Video Camera Calibration for Optical Densitometry

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Abstract

We describe an efficient technique by which to calibrate video cameras to record optical density (OD) from microscopic images. The method corrects for variation over the field of the bright and dark field intensities, does not assume a linear response of the camera to the incident intensity and requires a single calibration filter.

Key words optical density, camera calibration, shading correction, microscopic images.

1 Introduction

Techniques of microdensitometry for measuring the photographic or optical density of staining of microscopic sections are well established and accurate. The most common method to establish the integrated optical density of a sample of tissue, for example cell nuclei, is to sum the density measurements made using a pin-hole and photomultiplier tube scanned across the specimen mechanically. Whilst this method will usually result in higher accuracy it has the serious limitation of scanning relatively slowly. In contrast, video cameras can gather data very quickly and are cheap and convenient to use, particularly since the development of CCD array camera technology.

A number of authors [Donovan,, **?**, Levine,, Sánchez,] have reported the use of video cameras for densitometric measurements in a wide variety of applications. All these papers describe calibration techniques which either require a full set of neutral density filters or assume that the camera response is perfectly linear with respect to the incident light intensity. Furthermore these methods do not take account of shading (variation of illumination intensity over the imaged field) or the dark field response of the camera except

by restricting measurements to a small square in the center of the field, typically 1/4 to 1/16 th of the full image area or by a simple subtraction of the bright field value.

In particular the linearity of the camera response is widely regarded as essential [Sánchez,] for densitometry and indeed most measurement systems assume that the transmitted intensity is directly proportional to the camera output voltage. Although CCD array sensors may be accurately linear in themselves, the final measured value depends on the amplification within the camera, which often incorporates a γ adjustment, and on an analogue to digital conversion. In any case, as most digitising systems will digitise the analogue signal to only eight-bit grey-level resolution (*ie* 256 discrete levels) it is not always desirable to adjust the camera to operate linearly. There may be a significant advantage in increasing the resolution over parts of the intensity range at the cost of reducing it over others so that maximum useful information for a particular application may be encoded into a restricted digital range.

In this paper we report a calibration and image capture technique that applies to cameras with arbitrary response functions, properly taking account of shading and the dark field response of the camera. The method is quick, efficient and requires only a single neutral density filter which is to be regarded as a reference standard. In the next section we detail the theory of the method and in the following section show how the theory can be implemented as a simple and efficient calibration technique. The latter section also considers the treatment of uneven illumination or shading.

2 Theory

2.1 Camera Calibration

We wish to establish the dependence of the measured image value, g, on the light intensity, I, incident on the measuring system which can be taken to include the microscope from the objective to the camera, the camera and its internal electronics, the video transmission cable and finally the digitisation board in the host computer. The calibration is to be performed with respect to a reference density, $\delta\rho$, with transmission coefficient $\delta t = 10^{-\delta\rho}$. The experimental setup in highly schematic form is shown in figure 1 where the reference density can be inserted or removed from the light path as required. In figure 1 the camera is represented as a single detector and for convenience in the subsequent implementation

of the theory we convert the measured value to a value which goes to zero as the intensity goes to zero. This makes no difference to the following derivation but in the application of the method to video cameras it is important to take proper account of the dark-field image. Therefore we define

$$G(I) = g(I) - g(0),$$

and clearly $G(0) = 0$.

For each value I of the incident light intensity we can measure two values from the detector, G and G' by removing and inserting the reference density respectively. From the definition of the transmission coefficient we know that G and G' correspond to two values of intensity I and I' respectively, related by

$$I' = \delta t I = 10^{-\delta\rho} I.$$

By varying the incident intensity it is possible to generate the curve $G' = F(G(I))$ as a function of $G(I)$ parameterised by the (unknown) I. This curve will range from zero to the maximum measurable value which will depend on the system but is commonly 255.

