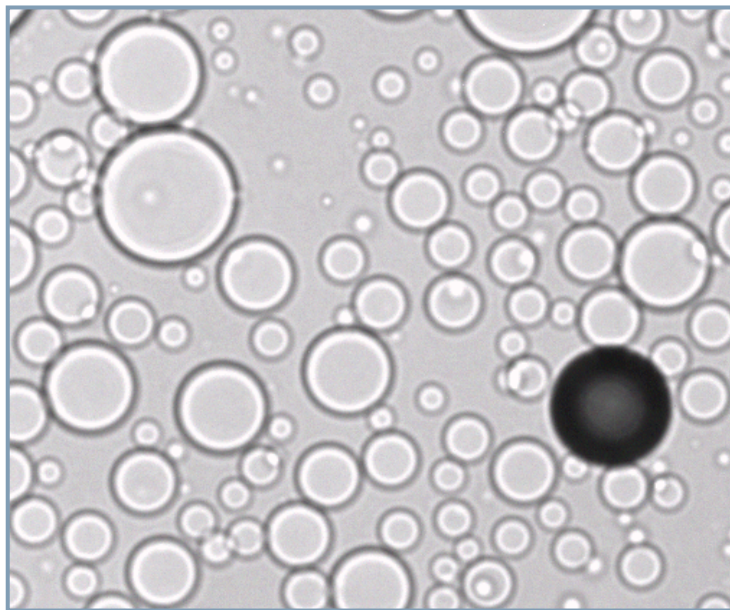


**CLEMEX**  
intelligent microscopy

**# 808**

## **Bubble Size Analysis**



Prepared by Myriam Savard Eng.  
Clemex Technologies Inc.  
For Nova Chemicals

### Sample Description

One sample composed of 90% Glycerin and 10% Mineral Oil was submitted for bubble analysis. The sample have to be mixed for two minutes before performing the analysis.

### Purpose of Analysis

To demonstrate that the Clemex Vision image analysis system can distinguish the bubbles from the glycerin and isolate the air bubbles from the oil ones. Size, shape, count and area percent measurements are requested.

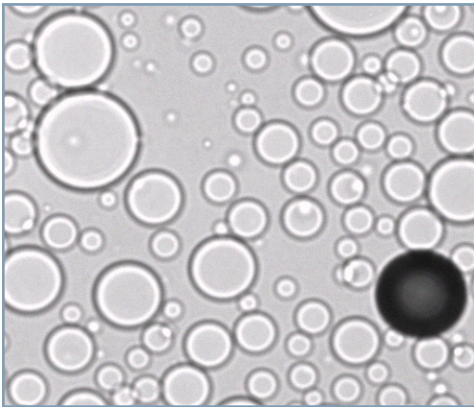


Figure 1: Part of the original image (0.6245 microns/pixel).

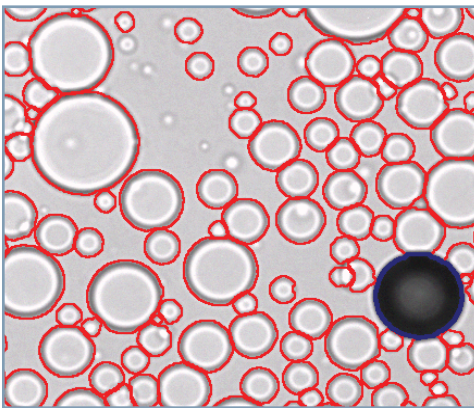


Figure 2: Outline of bubbles as measured in red (oil) and dark blue (air) bitplanes.

### Procedure<sup>1</sup>

Oil bubbles were binarized in red and air ones in cyan using Gray Threshold. Isolated oil bubbles were filled in and measured for their shape. Air bubbles were immediately suitable for all measurements. Then, all air bubbles were measured for their total area. The connected air bubbles were separated using automated tools, also making sure the bitplane representing them is the good size. Size and distribution measurements were then possible. An Opening of 5 cycles was applied on bubbles, eliminated ones that had 10 pixels in width or less.

1 Images taken during the procedure are available in appendix A

### Results

Two runs of 100 f elds each were performed on two zones of the slide, one 30 minutes after the mixing step, and the other 20 minutes later. Diameter, Area, Volume, Area Percent, Aspect Ratio, Roundness, Compactness, Roughness, Sphericity, Count, Density, Mean Spacing and Mean free path were obtained at each run.

Automated statistics and graphs were generated. Final results can be printed directly from Clemex Vision and were saved for further use. A customized report was built using the Report Generator module<sup>2</sup>. Raw data is linked to their respective objects for validation purposes. Raw data could also be exported in Excel format.

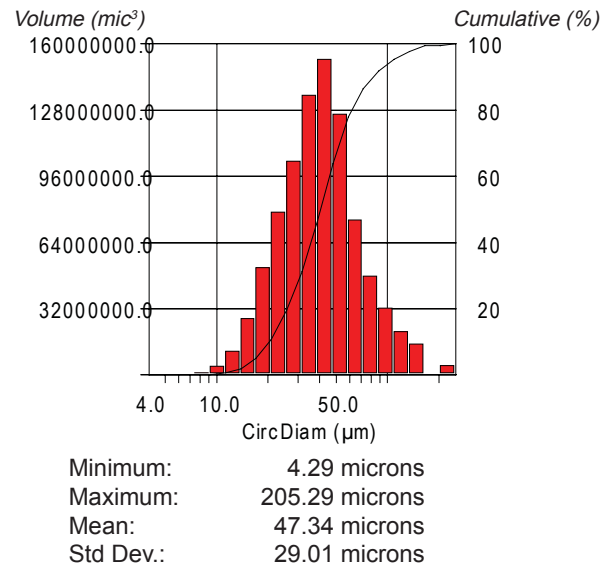


Figure 3: Oil bubbles diameter distribution and corresponding statistics.

### Equipment

Image Analysis System:	Clemex Vision PE
Microscope:	Leica DM LA
Objective/Magnification:	10x / 100x
Illumination:	Transmitted Light
Calibration:	0.6245 microns/pixel
Camera:	Clemex L 2.1M
Motorized Stage:	Marzhauser EK32IM 75x50mm
Stage Controller:	Clemex ST-2000

### Discussion

The main difficulty of this analysis was to detect all bubbles and still measuring their real size and shape. This problem was overcome measuring them in two steps. Consequently, the shape measurements were performed on the isolated bubbles only.

