

Virtual Cell Version 5.2

Tutorial II: FRAP with Binding

Creating a FRAP with binding BioModel

Introduction

This tutorial is a continuation of the first “FRAP” tutorial that 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. The bleaching reaction is modeled.
4. Both a nonspatial and a spatial simulation are demonstrated.

Following the Tutorial

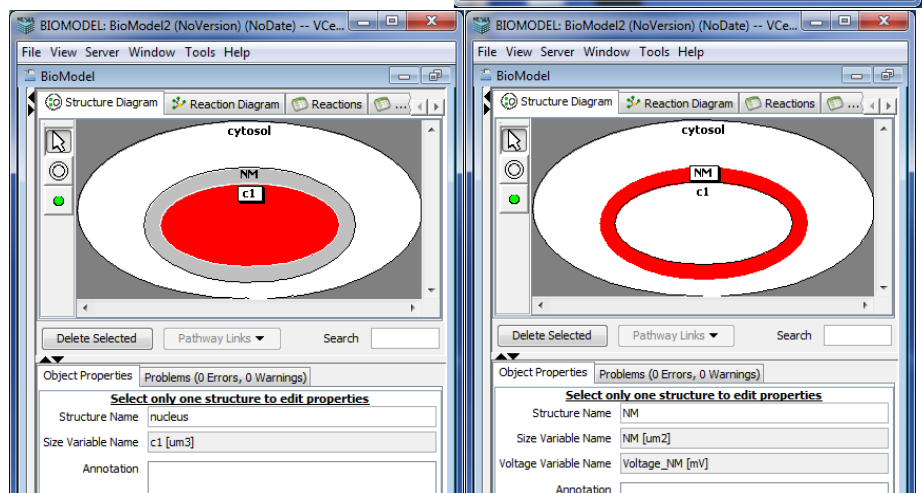
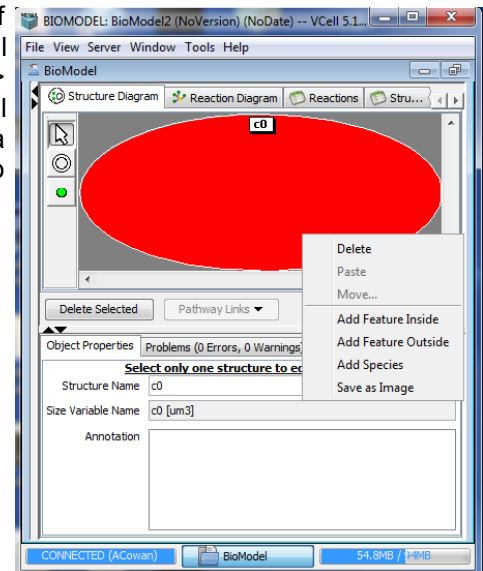
You can create your own BioModel and Application as you read through the tutorial or you may choose to load the public version of this model. To load the public BioModel tutorial, go to VCell Database> BioModels in the lower left panel and select Tutorials> Tutorial_FRAPbinding. There are two Applications (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, go to Structure Diagram at the top navigation bar, there you are presented with an undefined BioModel containing a single compartment. Select the compartment tool  and click in the compartment to create new compartment inside the existing one. Select the outer most circle and enter “cytosol” in the Structure Name text field in the Object Properties pane at the bottom of the page and press Enter. All names and expressions in the Virtual Cell are case sensitive. Be sure to note which case you use.

Click on the gray circle, this will turn red, and enter “NM” (Nuclear Membrane) into the Structure Name text field in the Object Property box at the bottom and press Enter. Click on the inner most circle, this will turn red, and enter in “nucleus” in the Structure Name text field at the bottom and press Enter.

