Virtual Cell Version 5.2

Tutorial I: FRAP

Creating a FRAP BioModel

Introduction

Fluorescence Redistribution After Photobleaching (FRAP) is a fluorescent optical technique used to measure the dynamic behavior of a molecule over time. Fluorescently labeled molecules are visualized through an epifluorescent or confocal microscope using low light excitation. Excitation light is pulsed at a high intensity in a defined region of interest in order to photobleach the fluorophores within the illuminated region. This creates a darkened area of photobleached molecules surrounded by fluorescently labeled molecules that are not photobleached. If the molecules are free to diffuse, bleached molecules will move out of the bleached region and unbleached (fluorescent) molecules will move into the bleached region, resulting in an increase in fluorescence intensity in the bleached region over time.

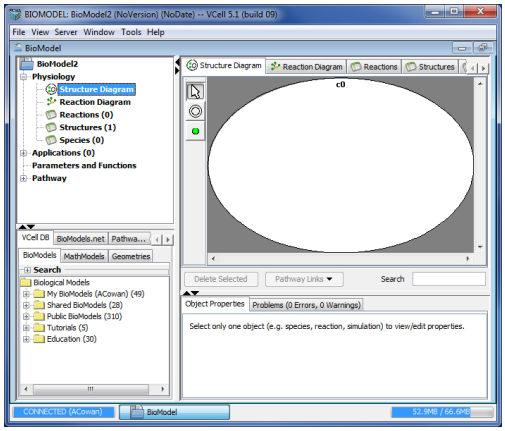
The fraction of molecules that are mobile (mobile fraction) and temporal characteristics of transport due to diffusion and/or unbinding from an immobile partner can be determined from FRAP experiments. How quickly the fluorescent molecules move into the photobleached region is a measure of either the diffusional mobility of unbound molecules or, if binding occurs, the exchange of bound and unbound molecules. In this tutorial you will create a model for a FRAP experiment of a freely diffusing species and simulate the results of the experiment performed in a 2 dimensional constructed geometry. A second tutorial is available to create a simulation of a FRAP experiment when binding to an immobile component occurs.

Following the Tutorial

You can create your own BioModel and Application as you read through the tutorial or you may choose to load the public version of this model. To open the public BioModel tutorial go to the bottom left Database Navigation pane and click on Tutorials>Tutorial_FRAP. The Application is FRAP, and the simulation results are saved as FRAP1. You cannot overwrite a public file, so you must save a copy under your own folder in order to make any changes to the BioModel.

Defining the biological model

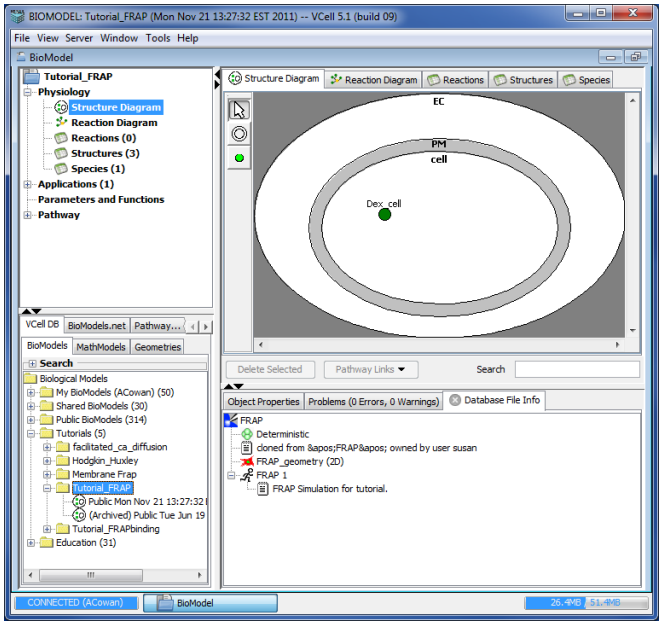
Creating and Defining Compartments



The biological model is defined as a collection of biochemical reactions acting on a set of molecular species localized in specific cellular structures. The cellular structures are defined as mutually exclusive compartments within the cells, as well as membranes that separate them. The compartments represent three-dimensional volumetric regions while the membranes represent two-dimensional surfaces separating the compartments. All structures can contain molecular species and a collection of reactions that describe the biochemical behavior of those species within that structure. Keep in mind when developing your model that compartments must be mapped to a specific cellular geometry before any quantitative simulation or analysis can be performed. This will be done in the Applications.