2 Both reports are available in appendix B.



## **Appendix A: Image Analysis Steps**



## Image Analysis Steps

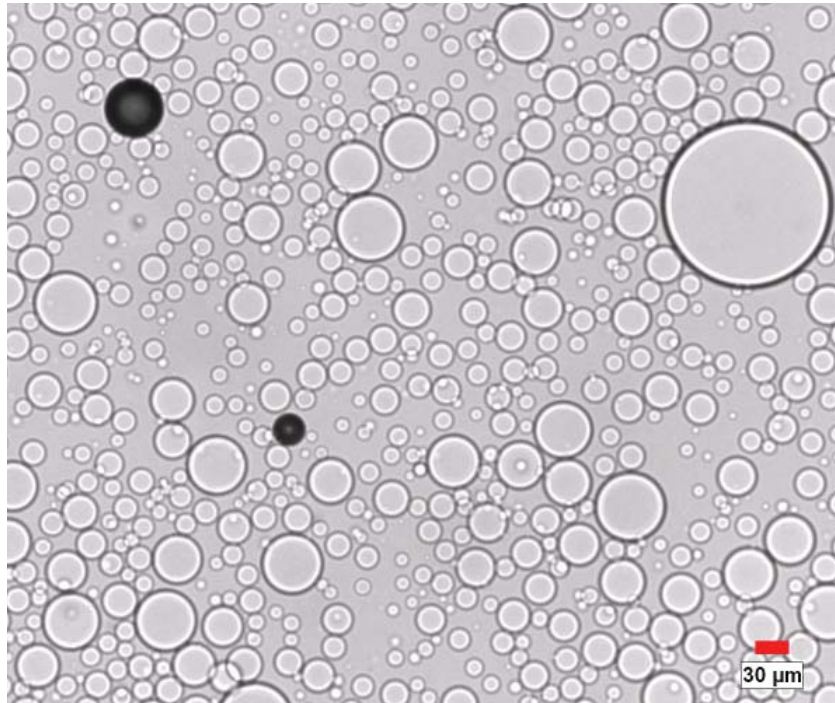


Image 1: Original image of a field containing some air bubbles (black). Magnification is 100x for a calibration factor of 0.6245 micron/pixel.

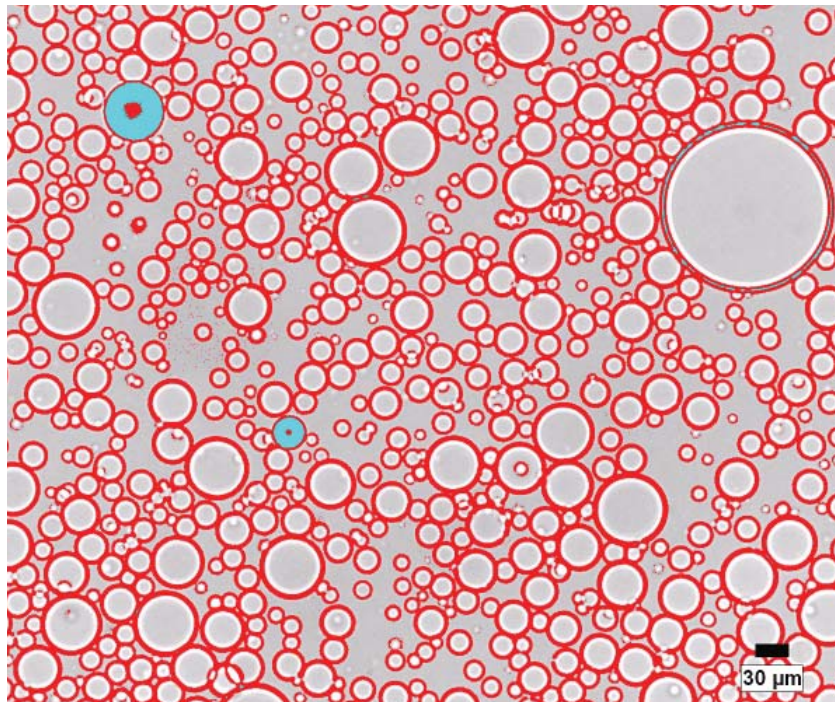
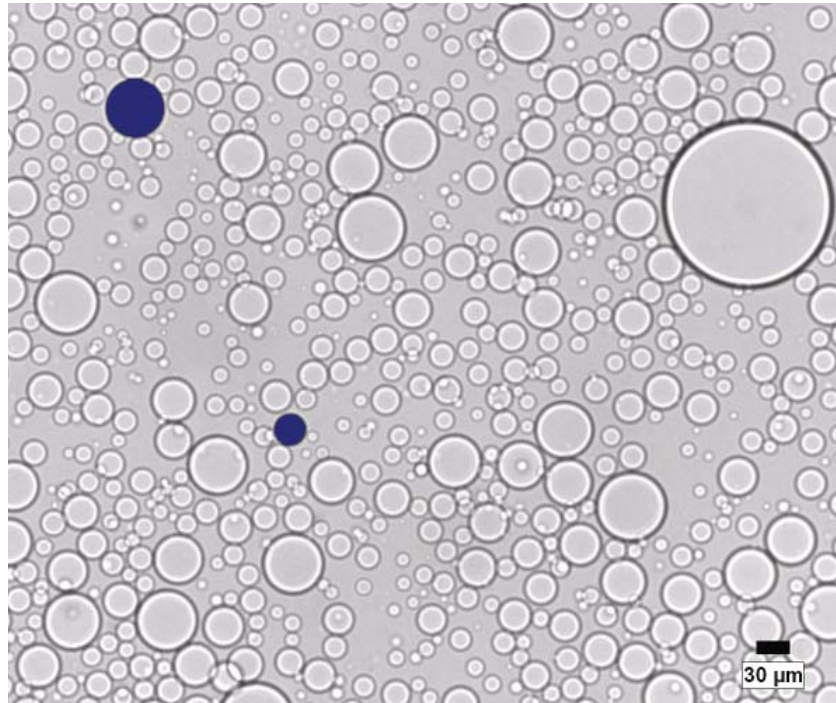
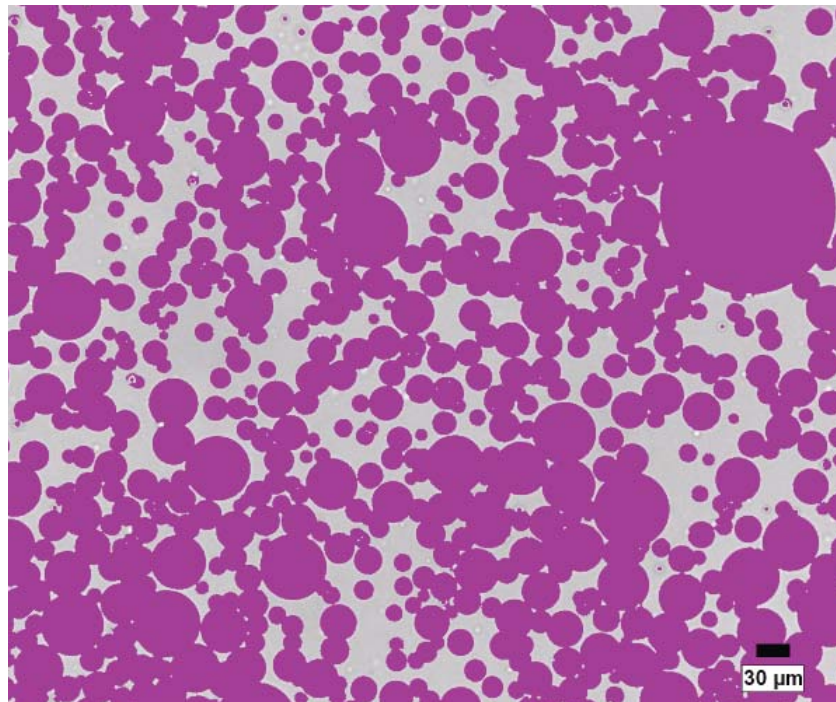


