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## Towards better characterisation and quantification of emulsification of silicone oil in vitro

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Complete List of Authors:	Chan, Yau Kei; University of Hong Kong, Department of Mechanical Engineering Czanner, Gabriela; University of Liverpool, Department of Biotatistics Shum, Ho Cheung; University of Hong Kong, Department of Mechanical Engineering Williams, Rachel; University of Liverpool, Department of Eye and Vision Science Cheung, Ning; National University of Singapore, Singapore Eye Research Institute Wong, David; Royal Liverpool University Hospital, St Paul's Eye Unit
Keywords:	silicone oil, emulsification, Coulter counter, laser light scattering

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2  
3 *Authors:*  
4

5 <sup>1,2</sup>Yau Kei Chan, <sup>3,4</sup>Gabriela Czanner, <sup>1</sup>Ho Cheung Shum, <sup>3</sup>Rachel L. Williams, <sup>5,6</sup>Ning  
6 Cheung and <sup>2,7</sup>David Wong  
7  
8  
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10  
11 *Affiliations:*  
12

13 <sup>1</sup>Department of Mechanical Engineering, Faculty of Engineering, University of Hong  
14 Kong  
15

16  
17 <sup>2</sup>Department of Ophthalmology, Li Ka Shing Faculty of Medicine, University of Hong  
18 Kong, Pokfulam, Hong Kong  
19

20  
21 <sup>3</sup>Department of Eye and Vision Science, University of Liverpool, Liverpool, United  
22 Kingdom  
23

24  
25 <sup>4</sup>Department of Biotatistics, University of Liverpool, Liverpool, United Kingdom  
26

27  
28 <sup>5</sup>Singapore Eye Research Institute, National University of Singapore, Singapore  
29

30  
31 <sup>6</sup>Centre for Eye Research Australia, University of Melbourne, Australia  
32

33  
34 <sup>7</sup>St Paul's Eye Unit, Royal Liverpool University Hospital, United Kingdom  
35

36 ***Title: Towards better characterisation and quantification of emulsification of***  
37 ***silicone oil in vitro***  
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39  
40  
41 *Corresponding Author:*  
42

43 Prof. David Wong  
44

45 St Paul's Eye Unit, 2z link, Royal Liverpool University Hospital, Prescot Street, Liverpool  
46 L7 8XP, [shdwong@gmail.com](mailto:shdwong@gmail.com), +44 79400186422  
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3 Purpose: Emulsification is related to complications arising from silicone oil (SO)  
4 tamponade. Currently there is no widely accepted method for testing the propensity of  
5 SO to emulsify that are physiologically realistic and quantitative.  
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11 Methods: We compared different ways of inducing emulsification namely vortex mixing,  
12 sonication and homogenisation. SO emulsification was quantitatively assessed using  
13 the Coulter counter and laser light scattering. The *in vitro* results are compared with the  
14 droplet size distribution profile of vitreous clinical washout. Conventional SO was  
15 compared with two novel SO blends with high-molecular-weight (HMW) additives  
16 (SO<sub>HMW2000</sub> and SO<sub>HMW5000</sub>).  
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26 Results: Of the three methods for inducing emulsification, homogenisation generated  
27 the most consistent emulsion samples with the smallest variance. The results from the  
28 Coulter counter measurement correlated strongly with the laser light scattering  
29 measurement within the range of 1 to 30 microns. The droplet size distribution profiles  
30 from human eyes were similar to that of emulsions generated *in vitro* by homogenisation.  
31  
32 The human size distribution profile were within the range of values obtained by the *in*  
33 *vitro* experiment. Compared to the conventional SO, the emulsion droplet counts for the  
34 new SO blends were significantly lower (SO<sub>HMW2000</sub> and SO<sub>HMW5000</sub> were 79% ( $\pm 17\%$ )  
35 and 49% ( $\pm 18\%$ ) of the SO<sub>2000</sub> and SO<sub>5000</sub> respectively;  $p = 0.03$  and  $p = 0.002$ ).  
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48 Conclusion: Emulsion generated *in vitro* by homogenisation has similar droplet size  
49 profile as human eyes filled with SO. Using this method to induce emulsion, SO blends  
50 with HMW additives demonstrated less propensity to emulsification with lower droplet  
51 counts compared to conventional SO with similar shear viscosity.  
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3 *Key words:* Silicone oil, Emulsification, Coulter counter, Laser light scattering  
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## 8 9 **Introduction**

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12 Silicone oil (SO) is widely accepted as a long-term tamponade, despite many known  
13 complications such as glaucoma (Honavar et al. 1999), inflammation (Theelen et al.  
14 2004), peri-oil proliferation (Lewis et al. 1988) and re-detachment (Falkner et al. 2001)  
15 after oil removal. Some of these complications are thought to be directly related to  
16 emulsification (Baino 2011). In recent years, attempts have been made to design new  
17 SO that is more resistant to emulsification (Williams et al. 2010). The concept is that  
18 adding a small amount of high molecular weight (2.5 million cSt, 423 kD) to  
19 conventional SO (1000 cSt, 37 kD) will increase not only the shear viscosity, but  
20 importantly the extensional viscosity thereby reducing the tendency to emulsify  
21 (Williams et al. 2010). Clinical comparison of the resistance between SO is fraught with  
22 difficulties. There are many patient dependent factors such as the degree of  
23 inflammation and the extent of blood-ocular-barrier breakdown that may influence the  
24 availability of surfactants in the eye. Emulsification depends on shear stresses applied  
25 on the SO bubble generated by eye movement (Chan et al. 2011). Using a model eye  
26 chamber simulating saccadic movements, we showed that surgical confounding factors  
27 such as the extent of fill and the presence of scleral indents might all influence SO  
28 emulsification (Chan et al. 2014). Individual patient and surgical confounding factors  
29 combine to make it impractical to carry out randomised clinical trials on every new  
30 proposed silicone oil. Potentially, in the near future there might be many silicone oils  
31 being introduced. If adding high molecular weight (HMW) components was effective in  
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3 reducing emulsification, then different conventional SO could be combined with different  
4 concentrations of HMW components to produce new oils with clinically useful properties.  
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6 Clearly, all other silicone based oils might also benefit from this modification and this  
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8 would include fluorinated SO and the heavy SO (Chan et al. 2014) which are mixtures  
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10 of SO with semi-fluorinated alkanes or alkenes. Consequently, in our opinion, there is  
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12 an important role for a standardised *in vitro* test for emulsification to screen new oils  
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14 before proceeding to clinical evaluations (Scott et al. 2005).  
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19 Previous experiments used various mechanical agitations to induce emulsification that  
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21 might not be physiological. (Savion et al. 1996; de Silva et al. 2005) The mechanical  
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23 force causing emulsification in the eye is mainly shear stress related to eye movements.  
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25 Shear stress acting on the liquid is the product of the viscosity of the liquid and the  
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27 shear rate. However, the mechanical agitation of both sonication and vortex mixing  
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29 might not provide constant shear rate to the silicone oil to cause emulsification. There is  
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31 a need to look for another method of mechanical agitation with constant shear rate for  
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33 the purpose of studying SO emulsification, that better mimics the conditions in the  
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35 human eye. In the past, we have used a SO-filled model eye chamber to study the  
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37 effect of eye movements and we estimated the maximum shear rate from stereotypical  
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39 saccades (Chan et al. 2011). If a standardised method of testing emulsification were to  
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41 be developed, ideally, it should take into account the physiological conditions that cause  
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43 emulsification to occur in the human eye (Chan et al. 2015).  
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50 We have recently used a Coulter counter to quantify emulsions removed from patients  
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52 (Chan et al. 2015). The majority of the droplets were too small to be observed even with  
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54 slit lamp bio-microscopy. The Coulter counter could accurately measure particles down  
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3 to 1  $\mu\text{m}$ . The peak of the particle size distribution profile could however not be obtained.

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5 We therefore did not know how many droplets there were smaller than 1  $\mu\text{m}$ . There is  
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7 still a need to find a way to characterise the size distribution profile more completely.

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9 This study aims to achieve two objectives: firstly, to find a consistent method of  
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11 mimicking emulsification in human eyes and secondly, to achieve a more  
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13 comprehensive quantification of SO droplets size and number.  
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## 16 17 18 19 20 **Materials and Methods**

### 21 22 Materials

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24 Five types of SO were used in this study. The shear viscosities of the SO are listed in  
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26 Table 1. SO<sub>1300</sub> was used to characterize the reproducibility of oil-in-water emulsions  
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28 generated by using different agitation methods. SO<sub>HMW2000</sub> and SO<sub>HMW5000</sub> were blends  
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30 of SO 1000 cSt with 5% and 10% of the high-molecular-weight (HMW) additive (423kD  
31  
32 polydimethylsiloxane) respectively. The shear viscosities of SO<sub>HMW2000</sub> and SO<sub>HMW5000</sub>  
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34 were around 2000 and 5000 cSt respectively. Conventional SO 1000 and 5000 cSt  
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36 were blended (at a ratio of 55% to 45%) to make a SO of 2000 cSt. We named this  
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38 blend SO<sub>2000</sub>. SO<sub>2000</sub> was compared with SO<sub>HMW2000</sub>. Similarly, conventional 5000 cSt oil  
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40 (SO<sub>5000</sub>) was used as the control oil and compared with SO<sub>HMW5000</sub>. SO<sub>1300</sub> (Arciolane  
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42 1300) was purchased from ARCAD, France. Apart from SO<sub>1300</sub>, all the other SO  
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44 samples were kindly donated by Fluoron GmbH, Germany.  
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### 51 52 Homogenisation

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3 The homogeniser (T10 Basic Ultra Turrax®, IKA®, Germany) along with the dispersing  
4 element was used to disperse the five types of SO (SO<sub>1300</sub> SO<sub>2000</sub>, SO<sub>HMW2000</sub>, SO<sub>5000</sub>  
5 and SO<sub>HMW5000</sub>) and generate oil-in-water emulsion under a controlled shear rate for 1  
6 minute. Two percent Pluronic® F68 (Life Technologies, USA) in phosphate buffered  
7 saline (PBS) was used as the aqueous phase. (Caramoy et al. 2010; Williams et al.  
8 2010; Caramoy et al. 2011; Caramoy et al. 2015) The volume ratio of SO<sub>1300</sub> to aqueous  
9 was 1:99. The small volume of SO to aqueous was intended to make sure that the SO  
10 phase was exhaustively dispersed to allow the fair comparison between various SO  
11 agents. The sample size of the emulsions for each SO agent was 6.  
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#### 25 Vortex mixing

