Throughput-speed Product Augmentation for Scanning Fiber-optic Two-photon Endomicroscopy

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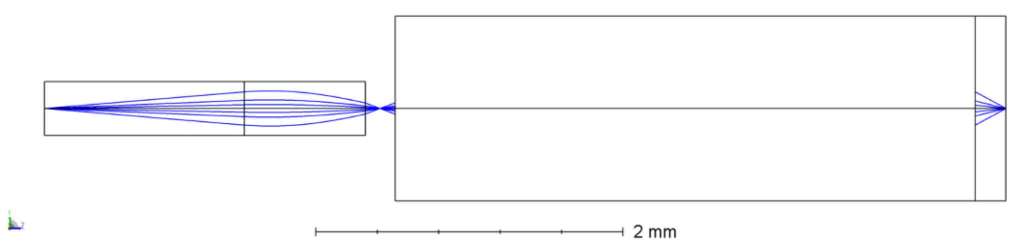
Supplementary Methods

ZEMAX setup for the excitation path

	Surf:Type		Comment	Radius	Thickness		Material	Coating	Semi-Diameter		Chip Zone	Mech Semi-Dia	Conic	TCE x 1E-6	Delta T	n0	Nr2	Nr4
0	OBJECT	Standard		Infini...	0.000		SILICA		0.000		0.000	0.175	0.000	-				
1	(aper)	Standard		Infini...	1.300		SILICA		0.150	U	0.000	0.175	0.000	-				
2	STOP (a	Gradient 5	IFRL-035-005-50	Infini...	0.789		GTAGNEU		0.175	U	-	-	0.000	0.000	1.000E-02	1.629	-3.384	6.360
3	(aper)	Standard		Infini...	0.095	V			0.175	U	0.000	0.175	0.000	0.000				
4		Standard		Infini...	0.100				3.941E-04		0.000	3.941E-04	0.000	0.000				
5		Coordinate Break			0.000		-		0.000		-	-		-	0.000	0.000	0.000	0.000
6	(aper)	Black Box Lens	GT-MO-080-0415-810....		<3.777>				0.600	U	-	-		0.000				
7	(aper)	Standard		Infini...	0.200		WATER		0.600	U	0.000	0.600	0.000	-				
8	IMAGE	Standard		Infini...	-				3.008E-04		0.000	0.600	0.000	0.000				



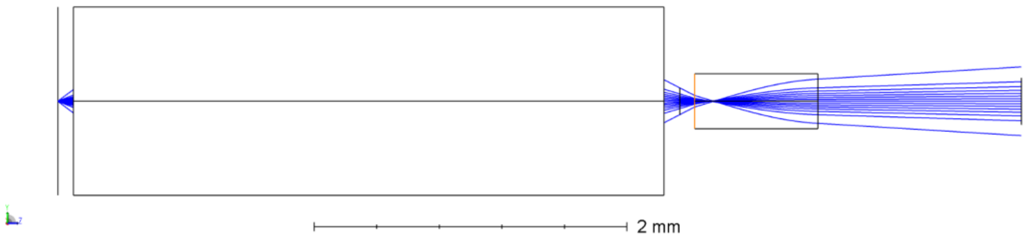
Supplementary Figure 1. ZEMAX setup for NA and off-axis focused spot radii analysis.

Shown in Supplementary Fig. 1 are the lens data and layout in ZEMAX corresponding to NA and focused spot radii analysis in Fig. 1C in the main text. Basically a short piece of silica rod is concatenated with a commercial GRIN rod lens (IFRL-035-005-50-570-NC, GRINTECH GmbH); the combination is then paired with a black box model of the 1.92 × micro objective (GT-MO-080-0415-810, GRINTECH GmbH). The initial beam NA injecting into the silica spacer is set to 0.12, matching the core NA of the DCF we used. A coordinate break surface (surface #5) introduces relative displacement between the GRIN rod lens and the micro objective, mimicking a deflected composite cantilever.

