

## Using the Network Tool

This program is for displaying a dynamic network diagram and corresponding time series plot of the output of the 3 loop Arabidopsis model (Locke *et al.*, 2005). The simulations can be dynamically altered to illustrate the effect of varying the model or environmental parameters.

To use the program, first place 'locke\_05b\_network.m', 'locke\_05b.prm', 'locke\_05b.sts' and 'Settings.set' in the same folder. If it did not happen automatically when you unzipped the archive, create a sub-folder called 'Parameters', and place 'Default.pv' in here. This file contains the default model parameters. Create another sub-folder called 'Environment' and place 'Default.env' in here. This file defines the cycle period and daylength. The program can now be run by simply typing 'locke\_05b\_network' at the MATLAB command prompt.

The user interface is divided into two parts. The left column is the simulation controls. The upper area controls the model parameters. The top list allows the user to select a parameters file, which contains the default values. One of these is supplied as above, but the user can create more using [Circadian Modelling](#), which can then be copied to the same folder as the default file.

The list below contains all the model parameters and their values. To alter one of these, select it with the mouse. Its value will then appear in the box below the list. You can then either type a new value or use the adjacent arrow buttons to increment or decrement the value by 10% of the default value. The 'D' button next to the '>' arrow restores the default value for the selected parameter. Any change is not applied however, until the 'Apply' button is clicked. The new value then appears next to its name. This is to allow a desired change at a very specific time during a simulation. The parameters can be altered either before or during a simulation.

The 'Environment' area allows the user to select an environment file, which defines the cycle period and daylength. One of these is supplied as above, but the user can create more using [Circadian Modelling](#), which can then be copied to the same folder as the default file.

The list below the file list contains a list of all environmental factors in the model. In the case of this model there is only one, white light, called 'White'. The button next to this list labelled 'P' toggles plotting of the light cycle on and off. The list below displays all the environmental parameters, with their values. To alter one of these, select it with the mouse. Its value will then appear in the box below the list. You can then either type a new value or use the adjacent arrow buttons to increment or decrement the value. The 'D' button next to the '>' arrow restores the default value for the selected parameter. Again the change is actually applied when 'Apply' is clicked. The new value then appears next to its name. The 'Pulse' button applies a light pulse when clicked during a simulation that is in a period of darkness. This allows an instant visualisation of the effect. The size and length of the pulse are defined in the list above.

