Forward-collected simultaneous fluorescence lifetime imaging and coherent anti-Stokes Raman scattering microscopy

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Abstract. We demonstrate the simultaneous collection and separation of femtosecond-laser-based forward-collected coherent anti-Stokes Raman scattering (F-CARS) and two-photon-excitation-induced fluorescence lifetime images (FLIM) using time-correlated single photon counting (TCSPC). We achieve this in a non-descanned geometry using a single multimode fiber without significant loss of light, field of view, and most importantly, TCSPC timing fidelity. In addition to showing the ability to separate CARS images from FLIM images using time gating, we also demonstrate composite multimodal epicollected FLIM imaging with fiber-collected F-CARS imaging in live cells.

Keywords: scanning microscopy; fiber optic applications; nonlinear optics; Raman spectroscopy; laser-induced fluorescence.

1 Introduction

Time-correlated single photon counting (TCSPC)-based fluorescence lifetime imaging microscopy (FLIM) is a powerful tool for studying structure and dynamics in live cells and tissues.[1,2] In particular, within the framework of Förster resonance energy transfer (FRET),[1,3] FLIM is used to resolve molecular interactions on the sub-10-nm length scale. There is a natural desire to integrate FRET or FLIM with the sub-diffraction-limited spatial resolution and sectioning capabilities of nonlinear optical microscopy modalities such as two-photon excitation fluorescence (TPEF), second-harmonic generation (SHG), and coherent anti-Stokes Raman scattering (CARS). Thus far, FRET has been combined with TPEF[1,3] and SHG,[4] but not with CARS. Compared with other nonlinear optical imaging methods, CARS microscopy offers a unique label-free modality that imaging cells and tissues based on their natural chemical composition. CARS microscopy is particularly suited to (and most often used for) imaging of lipid-rich tissue and cellular structures.[5] The combination of CARS and FRET would provide complimentary imaging modalities for probing molecular interactions in the vicinity of lipid-rich cellular architecture. A specific example would include studying the role of and interaction between intracellular lipid droplets and fluorescently tagged hepatitis-C virus (HCV) core protein by performing colocalization experiments, thereby elucidating the HCV infectious pathways. However, unlike TPEF, which is emitted isotropically, the coherent and phase-matched nature of the CARS process leads to a predominance of anti-Stokes emission in the forward direction.[6] Strictly speaking, no backward-directed CARS is expected in bulk homogeneous media. It has been found, however, that under tight focusing conditions, both subwavelength particles and interfaces emit epiderged CARS (E-CARS) signals.[7] Forward-collected CARS (F-CARS) from larger structures typically yields a signal two orders of magnitude greater than E-CARS.[6] It is worth noting that in highly scattering tissue, the presence of some backscattered F-CARS means that the backward-collected CARS signal often represents a mix of E- and F-CARS, which complicates subsequent image interpretation.[8] Establishing a FLIM-CARS microscope represents an important step toward conjoining FRET and CARS capabilities. Recently, Huser et al. demonstrated the incorporation of CARS with TCSPC and FLIM.[8,9] Because CARS is a nearly instantaneous process (on the order of the laser pulse duration), it is indistinguishable from the instrument response function (IRF) of the TCSPC apparatus and can thus be separated from the nanosecond-timescale fluorescence decay by time-gated analysis. However, efforts to combine FLIM with CARS have been hampered by configurations where signals are collected in the descanned backward direction. This is at odds with the maximal CARS signal being in the forward direction. One solution is to reflect the F-CARS back through the sample, collecting the signal via epidetection.[6-10] A second option is to use detectors in the forward direction without descanning. This can work well with large-area photomultiplier tubes (PMTs), but is problematic when using sensitive, ultrafast, photon-counting detectors such as single-photon avalanche photodiodes (SPADs) because of their small active area. Furthermore, this geometry adds considerable complexity to the microscope and requires nontrivial light shielding to enable high contrast detection.

