Single Molecule Tracking

Test Plan

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I. Abstract

Understanding the dynamic behavior of single molecules inside a living cell has many important applications in cell biology. Studies on the diffusional and viscoelastic properties of these molecules are necessary for determining how they interact with the cell membrane. Single Molecule Tracking (SMT) is one way to follow the molecule's path as it moves across a particular region. One option to see the SMT trajectories is by labeling molecules with quantum dots (QD) to distinguish them within a heterogeneous cell environment via fluorescence emission [1].

In this work, transient receptor potential cation channel subfamily V member 1 (TRP-V1) will be used as the single protein molecule of interest, which acts as an indicator of painful or thermal stimuli such as burning pain or heat [2] and is pertinent in pain medicine research such as chronic pelvic pain [3]. The overall goal of the project is to design a reproducible SMT procedure that will track the TRP-V1 molecules and investigate their diffusion-limited reactions and viscoelastic properties as a function of time. The data can then be used to assist in the modulation of TRP-V1 expression levels on the cell surface so that better thermal pain alleviation can be achieved.

IV. Testing Expectation

Based on our project, the primary objective is to be able to acquire the trajectories of Quantum Dots (QD) and Clathrin protein, on which we spend most time culturing, image processing and qualitatively analyze.

After getting the appropriately cultured Human Embryonic Kidney (HEK) cells, we are required to get the good and suitable images from Total Internal Reflection Fluorescence microscopy (TIRFM). There are two channels in each single image, which are QD and CLC (Clathrin Light Chain) channel. By applying "U-track" method, we can get the individual trajectory for each channel. Simultaneously, we are able to overlay the obtained trajectory with the consecutive molecular motion movies.

Accordingly, the main expectation we want to see is the approximate consistency of the actual motion tracking and trajectory we get.

V. Testing Criteria

- ✓ A good sequence of images captured from cultured HEK cells
  - The intensity of QD is homogeneous
  - There are enough QDs in the channel

- ✓ A good consistent tracking between the actual motion and trajectory
  - The noise has been decreased efficiently, there is few noises detected as QDs.
  - The QD motion has been detected correctly. There is no trajectory losing whereas the QD is still moving.
  - There is no other wrong non-molecular motion captured and tracked.

VI. Testing Measures

A. Image Resolution

The resolution of the image under the TIRFM is defined as the minimum distance between two points at which those points are still distinguishable from each other. So the good resolution will ensure us to acquire good image data even though two crossing particles are present. Meanwhile, the image clarity will be sharper rather than blurry.
The Rayleigh Criterion provides us the basic function to deal with this image resolution issue, which is denoted by: $d = \frac{\lambda}{2N.A}$; where d is the image resolution, λ is the wavelength of incoming light, and N.A. is the numerical aperture [4].

B. Light-Induced Photodamage

The cells exposed to the microscope's light for longer than 20 minutes will degrade, which is known as the light-induced photodamage.

C. Signal to Noise Ratio (SNR) & Blinking Rate

First of all, the application of TIRF can enhance the SNR when the QD-labeled molecules are located in the cell membrane.

Additionally, we can choose various excitation wavelength and intensity to get proper data acquisition. Different excitation wavelength will lead to different SNR and cellular

autofluorescence. At the same time, the intensity should be controlled at a appropriate value so that it still maintains good SNR.

Compared to other dyes or fluorescent proteins, QD has larger extinction coefficient that leads to the high brightness [5]. However, every coin has two sides. The blinking rate is an evitable problem for using QD as probe to detect the molecular motion and reconstruct the trajectory. Similarly, a proper excitation wavelength will ensure us to keep the blinking rate in an accepted range.

D. U-track method coefficient of correction

When using U-track method to image process, different images will have different tracking results with defaulted values for various parameters. We should adjust the coefficients for different scenarios in order to get satisfied trajectory.

