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FAST VIRTUAL DESTAINING OF IMMUNOCYTOLICAL SPECIMENS

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ABSTRACT

Microscopy-based diagnosis of certain diseases or infections, e.g. with human papilloma viruses (HPV) for the identification of high risk patients for cervical cancer, relies more and more often on immunocytochemical marker stains. These markers stain cells that exhibit a particular protein. In addition to one or more marker stains, pathologists need to simultaneously assess the morphology of the cell. Therefore the specimens are commonly also stained with a counter stain.

For a reliable computer assisted analysis of such multistained images a virtual destaining of all but one of the stains is required. This can be achieved by a specific color transformation. The transformation depends on the staining colors, and is therefore in general non-orthogonal.

In this paper we analyze the performance of this color space conversion. Based on a reformulation of the problem we develop faster implementations. We analyze the complexity of these implementations and demonstrate that with a marginal additional memory footprint the virtual destaining can be calculated 5 – 10 times faster than the conventional method. This, on the one hand, speeds up the automated analysis. More importantly, it enables pathologists to view the virtually destained slides and change the color transformation interactively at high frame rate.

Index Terms—immunocytochemical marker, cytology, color separation, virtual destaining, cancer diagnosis

1. INTRODUCTION

For certain types of cancer and their precursor stages it could be shown that these lesions can be highlighted on the cellular level by disease-specific proteins within the cells. To this end, cell specimens are taken from the patient, stained and investigated with a microscope. The visualization of such proteins depends on highly protein-sensitive and protein-specific stains. Over the recent years an increasing number of such so-called immunocytochemical marker stains have been developed.

For example, nearly all cervical cancers are caused by human papilloma viruses (HPV) [1]. This infection in turn is directly linked to an upregulation of a protein. A specific corresponding antibody is docked to this protein within the infected and altered cell and stained with immunocytochemical techniques. For the case of cervical brush smears, so-called PAP smears, it has been shown that the p16-antibody identifies infections by HPV with high sensitivity [2]. Since the cell specimens are obtained non-invasively with a brush, the immunocytochemical marker enables reliable identification of high-risk patients in screening scenarios. Only patients with HPV infection have to be examined further by cytopathological means and resampling in short time frame. Still, to cope with the high load of specimen, an automation for the investigation of microscopical slides is of great benefit.

Automated detection of marker positive cells, however, is facilitated if marker-positivity and nuclei morphology could...
be individually investigated. A new form of investigation would be available, if the pathologist could also investigate virtually destained slides. In [5], a method is proposed which algorithmically separates images obtained from microscopic slides into stain-specific channels (see Fig. 1). The stains are separated by estimating their optical densities and decomposing the image into stain specific contributions. The contribution from undesired stains are removed and a new image is reconstructed, now only showing the desired stain.

Processing large images in this manner is time consuming. In this paper we discuss performance improvements for the algorithm proposed by Ruifrok [5]. Using a lookup table (LUT) for the computation of the optical densities increases the performance by a factor of two. Yet the whole color separation process can be precomputed and stored in a LUT, which increases the performance by a factor of 10. However, the calculation of the latter LUT is expensive in terms of time and memory usage, and it has to be recomputed after changes of the lighting conditions and changes in the stain colors. We reformulate the algorithm and show that high speedup and fast update of a LUT can be achieved simultaneously. Performance and scalability of the algorithms are analyzed and measurement results about the performance are given.

2. METHOD

The image formation process of specimens in bright field light microscopy can be described by the Lambert-Beer law for the attenuation of light passing through the specimen. Let the sensor response to directly incident white light, not attenuated attenuation of light passing through the specimen. Depending on the optical density of the material and the stain the light attenuation is described by

\[ f_I(OD) := I_w \cdot e^{-OD}. \] (1)

The optical density \( OD \) in turn is linearly related to the concentration of the stain in the specimen. Therefore, Lambert-Beer’s law (1) allows to measure the overall optical density of particular stained characteristics of the specimens.

