PH-GFP Translocation
Virtual Cell Version 5.2

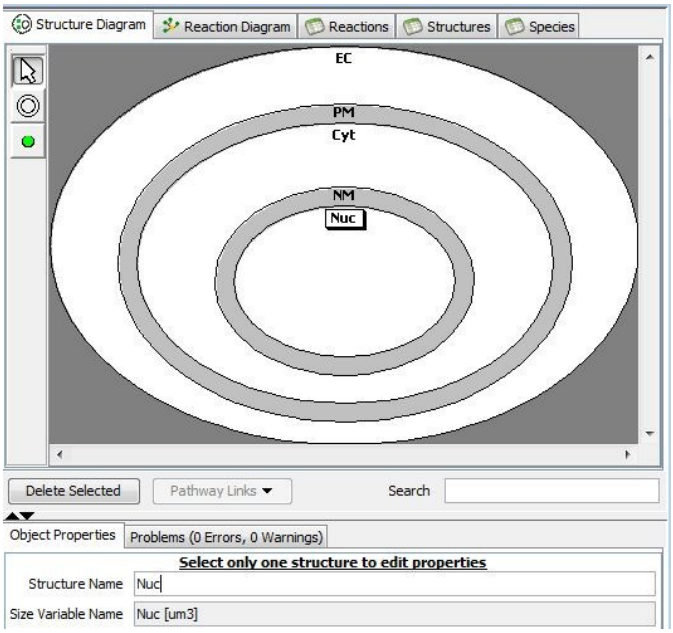
Introduction

This tutorial models the behavior of a fluorescent biosensor for IP3 release, as occurs during signaling events. The pleckstrin homology (PH) domain binds primarily to cytosolic IP3, but also binds to PIP2 in the membrane and can be tagged with the fluorescent molecule GFP. As PIP2 in the membrane is converted into diacyl glycerol and IP3, PH-GFP translocates from the membrane to the cytosol. This tutorial highlights the use of functions to create simulation results that can be compared to fluorescence images, in this case by creating a "species" that represents the sum of all GFP tagged species in the the cytosol. In addition, this tutorial shows how to add a time-dependent event to stimulate more production of IP3 after reaching an initial steady state, thus increasing the amount of IP3-PHGFP complex and altering the ratio of membrane vs cytosolic fluorescence. Finally, you will create a spatial geometry using analytic expressions.

Creating the BioModel

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. Cellular structures are defined as mutually exclusive compartments within the cells, as well as the 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 reactions that describe the biochemical behavior of those species within that structure. Keep in mind when developing your model that it must be mapped to a specific cellular geometry before any quantitative simulation or analysis can be performed.



When VCell is open, you will be presented with a new model that contains a single compartment named **c0.** Use the **structure tool** to click within c0 to create a new compartment and membrane. Use the structure tool again to click within the new compartment to create the final compartment and membrane.

Then, choose the **select tool** and click on the innermost compartment. Find where is says **Structure Name** in the **Object Properties** pane below. Change its name to **Nuc** (for nucleus). Then, select the membrane surrounding Nuc and change its name to **NM** (for nuclear membrane). Do the same for the next compartment and enter **Cyt** (for cytosol) as its name, and then enter **PM** (for plasma membrane) as the name of the outer membrane. The outermost compartment's name will become **EC** (for extracellular). Refer to the image on the right for visual guidance.

Creating Species

Select the species tool and click within the PM to create a new aspecies. Click within the PM again to create a second species. Choose the select tool and click one of the new species, and change its name to **PIP2_PM**. Click on the second species and change its name to **PIP2_PHGFP_PM**.

Select the species tool again and click within the Cyt compartment to create another species. Repeat this until there are four species in the Cyt compartment. Choose the select tool and choose one of the three new species. Change its name to **IP3_Cyt**. Choose another species and change its name to **IP3_PHGFP_Cyt.** Select the next species and name it **Stim**. Choose the last species and change its name to **PH_GFP_Cyt**.

