POLLEN DENSITY ANALYSIS

Sample Description

Two slides with pollen particles were submitted for analysis. Slide 10/06/08 was identified as sample 1, and slide 17/04/08 was named as sample 2.

Purpose of Analysis

Demonstrate the ability of Clemex Vision image analysis system can distinguish pollen particles and evaluate their density.

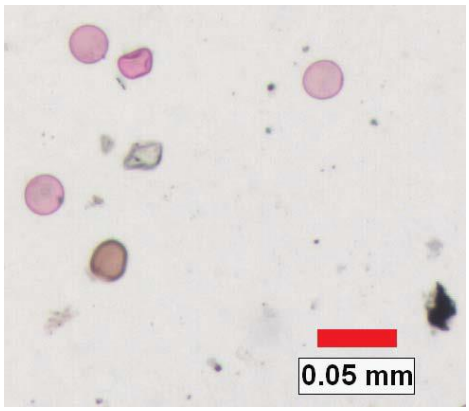


Figure 1: *Part of the original image (0.5983 µm/pixel).*

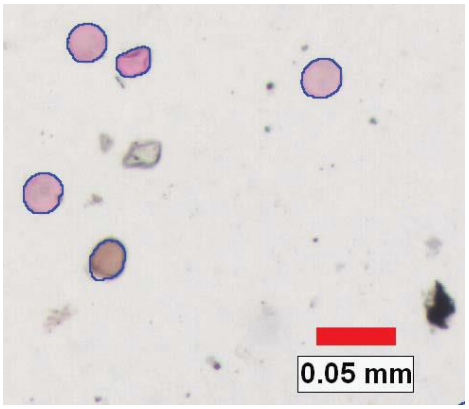


Figure 2: *Outlines of pollen particles as they were measured in blue bitplane.*

Procedure[1]

Most of the time, the pollen particles were found at different focus planes; consequently, a Multilayer-Grab was used to capture the images. The pollen particles were binarized into blue.

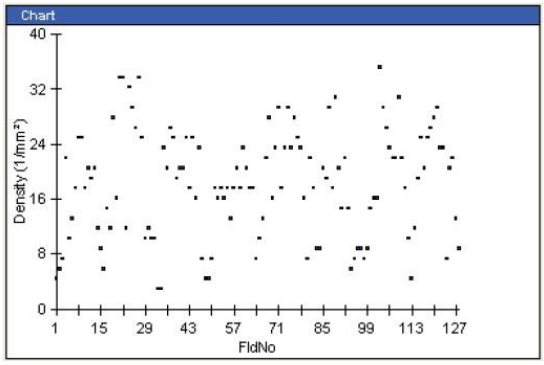
Binary operations were performed to eliminate features that were too rough, not sufficiently circular, too fuzzy, or very small. Slightly touching particles were separated prior to measurements.

Equipment

Image Analysis System:	Clemex Vision PSA
Microscope:	Leica DM LA with transmitted light
Camera:	Clemex L 2.0C
Magnification:	100X
Coupler C-mount:	0.7x
Stage:	Marzhauser EK32IM 75x50mm

Results[2]

Circular diameter and density were measured on each field. Many other measurements are available to quantify particles. Automated statistics and graphs were generated. Final results can be printed directly from Clemex Vision. Raw data are linked to their respective objects for validation purpose. Raw data can also be exported in Excel format.



Minimum:	2.934 1/mm²
Maximum:	35.214 1/mm²
Mean:	18.203 1/mm²
Std Dev.:	7.841 1/mm²

Figure 3: *Plot of the pollen density for each analyzed field of sample 1, and its corresponding statistics.*

1.Images taken during the procedure are available in appendix A
2. Complete results are available in appendix B

