Bee's Eye View: Using Multispectral Photography to Simulate Bee's View of Flowers in Natural Settings

by
Svea Bruslind

A THESIS

submitted to

Oregon State University

Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Zoology
(Honors Scholar)

Presented August 28th, 2023
Commencement June 2024

AN ABSTRACT OF THE THESIS OF

Svea Bruslind for the degree of <u>Honors Baccalaureate of Science in Zoology</u> presented on August 28th, 2023. Title: <u>Bee's Eye View: Using Multispectral Photography to Simulate Bee's View of Flowers in Natural Settings</u>.

Abstract approved:_____

Gail Langellotto

Scientists have long recognized that bees and other insects respond to floral color, including ultraviolet (UV) floral patterns that are invisible to the human eye. While previous research has focused on isolating and capturing these UV markings in laboratory settings, methods that could be used in natural settings needed to be improved. Digital photography presents a viable means to simulate aspects of bee vision more fully in natural settings. This paper details the hardware and methods needed to simulate the full-color range of bee vision via capture of the light wavelengths reflected by flowers, as seen by bees. We used a full spectrum converted mirrorless camera, a macro lens, and five specialized filters to capture floral images that simulate how bees perceive color in the field. This approach increases opportunities to explore the bee-vision of a diverse array of flowers, including those shaped by natural versus artificial selection. Our methods also provide novel outreach and engagement opportunities, by making it possible for people to better understand how bees see the world.

Corresponding e-mail address: bruslindsvea@gmail.com

Bee's Eye View: Using Multispectral Photography to Simulate Bee's View of Flowers in Natural Settings

by
Svea Bruslind

A THESIS

submitted to

Oregon State University

Honors College

in partial fulfillment of
the requirements for the
degree of

Honors Baccalaureate of Science in Zoology
(Honors Scholar)

Presented August 28th, 2023
Commencement June 2024

<u>Honors Baccalaureate of Science in Zoology</u> project of Svea Bruslind presented on August 28th, 2023.

APPROVED:

_____

Gail Langellotto, Mentor, representing Horticulture

_____

Andony Melathopoulos, Committee Member, representing Horticulture

_____

Jen Hayes, Committee Member, representing Horticulture

_____

Toni Doolen, Dean, Oregon State University Honors College

I understand that my project will become part of the permanent collection of Oregon State University, Honors College. My signature below authorizes release of my project to any reader upon request.

_____

Svea Bruslind, Author

Graphical Abstract

A series of specialized light filters were used in conjunction with a converted full spectrum camera mirrorless camera and macro lens to photograph *Nemophila menziesii* flowers in visible light, ultraviolet light, and in simulated "bee-vision".

Specifications table

Hardware name	Multispectral Photography
Subject area	Environmental, planetary, and agricultural sciences
Hardware type	Imaging tools
Closest commercial analog	No commercial analog is available
Open-source license	Creative Commons Attribution-ShareAlike license
Cost of hardware	USD 1,500.00
Source file repository	N/A

1. Hardware in context

Bees have compound eyes, made of small units called ommatidium. Each ommatidium is comprised of a corneal lens, a crystalline cone, and photoreceptors [1]. An ommatidium is also surrounded by a screening pigment, which absorbs incoming off-axis light. The photoreceptors have a light-sensitive portion called the rhabdom, which only receives light that passes through the small hole of the corneal lens [1]. Bees have three photoreceptor types, one for UV, one for blue light, and one for green light, rendering them capable of registering wavelengths ranging from 300nm-650 nm, with optimal color receptors peaking at 330nm, 430 nm, and 550 nm [2,3]. The majority of these color-recepting cells also have secondary sensitives

where the other cells absorb maximally, capturing a range of wavelengths beyond the peaks [2].

We use a full-spectrum camera capable of capturing wavelengths from 300nm-1150 nm. The wavelengths captured by the visible light filter combination are between approximately 380 nm and 700 nm, with optimal color receptors peaking at 500 nm [4]. For the ultraviolet (UV)-only filter combination, the range is approximately 300nm and 400nm, with optimal color receptor peaking at 350 nm [5,6]. Our goal was to achieve a spectral range of approximately 300-660nm, with optimal color receptors peaking at 350 and 500 nm [5,7]. We refer to this range as "Bee Vision" as it simulates a bee's perception of colors.

