### Kubelka-Munk theory applied to Flower Coloration Interpretation



Researchers from the University of Groningen, Netherlands are uniting the disciplines of biology and physics in the quantitative analysis of the reflectance spectra of flowers. The recent work on the Coloration of the Chilean Bellflower has important implications in the study of evolutionary biology and the relationship between plants and pollinators. Furthermore, this work contributes to our understanding of pigmentation and light scattering, as well as adding valuable reflectance spectra data for the *Nolana paradoxa*to the world's catalog of reference spectra data.

### **Applying Biology and Physics**

The application of the Kubelka-Munk layer stack theory to plant colorization analysis also has commercial and industrial implications related to materials, coatings, dyes, and paints; especially in the world of semiconductor fabrication and automobile and auto parts manufacturing.

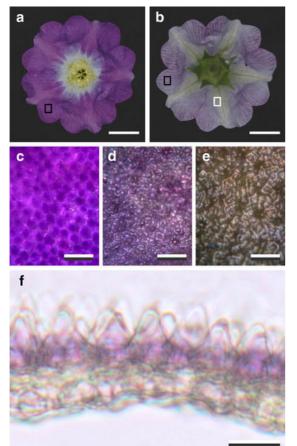
## Flower Petal Anatomy and the Stack Model

A great deal of effort has gone into studying the physical structure of plants, and in particular the colorization of flowers, in large part due to the relationship in signaling pollinators. Dr. Casper J. van der Kooi, professor of plant physiology at the Groningen Institute for Evolutionary Life, has done a tremendous amount of work to advance quantitative analysis of light interaction to explain flower coloration.

The petals of a flower are made up of several layers consisting of outer epidermal layers and inner pigmented and light scattering layers of the mesophyll. Pigment layers selectively absorb incident light within a specific wavelength, while light scattering structures and vacuoles backscatter the incident light in all directions, this diffuse reflectance creates a consistent visual effect when viewed from multiple angles.

In his recent work partnered with Dr. Doekle G. Stavenga, professor of computational physics at the Zernike Institute for Advanced Materials, University of Groningen, Dr. van der Kooi studied the Chilean Bellflower, Nolana paradoxa, which has a distinct color differentiation between the vivid, saturated purple of its adaxial (upper) surface and the unpigmented abaxial (lower) surface.

Previous efforts to explain the interaction of light within a plant to produce flower color relied on geometrical optics, however plant structures are not homogenous, making direct optical analysis inconvenient. Furthermore,



these methods require knowledge of essential optical parameters such as a refractive index and absorption coefficients of the component structures; however, optical constants of botanical samples such as these are simply unavailable in the current body of knowledge. Alternatively, the Kubelka-Munk theory for absorbing and diffusely scattering media allows the absorption and scattering coefficients to be derived from measured transmission and reflectance spectra.

Stavenga and van der Kooi have used this method with apparent success in previous work on a non-invasive method for estimating chlorophyll content using the Kubelka-Munk theory and treating a plant leaf as a stack of absorbing and reflective plates. In this related spectra analysis work sets the basis for future quantitative analysis and comparison of the coloration strategies of flowers.

### **Methods and Results**

To describe flower coloration, Drs. van der Kooi and Stavenga developed an optical model treating a flower petal as a stack of

layers, and then applied the Kubelka-Munk theory for diffuse scattering and absorbing media to the layers. This method uses the combined stack reflectance and transmission spectra and, with the number and relative thickness of the layers known, can estimate the reflectance and transmission spectra for each layer.

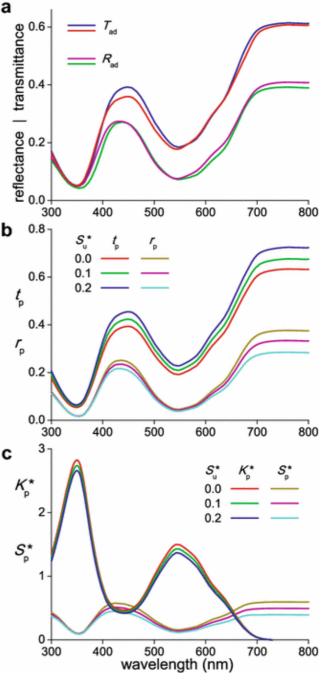
To measure the physical structure and distribution of pigments, these researchers examined cross sections of bellflower petal under magnification and found the *Nolana paradoxa* petals have a pigmented, strongly scattering adaxial mesophyll layer and an unpigmented moderately reflective abaxial layer.

To capture spectrophotometric measurements, researchers employed a deuterium-halogen lamp (AvaLight-D(H)-S) to deliver light via optical fiber to an integrating sphere (AvaSphere-50-Refl). With the corolla (the sum group of petals of a flower) positioned in the sphere and illuminated directionally, the reflected light was then captured by a second optical fiber and collected with the AvaSpec-ULS2048XL-USB2 spectrometer with a 2048 pixel backthinned CCD image sensor.

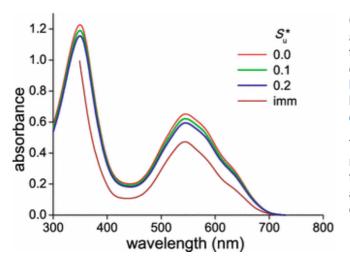
Stavenga and van der Kooi then interpreted this measured transmission and reflectance spectra for the corolla using the Kubelka-Munk absorbing stack layer model to estimate the transmission and reflectance spectra of the observed layers of the petal interior.

Subsequent measurements sought to confirm the results of applying the layer stack theory by measuring the

absorption spectra for the pigmented adaxial layer and the reflective properties unpigmented abaxial layer, individually. These subsequent experiments performed on the isolated layers appeared to confirm the spectral analysis derived from using the stack layer modeling.



# **Conclusion and Continuing Research**



Casper van der Kooi and Doekele Stavenga has greatly advanced our understanding of how plants use light. This team's growing body of work, together with other frequent contributors, has investigated the physical interaction of light that gives flowers their colorful appearance and how competition for pollinators affects the spectral display of flowers.

This research into the efficacy of the Kubelka-Munk method can have potentially wide-reaching impact on both the study of plants with potential agricultural applications, as well as potential relevance to other applications which concern stack metrology.

