Supplemental Document



Flexible method for generating needle-shaped beams and its application in optical coherence tomography: supplement

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Supplement DOI: https://doi.org/10.6084/m9.figshare.20145608

Parent Article DOI: https://doi.org/10.1364/OPTICA.456894

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24 1. Introduction

25 2. Methods

26 2.1 Principle



Fig.S1 The experimental demonstration of the focus shift function. The DOEs containing two 29 foci were fabricated and placed in OCT system (referring to Fig.S10). One focus is the original objective focus f, and the other is shifted to f_m according to the function P_m $(x,y,f_m,f) = (2\pi n/\lambda) \cdot \{ [f - \sqrt{f^2 - (x^2 + y^2)}] - [f_m - \sqrt{f_m^2 - (x^2 + y^2)}] \}$, where (x,y) is the planar coordinate, λ is the light wavelength, and n is the diffractive index of the 30 31 32 33 imaging space. (a, b) OCT B-scan images (x is the horizontal direction and z is the depth 34 direction) of the 0.8µm beads. In a B-scan, there are two separate bright layers due to the two 35 foci. The two focal planes were determined by the two intensity peaks and the interval 36 between the two peaks was measured to compare the designed shift $(f_m - f)$. The shifts in 37 the figures are 400µm and -300µm. (c) The experimental results are consistent with the 38 theoretical values in the range of [-400 μ m, 1200 μ m] with the errors between 1% and 5%, and 39 z = 0 is the original focal location.





42 Fig.S2 The method to allocate the pixels of the phase mask to different foci. This example 43 contains 9 foci and 1024×1024 pixels. (a) Every 3×3 contiguous pixels make up one unit cell 44 (four unit cells in the zoom-in view). The 9 pixels in one unit cell are assigned to the 9 foci, 45 for example, the pixel labeled with f1 means $L_1(x,y) = 1$ and $L_{m\neq 1}(x,y) = 0$ ($m \in \{1,2,\dots,9\}$ 46). The locations of the pixels associated with the same focus in different unit cells are 47 constant. For example, all the four f1 pixels are located in the top left corner of four 3×3 unit 48 cells, and $L_1(x,y) = 1$ is periodic in both X and Y directions. In this manner, each binary 49 function $L_m(x,y)$ is a two-dimensional periodic array. The periodic distributions introduce 8 50 apparent high-order peaks in the focal plane, generating 9 beams in total. (b) In order to 51 eliminate noisy high-order beams, the locations of the pixels assigned to a specific focus are 52 random in different unit cells. Since the spatially periodic property of $L_m(x,y)$ is broken, only 53 the zero-order beam at the center of the focal plane remains. The intensity ratio of the central 54 beam to the background is 102dB in the simulations. In real applications, the number of foci 55 can be huge, for example, 400 foci. (c) The amplitude of the zero-order peak is inversely 56 proportional to the foci number M. The background noise level also decreases with M. 57 Overall, the peak-to-background ratio (PBR) decreases with M, but PBR keeps a large value 58 even at M=400 (PBR>3100). Here, PBR is calculated as the ratio of peak intensity to the 59 average background intensity.



62 Fig.S3 The positions of the individual foci are optimized for the uniform axial intensity of the 63 generated beam. In this simulation example, the beam is composed of 81 foci with the length 64 of 300µm, and the lens is a 20x water immersion objective (UMPLFLN20XW, Olympus, 65 equivalent focal length = 9mm). (a) Two spatial distributions of the foci. In one, the foci are 66 uniformly distributed over the range 0-300µm with an equal interval (the objective original 67 focus is at z = 0), while in the second the foci locations are optimized by the algorithm listed 68 in e. (b) If the foci are uniformly distributed, the axial beam intensity is not uniform, which 69 creates maximums at the two ends of the beam. With optimized foci positions, the beam 70 displays uniform intensity distribution along the z direction. (c) A comparison of axial beam 71 intensities under the two conditions. With optimization, the intensity fluctuation is reduced 72 from 50% to 5%. (d) Optimization is also beneficial to narrow the maximum diameter of the 73 needle-shaped beam. (e) The algorithm developed in this study to uniform the beam axial 74 intensity by optimizing the foci positions. To accelerate the optimization of foci positions, we 75 only calculate the light distribution along the optical axis (x = 0 and y = 0, z has 1024 pixels) 76 and the number of iterations is fixed at 12. The computation time cost for 100 foci is 1.6s (I7-77 8700 3.2GHz, 64Gb RAM) or 1.4s (AMD 3945WX 4.0GHz, 160Gb RAM). For 400 foci, it is 78 5.5s (I7-8700 3.2GHz, 64Gb RAM) or 5.0s for 400 foci (AMD 3945WX 4.0GHz, 160Gb 79 RAM).

