DOI: https://doi.org/10.17816/morph.634185 EDN: CJEHQT

Automated Systems for Creating Tissue Microarrays in Oncomorphological Studies

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ABSTRACT

Tissue microarrays (TMAs) are a promising method for high-throughput analysis of archived tissue samples. Laboratories using conventional manual methods for creating TMAs face the challenge of increasing efficiency and standardization, which is particularly crucial for oncomorphological research and diagnostics. This can be achieved through the automation of the process.

This review focuses on the capabilities and advantages of automated systems for TMA creation over manual methods, with an emphasis on their application in the analysis of Ewing sarcoma and other undifferentiated round cell sarcomas. In the analyzed works, automated systems were used to extract and position tissue cores into recipient paraffin blocks, followed by histological and immunohistochemical analyses on the obtained TMA sections. Furthermore, the quality of the tissue microarray sections was evaluated. In these works, automated systems demonstrated high precision in positioning tissue cores, significantly accelerating TMA creation and improving the quality of the resulting sections.

Thus, automated systems for TMA creation offer significant advantages over manual methods, ensuring standardization and increasing the productivity of laboratory research. Automated systems allow for the efficient analysis of large sample sets, which is especially important for the validation of diagnostic and prognostic biomarkers. The published works highlight the need for further development and wider implementation of automated systems in oncomorphological research to enhance their efficiency and reproducibility.

Keywords: tissue microarrays; automation; oncomorphology.

To cite this article:
Parfenova IA, Eryshova SA. Automated Systems for Creating Tissue Microarrays in Oncomorphological Studies. *Morphology*. 2025;163(4):265–272. DOI: 10.17816/morph.634185 EDN: CJEHQT

Submitted: 10.07.2024 **Accepted:** 11.10.2024 **Published online:** 14.05.2025

DOI: https://doi.org/10.17816/morph.634185 EDN: CJEHQT

Применение автоматизированных систем для создания тканевых микроматриц в онкоморфологических исследованиях

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АННОТАЦИЯ

Тканевые микроматрицы — один из перспективных методов для высокопроизводительного анализа архивированных образцов тканей. Лаборатории, использующие традиционный ручной метод изготовления тканевых микроматриц (ТМА), сталкиваются с необходимостью повышения эффективности и стандартизации, что особенно важно для онкоморфологических исследований и диагностики. Достичь этого можно за счёт автоматизации процесса.

Настоящий обзор посвящён рассмотрению возможностей и преимуществ автоматизированных систем для создания ТМА по сравнению с ручным методом, с акцентом на их применение в анализе саркомы Юинга и других недифференцированных круглоклеточных сарком. В проанализированных работах автоматизированные системы использовали для извлечения и позиционирования тканевых цилиндров в парафиновые блоки-реципиенты, а на полученных срезах ТМА проводили гистологические и иммуногистохимические исследования. Кроме того, оценивали качество срезов тканевых микроматриц. В упомянутых работах автоматизированные системы показали высокую точность позиционирования тканевых цилиндров, что значительно ускорило процесс создания ТМА и улучшило качество готовых срезов.

Таким образом, внедрение автоматизированных систем для конструирования ТМА имеет значительные преимущества по сравнению с ручным методом, поскольку обеспечивает стандартизацию и повышает производительность лабораторных исследований. Автоматизированные системы позволяют эффективно анализировать большие серии образцов, что особенно важно для валидизации диагностических и прогностических биомаркеров. Опубликованные работы подчёркивают необходимость дальнейшего развития и более широкого внедрения автоматизированных систем в онкоморфологические исследования для повышения их эффективности и воспроизводимости.

Ключевые слова: тканевые микроматрицы; автоматизация; онкоморфология.

