Comparative Medicine
Copyright 2008
by the American Association for Laboratory Animal Science

Vol 58, No 3
June 2008
Pages 264–270

A Structured Light-based System for Scanning Subcutaneous Tumors in Laboratory Animals

Ibrahim Cem Girit,[1,*] Maria Jure-Kunkel,[2] and Kim W McIntyre[3]

Tumor size or volume is often the primary endpoint in preclinical efficacy studies of anticancer drugs. Efficient and accurate measurement of such tumors is crucial to rapid evaluation of novel drug candidates. Currently available techniques for acquiring high-throughput data on tumor volume are time-consuming and prone to various inaccuracies and errors. The laser-scanning technology we describe here provides a convenient, high-throughput system for tumor measurement that reduces interoperator variability and bias while providing automated data collection, processing and analysis.

Abbreviations: 3D, 3-dimensional

Cancer is the second-leading cause of death in the United States, with heart-related diseases being the first. Approximately 1.44 million new cases will be diagnosed in 2007, with 560,000 deaths.[1] Overall cancer death rates have decreased since 1990, mainly due to early detection of certain cancers such as prostate and breast, but cancer incidence for some tumor types has increased.[3] Given various changes in demographics, the incidence of cancer in the United States likely will double by 2050.[4] These statistics point to the urgent need of improved treatment modalities.

Identification and selection of potentially active anticancer agents has largely been based on screening large numbers of compounds, aided by rational drug design when the molecular structure of the target is known. Once compounds have been selected on the basis of specificity for the target and desired functional effect, the ultimate test for advancement of a compound to clinical evaluation is to show its safety and efficacy in animal tumor models. Subcutaneous human tumor xenograft models are used widely because they recapitulate many aspects of the biology of human tumors, including sensitivity to anticancer agents.[8] Tumors can be implanted subcutaneously in mice as fragments by the use of a trocar or as cell suspensions. The large subcutaneous space in rodents allows for continuous growth of the tumor.

The methods used by the industry to measure tumor characteristics vary in technical approach, versatility, performance and cost. General properties of these methods are summarized in Table 1.

Although many commercial and experimental devices are available for determination of tumor volume from 3-dimensional (3D) images, systems such as computed axial tomography, positron emission tomography, single-photon emission computed tomography, and magnetic resonance imaging are primarily designed to gather 3D information from multislice data. They are primarily designed for biological, chemical, and functional studies of animal models. In most cases, these devices are too large (even those developed especially for small-animal studies, that is, 'micro' versions of positron emission tomography, single-photon emission computed tomography, and magnetic resonance imaging) for use in an animal room. Because, they require extensive preparation efforts and expensive materials (radioactive sources, contrast agents, fluorescent chemicals, and so forth), these systems are impractical for high-throughput animal testing facilities.

Assessment and Measurement of Subcutaneous Tumors in Rodents

The caliper technique is the most commonly used method for large studies. Its cost is minimal, but it is the least accurate method among available techniques for this purpose. Caliper measurement requires no preparation time, and measurements take only 7 to 10 s per animal. Animals do not require any special restraint or anesthesia for measurement of tumor size. However, several factors influence the validity of caliper measurements. Sources of error include the measurement process itself: inadequacy of the volume formulas used to define tumors;[2] inconsistent measurement of tumor axes at subsequent examinations; differences in tumor measurement between different investigators; and the difficulty associated with measuring small tumors.

Generally only 2 dimensions of the tumor are measured to calculate the tumor volume, length (L) and width (W). These measurements also assume a certain tumor shape. Practice demonstrates that the tumor shapes are more or less variable (Figure 1). The justification usually cited for the method is that only the *relative* size of tumors matters. However, because the 2 axes along which size is measured are not documented and because different axes can be chosen by different operators (or the same operator) at each measurement, the reliability of even relative comparisons is questionable.

A study comparing 19 different formulae used in the literature[8] concluded that the ellipsoid volume formula based on the measurement of 3 axes was the most accurate for estimating tumor growth ($r = 0.93$). However, calipers are not designed to obtain an accurate measurement of the height of the tumor, therefore; only 2 dimensions are used for calculations of volume. This practice assumes that the tumor shape is an ellipsoid of rotation, for which

Received: 26 Oct 2007. Revision requested: 5 Dec 2007. Accepted: 8 March 2008.
[1]Biopticon Corporation, Plainsboro, NJ; [2]Oncology Drug Discovery, [3]Immunology Drug Discovery, Bristol-Myers Squibb, Research and Development, Princeton, NJ
[*]Corresponding author. Email: girit@comcast.net