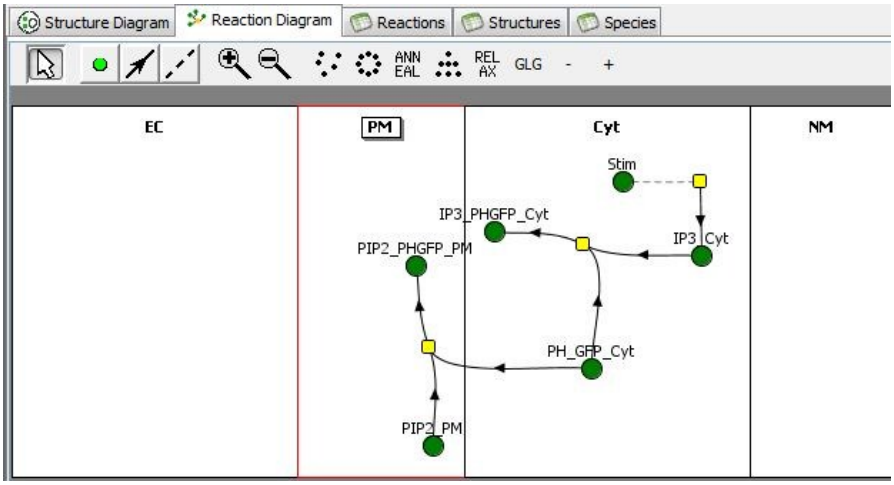
The structure diagram is now complete and it is time to move onto the reaction diagram.

Reaction Diagram

Creating the Reactions

You can view the reaction diagram either by clicking the **Reaction Diagram** tab above the structure diagram or by click-

ing **Reaction Diagram** in the navigation tree located in the upper left quarter of your VCell window.



First, you must reorganize your species. Choose the **select tool** and rearrange the green species so that their positions resemble the species in the right-hand image.

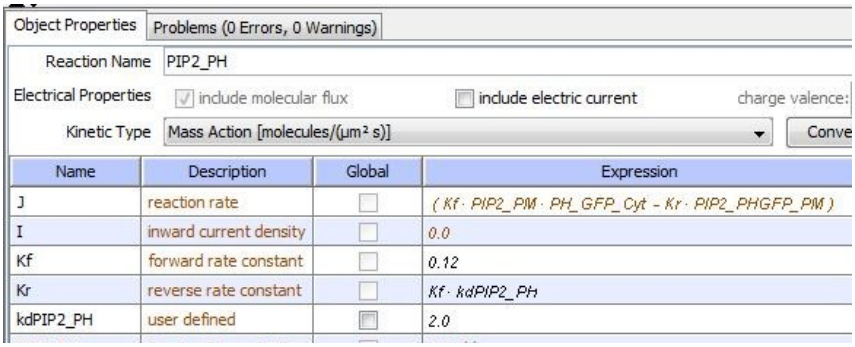
Next, you will create the reactions. Choose the **reaction tool** and click on the **PIP2_PM** species in the PM column. Then drag the line to **PIP2_PHGFP_PM** which is also in the PM column. This will create a reaction icon with PIP2_PM as a reactant and PIP2_PHGFP_PM as the product. To add **PH_GFP_Cyt** as a reactant, click on it and drag a line to the yellow reaction icon you just created.

To create the next reaction, click on **IP3_Cyt** and drag a line to **IP3_PHGFP_Cyt**. To add **PH_GFP_Cyt** to the reaction, click on it and drag a line to the newly created yellow reaction icon.

Lastly, to create the stimulus reaction, click anywhere on the white space in the Cyt Column. Then, drag a line to **IP3_Cyt.** This reaction will serve to stimulate the production of more IP3 once the concentrations reach a steady state for the first time. Select the catalyst tool and click on **Stim** and drag to the yellow reaction icon you just created. Your reaction diagram should look like the above picture.

Defining the Reactions

Now that you have created the reactions, it is time to define what kinetic type they are and at what rates they occur. Click on the first reaction you created, located in the PM Column and turn your attention to the **Object Properties** pane at the bottom.



