ARTICLE

Histopathological analysis of a large number of tissues using conventional techniques is both tedious and time-consuming. However, the introduction of tissue microarray technology means that it is now possible to study up to 1000 individual tissue samples on one glass microscope slide. Here, Matthew Ibbs considers an important new methodology.

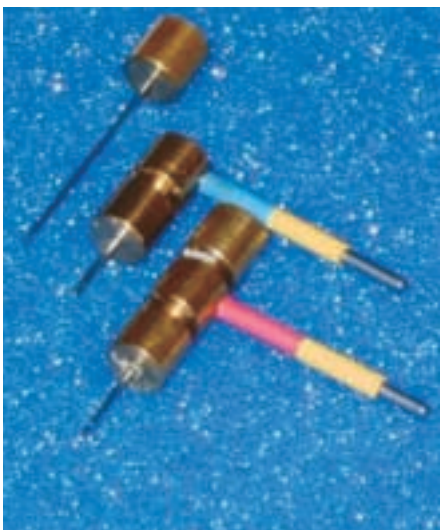
Tissue microarrays in histopathology

Until recently the research and development of new diagnostic tests in histopathology have always required considerable time, both from technical staff and from pathologists. A large number of specimens have needed to be processed into paraffin blocks, sectioned, stained and assessed microscopically before statistical analysis was possible. In recent years, however, along with the advent of microarray technologies for genomics and proteomics, histopathology research received a similar much-needed boost by the introduction of tissue microarray technology (TMA).[1]

There are three types of TMA block: tumour, progression and prognostic.[2] The tumour TMA block contains many different tumour types, making it a relatively quick and simple process to screen a range of tumour types for new or suspected biomarkers discovered during the course of genomic or proteomic studies. Progression TMA blocks contain samples that represent each stage of disease progression for a given tissue. Application of known biomarkers to such an array will show which markers are important and relevant at each stage of a disease. Prognostic TMA blocks comprise cores of tumour tissue for which there are known clinical outcome data; thus, they are used only in retrospective studies and are not suited to studies that require fresh material (eg proteomics). Prognostic TMA blocks can be used to show which biomarkers are indicative of specific clinical outcomes.

In 1998, Kononen *et al.*[1] described the first TMA. This new technique permitted the embedding of up to 1000 tissue specimens in a single histology block and has three main advantages over existing multi-tumour tissue blocks (MTTB).[3] Firstly, MTTBs comprise only 200–300 specimens at a maximum. Second, a specific specimen location or orientation within the histological sections cannot be guaranteed. Therefore, MTTBs are adequate only for testing new antisera, for which reactions and cross-reactions within tissues are being sought, but are inadequate for more detailed studies, sometimes involving many antisera, probes or primers, in which it must be possible to identify specific cases. Third, the TMA block can be used to create a reusable library or archive of cases with known characteristics and clinical outcomes (eg a single tumour type), while the MTTB's lack of orientation means that it is impossible to use it as an archive of valuable research material in this way.

> 'Tissue microarray blocks comprise up to 1000 cores of tissue, arranged perpendicular to the plane of sectioning'



Beecher Instruments produces both manually operated and automatic tissue samplers and is the market leader in the field.

Needles and pins

Tissue microarray blocks comprise up to 1000 cores of tissue, similar to those obtained by needle biopsy techniques, in a vertical position (ie arranged perpendicular to the plane of sectioning) within the block. The cores are extracted from donor paraffin-embedded tissue blocks and then inserted into a matrix of holes in a recipient block so that each core can be identified easily when the section is orientated correctly.

Most researchers select material from donor blocks by a review of haematoxylin and eosin (H&E)-stained slides. The areas of interest can be marked directly on the slide, which can be overlaid on the donor block in order to guide sampling and reduce the number inappropriate reactions in subsequent studies. However, some researchers have used other methods to identify material for selection. In one method, tumour-rich material was processed to paraffin specifically for TMA sampling after being identified in, and cut out from, frozen tissue.[4] In another example, completed TMA blocks were reviewed from H&E-stained sections and any inappropriate or unrepresentative cores were excluded from further analysis.[5] In the case of truly randomised population screening, this selection stage can be skipped.

When a section cut from a TMA is mounted on a microscope slide, a pattern of spots representing the tissue cores in the block is produced. In a review article published in 2001,[6] Olli Kallioniemi (a member of the group which originally published the TMA method) and co-workers gave a detailed description of the procedure used in array construction.

