Journal of Visualized Experiments
Reactive Inkjet Printing and Propulsion Analysis of Silk-based Self-propelled Micro-stirrers
--Manuscript Draft--

1   **TITLE:**
2   Reactive Inkjet Printing and Propulsion Analysis of Silk-based Self-propelled Micro-stirrers
3
4   **AUTHORS AND AFFILIATIONS:**
5   David A. Gregory[1], Piyush Kumar[1], Ana Jimenez-Franco[1], Yi Zhang[1], Yu Zhang[2], Stephen J. Ebbens[1],
6   Xiubo Zhao[1]
7
8   [1]Department of Chemical and Biological Engineering, The University of Sheffield, Sheffield, UK
9   [2]School of Engineering, University of Liverpool, Liverpool, UK
10
11   **Corresponding Authors:**
12   Xiubo Zhao   (xiubo.zhao@sheffield.ac.uk)
13   Stephen J. Ebbens   (s.ebbens@sheffield.ac.uk)
14   David A. Gregory   (d.a.gregory@sheffield.ac.uk)
15
16   **Email Addresses of Co-authors:**
17   Piyush Kumar   (pkumar2@sheffield.ac.uk)
18   Ana Jimenez-Franco (ajimenezfranco1@sheffield.ac.uk)
19   Yi Zhang   (yi.zhang@sheffield.ac.uk)
20   Yu Zhang   (yu.zhang@liverpool.ac.uk)
21

22   **KEYWORDS:**
23   self-propelled particles, autonomous motion, regenerated silk fibroin, catalase, hydrogen
24   peroxide, reactive inkjet printing, catalysis, micro-stirring, micro-motors, colloids, fluid dynamics
25
26   **SUMMARY:**
27   This protocol demonstrates the ability to utilize reactive inkjet printing to print self-motile
28   biocompatible and environmentally friendly micro-stirrers for use in biomedical and
29   environmental applications.
30
31   **ABSTRACT:**
32   In this study, a protocol for using reactive inkjet printing to fabricate enzymatically propelled silk
33   swimmers with well-defined shapes is reported. The resulting devices are an example of self-
34   propelled objects capable of generating motion without external actuation and have potential
35   applications in medicine and environmental sciences for a variety of purposes ranging from
36   micro-stirring, targeted therapeutic delivery, to water remediation (e.g., cleaning oil spills). This
37   method employs reactive inkjet printing to generate well-defined small-scale solid silk structures
38   by converting water soluble regenerated silk fibroin (silk I) to insoluble silk fibroin (silk II). These
39   structures are also selectively doped in specific regions with the enzyme catalase in order to
40   produce motion via bubble generation and detachment. The number of layers printed
41   determines the three-dimensional (3D) structure of the device, and so here the effect of this
42   parameter on the propulsive trajectories is reported. The results demonstrate the ability to tune
43   the motion by varying the dimensions of the printed structures.
44

INTRODUCTION:

Artificial self-propelled micro-stirrers (SPMSs) employ a variety of propulsion mechanisms to produce motion, which can be categorized as either chemical propulsion[1-6] or physical external propulsion. A common chemical propulsion mechanism is to use catalytic or enzymatic activity to either generate motion producing gradients or generate bubbles that impart momentum to the object when they detach. Previous studies have investigated several catalytic and chemical SPMSs, including polystyrene beads with platinum nanoparticles and chromium adsorbed on the surface[1], gold-platinum bimetallic Janus nano-rods[2], magnesium Janus micro-stirrers[3], micro-stirrers made of a magnesium core and titanium dioxide shell with embedded gold nanoparticles[4], and silk fibroin Janus micro-rockets with catalase embedded within the scaffold[5]. Physical propulsion mechanisms include magnetic[7,8], optical[9], and ultrasonic[10] propulsion systems, all being controlled by an external physical source. Depending on the intended application, SPMS size can range from a few nanometers to several hundreds of micrometers. Examples of potential applications of these abovementioned and other SPMSs are medical diagnosis of diseases with lab-on-a-chip devices[11], loading and in vivo targeted delivery of therapeutics[12], environmental remediation[3] (e.g., cleaning oil spills), and photocatalytic degradation of chemical and biological warfare agents, such as *Bacillus anthracis* and nerve agents[4]. Target application dependent, it is therefore desirable to be able to produce SPMSs that undergo specific trajectories such as long linear trajectories for transport challenges or rotational trajectories for micro mixing applications. The focus here is on rotational motion for stirring applications.

There is no single established method to fabricate SPMSs, but for medical and environmental applications, it is essential to use a material that is biocompatible, biodegradable, eco-friendly, readily available, cheap and allows easy fabrication of complex SPMSs without requiring sophisticated equipment. Regenerated silk fibroin (RSF) is one such material that fulfils all these parameters along with being also approved by the Food and Drug Administration (FDA).

Silk is a generic term used for several naturally occurring fibrous proteins, of which, the most commonly known is the one made by the larvae of silk moth, *Bombyx mori*, before its pupation. These cocoons are made of fibroin, a fibrous protein, stuck together with another protein called sericin. Silk fibroin (SF) has been found to have excellent mechanical properties, biocompatibility, and biodegradability[13], which makes it an ideal choice for fabricating SPMSs. SF exists in three polymorphic forms, namely, silk I, II, and III. Silk I is a water-soluble metastable form containing mainly helixes and random coils; silk II is a water-insoluble form containing mainly antiparallel β sheets of crystallized silk; and silk III is a threefold polyglycine II helical structure that exists at the water-air interface of the silk solution. Similar to other fibrous proteins, SF has repeating units of amino acid sequences. The naturally occurring SF of a cocoon consists of three main hexapeptide domains of such repeating units (GAGAGX), while X can be A, S, or Y. Through hydrogen binding, the (GA)n motifs from antiparallel β sheet structures further stack through van der Waals forces and form hydrophobic nano crystallizations[14,15].

Biocompatibility can be further improved by seeking to drive propulsion using naturally occurring enzymes to generate a concentration gradient or gas bubbles in a liquid medium (fuel).

89   Consequently, in this study, the enzyme catalase is used as the "engine" to generate propulsion
90   with hydrogen peroxide being used as the aqueous fuel medium. Catalase is an enzyme found in
91   almost all living organisms. It catalyzes the decomposition of hydrogen peroxide (H_2O_2) into water
92   and oxygen[16]. The release of oxygen bubbles from the enzyme sites of the SPMSs generates a
93   force onto the object causing it to undergo propulsion in the opposite direction of the bubble
94   release[5] (**Figure 1**).
95
96   In a catalytically driven SPMS, different positioning of the catalytic site results in different
97   propulsion behaviour and trajectories[1]. In pursuit of generating efficient micro-stirrers, it is
98   therefore, necessary to fabricate stirrers with well-defined geometric shapes and engine
99   positions and compare the different powers of the engine. Here, to facilitate these investigations,
100   it is described how regenerated silk fibroin SPMSs are fabricated at millimeter scale using reactive
101   inkjet printing (RIJ) technology. Inkjet printing is a non-contact method for the deposition of
102   materials. This makes the fabrication of small complex structures with high precision in
103   generating various shapes straightforward. RIJ takes place when two or more different reacting
104   materials are deposited and react on the substrate to produce the desired product material.
105   Therefore, SPMSs printed with one catalytic stirrer site off center gives the object asymmetry
106   that results in rotational motion. This approach also makes it simple to manufacture micro-
107   stirrers in a variety of shapes and design configurations defined by computer-aided design (CAD),
108   thus allowing easier and more accurate controllability on the desired movement during practical
109   applications. Finally, the ability of printing devices with varying thickness that exhibit different
110   propulsion properties is demonstrated.
111
112   This study provides a blueprint for manufacturing SPMSs with RSF at micrometer to millimeter
113   scale. The use of RIJ technology for manufacturing RSF micro-stirrers opens the door for the
114   highly versatile production of micro-stirrers from materials such as in situ produced scaffolds or
115   hydrogels, which are not otherwise able to be deposited or fabricated through other means such
116   as evaporation. After appropriate further functionalization (e.g., enzymes), these SPMSs can be
117   suitable for environmental remediation[3], such as cleaning biological toxins, organic pollutants,
118   and chemical and biological warfare agents[4].
119
120   **PROTOCOL:**
121
122   **CAUTION:** Please consult relevant material safety datasheets before use of hydrogen peroxide,
123   sodium carbonate, ethanol, calcium chloride, and methanol. Ensure to wear all appropriate
124   personal protective equipment including engineering controls while handling chemicals used in
125   this protocol.
126
127   **1.   Extraction of fibroin**
128
129   1.1.   Cut 5 g of cleaned silk cocoons into ~ 1 cm^2 small pieces using scissors.
130
131   1.2.   Boil 2 L of deionized (DI) water in a 2 L beaker on a magnetic hot plate under an extraction
132   hood.