Find where it says **Reaction Name** and enter **PIP2_PH**. Where it says **Kinetic Type**, select Mass Action [molecules/(µm² s)] from the drop down menu. Then, find where it says **Kf**, the forward rate constant, in the table and enter **0.12** into the box labeled **Expression**. Find where it says **Kr**, the reverse rate constant, and enter **Kf*kdPIP2_PH.** In this case, we define the forward rate constant in terms of the Kd for the reaction. (Note that this makes it easier to change the reaction rate in different simulations, based on assigning a Kd and assuming a diffusion limited reaction.) Enter **2** in the expression box for kdPIP2_PH. Refer to the image on the right if you need more guidance.

Next, click on the reaction where **IP3_Cyt** binds with **PH_GFP_Cyt** to create **IP3_PHGFP_Cyt**. Change this reaction's name to **IP3_PHGFP**. It's kinetic type should be Mass Action [µM/s] (recommended for stochastic application) by default; leave it as this. Find where it says **Kf** and enter **10** in the expression box. Find where it says **Kr** and enter **Kf*kdIP3PH**. Enter the value **0.1** for kdIP3PH.

Finally click on the stimulus reaction with on **IP3_Cyt** as a product. Change its name to **stim** and set its kinetic type to General [µM/s]. Find the row labeled **J** and enter **ksynth*stim** as the expression. Enter **1** in the expression box for

ksynth.

Explanation of Stim Reaction

The stim reaction will be used later to boost the concentration of IP3 in the cytosol after the species have reached a steady state. This will be done by creating an event protocol that turns on the reaction at a specified time (in this case, after a steady state is reached). The event will change stim's value to 1.0, changing the reaction rate from 1*0, or no reaction, to 1*1 for the specified times.

Creating the Steady State Application

You have completed everything necessary for the physiology, and now it is time to create the first application. Find where it says **Applications** in the navigation tree and click on it. Then, find where it says **Add New** and click it. Select **Deterministic** from the drop down menu.

This will create a new application called **Application0**. Right click this and select **Rename** from the drop down menu. Enter **steady state** as the new name.

To the right, you should be able to see the geometry of the application. This is a compartmental application, meaning all of the structures are mapped to a single compartment. We do not need to create any geometry for compartmental applications. We will use the default values for the volumes of the different cellular structures

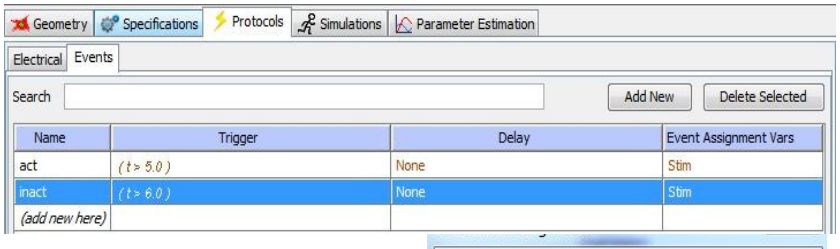
Specifications

Then, find the tab labeled **Specifications** above and click it. The **species** sub-tab should be open by default. This will list all of the species in your model. There should be a column labeled **Initial Condition**. Enter **0.1** in the initial condition box for **IP3_Cyt**. Enter **1.0** as the initial condition for **PH_GFP_Cyt**. Enter **120,000** (without comma) in the initial condition box for **PIP2_PM**. Leave the initial conditions for the other species at the default value 0.

Protocols

Find the **Protocols** tab and click it. The **Events** sub-tab should be open by default. You are going to use this to designate when the stimulus reaction is activated. The reactions in our simulation will reach steady state by 5 seconds, so you can set the event to begin at t>5.0 seconds and last for one second. Within protocols, an event is triggered when a Boolean expression changes from false to true. So to simulate a transient change, you will need two events, one to initiate the new value, and one to return to the original value. The process is explained below.

