NemaMetrix

Troubleshooting Guide

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Acquisition and Noise

Q: I'm experiencing high frequency noise in the chip. What can I do to resolve this?
Q: The worm signal is small or not appearing as expected; why is this?
Q: How can I unclog the chip channel?
Q: I'm using a Mac and the NemAquire software I downloaded will not launch. Why?
Q: I cannot import my data into LabChart using my Mac. Why?

Analysis Service

Q: I've received data back from the free Recording Analysis Service and I would like to know more about the confidence values. What explicitly do these mean?
Q: I just received data from the free Recording Analysis Service, and many of my recordings had no pumps. What does this imply and what can I do to get more quantitative data?

Q: I'm experiencing high frequency noise in the chip. What can I do to resolve this?

First, we'll isolate the problem by removing any worms from the chip. If you're not having high amplitude noise without worms, but are still experiencing problems with signal clarity, see The worm signal is small or not appearing as expected; why is this?

Make sure the channel is filled with buffer solution and check the filters in NemAcquire: the Highpass filter should be ON and the Notch filter should be OFF. With these settings and with no worm in the chip, the amplitude of noise should be no larger than 20 µV, peak to peak; around 10 µV is ideal for clean analysis. If you're having trouble getting the noise below 20 µV, attempt the steps below:

- Place the amplifier on the same surface as the ScreenChip Dock.
- Ensure that the cable from the ScreenChip Dock to the Amplifier is plugged in securely.
- Is the noise regularly interrupted? Does it seem to have a pattern or is it cyclical? Make sure that nothing on the lab bench or in the room is causing unnecessary vibrations.
- Try flowing buffer through the chip and verify that nothing is clogging the channel. If you're having trouble unclogging the chip, see How can I unclog the chip channel?

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- Sometimes fluid or bacterial growth in the exit tubing can act as an antenna. Try removing the exit tubing during a recording, or replacing the exit tubing if problems continue.
- Try using a new chip.
- If the amplitude of the noise continues to be above 20 µV, contact us at support@Nemametrix.com and we'll troubleshoot the system further.

Q: The worm signal is small or not appearing as expected; why is this?

If the signal amplitude is small or not visible, there are several things you can do to:

- Sometimes removing the exit post tubing from the chip during recording will increase the signal-to-noise ratio.
- Make sure the worm is sitting between the two electrodes in the chip channel. If the worm begins to slide out one end, the signal amplitude will decline or not be captured at all.
- The worm must be large enough for the channel. Worms that are too small for the chip will make a poor seal with the channel and the signal will be weak.
- Sometimes fluid or bacterial growth in the exit tubing can act as an antenna. Try removing the exit tubing during a recording, or replacing the exit tubing if problems continue.

Q: How can I unclog the chip channel?

The easiest way to unclog the channel is to fill a syringe with buffer solution and push the solution through the tubing in the entrance port until all eggs, bacteria or other waste is flushed from the channel. It is important to note that using too much pressure with a syringe can beak the bond between channel layers. If the chip is still clogged after using medium pressure in a syringe, use the vacuum pump and the steps below:

- Fill a new Eppendorf tube with buffer solution and use the vacuum pump to pull the buffer through the chip until it is cleared.
- Another option is to swap the tubing from the entrance port with the tubing and vacuum on the exit port, so that the vacuum pulls buffer solution through the chip going the opposite direction of normal flow.

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Q: I'm using a Mac and the NemAquire software I downloaded will not launch. Why?

Once you have downloaded the Mac version of NemAcquire, open the folder containing the downloaded file. Drag this file into the Application folder. The Applications Folder can be seen in a Finder window, or using the Applications icon in the bottom Dock. Once NemAcquire has been moved to the Applications folder, double-clicking on the NemAcquire icon will start the program.

Q: I cannot import my data into LabChart using my Mac. Why?

This question is specific to Mac users. If you are experiencing trouble importing the data into LabChart using a Windows computer, please contact our support team.

1. The first thing to do is check the recording file. Go to Finder and locate the file location.
2. Open the file using a text editor. The beginning of the file contains metadata entered by the user and some important data about the recording added by the software. After this section, there should be one column of data.
    - If there are two columns of data, open the file in excel and delete the first column of data.
    - If this is the case, open NemAcquire and go to the File Menu. Select Recording Options.
    - Uncheck the "Timestamp" option. This will prevent future recordings from adding this column and make import easier.
3. If you have only one column of data and are still having trouble importing the file into LabChart, open the recording file. Remove the metadata within the file up to the "channel_1" text. Now you should be able to import the data into LabChart for Mac.

Q: I've received data back from the free Recording Analysis Service and I would like to know more about the confidence values. What explicitly do these mean?

A confidence value is given to each recording that has been analyzed based on the recording's signal-to-noise ratio and the ease of analysis. We recommend reviewing any recording at a confidence value of six or less. The confidence values for a standard N2 control recording are detailed below. These constraints may be slightly different for mutants or with drug

treatments known to cause irregular waveforms and frequency. Signal-to-noise ratio, false positive and false negative rates remain the most important factor for determining the confidence value of any recording.

10

- Waveforms are easily visually identifiable
- Consistent pumping frequency and amplitude
- False positive and false negative rate < 1%
- Signal-to-noise ratio < 5%

8

- Waveforms are largely visually identifiable
- Small variations in pumping frequency and amplitude
- False positive and false negative rate < 5%
- Signal-to-noise ratio < 10%

6

- Some irregular waveforms
- Inconsistent pumping frequency and amplitude
- False positive and false negative rate < 10%
- Signal-to-noise ratio < 20%

4

- Many irregular waveforms
- Inconsistent pumping frequency and amplitude
- False positive and false negative rate < 20%
- Signal-to-noise ratio < 40%

2

- Pumps are not visually identifiable
- Detecting pumping frequency and amplitude is difficult
- False positive and false negative rate < 40%
- Signal-to-noise ratio < 40%

To get higher confidence values for recordings, make sure that you have low baseline noise amplitudes (See I'm experiencing high frequency noise in the chip. What can I do to resolve this?) and add lab notes about dead, abnormal, or poorly pumping worms.

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Q: I just received data from the free Recording Analysis Service, and many of my recordings had no pumps. What does this imply and what can I do to get more quantitative data?

Drug treatments or genetic mutations can alter worms' behavior and the resulting pharyngeal signal. This is a great thing! However, it does imply some subtlety when considering our data analysis service. We have very specific criteria for what constitutes a "pump" when we analyze data: to be identified as a "pump," the waveform must have both an apparent E and R spike, as well as a minimum interval between E and R spikes of at least 30 ms. Sometimes analyzed recordings are returned with 'no apparent pumps' because:

- The recordings lacked obvious E and/or R spikes
- Intervals between events identified as E and R spikes were too short (less than 30 ms)
- EPG signals could not be distinguished from noise.

Except for the latter case, when signal-to-noise ratio was poor, the finding that a worm produced no electrical events identifiable as pumps during a recording can be a positive result. For example, the anthelmintic drug ivermectin at 10 µM blocks pumping after about 10 min, so the absence of pumping indicates the drug was effective. When canonical pumps are not identified, there may still be important phenotypic signatures in the recordings such as missing E or R spikes, failed action potentials, failed pumps, etc. Therefore we recommend that investigators always review their recordings for more subtle phenotypes that our data analysis service is not setup to detect.

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