Hyperchromasia and Texture as effective features for analysis of malignancy in Pap smear Images

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Abstract

This work presents an approach for the analysis of abnormality in the cervical cells based on Texture and presence of Hyperchromasia, which are two important morphological features based on which one can distinguish between normal and abnormal cervical cells. The proposed approach is implemented in MATLAB®, a high level, interactive environment for data visualization/analysis/computation. This may help pathologist in identification of cervical cancer from Pap smear images and help in early diagnosis.

Keywords: Pap smear, Cervical cancer, Histogram, Gray-level Co-occurrence Matrix, Hyperchromasia

1. Introduction

Following breast cancer, cervical cancer is the second most prevalent cancers among women. According to a survey, one woman dies every seven minutes of cervical cancer and by 2025, it is estimated to be one death in every 4.6 minutes. There has been a regular campaign against cervical cancer for the last 30 years in India, but this has had little impact on the morbidity and mortality from the disease, with India ranking fourth worldwide [1]. Government of India has undertaken several cancer control program but these measures have not been effective in reaching the rural regions due untrained manpower, lack of infrastructure and lack of awareness[2]. So, early detection of cervical cancer may take a very precious role in preventing this disease. Some of the features for abnormality identification are the texture analysis and to check the presence of Hyperchromatic Crowded Groups (HCG). In medical imaging, images are used to gain maximum information for diagnostic purpose and those images are digital in nature. In the study of malignancy in cervical cancer Pap smear images are used. Pap smear test is a quick, simple and essentially painless test to detect abnormal cervical cells. These Pap smear images are used as an input for our approach which will study the texture and HCGs of the cervical cells and based on the parameters obtained, given samples can be classified into two groups i.e., normal class and abnormal classes.

2. Overview of Cervical Cancer

Cancer is a condition caused by uncontrollable growth of cells. Cervical cancer is a common cancer in women which is found in ectocervix, endocervix and in the transformation zone (Figure 1). Over 90% case of cervical cancer arises in transformation zone [7].

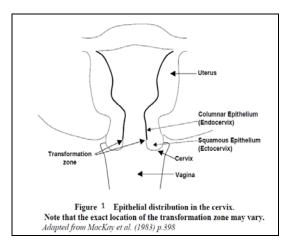


Figure 1. Epithelial Distribution of Cervix

There are many factors that lead to cancer:

- HPV (Human Papilloma Virus) infection
- Having multiple sex partners
- Giving birth before the age of 22
- Smoking
- Birth control pills.
- Low socio economic status
- Inadequate intake of folic acid

3. Texture and Abnormality

Texture is an important characteristic for the analysis of many types of images. But a standard definition of texture doesn't exist. But for a simple definition Image texture is a function of the spatial variation in pixel intensities (gray values), is useful in a variety of application and has been a subject of intense study by many researchers [4]. Using texture analysis various features like homogeneity, Contrast, Energy, Correlation and frequency of images can be calculated. The texture of images also refers to the appearance, structure and arrangement of the parts of an object within the image [3]

It can be observed that cancer cells are different from normal cervical cells as normal cells pose a homogeneous texture and the frequency distribution of the gray levels is also homogeneous. So analysis of textures of Pap smear image can take an important role in cervical cancer detection.

For our approach we have combined two texture analysis approaches:

- a) Histogram analysis combined with frequency calculation of the image pixels.
- b) Gray level Co-occurrence matrix (GLCM).

Both of these techniques belong to Statistical approach methods which mainly represent texture based on distribution and relationship between gray levels.

4. Hyperchromasia and Abnormality

Another morphological feature which can indicate the presence of abnormality is the presence of Hyperchromatic Crowded Groups (HCG). Hyperchromasia is nothing but presence of a cluster of cells which contain more than 15 cells. Following figure shows the presence of Hyperchromatic Crowded Group (HCG).