Find where it says **Add New** and click it. A new event called **Event0** will be created. Rename it **stimstart.** In the object properties pane below, find the line where it says **Trigger** and enter **(t>5.0)**. Then click on **Add Variable** and select **stim** from the drop down menu in the dialog box. Enter **1** in the expression field.

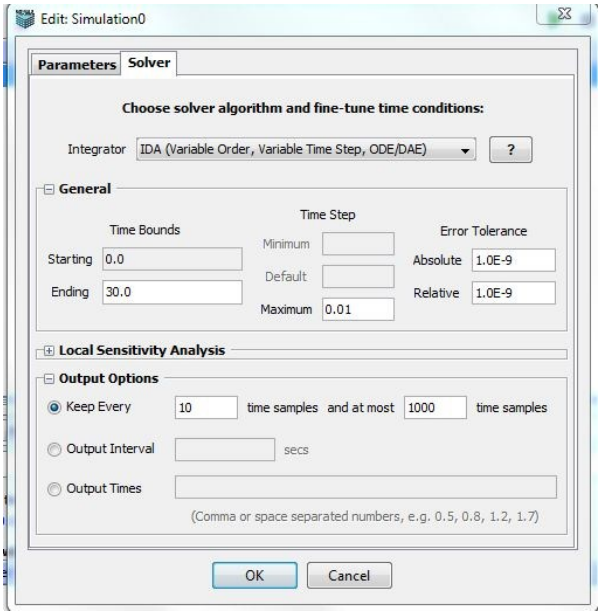


Next, create the second event by selecting **Add New** again. Rename this event **stimend**. In the object properties pane below, enter **(t>6.0)** as the **Trigger**. Then, click on **Add Variable** and select **stim** from the drop down menu and enter **0** in the expression field.

Running the Simulation

Click the **Simulation** tab (to the right of specification tab). Select the **New Simulation Tool**  to create a new simulation. You can rename it by double clicking in the name column and then entering the name you wish to give it, or leave it as the default name simulation0.

Then, with the simulation highlighted in blue, select the **Edit Simulation Tool** to open the edit simulation dialog box.



You will be presented with both a **Parameters** tab and a **Solver** tab. We can leave the parameter values as their default values and head to the solver tab. Find where it says **Integrator** and choose IDA (Variable Order, Variable Time Step, ODE/DAE) from the drop down menu.

Under **General**, set the ending time bound to **30** seconds. Under **Time Step** set **Maximum** to **0.01**.

Under **Output Options** select the **Keep Every** radio button. Enter **10** and **1000** into the left and right boxes, respectively.

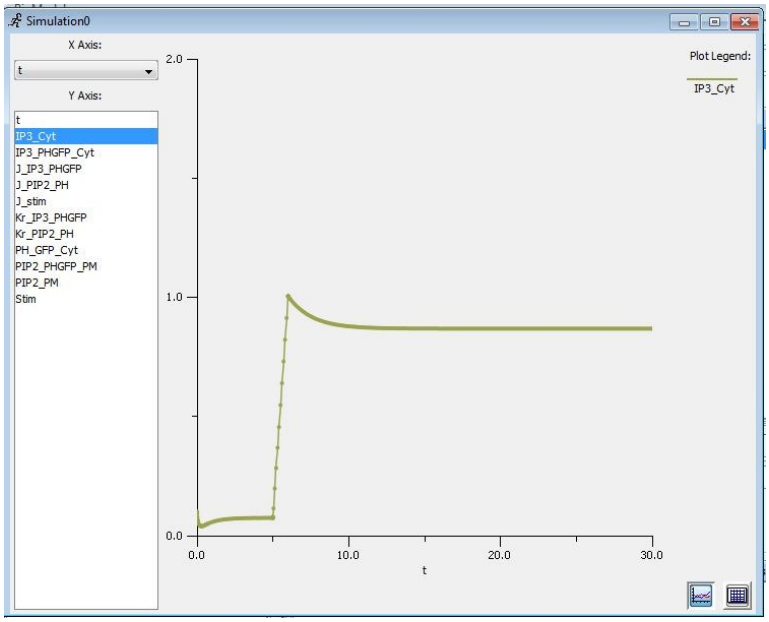
Press **OK** to finalize your settings and close the dialog box.

