Tissue microarrays: leaping the gap between research and clinical adoption

The use of tissue microarrays (TMAs) in the preclinical and translational research settings has become ubiquitous as they allow for high-throughput in situ biomarker analysis of hundreds of patient samples, with time and cost efficiency. Coupled with advanced imaging and image-analysis technologies that allow for objective and standardized biomarker expression assessment, TMAs have become critical tools for the development and validation of clinically meaningful biomarker diagnostic assays. However, their diagnostic use in the clinical laboratory setting is limited due to the need for conventional whole-section tissue assessment used for routine diagnostic purposes. In this article, after reviewing TMA basics and their translational and clinical research applications, we will focus on the use of TMAs for robust assay development and quality control in the clinical laboratory setting, as well as provide insights into how TMAs may serve well in the clinical setting as assay performance and quantification controls.

**KEYWORDS:** assay development | biomarker | clinical application | diagnostic testing | digital pathology | quality control | tissue microarray

Tissue microarrays (TMAs) have become a mainstay in preclinical and translational research, especially for the development of biomarker assays for characterization of disease. They allow for analysis of extremely small amounts of tissue, thus preserving valuable tissue blocks. At the same time, they dramatically increase the efficiency and cost-effectiveness of performing tissue-based studies [1, 2] by enabling the examination of 10s to 100s of different patient samples on the same slide. This approach also leads to greater reproducibility and comparability of patient samples since all samples are exposed to identical processing conditions [3]. By linking patient sample data to pathological and clinical data, including disease follow-up and treatment response, TMAs have expanded translational research, enabling large-scale biomarker and disease progression studies, leading to more informed and improved clinical hypotheses. Coupling TMAs with high-throughput and objective biomarker expression analysis with robust imaging technologies has led to further improvement in biomarker data that will ensure the continued use of TMAs, especially in the translational setting.

**TMA construction**

Placement of multiple tissues from multiple patients/organs in a single block/slide were described first in 1986 by Hector Battifora who termed it the ‘sausage’ tissue block [4], followed by description of another approach by Wan et al. in 1987 [5], but the process was laborious and time consuming. The use of TMAs did not become popular until Kononen et al. developed a mechanized version of TMA construction [6]. The use of TMAs has increased since then, with over 800 TMA-related publications in 2011 [7]. TMA construction has been reviewed previously [2, 3, 7–9]. However, in brief, TMAs are constructed by first selecting formalin-fixed paraffin-embedded (FFPE) tissue blocks of interest to be included in the TMA block to be constructed. Once cases have been selected, a pathologist reviews a hematoxylin and eosin (H&E) section from each sample block to identify (i.e., place circles around) regions of interest. For example, for tumors, the pathologist can select areas of invasive or noninvasive tumor (i.e., ductal carcinoma in situ in breast cancer), adjacent normal tissue, stroma, or all three, differentially, depending on the intended application. The circled H&E is then used as a guide to identify the coring location on the donor block. Once the core has been removed from the donor block, it is placed in the recipient block in a specific row/column orientation. Maintenance of this row/column information is absolutely vital for performing studies to enable linkage of pathology and clinical information [10]. Furthermore, although one to two cores can potentially be representative of the whole tumor [11, 12] it is good practice to include multiple cores (two to four) [13] from a single case to not only ensure disease representation, but also to
enable characterization of disease or biomarker heterogeneity, and to increase the likelihood of maintaining a large number of samples for analysis due to differential core thickness [14]. Two comprehensive reviews by Rimm et al. [10] and Franco et al. [15] discuss, in detail, considerations for TMA construction and utility from selection of blocks to statistical analysis considerations.

