doric

GETTING STARTED: Bundle-imaging Systems

Application Notes

Version 1.0.0

Contents

1

Getting Started

This chapter explains how to configure Doric Neuroscience Studio (DNS) for different bundle photometry systems, including Bundle-imaging Fiber Photometry System (BFMC)-Gen3, Bundle-imaging Fiber Photometry Cube with Targeted Optogenetics (BFTO), and Rotary Bundle-imaging Fiber Photometry (RBFMC).

1.1 Hardware Installation

To set up a bundle system from scratch, three manuals are required:

1. **Hardware manual:** Instructions for wiring and connecting the system components (link).
2. **Doric Neuroscience Studio (DNS) manual:** Detailed explanations of all icons, functions, and software features (link).
3. **Getting Started Application Note:** Step-by-step guidance for performing bundle photometry recordings in the lab.

1.2 Install Software

Doric provides three different software tools for separate functions, as listed below. The first two software are free and necessary, while the third is optional and available as a yearly license version:

1. ***Doric Neuroscience Studio (DNS):*** To operate Doric devices, you will need to install the ***Doric Neuroscience Studio*** (DNS) software on a Windows system. The software is regularly updated, and the latest versions are available for download on the Doric website. We recommend using the latest available version to have access to the latest features. As an initial step, download the software and install it on your computer.

   Note: Every time you install the software, do not forget to set software to High-Priority. This makes Windows prioritize DNS over any operations running in the background.

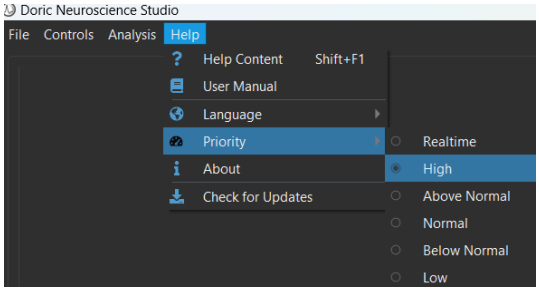

Figure 1.1: *Set Software to High-Priority.*

2. ***Doric Maintenance Tool (DMT):*** During installation, a secondary utility called the ***Doric Maintenance Tool*** (DMT) will also be installed automatically. Alternatively, you can download it on the Doric Lenses website. The DMT allows you to monitor the firmware version of your Doric devices and update the firmware when necessary.

   Note: If you have switched from DNS v5 to DNS v6, older devices will require a firmware update to be recognized by the new version of the software.

3. ***danse™:*** Lastly, the ***danse™*** software is used for post-recording data analysis. It allows you to open recorded files, access signal attributes, and compute $\Delta F/F$ for signal visualization. A yearly license version of danse is also available, which includes full analysis capabilities and behavioral analysis features, all without requiring any programming knowledge. You can watch all available tutorial videos on the Website. Do not hesitate to contact us at sales@doriclenses.com to request a free trial and an activation key.

Figure 1.2: *Doric Lenses Softwares*

1.3 Create a Fiber Photometry Configuration

Before creating the configurations, we recommend following the hardware manuals (section 1.1) to properly assemble and wire the setup first. Then take steps below to make the configuration.

Also note that this chapter provides a general overview of the software application. For more detailed information about individual icons within the software, please refer to the DNS manual.

1. Turn on the device(s) and open *Doric Neuroscience Studio* (DNS) on the computer that is connected to the device(s).

2. Once DNS is opened, the ***Device Selection*** window should automatically pop up. Here, you should see the console (NC500, BBC300 or BFMC-G3), and at least one BFPD camera(s). Select your console then click **Connect Device**. Once connected, close the window (as in Fig. 1.3).

NOTE:

- BFMC-G3 system has 2 BFPD cameras, both of them are supposed to appear in the **Device Selection** window.
- In **Device Selection** window, do not select the BFPD camera. This is because the camera is supposed to be used in slave-mode, as a channel configured in the console module.
- In **Device Selection** window, if you do not see a camera, most probably the camera driver is not installed properly. Refer to the troubleshooting chapter for more information or contact us at sales@doriclenses.com for support.
- The BBC300 and NC500 console may take a few seconds to appear on the list. If it does not show up immediately, double-check that the two ends of the USB cable are correctly connected to the USB ports, wait for a few seconds, and then click the **Refresh** button.

