Optimization of Bessel Beam for Light Sheet Microscopy

Paokuan Chin, Blake Madruga, Hector Garcia, Katsushi Arisaka

UCLA, Department of Physics and Astronomy

Introduction

Bessel Beam is a beam whose radial amplitude is the Bessel Function of the first kind (Fig. 1a & 1c), while a Guassian beam is defined in a similar way (Fig. 1b & 1d).

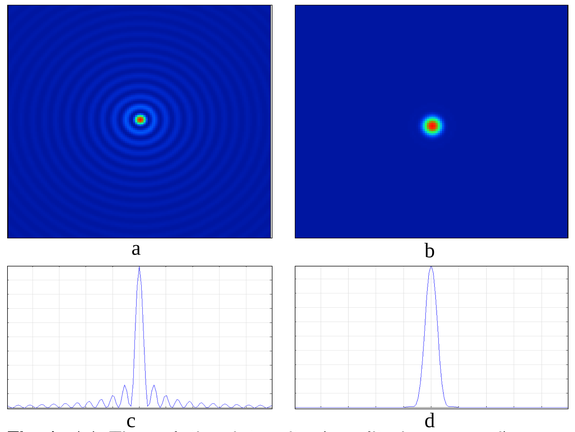

Fig 1: (a) The relative intensity (amplitude squared) cross section of a Bessel beam. (b) The intensity cross section of a Gaussian beam. (c) The relative radial intensity of a Bessel beam. (d) The relative radial intensity of a Gaussian beam. All plots are simulated by Zemax.

A Bessel beam does not diffract in free space as it propagates, i.e., it's central lobe remains in the same width (Fig 2a). In contrast, a Gaussian beam flares out as it propagates through space, up to a factor of $\sqrt{2}$ of its waist as it reaches its end (Fig. 2b). Furthermore, when a Bessel beam is obstructed, it reconstructs itself. It is for these reasons that we wish to incorporate Bessel beam into light sheet microscopy.

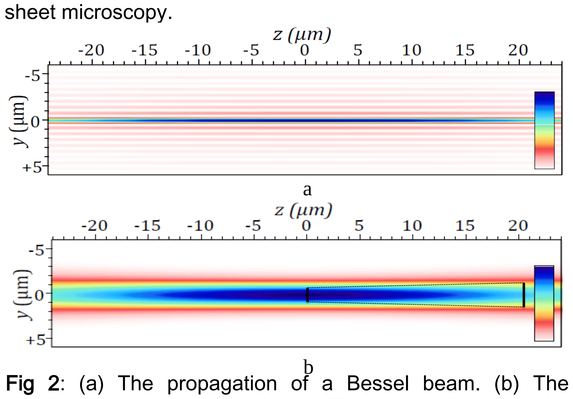

Fig 2: (a) The propagation of a Bessel beam. (b) The propagation of a Gaussian beam. The z axis represents the direction of propagation. The width (full width half maximum) of the Bessel Beam remains invariant, while that of the Gaussian beam expands, as indicated by the black lines.[1]

Ideally, a Bessel beam is created by colliding plane waves at a constant azimuthal angle (Fig 3), and so the beam would be infinitely long and still invariant in width. In practice, an approximate Bessel beam can be generated by passing a collimated (laser) beam through an axicon lens (Fig 4a).

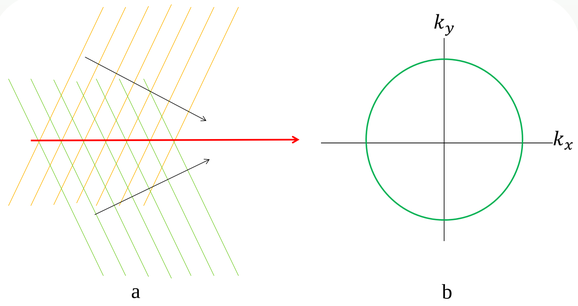

Fig 3: (a) An ideal Bessel Beam is created by colliding plane wave at constant angle. The black arrows indicate the k vectors, while the red arrow indicates the Bessel beam. (b) In k space, the colliding plane waves form a circle.

