

#5024 ABSTRACT

Background: High throughput screening through tissue microarray (TMA) analysis has begun to revolutionize the identification and evaluation of predictive and prognostic markers in breast cancer. A major limitation in this approach has been the fact that TMAs are interpreted manually with resulting interobserver, intraobserver and fatigue errors. A second limitation in TMA analysis is the prolonged time it takes for the evaluation to be completed. A third limitation is sampling error since each patient sample is limited to a single tissue core.

Materials and Methods: Any method of automated TMA interpretation must deal with the problem of tumor heterogeneity and resulting sampling error. Using novel epithelial recognition algorithms (ERAs) based on image enhancement, epithelial cell detection, stromal cell identification, epithelial area identification and artefact removal and specific recognition algorithms (SRAs) based on the imaging of a specific nuclear, cytoplasmic or membrane marker, eg., estrogen receptor (ER- α) combined with TMA scanning, automated TMA interpretation was carried out.

Results: Using novel ERAs based on clustering of tumor cells (pixel luminosities and densities) and mathematical formula (the Gaussian kernel), the shape of the tumor nuclei derived from their elongation ratio (ratio of long to short nuclear axes) and the size of the tumor nuclei, breast carcinoma cells could be identified and distinguished from background stroma and tissue artefacts from their scanned tissue core digital images. Using SRAs, which detect pixel colors and intensities for selective immunomarkers, eg, ER- α , immunocytochemical staining of only the breast carcinoma cells could be measured and recorded automatically. The speed of automated TMA interpretation increased 100 fold over manual interpretation. Repeat scanning and automatic interpretation of TMAs using these ERAs and SRAs in tandem revealed >98% reproducibility, effectively eliminating interobserver, intraobserver and fatigue errors. This automaticity of TMA interpretation fostered the creation of expanded TMA where 10 cores instead of 1 were created from each patient and used to manufacture an expanded TMA. Automated interpretation of this multiplex TMA increased marker accuracy 5X while maintaining speed / reproducibility of interpretation.

Discussion: Although this study used ER- α nuclear immunoreactivity as a prototype breast carcinoma marker, virtually any prognostic or predictive marker manifesting nuclear, cytoplasmic or membrane signals can be investigated by this combinatorial imaging approach utilizing ERAs and SRAs to fully automate breast cancer TMA interpretation. The advantages of this improved and rapid method of TMA interpretation may allow for the identification and validation of newer predictive and prognostic markers of breast cancer.

AUTOMATION OF BREAST CANCER TISSUE MICROARRAY INTERPRETATION THROUGH A COMBINATION OF EPITHELIAL AND SPECIFIC RECOGNITION ALGORITHMS

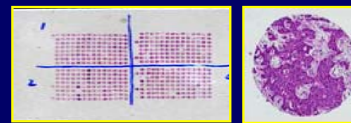
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BACKGROUND OF TMAs

Pathologists have also had to deal with the high throughput revolution which has taken center stage in genetics and molecular biology

- cDNA microarrays
- Protein microarrays
- Tissue microarrays (TMAs)

TMAs are supposed to foster HTS



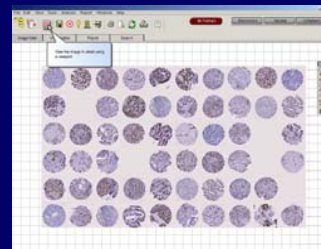
- TMAs allow for the the analysis of a large number of tissue samples
- TMAs standardize target evaluation
- TMAs permit the evaluation of many different targets
- The interpretation of TMAs has been manual and subjective and certainly not HTS



To date, because of their manual interpretation TMAs really have been a concept which is "Back to the Future".

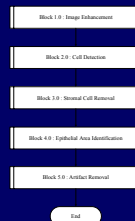
SCANNING OF TMAs

IMAGE ENHANCEMENT
Verifying image content
Contrast modification
Background removal



Once a slide has been digitized, its objects become a collection of pixels
Pixel values range from 0 to 255 in a gray scale image with 255 representing absolute white and 0 total black.

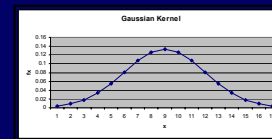
Color images consist of three planes corresponding to red, green, blue (RGB)
Many pathology images do not have pixel values that make effective use of the full range of pixel values (100-150). The visual appearance of an image can be improved by remapping the pixel values to take advantage of the full range of outputs.



CARCINOMA CELL IMAGE SELECTION

EPITHELIAL RECOGNITION ALGORITHMS (ERAs)

EPITHELIAL CELL DETECTION
Epithelial cells are more densely packed and darker than stromal cells
Hence their pixel intensity will be less than stromal cells



$$\text{Gaussian kernel } G(x) = \text{power}(2, -11.25 \cdot x^2 / (0.25 \cdot \pi)) \cdot (\text{Object} / \text{Object}) \cdot (\text{Object} / \text{Object})$$

$$\text{kernel size} = 1 + 2 \cdot \text{ceil}(2 \cdot \sum_{i=1}^{\infty} G(i))$$

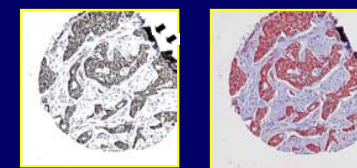
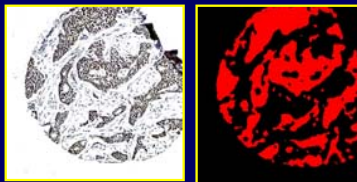
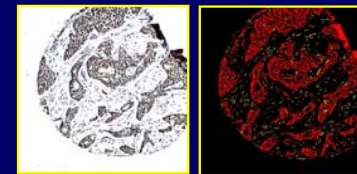
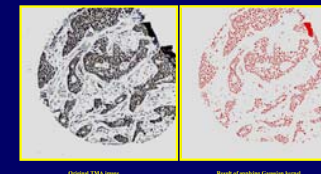
$$x = \text{kernel size} / 2$$

$$G(x) = G(x) \cdot G(x)$$

$$x = \text{kernel size} / 2$$

G = Gaussian value at x position
x = Pixel value at x

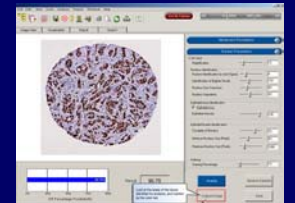
APPLYING THE GAUSSIAN KERNEL, CURVES OF SYMMETRY WILL BE DEFINED IN BOTH HORIZONTAL AND VERTICAL DIRECTIONS
A SELECTED PIXEL WILL BE CONSIDERED AS EPITHELIAL IF IT'S PIXEL INTENSITY IS LESS THAN 5 NEIGHBORING PIXELS IN ALL DIRECTIONS



ARTIFACT REMOVAL
Artifacts are usually very large and have a different type of texture than cells
We have found that the red plane of the image can be used to distinguish artifacts from cells
We will use a technique called the distribution of luminosity to determine if given pixels belong to artifact or not.

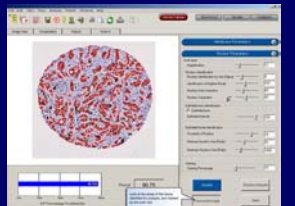
#5024 AUTOMATION OF TMA INTERPRETATION

SPECIFIC RECOGNITION ALGORITHMS (SRAs)



SRAs measuring nuclear immunoreactivity (brown nuclear pixels) quantitate ER- α

APPLICATION OF PREVIOUSLY DEVELOPED ERAs



ERAs AND SRAs WORKING IN TANDEM

