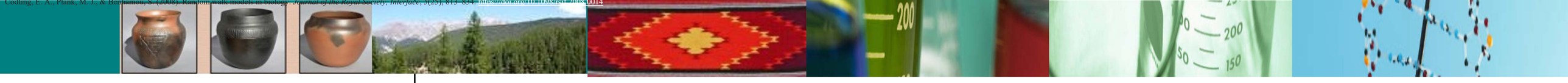

Analysis of T cell Movement within Lymph Nodes

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Abstract

T cells are lymphocytes that serve an important role in the immune system. The T cells we analyze originate from the lymph nodes of mice. The data comes from the University of New Mexico, where two-photon microscopy was used to record time and three-dimensional Cartesian coordinates of T cell locations.

We computed the speed, turning angle, and slopes from the squared displacement of T cell tracks to create distributions. Distributions illustrate the motion type (e.g. Brownian vs ballistic) and the speeds or angles that occur most frequently. We also computed p-values when comparing different T cell movies to quantify differences in T cell speeds.

Introduction

T lymphocytes are vital to maintaining the health of the organisms they inhabit. By fighting off bacteria, viruses, parasites, and cancer cells, T lymphocytes (T cells) are one of the most important components of an organism's body. T cells inhabit and patrol multiple types of tissue, but to activate, they must first encounter antigen-bearing dendritic cells located in the lymph nodes (LN); therefore, it is especially important to understand how T cells behave in the LN (Fricke et al., 2016). Many researchers have used two-photon microscopy to study the T cells of different tissues.

Brownian motion, Lévy walks, and correlated random walks (CRWs) have been proposed as ideal biological search models (Codling et al., 2008, as cited in Fricke et al., 2016). Many studies suggest that T cell motion in the LN is similar to a Brownian random walk or a Lévy walk. However Fricke et al. (2016) suggest that the movements of T cell step lengths and speeds in the lymph node are more consistent with a lognormal distribution. Previous work has found that T cells move at a median speed of 2.3 μm/min in live tissues, which is similar to speeds encountered in areas such as the skin, brain and lungs, but slower than the speed typically observed in the LN (Mrass et al., 2017). According to Katakai and Kinashi (2016), T cells located in the LN paracortex move with an average velocity of more than 10 μm/min. Fricke et al. (2016) found that T cell turning angles in the LN were not uniform but biased toward turning angles of less than 90°; this suggests T cells use a CRW, since Lévy walks and Brownian motion assume turning angle distributions are uniform. The LN environment also has a big impact on how a T cell moves. Research done by Fricke et al. (2016) shows that there are spots in the LN where T cells are more likely to be found, which have been referred to as "hot spots." In these hot spots, T cell movements tend to resemble Brownian motion.

By exploring the migratory patterns of T cells, this research will reveal more information about the immune system, which can then be applied to help scientific research in the medical field.

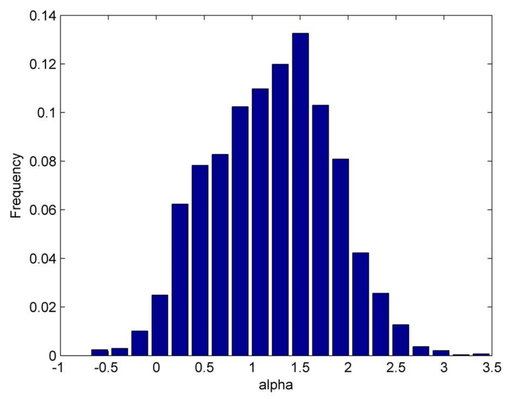
Squared Displacement

We find the distance squared and elapsed time t_i - t_1 between (x_i, y_i, z_i) and (x_1, y_1, z_1)

$$D_i^2 = (x_i - x_1)^2 + (y_i - y_1)^2 + (z_i - z_1)^2$$

$$dt_i = t_i - t_1$$

The linear regression line is then fit through the scatterplot of (dt_i, D_i^2) and the slope α of the line is used to determine if the movement is Brownian ($\alpha < 1$), Lévy ($1 \leq \alpha < 2$) or ballistic ($\alpha \geq 2$).



