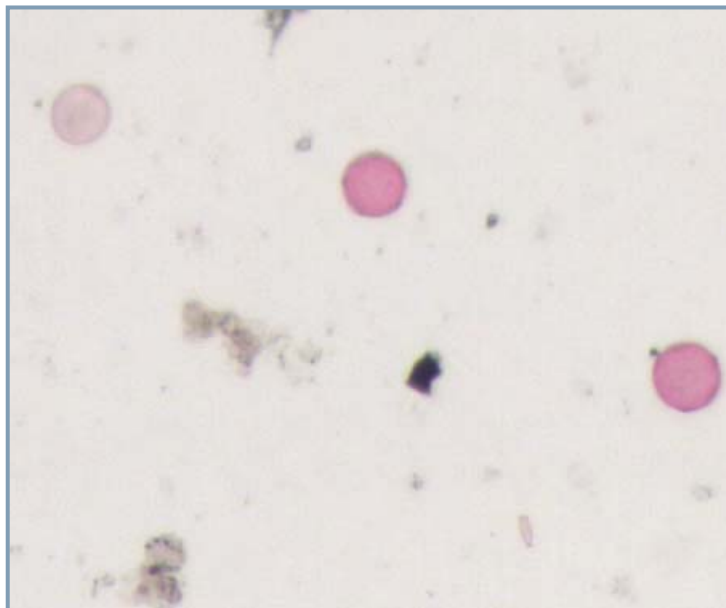


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Pollen Density Analysis



Prepared by Myriam Savard Eng.
Clemex Technologies Inc.
For Plymouth University

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 identified as sample 2.

Purpose of Analysis

To demonstrate that the Clemex Vision image analysis system can distinguish the pollen particles and evaluate their density.

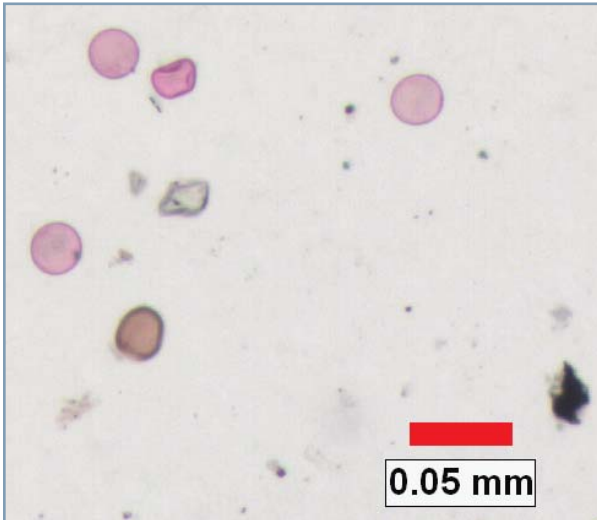


Figure 1: Part of the original image (0.5983 microns/pixel).

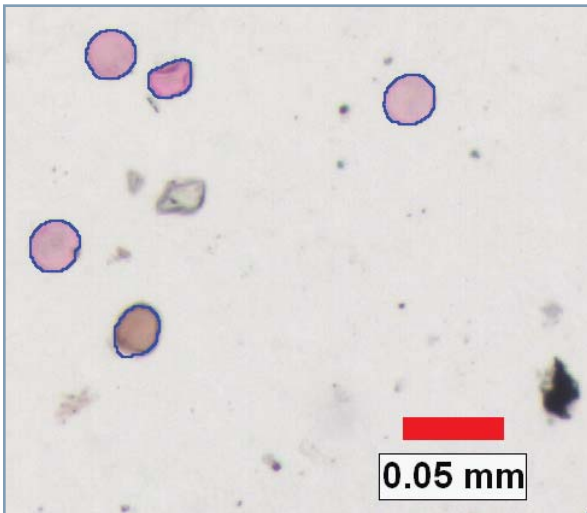


Figure 2: Outline of pollen particles as measured in blue bitplane.

Procedure¹

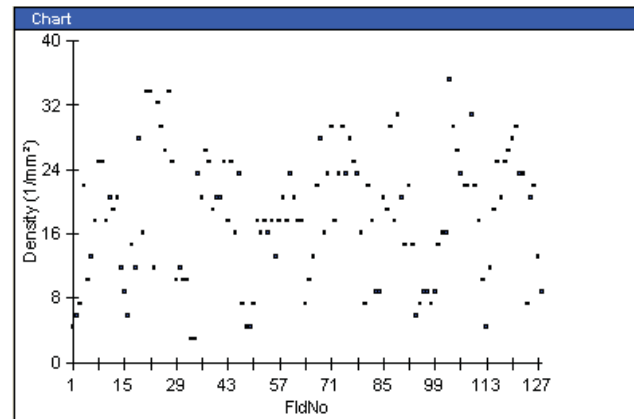
Most of the time, the pollen particles were found at different focus plans; consequently, a Multilayer-Grab was used to capture the images. Since the pollen particles were colored in pink, they were binarized in blue using the Color Threshold.

¹ Images taken during the procedure are available in appendix A

Some binary manipulations 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.