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28 The SO<sub>1300</sub> sample and the same volume of 2% Pluronic® F68 in PBS were added into  
29 a glass syringe. The oil/aqueous ratio (1:1) was accordance with a previously published  
30 study (Savion et al. 1996). The glass syringes were mounted on a vortex machine  
31 (Vortex-Genie 2, Scientific Industries, Inc., USA). The syringes were subjected to the  
32 highest speed of vortex mixing for 3 hours. The sample size of the emulsions for each  
33 SO agent was 8.  
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#### 43 Sonication

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46 The SO<sub>1300</sub> sample and the same volume of 2% Pluronic® F68 in PBS were added into  
47 a glass syringe. The oil/aqueous ratio (1:1) was in accordance with previously published  
48 studies (Caramoy et al. 2011; Caramoy et al. 2015). The glass syringe was immersed in  
49 an ultrasound water bath (2510DTH, Branson) and subjected to sonication for 1  
50 minute. The sample size of the emulsions for SO<sub>1300</sub> was 8.  
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### Human washout samples

We have previously published the results of using the Coulter counter to measure the size distribution profile of washings from patients collected during removal of oil (Chan et al. 2015). Briefly, after silicone oil was removed, a fluid air exchange was carried out and the fluid collected for analysis. There were 8 patients studied, 5 had 5000 cSt oil and 4 had 1300 cSt oil. These data were used to compare with that of emulsification generated by *in vitro* methods. The ethical committee from the Royal Liverpool Hospital granted us permission to study the washings from the patients.

### Particle measurement by Coulter counter

The emulsion samples generated by various methods of agitation from different SO were analysed using the Coulter counter (Multisizer® 4, Beckman Coulter, USA). In this study, the measuring probe with a 50µm aperture hole was used to provide a measurement range from 1µm to 30µm. The Coulter counter adopts the electrical zone sensing method of Coulter's principle (Edmundson 1966), which measures the size of non-conducting particles suspended in a fluid. The particle counter provided both number and size of particles suspended in the tested sample. The particle count of each sample presented herein was a mean value of 10 consecutive measurements.

### Particle measurement by laser light scattering method

The four SO emulsion samples (including SO<sub>2000</sub>, SO<sub>HMW2000</sub>, SO<sub>5000</sub> and SO<sub>HMW5000</sub>) generated by the homogeniser were also analysed using the laser light scattering method (Mastersizer 2000, Malvern, England). The method adopts the principle of laser light scattering and measures the size of particles in the suspension using laser



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3 diffraction (Sperazza et al. 2004). It covers the measurement range for the particle  
4 analysis from 0.02 to 2000  $\mu\text{m}$  in diameter and provides information on the particle size  
5 distribution within the suspension. The size distribution measurement for each sample  
6 presented herein was a mean value of 10 consecutive measurements.  
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13 Estimation of the size distribution of the *in vitro* SO emulsified droplets by extrapolating  
14 the measurements from Coulter counter to that of the laser light scattering method  
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19 The Coulter counter measurement provided the absolute number count of the SO  
20 droplets in the emulsion samples in the size range 1 and 30  $\mu\text{m}$  in diameter. The  
21 measurement using the laser light scattering method gave relative numbers and  
22 provided an overall percentage size distribution profile of the SO droplets in the  
23 emulsion samples in the size range 0.02 to 2000  $\mu\text{m}$  in diameter. The two measurement  
24 methods overlapped in the 1 and 30  $\mu\text{m}$  size range. The agreement of the two methods  
25 within this range was analysed.  
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37 Statistical Method  
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40 The Coulter counting method gave absolute numbers of droplets for every size interval  
41 whilst the laser light scattering gave relative numbers. The outputs from both the  
42 measurements were a droplet size distribution frequency table. The frequency was then  
43 expressed as a percentage of the total for a given size interval. (The number of droplets  
44 within a given size range divided by total number of droplets). We used the frequency  
45 tables to calculate variance and standard deviation in order to measure the spread of  
46 the data.  
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3 By using the Weibull nonlinear regression model as the mathematical model, the  
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5 outputs from the Coulter counter and laser light scattering were correlated between 1-30  
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7  $\mu\text{m}$ , in terms of relative numbers. After the best fitting model was found, we used the  
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9 model to examine the correlations and then used it to extrapolate the values from  
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11 Coulter counter (in %) for ranges 0 to 1  $\mu\text{m}$ . All calculations were done in software  
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13 Minitab 17, the models were fitted with Gauss-Newton optimisation algorithm.  
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18 Statistical significance between the differences of the emulsification of SO was  
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20 assessed using the Mann Whitney statistical test. The  $p$ -value  $< 0.05$  was considered to  
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22 be statistically significant.  
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## 26 27 28 **Results**

### 29 30 31 Methods of generating SO emulsion as measured by the Coulter counter

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34 Homogenisation yielded consistent results between the 6 samples. In contrast, both  
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36 vortex mixing and sonication generated oil-in-water emulsion without such consistent  
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38 result in terms of droplet count. Sonication in particular yielded very variable results.  
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41 (Table 2)  
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### 48 49 Size distribution of SO droplets generated by homogenisation measured by Coulter 50 51 counting and laser light scattering methods

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54 In Coulter counting measurement, the droplet size distribution profiles were similar for  
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56 emulsions from  $\text{SO}_{2000}$  and  $\text{SO}_{\text{HMW}2000}$  (Fig. 1a) and from  $\text{SO}_{\text{HMW}5000}$  and  $\text{SO}_{5000}$  (Fig. 1b).  
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3 Similarly, in laser light scattering measurement the droplet size distribution profiles of  
4 emulsions were similar between SO<sub>2000</sub> and SO<sub>HMW2000</sub> (Fig. 2a) as well as between  
5 emulsions from SO<sub>HMW5000</sub> and SO<sub>5000</sub> (Fig. 2b). Besides the similarity of the size  
6 distribution profiles, using laser light scattering methods, it was found that the smallest  
7 size of the droplets detected was between 0.63 – 0.71µm with a peak located in the  
8 range between 0.71 – 0.80µm. (Fig. 2)  
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### 22 Statistical modelling

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24 We plotted the percentages of one method against the other and explored their  
25 relationships with Weibull nonlinear regression model. (Fig. 3) It was found that the two  
26 results were highly correlated with each other under this model (as indicated by the red  
27 lines in Figure 3, R<sup>2</sup> = 0.978, 0.956, 0.986 and 0.978 respectively).  
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### 37 In vivo emulsion vs in vitro emulsion