1.3. Add 4.24 g of sodium carbonate gradually and slowly into the boiling water to avoid boiling over and let it dissolve with the help of a magnetic stir bar.

1.4. Wait until the solution starts to boil again and add the cut pieces of cocoons into the solution. Ensure that all the silk is submerged in the solution and keep the solution boiling under constant stirring for 90 min. Cover the beaker lightly with aluminium foil and top up with preheated DI water regularly to replenish water loss due to evaporation.

2. Drying of fibroin

2.1. Remove the extracted fibroin fibers from the sodium carbonate solution with a glass rod or spatula and wash 3x with 1 L of preheated DI water for each wash, gradually decreasing the temperature for every washing step (approximately 60 °C, 40 °C, and room temperature, 25 °C).

2.2. Spread out the fibroin fibers on a 750 mL borosilicate glass crystallizing dish and place it into a drying oven at 60 °C under atmospheric pressure and leave to dry overnight. Once dried, store the fibroin in a closed container at room temperature.

3. Dissolution of fibroin

3.1. Prepare a ternary solution (Ajisawa's reagent) containing 4.8 g of DI water, 3.7 g of ethanol, and 3.1 g of calcium chloride[17].

3.2. Place a two-neck round bottom flask (100 mL) into a water bath, made by filling a 750 mL borosilicate glass crystallizing dish with 600 mL of DI water, on top of a magnetic hot plate. Place the ternary solution inside the flask.

3.3. Place a thermometer in one of the necks to monitor the solution temperature accurately. Cover the other neck with aluminium foil to prevent drying out of the solution due to evaporation (or use a water-cooled reflux condenser). Heat up the solution to 80 °C.

NOTE: Ensure that the bulb of the thermometer is inside the solution.

3.4. When the temperature of the solution is stable at 80 °C, remove the aluminium foil and add 1 g of dried fibroin to the solution. Add a small magnetic stir bar to ensure that the solution is mixed well throughout the dissolution process. Cover the second neck again with aluminium foil to minimize evaporation but keep the system open. Leave to dissolve for 90 min.

4. Dialysis of fibroin solution

4.1. After 90 min of dissolution, leave the fibroin solution for 10 min to cool down to room temperature.

177   4.2.   Take one 15 cm long dialysis tube (molecular weight cut-off 12,000–14,000 kDa) and tie
178   a knot in one of the two ends. Wash it for a few minutes with running DI water from the tap.
179
180   4.3.   Open the other end and pour the fibroin solution inside. Using a metal clamp, close the
181   other end of the dialysis tube ensuring that the tube is closed as tightly as possible. Attach one
182   of the ends of the dialysis tube via a screw cap to an empty 30 mL plastic vial to allow the dialysis
183   tube to float in the water.
184
185   4.4.   Fill a 2 L beaker with 2 L of DI water and place the dialysis tube inside it. Change the water
186   at regular intervals. Check the conductivity of the water every time it is changed to follow the
187   dialysis process. The dialysis step finishes once the conductivity of the water is below 10 µS/cm.
188
189   NOTE: This process usually takes around 24–36 h with 5 changes of water.
190
191   4.5.   After dialysis is complete, cut one end of the dialysis tube with scissors and pour the
192   solution into a series of 1.5 mL tubes. Then, centrifuge for 5 min at 16,000 x g to remove any
193   particles inside the fibroin solution. Collect the supernatant in a 30 mL plastic vial and store it at
194   4 °C.
195
196   **5.   Determination of RSF solution concentration**
197
198   5.1.   Weigh a clean glass slide (W_1). Add 200 µL of silk solution (V_1).
199
200   5.2.   Leave the glass slide in an oven at 60 °C for 2 h.
201
202   5.3.   Weigh the glass slide again (W_2).
203
204   5.4.   Calculate the concentration of the silk solution (w/v) using the following formula:
205

206    $$Silk(w/v) = \frac{W_2 - W_1}{V_1}$$

207
208   **6.   Preparation of inks for printing**
209
210   6.1.   Prepare ink A (final volume 1.5 mL) by mixing the fibroin solution (40 mg/mL) and
211   polyethylene glycol 400 (PEG_{400}; 14 mg/mL) for printing the main body of the SPMSs.
212
213   6.2.   For printing the catalytic engine of the SPMSs, mix fibroin (40 mg/mL), PEG_{400} (12 mg/mL),
214   and catalase (6 mg/mL with catalytic activity of >20,000 units/mg) to make 1.5 mL of ink B.
215
216   6.3.   Prepare 1.5 mL of ink C by dissolving Coomassie brilliant blue (0.05 mg/mL) in methanol.
217

218 NOTE: Methanol is used for converting the fibroin random coils to rigid beta sheets by printing
219 ink C on top of ink A or ink B. Coomassie brilliant blue is used to provide a contrasting color of
220 SPMSs to aid auto tracking of the SPMSs during propulsion.
221
222 **7.   Reactive inkjet 3D printing**
223
224 NOTE: The inkjet printer used in these experiments is based on piezo actuated jetting devices
225 with glass nozzles. There are several commercially available inkjet printers for research that can
226 duplicate these functions.
227
228 7.1.   Use jetting devices with 80 µm nozzle diameter for printing the inks on a silicon substrate
229 placed on the stage at a working distance between the nozzle and the Si-wafer substrate of
230 around 5 mm. The geometric shapes of the SPMSs are digitally defined as a series of points of X-
231 Y coordinates in a spreadsheet file.
232
233 NOTE: The printer reads the coordinates serially and runs the printer accordingly. Each
234 coordinate point makes the printer jet once through the jetting device. Separate spreadsheet
235 files are created for inks A and B (see **Supplemental Files** [SPMS Main Body.xlsx and SPMS
236 Engine.xlsx]).
237
238 7.2.   Load the three inks (A, B, and C) into three reservoirs (1.5 mL each) and then adjust the
239 backpressure using the backpressure valve for each individual channel to ensure that the ink is
240 not dripping from the jetting devices.
241
242 NOTE: Three jetting devices on independent channels are needed.
243
244 7.3.   Adjust the jetting parameters (rise time 1, dwell time, fall time, eco time, rise time 2, idle
245 voltage, dwell voltage, echo voltage) for each channel to ensure that each ink gives a good stable
246 droplet formation (**Figure 2**).
247
248 NOTE: These parameters are jetting device and ink dependant and will need to be adjusted
249 accordingly.
250
251 7.4.   Print the silk fibroin ink layer-by-layer alternating with methanol on clean polished Si-
252 wafer substrates: stage 1, printing of ink A (main body); stage 2: printing of ink C (curing ink);
253 stage 3: printing of ink B (catalytic ink for engine sites); stage 4: printing of ink C (curing ink); stage
254 5: repeat stages 1-4 for desired layers required (e.g., 100).
255
256 NOTE: Two example designs for the 4 stages are included in **Supplemental Files**; SPMS Main
257 Body.xlsx is used for stage 1 and stage 2, and SPMS Engine.xlsx is used for stage 3 and stage 4.
258
259 7.5.   Print two batches of fibroin SPMSs with 200 layers and 100 layers thickness, respectively.
260

261   NOTE: The catalase engine is located on the side of one end of each stirrer. Thus, the stirrers have
262   one catalytic engine (see **Figure 1** red region).

263

264   7.6.   To remove the samples off the Si-wafers, immerse samples in DI water and gently agitate
265   until detachment occurs.

266

267   **8.   Data acquisition/tracking and trajectory analysis of self-propelled stirrers**

268

269   8.1.   Clean a glass petri dish (9 cm in diameter) with DI water ensuring that the surface is dust
270   free.

271

272   8.2.   Once clean and dry, add 10 mL of pre-filtered (0.45 µm) 5% w/v H_2O_2 into the petri dish
273   and leave to settle. Light up the bottom of the petri dish with a cool white light-emitting diode
274   (LED) light source and use a high-speed camera with macro zoom lens to capture the motion from
275   above. Save videos as .avi files.