A solution to this problem is to relay the forward-collected signal to off-board detectors with an optical fiber. The
advantages of fiber-coupling the forward-propagating signals are immediately evident: the small fiber core and strict coupling conditions inherently simplify light rejection of both scattered laser and ambient light; bulky detectors and their electronics can be kept away from the microscope, allowing easier sample preparation; and the fiber-coupled signals can be easily relayed to any number of detectors and spectrometers, greatly increasing the modality options of a fixed microscope setup. There have been real and perceived obstacles to this approach. First, coupling of the laser scanning signal to a submillimeter fiber core without untenable loss of light and image distortion is nontrivial. More important to TCSPC, however, is the need to relay photons with high timing fidelity. We feel that it is this last challenge that has largely prevented this approach from being widely adopted. A recent compromise has been the use of large fiber bundles for this purpose. This approach maintains some of the aforementioned advantages of fiber coupling, but the large bundle face can allow for the relaying of significant scattered light, and bundles further suffer loss of light from the inherent light rejection in the interstitial regions of the fiber packing. Finally, the bundle suffers from a significant amount of temporal broadening and skewing of photon arrival times as a function of coupling angle [numerical aperture (NA)].

In this paper we describe the simple modification of a commercial inverted microscope for collection of transmitted non-descanned light through a multimode optical fiber. We show that over 50% coupling and field of view (FOV) can be obtained, with minimal image distortion. Finally, we demonstrate that meters-long fiber can be used to relay TCSPC data with excellent timing fidelity, allowing for single- or multiple-detector FLIM + CARS imaging.

2 Experiments
2.1 Two-Photon Excitation Fluorescence and CARS Microscopy

A Ti:sapphire oscillator (Coherent Mira900) produces 60-fs pulses with a 80-MHz repetition rate, at a wavelength adjustable between 750 to 1000 nm. An 805-nm output was used in the experiments presented here. A variable beamsplitter (a half-wave plate followed by a polarizing beam cube) sends 200 to 250 mW to a commercial photonic crystal fiber (PCF) module (NKT Photonics, FemtoWhite CARS) for the creation of Stokes light to be used for CARS imaging, and the remainder (>1 W) comprises the pump beam used for TPEF, CARS, and SHG imaging modalities. A set of long-pass filters spectrally selects (λ > 950 nm) the Stokes supercontinuum before it is sent to matched SF-10 glass prisms for dispersion engineering, en route to a dichroic beam combiner (Chroma Technology cdxr950). The pump beam is likewise passed through a separate prism pair for dispersion engineering, before being sent to a computer-controlled delay stage and then recombined with the Stokes beam at the combiner. The combined beams are then routed to the microscope. An Olympus 40X 1.15 NA UAP0 water immersion lens with a cover slip correction collar is used as the objective. A 1050-nm component of the broadband Stokes spectrum is immediately evident: the small fiber core and strict coupling conditions inherently simplify light rejection of both scattered laser and ambient light; bulky detectors and their electronics can be kept away from the microscope, allowing easier sample preparation; and the fiber-coupled signals can be easily relayed to any number of detectors and spectrometers, greatly increasing the modality options of a fixed microscope setup. There have been real and perceived obstacles to this approach. First, coupling of the laser scanning signal to a submillimeter fiber core without untenable loss of light and image distortion is nontrivial. More important to TCSPC, however, is the need to relay photons with high timing fidelity. We feel that it is this last challenge that has largely prevented this approach from being widely adopted. A recent compromise has been the use of large fiber bundles for this purpose. This approach maintains some of the aforementioned advantages of fiber coupling, but the large bundle face can allow for the relaying of significant scattered light, and bundles further suffer loss of light from the inherent light rejection in the interstitial regions of the fiber packing. Finally, the bundle suffers from a significant amount of temporal broadening and skewing of photon arrival times as a function of coupling angle [numerical aperture (NA)].

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2.2 Microscope Geometry

An Olympus Fluoview 300 inverted laser-scanning microscope that is typically set up for descanned epidetection had the collection optics modified in both the forward direction and epidection. The fluorescence dichroic separator in the descanned epidection (after the confocal pinhole and within the FV300 scanning unit) was reengineered so that the descanned signal could be coupled to either the built-in large area PMTs or to the external detectors for TCSPC (Hamamatsu H5783-02). Time-resolved detection is achieved with the repetition rate of the Ti:sapphire laser serving as the time base. The scanning synchronization signal of the FV300 is used to coordinate timing of the TCSPC-based FLIM module (PicoHarp 300, Picoquant GmbH).

