

Protocol for taking shape analysis photos of Cod

- Leica Application Software (LAS) Version 4.12 is used with Leica camera DFC290 and Leica Stereomicroscope MZ6
- Images to be saved here: <O:\Torsk shape analysis>
Folders created by year, e.g. O:\Torsk shape analysis\2018
- A calibration image needs to be taken each time a set of images are taken and saved in the same folder. File name should include the magnification used e.g. cal_0_63x0_63x0_63_
- The following format is used for naming the images
"Fish ID_replicatenumber" where replicate number 1= a pair of otoliths; 2 = left otolith and 3 = right otolith
e.g. "7666117_1"
- Images are taken on a black background. A pair of otoliths are placed directly on the circular black felt mat. See image examples 1a, 2a and 3a
- Images are taken under reflected light, using LED "arm" lights
- LAS V4.12 Settings as follows:
 - "Input Options" there is a saved configuration which must be used, called **"Torsk Shape analysis 2017"** for both the calibration image and otolith images.
The saved settings are:
Exposure: 478.4
Gain: 1.0
Gamma: 2.0
 - Image formats to be used are :
Captured format: 2048 x 1536 Full Frame HQ
Live format: 2088 x 1550 Full Frame
Image type: Greyscale
 - Calibration settings to be used are:
Type: Measured
Configuration: "Torsk Shape analysis 2017"

