MATCHING TWO-DIMENSIONAL GEL ELECTROPHORESIS' SPOTS

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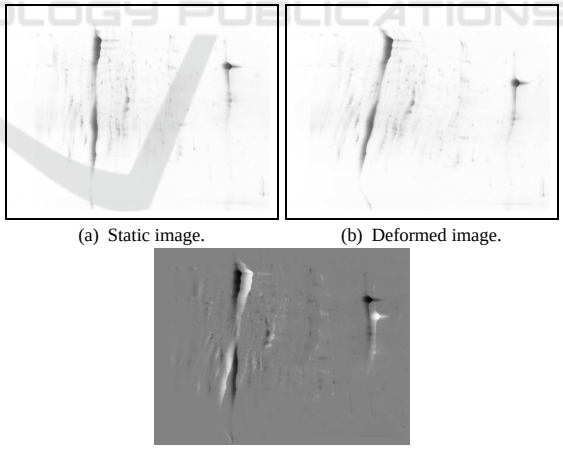
Abstract: This paper describes an approach for matching Two-Dimensional Electrophoresis (2-DE) gels' spots, involving the use of image registration. The number of false positive matches produced by the proposed approach is small, when compared to academic and commercial state-of-the-art approaches. This article contributes to solving one of the greatest bottlenecks in the 2-DE analysis pipeline.

1 INTRODUCTION

In the context of Proteomics and Genomics, differential analysis implies that two or more Two-Dimensional Electrophoresis (2-DE) gel images are compared in order to find the differences in terms of protein or DNA expression. Although it is possible to superpose two images and try to find their differences, it may not be the best approach. Distortions are present in the majority of the gels (Figure 1). This way, when two different images are superposed, the spots in one image will hardly be aligned with the spots in the other image (Figure 1(c)). Essentially because these distortions are mostly non-rigid, the problem of finding a correspondence between spots becomes an extremely difficult task.

State-of-the-art software packages are known for producing too many false positive matches (Rosengren et al., 2003; Srinark and Kambhamettu, 2008). The correction of false positive matches is an extremely time consuming task, being one of the main bottlenecks in the analysis of 2-DE gels.

The problem of matching two sets of spots can be put as a bipartite matching problem. However, the following section shows that bipartite matching algorithms may not be enough to achieve an acceptable matching solution. Thus, other approaches will also be considered, namely approaches that involve Image Registration.



(a) Static image. (b) Deformed image.

(c) Difference image.

Figure 1: Distortion between 2-DE gels. Figures 1(a) and 1(b) represent approximately the same protein expression, but are clearly different due to deformations.

2 BIPARTITE MATCHING

Matching protein spots between gels can be formulated as a bipartite matching problem. According to graph theory, a graph $G = (V,E)$ with vertices V and edges E is bipartite if there exists a partition $V = X \cup Y$ with $X \cap Y = \emptyset$ and $E \subset X \times Y$. A matching is a subset $M \subset E$ such that $\forall v \in V$ there is at most one edge in M that is incident on v. The size of the matching is given by the number of edges in M, and is defined as $|M|$. A perfect matching is a matching M where all the vertices are connected to one other

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vertex at most.

When the goal is to find the best matching between vertices, the problem can be posed as an assignment problem where each edge e is assigned a weight $w(e)$. The maximum-weight matching satisfies the condition that for any other M', $W(M') \leq W(M)$, where $W(M) = \sum_{e \in M} w(e)$. One of the most famous and reliable optimization algorithms that can be applied to find the maximum-weight in a bipartite matching is the Kuhn-Munkres algorithm (Kuhn, 1955), also called Hungarian Algorithm (HA).

Considering the spots in two different 2-DE gels, the goal is to match each corresponding spot in gel X to every other spot in gel Y. The spots are the vertices of the graph. A weighing function $w(e)$ based on the similarity between each inter-gel spot has to be defined.

