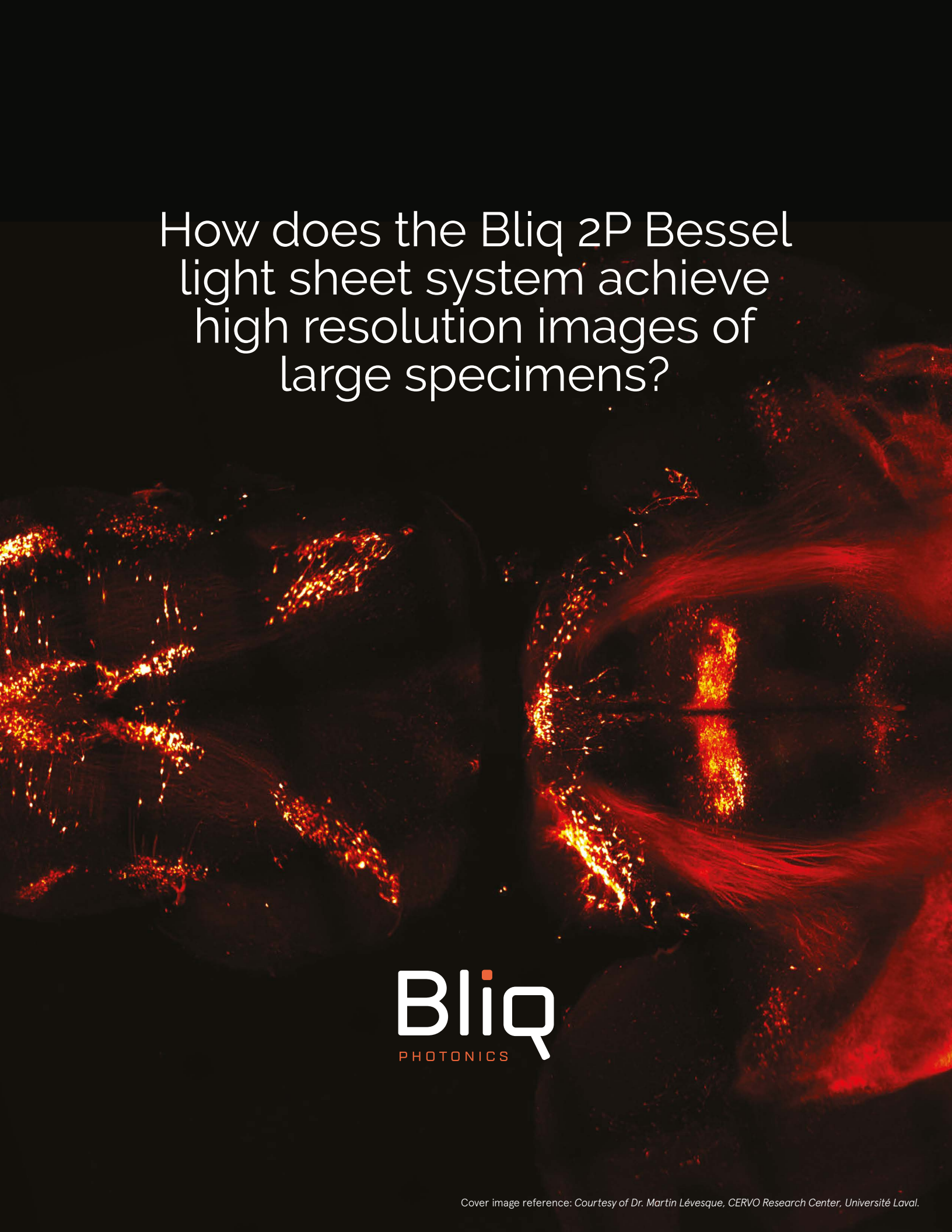
How does the Bliq 2P Bessel light sheet system achieve high resolution images of large specimens?



