Modified on 05-12-11 by KL&GG

Opt3d v10—a rough guide

General Overview

This program can provide spatial maps of optical data as well as the time course of activity at any particular location.

In Opt3d, the data are presented according to voxel rather than individual data channels. To achieve this, opt3d does the following:

1. Align the fiducial points from an MRI image with the fiducial points from a 3D-digitization file (.elp file). Because the source/detector locations are also included in the .elp files, this step puts the anatomical information and the montage information into the same coordinate system (defined by the intersection of the nasion and PA-PA planes).
2. Rescale the co-registered optode locations to fit the Talairach atlas coordinate system (the origin is the anterior commisure-AC).
   NOTE: Steps 1 and 2 can also be done outside opt3d (we use in-house software called coreg.m).
3. Define the path of detected light (i.e., banana) sampled by each source/detector pair.
4. For each voxel in Talairach space, average all channels whose banana passes through that voxel.

Using Opt3d

- ***Before* opening Opt3d**, you first need to create a number of files (detailed below).
- Open Opt3d and select the viewing size you prefer (View→Small, Medium, or Large)
- Load critical files into the program (File-->Open-->*each of the file types are listed separately*).
    - You can also edit them, if necessary (Edit-->*each of the file types are listed separately*).
- After everything is loaded, you can begin to look at the data (Display-->*Map, Time course, ROI, etc*) .
    - You will need to use the parameters file interactively to change the views, time scale, data type, etc.
    - See section on Looking at the Data
- The program can provide output ASCII files of:
    - The time course of activity at one location (i.e., voxel)
    - The time course of activity averaged across a set of voxels within an ROI
    - The data from a set of voxels within an ROI (voxels every 1 cm or every 5 mm)
    - See section on Saving the Data
    - Peak activity across time points (see Peak Analysis)
- To save the spatial maps (i.e., pretty pictures of the brain), you need to capture the screen (alt-print screen) and paste the image into powerpoint or some other software application.

Computer setup notes:

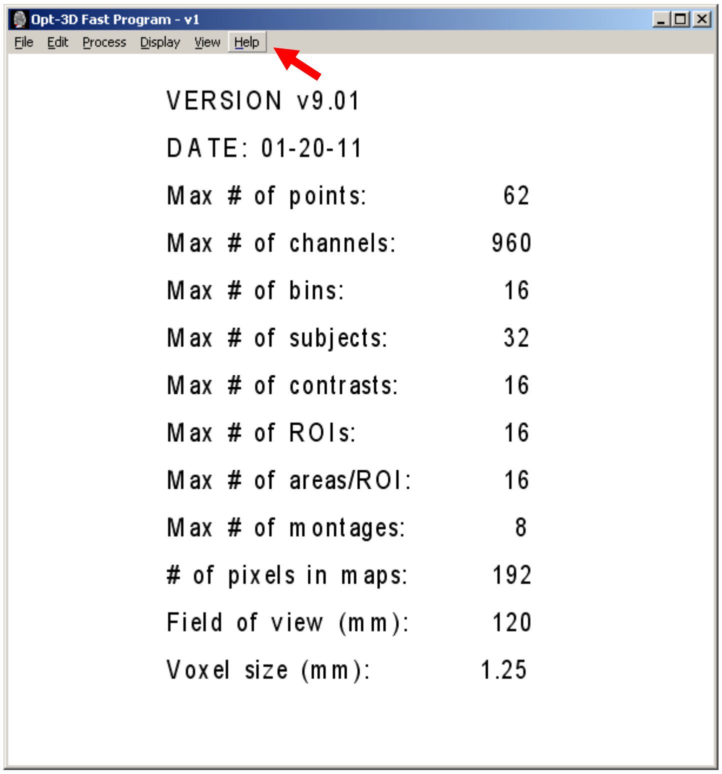
Currently, opt3d is only configured to run on a PC (Windows 2000 and up).

You will need to do the following to set up your computer to optimally run opt3d.

- **Create a temp folder at the level of the c: drive**
- Minimum of 256MB RAM, but you will be much happier if you have at least 2GB of RAM
- If you have a dual or quad core processor, you will be able to do other things on your computer while opt3d processes, which is quite nice because large data sets can take a little while to process.
- You may need to increase the file paging size for large data sets
- If the display plots a few spatial maps and then suddenly disappears, try the following:
  - On Windows 7
    - Control Panel → System and Security → System
    - On left sidebar list, click on Advanced System Settings
    - Click on the Settings button under “Performance”
    - Click on the Visual Effects Tab
    - Change the setting to “Adjust for best performance”
  - On Vista (and maybe earlier)
    - Control Panel → System
    - Click on the Advanced Tab
    - Click on the Settings button under “Performance”
    - Click on the Visual Effects Tab
    - Change the setting to “Adjust for best performance”

Before using Opt3d, check to see whether it can handle your data set

This can be done by going to “Help”.



```
VERSION v9.01
DATE: 01-20-11
Max # of points:        62
Max # of channels:     960
Max # of bins:          16
Max # of subjects:      32
Max # of contrasts:     16
Max # of ROIs:          16
Max # of areas/ROI:     16
Max # of montages:       8
# of pixels in maps:   192
Field of view (mm):    120
Voxel size (mm):      1.25
```

The Files: the big picture

The program needs a minimum of 4 files to run (and some of these files require additional files):

1. Parameters (*.prm)
2. Locations (*.loc)
    --calls the files for the template background brain (*.vmi and *.tai)
    --calls the montage files (*.mtg)
    --calls the 3-D digitization files (*.elp)
    --calls the talairach points files (*.tai)
3. Data (*.dat)
    --calls the data files (*.avg)

4. Design (*.des)

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5. OPTIONAL: Region of Interest (*.roi)
6. OPTIONAL: Align (*.ali)

Note:

The .prm file is created interactively from within the program, but the other files need to be created in a text editor before starting the program. Once loaded in the program though, they can be edited from within the program (usually – don't change the file name while editing within the program!).

