International Journal of Electrical, Electronic and Communication Sciences
ISSN: 2517-9438
Vol:4, No:11, 2010

Multichannel image mosaicing of stem cells

Alessandro Bevilacqua[1,2], Alessandro Gherardi[2], Filippo Piccinini[2]

***Abstract*—Image mosaicing techniques are usually employed to offer researchers a wider field of view of microscopic image of biological samples. a mosaic is commonly achieved using automated microscopes and often with one "color" channel, whether it refers to natural or fluorescent analysis. In this work we present a method to achieve three subsequent mosaics of the same part of a stem cell culture analyzed in phase contrast and in fluorescence, with a common non-automated inverted microscope. The mosaics obtained are then merged together to mark, in the original contrast phase images, nuclei and cytoplasm of the cells referring to a *mosaic* of the culture, rather than to single images. The experiments carried out prove the effectiveness of our approach with cultures of cells stained with calcein (green/cytoplasm and nuclei) and hoechst (blue/nuclei) probes.**

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

I. Introduction

A Mosaic is a compound image built through properly composing a high number of frames and warping (stitching) them into a common reference coordinate system, both spatial and tonal. The result consists of a single image of a greater resolution and spatial extent that represents a dense reconstruction of the structure of the scene. This is a technique widely used in microscopy, where by default the operator can watch just a small part of the object of interest, given by the microscope's Field Of View (FOV). The fluorescence analysis is often used to identify cell nuclei position (e.g. for counting purposes) or to study apoptosis. Normally, the cell cultures are stained with different fluorophores and the different excitation wavelengths are captured by microscope's filters. Practically speaking, after capturing images in phase contrast, the filter is switched to each different wavelength and the respective images captured accordingly. After that, images are merged together with the purpose to identify in the original images, for instance, nuclei position and cytoplasm [1]. The main difficulty to perform multichannel *mosaicing* in the same reference space is to have the same tracking capability for all the channels. This is yet more difficult with hand-held microscopes that do not provide any information about XY holder position. The examples reported in literature deals with single channel mosaicing when using non automated microscopes [2][3]. On the contrary, several examples of multichannel mosaicing are reported with automated microscopes, where for each channel images are taken when the holder is in the same *known* position. However, the image registration is performed with normal images (i.e., without fluorescence) and the same transform matrix is used for all the channels.

This work presents the method we conceived to achieve online a multichannel mosaic with a non automated microscope and to our knowledge this is the first attempt. Our mosaicing algorithm is based on a corner points tracker, where a "corner point" is defined as a small patch showing high photometric gradients, just like a "corner". Our tracker finds out all the corners in the image, these being independent from the objects' structure and suitable for general purpose applications. The microscopic images of cells in phase contrast contain objects (cells) with roughly uniform properties as far as the corner points are concerned. Therefore, after staining the cells by two different fluorophores, the microscopist can move the holder manually to build the mosaic in phase contrast. After that, the microscope's filter can be switched in a different position to capture the requested wavelength and a new mosaic is built in this new channel. This procedure is carried out for each channel. Making sure to cover more or less the same area as before, it is then possible to maximize the overlapping regions in the different channels.

This work is organized as follows. In Sect. II, we outline some representative examples of image mosaicing with and without automated microscopes. Sect. III describes the stages of our mosaicing algorithm. Finally, in Sect. IV we report and discuss the results achieved in our experiments, with images showing separate channels and multichannel information regarding mesenchymal stem cells. We draw some conclusions in Sect. V and give some hints for future works.

II. Previous Works

As a matter of fact, there are several methods that exploit automated (confocal) microscopes to build multichannel mosaics on line. In [4], the XY positions of all the images for each channel are the same, and known in advance because the confocal microscope employed is automated. Consequently, for each different channel the mosaic is built using the same transform matrix as in the absence of fluorescence. The same happens in [5], where the image consists of three channels, each of which is the emission of a different wavelength of light collected from as many fluorophores within the specimen.

