**S1 Table. Toy data - Mean and standard deviation, obtained by the SIPchart lookup approach and the eSIP fit approach.**

To estimate the error of the fit parameters and to compare the eSIP fit approach with the SIPchart lookup approach we generated toy data by calculating gauss profiles (Eq. 1) for layer and intensity profiles according to Eq. 5 for solution. Maximal photon counts varying from 1 to 1000 photons and applying the corresponding Poisson noise and an additional Gaussian noise corresponding to the readout noise of the Zeiss LSM Quasar detector at detector gain 700 was applied. The following parameters have been used: data points layer (z), from -10 to +10 µm with 0.2 µm spacing ; data points solution (z), from -5 to +5 µm with 0.1 µm spacing, from 6 to 50 µm with 1 µm spacing, from 55 to 200 µm with 5 µm spacing (same as in solution experiments); FWHM ($\omega_{FWHM}$), 1 µm; axial position ($z_0$), 0 µm; offset ($I_0$), 500 DL; photon conversion factor, 500 DL/photon, layer: skewness (s), -0.025, solution: length constant (LC), 0. We introduced an additional variation in the axial position ($z_0$) to avoid permanent coincidence of Gauss maximum and half-maximal values with pixel bins. In correlation to the settings in the presented work a 4x4 bin was used for analysis. Mean values and standard deviation (STD) were obtained from 500 simulations per intensity level. For the intensity (A) relative errors are provided as STD/mean. The error dependence with photon number is illustrated in S3 Fig. **SIPchart lookup approach, layer:** The Intensity (A) was found by searching for the brightest value. Since the z spacing is rather small compared to FWHM, A is notoriously too high at low photon counts. Contrary, for high photon counts it is too low. FWHM was determined by a line approximation between most outlaying data points above and the neighbouring data points below half maximal value. The axial position was found as the position of maximal intensity, so the accuracy directly correlates with z spacing. The offset value was estimated as average from ten values most distant from $z_0$. The skewness was obtained (according to Zwier et al., 2006) as skew = (b-a)/(a+b) (compare S3 Fig F), which deviates from the eSIP approach definition Eq. 2 by a factor of about -2. **eSIP fit approach, layer:** Parameters were obtained by fitting Eq. 1 to the toy data. The look up