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Article *in* Proceedings of SPIE - The International Society for Optical Engineering · March 2011

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DETECTING METASTASIS OF GASTRIC CARCINOMA USING HIGH-RESOLUTION MICRO-CT SYSTEM: IN VIVO SMALL ANIMAL STUDY

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ABSTRACT

Immunocytochemical and immunofluorescence staining are used for identifying the characteristics of metastasis in traditional ways. Micro-computed tomography (micro-CT) is a useful tool for monitoring and longitudinal imaging of tumor in small animal *in vivo*. In present study, we evaluated the feasibility of the detection for metastasis of gastric carcinoma by high-resolution micro-CT system with omnipaque accumulative enhancement method in the organs. Firstly, a high-resolution micro-CT ZKKS-MCT-sharp micro-CT was developed by our research group and Guangzhou Zhongke Kaisheng Medical Technology Co., Ltd. Secondly, several gastric carcinoma models were established through inoculating 2×10^6 BGC-823 gastric carcinoma cells subcutaneously. Thirdly, micro-CT scanning was performed after accumulative enhancement method of intraperitoneal injection of omnipaque contrast agent containing 360 mg iodine with a concentration of 350 mg I/ml. Finally, we obtained high-resolution anatomical information of the metastasis *in vivo* in a BALB/c NuNu nude mouse, the 3D tumor architecture is revealed in exquisite detail at about 35 μm spatial resolution. In addition, the accurate shape and volume of the micrometastasis as small as 0.78 mm^3 can be calculated with our software. Overall, our data suggest that this imaging approach and system could be used to enhance the understanding of tumor proliferation, metastasis and could be the basis for evaluating anti-tumor therapies.

Keywords: micro-CT, metastasis, gastric carcinoma, detecting, contrast enhancement

1. INTRODUCTION

Micro-CT has become a widely used tool for biomedical studies to enhance our understanding through observation high resolution anatomical structure information. Especially, with the development of advanced contrast agent, micro-CT could achieve high-contrast images with appropriate contrast agent or superior contrast enhancement method. However, these special contrast agents are very expensive for plenty of experimental applications. For example, Fenestra LC contrast for the contrast enhancement of small animals organs[1], blood vessel observation with the aid of AuroVist contrast agent[2]. Graham et al. reported a method of combination of intraperitoneally iohexol with Fenestra which provides better delineation of liver tumors than using Fenestra alone[3]. However, previous research have failed to consider the plenty of applications and cost-effective in practical experiments, it is only in blind pursuit of perfect contrast effect through several small animal experiments. In present study, we developed an omnipaque accumulative contrast enhancement method in the organs *in vivo* micro-CT, meanwhile, the performances have been evaluated via detection metastasis of gastric carcinoma by ZKKS-MCT-sharp micro-CT high-resolution micro-CT system. We aim to test the feasibility of the low cost contrast enhancement method for using of a large number of animal experiments. It is hoped that the question will be resolved with our proposed approach.

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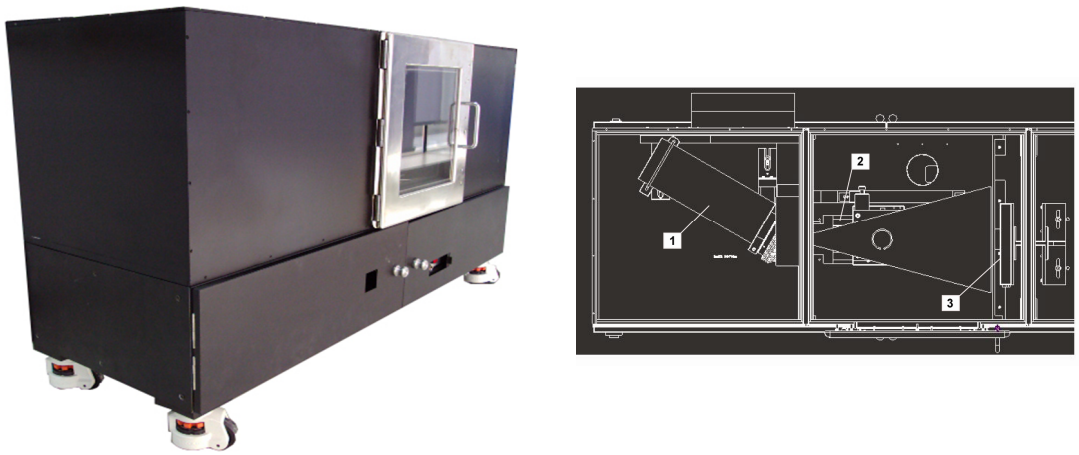
Medical Imaging 2011: Biomedical Applications in Molecular, Structural, and Functional Imaging, edited by John B. Weaver, Robert C. Molthen, Proc. of SPIE Vol. 7965, 79650L · © 2011 SPIE · CCC code: 0277-786X/11/$18 · doi: 10.1117/12.878410