276

277   NOTE: See **Table of Materials** for details of the equipment used.

278

279   8.3.   Wash the printed silk stirrers for 10 min by submerging them in DI water to remove any
280   unbound PEG_{400}. Carefully take one washed stirrer with the tip of a sterile syringe needle and
281   place it in the center of the petri dish. When the washed stirrer touches the H_2O_2 fuel, bubbles
282   start forming around the engine and circular motion of the stirrer is observed. When the system
283   appears stable (usually 10–30 s later), press **record** in the recording software to start capturing
284   the video.

285

286   8.4.   Perform tracking of the micro stirrers on a frame by frame basis, tracking each end of the
287   stirrers as indicated by points A and B in **Figure 3**.

288

289   NOTE: This can be done manually or with the aid of tracking software.

290

291   8.5.   From the obtained tracking data, calculate the instantaneous velocity between two
292   consecutive frames (e.g., 1 and 2) using the equation below and average the resulting velocities
293   from the entire sequence to obtain the mean instantaneous velocity.

294

295   8.6.   Further to this, calculate the angle of orientation φ. Then use the rate of change of φ to
296   determine the rotational velocity (**Figure 3**).

297

$$v_{inst} = \frac{\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}}{t_{frame}}$$

298

299   NOTE: When calculating instantaneous velocities from tracked image data, it is important that
300   the initial image of an object with known dimensions is taken to be able to calculate correct pixel
301   to micrometer values. These values will depend on the camera, objective, and distance used.

302 Depending on the type of printed particle, choose different tracking points for calculating the
303 velocity. For example, here tracking points A, B, and C (center of mass) are all used to determine
304 instantaneous velocities (**Figure 3**).
305
306 **9.   Characterization of SPMSs by SEM**
307
308 9.1.   Remove unused and used SPMSs from the Si-wafer or bulk solution and transfer them
309 onto 10 mm wide carbon sticky pads mounted onto aluminium scan electron microscopy (SEM)
310 stubs. Dry the samples in a drying oven for 10 min at 60 °C.
311
312 9.2.   Load the sample stubs onto the sputter coater stage. Sputter coat (argon plasma at
313 0.05 Torr) 50–100 nm of gold onto the samples, ensuring a homogeneous gold surface coverage
314 of the sample.
315
316 9.3.   Remove the sample stubs from the sputter coater and image in an SEM under vacuum
317 at 5.0 kV.
318
319 NOTE: Very high acceleration voltages can burn the silk and give rise to false features.
320
321 **REPRESENTATIVE RESULTS:**
322 After boiling the silk, it is expected that the dried fibers are around one third lighter than before,
323 indicating the successful removal of sericin. During the dissolution of the silk in Ajisawa's reagent,
324 the fibers should be completely dissolved, and a yellow viscous liquid should be recovered. After
325 dialysis, the silk solution should be less viscous but still show a slightly yellow color. If the silk has
326 turned into gel, this indicates that dissolution was not done successfully.
327
328 Stable droplets formed from the jetting devices will enable the higher definition of the printed
329 samples. **Figure 2** shows an example of a stable single droplet to give good printing results such
330 as the printed silk-stirrers shown in **Figure 4**. It is normal, depending how viscous the ink is, that
331 spreading occurs on the substrate.
332
333 Depending on the inkjet printers used and the droplet size, the distance between each printed
334 droplet needs to be adjusted in such a way that they overlap to generate connected lines. If the
335 droplets are too far apart, the printed structure will break up. In addition to this, if not enough
336 layers are printed, there is a chance of the micro-stirrers breaking when placed into fuel solution.
337 Once the stirrers have been removed off the substrate and washed, placing them into the
338 hydrogen peroxide fuel solution should instantly result in bubbles being formed. The success rate
339 of good bubble release depends strongly on the enzyme activity; if the enzyme activity is low,
340 fewer bubbles will form thus leading to poor propulsion results. **Figure 5** shows how the surface
341 morphology of the stirrers is altered due to the bubbles being released from the inner structures
342 generating small pores. A successful micro-stirrer will look similar to the ones that can be seen in
343 **Figure 6** and the two **Supplemental Videos S1** and **S2** respectively.
344

345 **Figure 6** shows still video frames of two representative, 100-layer (**Figure 6A**) and 200-layer
346 (**Figure 6B**) micro-stirrers in 5% H_2O_2 fuel. The red and green lines indicate the trajectories
347 tracked (see **Supplemental Videos S1** and **S2**). The rotational velocity can be determined by the
348 rate of change of orientation (ϕ, **Figure 3**) as shown in **Figure 7.** Comparison of 100-layer and
349 200-layer catalase doped micro-stirrers shows a distinctive increase in rotational velocity of ~0.6
350 fold from 60 ± 6 rpm to 100 ± 10 rpm (**Figure 7**).
351
352 **FIGURE LEGENDS:**
353 **Figure 1: Schematic illustration of the catalytic breakdown of hydrogen peroxide into water**
354 **and oxygen by catalase embedded in the stirrer's scaffold at desired locations (shown in red).**
355 The product oxygen bubbles provide the necessary propulsion for moving the stirrer.
356
357 **Figure 2: The time lapse images of the droplet formation of RSF from a jetting device (nozzle**
358 **diameter 80 μm).** The numbers below the images represent the time elapsed, in microseconds
359 (μs), since the initiation of jetting of the silk ink droplet.
360
361 **Figure 3: Schematic representation of particle tracking over two consecutive frames.** A and B
362 indicate tracking points and C indicates the center of mass. ϕ indicates the angle of orientation.
363 SPMS trajectory direction is indicated by the curved black arrow.
364
365 **Figure 4: Light micrograph of a freshly RIJ printed micro-stirrer (100 layers) before washing**. Red
366 box denotes catalase doped region (engine region).
367
368 **Figure 5: SEM images of the main body and catalase engine part of a SPMS after pores are**
369 **formed due to bubble release.** Pores can be clearly seen on the engine surface in the SEM images
370 of the SPMSs originating from the oxygen bubble release. (**A**) Silk micro-stirrers before exposure
371 to 5% w/v H_2O_2 fuel solution. (**B**) Silk SPMS after exposure to 5% w/v H_2O_2 fuel solution. Images
372 on the right are enlargements of the red regions.
373
374 **Figure 6: Video frames of two micro-stirrers in 5% fuel solution showing the trajectory over**
375 **time.** (**A**) 100-layer micro-stirrers. (**B**) 200-layer micro-stirrers.
376
377 **Figure 7: Comparison of angle of orientation (ϕ) for 100-layer (60 ± 6 rpm) and 200-layer (100**
378 **± 10 rpm) micro-stirrers.**
379
380 **Supplemental Video S1: Representative 100-layer self-motile micro-stirrer in 5% w/v H_2O_2**
381 **undergoing propulsion.**
382
383 **Supplemental Video S2: Representative 200-layer self-motile micro-stirrer in 5% w/v H_2O_2**
384 **undergoing propulsion.**
385
386 **DISCUSSION:**
387 A key feature of SPMSs produced in this protocol is the ability to quickly and easily design
388 different shapes and structures via RIJ of silk doped with enzymes such as catalase and achieve