parameter were used as start parameters for the fit. As expected, an improvement of the accuracy and lower standard deviations of the fit parameters were obtained. **eSIP fit approach, solution:** Parameters were obtained by fitting Eq. 5 to the toy data. The length constant LC was not fitted. The accuracy as deviation of the mean from the input parameter and the precision in terms of STD are illustrated in S3 Fig. Unless toy data are only provided for one most relevant parameter set, this demonstrates the quality of all approaches, whereas the eSIP fit approach for solution reaches at least results of the same quality as the SIPchart approach for layer.

| SIPchart look up approach, layer | | | | | |
|---|---|---|---|---|---|
| Intensity input [Photon] | Intensity (A) [Photon] | FWHM ($\omega_{FWHM}$) [µm] (input: 1 µm) | Axial position ($z_0$) [µm] (input: 0 µm) | Offset ($I_0$) [DL] (input: 500 DL) | Skewness (skew) (input: 0.0125*) |
| 1.00 | 1.14 (±16.78 %) | 0.91 ± 0.207 | 0.001 ± 0.183 | 499.8 ± 8.701 | 0.0242 ± 0.393 |
| 1.26 | 1.43 (±15.21 %) | 0.90 ± 0.191 | -0.004 ± 0.161 | 500.3 ± 8.299 | 0.0236 ± 0.364 |
| 1.58 | 1.77 (±13.92 %) | 0.91 ± 0.166 | -0.009 ± 0.158 | 499.8 ± 8.126 | 0.0378 ± 0.344 |
| 2.00 | 2.18 (±13.42 %) | 0.93 ± 0.149 | -0.002 ± 0.153 | 499.8 ± 8.076 | 0.0141 ± 0.334 |
| 2.51 | 2.69 (±12.25 %) | 0.95 ± 0.136 | -0.005 ± 0.145 | 499.7 ± 8.205 | 0.0207 ± 0.313 |
| 3.16 | 3.35 (±10.19 %) | 0.96 ± 0.126 | -0.012 ± 0.131 | 500.1 ± 8.272 | 0.0339 ± 0.291 |
| 3.98 | 4.19 (± 9.63 %) | 0.96 ± 0.108 | 0.004 ± 0.129 | 500.1 ± 8.454 | 0.0051 ± 0.280 |
| 5.01 | 5.17 (± 8.38 %) | 0.98 ± 0.090 | -0.002 ± 0.124 | 500.4 ± 8.308 | 0.0113 ± 0.262 |
| 6.31 | 6.48 (± 7.30 %) | 0.98 ± 0.086 | 0.000 ± 0.117 | 499.7 ± 8.015 | 0.0161 ± 0.246 |
| 7.94 | 8.10 (± 7.37 %) | 0.99 ± 0.080 | -0.002 ± 0.108 | 499.4 ± 8.138 | 0.0157 ± 0.224 |
| 10.00 | 10.23 (± 6.39 %) | 0.98 ± 0.070 | -0.002 ± 0.101 | 499.7 ± 8.625 | 0.0221 ± 0.207 |
| 12.59 | 12.75 (± 5.51 %) | 0.99 ± 0.060 | 0.002 ± 0.097 | 500.1 ± 8.570 | 0.0072 ± 0.198 |
| 15.85 | 15.99 (± 5.22 %) | 1.00 ± 0.055 | 0.005 ± 0.090 | 500.8 ± 8.246 | 0.0013 ± 0.185 |
| 19.95 | 19.99 (± 4.71 %) | 1.00 ± 0.050 | -0.005 ± 0.088 | 500.0 ± 8.280 | 0.0241 ± 0.178 |
| 25.12 | 25.20 (± 4.40 %) | 1.00 ± 0.045 | 0.007 ± 0.086 | 500.2 ± 8.161 | 0.0019 ± 0.175 |
| 31.62 | 31.45 (± 3.75 %) | 1.01 ± 0.038 | 0.002 ± 0.083 | 500.0 ± 8.132 | 0.0080 ± 0.168 |
