



Big Brother

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Video-based Data Acquisition for Circadian Biology

Big Brother is a video-based data collection system for circadian biology. Most conventional systems rely on switch closures triggered by running wheels, or on infrared beam crossings or burglar-alarm motion detectors. Such systems can be cumbersome to set up and run, and in some cases can be relatively insensitive. Big Brother tracks the distance travelled by each monitored animal using video cameras, reporting distance for each animal once per minute. The resulting data can be read by **ClockLab**, Actimetrics' program for circadian analysis.

Flexible. The image on each of 4 cameras can be divided into any number of zones, and the animal within each zone is tracked independently. Big Brother has tracked animals all up and down the phylogenetic tree, from drosophila to zebra fish to mice.

High temporal resolution. Big Brother locates each animal once every 2 seconds, calculates the distance travelled since the last time the animal was located, and adds the distance to the cumulative total for the current minute. At the end of each minute, the total distance is recorded and reset to zero. The tracker is highly sensitive, yet immune to noise.

Infrared Sensitive. Big Brother's cameras are infrared sensitive, so that tracking can be done under infrared or visible light, or on a light-dark cycle in which the lighting changes from one to the other.

Track up to 200 animals. A complete system includes a video acquisition board and 4 cameras. Up to 50 animals (or in some cases even more) can be tracked with each camera.

Big Brother runs under the **Windows 2000/XP** operating systems on all **PC-compatible** computers with a Pentium processor of at least 400 MHz clock speed. 128 MB of RAM (256 MB or higher is recommended). CCD cameras and video data acquisition board are provided, and require only the simplest of connections to set up.

Big Brother Installation

1) Install the Big Brother software, image acquisition card and drivers

- ***Before installing the video board***, load the NI-IMAQ software from the CD provided with the PCI-1408.
- Shut down the computer and plug the PCI-1408 board into the open PCI-slot that is closest to the processor.
- Run the Big Brother Installer from the CD.

2) Connect the camera(s)

- Connect camera 1 output connector to the "Video" input on the PCI-1408 using the BNC cable provided.
- Connect the video breakout cable to the PCI-1408. This cable has 4 BNC connectors on one end, and a 25-pin (printer-port style) connector on the other end. The 25-pin connector plugs into the corresponding connector on the 1408.

- Connect cameras 2-4 to the V1, V2 and V3 BNC connectors on the breakout cable
- Connect each camera to a 24VAC power supply using the two-stranded wire provided. Ignore the wire colors; polarity is unimportant since this is an AC power supply.
- Plug the power supplies into any 120V-60Hz outlet..

3) Test the cameras

- From Windows's START menu, select Programs->National Instruments IMAQ -> Explore IMAQ.
A Window entitled "Exploring - Measurement and Automation" should appear.
- In the list to the left of the window, open Measurement & Automation\Devices and Interfaces\IMAQ PIC-1407:img0\Channel 0: rs 170
- From the TOOLS menu, select IMAQ->grab. You should now see a real-time image from the camera. If no image appears, check the power and signal connections from the camera and video board. If you still have trouble, call National Instruments at 800-433-3488 and ask for help with NI-IMAQ.
- Repeat this procedure for each channel (camera).
- Once you have verified that the camera is working and properly connected to the image acquisition board, you may quit the camera test program by clicking the close box at the upper right of the window

5) Mount the cameras

Mount the camera securely above the center of the arrays of animal chambers. Be sure that the power and signal cables are secured so that they cannot be jostled during a trial. Any motion of the cable will move the camera ever so slightly, disrupting tracking.

6) Adjust the lighting

Lighting will vary with the application. For drosphila or zebrafish, the animals should be lit from below. We have had good success with the array of animal wells placed on a sheet of diffuser (translucent) glass. The light source, either infrared or visible are then placed below the diffuser. For mice, lighting should be from above.

Whether lighting from above or below, try to keep the level of lighting as even as possible across the entire image.

Note that the light level need not be over bright. Most cameras automatically adjust their sensitivity to the ambient light level.