It is proven in (Kuhn, 1955) that a maximum-weight matching is a perfect matching. This requires that each of the parties of the graph has the same number of vertices. 2-DE gels are unlikely to express the same exact number of spots, even if the feature detection step is perfect. This is easily solved by adding the number of missing vertices to the set that has them missing, and assigning an extremely high weight to connect to those vertices.

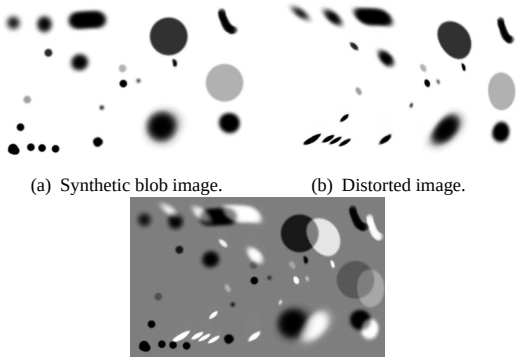
The complexity of the Hungarian Algorithm is $O(V^3)$. However, considering that manually correcting the results of a poor matching process between two 2-DE gel images may take several hours, the $O(V^3)$ cost in the HA is a minor drawback.

In order to extract the features (*i.e.* protein spots), the approach proposed in (dos Anjos et al., 2011) is used.

If each vertex v_i in the first party identifies the location of a spot in the first image, and each vertex v_j identifies the location of a spot in the second image, then, a cost function for each edge e could be the Euclidean Distance: $w(v_i, v_j) = \sqrt{(v_{ix} - v_{jx})^2 + (v_{iy} - v_{jy})^2}$. This weighing function produces a perfect match between Figures 2(a) and 2(b), however, if only one outlier is introduced, only $\approx 60\%$ of the spots are correctly matched. Therefore, a more robust weighing function that includes more information from the spots, namely the differences of distances d, areas a, volumes vo, circularities c, eccentricities ec, and orientations o, has to be defined:

$$\begin{aligned} w(v_i, v_j) \quad = \quad & \alpha_{\mathrm{d}} \|v_i, v_j\| + \alpha_{\mathrm{a}} \left| a_i - a_j \right| \\ & + \alpha_{\mathrm{vo}} \left| vo_i - vo_j \right| + \alpha_{\mathrm{c}} \left| c_i - c_j \right| \\ & + \alpha_{\mathrm{ec}} \left| ec_i - ec_j \right| + \alpha_{\mathrm{o}} \left| o_i - o_j \right| \quad (1) \end{aligned}$$

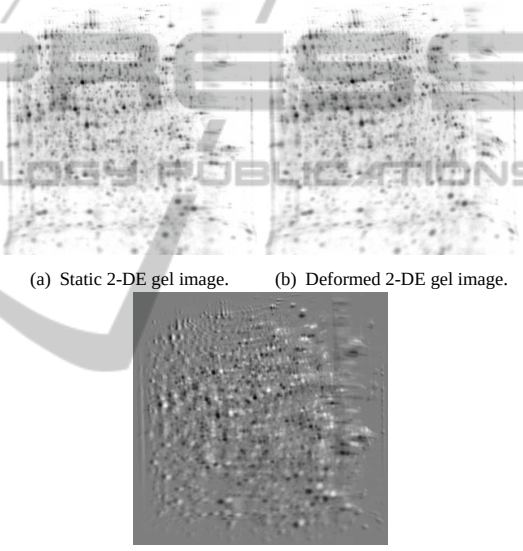
where the $alphas$ are the respective weights of each of the normalized components in w.



(a) Synthetic blob image. (b) Distorted image.

(c) Difference image.

Figure 2: Difference between synthetic images. A large distortion between images is present.



(a) Static 2-DE gel image. (b) Deformed 2-DE gel image.

(c) Difference image.

Figure 3: 2-DE gels images and difference image.

Applying the HA with this weighing function (Equation (1)), to Figures 2(a) and 2(b), produces a match without any errors. Moreover, the outlier is correctly identified. As reference, the weights were set to: $\alpha_{\mathrm{d}} = 0.65; \alpha_{\mathrm{a}} = 1.0; \alpha_{\mathrm{vo}} = 1.5$. The rest of the coefficients were set to zero.