Appendix A: Image Analysis Steps

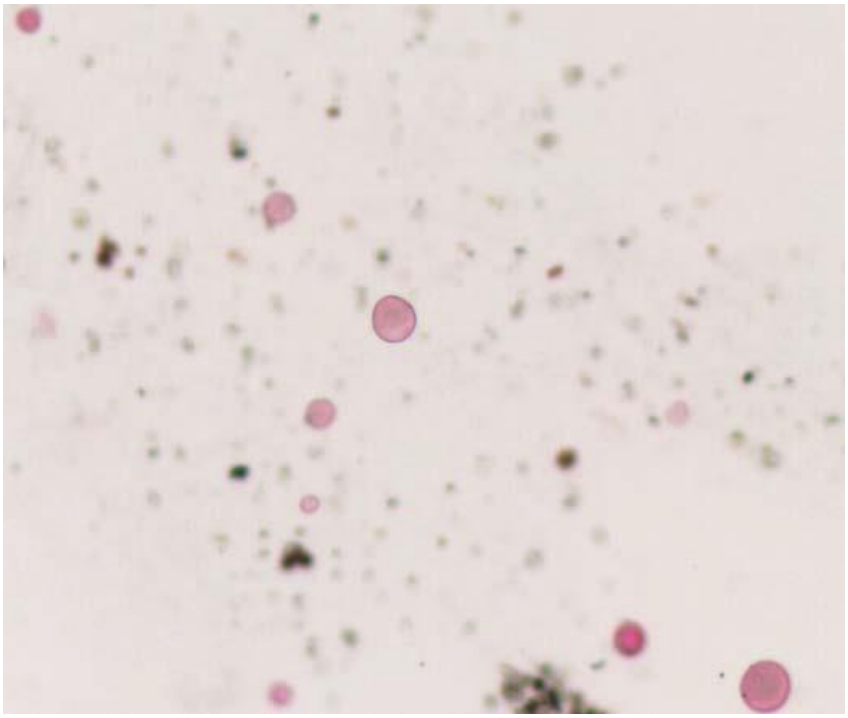


Image 1: Bottom layer of the multilayer grab.

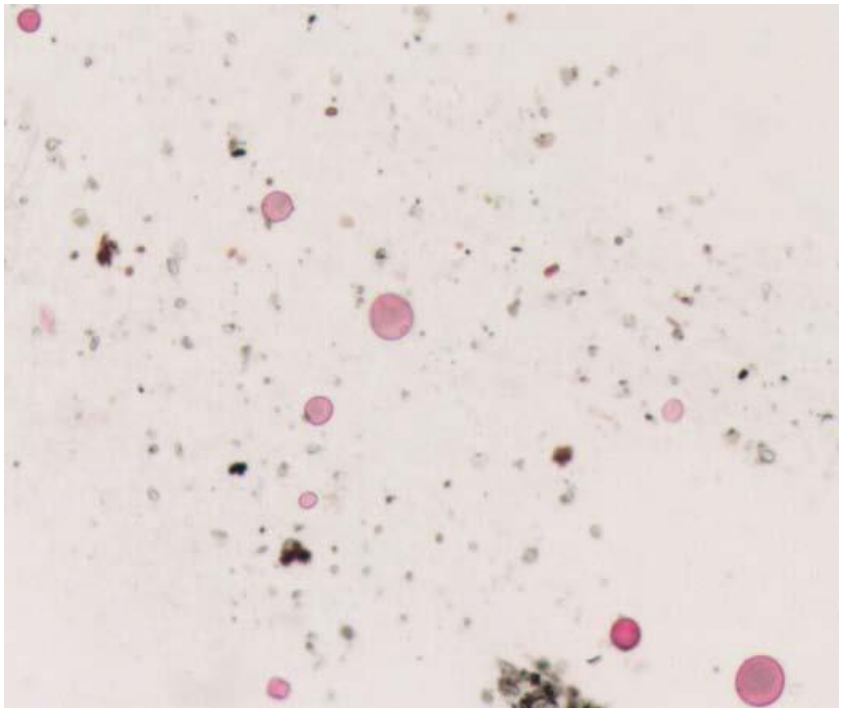


Image 2: Middle layer of the multilayer grab.