Advantages & disadvantages of TMAs

In addition to the clear advantage of TMAs with respect to the amount of tissue used and thus preservation of valuable tissue due to the relatively small amount of tissue required for construction, TMAs have advantages in several other key areas including reproducibility, analysis time, costs and applicability. As TMAs contain small cores representing all samples on a single slide, assay conditions are uniform across all samples, leading to greater reproducibility of results and reduced assay analysis time than individual slide analysis of each sample, and reagent costs are kept at a minimum since only one (or few) slides need to be analyzed. Additionally, tissue analysis methods that can be performed on whole tissue sections can be applied to TMAs, including immunohistochemistry (IHC) [16], immunofluorescence [17,18], FISH [19], chromogenic in situ hybridization [20], mRNA in situ hybridization [21,22] and miRNA in situ hybridization [23]. Furthermore, recent advances have enabled efficient extraction of DNA and/or RNA from TMA cores, enabling TMA technology to be coupled with advanced molecular testing [24].

With respect to assay development, quality assurance and, ultimately, clinical adoption, the primary subject of this review, these advantages are critical. Implicit with these advantages, TMAs enable efficient analysis across a wide range of biomarker expression levels. Advancement of quantitative methods in tissue will rely on the rapid and efficient analysis of many samples of differential expression levels.

Although the use of TMAs in the clinical laboratory as tools for primary diagnosis will most likely never be realized, several applications of TMAs from assay development through to quality assurance and assay control can be employed. Additionally, TMAs face the same challenges that FFPE-based sample analysis face, which includes how preanalytical variables (time to fixation, time in fixation and antigen oxidation/hydrolysis) affect sample quality, and, hence, analysis. However, TMAs are a great tool for assessment of the specific effects of these variables.

TMA applications

TMA applications are reviewed extensively in several published articles [8,15,25]. Although the goal of this review is to focus on the clinical laboratory application of TMAs, it is important to review some of the basic applications of TMAs. The most widely used application of TMAs is the large-scale cohort analysis of biomarker expression as a function of pathological characteristics and clinical outcome, looking at a single biomarker or a few biomarkers in a large number of cases [26] or biomarkers, based on literature searches [27].

However, as the genome and proteome become more studied and understood with ever-increasing complexity of regulation (e.g., miRNA), the importance of dissecting these pathways will become ever more critical for the development of meaningful biomarker assays and diagnostic tests. With this complexity comes the necessity for integration of multiple high-throughput technologies, enabling a funnel-down approach to biomarker assay development. TMAs serve as an invaluable tool where data from very high-throughput experiments (i.e., gene chip, mass spectrometry or reverse-phase protein arrays) can be validated in actual human tissue samples. A good example of this systems biology approach is provided by Faratian et al., where both in silico (mathematical modeling) and reverse-phase protein array approaches were used to dissect receptor tyrosine kinase pathways to identify several critical pathway members [28]. They were then validated on a TMA cohort consisting of 122 breast cancer cases that had been treated with trastuzumab.

Large co-operative research groups frequently take advantage of TMAs by placing samples from their large clinical trial cohorts. There are several examples of this, including the Breast Cancer International Research Group use of large TMAs to assess accuracy and reproducibility in large-scale clinical trials [29] and the ATAC trial. The ATAC trial employed TMAs to examine prognostic significance in 1125 patient samples using the 21-gene recurrence assay (OncoTypeDx®, Genomic Health, CA, USA) [30], as well as with a four-protein IHC assay [31]. The latter was subsequently validated on TMAs in the TEAM trial [32] and serves as an example of how validation of biomarker assays on TMAs translate to the clinical setting.

Beyond these typical oncology-focused applications, there have also been publications describing cell pellet-based TMAs [33], which have
applications for screening of cell lines, and for control inclusion, biopsy TMAs (such as bone marrow core biopsies or prostate needle core biopsies, reviewed by Datta and Kajdocsy-Balla [34]) as well as nononcology applications such as TMA-based analysis of Alzheimer’s disease [35] and analysis of xenografts [36]. Of particular interest is construction of cell-line TMAs as a discovery tool, specifically for interrogation of pathways related to drug response, since cell lines can be treated and highly controlled [37]. Further, cell-line TMAs can serve as assay controls for either assurance of assay performance or as calibration controls (see ‘Future perspective’ section).