We now define a sequence of values $\{G(I_i) \mid i > 0\}$ by repeated application of the function F *viz.*

$$G(I_i) = F(G(I_{i-1})),$$

G_0 is arbitrary and the sequence is terminated when $|G_i - G_{i-1}| < \Delta$. Δ should be determined by the noise characteristics, in this paper we used $\Delta = 1.0$. From above the sequence of values I_i are related by $I_i = \delta t I_{i-1}$ and by repeated application we establish

$$I_i = \delta t^i I_0,$$

which implies that we have calibrated the measured values G_i in terms of a single unknown intensity I_0 and the known transmission coefficient δt *i.e.*

$$G_i = H(\delta t^i I_0).$$

We can equally regard this as a function of density,

$$G_i = \overline{H}(\rho_0 + i\delta\rho).$$

This iteration can be understood in graphical terms as shown in figure 2. The curved line represents a typical measured set of (bright, dark) values of G. The diagonal line is

simply $G(I') = G(I)$. The iteration above corresponds to tracing the dashed horizontal and vertical lines to each new value in the sequence.

To fill in values of the response function G between those points determined from the curve at intervals of $\delta\rho$ we must invoke some additional constraint. The constraint can only arise from the nature of the physical device, for example that the device responds smoothly and monotonically with respect to intensity or exhibits a particular asymtotic behavior. We establish density values for all possible measured values by interpolating with respect to $log(G)$, which for most CCD cameras is approximately linear. We use cubic spline interpolation [Faux,].

Once the function $G(\rho_0 + i\delta\rho)$ has been established then optical densities are determined by measuring the response with and without the object of interest and then to subtract the corresponding densities. In practice this implies the storage of the bright-field image because the illumination is almost never sufficiently even that shading effects can be ignored. Clearly the method can equally determine transmission coefficients, but now the bright-field correction involves division rather than subtraction.

2.2 Glare

It should be noted that the calibration method described is concerned with the calibration of the camera and digitisation devices only, and takes no account of optical characteristics of the microscope, such as the glare.

A widely accepted model [Goldstein,] characterises glare by a parameter F defined as the intensity of additive glare as a fraction of the illuminating intensity. For many applications this model is appropriate and for completeness we include the appropriate formulae here. The parameter depends on the proportion of background illumination in the microscope field and nature of the sample to be measured. In many applications it is a reasonable approximation to assume that the sample occupies a negligible proportion of the field and the glare parameter can then be obtained for a particular microscope setup by measuring the *apparent* OD of a microscopic slide object (eg soot particle) which can be assumed to be black. Writing the apparent OD of the black object as ρ_b we have:

$$F = 10^{-\rho_b}$$

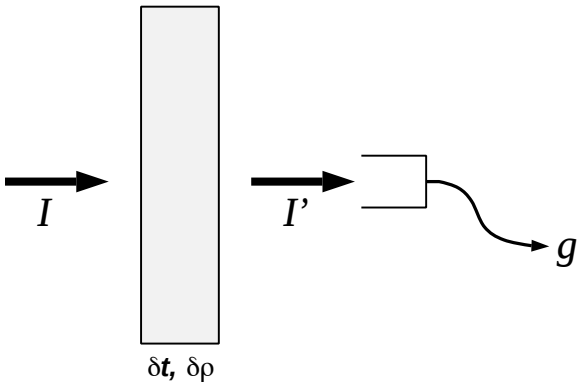


Figure 1: Schematic form of the camera calibration experimental setup..