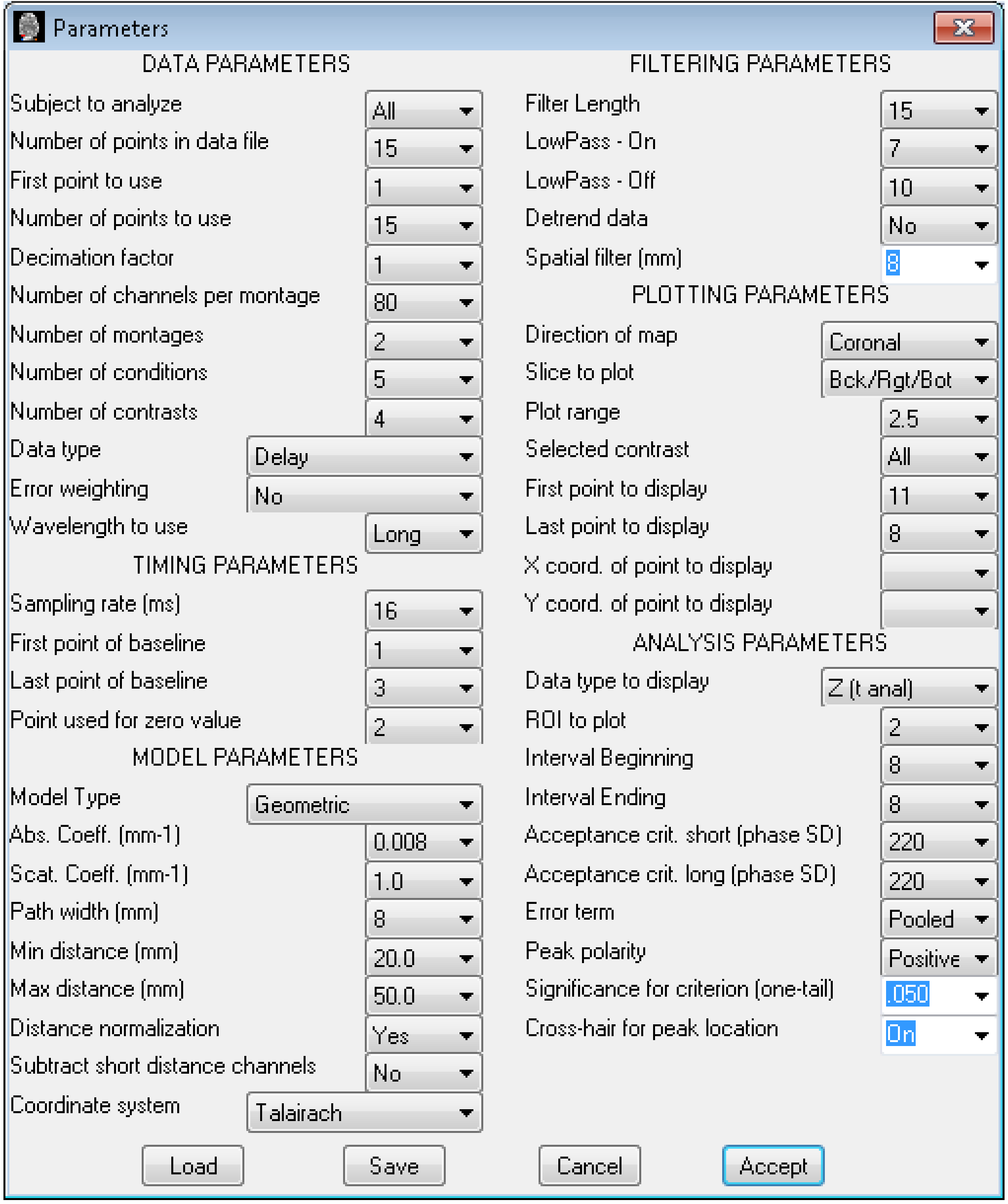
After loading all of the files, you then go to **Display** and select an option to plot the data. See Looking at the Data for an overview of the many ways to view the data.

The Parameters file

Parameters file (*.prm) Back to Page 1

The parameters file can be created interactively from within the program by selecting File→Edit→Parameters option. This will bring up the window seen below that can be edited.

These settings are used both for loading the data and for analyzing/displaying the data.



DATA PARAMETERS

Subject to analyze	All
Number of points in data file	15
First point to use	1
Number of points to use	15
Decimation factor	1
Number of channels per montage	80
Number of montages	2
Number of conditions	5
Number of contrasts	4
Data type	Delay
Error weighting	No
Wavelength to use	Long

TIMING PARAMETERS

Sampling rate (ms)	16
First point of baseline	1
Last point of baseline	3
Point used for zero value	2

MODEL PARAMETERS

Model Type	Geometric
Abs. Coeff. (mm-1)	0.008
Scat. Coeff. (mm-1)	1.0
Path width (mm)	8
Min distance (mm)	20.0
Max distance (mm)	50.0
Distance normalization	Yes
Subtract short distance channels	No
Coordinate system	Talairach

FILTERING PARAMETERS

Filter Length	15
LowPass - On	7
LowPass - Off	10
Detrend data	No
Spatial filter (mm)	8

PLOTTING PARAMETERS

Direction of map	Coronal
Slice to plot	Bck/Rgt/Bot
Plot range	2.5
Selected contrast	All
First point to display	11
Last point to display	8
X coord. of point to display	
Y coord. of point to display	

ANALYSIS PARAMETERS

Data type to display	Z (t anal)
ROI to plot	2
Interval Beginning	8
Interval Ending	8
Acceptance crit. short (phase SD)	220
Acceptance crit. long (phase SD)	220
Error term	Pooled
Peak polarity	Positive
Significance for criterion (one-tail)	.050
Cross-hair for peak location	On

Data parameters:

For the most part, these tell the program how to read the optical data (i.e., the *.avg files). To fill out this section, you need to know exactly how your data was collected (i.e., the Boxy settings) and how you segmented your data into event-related epochs (i.e., how you ran P_Pod).

- Subject to Analyze: Set this to ALL when running group-level statistics. Set it to a specific number (corresponding to the order of subjects in your .dat and .loc files) to look at a map or waveform for a given subject.
    - **NOTE:** when plotting single subject data, select "mean" or "t-score" for data type to display.
    - **NOTE:** This parameter does not control how many subjects will be loaded; this is actually controlled by the ".loc" file.
- Number of points in the data file: The total number of data points in your epoch (this can be found by opening up your *.avg file and counting the number of time points for 1 channel).
- First Point to Use/Number of points to use: This can be used when you have epochs that are greater than 62 points (maximum as of 5-6-11). Just put the starting point and then the total number of points you want to use for analysis. This will reset time point 1 to the specified starting point. Note that you will need to set your baseline and zero point with consideration of this new starting point.
- Decimation Factor**:** If you have too many points in your epoch to display AND you don't want to look at just a segment using the above option, then you can downsample your data with this option.
    - --The data are smoothed (by averaging neighboring points) before downsampling.
- Number of channels per montage: **If you are using the array style montage files (i.e., they have the word NEW at the top of the file) then you must select "Variable" from the menu.**
    - If your montage file is in the list format (a single vector) of channels with the same number of channels in each of your montages, then you will want to select that number from the pull-down menu. See (*.mtg) for more info.
- Number of montages: Set this to be equal to the number of *.avg files for each subject.
- Number of conditions: This refers to the number of trial types (or bins or conditions) in your *.avg files.
- Number of contrasts: This will update dynamically as you change your design file (*.des). It reflects the number of contrasts (or comparisons) specified in that file.
- Data type: Options are AC, DC, Delay (i.e., phase), Modulation (AC/DC). For the fast EROS signal, choose Delay.
    - "Combo" was an attempt at combining intensity and delay in hopes of improving signal to noise, but it doesn't, so we do not recommend using it.
- Error Weighting (yes/no): This can be used to standardize the channels for each subject.
    - Basically, for each channel it divides each time point in the subject average waveform by the standard deviation (across trials) associated with that time point. The net effect of this weighting procedure is that channels with lots of noise will contribute less to the overall grand average waveforms (because these channels will have large standard deviations in the denominator). This also means that short (superficial)

