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1 **TITLE:**
2 **Low-cost Custom Fabrication and Mode-locked Operation of an All-normal-dispersion**
3 **Femtosecond Fiber Laser for Multiphoton Microscopy**
4
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20 **KEYWORDS:**
21 fiber laser, femtosecond pulsed laser, multiphoton microscopy, low-cost, custom fabrication,
22 mode-locking
23
24 **SUMMARY:**
25 A method is presented to build a custom low-cost, mode-locked femtosecond fiber laser for
26 potential applications in multiphoton microscopy, endoscopy, and photomedicine. This laser is
27 built using commercially available parts and basic splicing techniques.
28
29 **ABSTRACT:**
30 A protocol is presented to build a custom low-cost yet high-performance femtosecond (fs) fiber
31 laser. This all-normal-dispersion (ANDi) ytterbium-doped fiber laser is built completely using
32 commercially available parts, including $8,000 in fiber optic and pump laser components, plus
33 $4,800 in standard optical components and extra-cavity accessories. Researchers new to fiber
34 optic device fabrication may also consider investing in basic fiber splicing and laser pulse
35 characterization equipment (~$63,000). Important for optimal laser operation, methods to verify
36 true versus apparent (partial or noise-like) mode-locked performance are presented. This system
37 achieves 70 fs pulse duration with a center wavelength of approximately 1,070 nm and a pulse
38 repetition rate of 31 MHz. This fiber laser exhibits the peak performance that may be obtained
39 for an easily assembled fiber laser system, which makes this design ideal for research laboratories
40 aiming to develop compact and portable fs laser technologies that enable new implementations
41 of clinical multiphoton microscopy and fs surgery.
42
43 **INTRODUCTION:**
44 Solid state femtosecond (fs) pulsed lasers are widely used for microscopy and biological research.

45   One typical example is the usage of multiphoton excitation (MPE) fluorescence microscopy,
46   where high peak power and low average power are desired to facilitate the MPE process while
47   minimizing photodamage mechanisms. Many high-performance solid-state lasers are
48   commercially available, and when combined with an optical parametric oscillator (OPO), the laser
49   wavelength can be tuned over a wide range[1]. For example, commercial oscillator–OPO systems
50   generate <120 fs pulse durations (typically with an 80 MHz pulse repetition rate) and >1 W
51   average power from 680 to 1,300 nm. However, the cost of these commercial tunable fs laser
52   systems is significant (>$200,000), and solid-state systems generally require water cooling and
53   are not portable for clinical applications.

55   Ultrashort pulsed fiber laser technology has matured in the past few years. The cost of a
56   commercial fs pulsed fiber laser is typically significantly lower than solid-state lasers, albeit
57   without the capability of broad wavelength tuning afforded by the solid-state systems mentioned
58   above. Note that fiber lasers can be paired with OPOs when desired (i.e., hybrid fiber–solid-state
59   systems). The large surface-to-volume ratio of fiber laser systems enables efficient air cooling[2].
60   Hence, fiber lasers are more portable than solid-state systems due to their relatively small size
61   and simplified cooling system. Further, fusion splicing of the fiber components reduces system
62   complexity and mechanical drift in contrast to the free-space alignment of the optical
63   components making up solid-state devices. All of these features make fiber lasers ideal for clinical
64   applications. In fact, all-fiber lasers have been developed for low-maintenance operation[3-5], and
65   all-polarization-maintaining (PM)-fiber lasers are stable to environmental factors including
66   changes in temperature and humidity as well as mechanical vibrations[2,6-8].

68   Here, a method is presented to build a cost-efficient fs pulsed ANDi fiber laser with commercially
69   available parts and standard fiber splicing techniques. Methods to characterize pulse repetition
70   rate, duration, and coherence (full mode-lock) are also presented. The resulting fiber laser
71   generates mode-locked pulses that can be compressed to 70 fs with a repetition rate of 31 MHz
72   and a wavelength centered at 1,060 to 1,070 nm. The maximum power output from the laser
73   cavity is approximately 1 W. The pulse physics of ANDi fiber lasers elegantly utilizes nonlinear
74   polarization evolution intrinsic to optical fiber as a key component of the saturable absorber[2,3,9-
75   11]. However, this means that the ANDi design is not easily implemented using PM fiber (although
76   an all-PM fiber implementation of ANDi mode-locking has been reported, albeit with low power
77   and ps pulse duration[12]). Thus, environmental stability requires significant engineering. Next
78   generation fiber laser designs, such as the Mamyshev oscillator, have the potential to offer
79   complete environmental stability as all-PM-fiber devices capable of an order-of-magnitude
80   increase in intracavity pulse energy as well as offering significant decreases in pulse duration to
81   enable applications that rely on broad pulse spectra[13,14]. Custom fabrication of these innovative
82   new fs fiber laser designs requires know-how and fiber splicing experience.

84   **PROTOCOL:**

86   **1. Splice single mode fibers (SMF)**

88   NOTE: Section 1 consists of general steps to splice SMFs. This is a non-essential, but

recommended, step for practicing fiber splices using inexpensive fiber. This step ensures proper performance of the splicing equipment before using more valuable fiber optic materials.

1.1. Cleave the first fiber.

1.1.1. Strip approximately 30 mm of the fiber with a fiber stripping tool. For fragile fibers (e.g., double clad fibers), a razor blade can be used to carefully peel off the buffer.

1.1.2. Use lint-free tissue with ethanol or isopropanol to clean the stripped fiber. A buzzing sound when wiping the fiber indicates that the fiber is sufficiently clean.

1.1.3. Place the fiber holder on the fiber cleaver. Make sure the blade, the fiber clamp of the cleaver, and the fiber holder are all clean. Cotton swabs with alcohol can be used to clean these parts of the cleaver.

1.1.4. Carefully load the fiber into the fiber holder. Leave approximately 25 mm of stripped, clean fiber at the free end for the cleaver to clamp.

1.1.5. Gently close the fiber clamp on the cleaver. To avoid extra tension applied to the fiber, reopen, and close the clamp so that the tension is released.

1.1.6. Press the "cut" button and the cleaver will automatically cleave the fiber.

NOTE: To ensure that the fiber remains clean, nothing should touch the fiber tip after cleaving.

1.1.7. Transfer the fiber holder to the fusion splicer. Use tweezers to move the piece cut off from the fiber to a sharps disposal container.

CAUTION: Hard tweezers and sharp tweezer tips can break the fiber. An appropriate tweezer for handling fiber optics should have plastic, rounded tips.

1.2. Cleave the second fiber.

1.2.1. Repeat step 1.1 on the second fiber with the other fiber holder. The two fibers to be spliced should be cleaved with the cleaved ends held opposing each other by the fiber holders within the fiber splicer.

1.2.2. Close the cover of the splicer.

1.3. Fusion splice the fibers.

1.3.1. Set up the parameters on the fusion splicer, including the core diameter, mode-field diameter (MFD), and cladding diameter. Set the alignment method to **Cladding**.

1.3.2. Press the start button, and the splicer will align automatically.

134

135 NOTE: It is possible to get error messages regarding a poor cleave shape or large cleave angle.
136 This is usually due to a poor cleave or contamination of the fiber after the cleave. If this occurs,
137 repeat the fiber cleaving procedure.

138

139 1.3.3. Press the start button at each stop to confirm the quality of the splice. The splice will be
140 done automatically.

141

142 1.3.4. Check the quality of the splice via the quality control checks performed by the splicer as
143 well as by using the camera view of the splice region. A good splice has a uniform cladding
144 boundary and uniform brightness along the fiber such that no splice juncture is visible.

145

146 NOTE: Fiber splicers often include optics to inspect the splice and to estimate the power loss
147 based on the measured geometry, shape, and light refraction through the fiber using a source
148 perpendicular to the fiber to view, image, and analyze the splice joint. Of course, this is only an
149 estimate, but it is sufficient in most cases. For identical fibers, the splicer will estimate this loss
150 as ~0 dB (i.e., no detectable loss). From previous results with the dissimilar fiber splices described
151 below, the splicer estimates of the power losses range from 0.07 dB (splice points B and C, **Figure**
152 **1**) to 0.3 dB (splice point D). These estimates most likely overestimate the loss due to mismatched
153 geometry and refraction of the dissimilar fiber optics, which falsely appear as defect objects.

154

155 1.3.5. Open the splicer cover, then open one of the fiber holders. The other fiber holder should
156 not be opened until the spliced fiber is removed from the splicer.

157

158 1.3.6. As an option, a fiber sleeve may be added to protect the splice. The heater on the splicer
159 may be used to mold the sleeve onto the fiber. Alternatively, a hot air gun may be used.

160

161 NOTE: If the two fibers are very long or attached to other components, the sleeve should be put
162 on one of the fibers before cleaving, and then it can be moved to the splicing point. The fiber
163 sleeve acts like a heat shrink tube in electronic circuits. It can be used to protect the splicing point
164 from a bending or pulling force. A fiber recoater may be used instead to recoat the splice point
165 for maximal protection of the splice point to mechanical damage, albeit at a significant additional
166 expense because this equipment must be purchased if it is not readily available.

167

168 **2. Assemble the fiber parts**

169

170 2.1. Splice the pump output fiber <1> with the pump input <2> of the pump signal combiner (see
171 the fiber laser diagram, **Figure 1**).

172

173 2.1.1. Follow section 1 to cleave and splice the fibers. Use the default setting of the program
174 BASIC I SP, with the exception of the fiber parameters (2-A and 2-B) that need to be input
175 manually. The splicing parameters that need to be entered can be found in **Table 1**.

176

177   2.2. Splice the combiner output <3> to the Yb-doped active fiber.

179   2.2.1. Follow step 1.1 to cleave the combiner output fiber <3>.

