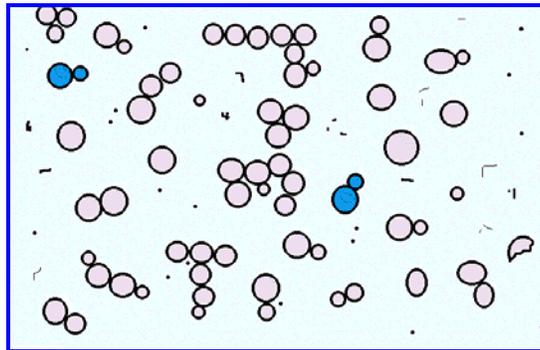




CLEMEX
Image Analysis Report

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Yeast Analysis

Sample Description

A sketch of yeast cells with some in gestation (budded).

Purpose of Analysis

Demonstrate the ability of the image analyzer to perform the following analyses:

- Percentage of dead cells versus living cells
 - Classify and count cells that are in gestation and those that are not in gestation
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Apparatus

Image Analysis System: Clemex Vision 2.1 software (1024)

Procedures



The calibration factor was adjusted to 10 $\mu\text{m}/\text{pixel}$ in order to produce realistic results from the drawing.

Dead cells were binarized using a *Color Threshold* instruction. The outline of the other cells were binarized by *Gray Thresholding* and then filled (*Fill*). Cells that were in contact with one another were separated by combining the outline with the full size objects (*Boolean Xor*). All the artifacts were removed (*Object Transfer by Limits*) and the original size of the cells was restored (*Zone, Invert, Boolean*). Dead cells were isolated into red bitplane using an *Object Transfer by Contact* instruction. Figure 1 shows the resulting bitplanes.

The smallest cells were isolated into pink bitplane using an *Object Transfer by Limits* instruction. They were temporarily considered as buds. Mother cells that were in contact with a bud were isolated from the others. In Figure 2, we can see mothers and their bud before being combined together for measurement purpose.

Isolated buds were transferred with the regular yeast cells. Using an *Object Transfer by Limits* based on a *Custom Measurement*, budded yeast cells that had a bud greater than one-half the size of the mother cell were sent back to the non-budded cells category. We can see bitplanes as measured in Figure 3.

Figure 4 represent the percentage of dead cells, budded cells and non-budded cells compared to all cells.

The most significant image¹ modifications and final results are as follows:

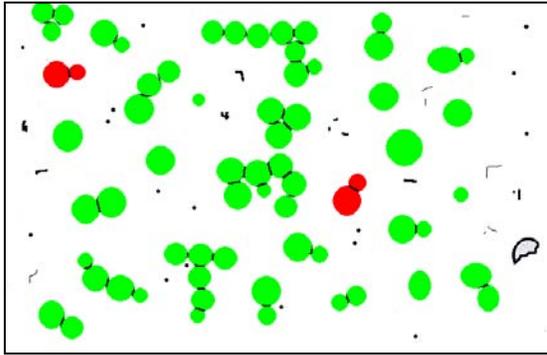


Figure 1: Separated cells with their full size (green). Dead cells were isolated in red and artifacts were eliminated.

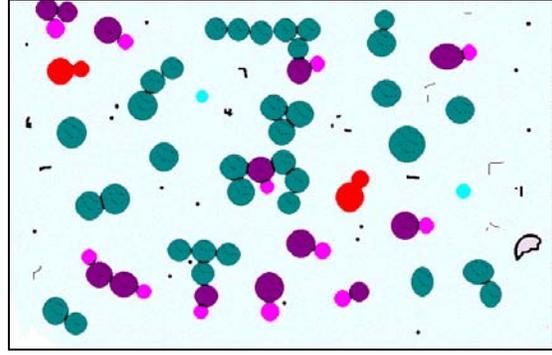


Figure 2: Isolated buds to be return to regular yeast cells category are in cyan, good buds are in pink, isolated yeast cells are in dark green and budded cells are in purple. Dead cells are in red.

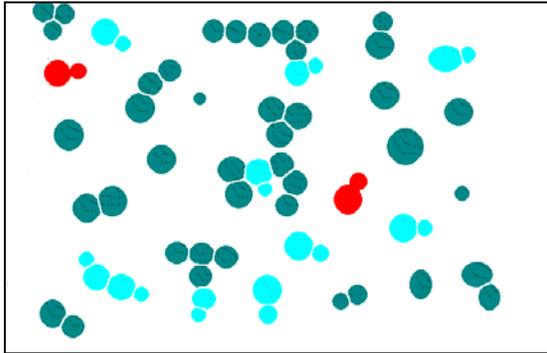


Figure 3: Budded yeast cells were attached to their corresponding buds into cyan bitplane. The mother and its bud were sent back to the regular yeast cells category (dark green) if the bud was greater than one-half the size of the mother cell.

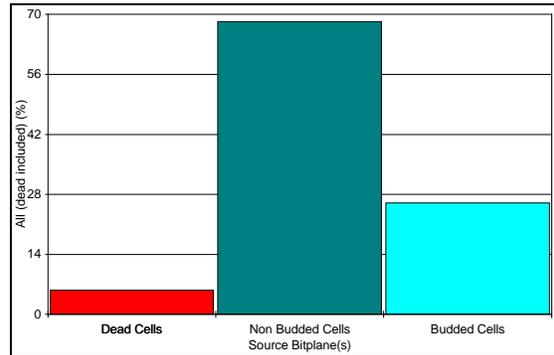


Figure 4: Percentage of isolated cells (isolated mothers) and percentage of cells in gestation (budded cells) compared to all cells (all mothers).

¹Note that some image characteristics may not be visible due to printing resolution.

Result Summary

	<i>Count</i>
<i>All cells including dead ones</i>	54
<i>All living cells</i>	52
<i>Dead cells</i>	2
<i>Budded cells</i>	10
<i>Non-budded cells</i>	42

Discussion



The analyzed image comes from a drawing that certainly not includes all possible sketches. A group including several mothers and buds all touching one to another was not represented here. If this sketch can be expected to occur in real life, results would have to be expressed differently.

Documentation on yeast application reports that a bud has to measure less than 50% of its mother area to be really counted as a bud. If it is not the case, the bud and the mother are counted as 2 distinct cells. According to this, working with an average on all isolated yeast cells to determine the maximum size for all buds is not correct. A big bud could be coupled with a small mother and counted as a unique gestation cell even if the bud would possibly be more than one-half the size of the mother cell.

To overcome this problem, a first buds classification was based on size ($< 50000 \text{ mic}^2$) and a second filter measured all budded cells to make sure that the current bud is not greater than one-half the size of the current mother. To achieve this second task, an *Object Transfer by Limits* based on a *Customized Object Measurement* was necessary.