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2P *BESSEL LIGHT SHEET SYSTEM*

| INTRODUCTION

Light sheet fluorescence microscopy (LSFM), also known as selective plane illumination microscopy (SPIM), is an emerging optical sectioning imaging tool that can be applied in a wide range of life science fields, such as developmental biology[1], neuroscience[2], cell biology[3], and plant development[4]. This powerful technique can be used to image large volume biological (fluorescent) specimens while preserving the tissue in its maximum physiological condition[5].

In a typical LSFM, a single slice of the sample is illuminated by a thin "sheet of light". The light emitted from the sample is then collected by a detection objective whose optical axis is generally orthogonal to the illumination axis, as shown in **Fig. 1**. To image the entire specimen, either the sample or the light sheet itself must be moved[6]. In both cases, having a large field of view (FOV), which is determined by the length of the beam, is a key factor for fast image acquisition.

The FOV and the axial resolution, which is determined by the illumination beam waist are closely related[7]. Therefore, the main limitation of LSFM is a decrease in axial resolution when imaging large specimens, especially in a conventional system where the illumination source is a Gaussian beam. However, the generation of a thinner light sheet comes at the cost of a shorter range over which the beam size remains narrow. (i.e., shorter Rayleigh range)[2,6]. For example, using a 10x objective with a NA of 0.3 can generate a light sheet with a thickness of 2 µm that remains uniform only over 150 µm[6].

The disadvantage of a short FOV is that image acquisition of large specimens can become time-consuming and difficult. In summary, it appears that in conventional Gaussian beam LSFM, it is difficult to achieve high axial resolution without compromising the FOV or to obtain a large FOV without decreasing the axial resolution. But is it possible to optimize both parameters?

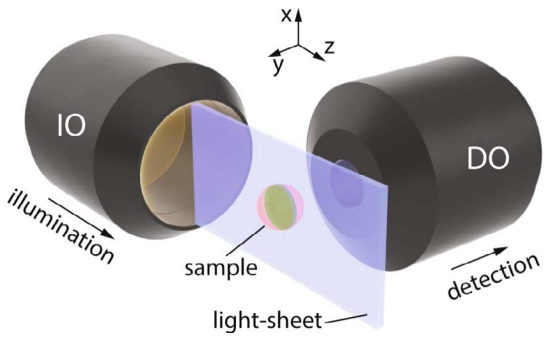

Figure 1. Principle behind light-sheet fluorescence microscopy (LSFM). The sample is placed near the illumination objective (IO) and illuminated by a light sheet; the fluorescence emission from the optical plane is detected by the detection objective (DO), which is oriented perpendicular to the illumination direction. The image is adapted from Kafian *et al.*, Scientific Reports, 2020[8].

In this paper, we explain how the Bliq SPIM can achieve an extended FOV (1.3 mm x 1.3 mm) while preserving high axial resolution (2 µm) by exploiting a Bessel beam and two-photon excitation instead of a Gaussian beam and single-photon illumination. But why using a Bessel beam and an infrared excitation?

Advantages of the Bessel beam over the Gaussian beam in LSFM

A Bessel beam is non-diffracting, with a cross-sectional profile of concentric rings. It can be generated from a diffracting Gaussian beam, by simply implementing a conical lens called an axicon in the laser path[9] as illustrated in **Fig. 2**.

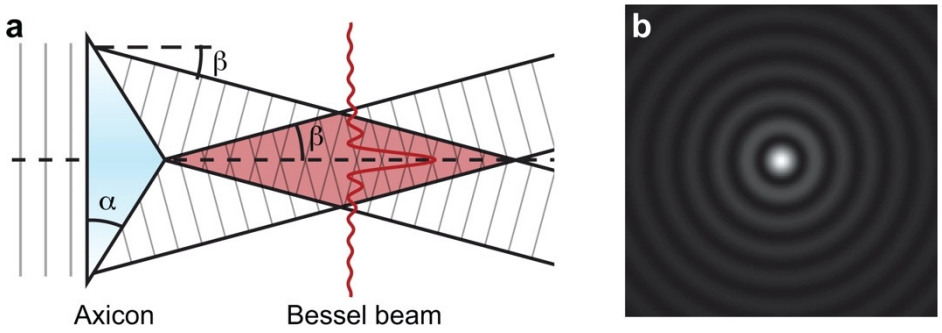

Figure 2. An axicon generates a non-diffractive Bessel beam. **a)** Illumination of the axicon (blue triangle), a conical lens of angle α, by a plane wave. The interference pattern after the axicon produces a Bessel beam that has a deviation angle β. The red trace shows the radial profile of the Bessel beam. **b)** Intensity profile of the Bessel beam. The image is adapted from Thériault *et al.*, Optics Express, 2013[9].

