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I. Installation and Initial Setup Instructions

1. Run the OmniPlexDemoV1Setup.exe program.
   a. Accept all default settings
   b. Note that the installer will configure the 'Microsoft Visual C++ 2005 Redistributable' package and install Sentinel System Drivers. Do not cancel out of them as they are required for the OmniPlex Demo software to run.
   c. The setup program will install the following files on the hard drive:
      i. C:\PlexonData\wideband.ddt [16 ch spike data riding on a 5 Hz sine wave; the sine wave is meant to represent field potential signals]
      ii. C:\PlexonData\demo16.px
      iii. C:\PlexonData\demo16.pxc
      iv. C:\Program Files\Plexon Inc\OmniPlex\OmniPlexServer.exe [also icon shortcut on desktop]
      v. C:\Program Files\Plexon Inc\OmniPlex\PlexControl.exe [also icon shortcut on desktop]
      vi. C:\Program Files\Plexon Inc\OmniPlex\Manual\OmniPlex_Demo_QuickstartGuide_V1.pdf [this document]

2. Run OmniPlex Server.exe ( ) program
   Note: Some operating systems may require that you run OmniPlex Server with ‘administrator’ privileges. If you have difficulty running the program by left double clicking on the icon, then RIGHT CLICK the icon and select “Run as administrator,” as shown below.

3. Navigate to the File | Open... menu and open the 'C:\PlexonData\demo16.pxs' file.
The following layout should then appear in the OmniPlex Server:
4. Run OmniPlex Control.exe ( ) program.
   Note: Some operating systems may require that you run PlexControl with ‘administrator’ privileges. If you have difficulty running the program by left double clicking on the icon, then RIGHT CLICK the icon and select “Run as administrator,” as shown below.

5. Navigate to the File | Open... menu and open the 'C:\PlexonData\demo16.pxc' file.
6. Press "Start Data Acquisition"
Data from the wideband.ddt file should then begin flowing through the system and appear in the PlexControl software:
II. Software Setup

1. Overview of different components within the OmniPlex Server design view:

   1 – Amplifier, 64 channel, (Set analog filter options in this view) [REPLACED WITH TEXT ADC EMULATOR IN DEMO VERSION]
   2 – A/D card, 64 channel [REPLACED WITH TEXT ADC EMULATOR IN DEMO VERSION]
   3 – Spike Separator module (set digital filtering parameters for spike pathway here)
   4 – LFP Separator module (set digital filtering parameters for Local Field Potential pathway here)
   5 – Digital Input module (set DI board for Mode 1 or Mode 3 here)
   6 – Thresholding module for spike detection
   7 – Spike Sorting module
   8 – Main Data Pool; data which is viewable in PlexControl and recordable to disk

2. Set appropriate analog high-pass (low-cut) filter values within the Amplifier module [NOT APPLICABLE IN DEMO VERSION OF SOFTWARE]
a. Right-click on rectangular box and select “Edit Device Options”

b. Select either the 0.05, 0.5, 3, or 100 Hz high-pass filter from the dropdown list for each bank of 16 channels.

3. Set high-pass (low-cut) digital filter values for the Spike Separator
   a. Right-click on rectangular box and select “Edit Device Options”
b. Enter the high-pass filter cutoff and number of poles for the spike digital high-pass (low-cut) filter. 150-400 Hz is typical, with a 4 pole filter.

![Spike Highpass Filter Settings](image)

4. Set low-pass (high-cut) digital filter values for the LFP (local field potential) Separator
   a. Right-click on rectangular box and select “Edit Device Options”
b. Enter the low-pass filter cutoff and number of poles for the local field potential (low-cut) filter, as well as the sampling rate for the LFP signals as they will be recorded to disk. 300-400 Hz is typical, with a 4 pole filter, and a 1000 Hz sampling rate.

