

Dynamic Virtual Analyzer and Simulator

A quick start guide v1.2

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About D-VASim

D-VASim stands for Dynamic Virtual Analyzer and Simulator. It is developed to analyze and simulate the genetic logic circuit models developed in the Systems Biology Markup Language (SBML) [1]. Practically, D-VASim can be used to simulate any bio-model available in SBML L3v1 (Level 3 version 1) core; however, it is solely designed for the simulations of genetic logic circuits, where user can apply external signals on the model during runtime.

D-VASim is developed on graphical programming language platform called LabVIEW (Laboratory Virtual Instrument Engineering Workbench) [2]. Besides graphical programming, textual programming language, like JAVA, is also used to integrate JSBML [3] library in it.

In this short document, you will learn how to use D-VASim (v1.2) to perform virtual laboratory experiments. This requires you to have pre-synthesized bio-model, in the form of SBML file, you want to test. This SBML can be obtained by creating a bio-model using any other bio-tool and generating its SBML L3v1 format.

Stochastic simulation algorithm (SSA) has been implemented to perform stochastic simulations of SBML models. Furthermore, D-VASim is also capable of simulating the deterministic behavior of a bio model by solving ordinary differential equations.

Latest version of D-VASim can be downloaded from <http://bda.compute.dtu.dk/downloads/>. The sample SBML models, included in the download package, are developed at the University of Utah by Myer's group [4]. The video demo of D-VASim can also be seen at <http://bda.compute.dtu.dk/user-manuals/>, which demonstrates the analysis of upper threshold value only. In D-VASim v1.2, the ability to analyze the upper and lower threshold values is included.

Conventions

The following conventions appear in this document:



This icon denotes a tip, which notifies you to advisory information.



This icon denotes an alert, which notifies you to important information.

bold

Bold text denotes items that you must select or click or enter the value in the software, such as open file option or running the simulation button or entering the value of simulation speed etc.

italic

Italic text denotes the name of a folder or a file path.

bold and italic

Bold and italic text denotes the name of a file.

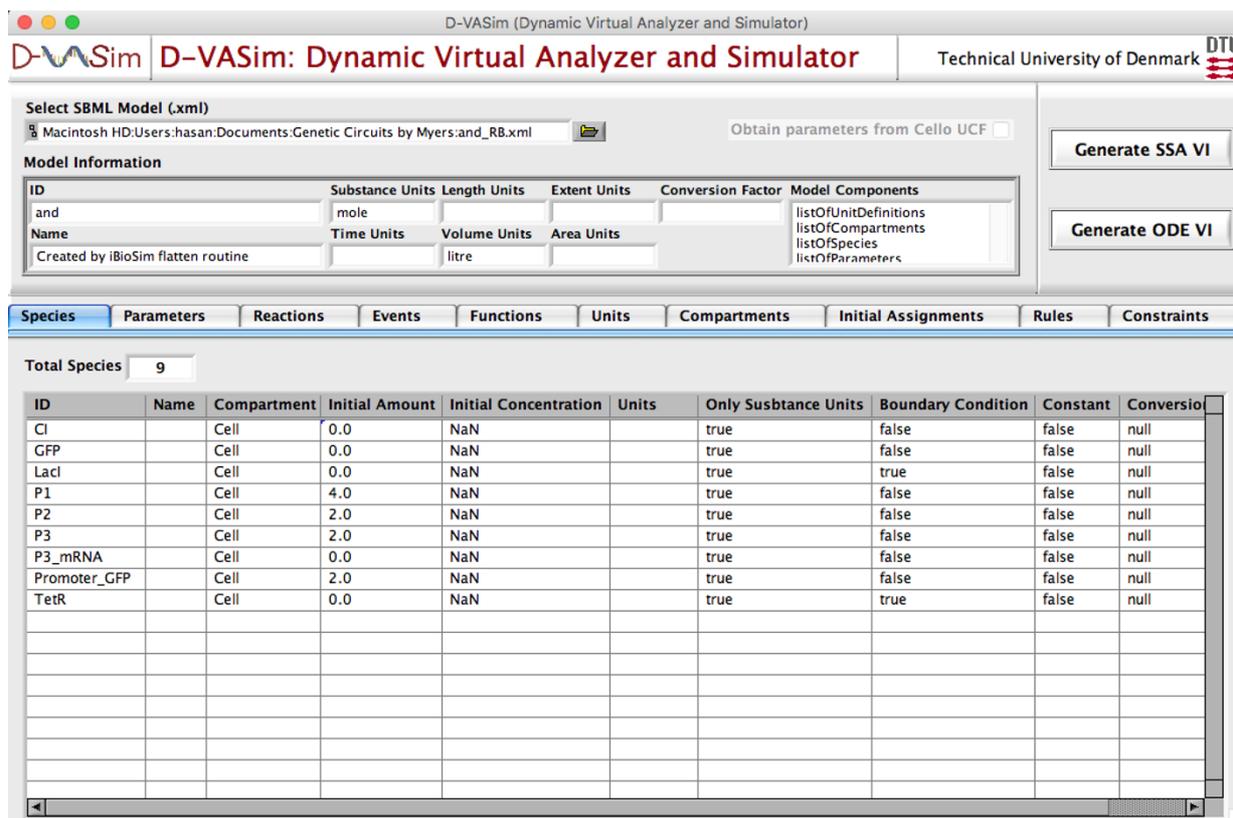


Figure 2. D-VASim showing the SBML components of a genetic AND gate model.

