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Vignetting correction by exploiting an optical microscopy image sequence

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***Abstract*— Vignetting is one of the most common problem that may affect digital imaging. The effect becomes particularly evident when images are stitched together to increase the camera's field of view (e.g., when building a mosaic), where it can lead to errors in automatic analyses. To correct the effect, the most common approach is to acquire an empty field image in advance that is used later to perform a flat field correction on every subsequently acquired image. However, in several cases, such as when dealing with off-line images or with real time acquisitions, this is not a viable option. The method we propose relies on a non parametric model to characterize in real time the vignetting function from the specimen itself, by using our foreground/background segmentation algorithm. The function is computed over a background built incrementally, detecting regions free of objects of interest. The experiments carried out using cell cultures and histological samples prove that our method yields results at least comparable to those achieved by using empty field.**

***Index Terms*— vignetting, microscopy, real time, flat field correction, mosaicing**

I. Introduction

DIGITAL imaging plays a major role in modern medicine thanks to the continuous progress of equipments during the last decades and its role is going to be even more prominent in the near future [1]. One of the most common problems that affect digital imaging based on CCD sensors is "vignetting", the radial falloff of image intensity propagating from the center of the optical axis, depending on the setup of the optical system [2].

Vignetting represents a problem for a wide class of automatic image analysis applications such as segmentation, tracking and rendering, but its effect is particularly notable in mosaicing, when two or more images are stitched together to increase the camera's Field Of View (FOV) [3] [4]. Besides, mosaicing fulfills an increasing demand arising from the need to understand the behavior of cell colonies and to study histological tissues [5] [6]. Unfortunately, images undergoing vignetting show seams in the stitched regions of mosaics, that could mislead visual analyses and introduce errors in the automatic ones.

The problem of vignetting has been extensively treated in the last years and several solutions have been proposed, mainly to correct mosaics, often with post processing operations (such as blending or spatial filtering), leaving the characterization of vignetting out of consideration. However, to preserve information and details, taking into account the vignetting characterization is mandatory [7].

The most trivial but common approach is acquiring an empty field image in advance [5] [8] [9]. However, several reasons could yield this image not be achievable. Simply, because images have been acquired by third parties and any empty field has not been taken. But also when dealing with real time mosaicing: for instance, in case of exploratory investigations carried out with microscope, it may happen that unexpectedly the researcher starts finding out the object of interest. It could be unfeasible to acquire an empty field starting from scratch and then retrieving the region of interest being mosaiced.

All the methods conceived to characterize vignetting from a single image can be classified into two categories. In the first class, the methods exploit complex object segmentation and recognition approaches to estimate the vignetting function [10], this being not suitable for real time applications. In the second class, methods employ priors on shape [11] or distribution [12] of the vignetting function, that are not suitable for general purposes. On the contrary, methods gathering information from image sequences may collect sufficient information that permit to characterize vignetting without requiring complex and computational demanding approaches. However, these methods either need several images [2] [3] or exploit parametric models suitable just as an earlier approximation, unless more specific information regarding optics is known in advance [4].

In this work, we present a general purpose approach that does not exploit any prior information about the camera or the system. Rather, it relies on a non-parametric method to both characterize vignetting and remove its effect in images acquired with an optical microscope. Our method is capable to extract all the necessary information from a sequence of images, even containing objects of interest. This makes our approach suitable to be used even *during* normal operator's inspection activities. Besides, the image sequence used as a bootstrap is not discarded, but subsequently included in the mosaic after being corrected from vignetting artifacts. The experimental results, carried out using cell cultures and histological specimens, which both cover the most relevant part of the biological routine examinations, prove that our method is capable to remove vignetting effectively. Besides offering a visual evaluation, we also propose a quantitative analysis.

This paper is organized as follows. Sect. II briefly discusses the state-of-the-art of the vignetting correction approaches. Sect. III describes the structure of our algorithm. Sec. IV analyses some experimental results proving the

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effectiveness of the solution we conceived. Closing remarks are given in Sect. V.

II. Previous Work

To correct and to characterize vignetting, often the easiest way is the preferred one: an empty field is acquired in advance. In [5], a reference image of the glass slide without any underlying tissue is acquired in advance and all the images are corrected by subtracting this reference. In [8], an ordinary white paper image is captured and used as the reference object. The vignetting function is calculated through normalization and the acquired images are corrected through a division by the vignetting function.