Consumption and efficiency

Although the careful selection of cases and the preparation of TMAs is time consuming – an estimated 30–70 blocks can be arrayed per hour manually, or 120–180 by automated means – the ability to place a large quantity of tissue samples on a single microscope slide and be able to identify each specimen with certainty has increased the speed and efficiency with which histological studies may be completed. For example, Richter *et al*.[7] were able to complete immunohistochemical interpretation of 2317 bladder carcinoma specimens in just four hours and fluorescence *in situ* hybridisation (FISH) scoring for the same array in just six days.

However, it should be borne in mind that the cost of preparing a TMA slide, comprising 1000 specimens, for immunohistochemistry is the same as that for a slide bearing a single conventional histological section. Furthermore, to complete a study of 1000 samples using conventional means consumes 1000 times the resources (antisera, primers etc). Speed and efficiency alone are insufficient reasons to adopt the TMA technique, as the results of such studies must be comparable with conventional methods in order to have any statistical meaning.

Numerous validation studies have now been carried out on for example breast,[8] gastric tissues,[9] colorectal tumours,[10, 11] lung[12] and bladder tissue.[13] These investigations verify that the results of TMA studies are statistically similar to results from studies using conventional histological methods, and agree with the author's own experience in Poland.[14]

> 'Histopathology research has received a much-needed boost by the introduction of tissue microarray technology'

In certain circumstances, however, the technology may fall short of requirements. For example, Merseburger *et al*.[15] found that the prognostic value of p53 and Bcl-2 could not be confirmed using the TMA technology. Markers expressed heterogeneously present a problem because they may be easily missed by sampling techniques – a problem that has been highlighted by other authors.[1,16] From these findings, it is evident that the problem cannot be avoided entirely, as it may also be related to inter-run variability in staining procedure; however, it can be reduced by increasing the number of samples included in the array, thus providing more tissue on which to base statistical analyses.

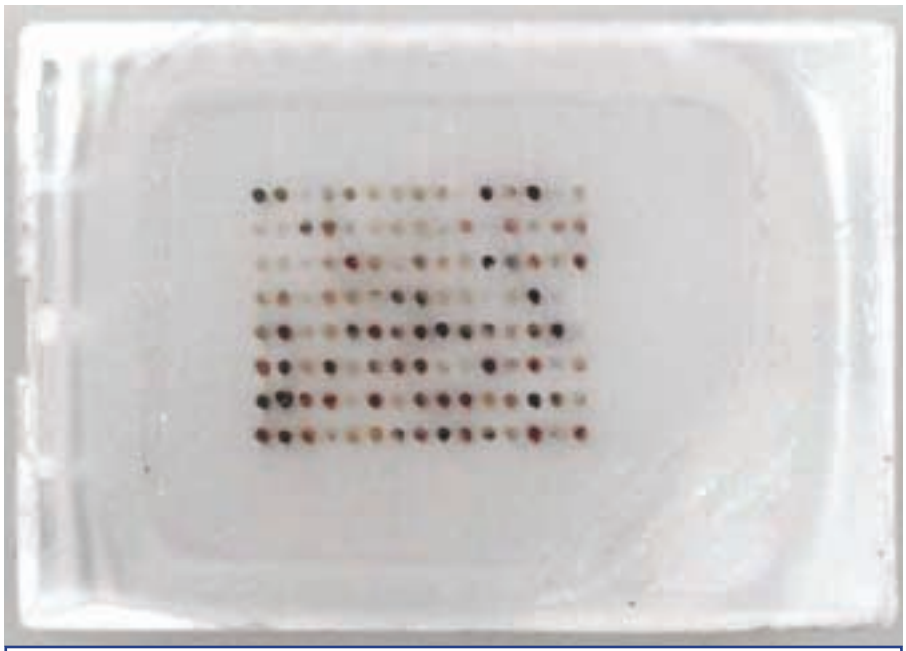
What an array!

