Bayesian Compensated Microscopy

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Abstract

We present a novel Bayesian compensated microscopy (BCM) technique designed for enhancing microscopy image quality. The proposed BCM technique provides a computational approach to jointly compensate for microscopy image degradations due to (1) optical aberrations, (2) illumination non-uniformities, and (3) imaging noise within a probabilistic framework. Experimental results based on a stained pathology sample of spleen tissue with leukemia demonstrate the effectiveness of the proposed BCM technique for the quality enhancement in microscopy imaging. The proposed BCM technique can lead to improved visualization of fine tissue structures as well as a more consistent visualization across the entire sample, which can be beneficial for accurate analysis and better interpretation of microscopy samples.

1 Introduction

Optical microscopy is the most widely used technique for observing and investigating cellular structures and properties of biological samples, and is a crucial tool for a wide range of applications such as pathology, water quality assessment, and neurobiology studies. The overall image quality obtained using optical microscopy is limited by a number of different factors, such as optical aberrations, illumination non-uniformities, as well as the imaging noise due to both the environment and the instrument itself. As such, techniques that can help mitigate or compensate for such image degrading factors can be greatly beneficial to accurate analysis and interpretation of microscopy samples.

A key factor in the degradation of microscopy image quality is the presence of optical aberrations, which reduces the contrast and resolution of microscopy instruments [1]. In general, any deviations of the light wavefront from its optimal shape leads to optical aberration which appears as a blurring or image distortion in acquired microscopy images. In microscopy imaging, different types of aberrations may arise due to: i) properties and design of microscopy system, and ii) the physical properties of the sample being imaged. Compensating for optical aberration is important for improving the functionality of microscopy imaging in different applications. Various hardware solutions have been proposed to mitigate image degradation due to optical aberrations, with adaptive optics [2] and wavefront shaping [3] showing the highest level of success. However, hardware-based aberration compensation methods require significant modifications to the optical microscopy configuration, and as such is complex, costly, and time consuming. As such, computational aberration compensation methods have become increasingly popular as they provide an easier and cheaper solution for reducing the negative effects of optical aberration [4, 5].

Another key factor in the degradation of microscopy image quality is illumination non-uniformities, where the light source used does not illuminate the entire sample evenly, with the most commonly seen example being poor illumination at the peripheries of the sample being imaged. The existence of illumination non-uniformities can negatively impact accurate assessment and analysis of the imaged sample; for example, the same tissue structures may appear differently at different locations in the sample based on the lighting at that location. In optical microscopy, illumination non-uniformity may originate from different sources such as misaligned optics, dust, nonuniform light sources, as well as vignetting. Various prospective and retrospective methods have been proposed for the illumination non-uniformity removal [6, 7]. Prospective methods need a reference image to estimate the correction surface, whereas retrospective approaches attempt to estimate the correction surface directly from the acquired microscopy image.

A third key factor in the degradation of microscopy image quality is imaging noise, which may originate from the instrument itself or the sample environment and condition (e.g., small dirt and dust) and can make it more challenging to proper visualize and interpret microscopy images. As such, a technique that can compensate for all of the aforementioned factors would be greatly beneficial in improving microscopy imaging quality.

In this study, a novel Bayesian compensated microscopy (BCM) technique designed for enhancing microscopy image quality. The proposed BCM technique provides a computational approach to jointly compensate for microscopy image degradations due to (1) optical aberrations, (2) illumination non-uniformities, and (3) imaging noise within a probabilistic framework.

2 Method

The proposed Bayesian compensated microscopy (BCM) technique can be described as follows. Let the formation of a microscopy image be modeled as a general forward problem,

$$g = D(f,h,b) + n \tag{1}$$

which shows the relationship between the measured microscopy image, g, and the compensated microscopy image, f, through a degradation function, D, which jointly accounts for the effect of optical aberration, h, illumination non-uniformities, b, as well as image noise, n. Using the model in Eq. 1, the problem of obtaining the compensated microscopy image f given g, h, and b can be modeled as an inverse problem,

$$f = D^{-1}(g,h,b). \tag{2}$$

Since this inverse problem cannot be solved in an analytical manner, alternative approaches to estimating the compensated microscopy image f is highly desired.