Image 2: First binarization showing detected air bubbles in cyan and the outline of oil bubbles in red. Binary operations will be performed to isolate each bubble for a correct measurement.

## Image Analysis Steps

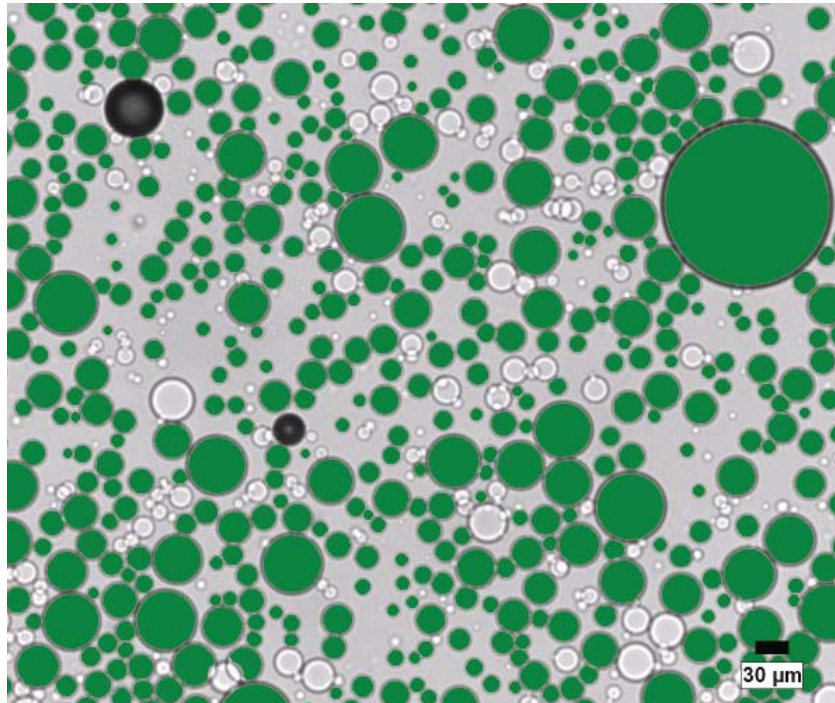


*Image 3: Air bubbles as measured for their size, shape, area percent and spacing.*

