Table S1. Imaging speed estimation

		Brain only	Brain in skull
Tile size (voxel)	x	900	900
	y	1600	1600
	z	250	150
Voxel size (μm)	x	0.35	0.35
	y	0.35	0.35
	z	1.0	1.0
Tile size (μm)	x	315	315
	y	560	560
	z	250	150
Tile imaging time (s)		30	20
Imaging speed ($\mu m^3/s$)		$1.5x10^6$	$1.3x10^6$
Imaging speed (voxel/s)		$1.2x10^7$	$1.1x10^7$
Overlap ratio	x	0.067	0.067
	y	0.063	0.063
	z	0.40	0.67
Volume (mm^3)		500	600
Estimated imaging time (day)		7.6	18.2

Table S2. Experimental ablation parameters

Ablation geometry	Effective ablation diameter	Task	Material	Fast axis scale factor	Slow axis scale factor	Z step size (μm)	Fluence (J/cm^2)	Fast axis speed (mm/s)	Slow axis step size (μm)	Speed ($μm^3/s$)	Speed (voxel/s)
Line	0.49 μm for fast axis and 134 μm for slow axis	Single pulse ablation test	Gelatin	-	-	-	10	-	-	-	-
		Continuous ablation test	Gelatin	3.1	0.75	5	10	1.5	100	$7.7x10^5$	$6.3x10^6$
			Skull	2.1	0.50	2	2	1.0	67	$1.4x10^5$	$1.1x10^6$
		In situ Imaging	Soft tissue	4.2	0.75	5	3*	2.0	100	$1.0x10^6$	$8.4x10^6$
			Skull	2.1	0.50	2	2*	1.0	67	$1.4x10^5$	$1.1x10^6$
Plane	75 μm for fast axis and 85 μm for slow axis	Continuous test	Gelatin	0.71	0.71	10	7	53	60	$3.2x10^7$	$2.6x10^8$

* Nominal ablation fluences are listed. During visual-based ablation refinement, the fluences for different materials were dynamically adjusted based on prior ablation result and the distance toward the target final ablation plane.

Table S3. Ablation speed estimation

		Coherent Astrella 1k (current)	Coherent Astrella 5k	Coherent Monaco
Repetition rate (Hz)		1×10^3	5×10^3	1×10^5 *
Sample volume (mm^3)	Brain	500		
	Skull	100		
Ablation speed ($\mu m^3/s$)	Brain	1.0×10^6	5.0×10^6	1.0×10^8
	Skull	1.4×10^5	7.0×10^6	1.4×10^7
Ablation time (day)	Brain	5.8	1.2	0.06
	Skull	8.3	1.7	0.08

*Untested. The ablation rate is limited by the resonance of the sloshing of fluid in the moving sample holder. The current container has a resonant frequency of 15 Hz, which limits the single-pulse cutting rate to 1×10^5 Hz or ten-times less than the 1.0 MHz pulse rate of the Monaco or equivalent laser. The maximum ablation rate may be further limited by bubble clearance and other issues.