181   2.2.2. Cleave the active fiber <4>.

183   NOTE: Because the active fiber <4> has an octagonal cladding, it does not fit the V-groove of the
184   fiber cleaver. Therefore, a simple cleave as described in step 1.1 will yield a relatively large cleave
185   angle. Thus, the following steps outline a special protocol to achieve a flat cleave angle using the
186   same equipment.

188   2.2.2.1. Follow section 1 to cleave and splice the active fiber <4> and a piece of 6/125 SMF. This
189   SMF is removed later and is not incorporated into the laser. Therefore, it is acceptable if the
190   quality of this cleave angle is poor. It is not important to obtain a flat cleave angle for this step.

192   2.2.2.2. Cut the SMF about 2 cm from the splicing point with a wire cutter.

194   2.2.2.3. Strip the entire length of the SMF, and strip the active fiber for another 0.5 cm. Now the
195   active fiber is capped with 2 cm of bufferless SMF.

197   2.2.2.4. Load the active fiber into the cleaver as in steps 1.1.3–1.1.5. Make sure only the SMF,
198   which has a circular cladding, is clamped by the fiber clamp.

200   2.2.2.5. Follow steps 1.1.6 and 1.1.7 to cleave the active fiber <4>. Since only the SMF is in the V-
201   groove, this cleave will yield a minimal cleave angle.

203   2.2.3. Follow step 1.3 to splice the fibers.

205   2.3. Roughly measure the total power output from the distal end of the active fiber <4>.

207   2.3.1. Cut the active fiber <4> at ~3 m from splice point <B>. Longer active fiber can be used for
208   a higher output power, but the repetition rate will be reduced due to the increase in the cavity
209   length.

211   2.3.2. Cleave the end <4C> as mentioned in step 1.1.

213   NOTE: Because the power measurement in the next step isestimated , it is unnecessary to use
214   the method mentioned in step 2.2.2.

216   2.3.3. Point the fiber towards the power meter and bring the fiber and power meter together
217   without physical contact.

219   WARNING: Putting the tip of the fiber too close to the power meter will possibly damage the
220   power meter sensor, as the light power is concentrated to a small point on the sensor. To avoid

221   this, use a minimum reliable pump power.

223   2.3.4. Read the power output from power meter. A large (>80%) efficiency throughput indicates
224   sufficient quality splices at points <A> and <B>.

226   NOTE: It is normal to have some power loss due to the absorption of the active fiber and due to
227   the inefficiency of the coupling method to the power meter as mentioned in steps 2.3.2 to 2.3.3.

229   2.4. Splice the active fiber <4> to the input <5> of the collimator <Col1>.

231   2.4.1. Follow step 2.2.2 to cleave the active fiber <4> on the end <C> to be spliced to the
232   collimator.

234   2.4.2. Cut the input <5> of collimator <Col1> to approximately 40 cm.

236   NOTE: The length of the passive fiber (<5>) should not be too long (>40 cm), because the
237   amplified pulse will broaden substantially in the time and the spectral domain due to increased
238   self-phase modulation (SPM) and group velocity dispersion (GVD) following passage through the
239   gain fiber (pulse amplification). These effects will increase the difficulty of pulse compression.

241   2.4.3. Follow steps 1.1 and 1.3 to cleave the collimator input <5> and splice the active and
242   collimator fibers.

244   NOTE: This splice <C> splice of a double clad fiber to an SMF may appear to be of a lower quality
245   than the prior splices. However, the actual performance depends only on the core alignment
246   because the pulse propagates within the core.

248   2.5. Splice fiber <6> of the second collimator <Col2> to the signal input fiber <7> of the combiner.

250   2.5.1. Follow section 1 to cleave and splice the fibers.

252   **3. Mount the fiber parts to the optical table**

254   3.1. Mount the pump laser to the optical table with screws and any necessary clamps.

256   3.2. Mount the pump signal combiner to the optical table with clamps. Thermal paste can be
257   used between the combiner and the table, because the optical table works as a heat sink for the
258   combiner.

260   3.3. Place the fibers on the table. Fibers 1, 2, 3, 5, 6, and 7 can be coiled individually to save space,
261   while the active fiber 4 should be either straight or coiled loosely with a radius of curvature >20
262   cm. Leave some space to access splice <C> for the next step.

264   CAUTION: A strong bend in the active fiber may cause the pump signal to escape the inner

265 cladding of the active fiber. This can lead to fatal burn points along the active fiber that will
266 require installing a new active fiber.
267
268 3.4. Apply the index matching gel to splice <C>. The index matching gel is used to guide the pump
269 light out of the active fiber in order to reduce the generation of heat and thermal damage at the
270 splice point. Note that there is no need to recoat the fiber. It is preferable to leave the fiber bare
271 and coated in index matching gel to minimize the risk of thermal damage.
272
273 3.5. Use optomechanical parts to mount and fix the two collimators <Col1> and <Col2> on the
274 optical table. The collimators should face each other with a separation of approximately 35 cm
275 to provide sufficient space for inserting the in-cavity free-space components.
276
277 **4. Assemble the free-space parts**
278
279 4.1. Turn on the pump laser. Set the power to 0.5 W (i.e., above the threshold for mode-locking
280 yet a safe power for aligning system components).
281
282 WARNING: At this point, the laboratory space must be Class IV laser certified, laser safety goggles
283 must be worn, and the personnel must have received Class IV laser training.
284
285 4.2. Use an infrared (IR) scope to check the splice point <C>. Apply the index matching gel on any
286 bright spots seen through the IR scope (indicative of potential points of thermal damage) in order
287 to help light escape at these risk points.
288
289 4.3. Adjust the position of the two collimators so that they are pointing directly at each other. An
290 IR viewing card can be used to assist centered beam alignment at the collimator entrance
291 apertures.
292
293 4.4. Mount a polarizing beam splitter (PBS) 6 cm away from <Col1>. Mount the sensor of a power
294 meter so that the power of the reflected laser output beam can be measured continuously. The
295 wavelength of the power meter should be set to 1,060 nm. A typical starting power reading with
296 0.5 W pump power is ~50 mW before alignment.
297
298 4.5. Adjust the screws on the collimator mounts to increase the reading of the power meter.
299 Continue making fine adjustments until the output power reaches a maximum value of about
300 150 mW, which indicates an excellent alignment.
301
302 NOTE: This step requires careful and patient adjustment, which is often time consuming. It is
303 most efficient to follow a systematic systematic procedure: First, rotate the two screws that
304 adjust the angle in the same direction (X or Y) on the two collimators, with one screw rotating
305 very slowly in one direction while the other is rotating fast to scan all reasonable angles. Keep
306 tracking the maximum reading from the power meter. Once the maximum power is found, switch
307 to the screws, adjusting to another direction. Repeat the slow rotate and fast scan described
308 above. Due to reflections from the lenses inside both collimators, it is possible to observe

309 multiple local maxima while aligning the collimators. The actual maximum power is much greater
310 (150 mW) compared with the local maxima (70 to 80 mW).

312 4.6. Mount the isolator 3 cm from <Col2>. Adjust the direction of the collimators again to align
313 the free space components and to maximize output power. The presence of the isolator may
314 slightly deflect the beam alignment, but the maximum output power is recovered by fine
315 adjustments to the collimators.

317 4.7. Mount the birefringent filter <BF>, a half wave plate <HWP> and two quarter wave plates
318 (<QWP1> and <QWP2>) to the corresponding positions shown in **Figure 1**. The birefringent filter
319 is sandwiched between two polarizers—one before (<PBS>) and one after (within the <ISO>)—
320 to create a sinusoidal band-pass filter effect. There must be a small (3°–5°) incident angle for the
321 <BF> in order to control the wavelength range. Tune the alignment of the collimators once more
322 until the output power reaches a maximum value.

324 **5. Set up extra-cavity components**

326 5.1. Splice all three ports of the splitter (**Figure 1**) with fiber optic connectors (FC) or
327 SubMiniature version A (SMA) connectors. The types of connectors depend on the input ports of
328 the photodiode and the optical spectrum analyzer (OSA). Splice steps are identical to those
329 described in section 1 above.

331 5.2. Connect one output of the splitter to the photodiode input port of the OSA and the other
332 output to the photodiode using FC connectors.

334 5.3. Connect the photodiode output port to the oscilloscope (OSC) with a Bayonet Neill–
335 Concelman (BNC) cable.

337 5.4. Connect the collimator <Col3> to the input port of the splitter.

339 NOTE: Using the connector to connect the splitter and <Col3> is for convenience. This connection
340 can be replaced with a splice if desired.

342 5.5. Remove the power meter sensor.

344 5.6. Mount the small mirror <M1> and the first compressor grating <G1> on the optical table. To
345 achieve maximum efficiency of the compressor gratings, use the power meter to monitor the
346 power of the first order maximum while adjusting the incident angle by rotating the grating.

348 NOTE: A rotational stage can be used to precisely control the rotation. As the loss due to the
349 incident angle offset is small, the rotational stage is not used here to reduce cost.