389   chemically driven motion via bubble propulsion[5]. This together with the high biocompatibility[18]
390   of these stirrers makes them highly desirable for future applications for both water remediation
391   challenges as well as for lab-on-a-chip applications for medical diagnosis devices.
392
393   Here, the propulsion ability is demonstrated by utilizing a simple line design with an engine part
394   printed to the side of it as shown in **Figure 1**. The red dots represent the catalytically active
395   catalase doped engine parts and the blue dots represent the inactive parts. To be able to
396   generate 3D shapes via RIJ, it is necessary to print multiple layers in order to generate a z-axes
397   height. Here, the numbers of layers deposited onto a Si-wafer were 100 and 200. By varying the
398   number of layers, a difference in propulsion velocity/rotation comparable to depositing double
399   the amount of material can be found. In order to have well-defined structures during inkjet
400   printing, it is important that the correct jetting parameters are selected in order to achieve a well-
401   defined droplet as shown in **Figure 2**. These parameters will vary according to the ink used and
402   the jetting devices. If the ink does not produce stable droplets, then the ink is most likely no
403   longer suitable for printing and is most likely starting to turn into gel. It is important to note that
404   the resolution limit strongly depends on the size of nozzle used, and smaller nozzles allow for
405   higher resolution and smaller structures/particles being printed.
406
407   An example of RIJ printed silk stirrer is shown in **Figure 4**, where the catalase doped engine part
408   (as indicated by the red marked region) can be seen attached to the side of the main body (also
409   see the schematic in **Figure 1** for details). The printed silk scaffold is a material that allows for the
410   fuel solution to diffuse throughout the entire 3D structure, and thus oxygen bubbles are formed
411   during the decomposition of hydrogen peroxide via catalase. The oxygen bubbles being released
412   generate micron-scale pores in the silk scaffold structure as can be seen by comparing the SEM
413   micrographs before exposure to H_2O_2 fuel (**Figure 5A)** and after H_2O_2 exposure (**Figure 5B)**. In
414   order to ensure that motion is due to the decomposition of H_2O_2 fuel but not surface tension
415   driven via the release of PEG_{400}, it is important that stirrers are initially immersed in water for a
416   period of at least 10 min and tested in DI water for surface tension motion prior to propulsion in
417   fuel solution.
418
419   The use of PEG_{400} allows for better release of bubbles from the silk surface[19] as previously
420   explained by Gregory et al.[5] but can also give rise to surface tension driven propulsion, which
421   may be desirable depending on the application as previously described[20]. This second mechanism
422   also gives the opportunity to produce SPMSs with two mechanisms that are time dependant and
423   can be advantageous for certain applications that would for example expect initial vigorous
424   stirring at the start followed by slower continued stirring for extended periods of time[20].
425
426   In conclusion, by using RIJ to produce autonomous self-propelled devices, a vast range of shapes
427   and sizes can be easily designed and printed. Silk as a base material for the devices gives
428   opportunities to easily encapsulate enzymes and other moieties into the structures giving the
429   possibility to add functions to these devices.
430
431

DISCLOSURES:
The authors have nothing to disclose.

REFERENCES:
1   Gregory, D. A., Campbell, A. I., Ebbens, S. J. Effect of Catalyst Distribution on Spherical Bubble Swimmer Trajectories. *The Journal of Physical Chemistry C.* **119** (27), 15339-15348 (2015).
2   Paxton, W. F. et al. Catalytic Nanomotors: Autonomous Movement of Striped Nanorods. *Journal of the American Chemical Society.* **126** (41), 13424-13431 (2004).
3   Gao, W. et al. Seawater-driven magnesium based Janus micromotors for environmental remediation. *Nanoscale.* **5** (11), 4696-4700 (2013).
4   Li, J. et al. Water-Driven Micromotors for Rapid Photocatalytic Degradation of Biological and Chemical Warfare Agents. *ACS Nano.* **8** (11), 11118-11125 (2014).
5   Gregory, D. A., Zhang, Y., Smith, P. J., Zhao, X., Ebbens, S. J. Reactive Inkjet Printing of Biocompatible Enzyme Powered Silk Micro-Rockets. *Small.* **12** (30), 4048-4055 (2016).
6   Ismagilov, R. F., Schwartz, A., Bowden, N., Whitesides, G. M. Autonomous movement and self-assembly. *Angewandte Chemie-International Edition.* **41** (4), 652-654 (2002).
7   Li, T. et al. Highly Efficient Freestyle Magnetic Nanoswimmer. *Nano Letters.* **17** (8), 5092-5098 (2017).
8   Barbot, A., Decanini, D., Hwang, G. in *2014 IEEE/RSJ International Conference on Intelligent Robots and Systems.* 4662-4667.
9   Xuan, M. et al. Near Infrared Light-Powered Janus Mesoporous Silica Nanoparticle Motors. *Journal of the American Chemical Society.* **138** (20), 6492-6497 (2016).
10   Garcia-Gradilla, V. et al. Functionalized ultrasound-propelled magnetically guided nanomotors: toward practical biomedical applications. *ACS Nano.* **7** (10), 9232-9240 (2013).
11   Baraban, L. et al. Catalytic Janus Motors on Microfluidic Chip: Deterministic Motion for Targeted Cargo Delivery. *ACS Nano.* **6** (4), 3383-3389 (2012).
12   Ghalanbor, Z., Marashi, S.-A., Ranjbar, B. Nanotechnology helps medicine: Nanoscale swimmers and their future applications. *Medical Hypotheses.* **65** (1), 198-199 (2005).
13   Lu, Q. et al. Water-Insoluble Silk Films with Silk I Structure. *Acta Biomaterialia.* **6** (4), 1380-1387 (2010).
14   Wilson, D., Valluzzi, R., Kaplan, D. Conformational Transitions in Model Silk Peptides. *Biophysical Journal.* **78** (5), 2690-2701 (2000).
15   Fink, T. D., Zha, R. H. Silk and Silk-Like Supramolecular Materials. *Macromolecular Rapid Communications.* **0** (0), 1700834.
16   Chelikani, P., Fita, I., Loewen, P. C. Diversity of structures and properties among catalases. *Cellular and Molecular Life Sciences CMLS.* **61** (2), 192-208 (2004).

