Multispectral digital lensless holographic microscopy: from femtosecond laser to white light LED

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Abstract. The use of femtosecond laser radiation and super bright white LED in digital lensless holographic microscopy is presented. For the ultrafast laser radiation two different configurations of operation of the microscope are presented and the dissimilar performance of each one analyzed. The microscope operating with a super bright white light LED in combination with optical filters shows very competitive performance as it is compared with more expensive optical sources. The broadband emission of both radiation sources allows the multispectral imaging of biological samples to obtain spectral responses and/or full color images of the microscopic specimens; sections of the head of a drosophila melanogaster fly are imaged in this contribution. The simple, solid, compact, lightweight, and reliable architecture of digital lensless holographic microscopy operating with broadband light sources to image biological specimens exhibiting micrometer-sized details is evaluated in the present contribution.

1. Introduction

The illumination of the samples with broadband light sources allows their spectral analysis and/or their full color imaging. As the sample has microscopic dimensions, multispectral microscopy methods have revealed valuable information of the specimens that no other imaging methodologies can achieve [1]. The achievements of the multispectral microscopy have been also sought with the emerging technology of digital holographic microscopy. The multispectral illumination of the samples has been utilized in digital holographic microscopy to provide full color images with micrometer resolution [2-5] or to enhance the features of the microscope itself [6]. Among the multispectral light sources, ultrafast lasers, with its associated broadband illumination and its intrinsic spatial coherence, have also been utilized in digital holographic microscopy [7-9]; the researches in these works have shown the possibility of doing multispectral digital holographic imaging of specimens with and without internal structure. Despite the good results reported by the use of the ultrafast lasers in digital holographic microscopy, their usually high economical cost and bulky geometry convert that multispectral approach in an exclusively in-lab methodology. With the aim of searching for a multispectral microscope with high degree of portability and ideally low-cost other options of broadband light sources should be explored.

Digital lensless holographic microscopy (DLHM) shows great portability [10] and a set-up that could be potentially of low-cost. DLHM has been developed up to the level that images with
micrometer spatial resolution can be achieved with the use of no lenses, at a low computational cost [11] and also basic multispectral applications of the technique have been presented [2, 4]. The simplicity of its architecture has encouraged the researches to utilize diverse light sources, as LED [4, 12, 13], discharge lamp [14], and of course ultrafast light sources [7-9]. In this contribution we want to summarize the main results that our group has achieved with the utilization of broadband light sources. We emphasize the efforts towards the development of a lightweight, low-cost, and portable multispectral microscopy device.

2. Digital lensless holographic microscopy (DLHM)

The possibility of getting rid of lenses to do microscopy brings great advantages: i) there are no optical aberrations associated with the microscope objectives and ii) the microscope could be less expensive, more lightweight and more portable. This possibility is brought into reality if the imaging process is carried out in a two-step methodology as was taught by Gabor [15-17]. The original Gabor’s idea was adopted by Kreuzer et al. [18] who added the power and versatility of the digital world to process the information recorded in a digital screen. This addition allows the overcoming of the flaws that prevented Gabor’s idea to be more attractive; the digital processing powers the elimination of the zero-diffracted order and the reduction of the nuisance introduced by the twin-images [11, 19]. This relatively new technology that merges the bright Gabor’s ideas with the power of the digital processing is named digital lensless holographic microscopy (DLHM).

In DLHM the sample is illuminated by the spherical waves produced by a point source. To produce the point source, the light source is focused down onto a pinhole with a diameter slightly larger than the illuminating wavelength \( \lambda \); the sample is placed at a distance \( z \) from the pinhole. On the surface of a digital screen (CCD or CMOS camera), located at a distance \( L \) from the point source, the waves scattered by the sample \( U_{\text{scat}} \) are superimposed with the portions of the spherical waves that travel with no perturbation \( U_{\text{ref}} \). The intensity recorded on the screen, called in-line hologram, is transferred to a computer for its further processing; see Figure 1. The intensity recorded on the screen with no object present is pixel-wise subtracted from the in-line hologram to produce the modified hologram \( \tilde{I}_r \) which does not carry the information of the zero-diffracted order:

\[
\tilde{I}_r = [U_{\text{scat}} \cdot r] + [U_{\text{scat}} \cdot r + U_{\text{ref}} \cdot r + U_{\text{ref}} \cdot r].
\]  

(1)