The different filter combinations allow for comparison across different modes of vision. The visible light photos provide the human perspective of the flowers, allowing for comparison across the different modes of vision in reference to how humans see. The UV-only photos isolate the UV wavelengths, allowing for comparison across the "Bee Vision" photos for what is purely UV reflectance, and what is a combination between the UV-Blue-Green wavelengths seen by bees.

This hardware is a combination of previous multispectral photography techniques, adapted for use in the natural environment to simulate the color aspects of bee vision more accurately. Over the years, multispectral photography techniques have been tested and refined; many different types of cameras have been used, including a color television camera with adapted lenses, spectrophotometers, and, more recently, digital single-lens reflex cameras, or DSLRs, and mirrorless cameras [8–10]. Recent research utilizes DSLR and mirrorless cameras modified with the removal of their infrared (IR) blocking filters, coupled with different types of UV-sensitive filters and lenses to capture the UV wavelengths visible to bees and explore the UV reflectance used by some flowers [9,11]. These previous techniques were all focused on capturing only UV reflectance, however, as they used full-spectrum cameras and UV-sensitive filters, combined with applied UV light. This isolates the UV reflectance present on the flowers but does not include other wavelengths, which is not an accurate simulation of how bees see color. These methods are not applicable in the field, as the full-spectrum camera is not capable of filtering out specific wavelengths by itself.

Our design combines a full-spectrum camera with specialized filters, to allow for the filtering out of specific wavelengths, and to narrow the spectrum being received by the camera. It can be used with natural light, which encompasses all the wavelengths visible by bees. This makes it closer to what colors bees are seeing, by including the blue-green portion of their spectrum, along with the UV.

2. Hardware description

We purchased a Sony™ α6000 mirrorless camera body that had already been modified for full spectrum photography by removing its internal hot mirror filter. This rendered it capable of capturing wavelengths ranging from 300 nm-1150nm (Fig. 1). This camera body was used with a Canon™ 50mm 1:35 FD macro lens and a Sony-to-Canon macro lens adapter (Fig. 2). Five 52mm x 2mm specialized filters sourced from UVIR Optics were used to control the wavelengths which reached the lens, and the specific combination of filters determined mode of vision was being replicated (e.g. Visible light, UV light, and "Bee Vision"). The five filters were: a Schott BG40 UV+Visual bandpass/IR Suppression filter (Fig. 3), a Hoya U-360 UV Bandpass/Dual Band IR filter (Fig. 4), a Schott UG5 UV + Blue/Green/IR Bandpass filter (Fig. 5), and two Schott S8612 AR Coated UV+Visual/IR Suppression filters (Fig. 6).

Together, the camera, lens, and filters:

- Allow the simulation of the full range of colors perceived by bees in the natural environment;
- Improves understanding of plant-pollinator interactions by providing a tool to compare bee visitation with a single suite of floral traits that may be apparent to bees, but unapparent to humans;
- Visualizes UV present on plants to better understand their potential function as signals to bees and other pollinators.

Fig. 1 Sony™ α6000 mirrorless camera body

Fig. 2 Canon™ 50mm 1:35 FD macro lens and a Sony-to-Canon macro lens adapter

Fig. 3 Schott BG40 UV+visual bandpass/IR suppression filter

Fig. 4 Hoya U-360 UV bandpass/dual band IR filter

Fig. 5 Schott UG5 UV+blue/green/IR bandpass filter

Fig. 6 Schott S8612 AR coated UV+visual/IR suppression filter

3. Design files summary

Design file name	File type	Open-source license	Location of the file
Design File 1	Figure	*Creative Commons Attribution-ShareAlike license*	Available with the article
Design File 2	Figure	*Creative Commons Attribution-ShareAlike license*	Available with the Article
Design File 3	Figure	*Creative Commons Attribution-ShareAlike license*	Available with the Article
Design File 4	Figure	*Creative Commons Attribution-ShareAlike license*	Available with the Article

DESIGN FILE 1: MAIN CAMERA BODY

The components of the main camera body are shown, with each component labeled, showing how they connect and where.