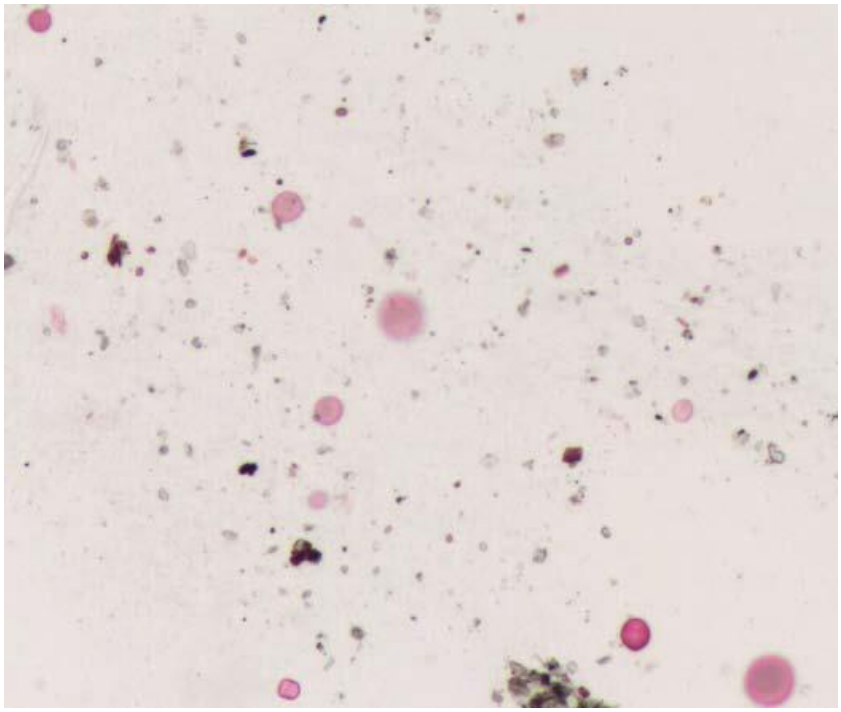


Image 3: Top layer of the multilayer grab

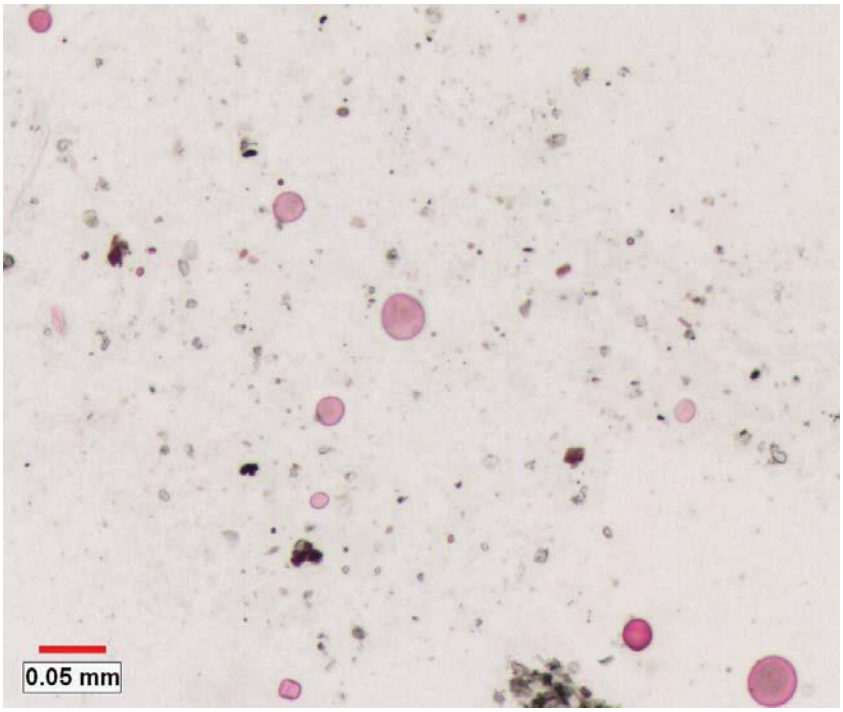


Image 4: Reconstituted image from 3 layers (100x)

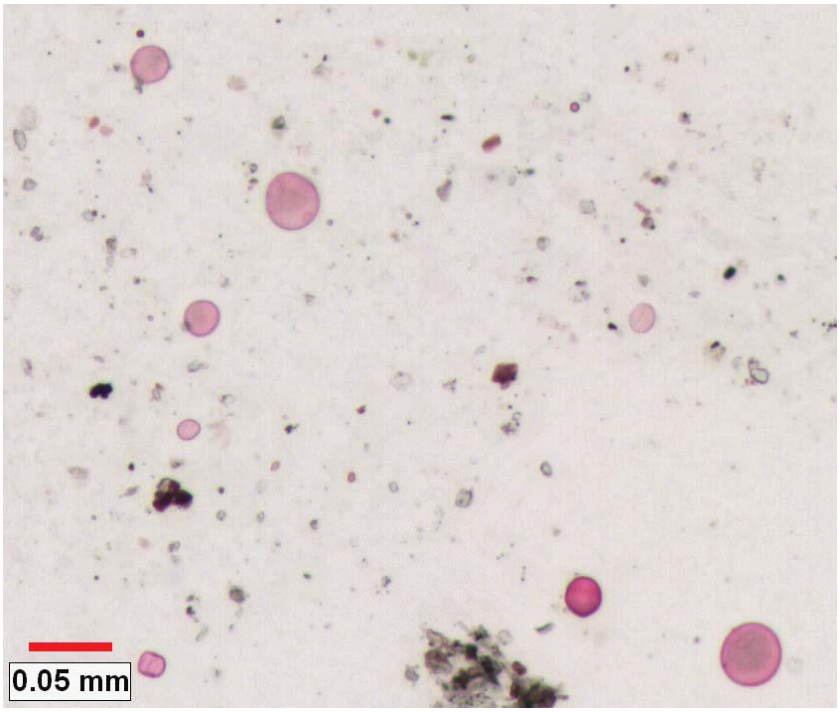


Image 5: Same as previous but with a closer look.

