Compensation of the Light Attenuation with Depth of Images Captured by a Confocal Microscope Using a MRF Deformation Model and Graph Cuts

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Abstract

We study series of fluorescent optical sections, i.e. three-dimensional (3D) biomedical images, captured by a confocal laser scanning microscope (CLSM). Fluorescent image intensities of optical sections from deep layers of the specimen are often darker than intensities of sections from the top layers due to absorption and scattering of both excitation and fluorescent light. To solve this problem we apply a Markov Random Field (MRF) model – including an efficient deformation model for tracking structures within the 3D images – for computation of optical flow. We approach the corresponding optimization problem by the graph cuts. Image intensities of optical sections are recomputed according to the found optical flow, since the flow gives us evaluation of their proper brightness. Finally, the light attenuation with depth is compensated by matching accumulative histograms of optical sections of the original series with respect to optical sections improved by the optical flow. By this approach we obtain an algorithm that is less sensitive to changes of structures within series (especially to their enlargement and diminishing) than algorithms based purely on histogram matching, warping or equalization.

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

1. Introduction

We apply a confocal laser scanning microscope (CLSM) for obtaining a series of fluorescent optical sections, i.e. a 3D digital representation, of a biological specimen through optical sectioning and scanning of 2D confocal planes.

Intensities of optical sections captured by a CLSM are often corrupted by light aberration and photobleaching, which results in the light attenuation with depth [DIA02]. As a consequence, images captured from deep layers of the specimen may be darker than images from top layers. This effect makes subsequent image analysis, segmentation, and 3D visualization of biological objects difficult.

We have developed a method for compensation of the light attenuation with depth using the dynamic histogram warping algorithm [CJK06]. However, this approach, similarly like approaches based on histogram matching or equalization, is less suitable for brightness compensation when depicted structures change within series of optical sections (especially, when the structures enlarge or diminish). In this case either the brightness compensation is insufficient or the amount of noise in images is undesirably increased.

We propose an alternative method for compensation of the light attenuation with depth. The method is inspired by recent development of models based on the Markov Random Fields (MRF), and development of global optimization algorithms, e.g. [KZ04].

The method estimates optical flow through the series of optical sections using an MRF deformation model [SKH07]. This model handles occlusions and deformations of structures caused by their changes among optical sections. The corresponding optimization problem is addressed by graph-cuts [BVZ01], [BK04].

By using the estimation of optical flow new intensity values of pixels in images of optical sections are computed. Since optimizing energies of MRF models represents an NP-complete problem, graph-cuts methods produce solutions which are close to the optimal ones. Thus the pixel intensities corrected by the optical flow may demonstrate discrepancies and partial corruption of structures.

Therefore, the light attenuation with depth is compensated by matching accumulative histograms of optical sections of the original series to accumulative histograms of optical sections improved by the optical flow. This approach gives us both improving global brightness of structures through estimation of optical flow and, at the same time, the preservation of local intensity patterns through subsequent matching of accumulative histograms.

The effectiveness of the method is demonstrated both on synthetic data, and real confocal data sets of terminal villus of human placenta.

2. Deformation model

In this section we describe a MRF model for the 2-dimensional deformation as proposed in [SKH07].

2.1. Energy minimization

Let $\mathcal{L} = \{1 \dots K\}$ be a set of labels. Let $G = (\mathcal{V}, \mathcal{E})$ be a graph with $\mathcal{E} \subseteq \mathcal{V} \times \mathcal{V}$ antisymmetric and antireflexive, i.e. $(s,t) \in \mathcal{E} \Rightarrow (t,s) \notin \mathcal{E}$. In the following we will denote by st an ordered pair $(s,t) \in \mathcal{E}$. Let each graph node $s \in \mathcal{V}$ be assigned a label $x_s \in \mathcal{L}$ and let a *labeling* be defined as $\mathrm{x} = \{x_s | s \in \mathcal{V}\}$. Let $\{\theta_s(i) \in \mathbb{R} | i \in \mathcal{L}, s \in \mathcal{V}\}$ be *univariate* potentials and $\{\theta_{st}(i,j) \in \mathbb{R} | i,j \in \mathcal{L}, st \in \mathcal{E}\}$ be *pairwise* potentials. Let *energy* of a labeling x be defined by:

$$E(\mathrm{x}|\theta) = \sum_{s\in\mathcal{V}} \theta_s(x_s) + \sum_{st\in\mathcal{E}} \theta_{st}(x_s, x_t), \quad (1)$$

where $\theta_s(\cdot)$ is also referred to as a *data term* an $\theta_{st}(x_s, x_t)$ as a *pair wise interaction term*. The probability distribution defined by $p(\mathrm{x}|\theta) \propto e^{(-E(\mathrm{x}|\theta))}$ is a Gibbs distribution, corresponding to a certain Markov Random Field. The problem of finding maximum a posteriori (MAP) configuration of this MRF corresponds to *energy minimization*: $\min_{\mathrm{x}} E(\mathrm{x}|\theta)$.

