Functional Specification

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Background

The DeForest Group utilizes a variety of light responsive materials to simulate the extracellular matrix in order to capture the dynamics in biology. The experimental photoresponsiveness of small molecules, proteins, etc. are used to generate plots and used to derive photokinetic rate constants. Typically, this is accomplished in GraphPad Prism; however, we only have GraphPad installed on one computer that we must all share.

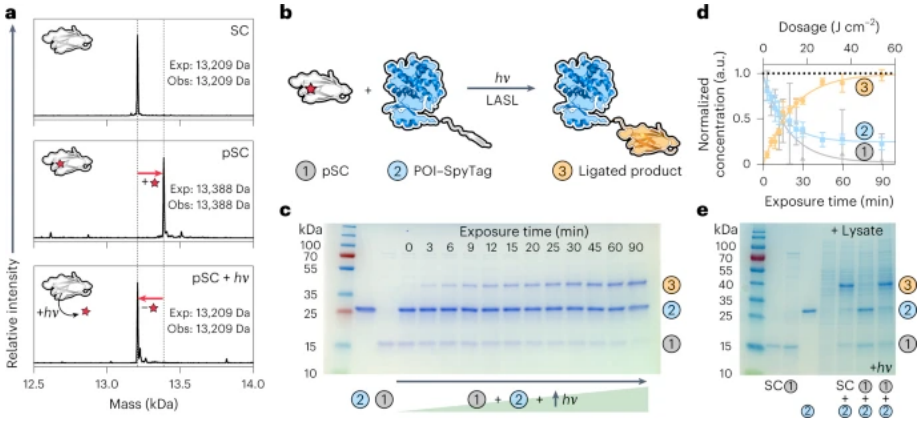

Figure 1. Example of photokinetic data produced from the DeForest group.

The first part of this tool provides two main functions. The first is a theoretical photokinetic calculator that can calculate the y values from input x values and kinetic rate constant from the user. This will allow to create a starting basis for calculating the length of time and intensity of light needed to produce a response in 3D as well as how patterned light exposure will affect experiments. The second main component is an experimental photokinetics calculator that allows users to input raw data from in solution photoactivation experiments to calculate a kinetic rate constant for activation of the biochemical cue and also get a plot.

The second part includes a stochastic model that simulates cell migration, but modified so that the spatial and timed presentation of a photoresponsive cue is incorporated into the model to influence cell movement, using a rho matrix that changes the initial input rm or motility of the cell. Migration assays are commonly used by researchers to study the effect of various biochemical and mechanical cues and for therapeutic development. Given the complexity of the extracellular matrix, tissue culture plastic and biomaterials, specifically hydrogels, have become a popular platform for probing and directing cell fate. Typically, variables such as the spatiotemporal presentation of the chemoattractant (concentration), cell seeding, and length of time for the

experiment are all variables that play a critical role in the outcome of experiments. However, optimizing all these conditions is difficult and time consuming.

Thus, the second python package is a more mathematical approach to cell migration assays in 2D (tissue culture plastic). For the 2D cell migration model, the user can input the spatial pattern of the chemoattractant, initial seeding densities of the cells, cell proliferation/cell death (which vary based on cell type), and length of time of the experiment.

User Profile

This tool is specifically developed for the DeForest Group but can be used by any researcher with a beginner coding background and is intended to be implemented in either a normal python notebook or a Colaboratory Python notebook. The user should be able to use pip install in python. The user will be able to input raw data in the form of an excel file and be able to call on the calculate_experimental function to calculate photokinetic data. In terms of the 2D model, the user will only have to specify constants such as cell proliferation, cell death, and length of time and then run the ChemotaxisSimulation.cell_movement to obtain data for a specific time point or ChemotaxisSimulation.simulate() to get an output video of a heat map of the cell migration over time.