  - channels will be weighted much more than long (deep) channels. Thus the procedure becomes insensitive to deep effects.
  - **NOTE:** Don't use this option when using the physical model.
- Wavelength to use: When running a dual wavelength experiment, this option allows you to selectively analyze the short or the long wavelength channels, or you can collapse them together by selecting "All".
  - Alternatively, you can select oxy or deoxy and the program will calculate oxy and deoxy hemoglobin concentration changes (i.e., using both wavelengths. As proposed by Boas et al. (2001). *NeuroImage, 13*, 76-90). This option should only be used with intensity data, as there is no well defined transformation of phase data into oxy- and deoxyhemoglobin concentration,

Timing parameters:
Opt3d keeps track of time in terms of sampling points. The sampling period for your study can be found in the header information at the top of the original Boxy data file (or in Boxy itself under Timing/Averaging Settings) or you can also determine this by looking at your *.avg files-the first column should be time in ms.

- Sampling rate (ms): If your sampling period is an integer, then you can select the appropriate number from the pull-down menu. If it is a fraction (e.g., 25.6 ms), then you need to select "Auto". The Auto feature uses the first column of the *.avg file to generate the sampling rate.
- First and last point of baseline: This will be used for baseline correction and should be specified in points.
- Point used for zero value: Typically, you will set this to the sample point which is closest to your time-locking event. To determine which point to use for the zero point, you can look at a .avg file and count all points up to and including the zero point. Alternatively, you can also check the pre-event window in p_pod. Basically, the duration of the pre-event window INCLUDES the event marker point. For example, if pre-event was 5 in p_pod, then the 5th sample point coincides with the event marker (and should show a time of zero in the .avg file).
  - **NOTE: When you analyze only a segment of your data, time (and sample points) will be relative to your newly specified "first point to use".**

Model Parameters:

- Model Type: The geometric option will produce discrete bananas, meaning that channels are given a weight for each voxel of 1 or 0 (in or outside the banana). The physical option will produce probabilistic bananas (fondly referred to as fuzzy bananas). With the physical model channels are given weights ranging between 0 and 1 for each voxel. In addition, the physical model incorporates the biphasic nature of the effect of scattering on delay measures.
- Absorption Coef: We typically set this somewhere between .008 and .01
- Scattering Coef: We set this differently for geometric (.8 to 1.0) and physical (2.0).
  - In reality, the product of these 2 numbers is what is important for the software. You can get a sense of the impact of these settings by plotting a slice (rather than a surface projection) and then looking at the depth of penetration that is modeled (indicated by the dark gray area). Selecting different absorption or scattering coefficients will produce small effects on surface projection maps.
  - Also, for the physical phase model, only the positive (deep) part of the banana is used for surface maps (but both positive and negative parts are considered for slices).
- Path Width: We usually set this to 8mm, but you can use this as a way to spatially filter the data. Use a wider path for smoother filtering.
- Min Distance (mm): Opt3d will disregard any channels whose source-detector distance is smaller than this number (e.g., the shorter distance channels may not be far enough apart to hit cortex and therefore will not contribute to the effects of interest and could be discarded).
- Max Distance (mm): Opt3d will disregard any channels whose source-detector distance is greater than this number (e.g., distances greater than 55mm are usually too noisy for signal detection).
  - By selecting appropriate min and max distances, it is possible to study the effect of source-detector distance on the optical results.
- Distance Normalization: This can be used to standardize the channels based on the source-detector distance. Basically, for each channel it divides each time point in the subject

average waveform by the source-detector distance for that channel. In some respects this is similar to the Error Weighting option, but uses distance rather than phase variability to rescale the channels. For phase, it can be considered as a tool for studying variations in the velocity of propagation of the photon density wave.
    - **NOTE:** Don't use this option when using the physical model.
- Subtract Short Distance Channels: This was designed to "correct" for movement artifacts (especially in the AC or DC intensity data) by subtracting a short channel waveform from all other channels (short <= 15 mm; the actual criterion used to define a short channel is the Min Distance parameter).
- Coordinate System: This option allows you to plot the data as Talairach or MNI (based on the same algorithm used by SPM99 to move between the two spaces).

Filtering Parameters:
If you haven't already filtered the data using P_Pod, you can use this module to do low-pass filtering. P_Pod filters the entire block of continuous data before signal-averaging. Opt3d filters the data after signal-averaging (i.e., on a much shorter epoch window), so filtering in p_pod is preferable.

- Filter Length: Set this to 3 or 5 to implement a boxcar filter. For a more sophisticated filter, this represents the weighted "tails" of the filter (in sampling points). The length should be at least twice the period of the highest frequency remaining in the data (e.g., if you are filtering down to 10 Hz, the filter length should be at least equal to the equivalent of 200 ms in sampling points).
- Low Pass – On: (In Hz) Frequencies below this value, will not be attenuated (0% reduction in amplitude).
- Low Pass – Off: (In Hz) Frequencies above this value will be removed (100% reduction in amplitude).
- Detrend Data: -- This will average the first 3 points and the last 3 points of the epoch, compute a straight line between these points, and then subtract it from the data.
    - A consequence of this is that there will be no variance in the first and last points, thus the pooled error variance will be lower than the "true" variance. This, of course, would also be an issue for the first and last points when using the separate error variance option.
- Spatial Filter: Uses a Gaussian filter with a 25 mm kernel (the # you specify here reflects the full width half max--fwhm).

Plotting Parameters:
You will work interactively with these parameters throughout the analyses. This is where you can select how to look at the data (both where and at what time point or points). Note that Opt3d always works with a surface (a slice or the brain surface).

- Direction of Map: Axial, coronal, or sagittal
- Slice to Plot: This interacts with the direction of the map to allow you to plot surface projections (options are at the very bottom of the pull-down menu) or individual slices.
- Plot Range: The color bar scale is thresholded and starts at +/- 2/3 of the value you set here. Autoscale works, but depending on your data it's not always that useful.
    - For T-scores or Z-scores, I usually start with a scale of about 2.5 or 3.0. If you use 3, then everything with a color has a Z of at least 1.96 corresponding to $p < .05$ for an uncorrected 2-tailed test.

