ANEUPLOIDY WITH 3D IMAGING AS A BIOMARKER FOR EARLY CANCER DIAGNOSIS

QUANTIFICATION OF RELATIVE CHROMATIN CONTENT IN FLOW CYTOMETRY STANDARDS USING 3D OPTM IMAGING TECHNIQUE

Nitin Agarwal[1], Alberto M. Biancardi[2], Florence W. Patten[3], Anthony P. Reeves[2], Eric J. Siebel[4]

[1]Department of Bioengineering and [4] Mechanical Engineering, University of Washington, Seattle,

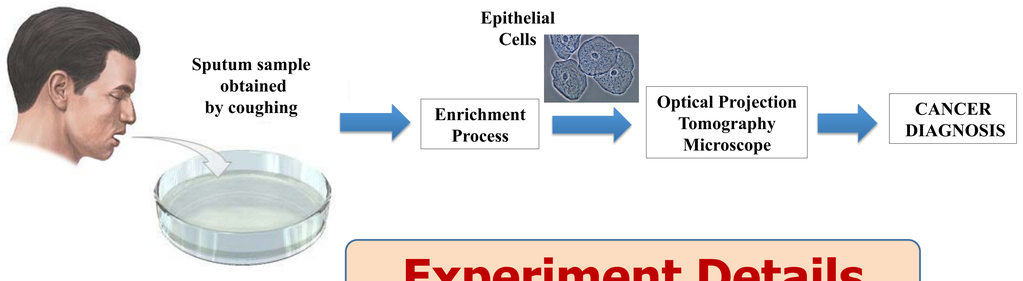
[2]School of Electrical and Computer Engineering, Cornell University, Ithaca, [3]VisionGate Inc., Phoenix

Introduction

- Cancer is the leading cause of death throughout the world. One of the common factors for its poor outcome is lack of accurate and efficient methods for early diagnosis.
- Conventionally, diagnosis is made by cytology of isolated cells or tissue. Computer-aided analysis of slides has also been used to automate this process [1].
- Cells and their nuclear chromatin features are three dimensional (3D) and hence analysis using two dimensional (2D) slides are unlikely to give accurate results for early cancer diagnosis.
- Optical Projection Tomography Microscope (OPTM) produces 3D images of cells and is being commercialized by VisionGate Inc. (Phoenix, AZ) as Cell-CT™. It reduces false negative rates of lung cancer detection by three-fold in a controlled comparison with 2D microscopy [2].
- In addition to 'site' and 'type' specific tests for cancer diagnosis, which are usually late, an important biomarker for early cytological diagnosis of any cancer is aneuploidy, abnormally high DNA content [3].
- Flow cytometry analysis (FCM) measures aneuploidy quite accurately for near diploid tumors though it requires a large tumor sample which is often not possible. Hence image cytometry analysis (ICA) is used as an alternative. ICA is not as sensitive to near diploid tumors as FCM. However, both are sensitive enough to detect high aneuploidy tumors [4].

Goals

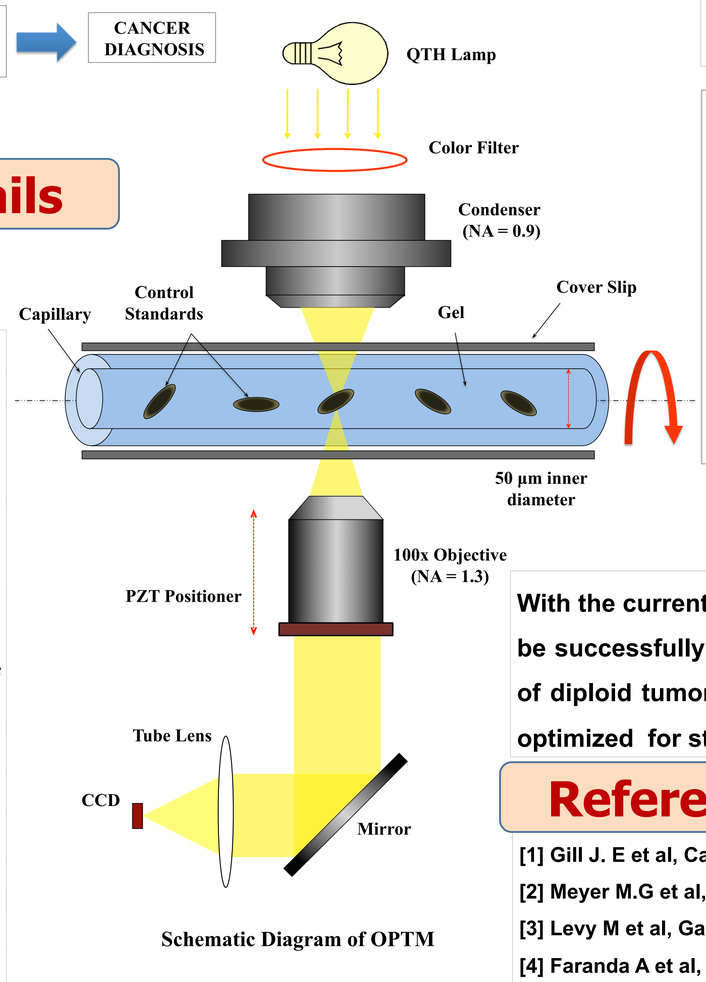
- To add the new feature of aneuploidy measurement to conventional cytological measures of nuclear morphology to make a more powerful and robust classifier for early cancer diagnosis. For example to diagnose lung cancer, sputum samples can be collected, enriched for epithelial cells using specific biomarkers and then run on OPTM for cancer diagnosis.



