We thank the reviewers for their opinions and suggestions and their critical examination of the manuscript. The response has been highlighted in the revised manuscript.

The authors focus on differentiating normal and cancerous tissues by using FTIR spectroscopy. The overlap of major absorbance bands of nucleic acids, carbohydrates and phospholipids in the mid-IR region would decrease the diagnostic potential of this technique. The authors used the colonic tissues as a model system to study the interference of macromolecules and eliminate RNA/DNA ratio as an improved diagnostic parameter for detecting colonic malignancies. This article is well written with good readability. Questions: 1.How much would RNA/DNA ratio improve the diagnostic accuracy? 2.Is any difference in characteristics of different stages of colonic abnormalities such as low grade intraepithelial neoplasia, high grade intraepithelial neoplasia and cancer when detected by FTIR spectroscopy ?

The RNA/DNA ratio is one of the parameters that has a near universal diagnostic potential across different types of malignancy (Sahu et al 2004, Mordechai et al 2004). Several works from our lab and elsewhere had established this during the earlier studies where the potential of FTIR spectroscopy/microscopy as a diagnostic technique was being examined. During malignancy accumulated glycogen or carbohydrates decrease in both Cervical intraepithelial neoplasia and cervical cancer and during this condition the RNA/DNA ratio is an effective parameter[31]. Thus, lowered interference from carbohydrates may indeed lead to improved nucleic acid signatures. We show cases of IBD in the present study and the variation. Observations with different grades of colonic malignancy (polyps and cancer) previously had shown that RNA/DNA is indeed an effective ratio. Histopathological features indicated the shrinking of the lumen or distortion of the lumen in crypt circle (morphology) evident of disappearing or altered carbohydrate metabolism[11].

1. Abstract (page 1) – Please write out what FTIR stands for, as audiences will vary in their knowledge of this field.

   The abstract has been rewritten as per the journal format and all changes included.
2. Materials and Methods (page 5) – From reviewing Figure 1, it is apparent the crypts that were examined were normal. It would serve the authors well to include a sentence that explicitly states this. It is a bit confusing initially, as the paper states tissue is taken from colon cancer patients.

   We have now included these details (Page 7, lines 16-22).
3. Materials and Methods (page 6) – Had any study patients been treated with neo-adjuvant chemotherapy or any other therapy that may have altered the background normal tissue (even if alterations are not readily seen on H&E stained sections)? If so, this should be mentioned.

   We have no information on this due to the existing protocols. But the behavior of normal tissues from different patients is similar indicating that this maybe a minor factor.
4. Spectral Analysis and Statistics (page 7) – The authors state 19 crypts were studied. How many patients were these crypts from? Were more than one crypt from the same patient studied; if so, were there any differences seen?

The normal crypts behaved similar across the different patients. More than one crypt were measured form a single patient based on the normal histological features. We used 19 crypts of human colonic tissues from patients that were of similar morphology, size and spectral features away from any abnormality to establish the initial spectral variation pattern A pathologist examined the tissue histology under microscope to ascertain that there was no influence of stromal material in the measurement sites in the biopsy and the region where measurement on crypts was undertaken was equivalent to what is observed in a normal colonic mucosa with regards to morphology. For the study using samples of IBD patients, measurements were made on crypts that were in

the region considered as having normal morphology as well as in regions where the disease was diagnosed by the expert pathologist. All diseased spectra presented are the average of spectra of that category of disease. Sub categories were not evaluated in this study and are a future prospectus. The difference spectra of IBD, normal and Crohn's represent the average spectra for each category from ten different patients subtracted from the average normal spectra. This is now described on page 26 legends to figures.

.We have added this now in Page8, lines 24-26.

5. Absorbance of nucleic acids in the crypts (page 13) – The authors compared normal crypts to colon cancer of "different grades." What were the grade and stage of each colon cancer case? Did the analysis vary according to grade or stage? If you blinded the researcher and only showed them the spectra, what is the likelihood they can identify cancer vs. normal? Is this possible using just your data? 6. The authors do not discuss the next steps for this study. Is there a plan to study cases with crypt architectural distortion (e.g. inflammatory bowel disease), inflammation or dysplasia (pre-malignant)?

We have not compared different grades of cancer in this study but have illustrated the principle using the samples from IBD patients. Within IBD the classes of IBD and Crohn's disease do show differences. With increasing evidence from different studies indicating common variations during malignancies, it is feasible to identify and classify the disease stage and grade based on spectral data. However the pathologist would still be needed for ascertaining that the measurements are made in the right region. While a pathologist's classification would still be subjective, a spectral data would help further lend a quantitative approach to the diagnosis. This has been the objective behind using computational methods that are free from human bias. Previously studies have indicated the potential of predicting diseases from biopsies[32]. While the disappearance of carbohydrates (Glycogen) an decrease in its signal in the FTIR spectra is a function of the differentiation process in the cervical

epithelium[33] and is elucidated by measurements in different zones , in the crypt the same analogy may be observed by varying the measurement using different aperture in the crypt circle. This establishes that biological variations can be manifested as chemical signatures and are the basis for the diagnostics. This is now discussed in the discussion section (Page 18 lines 7-20).

1: Sahu RK, Argov S, Salman A, Huleihel M, Grossman N, Hammody Z, Kapelushnik J, Mordechai S. Characteristic absorbance of nucleic acids in the Mid-IR region as possible common biomarkers for diagnosis of malignancy. Technol Cancer Res Treat. 2004 Dec;3(6):629-38. PubMed PMID: 15560721.

2: Mordechai S, Sahu RK, Hammody Z, Mark S, Kantarovich K, Guterman H, Podshyvalov A, Goldstein J, Argov S. Possible common biomarkers from FTIR microspectroscopy of cervical cancer and melanoma. J Microsc. 2004 Jul;215(Pt 1):86-91. PubMed PMID: 15230879.