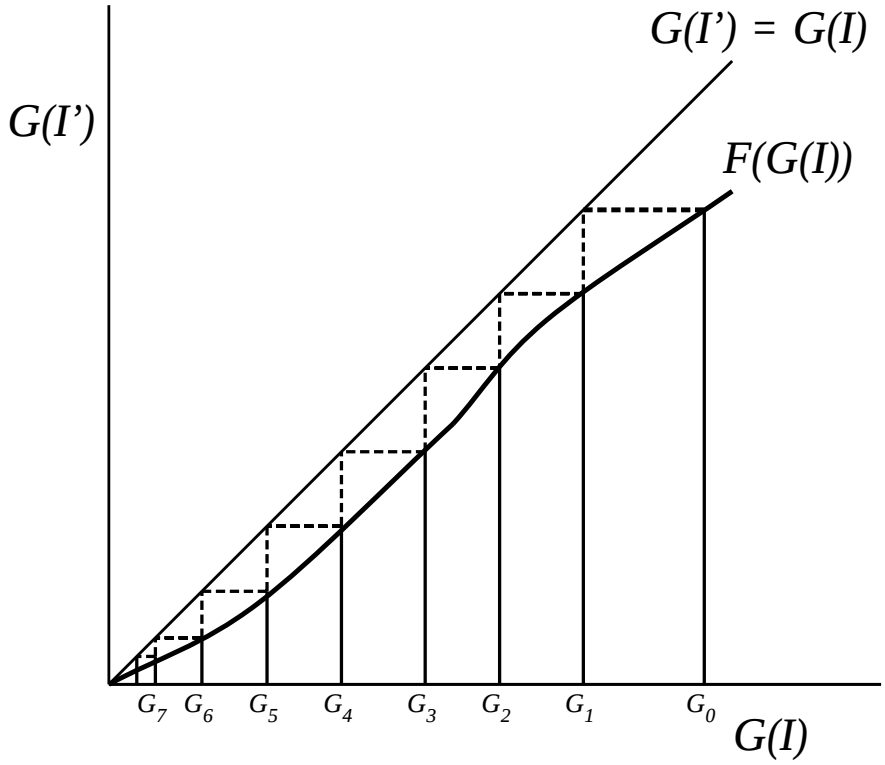


Figure 2: Diagramatic representation of the iterative scheme to establish the sequence of measured values that correspond to a geometric progression of intensity values or equivalently, equal increments of the reference density.

and the true OD, ρ_t of an object having an apparent OD ρ_a is given by

$$\rho_t = \log_{10}\left(\frac{1-F}{10^{-\rho_a}-F}\right).$$

This glare correction may be applied after the OD correction if required.

3 Methods

3.1 Hardware

The image digitisation and analysis was performed on a Sun (Sun Microsystems) 3/160 computer fitted with a Primagraphics "Virtuoso" framegrabber (Primagraphics Ltd, Royston, Herts., UK). The video cameras tested and compared in this work were a Pulnix TM86 and a Sony XC-77CE, both of which are CCD array cameras. The camera was mounted on a Leitz Ortholux microscope fitted with a standard bright-field condenser and a $\times 10$ objective lens. The Primagraphics framegrabber was capable of digitising the full video image to a spatial resolution of 768x576 pixels and a grey-level resolution of 8-bits, i.e. values ranging from zero to 255. The microscope illumination was powered by a Roband "Vareco 33-10" DC power supply. It is particularly important that the lamp voltage should be stable for accurate densitometry. The theory outlined above assumes constant spectral charateristics of the light source. In practice this is very difficult to control therefore we use monochromatic light by inserting a narrow band-pass filter in the light path. For these experiments we have used a BALZER interference filter with measured response peaked at 563 nm (green). For the experiments described below we used a Kodak Wratten neutral density filter of OD 0.1.

3.2 Calibration

To use the calibration method outlined above requires the determination of the graph of $G(I)$ against $G(I')$. This can be obtained using a single neutral density filter (possibly unknown value), plus any microscope slide with a reasonable variation of densities.

We assume that each of the elements of the array has a transfer function of the form

$$g_i(I) = a_i + f(b_i I),$$

where i is an element index and each may have a different dark-field value, a_i and a different shading, b_i. Variation in shading coefficient b_i arises from non-even illumination and from differences in individual CCD array elements for example their respective quantum efficiencies. The procedure to generate the points, $(G(I), G(I'))$, is:

1. digitise an entire dark field image by switching the microscope lamp off;

2. select a field on a slide that exhibits a reasonable range of densities and adjust the lamp level so that the brighter parts of the field almost saturate the digitised image;

3. digitise the field twice, without moving the slide, first without and then with the neutral density filter in the illumination path, [1] typically before the condenser. Subtract the dark-field image from each of these new images;

4. accumulate the values $(G(I), G(I'))$ by scanning through the two images, corresponding pixels from the two images are the required value pairs, $G(I')$ is the value from the image with the neutral density filter and $G(I)$ is the value from the image without the filter [2];

5. inspect the range of values extracted from the images; if parts of the response range are missing then adjust the lamp voltage and repeat from 3;

6. establish the values $G(\rho_0 + i\delta\rho)$;

7. select a starting value G_0 near the bright end of the range to correspond to the arbitrary offset density ρ_0 and tabulate the function $\rho(G)$ by the method outlined earlier.

