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

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Title: **Towards better characterisation and quantification of emulsification of silicone oil *in vitro***

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Purpose: Emulsification is related to complications arising from silicone oil (SO) tamponade. Currently there is no widely accepted method for testing the propensity of SO to emulsify that are physiologically realistic and quantitative.

Methods: We compared different ways of inducing emulsification namely vortex mixing, sonication and homogenisation. SO emulsification was quantitatively assessed using the Coulter counter and laser light scattering. The *in vitro* results are compared with the droplet size distribution profile of vitreous clinical washout. Conventional SO was compared with two novel SO blends with high-molecular-weight (HMW) additives ($SO_{HMW2000}$ and $SO_{HMW5000}$).

Results: Of the three methods for inducing emulsification, homogenisation generated the most consistent emulsion samples with the smallest variance. The results from the Coulter counter measurement correlated strongly with the laser light scattering measurement within the range of 1 to 30 microns. The droplet size distribution profiles from human eyes were similar to that of emulsions generated *in vitro* by homogenisation. The human size distribution profile were within the range of values obtained by the *in vitro* experiment. Compared to the conventional SO, the emulsion droplet counts for the new SO blends were significantly lower ($SO_{HMW2000}$ and $SO_{HMW5000}$ were 79% (±17%) and 49% (±18%) of the SO_{2000} and SO_{5000} respectively; $p = 0.03$ and $p = 0.002$).

Conclusion: Emulsion generated *in vitro* by homogenisation has similar droplet size profile as human eyes filled with SO. Using this method to induce emulsion, SO blends with HMW additives demonstrated less propensity to emulsification with lower droplet counts compared to conventional SO with similar shear viscosity.

Key words: Silicone oil, Emulsification, Coulter counter, Laser light scattering

Introduction

Silicone oil (SO) is widely accepted as a long-term tamponade, despite many known complications such as glaucoma (Honavar et al. 1999), inflammation (Theelen et al. 2004), peri-oil proliferation (Lewis et al. 1988) and re-detachment (Falkner et al. 2001) after oil removal. Some of these complications are thought to be directly related to emulsification (Baino 2011). In recent years, attempts have been made to design new SO that is more resistant to emulsification (Williams et al. 2010). The concept is that adding a small amount of high molecular weight (2.5 million cSt, 423 kD) to conventional SO (1000 cSt, 37 kD) will increase not only the shear viscosity, but importantly the extensional viscosity thereby reducing the tendency to emulsify (Williams et al. 2010). Clinical comparison of the resistance between SO is fraught with difficulties. There are many patient dependent factors such as the degree of inflammation and the extent of blood-ocular-barrier breakdown that may influence the availability of surfactants in the eye. Emulsification depends on shear stresses applied on the SO bubble generated by eye movement (Chan et al. 2011). Using a model eye chamber simulating saccadic movements, we showed that surgical confounding factors such as the extent of fill and the presence of scleral indents might all influence SO emulsification (Chan et al. 2014). Individual patient and surgical confounding factors combine to make it impractical to carry out randomised clinical trials on every new proposed silicone oil. Potentially, in the near future there might be many silicone oils being introduced. If adding high molecular weight (HMW) components was effective in

reducing emulsification, then different conventional SO could be combined with different concentrations of HMW components to produce new oils with clinically useful properties. Clearly, all other silicone based oils might also benefit from this modification and this would include fluorinated SO and the heavy SO (Chan et al. 2014) which are mixtures of SO with semi-fluorinated alkanes or alkenes. Consequently, in our opinion, there is an important role for a standardised *in vitro* test for emulsification to screen new oils before proceeding to clinical evaluations (Scott et al. 2005).

Previous experiments used various mechanical agitations to induce emulsification that might not be physiological. (Savion et al. 1996; de Silva et al. 2005) The mechanical force causing emulsification in the eye is mainly shear stress related to eye movements. Shear stress acting on the liquid is the product of the viscosity of the liquid and the shear rate. However, the mechanical agitation of both sonication and vortex mixing might not provide constant shear rate to the silicone oil to cause emulsification. There is a need to look for another method of mechanical agitation with constant shear rate for the purpose of studying SO emulsification, that better mimics the conditions in the human eye. In the past, we have used a SO-filled model eye chamber to study the effect of eye movements and we estimated the maximum shear rate from stereotypical saccades (Chan et al. 2011). If a standardised method of testing emulsification were to be developed, ideally, it should take into account the physiological conditions that cause emulsification to occur in the human eye (Chan et al. 2015).

We have recently used a Coulter counter to quantify emulsions removed from patients (Chan et al. 2015). The majority of the droplets were too small to be observed even with slit lamp bio-microscopy. The Coulter counter could accurately measure particles down

to 1 μm. The peak of the particle size distribution profile could however not be obtained. We therefore did not know how many droplets there were smaller than 1 μm. There is still a need to find a way to characterise the size distribution profile more completely.

This study aims to achieve two objectives: firstly, to find a consistent method of mimicking emulsification in human eyes and secondly, to achieve a more comprehensive quantification of SO droplets size and number.

Materials and Methods

Materials

Five types of SO were used in this study. The shear viscosities of the SO are listed in Table 1. SO_{1300} was used to characterize the reproducibility of oil-in-water emulsions generated by using different agitation methods. $SO_{HMW2000}$ and $SO_{HMW5000}$ were blends of SO 1000 cSt with 5% and 10% of the high-molecular-weight (HMW) additive (423kD polydimethylsiloxane) respectively. The shear viscosities of $SO_{HMW2000}$ and $SO_{HMW5000}$ were around 2000 and 5000 cSt respectively. Conventional SO 1000 and 5000 cSt were blended (at a ratio of 55% to 45%) to make a SO of 2000 cSt. We named this blend SO_{2000}. SO_{2000} was compared with $SO_{HMW2000}$. Similarly, conventional 5000 cSt oil ($SO_{5000)}$ was used as the control oil and compared with $SO_{HMW5000}$. SO_{1300} (Arciolane 1300) was purchased from ARCAD, France. Apart from SO_{1300}, all the other SO samples were kindly donated by Fluoron GmbH, Germany.

Homogenisation

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

Vortex mixing

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

Sonication

The SO_{1300} sample and the same volume of 2% Pluronic® F68 in PBS were added into a glass syringe. The oil/aqueous ratio (1:1) was in accordance with previously published studies (Caramoy et al. 2011; Caramoy et al. 2015). The glass syringe was immersed in an ultrasound water bath (2510DTH, Bransonic) and subjected to sonication for 1 minute. The sample size of the emulsions for SO_{1300} was 8.

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_{2000}, $SO_{HMW2000}$, SO_{5000} and $SO_{HMW5000}$) 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

diffraction (Sperazza et al. 2004). It covers the measurement range for the particle analysis from 0.02 to 2000 μm in diameter and provides information on the particle size distribution within the suspension. The size distribution measurement for each sample presented herein was a mean value of 10 consecutive measurements.

Estimation of the size distribution of the *in vitro* SO emulsified droplets by extrapolating the measurements from Coulter counter to that of the laser light scattering method

The Coulter counter measurement provided the absolute number count of the SO droplets in the emulsion samples in the size range 1 and 30 μm in diameter. The measurement using the laser light scattering method gave relative numbers and provided an overall percentage size distribution profile of the SO droplets in the emulsion samples in the size range 0.02 to 2000 μm in diameter. The two measurement methods overlapped in the 1 and 30 μm size range. The agreement of the two methods within this range was analysed.

Statistical Method

The Coulter counting method gave absolute numbers of droplets for every size interval whilst the laser light scattering gave relative numbers. The outputs from both the measurements were a droplet size distribution frequency table. The frequency was then expressed as a percentage of the total for a given size interval. (The number of droplets within a given size range divided by total number of droplets). We used the frequency tables to calculate variance and standard deviation in order to measure the spread of the data.

