Field-of-view extension and XY-drift correction in microscopy for large samples

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Abstract: We propose a method for sample XY-drift correction by means of feature detection and correlation analysis along with field-of-view extension for large sample images taken through a microscope with a motorized XY stage

Introduction

The ability to extend the field-of-view (FOV) of the specimen under study is very important in many biological applications [1, 2]. Imaging specimens larger than the microscope's field of view (FOV) can be accomplished by acquiring a grid of partially overlapping images and finally stitching the neighboring images (tiles) into a large mosaic i.e., reconstructing an image with extended FOV.

In order to obtain the tiles that will be stitched to reconstruct the region of interest (ROI), the sample is translated along the horizontal plane by a motorized XY-stage. When this procedure is repeated along different cycles (e.g. for time lapse imaging) an undesired sample XY-drift due to thermal fluctuations or mechanical vibrations may easily affect the localization precision.

One possibility to deal with this problem is by means of fiduciary markers like gold spheroids but it affects the image of the sample [3]. Another possibility is to compute the translation between the acquired images after each cycle, and try to correct it.

In the present work we propose a method based on feature detection and correlation analysis to correct the XY-drift and to perform the stitching of the corrected tiles to extend the field-of-view for large samples.Experiments were carried out using a Nymphaea vegetal sample (Amscope prepared slice).

Optical set-up and methods

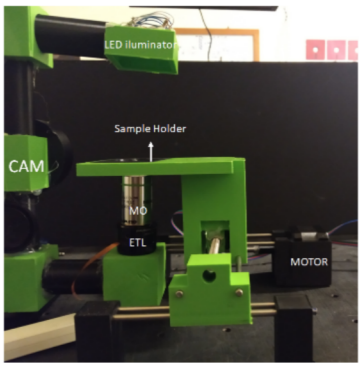
We used a low-cost 3D-printed brightfield inverted microscope with a XY-translation motorized stage. The optics of the microscope consists of a camera (CAM commercial camera), an electrically focus-tunable lens (ETL Optotune EL-10-30-C) and a microscope objective (MO $20/0.40$). The ETL allows fine tunning the in-focus plane [4–6]. The illumination is from above with a LED iluminator in an inverted configuration. The XY-translation motorized stage consists of two stepper motors (200 STEPS/REV, 600MM WIRE NEMA 23) and is driven by a microcontroller board (Arduino Leonard) from a computer. The microscope is shown in figure 1a.

Images of the sample were acquired during many cycles trying to return the platen to the same XY position and as expected there was an XY-drift introduced during the procedure that can be seen in fig 1b. To correct the drift, first the best translation was found by means of a feature-detection algorithm based on Speeded-Up Robust Features (SURF [7]) and then as a way to confirm and refine the result, the correlation between the reference image and the translated image was computed for translations in a neighborhood of the XY-translation estimated previously.Once the sample XY-shifts are corrected the stitching between overlapping neighboring images was performed (applying again the features method to previously corrected images).

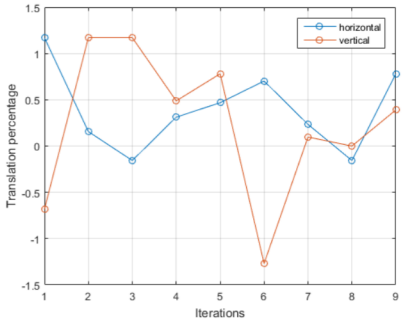
Experimental results

As expected, when the platen was cycled to return to the same position a sample XY-shift was introduced between the images. Figure 1b shows that there is a random behavior in the position of the images after each cycle and it was succesfully corrected by the proposed method as can be seen in figure 1c. Finally the stitching of the overlapping neighboring images was performed as shown in image 1c reconstructing the extended FOV as shown in figure 1d. The ability to correct XY-drift and obtain extended FOV by the methods described could have many applications in biological imaging. For example, in time-lapse experiments in cell and developmental biology, where images

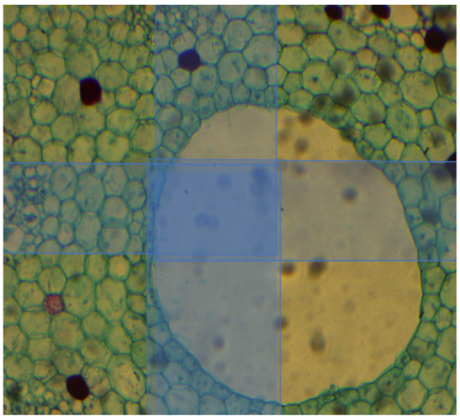
of multiple fields need to be taken repeatedly, and often assembled into large FOV, to explore processes such as large scale tissue dynamics and morphogenesis .



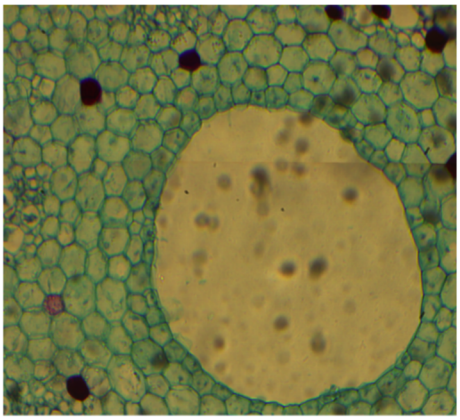
(a) 3D-printed microscope design



(b) Translation percentage as a function of iterations



(c) Overlapping of images



(d) Extended field of view Image

Fig. 1.

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