81 2.2 Simulations

82 Phase adjuster affects several characteristics of NB. Here we selected several simulation 83 experiments to reveal its role on shaping the NB profile. We use phase adjustors $Pa_m = PA \cdot$ 84 *m*, where the focus index *m* and *PA* is the coefficient.

The first example is a two-foci beam. The focus is F(x,y,z,f) = A(x,y,z,f)exp85 $[i \cdot P(x, y, z, f)]$, where A is the amplitude and P denotes phase distribution, referring to Eq. 86 87 (3) and Eq. (4) in the main text. Set the axial interval between the two foci as zero and the phase adjuster is zero, then the two foci are the same as F(x,y,z,f)/2, generating a single 88 bright focus F(x,y,z,f) with a FWHM diameter of 1.4µm, as shown in Fig.S4(a). Adding a 89 phase shift $\pi/2$ to the second focus, the combination is $F(x,y,z,f) \cdot 0.5 \cdot [1 + \exp(i \cdot \pi/2)]$, 90 and the amplitude is reduced to $A/\sqrt{2}$ and the diameter is unchanged, as shown in Fig.S4(b). 91 92 With the phase shift of π , the two foci counteract each other, as shown in **Fig.S4(c)**. It proves 93 that the combined optical field can be modulated by adjusting the phase difference between 94 the two spatially coincident foci.

95 Second, set the axial interval between the two foci as 4um. If the phase shift is zero, 96 Fig.S4(d), the two foci have the same phase distribution along the optical axis, the light filed 97 is $[F(x,y,z,f-2\mu m) + F(x,y,z,f+2\mu m)]/2$, and the diameter at the middle plane between 98 the two foci is $1.5\mu m$ (larger than the single-focus diameter $1.4\mu m$), given by 99 $[F(x,y,f,f-2\mu m)+F(x,y,f,f+2\mu m)]/2$. With a non-zero PA, the light filed is 100 $[F(x,y,z,f-2\mu m) + F(x,y,z,f+2\mu m) \cdot \exp(i \cdot PA)]/2$. By increasing the PA in Fig.S4(e-101 g), the optical field created by the interference of the two foci changes in terms of intensity, 102 diameter, and side lobes. With increasing PA, the diameter can reduce to be smaller than the 103 original objective focal size at the cost of the intensity, as shown in Fig.S4(e, f). With a larger 104 value of *PA*, donut focus may be generated, Fig.S4(g).

105 Third, we tested three closely-spaced foci with a 4µm interval. As illustrated in Fig.S5(a-106 c), a large value of PA can reduce beam diameter (even smaller than the Gaussian beam 107 focus) and the length of the three-foci beam. At a distant position, e.g., z=12.025mm in 108 Fig.S5(d-e), the intensity of the three-foci beam with a large PA is much weaker than that of 109 the one modulated by a small PA. In other words, more energy will leak into the neighboring 110 space when using a small PA. For a NB containing many foci, like the NBs in Fig.S10, with a 111 large PA, NB has a small diameter and the beam profile at a certain depth Z' is mainly 112 determined by the few local foci around Z' (barely affected by the distant foci), thus the NB 113 diameter is more uniform along z direction. On the contrary, a small PA leads to a large NB 114 diameter and makes it hard for the current optimization algorithm (Fig.S3) to simultaneously 115 achieve uniform axial intensity and uniform beam diameter along the depth direction. As 116 mentioned in Discussion section of the main text, both uniform intensity and uniform 117 diameter are promisingly achievable if we can develop a new design algorithm that fully leverages the design freedom of $f_1,...,f_M$ and $Pa_1,...,Pa_M$. 118

119 Next, based on the above simulated results, it is reasonable to surmise that a NB
120 composed of multiple foci can also be modulated by the phase adjuster in a similar way,
121 which has been testified in Fig.2. Conclusively, a large *PA* reduces the diameter at the cost of
122 side lobes and efficiency.



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125 Fig.S4 Simulations of two foci. The incident light is Gaussian beam (910nm, 4.6mm diameter 126 at $1/e^2$, intensity = 1), focused by a water immersion objective (focal length in water = 127 12mm). The two foci are spatially coincident in (a-c). (a) PA=0, beam diameter = 1.4 μ m, 128 maximum intensity = 4×10^6 ; (b) *PA*=0.5 π , beam diameter = 1.4 μ m, intensity = 2×10^6 ; (c) *PA* 129 $=1\pi$, intensity = 0. The two foci are separately fixed at Z=12mm-2µm and Z=12mm+2µm in 130 (d-g). (d) PA=0, beam diameter = 1.5µm, intensity = 3.3×10^6 , invisible side lobes; (e) PA=0.5 π , beam diameter = 1.3 μ m, intensity = 2.9×10⁶, invisible side lobes; (f) PA=1 π , beam 131 diameter = $1.0\mu m$, intensity = 0.4×10^6 , intensity ratio of the side lobe to the central main lobe 132 133 is 4%; (g) $PA=1.25\pi$, intensity = 1.4×10^5 , donut spot.



