Manual for the hyperspectral scanner*

N. E. Nevala[1] and T. Baden[1,2]

[1]School of Life Sciences, University of Sussex, United Kingdom

[2]Institute of Ophthalmic Research, University of Tübingen, Germany

*as described in Nevala and Baden 2018, bioRxiv

Overview

In this document we provide detailed instructions how to construct a complete hyperspectral scanner as presented in the accompanying paper, including a bill of materials (BOM) and use instructions. The Arduino code and SCAD files are provided on the project's GitHub page at https://github.com/BadenLab/3Dprinting_and_electronics/tree/master/Hyperspectral%20scanner.

Bill of Materials (BOM)

All details of the parts used and estimated costs are listed in Table 1. Without the protector case housing and waterproofing the costs are approximately £113, excluding the spectrometer. With the protector case housing and waterproofing the costs are around £340. In addition to these parts, a working laptop with the Arduino IDE (https://www.arduino.cc) and the spectrometer software (in our case from Thorlabs (https://www.thorlabs.com/software_pages/viewsoftwarepage.cfm?code=OSA) installed are required. For the analysis we provide instructions using IGOR Pro 7 Wavemetrics (https://www.wavemetrics.com/order/order_igordownloads.htm).

Circuit board

The overall operational and circuit logic is show in Figure 1. The circuit can be completed simply with all wires as indicated, or by organising pieces on a custom circuit board.

Table 1: Parts for hyperspectral scanner

	Supplier	Part number	Quantity	Unit price (£)	Full price (£)	Essential	Comments
Electronics							
Arduino Uno	Amazon		1	17.30	17.30	Yes	Any cheap clone is OK.
https://www.amazon.co.uk/Arduino-A000066-ATMEGA328-Microcontroller-Board/dp/B008GRTSV6/ref=sr_1_3?ie=UTF8&qid=1525263060&sr=8-3&keywords=arduino+uno							
MG90S Aluminum Metal Gear Servo Micro	Amazon		2	9.54	9.54	Yes	Comes in pack of 2.
https://www.amazon.co.uk/TowerPro-MG90S-Metal-Micro-Servo/dp/B00UB2ZBV0							
9V battery	RS Online		1	1.80	1.80	Yes	
https://uk.rs-online.com/web/p/9-volt-batteries/8264435/							
USB cable type A/B (From laptop to Arduino)	Amazon		1	1.89	1.89	Yes	
https://www.amazon.co.uk/Cable-Type-1-80-Connection-EDM/dp/B078ZPXSVS/ref=sr_1_16?s=diy&ie=UTF8&qid=1525263526&sr=1-16&keywords=USB+cable+type+A+B							
USB 2.0 cable A to Mini B (From laptop to spectrometer)	Amazon		1	2.50	2.50	Yes	
https://www.amazon.co.uk/rhinocables%C2%AE-Male-Cable-black-Lengths/dp/B076H1CJKF/ref=sr_1_11?s=computers&ie=UTF8&qid=1525263132&sr=1-11&keywords=USB+cable+type+A+B							
Toggle switch	Farnell	1MD1T1B1M1QE	1	2.41	2.41	Yes	
http://uk.farnell.com/multicomp/1md1t1b1m1qe/switch-dpdt-5a-120vac/dp/9473513							
5 pin Din Plug	Amazon	NE325	2	2.27	4.54	Yes	
https://www.amazon.co.uk/Plug-Nickel-Finish-Rubber-Boot/dp/B000LAPU4A/ref=sr_1_2?s=diy&ie=UTF8&qid=1525264155&sr=1-2&keywords=DIN+5+pin							
5 pin Din Chassis Socket	Amazon	NE328	2	1.66	3.32	Yes	
https://www.amazon.co.uk/pin-Chassis-Socket-Nickel-Finish/dp/B000LARV0Q/ref=sr_1_1?s=diy&ie=UTF8&qid=1525348842&sr=1-1&keywords=5+pin+Din+Chassis+Socket+Nickel+Finish							
BNC plug (male)	Farnell	4970	1	9.68	9.68	Yes	
http://uk.farnell.com/pomona/4970/test-lead-black-red-149-22mm-500vrms/dp/2526735?st=BNC%20plug%20male							
Wiring (any)	RS Online	331-862	100 cm	0.35	10.55	Yes	
https://uk.rs-online.com/web/p/hookup-equipment-wire/0331862/							
9V battery clip	Farnell	8459-0674	1	0.88	0.88	Yes	
http://uk.farnell.com/bud-industries/hh-3449/battery-retainer-clip/dp/1650667?st=9V%20battery%20clip							
SMB to BNC adapter cable			1			Yes	Included with spectrometer.

