High dynamic range microscope imaging based on exposure bracketing in full field OCT

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By applying the proposed high dynamic range (HDR) technique based on exposure bracketing, we demonstrate a meaningful reduction in the spatial noise in image frames acquired with a CCD camera so as to improve the fringe contrast in full field optical coherence tomography (FF-OCT). This new signal processing method thus allows improved probing within transparent or semi-transparent samples. The proposed method is demonstrated on 3 μm thick transparent polymer films of Mylar, which due to their transparency, produce low contrast fringe patterns in white-light interference microscopy. High resolution tomographic analysis is performed using the technique. After performing appropriate signal processing, resulting XZ sections are observed. Sub- μm sized defects can be lost in the noise which is present in images acquired with a CCD camera. With the proposed method, we show that by increasing the signal to noise ratio of the images, sub- μm sized defect structures can thus be detected. © 2015 Optical Society of America

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In microscopy, it is generally possible to control the type of illumination used. Goodman compared coherent and incoherent imaging according to several criteria (Ref. [1]). Two-point resolution is a criterion which has long been used as a quality factor to measure the ability of an optical system to resolve two very close point sources. But other effects of the system can also be considered in order to evaluate the quality. For example, the observation of a sharp edge is better for an incoherent imaging system than for a coherent system since for the coherent case, pronounced “ringing” due to diffraction appears on the edge. In addition, the use of coherent illumination is hampered by the appearance of speckle noise or diffraction patterns due to the fact that they are sensitive to optical imperfections such as small dust particles on the optics. However, the contrast of the image intensity distribution for coherent illumination is better than that for the incoherent case

which is relatively low. In interferometry, the contrast is an important factor since the phase information appears in the form of interference fringe patterns consisting of bright and dark fringes. For a given camera exposure time in which the details in dark fringes are visible, information is lost in the bright fringes that are overexposed. Conversely, for a given exposure time in which the bright fringes are not saturated, the dark fringes can be underexposed and information can likewise be lost in the noise. Many signal processing methods exist to decrease noise in images (Ref. [2]). One method consists of improving the contrast by increasing the dynamic range of the images, which using modern cameras is typically limited to 256 grey levels. The high dynamic range (HDR) technique is well-known in the field of photography. In microscopy, the HDR technique has been used in infrared imaging to enhance details on samples (Ref. [3], [4]). One of the HDR techniques consists in acquiring 8-bit depth images of a static scene with a camera using different exposure times, and then combining them to obtain a 10-bit or 12-bit depth image.

An HDR technique has been proposed for interferometry using coherent illumination in a Mach-Zehnder setup (Ref. [5]). In the field of microscopy, the contrast is generally better using coherent illumination (Ref. [1]). When applied to incoherent illumination, the HDR technique allows an improvement in contrast by increasing the bit depth of an image. The enhancement of the signal to noise ratio (SNR) can enable the detection of low contrast structures that are initially unobservable. In addition, this technique is useful for the observation of bright samples such as metallic structures because of their high reflectivity, or of transparent samples where the contrast is particularly low (Ref. [6]).

In this letter, an HDR technique for FF-OCT based on exposure bracketing is proposed, and illustrated by results obtained on 3 μm thick transparent polymer films of Mylar. This material is currently used as an insulator in electronics and microelectronics, as a material for cardiovascular prostheses and for plastic surgery and reconstructive ligament surgery. In all these applications it is important to be able to characterize the defects in the film as they have a large influence on the structural properties. We demonstrate that by using a combination of several images acquired with different exposure times, the dynamic range of the images can be increased, thus improving the SNR and the image contrast, leading to the detection of sub- μm details.

In the field of photography, the bracketing technique consists in acquiring several images of the same scene by using different camera settings and combining them in post processing. Bracketing is an efficient and useful way to obtain an adequate resulting image when it is laborious to define the right camera parameters to acquire the image

with a single shot. As the principle of the method resides in several acquisitions, it is commonly used for static situations. Bracketing can be performed in different ways by modifying various camera parameters, such as the exposure time, the gain or the white balance. However the technique most generally used is exposure bracketing (Ref. [7]). In photography, exposure defines the quantity of light per unit area reaching the sensor and is measured in lux seconds. It can be controlled by setting the shutter speed, the lens aperture and the scene illumination. As camera sensors have a limited exposure range or dynamic range (8, 10 or 12-bit depth), if the exposure is outside this range, the acquired image cannot be recorded accurately. Indeed, for an underexposed image details can be lost in some areas and conversely an overexposed image can contain saturated areas. It is sometimes difficult to find the right exposure adjustment for bright objects such as metallic objects in order for them to remain within sufficient range. The shutter speed or exposure time of a camera is defined as the length of time the shutter is open for each acquisition and is expressed in seconds. The quantity of light that reaches the camera sensor depends proportionally on this exposure time. The modification of the shutter speed alters the effect of the movement of an object on the image. Short exposure times are necessary to freeze the movement of moving objects, so avoiding blurring. In the case of non-moving objects, long exposures can be used, which is the reason why the technique of exposure bracketing is typically used for static scenes. It is useful for high contrast objects and with limited dynamic range media such as CCD sensors.