351 5.7. Mount the translational stage on the table. Mount the second compressor grating <G2> on
352 the translational stage. The distance between the gratings should be approximately 2 cm for

353 optimal compression with fine adjustment using the translational stage. Ensure that the gratings
354 are parallel.
355
356 5.8. Mount the compressor mirror <M2> on the optical table. This mirror should be vertical and
357 perpendicular to the moving direction of the translational stage.
358
359 5.9. Mount the rest of the mirrors, the beam splitter, and the collimator <Col3>. The alignment
360 will be adjusted later.
361
362 5.10. Turn on the pump laser. Adjust the pump level to less than 0.5 W.
363
364 5.11. Use an IR scope to check the splice <C>. Add index matching gel to any bright spots.
365
366 NOTE: Step 5.11 should be done regularly during the normal use of the laser.
367
368 5.12. Align the compressor.
369
370 5.12.1. Use an IR card to locate the beam, adjust the position of <M1>, and the compression
371 gratings so that the output beam traverses the pulse compression parts in the following
372 sequence: <M1>, <G1>, <G2>, <M2>, <G2>, <G1>, <M1>.
373
374 5.12.2. Tilt <M2> up slightly to raise the reflected beam, making it pass above the pulse picker
375 mirror <M1>.
376
377 NOTE: <M2> can be replaced by a retroreflector such that the pickoff mirror <M1> need not be
378 angled. That is, the reflected beam will be parallel to the incident beam, but displaced, using a
379 retroreflective mirror to simplify the setup.
380
381 5.13. Align the collimator with one output beam of the beam splitter.
382
383 5.13.1. Turn on the OSA and set the device to the **Power Meter** mode.
384
385 5.13.2. Adjust the angle of the mirror <M3> and the collimator to maximize the power input. The
386 power reading should be above -10 dBm.
387
388 **6. Attaining mode-locked performance with characterization of the laser pulse output**
389
390 6.1. Turn on the OSC and set the instrument to AC coupling mode with the trigger level set to 30
391 mV.
392
393 6.2. Move the OSA photodiode input fiber to monochromatic input. Set the device to **OSA** mode.
394
395 6.3. Lock the phase of the laser by adjusting the wave plates[15].
396

418
419 6.4. Acquire and analyze the radio frequency (RF) spectrum.
420
421 6.4.1. Disconnect the BNC cable from the OSC and connect it to the RF spectrum analyzer.
422
423 NOTE: Using a BNC tee adapter is not recommended, because the ground forms a closed loop,
424 which induces an echo within the circuit. The RF spectrum analyzer is not shown in **Figure 1**,
425 because it takes the same position as the OSC when in use.
426
427 6.4.2. Follow the RF spectrum analyzer instruction manual to locate the primary spectrum peak.
428 The approximate expected frequency can be calculated based on the time between two pulses
429 using the OSC.
430
431 6.4.3. Gently adjust the wave plates and birefringent filter to maximize the signal-to-noise ratio,
432 which is the height of the primary peak relative to the background.
433
434 NOTE: The mode-locking RF spectrum should be a single peak with no side lobes. For the best
435 imaging quality, the SNR should reach at least 70 dB. The spectrum on the OSA should be carefully
436 monitored, keeping track of the Batman spectral shape, to ensure the laser remains mode-
437 locked.
438
439 6.5. Follow the manufacturer instructions to align and operate the autocorrelator to measure the
440 pulse duration. The second output from the extra-cavity beam splitter can be used. Once the

441 pulse duration can be measured, carefully adjust the translational stage on which <G2> is
442 mounted to adjust the distance between the two gratings to tune the duration of the pulse.

443

444 NOTE: To ease alignment, it is best to mount mirror <M1> and <M2> separately from the two
445 gratings and the translational stage to which they are mounted. Also note that picosecond pulses
446 are observed as a broad pedestal along with a central fs pulse peak feature during partially mode-
447 locked operation[17].

448

449 6.6. Gradually increase the pump power above 0.5 W to find the maximum pump power. Powers
450 up to ~5W have been tested. Use the IR scope to constantly observe the active fiber <4>. If a
451 bright spot appears, the pump power is too high within the cavity, and it is likely to burn the
452 active fiber at this pump level.

453

454 NOTE: The maximum power of the system depends on the length of the active fiber and the
455 alignment of the in-cavity free-space components. The protocols described here reach output
456 powers up to 1 W without the appearance of bright spots or burns to the cavity, and this power
457 is more than sufficient for most imaging applications. Higher output powers were not tested but
458 may be possible, although multipulsing is likely to result[16-18].

459

460 **REPRESENTATIVE RESULTS:**
461 It is critical to verify mode-locked operation upon completion of the fiber laser fabrication
462 procedures. Signatures of optimal fs pulse generation and laser stability are as follows: First, the
463 output pulse may be sufficiently characterized by the instrumentation outlined in step 6. The
464 pulse spectrum output from the laser oscillator should be centered near 1,070 nm with the
465 characteristic cat-ear or Batman shape that indicates mode-locking as predicted by numerical
466 simulation of ANDi pulse physics[15] (**Figure 2A**). Although the characteristic spectrum is an
467 excellent indicator of pulse coherence, additional tests are warranted to ensure full mode-
468 locking, stability, and the expected laser performance. As a further diagnostic for mode-locking,
469 the pulse duration and pulse repetition power spectra are measured using the autocorrelator
470 and RF spectrum analyzer, respectively. A single peak without a pedestal is expected for both
471 measurements during mode-locked operation. During the autocorrelation measurements, the
472 grating pair can be tuned to achieve pulse compression. Pulse durations of 70 fs (full-width-half-
473 maximum) were measured (**Figure 2B**). This dechirped pulse duration approaches the estimated
474 transform limited compression of the present laser design: the transform limit is computed using
475 the measured pulse spectrum. Second, pulse stability may be tested by continuously monitoring
476 the average output power and the pulse spectrum. The power drift is less than ±3.5% over 24 h
477 (**Figure 2C**) without active cooling when the laser setup is mounted on a floating optical table
478 with vibration damping. This level of stability is sufficient for many imaging experiments. The
479 system then remains stable and self-starting for more than a week when powered down. The
480 free-space components undergo mechanical drift and the mode-lock is lost after several weeks,
481 but mode-locking can often be re-obtained by minor adjustments of the waveplates as outlined
482 in step 6.

483

484 Once mode-locking is verified, it is also important to test the imaging performance during

485 practical MPE and nonlinear microscopy experiments using simple test target and biological
486 samples. For example, the custom fiber laser output may be directed into a commercial laser
487 scanning microscope for two-photon excitation (2PE) fluorescence imaging (**Figure 3A**). Note that
488 the extra-cavity isolator, although lossy, is necessary to prevent back reflections from the
489 microscope optics from entering the laser oscillator. These back reflections often interrupt mode-
490 locking and fluorescence signal generation during imaging. Here, a test was conducted with a
491 commercial confocal laser scanning microscope and a descanned detector with a pinhole set to
492 the maximum size setting in order to increase the collected fluorescence signal. One simple test
493 sample for microscopy is the measurement of a fluorescent dye solution. A suggested first
494 microscopy experiment is to measure the fluorescent dye signal during adjustments of the pulse
495 power using a set of neutral density filters. This helps verify that the fluorescence signal is
496 quadratically dependent on the laser power delivered to the sample plane (**Figure 3B**), which is
497 the expected response for 2PE. Next, images of biological specimens may be collected
498 usingnonlinear 2PE tissue autofluorescence, for instance (see **Figure 3C**, an unstained, fixed brine
499 shrimp sample) as well as second harmonic generation (SHG) from collagen fibrils and 2PE of
500 extrinsic fluorescent stains (see **Figure 3D**, a freshly excised chicken tissue specimen stained with
501 rhodamine B). As an additional verification of 2PE, collected 2PE hyperspectral images of
502 multicolor fluorescent microsphere test targets were compared with hyperspectral images taken
503 by linear excitation with commercial diode lasers (**Figure 4**). The single-photon excitation and 2PE
504 fluorescence spectra were analyzed and compared for two of the microsphere colors
505 corresponding to two fluorescent dyes excited separately by commercial, continuous wave 514
506 nm and 594 nm lasers. The fluorescence spectra excited by the custom-built laser are identical
507 to the spectra taken with the commercial continuous wave lasers (single-photon excitation).
508 Collectively, these results indicate that the custom fs fiber laser generates pulses with sufficient
509 peak power and uniformity to generate 2PE fluorescence and SHG.
510
511 **FIGURE AND TABLE LEGENDS:**
512 **Figure 1: Schematic of the custom fiber laser and pulse characterization setup.** The numbered
513 black lines 1 and 2 indicate the pump laser output. The numbered black lines 3–7 indicate
514 intracavity fibers with the length of each fiber between the splice points indicated in meters. The
515 unnumbered black lines indicate extra-cavity fibers. The cross (x) marks indicate splice points.
516 The red lines are free-space light paths. The thick black line between the OSC and the photodiode
517 (PD) indicates a BNC cable. The RF spectrum analyzer, which takes the same position as the OSC
518 when being used, is not shown in the figure because the RF spectrum analyzer may be swapped
519 into the setup for the OSC using the BNC connector.
520
521 **Figure 2: The results of laser characterization.** (**A**) The spectrum of the output pulse from mode-
522 locking operation compared with numerical simulation. (**B**) The intensity autocorrelation signal
523 of the dechirped pulse compared to numerical simulation of the transform limit. (**C**) The output
524 power of the laser during two 24 h stability tests. (Adapted from Davoudzadeh et. al.[17])
525
526 **Figure 3: The results of MPE microscopy performance tests.** (**A**) Schematic of the custom-built
527 fiber laser with its output directed into a commercial confocal microscope. (**B**) The log-log plot
528 demonstrating the quadratic dependence of the MPE fluorescence signal as a function of laser

529   output power, measured using a solution of fluorescent dye. (**C**) 2PE autofluorescence image of
530   an unstained and fixed brine shrimp sample using the custom fs fiber laser. (**D**) SHG (cyan) of
531   collagen fibrils and 2PE fluorescence (magenta) of rhodamine B-stained cells from a freshly
532   excised chicken tissue using the custom fs fiber laser. Scale bars = 50 µm. (Adapted from
533   Davoudzadeh et. al.[17])

534

535   **Figure 4: A comparison of 2PE fluorescence using the custom fs fiber laser versus single-photon**
536   **excitation (1PE) using commercial diode lasers.** (**A**) A multichannel 1PE image of spectrally
537   distinct microbeads using several different diode lasers (Left; 1PE wavelengths are listed in nm.)
538   The fluorescent intensity profile of the same beads excited by a 514 nm diode laser (Middle) and
539   by the custom fs fiber laser (Right). Scale bars = 50 µm. (**B**) The normalized spectra of green (left)
540   and red (right) beads excited by the diode laser versus the custom fs fiber laser. (Adapted from
541   Davoudzadeh et. al.[17])

542

543   **Table 1**: **A summary of the parameters for the pump laser fiber splice point (A) as well as the**
544   **three intracavity fiber splice points (B–D).** Here the direction of light propagation is from the left
545   fiber to the right fiber. L = left fiber in splice joint; R = right fiber in splice joint; MFD = mean field
546   diameter.