3.3 Image Correction

The calibration procedure results in a conversion table from measured value to a density. To determine the density values in a particular image requires a corresponding dark-field (zero light) and bright-field (no object) image. Since the dark-field image clearly does not depend on the microscope optics or illumination, the dark-field image captured during

[1] The filter must not be placed in the image path since this might de-focus or shift the image

[2] In this case we have indexed the accumulating values by $G(I)$ and therefore $G(I')$ are averaged over corresponding points

calibration may be used for subsequent measurements. However the bright-field image must be re-captured whenever the slide, objective, condenser or lamp brightness are adjusted. The bright-field image is obtained as follows:

1. Set up the microscope for the slide of interest, using the required objective. Set the lamp brightness to give a good image on the video monitor. Close down the condensor field apperture so as to just enclose the required image frame and so minimise glare.

2. Move to an area of the slide which contains mostly clear field, and focus the objective and condensor on this region. Digitise several frames (we usually find three or four to be adequate) at different slide positions.

3. Compute the final bright-field image by selecting the brightest value across the captured frames at each pixel.

The bright-field may be used to correct subsequent images from the same slide as long as the microscope settings are not adjusted. To correct an image subtract the dark-field value, convert each pixel value to a density and subtract the bright-field density:

$$OD = \rho(g - g_d) - \rho(g_b - g_d),$$

where g is the measured value, g_d the corresponding dark-field value and g_b the corresponding bright field value.

3.4 Software

The methods we have outlined above have been incorporated into an interactive image capture program developed within the MRC Human Genetics Unit. The program is written in 'C' [Kernighan,] and the user interface implemented to run using revision 4 of the X11 [Gettys,] windowing system developed at the Massachusetts Institute of Technology. This provides the user with a graphical interface for all the operations required for calibration as well as frame averaging, region-of-interest selection, signal amplification (by summation) and contrast enhancement. With the software set up in this way the calibration process take about three minutes and is sufficiently convenient to allow recalibration for every digitisation session. At the MRC the program is used on a Sun 3/160 workstation with Primagrahics frame buffer and a Sun Sparcstation fitted with a Videopix video capture card. More details of the software are available from the authors.

4 Results

To demonstrate the application of the technique we have calibrated cameras from two manufacturers which were found to have different response functions and compared the resulting corrected images and measurements of neutral density filters. These two experiments are described below.

4.1 Image OD Conversion

The example slide used is a coronal section of a rat brain immunostained for tyrosine hydroxylase. The method we have detailed will work with any slide but the procedure is most efficiently undertaken using a slide with a wide range of densities. The two cameras were calibrated using a neutral density filter of OD 0.1 in each case. The example images cover approximately identical slide locations and the light level was adjusted to give visually acceptable uncorrected images in each case. Dark- and bright-field images are not shown but were obtained seperately for the two cameras by the methods described above.

In figure 3 we show two sets of curves. Figure 3(a) show the measured bright-dark curves for each camera. The Sony camera exhibits nearly linear response for the whole intensity range but with a lower sensitivity than the Pulnix. The Pulnix camera was adjusted to have a γ-value of less than one and results in a non-linear response curve. The density calibration curves calculated from these bright-dark values are shown in figure 3(b).

Figures 4 and 5 show the result of using these calibration curves on the raw uncorrected images. In each case the grey-level histogram of the image is shown in the inset graph. The histograms for the two uncorrected images show how different the responses of each camera are with the Pulnix exhibiting greater sensitivity at the low-light end of the range (note the histograms are drawn with increasing grey-value corresponding to increasing density). The corresponding histograms for the corrected images are nearly identical with small differences arising from different noise characteristics of the cameras and the fact that the two images are not exactly the same position or orientation.

4.2 OD Comparison

In order to determine the repeatability of OD measurements made by different cameras, the OD of a set of neutral density filters was measured using the Pulnix and Sony cameras.

