

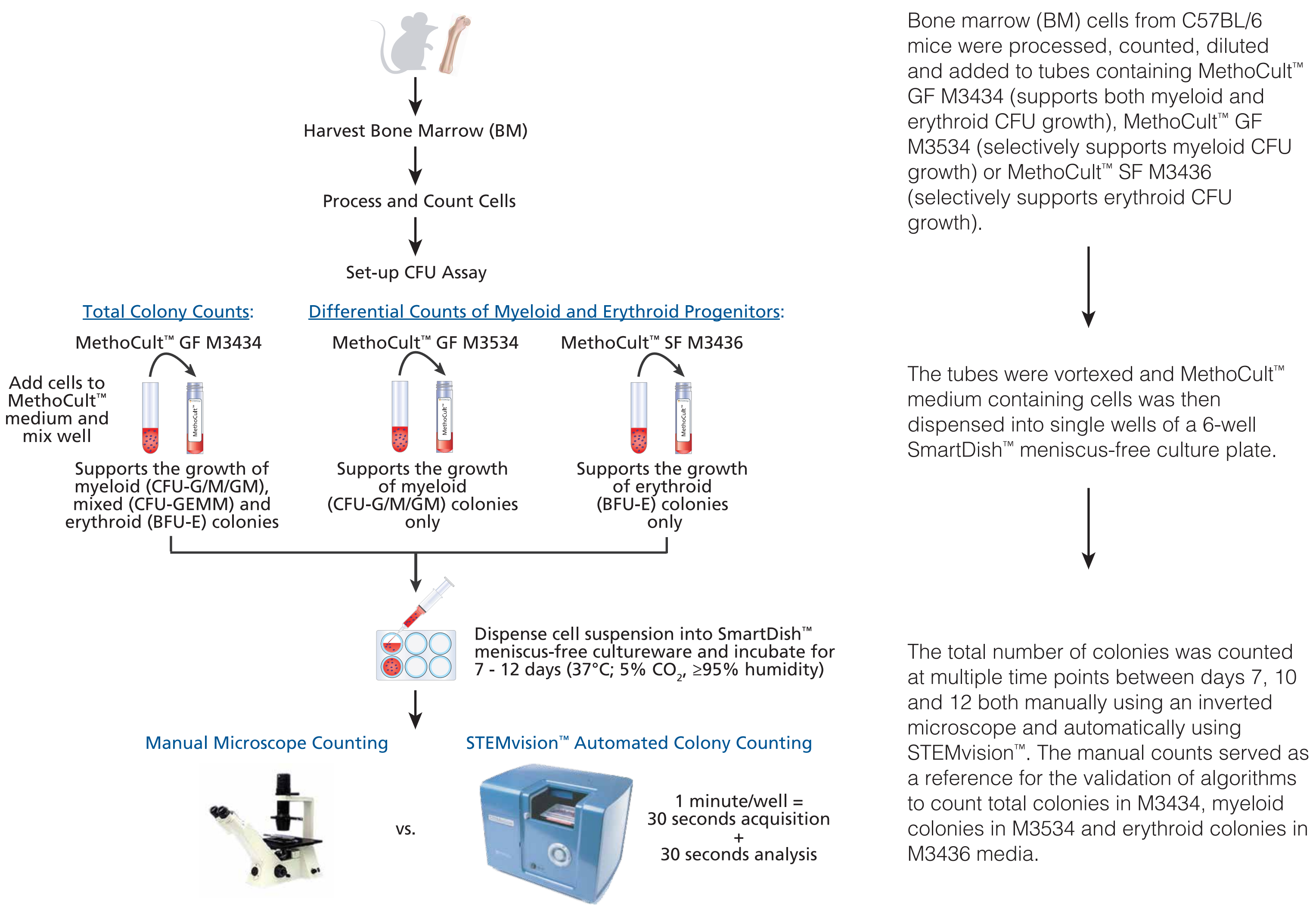
Automated Imaging and Analysis of Hematopoietic CFU Assays of Mouse Bone Marrow

