AMP03 Infrared system user’s guide

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Basics

For an introduction to the overall aspects of the use of infrared sensors to monitor cardiac activity in marine animals, (Burnett et al., 2013) offers an excellent overview including a technical description of the circuitry used in the amplifier component of the setup.

The setup described here consists of three components:

* An infrared sensor (Vishay CNY70) connected to a cable and RJ11 adaptor. When the sensor is placed correctly over the heart of a study animal and the adaptor connected to an amplifier (see below), it emits an infrared light which bounces off the internal structures inside the organism, and is picked up by the receiver element of the sensor. Changes in the position of internal structures during heartbeat contractions cause a change in the amount of light that is reflected. This in turn causes a change in voltage which is picked up by the amplifier circuit.
* An AMP03 amplifier. This is a commercially-produced version of the amplification circuit described in Burnett et al. (2013). The amplifier conditions the voltage signal from the infrared sensor, filters out high frequency noise and amplifies low-frequency signals. It has an on-off switch, an RJ11 socket which connects to the infrared sensor, and a BNC socket which connects to the Data Acquisition Device. There are two versions: the base model AMP-03 which takes two AA rechargeable batteries (these need to be replaced approximately every 10 hours of operation), or the AMP-03U, which takes its power from a USB port.
* A National Instruments USB6009 Data Acquisition Device (DAQ) (Figure 1). This receives the analog signal from the amplifier, converts it to a digital signal, and sends it to a laptop for acquisition and further processing. The DAQ consists of a series of analog I/O terminals used to connect to the amplifiers using BNC adaptors, some digital I/O terminals (which we don’t need), and a USB3 terminal to connect to a laptop. Multiple amplifiers can be connected to the DAQ at the same time to collect signals from multiple individuals at once.
* Female BNC to wire (pigtail) adapters: These are used to connect the screw terminals on the USB6009 with the amplfiers, and can be readily bought on Amazon.



Figure 1: National Instruments USB6009 amplifier connected to six BNC - wire adaptors

* Also required is a Windows (not Apple) laptop running National Instruments DAQExpress software, which can be downloaded here: <https://www.ni.com/en-us/support/downloads/software-products/download.daqexpress.html#348849>
* In order to view saved TDMS files in Excel you will also need the TDMS addon for Excel, which should be automatically installed as part of the above install of DAQExpress, but if not can be readily found online.

Step-by-step guide (first time use):

* Connect the USB6009 amplifier to your computer. It should give a prompt to open NI-DAQExpress, if not, manually open the program. You may need to log in using a NI user account.
* Once you are in, open the project “Periodic snail heartbeat monitoring”

**Hardware setup**

* The USB6009 supports two different types of analog input: Referenced single-ended, or differential double-ended. These are labelled differently but use overlapping terminals (see Figure 2).
* Using a combination of single and double-ended inputs, you can run up to six amplifiers off a single USB6009. However, this is somewhat tricky to setup, so unless you need to run more than four amplifiers at once I would recommend only using the differential inputs.
* To start, connect the wires from one of the pigtail adapter to the + and – terminals corresponding to double-ended input 0 (figure 2). Assuming you have purchased the same pigtail connectors I have used, the plastic-sheathed wire will need to go into the positive terminal, while the unsheathed (silver) wire will go into the negative terminal. Make sure they are tightly secured using a screwdriver.

Diagram

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Figure 2: Analog input terminal layout on the NI USB-6009

