

LabChart® Analysis Procedure

The sampling rate in LabChart® v7.3.7 was set to 100 kHz. Input from Channel 1 was turned on to accept output from the amplifier. The range for Channel 1 was set to -50 to 50 mV, and horizontal scaling to 50:1 for best visualization of spike activities. The time format was set to **From Start of File** in the **Display Settings** window of the **Setup** menu of the main toolbar in LabChart®. In the same **Display Settings** window, the **Always Seconds** checkbox was selected. Files were saved as LabChart® Data Files by selecting **Save As...** in the **File** menu of the LabChart® main toolbar.

Measurements were made and calculations were performed using the LabChart® Data Pad. The Data Pad was accessed by clicking the **Data Pad View** icon in the **Data Pad** menu. In the **Data Pad View** window, the title of **Column A** was clicked (the title was commonly represented as **A Channel 1 Mean**). In the **Data Pad Column A Setup** window, **Cyclic Measurements** was selected in the left interactive field and **Event Count** was selected in the right interactive field. **Channel 1** was selected from the **Calculate from source channel** dropdown menu. The **Only add if the selection includes the source channel** checkbox was unchecked, and the **Miniwindow** checkbox was unchecked. Clicking the **Options** button opened the **Cyclic Measurements – Data Pad** window (Fig. 13).

Clicking the **Customize** button brought up the **Cyclic Measurements Detection Settings (General – Sine shape)** window in which the noise level could be adjusted, and its effect on action potential detection could be observed in the **Preview of detected cycles** window. The value in the **Noise** textbox was set to whatever value produced the most accurate measurement of action potential count (usually between 0 and 10 mV), as determined through inspection. Once an appropriate noise level was set, the **OK** button was clicked. The **OK** button was then clicked in the **Cyclic Measurements – Data Pad** window and in the **Data Pad Column A Setup** window. The title of **Column B** was clicked and **Selection & Active Point** (in the left interactive field) and **Selection Duration** (in the right interactive field) were selected in the resultant window. The **Miniwindow** checkbox was unchecked. The **OK** button was then clicked in the **Data Pad Column B Setup** window.

The **Cell edit mode** icon, located in the upper-left corner of the **Data Pad View** window, was clicked and the cell in row 1 under **Column C** was selected. The following function was typed into the cell: =A1/B1. A1 represented the value of the event count calculation and B1 represented the selection duration. A window sometimes appeared asking if the user wanted to continue editing the cell. The **Yes** button was clicked to allow the function to be input. The title of **Column C** was right-clicked and **Rename Column** was selected. “Average Frequency Hz” was written in the **Column title** textbox and the **OK** button of the **Edit Column Title** window was clicked. The title of **Column C** (now “Average Frequency Hz”) was right-clicked again and **Show Miniwindow** was selected. The **Chart View** icon of the **Window** menu was clicked and the **Average Frequency Hz** miniwindow was resized and rearranged for better viewing (Fig. 14). The **Chart View** icon in the **Window** menu was clicked.

The time when the shutter switch was toggled to open was determined through inspection by setting the horizontal scaling to 1:1. A set of data was then selected by opening the **Commands** menu of the main LabChart® toolbar and clicking **Set Selection**. In the **Set Selection** window, the drop-down menu was set to **From Start of File**, the **As Seconds** checkbox was selected, and the **Active Point** checkbox was deselected. The time at which the shutter was opened was used for the **Start** textbox. A number of seconds sufficient to include the response to light was added to the time at which the switch was opened, e.g., 5 sec (cf. Results, Fig. 11), and this calculated time value was typed into the **End** textbox. The resultant value in the **Average Frequency Hz** miniwindow was recorded (Fig. 15). The same number of seconds used in the previous step was subtracted from the time recorded for the shutter opening. The resultant time value was typed into the **Start** textbox and the shutter opening time was input as the **End** value. The resultant value in the **Average Frequency Hz** miniwindow was recorded.

