REDUCING INTER-OPERATOR VARIABILITY USING A NOVEL 3D AND THERMAL MEASUREMENT SYSTEM WHEN MEASURING SUBCUTANEOUS TUMOURS IN MICE.

BioVolume Fuel3D, Genomics Institute of the Novartis Research Foundation

BIOVOLUME

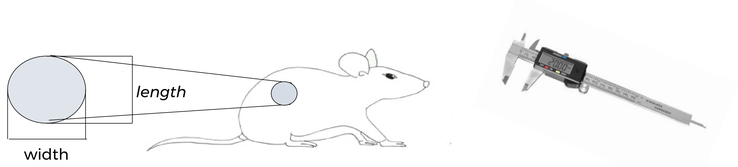
Christina Boykin (GNF), Jie Li (GNF), Cyrus Virata (GNF) , Celin Sanchez (GNF), Jennifer Anderson (GNF), Andrew Smith (Fuel3D), Jake Murkin (Fuel3D), Hui Wang (GNF), Ben Wen (GNF)

Genomics Institute of the Novartis Research Foundation

INTRODUCTION

Is there a problem with tumour measurement?

Measurement variability between technicians and scientists, when measuring subcutaneous tumours on mice with callipers, is a common concern that can cause limitations in experimental design and impact the ability to conduct studies in a concise manner. This lack of consistency between operators can introduce study delays or decrease the capacity of a facility, due to a single operator being required to perform all measurements within a specific study to circumvent any data inconsistencies that may arise as a result of differences in calliper measuring techniques.



How can this problem be addressed?

New techniques are now being developed with the aim of reducing this inter operator bias. BioVolume captures and utilises a combination of thermal, 3D and RGB photographic images in order to automatically measure the length, width, and height of a given tumour, in order to determine its overall volume.

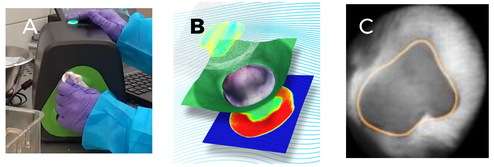
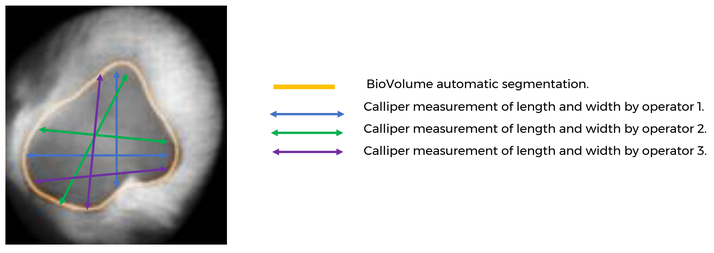


Fig 1. Process for capturing an image with Biovolume. (A) Scruffing & positioning a mouse for Biovolume measurement. (B) Biovolume Software combining 3D, RGB & Thermal data to measure the LWH of the tumour. (C) Thermal segmentation of the tumour from BioVolume software.



Design:

In this experiment, 3 operators at GNF were tasked with capturing measurements of subcutaneous tumours implanted within mice. This was completed with both callipers and BioVolume on the same day, for the duration of the study protocol. The process was then repeated over 2 additional experiments, totalling 3 separate studies.

The **primary goal** was to compare inter-operator variability of the 2 techniques to see if BioVolume outperformed callipers in the repeatability of measurements obtained by different operators.

A **secondary goal** of the experiment was to find out if collecting image data (thermal, 3D and RGB photographic image sets) using Biovolume provided additional traceability benefits to the users.

METHOD

3 Operators, anonymised with labels 101, 102 and 103, were chosen at random to take measurements of a single subcutaneous tumour implanted on the flank of individual mice, across 3 separate experiments. The animal type, number and measurement frequency are listed in Table 1.

Experiment Number	Mouse Strain	Sex	Number of mice	Number of Groups	Number of experimental days	Number of measurement sessions
1	C57 Black/6	Female	40	8	12	7
2	Balb/C	Female	64	8	7	3
3	Balb/C	Female	32	4	8	4

Table 1: Experimental design

For both techniques, length and width measurements were taken of the subcutaneous flank tumour on each of the mice. This was achieved by visually estimating the tumour at its longest length and a longest width measurement, taken at 90 degrees to the longest length when using callipers. The Biovolume System automatically measured the length and width to this standard. The length and width measurements were then used to calculate the tumour volume, using the formula:

$$Tumour\ Volume\ =\ \frac{\pi}{6}\ Tumour\ Length \times Tumour\ Width^2$$

Although Biovolume automatically measures length, width, and height, only length and width were used to compare with calliper measurements of length and width during this experiment. This is to ensure a direct like-for-like comparison of volume.

