Fast Algorithm for Matching of Image Pairs for Constant Brightness Applied to Stacks of Confocal Microscope Images

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Abstract

Fluorescent images captured by a confocal laser scanning microscope (CLSM) from deep layers of a specimen are often darker than images from the top layers due to absorption and scattering of both excitation and fluorescent light. These effects cause problems in subsequent analysis of biological objects. In a previous work [CJK06], we applied the DHW (dynamic histogram warping) algorithm of [CRH95] to brightness matching of CLSM image stacks. The algorithm takes about 3 sec to process one 256 gray-level CLSM slice on a 2.8 GHz CPU. To speed up processing of large series of confocal microscope images we propose an algorithm that executes about 100 times faster than DHW, while it yields comparable image quality. While our focus was on processing of large numbers of microscope images, the fast algorithm is applicable to other brightness matching tasks, such as for disparity map or depth evaluation in stereo vision, or for optical flow estimation in motion.

Categories and Subject Descriptors (according to ACM CCS) I.4.3 [IMAGE PROCESSING AND COMPUTER VISION]: Enhancement---Grayscale manipulation

1. Introduction

Confocal laser scanning microscope (CLSM) is a noninvasive tool that provides the possibility to obtain a series of fluorescent optical sections of a three-dimensional volume, representing an investigated biological specimen, through optical sectioning and scanning of 2D confocal planes.

Image intensities from a CLSM suffer from *light attenuation with depth* caused by light loss due to two main effects—*light aberration* and *photobleaching*. As a result, images captured from deep layers of the specimen are darker than images from superficial layers, which makes subsequent image analysis, segmentation, and 3D visualization of biological objects difficult.

We successfully addressed compensation of the light attenuation with depth in [CJK06] using the dynamic histogram warping algorithm (DHW) of [CRH95] for brightness matching. DHW processing of a single, 256 gray-level CLSM slice takes about 3 sec to on a Pentium 4, 2.8 GHz CPU. Driven by experiences of [CJK06], an idea for an algorithm that performs about 100 times faster than DHW has emerged and been verified recently, and will be presented here.

2. Arguments for an alternative solution.

[CRH95] formulates the problem of brightness matching in terms of *matching grayscale histograms* of two images.

For confocal microscopy, we actually *do not* require an exact *histogram matching*. We need to brighten dark images from a confocal stack, i.e. to make up for *light attenuation* due to varying depth of image layers. Compensation of light attenuation makes an image "equally bright" as some reference image. By equal brightness of two images we understand that the numbers of pixels having brightness values between *any* two levels b_1, b_2 should be approximately the same in both images. This can be achieved without exact matching of all histogram bins, which is anyway impossible to do when an image's brightness range needs to be stretched. We propose to *match brightness distribution* of an image over *all brightness ranges* instead.

3. Reformulation of the brightness matching problem.

For the reasons explained in the second paragraph, we will *reformulate the brightness matching problem* in terms of a quantitiy relevant to visual perception, i.e *brightness distribution* in an image, rather than in terms of a visually imperceivable statistical quantity providing only pointwise brightness information – the histogram.

For grayscale images we require the following:

- for *any* two different grayscale levels *k, l*, the number of pixels with intensities between the two levels *k, l* in the first image I_1 should *match* the number of pixels in the same intensity range of the second image, I_2
- brightness matching should *preserve the relative brightness* of the pixels, i.e. if in the *original image* I pixel p_1 is **brighter** than pixel p_2, then, in the *brightness-matched image* $\bar{I}$, the brightness-matched pixel $\bar{p}_1$ should be **brighter** or at least equally bright as brightness-matched $\bar{p}_2$.

Definition 1. Brightness matching of images.

We will say that the brightness of an image $\bar{I}$ *matches the brightness* of an image I_{ref} *within the resolution of the distribution function* S_{ref}, if, for ***any*** two brightness levels $\bar{b}_1, \bar{b}_2$, the number of pixels of $\bar{I}$ whose brightness lies between $\bar{b}_1, \bar{b}_2$, differs from the number of pixels of the reference image, I_{ref} whose intensities lie between $\bar{b}_1, \bar{b}_2$, by no more than the sum of two histogram values of I_{ref} at, or neighboring to, $\bar{b}_1, \bar{b}_2$.

