3D-Printed Autofocus Custom-Built Microscope for Extended Field-of-View Imaging of Large Biological Samples

Alejandro Silva*, **and Julia R. Alonso**

Instituto de Física, Facultad de Ingeniería, Universidad de la República, J. Herrera y Reissig 565, C.P. 11300.Montevideo, Uruguay.

* *asilva@fing.edu.uy*

Abstract: We propose a low-cost 3D-printed microscope with an electrically focus-tunable lens (ETL) and a motorized XY stage driven by a microcontroller board capable of autofocusing and extending the field-of-view (FOV) for large biological sample imaging.
© 2021 The Author(s)

Introduction

We propose a low-cost 3D-printed microscope with an electrically focus-tunable lens (ETL) and a motorized XY stage driven by a microcontroller board capable of autofocusing and extending the field-of-view (FOV) for large biological sample imaging.

When imaging a large biological sample through a microscope the objective need to be placed close to the sample leading to a limited field-of-view (FOV) that could be smaller than the region of interest (ROI) in the specimen under study. Then the ability to extend the FOV for imaging a larger view of the observed sample can be of great interest for many biological applications [1].

Another common problem in microscopy is the correction of the focus drift, which occurs due to various factors, including initial movement of the live sample, thermal drift and mechanical instability [2]. The focus drift can disrupt time lapse, therefore it is important to use automatic focal plane detection and stabilization systems [2, 3].

In the present work, we propose a low-cost custom-built 3D-printed autofocus motorized microscopy than can image specimens larger than the microscope objective's FOV by means of acquiring and stitching overlapping neighboring images usually known as tiles.

Motivation and related work

Optical set-up and experimental results

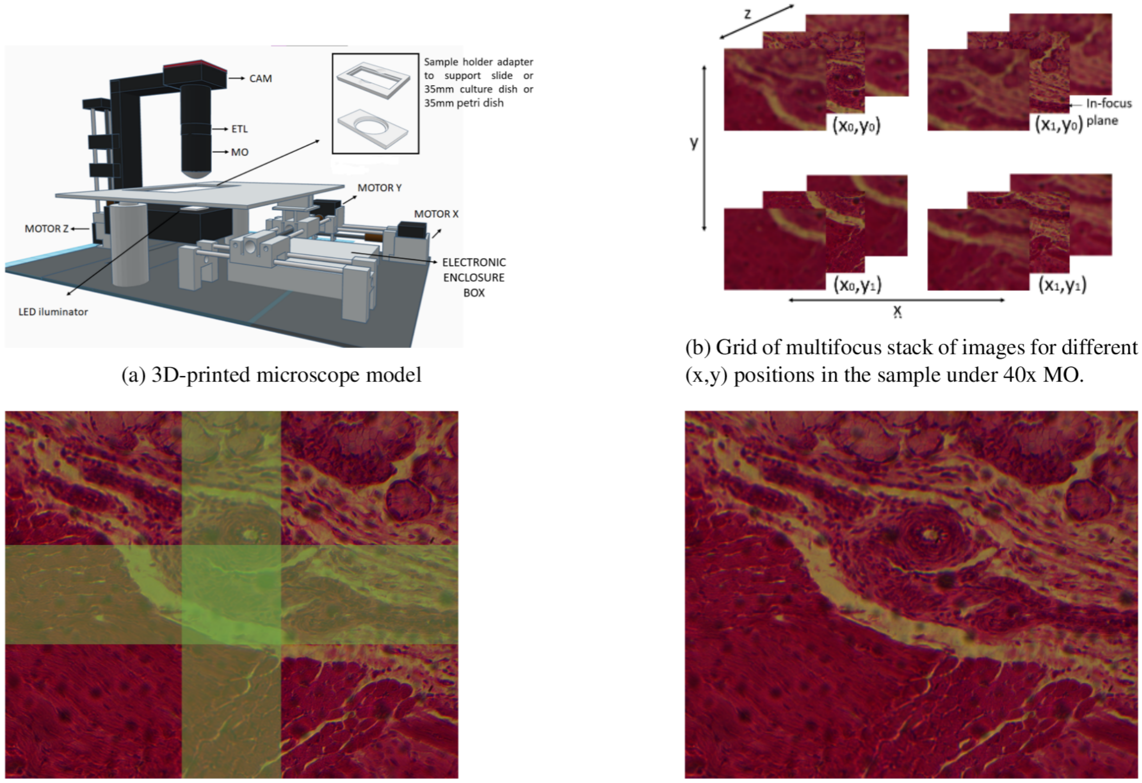
The 3D-printed microscope model is shown in figure 1a with a XY translational motorized stage, an extra motor for Z coarse positioning and the optical system made up of the camera, an electrically focus-tunable lens (ETL) and a microscope objective (MO). The ETL allows fine tunning the in-focus plane [4–6] and the biological samples are illuminated by transmission from below with a LED iluminator in an upright microscope configuration.