Figure 3(a) presents the image of one highly populated 2-DE gel. The same image is presented in Figure 3(b) with an arbitrary elastic deformation. It is possible to observe in Figure 3(c) that the gels' spots are not aligned.

Using as weights: $\alpha_{\mathrm{d}} = 2.5, \alpha_{\mathrm{a}} = 1.5, \alpha_{\mathrm{vo}} = 2.5$, at least, 103 spots were correctly matched, among a total of 476 detected. This was manually verified. Not only were the relatively close positioned spots matched but also the relatively far positioned ones. Nevertheless, the HA has limitations when the distortions in the im-

ages are extremely high. Additionally, the distortions present in the 2-DE images are mostly local and non-rigid, making it extremely difficult to find an optimum valid match. If these distortions are corrected, the number of matches may increase. This takes us to a different field: Image Registration – which is discussed in the next section.

3 IMAGE REGISTRATION

The goal of image registration is to find a geometric, or elastic transformation that makes one image similar to the other. Putting it more formally, the problem is the following: Given two images $S(\mathbf{i})$ and $D(\mathbf{i})$, where S is called the static image and D the deformed (also called reference and match, respectively), the goal is to find a transform T such that:

$$W = D(T(\mathbf{i})) \approx S(\mathbf{i}) \tag{2}$$

where W is the warped image.

In differential analysis the objective is to identify the differences and to quantify them, therefore, it may be dangerous to register one image into the other using some image registration methods. For example, Modersitzky (Modersitzki, 2004) completely warps a ball into a C shape using a fluid registration method.

3.1 Geometric Transform

Non-rigid transforms, also called deformable and elastic, allow relatively complex distortions in the image. These include stretching and curving of the image.

Affine transform, the most general transform that preserves parallelism between lines, is a non-rigid transform and is represented in homogeneous coordinates by:

$$\begin{bmatrix} x' \\ y' \\ 1 \end{bmatrix} = \begin{bmatrix} \theta_0 & \theta_1 & \theta_2 \\ \theta_3 & \theta_4 & \theta_5 \\ 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} x \\ y \\ 1 \end{bmatrix} \tag{3}$$

where θ_2 and θ_5 are the translations in x and y directions respectively.

The aforementioned transform preserves the straightness of the lines. Curved transforms, as the name points out, do not behave in a similar way. These include the polynomial transformations that can be expressed by:

$$x' = \sum_i^p \sum_j^{p-i} a_{ij} x^i y^j \quad \text{and} \quad y' = \sum_i^p \sum_j^{p-i} b_{ij} x^i y^j \tag{4}$$

where p is the order of the polynomial transformation. Quadratic and cubic polynomial transforms are the most used since they allow flexible enough transformations, and higher order polynomials tend to get unstable, specially at the borders of the images. Additionally, very small perturbations may lead to very unstable transformations in the image with a polynomial of higher order.

4 REGISTRATION WITH KNOWN CORRESPONDENCES

As previously seen, just by using the direct application of the HA to the weighed distances between correspondent spots in different gels, there was a substantial amount of correct spot matches between the gels. Image registration can be used to improve these results.

The idea is to select the best matches and use those matches to create a warping transform that hopefully will drag the other misplaced and mismatched spots to a corrected position in the gel image.

The proposed approach is the following:

1. Detect features;
2. Calculate the cost matrix;
3. Match using the Hungarian Algorithm;
4. Iterate until stability:
   (a) Select only very good markers ($\leq$ threshold);
   (b) Warp;
   (c) Recalculate the cost matrix;
   (d) Rematch;

In this approach, the image is not really being warped at each step. Because features have already been detected, only the coordinates of the center of the spots are recalculated, or warped. The objective is to match the protein spots in one gel image to the spots in the other gel image, instead of the whole image.