2.2. Product model

Let T^1, T^2 be sets of pixels and let $I^1: T^1 \mapsto [0,255]$, $I^2: T^2 \mapsto [0,255]$ be two greyscale images. Let labeling x with components $x_s = (x_s^1, x_s^2)$, $s \in \mathcal{V} = T^1$, be a 2D displacement field over T^1. Coordinates x_s^1 and x_s^2 denote x- and y- displacements of the pixel s. Let mapping D_x from image I^2 to image I^1 be defined by $(D_{\mathrm{x}} I)_s = I_{s+x_s}$. Let both coordinates take values from $\mathcal{L} = \{K_{\min}, \dots, K_{\max}\}$, thus variables $x_s = (x_s^1, x_s^2)$ take their values from the set $\mathcal{L}^2$.

Let $\theta_s(x_s) = (I_s^1 - I_s^2)^2 / 2\sigma_I^2$. This term corresponds to the statistical assumption of $p((D_{\mathrm{x}} I^2)_s - I_s^1 | \mathrm{x}) \propto \mathcal{N}(0, \sigma_I^2)$, $s \in \mathcal{V}$, which means that the deformed image $D_{\mathrm{x}} I^2$ is a noisy observation of image I^1 under fixed x assuming that Gaussian noise has variance σ_I^2. The usual setting for the interaction potential is

$$\theta_{st}(x_s, x_t) = \|x_s - x_t\|^2 / 2\sigma_I^2 = (x_s^1 - x_t^1)^2 / 2\sigma_I^2 + (x_s^2 - x_t^2)^2 / 2\sigma_I^2, st \in \mathcal{E}, \quad (2)$$

where $\mathcal{E}$ is the set of all horizontally and vertically neighbouring pairs of pixels. This term penalizes discontinuities in the deformation field x, so that close pixels are forced to go to close destinations (see Figure 1).

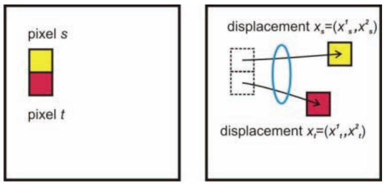

Figure 1: *Deformation model using MRF: data terms penalize colour deviation of single pixels from hypothesized destination, pair wise terms penalize spatial deviations.*

2.3. Block model

A drawback of the product model is that the interaction term $\|x_s - x_t\|^2 / 2\sigma_I^2$ assigns a nonzero penalty also to affine transformations. Especially, scaling is poorly handled. However, we need the deformation field to be locally affine, so that optical flow, corresponding to increasing (diminishing) objects, can also enlarge (diminish). Since discrete models are considered, it is possible to describe the deformation field locally by translations. In [SKH07] it is proposed to group pixels in blocks, and allow each block to have pixel wise displacements, see Figure 2.

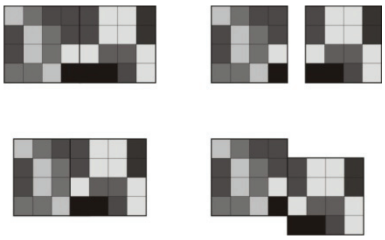

Figure 2: *Block model: left-top – two neighbouring blocks of 4×4 pixels; other images – examples of relative displacements. There are nine pixel wise relative displacements in total.*

Let the set T^1 be regularly subdivided into square blocks and let B be a set of these blocks. We assume the horizontal and vertical neighbourhood of the blocks. We let $\mathcal{V} \sim B$ and define the data term as

$$\theta_{st}(x_s, x_t) = \mathrm{Sim}\left(I_s^1, I_{s+(x_s,x_t)}^2\right)^2, \quad s,t \in \mathcal{V},\ st \in \mathcal{E}, \quad (3)$$

where I_s^1 is a fragment of image I^1 on block s; $s + (x_s, x_t)$ is a block s shifted by (x_s, x_t), and $\mathrm{Sim}(\cdot,\cdot)$ is the sum of squared differences across pixels of the corresponding image fragments. For all $st \in \mathcal{E}$ we set the interaction term to

$$\theta_{st}(x_s, x_t) = \begin{cases} 0, & x_s = x_t, \\ c, |x_s - x_t| \leq N, \\ \infty, |x_s - x_t| > N, \end{cases} \quad (4)$$

where $0 < c \ll 1/\sigma_I^2$ is a regularization term, and N is maximum allowed displacement of image fragments. The set of transformations with low penalty (less or equal to c) incorporates a certain range of affine transformations (e.g., it includes scale changes in the range 0.75–1.25, when blocks are 4×4 pixels and $N = 1$).