The Big Brother Main Window

File. Use this button to select or create a file into which the data are to be collected. The button brings up a standard file dialog for this purpose.

Data collection proceeds whenever the program is running. When a file has not been specified, the data are saved into a default file in the Big Brother program directory.

Data from all recorded cells are stored together in the data file. Use the Big Brother [File utility](#) to separate the data from each cell into its own ClockLab compatible file.

Camera. A poppupmenu for selecting which camera image is displayed in image window.

Row and Column. A small cursor shows the detected location of the animal in the selected image cell. The **Distance** indicator shows the accumulating distance for that cell.

Detection threshold. This value is used by the program to determine whether the animal has moved or not. To set the threshold

1. Begin by pointing the camera at your setup without any animals in place.
2. Be sure that the lighting on the setup is steady. Even slight shadows cast onto the apparatus by people moving about the room can degrade detection.
3. Watch the array of **motion** indicators just beneath the threshold control.
4. Adjust the threshold to the smallest value such that none of the motion indicators lights turn green over the course of several seconds. Too high a value will cause the program to miss some motion. Too low a value will cause false positives, that is, detected motion where none is present.
5. Once a value is chosen for the threshold, check to make sure that no false positives are reported on the other cameras as well.

Note that the program uses an algorithm to prevent changing shadows from being detected as motion of an animal within each cell of the image. But because the cells are small, the algorithm is not foolproof. Be very careful while adjusting the detection threshold not to cast shadows on the field of view. Even if you are standing between a white wall and the field of view, excess movement can cause significant shadows.

Show array. Displays the setup of the cell array for the currently selected camera.

Image error. Normally hidden. If an error occurs in the image acquisition process, the image error indicator will appear below the image window and give information about where the error occurred. The most common error occurs when a camera is not properly connected to the acquisition board or is not correctly connected to the power supply. In that case, the program will become sluggish to respond to the controls. Check the cameras and restart the program.

SuperVisor Settings

Universal settings

Cameras. Sets the number of cameras to be connected to the system. Up to 4 are permissible.

Array Size (x) and Array Size (y). Defines the number of cells in the cell array for each camera. As these controls are changed, the array grid in the image window will change accordingly.

Note that the number of cells is the same for each image. The sizes and locations of the cells within each image can vary, however (see below).

Frame Average. SuperVisor can average successive images in order to improve its detection in low-contrast or low lighting conditions. Increasing the number of frames averaged, however, can lead to blurring for fast-moving animals, and can slow down the data acquisition to be less than 2/sec. In general, start with **frame average** set to 1, and consult with Coulbourn Instruments if you are having trouble getting stable tracking.

Camera-specific settings

Camera Selector. Selects which image is currently being viewed, along with its cell array.

Field cursors. In the image window are 4 light-blue cursors (2 horizontal and 2 vertical lines). Drag these cursors to define the limits of the cell array, that is, the area in which animals will be located. If the cursors are not visible, use the **Find Cursor** button to bring them into view.

Image width. For each camera, enter the physical width of the whole field of view (not just the area defined by the cursors) from left edge to right edge, in centimeters. This tells allows the program to convert motion in pixel coordinates to motion in real coordinates.

Save and Exit. Saves the changes you have made and returns to the main program.

Cancel. Ignores any changes you have made and returns to the main program.

The Big Brother File Utility

Big Brother creates a single file containing all the data from all the cells recorded. The File Utility pulls out the data for each cell in turn and creates a data file that can be read by ClockLab. To use the File Utility:

Open the File Utility.

Click the **Select Data File** button and use the file dialog to select a Big Brother data file. Reading the file may take several seconds depending on file length and computer speed.

Once the file is read, the data from each cell can be viewed by using the three popupmenus (**Channel**, **Row** and **Column**) to the left of the data graph.

Click on the **Write Cell Files** button. The program will create the individual cell files, naming them according to the name of the data file and the cell camera, row and column. Clicking the **Cancel** button instead of the Write Cell Files button allows selection of another data file.

Click the **Exit** button to exit the program.