Definition 2. Preservation of relative brightness. Let I be an image. Let $b(p_i) \in \{0,1,...b_{\max}\}$ be the brightness of a pixel p_i in I. We'll say that a *brightness mapping* $f: I \to \bar{I}$ defined by:

$$\forall p \in I : \bar{b}(p) = g(b(p)) \qquad (3.1)$$

$\bar{b}(p_i) \in \{0,1,...b_{\max}\}$, *preserves relative image brightness*, if for any two pixels $p_1, p_2 \in I$ in the original image, and the homologous pixels $p_1, p_2 \in \bar{I}$ the following implications hold true:

$$\begin{aligned} b(p_1) < b(p_2) &\Rightarrow \bar{b}(p_1) \le \bar{b}(p_2) \\ b(p_1) = b(p_2) &\Rightarrow \bar{b}(p_1) = \bar{b}(p_2) \\ b(p_1) > b(p_2) &\Rightarrow \bar{b}(p_1) \ge \bar{b}(p_2) \end{aligned} \qquad (3.2)$$

Definition 3. Distribution function.

Let $h = \{h, h_1, ..., h_{b_{\max}}\}$ be the numbers of occurrence of respective grayscale values $\{0,1,...b_{\max}\}$. We define the *distribution function of an image* as a mapping $S : b \in \{0,1,...b_{\max}\} \to \{0,1,...,n_{pixels}\}$ such that

$$S(b) = \sum_{\substack{i \\ i \le b}} h_i \qquad (3.3)$$

Obviously, the distribution function is *monotonic* non-decreasing. From here on, we will assume that the reference image and the image being matched are the same size. This implies that the respective distribution functions *have the same final values*. These two properties greatly simplify the brightness matching task: for any value of *S,* we simply need to shift the corresponding brightness value until the value of *S* coincides with the value of S_{ref}.

Definition 4. Brightness matching of distribution functions.

Let S_{ref} be an image distribution function,

$$S_{ref} : b \in \{0,1,...b_{\max}\} \to \{0,1,...,n_{pixels}\}$$

Define an auxiliary boundary value $S_{ref}(-1) = 0$.

Let S be an image distribution function. We define a *brightness mapping g* of the image distribution function S onto S_{ref}, $g : b \to \bar{b}$, $b, \bar{b} \in \{0,1,...b_{\max}\}$ by the relationships

$$\begin{aligned} S(b) = S_{ref}(b) &\Rightarrow g(b) = b \qquad (3.4) \\ S(b) < S_{ref}(b) &\Rightarrow g(b) = b_{ref} : S_{ref}(b_{ref} - 1) < S(b) \le S_{ref}(b_{ref}) \\ S(b) > S_{ref}(b) &\Rightarrow g(b) = b_{ref} : S_{ref}(b_{ref}) \le S(b) < S_{ref}(b_{ref} + 1) \end{aligned}$$

Theorem 1. Let I_{ref} be an image with the image distribution function S_{ref}, I an image with the distribution function S, and $\bar{I}$ the map of I defined by (3.1), (3.4), with the image distribution function $\bar{S}$.

Let $\bar{b}_1 = g(b_1), \bar{b}_2 = g(b_2)$ be arbitrary brightness values mapped according to (3.4).

Let $N_{ref}(b_{1ref}, b_{2ref}) = S_{ref}(b_{2ref}) - S_{ref}(b_{1ref})$ and $\bar{N}(\bar{b}_1, \bar{b}_2) = \bar{S}(\bar{b}_2) - \bar{S}(\bar{b}_1)$ be numbers of pixels with brightness values in the half-open interval $(b_{1ref}, b_{2ref}\rangle = (\bar{b}_1, \bar{b}_2\rangle$ in the reference and mapped image I_{ref}, $\bar{I}$, respectively. Then

A. the brightness of the mapped image $\bar{I}$ with the distribution function $\bar{S}$ matches the brightness of the image I_{ref} within the resolution of S_{ref} as defined by Definition 1.

B. The matching error between $\bar{N}(\bar{b}_1, \bar{b}_2) = \bar{S}(\bar{b}_2) - \bar{S}(\bar{b}_1)$, the number of pixels in a brightness range of the *matched* image, and $N_{ref}(b_{1ref}, b_{2ref}) = S_{ref}(b_{2ref}) - S_{ref}(b_{1ref})$, the number of pixels in the corresponding brightness range of the *reference* image, is bounded by the following inequalities:

1. $S(b_1) < S_{ref}(b_1)$, $S(b_2) < S_{ref}(b_2)$:
$N_{ref}(b_{1ref}, b_{2ref}) \le \overline{N}(\overline{b}_1, \overline{b}_2) < N_{ref}(b_{1ref}, b_{2ref}) + h_{b_{1ref}} + h_{b_{2ref+1}}$

