Color measurements with the spectrometer

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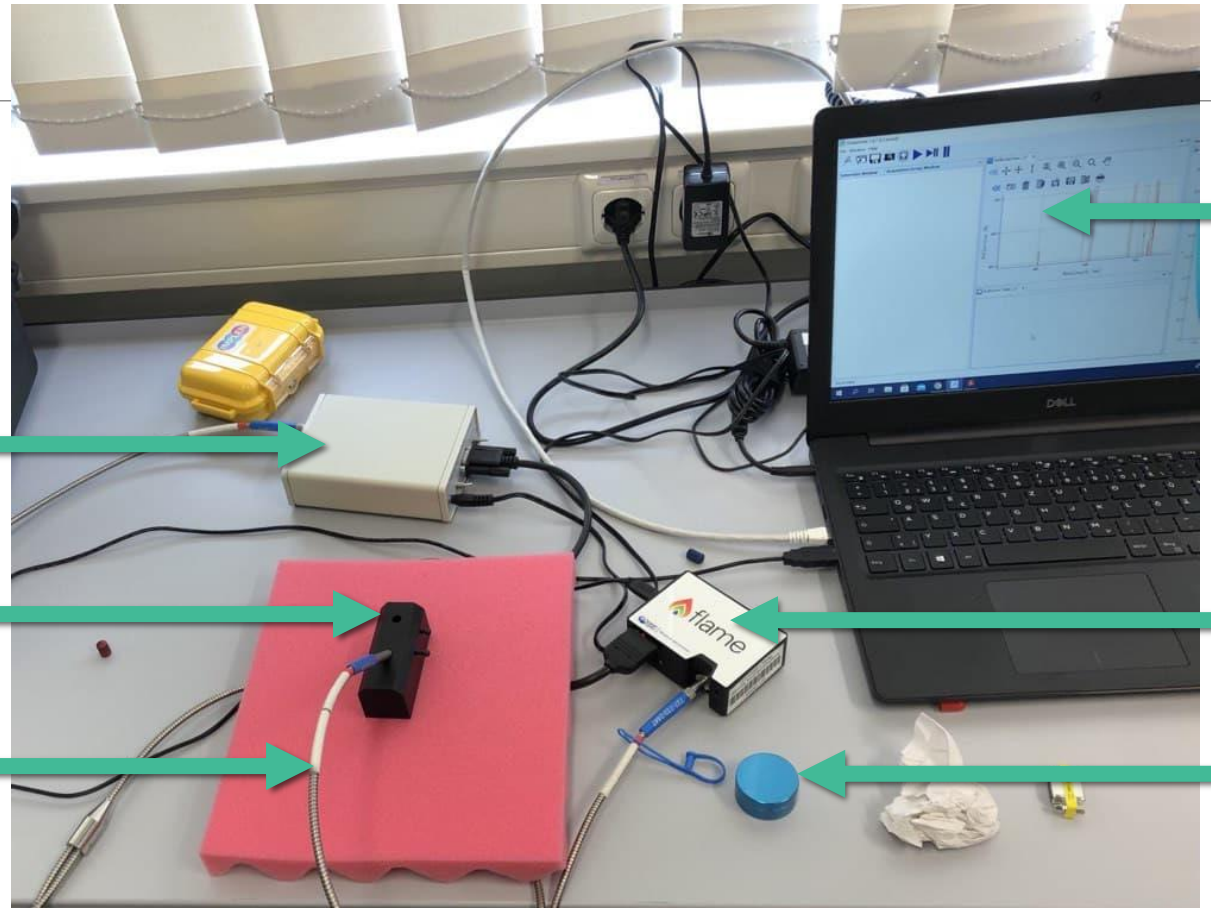
Setup



PX-2 Pulsed Xenon Lamp
(220-750nm)