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137 Fig. S5 Simulations of a 3-foci beam. The incident light is Gaussian beam (910nm, 4.6mm diameter at $1/e^2$, intensity = 1), focused by a 20x water immersion objective (focal length = 138 12mm). The Z positions of the three foci are 12mm-4 μ m, 12mm, 12mm+4 μ m. (a) PA=0, 139 beam diameter = 1.7µm, maximum intensity = 2.5×10^6 ; (b) PA=0.3 π , beam diameter = 140 141 1.5µm, intensity = 2.7×10^6 ; (c) PA=0.6 π , beam diameter = 1.2µm (Gaussian beam focus size is 1.4 μ m), intensity = 0.9 × 10⁶. (d-f) With increasing PA, the length of the beam, 142 143 described by FWHM of the axial intensity along the optical axis, becomes shorter (20µm, 144 14µm, and 10µm), and more energy is confined around the physical positions of the three 145 foci.

Step 1

- Choose the beam length L and determine the number of the foci M with the restriction of $RL \le L/(M-1) \le RL$, where RL stands for Rayleigh length of the objective.
- Select the position of the first focus, which is usually set at $f_1 = f$ or $f_1 = f L/2$, where f is the objective focal length, then the last focus location is $f_M = f_1 + L$. We used $f_1 = f$ for all the DOEs fabricated in this study.

Step 2

• Optimize the positions of the individual foci for the uniform axial intensity of the generated beam, as illustrated in Fig. S3.

Step 3

- The phase adjusters $Pa_1, ..., Pa_M$ are set at $Pa_m = PA \cdot m$ in this work, where m is the focus index $\in \{1, 2, ..., M\}$ and PA is a coefficient $\in [0, 2\pi]$.
- Scan *PA* within the range of $[0, 2\pi]$, thereby quantifying the beam characteristics under different *PA* values. Empirically, $0 < PA < 0.5\pi$ is the common range for NBs.

Step 4

- Select an appropriate PA for a design.
- Complete the phase pattern of the designed needle beam, as described in Fig. 1.

148 Fig.S6 The design procedure of a needle-shaped beam.

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150 2.3 Fabrication

151 The fabrication procedure is shown in Fig.S7. A blank fused silica wafer (4inch, 500µm 152 thickness) was first soaked in piranha solution (9:1 H₂SO₄:H₂O₂) for 20mins at 120°C for 153 cleaning. Then a 100nm thick aluminum (Al) layer was deposited onto one side of the wafer (750 seconds, Lesker Sputter). The Al layer serves as a heat conductor to improve the 154 downstream dry etching uniformity. With the coating tool (SVG Coat), a 1.6µm thick 155 156 photoresist (SPR3612, 2mm edge bead removal) was deposited onto the other side of the 157 wafer after being processed by Hexamethyldisilazane (HDMS, an adhesion promoter for 158 resists). The mask pattern was transferred onto the photoresist layer by direct laser writing 159 (MLA 150, Heidelberg; 405nm laser, 1 defocus, 50mJ/cm2 dosage). Next, the wafer was 160 baked at 110°C for 90s to harden the exposed photoresist then developed (SVG Developer). Then, the wafer was engraved by inductively coupled plasma (ICP) etching (ICP Dielectric 161 162 Etcher, Plasma Therm Versaline; the parameters were set as 450W ICP, 50W BP, 40CHF3, 2 163 O2, 5 mT, 4T He, 10°C electrode, 70°C liner, 90°C spool, 90°C lip). The etching rate was 164 2.3-2.5nm/s, measured using a dummy wafer. Following etching, the remaining photoresist 165 was removed by oxygen plasma (Plasma Resist Strip, Matrix). The lithography steps 166 described above were repeated three more times (the first round lithography for 1008nm, the second for 504nm, the third for 252nm, and the fourth for 126nm, combining to generate 16 167 168 heights). The Al layer was removed by first soaking the wafer in aluminum etchant (CMOS, 169 J.T. Baker) at 40°C for 20mins, and then cleansing in piranha solution (20mins at 120°C). 170 Finally, wafers were divided into separate DOEs using an Excimer laser (IX-255, IPG 171 Photonics; 193nm, under high fluence mode, 7mJ, VAT=35%, 4 repeated cycles).



