Results and Figures

Hotspot Analysis:

From the data files, spots that are visited by a unique number of T-cells are counted and the distribution of the visits is taken. The mean and standard deviation of each experiment is calculated. Any number of visits that is more than two standard deviations from the mean is what we are counting as hot spots.

We have 40 wild type data (WT) data and 18 CCR7 KO data (filtered). A way to compare the number of hotsopts in the CCR7 knockout experiments with the number of hotspots in the Wild Type experiments is to consider the ratio of the empirical data and simulated data of both WT and CCR7KO. We take the actual number of hotspots from the empirical data and calculate hotspot for the simulated data. For each corresponding experiment, we divide the empirical data with the simulated one. The scatter points in Figure 1 are the ratios of empirical and simulated data. We did the same thing for CCR7 knockouts.



Figure 1: This scatter plot shows the ratios of empirical and simulated vales for the hotspot analysis of wild type data (right) and CCR7KO data(left). Experiments are in the x-axis and ratios are in the y-axis. The red horizontal line is the mean of the ratios.

From both WT and CCR7 data, we can say that, difference between WT and CCR7KO is marginal. The mean ratio of WT is 1.8981 and CCR7 is 1.2399 which is also very close. We created a notched box-plot from the data which is shown in figure 2. We ran the Mann-Whitney U test and t-test between the data sets to test if they were statistically significant. The p-value indicates that they are marginally significant



Figure 2: This box plot shows the distribution of the data based on the ratios of hotspot analysis for WT and CCR7 data. The P-value for Mann-Whitney U test is .077584 and t-test is .049125. It shows that both data sets are statistically marginally different.

Mutual Information Analysis (T-DC):

From the data videos, we try to find the mutual information of red and green channels which are T cells and DCs accordingly. By calculating mutual information, we want to find out how much information can we gather about the T-DC interactions. We want to examine if T cells only be in the places where there is DCs. For our mutual information analysis, we want to calculate several values of mutual information between the green and red channels. We read in the images for all the time steps, in consecutive order and calculate several mutual information values. we also take each the red and green channel from the videos, and shuffle them, randomly, so that we can use this randomized image of the red or green frame in our mutual information calculations.

We calculated 6 values i.e. MI(R,G), MI(R,R), MI(R,RS), MI(G,G), MI(G,GS), MI(G,RS). MI(R,G) denotes the mutual information (MI) between the red channel and the green channel. MI(R,R) denotes the MI between the red channel with itself and so does the green channel with itself in MI(G,G). MI(R,RS) de-notes the MI between the red channel and that same red channel but shuffled. Same happens with green channel in MI(G,GS). MI(G,RS) is MI between green and shuffled red channel.

A screenshot of a cell phone

Description generated with high confidence

Figure 3: These plots show the mutual information analysis for 7 WT videos. Time steps is in the x-axis and MI is in the y-axis. Blue line shows MI(R,G), redline shows MI(R,R), green line shows MI(G,G), magenta line shows MI(R,RS), black line shows MI(G,GS) and cyan line shows MI(G,RS).

The two values MI(R,R) and (G,G) are what we use as our `upper bound' . They should be significantly larger than MI(R,G), because they show the mutual information between the same images. This means, if we have information about R (red channel), then there is already information available about itself. The two shuffled values MI(R,RS) and MI(G,GS) are our `lower bound' , and should be less than MI(R,G). This is because we are taking the mutual information between an image and its randomized self. So, if we have information about the original image, this doesn't tell us anything about a random image, so the mutual information should be very low. Figure 3 shows the MI analysis of wild type data (7 videos) and figure 4 shows the MI analysis of CCR7 data(12 videos). Both figures show the same characteristics as we have discussed here. However, the values for CCR7 are continuously lower than that of WT.

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Figure 4: These plots show the mutual information analysis for 12 CCR7 videos. Time steps is in the x-axis and MI is in the y-axis. Blue line shows MI(R,G), redline shows MI(R,R), green line shows MI(G,G), magenta line shows MI(R,RS), black line shows MI(G,GS) and cyan line shows MI(G,RS).

A close up of a map

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Figure 5: This box plot shows the distribution of the data based on the MI values for WT and CCR7 data. The P-value for Mann-Whitney U test is .0052393. It shows that the difference between both data sets is statistically significant.

* Normalized MI:

We calculate the normalized mutual information of the data by taking the ratio of mutual information between red and green channel with mutual information between green and green channel.

We use the normalized formula to be able to compare across experiments so it considers the internal entropy of the images that does not artificially change with the number of the cells.

* Relative MI:

We calculate the relative mutual information of the data which is a ratio of mutual information between red and green channel subtracted from shuffled red – green channel with mutual information between red and green channel.

We use the relative formula to be able to compare the mutual information with the randomly distributed cells.

Figure 6 and 7 show the mutual information, normalized mutual information and relative mutual information across experiments of both WT and CCR7KO data. The figures imply that the spatial relationship between WT and DC cells is more random than CCR7 KO cells and DCs.

It is to be noted here that we are now trying to find a combined formula for the normalized mutual information that will serve both purposes.

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Figure 6: These plots show the WT data analysis. Time steps is in the x-axis and Mi, normalized MI and relative MI are in the y-axis. Black line shows the value of MI, red line shows the value of normalized MI and cyan line shows the value of relative MI with time across experiments.

A screenshot of a cell phone

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Figure 7: These plots show the WT data analysis. Time steps is in the x-axis and Min normalized MI and relative MI are in the y-axis. Black line shows the value of MI, red line shows the value of normalized MI and cyan line shows the value of relative MI with time across experiments.

A close up of a map

Description generated with high confidence

Figure 8: This box plot shows the distribution of the data based on the normalized MI values for WT and CCR7 data. The P-value for Mann-Whitney U test is .09018. It shows that the difference between both data sets is not statistically significant.

A close up of a map

Description generated with very high confidence

Figure 9: This box plot shows the distribution of the data based on the relative MI values for WT and CCR7 data. The P-value for Mann-Whitney U test is .0011. It shows that the difference between both data sets is statistically significant.

Mutual Information Analysis (T-HEV):

For HEV red channel is HEV blood vessel and green channels are T cells.



Figure 10: These plots show the mutual information analysis for 4 HEV videos. Time steps is in the x-axis and MI is in the y-axis. Blue line shows MI (R,G), redline shows MI(R,R), green line shows MI(G,G), magenta line shows MI(R,RS), black line shows MI(G,GS) and cyan line shows MI(G,RS).



Figure 11: These plots show the comparison among MI, Normalized MI and relative MI of HEV data. Time steps is in the x-axis and MI, normalized MI and relative MI are in the y-axis. Blue line shows the value of MI, red line shows the value of relative MI and green line shows the value of normalized MI with time across experiments.



Figure 12: This box plot shows the distribution of the data based on the MI values for WT, CCR7 and HEV data. Mann-Whitney U test has been performed to calculate the statistical comparison.



Figure 13: This box plot shows the distribution of the data based on the normalized MI values for WT, CCR7 and HEV data. Mann-Whitney U test has been performed to calculate the statistical comparison.



Figure 14: This box plot shows the distribution of the data based on the relative MI values for WT, CCR7 and HEV data. Mann-Whitney U test has been performed to calculate the statistical comparison.

Mutual Information Analysis (T-FRC):

To be updated

T-DC distance analysis:

To be updated with Janie’s results