Virtual Cell Version 5.2

Tutorial: Multiple Applications of a Nuclear Transport Biomodel

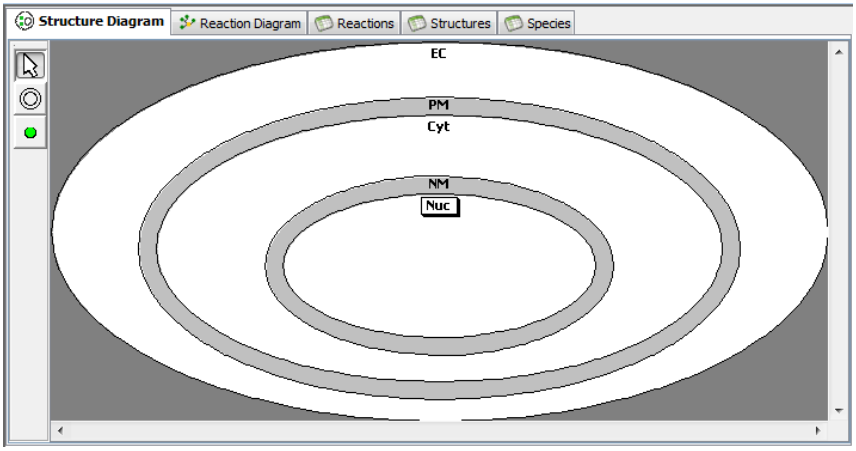
Creating multiple applications from a single biomodel

Introduction

This tutorial creates a Biomodel for Ran–associated cargo transport from the cytosol to the nucleus, consisting of the small GTPase Ran and a Cargo species that bind to create a RanCargo complex in the cytosol, which is then transported into the nucleus. Four different applications of the model are created and simulated: (1) a 3D spatial application that applies partial differential equations (PDE's) to a geometry created from a confocal 3D image stack; (2) a non-spatial compartmental model that uses ordinary differential equations (ODE's) to solve for concentrations in well-mixed compartments; (3) a non-spatial stochastic application consisting of probabilities associated with individual molecules in well-mixed compartments; and (4) a 3D spatial stochastic application following individual molecule trajectories in the 3D geometry. Open Tutorial_MultiApp from the Tutorials Folder in the BioModel Database or create a new model as you follow the tutorial.

Creating the Biomodel

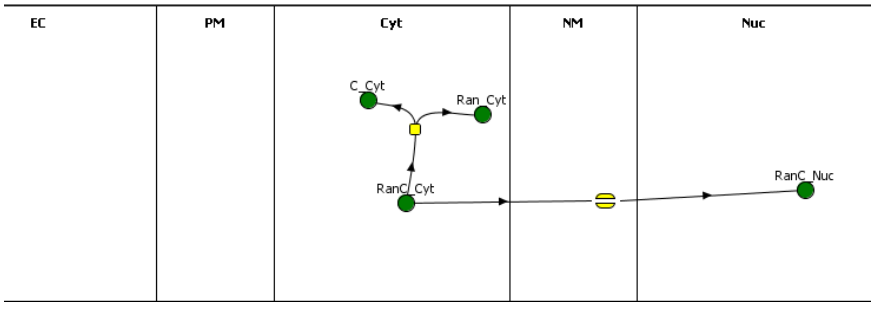
Creating and Defining Compartments



After opening a new model in VCell, select the **membrane tool**, , and click in the white circle. This creates a gray ring (membrane) that separates the diagram into two compartments. Select the **membrane tool** again and click within the inner white compartment to create another create another membrane and a third white compartment.

Using the **selection tool**, , click on the outermost white compartment. Look at the pane below the diagram, and find where it says **Structure Name**. Rename the structure EC (for extracellular). Use the selection tool again to click the outer membrane, and rename it PM (for plasma membrane). Repeat this process three more times to name the other compartments as they appear in the above picture (Cyt stands for cytosol, NM stands for nuclear membrane, and Nuc stands for nucleus).

Creating Species and Reactions



Above the structure diagram, Click on the **Reaction Diagram tab** to view the reaction diagram. The diagram will be an empty version of the picture . Using the line tool, ,drag a line from **Cyt** to **Nuc**. Two species (one in **Cyt** and one in **Nuc**) and a flux reaction are created. Select the species in the **Nuc** column. Rename it **RanC_Nuc**. Use the selection tool again and select the green species in the **Cyt** column. Rename it **RanC_Cyt**.

Create the binding reaction using the line tool; select the green species in **RanC_Cyt** and drag the line to anywhere within the **Cyt** column. A reaction icon is created. Use the line tool again and click on the reaction icon then drag a line

to anywhere in the **Cyt** column. This creates another species icon. Rename it **C_Cyt**. Next, use the selection tool to select the square reaction icon once more and drag another line to anywhere in the **Cyt** column. This creates the last species we need. Rename it **Ran_Cyt**.

Use the selection tool to select the flux icon in the NM column. You may leave its name as flux0 for this model. Make sure the **kinetic type** is set to Kinetic Type | General Flux Density (µM-µm/s) (it should be the default kinetic type).

Under **kinetic type**, there should be a table with a line named **J**. Find the box named **Expression** for this line and enter the following formula into the box:

Kflux*(RanC_Cyt-RanC_Nuc)

Additional lines should appear in the table after entering the formula. **Find the line named Kflux and enter 2 in its expression box.**

Next, use the selection tool to choose the square reaction icon in the **Cyt** column. You may leave its name as r0 for this model. It's **kinetic type** should be Kinetic Type | Mass Action [µM/s] (recommended for stochastic application) by default. The expression for this reaction should be **(Kf*RanC_Cyt-Kr*C_Cyt*RanC_Cyt)** by default and does not need to be changed.

Find the line named **Kf** and enter 1 in **the Expression** box. Find the line named **Kr** and enter 1000 in the **Expression** box.

