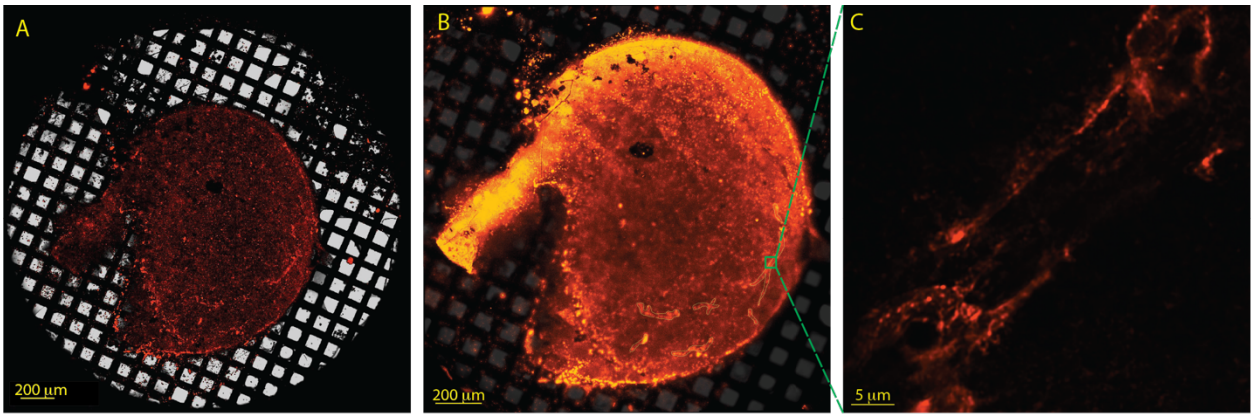

Figure 1. Cryo-FLM overview and ROIs definition for mouse brain tissue with astrocytes expressing tdTomato. All the images are acquired with a local Zeiss LSM900 equipped with an Airyscan 2 and Linkam cryo-stage. **(A)** Low-mag overview of the entire grid (2.5x) with brighfield and tdTomato channels. (**B)** 10x view with clearly identifiable astrocytes (overlay of the brighfield and tdTomato channels). Green rectangle highlights the defined ROI. **(C)** ROIs validation at high mag (100x, tdTomato).

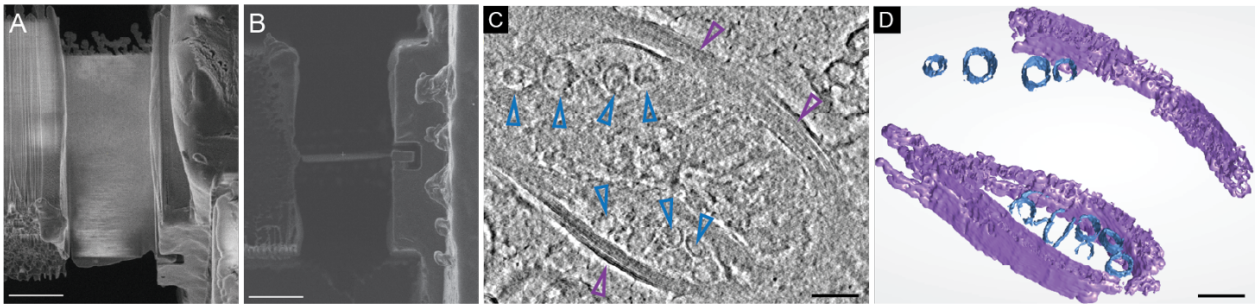

Figure 2. Example of the outputs obtained with our pipeline from a thick brain tissue sample. **(A)** Representative EB image of a finished lamella with a **(B)** corresponding IB view. **(C)** Tomographic slice showing myelinated axons (purple arrowhead) and vesicles (blue arrowhead) visualized through with a single training of neural network. **(D)** Segmentation of tomogram colored as in (C). Scale bars: **(A, B)** 5 µm, **(C, D)** 100 nm.