All the SBML components of a model can be analyzed here in a clean tabular form. For example, The **Specie** tab shown in Figure 2 depicts the total number of specie used in the genetic AND gate model along with their corresponding details. Similarly, the **Reactions** tab shown in Figure 3 contains the information related to the reaction kinetics of a model. D-VASim also generates the ordinary differential equations of a model, which can be seen in Figure 3. These equations are used to simulate the deterministic behavior of SBML model.

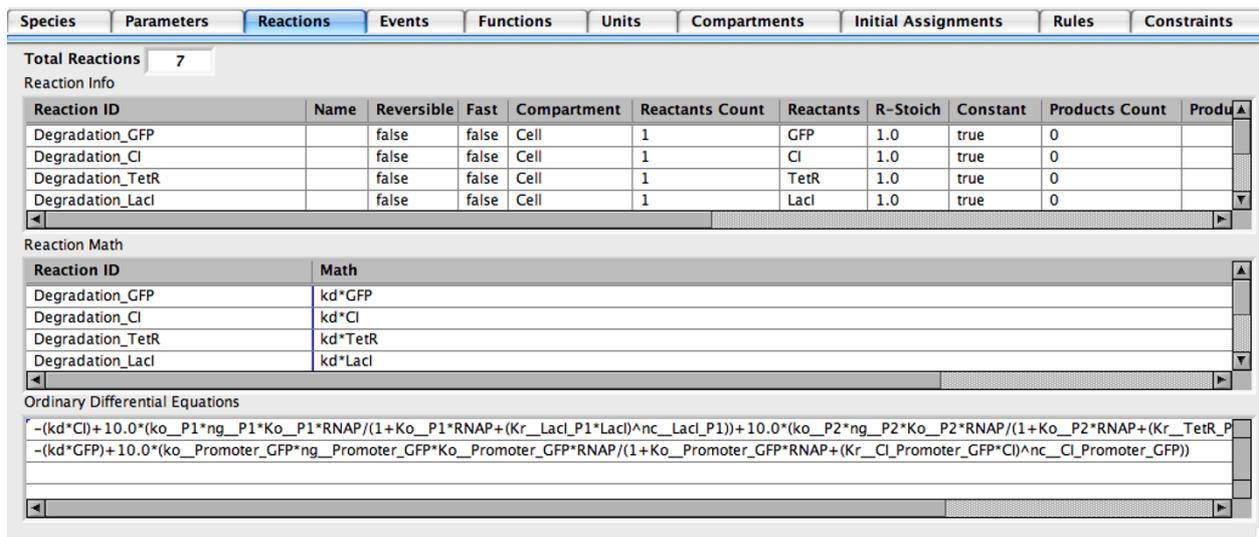


Figure 3. Reactions tab showing the reaction kinetics of a genetic AND gate model.

1.2. Generating a Virtual Laboratory Instrument

Once the components of SBML model are analyzed, a virtual environment for the interactive simulation can be generated simply by clicking on any of the two options, **Generate SSA VI** or **Generate ODE VI** shown at the top right corner of a tool. Now click **Generate SSA VI** button to generate a virtual instrument (VI) for stochastic simulations of a genetic AND gate model. This will bring up a virtual instrument shown in Figure 4.

