

Colour calibration for quantitative biological analysis: a novel automated multivariate approach

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Abstract

This work proposed a novel approach to practical use of digital photography for biological purposes.

Introduction

In biology, as well as in agriculture, colour images are acquired and analysed for several different purposes e.g. studying the animal behaviour [1] [2], distinguishing among animal related groups [3] [4] and plant science [5]. Nevertheless, it is fundamental for the analysis to have the control of the images both when they are taken (mostly by the standardization of the conditions during the image capture) and when they will be analysed. The illuminant and therefore the light source present when acquiring an image is crucial in determining the quality of obtained images. Different light sources present different emission spectra dominated by diverse wavelengths that affect those reflected by the object under analysis and therefore the quality of the image acquired by the sensor or perceived by human eye. Such dominant light kinds can be expressed in terms of Kelvin degree. The explanation of such a unit of measure is based on the concept of black body, being this an idealized object that absorbs all electromagnetic radiation that falls on it. The object, not being able to reflect any electromagnetic wavelength, appear totally black when is cold. Nevertheless it emits a temperature-dependent spectrum of light when warmed up termed black-body radiation and expressible as

Kelvin degree. This body can be simulated with an optimum approximation using a material made from a nickel-phosphorus alloy. The Planck law demonstrates as, for a black body, specifying the emission temperature, it is possible to define univocally the colour of the emitted radiation and vice-versa. Thus, following Planck law, light sources can be classified on the base of their colour temperature. Therefore, the calibration of acquired pictures is compulsory in order to discriminate samples or recognise colour patterns. In digital photography, with printing aims, a number of software are available for colour flow management from the acquisition to the print. Conversely, there are no practical standard methods of illumination and colour calibration to make pictures easily comparable for scientific purposes. In addition, the camera settings and its sensor's response to light, play a crucial role. Although some problems may occur when digital photography for objective colour quantification and pattern recognition is used inappropriately, the benefits it provides are several. Examples of advantages are: flexibility, low cost, accessibility, and the amount of information provided. Therefore this study aims to minimize the effects of illuminants and camera settings introducing a novel approach to colour image calibration based on automated multivariate analysis. This allows practical colour quantification in biological systems analysis.

Materials and methods

To diminish the colour variance among calibrated pictures the following camera characteristics and settings have to be adopted as previously reported [6]. The camera (Nikon Coolpix P600) provided high resolution (13.5 real MP) TIFF 8bit image (from RAW format) with good macro features and optical 4x NIKKOR lens. Manual white balance control, exposure and metering methods, were enabled. ISO sensibility was set to 100 to avoid noise appearance. The Gretamachbeth ColorChecker 24 colour-patch was used as reference standard while the relative software, ProfileMaker Pro 5.0 (PROM), was adopted as conventional calibration system.

MATLAB 7.1 R14 was used to perform the image calibration based on: i. second order polynomial interpolation (POLY2); ii. PLS (Partial Least Square) calibration. RGB declared values of the ColorChecker (24 patch) were used as y-block. The x-block was represented by the mean RGB value of the same 24 patch. Eight different light conditions were used to acquire pictures: 200 watt Tungsten bulbs; weakened Tungsten; flash; weakened flash; internal shadow; internal shadow slightly underexposed; external shadow; and finally, full sun (midday). These could represent unknown conditions of light colour temperature, thus the operativity of digital image acquisition.

The ColorChecker was displaced next to different biological samples on a black cardboard. For each condition, three consecutive images were acquired and the same 5 uniform Region Of Interest (ROI) belonging to biological objects (Fig. 2-A) were consequently extracted from each image. To quantify the efficiency of the different calibration systems (PLS, POLY2 and PROM) with respect to the original images (NONE) mean intra- and inter-euclidean distances were calculated. Intra-distances represent differences among mean ROI

values of the same illumination condition (triple). Inter-distances represent differences among mean ROI values of different illumination condition.

The colour calibration based on the PLS model was then applied to 3 biological case studies, for which pictures were taken in non-standardized light conditions. In these case studies 2 kind of colour checker were used: Gretamachbeth ColorChecker 24 patch, IFRAO standard scale 7 patch. 1st case: colour pattern of the squat lobster *Munida tenuimana* (Crustacea: Decapoda) caught at different depths of Mediterranean continental margins (400-1500 m). 2nd case: dorsal colour pattern of *Salamandra salamandra* (Amphibia: Urodela) a quite widespread European species having several subspecies recognisable on this parameter. 3rd case: *Anguilla anguilla* (Teleostea: Anguillidae) lateral body colouration to observe contrast between dorsal and ventral skins along the lateral line as an evidence of the developmental transition between yellow and silver eel stage. One image per case study was calibrated.

