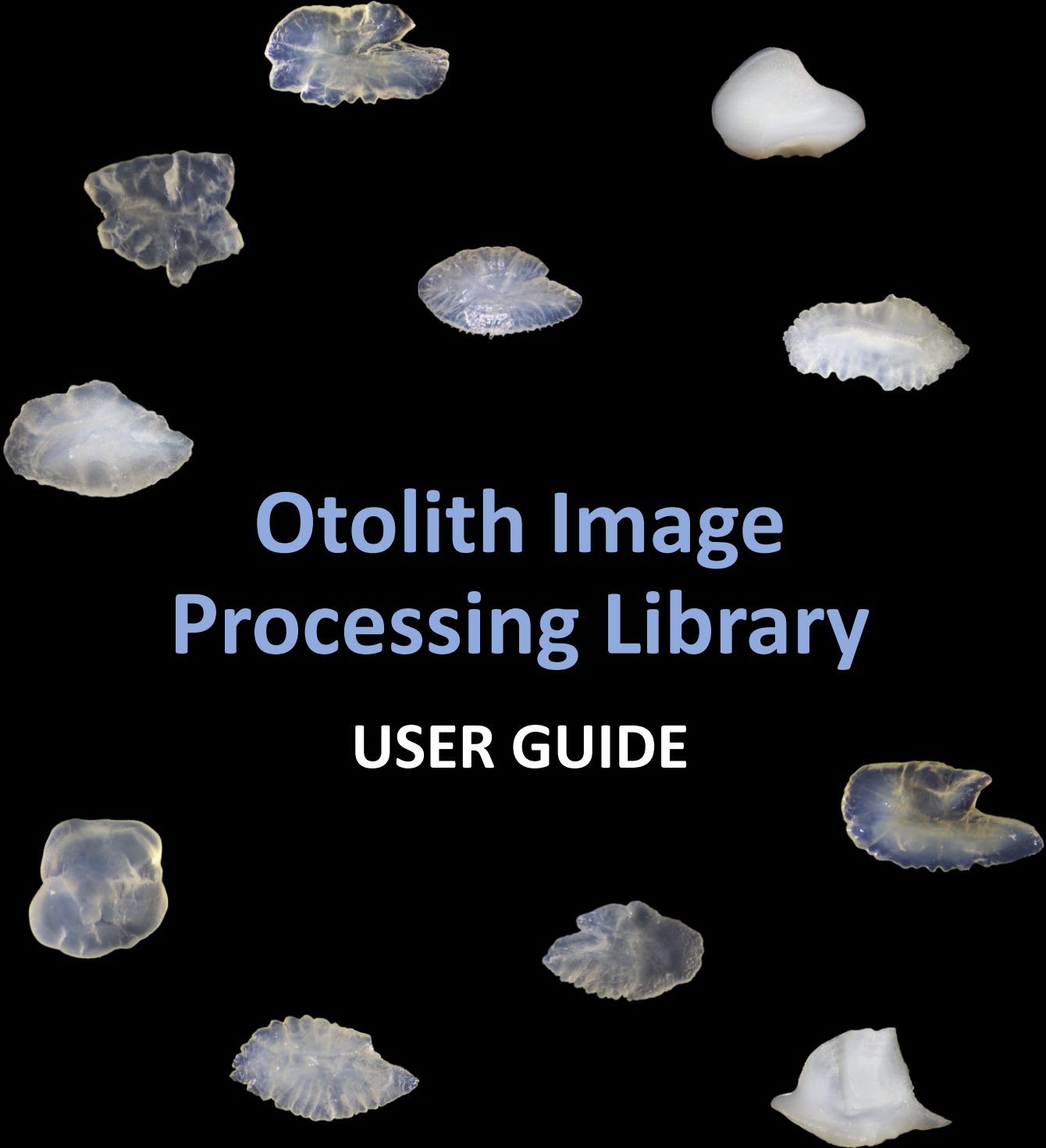




Otolith Image Processing Library

USER GUIDE



This guide is for use by researchers wishing to contribute otolith images to expand the OIPL library, or by those sampling unknown otoliths for identification by OIPL.

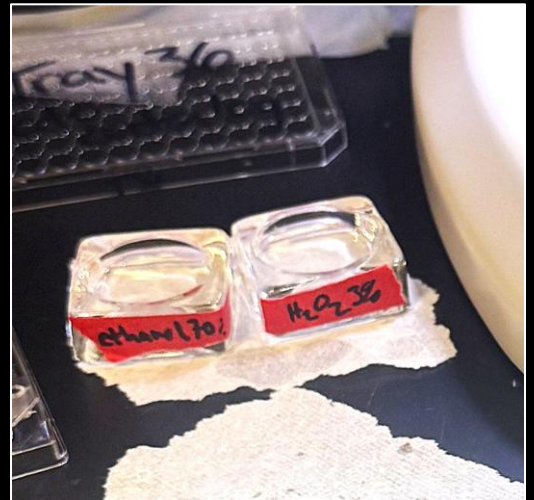
This guide describes procedures for cleaning and imaging otoliths.