Use Cases

The user can use this tool to obtain theoretical or experimental photokinetic data including plots, photokinetic rate constants, etc and also from and for 2D cell migration with an input to change migration rate.

Use Case 1: Theoretical photokinetic data:

- The user can input theoretical x values (in any units) and kinetic rate constant (unit must match x value unit) to obtain expected y values when input into the equation:

$$\boldsymbol{Y = Y_o + (plateau - Y_o)(1 - \exp(-k * x))}$$

  This is very useful to see what the expected curve/data should look like prior to running an experiment. Sometimes in the DeForest lab we run two to three time points instead of a larger range and it is good to anticipate what the next value should be, and if the experimental data does not match, typically this indicates there is a problem with the experimental set up.

Use Case 2: Calculating photokinetic rate constants and plotting from experimental data:

- If the user wants to obtain a graph and kinetic rate constant for their experimental data, the user can input their experimental time or light dosage conditions and corresponding y-values. This is useful as the calculation can be done very quickly and does not require the user to know how to interface with GraphPad, which can be tricky, or if they do not have access to the computer with GraphPad this is an alternative.
    - Photokinetic data should be obtained in a format with the time of exposure (minutes), light dosage (in mW cm^{-2}) , and raw output values. The user will need to indicate the number of replicates used in the experiment.

- The output will be a graph of the data and a kinetic rate constant. The user can also input a rate constant and obtain output values.

Use Case 3: Modeling 2D cell migration in response to a stimulus

- If the user wants to visualize 2D cell migration in response to different conditions such as cell type (cell types have varying characteristics, which can be modeled by changing the cell proliferation, death, movement rate inputs), size of the experiment, number of cells they are starting with and their orientation (e.g. for an experimental scratch assay usually cells are confluent on two sides of 2D area with no cells at the center, and their migration into the center is tracked. Another example are transwell migration assays

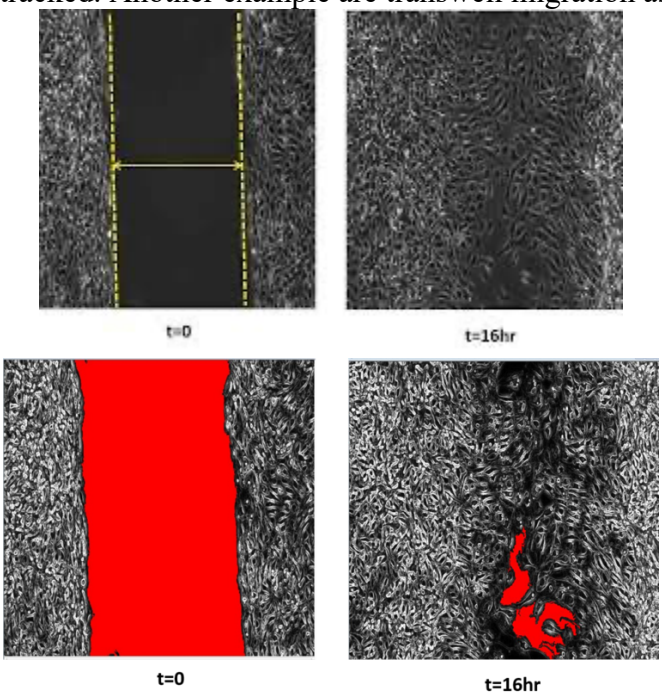

Figure 2: a) In vitro wound healing assay setup showing the area after scratch (t=0) and endpoint following migration and proliferation by cells (t= 16 hr). Red color indicates the area of after edge detection and image segmentation (Jonkman *et al.* 2014)

- In the 2D cell migration model the user can input the size of the area (nxm matrix where n =1 and m =1 is dimensionless, but the size necessary to contain 1 cell), the initial location/density of cells, cell proliferation, cell death, length of time of the experiment as well as spatiotemporal patterning of the chemoattractant.
- The output will be a heat map containing information of the density of cells based on the x,y dimension.