DESIGN FILE 2: MODE OF VISON— VISIBLE LIGHT
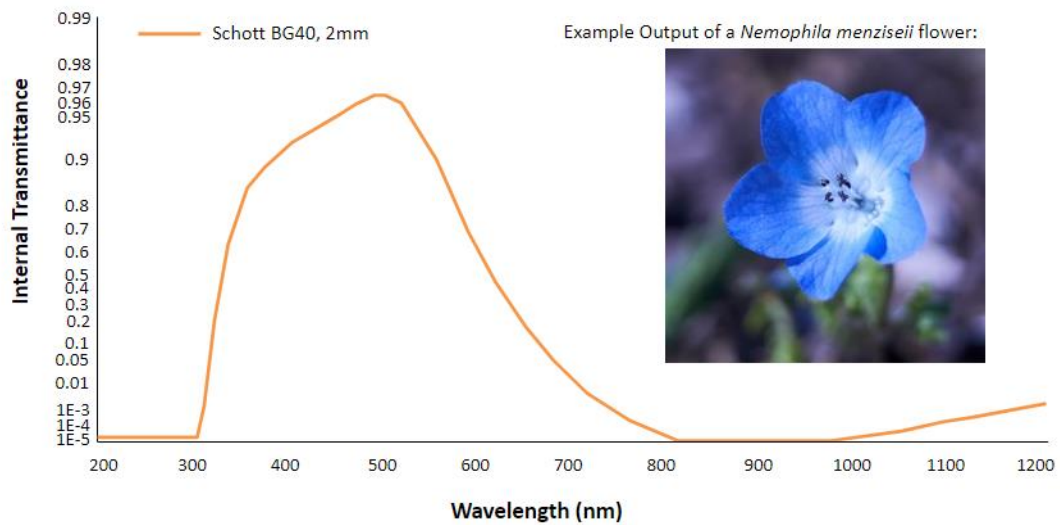
Components:

Camera, Adapter, & Lens   Schott BG40 Filter (2mm glass)

Internal Transmittance Diagram:

Graph showing the range of wavelengths transmitted through the Schott BG40 filter
into the camera sensor, adapted from Schott [4]. The photo provided shows the
resulting photograph from the filter and camera body combination.

DESIGN FILE 3: MODE OF VISON— ULTRAVIOLET LIGHT

Components:

Camera, Adapter, & Lenses
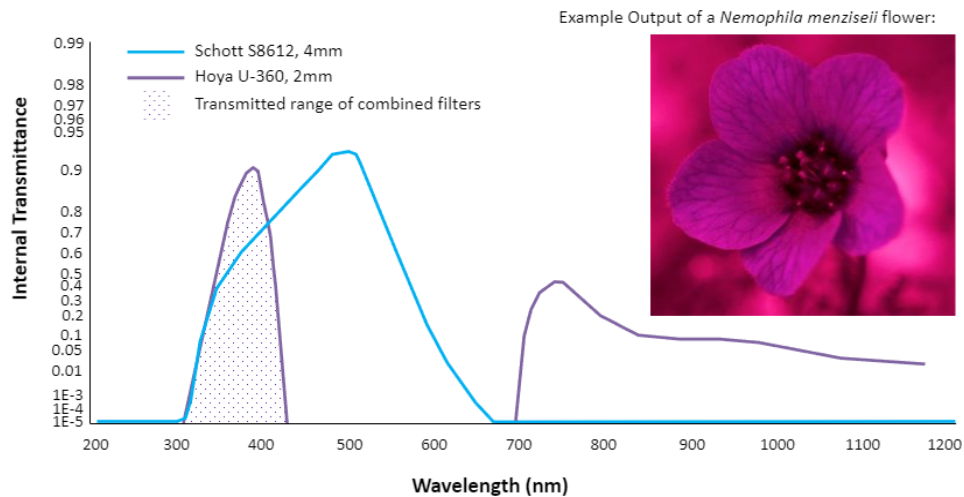

Two Schott S8612 Filters (4mm of glass total)

Hoya U-360 Filter (2mm glass)

Internal Transmittance Diagram:

Graph showing the wavelength range transmitted through the individual Hoya U-360 filter and the S8612 filters, as well as the transmitted range once the filters are combined, adapted from Hoya and Schott [5,6]. The photo provided shows the resulting photograph from the filter and camera body combination.