2. $S(b_1) > S_{ref}(b_1)$, $S(b_2) < S_{ref}(b_2)$:
$N_{ref}(b_{1ref}, b_{2ref}) - (h_{b_{1ref+1}} + h_{b_{2ref-1}}) \le \overline{N}(\overline{b}_1, \overline{b}_2) < N_{ref}(b_{1ref}, b_{2ref})$

3. $S(b_1) = S_{ref}(b_1)$, $S(b_2) < S_{ref}(b_2)$:
$N_{ref}(b_{1ref}, b_{2ref}) - h_{b_{2ref}} \le \overline{N}(\overline{b}_1, \overline{b}_2) < N_{ref}(b_{1ref}, b_{2ref})$

4. $S(b_1) = S_{ref}(b_1)$, $S(b_2) = S_{ref}(b_2)$: $\overline{N}(\overline{b}_1, \overline{b}_2) = N_{ref}(b_{1ref}, b_{2ref})$

5. $S(b_1) = S_{ref}(b_1)$, $S(b_2) > S_{ref}(b_2)$:
$N_{ref}(b_{1ref}, b_{2ref}) \le \overline{N}(\overline{b}_1, \overline{b}_2) < N_{ref}(b_{1ref}, b_{2ref}) + h_{b_{2ref+1}}$

6. $S(b_1) < S_{ref}(b_1)$, $S(b_2) < S_{ref}(b_2)$:
$N_{ref}(b_{1ref}, b_{2ref}) - h_{b_{2ref-1}} \le \overline{N}(\overline{b}_1, \overline{b}_2) < N_{ref}(b_{1ref}, b_{2ref}) + h_{b_{1ref-1}}$

7. $S(b_1) < S_{ref}(b_1)$, $S(b_2) = S_{ref}(b_2)$:
$N_{ref}(b_{1ref}, b_{2ref}) \le \overline{N}(\overline{b}_1, \overline{b}_2) < N_{ref}(b_{1ref}, b_{2ref}) + h_{b_{1ref-1}}$

8. $S(b_1) > S_{ref}(b_1)$, $S(b_2) = S_{ref}(b_2)$:
$N_{ref}(b_{1ref}, b_{2ref}) - h_{b_{1ref+1}} \le \overline{N}(\overline{b}_1, \overline{b}_2) < N_{ref}(b_{1ref}, b_{2ref})$

9. $S(b_1) > S_{ref}(b_1)$, $S(b_2) > S_{ref}(b_2)$:
$N_{ref}(b_{1ref}, b_{2ref}) - h_{b_{1ref+1}} \le \overline{N}(\overline{b}_1, \overline{b}_2) < N_{ref}(b_{1ref}, b_{2ref}) + h_{b_{2ref+1}}$

End of Theorem 1.

Sketch of proof: The proof relies on the definition of the brightness matching (3.4). To save space, the full proof will not be presented here.

Theorem 2. Let S_{ref} be an image distribution function, and I an image with the pertaining image distribution function S. The image mapping

$$f : I \to \bar{I}$$

defined by (3.1), (3.4), preserves relative image brightness. *End of Theorem 2.*

Sketch of proof: It suffices to proof that implications (3.2) hold true for any two *adjacent* brightness values, $b_i, b_i + 1$:

$$g(b_i) \le g(b_i + 1) \tag{3.5}$$

i.e. the brightness mapping g must prove to be monotonic non-decreasing. The claim (3.2) then holds for *any* brightness values by transitivity. The cases $S(b_i) >=< S_{ref}(b_i)$ and $S(b_i + 1) >=< S_{ref}(b_i + 1)$ may be arbitrarily combined in natural scenes, which yields 9 possible combinations. The rest of the proof relies once more on the definition of the brightness mapping (3.4). To save space, the proof will not be presented here at full length.

4. Implementation in MATLAB and ANSI C.

We implemented the algorithm as a target-independent ANSI C function. To allow use under MATLAB, we also provided a MATLAB wrapper, which – when compiled together with the algorithm using MATLAB's mex-command, can be called from anywhere in MATLAB.

The C implementation of the algorithm needs some 0.02 sec to match a single pair of 512x512 8 bit images, which is about 100 times faster than the DHW performance.