Fig.S7 The fabrication of the DOEs. (a) The fabrication process for the DOEs is based on four rounds of lithography. (b) Nine DOEs were fabricated on one wafer simultaneously. (c) The full-view microphotograph of one DOE (VHX-6000, Keyence). (d) A zoomed-in view of the DOE. (e) A close-up view of the individual 10µm×10µm pixels on the DOE surface. (f) The 16 height levels of a step structure (the red arrow in c) are measured by the profilometer (P2, Tencor). (g) The real 16 heights of 9 samples are tested and the errors are below 2%. STD stands for standard deviation, and CV is coefficient of variation.

182 3. Results

183 3.1 Beam profiles

184 Beam profiles were measured by a microscope system shown in Fig.S8. Its magnification is 185 44.4 when using 40x water immersion objective (UMPLFLN40XW, Olympus) for imaging 186 and is 11.1 with a 10x dry lens (LSM02-BB, Thorlabs). The axial scanning step size was 187 chosen between 1µm to 10µm to match different beam lengths. The beam diameter (FWHM) in a 2D image is calculated as $2 \times \sqrt{A_{FWHM}/\pi}$, where A_{FWHM} is the area where the beam 188 intensity is no less than the half of the maximum intensity (after background subtraction). As 189 190 show in Fig.S8(c), the cross section through the middle of the NB or focused Gaussian beam 191 is taken out to calculate the efficiency and sidelobe ratio. The sidelobe ratio is the peak 192 intensity ratio between the first sidelobe and the main lobe, and the needle-shaped beam (NB) 193 efficiency is evaluated by the ratio of the energy enclosed within the NB's central lobe to the 194 energy enclosed within the Gaussian focus spot. When calculating the efficiency, the focused 195 Gaussian beam is generated by the same objective used for NBs. In theory, the efficiency of 196 Gaussian beam is 100% since its focus contains all the incident energy (E_{IN}), $E_{GB} = E_{IN}$. In the 197 simulations, the input energy (the energy of the Gaussian beam before entering the objective) 198 is the same as the energy enclosed within the Gaussian focal spot, generating a 100% 199 efficiency. In the practical experiments of 3D beam profiles, the transmission efficiency of 200 the objective (EE_{OBJ}) is not 100%. Thus, the Gaussian spot energy is lower than the incident 201 energy. The energy of the Gaussian spot is $E_{GB} = E E_{OBJ} \times Input$ Energy. The energy of the 202 main lobe of the NB is $E_{NB} = EE_{OBJ} \times Input Energy \times E_{NB}$. So we use the energy of the 203 Gaussian focus as the reference to eliminate the effect of objective on energy transmission, 204 giving $E_{NB}/E_{GB} = (EE_{OBJ} \times Input Energy \times E_{NB})/(EE_{OBJ} \times Input Energy) = EE_{NB}$. 205



206 207 Fig.S8 The setup used to image the beam profiles. (a) This setup contains the three modules 208 of light source, the DOE system, and the imaging system. The light is produced by a 209 supercontinuum laser (SL, Superk Extreme, NKT Photonics). Lens L1 collimates the output 210 light from the single mode fiber (SMF). F1 is a bandpass filter (910±5nm, FB910-10, 211 Thorlabs) and F2 is a neutral density filter to reduce the power. L2 is the coupler (focal length 212 = 12mm, TC12APC-850, Thorlabs) to convey the laser into the downstream SMF. L3 is the 213 collimator with a focal length of 25mm and the output Gaussian beam has a diameter of 4.6mm. The homemade DOE aims to modulate the beam phase. The pair of L4 and L5 (focal 214 215 length = 60mm, AC254-060-B, Thorlabs) is a 4f system to transfer the phase pattern to the 216 back focal plane of the 20x water immersion objective OBJ1 (UMPLFLN20XW, Olympus). 217 The beam is focused by OBJ1 in the water of the tank and observed by another 40x water 218 immersion objective OBJ2 (UMPLFLN40XW, Olympus) with the lens L6 (focal length=200mm, AC254-200-B, Thorlabs). The beam is imaged on the CCD sensor (pixel size 219 220 = 5 μ m, WinCamD, Dataray). The imaging system is axially moved by a precise motor M 221 (Z812, Torlabs) to test the beam profiles at different depths. (b) A photo of the two objectives 222 and tank. (c, d) The sidelobe ratio of NB is the peak intensity ratio between the first sidelobe 223 and the main lobe, and the NB's efficiency is the ratio of the energy enclosed within the NB's 224 central lobe (white circle) to the energy enclosed within the Gaussian focus spot (= E_{NB}/E_{GB}).