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Introduction

The colony-forming unit (CFU) assay is the gold standard in vitro functional assay for measuring the number of progenitor cells in mouse hematopoietic cell populations and has numerous applications for basic research in hematopoiesis. In this assay, lineage-restricted and multi-potential progenitor cells are cultured in semisolid methylcellulose-based medium, such as MethoCult™, which contains specific combinations of cytokines that stimulate their proliferation and leads to the production of colonies of mature cells. Typically, mouse hematopoietic colonies are scored manually after 7 - 12 days using an inverted microscope. However, manual colony counting is somewhat subjective, time consuming and results often vary between individuals and laboratories. To address these problems we have developed new software for the STEMvision™ instrument that now allows automated counting of mouse CFU assays in different MethoCult™ media and at different time points. This automated system is faster, more accurate and more reproducible than manual colony enumeration using a microscope. In addition to colony counts, colonies are subdivided into four size classes as a qualitative measure of the proliferation potential of individual progenitors. The flexibility in the choice of assay end point (between days 7 - 12) and colony scoring criteria (size classes) allows adaptation of STEMvision™ for automated CFU assay counting in different applications.

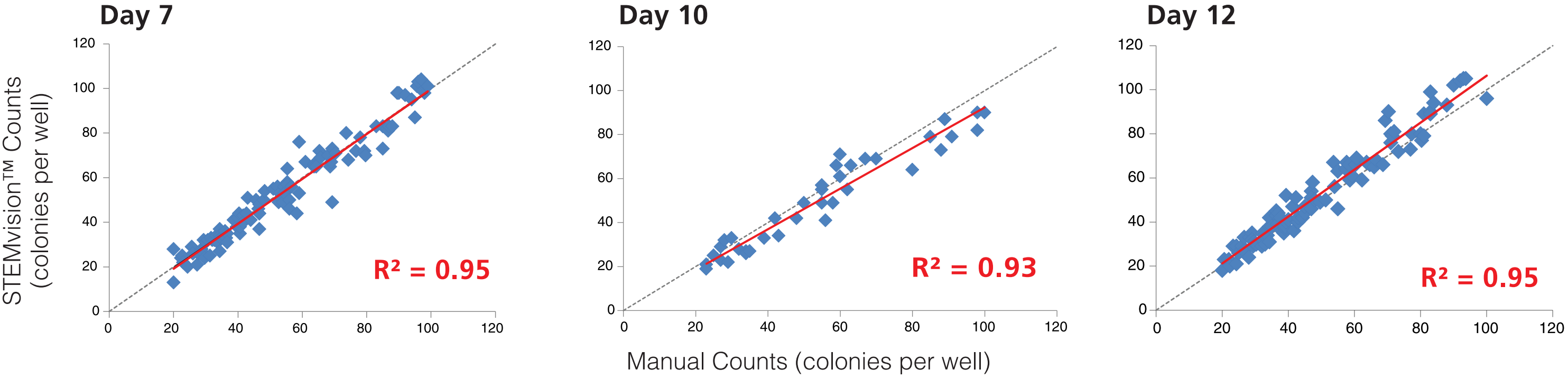
Materials & Methods



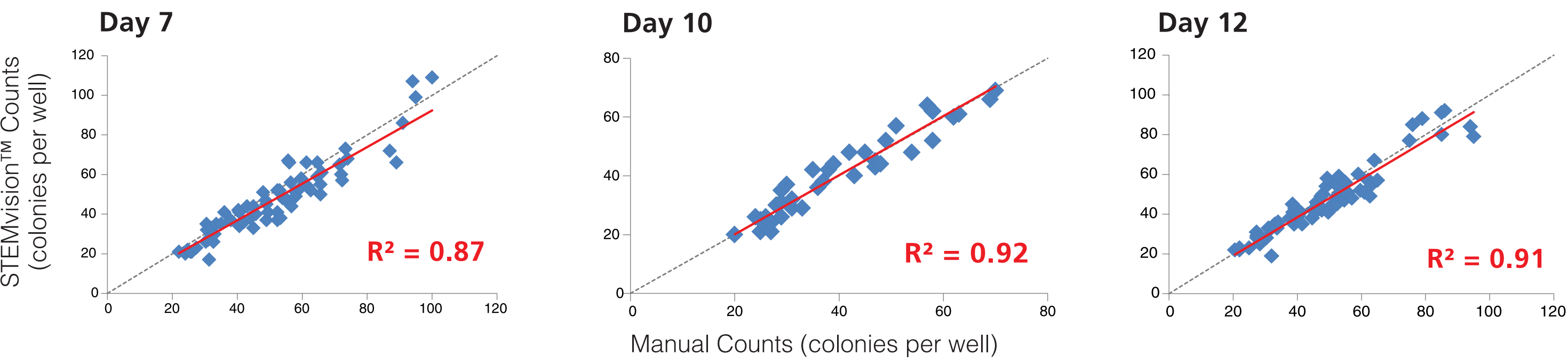
Results

Automated and Manual Counts of Total Mouse Colonies Are Highly Correlated

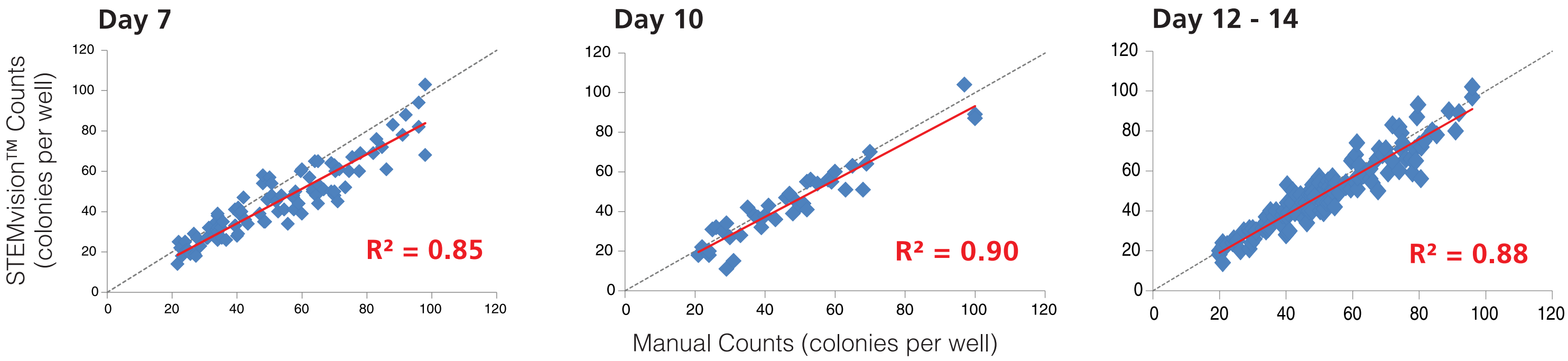
(A) MethoCult™ GF M3434: Myeloid and Erythroid Colonies