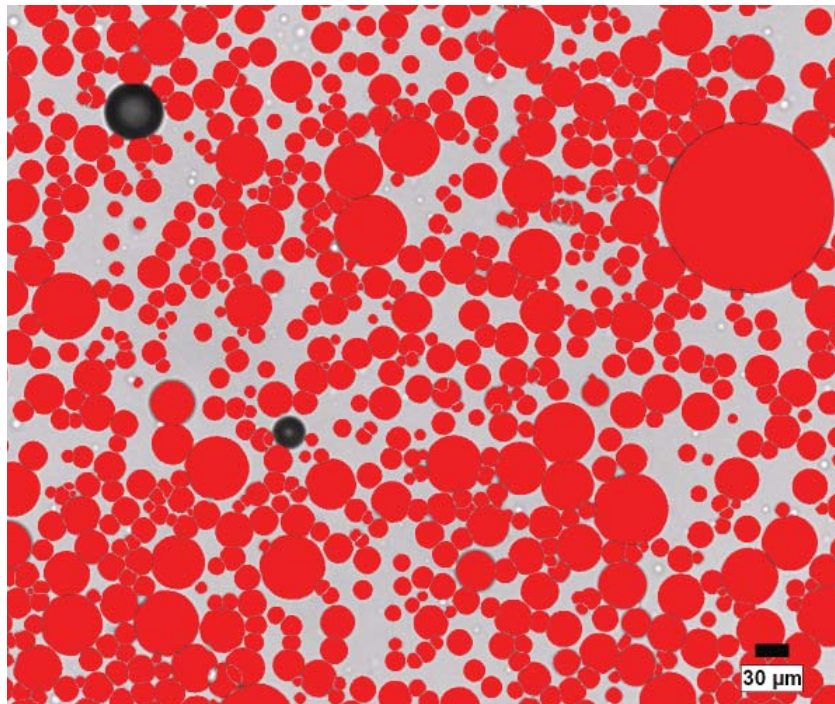


*Image 4: Oil bubbles as measured for their area percent.*

## Image Analysis Steps

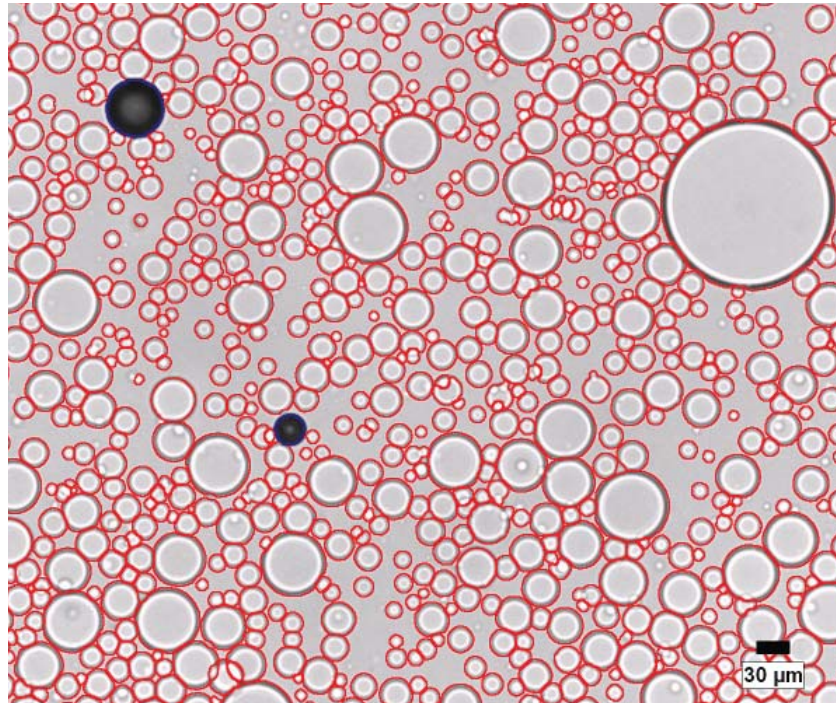


*Image 5: Air bubbles as measured for their shape. Only the isolated oil bubbles are measured at this step obtaining a more representative shape distribution than if the ones separated using the automated software tools would also be included.*

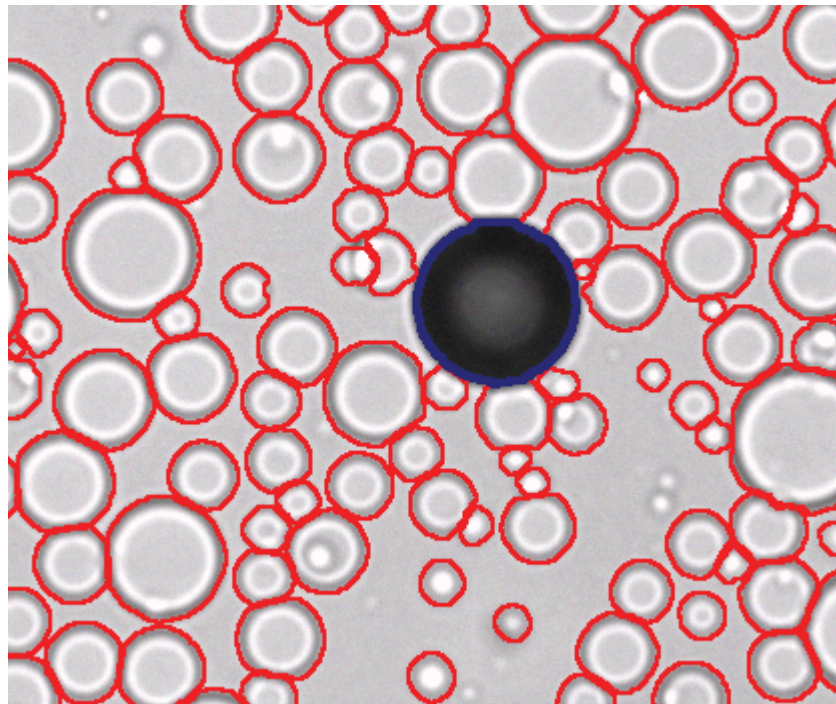


*Image 6: Connected oil bubbles were separated and were added to the isolated ones. All oil bubbles were grown back to their real size keeping one pixel spacing between them, and they were measured for their count, size and spacing. Features smaller than 10 pixels in width were eliminated.*

## Image Analysis Steps



*Image 7: Separated oil bubbles in red and air bubbles in dark blue all in outline view mode so we can see the original image through the bitplanes.*