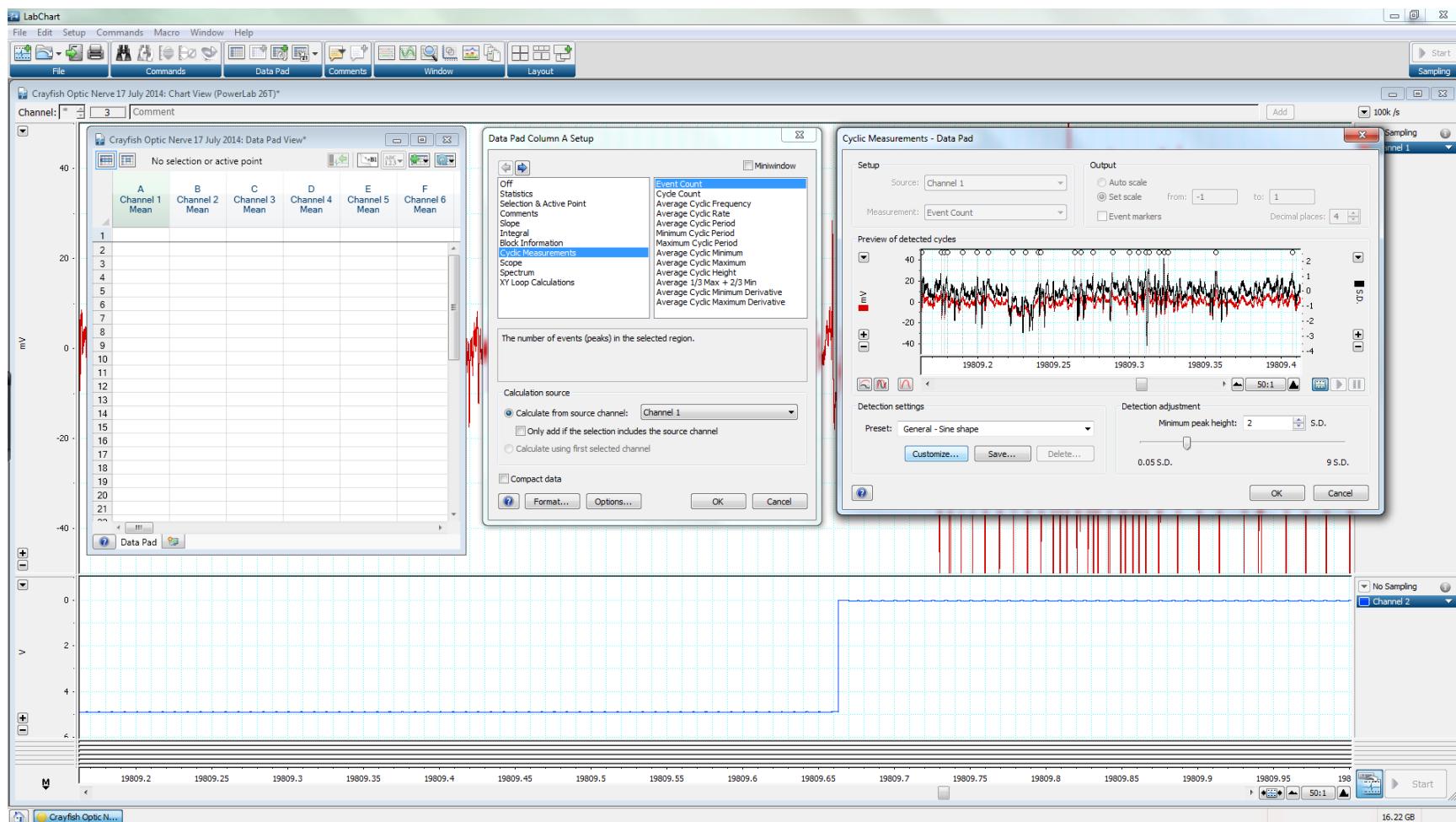


Figure 13. Screenshot taken within the LabChart® program showing the Data Pad, Column Setup, and Cyclic Measurements windows.