Unfortunately, an empty field image could be not at one's disposal. In [10], the vignetting function is characterized arising from the image itself. The method relies on a segmentation step to reveal areas with uniform scene radiance where gradual image intensity variations can be attributed to vignetting. Quality performance is strongly influenced by segmentation and it is very CPU intensive. An improved method is described in [11]. Here, no segmentation is required and this method achieves improvement in both performance and computing time. Despite that, the method relies on the center and the shape of the vignetting function is known a priori, thus limiting the applicability of the method in real contexts.

Nevertheless, due to noise and artifacts, characterizing the vignetting function from a single image is an ambitious goal. In [4] [2], methods are proposed to estimate the vignetting function starting from an image sequence. The authors rely on corner based tracking methods using several overlapping images. Both the methods are conceived for real world scenes and fail in case of unstructured scenes, such as cell cultures, that frequently show low characterizing corner points. In addition, both methods rely on priors such as the symmetry of the vignetting function, described by parametric models, that could not fit real cases. On the contrary, the non parametric model described in [3] starts from a sequence of partially overlapped images and it is of general purpose. The authors *need* correspondences of quite evenly distributed corners over the whole FOV: however, in practice overlapping images could not be feasible, due to scene evenness, and image registration could be hard to perform, accordingly.

III. Model and Method

Starting from the general camera image model proposed in [2] [3] [4], we can express a generic image $I(x)$ (where x is a lexicographically ordered vector of coordinate points) according to Eq. 1:

$$I(x) = r(G \cdot V(x) \cdot L(x)) \tag{1}$$

where r is the camera response function, G is the amplifier camera gain due to exposure, $V(x)$ is the spatial variant vignetting function, and $L(x)$ represents the scene intrinsic property. In case of outdoor images the scene intrinsic property $L(x)$ is the scene radiance. In brightfield microscopy $L(x)$ represents the transmitted light, whereas in phase microscopy it is the transmitted light spatially modified by the phase shift due to refractive index of the specimen. We consider r as being linear and spatial invariant, although it can be easily generalized to non linear functions [2] [4]. If $L(x)$ is spatially uniform, we can define an homogeneous image B according to Eq. 2:

$$B(x) = R \cdot G \cdot V(x) \cdot L \tag{2}$$

where R converts irradiance in gray levels. Supposing $G > 0$ and $V(x) \geq 1$, it is possible to estimate the vignetting function $V(x)$ by normalizing B for its minimum value, according to Eq. 3:

$$V(x) = \frac{B(x)}{min(B(x))} \tag{3}$$

Dividing an image $I(x)$ by $V(x)$ yields an undistorted (vignetting free) image, without any spatial tonal variations: this procedure is known in literature as *flat field correction*. We turn our attention to optical microscopy, where most of the routine examinations regard culture cells and histological samples. Each image is conceptually subdivided into two complementary regions: foreground and background ones. Here, the background (being the cover glass in histology and the culture medium in cell image analysis) is always quite uniform and it can be roughly considered as a single isotropic object.

Our method aims at detecting and extracting background regions from a sequence of images, captured randomly in real-time, to reconstruct a dense background from which to estimate the vignetting function. The estimation of the background of the specimen under analysis is then performed incrementally until enough information is gathered from the input images. As the background, we consider image regions in which the first derivative is quite low. A robust morphological opening is performed on the noisy estimation of the mask of background pixels M to prevent any foreground pixel to be included. We stop reconstructing the background when it covers at least $P\%$ of the image. The lacking data are replaced using interpolation, where applicable, and extrapolation. In particular, at the borders of possible holes we follow the gradient of the surrounding region, after that the gradient vanishes so to form a plateau. Where more images present background in the same position, we use redundant information to reduce the noise through a mean temporal filter. To regularize the reconstructed dense background we perform a high order polynomial fitting. At last, we can estimate the vignetting function by following Eq. 3.

The algorithm can be outlined as in Algorithm 1, where the values in the mask M are the central derivatives along rows and columns of images I_i and T_B represents the threshold of the gradient magnitude under which the image region is considered to be uniform and it depends on the curvature of the vignetting function.