In the proposed BCM technique, let us assume that the measured microscopy image, $G = \{G_s, s \in S\}$, and the compensated microscopy image, $F = \{F_s, s \in S\}$, are random fields in S, with S representing a set of locations in a discrete lattice $\mathcal{L}$, and $s \in S$ denote to a single location in $\mathcal{L}$. Furthermore, let $f = \{f_s, s \in S\}$ and $g = \{g_s, s \in S\}$ be realizations of F and G respectively. To solve the inverse problem of Eq. 2, the goal of proposed BCM technique is to estimate the compensated microscopy image, f, given the measured microscopy image, g, within a Maximum a Posteriori (MAP) framework,

$$\hat{f} = arg\,max_f\, P(f|g), \tag{3}$$

Using Bayes' theorem, Eq. 3 can be reformulated as,

$$\hat{f} = arg\,max_f\, P(g|f)P(f) \tag{4}$$

where $P(g|f)$ is the likelihood and $P(f)$ is the prior. In the proposed BCM technique, based on quantum photon emission statistics and taking optical aberration and illumination non-uniformity into account, the likelihood $P(g|f)$ can be modeled as,

$$P(g|f) = \prod_{s\in S} \frac{(D(f_s,h,b))^{g_s} e^{-(D(f_s,h,b))}}{g_s!} \tag{5}$$

The prior $P(f)$ models f as a nonstationary process with a nonstationary expectation $E(f_s)$ and variance τ^2,

$$P(f) = \prod_{s\in S} e^{-\frac{(f_s - E(f_s))^2}{2\tau^2}} \tag{6}$$

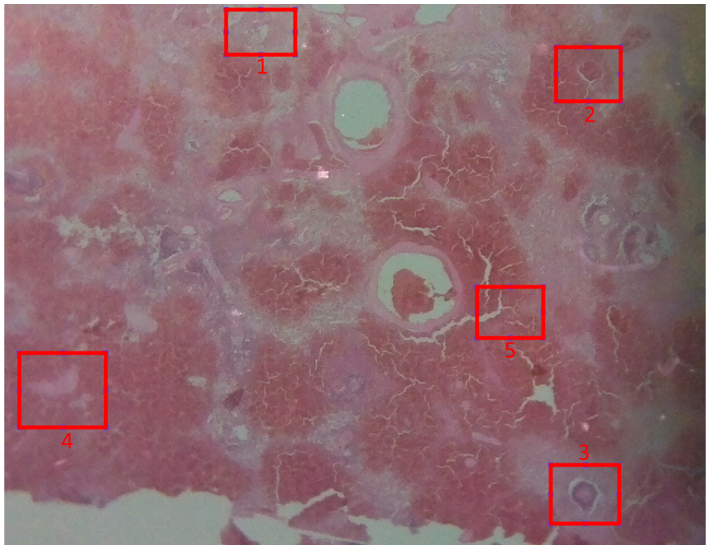
The aforementioned likelihood and prior are then used for maximizing the MAP problem in Eq. 3 to obtain an optimal estimate of the compensated microscopy image f.

3 Results

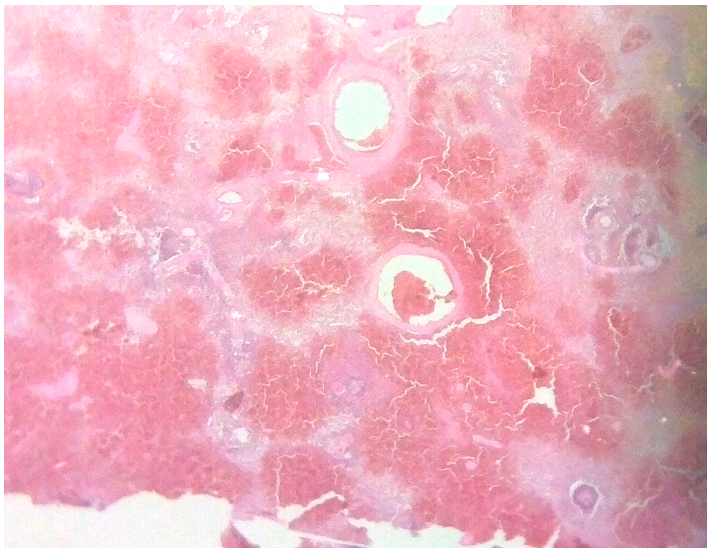
A stained pathology sample of spleen tissue with leukemia was used to evaluate the efficacy of the proposed BCM technique for

the purpose of improving microscopy image quality. The sample was imaged using a custom, compact digital microscopy system consisting of a cemented achromatic doublet lens with a focal length of $10mm$ and a diameter of $8mm$ placed in the optical path of a Raspberry Pi camera module. The spatial resolution of the digital microscope was determined to be $2.76\mu m$ by imaging the 1951 U.S. Air Force (USAF) target. By evaluating the proposed BCM technique on a custom, compact digital microscopy system powered by a Raspberry Pi, one can get a better sense of its ability to enable low-cost, low-power, field-portable microscopy, which would very beneficial for a wide range of applications such as on-the-fly sample analysis for water quality assessment or clinical sample testing in developing countries.