Results²

Density was measured on each field. Circular diameter measurement was also performed on each feature even though it was not requested. Many others measurements are available to quantify particles. Automated statistics and graphs were generated. Final results were printed directly from Clemex Vision and saved for further use. A customized report was built using the Report Generator module. Raw data is linked to their respective objects for validation purposes. Raw data could 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.

Equipment

Image Analysis System: Clemex Vision PSA
Microscope: Leica DM LA
Objective/Magnification: 10x / 100x
Coupler C-mount: 0.70x
Illumination: Transmitted Light
Calibration: 0.5982905 microns/pixel
Camera: Clemex L 2.0C
Motorized Stage: Marzhauser EK32IM 75x50mm
Stage Controller: Clemex ST-2000

Discussion

The main difficulty of this analysis was to detect the pollen particles without counting artifacts. The problem was overcome using a detection based on their color and by eliminating remaining artifacts using shape based filters.

² Complete results are available in appendix B.



Appendix A: Image Analysis Steps



Image Analysis Steps

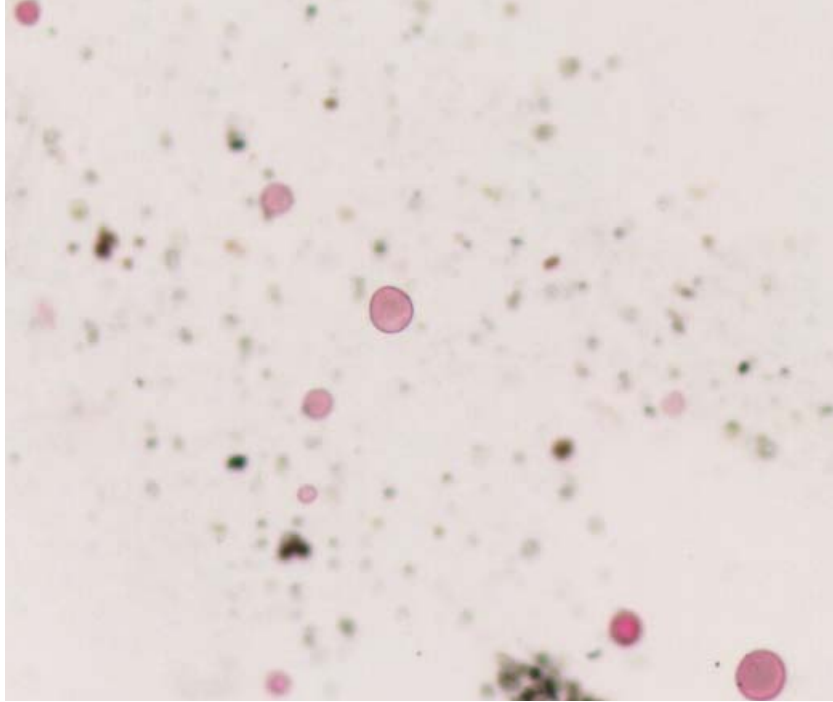


Image 1: Bottom layer of the multilayer grab.

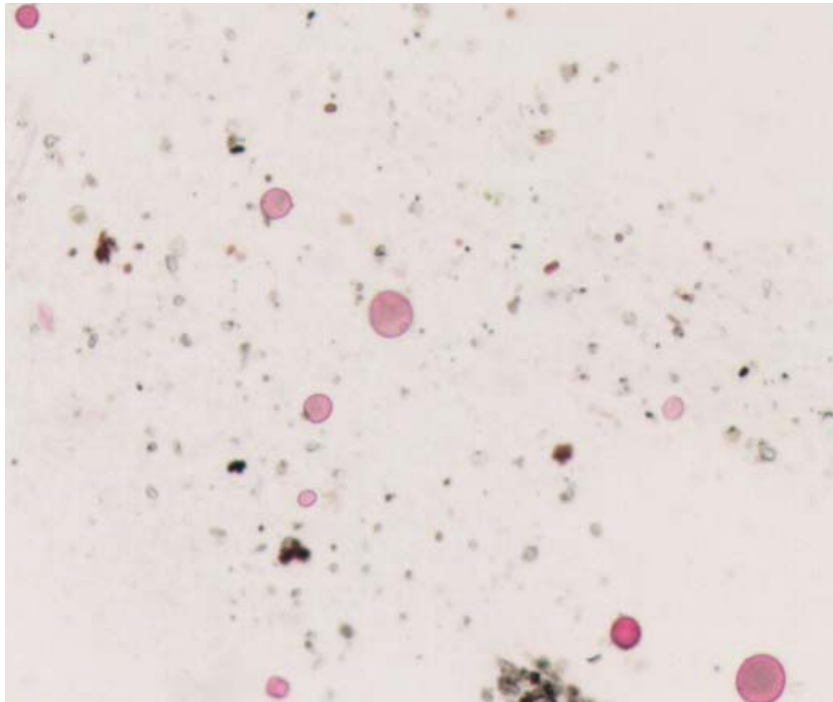


Image 2: Middle layer of the multilayer grab.

