High-resolution Microscopy with a Wide Field and an Extended Focus

Sri Rama Prasanna Pavani, Jigang Wu, and Changhuei Yang

Department of Electrical Engineering, California Institute of Technology, Pasadena, CA 91125, USA

pavani@caltech.edu , jigang@caltech.edu and, chyang@caltech.edu

Abstract: Resolution, field of view, and depth of field are tightly constrained parameters in traditional optical microscopes. In these microscopes, any increase in resolution necessarily comes with a decrease in depth of field, together with a decrease in the maximum possible field of view. Here, we propose a high resolution microscope with a wide field of view and an extended depth of field. Our system illuminates a thick sample with a series of non-diffracting Bessel beams, and images the sample on a sensor directly or with an inexpensive lens. By scanning the sample under investigation, the system proposes to acquire two dimensional images of large areas of the sample, while promptly showing intricate high resolution details, even those that are well outside the focal plane.

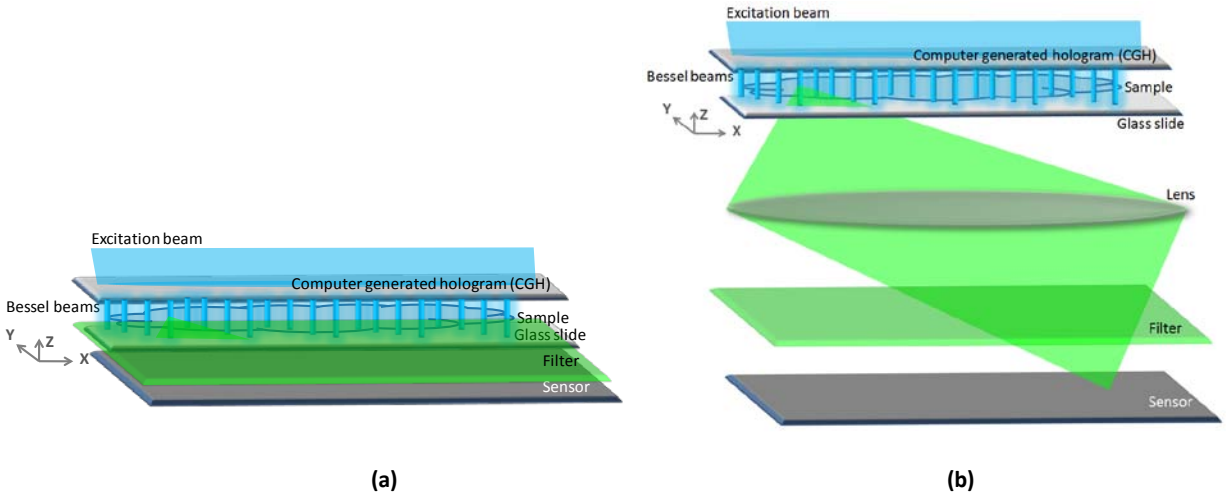
1. Introduction

With ever increasing forms and modalities, microscopes are proving to be powerful tools for exploring modern science. The gentleness of light has especially made an optical microscope a tool of choice to noninvasively probe living cells. Despite being mature tools, traditional optical microscopes do not have the ability to acquire high resolution images of large volumes of a sample. This inability is the consequence of a tradeoff between a microscope's resolution, its field of view, and its depth of field. While the resolution of a microscope defines the size of the smallest sample detail that can be discerned, the field of view defines the largest transverse (x,y) sample area that can be imaged, and the depth of field defines the maximum axial (z) sample thickness that can be imaged in focus. In conventional microscopes, any attempt to increase resolution fundamentally results in a decrease in the depth of field, and practically results in a decrease in the field of view. Today's microscopes are therefore restricted to acquire either low resolution images of large sample volumes, or high resolution images of small sample volumes.

In this report, we propose a system that has the ability to acquire high resolution images of large sample volumes. Unlike traditional microscopes, our system illuminates the sample with a series of non-diffracting Bessel beams that propagate through the sample with a constant spot size. The sample is then imaged on a sensor directly or with an inexpensive lens with a small numerical aperture. By scanning the sample through the series of beams, our proposed system has the ability to acquire high resolution images of large sample volumes.

2. Microscope with high-resolution, wide field of view, and a large depth of field

Our proposed device can be implemented as direct imaging scheme and projection imaging scheme. In the directly imaging scheme, a sample is illuminated by the Bessel beams and the transmission of the Bessel beams are detected directly by an imaging sensor (Fig. 1(a)). In the projection imaging scheme, the sample is imaged by a simple lens onto an imaging sensor (Fig. 1(b)). The imaging sensor can be a charge coupled device (CCD) or a complementary metal oxide semiconductor (CMOS) sensor. The sample is mounted on a translation stage for providing the scanning for imaging. To get an image, we can either do a raster scanning of the sample, or by using the similar scanning mechanism as in the optofluidic microscopy (OFM) [1], where the image can be acquired by simply doing a linear scanning.



