**Author's response to reviews**

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

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Responses to the Reviewer's Comments

Reviewer 1: (Dr Marianne Lorenzato)

The authors thank Dr Lorenzato for very helpful suggestions.

1. The reviewer is correct in pointing out that cells with DI>5N may be considered to lie outside the G2/M peak and a marker of instability only when the G0/G1 peak has a DI value of <1.25. In this study, no BAC had G0/G1 peak DI <1.25. We have now calculated cases with peak DI <2.25 that have >9Nvcells in the histogram. This data is now included and we have rewritten the paper to clarify this point.

2. A cut off value of 5% for >5N was chosen arbitrarily. This arbitrary number is now eliminated.

3. We have now detailed reasons for possible false negative results with flow cytometry and potential false positive results with image cytometry.

4. Presence of remaining tumoral area, on 5u HES stained sections, adjacent to the 50u section, used for flow cytometry analysis, was confirmed in all cases. This has been clarified in the text.

5. 42 blocs of BAC from 17 patients included up to 3 separate blocks from the same patient.

6. The sizes of the figures were about 1300 pixels × 800 pixels, with the resolution of 600 dpi.

7. In the abstract, we have substituted sensitive with reliable.
8. In table 1, the abbreviation N/A has been deleted.

Reviewer 2 (Dr Alfred Bocking)

The authors thank Dr Bocking for his very thoughtful comments.

1. We are very familiar with the "Consensus Reports on Diagnostic DNA-Image-Cytometry" by the European Society for Analytical Cellular Pathology (ESACP). That report deals with image cytometry in enzymatically dispersed cells and standards that the group has recommended for that technique. The report dismisses the use of DNA-image cytometry on sections as a diagnostic tool because of inherent technical problems. However, many of these problems have been eliminated or reduced with the newer imaging technology and standardization of tissue thickness.

2. The large CV in normal gastrointestinal mucosal cells may be related to their increased proliferative rate than non-dividing lymphocytes. Minimum number of cell counted is a function of cell selection. Issue of tissue thickness is now discussed in greater detail.

3. DNA image cytometry on enzymatically dispersed nuclei have certain advantages as pointed out by the reviewer. However, a major drawback of the use of dispersed cells is the fact that the technique fails to provide direct cellular correlation and contamination of the tumor cells with nonepithelial and non-tumor cells, unless tumor cells were selected by techniques such as laser capture microdissection. On the other hand, image cytometry on tissue sections has a distinct advantage of direct cellular correlation. Moreover, these data are stored permanently, and can be recalled and reassessed if needed.

4. A direct comparison of the results of DNA-image cytometry on dispersed nuclei and on sections is not currently available. In this manuscript, we have now discussed and addressed the technical issues and potential advantages and limitation of the two techniques.

5. We did not use histogram typing (like peridiploid, peritetraploid, x-ploidy or multiploid). Instead, we used actual DI values of the G0/G1 peaks. These data provides better quantitative information.

6. We have rewritten the manuscript to clarify all the important issues raised by Dr Bocking. We believe, high fidelity ICS provides an excellent method of DNA ploidy analysis in solid neoplastic conditions.