547

548   **DISCUSSION:**
549   The protocols outlined here synthesize know-how and expertise that have been common practice
550   in the laser physics laboratory for decades, but which is frequently unfamiliar to many biomedical
551   researchers. This work attempts to make this ultrafast fiber laser technology more accessible to
552   the broader community. The ANDi fiber laser design is well-established, as first developed in
553   seminal works by Wise and colleagues[3]. However, implementations of this technology by other
554   groups have sometimes resulted in reports of lasers that do not operate properly, illustrating the
555   need to further educate biomedical researchers in nontrivial aspects of pulse characterization
556   and mode-locked operation.

557

558   Note that custom laser fabrication and operation is generally not suitable for laboratories
559   unfamiliar with laser operation and safety. Laser safety training and consideration of hazards is
560   essential before attempting construction of a class 4 laser. Because the laser system is open,
561   there are two major reflection beams (coming from the compressor gratings and the in-cavity
562   PBS) and several minor reflections from other optics that need to be blocked. The free-space
563   components should be secured to a stable optical table in order to maintain the alignment. In
564   contrast, commercial lasers are always enclosed for safety and often utilize auto-alignment
565   mechanisms, making them easier and safer to operate.

566

567   As mentioned, the custom fs fiber laser presented here represents perhaps the best performance
568   that can be expected for an easily constructed system that minimizes material costs. The design
569   and quality of the splices is a critical factor for the laser efficiency, ease of fabrication, and
570   robustness to burn point damage. A low-quality splice cannot only reduce the pump output
571   efficiency, but also generate heat during operation and, thus, damage the cavity. To achieve high-
572   quality splices, one needs to make sure the fiber cleaver and splicer are clean. As mentioned

573 above, cotton swabs soaked with alcohol should be used to clean all working surfaces on a regular
574 basis. In addition, when large cleave angles (>0.3°) occur, it is strongly advised to recleave to
575 improve the splice quality.
576
577 Once mode-locked, the system is quite stable and remains self-starting during a period of more
578 than a week. In case of accidental perturbations in the system or mechanical drift of the free-
579 space components over time, the system will lose mode-locking, but the mode-locking laser can
580 often be easily recovered by slightly adjusting the wave plates. To maintain stable output,
581 temperature control of the active fiber is key. Therefore, the system is best used in an air-
582 conditioned room with minimal air flow near it. The system is relatively impervious to small
583 vibrations. In fact, the effect of mechanical vibration cannot be observed in both the temporal
584 and spectral domains if the system is put on a passive damped optical table. Touching the fiber
585 components of the oscillator will perturb the mode-lock, but mode-locking is recovered simply
586 by returning the fiber back to its approximate original position.
587
588 Finally, the compact form factor of fs fiber lasers is attractive for developing mobile clinical
589 systems. (e.g., mobile cart-based systems). While smaller in size compared to a solid-state laser,
590 the custom fiber laser design presented here contains several free-space components that
591 require alignment. This significantly limits the mobility of the system. It is possible to replace all
592 of these free-space components with fiber component analogs. Future work will include
593 development of new all-fiber laser designs using PM fiber to develop systems that are robust to
594 environmental changes.
595

603 **DISCLOSURES:**
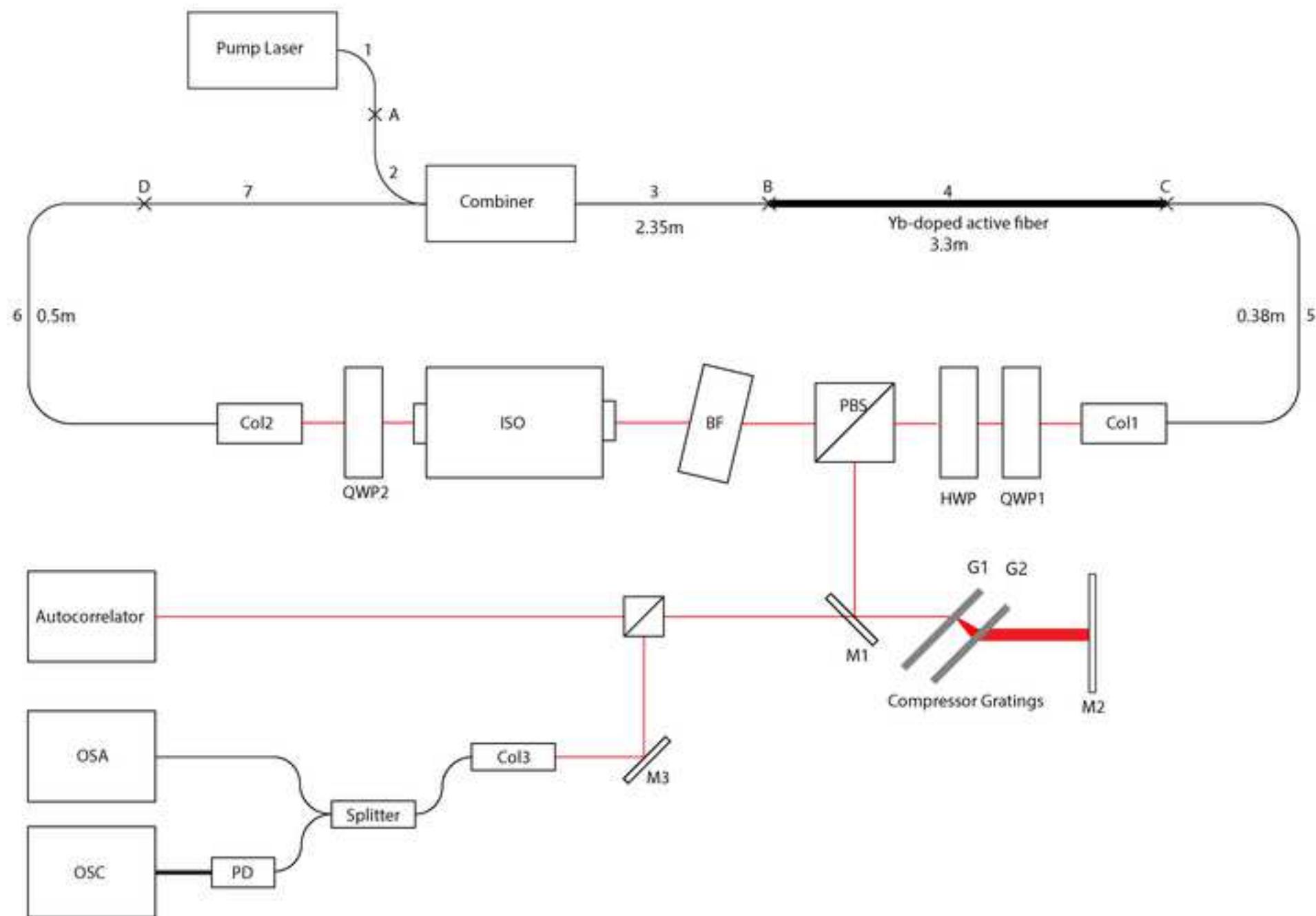604 The authors declare no competing interests.
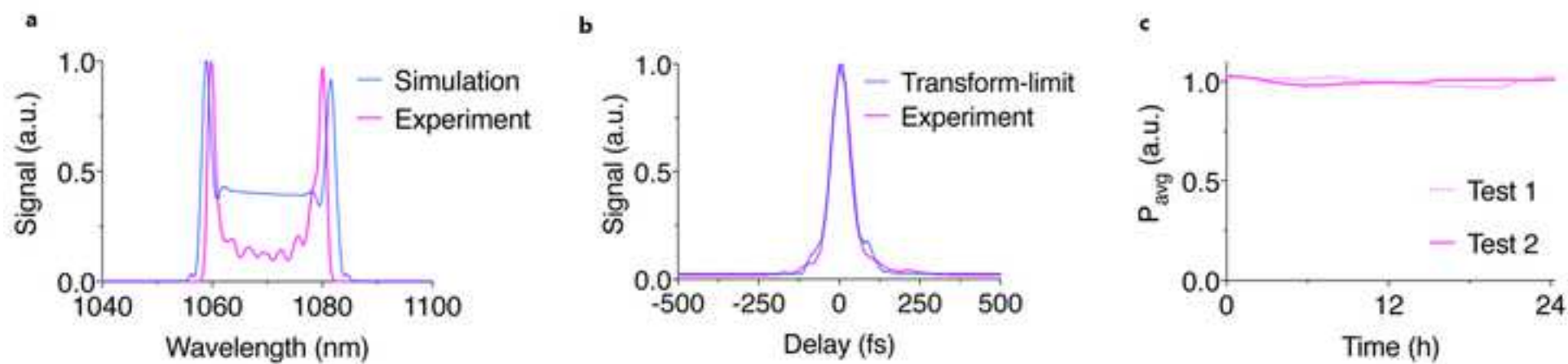605

