Technical Note: A Toy as Tool: A Low-Cost Image Analysis System for the Evaluation of Tumor Size in Experimental Small Animal Models

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ABSTRACT Image analysis systems are an essential tool in measurements of size of intraparenchymal tumors or lesions in experimental small animal models. Conventional image analysis systems are relatively expensive. We therefore compared the performance of a professional image analysis system with an inexpensive setup by evaluating tumor size in an orthotopic glioma mouse model. The maximum cross-sectional tumor area of H&E stained brain-slides of two groups of mice (treatment and control group) was measured by two independent investigators using a professional image analysis system (Leica DM IRB microscope) with the Leica Quantimet 500c software, and a low-cost-system (Intel QX3 microscope) with a non-commercial image analysis software. Mean tumor volumes were calculated and the results from each of the image analysis systems, investigators, and treatment effects were compared. The tumor volumes as measured with the low-cost and the professional system differed between −3.7 and +7.5% ($P = 0.69–0.99$). Measurements made by investigator A and B differed between −7.0 and +3.9% ($P = 0.69–0.88$). Treatment in all cases significantly reduced the tumor volume between 58.4 and 62.7% ($P = 0.0002$ or 0.0003), regardless of the investigator or the used image analysis system. We therefore conclude that the QX3 low-cost microscope in combination with a non-commercial image-analysis software represents an inexpensive solution to reliably analyze the size of regions of interest, if they provide a sufficient contrast. However, the low-cost setup due to its low resolution definitely limits a detailed analysis of histologic features. *Microsc. Res. Tech. 63:306–309, 2004.*

INTRODUCTION

Small animal models have become indispensable to evaluate the efficacy of experimental therapeutic strategies prior to their clinical application. Therefore, different in vivo settings have been developed to simulate different diseases like myocardial or cerebral infarction, metastatic spread of different kinds of tumors into different tissues, or orthotopic tumor models (Arnaud et al., 2003; Kunkel et al., 2001; Sampei et al., 2000; Taraboletti et al., 2002). All of these models have in common that the effect of a specific treatment is measured as the increase or decrease in size of a specific region of interest, e.g., a tumor, metastases, or an ischemic area.

The size of subcutaneously growing tumors can be easily quantified by sequential calliper measurements at any point during an experiment. When it comes to in vivo measurements of other regions of interest, which are not as easily accessible as subcutaneously growing tumors, expensive technical equipment, such as animal magnetic resonance scanners, are required. Those scanners are only available at very few institutions. Therefore, the animals are usually sacrificed at defined time points, and serial hematoxylin/eosin (H&E) stained sections of the tissue of interest are prepared. The size of the lesion or tumor can be evaluated using a standard light-optical microscope with a calibrated ocular grid, or even more simple, a ruler (Xu et al., 2003). However, it might be more accurate to use an image-analysis system to determine the maximum cross-sectional area of the object of interest. To do so, usually professional image analysis systems consisting of a video camera connected to a microscope and a personal computer are used. The costs for a complete image analysis system, or even only the costs to equip a pre-existing microscope with a camera and software, are considerable. Whereas a conventional light-optical microscope can be found in most institutions, the minimum costs for an upgrade can be estimated between $2,000–4,000. This does not necessarily include an image-analysis software, which will add additional costs of approximately $500–1,000.

As a less cost-intensive solution for accurate, fast, and easy analyses of histological sections would be desirable, we compared our laboratory's standard professional image analysis system with a particularly inexpensive microscope (Intel QX3), that was initially not intended for professional laboratory use and at the

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time of the study available for approximately $60. To further reduce costs, a freely available, non-commercial image analysis software was used in combination with the QX3 low-cost microscope.

In one of our previously published studies, we analyzed histological sections of xenografted mouse brains with our laboratory's standard professional image analysis system and found that tumor size was reduced significantly in the treatment group compared to the control group (Brockmann et al., 2003b). In the present study, we reanalyzed these tumor slides from the previous study first using our professional image analysis system, and second using the QX3 low-cost system. The results obtained by two independent investigators with both systems were compared for differences between the image analysis systems, the interobserver-variability, and for differences between the treatment and the control group when analyzed by both investigators with both systems.

