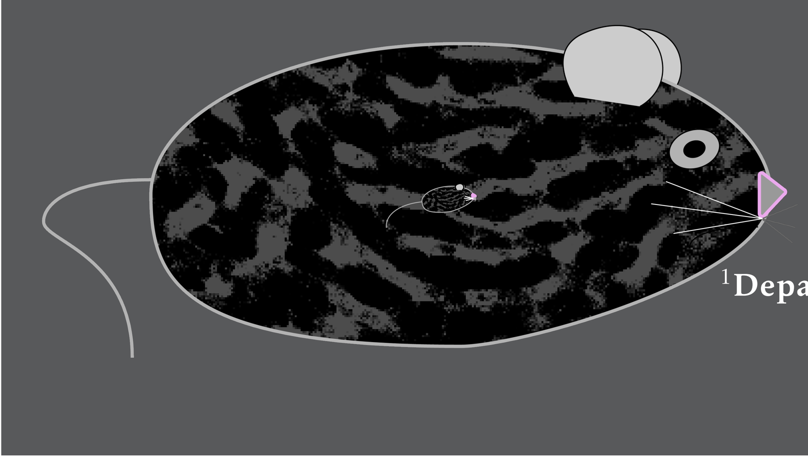

Improving the Analysis of T Cell Movement Data

Viktor S. Zenkov[1], James O'Connor[2], Ian A. Cockburn[2], Vitaly V. Ganusov[3]

[1]Department of Electrical Engineering & Computer Science, [3]Department of Microbiology, University of Tennessee, Knoxville [2]Department of Pathogens & Immunity, John Curtin School of Medical Research, Australian National University, Canberra

vzenkov@vols.utk.edu ORCID: 0000-0002-8864-1835

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Malaria and its foe are introduced.

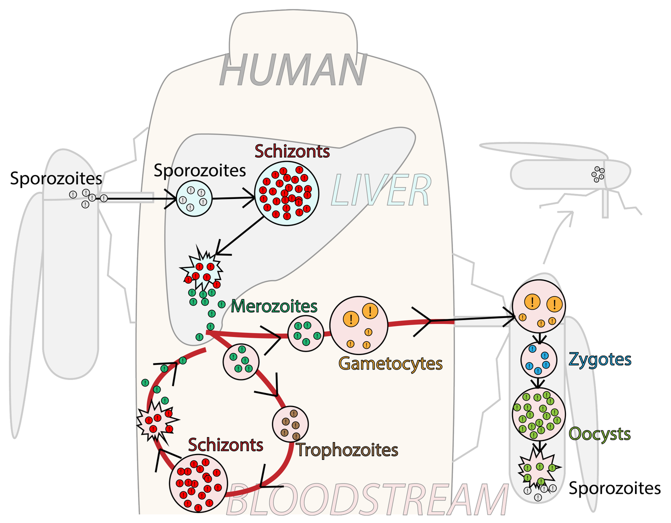

Figure 1: The lifecycle of malaria

- Malaria kills 500,000 people every year.
- Malaria parasites are injected into humans by mosquitos, then travel through the bloodstream to the liver, where they reproduce and cause harm.
- Vaccine-induced T cells combat malaria by eliminating malaria parasites during the liver stage.
- How T cells locate infection sites in the liver remains poorly understood.

The question:

- Are T cells moving **intentionally** toward parasites, or do they only kill parasites when they stumble upon them?

The null hypothesis:

- T cells move randomly without attraction to the parasite, hereafter called *without attraction*.