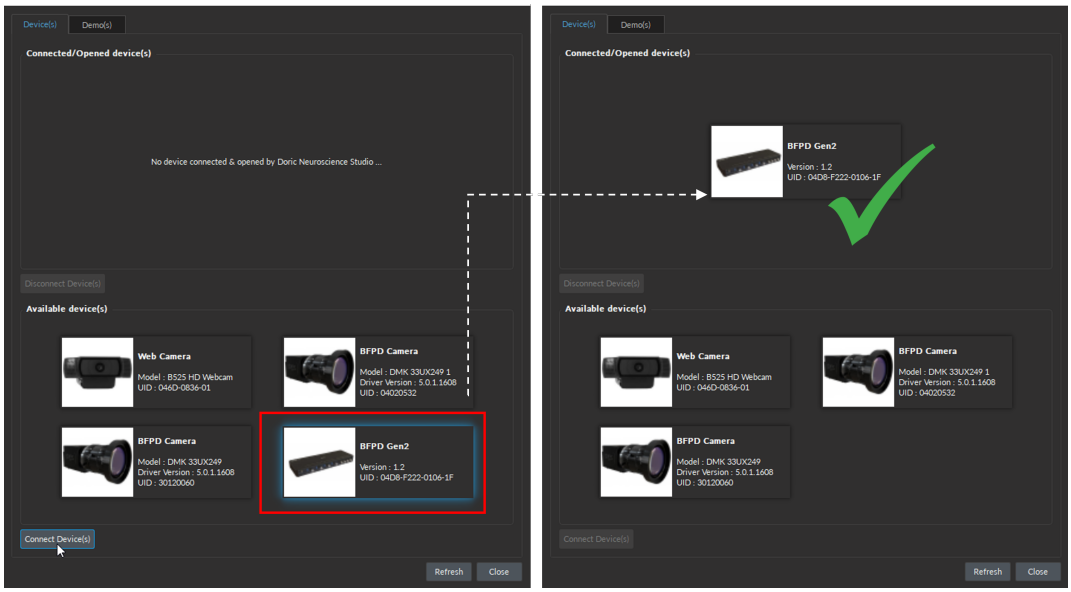

Figure 1.3: *Double click on the device of choice to connect it to DNS*

We will begin by making the photometry configurations.

3. Under console tabs, Click **Configurations** tab, select **Add Channel** (Fig. 1.4).

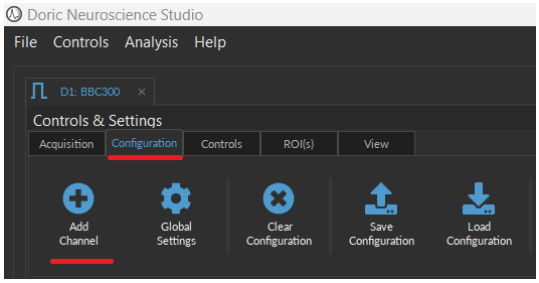

Figure 1.4: *Add Channel*

This will open a window that allows you to choose all photometry parameters. Here are the necessary ones exlained below with related points:

4. From the list on the left, select **BFMC** option, which is the photometry setting (Fig. 1.5).

5. Here you need to choose the **Preset Options** (Fig. 1.5). This drop-down box provides several options that automatically configure the camera and LED excitation timing to fit the application (1-color or 2-color). If you are using a BFTO or RBFMC bundle system, only the 1 CAM (camera) options will be available. If you are using the BFMC-G3, which has two cameras, the 2 CAM options will also become available.

   a) RBFMC and BFTO - one camera:
      - For green color photometry + isosbestic recording, select 1 CAM - 2 EXC / 2 Cycles.
      - For green color + isosbestic and red color photometry, select 1 CAM - 3 EXC / 3 Cycles.

   b) BFMC-G3 - one or two cameras.
      - For green color photometry + isosbestic recording, select 1 CAM / 2 EXC / 2 Cycles.
      - For green color + isosbestic and red color photometry, select 2 CAM /3 EXC / 3 Cycles. OR 2 CAM /3 EXC / 2 Cycles.

      Note: For BFMC-gen3 system, if you select the 2 CAM option, you will be able to increase the sampling frequency, as you will record green and red signals with different camera.