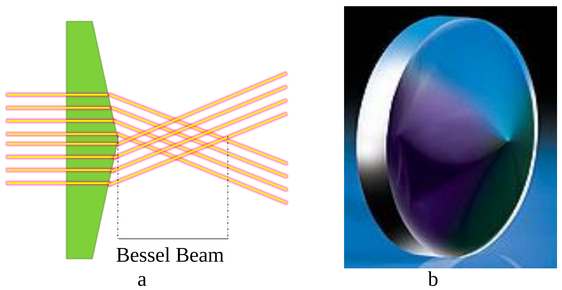

Fig 4: (a) An approximate Bessel beam can be created by shining collimated light at an axicon. (b) A physical axicon.[2]

To incorporate Bessel beam into light sheet microscopy, the beam needs to be shaped into the desired dimension. Typically, this can be achieved by manipulating the beam with lenses and an objective at the end. Furthermore, a galvanometer needs to be placed at the conjugate of the back focal plane of the objective to scan the beam in order to form a sheet.

Objective and Method

We wish to understand how lenses and objectives can shape the Bessel beam. For a Gaussian beam, it's a tradeoff between the width and the length of the beam. The longer the beam, the thicker it is. It all depends on the numerical aperture (NA) of the beam.

For Bessel beam, its length of the beam can be approximated by geometrically tracing parallel rays, while the width of the beam is calculated by Zemax, a software for optical simulation (Fig. 5). Specifically, it shows the cross-sectional point spread function (PSF) of the beam (Fig. 1). Though a Bessel beam is formed right after an axicon, its size can be manipulated through lenses. It is our goal to see exactly how lenses can affect the dimension of a Bessel beam. Then we may create one of desired dimension and incorporate it into light sheet microscopy. Note that these calculations are idealistic, as they take the incoming rays as perfectly parallel. In reality, a laser is used and it's rays are not parallel.

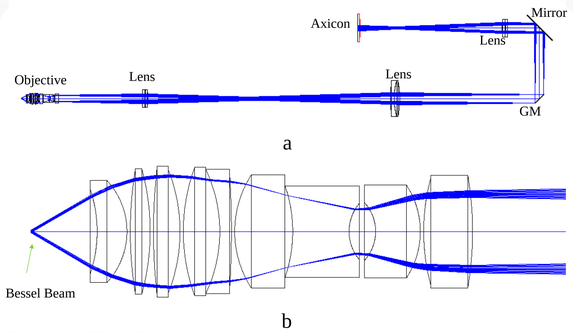

Fig 5: a) The Zemax layout of lenses and rays (blue lines).GM stands for Galvanometer. b) The objective is magnified here. The Bessel beam is at the leftmost tip of the rays. It is about 260 μm long.

Simulation Experiment and Analysis

After the Bessel beam, the rays of light become an expanding ring (Fig.4a). We define W_r to be the width of the ring, and D_r to be the diameter of the ring (measured from the center of the band of the ring). Lenses after the axicon must be placed in 4f configuration (i.e. their separation is the sum of their focal length). When passing through lenses, the rays alternate in forming Bessel beam and forming a thin ring, as is shown in Fig. 6.

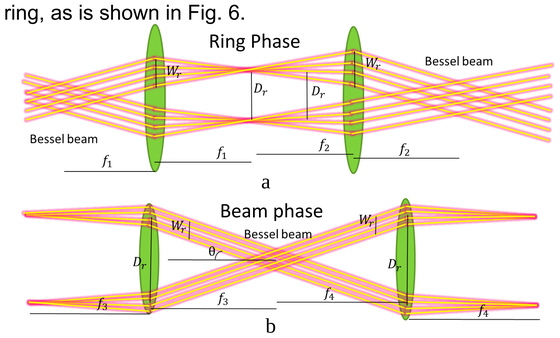

Fig 6: a) Between the lenses is when the rays form a ring. After and before the two lenses, the rays form the Bessel beam b) Between the lens is when the ring is focused and collapse into a Bessel beam. $n \sin\theta$ is the Na. Both diagrams show lenses in 4f configuration, where f indicates the focal length.