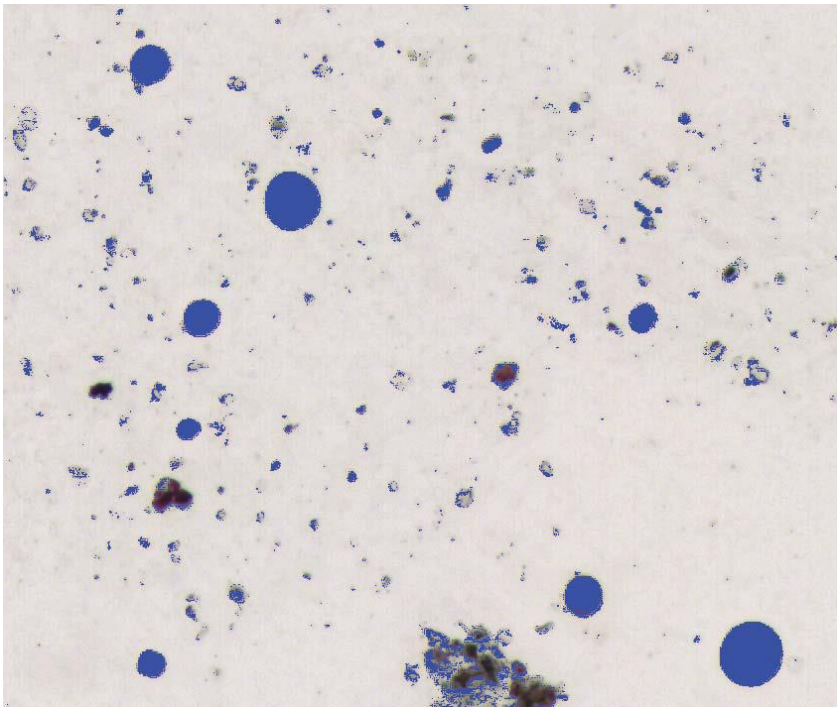


Image 6: Original MLG image as binarized using color thresholding.

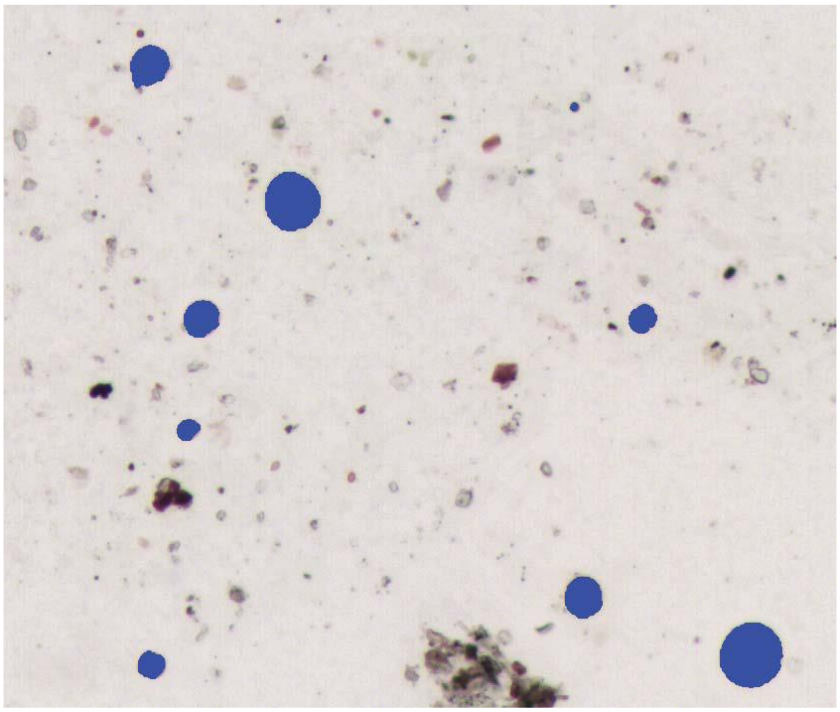


Image 7: Blue bitplane as cleaned from undesired detections.

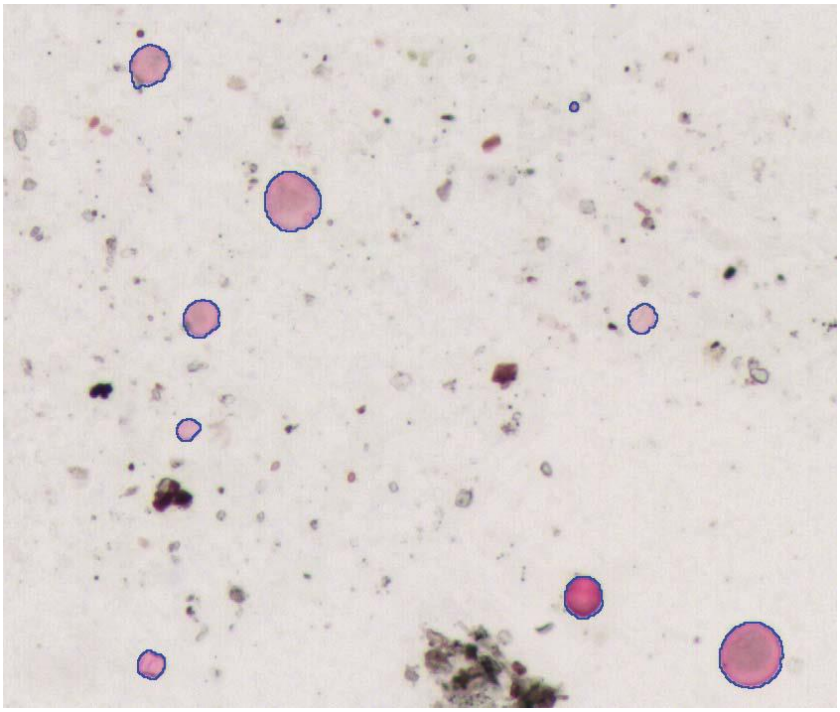
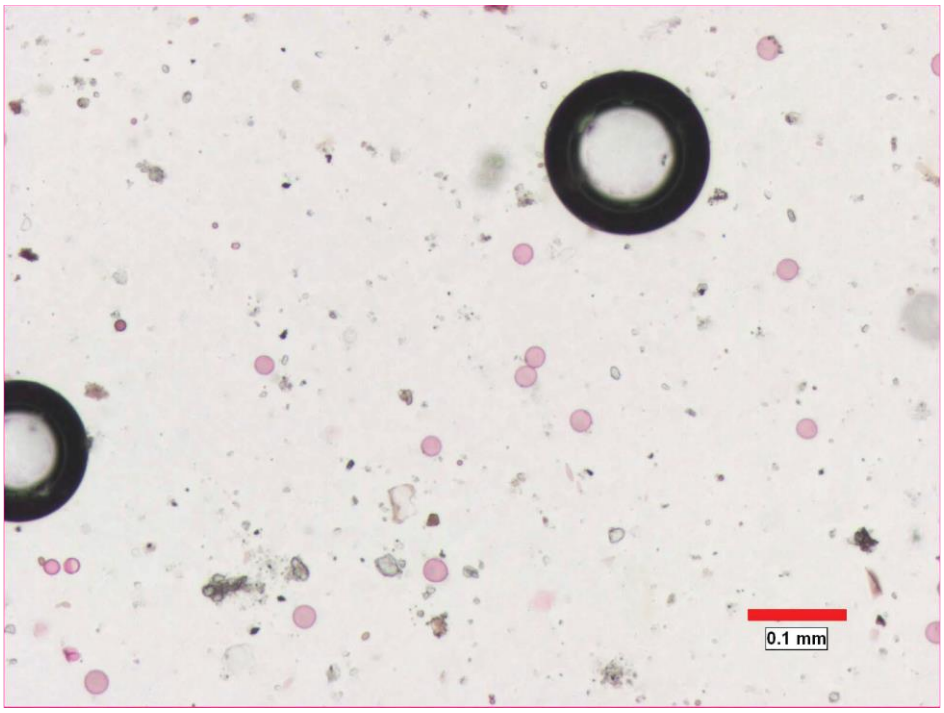
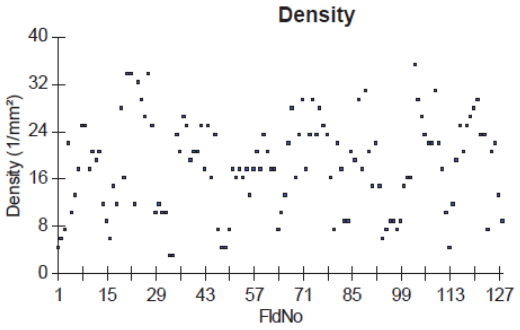


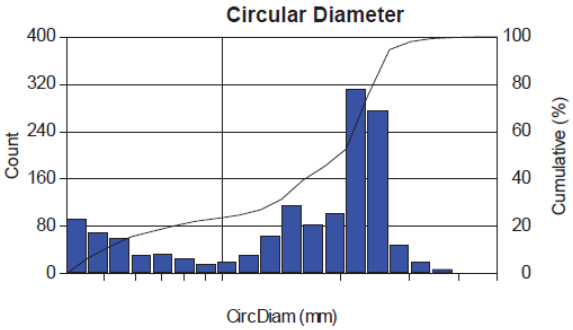
Image 8: Same as previous but in outline view mode.

Appendix B: Results

Sample #1 – 10 06 08







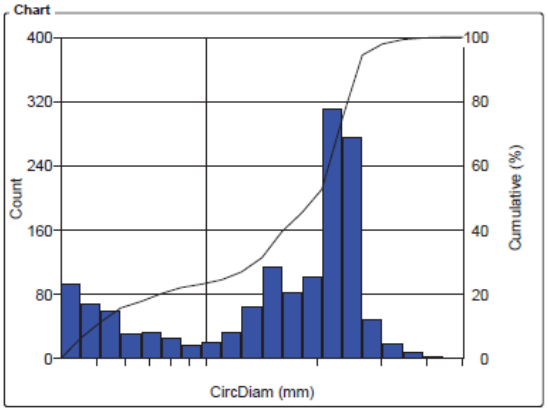
Min:	2.934	1/mm2	4.106	mic.
Max.:	35.214	1/mm2	40.178	mic.
Mean:	18.203	1/mm2	17.364	mic.
Std. Dev.:	7.841	1/mm2	7.790	mic.

