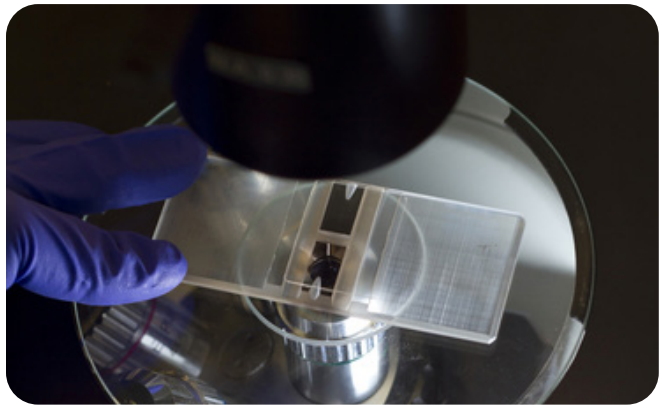
WHITEPAPER

Identifying and resolving the sources of hemacytometer counting error through automation

Introduction

The hemacytometer persists as the gold standard for laboratory cell counting. First utilized in 18th century France as a means to analyze patient blood samples, the hemacytometer has gone through a series of major developments over the past hundreds of years, creating a modern instrument that is more accurate and easier to use than its predecessors. The hemacytometer remains an integral part of all cell-based research, and yet sources of error inherent in its design and utilization persist. Those sources of error will be outlined here, followed by a discussion of how automation can be employed to eliminate many of these potential pitfalls.



Sources of hemacytometer error

1. **Human error (mixing, handling, dilution, miscalculation, and procedural errors made by humans)**
   a. In a study using five observers, errors due to operator and random error were 3.12%, and 7.8%, respectively.[3]
   b. James M. Ramsey performed an experiment to measure how sampling area and dilution factors affected variation in cell counts. He tested three area sizes (18, 9, and 4 mm^2) and two dilution factors (1:100 and 1:25). CVs increased as the sampling area decreased. Higher dilution factors also generated lower CVs.[4]
   c. Bane found that if the same operator was to count duplicate sperm samples, the variation in the results was attributed to 55% of sampling and pipetting error and 45% of chamber and counting error.[5] Freund and Carol published additional measurement to show that variation amongst different operators could be as high as 52% and there could be a 20% variation resulting from a single operator.[5]

2. **Need for multiple cell sample counts to ensure statistical accuracy**
   a. In 1907, John C. DaCosta stated that it was necessary to measure multiple drops of blood samples.[1]
   b. Nielsen, Smyth, and Greenfield concluded that in order to obtain an accuracy of 10%, 15%, and 20% in hemacytometer counting, the number of samples necessary were 7, 3, and 2, in addition to, 180, 200, and 125 cells counted per sample, respectively.[6]
   c. In 1881, Lyon and Thoma approximated the hemacytometer's standard error to be $\frac{1}{\sqrt{n}}$, where n was the number of cells counted.
   d. In 1907, William Sealy published his work counting brewer's yeast under the name of "Student", where he specifically calculated the statistical variation through experimentation and mathematical modeling, which resulted in the same equation $\frac{1}{\sqrt{n}}$.[7, 8]

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3. Need for uniform distribution of cells

a. In 1912, James C. Todd cited non-uniform distribution of cells as sources of error.[1]

b. Student also stated that there are two main sources of error, one was related to obtaining yeast samples that may not be representative of the bulk solution, and the error of random sampling where the cells were not uniformly distributed in the observation area.[7, 8]

c. 1947, an article was published on variation in cell density due to non-uniform distribution in the hemacytometer. The initial results showed that the area nearest and farthest from the entrance was 3.5% lower and higher than the average density, respectively.[9]

4. Instrumentation and material variation (grids, depth, coverslips, type of buffer, and pipettes)

a. The results showed experimental calculation of the error of the chamber and error of the pipet (CV %) were approximately 4.6% and 4.7%, respectively.[10]

b. In a study using five observers, errors due to pipettes and hemacytometers were 9.46% and 4.26%, respectively.[3]

c. In 1961, Sanders and Skerry concluded that the position of the coverslip could introduce a difference of 7.6%.[11]

d. This experiment measured the CV for each hemacytometer model with multiple dilution steps. As the dilution steps increased, the variation increased for the following models: Bürker-Türk (BT) (7.7%-12%), Thoma (6.6%-14.1%), and Makler (19.8%-23.6%).[12]

Resolving issues of the hemacytometer

With the development of computers, automation software, optics, fluorescent dyes, and precision manufacturing techniques and modern technologies such as fluorescent microscopy, flow cytometry, and image cytometry, automation has resolved many of the pitfalls associated with the hemacytometer.[13-25]

Automation resolves:

Human error - In order to resolve this issue, automation and robotics can replace manual liquid handling and counting operations.

Sampling errors - The more fields and cells counted, the lower the random error, but the more time required. By utilizing automated flow or imaging cytometers, thousands to millions of cells can be analyzed in a shorter amount of time, thus increasing efficiency and minimizing the random statistical error in the analysis.

Pipetting and dilution errors - These depend on the technical expertise of the operator. By employing automated pipettes or liquid handling systems, this error can be minimized.[26]

Material errors - Chamber error occurs due to variation between the different brands of hemacytometer, as well as variation within the same product line. This can also be resolved by using automated cytometers to increase sampling and minimize random error.

Non-uniform cell distribution - If a hemacytometer is not cleaned properly, or the coverslip not positioned correctly, variations are generated. These can be eliminated by cytometers that do not utilize chambers such as a flow cytometer. Cell samples that are inherently clumpy will still be difficult for automation. However, by using image cytometers, clumpy cells can be declustered using image analysis algorithms, which can improve the accuracy of cell counting.

Summary

The hemacytometer has been an essential tool in the field of biomedical research for hundreds of years, and it took many iterations to develop the device that researchers use today, yet it remains subject to many unavoidable sources of error. The use of modern automation has largely eliminated many of these sources of error, increasing the accuracy and efficiency of cell counting today.

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