Geometric Solution of Image Degradation by Diffraction in Lensless Sensing and Microscopy
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Abstract—This letter proposes a non-computational method of counteracting the effect of image degradation introduced by the diffraction phenomenon in lensless microscopy. The method builds upon the certainty that all the optical images (whether focused by lenses or not) are diffraction patterns and vice versa, which preserve the visual information up to a certain extent determined by the size of the point spread functions, like airy disks in some cases. A highly diverging beam can be exploited to reduce the spatial extent of these point spread functions relatively in the transformed projective space, which can aid us in the spatial unmixing of the visual information. The principle has been experimentally validated by the lensless imaging of red blood cells of diameter $\sim 6-9$ µm and a photolithography mask with features in micrometer scale. The important advantages of the proposed approach of shadow imaging is the improved depth of field and a drastic increase in the sensor to sample working distance.

Index Terms—Image degradation, Diffraction, Illumination, Sensing, Microscopy, Optical Fiber.

Shadow microscopy of the weakly absorbing samples with visible light has been discussed sometimes before [1], [2], [3]. The imaging system contains a light source to illuminate the object and an image sensor to record the object’s shadow. The restriction to deposit sample directly on the sensor’s surface, for preserving the high resolution limits its use to on-chip cell or tissue cultures only. For instance, in the paper of Yang et al, 2018, the sample to sensor distance is 5 µm only, which is the thickness of the protective coating on the sensor surface [1]. The fundamental problem that underlies lensless imaging is the scattering of the incident light by the sample and because of this phenomenon, every point in the object produces a cone of light which is projected as a point spread function (PSF) on the sensor. In incoherent illumination, this PSF can be approximated as a space-invariant Gaussian pattern whose variance depends on the sample to sensor distance [4]. Because of the very narrow frequency bandwidth support of this Gaussian pattern, the reconstruction problem is severely ill-posed [5] and high resolution reconstruction of the object is very difficult (and not reported satisfactorily as per authors’ literature survey). One solution is to use a coded mask in the beam path, so that the PSF is the magnified image of the mask itself (see [6], [7], [8]). Now by the choice of a mask of larger frequency bandwidth, a high resolution reconstruction of the object can be obtained using appropriate algorithms. An alternate solution is to use a coherent illumination, where the PSF naturally has a very large frequency bandwidth support unlike a Gaussian beam. This approach is very common for lensless microscopy and is very well-known by the name of “lensless or digital in-line holographic microscopy” sometimes shortened as LIHM or DIHM (see [2], [9], [10], [11] for details).

Unlike coded aperture imaging and LIHM, this letter does not consider solving the inverse problem for reconstruction of the object from the measurements. Instead this letter focuses on a geometric solution for preserving the information about the microscopic sample. A diffraction pattern is also an image of the sample (and vice versa [12]) which contains the visual information up to a certain resolution, determined by the spatial extent of the point spread function (PSF). Suppose there are two points in the object which are illuminated with a parallel beam of light. For a certain sample to sensor distance $z_2$, suppose the PSF has a radius $d$. We assume the spatial invariance of this PSF in our field of view for the sake of simplicity in this thought experiment. Now the signals from the individual points will start overlapping if the distance between these two points becomes less than $2d$ (see figure 1a). We can safely comment here that the half-pitch resolution limit is the radius of the PSF itself. (Please note that we are not considering the Rayleigh limit because the PSF here is an arbitrary function which will depend on the coherence of the...
light and the strength of unscattered part of the light. PSF shown in figure 1 is only indicative). Alternatively, if a high numerical aperture (NA) illumination i.e. a diverging beam is used to illuminate the same two points, a magnification \( M \) will be introduced in the image. In this case, the half pitch resolution limit is not the radius of the PSF \((d + \delta d)\) but the ratio of \((d + \delta d)\) and \(M\). In other words, the signals (or the centers of the patterns) from the two individual points are obtained at a larger separation, due to the different geometric projection angles for the different points (see figure 1b). The value of magnification is the ratio of distance between the light source and sensor plane \(z\) and the distance between the light source and object plane \(z_1\) i.e. \(M = \frac{z}{z_1}\) \[13\], \[14\]. Please note that this geometric magnification can be effectively exploited for microscopy here only if the magnification is large enough to counteract the loss of resolution due to the degradation introduced by the PSF.

A solid core photonic crystal fiber (PCF) of numerical aperture (NA) \(0.38 \pm 0.05\) and an effective mode field diameter of \(1.6 \pm 0.3\ \mu m\) (both the values at 780 nm respectively) has been used as an illumination source in this letter. This NA value corresponds to a half cone angle of 22.33 degrees. Please note that for a step index single mode optical fiber, the NA value is around 0.1, which corresponds to a half cone angle of 5.74 degrees only. The fiber is connected to a pigtailed laser diode of wavelength 830 nm. Some images have also been captured with illumination wavelengths 670 nm and 1310 nm for the generalization. Sample fixed on a glass slide is mounted on a micrometer stage to control its three-dimensional position precisely. This provides a mechanical control of both the magnification and the lateral scanning of the sample. A pinhole aperture of arbitrary diameter of \(< 1\) mm has been kept between the sample and the sensor to restrict the rays from the feature of interest only. This helps us to filter out any rays from the high scattering angles from the features outside our field of view.