In case of manual movements of the holder, there are very few examples of mosaicing applications and none of them seems to work with more than one channel. The authors in [2] do not use any configuration information to build the mosaic. However, the component microscopic images are one-channel and the mosaic is built in batch through global optimization using a genetic algorithm, this being not compliant with online requirements. In the work described in [3], the authors use Kanade-Lucas-Tomasi feature tracker (KLT) [6] to build a

[1] DEIS (Department of Electronics, Computer Science and Systems), University of Bologna; alessandro.bevilacqua@unibo.it
URL:http://cvg.deis.unibo.it
[2] ARCES (Advanced Research Centre on Electronic Systems), University of Bologna; {abevilacqua, agherardi, fpiccinini}@arces.unibo.it

single channel mosaic of 300 human skin images acquired *in vivo* with the hand-held dual-axes microscope and translational movements.

III. The mosaic algorithm

Image mosaicing in the field of microscopy can be performed by image registration techniques, exploiting a proper overlap between couples of captured images. The mosaic is built by aligning images with a geometric registration and achieving a seamless stitching using tonal registration, this being capable to work even in the presence of abrupt changes in lighting conditions.

A. Geometric registration

The registration model can be set to different kinds of transformations: translative, affine and projective, according to the design of the image acquisition system and the geometry of the object being imaged. In case of microscopy, due to the geometry of the system, the mapping between points on two consecutive images can be modeled primarily with a translative model, also assuming that the motion between frames is small. Couples of subsequent acquired images are aligned by detecting and matching (*tracking*) common structures (here, points) in a shared region by frame-to-frame (F2F) registration strategy. First, we identify trackable feature points in the current frame using the Shi/Tomasi algorithm [7]. These features are represented by large spatial image gradients in two orthogonal directions. Then, we find the corresponding locations of those features in the previous frame using a pyramidal implementation of the KLT [6], since it can achieve a high accuracy and its computational cost is compatible with a real-time application. It must be also noticed that feature points are independent from the shape of the objects being tracked, since the procedure exploits 3×3 patches of local gradients, thus being also suitable for general purpose. In case of a large displacement of the holder position, a fast initial guess based on a phase-correlation approach [8] is computed to guide the KLT tracker. The guess is used as a coarse estimation of the holder displacements, 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 RANSAC [9] robust estimator.

B. Image warping and blending

Our registration procedure is able to find the transformation matrix at sub-pixel level. Therefore, the image warping is based on interpolation techniques. Here we have chosen not to use blending mask in order to limit the information distortion. Images are warped into the mosaic frame through bi-linear interpolation by overwriting all the transformed pixels belonging to each image added to the mosaic. Images are also aligned tonally by using our methods described in [10] [11].

C. Image acquisition

As for image acquisition, the holder is moved *manually* to cover the region to analyze: a separate icon on our Graphical User Interface (GUI) keeps a color track of the path followed. After finishing the inspection with the first channel (e.g., in bright-field or in phase contrast), the filter on the optics is moved so to be sensible to the wavelength of another set of probes.

Objects in different channels show different gradient values, but in separate channels they are similar to each other. In case of "uniform" objects in the scene, where with *uniform* we mean with similar contrast, hence similar gradient values, the KLT works by finding all points with values roughly spread in the same order of magnitude. Therefore, while our feature tracking method works *independently* on the different channels, at present the problem is to register overlapping images coming from different channels. Accordingly, in order to be able to merge contents of the different channels, we need to have a common reference between the different mosaics. This is achieved by forcing the last image acquired with a given filter to be the first one of the new filter, without moving the stage holder.

IV. Experimental results

A. Data generation

The test bed is composed of a Nikon Eclipse TE2000-U inverted microscope, not equipped with a motorized precision stage, coupled with a mercury lamp to produce ultraviolet light and a set of filters to enable fluorescent imaging. A sequence of images, shooting Mesenchymal Stem Cell (MSC) cultures at a confluence level of 50%, has been acquired manually by moving the slider of the stage holder and given in input to our mosaicing algorithm. Various fluorescent indicators are available to study many aspects of the culture that are important from a physiological point of view. In our experiments, the MSC culture has been stained by two different type of fluorophores:

- HOechst stains (HO) for nuclei with excitation wavelength $\lambda_e = 400$ nm and emission wavelength of $\lambda_m = 488$ nm (blue)
- CAlcein/AM stains (CA) for living cells cytoplasm with excitation wavelength $\lambda_e = 488$ nm and emission wavelength of $\lambda_m = 530$ nm (green)

B. The mosaics

Different types of acquisition are performed: three sets of 4 images each have been acquired. The first set refers to Phase-Contrast (PC) acquisition, the second one uses CA and the third one uses HO.

