

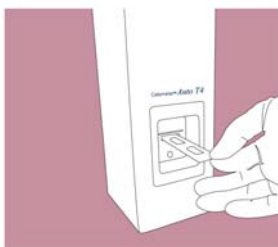


Cellometer® Auto M10

Counting Yeast, Human Platelet, and Algae

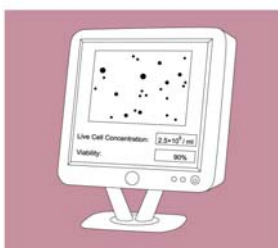
Summary

This application note provides some details on enhanced counting capability provided by Cellometer® Auto M10. Higher magnification images generated by Auto M10 enables more detailed analysis for heterogeneous populations with cells smaller than 5 μm . Cells which differ in size and morphology are identified and counted.



Introduction to Cellometer® Auto T4 and Auto M10

Cellometer® Auto T4 and Auto M10 are imaging instruments that acquire cell data from multiple locations of Cellometer® disposable counting chambers. They are connected to a computer via USB 2.0 cable. Cellometer® software automatically analyzes acquired cell images and measures cell concentration and viability.



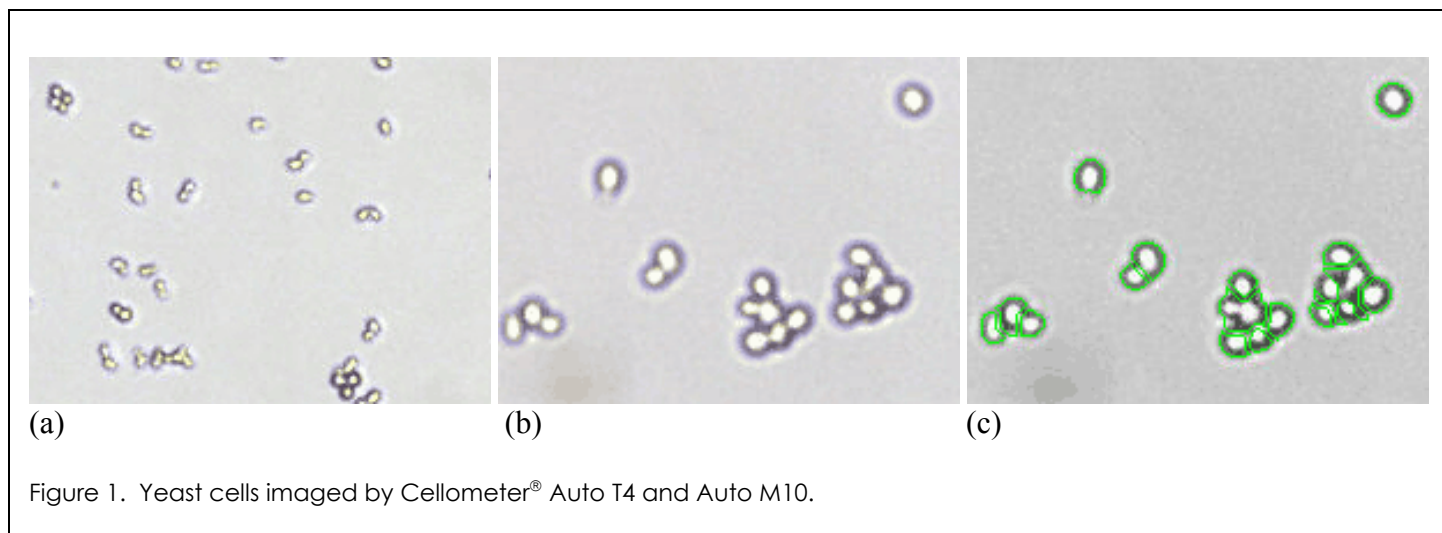
Simplicity and compact size make these systems an attractive product for automating cell counting operation. By using convenient cell type settings, the user can perform cell counting with high level of repeatability and accuracy. High quality disposable counting chambers, made of plastic materials, allow easy handling and disposal. Minimal sample amounts of 15 to 20 μl are used for counting to a total number much higher than possible by hand-count, thus reducing counting error. Crisp and intuitive graphic user interface assists simple operation.

