A novel method for preparation of tissue microarray

Han-Lei Dan, Ya-Li Zhang, Yan Zhang, Ya-Dong Wang, Zuo-Sheng Lai, Yu-Jie Yang, Hai-Hong Cui, Yan-Ting Jian, Jian Geng, Yan-Qing Ding, Chun-Hai Guo, Dian-Yuan Zhou

Abstract
AIM: To improve the technique of tissue microarray (tissue chip).

METHODS: A new tissue microarraying method was invented with a common microscope installed with a special holing needle, a sampling needle, and a special box fixing paraffin blocks on the microscope slide carrier. With the movement of microscope tube and objective stage on vertical and cross dimensions respectively, the holing procedure on the recipient paraffin blocks and sampling procedure of core tissue biopsies taken from the donor blocks were performed with the refitted microscope on the same platform. The precise observation and localization of representative regions in the donor blocks were also performed with the microscope equipped with a stereoscope.

RESULTS: Highly-qualified tissue chips of colorectal tumors were produced by a new method, which simplified the conventional microarraying procedure, and was more convenient and accurate than that employing the existing tissue microarraying instruments.

CONCLUSION: Using the refitted common microscope to produce tissue microarray is a simple, reliable, cost-effective and well-applicable technique.

INTRODUCTION
Tissue microarray (TMA, tissue chip) is one of the most important biochip technologies, following the gene chip and protein chip. This technology was first illustrated by Kononen et al.[1] in the magazine of Nature Medicine in 1998, which then has been working with the National Human Genome Research Institute. Illuminated by the idea of DNA microarray (gene chip), Kononen et al proposed a new method of tissue microarray to improve the conventional process of immunostaining and fluorescent in situ hybridization (FISH) of individual tissue sections, which was laborious, low-efficient, vulnerable to various factors affecting the experiments and poor comparability.

TMA technology is sharply distinguished from the methods illustrated by Battifora et al.[2] (1986) and Wan et al.[3] (1997), which involved manually randomized rearrangement of tissue specimens. The difference lies in that the spots on TMA chips are minute (0.6 mm-2.0 mm in diameter), the quantity is huge (up to 1000 arrays at present), the shape is regular and the arrays are ordinal. The results obtained from TMAs are more scientific and comparable while the research is more informative, efficient and requires less consumption of reagents. Besides, it paves the way to the standardization of inspection and automation of analysis, which eventually makes the molecular pathological research step forward on the way of standardization and advancement in technolog[4-7].

Nevertheless, the current facilities of tissue microarrays are not advanced as expected but are costly. Moreover, it is not convenient to observe and locate the exact sites in the donor tissue blocks, or to install, adjust and replace the holing and sampling needles. Thus, the whole procedure takes much time that restricts the wide application of this particular technology[8,9]. To make up the inadequacy, we proposed a new methodology of TMA in the following section, which is convenient, up-to-date and well applicable.

MATERIALS AND METHODS
Technical design
The key techniques of TMA included high-adhesive glass slide preparation, reliable tissue specimen acquisition, tissue core arrangement, fine sectioning and section transferring techniques, as well as tissue staining and molecular pathological technology. The core technology was the acquisition of reliable tissue specimen and arrangement of tissue cores, which involved tissue fixation, collection, dehydration, embedding, tissue array design and sampling procedures, especially the exact location of needed tissue, instruments and methods of sampling and puncture, etc. To avoid the inadequacy of the current TMA method, we proposed a new method for fabrication of TMAs through re-equipping the common microscope. With the precise mechanical controls and sampling site observation of the microscope, the new procedure simplified the holing, sampling and inserting process, increased reliabilities, and reduced costs and was ready for wide application.

