Published in final edited form as:

Lab Chip. 2011 August 21; 11(16): 2738–2743. doi:10.1039/c1lc20169g.

Combined reflection and transmission microscope for telemedicine applications in field settings

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Abstract

We demonstrate a field-portable upright and inverted microscope that can image specimens in both reflection and transmission modes. This compact and cost-effective dual-mode microscope weighs only ~135 grams (<4.8 ounces) and utilizes a simple light emitting diode (LED) to illuminate the sample of interest using a beam-splitter cube that is positioned above the object plane. This LED illumination is then partially reflected from the sample to be collected by two lenses, creating a reflection image of the specimen onto an opto-electronic sensor-array that is positioned above the beam-splitter cube. In addition to this, the illumination beam is also partially transmitted through the same specimen, which then casts lensfree in-line holograms of the same objects onto a second opto-electronic sensor-array that is positioned underneath the beam-splitter cube. By rapid digital reconstruction of the acquired lensfree holograms, transmission images (both phase and amplitude) of the same specimen are also created. We tested the performance of this field-portable microscope by imaging various micro-particles, blood smears as well as a histopathology slide corresponding to skin tissue. Being compact, light-weight and cost-effective, this combined reflection and transmission microscope might especially be useful for telemedicine applications in resource limited settings.

Introduction

Optical light microscopy provides a powerful tool that can be used for a broad set of applications in biomedicine. Bringing the same toolset to remote and resource limited locations has recently attracted significant attention of researchers, resulting in several technical advances in this field.^{1–28} Toward the same goal, here we demonstrate a dual-mode optical microscope (Fig. 1) that can provide both reflection and transmission images of specimens using a compact, light-weight and cost-effective imaging architecture. This field-portable digital microscope, weighing only ~135 grams (<4.8 ounces), uses a simple light emitting diode (LED) to uniformly illuminate the samples through a beam-splitter cube that is positioned above the object plane. Part of this illumination is reflected back from the sample and is imaged using two cost-effective lenses onto an opto-electronic sensor-array (*e.g.*, a complementary metal–oxide–semiconductor—CMOS chip) forming the reflection mode image of the specimen (see Fig. 1). The illumination light is also partially transmitted through the sample which forms lensfree in-line holograms^{3,4,7,9} of the samples onto a second CMOS chip that is positioned below the beam-splitter cube. Even though this LED illumination is only *partially* coherent (both temporally and spatially) these lensfree

transmission holograms can still be rapidly processed by iterative phase recovery algorithms,^{3,4,7} where an object support (*i.e.*, a 2D spatial mask that is generated using either the reflection microscope image or the digital back-projection of the raw hologram) is successively enforced at the sample plane to create phase and amplitude transmission images of the objects.

This dual-mode imaging architecture shown in Fig. 1 provides several advantages since the spatial information acquired from the reflection and the transmission images complement each other. First, the holographic transmission microscope (in its folded illumination geometry shown in Fig. 1) provides *both* phase and amplitude images of the samples, and therefore contains additional contrast mechanisms compared to a conventional bench-top reflection microscope. This can especially be very useful for imaging of weakly scattering objects that cast their signatures mostly in phase images. One example of this phase related contrast advantage has already been demonstrated for imaging and enumeration of water-borne parasites/bacteria in *e.g.*, drinking water.¹⁴ Other telemedicine related examples of the usefulness of this optical phase contrast created by lensfree transmission holographic microscopy include imaging of human malaria parasites in thin blood smears,¹¹ imaging of human sperm tails (toward evaluation of semen quality¹²), blood count, red blood cell volume measurements at the single cell level,⁹ *etc.*

Apart from phase contrast advantage, the same lensfree transmission geometry of our dual-mode microscope also exhibits a significant field-of-view advantage (*e.g.*, >20 fold) compared to a lens-based conventional bench-top microscope, which could especially be important for screening of rare signatures of, *e.g.*, parasites or bacteria.^{9,11,14}

In addition to these, the reflection mode image of the sample can also be used to improve the performance of our holographic image reconstruction (especially at high object densities) by providing a better spatial mask (*i.e.*, object support) for twin-image elimination or phase retrieval algorithms as outlined in Fig. 2.^{3,4} This enhances the contrast of the reconstructed phase and amplitude images of our lensfree transmission microscope, complementing the reflection mode image of the same sample acquired with our field-portable dual-mode architecture (Fig. 1). Furthermore, the reflection mode microscope widens the application areas of our field-portable microscope by enabling imaging of dense samples (such as histopathology/tissue slides), which is challenging for lensfree transmission imaging in general due to significant distortion of the waves transmitted through dense media.⁹

To provide its proof-of-concept, we imaged various micro-particles, blood smears as well as a histopathology slide (skin tissue) using the hand-held microscope shown in Fig. 1. While these micro-objects provided us the means to compare our field-portable microscope's image quality against a conventional bench-top microscope, they are also quite relevant to telemedicine needs. For instance blood samples and histopathology slides are widely imaged by health-care workers to screen various conditions including infections (*e.g.*, malaria, leprosy, botryomycosis), anemia, neutrophilia, neutropenia, poor nutrition, malignancies, drug toxicities, *etc.* Being cost-effective, compact, and light-weight (~135 grams) this dual-mode microscope could especially be useful for some of these telemedicine applications in, *e.g.*, developing countries.