Figure 1 shows the mosaic generated by the PC sequence. We can see how neither the low contrast of the MSC culture nor the moving debris present inside the medium represent a critical issue for our registration algorithm, which manages to register all the images correctly.

In Figure 2, the mosaic generated by the same MSC region after having switched the filter to match the Hoechst labels

Fig. 1. Mosaic for the PC sequence showing a region of the MSC culture.

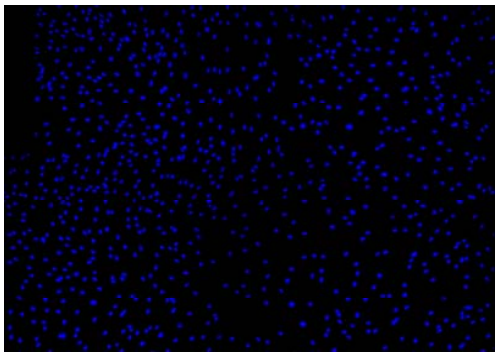

Fig. 2. Mosaic for the HO sequence depicting the blue nuclei of the MSC culture.

response is shown, providing that the last image of the PC sequence and the first of the HO sequence share the same position. Again, we can see how the presence of unstructured and untextured objects like the "blue dots" is not a matter of difficulty in detecting the real transformations between each couple of images. In order to make the registration more robust, a global morphological top-hat filter [12] has been applied to the image, preventing the small gradients due to the noisy background to be taken into account in the phase correlation process. As for the last sequence, Figure 3 shows

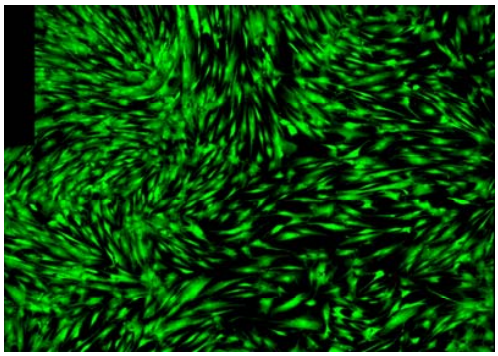

Fig. 3. Mosaic for the CA sequence showing the cytoplasmatic content of the living cells of the MSC culture.

the mosaic generated by the CA sequence emphasizing the cytoplasm content of the same portion of the MSC culture.

Once the fluorescent probes have been acquired in each set of images and the corresponding mosaics have been generated accordingly, the information coming from different probes can be fused in one image. Figures 4 and 5 show the

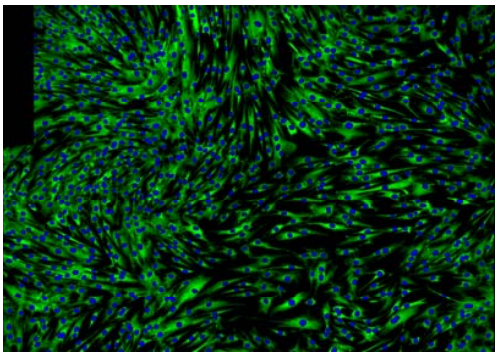

Fig. 4. Merge of the mosaics for the sequences CA-HO.

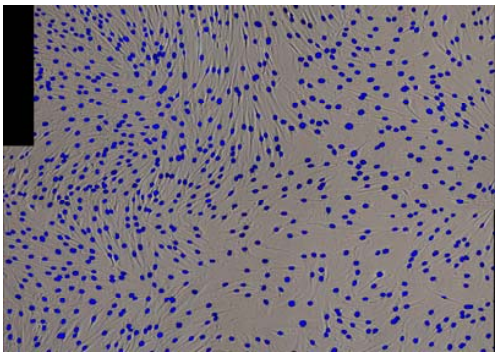

Fig. 5. Merge of the mosaics for the sequences CA-PC.

merging of the common regions of the mosaics generated by these sequences. In particular, Figure 4 shows the merging of nuclei (blue) and cytoplasm content (green) due to the CA-HO sequences, while Figure 5 shows the merging of nuclei (blue) and phase-contrast MSC visible contents. The merging of the different sequences is performed by weighing the content of the sequences. Of course, different weighing strategies may yield as many informative contents and this choice is left to the biologist analysing the culture.