CLEMEX

OBJM1 Count - CircDiam

Magn.: 100xa Calib.: 0.0006 mm/pixel Bitplane: Bitplane 1

Chart



Values

CircDiam Intervals (mm)		Count	%	Cumul%
0.004	- 0.005	92	6.55	6.55
0.005	- 0.005	69	4.91	11.46
0.005	- 0.006	60	4.27	15.73
0.006	- 0.007	31	2.21	17.94
0.007	- 0.007	33	2.35	20.28
0.007	- 0.009	26	1.85	22.14
0.009	- 0.010	16	1.14	23.27
0.010	- 0.011	20	1.42	24.70
0.011	- 0.013	32	2.28	26.98
0.013	- 0.014	64	4.56	31.53
0.014	- 0.016	115	8.19	39.72
0.016	- 0.018	82	5.84	45.55
0.018	- 0.021	102	7.26	52.81
0.021	- 0.023	311	22.14	74.95
0.023	- 0.027	276	19.64	94.59
0.027	- 0.030	48	3.42	98.01
0.030	- 0.034	19	1.35	99.36
0.034	- 0.039	7	0.50	99.86
0.039	- 0.044	2	0.14	100
0.044	- 0.050	0	0	100

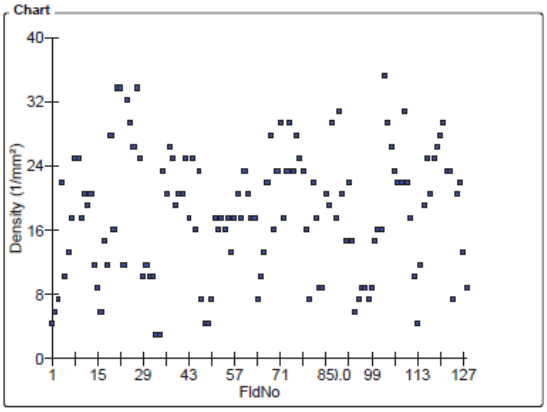
Statistics

Minimum:	0.00411	mm
Maximum:	0.04018	mm
Mean:	0.01736	mm
Std Dev.:	0.00779	mm
Sum:	24.39618	mm
Count:	1405	
Under:	0	
Over:	0	
Accepted:	100.00000	%
Field Count:	128	
Field Area:	0.59917	mm²
Total Area:	76.69352	mm²
D10:	0.00501	mm
D50:	0.02003	mm
D90:	0.02535	mm

FLDM2 Density - FldNo

Magn.: 100xa Calib.: 0.0006 mm/pixel Bitplane: Bitplane 1

Chart



0.1

Values

FldNo	Density (1/mm²)
1	4.402
2	5.869
3	7.336
4	22.009
5	10.271
6	13.205
7	17.607
8	24.943
9	24.943
10	17.607
11	20.541
12	19.074
13	20.541
14	11.738
15	8.803
16	5.869
17	14.672
18	11.738
19	27.878
20	16.140
21	33.747
22	33.747
23	11.738
24	32.279
25	29.345
26	26.410
27	33.747
28	24.943
29	10.271
30	11.738
31	10.271
32	10.271
33	2.934
34	2.934
35	23.476
36	20.541
37	26.410
38	24.943
39	19.074
40	20.541
41	20.541
42	24.943
43	17.607
44	24.943
45	16.140
46	23.476
47	7.336
48	4.402
49	4.402

Values - continued

FldNo	Density (1/mm²)
50	7.336
51	17.607
52	16.140
53	17.607
54	16.140
55	17.607
56	13.205
57	17.607
58	20.541
59	17.607
60	23.476
61	20.541
62	17.607
63	17.607
64	7.336
65	10.271
66	13.205
67	22.009
68	27.878
69	16.140
70	23.476
71	29.345
72	17.607
73	23.476
74	29.345
75	23.476
76	27.878
77	24.943
78	23.476
79	16.140
80	7.336
81	22.009
82	17.607
83	8.803
84	8.803
85	20.541
86	19.074
87	29.345
88	17.607
89	30.812
90	20.541
91	14.672
92	22.009
93	14.672
94	5.869
95	7.336
96	8.803
97	8.803
98	7.336
99	8.803
100	14.672
101	16.140
102	16.140
103	35.214
104	29.345
105	26.410
106	23.476
107	22.009
108	22.009
109	30.812
110	22.009
111	17.607
112	10.271
113	4.402
114	11.738
115	19.074
116	24.943
117	20.541
118	24.943
119	26.410
120	27.878
121	29.345
122	23.476