Results and discussion

The dual-mode microscopy concept presented in Fig. 1 and 2 was first tested using a smear of 4 μm metallic (opaque) particles placed on a regular glass slide. Fig. 3 presents both the transmission and reflection images of this sample acquired using our field-portable microscope shown in Fig. 1(a). For the holographic reconstruction results presented in Fig.

3(a), the object support (used for iterative phase recovery,³ see Fig. 2) is generated using the corresponding reflection microscope image of the same sample (refer to the Methods section for further details). The inset images in Fig. 3 also show two micro-particles that are touching each other and are clearly resolved. Notice also that the transmission microscope images of these micro-particles are entirely dark, while their reflection images show a rather different contrast, as expected from metallic (opaque) particles.

After these initial experiments, we then imaged a blood smear sample the results of which are summarized in Fig. 2 and 4. In Fig. 4(a) we show the raw lensfree hologram of the blood smear sample, where two zoomed regions are also highlighted in (b) and (c). These images illustrate the individual lensfree in-line holograms of the blood cells that are imaged using the transmission geometry of our dual-mode microscope shown in Fig. 1(a). Holographic reconstruction results for these regions are also shown in the same figure (top right images). Reflection mode images of the same regions (b and c) are also provided in Fig. 4, bottom right images, which nicely match to our lensfree transmission microscope images shown in the same figure. To better illustrate different contrast properties of the transmission and reflection microscopy modes, zoomed images of white and red blood cells are also shown in Fig. 4 bottom left images (A through D). Notice in these images that the phase and the amplitude of the reconstructed transmission images provide complementary contrast mechanisms (especially for sub-cellular nuclear features of white blood cells that are labelled with the Wright-Giemsa stain) when compared to the reflection mode images.

In our design, while lensfree transmission microscopy is rather useful in terms of providing a large imaging field-of-view (*e.g.*, $>20 \text{ mm}^2$) with a decent spatial resolution (*e.g.*, $<1 \text{ }\mu\text{m}$)^{7,10,11} it unfortunately suffers from one drawback, *i.e.*, for optically dense and connected objects (such as tissue slides), it starts to exhibit aberrations in its reconstructed transmission images. This in general is also true for any holographic transmission microscope that uses an in-line geometry. However, this partial limitation of our transmission geometry for dense connected objects can be entirely removed through the use of the reflection mode microscope that is embedded within the same architecture as shown in Fig. 1. To demonstrate its performance specifically for tissue imaging, in Fig. 5 we show a histopathology slide corresponding to skin tissue that is imaged using the reflection microscope of our dual-mode imager. The same figure also shows the image that is obtained using a conventional bench-top reflection microscope (10 $\times$ objective-lens; 0.25 numerical aperture—NA), which provides a decent match to our field-portable microscope image.

It is also important to note that this dual-mode microscope design can perform colour imaging in both transmission and reflection modes. For lensfree transmission holography, by using quasi-monochromatic illumination at three different wavelengths (*e.g.*, red, green and blue), we can sequentially obtain three different lensfree holograms of the specimen to synthesize a pseudo colour image.¹³ To implement this configuration in our field-portable microscope the quasi-monochromatic LED can be replaced by a 3 colour chip that has individually addressable red, green and blue LEDs in the same cost-effective package. The same sequential illumination scheme could also work for the reflection mode; however, achieving colour performance could be implemented easier using, *e.g.*, a wide-band visible LED together with a colour (*e.g.*, RGB) CMOS sensor-chip on the top.

Another point that we would like to discuss is that the reflection arm of our dual-mode field-portable microscope can potentially be converted into a fluorescent microscope with minimal changes to its architecture. For this purpose, we can use the same beam-splitter that is used in our design to pump the samples using, *e.g.*, an LED that has the correct excitation wavelength. As illustrated in Fig. 1, our handheld microscope has a modular design and we can easily change the installed LED to different colors for holographic transmission or

reflection mode imaging needs. To reject the reflected excitation light we can also use a fluorescent filter (*e.g.*, a thin-film interference or an absorption filter¹⁵) that is placed in front of the top CMOS sensor-chip to create the necessary dark-field background for fluorescent imaging.

