Recognition of Unstained Live *Drosophila* Cells in Microscope Images

Marko Tscherepanow\(^1\) Nickels Jensen\(^2\) Franz Kummert\(^1\)

\(^1\)Faculty of Technology, Bielefeld University  
P.O. Box 100 131, D-33501 Bielefeld  
{marko, franz}@techfak.uni-bielefeld.de

\(^2\)Faculty of Biology, Bielefeld University  
D-33594 Bielefeld  
nickels.jensen@genetik.uni-bielefeld.de

Abstract

In order to localise tagged proteins in living cells, the surrounding cells must be recognised first. Based on previous work regarding cell recognition in bright-field images, we propose an approach to the automated recognition of unstained live *Drosophila* cells, which are of high biological relevance. In order to achieve this goal, the original methods were extended to enable the additional application of an alternative microscopy technique, since the exclusive usage of bright-field images does not allow for an accurate segmentation of the considered cells. In order to cope with the increased number of parameters to be set, a genetic algorithm is applied. Furthermore, the employed segmentation and classification techniques needed to be adapted to the new cell characteristics. Therefore, a modified active contour approach and an enhanced feature set, allowing for a more detailed description of the obtained segments, are introduced.

1 Introduction

Since the beginning of the 20th century, the fruit fly *Drosophila* has been an object of intense study [10]. With their investigation of *Drosophila melanogaster*, Thomas Hunt Morgan and his co-researchers laid the foundations of modern genetics. But the interest in *Drosophila* has not waned, yet. Besides using fruit flies in order to examine the development of organisms [15], specialised cell lines are frequently utilised, for example, for studies concerning host–pathogen interactions [2].

In recent years, the complete nucleotide sequences of the genomes of various species including *Drosophila melanogaster* have been determined [1]. Although we have read the genetic message of these organisms, we still do not know its meaning. Based on hereditary information, proteins are formed, which are required for a great variety of functions such as assembling biological structures and controlling chemical reactions. Unfortunately, the functions of the majority of the genetically encoded proteins are unknown. The automatic subcellular localisation of proteins in live cells constitutes an important method for the acquisition of knowledge about these proteins [4]. Based on the localisation, conclusions about a protein’s function can be drawn. This knowledge cannot only be applied to the analysis of cell behaviour, but also to the investigation of diseases and the development of innovative remedies.

In order to perform such a localisation, fluorescence microscopy is frequently employed [4, 8, 11]. In this, the proteins are tagged using a fluorescent dye, e.g. the green fluorescent protein [20]. This causes the cell compartments containing these proteins to show up as bright image regions (see Figure 1).

Unfortunately, the cells are not necessarily visible. Hence, the position of the surrounding cells must be determined. But the subcellular localisation of proteins imposes special limitations on any potential cell recognition method: Firstly, such an approach must not influence the result of an investigation. Secondly, the quality of the applied fluorescence micrographs should not be diminished. Thus, only a small number of the published cell recognition methods are applicable in conjunction with protein localisation.

2 Related Work

In order to account for the limitations, which have to be considered within the context of automatic protein localisation in living cells, Section 2.1 introduces and evaluates basic microscopy techniques frequently used in conjunction with cell recognition approaches. As the choice for a recognition method strongly depends on the utilised microscopy technique, the application of several well-known approaches, which are discussed in Section 2.2, is impeded.

2.1 Microscopy Techniques

A large number of cell recognition approaches such as [6, 17, 27] employ phase contrast microscopy to increase the contrast of acquired images. It visualises the phase shift...
induced by the interaction with objects varying in thickness or refractive index. Since this microscopy technique requires special objectives that reduce the amplitude of incident light, the light from fluorescent objects would be attenuated as well. An alternation of the objective between the acquisition of the images used for protein localisation and cell recognition causes further problems, since it modifies the optical path. Consequently, an association of corresponding pixels of these images would be hampered.

Besides phase contrast microscopy, numerous approaches require special dyes [11, 14, 16]. If they were used within the context of protein localisation, they might interfere with the examined proteins or influence the cell state.

Bright-field microscopy, i.e. the direct observation of illuminated objects, is a widely-used method for cell observation. It is usually available without any special devices. But the resulting contrast is rather low, which necessitates more complex recognition techniques [12, 18, 19, 22].