When the software is initiated, you are presented with a BioModel document, with four panes that provide (clockwise from upper left): the overall model navigation tree; a full view of the selected model component (Reactions, Structure or Species); a detailed specification of the object properties for the selected model element; and available VCell and external database resources for finding models or model elements. On the left Navigation tree click on “Structure Diagram”. Select the

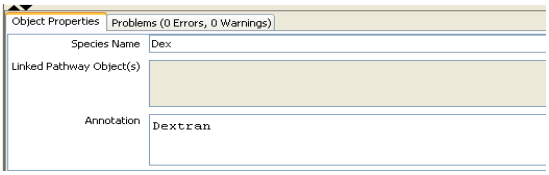
circle, ‘c0’, that appears in the pane to the right of the Navigation tree. Press once with the left mouse button, the compartment will turn red. On the bottom of the page in the Object Property box with the note: “Select only one structure to edit properties”, enter the name “EC” for extracellular in the Structure Name text field and press enter.



Select the compartment tool  and click in the extracellular compartment. A new compartment will be created. The innermost circle region will turn red. Click on the inner most circle again and a New Structure Dialog will appear at the bottom in the Object Property box. Type in “Cell” in the Structure Name text field for the inner most circle, then press enter. Select the membrane for the new compartment, the outer circle. Click once and it will turn red when selected. Type in “PM” (for Plasma Membrane) in the structure name text field for the outer circle, then press Enter.

Adding Species

Select the species tool  and click once in the Cell compartment. Select the pointer tool  and click on the circle you just created. On the bottom of the page a box will come up where you can edit the species. Under Species Name type “Dex”. In the Annotation text field type in “Dextran”.



The BioModel Physiology is complete and you should have a model that looks similar to the image. Save the model at this point before proceeding further. Go to File>Save As, and enter a unique name in the text field at the bottom, press SAVE.

The FRAP Application

Introduction

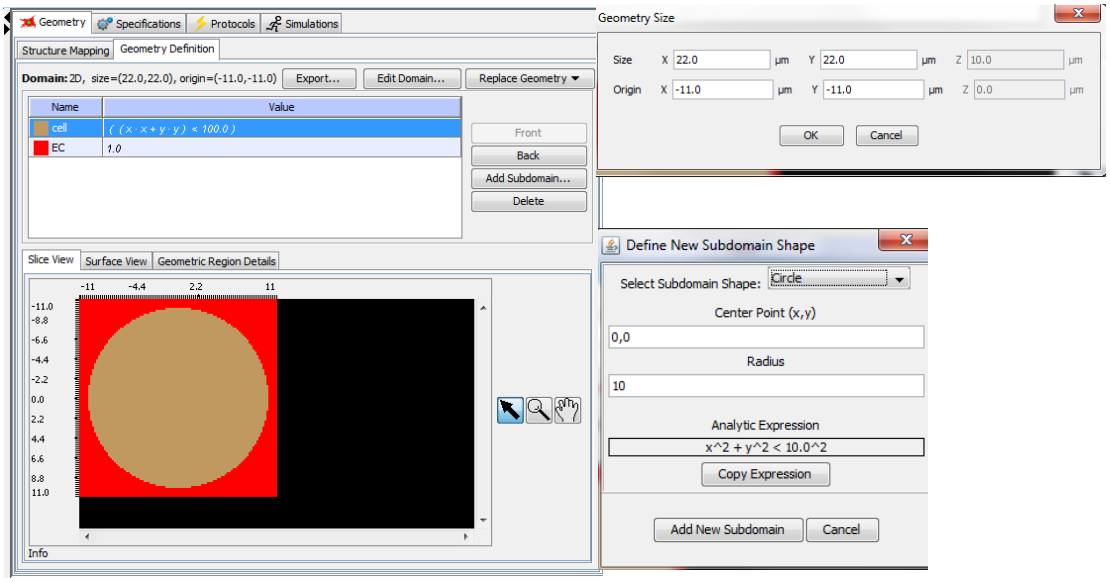
Each model being developed requires an Application, which consists of a detailed description of the cellular Geometry, Structure Mapping, Initial Conditions, and Reaction Mapping. The geometry represents the morphometry of a particular cell or portion of a cell. The geometry may be an imported image captured by various imaging modalities such as wide field, confocal, or electron microscopy. Analytic geometry may also be used to define very regular structures or symmetric cells.

