Activity 2

Re-referencing

For this next tutorial you will be using the **Demo1** tree. The data in this demo are from the Monster paradigm used during the data collection activity and discussed in the Boot Camp lecture. There will also be a description of the Monster paradigm and an explanation of the event code scheme in later tutorials. To view the first raw EEG file, double click on the **Demo1_Raw** node. If you do not see **Demo1_Raw** listed under the **Demo1** file header, press the + next to **Demo1**, which should produce a file called **Raw Data**. Press the + next to **Raw Data** and this should expose the **Demo1_Raw** node. Notice that you have 38 EEG channels. If you cannot see all 38 EEG channels, click on the  button until all channels are visible. The 38 channels include 32 EEG scalp sites labeled with the corresponding 10-20 electrode system label (FP1, Fz, etc.) and six external electrodes labeled EXG1-EXG6. EXG1-EXG6 correspond to the following positions:

EXG1- Horizontal EOG Right (HEOG_R)
EXG2- Horizontal EOG Left (HEOG_L)
EXG3- Vertical EOG Lower (VEOG_Lower)
EXG4- Vertical EOG Upper (VEOG_Upper)
EXG5- Left Mastoid (LM)
EXG6- Right Mastoid (RM)

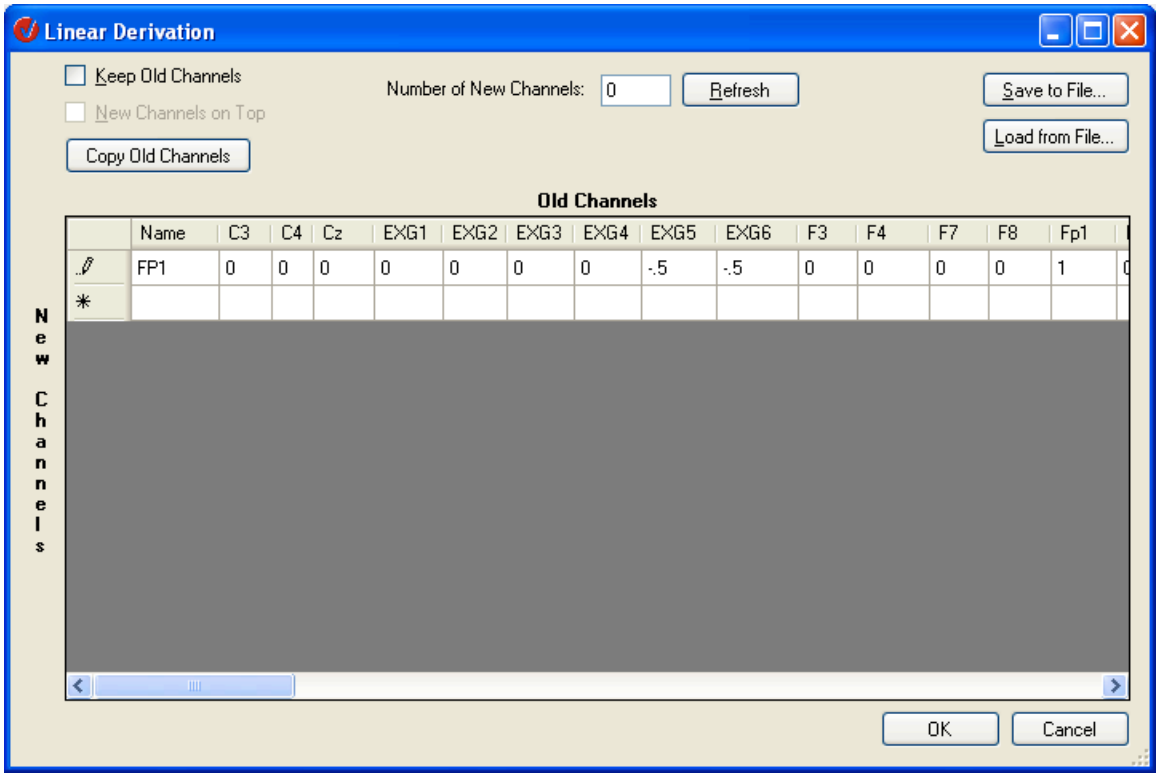
Once you have examined the raw EEG data, you are ready to start processing the data. Remember, it is always a good idea to thoroughly examine the raw EEG data from each subject before beginning data analysis. This allows you to identify any weird artifacts or segments of data that need to be excluded. This is especially important for re-referencing the data, because any artifacts present in the reference channels will be propagated to all of your channels when you re-reference the data.