2.1. Virtual destaining of immunocytological specimen

In the case of multiple stained specimens it is required to separate the images into stain specific channels. This enables measuring the optical density of each stain in the specimen individually. To this end Ruifrok [5] suggested a non-orthogonal color space transformation based on the measured color responses of the individual stains. For every acquired \( I = (I_R, I_G, I_B)^T \) vector, the corresponding optical densities \( OD \) are computed by inverting Eq. (1) given by

\[ f_{OD}(I) := f_I(OD)^{-1} = -\log(I/I_w), \] (2)

where \(^/\) is the component-wise division. Using this equation, RGB values corresponding to the individual stains can be transformed to their optical densities. The optical density vectors \( u, d, \) and \( n \) define the stain matrix \( \mathbf{M}_s \) as

\[ \mathbf{M}_s := (d, u, n). \] (3)

If only two stains are present on a slide, e.g., an immunocytochemical marker \( d \) (for desired) and counter stain \( u \) (undesired), the two vectors have to be complemented by a third vector \( n \) to span the RGB color space. For that case, we choose \( n = u \times d \) as the third spectral component. The matrix \( \mathbf{M}_s \) maps concentrations to optical densities. Consequently, the inverse \( \mathbf{M}_s^{-1} \) of \( \mathbf{M}_s \) transforms optical densities to the individual stain concentrations \( c_d, c_u, c_n \):

\[ c = \begin{pmatrix} c_d \\ c_u \\ c_n \end{pmatrix} = \mathbf{M}_s^{-1} f_{OD}(I). \] (4)

To virtually destain the image from one of the components (e.g., \( u \)) we set the corresponding concentration \( c_u = 0 \) by multiplication with the destain matrix \( \mathbf{M}_{dn} \) given by

\[ \mathbf{M}_{dn} = \begin{bmatrix} 1 & 0 & 0 \\ 0 & 0 & 0 \\ 0 & 0 & 1 \end{bmatrix}. \] (5)

The destain matrices \( \mathbf{M}_{du} \) and \( \mathbf{M}_{dn} \) are defined accordingly. A virtually destained slide can hence be calculated from the new concentration vector by reprojecting into optical densities with \( \mathbf{M}_s \) and converting from \( OD \) values to RGB values. The full virtual destaining is described by

\[ \hat{I}_{dn} = f_I (\mathbf{M}_s \mathbf{M}_{dn} \mathbf{M}_s^{-1} f_{OD}(I)). \] (6)

This can be written more compactly as

\[ \hat{I}_{dn} = f_I (\mathbf{M} f_{OD}(I)), \] (7)

where \( \mathbf{M} = \mathbf{M}_s \mathbf{M}_{dn} \mathbf{M}_s^{-1} = \mathbf{m}_{r,c} \) is a \( 3 \times 3 \) matrix for RGB images and \( r, c \in \{0, 1, 2\} \) are the row and column indices of the entries \( m_{r,c} \) in \( \mathbf{M} \).

This virtual destaining technique is well suited for stains with OD vectors \( d \) and \( u \) which are linearly independent and non-zero. Otherwise, and also when \( d \) and \( u \) are close to linear dependency, the inverse of the matrix \( \mathbf{M}_s \) is not defined or badly conditioned.

2.2. Fast implementations

In the original implementation acc. to Eq. (7), which we denote as “Ruifrok destaining” (A), all image pixels are individually converted to their optical densities, the undesired stain is removed and the result is transformed back to RGB values. To speed up this implementation we first note that the function \( f_{OD}(I) \) maps integer values to floating point numbers enabling an implementation with a lookup table (LUT). Given the sensor response \( I_w \) to the incident white light, all
This can be written as

\[ r \]

We now write Eq. (8) for one channel \( r \) giving

\[ \hat{I}_{dn} = I_w \cdot e^{M \cdot \text{log}(I_w/I_w)}. \]  

(8)

We now write Eq. (8) for one channel \( r \) giving

\[ \hat{I}_{d, r} = I_{w, r} \cdot e^{\sum_{c=0}^{2} m_{r, c} \cdot \text{log}(I_r/I_w, c)}. \]  

(9)

This can be written as

\[ \hat{I}_{d, r} = I_{w, r} \cdot 2^{c=0} e^{\text{log}(I_r/I_w, c)m_{r, c}}. \]  

(10)

