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.

Added

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% H2O2 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 (ɸ, **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 H2O2 fuel solution. (**B**) Silk SPMS after exposure to 5% w/v H2O2 fuel solution. Images on the right are enlargements of the red regions.

Editor: Please note that figure legends are slightly edited to include a title for each figure. Please review to ensure that they are correct.

They seem OK.

Please note:

Reference 20 has been updated as published a few days ago.

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. 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. 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.

|  |  |  |
| --- | --- | --- |
|  |  | 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**)

1. 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.