Requirement for automated & objective image-analysis tools

The development of next-generation tissue-based diagnostic tests in the clinical laboratory will be critically reliant upon automated imaging platforms coupled with robust and objective image-analysis tools, both for the whole-tissue clinical sample as well as TMAs. Likewise, the enhanced adoption of TMAs in the clinical setting will also be reliant upon such objective tools since manual assessment of TMA data is not only time-consuming, but also highly subjective, leading to issues with reproducibility [38,39].

There has been significant growth of semi-automated and automated objective methods for scoring of tissue (and unique programs geared for scoring of TMAs as well) in order to reduce intra- and inter-observer variability, and improve reproducibility of biomarker analysis. There are a variety of image-analysis software programs available for use in brightfield applications (H&E staining, chromagen-based IHC and chromogenic in situ hybridization, among others), as well as some for fluorescence applications (e.g., FISH and immunofluorescence among others), with some systems including both the image scanning/digitization and the algorithms for scoring that are described in more detail in a review by Rojo et al. [40] and listed in Table 1. A small number of image-analysis algorithms, such as for estrogen receptor (ER), progesterone receptor and/or human EGF receptor 2 (HER2) analysis (by Aperio [CA, USA] or Ventana [CA, USA]) have been US FDA-cleared for use in breast cancer. A more complete assessment of digital microscopy solutions beyond image-analysis has been reviewed by Rojo et al. [41] and Mulrane et al. [42,43].

Critical to all image-analysis systems is the ability to identify and/or differentiate regions of interest (image segmentation). For example, for assessment of ER expression, the tumor would have to be differentiated from surrounding normal and stromal elements. Once these regions have been identified, additional analysis, including morphological (i.e., positive nuclear identification) and protein expression analysis (IHC) can be performed. There are several different ways to accomplish image segmentation. The first, and most subjective, is to perform manual identification of regions by an operator circling those regions of interest through an imaging interface. The second is through feature-based or contextual compartmentalization whereby the image-analysis software is trained, based on morphological and/or histological features, to recognize areas of tumor and subsequent subcellular compartments (i.e., the nucleus). Three examples of this approach are Aperio’s PRECISION Image-analysis tools, Perkin Elmer’s (MA, USA) inForm™ and Definiens (Germany) Tissue Studio™. The third approach is through molecular identification of regions of interest (e.g., cytokeratin to identify areas of epithelium and differentiate from stromal components). Molecular identification of regions of interest does not require user training, but rather relies on objective image-analysis to differentiate molecular signal from background to define a compartment of interest (i.e., tumor nuclei). An example of this approach is Genoptix’s AQUA® technology.

Additionally, adoption of TMAs into the clinical setting will require standardization of imaging

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<tr>
<th>Manufacturer (location)</th>
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<td>3D Histech (Budapest, Hungary)</td>
<td>MIRAX HistoQuant™</td>
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<tr>
<td>Aperio (CA, USA)</td>
<td>Aperio Image Analysis Toolbox/Spectrum™/ PRECISION Image Analysis tools</td>
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<td>Caliper (a Perkin Elmer company, MA, USA)</td>
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<td>Genoptix (CA, USA)</td>
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<td>Leica Microsystems (Wetzlar, Germany)</td>
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and image-analysis such that assay results provide precise day-to-day and laboratory-to-laboratory results [44]. Without standardization, the use of TMAs and, thus, advanced assay technologies in the clinical laboratory will fall short.