3. Graph cuts – ∝-expansion algorithm

For optimization of the above described deformation model we apply the ∝-expansion algorithm [BVZ01]. It is an iterative graph cut-based technique that gives good solutions in practice.

Any labeling x can be uniquely represented by a partition of graph nodes $\mathrm{P} = \{\mathcal{P}_f | f \in \mathcal{L}\}$, where $\mathcal{P}_f = \{s \in \mathcal{V} | x_s = f$ is a subset of nodes which are assigned the label *f*. There is an obvious one to one correspondence between labelings x and partitions P.

Given a label ∝, a move from a partition P (labeling x) to a new partition P' (labeling x') is called an ∝-*expansion* if $\mathcal{P}_\propto \subset \mathcal{P}'_\propto$ and $\mathcal{P}'_f \subset \mathcal{P}_f$ for any label $f \neq \propto$. In other words, an ∝-expansion move allows any set of nodes to change the labels to ∝. The structure of the ∝-expansion algorithm is shown in Table 1.

1. Start with an arbitrary labeling x.
2. Set success ≔ 0
3. For each label $\propto \in \mathcal{L}$
    - 3.1. Find $\mathrm{x}' = \arg\min E(\mathrm{x}')$ among x' within ∝-expansion of x
    - 3.2. If $E(\mathrm{x}') < E(\mathrm{x})$, set $\mathrm{x} := \mathrm{x}'$ and success ≔ 1
4. If success = 1 goto 2
5. Return x

Table 1: *∝-expansion algorithm.*

The key step is 3.1. The algorithm is looking for the best labeling, but the size of the move space grows exponentially with the number of nodes, i.e. the number of image blocks in our case. Here, the graph cuts come in, and are applied. By the ∝-expansion algorithm one can reformulate the problem of finding the optimal labeling as a set of binary labeling problems that are solvable by graph-cuts [BVZ01].

4. Intensity matching of images using accumulative histograms

We will say that the intensity of an image I^1 *matches the intensity* of an image I^{ref} *within the resolution of an accumulative histogram* H^{ref}, if, for ***any*** two intensity levels b^1, b^2, the number of pixels of I^1 whose intensities lie between b^1, b^2, differs from the number of pixels of the reference image I^{ref}, whose intensities lie between b^1, b^2, by no more than the sum of two histogram values of I^{ref} at, or neighbouring to, b^1, b^2.

Let $h = \{h^0, h^1, \ldots, h^{b^{max}}\}$ be the numbers of occurrence of respective greyscale values $\{0,1,\ldots,b^{max}\}$. We define the *accumulative histogram of an image* as a mapping $H: b \in \{0,1,\ldots,b^{max}\} \to \{0,1,\ldots,n^{pixels}\}$ such that

$$H(b) = \sum_{i,i\leq b} h^i. \quad (5)$$

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

Let H^{ref} be a reference image accumulative histogram, $H^{ref}: b \in \{0,1,\ldots,b^{max}\} \to \{0,1,\ldots,n^{pixels}\}$. Define an auxiliary boundary value $H^{ref}(-1) = 0$. Let H be an image accumulative histogram. We define an *intensity mapping g* of the accumulative histogram H onto H^{ref}, $g: b \to b'$, $b, b' \in \{0,1,\ldots,b^{max}\}$ by the relationships

$$H(b) = H^{ref}(b) \Rightarrow g(b) = b,$$
$$H(b) < H^{ref}(b) \Rightarrow g(b) = b': H^{ref}(b'-1) < H(b) \leq H^{ref}(b'), \quad (6)$$
$$H(b) > H^{ref}(b) \Rightarrow g(b) = b': H^{ref}(b') \leq H(b) < H^{ref}(b'+1).$$

5. Description of our light attenuation compensation algorithm

The structure of the resulting algorithm is shown in Table 2.

1. Choose a reference image within the series of optical sections.
2. Compute optical flow among the reference image and optical sections using approaches described in Sections 2. and 3.
3. Compute new intensity levels in the optical sections according to found optical flows. This step estimates only the resulting brightness of images, but does not necessarily provide correct intensities of depicted structures due to NP-completeness of the underlying problem.

4. Using a method described in Section 4. compute resulting intensity levels by intensity matching of the original series of optical sections with respect to the series of optical sections found in Step 3 of this algorithm.

Table 2: *Light attenuation compensation algorithm.*

We implemented the algorithm in C++ as a plug-in module of the Ellipse software package (www.vidito.com) that is devoted to biomedical image processing and visualization.