Creating a Spatial Deterministic Application

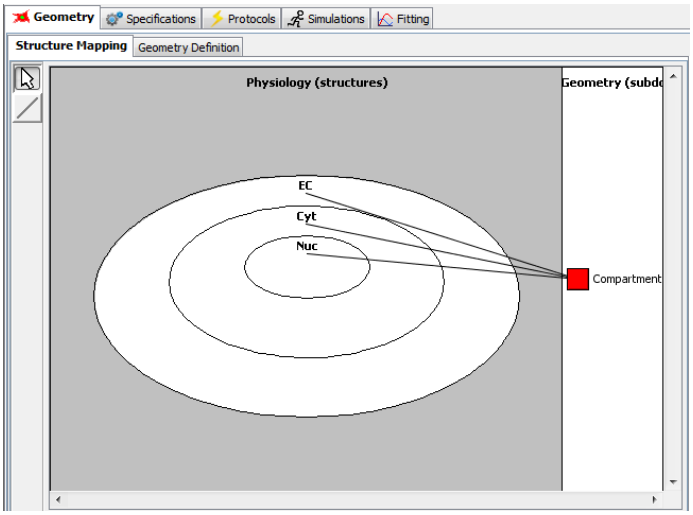
Create a new spatial application

In the left window navigation tree, select **Application**. The reaction diagram will be replaced with a blank window. From the top tabs in in the window select **Add New** with a drop down arrow. Click this and select **Deterministic**.

The name of the new Application is **Application0** by default. Change the name to **spatial deterministic** by right-clicking the **Application 0** and selecting **Rename**. In the left pane. Note that you must hit the Enter key to accept the new name. **Spatial deterministic** should appear under **Application** in the navigation tree. Click on this to open the application.

Select the **Geometry Definition** tab, then select and click on the **Add Geometry** button to show the drop-down menu. Select **New** from the drop-down menu.

Creating a geometry from an image file

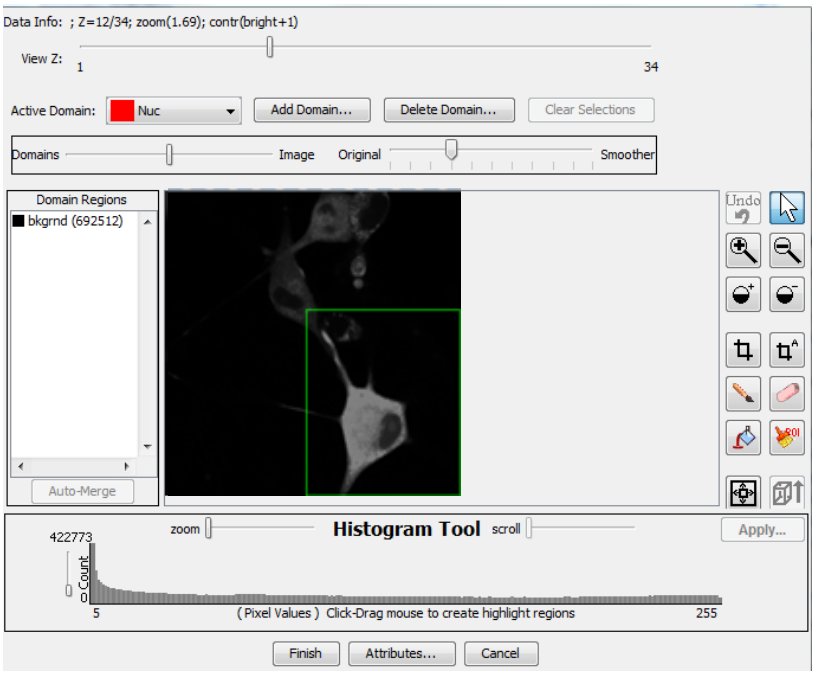


To import the image sets needed for this tutorial, you can download them from **www.vcell.org** under **User Guide** and scrolling down to the video tutorial section and clicking on **3D images for tutorial** next to Episode 2. If you have a slower internet connection that would make downloading them inconvenient, you can upload the final geometry from the public tutorial model [give name] geometry. After opening the folder with the images, a window will pop up asking if you want to import all of the images. Click **Import Z-Sections**. Before the pictures import, it will ask you to specify the resolution. Set the resolution to 50%.

When the images are uploaded, a new window should appear, and a prompt should ask if you want to and an empty domain or have it pre-segmented. Select **Add Empty Domain**. A new window should appear asking you to give the new region a name. Name it **Nuc**.

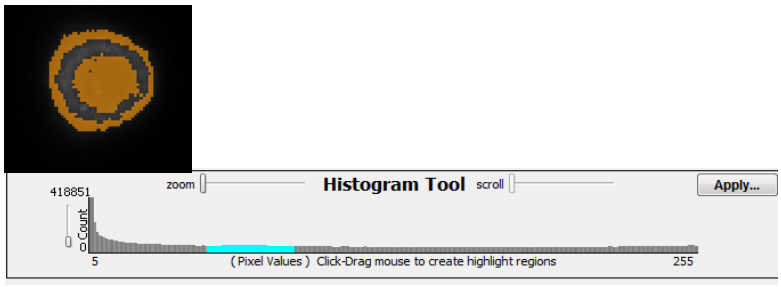
You should now see something similar to the image below. There should be a slider **View Z t**hat lets you navigate through the z-sections of the cell. You should see a dark circle within a lighter area appear and expand before fading out as you scroll through the slides. This is the nucleus.

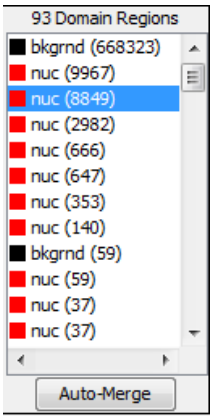
Using the crop tool,  , create a green box like the one in the above image to remove the extra space. Find the **Smoother** bar located above the image and set the slider to the second dash (this will smooth the pixel gradients and make editing the image easier). You can make the image bigger with the magnifying tool,  .