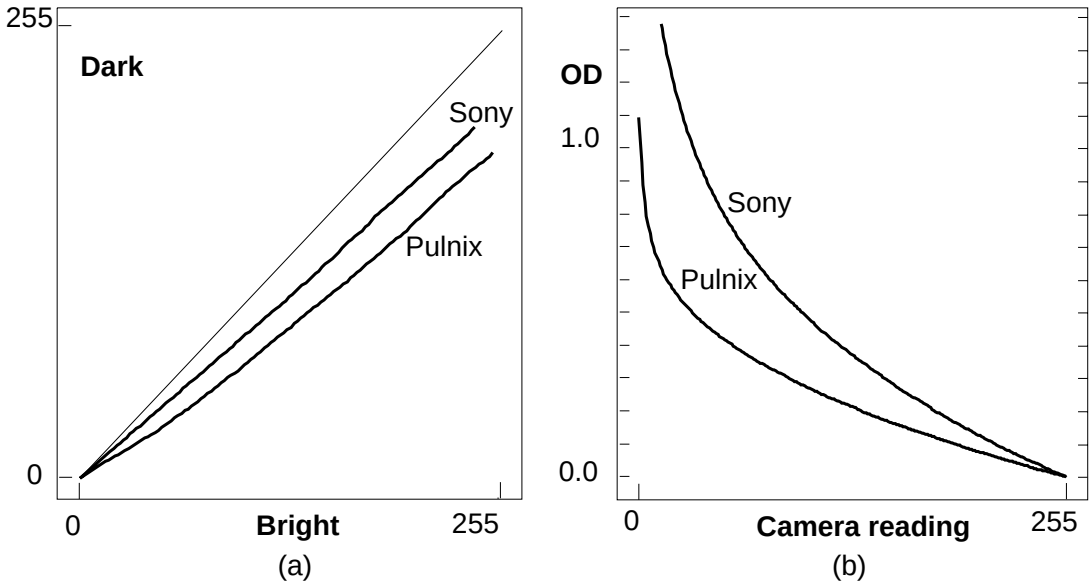


Figure 3: Calibration curves, (a) bright-dark curves for each camera, and (b) corresponding density calibration curves..

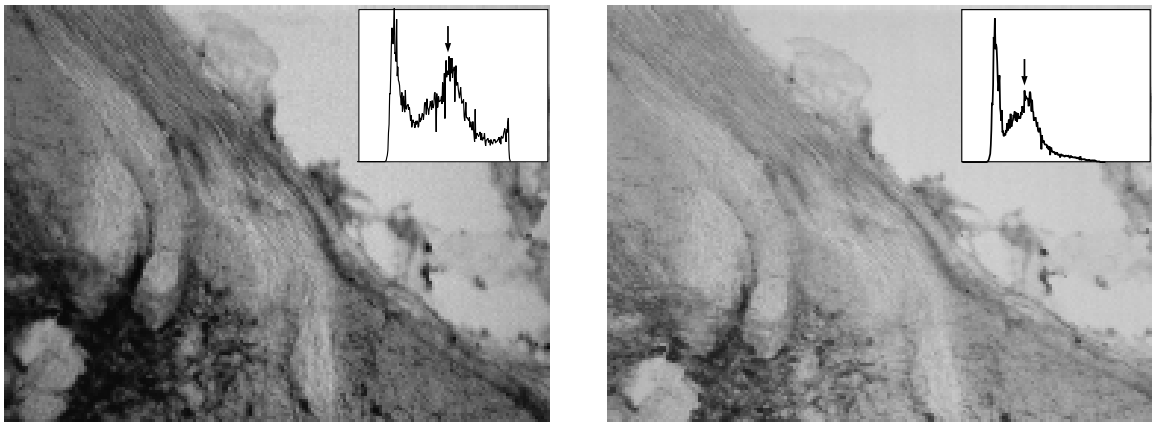


Figure 4: Uncorrected images and grey-level histograms (inset) for (a) Pulnix camera, and (b) Sony camera. The mean, standard deviation and peak position (as marked by the arrow) are (131.0, 49.9, 142) for the Pulnix and (95.8, 34.8, 103) for the Sony.

Each camera, was calibrated by the described procedure. Scaling was selected so that an OD of 1.0 was represented by a pixel value of 200.