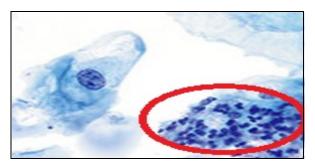


Figure 2. Presence of HCG (Source: University College of London Site [5])

5. Proposed Method

In this approach abnormality analysis is performed on 976 images which are collected from Dr B.Barooh Cancer Institute, Guwahati, website of University college of London (they provide nano-zoomer using which one can visualize the original slides online) [5] and MDE laboratory[6].

Sample collected from Dr B.Barooh Cancer Institute – 24 Sample collected from University college of London site – 35 Sample collected from MDE Lab – 917 The Pap smear images are in .jpg and .bmp formats.

Snapshot of a part of Image Database is shown in Figure 3-



Figure 3. Sample Database Collected from MDE Lab

5.1. Texture Analysis

5.1.1. Histogram Approach

The histogram of an image is the count of how many pixels in the image possess a given grey-level value. For an 8 bits image, this may be represented by a graph with an x coordinate ranging from 0 to 255 and a y coordinate representing the respective pixel count. Figure 4.b shows the histogram of the image in Figure 4.a.

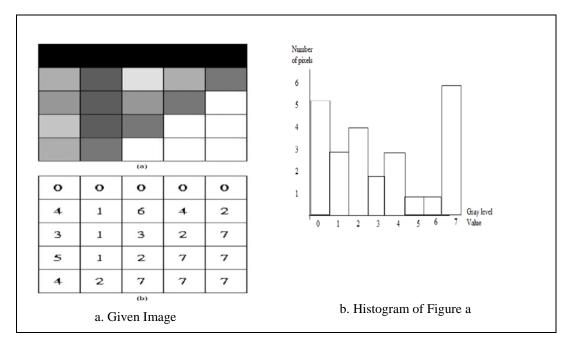


Figure 4. Histogram Representation of an Image

The following measures are taken in case of texture analysis –

- 1. The colored image is converted into gray scale image using rgb2gray function.
- 2. Histogram is evaluated using imhist function.
- 3. Frequency count is calculated from histogram which will give the occurrence of each pixel value having a particular gray level.
- 4. Then the frequency count is presented in a graph form where x co-ordinate represents the gray level and y co-ordinate represents the no of pixel having a particular gray level.

In case of an abnormal cervical cell the graph will have many maxima and minima points. But in case of normal cervical cell the graph will exhibit a smooth nature.

5.1.2 GLCM Approach

The gray-level co-occurrence matrix (GLCM) is a frequency matrix and a tabulation of how often different combinations of pixels brightness values (Gray levels) occur in an image. The GLCM indicates the frequency of a pair of pixels that are at "Exactly the same distance and direction of displacement vector" [4].

GLCM, c, is defined with respect to given (row, column) displacement h. And element (i, j), denoted c_{ij} , is the number of times a point having gray level j occurs in position h relative to a point having gray level i. Let Nh be the total number of pairs, then $C_{ij}=c_{ij}/NH$ is the elements of the normalized GLCM [8].

The co-occurrence probability provides a second-order method for generating texture features. These probabilities represent the conditional joint probabilities of all pair wise combinations of grey levels in the spatial window of interest given two parameters: Interpixel distance (δ) and orientation (θ). The probability measure can be defined as:

$$\Pr(x) = \left\{C_{ij} \mid (\delta, \theta) \right. \}$$
 Where Cij (the co-occurrence probability between grey levels i and j) is defined as:
$$Cij = \frac{Pij}{\sum\limits_{i,j=1}^G Pij}$$
 Where P_{ij} represents the number of occurrences of grey levels i and j within the given window, given a certain (δ, θ) pair;

Figure 5. Equation of Co-occurrence Probability [4]

G is the quantized number of gray levels. Orientation are of four types i.e. 0 degree, 45 degree, 90 degree and 135 degree. Figure 4 shows the orientation –