Then, with the simulation highlighted in blue, select the **run and save simulation tool** to run your simulation.

When your simulation is completed, select the **view results tool** to open the results dialog box.

Viewing the Results

You are going to view the results of this simulation to discover when a steady state is achieved, or when the concentration ceases changing and to determine the amount of change in IP3_cyt concentration caused by the stim reaction.



With **IP3_Cyt** selected, your results should resemble the image to the right. Notice how the concentration of **IP3_Cyt** reaches a steady state value as t approaches 5 seconds. Then, notice how the concentration of **IP3_Cyt** increases as the stimulus is activated and the reaction begins producing **IP3_Cyt**. Then, a second steady state is reached as time continues, this time at a much higher concentration than before.

Creating the Spatial Application

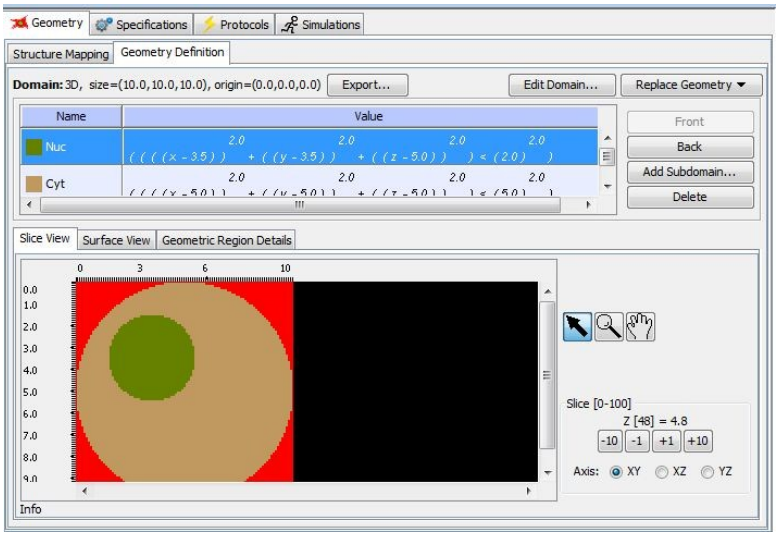
Now, you will observe steady state changes in a three dimensional spatial application. Click on **Applications** in the navigation tree panel and then click on **Add New** and choose **Deterministic** from the drop down menu. Right click the new application and select **Rename** and enter the name **Spatial.**

With the application created, it is time to create the geometry.

Creating the Geometry

Click on the **Geometry** tab and then select the **Geometry Definition** sub-tab. There should be a button labeled **Add Geometry** on the right. Click it and select **New** from the drop down menu. A dialog box will appear asking which type of geometry you would wish to use. Choose **Analytic Equations (3D)** and then press OK. Double click in the box labeled

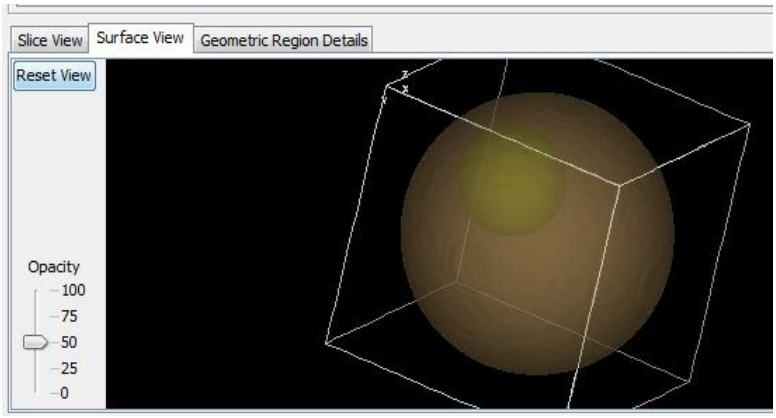
subdomain0 and rename it **EC** for extracellular.



