

# Automated Counting of Hematopoietic Colonies Reduces Assay Variability

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## Introduction

The hematopoietic colony-forming cell (CFC) assay is the standard for measuring the progenitor content of cord blood (CB) and other cell products for hematopoietic stem cell transplantation (HSCT). Colony enumeration is currently performed manually based on morphological criteria. The process is time-consuming and requires skilled operators to maintain consistent scoring criteria. Automation promises to reduce the component of assay variability due to scoring and save operator time. We have developed an imaging and analysis instrument (STEMvision™) for automated colony enumeration and classification.

## Methods

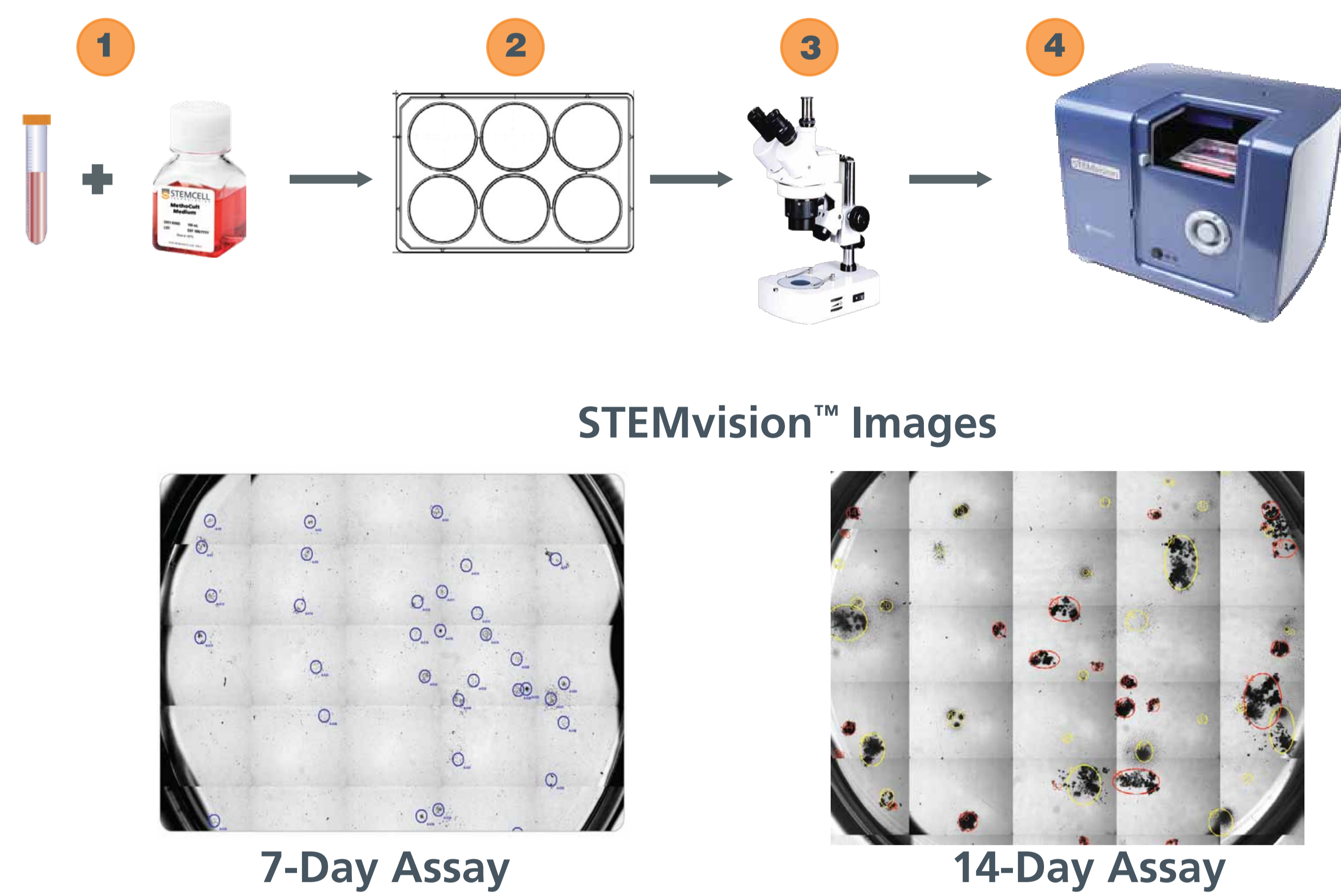
### CULTURE

A sample of cord blood cells is added to semisolid culture medium and plated into low-meniscus (SmartDish™) 6-well plates. The cells are then cultured for 7 days (MethoCult™ Express) or 14 days (MethoCult™ Optimum) following standard procedures.