A notable feature of the Bessel beam is that it is "self-healing," and this property leads to a significant reduction in shadowing, which is a challenge in LSFM. This effect is generated because certain absorbing/diffusing parts of the sample prevent the excitation light from reaching sites further away from the light source[10,11]. **Fig. 3** shows how the shadow is formed when using a Gaussian beam and how the Bessel beam is recovered after an isolated obstacle.

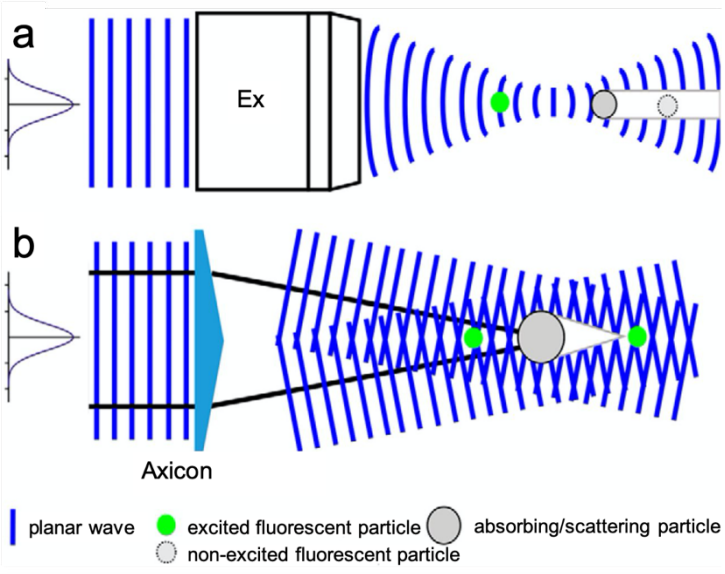

Figure 3. Shadowing effect with a Gaussian and a Bessel beam. **a)** A Gaussian beam is focused on a fluorescent particle through an excitation lens (Ex) and makes it excitable (green circle). An absorbing/diffusing particle (gray circle) blocks the light, and a fluorescent particle on the other side cannot be excited (smaller gray circle). **b)** For the Bessel beam (generated after the axicon), the beam profile is recovered after the obstacle, and the fluorescent particle behind can be excited. This image is adapted from Müllenbroich *et al.*, eNeuro, 2018[10].

Advantages of the Bessel beam over the Gaussian beam in LSFM

In addition to the reduction in shadowing, a remarkable advantage of using the Bessel beam over the Gaussian beam is to generate an extended FOV without compromising axial resolution[8,9], as shown in **Fig. 4**.

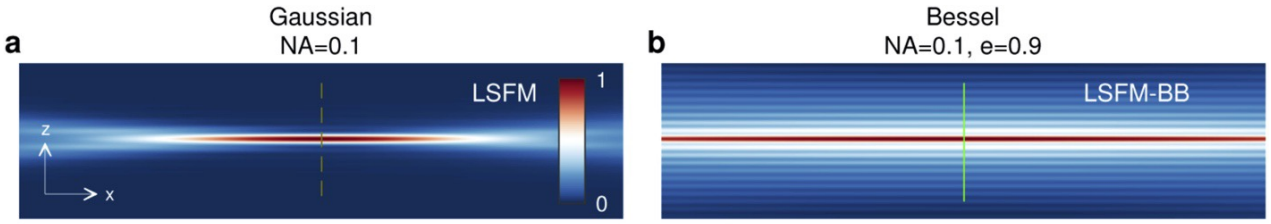

Figure 4. Simulated intensity profile of the LSFM using a) Gaussian beam and b) Bessel beam (BB) for an objective with a NA of 0.1. The image is adapted from Xiong *et al.*, Optics Express, 2020[11].

