Reviewer's report

Title: Comparison of DNA Histograms by Standard Flow Cytometry and Image Cytometry on Sections in Barrett's Adenocarcinoma

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Reviewer: marianne Lorenzato

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General
This article is well written even if the questions posed is not very new; the comparison of Flow and Image Cytometry for the DNA ploidy determination in cancer has been already studied in a lot of papers. However, the pathology chosen by the authors i.e. Barrett's Adenocarcinoma is quite original.

The methods used are appropriate and well described and the calibration methods for both techniques are well documented.

The data obtained are in accordance with the other findings available in the literature concerning this subject, however, the author have to be careful when they make conclusions concerning the high percentage of cells exceeding 5N and their high genetic instability. Such cells are of biological relevance in diploid tumors or in aneuploid tumors with an DI<1.25. Otherwise cells >5N belong to the G2/M fraction of the cell cycle of the aneuploid peak, and their presence with a high percentage is normal. In aneuploid tumors, cells with high genetic instability are cells with a DNA content higher than 9N (if the DI of the aneuploid peak is <2.25). So the conclusions concerning the presence of >5N cells have to be revised.

In the discussion, the reasons why false negative results are frequently observed with Flow Cytometry have to be detailed. Moreover, an additional thought concerning the possibility of false positive result obtained with Image Cytometry would be welcome with reference to the literature.

The conclusion concerning the usefulness of Image Cytometry for the DNA ploidy determination in cancer pathology is very interesting.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

1-If the authors want to make conclusions about the high rate of cells > 5N they have to consider only diploid tumors or aneuploid tumors with a very low DI (<1.25). See the general remarks. Why did they choose a cutoff value of 5%?

2-If the authors talk about high genetic instability the cells with a DNA content exceeding 9N have to be taken into account in case of aneuploidy.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1-The authors have to verify the presence of remaining tumoral areas on 3µ HES stained sections before and especially after the 50µ section performed for Flow Cytometry analysis.

2-The authors could explain how they obtain 42 blocs of BAC with 17 patients. Among the 12 patients with more than 1 bloc containing tumor, for some of them 3 or more blocs have been used?

3-The figures are very small and difficult to read when printed.

4-In the abstract, in the last sentence, the authors say that Image Cytometry is more sensitive than Flow Cytometry in detecting DNA... If they want to talk about sensitivity of a test, they have to calculate the sensitivity, specificity, negative and positive predictive values for each test.

Discretionary Revisions (which the author can choose to ignore)

In table 1, the abbreviation N/A has to be explained

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests