

A low-cost hyperspectral scanner for natural imaging above and under water

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Figures

Supplementary data

Abbreviations used. C₁₋₃: Chromatic axes 1-3; MWS: Middle Wavelength Sensitive; PC: Principal Component; PCA: Principal Component Analysis; PVC: Polyvinyl Chloride; RGBU: Red-Green-Blue-Ultraviolet; SNR: Signal-to-Noise Ratio; TTL: Transistor-Transistor Logic; UHI: Underwater Hyperspectral Imager; USB: Universal Serial Bus; UV: Ultraviolet

34 **ABSTRACT**

35 Hyperspectral imaging is a widely used technology for industrial and scientific purposes, but
36 the high cost and large size of commercial setups have made them impractical for most basic
37 research. Here, we designed and implemented a fully open source and low-cost hyperspectral
38 scanner based on a commercial spectrometer coupled to custom optical, mechanical and
39 electronic components. We demonstrate our scanner's utility for natural imaging in both
40 terrestrial and underwater environments. Our design provides sub-nm spectral resolution
41 between 350-1000 nm, including the UV part of the light spectrum which has been mostly
42 absent from commercial solutions and previous natural imaging studies. By comparing the full
43 light spectra from natural scenes to the spectral sensitivity of animals, we show how our
44 system can be used to identify subtle variations in chromatic details detectable by different
45 species. In addition, we have created an open access database for hyperspectral datasets
46 collected from natural scenes in the UK and India. Together with comprehensive online build-
47 and use-instructions, our setup provides an inexpensive and customisable solution to gather
48 and share hyperspectral imaging data.

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64 INTRODUCTION

65 Hyperspectral imaging combines spatial and detailed spectral information of a scene to
66 construct images where the full spectrum of light at each pixel is known¹. Commercial
67 hyperspectral imaging technology is used, for example, in food industry^{2,3}, agriculture^{4,5} and
68 astronomy¹. However, these devices are typically expensive, lack the ultraviolet (UV) part of
69 the spectrum and only few work under water. Moreover, many are bulky and must be attached
70 to a plane or other heavy machinery, which makes them unsuitable for most basic research.
71 Here, we present a low-cost and open source hyperspectral scanner design and demonstrate
72 its utility for studying animal colour vision in the context of the natural visual world.

73 Animals obtain sensory information that meets their specific needs to stay alive and to
74 reproduce. For many animals, this requires telling wavelength independent from intensity – an
75 ability widely referred to as colour vision. To study what chromatic contrasts are available for
76 an animal to see in nature requires measuring the spectral content of its environment (natural
77 imaging) and comparing this to the eye's spectral sensitivity.

78 Most previous work on natural imaging to study animal colour vision used sets of spectrally
79 narrow images generated by iteratively placing different interference filters within the range of
80 400-1,000 nm⁶⁻⁹ in front of a spectrally broad sensor array. So far, a major focus has been on
81 our own trichromatic visual system that samples the short (blue “B”), medium (green “G”) and
82 long (red “R”) wavelength (“human visible”) range of the electromagnetic spectrum^{6,8,10-12}.
83 However, across animals the number and spectral sensitivity of retinal photoreceptor types
84 varies widely. Perhaps most importantly, and unlike humans, many animals can see in the UV
85 part of the spectrum, which has not been included in available hyperspectral measurements
86 from terrestrial or underwater scenes. Johnsen et al. (2013, 2016)^{13,14} used an underwater
87 hyperspectral imager (UHI) to map the seafloor in an effort to identify structures and objects
88 with varying depth, but more shallow underwater habitats have not been studied in this way.
89 Finally, in 2013 Baden et al.¹⁵ used a hyperspectral scanner based on a spectrometer reaching
90 the UV spectrum of light and an optical fibre controlled by two servo motors. With their setup
91 it is possible to build hyperspectral images in a similar way to the design presented here, but
92 the system is both bulky and fragile. In addition, their setup cannot be easily waterproofed
93 because the point of light from the scene is guided with the optic fibre attached to the
94 spectrometer. Our design uses mirrors instead to overcome these shortcomings.

95 Here, we designed and built a low-cost open source hyperspectral scanner from 3D printed
96 parts, off-the-shelf electronic components and a commercial spectrometer that can take full
97 spectrum (350-1,000 nm), low spatial resolution (4.7°) images above and under water. With
98 our fully open design and instructions it is possible for researchers to build and modify their

99 own hyperspectral scanners at substantially lower costs compared to commercial devices
100 (~£1,500 for a spectrometer if unavailable, plus ~£113-340 for all additional components,
101 compared to tens to hundreds of thousands for commercial alternatives). We demonstrate the
102 performance of our system using example scans and show how this data can be used to study
103 animal colour vision in the immediate context of their natural visual world. We provide all raw
104 data of these and additional scans to populate a new public database of natural hyperspectral
105 images measured in the UK and in India ([https://zenodo.org/communities/hyperspectral-](https://zenodo.org/communities/hyperspectral-natural-imaging)
106 [natural-imaging](https://zenodo.org/communities/hyperspectral-natural-imaging)), to complement existing datasets^{16–18}.

107

108 **METHODS**

109 *Hardware design.*

110 The device is built around a trigger-enabled, commercial spectrometer (Thorlabs CCS200/M,
111 advertised as 200-1,000 nm but effectively useful above 350 nm). A set of two movable UV
112 reflecting mirrors (Thorlabs PFSQ10-03-F01 25.4 x 25.4 mm and PFSQ05-03-F01 12.7 x 12.7
113 mm) directs light from the scanned scene onto the spectrometer's sensor region via a pinhole
114 (see also Baden et al. 2013)¹⁵. To gradually assemble an image, an Arduino Uno
115 microcontroller (www.Arduino.cc) iteratively moves the two mirrors via servo-motors along a
116 pre-defined scan-path under serial control from a computer. At each new mirror position, the
117 Arduino triggers the spectrometer via a transistor-transistor logic (TTL) pulse to take a single
118 reading. An optional 9V battery powers the Arduino to relieve its universal serial bus (USB)
119 power connection. The entire set-up is encased in a waterproofed housing fitted with a quartz-
120 window (Thorlabs WG42012 50.8 mm UVFS Broadband Precision Window) to permit light to
121 enter. For underwater measurements, optional diving weights can be added to control
122 buoyancy. All internal mechanical components were designed using the freely available
123 OpenSCAD (www.OpenScad.org) and 3D printed on an Ultimaker 2 3D printer running Cura
124 2.7.0 (Ultimaker). For detailed build instructions including all 3D files and Arduino control code,
125 see the project's GitHub page at
126 [www.github.com/BadenLab/3Dprinting_and_electronics/tree/master/Hyperspectral%20scann](http://www.github.com/BadenLab/3Dprinting_and_electronics/tree/master/Hyperspectral%20scanner)
127 [er](http://www.github.com/BadenLab/3Dprinting_and_electronics/tree/master/Hyperspectral%20scanner).

128

129 *Scan-paths.*

130 Four scan paths are pre-programmed onto the Arduino control code: a 100 point raster at 6°
131 x- and y-spacing (60° x 60°), and three equi-spaced spirals at $r = \pm 30^\circ$ at $n=300, 600$ or $1,000$

132 points, respectively (Supplementary Figure 1). To generate spirals, we computed n points of
133 a Fermat's spiral:

$$134 \quad r = \sqrt{\theta \times n}$$

$$135 \quad \theta = \pi (3 - \sqrt{5})$$

136

137 where r is the radius and θ , in radians, is the "golden angle" ($\sim 137.5^\circ$). Next, we sorted points
138 by angle from the origin and thereafter ran a custom algorithm to minimise total path length.
139 For this, we iteratively and randomly exchanged two scan positions and calculated total path
140 length. Exchanges were kept if they resulted in path shortening but rejected in all other cases.
141 Running this algorithm for 10^5 iterations resulted in the semi-scrambled scan paths shown in
142 SFig. 1.

143

144 *Data collection.*

145 All recordings shown in this work used the 1,000-point spiral. Acquisition time for each scan
146 was 4-6 minutes, depending on the time set for each mirror movement (260-500 ms) and the
147 spectrometer's integration time (100-200 ms). These were adjusted based on the amount of
148 light available in the environment to yield an approximately constant signal-to-noise ratio
149 (SNR) between scans. In all cases, the scanner was supported using a hard-plastic box to
150 maintain an upright position. All outdoor scans were taken in sunny weather with a clear sky.
151 For details of the underwater measurement done in West Bengal India, see Zimmermann,
152 Nevala, Yoshimatsu et al., 2017¹⁹. In addition, we took a 180° RGB colour photograph of each
153 scanned scene with an action camera (Campark ACT80 3K 360°) or a $\sim 120^\circ$ photograph with
154 an ELP megapixel Super Mini 720p USB Camera Module.