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Fig.S9 The real beam profiles versus the simulations. (a) The real profiles of the Gaussian beam (910nm, 4.6mm diameter at $1/e^2$), focused by 20x water immersion objective. The experimental focal size is 1.6µm and the simulated size is 1.5µm. (b), The 300µm needleshaped beam (NB) with the phase adjusters $Pa_m = 0.040\pi \cdot m$ has a diameter of 3µm. Pa_m $= PA \cdot m$ and the focus index $m \in \{1, 2, ..., 81\}$. (c) The 300µm NB with $PA = 0.222\pi$ has a 1.2µm diameter that is smaller than the Gaussian focal spot. Sim., simulated; Exp., experimental.



235 Fig.S10 The real profiles of seven 300µm needle-shaped beams (NBs). (a) The real profiles. 236 The ZX scale bar is 25μ m×10 μ m and the XY scale bar is 10μ m×10 μ m. (b) The diameter 237 profiles. (c) The axial intensity profiles. The axial intensity of the NBs with diameters of 238 $2\mu m$, $3\mu m$, $4\mu m$, $5\mu m$, $6.5\mu m$ is basically uniform. The narrow NB (diameter = $1.2\mu m$ or 239 1.5µm) has a peak at the left end because the fabrication errors lead to an intensity 240 increasement at the objective focus (positioned at the left end of NB), which is comparable to 241 the NB intensity. Gaussian beam: 910nm, 4.6mm diameter at $1/e^2$. The objective: 20x water 242 immersion objective.



Fig.S11 The real profiles of the needle-shaped beams (NBs) with various sizes. (a) The real profiles. The ZX scale bar is $25\mu m \times 10\mu m$ and the XY scale bar is $10\mu m \times 10\mu m$. The maximum sidelobe ratios in the middle of the NB are 20% for 80µm×1.5µm NB, 17% for 450µm×1.5µm NB, 18% for 600µm×2µm NB, 11% for 700µm×3.5µm NB, and 17% for 1000μm×2.5μm NB. The number of the foci constituting one NB is 16 for 80μm×1.5μm NB, 100 for 450µm×1.5µm NB, 144 for 600µm×2µm NB, 169 for 700µm×3.5µm NB, and 196 for μ m×2.5 μ m NB. The values of PA for the above NBs are 0.210 π , 0.190 π , 0.104 π , 0.035π , and 0.129π respectively. (b) The diameter profile of the focused Gaussian beam. (c) The diameter profiles of all beams. (d) The axial intensity distributions. Gaussian beam: 910nm, 4.6mm diameter at $1/e^2$. The objective: 20x water immersion objective.



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256 Fig.S12 The real profiles of the needle-shaped beams (NBs) generated by non-immersion 257 objective. The is 10x objectives (focal length = 18mm, LSM02-BB, Thorlabs) was used as the 258 OBJ1 and OBJ2 in Supplementary Fig.8 with the same input Gaussian beam (910nm, 4.6mm 259 diameter). (a) The real profiles. The focused Gaussian beam has the Rayleigh length of 18µm 260 and a focal size of 3.5µm. The 600µm×7.5µm NB is contains 64 foci ($PA = 0.096\pi$, efficiency = 20%), which has a diameter profile changing between $4.5\mu m$ and $7.5\mu m$ within 261 262 its 600µm depth-of-focus (DOF). The 900µm×9µm NB has a diameter profile between 4µm 263 and 8.7µm within the 900µm DOF, containing 100 foci ($PA = 0.058\pi$, efficiency = 13%). 264 The maximum sidelobe ratios at the middle of NB are 5% for 600µm×7.5µm NB, 8% for 265 900μm×9μm NB. ZX scale bar, 35μm×20μm; XY scale bar, 20μm×20μm. (b) The axial 266 intensity profiles of the focused Gaussian beam and the two NBs. The uniformities of the two 267 NBs are both above 90%. (c) The diameter profiles. At the depth of 600µm, the focused 268 Gaussian beam has a diameter of $42\mu m$, while the $600\mu m \times 7.5\mu m$ NB has a diameter of $7\mu m$. 269 At the depth of 900µm, the diameter of the focused Gaussian beam is 76µm, and the diameter 270 of 900µm×9µm NB is only 8µm.