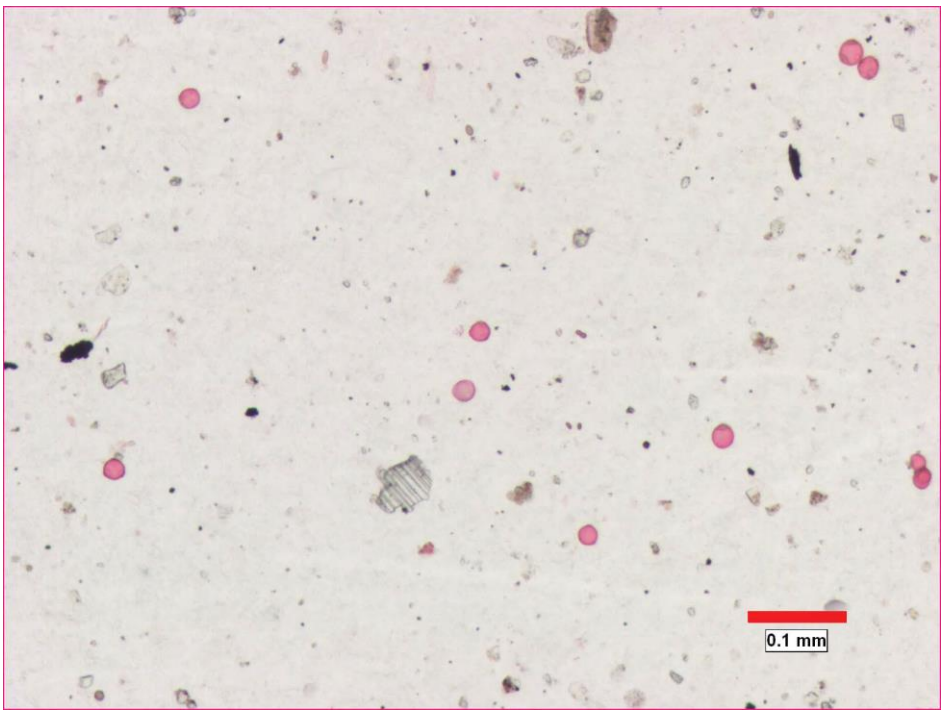
Values - continued

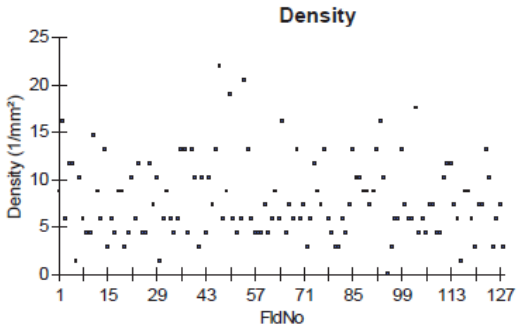
FldNo	Density (1/mm²)
123	23.476
124	7.336
125	20.541
126	22.009
127	13.205
128	8.803

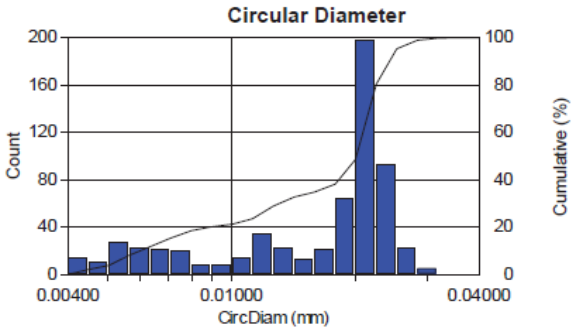
Statistics

Minimum:	2.93448	1/mm²
Maximum:	35.21378	1/mm²
Mean:	18.20296	1/mm²
Std Dev.:	7.84090	1/mm²
Count:	128	
Under:	0	
Over:	0	
Accepted:	100.00000	%
Field Count:	128	
Field Area:	0.68155	mm²
Total Area:	87.23858	mm²

Sample #2 – 17 04 08







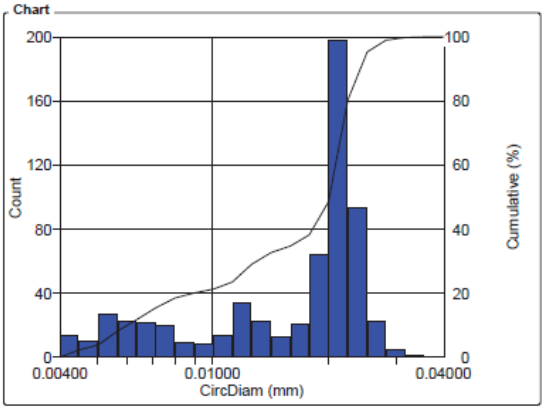
Min:	0.000	1/mm2	4.106	mic.
Max.:	22.009	1/mm2	31.823	mic.
Mean:	7.852	1/mm2	17.152	mic.
Std. Dev.:	4.111	1/mm2	6.754	mic.