155

156 *Data analysis.*

157 All data was analysed using custom scripts written in IGOR Pro 7 (Wavemetrics) and Fiji (NIH).
158 To visualise scanned images, we calculated the effective brightness of each individual
159 spectrum (hereafter referred to as "pixel") as sampled by different animals' opsin templates.
160 In each case, we z-normalised each channel's output across an entire scan and mapped the
161 resultant brightness map to 16-bit greyscale or false-colour coded maps, in each case with
162 zero centred at 2^{15} and range to 0 and to $2^{16}-1$. We then mapped each pixel onto the 2D plane
163 using a standard fish-eye projection. To map each spiral scan into a bitmap image, we scaled

164 a blank 150x150 target vector to $\pm 30^\circ$ (same as the scanner range), mapped each of n scanner
165 pixels to its nearest position in this target vector to yield n seed-pixels, and linearly interpolated
166 between seed-pixels to give the final image. The 150 x 150 pixel (60 x 60 degrees) target
167 vector was truncated beyond 30° from the centre to cut the corners which comprised no data
168 points. We also created hyperspectral videos by adding a 3rd dimension so that each pixel in
169 the 150 x 150 target vector holds a full spectrum. This way each video is constructed from 800
170 individual images where one frame equals to 1 nm window starting from 200 nm.

171

172 *Principal component analysis.*

173 For principal component analysis (PCA), we always projected across the chromatic dimension
174 (e.g. human trichromatic image would use 3 basis vectors, “red”, “green” and “blue”) after z-
175 normalising each vector.

176

177 **RESULTS**

178 The scanner with water-proofed casing, its inner workings and control logic are illustrated in
179 Figure 1. Light from the to-be-imaged scene enters the box through the quartz window (Fig.
180 1A) and reflects off the larger and then the smaller mirror, passing through a pinhole to
181 illuminate the active part of the spectrometer (Fig. 1B). To scan a scene, an Arduino script is
182 started via serial command from a computer to iteratively move the two mirrors through a pre-
183 defined scan path (Methods and Supplementary Video 1). At each scan-position, the mirrors
184 briefly wait while the spectrometer is triggered to take a single reading. All instructions for
185 building the scanner, including 3D part models and the microcontroller control code are
186 provided at the project's GitHub page at
187 [https://github.com/BadenLab/3Dprinting_and_electronics/tree/master/Hyperspectral%20scan](https://github.com/BadenLab/3Dprinting_and_electronics/tree/master/Hyperspectral%20scanner)
188 [ner](#).

189

190 *Scanner performance.*

191 In our scanner design, several factors contribute to the spatial resolution limit of the complete
192 system. These include spacing of the individual scan-points, angular precision of the servo-
193 motors, the effective angular size of the pinhole as well as the optical properties of the mirrors
194 and the quartz window. To therefore establish the scanner's effective spatial resolution, we
195 scanned a printout of 8.6° black and white bars in the mid-day sun using a 1,000-point spiral
196 (Fig. 2A, Supplementary Figure 1) and compared the result (Methods) to the original scene

197 (Fig. 2B, C). The difference between these two profiles approximately equates to a Gaussian
198 blur of 2.36° standard deviation, which effectively translates to $\sim 4.7^\circ$ as the finest detail the
199 scanner can reliably resolve under these light conditions. While this spatial resolution falls far
200 behind even the simplest commercial digital camera systems, our scanner instead provides
201 650 nm spectral range and sub-nm resolution that can be used to identify fine spectral details
202 in the scanned scene.

203 To illustrate the scanner's spectral resolution, we took a 1,000-point scan in the mid-day sun
204 of a blue door and red brick wall (Fig. 2D) and reconstructed the scene based on human red,
205 green and blue opsin templates²⁰ to assemble an RGB image (Methods, Fig. 2D). From this
206 scan, we then picked two individual "pixels" (blue and red dots) and extracted their full spectra
207 (Fig. 2E). Next, we illustrate the function with examples from terrestrial and underwater
208 scenes.

209

210 *Natural imaging and animal colour vision.*

211 The ability to take high-spectral resolution images is useful for many applications, including
212 food quality controls^{2,3}, agricultural monitoring^{4,5} and surface material identification from
213 space¹. Another possibility is to study the spectral information available for colour vision by
214 different animals. Here, our portable, waterproofed and low-cost hyperspectral scanner
215 reaching into the UV range allows studying the light environment animals live in. To illustrate
216 what can be achieved in this field, we showcase scans of three different scenes: a forest scene
217 from Brighton, UK (Figs. 3-5), a close-up scan of a flowering cactus (Fig. 6) and an underwater
218 river scene from West Bengal, India (Fig. 7). In each case, the estimated 60° field of view
219 covered by the scanner is indicated in the accompanying widefield photos (Fig. 3A, 4A, 6A,
220 7A). To showcase chromatic contrasts available for colour vision by different animals in these
221 scenes, we reconstructed the forest and cactus data with mouse (*Mus musculus*), human
222 (*Homo sapiens*), bee (*Apis mellifera*), butterfly (*Graphium sarpedon*), chicken (*Gallus gallus*
223 *domesticus*) and zebra finch (*Taeniopygia guttata*) spectral sensitivities (Fig. 5B, 6C). The
224 underwater scan was reconstructed based on zebrafish (*Danio rerio*) spectral sensitivity (Fig.
225 7B)²⁰⁻²⁵. In addition, we provide hyperspectral movies between 200 and 1,000 nm for these
226 three scenes, where each frame is a 1 nm instance of the scanned scene (Supplementary
227 Videos 2-4). These videos illustrate how different structures in the scene appear at different
228 wavelengths.

229 First, we used the data from the forest scene scan to compute how a trichromat human with
230 three opsins (red, green and blue) might see it (Fig. 3). To this end, we multiplied the spectra
231 from each "pixel" with the spectral sensitivity of each of the three corresponding opsins

232 templates to create “opsin activation maps” (red “R”, green “G” and blue “B”, Fig. 3A,
233 Methods), hereafter referred to as “channels”. These false-colour coded, monochromatic
234 images show the luminance driving each opsin across the scene. In this example, the R- and
235 G-channels clearly highlight the dark band of trees in the middle of the scene with varying light
236 and dark structures in the sky and on the ground. However, the B-channel shows mainly
237 structures from the sky but provides low contrast on the ground. To illustrate how these
238 channels can be used for our sense of colour vision, we combined them into an RGB image
239 (Fig. 3A, right).

240 To determine what chromatic structures are discernible with human spectral sensitivity, we
241 used principal component analysis (PCA) across the 3-dimensional RGB space by using the
242 R-, G- and B-channels as 3 basis vectors (Fig. 3B, C). In natural scenes, most variance across
243 space is driven by changes in overall luminance rather than chromatic contrasts^{6,9,10}. In this
244 type of data, the first principal component (PC1) therefore reliably extracts the achromatic
245 (greyscale) image content. From here it follows that all subsequent principal components
246 (PC2-n) must describe the chromatic axes in the image, in decreasing order of importance.
247 For simplicity, we hereafter refer to PC1 as the achromatic axis and PC2, PC3 and (where
248 applicable) PC4 as first, second and third chromatic axes, respectively ($C_{1,2,3}$). When applied
249 to the example scan of the forest scene with human spectral sensitivity, the achromatic image
250 with near equal loadings across the R-, G- and B-channels accounted for majority (97.7%) of
251 the total image variance (Fig. 3D-F), in agreement with previous work^{6,9,10}. This left 2.3% total
252 variance for the first and second chromatic axes C_1 and C_2 (Table 1). In line with Ruderman
253 et al. (1998)⁶, the chromatic contrasts emerging from PCA were R+G against B (C_1 , long- vs
254 short-wavelength opponency) and R against G while effectively ignoring B (C_2 , Fig. 3E). These
255 two chromatic axes predicted from the hyperspectral image matched the main chromatic
256 comparisons performed by the human visual system (“blue vs. yellow” and “red vs. green”).
257 To show where in the image different chromatic contrasts exist across space, and to facilitate
258 visual comparison between animals, we also mapped the chromatic axes into an RGB image
259 such that R displays C_1 , G C_2 and B C_3 . Since the trichromat human can only compute two
260 orthogonal chromatic axes ($n_{\text{Opsins}} - 1$), C_3 was set to 2^{15} (i.e. the mid-point in 16-bit) in this
261 example. These PC-based RGB images ignore the brightness variations of the achromatic
262 channel, therefore describing only chromatic information in a scene. This specific projection
263 allows a trichromat human observer viewing an RGB-enabled screen or printout to judge
264 where in a scanned scene an animal might detect dominant chromatic contrasts, even if that
265 animal uses more than three spectral cone types for colour vision. The power of this approach
266 can be illustrated when considering non-human colour vision based on the same dataset.

