# Tissue Microarray (TMA)

# 5

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## Contents



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## 5.1 Introduction and Purpose

The Tissue MicroArray procedure was developed by Kononen et al. [1] 10 years ago as a high-throughput tool to investigate a variety of biomarkers on tissue specimens [2]. To construct a TMA, small cores of tissues punched from donor paraffin blocks are transferred into an empty recipient block in arrayed fashion. Using these cores, samples from hundreds of different tissues or patients can be arrayed in a single paraffin block and analyzed by immunohistochemistry, in situ hybridization, or immunofluorescence.

As a research tool, TMAs are used predominantly for the simultaneous investigation of putative prognostic and predictive molecular targets in human cancer tissues [3]. They are also used for the in situ validation of candidate diagnostic markers identified in genomics and proteomics studies [4–7] and for the correlation of staining results with clinical endpoint [8, 9]. Moreover, the so-called progression TMAs are possible, in which cores of a single tissue type are used taking into account different stages of tumor development or different tumor grades. For example, a progression TMA for colon cancer could include normal colon, adenomas, with low and high-grade of dysplasia, as well as carcinomas [10].

TMAs can be also used in experiments aimed to determine whether a protein is expressed or not and to what extent in a wide range of different normal and/or lesional tissues. In addition, this technique can complement other proteomic methods with the advantage of studying the expression pattern of a specific protein with respect to cell compartments.

G. Stanta (ed.), *Guidelines for Molecular Analysis in Archive Tissues*,
DOI: 10.1007/978-3-642-17890-0_5, © Springer-Verlag Berlin Heidelberg 2011

### 5.1.1 General Considerations Regarding Tissue Microarray Design and Construction

Before sampling the tissues, it is fundamental to define in advance the questions that will be addressed by staining TMAs as it will have an impact on the sampling itself [11]. For example, if the main goal is to characterize the general expression pattern of a specific protein in a tissue, the sampling would be random; if the aim is to compare the expression of a protein between the lesional and the perilesional region of a tumor, the sampling would be done from the right area.

A potential problem in the construction of a TMA is the tissue heterogeneity, resulting in different gene and protein expression in different normal and/or tumor cells. The best way to overcome this intervariability of expression of the target is to take multiple cores of each area of interest from the same sample. Currently no sampling methods are available for standardization, but it seems intuitive that the more samples are taken, the more representative the staining results. Many studies seem to indicate that the results from triplicate TMA cores have up to 98% concordance with the result from full sections [12, 13]. A study by Goethals et al. [14] recommends at least four cores, whereas other authors have achieved more than 95% accuracy with only two cores [15]. There are technical reasons too for including more than one core for each case: some cores could be lost, e.g., during the sectioning. Moreover, in the case of low tumor cell density, doubling the number of cores per case could be necessary.

A particular point of debate refers to the core diameter with respect to tissue sampling. Most tissue arrayer instruments punch with a diameter between 0.6 and 2 mm, which is equivalent to a tissue area of 0.29–3.14 $mm^2$. Most of the published studies used the 0.6 mm diameter punches, as it allows preserving more source tissue and including a larger number of cores into the recipient block. Punches with a diameter of more than 0.6 mm are useful for tissues containing a large amount of fatty or connective component or for the study of the whole mucosa thickness, e.g., in the stomach.

Needles with a diameter of 3–5 mm allow a more rapid and precise manual microdissection of large tissue areas or cells of interest (see Chaps. 4 and 6). In this case, the tissue cores can also be used for molecular analyses, such as those performed in some IMPACTS laboratories. Once punched, the single cores are embedded in a new paraffin block, sectioned, and used for DNA, RNA, and protein extraction.

## 5.2 Protocol

### 5.2.1 General Considerations Regarding the Layout of Tissue Microarray

Although methods for the layout of a TMA have not been standardized so far, probably because different studies have different requirements, the following components seem to be essential in the TMA design.

To ensure unambiguous orientation and identification of individual cores within the TMA section, it is convenient to add one or more "orientation cores" in a specific position, generally outside the overall geometric margin of the array. Some researchers opted for the introduction of gaps; this empty core positions may help to orient the TMA without any confusion or doubts.

Control tissue cores can be also included in the array, placing them asymmetrically into the grid. These cores may serve both as internal "orientation cores" and as positive or negative internal experimental controls.

