

AutoRetic

Computerized automated grading
of reticulin fiber quantitation in
bone marrow

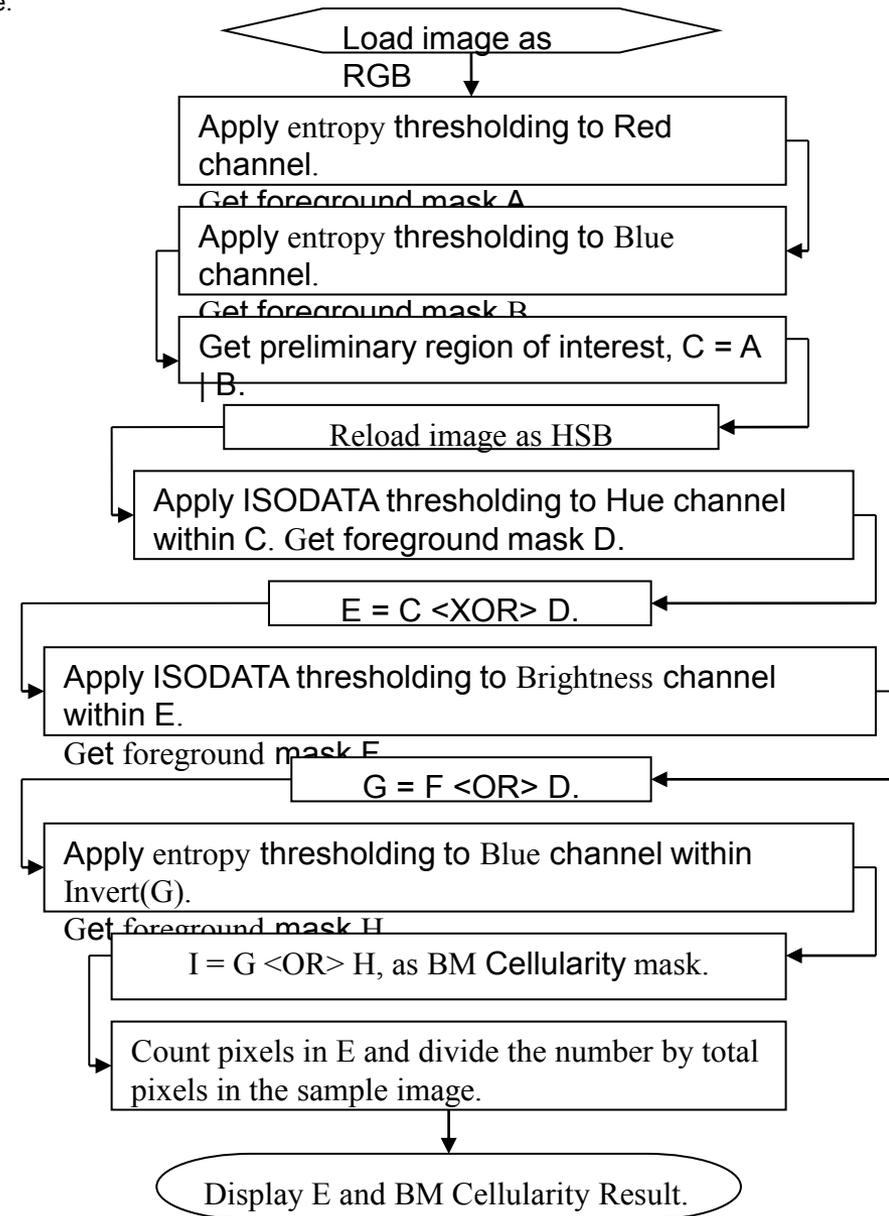
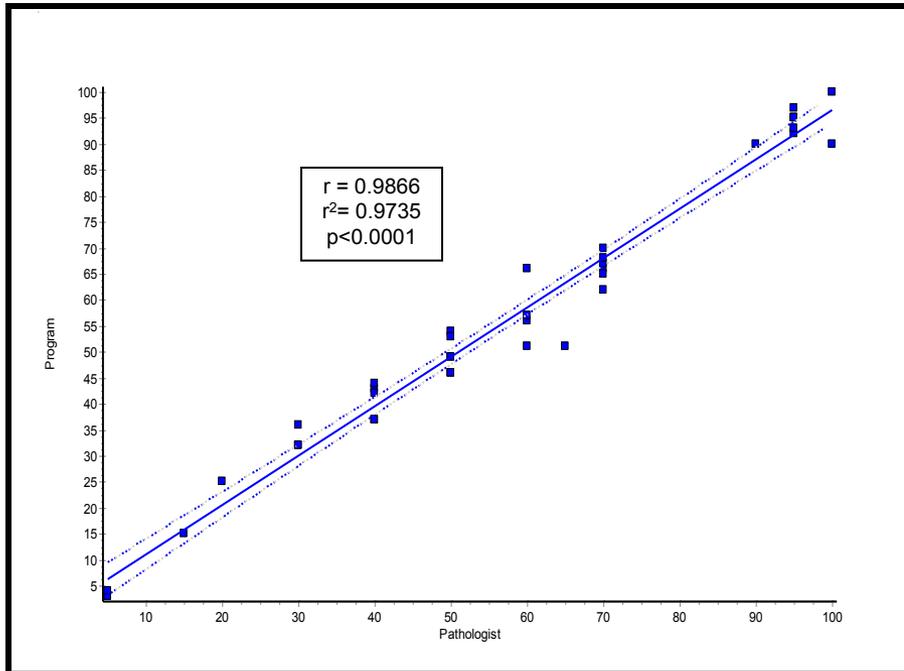
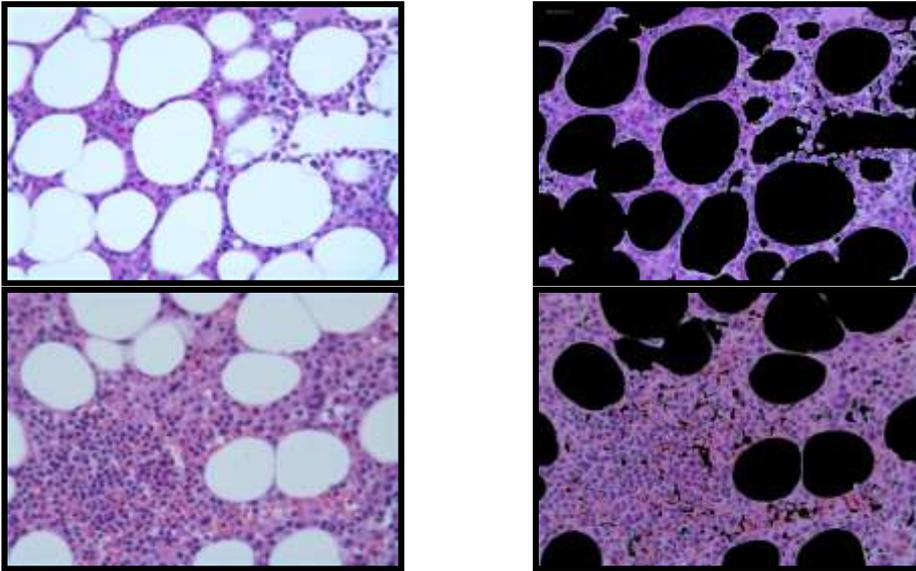
Statement of the Problem