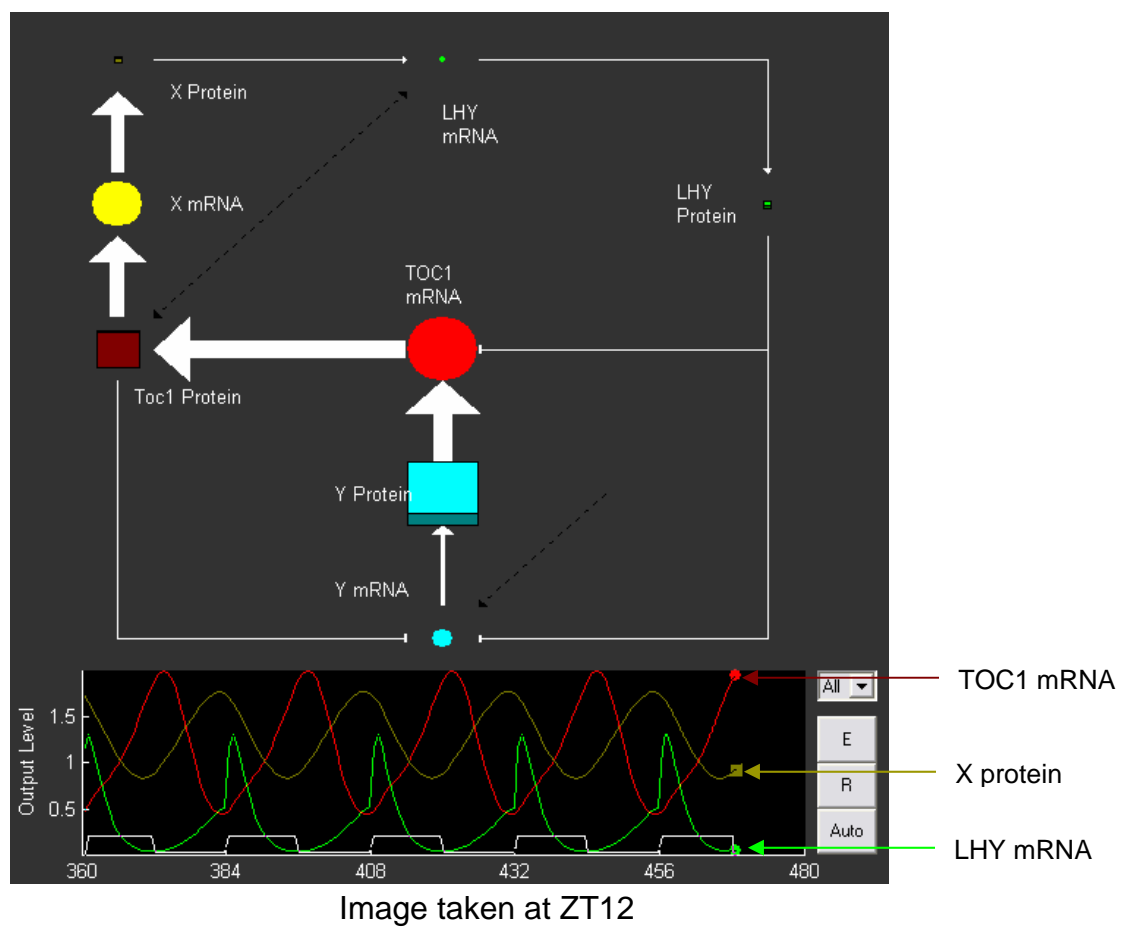
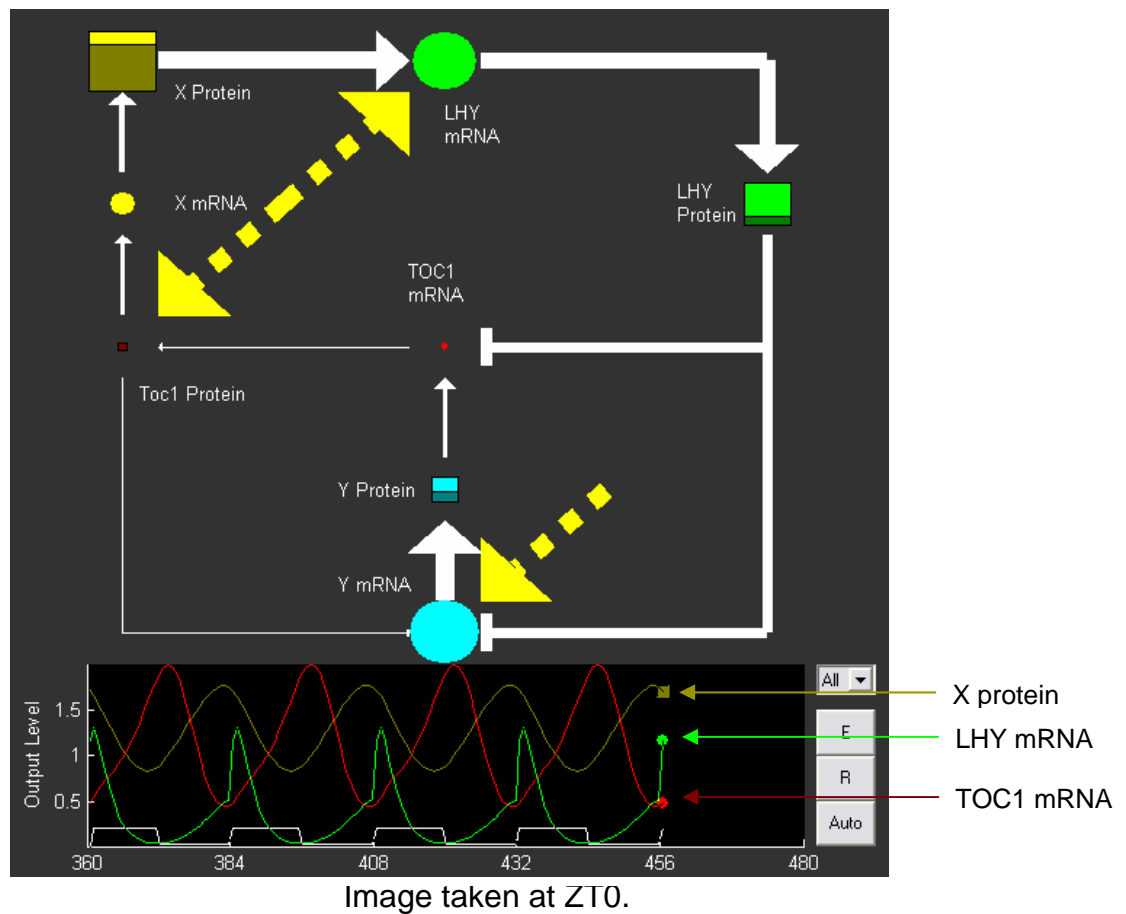
Below this area is a speed slider which alters the speed at which the simulation is run, by changing the frequency at which the plot is updated. Below this are 4 buttons. These are play, pause, stop and record.

