An Innovative Clearing Method for OPT Imaging of Murine Embryos

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Objective

Design a tissue clearing method that clears faster with minimal murine embryonic deformation and produces high-quality OPT images of its anatomy.

Background

- Fetal Alcohol Syndrome (FAS) impacts many individuals and is caused by the consumption of alcohol during pregnancy.
- Current imaging modalities are not sufficient to study the effects of FAS on the brain thoroughly
  - Optical Coherence Tomography (OCT) allows in vivo imaging to provide structural information, but not functional.
- Optical Projection Tomography (OPT) is an imaging method that can provide high-resolution, whole-body 3D images of murine embryos to observe the effects of FAS (Figure. 1).
- OPT requires the sample to be fully cleared, and current established clearing methods are prone to error, slow or non-effective.

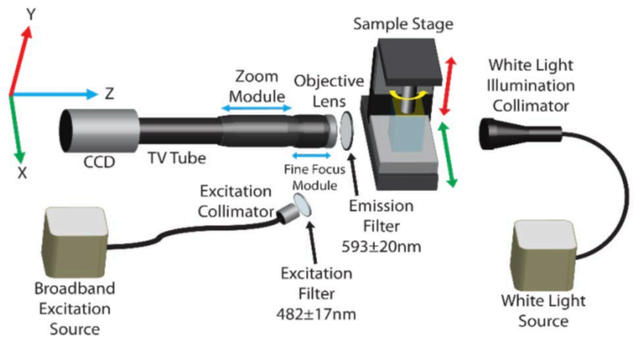

Figure 1: Set-up for OPT imaging

Acknowledgements

We would like to acknowledge Dr. Manmohan Singh and Jessica Gutierrez for the aid and expertise they provided towards the completion of this Capstone project. As well as Dr. Yuncheng Du, Dr. Jerome Schultz, and Dr. Ting Chen for their guidance.

Methodology

- Assessment of Previous Methods:
  - BABB was error-prone due to corrosive chemicals and difficult dehydration.
  - SCALE was very slow and showed no signs of proper clearing.
  - CUBIC seemed promising as it showed good clearing in 14 days, but improvements were needed to obtain better images. The CUBIC method was chosen.

- **NEWM:** Improvement of CUBIC
  - 3 criteria to change: increased temperature, increased concentration, and addition of ethanol to aid in clearing speed.
  - 8 trials were performed (N=2) where the changes were tested individually and in combination with each other.

- Quantitative Assessment: **Clearness Testing**
  - The clearness of the embryo was measured at days 2, 5, 7, 9, and 12 by shining a laser through the embryo, into a power meter and calculating the percentage of the total power of the laser that shines through each sample.

- Qualitative Assessment: **OPT Imaging**
  - A LabView program was used to obtain the OPT images (includes 720 2D images). The parameters used for imaging were an exposure time of 2000ms, excitation wavelength of 488 ± 2 nm, and the emission wavelength at 520 ± 20 nm.
  - Images were concatenated using MATLAB and reconstructed in NRecon (cross-sectional slicing and alignment). The artifacts were removed, and a 3D image of the embryo was displayed in Imaris.

Results

- The clearest trial was 39°C/40%/NoE, but the image that had the best visibility of internal structures was 37-39°C/40%/NoE.
- The 37-39°C/40%/NoE trial performed better than the control CUBIC method in both assesments.
- Errors in agarose making may have created bubbles in that trial that impacted its clearness score.

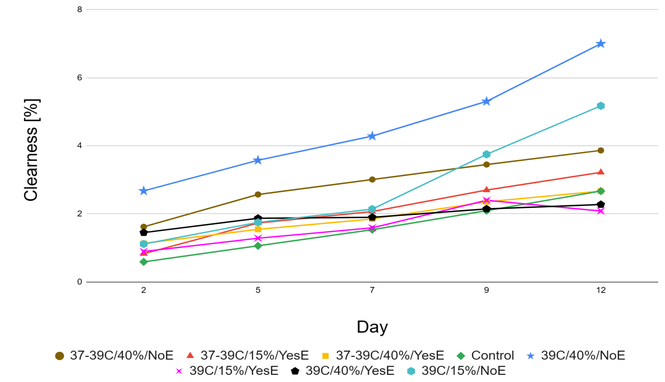

Figure 2: Clearness testing results for each trial.

Figure 3: Control CUBIC at 12 days

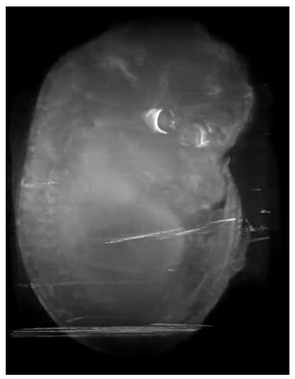

Figure 4: 37-39°C/40% Triton X-100/No Ethanol at 12 days

Conclusion

- A new clearing method (NEWM) was introduced.
- NEWM is a modified CUBIC protocol that increases Triton-X100 concentration to 40%. It clears faster and produces better images than the original.
- CUBIC and NEWM have both been established as suitable clearing methods for OPT Imaging.