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39 It was found that the droplet size distribution profile of *in vitro* emulsion generated by  
40 homogenisation was positioned as an outer envelope (Blue line) around the profile of  
41 the *in vivo* emulsion. (Fig. 4) This situation was present in both the SO groups (SO<sub>1300</sub>  
42 and SO<sub>5000</sub>). In other words, the individual *in vitro* profiles coincided with the maximum  
43 values of the individual *in vivo* profiles for the range of 1.5 µm and greater. All in all, the  
44 profiles of the *in vitro* samples were within the 2 S.D. of the profiles of the *in vivo*  
45 samples.  
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3 Estimated total droplets number by extrapolation (combining the results from the Coulter  
4 counter and laser light scattering)  
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9 Since the two methods strongly correlated to one another based on Weibull relationship,  
10 it was justifiable to extrapolate the data in order to determine the number of droplets  
11 smaller than 1 micron. The number of droplets between 0.5 and 1 micron were  
12 determined for the different oils using the Weibull model. It can be seen that the  
13 distribution profiles of the 4 different oils tested were similar. (Fig. 5)  
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24 Comparing conventional silicone oil with those with HMW additives (Coulter counting)  
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28 Two pairs of SO ( $SO_{2000}$  and  $SO_{HMW2000}$ ;  $SO_{5000}$  and  $SO_{HMW5000}$ ) with similar shear  
29 viscosities were tested. The emulsion generated from  $SO_{HMW2000}$  ( $16485 \pm 2806$ ) had a  
30 significantly lower droplet count than the emulsion from  $SO_{2000}$  ( $20777 \pm 3028$ ). ( $p = 0.03$ )  
31 (Fig. 6a). The total droplet count from emulsion  $SO_{HMW2000}$  was 79% ( $\pm 17\%$ ) of that of  
32  $SO_{2000}$ . Likewise,  $SO_{HMW5000}$  ( $28054 \pm 5168$ ) had a significantly lower droplet count than  
33  $SO_{5000}$  ( $58536 \pm 7473$ ). ( $p = 0.002$ ) (Fig. 6b) The total droplet count for  $SO_{HMW5000}$  was  
34 49% ( $\pm 18\%$ ) of that for  $SO_{5000}$ .  
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46 **Discussion**  
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48 Until recently, the only way of preventing emulsification is to choose highly purified SO  
49 with higher viscosities (Nakamura et al. 1990). The advent of small gauge surgery in  
50 recent years have provided impetus for choosing less viscous SO, simply because they  
51 are easier to inject and remove through smaller cannulas. New SO with HMW additives  
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3 claimed to be both more emulsification resistant and easier to inject and remove  
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5 (Williams et al. 2010) and this assertion was corroborated *in vitro* (Caramoy et al. 2011).  
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8 Nonetheless, others have observed early emulsification *in vivo* when these new SO  
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10 were used (Maier et al. 2011). There is therefore, justification to look again into whether  
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12 the claims for emulsification resistance could be substantiated.  
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16 The current evidence of laboratory-based experiments might be flawed. There are  
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18 several aspects to consider. Firstly, the method of generating emulsification has to be  
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20 valid. Mixing SO with water could potentially generate either oil-in-water or water-in-oil  
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22 emulsion. In the published images of a previous study using sonication, the droplets  
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24 were in the bottom of the oil phase suggesting that the droplets might be water-in-oil  
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26 (Caramoy et al. 2010). This result might be irrelevant clinically because the droplets  
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28 seen in patients are oil-in-water droplets. Secondly, the methods of generating  
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30 emulsification should yield consistent results. Our results showed that the methods of  
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32 vortex mixing and sonication were inconsistent. (Table 2) The same SO produced  
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34 widely varying results in terms of droplet numbers under identical conditions. Thirdly,  
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36 the SO should be dispersed totally in the aqueous phase. Whether the method of  
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38 generating emulsion is exhaustive will certainly affect the total number and the size of  
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40 the SO droplets. SO with a higher resistance to emulsification in theory should produce  
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42 droplets that are larger in size and fewer in number and *vice versa*. We believe that the  
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44 results using vortex mixing and sonication were inconsistent mainly because too much  
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46 oil was used (oil to water at the ratio of 1:1 in accordance to previous publications). At  
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48 the end of the agitation, there were clearly two phases seen with SO on top and  
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50 aqueous below. (Caramoy et al. 2011) In other words, not all the oil was dispersed.  
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3 From one experiment to another, the results using sonication and vortex might therefore  
4 reflect how much oil was successfully dispersed by the mechanical methods rather than  
5 measuring the resistance or readiness of the oil to emulsify. Homogenisation on the  
6 other hand is an established scientific method for generating emulsions. (Maa & Hsu  
7 1996) The use of oil to water in the ratio of 1 to 99 using homogenisation produced  
8 more consistent results. All the SO in each experiment was exhaustively emulsified  
9 leaving no bulk oil. We believe that this is the best way to make fair comparison the  
10 propensity of different SO to emulsify. The size distribution profiles of these *in vitro*  
11 emulsions generated by homogenization were similar to that of patients.  
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25 Emulsification is related to the presence of surfactants. In the eye, there are many  
26 surfactants present in the intraocular fluid which includes but not limited to different  
27 kinds of proteins, lipids and phospholipids.(Savion et al. 1996) We opted to use  
28 Pluronic® F68 as the model surfactant in this study because it is widely used as a  
29 standard in emulsification science. (Caramoy et al. 2010; Williams et al. 2010; Caramoy  
30 et al. 2011; Caramoy et al. 2015) Pluronic® F68 lowers the interfacial tension between  
31 SO and aqueous phase as effective as any surfactant that are present in the eye. It is  
32 important when comparing like with like that the ability to emulsify be standardised in  
33 concentration and effectiveness.  
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46 To date, the published results on resistance of the new SO with HMW additives relied  
47 on observation with the naked eye (Caramoy et al. 2011; Caramoy et al. 2015). We  
48 recently published quantification of washings from patients' eyes that had oil *in situ* for  
49 months using the Coulter counter (Chan et al. 2015). The majority of droplets were  
50 between 1-2  $\mu\text{m}$ . With slit-lamp biomicroscopy, we would not see discrete droplets or  
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3 cells less than 5  $\mu\text{m}$ , observing instead the scattering of light by these droplets as “flare”.  
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6 In this study, we also used the Coulter counter for droplet counting and sizing. Undiluted,  
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8 the concentration of emulsification in the aqueous might be high enough to be detected  
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10 by the laser light scattering method. However, the washings collected during SO  
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12 removal procedures were diluted by infusion fluids. Currently, it is technically difficult to  
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14 collect undiluted samples from the eye in sufficient quantity that would allow laser light  
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16 scattering to yield meaningful quantitative results.  
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20 Our study chose two methods to quantify droplet number. The Coulter counter allows a  
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22 precise particle counting of droplets. However, the Coulter counter has a lower limit of  
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24 measurement of 1  $\mu\text{m}$  in diameter. In our study we could not determine the peak of the  
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26 size distribution profile of the emulsion droplets, (Fig. 1) showing that there must be  
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28 droplets smaller than 1  $\mu\text{m}$  in diameter. Laser light scattering provides a very broad  
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30 range of sensitivity. The machine we used in the study (Mastersizer 2000) covered the  
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32 measurement range from 0.02 to 2000  $\mu\text{m}$  in diameter. This allowed us to obtain a more  
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34 complete size distribution profile of the emulsified droplets. (Fig. 2) Laser light scattering,  
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36 however, only gives relative not absolute numbers of droplets. The two methods of  
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38 measurements are based on different principles. The relationships have not been  
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40 previously explored mathematically. We were able to exploit the overlap in the range of  
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42 sizes (i.e. between 1 and 30  $\mu\text{m}$ ) to analyse the same sample. The graphs showed that  
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44 the correlation between the two methods to be very strong based on the Weibull  
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46 regression model. (Fig. 3) We could therefore justify extrapolating beyond the  
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48 measurement range of the Coulter counter based on the assumption that the Weibull  
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50 relationship we found would still hold if the Coulter counter was measurable in the range  
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3 between 0 and 1  $\mu\text{m}$ . We were particularly interested in the peak frequency of size  
4 distributions. Without the full characterisation of droplet distribution, we cannot  
5 confidently know if one emulsion compared to another had more or fewer droplets  
6 (Chan et al. 2015). After extrapolating, we could detect that the most numerous droplets  
7 were between 0.5 and 1 micron. (Fig. 3) There was a high degree of consistency  
8 between all SO tested as all plots have similar shapes. Using the statistical model to  
9 extrapolate below 1 micron revealed an important difference between the two methods.  
10 Figure 5 showed proportionately fewer droplets estimated by the calculation than the  
11 distribution indicated by laser light scattering method. This needs an explanation. We  
12 believe that this is due to one of the limitations of the Coulter Counter, which relies on  
13 the solutions to be suitably diluted. Otherwise, two or more droplets could pass through  
14 the aperture simultaneously and be counted as a single larger droplet. This known error  
15 is known as "coincidence". It seems that emulsification *in vitro* and in patients have  
16 many more small droplets. Coincidence is the likely explanation of why the Coulter  
17 Counter underestimated the number of small droplets. Coulter Counter is accurate over  
18 a narrower range, whilst laser scatter is widely acknowledged for being fast, accurate  
19 over a wider range.

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44 The high degree of correlation between the two methods suggested that using the  
45 Coulter counter alone without using the laser scatter method might be sufficient to  
46 reflect the overall propensity of an to emulsify. Significantly, though all 4 SO tested had  
47 very similar size distribution profiles the absolute numbers of droplets were different.  
48 Because the total volume oil emulsified was the same, the difference could only be  
49 accounted for by the more resistant oils having a few larger droplets and thus fewer  
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3 smaller droplets. This is in line with what was predicted if SO with HMW additives were  
4 more resistant to emulsification (Fig. 6).  
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9 There are limitations to our study. Firstly, with the use of homogeniser, we could only  
10 control the shear rate but not the shear stress. We know that the shear stress is a  
11 product of shear rate and shear viscosity. Therefore, with our experimental setup, we  
12 could have been applying up to five times more force to 5000 than to 1000 cSt. We  
13 were therefore, not able to compare SO with different shear viscosity using our  
14 experimental setup as direct comparison between the two would not be valid. **Secondly,**  
15 **the viscosity of SO is temperature dependent.(Romano et al. 2016) We have not**  
16 **measured the viscosity difference in between *in vitro* and *in vivo* environments though**  
17 **published figures indicate that the difference is small. Our *in vitro* experiments were**  
18 **carried out at room temperature whilst *in vivo* emulsification occurs at body**  
19 **temperature. However, we were careful to only compare the propensity of SO to**  
20 **emulsification *in vitro*. Therefore, the slight change of viscosity due to the temperature**  
21 **difference may not be so relevant. No direct comparison was made between *in vitro* and**  
22 ***in vivo* emulsification beyond the basic observation that the distribution profiles were**  
23 **similar.**  
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## 46 **Conclusion**

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48 In this study, we showed that homogenisation provided a consistent way to generate oil-  
49 in-water emulsion *in vitro*. We stressed the importance of exhaustively emulsifying the  
50 oils, ideally using principally only shear forces as this might be more physiological and  
51 yield size distribution similar to that in patients. Although the Coulter counter could only  
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3 count droplets within a narrow size range, the results correlated well with laser light  
4 scattering which has a widened range. The most numerous droplets seemed to be  
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6 between 0.5 and 1 micron in diameter. We also showed that SO with HMW additive  
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8 produced fewer emulsified droplets than conventional SO with similar shear viscosities.  
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## Legends

**Figure 1** Droplet size distributions of the emulsions from (a) SO<sub>2000</sub>, (b) SO<sub>HMW2000</sub>, (c) SO<sub>5000</sub> and (d) SO<sub>HMW5000</sub> generated by high shear homogenizer (measured by the Coulter counter)

**Figure 2** Droplet size distributions of the emulsions from (a) SO<sub>2000</sub> and SO<sub>HMW2000</sub> and (b) SO<sub>5000</sub> and SO<sub>HMW5000</sub> generated by high shear homogeniser (measured by the laser light scattering)

**Figure 3** The relationship between the measurements of Coulter counting and laser light scattering. CC, Coulter counting, LSC, Laser light scattering

**Figure 4** The size distribution profiles of emulsions from (a) SO<sub>1300</sub> and (b) SO<sub>5000</sub>. The red line and blue line indicate the mean size distribution profiles of *in vivo* and *in vitro* samples respectively.

**Figure 5** The extrapolation of the droplet size distribution profile for coulter counting (in %) for ranges between 0 and 1 µm.

