

# Working Report on the Use of Mathematical Morphology for Blood Smears Processing

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**Abstract**—Screening, reading manually from a slide, an activity which involves subjectivity and is tedious for the operator. The increasing number of drugs in testing makes it necessary to automate or semi automate the process. We propose an automated method for malaria thin blood smear inspection based on mathematical morphology tools operated on the grayscale image. The system is presented without a validation scheme and possibilities for the validation are proposed. Future work is outlined.

**Index Terms**—Mathematical Morphology, Microscopy, Drug Screening, Blood Count,

## I. INTRODUCTION

The development of new and effective drugs is a growing need in the modern world. In the developed countries aging populations with new chronic diseases represent a heavy load on the health system. In the poor countries diseases such as malaria is responsible for around 1'000,000 deaths per year. Furthermore, extended human mobility and the development of drug resistance makes old problems like tuberculosis resurface. According to the Pharmaceutical Research and Manufacturers of America Foundation (PhRMA) spending in R&D has risen exponentially in the later years and continues to rise. In 2002, PhRMA member companies invested an estimated \$32 billion on research to develop new treatments for diseases - an estimated 18.2 percent of domestic sales on R&D - a higher R&D to sales ratio than any other U.S. industry.

Although there is a great variety of drugs and active compounds being tested, with the number growing with the use of new and extended datasets (genomic, proteomic, etc...), the biotechnology and pharmaceutical industries find a bottleneck in clinical and preclinical research. It is still up to an expert to qualitatively assess large amounts of visual input, as live cell cultures or tissue samples, to determine the toxicity or effectiveness of a drug. In many cases an expert microscopy technician or pathologist must evaluate slight changes in the morphology and colour of cells by screening hundreds of them. This is a demanding work both physically and in time and gives high inter and intra observatory variability due

to different judgements, fatigue and diagnostic drift.

The present paper describes the techniques used in the development of an automatic cell counter based on giemsa stained images of plasmodium (malaria) infected blood smears. The aim is set the basis for a system that will return the parasitemia figure (number of infected blood cells over total red blood cell count) by correctly segmenting and separating erythrocytes and segment the plasmodium parasites within them. The parasites will be ready to be classified, either by a human operator or by an automatic system, as reported in [2]. The Work is divided in three parts. In procedure (II) we describe the work done in the cell and parasite segmentation. In Validation (III) we present the future work line for the validation of the method.

## II. PROCEDURE

### A. Cell Mask and Shading Correction

A large amount of literature has been dedicated to the automatic or semiautomatic segmentation and tracking of individual organs. Microscopy images and cell segmentation is an area in which much research is still to be done. In this type of images there is an undetermined amount of compact elements (cells), which must be separated from the background and individually identified.

In previous greyscale methods of separation and classification, erythrocytes are assumed either as already segmented or as having a characteristic intensity between that of the background (lighter) and that of the nucleus (darker) ([1] [2]). This does not hold for images in which staining or illumination is severely uneven, i.e. images that have to be equalized retrospectively due to the lack of calibration images or in which tincture has deposited. We have partially overcome this by the use of morphological illumination correction. Morphological correction is a non-linear filtering based on: a. objects in the scene are smaller than a given size and b. The shading correction may be represented as slowly changing (Low frequency) additive or multiplicative noise [3]. The background is found by doing morphological openings (if the objects are dark) or closings (if the objects are bright)

using a structuring element bigger than the object.

Normal peripheral red blood cells do not present large size variability, except for haematological medical conditions. One may assign the size of the structuring element by multiplying the normal radius (around 7  $\mu\text{m}$ ) by the magnification and the pixel resolution. [2] use greyscale granulometry to calculate the radius.

Results with and without shading correction are showed. If the object's size is big compared with the image, we will have a problem of sharp intensity transitions. Another disadvantage of the morphological correction is that if cells form clusters then the object size is actually bigger than the cell size, spurious edges will appear in the deepest parts of the cluster (Fig.1).