Then, click on the **Add Subdomain** button and select **Analytic** from the drop down menu. A dialog box will appear. Where it says **Select Subdomain Shape**, choose **Sphere** from the drop down menu. Enter 5,5,5 for its center point and 5 for its radius. Click **Add New Subdomain** to add the sphere to the geometry and close the dialog box. This will represent the cytosol, so rename the subdomain **Cyt.**

You will not be able to see the Cyt subdomain initially. To the right of the slice view, you should see a section under **Slice [0-100]** that contains four buttons: **-10, -1, +1, +10**. Click the **+10** button until the Cyt region is almost touching the perimeter of the red EC subdomain. You can use the –**1** and **+1** to fine tune which slice you want. This is merely for visual purposes, and the slice you are on will not affect the computations in the application.

Next, you must create the nuclear region. Click **Add New Subdomain** again, select **Analytic** from the drop down menu, and select sphere for the shape. Enter 3.5,3.5,5 for the center point and then 2 for the radius. Then, click **Add New Subdomain** to add it to the geometry and close the dialog box.



You may wish to view the geometry in **Surface View**, located above the display and to the immediate right of the **Slice View** tab. Surface View allows you to view your application in 3D. You can lower the opacity of the geometry to better see the nucleus.

Structure Mapping

Now, you must map the compartments from the physiology to the subdomains in the geometry. To do this, select the line tool ／ and then click on EC under **Physiology (structures)** and drag to the red EC box under **Geometry (subdomains)**. Then, click on Cyt in Physiology and drag to the tan Cyt box in Geometry. Lastly, click on Nuc in Physiology and drag to the green Nuc box in Geometry.

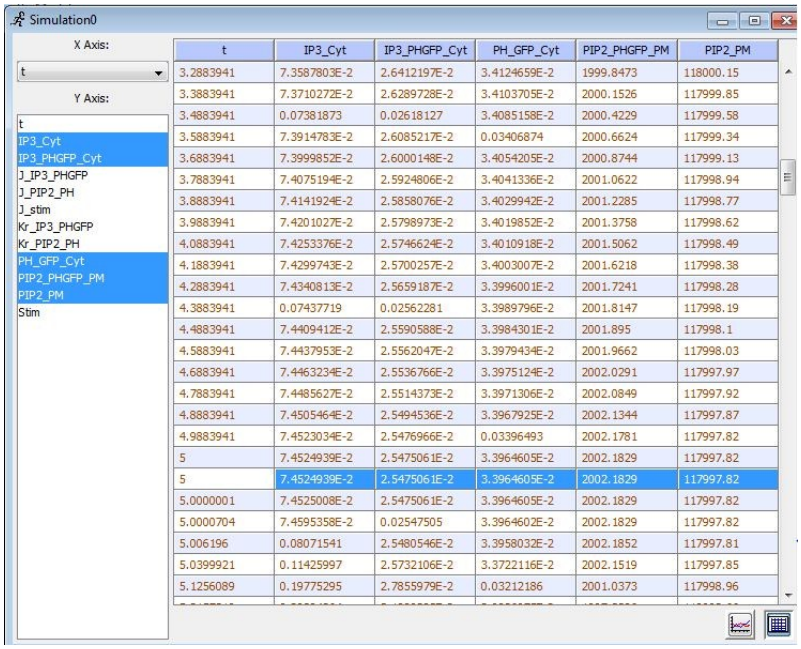
Specifications

Now, you will set the initial concentrations of the species. You already know that the species' concentrations reach a steady state at about 5 seconds, so you will copy the **steady state** simulation results at time t=5 seconds and use those as the initial conditions for this application.