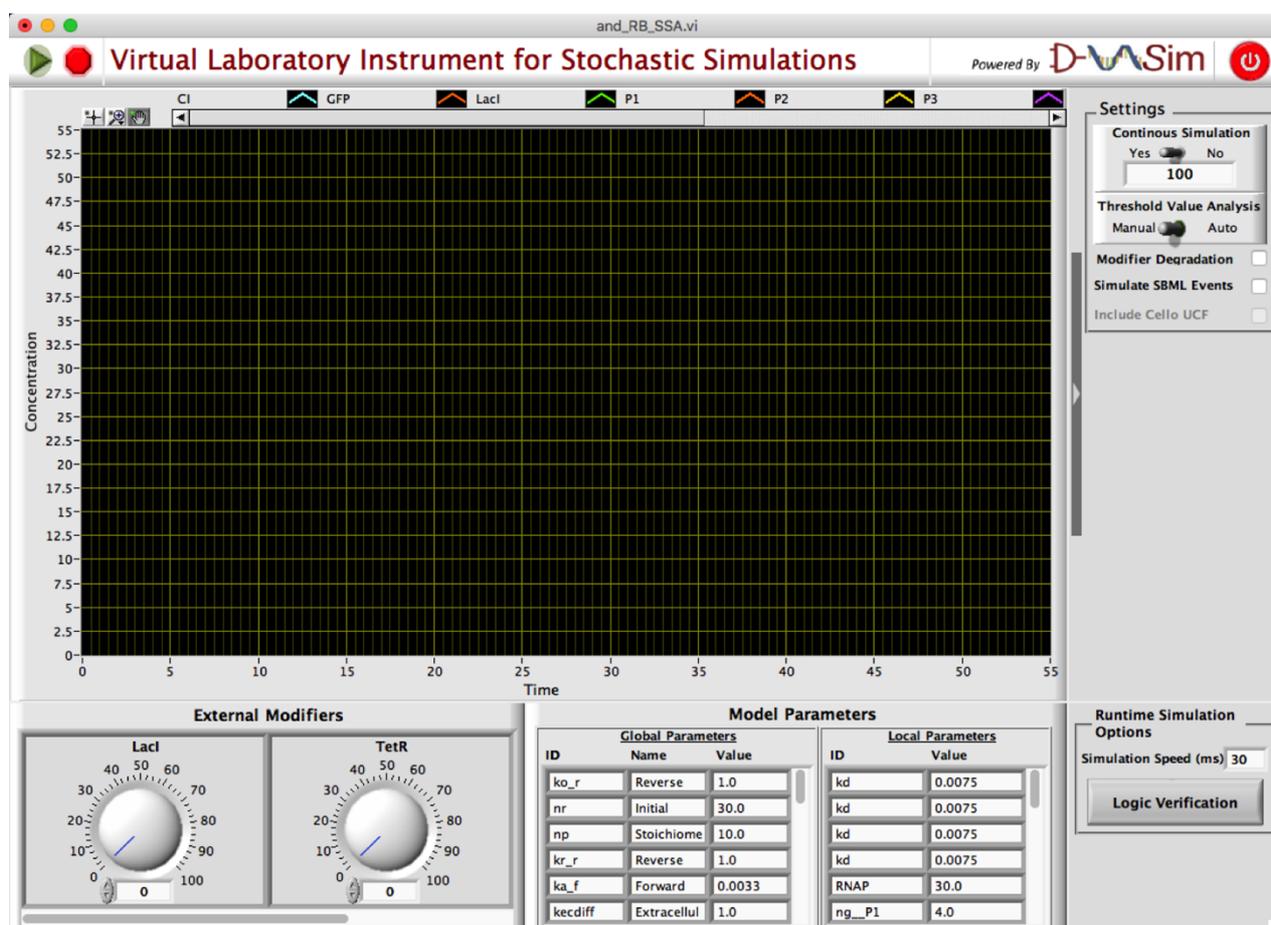


Figure 4. Virtual Instrument specifically for a genetic AND gate model.

Any instrument for stochastic simulation generated by D-VASim for the first time does neither reveal the names of graph legend nor the modifier controls, but only the parameters information. Pressing the **RUN** button (▶ shown at the top left corner) starts the simulation and makes the graph legends and modifiers visible.

2. Virtual Experimentation for Stochastic Simulations

The virtual instrument (shown in Figure 4) looks similar to a physical instrument, which can be used to interact with the model by tuning the input concentrations with the control knobs and observing the effects graphically.

Once the simulation is started after pressing the  button, user can increase or decrease the concentration of input (or external modifier) specie and observe its effects on the model during run time. This runtime interaction with the model gives user an insight of being in the lab performing wet-lab experiments.

Figure 5 shows the screenshot of the stochastic simulation of a genetic AND gate taken randomly after ~ 3050 time units has lapsed. This figure shows that unlike SBML events, which are predefined in the SBML file, user can interact with the model and change the concentration of input specie to any level and at any instant of time. In case if the concentration of input specie are required to be triggered to any specific level instantly, the numeric displays located beneath the control knobs can be used. As same as the user interact with the model by varying the input concentration using control knobs, the parameters can also be varied and their effects can be observed graphically during runtime.

When a **STOP** button ( located besides the run button at the top left corner) is pressed, screenshot of a graphical window is captured and saved inside *Sim_Results* folder located in the default application directory.

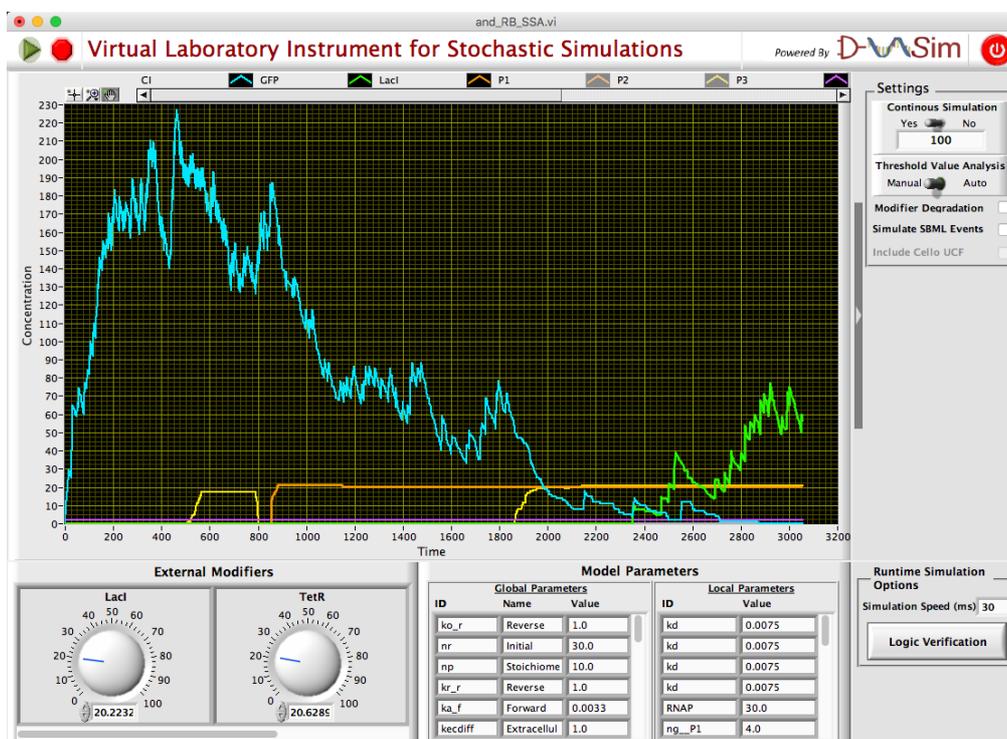


Figure 5. Screen capture of the stochastic simulation of a genetic AND gate.

Each image is saved by the following naming convention; “<model-name>_SSA_Screenshot.bmp”, where <model-name> is the name of a model. For example, the screenshot of a graphical window of a genetic AND gate model is saved as **and_RB_SSA_Screenshot.bmp**. The simulation traces of the entire session are also saved in the *Sim_Results* folder with the convention “<model-name>_SSADatalog.xls”. Pressing the **RUN** button again starts the simulation from the beginning.