*Image 8: Zoom in of the top left corner of previous image.*



## **Appendix B: Results**

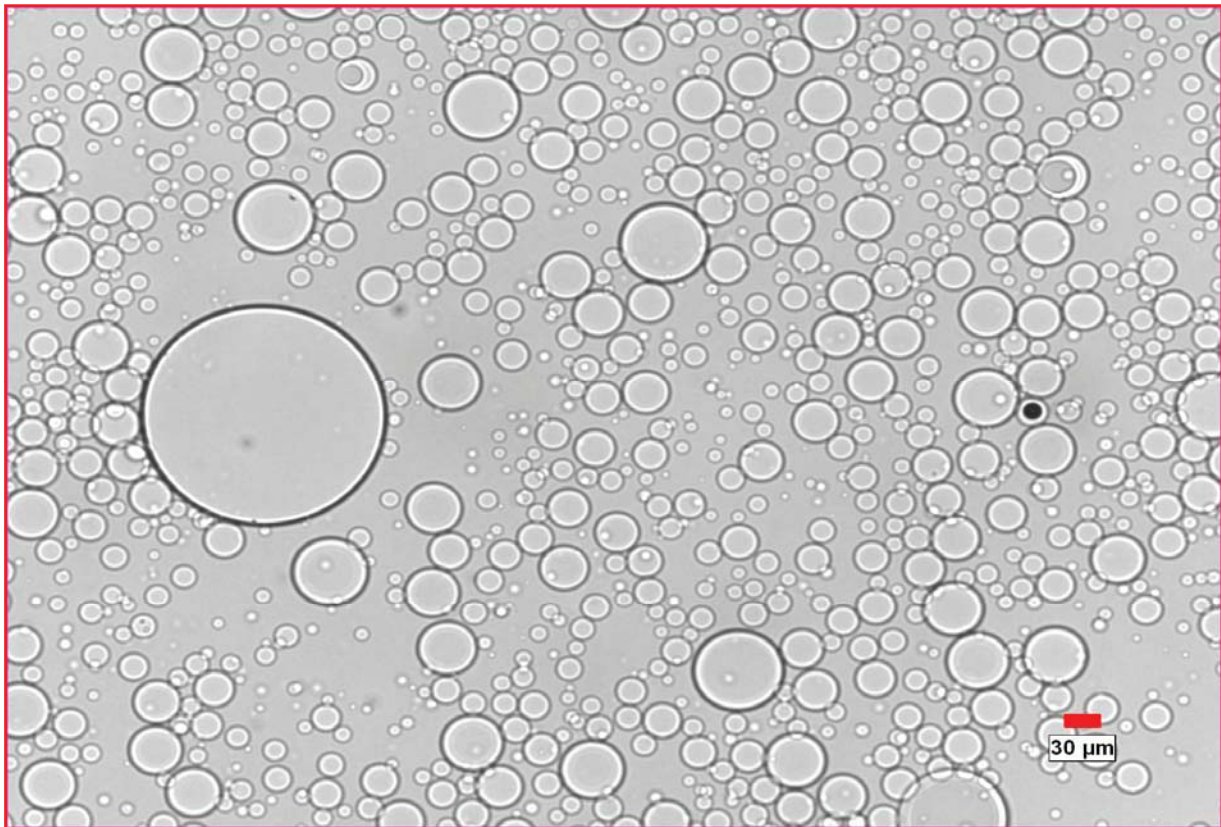


**Sample # 1 (after 30 minutes)**

### Particle Sizing Report

<b>Analysis:</b>	S1 30min	<b>Magnification:</b>	100x
<b>Date:</b>	2008-Dec-15	<b>Calibration:</b>	0.6245 µm/pixel
<b>Company:</b>	Clemex	<b>Total Fields:</b>	100
<b>Department:</b>	Laboratory ...)	<b>Total Area Covered:</b>	59295638 mic2
<b>User Name:</b>	Myriam Savard	<b>Count of all bubbles:</b>	86447
<b>Units:</b>	microns		

Figure 1: Typical field of view with the largest oil bubble found.

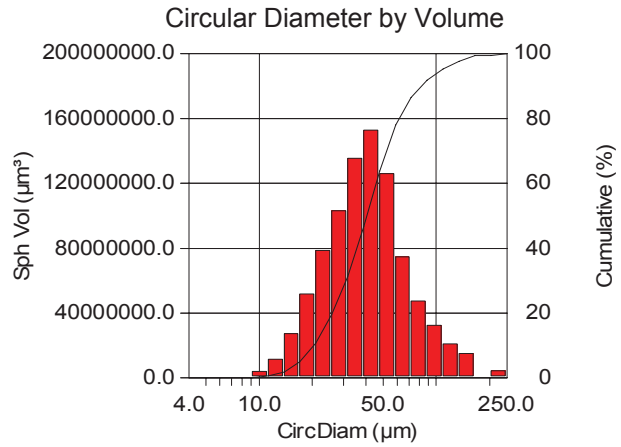
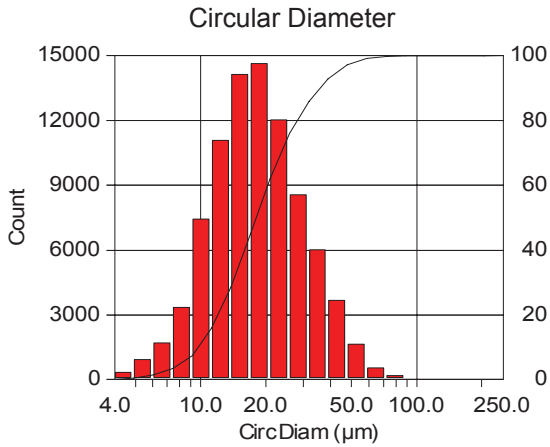


	Count	Dia- meter	Diameter (by vol.)	Area	Aspect Ratio	Round- ness	Area%	Mean Free Path *	Mean Spacing **	Density
Units	<i>n</i>	<i>mic</i>	<i>mic</i>	<i>mic2</i>	-	-	%	<i>mic</i>	<i>mic</i>	<i>/mm2</i>
<b>OIL</b>	86384	20.71	47.34	437	1.09	0.90	68.87	11.40	31.36	1457
<b>AIR</b>	63	34.74	99.11	1548	1.07	0.91	0.15	27465	27505	1.06

\* edge to edge

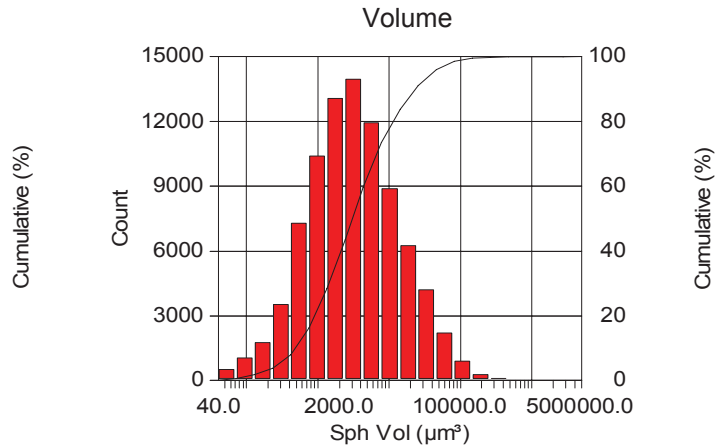
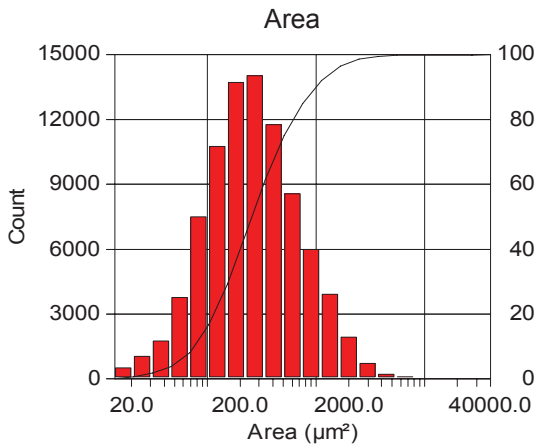
\*\* center to center