As with any new laboratory technology, benefits in efficiency must be weighed against financial outlay, and TMA technology is not the cheapest of procedures. The majority of published work on the subject has been completed with the aid of a precision instrument (Beecher Instruments, Silver Springs MD, USA).[1,6,8,9,11,12,15] Beecher Instruments produces both manually operated and automatic tissue samplers and is the market leader in the field. The cost of such instruments begins at just under $10,000, and it was with such equipment that Kononen *et al*. produced the first examples of TMA blocks.[1]

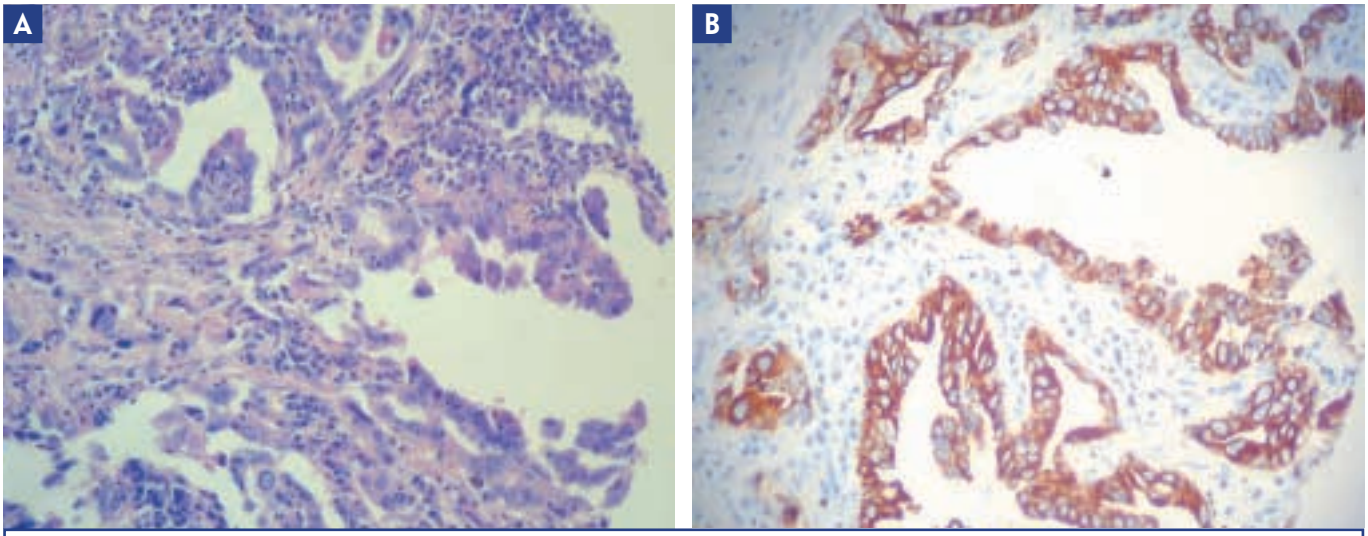
As it is necessary to archive all histopathology cases and keep paraffin blocks for a considerable length of time (usually many years), it would seem reasonable to produce a TMA that contained small samples of rare tumours for archival purposes, thus making the most of rare material and saving storage space. However, routine histopathology departments generally have neither the time nor the funds to invest in the production of such TMA blocks and often must release a very rare block of tissue for research purposes. The valuable material is often exhausted by the research project or the blocks are not returned to the laboratory that supplied them. Furthermore, although the production of a TMA block can be a reasonably rapid process, this is only the case if materials are readily available. In a routine setting, very rare or unusual specimens arrive only sporadically, making the rapid construction of a TMA-based tumour archive unrealistic in most cases.

It is possible to produce TMA-like blocks without the aid of an arraying machine and this could represent part of an answer to the problem. Where laboratories are reluctant to release an entire sample of rare material for research, a more modest form of array block, such as that produced by Gillet *et al*.,[17] could be used. Gillet and colleagues used an 11-gauge core-cut needle (2 mm diameter) to extract tissue from donor blocks. Using such a technique removes the need to purchase special equipment; however, the convenience and efficiency of the technique is partly reduced. Furthermore, the use of wider-gauged needles or punches can damage the donor blocks.

Gillet *et al*. embedded 328 cores, representing 157 cases, in 10 blocks; however, using an arraying machine, all of these cores could have been embedded in a single block. Hendriks *et al*.[10] described the use of a 0.6 mm punch, obtained from Beecher Instruments and generally supplied as a part of an arraying machine, but they did not mention the use of a precision instrument.It is possible that the authors used the needles to transfer tissue cores between the donor and recipient blocks. Use of such a narrow gauge needle would have permitted more tissue cores to be included per block than would have been possible with the system used by Gillet *et al*., but less than would have been possible with the automated system due to reduced accuracy in positioning.



Tissue microarray: cores of tissue inserted into a matrix of holes in a paraffin-wax block. Reproduced from Ref 12 by kind permission of the authors and of the publisher of the *Journal of Pathology* (©Pathological Society of Great Britain and Ireland).