Other parts

1150 Protector Case https://peliproducts.co.uk/1150-protector-case.html	Peli products UK	1150-000-110	1	48.50	48.50	For underwater	
UniBond Repair Power Epoxy Plastic https://www.amazon.co.uk/UniBond-Repair-Power-Epoxy-Plastic/dp/B003UGKTHK	Amazon		1	5.85	5.85	For underwater	
Lapurete's Silica Gel Packets Desiccant https://www.amazon.co.uk/Lapuretes-Desiccant-Regenerative-Humidity-Indicator-25%C3%9710g/dp/B01CHSA0JS	Amazon		1	9.14	9.14	For underwater	Comes in pack of 25.
AquaMate Fish Tank Sealant https://www.amazon.co.uk/Everbuild-LEUDBBNJS2125-AquaMate-Fish-Sealant/dp/B0012RRFCQ	Amazon		1	3.42	3.42	For underwater	
Multipurpose water repellent silicone grease https://www.amazon.co.uk/gp/product/B006O7HTDE/ref=oh_aui_detailpage_o09_s00?ie=UTF8&psc=1	Amazon		1	3.99	3.99	For underwater	
UVFS Broadband Window, Uncoated https://www.thorlabs.com/thorproduct.cfm?partnumber=WG42012	Thorlabs	WG42012	1	105.57	105.57	For underwater	
PVC Tube (19mm inner/25 mm outer diameter) http://pvctubeonline.co.uk/pvc_tube.htm	PVC Tube Online	PVC19mmT	3 m	10.85	10.85	For underwater	
Steel sheet CR4 | 250mm x 250mm x 1mm https://www.amazon.co.uk/Steel-sheet-CR4-250mm-1mm/dp/B00KRF9G4Y/ref=sr_1_7?ie=UTF8&qid=1526301901&sr=8-7&keywords=metal+sheet	Amazon		1	4.99	4.99	For underwater	Needs to be cut in smaller pieces
IP66 Weather resistant sealed 150*250*100mm ABS enclosure https://www.amazon.co.uk/gp/product/B01M8I26BV/ref=oh_aui_detailpage_o02_s00?ie=UTF8&psc=1	Amazon		1	13.55	13.55	For underwater	
2kg Empty and Filled Shot Bags (Lead weights) https://www.amazon.co.uk/1kg-Empty-Filled-Shot-Bags/dp/B00M1WK3BQ/ref=sr_1_1?s=sports&ie=UTF8&qid=1526302223&sr=1-1&keywords=1kg+2kg+3kg+Empty+and+Filled+Shot+Bags+1kg+2kg+3kg	Amazon		1	18.50	18.50	For underwater	
Square UV Enhanced Aluminum Mirror https://www.thorlabs.com/thorproduct.cfm?partnumber=PFSQ05-03-F01	Thorlabs	PFSQ05-03-F01	1	23.91	23.91	Yes	
Square UV Enhanced Aluminum Mirror https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_id=264	Thorlabs	PFSQ10-03-F01	1	35.19	35.19	Yes	
Screw for mirror holders			4			Yes	Included with micro-servos.

Total cost for only essential parts	£113.31
Total cost for full system	£337.67
Total cost for full system with spares	£347.87

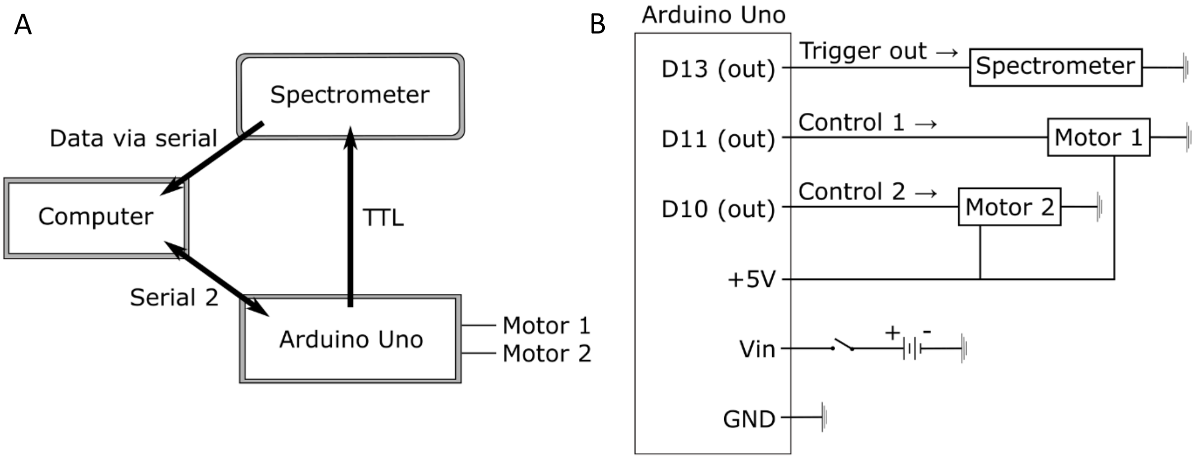

Figure 1: A, The operational logic and **B,** the circuit diagram.