- State of the art in pathology is estimation of many parameters
 - Bone marrow cellularity(Cell:Fat ratio) is estimated
 - Tissue immunohistochemistry positive ratio is estimated
 - Grading of reticulin is estimated

Goals

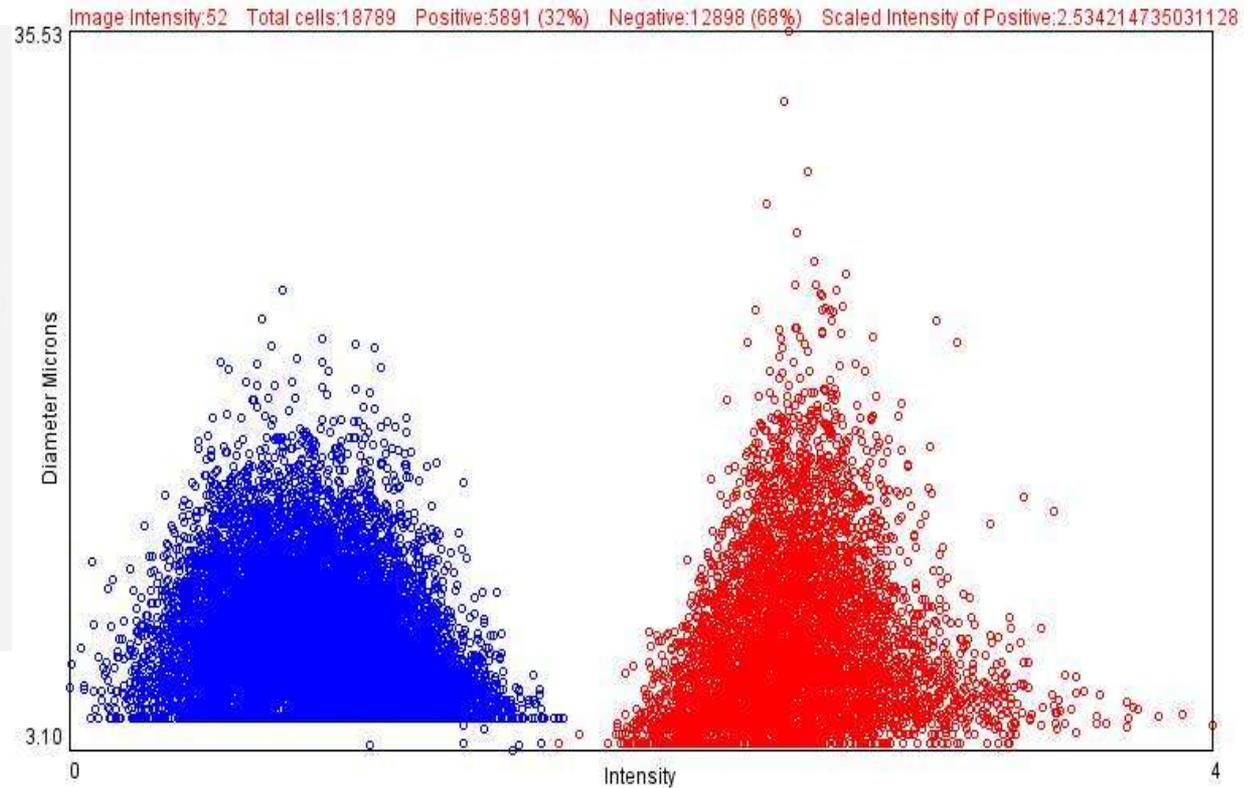
- Can computerized automation via creative algorithms minimize the subjectivity in some pathology determinations?
- Can these algorithms provide a standard foundation for objective determinations?
- Can the results of the above provide a quantitative results as a ratio, or a percentage or a grade?

AUTOMATED DIGITIZED IMAGES with cellularity results embedded in the digital image.



BM Cellularity Algorithm Flowchart

High Throughput Analysis of Tissue Antibody Microarray Using “Virtual Flow Cytometry” To Generate A Normally Distributed Immunohistochemistry Standard



1. High number of data points enough to generate a normal standard Gaussian cumulative frequency histogram with a “bell shaped” bivariate distribution of positive and negative objects representing staining results.
2. The bivariate distribution and its associated statistic could provide error rates and metrics for accuracy of segmentation and accuracy of immunostaining and an index of the quality of both.

Automated grading of the reticulin stain of bone marrows using AUTORETIC algorithm