Differential interference contrast (DIC) microscopy displays local gradients of the phase shift between two neighbouring points. The resulting images may exhibit a better contrast in comparison to bright-field images, but are more difficult to interpret [25, 26]. The optical gradient results in transitions from bright to dark pixels giving objects an almost three-dimensional appearance, which does not necessarily correspond to their real shape.

Bright-field microscopy is probably the most frequently applied microscopy technique. Therefore, we have decided to use bright-field images as the basis of our cell recognition method. In addition, we have employed differential interference contrast (DIC) images, since they might reveal details that are not visible in bright-field images. Both techniques can be used in conjunction with fluorescence microscopy.

2.2 Cell Recognition Approaches

The most common approach to cell recognition probably consists in thresholding [5]. It is often applied to nuclei rather than whole cells [13, 23]. As each cell usually has a single nucleus, which covers the major fraction of its volume, these tasks are roughly equivalent.

Thresholding requires a uniform and unambiguous distribution of pixel intensities, which does not occur either in bright-field or in DIC images that show a great variety of cell appearances. In addition, thresholding causes problems in separating adjoining objects, which have to be dealt with separately. Here for example, the distance transform and the watershed transform can be applied [13, 23]. Nevertheless, the prior binarisation of the image leads to a loss of information, which might be crucial for the determination of the objects’ exact boundaries.

As an alternative to thresholding, there are approaches that determine and link the edges of stained nuclei using geometrical constraints [16]. Unfortunately, these constraints do not necessarily reflect the shape of visible objects – especially if these objects partially overlap.

Since subcellular structures are to be analysed after cell recognition, a high magnification (60 ×, 1 µm equals 6.45 pixels) is required. So, the considered Drosophila cells (S2R+) [24] comprise between 3,000 and 25,000 pixels. Therefore, methods utilising rectangular patches in order to detect whole cells (cf. [12, 14]) cannot be employed, as the computational costs would be too high. So, for example, the approach proposed in [12] takes 1 to 8 minutes to recognise cells in relatively small images (640 × 480 pixels) using a patch size of 625 pixels on an Intel Pentium 4 processor operating at 1.6GHz.

Cells in bright-field and DIC microscope images are separated from other cells and the surrounding by their membrane. Consequently, it is beneficial to include information about it in the segmentation procedure. This can be accomplished by determining cell membrane pixels and linking them [3, 26]. But, in the case of images containing numerous cells of varying shape or size, it is difficult to obtain unambiguous solutions.

As an alternative to edge linking methods, parametric active contours, or rather snakes, have proven advantageous [17, 18, 27]. Besides exploiting gradient and image information, they allow for the incorporation of prior knowledge on cell features such as curvature and size without assuming a rigid model. Therefore, we decided to use a snake algorithm. As the basis of our work, we applied an approach enabling the automatic recognition of cells originating from the fall army worm Spodoptera frugiperda (Sf9) that we had introduced before. This method is divided into two principal steps: the localisation and segmentation of possible cells [18] as well as the classification of the resulting segments into cells and other image regions [19]. Nevertheless, our Drosophila melanogaster cells are noticeably more difficult to recognise than the Sf9 cells, which are separated from their surrounding by a clearly visible cell membrane (see Figure 2). Thus, extensive adaptations, in particular the in-
corporation of an additional DIC image by means of a genetic algorithm, a modified snake energy functional, and a more meaningful feature set were required (see Sections 3 and 4).

3 Localisation and Segmentation

In order to obtain initialisations for the segmentation procedure, several preprocessing steps must be performed (see Figure 3). At first, the image background is separated from areas containing objects such as cells. This is realised by examining local variations and subsequently applying a dynamic threshold. Then, probable membrane pixels are derived from the gradient magnitude image by means of an algebraic opening utilising linear structuring elements of a specific length \( l \). Pixels with an intensity larger than a small threshold \( \tau_m \) are considered as membranes. Based on these two images, the cells are localised; i.e. small regions within possible cells are determined – the cell markers. They have an approximate diameter \( d_m \), and constitute the initialisations of the actual segmentation procedure.