6. Results

In this section we show practical results obtained by the above described algorithm. To demonstrate its efficiency we compare it with the pure accumulative histogram matching described in Section 4.

Figure 3 exemplifies applying the light attenuation compensation algorithms to greyscale synthetic data set. The original data are formed by two circles the size of which decreases by 5%, and intensity levels of these circles decrease by 12% of the maximum intensity level between neighbouring images. Gaussian noise to simulate real data was added. When applying the histogram matching only, one can see the increase of noise level and disappearance of the inner circle in deep layers (right images). When the proposed algorithm is used, noise levels of corrected images are lower and the inner circles are better preserved than in the previous case.

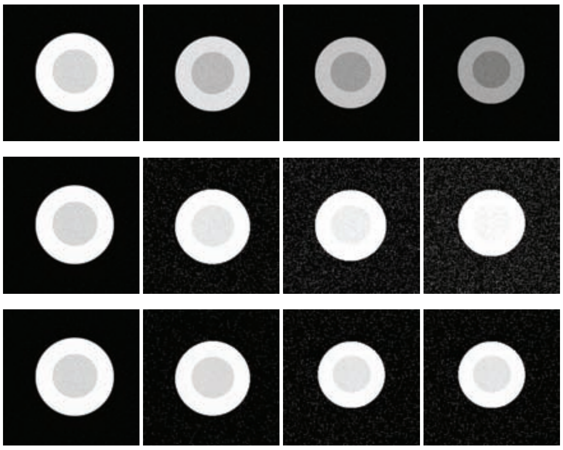

Figure 3: *Example of applying the light attenuation compensation algorithms to greyscale synthetic data set. Upper row: Original data; Middle row: Data set after applying the histogram matching; Bottom row: Data set after applying the described algorithm. In both cases the first image was the reference.*

Figure 4 shows using the algorithms to confocal data set of terminal villus of human placenta. Note the strong light attenuation with depth. When applying the described algorithm, one can see better preserving depicted structures in deep layers (right images) than in the case of histogram matching.

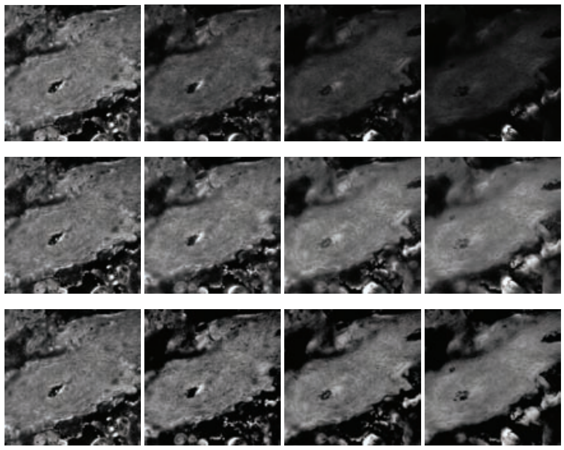

Figure 4: *Example of applying the light attenuation compensation algorithms to confocal data set of terminal villus of human placenta. Upper row: Original data; Middle row: Data set after applying the histogram matching; Bottom row: Data set after applying the described algorithm. In both cases the first image served as the reference.*

7. Conclusions

We presented a new and promising algorithm to compensation of the light attenuation with depth of images captured by a confocal microscope.

Supported by MSM6840770012, LC06063, GACR 102/08/0691.

References

[BK04] BOYKOV, Y., KOLMOGOROV, V.: An Experimental Comparison of Min-Cut/Max-Flow Algorithms for Energy Minimization in Vision. *IEEE PAMI*, 26 (September 2004), 1124–1137.

[BVZ01] BOYKOV, Y., VEKSLER, O., ZABIH, R.: Efficient Approximate Energy Minimization via Graph Cuts. *IEEE PAMI*, 20 (November 2001), 1222–1239.

[CJK06] ČAPEK M., JANÁČEK J., KUBÍNOVÁ L.: Methods for Compensation of the Light Attenuation with Depth of Images Captured by a Confocal Microscope. *Microscopy Research and Technique*, 69 (August 2006), 624–635.

[DIA02] DIASPRO A., editor: *Confocal and Two-Photon Microscopy, Foundation, Applications, and Advances*. New York: Wiley-Liss, Inc, 2002.

[KZ04] KOLMOGOROV, V., ZABIH, R.: What Energy Function Can Be Minimized via Graph Cuts? *IEEE PAMI*, 26 (February 2004), 147–159.

[SKH07] SHEKHOVTSOV, A., KOVTUN, I., HLAVÁČ, V.: Efficient MRF Deformation Model for Non-Rigid Image Matching. In *Proc. IEEE CVPR 2007* (2007), 1–6.