On-line detection of red blood cell shape using deformable templates

P.J.H. Bronkorst $^a$, M.J.T. Reinders $^b$, E.A. Hendriks $^{b,*}$, J. Grimbergen $^a$, R.M. Heethaar $^c$, G.J. Brakenhoff $^a$

$^a$ BioCentrum Amsterdam, Institute for Molecular Cell Biology, Amsterdam, Netherlands
$^b$ Information and Communication Theory Group, Faculty of Information Technology and Systems, Delft University of Technology, Mekelweg 4, 2626 CD Delft, Netherlands
$^c$ Laboratory of Clinical Physics and Informatics, Vrije Universiteit Amsterdam, Amsterdam, Netherlands

Received 16 December 1998; received in revised form 27 January 2000

Abstract

For the purpose of automating a clinical diagnostic apparatus to quantify the deformability of human red blood cells, we present an automated image analysis procedure for on-line detection of the cell shape based upon the method of parametric deformable templates. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Parametric deformable template; Red blood cell shape; Red cell deformability

1. Introduction

In 1995 we introduced a new method to study the shape recovery process of individual human red blood cells using multiple optical trapping (Bronkhorst et al., 1995). In this method, a red blood cell is deformed from a flat disc to a bent one using three optical traps (frame 1, Fig. 1). After removal of the traps, the cell relaxes back to its original flat shape and the time constant of this process is used as a measure of the deformability of the cell. Red cell deformability is one of the major determinants of the flow properties of the blood, and, therefore, an important factor regulating the blood circulation (Chien, 1987). Reduced red cell deformability increases whole blood viscosity and reduces oxygen transport to organs and tissues. The latter is the result of reduced perfusion or blockage of the small vessels of the microcirculation. These small vessels have diameters as small as 4 μm and the red blood cells (undeformed about 8 μm in diameter) need to deform considerably to enter them, indicating the importance of their deformability.

Standard techniques like filtration (Teitel, 1977) and ektacytometry (Bessis and Mohandas, 1975; Bronkhorst et al., 1995) for quantification of red cell deformability measure the mean deformability of a cell population. This means that sub-populations of less deformable cells can go unnoticed while these small amounts of cells may be responsible for circulatory problems. Techniques that measure the deformability of individual cells...
overcome this problem but are experimentally far more complex. Apart from our method, there are two main alternative techniques to measure the deformability of individual red blood cells: micropipette aspiration (Evans, 1989) and the rheoscope (Sutera et al., 1985). These techniques use different deformation methods, but both use manual analysis of images obtained through a microscope imaging system. Clinical applications of these methods have never been successful because the experiments were too elaborate, probably due to the lack of automation. The method using optical trapping, which we developed, is both sensitive to changes in red cell deformability (Bronkhorst et al., 1995) and well suited for automation and therefore has the potential to be successful in clinical use.

An important step in the automation of the measurement is automating the image analysis. Up till now, the image analysis was done manually and as shape parameter the angle between the two sides of the cell was measured interactively for every image of the sequence. The time course of this shape parameter was subsequently analyzed off-line to calculate its relaxation time constant. The manual analysis of a standard sample of 300 cells took on average 10 h.

Clinical applicability of the method can only be successful if the total measurement time (including image analysis) is acceptably short without the need of any additional actions from the user. Our goal is therefore to integrate a fully automatic image analysis procedure into the measurement procedure, so that the shape parameter versus time information is determined on-line. Apart from speed, the method needs to be robust in order to be able to deal with the naturally occurring variability in relaxation times, cell shape, and image quality. Reliability of the method in clinical applications requires that no more than 5% of the cells may have failed to produce a reliable relaxation time constant. Therefore, we aim to have a success-rate in the determination of the relaxation time of at least 95% for a standard (healthy) blood sample.