5. Set DI mode either to mode 1 (16 individual TTL lines) or mode 3 (16-bit strobed word input)
   a. Right-click on rectangular box and select “Edit Device Options”
6. Run the PlexControl program ( ).
7. Load appropriate PlexControl Configuration File (.pxc) using the File | Open menu command, or by clicking the “Open” menu button [optional]

   a. Create View Layout for Sources [optional; not required if loading a .pxc file]
8. Select Sort Method
   a. Click anywhere in the Waveforms window or the MultiWaveforms window to show Properties window for “Basic Sorting”
b. Scroll down Properties menu to “Sort Method” field, then left click to select sort method (choose either Templates, Lines, Boxes, Bands, or 2D polygon)

9. Start Data Acquisition
III. Adjusting Gains and Thresholds

A. Manual gain adjustment in the Properties table

(Important Note: this is analog gain in the Amplifier prior to separation of spikes and LFPs. Therefore the amplitude of the spike, LFP, and wide-band pathways will all be changed with a gain adjustment)

Note: To adjust gains on all the channels at the same time,
- Manually adjust the gain for Channel #1.
• Select the Gain cells for all the channels by Left Mouse Click on the “Gain” header. Then Right Mouse Click the Gain header and select “Set All Channels Like Topmost Selected Channel.”
B. Manual Threshold Adjustment (either in the Properties spreadsheet, or by left-clicking on the blue threshold line, dragging up or down, and then releasing)

Note: If the blue threshold line is not visible, then select the “View Options” (OPT) button and Click “Show Threshold”. You can also show/hide the Time-Voltage Grid as well as select the waveform update mode (Fade/Rolling/Erase) in this menu.
C. Automatic Gain and Threshold adjustment

a. Open the drop-down menu in the “Continuous for ‘Spike Separator’” window.

b. Select the buffer size to use by clicking the “SnapShot Options” button. 10 Seconds is a typical value to use.
c. Click the Auto Gain tab and select the method by which the Gain will be adjusted.

![Auto Gain Tab](image)

- Algorithm: Peak-based
- Set gain so that peak is at 0.7 of the total ADC range

![Threshold Setting](image)

d. Click the Auto Threshold tab and select the number of standard deviations away from the mean signal to set the threshold.

![Threshold Tab](image)

- Set the threshold at: 2.5 sigma
- Threshold Sign:
  - Positive
  - Negative
  - The sign of the mean signal
e. Click OK to exit the Continuous Snapshot Options menu.

f. Start collecting forward-looking snapshot by clicking the “Start Forward-looking Snapshot” button. Wait until the Snapshot has been fully acquired. The “STOP” button to the right of the “Start Snapshot” button will light up while the snapshot is being taken. When the snapshot has been fully acquired, the “STOP” button will fade to dark.

g. Click the “Perform Auto-Gain on Current Snapshot (All Channels)” button.
h. Click the “Perform Auto-Threshold on Current Snapshot (All Channels)” button.
IV. Spike Sorting

A. Modes for adding and viewing units

1. Using the Snapshot views to aid in setting up sorting parameters

   a) Open the “drop-down” menu in either the “Single channel waveforms” window or the 2D cluster window.

   [NOTE: Double left mouse click in the 2D Cluster window to toggle between the single channel 2D Cluster window and the multi-channel 2D Cluster window]
b) Select the buffer size to use by clicking the "SnapShot Options" button. 1000 spike waveforms per channel is typical. Then press OK to exit.

c) Press the "Backward-looking Snapshot" button to acquire and display the most recent 1000 spike waveforms that have crossed the threshold.
d) The most recent 1000 spike waveforms will be displayed in a static form, along with the menu toolbar for adding/redrawing/removing units (circled in red below). Add units [see the Methods for Spike Sorting below]
e) To return to Live mode after sorting after adding units, press the Show/Hide Snapshot button.
2. Setting up sorting parameters in live mode

   a) Open the “drop-down” menu in either the “Single channel waveforms” window or the 2D cluster window.