- Selected Contrast: You can select to plot ALL of your contrasts, or 1 at a time. For some analyses (e.g., cross-correlation, PCA), even if you select ALL, only the first contrast will be considered. To analyze another contrast, you must select it here.
- First and Last Points to display--You can use this 3 ways:
    - To display a map of 1 point in time, put the same number in both parameters (in points, not ms)
    - To display a movie across time, put the start and end points you desire for the movie.
    - To display a matrix of maps across several time points, put the last time point you want displayed in the "First Point to Display" slot and put the first time point in the "Last Point to Display" slot (i.e., reverse the order of the points).
        - With this option, you can only plot 1 contrast at a time (it will default to contrast 1, if ALL is selected).
    - **NOTE: If you are using the Interval Beginning and Ending in the Analysis Parameters section, you should set the first and last points to be equal.**
- X and Y coordinate of point to display—This can be used to go directly to the time course of a given location. You can also use this to specify the seed voxel for a cross-correlation analysis.
    - There are actually two ways to go to the time course waveform for a particular location.
        - You can right click on a location in the spatial map and this will take you directly to the corresponding waveform for that location.
        - You can determine the location of interest (X and Y coordinates), go to parameters and set those points, then go File and select Analyze→Timecourse.

Analysis Parameters:

- Data type to display: You have lots of options here.
    - **Means** – across subjects or for a given subject, depending on what you select for the "Subject to Analyze" parameter.
    - **T-scores** – across subjects or for a given subject, depending on what you select for the "Subject to Analyze" parameter. Notice that in Opt3D different voxels (and contrasts) may be based on a different number of subjects. This is taken into account for computing T-scores. However, the same T-score may correspond to different probabilities depending on the degrees of freedom. This may be a particular problem for border areas, when only a small number of subjects may contribute to the data (in fact, only locations with at least ¼ of the subjects, or at least 4 subjects, whichever is the least, are plotted). To correct this problem, use Z-score maps. Z(t anal) maps also allow application of random field theory to determine whether a particular value is significant, corrected for multiple comparisons.
    - **Consistency** – Uses a jackknife approach for calculating the correlation of each subject's average waveform with the grand average waveform for the remaining subjects (and then averaging the consistency scores across subjects). It can be plotted as an average across subjects or for a given subject. This differs from the mean value, T scores, or Z scores map because the entire epoch is considered for the analysis. However, no particular latency of effects is provided.
    - **Error Term** – The error term for the group level t-score (not available for individual subjects, but individual variability across trials can be found in the .avg and/or .avm files.

  - **Z(sqrt)** -- For each subject, contrast, and voxel, it first calculates the square root of the absolute value of each data point, preserving the sign (+ or -). Then it computes a t-score based on these new values and then finally converts those to Z-scores. This approach will de-emphasize the largest effects relative to the smallest effects and is most useful when you have small sample sizes (N < 10) with large variability. In this case, it tends to make distributions of the means more “normal” (or less “skewed”). With large N’s, this is not a problem because of the central tendency theorem.
  - **Z(boot)** -- The probability that the average of a random sample (with replacement) of individual subject means (with N equal to the sample size) is greater or smaller than 0 is computed (separately for each contrast and voxel), and then transformed into a Z score. This approach can be useful when the size of the signal of interest is not normally distributed across subjects (e.g., when you have outliers), but it takes much longer to compute.
    - **NOTE**: the correction for multiple comparisons based on random field theory cannot be applied to this analysis (as well as to the Z(sqrt) analysis).
  - **Z(t anal)** -- Z scores calculated from the p values for each t-test. The advantage of Z scores over the basic t-score plots is that the Z-scores will take into account the number of subjects going into each voxel. Because opt3d is location based, some subjects may not contribute data for every voxel that is plotted. Thus, when plotting t values, it is impossible to determine statistical significance by simply looking at the maps.
    - **NOTE**: Use this option to when you want to correct for multiple comparisons using the Random Field Theory.
  - **Latency** – This will plot a map where the color scale indicates latencies of peak activity. To use this option, you must also do the following:
    - Set “First Point to Display” and “Last Point to Display” to be equal to each other.
    - Set “Interval Beginning” and “Interval Ending” to reflect the range of time points you want to explore.
  - **Ampl/Latency** – Under construction as a useful way for estimating both amplitude and latency of a peak by fitting a cosinusoidal waveform to the data.
  - **Pulse Return** – For analyses of pulse data (only) time-locked to either the EKG or systole; **not to be used for EROS or NIRS experiments**.
    - Returns a latency corresponding to 30% of peak intensity (corresponds to the duration of systolic phase of pulse)

- ROI to plot—In the *.roi file you can define more than one region of interest. This parameter lets you select which one of those you want to plot. When you load a region of interest, it shows up as a green box on the spatial map, so you can see which region is being investigated. The current version of Opt3D only allows for box-like ROIs; also, it only allows for one ROI made up of only one box.
  - **TIP:** If you happen to put the bounding conditions in the wrong order (right to left instead of left to right), the program will still plot a green box that looks “correct”, but your peak measures (ex., for consistency or t-scores) won’t work. So, if you are getting all zeros down there for your peak measures, check your bounding box parameters. Also, the ROI must be defined in 3D, even if only surface maps are used.
- Interval beginning and ending—Designates the time interval you want to analyze. In theory it can be 1 point or a time series. You would set this as a time series for the following:

  - Mean amplitude measurement across time points (and plotted as mean, t-score, or z-score map)
  - Plot a latency map (color codes latencies of peak activity across space) for a certain interval only (to select the whole epoch, set the two values as the same);
  - Forward or Backward Cross Correlation (Display→Forward XCorr or →Backward XCorr): in this case it defines the section of data from the seed voxel used as a template (to select the whole epoch, set the two values as the same);
- Acceptance Criterion— Used to throw out bad (i.e., noisy) channels. The number relates to the standard deviation across trials for the delay (i.e., phase) measures. The .avg files generated with p_pod contain both the mean (first half of file) and the SE (second half) across trials for each time point (by bin and channel). Opt3d converts these SE scores to SD, and then averages them across the time series (and across bins or trial types). When you set a criterion, the program will ignore any channels whose standard deviation exceeds that criterion.
  - Determining the appropriate value for this parameter is somewhat tricky and will likely vary somewhat from experiment to experiment (and across different filter settings within an experiment).
  - As a general starting place, you might try using a criterion somewhere between 120 and 200 (in picoseconds).
- Error Term—Allows you to choose which type of error term (i.e., standard error across subjects) to use when calculating the t-scores.
  - Pooled: Uses the same error term for all time points in the epoch (error averaged across time-series).
  - Separate: Each time point has a separate error term.
- Peak Polarity—allows you to plot only positive, only negative, or both
  - But when you plot both, the peak value cross-hairs will always default to positive.
- Significance for Criterion—choose .05 for one-tailed criterion; .025 for two-tailedCross-hair—(toggle) marks (or not) the peak location (within the ROI if chosen) with a white cross.

