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Single Cell Track Analysis of Two Photon Microscopy on Th17 Cells in the Gut

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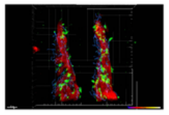
Introduction

Two-photon microscopy is a modern imaging technique for tracking single cells *in vivo* in order to analyze the migration and interaction behavior of cells under physiological conditions. Often, statistical analysis of these data are performed where mean quantities are computed from averaging over all cell tracks. As a consequence, all information about the cellular positions in the biological sample is averaged out. We show that important information in the imaging data can get lost in this way and that a single cell track analysis is required that takes cellular positions into account to correctly interpret the migration behavior of cells.



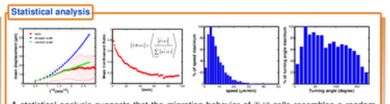
Contribution

We analyze two-photon microscopy data on T helper 17 (T_h17) cells from *in vivo* imaging in the gut of mice. These cells produce interleukin 17 (IL17) and are involved in the immune response against bacteria and fungi. Based on cell tracking data and the visualization of blood vessels in microvilli of the murine gut, we first perform a statistical analysis and arrive at the conclusion that the migration behavior of T_h17 cells resembles a random walk. However, a single cell track analysis reveals that the migration behavior is only seemingly random.

Method

Going beyound a statistical analysis, we performed a single cell analysis by recounstruction the dynamic blood vessel from the three-dimensional imaging data (z-stacks). We then determined the distance of individual T_h17 cells in each time point relative to the moving surface of the blood vessel.

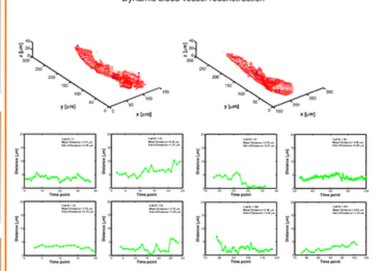
Statistical analysis



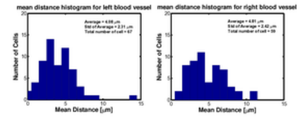
A statistical analysis suggests that the migration behavior of T_h17 cells resembles a random walk.

Single cell analysis

Dynamic blood vessel reconstruction



The dynamic analysis of single cell tracks shows that T_h17 cells are in fact not performing a random walk, but migrate in close proximity to the moving surface of blood vessels. In the most general sense, this investigation demonstrates the importance of single cell track analysis over statistical analysis for interpreting two-photon microscopy data.



Conclusion

Single cell analysis gives much more detailed information than statistical analysis. In this research we have analyzed single cells and found out that T cells approximately move along the dynamic blood vessels.

Acknowledgement

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