Supplementary Table 1

Reference	Lateral Localization Precision	Axial Localization Precision	3D/2D PSF used	Image Depth (μm)	Microscope Geometry	Method	Notes
Cella Zanacchi et al 2011[1]	~35 nm	~65-140 nm**	astigmatism	150	orthogonal objectives	Light sheet	1.8 μm light sheet thickness
Cella Zanacchi et al 2013[2]	~40 nm	-	2D	200	orthogonal objectives	Light sheet, 2 Photon	2.6 μm (1P), 2.8 μm (2P) light sheet thickness
Tehrani et al 2015[3]	41% improvement*	-	2D	50	single objective	AO	Genetic Algorithm for AO
Burke et al 2015[4]	~25 nm	~57 nm	astigmatism	6	single objective	AO	
Sigal et al 2015[5]	~20 nm	70 nm (resolution determined by section thickness)	2D	~70 (estimated from figure)	single objective	Serial ultra-thin sectioning	3D information gained from reconstruction of ultra-thin sections
Barna et al 2016[6]	6 nm***	41 nm***	astigmatism	10	single objective		Extensive protocols for 3D SMSN imaging
Legant et al 2016[7]	~8.9 nm	~41 nm	astigmatism	27.5	orthogonal objectives	Lattice Light Sheet	1.1 μm thick lattice light sheet
Huang et al 2016[8]	10 - 20 nm (resolution)	10 - 20 nm (resolution)	4Pi	10	opposing objectives	4PiSMSN with AO	
Tehrani et al 2017[9]	~146 nm (resolution)	-	2D	100	single objective	AO	Particle Swarm Optimization for AO
Present Work	~11 nm	~30 nm	astigmatism	30 (Brain), 95 (cavity)	single objective	AO and adaptive astigmatism	Nelder-Mead simplex AO and adaptive PSF engineering

* No numerical value given for localization precision or final image resolution
** Due to unavailability of AO, resolution varies at different depths
*** Measured at a depth of 5 μm

Supplementary Table 1: Comparison of depths and localization precisions or resolutions (resolutions (FWHM) is usually 2.35 times (or more) higher than precision values) from the current and previous approaches.

Supplementary Table 2

Figure and Panel	Frames/Z Step	Number of Steps	Number of Cycles	Total Frames	ROI Thickness (μm)	Centered Image Depth (μm)	Mean Lateral Localization Precision (nm)	Mean Axial Localization Precision (nm)	Mean Photon Counts	Mean Background	Number of Localizations
Figure 2	2000	7	11	154000	~2.4	~23.7	10.8	31.9	2304	91.6	1248019
S. Figure 7, Panel A	2000	8	6	96000	~2.8	~22.4	10.3	29.3	2288	86.3	489089
S. Figure 7, Panel B	1500	26	23	172500	~10	~9.0	10.0	26.4	2655	129.7	2767631
S. Figure 7, Panel C	2000	14	10	100000	~5.2	~18.6	10.9	30.8	2132	97.7	515184
S. Figure 7, Panel D	2000	14	9	88000	~5.2	~3.6	10.7	31.6	2166	100.9	858665
S. Figure 7, Panel E	2500	14	10	100000	~5.2	~21.8	11.1	29.3	2394	108	1082831
S. Figure 7, Panel F	2000	7	10	140000	~2.4	~13.7	11.4	33.8	2250	125.3	763919
S. Figure 7, Panel G	2000	4	12	96000	~1.2	~17.6	13.5	38.8	2136	141.1	582335
S. Figure 7, Panel H	2000	5	10	100000	~1.6	~25.3	13.8	39.8	2455	161.6	511664
S. Figure 7, Panel I	2000	7	8	112000	~2.4	~2.0	11.1	29.8	2236	122.1	1105847

Supplementary Table 2: Imaging parameters for the brain tissue data presented here. All images were recorded at 50 Hz and all axial steps were 400 nm. A minimum threshold of 800 photons was used during analysis. The data recorded for panels B-E used the stepping pattern described in **Online Methods**.

Supplementary Note 1: Synergistic approaches to increase super-resolution imaging depth

In order to reduce background fluorescence and therefore increase the signal to noise ratio of single molecule detections, light sheet illumination such as IML-SPIM[1] or two-photon activation IML-SPIM[2] has been demonstrated in SMSN imaging of tissues. IML-SPIM allows 3D localization of single molecules in thick tissue up to 150 μm using astigmatism, however, due to the unavailability of AO techniques, the resolution of 3D reconstructions deteriorates at greater depths (**Figure 1A-C, L, Supplementary Figures 3, 4, 11)** with a water immersion objective limiting the axial localization precision to ~140 nm (Ref. 1 in **Supplementary Table 1**). Two-photon activation allows activation of fluorescent probes within a single optical section providing a unique advantage in background fluorescence reduction and has been demonstrated through two-dimensional SMSN imaging of multiple optical sections to reconstruct images in 3D[2]. The potential combination of light-sheet illumination and activation or lattice light sheet as demonstrated in Legant et al, Nature Methods 2016[7] with the method developed here, will provide a significant improvement in imaging depth and capacity.