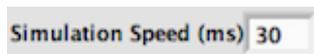
Alert – When the **STOP** button is pressed, the captured screenshot and the data log file overwrites any previously saved files of the same model.



Tip – The properties of graphical plots, including colors, can be changed by a left mouse click on the color of graph legend and selecting the desired menu.

2.1. Runtime Simulation Options

There are two runtime simulation options, available in D-VASim v1.2, shown at the bottom right corner of a VI in Figure 5. These two options are described below:



1. **Simulation Speed:** This option allows you to speed up or slow down the simulation by entering the numerical value in milliseconds.
2. **Logic Verification:** Obtain the Boolean logic exist in the model. Clicking this button prompts the user to input the threshold values of input and output specie, as shown in [Figure 8](#). When automatic [threshold value analysis](#) is performed, this box is auto-populated with the estimated threshold value. This option is disabled when the simulation is not running.



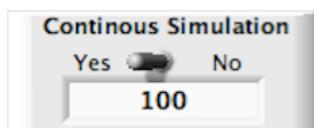
Alert – Make sure to apply all the possible input combinations each after the correct propagation delays. The results of boolean logic analysis will otherwise be incorrect.



Alert – Though, D-VASim v1.2 supports the logic verification of n-input genetic logic circuits, but the logic verification algorithm is tested for up to 3-input genetic circuits only. It is because the models for more complex circuits are not available.

2.2. Simulation Settings

The simulation settings are shown in the right sidebar, which contains the following five simulation options.



1. **Continuous Simulation:** User may specify here if they want to perform continuous simulation or for a specific interval of time. If switched to “No”, the box below can be used to specify the simulation time for which user wants to run the simulation. The default value of this switch is “Yes” meaning that the continuous simulation is always performed unless specified.

Modifier Degradation

2. **Modifier Degradation:** When checked, perform more realistic simulation by allowing the input concentrations to degrade over time.

Simulate SBML Events

3. **Simulate SBML Event:** When checked, simulate the events described in SBML file.



Tip – The triggering time and the assignment value of SBML events can be changed during run time until the time of event occurs. For example, if the triggering time of event is 1000 time units, the trigger time and the assignment value can be changed before 1000 time units has lapsed.

Threshold Value Analysis
Manual Auto

4. **Threshold Value Analysis:**

Manual – In this mode, VI assume that the threshold value of input specie and the propagation delays are already known.

Auto – When this mode is selected, VI estimates the threshold value based on the setting tab shown in Figure 6 below.

- **Start at** – Specify the initial concentration of input specie from which the analysis is required to be started. It is recommended to keep this value 0 for correct results.
- **Increment of** – This control allow you to set up the concentration value, which the tool increments during each iteration for checking threshold concentration.

For example: If you set

Start at: 0

Increment of: 5

Stop at: 15

Tool will check the threshold concentration for 4 different levels of concentration. i.e. 0 --> 5 --> 10 --> 15, for all possible input combinations.

Figure 6. Settings dialogue for automatic threshold value and propagation delay analyses.

- **Stop at** – Specify the concentration of inputs at which the threshold analysis should be stopped.
- **Assumed Time Delay** – Specify initially assumed time delay.

- **No. of iterations** – For how many times a user wants to verify if the estimated threshold value is consistent.
- **Verification RT** – For how long a user wants to verify the estimated threshold value for each no. of iterations mentioned above.
- **Settling Time** – It is also an assumed value, which specifies the time required by a model to get stable. During verification process, D-VASim triggers the concentration of inputs to the estimated threshold level once this time value has elapsed. Thus the verification starts once the input is triggered to its estimated threshold value and therefore it should run for enough amount of time to measure the propagation delay as well as the correctness of an estimated threshold value. Depending on the complexity of input-output stage, the value of “Verification RT” is suggested to be at least 3-4 times higher than the value of *Settling Time*.
- **Name of Output** – D-VASim now allows user to perform the threshold value and propagation delay analysis either for an entire circuit or for the intermediate circuit components. In this field, user can specify the name of output specie. The timing analysis is performed between the input specie, selected as control knobs (see section 2.2) and the output specie specified in this field.
- **% Acceptance of consistency** – Specify the minimum percentage of threshold value consistency a user wants.
Th High: If the chosen value is 90 for the AND gate genetic circuit, the tool give the results only when the average output is found to be 90% greater than the estimated threshold value.
Th Low: If the chosen value is 40 for the AND gate genetic circuit, the tool estimates the threshold value to be lower level when the size of an average output data above the estimated threshold value is found to be less than 40%.



Alert – It is an indication of wrong parameter selection if either tool takes too long to estimate the results or produce incorrect results. In this case user should consider increasing the value of “Verification RT” further higher (beyond 3-4 times) than the value of “Settling Time”. Though assuming large values of these parameters, including “Assumed Time Delay”, may increase the estimation time, but gives the better estimation.

When the tool finish estimating the threshold value with the desired minimum percentage of consistency, the results are displayed in the dialogue box as shown in Figure 7. The value of estimated propagation delay is the average value over all “No. of iterations” specified in the settings dialogue (Figure 6). The value enclosed in braces after the average estimated propagation delay specifies the standard deviation. In this figure, the output consistency for upper threshold level is found to be 100 % and that of lower threshold level is 23.1%.