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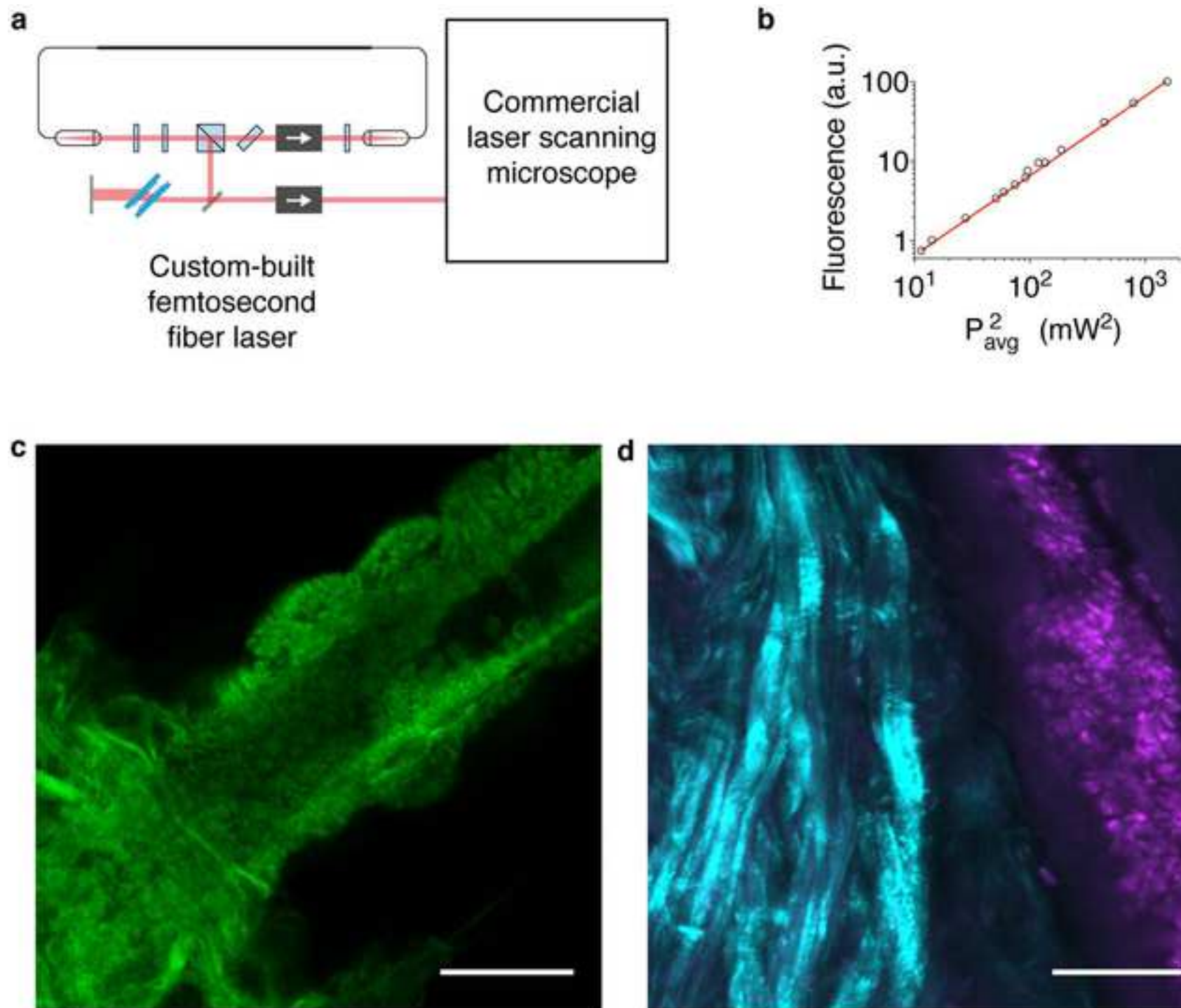616   *Express*. **7**, 3531–3542 (2016).

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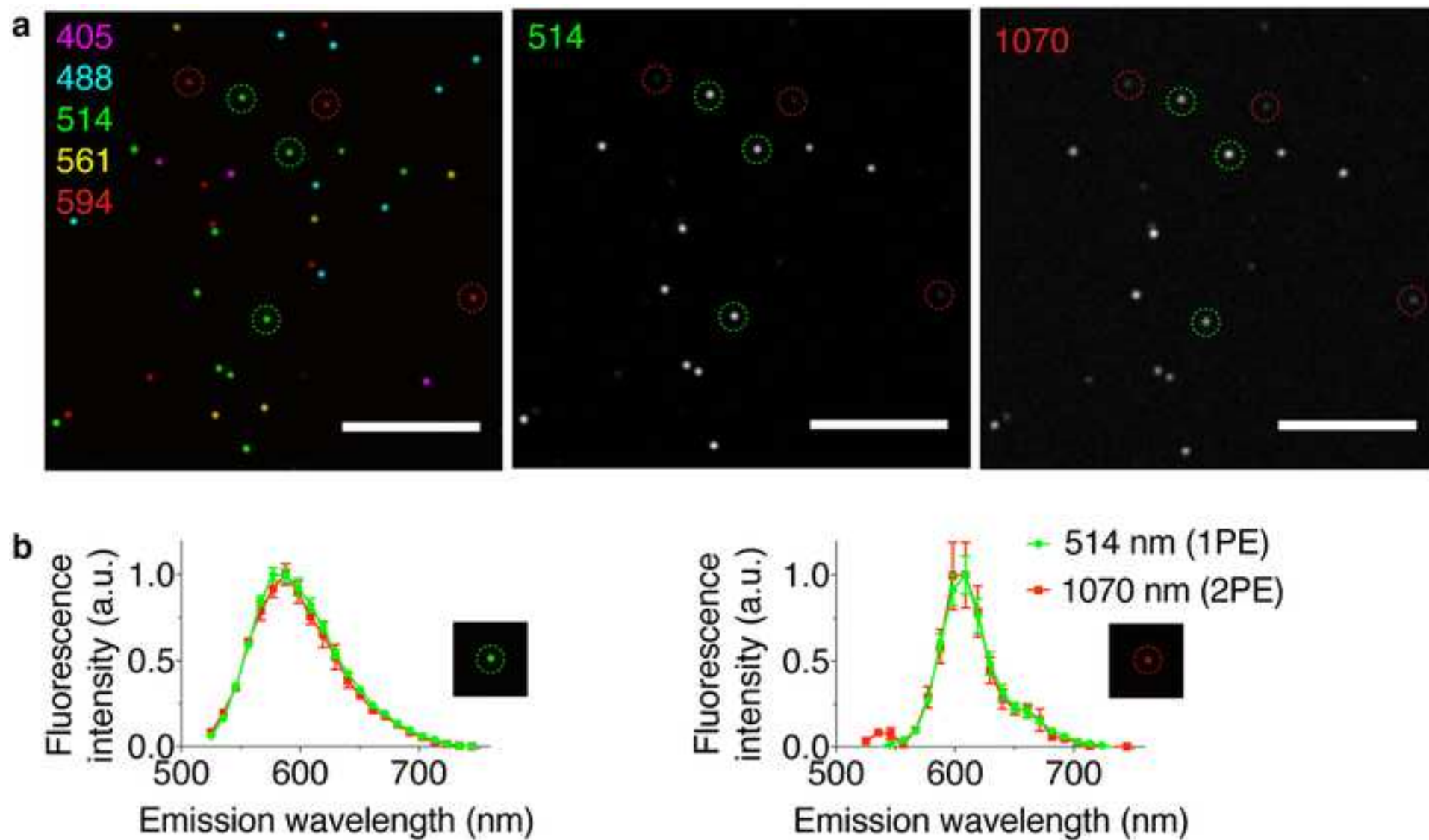646   (2010).

647

Figure1

Figure2

Click here to access/download;Figure;Figure2.bmp

a

Signal (a.u.)

Wavelength (nm)

— Simulation
— Experiment

b

Signal (a.u.)

Delay (fs)

— Transform-limit
— Experiment

c

P_{avg} (a.u.)

Time (h)

— Test 1
— Test 2

Figure3

Figure4 Click here to access/download;Figure;Figure4.bmp ±

a

405
488
514
561
594

514

1070

b

514 nm (1PE)
1070 nm (2PE)

Fluorescence intensity (a.u.)

Emission wavelength (nm)

Table1

Splicing point	A	B	C	D
Left fiber index	1	3	4	6
L coating diameter (µm)	250	250	250	250
L clad diameter (µm)	125	130	125	125
L core diameter (µm)	105	5	6	6
L MFD (µm)	105	4.8	7	6.2
Right fiber index	2	4	5	7
R coating diameter (µm)	250	250	250	250
R clad diameter (µm)	125	125	125	130
R core diameter (µm)	105	6	6	5
R MFD (µm)	105	7	6.2	4.8