By using the Weibull nonlinear regression model as the mathematical model, the outputs from the Coulter counter and laser light scattering were correlated between 1-30 μm, in terms of relative numbers. After the best fitting model was found, we used the model to examine the correlations and then used it to extrapolate the values from Coulter counter (in %) for ranges 0 to 1 μm. All calculations were done in software Minitab 17, the models were fitted with Gauss-Newton optimisation algorithm.

Statistical significance between the differences of the emulsification of SO was assessed using the Mann Whitney statistical test. The *p*-value < 0.05 was considered to be statistically significant.

Results

Methods of generating SO emulsion as measured by the Coulter counter

Homogenisation yielded consistent results between the 6 samples. In contrast, both vortex mixing and sonication generated oil-in-water emulsion without such consistent result in terms of droplet count. Sonication in particular yielded very variable results. (Table 2)

Size distribution of SO droplets generated by homogenisation measured by Coulter counting and laser light scattering methods

In Coulter counting measurement, the droplet size distribution profiles were similar for emulsions from SO_{2000} and $SO_{HMW2000}$ (Fig. 1a) and from $SO_{HMW5000}$ and SO_{5000} (Fig. 1b).

Similarly, in laser light scattering measurement the droplet size distribution profiles of emulsions were similar between SO_{2000} and $SO_{HMW2000}$ (Fig. 2a) as well as between emulsions from $SO_{HMW5000}$ and SO_{5000} (Fig. 2b). Besides the similarity of the size distribution profiles, using laser light scattering methods, it was found that the smallest size of the droplets detected was between 0.63 – 0.71μm with a peak located in the range between 0.71 – 0.80μm. (Fig. 2)

Statistical modelling

We plotted the percentages of one method against the other and explored their relationships with Weibull nonlinear regression model. (Fig. 3) It was found that the two results were highly correlated with each other under this model (as indicated by the red lines in Figure 3, R^2 = 0.978, 0.956, 0.986 and 0.978 respectively).

In vivo emulsion vs *in vitro* emulsion

It was found that the droplet size distribution profile of *in vitro* emulsion generated by homogenisation was positioned as an outer envelope (Blue line) around the profile of the *in vivo* emulsion. (Fig. 4) This situation was present in both the SO groups (SO_{1300} and SO_{5000}). In other words, the individual *in vitro* profiles coincided with the maximum values of the individual *in vivo* profiles for the range of 1.5 μm and greater. All in all, the profiles of the *in vitro* samples were within the 2 S.D. of the profiles of the *in vivo* samples.

Estimated total droplets number by extrapolation (combining the results from the Coulter counter and laser light scattering)

Since the two methods strongly correlated to one another based on Weibull relationship, it was justifiable to extrapolate the data in order to determine the number of droplets smaller than 1 micron. The number of droplets between 0.5 and 1 micron were determined for the different oils using the Weibull model. It can be seen that the distribution profiles of the 4 different oils tested were similar. (Fig. 5)

Comparing conventional silicone oil with those with HMW additives (Coulter counting)

Two pairs of SO (SO_{2000} and $SO_{HMW2000}$; SO_{5000} and $SO_{HMW5000}$) with similar shear viscosities were tested. The emulsion generated from $SO_{HMW2000}$ (16485±2806) had a significantly lower droplet count than the emulsion from SO_{2000} (20777±3028). (p = 0.03) (Fig. 6a). The total droplet count from emulsion $SO_{HMW2000}$ was 79% (±17%) of that of SO_{2000}. Likewise, $SO_{HMW5000}$ (28054±5168) had a significantly lower droplet count than SO_{5000} (58536±7473). (p = 0.002) (Fig. 6b) The total droplet count for $SO_{HMW5000}$ was 49% (±18%) of that for SO_{5000}.

Discussion

Until recently, the only way of preventing emulsification is to choose highly purified SO with higher viscosities (Nakamura et al. 1990). The advent of small gauge surgery in recent years have provided impetus for choosing less viscous SO, simply because they are easier to inject and remove through smaller cannulas. New SO with HMW additives

claimed to be both more emulsification resistant and easier to inject and remove (Williams et al. 2010) and this assertion was corroborated *in vitro* (Caramoy et al. 2011). Nonetheless, others have observed early emulsification *in vivo* when these new SO were used (Maier et al. 2011). There is therefore, justification to look again into whether the claims for emulsification resistance could be substantiated.

The current evidence of laboratory-based experiments might be flawed. There are several aspects to consider. Firstly, the method of generating emulsification has to be valid. Mixing SO with water could potentially generate either oil-in-water or water-in-oil emulsion. In the published images of a previous study using sonication, the droplets were in the bottom of the oil phase suggesting that the droplets might be water-in-oil (Caramoy et al. 2010). This result might be irrelevant clinically because the droplets seen in patients are oil-in-water droplets. Secondly, the methods of generating emulsification should yield consistent results. Our results showed that the methods of vortex mixing and sonication were inconsistent. (Table 2) The same SO produced widely varying results in terms of droplet numbers under identical conditions. Thirdly, the SO should be dispersed totally in the aqueous phase. Whether the method of generating emulsion is exhaustive will certainly affect the total number and the size of the SO droplets. SO with a higher resistance to emulsification in theory should produce droplets that are larger in size and fewer in number and *vice versa*. We believe that the results using vortex mixing and sonication were inconsistent mainly because too much oil was used (oil to water at the ratio of 1:1 in accordance to previous publications). At the end of the agitation, there were clearly two phases seen with SO on top and aqueous below. (Caramoy et al. 2011) In other words, not all the oil was dispersed.

From one experiment to another, the results using sonication and vortex might therefore reflect how much oil was successfully dispersed by the mechanical methods rather than measuring the resistance or readiness of the oil to emulsify. Homogenisation on the other hand is an established scientific method for generating emulsions. (Maa & Hsu 1996) The use of oil to water in the ratio of 1 to 99 using homogenisation produced more consistent results. All the SO in each experiment was exhaustively emulsified leaving no bulk oil. We believe that this is the best way to make fair comparison the propensity of different SO to emulsify. The size distribution profiles of these *in vitro* emulsions generated by homogenization were similar to that of patients.

Emulsification is related to the presence of surfactants. In the eye, there are many surfactants present in the intraocular fluid which includes but not limited to different kinds of proteins, lipids and phospholipids.(Savion et al. 1996) We opted to use Pluronic® F68 as the model surfactant in this study because it is widely used as a standard in emulsification science. (Caramoy et al. 2010; Williams et al. 2010; Caramoy et al. 2011; Caramoy et al. 2015) Pluronic® F68 lowers the interfacial tension between SO and aqueous phase as effective as any surfactant that are present in the eye. It is important when comparing like with like that the ability to emulsify be standardised in concentration and effectiveness.

To date, the published results on resistance of the new SO with HMW additives relied on observation with the naked eye (Caramoy et al. 2011; Caramoy et al. 2015). We recently published quantification of washings from patients' eyes that had oil *in situ* for months using the Coulter counter (Chan et al. 2015). The majority of droplets were between 1-2 μm. With slit-lamp biomicroscopy, we would not see discrete droplets or

cells less than 5 μm, observing instead the scattering of light by these droplets as “flare”. In this study, we also used the Coulter counter for droplet counting and sizing. Undiluted, the concentration of emulsification in the aqueous might be high enough to be detected by the laser light scattering method. However, the washings collected during SO removal procedures were diluted by infusion fluids. Currently, it is technically difficult to collect undiluted samples from the eye in sufficient quantity that would allow laser light scattering to yield meaningful quantitative results.

