Virtual Cell Version 4.8

Tutorial II: FRAP with Binding

Creating a FRAP with binding BioModel

Introduction

This tutorial is a continuation of the first “FRAP” tutorial which created a simulation of photobleaching a single fluorescent species in a single cellular compartment (cytosol), where photobleaching was assumed to be 100% in a defined region of the cell. Tutorial II is based on photobleaching experiments with the nuclear protein RAN, and adds the following additions to the scenario presented in Tutorial I.

1. There are both fluorescently labeled exogenous and unlabeled endogenous versions of the nuclear protein RAN.

2. RAN binds to an immobile component in the nucleus.

3. Both nuclear and cytoplasmic compartments are included, and there is a flux of RAN across the nuclear membrane separating the two compartments

4. Both a nonspatial and a spatial simulation are demonstrated.

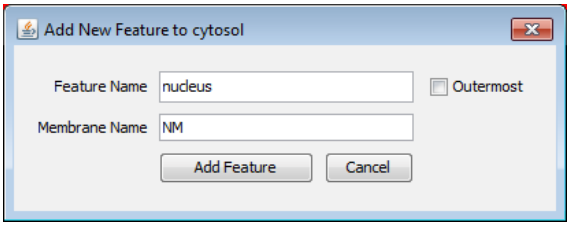
Following the Tutorial

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 load the public Biomodel tutorial to go File>Open>Biomodel… >Shared Models>tutorial>Tutorial_FRAPbinding. There are two Applications (Compartmental and Spatial) with saved simulation results. 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

When the software starts, you are presented with an undefined BioModel. Select the compartment once with the left mouse button, and then use the right mouse button to access the Properties menu or double-click the compartment with the left mouse button to access it. Enter "cytosol" in the Feature Name text field and press OK. All names and expressions in the Virtual Cell are case sensitive. Be sure to note which case you use.



Select the compartment tool  once and click in the cytosolic compartment. Type in "nucleus" in the Feature Name text field, and "NM" (Nuclear Membrane) in the Membrane Name text field; press Add Feature.

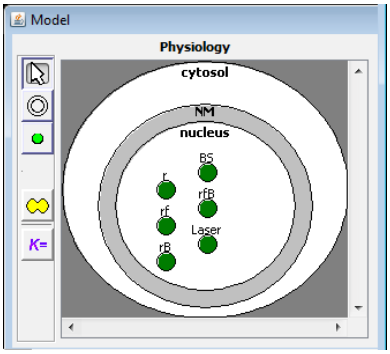
Adding Species

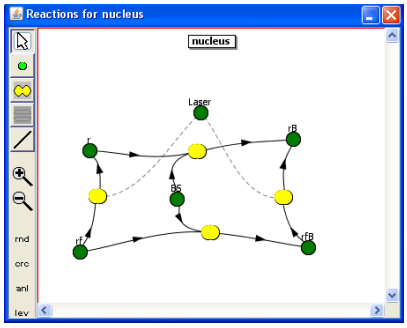
Select the species tool  and then click in the nuclear compartment once. In the Add New Species dialog, enter "r" in the Name text field. In the Annotation text field type in "RAN” and click Add.

Continue to use the species tool to add the following Species, using the abbreviations listed in the table. Your model should look similar to the picture when you have added all the species.

RAN	r
RAN-FITC	rf
RAN_bound	rB
Binding_sites	BS
RAN_FITC_bound	rfB
Light	Laser

You may want to save the model before proceeding with the reactions. Go to File>Save As and enter a unique name in the text field, press Save.





Defining Reactions

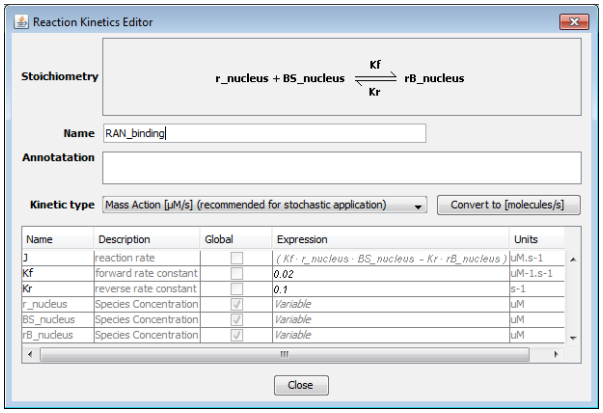
Click the arrow button to change the status of the mouse pointer. Select the nuclear compartment and then use the right mouse button to access the Reactions menu. Arrange the species in the Reactions dialog so that each is visible. You might want to use this image as a guide for organizing the reactions.