Figure 1. Schematic set-up of DLHM.
Because the preferred samples used in DLHM to work are considered weak scatters, the intensity of the scattered wave $|U_{\text{scat}}(\mathbf{r})|^2$ can be neglected [15]. As spherical illumination is utilized the presence of the twin-images, the terms in square brackets in equation (1), does not introduce any nuisance on the reconstructed images. Further discussion about the modified hologram $I_\mathbf{r}$ and the effects of the twin-images can be read in reference [11]. To recover the information gathered by the waves as they travel through the sample, $I_\mathbf{r}$ is numerically back-illuminated by a converging spherical wavefront that travels towards the pinhole. This reconstruction process can be correctly described by the Fresnel-Kirchhoff diffraction integral, which written to represent the wavefront propagation in DLHM turns into [11]:

$$U_{\text{scat}}(\mathbf{r'}) = \int_{\Delta \mathbf{r}} I_\mathbf{r} \exp \left\{ \frac{2\pi i}{\lambda} \left( \mathbf{r'} \cdot \mathbf{r} \right) / |\mathbf{r}| \right\} d\mathbf{r}$$

with the position vectors $\mathbf{r} = (x, y, L), \mathbf{r'} = (x', y', z)$ representing points at the digital screen and at the reconstruction plane, in that order.

3. Ultrafast laser-DLHM

The needed point source to illuminate the sample is generated in ultrafast laser-DLHM by focusing down onto a pinhole the light from a Ti:Sa laser of 12 fs operating a 80 MHz. The focusing element can be either an achromatic microscope objective (AMO) or a diffractive lens (DL), see panel (a) of Figure 2. As the AMO is utilized for generating the point source, the wavelengths that compose the spectrum of the broadband illumination of the source (panel (b) of Figure 2), generate an in-line hologram that corresponds to the intensity superposition of the individual holograms produced by each of the composing wavelengths $I'_\mathbf{r}, \lambda$ [9]:

$$I_\mathbf{r} = \int_{\Delta \lambda} I'_\mathbf{r}, \lambda \, \rho \, \lambda \, d\lambda$$

with $\lambda_0$ the mean wavelength and $\Delta \lambda$ the spectral width of the illuminating source. $\rho \, \lambda$ is the spectral power that weights the individual in-line holograms $I'_\mathbf{r}, \lambda$ which can be written as:

$$I'_\mathbf{r}, \lambda = |U_{\text{scat}}(\mathbf{r}, \lambda)|^2 + |U_{\text{ref}}(\mathbf{r}, \lambda)|^2 \pm |U_{\text{scat}}(\mathbf{r}, \lambda, U_{\text{ref}}(\mathbf{r}, \lambda) + U_{\text{ref}}(\mathbf{r}, \lambda, U_{\text{ref}}(\mathbf{r}, \lambda)|.$$
To eliminate possible intensity inhomogeneities and the zero-diffracted order, the intensity recorded by the digital screen with no sample present is pixel-wise subtracted from the in-line hologram to produce the intensity from the information of the sample is retrieved:

\[ \tilde{I} \mathbf{r} = \int_{\Delta \omega} \rho(\mathbf{r}, \lambda) \left[ \left| \tilde{I} \mathbf{r}, \lambda \right| - \left| I_{\text{ref}} \mathbf{r}, \lambda \right| \right] d\lambda. \] (5)

This intensity or incoherent superposition given by equation (5), which takes place due to the long integration time of the digital camera in comparison with the pulse duration, introduces the blurring of the reconstructed hologram as shown in panel (c) of Figure 2. No details of the sample are observed rather than the overall shape of it. The other option to produce the point source is the combination of the DL and the pinhole. This arrangement behaves as a tunable spectral filter where the selected wavelength is controlled by the distance from the DL to the pinhole and the spectral width is determined by the pinhole size and the numerical aperture of DL [8]. As the DL is utilized, such that from the spectrum of the femtosecond laser a pulse with 6 nm width is chosen (panel (d) Figure 2). The reconstructed image shows characteristics of the section of the head of the drosophila melanogaster fly that are not visible with the use of the AMO. In panels (c) and (e) of Figure 2, we have 2-time zoomed in similar regions to highlight the differences of the reconstructed images, as the AMO and the DL are utilized to produce the point source. Further details about the underlying phenomena about the coherence of the illuminating light that give rise to these two very different performances of the DLHM can be read elsewhere [8, 9].