RESULTS

Primary Goal - Inter-operator Variability

Growth Curves

Following the data collection, growth curves were plotted based on the calculated volume measurements of the three users, for both BioVolume and calliper results. An example can be seen in Figure 2, in which we see growth curves for each user for a single group in one of the three experiments. Demonstrating reduced measurement variability between the 3 operators when using BioVolume.

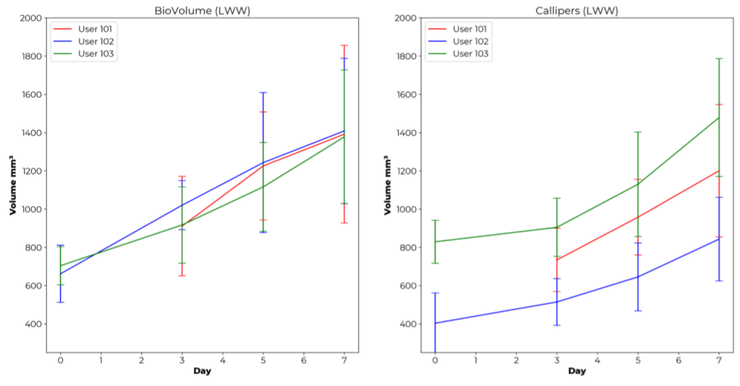
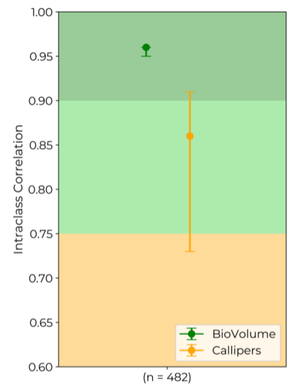


Figure 2: Average tumour growth curves for each of the three users in a single study group from experiment two. Error bars are 95% confidence intervals.

Intra-class correlation (ICC)



Technique	Group	ICC Score	95% CI
BioVolume	Excellent	0.96	(0.95 – 0.96)
Callipers	Good/Moderate	0.86	(0.73 – 0.91)

Table 2

The intra-class correlation (ICC 2,1) between the operator's tumour measurements was calculated to determine if this reduction in variability between operators was statistically significant. Intra-class correlation is widely used reliability index, which measures agreement between operators' measurements whilst accounting for other sources of variance.

A high ICC value corresponds to a high level of agreement between operators' measurements, where a value of 1 is considered perfect agreement.

Figure 2: ICCs Score comparing operators measures of the same tumour on the same day taken across all three experiments. Error bars are 95% confidence intervals.

Secondary Goal - Traceability of measurements

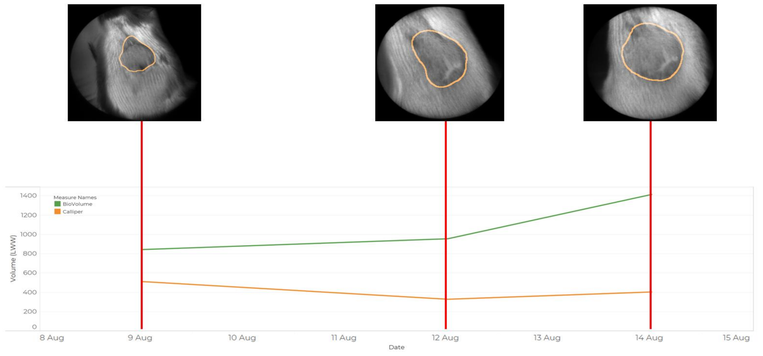


Figure 3: Figure 3: Growth curve of a single rodent in a single group in one of the three experiments, with thermal images of tumours.

CONCLUSIONS

The primary goal of the experiment, to compare inter-operator variability of 2 techniques and discover if BioVolume outperformed callipers, was achieved. BioVolume improved the repeatability of measurements between operators when measuring subcutaneous tumours. These findings were supported across all 3 studies, where comparable statistical results were achieved throughout.

The secondary goal, to discover if the collection of thermal, 3D and RGB image data sets using BioVolume provided additional traceability benefits was also found to be true. Providing the users with the ability to observe images of tumours retrospectively proved to be a valuable asset in assuring the validity of results obtained from the studies.

In summary, the reduction in measurement variability that BioVolume provides has the potential to improve the speed in which studies are carried out. It can remove the requirement for a single operator to perform all measurements throughout the course of a study. This also enables the capacity of a given facility to be increased, allowing a greater number of studies to be performed.

The higher, and statistically significant ICC score, for BioVolume over callipers further confirms increased agreeability between operators.