The arrangement of the interest cores within the TMA varies according to the type of study and each operator has to identify which design best suits his or her purposes. Ideally cores from the same donor block should not be positioned next to each other, since only random distribution ensures that results from individual cores are not statistically affected by technical blunders. However, random distribution of cores from the same donor block increases the workload and therefore becomes time consuming [11].

Because it is known that immunohistochemistry analyses on full tissue sections may show some staining artifacts at the tissue border, some researchers frame all the TMA with a "protection wall," a row of tissue cores that will not be subsequently analyzed [12].

## 5.2.2 Equipment

- *TMA Arrayer Instrument*[1]
- *Software interface* for the TMA design
- *Set of two punches of the preferred diameter*: one for the donor block and another one for the recipient block
- *A freshly poured empty paraffin block* (recipient block) of the desired size[2]
- *H&E slides* of the cases of interest on which the desired area for the sampling is marked
- *Donor blocks* of the cases of interest
- *Oven*
- *Glass slides*
- *Microtome*

## 5.2.3 Method

### 5.2.3.1 Array Design: Preparation of the Array Pattern

- Using specific software, define the geometrical parameters of the array: the paraffin block size, the needle diameter, the number of spots, and the distance between the spots. If necessary, rows, columns, or groups of empty spots can be introduced to separate the specimens.
- At this point, each donor block is linked to one or more TMA spots in order to create the map that will be followed during the TMA construction. It is sometimes possible to add a note for each donor block, recipient block, or spot that will be present in the final report.

### 5.2.3.2 TMA Construction: Allocation of Samples in the Array

- In this phase, the software facilitates the completion of the TMA construction. The digital camera is connected, and the homing of the automatic tray[3] and of the needle holder is performed.
- Usually, an operating flowchart guides every step of TMA construction. The steps include:
  1. Definition of the center of the tray.
  2. Insertion of the recipient block.
  3. Checking of the TMA template directly on the recipient block. If the position of the spots is not correct, it can be modified by moving the tray.
  4. Insertion of the first donor block.
  5. Choice of the method for the selection of punch area.[4]
  6. Selection of the punch areas.
  7. Sampling:
     - Prepare the hole in the recipient block
     - Take the sample from the donor block
     - Insert the sample in the prepared hole[5]
- Create the final report of the array in an Excel spread-sheet.

### 5.2.3.3 Assembling of the TMA

- Once completed, the TMA is placed upside down on a glass slide and into an oven at 40°C for about 30 min to facilitate binding of the donor cores with the paraffin wax of the block itself.
- The glass slide, attached to the TMA block, is used to level the block surface by gently pushing the cores into the block.
- After cooling, the block is ready for sectioning on a microtome[6] and for further analyses (Fig. 5.1).

[1]The methodology described here refers to the Galileo TMA CK 3500 Tissue Arrayer and to the IseTMA Software, from the Integrated System Engineering S.r.l. (Galileo TMA CK 3500 Tissue Microarrayer- Operating Manual) for the direct experience of the authors. Other valid arrayers are commercially available, such as the Veridiam Tissue Arrayer, the TMArrayer by Pathology Devices, and others.

[2]Air bubbles may be accidentally created within the paraffin block during the pouring and cooling procedures especially when metal molds are used; the formation of air bubbles can be minimized by using plastic molds.

[3]The automated tray ensures the precise positioning of the paraffin blocks while it is in use.

[4]The overlapping can be manual or digital. In manual overlapping, the reference glass slide is overlapped to the corresponding donor block and manually aligned with it looking at the monitor. In digital overlapping, the digital image of the reference slides is overlapped and aligned to the live donor block image.

[5]Repeat "Selection of the punch areas" and "Sampling" for each donor block, according to the design plan.

[6]The TMA block should be cut only by expert technicians as the TMA section needs to be cut and picked up from the hot water bath with great care to avoid distortion prior to aligning it in parallel with the edge of the glass slide to facilitate analyses.

