VOLUMETRIC MEASUREMENT OF TUMORS: MATHEMATICAL MODELS, ASSUMPTIONS, AND ERRORS

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Abstract
This activity involves the metrology, the science of measurement, of the determination of the volume of tumors by two different methods. Caliper measurements of the dimensions and volume from mass and density are done using a suitable substitute, which allows for a comparison of the two methods; and a number of literature-based ellipsoid models can be evaluated and compared. Google Sheets located in Google Drive, a free cloud-based application, will be used to handle data analysis and simulations of error behavior. Students will discover and explore the propagation of errors for the multivariable ellipsoid model!

Introduction
Mathematical modeling of experimental data with its inherited measurement error is a vital aspect of modern science and helps to unite the disciplines of mathematics and the sciences. How is the volume of a tumor or a peanut M&M's, serving as our similar-sized tumor surrogates, determined? Figure 1 outlines the methods for volume determination.

Measure the length (2a), width (2b), and depth (2c) using calipers and compute the volume assuming a perfect ellipsoid

$$V = \frac{4\pi}{3}abc$$

Measure the mass, m by using a balance (0.01g) and compute volume from peanut M&M density, D measurement
V = m/D

Measure the volume by water displacement in a wide-mouth graduated cylinder with 1-mL markings
$V_{M\&M's} = V_{final} - V_{initial}$

Figure 1 - Finding the Volume of a Peanut M&M

Now in the biomedical world of tumors, making a direct measurement or determination may not be an easy task (Ayers et al., 2010; Feldman et al., 2009; Wallace, 2017).

In this activity, the ellipsoid volume determined by caliper or from image processing to derive the dimensions will be examined. Caliper measurements on surface tumors usually lacked a depth measurement; hence, approximation models evolved in a variety of forms. We explore the dimensional measurements and their possible experimental error, first by actual caliper measurements of peanut M&M's, comparison with three different approximation models, and then by a variety of pre-built Google Sheets spreadsheet simulations to examine error behaviors. Lastly students can discover the propagation of error (total error > sum of individual errors) for this multivariable system.

The activity will be done in small groups, with the following objectives:
- Measure the three dimensions of peanut M&M's, serving as our similar-sized tumor surrogates, using vernier calipers. Compute the volume assuming perfect ellipsoids. Caliper instructions are provided as well as an introduction to ellipsoids.
- Measure the mass by using a balance (0.01g) and compute the volume by using the measured density of peanut M&M's. (We determined the average density of peanut M&M's as described in the "Experimental Measurements" Section.)
- Groups will pool data in Google Sheets to construct a comparison plot of the computed volumes and include the y = x line. This is a common way to compare methods. A class discussion of the pooled data will ensue. (Instructors could use the chat function in Google Sheets to do a guided online discussion by the groups.)
- Examine some mathematical models in the scientific literature used by biologists to determine tumor volume based on a number of different assumptions. Here we compare results to a perfect ellipsoid volume and calculate the percentage error in a prebuilt Google Sheets spreadsheet. The validity of the assumptions are evaluated.
- Using the surrogate tumor or peanut M&M caliper data, compute the volumes for the various assumption models and generate a comparison plot with an y = x line and interpret the results where x and y are volumes from various methods.
- Explore the behavior of real data for tumors measured by two different methods to try to derive the possible errant results between methods. Determine if there is a systematic error and, if so, whether it is constant or proportional. A simulation in Google Sheets for a spherical tumor is provided to allow students to explore error behavior.
- Investigate the possibility that random error can generate the error by numerical experimentation using a Google Sheets simulation.
- Discover the propagation of error in the multivariable measurements in the determination of the ellipsoid volume by numerical experimentation using another Google Sheets simulation. Students will discover that the total error is greater than the individual errors of the three dimensions measured (all the computations are done by the simulation)! This is a conceptual approach with glass box computations (students can explore the math/formulas if desired or guided by instructor) for novice learners.

To view the Google Sheets spreadsheet, go to https://goo.gl/Xcdn8y (case sensitive). Anybody can view; however, to get an interactive version (sign into your Google account), go to File on the Menu bar and then select Make a copy…

The latter part of this activity is based on Wallace (2017), who stressed the importance of considering measurement error. However, it has been strengthened in its scientific content and made more simulation-based for student investigation.

