Title: **Visual perception of colourful petals reminds us of classical fragments**

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Text

Colour has attracted the interest and attention of many of the most gifted intellects of all time. Ideas of early thinkers were not -and could not have been- grasped on a scientific level without knowledge of a kind that lay far in the future. One character that is being considered is the colourful surfaces of living tissues, which could hardly have been visualized without a corresponding reference to the microscale parallel. Millions of years before man made manipulated synthetic structures, biological systems were using nanoscale architecture to produce striking optical effects[1]. Here we show the microsculpture of the adaxial surface of flower petals from the asphodel, the Stork's-bill and the common poppy by using optical, scanning electron and atomic force microscopy. Microsculpture has been studied in leaves[2, 3, 4, 5] and pollen grains[6] of higher plants. To the best of our knowledge imaging and nanoscale morphometry of petals has not been reported hitherto. Our findings

on flower petals' microsculpture may be linked with aspects on colour revealed from ancient literature.

Empedocles, Democritus, Plato, Aristotle, Theophrastus, Descartes, Hooke, von Fraunhofer, Newton, Leonardo da Vinci (his notes reveal knowledge of Theophrastus text on colours), Goethe (his books reveal knowledge of Leonardo's ideas about colours), Hegel, Schopenhauer, Young, Maxwell, Helmholtz, Hering and Schrödinger all have been intrigued by colour and have contributed to our knowledge of it. Also, colour had always been an interesting subject for philosophers, although their ideas could be neither confirmed nor disproved for nearly two thousand years. Democritus (460-370 B.C.) regarded colours as the visually perceived result of various shapes, orderings, and positions of atoms and he conceived of colours as quantities of energy (light) that reveals translucence as an incorporeal property ranging from bright to dark. Plato (427-347 B.C.) wrote that colours are generated through the interaction of certain defined kinds of elemental particles and visual organs[7, 8]. According to Aristotle (384-322 B.C.) colour is a visible corporeal quality[9]. The Pythagoreans named the corporeal surface colour; colour is either in the limits (of bodies) or is the limit itself[9]. Theophrastus (370-288 B.C.) was the first to come up with a description of colour[10, 11] According to Zeno (332-261 B.C.) colours constitute the first determinant of the form of matter[11], this extraordinary statement stands furthermore isolated among the statements about colour and has received very little attention[12, 13]. Newton (1642-1726) states that wavelength composition of a light beam serves to define its colour, i.e. waves of length 400 to 450 nm are violet, 450 to 480 blue, 480 to 560 green, 560 to 590 nm yellow, 590 to 620 nm orange and 620 to 800 mm red. So pervasive is this doctrine in contemporary life that the existence of other explanations of colour is considered a matter for history of science[11, 14, 15]. In the

ongoing development of colour theory over centuries, Johann Wolfgang von Goethe (1749-1832) rejuvenated classical, philosophical ideas on colour[16].

In the plant kingdom, pigments of flowers can be exploited as sources for natural pigments and structural patterns. It is likely that natural systems offer technologically unrealised photonic structures and design protocols[2], while modern industry is conceived and nurtured largely by the demands for colour[17].

Flowering plants known from antiquity[18] (presented by Theophrastus, named by C. Linnaeus and collected by J. Sibthorp[18]) rely on a remarkable visual strategy in order to attract pollinators. It is likely that colourful flowers have co-evolved with the way pollinators see them[19, 20], while floral colour has been used by pollinators as a predictor of nutritional rewards[21, 22, 23] and warmth[24].

We present microscopic images of the adaxial surfaces of off-white petals (Fig. 1a) from *Asphodelus ramosus* L. (asphodel), of crimson petals (Fig. 1b) from *Erodium malacoides* (L.) L' Hir. (Stork's-bill) and red petals (Fig. 1c) from *Papaver rhoeas* L. (common poppy), consisting of two-three individual layers of cells, approximately 2–30 μm thick[25, 26].Cuticle folds of the surface of petals were observed by using scanning electron (SEM) and atomic force microscopy (AFM). To perform our work we relied on a high resolution tool in order to detect the first topographical information of petal surfaces.

Images of the adaxial surface of petals from the asphodel flower were obtained by using optical microscopy (Fig. $1a_1$), showing mature stomata. In SEM image, nearly parallel cuticle folds were observed (Fig. $1a_2$). AFM imaging, over a 4 μm^2 scan area reveals a periodicity of cuticle folds (Fig. $1a_3$) and a detailed surface relief (Fig. $1a_4$). According to Theophrastus[10] what is smooth is white and all smooth surfaces are brilliant[9]; but brilliant substances must also have open passages and be

translucent[10]. Democritus calls white smooth and black rough and he refers to the shape of the atomic figures[13].