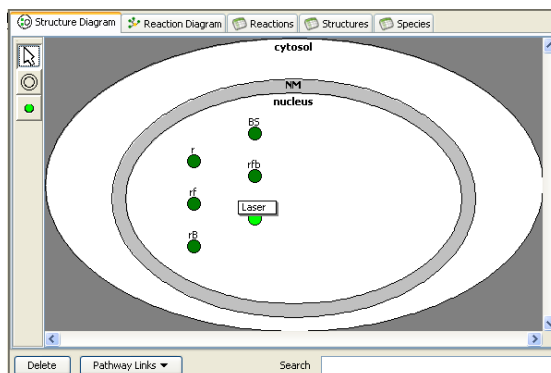


Adding Species

Select the species tool  and then click in the nuclear compartment once. Click on the Species (circle) just created to select it and access the Object Property box that appears at the bottom of the page where you can enter in Species Name and Annotation. Enter "r" in the Species Name text field. In the Annotation text field type in "RAN" and click Enter.

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


Annotation	Name
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 at the bottom, press Save.

Defining Reactions

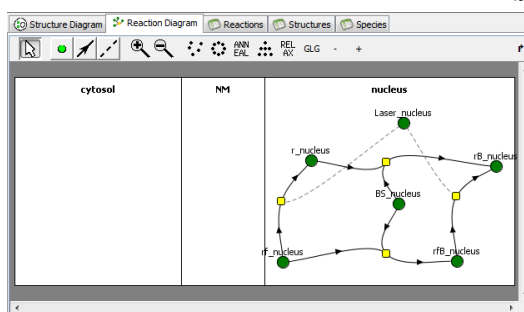
Click on Reaction Diagram at the top of the navigation bar. Using the arrow button click on the nucleus compartment. Arrange the species in the box so that each is visible. You might want to use this image as a guide for organizing the reactions.

Using the Reaction Connection tool  connect the species to each other using the picture and table as a guide on how to connect them. Make sure the arrows are pointing in the same way as the picture. Between each connection will be a reaction icon in yellow.

Select the Set a Catalyst tool  and connect the Laser species to the two reaction icons (bleaching 1 and bleaching 2) as shown in the picture. The laser is acting as a catalyst for the bleaching reactions.

Once the five species are connected go back to the arrow button and click on the first reaction icon (yellow

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



square) between r and rB, at the bottom there will be a table where you can change the Reaction Name and edit reaction kinetics. Alternatively, select Reactions in the upper left navigation or via the window tab. The Reactions view provides a table of all created reactions. Double click in the Name field for each reaction to change the names to those shown in the table (Be sure to note which case you use.) This name will appear when the reaction icon is selected.

Select the RAN_binding reaction In the Object Property box at the bottom 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"

Reaction	Name	Structure	Kinetics
BS_nucleus + r_nucleus -> rB_nucleus	RAN binding	nucleus	MassAction
rB_nucleus -> rB_nucleus	bleaching2	nucleus	General
rf_nucleus + BS_nucleus -> rfB_nucleus	RAN-FITC binding	nucleus	MassAction
rf_nucleus -> r_nucleus	bleaching1	nucleus	General
(add new here, e.g. a+b->c)			

Name	Description	Global	Expression	Units
J	reaction rate	<input type="checkbox"/>	$(K_f \cdot BS_nucleus \cdot r_nucleus - K_r \cdot rB_nucleus)$	uM.s-1
Kf	forward rate constant	<input type="checkbox"/>	0.02	uM-1.s-1
Kr	reverse rate constant	<input type="checkbox"/>	0.1	s-1
r_nucleus	Species Concentration	<input checked="" type="checkbox"/>	Variable	uM
BS_nucleus	Species Concentration	<input checked="" type="checkbox"/>	Variable	uM
rB_nucleus	Species Concentration	<input checked="" type="checkbox"/>	Variable	uM

and press Enter. Double click the Expression text field for Reverse Rate Constant Kr and enter “0.1” and press Enter.