   [NOTE: Double left mouse click in the 2D Cluster window to toggle between the single channel 2D Cluster window and the multi-channel 2D Cluster window]
b) Left click the “Edit Units” button
c) The menu toolbar for adding/redrawing/removing units will appear. Add units as necessary [see the Methods for Spike Sorting below]

d) Click the “Show all/Current unit” button to display all sorted units on a channel or only the currently select unit in the Units window.
e) To select a particular unit (unit a, unit b, unit c, etc.), left mouse click on the corresponding window in the Units window.
B. Methods for Sorting

• Note 1 – See section above regarding how to select different from among the different sort methods)
• Note 2 – The examples below show how to set up the sorting parameters in live mode (see above). The procedures are almost identical for setting up the parameters while viewing a snapshot.
• Note 3 – Data acquisition must be stopped before the sorting method can be changed.

1. Sorting using Lines

   a) Click the drop-down menu button in the Single Waveform window

   ![Image of Single Waveform window showing sorting options]

   b) Click the edit units button
c) Click the define new unit button
**d)** Left click in the waveform display, drag the mouse, then release the mouse to create a line.
e) Click the Add Line button to add additional lines in the same manner. Waveforms which cross all the lines are Sorted as a unit.
f) Additional lines may be added to units in the same method, and additional units may be added using the “Add Unit” button.
2. Sorting using Templates
   
a) Click the drop-down menu button in either the Single Waveform window or the 2D or 3D cluster window

b) Click the "Edit Units" button in the corresponding window
c) Click the “Define New Unit” button and either circle a cluster (in the 2D or 3D cluster view) or cross waveforms (in the single channel waveforms view. Hold down the left mouse button, draw the circle around the cluster or line across the waveforms, and then release the left mouse button.
d) Add additional units in the same fashion

e) Click on the “Show All/Current Units” button to view one or all of the templates.

f) Select Unit A template and adjust the tolerance fit appropriately (moving slider left or right).
g) Repeat for Unit B, C, etc.

h) You can move individual components of the template up or down by clicking on a piece of the template and moving up or down (optional).

i) Click the “Edit Units” button to remove the display of the templates and the unit editing toolbar.
3. Sorting using 2D Polygons

a) Click the drop-down menu button in the 2D cluster window (note: left double click to toggle between the multichannel 2D cluster window and the single channel 2D cluster window)
b) **Click the “Edit Units” button**
c) Click the “Define New Unit” button and circle a cluster by clicking the right mouse button, dragging the mouse around a cluster, then releasing the mouse. The resulting contour may be moved by left mouse clicking on it and dragging it to a different location.

\[\text{Diagram showing cluster selection and movement.}\]

d) Repeat the above procedure to add more units. Click the “Show All/Current Unit button to view the 2D contours for either all the units or only the selected units.
4. Sorting using Bands
   
a) Click the drop-down menu button in either the Single Waveform window or the 2D or 3D cluster window

b) Click the "Edit Units" button in the corresponding window
c) Click the "Define New Unit" button and either circle a cluster (in the 2D or 3D cluster view) or cross waveforms (in the single channel waveforms view. Hold down the left mouse button, draw the circle around the cluster or line across the waveforms, and then release the left mouse button.

d) A band will be displayed in the waveform window. The center blue line represents the mean of the band, and the upper and lower yellow squares represent the min and max of the band at each time point. Only waveforms which stay in between the max and min of the band at each time point will be sorted as the unit.
e) The center, min, and max of the band at each time point may be adjusted by left clicking on the small square and dragging the square up or down.
5. Sorting using Boxes

   a) **Click the drop-down menu button in the Single Waveform window**

   b) **Click the edit units button**
c) Click the define new unit button
d) Two yellow boxes will appear (one solid, one dashed). Move the two boxes by left clicking and dragging them into position. Waveforms which enter both boxes will be sorted as a unit.
e) Repeat the above procedure to add more boxes. Press the Show All/Current Unit button to show boxes for either all the sorted units or just the selected unit.
6. **Automatic Spike Sorting using Valley Seeking Algorithm**

[To be written]
V. Manipulating windows and views

- Use the “SHIFT”+ Left Mouse to shift the 2D and 3D cluster display in the X, Y plane
- Use the mouse wheel to zoom in and out in the 2D and 3D cluster display
- Left click and drag to rotate the 3D cluster display
VI. Recording

To determine how recording is initiated and terminated, select the Recording Control tab within the Global Options menu.

