

Introduction: Tissue microarrays (TMAs) have emerged as the method of choice for evaluating clinical materials in the context of biomarker development for a number of reasons:

- TMAs are optimally suited for large-scale *in situ* tissue analyses
- Utilizing TMAs allows preservation of valuable tissue resources by avoiding cutting traditional tissue sections
- TMAs can be constructed from various materials – formalin and fresh frozen tissues, experimental tissues, cell lines and xenografts
- TMAs offer a high degree of standardization, staining optimization and more vigorous quality control can be achieved more effectively.

Evaluation and interpretation of TMAs constitutes a significant challenge. In this presentation we provide evidence to support the claim that automated laser scanning analysis has a complementary and equal (and often superior) quality to the analysis that can be achieved by a pathologist.

TMA preparation: Preparation of tissue microarrays demands careful analysis of starting material by a pathologist to locate areas of interest (tumor), with triplicate plugs usually being included. A 360-spot TMA is typically constructed during a five-day period by an experienced pathologist and array technician.

In this study we used chromatically and fluorescently stained 108-element TMAs containing cores of normal breast tissue and breast adenocarcinoma. Each case was represented by three spots from a tumor sample and in some cases by three spots from a normal counterpart area.

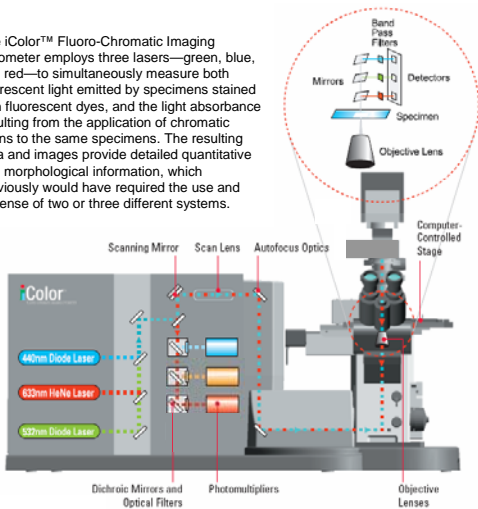
Workflow scenarios:

Manual pathologist evaluation: The chromatic TMA was analyzed by an experienced pathologist on a spot-by-spot and row-by-row basis under 100x magnification. This process consisted of the following steps: (1) overall examination of the TMA, with quality control for each individual array performed immediately after assembling and staining to allow verification that each spot represented the initial diagnosis, (2) individual element scoring for PR+/-, ER+/-, and HER2/Neu staining intensity (0-3); (3) digital camera imaging of representative spots, and entering special comments and results into a computer database in Excel format; and (4) data analysis and reporting.

Automated TMA analysis: The automated analysis workflow consisted of the following steps: (1) selecting and setting the appropriate fluorescence and light absorption signals to be used in the analysis; (2) performing a high-speed overview scan of the TMA, typically at 5 micron resolution; (3) automated identification of TMA core elements; (4) review (and editing if necessary) of identified core elements; (5) high-resolution analysis of the individual core elements; (6) evaluation of the results in the iBrowser[®] Data Integration software; and (7) data export to a spreadsheet program.

iColor™ Fluoro-Chromatic Imaging Cytometer for automated TMA analysis:

The iColor™ Fluoro-Chromatic Imaging Cytometer employs three lasers—green, blue, and red—to simultaneously measure both fluorescent light emitted by specimens stained with fluorescent dyes, and the light absorbance resulting from the application of chromatic stains to the same specimens. The resulting data and images provide detailed quantitative and morphological information, which previously would have required the use and expense of two or three different systems.



Traditional Manual Pathology Evaluation

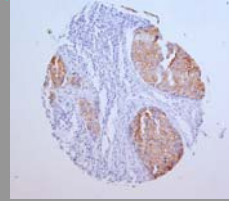
Initial examination of TMA

Pathologist initially examines TMA for quality of staining and accuracy of core selection

Time required: 1 hour or more

Relatively fast visualization, but:

- No documentation
- Even at 10X, magnification orientation is easily lost
- Review one array at a time



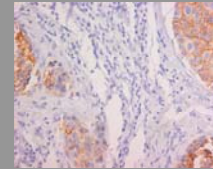
Scoring of TMA core elements

Pathologist manually scores individual TMA elements

Time required: 6 hours or more per parameter, per observer

Analysis of complex morphology – however:

- Multiple observers are required for consensus
- Scoring is semi-quantitative and subjective
- Human eye is not sensitive to fine differences in color intensity
- Lengthy process and extended periods of high concentration may lead to errors

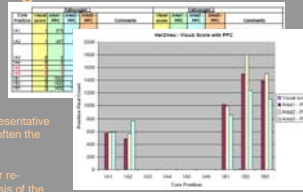


Reporting and documenting results

Pathologist archives data

Time required: 4 hours or more

- Initially very few “representative” elements are documented on camera (a compromise to save time).
- Process is lengthy if documentation and representative pictures are required for all core elements – often the case for scientific data analysis.
- There is often a need for additional images or re-analysis, entailing archive search, new analysis of the array by an experienced pathologist, and documentation.



Results and conclusion:

- Manual analysis of the 108-element TMA required at least 5-6 hours of uninterrupted, focused investigation by a trained pathologist. (Time refers to scoring only.) It is a highly demanding and a slow process, even for experienced practitioners, and some errors are inevitable.
- Automated TMA analysis is rapid. The initial scans are completed in 10–20 minutes per array, and editing is accomplished in 5 minutes. High-resolution scans require 1 hour of walk-away analysis time.
- Chromatically or fluorescently stained samples may be analyzed.
- In a previously described study, excellent correlation was shown between Pathologist's scoring and automated analysis of a CAP survey Her2/Neu-expressing TMA.
- The most critical step of automated TMA analysis is selection of the area to be analyzed. Automated analysis required a minimal degree of technician intervention for review and editing of TMA cores prior to high-resolution rescanning.
- The same chromatically stained slides which are optimal for conventional manual interpretation can be utilized in LSC analysis. Additionally, multiple fluorescent dyes can be used, allowing improved quantification of immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH) TMAs.
- Automated TMA analysis by laser scanning cytometry offers fast, objective processing with the supporting image data archived in an accessible format.

Benefits of automated scanning warrant implementation of a new paradigm in TMA analysis.

Automated TMA Analysis by iColor™ Fluoro-Chromatic Imaging Cytometer

Initial examination of TMA

iColor performs scout scan to identify TMA elements

Time required: 10 minutes (with option for observer to review images)



- Documented visual assessment of overall array
- Digital TMA map
- Walk-away analysis of up to 180 microscope slides

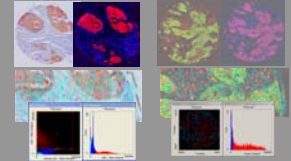


Analysis of TMA core elements

iColor derives data from laser-scan images, automatically scores TMA elements

Time required: 1.5 hours (full walk-away analysis)

- Quantitative data and morphology produced simultaneously
- Traditional IHC and fluorescently-labeled TMAs can be analyzed on the same instrument.
- Sophisticated algorithms for tissue autofluorescence correction can be applied.
- Objective analysis with high sensitivity and reproducibility.
- Embraces emerging reagent technologies: antibodies, Q-dots, etc.



Reporting results and archiving data

iColor instantaneously archives data

- Results can be reported as units of fluorescent intensity, laser light absorption, or according to traditional 0 to 3+ scoring system.
- Fully documented analysis, from TMA overview to final results