### COUNTING

Microscope counts were obtained by viewing colonies on an inverted microscope. Image counts were obtained by counting colonies based on an image from the automated instrument (STEMvision™). Automated counts were performed by imaging and analysis on automated instruments. For each counting method, a number of independent counts were performed on each sample. For microscope and image counts, independent counts are defined as a counts performed on a given sample by different people. For automated counts the independent counts are performed by imaging and analyzing with different instrument units. Microscope counts were performed by 2-4 people. Image counts were performed by 2-5 people. Automated counts were performed on 2-4 units of the automated instrument.

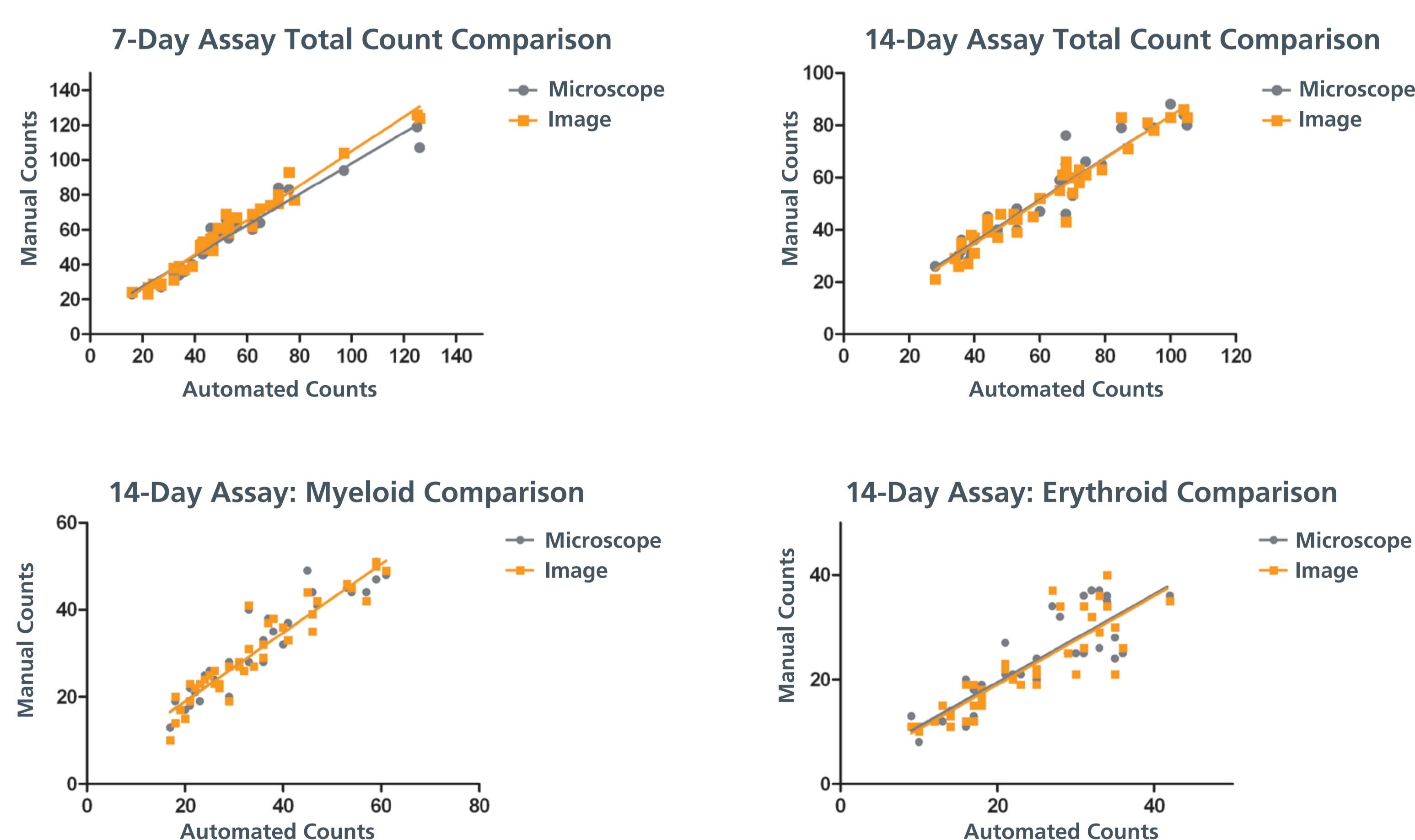
FIGURE 1: Culture and Analysis Workflow



1. A sample of cord blood cells is added to MethoCult™ Express (7-day assay) or MethoCult® H4034 Optimum (14-day assay) medium.
2. The medium is plated in 35mm 6-well SmartDish™ low-meniscus cultureware.
3. Microscope Counts: Colonies are counted manually by 2-4 experienced operators using a standard inverted light microscope.
4. Automated Counts: Colonies are counted automatically using the STEMvision™ instrument and software.
5. Image Counts: Colony images are counted manually by 2-5 experienced operators.