Image Analysis Steps

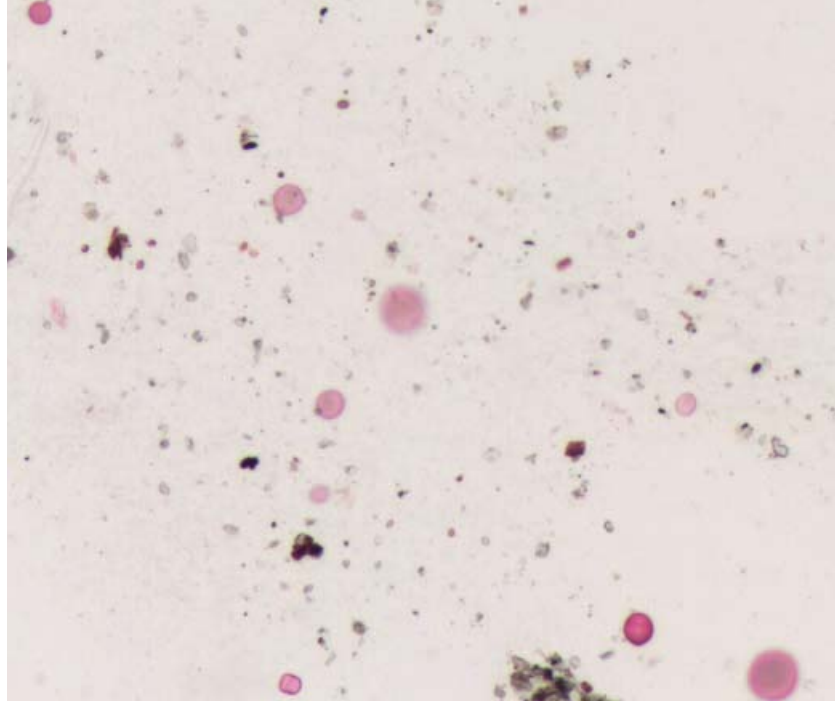


Image 3: Top layer of the multilayer grab.

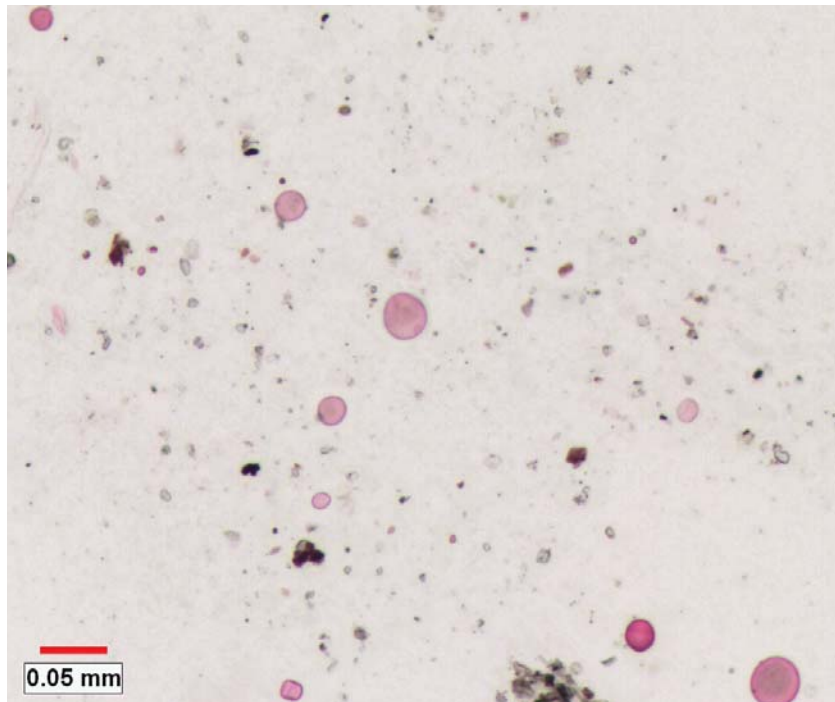


Image 4: Reconstituted image from 3 layers (100x).