Our first goal in this activity will be to re-reference the EEG channels to the average of the left and right mastoid channels. Our second goal will be to create a bipolar HEOG channel (HEOG_R minus HEOG_L) and a bipolar VEOG channel (VEOG_Lower minus VEOG_Upper). Creating bipolar channels for the EOG signals will help in the identification of eyeblink and eye movement artifacts, as you will see in the Artifact Rejection tutorial.

The data files we will be using throughout these demos were collected with the Biosemi Active-Two system. Unlike traditional EEG systems that record data in a *differential* mode, the Biosemi system records in what is known as *single-ended* mode. This means that each site is recorded as the potential between that site and the common mode sense (CMS) electrode site (which is analogous to a ground electrode). Conventional systems record the potential between an active site and the ground electrode and the voltage between a reference site and a ground electrode, and then electronically form the difference between the active and reference sites online. With the Biosemi system we perform this subtraction offline during data analysis. First let's re-reference the data recorded in single-ended mode. Later we will discuss how to re-reference data collected in differential mode.

Double-click on the **Demo1_Raw** file. To re-reference the data, select **Transformations > Dataset Preprocessing > Linear Derivation**. The Linear Derivation command is used to create new EEG channels that are linear (summed and scaled) combinations of the existing EEG channels. However, this way of referencing data can be prone to mistakes and can be rather tedious to set up, particularly if you are using a dataset with many channels. However, manually creating re-referenced data using the linear derivation tool is a great exercise to show precisely what is being done to the data during re-referencing. Therefore, we will work through the logic of re-referencing our data for one channel of EEG using the linear derivation tool to work through the math involved. Let's do this for the FP1 channel.

Click on the button *Load from File,* and when it prompts you for the text file, load **C:\Bootcamp_Analyzer2\workfiles\Reference_FP1_sem.txt**. The file should look like this:



Notice that the leftmost column of the spreadsheet contains the names of the output channels (i.e., the channels that we will create). The top row contains the names of the input channels (i.e., the original channels from the **Demo1_Raw** file). Each cell in the matrix contains a number that indicates the scaling of the input channel that will be used in computing the output channel. In this case, we are re-referencing channel FP1 to the average of the left and right mastoids. Most of the values in the matrix are set to 0, but if you look closely you will see a 1 wherever a particular electrode site in the input channel column is also present in the output channel column, in this case channel FP1. In the EXG5 and EXG6 columns (which represent the left mastoid and right mastoid, respectively), you will see that each of these columns has a coefficient of -0.5. Let's work through the logic of this. Originally, the FP1 electrode site was

recorded in single-ended mode as the potential between FP1 and CMS. Now we want to re-reference FP1 to the left and right mastoids by subtracting the average of channels EXG5 and EXG6 from channel FP1. Thus, we want to compute the following:

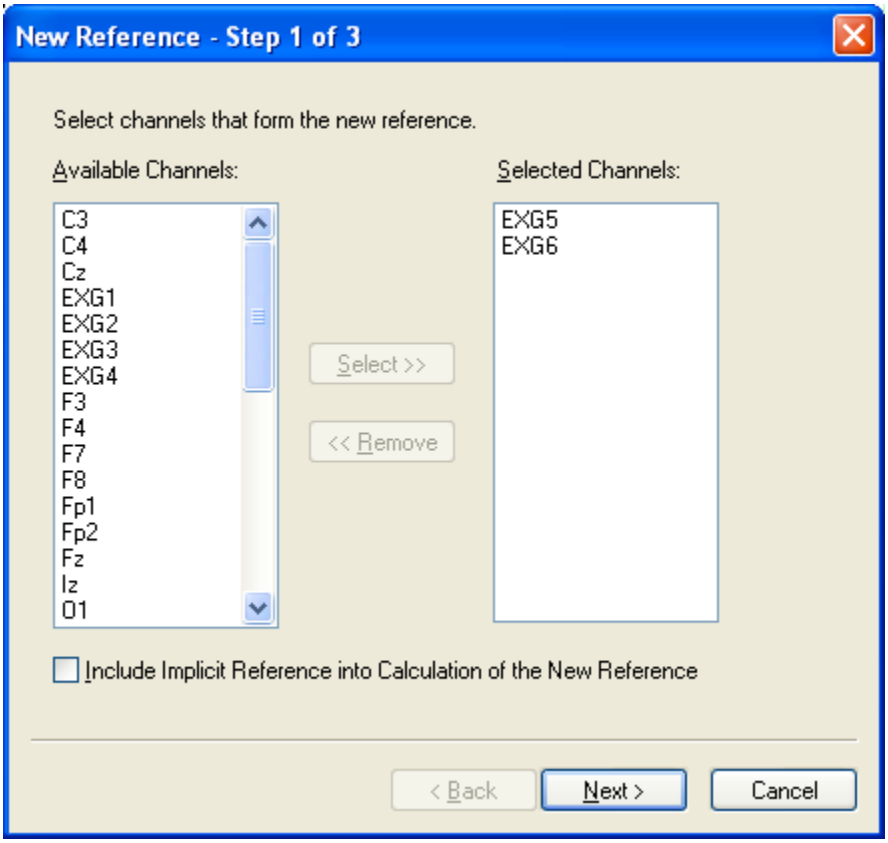
$$FP1_{referenced} = FP1_{original} - [(EXG5 + EXG6) \div 2]$$

which is the same as:

$$FP1_{referenced} = FP1_{original} + (-0.5 \times EXG5) + (-0.5 \times EXG6)$$

Click OK and a new node will appear under **Demo1_raw** labeled **Linear Derivation**. Re-label this new node **Demo1_FP1_sem**. FP1 has now been re-referenced to the average of the two mastoids (i.e. EXG5 and EXG6). Of course, we need to re-reference all of our data channels and not just FP1. Now that we understand how re-referencing works, instead of doing this using the linear derivation method for each channel of data, we will re-reference the entire data set in one step with the re-referencing option in Analyzer. Let's go back to our original unreferenced raw EEG data, node **Demo1_Raw**.

Go to **Transformations > Dataset Preprocessing > Channel Preprocessing > New Reference**. The window should look like this:



Select the two mastoid channels (EXG5 and EXG6). You will see that there is a checkbox for whether to include an implicit reference into the calculation of the new reference. The *Include Implicit Reference into Calculation of the New Reference* button should be unchecked for re-referencing data collected in single-ended mode, because there is no real implicit reference channel. Click *Next*.

In the next window you need to indicate the channels to which you would like the new reference to be applied. We will re-reference all 32 of the EEG data channels, but we will not re-reference the external eye or mastoid channels or the Status channel (which contains the event code information). Click *Next*.

It will then ask you the name of the new channel (leave this blank). Click *Finish* and a new node will be created named **New Reference.** Rename this node to **Demo1_ref_sem**. Now we have re-referenced the EEG data channels. Before we create the bipolar EOG channels, let's work through the logic of re-referencing for data collected in differential mode, which many of you may be using in your own labs.

Re-referencing Data in Differential Mode

Unlike the Biosemi system, most systems record the data online as referenced to a specific site, like a mastoid, an earlobe, or the nose. If the data are originally collected with a reference on one side of the head (like a mastoid or earlobe), it is usually desirable to re-reference the data offline to the average of sites over both sides of the head (e.g., to the average of the left and right mastoids). This is conceptually the same as the process we just completed in which we re-referenced offline to the average of the two mastoids, but we need to take into account that the data are already referenced to one of the mastoids. How exactly does this work?