267 Unlike humans, many animals use the ultraviolet (UV) part of the spectrum for vision^{26,27}. To
268 illustrate how the addition of UV-channel can change available chromatic information, we next
269 performed the same analysis for a tetrachromatic zebra finch (Fig. 4). This bird uses four,
270 approximately equi-spaced opsins (red, green, blue and UV), which in addition are spectrally
271 sharpened with oil droplets²³. As before, the monochromatic opsin-channels (RGB and “U” for
272 UV, Fig. 4A) appeared with R- and G-channels showing structures both in the sky and on the
273 ground while B- and U-channels mainly highlighted the sky. We next computed the principal
274 components across the now four opsin channels (Fig. 4B-F).

275 This time the achromatic axis explained only 92.5% of the total variance leaving 7.5% for
276 chromatic comparisons, which now comprised three chromatic axes (C_{1-3} , Table 1). As with
277 humans, the most important chromatic axis compared long- and short-wavelength channels
278 (C_1 , R+G against B+U, single zero crossing in Fig. 4E). C_2 was also similar to the human
279 version by comparing R- and G-channels, but in addition paired the R-channel with the UV
280 and the G-channel with the blue (two zero crossings). While the spatial structure highlighted
281 by C_1 was similar to that of the human, C_2 picked up additional details from the ground (Fig.
282 4D). Finally, C_3 (R+B against G+U) highlighted additional structures in the scene that are
283 largely invisible to the human observer.

284

285 *An animal's opsin complement dictates discernible chromatic contrasts.*

286 To further survey how an animal's opsin complement can affect the way chromatic details are
287 detectable in complex scenes, we compared data from the forest scene (Fig. 5) to a close-up
288 scan of a flowering cactus (Fig. 6) and filtered each using different animals' spectral
289 sensitivities: a dichromat mouse, a trichromat human and bee and a tetrachromat butterfly,
290 chicken and zebra finch. In these scenes, the order of the chromatic axes was largely stable
291 across opsin complements used (PC1 – achromatic, C_1 – long vs short wavelengths, C_2 –
292 R+U vs G+B, C_3 – R+B vs G+U), and here we only show the achromatic and C_{1-3}
293 reconstructions alongside the PC RGB images (Fig. 5A and 6B) next to the spectral sensitivity
294 of each animal (Fig. 5B and 6C). In each case, the number of chromatic channels shown
295 corresponds to the number of an animal's cone types minus 1.

296 The chromatic axes usable by different animals revealed diverse spatio-chromatic structures
297 from both scenes (Fig. 5 and 6). Across all animals compared, while C_1 still reliably highlighted
298 a long- vs. short-wavelength axis, the exact image content picked up along C_{1-n} varied between
299 opsin complements (Fig. 5A and 6B). For example, in the cactus scene the C_1 for the chicken
300 highlighted spatial structures in the image that other animals instead picked up with C_2 . A
301 similar difference was also seen in the forest scene, where C_2 and C_3 in butterfly showed

302 structures that were captured in the inverse order in the chicken and zebra finch (Fig. 5A). In
303 addition, humans and butterflies had more consistent arrangement and structures in chromatic
304 axes between each other than with other animals, possibly due to their similarly overlapping
305 spectral sensitivities of the green and red cones.

306 For all animals in both scenes, the achromatic image content captured at least 91.9% of the
307 total variance, leaving 1.4-8.1% for the chromatic axes (Table 1). For the forest scene, the
308 addition of opsin-channels increased the amount of variance explained by the chromatic axes,
309 and in particular for animals with widely spaced spectral channels (e.g. with chicken and
310 butterfly, Table 1). In general, more chromatic details was discerned with more cones,
311 especially when these cones had low-overlap spectral sensitivities covering a wide range of
312 the natural light spectrum (e.g. from around 350 nm to over 600 nm as with zebra finch).
313 Moreover, spectral sharpening of the opsin peaks through the addition of oil droplets (chicken
314 and zebra finch) brought out further details and higher chromatic contrasts in the scanned
315 scene. The order of importance for the chromatic axes that optimally decompose scans
316 depended strongly on the set of input vectors – the spectral shape and position of the animal's
317 opsins.

318

319 *Hyperspectral imaging under water.*

320 As light travels through the water column, water and dissolved particles absorb both extremes
321 of the light spectrum making it more monochromatic with increasing depth^{9,28}. Mainly because
322 of this filtering and scattering, underwater light environments have spectral characteristics that
323 differ strongly from terrestrial scenes. To illustrate one example from this underwater world,
324 we show a scan from a shallow freshwater river scene (Fig. 7A) taken in the natural habitat of
325 zebrafish (*Danio rerio*) in West Bengal, India¹⁹. The data was analysed based on the spectral
326 sensitivity of the tetrachromatic zebrafish with red, green, blue and UV sensitive cones (Fig.
327 7B)^{21,26}. In this example, the monochromatic R-, G-, and B-channels picked up different
328 dominant spatial structures in the scene, while the U channel appeared more “blurry” with only
329 small intensity differences around the horizon (Fig. 7C). Here, the total variance explained by
330 the chromatic axes C_{1-3} (14.7%, Fig. 7F) was higher compared to the two terrestrial scenes.
331 C_1 compared long (R+G) and short (B+U) wavelengths between upper and lower parts of the
332 scene (Fig. 7D, E) that arose from spectral filtering under water. Finally, C_2 and C_3 brought
333 out further details that probably correspond to pieces of the imaged vegetation.

334

335 *An open database for natural imaging.*

336 Based on these and other additional scans above and under water from around the world (for
337 example, see Zimmermann et al., 2017¹⁹) we created an open access database online
338 (<https://zenodo.org/communities/hyperspectral-natural-imaging>). All measurements in the
339 database are taken with the hyperspectral scanner as described here.

340

341 **DISCUSSION**

342 We have designed and implemented an inexpensive and easy-to-build alternative to
343 commercial hyperspectral scanners suited for field work above and under water. Without the
344 spectrometer (~£1,500), the entire system can be built for ~£113-340, making it notably
345 cheaper than commercial alternatives. In principle, any trigger-enabled spectrometer can be
346 used for the design. Alternatively, spectrometers can also be home-built^{29,30} to further reduce
347 costs.

348 The spatial resolution of the scanner with the 1,000-points scan (~4.7°), though substantially
349 below that of most commercial camera systems, is close to the behavioural resolution limit of
350 several model-animals like zebrafish larvae (~3°)³¹ or fruit flies (~1-4°)³². Notably, most animal
351 visual systems inherently combine a low-spatial resolution chromatic representation of the
352 visual world with a high-spatial resolution achromatic representation³³⁻³⁵. As such, our system
353 can likely also give useful insights into the chromatic visual world of animals with much more
354 highly resolved eyes. The spatial resolution of our system could principally be further
355 improved, for example by using a smaller pinhole in combination with higher-angular-precision
356 motors. However, the amount of natural light for vision is limited, especially when imaging
357 under water where light is quickly attenuated with increasing depth. As a result, higher spatial
358 resolution in our system would require a substantially increased integration times for each
359 pixel. This would result in very long scan-durations, which is unfavourable when scanning in
360 quickly changing natural environments.