2. MATERIALS AND METHODS

2.1 Overview of system

We adopted a high-resolution micro-CT system (ZKKS-MCT-sharp, Guangzhou Zhongke Kaisheng Medical Technology CO., Ltd, China) which is jointly developed by Guangzhou Zhongke Kaisheng Medical Technology CO., Ltd, Institute of Automation, CAS and Xidian University. High-quality three-dimensional anatomic structure information can be achieved based on advanced hardware setting and previous algorithm research by our group. Imaging system and corresponding reconstruction algorithm focus on hardware system construction and experimental test[4-6],and Feldkamp-Davis-Kress (FDK) cone-beam reconstruction algorithm on GPU acceleration scheme[7-8]and other algorithm improvement[9] and performance evaluation[10]. Our spatial resolution can reach to about 35μm at tested by phantom[5] (micro-CT phantom, QRM, Germany).



(a) (b)

Figure 1. High-resolution ZKKS-MCT-Sharp micro-CT imaging system.
(a) ZKKS-MCT-Sharp micro-CT imaging system. (b) Top view of the assembly of micro-CT system (1) X-ray tube. (2) X-ray detector. (3) Precise electronic driving translation stage

The micro-CT system is shown as Figure 1(a). It is performed by employing an X-ray tube (UltraBright X-ray tube, OXFORD INSTRUMENTS, X-ray technology. Inc, CA) with a focal spot size continuously adjustable from 13 to 40 μm which is accompanied by a CMOS flat panel X-ray detector (HAMAMATSU C7942CA-02, Japan) incorporating a 2240×2344 active pixel array with a 50 μm pixel pitch. An imaging chamber is setting between the X-ray tube and detector, and the sample holder can move on a precise electronic driving translation stage to change magnification rate. Figure 1(b) shows the assembly of our micro-CT system. The data are transferred to the host computer by a frame grabber card (IMAQ PCI-1424, National Instruments, TX) in the system. The Feldkamp-Davis-Kress (FDK) cone-beam reconstruction algorithm is integrated on acceleration scheme with commodity GPU accelerator card. Thus, it can achieve high-speed and high-quality three-dimensional reconstruction anatomy for 500 slices of the 512×512 projection images within 10 seconds. In this proposed contrast enhancement experiments, all the scan parameters setting are shown in Table 1.

Table 1. The scan parameters setting of micro-CT system

Tube voltage	Power	Trigger mode	Binning	Projection
60 kVp	50 watt	internal	1	1 frame/0.72°

2.2 Low cost contrast enhancement method

Animal models construction

All animal procedures were in accordance with Shanghai Institute of Materia Medica, Chinese Academy of Sciences approved animal protocol. In order to acquire metastasis animal models of gastric carcinoma, Eight-week-old BALB/c NuNu nude mouse models were established through inoculation 2×10^6 BGC-823 gastric carcinoma cells subcutaneously and remained in the mouse model for 4 weeks after inoculation. The animal models were best performed imaging that should maintain on a non-chow, vegetable or liquid food diet for 24-48 hours prior to study to minimize imaging artifacts due to minerals that are found in rodent chow.