4. White light LED-DLHM

The performance of the ultrafast laser-DLHM shows two distinctive performances of this microscopy methodology. While the overall shape of the sample can be reproduced by generating the point source with the AMO, details of the structure of the sample can be recovered by creating the point source with the DL. However, neither of these two options to generate the illuminating broadband source show any promise towards the design of a portable and low-cost multispectral microscope. The use of a super-bright white light LED as illuminating source supplies an alternative path to develop a portable and low-cost multispectral microscope. In the white light LED-DLHM, the needed point source to illuminate the sample is generated by focusing down the light from the LED onto a pinhole, as shown in panel (a) of Figure 3. A regular of these LEDs has a spectral width ranging from 400 nm to 750 nm. This feature allows for using the appropriate bandpass filters to obtain the required illumination for the wavelength of interest. In the case of RGB imaging, one could think a further simplification by using directly a RGB or a tri-LED, however the individual spectral width for each wavelength of at least 30 nm spoils this option [4]. To test the performance of the white light-DLHM, we have initially recorded a set of red, green, and blue holograms to produce a full color image of a section of the head of a drosophila melanogaster fly. In our experiment, the light emitted by the super-bright white-light LED (LXML-PWN2 Luxeon®) was initially collimated. The collimated light was filtered spectrally by means of three bandpass filters from the company Thorlabs®, 632.8 ± 0.2 nm, 532 ± 1 nm and, 405 ± 10nm; the filters were mounted on a motorized wheel. To produce the illuminating point source for each wavelength, the corresponding beam was focused down onto a pinhole of 1 µm in diameter by means of a AMO. The use of this microscope objective minimizes the waist variation of the focused beams for the different wavelengths and therefore smoother spherical waves were produced. The pinhole spatial filters the impinging light on it; the combination of the spectral and the spatial filtering tailors the light of the super-bright white-light LED to have the coherence properties demanded by DLHM to work [11, 13, 20]. The complete experimental set-up is illustrated in panel (a) of Figure 3. The wheel is synchronized with the recording device to record individual hologram for the chosen wavelengths.
Figure 3. White light LED digital lensless holographic microscope. (a) Schematic set-up for white light LED DLHM. (b) Reconstruction of the green channel of the RGB holograms. (c) Full RGB image of the reconstructed image for a section of the head of a Drosophila Melanogaster fly.

To illustrate the individual reconstructed images, in panel (b) of Figure 3 we show the green channel of the set of images. The fusion of the red, green, and blue reconstructed images is shown in panel (c) of Figure 3. The full color image of the specimen after the correct fusion show details in the range of micrometers. The zoomed-in encircled area in the top shows a hair that measures of the order of 2 μm. At the lower right corner of the same panel the zoomed-in area shows the omatidia of the eye of the fly which are in the order of 3 to 2 μm. The quality of the full color reconstructed image in panel (c) validates the feasibility of performing multispectral microscopy with a low-cost, lightweight, and potentially portable device. In similar way as shown in this application for the RGB image, further number of wavelengths can be utilized to have a more complete spectral analysis of the sample while the micrometer-sized resolution with a low-cost, lightweight and potentially portable multispectral microscope is kept.

One key factor to consider in the operation of the white light DLHM is the amount of power available after the spatial filtering process of the LED light. The LED device we have used has a radiant power of the order of 0.5 mW. After the collimation and focalization processes, over the surface of the pinhole we drive an irradiance of about 0.2 MW/cm². In contrast, as the femtosecond laser is utilized, that irradiance is of the order of 2.5 TW/cm². Fortunately, the versatility provided by the digital recording and the numerical processing, powers this technology with the needed tools to equalize the recorded holograms. The equalization process avoids any perturbations that could be introduced by the dissimilar values of available irradiance upon the sample and hence over the recording device. This claim can be verified in the content of this paper, where the quality of the reconstructed holograms for both types of light sources is fully understood from the coherence properties of the sources rather than irradiiance basis analysis would be necessary.
5. Conclusions
The use of multiple wavelengths in microscopy imaging systems allows the study of the spectral response of the observed specimen. That spectral response can be evaluated via digital lensless holographic microscopy (DLHM), one the simplest microscopy methodologies. The simplicity, robustness, and reliability of DLHM ease the utilization of broadband light sources to accomplish the multispectral imaging of microscopic samples with micrometer resolution and without the need of lenses. The use of no lenses to image the sample eliminates any chromatic or optical aberration inherited from the lenses as well as allows the development of lightweight devices.

