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Application of ImageJ for processing Shilajit exposed PBMC images

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ABSTRACT

Objective: In this study, application of ImageJ for processing Shilajit exposed PBMC images were studied.

Methods: In present study experiment was designed with human PBMC treated with Shilajit in high concentration (18mg/ml).Digital images were taken after one hour exposure with Shilajit and image processing steps were implemented.

Results: Acquired images from Shilajit exposed human PBMC had low contrast and substantial background noise as media was mixed with Shilajit. So images were processed by adjusting brightness and contrast, applying median filter, thresholding and watershed algorithm.

Conclusion: A high concentration of shilajit (18mg/ml) was detrimental to human PBMC. ImageJ can be efficiently used to process and extract information from low resolution images.

Keywords: ImageJ, Shilajit, PBMC

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INTRODUCTION

One of the most powerful methods in cell culture experiment is the visual analysis of a sample. But visual analysis of many samples is time-consuming, sometimes subjective and biased as well as, nonquantitative. This issue can be resolved by collecting digital images and processing them with image analysis tool to extract information. It has several advantages over tedious manual visual analysis including speed, quantitative and reproducible results. Numerous commercial and free software packages exist for image analysis but many of them are expensive and only suitable for very specific analysis1. ImageJ is a public domain Java image processing and analysis program inspired by NIH Image. It runs in different platforms like Windows, Mac and Linux. ImageJ is designed with an open architecture which provides extensibility via Java plugins. These plugins make it possible to solve almost any image processing or analysis problem [1-5].

Effect of phytochemicals or phytoextracts is usually investigated through cell cytotoxicity or other suitable assays. Visual examination of cells exposed to phytochemicals is very important to understand the effect. But it becomes really difficult to extract information by visual observation where media is mixed with phytochemical extract which is intensely colored. In present study we have designed our experiment with human PBMC treated with Shilajit (an ayurvedic drug) as effect of that drug in high concentration on human PBMC is not well investigated according to the published literature. Digital images were taken after one hour exposure with Shilajit and image processing steps were implemented. Shilajit which is a sticky, tar-like pale brown to black resin substance with a distinctive pungent odour has been used as a traditional ayurvedic medicine in India and other Asian countries. It is found to have many health benefits such as – anti-oxidant, anti-aging and anti-inflammatory properties [6-8].PBMC is widely used in screening assays with phytochemicals so we chose PBMC as our model system for study.

MATERIAL AND METHODS

Experimental Protocol:

Human blood was obtained from registered blood bank in the city. All chemicals were obtained from Sigma Aldrich. 20 mL of blood obtained from the Blood Bank was used to isolate PBMCs using gradient centrifugation with equal volumes of Histopaque 1077 for 30 min at 1500 rpm. Harvested PBMCs collected from the interface were washed three times with phosphate buffered saline for 10 min at 1000 rpm. After final wash step, the cells were resuspended in RPMI-1640 [9-11]. For this experiment 180mg of commercially available Shilajit was weighed and was dissolved in 1mL of 1X PBS. The stock concentration was 180mg/mL. The stock was then diluted to 1:10, diluent as 1X PBS. Shilajit solution (18mg/ml) was added in PBMC cell suspension. This was incubated for one hour at room temperature and the cells were then observed under a phase contrast microscope. The objective of the phase contrast microscope was kept at 20X and the eyepiece was kept at 10x.A control was taken and was also incubated at the same condition as the tests. Experimental designs were set in triplicate.

Image processing workflow:

The first step in image analysis is to acquire images for image processing. After one hour of incubation images were captured using digital SLR camera. Next, the images were processed to reduce noise and to enhance the contrast between features. Raw image was opened in ImageJ interface and brightness and contrast was adjusted. Careful optimization of the settings during image processing can dramatically improve the success of subsequent steps. Images were converted to 8 bit. An 8-bit pixel, which is probably the most common type, allows for 256 intensities of gray. Median Filtering which is a type of transformation that modifies pixel intensity values was used to reduce noise of the image. Next threshold of the image was adjusted to account for differences in the intensities of pixels in different regions of the image. Morphological processing like dilation was applied. Dilation is the operation in which an object is expanded (or broadened) through its borders. Images were dilated and then watershed algorithm was applied for efficient segmentation. Specified set of operations has to be manually crafted for each specific image [12].

RESULTS AND DISCUSSION

Standard image analysis protocol requires high-quality images specifically optimized for image processing. In present experimental study visual analysis of cells under microscope was extremely difficult as there was hardly any contrast between cells and background media. Acquired images from Shilajit exposed human PBMC had low resolution, low contrast and substantial background noise as media was mixed with Shilajit. So in next step, the images were processed to reduce noise and to enhance the contrast between features. Brightness and contrast was adjusted to make sure there was some difference between the cells and media colour. Images were converted to 8 bit and threshold of the image was adjusted. Application of median filter, changing the image threshold and binarization followed by application of morphological processing like image dialation, were able to identify cells from the background. Finally watershed algorithm was used to carve out cells from debris. Fig 1(a) & (b) shows result of image processing with control and Shilajit exposed human PBMC.

