Recent advances in the field of human molecular genetics have revealed gene-based disease mechanisms in many areas of medicine. The study of new prognostic and diagnostic markers in large numbers of clinical specimens is an important step in translating the new findings from basic science to clinical practice.

The investigation of the pathogenesis and progression of diseases such as cancer has been revolutionized with the increased use of new molecular biology techniques. Elucidating the fundamental molecular mechanisms that are involved in the stepwise progression from normal tissues to malignant tumors is essential in our knowledge of cancers, and should ultimately lead to improved methods of detection, treatment, and cures for cancers.

Studies on clinical tissue have identified multiple novel markers, primarily at the gene level. The validation of these markers using the standard histopathological techniques is time consuming, and labor intensive and costly, particularly when multiple markers are tested on numerous specimens.

Tissue microarray is a recent innovation in the field of pathology. A microarray contains many small representative tissue samples from hundreds of different cases assembled on a single histologic slide, and therefore allows high throughput analysis of multiple specimens at the same time. Tissue microarrays are paraffin blocks produced by extracting cylindrical tissue cores from different paraffin donor blocks and re-embedding these into a single recipient (microarray) block at defined array coordinates. Using this technique, up to 1000 or more tissue samples can be arrayed into a single paraffin block. It can permit simultaneous analysis of molecular targets at the DNA, mRNA, and protein levels under identical, standardized conditions on a single glass slide, and also provide maximal preservation and use of limited and irreplaceable archival tissue samples. This versatile technique, in which data analysis is automated, facilitates retrospective and prospective human tissue studies. It is a practical and effective tool for high-throughput molecular analysis of tissues that is helping to identify new diagnostic and prognostic markers and targets in human cancers, and has a range of potential applications in basic research, prognostic oncology and drug discovery. This article summarizes the technical aspects of tissue microarray construction and sectioning, advantages, application, and limitations.
ported 20 years ago by Battifora who described a “sau-
sage block” method in which he wrapped 1 mm thick
‘rods’ of different tissues in a sheet of small intestine
which was then embedded in a paraffin block and
from which numerous sections were cut and exam-
ined.10 The array format was first conceived by Wan
and colleagues in 1987.11 Although this technique
confers the significant advantage of simultaneously
examining multiple tissue specimens under identical
conditions, the inability to satisfactorily identify in-
dividual ‘rods’ limited any meaningful interpretation.
These limitations were addressed subsequently, and in
1998, Kononen et al invented a device for rapid and
accurate construction of tissue microarrays in a man-
er that is easily accessible to most pathology labs. The
invention of this device and its commercialization led
to a dramatic increase in the popularity and utility of
the technique.12

Tissue microarray technique
Microarray is a technique for organizing minute
amounts of biological samples on a solid support.13
Tissue microarrays are composite paraffin blocks con-
structed by extracting cylindrical tissue core “biopsies”
from different paraffin donor blocks and re-embed-
dding these into a single recipient (microarray) block
at defined array coordinates.14,15 At first, the donor
blocks (invariably stored paraffin blocks) are retrieved
and sectioned to produce standard microscopic slides
that are stained with hematoxylin and eosin. An ex-
perienced pathologist examines the slides to mark the
area of interest, which is commonly an area of cancer
(Figure 1), after which the samples can be arrayed.4

A tissue microarray instrument (for example,
Beecher Instruments, Winsconsin, USA, www. beecher-
insstruments.com) is used to acquire a tissue core from
the donor block.16 This core is then placed in an empty
paraffin block—the recipient block (Figure 2).16 The
current Beecher Instruments arraying device is designed
to produce sample circular spots that are 0.6 mm in di-
ameter at a spacing of 0.7–0.8 mm.17 The surface area
of each sample is 0.282 mm², or in pathologists’ terms,
about the size of 2–3 high power fields. The number of
spots on a single slide is variable depending on the array
design; the current comfortable maximum with the 0.6
mm needle is about 600 spots per standard glass micro-
scope slide.18 The core is placed at a specifically assigned
coordinate (X-Y guide), which is accurately recorded,
typically on a spreadsheet, such as Microsoft Excel.4 The
sampling process can then be repeated many times
from different donor blocks until hundreds, or even
thousands, of cores are placed into one recipient block,
producing the final tissue microarray block (Figure 2).
Using a microtome, 5 µm sections are cut from the tis-
te microarray blocks to generate tissue microarray
slides for molecular and immunohistochemical analyses
(Figure 3).16