IHCFLOW tm GreenGreat Inc., **Zhong, E. Cualing, H.**

- **Background**

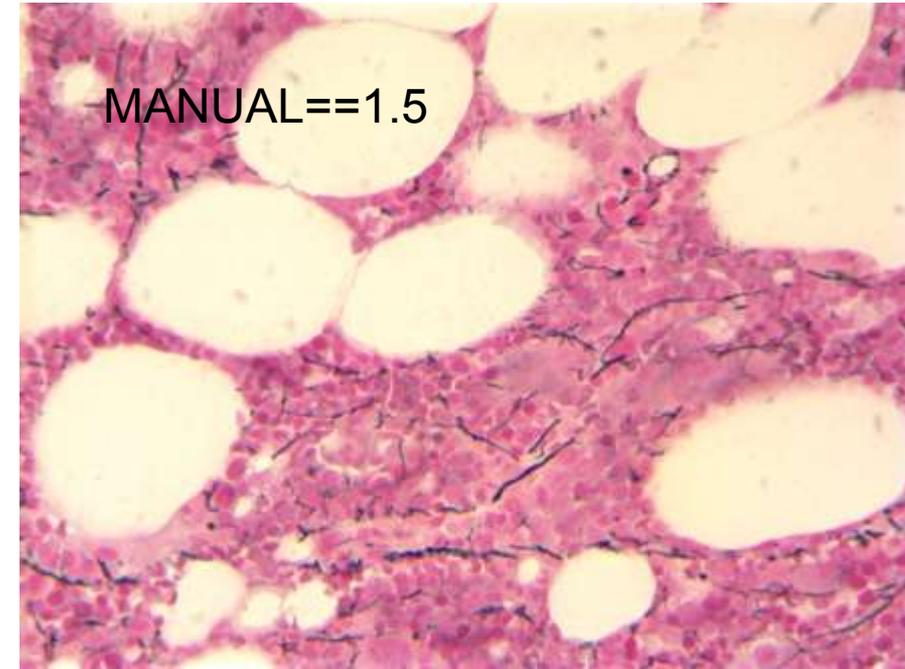
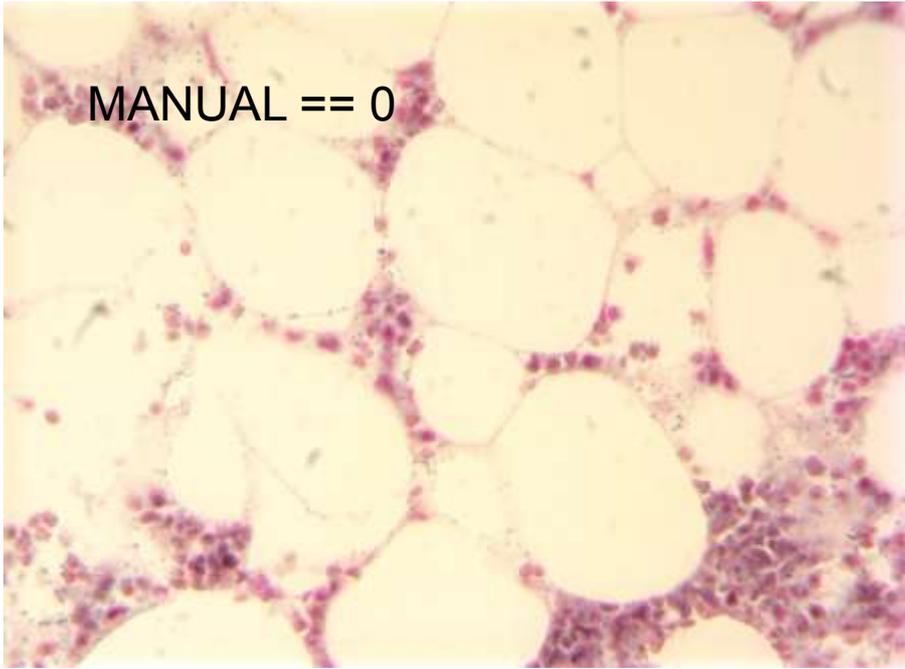
- Determining the grade of reticulin fibrosis in the bone marrow is currently performed subjectively using manual and visual grade assignment by hematopathologists with
- unavoidable intra- and interobserver bias. Because of this inconsistency, a rapid, accurate, reproducible computerized algorithm is needed. Here, we describe a novel AUTORETIC software in comparison with the pathologist manual grading.