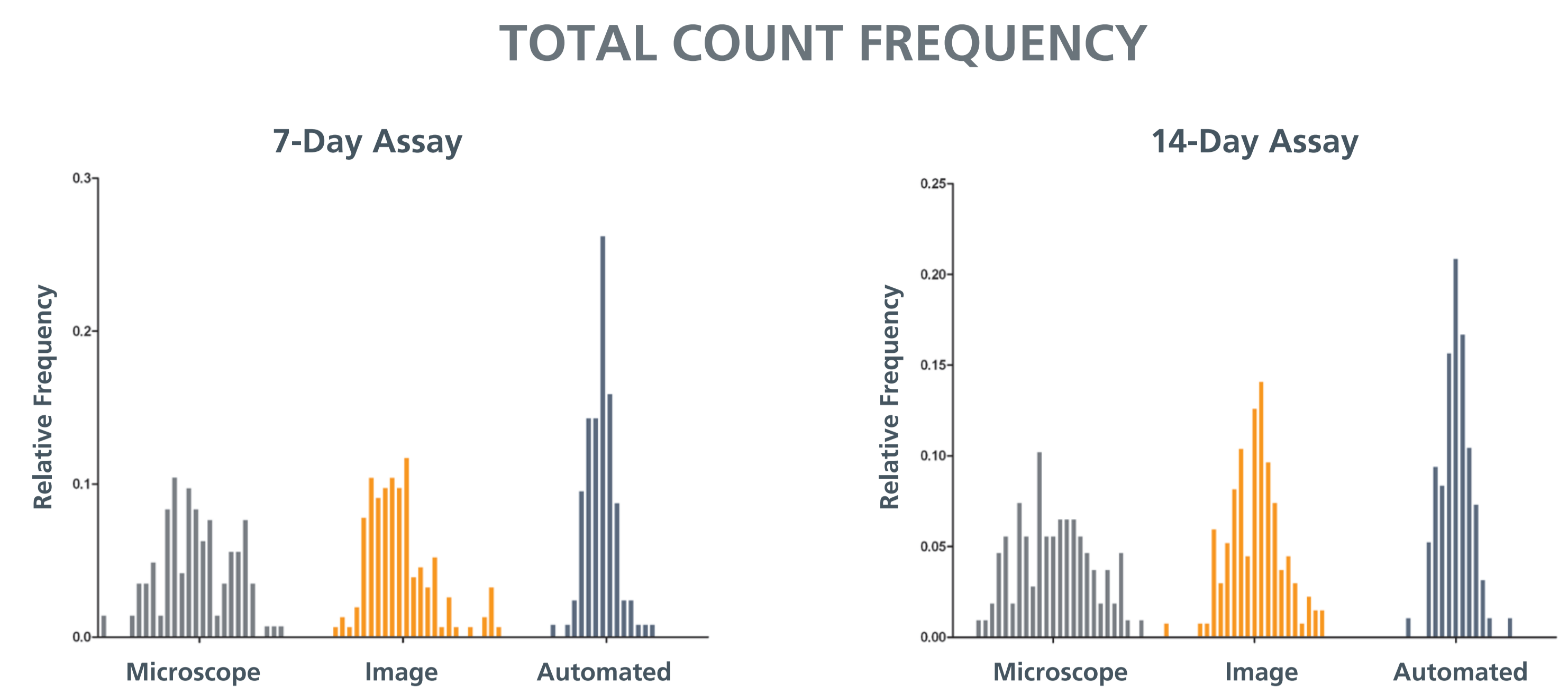
## Results

FIGURE 2: Automated and Manual Total Colony Counts are Highly Correlated



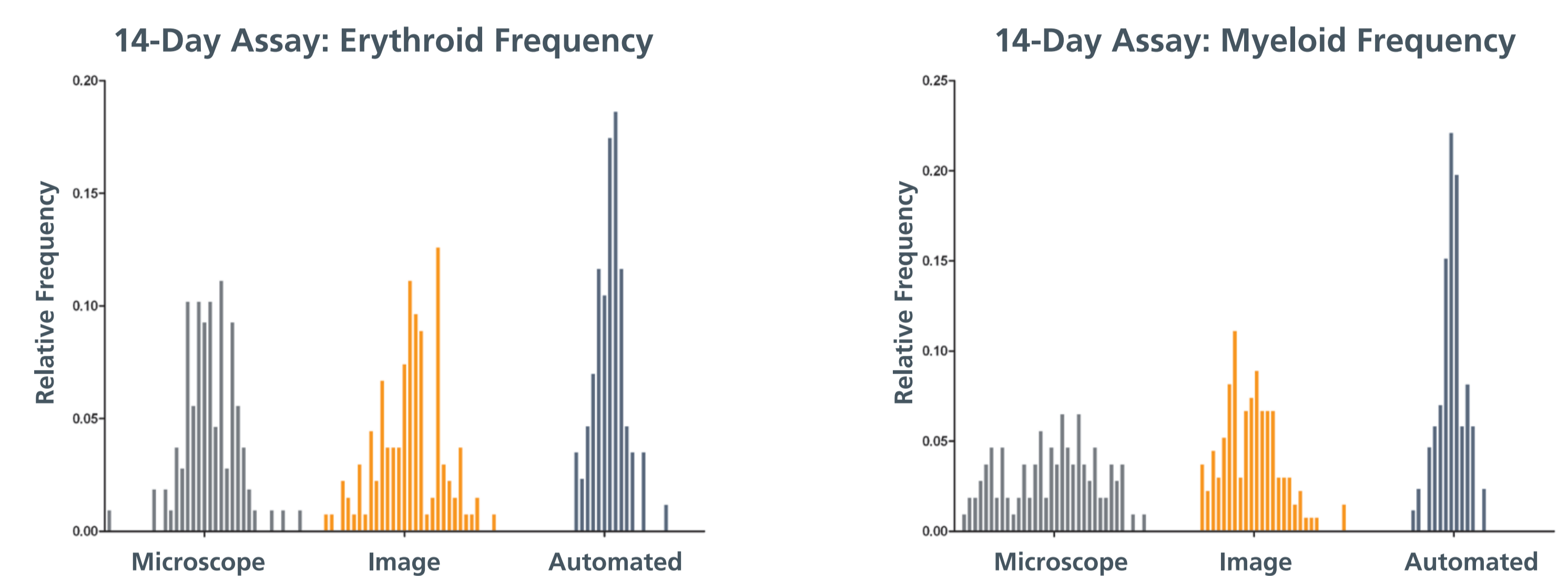
These plots show the average colony counts for the manual microscope and image counts as a function of the automated counts for the same samples. For the 7-day assay (n=36 samples) the automated counts are highly correlated to both the microscope and image counts ( $r^2 > 0.95$ ) and about 5% higher than both counting methods on average (significant difference in rank sum with  $P < 0.05$ ). For the 14-day assays (n=39), the total automated counts are highly correlated to the manual counts ( $r^2 > 0.90$ ) and about 18% higher than both the microscope and image counts (significant difference in rank sum with  $P < 0.05$ ). Erythroid and myeloid colony subsets are also highly correlated to the manual counting methods ( $r^2 > 0.85\%$ ). The automated counts are higher than the manual counts for both erythroid and myeloid subsets (about 15% and 20% higher, respectively). The higher average values for the automated system are likely due to scoring large multi-cluster myeloid colonies as multiple individual colonies and more effective detection of erythroid colonies.

