### Fwd: Freezing and coating

Please see email from Dr Smith which proves to him that our Nanobubble count is real and does not include other particles.

Begin forwarded message:

From: Steve Ward-Smith Subject: Freezing and coating Date: 16 March 2018 at 14:42:00 GMT

Dear Tony,

We froze then defrosted some of the water we generated. Counts then back to similar levels as the blank, so killed all the bubbles. This proves to any doubters that it is indeed bubbles not any other particulate contamination.

Have you any ideas on easy ways to generate coated bubbles? We have another technology for bubble measurement but have a suspicionit works best when bubbles are coated. Was thinking generation in soapy water, but would that form a foam? Or a protein solution? All ideas welcome..

Regards, Steve

Stephen Ward-Smith Key Account Manager



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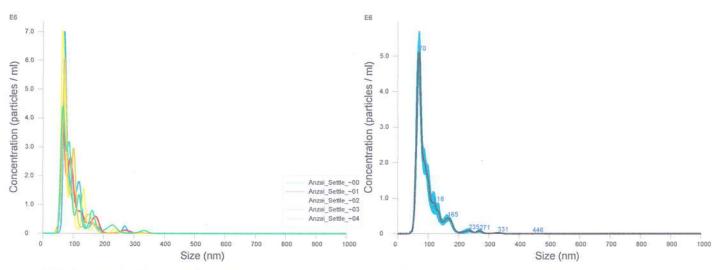
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Compary registered in England No. 1020602 Registered office as per above.

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### Anzai Settle time





### Averaged FTLA Concentration / Size for Experiment: Anzai Settle time

Error bars indicate + / -1 standard error of the mean

Anzai\_Settle\_time\_00 Anzai\_Settle\_time\_01 Anzai\_Settle\_time\_02 Anzai\_Settle\_time\_03 Anzai\_Settle\_time\_04

Included Files

### Details

NTA Version:

Script Used:

Operator: Pre-treatment: Sample Name: Diluent: Remarks:

Time Captured:

NTA 3.2 Dev Build 3.2.16 13:03:23 09/03/2018

SOP Standard Measurement 12-36-44PM 09~

### Results

Stats: Merged Da	ta
Mean:	96.3 nm
Mode:	69.5 nm
SD:	42.3 nm
D10:	61.9 nm
D50:	80.4 nm
D90:	151.4 nm

Stats: Mean +/- Standard Error

Stats. Mean +/- Standard	EITOI
Mean:	96.2 +/- 2.8 nm
Mode:	68.4 +/- 1.4 nm
SD:	41.1 +/- 3.5 nm
D10:	62.5 +/- 1.4 nm
D50:	80.4 +/- 2.6 nm
D90:	149.4 +/- 7.2 nm
Concentration (Upgrade):	1.87e+008 +/- 3.76e+006 particles/ml
	22.6 +/- 0.6 particles/frame
	28.2 +/- 1.1 centres/frame

Concentration measurements may require some caution due to noise See summary file for more info

### Camera Type: Laser Type:

**Capture Settings** 

Camera Level: Slider Shutter: Slider Gain: FPS Number of Frames: Temperature: Viscosity: Dilution factor: Syringe Pump Speed:

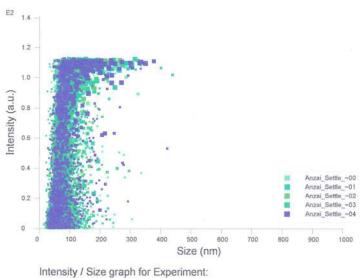
sCMOS Blue405 Manual settings used 1300 512 25.0 2248 23.5 - 23.7 °C (Water) 0.915 - 0.919 cP Dilution not recorded 80

### Analysis Settings

Detect Threshold: 6 Blur Size: Auto Max Jump Distance:

Auto: 14.3 - 16.0 pix

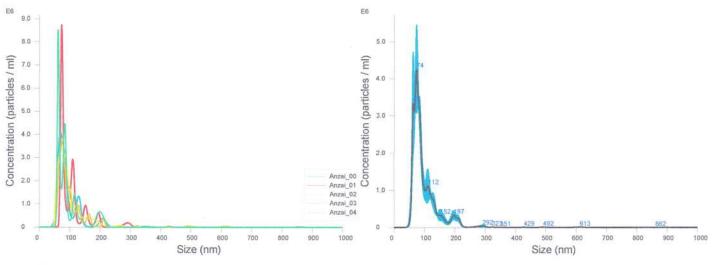
## Anzai Settle time



Anzai\_Settle\_time\_

### Script Used: (Full Text):

SOP Standard Measurement 12-36-44PM 09Mar2018.txt



FTLA Concentration / Size graph for Experiment: Anzai

### Averaged FTLA Concentration / Size for Experiment: Anzai\_ Error bars indicate + / -1 standard error of the mean

### Included Files

Anzai\_00 Anzai\_01 Anzai\_02 Anzai\_03 Anzai 04

### Details

Operator:

Sample Name: Diluent: Remarks:

**Capture Settings** 

NTA Version: NTA 3.2 Dev Build 3.2.16 Script Used: SOP Standard Measurement 12-36-44PM 09~ Time Captured: 12:36:54 09/03/2018 Pre-treatment:

### Results

Stats: Merged Data	
Mean:	99.1 nm
Mode:	73.4 nm
SD:	51.8 nm
D10:	61.6 nm
D50:	81.4 nm
D90:	154.1 nm

#### Stats: Mean +/- Standard Error

Mean:	99.2 +/- 2.6 nm
Mode:	70.7 +/- 2.6 nm
SD:	51.0 +/- 4.8 nm
D10:	62.8 +/- 1.9 nm
D50:	81.8 +/- 0.9 nm
D90:	157.6 +/- 7.6 nm
Concentration (Upgrade):	1.80e+008 +/- 4.51e+006 particles/ml
	21.9 +/- 0.5 particles/frame
	27.8 +/- 0.4 centres/frame

Concentration measurements may require some caution due to noise See summary file for more info

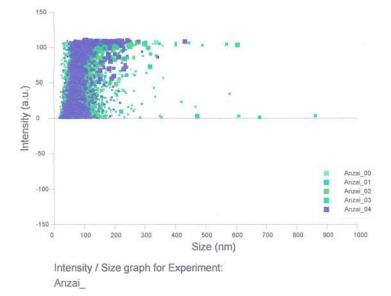
Camera Type:
Laser Type:
Camera Level:
Slider Shutter:
Slider Gain:
FPS
Number of Frames:
Temperature:
Viscosity:
Dilution factor:
Syringe Pump Speed:

sCMOS Blue405 Manual settings used 1300 512 25.0 2248 22.7 - 23.2 °C (Water) 0.926 - 0.938 cP Dilution not recorded 80

#### Analysis Settings

Detect Threshold: 6 Blur Size: Auto Max Jump Distance:

Auto: 12.8 - 16.0 pix

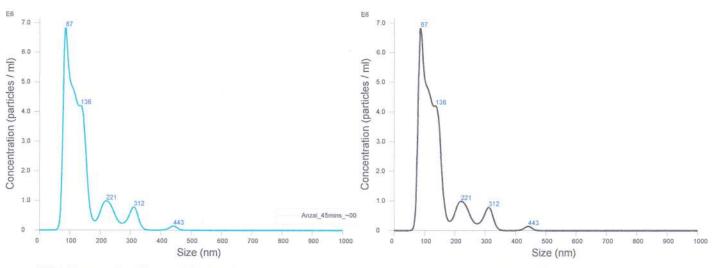


### Script Used: (Full Text):

SOP Standard Measurement 12-36-44PM 09Mar2018.txt

### Anzai

### Anzai 45mins



Results

Mean:

Mode:

SD:

D10:

D50:

D90:

Stats: Merged Data

FTLA Concentration / Size graph for Experiment: Anzai\_45mins\_

### Averaged FTLA Concentration / Size for Experiment: Anzai\_45mins\_

Error bars indicate + / -1 standard error of the mean

141.3 nm

86.2 nm

68.0 nm

82.4 nm

119.6 nm

239.3 nm

### Included Files

Anzai\_45mins\_00

### Details

NTA Version:NTA 3.2 Dev Build 3.2.16Script Used:SOP Standard Measurement 02-14-44PM 09~Time Captured:14:15:31 09/03/2018Operator:Pre-treatment:Sample Name:Jiluent:Diluent:Remarks:

### **Capture Settings**

Camera Type:	sCMOS
Laser Type:	Blue405
Camera Level:	14
Slider Shutter:	1259
Slider Gain:	366
FPS	25.0
Number of Frames:	1498
Temperature:	23.1 °C
Viscosity:	(Water) 0.9 cP
Dilution factor:	Dilution not recorded
Syringe Pump Speed:	80