Let's imagine that we collected our data online referenced to the left mastoid. This means that each of our data channels and the right mastoid were all collected as the difference between the data channel and the left mastoid. For the Fp1 site, for example, this is literally equal to Fp1 – Left Mastoid.

Now we want to re-reference our data so that each channel is re-referenced to the average of the two mastoids. If you work through the algebra, it turns out that in order to re-reference our data to the average of the left and right mastoids, you simply need to subtract out half of the right mastoid. If you would like to work through this algebraically, the equations can be found on page 108 of Luck, 2005 or in your boot camp lecture notes.

To see how this works, go to **Transformations > Dataset Preprocessing > Linear Derivation** and load the text file **C:\Bootcamp_Analyzer2\workfiles\Reference_FP1_dm.txt**. You can see that for the new channel FP1 there is a -0.5 for EXG6 (right mastoid) and a 1 for FP1 just like we had for the data collected in single-ended mode. However, because in this example the data were collected (hypothetically) as referenced to the left mastoid online, there is a zero in the EXG5 spot (remember, the data were already collected against this reference online, hypothetically).

With this derivation we will end up with FP1 re-referenced to the average of the right and left mastoid.

Once again, we can do this for all of our EEG channels in one step using the re-referencing tool in Analyzer (feel free to hit *Cancel* on the linear derivation for FP1). Make sure that you have selected the unreferenced raw data, **Demo1_Raw,** and then go to **Transformations > Dataset Preprocessing > Channel Preprocessing > New Reference**.

This time you will just select the right mastoid as a reference (EXG6). You will also need to make sure that you DO check the box for including an implicit reference into the calculation of the new reference, since these data were (hypothetically) referenced online to the left mastoid. Checking this box means that Analyzer will subtract out half of EXG5 from the EEG channels to which you choose to apply the selected reference. Leaving this box unchecked would mean that Analyzer would subtract out the entire channel from each of the selected EEG channels, and that would not result in an average mastoid reference. Again, we will re-reference all 32 of the EEG data channels. Rename the new node to **Demo1_ref_dm**.

