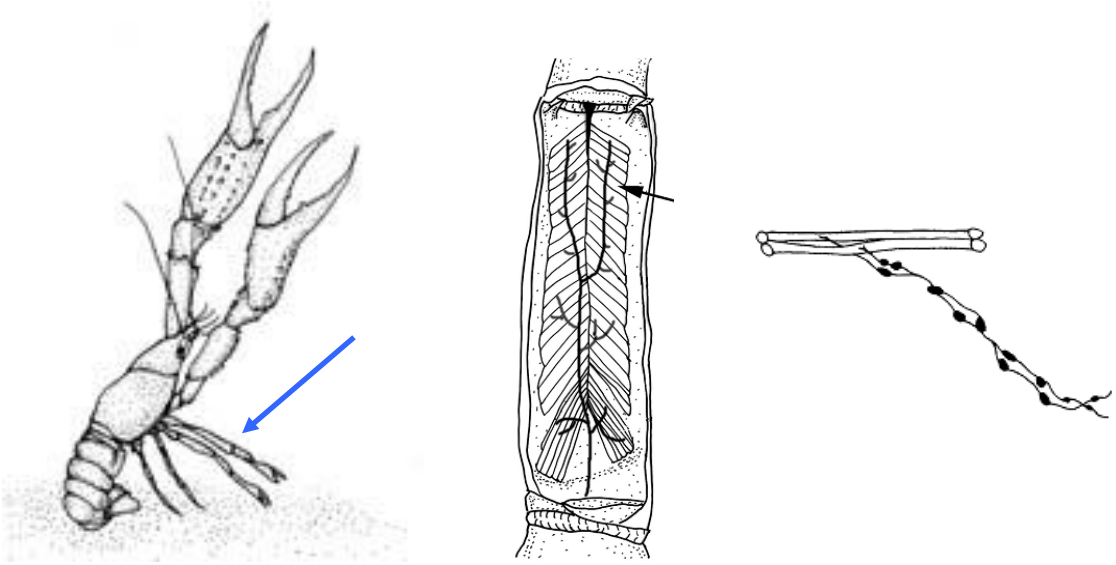
**Title: Stereology in a biological context with the integration of mathematics, design and modeling.**

Quick idea: The idea is to bridge biology and math (particularly geometry) with a thematic approach to a practical problems faced in biological imaging.

Topic: Workshop

The stereology of the serial sections is to be considered in how scientists use approximations and estimates to obtain area and volume of objects. In addition, estimates of error, involved with measures, is considered to teach students' measurement error is a part of science and one needs to be aware of such errors for estimating a range of potential true measures. This topic of error analysis is not commonly tackled in the 3-D rendering of many microscopic imaging of structures but it is essential to consider in order to quantify changes which may take place in order to assess the significance of an experimental alteration. Unfortunately there are many erroneous reports of comparative studies and of biological structural differences due to experimental conditions in which the investigators did not consider the degree of error in the measurements. These reports will be highlighted by student engagement in their own inquiry based analysis. A story line is provided to the modules so that students will take the role of a scientist to calculate dimensions of the objects in question from serial sections of different thickness and orientation. Physical model building is emphasized to obtain 3D views and to estimate real area and volume of specimens in question.

**Biology -- Neuromuscular synapse**



**1. one math problem is a statistical one but very simple (algebra)**
There are papers (scientific ones that can be used as a reference)

Indexing the strength of communication from a nerve to another nerve is very important in medical research. How to make stronger communication in disease states or to block it for treatment of epilepsy.
**A. Quantal Analysis:** Evoked excitatory postsynaptic currents are recorded and analyzed to determine the mean quantal content (*m*). In each synaptic current recording, a trigger artifact and a nerve spike can be visualized which indicates nerve stimulation. Mean quantal content can be determined by direct counts (*mco*):

Direct counts (*mco*)=[3(0)(#of failures)+(1)(# of single events)+(2)(# of double events)...] / the total number of sweeps.