Based on a single objective geometry and HiLo light-sheet illumination, we demonstrated that our approach leads to improved axial localization precision which we hope could be easily adopted without needing a special optical design (**Supplementary Table 1**). We expect the combination of the developed method here with sophisticated illumination/detection modalities such as lattice light-sheet[7] or interferometry based SMSN[8,10,11] could further improve the 3D resolution achievable due to the significantly thinner illumination profile of the lattice light-sheet than HiLo and the increased axial

information content of single molecules detections (through 4Pi/interferometric detection of single molecules).

Simultaneously, advanced tissue clearing[12] and expansion techniques[13] provide synergistic mechanisms in significantly reducing fluorescence background and scattering. These novel techniques could be further combined with this development to correct aberration-induced resolution deteriorations and reconstruction inaccuracies for 3D-SMSN imaging. While at the same time, the significantly increased sample thickness in expansion microscopy together with the required single molecule switching buffer environment could potentially lead to increased aberrations that need to be corrected for reliable 3D imaging.

Supplementary Note 2: Comparison of data with and without AO correction

There are several subtle key differences between **Figure 1M** and **Figure 1N** that highlight the improvement using the developed approach for imaging thick specimens. The axial resolution of an uncorrected PSF as demonstrated is significantly more stretched along the axial dimension than a corrected PSF (**Figure 1A-F and Supplementary Figure 4**). This distortion results in a cell or ROI appearing “thicker” than it actually is as the stretching effect makes the PSF detectable through a larger axial range and therefore requiring imaging over a larger axial range. In addition, aberrations, especially spherical aberrations, inhibit the PSF shape modulation at different focal positions (**Figure 1J, K and Supplementary Figures 3, 5**) and this effect can significantly reduce axial information content in each detected PSF resulting in image artifacts which can be visualized in cross-sections in the axial direction. This

aberration has several effects visible in localized data (**Figure 1M**); 1) reconstructed super-resolution volumes appear as stripes in the axial dimension and 2) The PSF (axially stretched by spherical aberrations) leads to localization of the same set of single molecules from multiple optical sections however with little axial information as their shape modulated weakly with their axial positions (appeared as “in focus”). This is observed in **Figure 1M**. When AO and AA are implemented, modulation of the PSF improves localization precision, and in addition, optical sections will now detect different subsets of molecules at each depth.

Supplementary Note 3: Super-resolution reconstruction of amyloid beta plaques

The first examination of the fine structure of amyloid plaques using super-resolution techniques in humans and animal models of AD was reported by Yuan et al in 2016[14]. The published study found that plaques are 'trimmed' by surrounding microglial cells and allowed classification of the plaques into different classes based on their structure and provided a clear demonstration of the power of super resolution microscopy to uncover previously unappreciated plaque dynamics. Examination of the images in Yuan et al in 2016[14] and those of the present study provide a demonstration of the potential use of super-resolution techniques to shed light on exactly how the microglial cells remodel the different amyloid fibril types and how disease related mutations affect these processes and plaque phenotypes.

In the presented data, Beta Amyloid fibrils size ranges drastically as shown in **Supplementary Figure 7** and studies using thin slices[14] could potentially bias towards relatively thin clusters. We chose to demonstrate our approach in 30 μm thick samples,

in part, due to the relative size of beta amyloid plaques (most are <20μm) (**Supplementary Figures 7, 9 and 10**).

Amyloid plaques were previously known to be structurally heterogenous, based on their differential reactivity with various antibodies and chemical probes and our developed methods now allows us to monitor the morphology of the plaques and their evolution during disease progression. Plaque remodeling is mediated by glial cells and further studies with multi-color imaging will permit simultaneous visualization of these interactions. Importantly, the ability to resolve nanoscale structures within the brain will also allow structural determination of how vascular amyloid deposits are distinguished from those in the parenchyma.

Supplementary Note 4: Alignment of Deformable Mirror

The two most critical criteria in proper DM alignment are 1) the signal diameter matches the working diameter of the DM and 2) the DM be laterally centered and placed at the pupil plane of the signal. In **Supplementary Figure 13**, the lenses, L_1-L_3, were carefully chosen so that the diameter of the magnified pupil matches the diameter of the DM as closely as possible, with the focal length, L_3, long enough to minimize the angle of incidence at the DM and to accommodate the geometry (physical size) of the system. Subsequent lenses magnify the signal to satisfy the Nyquist criterion (120 nm pixel size here).

Proper DM placement requires the identification of the pupil plane. This was achieved through a well aligned 4*f* system. A set of small pinholes placed at either the sample plane or an intermediate image plane spatially filters illumination light. The pupil plane is

located where the signals overlap (points converge) and the DM should be placed at this position. Fine tuning of the DM position is achieved by imaging fluorescent beads. Out-of-focus beads reveal whether the signal is clipped at the edge of the DM (assuming no vignetting in other locations). The DM is adjusted depending on how the signal is clipped. Clipping on the edge requires the DM to be re-centered, while uneven clipping along the axial direction requires adjustment of the DM along the optical path. Overfilling the DM results in a loss of signal reducing the NA of the system. Underfilling the DM results in unused actuators limiting the effectiveness of the AO correction.

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