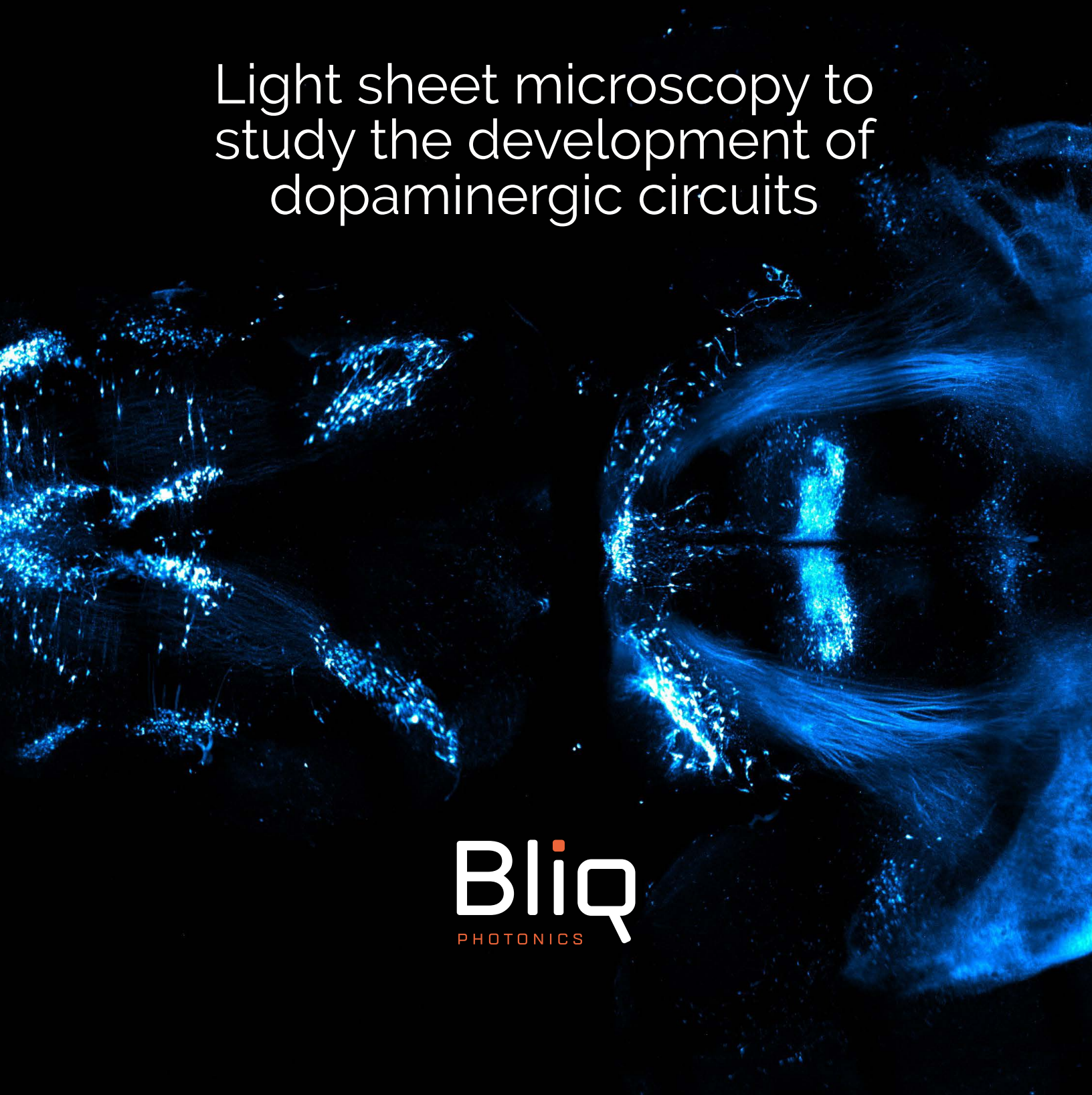
Light sheet microscopy to study the development of dopaminergic circuits



