Analysis on Diffusivity of IQGAP1 in Jurkat T-Cells

Will Heffernan

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Abstract

IQGAP1 is a protein in the IQGAP family that is known to interact with a variety of intracellular molecules such as cytoskeletal components to living cells, pertaining to a diverse array of biological functions. The purpose of this project is to investigate the effect of cytoskeletal components on the diffusivity of this particular protein within Jurkat T-cells. While few meaningful conclusions can be drawn from the data and figures created so far, continuing image analysis through various softwares will likely prove fruitful in determining how different drugs affect the diffusivity of IQGAP1 within cells. Further research on the interactions of IQGAP1 as well as influential conditions on the protein should be pursued in order to find practical applications of the currently known functions of IQGAP1. As the semester ends, I plan to continue work on this project in order to further analyze data from the lab and draw more meaningful conclusions in regards to how the protein's diffusivity is affected under different conditions.

Introduction

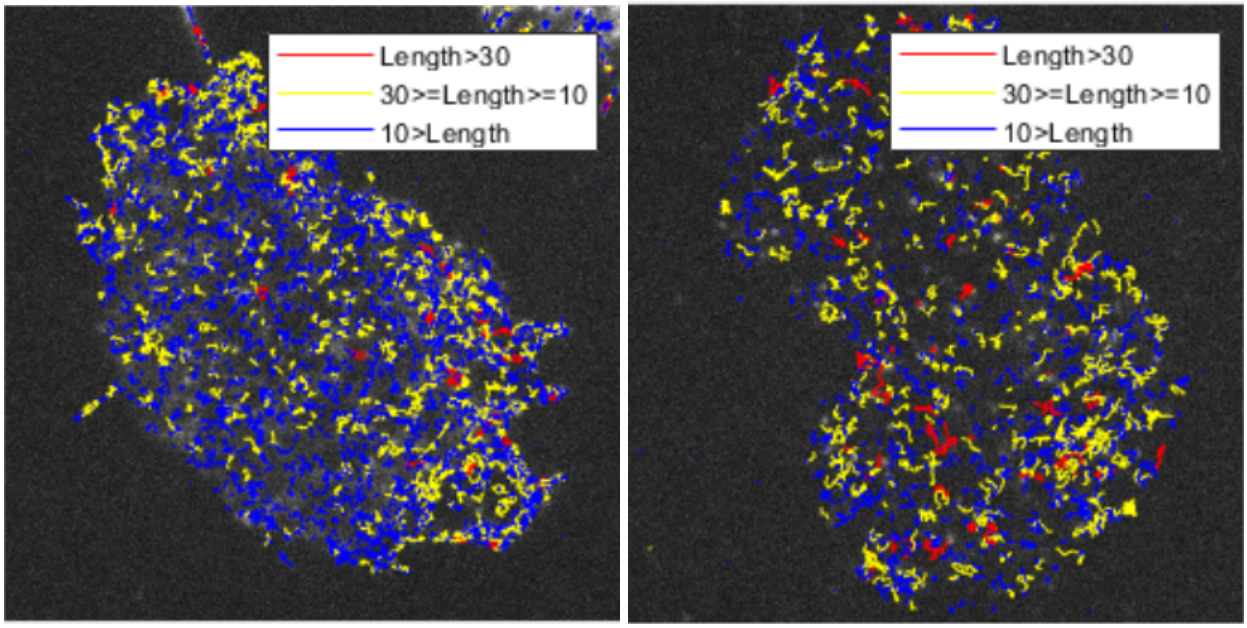
IQGAP1 is a member of the IQ motif containing GTPase protein family, alongside IQGAP2 and IQGAP3. IQGAP1 in humans is currently known to interact with signaling molecules as well as cytoskeleton components so as to regulate cell morphology and motility (NCBI, 2021). Existing literature on IQGAP1 reveals some clear correlations between its function or lack thereof and the proliferation of cells. The protein has been reported to have some relationship to the growth and metastasis of esophageal squamous cell carcinoma as well as to a proliferation arrest in vascular

smooth muscle cells (Grabowska, W. et al., 2020). Here, we used a particle tracking technique to extract the diffusivity of IQGAP1 molecules under some drug treatment of single Jurkat T cells.

