Outpatient Cytopathology® center

TOPICS IN CLINICAL FNA

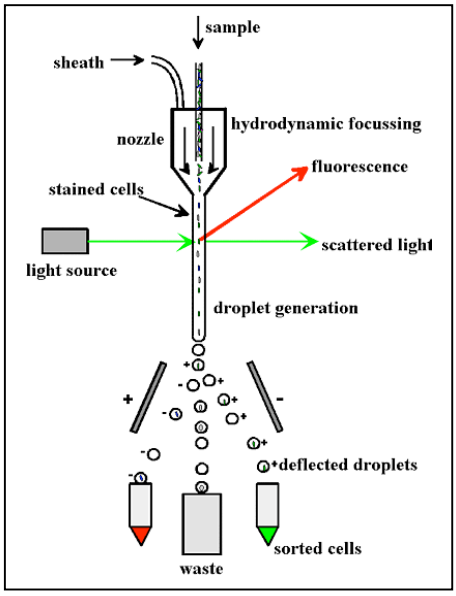
Aspiration Biopsy Cytology March 2005

# FLOW CYTOMETRY – WHY IS IT SO USEFUL?

Flow cytometry is a technique of automated cell analysis that allows cells to be studied based on cell surface characteristics. The technique, developed in the 1970's, has rapidly become an essential tool in the clinical laboratory. Its major clinical application is the diagnosis of hematologic and lymphoid malignancies, but a wide variety of other applications also exist.

**Basic Principles**

*Cytology* and *cytometry* are different, but complementary, techniques. Cytology examines how cells "look" on a flat slide under a microscope. Cytometry, on the other hand, examines cells in a liquid medium. The *cells flow* past a detector that *measures* various structural characteristics (hence, *flow cytometry).*




Specifically, cells suspended in a liquid medium flow individually through a sensing area. As each cell passes through this area, electrical or optical signals are generated. These signals – which may be fluorescence, light scatter, light absorbance, or electrical resistance of the cells – are accurately measured. These physical measurements are translated into cellular properties such as cell size and viability. Nucleic acid levels, or the presence of enzymes or specific antigens, can be rapidly analyzed.

Flow cytometry has become an essential tool in the diagnosis of hematologic and lymphoid neoplasia. Indeed, treatment strategy often depends on the type of antigenic parameters found on the tumor cells.

**Flow Cytometry and FNA**

Cellular material is obtained for flow cytometry analysis through fine needle aspiration biopsy or surgical biopsy. Fine needle aspiration (FNA) biopsy is particularly well suited for obtaining tissue for flow cytometry studies. At the Outpatient Cytopathology Center, flow cytometry is used routinely in the work-up of patients with lymphoid malignancies.

Case example: A patient presents with an enlarged lymph node or soft tissue mass, and a FNA biopsy is performed with a 25-gauge needle. If immediate interpretation of the aspirated specimen is suspicious for lymphoma, additional material is obtained for flow cytometry studies. Using a monoclonal antibody technique, flow cytometry determines cellular antigens of the lymphoid cells. The immunophenotypic (antigenic) characteristics of the cells are then correlated with the cytologic features. In most cases, the sub-type of lymphoma can be determined from the fine needle aspiration biopsy specimen.

FNA biopsy combined with flow cytometry is successful in evaluating lymphoma and leukemia in 75-90% of cases. False negatives result from tumor necrosis or sclerosis, partial tissue involvement by malignancy, and some rare T-cell non-Hodgkin's lymphomas (NHL).

Studies report accurate sub-classification of NHL by flow cytometry using FNA samples in the range of 71-77%. There are now many peer reviewed articles in the current literature which advocate fine needle aspiration biopsy and flow cytometry as the method of choice for diagnosis of lymphoma. With this approach, many patients no longer need to undergo an excisional biopsy, but can be referred immediately for treatment.

**Clinical Applications**

Cellular antigens are identified with flow cytometry using fluorochrome-labeled monoclonal antibodies. This is called immunophenotypic analysis and is critical in the initial diagnosis and classification of acute leukemias, chronic lymphoproliferative diseases and malignant lymphomas.

Flow cytometry can determine if a monoclonal population of B- or T- cells is present. It can also aid in rendering a specific diagnosis, if classic antigenic marker patterns are present on the cells of interest. In addition, immunophenotypic analysis provides prognostic information. In the future, flow cytometry will be used to analyze apoptosis, multi-drug resistance, leukemic-specific proteins (chimeric proteins), cytokine receptors and other parameters that may lend additional diagnostic and prognostic information.

Flow cytometry can detect the persistence of malignant cells in the bone marrow or in other tissues of patients with hematologic malignancies after treatment. Flow cytometry can have detection limits up to $10^{-2}$ to $10^{-4}$ cells, and can detect recurrent cancer before conventional morphologic changes. It is believed that these residual malignant cells are the source of disease relapse in many patients. This is currently an area of active research. For example, a leukemic patient in remission has a cerebral spinal fluid (CSF) test performed to evaluate for recurrences due to some new clinical symptoms. If the CSF contains cells with antigens to CD10, CD34 and TdT (a marker of immature T and B lymphoblasts), then this antigenic pattern is diagnostic of recurrent leukemia because these markers are not normally present in the CSF.


Dunphy CH. Applications of Flow Cytometry to Diagnostic Hematopathology. *Advance* ; 2003:19-21.

Schmitz JL. Theory, Clinical Applications of How Flow Cytometry. *Advance Laboratory*; 2003:32-36.


## Company Profile

OUTPATIENT CYTOPATHOLOGY CENTER (OCC) is an independent pathology practice that specializes in performing and interpreting fine needle aspiration biopsy specimens. OCC is accredited by the College of American Pathologists. The practice was established in 1991 in Johnson City, Tennessee. Patients may be referred for aspiration biopsy of most palpable masses as well as for aspiration of non-palpable breast and thyroid mosses that can be visualized by ultrasound. OCC is a participating provider with most insurance plans. Our referral area includes patients from Virginia, West Virginia, North Carolina, South Carolina and Georgia.

### Dr. Rollins

**SUSAN D. ROLLINS, M.D., F.I.A.C.** is Board Certified by the American Board of Pathology in Cytopathology, and in Anatomic and Clinical Pathology. Additionally, in 1994 she was inducted as a Fellow in the International Academy of Cytology. She began her training under G. Barry Schumann, M.D. at the University of Utah School of Medicine, subsequently completed a fellowship in Cytopathology under Carlos Bedrossian, M.D. at St. Louis University School of Medicine, and has completed a fellowship in Clinical Cytopathology under Torsten Lowhagen, M.D. at the Karolinska Hospital in Stockholm, Sweden. The author of numerous articles in the field of cytopathology, Dr. Rollins also has served as a faculty member for cytopathology courses taught on a national level.

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**JANET F. STASTNY, D.O.** is Board Certified by the American Board of Pathology in Anatomic Pathology and has specialty boards in Cytopathology. She completed a pathology residency at the University of Cincinnati and subsequently a one-year fellowship in cytopathology and surgical pathology at the Virginia Commonwealth University / Medical College of Virginia. She was on the faculty at the University for 7 years specializing in gynecologic pathology and cytopathology. She has written numerous articles in the field of cytopathology and gynecologic pathology and has taught cytopathology courses at national meetings. She is currently involved on national committees dealing with current issues concerning the practice of cytology.