Format for ANSWERING REVIEWERS

August 22, 2013

Dear Editor,

I, along with my coauthors, would like to re-submit the attached manuscript entitled "**Efficacy of a novel auto-fluorescence imaging system with computer-assisted color analysis for assessment of colorectal lesions**" as a brief article. The manuscript ID is ESPS Manuscript NO: 3904.

The manuscript has been carefully rechecked and appropriate changes have been made in accordance with the reviewers' suggestions. The responses to their comments have been prepared and attached herewith. The revised manuscript has been checked by a native English speaker to address any concerns regarding the language quality of our manuscript.

We thank you and the reviewers for your thoughtful suggestions and insights, which have enriched the manuscript and produced a more balanced and better account of the research. We hope that the revised manuscript is now suitable for publication in your journal.

Title: Efficacy of a novel autofluorescence imaging system with computer-assisted color analysis for assessment of colorectal lesions.

Authors: Hiroko Inomata, Naoto Tamai, Hiroyuki Aihara, Kazuki Sumiyama, Shoichi Saito, Yomohiro Kato, Hisao Tajiri.

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 3904

The manuscript has been improved according to the reviewers' suggestions, as follows:
1 The format has been updated.

2 The following revisions have been made according to the suggestions of the reviewers:

(1) The content of "Methods" in the "Abstract" should be present in a concise description.
Answer:
In accordance with your suggestion, we have revised and shortened the Methods section of the Abstract and made it more concise.

(2) Regarding the "Histological assessment", the authors should make a description of who are responsible for the diagnosis?
Answer:
A pathologist at our hospital provided the final pathological diagnosis. We have added this information to the Materials and Methods section.

(3) In "Statistical analysis", what's the SD?
Answer:
SD means standard deviation. We have added this information to the Materials and Methods section.

(4) In "Results", the sentences " A total of 191 patients were recruited for this study. Ninety patients refused to participate to this study, 9 patients had the lesions diagnosed as SSA/P pathologically and 5 patients had been diagnosed as IBD." are difficult to readers. Especially for the case numbers, please explain how to get the final number of 88.
Answer:
As the reviewer noted, our description was confusing, and the number of patients who refused to participate in this study was misreported. We have corrected and revised the description in the Results section accordingly.

(5) The "Table 1" should be modified and displayed in a separate page.
Answer:
Because the information provided in Table 1 was also described in the Results section, we have chosen to delete the original Table 1 from the manuscript.

(6) How about the difference in efficiency of this novel AFI system with computer-assisted color analysis between trainees and experts?
Answer:
In this study, colonoscopy and determination of the region of interest were performed by an experienced endoscopist. Therefore, we had no data on which to base comparison of the efficacy of color tone intensity analysis between non-experts and experts. The expert-only nature of this study should be considered a limitation. We have mentioned this limitation of the study in the Discussion section.

(7) On exclusion criteria, it might be more comprehensive for a reader with less knowledge on colonic polyps if the authors could explain why serrated polyps needed to be excluded.
Answer:
At present, the histological diagnostic criteria for SSA/P are controversial in Japan; therefore, we excluded SSA/P lesions from this study. We have added this information to the Materials and Methods section.

(8) Each Figures' legend should be complete by itself. For examples; What are differences between the two pictures in the Fig 1. The abbreviation ROI on Fig 2 might be replaced by the full words. Fig 3 is interesting but the legend contained no details about what program and what is it doing? 3. The ROC curves did not depict any cut-off points, but rather area under the curve. (see the legends). And it would be better if they were stated that 'The ROC curve sensitivity and 1-specificity of G/R ratio in discriminating between ... and..."
Answer:
As the reviewer noted, our initial figure legends were not satisfactory. Therefore, we have revised all of the figure legends to make them more readable and to omit

abbreviations.

(9) The authors should add further explanations to the figure legend with respect to the image in Figure 1. 2. Physical principles regarding this procedure should be explained in "materials and methods" section.
Answer:
As we stated above, we have revised all figure legends for readability and have omitted abbreviations (including that of Fig. 1.2). Additionally, we have added a description of the physical principles behind the AFI system and of real-time color intensity analysis to the Introduction section.

(10) This article is interesting in novelty for computer-assisted color analysis of colorectal lesions using a new system.
Answer:
Thank you for your comment.

(11) The mechanism and features of the new system should be introduced briefly for readership of the journal.
Answer:
There are two main differences between the former AFI system (EVIS LUCERA CLV-260SL, EVIS LUCERA CV-260S; Olympus Medical Systems, Tokyo, Japan) and second-generation AFI systems. The first major improvement is a brighter lamp that enables a higher frame rate, even though the overall brightness of the endoscopic images is equal to that from first-generation AFI systems. Therefore, there is less flickering and color splitting in the endoscopic images. The second major improvement is in the image-processing algorithm, specifically the noise-reduction algorithm, which results in higher-resolution images with less noise interference.
We have added this information to the Introduction section.