* Next, connect an AMP-03 amplifier to the USB6009 using the BNC adaptor.
* Plug a sensor into the amplifier and turn the amplifier on. You should see a red light on the amplifier, and a green light which will flash on and off when you move something in front of the sensor head.
* Moving back to the computer, navigate to File > New > Analog Input.
* By default, the program will create an input for input 0 (the box with the yellow tab at the bottom of the screen) and you should now be able to see the voltage signal from the sensor visualized on the screen.
* In the top left corner of the screen, there is a “play” and a “stop” button, as well as a “record” button which can be used to make short recordings
* If you hold your finger lightly over the sensor, you should be able to detect your pulse on the screen. This is a very good way to check if the sensor is working correctly (because the signal given by a human pulse is very similar to that given by a snail’s heartbeat), and to check if there is any excess noise on the signal.
* If there is noise, a slight twist of the BNC connectors can often rectify this; if not, check that the wires are correctly screwed into the terminals. Don’t worry if you get a noisy signal when there is no object in front of the sensor; this is completely normal!
* Now that you have confirmed that the setup is working, some small alterations to the setup of the program should be made:
  + First, navigate to the Channel tab on the right side of the screen. Change the values of “Voltage input” from -20 and +20 to 0 and +4, respectively. This is because the voltage returned by the sensor never goes outside of these values, so you are essentially changing the scaling of the y axis to display the maximum breadth of the data
  + Second, the sample rate needs to be reduced, because the default value used by the software (20KHz) is way too high for what we need and will result in infeasibly large files.
  + To do this, navigate to the Task tab, unselect “Auto Managed Timing”, and change the desired sample rate from the default value of 20 KHz to a much lower value- I normally use 40 Hz but any value between 20-100Hz should be fine – note that to get a rate of 40Hz you will need to enter a value of “0.04” because it will initially expect the value to be given in KHz.

* You can now add more inputs by plugging in extra BNC adaptors and amplifiers to the other terminals, and then selecting “create new input” at the bottom of the screen. Note that if you add any single ended inputs, you may need to change the terminal configuration in the Channel tab. Also, you will need readjust the Voltage input values for each new input you add.
* You have the option to show or hide each input by right clicking on their respective box.
* Although you have the option to rename your inputs, I would strongly recommend setting them up so they are labelled 0,1,2,3,4… etc. from left to right, even if the name you give them doesn’t directly correspond to the terminal they are plugged into. This is because the scripts I have written for downstream processing of the files currently expect them to be in this order (although I might change this)
* The “play” and “stop” buttons at the top of the screen can be used to play and pause the signal. To do basic recordings, you can use the “Record” button; the file generated can be exported as a .csv file if needed.
* Once you have the program set up with all of the inputs you need, I’d recommend saving the project file for future use.

**Attaching the sensor to a snail**

* For snails with roughly “conventional” shell shapes, i.e. Littorinids, whelks, drills, and topshells (although no guarantees with the latter latter are anatomically a bit different), Figure 3 shows good sensor placement to pick up the heartbeat for most individuals. However, this is often a question of trial and error as individuals with differently shaped shells / different growth stages may vary a lot in terms of where you will get the best signal.
* As a general rule, a good heartbeat signal is indicated by a REPEATING pattern. The actual strength of the signal in terms of amplitude is not important (as long as the signal is not so weak that you risk losing it completely). The strength of the signal will vary based on a lot of factors that have nothing to do with the physiology of the organism – shell thickness and colouration, sensor placement and sensor quality, so we can’t get any useful information from the signal strength or quality. Remember that the only useful information we can get from this technique is heartbeat frequency; no other characteristics of the signal should be used
* Some good examples of different signal types to look out for are given in Burnett et al. 2013 (reference at end of document)
* To provide a temporary attachment of the sensor to the animal, we use dental wax, which is easily available online. Placing a thin layer of wax on the head of the sensor will enable you to directly stick the snail to the sensor, repositioning it until you get a good heartbeat signal.
* For a more permanent attachment, a thin layer of gel superglue can then be placed around the perimeter of the sensor forming a seal between it and the snail. This will be sufficient to hold the snail in place during the trial, but will still allow for the snail to be detached at the end of the trial by applying lateral force. I have found that the best glues to use are either Loctitie Ultragel Control, or Loctite Powerflex gel, as these provide a strong, flexible bond but also don’t appear to irritate the snails as much as other brands.
* Once you have added the glue, leave the sensor with snail attached out of the water for around 15 minutes to allow the glue to harden, before placing the sensor head in water. Make sure that the other end of the sensor (the end that plugs into the amplifier) always stays dry.
* Generally, we leave the snails in water attached to the sensors overnight to recover from the stress of handling/ irritation caused by the superglue fumes, before using them in trials the next day.