FIGURE 3: Automated Counting Shows Reduced Variability



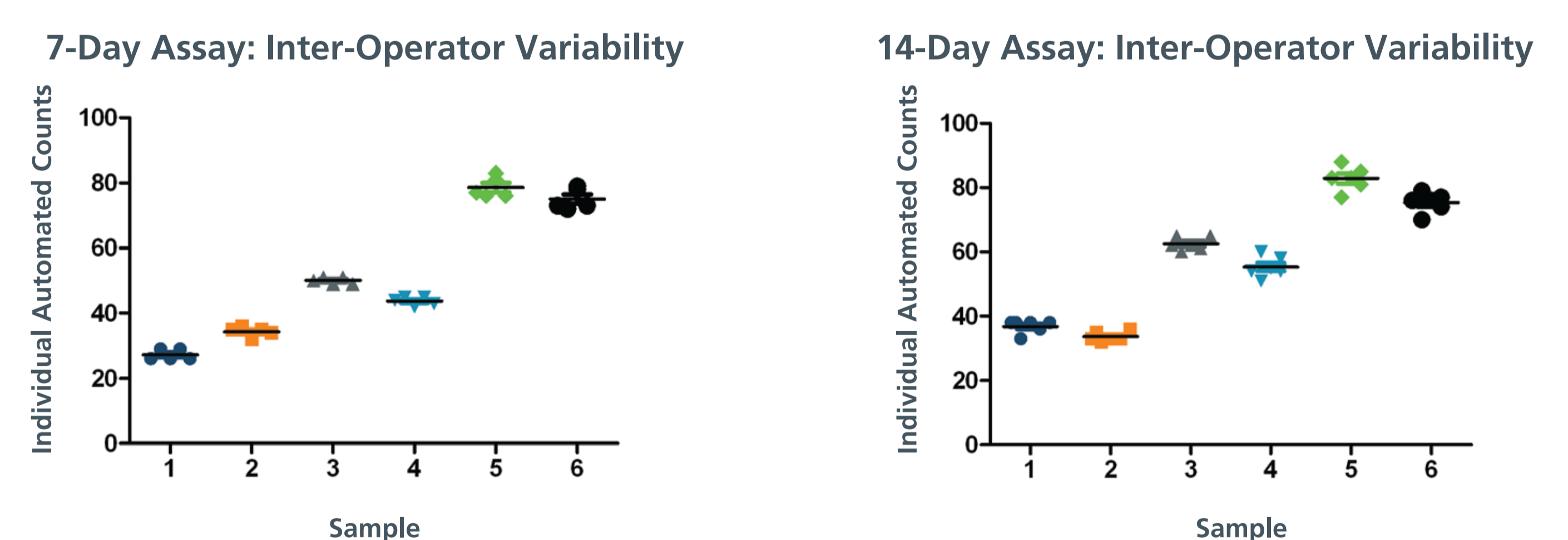
For each counting method, a histogram shows the frequency distribution of the normalized total colony counts. The colony counts were normalized by dividing each independent count by the average colony count for that sample. Histograms are shown for manual microscope counts, manual image counts and automated counts for both 7-day and 14-day assays. For the 14-day assay, the coefficient of variance (CV) for the automated counts (4.4%) is significantly lower (F test to compare variance,  $P < 0.05$ ) than for the microscope and image counts (11 and 7.9% respectively). Likewise, for the 7-day assay the CV for the automated counts (4.2%) is significantly lower than for the microscope and image counts (10% and 9.2% respectively). The variability in the image counts is significantly lower than the microscope counts for the 14-day assay.

CFU-GM AND BFU-E COLONY COUNT FREQUENCY



For each counting method, a histogram shows the frequency distribution of the normalized total colony counts. The colony counts were normalized by dividing each independent count by the average colony count for that sample. Histograms are shown for manual microscope counts, manual image counts, and automated counts for CFU-GM and BFU-E in the 14-day assay. When counting CFU-GM the coefficient of variance (CV) for the automated counts (5.2%) is significantly lower (F test to compare variance,  $P < 0.05$ ) than for the microscope and image counts (16.5 and 10.2%, respectively). Likewise, when counting BFU-E the CV for the automated counts (5.6%) is significantly lower than for the microscope and image counts (9.6% and 11.4%, respectively). The variability in the image counts is significantly lower than the microscope counts for the CFU-GM colonies.

FIGURE 4: Inter-Operator Variability



To isolate inter-operator variability from inter-instrument and operator variability, individual operators imaged and analyzed 6 cultures independently on a single instrument. The plot of average total colony count versus individual counts for the 7-day (5 operators) and 14-day assays (6 operators) show that variability is low for both assay types, with a CV of 4.1% for the 7-day assay and 4.3% for the 14-day assay. This variability is similar to the variability for the automated assay as a whole, suggesting either that residual variability is operator dependent, or that repeat measurements of a culture lead to variability due to changes in colony morphology after prolonged handling.

## Conclusions

- Automated counting reduces variability between operators relative to manual counting.
- Manual counting from whole well images can reduce variability between operators relative to microscope counts.
- Automated counting provides a basis for reducing inter-laboratory variability in assessing hematopoietic progenitor frequency in cord blood samples.
- Automated counting is effective and thus can be used to eliminate time required to score colonies manually.