To generate the date in Fig. 1C in the main text, the length of the silica spacer is varied from 1.1 mm to 1.6 mm (in 0.05 mm step size). For each silica spacer length, first the separation between the GRIN rod lens and the back focal plane of micro objective (surface #4 here) is fine-tuned for optimal on-axis focus at the designed sample-side focal depth (i.e. 200 μm in water) of the micro objective. Then tip displacement up to 300 μm in amplitude is introduced via the coordinate break surface, and the resultant off-axis focused spot radii are reported. A custom macro written in ZEMAX programing language (ZPL) automates the whole process and streams output to a text file for further analysis.

ZEMAX setup for the emission/collection path

	Surf:Type		Comment	Radius	Thickness	Material	Coating	Semi-Diameter		Chip Zone	Mech Semi-Dia	Conic	TCE x 1E-6	Delta T	n0	Nr2	Nr4
0	OBJECT	Standard		Infini...	0.100	WATER		0.000		0.000	0.600	0.0...	-				
1	(aper)	Black Box Lens	GT-MO-080-0415-810 reverse.ZBB		<3.777>			0.600	U	-	-		0.000				
2		Standard		Infini...	0.000			0.137		0.000	0.137	0.0...	0.000				
3	STOP (aper)	Standard	Fixed WD of mini-OBJ	Infini...	0.100			0.500	U	0.000	0.500	0.0...	0.000				
4		Standard	Optimal Spacer WD	Infini...	0.095			0.085		0.000	0.085	0.0...	0.000				
5	(aper)	Gradient 5	IFRL-035-005-50	Infini...	0.789	GTAGNEU		0.175	U	-	-	0.0...	0.000	1.000E-02	1.629	-3.384	6.360
6	(aper)	Standard		Infini...	1.300	SILICA		0.150	U	0.000	0.150	0.0...	-				
7	IMAGE	Standard		Infini...	-			0.000	U	0.000	0.150	0.0...	0.000				



Supplementary Figure 2. ZEMAX setup for on-axis collection efficiency analysis. Note that although silica spacer is included in the lens data, ray tracing is terminated at the right-end surface of the GRIN rod lens (IFRL-035-005-50-570-NC, GRINTECH GmbH). Further ray tracing within the silica spacer is carried out by a home-developed MATLAB script which takes care of total internal reflection (TIR) of skew rays on the cylindrical silica-air interface [1] in the spacer.

The ZEMAX beam path is then reversed for collection efficiency analysis, with the on-axis case shown in Supplementary Fig. 2. This time the first surface (surface #0 here in the lens data table) corresponds to the superficial tissue surface, and the distance from tissue surface to the front end face of the micro objective (surface #1 here) is varied from 0 µm to 150 µm, corresponding to a point source depth from 200 µm to 50 µm, as shown in Fig. 1D in the main text.

For each point source depth, the scattered fluorescence photons can emerge from the tissue surface at a random radial radius ρ with a random pointing vector determined by a polar angle $\alpha < 90$ degree and an azimuthal angle $\theta \in [0,2\pi)$, all relative to the optical axis of the system, as given by Monte Carlo simulation. Here we sampled the radius ρ from 2 µm to 498 µm with a step size of 4 µm (totally 125 samples), the azimuthal θ from 0 to 345 degree with a step size of 15 degree (totally 24 samples), and the polar angle α from 3 to 87 degree with a step size of 6 degree (totally 15 samples). Each initial ray determined by (ρ, θ, α) is then traced in ZEMAX up to the right end face of the GRIN rod lens, where its lateral location and direction cosines are logged. This process is automated by another custom macro written in ZEMAX programing language (ZPL).

We have also simulated the collection efficiency for off-axis scenarios, where a lateral displacement is introduced between the cantilever and the micro objective (up to 300 µm as in Fig. 1C). The resultant collection efficiencies turned out quite similar to the on-axis case. This is understandable since the epi-multiphoton-fluorescence collection is a non-imaging process and thus more immune to field-related aberrations. Thus in Fig. 1D of the main text we included only the on-axis collection efficiency for conciseness.

All ZEMAX files, ZPL macros, and MATLAB scripts for ray tracing in silica spacer are available upon request.