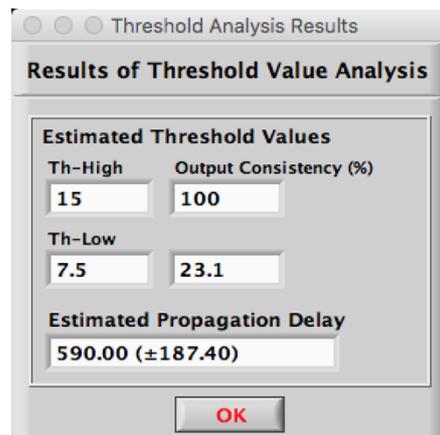


Figure 7. Dialogue box indicating the estimated results of threshold value and propagation delay analysis.

The results are stored in the current application directory with the following naming convention; “<model-name>_SSA-TP-Analysis.txt”, where <model-name> is the name of a model. For the case of genetic AND gate, the results will be saved as *and_RB_SSA-TP-Analysis.txt*.

User can now perform the runtime interactive simulations and perform logic analysis, by pressing the [Logic Verification](#) button shown in [section 2.1](#). It will pop up a window (Figure 8) where user can specify the upper threshold values of a circuit. As mentioned before, if the automatic threshold value analysis is performed, the list of threshold values is auto populated as shown in Figure 8. When all the threshold values are entered, a new window (Figure 9) pops up indicating the logic function, in terms of a Boolean expression, extracted from the genetic logic circuit model. This window also contains the entire simulation data as analog data points and their corresponding digital data according to the defined threshold values. If all the input combinations are applied correctly during the simulation, a message in green text (Figure 9 (a)) will appear showing the percentage fitness of the boolean expression in the acquired simulation data. If all the possible input combinations are not applied, a boolean expression may appear with the message in red text (Figure 9 (b)) indicating that all input combinations are not applied correctly. Boolean expression box may also appear blank when all the input combinations are not applied correctly. A correct set of input combinations is the one in which all the possible input combinations are applied each after the minimum propagation delay.

D-VASim also indicates the time, in seconds, required to estimate the boolean logic from the analog simulation data. This estimation time depends on the size of the data as well as on the number of inputs of a circuit.

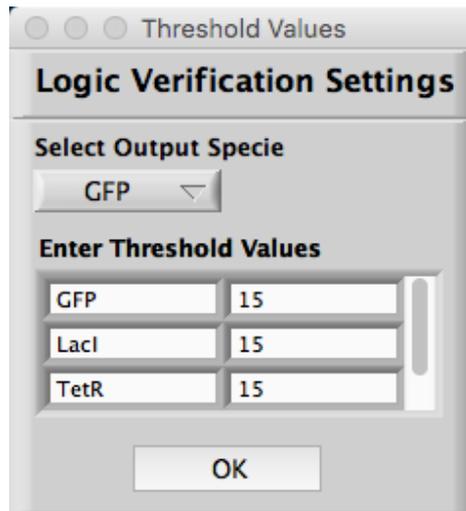
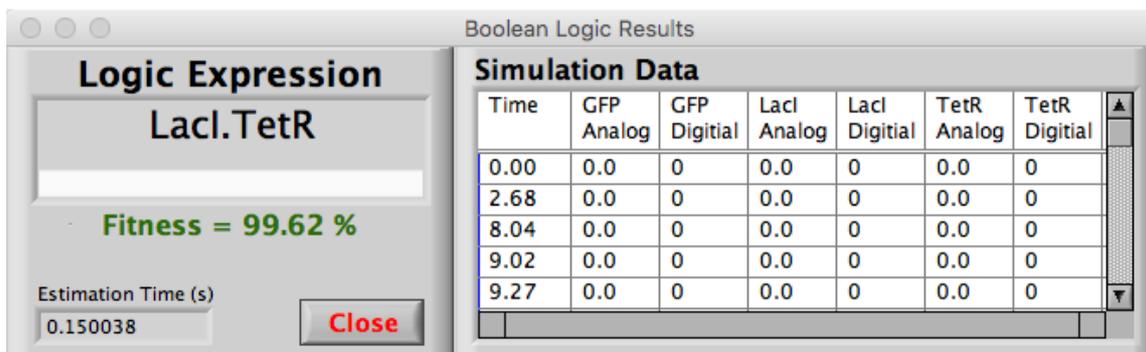
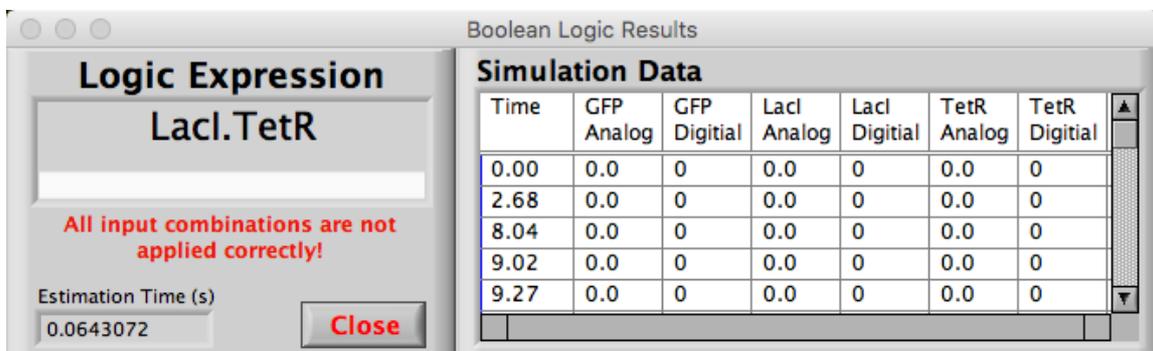


Figure 8. Dialogue box to set up the threshold values for Boolean logic analysis.



(a)



(b)

Figure 9. Boolean logic extracted from the analog simulation data along with its corresponding digital data.