361 Spatial resolution aside, the spectral range and detail of our scanning approach far exceeds
362 the spectral performance of interference filter-based approaches, as used in most previous
363 hyperspectral imaging studies^{6,8,9,17,36}. This difference may be crucial for some questions. For
364 example, zebrafish have four opsin-genes for middle wavelength sensitive (MWS) cones
365 (“green cones”) that are used in different parts of the retina and are separated in spectral
366 sensitivity by few nanometres^{22,37}. Most interference filter setups use relatively broad spectral
367 sensitivity steps and would therefore miss small details in the natural scenes that could be
368 picked up with slightly different spectral sensitivities of different opsins. By choosing individual
369 “pixels” and the spectra they hold, it is possible to analyse fine details in complex scenes that
370 animals can use for colour vision. This can be done already with very coarse spatial resolution

371 to reveal structures that otherwise would remain undetected. In agreement with previous
372 studies, we have shown how principal component analysis aids to separate achromatic and
373 chromatic information in natural images^{6,9}. Here, PCA across the chromatic channels
374 highlights spatio-chromatic aspects in the scene that may be useful for vision. Perhaps not
375 surprisingly, this reveals major, overall trends in landscapes (Figs. 3-5) with short wavelength
376 dominated sky and long wavelength dominated ground. This is true also for the underwater
377 habitats (Fig. 7), where light spectrum in the water column transforms from “blue-ish” short
378 wavelength dominated to “red-ish” long wavelength dominated with increasing depth¹⁹. The
379 PCs can also highlight details in complex scenes that might otherwise stay hidden but that
380 may be important for animals to see in their natural habitats.

381

382 **CONCLUSION**

383 We have shown how our simple, self-made scanner can produce hyperspectral images that
384 can be used to study animal colour vision. We have also started to populate an open database
385 of hyperspectral images from various natural scenes
386 (<https://zenodo.org/communities/hyperspectral-natural-imaging>). In the future, it will be
387 interesting to survey a more varied set of habitats and, for example, to compare how closely
388 related animal species living in different habitats have evolved with varying visual abilities.
389 This could also include variations of the presented design, for example to scan larger fields of
390 view, or a time-automation mode by which the same scene can be conveniently followed over
391 the course of a day. We will be pleased to facilitate other’s additions to the design through a
392 centralised project repository
393 ([www.github.com/BadenLab/3Dprinting_and_electronics/tree/master/Hyperspectral%20scan](http://www.github.com/BadenLab/3Dprinting_and_electronics/tree/master/Hyperspectral%20scanner)
394 [ner](http://www.github.com/BadenLab/3Dprinting_and_electronics/tree/master/Hyperspectral%20scanner)) and hope that in this way more researchers will be able to contribute to building a more
395 global picture of the natural light available for animal vision on earth.

396

397

398 **FIGURE LEGENDS**

399 **Figure 1. A Hyperspectral scanner for low-cost natural imaging.**

400 **(A)** The waterproof casing with a window (white asterisk) for light to enter. The PVC tube on
401 top protects the cables to the computer. **(B)** Internal arrangement of parts: the spectrometer,
402 Arduino Uno microcontroller, 9V battery, two servo motors (Motors 1 and 2) with mirrors
403 attached to them and a pinhole. Light reaches first the larger mirror underneath the window of

404 the casing, reflects to the smaller mirror and from there through the pinhole to the
405 spectrometer's sensor. Light deflected off the first mirror is partly shadowed by the edges of
406 the casing, which creates dark stripes at the horizontal edges of the scanned images when
407 the box is closed. These edges are cropped in the presented example scans (Figs. 2 and 7).
408 Spectral filtering by the quartz window was corrected for in postprocessing (Supplementary
409 Figure 2). **(C)** Operational logic. The scanning path is uploaded to the Arduino from the
410 computer via Serial 2 connection to define the motor movements. After each movement the
411 spectrophotometer is triggered via TTL to take a measurement and send the data to the
412 computer via serial. The ongoing state of the scanning path is fed from the control circuit to
413 the computer. **(D)** Circuit diagram.

414

415 **Figure 2. Scanner performance.**

416 **(A-C)** A printout of 8.6° black and white bars (A) was scanned with a 1,000 point spiral
417 scanning path (B) to estimate the scanner's spatial resolution. In (C), the average brightness
418 (red) as indicated in (B) is plotted on top of the idealised brightness profile (black). **(D)** An
419 action camera picture of the blue door + red brick wall measured outdoors and an RGB
420 representation image of the scan when using opsin templates from human spectral sensitivity.
421 Blue and red dots in the RGB representation refer to the two points used to show examples of
422 individual spectra in **(E)**.

423

424 **Figure 3. An example data set of the forest scene with human spectral sensitivity.**

425 **(A)** A 180° photo of the forest scene with an approximate 60° scanner covered area (left). On
426 the right, monochromatic R-, G- and B-channels were constructed from the scanned data by
427 multiplying spectra from each pixel with the opsin templates (see Fig. 5B, 6C). The RGB image
428 shows the reconstruction built based on the opsin channels. The different colour appearance
429 of this RGB reconstruction compared to the photograph is due to differential colour-channel
430 equalisations in the two images. **(B)** Pixels from the R-, G- and B-channels aligned in the order
431 of the measurement with an arrow on the right indicating the direction of the principal
432 component analysis (PCA). **(C)** Achromatic and chromatic axes C_{1-2} aligned in the same order
433 as in the previous image, and then reconstructed back to images in **(D)** to add the spatial
434 information. The RGB image shows C_1 in red and C_2 in green (blue set to constant brightness).
435 **(E)** Loadings from achromatic and chromatic axes, bars illustrating the amount of input from
436 each opsin channel. **(F)** The cumulative variance explained (%) for each axis.

437

438 **Figure 4. The forest scene with zebra finch spectral sensitivity.**

439 **(A)** A still image of the forest scene with the approximated 60° scanner covered area,
440 monochromatic opsin channels (R, G, B, U) and an RGB reconstruction where R is shown as
441 red, G as green and B+U as blue. **(B-F)** As in Fig. 3, with an addition of the UV channel (U) in
442 all images. The RGB image in (D) displays C₁ in red, C₂ in green and C₃ in blue.

443

444 **Figure 5. PC reconstructions of the forest scene.**

445 **(A)** Achromatic and chromatic PCA reconstructions from the forest scene data for a mouse
446 (*Mus musculus*), a human (*Homo sapiens*), a bee (*Apis mellifera*), a butterfly (*Graphium*
447 *sarpedon*), a chicken (*Gallus gallus domesticus*) and a zebra finch (*Taeniopygia guttata*) and
448 PC RGB pictures. The number of chromatic axes equals to the number of cone types minus
449 1. Again, the PC RGB picture is constructed from chromatic axes C_{1-n}. In PC RGB, the C₁ is
450 shown as red, C₂ as green and C₃ as blue. **(B)** Opsin absorption curves showing the spectral
451 sensitivity of the cones for each animal. The pink, blue, green and red curves correspond to
452 UV, blue, green and red sensitive opsins, respectively.

453

454 **Figure 6. PC reconstructions of the flowering cactus.**

455 **(A)** A 120° photo of the scanned scene with a flowering cactus and the approximate 60°
456 window (black circle) the scanner can cover. **(B)** Reconstructions for the chromatic axes C_{1-n}
457 and PC RGB images and the absorption curves for each animal as in Fig. 5.

458

459 **Figure 7. An underwater scene from India with zebrafish spectral sensitivity.**

460 **(A)** A 180° photo of the scanned underwater river scene from West Bengal, India, and the
461 approximate 60° scanner covered window. **(B)** The zebrafish opsin complement. **(C)** The
462 monochromatic opsin channels (R, G, B, U) and the RGB reconstruction as in Fig. 4. **(D)** The
463 achromatic and chromatic axes reconstructed back to images to show where in the scene
464 information based on each axis can be found. **(E)** Loadings from each opsin channel as
465 explained in Fig. 3E. **(F)** The cumulative variance explained (%) for each axis.

466

467 **Table 1. The total variance explained by chromatic axes C1-n in the forest and cactus**
468 **scans.**

469

470 **Supplementary Figure 1. Four scanning paths created with the Fermat's spiral across**
471 **the 60° area.**

472 **(A)** 100 points square, **(B)** 300 points spiral **(C)** 600 points spiral **(D)** 1000 points spiral.

473

474 **Supplementary Figure 2. Light spectrum with and without the box.**

475 **(A)** Spectrometer readings of a clear daylight sky taken through the spectrometer's fibreoptic
476 (orange) or through the complete optical path of the scanner (black, i.e. 2 mirrors and a quartz
477 window, though lacking the fibreoptic). When purchased, the spectrometer is calibrated with
478 the fibreoptic attached. Accordingly, we computed the corresponding correction curve and
479 applied it to all scanner data presented throughout this work **(B)**.