Equipments and articles
Following equipments and articles were required: a refitted common light microscope; a set of holing, sampling needles and related components (China patent No. 03113734.2), which were installed in the objective holde; a special paraffin-fixing box (China patent No. 03113733.4) placed on the slide carrier; turn buckles and screw nuts fitted by the microscope pillar for controlling the moving range of object stage or the lens; the buckles for fixing glass slide carrier; sufficient paraffin-
embedded donor and recipient blocks; a temperature controlling oven; adhesive-coated glass slides; a tissue sectioning and transferring system as well as instruments, equipments and reagents of routine staining and molecular pathology. A stereomicroscope, a scanning apparatus and a computer automation analysis system were needed if available.

**Tissue sample acquisition**

This method could be applied in fabrication of paraffin embedded tissue microarrays. We took surgically or endoscopically sectioned fresh tissue, and banked paraffin-embedded tissue blocks as the specimen. The acquisition procedure included tissue fixation, collection, dehydration, hyaline and paraffin embedding, which were basically not different from the routine ones. However, to ensure the quality of TMA, procedures such as the use of fixation solutions and fixing time should be standardized and mRNA in the specimen should be preserved as much as possible.

**Paraffin TMA block construction**

This was one of the key techniques in the new procedure. Illustrated in Figure 1(A, B, C, D), the basic process included holing in the recipient blocks, location on the donor blocks, tissue core sampling and inserting. A. Holing A recipient block is fixed in the paraffin-fixing box, and the holing needle is tuned to the working place, and punched into the exact site of the recipient block and then is drawn back with adjustment of the rotating screw. Then the core needle is pressed to squeeze out the paraffin in the needle. B. Location A donor block is fixed in the paraffin-fixing box, and the stereomicroscope lens is tuned to the working place, then the exact sampling site is observed and fixed in the center part of the microscopic vision field. C. Sampling The sampling needle is tuned to the working place, and inserted into the selected site of donor block, and retrieved with the adjustment of rotating screw. D. Inserting With the microscope stage moving, the recipient block moves under the sampling needle. When the sampling needle moves exactly above the recipient hole, the core needle is pressed to squeeze out the tissue core and then inserted it into the recipient hole.

**Sectioning, transferring and staining or molecular pathological detecting**

After insertion of all the samples, the recipient block was taken out from the fixing box and heated up to 58-65°C and the tissue core was flattened by a glass slide and then cooled down. The next procedures were sectioning, tissue slice transferring and routine staining (HE) or PCR, immunofluorescent in situ hybridization, etc. (Figures 3-4).

**RESULTS**

Employing the above method, high-quality tissue chips of colorectal tumors were successfully produced. Figure 3A is an image of the paraffin embedded tissue core arrays, which was 24 mm×35 mm in size. Every 3 core-specimens were obtained from each paraffin-embedded biopsy specimen, resulting in 111 cores from 35 colorectal tumor biopsy specimens (including colorectal adenocarcinoma, adenoma, non-adenoma polyps) and 2 normal colorectal tissue specimens. Each core was 1.3 mm in diameter and 0.7 mm in spacing.

The tissue micro-array of colorectal tumors on a glass slide (HE staining) is displayed in Figure 3B. The blank spot (dot array) on the left-hand corner was the location mark, and the blank spot in the center part was lost during sectioning and
staining, which made a rate of 0.8% non-evaluable core biopsy sections. The selected spots in Figure 3B were magnified (X8) in Figure 3C under a stereoscope and the dot arrays were in good order, sharp bordered, not crushed or deformed. All the arraying tissue specimens were representative of the interest regions, which coincidently met the research demands. Good staining quality was also attained on the same array sites in different slides stained with HE, immunohistochemistry and others respectively. The results were more comparable and sensitive on the chip slide as shown in Figure 4. Further detailed analyses would be reported soon.