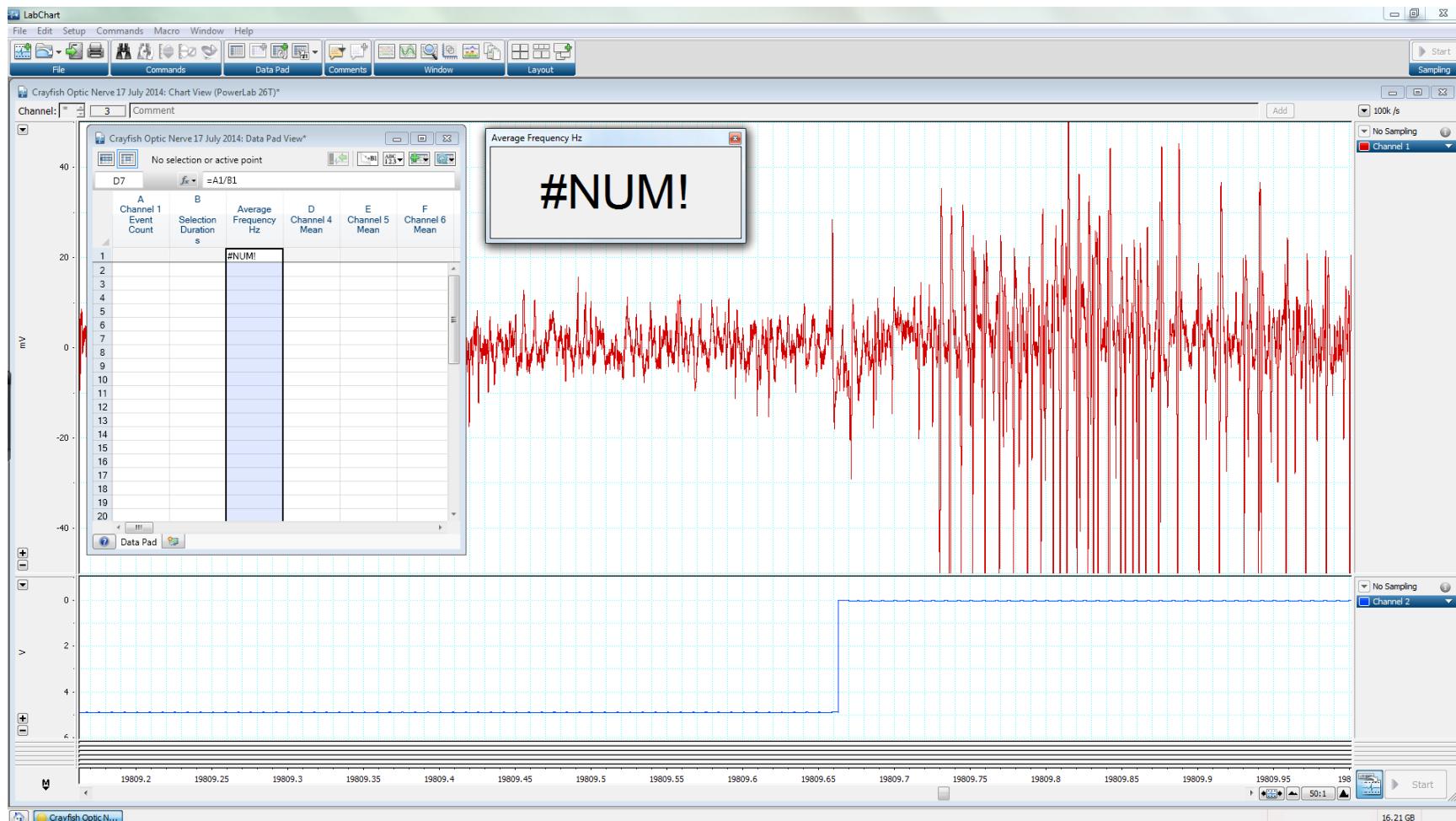


Figure 14. Screenshot showing the Cell edit mode and Average Frequency Hz miniwindow in LabChart®.