Reflection Probe Holder
for 6.35mm probes

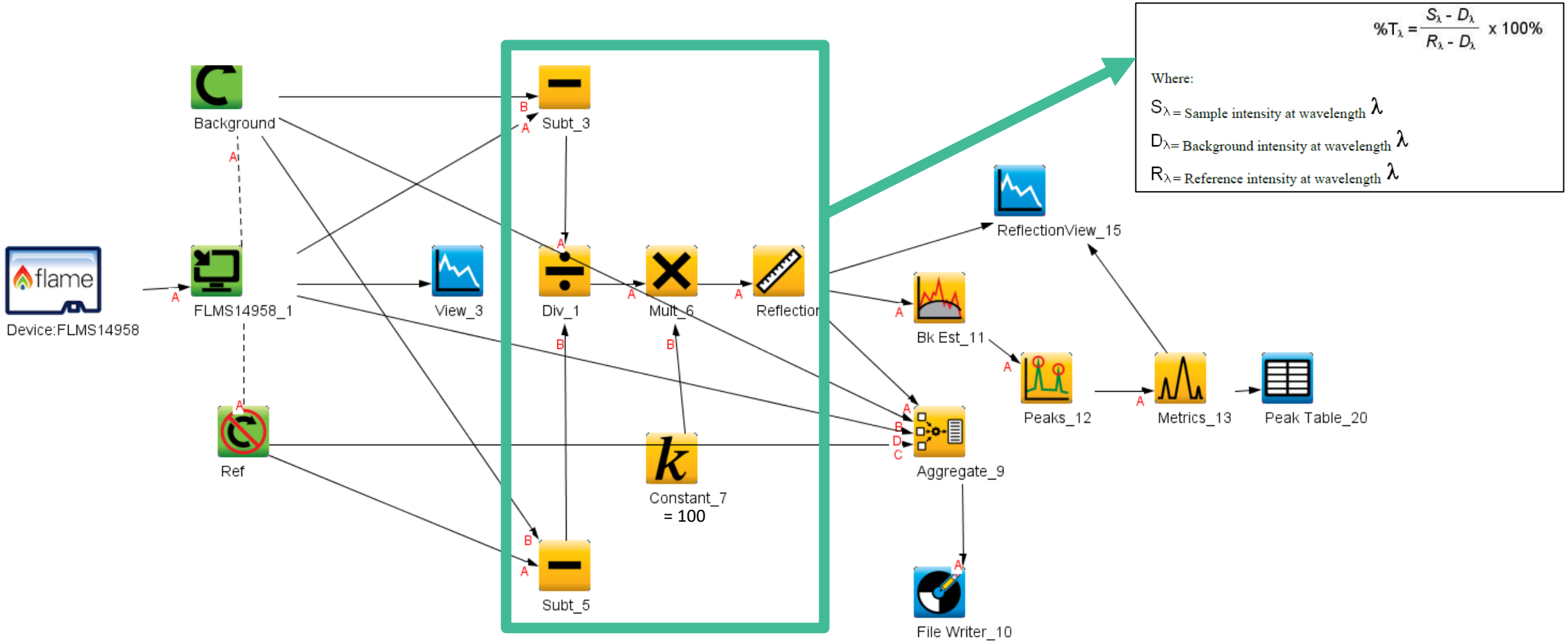
“QR400-7-UV-BX”
Reflection probe
(at 45° angle)

OceanView software

Spectrometer
“FLAME-S-UV-VIS”

Diffuse
Reflectance Standard

Workflow for measuring color (reflectance)



How to measure

1. Dark background reference

- Uncheck „Enable lightsource“ in the software.
- Close all opening in the probe holder
- click  to take the background reference

2. White reference

- enable light again
- place diffuse standard under probe
- click  to take the white reference

3. Reflectance measurement (color)

- place fruit under the probe holder
- press  to save reflectance measurement

 = File writer: click after calibration and before reflectance measurement (remember to change the file name before!!)