Как цитировать:
Парфенова И.А., Ерышова С.А. Применение автоматизированных систем для создания тканевых микроматриц в онкоморфологических исследованиях // Морфология. 2025. Т. 163, № 4. С. 265–272. DOI: 10.17816/morph.634185 EDN: CJEHQT

Рукопись получена: 10.07.2024 **Рукопись одобрена:** 11.10.2024 **Опубликована online:** 14.05.2025

DOI: https://doi.org/10.17816/morph.634185 EDN: CJEHQT

自动化系统在肿瘤形态学研究中用于创建组织微阵列的应用

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摘要

组织微阵列（tissue microarray, TMA）是高通量分析归档组织样本的前景方法。使用传统手工方法制作TMA的实验室面临提高效率和标准化的需求，这对于肿瘤形态学研究和诊断尤为重要。通过自动化过程可以实现这一目标。

本综述讨论了自动化系统在创建TMA中的优势与传统手工方法的对比，重点介绍它们在分析尤文肉瘤和其他未分化圆细胞肉瘤中的应用。分析的研究中，自动化系统用于提取和定位组织柱至石蜡接收块，并在获得的TMA切片上进行组织学和免疫组化分析。此外，还评估了组织微阵列切片的质量。在这些研究中，自动化系统显示出高精度的组织柱定位，大大加快了TMA的制作过程，并提高了最终切片的质量。

因此，自动化系统在构建TMA中的应用相比手工方法具有显著优势，因为它保证了标准化并提高了实验室研究的生产力。自动化系统能够有效分析大量样本，这对于验证诊断和预后生物标志物尤为重要。已发表的研究强调了进一步发展和更广泛应用自动化系统在肿瘤形态学研究中的必要性，以提高其效率和可重复性。

关键词：组织微阵列；自动化；肿瘤形态学。

To cite this article:
Parfenova IA, Eryshova SA. 自动化系统在肿瘤形态学研究中用于创建组织微阵列的应用. *Morphology*. 2025;163(4):265–272.
DOI: 10.17816/morph.634185 EDN: CJEHQT

收到：10.07.2024 **接受：**11.10.2024 **发布日期：**14.05.2025

INTRODUCTION

The relevance of implementing automated systems for constructing tissue microarrays (TMAs) in oncomorphological research is driven by the need to improve the accuracy and efficiency of high-volume tissue sample analysis. TMAs enable simultaneous evaluation of dozens to hundreds of samples on a single slide, which significantly accelerates diagnostic and investigative processes in oncology [1]. The TMA technique proposed by Battifora in 1986 is widely used for validation of diagnostic and prognostic biomarkers, quality control in immunohistochemical (IHC) analysis, and other scientific applications [2, 3].

The challenges of traditional manual TMA construction are largely related to the labor-intensive nature of the process, high operator skill requirements, and considerable time consumption [4]. Automation of TMA construction overcomes these challenges owing to high precision, substantial time savings, and reduced risk of cross-contamination [5, 6]. However, despite the clear advantages of automated systems, their implementation in clinical practice in Russia remains limited due to the absence of regulatory certification [7].

Published studies have confirmed the effectiveness of automated platforms for TMA construction. For example, automated systems have been successfully applied for biomarker evaluation across multiple tumor types [3], ensuring high accuracy. Documented savings of time and resources make automated systems attractive for laboratories with a high sample load and substantial workload demands [5].

Thus, the introduction of automated systems for TMA construction is a major step forward in improving efficiency and standardization within oncomorphological research. Nevertheless, full integration of these systems into clinical practice requires resolution of issues related to registration and standardization of their use in Russia [7].

This review summarizes the capabilities and advantages of automated systems for TMA construction compared with manual methods, with emphasis on their application in the analysis of Ewing sarcoma and other undifferentiated round cell sarcomas.

EXPERIENCE IN THE APPLICATION OF AUTOMATED SYSTEMS FOR TISSUE MICROARRAY CONSTRUCTION

Automated systems for TMA construction are high-technology devices that enable precise and rapid fabrication of microarrays from tens to hundreds of tissue samples [4]. The main modules of such systems include: a unit for automated extraction of tissue cores from donor paraffin blocks and their transfer into recipient blocks (see Fig. 1); a high-precision micromanipulator for positioning of tissue cores; a tissue core depth-control system (laser sensor or mechanical probe); and dedicated software for building a virtual TMA layout [8].

