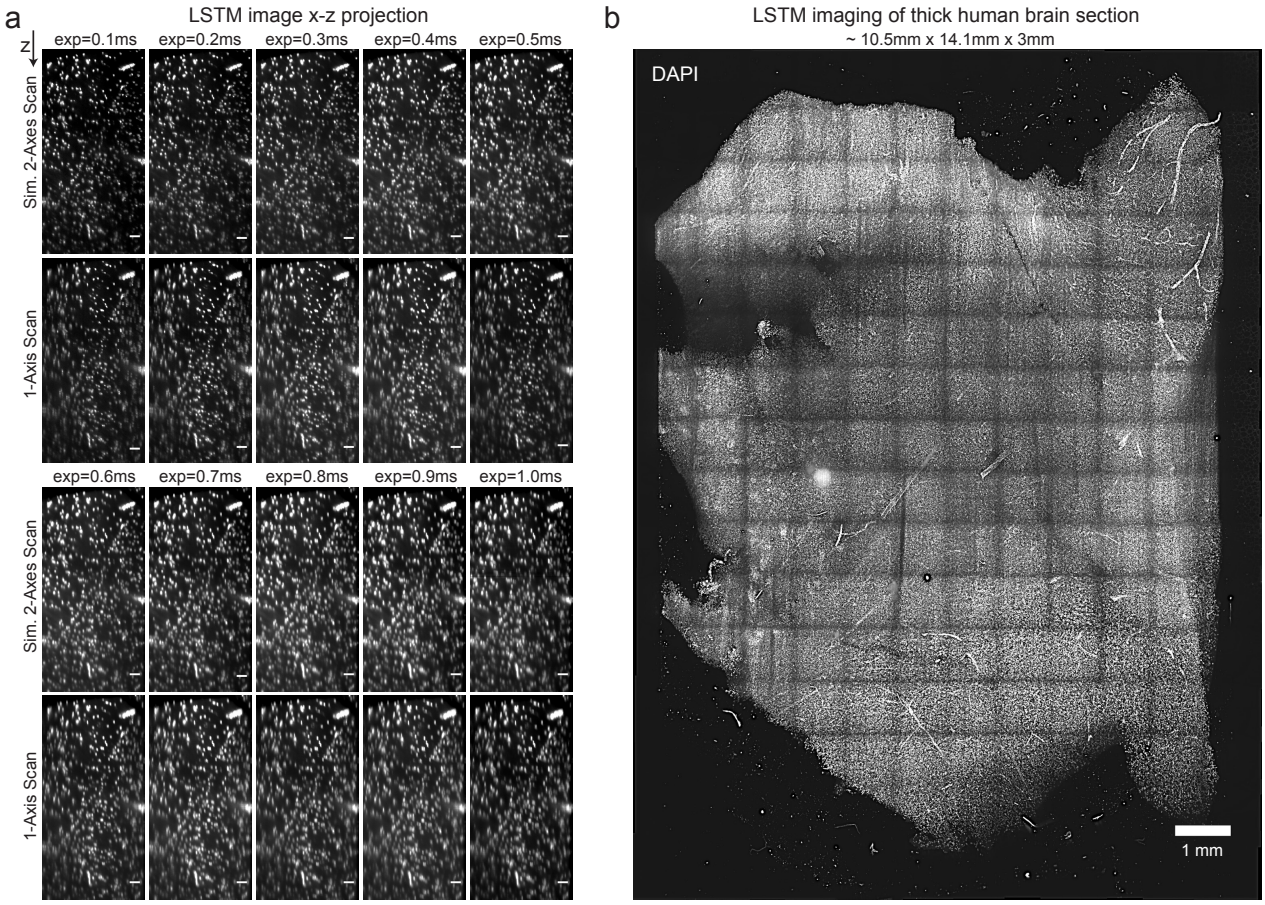
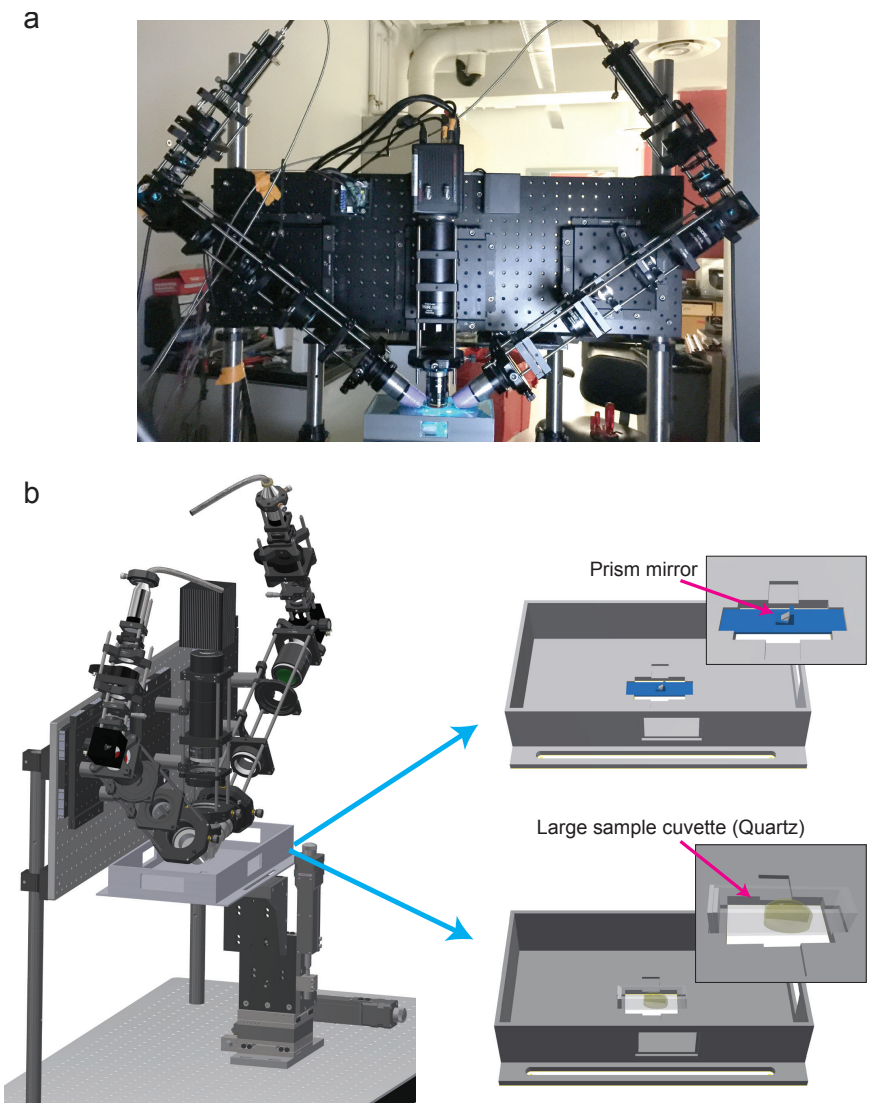
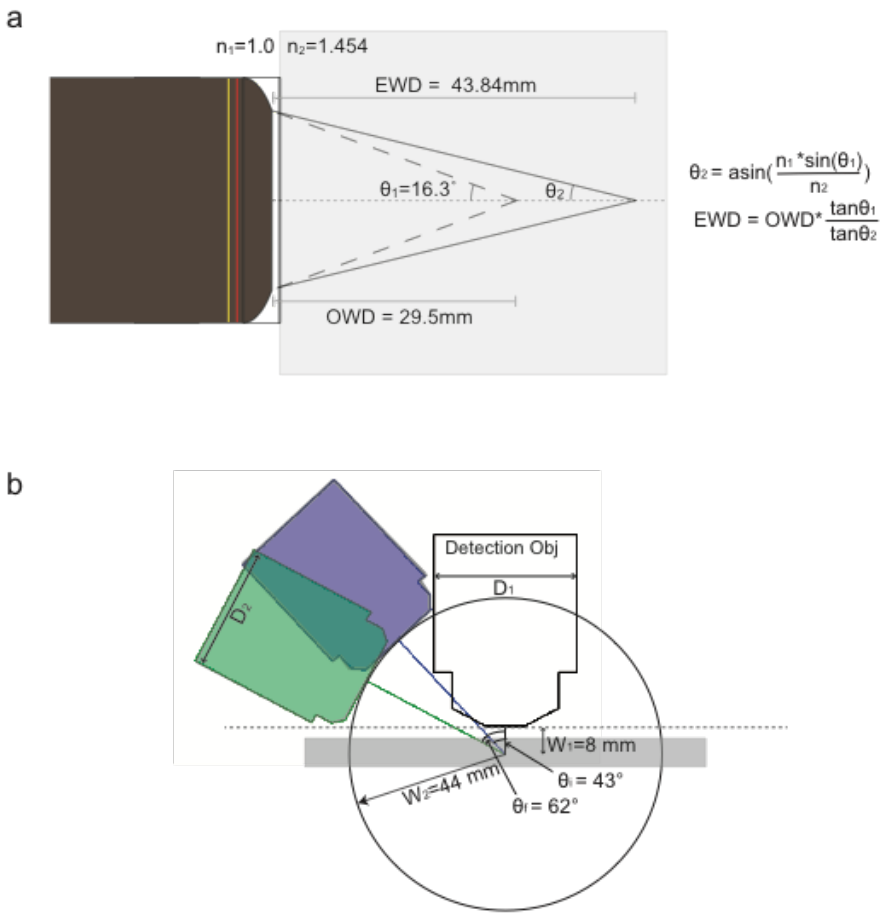


