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On-line image mosaicing of live stem cells

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***Abstract*—Image mosaicing is a technique that permits to enlarge the field of view of a camera. For instance, it is employed to achieve panoramas with common cameras or even in scientific applications, to achieve the image of a whole culture in microscopical imaging. Usually, a mosaic of cell cultures is achieved through using automated microscopes. However, this is often performed in batch, through CPU intensive minimization algorithms. In addition, live stem cells are studied in phase contrast, showing a low contrast that cannot be improved further. We present a method to study the flat field from live stem cells images even in case of 100% confluence, this permitting to build accurate mosaics on-line using high performance algorithms.**

***Index Terms*—microscopy, image mosaicing, stem cells**

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I. Introduction

In the past years, digital image processing has represented the first attempt by biologists to exploit computer vision in the life sciences. Nowadays, the increasing processing power available at a low cost in every bio-lab has open the doors to automated image analysis. In particular, it has been possible to increase the field of view of microscopes by stitching together more overlapping images of the same culture. This technique, known as *image mosaicing*, is usually embedded in the software tool provided with expensive automated microscopes. Here, the motorized XY-tables can be used to create mosaicing even from thousands of individually captured histological images in [1], for instance to increase the significance of the statistical measures or to study global properties of stem cell cultures [2]. To our purposes, the mosaicing technique can be roughly described as a two-stage approach. In the first stage, each image is normalized with respect to uneven illumination changes using a *background* image containing no objects of interest. This stage is also known as *flat field* correction. After that, common parts of different images are used to derive the proper transformation between images so to stitch them together. This is often accomplished by looking in the *foreground* of the shared parts for common features referring to the objects of interest. In addition, the foreground is usually obtained by differencing each acquired image with the background.

Accordingly, the flat field correction plays a crucial role in order to have normalized images. Of course, the easiest way to achieve a background is to acquire images when the field of view is free of objects of interest [3]. However, such an image could not be at one's disposal and many authors [4] simply take the light unbalance into account at the image difference stage. Then, some authors build a model of the background using a generic polynomial approach [5], exploiting *a priori* information regarding either the light field of the specific microscope (such as the light pattern [1]) or the cell properties (such as the border gradients in [6] or simply the cell localization as in [7]). The work presented in [8] addresses a non-parametric and general purpose approach to build automatically a background image stemming from a sequence of images where the background is somehow present. However, this method cannot work in the presence of images with lack of background.

The novelty of our approach consists in using the live stem cells present in the field of view of the microscope as natural markers to estimate the light field. In addition, it is worth remarking that we have not any need to distinguish between background and foreground (objects of interest). Accordingly, we do not use any segmentation algorithm and we do not need to know where the cells are. We just require to have more images at our disposal where the cells are randomly distributed.

This work is organized as follows. Sect. II describes the stages of the image registration problem and the algorithm we implemented. Extensive experimental results are discussed in Sect. III, where two image sequences of live stem cells with a confluence of 50% and 100% are analysed and the results are compared with a background of "ground-truth". Conclusions are drawn in Sect. IV and some hints for future directions are given.

II. The image registration problem

Image mosaicing in the field of microscopy can be performed in different ways, depending on whether or not the microscope has an automated stage holder. Modern automated microscopes usually are available with the option of performing image mosaicing by using known translation of the motorized stage. Through this expensive option, the mosaic is composed by stitching the images according to the known relative positions. Otherwise the stage holder is positioned manually: in this case, the mosaic can be built by image registration techniques, exploiting a certain overlap between the captured images. In any case, the problem is twofold: aligning images from a geometric point of view and achieving a seamless stitching even from abrupt changes in lighting conditions. The former can be solved by a geometric registration, whereas the latter requires a tonal registration.

A. Geometric registration

The model that governs the registration depends on the physical properties of the imaged objects as well as on the kind of motion the overall system undergoes. In case of microscopy,

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due to the geometry of the system, the mapping between points on two consecutive images can be modeled with a translative model.

After choosing the proper geometrical model, images need to be aligned by detecting and matching features in a common overlapping region. As far as the features to be tracked in the acquired images are concerned, we have chosen the Kanade-Lucas-Tomasi feature tracker (KLT) [9], [10], since it can achieve a high accuracy and its computational cost is compatible with the real-time constraints.

A fast initial guess, based on a phase-correlation approach [11], is computed to guide the KLT tracker in case of large displacements of the holder position. The phase correlation guess is used as a coarse estimation of the holder displacement, this granting additional benefits in terms of robustness and performance.