Table 1. Comparison of tumor size measurement techniques

Technology	Special requirements	Accuracy (mm)	Speed (min)
Caliper	None	not applicable	>0.3; no prep time
Microcomputed axial tomography	Anesthesia, radioactive tracers	0.05	5–30; prolonged prep time
Magnetic resonance imaging	Anesthesia, contrast agents	0.025	>10; prolonged prep time
Micro positron emission tomography	Anesthesia, radioactive tracers	>1.85	5–15; prolonged prep time
Micro single-photon emission computed tomography	Anesthesia, radioactive tracers	>0.5	>30–240; prolonged prep time
Ultrasonography	Anesthesia	0.030	5–10
Structured light scanner	None	<0.3	0.05; no prep time

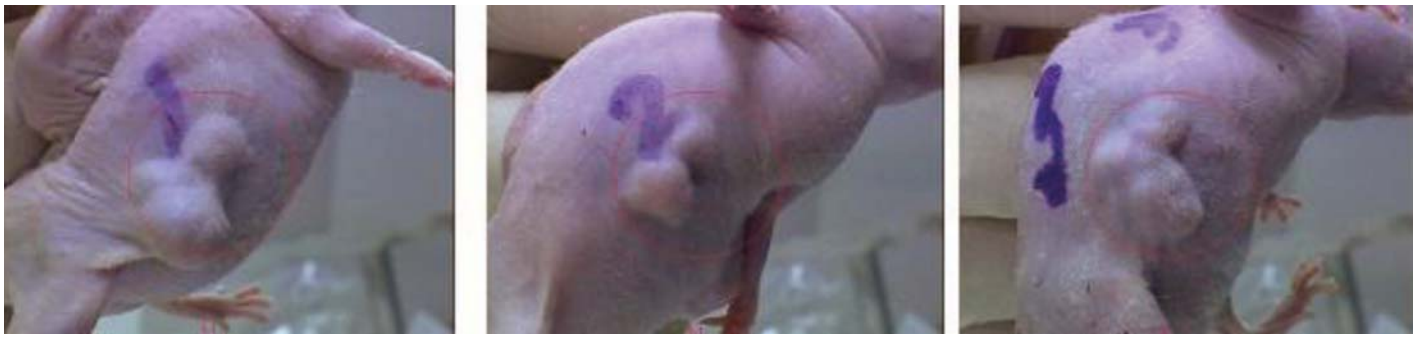

Figure 1. Examples of subcutaneous tumors.

the volume formula is $\pi/6 \times L \times W \times H$. Given the assumption that the height of the tumor is the same as the width or length of the tumor and approximating $\pi/6$ to 0.5 or 0.4, this formula is then reduced to a generally accepted one: 0.5 (or 0.4) $\times L \times W \times W$. The commonly used coefficients 0.4 and 0.5, therefore, are 24% and 5% different than the real coefficient, $\pi/6$, respectively. Even though this coefficient should be defined as $\pi/6$ instead of 0.5 (or 0.4), it is acknowledged that the actual shape of tumors is in general more complex than a simple ellipsoid of rotation.[2] Therefore uncertainty regarding tumor shape can introduce at least 25% error in volume measurements. Additional errors as high as 27% were introduced by a single operator performing repeated caliper measurements when small masses were measured.[5] These errors are due to the arbitrary choice of measurement axes and deformation of the tumor by the measuring instrument.

Tumor shape does not follow the initial semiellipsoid shape as the mass grows. Toward the end of measurement period, a tumor's shape deviates highly from an ellipsoid. Therefore the measurements obtained toward the end of tumor growth surely would lead to larger volume errors for differences between the control and treatment groups.

The accuracy in volume measurements becomes important given that the practice of taking the mean of tumor measurements in a group of animals may be statistically flawed, because the data points on the growth curve are not statistically independent: the largest tumor in a group of animals on one day of measurement will probably still be the largest on the next day of measurement.[7]

Other possible sources of errors are the extensive involvement of human operators in recording and processing the measurement data (transcription errors) and the use of invalidated and simple spreadsheets to store and analyze data (template manipulation).

Finally, for necrotic tumors, the volume of missing mass cannot be taken into account by caliper measurements. Because of this limitation in caliper-based measurements, mice with such tumors sometimes are discarded at later stages of tumor model studies, causing not only loss of valuable time but also increased cost of preclinical cancer studies.

In some instances, researchers use electronic calipers to record measurements directly to a data file spreadsheet, eliminating errors in the transfer of data.[6] Although this method eliminates the errors associated with transcribing results to a computer, it does not resolve all of the problems mentioned earlier.

An ideal solution would not only eliminate the sources of measurement and analysis errors, but also increase the productivity and efficiency of the process. The optical method we propose here is a noncontact method, yielding more reliable measurements by avoiding the problems listed earlier. Our method permits researchers to visually compare tumors at different stages of their development. It does not require anesthetizing, and does not require administration of radioactive compounds, contrast agents, and other chemicals. Our method also supports immediate automated input of all the data into computer databases, which in turn enables automated statistical analysis of data, visual comparison of results, and automatic generation of reports, as does any other *dedicated* computer software.