Markers are detected and selected and the coefficients for a polynomial transform (Equation (4)) are calculated. The degree of the polynomial transform is limited by the number of detected points, therefore, higher order polynomial transforms were preferred until a maximum degree of 3. The intensity levels resulting from the warping are simply ignored since the objective here is just to correct the positions of the spots.

The weights used in this matching were the same as the previous ones: $\alpha_d = 2.5$; $\alpha_a = 1.5$; $\alpha_{vo} = 2.5$. Two new parameters are additionally used, namely t_d and t_c, that define a threshold for the maximum allowed distance for a spot to be accepted as a good

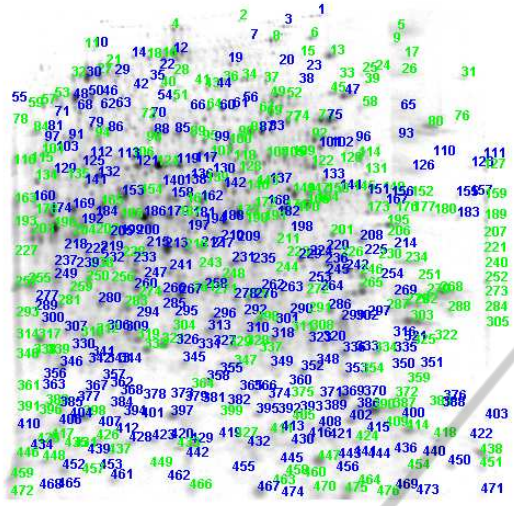


Figure 4: Matched with iterative registration. Blue numbers represent reliable matches.

match, and the maximum allowed cost for a match to be considered valid, respectively. The values set for these thresholds were: $t_d = 0.09; t_c = 0.1$.

By using the presented approach, we can get a minimum of 255 correct matches as shown in Figure 4, in contrast to 103 in the previous attempt. This represents an increase of more than 100%. The blue markers are the matches accepted as having an acceptable quality. The green markers are markers that are not considered reliable for being at a relatively far Euclidean distance from the match, although many can also be found as correct matches.

Although there is a great improvement, the results obtained from matching "naturally" deformed images is far from acceptable. A more advanced approach is presented in the next section as an attempt to improve these results.

5 REGISTRATION WITHOUT KNOWN CORRESPONDENCES

For two images, it may be acceptable to ask the user to manually place landmarks in order to allow the calculation of the transformation. For large amounts of images, that is not a suitable approach. For the global misalignment correction, an automatic parametric affine transformation was chosen. Also, in order to correct local distortions, non-parametric deformable registration is considered in this section.

5.1 Correction of Global Distortions

When a 2-DE gel is placed in the scanner, the position of the gel will hardly be the same as the previously scanned gels. Rotation and translation are the most common differences between gels, and these are global deformations. Before any other attempt of matching relative nearby spots between gels, these global distortions should be corrected.

Parametric image registration deals with the problem of finding the transform's parameters, in order to minimize a similarity measure or error function.

Let us start by choosing the SSD as the similarity measure. Thus, the problem is to find the parameters Θ that minimize the following error function:

$$E(S, D(T(\Theta))) = \sum_{i}^{N} (S(\vec{x}_i) - D(T(\Theta, \vec{x}_i)))^2 \quad (5)$$

where Θ is a set of K parameters $\Theta = \{\theta_0, \theta_1, \ldots, \theta_k, \ldots, \theta_K\}$ belonging to the transform that warps D. E will stabilize and, therefore, should be minimum when $\frac{\partial E}{\partial \theta_k} = 0$, for all k.

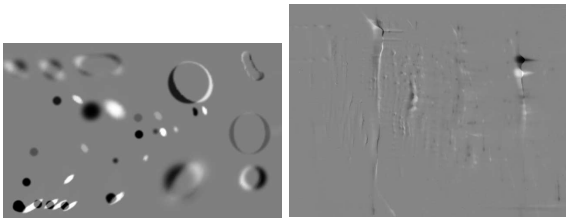
The error can be minimized by either Gradient Descent, Gauss-Newton, or Levenberg-Marquardt optimization (Levenberg, 1944; Marquardt, 1963). Since the latter has become a standard technique for nonlinear least-squares problems and can be thought of as a combination of Gradient Descent and Gauss-Newton, this is the method used in the proposed approach.