![Recording Control Window](image)

<table>
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<tr>
<th>Options</th>
<th>Details</th>
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</thead>
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<tr>
<td><strong>Start Recording</strong></td>
<td><strong>From GUI Only</strong></td>
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<td><strong>Occurrence(s) of an Event:</strong></td>
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<td></td>
<td><strong>Source:</strong> #7: &quot;Single-bit Events&quot;</td>
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<td><strong>After</strong> 0::0::0::0 Hours::Mins::Secs have elapsed</td>
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<td><strong>Pause Recording</strong></td>
<td><strong>From GUI Only</strong></td>
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<td><strong>Occurrence(s) of an Event:</strong></td>
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<td><strong>Source:</strong> #7: &quot;Single-bit Events&quot;</td>
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<td><strong>After</strong> 0::0::0::0 Hours::Mins::Secs have elapsed</td>
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<td><strong>Stop Recording</strong></td>
<td><strong>From GUI Only</strong></td>
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<td><strong>Occurrence(s) of an Event:</strong></td>
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<tr>
<td></td>
<td><strong>After</strong> 0::0::0::0 Hours::Mins::Secs have elapsed</td>
</tr>
</tbody>
</table>

- **Note:** every Resume Recording starts a 'Frame'
- **Immediately Pause after Starting Recording**
- **After Stopping, Allow Restart Recording to a New File**
A. Start/Stop Recording from software

To control the start/pause/stop recording from the tool bar buttons in the PlexControl software (i.e., from the Graphical User Interface or GUI), select the "From GUI ONLY" option in the software.
B. Start/Stop Recording from a hardware trigger line

Recording can be initiated with any digital event.

For instance, to Start recording when RSTART=True and Stop recording when RSTART transitions from True to False (and an RSTOP event is generated):

a. Select the “After” radio button in the “Start Recording” and “Stop Recording” sections
b. Select “Other Events” from the Source drop-down menu

c. In the Start Recording section, select “RSTART” for the Channel.
   In the Stop Recording section, select “RSTOP” for the Channel.

Note: An “RSTOP” event is automatically created whenever the “RSTART” hardware trigger line transitions from True to False.
C. Enabling/Disabling Channels, and Selecting data to record to file
Enabled:

Disabling channels (unchecking the box in the Enabled column in the Properties Spreadsheet) removes the particular channel from the display, and prevents the selected and all the associated filtered signals (spike waveform segments, continuous wideband signal, continuous spike signal, and continuous LFP signals) from being recorded to file.

Rec Spks: Checking this box will result in thresholded spike waveform segments being recorded to file.

Rec Wdb: Checking this box will result in the wideband signal being recorded to file.

Rec SpkCnt: Checking this box will result in the continuous spike signal being recorded to file.

Rec LFP: Checking this box will result in the low-pass LFP signal to be recorded to file.
VII. Referencing

- Note: Note that the referencing described here is analog referencing prior to A/D conversion, and therefore apply to the wide-band signal prior to separation of spike and lfp signals with digital filtering.
- References on any electrode channel may be changed by using a drop-down list in the “Properties” table.

### Properties Spreadsheet for 'Sorted Spikes'

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<th>Gain</th>
<th>Reference</th>
<th>Threshold%</th>
<th>Num Units</th>
<th>Rec Spks</th>
<th>Rec Wdb</th>
<th>Rec SpkCnt</th>
<th>Rec LFP</th>
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### AMP Gnd (Hard Gnd in above picture)

Amplifier Ground (not directly connected to ground on headstage). Note: The AMP Gnd is not recommended for standard use, as it may not be as effective at common mode rejection as the other reference options.