Name of Material/ Equipment	Company	Catalog Number
Adapters, mirrors, posts, mounts, and translational stage (optomechanics)	Thorlabs	TR6-P5 (3x), AD12NT (2x), PFSQ20-03-M01, PFSQ05-03-M01, KMS, KM100C, KM100CL, KM200S, LT1, LT101, UPH2-P5, UPH3-P5 (2x)
Advanced optical fiber cleaver	AFL	CT-100
Autocorrelator	Femtochrome	FR-103XL/IR/FA/CDA
Beamsplitter mount	Thorlabs	BSH1/M
Factory fusion splicer	AFL	FSM-100P
Fiber collimators	OZ Optics (Canada)	LPC-08-1064-6/125-S-1.6-7.5AS-60-X-1-2-HPC
Fiber-coupled,high-speed photodiode detector	Thorlabs	DET08CFC
Free-space isolator	Thorlabs	IO-5-1050-HP
Free-space isolator	Thorlabs	IO-3D-1050-VLP
Half waveplate	Union Optics (China)	WPZ2312
High power multimode fiber pump module	Gauss Lasers (China)	Pump-MM-976-10
High power pump and signal combiner	ITF Technology (Canada)	MMC02112DF1
Index matching gel	Thorlabs	G608N3
Optical spectrum analyzer	Keysight	Agilent 70951B
Oscilloscope	Keysight	Agilent 54845A
Passive double clad fiber(5/130 µm)	ITF Technology (Canada)	MMC02112DF1
Polarizing beamsplitter	Thorlabs	PBS253
Quarter waveplates	Union Optics (China)	WPZ4312
Quartz birefringent filter plate	Newlight (Canada)	BIR1060
RF spectrum analyzer	Tektronix	RSA306B
Single mode fiber (6/125 µm)	OZ Optics (Canada)	LPC-08-1064-6/125-S-1.6-7.5AS-60-X-1-2-HPC
Single mode fiber coupler	AFW (Australia)	FOSC-2-64-30-L-1-H64-2
Transmission diffraction grating 1	LightSmyth	T-1000-1040-3212-94
Transmission diffraction grating 2	LightSmyth	T-1000-1040-60x12.3-94
Waveplate rotation mount	Thorlabs	RSP1/M
Ytterbium-doped single mode double clad fiber	Thorlabs	YB1200-6/125DC

Comments/Description

Standard optical components

3x

2x

3m, Included with combiner

2x

1m, Included with collimators

For compressor
For compressor
4x
3m

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Article Title: Low-Cost Custom Fabrication and Mode-Locked Operation of an Al-Normal-Dispersion Femtosecond Fiber Laser for Multiphoton Microscopy

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August 2, 2019

Philip Steindel, PhD
Review Editor, JoVE

RE: Manuscript JoVE60160 titled "Low-Cost Fabrication and Mode-Locked Operation of an
        All-Normal-Dispersion Femtosecond Fiber Laser for Multiphoton Microscopy"

Dear Dr. Steindel:

On behalf of my co-authors and myself, we are grateful to you and the Reviewers for constructive comments to clarify important details that need to be presented in more depth to help readers reproduce the presented laser. As detailed below, we have made a number of changes to improve the manuscript. The new revisions are summarized below (in blue font) and highlighted in the revised article file with tracked changes.

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A thorough proofread has been performed with corrections of spelling and grammar.

(2) Please ensure that the manuscript is formatted according to JoVE guidelines...

The manuscript has been reformatted following instructions form the Editor.

(3) JoVE cannot publish manuscripts containing commercial language…

Commercial language has been removed from the manuscript and all commercial products are listed in the Table of Materials and Reagents.

Protocol:
(1) …Please highlight 2.75 pages or less of the Protocol…that identifies the essential steps of the protocol for the video...

The Protocol total length is ~8 pages. Yellow highlight in the revised manuscript indicates the steps to be filmed/visualized (~2.75 pages). We anticipate that these particular steps (fiber splicing and achieving a mode-lock) will be most impactful to the community. In fact, we would have greatly appreciated such a resource ourselves when we first began this project!

(2) For each protocol step/substep, please ensure you answer the "how" question…

The revised manuscript now describes how to do each step/substep and additional notes on "tricky" aspects are now often included for several key substeps.

Figures and Tables:
(1) Please remove the embedded figures from the manuscript.

The embedded figures have been removed from the manuscript.

(2) Please remove the embedded table from the manuscript. All tables should be uploaded separately to your Editorial Manager account in the form of an .xls or .xlsx file.

The embedded table has been removed from the manuscript.

(3) Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints.

Here is a link to the editorial policy that allows re-print of the figures from our open access *Scientific Reports* article (Davoudzadeh N, Ducourthial G, Spring BQ. Custom fabrication and mode-locked operation of a femtosecond fiber laser for multiphoton microscopy. *Sci Rep* **9**: 4233, 2019):
https://www.nature.com/srep/about/open-access

(*4) Figure 4A: Please define the scale bars in the legend.*

The length of the scale bars is now defined in the legend.

Table of Materials:
(1) Please ensure the Table of Materials has information on all materials and equipment used, especially those mentioned in the Protocol.

All materials and equipment used is listed explicitly in the revised Table of Materials.

<u>Reviewer #1:</u>
(1) There is no information about the splicing program parameters. This setup involves splicing of dissimilar fibers, and double-clad fibers to single-clad fibers. The authors should mention about the parameters of the used program in the Fujikura splicer.

We apologize for not providing more details and we intend to show these steps explicitly in the video portion of the article. Nevertheless, the Review raises an important point that more detail should appear in print as well.

The revised Table 1 outlines the key parameters for all four fiber splices involved in fabrication of the fs fiber laser.

In addition, as the Reviewer suggests, we provide explicit details in the revised step 2.1.1 regarding the splicer program used for splicing dissimilar fibers:

(2) Double-cladding fibers should be recoated after splicing with low index polymer, there is no information about that in the protocol about using a recoater.

We thank the Reviewer for raising an important point of potential confusion. For the fiber splice point C, index matching gel should be applied to guide the pump signal out of the fiber (light escape to prevent burn damage points). Therefore, there is no need to recoat as now indicated in the revised step 3.4:

Apply index matching gel to splice <C>. The index matching gel is used to guide the pump light out of active fiber in order to reduce the generation of heat and thermal damage at the splice point. Note that there is no need to recoat the fiber. Rather it is preferable to leave the fiber bare and coated in index matching gel to minimize the risk of thermal damage.

(3) I couldn't find any information about the lengths of the fibers, which is critical for obtaining the performance. What is the length of each section? At least the length of the Yb-doped fiber should be given.

We apologize for the omission and thank the Reviewer for catching this. The length of each fiber within the oscillator circuit is now indicated in the revised Figure 1. Note that the lengths of fibers connected to components auxiliary to the oscillator (pump laser, combiner, collimators etc.) are not critical and we use the original fiber lengths provided by the manufacturers for flexibility and convenience.

Also, the following additions were made to the revised Protocol:

2.3.1 Cut the active fiber <4> at approximately 3 m from splice point <B>. Longer active fiber can be used for a higher output power, but the repetition rate will be reduced due to the increase in the cavity length.

2.4.2   Cut the input <5> of collimator <Col1> to approximately 40 cm.
Note: The length of the passive fiber (<5>) should not be too long (> 40 cm), as the amplified pulse will broaden substantially in the time and the spectral domain due to increased self-phase modulation (SPM) and group velocity dispersion (GVD) following passage through the gain fiber (pulse amplification). These effects will increase the difficulty of pulse compression.

(4) What is the distance between collimators?

The space between collimators is ~35 cm as now state in the revised step 3.5:

3.5 Use optomechanical parts to mount and fix the two collimators <Col1> and <Col2> on the optical table. The collimators should face each other with a separation of approximately 35 cm to provide sufficient space for inserting the in-cavity free-space components.

(5) There is no information about the compressor - angle of incidence on the gratings, and separation between the gratings which gives the best compression ratio. Without this information the results are unreproducible.

The Reviewer raises an excellent point of difficulty in reproducing the pulse compression. In practice, the placement of the gratings is an optimization problem that requires careful

alignment. The revised sections 5.6, 5.7, 5.12 and 6.5 detail an efficient approach to align and optimize the gratings for optimal laser throughput and pulse compression:

5.6 Mount the small mirror <M1> and the first compressor grating <G1> on the optical table. To achieve maximum efficiency of the compressor gratings, use the power meter to monitor the power of the first order maximum while adjusting the incident angle by rotating the grating.

Note: A rotational stage can be used to precisely control the rotation. As the loss due to the incident angle offset is small, the rotational stage is not used here to reduce cost.

5.7 Mount the translational stage on the table. Mount the second compressor grating <G2> on the translational stage. The distance between the gratings should be approximately 2 cm for optimal compression with fine adjustment using the translational stage. Ensure that the gratings are parallel.

5.12   Align the compressor

5.12.1   Use an IR card to locate the beam, adjust the position of <M1> and the compression gratings so that the output beam traverses the pulse compression parts in the following sequence: <M1>, <G1>, <G2>, <M2>, <G2>, <G1>, <M1>.

6.5 Follow the manufacturer instructions to align and operate the autocorrelator to measure the pulse duration. The second output from the extra-cavity beam splitter can be used here. Once the pulse duration can be measured, carefully adjust the translational stage on which <G2> is mounted to adjust the distance between the two gratings to tune the duration of the pulse.

Note: To ease alignment, it is best practice to mount mirror <M1> and <M2> separately from the two gratings (and the translational stage to which they are mounted). Also note that picosecond pulses are observed as a broad pedestal along with a central fs pulse peak feature during partially mode-locked operation.[17]

(6) The mirror M2 is a flat mirror which is tilted - this is not the best idea, it is much more efficient to use a hollow roof retroreflector instead. With a tilted mirror, the reflected beam does not travel the same path as the incident beam.

We thank the Reviewer for the suggestion. The revised section 5.12.2 contains a note indicating that a retroreflective mirror may be used as a more elegant solution:

5.12.2 Tilt <M2> up slightly to raise the reflected beam, making it pass above the pulse picker mirror <M1>.

Note: <M2> can be replaced by a retroreflector such that the pickoff mirror <M1> need not be angled. That is, the reflected beam will be parallel to the incident beam, but displaced, using a retroreflective mirror to simplify the setup.

(7) The list of materials does not include optomechanics (mounts for optics, collimators, gratings), I am not sure if they are included in the budget? A stable grating mount can be expensive, as well as kinematic mounts for mirrors/collimators, micrometer stages which aid the alignment of the collimators, etc. This is not mentioned in the text.

Good point and, yes, a complete optomechanics part list (with part numbers) is included in the List of Materials. The cost of these materials is also included in the budget.