METHODS

Determination of Tumor Size

Paraffin sections from mice bearing orthotopic glioma grafts as previously published (Brockmann et al., 2003b) were reanalyzed. Using a guide screw system (Brockmann et al., 2003a; Lal et al., 2000), one group of mice was treated by daily intratumoral injections of an anti-tumor agent (treatment group), the second group received daily injections of buffer solution only (control group). Serial coronal sections (5 μm thick) of the mouse brains were H&E stained. The maximum cross-sectional area of the intracranial glioblastoma xenografts was determined using our laboratory's standard professional image-analysis system (Leica Quantimet 500; Leica, Hamburg, Germany) and the low-cost Intel Play QX3 Computer Microscope (Intel Corporation, Santa Clara, CA). The measurements were performed by two independent investigators using both image analysis systems.

Our standard professional image analysis system consisted of a JVC TK-1280E-colour-video-camera (JVC, Friedberg, Germany) that was attached to a Leica DM-IRB inverted research microscope (Leica, Bensheim, Germany) and connected to a personal computer fitted with a special framegrabber-card driven by Leica's Quantimet 500c software. All measurements (including calibration) were conducted at 30× magnification. The software was calibrated to a standard-scaled-slide (2 mm). Afterwards, the tumor sections were placed under the microscope and the tumor area [mm^2] was assessed using the Quantimet 500c software.

The low-cost image-analysis system consisted of an Intel Play QX3 Computer Microscope (Fig. 1A) that was connected to a personal computer's USB-port (Universal Serial Bus) with no additional framegrabber-card required. Acquisition of images was performed using the software-interface that was supplied with the microscope. Images of the standard-scale (2 mm) and the tumor sections were saved at 10-fold magnification, exported in bitmap format and saved to the computer's hard drive. Then the images of the standard-scale and of the tumor slides were opened using the UTHSCSA Image Tool software (University of Texas Health Science Center San Antonio Image Tool Ver. 3.00), which is available as freeware for Internet download (Wilcox et al., 2002). The software was calibrated to the image of the standard measure-scale and maximum tumor areas (mm^2) were determined.

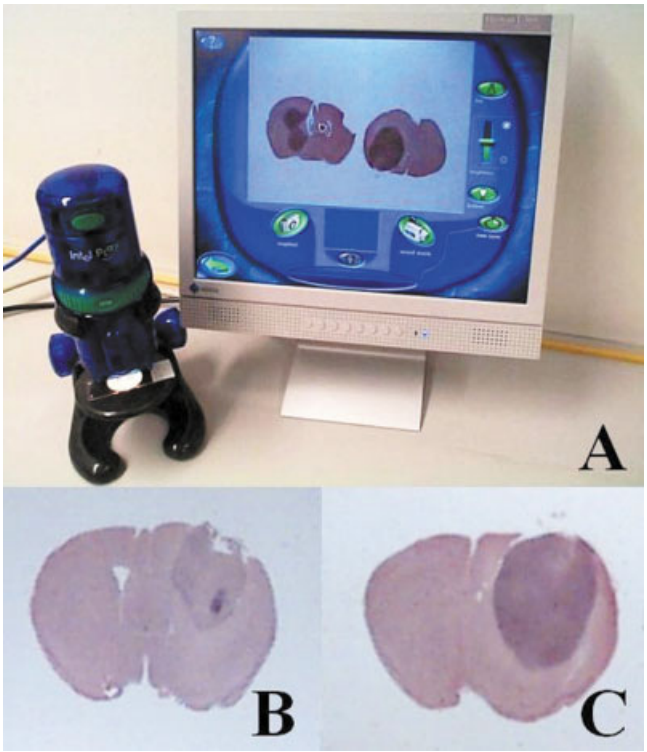

Fig. 1. **A:** The low-cost image analysis system (Intel Play QX3 Computer Microscope) is connected to a standard personal computers USB-port. For analysis of translucent objects, the bottom light has been turned on. Magnifications (10×, 60×, 200×) can easily be changed by manually rotating a barrel, which contains three lens tubes. The software provided with the microscope is active and shows a histological sample slide on the monitor. B+C, representative size examples of intracerebrally grown tumors from mice of the treatment group (**B**) or the control group (**C**). Paraffin-embedded sections were stained with hematoxylin-eosin. Images were taken at 10× magnification using the QX3 low-cost system and the supplied software. The low-resolution CMOS-sensor (Complementary Metal Oxide Semiconductor) of the low-cost system results in moderately blurred images, which compromises thorough analysis of histological details, but allows analysis of the maximum cross-sectional tumor area. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

In both setups, the tumor volume was estimated using the formula: volume = (square root of maximal tumor cross-sectional area)^3.