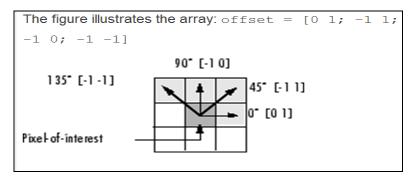


Figure 6. Orientation (θ) [8]

θ is represented as offset which is a p-by-2 array of integers specifying the distance between the pixel of interest and its neighbor. Each row in the array is a two-element vector, [row_offset, col_offset], that specifies the relationship, or *offset*, of a pair of pixels. row_offset is the number of rows between the pixel-of-interest and its neighbor. col_offset is the number of columns between the pixel-of-interest and its neighbor. Because the offset is often expressed as an angle, the following table lists the offset values that specify common angles, given the pixel distance D.

Angle	Offset
0	[0 D]
45	[-D D]
90	[-D 0]
135	[-D -D]

For our approach we considered D value as 1.

One example of GLCM calculation is shown below:

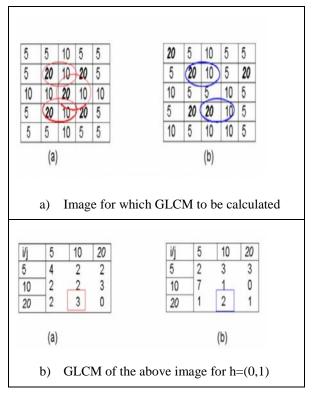


Figure 7. Example of GLCM Calculation [4]

Steps used to identify the texture properties using GLCM are as follows –

- a) Convert the rgb image to gray scale image using rgb2gray function.
- b) Construct the GLCM using function graycomatrix.
- c) Extracting properties like homogeneity, Contrast and Energy using function graycoprops.
- d) Analysis of output data and drawing a conclusion.

List of features extracted are shown in the following table –

Table 1. Feature that can be Extracted from GLCM

PARAMETER	DESCRIPTION	FORMULA
CONTRAST	Returns a measure of the intensity contrast between a pixel and its neighbor over the whole image. Range = [0 (size(GLCM) - 1) ²]	
	The Contrast is expected to be low if the gray levels of each pixel pair are similar. Contrast is 0 for a constant image.	$\sum_{i,j} i-j ^2 P(i,j)$
HOMOGENEITY	Measures the local homogeneity of a pixel pair. The Homogeneity is expected to be large if the gray levels of each pixel pair are similar. Range = [0 1]	$\sum_{i,j} \frac{P(i,j)}{1+ i-j }$
CORRELATION	Returns a measure of how correlated a pixel is to its neighbor over the whole image. Range = [-1 1] Correlation is 1 or -1 for a perfectly positively or negatively correlated image. The Correlation is expected to be high if the graylevels of the pixel pairs are highly correlated.	$\sum_{i,j} \frac{(i-\mu i)(j-\mu j)P(i,j)}{\sigma_i \sigma_j}$
ENERGY	Measures the number of repeated pairs. The Energy is expected to be high if the occurrence of repeated pixel pairs is high. Energy is 1 for a constant image.	$\sum_{i,j} P(i,j)^2$

For our approach we are considering Homogeneity, Contrast and Energy.

5.2. To Check the Presence of HCG

For detecting the Hyperchromatic Crowded Groups following measures are taken into consideration.

- 1. Select the image for which HCG to be identified.
- 2. Crop the particular region of interest (ROI), i.e. the portion which is dark and crowded.
- 3. Find the number of nucleus inside the ROI.
- 4. If number_of_nucleus>15 then "Hyperchromasia is present" and if <15 then "Hyperchromasia is absent".

Presence of Hyperchromasia leads to abnormality in cervical cells.