Our study chose two methods to quantify droplet number. The Coulter counter allows a precise particle counting of droplets. However, the Coulter counter has a lower limit of measurement of 1 μm in diameter. In our study we could not determine the peak of the size distribution profile of the emulsion droplets, (Fig. 1) showing that there must be droplets smaller than 1 μm in diameter. Laser light scattering provides a very broad range of sensitivity. The machine we used in the study (Mastersizer 2000) covered the measurement range from 0.02 to 2000 μm in diameter. This allowed us to obtain a more complete size distribution profile of the emulsified droplets. (Fig. 2) Laser light scattering, however, only gives relative not absolute numbers of droplets. The two methods of measurements are based on different principles. The relationships have not been previously explored mathematically. We were able to exploit the overlap in the range of sizes (i.e. between 1 and 30 μm) to analyse the same sample. The graphs showed that the correlation between the two methods to be very strong based on the Weibull regression model. (Fig. 3) We could therefore justify extrapolating beyond the measurement range of the Coulter counter based on the assumption that the Weibull relationship we found would still hold if the Coulter counter was measurable in the range

between 0 and 1 μm. We were particularly interested in the peak frequency of size distributions. Without the full characterisation of droplet distribution, we cannot confidently know if one emulsion compared to another had more or fewer droplets (Chan et al. 2015). After extrapolating, we could detect that the most numerous droplets were between 0.5 and 1 micron. (Fig. 3) There was a high degree of consistency between all SO tested as all plots have similar shapes. Using the statistical model to extrapolate below 1 micron revealed an important difference between the two methods. Figure 5 showed proportionately fewer droplets estimated by the calculation than the distribution indicated by laser light scattering method. This needs an explanation. We believe that this is due to one of the limitations of the Coulter Counter, which relies on the solutions to be suitably diluted. Otherwise, two or more droplets could pass through the aperture simultaneously and be counted as a single larger droplet. This known error is known as “coincidence”. It seems that emulsification *in vitro* and in patients have many more small droplets. Coincidence is the likely explanation of why the Coulter Counter underestimated the number of small droplets. Coulter Counter is accurate over a narrower range, whilst laser scatter is widely acknowledged for being fast, accurate over a wider range.

The high degree of correlation between the two methods suggested that using the Coulter counter alone without using the laser scatter method might be sufficient to reflect the overall propensity of an to emulsify. Significantly, though all 4 SO tested had very similar size distribution profiles the absolute numbers of droplets were different. Because the total volume oil emulsified was the same, the difference could only be accounted for by the more resistant oils having a few larger droplets and thus fewer

smaller droplets. This is in line with what was predicted if SO with HMW additives were more resistant to emulsification (Fig. 6).

There are limitations to our study. Firstly, with the use of homogeniser, we could only control the shear rate but not the shear stress. We know that the shear stress is a product of shear rate and shear viscosity. Therefore, with our experimental setup, we could have been applying up to five times more force to 5000 than to 1000 cSt. We were therefore, not able to compare SO with different shear viscosity using our experimental setup as direct comparison between the two would not be valid. Secondly, the viscosity of SO is temperature dependent.(Romano et al. 2016) We have not measured the viscosity difference in between *in vitro* and *in vivo* environments though published figures indicate that the difference is small. Our *in vitro* experiments were carried out at room temperature whilst *in vivo* emulsification occurs at body temperature. However, we were careful to only compare the propensity of SO to emulsification *in vitro*. Therefore, the slight change of viscosity due to the temperature difference may not be so relevant. No direct comparison was made between *in vitro* and *in vivo* emulsification beyond the basic observation that the distribution profiles were similar.

Conclusion

In this study, we showed that homogenisation provided a consistent way to generate oil-in-water emulsion *in vitro*. We stressed the importance of exhaustively emulsifying the oils, ideally using principally only shear forces as this might be more physiological and yield size distribution similar to that in patients. Although the Coulter counter could only

count droplets within a narrow size range, the results correlated well with laser light scattering which has a widened range. The most numerous droplets seemed to be between 0.5 and 1 micron in diameter. We also showed that SO with HMW additive produced fewer emulsified droplets than conventional SO with similar shear viscosities.

Legends

Figure 1 Droplet size distributions of the emulsions from (a) SO_{2000}, (b) $SO_{HMW2000}$, (c) SO_{5000} and (d) $SO_{HMW5000}$ generated by high shear homogenizer (measured by the Coulter counter)

Figure 2 Droplet size distributions of the emulsions from (a) SO_{2000} and $SO_{HMW2000}$ and (b) SO_{5000} and $SO_{HMW5000}$ 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_{1300} and (b) SO_{5000}. 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) $SO_{HMW2000}$ relative to SO_{2000} and (b) $SO_{HMW5000}$ relative to SO_{5000} 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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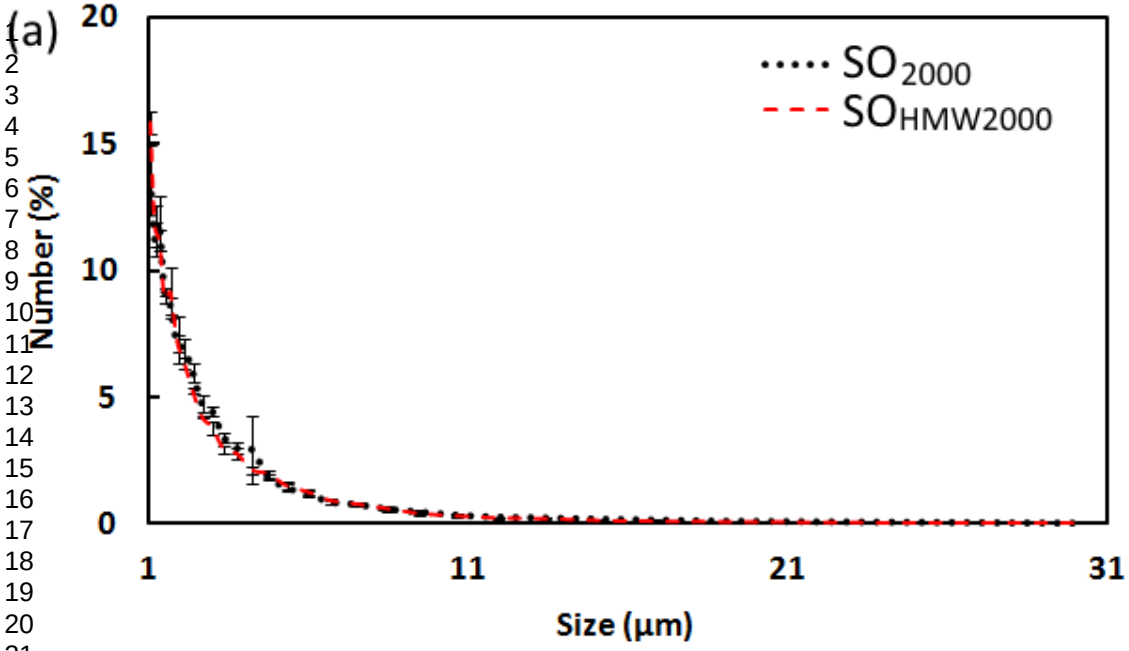
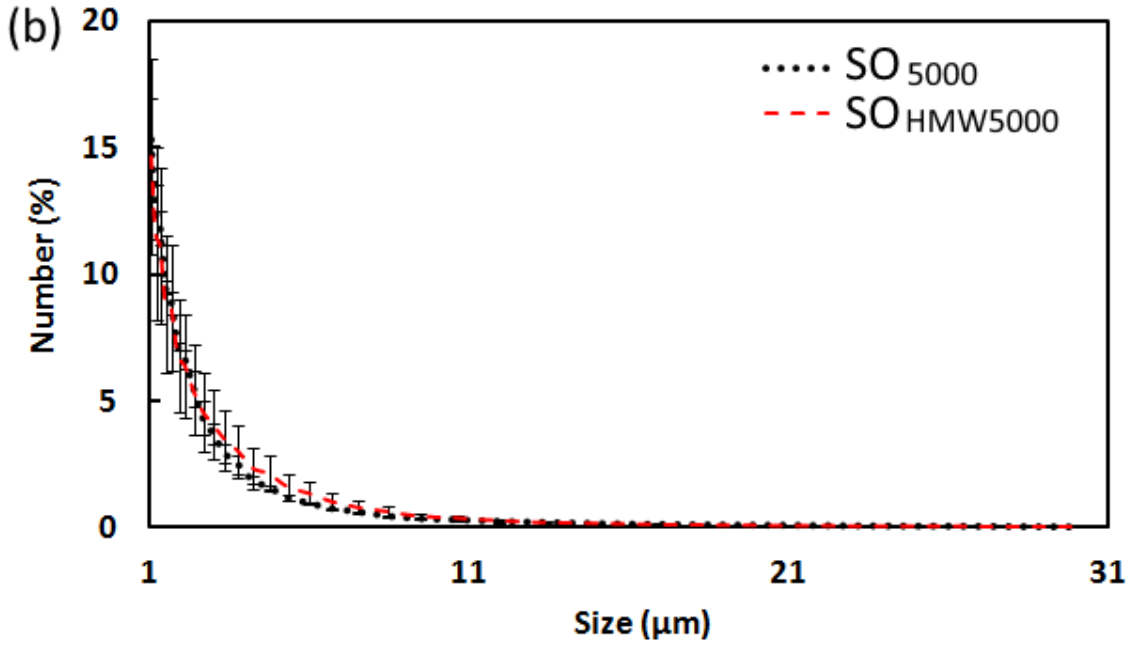
Table 1 Shear viscosities of silicone oil samples (Chan et al. 2011)