At the bottom of the window is a gray **Histogram Tool**. Select areas of the histogram to highlight by clicking and dragging. This will highlight areas of the diagram.

Try to select an area of the histogram like the one shown below. Use the navigation slider at the top of the window to see if the nucleus has been highlight entirely, and it is completely separated from any other highlighted parts. **It is OK if more than just the nucleus is highlighted—this can be fixed.**



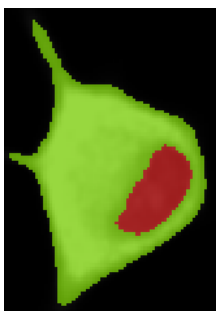


Once you've correctly highlighted the nucleus, click the **Apply…** button above the histogram. A new window will pop asking to update the domain or create a new one. Select **Update Domain**.

To the left of the image, a **Domain Regions** pane details all of the domain regions, with **bkgrnd** as the top region. Use the **selection tool** to click the nucleus in the image its domain should be highlighted in the pane to the left. Vice versa, selecting the domain in the pane highlights it in orange in the image. If when you click on the nucleus, another part of the image lights up alongside the nucleus (it will look like the image to the right—note how the outer cell membrane is highlighted along with the circular nucleus), click on **Delete Domain**. A window will pop up, and you must click **Delete Only Current Domain**. Restart the above process using the histogram tool, this time selecting a smaller section.

Once you have succeeded in highlighting the nucleus correctly, bring your attention to the **Domain Regions** pane to the left of the image. When you click on the nucleus in the image, one of these regions should light up. All of the other regions besides this one and **bckgrnd** at the top of the list must be auto-merged, a process explain in the next paragraph.

Now, select every domain **except bckgrnd and the correct nucleus domain** and click **Auto-Merge**. The easiest way to do so is to select all of the domains using Ctrl+A and then clicking "bckgrnd" and the nucleus' domain while still holding down Ctrl .

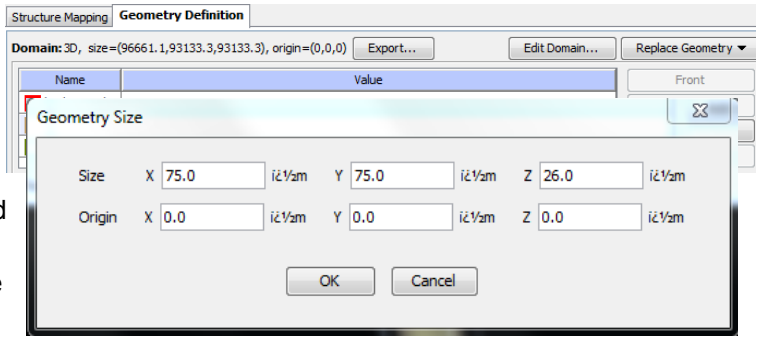


Next, click the **Add Domain…** button and enter the new domain name as **Cyt.** Using the histogram again, select from about where the histogram in the top right corner starts and drag to the end of the histogram. Click **Apply…**, then **Update Domain**, and then **Keep existing Domain Regions when overlapping**.

Most of the cell should be highlighted green now. Click on the cytosol surrounding the red nucleus to highlight this region in the **Domain Regions** pane to the left. Auto-Merge every region **besides** this one, bckgrnd, and the nucleus' region.

Click **Finish** at the bottom of the window and a prompt will appear saying some parts of the image have not been assigned a domain. Click the **Assign as default "background"** option. Another prompt should appear asking about adding borders to the image; select the **Add empty border** option.

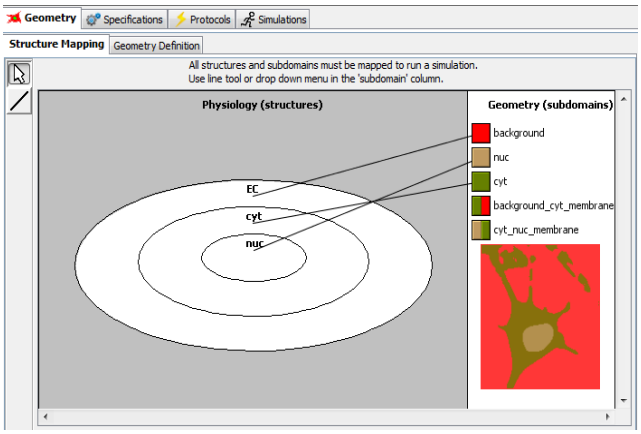
You will be brought back to the **Geometry** tab of your application. Find the **Edit Domain…** tab and click it. This opens a new window called **Geometry Size.**



The x (length), y (height), and z (width) coordinates may need to be adjusted from the default decimals VCell assigned to them. Enter 75 in for the x and y coordinates and 26 for the z coordinate. Click **OK**. These numbers change the size and depth of your geometry, and you may change them as necessary.

Mapping compartments to Domain Regions

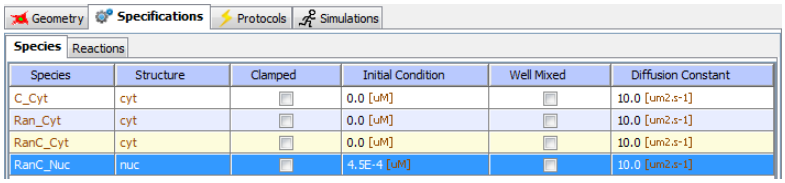
Now, you must assign the compartments you created in your model (Physiology) to the geometry domains you created when you segmented your images. Click on the **Structure Mapping** tab and you should see something similar to the image to the right.



Using the line tool,  , drag a line linking the EC compartment to the red background box. Then, drag a line from the Cyt compartment in the Physiology to the green Cyt box. Lastly, drag a line from the Nuc compartment to the tan Nuc box. You must draw from the Physiology to the Geometry.

