_0}, A_{ij,j\in N_i}) = \max_{\omega_\lambda \in \Omega} P(\theta_i = \omega_\lambda \mid x_{j,j\in N_0}, A_{ij,j\in N_i}) \quad \text{Eq.1}$$

In [11], it has been proven that under certain often adopted assumptions, the conditional probabilities in this equation can be expressed in a form that indicates that a relaxation *updating rule* would be an appropriate method for finding the required maximum. The proposed updating rule in [11] is:

$$P^{(n+1)}(\theta_i = \omega_{\theta i}) = \frac{P^{(n)}(\theta_i = \omega_{\theta i}) Q^{(n)}(\theta_i = \omega_{\theta i})}{\sum_{\omega_\lambda \in \Omega} P^{(n)}(\theta_i = \omega_\lambda) Q^{(n)}(\theta_i = \omega_\lambda)} \quad \text{Eq.2}$$

Where $Q^{(n)}$ is derived from the following formula:

$$Q^{(n)}(\theta_i = \omega_\alpha) = \prod_{j\in N_i} \sum_{\omega_\beta} P^{(n)}(\theta_j = \omega_\beta) p(A_{ij} \mid \theta_i = \omega_\alpha, \theta_j = \omega_\beta) \quad \text{Eq.3}$$

Furthermore $p(A_{ij} \mid \theta_i = \omega_\alpha, \theta_j = \omega_\beta)$ is known as the compatibility coefficient. The compatibility coefficient is used in order to quantify the compatibility of matching θ_j to ω_β. This compatibility coefficient is defined in [11] as:

$$p(A_{ij} \mid \theta_i = \omega_\alpha, \theta_j = \omega_\beta) = \frac{1}{\prod_{k=1}^{m2} \sqrt{2\pi}\sigma_k} e^{-\sum_{k=1}^{m2} (A_{ij}^{(k)} - A_{\alpha\beta})^2 / (2\sigma^2{}_k)} \quad \text{Eq.4}$$

This recursive procedure will terminate after 200 iterations.

In order to translate the probabilistic relaxation to point matching problem let us consider an easy example:

In this example one wants to match the points of set A which contains two points, to the points of set B which contains three points. These two sets are shown in Figure 3.

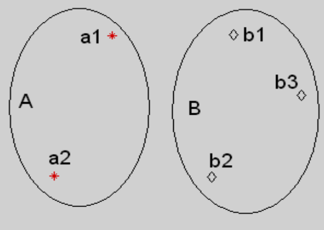

Figure 3: Two sets of Points

The matching problem can be reduced to finding a matching matrix, M, which corresponds the members of set A to the members of set B. Therefore, by considering the Null node[4] in both sets, this matching matrix will have the following configuration:

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[3] It means that the labels of more than one object are allowed to have the same value. This property of the algorithm is not desired in the 2D gel matching problem. Therefore it should not be considered in the following of this paper.

[4] The Null node is used in order to correspond to nodes which do not have any correspondence to the real data.

$$\begin{bmatrix} a1 \\ a2 \\ NullA \end{bmatrix} = \begin{bmatrix} M_{11} & M_{12} & M_{13} & M_{14} \\ M_{21} & M_{22} & M_{23} & M_{24} \\ M_{31} & M_{32} & M_{33} & M_{34} \end{bmatrix} \begin{bmatrix} b1 \\ b2 \\ b3 \\ NullB \end{bmatrix}$$

In fact $M_{i\alpha}$ indicates the probability of matching a_i to b_α. In other words it is equivalent to $P(\theta_i = \omega_\alpha)$ in the above-mentioned notations. Each of the components of this matrix is calculated using the updating rule of Equation 2.

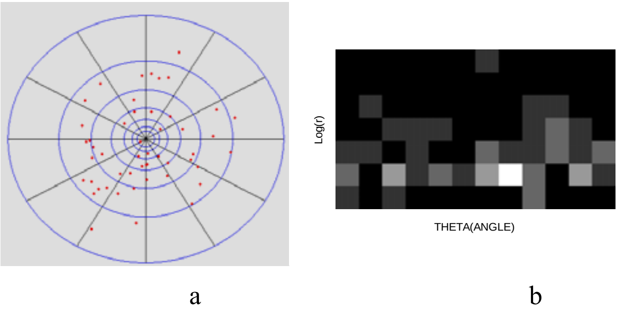
In the remainder of this section a brief introduction to shape context and the initial matching matrix is given.