### B. Nucleated Elements Segmentation

Bright spots in the image correspond to depositions of giemsa in elements with a nucleus (ADN) such as white cell nucleus (not considered in the present work), malaria parasites or grunge. Red blood cells are not nucleated. The segmentation of these nucleic elements is important from a medical point of view to identify the parasite type, state of development and degree of infection. In bone marrow blood samples white blood cells are usually the main interest zone and extensive literature has been devoted to their segmentation and classification. From the image analysis point of view to "remove" these zones in the analysis of the image will "highlight" the erythrocytes statistical and topological features, making their segmentation and classification robust.

Immature plasmodium parasites (those that have not completely invaded the cell or ruptured it) may be regarded as small dot-like or slightly elongated elements. These elements may be segmented by closing the image with line structuring elements approaching the cell radius in different orientations. The filtering scheme is explained in [4] and consists on choosing the minimum of

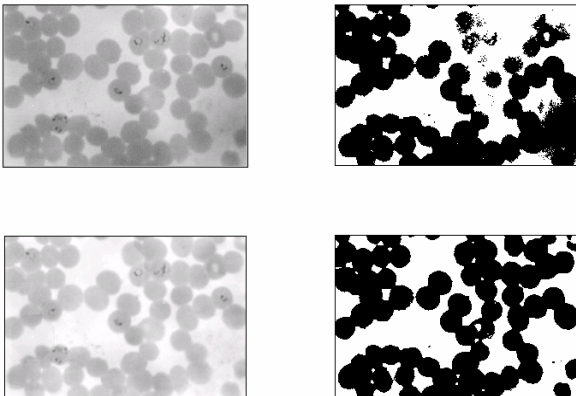


Fig.1 Up, Original Image and binarization. Down, Image after shading Correction

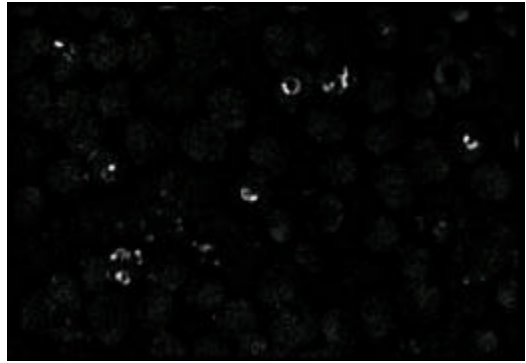


Fig. 2 Morphologically filtered parasites

the values from the multidirectional closings at each point. The results are shown in Fig 2.

The difference image between the filtered and original image will present low and extended noisy behaviour (due to texture) and high-localized values due to nucleated elements. Either by combining automated intensity and size elements or by applying high thresholds parasites in the image are localized.

### C. Morphological Separation

Overlapped and cluttered cells are an inevitable, unsolved, and usually ignored problem in blood slide analysis. It is up to the technician to choose an ideal work area in the smear where the cells are neither too cluttered nor too dispersed. In the more dispersed area the cells extend due to the lack of pressure and lose characteristic morphology and in the cluttered area they are indistinguishable one from the other. [5] have proposed automated criteria for the choosing of an ideal area. Our approach has been to use the morphology of the background-cell border as an initial approach to the cells forms, using a priori knowledge of the cell. We later make use of local information, such as edges or greyscale connectivity, in a top down segmentation scheme to refine the classifying and find cells deeper down the cluster.

The watershed algorithm has been widely used as it subdivides the image in catchment basins and clusters together pixels based on spatial proximity and similarity of the gradient. We have applied the unmarked watershed algorithm to the distance image transformation of the complement of the cell mask. In trivial cases (single cells or extremely acute bottlenecks) it will produce a proper separation of the cells, usually it will over segment. It will, however, group borders that define convex regions as local maxima in a distance image are the centres of a locally convex regions, using convex in the traditional sense for an Euclidean 2D space. Regions that agree with certain shape criteria (i.e. compactness, size, topological constraints) are then used to approximate ellipses. Circles have also been used due to ease of parameterisation, but they may be seen as special cases of ellipses and in



Fig.3 Watershed Pixel Classification

general do not make a good approximation.

#### D. Ellipse Approximation

Ellipse approximation is a difficult problem by itself and goes beyond the scope of this report. We have used the algorithm proposed by [6]. The points employed in the approximation are the points pertaining to the cell-background border, as they retain the larger amount of information. Fig shows the results of the ellipse approximation to the cell. Only the axis that are within the rheological characteristics of normal red blood cells are considered.