**Oil bubbles - Size**



Minimum: 4.29  
 Maximum: 205.29  
 Mean: 20.71  
 Std Dev.: 11.29  
 D10: 9.84  
 D50: 17.98  
 D90: 35.31  
 Count: 86384

4.29  
 205.29  
 47.34  
 29.01  
 20.42  
 40.56  
 82.45  
 86384

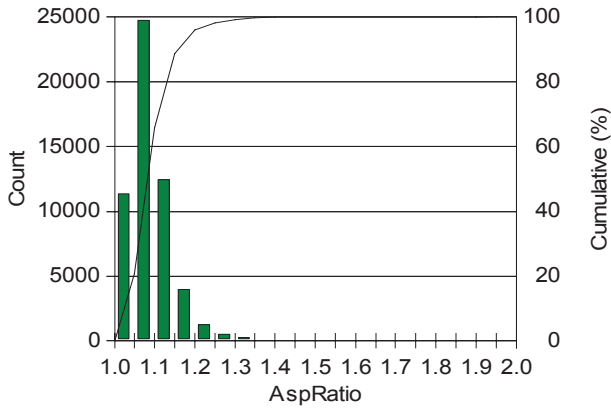


Minimum: 14.43  
 Maximum: 33101.27  
 Mean: 436.82  
 Std Dev.: 618.37  
 D10: 76.05  
 D50: 254.04  
 D90: 979.49  
 Count: 86384

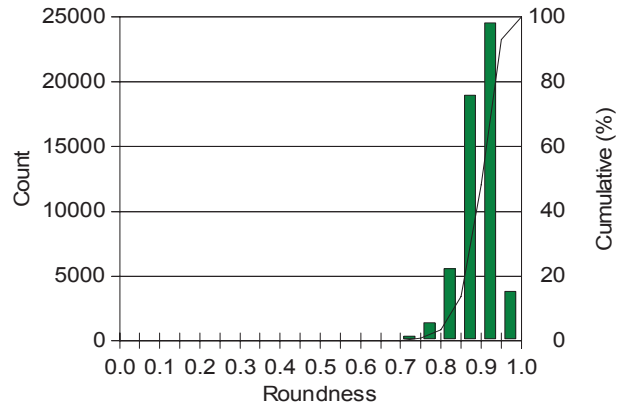
41.23  
 4530340.00  
 10278.28  
 37118.95  
 498.87  
 3045.87  
 23060.23  
 86384

## Oil bubbles - Shape

### Aspect Ratio



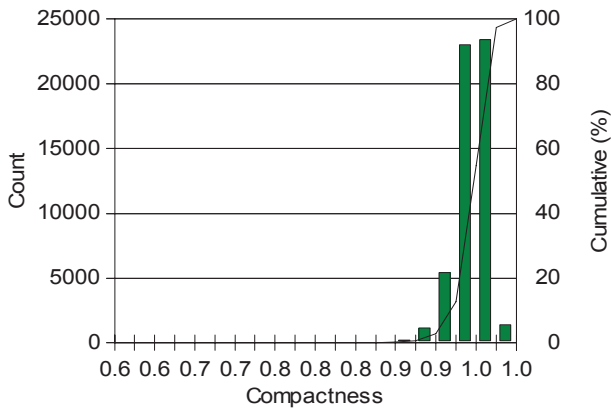
### Roundness



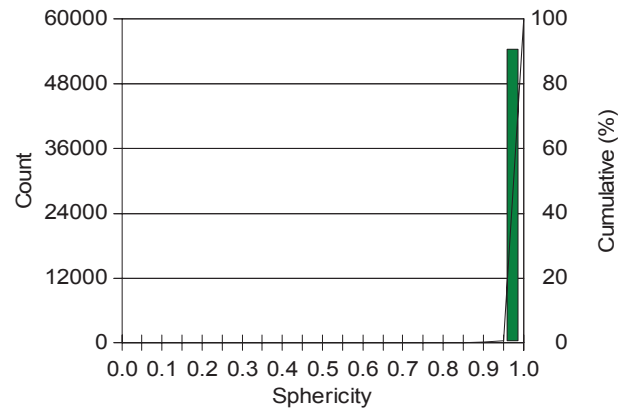
Minimum: 1.01  
 Maximum: 1.94  
 Mean: 1.09  
 Std Dev.: 0.05  
 D10: 1.04  
 D50: 1.08  
 D90: 1.16  
 Count: 54801

0.44  
 0.99  
 0.90  
 0.04  
 0.84  
 0.90  
 0.95  
 54801

### Compactness



### Sphericity



Minimum: 0.67  
 Maximum: 0.99  
 Mean: 0.96  
 Std Dev.: 0.02  
 D10: 0.94  
 D50: 0.96  
 D90: 0.97  
 Count: 54801