(a) Percentage fitness appears when all the input combinations are properly applied.

(b) Error message appears when all the input combinations are not applied correctly before trying to estimate the boolean logic of a circuit.

2.3. Additional features of Stochastic Simulation in D-VASim

In D-VASim v1.1, two new features have been introduced, which allows user to:

- 1) Create control knobs of specie manually. This not only helps user to vary the concentration of selected specie during runtime, but also perform the timing analysis between these specie and the specified output.
- 2) View the mixed signal waveforms (digital and analog) for better timing analysis.

The control knobs for the input specie can be created by dropping down the **Options** menu and selecting **External Inputs** as shown in Figure 10(a). When **External Inputs** is selected, a pop-up window is appeared as shown in Figure 10(b). The user can drag the specie name from left-hand box to right-hand box in order to create its control knob. In Figure 10(b), LacI and TetR specie are selected whose control knobs are created as shown in Figure 4. These control knobs can only be created when the simulation is not running.

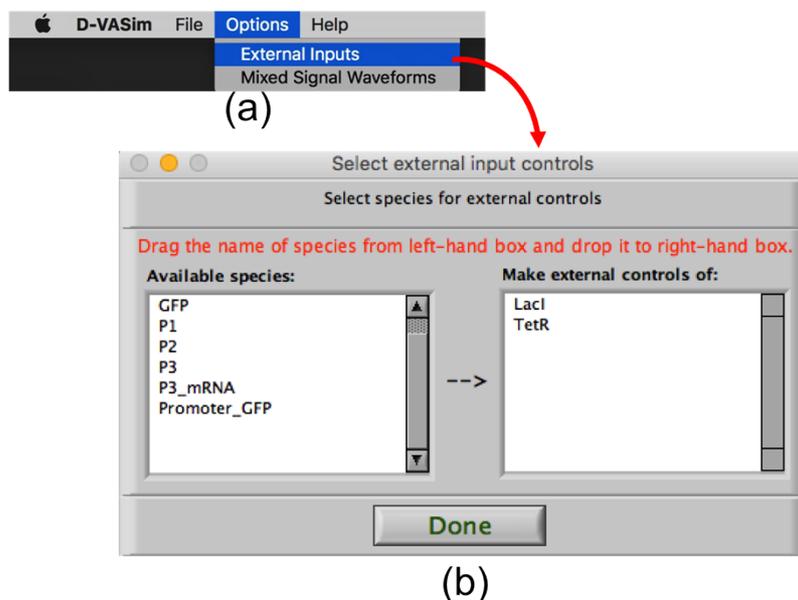


Figure 10. Process of creating control knobs for specie. (a) Select **External Inputs** from **Options** dropdown menu. (b) Drag the name of specie from left-hand to right-hand box to create its control knob.



Figure 11. Mixed signal waveforms of genetic AND gate.

Similarly, after performing interactive stochastic simulation of a model in the D-VASim virtual laboratory environment, the mixed signal waveforms for timing analysis can be viewed by selecting **Mixed Signal Waveforms** option from the **Options** dropdown menu. Figure 11 shows the window of mixed signal waveforms generated for the simulation traces of genetic AND gate shown in Figure 5. This figure shows the analog and digital traces, in parallel, for genetic AND gate. The digital curves are generated based on the estimated threshold value shown in Figure 7. This value is automatically extracted if the auto threshold value analysis is performed, otherwise user can enter the threshold value and **Reset** the curves. **Capture Graph** button takes the screen-shot of waveforms and save it inside *Sim_Results* folder under the default application directory. Each image is saved by the file name **MixedSignalWaveforms.bmp**. The blank vertical box shown at the right-hand side in Figure 11 allows user to create cursors (see section 4 for more details about how to create cursors).



Alert – When the **Capture Graph** button is pressed, the captured screenshot overwrites any previously saved files of the same model.



Tip – The properties of graphical plots, including colors, can be changed by a left mouse click on the color of graph legend and selecting the desired menu.

3. Virtual Experimentation for ODE Simulations

Similar to generating virtual instrument for stochastic simulations, the virtual instrument for deterministic simulations can also be generated by clicking on **Generate ODE VI** button depicting in Figure 2.

When the  button is pressed, the deterministic simulation results of a genetic AND gate for 6000 time units are appeared as shown in Figure 12. As same as in the case of stochastic simulation, user can change the concentration of external input specie using control knobs for deterministic simulation also. However, every single change in the input concentration or parameters results in the execution of selected simulation algorithm for the defined interval of time and plots the new set of curves.

