**Automated Voxel Placement: Step-by-step instructions**

*Version: 5.0; September 2016*

*Citation: Woodcock EA, Arshad M, Khatib D, & Stanley JA (2018). Automated Voxel Placement: A Linux-based Suite of Tools for Accurate and Reliable Single Voxel Co-registration. JNPN.*

*Dependencies:*

* Matlab (Linux version), FSL, Freesurfer, new\_bc (www.pixelbeat.org), dcm2nii (http://www.mccauslandcenter.sc.edu),
* Matlab scripts: GetEulerAngles, round2dp, makeFslXfmMatrix, rad2deg

***Instructions to create a template voxel location (‘avp\_create’):***

1. *Note: execute scripts on a computer that will be available at the scanner at the time of each subject scan. The user can specify the location of required folder/files in the ‘avp\_config.txt’ document.*
2. Copy all avp scripts to the location of your linux workspace and type: “chmod u+x [directory]/avp\_create” for each script (‘avp\_coregister’ and ‘avp\_overlap’). Scripts are now executable.
3. Place a high resolution T1-weighted nifti file (e.g. MNI template brain or prior subject scan) in the present working directory and name it ‘template.nii.gz’. The present working directory will be referred to as the ‘home’ folder in these instructions.
   1. The ‘template.nii.gz’ should reflect the subject population you will be recruiting for that research study (e.g. use an adult template brain for a neuroimaging study on adult research subjects). The accuracy of voxel coregistration in ‘avp\_coregister’ can be improved by using a ‘template.nii.gz’ image that is approximately the same anatomical size as your research subjects. This is especially important for voxel locations positioned at tissue boundaries (e.g. cortex).
   2. The ‘template.nii.gz’ can be an image of any subject population (e.g. children, adults, rodents, etc.) or anatomy (e.g. brain, abdomen, etc.). Moreover, each research study can have a different template image (i.e. the template image is study-specific).
4. Launch ‘avp\_create’ in a Linux terminal in your ‘home’ folder
5. Enter the following voxel information (**comma-separated**) when prompted:
   1. Voxel center coordinate in pixel space (e.g. “143,162,94”)
   2. Voxel dimensions in millimeters (e.g. “15,20,15”)
   3. Voxel angulations/rotations in degrees (e.g. “12,13,25”)
   4. Enter the voxel name and 3-letter study name (e.g. “dlpfc,nss”) [study name MUST be exactly 3 letters]
   5. Enter the image orientation (e.g. “L,A,S”)
6. ‘avp\_create’ will generate a voxel with those attributes in the template image and display the voxel and template image in FSLview.
7. Appraise the voxel position. Are you satisfied with its placement? Close FSLview.
8. Is the voxel placement optimal? Respond ‘y’ or ‘n’.
9. If no (‘n’), relaunch ‘avp\_create’ and make adjustments to the voxel parameters.
10. If yes (‘y’), a file entitled ‘volume\_locations.txt’ will be created in the ‘home’ directory and will be appended with the voxel attributes.

***Instructions for coregistration of a template voxel to subject space (‘avp\_coregister’)***

1. *Note:* ‘avp\_create’ will automatically create all folders required for ‘avp\_coregister’. ­­
2. After the T1-weighted structural scan (MPRAGE), place all subject dicom (or .ima) files into the ‘dicom\_files’ folder - located as a sub-folder of the ‘home’ directory.
3. Run ‘avp\_coregister’ in the ‘home’ folder. ‘avp\_coregister’ will prompt you to enter the 3-letter study code (e.g. “nss”), 4-number subject id (e.g. “1001”), and 1-letter time point (e.g. “a”) associated with each subject scan.
4. Select the template voxel from the list (each template voxel was previously created using the ‘avp\_create’ script).
5. ‘avp\_coregister’ will determine the location and angulation/rotation values needed to place the template voxel in the linearly-equivalent approximate location in subject space. We recommend shimming (e.g. FASTESTMAP) the approximate voxel location while ‘avp\_coregister’ is processing.
6. Enter the voxel dimensions, center coordinate, and rotation angles in the scanner computer to prescribe the voxel in the current subject space.
7. Press ‘enter’ on the computer that executed ‘avp\_coregister.’ Two voxels will be displayed in FSLview. The red voxel is the coregistered volume (i.e. ‘FLIRT’) while the blue volume was created using the calculated values that were just entered into the scanner. The FSLview voxels should overlap and match the wireframe voxel previewed on the scanner computer.
8. If the voxels do not overlap or do not match the wireframe preview on the scanner computer, there was a problem. Troubleshoot errors. If the problem cannot be resolved, please email first author.
9. If the voxels overlap, ‘avp\_coregister’ worked properly - collect your single voxel measurements.

*Note: ‘avp\_create’ and ‘avp\_coregister’ were developed using a Siemens system and may not work properly on other systems. Test prior to enrolling research subjects.*

*Instructions for calculating geometric voxel overlap (‘avp\_overlap’)*

1. Copy one dicom header file for each research subject at each time point into the ‘rda\_files’ directory (default location: /studies/[study\_name]/rda\_files; location can be specified in the ‘avp\_config.txt’ file). Each RDA file should be named using the format: “3-letter study name””4-number subject ID””1-letter time point”\_”voxel name”. For example: ‘nss1001a\_dlpfc.rda\_hdr’
2. Run ‘avp\_overlap’ from the ‘home’ directory.
3. Select the study and voxel (from the list generated) for which you want to calculate percentage of voxel overlap.
4. *Note:* Processing time is dependent on number of subjects and time points – total processing time may be substantial (>15 minutes).
5. Text files will be generated with results for between-subject 3D geometric voxel overlap at each time point, and if the data exist, within each subject across time points (i.e. longitudinal studies) for five different preset pixel thresholds (0.55, 0.65, 0.75, 0.85 and 0.95; see manuscript for description). In addition, tissue voxel composition (gray vs. white matter) and coefficient of variation percentage will also be calculated. The user must select an appropriate pixel threshold for each voxel prior to interpreting voxel placement accuracy and reliability. The user should select the pixel threshold that is closest to, without exceeding, 100% of the total volume size (labeled ‘Percent\_Total\_Voxel’ in the ‘Overlap\_Summary.txt’ file) and report overlap values using that pixel threshold for all calculations.

*Note: A voxel that is orthogonal to the FOV will result in identical overlap values for each pixel threshold.*