Setting specifications for the Application

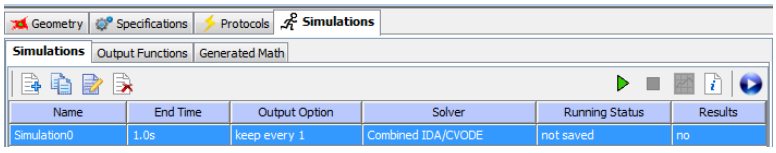
Now you need to specify conditions that will apply to all simulations of this specific Application. Select the **Specifications** tab, find the line for the species **RanC_Nuc** and enter 0.00045 into the **Initial Condition** box as the initial concentration in micromolar. (Note that you will be able to modify initial conditions separately within any given simulation of this Application). VCell will automatically convert decimals into scientific notation. The diffusion constants do not need to be changed from the default of 10 for this model.



Creating Simulations of Applications

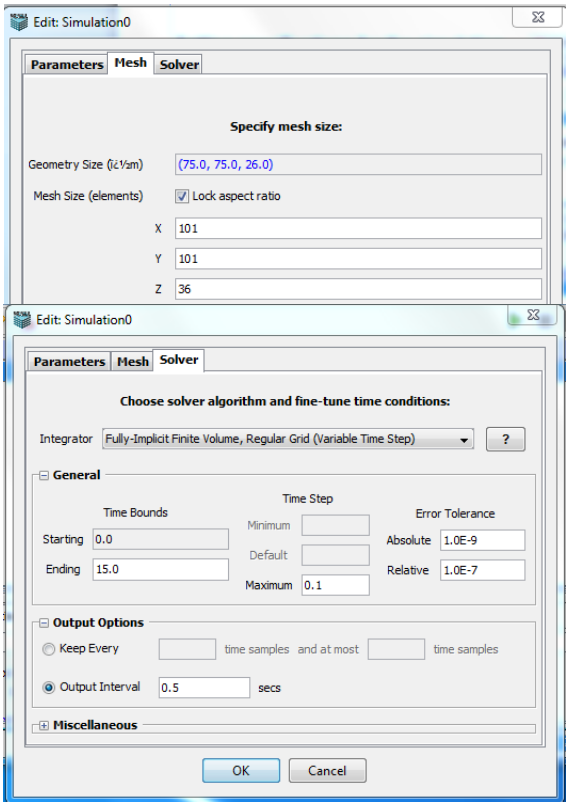
Create a new simulation

Select the **Simulations** tab, then use the **New Simulation tool**,  , to add a simulation to the list. You may leave its name as **Simulation0** for this model.



You can save your work at any time; once you create a simulation, before you save it is useful to click on the **Generated Math** tab and then click the tab that says **Refresh Math**. This ensures any changes you made to your model throughout this tutorial matches the math used to compute the simulation.

Now that the simulation has been created, it is time to edit it. Click on the **Edit Simulation tool**, , to open the **Edit: SImulation0** window.



Select the **Mesh** tab to define the number of mesh elements that will be used to compute the simulation. Make sure the **Lock aspect ratio** box is checked. This keeps the x, y, and z dimensions proportional if any one of the three is changed. Enter 101 into the box for x and the y and z co-ordinates should change to match the ones in the image to the right.

Next, select the **Solver** tab. The **Integrator** should be set to **Fully-Implicit Finite Volume, Regular Grid (Variable Time Step)** by default, but change it to this setting if it is not.

Change the **Ending** Time Bound to 15 seconds.

Change the **Output Interval** to 0.5 seconds.

Click OK to save these changes to the **Mesh** and **Solver**.

A warning window may appear saying the simulation could be too large. Choose to run the simulation anyway.

Now all that is left is to run your simulation and view the results.

Click the Run button, , to begin running your simulation. This will save your model and send the job it to the VCell servers to be run.

The box labeled **Running Status** details the progress of your simulation and shows when it has been completed.

If your simulation should fail to complete, it could be because of the mesh size. You can try a different mesh size (say, 90 for x to adjust y and z accordingly) and run it again. Keep changing the mesh size as needed until your simulation is able to complete.

View Results

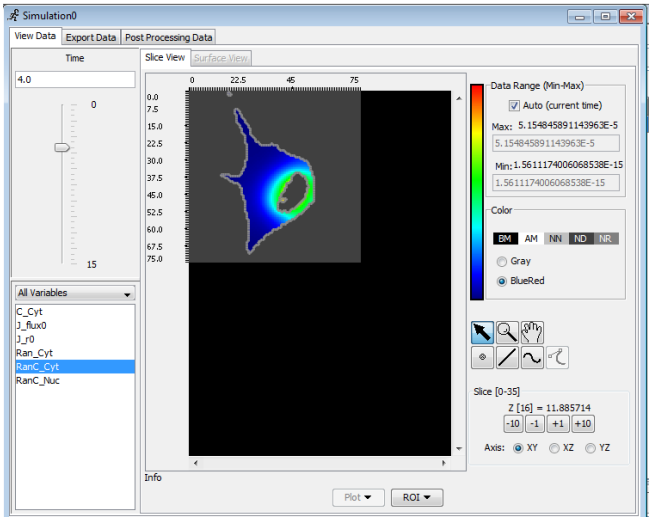
Results can be viewed as soon as there are results on the server; you do not need to wait for the simulation to finish.

Highlight Simulation0 and click the **Simulation Results** button, , to view the data generated by your simulation.

A **Slice navigator** is in the lower right corner. By clicking any of –10, -1, +1, +10, you can move up and down the Z-slices. Pick a slice that allows you to see as much of the nucleus and cytosol as possible.

A **Time Selector** Slider is in the upper left. When the slider is selected, the up/down keyboard arrows can be used to increment the time step in view.

The **Variables** pane in the lower left lists all of the species used in your simulation. Click on a desired variable to diplay that concentration in the results. In Slice View only one variable at a time can be displayed.