The segmentation is performed by means of active contours. Since our approach aims at complete independence from user interactions while processing images, special requirements have to be fulfilled. In order to enable the growing of the snakes, the minimum distance to the corresponding cell marker is incorporated. Equation (1) shows the employed energy functional \( E_{\text{snake}}^* \), which assesses the snake represented by a parametric curve with arc length \( s \). During the segmentation, \( E_{\text{snake}}^* \) is minimised.

\[
E_{\text{snake}}^*(x(s), y(s)) = \int_0^1 \left[ \alpha E_{\text{cont}}(x(s), y(s)) + \beta E_{\text{curv}}(x(s), y(s)) \\
+ \gamma \left( E_{\text{dist}}(x(s), y(s)) \right) E_{ao}(x(s), y(s)) \\
+ \delta \left( E_{\text{dist}}(x(s), y(s)) \right) E_{\text{dist}}(x(s), y(s))^\kappa \right] ds \quad (1)
\]

\( E_{\text{cont}} \) and \( E_{\text{curv}} \) control the continuity and curvature, respectively. \( E_{ao} \) represents the cell membrane image and \( E_{\text{dist}} \) the distance from the initial contour. As the energies are minimised, the image as well as the distance have to be inverted. Thus, a distance \( \Delta_{\text{max}} \) limiting the growth of the snake is required.

The parameters \( \alpha, \beta, \gamma, \) and \( \delta \) control the influence of the energy terms. With this, \( \gamma \) and \( \delta \) are modified dependent on \( E_{\text{dist}} \) (cf. Equations 2 and 3). Their base values are denoted \( \gamma_0 \) and \( \delta_0 \), respectively. Moreover, \( \kappa \) enables a non-linear modification of the distance energy, for instance, in order to account for high intracellular gradient magnitudes.

\[
\gamma \left( E_{\text{dist}}(x(s), y(s)) \right) = \gamma_0 \cdot \frac{\Delta_{\text{max}} - E_{\text{dist}}(x(s), y(s))}{\Delta_{\text{max}}} \quad (2)
\]

\[
\delta \left( E_{\text{dist}}(x(s), y(s)) \right) = \delta_0 + \gamma_0 - \gamma \left( E_{\text{dist}}(x(s), y(s)) \right) \quad (3)
\]

In the case of a snake having a great distance to its initialisation, \( E_{\text{dist}} \) is small. Therefore, high pixel values near the cell markers within the cells are suppressed due to Equation (2). Equation (3) ensures that the extending force is reduced if the snake reaches a distance from its cell marker where the probability of membrane pixels is high. Additionally, background pixels receive a high value of \( E_{\text{dist}} \), in order to avoid an extension of the snake in this region.

If \( \kappa \) is set to one, this method equals the original segmentation technique for Sf9 cells [18]. But due to the new cell characteristics and the consideration of two instead of one
microscope image, the relevant parameters must be chosen in a different way (cf. Sections 3.1 and 3.2). The corresponding results are contrasted in Section 3.3.

3.1 The Original Segmentation Method

A total of seven parameters must be set in order to employ the original approach proposed in [18] to an image. As only the relation of the energy weights is relevant for the greedy approach used, $\alpha$ can be chosen arbitrarily. $\beta$, $\gamma$, and $\delta$ are determined by sampling and a subsequent exhaustive search. $\Delta_{\text{max}}$ is set to 120% and $d_{\text{m}}$ to 5% of the maximum cell radius. $\tau_{\text{m}}$ equals a small fraction (one fourth) of the maximum pixel intensity so as to suppress background noise after the algebraic opening. Finally, the length $l$ is derived automatically from manually extracted cells, which are referred to as cell masks.

3.2 Adaptation to Drosophila Cells

The application of the original active contour approach to bright-field images of S2R+ cells resulted in significantly worse segmentations in comparison to Sf9 cells (cf. Section 3.3). Consequently, several crucial adaptations had to be performed. Firstly, the segmentation is computed in two corresponding images: a bright-field image and a DIC image (see Figure 4). Each of these images reveals details that are not visible in the other. DIC microscopy increases the overall contrast but results in discontinuous intensity levels of cell membranes, as local gradients are displayed. On the other hand, the cells are less visible in bright-field images, but pixels showing cell membranes exhibit a lower variance.