Printing 3D parts

All 3D printed parts were designed using OpenSCAD which is freely available at (http://www.openscad.org/). All scripts are provided on the GitHub. The precise measurements of the printed parts are designed to fit the commercial protector case used (see Table 1, Fig. 2C) and a Thorlabs CCS200 spectrometer. If other types of cases, spectrometers or components are used, measurements for the base (Fig. 2A) should be adjusted accordingly. In addition to the base, other essential 3D printed parts for the scanner are the mirror holders attached to the two micro-servo motors and a pinhole placed in front of the spectrometer sensor (Fig. 2D). For the waterproofed version, tube and window holders are also needed (Fig. 2E and F). The dimensions of these are determined by the size of the window and tube used and should be adjusted accordingly if other versions are used. Before starting to assemble the scanner or the waterproof casing, make sure to have all the necessary components and 3D printed parts ready and in correct size.

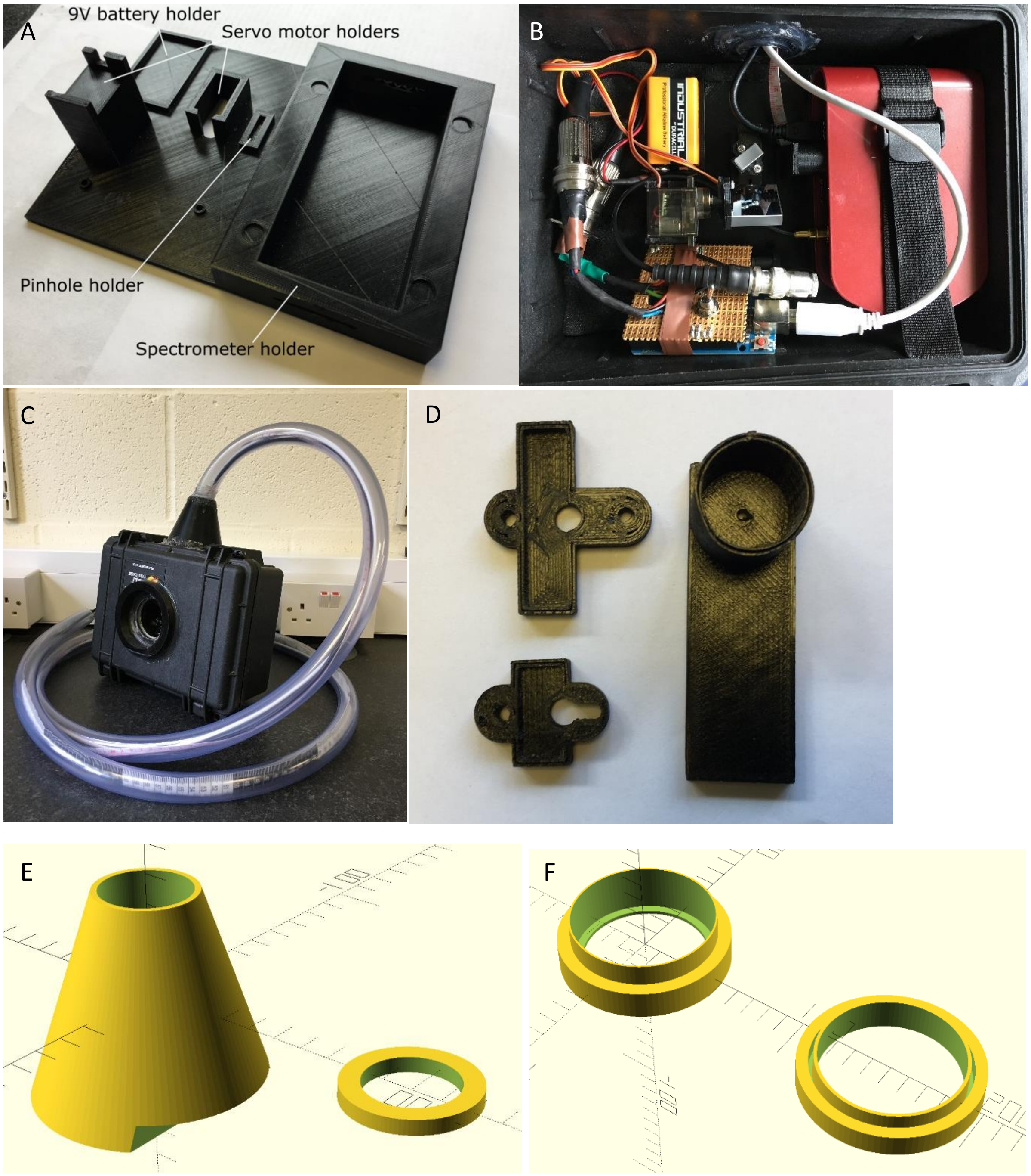

Figure 2: A, 3D printed base showing slots for 9V battery, two servo motors, a pinhole and a spectrometer. The higher servo motor holder should be the one holding the big mirror and the lower one holding the smaller mirror. In addition, we drilled two small slits to use a strap to secure the spectrometer to the base. **B,** The base inside the protector case with all the components. **C,** The complete scanner with waterproofed housing. **D,** 3D printer mirror holders and a pinhole. **E,** Screenshot of the 3D parts for the outside (left) and the inside (right) support parts for the tube. **F,** Screenshot of the 3D parts for the base (left) and the cap (right) parts of the window holder.

Assembling the scanner (after the electronics are assembled)

1. Position the circuit and Arduino as shown above. This can be taped down if desired.
2. Attach the mirror holders to the servo-motors with screws that come with the servo-motors.
3. Place the servo-motors on the base. The servo-motor with larger mirror holder should be placed on the more elevated motor holder (Fig. 2B). Attach mirrors to the servo-motors. The holders should be tight enough to hold the mirrors without additional support, but if not, a small dot of the sealant can be used to glue them into place.
4. Attach the 9V battery to the base and connect it with the circuit board.
5. Add the spectrometer and mount the pinhole in front of it.
6. Connect the power cords between laptop and Arduino + spectrometer, and the trigger cord between spectrometer and Arduino.

Waterproofing the protector case

