

Clearing the way: Non-toxic clearing and labeling with fluorescent REAfinity™ antibodies for enhanced 3D visualization of tissues and organs

K. Bigott¹, J. Werheid¹, K. Gerhold¹, F. Küster¹, D. Barleben¹, D. Bessa-Neto¹, D.A. Yushchenko¹, L. Haalck¹, G. Ebel¹, C. Dose¹, A. Bosio¹, M. Jungblut¹, L. Nolte¹, D. Eckardt¹

¹ Miltenyi Biotec GmbH, Bergisch Gladbach, Germany.

Abstract

Generation of a 3D cellular topography of biological specimens is often essential to understand cellular and molecular processes. Especially, spatial visualization of heterogenous protein expression in solid tumor samples is considered important for advanced phenotyping and potential diagnostic applications. However, for a long time, biologists were limited to the visualization of thin tissue sections, which provides limited cellular context. Furthermore, sectioning is time-consuming and often prone to artifacts, like ripping and folding. While clearing methods in combination with light-sheet fluorescence microscopy have emerged as powerful tools for 3D reconstruction of tissues, most clearing techniques involve toxic reagents which require special safety provisions and generate extra effort for sample preparation as well as imaging.

To address these shortcomings, we developed a novel clearing workflow based on non-toxic reagents that allows for efficient 3D imaging of various biological samples, including mouse brain, stem cell-derived organoids, tumor samples, and tumor spheroids. For blood-rich organs and tissues, we developed an efficient depigmentation module in order to increase light transmission and reduce autofluorescence. Towards 3D imaging, we combined our clearing procedure with whole-mount immunostaining of mouse brain hemispheres, human tumor samples and organoids using Miltenyi Biotec's REAfinity™ antibodies coupled to newly developed Vio® dye variants optimized for application in light-sheet microscopy. REAfinity™ antibodies are recombinant antibodies engineered to provide high specificity, purity, and superior lot-to-lot consistency compared to hybridoma-derived antibodies. Vio® dyes are a new generation of fluorescent dyes characterized by remarkable brightness, photostability and highly resistant against organic solvents during clearing procedure. By combining the advantages of REAfinity™ antibodies and Vio® dyes, as well as by optimizing conjugation of antibodies with Vio® dyes, we developed staining reagents that significantly reduce staining duration and are thus ideally suited for whole-mount tissue labeling, multiplexing, and imaging with light-sheet microscopy. In the future, our conjugate portfolio will continuously grow with increasing target specificities and development of additional Vio® dye varieties. Our data indicates highly efficient labeling of the vasculature, different neuronal subtypes, astrocytes, and oligodendrocytes in adult mouse brains, as well as bright staining of neural progenitors and proliferative cells in cerebral organoids. Furthermore, carcinoma cells and different leucocyte subtypes were visualized in solid tumors. After data acquisition, our in-house developed postprocessing pipeline enables the elimination of noise, intensity inhomogeneities, and striping artifacts. This enables the ideal preparation of 3D data for subsequent data visualization and quantification.

In summary, we developed a robust, fast, and non-toxic clearing procedure combined with bright antibody conjugates for optimal visualization of tissue in 3D, paving the way for a better understanding of tissue structures and disease mechanisms.