Angle distribution

First, we find the vectors using the last 3 positions of a T cell.

Vector 1	Vector 2
$Vx_1 = x_2 - x_1$	$Vx_2 = x_3 - x_2$
$Vy_1 = y_2 - y_1$	$Vy_2 = y_3 - y_2$
$Vz_1 = z_2 - z_1$	$Vz_2 = z_3 - z_2$

We then calculate the dot product with the following equation:

$$Dot\ Product = (Vx_1 \cdot Vx_2) + (Vy_1 \cdot Vy_2) + (Vz_1 \cdot Vz_2)$$

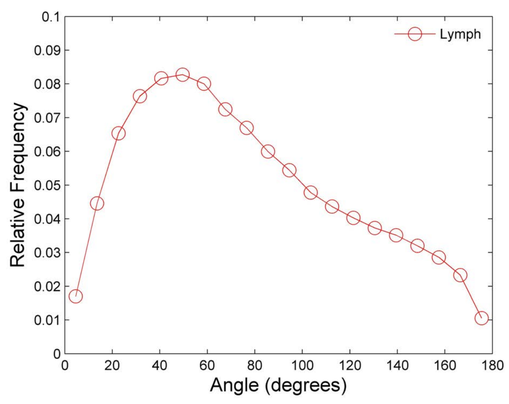
Next, we calculate the magnitudes of the two vectors.

$$|V1| = \sqrt{(Vx_1)^2 + (Vy_1)^2 + (Vz_1)^2}$$

$$|V2| = \sqrt{(Vx_2)^2 + (Vy_2)^2 + (Vz_2)^2}$$

Lastly, we calculate the angle by computing the arccosine of the dot product divided by the product of the two magnitudes:

$$\theta = COS^{-1}\left(\frac{Dot\ Product}{|V1||V2|}\right)$$



Speed distribution

The distance between two positions in a T cell track is found using the formula

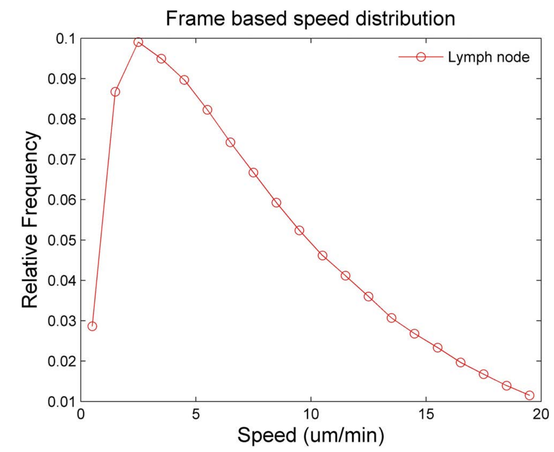
$$D_i = \sqrt{(x_{i+1} - x_i)^2 + (y_{i+1} - y_i)^2 + (z_{i+1} - z_i)^2}$$

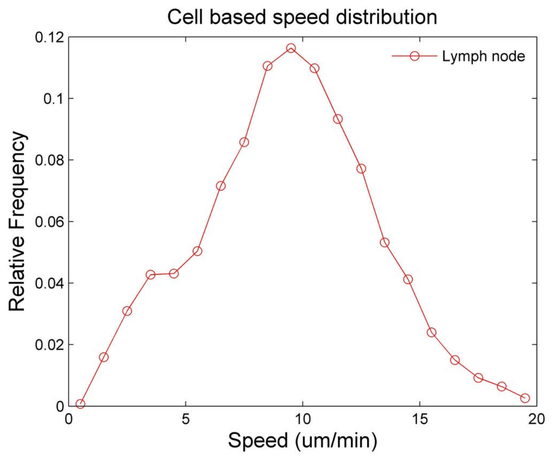
where the coordinates (x_i, y_i, z_i) refer to the first position and the coordinates $(x_{i+1}, y_{i+1}, z_{i+1})$ refer to the second position. The frame-based speed is then calculated using $\text{Speed}_{\text{frame}} = \frac{D_i}{t_{i+1} - t_i}$

where $t_{i+1} - t_i$ is the time difference between the two positions.