**Figure 6** Droplet count of the emulsion from (a) SO<sub>HMW2000</sub> relative to SO<sub>2000</sub> and (b) SO<sub>HMW5000</sub> relative to SO<sub>5000</sub> generated by homogenisation (measured by the Coulter counter) (Mann Whitney test, \*; *p*-value < 0.05; \*\*, *p*-value < 0.01; n = 6)

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**Table 1 Shear viscosities of silicone oil samples (Chan et al. 2011)**

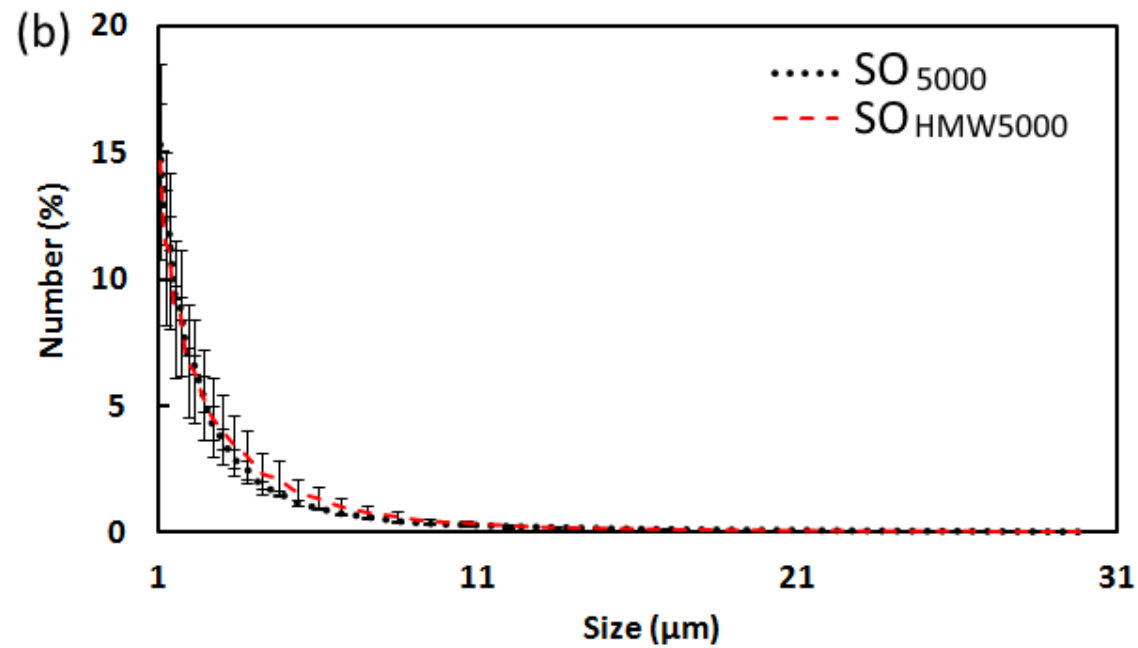
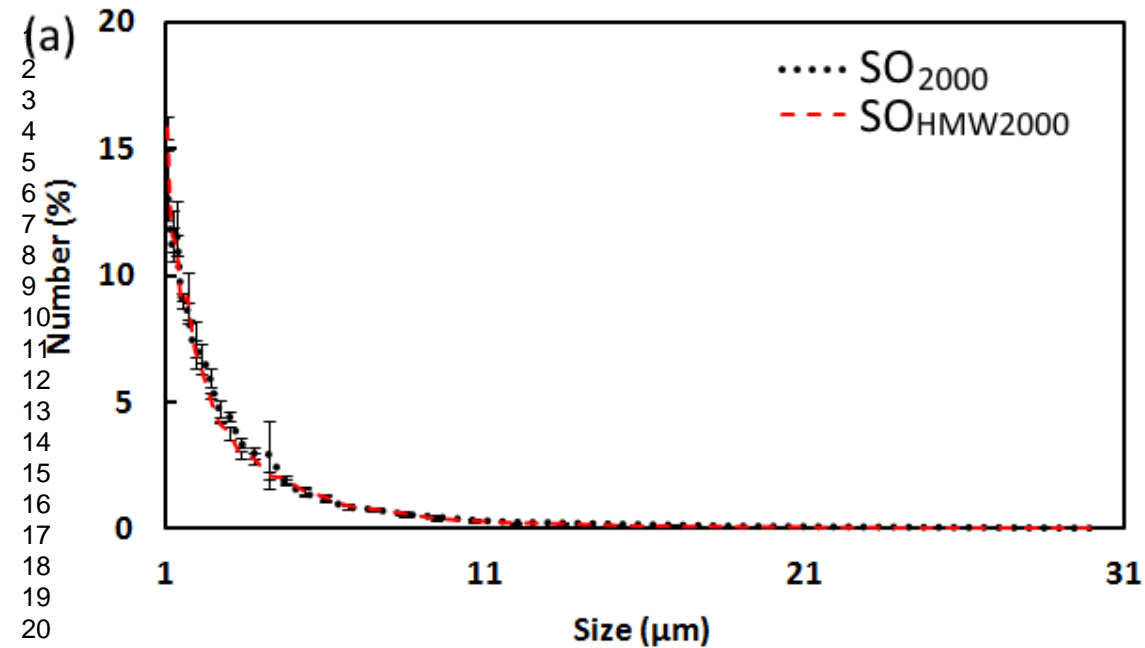
Silicone oil (SO)	Shear viscosity at 25°C/ (cSt)
SO <sub>1300</sub>	1300
SO <sub>2000</sub>	2141
SO <sub>HMW2000</sub>	2189
SO <sub>5000</sub>	4910
SO <sub>HMW5000</sub>	5090

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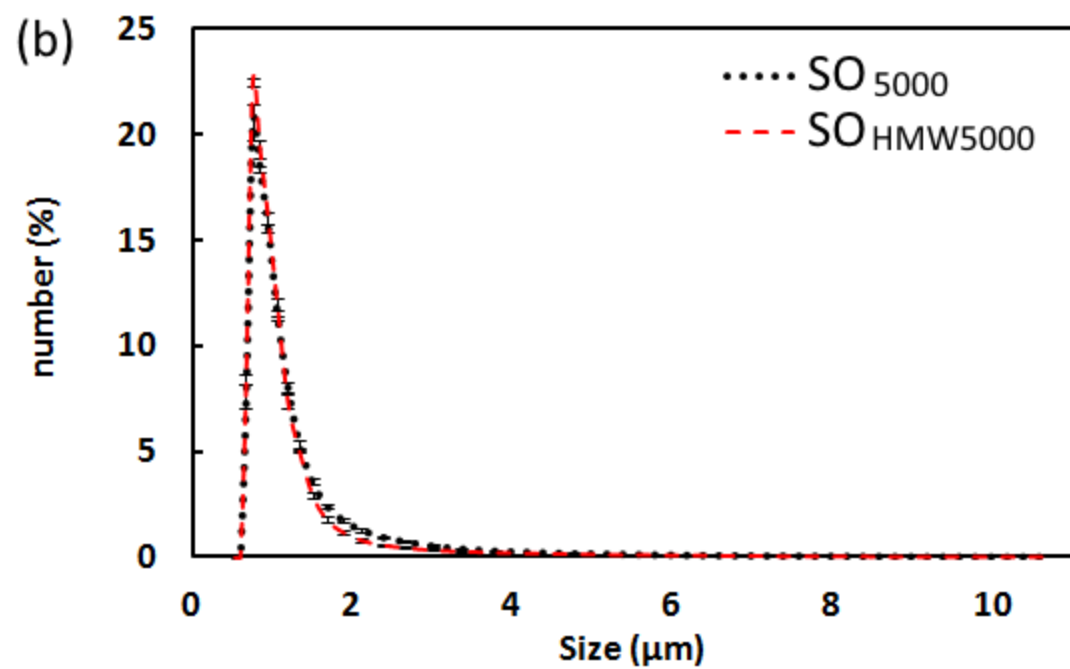
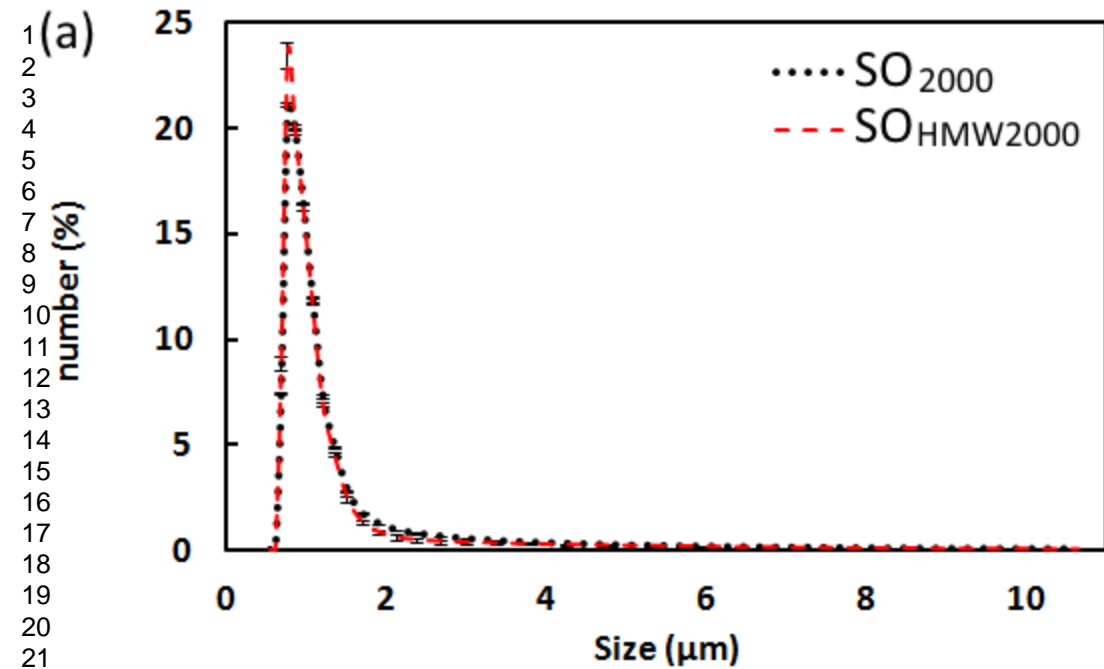
**Table 2 The variation of droplet count in *in-vitro* SO<sub>1300</sub> emulsions generated by the three methods of agitation**