Contrast enhancement strategy

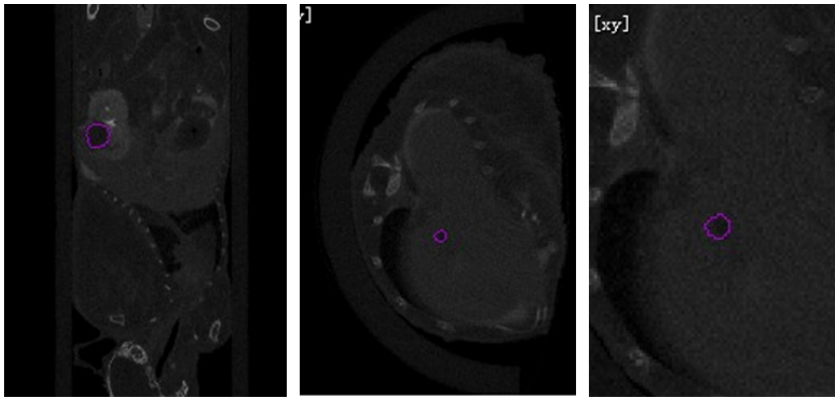
As we all know, omnipaque is widely used in clinical radioactive imaging. It is safe enough for low toxicity than special animal contrast agents. However, omnipaque allows contrast enhancement in murine animal within very short time after post-injection since its high heart beat and quick elimination with traditional tail vein injection. Therefore, Common omnipaque (350mg I/ml, GE Healthcare, China) was administrated through the route of intraperitoneal injection and was absorbed by organ tissue via continued accumulation. The administrations were performed at a dose of 90 mg Iodine every time for 4 times within 15 hours as shown in Table 2.

Table 2. Administration of omnipaque for micro-CT imaging

Number	Size of dose (Iodine: mg)	Time Point 0 point at the first injection	Route of administration	Injection method
1	90	0 hour	intraperitoneal	Multi-site injection
2	90	10 hours	intraperitoneal	Multi-site injection
3	90	13 hours	intraperitoneal	Multi-site injection
4	90	15 hours	intraperitoneal	Multi-site injection

3. MOUSE EXPERIMENT

In this mouse experiment, a random living BALB/c NuNu nude mouse was selected as the research object to evaluate the performance of the contrast enhancement method. The anesthetized mouse was placed on the warmed holder with its head positioned and secured in an anesthesia mask connected gaseous anesthesia (isoflurane 2 %, oxygen $0.3l\ min^{-1}$) to sustain sedation during imaging. Upon obtaining the scout view, the desired whole body region or an organ was selected as the anatomic landmark for image acquisition. Three typical slices of contrast enhancement effects with the common omnipaque contrast agent were shown in Figure 2, we can observe the pink metastasis mass located in left kidney, micrometastasis lesions in hepatic lobes. In this experiment, the scan parameters of micro-CT system were set as list in Table 1. After performing the semi-automatic segmentation of every slice, the three-dimensional results about the metastasis of gastric carcinoma were shown in Figure 3. The metastasis mass was clearly visualized in violet color in Figure 3. Furthermore, the volume of tumor could be calculated by our micro-CT software based on the integration of tumor voxel size. This approach could be used to assess tumor proliferation, metastasis and could be an effective tool for evaluating anti-tumor therapies.



(a) (b) (c)

Figure 2. The typical slices of contrast enhancement with the common omnipaque contrast agent, (a) a metastasis located in the left kidney, (b) a micrometastasis located in a hepatic lobe, (c) the amplified image of the micrometastasis of (b). (The pink region is the metastasis of gastric carcinoma)