This paper introduces an automatic approach to the required image analysis that meets the above requirements. The method exploits prior knowledge about the shape and the possible deformations of the cell. Based on the automatically retrieved shape parameters an optimal relaxation parameter is calculated. Although alternatives were studied, it turned out that the angle remained the best choice because it changes over a large range during relaxation (90–180°) and is invariant to cell size and location. Furthermore, the time course of the angle versus time data can be described by a single exponential, which makes the

---

**Fig. 1.** Example of a time sequence of a red blood cell shape recovery (frames are 20 ms apart) that constitutes a set of 10 128 x 128 grayscale (0–255) images. The first image is taken just before the traps are turned off. The traps are visible as white dots in a triangular configuration (approximately 90°).
calculation of the relaxation time constant straightforward.

First, in Section 2, we review a number of possible image analysis algorithms in order to automatically retrieve the cell shape and we motivate our choice for parametric deformable templates. Details about this method as well as the exact definition of the prior shape knowledge are presented in Sections 3 and 4. Section 5 then elaborates on the results of the image analysis on a large number of test cells and presents the achieved success-rates. Finally, we end with a discussion.

2. Analyzing a cell shape from images

This section describes possible solutions adopted from the Computer Vision Society for the apparent image analysis problem. First, however, we give some background on the acquisition of the imagery.

2.1. Image acquisition

The shape recovery of the red blood cell is viewed by a microscope imaging system using phase contrast illumination \(100 \times\) Zeiss objective, NA \(\approx 1.4\). The images are recorded using a CCD-camera (Grundig Electronic, Germany) and frame-grabber (dt3155, Data Translocation, USA) system. The relevant window of \(128 \times 128\) pixels (0–255 grayscale) is selected and stored as a sequence of 10 images and serves as the input of the image analysis algorithm. Even and odd fields of the video signal are used separately meaning that the images are 20 ms apart in time, i.e., a 50 Hz sampling frequency. Fig. 1 shows an example of such a sequence. The first image of the sequence is the last video field where the traps, that were responsible for the deformation, were visible.

The phase contrast microscopy results in images where the cell membranes are highlighted with a relatively high contrast (Fig. 1). However, an additional effect is that the contours are relatively broad and that there is a halo present around the cell. Furthermore, biological and experimental variations cause significant variations in the intensity distributions of the images between cells. One of the results of these effects is that the cell membrane is not always entirely visible which generally results in non-closed contours at the right-hand side of the cell.

2.2. Image analysis solutions

The image analysis algorithm has to be able to automatically detect the shape with just enough detail to obtain the relaxation shape parameter with enough accuracy for our purpose. Because cell shapes are restricted, the image analysis can exploit the prior known geometric information in order to increase robustness and computation time.

The typical image features like non-closed contours, highly variable contrast and intensities, cause it to be a not-straightforward image analysis problem. In general, the phenomenon of the non-closed cell contours excludes all methods that search for or need closed contours. At first glance, a Hough Transform method (Illingworth and Kittler, 1988) to obtain the two orientations appears to be the obvious choice. Although such a method is robust, non-relevant regions within the image with variable orientations contributed significantly and make the method unreliable. Automatic pre-processing of the images turned out to be unsuccessful due to the variability in contrast and/or intensity of the non-relevant features. A generalized Hough Transform using a parameterization was considered unsuitable because it needs too much computation time due to the complex shape and, therefore, large number of parameters.

A group of methods that use only part of an image (thus potentially less computational intensive), and where cell contours need not be closed, is that of active contours (Jain et al., 1996; Kass et al., 1988). In these methods, the shape of an object is found based upon an a priori shape description (e.g. prototype template) which is then deformed by a process of applying shape transformations in order to obtain the best match with the object in the image. Two types of active contours can be distinguished: (1) free form, known as snakes (Kass et al., 1988), and (2) parametric, known as deformable templates (Yuille et al., 1992; Staib and Duncan, 1992). In the free form
active contour, there is no global structure imposed and the shape is determined by local constraints of continuity and smoothness. This makes it more sensitive to local irregularities (e.g., noise) as compared to the parametric template and, therefore, less useful for our problem.