Microscopical appearance of an individual core in a tissue microarray: a) stained with haematoxylin and eosin, b) immunostaining for CAM 5.2.

Automation and deconvolution

Tissue microarray technology can produce vast amounts of data in a very short time,[7] and this must be processed and analysed. In this respect, the matrix arrangement of samples within a TMA block facilitates the use of computerised systems. The arrays can be constructed in an entirely automated manner, and results from TMA slides can also be collected using computer-steered image capture systems.

Image capture and analysis software such as the Bliss system (Bacus Laboratories, Lombard IL, USA) or the ACIS system (Chroma Vision Medical Systems, San Juan Capistrano CA, USA), although expensive, provide the opportunity to have TMA slides scanned and graded in an entirely automatic fashion. Such software systems can often grade the level of staining intensity into more than 200 levels, a degree of accuracy that is impossible for a human observer but a facility that can reduce the likelihood of intra-observer variability to negligible levels.

Another advantage of image capture and analysis systems is that the images collected by the system can be catalogued and saved to a database for recall at a later date. The database can also link the saved images with other textual and graphical data recorded for an individual case so that all available materials can be recalled simultaneously for review. Such a system was employed in a study into the application of web-based analysis of TMA image data.[20]

Other software programs designed for use with microarrays can be downloaded from internet sites free of charge, and have been described by Liu *et al*.[21] The authors described software they used to process data obtained from complementary DNA (cDNA) microarrays and later used it to process TMA data. Such software includes a data reformatting system, TMA-Deconvoluter, which is designed to solve the problem of translating large amounts of three dimensional TMA data (two dimensions being the matrix arrangement of arrayed material and the third being the multiple sets of data derived for the same array through use of multiple antisera, probes or primers) from workbooks (Microsoft, Excel) into a two-dimensional spreadsheet-type format. The deconvoluter produces a table of results that is more readily handled by common statistical programs and spreadsheets than the original TMA data.

> 'Data reformatting solves the problem of translating large amounts of three dimensional TMA data comprising that derived from the matrix arrangement and that from the use of multiple antisera, probes or primers'

Future expression

Tissue microarray technology provides a promising method for the rapid delivery of results in histopathological research. However, in cases where the prognostic significance of findings is important, it remains unreliable because of problems related to the heterogeneity of antigen expression. In such cases, conventional histology must remain the gold standard for the foreseeable future.

It is probable that with the falling costs of technology, and increased competition between developers, TMA will become an easily affordable, justifiable and commonly used tool for archiving rare or scarce material and for inter-laboratory quality assurance procedures in routine histopathology laboratories. This may lead to more research work being carried out in smaller centres. ■

REFERENCES

1 Kononen J, Bubendorf L, Kallioniemi A *et al*. Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med* 1998; **4**: 844–7.
2 Simon R, Malacher M, Sauter G. Tissue microarrays. *Biotechniques* 2004; **36**(1): 98–105.
3 Battifora H. The multitumor (sausage) tissue block: novel method for immunohistochemical antibody testing. *Lab Invest* 1986; **55**(2): 244–8.
4 Frierson HF, Moskaluk CA, Powell SM *et al*. Large-scale molecular and tissue microarray analysis of mesothelin expression in common human carcinomas. *Hum Pathol* 2003; **34**(6): 605–9.
5 Hedvat CV, Hegde A, Chaganti RSK *et al*. Application of tissue microarray technology to the study of non-Hodgkin's and Hodgkin's lymphoma. *Hum Pathol* 2002; **33**(10): 968–74.
6 Kallioniemi OP, Wagner U, Kononen J, Sauter, G. Tissue microarray technology for high-throughput molecular profiling of cancer. *Hum Mol Genet* 2001; **10**(7): 657–62.
7 Richter J, Wagner U, Kononen J *et al*. High-throughput tissue microarray analysis of cyclin E gene amplification and overexpression in urinary bladder cancer. *Am J Pathol* 2000; **157**(3): 787–94.
8 Camp RL, Charette LA, Rimm DL. Validation of tissue microarray technology in breast carcinoma. *Lab Invest* 2000; **80**(12): 1943–9.
9 Gulmann C, Butler D, Kay E, Grace A, Leader M. Biopsy of a biopsy: validation of immunoprofiling in gastric cancer biopsy