Select the bleaching 1 reaction icon to access the Object Property and edit the reaction. Select General [μM/s] for the Kinetic type and enter the following in the Expression text field for the Reaction Rate equation:

$$(Vmax \cdot rf \cdot Laser \cdot ((t > 1.0) \& \& (t < 1.5)))$$

After pressing Enter to accept the equation, 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.

Select bleaching 2 reaction icon to edit the Object Properties. Select General [μM/s] for the Kinetic type and enter the following Reaction Rate Equation in the Expression text field:

$$(Vmax2 \cdot rfB \cdot Laser \cdot ((t > 1.0) \& \& (t < 1.5)))$$

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.

Object Properties	Problems (0 Errors, 6 Warnings)
Reaction Name	bleaching 1
Kinetic Type	General [μM/s]

Name	Description	Global	Expression	Units
J	reaction rate	<input type="checkbox"/>	$Vmax2 \cdot rfB_nucleus \cdot Laser_nucleus \cdot ((t > 1.0) \& \& (t < 1.5))$	uM.s-1
Vmax2	user defined	<input type="checkbox"/>	50.0	uM-1.s-1
rfB_nucleus	Species Concentration	<input checked="" type="checkbox"/>	Variable	uM
Laser_nucleus	Species Concentration	<input checked="" type="checkbox"/>	Variable	uM
t	time	<input checked="" type="checkbox"/>	Variable	s

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

Access the Object Property 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 Applications.

Object Properties	Problems (0 Errors, 6 Warnings)
Reaction Name	RAN_FITC_binding
Kinetic Type	Mass Action [μM/s] (recommended for stochastic application)

Name	Description	Global	Expression	Units
J	reaction rate	<input type="checkbox"/>	$(K_f \cdot rf \cdot BS - K_r \cdot rfB)$	uM.s-1
Kf	forward rate constant	<input type="checkbox"/>	0.02	uM-1.s-1
Kr	reverse rate constant	<input type="checkbox"/>	0.1	s-1
BS	Species Concentration	<input checked="" type="checkbox"/>	Variable	uM
rf	Species Concentration	<input checked="" type="checkbox"/>	Variable	uM
rfB	Species Concentration	<input checked="" type="checkbox"/>	Variable	uM

Creating Applications and Running Simulations

Introduction to Applications

Each model 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.

For this model you will initially create a Compartmental Application (default) where the BioModel is mapped to a single point. 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) that are 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. In the Compartmental Application, the species “light” that catalyzes the bleaching reaction is always 0, so no bleaching occurs. Rather, in the Compartmental Application we allow the binding reaction to reach steady state.

The steady state levels of the species that participate in the binding reactions (taken from the final time point of the Compartmental Application) will then be used as initial conditions in a Spatial Application. In the Spatial Application, you will map the BioModel to a two dimensional Geometry associated with the application. These models are solved using partial differential equations solved for each a mesh. The bleaching reaction will be invoked for a specific region of the nucleus, and resulting redistribution of species followed within the geometry.

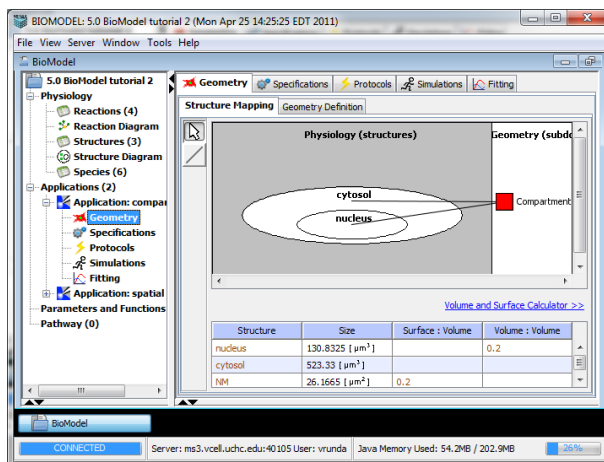
Creating the Compartmental Application

Compartmental Application and Geometry

On the left navigation bar click on Application, to the right click Add New and select Deterministic. Double click under the Name field and enter in “compartmental”, press Enter. Click “Application: compartmental” on the left to open 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