The forward-propagating signal pathway has been modified to enable collection through a multimode fiber. The signal is collected by a long-working-distance, 0.55-NA condenser. Using a 45-deg mirror, the signal is then directed through dichroic filters to a high-NA aspheric lens (Thorlabs, C330TM-E-A), which collects the signal into a standard multimode fiber (Thorlabs FT600EMT, 600-μm core, 0.39 NA). The fiber can be used to direct the signal to any desired detector, but for CARS microscopy a red-sensitivity-enhanced PMT (Hamamatsu R3896) is typically used. A photo of this adapter and a schematic of the coupling optics train are provided in Fig. 1. Under typical operation, we collect the CARS signal in the forward direction while forward-propagating SHG and TPEF signals (as well as both Stokes and pump pulses) are blocked by bandpass filters.

2.3 Sample Preparation

In this article we show images of African violet pollen, octadecene oil drops in fluorescein-dyed water, and live human hepatoma cell line (HuH-7). Unstained pollen samples were dry-extracted from flower stamens and suspended in water. For a sample of oil droplets in a fluorescing background, we continually diluted samples of fluorescein in water until TPEF signals in the forward direction were roughly matched in intensity to
3 Results and Discussion

3.1 The Optics of Fiber Coupling Nondescanned Light

The key to coupling the nondescanned light into the fiber in the forward direction with minimal field-of-view aperturing or distortion is to reimage the back aperture conjugate plane of the objective. To enable coupling, this collimated beam must have a spot size smaller than the fiber core. Depending on the initial position in the sample, the incoming beam will impinge on the fiber at an angle, and for efficient coupling this must be contained within the NA of the fiber [see Fig. 1(a)]. The optimal coupling geometry represents an optimization of the fiber core diameter, the numerical aperture of the fiber, the condenser to a 2.5-mm-sized image plane. A 21.9-deg incoming angle, representing a NA of 0.37, which corresponds to an incoming angle of 21.9 deg, representing a NA of 0.37, which is with the 0.39 NA of the multimode fiber. Thus, the key to coupling our microscope images into this specific fiber. With ideal alignment and optical placement we thus expect little loss in field of view over the entire scan range. In practice, slight imperfections in the placement of the fiber and the coupling asphere, as well as in microscope alignment, can lead to significant loss in the FOV. One particular culprit for loss of FOV is the placement of the fiber face away from the objective back aperture conjugate plane: the fiber face seems to be at an “infinity space” because all the beams are collimated. However, because the beams are angled into the fiber (coming together at the face), one would get some loss in the beams from the periphery of the FOV if the fiber is not exactly located at the appropriate conjugate plane. For example, we estimate that if the asphere is misaligned by 1 mm (axially), there will be noticeable loss of light from the edges of the FOV (but not from on-axis). It is important to reiterate that we are not imaging the sample plane onto the fiber face, as one would onto a camera. Thus, at the position of the fiber face, the signal looks like a stationary spot with a changing incoming angle, rather than a smaller, raster-scanning spot as it appears away from this plane.

Figure 2 presents a comparison of different possible collection geometries. In the forward direction, either a fiber was used

CARS signals from octadecene. We then dispersed a droplet of octadecene into the solution, forming droplets a few microns in diameter, and set this on a microscope slide and cover slip. Fine tuning of the relative CARS and TPEF signals was obtained by minor adjustment of Stokes power. HuH-7 cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 100 nM nonessential amino acids, 50 U/ml penicillin, 50 µg/ml streptomycin, and 10% (v/v) fetal bovine serum, as detailed elsewhere.17

![Figure 1](image1.png)

Fig. 1 (a) Fiber-coupling geometry (not to scale): obj: objective; smpl: sample plane; cond: condenser; adpt: fiber coupling adapter; mirr: mirror; asp: aspheric lens; and fib: multimode fiber. Cyan dashed line and magenta dashed line denote objective back aperture conjugate planes and sample conjugate planes, respectively. (b) and (c) Annotated photos of microscope with fiber-coupling adapter beside and affixed to the condenser housing, respectively. (Color online only.)

