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Detection of felt tip markers on microscope slides

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ABSTRACT

Sensitivity and specificity of conventional cytological methods for cancer diagnosis can be raised significantly by applying further adjuvant cytological methods. To this end, the pathologist marks regions of interest (ROI) with a felt tip pen on the microscope slide for further analysis. This paper presents algorithms for the automated detection of these ROIs, which enables further automated processing of these regions by digital pathology solutions and image analysis. For this purpose, an overview scan is obtained at low magnification. Slides from different manufacturers need to be treated, as they might contain certain regions which need to be excluded from the analysis. Therefore the slide type is identified first. Subsequently, the felt tip marks are detected automatically, and gaps appearing in the case of ROIs which have been drawn incompletely are closed. Based on the marker detection, the ROIs are obtained. The algorithms have been optimized on a training set of 82 manually annotated images. On the test set, the slide types of all but one out of 81 slides were identified correctly. A sensitivity of 98.31\% and a positive predictive value of 97.48\% were reached for the detection of ROIs. In combination with a slide loader or a whole slide imaging scanner as well as automated image analysis, this enables fully automated batch processing of slides.

Keywords: Multi modal cell analysis, k-means clustering, Otsu thresholding, EM-Algorithm, closing gaps

1. INTRODUCTION

Sensitivity and specificity of conventional oral cytology are reported to be 91.3\% and 95.1\% respectively.\textsuperscript{1} The diagnostic performance can be increased significantly by applying adjuvant methods. The workflow in the so-called Multimodal Cell Analysis (MMCA) is as follows: Cells from suspicious lesions are obtained non-invasively, for example by a brush smear. The cells are deposited on a glass slide, fixed and stained according to Papanicolaou. With this stain, the pathologist analyzes the cell and nucleus morphology. The pathologist marks regions with morphologically suspicious cells with a felt tip pen on the glass slide. The slides are restained using the Feulgen reaction, which allows a quantitative measurement of the nuclear DNA content of the suspicious cells. This increases sensitivity to 95.1\% and specificity to 100\%.\textsuperscript{1} Finally, the slide is restained with the AgNor stain, which marks "protein fabrics" in the nucleus, the so called nuclear organizer regions. In rapidly proliferating cancer cells, the number of these protein fabrics is increased. If this whole analysis sequence is applied, perfect sensitivity and specificity are reached.\textsuperscript{2}

In order to reduce the high workload of visual analysis for the pathological expert, both DNA Image Cytometry and AgNor analysis can be performed by image processing.\textsuperscript{3,4} Up to now, the ROIs for scanning have to be set up by manual interaction, which implies additional effort. More importantly, manual interaction is required before each individual slide is scanned. This hampers batch or overnight scanning and results in a low degree of capacity utilization of these devices.

We therefore present algorithms for the automated detection of felt tip markers on glass slides. These algorithms contain methods to identify the slide types from different manufacturers and the felt tip markers, to close incomplete felt tip markers and extract the regions of interest for scanning.

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2. METHODS

2.1 Equipment and Material

A Motic BA600 motorized microscope with a MotiCam285A RGB camera (1360×1024 resolution) was used for image acquisition. Overview scans of 163 slides with felt tip markers were acquired by scanning the slide at 2× magnification and stitching the images. Such an overview scan comprises 1541×489 pixels, where one pixel in the image corresponds to 0.051 mm on the slide. Six slide types were scanned: slides without manufacturer’s name, Motic slides, UniMark slides, Starfrost slides and two different slide types from Tharmac. Red, blue and green felt tip markers with a thickness of 0.6 mm were used for circumscribing ROIs. The regions of interest in the overview scans were manually annotated by marking the central part of each region and by assigning the slide type.

2.2 Detection of the Slide Type

In clinical practice, a pathological institute uses different slide types. Also it receives slides from various sites which again might use different types of slides. Figure 1 shows a selection of glass slides. The slides might contain the manufacturers name or already specify a region where cells are deposited. Parts of the name on the slide might be confused with felt tip markers (for instance the "R" from Tharmac, Fig. 1b). Felt tip markers should only be detected in the region where cells were deposited (also Fig. 1b). Alternatively, if no felt tip marking is present in this region, it needs to be scanned completely (Fig. 1c). All this must be taken into consideration during felt tip marker detection. Therefore, the slide type and the region in which the felt tip markers should be detected need to be identified first.

Detection of the slide type is realized by once scanning empty reference slides of each type. An expert then annotates two kinds of regions:

- The recognition region: A region comprising the features of the slide which are characteristic for the slide type. This region contains for instance the manufacturers name and markings for depositing cells. However the label region containing the case number is excluded, as this region is specific for the individual slide, but not the slide type.