A blank area of an arbitrary slide was selected, and after setting the lamp brightness to avoid saturation, a bright field was captured. Each neutral density filter was inserted in the illumination path, an image captured then corrected to optical density with reference to the bright field image and appropriate calibration table. The OD of the filters were recorded as the mean pixel value, after rescaling (ie division by 200) and are shown in table 1. Note, the actual values measured depend on the accuracy of the 0.1 OD filter used in the calibration process.

The correlation coefficient between the two Pulnix and Sony readings is 0.9950. Notice that it is the two higher OD readings which differ the most (with these points excluded, the correlation is 0.9996). We believe this is because the Sony camera has a near linear response and therefore has low resolution of high optical densities. This is especially apparent for 8-bit digitisation. The pulnix camera has $\gamma < 1.0$ (ie it is more sensitive at low intensity levels) giving it greater discrimination for higher OD measurements.

5 Discussion and Conclusion

The two images shown in figure 4 were obtained with two different cameras which were found to have very different response functions. The calibration technique descibed in this paper has been applied in each case using in-house software and takes about three minutes for each camera. Once calibrated then by capturing a bright field image it is possible to estimate the true optical density on the slide although in this case we have not corrected for glare. The corrected images for both cameras shown in figure 5 illustrate how well the method works. There are differences between the images arising from digitisation artefacts and as explained earlier it may be advantageous within any particular application not to set the camera to respond linearly with respect to intensity.

In many applications, for example the determination of cell ploidy, it is important to increase the density resolution at the dark end of the scale and so choosing $\gamma \approx 0.5$ provides better resolution of the objects of interest.

In summary we have developed a convenient and accurate technique to calibrate video cameras for microscopic applications. The method relies only on the definition of optical

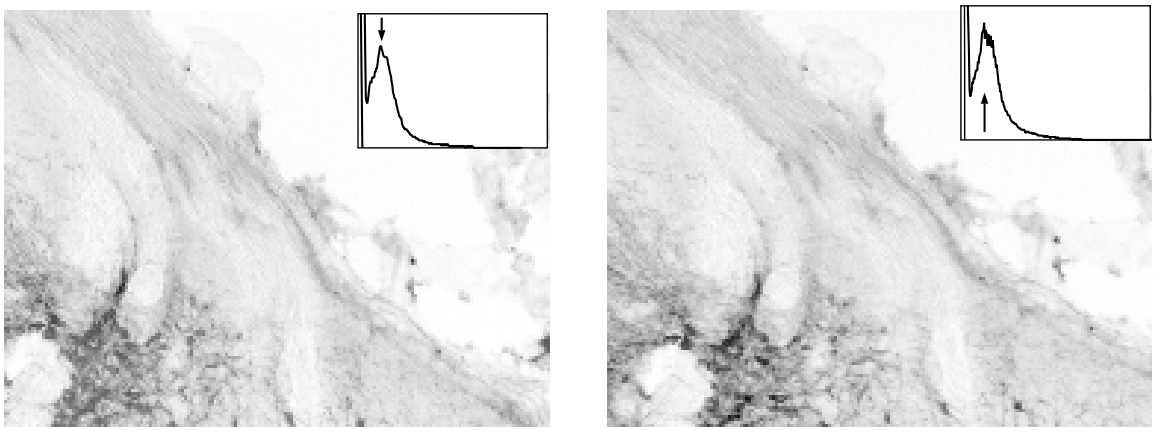


Figure 5: OD corrected images and grey-level histograms (inset) for (a) Pulnix camera, and (b) Sony camera. The mean, standard deviation and peak positions are now (31.9, 28.2, 25) and (32.8, 28.9, 26) respectively.

Nominal	Pulnix	Sony
0.1	0.100	0.096
0.2	0.213	0.206
0.3	0.310	0.308
0.4	0.465	0.461
0.5	0.502	0.514
0.6	0.671	0.662
0.7	0.777	0.786
0.8	0.831	0.845
0.9	0.902	0.957
1.0	1.003	1.141

Table 1: OD of neutral density filters measured with the Pulnix and Sony cameras. The filters used were Kodak Wratten gelatin neutral density filters with nominal densities as shown.

density and can be applied to any camera with any response function, in particular it is not necessary to assume linearity. The only additional equipment requirement for a digitising system is a single neutral density filter and the calibration can be undertaken using typical slides for any application.

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