Images acquired for a given set of conditions (same gain, same illumination...) with different exposure times can then be combined in post processing to obtain a high dynamic range (HDR) image. The HDR technique has already proposed for interferometry by J. Vargas et al. using a coherent illumination source in a Mach-Zehnder setup (Ref. [5]) and was then applied in a phase-shifting algorithm in order to increase the quality of the phase recovered (Ref. [8]). The method we propose here is similar to that used in Ref. [5] but is applied in white-light interferometry and uses different weights in the formula, that are initially modulation maps, to create the HDR images. The result of the new technique is to produce fully quantified increased depth images.

In this letter, we propose the hybrid full depth HDR technique applied to full-field optical coherence tomography (FF-OCT) typically used to measure the thickness and internal structures of transparent and diffusing layers by the detection of multiple fringe envelope signals. In these applications of FF-OCT, the signal to noise ratio can be very low. The contrast of the interference fringes is therefore a very important factor, hence the interest of improving it by this proposed HDR technique. The weights are obtained after calibration of the system, which consists in varying the exposure time of the camera and measuring the mean value of the pixel intensities on a flat image acquired by the sensor. The response can be approximated by a linear equation, as illustrated in Fig. 1.

Fig. 1. Calibration of the system: CCD camera intensity response versus exposure time variation.

Then, the curve is inverted laterally and follows an affine equation given by:

$$w_i = -7.87 \cdot 10^{-5} \cdot i + 1.13 \quad (1)$$

where i is the corresponding exposure time in μs . The resulting weights w_i are then used to enhance underexposed areas and reduce overexposed areas. Table 1 shows the values of w_i obtained for exposure times of 1600 μs to 12760 μs in steps of 2480 μs .

Table 1. Weights corresponding to each exposure time.

Exposure time (μs)	1600	4080	6560	9040	10280	12760
w_i	1.00	0.81	0.62	0.42	0.32	0.13

After having acquired several standard dynamic range (SDR) images of 8-bit depth with different exposure times, an image with enhanced dynamic range can thus be obtained with:

$$I_{HDR} = \sum_i I_i \cdot w_i \quad (2)$$

where I_i is the image with a given exposure time. Indeed, the brightest pixels in images acquired with high exposure times are saturated and their values are set to 255. However these pixels have a relevant value on the other images with lower exposure times. Thus, by applying Eq. (2), the value of these border value pixels can be approximated by compensation.

The system used is an adapted Leitz-Linnik interference microscope composed of x50 objectives (NA = 0.85) and cube beam splitter. The image acquisition is performed with a Basler avA1000-100gc GigE CCD camera with a Giga Ethernet connection. The sample is mounted on a piezoelectric table (PIFOC, from PI) for Z-scanning. Acquisition and processing is carried out on a PC equipped with an Intel® Xeon® CPU processor (2.40 GHz, 8 Go RAM) with a Windows 7 (64 bits) operating system. The software used to control the acquisition is written in LabVIEW (version 2014, 64 bits, NI). The technique of white-light coherence scanning interference used is described in (Ref. [9]) and allows the building up of an XYZ image stack. The results obtained on the XZ images after 2D processing enable the observation of the rear and front sides of a transparent layer. Due to the transparency of the sample, the contrast obtained in the images is low and some details are hardly observable. Applying the proposed HDR technique makes it possible to obtain images with less noise and an improved contrast. After image acquisition, the post processing is performed with software written in MATLAB (version 2013) to create the HDR images.

To demonstrate the improvements produced by the proposed image acquisition and post processing, a study of noise quantification has been carried out. By observing the effects of the exposure bracketing on a line profile of an HDR flat image compared to an SDR flat image, a decrease in the spatial noise can be observed. The following results were obtained on a transparent polymer film of Mylar with a thickness of 3 μm for a camera exposure time from 1600 μs to 12760 μs in steps of 1240 μs , corresponding to an acquisition of 10 images. The SNR images used for the comparisons in this section are those obtained with the exposure time of 9040 μs in which no pixels are saturated.

Table 2. SNR values of a line profile on SDR and HDR images.