Since the parallel segmentation of two microscope images as well as the incorporation of the exponent $\kappa$ in the energy functional (see Equation 1) increase the number of parameters to be set, an exhaustive search is not possible. Therefore, we resorted to a genetic algorithm, in order to reduce the computational load. Genetic algorithms employ biologically motivated mechanisms such as selection, mutation, and recombination to a set of possible solutions [7]. The characteristics of these solution candidates, referred to as individuals, are encoded by their genome. For our task, the genome $g_i$ of an individual $i$ consists of the relevant parameters for the segmentation of both types of images (see Equation 4).

\[
g_i = \left( \beta_{\text{bf}}, \gamma_{\text{bf}}, \delta_{\text{bf}}, \Delta_{\text{max}}^{\text{bf}}, \kappa_{\text{m}}, \tau_{\text{m}}, d_{\text{m}}^{\text{bf}}, \beta_{\text{dic}}, \gamma_{\text{dic}}, \delta_{\text{dic}}, \Delta_{\text{max}}^{\text{dic}}, \kappa_{\text{m}}, \tau_{\text{m}}, d_{\text{m}}^{\text{dic}} \right)
\]

In order to enable the application of a genetic algorithm, the quality of the segmentation must be assessed first. Here, we utilise the shape-dependent error measure $d_j$ which was introduced in [18]. It is proportional to the maximum distance $d_{j,\text{max}}$ of an automatically determined segment $j$ to a reference region, which is called a cell mask (see Equation 5). The distance $d_{j,\text{max}}$ is divided by the size of the cell represented by the length $b_j$ of a segment’s semi-major axis, which is computed using ellipse fitting. So the comparison of cells with different sizes is facilitated.

\[
d_j = \frac{d_{j,\text{max}}}{b_j}
\]

Since each cell is visible in both of the considered images, one segment has to be chosen for the evaluation. Furthermore, in order to recognise a cell, its correct segmentation in one image is sufficient. Hence, we decided to select the minimum error (cf. Equation 6).

\[
d_{j,\text{both}} = \min\left(d_{j,\text{bf}}, d_{j,\text{dic}}\right)
\]

Based on $d_{j,\text{both}}$, the evaluation of an individual $i$ becomes possible. Each individual is associated with a fitness value $f(g_i)$ computed according to Equation 7. This fitness function penalises high segmentation errors and, weighted by the constant $c_{\text{m}}$, the number of undetected cells, as these cells cannot contribute to the segmentation error. Therefore, the genetic algorithm tends to select individuals performing an accurate segmentation of a large number of cells.

\[
f(g_i) = 1 - \frac{1}{n_c} \sum_{j=1}^{n_c} d_{j,\text{both}} - c_{\text{m}} \left(1 - \frac{n_c}{n}\right)
\]

In order to cope with slight differences in the fitness values of the population, rank-based selection is applied. Furthermore, arithmetic cross-over\(^1\) and mutation\(^2\) for continuous-valued genes are utilised [7], as the genome consists of a sequence of numeric values representing specific parameters of the approach.

\(^1\)cross-over probability: 0.1
\(^2\)changes sampled from $\mathcal{N}(0, 0.025^2)$
3.3 Results

In order to evaluate our approach, mean, standard deviation, and median of the segmentation error $\sigma_{\text{both}}^2$, as well as the numbers of determined cell markers and localised cell masks, were compared with the results of the original segmentation method based on 20 image pairs ($1344 \times 1024$ pixels). From these images, 489 cells had been extracted manually. As the original technique is only able to process single images, independent experiments using either bright-field (BF) images or DIC images were conducted. In addition, investigations of the segmentation using an alternative value for $\kappa$ were carried out.

All examinations were performed applying cross-validation. The parameters for the original method were determined for 19 images and tested on the remaining one. In contrast, the genetic algorithm was evaluated using five sets of four images each: four training sets and one test set. So the computational effort for the optimisation decreased. The final values, which are shown in Table 1, were then obtained by averaging over subsequent trials using different test and training images.