If your students are new to considering errors, direct them to our Ruler and Measurement Error interactive Excel spreadsheet (Sinex, 2012). Further discussion on measurement errors, the types of errors, and how they influence mathematical modeling results can be found in Sinex (2005), Sinex (2013), and Sinex (2018). For a general approach starting with the basics to metrology in medicine, see the review article by Squara et al. (2015).

Experimental Measurements

Data were collected by the three methods that were outlined in Figure 1. Instructions for using the caliper are included on the "measurements" tab. Figure 2 shows the comparison plots for volume by the caliper versus volume by water displacement on the top and volume by the caliper versus volume from density on the bottom. The density of peanut M&M's was found to be 1.22 ± 0.044 g/mL at the two sigma level for ten trials of six peanut M&M's determined by water displacement; yielding a coefficient of variation of 3.5% . Sixty peanut M&M's were measured by the authors to produce each plot. The y = x line (slope = 1, y-intercept = 0) on each plot would represent perfect agreement between the two methods being compared. This data is pooled by student groups into a shared with anyone spreadsheet where they have been given editing privileges. This is done on the "peanut M&M data" tab. How do the three measurements outlined in Figure 1 compare?

For the volume by the caliper versus volume by water displacement (Figure 2, upper graph), there is considerable scatter in the data plus the slope is less then one and a sizable y-intercept occurs. The large random variation is due to determination of a small volume of water displaced by a single peanut M&M. This in turns causes the large variation in both the slope and y-intercept.   For the volume by the caliper versus volume from density (Figure 2, lower graph), the two methods are pretty much in agreement. The small amount of scatter is most likely due to the imperfection of the assumed perfect ellipsoid volume for the peanut M&M's. See Table 1 for a summary.
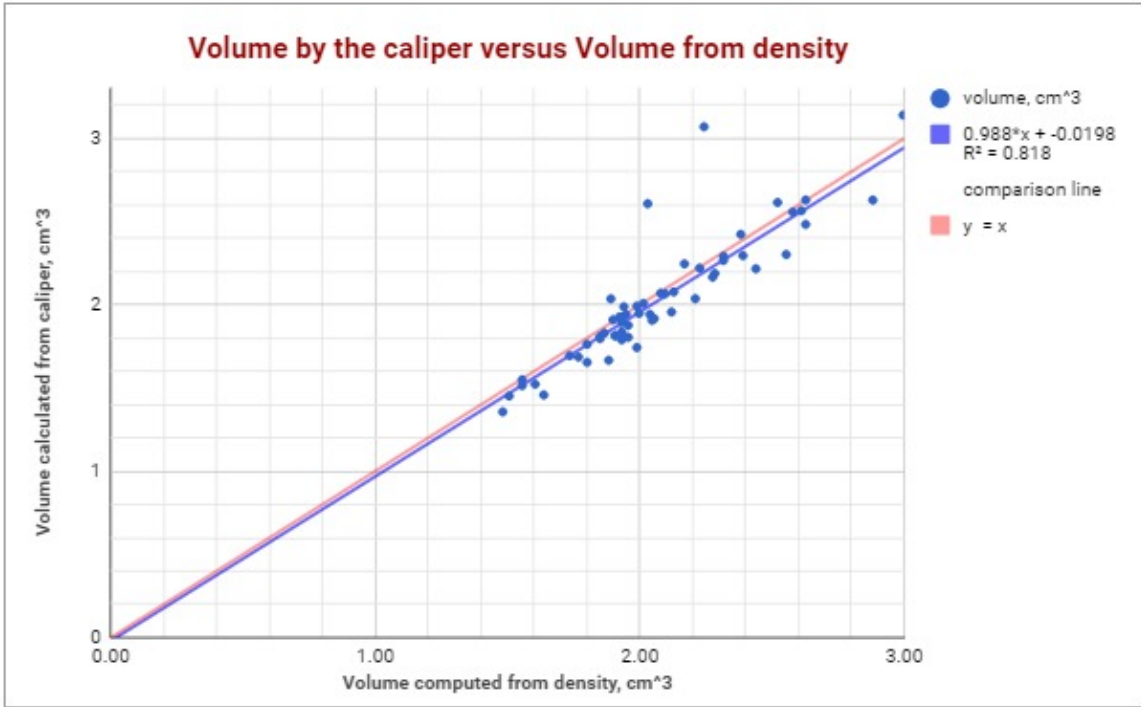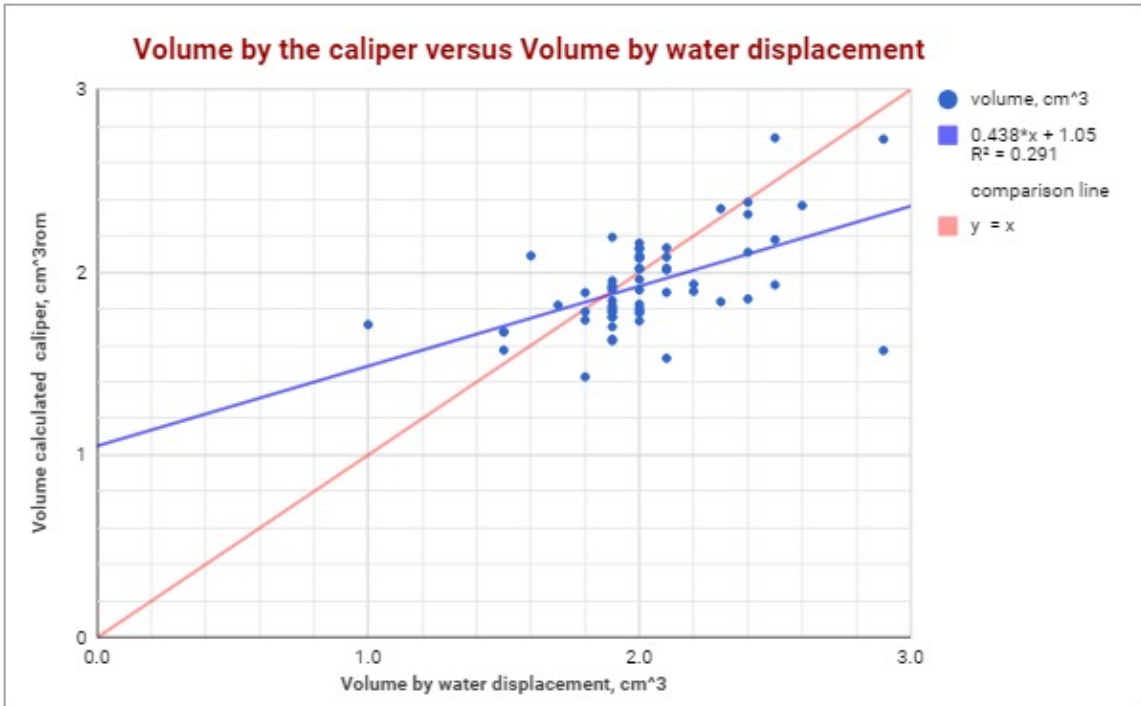
Figure 2 - Measurement Methods Comparison Plots

Table 1 - Results from Method Comparison

quantity	y = x line	$V_{caliper}$ vs. $V_{water\ displaced}$	$V_{caliper}$ vs. $V_{from\ density}$
slope	1	0.438	0.988
y-intercept	0	1.05	-0.0198
r-square	1	0.291*	0.818*
comment	Perfect agreement	Vast scatter in data which influences the slope and y-intercept	Almost perfect with some random variation!

*significant at P = 0.05 for 60 measurements

The basics of pooling data and sharing in Google Sheets is described in Sinex et al. (2016).

At the very least, the caliper measurements to calculate the ellipsoid volume need to be collected for a sample of 25 or more peanut M&M's.

Examining the Biomedical Approximation Models
Why use approximation methods? Caliper measurements of visible subcutaneous tumors are a common inexpensive technique to examine tumor growth and response to drug and/or therapy treatments. Ultrasound imaging has drawn the caliper method into question (Ayers et al., 2010; Faustino-Rocha et al., 2013). Here we examine only three of many approximation methods in the literature compared to perfect ellipsoid as outlined in Table 2.

