IHCFlow Imaging Laboratory

Validation of Rapid, Software-based Automated Determination of Bone Marrow Cellularity-A Viewer Function of Digital Pathology

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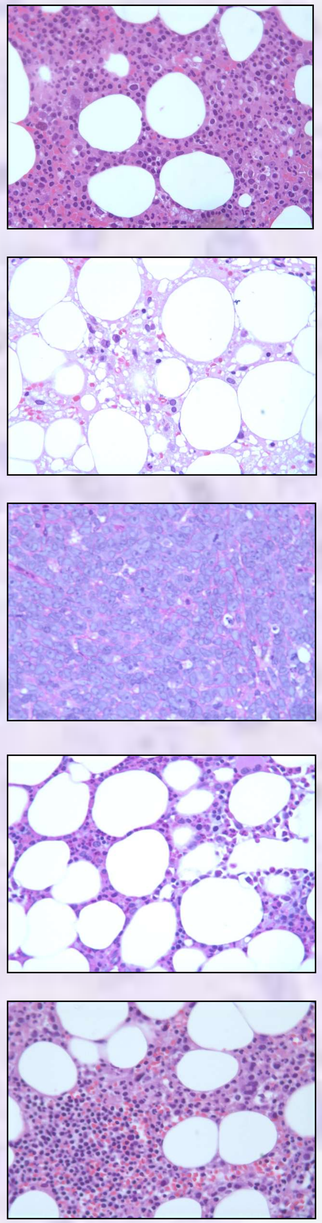
Background: Determining bone marrow cellularity is often a subjective estimation with great interobserver variation. A rapid, accurate, reproducible method would be desirable for pathologists who regularly examine bone marrow biopsies.

Design: Using public domain image analysis software ImageJ (NIH W Rasband, v1.34m) and advanced user-defined algorithms provided by iHCFlowGreenGreat, we examined 37 bone marrow core biopsies stained with hematoxylin and eosin and periodic acid shift stains obtained by 4 pathologists using 4 different microscopes color CCD setup. Normal marrows and those involved by both benign and malignant processes including anemia, status post chemotherapy, myelodysplastic and myeloproliferative disorders, leukemias, and lymphomas are examined. The raw images and the corresponding segmented marrow cellularity are stored for evaluation. The images were either PAS or H and E stained and either 20x or 40x. All bone trabeculae are excluded by each operator. Analysis takes 2-3 seconds using a Pentium 1.6 Gigahertz CPU, with a conventional color CCD camera and the Twain facility software.

Result: Nine out of ten cases examined demonstrated 100% accuracy in determining cellularity using the image analysis software. The correlation coefficient of pathologist and Bone Marrow Cytometer using linear regression (Instat Graphpad) is r=0.9866, p<0.0001 with four outliers in 37 cases. Initial problematic images, hypo and hypercellular marrows, are resolved with nuclear segmentation in the hypocellular marrows and accurate nuclear and cytoplasmic segmentation in the hypercellular marrows. The hypocellular marrow at day 14 post chemotherapy was segmented including the serous stroma and extracted nucleated cells only. Updated system software has increased accuracy with better correlation with hypocellular specimens (using *BM Cellularity 2*) and hypercellular specimens (using *BM Cellularity 3*).

Conclusion and applications: We found the automated image analysis technique to be useful, rapid and accurate way to extract bone marrow cellularity and provide a cell to fat ratio. We believe an accurate, objective, rapid measurement of bone marrow cellularity would be beneficial to practicing pathologists. This technology could be used in diagnosis and monitoring of patients pre/post chemotherapy, pre/post bone marrow transplant, setting baseline cellularity, determining tumor burden (i.e. multiple myeloma, lymphoma, leukemia). The system could be embedded into the scope, allowing for simultaneous extraction of bone marrow cellularity while making a diagnosis.