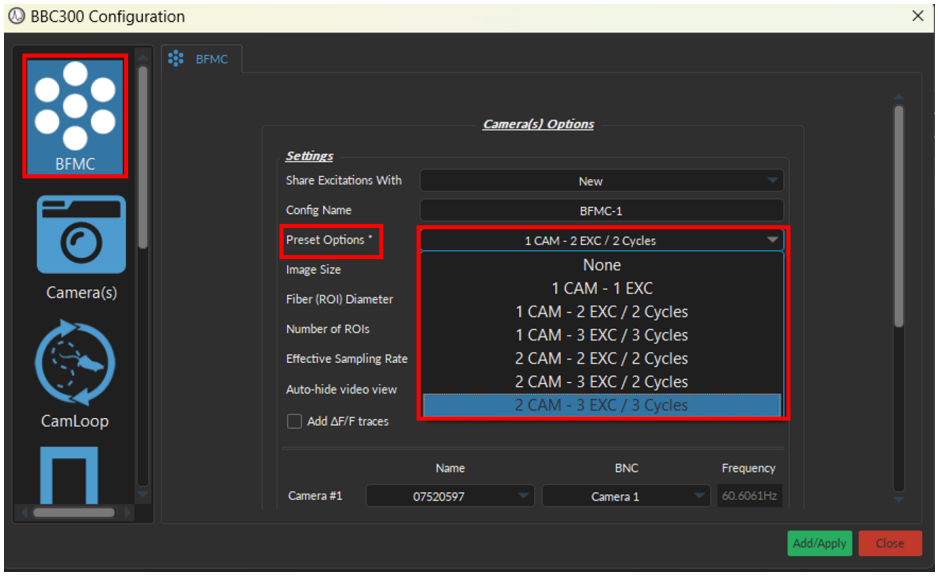

Figure 1.5: *Add Channel*

6. Next step is to choose the **Image Size**, which changes the FOV of the camera (zoom in/out). When imaging more fibers, a larger FOV is necessary to ensure all ROIs can be imaged in one frame (Fig. 1.6).

Note: When you are imaging small number of fibers, reduce the image size to reduce computational resources on Windows System processing during data acquisition (Fig. 1.11).

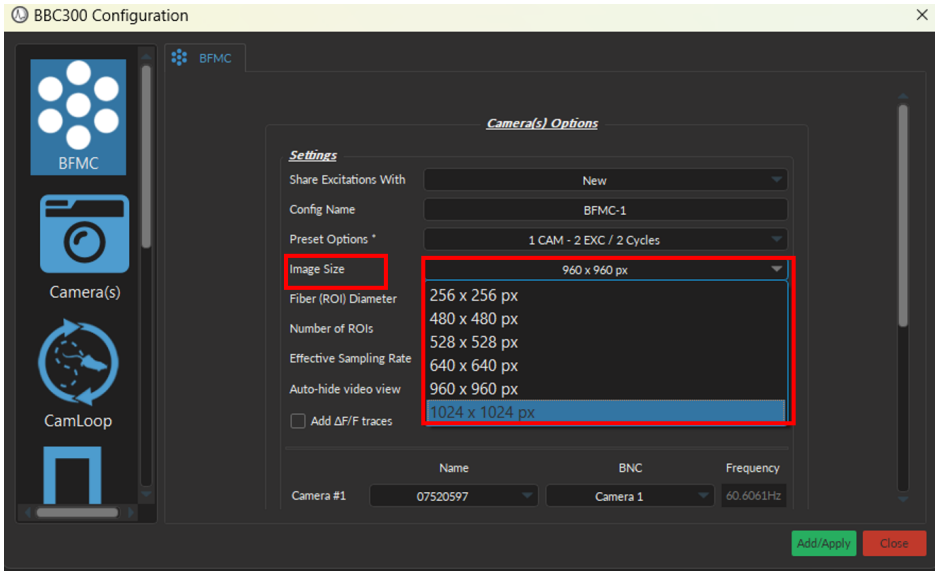

Figure 1.6: *Add Channel*

7. **Fiber (ROI) Diameter:** In this drop-down box, select the actual size of the fibers. This will define ROI circles diameter that will be drawn over the live camera view and insure each fiber has an identical diameter.

   If you are not sure, check your patch cords and cannula, the fiber diameter is usually labeled on them. In photometry, most clients commonly use 400 µm or 200 µm diameter fibers.

8. **Number of ROIs:** Here, select from how many brain sites/animals you want to record the signal. This will define the total number of ROI circles (with identical diameters) that will be drawn over the live camera view.

9. **Effective Sampling Rate:** According to the settings you selected so far, a number of sampling rate options will be unlocked here (Fig. 1.7).

To increase the sampling rate, you can (1) reduce the image size, and/or (2) reduce the number of excitations used (in preset options).