Locations file format (*.loc)

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This file contains the information for the template background brain on which the data are displayed. It also contains subject specific information for alignment purposes and translation from channel to voxel space. Besides providing all the spatial information needed for 2- and 3D reconstruction, this file also defines the number of subjects in the analysis.

There are 2 possible formats for this file. The format depends on whether the co-registration and Talairach transformation occur in opt3d (in which case you use format 1) or with the Coreg script (format 2).

1. Using opt3d for co-registration and tai transformation:

Ex.

c:\data\rel\opt-crd\brain2.vmi (a segmented volumetric MRI file created with EMSE)
c:\data\rel\opt-crd\brain2.tai (the talairach points for the above brain)
8 4 (needs to sum to the total number of digitized points – usually # sources and # detectors)
1 (there's always a "1" before each of the following 3-line sets…these are usually subject-specific)
c:\data\rel\reg\rel.mtg (the montage file)
c:\data\rel\reg\rel001.elp (the *.elp file containing the 3d digitization of the source/detector locations)
c:\data\rel\reg\001ac_121202.tai (the Talairach points based on the MRI)
1
c:\data\rel\reg\rel.mtg
c:\data\rel\reg\rel501.elp
c:\data\rel\reg\501ac_022403.tai.

2. Using COREG first to create .tol files, and then loading these files into opt3d

Ex.

`c:\data\brain2.vmi`
`c:\data\brain2.tai`
`159 64` (needs to sum to the total number of digitized points – usually # sources and # detectors)
`2` (there's always a "2" before each of the following 2-line sets)
`c:\data\psd\reg\psd135_good.mtg` (the montage file)
`c:\data\psd\reg\psd135.tol` (the digitized locations in Talairach space – output of COREG)
`2`
`c:\data\psd\reg\psd146_good.mtg`
`c:\data\psd\reg\psd146.tol`
`2`
`c:\data\psd\reg\psd147_good.mtg`
`c:\data\psd\reg\psd147.tol`
`2`
`c:\data\psd\reg\psd155_good.mtg`
`c:\data\psd\reg\psd155.tol`

SEE SUBFILES FOR .LOC for details on the structure of the subfiles going into these lists

Data file format (*.dat) Back to Page 1

This contains the path and name of all of the averaged data files. The rows should be in the order Subject x Montage (slowest-to-fastest).

It must list a number of files equal to the number of subjects (as defined by the .LOC file) times the number of sessions or montages (as defined in the parameter table). **Note: the order of the subjects must be identical to that used in the .LOC file.**

In the following example, there were 7 subjects and each subject was run through 2 montages (a, b)

Ex.

```
c:\data\anc\pc00-00\ALLanc071a.avg
c:\data\anc\pc00-00\ALLanc071b.avg
c:\data\anc\pc00-00\ALLanc086a.avg
c:\data\anc\pc00-00\ALLanc086b.avg
c:\data\anc\pc00-00\ALLanc089a.avg
c:\data\anc\pc00-00\ALLanc089b.avg
c:\data\anc\pc00-00\ALLanc096a.avg
c:\data\anc\pc00-00\ALLanc096b.avg
c:\data\anc\pc00-00\ALLanc105a.avg
c:\data\anc\pc00-00\ALLanc105b.avg
c:\data\anc\pc00-00\ALLanc110a.avg
c:\data\anc\pc00-00\ALLanc110b.avg
c:\data\anc\pc00-00\ALLanc111a.avg
c:\data\anc\pc00-00\ALLanc111b.avg
```

Design file format (*.des)

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This file contains the contrast weights for analyzing the data. Each row represents a subject (in the same order as in the .LOC and .DAT files), and each column represents a weight given to a specific condition (or trial type or bin) in the .AVG files.

This matrix must be preceded by a line with the number of contrasts, and a series of lines each containing a label (max 8 characters) describing each contrast.

Example experiment:
4 subjects
3 conditions (also referred to as bins or trial types)
--in the order: warmup, frequent, rare

To test the frequent condition versus baseline (or hypothetical zero):
Example file format:

1	(number of contrasts)
Freq	(label for contrast 1)
0 1 0	(contrast weights for subject 1)
0 1 0	(contrast weights for subject 2)
0 1 0	(contrast weights for subject 3)
0 1 0	(contrast weights for subject 4)

--warmup gets a weight of 0
--frequent gets a weight of 1
--rare gets a weight of 0

To test the difference between 2 conditions (in this case Frequent minus Rare)

```
1
Freq>Rare
0 1 -1
0 1 -1
0 1 -1
0 1 -1
```

You can also do more than 1 contrast at a time by listing several on 1 line (up to 16 usually).

```
3                ( change this first number to reflect the number of contrasts)
Freq>Rare
Freq
Rare
0 1 -1  0 1 0  0 0 1
0 1 -1  0 1 0  0 0 1
0 1 -1  0 1 0  0 0 1
0 1 -1  0 1 0  0 0 1
```

-Contrast 1: Frequent – Rare (0 1 -1)
-Contrast 2: Frequent vs. baseline (0 1 0)
-Contrast 3: Rare vs. baseline (0 0 1)

For contrasts between subjects (i.e., group effects)—assuming your data list (*.dat) is in the order group x subject x montage
Example file format:

```
2
Fgrp1>2
Rgrp1>2
0  1 0  0 0  1    (contrast weights for subject 1 of group 1)
0  1 0  0 0  1    (contrast weights for subject 2 of group 1)
0 -1 0  0 0 -1    (contrast weights for subject 1 of group 2)
0 -1 0  0 0 -1    (contrast weights for subject 2 of group 2)
```

-Contrast 1: Group 1 > 2 for the Frequent condition
-Contrast 2: Group 1 > 2 for the Rare condition

**This contrast weighting procedure is quite flexible. I've only listed a few of the MANY possibilities here. For instance a trend analysis can be set up by using linear weights (such as -3,-1,1,3) and quadratic weights (such -1,1,1,-1), orthogonal contrasts can be set up using orthogonal sets of weights (such 2,-1,-1 and 0,1,-1), and so on (notice that weights need not be equal to -1, 0, or 1). However, if the sum of the weights is not equal to 0, the result will also include a "mean" component.

Region of interest file format (*.roi)—OPTIONAL

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This file defines a 3-dimensional region of interest (or bounding box). It is necessary for doing consistency maps and for returning the peak values and locations. Although you can list more than 1 region in this file, the analysis is limited to 1 region at a time. You can select which region to use in the parameter file.

Ex.