(B) MethoCult™ GF M3534: Myeloid Colonies



(C) MethoCult™ SF M3436: Erythroid Colonies



(D) Correlation Statistics

MethoCult™ Medium (Colonies Supported)	Day Counted	Slope, 95%CI*	Correlation Coefficient (R²)	No. CFU Assays
MethoCult™ GF M3434 (Myeloid & Erythroid)	7	0.99 (0.97 - 1.01)	0.95	104
	10	0.92 (0.89 - 0.96)	0.93	38
	12	1.06 (1.05 - 1.08)	0.95	99
MethoCult™ GF M3534 (Myeloid)	7	0.92 (0.89 - 0.95)	0.87	67
	10	1.01 (0.98 - 1.04)	0.92	40
	12	0.96 (0.94 - 0.99)	0.91	68
MethoCult™ SF M3436 (Erythroid)	7	0.86 (0.83 - 0.88)	0.85	96
	10	0.93 (0.89 - 0.97)	0.90	40
	12 - 14**	0.95 (0.93 - 0.97)	0.88	131

* CI: Confidence Interval

** Mouse CFU assays of erythroid progenitor cells plated in M3436 should be counted between 10 to 14 days.

Figure 1: Correlation between manual and automated STEMvision™ counts of the total number of combined myeloid and erythroid colonies in MethoCult™ GF M3434 medium (A), the number of myeloid colonies in MethoCult™ GF M3534 medium (B), and the number of erythroid colonies in MethoCult™ SF M3436 medium (C), each counted after 7, 10 and 12 days of culture. Note that colony sub-types (e.g. CFU-G vs CFU-M) within individual cultures are not determined in these assays. Automated colony counts produced by STEMvision™ were compared with the average manual counts from one to three expert staff members. Gray dashed lines represent a theoretical perfect linear correlation between manual and automated counts. Red solid lines represent the actual linear correlation between manual and automated counts. The correlation coefficient (r²) between manual and automated counts is shown in red. The table provides an overview of detailed statistics comparing manual and automated counts for all three media and each time point (D).