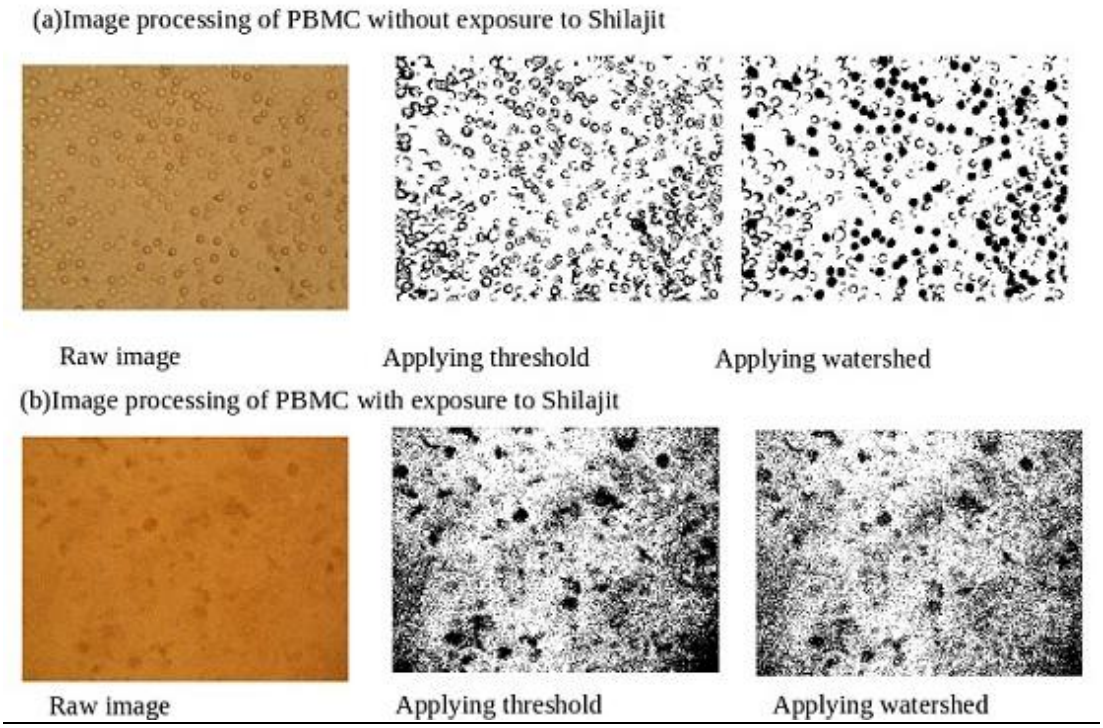

Figure 1: Image processing workflow with results for Control (a) and Shilajit exposed human PBMC (b)

After processing images of control PBMC and PBMC exposed to high concentration of of Shilajit it was observed that high concentrations of shilajit was detrimental to human PBMC. Very few intact PBMC were observed after one hour of exposure and there was lot of cell debris. So ImageJ can be efficiently used to process and extract information from low resolution images.

CONCLUSION

High concentrations of shilajit (18mg/ml) were detrimental to human PBMC. ImageJ can be efficiently used to process and extract information from low resolution images.

REFERENCES

1. Murphy RFE, Meijering, Danuser G, “Special issue on molecular and cellular bioimaging”. IEEE Trans. Image Process. 2005; 14:1233-1236.
2. Tiago Ferreira and Wayne Rasband, ImageJ User Guide IJ 1.46r, 2012.
3. Caroline A. Schneider, Wayne S. Rasband, Kevin W. Eliceiri, “NIH Image to ImageJ: 25 years of Image Analysis” Nat Methods. 2012, 9(7):671–675.
4. Girish V, Vijayalakshmi A, “Affordable image analysis using NIH Image/ ImageJ” Indian J Cancer, 2004; 4(1):47
5. Helmy and Abdel Azim, “Efficacy of ImageJ in the assessment of apoptosis” Diagnostic Pathology, 2012; 7:15.
6. Acharya SB, Frotan MH, Goel RK, Tripathi SK, Das PK, "Pharmacological actions of Shilajit". Indian Journal of Experimental Biology, 1988:26(10):775–7.
7. Goel R.K, Banerjee R.S, Acharya S.B, "Antiulcerogenic and antiinflammatory studies with shilajit". Journal of Ethnopharmacology, 1990; 29(1):95–103.
8. Ghosal S, Singh SK, Kumar Y, Srivastava R, Goel RK, Dey R, Bhattacharya SK, "Anti-ulcerogenic activity of fulvic acids and 4′-methoxy-6-carbomethoxybiphenyl isolated from shilajit". Phytotherapy Research, 1988; 2(4):187.
9. Freshney RI, Culture of Animal cells: A Manual of Basic Technique. NJ: John Wiley & Sons Inc., 2005.
10. Cell culture Basics Handbook, Thermo Fischer Scientific Inc. 2015.
11. Ficoll-Paque PLUS Handbook: For in vitro isolation of lymphocytes, Amersham Biosciences, 2001.
12. Adrienne H.K. Roeder, Alexandre Cunha, Michael C. Burl and Elliot M. Meyerowitz, “A computational image analysis glossary for biologists” Development, 2012; 139:3071-3080.