| 39.81 | 39.66 (± 3.45 %) | 1.01 ± 0.035 | -0.000 ± 0.077 | 499.4 ± 8.272 | 0.0135 ± 0.155 |
| 50.12 | 50.00 (± 3.16 %) | 1.00 ± 0.031 | 0.003 ± 0.075 | 500.7 ± 8.371 | 0.0095 ± 0.152 |
| 63.10 | 62.94 (± 2.82 %) | 1.01 ± 0.029 | 0.004 ± 0.071 | 500.0 ± 8.005 | 0.0050 ± 0.141 |
| 79.43 | 79.11 (± 2.63 %) | 1.01 ± 0.027 | 0.001 ± 0.071 | 499.5 ± 8.521 | 0.0108 ± 0.141 |
| 100.00 | 99.29 (± 2.38 %) | 1.01 ± 0.023 | -0.003 ± 0.064 | 500.0 ± 8.586 | 0.0185 ± 0.128 |
| 125.89 | 125.14 (± 2.12 %) | 1.01 ± 0.021 | 0.001 ± 0.063 | 499.7 ± 8.460 | 0.0115 ± 0.124 |
| 158.49 | 157.13 (± 1.95 %) | 1.01 ± 0.019 | -0.003 ± 0.064 | 499.9 ± 8.019 | 0.0202 ± 0.126 |
| 199.53 | 197.92 (± 1.79 %) | 1.01 ± 0.017 | 0.006 ± 0.063 | 500.0 ± 8.206 | 0.0007 ± 0.125 |
| 251.19 | 248.96 (± 1.69 %) | 1.01 ± 0.015 | -0.003 ± 0.064 | 499.6 ± 8.583 | 0.0193 ± 0.125 |
| 316.23 | 313.63 (± 1.59 %) | 1.01 ± 0.014 | 0.000 ± 0.061 | 500.0 ± 7.875 | 0.0121 ± 0.118 |
| 398.11 | 394.77 (± 1.47 %) | 1.01 ± 0.013 | -0.003 ± 0.061 | 500.2 ± 8.161 | 0.0176 ± 0.118 |
| 501.19 | 497.08 (± 1.34 %) | 1.01 ± 0.011 | -0.002 ± 0.060 | 500.1 ± 8.071 | 0.0160 ± 0.119 |
| 630.96 | 625.35 (± 1.19 %) | 1.01 ± 0.010 | -0.003 ± 0.059 | 500.3 ± 7.979 | 0.0183 ± 0.115 |
| 794.33 | 787.33 (± 1.13 %) | 1.01 ± 0.009 | 0.002 ± 0.056 | 499.8 ± 8.113 | 0.0083 ± 0.109 |
| 1000.00 | 991.74 (± 1.05 %) | 1.01 ± 0.008 | 0.002 ± 0.056 | 500.7 ± 8.281 | 0.0100 ± 0.110 |

| eSIP fit approach | | | | | |
|---|---|---|---|---|---|
| Intensity input [Photon] | Intensity fit (A) [Photon] | FWHM ($\omega_{FWHM}$) [µm] (input: 1 µm) | Axial position ($z_0$) [µm] (input: 0 µm) | Offset ($I_0$) [DL] (input: 500 DL) | Skewness (s) (input: -0.025) |
| 1.00 | 0.98 (±16.27 %) | 0.93 ± 0.113 | 0.011 ± 0.089 | 499.8 ± 2.655 | -0.0051 ± 0.154 |
| 1.26 | 1.24 (±14.15 %) | 0.93 ± 0.098 | 0.000 ± 0.078 | 499.9 ± 2.475 | -0.0269 ± 0.138 |
| 1.58 | 1.58 (±12.19 %) | 0.94 ± 0.083 | 0.003 ± 0.068 | 499.7 ± 2.607 | -0.0170 ± 0.122 |
| 2.00 | 2.01 (±11.34 %) | 0.94 ± 0.077 | 0.000 ± 0.060 | 500.3 ± 2.758 | -0.0176 ± 0.112 |
| 2.51 | 2.53 (± 9.41 %) | 0.95 ± 0.060 | 0.001 ± 0.051 | 499.7 ± 2.700 | -0.0207 ± 0.096 |
| 3.16 | 3.19 (± 7.93 %) | 0.96 ± 0.055 | -0.003 ± 0.045 | 499.9 ± 2.653 | -0.0274 ± 0.084 |
| 3.98 | 4.01 (± 7.16 %) | 0.97 ± 0.049 | 0.001 ± 0.041 | 499.8 ± 2.934 | -0.0248 ± 0.074 |
| 5.01 | 5.04 (± 6.31 %) | 0.97 ± 0.041 | -0.002 ± 0.036 | 500.1 ± 2.951 | -0.0243 ± 0.061 |