Methodology

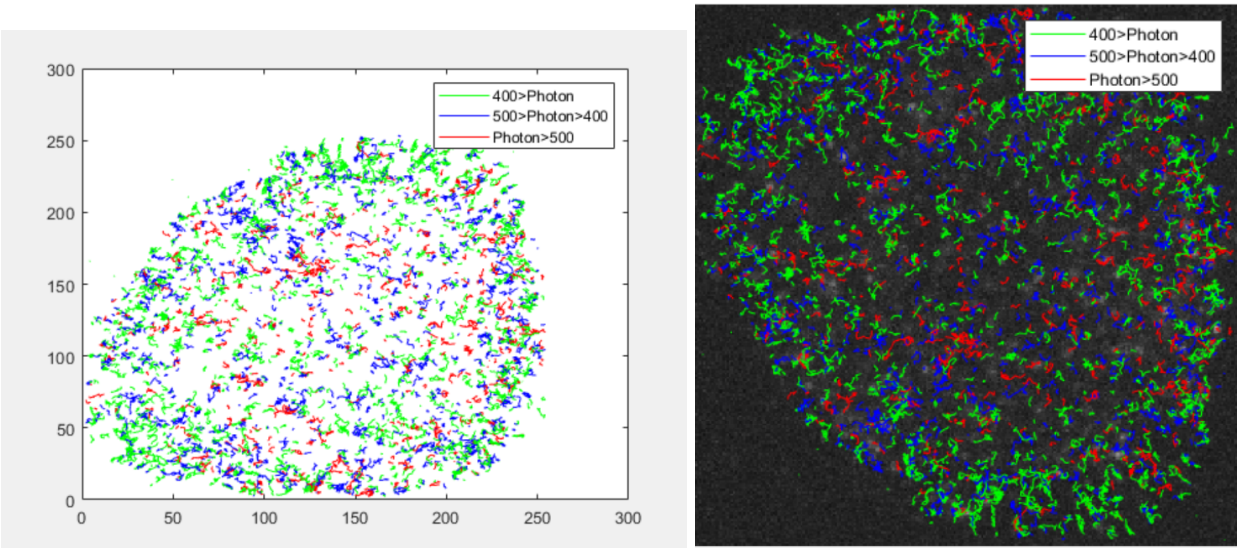
To allow for creation of the IQGAP1 protein, Jurkat T-cells were first transfected with GFP-IQGAP1. Various drug conditions were imposed on separate Jurkat cells in order to later analyze the effects these drugs have on the diffusivity of IQGAP1. Images of the cells under different drug conditions were then obtained using a microscope with total internal reflection fluorescence (TIRF). The TIRF images were then converted into .tif files to allow for processing through MATLAB. Following this conversion, a particle detection script under the name Ivan_Peter_Nick_SPTLM_100X_w_1_5X was used in MATLAB to obtain data including the x-y position and number of photons of IQGAP1 molecules, among other outputs. Following particle detection, binary masks of the cells were created using Fiji in order to exclude any particles that do not reside within a cell. In the first stage of analysis, area of cells were plotted with respect to frame number as well as the x and y values for the centroid of the cell. Various functions in MATLAB were then used to plot tracks over cell images, color coded by their track length and number of photons. Isolated images of singular tracks were also plotted to allow for more in depth looks at individual tracks.