### Analysis Settings

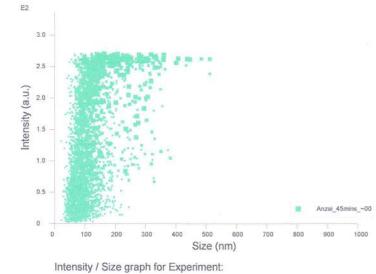
Detect Threshold:	6
Blur Size:	Auto
Max Jump Distance:	Auto: 12.7 pix

### Stats: Mean +/- Standard Error

141.3 +/- 0.0 nm
86.2 +/- 0.0 nm
68.0 +/- 0.0 nm
82.4 +/- 0.0 nm
119.6 +/- 0.0 nm
239.3 +/- 0.0 nm
4.99e+008 +/- 0.00e+000 particles/ml
60.7 +/- 0.0 particles/frame
63.7 +/- 0.0 centres/frame

## Anzai 45mins



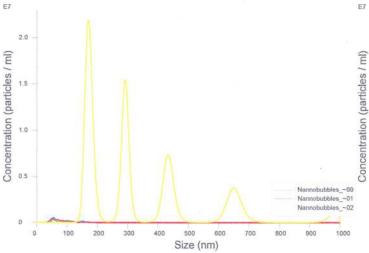


Anzai\_45mins\_

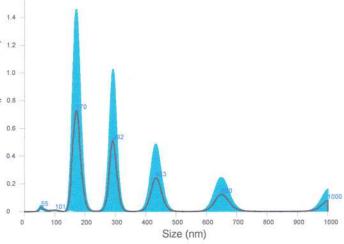
### Script Used: (Full Text):

SOP Standard Measurement 02-14-44PM 09Mar2018.txt

## Nannobubbles



FTLA Concentration / Size graph for Experiment: Nannobubbles\_



Averaged FTLA Concentration / Size for Experiment: Nannobubbles\_ Error bars indicate + / -1 standard error of the mean

337.6 nm

169.9 nm

204.4 nm

160.4 nm

286.9 nm

### Included Files

Nannobubbles\_00 Nannobubbles\_01 Nannobubbles\_02

### Details

NTA Version: Script Used: Time Captured: Operator: Pre-treatment: Sample Name: Diluent: Remarks:

### **Capture Settings**

Camera Type:	sCMOS
Laser Type:	Blue405
Camera Level:	16
Slider Shutter:	1300
Slider Gain:	512
FPS	25.0
Number of Frames:	2248
Temperature:	23.0 - 23.1 °C
Viscosity:	(Water) 0.928 - 0.930 cP
Dilution factor:	Dilution not recorded
Syringe Pump Speed:	100

### Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 7.1 - 31.6 pix

NTA 3.2 Dev Build 3.2.16 SOP Standard Measurement 12-09-11PM 09~ 12:09:17 09/03/2018

D90:	649.4 nm
	-
Stats: Mean +/- Standard	Error
Mean:	169.7 +/- 86.5 nm
Mode:	92.3 +/- 38.9 nm
SD:	90.2 +/- 56.5 nm
D10:	86.3 +/- 38.0 nm
D50:	147.0 +/- 70.7 nm
D90:	305.3 +/- 172.8 nm
Concentration (Upgrade):	6.12e+008 +/- 5.94e+008 particles/ml
	17.6 +/- 15.7 particles/frame
	28.9 +/- 26.7 centres/frame
Concentration measureme	ents may be unreliable

See summary file for more info

Results

Mean:

Mode:

SD:

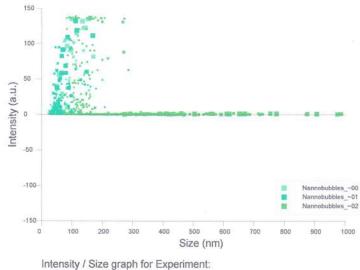
D10:

D50:

Stats: Merged Data

### Nannobubbles



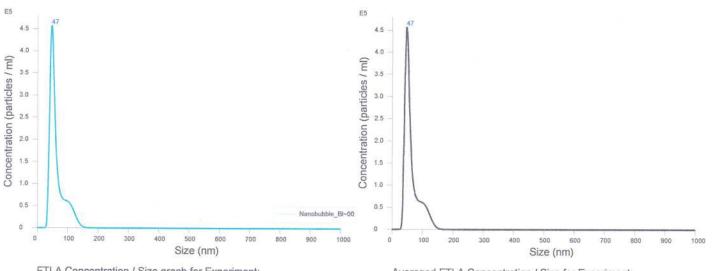


Nannobubbles\_

### Script Used: (Full Text):

SOP Standard Measurement 12-09-11PM 09Mar2018.txt

### Nanobubble Blank





### Averaged FTLA Concentration / Size for Experiment: Nanobubble Blank Error bars indicate + / -1 standard error of the mean