Select the reaction icon  and then click once in the Reaction area. Create a total of 4 reaction icons by clicking three more times. Arrange the reaction icons as in the picture above while your mouse pointer is at arrow status. Select the reaction icon, one at a time, and use the right mouse button to access the Properties option that will open the Reaction Kinetics Editor. Change the name in each editor to reaction names shown in the table(Be sure to note which case you use.) This name will appear when the reaction icon is selected.

Name:	Reactants:	Product:
RAN_binding	r BS	rB
bleaching 1	rf	r
bleaching 2	rfB	rB
RAN_FITC_binding	rf BS	rfB

Use the line tool to connect the species to the reaction icons as in the image above. Always draw the connection line from the species to the reaction or flux icon. Several relationship options will appear along the line with slight adjustments. “Reactant, Product, Catalyst” will determine relationship of the species to the reaction kinetics. Choose the right relationship type to make a proper connection.

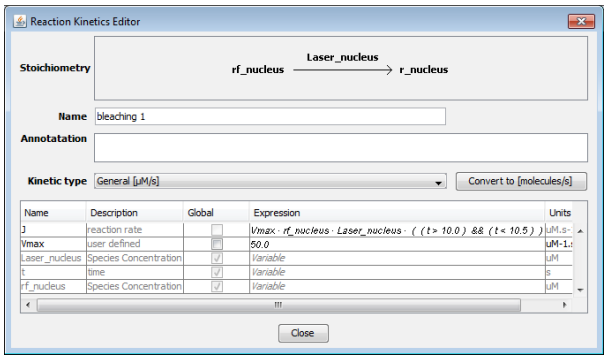
The reaction icon is set up such that the left side of the icons is for reactants and the right side is for products. You have to drag to the middle of the reaction icon with the Laser species. The laser is acting as a catalyst for the bleaching reactions.



Select the RAN_binding reaction icon and double-click or use the right mouse button to access the Properties option. Select Mass Action for the Kinetic Type from the selection list. Double click the Expression text field for Forward Rate Constant Kf, and enter "0.02". Double click the Expression text field for Reverse Rate Constant Kr and enter "0.1". Close the Editor when you have finished.

Open the Reaction Kinetics Editor for the bleaching 1 reaction icon. Select General [µM/s] for the Kinetic type and enter the following in the Expression text field for the Reaction Rate equation:

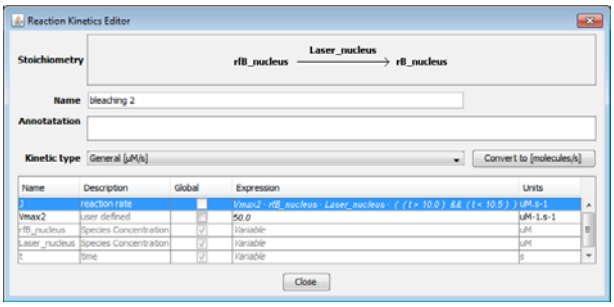
(Vmax*rf_nucleus*Laser_nucleus*((t>10.0)&&(t<10.5)))



After press Enter to accept the equation, the Vmax will appear as a parameter. Double click the Expression text field for Vmax and type in "50.0". Press Enter to accept the value. This equation defines the bleaching period as .5 seconds, starting after 10 seconds.

Open the Reaction Kinetics Editor for the bleaching 2 reaction icon. Select General [µM/s] for the Kinetic type and enter the following Reaction Rate Equation in the Expression text field:

(Vmax2*rfB_nucleus*Laser_nucleus*((t>10.0)&&(t<10.5)))

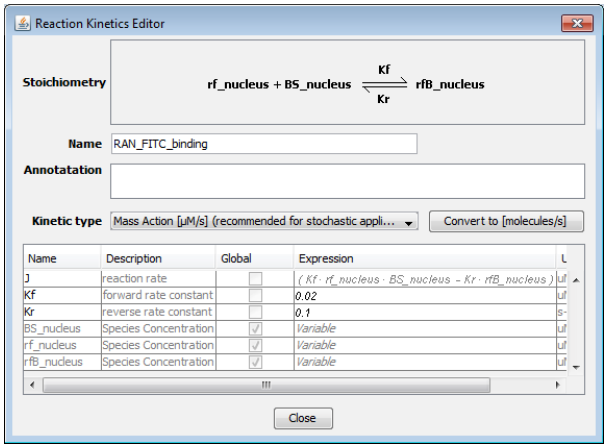


Vmax2 will appear as a parameter. Double click the Expression text field and type in "50.0". Press Enter to accept the value. This equation defines the bleaching period as .5 seconds, starting after 10 seconds.