476 17   Ajisawa, A. Dissolution of silk fibroin with calciumchloride/ethanol aqueous solution.
477   *Journal of Sericultural Science of Japan.* **67** (2), 91-94 (1998).
478 18   Zhang, Y., Gregory, D. A., Smith, P. J., Zhao, X. in *Printing For Fabrication (NIP)*   406-409
479   (Society for Imaging Science and Technology, Manchester, 2016).
480 19   Gregory, D. A., Zhang, Y., Smith, P. J., Ebbens, S. J., Zhao, X. in *Printing For Fabrication*
481   *(NIP)*   452-456 (Society for Imaging Science and Technology, Manchester, 2016).
482 20   Zhang, Y. et al. Reactive Inkjet Printing of Functional Silk Stirrers for Enhanced Mixing and
483   Sensing. *Small.* e1804213, (2018).
484
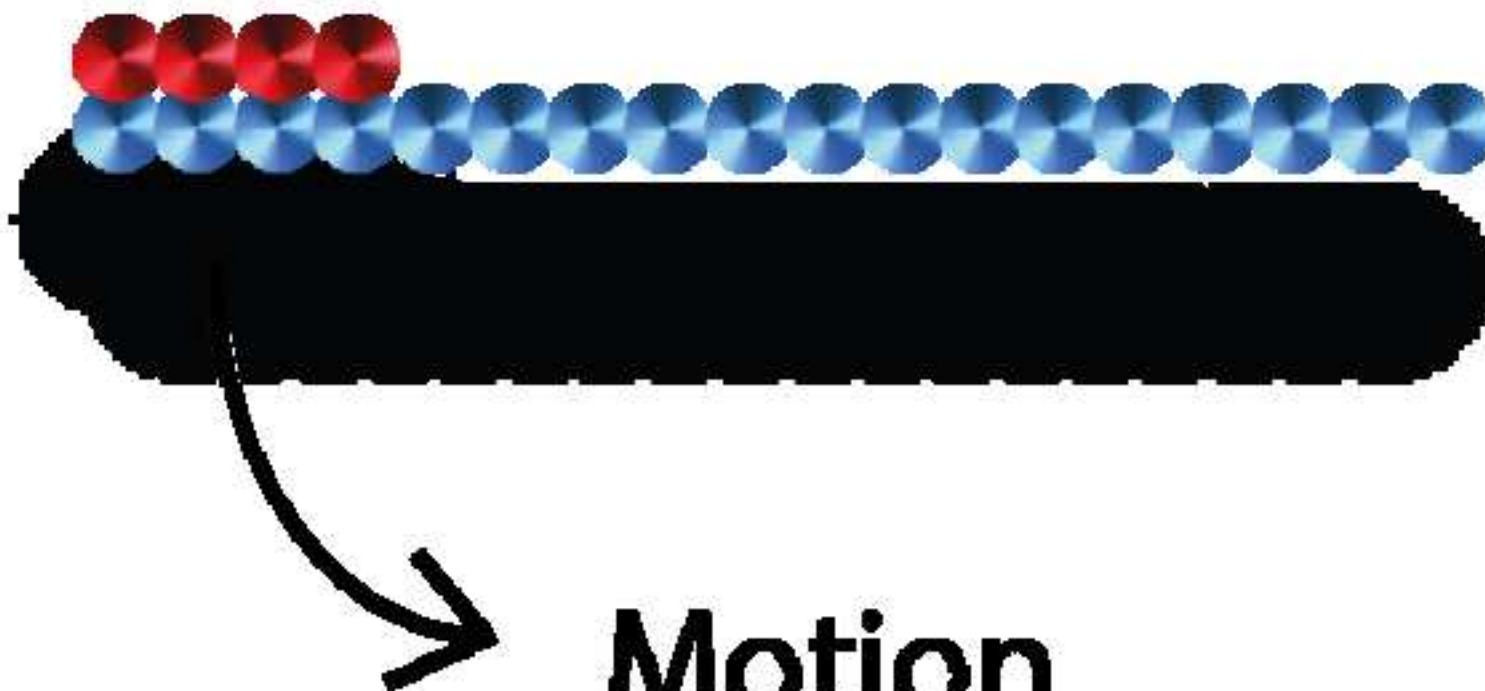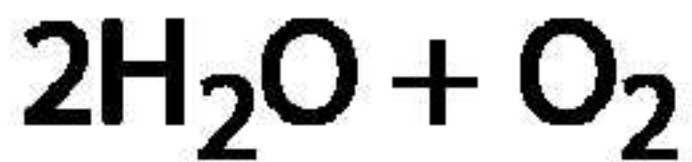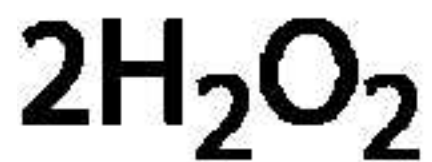
Figure 1
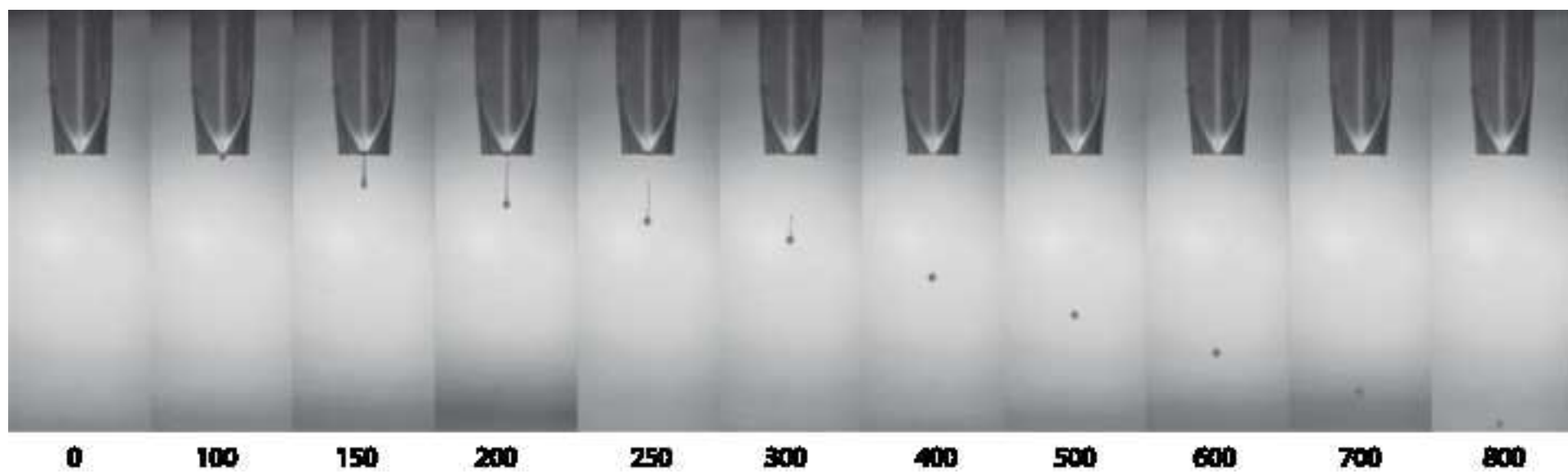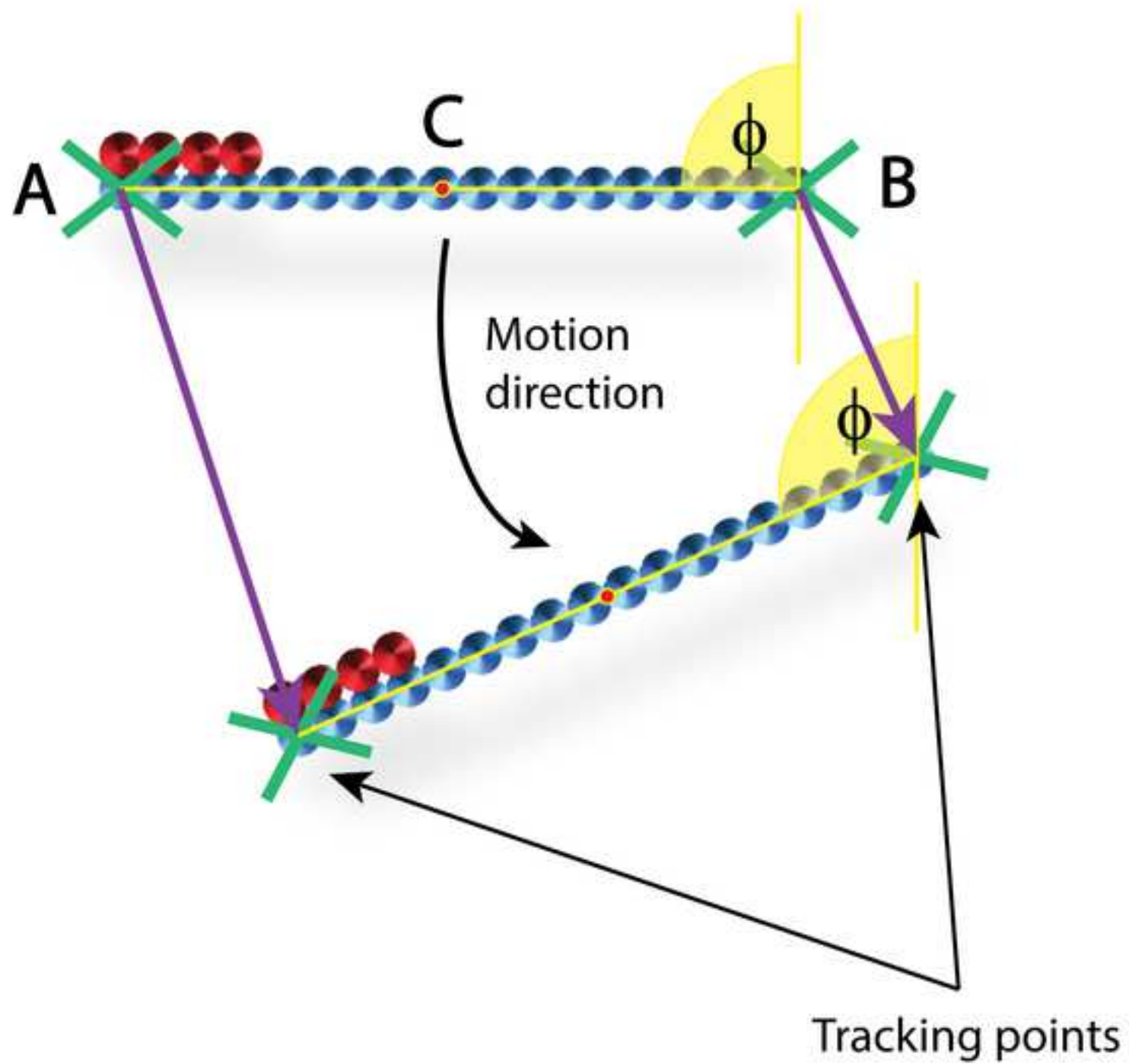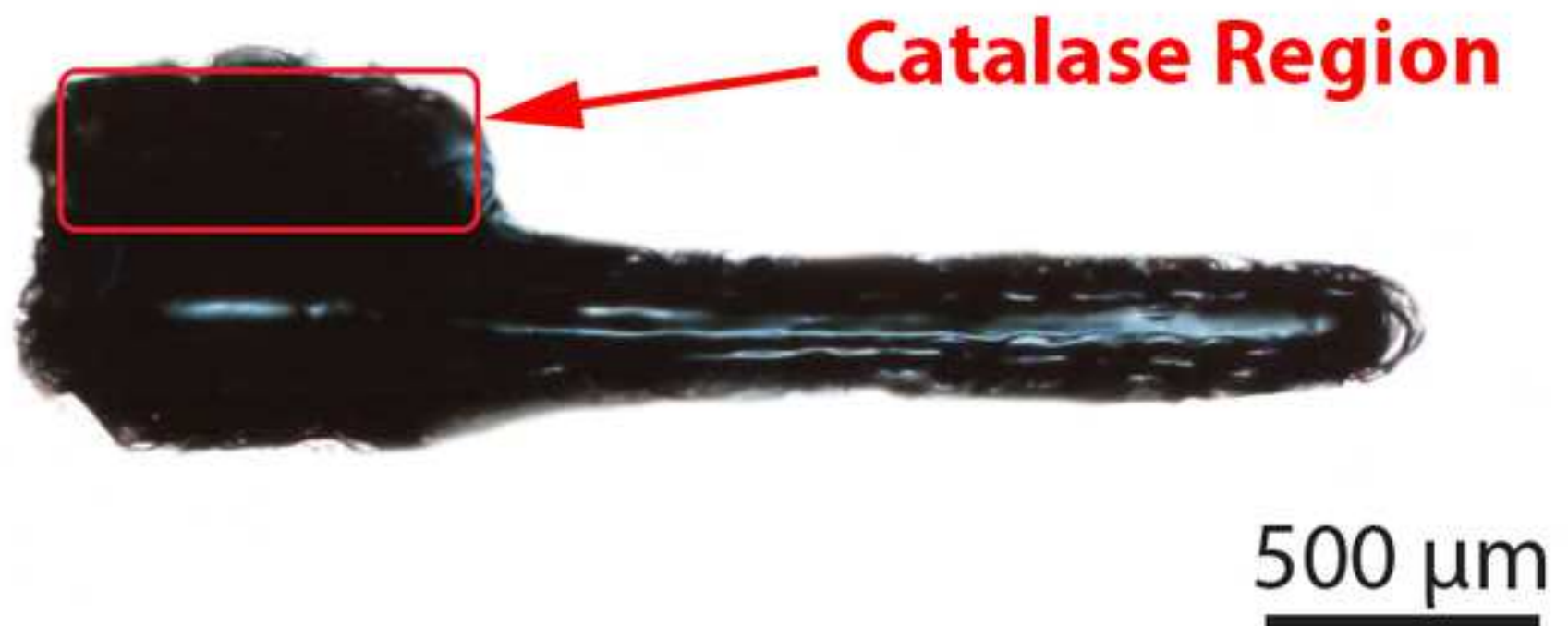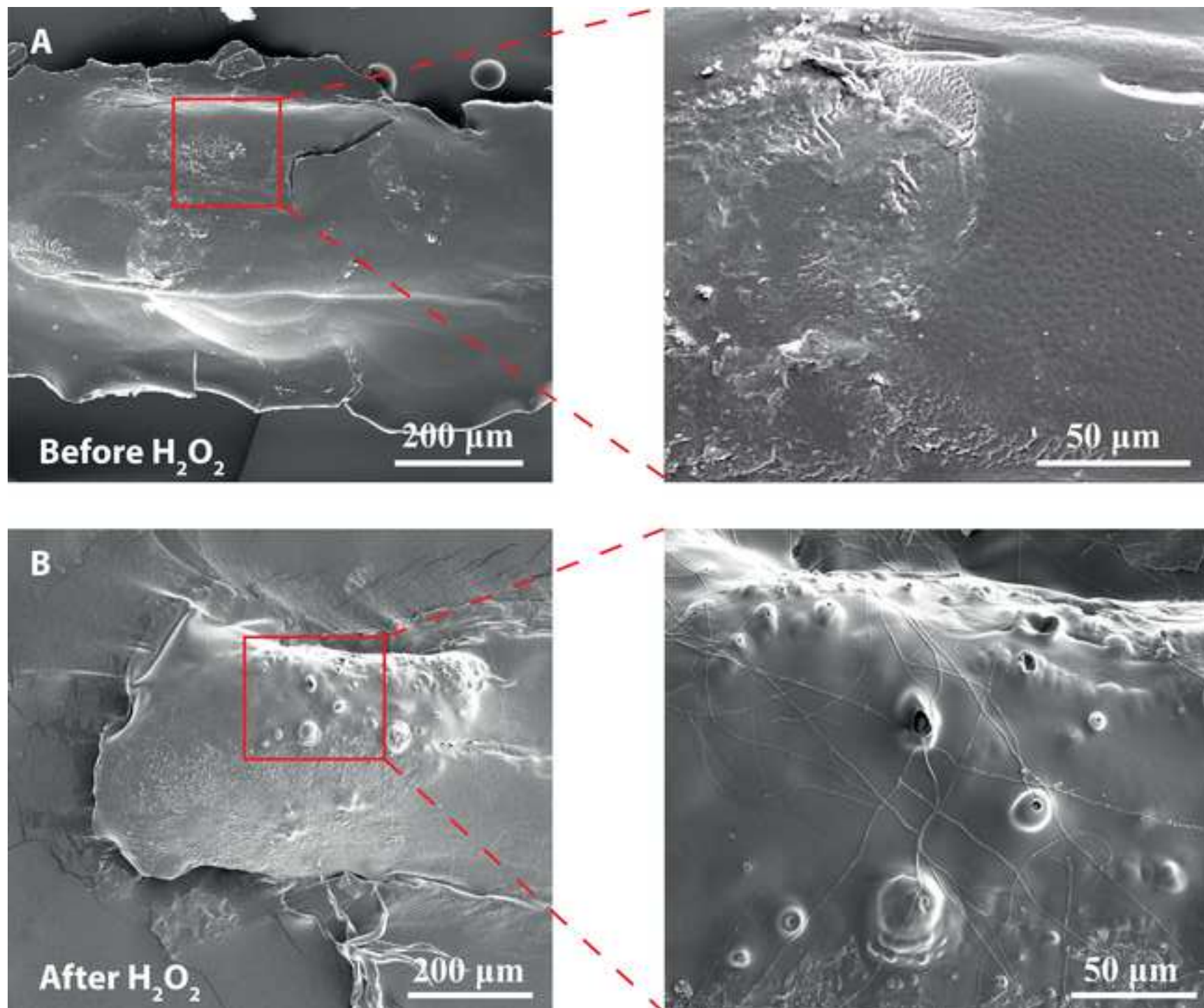
$$2H_2O_2 \qquad 2H_2O + O_2$$