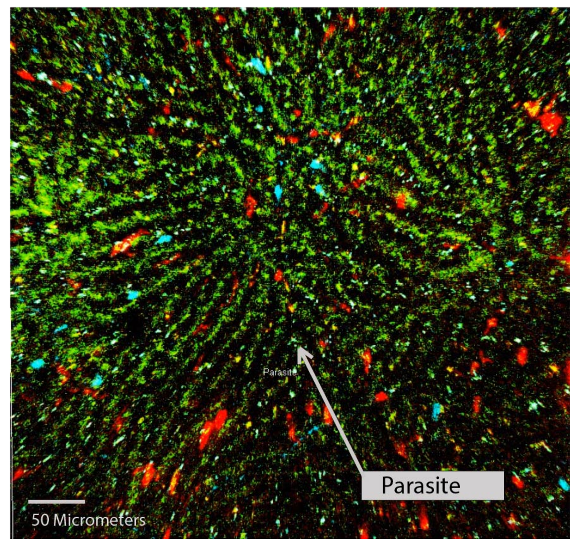

Figure 2: Liver area imaged with intravital microscopy. specific T cells/control cells/hepatocytes/sinusoids(blood vessels)

I use data collected from a designed experiment.

A mouse was injected with 5×10^6 OT-1 (antigen specific) cells stained with cell trace violet and 5×10^6 TCRP14 (non-antigen specific) cells stained with CMTPX Red. After 2 hours 5×10^6 Plasmodium Berghei CS[5M] sporozoites were injected into the mouse. The mouse was imaged with intravital microscopy 3 hours later. Individually imaged areas contained around 20-30 T cells and control cells and one parasite.

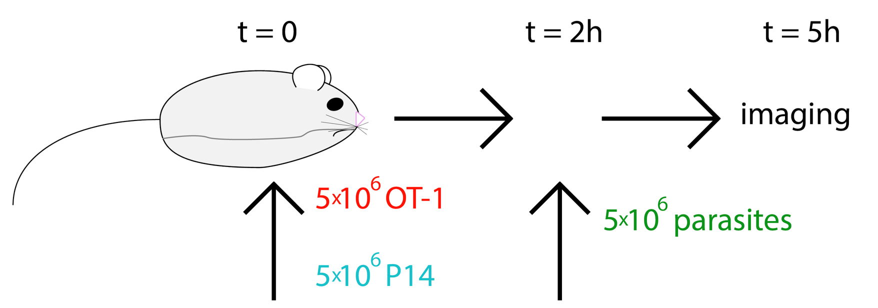

Figure 3: Details of the experimental design

I perform a hypothesis test for each metric below.

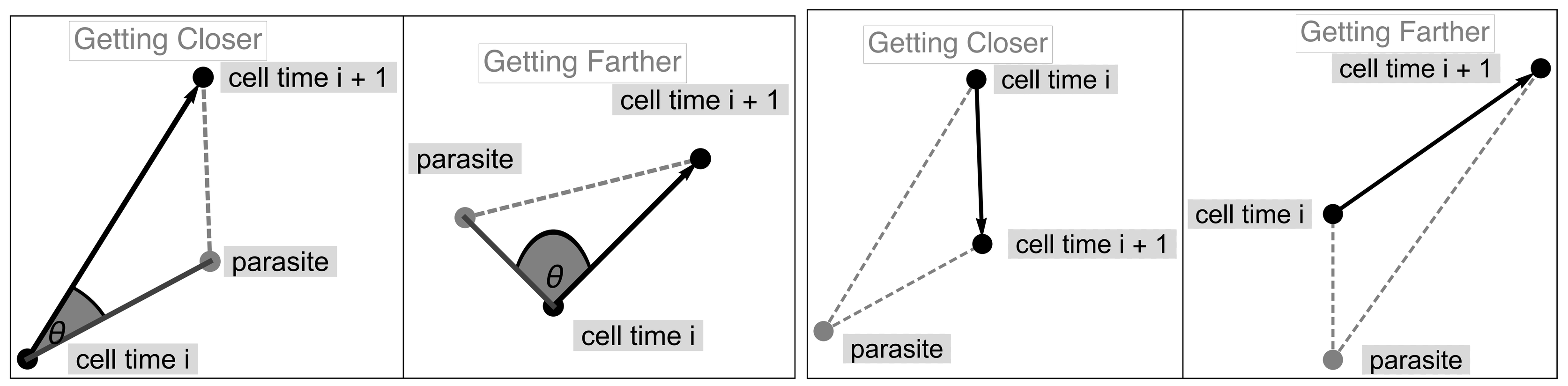

Figure 4: A) The angle metric B) The distance metric

The two tests' results are significantly different.