There are two separate bleaching reactions to account for bound and unbound RAN.

Open the Reaction Kinetics Editor for the RAN_FITC_binding reaction icon. Select Mass Action for the Kinetic Type and enter "0.02" for a Forward Rate, Kf, and "0.1" for a Reverse Rate, Kr.

Once all the reactions have been entered, be sure to note which case you use as all names and expressions in the Virtual Cell are case sensitive, save the model and proceed to Application.



The FRAP binding Applications

Introduction

In this model you will initially create a Compartmental model where the BioModel is mapped to a single point. Next, in the Spatial model you will map the BioModel to a two dimensional Geometry. After setting up both models you will be running the simulations and looking at the results. The results for the Compartmental model will give you an initial idea about how your model is performing.

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 captured experimentally by various imaging modalities such as wide field, confocal, or electron microscopy. Images can then be imported into the virtual Cell (see chapter 6.3 in the User Guide).Analytic geometry may be used to define very regular structures or symmetric cells.

A Compartmental model represents a single point simulation based on the defined physiological model and the geometric assumptions. Structures are assigned Volume (μm^3) and Surface (μm^2) which is used to calculate the Surface to Volume Ratio and Volume Fraction. Compartmental models are not spatially resolved. The compartmental models are solved using nonlinear ordinary differential equations. These equations are generally computed within seconds.

Creating the Applications

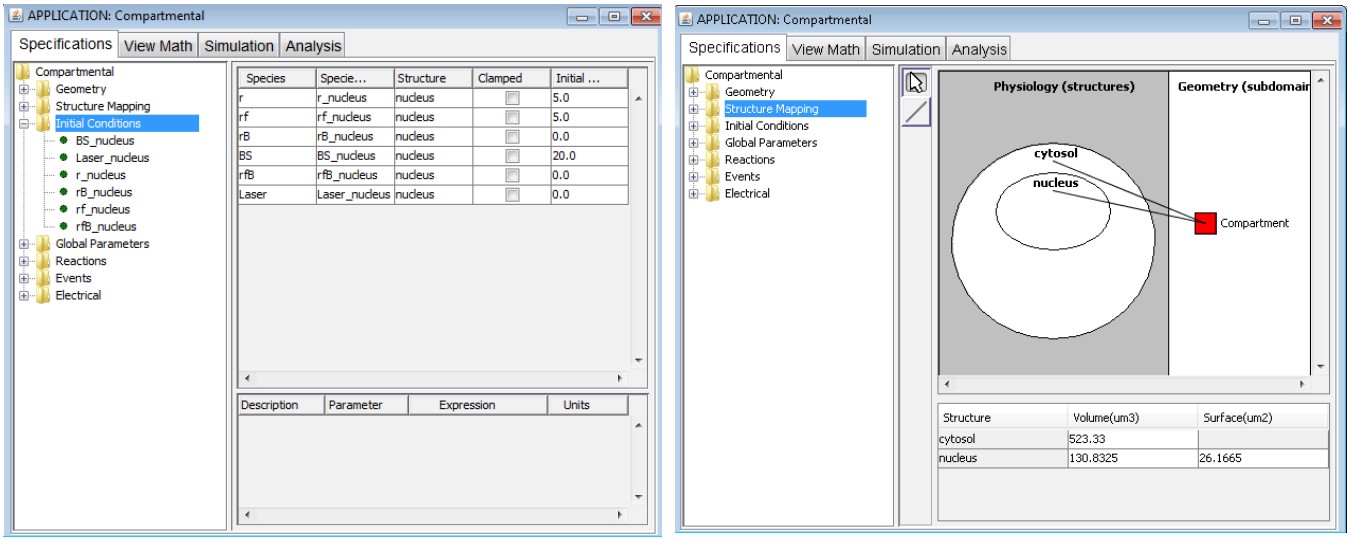
Compartmental Application and Geometry

In the Application panel of the BioModel go to Application>New>Deterministic Application and enter

"compartmental" for the Application name. The Application will initialize with the Structure Mapping panel. The compartmental model is the default model and is automatically mapped to a single compartment that includes both the cytosol and the nucleus. Thus it is not necessary to create a new geometry.