5.1.1 Tests

Figures 5(a) and 5(b) show the resulting difference images of automatically registering images in Figure 2(b) to 2(a), and 1(b) to 1(a), respectively. Compare Figure 5(a) with Figure 2(c). Also, compare Figure 5(b) with Figure 1(c). It is obvious that the global registration method corrected the global affine distortions on both images. One of the great advantages of calculating the distortion as global is that the number of parameters that have to be estimated for the transformation is relatively small. The disadvantage is that local distortions are not corrected, and 2-DE gel images are also prolific in these kind of distortions. Therefore, these resulting global transforms can be used as a very good initial seed for a registration method that handles local distortions.

5.2 Correction of Local Distortions

In order to take care of local distortions, automatic non-parametric image registration was chosen since parametric transformations do not perform well for local distortions (Goshtasby, 1986). Image registration without known correspondences is an ill-posed problem. A mathematical problem is ill-posed if its



(a) Synthetic image. (b) 2-DE image.

Figure 5: Differences after affine registration.

solution is not unique, does not exist, or does not depend continuously on the data (Hadamard, 1902).

An approach to solve ill-posed problems is based in the regularization theory. Generally, it involves the inclusion of constraints on the possible solutions. Constraints are, usually, imposed by a regularizer. The registration method presented in the previous section did not need an explicit inclusion of any kind of regularizer, since the solutions were implicitly regularized by properties of the space. The most common constraint used in the regularization of image processing problems is smoothness. Regularization theory involves the use of two terms:

- the error term E;
- the regularization term R.

The error term is usually the sum of squared differences, rewritten as follows:

$$E_{\mathrm{SSD}}(S,D,T) = \sum_{i}^{N} (S(\vec{x}_i) - D(T(\vec{x}_i)))^2 \qquad (6)$$

and the regularization term:

$$R(T) = \|\nabla T\|^2 \qquad (7)$$

The regularizer can be seen as a term that will prevent abnormal transformations from occurring or, in other words, a term that penalizes certain exaggerations in the transformation.

Consequently, the total error to be minimized can be expressed as:

$$E_t(S,D,T) = \sum E(S,D,T) + \lambda R(T) \qquad (8)$$

with λ being a value that controls the trade-off between data fitness and transformation smoothness.

If the transformation caused by T in $\vec{x}$ is represented by $\mathbf{u}(\vec{x})$, or $T(\vec{x}) = \vec{x} + \mathbf{u}(\vec{x})$, then the problem can be posed in a variational way. Therefore, the objective now is to find the $\mathbf{u}$ that minimizes $E(S,D,\mathbf{u})$. In other words, we want to minimize the following error function:

$$E_t(S,D,\mathbf{u}) = \sum_{i} E(S,D,\mathbf{u}) + \lambda R(\mathbf{u}) \qquad (9)$$

When E_t is minimized, then $\mathbf{u}$ satisfies the Euler-Lagrange equation:

$$\nabla(E(S,D,\mathbf{u})) + \lambda \nabla R(\mathbf{u}) = 0. \qquad (10)$$

An approach presented in (Knut Conradsen, 1992) tries to find the best $\mathbf{u}$ using an approximation to the cross-correlation as matching criteria. In the cited source, an algorithm to match two 2-DE gel images of 512×512 pixels is presented. The method proposed by the author estimates a transformation that maps one 2-DE gel image to the other by using the intensity of the pixels as main criteria. For each of the pixels in one gel image, a disparity vector is estimated or, in other words, the distance between coordinates of the pixel in one gel and the best candidate position on the other is calculated. The best candidate is identified by the local arrangement of gray levels for the neighbor pixel in the other gel, and the matching is done by cross-correlating the two gels. The different maxima of the cross-correlation function will produce the disparity vectors. These vectors are used to warp the second gel so that it matches the first as well as possible.