Click on the **Steady State** application in the navigation tree panel. Click on the **Simulations** tab and then click on **view results** . Switch from plot view to table view by click the **data spreadsheet tool** . Select the species **IP3_Cyt, PH_GFP_Cyt, IP3_PHGFP_Cyt, PIP2_PM,** and **PIP2_PHGFP_PM**. Scroll down the table until you see a date for the time at 5 seconds or close to 5 seconds (since the species have reached steady state, their concentrations are the same at multiple time steps before 5 seconds).

Highlight the concentrations at that row and right click. Select **Copy**. Then, return to the **Spatial** application and click on the **Specifications** tab. Highlight everything using ctrl+a and then right click. Select **Paste**. A dialog box will appear

prompting you to select which concentrations you wish to copy over. Choose **Select All** and then press **OK**.



t	IP3_Cyt	IP3_PHGFP_Cyt	PH_GFP_Cyt	PIP2_PHGFP_PM	PIP2_PM
3.2883941	7.3587803E-2	2.6412197E-2	3.4124659E-2	1999.8473	118000.15
3.3883941	7.3710272E-2	2.6289728E-2	3.4103705E-2	2000.1526	117999.85
3.4883941	0.07381873	0.02618127	3.4085158E-2	2000.4229	117999.58
3.5883941	7.3914783E-2	2.6085217E-2	0.03406874	2000.6624	117999.34
3.6883941	7.3999852E-2	2.6000148E-2	3.4054205E-2	2000.8744	117999.13
3.7883941	7.4075194E-2	2.5924806E-2	3.4041336E-2	2001.0622	117998.94
3.8883941	7.4141924E-2	2.5858076E-2	3.4029942E-2	2001.2285	117998.77
3.9883941	7.4201027E-2	2.5798973E-2	3.4019852E-2	2001.3758	117998.62
4.0883941	7.4253376E-2	2.5746624E-2	3.4010918E-2	2001.5062	117998.49
4.1883941	7.4299743E-2	2.5700257E-2	3.4003007E-2	2001.6218	117998.38
4.2883941	7.4340813E-2	2.5659187E-2	3.3996001E-2	2001.7241	117998.28
4.3883941	0.07437719	0.02562281	3.3989796E-2	2001.8147	117998.19
4.4883941	7.4409412E-2	2.5590588E-2	3.3984301E-2	2001.895	117998.1
4.5883941	7.4437953E-2	2.5562047E-2	3.3979434E-2	2001.9662	117998.03
4.6883941	7.4463234E-2	2.5536766E-2	3.3975124E-2	2002.0291	117997.97
4.7883941	7.4485627E-2	2.5514373E-2	3.3971306E-2	2002.0849	117997.92
4.8883941	7.4505464E-2	2.5494536E-2	3.3967925E-2	2002.1344	117997.87
4.9883941	7.4523034E-2	2.5476966E-2	0.03396493	2002.1781	117997.82
5	7.4524939E-2	2.5475061E-2	3.3964605E-2	2002.1829	117997.82
5	7.4524939E-2	2.5475061E-2	3.3964605E-2	2002.1829	117997.82
5.0000001	7.4525008E-2	2.5475061E-2	3.3964605E-2	2002.1829	117997.82
5.0000704	7.4595358E-2	0.02547505	3.3964602E-2	2002.1829	117997.82
5.006196	0.08071541	2.5480546E-2	3.3958032E-2	2002.1852	117997.81
5.0399921	0.11425997	2.5732106E-2	3.3722116E-2	2002.1519	117997.85
5.1256089	0.19775295	2.7855979E-2	0.03212186	2001.0373	117998.96

Now, you must set the initial condition for **Stim**. First, check the box marked **Clamped**. This causes VCell to treat Stim as a constant, and not a variable, which allows you to create a Boolean expression for when it is activated. Enter the Boolean into the field for **Initial Condition**:

0+1*((t>5.0)&&(t<6.0))

This expression causes the concentration of Stim to be 0 at all times except from 5 seconds to 6 seconds, when its concentration will be 1. This changes the Stim reaction, **ksynth*Stim**, from 1*0 to 1*1, activating the synthesis reaction and production of IP3.