Shape context

For a point p_i in the set of points, the coarse histogram h_i of the relative coordinates of the remaining *n-1* points can be computed as below:

$$h_i(k) = \#\{q \neq p_i : (q - p_i) \in bin(k)\} \qquad \text{Eq.5}$$

This histogram is known as the *shape context* of point p_i. The descriptor should be more sensitive to differences in nearby points. Therefore usually a log-polar coordinate system is used. An example of a series of points and also the shape context histogram of the central point are shown in Figure 5.



a b

Figure 4: a: The points and their locations in different bins b: The shape context histogram that corresponds to figure a (The black pixels represent 0)

Initial Matching Matrix

The initial matching matrix should have a relationship with the attribute (shape context) of the points. Therefore the χ^2 distance between the histograms of two points in scene and model images is used. If we denote the two K-bin histograms by g(k) and h(k) the χ^2 distance between those two points is given by:

$$\chi^2(h(k), g(k)) = \frac{1}{2}\sum_{k=1}^{K}\frac{[g(k) - h(k)]^2}{g(k) + h(k)} \qquad \text{Eq.6}$$

The higher the value of χ^2 the less probable the two selected points of scene and model to be matched. Therefore the matching matrix can be initialized by:

$$M_{i\alpha}{}^{0} = \frac{1}{\chi^2(i(k), \alpha(k))}$$

In order to make the sum of each row in the initial matching matrix equal to 1, the values of M should be normalized. Therefore each row of the matching matrix (without considering the row and column that corresponds to the Null node) is given by:

$$\hat{M}_{i\alpha}{}^{0} = \frac{M_{i\alpha}{}^{0}}{\sum_{\alpha} M_{i\alpha}{}^{0}}$$

Furthermore, the initial probability of matching each node to the Null node and also the compatibility coefficient of matching each node to Null node are defined in [11] as.

Another problem that should be considered is the matching of many-to-one that is allowed in the algorithm proposed in [11]. This problem is solved as below:

Consider two sets of points with *K* points in one set and *L* points in the other set. Therefore the matching matrix will be a *(K+1)×(L+1)* matrix. After finding the finalized matching matrix, point *i* matches to point *j* if:

$$(M_{ij} = \max_{i=1 \longrightarrow K+1} M_{ij}) \,\&\, (M_{ij} = \max_{j=1 \longrightarrow L+1} M_{ij}) \Rightarrow i \xleftrightarrow[\textit{Matches}]{} j$$

In other words the largest argument in both column and row corresponds to the two points that belong to that desired column and row.

2.3 Thin Plate Splines

Registration techniques, based on corresponding features (landmarks), are the most practical tools for aligning two images. The geometrical transformation functions that employ landmarks can be based on different non-linear functions such as radial basis functions. The registration problem in this theory is considered as an interpolation problem in which the interpolating function is equivalent to the mapping function and different parameters of the function are computed using the landmarks.

The problem of minimizing the bending energy and at the same time satisfying the interpolation conditions is known as *thin plate splines (TPS),* which is an appropriate physical model of steel plates that are under stress. The bending energy is defined as:

$$E_B = \iint_{R^2} \left(\left(\frac{\partial^2 f}{\partial x^2} \right)^2 + 2\left(\frac{\partial^2 f}{\partial x \partial y} \right)^2 + \left(\frac{\partial^2 f}{\partial y^2} \right)^2 \right) dxdy \qquad \text{Eq.7}$$

It has been shown in [12] that the unique radial basis function that minimizes the bending energy has the form of:

$$U(r) = r^2 \log r^2 \qquad \text{Eq.8}$$

The radial basis functions are suitable non-linear transformations for interpolating scattered data [12]. Therefore it is an appropriate choice for the problem of aligning the 2 dimensional electrophoresis images in which the extracted points are randomly distributed.

The algebra of the thin plate splines for Arbitrary Sets of Landmark is given as below:

We have two sets of corresponded points and we are concerned in finding a mapping function based on the introduced RBF function in equation 8. Let’s assume the two sets of two dimensional corresponded points as $\{\underline{b}_i\}_{i=1}^N$ and $\{\underline{c}_i\}_{i=1}^N$ the mapping interpolation function is defined as below:

$$f(\underline{x}) = \sum_{i=1}^{N} w_i U(|\underline{x}-\underline{b}_i|) + \underbrace{a_0 + a_1 x_{1x} + a_2 x_{2y}}_{p(\underline{x})} = \sum_{i=1}^{N} w_i U(|\underline{x}-\underline{b}_i|) + p(\underline{x})$$

The aim of the interpolation problem is to calculate w_i, a_0, a_1, a_2 coefficients. These coefficients can be computed using two assumptions.

1. The correspondences that exist between two sets of points. In other words it is expected that:

$$f(\underline{b}_i) = \underline{c}_i, i = 1, 2, ..., N$$

2. Polynomial reproduction. In other words if the ideal mapping interpolation function is a polynomial transformation, this can be reproduced by the mapping interpolation function.

