Alpha Metrix

MANUAL TISSUE ARRAYERS MTA-1 AND MTA-2

Individual Microgenomic Solutions

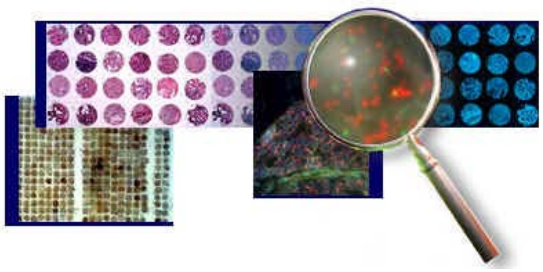
What are Tissue MicroArrays

Tissue microarrays are a method of relocating tissue from conventional histologic paraffin blocks such that tissue from multiple patients or blocks can be seen on the same slide. This is done by using a needle to biopsy a standard histologic sections and placing the core into an array on a recipient paraffin block. This technique, originally described by in 1987 by Wan, Fortuna and Furmanski in Journal of Immunological Methods. They published a modification of Battifora's "sausage" block technique whereby tissue cores were placed in specific spatially fixed positions in a block. The technique was popularized by Kononen and colleagues in the laboratory of Ollie Kallioneimi after a publication in Nature Medicine in 1998. This technology should not be confused with DNA microarrays where each tiny spot represents a unique cloned cDNA or oligonucleotide. In tissue microarrays, the spots are larger and contain small histologic sections from unique tissues or tumors.

What are the Advantages?

There are numerous advantages to this technology including:

- amplification of a scarce resource,
- experimental uniformity
- decreased assay volume.
- does not destroy original block



A standard histologic section is about 3-5mm thick, with variation depending on the submitting pathologist or tech. After use for primary diagnosis, the sections can be cut 50-100 times depending on the care and skill of the sectioning technician. Thus, on average, each archived block might yield material for a maximum of 100 assays. If this same block is processed for optimal microarray construction it could routinely be needle biopsied 200-300 times or more depending on the size of the tumor in the original block (Theoretically it could be biopsied 1000's of times based on calculations of area, but empirically, 200-300 is selected as a conservative estimation) Then, once tissue microarrays are constructed, they can be judiciously sectioned in order to maximize the number of sections cut from an array. The sectioning process uses a tape-based sectioning aid (from Instrumedics Inc.) 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 5mm array block) represented as 0.6 mm disks of tissue. Thus this technique essentially amplifies (up to 10,000 fold) the limited tissue resource.

MTA-2

Using this technology, each tissue sample is treated in an identical manner.

Like conventional formalin-fixed paraffin embedded material, tissue 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 sections can have substantial slide to slide variability associated with processing 300 slides (for example, 20 batches of 15 slides). The tissue microarrays allow the entire cohort to be analyzed in one batch on a single slide. Thus reagent concentrations are identical for each case, as are incubation times and temperatures, wash conditions, and antigen retrieval, if necessary.

Another significant advantage is ,that only a very small (a few µl) amount of reagent is required to analyze an entire cohort. This advantage raises the possibility of use of tissue microarrays in screening procedures (for example in hybridoma screening), a protocol that is impossible using conventional sections. It also saves money when reagents are costly.

MTA-1

Finally, 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 block. Then upon subsequent sectioning, it is still possible to make a diagnosis, even though tissue has been taken for array-based studies.

Building a Tissue Array

General Principle

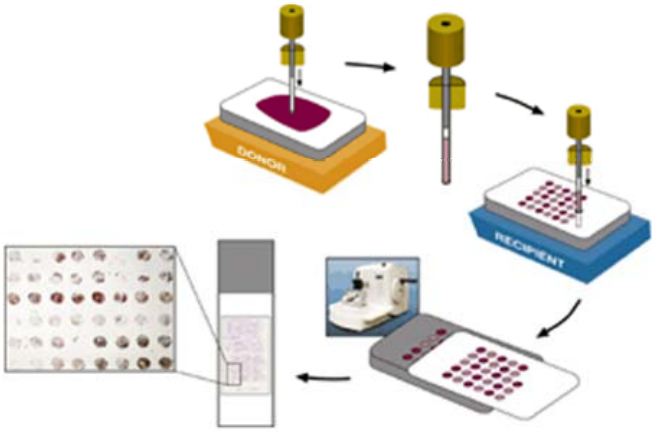
In a virgin paraffin block, named recepient block, a cylindrical hole is made with the Tissue Arrayer.

A core is sampled in the sample block, named donor block, at the point of interest. This sample core is transfered into the recepient block and inserted into the hole made before.

By repeating this operation from many various tumor blocks, you build the Tissue Array block.

