Quantitative Image Analysis of Ki-67 Immunohistochemistry Compared with Manual Pathologist Analysis in Breast Cancer

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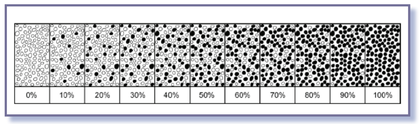
Introduction

Determining Ki-67 percentage in a nuclear IHC quantitative sample is complicated by tumor type, observer variability, staining intensities, bias and workload.

Manual analysis protocol calls for cell counting. CAP Breast Biomarker templates provide a visual aid for estimating percentage of positive cells.



The goal of this IRB approved/exempt study was to compare accuracy of manual analysis by counting/visual estimation vs. computer aided image analysis in Ki-67 breast cancer.

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GenASIs HiPath has been provided by Applied Spectral Imaging Inc. - Carlsbad, CA www.spectral-imaging.com

Methods

GenASIs HiPath functions as an adjunct tool to the microscope

- **Location:** Spartanburg Medical Center, Spartanburg SC
- **Control Group:** 100 slides stained with CONFIRM anti-Ki-67 (30-9) Rabbit monoclonal Primary Antibody (Ventana) scored in years 2012-2013
- **Equipment:** Author's microscope (Manual, Olympus BX43), equipped with halogen or LED light source, camera adapter with a 1x C-Mount & vendor provided (Applied Spectral Imaging - Carlsbad, CA) GigE high resolution, colour CCD Camera & Image Analysis software (GenASIs HiPath)
- **Method:** Select frames from the control group were acquired on author's microscope with CCD camera & analyzed using GenASIs HiPath. Average cell count per sample was 1,786
- **Test Parameters:** Slides scored by manual analysis as 1%-14% were characterized as low proliferation, and slides scored by manual analysis as equal or greater than 15% were characterized as expressing the proliferation marker. In addition to the cutoff of 15%, the study looked at the actual scores to determine overall accuracy of pathologist manual analysis and GenASIs HiPath image analysis.

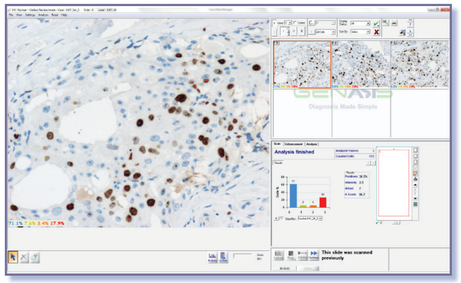
Results

Manual Scoring < 15%

- Manual analysis control group scored 35 slides as ≤14%
- GenASIs Hipath Image Analysis concordance was 30/35 slides (86%)
- Manual analysis revised 4 cases to scores equal or higher than 15%
- Final concordance was 34/35 slides (97%).

Scoring of Ki-67 Proliferation Discordance & Revision

Case	Initial Manual	GenASIs HiPath	Revised Manual
35	8%	36%	40%
28	10%	32%	30%
81	8%	18%	12%



Case # 28

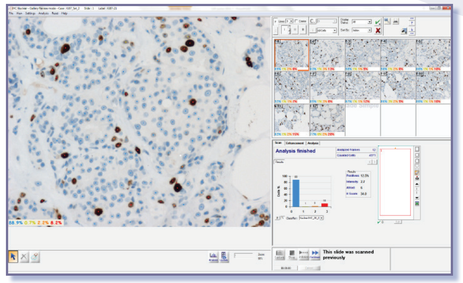
Image Analysis Score: 32%
Initial Manual Score: 10%
Revised Manual Score: 30%

Manual Scoring => 15%

- Manual analysis control group scored 65 slides as ≥15%
- GenASIs HiPath Image Analysis concordance was 61/65 slides (94%)
- No cases were revised
- Cases with discordance were scored by GenASIs HiPath slightly below 15%

Scoring of Ki-67 Proliferation Discordance

Case	Initial Manual	GenASIs HiPath
44	15%	12%
21	15%	13%
36	15%	13%
5	20%	14%



Case # 21

Image Analysis Score: 13%
Manual Score: 15%

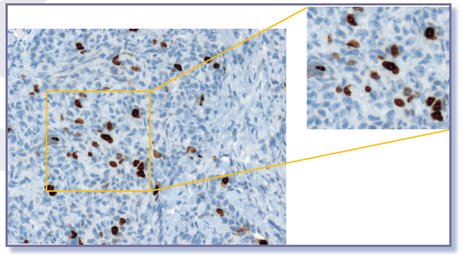
Conclusions

For Ki-67 IHC samples, GenASIs HiPath is useful as an adjunct tool to measure percentage of cell staining.

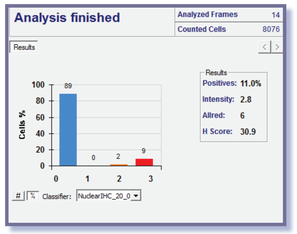
The image analysis improved the accuracy of the low proliferation and results were revised by the pathologist.

Discussion

- Useful for quantitative immunohistochemistry, particularly breast cancer panel
- Reports number of analyzed cells and frames
- Captures select frames and regions of interest
- Performs statistical analysis for each frame, regions of interest within frames, as well as composite scoring for all frames
- Technology that connects personal microscope and PC is a convenient method



Precise Cell Segmentation Within Selected Region of Interest



Various Statistical and Analysis Display