The automated TMA fabrication process begins with uploading the virtual layout of core placement into the recipient block. Modern TMA design software allows specifying diameter of tissue cores, distance between cores, their number and arrangement, and assigning individual annotation to each sample [4]. Based on the virtual layout, the device automatically forms holes of a predetermined diameter in the recipient block for placement of each sample (see Fig. 2).

The operator then sequentially loads donor paraffin blocks into the instrument and, using the micromanipulator, positions the punch needle over the region of interest selected in each sample based on the corresponding hematoxylin–eosin–stained control section (see Fig. 2) [9]. The device automatically extracts a tissue core of the predefined diameter (typically 0.6–2 mm) from the donor block and transfers it into the corresponding hole of the recipient block. The depth-control system ensures precise placement of each tissue core at a uniform level, which is particularly important for obtaining high-quality sections [10].

After completion of core collection, the recipient block is remelted to secure the cores in place and then cooled. The resulting TMA block is sectioned at 4–5 μm thickness and can be used for histological and IHC studies, as well as for fluorescence *in situ* hybridization (FISH) [11]. Examples of micropreparations produced using this technology are shown in Fig. 3.

An important aspect of TMA construction is the height of tissue cores. To preserve the original diagnostic material, additional paraffin blocks from the same case are often used for TMA preparation. This is standard practice that allows studies to be conducted without compromising primary diagnostics.

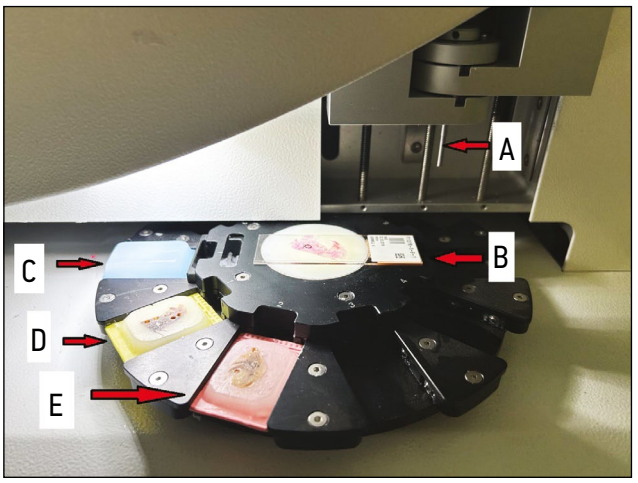

Fig. 1. Device for automatic punch needle positioning: A, punch needle; B, histological slide with marked area of interest, stained with hematoxylin and eosin, placed in the center of the rotator for positioning; C, recipient block; D, donor block 1; E, donor block 2.