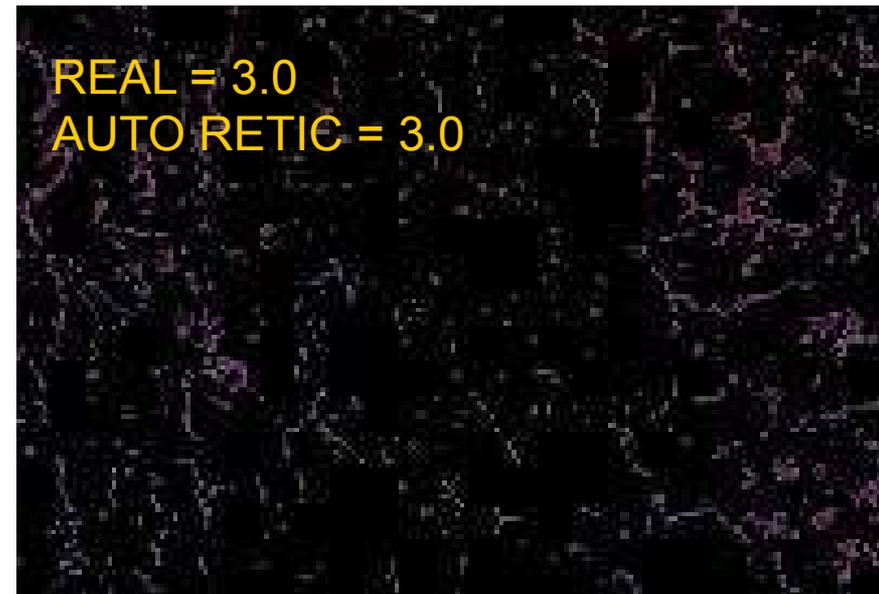
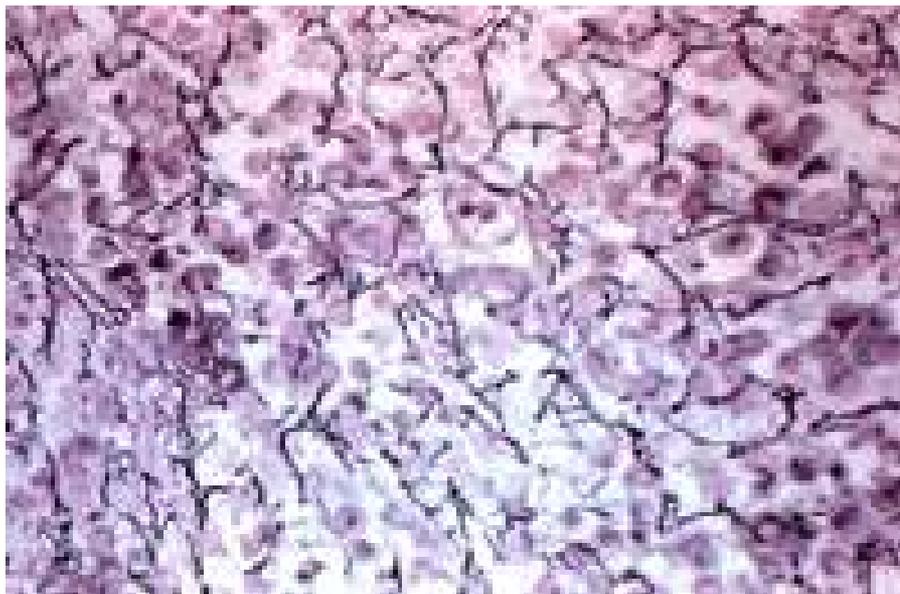