480

481 **Supplementary Video 1.**

482 A video demonstrating the mirror movements and how light is guided to the spectrometer
483 through them.

484

485 **Supplementary Videos 2-4.**

486 Hyperspectral reconstructions of the three scanned scenes presented in this work, with each
487 frame corresponding to a 1 nm instance.

488

489 **Author contributions**

490 The scanner was conceived and implemented by NEN and TB. Data was analysed by NEN
491 using custom scripts written by TB and modified by NEN. The paper was written by NEN with
492 help from TB.

493

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500

501 Declaration of Interests

502 The authors declare no competing interests.

503

504

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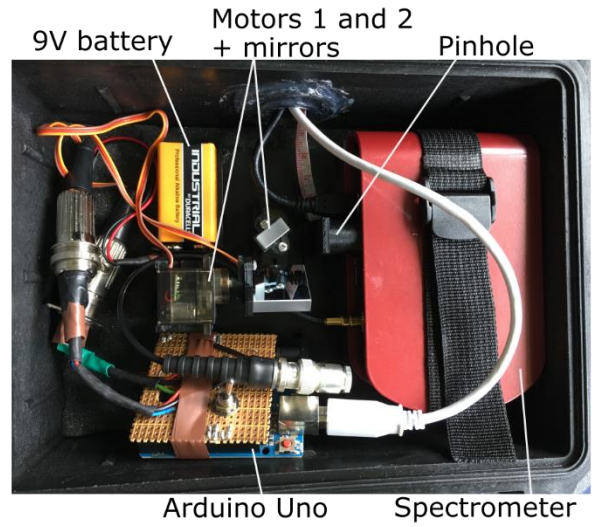
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Figure 1. A Hyperspectral scanner for low-cost natural imaging.

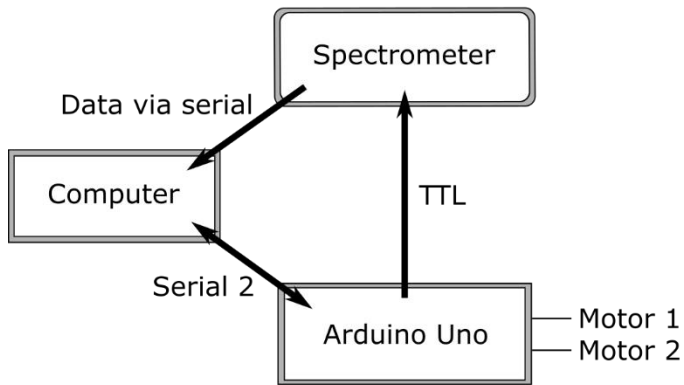
A



B



C



D

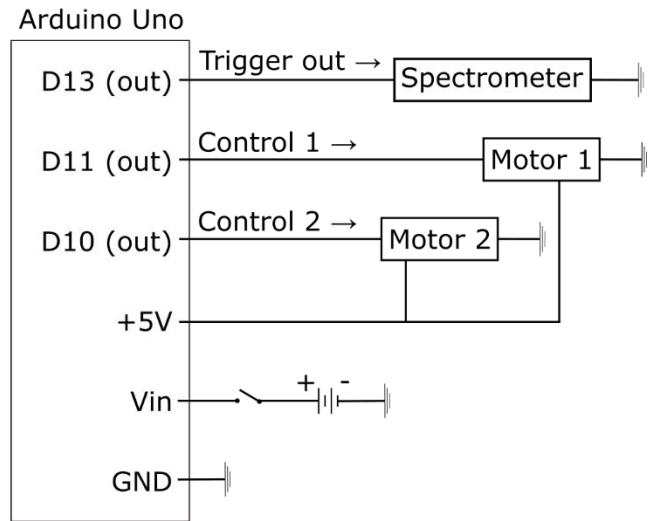


Figure 2. Scanner performance.

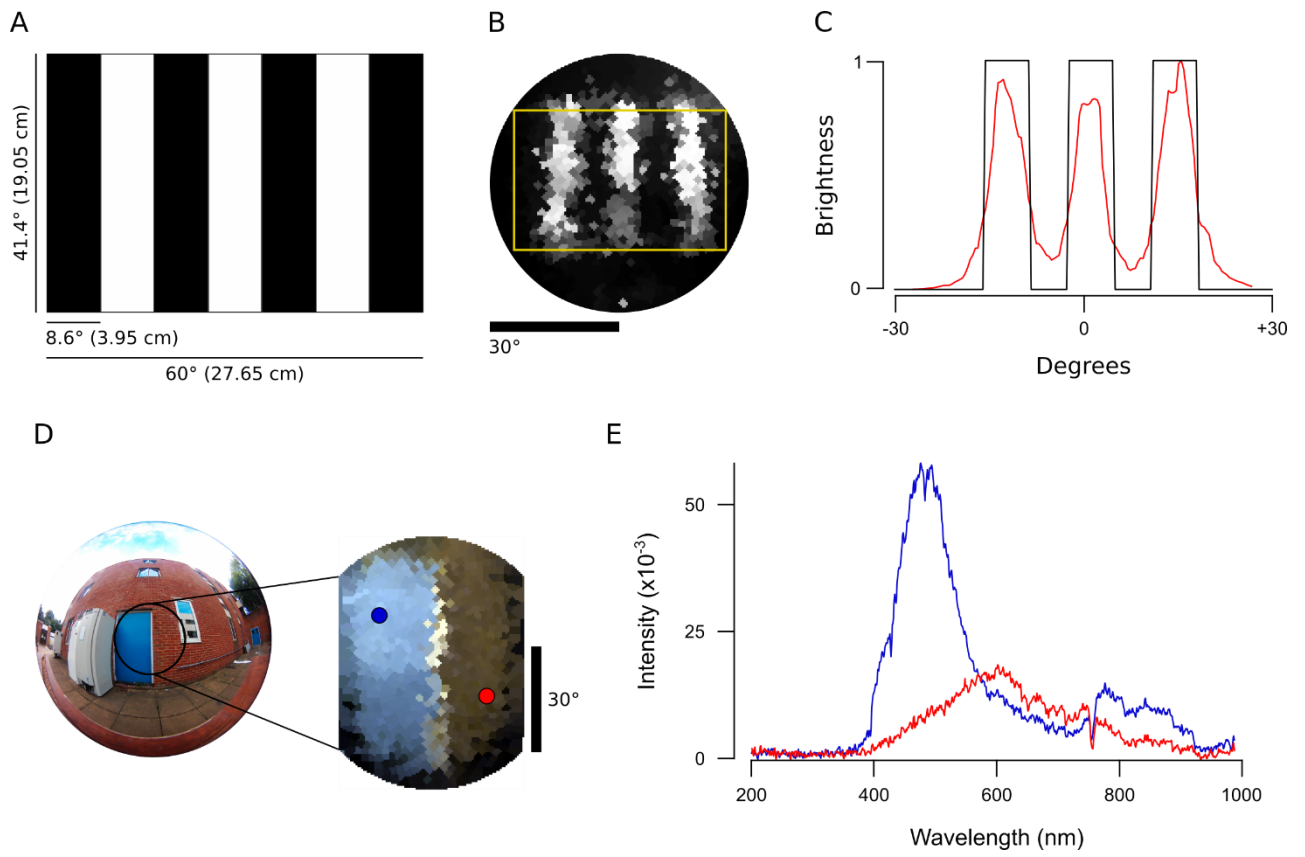


Figure 3. An example data set of the forest scene with human spectral sensitivity.