An acquired microscopy image of the spleen tissue with leukemia is shown in Fig.1(a). A number of observations can be made from this acquired microscopy image. First, it can be observed that tissue detail and contrast become increasingly degraded as approach the peripheries of the imaged tissue due to optical aberration. It can also be clearly observed that the acquired image not only appears dark in general, but also exhibits noticeable illumination non-uniformities (most noticeable in the top-right corner). Furthermore, the presence of imaging noise can be observed in the homogeneous regions.



(a) Original



(b) BCM

Fig. 1: (a) Acquired microscopy image of the spleen tissue with leukemia; (b) numerically compensated microscopy image using proposed BCM technique.

The resulted numerically compensated microscopy image using the proposed BCM technique using the same sample is shown in Fig.1(b). As can be seen from this figure, the produced numerically compensated image in Fig.1(b) exhibits a noticeable improvement in both tissue contrast and detail compared to the acquired microscopy image in Fig.1(a). The produced numerically compensated image in Fig.1(b) also appears noticeably brighter and exhibits less illumination non-uniformities when compared to the acquired microscopy image in Fig.1(a), which is most noticeable in the top-right corner and makes it easier to visualize different tissue details and structures consistently across the sample.

To better visualize the improvements gained using the proposed BCM technique, several regions are chosen from different locations (with the regions marked with red squares in Fig.2(a)) and zoomed in for visualization in Fig.2. A number of important observations can be made from the zoomed-in regions. First, it can be seen from the zoomed-in-regions in Fig.2(a,c,e,g) that these areas are appear to be more blurry and with lower contrast in the original image acquisition due to optical aberration. Looking at the zoomed-in regions from the compensated image using BCM, shown in Fig.2(b,d,f,h), it can be observed that there is a noticeable improvement in tissue detail and contrast, with different cellular structures as well as cell boundaries more clearly observable and sharper in the produced numerically compensated image obtained using BCM. Furthermore, it can be observed that the homogeneous regions in the zoomed-in regions of the produced numerically compensated image obtained using BCM exhibit noticeably less imaging noise. As such, these results demonstrate the noticeably improved image quality that can be achieved using the proposed BCM technique, allowing for better and more consistent visualization of fine tissue structures and features of the biological tissue. 1em