Image Analysis Steps

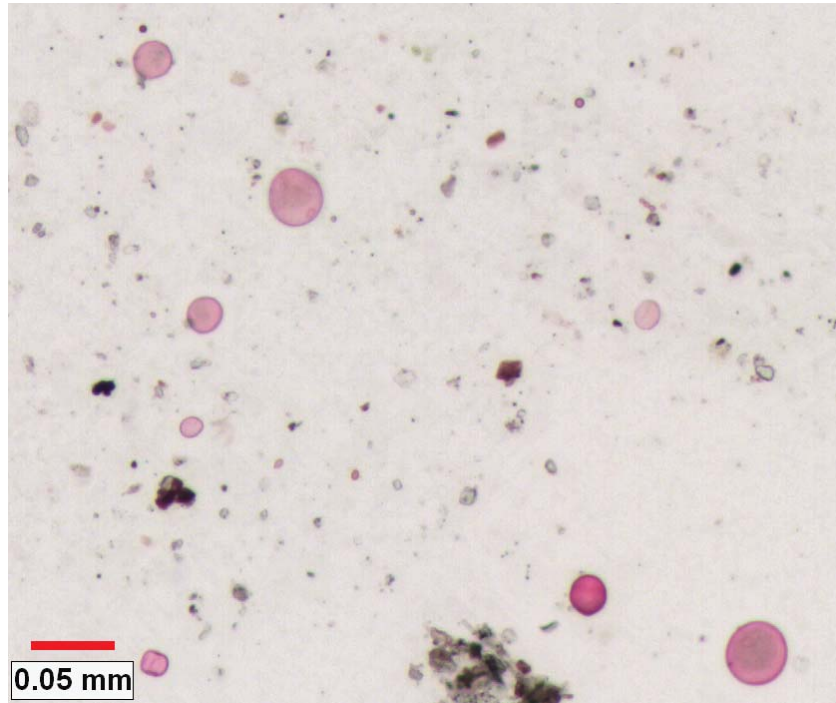


Image 5: Same as previous but with a closer look.

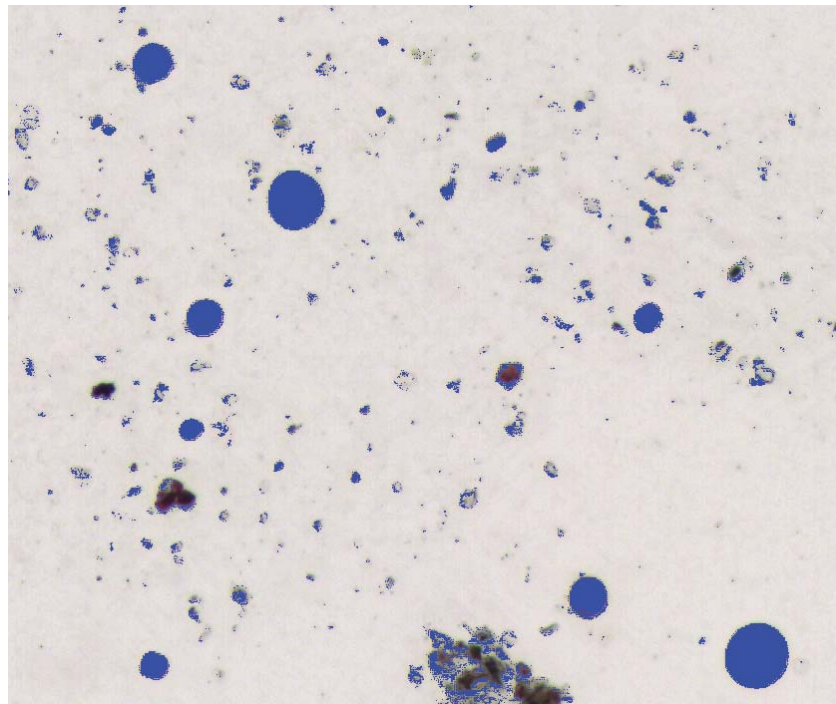


Image 6: Original MLG image as binarized using color thresholding based on pink coloration.

Image Analysis Steps

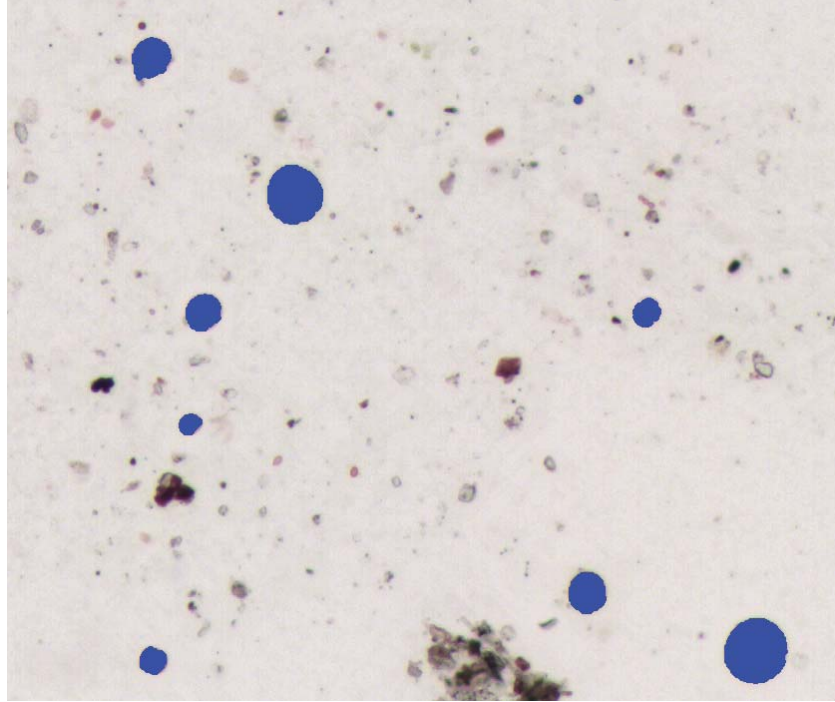


Image 7: Blue bitplane as cleaned from undesired detections.