- Running the same binomial test with $p = 0.5$ on angles and distances gives the results shown here.
- Paired tests show that these results are significantly different.
- Previous analysis suggests the distance metric might have additional complexity, so I **investigate the distance metric**.

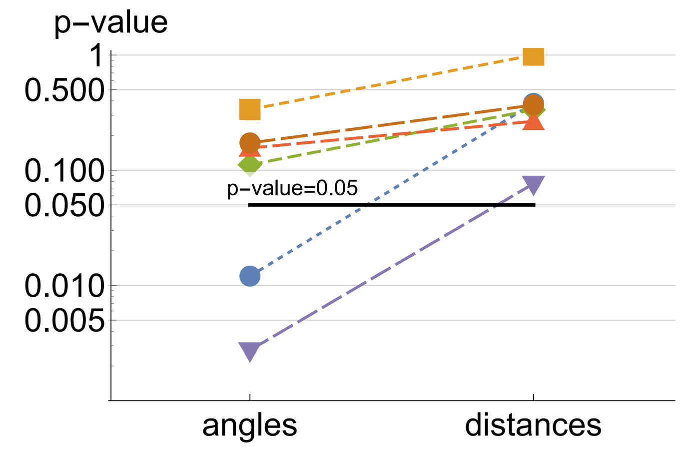

Figure 5: The paired T test gave a p-value of 0.0277.

Calculate the probability of "getting closer" if the cell moves *without attraction*.

- For any movement, let the cell be at a distance $x > 0$ from the parasite and let it move a distance $r \geq 0$.
- Consider two spheres, one around the cell with radius r and the other around the parasite with radius x and its edge touching the cell.
- The cell will move to a position on the surface of the sphere of radius r on its next move. A representation of this in two dimensions is shown to the right.

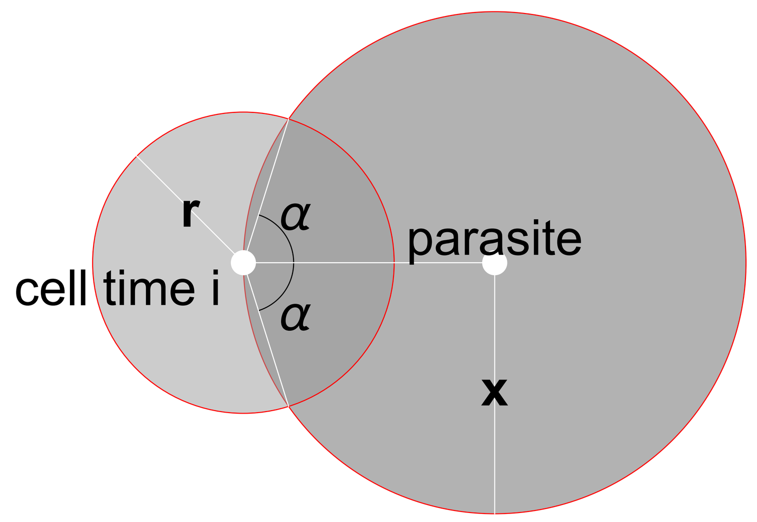

Figure 6: $r < 2x$

- Positions inside (outside) the sphere of radius x correspond to the cell getting closer to (farther from) the parasite.
- If the direction is chosen *without attraction*, then the probability that the cell gets closer is the surface area of the portion of the sphere of radius r which corresponds to the cell getting closer divided by the surface area of the sphere of radius r, which is $\frac{2\pi r^2(1-\cos\alpha)}{4\pi r^2} = \frac{2\pi r^2(1-\frac{r}{2x})}{4\pi r^2} = \frac{1}{2} - \frac{r}{4x} \leq \frac{1}{2}$, with equality happening when $r = 0$, which corresponds to the cell remaining in place.
- For x large or r small, the probability approaches $\frac{1}{2}$.
- For x small or r large, we have $r > 2x$ and the probability becomes negative. This situation is shown to the right. The cell gets farther from the parasite, so the probability of the cell getting closer is 0. Thus the probability is set to 0 when $r > 2x$.