It should be noted that, *p(x)* is added to the RBF function in order to add the ability of reproducing the affine transformation. For more details about computing the coefficients the reader is referred to [13] and [14].

Since the interpolation function in digital images maps $N^2 \to R^2$, therefore it is necessary to use another interpolation function to find the grey-level of the new transformed points. In this project a bicubic interpolation function is used for finding the grey-level of the new transformed points. In this scheme the grey-levels of the sixteen nearest discrete points to the transformed point are utilized for computing the grey-level of the transformed point.

3. Results

Implementation of the proposed technique improves the mutual information quantity between the two images by 24% and improves the correlation coefficient between the two images by 31%. Furthermore in order to perceive the alignment of the two images a special color scheme is used. In this scheme, the two grey-level images are combined into one color image. The green component of this color image is the first image and the red and blue components are the second image. In other words green is controlled by the first image and the blue and red components are controlled by the second image. This means that the structures will appear as gray or black, if they coincide in both images, otherwise they will appear as green or magenta. It should be noted that this way of evaluating the registration is just for demonstration.

Figure 5 is the combination of the two images before registration and figure 6 is the combination of the two images after the registration. The number of black dots is an indication of the alignment between the two images.

4. Conclusions

In this paper a new technique for aligning the 2 dimensional electrophoresis images was proposed. The designed algorithm consists of different parts including segmentation, point matching, geometrical and grey level transformations. The novel part of the algorithm is the new point matching technique. The results of the designed algorithm were reasonable and acceptable. Although the following suggestions may improve the results:

1. Using a more accurate algorithm for segmentation that minimizes the number of outliers in the extracted data. Furthermore considering some challenges like segmentation of the proteins that are over-lapped, or designing an algorithm that is not sensitive to the size of the blobs may make the algorithm more applicable.
2. Using a point matching scheme that finds the correspondence between the extracted points as accurate as possible. Considering some problems like null nodes and the problem of matching many-to-one may improve the accuracy.
3. Designing a geometrical transformation that is robust to the outliers. In other words the outliers do not affect the whole geometrical transformation
4. Assessing the physical reasons of the deformation of the images and model these deformations in a mathematical manner can be a valuable research. In fact if we can model the deformations by considering the physical causes, the geometrical transformation can be more accurate and more efficient

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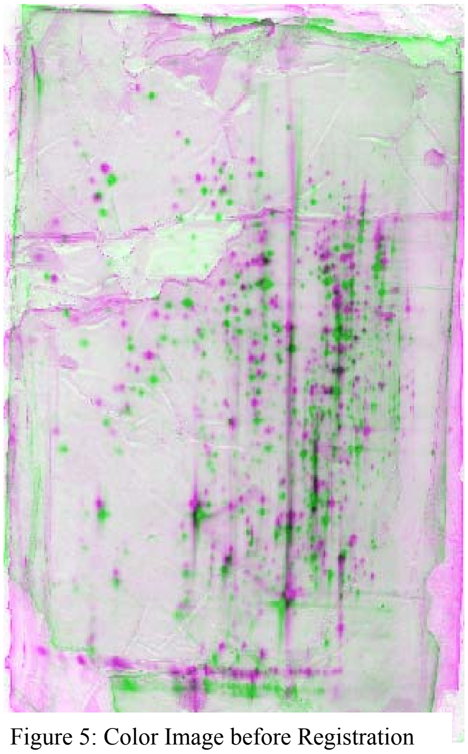
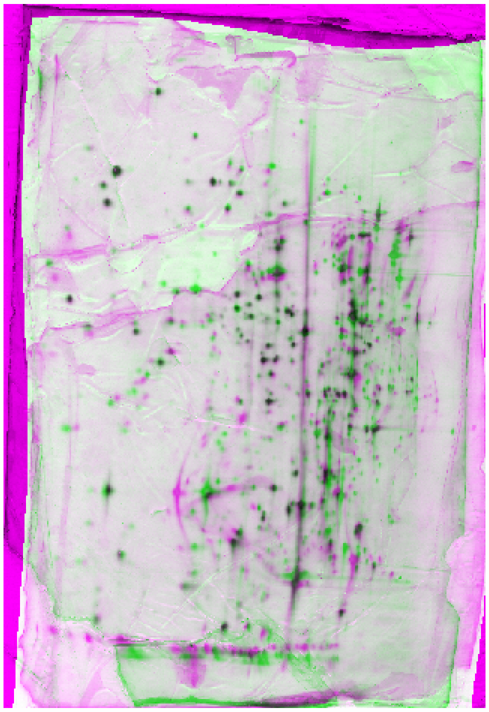


Color Image before Registration

Figure 5: Color Image before Registration



Color Image of the Registered Images

Figure 6:Color Image of the Registered Images