1. To insert the front window, drill a 75 mm diameter hole to the front panel of the case (Fig. 3). Insert the base part of the window holder to the inner side of the case.
2. Carefully add two-component glue around the window (be careful not to smear the front or back parts of the window with glue) and insert the window to the holder. There should be enough glue all around the window to prevent any water to leak in! Carefully remove any extra glue from the window.
3. Add two-component glue to the outer rim and the groove of the cap part and press it firmly against the base part. Leave to dry.
4. Use fish tank sealant to carefully cover all possible seams inside and outside. Be careful not to leave any sealant on the window. Let the sealant dry over night or until it is completely set.
5. Confirm that the window inserted is waterproof before continuing to the next part.
6. Drill a 26 mm diameter hole as shown in Fig. 3 for the cables/tube. The hole should be positioned in the middle of the panel at the intended top of the scanner. Pass the tube through the hole, the tube should be a very tight fit.
7. Take the 3D printed inside support for the tube and apply two-component glue on one side of the part. Press the part firmly against the wall inside the box around the tube hole.
8. Take the 3D printed outside support for the tube and apply two-component glue on the wider bottom part of it. Press firmly against the outside part of the wall around the tube hole (the stepped edge should go on top of the lid only a little bit).
9. Leave to dry.
10. Take the tube and apply a large amount of sealant around the outer part of it for approximately 5 cm length. Push the tube through the holders until the edge of the tube is evenly levelled with the inside tube holder (so that no additional tube projects inside the case).

11. Apply ample sealant between the outside tube holder and the tube. The sealant can “overflow” a little bit, but make sure to leave an even surface. Apply a thick but smooth layer of sealant to all possible seams around the inside and outside tube holders (the inside tube holder can be “covered” with silicone as long as there is enough space for the cords to get through the tube). Leave to dry over night or until the sealant is completely dry.
12. Confirm that the box is waterproof before continuing to the next part.
13. Pass the power cord for Arduino and the spectrometer trigger cord through the tube (alternatively this can also be done before inserting the tube into its place).
14. To further improve the waterproofing, we also added a light layer of grease in the seams of the case. Appropriate care and testing should be completed before the case is placed under water with electronics and spectrometer inside.