Results and Discussion

Figure 1 presents the original and the PLS calibrated images of the three biological case studies.

Table 1 shows as intra-distances among pictures taken in sequence at the same illuminant conditions are always lower than the inter-ones. Both the new approaches proposed (PLS and POLY2) allow achieving a better calibration with respect to the conventional software (PROM). The distances are lower were the colouration of the sample are more homogeneous (ROI1= Leave; ROI2=dark background).

	Mean Intra-Distance				Mean Inter-Distance			
	NONE	PLS	POLY2	PROM	NONE	PLS	POLY2	PROM
ROI 1	2.7	1.2	1.1	2.2	24.5	6.6	4.6	17.3
ROI 2	11.0	8.3	8.3	9.9	38.6	14.8	15.0	28.7
ROI 3	11.3	6.6	6.7	8.8	43.1	14.9	15.1	27.4
ROI 4	11.9	8.5	8.0	9.2	40.9	20.4	19.2	23.9
ROI 5	7.6	3.8	3.4	6.5	25.4	8.9	7.4	19.7

Tab. 1. Mean intra- inter-Euclidean distances among RGB mean ROI values; the scale reported is 0-255.

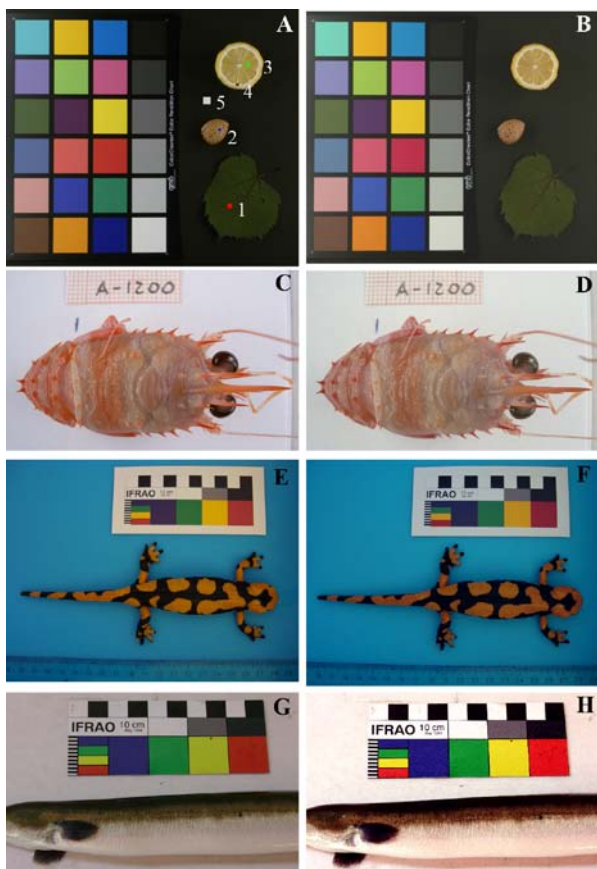


Fig. 1. **A.** Original image used to build the models (coloured squares represents the object ROIs reported in Table 1: 1. red ROI on a leaf, 2. blue ROI on an almond, 3. green ROI on the lemon segment, 4. brown ROI on the lemon flavedo, 5. pale gray ROI on the dark background). **B.** Original image used to build the models after PLS calibration. **C.** Original image of *Munida tenuimana*. **D.** PLS calibrated image of *Munida tenuimana*. **E.** Original image of *Salamandra salamandra*. **F.** PLS calibrated image of *Salamandra salamandra*. **G.** Original image of *Anguilla anguilla*. **H.** PLS calibrated image of *Anguilla anguilla*.

Conclusions

Digital technology in studying animal coloration is a potentially highly powerful method to avoid some of the drawbacks of other techniques. With market increased representation of digital photography products at low cost, is growing the interest for the application of such instruments. Although its feasibility digital photography can not be used without to its technicalities.

The proposed methods of colour calibration (POLY2 and PLS) allow the quantitative investigation of biological samples reducing the colour variance due to external factors (sensors,

camera settings and illuminations). It was demonstrated the occurrence of variance due to the sensor (intra-distances). Operators should be aware to the attention needed for image acquisition: choosing proper illumination (also in field operations), camera settings (as proposed by [6]) and using colour charts.

Finally, the process was fast and comparable to other available methods, with potentially hundreds of images taken in a day, quickly calibrated with a custom platform such as MATLAB environment. Detailed and complex measurements of traits associated with colour can be undertaken rapidly, with measurements and calculations that would normally be painstakingly undertaken by hand, including morphometric measurements and shapes analysis, such as Fourier analysis [7].

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