(a) **(b)**

Figure 1: High-resolution microscope with a wide field of view and a large depth of field

The Bessel beam can be implemented by using a custom designed computer generated hologram (CGH) that produces a series of well separated Bessel beams, upon being illuminated by a plane wave (excitation beam). Bessel beams are non-diffracting beams. In other words, their beam width remains constant with propagation. While it is not possible to generate strictly non-diffracting beams, CGHs can be designed to generate beams that do not diffract within a limited region of space. A side by side comparison of a Bessel beam and a focusing spherical wave with 0.3 numerical aperture is shown in Figure 2. The CGH in our proposed device is computationally designed by interfering a conical wave front with a plane wave. The spot size and the focal plane of the Bessel beams are controlled by adjusting the width and the peak phase retardation of the conical wave front. By spatially shifting and summing these interference patterns, a series of Bessel beams can be generated.

The other way to implement the Bessel beams is to use a two dimensional microaxicon array. As we know, the axicon is a conventional way to generate Bessel beam. The fabrication of the 2D microaxicon array can be implemented by multilayer lithography or etching processes. The efficiency of the microaxicon array will be better than binary CGH since there's no multiple diffraction.

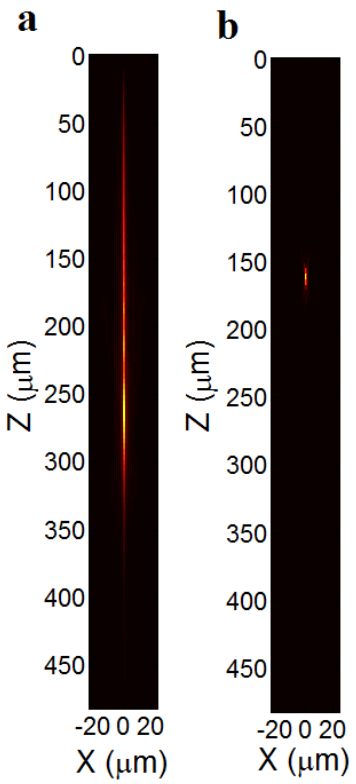


Figure 2. Comparison of (a) Bessel beam and (b) a focusing spherical wave with 0.3 numerical aperture

The single-lens imaging module projects an image of the illuminated regions of the sample on the sensor. An appropriate filter may be used on top of the sensor in the case of fluorescence imaging. By using an imaging lens with numerical aperture (NA) much smaller than that of the Bessel beams, we make the depth of field of the imaging lens to match or exceed the maximum non-diffracting range of the Bessel beams. Consequently, when the lens is positioned such that its object plane is located half way through the Bessel beams, the entire sample thickness illuminated by the Bessel illumination module is imaged sharply in-focus on the sensor. It is worth noting that the resolution of our system is limited only by the spot size of the Bessel beams, and not on the resolution of the imaging lens. It suffices for us to use a low NA imaging lens whose resolution is as large as the distance of separation between two adjacent Bessel beams. Interestingly, such a low NA imaging lens also permits us to acquire aberration-less images of a much larger region of the sample than what would have been possible with a traditional microscope with a similar resolution.

At any given time, the information from the sensor corresponds to a set of discrete transverse (X,Y) points on the sample, with an extended depth (Z) range. A raster scan of the sample, while continuously

acquiring images from the sensor, enables us to sample all transverse points on the sample, and consequently create a continuous high-resolution 2D image of the sample with a wide field of view and an extended depth of focus.