DESIGN FILE 4: MODE OF VISON— "BEE VISION"

Components:

Camera, Adapter, & Lenses
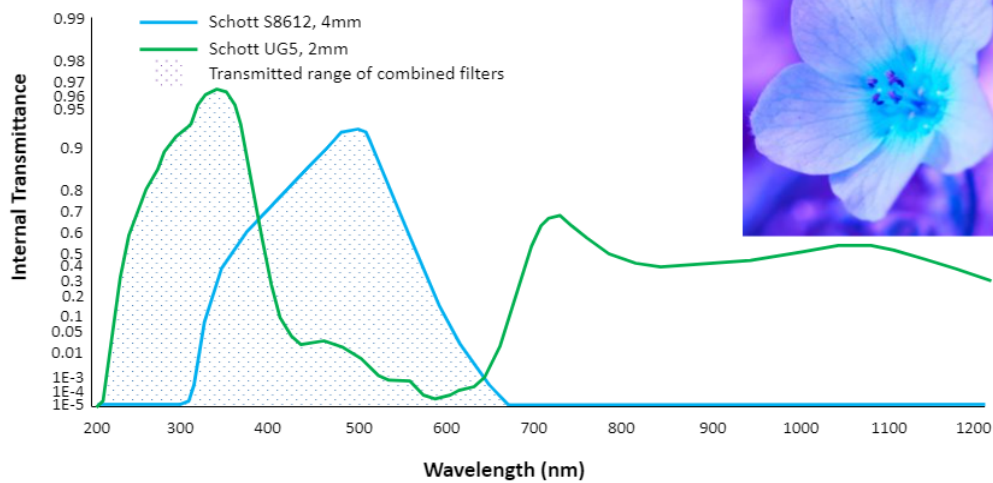

Two Schott S8612 Filters (4mm of glass total)

Schott UG5 Filter (2mm glass)

Internal Transmittance Diagram:

Graph showing the wavelength range transmitted through the individual Schott UG5 filter and the S8612 filters, as well as the transmitted range once the filters are combined, adapted from Schott [5,7]. The photo provided shows the resulting photograph from the filter and camera body combination.

4. Bill of materials summary

Designator	Component	Number	Cost per unit-currency	Total cost-currency	Source of materials	Material type
Design File 1	SonyTM α6000 mirrorless camera body	1	USD 667.00	$667.00	Link	Composite
Design File 1	Canon^{TM} 50mm 1:35 FD macro lens	1	USD 103.50	$103.50	Link	Composite
Design File 1	Fotasy FD-FE Sony to Canon Macro Lens Adapter	1	USD 14.69	$14.69	Link	Metal
Design File 2	Schott BG40 UV+Visual bandpass/IR Suppression filter	1	USD 69.95	$69.95	Link	Composite
Design File 3	Hoya U-360 UV Bandpass/Dual Band IR filter	1	USD 73.45	$73.45	Link	Composite
Design File 4	Schott UG5 UV + Blue/Green/IR Bandpass filter	1	USD 89.45	$89.45	Link	Composite
Design Files 3 and 4	Schott S8612 AR Coated UV+Visual/IR Suppression filter	2	USD 79.95	$159.90	Link	Composite
Inserted into camera body	Sandisk 64GB memory card	1	USD 16.90	$16.90	Link	Composite
Inserted into camera	RAVPower SONY NP-FW50 battery pack	1	USD 33.98	$33.98	Link	Composite
Optional attachment to the camera	Camopro 70-inch tripod	1	USD 32.99	$32.99	Link	Composite
Storage for camera	Caden Camera backpack	1	USD 29.99	$29.99	Link	Composite
SD card reader/writer	Staples 17-in-1 card reader/writer	1	USD 14.95	$14.95	Link	Composite
Storage for files	LaCie Rugged Thunderbolt 1 TB external hard drive	1	USD 136.54	$136.54	Link	Composite

5. Build instructions

To remove the hot mirror filter from the camera body, two possible options are to buy a new camera body and have it sent to a specialist for removal of the filter or buy a refurbished camera body where its hot mirror filter has already been removed. Once the camera is in hand, insert a memory card into the camera body's card slot, and one charged battery into the battery slot in the camera. We used a 64GB Sandisk memory card. Attach the Canon^{TM} 50mm 1:35 FD macro lens to the Fotasy FD-FE Sony to Canon Macro Lens Adapter as shown in Design File 1. Attach the Fotasy FD-FE

Sony to Canon Macro Lens Adapter to the SonyTM α6000 mirrorless camera body, as shown in Design File 1. Attach the filter(s) to the front of the Canon^{TM} 50mm 1:35 FD macro lens, according to the mode of vision being photographed as shown in Design Files 2-4.