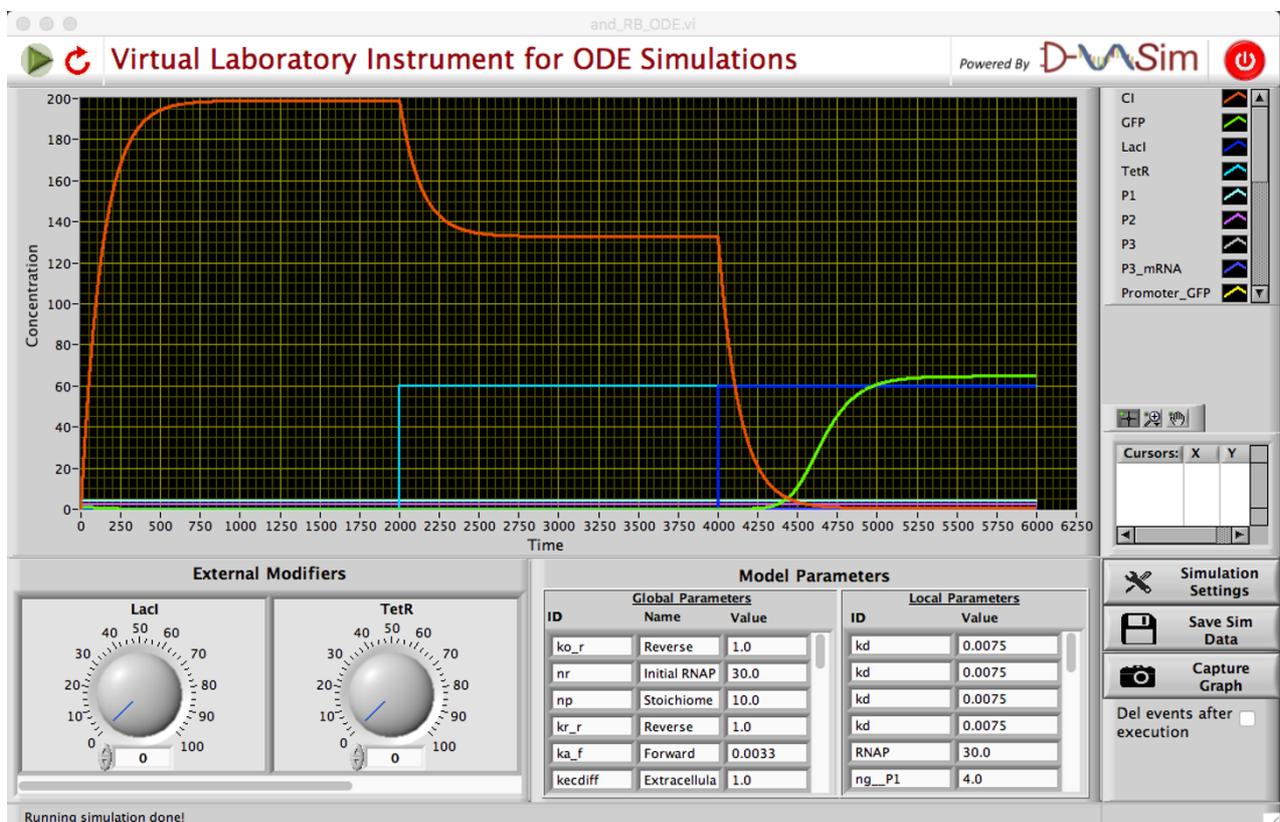


Figure 12. Screen capture of the deterministic simulation of a genetic AND gate.

The horizontal *status bar* is shown at the bottom in Figure 12. It shows the status of the actions being performed in this VI for ODE simulations. For example, pressing the run button shows the status of simulation as shown in Figure 13 below. The progress bar shown at right-hand side depicts the percentage of results currently being obtained. The plot is visible once the progress bar reaches to 100 %.



Figure 13. Screen capture of the status bar showing that the VI is running simulation. The progress bar shown at right-hand side indicates that the 65% of simulation data is obtained.

When a **RESET** button (🔄) button is pressed, screenshot of a graph window and the simulation data is captured and saved under *Sim_Results* folder located in the default application directory. Each image is saved by the following naming convention; “<model-name>_ODE_Screenshot.bmp”, where <model-name> is the name of a model. For example, the screenshot of a graphical window of a genetic AND gate model is saved as *and_RB_ODE_Screenshot.bmp*. The simulation traces of the entire session are saved with the naming convention; “<model-name>_ODEDataLog.xls”. Pressing the **RUN** button again starts the simulation from the beginning.

Alternatively, the simulation data and the graphical plots can be stored by pressing the buttons shown in Figure 14(a) and 14(b) respectively.



Figure 14. Available options in ODE VI to (a) Save simulation data. (b) Capture graph window.



Alert – When the **RESET** or **Save Sim Data** or **Capture Graph** button is pressed, the captured screenshot and the data log file overwrites any previously saved files of the same model.



Tip – The properties of graphical plots, including colors, can be changed by a left mouse click on the color of graph legend and selecting the desired menu.

3.1. Simulation Settings

The **Simulation Settings** button (shown in Figure 15(a)) helps user to set the desired simulation parameters. A settings window, shown in Figure 15(b), is popped-up when user clicks on the **Simulation Settings** button.