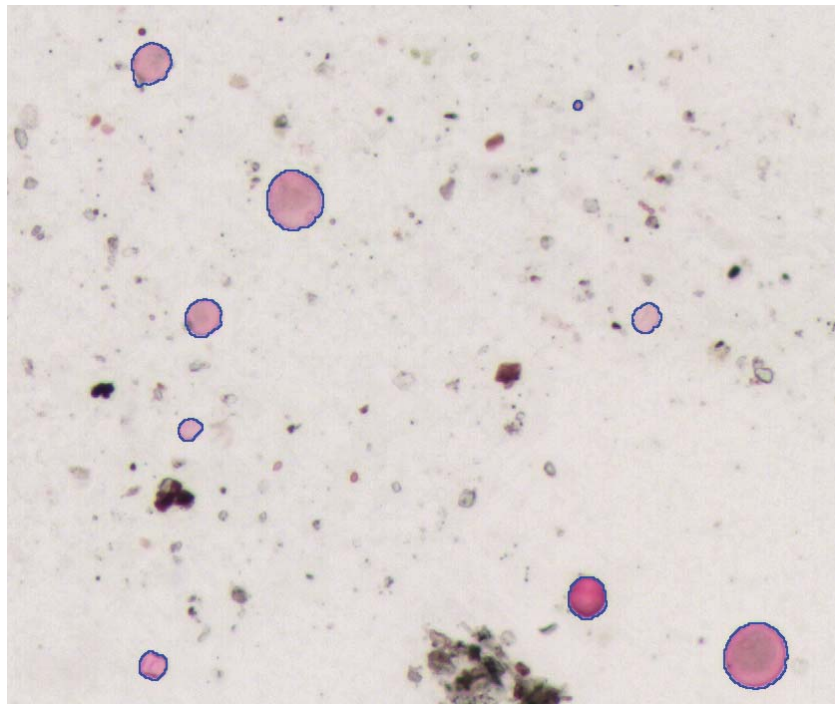


Image 8: Same as previous but in outline view mode.



Appendix B: Results



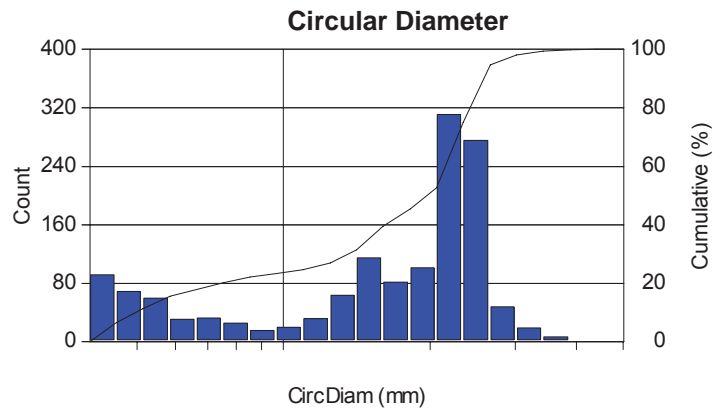
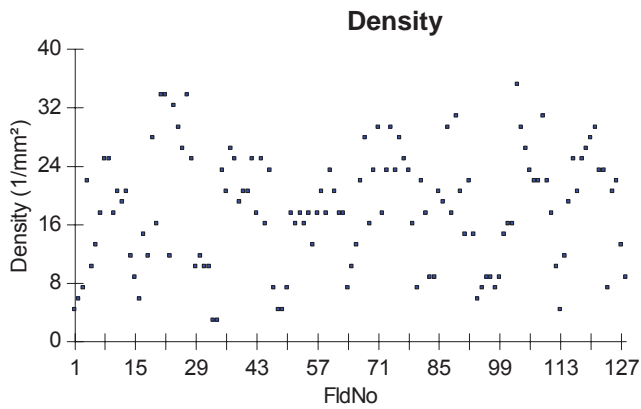
Sample # 1 - 10 06 08

- 1 - Report from Report Generator**
- 2 - Printed results from Clemex Vision**

Pollen Content Analysis

| | | | |
|--------------------|---------------------------------|-------------------------|-----------------------------------|
| Date: | 2008-Oct-07, 2:33:09 PM -04'00' | Magnification: | 100xa |
| Company: | Clemex | Calibration: | 0.5983 $\mu\text{m}/\text{pixel}$ |
| Department: | Laboratory | Units: | millimeters |
| User Name: | Myriam Savard | # Fields: | 128 |
| Sample ID: | S1 - 10/06/08 | Particles Count: | 1588 |

Figure 1: Field having the greater density.



Min: 2.934 1/mm²
Max.: 35.214 1/mm²
Mean: 18.203 1/mm²
Std. Dev.: 7.841 1/mm²

4.106 mic.
 40.178 mic.
 17.364 mic.
 7.790 mic.