These results demonstrate that STEMvision™ can accurately and reproducibly count the total number of mouse hematopoietic colonies in different MethoCult™ media and at different time points.

Automated Colony Counting is More Reproducible Than Manual Counting

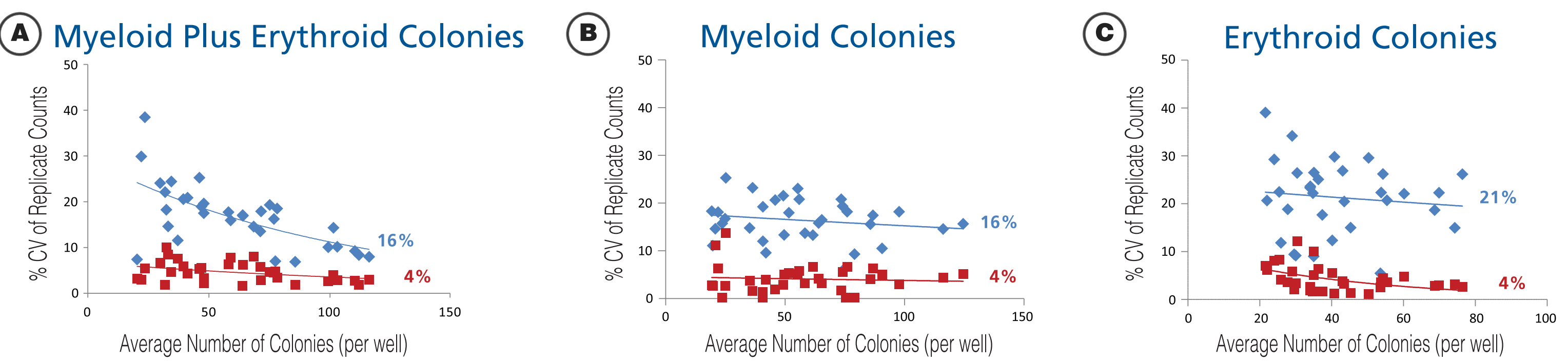


Figure 2: The coefficient of variation (CV) for total colony counts in 12-day CFU assays in MethoCult™ GF M3434 (supports myeloid and erythroid colonies) (A), GF M3534 (supports only myeloid colonies) (B), and SF M3436 (supports only erythroid colonies) (C). The variability of replicate colony counts, expressed as CV, was plotted against the average total number of colonies in individual CFU assay wells (n = 34). The results show the variability of manual microscope counts produced by five experienced operators (blue diamonds) and between automated counts obtained by repeated imaging of the same culture on three to four separate STEMvision™ instruments (red squares).

The average CV values for automated counts produced by STEMvision™ were much lower (4%) than the CV of manual counts (16%) for all three types of colonies.

Colonies are Subdivided Into Four Classes Based on Size and Density Allowing Progenitors with Different Proliferation Potentials to be Counted Separately