Table 2 - Approximation Methods

Method	Formula	Comment
Perfect ellipsoid volume formula	$V = (4\pi/3)abc$	Standard of comparison
Xenograft volume measures a & b	$V = (4\pi/3)ab^2$	Assumes a prolate ellipsoid
Xenograft volume with $\pi \sim 3$	$V = 4ab^2 = LW^2/2$	Produces simpler formula
Feldman et al. (2009) equation	$V = 0.613\ (\sqrt{LW}/2$	Derived experimentally

Do the various approximations cause any bias (high or low percent errors) in the results? In Figure 3, the three dimensions are entered in the yellow cells and four models are computed. The percent error is calculated assuming the ellipsoid model as a basis for comparison.

$$percent\ error\ = \frac{approximatation\ model\ -\ ellipsoid\ model}{ellipsoid\ model} \times 100$$

Errors will be positive if approximate model > ellipsoid model or negative if approximate model < ellipsoid model. The sign of the error gives the direction of the error and is very useful when trying to deduce the cause of the error.

The "comparing models" tab allows students to explore the error in the three methods. The errors for the xenograft and xenograft with $\pi \sim 3$ models are small if the tumor is a prolate ellipsoid and the Feldman et al. (2009) model is a sizable negative error. If the tumor is not a prolate ellipsoid, the errors are all large.
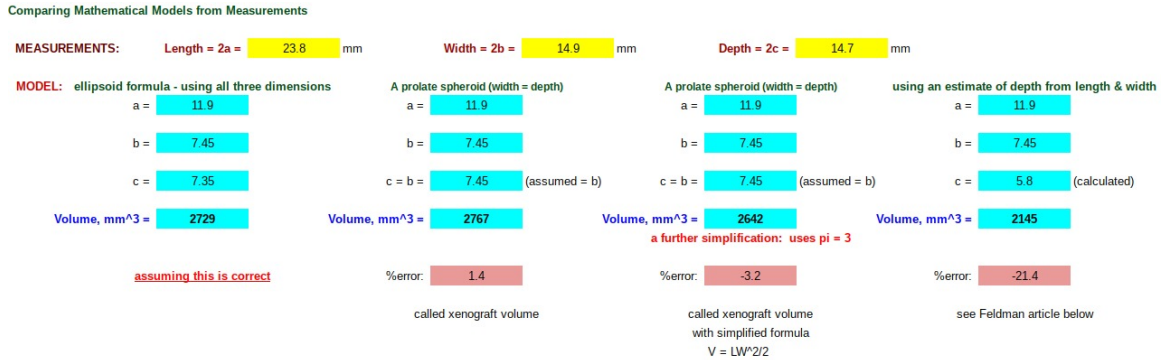

Figure 3 - The "Comparing Models" Tab

Using the caliper measurements of the peanut M&M's, a comparison plot of the three approximation methods compared to the calculated perfect ellipsoid volume can be constructed as illustrated in Figure 4. The closest to the y = x line is the xenograft with $\pi \sim 3$ as noted by Faustino-Rocha et al. (2013) in their comparison of six approximation models. The Feldman et al. (2009) model has the greatest error. Now, can we identify the type of error?
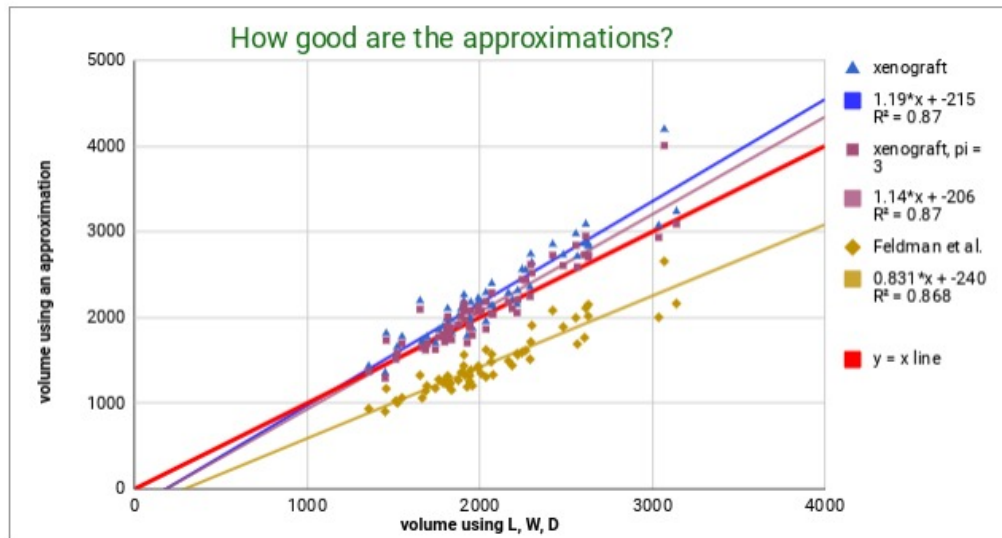