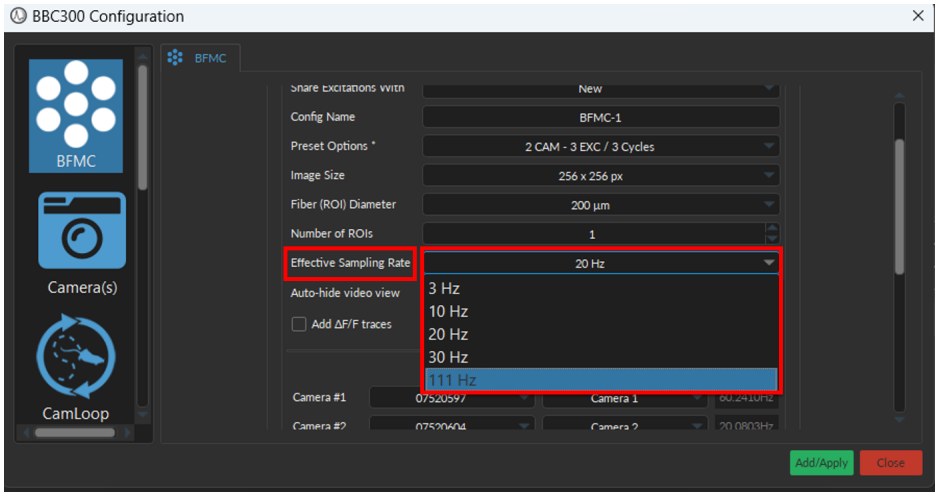

Figure 1.7: *Sampling Rate*

10. Ensure the camera/LED are properly assigned according to the hardwiring of the system.

    So far, we selected the settings of photometry recording. According to your parameters, software will automatically display two (for 2 EXC / 2 Cycles) or three (for 3 EXC / 3 Cycles) **Excitation #** channels in the box below (Fig. 1.8). There are few things to double check and ensure here:

    a) Ensure that the system is wired in the standard configuration, as described in detail in the hardware manuals. In this configuration, EXC#1 corresponds to the isosbestic signal, EXC#2 to the green signal, and EXC#3 to the red signal (Fig. 1.8).

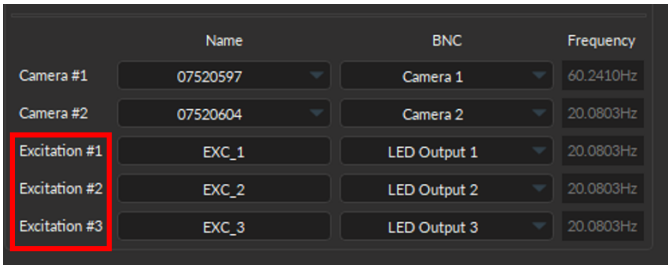

Figure 1.8: *Excitation Channels*

    b) If you are using a BFMC-G3 system, which includes two imaging cameras, and you plan to record green and red photometry, it is recommended that the isosbestic and green channels are recorded on one camera, while the red channel is recorded on the second camera. According to the **Show Timing Preview** window, double check the cycles (Fig. 1.9).

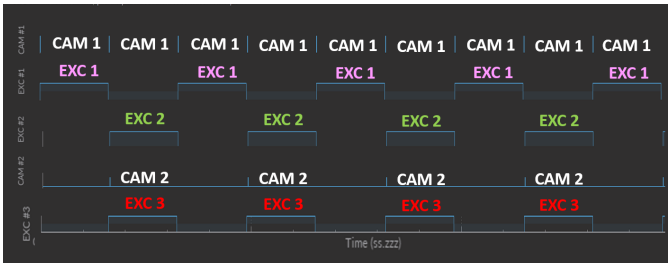

Figure 1.9: *Show Timing Preview*

This setup also simplifies data analysis, particularly when calculating $\Delta F/F$ in Danse Analysis software using the **"Photometry $\Delta F/F$ "** function.

11. The **Signals** box, displays the default name and color for each excitation channel. If you have followed the standard wiring, you can rename and adjust the colors as follows: EXC #1: Isosbestic with a pink color box, EXC #2: Green with a green color box, and EXC #3: Red with a red color box (Fig. 1.10).

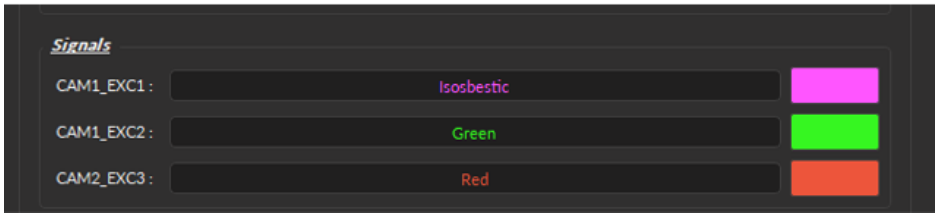

Figure 1.10: *Signals Name and Color Change*

Lastly, if you are interested to view live $\Delta F/F$ calculation in the acquisition window, do not forget to select **Add $\Delta F/F$ traces**.