Supplementary Figure 1. LSTM optical sectioning. (a**)** x-z maximum intensity projections of an image stack acquired form a Human brain slice, shown in (b), stained with DAPI, with camera exposure times ranging from 0.1 to 1 millisecond in two different scanning modes (LSTM 1-AS and LSTM 2-AS). 2-AS mode allows for uniform planar illumination for achieving quantitative imaging. All scale bars are 100 microns. (b) LSTM imaging of a large thick section of cleared human brain tissue (~10.5mm x 14.1mm x 3mm) stained with DAPI. 0.5 ms exposure settings were used to acquire this dataset. Scale bar is 1 mm.

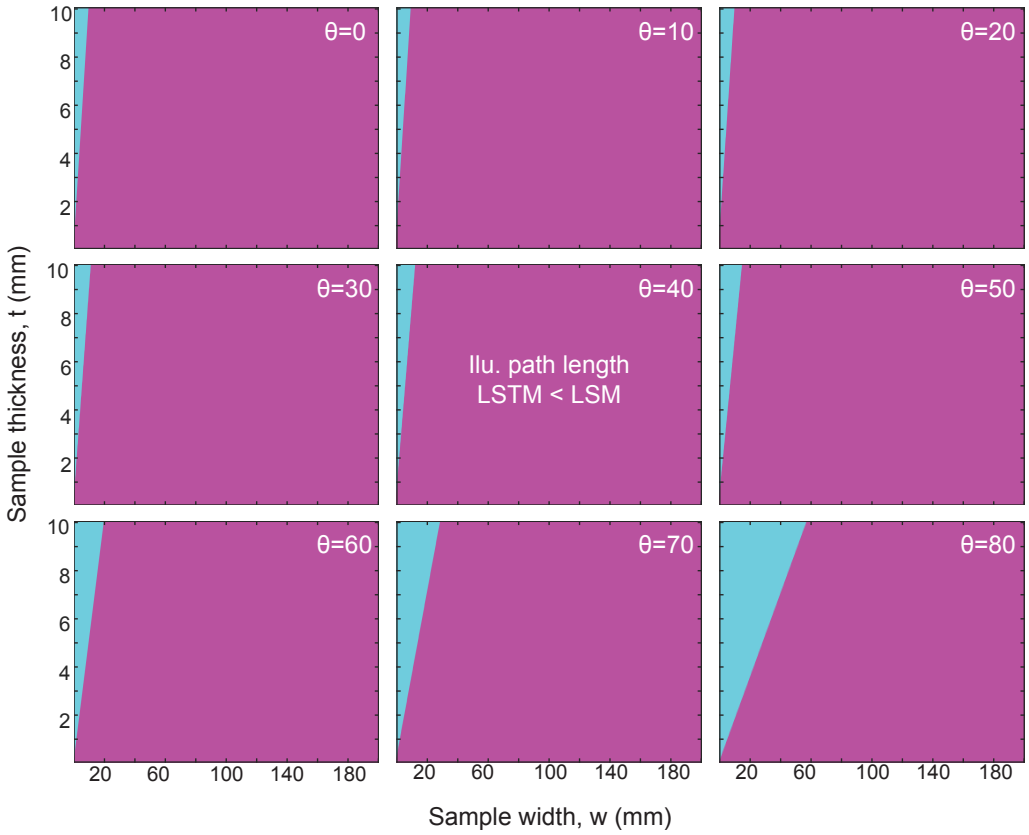


Supplementary Figure 2. LSTM microscopy implementation. (a) Picture of the physical LSTM microscope setup. (b) Side view of the 3D model of LSTM and the sample mounting system. The 3D printed sample chamber is designed to accommodate large biological samples while still allowing the objectives to be immersed in the immersion liquid. Two transparent glass windows, located on the lateral sides, facilitates positioning of the sample under the microscope. An additional window is realized at the bottom part of the chamber to allow illumination light to pass through. An adapter was used to mount a prism mirror at about 10° from the normal surface, to facilitate the optical alignment of the system.



Supplementary Figure 3. Geometric constraints of LSTM implementation. (a) Schematics representing approximate calculations of the effective working distance (EWD) of the air illumination objective (Olympus Macro 4x/0.28NA/29.5WD Air) when used in immersion liquid (refractive index 1.454). Original working distance (OWD) is the working distance in air according to the objective specifications. A thin quartz coverslip and a 3D printed cap were used to seal the illumination objectives. EWD was