Increasing the accuracy of measurement-based results will improve the scientific integrity of small-animal model studies by eliminating intrinsic and operator-dependent measurement and transcription errors. The gain in productivity can translate itself in many ways: improving the value of the operation by decreasing manual and repetitive tasks, thus allowing researchers to focus efforts on higher value areas; by accommodating more studies in the same time period; reducing labor costs; and allowing for cross-laboratory validation of experimental results.

Concepts in Systems Development

Imaging problem definition. The goal of our work has been to develop an imaging system to record and measure the 2D projection and 3D surface structures, such as subcutaneous tumors, on test animals' bodies without contact. The system must be capable of extracting the absolute (*x, y, z*) coordinates of points from the sensed animal body surface. In the case of measurements of flat lesions, the absolute (*x, y*) coordinates will be sufficient.

The following major digital image tasks are involved in setting up and using the system:

1) Calibrating the imaging system—a necessary procedure for the recovery of the absolute (*x, y, z*) coordinate information and (*x, y*) projection shape;

2) Sensing the 3D surface—the (*x, y, z*) map of the surface of interest is retrieved;

3) Isolating the object(s) on the surface of interest—by analyzing the (*x, y, z*) map retrieved in Step 1 and (*x, y*) for projection; and

4) Calculating the volume or area of the object(s).

To achieve these objectives, an appropriate and efficient image processing technique for quick and accurate measurements is needed. The following section outlines our efforts to develop this technique.

Scanner design considerations. Various structured light pattern techniques were studied for their suitability for tumor measurements. The moving line technique over a grid pattern (dots or lines, colors, and so forth) was chosen because of the difficulty in recognizing the start of patterns generated by using other techniques.[5] If the initial pattern is missed, for example, because of shadowing effect, no surface formation or volume calculation can be carried out. Such techniques therefore use extra reference patterns or sensors to overcome this difficulty. However, the moving structured pattern technique eliminates the problem of recognizing the start of patterns because patterns coordinates always are known: they are created under computer control. A potential drawback of the moving stripe approach is that it takes multiple video frames to capture an object's geometry. Our tests show that about 150 to 250 laser stripe positions provide a sufficient dataset to cover tumors surface with sufficient accuracy along the *y* direction. Processing this number of frames by the moderately high-speed digital cameras we used takes 3 s or less.

A parallax-based laser stripe method[5] was used for the scanner design. The laser projects a stripe on the object and the image of the stripe is captured by a video camera. Parallax between the laser stripe source and the video camera is used to determine distance to the object. The advantage of the laser stripe–video camera system is that careful calibration of the laser stripe source position relative to the camera permits precise calculation of the distance. The laser stripe is moved to another nearby position, and the object is scanned again.

Laser Scanner

The structured light scanner consists of the scanner head, a stand, and the electronic control module (Figure 2 A). The scanner head contains the laser light source and integral camera. The head can be attached to a portable stand, but it also can be mounted to a wall or laboratory hood. The portable unit is ergonomic in that it can move and rotate around multiple axes, providing comfort and ease-of-use for virtually any operator.

To simplify reproducible placement of the tumors within the optical limits of the scanner, a stationary platform (or 'mask') with a 30-mm diameter hole placed in optical axis at 10 cm from the camera (Figure 2 A) is used. When the mouse is placed under this mask, the tumor and surrounding healthy skin are exposed to the camera through the circular opening, providing a well-defined geometry for tumor segmentation. Figure 2 B shows a tumor and the surrounding healthy skin as seen by the camera through the mask. Masks with different diameters can be used for mice or rats to accommodate tumors of different sizes. Masks also can be made of materials that can be sterilized easily.

The electronics box contains the necessary control modules. Two cables connect the scanner to a computer: a Firewire cable for image-related data and a USB cable for control. In addition, the scanner head is connected to the control box by 2 cables.

Algorithms for calculation of tumor volume. The scanner defines a set of surface points on the skin of the mouse, which are described in terms of their (*x, y, z*) coordinates. The healthy mouse skin surface and tumor surface should be recognized as distinctively separate from each other. Although determining tumor boundaries by eye and tumor volumes by plethysmometry are straightforward, automating the process by software-based tumor scan analysis requires sophisticated algorithms. The input to the algorithm is a point cloud, a set of coordinates (*x, y, z*) delineating the tumor and surrounding area. The program first processes the raw point cloud data into a form suitable for analysis. The program then determines the boundary of the tumor, excluding noncancerous tissue. The tumor volume is calculated from the raised surface above the tumor, geometrically 'cut off' by an interpolated plane based on the healthy skin around it. This calculation requires defining parameters such as the plane's position and orientation. The algorithm following outlines the necessary computational steps.