12. When all changes are applied, click **Add/Apply** key, on the bottom right, and close the configuration window.

1.4 ROI Settings and Power Measurement

In this section, we will initially find the fibers to draw ROIs, then we will adjust the resolution of the image, if ever needed, next we will adjust the power of each fiber.

Step 1: Make sure all fibers are in the field of view

1. Initially, make sure all components are ON and that the sample patch cord is connected. In the **Acquisition** window, click **Live**. You should see excitation light coming out of the patch cord. If this is the case, hold the tip of the patch cord toward a strong light source, such as the light bulbs in the lab. In the **Acquisition** window, under **BFMC View**, you should see your fibers.
2. If the image size is very large and includes a lot of empty space in addition to the fibers, try reducing the **Image Size** parameter in the configuration (Fig. 1.11). This will help facilitate data processing by PC. To modify the **Image Size**, click one of the **Camera Settings** icons on the right side of the **Acquisition** window to open the settings window (Fig. 1.12).
3. If you cannot see all the fibers, or if some fibers appear cut off in the middle, take the following two steps:
   - First, make sure the **Image Size** is large enough to include all the ROIs in the field of view. To modify the **Image Size**, click one of the **Camera Settings** icons on the right side of the **Acquisition** window to open the settings window (Fig. 1.12).
   - Second, adjust the field of view offset along the X and Y axes. To do this, switch to the **Control** tab. Disable **Auto Center**, then adjust the X and Y offset until all fibers are within the field of view (Fig. 1.13).

Figure 1.11: *Adjusting BFPD View*

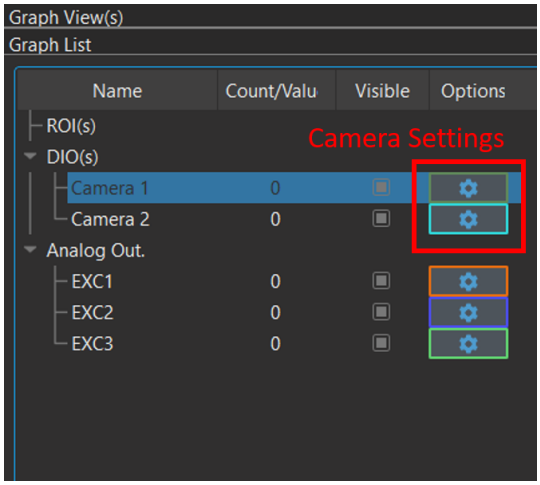

Figure 1.12: *Access Image Size*

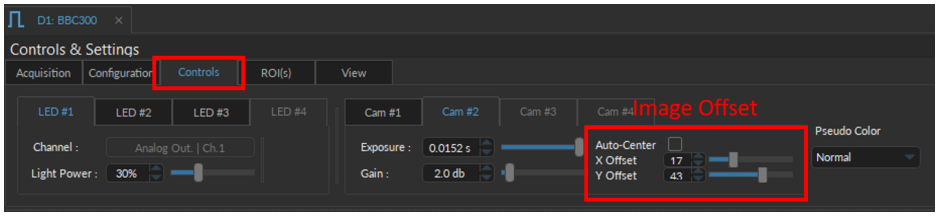

Figure 1.13: *Adjusting Image Offset*

Step 2: Adjust ROIs and label the patch cord outputs

1. Now that you are able to see the fibers, we need to identify which branching patch cord output corresponds to each ROI and fiber in the image. First, in the DNS software, click **Live**. Then select one patch cord output and gently move without disturbing the others. In the **Acquisition** window under **BFMC View**, you will see that the corresponding fiber image begins to blink while the others remain stable.

    a) If you are using a BFTO system, the branching patch cord outputs are labeled with numbers. Match these numbers by dragging the ROI with the same number and placing it over the corresponding fiber image (Fig. 1.14).

    b) If you are using a BFMC bundle system, the branching patch cords are not labeled. In this case, label each branch manually in the lab and assign a number to each one. Then drag the ROI with the matching number and place it over the corresponding fiber image (Fig. 1.14).

2. After all ROIs are properly positioned, switch to the **ROIs** tab, and select **Editing Locked** to lock the ROIs in place (Fig. 1.15). This will prevent accidentally moving the ROIs.

    Note that at anytime, you can unlock and readjust the ROI position. You can also DELETE an ROI by first selecting it (dotted line) and then using the “delete” key on your keyboard..