In this work we have presented the use of a femtosecond-laser and a super bright white LED as light sources in DLHM. With both light sources we have shown that full color images and the spectral response of the sample can be obtained with this simple imaging methodology. Sections of the head of a drosophila melanogaster fly or fruit fly have been imaged with the ultrafast-DLHM and the white light LED-DLHM. The results shown in this contribution are good examples of the versatility of DLHM in the use of light sources which could worth from as large as thousands to as little as cents of dollars. The latter option paves the road to the development of a low-cost, lightweight, low computational cost, and potentially portable multispectral lensless holographic microscope.

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References
[1] M. E. Dickinson, G. Bearman, S. Tille, R. Lansford, and S. E. Fraser, 2001, Multi-spectral imaging and linear unmixing add a whole new dimension to laser scanning fluorescence microscopy, Biotechniques 31.
[2] J. Garcia-Sucerquia, 2012, Color lensless digital holographic microscopy with micrometer resolution, Opt. Lett. 37, 1724-1726.
[3] F. Dubois and C. Yourassowsky, 2012, Full off-axis red-green-blue digital holographic microscope with LED illumination, Opt. Lett. 37, 2190-2192.
[4] J. P. Ryle, S. McDonnell, and J. T. Sheridan, 2011, Lensless multispectral digital in-line holographic microscope, Journal of Biomedical Optics 16, 126004-126004.
[5] S. Seo, T. W. Su, A. Erlinger, and A. Ozcan, 2008, Multi-color LUCAS: Lensfree on-chip cytometry using tunable monochromatic illumination and digital noise reduction, Cellular and Molecular Bioengineering 1, 146-156.
[6] M. K. Kim, 1999, Wavelength-scanning digital interference holography for optical section imaging, Optics Letters 24, 1693-1695.
[7] M. Brunel, H. Shen, S. Coëtmemelc, D. Lebrun, and K. Ait Ameur, 2012, Femtosecond digital in-line holography with the fractional Fourier transform: application to phase-contrast metrology, Appl. Phys. B 106, 583-591.
[8] O. Mendoza-Yero, E. Tajahuercue, J. Lancis, and J. Garcia-Sucerquia, 2013, Diffractive digital lensless holographic microscopy with fine spectral tuning, Opt. Lett. 38, 2107-2109.
[9] O. Mendoza-Yero, A. Calabuig, E. Tajahuercue, J. Lancis, P. André, and J. Garcia-Sucerquia, 2013, Femtosecond digital lensless holographic microscopy to image biological samples, Optics Letters 38, 3205-3207.
[10] S. K. Jericho, P. Klages, J. Nadeau, E. M. Dumas, M. H. Jericho, and H. J. Kreuzer, 2010, In-line digital holographic microscopy for terrestrial and exobiological research, *Planetary and Space Science* **58**, 701-705.

[11] J. Garcia-Sucerquia, W. Xu, S. K. Jericho, P. Klages, M. H. Jericho, and H. J. Kreuzer, 2006, Digital in-line holographic microscopy, *Appl. Opt.* **45**, 836-850.

[12] P. Petruck, R. Riesenberg, and R. Kowarschik, 2012, Partially coherent light-emitting diode illumination for video-rate in-line holographic microscopy, *Appl. Opt.* **51**, 2333-2340.

[13] J. Garcia-Sucerquia, 2013, Noise reduction in digital lensless holographic microscopy by engineering the light from a light-emitting diode, *Appl. Opt.* **52**, A232-A239.

[14] P. Petruck, R. Riesenberg, and R. Kowarschik, 2012, Optimized coherence parameters for high-resolution holographic microscopy, *Appl. Phys. B* **106**, 339-348.

[15] D. Gabor, 1948, A new microscopic principle, *Nature* **161**, 777–778.

[16] D. Gabor, 1949, Microscopy by reconstructed wave-fronts, *Proc. R. Soc. London A* **197**, 454.

[17] D. Gabor, 1951, Microscopy by reconstructed wave fronts: II, *Proc. Phys. Soc. London B* **64**, 449.

[18] H. J. Kreuzer, H. W. Fink, H. Schmid, and S. Bonev, 1995, Holography of holes, with electrons and photons, *Journal of Microscopy* **178**, 191-197.

[19] J. Garcia-Sucerquia, D. C. Alvarez-Palacio, M. H. Jericho, and H. J. Kreuzer, 2006, Comment on "Reconstruction algorithm for high-numerical-aperture holograms with diffraction-limited resolution", *Optics Letters* **31**, 2845-2847.

[20] D. Alvarez-Palacio and J. Garcia-Sucerquia, 2010, Digital in-line holographic microscopy with partially coherent light: Micrometer resolution, *Revista Mexicana de Fisica* **56**, 445-448.