Statistics

The differences in tumor volume measured by investigator A and investigator B using the Leica Quantimet 500c system or the Intel Play QX3 Computer Microscope in combination with the UTHSCSA image analysis software were analyzed.

To determine the "inter-observer" difference between investigator A and B, the groups were matched for treatment (anti-tumor agent or buffer solution) and the microscope used. The results obtained by investigator A were set to represent 100%. The differences between investigator A and B were calculated as the percentage of the volumes measured by investigator B compared to the results of investigator A. The results were analyzed using an unpaired *t*-test.

TABLE 1. Comparisons of tumor volume [mm³] measurements by investigator A and investigator B using the Leica Quantimet 500c system or the Intel Play QX3 computer microscope in combination with the UTHSCSA image analysis software[1]

	Leica	QX3	Microscope variability
Control group			
Investigator 1	23.3 ± 8.9	22.5 ± 8.4	−3.7% (P = 0.83)
Investigator 2	21.7 ± 7.9	23.3 ± 8.9	+7.5% (P = 0.69)
Inter-investigator variability	−7.0% P = 0.69	+3.9% P = 0.83	
Treatment group			
Investigator 1	8.7 ± 3.7	9.4 ± 3.8	+7.3% (P = 0.71)
Investigator 2	9.0 ± 4.0	9.0 ± 3.7	−0.2% (P = 0.99)
Inter-investigator variability	+3.2% P = 0.88	−4.0% P = 0.82	

[1]Shown are the results for tumors of the control group, and the size of tumors in mice of the treatment group. The Leica system and investigator A were set to 100%. No significant differences within the treatment or the control group were detected comparing the measurements made by investigator A and B, or the data acquired with the low-cost QX3 computer microscope and our laboratory's standard professional Leica Quantimet 500c system.

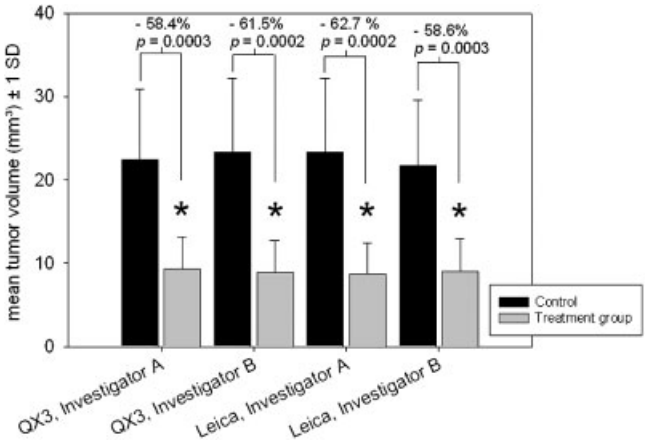

Fig. 2. Differences between the treatment and the control group as analyzed by two independent investigators using the QX3 low-cost system or our standard professional image analysis system (Leica). In all cases, treatment resulted in a significant reduction of tumor size compared to the control group. The mean tumor volume was reduced by 58.4–62.7% with similar P values, thus demonstrating independence of the results from the investigator or the image analysis system used.

To determine the differences between our standard professional system (Leica) and the low-cost system (Intel QX3), the results obtained with the professional system were set to 100% representing our laboratory's standard technique. The measurement groups were matched for treatment and investigator, and the difference between both systems was analyzed as the percentage of values obtained by the low-cost system compared to our professional image analysis system. The results were analyzed using an unpaired t-test.

Finally, the groups were matched for investigator and image analysis system, and the effects of the treatment (anti-tumor agent vs. buffer solution only) upon reduction of tumor size were determined. An unpaired t-test was used to compare the effect of treatment to the control group. In all cases P values < 0.05 were considered to be statistically significant.

RESULTS

Table 1 shows the inter-investigator-variability between investigator A and investigator B, which ranged between −7.0 and +3.9%. No statistically significant difference was found (P = 0.69–0.88). Similarly, the differences between the professional and the low-cost system ranged between −3.7 and +7.5%, which again was not a significant difference between both systems (P = 0.69–0.99; Table 1). When the effect of the different medications upon tumor size was analyzed, the treatment group presented with a significantly reduced mean tumor volume. The treatment effect ranged between 58.4–62.7% and could be demonstrated regardless of the investigator and the image analysis system used (Fig. 2). The significance level remained unchanged for the different evaluations (QX3+Investigator A: −58.4% (P = 0.0003), QX3+Investigator B: −61.5% (P = 0.0002), Leica+Investigator A: −62.7% (P = 0.0002), Leica+Investigator B: −58.4% (P = 0.0003).