Figure 4 - Comparison Plot for Approximation Methods and Perfect Ellipsoid Volume

Error Analysis via Spreadsheet Simulation

Wallace (2017) presents a small sample of real ellipsoid tumor data taken from Ayers et al. (2010). The Ayers et al. (2010) paper had the xenograft volume measured by three independent observers and the true tumor volume determined by mass and water displacement. Figure 5 illustrates their results graphically with a fairly high r-square value and shows the y = x comparison line. The data shows a considerable amount of scatter despite the r-square value. This is also seen in the large variation in the percent error that is both positive and negative. To consider the mean percent error, the magnitude or absolute value of the error is averaged.

Wallace (2017) suggests there is a trend in the percent with tumor volume. The behavior of percent error and considering the scatter of the data points suggests that the data contains a large amount of random error, which also randomly changes the slope and y-intercept.
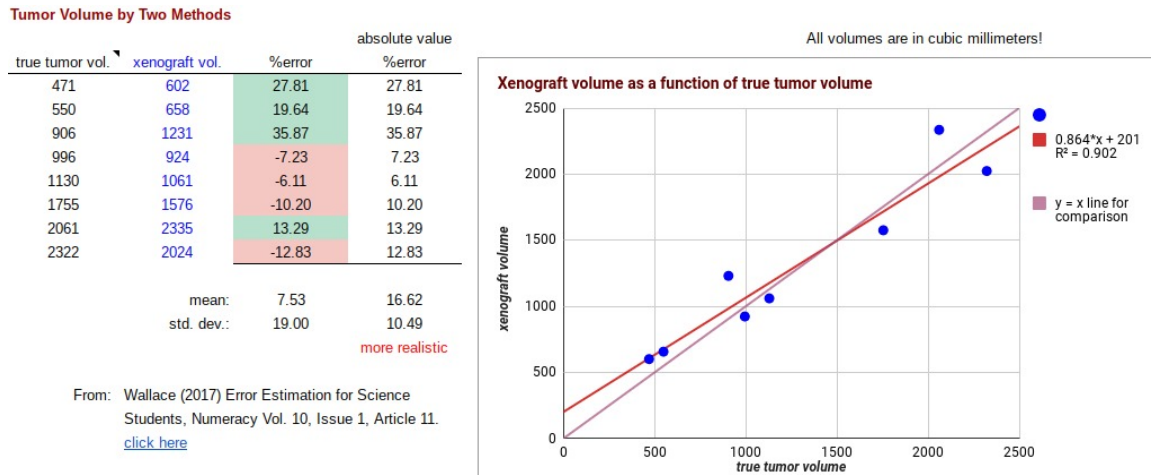
Tumor Volume by Two Methods

true tumor vol.	xenograft vol.	%error	absolute value %error
471	602	27.81	27.81
550	658	19.64	19.64
906	1231	35.87	35.87
996	924	-7.23	7.23
1130	1061	-6.11	6.11
1755	1576	-10.20	10.20
2061	2335	13.29	13.29
2322	2024	-12.83	12.83
	mean:	7.53	16.62
	std. dev.:	19.00	10.49
			more realistic

From: Wallace (2017) Error Estimation for Science Students, Numeracy Vol. 10, Issue 1, Article 11. click here

All volumes are in cubic millimeters!