#### E. Edge Adding

To this point we have only used a global characteristic, intensity, to classify the cells. Local characteristics, such as edges may help finding new cells and refining the approach. The initial use of an edge finder algorithm has not been pursued as many false edges are detected, mainly in the highly textured background and in the inner pallor of the red blood cells. Because of this we use only the edges found in the vicinity of the calculated ellipses. These edges are included in a new distance transformation

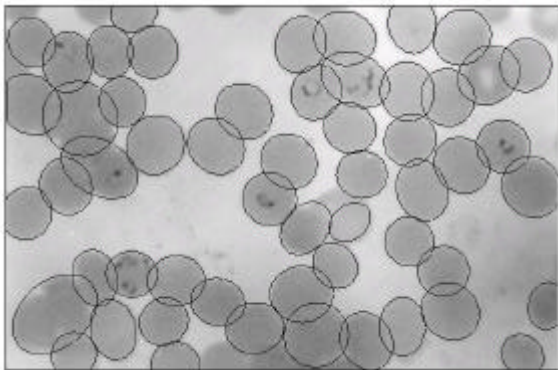


Fig. 4 Cell Separation. Some cells are misclassified together

when they serve to separate cells that exceed the size constraint. The results of this processing are shown in Fig. 5.

### III. VALIDATION AND FUTURE WORK

The refinement scheme may be repeated until idempotence. However, it is not guaranteed that it will converge to the desired answer. The main risk lies in separating a correctly segmented cell. We see that a local validation scheme is necessary, not only as an inner-parameter, but to define an optimal segmentation.

[7] define energy functions to be minimized for microscopic images. They try to minimize the energy functions by region splitting and merging and using an energy based on a a priori training model

$$E = E_{color} + aE_{area} + bE_{deform}. \quad (1)$$

$E_{color}$  is a region color compatibility term,  $E_{area}$  is a region/model overlap term,  $E_{deform}$  is the deformation energy term. [4] uses cost functions are based in Fuzzy rule relaxation and region similarities. These methods are slow and require the definition of step sizes. Future work in this area requires us to define a validation based on the cell and its vicinity. This must include: Feature (i.e. intensity, texture) homogeneity within the proposed cell, feature distinction with the background, level of connectivity of the cell with neighbouring cells, deformability based on cell physical characteristics and neighbouring cell positions, and edge localization. Both the recently defined greyscale connectivity and active seem to be the best suited to give an elegant and compact solution.

It must be added that original images were coloured. No attempt has been made to classify the objects either by colour or texture. Our aim is to develop a work methodology in which further regional information can be smoothly added to the morphological background.

### IV. CONCLUSIONS

We have presented an automated method for red blood cell counting declustering and plasmodium segmentation. Our method uses techniques based on mathematical morphology for cell size calculation, shading correction and to create a first approach to the cells form.

A method to approach the cell contour to an ellipse and the use of edge map info in the vicinity of this ellipse to refine it where explained.

Future approaches for the problem were outlined.

There is no model, of which we are aware of, that currently satisfactory describes blood cells for image proc-

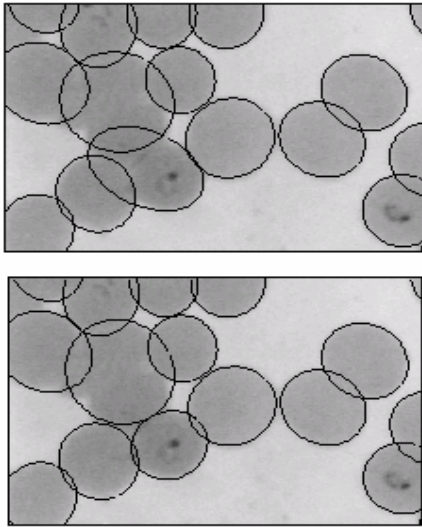


Fig. 5 Region Fine tuning by applying edge information.

essing uses. Descriptions are well suited for human operators but too loosely stated for machine operation. The present work tries to approach to a parameter free description of cells that may be expanded to other microscopic images.

The segmentation of microscopical images is neither trivial nor straightforward due to the natural irregularity

of biological structures. General machine vision algorithms and methodologies (like edge or corner detection) must be revisited in order to make them applicable to microscopic analysis.

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