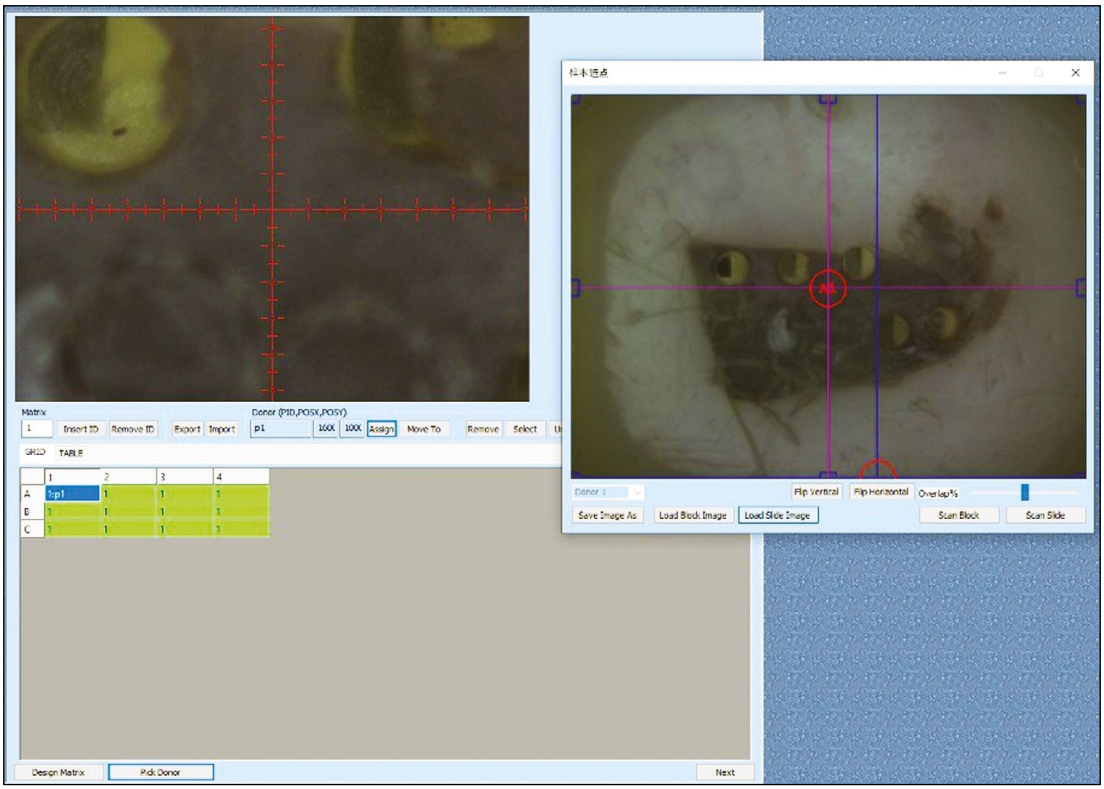

Fig. 2. Software-guided punch needle positioning.

In our laboratory, an automated TMA construction system is used for both research and clinical-diagnostic purposes[1]. In particular, we constructed a TMA from 106 Ewing sarcoma cases [12] and other undifferentiated round cell sarcomas (*BCOR*-altered sarcoma and *CIC*-rearranged sarcoma). For each case, two representative tumor tissue cores 1 mm in diameter were selected and transferred into recipient paraffin blocks. TMA sections were stained with hematoxylin and eosin to assess morphological features. For differential diagnostic algorithm development, IHC studies were performed on an automated immunostainer Ventana BenchMark ULTRA (Roche, Switzerland) using antibodies to markers relevant for phenotypic characterization of Ewing sarcoma and other undifferentiated round cell sarcomas: CD99 (Cell Marque, US), NKX2.2 (BioSB, US), BCOR (Cell Marque, US), SATB2 (Santa Cruz, US), TLE1 (Cell Marque, US), WT1 (Cell Marque, US), ETV4 (Invitrogen, US), desmin (Cell Marque, US), myogenin (Cell Marque, US), MyoD1 (Cell Marque, US), and pancytokeratin (Ventana, US). Since the study was retrospective, the immunohistochemical antibody panel was selected considering that the most common variants of round cell sarcomas were included. The quality of TMA sections and staining results was independently evaluated by two pathologists.

The use of an automated TMA construction system in our study enabled efficient analysis of a large cohort (106 cases) of Ewing sarcoma and other undifferentiated round cell sarcomas, and made it possible to identify novel combinations of diagnostic markers. IHC results demonstrated differences in marker expression between Ewing sarcoma, *BCOR*-altered sarcoma, and *CIC*-rearranged sarcoma. Co-expression of CD99 and NKX2.2, characteristic of Ewing sarcoma, was observed in 94% of cases, whereas this combination was not detected in other tumor types. *BCOR*-altered sarcoma was distinguished by co-expression of BCOR, TLE1, and SATB2 in 80% of cases. *CIC*-rearranged sarcoma was characterized