<table>
<thead>
<tr>
<th>method</th>
<th>$n_m$</th>
<th>$n_c$</th>
<th>$\mu$</th>
<th>$\sigma$</th>
<th>$m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>original, BF</td>
<td>1660</td>
<td>467</td>
<td>0.266</td>
<td>0.156</td>
<td>0.222</td>
</tr>
<tr>
<td>original, $\kappa=2$, BF</td>
<td>1660</td>
<td>467</td>
<td>0.214</td>
<td>0.132</td>
<td>0.169</td>
</tr>
<tr>
<td>original, DIC</td>
<td>1190</td>
<td>443</td>
<td>0.250</td>
<td>0.149</td>
<td>0.214</td>
</tr>
<tr>
<td>original, $\kappa=2$, DIC</td>
<td>1190</td>
<td>443</td>
<td>0.215</td>
<td>0.133</td>
<td>0.185</td>
</tr>
<tr>
<td>gen. algorithm</td>
<td>1906</td>
<td>469</td>
<td>0.153</td>
<td>0.101</td>
<td>0.119</td>
</tr>
</tbody>
</table>

Table 1. Number of determined markers $n_m$, number of localised cells $n_c$ (max.: 489), as well as mean $\mu$, standard deviation $\sigma$, and median $m$ of the segmentation error.

Intriguingly, the number of cell markers yielded by the method using the genetic algorithm is much lower than the sum of the markers found by the original approach in bright-field and DIC images. As a result, the total computational effort for processing these images in parallel is only moderately higher than for the usage of either image type.

The segmentation results of the original approach are not satisfying; either in bright-field or in DIC images. By comparison, mean errors between 0.09 and 0.14 were observed using Sf9 cells [18]. Values of $\kappa$, which are higher than 1.0, entail a decreased importance of intracellular structures during the segmentation. So, the segmentation errors of the original approach could be reduced with respect to both types of images using $\kappa=2$. However, only the genetic algorithm enabled results lying in the range known from Sf9 cells. The median, in particular, indicates that the majority of these cells is segmented properly.

4 Classification

After the segmentation, the resulting segments in each image, bright-field and DIC, need to be analysed. Some segments represent real cells whereas others do not. Therefore, the segments are classified as ‘cells’ and ‘non-cells’, respectively. Unfortunately, it is not possible to acquire representative training samples for every kind of non-cell which might occur. Hence, the employed classifier must be capable of rejecting unknown samples, which are not similar to the training data. The simplified fuzzy ARTMAP (SFAM) [21] has proven advantageous for this purpose and has performed even better than a support vector machine-based approach [19]. It is introduced in Section 4.2. The employed features and the methods utilised for generating appropriate datasets are addressed in Section 4.1.

4.1 Features and Datasets

The original feature set, which was tailored to the recognition of Sf9 cell, encompasses some shape features and numerous histogram-based features like statistical moments and quantiles. They are computed for several segment-specific image regions: the whole segment, its core, and two overlapping tubes centred around the segment’s contour with radii of 5% and 10% of the mean cell diameter, respectively. In order to enable the usage of S2R+ cells, these image regions had to be modified (see Figure 5).

Intriguingly, the number of cell markers yielded by the method using the genetic algorithm is much lower than the sum of the markers found by the original approach in bright-field and DIC images. As a result, the total computational effort for processing these images in parallel is only moderately higher than for the usage of either image type.

Because of the higher number of considered image regions, 144 instead of 107 basic features are computed. These feature sets were reduced in three steps according to the original classification approach; i.e. correlation analysis, principal component analysis, and independent component analysis [9] were performed consecutively.
Since the manual segmentation of cells, which are required as training data, is very laborious, the number of training samples was increased as suggested in [19]. Several images were segmented using different values for the snakes’ energy weights. Provided a resulting segment showed a high similarity to the corresponding manually extracted cell (error<10% of the cell’s size), it was considered as cell as well. In addition, very dissimilar segments (error>33%) were regarded as non-cells. Here, we apply \( d_j \) as error measure whereas the original method employs the minimum of \( d_j \) and the fraction of incorrectly segmented pixels [19], which is more sensitive to variations and results in the rejection of numerous properly segmented S2R+ cells. Besides this automatic generation of non-cell segments, examples of non-cells were manually selected in segmented images. So, manually as well as automatically acquired samples were available.

### 4.2 The Applied Classifier

The SFAM enables fast on-line learning of new inputs [21]. This is important, as new data are to be integrated during the application of our approach. An SFAM network encompasses three layers (see Figure 6).

