

3D VISUALIZATION OF GAMMA DELTA T CELLS IN MOUSE VAGINA USING TISSUE CLEARING METHODS

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The spatial distribution of immune cells in their native environment is crucial for their functions. Frequently, however, the exact location of cells is difficult to assess due to lack of methods allowing to image the whole tissue without sectioning. Thus, the immune surveillance of reproductive mucosa is poorly characterized as it is relatively thick and its thickness changes during the hormonal cycle, which further hinders the analysis. Recently, several tissue clearing methods have been developed for 3D imaging of thick specimens. However, none of these techniques has been tested on the vaginal wall. We have applied two methods: CUBIC [1] and ScaleS [2] to find a clearing method suitable for visualization of vaginal $\gamma\delta$ T lymphocytes which are the key immune cells engaged in epithelial homeostasis control.

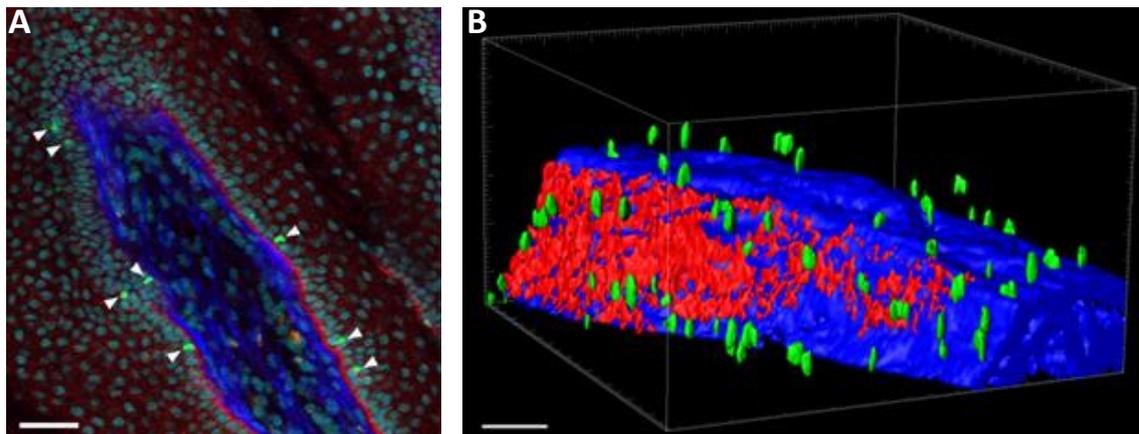


Figure 1: Mouse vagina after CUBIC clearing. A. Cross-section of vaginal epithelium and stroma. Arrowheads point to $\gamma\delta$ T cells. B. 3D rendering of vaginal wall. Green, $\gamma\delta$ T cells; red, laminin; blue, collagen; cyan, DAPI. Scale bar 50 μ m.

The imaging of transparent tissues was performed on a Leica SP8 confocal microscope using single and two photon excitation, second harmonic generation was used to visualize collagen [Figure 1]. Image processing revealed that GFP⁺ $\gamma\delta$ T cells reside in epithelium above but in close proximity to immunofluorescently-labeled laminin and collagen-rich stroma. Pros and cons of using both methods for clearing the reproductive tract will be discussed.

References: [1] Susaki E.A.; et al., *Cell* 157, 726–739 (2014); [2] Hama H; et al., *Nat. Neurosci.* 18, 1518–1529 (2015).

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