2	(number of regions to be defined)
1 MiddleRegion	(1 and a name for the defined region)
1. 60. 70. -47. -12. -3. 19.	(1. plus the coordinates—Lft/Rt, Back/Front, Bot/Top—separated by “. ”)
1 BackRegion	
1. 60. 70. -70. -50. -3. 19.	

This file can also be used to contrast 2 regions of interest (say left vs right motor cortex). To achieve this, you need to define 2 regions of interest under the header label and use the first number in each line as the contrast weight. However, this option only works for the ROI analysis (and not for the maps, where only the first box will be considered).

Ex.

```
1
2 Left - Right
1. -40. -20. -15. 15. 40. 80.
-1. 20. 40. -15. 15. 40. 80.
```

Alignment file format (*.ali)—OPTIONAL

Including this file determines a shift of the individual subject maps to align them to a particular spatial locationdefined in Talairach (or MNI) space. This spatial location may be chosen to reflect a particular structural (e.g., the origin of the calcarine fissure) or functional (e.g., a peak point selected using a localizer task) information about each subject. Each row contains the X (Left-Right), Y (Posterior-Anterior), Z (Inferior-Superior) coordinate for a given subject. The order of the rows should match the order of the subjects in the .dat and .loc files.

For surface projections, the orientation of the projection can be ignored (i.e., the example below was used for a coronal projection and therefore the Y dimension was zeroed out).

Ex.

```
3       0       -1
-5      0       4
2       0       -1
0       0       -5
3       0       1
0       0       0
-3      0       0
2       0       -17
9       0       0
```

Display Menu (Looking at the data) Back to Page 1

There are lots of different ways of looking at the data. For the most part, these are determined by a combination of the Display menu selection and how you set the *.prm file (some of the details of how to achieve different views are also covered in the parameters section).

Display→Map (grid background) OR **Display→Figure** (black background)
--MOST COMMON

- You can plot spatial maps for specific contrasts as:
    - **t-scores**
    - **means**
    - **Consistency** (for this one, you will need to first define and load an ROI bounding box)
        - Consistency uses a jackknife approach for calculating the correlation of each subject's average waveform with the grand average waveform for the remaining subjects (and then averaging the consistency scores across subjects)
    - **Z(t anal)** is a plot of Z scores calculated from the p values for each t-test. The advantage of Z scores over the basic t-score plots is that the Z-scores will take into account the number of subjects going into each voxel. Because opt3d is location based, some subjects may not contribute data for every voxel that is plotted, therefore when plotting t values, it is impossible to determine statistical significance by simply looking at the maps
        - If you load an ROI before plotting, the resulting map will also give you more information, such as the correction for multiple comparisons, peak Z and its location, etc.
    - **Z(boot)** is based on the outcome of a bootstrap analysis (for every voxel) which provides a p-value that is then converted to a z-score for plotting. This approach can be useful when the size of the signal of interest is not normally distributed across subjects (e.g., when you have outliers), but it is slow. Also, it always uses "separate" error terms.
    - **Z(sqrt)**: For each subject, it first calculates the square root of the absolute value of each data point, preserving the sign (+ or -). Then it computes a t-score based on these new values and then finally converts those to Z-scores. This approach will de-emphasize the largest effects relative to the smallest effects and is most useful when you have small sample sizes (N < 10) with large variability.
    - **Error term**: plots the standard error across subjects (i.e., the error term for the t-tests)
    - **Latency**: Instead of plotting 1 moment in time, it will plot the location of peak activity (above some threshold) across a range of times such that your color bar will now represent latencies of peak activity.
        - You will also need to:
            - Set Interval Beginning and Interval Ending
                - If these are equal, it will consider the entire epoch
                - smaller ranges tend to work better
            - You may also want to adjust the scale
- You can view the spatial maps:
    - Sagittally
    - Axially (Direction of map parameter)
    - Coronally

  - On individual slices (Slice to plot parameter)
  - As a surface projection (Slice to plot parameter)
- You can plot the spatial map:
  - for 1 time point
  - as a movie (see Time points to Display)
  - as a series of time points (1 map for each) –max of 16 time points
  - as a mean over a time interval (see Interval Beginning and Interval Ending)
- You can get the peak t-score, mean, Z, etc and it's location
  - This is calculated automatically when you load an ROI bounding box and plot the spatial map
  - This info appears at the bottom of the spatial map (for whichever value—t, mean, Z etc.—that you designated with the Data Type to Display parameter).
  - It also reports the mean for the whole ROI
- You can find out the Talairach (or MNI) coordinates of a particular point on the spatial map
  - Left click on the location
- You can get the time course of activity at a particular location by right clicking on the map
  - Right click on a location and this will take you directly to the waveform associated with that location
  - Or see next Display feature

Display→Timecourse

- If you have plotted a map or series of maps and then go directly to Display→Timecourse, you will get a waveform associated with the last plotted map. If you are using an ROI, the voxel plotted will be the voxel of peak activity within the ROI (for the last map).
  - Note: without an ROI, the default voxel to plot as a time course will be the origin for that view (within +/- 1 mm). Sometimes, this voxel has data in it, in which case it will plot a waveform. Other times, this voxel will not contain data, and then the program will not plot anything.
- If know which voxel you would like to plot as a waveform, you can plug in the coordinates in the .prm file (X and Y point of coordinate to display) and then Display→Timecourse.

Display→ROI

- Use this option when you want to see the waveform corresponding to the average of all voxels within your ROI.

Display→Ind. Subjects

- Provides a plot of individual subject mean time courses.
- These are not plotted as waveforms per se, rather they are color coded bar plots.
  - The color bar should be used only as a general reference because each subject plot is autoscaled to account for across subject variability.
- The subjects are stacked in order of magnitude of response at the time point specified in the Time points to Display parameter.
- The subject numbers indicate the relative position of the subject in the .LOC file.
- Some subjects may not have values for a particular point.