Parametric surface algorithm. This algorithm fits a parametric surface to the skin surrounding the tumor through repeated application of least-squares fitting, such that tumors are defined as outliers above the parametric surface. The data are incorporated into the model, starting from the border of the surface and successively moving inward to the center. The steps of calculations are:

1. Initialize the system by setting
    a. the scanner data noise boundary,
    b. the radius of the hole in the mask,
    c. the lowest depth below the mask to be considered valid data,
    d. the number of border pixels to exclude,
    e. a z-score statistic to determine to boundary of the tu mor, and
    f. the number of iterations used for the estimation pro cess.
2. Read point cloud data representing data points on the surface of the tumor within the mask.
3. Convert the point cloud data to a height map.
4. Least-square fit these data to the function

$$h(i, j) = c1*i2 + c2*j2 + c3*i + c4*j + c5,$$

where *i* and *j* are indices of data points along *x* and *y* direction, and c_k are coefficients to be determined.

5. Calculate the center and pixel density of the surface within the mask in *x*, *y* and *z* directions

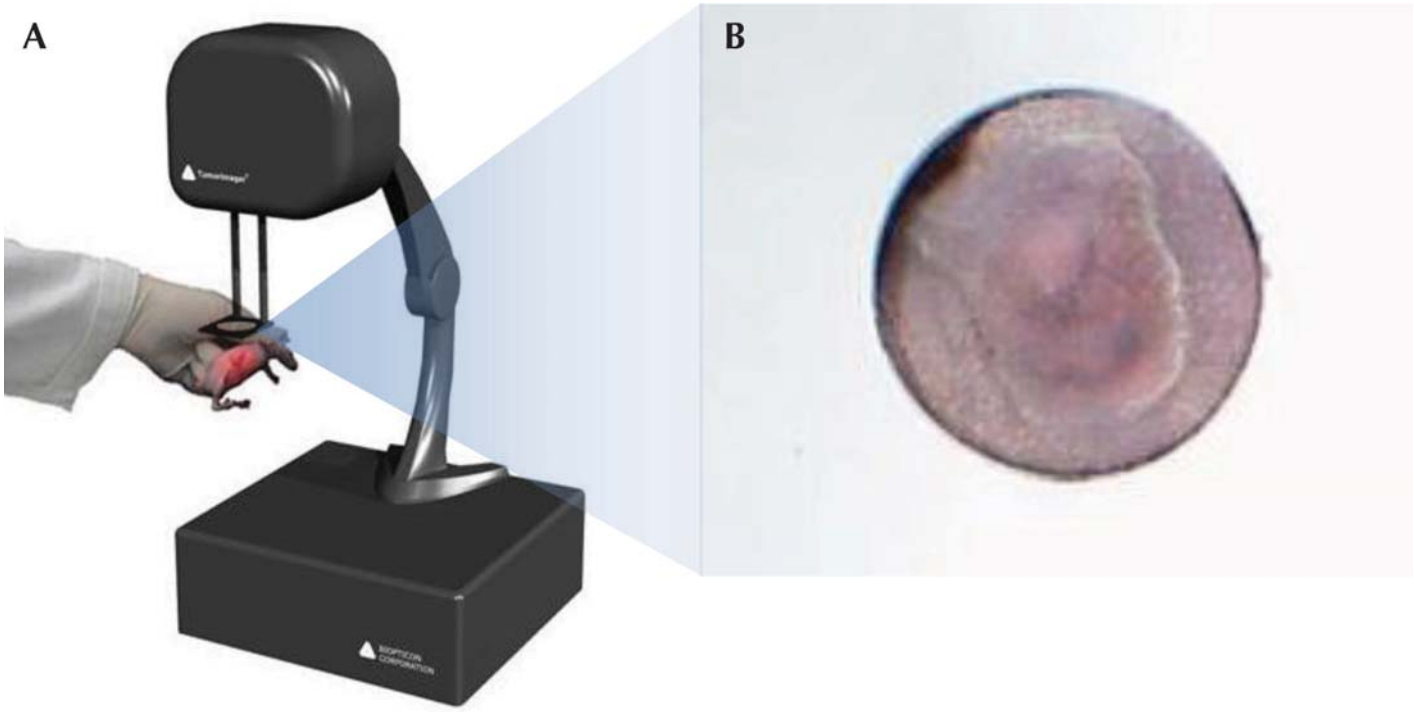

Figure 2. (A) The structured light scanner. (B) Tumor as seen through the measurement mask.

6. Remove the data below the mask.
7. Fit the parametric function

$$h(i, j) = p1*i2 + p2*i*j + p3*j2 + p4*i + p5*j + p6$$

to data representing the surface surrounding the tumor
8. Identify the outliers by using this stochastic model iteratively.
9. Calculate the tumor volume as the difference between 2 surfaces within the outliers.

Measurements

Reference measurements. Before the tumor segmentation and volume calculation algorithms were applied on real tumors, they were first evaluated on a variety of tumor phantoms with known volumes (as measured by a plethysmometer) made out of sculptor's clay (Figure 3). These tumor phantoms were modeled after real tumors and represented a large variation in shape and size.