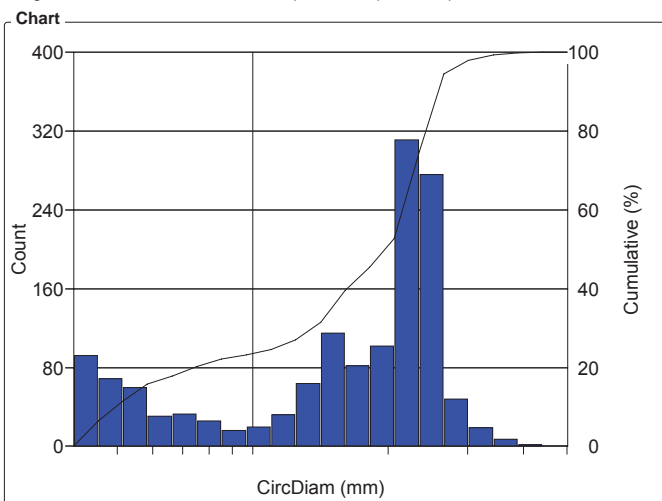
Pollen 100x S1

Organization: **Clemex**
 Department: **Laboratory**
 User: **Myriam Savard**

Date & Time: **08/10/2008 14:21:20 PM**
 Sample: **S1 - 10/06/08**

OBJM1 Count - CircDiam

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

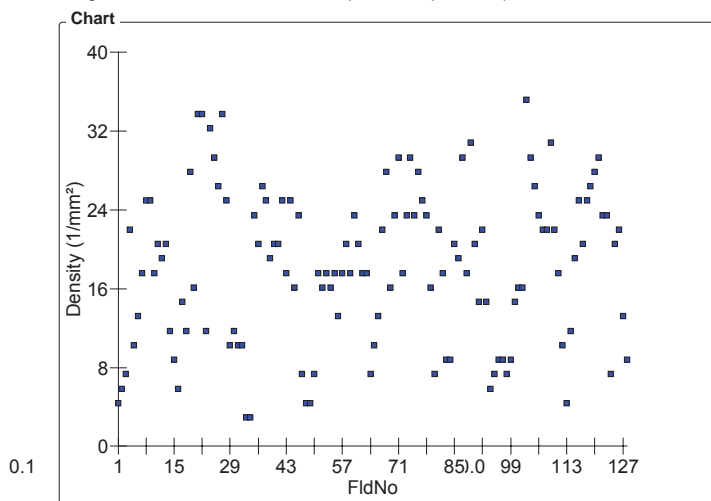


| CircDiam Intervals (mm) | Count | % | Cumul% |
|-------------------------|-------|-------|--------|
| 0.004 - 0.005 | 92 | 6.55 | 6.55 |
| 0.005 - 0.005 | 69 | 4.91 | 11.46 |
| 0.005 - 0.006 | 60 | 4.27 | 15.73 |
| 0.006 - 0.007 | 31 | 2.21 | 17.94 |
| 0.007 - 0.007 | 33 | 2.35 | 20.28 |
| 0.007 - 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 | mm ² |
| Total Area: | 76.69352 | mm ² |
| 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



| FldNo | Density (1/mm ²) |
|-------|------------------------------|
| 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 |

Pollen 100x S1

Organization: **Clemex**
 Department: **Laboratory**
 User: **Myriam Savard**

Date & Time: **08/10/2008 14:21:21 PM**
 Sample: **S1 - 10/06/08**

Values - continued

| FidNo | Density (1/mm ²) |
|-------|------------------------------|
| 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

| FidNo | Density (1/mm ²) |
|-------|------------------------------|
| 123 | 23.476 |
| 124 | 7.336 |
| 125 | 20.541 |
| 126 | 22.009 |
| 127 | 13.205 |
| 128 | 8.803 |

Statistics

| | | |
|--------------|-----------|-------------------|
| Minimum: | 2.93448 | 1/mm ² |
| Maximum: | 35.21378 | 1/mm ² |
| Mean: | 18.20296 | 1/mm ² |
| Std Dev.: | 7.84090 | 1/mm ² |
| Count: | 128 | |
| Under: | 0 | |
| Over: | 0 | |
| Accepted: | 100.00000 | % |
| Field Count: | 128 | |
| Field Area: | 0.68155 | mm ² |
| Total Area: | 87.23858 | mm ² |