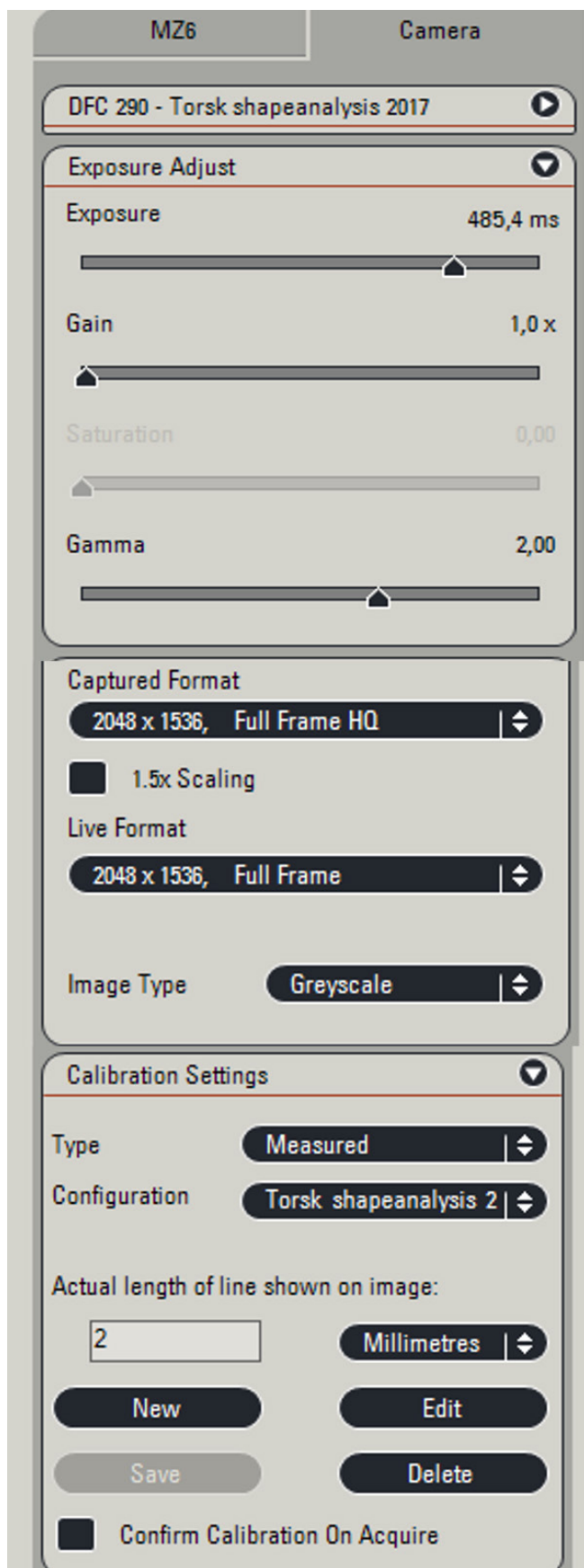


Figure 1. LAS settings

- 1. Set Capture location before you take images**
- 2. Name the image before you take the image**
- 3. Take a calibration image and annotate with scale bar, image name and date**
- 4. Convert from colour to an 8 bit image**
After the image has been acquired it will open in "Process" mode. Under the "Enhance" tab you click on "Convert Colour to Greyscale" and the "Apply". Choose "Replace" from the options

To take a picture of a pair of otoliths

- 1. LAS settings are the same as when taking the calibration image**
- 2. Save all otolith images in the same folder as the calibration image**
- 3. Name each image before you take the image**
- 4. Focus if necessary**
- 5. Acquire image**
- 6. Convert from Colour to 8 bit**

Orientation of Cod otoliths for shape analysis images

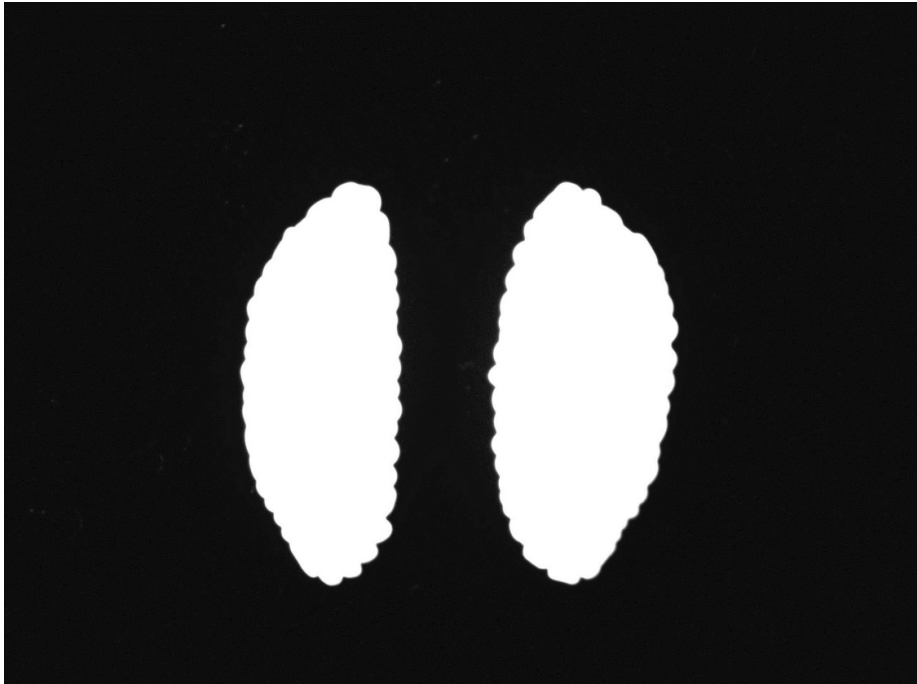


Figure 1a. Shows correct orientation and contrast for a pair of cod otoliths to be used for shape analysis