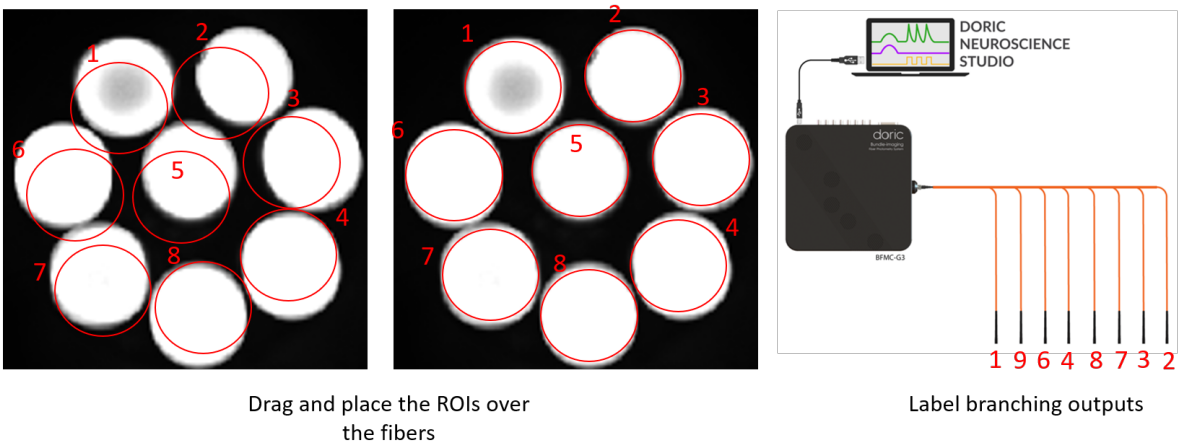

Figure 1.14: *Adjusting ROIs over the Fibers*

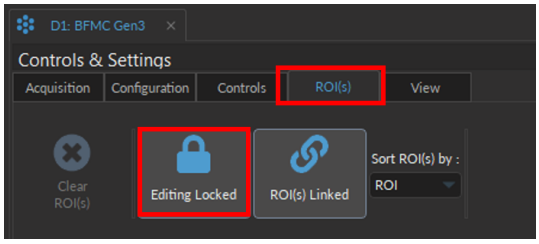

Figure 1.15: *Lock the ROIs*

Step 3: Adjust the power of each light

Before running the experiments, it is a good idea to measure the power of each light, and makes sure there is sufficient power. It is important to measure each light separately.

Note: If you are planning to use the system with a rotary joint, we suggest you also connect the rotary joint and measure the power the at the output before delivering to the animal.

Here are instruction for power measurement:

1. Go to the **Control** tab (Fig. 1.16).
2. Set the **Light Power** of LED #2 and #3 to 0%. In this case, only LED #1 will generate light (Fig. 1.16).

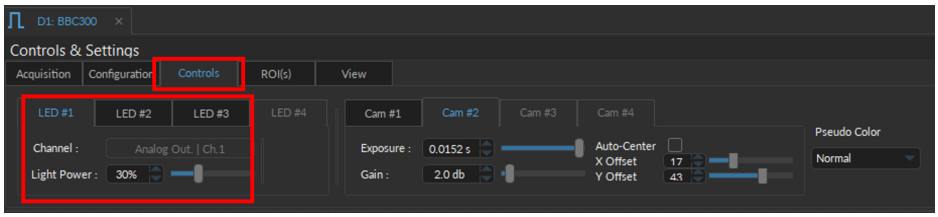

Figure 1.16: *Change LED light Power*

3. In the **Acquisition** tab, run the system in **Live** mode and then measure the output power of the branching patch cord outputs using a power meter. We recommend keeping a record.

Note: If the system has been wired following the standard configuration, LED #1 corresponds to the isosbestic LED (purple light). Make sure to update wavelength on the power meter settings when taking the measurement.

4. If the measured power is too high, go back to the **Control** tab and reduce the LED #1 **Light Power** Percentage. Repeat step #2 and #3 until the desired power is reached. Note down the ideal % for that LED.

5. Repeat the same process for other LEDs.

6. Once you've determine the ideal % for each LED one at a time, remove the 0% and adjust the two remaining LEDs according to the value determined earlier.

Note: If you are using a $400\,\mu$m, $0.57\,\mathrm{NA}$ patch cord and rotary joint,usually between 30–80 μW is where you can find the best power.

7. Now the configuration file is ready, you can save this configuration file. To save it, in the **Configurations** tab, select **Save Configuration**, then select an appropriate name and save it. In the future, simply open the saved configuration file to record data (Fig. 1.17).

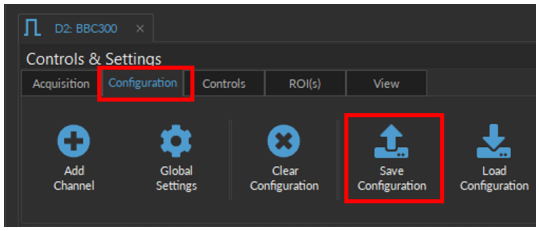

Figure 1.17: *Save Configuration*

1.5 Data Acquisition in DNS

Once the configuration is complete, proceed to the **Acquisition** tab in the console to begin data recording.