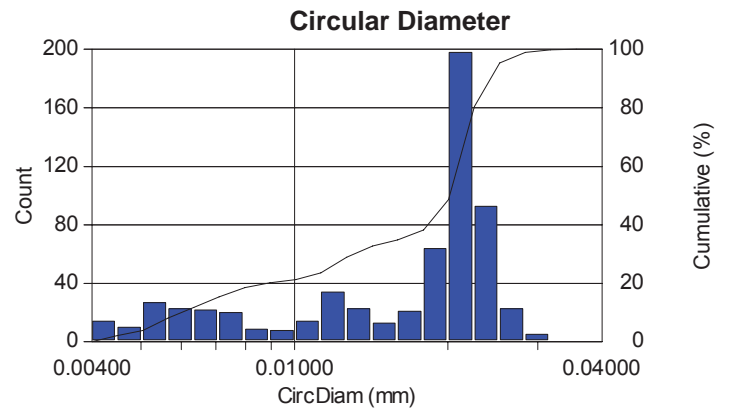
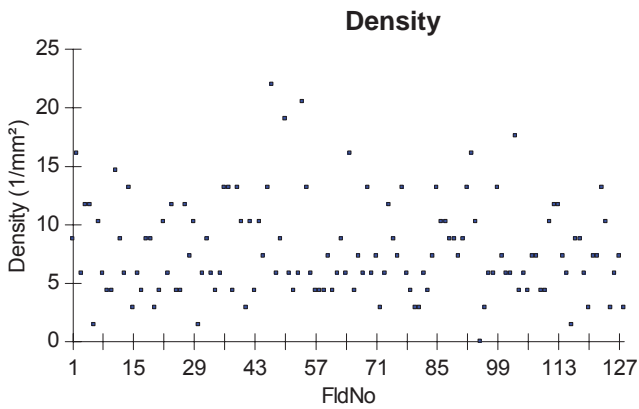
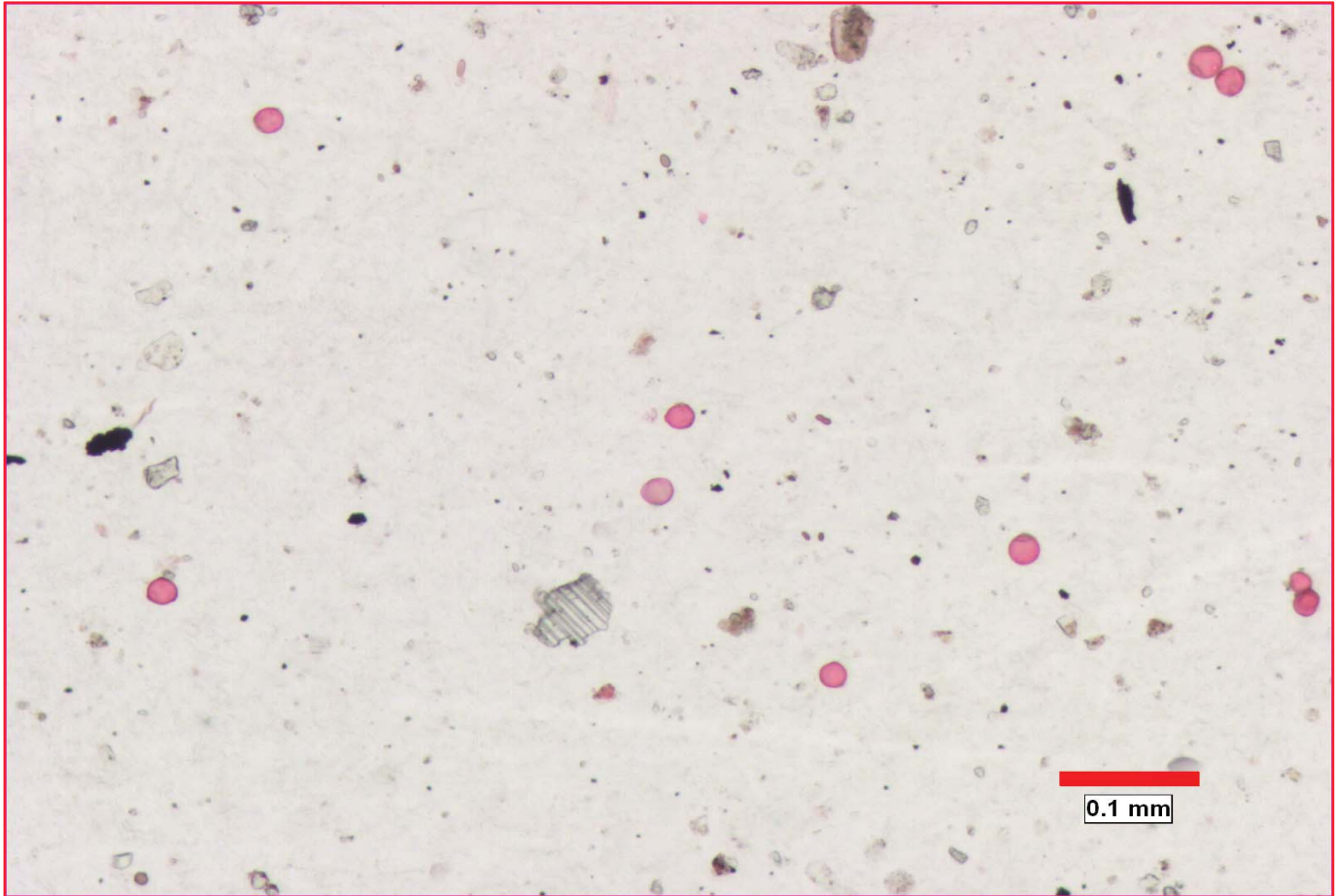
Sample # 2 - 17 04 08