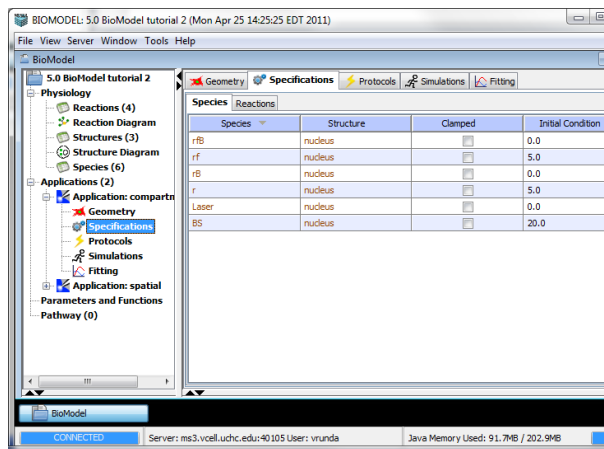
In the Structure Mapping folder, we enter in Volume and Surface sizes for the nucleus and the Volume size of the parent feature, the cytosol. Select the text field in the Size column for cytosol, enter 523.33. This is the volume of a cell that is spherical with a 10 μm diameter. Then select the text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




Initial Conditions


Select the Specifications tab on the top navigation bar. Double click in the Initial Conditions text field for RAN (r) and enter an initial concentration of 5.0. Press Enter to accept the value. 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.



Compartmental Simulation and Results


Select the Simulation tab from the top navigation bar and press the New Button . In the table in the middle of the Simulations dialog, a simulation with a default name will appear. Double click the Name text field and type in your own simulation name.

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 Solver tab has various integrators that can be used. For this example, choose any one other than the default integrator. The default Time Step should be 0.1; leave it as that value. The ability to change the Time Step default depends on the integrator choice. Press OK to accept the conditions and to close the dialog.

Make sure your simulation is still selected when you press

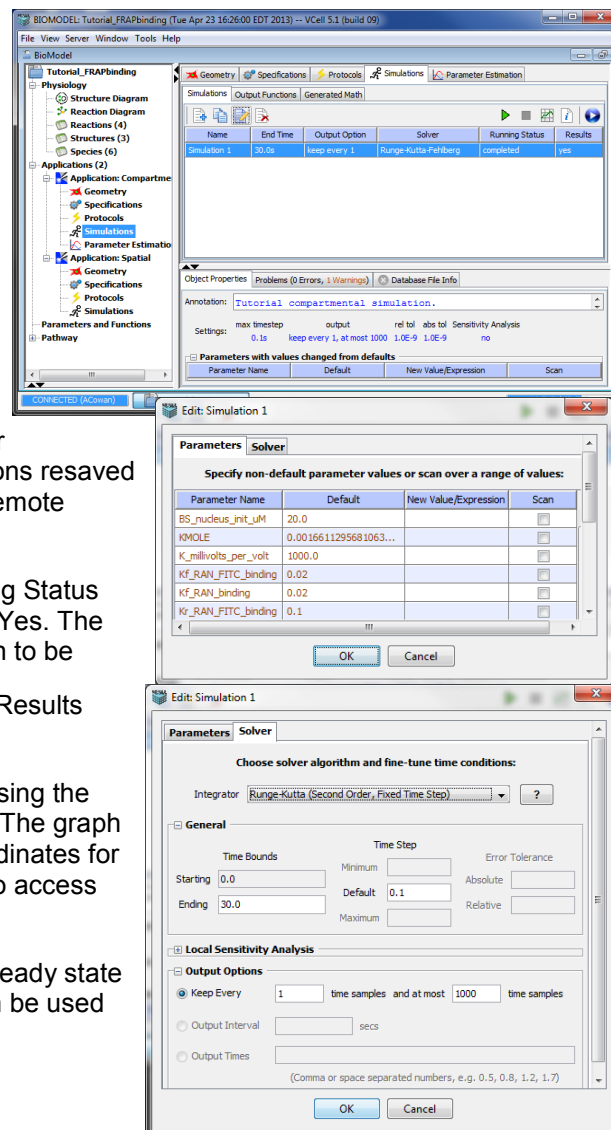
the Run Simulation button  to initiate the simulation. Your model will automatically be resaved with the new run conditions resaved and the simulation will begin. The results are stored on the remote database server.