A median smoothing is applied at each level of the pyramid, and together with the scaling process, these act as a regularizer.

6 REGISTRATION-BASED MATCHING

Some modifications were made to the algorithm presented in (Knut Conradsen, 1992). First, it was adapted to work with any image size. As some artifacts could be seen at the end of the registration process, another improvement consists in the use of the original image and the cumulative displacement matrices when warping at the intermediary steps, instead of always using the same warped image with the intermediate matrices. Also, cubic B-Spline interpolation replaced cubic interpolation. Images with more complex distortions were not being correctly registered, therefore, a parameter was introduced in order to allow more iterations at each level of the pyramid. These changes introduced considerable improvements at the cost of more computational complexity.

In the proposed approach, the intention is to find correct positions for each spot in order to allow a better matching. Therefore, what matters are the transforms and not the warped images.

We proposed the following approach:

1. Normalize the images;

2. Global Affine Registration;
3. Full image registration using the previous calculated transform as seed;
4. Detect features in the original images;
5. Correct the spots' coordinates using the transform calculated in step 3;
6. Match features with corrected coordinates;

After the results of the affine global and full registrations, the features in the original images are detected using the approach proposed in (dos Anjos et al., 2011). As mentioned before, position, area, volume and other descriptors are extracted from the spots. Nevertheless, only the coordinates of the deformed image are corrected by using the total displacement field calculated from the image registration process.

The total displacement $\mathbf{u}_t(\vec{x})$ for the position of each detected spot is the following:

$$\mathbf{u}_t(\vec{x}) = \vec{x} - ((A\vec{x}+b) + \mathbf{u}(\vec{x})) \qquad (11)$$

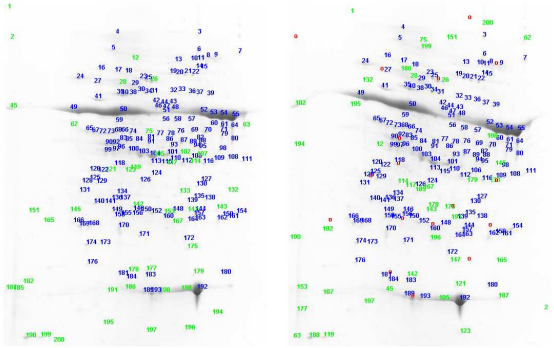
where $(A\vec{x}+b)$ is the affine transform calculated as described in Section 5.1, and $\mathbf{u}(\vec{x})$ is the displacement for each coordinate as calculated in (Knut Conradsen, 1992) with the proposed improvements.

This way, position can be given a much higher weight in the cost function as it is supposed to be more reliable. Another improvement, relatively to the previous presented approach is the replacement of the detected volume by the volume of the spot weighed by a Gaussian centered at the spot. Therefore, instead of relying only on the detected volume, one relies on the volume and on the context defined by the spot's immediate surroundings. Therefore, even having lower representation, the information surrounding the spot is included, contributing for a better match. Additionally, a threshold t_{ed} was created in order to immediately exclude the possibility of matches that are at a very far position from the corresponding spot. Finally, spot matches not respecting this threshold are set to ∞. Only then, the cost matrix is processed by the HA.

7 RESULTS

All the parameters were the same for the complete set of images. Distance and (new) volume, for the weighing function were set to: $\alpha_d = 1.0$; $\alpha_{\text{vo2}} = 1.0$. All the other weight coefficients were set to zero. Moreover, $t_d = 0.35; t_c = 0.35; t_{\text{ed}} = 35$, where t_{ed} is not normalized as it is used during the construction of the cost matrix.

