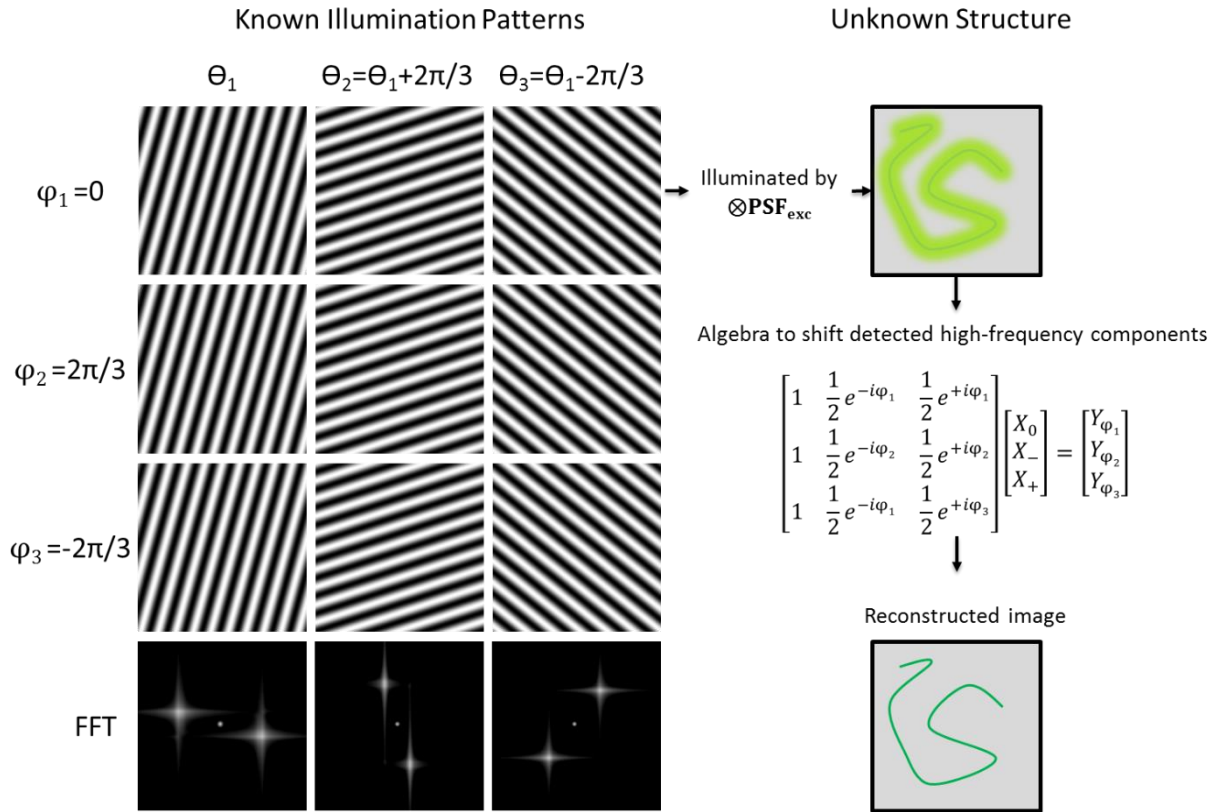


Supplementary Material

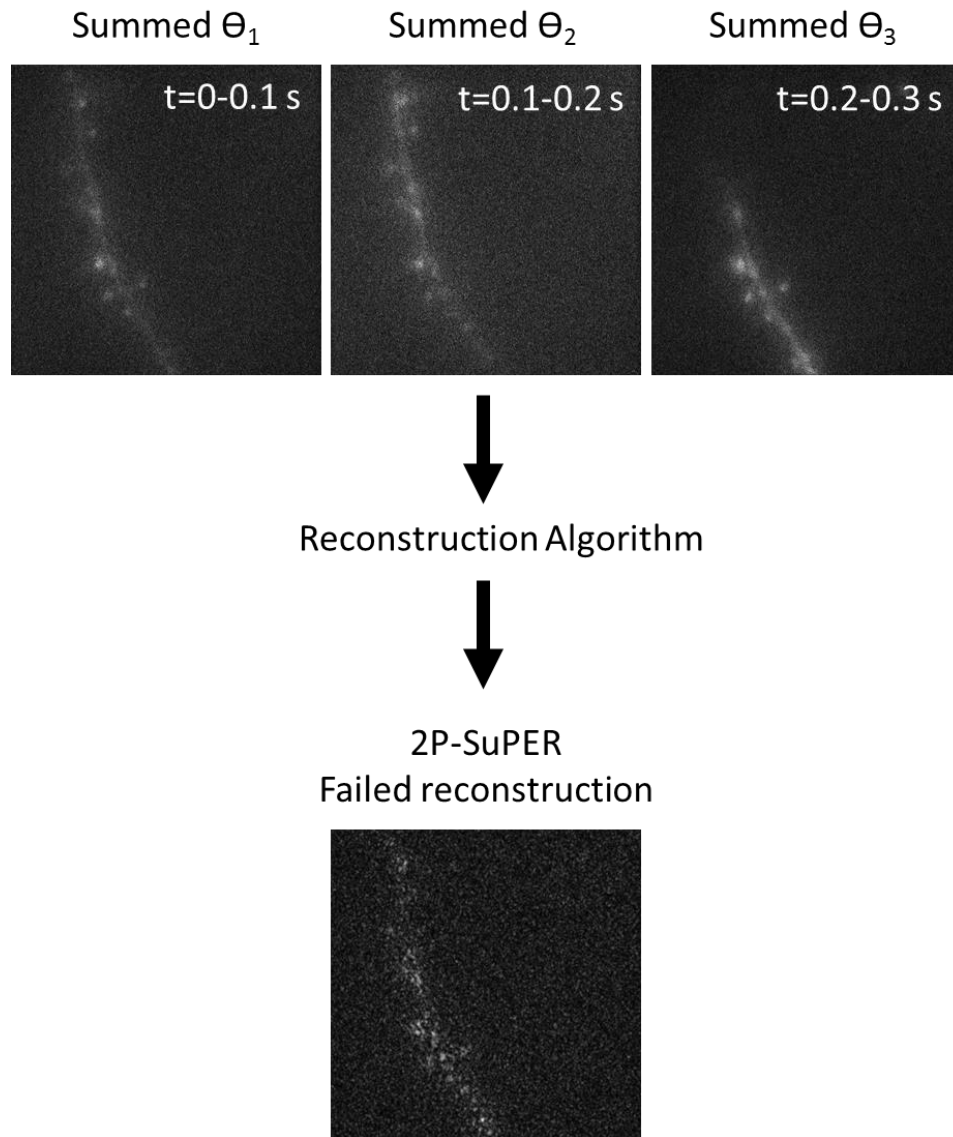
In vivo super-resolution imaging of neuronal structure in the mouse brain

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Supplementary Figure 1. Illustration of structured illumination reconstruction concept. A total of nine known illumination patterns used at 3 equally space angles (Θ) 120° apart, phase shifted (φ) twice at a step of $2\pi/3$ (left). The corresponding frequency pattern contains information about the excitation pointspread function (PSF_{exc}) and patterned illumination structure and can be found by calculating the fast Fourier transform (FFT) of the image (bottom left). The high frequency components of the image appear as two bright spots on the outer image of the FFT. Since the illumination structure is known, it is possible to solve and shift the modulated frequency components (X_0, X_-, X_+) from each FFT to their corresponding place using matrix algebra and the vectorized expression of the corresponding image ($Y_{\varphi_1}, Y_{\varphi_2}, Y_{\varphi_3}$) (right). Reconstructing the high frequency components leads to an image with approximately a 2-fold resolution enhancement.



Supplementary Figure 2. Example of reconstruction artifacts due to motion during the 9 frame acquisition of an *in vivo* dendrite in the mouse brain at a single imaging plane. Breathing and heart beating causes micro-motion of the target during imaging. When the target is not sufficiently immobilized during all 9 frame acquisitions, 2P-SuPER microscopy fails to correctly reconstruct the image. It was necessary to reduce *in vivo* motion using a silicon elastomer and coverslip for successful reconstruction using 2P-SuPER microscopy.