| Ref A | Buffered Ground (input pin #9) on HST/8o50-G1-GR headstage (the ch 1-8 headstage) |
|       | Reference electrode (input pin #9) on HST/8o50-G1-TR headstage (the ch 1-8 headstage) |
|       | Buffered Ground (input pin 11) on HST/16V-G20 headstage |
|       | Buffered Ground (input pins 18 and 19) on HST/32V-G20 headstage |
- Buffered Ground (input pin #9) on HST/8o50-G1-GR headstage (the ch 9-16 headstage)
- Reference electrode (input pin #9) on HST/8o50-G1-TR headstage (the ch 9-16 headstage)
- Reference electrode (input pin 1) on HST/16V-G20 headstage
- Reference electrodes (input pin 1 and 36) on HST/32V-G20 headstage. Recommended to tie inputs pin 1 and 36 together to one reference electrode

<table>
<thead>
<tr>
<th>Ref</th>
<th>Description</th>
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<td>Programmable reference electrode #1, selected from the “REFERENCES” table</td>
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<td>Programmable reference electrode #5, selected from the “REFERENCES” table</td>
</tr>
</tbody>
</table>

- Use the “View | References” menu command to bring up the “Reference” spreadsheet window.
- Use the drop-down lists to choose which recording electrode you wish to serve as Reference electrode 1 (REF1), Reference electrode 2 (REF2), etc. You may chose up to 5 different programmable reference electrodes.

Consequences of using a Local Reference electrode, and method for using a local reference electrode and recording LFP signals.

- Note that since the analog referencing applies to the wide-band signal prior to A/D conversion and the separation of spike and LPF signals, applying a local reference may result in the elimination of the LFP signal. LFPs are typically recorded using a distant reference electrode.
- You may use a local reference electrode AND still record LFP signals from a distant ground in the OmniPlex system by sending the wide-band signals from a given headstage into two amplifier channels using jumper adaptors included with your OmniPlex system. The local reference electrode can be applied to the first amplifier board channels (e.g. ch 1-16) and not the second (e.g. ch 17-32). In this way, ch 1-16 will have a spike signal with local referencing, while ch 17-32 will have a wide-band signal with a distant reference or ground. To this, you will require (1) a hardware jumper (shown below) and (ii)
an appropriate .pxs file which supports duplicate wide-band signals, interpreting half the channels as spike and the later half as wide-band.

**Jumpering multiple Amplifier boards.**

![OmniPlex amplifier with jumper connecting the amplifier input of ch 1-16 and ch17-32 (top to boards). In this case, a local reference could be used on ch 1-16 but not on ch 17-32. The spike signals for the 16 electrodes would be obtained from ch 1-16 and the LFP and Wideband signal from ch 17-32. Channels can be renamed in the OmniPlex software, if desired.](image-url)
The above picture shows an OmniPlex amplifier with jumpers connecting ch 1-16 with ch 17-32 (top two boards), and connecting ch 33-48 with ch 49-64 (bottom two boards). In this case, a local reference could be used on ch 1-16 and 33-48 but not on ch 17-32 and ch 49-64. The spike signals for the 32 electrodes would be obtained from ch. 1-16 and 33-48, and the LFP and Wideband signals from ch 17-32 and ch 49-64. Channels can be renamed in the OmniPlex software, if desired.

**Note that hardware jumpers are included with each OmniPlex system.**

VIII. General Options

The General Options menu is accessed via the Global Options menu.
IX. Arranging windows within PlexControl

- Windows within the PlexControl program may be resized, repositioned, and pulled off as separate windows. To do these options, simply left click on the title bar of the window you wish to relocate, drag it to a new location, then release the left mouse button.
- If you wish to return to the original default window layout, you may either
  - Use the Window | Layout | Reset to Default Layout option, or
  - Click the View Layout for Sources in the Tasks/System window
X. Hardware Components [APPLICABLE FOR NON-DEMO VERSION OF OMNIPLEX]

A. Main Unit:

1. PC Link (connects to PCI board in computer)
2. Timing Control
a. Unused connector
b. TTL digital output trigger for currently selected sorted channel (to send to oscilloscope or audio monitor)
c. Continuous Spike Analog Output of currently selected channel (to send to oscilloscope or audio monitor)

