Published in final edited form as:

J Microsc. 2011 February ; 241(2): . doi:10.1111/j.1365-2818.2010.03443.x.

Restoration of distorted colour microscopic images from transverse chromatic aberration of imperfect lenses

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Summary

An algorithm is presented for restoration of colour microscopic images with distortions from imperfect microscope lenses having transverse chromatic aberrations, resulting in a magnification that slightly varies with wavelengths or colours. The differential of each colour component image is computed as the difference between the component image and its slightly magnified version. The absolute values in the differential component images are generally higher at the edges where greater discontinuities occur. The two cross-correlation functions of the absolute differentials between red and green colours and between red and blue colours are then computed. The maximum in the two cross-correlation functions were sought, respectively, and the cross-correlation delays were then calculated. The two cross-correlation delays were used to determine dispersions and to realign the three colour components. Results of real microscopic images are provided. The restored image and the original are compared both visually and quantitatively in terms of the estimated entropies measured for the degree of concentrations using vector distributions.

Keywords

Image restoration; microscopy imaging; microscope lens; transverse chromatic aberration

Introduction

Chromatic aberrations are common with any single thin lens because different colours of light propagate at different speeds within a medium in which the refractive index is wavelength-dependent. This phenomenon is known as dispersion. Chromatic aberration can be corrected by combining positive and negative lens elements that have different colour dispersion properties (Clarke & Eberhardt, 2002; Davidson & Abramowitz 2002; Sheppard 2007; Wolf, 2007).

Oblique light leads to transverse chromatic aberration, also known as lateral colour, which refers to a sideward focus resulting in an image magnification that is wavelength-dependent. Although this phenomenon can be reduced by careful correction using a combination of additional lenses, it may still occur when imperfect lenses fail to produce perfectly focused images. A manual method for reducing the transverse chromatic aberration is implemented as the PTShift procedure using Photoshop software. With the PTShift function, a user can

manually adjust the colour shift values while viewing and monitoring the reconstructed image until a visually satisfactory image appears. Although minor transverse chromatic aberration is acceptable to most pathologists when viewing diagnostic specimens, it may significantly affect the quantitative results of the image segmentation and analysis algorithms that are based on the vector clusters (Wu *et al.*, 2008). In this short communication, we describe an algorithm for restoration of images having transverse chromatic aberrations due to imperfect microscope lenses.

Materials and methods

Let the original colour image of size $N_1 \times N_2$ be represented by a two-dimensional discrete sequence of vectors $\mathbf{x}(n_1, n_2) = (x_r(n_1, n_2), x_g(n_1, n_2), x_b(n_1, n_2))^t$, for $n_1 = 0, 1, \dots, N_1 - 1$ and $n_2 = 0, 1, \dots, N_2 - 1$, in which the superscript t denotes the transposition of vectors. Let the three continuous RGB (red, green and blue) components, $\tilde{x}_r(t_1, t_2)$, $\tilde{x}_g(t_1, t_2)$ and $\tilde{x}_b(t_1, t_2)$, for $0 \leq t_1 \leq N_1 - 1$ and $0 \leq t_2 \leq N_2 - 1$, be the interpolations from the discrete components, $x_r(n_1, n_2)$, $x_g(n_1, n_2)$ and $x_b(n_1, n_2)$, for $n_1 = 0, 1, \dots, N_1 - 1$ and $n_2 = 0, 1, \dots, N_2 - 1$, respectively, by

$$\tilde{x}_r(t_1, t_2) = \frac{\sum_{m_1=0}^1 \sum_{m_2=0}^1 a(m_1, m_2) x_r(\lfloor t_1 \rfloor + m_1, \lfloor t_2 \rfloor + m_2)}{\sum_{m_1=0}^1 \sum_{m_2=0}^1 a(m_1, m_2)}, \quad (1)$$

$$\tilde{x}_g(t_1, t_2) = \frac{\sum_{m_1=0}^1 \sum_{m_2=0}^1 a(m_1, m_2) x_g(\lfloor t_1 \rfloor + m_1, \lfloor t_2 \rfloor + m_2)}{\sum_{m_1=0}^1 \sum_{m_2=0}^1 a(m_1, m_2)} \quad (2)$$

and

$$\tilde{x}_b(t_1, t_2) = \frac{\sum_{m_1=0}^1 \sum_{m_2=0}^1 a(m_1, m_2) x_b(\lfloor t_1 \rfloor + m_1, \lfloor t_2 \rfloor + m_2)}{\sum_{m_1=0}^1 \sum_{m_2=0}^1 a(m_1, m_2)}, \quad (3)$$

in which the floor operation $\lfloor \cdot \rfloor$ outputs the largest integer less than the variable, the

coefficients $a(0, 0) = 1/\sqrt{(\Delta t_1)^2 + (\Delta t_2)^2}$, $a(0, 1) = 1/\sqrt{(\Delta t_1)^2 + (1 - \Delta t_2)^2}$,