272 3.2 Needle-shaped beams in OCT

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276 Fig.S13 The OCT imaging system. Light source. The light is from a supercontinuum laser (SL, Superk Extreme, NKT Photonics). Lens L1 collimates the output light from the single 277 278 mode fiber (SMF). F1 is a long-pass filter (800nm, FELH0800, Thorlabs) and F2 is a short-279 pass (1050nm, #64-338, Edmund) filter. L2 is the coupler (focal length = 12mm, TC12APC-280 850, Thorlabs) to convey the laser into the downstream SMF. Reference arm. L3 is the 281 coupler (focal length = 25mm, TC25APC-850, Thorlabs). HWP is a half-wave plate 282 (WPH05ME-980, Thorlabs) to reduce the polarization disparity between the reference arm 283 and sample arm. A1 is the adjustable aperture (SM1D12C, Thorlabs) to change the power. M 284 is the mirror (solid prism retroreflector, PS975M-B, Thorlabs) to reflect the input laser. PBS 285 is a polarizing beam-splitter cube (CCM1-PBS253/M, Thorlabs) to eliminate the reflected 286 light perpendicular to the input, which is induced by the mirror and can generate ghost images 287 in OCT. TS is a translation stage (CT1, Thorlabs) to adjust reference arm length. Sample arm. 288 L4 is the collimator with a focal length of 25mm and the output Gaussian beam has a 289 diameter of 4.6mm. The homemade DOE aims to modulate the beam phase. The pair of L5 290 and L6 (focal length = 60mm, AC254-060-B, Thorlabs) is a 4f system that transfers the phase 291 pattern to the back focal plane of the 20x water immersion objective OBJ (UMPLFLN20XW, 292 Olympus). GM is a two-dimensional galvo mirror (OCTP-900, Thorlabs) to scan the sample. SA stands for sample. Spectrometer. The spectrometer is from a commercial OCT 293 294 (Ganymede OCTP-900, Thorlabs) with the detection range from 810nm to 1010nm. L, lens; 295 G, grating. FC is the fiber coupler (TW930R5A2, Thorlabs) to connect the above four parts. 296 Software. The Thorlabs Ganymede imaging software.

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298 OCT setup. As shown in Fig.S10, our OCT system is based on a commercial OCT 299 instrument (Ganymede OCTP-900, Thorlabs) and the light source is centered at 910nm with a 300 bandwidth of 200nm (EXR-9 continuum laser, NKT Photonics). Its acquisition rate is 30,000 301 A-scans/s. The imaging depth is 2mm in air or 1.5mm in water with 1024pixels, and the 302 experimental axial resolution is 3 pixels (FWHM in linear scale). Its original lateral resolution 303 depends on the objective, which is 1.6µm with a 20x water immersion objective 304 (UMPLFLN20XW, Olympus) and 4µm with a 10x dry objective (LSM02-BB, Thorlabs). The 305 laser power entering the objective aperture is controlled below 5mW. Since our system 306 utilizes the same optical path (the sample arm) to illuminate the sample and collect the light 307 (common path), both the incident and the backscattered lights pass through the DOE (double-308 passing, collected light intensity \propto the square of DOE efficiency). The OCT signal intensity 309 is proportional to the amplitude of the light from the sample arm (OCT intensity \propto 310 [amplitude of sample arm signal \times amplitude of reference arm signal]). That means signal

311 intensity is linear to the square root of sample arm intensity also linearly modulated by DOE 312 efficiency. We tested the NBs in Fig.2 with the OCT system by scanning a metal plate in 313 water. As listed in **Table S1**, the results prove that the signal intensity is roughly proportional 314 to DOE efficiency. The sensitivity is measured as the ratio of metal plate intensity to the 315 background intensity (the blank area containing no sample). The ideal point-spread function (PSF) is the same as the intensity profile of the beam. The system point-spread function 316 317 $(PSF)^1$ is the product of the light illumination PSF_1 and the light collection PSF_C . The double-318 passing configuration gives PSF_I and PSF_C the same amplitude profile of needle-shaped beam 319 PSF_{NB} , thus the OCT system PSF_{DP} is the same as the intensity profile of needle-shaped beam 320 $(=|PSF_{NB}|^2)$. Another possible configuration is the single-passing one, where the DOE is only used for light illumination and an independent light collection channel is required to detect 321 322 the lights back-scattered from the sample. Its PSF_{SP} is the product of PSF_{NB} and PSF_{GB} , where 323 PSF_{GB} is the 3D Gaussian amplitude profile shaped by the confocal gate of the light collection 324 fiber via the objective. Apparently, $PSF_{SP} = |PSF_{NB} \times PSF_{GB}|$ makes the axial intensity 325 reduce as well as the lateral resolution degrade with the distance to the objective focal plane. 326 Also, double-passing configuration used in this work can suppress side lobes, since PSF_{DP} is 327 proportional to the intensity profile of NB while PSF_{SP} is proportional to the amplitude profile 328 of NB. 329

