Automated Objective Determination of Percentage of Malignant Nuclei for Mutation Testing

Hollis Viray[1], Madeline Coulter[1], Kevin Li[1], Kristin Lane[2], Aruna Madan[1], Kurt Schalper[1], Kisha Mitchell[1], Clifford Hoyt[2], and David L. Rimm[1]

[1]Department of Pathology, Yale University School of Medicine, New Haven, CT; [2]Caliper Life Sciences, Hopkinton, MA

Abstract

Background: DNA mutations detected in tumors are a critical companion diagnostic test for some new targeted therapies. The accuracy of mutation detection depends on the sensitivity of the assay and on the percentage of tumor cells in the sample. Currently, the malignant cell percentage is judged by eye resulting in a large variation of estimated percentages. This aspect of DNA mutation testing can potentially be standardized by developing a computer algorithm capable of objectively determining the percentage of malignant nuclei in an image of tumor tissue.

Methods: H&E images from colon adenocarcinoma cases were selected for algorithm development and testing. To create a criterion standard for evaluating algorithm accuracy, the nuclei in each image were classified as malignant or benign and counted by a technician, then reviewed by a pathologist. Using inForm software (Caliper Life Sciences), an algorithm was developed to calculate the percentage of malignant cells in a single field of view based on feature extraction involving tissue stain optical densities and morphology. Example regions defining malignant and benign nuclei from 25 cases were used to train the algorithm. The algorithm was subsequently validated on a separate set of 100 images from a tissue microarray.

Results: Among the training images, Algorithm #9 had a median deviation from the manually counted percentage of malignant nuclei of 5.4%. The algorithm differed from the criterion standard by less than 5.0% on 11 (44.0%) of the 25 training images. For 17 (68.0%) of the training images, Algorithm #9 differed by less than 10.0% from the criterion standard. In the validation set, the algorithm deviated from the criterion standard by a median of 6.2%. 47 (47.0%) of the validation images deviated by less than 5.0% and 58 (58.0%) deviated by less than 10.0%.

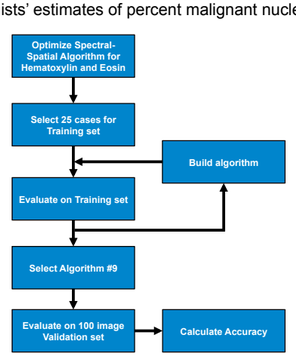
Conclusion: This method represents an exploratory example with future potential to be used as a tool to assist in determining the percent of malignant nuclei present in a tissue sample. Further validation of this algorithm or an improved algorithm may have value to more accurately assess percentage of malignant cells for future companion diagnostic mutation testing.

Background/ Materials & Methods

Background: DNA mutation assessments on patient tumor samples are a critical companion diagnostic test for some new targeted therapies. Mutation detection is limited by the sensitivity of the assay used and the percentage of malignant cells present in the tissue sample analyzed. Currently, the percentage of malignant nuclei is estimated by eye resulting in a large variation of pathologist assessment. This aspect of DNA mutation testing can potentially be standardized by developing a computer algorithm capable of objectively determining the percentage of malignant nuclei in an image of tumor tissue.

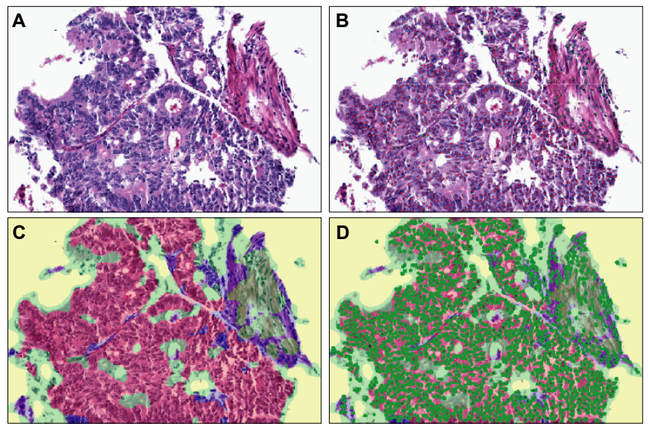
Methods: Using inForm software (Caliper Life Sciences), an algorithm was optimized to analyze tissue spectral and spatial data based on H&E staining of colon adenocarcinoma cases. Regions from 25 cases were used to train the algorithm to define areas with malignant nuclei, benign nuclei, necrosis, and blank space, then count the nuclei within each tissue area allowing for the calculation of percent malignant nuclei. To create a criterion standard for evaluating algorithm accuracy, the nuclei in each image were also classified as malignant or benign, counted by a technician, then reviewed by a pathologist. Algorithm #9 was selected as the optimal algorithm. To further evaluate the accuracy of Algorithm #9, 100 additional colon adenocarcinoma cases from a tissue microarray (TMA) were selected by a pathologist. Algorithm performance on the validation set was evaluated in relation to the criterion standard and to three pathologists' estimates of percent malignant nuclei.