| 6.31 | 6.36 (± 5.29 %) | 0.97 ± 0.036 | 0.002 ± 0.032 | 499.8 ± 2.728 | -0.0200 ± 0.054 |
| 7.94 | 8.00 (± 4.97 %) | 0.98 ± 0.034 | 0.002 ± 0.028 | 499.8 ± 2.760 | -0.0242 ± 0.049 |
| 10.00 | 10.08 (± 4.18 %) | 0.98 ± 0.029 | 0.002 ± 0.026 | 499.5 ± 2.966 | -0.0241 ± 0.043 |
| 12.59 | 12.70 (± 3.96 %) | 0.98 ± 0.026 | 0.000 ± 0.022 | 499.5 ± 2.760 | -0.0239 ± 0.038 |
| 15.85 | 15.96 (± 3.44 %) | 0.98 ± 0.022 | 0.000 ± 0.019 | 499.9 ± 2.815 | -0.0246 ± 0.033 |
| 19.95 | 20.03 (± 3.11 %) | 0.99 ± 0.021 | 0.002 ± 0.017 | 499.8 ± 2.982 | -0.0238 ± 0.029 |
| 25.12 | 25.32 (± 2.80 %) | 0.99 ± 0.018 | 0.002 ± 0.015 | 499.9 ± 2.902 | -0.0233 ± 0.025 |
| 31.62 | 31.70 (± 2.43 %) | 0.99 ± 0.016 | -0.000 ± 0.014 | 499.5 ± 2.852 | -0.0245 ± 0.023 |
| 39.81 | 39.99 (± 2.09 %) | 0.99 ± 0.014 | 0.000 ± 0.012 | 499.5 ± 2.755 | -0.0242 ± 0.020 |
| 50.12 | 50.30 (± 1.99 %) | 0.99 ± 0.013 | 0.001 ± 0.011 | 499.9 ± 2.815 | -0.0243 ± 0.018 |
| 63.10 | 63.37 (± 1.65 %) | 0.99 ± 0.010 | 0.001 ± 0.010 | 499.4 ± 2.764 | -0.0239 ± 0.015 |
| 79.43 | 79.78 (± 1.45 %) | 0.99 ± 0.010 | 0.000 ± 0.009 | 499.8 ± 2.810 | -0.0250 ± 0.014 |
| 100.00 | 100.32 (± 1.35 %) | 1.00 ± 0.008 | -0.000 ± 0.008 | 499.7 ± 2.944 | -0.0252 ± 0.012 |
| 125.89 | 126.24 (± 1.16 %) | 1.00 ± 0.008 | 0.001 ± 0.007 | 499.6 ± 2.886 | -0.0246 ± 0.012 |
| 158.49 | 158.82 (± 1.12 %) | 1.00 ± 0.007 | 0.001 ± 0.006 | 499.5 ± 2.861 | -0.0243 ± 0.010 |
| 199.53 | 199.92 (± 0.95 %) | 1.00 ± 0.006 | 0.000 ± 0.005 | 499.4 ± 2.982 | -0.0244 ± 0.009 |
| 251.19 | 251.53 (± 0.86 %) | 1.00 ± 0.005 | 0.001 ± 0.005 | 499.5 ± 3.102 | -0.0239 ± 0.007 |
| 316.23 | 316.59 (± 0.76 %) | 1.00 ± 0.005 | 0.000 ± 0.004 | 499.5 ± 2.865 | -0.0247 ± 0.007 |
| 398.11 | 398.61 (± 0.64 %) | 1.00 ± 0.004 | 0.000 ± 0.004 | 499.6 ± 2.889 | -0.0244 ± 0.006 |
| 501.19 | 501.77 (± 0.60 %) | 1.00 ± 0.003 | 0.000 ± 0.003 | 499.3 ± 2.852 | -0.0248 ± 0.005 |
| 630.96 | 631.33 (± 0.50 %) | 1.00 ± 0.003 | -0.000 ± 0.003 | 499.4 ± 2.772 | -0.0251 ± 0.005 |
| 794.33 | 794.73 (± 0.44 %) | 1.00 ± 0.003 | 0.000 ± 0.003 | 499.7 ± 2.745 | -0.0249 ± 0.004 |
| 1000.00 | 1000.81 (± 0.43 %) | 1.00 ± 0.003 | 0.000 ± 0.002 | 499.6 ± 2.968 | -0.0245 ± 0.004 |

| eSIP fit approach Solution | | | | |
|---|---|---|---|---|
| Intensity input [Photon] | Intensity fit (A) [Photon] | FWHM ($\omega_{FWHM}$) [µm] (input: 1 µm) | Axial position ($z_0$) [µm] (input: 0 µm) | Offset ($I_0$) [DL] (input: 500 DL) |