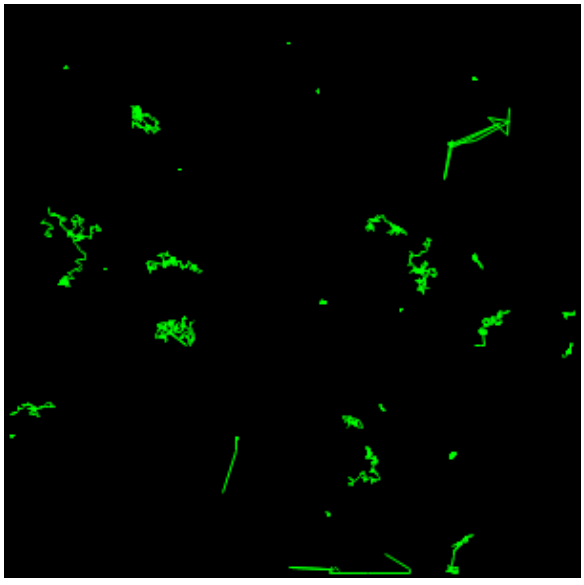
VII. Incremental Testing

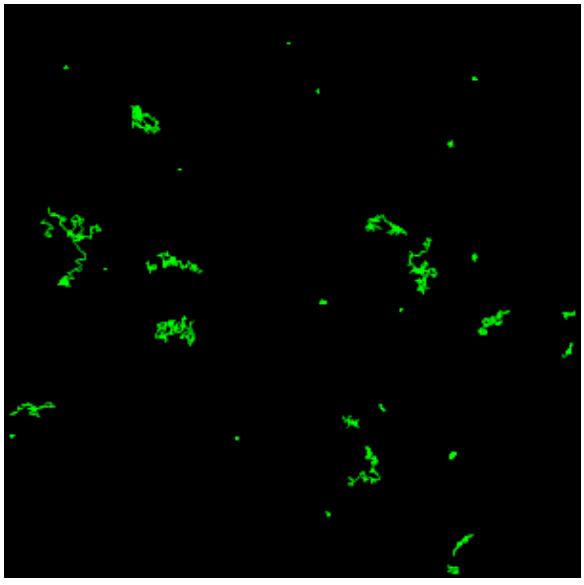
1) Excitation wavelength
   - 350-455 nm; ultraviolet-blue region
   - 455-492 nm; blue region
   - 530-580 nm; green-orange region
   - 620-780 nm; red region

2) Excitation intensity
   - 0.1 kw/cm^2
   - 0.5 kw/cm^2
   - 1.0 kw/cm^2
   - 1.5 kw/cm^2

3) Exposure time
   - 5 minutes
   - 10 minutes
   - 15 minutes
   - 20 minutes
   - 25 minutes

4) U-track method coefficient of correction
   - Value of subtract background: 100, 200, 300, 400
   - Maximum gaping time: 3, 4, 5
   - Value of linear motion: 0, 1, 2
   - Alpha values for detection: 0.01, 0.001, 0.0001, 0.00001, 0.000001





Above is a comparison before and after correcting some coefficients in U-track method, including subtracting more values from the background to decline the noise effect, narrowing down the maximum values for gaping time and turning off any linear motion in this tracking. Obviously, we can find out that the nonsense trajectory is eliminated in the second figure when we adjust the coefficient into proper values.

VIII. Necessary Software

- Imagej
  This software is used to separate two channels into individual channel, the contrast/ brightness and Gaussian blurr functions helps us to see the motion clearly. What's more, we can overlay the obtained trajectory onto the movie of molecular motions to figure out the consistency dynamically.

- MATLAB
  It is the main analytical software, on which U-track method applies. We can reconstruct the detected trajectory through it. Also, we edit the parameters in it to acquire better tracking result.

- LabVIEW
  Basically, it will display the whole dataflow process of our project. Its graphical programing will illustrate each single QD motion intuitively based on the data of coordinate information and intensity values derived from U-track method.

- Origin
  Right now, Origin is very suitable to deal with a bunch of data for all frames of images. We can cut out the data we don't want. For example, after going through the values of all QD motions, we can choose the most evident QD to draw a single tracking trajectory and overlay onto the movies to dig out more in detail.

Reference

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