330 Image acquisition. For the experiments in Figs.3-5, pixel number in both the X and Y 331 directions was chosen as 512 (Field-of-View = 0.125mm×0.125mm, 1mm×1mm, 332 0.5mm×0.5mm, respectively). To enhance the detection sensitivity, the A-scan at every point 333 was repeated successively four times and the averaged data were used for image 334 reconstruction. The acquisition time for one 3D volume is around 45s. In the reconstructed 335 3D images, a 2D Gaussian filter with a standard deviation of 2 pixels was applied for every 336 XY layer to reduce the speckle noises. For the dynamic imaging of drosophila larva, no A-337 scan average was applied in order to achieve a high frame rate (12 B-scans/s). The scanning 338 range is 4mm in the first section of Supplementary Video and 1mm in the second and third 339 sections, and the number of scanning steps was 2048 for all the three sections. All B-scans 340 were processed by a 2D Gaussian filter with a standard deviation of 2 pixels to reduce speckle 341 noise. All OCT images were presented in log10 scale. In a single 2D OCT image, the 342 intensity scale was dynamically adjusted according to the maximum intensity and the 343 minimum intensity (or background) in the image.

344

345 Beads imaging. The phantom used to characterize the needle-shaped beams in OCT was 346 made by uniformly distributing 0.8µm polystyrene (PS) beads (TP-08-10, Spherotech) in 347 ultrasound gel (Aquasonic 100, Parker). The phantom was degassed with a centrifuge (15mins at 15,000rpm, ST16-R, Thermo) with bead concentration around 2×10⁵/mm³. The 348 349 beam resolution in OCT system was tested with the 0.8µm polystyrene beads. The resolution 350 at specific depths was determined by the average diameter (FWHM in linear scale) of bead 351 profiles. Each XY layer containing beads in a volumetric image was measured to determine 352 the complete resolution profile of the target beam. Within each depth, 200-300 beads were 353 averaged to give reliable results. Additionally, 20 samples were averaged for measuring one 354 sidelobe ratio.

¹ Stelzer, E.H. and Lindek, S., 1994. Fundamental reduction of the observation volume in far-field light microscopy by detection orthogonal to the illumination axis: confocal theta microscopy. Optics Communications, 111(5-6), pp.536-547.

Beam	Theoretical efficiency	Efficiency measured by beam profiler	Relative intensity of metal plate in OCT	Intensity (I) of metal plate in OCT	Standard deviation (STD) of background intensity in OCT	Sensitivity (I/STD) measured with metal plate in OCT	Sensitivity measured with metal plate in OCT/dB
Gaussian beam	100%	100.00%	100.00%	683600	180	3798	36
300µm×1.2µm NB	0.10%	0.30%	1.90%	12000	158	76	19
300µm×1.5µm NB	0.30%	0.50%	2.30%	16000	163	98	20
300µm×2µm NB	1%	2%	4%	26000	170	153	22
300µm×3µm NB	4%	4%	7%	45000	170	265	24
300µm×4µm NB	9%	9%	10%	70000	175	400	26
300µm×5µm NB	15%	22%	23%	160000	172	930	30
300µm×7µm NB	13%	20%	14%	95000	177	537	27
358	•	*	•	*	•	•	•

356 Table R1 The quantitative characteristics of needle-shaped beams357



360 Fig.S14 The XY images of 0.8µm microbeads captured by the OCT with the focused 361 Gaussian beam (20x water immersion objective), 80µm×1.5µm NB, and 300µm×3µm NB. 362 The XY images at the depths from -160µm to 160µm with 20µm interval are showed. The 363 Gaussian beam only produces high resolution image at z = 0, and the beads become barely 364 distinguishable beyond $z < -40 \mu m$ and $z > 80 \mu m \times 1.5 \mu m$ NB is able to clearly reveal 365 the individual beads in the depth range between -40µm and 40µm, and still can profile beads 366 at the depths of -60µm and 60µm. Outside the aforementioned depths, the XY-images are 367 completely out of focus and contain only noise. 300µm×3µm NB effectively detects single 368 beads for the whole depth range from -160µm to 160µm. Since NBs have sidelobes, the bead 369 profiles may have sidelobes at some specific depths. 80µm NB produces sidelobes in the XY-370 images from $z = -20 \mu m$ to $z = 20 \mu m$, and $300 \mu m$ NB does from $z = -40 \mu m$ to $z = 40 \mu m$. The sidelobe ratios (SRs) are listed below the corresponding XY-images. The sidelobes have 371 372 limited effect on distinguishing closely adjacent beads, for example, the boundaries among 373 the adjacent beads marked by the red arrows are easily legible. GB, Gaussian beam; NB, 374 needle-shaped beam; WI, water immersion; SR, sidelobe ratio. Scale bar (in the last XY-375 image), 10µm. The filed-of-view is 50µm×50µm.