Step 3 of this approach comprise of two steps:

a) Preprocessing - The step is to perform pre-processing operations on the input Pap smear image. The input image is a colored image which is in RGB mode. As is known that the color images are difficult to process so we convert the image to

grayscale image using rgb2gray function in matlab. Then we adjust the contrast of the image using imadjust function so that we can get a uniform background. It also helps in further processing of the image. Next Gaussian filter is applied on the image. After that the histogram of the image is obtained using imhist function, which helps in finding a suitable threshold for grayscale image to binary conversion using im2bw function of the image so that we can perform the morphological operation for feature extraction. Finally median filter is applied on the output image so that it can remove the salt and pepper noise present in the image.

b) Morphological Operations – The next step is to perform the morphological operations so that proper segmentation of the image can be done. Firstly the opening is performed using imopen function of matlab. This will remove the thin protrusions and also help in finding two separate nucleuses which are slightly connected. The complement operations are performed using imcomplement function. In matlab the object are set of white pixels that are connected to each other [10]. So to extract different features we have to give a value of 1 to all the pixels corresponding to a nucleus. But the main difficulty for proper segmentation is presence of unwanted components like WBC and other cells. So we have to take good measures to remove those components. In our approach we performed morphological erosion using imerode function of matlab which is followed by Median filter of the image. Again all the areas are cleared using bwareaopen function to remove the entire object having less than 100 pixels. This will remove the WBC s present. Finally we have to fill up the holes using imfill function. Then no of objects are counted and this total count will give no of nucleus present in the image.

6. Results and Discussion

6.1. Texture Analysis

6.1.1 Histogram Approach

Texture of Normal Pap smear sample: The samples images taken as an input to the system (Only 6 images are shown here) are shown in the following table –

Table 2. Sample Normal Pap Smear Images taken for Frequency Calculation using Histogram Approach

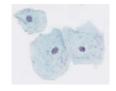


Sample 1 (Source: Dr B.Barooh

Cancer Institute)

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Sample 2 (Source: Dr B.Barooh Cancer Institute)



Sample 3 (Source: University College of London Site [5])

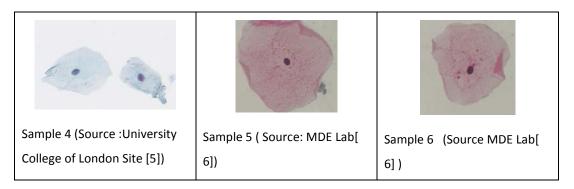
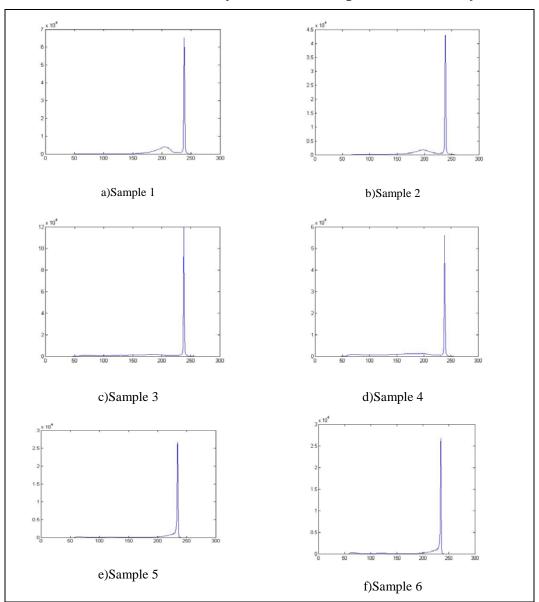


Table 3. Output of The System For The Images Of Table 1. In Frequency Graphs Of Normal Sample, X Co-Ordinate Shows The Gray Levels And Y Co-Ordinate Shows The Probability Of Pixels Having A Particular Gray Levels



Texture of Abnormal Pap smear sample

Table 4. Sample Abnormal Pap Smear Images taken for Frequency Calculation using Histogram Approach