**Assay development**

- **Quantitative optimization**

  Tissue-based assay optimization is typically performed using a few (<10) representative whole-tissue samples to ensure assay specificity and sensitivity. This is typically a subjective process where the operator decides, by eye, the optimal assay conditions. However, with the advent of TMAs coupled with quantitative and objective tissue-based methodologies, the ability to optimize assays objectively and quantitatively has been realized [Christiansen J et al. Quantitative and objective methodologies for immunohistochemistry assay development and quality control (2013), Manuscript in preparation]. Although this approach was developed using AQUA technology, it is applicable to all quantitative and robust image-analysis approaches. As discussed earlier, the distinct advantage of TMAs is the ability to array hundreds of different tissue samples on a single slide, with these hundreds of samples representing not only a wide range of analyte expression, but also a range of tissue types and histologies. The goal of any assay optimization is to maximize specificity and sensitivity; in this application we strive to maximize the dynamic range of the assay between signal (sensitivity) and noise/background (specificity) such that the assay is in the linear range for as many samples as possible. In much the same way that quantitative immunoassays such as ELISA are optimized, the use of TMAs together with quantitative assay approaches enable the same process to be performed in tissue.

  An idealized example is presented in Figure 1 where multiple assay conditions (i.e., antibody concentration for quantitative IHC assays) can be tested using multiple serial sections of the same TMA block [Christiansen J et al. Quantitative and objective methodologies for immunohistochemistry assay development and quality control (2013), Manuscript in preparation]. As TMAs represent a range of differentially expressing samples, a dynamic range can be calculated whereby the high-level expressing cases are compared with the low level or negative-expressing cases (red dashed line in Figure 1). By selecting the assay condition that provides the optimal dynamic range, it is ensured that the assay will provide the greatest resolution for quantitatively differentiating samples. Interestingly, the assay condition that provides the visually 'best' condition is different (typically 0.5–2 logs differential in assay concentration) to that for the quantitatively 'best' condition. Furthermore, depending on TMA design, the data obtained can be further used for disease characterization.

- **Performance & precision**

  Once assays have been objectively and quantitatively optimized, TMAs afford the ability to assess performance and precision of an assay since a population of samples can be assessed for day-to-day reproducibility with time and cost efficiency in a highly reproducible format. As discussed earlier, the future use of TMAs will be for quantitative assays rather than categorical type assays (i.e., traditional IHC) and, as such, assessment of performance and precision assessment of tissue-based assays can be achieved on a more rigorous and quantitative level. With continuous ‘scale’-type data, linear regression analysis can be performed for assessment of assay performance through comparisons of correlation coefficients and slope of the line. For assay precision, the percentage coefficient of variation can be determined across a single sample for multiple assays runs and days to provide an overall assay precision assessment. Performance and precision criteria can be established whereby assays can be qualified as strongly, moderately or weakly reproducing assays [Christiansen J et al. Quantitative and objective methodologies for immunohistochemistry assay development and quality control (2013), Manuscript in preparation] by assigning cut-offs to correlation coefficients (i.e., Pearson’s R >0.9 for strong performance), slope (i.e., 1.9–1.1 for strong performance) and the percentage coefficient of variation (i.e., <10% for strong precision).

- **Other TMA applications for assay validation**

  Tissue analytes are differentially susceptible to preanalytical variables that affect tissue-based assays. TMAs serve as great tools for assessment of analyte stability and provide an opportunity to develop novel biomarkers for assessment of the effects of preanalytical variables. TMAs can also be utilized for exploring the impact of different tissue processing and fixation conditions, in addition to analysis of inter-laboratory and observer variability [45–47]. Standardization of fixation conditions is imperative to ensure the highest quality and accurate assay data [48], and TMAs have been used to examine the effects of time to fixation [49,50]. This TMA tool can
be used to validate assays for analyte stability as a function of time to fixation, as well as provide the opportunity to develop normalization and/or go/no-go controls for clinical laboratory tissue samples, for general or specific assay requirements.

Furthermore, the quality of a TMA slide (or any FFPE sample slide) may decrease due to the time between FFPE sample sectioning and staining, which can result in potential false negatives [51–53], but not due to long-term storage of samples in paraffin blocks [54]. However, some studies have found that while the percentage of positive cases decreases, most associations were found in both fresh and older sections [55]. This also may impact analysis of mRNA in situ hybridization where there may be issues with sensitivity of the probe based on sample and/or section age, and RNA quality, but is less of a concern as far as analysis of DNA by FISH, since DNA is more stable, although protocol enhancements for TMAs may be utilized [56,57].