Once the tracker has found enough reference points in the common region, the transformation matrix H is estimated according to the given model by using a robust estimator (RANSAC [12]).

The proposed image mosaicing algorithm uses a frame-to-frame (F2F) registration strategy where differences among temporally adjacent frames are meant to be small in both geometric distortions artifacts and lighting conditions, hence feature correspondences are more reliable, thus resulting in a highly accurate alignment.

B. Flat field correction

Once the geometric image registration problem is solved, tonal misalignment between the images composing the mosaic can still be present. In particular, differences in lighting condition may affect either the brightness or the color content of subsequent images. Also, since the light field of the microscope is not perfectly flat, a vignetting effect may prevent a seamless stitching of the images composing the mosaic. In order to overcome these problems, a flat field correction is applied to each processed image. Here we perform the flat field correction be means of a light field estimation achieved by taking into account the *whole* image content, disregarding between background and foreground. To this purpose, our approach just requires that there are more images showing a different random spatial distribution of live cells, without any bound to the cells' confluence, that can be even of 100%. In fact, since all the cells have a similar photometric aspect, they act as natural *uniform* markers whose changes in colors (or gray levels) mostly depend on their position within the field of view. In practice, the light field estimation algorithm works by analyzing the first N images of the sequence, through the following three steps:

1) Estimation of a robust image $B = median(I_i), \quad i = \{1..N\}$by a temporal median filter of each pixels in the sequence.
2) Averaging filter on B
3) Light field estimation $LF = B - min(B)$

The first step is needed since it detects the most common pixel values in each position while rejecting outliers, due to the presence of moving residue of the cells on the culture medium. The second step is used to smooth the estimated background image, whereas the third step estimates the final light field as the topmost structure of the background. The flat field correction applied to each image I is then $I_{FF} = I - LF + mean(LF)$.

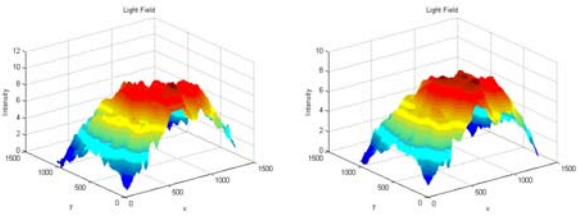
C. Image warping

After that geometric registration and tonal alignment between the images are computed, the mosaic is composed by warping images into a common reference frame according to the geometric transformations H. Differently from what that common use is, we do not employ blending mask so to manage to better evaluate the robustness of our method in the stitching regions in terms of geometric and tonal alignment. Images are warped into the mosaic frame at a sub-pixel level through bilinear interpolation by simply overwriting all the transformed pixels.

III. Experimental results

The test bed is composed of a Nikon Eclipse TE2000-U inverted microscope, not equipped with a motorized precision stage. Two sequences of images taken from Mesenchymal Stem Cell (MSC) cultures at a confluence level of 50% (S1) and 100% (S2) respectively, are acquired manually by moving the slider of the stage holder and given in input to our algorithm, which extracts the light field and updates in real-time the image mosaics. The microscope imaging mode is set to phase contrast acquisition, and the acquired images show a very poor contrast due to the MSC cultures being analyzed.

The tonal alignment algorithm on the set of images of sequence S1 produces a light field background shown in Figure 1(a).



(a) (b)

Fig. 1: The light field extracted from images of sequence S1 (a) and the corresponding ground-truth (b).

In Figure 1(b), the corresponding ground-truth light field is shown. The latter has been achieved by averaging a sequence of empty field acquisitions on a specimen containing nothing but the culture medium (i.e. without any cell). As it can be seen, the algorithm is able to reproduce the same light field background even in the presence of foreground objects.

As for the sequence S2, an image is shown in Figure 2(a) together with the extracted light field (b). Here we can appreciate the effects of the light field compensation performed by our algorithm, in spite of the cells density being very high.

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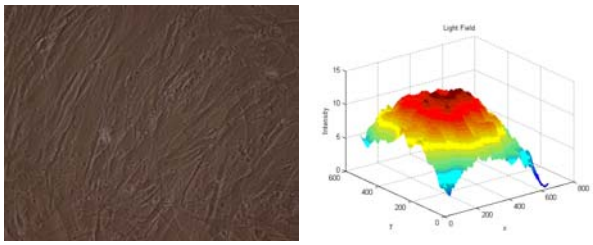

(a) (b)

Fig. 2: An image extracted by sequence S2 (a) and the corresponding extracted light field (b).