CLEMEX

OBJM1 Count - CircDiam

Magn.: 100xa Calib.: 0.0006 mm/pixel Bitplane: Bitplane 1

Chart



Values

CircDiam Intervals (mm)			Count	%	Cumul%
0.004	-	0.004	14	2.25	2.25
0.004	-	0.005	10	1.61	3.86
0.005	-	0.006	27	4.34	8.20
0.006	-	0.006	23	3.70	11.90
0.006	-	0.007	22	3.54	15.43
0.007	-	0.008	20	3.22	18.65
0.008	-	0.009	9	1.45	20.10
0.009	-	0.010	8	1.29	21.38
0.010	-	0.011	14	2.25	23.63
0.011	-	0.013	34	5.47	29.10
0.013	-	0.014	23	3.70	32.80
0.014	-	0.016	13	2.09	34.89
0.016	-	0.018	21	3.38	38.26
0.018	-	0.020	64	10.29	48.55
0.020	-	0.023	198	31.83	80.39
0.023	-	0.025	93	14.95	95.34
0.025	-	0.028	23	3.70	99.04
0.028	-	0.032	5	0.80	99.84
0.032	-	0.036	1	0.16	100
0.036	-	0.040	0	0	100

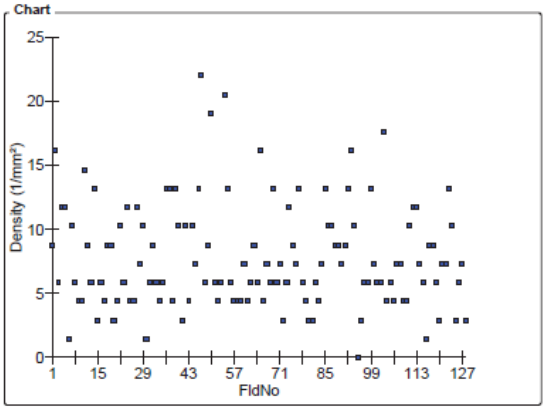
Statistics

Minimum:	0.00411	mm
Maximum:	0.03182	mm
Mean:	0.01715	mm
Std Dev.:	0.00675	mm
Sum:	10.66866	mm
Count:	622	
Under:	0	
Over:	0	
Accepted:	100.00000	%
Field Count:	128	
Field Area:	0.59917	mm²
Total Area:	76.69352	mm²
D10:	0.00589	mm
D50:	0.02025	mm
D90:	0.02382	mm

FLDM2 Density - FldNo

Magn.: 100xa Calib.: 0.0006 mm/pixel Bitplane: Bitplane 1

Chart



Values

FldNo	Density (1/mm²)
1	8.803
2	16.140
3	5.869
4	11.738
5	11.738
6	1.467
7	10.271
8	5.869
9	4.402
10	4.402
11	14.672
12	8.803
13	5.869
14	13.205
15	2.934
16	5.869
17	4.402
18	8.803
19	8.803
20	2.934
21	4.402
22	10.271
23	5.869
24	11.738
25	4.402
26	4.402
27	11.738
28	7.336
29	10.271
30	1.467
31	5.869
32	8.803
33	5.869
34	4.402
35	5.869
36	13.205
37	13.205
38	4.402
39	13.205
40	10.271
41	2.934
42	10.271
43	4.402
44	10.271
45	7.336
46	13.205
47	22.009
48	5.869
49	8.803

Values - continued

FldNo	Density (1/mm²)
50	19.074
51	5.869
52	4.402
53	5.869
54	20.541
55	13.205
56	5.869
57	4.402
58	4.402
59	4.402
60	7.336
61	4.402
62	5.869
63	8.803
64	5.869
65	16.140
66	4.402
67	7.336
68	5.869
69	13.205
70	5.869
71	7.336
72	2.934
73	5.869
74	11.738
75	8.803
76	7.336
77	13.205
78	5.869
79	4.402
80	2.934
81	2.934
82	5.869
83	4.402
84	7.336
85	13.205
86	10.271
87	10.271
88	8.803
89	8.803
90	7.336
91	8.803
92	13.205
93	16.140
94	10.271
95	0
96	2.934
97	5.869
98	5.869
99	13.205
100	7.336
101	5.869
102	5.869
103	17.607
104	4.402
105	5.869
106	4.402
107	7.336
108	7.336
109	4.402
110	4.402
111	10.271
112	11.738
113	11.738
114	7.336
115	5.869
116	1.467
117	8.803
118	8.803
119	5.869
120	2.934
121	7.336
122	7.336

Values - continued

FldNo	Density (1/mm²)
123	13.205
124	10.271
125	2.934
126	5.869
127	7.336
128	2.934

Statistics

Minimum:	0.00000	1/mm²
Maximum:	22.00861	1/mm²
Mean:	7.85203	1/mm²
Std Dev.:	4.11137	1/mm²
Count:	128	
Under:	0	
Over:	0	
Accepted:	100.00000	%
Field Count:	128	
Field Area:	0.68155	mm²
Total Area:	87.23858	mm²