![Figure 2](image2.png)

Fig. 2 TPEF images of African violet pollen in water. (a) Forward-collected nondescanned detection with a PMT placed directly at a conjugate plane of the objective back aperture. All room lights are turned off and the microscope is covered by a black shroud. (b) Forward-collected nondescanned detection through a multimode fiber placed at the same location, directly imaging the effect of loss of FOV. The microscope is uncovered, and considerable ambient room light is present. Slight changes in field of view (FOV) compared to (a) are due to a small drift in focal plane. (c) Concurrent descanned epifluorescence imaging onto built-in detectors, for comparison. Ambience lighting conditions are the same as in (b); FOV is identical to (a). (d) Overlaid images of concurrently collected epifluorescence images (green) and fiber-coupled CARS at 2880 cm⁻¹ (red) showing the quality of image obtained near the center of the microscope FOV. (e) A measure of coupling efficiency and FOV aperturing, obtained by comparing TPEF signals from a dilute fluorescein sample. A fiber-coupled image [as in (b)] is divided by a background-subtracted direct-PMT-detected image [as in (a)] showing a uniform 50% to 55% coupling region that extends over a diameter of ~90 µm, or 26% of the original FOV. (Color online only.)
to collect the light or the fiber was removed from the adapter
and a PMT was placed in the same spot. To compare the two
descanned images in the forward direction with an optimized
detection geometry, we also include a simultaneously obtained
image from the built-in, descanned, backward-collected path.
As would be expected, the three images are very similar, but do
illustrate some subtle differences of import. One of the predicted
advantages of proper fiber coupling is the rejection of scattered
laser and ambient light because of the strict coupling conditions
into the fiber. This should yield enhancements similar to those
achieved by the confocal pinhole in the epifield; however,
because this is not truly confocal, epideected descanned signals
still demonstrate the highest stray light rejection. As can be seen
in Fig. 2, for the forward-collected signals the fiber-coupled
geometry shows considerably better background light rejection
than the direct-PMT coupling. It is important to note that the
direct-PMT coupling images were taken with the room lights
off and with the microscope covered with a black cloth shroud,
and the fiber-coupled images were taken in the presence of sig-
ificant ambient room lights and with the shroud removed. With
this amount of ambient light, a direct PMT image is completely
saturated by background light (not shown). This configuration,
therefore, cannot be used for imaging at any gain setting. This
may seem a minor consideration when choosing collection ge-
ometry, but near-complete relaxation of ambient and scattered
light rejection requirements results in considerably enhanced
microscope usability. Thus, by using a simple multimode fiber
collection geometry we are able to obtain quasi-confocal imag-
ing with minimal free-space optical components in an economic
nondescanned geometry. The ease of light shielding with a fiber-
coupled scheme is of particular advantage when conducting sin-
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coupled scheme is of particular advantage when conducting sin-
ngle photon-counting detection such as TCSPC (as discussed
below).

There are disadvantages to fiber-coupling laser-scanned mi-
roscope signals. Primarily, as mentioned above, deviations
from proper optical geometry can lead to significant loss in
both FOV and transmitted light intensity. A comparison of the
images in Figs. 2(a) and 2(b) shows a perceptible narrowing in
the total FOV. The coupling performance of our system is best
seen, however, by taking a ratio of free-space to fiber-coupled
images of a uniform sample, as shown in Fig. 2(e). This figure
demonstrates that we experience a uniform 45% to 55% inten-
sity coupling loss over a FOV of 90 μm (26% of the image), and
75% loss beyond a FOV of 200 μm in diameter. These values
are only representative for our choice of fiber, condenser, and
coupling aspheric lens. It is likely that further optimisation of
these parameters will yield considerably improved performance
beyond what we show here. Nonetheless, this FOV restriction
and minor coupling loss do not prove an impediment in the typ-
cal laser-scanning microscopy performed in our laboratory, as
demonstrated by our high-resolution multimodal imaging of an
~60 μm-sized pollen grain, shown in Fig. 2(d).

3.2 Timing Fidelity and Modal Dispersion in
Multimode Fiber

An important barrier to the wider adoption of fiber-routed sig-
als for TCSPC was the perceived loss of timing fidelity when
considering light propagation in a fiber to be governed by total
internal reflection.11 A simple analysis of the timing spread due
to geometric propagation in a glass rod that guides by simple
total internal reflection is given by

$$\Delta t = \frac{nl}{c} \left[ \frac{1}{\sqrt{1 - NA^2/n^2}} - 1 \right], \quad (1)$$