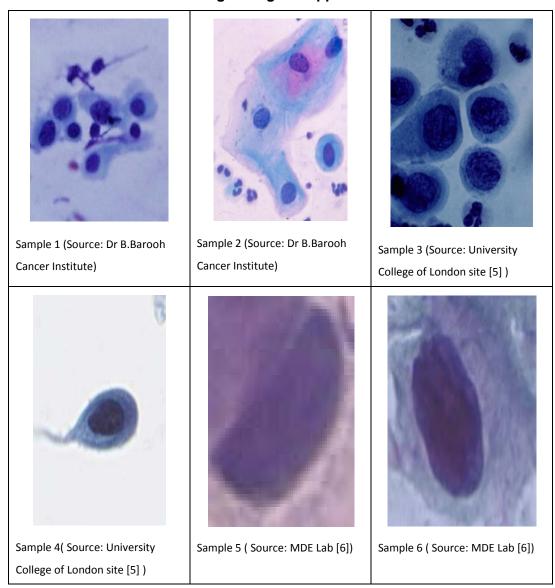
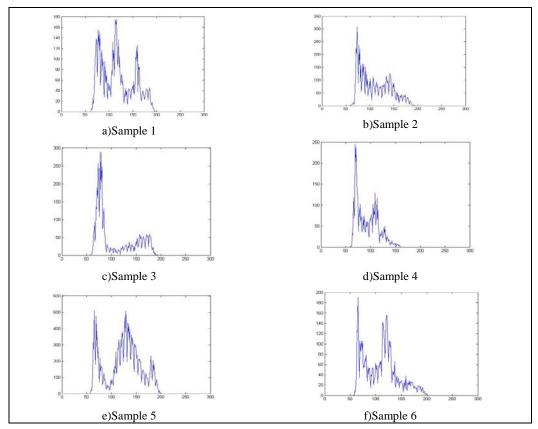


Table 5. Output of the System for the Images of Table 4. In Frequency Graphs of Normal sample, X co-ordinate shows the Gray Levels and y co-ordinate shows the Probability of Pixels having a Particular Gray Levels



From our observations it can be concluded that Normal Pap Smear samples graphs are almost similar and have maxima points in the range from 200-250. And the graph is smooth in nature. But from abnormal Pap smear samples we can observe that the graph is having many maxima and minima points with lots of fluctuation having no specific range where it will have its maximum points. There is no similarity among the graphs.

6.1.2. GLCM Approach

Normal sample of 20 Pap smear images that are taken into consideration is in the following figure –



Figure 8. Normal Pap Smear Image Samples given as an Input To The System for Homogeneity, Contrast and Energy Calculation (Source: MDE lab [6])

Abnormal sample of 20 Pap smear images that are taken into consideration is in the following figure –

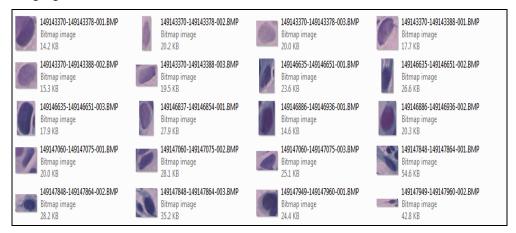


Figure 9. Abnormal Pap Smear Image Samples given as an Input to the System for Homogeneity, Contrast and Energy calculation (Source: MDE lab [6])

Homogeneity Measures: Only 40 samples are shown here where 20 are normal and 20 are abnormal samples.