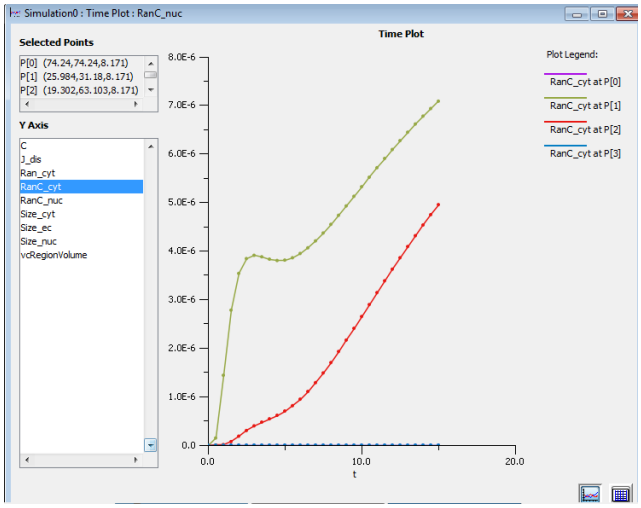
The panel on the upper right provides alternative look up tables to display concentration data at each pixel, and either automatic scaling or user defined upper and lower bounds for visualization.

A variety of graphs or tabulations of the data are available (use the online Help (top line of main window, select Help). Use the dot tool, , to select one or more points in the cell. Click the **Plot** button and select **Time** from the drop down menu to view the graph of species concentration vs time at that point(s). Shift with left mouse can be used to select multiple variables for dispay on

the same graph. You can switch between tabular or graphical form using the  icons on the bottom right.

The **Time Plot** should look similar to the one on the right, although your graph may differ based on the points you chose.

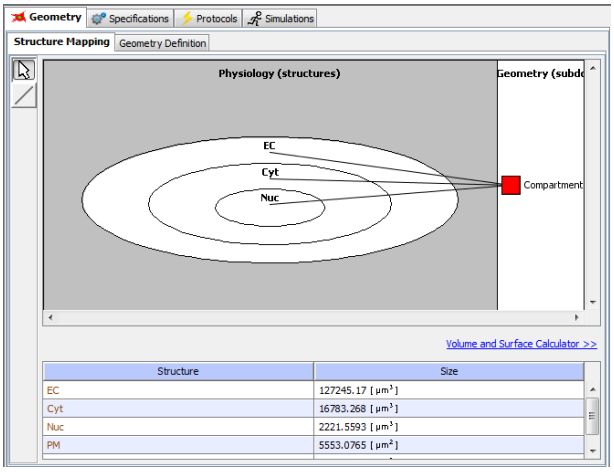
You can choose to display the concentrations for differing species by clicking them in the **Y Axis** pane on the left.



Creating a second Non-Spatial Stochastic Application of the same BioModel

Create a new application of the same BioModel.

You have just finished your first application, a spatial deterministic application, for this bioModel. This VCell supports four types of applications, and next you will create a Non-spatial stochastic application of the BioModel. Stochastic applications are required when the number of molecules is low enough that noise from random events can significantly affect the number of particles in a compartment or region. Thus, in general a number of individual simulation runs is computed and the average behavior determined.

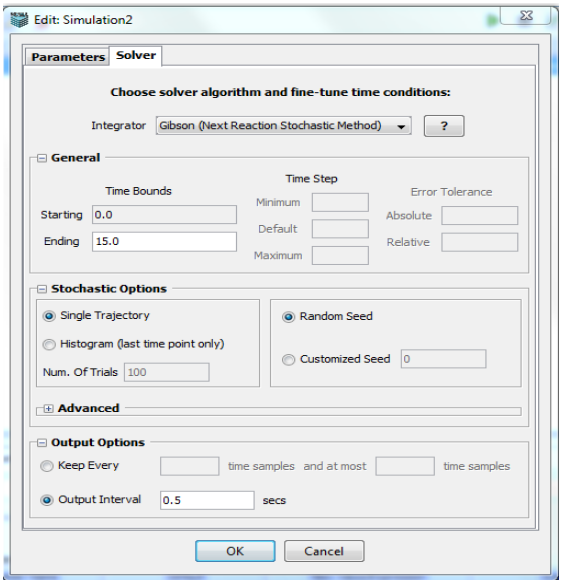


Right click your **spatial deterministic** application and select **Copy as -> Nonspatial -> Stochastic**. Right click this application once it is created and select **Rename**. Name the application **non-spatial stochastic**.

Select the **Non-spatial stochastic** application to view it in the main workspace. Select the **Geometry** upper tab and **Structure Mapping** lower tab.

Since this application was copied from the **spatial deterministic** application, the **Structure Sizes** (blue table at bottom of right hand image) are set to match the sizes of the image you used previously. There is no need to change them for this application. The structures all map to a single compartment.

Now select the **Specifications** tab, then the Species tab within the specifications pane. The initial conditions will be reported in concentration units and will retain the value of 4.5E-4 from the spatial deterministic simulation. Click the Number of Particles radio button to see the number of particles rather than concentration; based on the volume of the nucleus defined in the geometry VCell calculates the total number of particles that will reflect the original concentration; in this case the number of particles is 387 (This will most likely be different than the number in your model).



Running a stochastic simulation with parameter scanning.

Select the **Simulations** tab at the top of the window. Then, click the **new simulation** tool,  , to add a simulation. Change the name of the simulation to For Data.

Next, click the **edit simulation** tool,  to open the **Edit: Simulation** window and click on the **Solver** tab.