The parametric deformable template is more suited for our problem. The a priori information about the cell shape is used to describe the desired cell shape. Next, the supposed shape is put into an attractor field build from the image features of the measured cell, such as local intensities and/or gradients. The position in the attractor field is interpreted as an energy function that is minimized to find the exact shape of the recorded cell.

The computational costs can be kept low by minimizing only over a small number of parameters that describe the template. Using only a few shape parameters additionally ignores local irregularities and, therefore, increases the robustness. However, inherent to all active contour methods, it uses a sub-optimal optimization scheme and hence does need a good set of initial values for its parameters as a starting-point in order to avoid local minima. Fortunately, in our case, the optical traps in the first image can be identified relatively easy and consequently serve to initialize the analysis.

Another active contour method, denoted by active shape models, has been introduced by Cootes et al. (1995). This method tries to combine the advantages of both active contour types. By using a learning set of possible shapes, this method learns the local distributions of the points on the contour. These local distributions are then used as restrictions on the movements of the points during contour detection. By reducing the dimensionality of these distributions using PCA, these restrictions can be parameterized. The resulting parameters consequently define the shape of the contour. Although this technique automatically learns the shape of the subject and the allowed deformations, we decided not to use this technique for the following reasons. For one, it is still necessary to identify the points that are essential for a robust contour detection. For example, in our application, the points on the right sided contour of the cells are quite uninformative and should be left out of the model. Effectively, this makes that the automatic learning scheme of active shape models still has to be tailored quite extensively. Another important reason was that eventually we are interested in the relaxation parameter of the cell shape being the angle between the two lines approximating the left side of the cell. This prior knowledge is exploited most in the deformable template method in which this knowledge can directly be incorporated. For all other methods an additional line fitting would be necessary. Further a reason not to choose for point representations is that the cells are highly flexible during the cell relaxation. Probably this would lead to the necessity of a separate contour model for each of the relaxation stages. Having a parameterization of the bending behavior excludes this necessity. Additionally, the parameterized method allows for more easy incorporation of global, area based, image characteristics in the energy function.

3. Parametric deformable template

3.1. The method

The key element in the parameterized deformable template technique is that the prior knowledge about the shape and appearance of the object is exploited as much as possible. First of all, the shape $S(\Theta)$ is parameterized using $K$ parameters: $\Theta = (\theta_1, \theta_2, \ldots, \theta_K)$. Generally, one chooses, for computational and accuracy reasons, the simplest model that still is capable of describing enough shape detail.

Given such a parameterization additional knowledge about the range of values that those parameters can take can be embedded into the template description. These constraints are formulated as a cost function depending on the parameter set: $C_i(\Theta)$. For example, when two parameters should be equal in size such a cost function can look like: $C_i(\Theta) = |\theta_1 - \theta_2|$. Generally, all constraints can be summed into an energy term that specifies how well the shape matches these (internal) constraints

$$E_{\text{int}} = \sum_i C_i(\Theta).$$ (1)
To be able to find the shape of the object in the image one has to incorporate knowledge about the appearance of the object and the actual measured values into the template description. Generally, one knows that a certain image characteristic, \( \phi_j(x,y) \), of the image, \( i(x,y) \), should be as high as possible along the contour of the object. More explicitly, this can be expressed in a cost function

\[
C_i^F(S(\Theta)) = - \int_{s \in \partial S(\Theta)} \phi_j(p(s)) \, ds
\]

where \( \partial S(\Theta) \) denotes the contour elements, \( s \) the line parameterization of the contour, and \( p(s) = (x(s), y(s)) \) denotes the position of a contour element in the image. Note that maximizing \( \phi_j(x,y) \) causes the minus sign in \( C_i^F(S(\Theta)) \).