Finally, we would like to emphasize that, in its folded illumination geometry (Fig. 1), our lensfree transmission holography scheme has relatively lower spatial resolution when compared to some of our earlier lensfree transmission imaging results.³ The main reasons behind this are multiple reflection related artefacts and additional spatial aberrations that are introduced by different surfaces of the beam-splitter cube that is employed in our dual-mode microscope. Such a beam-splitter cube is needed to look at the same object field-of-view in both reflection and transmission geometries as shown in Fig. 1 and was not utilized in our earlier transmission microscopy designs, which relatively improved the quality of their reconstruction results compared to this dual-mode microscope.

Methods

Reflection mode microscope design

Our reflection mode microscope design contains two lenses. The one that is placed closer to the object plane has a focal length of $f_1 = 10$ mm (Thorlabs—LA1116, NA = 0.28) and the second lens has a focal length of $f_2 = 50$ mm (Thorlabs—LA1213, NA = 0.125). The distance of the object plane from the first lens is ~ 10 mm, whereas the distance between the two lenses is set to be ~ 1 mm. The second lens is positioned at a distance of ~ 50 mm from the top sensor-chip (CMOS; Model# MT9P031, Micron Technology; pixel size: $2.2 \mu\text{m}$, 5 Mpixels). This simple and compact design provides us with $5\times$ magnification ($f_2/f_1 = 5$) between the object and the sensor planes, such that the effective pixel size at the object plane becomes $2.2 \mu\text{m}/5 = 440$ nm. Here, we should emphasize that rather than using a standard long-working distance objective-lens, we preferred to create a much simpler imaging architecture for the reflection arm of our field-portable microscope, achieving a lighter, more compact and cost-effective design.

As for the light source, an LED (SunLED Corporation, Part# XLFB11W; center wavelength: 467 nm, bandwidth: 22 nm) is used to illuminate the beam splitter cube (Thorlabs, Part# BS010—10 mm length) from the side as shown in Fig. 1. The light that is reflected from the sample is then collected through the same beam-splitter cube forming reflection images of the objects onto the CMOS chip located at the top of our microscope.

Transmission mode microscope design

The LED source is combined with a color filter of ~ 10 nm bandwidth, and is butt-coupled to a 0.1 mm pinhole/aperture without the use of any focusing or alignment optics, illuminating the entire object field-of-view of $\sim 24 \text{ mm}^2$, which is the active area of the bottom CMOS sensor chip (see Fig. 1). Reduction of the illumination bandwidth of the LED and use of a 0.1 mm pinhole enabled us to tune the temporal and the spatial coherence properties of our holographic transmission microscope, respectively, such that holographic fringes corresponding to a numerical aperture of ~ 0.1 to 0.2 can be physically created and sampled at the CMOS chip surface. The effective “folded” distance between the pinhole and the sample planes (z_1) and the distance between the sample and the sensor planes (z_2) are chosen to be ~ 5 cm and < 1 mm, respectively. Both the illumination LED and the CMOS sensors are powered through a USB connection from the side. The sample to be imaged is loaded into the microscope within a mechanical tray from the side (see Fig. 1(a)).

Holographic reconstruction process using the reflection mode image as a mask

The method of reconstructing transmission holographic images is summarized in Fig. 2. First, both the raw holographic and the reflection microscope images are acquired. In order to eliminate the twin image artefact in lensfree holograms,^{3,7} the object support (*i.e.*, the mask) has to be estimated. Creation of this spatial mask based on our reflection microscope image involves a two-step process. First, the reflection microscope image is converted to a binary image using a suitable threshold. After this stage, the acquired mask mostly covers the support of the high contrast regions of the objects. However, various smaller areas that belong to the object support may not be contained at this stage of the mask due to, *e.g.*, large variations in the image intensity within the object boundaries. To mitigate this problem, these missing regions in the mask are filled (without adding large unwanted areas to it) by detecting small areas/features that have high spatial eccentricity. After this step, the mask is also filtered by a 2D Gaussian function to smoothen its edges, which avoids the introduction of spatial artefacts in our iterative hologram reconstruction process.³

Digital deconvolution of the reflection mode images

In order to enhance the contrast and the resolution of our reflection mode images, we performed image deconvolution using an open source software (DeconvolutionJ plug-in for ImageJ²⁹), which is based on regularized Wiener filtering. In this algorithm, which is performed in the spatial frequency domain, the input images are deconvolved by the point spread function (PSF) of the imaging system, while a regularized Wiener filter is used to prevent amplification of high frequency noise.³⁰ For our reflection microscope images, we used a calculated PSF for the deconvolution process, which was assumed to have a Gaussian profile with a full-width-at-half-maximum (FWHM) of $\sim 1.5 \mu\text{m}$.