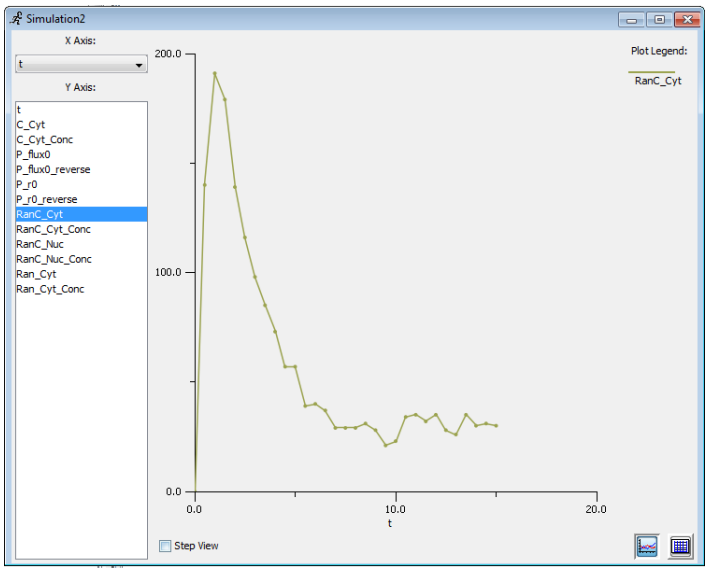
The default **Integrator** should be **Gibson (Next Reaction Stochastic Method)**. Leave it as this for the simulation.

Under **Time Bounds**, set the **Ending** to 15 seconds.

Under **Output Options**, set the **Output Interval** to 0.5 seconds.

Click **OK** to finalize these changes.

Click on the **run simulation** tool, , to begin running the simulation. The box labeled **Running Status** will inform you of the simulation's progress.



View Results

When the simulation has completed, click on the **simulation results** tool, , to view the results. The results window in this type of application is a simple graph of concentration vs time. The concentration of RanC (complex) in the cytosol will be used as "experimental data" in the next part of the tutorial. In the **Y Axis** pane on the left, select the species **RanC_Cyt_Conc** and it's concentration graph should appear that will be similar (but not identical, because every stochastic simulation will vary because of random noise) to that shown here.

Click on the **show data** tool, , to view the concentrations as a spreadsheet. Right click the data in the spreadsheet and select **Copy All**.

Paste this data in Microsoft Excel or another spreadsheet creating software and then save the spreadsheet as a **CSV file**. Make sure you can access this file easily as you will need it for the next simulation.

Creating a Non-Spatial Deterministic (ODE) Application and estimating parameters using COPASI parameter estimation tools.

Create a third application of the BioModel

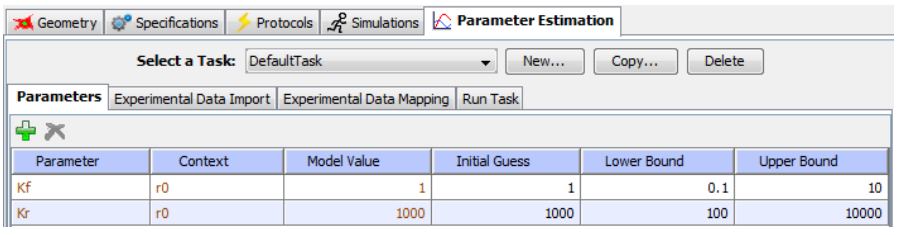
The next application you will create will be deterministic non-spatial. Create this application by right-clicking the **spatial deterministic** application and selecting **Copy As -> Non-Spatial -> Deterministic**.

The name of the new application will appear as **Copy of spatial Deterministic**. Rename the application by right-clicking and selecting **Rename**. Name the copied application **non-spatial deterministic;** note that you must hit the Enter key to accept the new name.

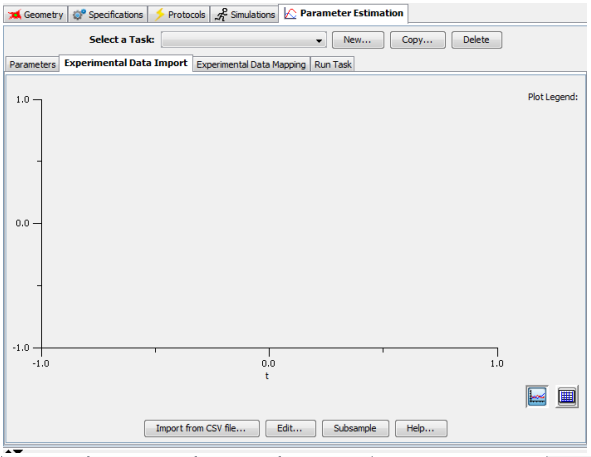
Click the **Specifications** tab to see that the initial concentration of **RanC_Nuc** is still 0.00045 from the previous application.

Importing experimental data for parameter estimation

Clicking on the **Parameter Estimation** tab will bring you to the parameter estimation workspace, shown to the right.



Click on the **Add tool**, , to open a new window with a list of parameters to add to the application. Add **Kf** and **Kr** as the parameters (You will need to click the **Add tool** each time you want to add a parameter). VCell will automatically put in an initial guess for the parameters. You may leave them as is.



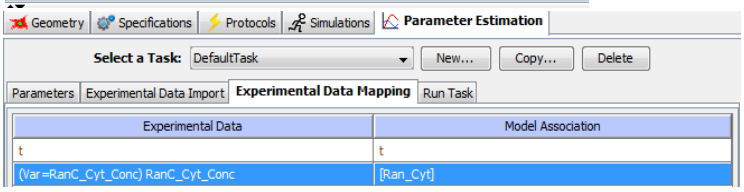
Click on the **Experimental Data Import** tab and it should lead you to a window similar to the image below. The experimental data that you need is in the spreadsheet that you saved from the previous simulation.

Click on the **Import from CVS file…** tab. Find the data where you saved it on your computer and select **Open**.