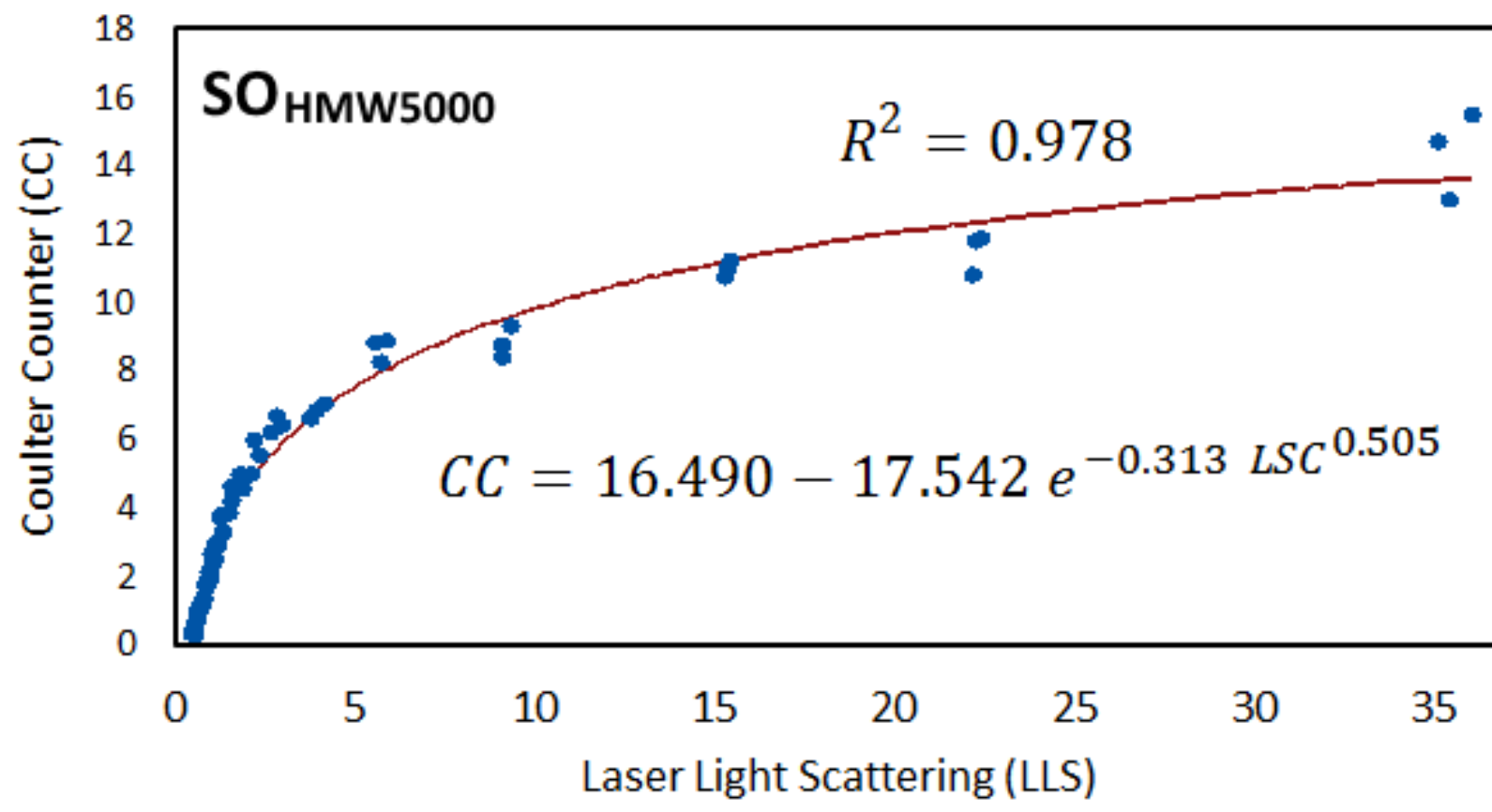
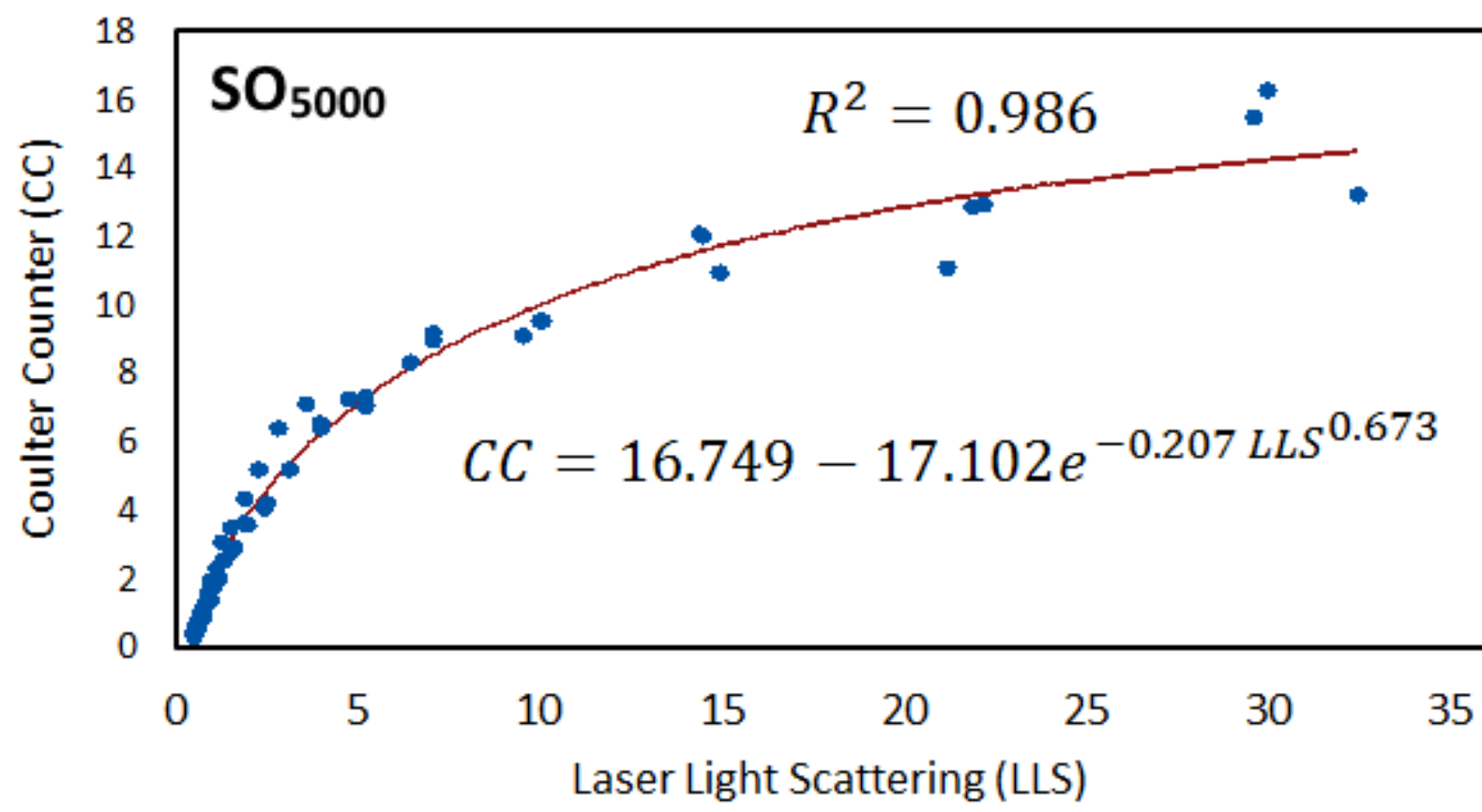
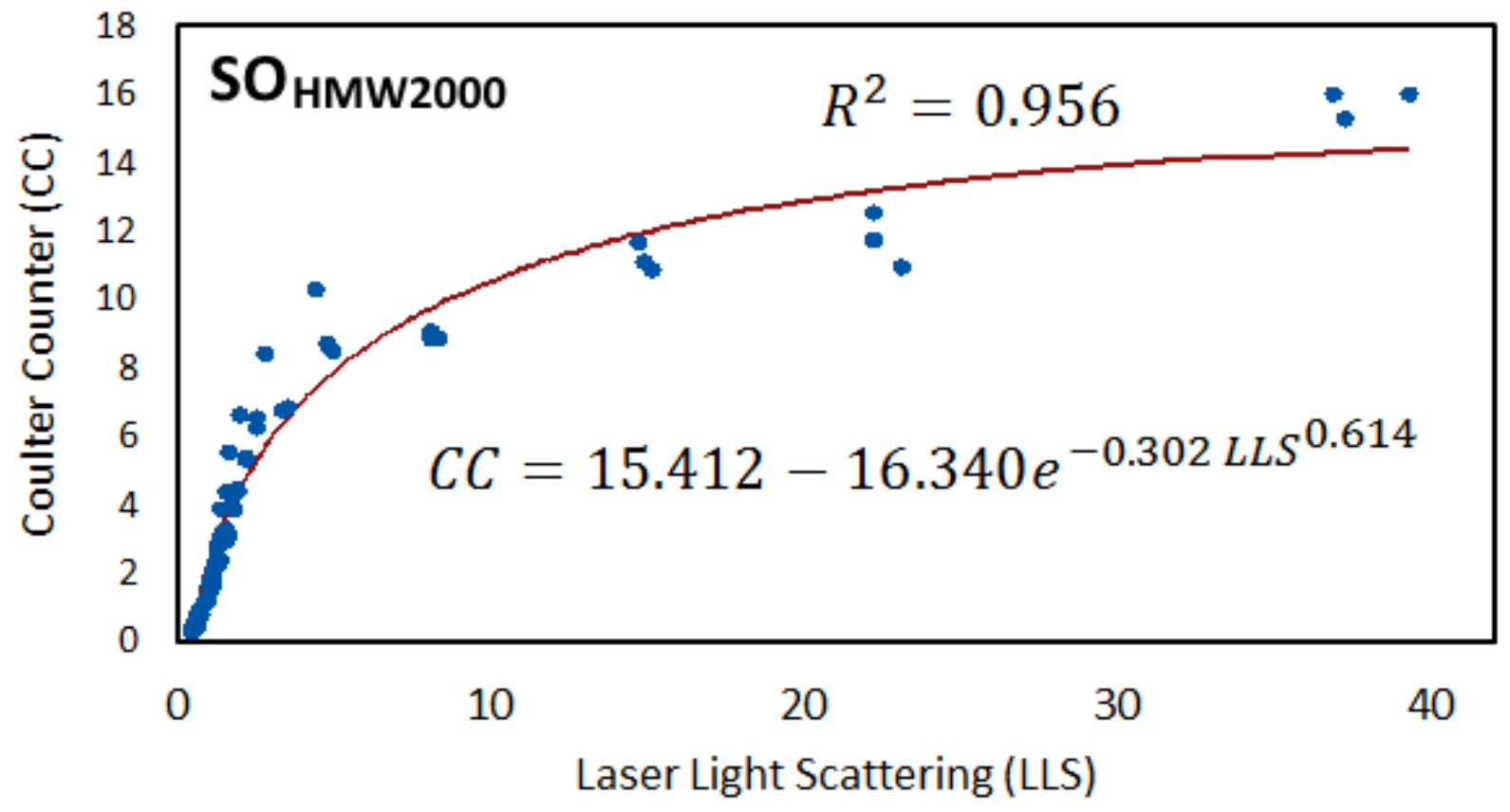
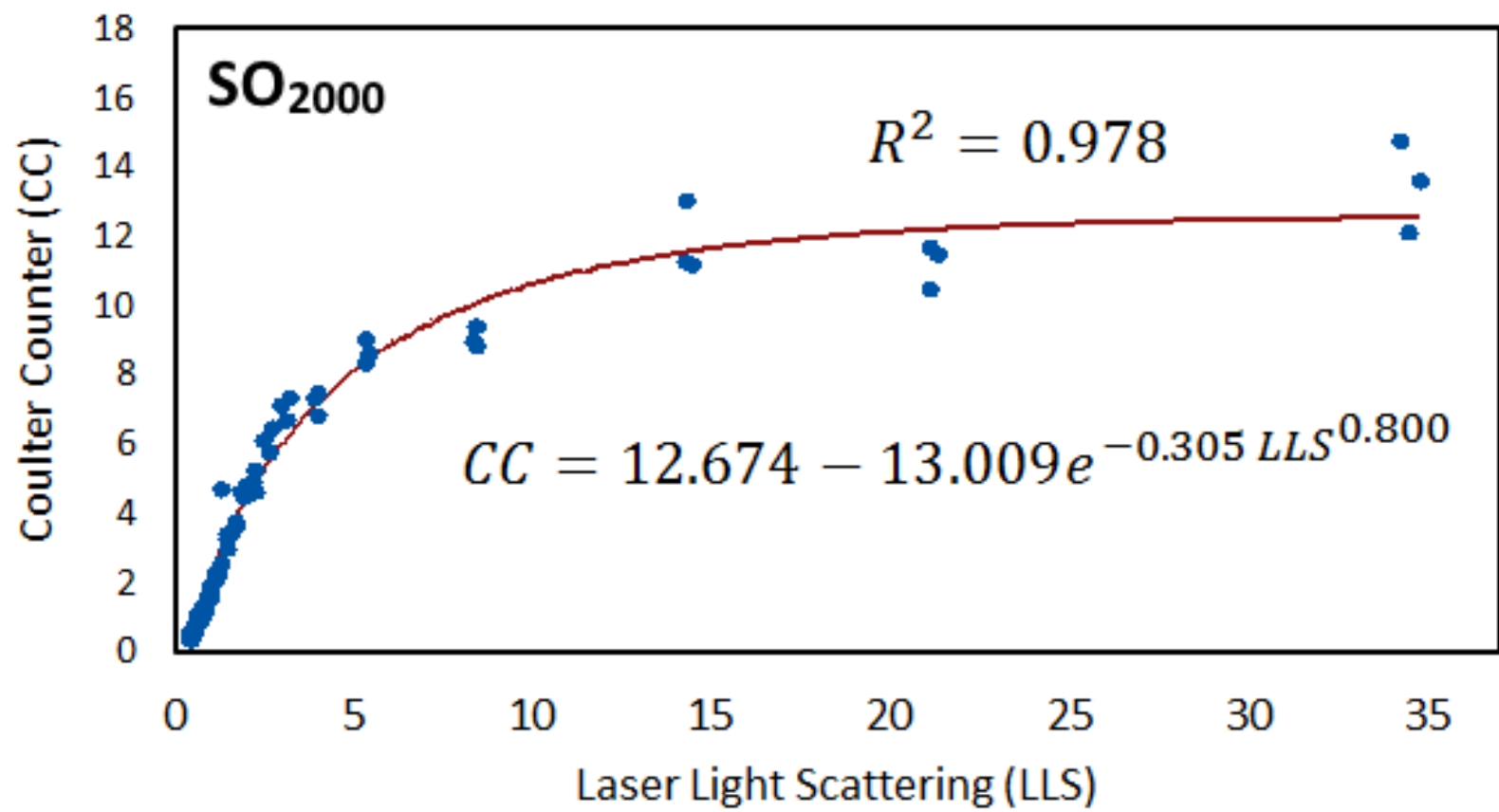
Samples	Vortex	Sonication	Homogenisation
1	2493	3126	10191
2	5794	11859	9990
3	3621	211817	9776
4	5855	35400	9709
5	7817	19830	8013
6	8456	87057	7701
7	1271	908	/
8	11072	80930	/
Mean ± S.D.	5797 ± 3276	56366 ± 71089	9230 ± 1081