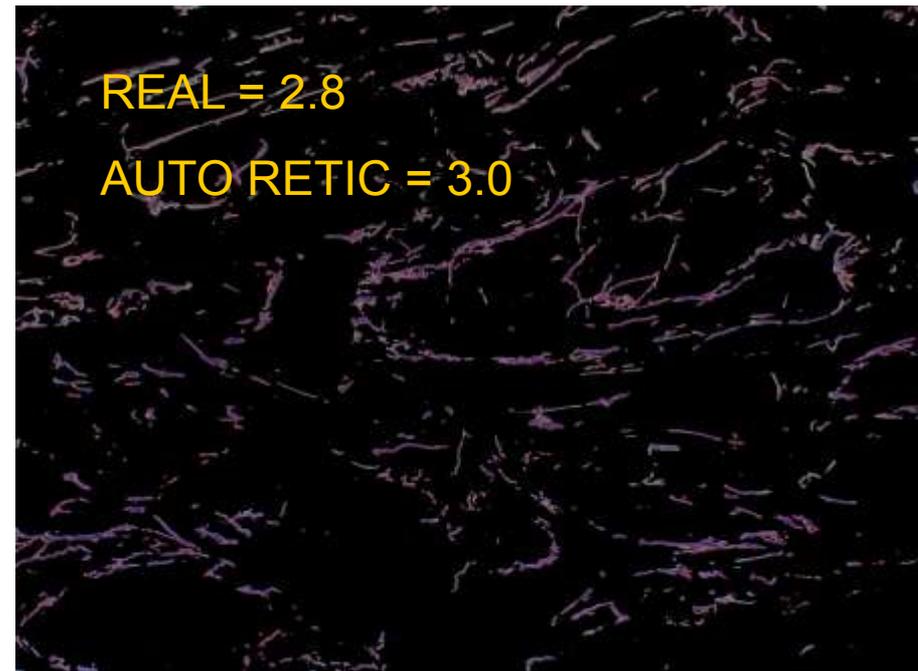
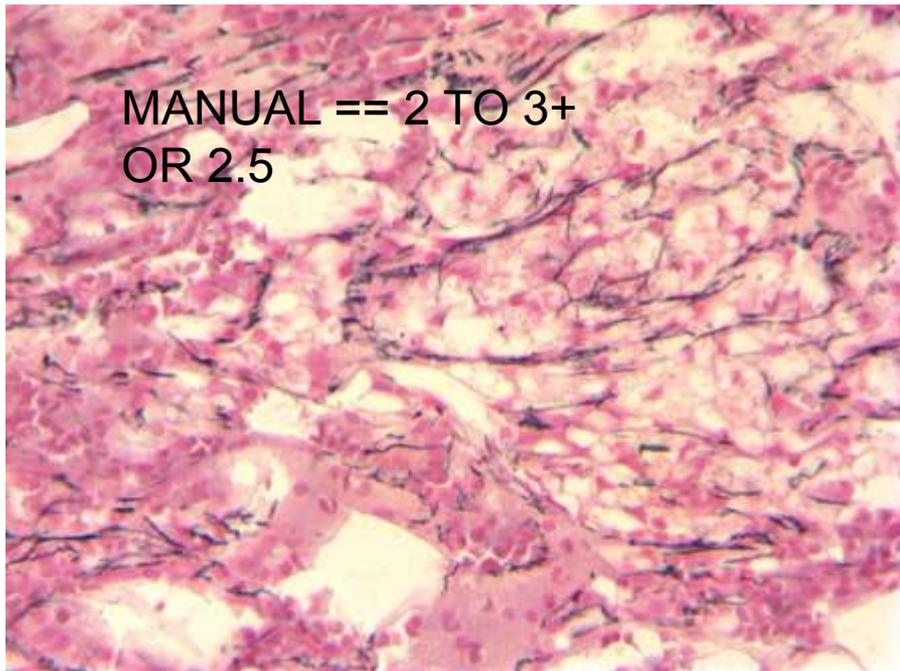
Materials and Methods