Besides maximizing characteristics over the contour of the shape, parametric deformable templates especially allow for including appearance knowledge about areas other than the contour, for example over an area spanned by the shape

\[
C_i^E(S(\Theta)) = - \int_{A \in S(\Theta)} \phi_j(x,y) \, dA
\]

Generally, all these appearance models are then summarized into an external energy term

\[
E_{\text{ext}} = \sum_i C_i^E(S(\Theta)).
\]

Thus, the match between the prior knowledge about the shape and appearance of the object and the measured image is expressed by an energy function that is the sum of the above internal and external energy terms

\[
E = E_{\text{int}} + E_{\text{ext}}.
\]

Finding the shape of the object in a given image can now be defined as a minimization of this energy function \( E \) with respect to the shape parameters \( \Theta \). When having reasonable initial estimates of the parameters \( \Theta \), this minimization can be solved using a standard gradient descent method, i.e.

\[
\Theta^{n+1} = \Theta^{n} + \frac{\partial E}{\partial \Theta} \bigg|_{\Theta^{n}}
\]

where \( \Theta^{n} \) represents the parameters at the \( n \)th iteration of the minimization scheme, and \( \eta \) is a weighting parameter. This requires, however, the calculation of the partial derivatives of the energy function with respect to each of the parameters \( \theta_i \). Instead, we have used Powell’s minimization scheme (Press et al., 1994) to perform this multidimensional minimization. This procedure minimizes the energy function, \( E \), for each parameter, \( \theta_i \), successively, until an overall minimum is found. The minimization is done without using an analytical expression of the first derivative. Thus, for each parameter \( \theta_i \) separately, the energy function is minimized along the direction of its unit vector \( e_i \). The parameter \( \Theta \) is then updated according to

\[
\Theta^{n+1} = \Theta^{n} + \lambda e_i,
\]

where \( \lambda \) is chosen such that it minimizes \( E(\Theta + \lambda e_i) \). The latter line minimization can easily be performed by evaluating the energy function at different points, e.g.

\[
\lambda^{k+1} = \lambda^{k} + \eta \frac{\partial E(\Theta)}{\partial \theta_i} \bigg|_{\Theta^{k}}
\]

\[
= \lambda^{k} + \eta \frac{E(\Theta^{k} + \Delta e_i) - E(\Theta^{k} - \Delta e_i)}{2 \Delta e_i}
\]

with \( \Theta^{k} = \Theta^{n} + \lambda^{k} e_i \), until

\[
E(\Theta^{n} + \lambda^{k+1} e_i) > E(\Theta^{n} + \lambda^{k} e_i).
\]

### 3.2. Defining template prototype and energy function

When using the parametric deformable templates to automatically detect deformed blood cell shapes it is thus necessary to define an appropriate parameterization of the shape (or template prototype) and according appearance model (energy function). Because the definition of both the parameterization and the energy function are coupled, we used the following strategy to come up with the template description. Firstly, an appropriate parameterization is searched, keeping the energy function simple and fixed. Secondly, the
parameterization is kept fixed and an optimal appearance model is searched. The simple appearance model that is assumed when looking for an optimal parameterization states that the cell membranes (its contours) will appear with a relatively high contrast in the images due to the phase contrast microscopy. The discretized energy function then simply becomes

\[ E = E_{ext} = \sum_{s \in \mathbb{S}(\theta)} i(p(s)), \]

where \( i(p(s)) \) represents the image intensity at position \( p(s) = (x(s), y(s)) \) along the contour \( \partial S(\theta) \).

### 3.2.1. Parameterization

Finding an optimal parameterization of the blood cell shapes implies finding an optimal balance between accuracy, robustness, and complexity. Obviously, when the parameterization is not accurate enough, important shape changes cannot be tracked. However, when the parameterization becomes too accurate (more parameters) the estimation process becomes less robust against varying imaging conditions, like noise, as well as that it would require much more computation time.