estimated to be 43.84mm. (b) Geometric constraints calculation for the co-arrangement of the illumination and detection objectives. The two boundary conditions are shown in blue and green shading of the illumination objective. For the upper bound limit (blue), the relationship among different parameters is defined by the equation $W2 * \sin(\theta i) = \frac{D1}{2} + D2 *$

$\frac{\cos(\theta i)}{2}$. And the lower bound limit (green) by $W2 * \cos(\theta f) = W1 + D2 * \frac{\sin(\theta f)}{2}$. W1 and W2 are the effective working distance of detection and illumination objectives respectively. D1 and D2 are the diameters of the detection and illumination objectives respectively. θi and θf are the angular position of upper and lower bounds respectively. For Macro 4x/0.28NA/29.5mmWD as illumination objective and 10X/0.6NA/8mmWD as detection objective, the calculated θi and θf are 43.32° and 63.37° respectively. This range served as a starting point during the optical alignment of the system.



Supplementary Figure 4. Comparison of illumination path lengths in LSTM and LSM. The graphs compare the illumination path lengths required for imaging a sample of given width (w, the shortest lateral dimension) and thickness (t). The ratios of illumination path lengths were calculated (using $w/(\frac{t}{\cos(\theta)})$) for different angular arrangements and converted to a binary heat map. Magenta and Cyan regions mark the combinations of w and t for which the illumination path length was smaller in LSTM and LSM, respectively.

Supplementary Videos.

Supplementary Video 1. Comparison of image volumes acquired with LSTM in 1-AS and 2-AS modes. The 3D rendering visualizes the image stacks acquired from the same sample (human brain section stained with DAPI) with LSTM in 1-Axis Scan (1-AS) and simultaneous 2-Axes Scan (2-AS) mode.