As suggested by the figure, when the light is in the ring phase (Fig.6a), W_r first contracts and then expand. The ratio of the initial W_r to the final W_r is simply f_2/f_1, while D_r doesn't change. On the other hand, in beam phase (Fig.6b), W_r doesn't change, but the ratio of intial D_r to the final D_r is f_4/f_3. Furthermore, in the beam phase, it can be shown geometrically that the length of the beam is $W_r \cot\theta$, or in terms of NA

$$L = W_r \frac{\sqrt{n^2 - NA^2}}{NA} \qquad (1)$$

Next, the other parameter of the Bessel beam is its width. We conjecture that NA will have an effect on it, as with a Gaussian beam. In Zemax, we simulated Bessel beam of different NA/n. The result is Fig.7.

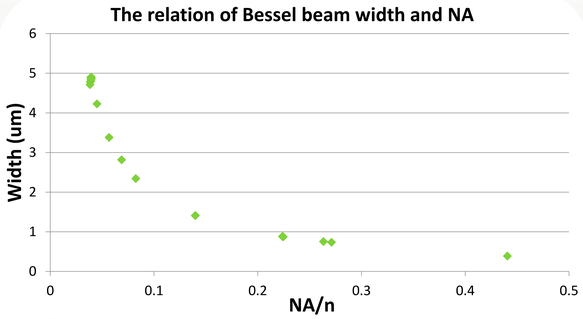

Fig 7: In Zemax simulation, numerous NA/n are used to form the Bessel beam. The lens system is in air, so n is 1. The width is defined as the FWHM of the PSF (Fig.1). We conclude that the width is inversely proportional to the square of NA/n

On the left are a cluster of data points that have similar NA/n but different L (i.e. beam length varies). They all have similar width, proving that L is decoupled from W (width). Furthermore, we take the inverse square of NA/n and plot it against W, and the result shows a line. Thus we conclude

$$W \propto \left(\frac{n}{NA}\right)^2 \qquad (2)$$

Microscope Setup and the Next Step

With the knowledge of Bessel beam shaping, we have setup a sheet illumination microscope that features a sheet area of 1mm order and sheet thickness of 1μm order. Such dimension can never be achieved with a Gaussian beam. The image of the beam is shown in Fig. 8, while the setup of the microscope is shown in Fig. 9. We have been able to image an 1mm^3 transparent brain[3], as shown in Fig 10.

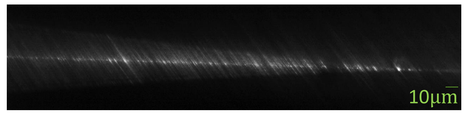

Fig 8. An image of the Bessel beam take from the side. The scattering media is water

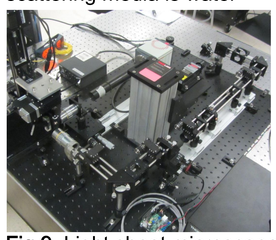

Fig 9: Light sheet microscope using Bessel beam

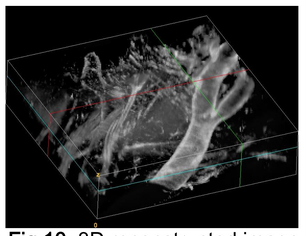

Fig 10. 3D reconstructed image of a brain.

Conclusion

We have found the techniques for Bessel beam shaping, as indicated by Fig. 6. The parameters that govern the dimensions of the beam are quantified by equation (1) and (2). With these knowledge, we are heading to a field in light sheet microscopy that Gaussian beams cannot achieve.

1. Fahrbach, Florian Olivier. 2013. *Microscopy with Self-Reconstructing Beams.* Albert-Ludwigs-Universitat Technische Fakultat Institut fur Mikrosystemtechnik
2. Picture from Edmund Optics. http://www.edmundoptics.com/optics/optical-lenses/aspheric-lenses/plano-convex-pcx-axicons/3364
3.Brain sample provided by Laurent Bentolila of CNSI light microscopy.