In summary, the use of a non-diffractive beam such as the Bessel beam in LSFM provides a large FOV and high axial resolution while minimizing shadowing. These features are crucial, especially for imaging large biological specimens and for achieving fast and robust acquisition.

Advantages of two-photon over single-photon Bessel LSFM

Imaging with a single-photon Bessel beam can suffer from poor contrast because much of the laser power is carried in the side lobes of the intensity profile and not in the central lobe that illuminates the focal plane[7]. As a result, on both side of the focal plane, the fluorophores are excited and an out-of-focus signal is generated. A solution to overcome this problem is to exploit a two-photon excitation instead of a single-photon excitation; the side lobes are thus suppressed (**Fig. 5**).

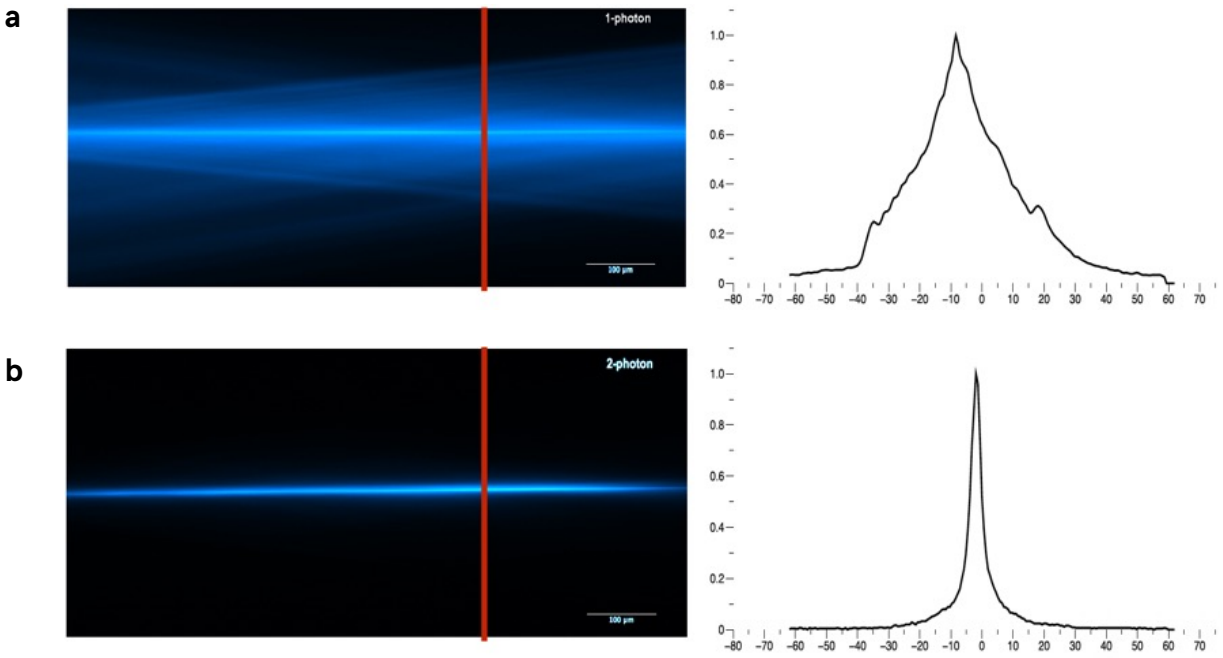

Figure 5. Side view of a fluorescence excitation generated by **a)** single-photon and **b)** two-photon Bessel beam. The image is adapted from Akitegetse C., 2021, PhD thesis, University of Laval, Quebec, Canada.

In the following, we present the working principle of the Bliq SPIM system that takes advantage of the Bessel beam and two-photon excitation.