Once the simulation has generated some results, the Running Status field will display Completed and the Results field will display Yes. The simulation must be selected for the Simulation Results button to be

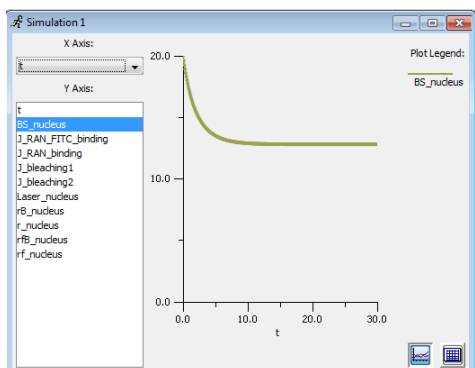
active. Press the Simulation Results button  to open the Results dialog.

You can select how you want the graph constructed by choosing the parameters of the X and Y-axes from the left navigation bar. 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 Settings dialog for additional graphing options.


The results of the compartmental simulation will define the steady state distribution of species in the binding reaction, which can then be used as initial conditions for the spatial application.



Export Features



For Compartmental Applications, 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.

Spatial Application

Spatial Geometry

To create the spatial application, select the compartmental application from the left side navigation bar and in the right click menu, select copy. Right click on the new application, select "Rename" and type in "spatial" as the new name. In the Spatial Application, select the Geometry Definition tab at the top. On the right hand side click "Add Geometry" and select "New..." from the drop down menu. Select "Analytic 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 to create an additional subdomain. From the drop down menu, choose Circle for the Subdomain Shape, (0,0) for Center and 10.0 for Radius.

The equation will automatically appear in Analytical 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 next to “nucleus” and enter the following equation that defines a circle.


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

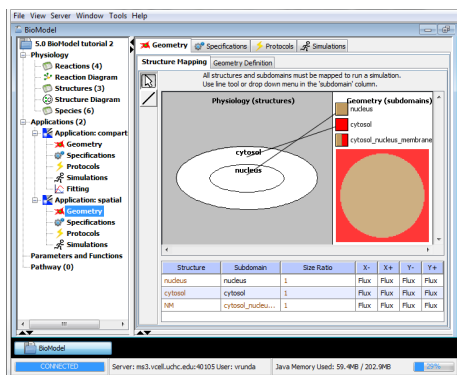
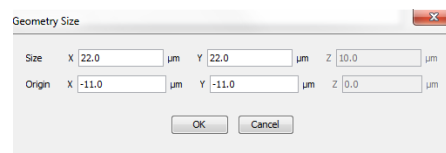
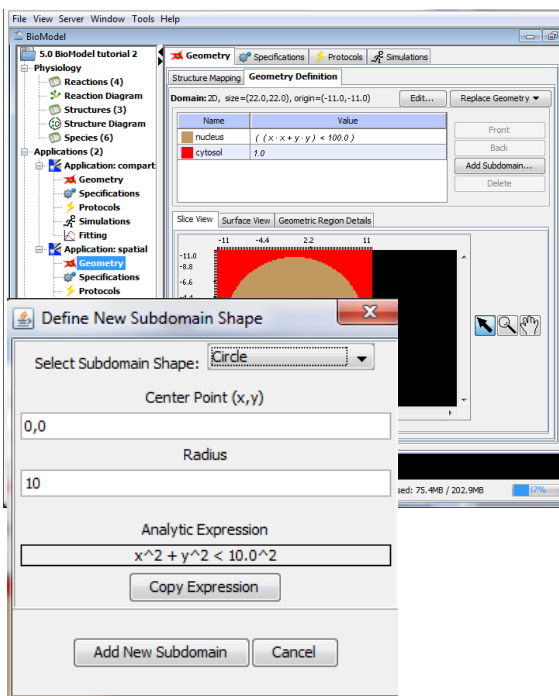
Press Enter after entering the equation. The two subdomains that you create will represent the cytosolic and nuclear compartments.