Results



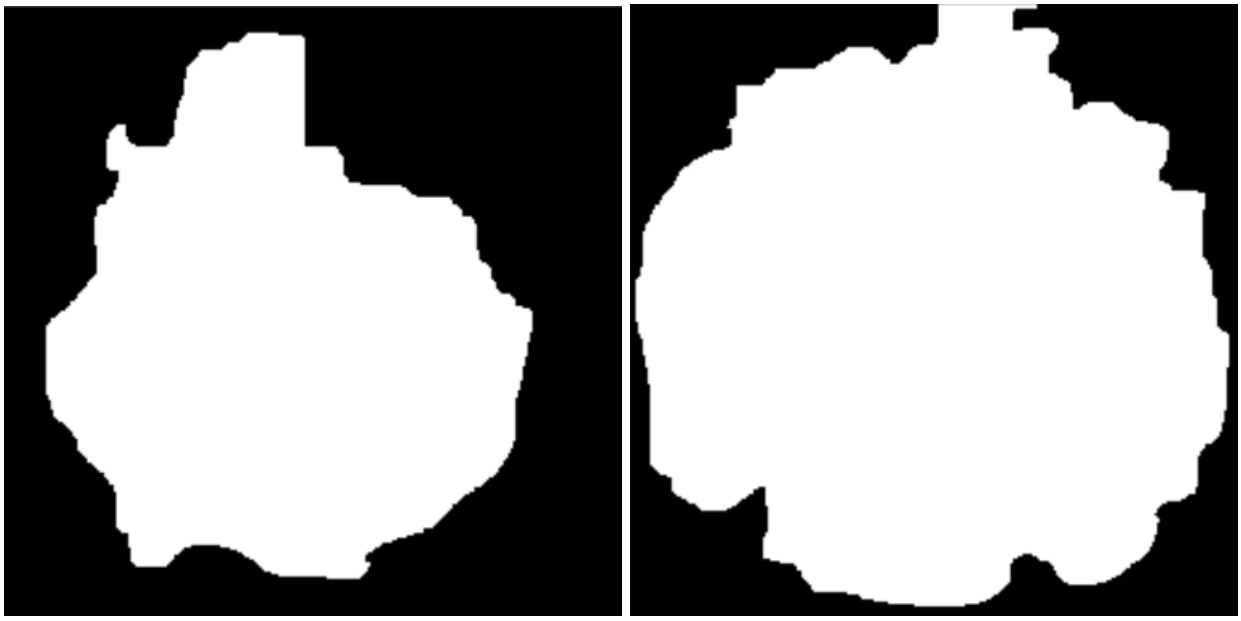
Two plots of cell tracks colored by their lengths in respect to number of frames.

Seen above are tracks superimposed and plotted over the image of the cell from which they were detected and colored according to their length. These particular cells contained little to no tracks longer than 30 frames, however, they seem to exhibit longer tracks near the edge of the cell. This is especially noticeable from the figure on the left from qualitative analysis. To determine if the concentration of track lengths among certain areas of the cell is significant, statistical analysis should be used.



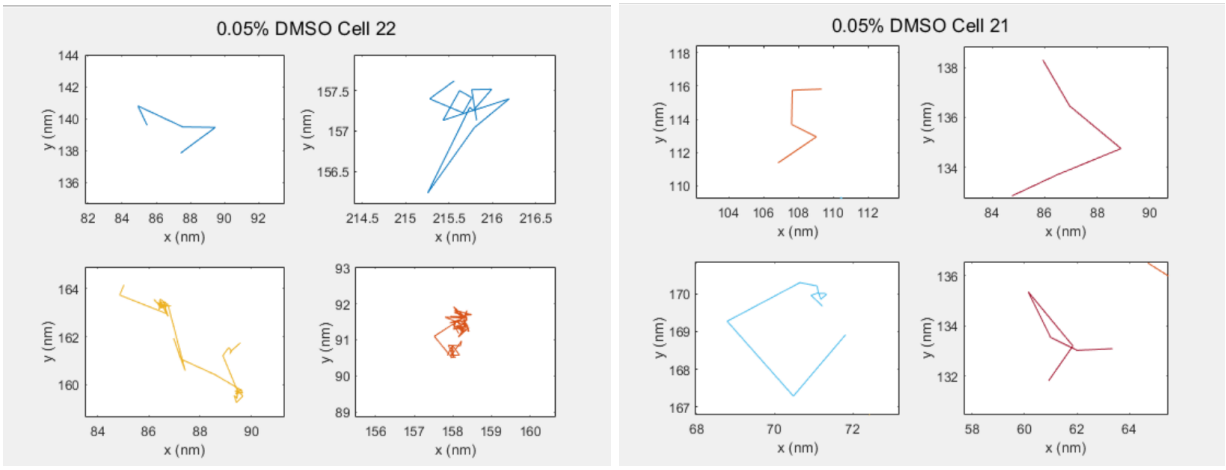
Tracks plotted and colored according to the number of photons detected (left) and tracks superimposed and then plotted on the original cell image, also colored according to number of photons detected (right).