**DISCUSSION**

As one of the three most important biochip technologies, tissue microarray has been accepted as a large-scale, parallel molecular analysis just like gene and protein chips[3-6,10-15]. The results were more scientific, comparable, informative and efficient, and it needed less consumption of reagents[16-23]. The difference lies in that the dot arrays of tissue chip were specimens from hundreds of different tissues and could show the features of morphology and expressions of DNA, RNA or proteins *in situ* associated with clinical endpoints at the same time. And the procedure was less complex and the results were more clinically applicable. Thus TMA is more feasible in the functional researches of gene and protein, especially in researches of gene or protein profiles in different tissues or populations. Therefore TMA has become one of the most important methods in functional genomic and proteonomic researches in the post-genomic era and, it has been expected to have wide applications in molecular pathology, drug discovery, monitoring of hygiene and environment, as well as national defense researches (Table 1)[24,25].

However, the present technology of TMA is still not satisfactory. On the one hand, the instruments were not very advanced but costly. On the other hand, the present procedure took time and was not convenient to observe and locate the exact sites in donor tissue blocks[8,9,26-32]. The new method to produce tissue microarray with refitted common microscope we presented here has the following advantages. (1) With the precise mechanical control of common microscope, the holing and sampling procedures in the paraffin recipient block and donor block are simpler and more accurate. (2) The punching procedure of holing and sampling needles is controlled by the adjustment of microscope stage or lens regulating screw, which frees us from direct finger operation and avoids needle gliding or trembling. This method is less laborious and easier and more accurate to control the holing and sampling depth. (3) The working place of holing and sampling needles and object lens changes with the rotation of objective holde and thus is easier to replace the needles and more convenient to apply the single-use holing and sampling needles in order to maintain a higher accuracy. (4) With this new method, the sampling sites can be easily and accurately observed and located under a microscope or a stereomicroscope and thus spares the efforts of marking the regions to punch on the face of the donor block and without

**Table 1** Comparison of gene, protein and tissue chips

| Substrate | Gene chip | Protein chip | Tissue chip |
|-----------|-----------|--------------|-------------|
| Probes    | Definitive DNA or RNA (Oligo or cDNA) | Definitive proteins (antigens) antibodies, etc. | Many tissue samples |
| Target material | Labelled DNA or RNA (Cy3 and Cy5-tyramide) | Labelled antigens or antibodies | Specific antigens or antibodies, as well as labelled DNA or RNA sequences |
| Target material | Various sequences of DNA or RNA | Multiple kinds of proteins | Morphology and DNA, RNA and proteins |
| Superiorities | Analyzing numerous genes simultaneously in the same sample | Analyzing numerous proteins simultaneously in the same sample | Profile of genes or proteins in numerous tissues or populations |
| Applications | Large-scale, parallel analysis of genetic alterations, functions and drug researches at DNA and RNA level | Large-scale, parallel analysis of concentrations, functional activities or interactions of proteins | Large-scale, parallel in situ analysis of DNA, RNA and proteins associated with clinical endpoints in hundreds of cell types or tissues at one time |

**Figure 4** Photographs of different tissue array elements stained with HE(A) and immunohistochemistry (B: P53, C: PCNA).
eyeballing, as is the case with the Beecher’s instruments.
(5) The whole process of holing, locating and sampling is performed with the same instrument on the same platform and thus spares uses of other platforms and instruments. As TMA involves a number of related technologies and sciences, including engineering, surface physics and chemistry, pathology, molecular biology and information science, to produce high-quality tissue biochips, consideration should be also taken into the design of arrays, the qualities of holing and sampling needles, sectioning and transferring techniques, staining and other molecular pathological techniques. To improve the density and precision of arrays, the needles should be more minute in diameter and sharper in point. The arrangement of arrays should be in accordance with the research objectives and the computer imaging, and statistical tools should be provided for efficient management of a large amount of data generated from this high-throughput approach[33-35].