Motion

Figure 2

Figure 3

A   C   φ   B

Motion direction

φ

Tracking points

Figure 4

Catalase Region

500 μm

Figure 5

A

Before H$_2$O$_2$

200 μm

50 μm

B

After H$_2$O$_2$

200 μm

50 μm

Figure 6

Figure 7

100 Layers

200 Layers

Video S1
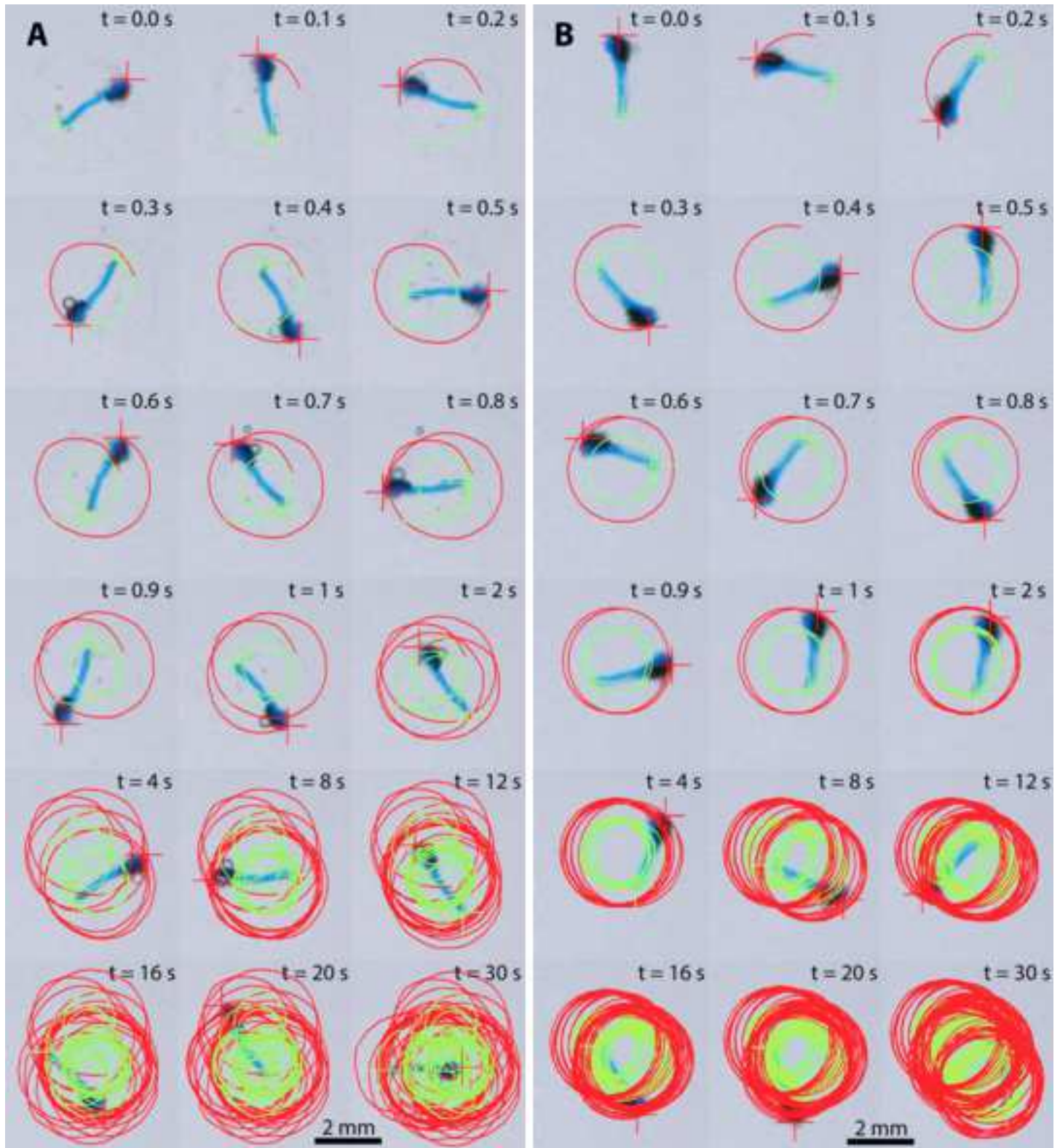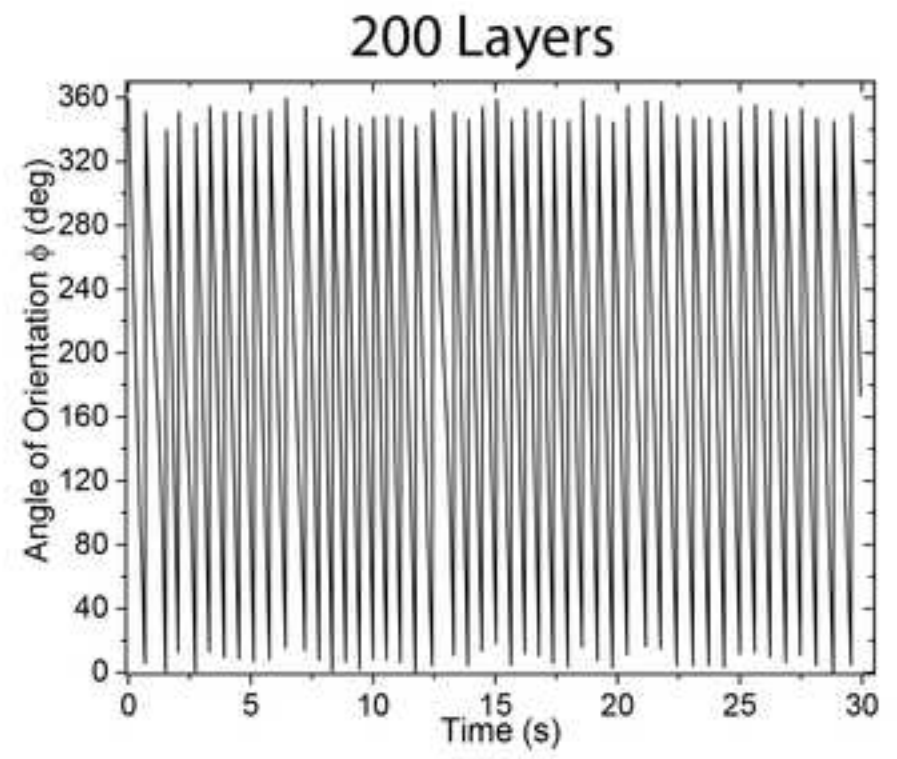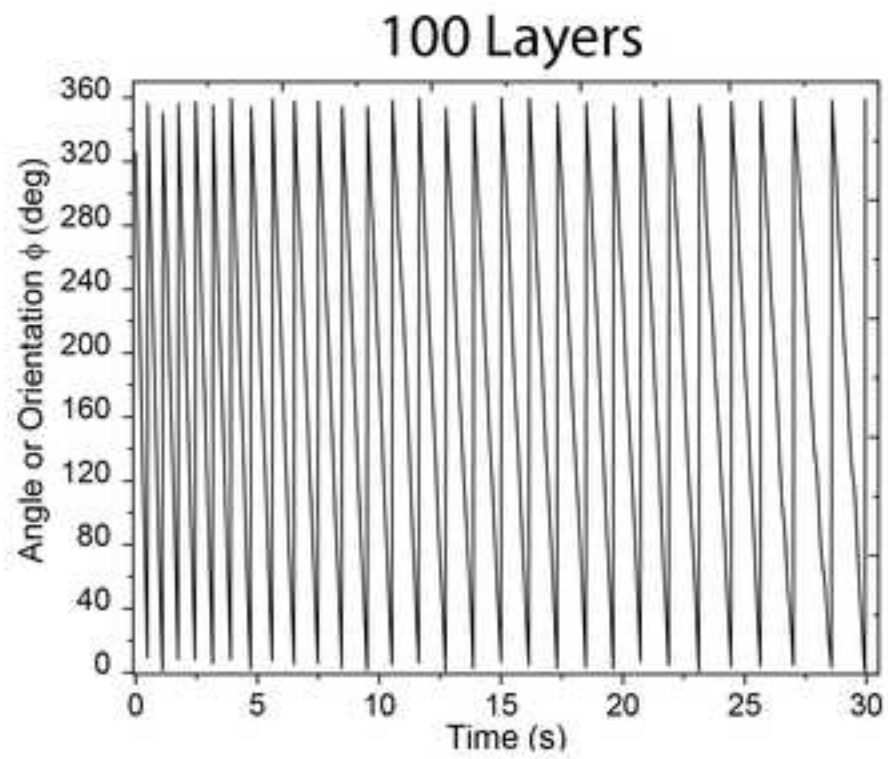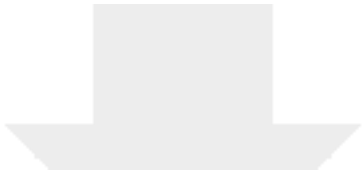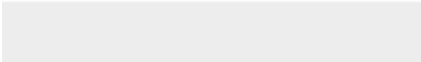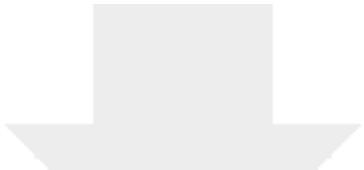
Click here to access/download
Video or Animated Figure
Video 1 (100 Layers).mp4

Video S2

Click here to access/download
Video or Animated Figure
Video 2 (200 Layers).mp4

Table of Materials
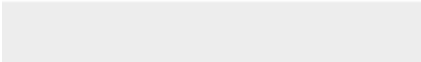
Name of Material/ Equipment	Company	Catalogue Number
Sodium Carbonate	Alfa Aesar	11552
Calcium Chloride	Fluka Analytical	C1016
Ethanol	Fisher Scientific	10542382
PEG-400	Aldrich Chemistry	202398
Catalase	Sigma Life Science	E3289
Methanol	Acros Organics	268280025
Hydrogen Peroxide	Sigma Aldrich	31642
Silk	Southwest University, China	NA
Dialysis Tubes	Sigma Aldrich	D9777
Fisherbrand Hoffman Clips	Fisher Scientific	12744396
Si-Wafer	Sigma Aldrich	647535
Balance	OHAUS Pioneer	PA214C
Conductivity meter	Mettler Toledo	FG3
Centrifuge	Thermo Scientific	10355052
Hotplate	Stuart	US152
Camera	PixeLink	PL-D732CU-T
Lens	Navitar	Navitar 1-60135
Jetting Devices	Microfab Technologies Inc.	MJ-AT-01-40-8MX MJ-AT-01-80-8MX
Lightpad	AGPTEK	UN-HL0245-EUUN
Pipettors	Eppendorf	3123000063 3123000055
Microscope	Nikon	LV100ND
SEM	Fei	F50