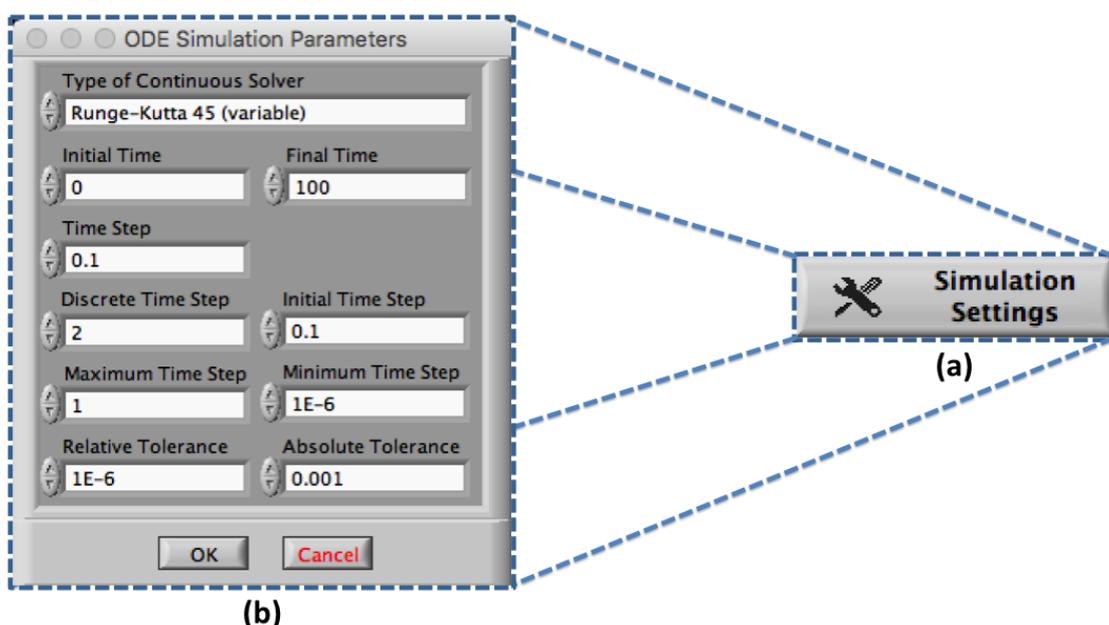


Figure 15. ODE simulation properties (a) Simulation settings option. (b) Window of simulation settings.

D-VASim VIs for ODE simulation supports the following ten continuous solvers:

1. Runge-Kutta 1 (Euler)
2. Runge-Kutta 2
3. Runge-Kutta 3
4. Runge-Kutta 4
5. Runge-Kutta 23 (variable)
6. Runge-Kutta 45 (variable)
7. BDF (variable)
8. Adams-Moulton (variable)
9. Rosenbrock (variable)
10. Discreet States Only

The parameters shown in Figure 15(b) are set as the default parameters. There is one other option available at the right bottom of ODE VI, which is described below

Del events after
execution

Del events after execution: When marked checked, the events are deleted internally after executing once. This helps increasing the simulation speed by avoid checking the executed events repeatedly.



Tip – If the desired simulation results are not obtained, try simulating the model again by checking or unchecking the option “Del events after execution”.

4. General Software Options and Tips

Following are some general tips and options for both stochastic and ODE virtual instruments generated by D-VASim.



Tip – Hover mouse over simulation options to pop up the tip strip with a short description.



Tip – Pressing keys CMD+SHIFT+H (on MAC OS) and CTRL+H (on Windows OS) open the context help.



Tip – User can right click on the graph window to get the runtime shortcut menu shown in Figure 16 below.

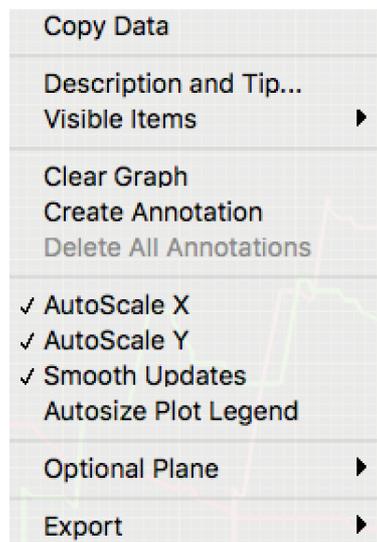
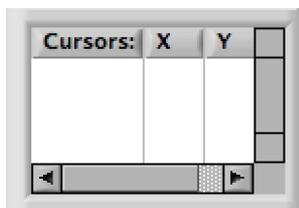


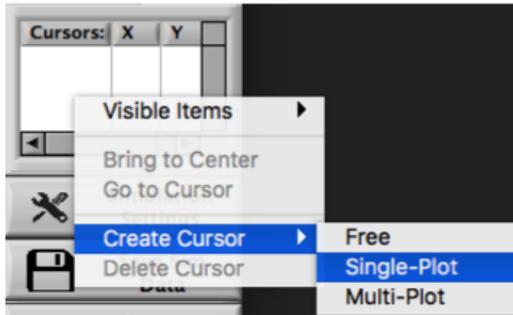
Figure 16. Runtime shortcut menu of graph window.



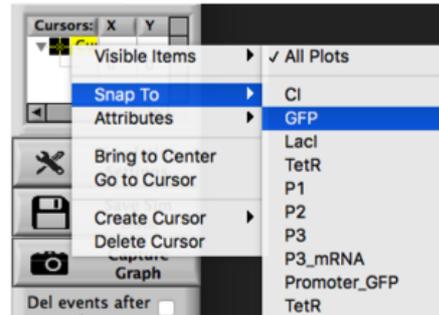
Graph Palette: This option can be used to zoom-in, zoom-out, expand graph etc.



Cursor Legend: With the help of this box, cursors can be set to track the values of desired specie. For example, single-plot cursor can be set to monitor GFP specie by **right clicking** in the Cursors window and then selecting **Single-Plot** from **Create Cursors** option, as depicted in Figure 17(a). Once the cursor is created, it can be snap to GFP specie by **right clicking** on the created cursor and then go to **Snap To** option and select **GFP**. These steps are shown in Figure 17(b).



(a)



(b)

Figure 17. Steps of creating cursors on specie.

Finally, similar to any physical instrument, virtual instruments powered by D-VASim also have emergency shut down button (🔌) shown at the top right corner of a virtual instrument. This button can be used to stop and shut down the instrument instantly.

References

- [1]. Systems Biology Markup Language, http://sbml.org/Main_Page.
- [2]. National Instruments LabVIEW, <http://www.ni.com/labview/>.
- [3]. Dräger A, Rodriguez N, Dumousseau M, Dörr A, Wrzodek C, Le Novère N, Zell A, and Hucka M. **JSBML: a flexible Java library for working with SBML**. *Bioinformatics* (2011), 27(15): 2167–2168.
- [4]. Chris J. Myers. *Engineering Genetic Circuits*. Chapman & Hall/CRC Press, July 2009.