Supplementary Video 2. 3D model of LSTM implementation. The 3D modelling and rendering was performed using Autodesk Inventor 2017, and the animation was performed using Autodesk Fusion 360 2017 and Matlab. The components labelled are LS (laser source), collimator, ND (neural density) filter mount, iris, ETL (electrically tunable lens), slit, CL (cylindrical lens), galvo scanner, SL (scan lens), iris and TL (tube lens)

Supplementary Video 3. High-resolution LSTM imaging of intact *Thy1-eYFP* mouse central nervous system. The bounding box for the entire sample is 11.8mm x 27.6 mm x 5.2 mm, and for the sub-volume shown is 5.1mm x 3.1mm x 3.5mm. The raw data was down-sampled 2x2 fold (to make the volume rendering feasible) for the sub-volume rendering.

Supplementary Video 4. High-resolution LSTM imaging of a large tissue of *Thy1-eYFP* mouse brain. The bounding box is 9.6mm x 13.5 mm x 5.34 mm. The raw data was down-sampled 4x4 fold to make the volume rendering feasible.

Supplementary Video 5. Visualization of an image stack of vasculature stained rat brain tissue. The Video visualizes an image stack acquired from a large rat brain slice (stained for vasculature with tomato lectin) using LSTM in 2-AS mode. The bounding box is 1mm x 1mm x 5mm.

Table S1. LSTM parts list.

Table S1. Parts list of aLSM; related to Figure 2.

Source	Cat. Number	Qty	Description
Detection unit			
Thorlabs	CXY2	1	60 mm Cage System Translating Lens Mount for Ø2" Optics
Thorlabs	LCP90F	1	60 mm Removable Cage Plate
Thorlabs	SM2A20	1	SM2 - M38 ADAPTER FOR NIKON TUBE LENS
Thorlabs	SM2L30-SM2	2	SM2 Lens Tube, 3" Thread Depth, One Retaining Ring Included
Thorlabs	LCP09	2	60 mm Cage Plate with Ø2.2" Double Bore for SM2 Lens Tube Mounting
Thorlabs	ER10	4	Cage Assembly Rod, 10" Long, Ø6 mm
Thorlabs	SM1A1	1	Adapter with External SM05 Threads and Internal SM1 Threads
Thorlabs	SM2A31	1	Adapter with External C-Mount Threads and Internal SM2 Threads
Hamamatsu	C13440	1	Digital camera
Custom	ObjAdater	1	Custom made Adapter from SM2 to M34 threading
Thorlabs	SM2V10	1	Ø2" Adjustable Lens Tube, 0.81" Travel
Olympus	XlPlan N, 10X	2	Oil immersion Objective
Thorlabs	LCP01B	2	60 mm Cage Mounting Bracket
Thorlabs	RS2	2	Ø1" Pillar Post, 1/4"-20 Taps, L = 2", 8-32 Adapter Included
Thorlabs	TBB0606	2	Large-Area Translation Stage, 6" x 7.66"
Two Illumination units			
Thorlabs	SM2V10	2	Ø2" Adjustable Lens Tube, 0.81" Travel
Custom	ObjAdater	2	Custom made Adapter from SM2 to M34 threading
Olympus	Macro 4x/0.28NA/29.5WD	2	Air Objective
Thorlabs	SM2A20	2	SM2 - M38 ADAPTER FOR NIKON TUBE LENS
Thorlabs	CXY2	2	60 mm Cage System Translating Lens Mount for Ø2" Optics
Thorlabs	LCP09	2	60 mm Cage Plate with Ø2.2" Double Bore for SM2 Lens Tube Mounting
Thorlabs	LCP01B	4	60 mm Cage Mounting Bracket
Thorlabs	RS2	4	Ø1" Pillar Post, 1/4"-20 Taps, L = 2", 8-32 Adapter Included
Thorlabs	TBB0606	4	Large-Area Translation Stage, 6" x 7.66"
Thorlabs	ER05	8	Cage Assembly Rod, 1/2" Long, Ø6 mm
Thorlabs	LCP02	6	30 mm to 60 mm Cage Plate Adapter, 8-32 Tap
Thorlabs	LJ1695RM-A	2	Ø1", N-BK7 Mounted Plano-Convex Round Cyl Lens
Thorlabs	CRM1L	2	Cage Rotation Mount for Ø1" Optics, Double Bored with Setscrew, 8-32 Tap
Thorlabs	CP20S	2	30 mm Cage System Iris, Ø20.0 mm Maximum Aperture
Thorlabs	CP90F	2	30 mm Removable Cage Plate, Front and Back Plate, Internal SM1 Threading
Thorlabs	CXY1	2	30 mm Cage System, XY Translating Lens Mount for Ø1" Optics