Kernel adapted Monte Carlo code

We used the original C++ code available online (https://omlc.org/software/mc/mcml/) for Monte Carlo simulation of scattered photons in biological tissues . Since the original code was designed to trace an incident photon launched from outside the phantom, we adapted the code following instructions in Chapter 11 of the online manual (available on the same webpage above) to simulate a buried source. In specific, we changed the `ReadParm` function in the file "mcmlio.c" to read the source depth, and the `LaunchPhoton` function in the file "mcmlgo.c" to initialize an isotropic fluorescence photon.

The adapted functions are listed below for reference.

```
/****************************************************
************\
* Adapted ReadParm() function in mcmlio.c
* It called a new function named ReadSrpDepth() as
given below
* Read in the input parameters for one run.
\****************************************************
************/
void ReadParm(FILE* File_Ptr, InputStruct * In_Ptr)
{
  char buf[STRLEN];

  In_Ptr->Wth = WEIGHT;

  ReadFnameFormat(File_Ptr, In_Ptr);
  ReadNumPhotons(File_Ptr, In_Ptr);
  ReadSrcDepth(File_Ptr, In_Ptr);
  ReadDzDr(File_Ptr, In_Ptr);
  ReadNzNrNa(File_Ptr, In_Ptr);
  ReadNumLayers(File_Ptr, In_Ptr);

  ReadLayerSpecs(File_Ptr, In_Ptr->num_layers,

        &In_Ptr->layerspecs);
  CriticalAngle(In_Ptr->num_layers,
&In_Ptr->layerspecs);
}
```

```
/****************************************************
***********\
* New function introduced into mcmlio.c
* Read in the next line to get the depth of buried
source
\****************************************************
***********/
void   ReadSrcDepth(FILE   *File_Ptr,   InputStruct
*In_Ptr)
{
        char buf[STRLEN];
        /** read in depth of buried source point **/
        strcpy(buf, FindDataLine(File_Ptr));
        if(buf[0] == '\0')
                nrerror("Reading  depth  of  buried
source.\n");

        // %lf is needed for scanning a double with
scanf
        sscanf(buf, "%lf", &In_Ptr->source_z);

        if(In_Ptr->source_z < 0)
                nrerror("Negative           source
depth.\n");
}
```

```
/****************************************************
*********\
* Adapted LaunchPhoton() function in mcmlgo.c
* Initialize a photon packet.
\****************************************************
*********/
void LaunchPhoton(        double Rspecular,
                          LayerStruct            *
                          Layerspecs_Ptr,
                          PhotonStruct * Photon_Ptr,
                          double srcDepth)
{
        // There is no initial reflection-induced
loss of weight for a buried source.
        if (srcDepth > 0.0) {
                Photon_Ptr->w = 1.0;
        }
        else {
                Photon_Ptr->w   = 1.0 - Rspecular;

        }

        Photon_Ptr->dead = 0;
        Photon_Ptr->layer        = 1;
        Photon_Ptr->s            = 0;
        Photon_Ptr->sleft= 0;

        Photon_Ptr->x            = 0.0;
        Photon_Ptr->y            = 0.0;
        // needs to change this one
        // Photon_Ptr->z         = 0.0;
        Photon_Ptr->z            = srcDepth;
        Photon_Ptr->ux   = 0.0;
        Photon_Ptr->uy   = 0.0;
        Photon_Ptr->uz   = 1.0;

        if((Layerspecs_Ptr[1].mua == 0.0)
                && (Layerspecs_Ptr[1].mus == 0.0))
        { /* glass layer. */
                if (srcDepth > 0.0) {
                        nrerror("The        source
shouldn't be buried in glass layer!\n");
                }
                else {
                        Photon_Ptr->layer
        = 2;
                        Photon_Ptr->z    =
Layerspecs_Ptr[2].z0;
                }
        }

        // To simulate a buried isotropic source,
scatter the launched photon immediately
        if (srcDepth >= 0.0 )
        {
                Spin(0.0, Photon_Ptr);
        }
}
```

References

[1] A. S. Ang, Q. M. Sugon, and D. J. McNamara, "Skew ray tracing in a step-index optical fiber using geometric algebra," *Appl. Opt.*, vol. 54, no. 12, pp. 3764-3773, 2015