To begin a simulation, click the play button. The network diagram will begin to oscillate. Each mRNA is represented by a coloured circle, and each corresponding protein by a square of the same colour. These vary in size according to the level of the species they represent. Size is determined by taking the maximum and minimum values for each species over the previous two cycle periods, and comparing the current size to these. The protein squares are partly shaded in a darker colour. The size of the block represents total protein in the cytoplasm and nucleus, and the shaded area represents the proportion which is in the nucleus, and the unshaded area the proportion in the cytoplasm. The excitatory and inhibitory arrows also oscillate in thickness. These represent the changing strengths of interactions between the species, determined in the same way as above. The yellow arrows represent the light cycle. The brightness of the yellow represents the current light value, either 'On value' or 'Off value' from the environment parameters list, as a proportion of 'On value'. The thickness of the light arrows represent the strength of their input, which is determined not only by light value, but also by the level of Protein P (see model equations). This species is not shown in the network diagram.

Clicking on one of the mRNA circles or protein squares toggles the display of this species on the time series plot. Note that for proteins, it is combined cytoplasmic and nuclear levels which are plotted, and they are plotted in the shaded colour to distinguish them from mRNA plots in the brighter colour. mRNAs and Proteins are also distinguished by the use of circular and square markers respectively. Some examples are shown below.

The upper image was taken just after dawn. The light arrows are at their maximum size. The level of LHY mRNA is approaching its maximum, as can be seen by the large green circle, and by the time series plot. Hence the thick arrow connecting LHY mRNA to LHY protein, representing high levels of translation. Protein levels are already moderately high and are acting to inhibit TOC1 and Y translation. TOC1 mRNA and protein are both at very low levels, so the impact of TOC1 on its targets X and Y is low, hence the thin arrows. Levels of X mRNA are past their peak and quite low, but X protein is at its peak value. This can be seen very well in the time series plot. X is therefore strongly activating LHY transcription. Y mRNA is at its peak, but protein levels have yet to build up, hence it is only weakly activating TOC1 production.

The lower image was taken just after dusk. The light arrows are at their minimum size, and are black to indicate that 'Off value' is zero in this simulation. LHY mRNA and protein are at their minimum values, hence there is only very low level of repression of TOC1 mRNA, which is at its maximum, with TOC1 protein approaching its maximum. This has led to activation of X mRNA, but X protein levels have yet to build up. This can also be seen in the time series plot, which shows X protein at its minimum. Protein Y levels are at their peak, hence strong activation of TOC1 transcription, but mRNA levels have declined.



The white square wave is the L:D cycle, which can be switched on and off as described above.

The buttons beside the axes are for changing the axis scales and are used as follows. Firstly, select from the list the axis you wish to change, X, Y, or All. Now click 'E' to enlarge the selected axis. This expands the axis by two. 'R' reduces the selected axis by 2. 'Auto' only works for the Y axis and attempts to scale the axis to fit the data. This works by looking at the last two cycle periods worth of data to determine the maximum and minimum values

The record button is to allow users to make their own pre-recorded simulations in the form of an avi file. Click the record button, either before or during a simulation, which should then turn red to indicate record mode. When you wish to stop recording, click record again or click stop to end the simulation. A new file called 'recordingXX.avi' will then be created in the program folder.

Note that in order to preserve the frame size, it is essential not to re-size the border whilst recording.

Note that the speed of the simulation slows down considerably whilst recording. This is due to high demand on the CPU. In theory, the user is meant to be able to dynamically alter the model parameters and environment during recording, as during normal play mode. However, in our experience due to the high demand on the CPU the controls become unresponsive, and the program can occasionally crash. It may work well though on a very fast machine (ours is 2GHz, 1Gb RAM). There is also a delay of up to several minutes when stopping the simulation after having made a recording as the avi file is created.