### Included Files

Nanobubble\_Blank\_00

### Details

NTA Version: NTA 3.2 Dev Build 3.2.16 Script Used: SOP Standard Measurement 11-40-23AM 09~ Time Captured: 11:40:29 09/03/2018 Operator: Pre-treatment: Sample Name: Diluent:

### **Capture Settings**

Remarks:

Camera Type:	sCMOS
Laser Type:	Blue405
Camera Level:	16
Slider Shutter:	1300
Slider Gain:	512
FPS	25.0
Number of Frames:	1498
Temperature:	21.2 °C
Viscosity:	(Water) 1.0 cP
Dilution factor:	Dilution not recorded
Syringe Pump Speed:	100

### Analysis Settings

Detect Threshold:	5
Blur Size:	Auto
Max Jump Distance:	Auto: 14.4 pix

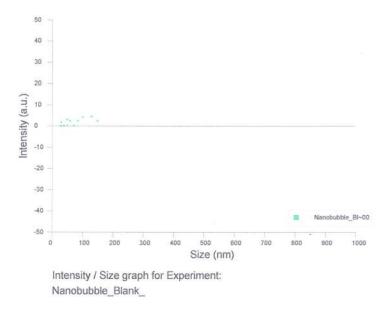
## Results

Stats: Merged Data	
Mean:	63.5 nm
Mode:	46.7 nm
SD:	24.8 nm
D10:	40.1 nm
D50:	53.2 nm
D90:	103.4 nm

#### Stats: Mean +/- Standard Error

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Mean:	63.5 +/- 0.0 nm
Mode:	46.7 +/- 0.0 nm
SD:	24.8 +/- 0.0 nm
D10:	40.1 +/- 0.0 nm
D50:	53.2 +/- 0.0 nm
D90:	103.4 +/- 0.0 nm
Concentration (Upgrade):	1.35e+007 +/- 0.00e+000 particles/ml
	0.6 +/- 0.0 particles/frame
	1.4 +/- 0.0 centres/frame
Concentration measureme	ents may be unreliable
See summary file for more	e info

## Nanobubble Blank



### Script Used: (Full Text):

SOP Standard Measurement 11-40-23AM 09Mar2018.txt

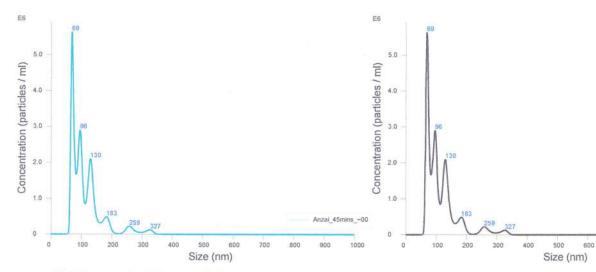
### Anzai 45mins 10x

700

800

900

1000



FTLA Concentration / Size graph for Experiment: Anzai\_45mins\_10x\_

### Included Files

Anzai\_45mins\_10x\_00

### Details

NTA Version: NTA 3.2 Dev Build 3.2.16 Script Used: SOP Standard Measurement 02-14-44PM 09~ Time Captured: 14:27:36 09/03/2018 Operator: Pre-treatment:

### Capture Settings

Sample Name:

Diluent:

Remarks:

Camera Type:	sCMOS
Laser Type:	Blue405
Camera Level:	16
Slider Shutter:	1300
Slider Gain:	512
FPS	25.0
Number of Frames:	1498
Temperature:	23.2 °C
Viscosity:	(Water) 0.9 cP
Dilution factor:	Dilution not recorded
Syringe Pump Speed:	80

#### **Analysis Settings**

Detect Threshold:	6
Blur Size:	Auto
Max Jump Distance:	Auto: 14.3 pix

Averaged FTLA Concentration / Size for Experiment: Anzai\_45mins\_10x\_ Error bars indicate + / -1 standard error of the mean

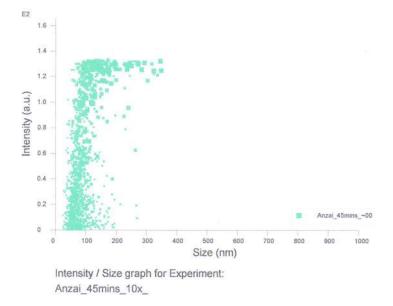
### Results

Stats: Merged Data	
Mean:	113.3 nm
Mode:	68.7 nm
SD:	53.4 nm
D10:	66.6 nm
D50:	96.2 nm
D90:	176.7 nm