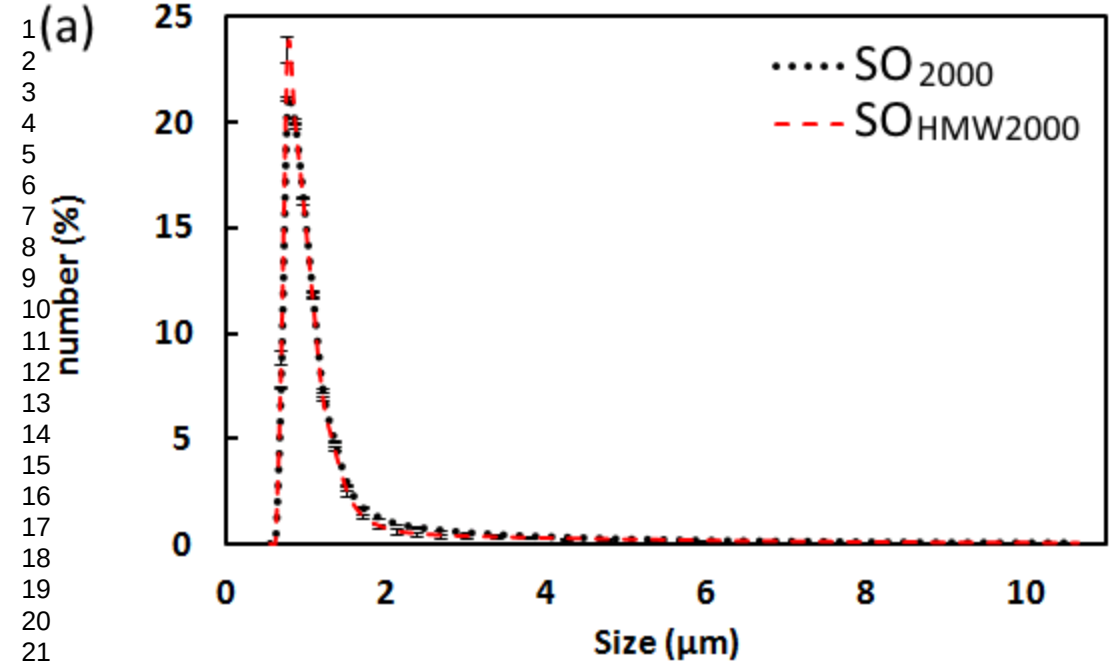
Silicone oil (SO)	Shear viscosity at 25°C/ (cSt)
SO_{1300}	1300
SO_{2000}	2141
$SO_{HMW2000}$	2189
SO_{5000}	4910
$SO_{HMW5000}$	5090

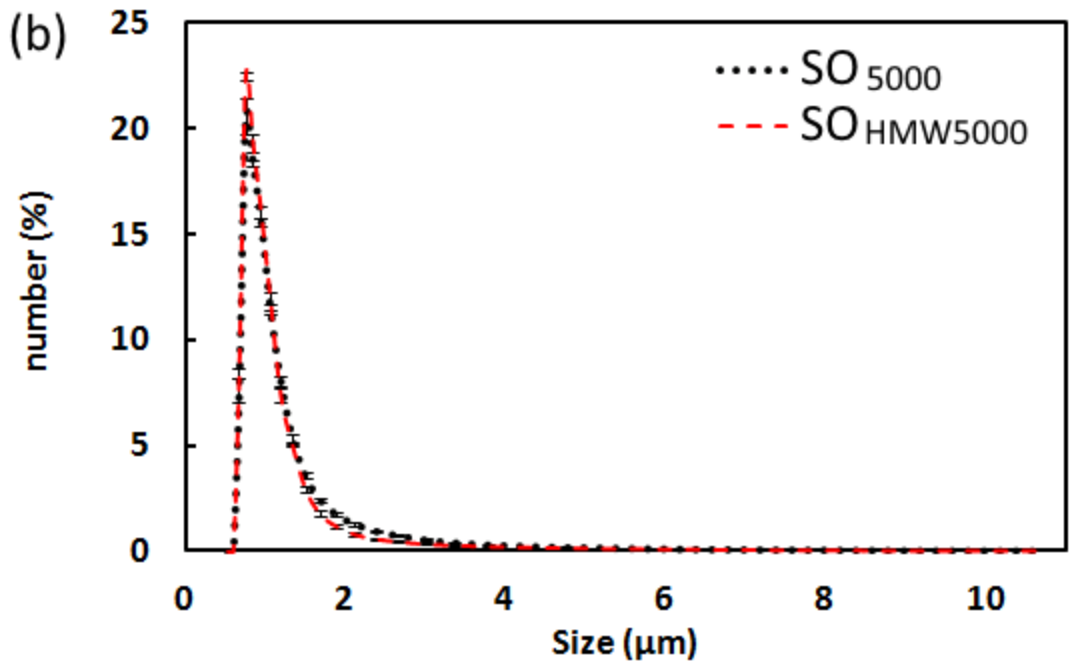
Table 2 The variation of droplet count in *in-vitro* SO_{1300} emulsions generated by the three methods of agitation