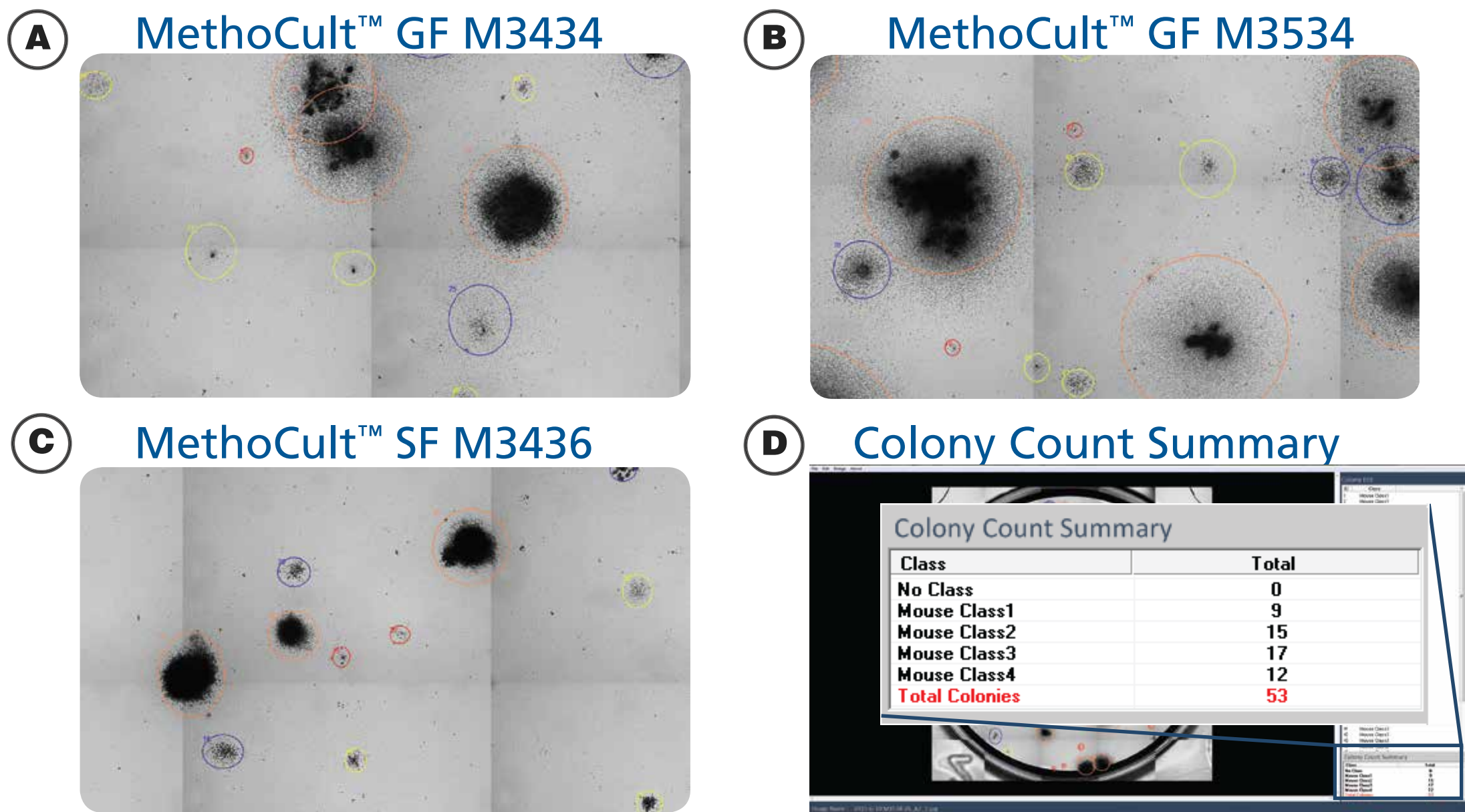


Figure 3: Representative STEMvision™ images showing size classification of colonies derived from mouse BM progenitors after 12 days of culture in MethoCult™ GF M3434 (A), MethoCult™ GF M3534 (B) or MethoCult™ SF M3436 (C) media. Red circles identify the smallest colonies - size class 1, yellow circles - size class 2, blue circles - size class 3, and orange circles identify the largest colonies - size class 4. Example of STEMvision™ CFU assay viewing software (D). The distribution of colonies in different size classes is summarized in a table.

The size class feature allows the user to define a threshold for colony counting; e.g. very small colonies in class size 1 that typically contain <50 cells can be excluded from total colony counts if desired.

Examples of Automated Colony Counts and Changes in Size Classification of Individual Colonies Over Time