$a(1, 0) = 1/\sqrt{(1 - \Delta t_1)^2 + (\Delta t_2)^2}$, $a(1, 1) = 1/\sqrt{(1 - \Delta t_1)^2 + (1 - \Delta t_2)^2}$, $t_1 = t_1 - \lfloor t_1 \rfloor$ and $t_2 = t_2 - \lfloor t_2 \rfloor$.

If the magnification for the red component, $x_r(n_1, n_2)$, is changed by a very small value such that the domain of the component image increases from $N_1 \times N_2$ to $(N_1 + 2) \times (N_2 + 2)$ in which $\ll (N_1 - 1)/2$, the larger image

$$\tilde{x}_{r,\Delta}(t_1, t_2) = \tilde{x}_r\left(\frac{\Delta(c_1 - t_1)}{(N_1 - 1)/2} + t_1, \frac{\Delta(c_2 - t_2)}{(N_2 - 1)/2} + t_2\right), \quad (4)$$

for $-t_1 \leq t_1 \leq N_1 - 1 +$ and $-t_2 \leq t_2 \leq N_2 - 1 +$, which has the same centre in which the original image is located $(c_1, c_2) = ((N_1 - 1)/2, (N_2 - 1)/2)$. The absolute differential between the red image component and that of its adjusted magnification is

$\varepsilon_r(n_1, n_2) = |\tilde{x}_{r,\Delta}(n_1, n_2) - x_r(n_1, n_2)|$, for $n_1 = 0, 1, \dots, N_1 - 1$ and $n_2 = 0, 1, \dots, N_2 - 1$.

The other two absolute differentials can also be obtained in similar ways as

$\varepsilon_g(n_1, n_2) = |\tilde{x}_{g,\Delta}(n_1, n_2) - x_g(n_1, n_2)|$ and $\varepsilon_b(n_1, n_2) = |\tilde{x}_{b,\Delta}(n_1, n_2) - x_b(n_1, n_2)|$, respectively.

The matrices, $r(n_1, n_2)$, $g(n_1, n_2)$ and $b(n_1, n_2)$, are the absolute differences between the colour components and their slightly magnified versions, respectively. Thus, they are generally higher at the edges of the respective component images. For the colour microscopic images of specimens, the edges of the three individual colour components may match well. We define the cross-correlation function between $r(n_1, n_2)$ and $g(n_1, n_2)$ as

$$r_{r-g}(\delta) = \begin{cases} \sum_{n_1=0}^{N_1-1} \sum_{n_2=0}^{N_2-1} \varepsilon_r(n_1, n_2) \tilde{\varepsilon}_{g,\delta}(n_1, n_2), & \text{if } \delta \geq 0, \\ \sum_{n_1=0}^{N_1-1} \sum_{n_2=0}^{N_2-1} \tilde{\varepsilon}_{r,-\delta}(n_1, n_2) \varepsilon_g(n_1, n_2), & \text{if } \delta < 0, \end{cases} \quad (5)$$

in which

$$\tilde{\varepsilon}_{g,\delta}(n_1, n_2) = \tilde{\varepsilon}_g\left(\frac{\delta(c_1 - t_1)}{(N_1 - 1)/2} + t_1, \frac{\delta(c_2 - t_2)}{(N_2 - 1)/2} + t_2\right)_{t_1=n_1, t_2=n_2} \quad (6)$$

and

$$\tilde{\varepsilon}_{r,-\delta}(n_1, n_2) = \tilde{\varepsilon}_r\left(\frac{-\delta(c_1 - t_1)}{(N_1 - 1)/2} + t_1, \frac{-\delta(c_2 - t_2)}{(N_2 - 1)/2} + t_2\right)_{t_1=n_1, t_2=n_2}. \quad (7)$$