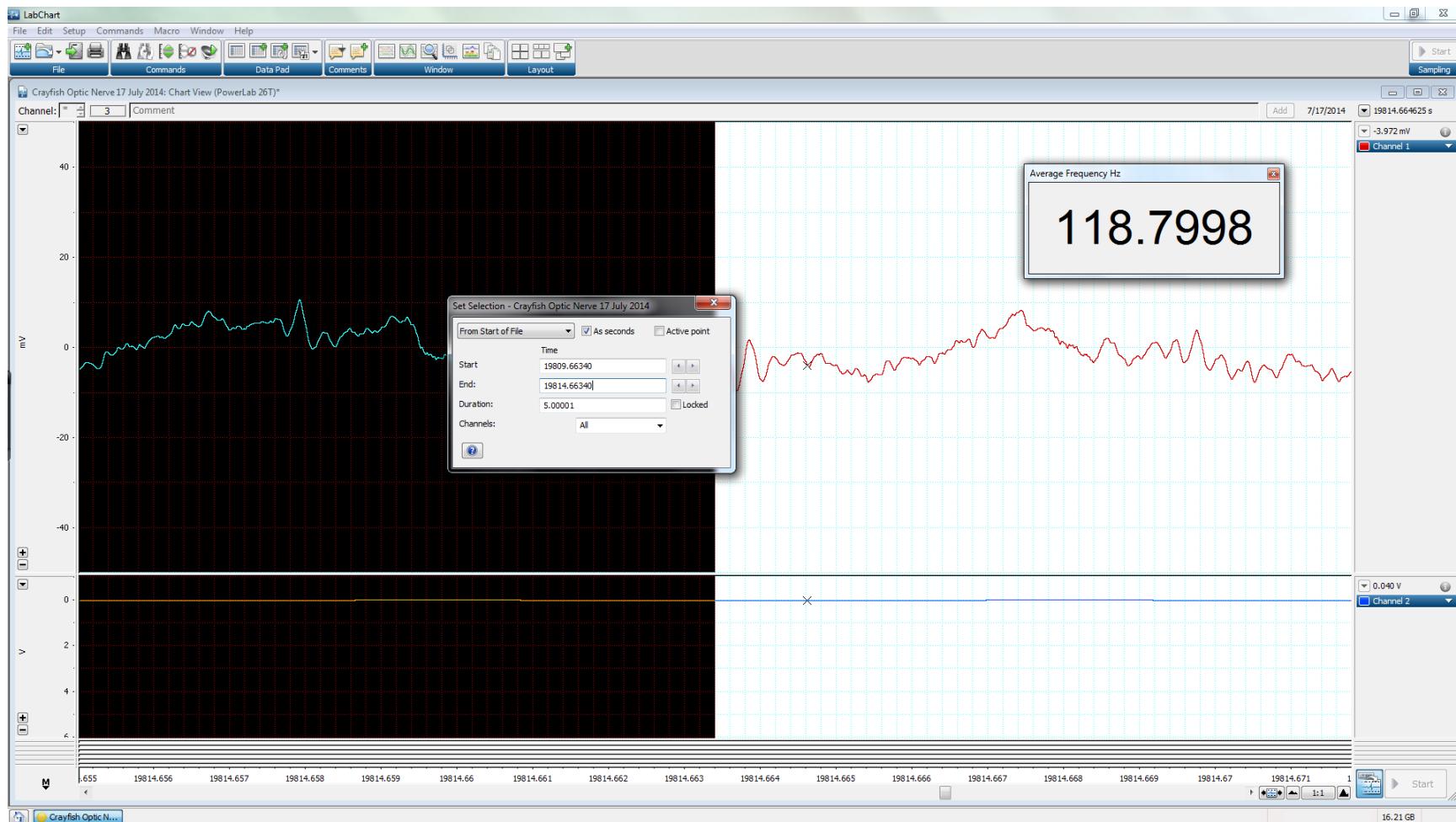


Figure 15. Screenshot taken within LabChart® displaying how to use the Set Selection window and observe values displayed in the Average Frequency Hz miniwindow.