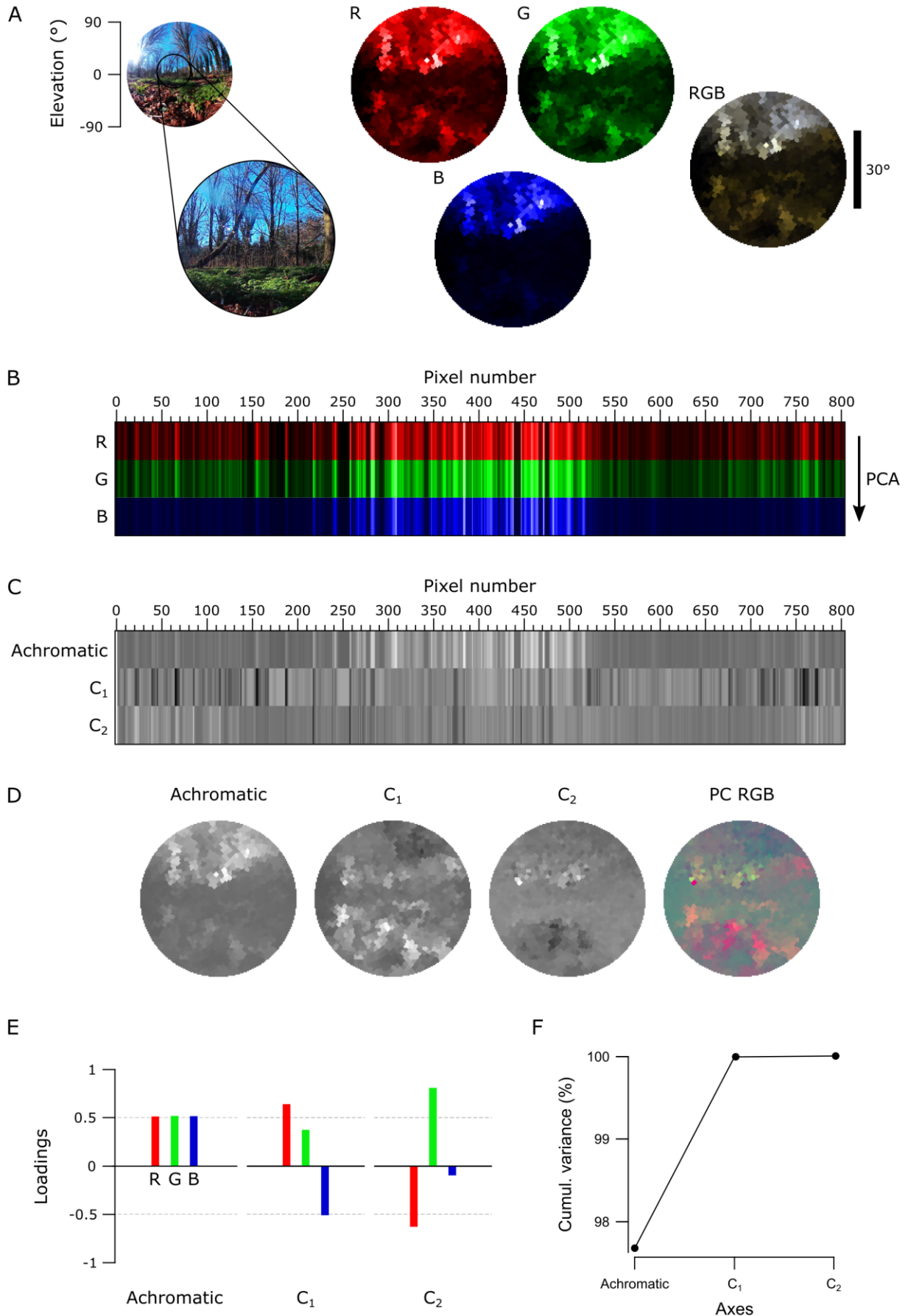


Figure 4. The forest scene with zebra finch spectral sensitivity.

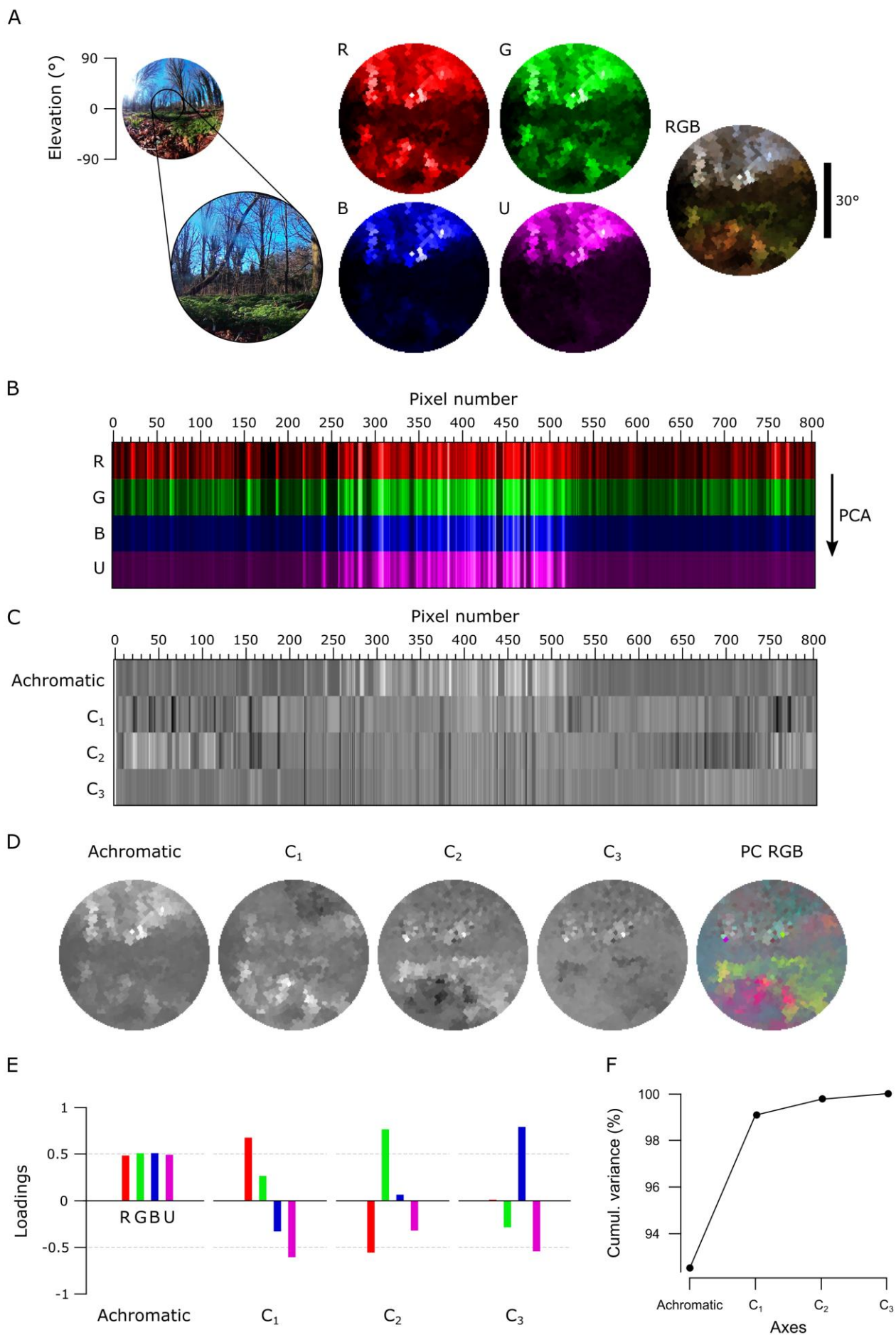


Figure 5. PC reconstructions of the forest scene.