REFERENCES
1 Kononen J, Bubendorf L, Kallioniemi A. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 1998; 4: 844-847
2 Battifora H. The multitumor (soupsage) tissue block: novel method for immunohistochemical antibody testing. Lab Invest 1986; 55: 244-248
3 Wan WH, Fortuna MB, Furmanski P. A rapid and efficient method for testing immunohistochemical reactivity of monoclonal antibodies against multiple tissue samples simultaneously. J Immunol Methods 1997; 298: 121-129
4 Hsu FD, Nielsen TO, Alkushi A. Tissue microarrays are an effective quality assurance tool for diagnostic immunohistochemistry. Mod Pathol 2002; 15: 1374-1378
5 Kallioniemi OP. Biochip technologies in cancer research. Annu Rev Med 2001; 53: 142-147
6 Stears RL, Martinsey T, Schena M. Trends in microarray analysis. Nat Med 2003; 9: 140-145
7 Bittker CA, Sherlock G, Parkinson H, Rocca-Serra P, Brooksbank C, Causton HC, Cavaleri D, Gaasterland T, Hingamp P, Holstegae R, Ringwald M, Spellman P, Stoekelck CJ, Stewart JE, Taylor R, Braza V, Quackenbush J. Microarray Gene Expression Data (MGED) Society. Standards for microarray data. Science 2002; 298: 539
8 Hoos A, Cordon-Cardo C. Tissue microarray profiling of cancer specimens and cell lines: opportunities and limitations. Lab Invest 2001; 81: 1331-1338
9 Merseburger AS, Kuczky MA, Serth J, Bokemeyer C, Young DY, Sun L, Connelly RR, McLeod DG, Mostofi FK, Srivastava SK, Stenzel A, Moul JW, Sesterhenn IA. 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: 223-228
10 Torhorst J, Buercher C, Kononen J, Haas P, Zuber M, Kochli OR, Mross F, Dietrich H, Moch H, Mihatsch M, Kallioniemi OP, Sauter G. Tissue microarrays for rapid linking of molecular changes to clinical endpoints. Am J Pathol 2001; 159: 2249-2256
11 Wang HT, Kong JP, Ding F, Wang XQ, Wang MR, Liu LX, Wu M, Liu ZH. Analysis of gene expression profiles induced by EMP-1 in esophageal cancer cells using cDNA Microarray. World J Gastroenterol 2003; 9: 392-398
12 Xu SH, Qian LJ, Mou HZ, Zhu CH, Zhou XM, Liu XJ, Chen Y, Bao WY. Difference of gene expression profiles between esophageal carcinoma and its precancerous epithelium by gene chip. World J Gastroenterol 2003; 9: 417-422
13 Zhou J, Zhao LQ, Xiong MM, Wang XQ, Yang GR, Qiu ZL, Wu M, Liu ZH. Gene expression profiles at different stages of human esophageal squamous cell carcinoma. World J Gastroenterol 2003; 9: 915
14 Zhu H, Bilgin M, Bangham R, Hall D, Casamayor A, Bertone P, Lan N, Jansen R, Biedlingmaier S, Houfek T, Mitchell T, Miller P, Dean RA, Gerstein M, Snyder M. Global analysis of protein activities using proteome chips. Science 2001; 293: 2101-2105
15 Rao J, Seligson D, Hennstree GP. Protein expression analysis using quantitative fluorescence image analysis on tissue microarray slides. Biotechniques 2002; 32: 924-926
16 Van ’t Veer LJ, De Jong D. The microarray way to tailored cancer treatment. Nat Med 2002; 8: 13-14
17 Kim WH, Rubin MA, Dunn RL. High-density tissue microarray. Am J Surg Pathol 2002; 26: 1236-1239
18 Bubendorf L, Nocto A, Moh H, Sauter G. Tissue microarray (TMA) technology: miniaturized pathology archives for high-throughput in situ studies. J Pathol 2001; 195: 72-79
19 Rimm DL, Camp RL, Charette LA, Costa J, Olsen DA, Reiss M. Tissue microarray: a new technology for amplification of tissue resources. Cancer 2001; 7: 24-31