As a first approach we only model the shape of the left side of the cell contour because only this side has consistently the highest contrast and, moreover, contains the desired relaxation information. In finding the optimal parameterization, a number of different shapes were tried to describe the bending of the left cell contour: a parabola segment \((3 + 2 = 5 \text{ parameters})\), an ellipse segment \((4 + 2 = 6 \text{ parameters})\), and a set of two lines \((4 + 2 = 6 \text{ parameters})\). All shapes were cut off at two outer points where two circle segments with a fixed radius were attached to the shape, with a continuous and smooth connection. These “hooks” fixed the templates to the top and bottom ends of the cell contour without increasing the number of template parameters. This proved necessary for any template fit to converge.

Preliminary experiments with these shapes showed that the template of two lines did not match the cell shape properly. The parabola and ellipse matched the shape reasonably well for small angles but rather poorly for the larger angles. The success-rate for obtaining a reasonable template fit was about 80% (i.e., in 80% of the test images a reasonable template fit was found). However, the determination of the desired angle from these template functions proved not reliable causing the success-rate in the determination of the relaxation time constant to be as low as 30%.

Based on these experiments we came up with a more complex template prototype. A suitable one was found in the form of the “rounded-V” shape based upon the observation that the cell shape of a deformed red blood cell resembles a “V” on its side. This shape, see Fig. 2, allows to represent the bent of the cell contour accurately by the angle

![Fig. 2. The cell template consists of two line segments (II and IV) and three circle segments (I, III, and V). All elements of the template are depicted as thick bold lines. The seven variable parameters of the template are \((x_0, y_0)\), \(y_{\text{start}}\), \(y_{\text{stop}}\), \(a_A\), \(a_B\), and \(R\). There are two pre-fixed parameters: the radius \(r = 10\) pixels of the top and bottom circle segments, and the starting angle of the top and bottom circle segments \(\psi = 60^\circ\). Additionally, there is one appearance model parameter: distance \(d = 5\) pixels, indicating the neighborhood at which the template also has to be evaluated. The other variables depend upon the fixed and variable ones and are used only for reasons of clarity.](image-url)
between the 'legs' of the V as well as the length of the cell sides. Additionally, by rounding the ends of the shape, the smoothness of the cell boundary is adequately modeled. The shape, therefore, exist of five line pieces, two representing the legs of the V (segments II and IV), two representing the rounding at the end of the V-legs (segments I and V), and one rounding at the heart of the V (segment III). In total the shape is represented by seven free parameters, see also Fig. 2.

1. Legs of the V, segments II and IV. The angle between the two sides of the cell is equal to the angle between the legs of the V. These legs are represented by two lines (II and IV) that have orientations \( \alpha_d \) and \( \alpha_b \) with respect to the positive x-axis, respectively

\[
\begin{align*}
\text{II :} & \quad \left\{ \begin{array}{l}
x = x_d + \frac{1}{\tan \alpha_d} (y_d - s), \\
y = s, \\
s \in [y_{\text{start}}, y_d], \\
x = x_b + \frac{1}{\tan \alpha_b} (s - y_b), \\
y = s, \\
s \in [y_b, y_{\text{stop}}],
\end{array} \right.
\end{align*}
\]

where \( y_{\text{start}} \) and \( y_{\text{stop}} \) define the length of each of the line segments. \((x_d, y_d)\) and \((x_b, y_b)\) are two points that connect the line segments with the rounded heart of the V (segment III), chosen such that the connections are both continuous and smooth.