BliQ PHOTONICS

Cover image reference: *Courtesy of Dr. Martin Lévesque, CERVO Research Center, Université Laval.*

CASE STUDY

LIGHT SHEET MICROSCOPY TO STUDY THE DEVELOPMENT OF DOPAMINERGIC CIRCUITS

It has become clear that to understand complex biological systems of higher organisms, organs must be analyzed in their three-dimensional aspects. However, biological specimens are inherently opaque and due to their high lipid composition, which causes strong light scattering, it becomes a real challenge to image them clearly in depth. Fortunately, the growing number of clearing and labeling techniques for large-scale tissues now make it possible to extract detailed 3D structural and molecular information from tissues or whole organisms and to obtain clear images of subcellular structures.

Novel developments in imaging methods are important in overcoming different imaging challenges. Within this framework, light sheet fluorescence microscopy (LSFM) has proven to be an excellent three-dimensional optical imaging technique to study biological processes and observe intact specimens at high spatio-temporal resolution.

Light sheet microscopy is generally performed using a visible Gaussian beam scanned in parallel to a camera detector plane. Since the depth of field and the beam waist are closely related, the technology becomes challenging when there is a need to image bigger samples and increase the field of illumination (determined by the beam length), without compromising on the axial resolution (determined by the light sheet thickness). To overcome this challenge, Bliq Photonics has developed a unique light sheet system, using an axicon lens to form a thin needle-shaped beam (Bessel beam) that produces a light sheet when swept at a very high speed along its axis. The main advantage of this strategy resides in its capacity to create a light sheet of uniform thickness along its longitudinal profile and thus provide large volume images with an isotropic subcellular resolution. Another attractive feature of Bessel beam imaging is that the illumination profile remains undisturbed when there is an obstruction within the sample, which reduces shading compared to standard Gaussian beam technology and ensures that no data is lost. Furthermore, the system utilizes a two-photon laser to reduce phototoxicity and photodamage, as well as to penetrate deep into the specimens and remove the contribution of secondary lobes (out-of-focus signal adjacent to the focal plane) found with single-photon Bessel beams.

Studying entire rodent brains at the scale of neuronal circuits is met with the aforementioned imaging challenges. With innovative brain clearing techniques and single-cell labeling, it is now possible to trace an individual neuron from the cell body to the end of its axon. However, choosing the right imaging method and understanding its advantages and limitations can be a difficult and time-consuming process. The laboratory of Martin Lévesque at the CERVO Research Center in Québec City, Canada has chosen to benefit from the advantages of the Bliq's Bessel light sheet system in their exploration of mouse dopaminergic circuit development from embryonic to adult stages. They found that this technology is very versatile with compatibility across diverse types of samples like organoids, brains, or whole rodent embryos while providing fast image acquisition of large samples at high resolution.

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In their publication: Transcriptional repression of Plxnc1 by Lmx1a and Lmx1b directs topographic dopaminergic circuit formation, the team investigated the mechanisms controlling the development of the dopaminergic neurons. One part of their investigation was to understand the dopaminergic innervation to the striatum after a specific conditional knockout. At first, they used 60 µm thick coronal slices of mouse brain. After immunostaining against tyrosine hydroxylase (TH) to reveal the dopaminergic axons in the striatum, the group acquired images using a confocal microscope. They were able to observe a different pattern of innervation into the striatum (fig. 1.). Analysis revealed a defect in dopaminergic axonal arborization of the dorsal striatum but did not provide complete information on the spatial organization.

The use of a coronal section allowed the team to observe dopaminergic axonal projections with a dorsal-ventral and medio-lateral view. To specifically study antero-posterior organization in the striatum, the group had two options. The first was to use coronal sections of the entire striatum, followed by immunostaining for all sections and imaging on a confocal microscope or slide scanner to achieve faster acquisition. Then, to obtain the antero-posterior organization, they would have to perform a 3D reconstruction with all the coronal section images. This approach has some limitations, including the reproducibility of staining in all sections of the striatum. In addition, the group would have to make sure to collect all sections from the same brain, so as not to miss antero-posterior information of axonal arborization. Given these constraints, the group decided on an alternative that involved Bliq's two-photon Bessel beam light sheet system.