| 1.00 | 0.91 (± 3.58 %) | 0.93 ± 0.267 | 0.043 ± 0.110 | 500.6 ± 4.193 |
| 1.26 | 1.18 (± 2.66 %) | 0.93 ± 0.218 | 0.038 ± 0.085 | 500.4 ± 4.173 |
| 1.58 | 1.51 (± 2.21 %) | 0.93 ± 0.183 | 0.033 ± 0.071 | 500.1 ± 4.397 |
| 2.00 | 1.93 (± 1.90 %) | 0.94 ± 0.156 | 0.028 ± 0.063 | 500.3 ± 3.949 |
| 2.51 | 2.44 (± 1.65 %) | 0.94 ± 0.128 | 0.018 ± 0.051 | 500.3 ± 4.243 |
| 3.16 | 3.09 (± 1.42 %) | 0.93 ± 0.114 | 0.015 ± 0.045 | 500.1 ± 4.073 |
| 3.98 | 3.91 (± 1.23 %) | 0.94 ± 0.101 | 0.012 ± 0.039 | 500.2 ± 4.200 |
| 5.01 | 4.95 (± 1.03 %) | 0.95 ± 0.084 | 0.007 ± 0.034 | 499.9 ± 4.177 |
| 6.31 | 6.24 (± 0.97 %) | 0.96 ± 0.077 | 0.009 ± 0.030 | 500.2 ± 4.109 |
| 7.94 | 7.88 (± 0.86 %) | 0.96 ± 0.070 | 0.004 ± 0.026 | 499.5 ± 4.335 |
| 10.00 | 9.93 (± 0.70 %) | 0.96 ± 0.058 | 0.004 ± 0.023 | 499.8 ± 4.366 |
| 12.59 | 12.53 (± 0.64 %) | 0.96 ± 0.052 | 0.001 ± 0.020 | 499.6 ± 4.440 |
| 15.85 | 15.79 (± 0.58 %) | 0.97 ± 0.044 | 0.001 ± 0.018 | 499.9 ± 4.274 |
| 19.95 | 19.90 (± 0.53 %) | 0.97 ± 0.041 | 0.000 ± 0.016 | 499.7 ± 4.069 |
| 25.12 | 25.05 (± 0.46 %) | 0.97 ± 0.034 | -0.000 ± 0.013 | 499.5 ± 4.356 |
| 31.62 | 31.58 (± 0.45 %) | 0.98 ± 0.031 | -0.000 ± 0.012 | 499.8 ± 4.475 |
| 39.81 | 39.73 (± 0.35 %) | 0.98 ± 0.028 | -0.000 ± 0.011 | 499.5 ± 4.501 |
| 50.12 | 50.04 (± 0.33 %) | 0.99 ± 0.024 | -0.001 ± 0.010 | 499.8 ± 4.423 |
| 63.10 | 63.01 (± 0.27 %) | 0.99 ± 0.022 | -0.001 ± 0.009 | 499.6 ± 4.661 |
| 79.43 | 79.36 (± 0.26 %) | 0.99 ± 0.019 | -0.000 ± 0.008 | 499.4 ± 4.182 |
| 100.00 | 99.92 (± 0.24 %) | 0.99 ± 0.017 | -0.001 ± 0.007 | 499.4 ± 4.410 |
| 125.89 | 125.81 (± 0.22 %) | 0.99 ± 0.016 | -0.001 ± 0.006 | 499.5 ± 4.697 |
| 158.49 | 158.38 (± 0.18 %) | 0.99 ± 0.013 | -0.000 ± 0.005 | 499.7 ± 4.483 |
| 199.53 | 199.42 (± 0.16 %) | 0.99 ± 0.012 | -0.001 ± 0.005 | 499.3 ± 4.427 |
| 251.19 | 251.08 (± 0.15 %) | 0.99 ± 0.009 | -0.001 ± 0.004 | 499.0 ± 4.662 |
| 316.23 | 316.14 (± 0.13 %) | 1.00 ± 0.009 | -0.001 ± 0.004 | 499.1 ± 4.644 |
| 398.11 | 398.03 (± 0.12 %) | 1.00 ± 0.008 | -0.001 ± 0.004 | 498.9 ± 4.824 |
| 501.19 | 501.15 (± 0.10 %) | 1.00 ± 0.007 | -0.000 ± 0.003 | 499.0 ± 4.597 |
| 630.96 | 630.86 (± 0.09 %) | 1.00 ± 0.006 | -0.000 ± 0.003 | 498.8 ± 4.732 |
| 794.33 | 794.26 (± 0.08 %) | 1.00 ± 0.006 | -0.000 ± 0.003 | 498.8 ± 4.654 |
| 1000.00 | 999.89 (± 0.07 %) | 1.00 ± 0.005 | -0.000 ± 0.002 | 499.1 ± 4.886 |