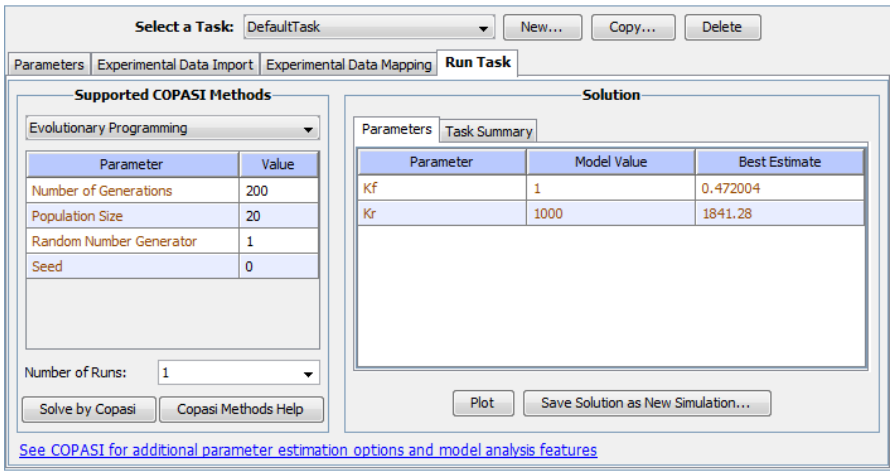
A graph of the data should appear in the window.

Experimental Data Mapping

Now, click on the **Experimental Data Mapping** tab.



One of the **Experimental Data** variables be unmapped (it will be the one that's not **t**—the time). Click on its line to highlight it. Then, select the **Map Experimental Data…** tab located at the bottom. A new window should pop up with a list of species to map the variable to. Select **[RanC_Cyt]** from the list and then click **OK** to finalize the decision.



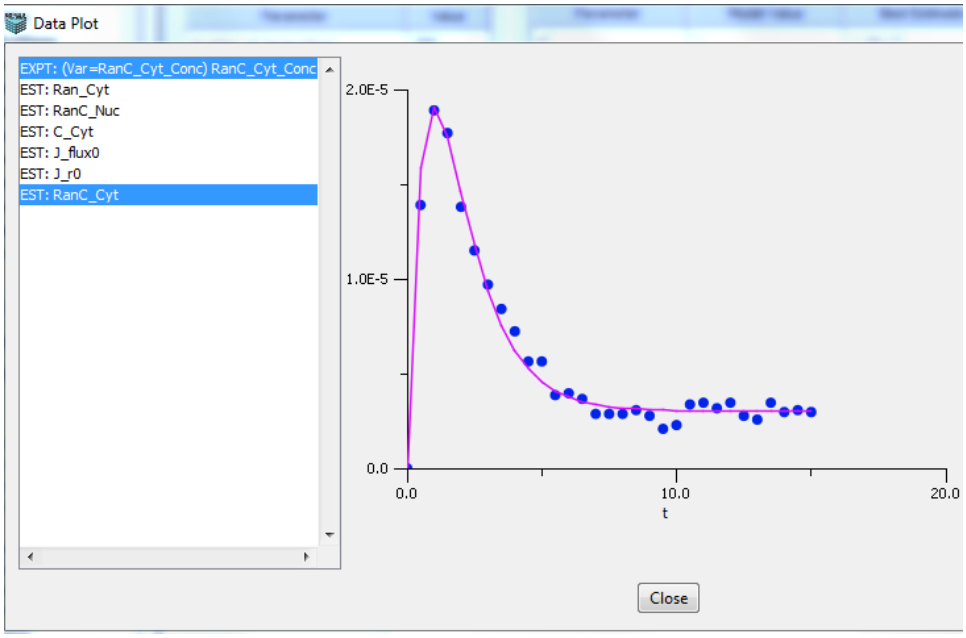
Running the estimation

Select **Run Task** from within the Parameter Estimation workspace. The default **Supported COPASI Method** should be **Evolutionary Programming**. Leave it as this. The parameter values do not need to be changed in this application.

Click on the **Solve by Copasi** tab, and it should start performing the task automatically.

When it is finished, click on the **Plot** tab to see if the parameter estimations are a good fit.

This should open up a new window titled **Data Plot** that resembles the image below.



Comparing the data

The blue dots are the experimental data from the previous application and the pink line is VCell's estimate for how the concentration of RanC_Cyt changed over time. As the image shows, the estimate is a good fit for the data.

Creating the next application

Creating the next application follows a similar process to the previous one. Find the application you labeled **Spatial deterministic** and right click it. Select **Copy As -> Spatial -> Stochastic**. The application you just created will be named **Copy of Spatial deterministic**. Right click it, select **Rename**, and name it **Spatial Stochastic.**.

Click on the **Specifications** tab near the top of the window, then the Species tab within the Specifications.

Since this application is stochastic, you can delineate the number of particles you want in addition to the concentration. VCell will automatically calculate the concentration for a species that you enter the number of particles for, and vice versa.

Click on the radio button for **Number of Particles**. Find the line labeled **RanC_Nuc** and enter 1000 into the **Initial Condition** box.

Preparing the Simulation

Now, click the **Simulations** tab at the top of the window. Click on the **new simulation** tool, , to add a simulation. You may leave its name as the default **Simulation1** for this application.

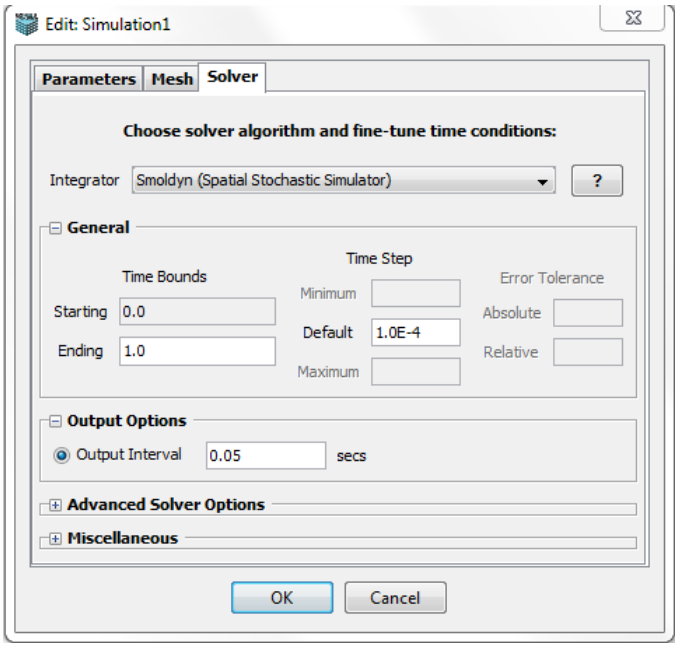
Next, click on the **edit simulation** tool, , and a window titled **Edit: Simulation** should pop up. Click on the **Solver** tab.