DISCUSSION

Professional image analysis systems can be considered relatively inexpensive compared to other laboratory devices. Nevertheless, a considerable amount of money still has to be invested in order to purchase one of those systems that usually consist of a number of highly specialized components. First of all, a light-microscope fitted with an adapter to connect a camera to it is a prerequisite as well as a digital or conventional camera connected to the microscope. The camera itself in most cases is connected to an interface card, a so-called framegrabber, which again is mounted in the PCI (Peripheral Component Interconnect) or ISA (Industry Standard Architecture) slot of a personal computer. Finally, an image-analysis software is needed to perform the measurements. Assuming that an appropriate microscope is available, the minimum costs for the remaining equipment needed to assemble a low-quality image-analysis system range around $2,000–4,000. This does not necessarily include an image-analysis software, which may cost an additional $500–1,000. When particularly needed for highly specialized implementations, as in the underlying study, a less cost-intensive solution would be desirable.

The Intel Play QX3 Computer Microscope is available for approximately $60 and was originally designed for children as a tool to provide easy-to-use photomicrography at an affordable price. The QX3 uses a CMOS-sensor with a CIF (Common Interchange Format) resolution of 352×288 pixels. However, the camera hardware is set up to transmit only a subset of the sensor array (320×240) centered in the middle of the array, which prevents a degradation of the color quality of the pixels at the perimeter and reduces lens distortion, for which pixels along the outer edge are more susceptible (Jelinek et al., 2001). The microscope comes with three preset magnification options, which are 10×, 60×, and 200×. It offers a manageable size and a weight that allows the researcher to transfer the microscope easily to another laboratory or even to take the microscope with him in order to scan larger numbers of slides anywhere. A software to view, capture, and export images is provided with the microscope. The images can be exported using bitmap or jpg (Joint Photographic Expert Group) format for subsequent

data analyses and can be stored digitally. The computer microscope relies on a single USB-port connection for power supply and data transmission and there is no need to install any additional frame-grabber-cards, thus improving its ease of use, reducing costs, and offering its use in combination with a standard notebook computer.

The differences in the tumor volumes, analyzed by the low-cost and our laboratory's standard professional image analysis system, ranged between −3.7 and +7.3% and the measurements of both investigators differed between −7.0 and +3.9%, which was not significant. Because the variations between both microscope setups and the inter-observer variance are similar, we suppose that the differences between the low-cost and the professional system are not attributable to differences between both systems. This is supported by the results we obtained from comparing the effects of treatment, which in all variations of investigator and used image analysis system showed similar results with only slight deviations and almost equal *P* values. Therefore, the QX3 in combination with the non-commercial scientific image analysis software as provided by the UTHSCSA can be considered a sufficient tool at least for image analysis of tissues with a high contrast, such as glioblastoma xenografts and normal brain as presented in our study. In our study, the reduced image resolution of the low-cost setup (QX3/Image Tool) is likely to be limited by the microscope itself rather than the image analysis software. Therefore, a cross-performance analysis comparing the combination of the low-cost microscope with the Quantimet 500c-software and vice versa was not performed. However, the possibility should be kept in mind that a "bottleneck" in image analysis determining the image quality may be the result of both the performance of the microscope as well as the processing software.

The most striking limitation of the low-cost system certainly lies in its restriction to display images with a resolution as low as 320 × 240 pixels, which results in moderately blurred images and hampers a thorough assessment of histological details (Fig. 1B,C). Furthermore, the low resolution can make differentiation of tumor tissue from the surrounding brain difficult, especially in the rare occasion that H&E staining produces a low contrast. Therefore, a good and strong H&E stain is crucial in the evaluation of tumor size using the low-cost system (Fig.1B,C). Increasing the magnification from 10× to 60× in order to compensate for the lack of contrast can result in an incomplete depiction of the tumor, which in many cases exceeded the field of view (FOV) in the present study. Routines provided by the image analysis software to enhance contrast did not result in an improved assessability of the images with a faint tumor/brain-contrast. Software tools offered by other programs like Photoshop (Adobe Systems, San Jose, CA) only slightly increased the contrast. Another problem associated with the QX3's low resolution is a more difficult calibration, because the used standard-measure-slide is, like the tumor slides, blurred at 10× magnification. Increasing magnification to 60× improves the image quality of the standard measure, thus easing calibration. But as described above, tumors often exceed the FOV at 60× magnification, which makes analysis of these tumors impossible. Finally, working with the low-cost system is slightly more time-consuming because of the need to scan the images and export and reload the data files prior to the measurements.