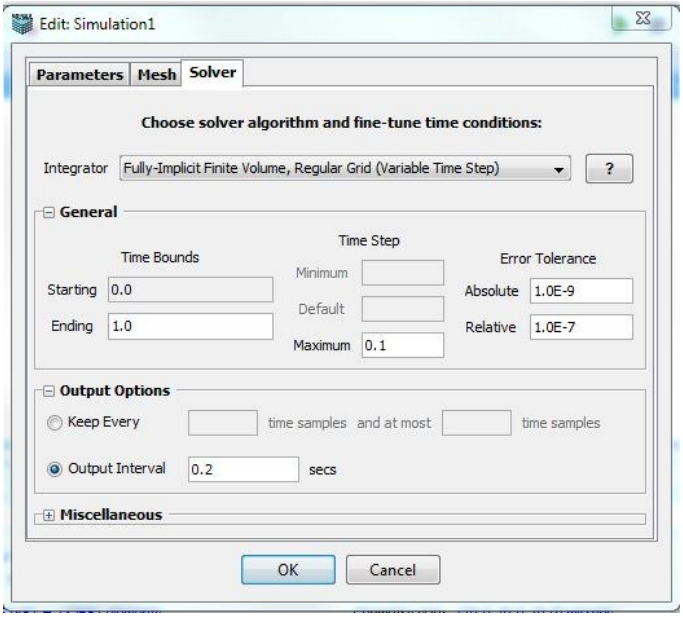
The **Diffusion Constants** should be the following:

Name	Diffusion Constant
IP3_Cyt	10
IP3_PHGFP_Cyt	10
PH_GFP_Cyt	10
PIP2_PM	0.1
PIP2_PHGFP_PM	0.1

Creating the Simulation

Click on the **Simulations** tab. Create a new simulation using the **Add New Simulation** tool and then click on **Edit Simulation** . You will be taken to a dialog box with **Parameters**, **Mesh**, and **Solver** tabs. You can leave the parameters as their defaults and head to the **Mesh** tab. Make sure **Lock Aspect Ratio** is checked and then change the value for X to **31.** Y and Z should change accordingly (also to 31).

Click on the **Solver Tab**. The default integrator should be Fully-Implicit Finite Volume, Regular Grid (Variable Time Step) . Leave it as this. Change the **Ending Time Bound** to 20 seconds. Leave the **Time Step** as 0.1 and do not change the **Error Tolerance** values. Change the **Output Interval** to 0.2 seconds.



Click **OK** to finalize your settings and close the dialog box.

Next, we must create a function. The purpose of this function is to better represent fluorescence in the model. VCell reports each species' concentration separately in the results, but when viewing the model under a real life microscope, you would see all fluorescence. This function will allow you to add the concentrations of the different fluorescent species in your application and report them as a single concentration throughout the cell.

Creating the Function

Click on the **Output Functions** sub-tab. Click the **Add Function** button. A dialog box will appear prompting you to name the function and give it an expression. Enter **Fluorescence** as the name and enter the following as the expression:

IP3_PHGP_Cyt+PH_GFP_Cyt

Click the **Next>>** button to specify which compartment the function applies too. The default compartment should be Cyt. Leave it as this. Click **Finish** to create your function and close the dialog box. Head back to the **Simulations** sub-tab.

Click **Run and Save Simulation**  to start your simulation.

View Results

Once your simulation has completed running, you may click the **View Results tool**  . The concentrations of your species as well as your fluorescence function will be available to display. Click the **Point tool** to select point(s) you wish to view the concentrations of and then click **Plot** at the bottom of the results window.

Notice how the concentration of the species begins at a steady because you pasted the steady values from the previous application as the initial conditions for this spatial application. At time t=5 seconds, you can see the activation of stim and the increase of IP3 concentration. You can also see how the new steady state is reached as t progresses beyond 10 seconds.

The time plot is also useful for finding the maximum and minimum values of species' individual concentrations. This can be used to adjust the min and max of the **Data Range** (top right of the results window). You may wish to do this so the colors more accurately display gradients.