The Tissue Array block may count few tens up to a thousand of sample cores, upon core size and experiment requests.

The Tissue Array block, once completed, can be cut on a standard microtome as with any sample paraffin block. Each section, fews microns thick, includes all the sample cores in slice. The Tissue Array slice can be transferred and mounted on a microscope slide to be stained along standard procedures like HES, immunostains, FISH or PCR



Experiment plan preparation

It is needed to have replicates in the block. Indeed, through the many steps of the procedure, cores can be lost. Replicates guarantee that user will have at least one core which can be explored. Moreover, pathogenic or normal tissues are frequently heterogeneous.

Organization of the Tissue Array on the slide is function of the number of different samples to be analyzed simultaneously, the number of replicates and the tissue array analysis method used.

A manual analysis will be facilitated with a block organization where blocks gather only few spots like shown here

Biopsy	Position	X	Y	Grade
125322	A1	0,000	0,000	
215563	A2	0,700	0,000	
15665	A3	1,400	0,000	
1236565	A4	2,100	0,000	
51651	A5	2,800	-0,700	
3565	B1	0,000	-0,700	
31256	B2	0,700	-0,700	
14785	B3	1,400	-0,700	
21669	B4	2,100	-0,700	

beside to help users orientating him self into the Array.

The building of the Tissue Array is also facilitated by the use of a table prepared before starting the building and detailing : sample ID, sample spot position (A1, A2, …C3…), X and Y corresponding coordinates in microns. Additional columns may be used to allow results data input.

Selecting the samples,locating the zone of interest

Tissue Array can be generated as well from archive blocks as from newly made blocks.

The technique has been validated on paraffin embedded tissues. Recent works have shown validity of the principle on frozen tissues as well however still to be improved.

The donor block, if new, must first be cut until the level of interest is reached.

The punching zones are located and marked (with a pen for example) under the microscope on a freshly prepared HES slide (or other staining).

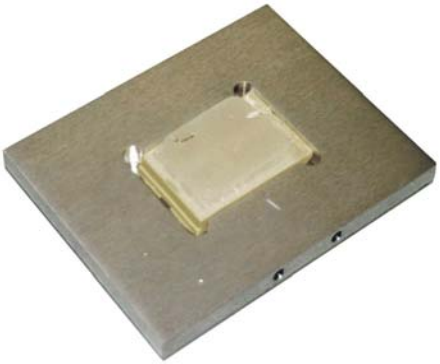
Donor Block

The slide is then superimposed to the donor block to approximately locate the zones to punch.

Small tumors or more precise punchings on cell groups fro example may require more precautions.

Preparing the recipient block

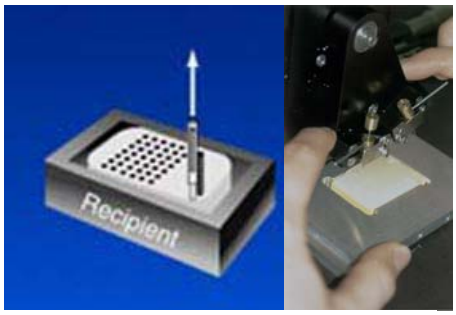
The Tissue Array is build in a virgin paraffin block. The block can be tracked by a barcode label on the cassette.



Recipient block in its rack

Following the experimental plan and with the micrometers on the Tissue Arrayer, the user can precisely position the puncher. A recipient hole is realized. Usually, for precision reason, recipient holes are made one by one. Removable racks for recipient blocks allows the building of many Tissue Array blocks simultaneously, either multiple users, either different samples...

The recipient block racks are made of solid stainless steel and include screws to firmly fix the recipient block.



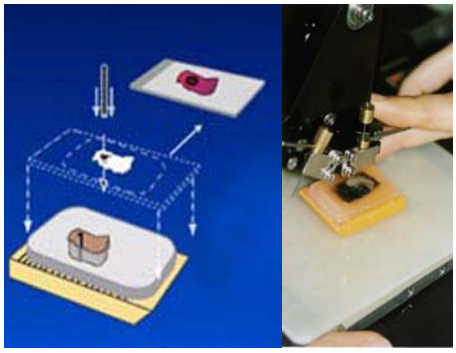
Punching into the recipient block

Sampling the core into the sample

The donor block is placed on a specific donor block bridge rack that allows the user to place the donor block right undernieth the puncher while still leaving the recipient block in place. A score is sampled into the block at the point located through the HES slide observation. The core is taken away by the puncher.

The Tissue Arrayer allows you to sample cores which sizes can be 600, 1000, 2000 or 4000 µm. Larger the core, the more it is informative however the less you can put onto a slide.

600 µm is the most frequently used size because it allows high numbers of samples while still preserving histological significance.

