Fourier Ptychography Microscopy Resolution Improvement Employing Refocusing

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Abstract: Fourier ptychography is a computational imaging technique that is used to overcome the physical limitations in determining the spatial resolution of an optical system by combining a large number of low resolution images. The low resolution images are acquired using programmed illumination from an array of light sources, thus enabling the scanning of the k-space, which is the reciprocal space of the real domain. The use of this approach would improve the resolution when biological samples from patients are analyzed in multiparametric chambers. When an off-center light source is used at an oblique incidence angle, the optical path length changes thus defocusing the expected image. The common depth of focus of microscopes is a few micrometers and when a chamber of thickness from 0.5-1.0 cm is used, an adjustment of the focusing is needed. Here in this work, we report Fourier ptychography using LED illumination and an improved image quality is acquired when refocusing is implemented.

1 INTRODUCTION

Acquisition of high resolution images is essential in medical imaging and this is facilitated by an optimal combination of high-end optical systems and computational imaging. Costly machineries often have bench-top design that are sturdy and need the associating components to adapt to their design. The development of the experimental instruments has enabled the measurement of several vital parameters of patients in the same setup. In this case, in order to use a small amount of diagnostic specimen (saliva, blood etc) microfluidic platforms are used (Chmayssem *et al*., 2021). To monitor the health state of cells under different stress conditions microscopy is employed. To increase the resolution of microscope intuitive modifications have been applied in the illumination such as replacing the light source by a LED array (Zheng et al., 2011) and then later this LED array enabled illumination is used to scan the reciprocal space (k-space) of the light propagation. This technique has led to the development of Fourier ptychography that enables the increase of the resolution of the acquired images in both phase and amplitude (Zheng et al., 2013). This technique can overcome the physical limitations of the microscope as it can increase the depth of focus, thus enabling a broader range of focused sample. Tian *et al*. (2014) implemented multiplexed illumination to reduce the number of images to be acquired thus optimizing the runtime of the experiment and the time needed to run the reconstruction algorithms when acquiring the final image. This latter contribution would prove valuable in case when one is using microfluidic platforms and monitoring is conducted by microscopes. If a biological sample is undergoing some change because of some induced stress, then one would have to acquire all the images and then analyse them in a short time. The work reported so far in the literature has been the proof of principle and is applied on bare samples that are attached on microscope glass slides. Even when applied on simple glass slides in a multiplexed mode, some of the images could diverge out of focus as the depth of focus is limited. This happens as the optical path length in air surrounding the sample (on the side of the incoming light illumination and on the side of the outgoing modified light past the sample) is different when different LEDs are turned on. In this case the optical path length is comparable to the geometric

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path length. When microfluidic chambers are used, their larger than one index of refraction (comparing to air) leads to an increase of the difference between the optical path length and the geometric path length. Considering that a single LED source results in a normal incidence to the sample, all the other secondary LED illumination sources have an oblique incident angle and this brings to the attention the need to refocus the sample. At the same time one may consider that the microfluidic chambers do not have a perfect width across the volume as few micrometers of difference in thickness may be inevitable. This constitutes another source of the undesired defocusing. At the same time, complex fluids that are used in the microfluidic channels may exhibit varying index of refraction as a result of mixing with different densities of biological samples and this will then change the optical path length. All these arguments reminds one on the need of careful refocusing before each image acquisition. Here in this work we compare the performance of Fourier ptychography reconstruction as the focus control is implemented each time we acquire an image. We observe that a refocusing improves the quality of the reconstructed image.

2 THEORY AND EXPERIMENTAL DESIGN

The main limitation in medical imaging using optical microscopy is the spatial resolution. Under the visible light spectrum, it is not possible to observe images of objects smaller than half of the wavelength of the incident light source (0.4 to 0.7 μm). Furthermore, in live cell examination, unsuccessful results may be obtained because often contrast is low. Staining with selective dyes, which are used to increase the contrast, may modify the sample and introduce other structural features that are not present in the specimen. With all the associating challenges, there is a continuous increase in the number of patients being examined using microscopic techniques rather than other diagnosis methods, as they are consistently accurate and of low cost with the latter being essential in resource-limited settings. An increase in the demand for digital microscopes in the current coronavirus pandemic has been observed. Some of the greatest innovators that operate in the market of digital portable microscopes include Carl Zeiss AG, Olympus Corporation, Keyence Corporation, Nikon Corporation, Leica Microsystems etc. These benchtop commercial microscopes can be used to implement Fourier ptychography.