Tumor phantoms were first measured by using a Fowler Slyvac caliper, and their volumes were calculated by using the formula, $0.5 \times L \times W \times W$, where L and W are the length and width of the tumor, respectively. Each tumor phantom then was scanned. The number of scan lines was set to 120 to 190 lines per scan, providing resolution of 0.15 to 0.25 mm along scan direction. The accuracy of the volumes determined by the laser scanning technique was compared with those determined by use of a plethysmometer (model 7140, Ugo Basile, Comerio, Italy). We also compared the volumes of the tumor phantoms as obtained with the standard caliper technique.

After all the measurements were completed, the difference between each of the measured values and its actual size as determined by plethysmometry was calculated for each tumor. The difference (mean ± 1SD) between the values derived from the plethysmometer and those from the scanner (–0.03 ± 0.01 mm^3) was markedly smaller than that between the plethysmometer and caliper (–0.20 ± 0.06 mm^3). The slope of the linear regression line (1.02, $R^2 = 0.995$) for the volumes from the scanner versus the plethysmometer (Figure 4) suggests near perfect correlation between the measurements obtained by these 2 techniques. In contrast, caliper measurements appear to overestimate the 'true' volumes of the tumor phantoms as determined by the plethysmometer (slope, 1.328). The caliper data also show a tendency toward greater variability (scatter) compared with plethysmometer measurements ($R^2 = 0.901$).

Tumor measurements. No animals were purchased or used solely for the purposes of this study. All animals used for the evaluation and testing of the scanner were animals already enrolled in studies conducted for routine drug discovery research purposes at our institution. These studies have been reviewed and approved by our institutional animal care and use committee. The studies are conducted in a fully AAALAC-accredited facility. The technique described here does not require anesthetization or specialized restraint of animals. Female BALB/c athymic mice (*nu/nu*), 6 to 7 wk old, were purchased from Harlan Sprague Dawley (Indianapolis, IN). L2987 (pulmonary adenocarcinoma) and GTL16 (gastric carcinoma) cells were passaged subcutaneously as tumor fragments by using 13-gauge trocars. Measuring of subcutaneous tumors was initiated once tumors reached 50 to 100 mm^3 in size (approximately 10 to 20 d after implantation).

Tumors were measured first with calipers and then were scanned by the same operators, as described earlier. Animals were scanned by placing them under the mask and activating the scanning sequence. If an animal moved during the scan, it was rescanned. The volume of the measured tumor was calculated automatically by the program and was displayed on the computer screen. The operator could check whether a tumor was scanned

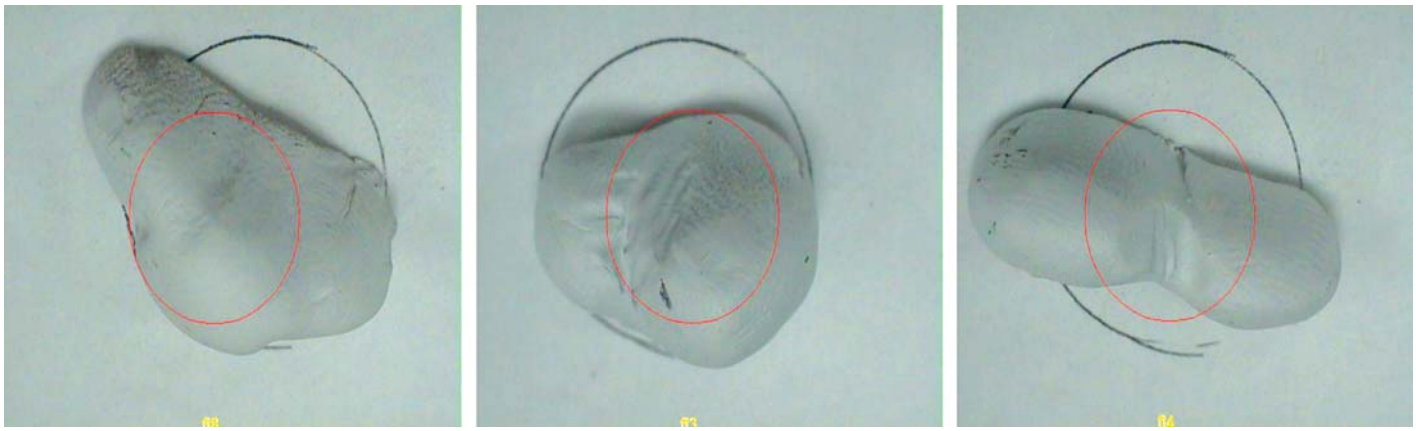

Figure 3. Samples of tumor phantoms.