Figure 1. Method for Development of Automated Assessment of Percent Malignant Nuclei



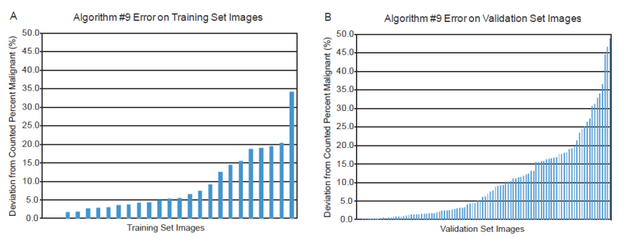
Results

Figure 2. Images Depicting Algorithm #9 Function



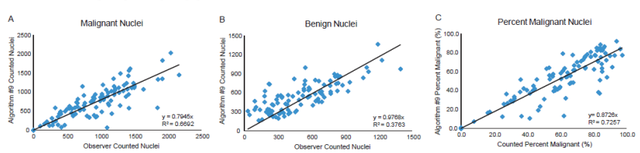
Representative Images of Algorithm #9. H&E of a colon adenocarcinoma case **(A)**. Nuclei marked with red and green dots to obtain a criterion standard **(B)**. Algorithm #9 tissue segmentation map identifies regions of tissue as malignant (red), benign (green), or necrotic (blue) **(C)**. Algorithm #9 object count image highlights individual nuclei in bright green circles **(D)**.

Figure 3. Algorithm #9 Error Distribution



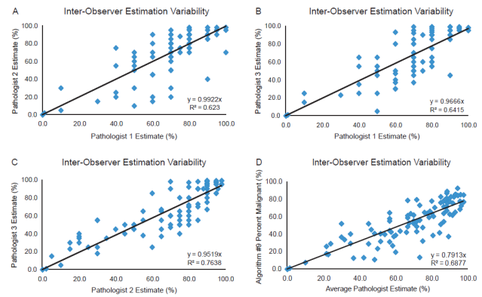
Algorithm Error Frequency. Algorithm #9 error was evaluated as the deviation of the algorithm-determined percentage of malignant nuclei from the manually-counted percentage on the training set **(A)** and on the validation set **(B)**.

Figure 4. Algorithm #9 Nuclear Count and Percent Malignancy Performance



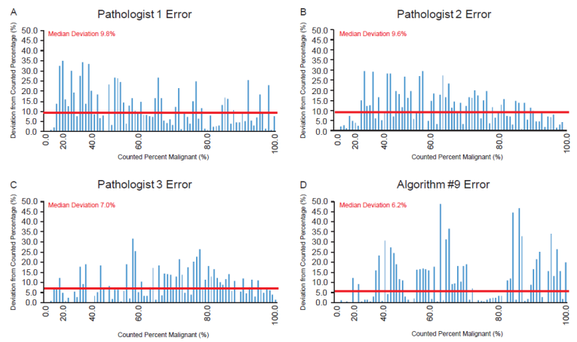
Algorithm Nuclear Counts. Total nuclei identified by Algorithm #9 plotted versus the observer counted values for total malignant nuclei **(A)**, total benign nuclei **(B)**, and percentage of malignant nuclei **(C)** for the validation set images.

Figure 5. Inter-Observer Variability between Pathologists



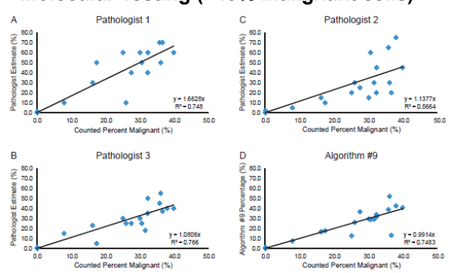
Pathologist Estimate Agreement. Individual pathologists' estimates of percent malignant nuclei plotted versus each other **(A-C)**. Average pathologist estimate plotted versus Algorithm #9-determined percent malignant **(D)**.

Figure 6. Error Distribution Between Pathologist Estimation and Count



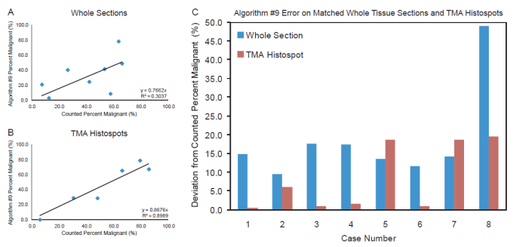
Comparison of Pathologist and Algorithm #9 Error. Pathologist estimation and algorithm error shown in order of increasing tumor content **(A-D)**. Median deviation for each observer/technique shown with a red line.

Figure 7. Estimation Accuracy in the Critical Low Range for Molecular Testing (<40% malignant cells)



Algorithm and Pathologist Accuracy on Low Tumor Content. Pathologist estimates and Algorithm #9 results plotted versus the criterion standard for images containing less than 40.0% malignant nuclei **(A-D)**.

Figure 8. Comparing TMA to Whole Section Data



Algorithm Performance on Whole Tissue Sections. Algorithm #9 percentages on whole tissue sections **(A)** and corresponding TMA histospots **(B)** plotted versus the counted percent malignant. Deviation from the counted percent malignant is shown for matched cases of whole sections and TMA histospots **(C)**.

Conclusions

- A computer algorithm using inForm software (Caliper Life Sciences) for image spectral/spatial analysis provides a tool for objectively determining the percentage of malignant nuclei present in a tissue section.
- Algorithm #9 is comparable to pathologist estimation accuracy for determination of percentage of malignant nuclei.
- Algorithm #9 appears to be highly dependent on the H&E stain since performance on separately stained whole sections is substantially worse than performance on TMA histospots in the training and validation sets with the same H&E stain.
- Further algorithm development and validation is needed to increase accuracy for future assistance in companion diagnostic mutation testing.

References

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C. S. Karapetis, et al., K-ras *Mutations and Benefit from Cetuximab in Advanced Colorectal Cancer.* N Engl J Med. 2008.

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