- 1 - Report from Report Generator**
- 2 - Printed results from Clemex Vision**

Pollen Content Analysis

| | | | |
|--------------------|---------------------------------|-------------------------|-----------------------------------|
| Date: | 2008-Oct-07, 3:38:47 PM -04'00' | Magnification: | 100x |
| Company: | Clemex | Calibration: | 0.5983 $\mu\text{m}/\text{pixel}$ |
| Department: | Laboratory | Units: | millimeters |
| User Name: | Myriam Savard | # Fields: | 128 |
| Sample ID: | S2 - 17/04/08 | Particles Count: | 685 |

Figure 1: Field having the greater density.



| | | | | |
|-------------------|--------|-------------------|--------|------|
| Min: | 0.000 | 1/mm ² | 4.106 | mic. |
| Max.: | 22.009 | 1/mm ² | 31.823 | mic. |
| Mean: | 7.852 | 1/mm ² | 17.152 | mic. |
| Std. Dev.: | 4.111 | 1/mm ² | 6.754 | mic. |

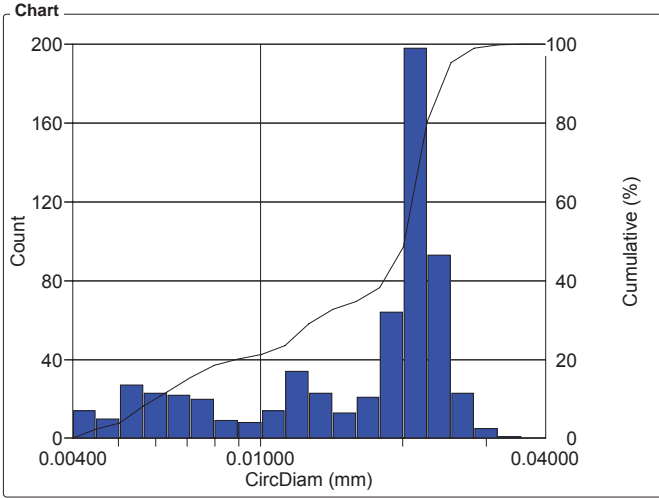
Pollen 100x

Organization: **Clemex**
 Department:
 User: **Myriam Savard**

Date & Time: **08/10/2008 14:22:58 PM**
 Sample:

OBJM1 Count - CircDiam

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



Values

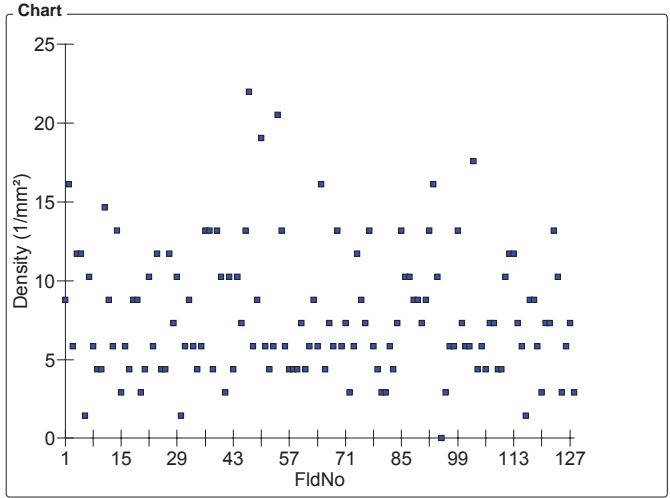
| CircDiam Intervals (mm) | Count | % | Cumul% |
|-------------------------|-------|-------|--------|
| 0.004 - 0.004 | 14 | 2.25 | 2.25 |
| 0.004 - 0.005 | 10 | 1.61 | 3.86 |
| 0.005 - 0.006 | 27 | 4.34 | 8.20 |
| 0.006 - 0.006 | 23 | 3.70 | 11.90 |
| 0.006 - 0.007 | 22 | 3.54 | 15.43 |
| 0.007 - 0.008 | 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 | mm ² |
| Total Area: | 76.69352 | mm ² |
| 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



Values

| FldNo | Density (1/mm ²) |
|-------|------------------------------|
| 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 |

Pollen 100x

Organization: **Clemex**
 Department:
 User: **Myriam Savard**

Date & Time: **08/10/2008 14:22:58 PM**
 Sample:

Values - continued

| FldNo | Density (1/mm ²) |
|-------|------------------------------|
| 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/mm ²) |
|-------|------------------------------|
| 123 | 13.205 |
| 124 | 10.271 |
| 125 | 2.934 |
| 126 | 5.869 |
| 127 | 7.336 |
| 128 | 2.934 |

Statistics

| | | |
|--------------|-----------|-------------------|
| Minimum: | 0.00000 | 1/mm ² |
| Maximum: | 22.00861 | 1/mm ² |
| Mean: | 7.85203 | 1/mm ² |
| Std Dev.: | 4.11137 | 1/mm ² |
| Count: | 128 | |
| Under: | 0 | |
| Over: | 0 | |
| Accepted: | 100.00000 | % |
| Field Count: | 128 | |
| Field Area: | 0.68155 | mm ² |
| Total Area: | 87.23858 | mm ² |

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