(8) There is no information about the birefringent filter, why is it for? Is it a bandpass filter? What is the angle of incidence?

Excellent query and the Reviewer is correct. The following was added to step 4.7 to clarify the role of the birefringent plate:

> **4.7** Mount the birefringent filter <BF>, a half wave plate <HWP> and two quarter wave plates <QWP1> and <QWP2> to the corresponding positions shown in Fig. 1. The birefringent filter is sandwiched between two polarizers—one before (<PBS>) and one after (within the <ISO>)—to create a sinusoidal band-pass filter effect. There must be a small (3° to 5°) incident angle for the <BF> in order to control the wavelength range. Tune the alignment of the collimators once more until the output power reaches a maximum value.

(9) What is the output power of the laser?

The maximum output power we have tested is 1 W, as mentioned in the Introduction (line 77 of the revised submission). To clarify, a new step (6.6) has been added to address the output power adjustment and maximum level:

> **6.6** Gradually increase the pump power (above 0.5 W) to find the maximum pump power (we have tested pump powers up to ~5 W). Use the IR scope to observe the active fiber <4> constantly. If a bright spot appears, the pump power is too high within the cavity and it is likely to burn the active fiber at this pump level.
>
> Note: The maximum power of the system depends on the length of the active fiber and the alignment of the in-cavity free-space components. The protocols described here reach output powers up to 1 W without the appearance of bright spots or burns to the cavity, and this power is more than sufficient for most imaging applications. Higher output powers were not tested but may be possible, although, multipulsing is likely to result[16-18].

(10) Is the mode-locking regime self-starting or does it require time-consuming alignment of the waveplates to initiate the mode-locking? It would be good to warn the reader and potential user that this kind of laser (mode-locked via nonlinear polarization rotation) is generally not self-stating and requires re-alignment.

This system is self-starting from oscillator fluctuations and once mode-locked the laser remains stable for several weeks. To clarify this, the following paragraph was added to the Discussion:

> Once mode-locked, the system is quite stable in our experience and remains self-starting during a period of more than a week. In case of accidental perturbations of the system, or mechanical drift of the free space components over time, the system will lose mode-locking, but one can often easily recover the mode-locking laser by slightly adjusting the wave plates. To maintain stable output, temperature control of the active fiber is key. Therefore, we suggest operating the system in an air-conditioned room with minimal air flow near the system. The system is relatively robust to small vibrations. In fact, the effect of mechanical vibration cannot be observed in both temporal and spectral domain if system is put on a passive damped optical table. Touching the fiber components of the oscillator will perturb the mode-lock, but mode-locking is recovered simply by returning the fiber back to approximately its original position.

(11) It should be mention how sensitive the laser is to fiber movement, external disturbances like touching or bending of the fiber, vibrations, etc.

This is addressed in part in the response above. Generally, the system is robust in a normal lab environment if left undisturbed (but certainly not portable or mobile). To emphasize this

important point as an area for future development, the following paragraph concludes the revised Discussion:

> Finally, the compact form factor of fs fiber lasers is attractive for developing mobile clinical systems; *e.g.*, mobile cart-based systems. While smaller in size compared with a solid-state laser, the custom fiber laser design present here contains several free-space components, which require alignment. This significantly limits the mobility of the system. It is possible to replace all of these free-space components with fiber component analogs. Future work will include development of new all-fiber laser designs using PM fiber to develop systems that are robust to environmental changes.

(12) The Authors write in the abstract: "methods to verify true versus seemingly (partial or noise-like) mode-locked performance are presented". I don't know what does it mean "partial mode-locking"? I also didn't find in the manuscript the method how to distinguish noise-like pulse (NLP) mode locking from fundamental continuous mode-locking. The features of NLP are very characteristic so it is not so difficult to find the criteria, they should be clearly described in the text (e.g. the shape of NLP optical spectrum, the characteristic shape of NLP autocorrelation, etc.). The Authors should also answer the question how likely it is to obtain NLP in the presented laser.

Thank you for raising another important potential point of confusion. Partially mode-locking is another term for noise-like pulse (NLP) mode-locking and refers to partial, but not full, mode coherence. This key point will be the major subject of the video portion of this contribution, and our prior publication (Davoudzadeh et al. *Sci Rep* **9**: 4233, 2019) details characteristic features of NLP vs. full mode-locking. The revised manuscript now describes several of characteristic working modes of an all-normal-dispersion laser (which will be fully illustrated in the video contribution) in notes added to steps 6.3 and 6.5:

> Note: There are several characteristic modes of the laser operation that can be distinguished by observing the OSA: 1. One or two narrow (~1 nm) peaks. These are amplified spontaneous emission (ASE). 2. A wide (~50 nm) noisy peak with broken lines appearing randomly. This is a partial mode-lock (PML) spectrum. In this noise-like pulse mode the intensity and duration of each pulse varies, which results in poor image quality (unless one integrates the pulse fluctuations over longer pixel residence times).[13] 3. One ASE peak with a very noisy background consisting of many low-amplitude peaks. This is a non-mode-locked Q-switching mode. When in this mode, mode-locking can often be achieved by rotating <QWP1> over a small angle. 4. The "Batman" shaped mode-locking spectrum. The "ears" typically have different amplitudes with a flat spectrum between the sharp edge features. Reference 13 provides detailed measurements and illustrative results for each of these modes of operation.

> Note: For partially mode locked operation, picosecond pulses are observed as a broad pedestal along with a central fs pulse peak feature.[13]

Reviewer #2:
(1) Line 75: "A limitation of ANDi is that the pulse physics is incompatible with PM fiber such that environmental stability requires significant engineering." Literature(s) should be cited here to support this statement.

We thank the Reviewer for highlighting an incomplete thought. This passage has been revised as follows with citations:

> The pulse physics of ANDi fiber lasers elegantly utilizes nonlinear polarization evolution intrinsic to optical fiber as a key component of the saturable absorber.[2,3,9-11] However, this means that the

ANDi design is not easily implemented using PM fiber (although an all-PM fiber implementation of ANDi mode-locking has been reported, albeit with low power and ps pulse duration[12]) such that environmental stability requires significant engineering.

(2) Line 93: "1.1.2 Use lint-free tissue to clean the stripped fiber." Is ethanol needed here for cleaning the fiber?

Good point and we thank the Reviewer for catching this omission. The revised step 1.1.2 now reads:

Use lint-free tissue with ethanol or isopropanol to clean the stripped fiber. A buzz sound when wiping the fiber indicates that the fiber is sufficiently clean.

(3) Line 123: "A good splice has a uniform cladding boundary and uniform brightness along the fiber such that no splice juncture is visible." To the reviewer's knowledge, the fiber splicers generally have a loss estimation after finishing each splicing. Could this loss estimation help quantitatively judge the quality of the splicing point? (e.g. the splicing is acceptable if the loss is <0.03 dB).

Yes, the Reviewer is correct that fiber splicers often include optics to inspect the splice and to estimate the power loss based on the measured geometry/shape and light refraction through the fiber using a source perpendicular to the fiber to view the splice joint. Of course, this is only an estimate but sufficient in most cases. To clarify this fundamental point, the following note has been added to the revised step 1.3.4, which introduces basic fiber splicing techniques:

Note: Fiber splicers often include optics to inspect the splice and to estimate the power loss based on the measured geometry, shape and light refraction through the fiber using a source perpendicular to the fiber to view, image and analyze the splice joint. Of course, this is only an estimate but sufficient in most cases. For identical fibers the splicer will estimate this loss as ~0 dB (no detectable loss). In our experience, for the dissimilar fiber splices described below, the splicer estimates of the power losses range from 0.07 dB (splice points B and C, Fig. 1) to 0.3 dB (splice point D). These estimated losses most likely overestimate the loss due to mismatched geometry and refraction of the dissimilar fiber optics, which falsely appear as defect objects.

(4) Line 127: The authors mentioned that a fiber sleeve could be used to protect the splicing point. How about recoating the splicing point with a fiber recoater? Is this better or worse comparing with fiber sleeve?

This is a good query and is addressed in part above in the response to Reviewer #1's point (2). (At splice point D, index matching gel must be applied to guide the pump signal out of the fiber). At other splicing points, sleeves and recoaters can help prevent mechanical damage and breaks at the splice joints. However, recoaters add significant cost and here a goal was to minimize cost. Nevertheless, this point is now clarified in the revised step 1.3.6:

Note: If the two fibers are very long or attached to other components, the sleeve should be put on one of the fibers before cleaving, and then it can be moved to the splicing point. The fiber sleeve acts like a heat shrink tube in electronic circuits. It can be used to protect the splicing point from bending or pulling force. It is worth noting that a fiber recoater may instead be applied to recoat the splice point for maximal protection of the splice point to mechanical damage, albeit at a significant additional expense to purchase this equipment if not available.

(5) Line 163: "A large (>80%) efficiency indicates good splices at point and ." What caused this 20% loss? Is it mainly caused by the absorption of gain fiber or the mismatch between different fibers?

Yes, exactly right. Also, since light coming out of the end of active fiber is not collimated, there might be a loss due to limited sensor area of the power meter. To clarify this, the following note was added to step 2.3.4:

> Note: It is normal to have some power loss due to the absorption of the active fiber and due to the inefficiency of the coupling method to the power meter as mentioned in steps 2.3.2 to 2.3.3.

(6) Line 179: "while the active fiber 4 should be either straight or coiled loosely with radius of curvature >20 cm." Will it significantly influence the pulse property (or say mode locking status) if the active fiber was tightly coiled or strongly bended?

Good question. The inner cladding of the active fiber can be considered to be a multimode fiber. Then, a large bending angle may cause the pump signal to escape the coating. The following sentence was added to the revised step 3.3 to clarify this important point:

> Caution: A strong bend in the active fiber may cause the pump signal to escape the inner cladding of the active fiber. This can lead to fatal burn points along the active fiber that will require installing a new active fiber.

