



Use of Automated Image Cytometry in the Diagnosis of Lung Cancer using Bronchial Washings

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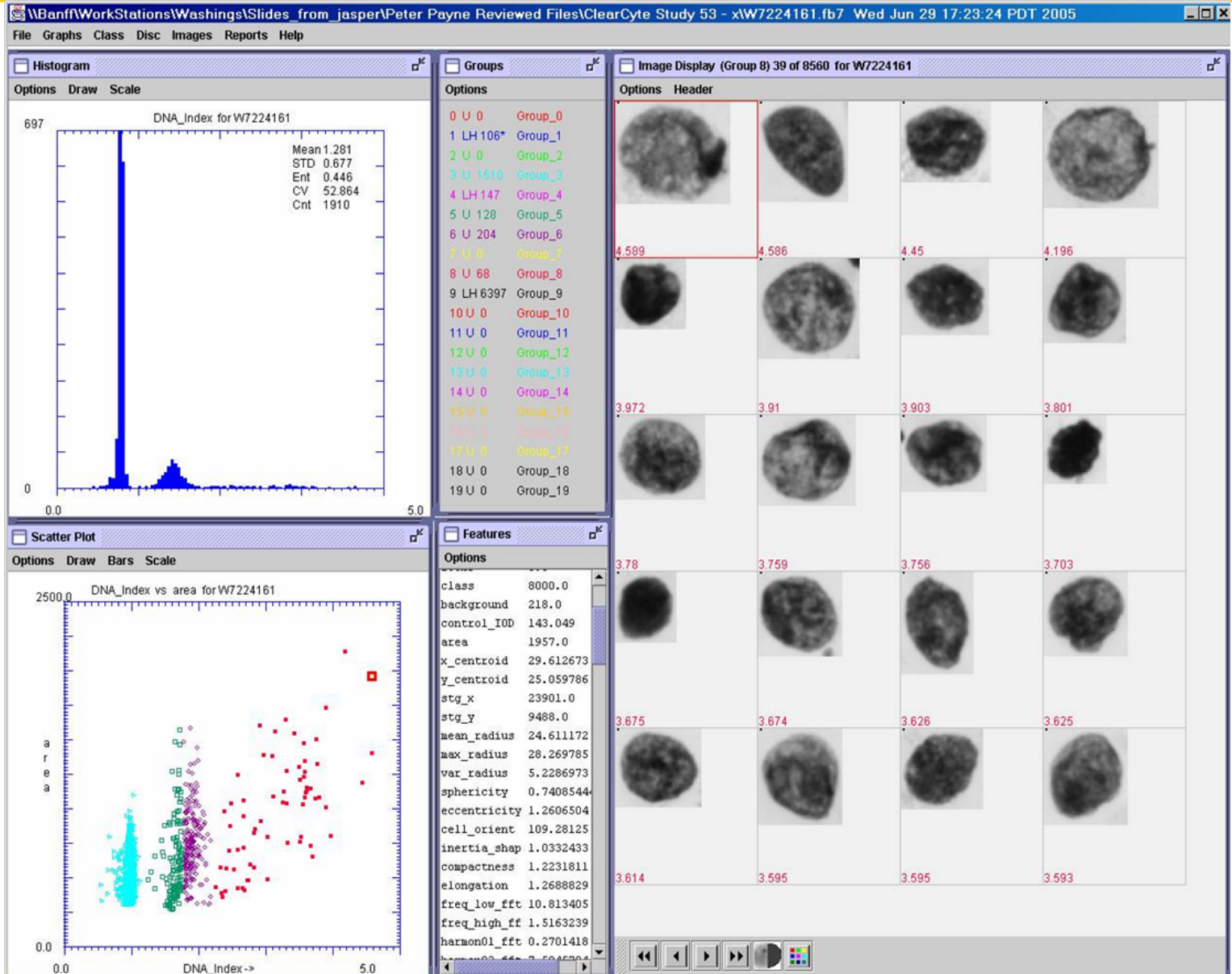
The diagnosis of lung cancer from bronchial washings by means of conventional cytology has some methodological shortcomings. A review of publications on the subject reports an average sensitivity of 48% for centrally located tumours and 43% for peripheral tumours^{1,2}. Therefore there is a need for more sensitive methods and technologies to diagnose patients suspected of having lung cancer. For this purpose we investigated the quantitative cytology system **ClearCyte™** (Perceptronix Medical Inc., Vancouver, Canada) which utilizes automated image cytometry of thousands of exfoliated cell nuclei stained with Feulgen DNA stoichiometric stain and allows subsequent visual interactive review of atypical nuclei by a cytologist.





Bronchial washing samples were taken during bronchoscopy, placed into collection jars with fixative, processed and deposited on microscope slides using a Cytospin 4 centrifuge (Thermo-Shandon, Pittsburgh, PA). Three slides from each sample were stained with haematoxylin and eosin and underwent conventional cytological assessment. Another three slides from each sample were stained with the DNA stoichiometric Feulgen stain and scanned using the **ClearCyte™** image cytometer. The cytometer consists of a base microscope with an automated XYZ stage, automated slide loader, high-resolution CCD camera and computer with monitor. The images of relevant cell nuclei from the bronchial washings were selected and separated from debris by an automated, algorithmic process and the normalized DNA amount (ploidy) and nuclear morphology features of the cells were calculated. Images of measured cells with aneuploid DNA content were displayed on the computer monitor for manual review. The cytologist, who was blinded from the institutional diagnosis, subsequently cleaned suspicious cell galleries of the remaining debris and reviewed the diagnostic cells (cells with a DNA Index exceeding 2.25) directly under the **ClearCyte™** microscope.