Thorlabs	CP12	2	30 mm Cage Plate, Ø1.2" Double Bore for SM1 Lens Tube Mounting
Thorlabs	LCP01	4	60 mm Cage Plate, SM2 Threads, 0.5" Thick, 8-32 Tap (Two SM2RR Retaining Rings Included)
Thorlabs	CLS-SL	2	Scan Lens with Large Field of View, 400 to 750 nm, EFL=70 mm
Thorlabs	ER18	6	Cage Assembly Rod, 18" Long, Ø6 mm
Thorlabs	LCP50S	2	60 mm Cage System Iris, Ø50.0 mm Maximum Aperture
Optotune	EL-16-40-TC-VIS-5D-C	2	Electrically Tunable Lens
Thorlabs	ER4	18	Cage Assembly Rod, 4" Long, Ø6 mm
Thorlabs	VA100C	2	30mm Cage System Adjustable Slit, 8-32 Tap, Imperial Micrometer
Thorlabs	GVS001	2	1D Galvo System, Silver-Coated Mirror, PSU Not Included
Thorlabs	GCM001	2	1D Galvo 30 mm Cage System Mount
Omicron	Custom	2	Collimator with ~10 mm bead diameter output
Omicron	SOLE-6	1	LASER engine with 4 lines: 405, 488, 561, 647 nm
Mounting Base			
Thorlabs	MB1236	1	Aluminum Breadboard 12" x 36" x 1/2", 1/4"-20 Taps
Thorlabs	RS12	4	Ø1" Pillar Post, 1/4"-20 Taps, L = 12", 8-32 Adapter Included
Thorlabs	C1001	4	Post Mounting Clamp for Ø1" Post
Motorized XYZ Stage and sample mounting			
Thorlabs	LNR50S	3	50 mm (1.97") TravelMax Translation Stage, 1/4"-20 Taps
Thorlabs	LNR50P3	1	XY Adapter Plate for LNR50 TravelMax Stages, Imperial Hole Spacings
	LNR50P2	2	Right-Angle Bracket for LNR50 TravelMax Stages, Imperial Threads
Custom	Sample chamber	1	Custom made 3D printed sample chamber
Controls & electronics			
National Instruments	PXIe-PCIe8381	1	Control PCIe card for PXI chasis
National Instruments	NI PXIe-1028	1	PXI express chasis
National Instruments	7852R	1	FPGA card
National Instruments	7842R	2	FPGA card
Thorlabs	GPS011	1	Galvo System Linear Power Supply
Thorlabs	LEDD1B	2	T-Cube LED Driver with Trigger Mode, 1200 mA
Thorlabs	BSC203	1	BSC203 - Three-Channel APT™ Benchtop Stepper Motor Controller
Markettech	SOLE-6	1	6 Laser Light Engine with 4 wavelengths
Serversdirect	Custom	1	Custom workstation with Supermicro X10DRHCT Motherboard, 24 TB SSD Hard Disks (EVO 850), 256 GB RAM (Crucial RDIMM DDR4), Quadro K4200 Graphics card.