## Text S3. Theoretical Point Spread Function of HSM

In the HSM, the excitation path is the same as a line scanning confocal microscope. On the sample plane, assuming that a single fluorophore is at point (0,0,*z*), the excitation laser line is along the *y*-axis at position . The excitation field at the fluorophore is

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We use for magnitude point spread function, and for intensity point spread function. is a line spread function, assuming the intensity along *y*-axis is uniform. In the emission path, after light passes through the slit, the emission field at slit will be dispersed along *x*-axis by spectrometer at the image plane. The field at image plane is generated by shifting the slit field along *x*-axis (spectral dimension) and multiplying each shifted field by the spectral function at the corresponding wavelength. We define the spectral function as , which depends on the spectrum of the sample. The field at position on image plane is

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 is the wide field emission point spread function, and is the slit shape function along x axis, which is a rectangular function with the width equal to the slit width . The above expression is the convolution of with . Combining the excitation and emission fields gives the magnitude point spread function of the HSM

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 is the wide field excitation line spread function. is a 4D point spread function, with spatial dimension , and spectral dimension . The intensity point spread function is

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