Epidermal cells of crimson *Erodium* flower petals display thickenings of the cell wall at their base in a radial distribution (Fig. $1b_1$) and the rounded cells have a conical-papillate shape (Fig. $1b_2$) that resembles images obtained by AFM (Fig. $1b_3$) and the surface relief is shown in Figure $1b_4$. According to Theophrastus crimson comes from white, red and black[10]; for thus it makes an appearance delightful to the senses, while its brilliance and lustre testify to the presence of white[13, 15]. Aristotle proposed purple as the strongest colour-energy after light itself, while warmth is associated with red[27]. In the nanoworld, a particle with a diameter of about 100 nm appears purple-pink, but the colour shifts to red for particles with diameters of around 20 nm[28].

The adaxial surface of petals of *Papaver* (Fig. 1c) viewed by optical microscope possesses oblong, red, epidermal cells with anticlinal undular walls (Fig. $1c_1$). Cuticle folds of petals form an irregular pattern, as is apparent in SEM (Fig. $1c_2$), in AFM imaging (Fig. $1c_3$) and from the surface relief (Fig. $1c_4$). According to Theophrastus red is composed of atoms whose form is the same as, but larger than, that of things which are warm[10].

Colour reflects the state of the object to which it belongs and it depends on a relationship between light and the corporeal quality of the matter. However, “true” and “pure” colours are never seen[27, 29]. It also seems likely that the perception of colour has been influenced by qualities and temperaments being associated with colours. In addition, colour can be seen on a surface, as a spirited thing and the word to describe it was often fittingly applied as an adjective meaning something related to the colour itself. On the other hand, colourful plant pigments concentrated in vacuoles

and plastids are dependent on a mixture of elements in the cells, and in the environment (e.g. pH, heat and moisture). Therefore, it might be an oversimplification that a wavelength of light determines a colour.

Methods Summary

Fully expanded, turgid petals were harvested from flowers of *Asphodelus ramosus* (Asphodelaceae), *Erodium malacoides* (Geraniaceae) and *Papaver rhoeas* (Papaveraceae) plants growing under field conditions (38°14.3´N, 23°47.8´E) during spring and then were carefully transferred to the laboratory. Fresh tissue samples were cut and the surface sections were directly examined with a Zeiss Axioplan II microscope (Zeiss, Oberkochen, Germany) equipped with a camera, using Kodac colour 400 film. Petal samples were carefully cut in 2–4 mm^2 pieces and fixed in 3 % glutaraldehyde in phosphate buffer at pH 7 at room temperature for 2 h. The tissues were then postfixed in 1 % OsO_4 in the same buffer at 4 °C for 4 hours and dehydrated in a graded acetone series. The dehydrated tissue samples were critical point dried, mounted with a double adhesive tape on stubs, sputter coated with gold[30] and observed with a scanning electron microscope (JEOL 6300 SEM, Japan); SEM pictures were digitally recorded. Floral tissues, in their natural state, are very sensitive to the mechanical pressures of atomic force microscopy (AFM); thus, a mild method of fixation was performed on petals by using glutaraldehyde, paraformaldehyde and osmium tetroxide. Segments (4 mm^2) were cut from the adaxial petal surface and were immersed in a solution of 2 % glutaraldehyde plus 2% paraformaldehyde, in 0.1 M sodium cacodylate buffer (SCB), at pH 7.2, for 2.5 h, at room temperature. The plant material was washed three times by immersion in SCB for 5 min, and postfixed in 2 % OsO_4 for 5 h at 4°C. It was then washed three times in SCB for 30 min (each

time) at room temperature. The tissues, immersed in the buffer solution, remained at 4°C until they were to be studied. In order to quantify surface profiles of petals from topographic images, the stained tissues were screened in tap mapping atomic force microscope (TM-AFM, Multimode SPM, Veeco, USA) and various parameters were analysed and processed by using the software package Nanoscope III (Veeco, USA).

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Figure Legend

Figure 1| **Morphometry of flower petal surface (**open square indicates the area of the petal adaxial surface obtained by using microscopes). a. *Asphodelus ramosus*' off-white surface obtained by optical microscope (a_1), SEM (a_2) and TM-AFM (a_3), with profile view of the line section (a_4). b. *Erodium malacoides*' crimson surface obtained by optical microscope (b_1), SEM (b_2) and TM-AFM (b_3) image, with profile view of the line section (b_4). c. *Papaver rhoeas*' red surface obtained by optical microscope (c_1), SEM (c_2) and TM-AFM (c_3), with profile view of the line section (c_4).

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