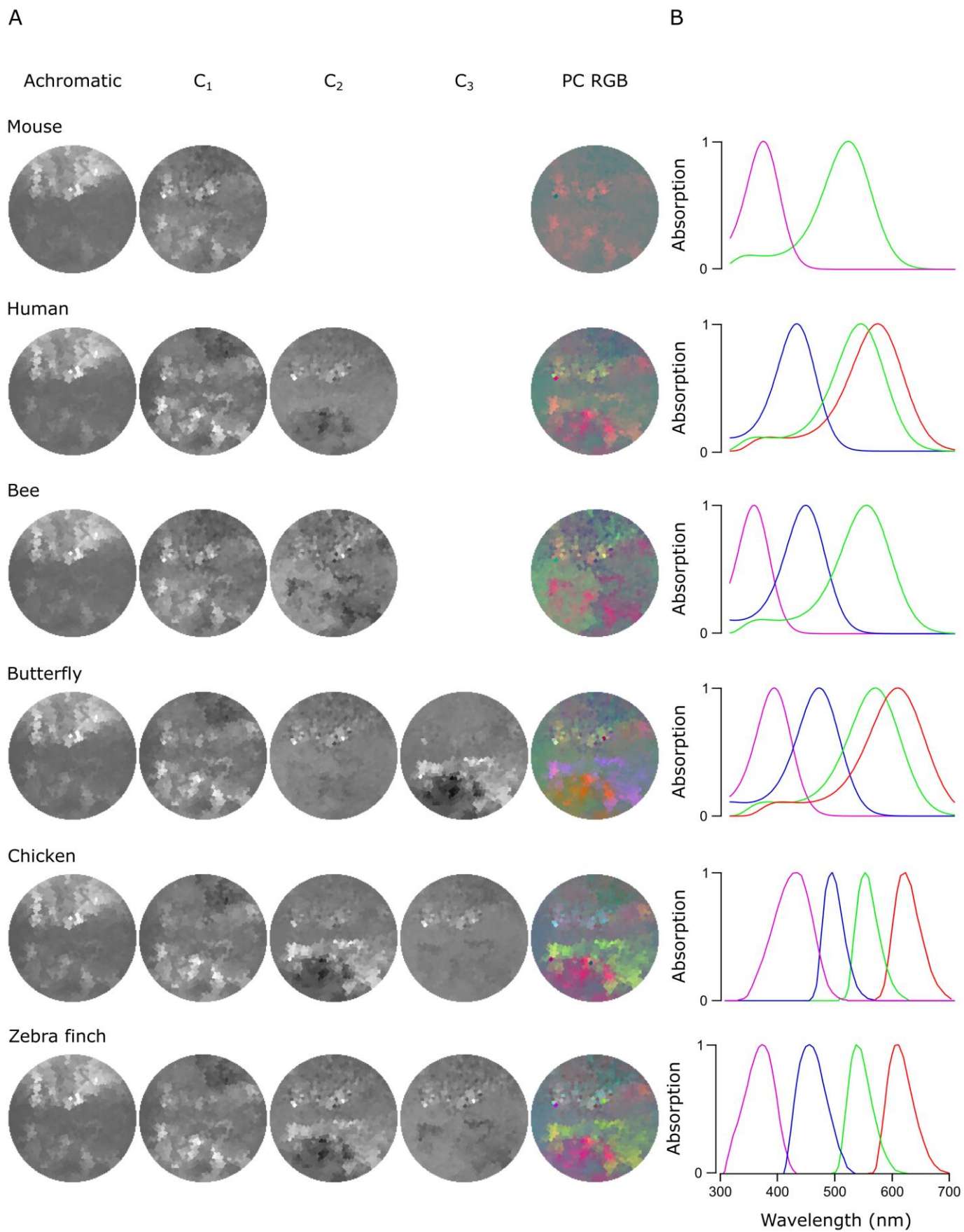
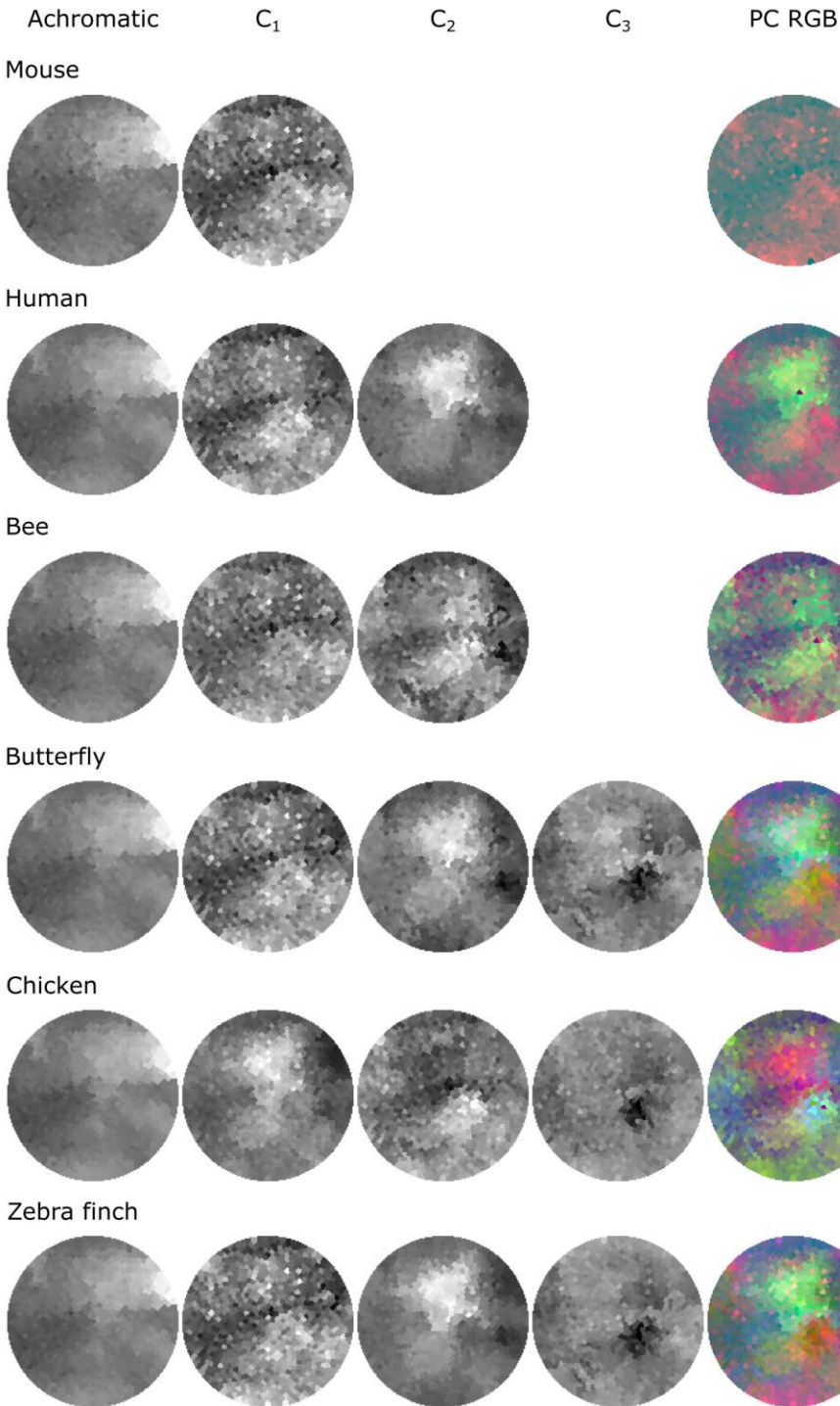


Figure 6. PC reconstructions of the flowering cactus.