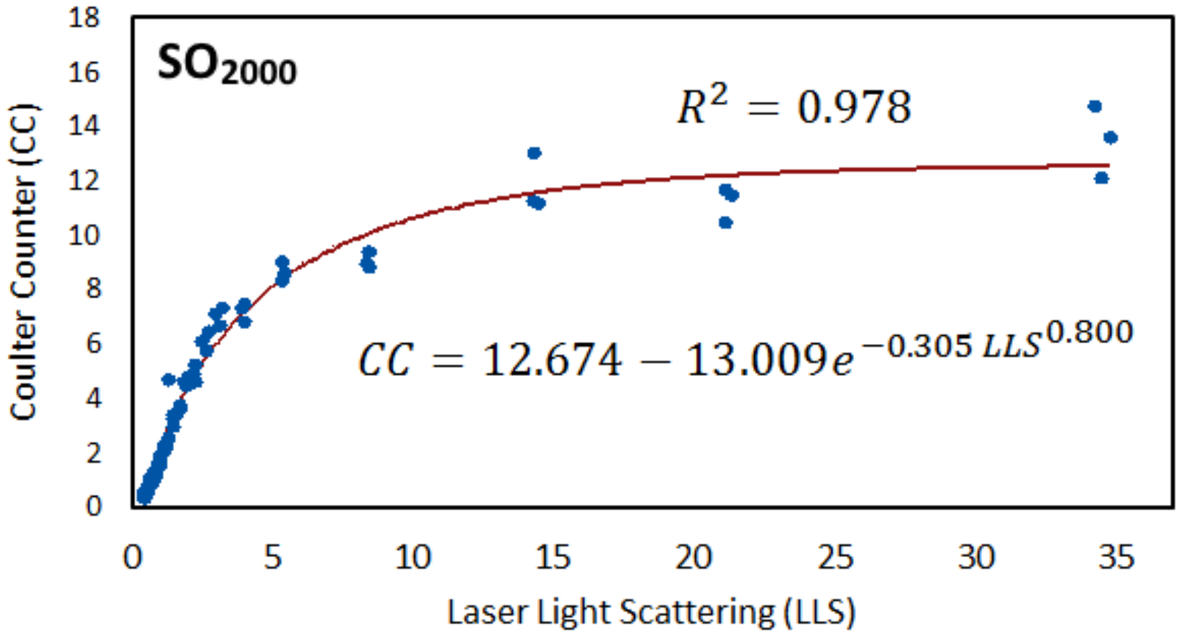
Samples	Vortex	Sonication	Homogenisation
1	2493	3126	10191
2	5794	11859	9990
3	3621	211817	9776
4	5855	35400	9709
5	7817	19830	8013
6	8456	87057	7701
7	1271	908	/
8	11072	80930	/
Mean ± S.D.	5797 ± 3276	56366 ± 71089	9230 ± 1081