Structure Mapping

In the Structure Mapping folder, we enter the Volume and Surface sizes for the nucleus and the Volume size of the parent feature, the cytosol. Select the text field in the Volume (μm^3) column for the cytosol, enter 523.33. This is the volume of a cell that is spherical with a 10μm diameter. Then select the text field in the Volume (μm^3) column for the nucleus, enter 130.8325 and for the Surface (μm^2) enter, 26.1665.



Initial Conditions

Select the Initial Conditions folder. Double click in the Initial Conditions text field for RAN (r) and enter an initial concentration of 5.0. Press OK to accept the value and to dismiss the dialog. Do the same for RAN-FITC (rf), enter a value of 5.0 and for Binding sites (BS) enter a value of 20.0.

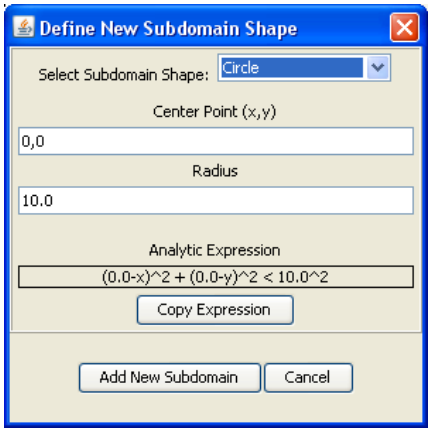
You may want to resave your model at this point and proceed to Compartmental Simulation. This can be done by going to File>Save...

Spatial Application

Spatial Geometry

To create the spatial application, select the compartmental application from the right side of the biomodel and in the right click menu, select copy. A dialogue box will pop up allowing the user to name this new application as “spatial”. In the Spatial Application, select the geometry folder and click Create New Geometry. When the Geometry Type window appears, select Analytical Equations (2D). You will be presented with the Geometry document which contains a single subdomain viewable in the Geometry Editor. Double click the Name text field for the subdomain and enter "cytosol". Keep cytosol value at 1.0.

Press Add Subdomain one more time to create an additional subdomain. From the drop down menu, choose Circle for Subdomain Shape, (0,0) for Center and 10.0 for Radius.

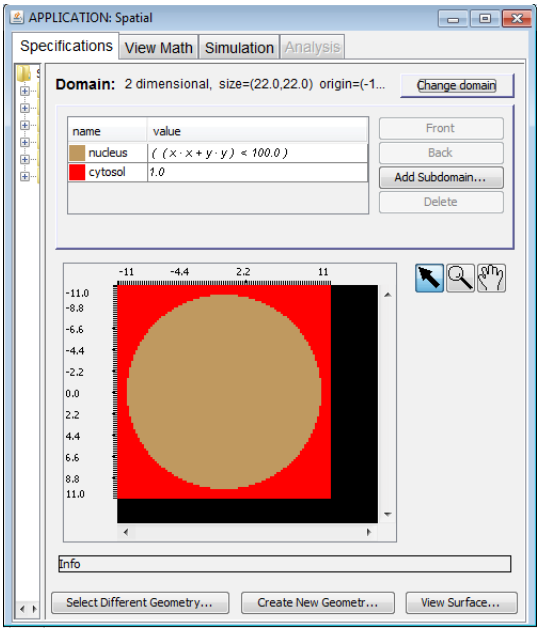


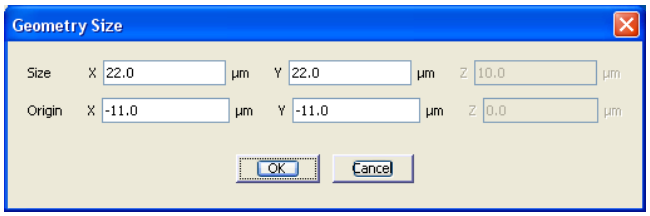
The equation will automatically appear in Analytic Expression. Click Add New Subdomain to confirm it. Rename the new subdomain as “nucleus".

You can also choose Manual for Subdomain Shape and edit the equation directly in Geometry Editor. To do this double click the Value text field and enter the following equation that defines a circle:

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

Press Enter after entering the equation. The two subdomains that you create will represent the cytosolic and nuclear compartments.