RAW IMAGES



SEGMENTED IMAGES

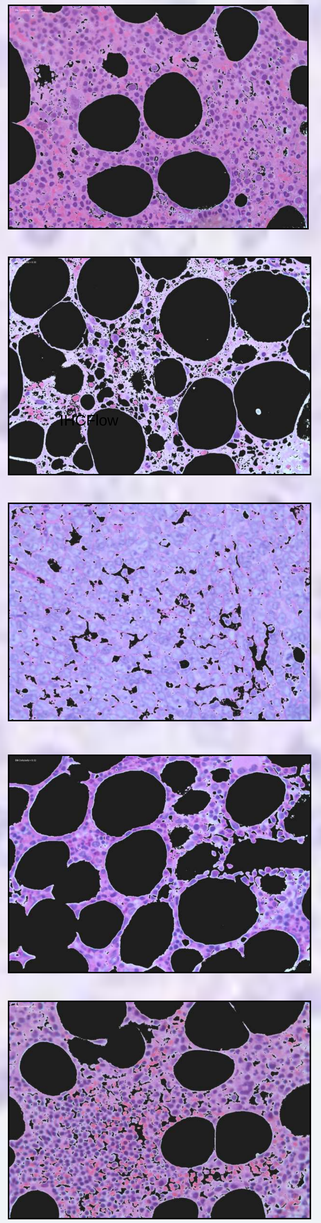
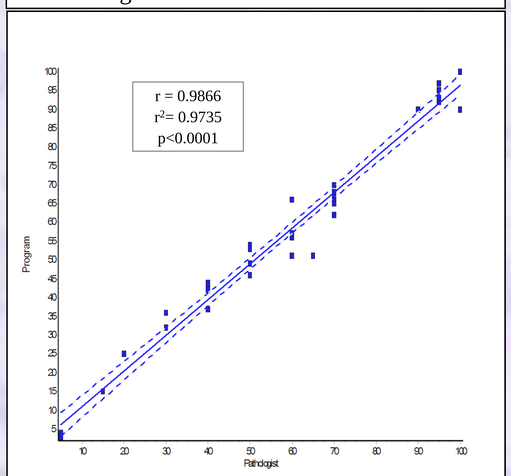


Table 1: Raw Data Comparing Pathologist Estimates to Automated Calculation

CASE #	STAIN	PATHOLOGIST	PROGRAM	DIAGNOSIS
1	H&E	60	66	ET
2	H&E	50	49	NHL
3	PAS	60	56	NEGATIVE
4	PAS	30	32	NEGATIVE
5	PAS	60	57	ANEMIA
6	PAS	65	51	CML
7	H&E	100	100	MDS
8	PAS	95	95	MDS
9	H&E	5	3	DAY 14
10	PAS	5	4	DAY 14
11	PAS	90	90	ALL
12	PAS	100	90	ALL
13	PAS	50	46	ANEMIA
14	PAS	40	43	ANEMIA
15	PAS	50	54	MM
16	PAS	30	36	ANEMIA
17	H&E	40	44	ANEMIA
18	H&E	40	37	NHL
19	H&E	20	25	RECOVERY
20	H&E	60	51	RECOVERY
21	H&E	40	37	MM
22	H&E	50	46	MM
23	H&E	70	70	NEGATIVE
24	H&E	30	32	NEGATIVE
25	H&E	40	42	NHL
26	H&E	70	66	NHL
27	PAS	70	62	NHL
28	PAS	70	67	NHL
29	H&E	50	49	NHL
30	H&E	50	53	PV
31	PAS	15	15	MDS
32	H&E	95	97	MDS
33	PAS	95	92	MDS
34	PAS	95	95	MDS
35	PAS	95	93	MDS
36	PAS	70	68	MDS
37	H&E	70	65	NHL

Figure 1: Linear Regression of Correlative Results



***Summary of the Software Algorithm for* Bone Marrow Cellularity**

Tissue Cytometry

1. Given *x*, *y* as the pixel spatial coordinate in the image frame of an arbitrary image *A* with RGB information representing the original color image.
2. This image is subjected to contrast intensity enhancement using red and blue CCD channels.
3. The Red channel is used to enhance blue nuclei and Blue channel is used to enhance brown stained cell objects.
4. Objects masks of same class of cells selected using automated thresholding by isodata.
5. Calculation of the parameter x for entropy mode thresholding.
6. Secondary extraction of dominant hue and dominant intensity from above masks using combined percentile, isodata, and entropy thresholding.
7. The masks are refined by thresholding only in hue and intensity using entropy x mode with prior removal of pixel singularities.
8. More refinement of the masks using linking and filling voids.
9. Display of segmented image with color overlay.

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References:

Cualing HD, Zhong E, Moscinski L. "Virtual Flow Cytometry" of Immunostained Lymphocytes on Microscopic Tissue Slides: *i*HCFlow™ Tissue Cytometry. *Cytometry.* 2007;72B:63-76.

Cualing HD. Automated Analysis in Flow Cytometry. Cytometry. 2000;42:110-3.