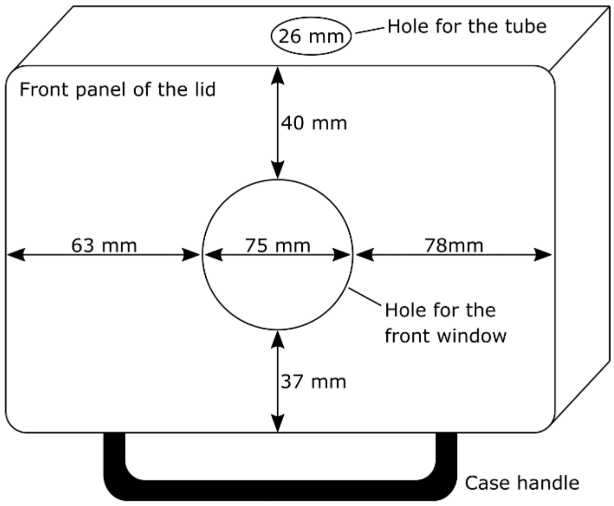

Figure 3: Positions of the holes for the window on the front panel of the case lid and the tube.

Assembling the scanner inside the protector case

1. Leave approximately a 1.5 – 2 cm thick layer of foam that comes with the case at the bottom. Place the base with all components attached to it on top of the foam. For underwater measurements we added scrap metal plates underneath the foam to decrease its buoyancy to the point where it would robustly sit on a riverbed at ~50 cm depth. In addition, or alternatively, lead weights can be used.
2. Attach the power cords to Arduino and spectrometer, and the trigger cord between the two.
3. Place the base so that the centre of the bigger mirror is centred relative to the front panel window. Secure the position using leftover foam pieces. Additional foam should also be applied carefully on top of the spectrometer and the other parts of the base (but not the mirrors) to prevent any additional movement when the scanner is turned to upright position. If necessary, the spectrometer can also be attached to the base with straps or tape.
4. Close the lid. Check that the big mirror is not touching the front window.

5. To hold the scanner in an upright position, we used an additional hard plastic box (Fig. 4).

6. To prevent condensation inside the box, we recommend using humidity absorbing Silica Gel Packets inside the scanner box.

Figure 4: A hard plastic box supporting the upright position of the scanner. The edge of the plastic box had to be cut to prevent covering of the window of the scanner.

Operating the scanner

1. Connect the Arduino and the spectrometer to the laptop. Turn the battery switch on Arduino in to “ON” position. This provides an additional power source for the Arduino from the 9V battery.

2. Launch both Arduino IDE and Thorlabs spectrometer software on the laptop.

3. Create an empty folder called “Spectra” on the Desktop.

4. Preparing the Thorlabs spectrometer software (these steps should be re-done each time the software is started as it does not store these preferences):

    a. Under the “Sweep” bar, choose “Trigger Mode: Software” and take one single test measurement by pressing “Single” (Fig. 5). A single sweep spectrum should appear on the screen.

    b. Under “File”, choose “Export trace” and save the test file in the “Spectra” folder created earlier. Choose “text file” in the “save as type”. Press “save”.

    c. On the window popping up, choose “comma” for the “Separate Columns by”. Press “Ok”. Now all the files for the actual measurements will be saved as text files and columns will be separated by commas. Delete the test file from the “Spectra” folder before doing any measurements!

    d. Change the “Trigger Mode” to “External” before starting a measurement.

   e. Choose a desired Integration Time (in ms, Fig. 5). Note: this must be smaller than the “Sampling time” in Arduino script, which defines the mirror movement intervals. Typical values used are approximately 100-200 ms for the Integration time and 260-500 ms for the Sampling time.

5. Preparing the Arduino script:

   a. Upload the “servo*.ino” script to the Arduino using the Arduino IDE (if in doubt how to do that, consult the Arduino online help). Choose a desired sampling time (in ms, Fig. 6). Note: this must be longer than the “Integration time” on the Thorlabs software.

   b. Press “Save” and “Upload”. Once complete, open the serial monitor.

   c. By moving the mirrors with AWSD commands (see “Operating the Arduino code”), move the mirrors until you can see the pinhole hole in the centre of each mirror by looking straight down at the bigger mirror through the box window. Enter these mirror positions as the X- and Y-offset values in the script (Fig. 6). Save and upload again. The mirrors should ideally be re-aligned like this every time before starting a measurement, and certainly if the box if substantially moved or reconnected.

   d. Choose a desired scan mode.

6. Go back to the Thorlabs software. Under “File”, choose “Auto Save”. Output directory should be set to the “Spectra” folder. Set naming of the files as wanted and File Format to “text”. Press “Ok”. In the pop-up window choose “Ok”.