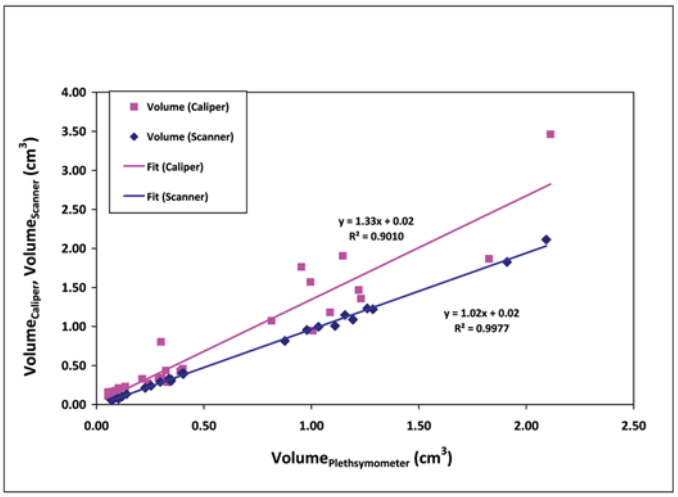

Figure 4. Results of tumor phantom measurements by three methods.

properly by viewing its 3D image displayed on the computer screen.

The whole process took about 3 to 5 s for each mouse. The measured value was displayed on a graph to show the growth of the tumor over time (for repeated measurements) and was saved directly to a database.

Tumor segmentation and subsequent volume calculations were done in real time by using custom software, and the scanned tumor data were displayed in 3D and saved for further analysis. Figure 5 A shows a scanned image of a tumor, which is a rendered as a point cloud image with each dot representing *x*, *y*, and *z* coordinates of points on the tumor surface. Figure 5 B shows a 3D representation of the segmented area of a necrotic tumor. Although this tumor is necrotic, the crater-like region within the tumor and the surrounding tissue are properly defined by the segmentation algorithms.

To judge the accuracy of the scanning and caliper measurements, we again compared them with measurements obtained by volume-displacement plethysmometry after dissection of the tumor mass. The tumors were excised at their base, including the skin covering the tumor, and the volumes were measured by complete submersion in the plethysmometer used previously for the tumor phantoms. Although plethysmometry obviously cannot be used in daily characterization of tumors, it can be used as a 'gold standard' method because it is direct and simple and its accuracy is operator-independent. Triplicate determinations were obtained for each tumor, and the average volume was used in further comparisons. Tumor volumes also were determined from caliper measurements taken prior to tumor excision.

A comparison of the 3 measurement techniques is shown in Figure 6. The deviations of the volume results for the caliper and scanning methods from the plethysmometer results are plotted in Figure 7. For the graph labels the same nomenclature for tumor phantoms was used. The difference (mean ± 1SD) between the scanner- and plethysmometer-derived values (–0.02 ± 0.03 mm^3) again is smaller than that between the caliper and plethysmometer (–0.36 ± 0.09 mm^3). These results show that the scanning method yields more accurate results than does the caliper method. The caliper method has a considerable bias (average deviation) as well as greater variability. For the reasons discussed in the section on assessment of tumor measurements, caliper measurements overestimate tumor volumes. In contrast, the measurement bias is considerably reduced for the scanner method. These comparisons have since been extended to more than 200 tumors, with similar results.

Next, the variability in tumor measurements among 3 investigators was examined. Each investigator independently measured the tumors on 8 mice, first with calipers and then again with the scanner. The calculated volumes were compared with those obtained by plethysmometry. The compiled data demonstrate a high degree of interoperator variability among the tumor volumes determined by the caliper technique (0.15 ± 0.06 mm^3) compared with that for the scanner method (0.05 ± 0.03 mm^3). Conversely, the scanner technique yielded a less variable dataset among the 3 different operators.

Discussion

Like any new technology, the scanner method has potential areas of concern to be addressed. Although none of these issues at the moment affect the integrity of tumor volume measurement, they should nevertheless be understood. The first area of concern is animal alignment. For the segmentation and volume calculation algorithms to function properly, the tumor to be scanned should be centrally positioned within the laser path and the camera's field of view. This placement is achieved by aligning tumors