Algorithm 1 vignetting function estimation

1. *while* estimated background region $< P\cdot$ size(I)
2. $M \leftarrow$ background's mask of image I_i
3. *foreach* (x) in I_i
4. *if* $M(x) < T_B$
   $M(x) = 1$
   else
   $M(x)$ is void
5. $B_i(x) \leftarrow I_i(x)\cdot$ morphological_opening(M)
6. $i = i + 1$
7. $B(x) \leftarrow$ fitting(temporal_mean($B_i(x)$))
8. $V(x) = \frac{min(B(x))}{B(x)}$

Once the vignetting function is estimated, the flat field correction is performed for each acquired image to remove the vignetting effect.

IV. Experimental Results

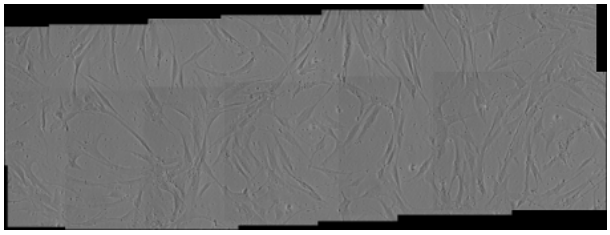
The experiments aim to assess the improvement our method can yield in terms of images' vignetting removal by comparing mosaics obtained through our estimated vignetting function with those generated without using our method. To provide a numerical assessment of results we have used two different metric indexes, widely employed in literature [8] [13] [12]: the Signal to Noise Ratio (SNR), the basic index in signal analysis and the Universal Quality Index (UQI) [14], the latter representing image quality more closely to the human visual perception.

The experimental results have been performed on several test images sequences, acquired using different cameras and microscopes. Here we only show the results performed on two out of the many image sequences analyzed, chosen as representative classes of cells and histological samples, respectively. Both have been acquired in phase contrast with a magnification factor of $100\times$ by using a non-automated inverted microscope (Nikon Eclipse TE2000-U), equipped with a digital camera (Nikon DXM1200). All the images acquired are 640×512 pixel in size. G has been kept constant for all the images of each sequence. As for the parameters of Algorithm 1, extensive assessments have proved that $T_B = 4$ is fair even with strong curvatures and $P = 90\%$ is good for all the sequences. However, this is not a too sensitive parameter: a higher threshold simply would slow the method down, requiring more images.

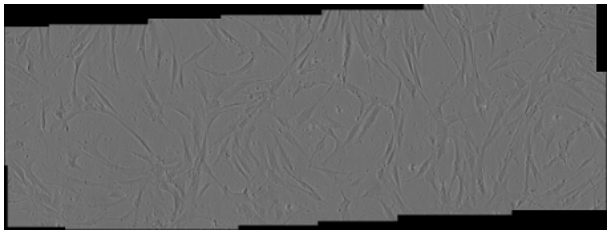
As the first step, we have acquired the sequence of empty field images used to built the vignetting function of ground truth ($V_{GT}(x)$) according to Eq. 3. Following, the two sequences of overlapped images have been acquired. It is worth remarking that our algorithm does not require any image overlapping and this is performed for measuring purposes only, to achieve the mosaics. The "stem-cells" sequence, hereinafter S1, is composed of 12 images representing mesenchymal stem cells with an approximate confluence of 50%, where the content of the cells is uniform at human sight and the contrast is nearly absent. The "histological" sequence, hereinafter S2, is a set of 15 images acquired from a histological sample, showing a higher dynamic range, with well defined contrast and object's contours. Then, we have estimated the vignetting function $V_{S1}(x)$ and $V_{S2}(x)$ from the two image sequences, by following the steps of Algorithm 1. In our experiments, before registering images in the mosaics, they all have been used to estimate the vignetting function of the sequences: for S1 and S2, the lack of background is below 2% and below 1%, respectively.

A wide amount of methods has been proposed in literature to built mosaics of images acquired with non automated microscopes [5] [6] [7] [15] [16]. We chose [16] because it allows to build a mosaic incrementally *during* the manual motion of the microscope holder.