Because the edges or discontinuities between different objects likely happen in the same place within different colour component images, the correlation function should be high when the two errors are matched and thus have a high cross-correlation. Therefore, the peak in $r_{r-g}(\delta)$ is sought over the variable δ . Similarly, we can also obtain the cross-correlation function of $r_{r-b}(\delta)$ such as

$$r_{r-b}(\delta) = \begin{cases} \sum_{n_1=0}^{N_1-1} \sum_{n_2=0}^{N_2-1} \varepsilon_r(n_1, n_2) \tilde{\varepsilon}_{b,\delta}(n_1, n_2), & \text{if } \delta \geq 0, \\ \sum_{n_1=0}^{N_1-1} \sum_{n_2=0}^{N_2-1} \tilde{\varepsilon}_{r,-\delta}(n_1, n_2) \varepsilon_b(n_1, n_2), & \text{if } \delta < 0, \end{cases} \quad (8)$$

in which

$$\tilde{\varepsilon}_{b,\delta}(n_1, n_2) = \tilde{\varepsilon}_b\left(\frac{\delta(c_1 - n_1)}{(N_1 - 1)/2} + n_1, \frac{\delta(c_2 - n_2)}{(N_2 - 1)/2} + n_2\right). \quad (9)$$

We seek the peaks of the two correlation functions, $r_{r-g}(\delta)$ and $r_{r-b}(\delta)$, respectively, and find the corresponding locations at $\delta_{r-g} = \max_{\delta}^{-1} \{r_{r-g}(\delta)\}$ and $\delta_{r-b} = \max_{\delta}^{-1} \{r_{r-b}(\delta)\}$.

With the adjustments calculated, we then reconstruct the colour image by magnifying two of the three components and retaining the third. If both $r_{r-g}(\delta)$ and $r_{r-b}(\delta)$ are positive, the red component remains unchanged. Otherwise, either the green or blue component remains the same depending on which of the two is smaller between $r_{r-g}(\delta)$ and $r_{r-b}(\delta)$. Without loss of generality, we presume that both $r_{r-g}(\delta)$ and $r_{r-b}(\delta)$ are negative and $r_{r-g}(\delta) > r_{r-b}(\delta)$ as is the case with the images in the current study. The restored colour image is reconstructed by

$$\mathbf{y}(n_1, n_2) = (y_r(n_1, n_2), y_g(n_1, n_2), y_b(n_1, n_2))^t, \quad (10)$$

in which and

$$y_r(n_1, n_2) = \tilde{x}_r\left(\frac{-\delta_{r-b}(c_1 - n_1)}{(N_1 - 1)/2} + n_1, \frac{-\delta_{r-b}(c_2 - n_2)}{(N_2 - 1)/2} + n_2\right), \quad (11)$$

$$y_g(n_1, n_2) = \tilde{x}_r \left(\frac{(\delta_{r-g} - \delta_{r-b})(c_1 - n_1)}{(N_1 - 1)/2} + n_1, \frac{(\delta_{r-g} - \delta_{r-b})(c_2 - n_2)}{(N_2 - 1)/2} + n_2 \right) \quad (12)$$

and

$$y_b(n_1, n_2) = \tilde{x}_b(n_1, n_2), \quad (13)$$

for $n_1 = 0, 1, \dots, N_1 - 1$ and $n_2 = 0, 1, \dots, N_2 - 1$.

The algorithm is summarized in the flow chart in Fig. 1. The input image is deconstructed into the three separate RGB components. Each component is slightly magnified and then re-sampled from the interpolated analog image back into the discrete image. The red component is magnified by Eqs (1) and (4), followed by an analog-to-digital procedure sampling at the integer grids as $t_1 = n_1$ and $t_2 = n_2$. The other two colour components are processed in the same way. The difference between input and output for magnifying and re-sampling each block is calculated and its absolute value is then computed. Two correlation functions between red r and green g , and between red r and blue b are calculated, respectively. We can find the two bias values of $r-g$ and $r-b$ from the two separate correlation functions. The modified colour components are obtained from Eqs (11) to (13). The restored colour image is the colour image composed of the new colour RGB components.