As it will be demonstrated, one of the great advantages of this approach is that it is very difficult to find a false match between the markers presented as valid markers, meaning that the presence of false matches is extremely low, or non-existent. Only markers presented in blue are matches that respect all the defined thresholds. Green markers have found a match in the HA but do not respect either t_d or t_c. Finally, the red markers did not find any match in the HA, being considered as outliers.



(a) 200 spots found. (b) 217 spots found.

Figure 6: Figures 1(a) and 1(b). Valid matches: 155.

Figure 6 shows the result of the suggested matching process used to match the spots of Figures 1(a) and 1(b).

A very popular approach used in feature matching is Shape Context (Belongie and Malik, 2000). It basically consists of analyzing the spacial relationship between points.

Shape contexts use mainly four parameters. The first parameter defines the number of radial bins for the creation of the histograms (set to 10), the second is the number of theta bins that defines how many slices should the histograms be divided in (set to 24), and the minimum and maximum widths of the bins (set to 1 and 100, respectively).

Table 1 presents a comparison of the results of the matches using the proposed approach and matching with shape contexts.

It is clear that the proposed approach is doing a better job than the shape context approach. The number of false matched spots is extremely low when using the proposed approach. This is highly desirable because it avoids the necessity of a laborious manual intervention to correct the results.

PDQuest is considered as one of the best software packages in matching 2-DE gel images (Rosengren et al., 2003). Despite not directly comparable with PDQuest, or with any other commercial software, because the detected spots are not exactly the same as the detected by our approach, results from matching the same images used in Table 1 are presented in Table 2 using PDQuest Version 8.0.1 build 055.

Although the number of false matched spots is

Table 1: Comparison between the detection of the proposed approach and shape context approach.

Spot Det		**Proposed**				**Shape Context**			
Stat	**Def**	**Mat**	**Min %**	**F.P.**	**% F.P.**	**Mat**	**Min %**	**F.P.**	**% F.P.**
341	374	238	69.79%	0	0.00%	272	79.77%	≥ 35	$\geq 10.26\%$
200	217	155	77.50%	0	0.00%	130	65.00%	≥ 20	$\geq 10.00\%$
97	114	56	57.73%	1	1.03%	52	53.61%	≥ 15	$\geq 15.46\%$
49	44	32	72.73%	1	2.27%	36	81.82%	≥ 12	$\geq 27.27\%$
422	398	312	78.39%	2	0.50%	315	79.15%	≥ 30	$\geq 07.54\%$
160	207	79	49.38%	2	1.25%	118	73.75%	≥ 20	$\geq 12.50\%$
160	195	41	25.63%	7	4.38%	48	30.00%	≥ 07	$\geq 04.38\%$
160	260	104	65.00%	1	0.63%	117	73.13%	≥ 10	$\geq 06.25\%$
160	278	92	57.50%	0	0.00%	112	70.00%	≥ 15	$\geq 09.38\%$
160	249	54	33.75%	6	3.75%	88	55.00%	≥ 10	$\geq 06.25\%$
		Avg:	58.74%	**Med:**	1.37%	**Avg:**	66.12%	**Med:**	$\geq 13.99\%$

Table 2: Matching with PDQuest.

Spot Det		**PDQuest**			
Stat	**Def**	**Mat**	**Min %**	**F.P.**	**% F.P.**
154	331	123	79.87%	0	0.00%
174	180	137	78.74%	7	4.02%
91	58	52	89.66%	4	6.90%
45	31	29	93.55%	1	3.23%
332	293	209	71.33%	5	1.71%
137	154	78	56.93%	3	2.19%
137	146	60	43.80%	8	5.84%
137	175	95	69.34%	0	0.00%
137	214	100	72.99%	0	0.00%
137	249	58	42.34%	4	2.92%
		Avg:	69.85%	**Med:**	3.65%

also low, when compared to the proposed approach, it is possible to conclude that a lot of post-processing will be needed. First, many undetected valid spots have to be manually added and, then, they need to be re-matched. Nevertheless, the matching is also very good.

In summary, an effective spot matching approach is proposed in this article. These results are outperforming the results obtained from important academic and commercial approaches.

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