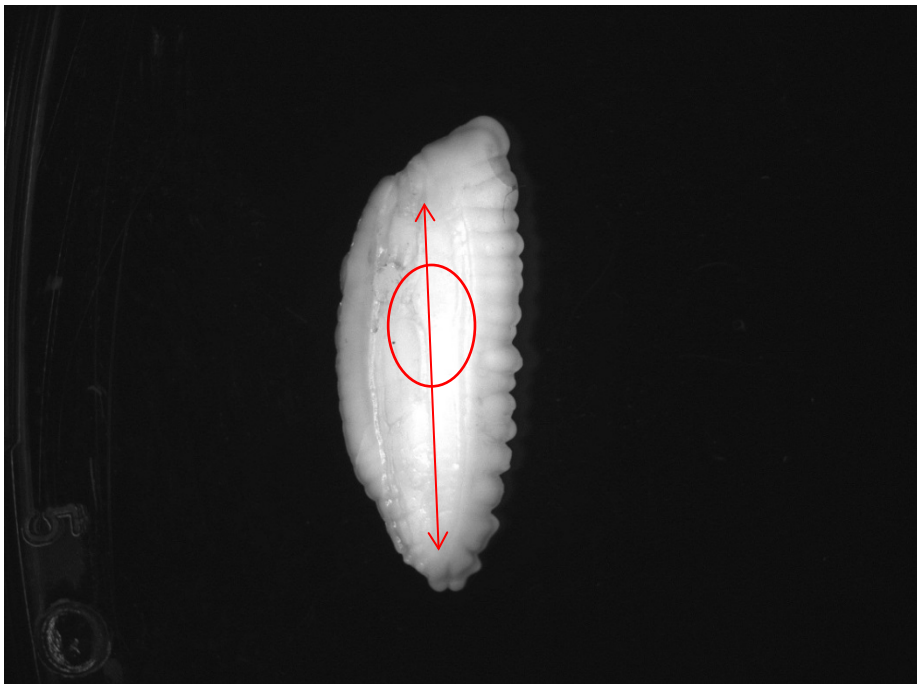


Figure 2a. Correct orientation. The red line is to show where there is a line along the otolith which ends at the centre point of the otolith. This centre point should be placed at the bottom of the image. The red circle shows the curve in the line which should always be closer to the top and not the bottom of the image.

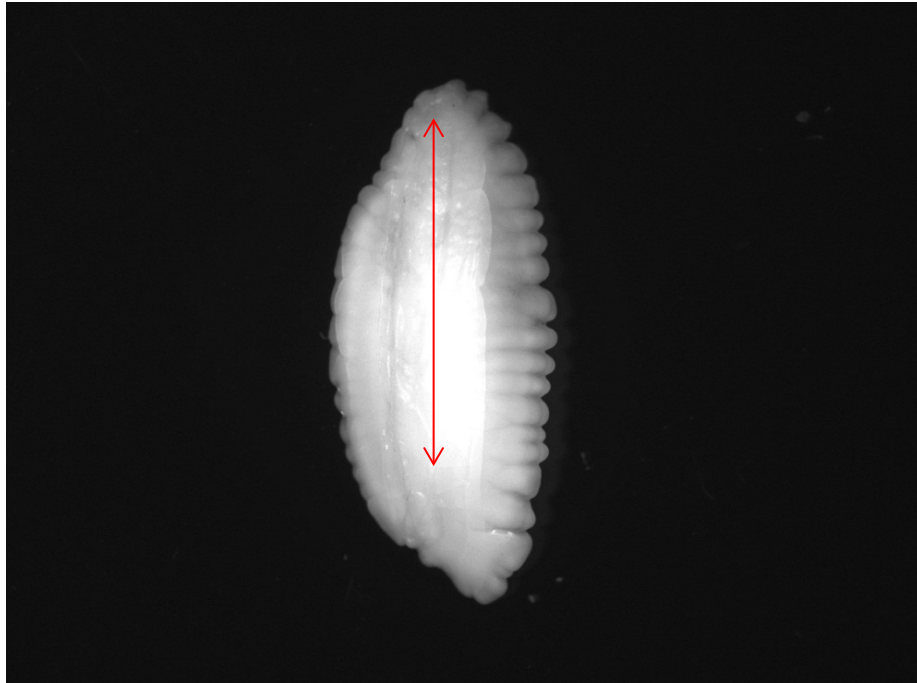


Figure 3a. Incorrect orientation. The red line ends at a point which should be at the bottom of the image.