2.1 Theory and Mathematical Apparatus

Fourier ptychography is an excellent example of how one can apply algorithms in both the real (x-space) and the reciprocal domains (k-space) to improve the spatial resolution (micrometer scale). The information in the reciprocal domain (with the unit of m^{-1}) is indirectly collected as the k vector - denoting the direction of the light illumination to the camera sensor – can be selected for each image by turning on LED one by one. It uses objective lens with small numerical aperture and is able to increase the field of view. This method requires no manual scanning of the specimen plane, since a LED array is used as an illumination source. So instead of using a narrow beam of light to illuminate the sample, different angle illuminations are provided by programming the LED array to turn on individual LEDs without having to move any part of the physical system.

2.2 Experimental Design

The major construct is the combination of the programmable LED array, sample, light gathering system, and the CMOS camera. As mentioned earlier LED array is placed sufficiently below the sample so that the illumination is considered to be spatially coherent. In order to achieve this, the sample must be lifted at a fixed distance of few centimeters above the fixed LED matrix. This is done by means of metric adapter plates fixed on the metric breadboard using a 90° metric adapter that glues them together. 32x32 LED array will be used with a spacing of 4 mm between neighboring LEDs. This spacing is convenient to cover the sample area and to clearly define the changes and shifts in each data measurement. The structure of the experimental setup is shown in Figure 1. A magnifying lens (model number 378-805-3) with a relatively small numerical aperture is used with. It is a 50x magnifying lens with infinity correction.

This means that the image is directly passed to the camera sensor without being diffracted and modified inside the path from objective to the sensor of the camera. In order to perform the bright field refocusing that will correct for geometrical shifts and phase aberrations that degrade the resolution of the final image, the focus will be adjusted manually as

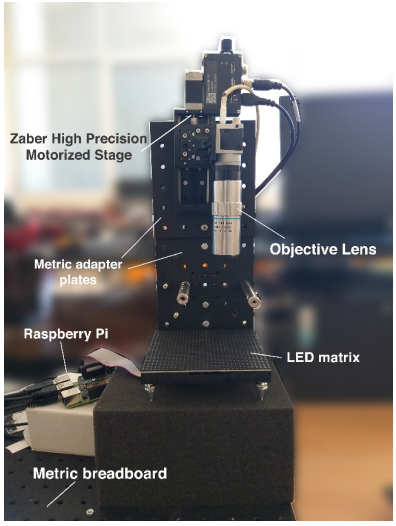

Figure 1: Experimental setup used in this work

proposed by using a step-controlled motor with high precision. The camera together with the lens is mounted to the Zaber motor (Zaber Technologies Inc. with a resolution of 0.0476 μm) that is responsible for controlling the up and down movements of the sample each time the illumination angle hence the change of focus occurs.

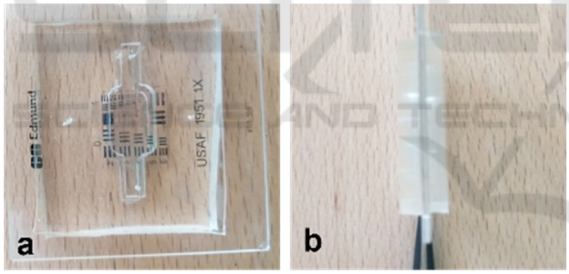

Figure 2: Microfluidic chamber material with width of 5 mm. a) Front view of the chamber enclosing USAF 1951 b) side view of the microfluidic chamber.

Its own software controls this motor while the LEDs are turned on sequentially by using a raspberry pi and a python script that identifies how the LEDs are turned on and the time slots for each of them being turned on. To acquire the images a CMOS sensor camera is used. It imposes a lot of advantages compared to CCD cameras where the most important one was the faster data acquisition rate. These cameras have lower cost compared to other cameras that may be used in modern digital microscopes. The USAF 1951 that facilitates a quantitative analysis is enclosed inside a double-sided microfluidic chamber as shown in Figure 2. The use of the chamber increased the working distance by 1 mm.

3 RESULTS

The output of the algorithm is a reconstructed image with higher resolution, one image per each dataset composed of 25 images. The details become more significant at the end of the preset number of iterations. The image with and without focus for each dataset are compared with each other by means of algorithms that calculate the contrast and the sharpness of each output. Beginning with the first and second dataset '5x5 LEDs with USAF 1951 with and without refocusing', the synthesized numerical aperture of Fourier Ptychography is 0.9363 and the reconstructed image is displayed below in Figure 3 together with the central raw image with maximum supposed intensity.