Display→PCA/time

- Computes a temporal PCA using all voxels and subjects (for a particular orientation).
- To use:
    - Set the Interval Beginning and Interval Ending
        - If these are equal, it will consider the entire epoch

    - It will first plot the time course of the principal components (those explaining 80% of the variance or up to 16 components)
    - Click the mouse button and it will plot spatial maps (using Z scores) corresponding to those components.
- Generally speaking, PCA can be an effective way of reducing multiple comparisons by narrowing the temporal regions explored.
- The component loadings are printed in c:\temp\pca.lst
- The component scores for the peak locations for each component can be saved using the Save => PCA component scores

Display→Forward XCorr
Display→Backward XCorr

- For each subject, it computes lagged cross-correlations between a seed voxel and all other voxels (for a particular orientation); it will also present a set of maps (in Z scores) for each lag, computed across subjects (based on Fisher-transformed cross-correlations from each subject)
- To use:
    - Set the Interval Beginning and Interval Ending
        - This will set the interval used for the seed waveform
        - If these are equal, it will consider the entire epoch
    - Set the seed with X and Y point of coordinate to display
        - If setting a seed that is in the same orientation as the voxels being explored, then set the exact voxel.
        - If you would like to have a seed from one hemisphere crossed with voxels in the opposite hemisphere (or if you would like to cross-correlate with some other measure like an ERP waveform), then you chose "external" for X and Y and supply an ASCII file that contains the desired seed timeseries for each subject.
            - Format: rows x columns = time x subject
    - Set the orientation
    - You may also want to set an ROI to get statistical criteria and peak locations.
    - The number of lags will be ½ the total number of points in your epoch or 16 maximum.
- Forward versus backward cross-correlations
    - This depends on the logic behind your seed and refers to positive versus negative lags.
    - If you want to know what regions *followed* activation of your seed voxel, choose Forward (shifting the seed waveform in a positive direction).
    - If you want to know what activations may have *preceded* activation of your seed, choose Backward (shifting the seed waveform in a negative direction).

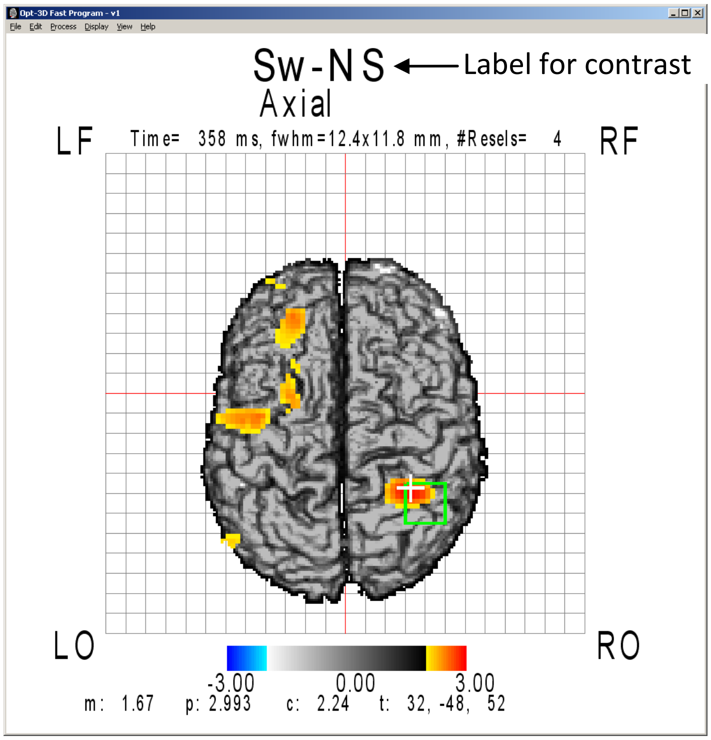
Display→Peak Analysis

- A method for creating an output file that will contain (among other things) a list of peak points, along with their criterion values and coordinates.
- The file will include values for all ROIs, all contrasts, and all time points specified and put them into 1 big file.
- To use:
  - Load an ROI file with up to 9 regions of interest
  - Make sure your design file has all of the contrasts you want peaks for
  - Set Time points to Display to be the time range you want values for (in the order early point to late point…like you're asking for a movie of the time points).
  - Now click Display→Peak Analysis and it will prompt you for a file name. It will be an ASCII comma delimited file.
- At the top of the file will be some header information. The first section will be a short-hand version of your parameter settings (.prm). Next will be a section listing the contrast weights that were used. Then there will be a large section that starts just after the word "RESULTS" that contains all of the critical info.
- The data columns are labeled, but here is list of the outputs (in order):
  - ROI# (order in the .roi file)
  - ROI Name
  - Contrast # (order in the .des file)
  - Contrast Name
  - Time (in ms)
  - Mean amplitude across the whole ROI (avg of all voxels in ROI)
  - FWHM for X dimension
  - FWHM for Y dimension
  - # of Resels
  - Peak Z value
  - Criterion Z value
  - X, Y, Z coordinates of the peak location (3 columns)
  - X, Y, Z coordinates defined in your .roi file (edges of 3 dimensional box…6 columns)
- The rows are nested Slowest-to-fastest: ROI x contrast x time.

Plot Features

A basic spatial map will look something like this (axial image plotting z-scores):

- Dark gray area indicates regions sampled by at least ¼ of the total number of subjects
- The green box is the ROI (it will only appear if you load an .ROI file)



Time= 358 ms, fwhm=12.4x11.8 mm, #Resels= 4

m: 1.67 p: 2.993 c: 2.24 t: 32, -48, 52

Correction for multiple comparisons

To use:

- Set Data Type to Display parameter to Z(t-anal))
- Load an ROI
- Then Display→Map or Display→Figure

Output above each figure:

- fwhm: an estimate of the smoothness of the data in x and y dimensions; can also be considered as a measure of spatial resolution.
- #RESELS: an estimate of the number of “independent” values (or degrees of freedom) within the ROI

Output below each figure:

- m: average value of the DV (delay, etc.) in the ROI (this is average of the Z scores for each point, not the Z score of the average!)
- p: peak value of the DV (delay, etc.) in the ROI (as standard Z scores)
- c: minimum criterion for determining whether a particular value in the ROI is statistically significant (.05, one-tailed or .025, two-tailed – set Significance for Criterion)
- **NOTE:** The criterion value is sometimes reported by opt3d as being lower than the p<.05 level for a 1-tailed test (which is 1.69 for Z-scores). This can happen when the number of resels is computed to close to 1.

When you plot an ROI, the software incorporates some of the statistical analyses included in SPM99 for evaluating the significance of effects in brain image maps. SPM99 applies tools derived from a theory called Gaussian Field Theory. This can be used to evaluate the probability that any value within a statistical map is greater than a certain criterion, keeping into account the fact that the different points of the map are correlated. This correlation between data points is also called "smoothness." Kiebel et al. (NeuroImage, 10, 756-766, 1999) describe a robust method for estimating the smoothness of a particular statistical map (using residuals from a generalized linear model), and also report how to compute the probability values according to the Gaussian Field Theory – this method has been incorporated in SPM99, which is considered the gold standard for robust statistical analysis of brain imaging data. The same logic and quantitative methods are incorporated in Opt3d (although without some of the options available in SPM99, thus simplifying the program and the display). Also, Opt3d applies this to two-dimensional maps, rather than 3D data as in SPM99.

Saving the data

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To save the spatial maps or waveform images, you need to capture the screen (alt-print screen) and paste the image into powerpoint or use some other method for saving the images. The last plot can also be saved in bitmap format into a file using the Save=>Image command.

