

easy Cell CLEM: eC-CLEM a plugin to register correlative data

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1. INTRODUCTION

Melanin biosynthesis occurs in melanocytes, a specialized cell dedicated to melanin pigment production and responsible for tissue pigmentation. Skin melanocytes exploit their endosomal system and associated machineries to generate a Lysosome Related Organelle (LRO), the melanosome, where melanin is synthesized and stored. Melanosome originates from early sorting endosome and matures through a sequential supply of melanogenic cargoes. Using Alexa-labelled transferrin and GFP-tagged proteins involved in formation of endosomal carriers, we decipher the mechanism of cargo delivery to melanosomes that underlie the pathophysiology of albinism [1].

Melanosome biogenesis is addressed *in vivo* in skin melanocytes and in reconstructed human epidermis using our advanced Correlative light and electron microscopy (CLEM) workflow [2]. CLEM is taking an increasing part in imaging for electron microscopy laboratories and many approaches are developed to address a large variety of biological specimen and phenotypes. Yet, aligning the light microscope images with the electron micrographs remains common to all CLEM approaches. We have developed easy Cell CLEM (eC-CLEM) as a new and intuitive plugin to allow any scientist to align its CLEM images without any image processing prerequisite or mathematical background. We rely on the user expertise to identify corresponding landmarks between the different images to align the two registered images. Our plugin functions for two or three dimension datasets and contains some internal routines to pre-process the data and improve the signal to noise ratio, thereby facilitating the user's task.

No mathematical background is required to manipulate the plugin and the code is open source to allow increasing the plugin improvement on a long term.

2. RESULTS

2.1 A simple GUI

To design the plugin, we used the open-source software Icy [3]. The intuitive interface with several display controls at hand makes the images manipulation very rapid and user-friendly. We simplified the graphical user interface (GUI) to the strict minimum to avoid distracting the user or disturb him with complex mathematical parameters.

The graphical user interface offers to work in two dimensions (2D) or in three dimensions (3D), and options appear accordingly. We supposed that most application would require registering fluorescent images on electron micrographs, but the choice is left to the user. Applying a previously computed transformation allows to align large datasets (time course, multichannel for example) according to the chosen image. This saves a significant processing time when large datasets are meant to be registered.

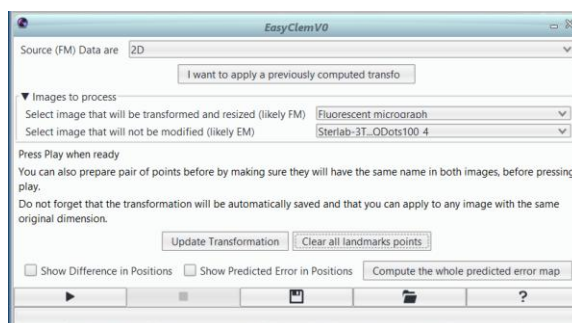


Figure 1. The graphical user interface is kept simple.

2.2 Registration error calculation

The registration is based on landmarks seeding by the user. Each supplementary fiducial is used to recalculate the localization accuracy of all landmarks [4]. Thereby, it becomes possible to locally assign the alignment accuracy of a pin-pointed feature. A global error map can also be calculated to illustrate the global alignment accuracy of the image. It may be extrapolated to the deformation induced to the specimen between the various transformation steps between the light and the electron microscope.

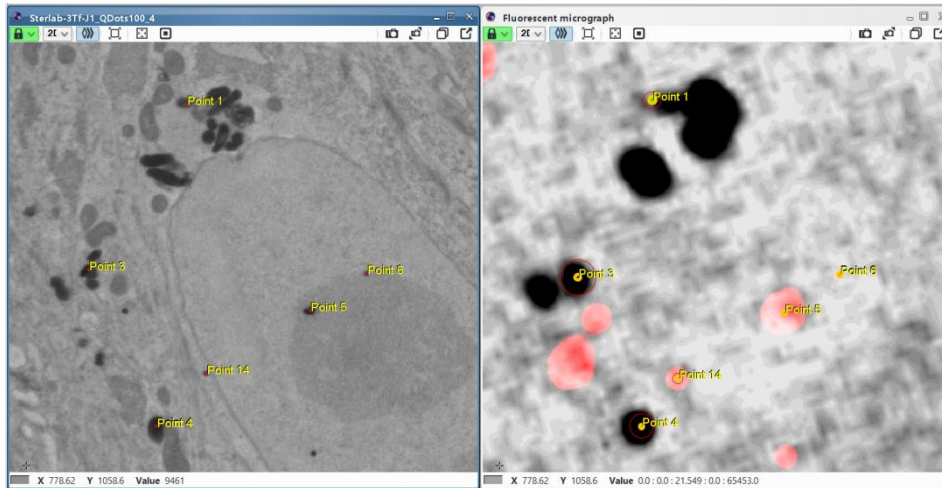


Figure 2. Each fiducial is manually identified and the localization accuracy is displayed on the transformed image to highlight the local assignment error. Point 3 is most probably mis-targeted while point 6 is accurately centered.

3. CONCLUSION

We have created a simple plugin, dedicated to image registration for non image-analysts. Our goal was to keep the biological expertise into the scientist hands, without requiring advance image processing knowledge. The plugin is freely available on the Icy website.

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