2. Rounded heart of the V, segment III. The line segments are connected by a circular segment (III) with a variable radius \( R \). The location of the template in the \( x \)-\( y \) plane is represented by the ultimate left point of the circle segment \((x_0, y_0)\).

\[
\begin{align*}
\text{III :} & \quad \left\{ \begin{array}{l}
x = x_0 + R + R \cos(s), \\
y = y_0 - R \sin(s), \\
s \in [90 + \alpha_d, 270 - \alpha_b].
\end{array} \right.
\end{align*}
\]

3. Rounded ends of the V, segments I and V. The top line segment (II) starts at the \( y \)-coordinate \( y_{\text{start}} \) and the bottom line segment (III) ends at \( y_{\text{stop}} \). At both ends of the two line segments \((y_{\text{start}}, y_{\text{stop}})\), two 'hooks' are inserted to represent the bents of the cell at its ends and to keep the template from shrinking (ensuring a more accurate fit). The "hooks" are added in the form of two circular segments (I and V) with a fixed radius \( r \) and \( \psi \) (see Fig. 2)

\[
\begin{align*}
\text{I :} & \quad \left\{ \begin{array}{l}
x = x_1 + r \cos(s), \\
y = y_1 - r \sin(s), \\
s \in [\psi, 90 + \alpha_d],
\end{array} \right. \\
\text{V :} & \quad \left\{ \begin{array}{l}
x = x_2 + r \cos(s), \\
y = y_2 - r \sin(s), \\
s \in [-90 + \alpha_b, \psi],
\end{array} \right.
\end{align*}
\]

where \((x_1, y_1)\) and \((x_2, y_2)\) are the centers of the circular hooks and are chosen such that the connection between the hooks and the line segments are continuous and smooth.

Because we enforced all connections to be continuous and smooth (continuous derivative) the template is uniquely defined by nine parameters. Seven of these parameters are variable when fitting the template, these are \((x_0, y_0, \alpha_d, \alpha_b, y_{\text{start}}, y_{\text{stop}}, R)\). Two of them are kept fixed because they can be estimated relatively robustly from the trap points, these are \((r, \psi)\). The angle between the two legs of the V, equal to \( \alpha_d + \alpha_b \), is the desired relaxation parameter.

3.2.2. Appearance model

The above derived template prototype proved to be a good match for a sufficient large range of deformation angles and proved robust for local irregularities of the cell contour. Recall that the appearance model that was assumed when looking for an optimal parameterization stated that the cell membranes (its contours) would appear with a relatively high contrast in the images. For the chosen parameterization that becomes

\[
E^i_{\text{ext}} = \frac{w_1}{N_{\text{II}} + N_{\text{IV}}} \sum_{z \in \text{II,IV}} i(p(s)) + \frac{w_2}{N_{\text{I}} + N_{\text{V}}} \sum_{z \in \text{I, V}} i(p(s)) + \frac{w_3}{N_{\text{III}}} \sum_{z \in \text{III}} i(p(s)),
\]

where \( N_{\text{I}} \) stands for the number of pixels along segment \( i \), and \( w_i \) are weights that control the importance of each term.

Experiments, however, showed that when using this template, the angles are still systematically underestimated at larger angles. By changing the
appearance model this systematical behavior can be adjusted.

First of all, we tried to broaden the thickness of the cell contour to 3 pixels instead of 1, as well as some image pre-processing (e.g. smoothening the image). Neither of these two did improve the fit. After careful observation of the cell images, one notices that the high intensity of the contour always comes with low intensities near the cell contour. Hence, besides maximizing the intensities along the cell contour one can also strive towards minimizing the intensities on both sides of the cell contour at some distance. This can be achieved by adding the following term to the energy function:

\[
E_{\text{ext}}^2 = \sum_{k=1}^N \sum_{s \in \Omega_{\text{III,IV}}} i(p(s) + d n(s)) + \sum_{k=1}^N \sum_{s \in \Omega_{\text{III,IV}}} i(p(s) - d n(s)),
\]

where \( n(s) \) represent the normal on the contour \( S(\Theta) \) at position \( p(s) \). The first term sums over the area outside the cell while the second term sums over the area inside the cell.