Quality assurance

For the same reasons TMAs are great tools for assay development and validation, they are starting to become well-used tools for clinical laboratory quality assurance [58–61] by providing a much simpler means to assess intra- and inter-laboratory reproducibility on both a qualitative (positive/negative) and potentially quantitative level. The College of American Pathologists [10] is implementing the use of TMAs for laboratory proficiency testing. A specific study by Fitzgibbons et al. [60] and highlighted by the College of American Pathologists demonstrates the use of TMAs to assess reproducibility of HER2 IHC staining and interpretation, as well as FISH testing across 243 laboratories. Each laboratory received 80 cases across eight TMA blocks to stain by HER2 IHC and interpret using their own standard procedures for determination of HER2 positive/negative status. The study showed that “70% of the samples had 90% concordance among laboratories, suggesting [TMAs] potential utility as consensus specimens for methodologic validation and controls” [60].

Another study by Terry et al. used TMAs as part of an implementation of the Canadian External Quality Assurance program for breast cancer testing [61]. The study included TMAs with 38 independent samples tested across 18 laboratories. The results of the study showed that laboratories had a strong positive agreement (>0.8 by kappa interobserver statistics) in 85% of cases. One of the advantages of TMAs, as pointed out by this study, is rapid testing turnaround time and bioinformatics (statistical analysis).

The limitation in using TMAs for quality assurance purposes in the clinical laboratory is that it does not provide the real-world variability of whole-tissue section (WTS) analysis since comparison of single cores usually only entails evaluation of a single 20× field-of-view (FOV), whereas WTS evaluation can sometimes have hundreds of FOVs for evaluation. Typically, a pathologist will not evaluate all FOVs for a given clinical specimen, thereby introducing variance in terms of laboratory-to-laboratory assessment of WTS. Thus, although TMAs are a great tool for overall assessment of laboratory performance for a given assay or test, proficiency testing may also need to be performed using WTS.

Next-generation applications of TMAs in the clinical laboratory

TMAs have been established as a robust and dynamic research tool, especially in the translational space, where the ability to assay hundreds of cases on a single slide is advantageous both in terms of time and cost savings, but also as an analytically superior method. Their use and adoption in the clinical laboratory, however, has
been quite limited, most likely due to TMAs not being sufficient for making a clinical diagnosis, since there is not enough tissue to ascertain contextual features of diseased tissue or to capture the heterogeneity within a sample. Nonetheless, by taking advantage of the reproducibility, as well as time- and cost-effectiveness, TMAs serve well as tools to assess a clinical laboratory’s overall performance with respect to specific assays.

However, further adoption of TMAs into the clinical laboratory is likely to be driven by and, to some extent, dependent upon, two main factors. First, the increasing need for and clinical utility of truly quantitative diagnostic tissue-based assays and, second, the adoption of digital pathology and robust objective image-analysis tools for assessment and quantification of assay results. Both of these factors have been reviewed here as drivers for TMA use due to the necessity for cost- and time-effective robust assay and imaging controls.

- **TMAs as quantitative assay controls in the clinical laboratory**

As tissue-based assays become more quantitative and as these assays begin to be adopted into the clinical laboratory for objective assessments, there will be a critical need for quantitative assay controls as differentiated from qualitative controls (simply positive/negative). As outlined above, a distinct advantage of TMAs is that they provide a methodology for the assessment of tens to hundreds of samples that represent a wide range of expression and/or pathological/clinical characteristics. In order to control for a quantitative assay, a wide range of expression levels will need to be analyzed. A simple positive or negative control will not suffice for a truly quantitative assay. For example, for immunoassays such as ELISA, a range of controls is used to establish a standard curve. As a first approximation, TMAs of small sample size representing a range of biomarker expression could be used as simple quantitative assay controls (Figure 2). The TMA could be placed on the actual clinical sample slide to not only serve as an assay control, but also as a reagent control to ensure equivalent and appropriate treatment of the individual clinical sample. The TMA samples could either be tissue or clonal cell lines. The advantage of clonal cell lines is the homogeneity of expression compared with tissue. Additionally, the use of pure protein is also a viable option.