Brief Descriptions of Cellometer® Auto T4 and Auto M10

Model #	Description	Most Suitable Cell Size
Auto T4	Cellometer® automatic cell counter, two counting locations, standard magnification.	$\geq 5 \mu\text{m}$
Auto M10	Cellometer® automatic cell counter, four counting locations, higher magnification.	2 – 12 μm

Imaging Yeast using Auto T4 and Auto M10

Images in Figure 1 depict difference in magnification of Auto T4 and Auto M10. Baker's yeast was loaded into a Cellometer[®] counting chamber. First, the chamber was imaged by an Auto T4, as shown in Figure 1 (a). The same counting chamber was then loaded into an Auto M10 cell counter. Figure 1 (b) shows the yeast cells, imaged with AutoM10, with much more details than those in Figure 1 (a). Given the detailed image data, the de-cluster software feature is activated to count clumpy yeast cells, as shown in Figure 1 (c). Green circles indicate counting individual yeast cells.

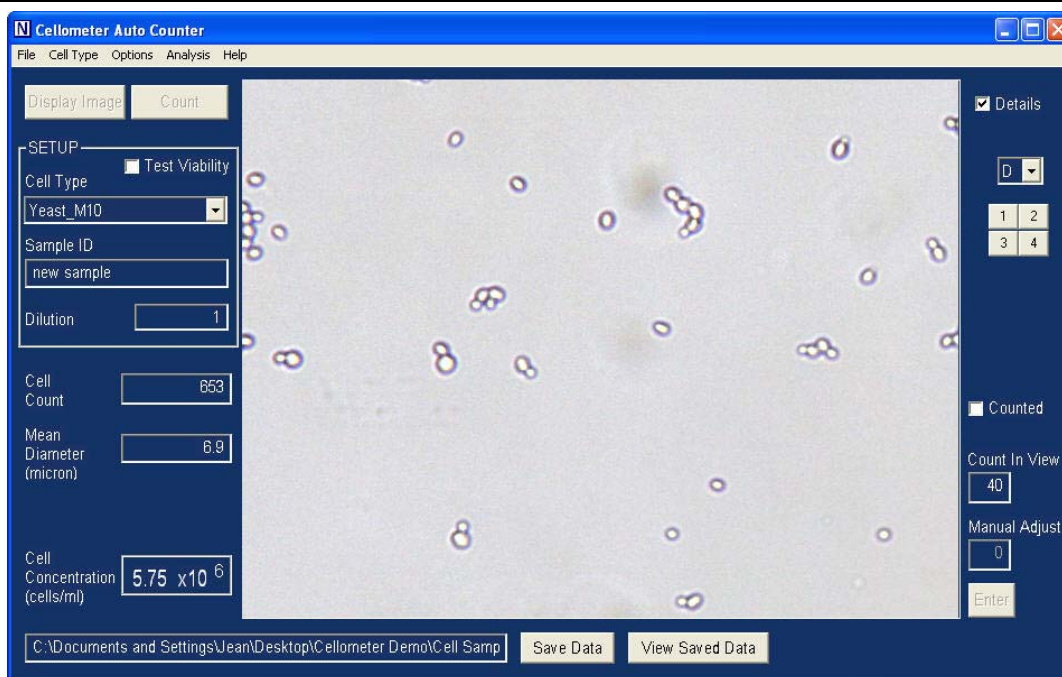


Counting Yeast

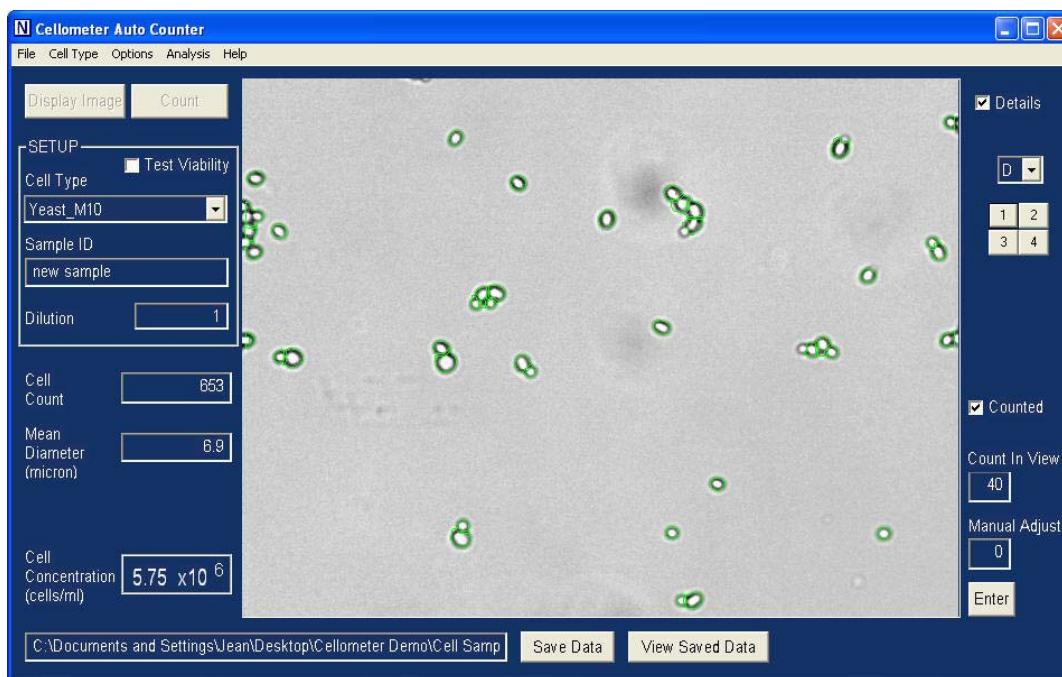
As shown above, higher magnification Auto M10 provides sufficient resolution for the Cellometer[®] software de-cluster function for the clumpy yeast cells. The Auto M10 is selected for the following experiment of counting yeast cells.

Experimental procedure:

1. Prepare yeast sample.
2. Dilute samples with PBS at different dilution factors: 4, 8, 16, 32, 64, and 128.
3. Pipette 20 μ l of solution into sample introduction port of a disposable counting chamber.
4. Insert loaded counting chamber into Auto M10.
5. Run Cellometer[®] software to obtain cell concentration results.



(a)



(b)

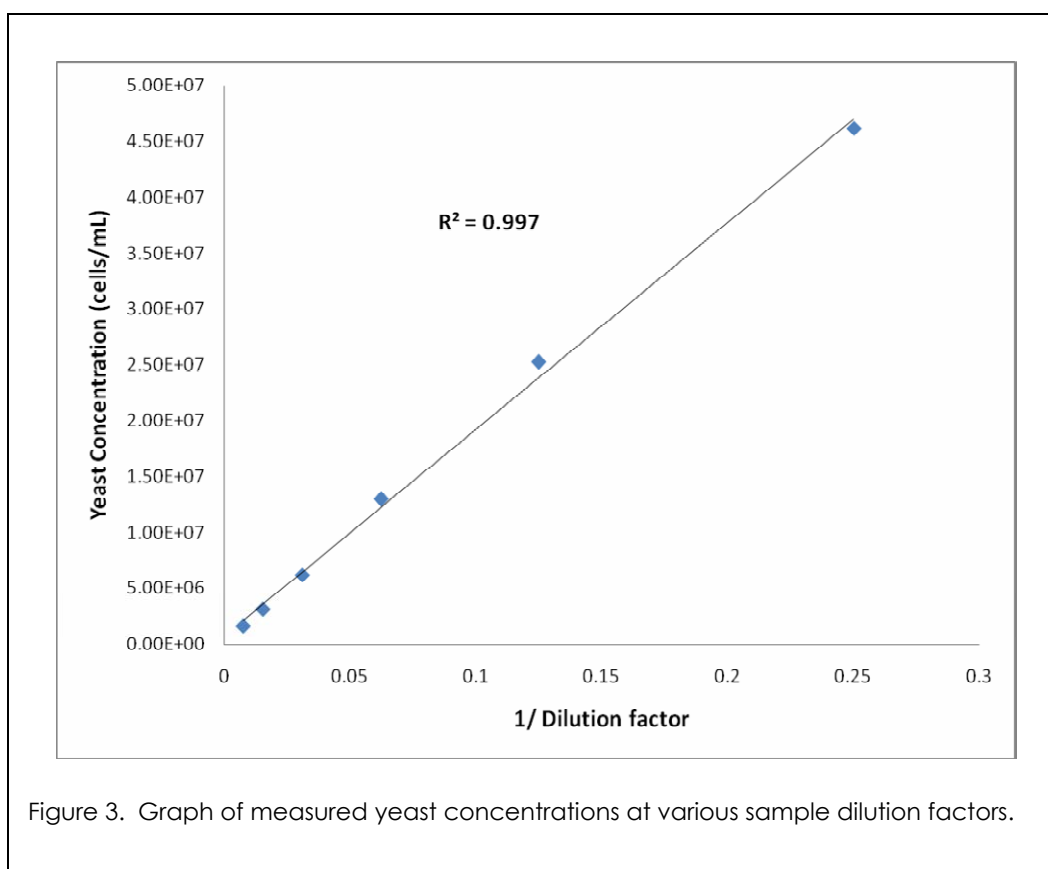
Figure 2. Cellometer® Auto M10 images of yeast cells, (a) yeast cells, and (b) counted yeast cells.

