Letter to the Editor

Dear Editors,

We read with great interest the article entitled "Comparing variability in measurement of subcutaneous tumors in mice using 3D thermal imaging and calipers."[1] This research aimed to determine whether the previously observed lower variability on interoperator repeats in the measurement of subcutaneous tumors in mice could be reproduced in a larger dataset.[1] We congratulate the authors for this original article and have several positive comments. The study evaluated a high number of samples. A total of 6,532 individual subcutaneous tumors from 27 laboratories across 289 studies and 153 operators were evaluated. The subcutaneous tumors were established in the flank area of 20 mouse strains (C57BL/6, Balb/C, R2G2, Nude, NSG) by inoculation of over 100 cell lines (MC38, TC-1, CT26, LNCaP, U87, 4T1, HT-29, PC3) and patient-derived xenografts. The tumors were scanned by 3D and thermal imaging system (3D-TI) and were measured multiple times by different operators using callipers. The tumor boundary was automatically determined by the 3D-TI software in a process known as automatic segmentation and automatically measured algorithmically by the 3D-TI system.1 The methods used for tumor measurement in this article are replicable, but 3D-TI is an expensive tool as compared with calipers.

As the authors stated, "In vivo preclinical oncology studies rely on accurate and reproducible measurement of tumors for making conclusions about tumor growth kinetics and drug efficacy." Our research group has measured chemically-induced (methyl-N-nitrosourea, or MNU) mammary tumors in female Sprague–Dawley rats to assess the effects of long-term exercise training or the antihistamine drug ketotifen on tumor development.[2,3] The mammary chains were palpated once a week to detect tumor development, and tumors were evaluated once a week by using ultrasonography B-mode with a Logiq P6 (GE Healthcare, Chicago, IL) device, a 10-MHz linear probe, and a standoff pad. The ultrasonographic images were recorded, and the tumors were measured by setting the electronic cursors that were integrated into the ultrasound device at the borders of the tumor. The length (L), width (W) and depth (D) of tumors were measured. Furthermore, tumor L and W were measured using an external caliper. Calipers are a time-efficient and inexpensive tool as compared with imaging modalities. We next used different formulas to calculate tumor volume from these measurements and compared the calculated tumor volumes with the real tumor volume calculated by water displacement by immersing each tumor in a beaker with saline solution to determine which formulas gave the most accurate result. We found that caliper and ultrasonography measurements were significantly correlated, but that tumor volume varied substantially based on the formula used. Because the mammary tumors seemed to have an oblate spheroid geometry, the most accurate volume was obtained by using the formula $V = (W2 \times L)/2$ for caliper measurements and the formula $V = (4/3) \times \pi \times (L/2) \times (L/2) \times (D/2)$ for ultrasonography measurements.[3]

Some years later, in other work using chemically induced mammary cancer in rats,[2] we confirmed the data obtained in our previous work. We confirmed that mammary tumors grow as oblate spheroids. We also verified that beyond volume evaluation by water displacement, the determination based on tumor weight is the most accurate way to evaluate tumor volume after euthanasia or tumor excision. We also confirmed that the formula $V = (4/3) \times \pi \times (L/2) \times (L/2) \times (D/2)$ provides the most accurate evaluation of in vivo mammary tumor volume.[2]

In conclusion, all of these studies contributed and added value to the study of subcutaneous tumors in animals and may be useful in addressing the effects of lifestyle and natural or chemical compounds on cancer development.

Sincerely,
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References

1. **Brough DW, Murkin JT, Amos HE, Smith AI, Turley KD.** 2022. Comparing variability in measurement of subcutaneous tumors in mice using 3D thermal imaging and calipers. Comp Med **72:**364–375. https://doi.org/10.30802/aalas-cm-22-000033.
2. **Faustino-Rocha AI, Gama A, Oliveira PA, Alvarado A, Fidalgo-Gonçalves L, Ferreira R, Ginja M.** 2016. Ultrasonography as the gold standard for in vivo volumetric determination of chemically-induced mammary tumors. In Vivo **30:**465–72.
3. **Faustino-Rocha A, Oliveira PA, Pinho-Oliveira J, Teixeira-Guedes C, Soares-Maia R, da Costa RG, Colaç B, Pires MJ, Colaç J, Ferreira R, Ginja M.** 2013. Estimation of rat mammary tumor volume using caliper and ultrasonography measurements. Lab Anim (NY) **42:**217–224. https://doi.org/10.1038/laban.25

Response

Dear Dr Ana Faustino-Rocha and Dr Paula Oliveira,

Thank you for the kind words on our publication and for sharing your findings. Not only is the 3D-TI more reproducible, but it is also more accurate. The improvement in accuracy stems from the use of tumor height in the volume formula instead of using a width as a proxy for the height. This seems to line up with your findings as well in which using the tumor depth (height) gives the most accurate tumor volumes when compared with excised tumor weights.

We have also performed analysis comparing 3D-TI tumor volume with excised weights and MRI and have found that 3D-TI is ~8X more accurate than callipers on average. We have also performed extensive analysis looking at tumor prominence (height/width) and how this can vary across cell line and size. This analysis is currently in pre-print at https://www.biorxiv.org/content/10.1101/2022.09.29.510123v1 and will be published soon.

Kind regards,
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