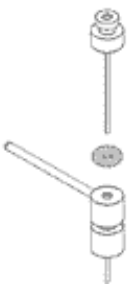


Selection of core

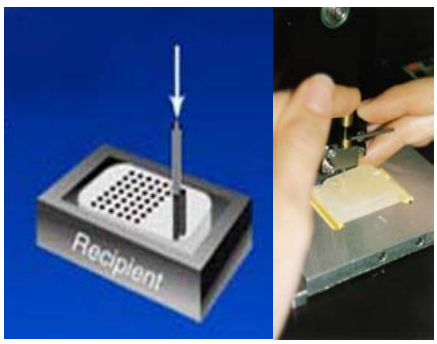
Transfering the sample core into the recipient

The sample puncher is pushed down close to the block surface, rightup the hole made before.

The micrometers ensure precise positioning of the assembly. Once the alignment is visually verified, the core is pushed out of the puncher into the hole with the stylet. The operation is repeated for each new sample core.



Once completed, the Tissue Array block is warmed up at 37°C for 10 to 15 minutes to allow the cores and the paraffin walls to get more cohesive for easier sections cutting and cores recovery.



Transfer of core

Transfer of the Tissue Array section and staining

The Tissue Array block can be classically cut into sections of a few microns on a standard microtome and afterwards mounted on superfrost slides for good adherence of the tissue cores.

Users frequently observe that while cutting Tissue Arrays blocks, cores are folded or even not cut properly and losses of up to 20 % are not rare.

Using an adhesive PSA tape eases tissue cores recovery and significantly decreases the losses while improving tissue array section geometry on the slide which facilitates further analysis under the microscope or by the imaging system Spot Browser.

Once the tissue array section is transferred to the slide, it can be stained with various standard protocols like HES, IHC, ISH and FISH without special precautions.

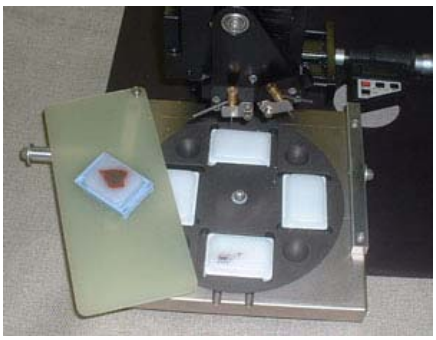
Depending on Tissue Array block thickness, the preparation of multiple similar slides from the same block enables large multi-parameter studies.


Tissue Array transfer to the slide

The must accessories for successful Tissue Arrays

Block Caroussel

Recipient block indexer (image), a rotating recipient block holder that holds four blocks at a time and speeds up the concurrent production of multiple blocks.



Depth Stop Kit

Depth stop kit includes 5 positive depth stops in 1-mm increments (from 2 to 6 mm) for sampling the donor block. Kit also includes a companion set of depth stops for holding the donor punch just above the recipient block when depositing tissue. Use of preset stops enables simple control of the length of the tissue core, providing more consistent results and less waste. Excellent operation tool for experienced users as well as training aid for new users

Punches for the manual arrayer

Punches are sold as matched pairs One for the donor block and another one for the recipient block.

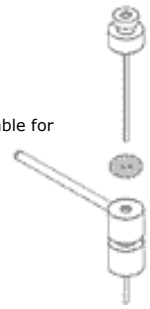
Sets of 10 punches are also available.

Punches available for

0,6 mm

1,0 mm

1,5 mm



The observation magnifying lamp

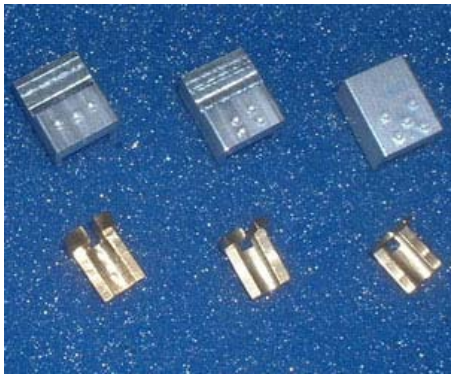
Building a Tissue Array block is a tedious and meticulous work on small objects weakly constrasted like the paraffin cores.

The magnifying lamp Wave Plus ensures an homogeneous and adjustable light to point out the cores and holes in paraffin.

The lamp includes a rectangular magnifying glass of 3 dioptries which shows very few deformation on the sides unlike circular magnifying glasses. It brings significant comfort to the Tissue Arrayer users.

The PSA Tape Transfer System

Tissue Array block is even more precious than your sample block from which it is originated. Tape Transfer System prevents wasting your tissue array blocks when trimming into it for section preparation. It allows you to maximize the use of your tissue array block. See our web site for more information at www.alphametrix.de



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