# Utilizing Intraoperative Flow Cytometry to Accurately Characterize Bladder Cancer Cells

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## Abstract

Bladder cancer is a serious health concern. The first line of treatment is transurethral resection, and a precise evaluation of the tumor margins may call for full tumor removal. Cancer cells frequently display proliferative potential and genomic instability. Intraoperative Flow Cytometry (iFC), a next-generation margin evaluation tool for assessing DNA content, has previously been shown to be useful in the detection of several malignancies. In the current study, we looked into the potential utility of iFC for identifying bladder cancer following surgery. The entire iFC evaluation process takes 3 minutes to 5 minutes per sample and includes a two-step analysis that includes calculating the DNA-index and tumor-index. 24 hyperploid and one hypoploid tumors were first discovered through DNA-index computation. Furthermore, tumor samples can be identified from normal cells by virtue of their significantly increased proliferation potential, according to results of cell cycle analysis and Tumor-index computation. The pathology assessment was used as the benchmark for iFC evaluation, and it was found that our methodology has a 98% accuracy rate in identifying the presence of cancer cells in a particular sample. Our findings suggest future examination of the utility of iFC as a cutting-edge technique for evaluating malignancy during transurethral resections.

**Keywords:** Cancer • Bladder cancer • Flowcytometry • Surgical treatment • Surgical oncology

## Introduction

A set of illnesses known as cancer involve abnormal cell proliferation and have the ability to invade or spread to different bodily regions. In contrast to this, benign tumors do not spread. A lump, unusual bleeding, a persistent cough, unexplained weight loss, and a change in bowel habits are all potential warning signs and symptoms. These signs of cancer may be present, but there may be other causes as well. Humans are susceptible to over 100 different malignancies.

About 22% of cancer fatalities are related to tobacco usage. An additional 10% of cases are brought on by obesity, a bad diet, a lack of exercise, or excessive alcohol consumption. Other concerns include exposure to ionizing radiation, certain diseases, and environmental contaminants. Infections include *Helicobacter pylori,* hepatitis B, hepatitis C, human papillomavirus infection, Epstein-Barr virus, and Human Immunodeficiency Virus (HIV) are responsible for 15% of cancer cases in the developing world. These elements influence a cell's genes, at least in part. Typically, it takes a lot of genetic alterations for cancer to manifest. Inherited genetic flaws cause cancer in 5%-10% of cases. A few warning signs and symptoms as well as screening tests can help diagnose cancer. A biopsy is then often used to validate the results of subsequent imaging studies. By quitting smoking, maintaining a healthy weight, consuming less alcohol, eating lots of fruits, vegetables, whole grains, and resistant starches, getting immunized against specific infectious diseases, consuming less processed meat and red meat, and avoiding excessive sun exposure, you can lower your risk of getting some cancers. Screening is helpful for cervical and colorectal cancer early detection. There is debate concerning the advantages of breast cancer screening. Radiation therapy, surgery, chemotherapy, and targeted therapy are frequently used to treat cancer. An essential component of care is the control of pain and symptoms. People with advanced sickness should prioritize palliative care. The type of cancer and the degree of the disease when treatment begins determine the likelihood of survival. Around 90.5 million people worldwide had cancer in 2015. Globally, there were 10 million cancer-related fatalities in 2019 and 23.6 million new instances of the disease per year, suggesting rises of 26% and 21% over the previous ten years, respectively.

Lung, prostate, colorectal, and stomach cancer are the most prevalent forms of cancer in men. Breast cancer, colorectal cancer, lung cancer, and cervical cancer are the most prevalent forms in females. In terms of the total number of new instances of cancer each year, skin cancers other than melanoma would make up about 40% of the cases. Except in Africa, where non-Hodgkin lymphoma is more prevalent, acute lymphoblastic leukemia and brain tumors are the most common cancers in children. Around 165,000 kids under the age of 15 received cancer diagnoses in 2012. Age considerably raises the risk of cancer, and several malignancies are more prevalent in industrialized nations. As more individuals live to old age and as lifestyle changes take place in the developing countries, rates are rising. As of 2010, it was projected that the annual economic expenses of cancer worldwide was US$1.16 trillion. With more than 1 death per 1000 people each year worldwide, cancer is still the top cause of death in humans. According to the Global Cancer Observatory, bladder cancer is the most prevalent urological cancer in men's urinary tracts, occurring two to six times more frequently in male patients than in female patients and accounting for 440,864 cases and 158,785 deaths in male patients in 2020. Urothelial carcinomas, which are bladder cancers that originate from the lining of the urinary tract, account for 90%-95% of bladder cancer cases, while squamous cell carcinomas, which come from the cells that line the bladder, and adenocarcinomas, which come from glandular cells, account for 4% and 2% of cases, respectively. According to the extent of invasion, bladder cancer cases are either classified as Non-Muscle-Invasive Bladder Cancer (NMIBC), which have a lower risk of metastasizing and a better prognosis, or as Muscle-Invasive Bladder Cancer cells (MIBC), which have a higher degree of invasiveness and can spread to other organs. The majority of BC patients are de novo diagnoses, with a 5-year survival rate at local illness of less than 60% and less than 10% in distant metastases. BC instances may emerge through remission of NMIBC cases. NMIBC and MIBC can be further divided into the subtypes of basal- and luminal-like, and by the cancer genome atlas into luminal-papillary, infiltrated, squamous, neuronal/small cell, and luminal/genomically unstable carcinoma. These divisions are possible due to distinctive histopathological features and heterogeneity resulting from differentially expressed genes.