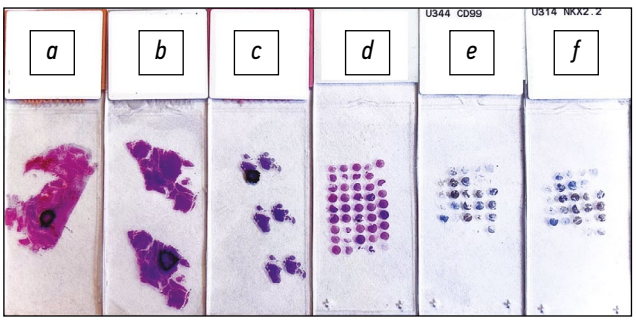

Fig. 3. Overview of the microslides: *a–c*, microslides with marked areas of interest, stained with hematoxylin and eosin; *d*, microslide with tissue microarray, stained with hematoxylin and eosin; *e*, *f*, microslides stained by immunohistochemistry for CD99 and transcription factor NKX2.2, respectively.

[1] At the time of preparing this publication, automated tissue microarray construction instruments do not have regulatory registration in Russia.

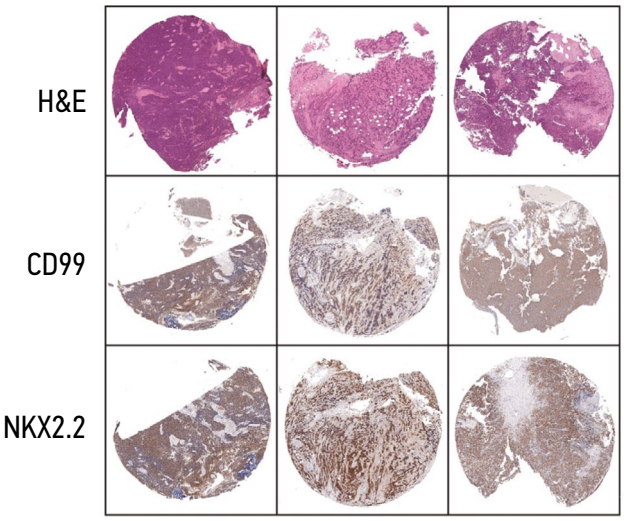

Fig. 4. Findings in patients with Ewing sarcoma: H&E, slides stained with hematoxylin and eosin, ×40; CD99, slides stained with immunohistochemistry for CD99, ×40; NKX2.2, slides stained with immunohistochemistry for transcription factor NKX2.2, ×40. Only slides positive for antibodies against CD99 and NKX2.2 markers are presented, ×40.

by co-expression of ETV4 and WT1, although this was identified in only 25% of cases. Based on these results, a differential diagnostic algorithm was developed for tumors both within this group and in comparison with other round cell neoplasms [12].

ADVANTAGES AND DISADVANTAGES OF AUTOMATED SYSTEMS

The implementation of automated systems for TMA construction opens new opportunities for standardization and acceleration of oncomorphological studies. Compared with manual TMA fabrication, automated production offers several advantages [6]:

- Higher accuracy of tissue core sampling due to high-precision mechanics and depth-control systems [3];
- Substantial time savings for highly trained personnel, especially when processing large sample volumes [5];
- Reduced risk of cross-contamination between samples and tissue damage due to minimization of manual manipulation [7];
- Flexible TMA design with the ability to select various core parameters and spatial layout using specialized software [4];
- Individual labeling and annotation of tissue cores allowing subsequent correlation of morphologic features, IHC, and FISH results [13].

Notably, TMAs serve as a universal control. Although not all samples will yield a positive reaction with antibodies against markers relevant to a specific diagnostic case, the value of TMA lies in the ability to simultaneously analyze numerous specimens, which is particularly important for large-scale studies.

The use of automated systems requires consideration of additional time expenditures for programming and instrument maintenance. Nevertheless, our experience shows that with appropriate workflow organization and regular utilization, automated systems allow substantial optimization of laboratory processes, particularly when handling large volumes of samples.