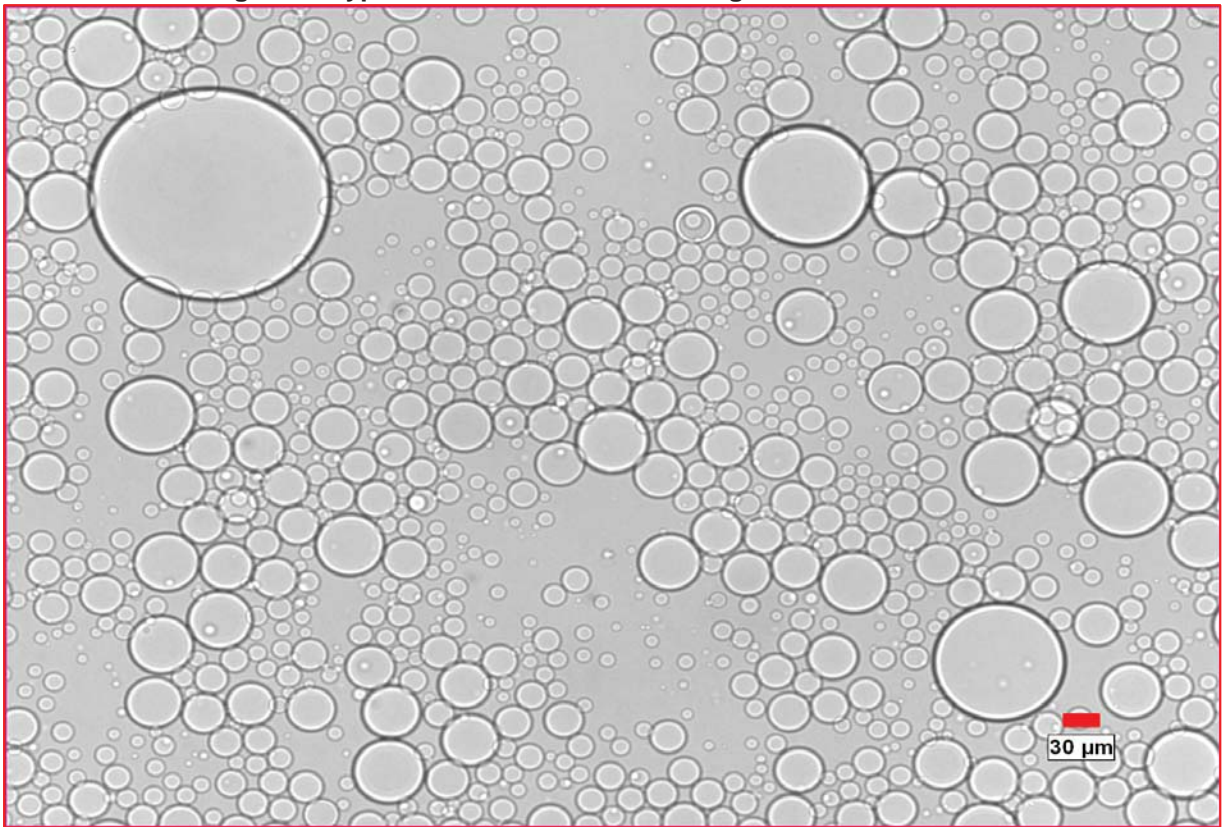
0.46  
 1.00  
 1.00  
 0.01  
 0.99  
 1.00  
 1.00  
 54801

**Sample # 2 (after 50 minutes)**

Particle Sizing Report

<b>Analysis:</b>	S2 50 min.	<b>Magnification:</b>	100x
<b>Date:</b>	2008-Dec-15	<b>Calibration:</b>	0.6245 µm/pixel
<b>Company:</b>	Clemex	<b>Total Fields:</b>	100
<b>Department:</b>	Laboratory (...)	<b>Total Area Covered:</b>	59295638 mic2
<b>User Name:</b>	Myriam Savard	<b>Count of all bubbles:</b>	86910
<b>Units:</b>	microns		

Figure 1: Typical field of view with the largest oil bubble found.

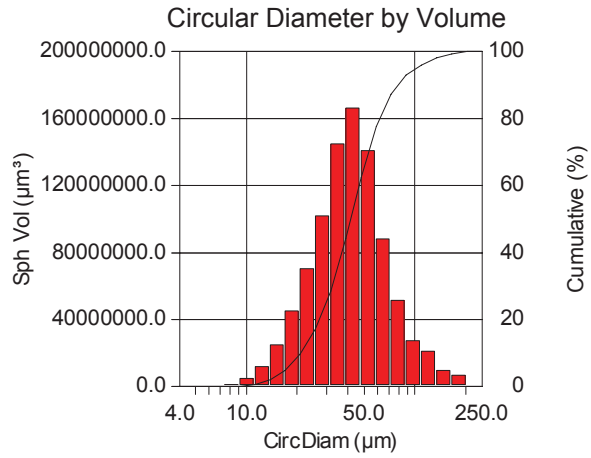
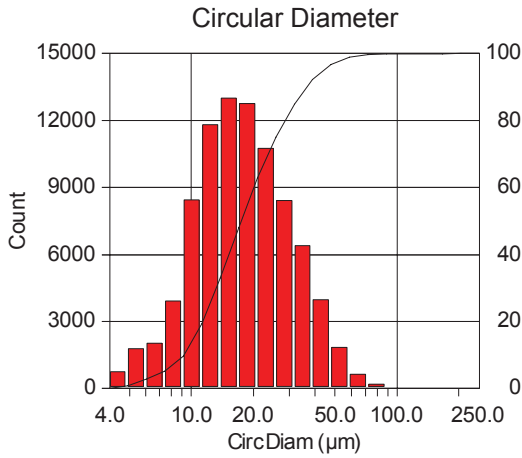


	Count	Dia- meter	Diameter (by vol.)	Area	Aspect Ratio	Round- ness	Area%	Mean Free Path *	Mean Spacing **	Density
Units	<i>n</i>	<i>mic</i>	<i>mic</i>	<i>mic2</i>	-	-	%	<i>mic</i>	<i>mic</i>	<i>/mm2</i>
<b>OIL</b>	86851	20.48	47.43	440	1.09	0.89	69.35	11.23	31.55	1465
<b>AIR</b>	59	35.47	98.11	1555	1.06	0.92	0.15	28137	28179	1.00