As the logarithm is the inverse of the exponential, the equation can now be simplified to

\[ \hat{I}_{d, r} = I_{w, r} \cdot 2^{c=0} \left( \frac{I_r}{I_w, c} \right)^{m_{r, c}}. \]  

(11)

The exponents \( m_{r, c} \) in Eq. (11) are the elements of the matrix \( M \) and therefore depend on the optical densities \( d, u \) and \( n \) of the stains. The values for every product term \( (I_r/I_w, c)^{m_{r, c}} \) can now be pre-calculated and stored in a LUT. For a RGB image, for instance, this requires the computation of 9 LUTs. The RGB values for a virtually destained slide are then computed by looking up the factors in Eq. (11) in the corresponding LUT and multiplying them with each other and \( I_w \). We denote this algorithm as “Power destaining” (D).

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>double (64 bit)</th>
<th>complexity</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A) Ruifrok destaining</td>
<td>2660 ms</td>
<td>( \mathcal{O}(N_c \cdot 2^{N_b}) )</td>
</tr>
<tr>
<td>(B) OD-LUT calculation</td>
<td>&lt; 1 ms</td>
<td>( \mathcal{O}(N_c \cdot 2^{N_b} \cdot N_c) )</td>
</tr>
<tr>
<td>Ruifrok destaining OD-LUT</td>
<td>1460 ms</td>
<td></td>
</tr>
<tr>
<td>(C) Full-LUT calculation</td>
<td>2920 ms</td>
<td>( \mathcal{O}(N_c \cdot 2^{N_b} \cdot N_c) )</td>
</tr>
<tr>
<td>Ruifrok destaining Full-LUT</td>
<td>253 ms</td>
<td></td>
</tr>
<tr>
<td>(D) Power-LUT calculation</td>
<td>&lt; 1 ms</td>
<td>( \mathcal{O}(N_c^2 \cdot 2^{N_b}) )</td>
</tr>
<tr>
<td>Power destaining</td>
<td>545 ms</td>
<td></td>
</tr>
</tbody>
</table>

Optical densities which the sensor is able to capture can be pre-calculated with \( f_{OD}(I) \) from Eq. (2). For an 8 bit image, for instance, these are 256 floating point numbers for every color channel. The optical densities from the LUT are then used for the further computation of the virtually destained image in Eq. (7). We call this implementation “Ruifrok destaining OD-LUT” (B). Note, however, that for transforming the optical densities back to RGB values with \( f_I(OD) \), a straightforward LUT implementation cannot be given, since this function maps floating point numbers to floating point numbers.

Still, since Eq. (7) maps each RGB vector to a floating point RGB vector, it is possible to pre-calculate the destaining results for all RGB vectors. The calculation of this LUT depends on \( I_w, u, d, \) and \( n \). This LUT implementation maps the input for Eq. (7) directly to the color image output and consequently requires the computation of Eq. (7) for all possible combinations of camera sensor responses. We denote this implementation as “Ruifrok destaining Full-LUT” (C).

The computation of the Full-LUT has a high complexity (see Section 3). To achieve a lower complexity we reformulate the color separation algorithm. First, we rewrite Eq. (7) with \( f_{OD} \) and \( f_I \)

\[ \hat{I}_{dn} = I_w \cdot e^{M \cdot \text{log}(I_w/I_w)}. \]  

(8)

We now write Eq. (8) for one channel \( r \) giving

\[ I_{d, r} = I_{w, r} \cdot e^{\sum_{c=0}^{2} m_{r, c} \cdot \text{log}(I_r/I_w, c)}. \]  

(9)

This can be written as

\[ I_{d, r} = I_{w, r} \cdot 2^{c=0} e^{\text{log}(I_r/I_w, c)m_{r, c}}. \]  

(10)

As the logarithm is the inverse of the exponential, the equation can now be simplified to

\[ I_{d, r} = I_{w, r} \cdot 2^{c=0} \left( \frac{I_r}{I_w, c} \right)^{m_{r, c}}. \]  

(11)

The exponents \( m_{r, c} \) in Eq. (11) are the elements of the matrix \( M \) and therefore depend on the optical densities \( d, u \) and \( n \) of the stains. The values for every product term \( (I_r/I_w, c)^{m_{r, c}} \) can now be pre-calculated and stored in a LUT. For an RGB image, for instance, this requires the computation of 9 LUTs. The RGB values for a virtually destained slide are then computed by looking up the factors in Eq. (11) in the corresponding LUT and multiplying them with each other and \( I_w \). We denote this algorithm as “Power destaining” (D).