Experiment Details

Image Acquisition

- Flow cytometry standards, chicken erythrocyte nuclei (CEN), trout erythrocyte nuclei (TEN) and triploid trout erythrocyte nuclei (TTEN) were used as their DNA indices are know.
- A FACS scan was done before fixing, to ensure that they were in their non-dividing state. These nuclei were stained with acidic thionin using suspension technique for 30 and 15 mins and were compared to the standard hematoxylin staining.
- Using an optical gel having similar refractive index as that of capillary, these stained nuclei were inserted into the capillary via a syringe. Pressure was applied to advance the nuclei between the condenser and the objective. OPTM then acquired 500 2D transmission images (pseudo projections) while the cell was being rotated at an angular division of 0.72 degrees. A 3D optical image of stained nuclei was reconstructed using filtered backprojection, similar to Computed Tomography (CT).

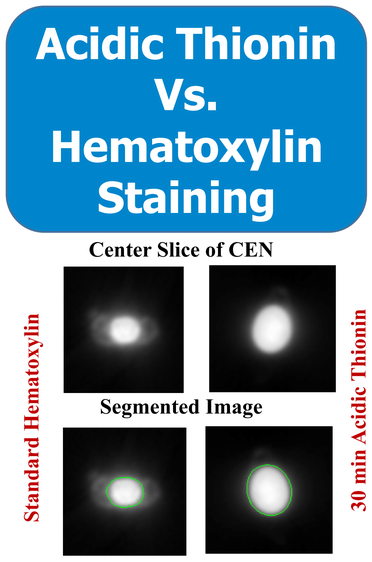


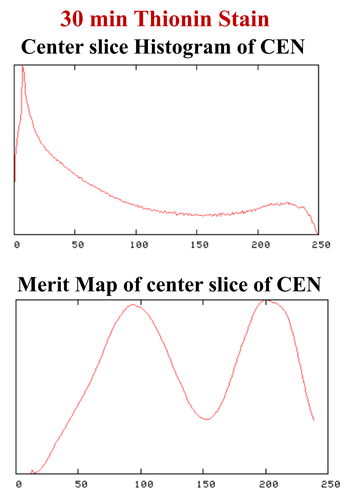
Schematic Diagram of OPTM

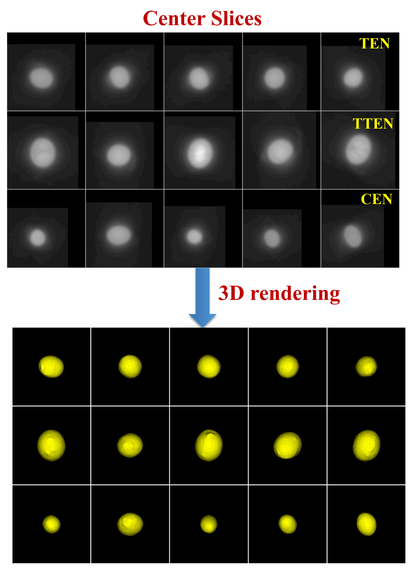
Image Analysis

- 3D segmentation algorithm which segments the nucleus from the background was developed. Each image intensity in the center slice was given a merit value and a merit map was plotted for all these intensities. The peaks in the merit map served as the threshold for segmentation. For a cell, the first peak segments out the cytoplasm and second one the nucleus. However for these standards, the first peak only segmented out the nucleus as there was no cytoplasm.
- Ratio of DNA indices were compared for 50 nuclei of each of the three flow cytometry standards. To calculate the DNA index, mean of the ratio of individual observations and their standard deviations were computed.

Acidic Thionin Vs. Hematoxylin Staining

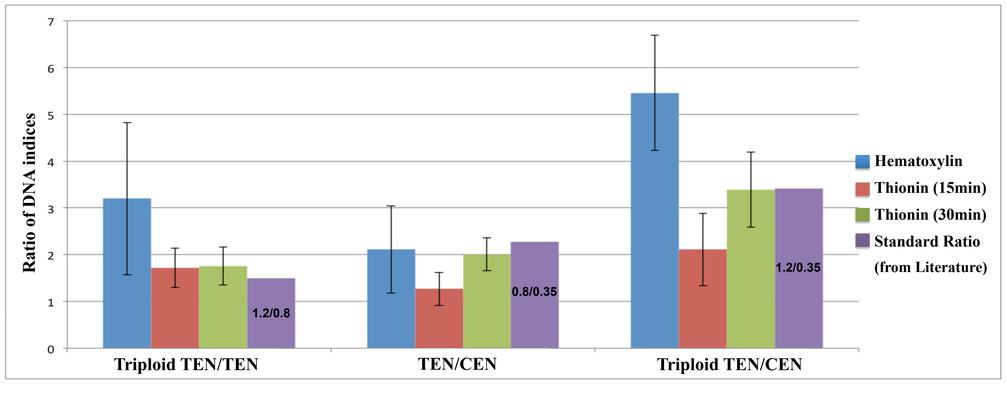






Results

Mean error was the least in 30 min of thionin staining, making it to be most quantitative among all three staining procedures.



Conclusion and Future Work

With the current procedure, a cancer cell having a DNA index of 1.35 or more (35% mean error) will be successfully identified by the OPTM. To increase the sensitivity of the procedure for detection of diploid tumors, a mean error of 10% has to be achieved [5]. Thionin staining could be further optimized for stociometricity. Improvement in 3D segmentation could also lower the error.

References

[1] Gill J. E et al, Cancer Research 38(7) 1893-98 (1978).

[2] Meyer M.G et al, Pattern Recognition 42 141-146 (2009).

[3] Levy M et al, Gastroenterology 142 1112– 1121 (2012).

[4] Faranda A et al, Analytical and Quantitative Cytology and Histology 19(4) 338-344 (1997).

[5] Blocking A et al, Analytical Cellular Pathology 8 67-74 (1995).