Results

Figure 2(a) shows a corner portion (for $0 \leq n_1 < N_1/4$ and $0 \leq n_2 < N_2/4$) taken from an original image with size $N_1 \times N_2 = 1600 \times 1200$ acquired using a Nikon Labophot-2 Light microscope (Morrell Instruments, Melville, NY, U.S.A.) and a Coolpix MDC lens of achromatic Plan 40 with aperture 0.7 and working distance 0.61 mm. The actual metric size of the original image is measured as $0.2119 \times 0.1589 = 0.0337 \text{ mm}^2$. The tissue is a $5 \mu\text{m}$ brain section that has been formalin-fixed and paraffin-embedded. Immunohistochemical staining was performed after the tissue was deparaffinized and rehydrated prior to antibody incubation. Antibody detection was performed using an ImmPRESS Ig Kit from Vector Laboratories (Burlingame, CA, U.S.A.) followed by diaminobenzidine and then counterstained with hematoxylin. The immunohistochemical-stained image shows a microglial cell cytoplasm (brown colour) with the nucleus stained blue by hematoxylin, and neuropil regions appearing either light blue or grey. The transverse chromatic aberrations or lateral colours are more obvious at the edges of the image where a high degree of discontinuity exists among the different objects. The microglial cell cytoplasm area that appears significantly different in the brown colour may have extra false red regions along the edges on one side because of transverse chromatic aberrations.

In this study, the algorithm is implemented in the Matlab (The MathWorks Inc., Natick, MA, U.S.A.) environment that provides ready-to-use mathematical functions and file reading as well as writing procedures for images in the standard format. The individual RGB colour components of the original image are shown in Figs 3(a)–(c). The absolute differentials of the three colour components, $r(n_1, n_2)$, $g(n_1, n_2)$ and $b(n_1, n_2)$, for $0 \leq n_1 < N_1/4$ and $0 \leq n_2 < N_2/4$, are shown in Figs 3(a)–(c). The cross-correlation function, $r_{r-g}()$, of the absolute differentials between $r(n_1, n_2)$, as shown in Fig. 3(a), and $g(n_1, n_2)$, as shown in Fig. 3(b), for $0 \leq n_1 < N_1/4$ and $0 \leq n_2 < N_2/4$, as shown in Fig. 4(a). The maximum is at $r_{r-g} = -3.75$. Figure 4(b) shows $r_{r-b}()$, the cross-correlation function of the absolute differentials between $r(n_1, n_2)$ and $b(n_1, n_2)$. The maximum is at $r_{r-b} = -6.25$. The cross-correlation delays for the image are computed as $r_{r-g} = -3.75$ between red and green,

and $r-b=-6.25$ between the red and blue differentials. Because the red has the longest wavelength and the blue has the shortest, many objects with sharp edges have an extra thin red area along one side and a blue area on the other side as shown in Fig. 2(a). The restored image obtained from the Eqs (3)–(6) is shown in Fig. 2(b), in which the false red and blue areas due to the transverse chromatic aberrations of the lens are corrected and suppressed.

Figure 5 shows the comparison of distributions between the original and restored images. Figure 5(a) and (a') are the joint distributions between red on the vertical axis and green on the horizontal axis of the original and restored images, respectively. The distribution in (a') is more concentrated because of the mismatch of colours that spreads out the vectors from the resulting clusters and are thus corrected. Similar results are shown in (b) and (b') for the vector distributions between the red and green components of the original and restored images, respectively, as well as in (c) and (c') for the distributions between the green and blue colour components of the original and restored images, respectively.

To quantitatively evaluate the concentrations, the entropy is computed for each distribution in Fig. 5. Entropy is a measure of the information content of a signal (Beirlant *et al.*, 1997; Wu & Barba, 1998). Although higher entropy is associated with randomness or uncertainty, lower entropy is an expression of simplicity or certainty. Those distributions having a wide spread of vectors due to lateral colours may in fact have larger entropies. Entropy is reduced by moving the stray vectors back to the clusters where they belong.

The entropy is estimated based on the histograms, $f(l_1, l_2)$, for $l_1 = 0, 1, \dots, 255$ and $l_2 = 0, 1, \dots, 255$, by $H = - \sum_{l_1=0}^{255} \sum_{l_2=0}^{255} f(l_1, l_2) \log f(l_1, l_2)$. Table 1 has listed the estimated entropies from the histograms in Fig. 5 and shows that the restored image has lower entropies than the original image for each and every comparison.

Figure 6 shows three additional pairs of highly magnified images placed side-by-side for easier comparison. On the left are three original images with marked colour aberrations in the obvious blue and red areas that appear on the opposite sides of cells and other small objects; on the right panel are their respective restored images with less intense chromatic aberrations. Comparing the blue areas denoted by black arrows and the red areas denoted by the white arrows in the original images as well as those areas denoted by the corresponding arrows, it is shown that the chromatic aberrations are markedly reduced.

For easier comparison, we demonstrate in Fig. 7 the results of an image of simple lines. Figure 7(a) is an original image of size 256×256 from a calibration slide with scale lines. The image is the upper left corner portion of a larger image of size 1600×1200 . Figures 7(b) and (c) show the restorations of the proposed algorithm and the manual method, respectively. In the manual method, the restoration is achieved by selecting the best result from the manual shifting of the colour components. It is observed from Figs 7(b) and (c) that the areas pointed to by the arrows, that both methods can produce comparable results.

