A simple rapid process for semi-automated segmentation of mouse brain from magnetic resonance images of the whole mouse head

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Supplemental Data: Instructions for running the skull stripping program

Once you have downloaded and installed the dependencies as described in the README document, you can begin using the program. You will need to use a Unix-based computer. An Apple computer running in Terminal, or a Linux/Windows-PC are both compatible with this processing environment.

1. Create a new directory. Copy the program, skullstripper.py, together with the images to be masked as well as the reference image together with its high-quality reference mask. Within the same new directory create another folder named "Mask" to receive the masks as they are generated.

Note that the reference mask and image should be from the same dataset with the same imaging parameters, including voxel sizes. Check that the "header" information is identical for all images. Images should be in nifty (.nii) format, and oriented similarly.

2. To generate a mask for a single target image:
At the prompt, use the following skullstripper command:

```
'python ./skullstripper.py -in <IN.nii> -ref <REF.nii> -refmask <REFMASK.nii> -out <OUTFILE.nii> -thr <threshold value>'
```

Where "IN" is the file name of the target image; "REF" is the name of the reference head image; "REFMASK" is the name of the reference mask, and "OUTFILE.nii" will be the name of the resulting mask for the target image. The <> text in this command should be modified to represent the names of the images in your new directory. You can cut and paste the image names into the command from that directory.

After entering the command, press "enter" to initiate processing. The program will then put out a stream of text, and the new mask will appear in the new directory with the new mask labeled <OUTFILE>.nii. The procedure takes less than a minute.

3. To extract the brain from a set of multiple images:
This program also works well to create masks for multiple images taken at different times of the same animal; or for multiple anatomically similar animals when captured with the same imaging parameters, using a single reference image and its mask. All images to be processed should be placed in a single directory. To receive the new masks, an empty directory within the image directory should be created, entitled "Mask".

To batch masks for multiple images, we use the 'for' loop command:

```
for f in *.nii ; do python skullstripper.py –in $f -ref
<reference volume> -refmask <reference mask> -out Mask
${f}_mask –thr <threshold value>; done
```

The "f" is a random letter that you are designating to everything that ends in ".nii". Thus, in this case, all images with the extension .nii in the same directory will be used as input, and the output will be deposited in the folder “Mask” with the same name as the input image appended by “_mask”.

The output masks will be placed in the “masks” directory , the stripped images will be placed in the “stripped_images” directory and the images will keep the same name as the raw images just with “_stripped” appended to the end.