The default integrator should be **Smoldyn (Spatial Stochastic Simulator)**. Leave this as it is for this simulation.

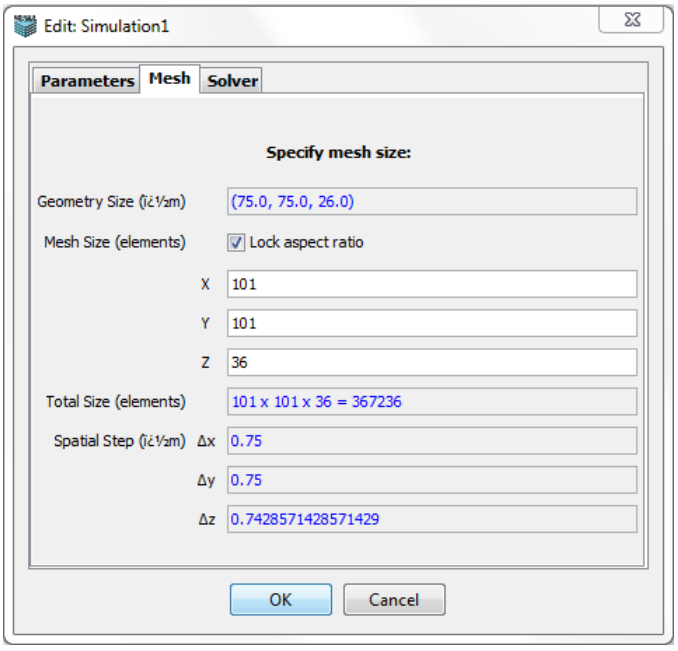
Under **Time Bounds**, change **Ending** to 10 seconds.

Under **Output Options**, change the **Output Interval** to 0.5 seconds.

Remember, do not press enter to finalize your entries, as this will prematurely close the window without saving your changes. Wait until you have changed everything you need to and then click **OK** to finalize your changes.



Click the **edit simulation** tool again and select the **Mesh** tab. Make sure the **Lock aspect ratio** box is checked, make sure the white box next to the X variable reads 101. If it does not, change it to 101 and VCell will automatically adjust the other two variables to maintain the proper proportions.

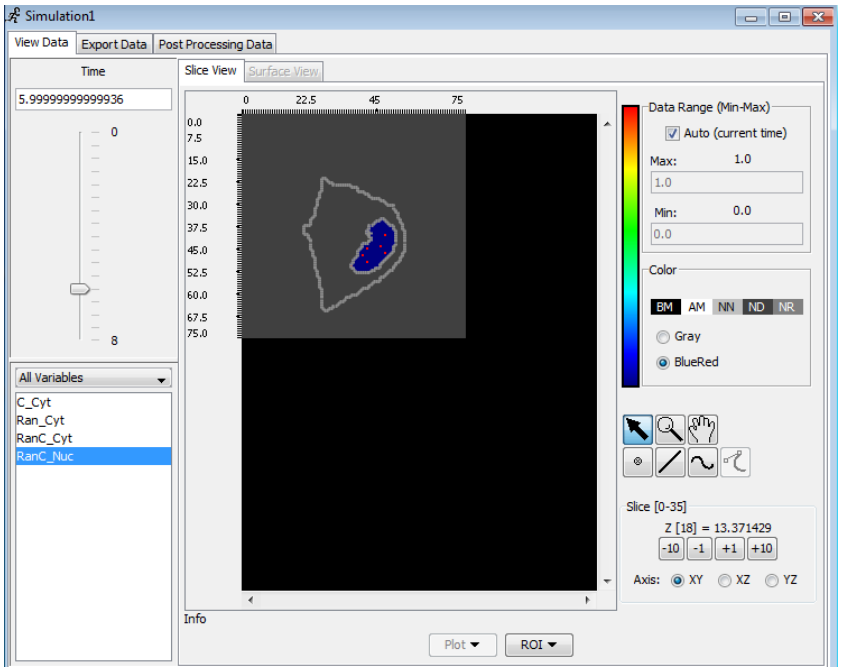


Now that the simulation has been set up, it is time to run it. Click on the **Run Simulation** tool, , to start the simulation. The **Running Status** box should inform you of the simulation's progress.

NOTE This simulation takes a long time to complete running

However, you can view the results that VCell has completed up to the current point before the simulation has finished completely. Click on the **simulation results** tool, ,to view your results so far.

Viewing your results



Clicking **simulation results** , will take you to a new window that looks similar to the one on the right

The first thing you should notice is the **Slice navigator** in the bottom right corner. You can navigate through the slices by clicking +10, +1, -10, or –1.

Choose a slice that lets you see a good portion of the nucleus and a lot of the cytosol, similar to the image on the right.

The next tool you should notice is the **Time Slider** in the upper left. You can drag the pointer up and down to see how the concentrations of the species change over time.

Next, in the upper right, is the color key that lets you interpret that concentrations of the species.

In the bottom left is a pan titled **All Variables**. Clicking the species in here will highlight them in the slice image. They will appear as colored dots, where each dot represents particles of the species.

By navigating through the slices at different times, you can see how the particles move throughout the cell.

To view a real time movie of all the individual particles, highlight the simulation and click on the **Real Time Particle Viewer** icon, . The Paticle Viewer may take some time to load. Particles of each species in the model will appear as a different color.