6. Operation instructions

Photograph on sunny days, without cloud cover over the floral area. Photograph between the hours of 0900 H and 1600 H (PST), with prime photography hours between 1100 H and 1400 H, when the sun is directly or nearly overhead. Prioritize times when flowers are open and completely exposed to the sun. Days with little to no wind are preferred, as it makes it easier to capture flowers in the same or similar positions across the different filter combinations, to allow for comparison between modes of vision. Temperature is not a concern, except in terms of human health and safety.

Next, establish the camera position. Orient the camera as close as possible to the flower, to capture the details and limit potential background interferences. Attach the tripod if necessary. Adjust the filter combinations according to the mode of vision being photographed, as shown in Design Files 2-4. The order of how the filters are attached does not affect the output image.

When photographing in visible light attach the filter shown in Design File 2 and set the aperture to an F stop of 3.5, shown in Fig. 7, (the lowest setting for the camera being used) to allow as much light as possible into the lens and create a lower depth of field. Set the ISO to 100 (the lowest ISO for the camera being used), shown in Fig. 8, to keep the bright sun from over-exposing the photo, and to simulate visible light. Set the shutter speed to between 1/1000-1/1500s, shown in Fig. 8, depending on the sun's intensity with a higher speed for a brighter day, again to simulate visible light and keep the photo from over-exposing (Fig.9). Take several test photos and adjust settings as needed to obtain photos that are as close to visible light as possible. Once the settings are adjusted, take several photos. To better allow for cross-comparisons between photographic modes, maintain the camera position and switch the filters to the next mode of vision being photographed.

When photographing in Ultraviolet (UV) light, attach the filters shown in Design File 3, set the aperture at an F stop of 3.5, shown in Fig. 7, and set the ISO to the highest setting of 25600, shown in Fig. 8. A higher ISO is necessary as the lack of other wavelengths reduces the available light and makes a higher sensitivity necessary to achieve a usable image. Drop the shutter speed to 1/150-1/200s, shown in Fig. 8, to accommodate the lower light conditions. Once the flower and its details are visible and not underexposed, as shown in Fig. 10, take the photos. Maintain the camera position and switch the filters to the next mode of vision being photographed.

When photographing in "Bee Vision" attach the filters shown in Design File 4 and set the aperture to an F stop of 3.5 (Fig. 7). Drop the ISO to a setting between 10000 and 25600, shown in Fig. 8, depending on the strength of the light for the day. For lower light days, set to a higher ISO, for a higher sensitivity. For brighter days, set to a lower ISO, for a lower sensitivity. Increase the shutter speed to 1/300-1/400s, shown in Fig. 8, to simulate the speed at which a bee would see and approach a flower. Once the settings are adjusted so that no areas are overexposed, as shown in Fig. 11, take the photos.

Review the photos on the camera, and if satisfactory, collapse the equipment and stow it away in appropriate storage containers. Transfer the photos from the memory disk for long-term storage in their RAW format onto a hard drive. We used a LaCie Rugged Thunderbolt 1 TB external hard drive. Sort images and store as appropriate, with labeling for all files.

Fig. 7 Moving the wheel control indicated above controls the F Stop setting.

Fig. 8 Scrolling the wheel control changes the shutter speed. Pressing down on the wheel once on the side that says ISO and scrolling the wheel will change the ISO settings.
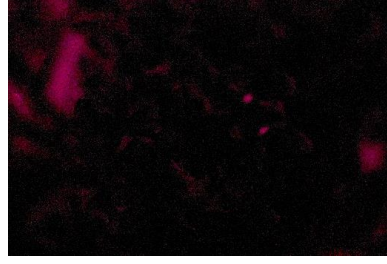
Fig. 9 Overexposed flower in Visible Light

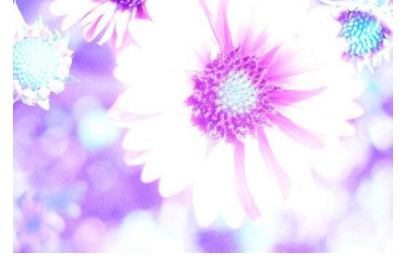Fig. 10 Underexposed Flower in UV light

Fig. 11 Overexposed flower in "Bee Vision"

7. Validation and characterization

This hardware is usable for exploring bees' color perception of the world, as well as the UV reflectance displayed by some plants. This hardware would make it possible to visualize the UV reflectance, as well as track them over time, allowing more insight into the uses of UV reflectance by plants, and possible impacts and effects. The glass used in the filters had already been validated by the companies, Hoya and Schott, as shown by the graphs in Design file 2-4, which were adapted from information pulled from the Hoya and Schott websites [4–7]. The filters were also validated by the filter manufacturer, UVIR Optics, who tested the filters before selling them.