Press the Edit button, at the top right corner, 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. Press OK at the bottom 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. Draw a line from the nucleus in the physiology model to the color that represents the nuclear space in the geometry diagram to map nucleus to nucleus; then use the same process to map cytosol to cytosol. You need to reselect the line tool each time you do a mapping, and you need to map from 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.

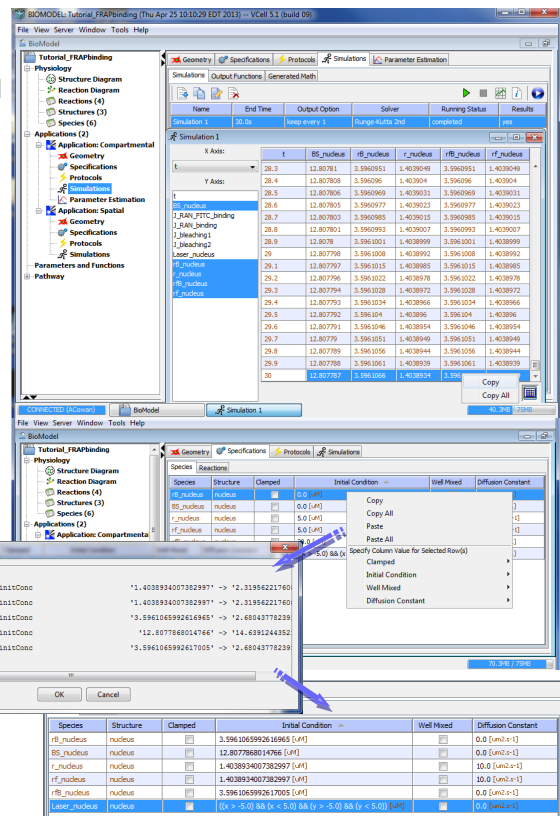


Initial Conditions

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 Specifications tab of the Application. We will use the final concentrations of the species in the compartmental application as initial conditions in the Spatial Application. First, go to the Simulation in the Compartmental Application, click on the simulation name in the main table to

select and click on the Results button  to retrieve the results.

Select the table view . In the Y Axis list, use control with click to select the BS_nucleus, rB_nucleus, r_nucleus, rB_nucleus and rf_nucleus species. Data for all these species will appear in the table. Scroll to the last row (longest time point in the simulation), select the values in the row (t = 30s) right click and choose Copy. Close the table. Select the Specifications for the Spatial model in the left navigation tree. Select anyone of the text fields in the Initial Conditions column, and click the right mouse button to bring up the menu and select Paste All. In the pop-up menu, check the boxes for all species you want to paste (or check the Select all box) and select OK. The values will be pasted into the initial conditions for the selected species.



The top screenshot shows the 'Simulation Results' table with columns for Name, End Time, Output Option, Solver, Running Status, and Results. The bottom screenshot shows the 'Initial Conditions' dialog box with a table for Species, Structure, Clamped, Initial Condition, Well Mixed, and Diffusion Constant. The 'Initial Condition' column is being edited with a paste operation.

Because this is a Spatial application additional values need to be entered. In the Diffusion Constant text field for RAN (r_nucleus) enter 10; likewise for RAN-FITC (rf_nucleus). These should be the default values. Make sure the Diffusion constants for all other species are 0.