Xenograft volume as a function of true tumor volume

0.864*x + 201
R² = 0.902

y = x line for comparison

Figure 5 - Some Real Tumor Data and Error

Using numerical simulation, can we generate results from just random error? Go to the "volume data and random error" tab and set the random error in the yellow cell. By using the CRTL + R keys generate random error in the true volume, and do this repeatedly, and observe the scatter and r-square value, the slope, and the y-intercept. See if you can match the trend in the original data. In Figure 6, an example of matching the trend is shown and demonstrates that random error in the xenograft model (i.e. - the caliper measurement) causes most of the error in the volume determination.
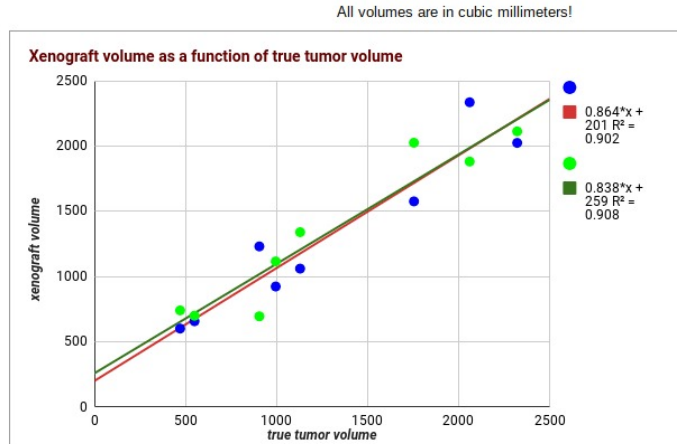
Figure 6 - "Volume Data and Random Error" Tab

Systematic error causes a bias in results and it can be either positive (high) or negative (low). Systematic error can be of a constant or proportional nature as well as outlined in Table 3.

Table 3 - Volume of a Sphere and its Error in the Radius, r

Error	Random	Systematic - Constant	Systematic - Proportional
Result	r ± noise causes scatter in data	r + k where k can be either positive or negative	r(1+k) = r + kr where k can be either positive or negative

We explore this for the volume of a spherical object in Figure 7. The behavior of the absolute error or difference and the relative error or percentage error are shown in tabular and graphical forms for both the radius and computed volume. On the "spherical volume error" tab, students can set the either a constant or proportional systematic error in the radius and explore behavior as illustrated in Figure 7.
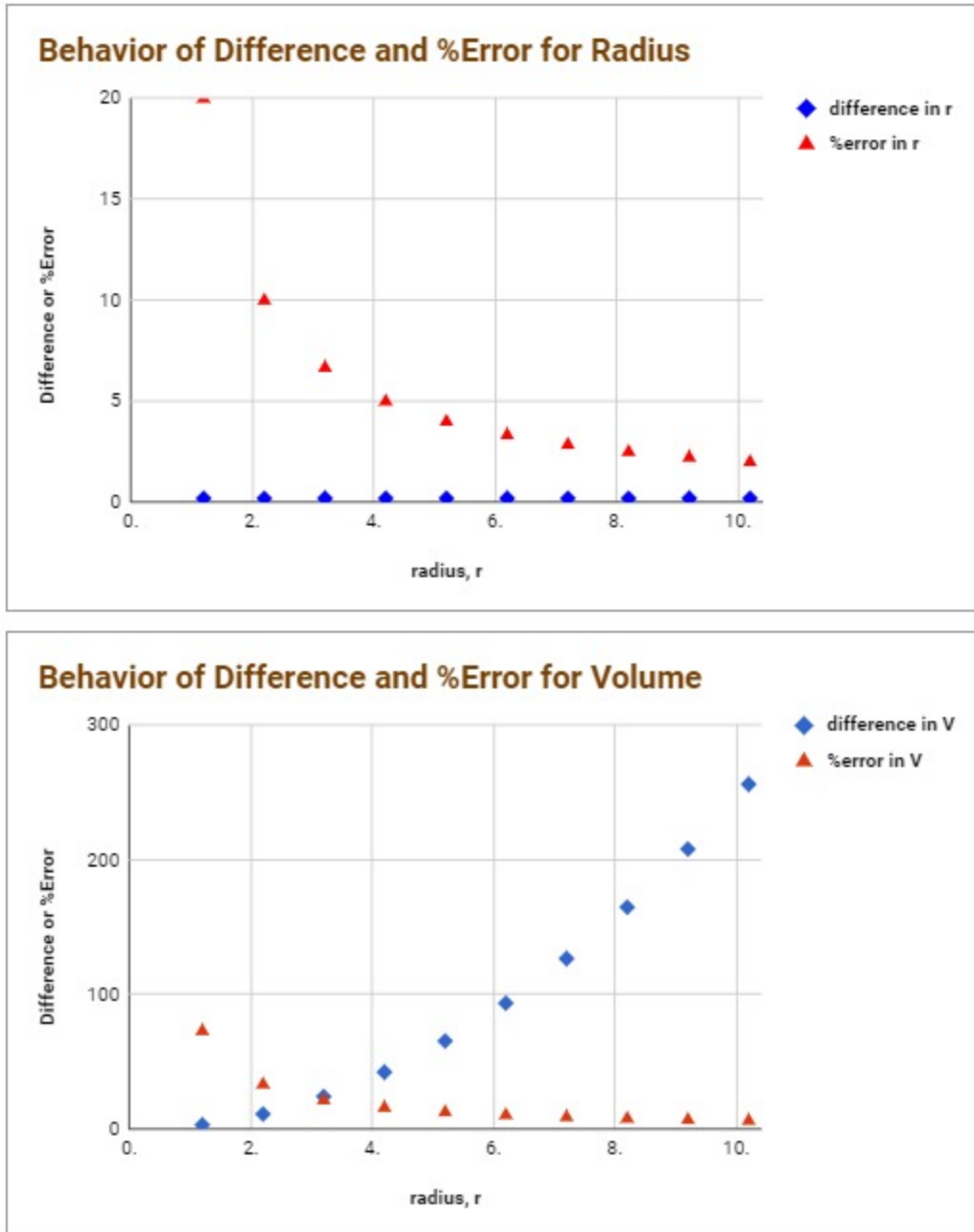
Figure 7 - Spherical Volume Error - For Constant Error in Radius