Step 1: Data recording

For real-time signal monitoring, use the **Live** option. To record and save data, click the **Record** button. Before saving, you can specify the destination folder and file index using the **Saving Options** button (Fig. 1.18).

In the **Saving Choices** box, note that the first option, **DIO and timing Data (Camera(s) and LED Excitation(s))**, will save all photometry signals, LED excitation signals, and camera feeds. To keep the files size small and manageable, we strongly recommend to skip saving camera feed by selecting the second option **Camera(s)/Video Feed(s)**.

Lastly, the **Auto Concatenate datasets created** is a safety check. In fact, when the recorded file gets too large, bigger than 30 GB, to speed up PC memory processing, software will automatically split data into smaller files. When the recording is finished, if option **Auto Concatenate datasets created** is selected, it will merge all the spitted files into a single one. If not selected, it will save data as multiple files.

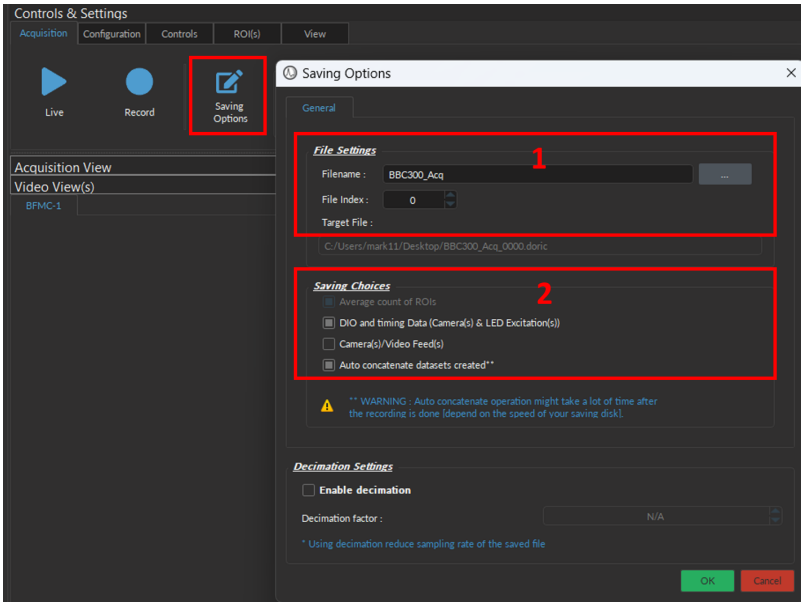

Figure 1.18: *Saving Options*

Step 2: Acquisition Window

The image below, displays the Acquisition window, when you are recording a bundle photometry signal (Fig 1.19). **Note** that the signals displayed in the image are not real experimental data; they were recorded from ambient light.