Conclusions

We presented a compact and cost-effective dual-mode microscope that can provide both transmission and reflection images of various specimens within the same light-weight design (~ 135 grams). This field-portable microscope utilizes a simple LED for illumination and captures a reflection mode image of the sample based on two cost-effective lenses and a CMOS sensor-array. The illumination light, after a folded propagation distance of $z_1 \approx 5$ cm, picks up partial spatial coherence that is sufficiently large to record lensfree in-line transmission holograms of the objects onto a second CMOS sensor-array that is located at the bottom of the same microscope design. Rapid digital processing of these lensfree in-line holograms permits reconstruction of phase and amplitude transmission images of the same samples, complementing the spatial information provided by the reflection mode images. We tested the performance of this field-portable dual-mode microscope by imaging various particles, blood smear samples as well as a histopathology slide corresponding to skin tissue. Being rather compact and light-weight, this cost-effective microscope might provide a useful tool for telemedicine applications in resource limited environments.

Acknowledgments

A. Ozcan gratefully acknowledges the support of NSF CAREER Award, the ONR Young Investigator Award and the NIH Director's New Innovator Award (DP2OD006427) from the Office of The Director, NIH. The authors also acknowledge the support of the Bill & Melinda Gates Foundation, Vodafone Americas Foundation, and NSF BISH program (under Awards # 0754880 and 0930501).

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Fig. 1.

(a) A working prototype and (b) a schematic diagram of our dual-mode microscope are illustrated. This compact and cost-effective microscope weighs only ~135 grams (<4.8 ounces) and can achieve both transmission and reflection mode imaging of specimens. For illumination it utilizes a simple LED, filtered by a pinhole (~100 μm), which is partially reflected from the sample, creating the reflection image of the specimen onto a CMOS chip located at the top of the microscope. The illumination light is also partially transmitted through the sample, which casts lensfree holograms of the objects onto a second CMOS chip, located at the bottom of the same microscope assembly. These lensfree in-line holograms are then rapidly processed to create transmission images (both phase and amplitude) of the same samples. Two mechanical scanners are also used in our design to be able to shift/control the imaging field of view of the reflection-mode microscope. Both the illumination LED and the CMOS sensors are powered through a USB connection from the side. BS: beam-splitter cube; LED: light emitting diode; CMOS: complementary metal-oxide-semiconductor; USB: universal serial bus.

Fig. 2.

Our dual-mode microscope captures two independent images of the sample: using the top CMOS chip, we capture a reflection mode image equivalent to a $5\times$ objective-lens; and using the bottom CMOS chip we capture a lensfree transmission in-line hologram of the same sample. After digital processing, the reflection image can also be used to create an object support function (*i.e.*, a 2D mask) for reconstruction of the acquired lensfree holograms. For this reconstruction procedure, the 2D object support is successively enforced at the object plane while the missing holographic phase is being iteratively recovered.³ After convergence is achieved (typically taking 10–15 iterations and <1 second)^{3,7} amplitude and phase transmission images of the specimen are reconstructed as illustrated in the figure. Notice how the same imaging field-of-view exhibits different contrast for the holographically reconstructed images compared to the reflection mode image. The object in this case is a whole blood smear sample that is stained with the Wright-Giemsa staining kit³ (Fisher Diagnostics).

Fig. 3.

Transmission (a) and reflection (b) images of 4 μm diameter opaque particles (smeared on a glass slide) are shown. These images are acquired using the field-portable dual-mode microscope shown in Fig. 1(a). Refer to the Methods section for further details on each microscopy modality.

Fig. 4.

(a) Raw holographic image of a blood smear sample that is acquired using the bottom arm (transmission-mode) of the field-portable microscope shown in Fig. 1(a). (b and c) Cropped regions from the raw holographic image shown in (a). Lensfree in-line transmission holograms of the cells are visible in these zoomed images. On the top right, the reconstructed images for these two regions of interest (b and c) are shown. At the bottom right, our reflection microscope images of the same regions (b and c) are illustrated, providing a decent match to the transmission microscope images of the same regions. On the bottom left, several zoomed regions (A–D) taken from our transmission and reflection images are also provided, highlighting different contrast mechanisms present in the reconstructed phase and amplitude as well as the reflection microscope images. Note that the blood smear sample of this figure is processed with the Wright-Giemsa staining kit following the same protocol outlined in ref. 3.

Fig. 5.

Comparison of our field-portable dual-mode microscope (Fig. 1a) against a conventional bench-top microscope (10× objective-lens; 0.25 NA) is provided for a histopathology slide (skin tissue) that is imaged in reflection mode.