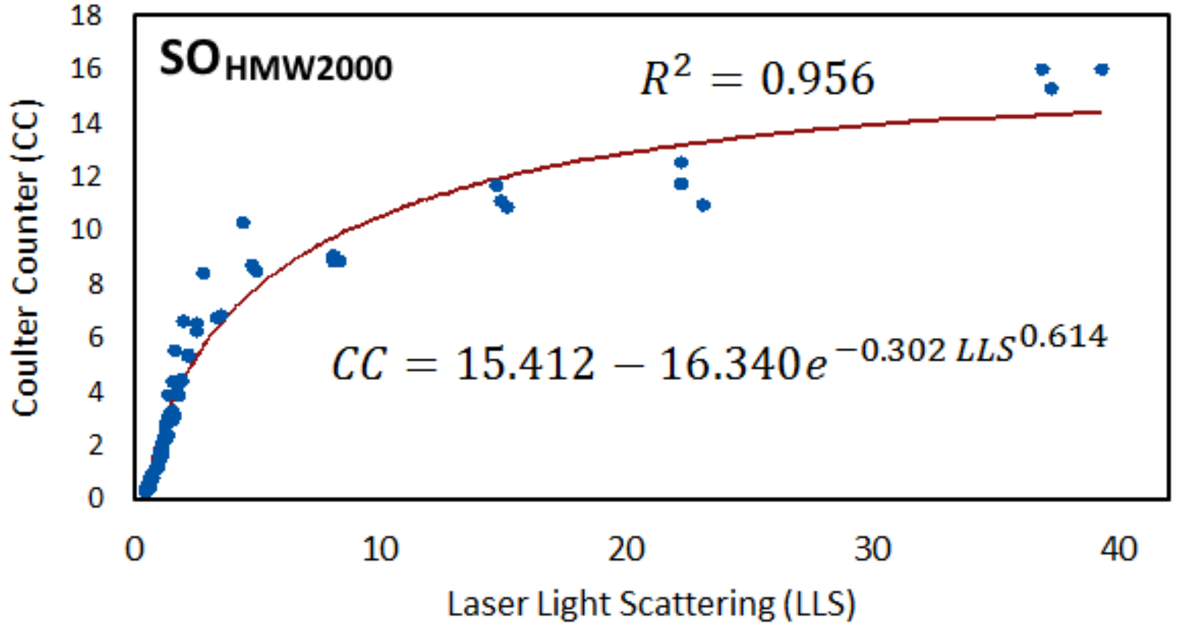

(a)
SO2000
SOHMW2000
Number (%)
Size (µm)
0
5
10
15
20
1
11
21
31

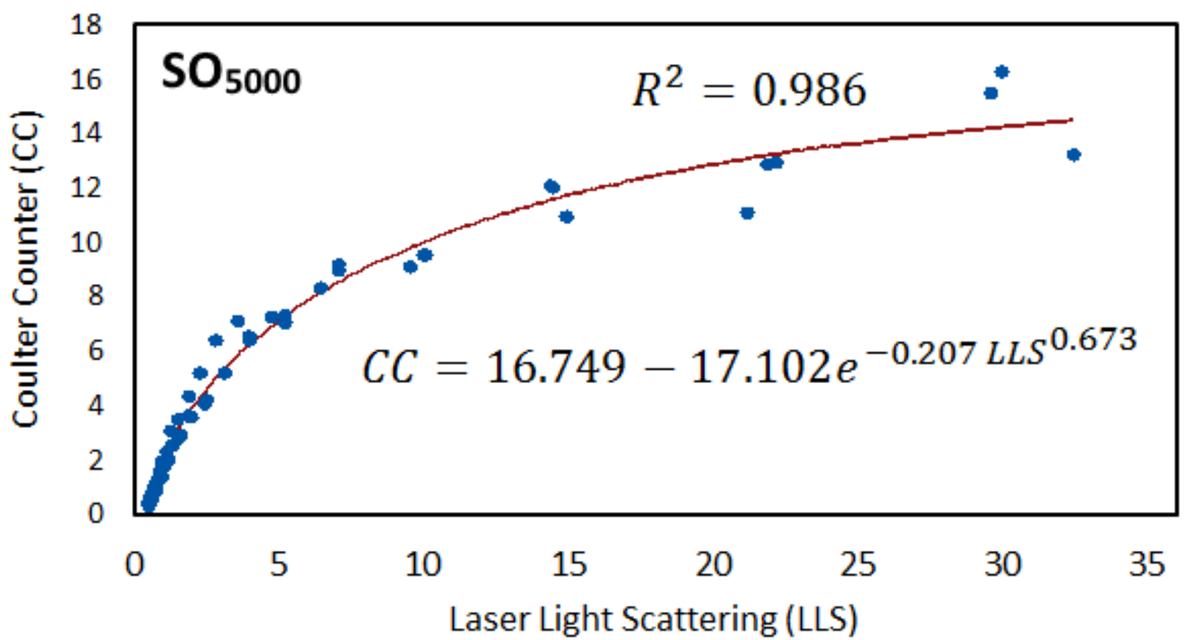

(b)
SO 5000
SO HMW5000
Number (%)
Size (µm)
0
5
10
15
20
1
11
21
31

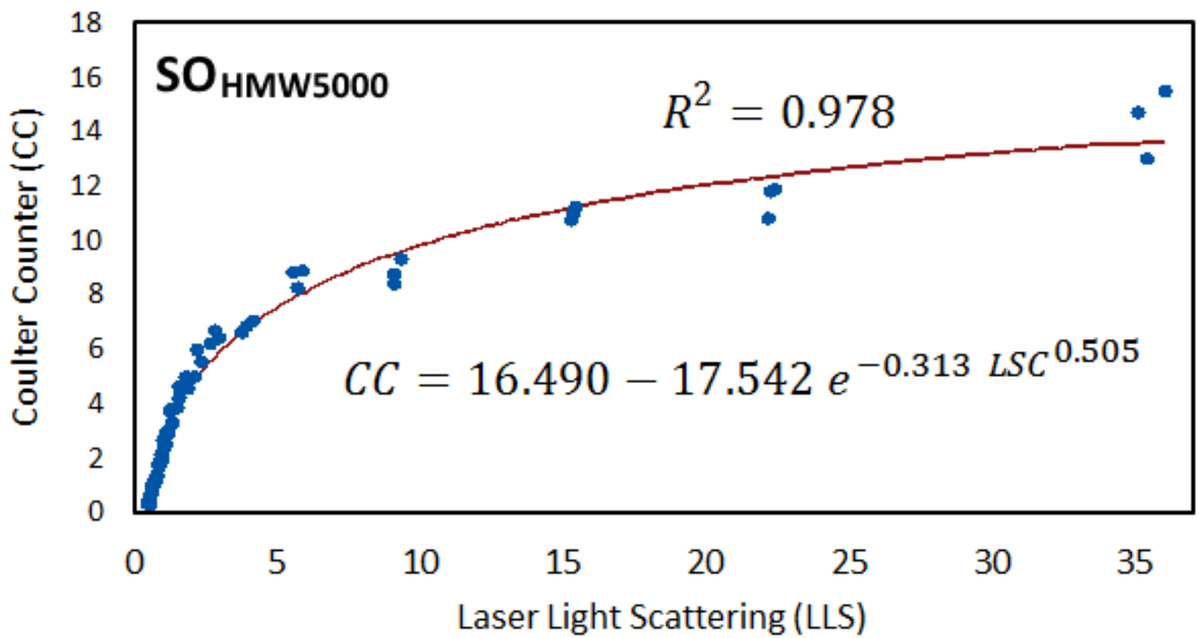

(a)
25
20
15
10
5
0
number (%)
0
2
4
6
8
10
Size (µm)
SO 2000
SO HMW2000

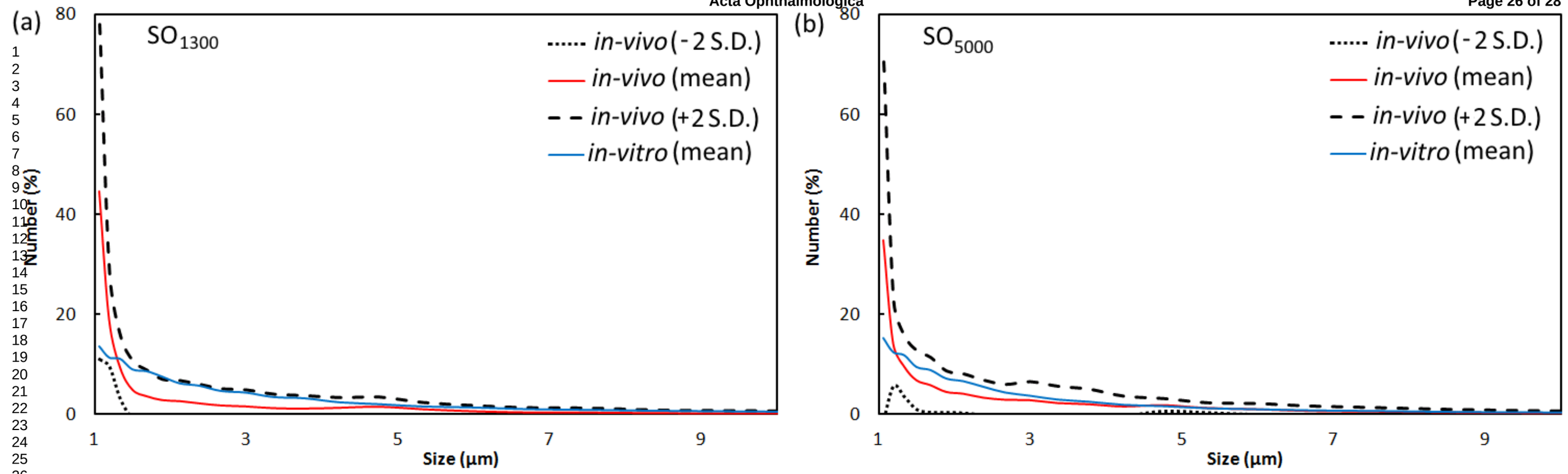

(b)
25
20
15
10
5
0
number (%)
0
2
4
6
8
10
Size (µm)
SO 5000
SO HMW5000

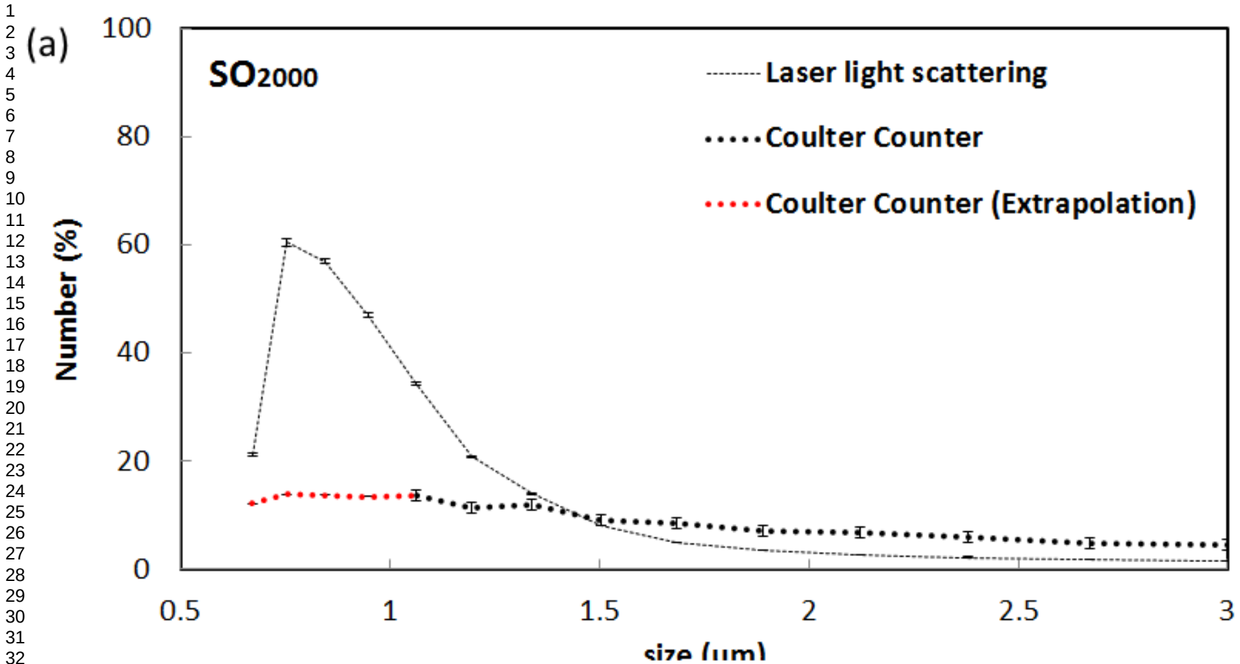

SO2000
$R^2 = 0.978$
$CC = 12.674 - 13.009e^{-0.305\,LLS^{0.800}}$
Coulter Counter (CC)
Laser Light Scattering (LLS)