Working principle of the Bliq SPIM

The Bliq LSFM is an open horizontal SPIM configuration that can be adapted to image a biological sample up to 4cm x 7cm x 10cm in size. The sample is only illuminated from one side, which makes the design relatively simple and avoids post-processing of the images (i.e., alignment of the images from both sides). An axicon lens is integrated into the excitation light path to generate an elongated Bessel beam (2.6 mm, twice the FOV length) across the sample. The Bliq LSFM uses a multiphoton laser to eliminate the out-of-focus signal from the side lobes of the Bessel beam. In order to form the light sheet, the beam is swept along its axis at very high speed by a Galvo mirror. The schematic of the optical system is shown in **Fig. 6**.

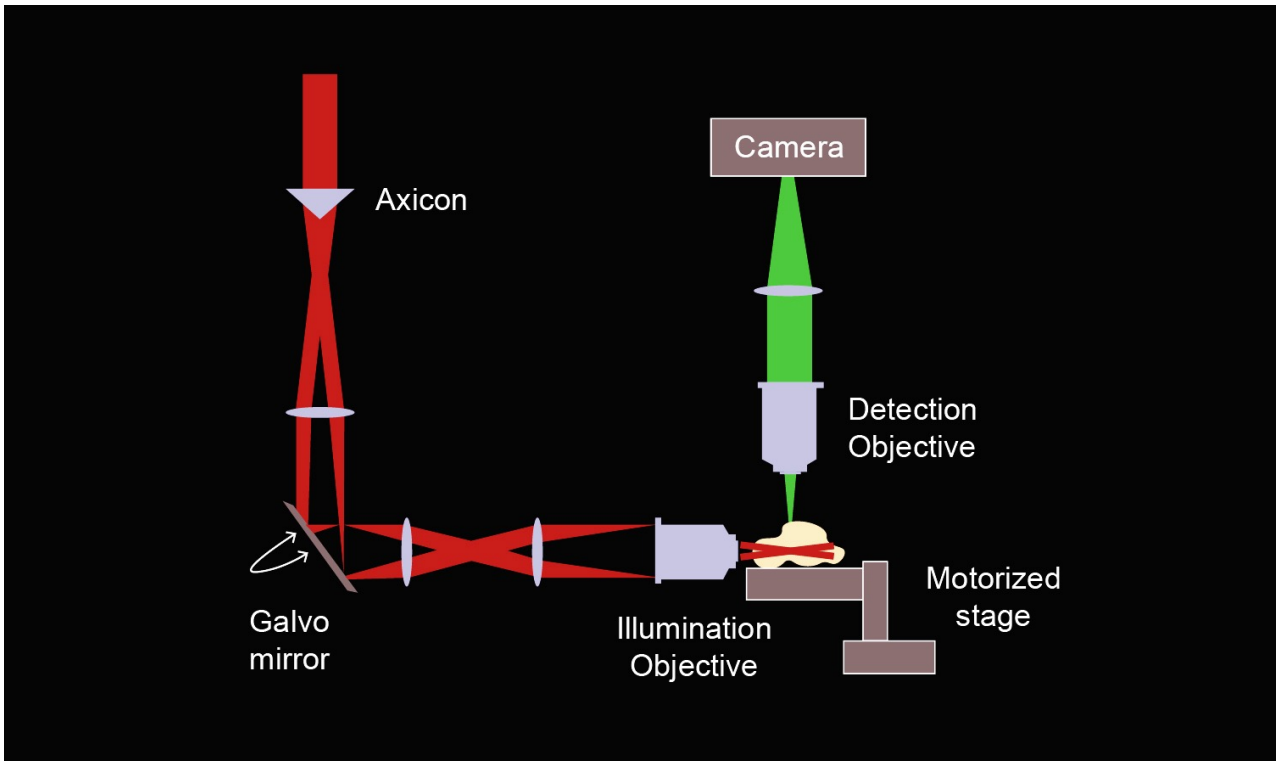

Figure 6. Diagram of the Bliq 2P Bessel beam light sheet system. The image is adapted from Akitegetse C., 2021, PhD thesis, University of Laval, Quebec, Canada.

In conclusion, the main advantages of the Bliq SPIM reside in its ability to create a light sheet of uniform thickness (2 µm axial resolution) over a large FOV of 1.3mm x 1.3 mm and thus to deliver large volume images with isotropic subcellular resolution (650 nm lateral resolution).

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