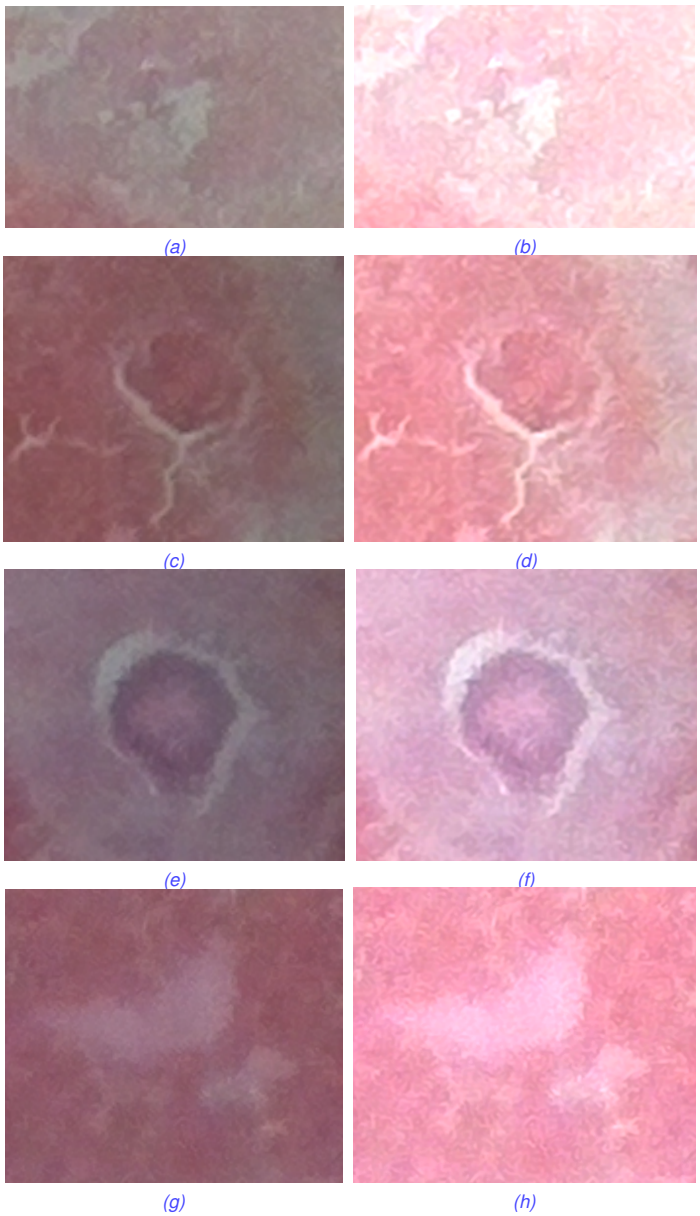
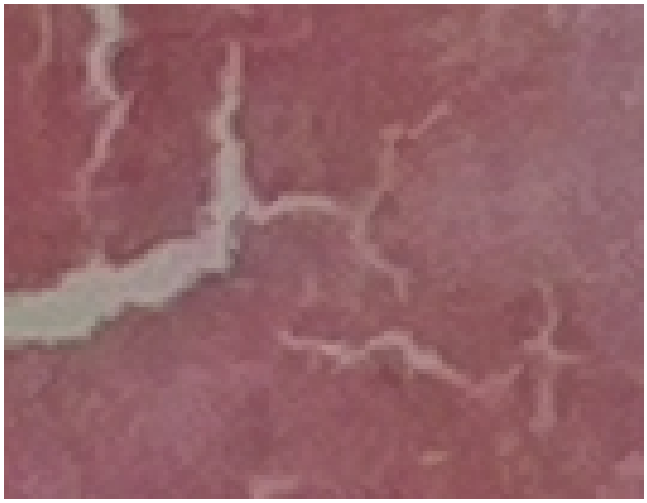
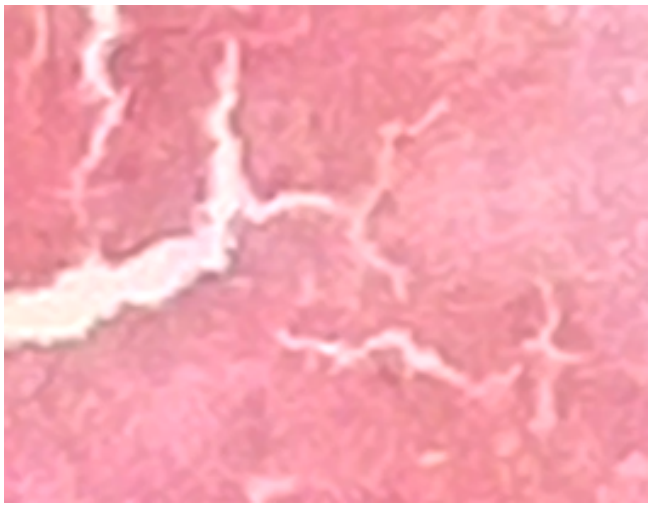


Fig. 2: Zoomed-in regions of the locations marked with red squares in Fig2(a). (a,c,e,g) Zoomed-in regions for the original microscopy image of a spleen tissue with leukemia, (b,d,f,h) Zoomed-in regions for the numerically compensated microscopy image using proposed BCM technique.



(a)



(b)

Fig. 3: Double zoomed-in region for the location marked with the red square #5 in the original microscopy image of Fig. 1(a).

A double zoomed-in region for the location marked with the red square #5 is also shown in Fig. 3 to clearly show the performance of proposed BCM technique in terms of image quality improvement.

4 Conclusion

In this study, we introduced a new Bayesian compensated microscopy technique for enabling enhanced microscopy image quality. The proposed technique takes into account optical aberrations, illumination non-uniformities, and imaging noise within a Maximum a Posteriori framework to obtain the best estimate of the compensated microscopy image. Experimental results show that the proposed technique can produce compensated microscopy images with noticeably improved tissue contrast and detail, greater visual consistency across the imaged sample, as well as reduced presence of imaging noise. Given that this technique does not require any hardware modifications, it can be a potentially powerful tool for enabling the proliferation of low-cost, low-power, field-portable microscopy in a wide range of applications.

Acknowledgments

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