Some things to keep in mind

- Consistent labelling (e.g., sp1-i1-r1)
  - Edit file name with every new measurement
- Take new calibration after all replicates for an individual
- Clean reflection probe (detector) when needed
- Exclude external light from the measurement

Processing reflectance spectra

HOW TO CALCULATE HUE, CHROMA AND BRIGHTNESS FOR OBJECTS (COLOR)

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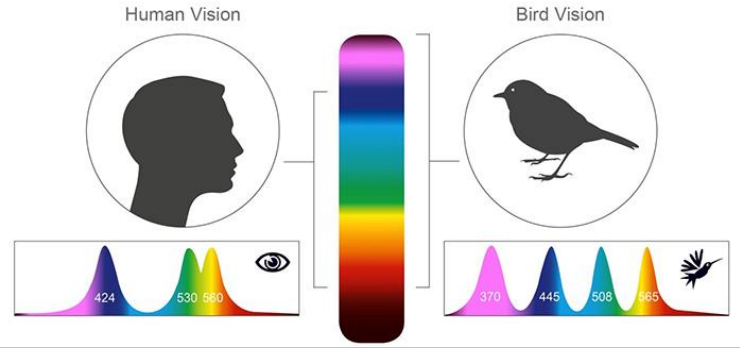
Color is…



- *„…the visual perceptual property deriving from the spectrum of light interacting with the photoreceptor cells of the eyes“ (wikipedia)*
- *… all frequencies of the visible light spectrum that are reflected back from the object into the observer’s eye*

- depends on the type and sensitivity of different types of cone cells in the retina
- **Human** visible spectrum 380 - 750 nm
  (3 types of cones: blue, green, red)
- **Bird** visible spectrum 300 – 750 nm
  (4 types of cones: UV, blue, green, red)

Reflectance

The reflectance spectrum shows the distribution of photons that can be potentially reflected towards the eye/detector at each wavelength

Reflectance (R [%]): relative measure, depends on the spectrum of the incident light

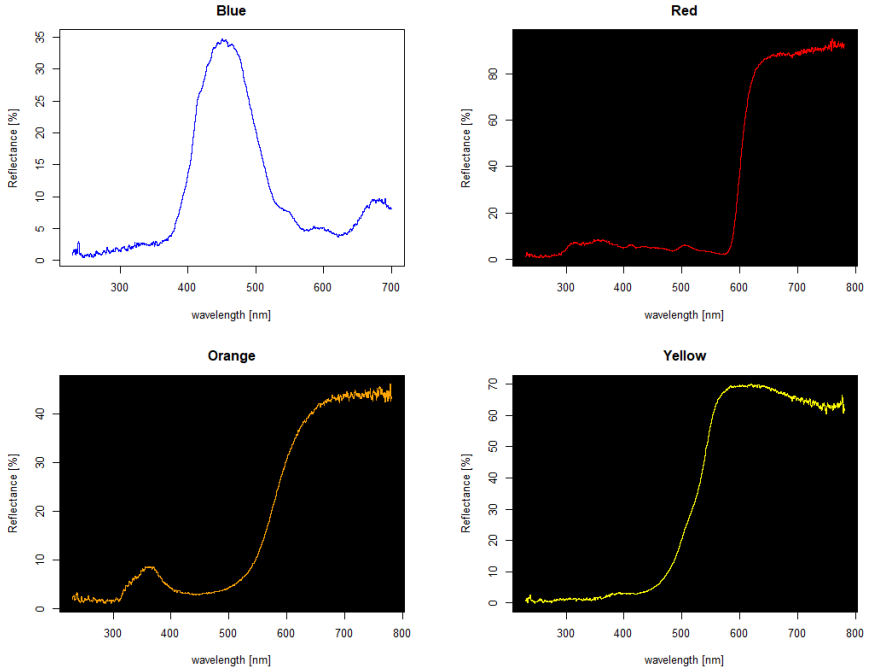
Transmission or Reflectance → $\%T_\lambda = \frac{S_\lambda - D_\lambda}{R_\lambda - D_\lambda} \times 100\%$

Where:

S_λ = Sample intensity at wavelength λ ← Measurement

D_λ = Background intensity at wavelength λ ← Dark calibration

R_λ = Reference intensity at wavelength λ ← Light calibration



Properties of color

Brightness

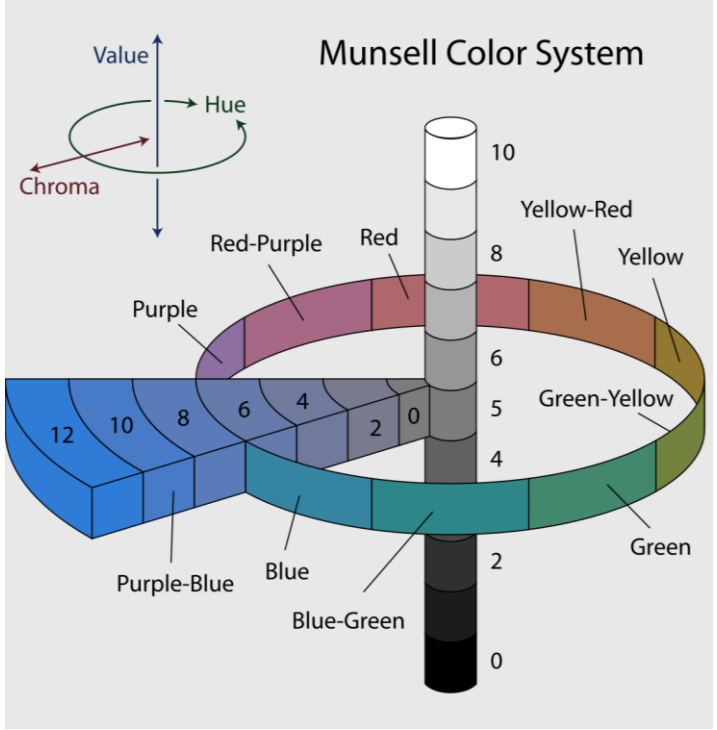
- = average or total intensity of reflected wavelengths
- the higher the reflectance amplitudes, the brighter it is perceived
- $R_{average}$

Hue

- = color that is predominantly perceived by the observer
- peak wavelength (λ at R_{max})

Chroma

- = saturation, function of how rapidly R% changes horizontally along wavelengths
- steeper slopes indicate higher saturation
- $\frac{R_{max} - R_{min}}{R_{average}}$