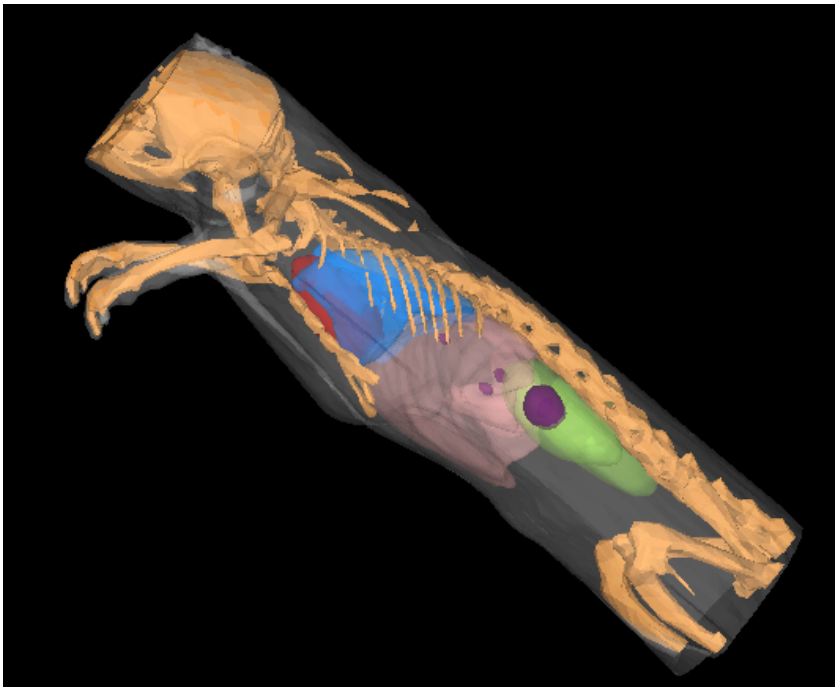


Figure 3. The result of three-dimensional results about the metastasis (the gold, red, blue, pink, green region refer to bone, heart, lung, livers and kidney tissue, respectively, and the violet region refers to several metastasis of gastric carcinoma)

4. RESULTS

In the present study, our micro-CT can be a useful tool for evaluation and monitoring the development of gastric carcinoma *in vivo*. The micrometastasis of gastric carcinoma distribute in the left kidney and hepatic lobes, the volume of metastasis is 20.27 mm^3 in kidney and 1.12 mm^3, 1.11 mm^3 and 0.78 mm^3 in the hepatic lobes through our micro-CT software. The actual volume and location of the metastasis were verified through autopsy. The low-cost common omnipaque contrast is effective to enhance the contrast between the organs and the tumor tissue with appropriate method.

Overall, our data suggest that this micro-CT system and imaging approach could be used to understand tumor proliferation, metastasis better and assess anti-tumor therapies.

5. DISCUSSION AND CONCLUSION

We have developed high-resolution micro-CT system (ZKKS-MCT-sharp, jointly developed by Guangzhou Zhongke Kaisheng Medical Technology CO., Ltd, Institute of Automation, CAS and Xidian University) which can provide high-quality three-dimensional anatomic structure information based on Feldkamp-Davis-Kress (FDK) cone-beam reconstruction algorithm on commodity GPU using an acceleration scheme. The present work concentrates on a novel low-cost contrast enhancement based on micro-CT system and algorithm platform mentioned above. The experimental data of the metastasis of gastric carcinoma BGC-823 in the whole body of the small animal suggest that the diameter less than 0.1 mm of micrometastasis can be detected with the aid of ZKKS-MCT-sharp micro-CT system together with an appropriate contrast enhancement method. At the same time, we find gastric carcinoma BGC-823 spread to right kidney and hepatic lobes from the primary location of tumor subcutaneous inoculation or distal tail vein injection. In the experimental process, it is an effective method that we adopted a low cost accumulative contrast enhancement in the organs and metastasis with several point-in-time injection of common omnipaque contrast agent.

We have to point out that some limitations of this study as following: 1) multi-site and several injections for one mouse bring much more work than special contrast one injection. 2) We could not guarantee the consistency of contrast enhancement effect in a large number of mice, it maybe reach about 90% success rates through previous data. However, this problem could be solved if we improve the injection technology.

In conclusion, *In vivo* micro-CT imaging is used for monitoring the tumor metastasis and proliferation. Especially, it can be performed in longitudinal imaging *in vivo* and avoid performing an autopsy of a large number of mice. A practical contrast enhancement method was adopted in the small animal *in vivo* experiment. The monitoring and analysis methods are effective in the practical application.

ACKNOWLEDGMENTS

This work is supported by the Program of the National Basic Research and Development Program of China (973) under Grant No. 2011CB707702, CAS Hundred Talents Program, the National Natural Science Foundation of China under Grant Nos. 81090272, 81000632, 30900334, the Shaanxi Provincial Natural Science Foundation Research Project under Grant No. 2009JQ8018, the Fundamental Research Funds for the Central Universities.

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