tissue microarrays. *Histopathology*. 2003; **42**: 70–6.
10 Hendriks Y, Franken P, Dierssen JW *et al*. Conventional and tissue microarray immunohistochemical expression analysis of mismatch repair in hereditary colorectal tumors. *Am J Pathol* 2003; **162**(2): 469–77.
11 Jourdan F, Sebbagh N, Comperat E *et al*. Tissue microarray technology: validation in colorectal carcinoma and analysis of p53, hMLH1, and hMSH2 immunohistochemical expression. *Virchows Arch* 2003; **443**(2): 115–21.
12 Leversha MA, Fielding P, Watson S, Gosney JR, Field JK. Expression of p53, pRB, and p16 in lung tumours: a validation study on tissue microarrays. *J Pathol* 2003; **200**: 610–9.
13 Nocito A, Bubendorf L, Maria Tinner E *et al*. Microarrays of bladder cancer tissue are highly representative of proliferation index and histological grade. *J Pathol* 2001; **194**(3): 349–57.
14 Ibbs MR, Kurzawa PJ, Breborowicz J. Immunohistochemical profiling of adenocarcinomas of known and unknown origin using a tissue microarray. *Pol J Pathol* 2004; **50**(2): 19.
15 Merseburger AS, Kuczyk MA, Serth J *et al*. Limitations of tissue microarrays in the evaluation of focal alterations of bcl-2 and p53 in whole mount-derived prostate tissues. *Oncol Rep* 2003; **10**(1): 223–8.
16 Schraml P, Kononen J, Bubendorf L *et al*. Tissue microarrays for gene amplification surveys in many different tumor types. *Clin Cancer Res* 1999; **5**(8):1966–75.
17 Gillett CE, Springall RJ, Barnes DM, Hanby AM. Multiple tissue core arrays in histopathology research: a validation study. *J Pathol*. 2000; **192**: 549–53.
18 Ayala G, Wang D, Wulf G *et al*. The prolyl isomerase Pin1 is a novel prognostic marker in human prostate cancer. *Cancer Res* 2003; **63**(19): 6244–51.
19 Jubb AM, Landon TH, Burwick J *et al*. Quantitative analysis of colorectal tissue microarrays by immunofluorescence and *in situ* hybridization. *J Pathol* 2003; **200**(5): 577–88.
20 Bova GS, Parmigiani G, Epstein JI, Wheeler T, Mucci R, Rubin MA. Web-based tissue microarray image data analysis: initial validation testing through prostate cancer Gleason grading. *Hum Pathol* 2001; **32**(4): 417–27.
21 Liu CL, Prapong W, Natkunam Y *et al*. Software tools for high-throughput analysis and archiving of immunohistochemistry staining data obtained with tissue microarrays. *Am J Pathol* 2002; **161**(5): 1557–65.

Matthew R Ibbs CSci FIBMS is a biomedical scientist working in the Department of Histopathology, Wielkopolska Oncology Centre, Poznan, Poland.

IBMS Biomedical Science Congress 2005 Call for Short Papers and Posters

Biomedical science students and university lecturers are invited to present a project, review or case study, as a Short Paper or Poster, at the next Biomedical Science Congress to be held at the International Convention Centre in Birmingham, from Monday 26 to Wednesday 28 September 2005.

ADVANTAGES TO STUDENTS
- Improve your CV
- Earn CPD credits
- Day delegate fee waived

ADVANTAGES TO UNIVERSITY
- Influence the QAA
- Promote your university
- Research selectivity

The presenter's delegate fee will be waived for the day of presentation

For further details please contact: Alice Madziwa, IBMS, 12 Coldbath Square, London EC1R 5HL, tel 020 7713 0214 (ext 138) or email congress@ibms.org.

Application forms are available to download from the IBMS website at www.ibms.org

Congress schedule for Short Papers and Posters

POSTERS

Monday 26 September
No Posters

Tuesday 27 September
Cytopathology
Haematology
Medical microbiology
Virology

Wednesday 28 September
Cellular pathology
Clinical chemistry
Education/Management
Immunology
Transfusion science

SHORT PAPERS

Monday 26 September
No Short Papers

Tuesday 27 September
Cytopathology
Haematology
Medical microbiology
Virology

Wednesday 28 September
Cellular pathology
Clinical Chemistry
Immunology
Transfusion science

DEADLINE FOR SUBMISSION OF SHORT PAPERS AND POSTERS IS TUESDAY 31 MAY

Are you receiving the free IBMS *eNewsletter?*

Visit the home page at **www.ibms.org** and add your email address for news, science event reports, press releases, useful website links and information on professional issues. Alternatively, email **website@ibms.org** with 'newsletter' in the subject field.