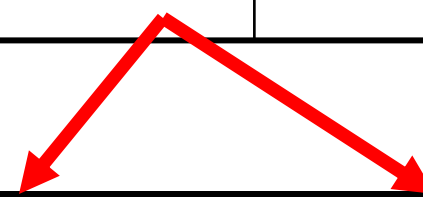
The patient data comprised 213 high-risk patients who underwent diagnostic bronchoscopy procedures. 58 of those patients were confirmed to have lung cancer by histopathology, while 155 were negative for lung cancer. None of the patients included in the study had any prior clinical history of cancer.

Positive Cases:
N = 58

Negative Cases:
N = 155



	Sensitivity (N)	Specificity (N)
Manual Cytology	63.8% (58)	100.0% (155)
Quantitative Cytometry	74.1% (58)	96.1% (155)



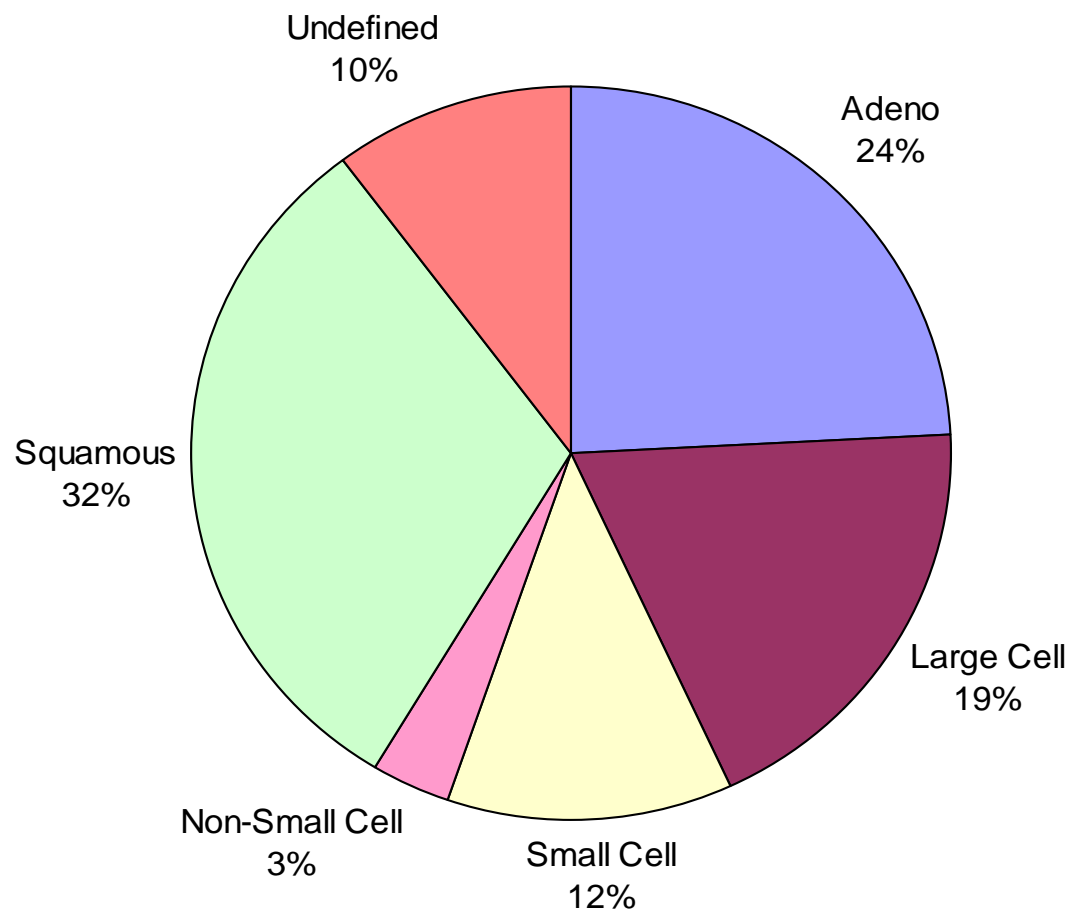
	Sensitivity (18) Peripheral Lesions	Sensitivity (40) Central Lesions
Manual Cytology	33.3% (18)	77.5% (40)
Quantitative Cytometry	66.7% (18)	77.5% (40)



Quantitative analysis of Feulgen-stained bronchial washings using the **ClearCyte™ image cytometer with subsequent cytologist review produced the same sensitivity as conventional cytology for central tumors (77.5%). However, for peripheral tumors there was a substantial benefit in performing quantitative cytological analysis. Cytologist review using the **ClearCyte™** system reached a sensitivity of 66.6% over 33.3% for conventional cytology. The specificity remained comparable for both modalities.**



Breakdown of Cancer Types (N = 58)





Of particular interest to the study were 5 patients for which all the traditional diagnostic procedures using samples obtained during bronchoscopy (histopathology of forceps biopsy, cytology of brushings and washings) returned as malignancy not found, whilst quantitative analysis of the Feulgen-stained washing specimens obtained at the same time as the other specimens produced positive for cancer results. Upon follow-up all 5 patients were diagnosed with cancer either by more invasive diagnostic procedure (FNA) or finally by open biopsy. These patients were not included in the summary of study results above.



1. **Rivera MP, Detterbeck F, Mehta AC. Diagnosis of Lung Cancer. *Chest* 2003;123:129s-136s.**
2. **Schreiber G, McCrory DC. Performance Characteristics of Different Modalities for Diagnosis of Suspected Lung Cancer. *Chest* 2003;123:115s-129s.**