Creating the Application

In the BioModel Navigation tree click on Applications (if necessary, first click on the adjacent plus sign to expand the Applications tree). In the right pane, above the table column headers (Name, Type, Annotation), click on the Add New drop down menu and select Deterministic. Double Click the title under the Name Field and change it to *“FRAP”* and press enter.

Spatial Analytical Geometry

On the left Navigation tree click on Application: FRAP under the Applications field; This should open you to the Geometry tab of the top navigation bar in the main workspace pane. On the secondary navigation bar click on Geometry Definition, to the right is a drop down menu titled Add Geometry (if the application already has a geometry, the button reads Replace Geometry) select New< Analytic Equations (2D) and click OK. The Geometry document will open with a single compartment automatically created in the Geometry Editor. This will be used as the outermost spatial region and will be mapped to the extracellular (EC) space represented in our physiology. Double click the Name text field (“subdomain0”) on the top for the subdomain. Type in “EC” and press Enter. Leave the value at 1.0.



To represent the spatial region for the cellular compartment, we will add a circle. Click Add Subdomain to the right, and select Analytic from the drop down menu. A new window titled Define New Subdomain Shape will pop up. Under Select Subdomain Shape, choose Circle, (0,0) for Center Point and 10.0 for Radius.

The equation will automatically appear in Analytic Expression. Click Add New Subdomain to confirm it. Double click the name text field for the new subdomain; type in “Cell” and press Enter.

You can also edit the Subdomain Shape directly in Geometry Editor. Above the circle, double click the value text field next to “Cell"; enter the following equation to define a circle:

((x*x+y*y)<100.0)

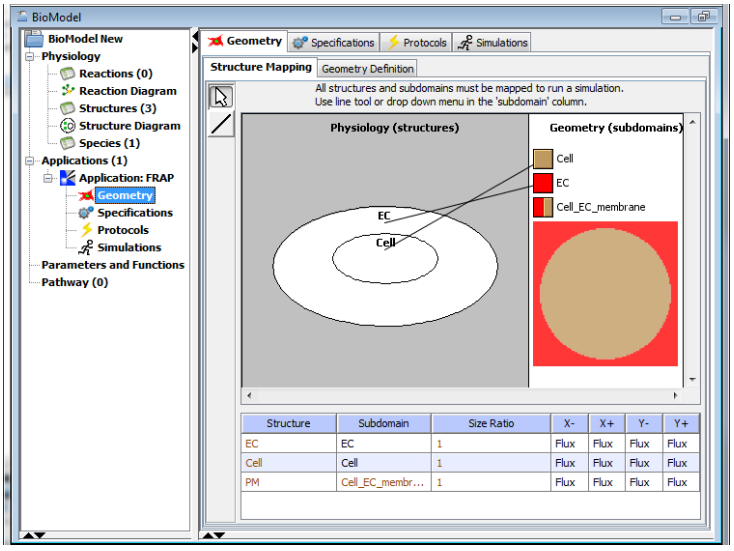
The two subdomains represent the extracellular (EC) and cytosolic (Cell) compartments.

To define the unit size of the domain of the geometry, we need to specify the Geometry Size. In the upper right corner click the Edit Domain button to access the Geometry Size dialog. Enter “22” in the X and Y size text fields, and enter “-11” in the X and Y origin text fields. Press OK to accept the values and to close the window.