### Stats: Mean +/- Standard Error

otats. Mean 17- Otanuaru	LIIUI
Mean:	113.3 +/- 0.0 nm
Mode:	68.7 +/- 0.0 nm
SD:	53.4 +/- 0.0 nm
D10:	66.6 +/- 0.0 nm
D50:	96.2 +/- 0.0 nm
D90:	176.7 +/- 0.0 nm
Concentration (Upgrade):	2.11e+008 +/- 0.00e+000 particles/ml
	26.0 +/- 0.0 particles/frame
	30.9 +/- 0.0 centres/frame

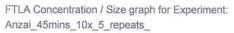
## Anzai 45mins 10x



### Script Used: (Full Text):

SOP Standard Measurement 02-14-44PM 09Mar2018.txt

#### E6 E6 7.0 4.0 Concentration (particles / ml) Concentration (particles / ml) 3.5 3,0 2.5 2.0 1.5 Anzai\_45mins\_~00 1.0 Anzai\_45mins\_-01 Anzai\_45mins\_-02 Anzai\_45mins\_-03 1.0 0.5 Anzai 45mins -04 0 0 500 0 100 400 600 700 1000 200 300 800 900 Size (nm)



### Included Files

Anzai\_45mins\_10x\_5\_repeats\_00 Anzai\_45mins\_10x\_5\_repeats\_01 Anzai\_45mins\_10x\_5\_repeats\_02 Anzai\_45mins\_10x\_5\_repeats\_03 Anzai\_45mins\_10x\_5\_repeats\_04

### Details

NTA Version:NTA 3.2 Dev Build 3.2.16Script Used:SOP Standard Measurement 02-32-49PM 09~Time Captured:14:32:59 09/03/2018Operator:Pre-treatment:Sample Name:Jiluent:Diluent:Remarks:

### Capture Settings

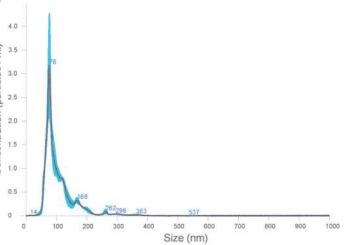
Camera Type:	sCMOS
Laser Type:	Blue405
Camera Level:	16
Slider Shutter:	1300
Slider Gain:	512
FPS	25.0
Number of Frames:	2248
Temperature:	23.5 - 23.7 °C
Viscosity:	(Water) 0.916 - 0.920 cP
Dilution factor:	Dilution not recorded
Syringe Pump Speed:	80

### Analysis Settings

Detect Threshold:	6
Blur Size:	Au
Max Jump Distance:	Au

uto uto: 14.3 - 27.6 pix

## Anzai 45mins 10x 5 repeats



Averaged FTLA Concentration / Size for Experiment: Anzai\_45mins\_10x\_5\_repeats\_ Error bars indicate + / -1 standard error of the mean

### Results

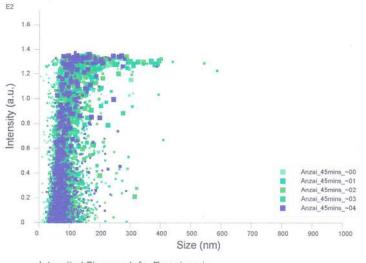
Stats: Merged Data	1
Mean:	106.0 nm
Mode:	75.4 nm
SD:	51.8 nm
D10:	64.5 nm
D50:	86.9 nm
D90:	169.4 nm

#### Stats: Mean +/- Standard Error

Mean:	106.3 +/- 3.4 nm
Mode:	77.5 +/- 3.1 nm
SD:	51.2 +/- 4.4 nm
D10:	64.4 +/- 1.1 nm
D50:	87.0 +/- 2.9 nm
D90:	172.0 +/- 4.8 nm
Concentration (Upgrade):	1.25e+008 +/- 6.44e+006 particles/ml
	15.0 +/- 0.6 particles/frame
	18.4 +/- 0.2 centres/frame

Concentration measurements may require some caution due to noise See summary file for more info

## Anzai 45mins 10x 5 repeats



Intensity / Size graph for Experiment: Anzai\_45mins\_10x\_5\_repeats\_

Script Used: (Full Text):

SOP Standard Measurement 02-32-49PM 09Mar2018.txt