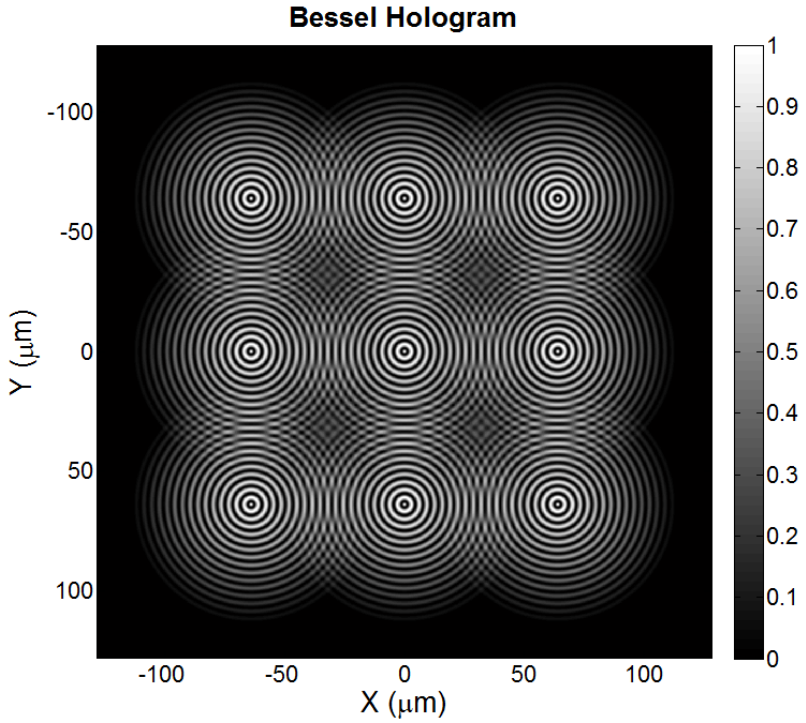


Figure 3: Amplitude hologram that generates 9 bessel beams

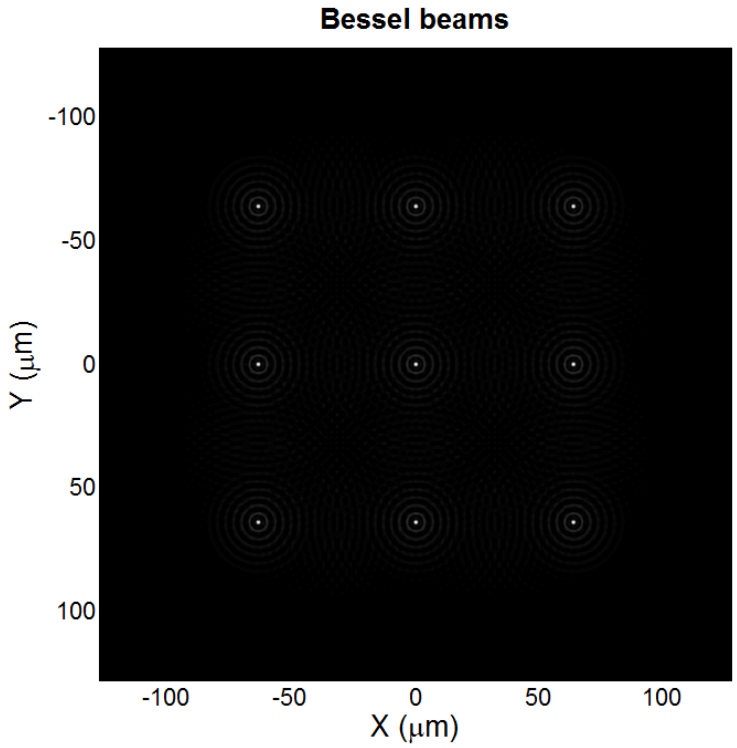


Figure 4: Bessel beams generated by the amplitude hologram in Fig. 3

3. Example of a computer generated hologram for generating Bessel beams

Figure 3 shows an example of a CGH that generates nine Bessel beams with the following parameters: spot size = 1 μm, separation between beams = 64 μm, CGH-focus distance = 160 μm, and wavelength = 0.5 μm. When illuminated with a spatially coherent beam, the CGH forms nine Bessel beams after an axial distance of 160 μm from it (Figure 4).

4. Fabrication of Bessel computer generated holograms

Depending on resolution requirements, amplitude computer generated holograms (such as Fig. 3) can either be printed with grayscale graphics printers, with photoplotters as halftone images, or can be fabricated as chrome/iron oxide binary photomasks.

Typically, the printing resolution requirement scales with the desired resolution of our system. For example, to generate Bessel beams with 1 μm spot size, the smallest feature size in our CGH is of the order of 1 μm. In this case, the resolution of printing technique used should be at least 1 μm. Such high resolution CGHs may be fabricated as binary chrome/iron oxide photo masks. While it might be expensive to fabricate the master chrome mask, copies of the master can be readily recorded either as optical holograms, or be duplicated using standard photolithography techniques.

Conclusion

We have proposed a microscope that has the ability to acquire high resolution images with a large field of view and an extended depth of field. By decoupling the tradeoff between resolution, field of view, and depth of field, our microscope proposes to enable biologists to investigate large volumes of sample, while simultaneously being able to discern intricate high resolution details.

References:

1. X. Heng, D. Erickson, L. R. Baugh, Z. Yaqoob, P. W. Sternberg, D. Psaltis, and C. H. Yang, "Optofluidic microscopy - a method for implementing a high resolution optical microscope on a chip," Lab on a Chip **6**, 1274-1276 (2006).