(7) Line 185: "They should face each other and the distance between them is approximately 35 cm." Does this 35 cm spatial distance match the working distance of the collimators the best? How much coupling efficiency between the collimators could you achieve in such a distance?

No, the 35 cm is simply the minimum space of clearance required for the free-space components, and the coupling efficiency is not easy to measure precisely without coupling the collimator fiber to the power meter. Here, we simply maximize the power meter readings (as specified in step 4.5). The rationale for the 35 cm is now clarified in the revised step 3.5:

> **3.5** Use optomechanical parts to mount and fix the two collimators <Col1> and <Col2> on the optical table. The collimators should face each other with a separation of approximately 35 cm to provide sufficient space for inserting the in-cavity free-space components.

(8) Line 200: "Keep adjusting until the output power reaches a maximum value of about 150 mW." The wavelength setting of the power meter should be pointed out here. It would be nice (but not mandatory) if the authors could show some small tricks on how to quickly get a good alignment which 'is often time consuming'.

These are excellent points and the following revisions have been made to steps 4.4 and 4.5 to specify the wavelength setting and to aid readers in efficiently aligning the collimators:

> **4.4** Mount a polarizing beam splitter (PBS) 6 cm from <Col1>. Mount the sensor of a power meter so that the power of the reflected laser output beam can be measured continuously. The wavelength of the power meter should be set to 1060 nm. A typical starting power reading with 0.5 W pump power is ~50 mW before alignment.

> **4.5** Adjust the screws on the collimator mounts to increase the reading of the power meter. Continue fine adjustments until the output power reaches a maximum value of about 150 mW, which indicates an excellent alignment.

Note: This step requires careful and patient adjustment, which is often time consuming. It is most efficient to be systematic as follows. First rotate the two screws that adjust the angle in the same direction (x or y) on the two collimators, with one screw rotating very slowly in one direction while the other rotating fast to scan all reasonable angles. Keep tracking the maximum reading from the power meter. Once the maximum power is found, switch to the screws adjusting another direction and repeat the slow rotate and fast scan outlined above. Due to reflections from the lenses inside both collimators, it is possible to observe multiple local maxima while aligning the collimators. The actual maximum power is much greater (150 mW) compared with the local maxima (70 to 80 mW).

(9) Line 234: "…in the following sequence: , first grating, second grating, , second grating, first grating, ." It will be more concise if the authors could write the "first grating" and "second grating" into abbreviation, e.g. and .

Thank you for the suggestion. In the revised step 5.12, the two gratings are now indicated as <G1> and <G2>, and the sequence has been updated accordingly (with the labels also updated in Fig. 1):

5.12 Align the compressor

5.12.1 Use an IR card to locate the beam, adjust the position of <M1> and the compression gratings so that the output beam traverses the pulse compression parts in the following sequence: <M1>, <G1>, <G2>, <M2>, <G2>, <G1>, <M1>.

(10) Line 231: In section "5.12 Align the compressor", the description is over simplified. According to the reviewer's experience, aligning the compressor with high efficiency (e.g. 94%^4 for double pass transmission grating pair) and good output beam quality is not so easy, especially for new comers. More details about "how to optimize the incident angle?", "how to ensure the gratings are parallel?", "how much efficiency is acceptable?" and so on should be added.

We agree with the Reviewer's concern and, in fact, Reviewer #1 also raised this concern. Please see the response to Reviewer #1's point (5) above for detailed changes.

(11) Line 242: When trying to mode lock the laser, is there any phenomenon that indicates your waveplate combination is close to or far away from the mode locking point? For example, sometime there will be many glitches on the optical spectrum, but sometimes only a CW peak.

This key question will be more fully addressed in the video component of this submission. Please also refer to Reviewer #1's point (12) for details regarding changes to the manuscript text. Essentially once one becomes familiar with the characteristic modes, it is indeed possible to recognize when the waveplate combination is close to the mode-lock and fine adjustments can be made. As will be illustrated in the video, Q-switching first appears during a larger sweep of the waveplates, and once found it is possible to achieve mode-locking by fine adjustments.

(12) Line 259: What does the RF peak look like when mode locking? Should it be very narrow in linewidth and very stable in SNR? Or something else?

The following note was added to the revised step 6.4.3 to clarify this point:

6.4.3 Gently adjust the wave plates and birefringent filter to maximize the signal to noise ratio, which is the height of the primary peak relative to the background.

(13) Line 283: "The power drift is less than ±3.5% over 24 hours without active cooling, which is suitable for many imaging experiments." Some examples of imaging experiments should be given (cited) here.

The imaging results shown in Figure 3 are good examples. After all, the proof of a properly functioning laser with sufficient peak pulse power is acquisition of high quality nonlinear images. The laser system is capable of generating two photon excited autofluorescence (Fig. 3c), second harmonic generation and two photon excited fluorescence using exogenous dyes (Fig. 3d). The last paragraph of the revised Representative Results provides further details:

Once mode-locking is verified, it is also important to test the imaging performance during practical MPE and nonlinear microscopy experiments using simple test target and biological samples. For example, the custom fiber laser output may be directed into a commercial laser scanning microscope for two-photon excitation (2PE) fluorescence imaging (Fig 3a). Note that the extracavity isolator, although lossy, is necessary to prevent back reflections from the microscope optics from entering the laser oscillator. These back reflections often interrupt mode-locking and fluorescence signal generation during imaging. Here, we applied a commercial confocal laser scanning microscopospe and used a de-scanned detector with a pinhole set to the maximum size setting in order to increase the collected fluorescence signal. One simple test sample for microscopy is a solution of fluorescent dye. A suggested first microscopy experiment is to measure the fluorescent dye signal during adjustments of the pulse power using a set of neutral density filters. This facilitates verifying that the fluorescence signal is quadratically dependent on the laser power delivered to the sample plane (Fig 3b), which is the expected response for 2PE. Next, images of biological specimens may be collected utilizing, for instantce, nonlinear 2PE tissue autofluorescence (Fig 3c, an unstained, fixed brine shrimp sample) as well as second harmonic generation (SHG) from collagen fibrils and 2PE of extrinsic fluorescent staines (Fig 3d, a freshly excised chicken tissue specimen stained with rhodamine B). As an additional verification of 2PE, we also collected 2PE hyperspectral images of multicolor fluorescent microsphere test targets compared with hyperspectral images taken by linear excitation with commercial diode lasers (Fig 4). The single-photon excitation and 2PE fluorescence spectra were analyzed and compared for two of the microsphere colors (corresponding to two fluorescent dyes excited separately by commercial, continuous wave 514 nm and 594 nm lasers). The fluorescence spectra excited by the custom-built laser are identical to the spectra taken with the commercial continuous wave lasers (single-photon excitation). Collectively, these results indicate that the custom fs fiber laser generates pulses with sufficient peak power and uniformity to generate 2PE fluorescence and SHG.

(14) Line 284: How much is the threshold power for self-starting?

The threshold depends on the length of the fiber and cavity. For our laser, the threshold is between 0.3 W and 0.4 W. We typically use 0.5 W for alignment and mode-locking as mentioned in step 4.1:

4.1 Turn on the pump laser. Set the power to 0.5 W (above the threshold for mode-locking yet a safe power for aligning system components).

(15) In general, single mode pump diodes are usually used for oscillator, while multimode pump diodes + double cladding fibers for amplifiers. In this manuscript, however, the authors use a multimode diode to pump the oscillator, and the working power is only 0.5 W which could be

easily offered by a single mode pump diode. Why do the authors use a multi-mode pump laser for oscillator? Is there something special?

The Reviewer may have missed the point that 0.5 W is chosen only for alignment and testing purposes. As the maximum output of the system is greater than 1W, a pump with >5W power is needed. To help clarify, the new section 6.6 describes a procedure for achieving the maximum output power of the system as mentioned above in response to Reviewer #1's point (9):

> **6.6** Gradually increase the pump power (above 0.5 W) to find the maximum pump power (we have tested pump powers up to ~5 W). Use the IR scope to observe the active fiber <4> constantly. If a bright spot appears, the pump power is too high within the cavity and it is likely to burn the active fiber at this pump level.
>
> Note: The maximum power of the system depends on the length of the active fiber and the alignment of the in-cavity free-space components. The protocols described here reach output powers up to 1 W without the appearance of bright spots or burns to the cavity, and this power is more than sufficient for most imaging applications. Higher output powers were not tested but may be possible, although, multipulsing is likely to result[16-18].

(16) Line 299: "The fluorescence spectra excited by the custom-built laser are identical to the images taken with the commercial continuous wave laser." How much is the commercial CW laser? One feature of the custom-built ANDi laser is "Low cost", is it cheaper than the commercial CW laser? In addition, it seems the way to decrease cost in this manuscript is to use commercially available components. Is there any other way to further decrease the cost?

Here, the point is not how much the custom fs fiber laser cost compares to commercial CW lasers. The point is that the fs fiber laser costs less than typical commercial solid-state fs lasers. The commercial CW laser is relatively inexpensive but cannot be applied for nonlinear imaging. Here it is included simply to validate that the custom fs fiber laser is performing as expected to generate nonlinear (two-photon excited) fluorescence signal.

(17) In the manuscript there is only one paragraph discussing the imaging performance of the laser. However, the figures of imaging results have larger amount and take more space than the laser's own characteristics. Therefore, I think the article structure is a little bit unbalanced. One possible way is that the authors could expand a little more discussion about their advantages on imaging experiments.

We have added additional details to describe the performance of the imaging experiments in the Representative Results section as mentioned in response to Reviewer #2's point (13).

Sincerely,

Bryan Q. Spring