5. Results of brightness matching of images from confocal microscopy.

Some results of the fast algorithm tested on two different confocal image stacks consisting of 42 and 57 images, respectively, are presented in Fig.1 and Fig.2.

6. Extension for an arbitrary reference distribution function.

The formulation of Theorem 2 only assumes matching of an image to a *reference distribution function*, not to *another image*. The reference can therefore be constructed artificially, without a particular reference image, using a much more general algorithm, as we did e.g. in [CJK06].

7. Conclusions.

We presented a new problem formulation of brightness matching of image pairs in terms of matching of *brightness level ranges*, rather than in terms of matching *individual histogram values*. We proposed a fast algorithm to solve our brightness matching formulation.

For our algorithm, we provided an error bound on the brightness-matching error between a matched image and the reference image.

We proved that our brightness matching algorithm preserves the relative brightness in the matched image.

Empirically, we have shown that a C implementation of our algorithm executes about 100 times faster than the DHW algorithm we used previously.

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References

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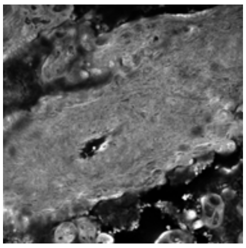
[CRH95] COX I., ROY S., HINGORANI S.: Dynamic Histogram Warping of Image Pairs for Constant Image Brightness, *IEEE Int. Conf. on Image Processing,* (1995), `http://www.adastral.ucl.ac.uk/~icox/`



reference image

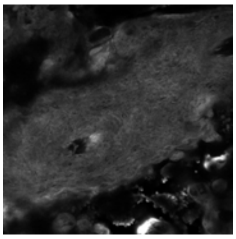


image #20 from the confocal stack **before** brightness matching

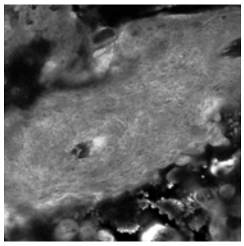


image #20 from the confocal stack **after** brightness matching

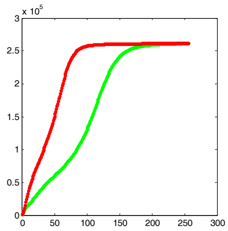


image distribution functions of image #20 (red) and reference image (green) **before** brightness matching

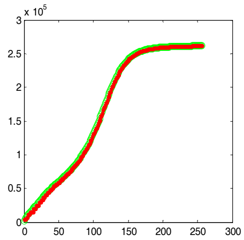
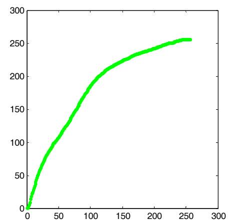


image distribution functions of image #20 (red) and reference image (green) **after** brightness matching

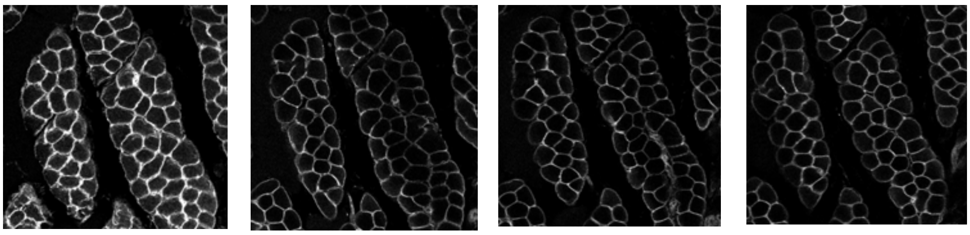


monotonic brightness mapping function g defined by (3.4) for image #20 and reference image

Figure 1: *Brightness matching of an image pair showing the original matched distribution functions, as well as the brightness mapping function g.*

reference image | image #25 | image #40 | image #57

images **before** brightness matching



images **after** brightness matching

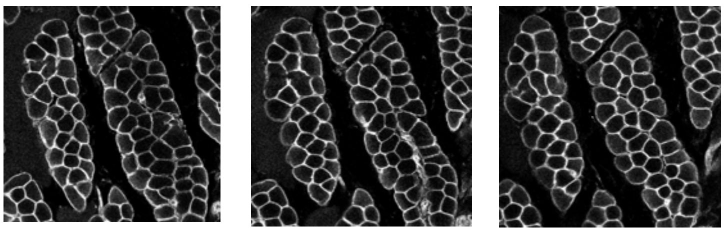


Figure 2: *Brightness matching of a series of images pair showing the original matched distribution functions, as well as the brightness mapping function g.*