7. Under “Sweep”, press “Repeat”. Ensure that the “spectra” folder on the Desktop is empty.

8. Go back to Arduino Serial Monitor and choose “P” followed by pressing Enter to run the scan.

9. Measured scans should appear in the spectra folder. After the scan is finished, move the data to a separate folder before taking any more measurements to avoid mixing up the data.

10. If the scanner is measuring fewer or extra points than the scan mode is indicating, integration and/or sampling time should be adjusted until correct number is achieved. However, with each scan mode one additional point is always included (e.g. with “Spiral 1000” mode correct number of scanned points is 1001, with “Spiral 600” mode correct number of scanned points is 601 etc.). The first sweep should always be excluded from the analysed data (just delete it).

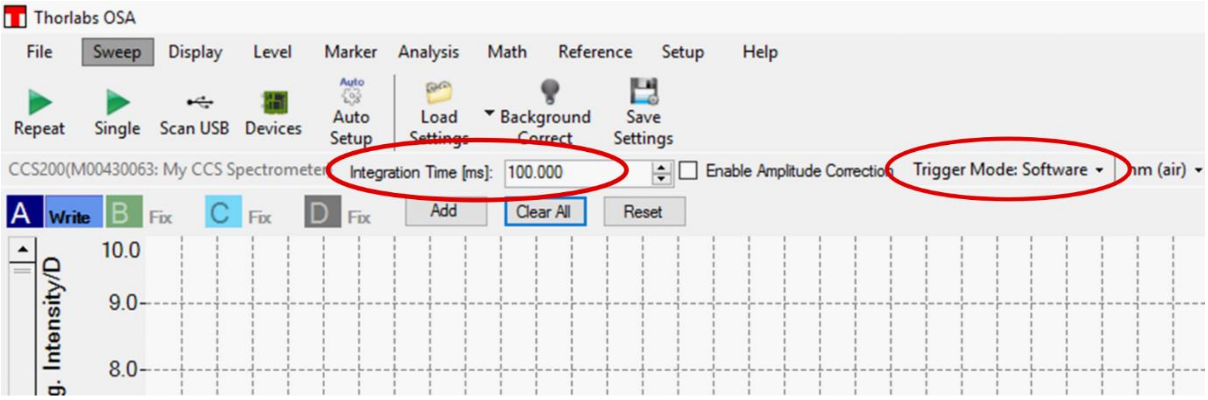

Figure 5: A screenshot of the Thorlabs software for the spectrometer highlighting where the Integration time and the Trigger mode can be changed.

Figure 6: A screenshot of the Arduino IDE showing where the X- and Y-offsets and the sampling time can be modified.

Operating the Arduino code

In the serial command window, the below commands (followed by ”Enter”) can be used:

Z – Set both servo-motor offsets to the Xoffset and Yoffset values as defined in the top of the Arduino script.

D – Calibrate X servo-motor to right.

A – Calibrate X servo-motor to left.

W – Calibrate Y servo-motor upwards.

S – Calibrate Y servo-motor downwards.

Q – Cycle through the different scan modes (explained below, Fig. 7).

R – Send 50 triggers without changing the position of the servo-motors (useful for testing).

T – Send one individual trigger without changing the position of the servo-motors (useful for testing).

P – Execute the selected scan mode.

Scan modes

Figure 7 shows the pattern and path of the four possible scan modes (a 100 points square and 300, 600 or 1000 points spirals, Fig. 7). Each of them covers the same 60-degree area with different angular spacing. The paths covering all the measuring points are optimised to achieve a minimal path length.