![Image of Three-layered structure of the SFAM. The layers are denoted by F0, F1, and F2.](image)

The first layer \( F0 \) performs a suitable encoding of the input vector \( x(t) \) called complement coding. The resulting vector \( x^{F1}(t) \) constitutes the input of the subsequent layer \( F1 \). The nodes of the output layer \( F2 \) are linked to all nodes of the \( F1 \) layer. The corresponding weights \( w^{F2}_j(t) \) define hyper-rectangular subspaces of the input space – the categories. In addition, each \( F2 \) neuron is associated with a class label. As the SFAM is an incremental network, there are neurons, which are not in use but required for an extension of the network – the uncommitted nodes.

After a new training sample \( x(t) \) has been presented and complement coded, the \( F2 \) nodes are activated according to Equation 8.

\[
z^{F2}_i(t) = \frac{|x^{F1}(t) \wedge w^{F2}_j(t)|}{\alpha + |w^{F2}_j(t)|} \tag{8}
\]

The parameter \( \alpha \) should be set slightly higher than zero. This enables small categories to be preferred to large ones. ‘\( \^ \)’ symbolises a point-wise minimum operation. The norm \( | \cdot | \) for \( n \)-dimensional vectors \( x \) is defined as follows:

\[
|x| = \sum_{k=1}^{n} |x_k| \tag{9}
\]

After all \( F2 \) nodes have been activated, the best-matching category corresponding to the node \( j \) with the highest activation and the correct class label is selected. But it is only allowed to grow and enclose the new input if the vigilance criterion is fulfilled (see Equation 10). Thus, the category size is limited by the vigilance parameter \( \rho \).

\[
\frac{|x^{F1}(t) \wedge w^{F2}_j(t)|}{|x^{F1}(t)|} \geq \rho \tag{10}
\]

Assuming neuron \( j \) was not able to fulfil the vigilance criterion, its activation is reset, a new best-matching node is chosen and its weights are adapted. If no suitable node is available, an uncommitted node is selected and associated with the input’s class label.

In order to classify new patterns, the activation of all \( F2 \) nodes is computed and the output of the best-matching node \( y_j(t) \) is set to its class label. The other \( m-1 \) outputs are set to \(-1\). A classification result \( c(t) \) can then be determined:

\[
c(t) = \max_{i=1,...,m} y_i(t). \tag{11}
\]

For our task, a measure specifying whether an input is known or unknown is required. Furthermore, it would be beneficial to have information on the degree of knowledge; e.g. in order to merge the results from bright-field and DIC images. The activation \( z^{F2}_i(t) \) cannot fulfil this task, since it varies depending on a category’s size (cf. Equation 8). Therefore, Equation 12 is employed. Here, \( z^{F2}_i(t) \) corresponds to the distance from an input to category \( i \).

\[
z^{F2}_i(t) = |x^{F1}(t) \wedge w^{F2}_j(t)| - w^{F2}_j(t) \tag{12}
\]

The minimum value \( z^{F2}_{i_{\min}}(t) \) of \( z^{F2}_i(t) \) over all nodes \( i \) indicates the degree of knowledge about an input. Assuming \( z^{F2}_{i_{\min}}(t)=0 \), the input is completely known. Higher values correspond to less knowledge. However, an input which is close to a category is likely to be representable by it. Therefore we have introduced a threshold \( \tau \) which denotes the maximum distance an input is considered as being known.

If an input is to be classified, the outputs \( y_i(t) \) of all \( F2 \) nodes \( i \) are set to \(-1\). Then, the input is complement coded and all \( F2 \) neurons are activated according to Equation 12.
In the case that $t_\text{min}^2(t)$ is smaller than $\tau$, the output $y_i$ of the corresponding neuron $i$ receives its class label. Otherwise, $y_i$ remains unchanged. $c(t)$ now yields a class label for known inputs and $-1$ otherwise (see Equation 11).

### 4.3 Classification Results

The evaluation of our classification approach was performed using 489 images of single cells (cf. Section 3.3). In addition, non-cell segments that had been manually selected in bright-field (961) as well as DIC (1,144) images were utilised. Since the resulting number of training samples was too small, about 40,000 additional segments depicting both cells and other image regions were generated automatically (see Section 4.1).