Discovering the Propagation of Error

The determination of the volume from the three radii of the ellipsoid is a multivariable process. Each of the three radii have an error in their measurement. Is the total error in the volume the sum of the three individual radii measurements? Figure 8 shows the "ellipsoid volume I" tab which allows investigating proportional error by setting the three ellipsoid radii (a, b, and c) and addressing a variety of combinations of errors.

Volume of an Ellipsoid I

These errors are all proportional systematic errors!

%error	Quantity	length a	width b	depth c	volume V	absolute error difference	relative error %error		sum
--	errorless	30.00	20.00	10.00	25132.7	0.0	0.0		
10	error in a	33.00	20.00	10.00	27646.0	2513.3	10.0		
10	error in b	30.00	22.00	10.00	27646.0	2513.3	10.0		
	combine two rows above	33.00	22.00	10.00	30410.6	5277.9	21.0		
10	error in c	30.00	20.00	11.00	27646.0	2513.3	10.0		
0	error in a & b	30.00	20.00	10.00	25132.7	0.0	0.0		
0	error in a, b, & c	30.00	20.00	10.00	25132.7	0.0	0.0		
--	error in a, b, & c (rows 8, 10, and 14)	33.00	22.00	11.00	33451.7	8318.9	33.1		30.0

Figure 8 - "Ellipsoid Volume I" Tab - Proportional Error

The "ellipsoid volume II" tab (not shown) allows investigating constant errors. Students will discover that the total error is always greater than the sum of the individual errors. This is a nice way to introduce students to the propagation of errors, without the use of higher level of mathematics. This is a conceptual approach with glass box computations (students can explore the math/formulas if desired or guided by instructor) for novice learners. For those diehards interested in more on the propagation of errors, see Chapter 3 of Taylor (1997).

Going Further
The medical community uses a Bland-Altman plot for method comparison. This is a plot of the difference between the two methods as a function of the average of the two methods (Bland & Altman, 2003). This should be an easy set of computations for student to do and then produce a scatter plot. Students would then need to add three horizontal lines (plot multiple points in Google Sheets), the mean of the differences, the mean + 2 standard deviations, and the mean - 2 standard deviations.