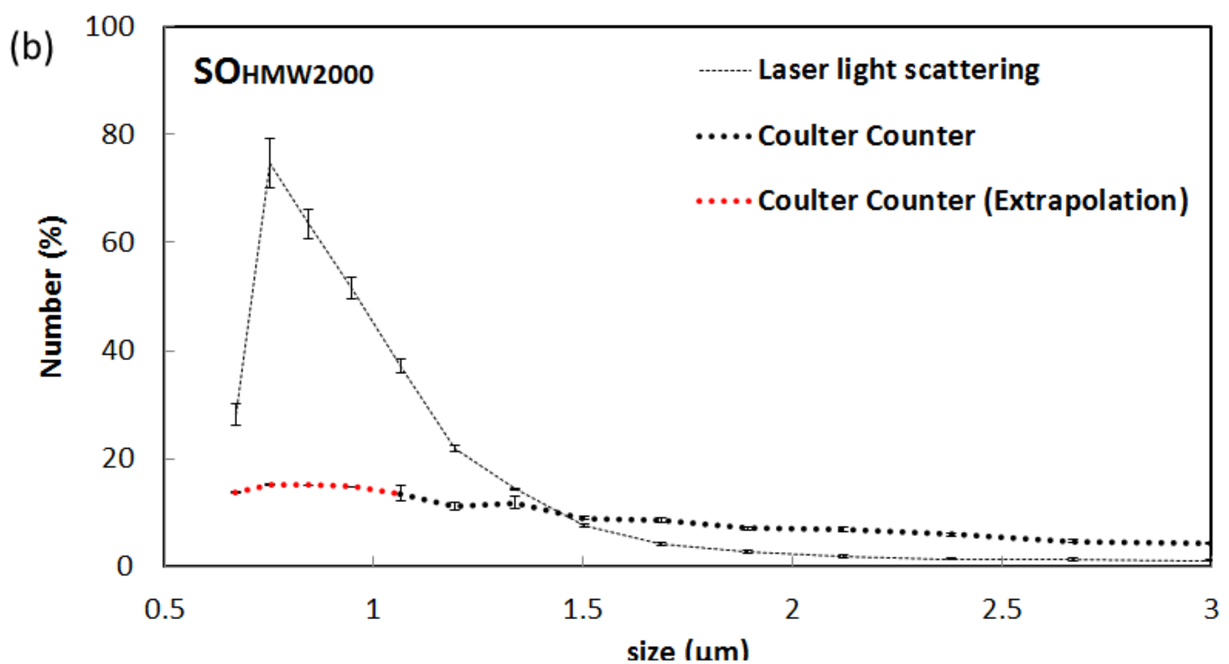

SOHMW2000
$R^2 = 0.956$
$CC = 15.412 - 16.340e^{-0.302\,LLS^{0.614}}$
Coulter Counter (CC)
Laser Light Scattering (LLS)

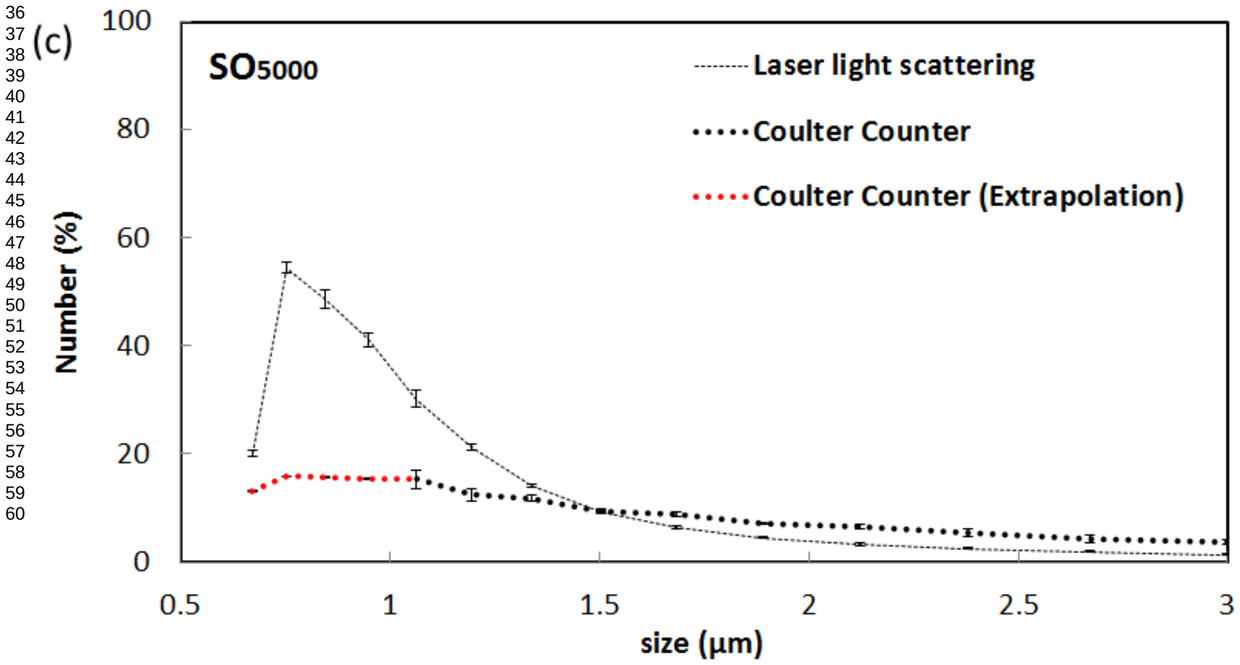

SO5000
$R^2 = 0.986$
$CC = 16.749 - 17.102e^{-0.207\,LLS^{0.673}}$
Coulter Counter (CC)
Laser Light Scattering (LLS)

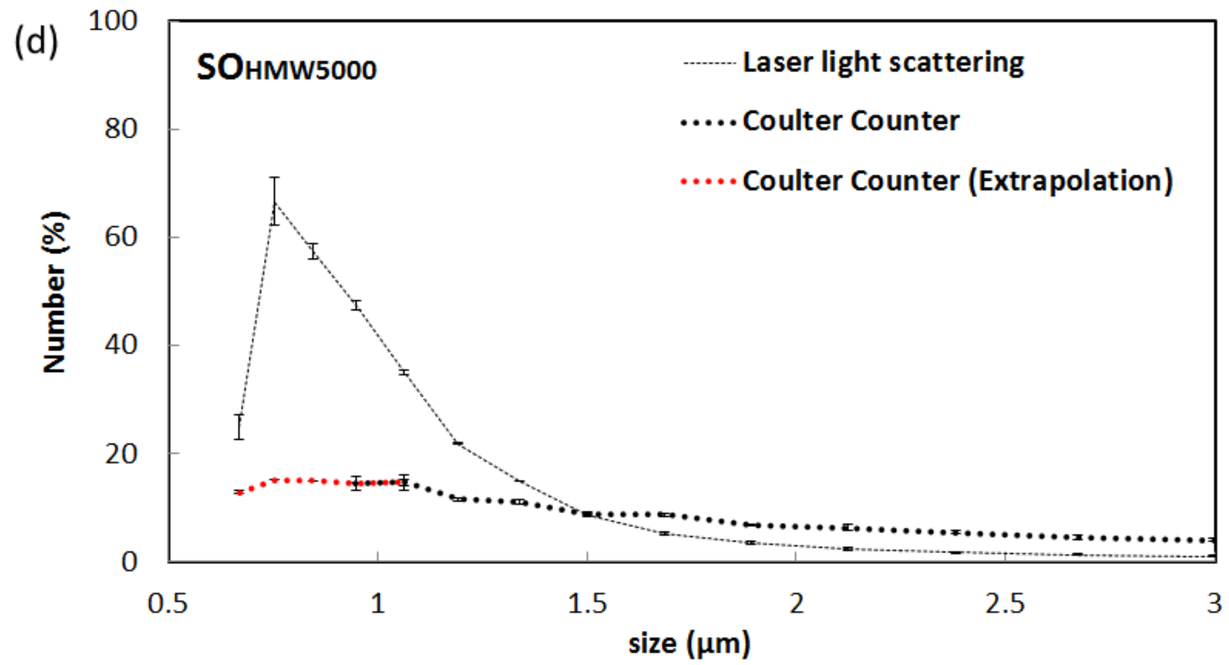

SOHMW5000
$R^2 = 0.978$
$CC = 16.490 - 17.542\,e^{-0.313\,LSC^{0.505}}$
Coulter Counter (CC)
Laser Light Scattering (LLS)