This approach is outlined in Figure 2. As one level of control, these TMAs could serve as go/no-go decision points for the assay and/or individual clinical samples. Based on established criteria (e.g., correlation coefficient and slope of the line) compared with a reference standard, a decision could be made with respect to the validity of the assay. Two idealized examples are provided in Figures 2B & 2C as successful and failed assay runs, respectively. Notice for the successful assay, the correlation coefficient and slope both approach 1, indicating strong performance of the assay with respect to established data. However, for the failed control, although the correlation coefficient approaches 1, the slope of the line is 0.5, indicating a fundamental assay performance error, resulting in much higher scores than expected. The use of the clinical sample data could thus result in a false positive. As a second level of control and in the absence of a standardized imaging platform, this type of control could also be used as a normalization control.

![Figure 2. Using tissue microarrays as assay controls for clinical sample testing.](image)
whereby the equation of the line could be used to normalize the clinical sample score to a reference standard, provided the performance of the assay was within acceptable limits.

**TMAs as quantitative calibration controls in the clinical laboratory**

The final level of control for tissue-based assays would be a control that enabled calibration to an absolute concentration of the analyte based with respect to a gold standard. In tissue, directly obtaining an *in situ* absolute level of an analyte is theoretically not possible without grinding the tissue up to perform an *in vitro* assay such as an ELISA. However, by coupling tissue and/or cell-line TMAs with *in vitro* assays such as ELISA, western blot or reverse transcriptase-PCR, a calibration or standard curve could be derived.

**Figure 3. Example of using cell lines and tissue microarrays to construct a standard curve for the conversion of relative expression scores into absolute concentrations.** (A) Depicts correlating western blot analysis of protein concentrations in cell lines with (C) quantitative immunofluorescence of cell lines in tissue microarray format (AQUA® scores [y-axis] and pg ER/µg total protein [x-axis]). With this correlation, an equation was derived, allowing for the conversion of tissue-based AQUA scores into protein concentrations. (D) Using test arrays, the lower limit of quantification was determined at 2 pg ER/µg total protein. (E) Visual confirmation of lower limit of quantification. Scale bars represent 100 µm. CK: Cytokeratin; DAPI: 4',6-diamidino-2-phenylindole; ER: Estrogen receptor; rER: Recombinant estrogen receptor. Reproduced with permission from [62].
such that assay scores from individual clinical tissue samples could be converted to absolute concentration or levels. Regarding blood-based clinical assays (i.e., glucose or cholesterol), there is an expectation of not only obtaining a quantitative objective number (i.e., concentration), but also that this number is commonly understood and standardized. This is not the case currently with tissue-based assays; however, the future of clinical tissue assays and TMAs could be the means to drive this achievement.

This concept was demonstrated by Welsh et al. (Figure 3) [62] and outlined in Figure 4. Welsh et al. used commercially available and engineered cell lines with increasing calibrated concentrations of estrogen receptor (a critical biomarker for the effective treatment of breast cancer [63]). With known concentrations (as determined by quantitative western blotting; Figure 3A), expression scores for these cell lines were calculated using AQUA technology in conjunction with a cell-line TMA (Figure 3B) and subsequently correlated to absolute protein concentration to derive a standard curve with a conversion equation (Figure 3C). Welsh et al. went on to determine the lower limit of quantification using a test series of tissues (Figure 3D) and validated their findings with respect to breast cancer outcome.