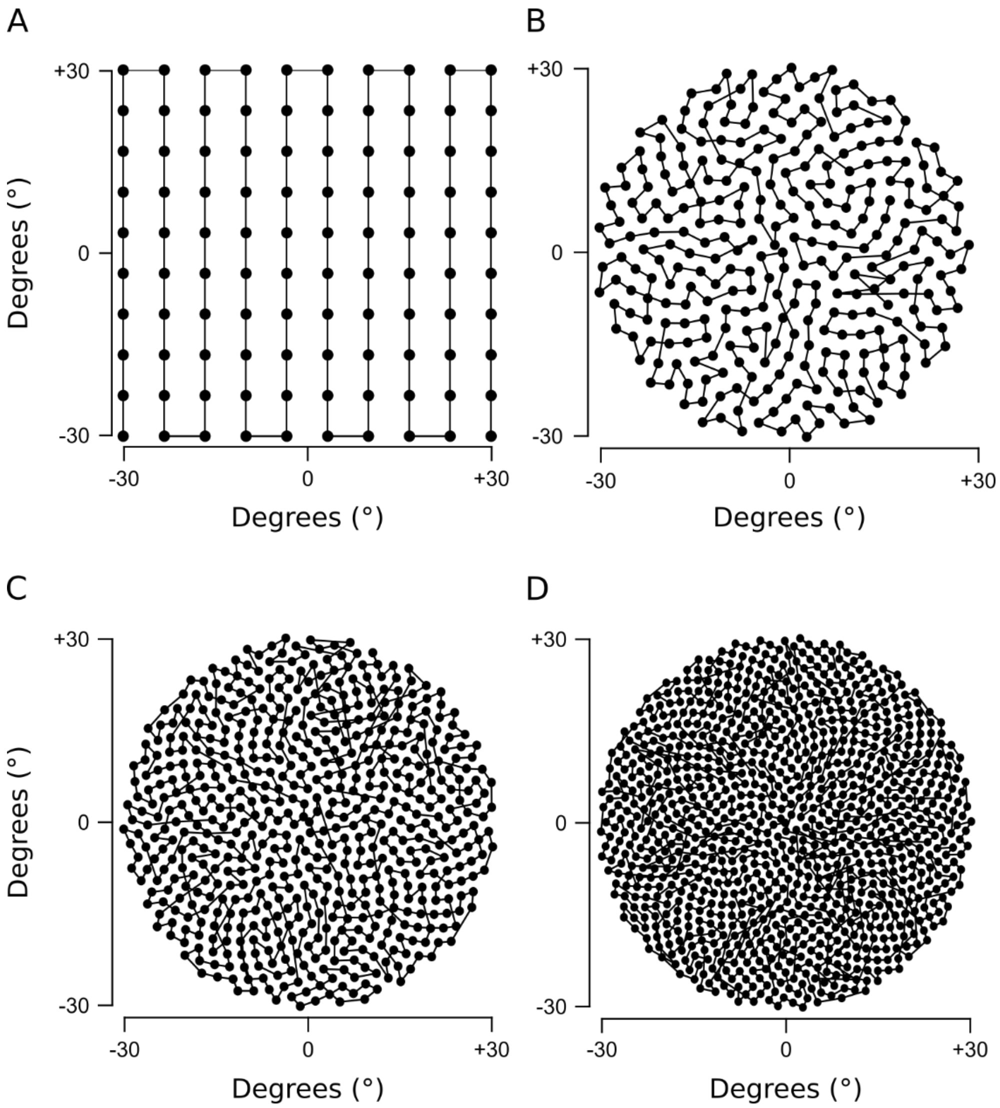

Figure 7: The 4 possible scan modes. **A,** 100 points square. **B,** 300 points spiral. **C,** 600 points spiral. **D,** 1000 points spiral.

Analysis in Igor Pro

1. Download the Scanner_empty.pxp Igor experiment file and the Scanner_v15.ipf Igor script file from the project's GitHub page.
2. Save the Scanner_empty.pxp file with a different file name.
3. Create a new folder under "root". Each individual complete scan requires a separate folder. Move the red arrow next to the "root" folder and drag it to point to your (currently empty) measurement file.
4. Open the folder where the raw data from the scan is. Select all the files but <u>leave the first point out</u>. By selecting the first selected file (the second file all in all), drag all the files to Igor. For each pop-up window, select "Load". Note that each individual file creates two waves in Igor (one of them holding the data, one holding the wavelengths).

   IMPORTANT: When dragging the individual "scan pixels" into Igor, make sure that you highlight the full array and then click and drag the <u>first</u> measurement to load the entire dataset at once but in order. Otherwise Igor may load them in the wrong order (it will just auto-name them wave0, wave1, wave2 etc so you will not know it did this unless you check).

5. Choose File – Open File – Procedure, and choose the Scanner_v15.ipf file to open the script in the experiment.
6. Run the function "Collect(DataFolder)", where DataFolder is replaced with the name of your folder holding the data in Igor. This removes every other wave from the measurement folder (the wavelength waves) and creates one wave with all the data in it.
7. Choose the desired animal you want to analyse your data with. In the script, set the "Chromat type" (Fig. 8) to the number of opsins the animal uses (see the folder for the opsin types in the Data Browser). For example, zebra finch have 4 different opsins, so the Chromat type is set to 4.
8. If using the waterproofed casing, the edges of the image can be cropped in "XEdgeCrop_deg" (= 0 to 30 degrees) to get rid of the shadowing caused by the case (Fig. 8).
9. The image can be rotated 90 degrees using the "flipflop" variable (=0 or 1) in case the scanner is used in any other position than showed in Figs. 2C and 4.
10. Run the analysis using function Analyse(DataFolder,Scanpath,species,display_stuff). As an example, the function can be written as Analyse("Cactus","Scanpaths:Spiral1000_30deg","Zebrafinch_oil",1) where "Cactus" is the name of the data folder, "Scanpaths:Spiral1000_30deg" is the scan path used to collect the data, "Zebrafinch_oil" is the name of the folder under "Opsins" folder holding the desired opsin templates for zebra finch and "1" (or alternatively "0") will display or not display the image after analysis. An example of the result after analysis is shown in Fig. 9.