where $n$ is the glass index, $l$ is the rod length, $c$ is the speed of
light in vacuum, and $NA$ is the numerical aperture of the rod.12 For
example, 2 m of multimode fiber (Thorlabs FT600EMT) with
$n = 1.457$ and $NA = 0.39$ would lead to 370-ps timing uncer-
tainty, which may be significant for many FLIM applications.
This concern has been reinforced by TCSPC measurements with
large fiber bundles that have shown a strong relationship between
photon arrival time and the NA of the transmitted light.13 Fiber
bundles suffer from the added problem that the large bundle face
and collection NA does not provide the same spatial filtering as
that of a smaller fiber. Nonetheless, fiber bundles have recently
begun finding a use in TCSPC-based microscopy.14 Unlike in a
glass rod, a pulse propagating in a clad fiber is mainly broadened
by modal dispersion and not the simple propagation model that
leads to the temporal spread predicted by Eq. (1). For multi-
mode (and single-mode) fiber, waveguide dispersion leads to
a more complicated spread in pulse dispersion, but the maximum
spread in arrival times is simple to estimate18:

$$\Delta t_{\text{max}} = \frac{n_{\text{core}}}{c} \frac{\delta}{1 - \delta}, \quad (2)$$

and

$$\Delta t_{\text{rms}} \approx 0.5 \Delta t_{\text{max}}. \quad (3)$$

where $\delta$ is the difference between the index of the cladding and
core and $l$ is the length of the fiber. For a 2-m length of our
multimode fiber, $\delta = 0.053$, and thus we would expect at most
270 ps of spread. This value is similar to that found from Eq. (1),
which explains why multimode fibers are often treated like sim-
ple light pipes. To get the maximum 270 ps of spread, however,
would mean that we can simultaneously excite all of the modes
of the fiber. In our coupling scheme, collimated light of a diame-
ter similar to that of the core is incident onto the fiber, and we may
thus expect to excite only a small subset of the allowed modes
of propagation.18 This contrasts with a simple total-internal-
reflection model, which would imply that utilizing the full NA
of the fiber must lead to the full spread in arrival times.

We have modified our microscope for descanned epideected
FLIM by picking off the signal just prior to incidence on the
built-in PMTs and routing it to an externally mounted fast PMT
connected to a commercial TCSPC timing electronics module,
as described in Sec. 2.2. We also coupled the fiber to one of
these detectors to test the effect of fiber collection on TCSPC
timing fidelity and to provide the first demonstration of joint
time-gated TCSPC FLIM/nondescanned F-CARS modalities.
Because CARS is an instantaneous process compared with PMT
response time, it is a good measure of the IRF of our system.
Thus, by studying the timing variation and spread across the
FOV on a homogenous CARS sample, we are able to ascertain
the impact of using a multimode fiber on our IRF. Furthermore,
by using a sample—as opposed to directly relaying laser light
to the detector—we can establish an IRF image, which gives us
visual insight into the effects of fiber coupling on arrival time
spread. Figure 3 presents an analysis of the photon arrival times
of CARS signals from a sample of pure octadecene. We parse
the CARS image FOV into seven radial segments to explore the hypothesis that photon arrival times will increase monotonically across the FOV due to the excitation of a larger proportion of higher modes in the fiber. Figure 3(b) presents the averaged CARS signal from each radial section and shows that while there is a monotonic signal delay as a function of radial position in the FOV, this delay is less than 50 ps at the edge of the image. This represents a minimal timing delay within our TCSPC timing bin resolution of 32 ps. Thus, a 2-m-long multimode fiber does not introduce significant timing variation across the 350-μm-diameter FOV considered here. In addition, as shown in the inset of Fig. 3(b), the FWHM of the IRF does not significantly broaden across the image. At the periphery of the image, a maximum shift of 46 ps and a maximum broadening of only 17 ps is observed. This timing spread is very small compared to the 270-ps spread one could expect from the excitation of the full range of allowed fiber modes. Although for most FLIM applications the time spread introduced by fiber coupling is negligible, for high-timing-resolution TCSPC applications such as determining short lifetimes, good signal-to-noise measurements of the IRF are important. Typically, the IRF is treated as a constant across the image, and it is collected as a global parameter to be deconvolved from the position-dependent TCSPC image. In our case, one could construct an IRF map for pixel-by-pixel deconvolution. The FLIM signal can then be extracted directly by calibrating against such an IRF map. For example, a CARS arrival time map taken from pixel-by-pixel analysis of the arrival histogram is presented in Fig. 3(c), and similar analysis could be useful when using a more dispersive fiber. The radial symmetry of the IRF map further allows averaging of pixels within ring segments to provide improved signal-to-noise in an IRF map.