Discussions and conclusions

Oblique light leads to transverse chromatic aberration that refers to the sideward focus resulting in an image magnification that is wavelength-dependent. In a microscope, multiple lenses are used to compensate for the chromatic aberration. While an ideal optical image-forming system with correct compensation can produce sharp colour images with little chromatic aberration, a microscope with a less than perfect lens may yield images with severe distortions in which the conjugate colour image components are separated transversely. To restore the degraded images from such imperfect microscopes, an algorithm based solely on the acquired images has been devised and is herein presented.

The first step is to calculate the absolute differentials of the colour components. The differential of a colour component is defined as the difference between the component image and its slightly magnified version using an interpolation procedure. The absolute values in the differential component images are generally higher at the edges where higher discontinuities occur. The second step is to compute the two cross-correlation functions of the absolute differentials between red and green colours and between red and blue colours. The maximum in the two cross-correlation functions were sought, respectively, and the cross-correlation delays were then calculated. The third step is to use the two cross-correlation delays to determine the dispersions and to then realign the three colour components. Results of real microscopic images are provided and compared. The estimated entropies are generally lower after the restorations, implying that the vectors are more concentrated after processing.

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Fig. 1.
The proposed algorithm to correct distorted images is presented earlier.

Fig. 2.

Comparison between the original and restored images. Panel (a) is the upper-left corner section (digital size 400×300 , metric size $0.053 \times 0.0397 \text{ mm}^2$) of the original image (size 1600×1200 , metric size $0.2119 \times 0.1589 \text{ mm}^2$) using a Nikon Labophot-2 Light microscope. The image is of a $5 \mu\text{m}$ thick formalin-fixed paraffin-embedded brain section. The areas with sharper edges or discontinuities have extra false red areas (as denoted by white arrows) along boundaries on one side and blue areas (as denoted by the black arrows) along boundaries of the opposite side. Panel (b) is the restored image: the false red and blue colours on the opposite sides of each object in the original image are now less intense.

Fig. 3.

Colour components and their differentials with the small increment of $\Delta = 2$ in the magnifications. Panel (a) is the red component $x_r(n_1, n_2)$, panel (b) represents the green component $x_g(n_1, n_2)$ and panel (c) the blue component $x_b(n_1, n_2)$; panel (a) is the absolute of the red differential, $|x_r(n_1, n_2)|$, panel (b) is the absolute of the green differential, $|x_g(n_1, n_2)|$ and panel (c) is the absolute of the blue differential, $|x_b(n_1, n_2)|$.

Fig. 4.

Panel (a) is the cross-correlation function of the absolute differentials between the red and green colours, maximum at $r_{-g} = -3.75$, whereas panel (b) is the cross-correlation function of the absolute differentials between the red and blue colours, maximum at $r_{-b} = -6.25$.

Fig. 5.

Panels (a)–(c) are the distributions of the paired components of the original colour image in Fig. 2(a): (a) red (vertical axis)—green (horizontal axis), (b) red—blue and (c) green—blue; (a)–(c) Distributions of the paired components of the restored image in Fig. 2(b): (a) red—green, (b) red—blue and (c) green—blue, respectively. The narrower distributions occurring in the restored image imply higher concentrations in the colour space. Their respective entropy estimations are listed in Table 1.

Fig. 6.

Three high magnification comparisons between the original images and their restored ones that are placed side-by-side. On the left are three original images with severe colour aberrations with obvious blue areas (as pointed by black arrows) and red areas (as pointed by white arrows) on opposite sides of cells and other small objects. On the right are their restored images with less intense chromatic aberrations (see the corresponding areas pointed by the black and white arrows).

Fig. 7. Comparison of results on a line image. (a) Original image of simple lines from a calibration scale slide, (b) restored by the proposed algorithm and (c) manually restored. Some areas with larger chromatic aberrations in the original image in (a) are denoted by the black and white arrows. The corresponding areas in (b) and (c) are also denoted by arrows for easier visual comparison.

Table 1

The entropies estimated from the six two-dimensional distributions in Fig. 5, respectively.

		Red-green		Red-blue		Green-blue
Original image	(a)	8.3302	(b)	8.1178	(c)	7.6969
Restored image	(a)	7.9637	(b)	7.6198	(c)	7.5765