As for the geometric registration error measured in the common region of the mosaics, it yields a displacement between the mosaics which is below one half of a pixel, this resulting in a very good geometric alignment.

V. Conclusions

Our research is the first one addressing the registration of mosaics of mesenchymal stem cells with non automated microscopes. Images have been acquired with a camera coupled with an inverted microscope in phase contrast and in fluorescence, after the culture cells have been stained by Calcein and Hoechst to emphasize nuclei and cytoplasm. While the same registration algorithm is employed for every channels, one image for each couple of mosaics must share

the same XY position: the last image of a channel must be the first of the new one. The experiments carried out with small sets of four images for each channel show an overall registration error below half a pixel.

The next step of this research work is trying to relax the constraint of having the last position of a given channel as the first one of the next probe. This means to devise a inter-channel registration method also using image fusion techniques.

ACKNOWLEDGMENTS

We would like to thank the biologist Dr. Enrico Lucarelli and his staff of the Bone Regeneration Laboratory (BRL) of the Rizzoli Orthopedic Institute (Bologna, Italy) for providing the data used in this work.

REFERENCES

[1] I. Tognarini, S. Sorace, R. Zonefrati, G. Galli, A. Gozzini, S. C. Sala, G. Thyrion, A. Carossino, A. Tanini, C. Mavilia, C. Azzari, F. Sbaiz, A. Facchini, R. Capanna, and M. Brandi, "In vitro differentiation of human mesenchymal stem cells on ti6al4v surfaces," *Biomaterials*, vol. 29, pp. 809–824, 2008.

[2] K. S. Fubito Toyama and J. Miyamichi, "Image mosaicing from a set of images without configuration information," in *Proceedings of the* 17^{th} *International Conference on Pattern Recognition (ICPRŠ04), Cambridge UK, August 23*, vol. 2, 2004, pp. 899–902.

[3] K. Loewke, D. Camarillo, W. Piyawattanametha, D. Breeden, and K. Salisbury, "Real-time image mosaicing with a hand-held dual-axes confocal microscope," in *Proceedings of the SPIE Conf. on Endoscopic Microscopy III, San Jose, CA, USA, January 20*, vol. 6851, 2008, pp. 1–9.

[4] Y. Pang, A. A. Ucuzian, A. Matsumura, E. M. Brey, A. A. Gassman, V. A. Husak, and H. P. Greisler, "The temporal and spatial dynamics of microscale collagen scaffold remodeling by smooth muscle cells," *Biomaterials*, vol. 30, no. 11, pp. 2023–2031, Apr. 2009.

[5] H. S. Kim, J. P. Schulze, A. C. Cone, G. E. Sosinsky, and M. E. Martone, "Multi-channel transfer function with dimensionality reduction," in *Conference on Visualization and Data Analysis, IS&T/SPIEŠs International Symposium on Electronic Imaging*, 2010, pp. 1–12.

[6] J. Y. Bouguet, "Pyramidal implementation of the Lukas Kanade feature tracker: Description of the algorithm," *In Intel Research Laboratory, Technical Report*, pp. 1–9, 1999.

[7] J. Shi and C. Tomasi, "Good features to track," in *Proceedings of Computer Vision and Pattern Recognition*, 1994, pp. 593–600.

[8] H. Foroosh, J. B. Zerubia, and M. Berthod, "Extension of phase correlation to subpixel registration," *IEEE Transactions on Image Processing*, vol. 14, no. 1, pp. 12–22, 2002.

[9] M. A. Fischler and R. C. Bolles, "Random sample and consensus: A paradigm for model fitting with application to image analysis and automated cartography," *Comm. of the ACM*, vol. 24, no. 6, pp. 381–395, 1981.

[10] A. Bevilacqua, A. Gherardi, L. Carozza, and F. Piccinini, "Semi-automatic background detection in microscopic images," in *International Conference on Biological Science and Engineering (ICBSE), Venice, Italy, November 24-26*, 2010.

[11] P. Azzari and A. Bevilacqua, "Joint spatial and tonal mosaic alignment for motion detection with ptz camera," *Lecture Notes in Computer Science*, vol. 4142, pp. 764–775, 2006.

[12] E. Dougherty, *An Introduction to Morphological Image Processing*. SPIE-International Society for Optical Engine, Feb. 1992.