As demonstrated by Ly et al., the fact that CARS is indistinguishable from the IRF in a TCSPC experiment allows for cocollection and subsequent time-gated separation of CARS and FLIM on a single detector. We demonstrate this modality on a pure F-CARS signal coupled through a fiber while simultaneously performing FLIM. Figure 4 presents separation of CARS signals in oil from a TPEF background of fluorescein in aqueous solution. The signal from the oil droplets arrives within the IRF time, whereas those from the aqueous solution arrive with an exponential decay time of 3.4 ns, as shown in Fig. 4(a). Figure 4(b) shows an image of all photons arriving over a 300-ps timescale about the IRF peak (at 1.22 ns) and represents the CARS image. Figure 4(c) shows an image of all photons arriving 300 ps after this time (1.55 to 12.5 ns) and represents the TPEF image. In addition, a region in the FOV that could look like an oil drop in the FLIM image is absent from the CARS image and is identified as an air bubble.
As a first step toward conducting future FRET + CARS experiments, we demonstrate concurrent two-channel TCSPC-based FLIM and CARS imaging of live cells stained with Hoechst3342 (Hoechst) and DiOc6(3) (DiO). One of the channels is epifluorescent FLIM and the other is simultaneous fiber-coupled F-CARS imaging onto a standard PMT. Hoechst is a nucleus-staining dye, and DiO typically stains the ER in the cytoplasm. HuH-7 cells are selected for their high lipid droplet content, which is imaged by CARS. Figure 5 shows the resulting CARS and FLIM images. Strong CARS signals are seen from lipid droplets in the cytoplasm. A lifetime map reveals the Hoechst in the nucleus to have an average fluorescence decay lifetime of 1.6 ± 0.4 ns and that of DiO in the cytoplasm to be <1 ns. Interestingly, we have found that DiO displays a wide range of lifetimes in the cytoplasm, ranging from 0.4 to 1.0 ns depending on its decay lifetime of intracellular DiO dye. The nuclear-bound Hoechst lifetime reported here is in good agreement with values in the literature.19,20

4 Conclusion

We have presented the novel combination of FLIM and true forward-collected CARS microscopy. We also presented single-detector F-CARS + FLIM imaging using time-gated TCSPC analysis. To accomplish this, we developed a small adapter for an inverted laser-scanning microscope that fits on the condenser and couples light into a standard multimode fiber for routing to off-board detectors. We demonstrated the advantages of fiber-coupling laser-scanning microscope signals: significant reduction in the need to regulate stray light; improved flexibility in microscope design and detector placement; and the ease of collecting nondescanned light for high-time-resolution TCSPC applications. We outlined key considerations for choosing a proper fiber-coupling geometry in a laser-scanning microscope and provided suggestions for how to do this. We have furthermore aimed to show, in detail, the minor effects that fiber dispersion has on TCSPC timing, as well as a way to calibrate high-timing-resolution measurements for those effects that may be present. We have found that with a 2-m-long 0.60-mm-diameter-core 0.39-NA multimode fiber we get no more than 50 ps of pulse delay spread across a 350-μm-diameter image (the instrument response function of our setup is 200 ps). We determined that broadening of the IRF is considerably less than the pulse delay spread, indicating that light from a given location in the sample only excites a narrow and well-defined subset of fiber modes. Although we highlight the use of fiber coupling for forward-detected light—mostly because of our interest in CARS, which is mainly forward propagating, and because commercial confocal inverted microscopes are well equipped for epidection—fiber coupling of nondescanned epifluorescent light poses no additional difficulties. Drawbacks to coupling though multimode fiber are a modest reduction in the field of view and coupling losses in transmission. With our unoptimised coupling geometry, we are able to achieve >50% transmission in the center of the image and a flat-intensity field of view over 25% (90-μm diameter) of the image. Imaging beyond this FOV suffers from gradual apodization of light but remains spatially undistorted. We suggest that the drawbacks of using a multimode fiber can be further mitigated with the use of a larger-core graded-index fiber, which is expected to display even lower modal dispersion.

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