### 3. Analysis of the Implementations

Let \( N \) be the number of pixels in the image, \( N_c \) the number of color channels, and \( N_b \) the number of bits of the quantized camera output (yielding \( 2^{N_b} \) possible sensor outputs).

The overall algorithm as given by Eq. (7) is \( \mathcal{O}(N) \). Therefore, all implementations (A)-(D) given in Section 2 are implementational improvements (measured in Section 4) and do not change the order of the algorithm.

However, the computation of the lookup tables differs for the presented approaches and thus leads to different computational complexities and memory requirements for the LUTs.

(A) Ruifrok destaining: This implementation does not exploit LUTs and thus requires no additional computation time or memory. However, this implementation has the computationally most expensive inner loop.

(B) Ruifrok destaining OD-LUT: For every of the \( 2^{N_b} \) possible camera outputs, an optical density has to be computed. As the \( I_w \) differs for every color channel, such a LUT has to be calculated for every color channel, thus the complexity is \( \mathcal{O}(N_c \cdot 2^{N_b}) \).

(C) Ruifrok destaining Full-LUT: The number of all possible sensor combinations, for which the LUT is precomputed, is given by \( (2^{N_b})^{N_c} \). Every entry of the LUT contains \( N_c \) values, therefore the complexity and memory footprint for the Full-LUT is \( \mathcal{O}(N_c \cdot 2^{N_b} \cdot N_c) \). For instance for an 8 bit RGB camera, the Full-LUT consists of \( 256^3 \approx 16.7M \) entries of floating point RGB triplets. Consequently, this Full-LUT using double precision floating point (8 Byte) requires \( 256^3 \cdot 3 \cdot 8 \text{ Byte} \approx 400 \text{ MB of memory} \).
4. EXPERIMENTS AND RESULTS

We acquired images of immunocytochemically stained cervical smears, which have been stained with hematoxylin and the p16-marker (REAL-Kit from DAKO, Glostrup, Denmark with DAB (diaminobenzidine) as brown colorization), with a Nikon Ti-E microscope, 10x objective (NA = 0.3) and equipped with a Baumer TXG50c 5 Megapixel RGB camera. All images were acquired in 8 bit mode and all algorithmic variants have been implemented in C with double precision (64 bit) floating point. The calculations have been carried out on a notebook with Intel Core2 Duo CPU (T9400) at 2.53GHz and 4GB RAM. Timing results are given as the mean calculation time over 100 runs and are given in Table 1. Image results are shown in Fig. 1 and Fig. 2.

5. DISCUSSION

In this paper we have analyzed the color separation algorithm by Ruifrok [5]. We reformulated the algorithm and demonstrated that for the microscope with imaging described by Lambert-Beer’s law, virtual destaining can be achieved by calculating a product of one factor for each color channel.

The fastest color separation can be achieved with LUT for every possible RGB color vector. However, this Full-LUT requires a huge amount of memory even for common 3-channel 8 bit images. Since the memory footprint increases exponentially with the channel count and the bit depth, it becomes infeasible for multispectral cameras or higher bit depth. The power destaining, on the other hand, only requires a moderate amount of memory for the lookup tables and achieves almost the same speedup. Moreover, recalibrating the color separation or when interactively adapting the color vectors of the underlying stains is fastest with the power destaining implementation, since for this setting the overall time of LUT calculation and color separation has to be as low as possible.

The joint speedup of the power destaining implementation for calibration and color separation thus enable interactive color selection and live screen display of virtually destained slides. To this end, the pathologist selects stain colors and white point by clicking on the corresponding points in a live image from the photo microscopic system. The virtually destained slide with the recalibrated parameters is then shown with high frame rate. For instance, we achieve a frame rate of ≈7 fps at screen resolution (1.2 Megapixel).

All algorithms are suitable for GPU implementation, which we will investigate next. Finally, we point out that the current algorithm is given for the special case of transmission light microscopy imaging under Lambert-Beer’s law.

6. REFERENCES