Fig. 1 shows the mosaics referring to S1. Fig. 1 (a)



(a)

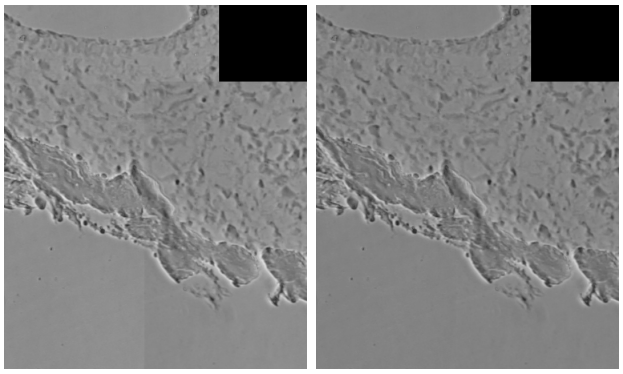


(b)

Fig. 1. Mosaics from the sequence S1. In (a), images are simply stitched together, while in (b) the mosaic is obtained with our vignetting correction.

refers to the mosaic built by stitching together the original (uncorrected) images. The effects of vignetting are evident: stitching regions are markedly visible. On the contrary, no seems are visible in the mosaic built using our vignetting correction (Fig. 1 (b)). Also, visually they are indistinguishable from those built using the ground truth $V_{GT}(x)$ (here not shown). Results for S2 are similar: two images' details referring to the same region have been extracted from the mosaics achieved without and with our correction method and shown in Figs. 2 (a) and 2 (b), respectively.

To numerically measure the quality of our vignetting correction, SNR and UQI have been evaluated in the overlapping area of the mosaics. Table I reports the outcome of this comparison. As expected, both the indexes confirm that the vignetting correction yields an improvement. On the other side, the value obtained with both the indexes are comparable when considering $V_{GT}(x)$ and our vignetting functions. In general, SNR is always better in S1 than S2, this probably due to the cell images being naturally more smoothed. The wider photometric range of S2 is probably the cause of an overall greater amount of noise that worsens the SNR, although slightly. At the same time, this points out a better



(a) (b)

Fig. 2. Two details of the same region, extracted from the mosaics obtained with sequence S2. In (a), images are simply stitched together and vignetting effect is markedly visible. In (b), the mosaic obtained with our vignetting correction proves the effectiveness of our method to eliminate seams.

TABLE I

NUMERICAL EVALUATION OF MOSAICS' QUALITY

Mosaic set and IF used in correction	SNR	UQI
stem-cells (simple stitching)	27.80	0.82
stem-cells ($V_{GT}(x)$)	30.74	0.88
stem-cells ($V_{S1}(x)$)	30.91	0.89
histological sample (simple stitching)	27.66	0.94
histological sample ($V_{GT}(x)$)	30.60	0.96
histological sample ($V_{S2}(x)$)	30.39	0.96

contrasted image that is probably the reason why the UQI is always better for S2. Moreover, the improvement yielded by vignetting correction has been more effective for S1: in fact, the errors are more evident in low contrast images and the correction accordingly.

V. CONCLUSION AND FUTURE WORK

In this work, we present a non parametric vignetting estimation method suitable to work on line for microscopic image analysis. It has been conceived to be used in case the empty field is not available (for instance, on sequences previously acquired). While most of the methods in literatures rely on empty field images or complex matching methods exploiting sequences of images, our approach performs a background/foreground segmentation by extracting quite uniform regions from the sequences of images, even during normal inspection work. Also, we do not make any assumption regarding properties of the vignetting function (e.g. symmetry) and the linearity of the sensor's response function is not a requirement. The effectiveness of our method has been assessed by correcting images of histological and cell cultures' specimens that both cover the most common microscopic routine examinations. The vignetting function has been estimated from the specimen itself by using our method and, for comparison purposes, from empty field images. Couples of mosaics built with simple stitching and using these corrections have been compared together. The results analysed by using common error indexes and quality metrics prove that our method performs at least as good as the *empty field*-based methods.

As for the improvements, we are working on relaxing the termination condition of the algorithm, by using the distribution of holes rather than a global threshold. In addition, new error metrics are being studied in order to capture the improvements achieved by our method, from both a quantitative and a perceptual point of view.

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