Properties of color in the reflectance spectrum

Brightness

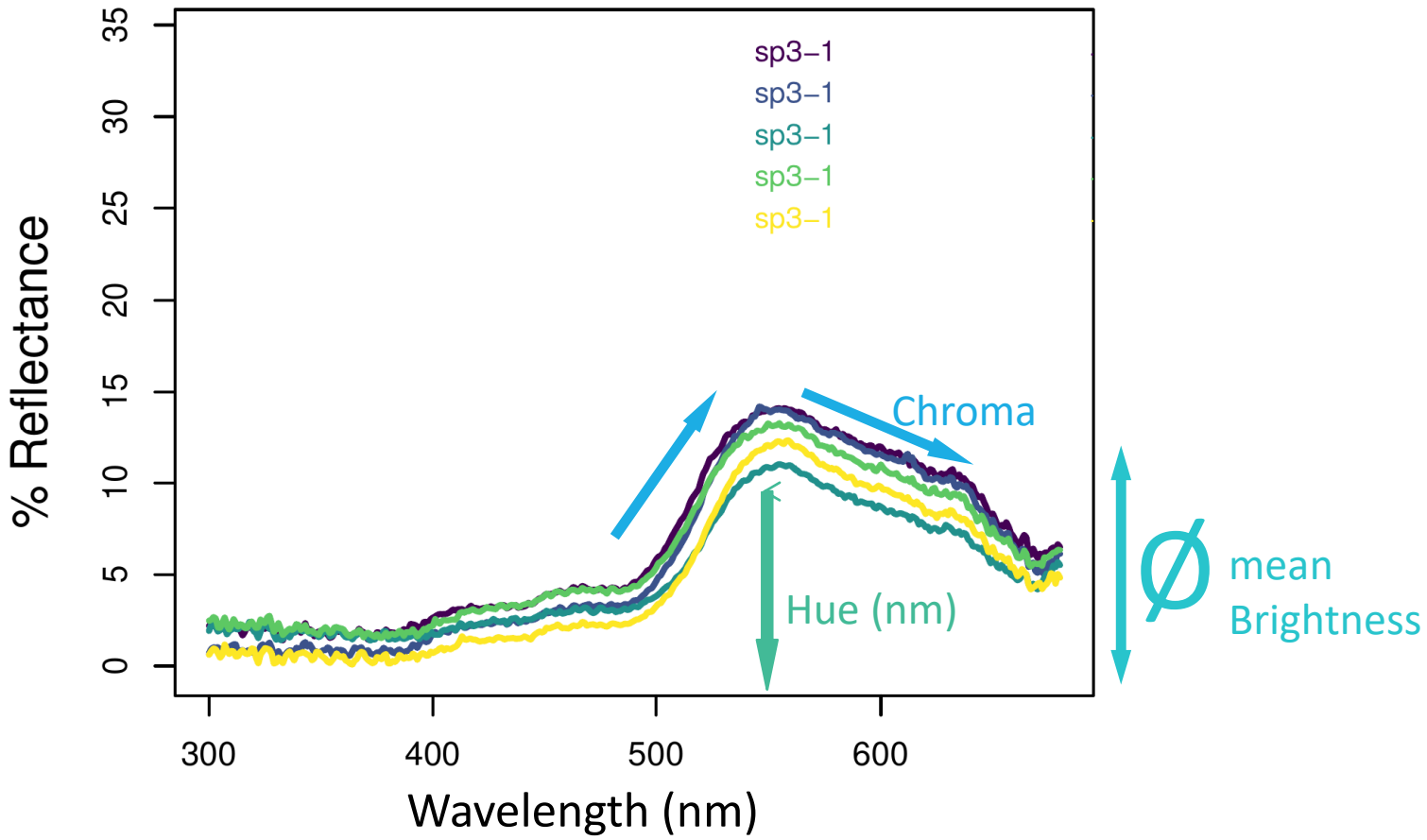
- $R_{average}$

Hue

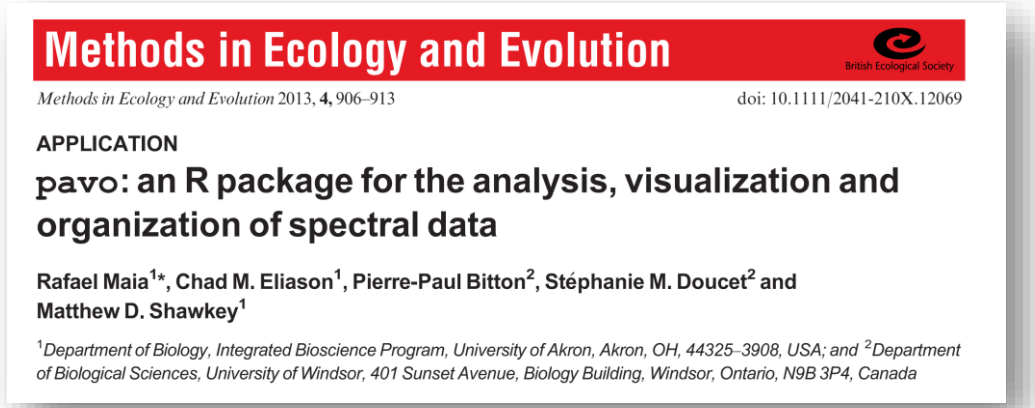
- peak wavelength (λ at R_{max})

Chroma

- $\frac{R_{max} - R_{min}}{R_{average}}$



Computing colorimetric variables in R



Methods in Ecology and Evolution

British Ecological Society

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APPLICATION

pavo: an R package for the analysis, visualization and organization of spectral data

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A) Calculating brightness, hue and chroma

1) For replicates individually

First, we have to subset the reflection data into species data frames for the separate calculation of the metrics. Pavo has a modified version of subset() which uses partial matching of strings. You can indicate any sequence of letter/signs/numbers that you would like to filter for in the column names of your reflection measurement.

