Supplementary Table 1 | Summary of application-oriented imaging modalities and techniques.

Technology	Basic Mechanism	Advantages	Limitations	Exemplary Applications	Ref.
A. Transparent preparations (Hydra, worm, zebra-fish, Drosophila larva, superficial layers of cortex, etc.)					
A1. Wide field imaging (Fluorescence imaged with camera: CCD or scientific CMOS camera)					
Standard epi-fluorescence	One-photon, wide-area excitation on sample.	Most basic fluorescence microscope; Large FOV; Commercially mature.	No optical sectioning; Out-of-focus regions fluorescing and contributing to background.	Immunohistochemistry in brain slides; *In vivo* fluorescence inspection; *In vivo* mesoscale (low spatial resolution) intrinsic / calcium / voltage imaging.	11-14
Spinning disk confocal [closely related to confocal laser scanning microscopy (CLSM)]	Multiple one-photon excitation focal spots scanned across sample in 2D; Out-of-focus fluorescence rejected by pinholes upon detection.	Optical sectioning; Commercially mature.	Out-of-focus regions fluorescing and suppressed at detection, wasting excitation energy in those regions.	Immunohistochemistry in brain slides; *In vivo* fluorescence inspection; Functional imaging in samples not sensitive photodamage.	15, 17
Light-sheet	A thin one/two-photon light-sheet excitation, injected to the sample in an orthogonal direction to the dection.	Optical sectioning; Efficient usage of excitation photons; Large FOV; Commercially available.	Sophisticated alignment; Extra objectives required.	Functional circuit mapping across extended brain volume.	3, 19-23
Temporal focusing (TF)	A two-photon excitation plane on sample, with its tight axial confinement enabled by geometric dispersion at out-of-focus regions.	Wide-field scanless two-photon; Tight optical sectioning over large FOV; Spatially patterned illumination when combined with a spatial light modulator (SLM).	High laser peak power required for excitation of the entire plane.	Retinal neural activity; Synapse dynamics in superficial cortical layers.	28, 29
Extended depth of field (EOF) holographic	Holographic patterned multiple two-photon excitation focal spots imaging targeted cells in 3D; Fluorescence detected by a wavefront-coded camera across extended depths.	Simultaneously imaging multi-sites in 3D.	Number of simultaneous imaging spots limited by laser power and camera dynamic range.	Synapse activity across dendritic arbor in superficial cortical layers.	34, 35
Light-field (LF)	One-photon, whole volume excitation on sample; Camera with microlens array to collect spatial and angular inforamtion of emitted fluorescence.	Simultaneously imaging whole volume in 3D.	Complicated image deconvolution and optimization; Tradeoff between lateral / axial resolution and axial imaging range.	The sophisticated data reconstruction procedure may need to be further optimized for practical applications.	37
B. Scattering preparations (cortex or deep brain regions in rodents, cats, songbirds, non-human primates, etc.)					
B1. Two-photon laser scanning microscopy (Fluorescence detected with PMT)					
Standard two-photon laser scanning microscopy (2PLSM)	A single two-photon focal spot raster scanned across sample. Non-descanned detection with a PMT.	Resistant to scattering; Deep layer imaging; Reduced phototoxicity (compared to confocal microscopy). Commercially mature.	Expensive two-photon lasers; Limited pixel rates.	Functional connectivity between cortical layers; Excitatory and inhibitory circuits; Cortical response to sensory stimulus; Neural activity during behavior; Neurovascular coupling; Spines formation.	4, 5, 38, 39
Fast 3D imaging: Piezo-controlled objective	Axial position of objective controlled by piezo.	Easy setup and readily attached to two-photon laser scanning microscopes.	Mechanical movement introduced; Relatively slow speed.		41