Despite the clear advantages of using automated systems for TMA construction, obtaining valid results requires adherence to several methodological considerations: mapping should be performed using tissues structurally distinct from the target material [3]; tissue cores must be selected with attention to tumor heterogeneity to ensure representativeness [2]; a sufficient number of tissue cylinders per case (at least 2–3) should be included [14]; staining and interpretation protocols must be standardized [5, 15].

Experience in our laboratory demonstrates that implementation of automated systems for TMA construction substantially optimizes the simultaneous production of high-quality sections from a large number of specimens for all major types of morphological studies.

CONCLUSION

Tissue microarrays represent a modern oncomorphology tool that enables efficient assessment of large archival sample series for validation of diagnostic and prognostic biomarkers, both in research settings and for immunohistochemistry quality control.

Automation of TMA construction using dedicated systems ensures standardization of the pre-analytical phase and increases laboratory throughput compared with manual methods.

Further development and wider implementation of automated systems for TMA construction will enhance the efficiency of oncomorphological research and accelerate translation of scientific advances into clinical practice.

ADDITIONAL INFORMATION

Author contributions: I.A. Parfenova: conceptualization, methodology, writing—original draft, writing—review & editing; S.A. Eryshova: conceptualization, methodology, formal analysis, writing—original draft. All the authors approved the version of the manuscript to be published and agreed to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Acknowledgements: The authors express their gratitude to Dr. Ilya V. Sidorov, pathologist at the Pathology Department of the Dmitry Rogachev National Medical Research Center of Pediatric Hematology, Oncology, and Immunology, and Dr. Dmitry M. Konovalov, Head of the Pathology Department at the same institution, for providing clinical

material and valuable consultations during the research in patients with undifferentiated round cell sarcomas. Thanks to their expert knowledge and support, this work was successfully completed.

Funding sources: No funding.

Disclosure of interests: The authors have no relationships, activities, or interests for the last three years related to for-profit or not-for-profit third parties whose interests may be affected by the content of the article.

Statement of originality: No previously obtained or published material (text, images, or data) was used in this study or article.

Data availability statement: All data obtained in this study are available in this article.

Generative AI: No generative artificial intelligence technologies were used to prepare this article.

Provenance and peer review: This paper was submitted unsolicited and reviewed following the standard procedure. The peer review process involved two external reviewers, a member of the Editorial Board, and the in-house scientific editor.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. И.А. Парфенова — определение концепции, написание черновика рукописи, пересмотр и редактирование рукописи; С.А. Ерышова — определение концепции, проведение исследования, написание черновика рукописи. Все авторы одобрили рукопись (версию для публикации), а также согласились нести ответственность за все аспекты работы, гарантируя надлежащее рассмотрение и решение вопросов, связанных с точностью и добросовестностью любой её части.

Благодарности. Авторы выражают благодарность врачу-патологоанатому патологоанатомического отделения ФГБУ «НМИЦ ДГОИ им. Дмитрия Рогачёва» МЗ РФ Сидорову Илье Владимировичу и заведующему патологоанатомическим отделением ФГБУ «НМИЦ ДГОИ им. Дмитрия Рогачёва» МЗ РФ Коновалову Дмитрию Михайловичу за предоставление клинического материала и ценные консультации в ходе проведения исследования пациентов с недифференцированными круглоклеточными саркомами. Благодаря их экспертным знаниям и поддержке удалось успешно выполнить данную работу.

Источники финансирования. Отсутствуют.

Раскрытие интересов. Авторы заявляют об отсутствии отношений, деятельности и интересов за последние три года, связанных с третьими лицами (коммерческими и некоммерческими), интересы которых могут быть затронуты содержанием статьи.

Доступ к данным. Редакционная политика в отношении совместного использования данных к настоящей работе не применима, новые данные не собирали и не создавали.

Генеративный искусственный интеллект. При создании настоящей статьи технологии генеративного искусственного интеллекта не использовали.

Рассмотрение и рецензирование. Настоящая работа подана в журнал в инициативном порядке и рассмотрена по обычной процедуре. В рецензировании участвовали два внешних рецензента, член редакционной коллегии и научный редактор издания.

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