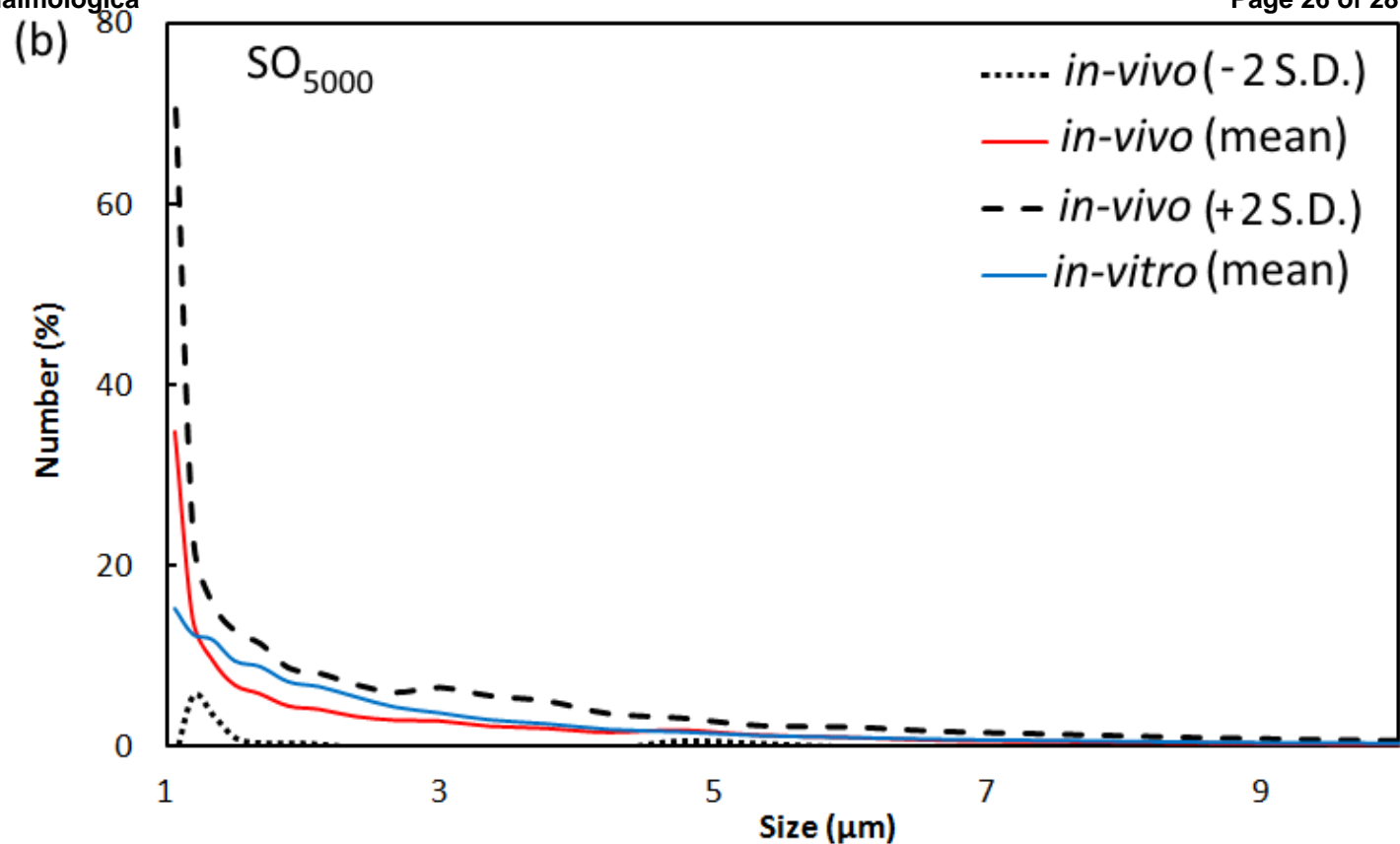
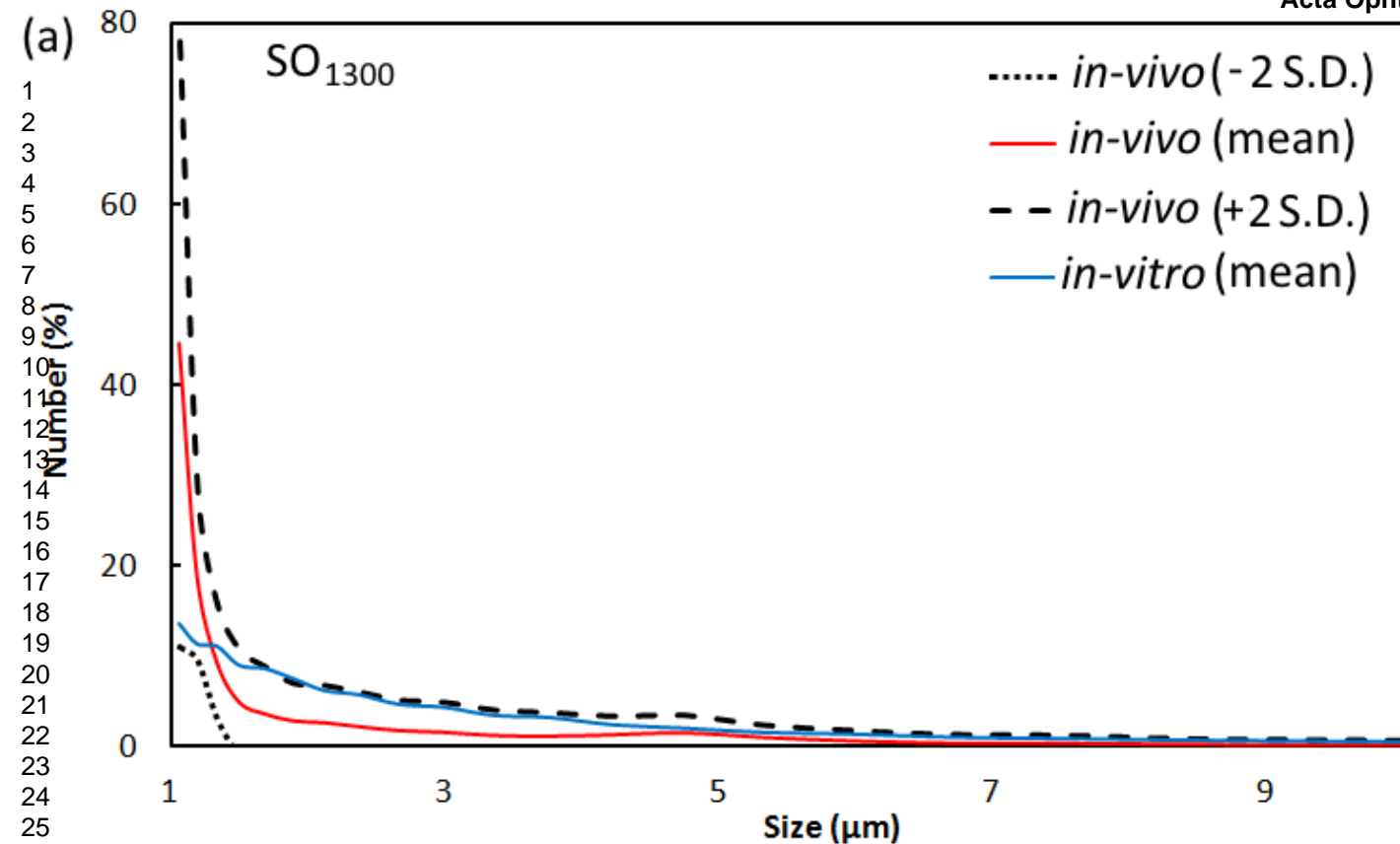
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