376 3.3 Skin imaging

Human tissue specimens that would otherwise have been discarded during surgical excision of skin growths were collected, placed in keratinocyte media, and stored at 4°C for an average of four hours before being transported to our lab. The specimens were stored in 10% formalin solution (VIP-Fixative, Scigen) at 4 °C in the laboratory refrigerator after being embedded in agar gel in Petri dishes. Informed consent was obtained from all subjects. All experimental protocols were approved by the Stanford Institutional Review Board (Protocol #48409), and all methods were carried out in accordance with relevant guidelines and regulations.

384 385



Fig.S15 The surface projections of the 3D human skin images captured with Gaussian beam (20x water immersion lens) and 300µm×3µm NB. a, With Gaussian beam imaging, only the right upper region is in focus and the rest are blurry and dark. b, With 300µm×3µm NB, the details over the entire surface projection are revealed. Some comparisons are indicated by the red arrows. Scale bar, 200µm; XY-FOV, 1mm×1mm.



393	Fig.S16 The XY images of human skin epidermis at the depths from $z = 20 \mu m$ to $z =$
394	100µm. (a) Gaussian beam was focused (by 20x water immersion lens) at 90µm depth. (b)
395	80μ m×1.5 μ m NB started at $z = 20\mu$ m and ended at $z = 100\mu$ m. (c) The all-in-focus images
396	were captured by axially focusing Gaussian beam at different depths. The XY-images of
397	80μ m×1.5 μ m NB are coincident with the all-in-focus images from $z = 20\mu$ m to $z = 100\mu$ m
398	(e.g., both the cells marked by the red and yellow arrows are consistent), while the reliable
399	range for Gaussian beam is from $z = 80 \mu m$ to $z = 100 \mu m$ (e.g., only the cells marked by the
400	red arrows are consistent, but the cells marked by the yellows are not). Scale bar, 100µm;
401	XY-FOV, 0.5mm×0.5mm. NB, needle-shaped beam; FOV, field-of-view.

402 3.4 Dynamic imaging of drosophila larva

In live imaging experiments, we used 3rd instar larvae of the standard laboratory wild-type
Drosophila melanogaster (Canton-S strain). Flies were raised on cornmeal agar media with a
12h light/dark cycle at 25°C and 50% relative humidity. All experimental protocols were
approved by the Stanford Institutional Review Board (Protocol #48409), and all methods
were carried out in accordance with relevant guidelines and regulations. Visualization 1
shows dynamic imaging of heartbeat, digestive system, and muscle motion.









412 Fig.S17 The images of 0.8µm microbeads captured by the OCT with Gaussian beam (10x dry 413 objective, LSM02-BB, Thorlabs) and 700µm×8µm NB. The beam profiles are given in 414 Supplementary Fig.9. 700µm×8µm NB here and 600µm×7.5µm NB in Supplementary Fig.9 415 were generated by the same diffractive optical element. The size changes because the ultrasound gel used to contain the microbeads has a refractive index about 1.33, which is 416 417 larger than that of air (=1). (a) The B-scan images. The depth range where the beads are 418 clearly imaged is 280µm for Gaussian beam and 880µm for 700µm NB. S, the sample 419 surface. Scale bar, 50µm. (b) The resolution profiles. 700µm×8µm NB has a resolution 420 varying between 4.5µm (at the ends of the beam) and 8µm (in the middle). The resolution of 421 the Gaussian beam is down to 4µm but increases rapidly to 9.6µm at 230µm depth, and the

422 beads become indistinguishable in the depths deeper than 230µm due to its resolution loss. (c) 423 XY images. In Gaussian imaging, the beads located between z = 0 and $z = 200 \mu m$ can produce complete circular profiles. For 700 μ m NB, the range is from z = 0 to $z = 700\mu$ m. 424 425 (d) The peak-to-background ratios (PBRs) along depth and (e) the signal-to-noise ratios (SNR) in 426 the 3D bead images. In the depth range from 200µm to 800µm, the NB outperforms the 427 focused Gaussian beam. PBR = (peak intensity - average background intensity) ÷ average background intensity, SNR = (peak intensity - average background intensity) ÷ standard 428 429 deviation of background intensity. Scale bar, 50µm.

431 4. Discussion and conclusion



Fig.S18 The second configuration of OCT sample arm. (a) The sample arm in Supplementary
Fig.10can use another configuration, where DOE is directly placed on the top of the objective
(OBJ) and just beneath the Galvo mirror (GM) scanner. Under this configuration,
700µm×8µm NB was tested to image 0.8µm microbeads. (b) The B-scan image of 0.8µm
microbeads. S, the sample surface. Scale bar, 50µm. (c) The XY images. Scale bar, 50µm.
Referring to Supplementary Fig.13, the bead images taken with this configuration coincided
with those using the previous configuration.