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Introduction

The optical clearing of the murine cardiac tissue has always been a challenging goal to obtain successful t3D reconstructions of entire hearts. Typically, the developed protocols are targeted at the clearing of the brain; cardiac tissue requires proper arrangements to the original protocols, since it displays a number of differences, which are usually tough and time-consuming to figure out. At the beginning of this year, I employed the most efficient optical clearing protocols and I optimised the new SHIELD methodology for its application on the cardiac tissue. In particular, I focused on comparing the tissue features of the hearts cleared with different approaches (uDISCO, CLARITY and SHIELD).

Methods

The hearts have been isolated from anesthetized animals, cannulated and perfused with PBS and PFA 4% in PBS (pH 7.6). Optimised CLARITY, SHIELD and uDISCO protocols have been performed on control hearts, which have been subsequently stained with Wheat Germ Agglutinin (WGA)-Alexa Fluor 633 for cellular membrane detection. Whole cleared hearts were examined using a custommade fluorescence light-sheet microscope, able to rapidly acquire images of a mesoscopic FOV with micron-scale resolution (MesoSPIM) (Figure 1).

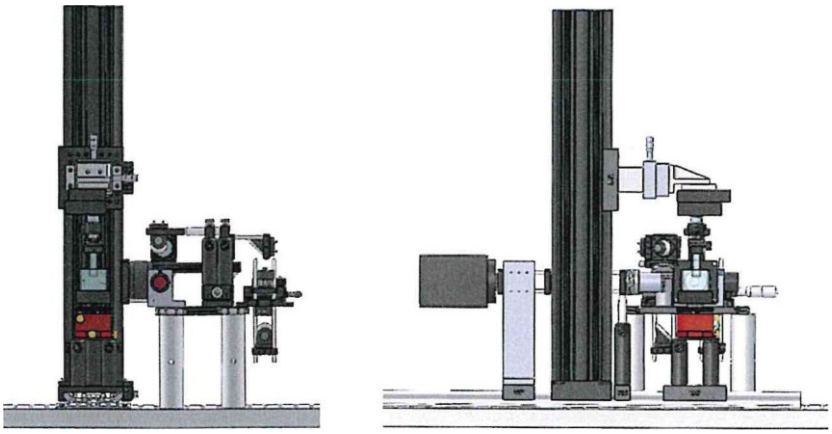

Figure 1: MesoSPIM Front view.

Results

We observed that, in terms of tissue preservation and integrity, the SHIELD procedure is the most successful one when applied on entire hearts, and it also offers a good compromise in terms of clearing timings; moreover, it is very easy to perform. However, due to the compactness

maintained by the tissue, it is very challenging to obtain homogeneous staining of the entire organ, with even the smallsized fluorescent dyes (e.g. WGA) remaining confined to the surface of the tissue (Figure 2). Due to the actual staining issue in SHIELD-cleared hearts, we can state that, currently, the CLARITY protocol is the most complete and finalising approach to obtain 3D reconstructions of fluorescently labelled organs.
Finally, I have performed the optimised approach on a pathological mouse model of arrhythmogenic cardiomyopathy (AC) and on control hearts, which will be stained, imaged and the structural features of the tissue will be analyse to understand the structural remodelling occurring in pathology.

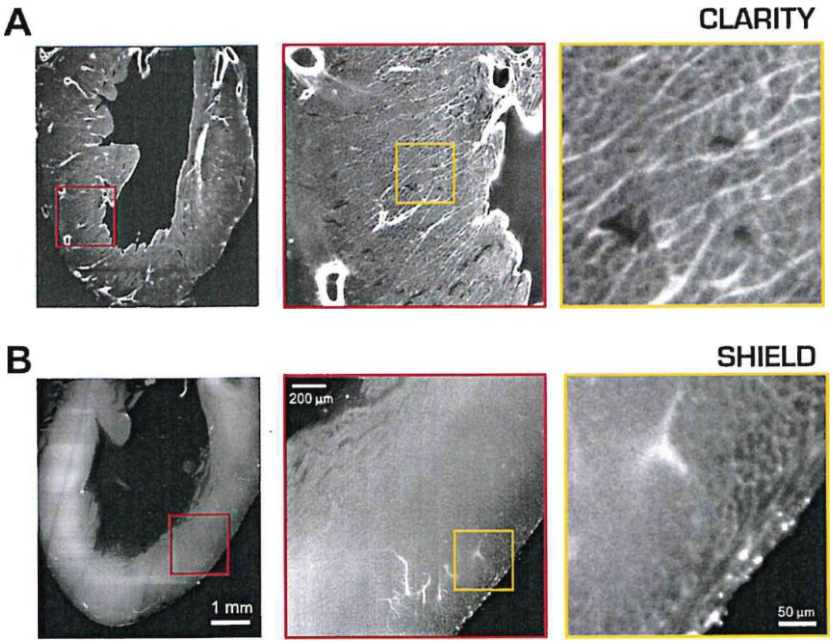

Figure 2: CLARITY (A)- and SHIELD (B)-cleared hearts, labelled with WGA — Alexa Fluor 633 and acquired with our custom-made MesoSPIM.

Publications:

- Camilla Olianti, Francesco Giardini, Erica Lazzeri, Irene Costantini, Ludovico Silvestri, Raffaele Coppini, Elisabetta Cerbai, Francesco S. Pavone, Leonardo Sacconi "Optical clearing in cardiac imaging: A comparative study", Progress in Biophysics and Molecular Biology, 2021, ISSN 00796107, https://d0i.org/10.1016/j.pbiom01bi0.2021.07.012.

- Camilla Olianti , Francesco Giardini , Erica Lazzeri , Giada Beconi, Irene Costantini, Ludovico Silvestri, Elisabetta Cerbai, Francesco S. Pavone, Leonardo Sacconi "Mesoscopic optical imaging of whole mouse heart", Joumal of Visualised Experiments (JoVE), in press.