To gain the benefits of the light sheet microscope, the team first cleared the brains of control and cKO mice using the iDISCO protocol. TH-immunostaining was then performed and combined with Alexa Fluo 594 to reveal the dopaminergic axons in the striatum. Using a single mouse brain (control or cKO), the group was able to visualize the entire striatal dopaminergic arborization at once with a high axial resolution of less than 2 µm (fig. 2).

This innovative two-photon Bessel light sheet solution allowed the Levesque lab to overcome the difficulties of rapidly scanning large samples at high resolution while obtaining satisfactory three-dimensional results of whole rodent brains. Light sheet microscopy is currently used by the team to study dopaminergic neurons in the context of Parkinson's disease. The group will face other challenges, but the versatility and uniqueness of the Bliq system will be key to overcoming the constraints of these new studies that include finding the best imaging conditions to study this rare but widespread disease.

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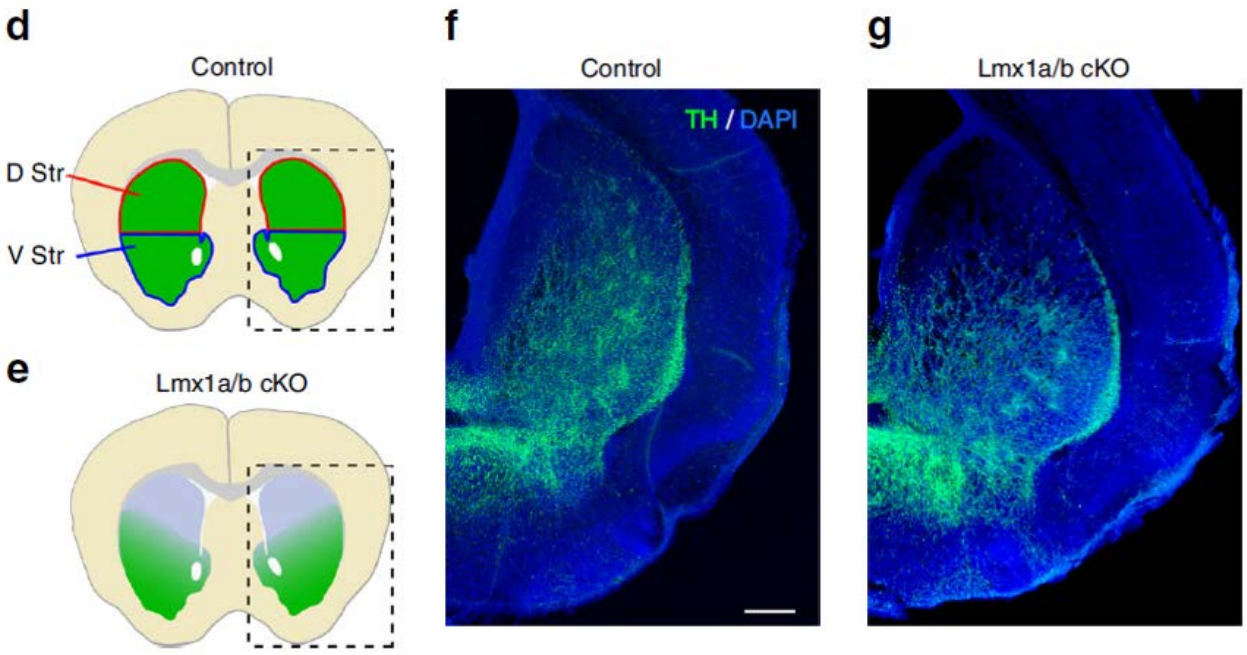

Fig. 1. Characterization of the phenotype of Lmx1a/b double conditional mutant mice at P1. **d, e** Schematic representations of axonal innervation in the striatum in control and Lmx1a/b cKO mice brains at P1. **f, g** Representative confocal images of control and Lmx1a/b cKO mice brain sections showing a loss of dopaminergic innervation in dorso-posterior striatum for Lmx1a/b cKO mice (TH in green and DAPI in blue).