Text output options (under File→Save menu):

- Point: Provides data from 1 voxel
- Average: Not functional
- ROI Analyis: Provides data from the average across all voxels defined in the ROI
- Data Matrix Low Res: Provides data from voxels within the ROI (a point every 1 cm)
- Data Matrix Hi Res: Provides data from voxels within the ROI (a point every 5 mm)
- Data Matrix Ultra Hi Res: Provides data from voxels within the ROI (a point every 2.5 mm)
- Cross-correlation peaks
- Component scores peaks

There is also an option for saving a slice. The output is a file that can be read back into Opt3d. Basically, it contains the means and standard errors for every time point for a given orientation and set of contrasts. To use it, you need to load a .prm and a .loc file before loading the slice file (.slc).

- Advantage: Loading a .slc file is sometimes faster than loading the data and running the first analysis (especially for very large data sets). Therefore it can be a quick route to getting plots of every time point or plots for a common set of contrasts.
- Disadvantages: You cannot change any of the following after loading a slice:
    - Timing parameters
    - Model parameters
    - Filtering parameters
    - Direction of map or slice to plot
    - Acceptance Criterion
    - Data type (i.e., can't change from Delay to Intensity)
    - You also can't add new contrasts
- So, once you've loaded a .slc file, the things you CAN do are:
    - look at every time point
    - can change the analysis (e.g., from Z to a latency map)
    - change the ROIs (if you load one)

Format of the output file for 1 voxel in a t-score map

The output file gives you quite alot of information. Here's a general rundown of what it gives you (in the order it will appear in the output file).

1. The Talairach coordinates of the location you requested.
    a. In the example below, I was using a sagittal surface projection. This is indicated by "l-r=100".
2. A list of the contrasts that were in your *.des file and the number of subjects included in each of those contrasts.
3. The consistency values. Each row of values corresponds to a contrast condition and these values will only be returned if you had loaded a ROI file before outputing the data.
    a. The first number is the average consistency across subjects (zero's are included in the averages).

    b. The second number is the t-score associated with that consistency value (this can be used to determine whether the consistency correlation was statistically significant).
    c. The remaining numbers are each subject's consistency values (i.e., correlation of subject X with the remaining subjects, for that particular location).
4. The grand average waveforms—mean values.
5. The standard error for that waveform.
6. The grand average waveforms—t-score values.
7. Individual subject averages—mean values (grouped by contrast condition).
8. Channels that were included for each subject's waveform.
    a. **NOTE:** when using the physical model, the channel information does not reflect the weights used for averaging channels together.

Format of the output file for 1 voxel in a z-score map

The output is almost the same as the t-score output, except the following:

- It does not provide the timecourse of means (for indiv subjects nor group avgs)
- It provides the time course of z values rather than t values
- It provides the time course of probability values rather than the standard errors

Format of the output file for low, high, or ultrahigh resolution matrix

The output will be the same as the z or t output files, but it will just have multiple points appended to it. Note: this will be a VERY LARGE file. ☺

Format of subfiles that are called by the .loc file Back to Page 1

Subfiles for *.loc

*.mtg files—An array specifying # of channels, channel labels, source/detector locations, wavelengths, and modulation frequency of the hardware

The specific names given to the sources (ex. RA01) and detectors (ex. LB01) is determined by the output file of the digitization software (for CNL it is Polhemus/Locator--the .elp file). The order of the source/detector pairs is determined by your montage setup in Boxy and is generally referred to as the order of the "data channels".

Format for the .mtg file:
Header: row 1 -- NEW
Row 2 -- number of channels for each montage
Columns:
Channel counter (cumulative across montages)
Actual channel number in raw data set (cumulative across montages)
Source location
Detector location
Wavelength
Modulation Frequency

Example of first part of a file (this is from a good.mtg generated after channel selection in p_pod):

```
NEW
51 61 51 63 65 53 65 54
1     1     RA01  LB01  830   110
2     2     RA04  LB01  830   110
3     3     RA06  LB01  830   110
4     6     LD01  LB01  830   110
5     7     LD04  LB01  830   110
6     13    RA01  LB03  830   110
7     14    RA04  LB03  830   110
8     15    RA06  LB03  830   110
9     16    RA08  LB03  830   110
10    17    RA11  LB03  830   110
11    18    LD01  LB03  830   110
12    19    LD04  LB03  830   110
13    20    LD07  LB03  830   110
14    30    LD01  LF02  830   110
15    31    LD04  LF02  830   110
16    32    LD07  LF02  830   110
17    33    LH01  LF02  830   110
18    34    LH03  LF02  830   110
19    35    LH04  LF02  830   110
```

Subfiles for *.loc (con’t) Back to Page 1

*.elp—File generated by the Locator Program and Polhemus. It contains the 3d spatial information for the location of the sources and detectors in relation to the fiducial points.

*.tai—A list of coordinates indicating the fiducial points and the extremities of the brain in AC/PC space (CNL lab uses AFNI for finding these points on the MR images). It is a basic text file with the following format:

Format as of current (11-06-10) .tai files:

```
UofI new scaling  %NOTE: no spaces in “UofI”!
RAI               %The next 3 lines refer to AFNI native space info
191 236 171       %Number of voxels in each dimension L-R, A-P, I-S
1.00 1.00 1.00    %Voxel size in mm
AC Superior Edge
92  95  70        %voxel location in AC-PC space A-P, L-R, I-S
PC Inferior Edge
125   95   70
Most Anterior Point
30  111   94
Most Posterior Point
199  109   61
Most Superior Point
121   79  148
Most Inferior Point
94  53  34
Most Left Point
129   23   59
Most Right Point
128  168   63
Nasion
12  100   70
Left Preauricular
102   18   37
Right Preauricular
106  174   35
```

*.tol—This is a file generated by the COREG program (Matlab script for co-registration and Talairach transformation of the elp src/det digitized locations). It contains the digitized labels and the X, Y, Z coordinates of the digitized points in Talairach space (L-R, P-A, I-S)

LB01	-25.757576	84.677419	13.361111
LB04	-21.636364	54.193548	59.611111
LB07	-20.606061	-1.769231	85.305556
LB10	-15.454545	-54.805195	87.361111
LB13	-14.424242	-99.948052	48.305556
LD02	-41.212121	75.645161	16.444444
LD05	-40.181818	65.483871	38.027778
LD11	-38.121212	21.451613	69.888889
LD16	-39.151515	-23.000000	83.250000
LD20	-39.151515	-58.909091	79.138889
LD23	-38.121212	-81.480519	62.694444

Just to make things as confusing as possible, each of the files we use for location information uses a different coordinate system. Here is a handy reference to help you keep it all straight:

	x	**y**	**z**
ELP files	P-A	R-L	I-S
TAI files	A-P	L-R	I-S
ROI files	L-R	P-A	I-S
Talairach Coordinates	L-R	P-A	I-S