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		Electrically tunable lens (ETL)	Liquid lens shape changed to perform refocusing.	Low cost.	Relatively slow speed.		42
		Spatial light modulator (SLM)	Programmable SLM to change the light wavefront (beam divergence) to perform refocusing.	High speed; Capable for wavefront correction and beam multiplexing.		High speed volumetric imaging; Neural circuits dynamics across multiple cortical layers.	44
		Ultrasound lens (UL) / Tunable acoustic gradient index of refraction (TAG) lens	Standing acoustic wave across the lens to spatially modulate its refractive index to perform refocusing.	Extremely high speed in axial scan.	Must continuously scan in axial direction.		45
		Remote focusing	An auxiliary objective and a scanning mirror combined as a retroreflective unit to change the light wavefront (beam divergence) to perform refocusing.	Minimized optical distortion.	Sophisticated alignment; An extra objective and custom-designed parts required; Scanning mirror should sustain focused high power laser pulses.		47, 48
		Acousto-optic deflectors (AOD) for random access	Traveling acoustic wave across the glass to modulate its refractive index to create periodic/chirped diffractive grating to deflect/refocus light.	Extremely high speed beam hopping.	High insertion loss and expensive; Non-trivial realignment if excitation wavelength changed.	Neural signal dynamics in whole dendrite arbor across multiple cortical layers; Correlation between activity in global 3D network and individual cells.	50, 51, 53
	Large FOV		Special designed optics; Large aperture objectives with low magnification and high NA.	Extremely large field of view.	Customer-designed aberration correction optics and objective required.	Detailed circuitry across different cortical regions; Multiple orientation columns in primary visual cortex of cats and non-human primates.	54-57
	B2. Multiplexing technology in two-photon microscopy (high throughput)						
	Temporal multiplexing		Laser pulses interleaved in time and scanned across different regions in sample.	Fast imaging multi-region/multiplane in 3D.	Sophisticated optical alignment, setup and data acquisition hardware.	Information flow between different cortical regions	58
	Multifocal microscopy with multianode PMT		Multiple beams scanned different regions in a 2D grid. Fluorescence from each excitation beam detected by one anode in multianode PMT.	Fast imaging multi-region in 2D.	Cross-talk when imaging deep layers due to tissue scattering.	Combined with large FOV microscope for fast mesoscale imaging with cellular resolution.	59
	Wavelength multiplexing		Multiple laser beams with different wavelengths to excite different fluorophores in sample.	Image different cell types simultaneously.	Optical parametric oscillators (OPOs) or extra two-photon lasers required.	Activity of different cell types (e.g. pyramidal cells / interneurons / astrocytes).	62
	Code multiplexing		Holographic patterned multiple beams imaging targeted cells. Each beam tagged with different temporal features, used to unmix the PMT detected signal.	Simultaneously imaging multi-site in 3D; No scanning required.	Tradeoff between total number of simultaneously imaging spots and temporal resolution.	Synapse dynamics in deep cortical layers.	64
	Holographic multiplane		Holographic patterned multiple beams scanned across different planes in sample. Source separation algorithm to unmix the PMT detected signal.	Fast imaging multi-region/multiplane in 3D; High flexibility; Simple optical setup.	Total number of simultaneous imaged regions/planes limited by labelling sparsity.	Activity correlations between different cortical layers; Relations between upstream interneurons and downstream activity and synchrony.	44

	Engineered PSF		PSF (Wavefront) engineered excitation beam (e.g. laterally- or axially-extended) scanned across sample. Signal recovered with statistical algorithms.	Fast imaging speed.	High laser peak power may be required for the desired beam profile. Sample may be required to be sparsely labeled.	Combined with large FOV microscopes for fast mesoscale imaging with cellular resolution.	66, 69, 70
	B3. Deep brain imaging						
	Adaptive optics (AO)		SLM or deformable mirror (DM) to shape the light wavefront to counteract the light scattering effect in sample.	Access to deep layers with high quality optical signal.	Limited correction field of view.	Dynamics of fine structures (e.g. spines, boutons) in deep brain regions	76-79, 81, 82
	Three-photon		Longer wavelength light encounters less light scattering.	Resistant to light scattering; Access to deep layers.	Low three-photon absorption coefficient resulting in a requirement of higher laser power or lower frame rate; Three-photon laser required.	Deep layers (e.g. cortical layer 5, 6 and hippocampus) imaging for an intact brain.	72, 83
	Gradient-index (GRIN) lens assisted		The GRIN lens serving as a relay lens and objective to deliver and focus light deep into the brain.	Access to deep layers.	Highly invasive to the brain.	Functional imaging of hippocampus, thalamus and hypothalamus; Dendritic spines in CA1.	84, 87
	Micro-prism assisted		Micro-prism inserted in the brain converting axial planes into horizontal planes for imaging.	Simultaneous imaging across all cortical layers in a single FOV.	Highly invasive to the brain.	Inter- and intralaminar cortical dynamics and information flow; Neural activity in medial prefrontal cortex, or similar regions buried inside the cortical sulci	85, 86
	Specially designed collection optics		Specially designed optics to collect the fluorescence that escapes from the collection cone of objective.	Enhance the signal to noise ratio.	Specially designed collection optics.	Deep layers imaging for an intact brain.	73
C. Imaging freely behaving animals							
	Wide-field epi-fluorescence	Integrated micro-scope	Integrated one-photon excitation light source, optics, camera into a miniaturized device.	Highly integrated, all-in-one system; Commercially available.	Large footprint device mounted on mouse head.	Brain (e.g. cortex, hippocampus) activity during sensory, cognitive and motor tasks.	88, 89
		Fiber-scope (fiber-bundle / multicore fiber)	Fiber bundle/ multicore fiber used to couple light between a one-photon bulk microscope and a miniaturized objective on the brain.	Capable to project patterns and advanced imaging schemes such as structured illumination.	Bulky bench-top optics required at proximal end; Reduced resolution due to pixilation of image.	Influence of interneurons to the local neural network during animal behaviors; Simultaneous imaging and photostimulation in freely behaving animals.	90, 91
	Two-photon scanning	Miniature micro-scope	Integrated optics, scan engines (and PMT) into a miniaturized device, with two-photon excitation (and fluorescence) transmitted by special fibers such a photonic-crystal fiber (PCF).	Optical sectioning capability; Minimal pulse dispersion and self-phase modulation.	Large footprint device mounted on mouse head.	Technologies need to be further developed for practical applications.	92-95
		Fiber-bundle / multicore fiber	Fiber bundle/ multicore fiber used to couple light between a two-photon bulk microscope and a miniaturized objective on the brain.	Optical sectioning capability; Laser scanning at proximal end; Mechanical flexibility.	Reduced resolution due to pixilation of image; Dispersion compensation required; Self-phase modulation at high pulse energies.		96