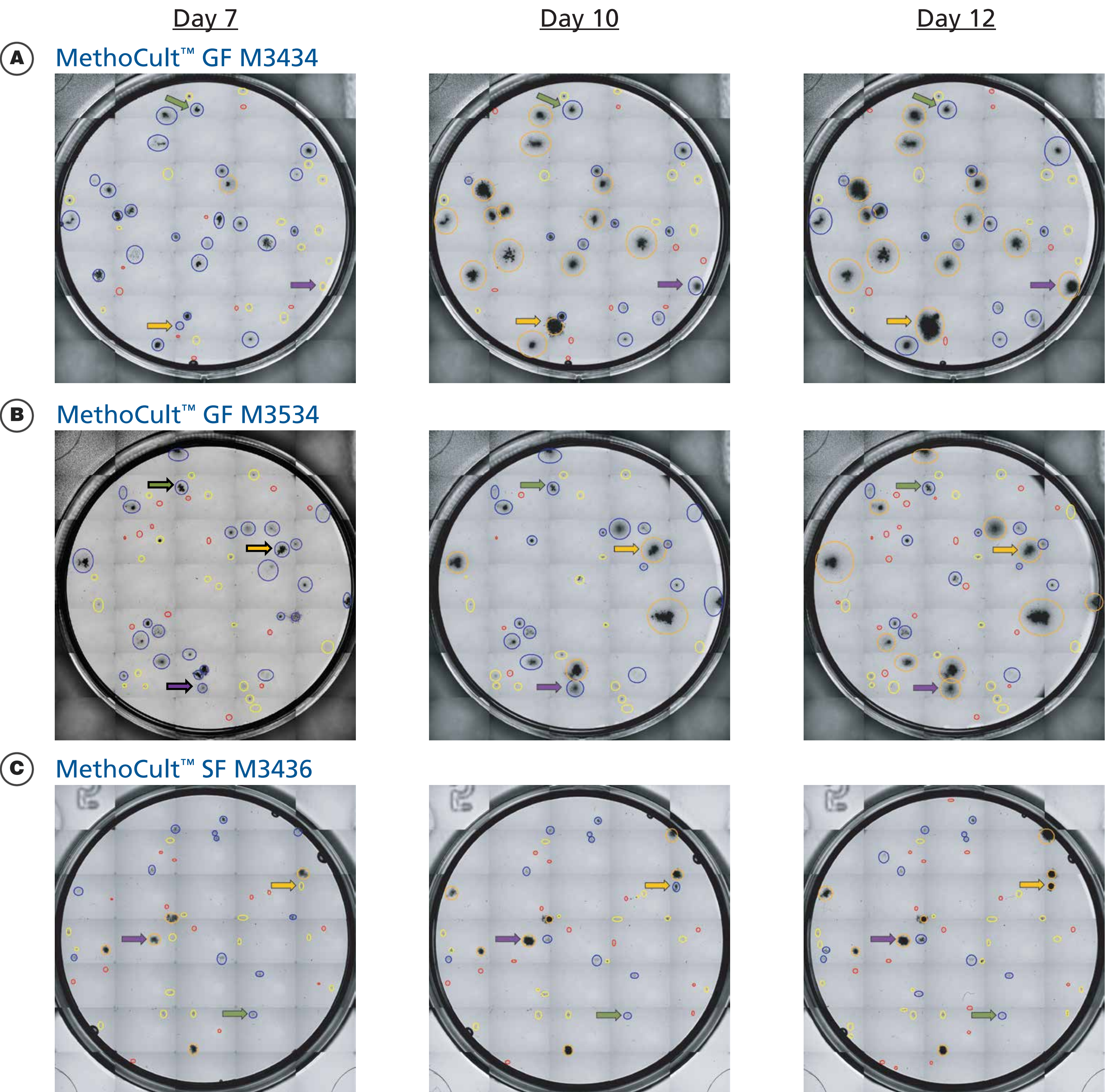


Figure 4: Representative STEMvision™ images of the same culture followed for up to 12 days. Images were analyzed using the STEMvision™ Mouse Bone Marrow Analysis Packages (i.e. software) designed for use with MethoCult™ GF M3434 (A), MethoCult™ GF M3534 (B) or MethoCult™ SF M3436 (C) media. Red circles identify the smallest colonies - size class 1, yellow circles - size class 2, blue circles - size class 3, and orange circles identify the largest colonies - size class 4. Highlighted with the arrows are colonies with distinct proliferation potentials whose size remained relatively stable or changed over time. Each arrow indicates the same colony on day 7, 10 and 12 of culture.

Conclusions

- Automated STEMvision™ counting of total colonies in CFU assays of mouse bone marrow cells is highly correlated to manual counting by expert users.
- Automated colony counting significantly reduces the variability of the CFU assay compared to manual counting.
- Subdivision of colonies into four size classes allows for customization of colony counting criteria and tracking of colony growth over time.
- Automated counting is fast: Image acquisition and analysis take approximately 1 min per well (30 seconds acquisition plus 30 seconds analysis).
- The flexibility in the choice of assay type (i.e. MethoCult™ formulation), the day of colony counting (between days 7 - 12) and colony counting criteria (size classes) allows adaptation of the STEMvision™ platform to different research applications.

For information on how to use STEMvision™ for counting of CFU assays of human bone marrow, peripheral or cord blood cells, see us at STEMCELL Technologies' booth.