20 Moch H, Kononen T, Kallioniemi OP, Sauter G. Tissue microarrays: what will they bring to molecular and anatomic pathology? Adv Anat Pathol 2001; 8: 14-20
21 Moch H, Schraml P, Bubendorf L, Mirlacher M, Kononen J, Gas- ter T, Mihatsch MJ, Kallioniemi OP, Sauter G. High-throughput tissue microarray analysis to evaluate genes uncovered by cDNA microarray screening in renal cell carcinoma. Am J Pathol 1999; 154: 981-986
22 Nocto A, Kononen J, Kallioniemi OP, et al. Tissue microarrays (TMAs) for high-throughput molecular pathology research. Int J Cancer 2001; 94: 1-5
23 Andersen CL, Hostetter G, Grigoryan A, Sauter G, Kallioniemi A. Improved procedure for fluorescence in situ hybridization on tissue microarrays. Cytoometry 2001; 45: 83-96
24 Packeisen J, Buercher H, Krich R, Boeker W. Tissue microarrays: a new approach for quality control in immunohistochemistry. J Clin Pathol 2002; 55: 613-615
25 Parker RL, Huntsman DG, Lesack DW, Cuples JB, Grant DR, Akbari M, Gilks CB. Assessment of interlaboratory variation in the immunohistochemical determination of estrogen receptor status using a breast cancer tissue microarray. Am J Clin Pathol 2002; 2: 723-728
26 Chen W, Foran DJ, Reiss M. Unsupervised imaging, registration and archiving of tissue microarrays. Proc AMIA Symp 2002: 136-139
27 Chung GG, Kielhorn EP, Rimm DL. Subjective differences in outcome are seen as a function of the immunohistochemical method used on a colorectal cancer tissue microarray. Clin Colorectal Cancer 2002; 1: 237-242
28 Rubin MA, Dunn R, Strawderman M, Pienta KJ. Tissue microarray sampling strategy for prostate cancer biomarker analysis. Am J Surg Pathol 2002; 26: 312-319
29 Hendriks Y, Franken P, Dierssen JW, De Leeuw W, Wijnjes J, Dred E, Tops C, Breuning M, Brocker-Vriends A, Vanse H, Fodeck R, Mornau H. Conventional and tissue microarray immunohistochemical expression analysis of mismatch repair in hereditary colorectal tumors. Am J Pathol 2001; 159: 315-317
30 Wang Y, Wu MC, Shan JS, Zhang W, Wu WQ, Guan XY. Prognostic significance of c-myc and BAI1 amplification in hepato- cellular carcinoma. A broad survey using high-throughput tissue microarray. Cancer 2002; 95: 2346-2352
31 Gancberg D, Di Leo A, Rouas G, Jarvinen T, Verhest A, Isola J, Picart MJ, Laranom M. Reliability of the tissue microarray based FISH for evaluation of the HER-2 oncogene in breast carcinoma. J Clin Pathol 2002; 26: 469-477
32 Sugita M, Geraci M, Gao B, Powell LW, Hirsh FR, Johnson G, Lapadat R, Gabrielsson E, Brammes R, Bunns PA, Franklin WA. Combined use of oligonucleotide and tissue microarrays identifies cancer/ testis antigens as biomarkers in lung carcinoma. Cancer Res 2002; 6: 3971-3979
33 Liu CL, Prapong W, Natkunam Y, Alizadeh A, Montgomery K, Gilks CB, van de Rijn M. Software tools for high-throughput analysis and archiving of immunohistochemistry staining data obtained with tissue microarrays. Am J Pathol 2002; 161: 1557-1565
34 Rubin MA, Dunn R, Strawderman M, Pienta KJ. Tissue microarray sampling strategy for prostate cancer biomarker analysis. Am J Surg Pathol 2002; 26: 312-319
35 Boguski MS, McIntosh MW. Biomedical informatics for proteomics. Nature 2003; 422: 233-237

Edited by Zhu LH and Wang XL