Optical Projection Tomography (OPT)

Mouse Imaging Centre OPT device:

Our system was custom built at MICe by Michael Wong and Jun Dazai. Design and implementation details can be found in their 2013 Plos ONE paper.

OPT Sample Preparation

- Please see preparation instructions here: OPT sample preparation

OPT Sample Imaging

- General imaging instructions are as follows:

1. Please see above **OPT Sample Preparation** section for instructions on how to prepare your sample for imaging
2. Once your sample is embedded in agar and has been sufficiently cleared in BABB (ie. agar and sample are transparent), it is ready for imaging
3. Remove the agar-embedded sample from the vial and place it on a piece of clean gauze - pat the agar with gauze to remove excess BABB
    - wrap the agar in the gauze and allow to sit for at least 30 minutes
4. Take a clean metal chuck (wipe surface with methanol) and apply a small amount of gel super glue (blue container) - enough for a thin layer - over the top of the chuck. Allow to sit for 5 minutes so the glue becomes tacky.
5. Place agar-embedded sample on to the top of the metal chuck - allow to set for at least an hour
    - you can prepare mutliple samples/chucks at one time
6. While you wait, turn on the following OPT components
    - **UV light source (this will take several minutes to warm up)**
    - **microscope controller**
    - **CCD camera**
    - **white light source**
7. On OPT computer, double click the **OPT Scanner 3.0 with shutter.vi** icon to open the imaging software
8. Open the door to the OPT system
    a. make sure that the glass cuvette is 80% filled with BABB. If not, it may be time to clean the cuvette and replace with fresh BABB
9. On the imaging software interface, press the white arrow on the top right of the screen - you will notice that the stage will begin to move
10. Set **Exposure** value at 25 and press the **Update** button
11. Place the sample and chuck upside down onto the steel disk mount for the rotation stage - the metal chuck has a magnet in it and will remain secure
12. Lower the sample into the BABB cuvette by adjusting the **vertical position** value on the OPT software (try 5 to start). Press enter.
13. Once you are happy with the vertical position of your sample, adjust the zoom so that your sample takes up most of the viewing window (try 7000 to start). Press the **GO** box after making any changes.
14. Centre your sample (using forceps - touch only the metal chuck) on the green visualization line in the middle of the viewing window.
15. Change rotation value incrementally and adjust sample as needed to make sure it is centred at every angle
16. Turn off **white light source**
17. Close door to OPT system
18. Change **Exposure** value to 500
19. Adjust **UV light source** gain value to 100% (by pressing the 'UP' botton on the box)
20. On the OPT software interface, make any necessary adjustments to the **Focus** (start at 18 - focus is changed automatically when this value is edited)
21. Standard scanning parameters set already are:
    a. Step Size: 0.3
    b. Avg: 1
    c. # of views: 1200
22. Enter the name of your sample in the **File Name** field (it is recommended you add an underscore after the name ex. filename_)
23. When ready to scan:
    a. press the **Acquire Dark Field** button
    b. press the **Update** button
    c. wait for the machine to acquire 10 dark field images (the sample will be raised and then returned back to its original position)
    d. press **Scan** and then **Update**
24. Each scan takes approximately 25 minutes

OPT Reconstruction

Step 1 - Converting binary files to tif files

1. Before your image can be reconstructed, the binary files produced by the OPT scan must be converted to tif files.
2. On the same computer you used to image your samples, open the **MATLAB (R2009b)** icon
3. When MATLAB is open, make sure to set the current folder as C:\Documents and Settings\Jun\Desktop\Matlab\OPT Script
4. In the command window type **postOPTnew**
5. The script will prompt you to enter the number of data sets to be processed - enter that value
6. Then enter the File names of each of your samples as you named them during the scan
7. Press enter after each File name
8. The tif files will then transfer to the reconstruction computer (across from the micro CT)
9. Record the **pixel size** (microns) of the image (you will need this for **Step 3**)

Step 2 - Producing reconstructed tif files

1. On the OPT reconstruction computer, open the Nrecon folder on the Desktop

2. Double click the **GPUReconServer** icon and then the **Nrecon** application icon
3. Press the open folder icon at the top left of the Nrecon page
4. Find the folder with your files, double click any of the 1199 tif files
5. Adjust the position of the green line across the sample (for embryos, this would be across the heart)
6. In the Reconstruction tab on the top right of the screen, select the **Settings** tab
    - adjust the **misalignment compensation** value until the sample in the viewing window appears in focus
    - adjust the **Ring artifact reduction** value to 4
7. Back at the **Start** tab, press the **Fine tuning** box
    - select **Post-alignment option** and select the following parameters:
        - **Number of trials:** 5
        - **Parameter step:** 1
    - Press **Start** button
    - Use the up and down arrows at the top of the screen to select the best image (this should be the one that looks most focused)
8. Repeat step 7 above but change the **Parameter step** size to 0.5
    - Again, use the up and down arrows to select the best focused image
9. Note: If you are unable to find a focused image it may be that your sample moved during the scan. In this case, you will have to rescan your sample
10. Once you are satisfied with your misalignment compensation setting, press the **Add to batch** button
11. Repeat for each of your samples
12. Once all your samples have been added to the batch, press **Start batch** on the bottom left of the screen
13. Once the batch is completed, you will find the reconstructed tif files in your sample folder
14. These will have to be transferred to your hpf directory (the OPT reconstruction computer has very little available memory to store images)

Step 3 - Producing a 3D mnc file

1. **Step 1-**9 of the reconstruction process will output a **pixel size** in microns to be used in the below script
2. In a terminal, in the directory with the reconstructed tif files, run

```
for i in filename__rec*.tif; do convert $i GRAY:-; done | rawtominc -transverse -short -unsigned -xstep pixel_size/1000 -ystep pixel_size/1000 -zstep pixel_size/1000 -xstart 0 -ystart 0 -zstart 0 -2 -clobber /path/to/output/dir/filename.mnc 1023 1024 1024
```

3. Open the reconstructed mnc file in **OCCIViewer** to view your 3D image