A product similar to the QX3 is the so-called "MIC-D" inverted digital microscope manufactured by Olympus, which, like the QX3, is connected via a computer USB-port and comes with a software for acquisition of images. The MIC-D has not been tested by the authors, but it can be assumed that this microscope closes the gap between the described low-cost solution (QX3) and other professional image analysis systems. The noticeable advantage of the MIC-D is its improved CMOS image sensor, which allows a resolution of more than 300,000 pixels, which is a more than 4× higher resolution than that of the QX3. Another advantage of this system is the increased number of zoom magnification-steps, which is made possible by a completely different microscope construction and potentially could help to achieve optimal magnification levels. Unfortunately, the MIC-D also closes the gap regarding the costs, as the MIC-D is more than 10 times the cost of the QX3, ranging between $700–900. As for the microscope system, there also may be some alternative for image analysis softwares. Programs currently available for free as Internet downloads are *NIH Image* (Macintosh), *Scion Image for Windows*, *VayTek Image* (Windows), or *Image/J*, which is system-independent as a Java program.

In conclusion, the combination of the QX3 microscope and a non-commercial image-analysis software proved to be a sufficient, easy-to-use, and particularly inexpensive tool to accurately determine the size of well-delineated regions of interest, as demonstrated in this study. However, due to the low resolution of the QX3, its use remains limited to the analysis of well-delineated, high-contrast tissues.

REFERENCES

Arnaud C, Godin-Ribuot D, Bottari S, Peinnequin A, Joyeux M, Demenge P, Ribuot C. 2003. iNOS is a mediator of the heat stress-induced preconditioning against myocardial infarction in vivo in the rat. Cardiovasc Res 58:118–125.

Brockmann MA, Lamszus K, Westphal M. 2003a. Improved method for the intracerebral engraftment of tumour cells and intratumoural treatment using a guide screw system in mice. Acta Neurochir (Wien) 145:777–781.

Brockmann MA, Papadimitriou A, Brandt M, Fillbrandt R, Westphal M, Lamszus K. 2003b. Inhibition of intracerebral glioblastoma growth by local treatment with the scatter factor/hepatocyte growth factor-antagonist NK4. Clin Cancer Res 9:4578–4585.

Jelinek L, Peters G, Okuley J, McGowan S. 2001. Dissection of the Intel Play QX3 Computer Microscope. Intel Technol J 5:1–10.

Kunkel P, Ulbricht U, Bohlen P, Brockmann MA, Fillbrandt R, Stavrou D, Westphal M, Lamszus K. 2001. Inhibition of glioma angiogenesis and growth in vivo by systemic treatment with a monoclonal antibody against vascular endothelial growth factor receptor-2. Cancer Res 61:6624–6628.

Lal S, Lacroix M, Tofilon P, Fuller GN, Sawaya R, Lang FF. 2000. An implantable guide-screw system for brain tumor studies in small animals. J Neurosurg 92:326–333.

Sampei K, Goto S, Alkayed NJ, Crain BJ, Korach KS, Traystman RJ, Demas GE, Nelson RJ, Hurn PD. 2000. Stroke in estrogen receptor-alpha-deficient mice. Stroke 31:738–743.

Taraboletti G, Micheletti G, Rieppi M, Poli M, Turatto M, Rossi C, Borsotti P, Roccabianca P, Scanziani E, Nicoletti MI, Bombardelli E, Morazzoni P, Riva A, Giavazzi R. 2002. Antiangiogenic and antitumor activity of IDN 5390, a new taxane derivative. Clin Cancer Res 8:1182–1188.

Wilcox D, Dove B, McDavid D, Greer D. 2002. UTHSCSA Image Tool for Windows 3.0. http://ddsdx.uthscsa.edu/.

Xu S, Gade TP, Matei C, Zakian K, Alfieri AA, Hu X, Holland EC, Soghomonian S, Tjuvajev J, Ballon D, Koutcher JA. 2003. In vivo multiple-mouse imaging at 1.5 T. Magn Reson Med 49:551–557.