In lensless in-line holographic microscopy (LIHM) at unit magnification, the pixel-pitch of the image sensor determines the limit of resolution (along with some other factors), unless some sub-pixel super-resolution technique is employed. So a high resolution image sensor is an essential requirement in LIHM. In the proposed method, the high resolution of the image sensor (i.e. small pixel-pitch) is of little interest but a significantly large sensing area is the essential requirement.
Fig. 4. Projections of RBCs using the proposed method at different magnifications, pixel-pitch is $\sim 18 \mu m$ for all the images corresponding to the proposed method. (a-c) at magnification $\sim 100$ under three different illumination wavelengths. (d) at magnification $\sim 150$, wavelength 830 nm. (e-f) at magnification $\sim 200$, wavelength 830 nm. (g-h) at magnification $\sim 200$ with pseudocolor, wavelength 830 nm. (i) at magnification $\sim 100$ visualized using pseudocolor and mesh plot, blue denotes high intensity and red denotes low intensity, wavelength 830 nm. (j) under bright-field microscope with 40x objective lens and broadband illumination. (k) under atomic force microscope.

Fig. 5. (a) RBC under a bright field microscope with 100x objective lens and broadband illumination. (b) RBCs under an atomic force microscope showing the height using color.

The reason can be understood with this instance: if a $25 \mu m$ feature is magnified by 200 times, it will we projected on a 5 mm sensor area. In this letter, a lead-oxysulfide vidicon image sensor of 9.5 mm (vertical) $\times$ 12.7 mm (horizontal) sensing area has been used. The horizontal and vertical resolutions of this image sensor is around $18 \mu m$ both (9.5 mm/525 vertical raster scans and 12.7 mm/700 horizontal TV lines).

To experimentally demonstrate the principle discussed in this letter, we first show two diffraction patterns captured at magnifications around 1 in the figures 3a and 3b. These figures show the projections of isolated red blood cells (RBCs) of diameter $\sim 6-9 \mu m$. For these two figures, the spatial extent of the PSFs is much larger than the size of object under observation and hence no visual information can be obtained directly by looking at these projections. Reconstructions from these diffraction patterns (using angular spectrum method [15] based on the principle of in-line holography) have also been included (see figure 3c and 3d), to assist the reader in recognizing the degradations introduced in these images by the diffraction phenomenon. Next we introduce a magnification of around 100 times using the optical setup and imaging geometry shown in figure 2 and the results have been shown in figures 4a to c. Unlike figure 3, isolated RBCs can be observed easily in these images. This change in the visual appearance of the diffraction pattern and the spatial unmixing of the signals from individual RBCs in the latter images is the...
direct experimental validation of the principle presented in this letter. Figures 4e to h correspond to an optical magnification of around 200 times obtained using the same imaging geometry, now a single RBC image extends to a length of around ∼1.5-2 mm on the image sensor. For instance, for the figure 4e, the digital resolution is 96×104 and pixel pitch is ∼18 µm (pixel pitch is same for all the images acquired with setup shown in figure 2). In these images, even the well-known concave shaped morphology of the RBCs [16] can be undoubtedly observed. Please note that the light source and the image sensor are fixed, only the sample's position is changed to control the magnification. As the magnification is increased, the field of view decreases proportionally. Figure 5 shows the diameter and the thickness of an RBC measured in a bright field microscope and an atomic force microscope as the gold standard methods.

In figure 6, images of a photolithography mask with features of dimensions ∼600 nm (first bright vertical line like feature from left), ∼3 µm (next dark vertical feature) and ∼1 µm (second bright vertical line like feature) have been shown for the further validation of the imaging principle and resolution. A full pitch resolution of around ∼2-3 micrometers can be anticipated for the proposed method, from the images of the red blood cells and this photolithography mask.

The images captured using this imaging geometry have one-point perspective present, due to the depth dependent magnification. One advantage of this method is that the depth of field is not limited to few microns (or less than a micron) like in lens based microscopy operated at similar magnifications. Also the sensor to sample distance (in lensless shadow microscopy) is increased from the micrometer scale [11] to the centimeter scale, this eliminates the restriction to deposit sample on the sensor surface. The optical fiber tip was in a close proximity of the sample (∼50-200 µm), this can be handled using an appropriate instrumentation. Please note that the stochastic vibration of the cleaved end of the optical fiber tip is also a factor contributing to the blurring of images. At last, the utility of this principle can be found in the design and development of cell counters, flow cytometry, imaging in microfluidics, crack detection and in the development of microscopes.

In conclusion, the image degradation introduced in the lensless shadow microscopy by the diffraction phenomenon can be suppressed by changing the illumination strategy. Geometric magnifications of large values obtained using a large numerical aperture light source, can help to us perform lensless imaging without involving any computational reconstruction step. Sub-pixel resolution has been clearly demonstrated, as the pixel-pitch of the image sensor used was ∼18 µm and the sample being captured was of size ∼6-9 µm or of further smaller size. Sensor to sample distance increased drastically from a few micrometers to around a centimeter using the proposed principle.

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