Plot Yeast Concentration versus Sample Dilution

Table 1 and Figure 3 show data from a set of yeast dilution measurements. A total of 6 samples with two-fold dilutions are used to generate the plot. The result shows excellent linearity for concentration versus sample dilution.

Table 1. Measured yeast concentrations using the Auto M10.

date time	cell type	sample name	total cell count	mean diameter	concentration
6/21/2007 15:25	Yeast_M10	Yeast -1	1324	5.5	4.62E+07
6/21/2007 15:23	Yeast_M10	Yeast -2	1087	5.5	2.53E+07
6/21/2007 15:21	Yeast_M10	Yeast -3	1482	6	1.30E+07
6/21/2007 15:19	Yeast_M10	Yeast -4	716	5.3	6.23E+06
6/21/2007 15:32	Yeast_M10	Yeast -5	355	6.5	3.12E+06
6/21/2007 15:35	Yeast_M10	Yeast -6	191	5.6	1.67E+06



Count Human Platelet

The higher magnification Auto M10 is also used to count human platelets, using the following procedure:

1. Prepare cell sample: fresh PBMC.
2. Dilute samples with PBS at a total of 5 two-fold dilutions.
3. Pipette 20 μ l of solution into sample introduction port of a disposable counting chamber.
4. Insert loaded counting chamber into Auto M10.
5. Run Auto M10 software to obtain cell concentration.
6. Plot Platelet concentration versus 1/dilution factor.

Fresh PBMC samples contain platelets, which is used in this experiment to count platelet. Figure 4 shows an example of platelet cells from fresh human PBMC sample, taken by Cellometer[®] model Auto M10. In this case, platelets are identified by size and counted, while PBMC are excluded. Platelets are identified and indicated by green circles. The yellow circle indicates the larger PBMC cells, which are not counted.