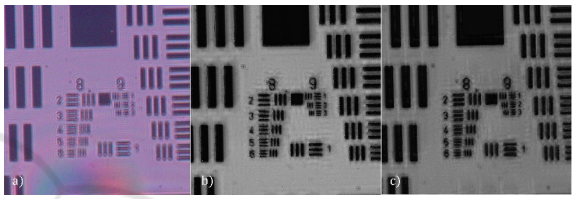

Figure 3: a) Original image captured with central LED, b) Reconstructed image without applying focus adjustment c) Reconstructed image with focus adjustment.

Computational power is used to determine the image quality of both reconstructed images with the refocus method and without. The measured parameters are Brightness, Contrast, Sharpness, PIQE (Perception based Image Quality Evaluator) and NIQE (Natural Image Quality Evaluator). The respective values are displayed in Table 1:

Table 1: Image quality measurement parameters.

	Brightness	Sharpness	PIQE	NIQE
Original Image	127.90	3.39	6.18	3.71
Rec. Img NR	102.56	8.04	26.83	6.19
Rec. Img WR	86.60	7.45	26.15	5.95

The other datasets produced similar results, but since they were turned into grayscale during the reconstruction process, it was more difficult to observe the differences caused by effect of refocusing the image. Another important aspect of FPM is the effect of thickness, or the consideration of the third dimension of the sample. Since Fourier algorithm takes into account the slightest detail of the image, the angular illumination will cause some aberrations to the obtained data. The effect of such thickness was discussed in Chapter 4, and the experiment results are also analyzed. Theoretically the glass specimen

imposed an error to the data acquisition process of nearly 1 percent, which can or cannot be negligible. This depends in the application of FPM which is about slightest details in minute length scale. In case of micro fluidic chamber of thickness nearly 4 mm per each side, the error was very significant when compared to the same sample without the chambers. The thickness effect completely distorted the signal of the image, which was reconstructed with a not very pleasant resolution. So, this must also be taken into consideration when using different types of samples with varying thickness.

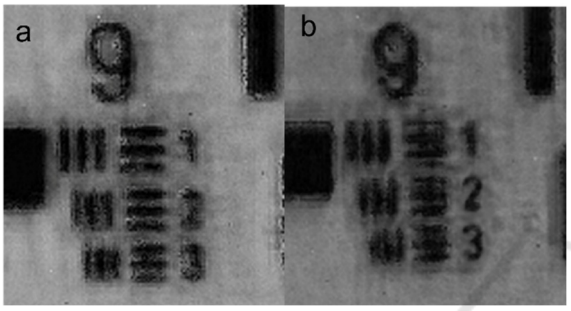

Figure 4: Reconstructed image a) without refocus, b) with refocus.

4 CONCLUSIONS

In this work we proposed a different approach to the increment of reconstructed image resolution, by adjusting the focus manually using a geometrical approach to solve the problem. The relevant concepts to the theoretical approach of the proposed solution were also explained. Theoretically the work proves to be successful, relying also in the computational power to resolve the adjustment of image resolution. When estimating the performance of an experiment that is conducted for the first time, there may be a lot of space for improvement. However, assuming the aberrations arising from moderate conditions in which the experiment was realized, the results proved the point even though not much significant improvement was made in the resulting images. In the reconstruction process, much less raw images are used, preventing the computer from drowning in unnecessary data of low to none important information of the phase and amplitude (including darkfield images as well). Reducing the number of images to 25 was not based on any logical fact rather than experience in image reconstruction. The illuminating source was enough to produce electromagnetic waves with oblique incidence, however in practice illumination is not coherent, which leads to images having reduced spatial coherence. The NA was increased from the synthetic NA produced in the algorithm of FP and the image resolution was increased up to 4 times. This could be further improved by increasing the number of captured images which comes with expense in the computational run time and large number of iterations required.

The effect of refocusing became much clear during the data analysis process, where the refocused raw data managed to produce an image of a higher quality and resolution. The manual adjustment was made step by step in order to carefully observe the change in the shape and 3D effect of each captured image. Even though it took a few minutes of adjustment for 25 images, the pattern of motion can be translated into an algorithm and run by the Zaber Console Software in order to perform the active refocusing in an automated way. Another important aspect that was discussed in the fourth chapter was the thickness of the sample. According to the theoretical analysis, it imposed no notable error for the glass sample. However, this was not the case for the practical aspect, where the resolution was decreased significantly.

The purpose of this study was to introduce a different path towards the automatization of the microscope with the help of Artificial Intelligence. The corrections that are made physically before the data acquisition reduce the computational time by performing just as well, not even better. Active focusing is in its early stages and still requires the expertise of biologists and scientists that can train the AI in the appropriate way.

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