Hide

```
refl_sp1 <- subset(specs, "sp1") # based on species id in names in reflectance df
refl_sp2 <- subset(specs, "sp2")
refl_sp3 <- subset(specs, "sp3")
```

Next we will calculate brightness, hue and chroma using the *summary()* function for each replicate (we can also do it for each species or each individual tree that was sampled, see below).
If we use the *subset = T* argument inside the function, it will automatically calculate the three desired variables. There is a diversity of other measures the function can calculate but those three are the most commonly used in comparative studies.

Hide

```
# again we cut out some more of the spectral noise (needed here. may not be needed for your data. Remove wlmin/wlmax argumen
ts if not needed)

col_sp1 <- summary(refl_sp1, subset =T, wlmin = 300, wlmax= 680)
```

Hide

```
col_sp2 <- summary(refl_sp2, subset =T, wlmin = 300, wlmax= 680)
```

Hide

```
col_sp3 <- summary(refl_sp3, subset =T, wlmin = 300, wlmax= 680)
```

* Change species/samples labels and folder structure for your own purposes from the provided R script
* script includes some techniques for visualization

Helpful information

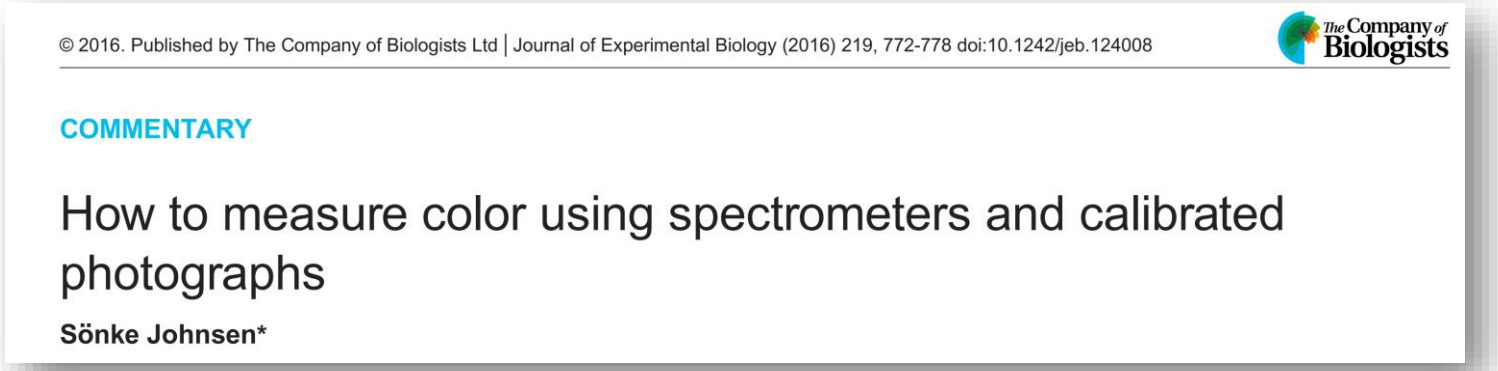
pavo package:

- https://rafaelmaia.net/pavo/articles/pavo.html (tutorial)
- https://rdrr.io/cran/pavo/ (description of functions)

Information about the formulas and arguments in R and *pavo* can be found on their help page:

- Type ?[your function]() into the R console, where [your function] has to be replaced with the function you want to read more about: e.g., ?summary.rspec()

Information about the difficulties of measuring color with a spectrometer: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COMMENTARY

How to measure color using spectrometers and calibrated photographs

Sönke Johnsen*