Validation for this technique is lacking, however previous literature suggests a possible method that could be used [9]. The flower's UV reflectance could be calculated according to pixel color values. Each photo could be analyzed by an image processing software, such as ImageJ, that registers each pixel's color values, and reports upon the pixels that register in color values corresponding to UV. This would allow for validation of the technique, by showing whether it is truly capturing UV wavelengths, and thus, UV signaling.

It should be noted, however, that this technique is useable for simulating bees' perception of colors, but it is not useable to simulate bees' perception of shapes and contrast, as well as the overall resolution of bee vision. Therefore, this technique cannot be used to simulate all aspects of bees' vision and should only be used to explore bees' color perception of the world and to explore UV signals in the natural world.

Capabilities
- Resolution 6000 x 4000
- Max shutter speed 1/4000 sec
- ISO range 100-25600
- Minimum aperture 32
- Maximum aperture 3.5
- Focal length 50 mm
- Wavelength range 300-1150

Ethics statements

The authors report no ethical concerns.

CRediT author statement

Svea Bruslind: Methodology, Investigation, Writing- Original Draft, Writing-Review and Editing *Jen Hayes*: Conceptualization, Methodology, Visualization, Funding Acquisition, Writing- Original draft, Writing- Review, and Editing *Gail Langellotto*: Supervision, Project Administration, Resources, Funding Acquisition, Writing- Review and Editing.

References:

[1] H. Somanathan, A. Kelber, R.M. Borges, R. Wallén, E.J. Warrant, Visual ecology of Indian carpenter bees II: adaptations of eyes and ocelli to nocturnal and diurnal lifestyles, J. Comp. Physiol. Neuroethol. Sens. Neural Behav. Physiol. A. 195 (2009) 571–83. https://doi.org/10.1007/s00359-009-0432-9.

[2] R. Menzel, M. Blakers, Colour receptors in the bee eye ? Morphology and spectral sensitivity, J. Comp. Physiol. 108 (1976) 11–13. https://doi.org/10.1007/BF00625437.

[3] F.R. Prete, Complex Worlds from Simpler Nervous Systems, MIT Press, 2004.

[4] BG40 | SCHOTT Advanced Optics US, (n.d.). https://www.us.schott.com/shop/advanced-optics/en/Matt-Filter-Plates/BG40/c/glass-BG40 (accessed August 21, 2023).

[5] S8612 | SCHOTT Advanced Optics US, (n.d.). https://www.us.schott.com/shop/advanced-optics/en/Matt-Filter-Plates/S8612/c/glass-S8612 (accessed August 21, 2023).

[6] Ultraviolet Transmitting, Visible Absorbing Filters (U) – Hoya Optics, (n.d.). https://hoyaoptics.com/colored-glass-filters/ultraviolet-transmitting-visible-absorbing-filters-u/ (accessed August 21, 2023).

[7] UG5 | SCHOTT Advanced Optics, (n.d.). https://www.schott.com/shop/advanced-optics/en/Matt-Filter-Plates/UG5/c/glass-UG5 (accessed August 21, 2023).

[8] T. Eisner, D.J. Aneshansley, M. Eisner, Ultraviolet Viewing with a Color Television Camera, BioScience. 38 (1988) 496–498.

[9] F. Napoleone, D. Manzini, S. Burrascano, How to measure flower ultraviolet

reflectance using digital photography, Appl. Veg. Sci. 25 (2022) e12648. https://doi.org/10.1111/avsc.12648.

[10] J.E. Garcia, A.D. Greentree, M. Shrestha, A. Dorin, A.G. Dyer, Flower Colours through the Lens: Quantitative Measurement with Visible and Ultraviolet Digital Photography, PLOS ONE. 9 (2014) e96646. https://doi.org/10.1371/journal.pone.0096646.

[11] C. Verhoeven, Z.-X. Ren, K. Lunau, False-colour photography: a novel digital approach to visualize the bee view of flowers, J. Pollinat. Ecol. 23 (2018) 102–118. https://doi.org/10.26786/1920-7603(2018)11.