A



B



C

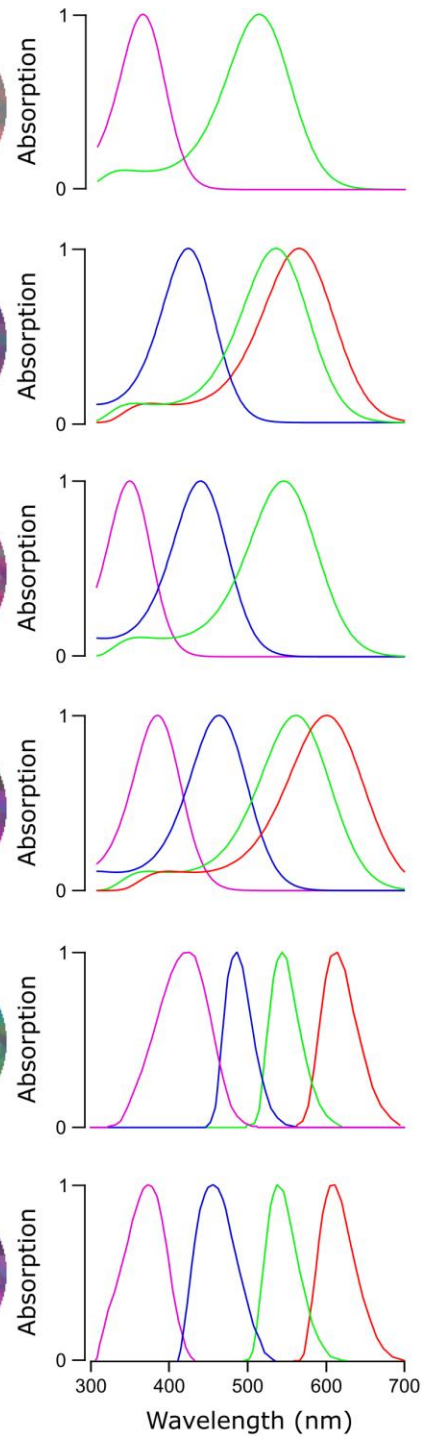
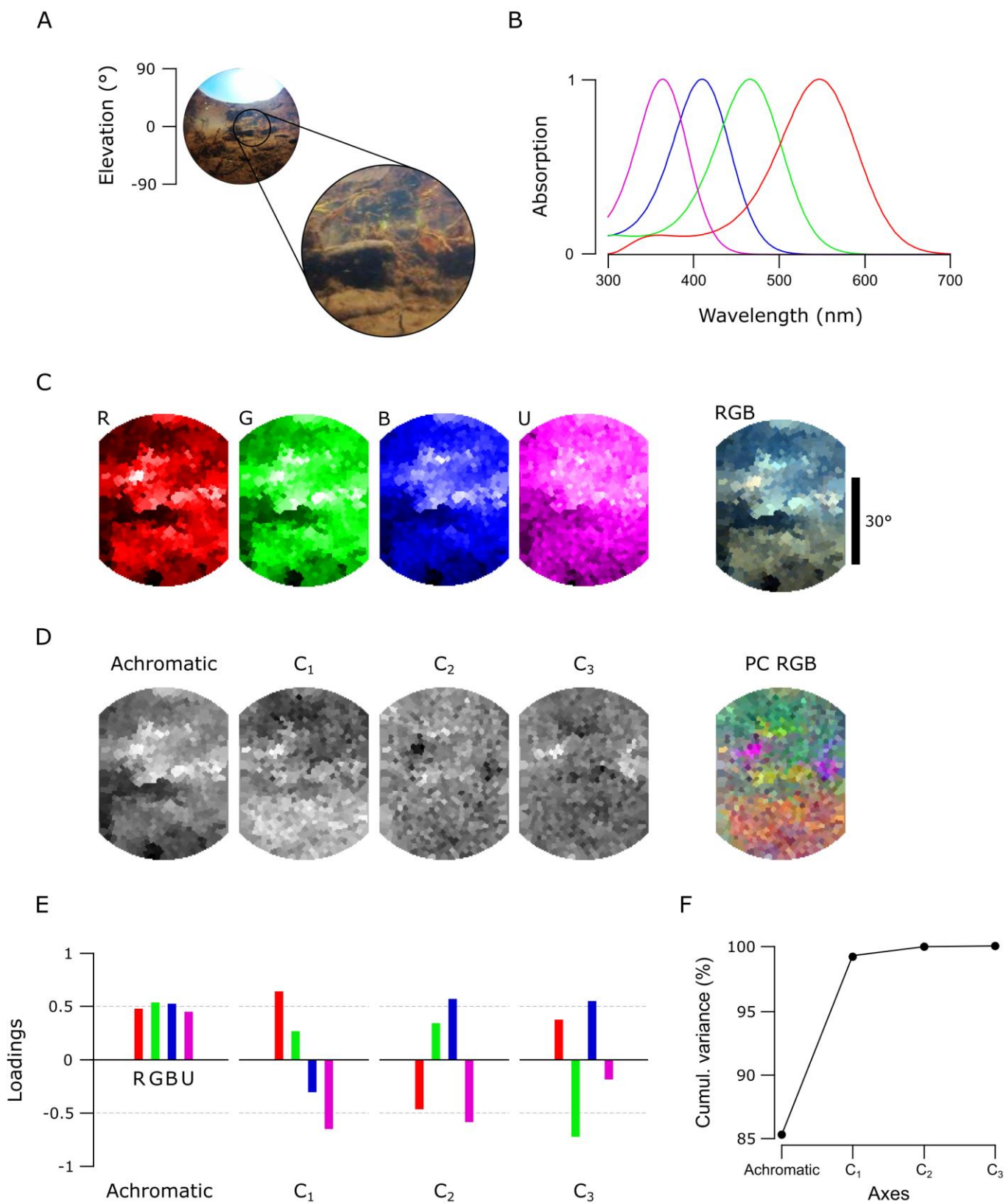


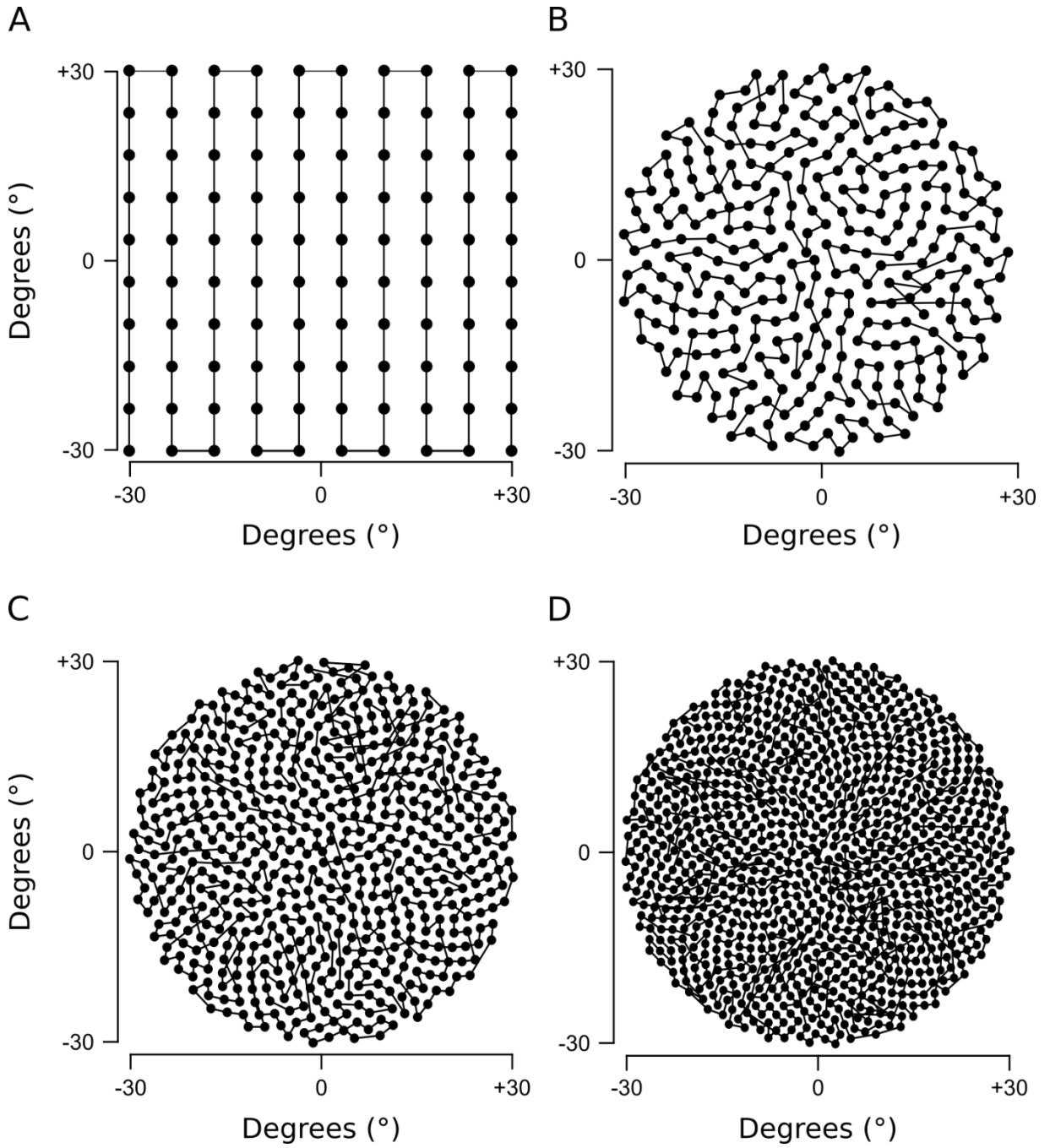
Figure 7. An underwater scene from India with zebrafish spectral sensitivity.



Tabel 1. The total variance explained by chromatic axes C1-n in the forest and cactus scans.

Variance explained by chromatic axes C _{1-n} (%)		
	Forest (Fig. 5)	Cactus (Fig. 6)
Mouse	2.6	8.0
Human	2.3	1.4
Bee	3.9	8.1
Butterfly	3.8	3.8
Chicken	6.7	2.9
Zebra finch	7.5	6.5

Supplementary Figure 1. Four scanning paths created with the Fermat's spiral across the 60° area.



Supplementary Figure 2: Light spectrum with and without the box