Wallace (2017) suggests that compression of the tumor as a source of error. Three compression models (Figure 9) can be developed and students can set-up the computations. This would make a nice online collaborative-pair computational spreadsheet project (Sinex et al., 2017). These models, which start as a spherical tumor, are provided on a separate Google Sheets spreadsheet - https://goo.gl/4mmKud (case sensitive). Anybody can view; however, to get an interactive version (sign into your Google account), go to File on the Menu bar and then select Make a copy… The resulting spreadsheets are interactive, as the %compressed can be adjusted to see how it influences error.

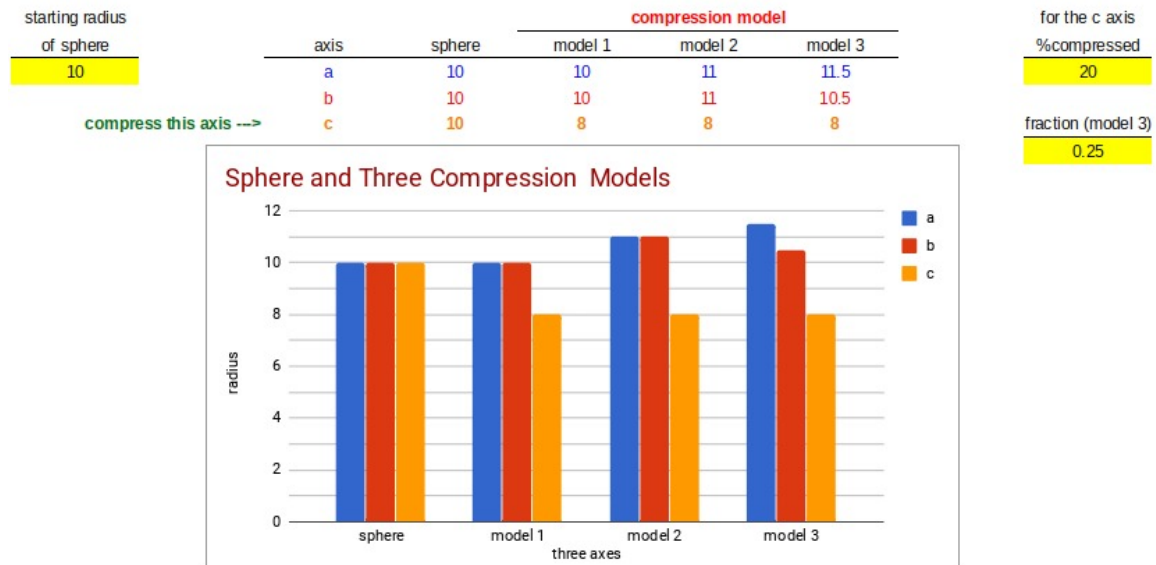| starting radius of sphere		axis	sphere	compression model model 1	model 2	model 3	for the c axis %compressed
10		a	10	10	11	11.5	20
		b	10	10	11	10.5	
compress this axis --->		c	10	8	8	8	fraction (model 3) 0.25

Figure 9 - Compression Models

Consider having an open-ended assessment question as a discussion by individuals, groups or the whole class in Google Docs. Explain how the fact that tumors are not perfect ellipsoids influences the error. Is this imperfection random or systematic? Justify your response!

Some Final Thoughts

Via peanut M&M's as a surrogate for tumors, we provide an authentic task to get students involved in metrology, the science of measurement. Students are introduced to the use of vernier calipers and the multivariable ellipsoid geometry. Using Google Sheets, a large data set can be obtained by pooling small group data, a variety of pre-built interactive spreadsheets or simulations allow students to explore concepts dealing with measurement, its validation by comparison to another measurement technique, its error, and the use of approximations models found in the biomedical literature. Students also discover the propagation of errors in multivariable systems.

Students use their computational and graphical spreadsheet skills to address a variety of tasks. They ponder questions using a number of spreadsheet simulations that requires numerical experimentation. All-in-all an engaging pedagogy is provided. Plus in all our efforts to use the collaborative nature of Google Sheet, students and inservice teachers have given in high marks.

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Classroom Resources

Interactive Ellipsoid, http://demonstrations.wolfram.com/Ellipsoid/ (assessed May 2018).

How to Read a Vernier Caliper,
http://iwant2study.org/lookangejss/01_measurement/ejss_model_AAPTVernierCaliper/AAPTVernierCaliper_Simulation.xhtml, (assessed May 2018).