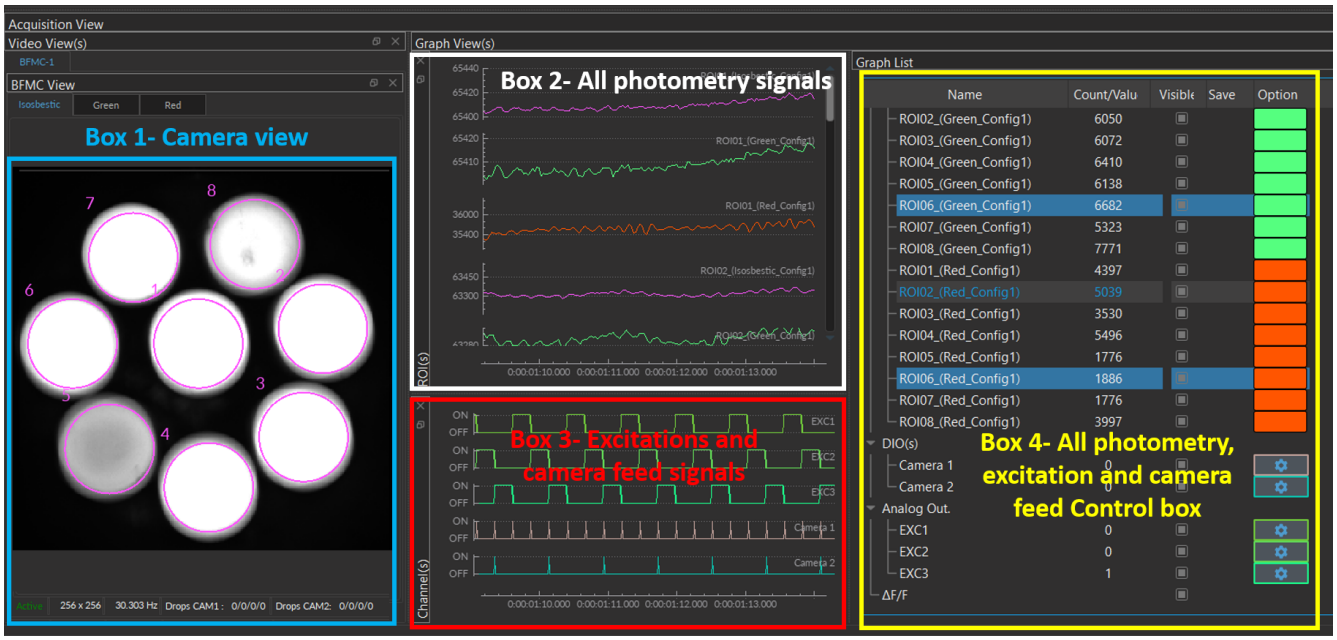

Figure 1.19: *Acquisition Window*

Below are descriptions of each highlighted box:

1. **Box 1:** This box contains multiple BFMC camera view tabs, depending on the configuration you selected. Here, you should see the fibers and the signals coming from the brain through each fiber.

   Note: If the patch cord is connected to the animal but you see a black frame with no visible fibers, try the following steps:

   - Check whether the camera is actively recording. If it is the case, a green "Active" indicator will appear under the camera view. If not, refer to troubleshooting chapter for solutions.

- Check the gain range and increase it to 2 db to see if a signal appears. To access gain settings, open **Controls** tab, and use the slider to adjust gain range (Fig 1.20).

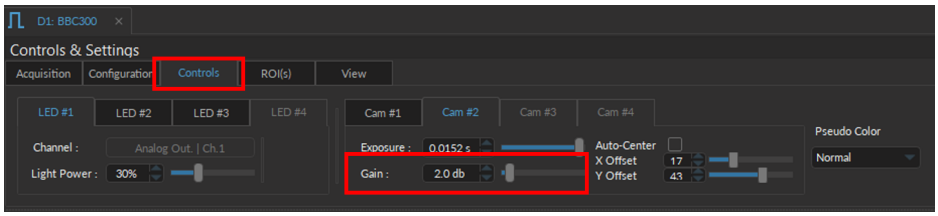

Figure 1.20: *Adjusting the Gain*

- You can also gradually increase the LED power and go live to see whether the signal becomes stronger.
- If none of the above helps, verify that the biosensor expression is adequate and that the cannula fiber is implanted close to the target brain region (within 100 µm distance).

2. **Box 2:** This box displays all de-interleaved photometry signals, including isosbestic, green, and red signals from all ROIs. By default, signals are sorted by ROI number. To change the sorting order, right-click on one of the signals and select from the available sorting options (Fig 1.21).

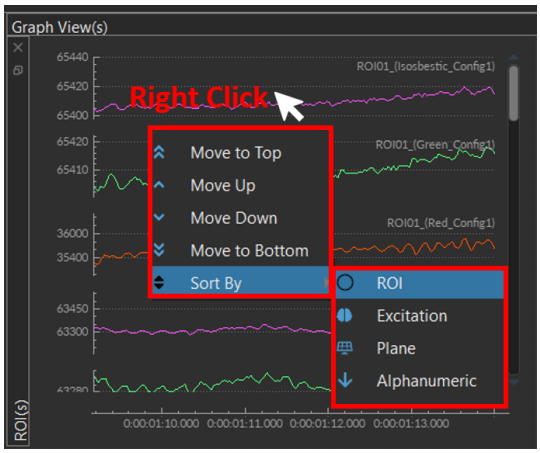

Figure 1.21: *Signal Sorting*

3. **Box 3:** This display box shows all LED excitation signals as well as the camera recording feed.

   Note You can close this display window to create more space for viewing the ROI signals. Closing this display will not affect data recording, and the information will still be saved in the data file.

4. **Box 4:** This box lists all ROI signals, cameras, and LED excitations. By default, all signals are visible, but you can deselect any signal to hide it from the display boxes. Hiding signals does not prevent them from being saved in the data file.

Note Signal saturation is uncommon in bundle photometry systems. However, it is important to avoid overstimulating biosensors with excessively high light power, as this can cause photobleaching of biosensors and distort the baseline of the recorded signals.

2

Support

2.1 Contact us

For any questions or comments, do not hesitate to contact us by:

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