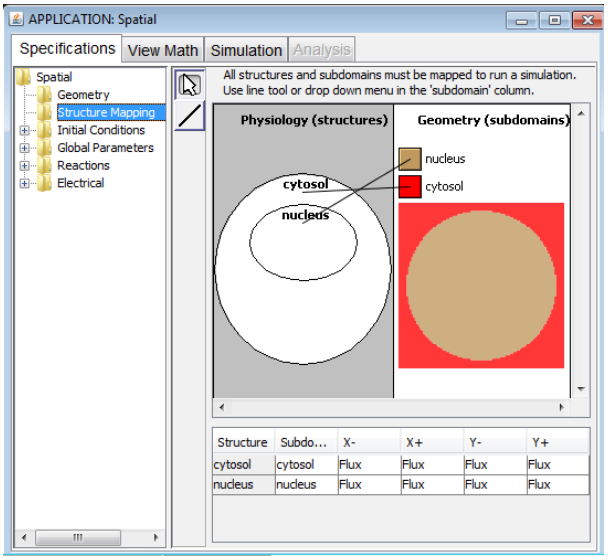




Press the Change domain button to access the Geometry size dialog. Enter "22" in the X and Y size text fields, and enter "-11" in the X and Y origin text fields. Press OK to accept the values and to close the window.

If the circle you just defined is not visible, make sure to select the nuclear volume and press Front to bring it in front of the cytosolic volume. The Front and Back buttons set the positioning of the subdomains. It is important to arrange the subdomains in the list so they are calculated in the proper order. If a subdomain is hidden or unreachable, it will not be calculated.

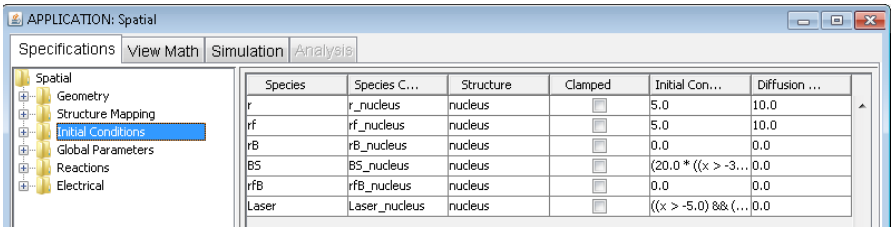
Structure Mapping



In the structure mapping folder, use the line tool  to map the physiology model to the geometric representation. Map extracellular to extracellular and cytosol to cytosol. You need to reselect the line tool each time you do a mapping, and you need to map from the physiology to the geometry. The sizes associated with the geometry are used for Volume and Surface for each feature are taken from the resolved geometry.

Initial Conditions

Since the application was copied from the original Compartmental application, all of the values previously entered for initial conditions were carried over. The conditions and diffusion coefficients are entered in the Initial Conditions tab of the Application. Select the Initial Conditions tab. Enter the conditions for each species in the Initial Conditions text field. The Initial Conditions text field for RAN (r_nucleus) should contain a value of 5.0 (i.e. 5 micromoles)entered previously. With this being a spatial



application additional values need to be entered. In the Diffusion Constant text field for RAN (r_nucleus) enter 10.

In the same manner (if the values are not already present) enter the same values for RAN-FITC (rf_nucleus) as you did for RAN: 5.0 for an Initial Concentration and 10.0 for the Diffusion Constant. For Binding_sites (BS) , double click the Initial Condition text field enter the value 20.

In the Initial Condition text field for the Laser enter in the following equation, which creates a rectangular field in the nucleus.

((x>-5.0)&&(x<5.0)&&(y>-5.0)&&(y<5.0))