\* edge to edge

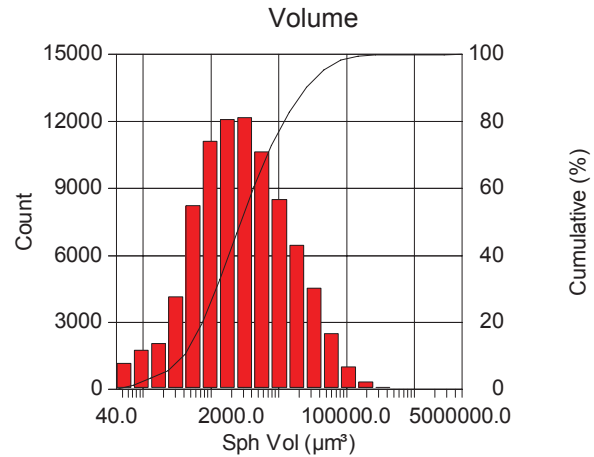
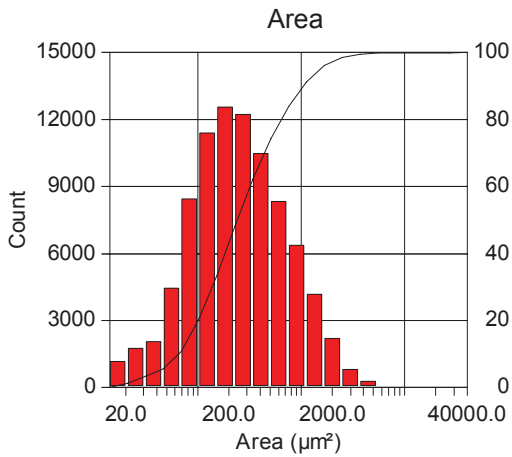
\*\* center to center

**Oil bubbles - Size**



Minimum: 4.29  
 Maximum: 198.75  
 Mean: 20.48  
 Std Dev.: 11.84  
 D10: 9.14  
 D50: 17.44  
 D90: 36.31  
 Count: 86851

4.29  
 198.75  
 47.43  
 27.43  
 21.13  
 41.68  
 78.42  
 86851

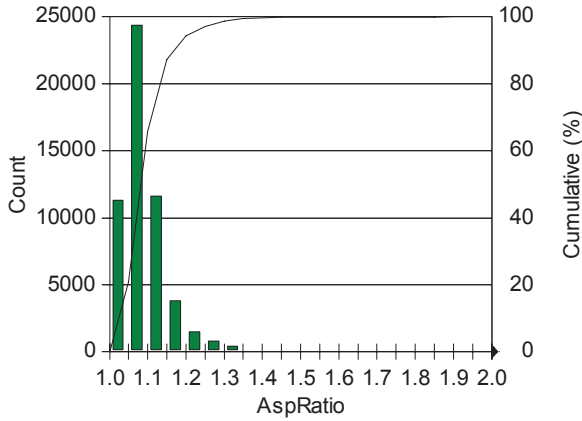


Minimum: 14.43  
 Maximum: 31023.74  
 Mean: 439.73  
 Std Dev.: 630.51  
 D10: 65.58  
 D50: 238.81  
 D90: 1035.70  
 Count: 86851

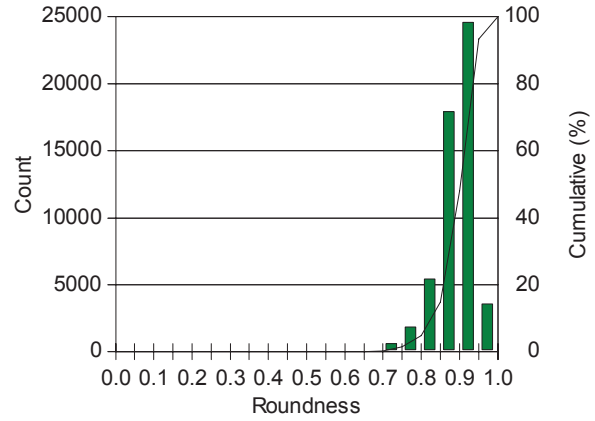
41.23  
 4110598.25  
 10574.42  
 36137.00  
 399.48  
 2776.12  
 25073.64  
 86851

**Oil bubbles - Shape**

**Aspect Ratio**



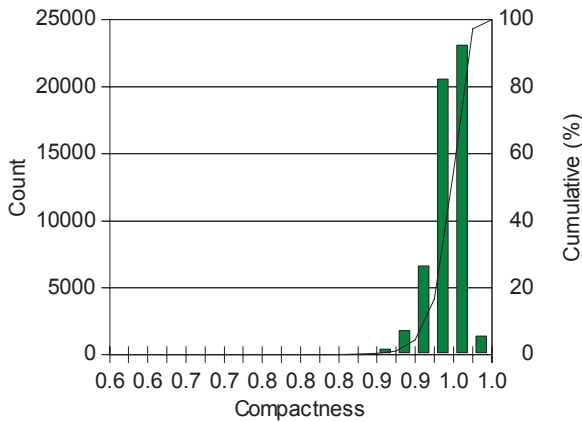
**Roundness**



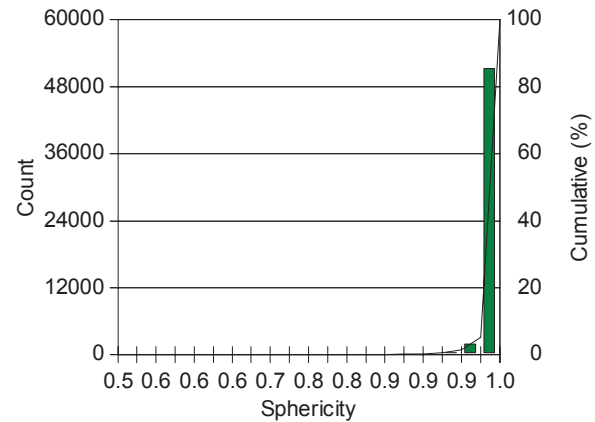
Minimum: 1.01  
 Maximum: 1.89  
 Mean: 1.09  
 Std Dev.: 0.06  
 D10: 1.04  
 D50: 1.08  
 D90: 1.16  
 Count: 54146

0.43  
 0.99  
 0.89  
 0.05  
 0.83  
 0.90  
 0.94  
 54148

**Compactness**



**Sphericity**



Minimum: 0.64  
 Maximum: 0.99  
 Mean: 0.95  
 Std Dev.: 0.02  
 D10: 0.93  
 D50: 0.96  
 D90: 0.97  
 Count: 54148

0.51  
 1.00  
 1.00  
 0.02  
 0.99  
 1.00  
 1.00  
 54148

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