As various cells may exhibit varied responsiveness to anti-cancer medications, immunohistological classification and the molecular characteristics in bladder carcinomas are a cornerstone in assessing and addressing subsequent therapy plan. Although bladder cancer is typically found in its early stages (7 cases out of 10 cases), making it a candidate for tumor excision, only 15% to 70% of cases relapse after a year. The 5-year survival rate for in situ cancers after successful removal is 94%, but it drops to 6% in cases of metastatic disease.

Since it has been anticipated that more than 80% of the >15 million cancer cases in 2015 were candidates for surgical treatment, surgical management is the first-line treatment for 4 out of 5 cancer patients. With a prediction that > 45 million surgical procedures will be conducted for tumor removal by 2030, low post-operative mortality and morbidity rates, as well as the possibility of total tumor removal, are among the key benefits of surgery. The typical procedure for diagnosing and treating non-invasive bladder cancer is transurethral resection. The precise characterization of cancer cells is necessary for both the diagnosis and ongoing therapeutic management of the patient because the effectiveness of the procedure depends on a number of factors. With numerous uses in cancer, including immunophenotyping, characterization of hematological malignancies, exposing quantifiable residual illness, ploidy evaluation, and cell cycle assessment, flow cytometry is one of the most efficient single-cell analytic techniques. A full tumor removal may be possible with the use of Intraoperative Flow Cytometry (iFC), a quick, highly sensitive, and reasonably priced technique that has the ability to characterize tumor biology and margin status. Intraoperative flow cytometry has been used by our team, initially for brain tumors. The methodology has been further standardized and employed in numerous forms of malignancy, including head and neck, breast, liver, pancreas, colorectal, as well as gynecological neoplasms, based on the excellent diagnostic capability of iFC.

In conclusion, iFC is an effective educational tool for differentiating tumor cells from nearby healthy tissue in a variety of malignancies, resulting in the best possible excision and prognosis for remission. To the knowledge, this trial represents the first time iFC has been used to treat urological and bladder cancer. For the best results in sample collection and analysis during transurethral resections, we improved earlier iFC techniques. According to our results, iFC can characterize cancer quickly and accurately (98.1% of the time), and this method merits future investigation in larger research.

## Discussion

Unquestionably, the best technique for determining DNA content at the cellular level is flow cytometry. The fact that FC can be used on most tissues and cell lines originating from a variety of cancer types, including bladder cancer, is one of its key benefits. Other benefits include the analysis's speed, precision, and cost-effectiveness. The use of FC in the treatment of cancer includes, among other things, the development of novel therapies and the screening of drugs as well as diagnostic intraoperative FC analysis. The proper characterization of tumor biology and the assessment of resection margin status are both aided by the intraoperative use of FC. Our research team is examining the best strategy to make iFC a common next-generation margin evaluation tool. With a high accuracy that is typically above 90%, iFC has been effectively used in surgical procedures addressing malignancies of the brain, head and neck, breast, gynecological, hepatobiliary, and colorectal up to this point.

The current work expands the use of iFC in bladder cancer analysis during transurethral resections and shows that it characterizes bladder cancer cells with a high degree of accuracy (>98%). The test displays a 100% Negative Predictive Value (NPV) and a 96.20% Positive Predictive Value (PPV).

Transurethral resection can be used to treat tumors that are detected early, although the median survival time after an invasive UC recurrence is still only 5.6 months. This idea emphasizes the significance of early bladder cancer detection and therapeutic intervention. For post-operative care and to increase survival, an accurate tumor characterization is essential. We verified that the iFC methodology can be adapted to assess and describe malignancy during bladder cancer surgery based on DNA content analysis. Our unique methodology made sample collecting and analysis possible. As a result, evaluation of DNA-index and proliferation analysis of the resected tissue would be a powerful ally in evaluating healthy tumor margins coupled with accurate identification of histological subtypes by pathology. According to theory, iFC may also help to stop the spread of lymph node metastases and muscle-invasive illness, thereby enhancing oncological outcomes. A pilot analysis in selected samples, which evaluated the margin state of five patients and correctly demarcated their margin status using the gold standard pathology examination, has confirmed the usefulness of iFC in this type of surgery.

Bladder cancer is known for having genomic instability, which results in abnormalities in cell cycle and growth regulation, giving birth to malignant transformation, and maintaining proliferation. In fact, the Ras-Mitogen-Activated Protein Kinase (MAPK) signal transduction pathway is changed in non-invasive carcinomas, but retinoblastoma and *p53* are two of the most often mutated tumor suppressor genes found in invasive cancers. Chromosomal abnormalities, specifically polyploidy and hypoploidy, are known to contribute to carcinogenesis and serve as a prognostic and predictive marker for cancer cells. Based on DNA-index calculations, we found aneuploidy in roughly 50% of the cancer cases in our study. Additionally, an induction of the tumor-index was found to be a result of the bladder cancer cells' ongoing proliferative signaling. We think that the high sensitivity and specificity of iFC analysis may be due to the genomic instability, which is a defining characteristic of bladder cancer cells.

## Conclusion

Since surgical removal is typically the first course of treatment for cancer, total tumor eradication is the ultimate objective in the discipline of surgical oncology. The precise characterization of a tumor sample in order to define resection margins is a requirement for this. Additionally, the intraoperative tumor biology representation might provide important information for future clinical care. The significance of iFC as a tool in transurethral resection that provides a quick and accurate characterization of cancer cells as well as a depiction of tumor biology is first brought to light in our report. These findings require further analysis and confirmation in multicenter trials with bigger populations.

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