Table 2 contrasts the results obtained by the original classification method proposed in [19] with our approach utilising both bright-field and DIC images. Although the training of the classifiers took place using manually and automatically generated samples, the evaluation is solely based on manually determined segments, as their biological relevance is higher. Therefore, all results were obtained by means of 10-fold cross-validation with respect to the manually extracted segments insofar as each test set encompassed only cells which had not been used for training.

<table>
<thead>
<tr>
<th>method</th>
<th>features</th>
<th>fnr</th>
<th>fpr</th>
<th>acc</th>
</tr>
</thead>
<tbody>
<tr>
<td>original, BF</td>
<td>24</td>
<td>0.513</td>
<td>0.033</td>
<td>0.805</td>
</tr>
<tr>
<td>original, DIC</td>
<td>28</td>
<td>0.470</td>
<td>0.016</td>
<td>0.848</td>
</tr>
<tr>
<td>adapted, BF</td>
<td>33</td>
<td>0.247</td>
<td>0.058</td>
<td>0.878</td>
</tr>
<tr>
<td>adapted, DIC</td>
<td>37</td>
<td>0.282</td>
<td>0.032</td>
<td>0.893</td>
</tr>
</tbody>
</table>

Table 2. Classification results. False negative ratio (fnr), false positive ratio (fpr), and total accuracy (acc) for the respective classifiers.

Besides the classification errors, Table 2 shows the numbers of features used. These numbers resulted from the feature reduction step outlined in Section 4.1. The basic feature sets comprised 107 (original approach) and 144 (adapted technique) features, respectively. As reported in [19], the feature reduction caused a slight improvement of the classifiers’ accuracies.

In contrast to its application to Sf9 cells where accuracies up to 95% were reported, the original approach achieved only moderate results. This is likely to originate from the segmentation procedure, whose errors are increased due to the more complex appearances of the considered S2R+ cells. Our modified feature set seems to compensate for this effect to a certain degree, but not completely. As we apply two classifiers in parallel, this constitutes no drawback. It rather results in an increased importance of the false positive ratios (fprs) which equal the resulting error when added together in the case of both microscopy techniques being combined. However, even using this pessimistic estimation, the fpr does not reach values higher than 10%. More realistically, smaller false positive ratios can be expected, as only a subset of the observable objects is found in each image. So, our classifiers can be employed for cell recognition in the context of protein localisation. Admittedly, our method caused an increment of the fpr. But these fprs can be justified, as the original classification method leads to extremely low recognition rates (see Section 5) caused by very high false negative ratios (cf. Table 2).

### 5 Overall Performance

After the classification, the segments obtained from both images need to be combined in order to ensure that one cell is represented by not more than one segment. Therefore, segments, which were classified as cells and cover similar image regions, are associated. Then, the segment yielding the lowest distance $z^2_{\text{min}}(t)$ is selected (see Figure 7).

On the whole, 76.9% of the 225 reference cells, which had been manually extracted from ten test image pairs (1344×1024 pixels), were found by means of the suggested method; i.e. a sequential application of the proposed localisation, segmentation, and classification techniques. With this, the processing of an image pair took about 26.5s on an AMD Athlon 64 CPU (2GHz) and the mean segmentation error amounts to $\mu_{\text{class}}=0.141$. Using $d_j<12.5\%$ as criterion for the generation of additional training data, even 81.3% of the cells were recognised. Unfortunately, this modification caused a rise of the fprs by 100%. Therefore, it was rejected.

In contrast, the original approach that solely applies bright-field images recognised only 36.0% of the test cells. This is mainly a result of the bad segmentation, since inaccurately segmented cells are rejected as non-cells. On the other hand, the average error of the recognised cells is rather low ($\mu_{\text{class}}=0.131$).
6 Conclusion

We have presented a fast method (cf. Sections 2.2 and 5) for recognising unstained living Drosophila cells that are of the utmost interest for biological research. It is based on methods developed for the analysis of bright-field images containing Sf9 cells. But the original methods were noticeably outperformed with respect to the new cell type. Considering the irregular shape and structure of various cells, the obtained recognition rate of 76.9% is very good and totally sufficient, as each image pair contains multiple cells (usually more than 20). Based on our approach, new techniques for the automatic subcellular localisation of proteins in S2R+ cells can be developed in the future.

References