New technologies are under development that may
allow as many as 2000 or more sections per slide. Using
this method, an entire cohort of cases can be analyzed
by staining just one or two master array slides, instead
of staining hundreds of conventional slides.18 Yet each
spot on the array is similar to a conventional slide in
that complete demographic and outcome information is
maintained for each case so that rigorous statistical an-
alysis can be done as rapidly as the arrays are analyzed.15

Advantages and applications of tissue microarrays
There are numerous advantages of tissue microarray
over standard techniques, including:

Amplification of a scarce resource. A standard histo-
logic section is about 3–5 mm thick, with variation de-
pending on the submitting pathologist or technician.
After use for primary diagnosis, the sections can be cut
50–100 times depending on the care and skill of the sec-
tioning technician. Thus, on average, each archived block
might yield material for a maximum of 100 assays.13 If
this same block is processed for optimal microarray con-
struction it could routinely be needle biopsied 200–300
times or more depending on the size of the tumor in the
original block. Once tissue microarrays are constructed,
they can be judiciously sectioned to maximize the number of sections cut from an array. The sectioning process uses a tape-based sectioning aid (from Instrumedics Inc., Missouri, USA, www.instrumedics.com) that allows cutting of thinner sections. Optimal sectioning of arrays is obtained with about 2-3 µm sections. Thus, instead of 50-100 conventional sections or samples for analysis from one tissue biopsy, the microarray technique could produce material for 500,000 assays (assuming 250 biopsies per section times 2000 2.5-µm sections per 5 mm array block) represented as 0.6 mm disks of tissue. Thus this technique essentially amplifies (up to 10,000 fold) the limited tissue resource.\(^{15}\)

**Simultaneous analysis of very large numbers of specimens.** Tissue microarrays provide high throughput data acquisition. For instance, if a tissue microarray block containing 1000 cores is cut 200 times, this allows for 200,000 individual assays.\(^{19,20}\)

**Experimental uniformity.** With this technology, each tissue sample is treated in an identical manner and microarrays are amenable to a wide range of techniques, including histochemical stains, immunologic stains with either chromogenic or fluorescent visualization, in situ hybridization (including both mRNA ISH and FISH), and even tissue micro-dissection techniques. For each of these protocols, conventional procedures can have substantial slide-to-slide variability associated with processing 300 slides (for example, 20 batches of 15 slides). Tissue microarray allows the entire cohort to be analyzed in one batch on a single slide. Thus, variables such as antigen retrieval, temperature, incubation times, washing procedure, and reagent concentration are standardized for the entire cohort.\(^{21-23}\)

**Decreased assay volume, time and cost.** As only a very small amount of reagent (a few µL) is required to analyze an entire cohort, less laboratory personnel are required to perform the experiments. This method has proven to be extremely efficient, of shorter duration, and cost effective, especially with expensive reagents.\(^4\) This advantage raises the possibility of use of tissue microarrays in screening procedures.

**Does not destroy original block for diagnosis and thus conserves valuable tissue.** There are occasions where the original block must be returned to the patient or donating institution. In these cases the block may be cored a few times without destroying the original tissue block. Then, upon subsequent sectioning, it is still possible to make a diagnosis, even though tissue has been taken for array-based studies.

Tissue microarray has proved to be an effective and efficient tool for assessing quality assurance programs such as intra- and interlaboratory variation in immuno-histochemical and molecular studies. A tissue microarray block can be created from numerous tissue specimens, then sectioned and unstained slides distributed to different laboratories, with each laboratory doing immunostains or molecular tests. Thus, tissue microarray can facilitate the standardization of immunohistochemical, fluorescence in situ hybridization, and other molecular assays, so that results are reproducible between laboratories. The tissue microarray also can be used in internal quality control (eg, quarterly or monthly) to ensure that there is no drift of staining results or reporting within a laboratory. It can also be used for optimization of diagnostic reagents such as monoclonal antibodies and gene probes.\(^5\) It is believed that the tissue microarray is an improvement over the current practice of using a single strong positive control for quality.\(^{24-26}\)

**Tissue microarray facilitates rapid translation of molecular discoveries to clinical applications.** The technique has been applied to tumor research (gliomas, breast tumors, lung cancer among others). The development of novel biochip technologies has opened up new possibili-
Tissue microarray is a practical and effective tool for high-throughput molecular analysis of tissues that is helping identify new diagnostic and prognostic markers and targets in human cancers. It has varying degrees of research use and offers a range of potential applications.
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