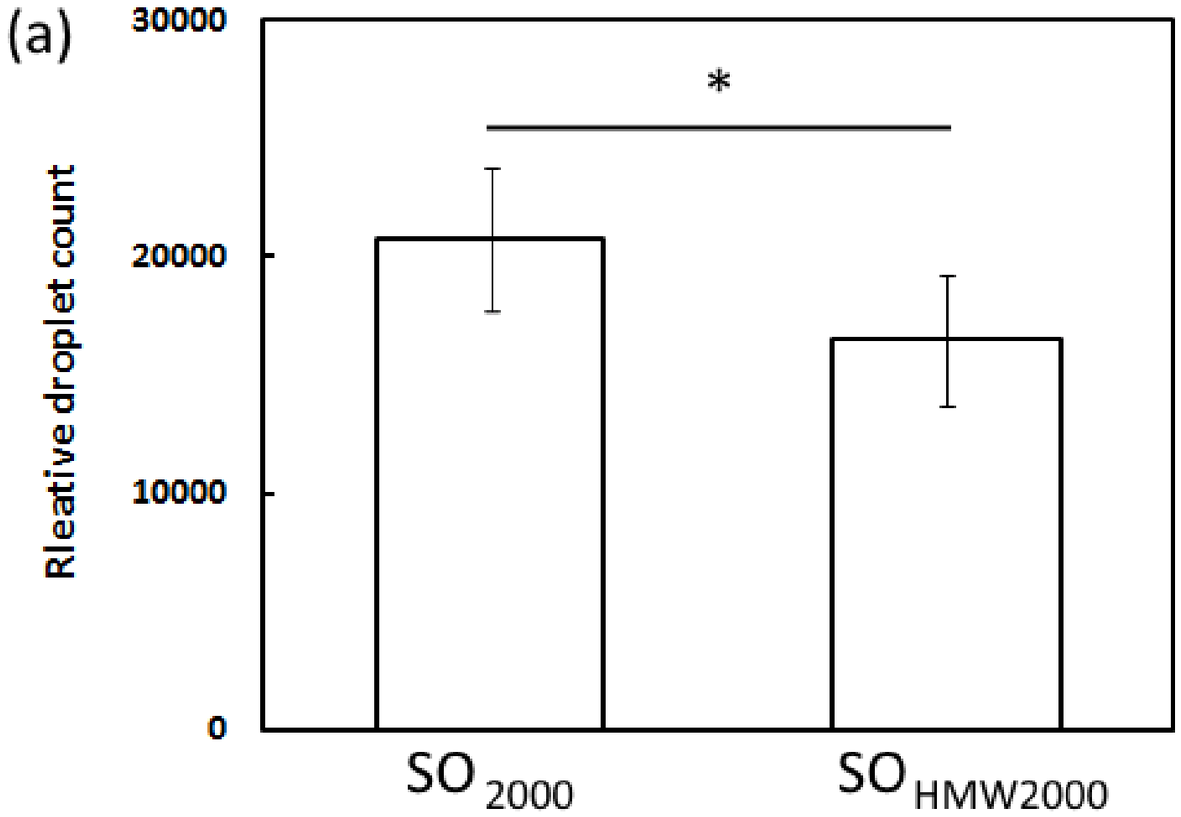

(a)
SO1300
in-vivo (-2 S.D.)
in-vivo (mean)
in-vivo (+2 S.D.)
in-vitro (mean)
Number (%)
Size (µm)
(b)
SO5000
in-vivo (-2 S.D.)
in-vivo (mean)
in-vivo (+2 S.D.)
in-vitro (mean)
Number (%)
Size (µm)

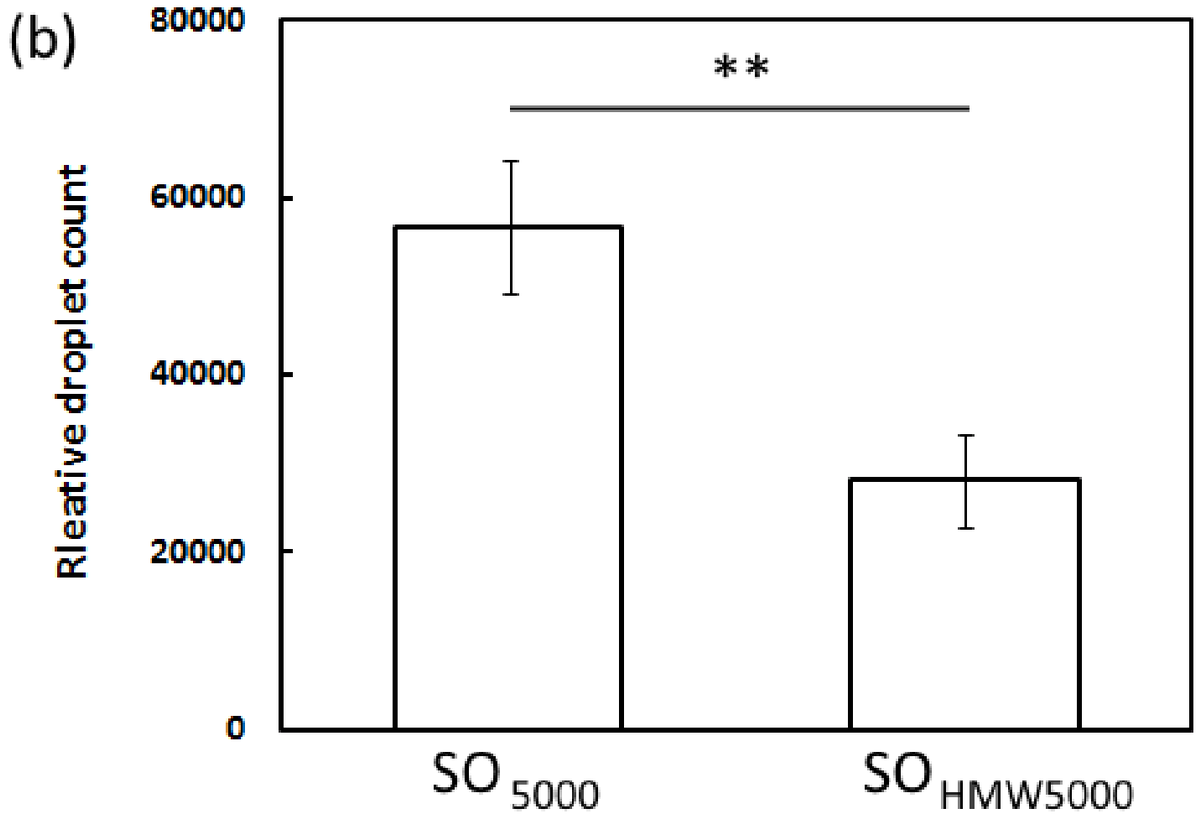

(a)
SO2000
Laser light scattering
Coulter Counter
Coulter Counter (Extrapolation)
Number (%)
size (um)


(b)
SOHMW2000
Laser light scattering
Coulter Counter
Coulter Counter (Extrapolation)
Number (%)
size (um)


(c)
SO5000
Laser light scattering
Coulter Counter
Coulter Counter (Extrapolation)
Number (%)
size (µm)


(d)
SOHMW5000
Laser light scattering
Coulter Counter
Coulter Counter (Extrapolation)
Number (%)
size (µm)


(a)
30000
20000
10000
0
Rleative droplet count
*
SO 2000
SO HMW2000


(b)
80000
60000
40000
20000
0
Rleative droplet count
**
SO 5000
SO HMW5000