- A test and control group of images are compared.
- Test group has 65 patients, 23-80 years of age, M:F ratio 1:1.6 with various hematological diseases. The biopsy reticulin staining was performed using VENTANA Nexus automated system. A 20x digital color image was captured: average of 3 frames (1-7) per case. The results of manual grading by two pathologists are compared with the automated grading by AUTORETIC software that was developed and provided by IHCflow/GreenGreat corp. using proprietary algorithm.
- Control group of similar diseases, with marrow biopsy stained with a non-automated reticulin technic, using a different microscope CCD setup, is graded by a different pathologist, but with images likewise run in AUTORETIC to compare reproducibility.
- Grading was performed based on Bain's criteria. (BJ, Bain, Jul 2001, Bone Marrow Pathology textbook).

Criteria for Reticulin Grading

- 0 No reticulin fibers
- 1 Occasional fine individual fibers and foci of a fine fiber network
- 2 Fine fiber network throughout most of the sections, no coarse fibers
- 3 Diffuse fiber network with scattered thick coarse fibers, occasional megakaryocytes encircled
- 4 Diffuse often coarse fiber network with back to back fibers and many cells including megakaryocytes encircled





RESULTS

- The AUTORETIC grading finished in 2-3 seconds with continuous rounded to 0+, 1+ 2+ 3+, 4+ output. Agreement between the pathologist and computer was judged true if the difference between grades is 0.5 or less.
- Results demonstrate positive strong correlation between the manual grading and computer grading using our test and control groups.
- In the test group, the mean reticulin manual grading is 1.8 (95% CI 1.58 - 2.07) vs AUTORETIC mean of 1.734 with 95% CI(1.47 - 1.99) with no significant difference between the mean(SD). The correlation was high with r 0.8699 (nonparametric Spearman $p < 0.0001$).
- In the control group, the correlation was likewise high with r 0.7687(Spearman, 95% CI 0.71-0.82).

CONCLUSION

- In conclusion, the mean and SD for manual and AUTORETIC program are similar and they highly correlate with each other, indicating the software is a rapid, accurate and reproducible computerized technic that will be objective and useful in clinical bone marrow analysis. In addition, the program is robust and adaptable to variability of conditions and is applicable to bone marrow stained manually or automatically, digitized in a different microscope, or scored by a different user.