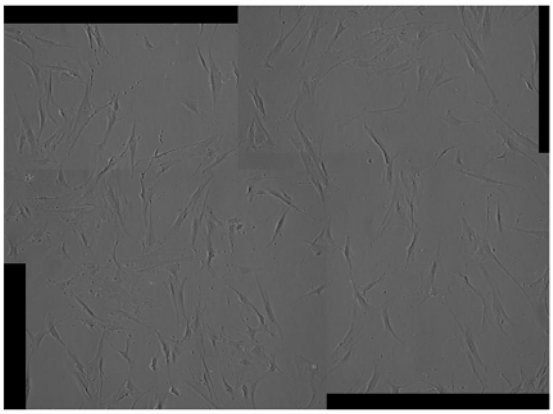

Fig. 3: A mosaic of 4 frames belonging to sequence S2, without tonal registration.

In Figure 3, the resulting mosaic referring to the sequence S1, and generated by our registration method without using the tonal alignment algorithm, is shown.

As we can see, the effects of the uneven light field is clearly visible. Composing the images without the flat field correction emphasizes the seam and the vignetting effects preclude a pleasant visualization of the composed cell culture. However, we can exploit these artifacts to visually appreciate how the images' geometric transformations have been correctly computed by our registration algorithm. Cell structures on the image borders appear correctly aligned from a geometric point of view, leading to a mosaic free of visual distortions. To further address the quality of the registration algorithm from a numerical point of view, we quantify the registration performances by evaluating a proper metric on a Region of Interest (ROI) of the mosaic: the Mean Square Error (MSE) defined according to Eq. 1:

$$MSE = \frac{\sum_x \sum_y (I(x,y) - R(x,y))^2}{N} \qquad (1)$$

where R is the ROI of the original image unaffected by registration artifacts, I is the corresponding ROI of the mosaic and N is the number of pixels in the ROI.

The result on the same set of images mosaiced by using the flat field correction algorithm is shown in Figure 4. As we can see, by applying the tonal alignment through the flat field correction a seamless stitching between the images is produced. This is an important aspect to be considered when biologists need to visually evaluate the cells culture. Nevertheless, producing a tonal aligned mosaic is also a crucial aspect when the output image have to be further analyzed by image analysis measurement tools, since fictitious image gradients along the stitching regions, or simply a global thresholding operation, may produce unwilling results.

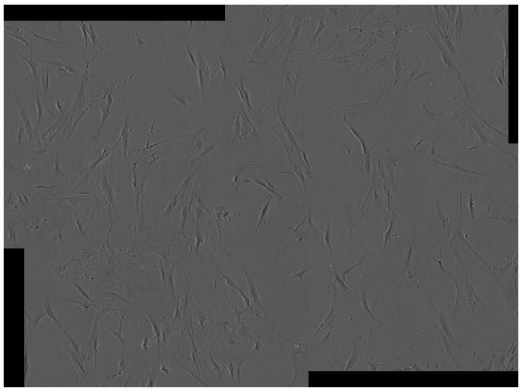

Fig. 4: A mosaic with tonal registration, made of 4 frames of the stem-cells sequence.

Table I resumes the results referring to the proposed mosaicing algorithm, expressed in terms of MSE values when evaluated over a ROI of overlapping regions on the first frame.

Table I: MSE results for sequence S1

Sequence	MSE
S1 w/o tonal	21.27
S1 with tonal	14.05

It is worth noticing how the evaluated metric produces a smaller value when using our flat field correction algorithm: a MSE of 21.27 is attained by using geometric alignment only, whereas a MSE of 14.05 is achieved with both geometric and tonal registrations.

IV. Conclusions

We have presented a mosaicing algorithm for live stem cells, capable to work on-line by using local image registration. The main problem faced in this work has been that of recovering automatically the microscope's light field, in those cases where either an empty field could not be available or the image does not even show any background region to be used as a partial reference. An innovative algorithm has been devised to recover the microscope's light field by exploiting the stem cells as natural markers. The light field thus attained is then used to correct the images acquired so that they show even illumination. This permits to eliminate the seam signs in the mosaics, in correspondence of the composing image borders. Experimental results have been successfully carried out using images of live stem cell cultures at difference confluences, even considering a value of 100%.

We are now examining more kinds of cells, in order to assess to feasibility to apply our method to other cells as well, or or biological samples.

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