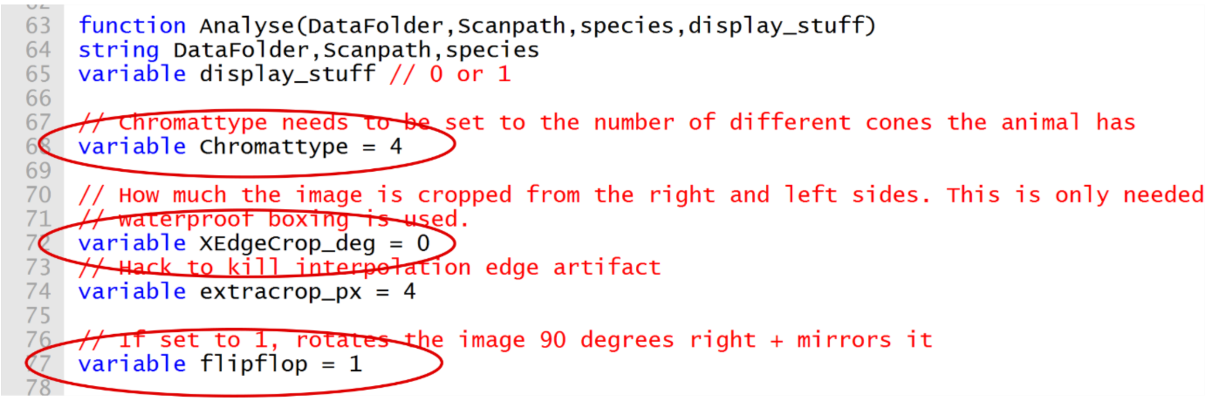

Figure 8: A screenshot of the Igor script showing where the Chromat type, Edge cropping and flipping the scan can be modified.

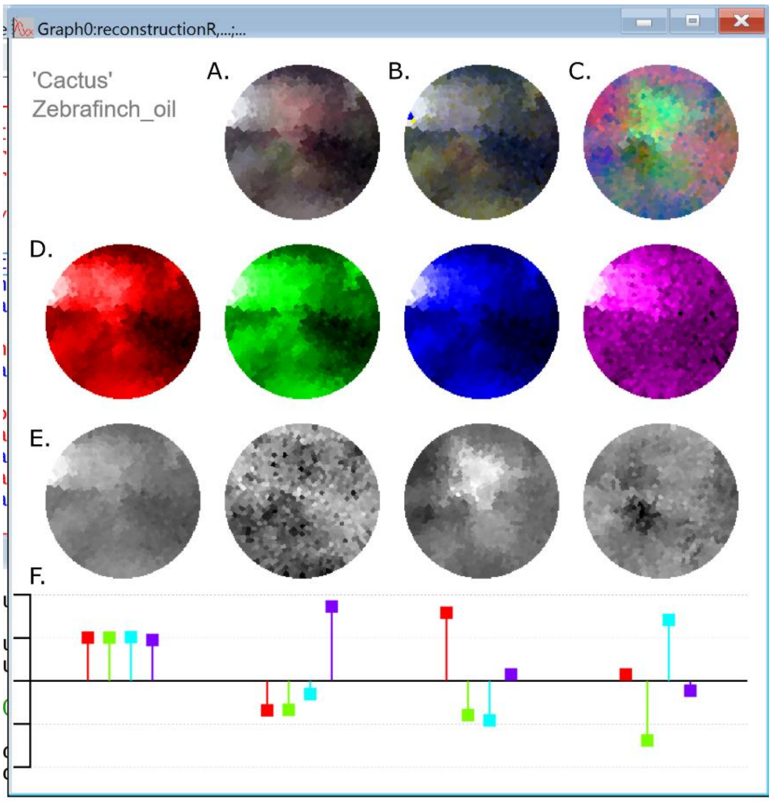

Figure 9: An example result graph from Igor after analysis. **A,** RGB (Red, Green, Blue) reconstruction of the monochromatic opsin channels from **D. B,** Combination of the first 3 opsin maps (from D) as an RGB image. **C,** RGB reconstruction of the principal components in **E. F,** Loadings explaining how much information is needed from each opsin channel in D.