Homogeneity 0.9189 1 0.8952 0.8844 0.8952 0.8752 3.55 1 0.9189 0.9093 0.9093 3.6564 3.496 0.8907 0.8629 0.8907 0.8517 3.6949 0.945 0.9142 0.9215 0.9142 3.4175 0.8635 0.8483 0.8635 0.8422 0.9664 0.9479 0.9533 0.9509 3.8185 0.8886 0.883 0.8886 0.8458 3,506 0.9501 0.922 0.9358 0.932 3.7399 0.8872 0.8778 0.882 0.8644 3.5114 5 0.9489 0.9186 0.9275 0.9207 3.7157 0.8651 0.8673 3.3821 6 0.9442 0.9108 0.92 0.9164 3.6914 0.8131 0.8366 0.8651 0.8929 0.915 0.8669 3.5399 0.935 0.9 0.9074 0.9018 3.6442 3.5734 0.9574 0.9402 0.9436 3.7772 0.8728 0.8899 0.9109 0.8998 0.936 0.888 0.8873 0.8866 3.5231 0.9533 0.9324 0.9395 0.928 3.7532 0.8612 10 0.9412 0.9355 10 0.8392 0.8833 0.8919 0.8743 3 4887 0.9569 0.9316 3.7652 11 11 0.9484 0.9508 0.9376 3.7968 0.831 0.8811 0.8037 0.8962 3.412 0.96 12 0.8202 0.8782 0.8921 3.4601 12 0.9629 0.9442 0.9404 0.943 3,7905 0.8696 3.6903 13 0.9426 0.9119 0.9225 0.9133 13 0.8056 0.83 0.878 0.8759 3.3895 0.8747 0.9499 0.9215 0.9271 0.9234 3.7219 14 0.8168 0.8937 0.8754 3.4606 15 0.8208 0.8989 0.8226 0.892 3,4343 15 0.9516 0.9257 0.9314 0.9221 3.7308 16 0.9533 0.9324 0.9395 0.928 3.7532 16 0.8024 0.8809 0.8972 0.8675 3,448 17 17 3.4788 0.9569 0.9316 0.9412 0.9355 3.7652 0.8156 0.8852 0.9039 0.8741 18 0.96 0.9484 0.9508 0.9376 3.7968 18 0.8966 0.8478 0.8759 0.8519 3.4722 19 0.9629 0.943 0.9442 0.9404 3.7905 19 0.8234 0.895 0.8085 0.8858 3.4127 20 0.9536 0.9332 0.9326 3.7454 20 0.842 0.8989 0.8186 3.3715 0.9664 0.9479 0.9533 0.9509 3.8185 0.8952 0.8929 Maximum Maximum 0.915 0.8998 3,5734 0.9189 0.9 0.8024 Normal samples Abnormal samples

Table 6. Homogeneity Value of 20 Normal and 20 Abnormal Samples

From the observation of Homogeneity it is observed that homogeneity of normal sample is in the range of 0.9-1.0 and sum generally fall in the range of 3.6-4.0. In case of abnormal sample the homogeneity value is in the range 0.7-0.9 and range of the sum is 3.2-3.6. Here sum indicates the summation of homogeneity values for all the orientations.

Contrast measures:

Table 7. Contrast Values of 20 Normal and 20 Abnormal Samples

		Contrast						Contrast						
ormal	Sample no		45	90	135	SUM	Abnormal Sample no	0	45	90	135	SUM		
	1	0.1205	0.1824	0.1625	0.1821	0.6475	1	0.2792	0.2651	0.222	0.296	1.062		
	2	0.1103	0.1723	0.1574	0.1677	0.6077	2	0.2235	0.2838	0.2224	0.3205	1.050		
	3	0.1072	0.1569	0.1489	0.168	0.581	3	0.2074	0.3214	0.2818	0.3585	1.169		
	4	0.1197	0.1824	0.168	0.1868	0.6569	4	0.2102	0.2736	0.2547	0.4298	1.168		
	5	0.0671	0.105	0.0938	0.0988	0.3647	5	0.2733	0.2577	0.2538	0.3623	1.147		
	6	0.1	0.1604	0.1288	0.1378	0.527	6	0.2774	0.2829	0.298	0.4094	1.267		
	7	0.1027	0.1679	0.147	0.163	0.5806	7	0.2088	0.2328	0.2712	0.2063	0.919		
	8	0.1117	0.1792	0.1605	0.1674	0.6188	8	0.2694	0.323	0.2972	0.4019	1.291		
	9	0.1047	0.1671	0.1481	0.1567	0.5766	9	0.2752	0.2447	0.2723	0.2271	1.019		
	10	0.13	0.1221	0.1854	0.1972	0.6347	10	0.2871	0.2137	0.2245	0.3726	1.097		
	11	0.0939	0.1395	0.1237	0.1524	0.5095	11	0.299	0.2277	0.2791	0.2771	1.082		
	12	0.1004	0.1581	0.1461	0.1539	0.5585	12	0.2892	0.2756	0.2333	0.4009	1.19		
	13	0.0968	0.1501	0.1383	0.1586	0.5438	13	0.199	0.2227	0.2787	0.2617	0.962		
	14	0.0865	0.1431	0.1201	0.1316	0.4813	14	0.2124	0.2083	0.2866	0.2074	0.914		
	15	0.0806	0.1051	0.1004	0.1349	0.421	15	0.2486	0.2068	0.2405	0.2096	0.905		
	16	0.0742	0.1151	0.112	0.1202	0.4215	16	0.21	0.2343	0.3421	0.2345	1.020		
	17	0.1231	0.1861	0.1599	0.1852	0.6543	17	0.2775	0.3149	0.2712	0.3624	1.22		
	18	0.0929	0.134	0.135	0.1483	0.5102	18	0.2887	0.3023	0.2392	0.2633	1.093		
	19	0.1197	0.1883	0.1811	0.1996	0.6887	19	0.1937	0.3341	0.3292	0.3193	1.176		
	20	0.0981	0.1212	0.0877	0.1235	0.4305	20	0.289	0.3214	0.299	0.41	1.319		
	MAXIMUM	0.13	0.1883	0.1854	0.1996	0.6887	MAXIMUM	0.299	0.3341	0.3421	0.4298	1.319		
	MINIMUM	0.0671	0.105	0.0877	0.0988	0.3647	MINIMUM	0.1937	0.2068	0.222	0.2063	0.905		
	,	ı)Normal	sample	es.			h)Abnorm	al sam	nles				

After observing 976 images (20 of them are shown here) it can be concluded that the contrast values is in the range of 0.08-0.2 and sum will be in the range of 0.3-0.85 for normal samples. In case of abnormal samples the contrast range is 0.2-0.5. Sum will be the range of 0.85-1.5. Here sum indicates the summation of contrast values for all the orientations.

Energy measure:

Table 8. Energy Values of 20 Normal and 20 Abnormal Samples

	ENERGY						ENERGY						
Normal	Sample no		45	90	135	SUM	Abnormal Sample no	0	45	90	135	SUM	
	1	0.2735	0.2494	0.2576	0.2496	1.0301	1	0.1815	0.1697	0.1717	0.164	0.686	
	2	0.2999	0.2767	0.2823	0.2789	1.1378	2	0.1276	0.1177	0.1276	0.1132	0.486	
	3	0.2785	0.2597	0.2648	0.2628	1.0658	3	0.1246	0.1039	0.11	0.1013	0.439	
	4	0.3387	0.3123	0.3242	0.3208	1.296	4	0.1639	0.158	0.1403	0.1536	0.615	
	5	0.2514	0.2255	0.2321	0.2269	0.9359	5	0.1677	0.1514	0.154	0.1483	0.621	
	6	0.2844	0.2525	0.2597	0.2578	1.0544	6	0.1365	0.1138	0.1134	0.1027	0.466	
	7	0.3426	0.3141	0.3223	0.3193	1.2983	7	0.1275	0.1244	0.1342	0.1143	0.500	
	8	0.2609	0.2301	0.2367	0.2323	0.96	8	0.1607	0.159	0.1702	0.1514	0.641	
	9	0.2815	0.2667	0.2684	0.2625	1.0791	9	0.1566	0.1436	0.1565	0.1459	0.602	
	10	0.2424	0.221	0.2254	0.2225	0.9113	10	0.1496	0.1437	0.1549	0.1349	0.583	
	11	0.3357	0.3073	0.3129	0.3035	1.2594	11	0.1193	0.1133	0.1202	0.1086	0.461	
	12	0.3152	0.2963	0.3019	0.2928	1.2062	12	0.1169	0.1115	0.1084	0.0952	0.43	
	13	0.2991	0.2746	0.2831	0.2774	1.1342	13	0.1676	0.1531	0.1613	0.1551	0.637	
	14	0.3977	0.3764	0.3772	0.3732	1.5245	14	0.1728	0.1622	0.1669	0.161	0.662	
	15	0.2952	0.2759	0.2744	0.27	1.1155	15	0.1494	0.1351	0.1403	0.1287	0.553	
	16	0.2498	0.2245	0.2272	0.2205	0.922	16	0.1891	0.1745	0.1788	0.1694	0.711	
	17	0.2987	0.2745	0.2826	0.2726	1.1284	17	0.1702	0.1506	0.1577	0.15	0.628	
	18	0.349	0.3286	0.3349	0.328	1.3405	18	0.1284	0.1109	0.1202	0.1148	0.474	
	19	0.2909	0.2764	0.2838	0.2789	1.13	19	0.1146	0.1986	0.1976	0.1872	0.69	
	20	0.3111	0.2941	0.298	0.2941	1.1973	20	0.1946	0.1691	0.177	0.1741	0.714	
	MAXIMUM	0.3977	0.3764	0.3772	0.3732	1.5245	MAXIMUM	0.1946	0.1986	0.1976	0.1872	0.714	
	MINIMUM	0.2424	0.221	0.2254	0.2205	0.9113	MINIMUM	0.1146	0.1039	0.1084	0.0952	0.43	
		Normal 3	Sample	;			Abnormal Sample						

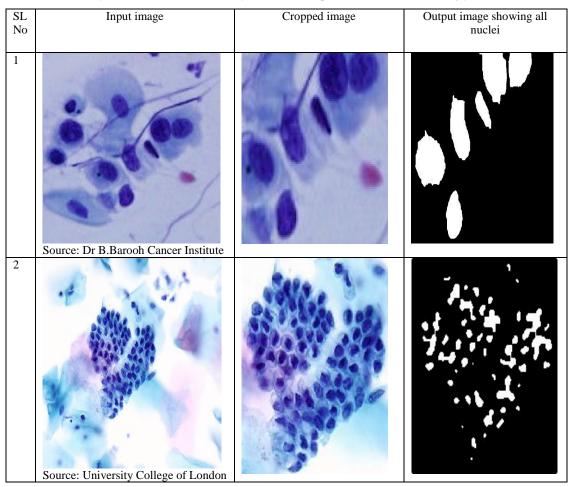
It can be concluded that Energy of normal sample is in the range of 0.2-0.4 and sum is in the range of 0.8-1.7. For Abnormal sample the energy is in the range of 0.08-2.0 and sum is in

the range of 0.3-0.8. Here sum indicates the summation of energy values for all the orientations.

6.2. To Check the Presence of HCG

The steps to check the presence of HCG is already explained above. Now the outputs are shown below (only two cases one for normal and another for abnormal sample is shown) –

Table 9. Snapshots of all the Output showing the Presence of Hyperchromasia



Observation 1:

Discussion 1: (According to SL no 1 from above table)

Here number of nuclues = 6.

Hence, Hyperchromasia is absent.

Observation 2:

Discussion 2: (According to SL no 2 from above table)

 $Number_of_nuclues = 52$

Hence, Hyperchromasia is present.

7. Conclusion

It can be concluded that texture analysis is one of the most important feature for abnormal and normal cervical cell classification. It can help in early detection of cervical cancer. Medical images have various limitations such as low quality, presence of noise and human error in interpretation [9]. So this type of automatic analysis may help the pathologist to a great extent. But this system totally depends upon the quality and contrast of the image. Some idea for further studies may include considering the whole Pap smear image for analysis at the same time instead of taking one cell at a time from that image.

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