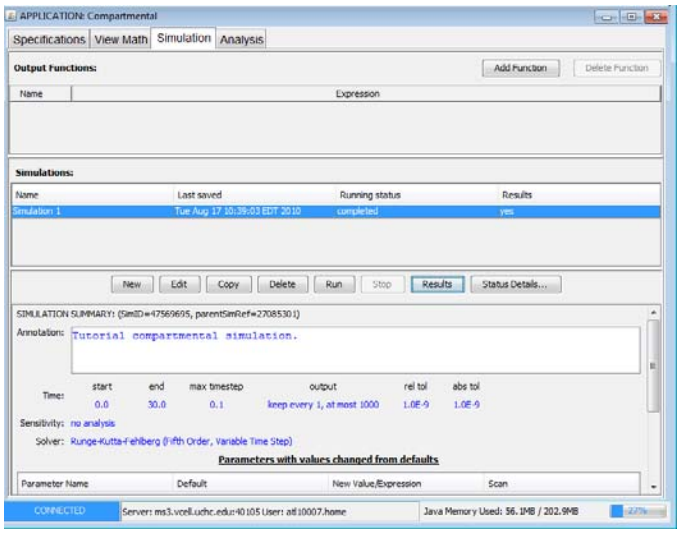
Reaction Mapping

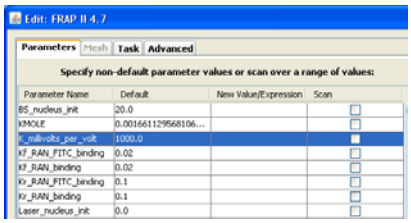
Leave the Reaction Mapping in the default settings. The reactions should all be enabled. Fast kinetics is used when one reaction occurs on a much faster time scale than the majority of the other reactions, and should not be enabled in this case.

Running the FRAP Binding Compartmental and Spatial Simulations

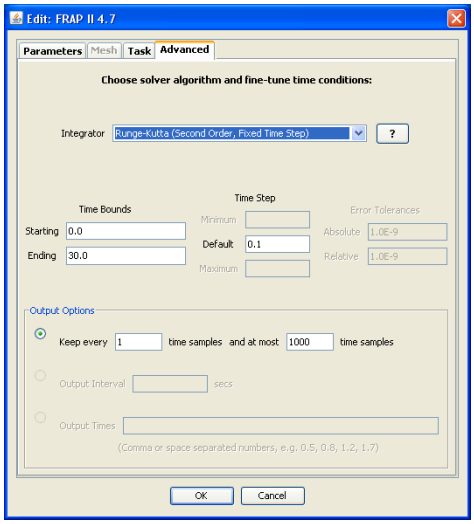
I. Compartmental Simulation and Results

Reopen the Compartmental application from the application window to the right of the BioModel. Select the Simulation tab and press the New button. In the middle panel of the Simulations dialog, a simulation with a default name will appear. Double click the Name text field and type in a simulation name. You can add additional notes regarding the simulation in the Comments text field if you choose to do so.





Press the Edit button to access the Parameters tab that lists all the parameters in the model and their corresponding default values. You can change the values and run new simulations without having to rebuild a new model.

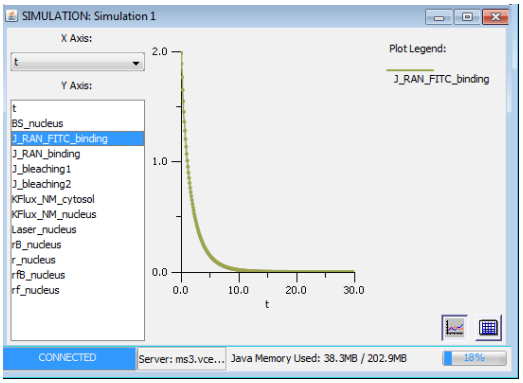


To change a default value, double click the New Value/Expression text field and enter in a new value. Altered values appear in red text. In this tutorial we will not alter any values.

The Advanced tab has various integrators that can be used. For this example, choose any one other than the default integrator. Then change the Time Step from the default of 1.0 to 0.1.The ability to change the Time Step default depends on the integrator choice.

Make sure your simulation is still selected when you press the Run button to initiate the simulation. Your model will automatically be resaved with the new run conditions and the simulation will begin. The results are stored on the remote database server.

Once the simulation has generated some results, the Status field will display Complete and the Results field will display Yes. The simulation must be selected for the Results button to be active. Press the Results button to open the Results dialog.

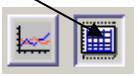


You can select how you want the graph constructed by choosing the parameters for the X and Y axes. The graph is interactive; put your mouse over the graph to see the coordinates for each data point on the curve. Press the right mouse button to access the Plot setting dialog for additional graphing options.