The appearance model for the changing blood cells thus becomes

\[
E = E_{\text{ext}}^1 + E_{\text{ext}}^2.
\]

The weights were set as follows:

\[
w_1 = w_3 = \begin{cases} 2, & (y - y_0) \leq 4, \\ 1, & \text{else}, \end{cases} \]

\[
w_2 = 2,
\]

\[
w_4 = 1,
\]

\[
w_5 = \begin{cases} 0.5, & y \in [y_{\text{start}} + 1/2(y_y - y_{\text{start}}), \\ y_y + 1/2(y_y - y_{\text{stop}})], \\ 0, & \text{else}. \end{cases}
\]

4. Matching the template to the image

Once the template is defined, i.e. its parameterization and its appearance model are set, we only need a strategy to match the template against the images of the changing blood cells. As already indicated in Section 3.1, finding the shape of a cell in the image is done by minimizing the energy function with respect to the set of free shape parameters. Additional to this minimization scheme, one needs a correct initialization and evaluation procedure to make the matching strategy successful.

4.1. Minimization

Matching the template with a cell in the image is done by minimizing the energy function \( E \) in Eq. (16), with respect to the seven free shape parameters, i.e. \( x_0, y_0, z_A, z_B, y_{\text{start}}, y_{\text{stop}} \). R. We adopted Powells minimization scheme (Press et al., 1994) to perform this multi-dimensional minimization (see also Section 3.1).

4.2. Accuracy

This scheme does not provide us with information about the accuracy of the obtained fit results. Fortunately, we are more interested in the accuracy of the relaxation time constant then the shape fit. The relaxation time constant accuracy can be measured by analyzing the time behavior of the estimated (shape) angle \( (x_A + x_B) \). Because an exponential decay of this angle is expected, the fitting error of the data from one experiment with an exponential curve provides us with a reliable measure of the accuracy.

4.3. Initialization

A downhill minimization can easily be trapped in a local minimum. Therefore, one should select such initial values for the shape parameters that the chance of finding a global minimum increases. Hence, the method of deformable templates requires a good initial guess for the shape, size, and location of the object.

We use the coordinates of the optical traps to obtain this initial set. This is possible because we know that the cell is located at the trap locations and is unlikely to have moved more than a few pixels. Furthermore, because these traps were responsible for the deformation of the cell, we can also use them to approximate the initial value of the deformation angle.
The trap coordinates are determined prior to a measurement and are therefore known. Using this information, an acceptable set of initial values for the parameters is found for the first image of the sequence. For the following images, the results from the previous image are used as initial set of parameters.

4.4. Evaluation

Experiments on a large number of cells showed that even for a matching strategy that exploits initial knowledge about the trap locations, it was possible for the template to be matched against the halo of the cell contour, i.e. still a local minimum was found. In some situations these misfits could be overcome when using slightly different initial parameters. It thus is important to detect when a fit fails. Apart from being able to re-try the fit with adapted initial parameters, unreliable fits can be eliminated from further analysis. Because the fit-error does not give reliable information about the accuracy of the fit, a fit-testing procedure was developed. The testing procedure consists of a number of checks that are based upon the prior information about the cell shape and the shape recovery process.

Two types of checks are performed on the results of a template fit: out-of-range errors and shape errors. An out-of-range error occurs if the parameters have obtained such values that the template (or part of it) falls outside the boundaries of the image. A shape error occurs if one or more parameters have obtained values that are unrealistic with respect to the a priori information about the cell shape and the shape recovery process. Examples of errors of the second type are: small angles (<80°), too large or too small cell size, or large shifts in the location of the cell. If errors of the second type occur, the template fit is retried with a slightly different set of initial values, a shifted template or a slightly larger or smaller template. In most cases a reliable fit is found after one or two retries.

Additionally, the results are checked by evaluating the exponential fit of the estimated bending angle for the different time instances. In those cases where the estimated angles may have gone wrong (indicated by large exponential fit errors), the user is asked whether the measurement is acceptable or not. Although such interaction is against our goal to have a completely automatic procedure, it proved necessary to avoid too many unnecessary failures.

The complete matching strategy then becomes like in Fig. 3.

Fig. 3. Matching strategy.