The XY motorized translation stage is driven by a microcontroller board providing the required travel range for both coarse and fine positioning of the specimen. Besides 3D-printed sample holder adapters to the XY stage makes it possible to work with slides or 35mm culture or 35mm petri dishes.

The design of the proposed microscope allows its relatively simple transformation from the upright microscope configuration to an inverted one which is of interest for time-lapse studies of entire cell cultures in multiple imaging modalities, furthermore the ability to monitor cells in real time and for long periods of time is important in many different biological applications, as well as screening for new drugs or tests of the proliferative and invasive dynamics of tumor cells [7].

As a proof of principle of the performance of the proposed microscope we used a dog esophagus preparation from an educational set of biological samples. In figure 1b we show stacks of images acquired at different (x,y) positions of the motorized XY stage to obtain neighboring images with overlapping FOV (e.g., one third of the horizontal size of the image) as seen in figure 1c. The sample can be kept in-focus without any mechanical movement by means of the ETL focal distance modulation with an applied current and an autofocusing algorithm based on a focus measure (FM). The FM is a function of depth, and its maximum value must be in the focal plane [1]. For each position of the stage (x, y) this FM function is fit to the data. Thus obtaining the image in focus for that position, which will be used for the stitching.

Since each image has a known X and Y overlap with its neighbors, the stitching algorithm only needs to consider the overlapping regions between neighboring images to compute the features using a feature-detection algorithm based on Speeded-Up Robust Features (SURF) [8] and to find matching pairs between the different images. Then the best transformation for those points is calculated. The resulting extended FOV image is shown in figure 1d with a good stitching accuracy.



(a) 3D-printed microscope model

(b) Grid of multifocus stack of images for different (x,y) positions in the sample under 40x MO.

(c) Stitching overlapping neighboring image tiles

(d) Extended FOV image

Fig. 1.

Funding. Agencia Nacional de Investigación e Innovación ANII (Grant number FMV_2019_1_156126).

Acknowledgments A. Silva acknowledges a scholarship from Agencia Nacional de Investigación e Innovación ANII.

References

1. J. Chalfoun, M. Majurski, T. Blattner, K. Bhadriraju, W. Keyrouz, P. Bajcsy, and M. Brady, "Mist: accurate and scalable microscopy image stitching tool with stage modeling and error minimization," *Scientific reports*, vol. 7, no. 1, pp. 1–10, 2017.
2. Z. Wang, M. Lei, B. Yao, Y. Cai, Y. Liang, Y. Yang, X. Yang, H. Li, and D. Xiong, "Compact multi-band fluorescent microscope with an electrically tunable lens for autofocusing," *Biomedical optics express*, vol. 6, no. 11, pp. 4353–4364, 2015.
3. M. Bathe-Peters, P. Annibale, and M. Lohse, "All-optical microscope autofocus based on an electrically tunable lens and a totally internally reflected ir laser," *Optics express*, vol. 26, no. 3, pp. 2359–2368, 2018.
4. J. R. Alonso, A. Fernández, G. A. Ayubi, and J. A. Ferrari, "All-in-focus image reconstruction under severe defocus," *Optics letters*, vol. 40, no. 8, pp. 1671–1674, 2015.
5. J. R. Alonso, A. Silva, and M. Arocena, "3d visualization in multifocus fluorescence microscopy," in *Three-Dimensional Imaging, Visualization, and Display 2019*, vol. 10997, p. 109970Q, International Society for Optics and Photonics, 2019.
6. J. R. Alonso, A. Silva, and M. Arocena, "Computational multimodal and multifocus 3d microscopy," in *Unconventional Optical Imaging II*, vol. 11351, p. 1135110, International Society for Optics and Photonics, 2020.

7. M. Arocena, M. Landeira, A. Di Paolo, A. Silva, J. Sotelo-Silveira, A. Fernández, and J. Alonso, "Using a variant of coverslip hypoxia to visualize tumor cell alterations at increasing distances from an oxygen source," *Journal of cellular physiology*, vol. 234, no. 10, pp. 16671–16678, 2019.
8. L. Juan and G. Oubong, "Surf applied in panorama image stitching," in *2010 2nd international conference on image processing theory, tools and applications*, pp. 495–499, IEEE, 2010.