Both of the above figures show tracks from a single cell plotted and colored based on the number of photons detected in each track. Based on the visual observation of this particle cell, my impression is that the detected particles with lesser number of photons reside in the periphery of the cell. This provides an insight that the threshold of 400 number of photons could be an ideal threshold for assessing the distinct particles with respect to the region of the cell. There seems to be a random distribution of tracks corresponding to their photon number, with most of the tracks having less than 500 photons, as seen from the very small amount of red colored tracks but randomly distributed.



Two binary masks traced from cells used in the experiment.

The figures above display two binary masks traced from cells that were previously imaged using IRM. The white region corresponds to the area of interest whereas in the black region any outlier particles will be excluded. Later on in the project, processed images such as these may be used for a refined analysis, limited to within the masked region.



Magnified tracks from a cell treated with 0.05% concentration of DMSO.

Next, I sought to determine If the modes of the motion of tracks are similar. The figure above shows that trajectories exhibit different modes of motion including slow, intermediate and

fast.. A variety of directions and shapes are created from the movement of particles within the cell. An interesting development may be what causes these physical differences in tracks, whether it be the drug condition imposed on the cell or other factors.

Discussion

This project, although specific to the diffusion of IQGAP1, holds great significance to the progression of knowledge in physical, cellular processes. It is possible that the diffusion of IQGAP1 within a cell may promote or inhibit its interactions with other intracellular molecules. As it pertains to disease, IQGAP1 has been seen to promote angiogenesis, and thus, the proliferation and metastasis of cancerous cells when the protein is overexpressed (Li et al., 2018). Anti-angiogenesis is a developing cancer treatment so inhibiting the expression of IQGAP1 may prove crucial to improving this particular strategy for mitigating the progression of cancer in humans. Additionally, IQGAP1 has been reported to interact directly and indirectly with a plethora of proteins related to the scaffolding, nuclei, and cytoskeleton in cells (White, C. et al, 2011). Further research on the functions of IQGAP1 in tandem with this project on diffusivity of the protein has great potential in terms of practical applications to cancer treatment as well as dysfunction in intracellular signaling.

In reflection upon my involvement in this project, I plan to continue analysis on the effects that various drugs have on the diffusivity of IQGAP1 within the Jurkat T-cells. Working more with softwares such as Fiji and MATLAB should allow me to follow through on desired techniques of image analysis as well as find other methods of analysis that could prove valuable to the ends of the project. Something else I would like to work on next semester is verifying the accuracy of a macro-particle-detection script in order to expedite the process of obtaining data. In the interest of refining analysis, creating a script to automate binary masking could also prove

valuable to expediting image processing. My next step in this project is to continue work on bee swarm-style charts with evenly distributed x-values corresponding to track length on the y axis. Individual bee swarms will constitute a larger swarm chart displaying track lengths for multiple conditions imposed on the Jurkat cells. These swarm charts will allow for qualitative as well as statistical analysis of how each drug condition affects the track length, and thus the diffusivity of IQGAP1. I believe this particular method of analysis will allow for more meaningful conclusions in relation to the purpose of the project.

References

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