	SDR image	HDR Image
Mean value	159.2	531.9
Peak-to-valley	20	35
Standard deviation	3.9	6.5
SNR (dB)	32.3	38.2

By calculating the signal to noise ratio (SNR) of both line profiles, an improvement of almost 20% in the SNR of the HDR image can be noted, in comparison to the SNR of the SDR image, as given in Table 2. Even though such an improvement could also be obtained by the simple

averaging of 4 images of the same scene with the same exposure time, the proposed HDR technique allows the revealing of saturated pixels in addition to the SNR improvement. The proposed HDR technique also allows an improvement of the dynamic range of the images, as illustrated in Fig. 2 in which the histograms of the images acquired with exposure times of 1600 μs , 6560 μs and 12760 μs , as well as the histogram of the resulting HDR image are represented. It can be first observed that the dynamic range of the HDR images is increased to a depth of 10-bits. In addition, the histograms of an underexposed image with an exposure time of 1600 μs (histogram A) and an overexposed image with an exposure time of 12760 μs (histogram C) are respectively saturated to 18 and 255 gray levels. The histogram of the corresponding HDR image obtained by combining 10 exposure times is not saturated thanks to the compensation of the pixels in the intermediate exposure times.

Fig. 2. Histograms of images acquired with exposure times of 1600 μs , 6560 μs and 12760 μs and of the resulting HDR image.

As the proposed technique allows an improvement in the SNR of the images, this leads to an improvement in the contrast of the image and thus to the observation of μm -sized structures, initially lost in the noise. This can be observed for certain positions within the thickness of the layer where defects can be distinguished in the HDR image in Fig. 3(b), whereas they are not visible in the SDR image in Fig. 3(a).

Fig. 3. Detail enhancement in XY HDR images. (a) An SDR image, and (b) the corresponding HDR image using hot colormap of MATLAB®.

By observing the fringe signals from a pixel in an XY image from the XYZ image stack, an improvement of the signals can be noted in the HDR images, as shown in Fig. 4(b). The red curve corresponds to the fringe envelope obtained after removal of the local average of the raw fringes in grey along the z-axis and low pass filtering the fringe signal (Ref. [9]). On the HDR fringe signal, three envelope peaks can clearly be detected, the right hand peak being hardly visible on the SDR fringe signal due to the presence of noise. The first peak from the left corresponds to the front side of the transparent sample, the second one to a structure contained within the layer and the last one to the rear interface.

Fig. 4. Fringe interferograms and envelopes for (a) a SDR and (b) an HDR image on a pixel in a stack of XY images.

The improvement in the envelope of the fringe signal has beneficial repercussions on the XZ images. Indeed, as shown in Fig. 5(a) and (b), a structure contained in the transparent layer (in the circle) can be detected in the HDR images. These XZ images are shown using the jet colormap proposed by MATLAB®, developed by National Center for Supercomputer Applications (NCSA) and based on the classical rainbow color map. The contrast $([I_{\text{max}} - I_{\text{min}}]/[I_{\text{max}} + I_{\text{min}}]) \times 100$ obtained in the areas contained in the circles is equal to 37% for the SDR XZ image and 100% for the HDR XZ image. In addition, the SNR calculated along the horizontal line profile in the SDR image in Fig 5(a) is 38 dB whereas in the HDR image in Fig 5(b), the SNR is 44 dB, i.e. there is an increase of 6 dB, corresponding to an improvement of a factor of 2.

Fig. 5. Detection of a structure in an HDR XZ image. (a) An XZ SDR image, and (b) the XZ corresponding HDR image using jet colormap of MATLAB®.

By observing a line profile across a structure observed within the layer, as illustrated in Fig. 6(a), it can be noted that the contrast has been improved for the HDR image. The corresponding histograms in Fig. 6(b) and (c) show that the structure contained in the layer is lost in the noise for the SDR image which has a value of around 65 gray levels, whereas the HDR image shows up three peaks in addition to the peak of the noise, corresponding to the front side, the rear side of the layer and the structure contained in the polymer.

Fig. 6. Observation of a vertical line profile. (a) Cross sections for a SDR and an HDR images; (b) Histogram of the line profile of a SDR image; (c) Histogram of the line profile of a HDR image.

In addition, an improvement in the lateral resolution of the system can be observed after applying the proposed HDR technique. This effect is demonstrated in Fig. 6, by measuring the lateral distance at the edges of the structure between the 90% and 10% points of the intensity. In the example in Fig. 7(c) and (d), the lateral resolution has been improved by 540 nm to a value of 480 nm for the right side and by 20 nm to a value of 380 nm for the left side, slightly better than the lateral optical resolution of 0.45 μm.

Fig. 7. Spatial resolution improvement. (a) Line profile on the SDR image and (b) on the HDR image using jet colormap of MATLAB®; (c) Cross section of the SDR image; (d) Cross section of the HDR image.

In this letter, we have proposed a hybrid full depth HDR image processing technique for FF-OCT using incoherent illumination to improve the performance in the detection sensitivity of internal structures and details in the tomographic sectioning of transparent layers. Thanks to contrast enhancement and an increase in the SNR by a factor of 2, structures of about 1 μm in diameter contained in the layer and initially lost in noise are thus made visible with the proposed method. In addition, the lateral resolution of the system is improved to a near-diffraction limited value of 0.45 μm.

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