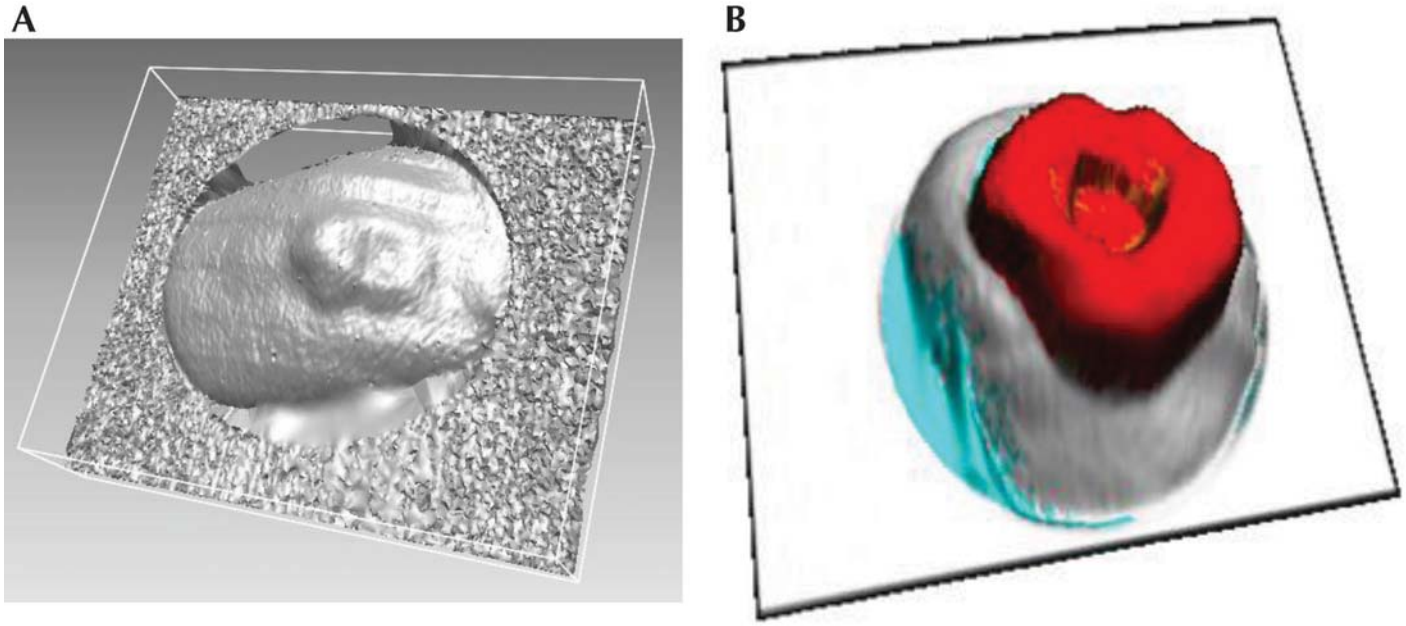

Figure 5. (A) A scanned tumor through the mask. (B) A scanned necrotic tumor. The tumor proper is shown in red, and the surrounding healthy skin in gray.

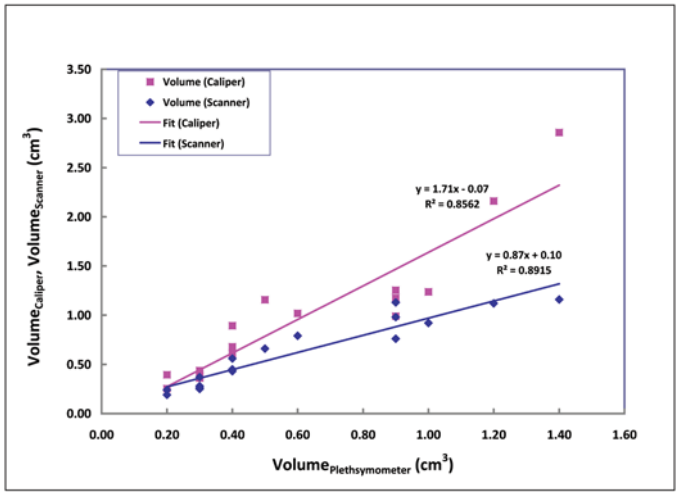

Figure 6. Results for tumor volume measurements.

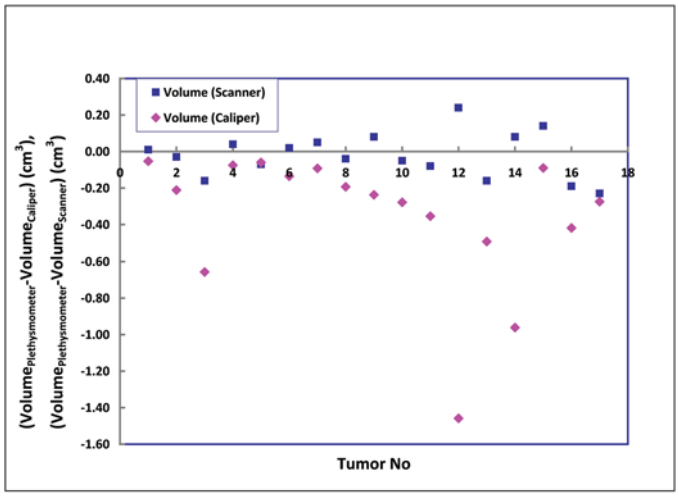

Figure 7. Deviations of measurements from actual values (that is, those obtained by plethysomometry).

through a physical mask (Figure 2 B). All of our tests discussed here were carried out using this mask. To reduce scanning errors tumors should be positioned in the middle and at the same level of the mask. Tumors scanned at obscured angles result in incorrect volume estimations.

Shadowing effect is another concern that can affect the accuracy of the data obtained. Due to the height of the tumor, in some cases, parts of the tumor that lie behind the laser's line of view are not scanned completely. Our measurements show that such regions tend to be small in comparison to the overall surface that is being scanned. These regions are therefore easily 'patched' by interpolation by means of standard surface-fitting methods. Because the surface fitted is small and flat, the procedure used is robust. This factor is not considered to be a major source in the calculation of the tumor's volume.