Export Features

Data can be copied from the data view and pasted into additional graphing programs such as Excel. In the Graph window the user must select the desired variables using the Shift+click or Ctrl+click

commands. Once selected the user can switch to the data view by clicking on show data icon

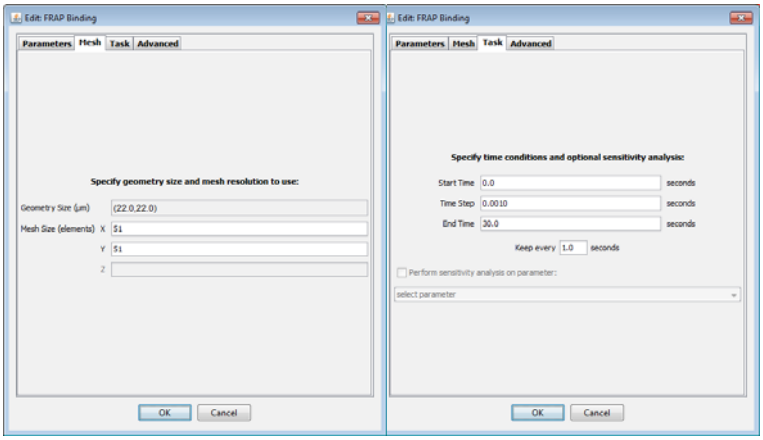


in the bottom right of the window. Once in this view the data can be selected and copied using the copy command or shortcut key. See Chapter 9 of the user guide, Exporting Simulation Results, for more information.

II. Spatial Simulation and Results

Reopen the spatial application from the application frame in the Model window to the right of the physiology. Press the Simulations tab on the Application dialog and press the New button. Double click in the Name text field to name the simulation.

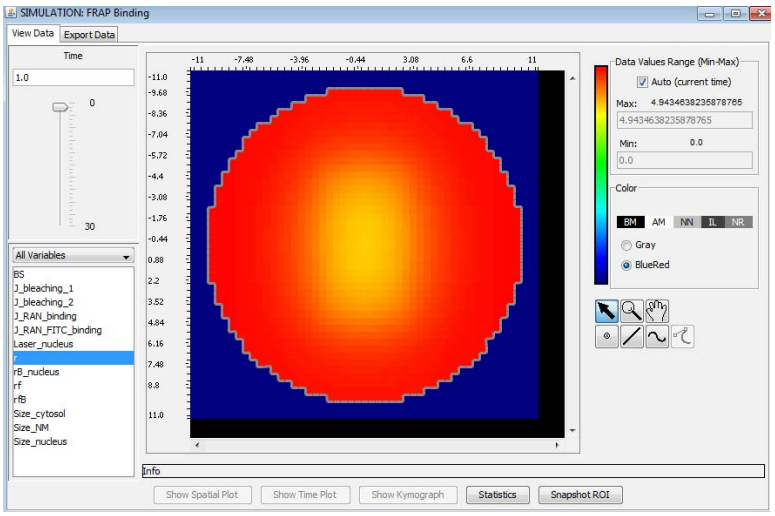
Press the Edit button to access the Mesh and Task tabs. Select the Mesh tab, enter "51" for the X and Y dimensions for the Mesh Size. The Geometry Size, which was defined in the Geometry Editor, should be listed as (22.0, 22.0).



Start Time: 0
Time Step: 0.001
End Time: 30.0
Keep Every: 1 sec

Press the Task tab to define the run conditions for the simulation, as described in the table. Press OK to accept the conditions and to close the dialog.

Make sure your simulation is still selected when you press Run to initiate the simulation. Your model will automatically be resaved with the run conditions and the simulation will begin. The results from the simulation are automatically stored on the remote database server.



Once results have been generated, press the Results button to access the Results dialog. The simulation must be selected to activate the Results button.

Use the scroll bar, on the left side of the Results dialog, to change the time interval or enter a time in the Time text field and press Enter. You can drag the scroll bar or select it and then use the up and down arrows on

your keyboard to step through the time points.

You can display your results in either a Gray or a Blue-Red color map. You may toggle between auto and manual scaling. 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 Point  tool to generate a Time Plot, and the Line  and Spline  tools to generate a Spatial Plot.

You can choose between displaying your results as a plot and viewing the data values. Press your right mouse button, while over the graph, to access the Plot Settings dialog.

Export Features

Click on the Export Data tab to display the export features. Select the variable(s), time interval and data region(s) you wish to export. Files may be exported as Comma delimited ASCII files, QuickTime move files, GIF89a image files, Animated GIF files and several other formats. See Chapter 9 of the user guide, Exporting Simulation Results, for more information.