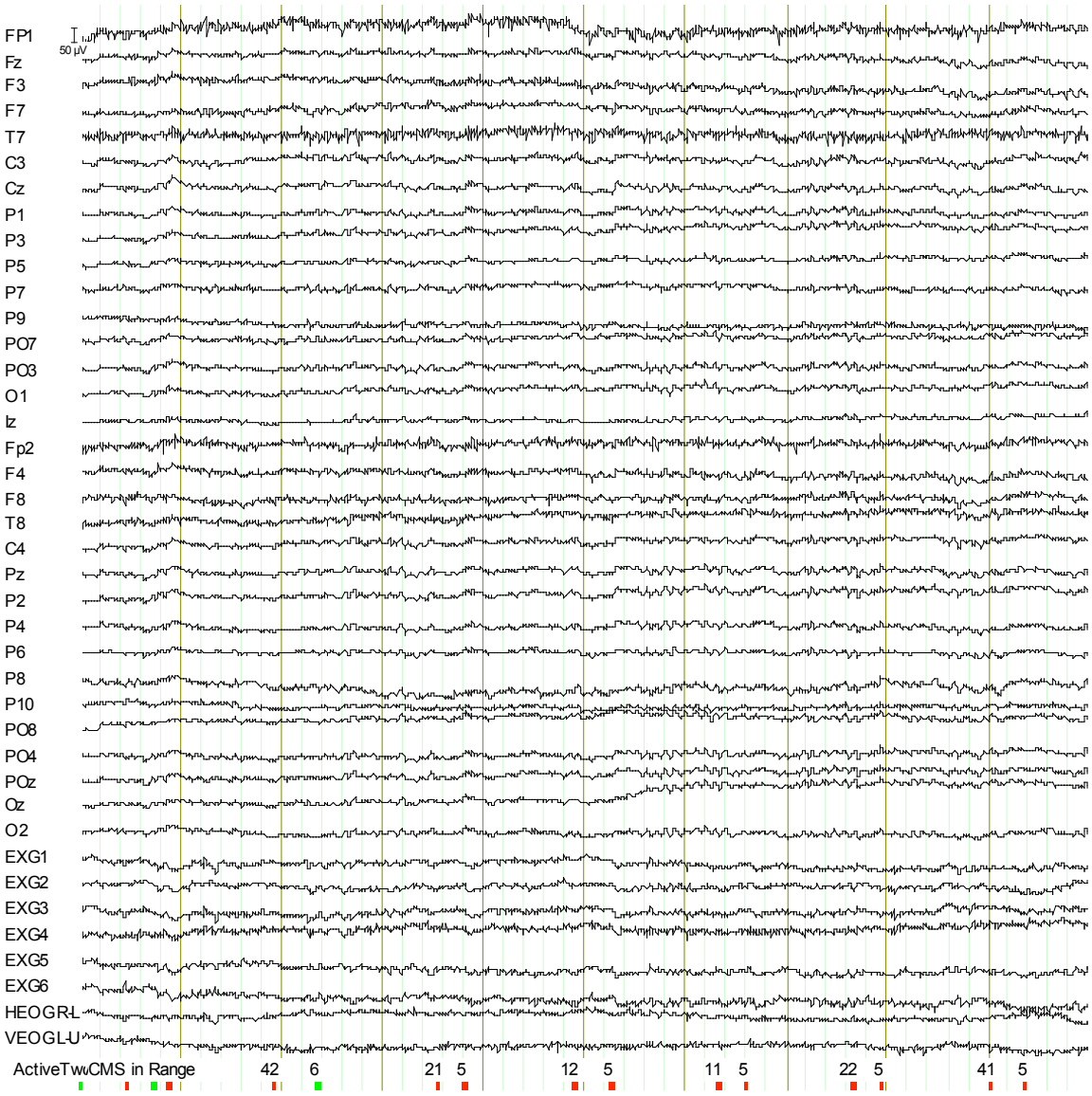
Bipolar channels

In addition to referencing the scalp sites to the average of the mastoids, you may also want to create bipolar channels from the ocular electrodes. We will discuss the usefulness of these bipolar channels more in the tutorial on artifact rejection.

Double click on **Demo1_ref_sem** and select **Transformations > Dataset Preprocessing > Linear Derivation** and load file **C:\Bootcamp_Analyzer2\workfiles\Bipolar.txt**.
If you scroll down to the bottom of the Linear Derivations window, you will see a channel labeled HEOGR-L, which is created by taking channel EXG1 minus channel EXG2 (HEOG_R minus HEOG_L). This will create a bipolar channel. By creating a channel like this, all of the EEG that is the same at the two HEOG sites will be subtracted away, making it easier to see the horizontal eye movements, which are opposite in polarity at the HEOG_L and HEOG_R sites (this will become more important later on in the tutorials).

In addition to the bipolar HEOG channel, we have also created a bipolar VEOG channel (VEOGL-U). This channel is the subtraction of VEOG_Lower minus VEOG-Upper (EXG3 minus EXG4). This subtracts away all of the brain activity in common to these two sites, leaving only the difference (which is large for blinks, which are opposite in polarity below versus above the eyes).

Click OK and a new node will appear under the **Demo1_ref_sem** labeled **Linear Derivation**. Rename this new node **Demo1_ref_sem_bp.** The re-referenced data should look like the screen shot below:



Next try to re-reference the data for **demo2.** Since we have already done this on the first demo, you can click on the **Demo1_ref_sem** node and drag it down onto **Demo2_Raw**. One of the most convenient features of Analyzer is that when you click on something in a tree and drag it onto a different node, it will replicate the operation you performed on the first node on the second node. Very cool! Another nice feature of Analyzer is that it keeps track of the manipulations that you have performed. This information is available if you right click on the history node and select *Operation Infos*. Another way to keep track of all the operations you perform on the data is to rename the nodes with more informative names, which is what we have started to do. Go ahead and rename the referenced nodes under **demo2** with **Demo2_ref_sem** and **Demo2_ref_sem_bp.**

Now you have re-referenced both data sets and you are ready to move on to the next tutorial.