- The detection region: Region in which felt tip markers should be detected.

These annotated slides constitute a slide reference database. For determining the type of an analysis slide, the normalized correlation of this slide with each slide from the reference database is computed, and the slide type which yields the highest correlation is assigned. The detection region of the reference slide is then transferred to the slide to be analyzed.
2.3 Detection of Felt Tip Markers

Next, the felt tip markers in the detection region are identified. In total, three algorithms have been used: A straightforward approach is to apply Otsu’s thresholding algorithm on the gray level image of the slide. If the cell density on a slide is high, the stained cells are also visible in the overview scan (see Fig. 1a). As the felt tip markers on an individual slide have the same color and differ from cells by a higher saturation, transforming the RGB image to the HSV-colorspace enables further possibilities for analysis. Therefore in another approach, a Gaussian distribution is fitted to all saturation values from the image below a value of 0.2 using an expectation maximization approach. Pixels are then marked as felt tip markers if they fall into the $1.64 \sigma$ neighborhood of the distribution (containing 90% of all samples). The third approach considers the hue and saturation component at the same time. A k-means clustering approach aims to detect two clusters, one containing the felt tip markers (high saturation, and a relatively constant hue) and one containing the rest of the image. The slide is segmented based on the assignment to these two clusters.

After detection, a post processing step is used for smoothing the detected markers and removing detected objects which are too small, too ragged or too thick to be felt tip markers: Smoothing is achieved by morphological dilation, using a circular structure element with 0.25 mm diameter followed by morphological erosion by 0.1 mm. The structure element for dilation is larger to create a small gap between felt tip marker and ROI. This ensures that the felt tip markers are not included in the fields of view during the final scanning of the slide. Felt tip marker objects smaller than 3 mm$^2$ are removed. The raggedness of the detected felt tip markers is quantified by computing the variance of the distance from each boundary point to the centerline of detected felt tip markers. If this variance is above 0.15, the object is removed. The same is true if the 90% quantile of these distances is above 1 mm. The thresholds for post processing have empirically been found on the training set.

In some cases, no felt tip marker is present in the detection region (Fig. 1c). This case is identified by checking if the threshold found by Otsu’s thresholding method is above 0.7 (double valued gray image) or if the total area of detected felt tip markers is lower than 5 mm$^2$. In both cases, the whole detected region is used for scanning and the intended ROI has already been found. But if felt tip markers are detected, the final output of the detection algorithm is the a binary mask where all detected pixels are marked as positive. This result is processed further for extracting the ROIs. Figure 2 displays the slide from Fig. 1b where the detection region and detected felt tip markers are shown.

2.4 Closing Boundary Gaps

In some cases, the ROI is not only not circumscribed by felt tip markers, but also by the boundary of the slide (see Fig. 3a for an example). In order to fully circumscribe the ROIs, these gaps need to be closed. Therefore, segments which belong to detected felt tip markers and at the same time touch the boundary of the detection region are extracted. The centroid of these segments is chosen as a point representing this segment. For all possible pairs of two such points, starting from the first point, the boundary of the detection region is traced into the direction of the second point. If the distance traced is smaller than 2 cm and if the two points belong to
Figure 3: Closing boundary gaps: In (a) an example is shown where the ROI is circumscribed by felt tip markings and the slide boundary at the same time. (b) Zoom of the square region in (a), demonstrating the closing of the boundary gap: Starting from the point $P_1$, representing one segment touching the boundary, the boundary is traced (yellow line with white arrows). As $P_2$ is close enough, the boundary is also marked as positive in the detection mask.

the same segment, they are connected. The pixels which connect these two points are also marked as positive in the detection mask.

2.5 Closing Incomplete Felt Tip Markers

Additional gaps in the detected felt tip markers might occur if the manual drawing is incomplete or if the color of the felt tip is too weak to be detected (see Fig. 4a). Three methods for closing these gaps are investigated:

In the morphological approach, morphological dilation by 1 mm is used, with the aim to connect objects which are close to each other. After dilation, the morphological skeleton is computed. If the dilation connects two disjoint objects, they are also connected in this skeleton. All pixels of original detection or the skeleton are therefore marked as positive in the detection mask. However, it might be more specific to close boundaries only in their predominant direction. In the linear approach, a line is fitted to the end points of the detected felt tip markers. The line is extended by 1 mm, and if another detected object is found within this range, the gap is closed by marking the pixels of the line as positive in the detection mask. Often, ROIs are circular or ellipse shaped. In the ellipse fitting approach, two endpoints of detected felt tip markers are connected by an ellipse which is fitted to the end segments. To this end, starting from the end points $e_1$ and $e_2$, the centerline of the detected felt tip markers is traced back by 5 mm. The points at every 0.5 mm are extracted, and an ellipse is fitted to these segments using the algorithm from Halir et al. If the distance between the ellipse points and the end points $e_1$ and $e_2$ is below 1 mm, the ellipse segment between $e_1$ and $e_2$ is marked as positive. Figure 4b exemplary shows how the ellipse fit can be used to close an incomplete felt tip marking.