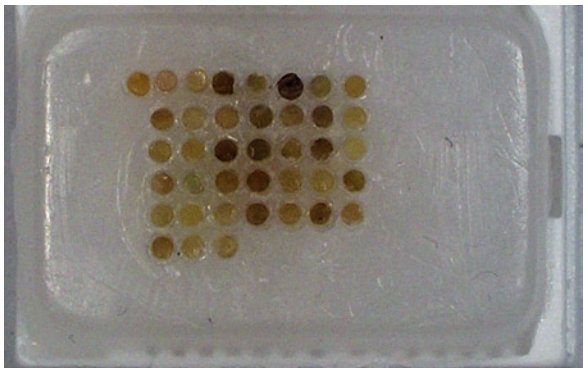

**Fig. 5.1** Representative picture of a TMA block in which 1.5 mm cores have been inserted from selected donor blocks

#### 5.2.3.4 Evaluation of the Results

Generally, TMA sections are read serially using a conventional light microscope. However, keeping track of the position of each core can sometimes become very difficult when many spots are present in one slide. Open source types of software have been developed and are available, for example from Stanford University (http://genome-www.stanford.edu/TMA/) and Johns Hopkins University, (http://tmaj.pathology.jhmi.edu/) to make digital scanning and image for the evaluation of the results.

## References


1. Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G, Kallioniemi OP (1998) Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 4(7): 844–847
2. Yan P, Seelentag W, Bachmann A, Bosman FT (2007) An agarose matrix facilitates sectioning of tissue microarray blocks. J Histochem Cytochem 55(1):21–24
3. Egervari K, Szollosi Z, Nemes Z (2007) Tissue microarray technology in breast cancer HER2 diagnostics. Pathol Res Pract 203(3):169–177
4. Bubendorf L, Kolmer M, Kononen J, Koivisto P, Mousses S, Chen Y, Mahlamaki E, Schraml P, Moch H, Willi N, Elkahloun AG, Pretlow TG, Gasser TC, Mihatsch MJ, Sauter G, Kallioniemi OP (1999) Hormone therapy failure in human prostate cancer: analysis by complementary DNA and tissue microarrays. J Natl Cancer Inst 91(20):1758–1764
5. Dube V, Grigull J, DeSouza LV, Ghanny S, Colgan TJ, Romaschin AD, Siu KW (2007) Verification of endometrial tissue biomarkers previously discovered using mass spectrometry-based proteomics by means of immunohistochemistry in a tissue microarray format. J Proteome Res 6(7):2648–2655
6. Hewitt SM (2006) The application of tissue microarrays in the validation of microarray results. Meth Enzymol 410:400–415
7. Skacel M, Siva A, Xu B, Tubbs RR (2007) From array to array: confirmation of genomic gains and losses discovered by array-based comparative genomic hybridization utilizing fluorescence in situ hybridization on tissue microarrays. J Mol Histol 38(2):135–140
8. Kallioniemi OP, Wagner U, Kononen J, Sauter G (2001) Tissue microarray technology for high-throughput molecular profiling of cancer. Hum Mol Genet 10(7):657–662
9. Venkataraman G, Ananthanaranayanan V (2005) Tissue microarrays: potential in the Indian subcontinent. Indian J Cancer 42(1):9–14
10. Chen WC, Lin MS, Zhang BF, Fang J, Zhou Q, Hu Y, Gao HJ (2007) Survey of molecular profiling during human colon cancer development and progression by immunohistochemical staining on tissue microarray. World J Gastroenterol 13(5):699–708
11. Parsons M, Grabsch H (2009) How to make a tissue microarray. Diagn Histopathol 15(3):142–150
12. Hoos A, Cordon-Cardo C (2001) Tissue microarray profiling of cancer specimens and cell lines: opportunities and limitations. Lab Invest 81(10):1331–1338
13. Sapino A, Marchiò C, Senetta R, Macrì L, Cassoni P, Ghisolfi G, Cerrato M, D'Ambrosio E, Bussolati G (2006) Routine assessment of prognostic factors in breast cancer using a multicore tissue microarray procedure. Virchow Arch 449(3):288–296
14. Goethals L, Perneel C, Debucquoy A, De Schutter H, Borghys D, Ectors N, Geboes K, McBride WH, Haustermans KM (2006) A new approach to the validation of tissue microarrays. J Pathol 208(5):607–614
15. Camp RL, Charette LA, Rimm DL (2000) Validation of tissue microarray technology in breast carcinoma. Lab Invest 80(12):1943–1949