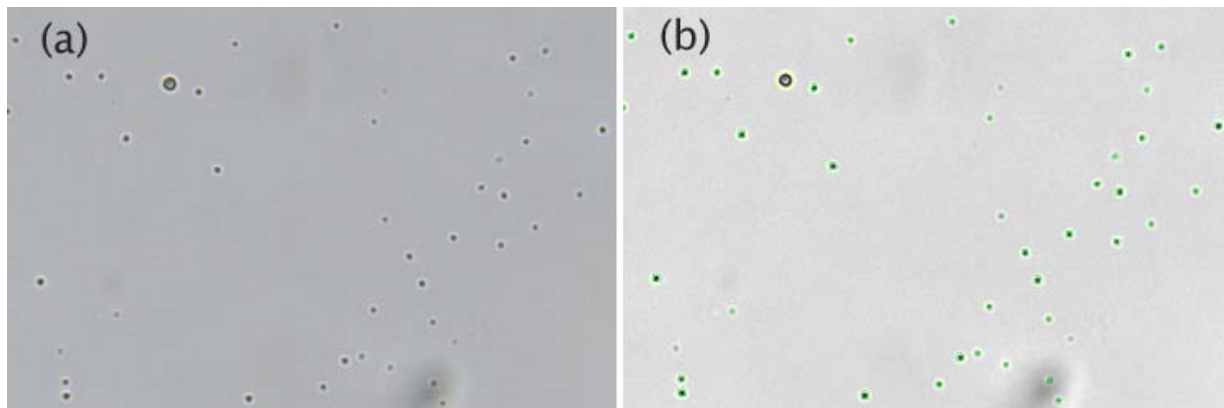


Figure 4. Images of fresh human platelets, (a) raw image, and (b) after counting. The counted platelets as indicated by green circles. The yellow circle indicates a much larger PBMC, which is not counted.

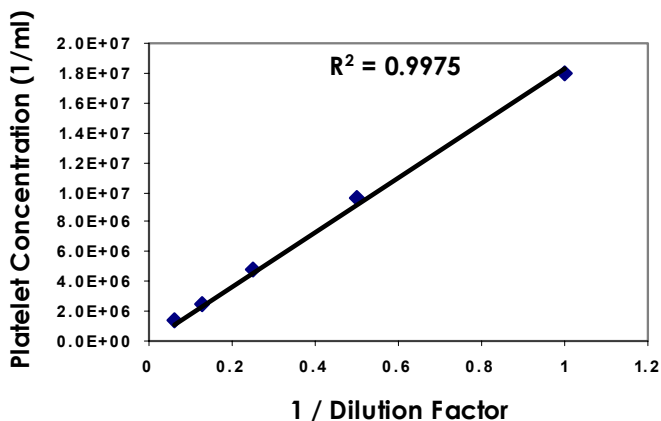


Figure 5. Measured platelet concentrations at various sample dilution factors. The result shows excellent linearity for concentration versus sample dilution.

Counting Algae using Auto M10

Auto M10 has also been used to count smaller sized algae, with typical image and counted results shown in Figure 6.

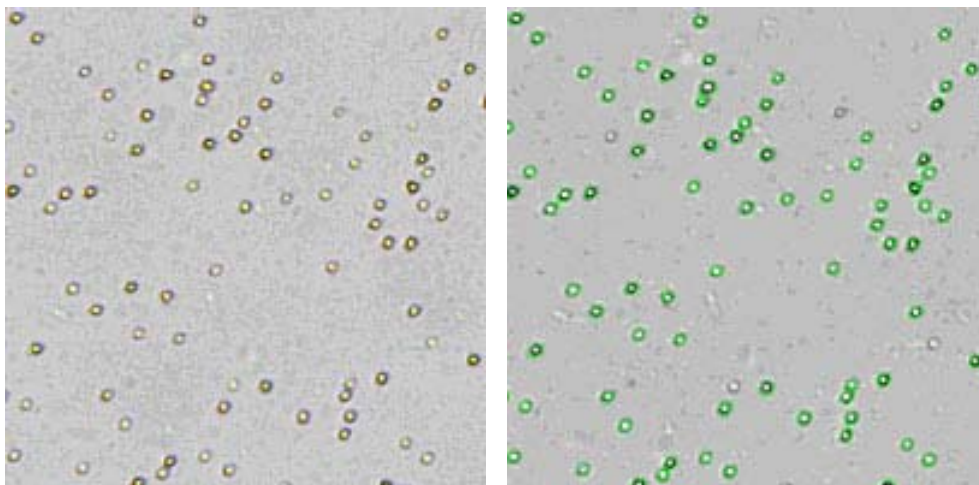


Figure 6. Images of algae cells: (a) raw image; (b) counted cells circled in green. Mean cell size: 3.8 μm . Algae concentration = 2.0×10^7 /ml.