Multi-sample automation of the CLARITY technology for the processing of 3D volumes of tissue

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ABSTRACT

The current technologies utilized for preclinical and clinical drug development in cancer is largely dependent upon the 2-dimensional (2D) analysis of thin Formalin-Fixed Paraffin-Embedded (FFPE) tissue sections (5-10 µm). However, the importance of understanding cellular phenotypic information combined with three-dimensional (3D) spatial analysis of tissues has recently evolved. In recent years, several clearing techniques, such as CLARITY, have been developed and modified as a means to image and elucidate these volumetric tissues. Most of these techniques have employed chemical approaches to improve tissue clearing, while inadvertently affecting the tissue integrity on a microscopic or macromolecular level. Our previous work with CLARITY has demonstrated how the tissue hydrogel matrix (HM) is able to maintain its structural integrity overall. Yet, some of the most noted barriers to employing this technique has been the lengthy processing times, and the lack of robust 3D spatial analysis software. We sought to address these issues through the development of an automated clearing and staining platform for CLARITY processed tissues with a proprietary 3D imaging engine employing artificial intelligence and machine learning techniques. All experiments were performed with the CLARITY technique using FFPE-embedded tissues that were cleared with a 250nm isotropic clearing buffer. Evaluation of the clearing module was assessed using a passive clearing (diffusion-based) approach before sample staining. The effectiveness of the staining module was assessed using passive clearing tissues that were actively stained with the developed respective module, followed by standard imaging. The imaging data was then processed using the proprietary software to perform segmentation, classification, and quantitative spatial analysis. We were able to demonstrate successful clearing and staining in both normal and malignant breast tissues. This was a significant reduction in the time associated with the standard passive clearing and staining protocol. In short, we were able to demonstrate how the tissue sample was processed from biopsy punch sample to 3D reconstruction in sample registration for sample reconstruction, but also maintains the benefit of multiple interrogation of a single sample. Although passive clearing and 3D analysis are still in their infancy from a technology perspective, our tissue sample using these approaches provides as much volumetric information as 200 FFPE sections, while also maintaining key spatial information.

1. THE CLARITY TECHNOLOGY WORKFLOW

1.1. Sample Preparation

1.2. Automated Passive Clearing

1.3. Automated Active Clearing

1.4. Automated Active Staining

1.5. 3D Software Analysis Workflow

2. ACTIVATING CLEARING MODULE

2.1. Automated Passive Clearing

2.2. Automated Active Clearing

3. ACTIVATING STAINING MODULE

3.1. Automated Passive Staining

3.2. Automated Active Staining

4. 3D SOFTWARE ANALYSIS WORKFLOW

5. SUMMARY

- Our active clearing and staining modules resulted in a significant reduction in the time associated with the standard passive clearing and staining procedure.
- Technologically, volumetric clearing and 3D analysis are still in their infancy; however, our automated platform demonstrates the viability of these proprietary software and methodologies.
- The development of our end-to-end multi-sample clearing and staining platform, with individualized sample chambers, not only removes the laborious sectioning and tissue cross-contamination, but also the need of sample registration for 3D reconstruction.

Figure 1: A 4D image of activated breast cancer needle core biopsy demonstrating 20x magnification using a Live/Dead dual-stained breast carcinoma cell line (center), and a DAPI-only cell line (right).

Figure 2: A 4D image of a CLARITY processed mouse kidney and mouse brain, Histone H3 (red), DAPI (blue).

Figure 3: A normal mouse kidney biopsy punch sample before passive clearing (left), after passive clearing (center), and actively stained (right) with DAPI (blue) and MARIS visualization after cortical imaging was complete.

Figure 4: A 3D image of an activated breast cancer needle core biopsy demonstrating 20x magnification using a Live/Dead dual-stained breast carcinoma cell line (center), and a DAPI-only cell line (right).

Figure 5: A normal mouse kidney biopsy punch sample before passive clearing (left), after active clearing (center), and actively stained (right) with DAPI (blue). Right image: Normal mouse kidney (top), CLARITY (bottom), and DAPI (blue).

Figure 6: A 4D image of an activated breast cancer needle core biopsy demonstrating 20x magnification using a Live/Dead dual-stained breast carcinoma cell line (center), and a DAPI-only cell line (right).