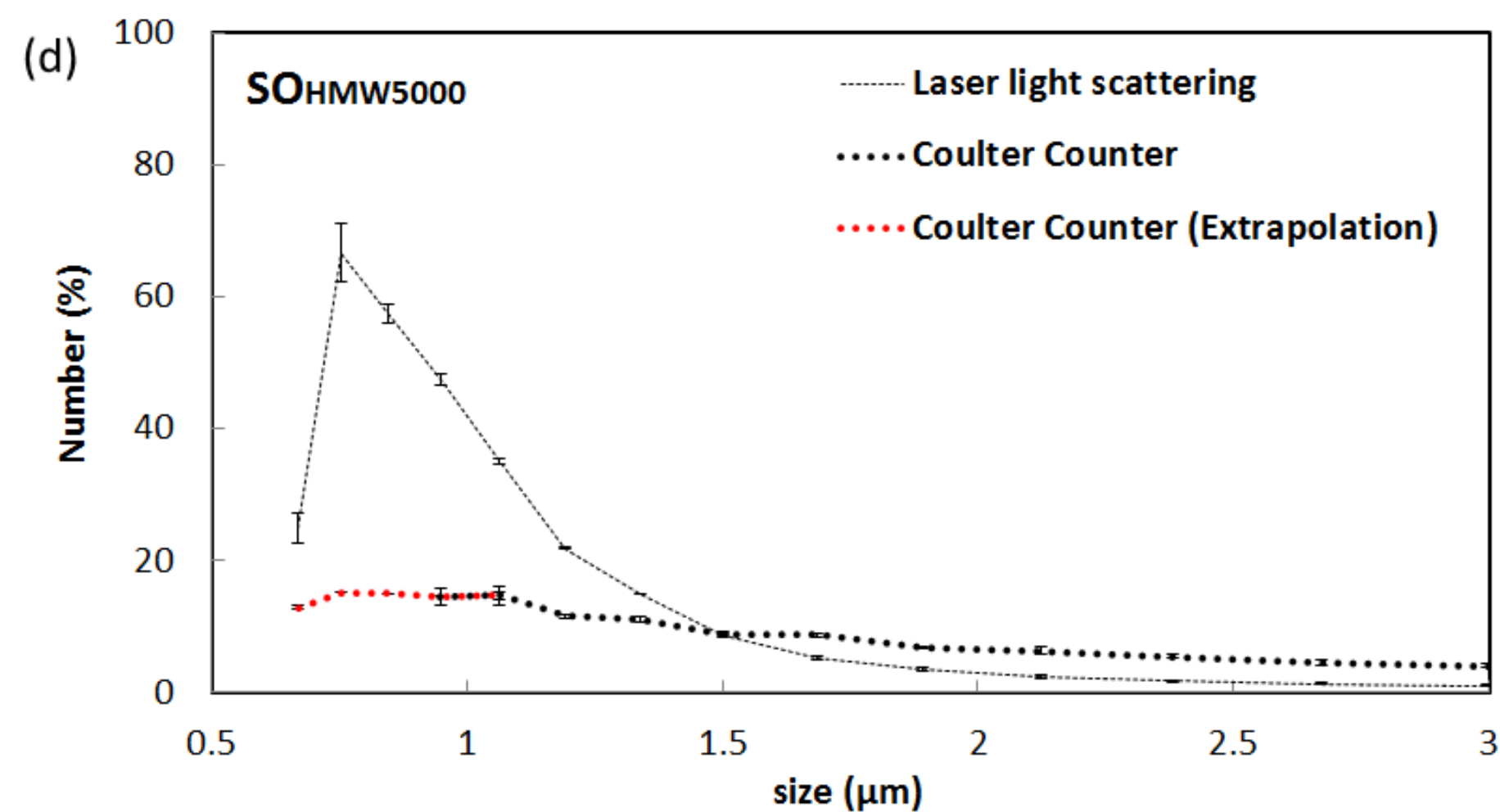
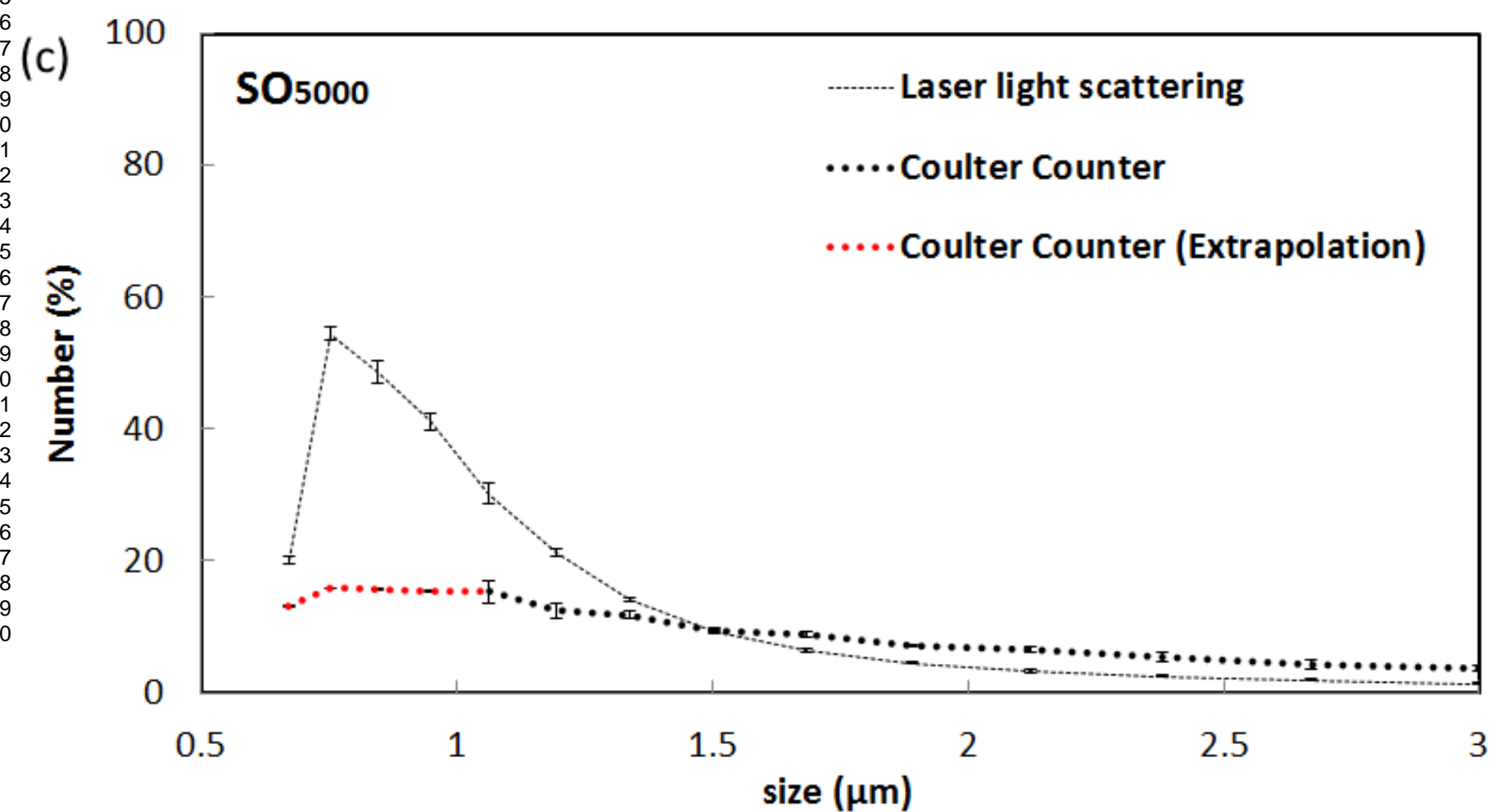
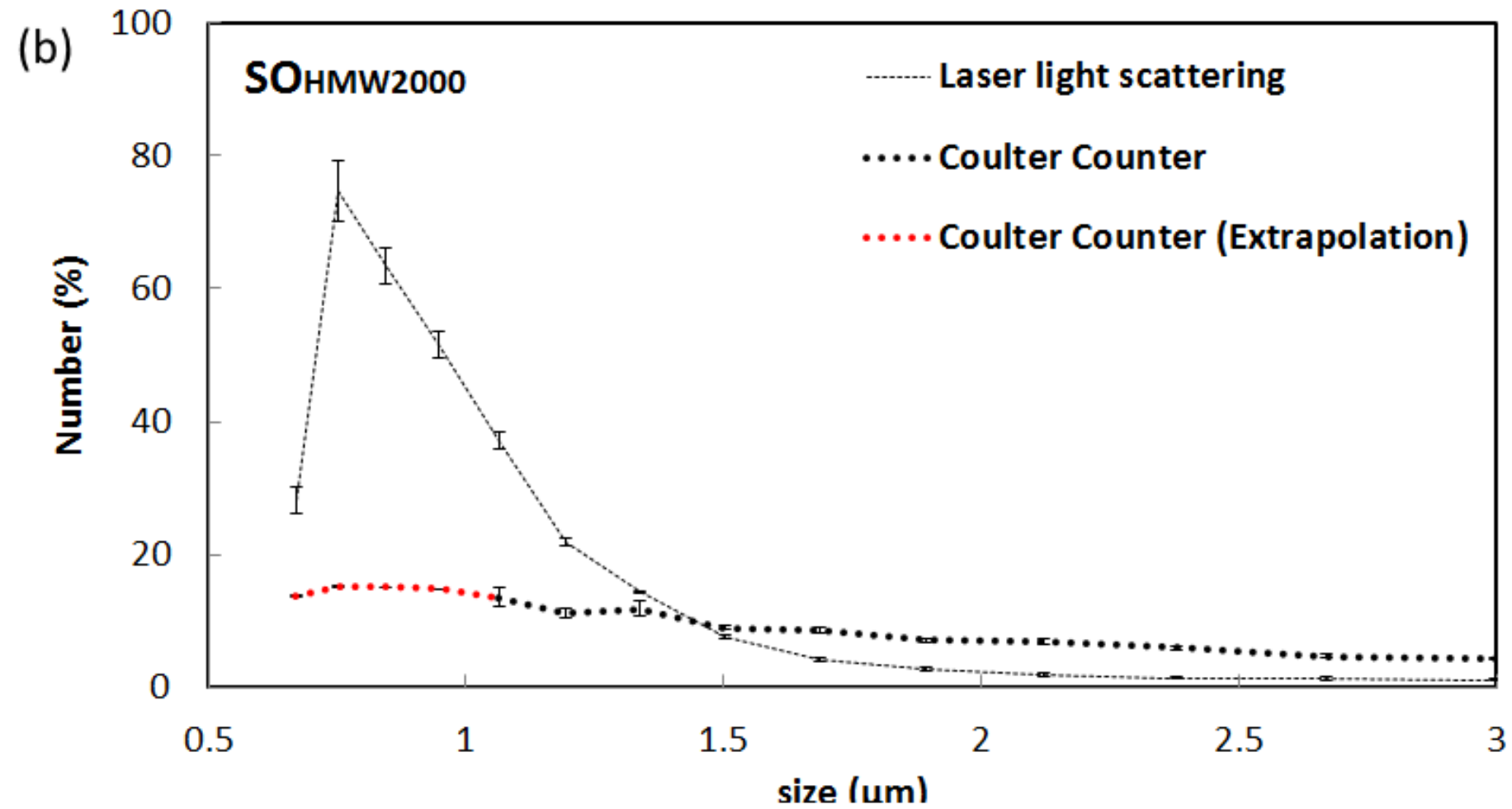
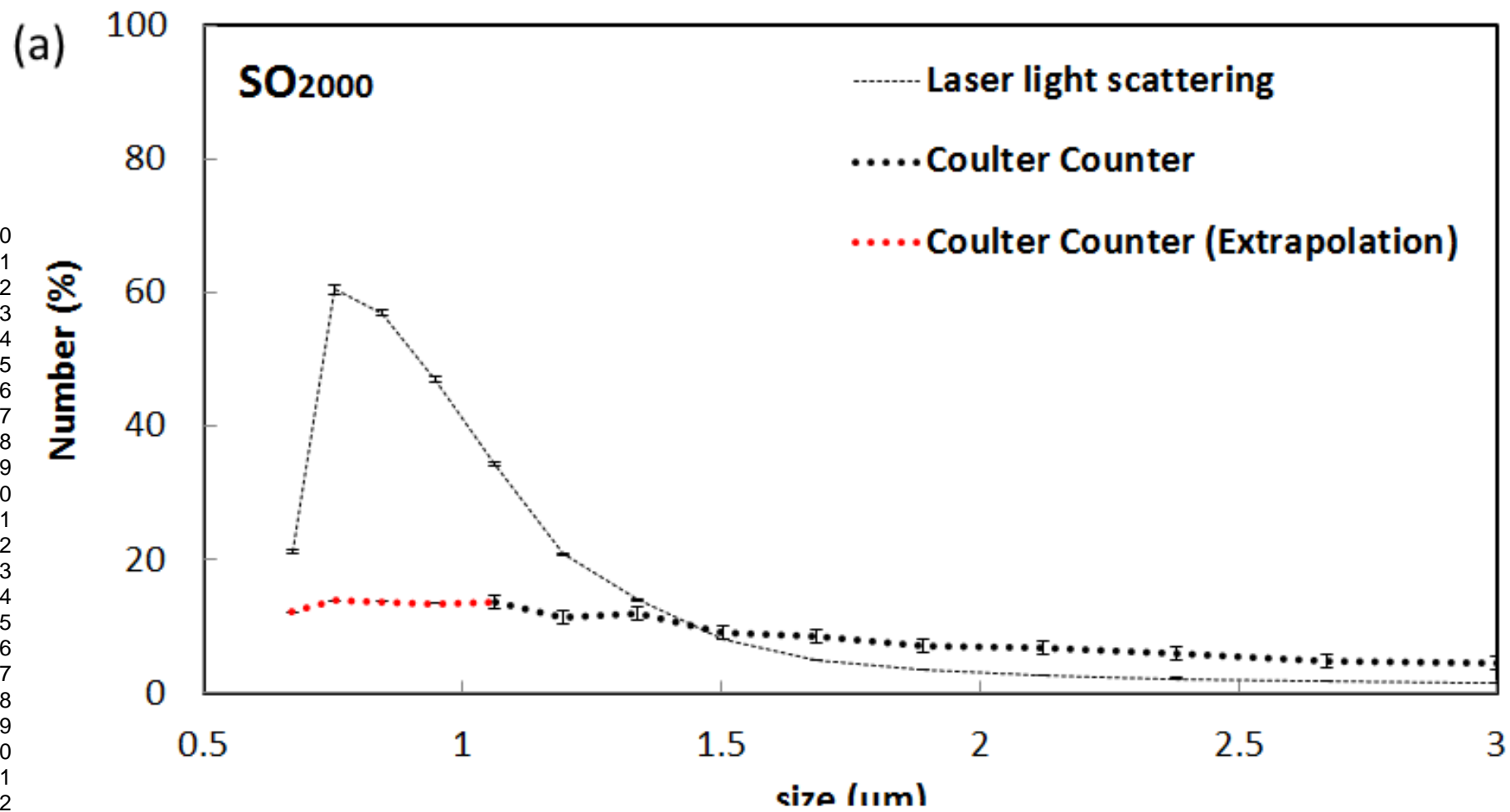