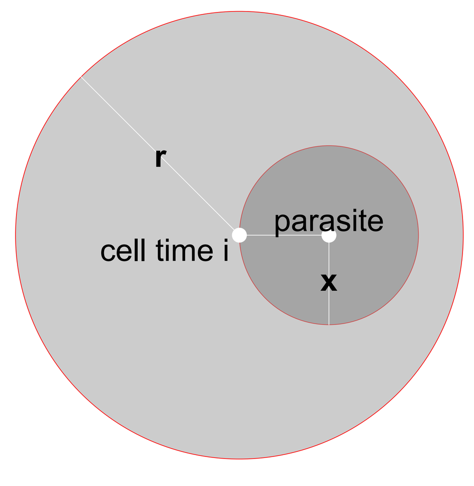

Figure 7: $r \geq 2x$

For the distance metric scenario, we now have the actual probability that a cell gets closer to the parasite, assuming that the cell moves *without attraction*.

I then reanalyze the data.

- Instead of $p = \frac{1}{2}$ in the distance metric scenario, the probability of a cell moving *without attraction* closer to the parasite is $p = \frac{1}{2} - \frac{r}{4x}$, which we associate with a Bernoulli random variable.
- The test statistic under the null hypothesis is the sum of these non-identically-distributed Bernoulli random variables, which has a Poisson binomial distribution.

I overcame hurdles.

- The straightforward programming of the Poisson binomial distribution runs inefficiently.
- I found several approximations for the Poisson binomial distribution in the literature, one of which I used below.

The new results are beneficial.

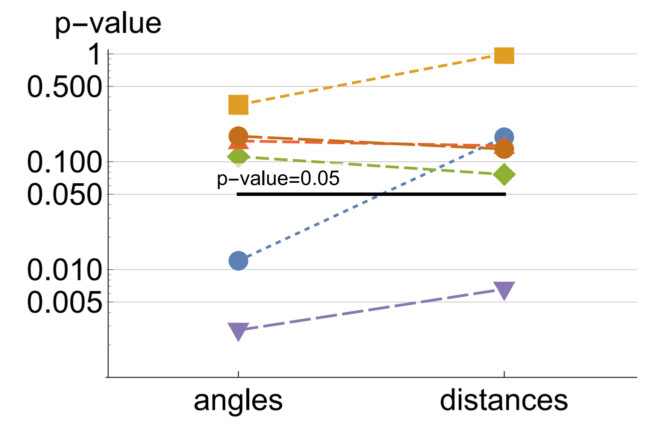

Figure 8: The paired T test gave a p-value of 0.834.

- Running binomial tests with $p = 0.5$ on angles, and Poisson binomial tests on distances, gives the results shown above.
- Paired tests show that these results are **not** significantly different.
- We may now find data suggesting attraction which did not previously suggest attraction.
- The distance metric may be more intuitive than the angle metric, so increasing its accuracy makes it more appealing.
- This test improvement can be incorporated into other cell movement investigations.

There are further steps to take to identify attraction.

- Our analyses so far have assumed that cells move in an open space; in reality they move constrained to a tube structure called sinusoids.
- I have started collecting data about sinusoids. I will consider how my metrics and tests apply to constrained cell movement, make adaptations, and more accurately measure attraction!

NIH Grant R01 GM118553 to Vitaly V Ganusov. Resources: ∘ Cockburn, Ian A., et al. "In vivo imaging of CD8⁺ T cell-mediated elimination of malaria liver stages." *PNAS*, vol. 110, 2013, pp. 9090-9095. ∘ Soon, Spario Y. T. "Binomial Approximation for Dependent Indicators." *Statistica Sinica*, vol. 6, 1996, pp. 703-714. ∘ World malaria report 2017. Geneva: World Health Organization; 2017.