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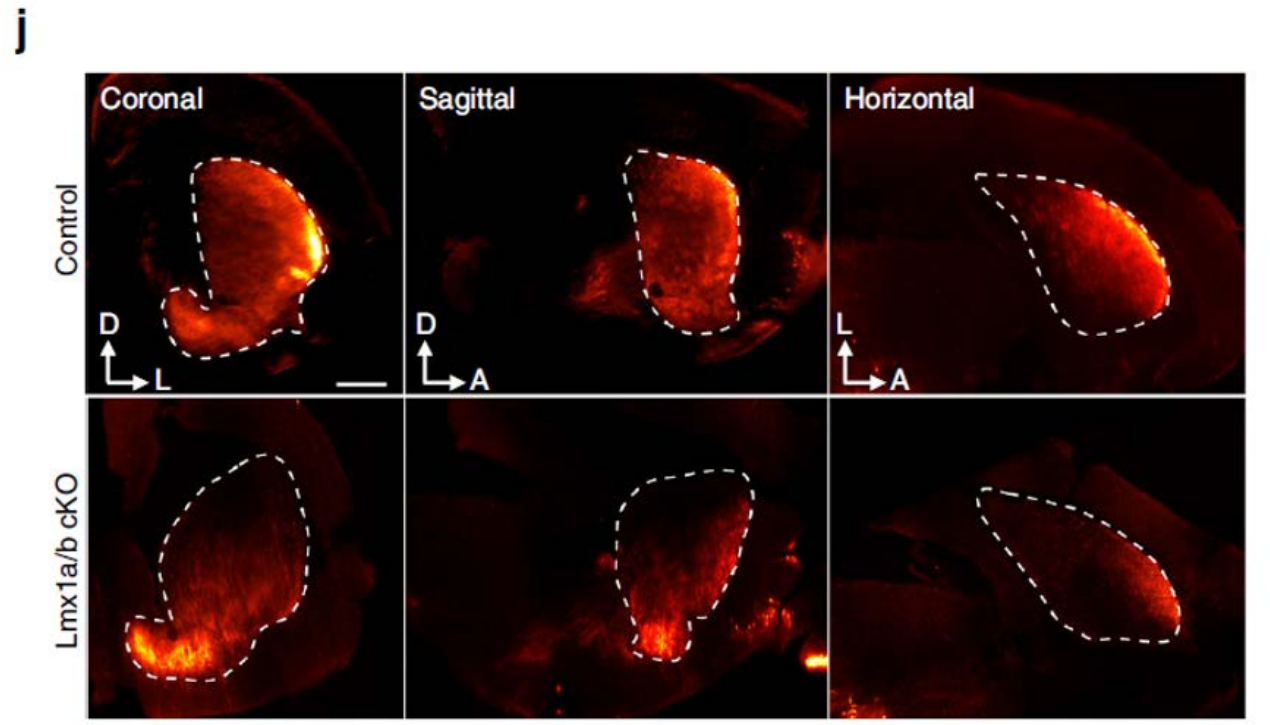

Fig. 2. Characterization of the phenotype of Lmx1a/b double conditional mutant mice with iDISCO at P1. Light sheet scans of the TH immunostaining in the striatum of iDISCO-cleared brains from control and Lmx1a/b cKO mice. The panels show single optical planes in coronal and reconstructed sagittal and horizontal planes showing the lack of TH axons in the dorsal striatum of the Lmx1a/b cKO mice brains. Dotted lines delineate the border of the striatum. D, dorsal; L, lateral.

Bliq Photonics is a Canadian company founded and based in Quebec City, that designs and manufactures imaging systems for life science applications. Our growing portfolio, backed by patented technologies, supports a variety of *in vivo* experiments. Our technologies include multiphoton, confocal and light sheet systems, real-time volumetric imaging and super-resolution.

Bliq's team understands customer vision, and crafts each imaging and multiphoton solution to meet high standards of usability and reliability.

Fueled by many years of microscopy experience we know that support and collaboration begins with a commitment to each customer.

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