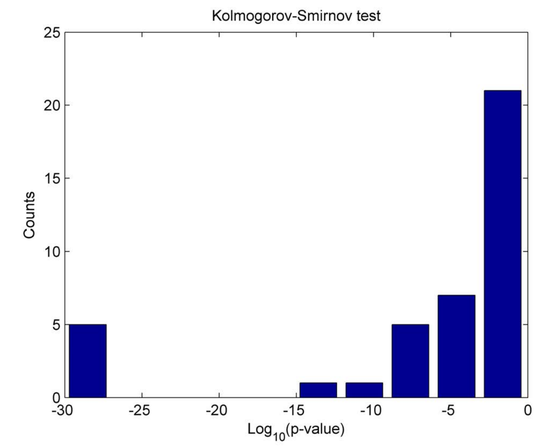
The cell-based speed is calculated using $\text{Speed}_{\text{cell}} = \frac{\sum D_i}{t_n - t_1}$.

Distributions are then created using the frame-based and cell-based speeds.





P value comparison



The cell-based speed distribution of T cells can be heterogeneous as shown in the plot of p-values which were generated by comparing a mean distribution with a movie distribution using the Kolmogorov-Smirnov test in R.

Discussion

The squared displacement slope distributions show that the T cells in the lymph node exhibit Brownian, Lévy, and ballistic motion. The turning angle distribution confirms that the motion is heterogenous. The turning angle distribution peaks at 50 degrees and is not uniform. A uniform distribution would be expected if the T cells moved using Brownian motion.

We also note that the speed distributions vary depending on whether the cell-based or frame-based speeds are used. The frame based distribution peaks at ~2.5 um/min with a long tail while the cell-based distribution peaks at ~10 um/min and exhibits greater symmetry.

The p-value plot shows that the cell-based speed distribution of some movies are very different from the mean distribution.

Conclusion

We have created distributions of squared displacement slopes, turning angle distributions, and frame-based and cell-based speed distributions based on two-photon microscopy data from T cell movement in the lymph nodes of mice.

Our analysis will help understand how T cells move within the lymph node and will serve as a baseline for comparisons with T cell movement in other tissues.

References

Codling, E. A., Plank, M. J., & Benhamou, S. (2008). Random walk models in biology. *Journal of the Royal Society, Interface*, *5*(25), 813-834. https://doi.org/10.1098/rsif.2008.0014.

Fricke, G. M., Letendre, K. A., Moses, M. E., & Cannon, J. L. (2016). Persistence and Adaptation in Immunity: T Cells Balance the Extent and Thoroughness of Search. *PLoS Computational Biology*, 12(3), e1004818. https://doi.org/10.1371/journal.pcbi.1004818

Katakai, T., & Kinashi, T. (2016). Microenvironmental Control of High-Speed Interstitial T Cell Migration in the Lymph Node. *Frontiers in Immunology*, 7, 194. https://doi.org/10.3389/fimmu.2016.00194

Mrass, P., Oruganti, S. R., Fricke, G. M., Tafoya, J., Byrum, J. R., Yang, L., Hamilton, S. L., Miller, M. J., Moses, M. E., & Cannon, J. L. (2017). ROCK regulates the intermittent mode of interstitial T cell migration in inflamed lungs. *Nature Communications*, 8(1), 1010. https://doi.org/10.1038/s41467-017-01032-2

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