Conclusion
TMAs are a potentially valuable tool that have grown in popularity over the last two decades, especially in preclinical and translational research. Clinical adoption of TMAs will occur on three levels. The first level is for assay development and validation, where TMAs can be used as initial tools to optimize and validate clinically relevant assays. The second level is quality assurance where TMAs are starting to be used to assess intra- and inter-laboratory assay reproducibility. The third and final level will be development of robust and quantitative assay controls. Driving clinical adoption of TMAs will depend on the development of more quantitative tissue-based assays that are clinically relevant, as well as adoption of digital pathology and objective image-analysis tools. Taken together, the use of TMAs in the clinical laboratory will become ever more valuable in the future as the science and technology of tissue-based assays progresses.

Future perspective
As TMAs have become the predominant tool in the translational and clinical research laboratory for high-throughput assessment and interpretation of biomarker expression, we anticipate that TMAs will become more prevalent in the clinical laboratory space. This will coincide with the development of quantitative in situ assays and technologies that require more quantitative-type controls that mimic the real-world clinical sample or tissue. It is this use that will enable TMAs to gain prominence in the clinical laboratory. Additionally, if measuring analytes in situ is to become like measuring analytes in blood or serum, sample-relevant quantitative calibrators will also have to be developed and the easiest medium for this development will be TMAs. Furthermore, due to their ease and efficiency of use, TMAs should gain adoption even for simpler assays (i.e., chromogen-based IHC) as laboratory-to-laboratory quality control tools that will enable the assurance of reproducible clinical results critical for the accurate diagnosis of patients.

Financial & competing interests disclosure
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Tissue microarrays (TMAs) have become ubiquitous in the research setting for high-throughput in situ biomarker analysis due to the ability to analyze hundreds of samples on a single slide with cost- and time-effectiveness. Development of digital imaging and image analysis have allowed for improvements in biomarker assessments in the translational setting. Clinical diagnostic use of TMAs is limited due to the limited sample size; however, they have been used for quality assurance purposes in the clinical setting, such as inter- and intra-laboratory concordance.

**TMA applications**

- Methods that can be applied to whole-tissue sections are applicable to TMAs including DNA-, RNA- and protein-based assessments.
- The most prevalent use of TMAs is large-scale cohort analysis of biomarker expression in the context of pathological characteristics and/or clinical outcome.
- TMAs are subject to the same limitations of other sample analyses with regard to variable preanalytic conditions of the samples used.

**The requirement for automated & objective image analysis tools**

- Further adoption of TMAs into the clinical setting will likely rely on objective analysis to reduce subjectivity and to produce time-effectiveness, as well as to provide standardization and precise results.
- There are a variety of image analysis software programs available for brightfield as well as fluorescence applications for TMA analysis; image analysis is typically performed using methods of manual identification, feature- or contextual-based compartmentalization using morphological features, and/or differentiation of specific regions/compartments of interest.

**Assay development**

- Optimization of immunohistochemistry assays has typically been a subjective, manual process, which can be improved using objective and quantitative approaches with TMAs.
- Assessment of assay reproducibility can be performed rapidly and efficiently using TMAs for intra- and inter-laboratory comparisons.

**Quality assurance**

- TMAs have been adopted for use in clinical laboratory proficiency testing by organizations such as the College of American Pathologists and the Canadian External Quality Assurance program for breast cancer testing.
- Limitations to TMAs do exist as they lack heterogeneity seen in whole-tissue sections, yet they serve as a useful tool for laboratory assessment purposes.

**Next-generation applications of TMAs in the clinical laboratory**

- Further adoption of TMAs into the clinical laboratory is likely to be driven by adoption of both digital pathology and/or the use of quantitative tissue-based assays.
- TMAs can serve as useful tools for quantitative assay controls in the future due to their ability to represent a wide range of biomarker expression and/or tissue characteristics on a single slide.
- Use of TMAs with concentration-based controls would bring further objectivity and standardization to tissue-based biomarker assays.

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Papers of special note have been highlighted as:
- of interest
- **of considerable interest**


Describes the importance of digital

**Reviews the use of TMAs as tools for the
development and validation of predictive
biomarkers.**


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