ACCURATE MOSAICING OF CELL IMAGES ACQUIRED WITH NON AUTOMATED EQUIPMENT

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ABSTRACT

One of the most important requirements for *in-vitro* cell analysis is the ability to visualize a large part of a cell culture with an elevated level of detail. The creation of image mosaics is a useful process for extending the field of view and preserving the resolution of an image. The methods in the field make use of holder known displacements to achieve this goal. Moreover, mosaics are usually built off-line. Our proposed method is able to create a mosaic of microscopic images of cells in real time, without relying on acquisitions performed by automated equipment and without requiring a uniform light field. The accuracy of the mosaic is assessed by measuring the intensity values of a region of interest (ROI) before and after registration.

OBJECTIVES

- Flat Field Correction
- Geometric and Tonal Registration
- Cells High-Throughput Analysis
- Mosaic Quality Measure

INTRODUCTION

Image mosaicing, also known as image stitching, has a wide range of applications in different fields, from panoramic image generation through several individual photographs, to digital terrain generation of a larger area. In biology, mosaicing techniques are used for extending the field of view of the microscope, for imaging a wider area of the cell culture. In this field, one of the greatest needs of biologists studying the behavior of cells *in-vitro* is the possibility to obtain detailed images in order to deduce and report information on the whole culture instead of only analyzing a part of it. This problem is solved in microscopes with a high level of automation, despite their availability is limited to some research laboratories only. Our algorithm can be used with any optical microscope, even when the shift of the holder is not automated. In this study, we propose algorithms for automatically estimating, in real time, a globally optimal geometric and photometric transformation for mosaicing cells images. The images are acquired manually in phase contrast mode and have a very poor contrast due to the Mesenchymal Stem Cells being imaged. Since in most of the cases the light field is non-constant, the algorithm performs also a tonal registration. This transformation is used to balance variations in intensity due to inhomogeneous illumination within and across images. Then, the recovered geometric transformation maps all images in the same target space thus obtaining a large image of the entire cell culture. To assess the accuracy of the final mosaic, the intensity values of pixels in a ROI are compared before and after registrations.

Fig.1a

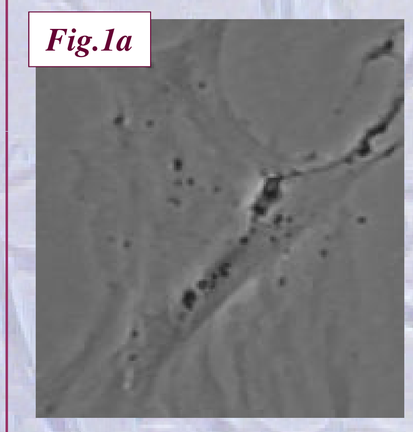

Fig.1b

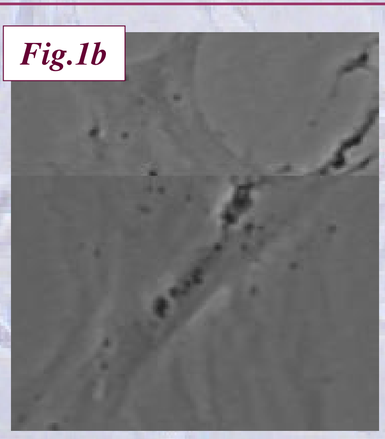

Fig.1c

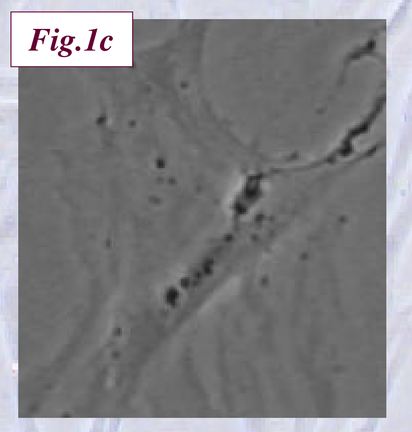

Fig.1a: ROI from the original image. Fig 1b: Mosaic's ROI after only geometric registration (F2F). Without tonal alignment it is simple to see the frames stitching region. Fig 1c: same ROI after our improvements introduced by tonal and Frame-To-Mosaic registrations.

MATERIAL & METHODS

Cell images were acquired using a Nikon Eclipse TE2000-U optical microscope that was not equipped with a motorized precision stage. The percentage of overlap area is in the range of 15-50%. The algorithm was implemented in C/C++ and run on a consumer PC (Intel 1.60 GHz, 1 GB RAM) in real time. To prevent a vignetting effect, the images must undergo a tonal registration. Firstly, a bootstrap sequence is acquired for detecting the light field of the microscope by processing them through three sequential stages of median filtering (MF): the first is performed spatially on each acquired image, the second is a temporal MF between all the images at each corresponding pixel's position, and finally another spatial MF on the resulting image. This processing produces a Gaussian-like distribution that represents an effective approximation of the illumination field of the light source. The subsequent images are then corrected by subtracting this distribution, resulting in an almost flat light field. Secondly, a following histogram specification which targets the histogram of the first image before being processed makes all the images in the same dynamic range. After the tonal registration, each image acquired is geometrically registered in a common reference frame (the first image, in our case). A Lukas Kanade Tomasi feature tracker has been used with an affine geometrical model (Frame-To-Frame registration, F2F). To increase the accuracy, a Frame-To-Mosaic registration (F2M) is also performed: the final transformation matrix is composed by the F2F registration matrix and the amount of correction introduced by registering the current unregistered frame with the overlapping area of the mosaic. Clearly speaking, this amount is the identity matrix in case of perfect F2F registration. Accuracy of the resulting mosaic is assessed by comparing the Mean Squared Error (MSE) of intensity values in a region of interest (ROI) of N pixels according to:

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

where R is the ROI of the original unregistered image and I the corresponding ROI of the mosaic.

RESULTS

Tab.1	F2F (FIG 1B) – ORIGINAL IMAGE (FIG. 1A)	TONAL AND F2M (FIG 1C) – ORIGINAL IMAGE (FIG. 1A)	TONAL AND F2M (FIG 1C) - ORIGINAL IMAGE FLAT FIELD
MSE	108	19	17

Results in Table 1, show how the MSE computed with the classic F2F registration (*Fig. 1b*) is far higher compared with our method (Tonal and F2M registration, *Fig. 1c*). Also, when comparing our method with the original flat-filed correct image, the MSE value decreases to 17. It should be noted that the high values of MSE are due to small debris moving within the medium of the cell's culture.

CONCLUSIONS

A robust image mosaicing method has been devised in order to cope with non automated microscopes. Our method can improve classical cell's mosaicing techniques both in terms of geometric alignment and tonal registration, and in contrast to what is usually available, to produce real-time mosaics of cells. This can enable legacy laboratory instrumentation to high-throughput analyses. Future improvements can deal with the light field detection algorithm, by carefully choosing which images have to be used in the bootstrap phase.

BIBLIOGRAPHY

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