3. Digital Input board
   a. Port A (primary digital input pathway)
   b. Port B (secondary digital input pathway)

4. Amplifier link; connect to back of Amplifier

5. 64 channel A/D board

6. OmniPlex Analog Input
   a. Ch 1-16 input
   b. Ch 17-32 input
   c. Ch 33-48 input
   d. Ch 49-64 input (can be connected either directly to bottom board in Amplifier, or to BNC Interface as shown above)

7. BNC Interface
   a. P2 connector - for interfacing BNC inputs to ch 49-64 of A/D system
   b. J2 jumpers – for connecting individual BNC connectors to pins on the P2 connector
   c. P1 connector – for interfacing BNC inputs to ribbon cable from Amplifier
   d. J1 jumpers – for connecting individual BNC connectors to pins on the P1 connector

B. Amplifier-OmniPlex Analog Input-BNC Interface wiring examples.
Example #1: Input 64 channels from Amplifier

- Use four ribbon cables to connect all four Amplifier boards into four connectors on the OmniPlex Analog Input board.
- The BNC Interface will not be used in this case.

Example #2: Input 62 channels from Amplifier (leaving Amplifier channels #63 and #64 unused) and 2 channels via BNC inputs (shown in picture above)

- Use three ribbon cables to connect top three Amplifier boards to ch 1-16, 17-32, and the 33-48 connectors on the OmniPlex Analog Input board.
- Use one ribbon cable to connect the bottom Amplifier board to the P1 connector on the BNC Interface
- Use the short grey ribbon cable to connect the P2 connector on the BNC Interface to the ch 49-64 connector on the OmniPlex Analog Input board.
- All 16 J2 jumpers should be connected
- The top 14 of the J1 jumpers should be connected, to send channels 49-62 through to the P2 connector and the A/D system. The bottom two J1 jumpers should be disconnected (so that amplifier channels #63 and #64 to not interfere with the signals sent into BNC connector #15 and #16).
- Connect the two external signals into BNC connectors #15 and #16.

C. Digital Input
### Port A:

<table>
<thead>
<tr>
<th>Pin #</th>
<th>Function</th>
<th>Pin #</th>
<th>Function</th>
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<tbody>
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<td>Data_1</td>
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<td>Data_16</td>
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<td>Data_12</td>
<td>25</td>
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[72]
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</tr>
<tr>
<td>13</td>
<td>Data_13</td>
<td>26</td>
</tr>
</tbody>
</table>

Digital Inputs are configured by right-clicking on the “Plexon Digital Input” module in the OmniPlex Server window and select “Edit Device Options...”.
High = +5V  
Low = 0V

Mode 1 – 16 individual TTL lines  
Mode 3 – 16 bit strobed word, read when strobed line = True

RSTART – used for remote start for recording  
RSTOP – Automatically inserted event when RSTART line transitions from True to False.

D. Amplifier:
Amplifier, Front View:

Amplifier, Back View:
1. Headstage Power switch (Provides +/- 2.5V power to headstages when ON)
2. Headset type switch: Set to 1X if using unity-gain headstages, or 20X if using 20x gain headstages. Adjusts fixed gain so that net overall hardware fixed gain is 250x prior to programmable analog gain modules.
3. Ground – put in right-most position (atism)
4. Ground Jack:

   Connect pin end of ground cable (4a in picture below) to Ground Jack, and connect alligator clip end of ground cable (4b in picture below) to any conductive material outside the animal’s skull that may cause noise (stereotaxic apparatus, metal tables or e-phys chasses, etc.

5. Input connector for ch 1-16 (26 pin female harwin connector, connect to male harwin connector on headstage cable)
6. Input connector for ch 17-32
   " "
7. Input connector for ch 33-48
   " "
8. Input connector for ch 49-64
   " "
   a. White dots on headstage cable facing up
   b. For 8-ch headstage cables, put first cable in right-most position, put second cable in left-most position
9. Headstage power light indicators
10. Amplifier link connector – to connect amplifier with "AMP LINK" board in main unit.
11. Power indicator light – on when amplifier is receiving power from the main unit.
12. Output connector for ch 1-16
13. Output connector for ch 17-32
14. Output connector for ch 33-48
15. Output connector for ch 49-64