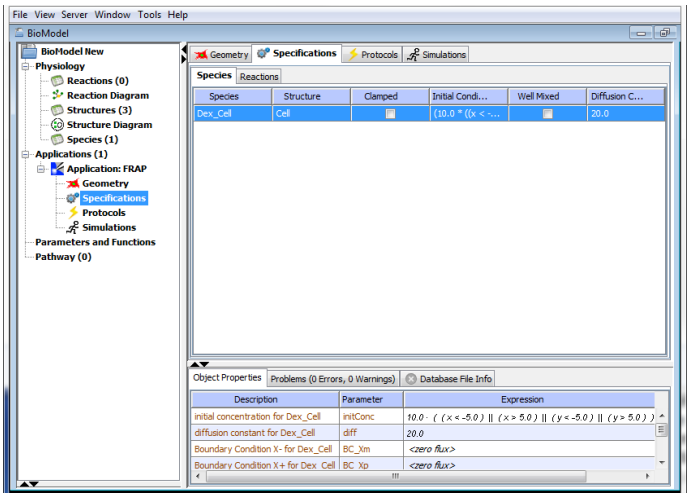
If the circle you have just defined is not visible, select the Cell volume and press Front to bring it in front of the extracellular volume.

Structure Mapping

Change from the Geometry Definition folder to the Structure Mapping folder by clicking on Structure Mapping on the secondary navigation bar. Use the line tool  to map the physiology model to the geometric representation. Map EC to EC and cell to cell. You need to reselect the line tool each time you do mapping, and you need to map from the physiology to the Geometry.



Initial Conditions



Select the Specifications tab from the top navigation bar. Select Dex; at the bottom a table will appear, type the following equation in the Expression field for the initial concentration of Dex.

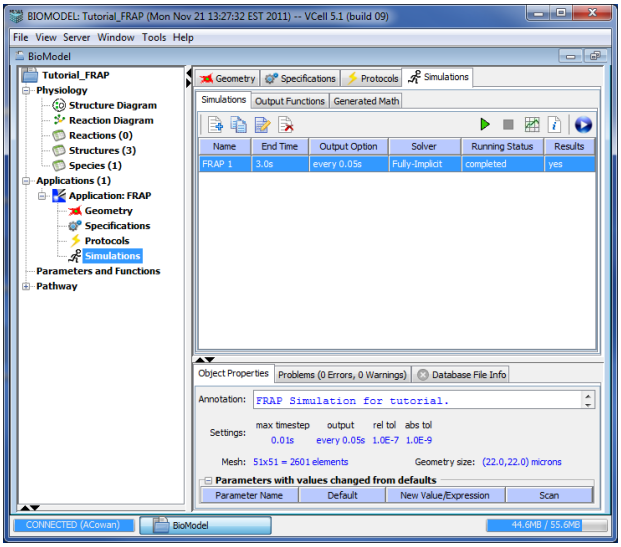
(10.0*((x<-5.0)||(x>5.0)||(y<-5.0)||(y>5.0)))

Press Enter on your keyboard to accept the equation. This equation defines the location in x,y coordinates where the initial concentration of Dex is 10. Where the concentration is not set to 10.0, the concentration is 0. Thus, we set both the initial concentration of Dex and the bleach region (where Dex is 0) in a single Boolean statement.

Enter 20 in the Expression field for Diffusion Constant.

Note that Reaction Mapping does not apply to this model since there aren't any reactions described. Resave your model before proceeding to the simulation.

Running a FRAP Simulation and Generating Results



Simulations

Select the Simulations tab from the top navigation bar.

Use the New Simulation button to start a new simulation.

In the Simulations table, a simulation with a default name will appear. Double click the Simulation Name text field, and enter a name.

Make sure the Simulation name is selected; press the Edit Simulation button to access the additional runtime features: Parameters, Mesh, and Solver.

Select the Mesh tab. Enter "51" for the X and Y dimensions for the Mesh Size. The Geometry Size should be listed as (22.0, 22.0).