5. Experiments

The presented template fit method matches the cell shapes well within the range of angles that occurred in experiments. Fig. 4 shows the template fits for the different cell images of Fig. 1 and in Fig. 5 the angles versus time are plot from which the relaxation time constant is obtained by fitting an exponential curve through the data. The image analysis proves to be sufficiently robust for image variations as can be seen in Fig. 6, where extreme examples and their template fits are shown. For the automatic analysis, the measurement error is in the order of 3°.

We analyzed the shape recovery of about 900 healthy cells (8100 angles). The success-rate in the determination of the angle is 96.4%, where successful detection is defined whenever an automatically detected angle, by visual inspection,
corresponds to the cell shape. Of the failed angles (3.6%), about 50% is detected as such (≈1.7% of all angles) and eliminated from further analysis. The remaining faulty angles (1.9%) were not detected. Effectively this means that the success-rate of the determination of the angle is 98.1% because a detected failure can be considered successful from the point of view of the success of the image analysis procedure.

More important for applicability of the image analysis procedure, is the success-rate of the determination of the relaxation time constant from the angle versus time data. In the analysis of the 900 cells from above, the success-rate was 95.7%. The group of failures (4.3% of the cells) contains the cells where too many angle estimates have failed but also the cells where the variability of the angles was too large to be able to obtain a reliable result from the exponential fit. In 2.8% of the cells this failure was detected and therefore the effective success-rate of the method as a whole is 98.5%.

For a subset of 80 cells, all angles were analyzed both manually and automatically and the angles were compared. On an average, the methods give the same angles although both have a large variance. The correlation between the two methods is depicted in Fig. 7. The correlation coefficient is 0.906 ± 0.007. Although in theory this coefficient should be equal to 1, this is an acceptable result due to the unreliability of the manual analysis (measurement error about 5° instead of 3° for the automatic analysis). It was e.g. seen that in manual analysis, the right side of the cell was used unintentionally in the determination of the angle resulting in slightly different angle. This is visible in Fig. 7, where at larger

Fig. 4. The same relaxation as in Fig. 1, now with the fitted template (white). The gray lines indicate the location where the neighborhood is taken. In the first image, the initial template is depicted as well as the traps (white dots) from which the initial template is obtained.

Fig. 5. The measured angle \( (a_x + a_y) \) versus time plot for the cell of Fig. 4. From this plot the relaxation time constant \( \tau \) is obtained by fitting an exponential curve \( a(t) = (180 - a(0)) \exp(-t/\tau) + a(0) \) through the data. For this cell the relaxation time constant \( \tau \) is 138 ± 4 ms.
angles relatively many points are located below the line.

The high success-rate is achieved because in dubious cases (about 5% of the cells), interaction of the user was required in judging whether or not the fit had succeeded. The calculation time is about 10 s on a 133 MHz Pentium PC XPS P133c. On an average, a sample of 300 cells, takes about 1 h to analyze, which is an improvement of 90% compared to the 10 h for the manual analysis. These calculation times can be significantly reduced when the software is optimized with respect to speed and hardware is used for the computations.

6. Discussion

The parametric deformable template fit method as we presented in this paper for the automatic image analysis of the red blood cell shape recovery measurement, proves to be very successful. The method is automatic, robust, fast and accurate enough for our purpose. Furthermore, the measurement time decreased by 90%. The image analysis to obtain the shape parameter (deformation angle) has a success-rate of 96.4%.

For the purpose of automation of the method, the success-rates of the determination of the relaxation time constant from the angles are more important than the success-rates of the determination of the angles itself, provided failures are detected and eliminated from the calculation of the relaxation time. The success-rate for the determination of the time constant, proved to be 95.7% (effective even 98.5%) which meets our goal of at least 95%.

In conclusion, we presented the solution to the problem of automating the measurement of the red cell deformability using optical trapping, that meets our goals concerning speed,
robustness, and accuracy and it is currently successfully implemented in an on-line image analysis process.

References


