by the bone marrow niche consisting of numerous proteins, proteoglycans, cytokines and growth factors. One of the chondroitin sulfate proteoglycan, i.e., Versican (VCAN) has gained consideration in the context of solid tumors where in it has been shown to promote tumor progression. The importance of VCAN has been considered to identify its involvement in association with MM. The regulation of VCAN could be achieved by non-coding RNAs, i.e., microRNAs whose relative expression has also been assessed. **Materials and Methods:** 30 newly diagnosed MM patients and 20 controls were recruited. The bone marrow aspirate was collected from the study subjects followed by isolation of Bone Marrow Mononuclear Cells (BMMNCs). In a representative sample population (n=10), Bone Marrow Stromal Cells (BMSCs) was harvested from the BMMNCs by primary culture. RNA was isolated from both BMSCs and BMMNCs to investigate the relative mRNA expression of VCAN and its four isoforms (V0, V1, V2 & V3) along with the relative microRNA expression of miR-144, miR-199a3p and miR-203. The spearman correlation analysis was performed to determine the interrelationship, if any, between VCAN and microRNA. **Results:** The relative mRNA expression of VCAN and its isoforms (V0 and V1) were found to be significantly higher ($p<0.01$) in MM patients as compared to controls in both BMMNCs and BMSCs with higher expression in BMSCs in comparison to BMMNCs. The relative microRNA expression of miR-144, miR-199a3p and miR-203 were significantly reduced ($p<0.05$) in patients in both BMMNCs and BMSCs with BMSCs having lower expression than the levels in BMMNCs. Upon spearman correlation analysis, microRNA levels were found to have negative correlation with VCAN and its isoforms in both BMMNCs and BMSCs signifying the inverse relation in their expression. **Conclusion:** Augmented levels of VCAN and its isoforms in the bone marrow of patients especially in BMSCs imply their involvement in the bone marrow microenvironment of MM which could be exploited as a therapeutic target for the treatment of the malignancy. The negative correlation of microRNA expression indicates the plausible involvement of these microRNAs in regulation of VCAN in MM. Thus, targeting VCAN using microRNAs could be established as the functional therapeutic approach in MM.

## PS-176

### Stain Color Normalization and Segmentation of Plasma Cells in Microscopic Images as a Prelude to Development of Computer Assisted Automated Disease Diagnostic Tool in Multiple Myeloma

*Ritu Gupta,[1] Pramit Mallick,[2] Rahul Duggal,[2] Anubha Gupta,[2] Ojaswa Sharma[2]*

*[1]All India Institute of Medical Sciences, New Delhi, New Delhi, Delhi; [2]Indraprastha Institute of Information Technology, Delhi, India*

**Background:** Normalization and segmentation of cells is a precursor to cell analysis required for developing any computer assisted automated disease diagnostic tool. In this work, we address stain color normalization and cell segmentation from microscopic images stained with Jenner-Giemsa stain for multiple myeloma (MM). **Materials & Methods:** Images were captured from the slides of patients with MM and stained using Jenner-Giemsa stain. The proposed method of stain normalization carried out white balancing for illumination correction, stain vector correction, and stain quantity correction. Stain vector correction deals with errors caused due to variations in the stain chemical over time and batches. This was achieved via singular value decomposition (SVD) method. Here, a given RGB) image was first converted to the Optical Density (OD) space and reshaped to a 3xN matrix (with N no. of pixels and Red, Green, and Blue channels). SVD of this matrix is computed and all singular vectors of the input query image are aligned with the stain basis vectors of the reference image. Correspondingly, all intensity values are appropriately rotated in the OD space. Lastly, stain quantity correction is achieved via histogram percentile matching. The cell segmentation is carried out using modified multiphase level set formulation with three stages. In stage-1, intensity probability density functions (pdfs) of plasma cell (PC) nucleus, PC cytoplasm, unstained cells, and background are modeled in RGB, HSV, and Lab color spaces, and unstained cells are removed. In stage-2, regularization terms are added based on the pdfs to the multiphase level set formulation. In stage-3, watershed along with circle Hough transform are applied to segment cell clusters. To reduce false positives, Gaussian mixture modeling is used to filter out unwanted cells. **Results:** We tested our stain color normalization method on many MM images with one image as the reference. Both qualitative and quantitative results demonstrate that the proposed method efficiently normalizes stain color variations. The segmentation pipeline is tested on 50 MM images consisting of 165 single/isolated cells and 21 clusters. Our proposed method segmented 141 single cells and 16 clusters successfully, and had only one false positive cell. Compared to the other methods of intensity thresholding and conventional multiphase level set, the proposed pipeline is robust and provides greatly improved results. **Conclusion:** This work presents a method to normalize variations in stain color of MM images stained with Jenner-Giemsa stain and a 3-stage pipeline for efficient cell segmentation. The proposed methods lead to effective stain normalization and segmentation of plasma cells from microscopic images of multiple Myeloma. This is a step forward for the development of computer assisted automated analysis of microscopic images of MM.

## PS-177

### Immunophenotyping Patterns of Plasma cells in Plasma Cell Proliferative Disorders

*Ritu Gupta,[1] Meetu Dahiya,[1] Nitin Mathur,[1] Lalit Kumar,[1] Atul Sharma,[1] Gurvinder Kaur,[1] Varun Shekhar[1]*

*[1]All India Institute of Medical Sciences, New Delhi, New Delhi, Delhi*

Presence of normal plasma cells (PC) and preserved B-cell compartment in the bone marrow of patients with plasma cell