The OIPL uses machine learning models to compare images of unknown otoliths to those in our library, returning the best matches to the user. If the unknown otolith is weighed, the OIPL will also return the estimated size of the fish from which the otolith came, based on fish length-otolith weight regressions developed as part of the library.

<https://www.disl.edu/research/oipl/>

Cleaning

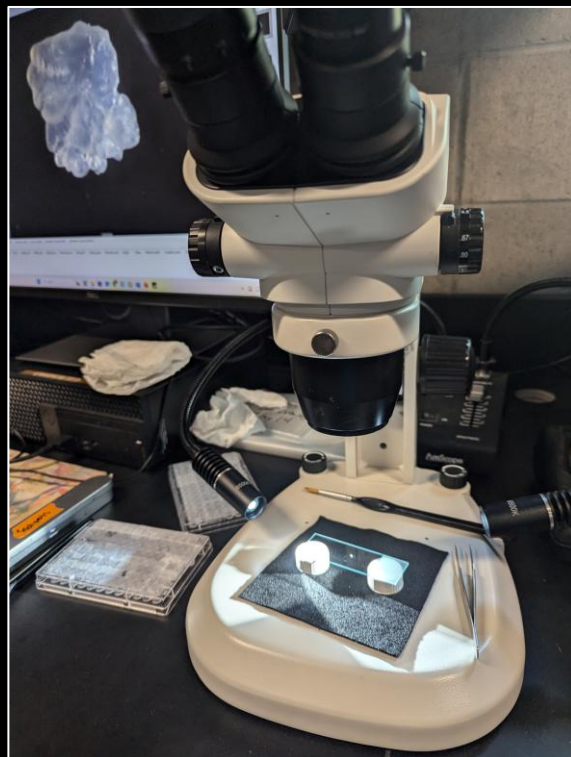
- Target the largest pair of otoliths. The OIPL is primarily sagitta, but in some species the lapillus or asteriscus can also be large.
- Upon removal from fish or stomach contents, place immediately into 12% hydrogen peroxide. Do not allow tissue to dry onto otolith before this step.
- **Do not use bleach** for this step, it will penetrate the otolith and turn it chalky during storage.



- After 1-2 minutes, inspect the otolith (under a microscope if needed). Tissues should float away from the otolith, but may need prodding with forceps to remove any remaining tissue. If tissue still remains, blot and gently rub with paper towel and return to peroxide.
- Once the otolith is free of tissue, transfer to 70% ethanol for 1-2 minutes. This rinses off the peroxide and allows for rapid drying of the otolith.
- Transfer otolith onto a clean glass slide to dry. It is now ready for imaging or storage.

Imaging

- We image otoliths using a camera mounted on a dissecting microscope, but a cell phone held to the eyepiece will work.
- The imaging stage comprises a piece of black felt or cloth and a stand of a clean glass slide resting on two 20ml vial lids.
- The imaging stage allows for the otolith to be in focus over an out-of-focus black background.
- Angling the light source from the side reduces glare off the otolith and any glare from the black background



- Orient the otolith sulcus up, aligned horizontally.
- Zoom in as close as possible while maintaining a crisp outline with minimal blurring.



- Accepted image file formats for the OIPL are png, jpg, tiff

Weighing

- We have developed otolith weight-fish length regressions for many species in the OIPL.
- For species positively identified by the OIPL, enter the otolith weight (in mg) and OIPL will return the estimated total length (in mm) of the fish the otolith came from, if it is within the weight range used to develop the regressions.
- Use a microbalance to measure otolith weight to the nearest 0.001 mg.
- When otoliths are located as a pair encased within the otic capsule, they can be weighed as a pair and the average weight entered.



- DO NOT weigh highly digested otoliths. They will have lost mass, and fish size will be underestimated.

Tips

- Otolith handling – we use fine forceps and dissecting brushes to handle and manipulate otoliths
- 3% peroxide will work, but 12% works much better
- 96-well plates are good for otolith storage – but ensure you carefully tape over the wells to keep otoliths in their specified well locations
- Clean otoliths immediately and image within a few days to reduce likelihood of otoliths becoming chalky during storage.



Enquiries

Ronald Baker
rbaker@disl.org