As several different situations might occur, the combination of these algorithms is also examined.
Figure 4: Closing incomplete felt tip markers: In (b) it is shown how the gap within the square region of (a) is closed. The end points of the centerline of the detected felt tip markers are marked by magenta crosses. From there, the centerline is traced back and points on the way are extracted (cyan crosses). An ellipse (yellow) is fitted to these points and used for closing the detected felt tip markings.

2.6 Extracting Regions of Interest

After the detection of felt tip markers, and closing gaps at the boundary and due to incomplete felt tip markers, all ROIs should be circumscribed by a detected region. The regions of interest are extracted by applying a flood fill operation on the detected objects and subtracting the detected objects from this mask. Regions which are smaller than 0.75 mm² are non-valid ROIs and are therefore removed by a post processing step.

2.7 Evaluation Methods and Optimization of Parameters

For evaluating the detection of the slide type, the correct classification rate is used. For evaluating the detection of ROIs, a region of interest is considered to be detected (true positive) if it fully covers the manual annotation of this region. A detected region is marked as false positive if it does not fully cover any manual annotation. Two kinds of error are possible: If a region has been missed, the slide needs to be scanned again. If a superfluous region has been found, this unnecessarily increases the scanning time. The percentage of correctly identified regions is assessed by the sensitivity, and the percentage of detected regions which are truly positive by the positive predictive value (PPV).

The 163 scanned slides were separated into a training set of 82 and a test set of 81 slides. The parameters for the detection algorithm and the best combination of the algorithmic alternatives have been optimized by an exhaustive search on the training set. The F-Score, the harmonic mean of sensitivity and positive predictive value, is used as optimization criterion.
3. RESULTS
On the training set, the slide type could be identified in all cases. The best performance for detecting ROIs is achieved when using the following configuration:

- Detection of felt tip markers: Gaussian model on saturation values
- Connect parts touching the boundary: Activated
- Close gaps: Ellipse fit and linear model

The performance achieved is an F-Score of 98.83%, whereupon the sensitivity was 99.22% and the positive predictive value was 98.45%. Applied on the test set, all but one slide type could be identified correctly. Sensitivity and positive predictive value were 98.31% and 97.48%, respectively. Figure 5 shows examples of ROI detection.

4. DISCUSSION AND CONCLUSIONS
We have presented and optimized algorithms for extracting the regions of interest marked on microscope glass slides. The detection of these ROIs is relevant for the batch processing of cytological slides by image analysis. A two-step approach is used: First, the slide type is detected to identify slides from different vendors. Determining the slide type is based on the normalized correlation with slides from a manually annotated reference database. The correct classification rate for distinguishing six slide types on the test set was 98.77%. After the slide type has been determined, regions of interest are automatically detected in the second step. For this purpose, several
strategies for detecting felt tip markers and closing gaps have been implemented and optimized. Applied on the test set, it achieved a sensitivity of 98.31% and a positive predictive value of 97.48%. Figure 5 shows examples of successful detection of ROIs, and cases where the algorithm failed.

In the following, the algorithm’s failures are analyzed: Two relevant ROIs have been missed: In one case this was a direct consequence of the fact that the slide type was classified incorrectly and that the relevant ROI was not inside the detection region of the wrong slide type. In another case, an incomplete felt tip marker was not closed correctly because the endpoint at the right-hand side of the gap turns to the inside of the ROI and thus is too far away from the ellipse fit (Sec. 2.5 and Fig. 5f). Also, two superfluous regions have been detected: There are cases where the felt tip markings of two individual markings touching each other also touched the boundary of the detection region (Fig. 5e). Then the algorithm for closing the boundary gaps caused the detection of a spurious ROI. In all other cases, the ROIs were detected correctly.

To conclude, the presented algorithm enables the batch scanning of several slides using a microscope system with automated slide loader. Thus it greatly increases the degree of utilization for digital pathology solutions. As future work, we are planning to evaluate the algorithms on other scanners. In whole slide imaging systems overview scans are usually obtained by a macro camera. The resolution of these scans is lower as the images acquired for this work. It shall be examined if the high performance can be retained at lower resolution as well.

REFERENCES