Comments/Description

anhydrous, 99.5%, granular
anhydrous, >93%, granular
HPLC grade
average Mn 400, tetramer mol wt ~250 kDa
>20K units
HPLC grade

30% (w/w)
Raw Cleaned Silk Cocoons, Bombox Mori
Cellulose, avg, flat width 25 mm, Typical
molecular weight cut-off = 14000

Clips used to close the ends of the dialysis tubes

Used as printing substrate

Analytical Balance
Mettler Toledo FiveGo Portable conductivity
meter

Heraeus Biofuge fresco sold by Thermo Scientific
Stuart US152 Magnetic Stirrer
High Speed Colour Camera
Macro Zoom Lens
80um nozzle diameter Jetting device
80um nozzle diameter Jetting device
Light for the swimming experiment
single-channel, variable, 100 – 1,000 µL, blue
single-channel, variable, 20 – 200 µL, yellow
Manual, upright microscope
Used for Scanning electron micrographs

jove
JOURNAL OF
VISUALIZED EXPERIMENTS

1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT - UK

Title of Article:	Reactive Inkjet Printing and Propulsion Analysis of Silk-Based Self-Propelled Micro-Stirrers
Author(s):	David Alexander Gregory, Piyush Kumar, Ana Jimenez-Franco, Yi Zhang, Yu Zhang Steve J. Ebbens, Xiubo Zhao

Item 1: The Author elects to have the Materials be made available (as described at http://www.jove.com/publish) via:

☐ Standard Access   ☑ Open Access

Item 2: Please select one of the following items:

☑ The Author is **NOT** a United States government employee.

☐ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

ARTICLE AND VIDEO LICENSE AGREEMENT

1.   **Defined Terms.** As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution 3.0 Agreement (also known as CC-BY), the terms and conditions of which can be found at: http://creativecommons.org/licenses/by/3.0/us/legalcode ; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of The Journal of Visualized Experiments; "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion

of the Article, and in which the Author may or may not appear.

2.   **Background.** The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3.   **Grant of Rights in Article.** In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4 and 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

612542.6   For questions, please contact us at submissions@jove.com or +1.617.945.9051.

4. **Retention of Rights in Article.** Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. **Grant of Rights in Video - Standard Access.** This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. **Grant of Rights in Video - Open Access.** This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats.

7. **Government Employees.** If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. **Protection of the work.** The Author(s) authorize JoVE to take steps in the Author(s) name and on their behalf if JoVE believes some third party could be infringing or might infringe the copyright of either the Author's Article and/or Video.

9. **Likeness, Privacy, Personality.** The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

10. **Author Warranties**. The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

11. **JoVE Discretion.** If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole

![jove logo] 1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

ARTICLE AND VIDEO LICENSE AGREEMENT - UK

discretion andwithout giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

12.   **Indemnification.** The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in JoVE or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contaminationdue to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or
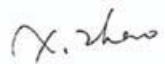
decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

13.   **Fees.** To cover the cost incurred for publication, Jo VE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

14.   **Transfer, Governing Law.** This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement is required per submission.

CORRESPONDING AUTHOR

Name:
| Xiubo Zhao |

Department:
| Department of Chemical and Biological Engineering |

Institution:
| University of Sheffield |

Title:
| Lecturer |

Signature: | *X. Zhao* | Date: | 30.08.2018 |

Please submit a **signed** and **dated** copy of this license by one of the following three methods:
1. Upload an electronic version on the JoVE submission site
2. Fax the document to +1.866.381.2236
3. Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02140

Rebuttal Document Rev. 2

Dear Dr Cao we would like to thank you for your comments and hope these revisions will meet your approval. Kind Regards

David

Editorial comments:
1. The editor has formatted the manuscript to match the journal's style. Please retain the same.
2. Please convert centrifuge speeds to centrifugal force (x g) instead of using rpm (see line 192).

This has now be altered to 16000 g

3. Please upload the printing and tracking program files to your editorial manager account as Supplementary Coding Files.

Dear Dr Cao,

As this protocol really focuses on the experimental and not software, we have altered this accordingly. We have added an extra figure to explain the calculations and added an equation. This should help illustrate how to go about the whole process. We hope this revision is acceptable.

We are not able to publicly share our home-built software due to IP restrictions however we have happy to include a contact where interested parties can directly contact us for tracking software if interested in collaborations.

4. Please address specific comments marked in the attached manuscript.

The comments have been edited accordingly:

Editor: I referenced Figure 1 here, because otherwise Figure 2 is introduced before Figure 1. Please confirm if it is acceptable or update the figure numbers.

This is fine.

Editor: Please convert centrifuge speeds to centrifugal force (**x g**) instead of revolutions per minute (rpm).

13000 rpm has been altered to 16000 g

Editor: Please specify the substrate used. Si-wafer substrate?

This is correct

Editor: Please specify the parameters adjusted.

Adjust the jetting parameters (rise time 1, dwell time, fall time, eco time, rise time 2, idle voltage, dwell voltage, echo voltage)

Editor: Is this set in the program?

WE have added a note and additional files to illustrate the design of the SPMSs

Note: 2 example designs for the 4 stages have been added to the supporting information; SPMS Main Body.xlsx (used for Stage 1 and 2) and SPMS Engine.xlsx (used for Stage 3 and 4)

Editor: Can this be combined with section 7?

This has been combined with section 7:

Editor: Please write the text in the imperative tense or include as a Note.

This has been included as a note:

NOTE: The catalase engine is located on the side of one end of each stirrer. Thus, the stirrers have one catalytic engine (see **Figure 1** red region).

Editor: These belong to the Results section. Please consider moving them to the Results section.

Has been moved and edited to suit the results section:

Figure 6 shows still video frames of two representative, 100-layer (**Figure 6A**) and 200-layer (**Figure 6B**) micro-stirrers in 5% H_2O_2 fuel. The red and green lines indicate the trajectories tracked (see **Supplemental Videos S1** and **S2**). The rotational velocity can be determined by the rate of change of orientation (ϕ, **Figure 3**) as shown in **Figure 7.** Comparison of 100-layer and 200-layer catalase doped micro-stirrers shows a distinctive increase in rotational velocity of ~0.6 fold from 60 ± 6 rpm to 100 ± 10 rpm (**Figure 7**).

Editor: Please rephrase this statement to be more clear.

Has been rephrased for clarity:

Figure 5: SEM images of the main body and catalase engine part of a SPMS **after pores are formed due to bubble release. Pores can be clearly seen on the engine surface in the SEM images of the** SPMSs originating from the oxygen bubble release**. (A)** Silk micro stirrers before exposure to 5% w/v

20   Zhang, Y. et al. Reactive Inkjet Printing of Functional Silk Stirrers for Enhanced Mixing and Sensing. Small. doi:10.1002/smll.201804213 1804213, (2018).

The following section has been adjusted to address the initial editorial comment 3.

1.1.   Perform tracking of the micro stirrers on a frame by frame basis, tracking each end of the stirrers as indicated by points A and B in (**Figure 3**).
      Note: This can be done manually or with the aid of tracking software.

1.2.   From the obtained tracking data calculate the instantaneous velocity between two consecutive frames (e.g. 1 and 2) using Equation 1 and average the resulting velocities from the entire sequence to obtain the mean instantaneous velocity.

$$v_{inst} = \frac{\sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2}}{t_{frame}}$$

Eq. (1)

NOTE: When calculating instantaneous velocities from tracked image data it is important that the initial image of an object with known dimensions is taken to be able to calculate correct pixel to um values. These values will depend on the camera, objective and distance used. Depending on your type of printed particle you might want to choose different tracking points for calculating the velocity, e.g. here tracking points A, B and C (centre of mass) were all used to determine instantaneous velocities. (**Figure 3**)

2.   Further to this, calculate the angle of orientation ϕ, the rate of change of orientation is then used to determine the rotational velocity. (**Figure 3**)

An extra figure has been added and the other figures renumbered.

Figure 3: Schematic representation of tracking the particle over two consecutive frames, A and B indicate tracking points C indicates the centre of mass. Φ indicated the angle of orientation. Motion direction is indicated by the curved black arrow.

Click here to access/download
Supplemental Coding Files
SPMS Main Body.xlsx

Click here to access/download

Supplemental Coding Files

SPMS Engine.xlsx