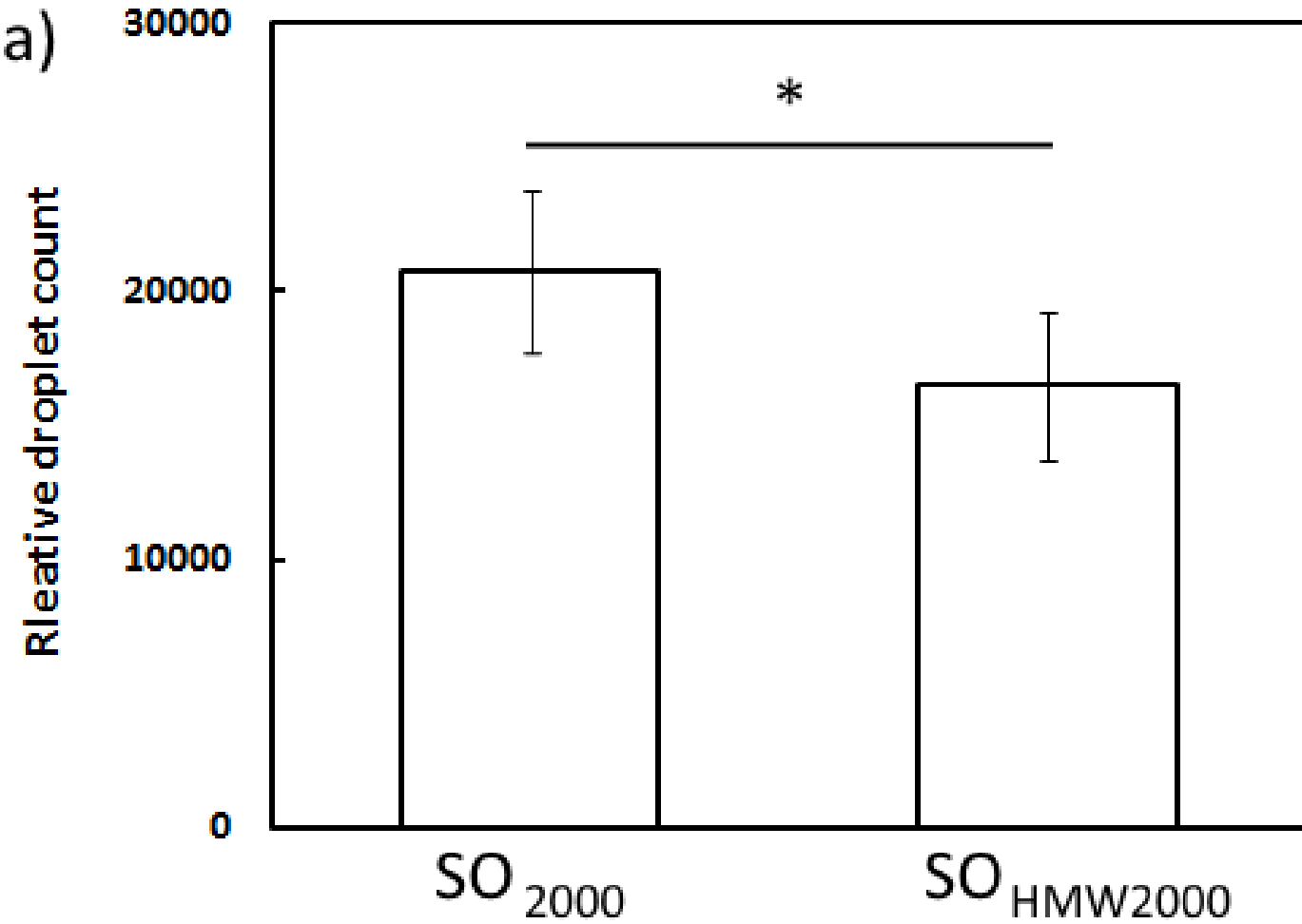




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