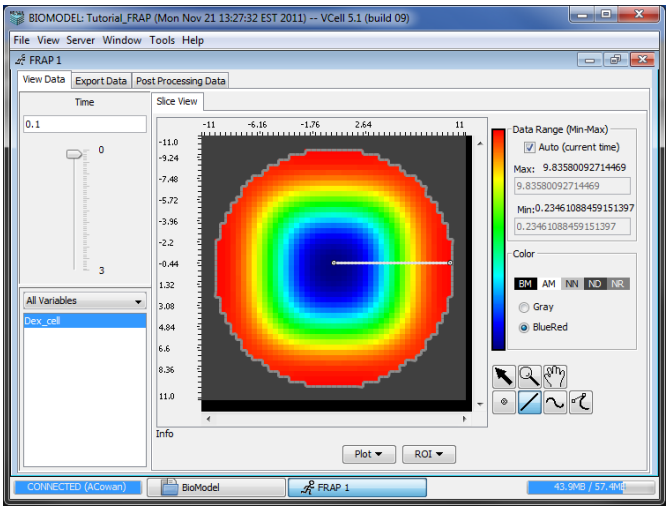
Select the Solver tab to set the Time Bounds to Ending 3.0, Time Step to Maximum 0.01; in Output Options select the Output Interval radio button and enter and Output Interval of 0.05 seconds.

Press OK to accept all the entries and to close the Edit dialog.

Select the simulation and press Run Simulation to initiate the simulation. Your model will automatically be saved with the run conditions and the simulation will begin.

Once results have been generated, press Simulation Results to see them. You may have to reselect the Simulation in order to activate the Simulation Results button. Use the scroll bar on the left side of the results dialog to change the time interval to 0.1 or enter the time in the Time text field and press Enter to duplicate the image here.

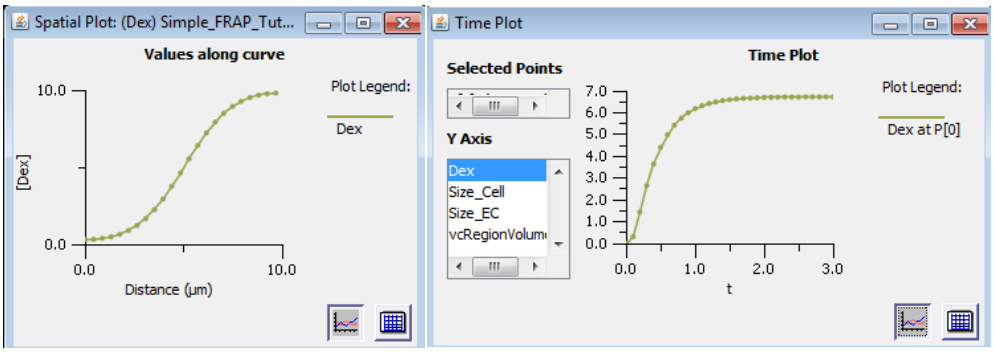
You can display the results in either Gray or a Blue-Red color map. You can also toggle between auto and manual scaling. Uncheck the Auto box and enter values in the Min and Max text fields for manual scaling. Remember to press Enter to accept the value and to update the image display.

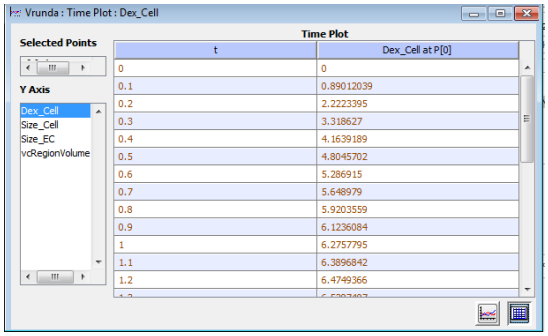


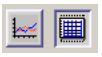
Use the Line  or Spline  tools to define regions of interest for a Spatial plot. Select Spatial under the Plot drop down menu at the bottom of the page to view the graph.

Use the Point  tool to define a point for a Time plot. Select Time under the Plot drop down menu at the bottom of the page to view the graph.

Right click while over the graph to display the Plot Settings dialog. Select your options for Auto Scale, Stretch, Draw nodes, Show crosshairs, and Snap to nodes. Press OK to accept your options and to close the dialog.





You can also view the data values by pressing the Show Values icon  . You can copy the values directly into a tab delimited spreadsheet. Use ctrl C to copy individual cells and ctrl K to copy all the data values. You can also right-click a cell to bring up a menu and press Copy to copy the individual cell or press Copy All to copy all data values.

Export Features

Click on Export data tab at the top of the window to access the export dialog. Depending on your model, you can export data from a single time point or range of time points, and a single variable or several variables, in a variety of different formats. Please see the User Guide, Exporting Simulation Results, for additional information.