(12) The technical and scientific basis needs explaining of the color tone analysis to predict the depth of invasion.
Answer:
If colorectal neoplasia invades the submucosal layer, which contains autofluorescent substances including collagen, nicotinamide adenine dinucleotide hydrate (NADH), flavin adenine dinucleotide, lysosome granules, and porphyrin, it is expected that the autofluorescence will attenuate. Therefore, we hypothesized that the use of the AFI system would permit diagnosis of the depth of invasion. To verify our hypothesis, we evaluated the effectiveness of the novel AFI system in determining the depth of invasion of colorectal neoplasia.
We have added this information to the Introduction section.

(13) A table is suggested to compare the results of endoscopic diagnosis with that of histological diagnosis of the lesions.
Answer:
We have provided endoscopic diagnosis results on the histology of the colorectal lesions that were obtained by magnifying endoscopy. Additionally, in Table 1, we compare the results obtained using computer-aided diagnosis and endoscopic diagnosis.

(14) Figures are redundant and should be simplified. Figure legends should be more detailed for the readers to know what is demonstrated. For example, in Figure 1, what are the features that the authors want to show?

Answer:

As the reviewer noted, our figures and figure legends were not informative. We have revised the figures and all figure legends to make them more readable and informative without using abbreviations (including Fig. 1).

(15) Because of a lack of SM deep cancers, PPV for distinguishing SM deep cancers was extremely low. It was difficult to agree with their conclusion only from this data.

Answer:

As the reviewer points out, the number of SM deep lesions examined in this study was very limited. Therefore, the paucity of SM deep lesions should be considered a limitation of the study, as we mention in the Discussion section.

In addition, we revised the conclusion of the Discussion section as follows:

Color tone analysis, which is an objective and time-saving method, may offer the potential to determine the depth of invasion of colorectal neoplasms, although the number of SM deep lesions examined in the present study was limited. Further studies are needed to determine the usefulness of color tone analysis in estimating the depth of invasion of colorectal neoplasms.

(16) How did the authors calculate sample size?

Answer:

This is a first report evaluating color tone intensity analysis using a novel AFI system for distinguishing colorectal neoplastic lesions from non-neoplastic lesions. Therefore, it was difficult to predict the efficacy of the novel AFI system to calculate the sample size for this study. The lack of a sample size calculation in the design of this study should be considered a limitation; we have added this information to the Discussion section.

(17) Magnifying endoscopy with NBI and CE using crystal violet was performed to determine the treatment strategy, finally. Could the authors show us the comparison data between magnifying endoscopy and AFI with computer-assisted color analysis?

Answer:

We have provided endoscopic diagnosis results for the depth of invasion of the colorectal lesions obtained by magnifying endoscopy. Moreover, we have compared the results obtained by computer-aided diagnosis and by endoscopic diagnosis in Table 2.

(18) In this study, polyps ≥ 5 mm were enrolled. According to ASGE statements, such polyps were candidates for resection, irrespective of histology. Polyps < 5 mm were adequate as candidates to determine polyps which didn't need to be removed.

Answer:

As the reviewer noted, lesions < 5 mm in size were excluded from this study because the treatment strategy and guidelines for such polyps have not yet been established in Japan. However, the sizes of the lesions included in this study

should be considered a limitation, and we have included this point in the description of the study limitations in the Discussion section.

(19) The authors have used NBI without magnification or chromoendoscopy before color-tone sampling from ROI. It would be helpful to provide data regarding the comparison with diagnostic accuracy (especially, depth of invasion) of NBI with/without magnification.
Answer:
We have now provided the endoscopic diagnosis results for the depth of invasion of colorectal lesions using magnifying endoscopy with NBI and chromoendoscopy. In addition, we have compared the results obtained using computer-aided diagnosis with those obtained using endoscopic diagnosis in Table 2

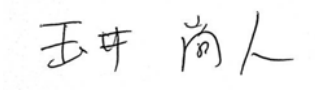
(20) The authors selected the ROI determined using chromoendoscopy for color tone sampling. However, the authors didn't comment in detail which ROI was chosen. In addition, diagnostic accuracy for predicting histology according to the shape or size of the lesions was different? What is the difference from another studies using quantitative intensity of fluorescence?
Answer:
We have added a description of how the region of interest is determined to the Materials and Methods section. We have also compared the diagnostic accuracy between protruded type lesions and flat elevated/depressed type lesions and have provided the results in Table 1.

There are two major differences between the present report and the previous report. First, the AFI system used in this study is a novel AFI system that allows the use of higher-resolution imaging with less noise interference; the AFI system used in the previous reports was an earlier-generation system. Second, the present study is the first report to evaluate the efficacy of color tone intensity analysis in predicting depth of invasion.
We have added this information to the Discussion section.

3 The references and typesetting were corrected.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,



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