Because they are not anesthetized, animals sometimes move during the measurements. However, because the entire measurement process takes less than 5 s to perform, an animal that moves during a scan can simply be rescanned. The motion of the animal is detected not only by the operator but is confirmed by a blurred image of the scanned tumor displayed on the computer monitor.

The handling of animals does not negate the noninvasiveness of this method, because any method of tumor measurement requires some degree of animal manipulation. However, because contrast agents, anesthetics, and radioactive materials are unnecessary and because the scanning time is short, this technique is mini-

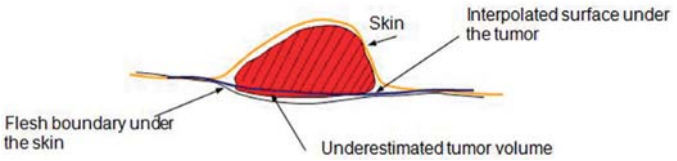

Figure 8. Underestimated tumor volumes.

mally stressful to the animals. Therefore potential animal motion during tumor measurement is only a minor inconvenience.

Because images are obtained only for the external surface of the tumor, our scanning technique cannot measure the volume of any part of the tumor below the interpolated plane of the surrounding healthy skin. If the tumor passes through this interpolated plane, then the volume of the tumor will be underestimated (Figure 8). Our careful study of excised tumors showed that this drawback is a concern only for very large tumors (volume exceeding 2.5 cm^3). In these rare instances, the large tumor mass compresses the underlying tissue and a portion of the mass extends beneath the interpolated plane of the healthy skin. For tumors whose volume was greater than 2.5 cm^3, the discrepancy between the scanner volume and the true (plethysmometer) volume was less than 2%. This feature is considered another minor limitation of the technique.

The technique described here was tested only on hairless mice; tests on other mouse strains, especially those with fur, are planned. Tumors on rats can be measured as long as they are small enough to fit within mask opening (30 mm). A larger mask with 35 mm can be used to allow measurement of larger tumors.

For the volume measurement algorithm to work, tumors should be surrounded by healthy skin so that the surface under the tumor can be interpolated. Therefore, tumors that are on a part of the body obstructed by protrusions, such as body parts, or that lack healthy surface around them due to the large size of the tumor cannot be measured accurately with our scanning system. For such tumors, the boundary definition and extrapolation of healthy skin surface surrounding tumor will fail, resulting in incorrect volume estimations.

In conclusion, we have developed a novel, rapid, and accurate structured light scanning technology for determination of the volumes of subcutaneous tumors in experimental animals. This technique overcomes many of the limitations of other commonly used methodologies (for example, mechanical calipers), although it has a few limitations of its own. This compact platform provides the operator with an immediate 3D representation of each scanned tumor. The supporting software archives the tumor image for future review and graphs the tumor's calculated volume. The laser system described here represents a considerable step forward in improving the ease, accuracy, and throughput of small-animal, preclinical cancer-efficacy studies.

Acknowledgments

We thank the staff of the Departments of Oncology Drug Discovery and Immunology Drug Discovery, Bristol Myers–Squibb; Gennaro Dito, Robert Jeyaseelan, Gregg Masters, David J Schuster, and Tracy L Taylor for the help in taking data for the experiments; Edward Kuspiel, Daniel Batalla, Walter Ruediger, and Robert Ruediger of the Automation and Technology Department for help in building the scanner; and Dr. Scout Chou of Charles River Laboratories for carefully reading the manuscript.

References

1. **Cancer Facts and Figures** [Internet]. Atlanta: American Cancer Society; c2007. Available from: http://www.cancer.org/docroot/STT/content/STT_1x_Cancer_Facts__Figures_2007.asp.
2. **Davis ID.** 1997. A method for minimizing the effects of biologic diversity on the measurement of tumor growth rates. Contemp Top Lab Anim Sci **36:**80–85.
3. **Edwards B.** 2002. The annual report to the nation on the status of cancer, 1973–1999, featuring implications of age and aging on the US cancer burden. Cancer **10:**2766–2792.
4. **Euhus DM, Hudd C, LaRegina MC, Johnson FE.** 1986. Tumor measurement in the nude mouse. J Surg Oncol **31:**229–234.
5. **Klette R, Schluns K, Koschan A.** 1998. Computer vision, three dimensional data from images. New York: Springer.
6. **Labcat.** 2001. Innovative programming associates. Princeton (NJ): Labcat.
7. **Steel GG.** 1978. Growth kinetics of tumours: cell population kinetics in relation to the growth and treatment of cancer. Oxford: Oxford University Press. p 25.
8. **Tomayko MM, Reynolds CP.** 1989. Determination of subcutaneous tumors in the athymic (nude) mice. Cancer Chemother Pharmacol **24:**148–154.