**A hand holding a black wire

Description automatically generated** A hand holding a black object

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Figure 3: Sensor placement over the heart of a typical marine snail (Littorina saxatilis)

**Recording trial data**

* The “Record” function in NI-DAQ Express is fine for making individual recordings of snail heartbeats for short durations. However, for longer term ramping experiments, it is better to switch to a system that can automatically record data over set durations and at set intervals. For this, I created a virtual instrument in NI-DAQ Express based on one of the demo files that came with the software, which will do this for us.
* To start, open up the project “Periodic snail heartbeat monitoring”.
* Within the project, you will need to go to file>new> analog input and set up your analog input program as I instructed earlier. Alternatively, you may be able to drag and drop the program you made earlier into the “Periodic snail heartbeat monitoring” folder on your desktop, and if should turn up in he project files bar on the left side of your screen
* Once you have set this program up, make sure you save it within the “Periodic snail heartbeat monitoring” Project folder.
* Next, open up “Periodic logging.gvi” which should be located under project files.
* Your display should now look like Figure 4 (make sure “panel” is selected in the middle top part of the screen”):

A screenshot of a computer

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Figure 4: Periodic logging Virtual instrument display.

* To start logging data:
  + First, Change the “DAQmx Analog Input Task” to show the analog input program you just set up. Note you must have saved the program file in order for it to include any recent changes when you come to start the logging.
  + Next, change “path” to specify a file path (i.e. a folder) where you want your data to be saved.
  + Next, type something into “group”. This will become the file name of each of the files generated, to which will be added a date and timestamp (so for instance, a group name of “PrjUro\_1\_12up\_NCSC” will result in a list of files named PrjUro\_1\_12up\_NCSC\_20230413\_135746, PrjUro\_1\_12up\_NCSC\_20230413\_140746, etc….)
  + Next, change “Logging interval” to specify the interval between successive files that you want. Generally, if doing a ramping experiment you may want to continually collect data throughout the trial; however, this results in very long files that are very hard to read later on, so I recommend chopping your data into 5 or 10 minute long files. This will make downstream processing and calculations of heartrates over time a lot more straightforward.
  + Lastly, select the duration of each recording by changing “Logging duration”. Note that if you want to log heartrates continually during the trial but want to save the files in 10 minute chunks, you need to set the logging duration to a value slightly lower than the logging interval. This is because the program needs a few seconds after each recording to save the data, before the next recording can begin. For example if you set the logging interval at 10 minutes, you should set the logging duration at a slightly lower value, say 9.7 minutes. This does mean that you will lose a few seconds of data every ten minutes, unfortunately, but this isn’t a major issue from a data analysis perspective.
  + Once you have changed these settings, hit the “Run” button (green arrow) to start your recording. TDMS files containing the heartbeat recordings will then start being saved in your specified folder, with the file that you are currently recording being shown in the “Log File” panel at the bottom of the screen. The individual heartbeat traces should appear in the two boxes in the center of the screen. The top box contains the “real” data that is actually being saved in the TDMS files, while the bottom box gives a low-pass filtered version of the signal – I include this because it represents what the data will look like if you clean the signal later in R when you come to process the heartrates (more on this later)….
  + To stop the recording, hit the “Stop” button. Any files that are midway through recording will be saved to that point.

**Processing the heartrate data (To be added)**

References

* Burnett, N. P., Seabra, R., de Pirro, M., Wethey, D. S., Woodin, S. A., Helmuth, B., Zippay, M. L., Sarà, G., Monaco, C., & Lima, F. P. (2013). An improved noninvasive method for measuring heartbeat of intertidal animals. Limnology and Oceanography: Methods, 11(FEB), 91–100. https://doi.org/10.4319/lom.2013.11.91