In the Initial Condition text field for the Laser enter in the following equation, which will restrict the activity of the laser to a rectangular area within 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.

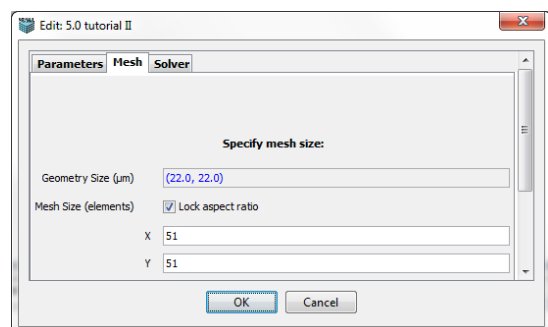
Running the FRAP Binding Spatial Simulations

Specifying Simulation Conditions

Press the Simulations tab on the top navigation bar and press the New button. Double click in the Name text field to name the simulation.

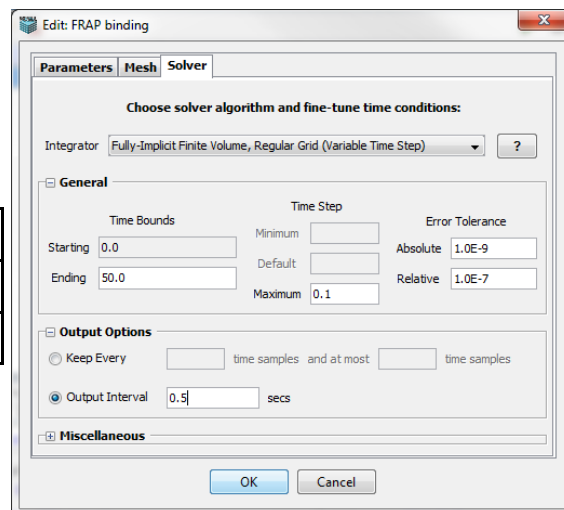
Save the model.

Press the Edit button  to access the Parameters, Mesh, and Solver 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).




Press the Solver tab to define the run conditions. Select the Fully-Implicit Finite Volume, Regular Grid solver and set run times as described in the table. Press OK to accept the conditions and to close the dialog.


Start Time: 0
End Time: 50.0 (sec)
Output Interval: 0.5 sec



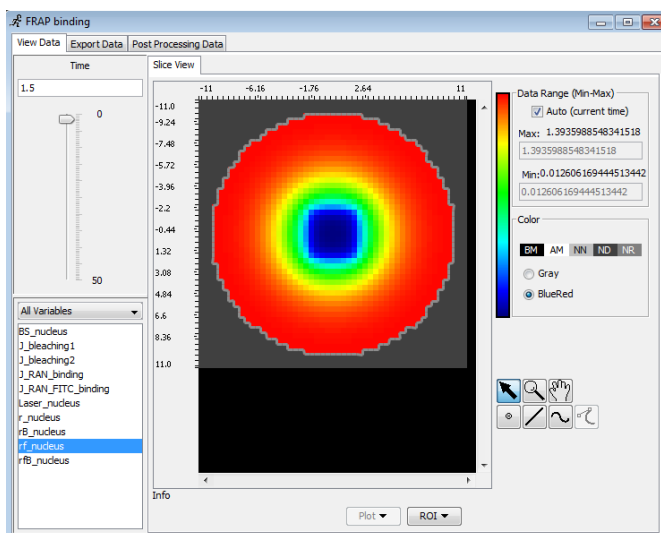
Results

Again, make sure your simulation is still selected when you press the Run Simulation  button 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 stores on the remote database server.




Once results have been generated, press the

Simulation Results  button to access the Results dialog. The simulation must be selected to activate the Simulation 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 Gray or BlueRed 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 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 movie files, GIF89a image files, Animated GIF files and several other formats. See Chapter 9 of the user guide, Exporting Simulation Results, for more information.