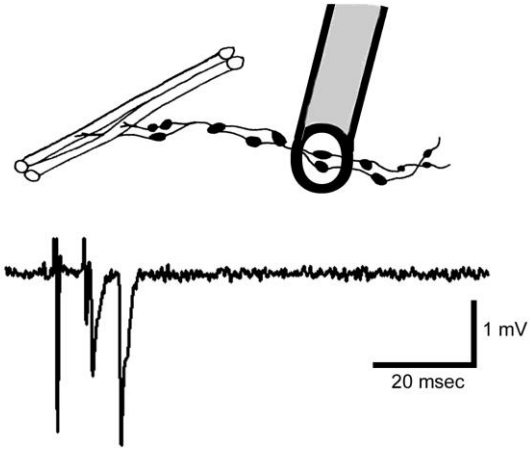



**Figure 1: An electrode placed over a nerve terminal and a recording of 2 events of transmitter release. To determine mean quantal content ($m_{co}$) one can directly count the number of evoked events: The trace shows two evoked events. See Methods for the equation to calculate $m_{co}$.**

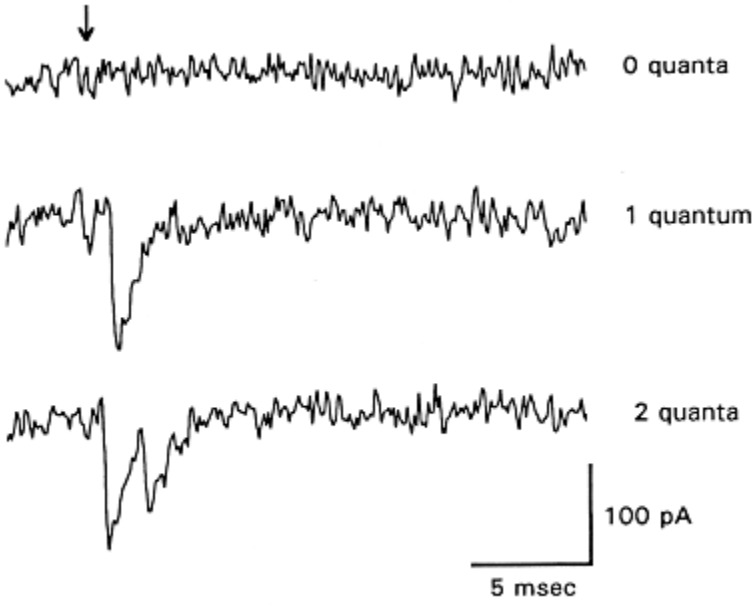
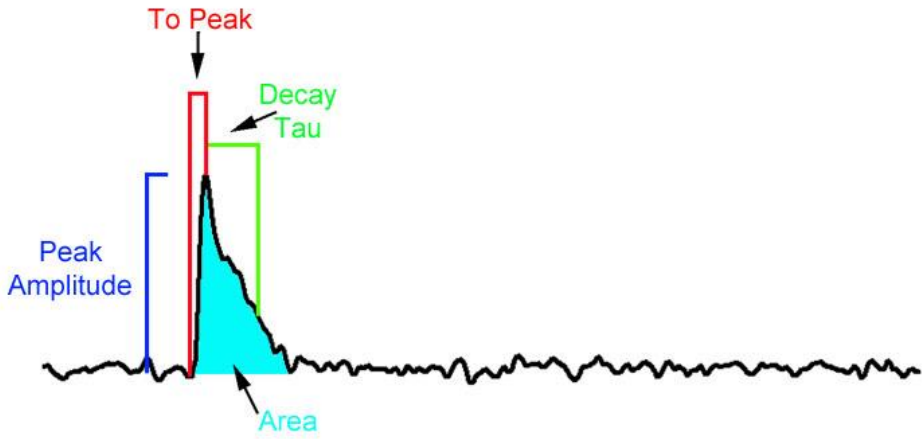



**Figure 2: 1st event is a failure, 2nd is a single and the 3rd event is a double.**

An alternative approach is to measure the area under the curve for a period of time which encompasses all evoked events, even ones with substantial latency jitter. There is some variation to quantal events that can arise due to presynaptic as well as postsynaptic factors. Differences in sizes and shapes of the individual quantal events may indicate multiple synaptic sites at work. Thus, for example, analysis of cluster for peak amplitude and area under the curve and can produce precise estimates of types of events.




2.

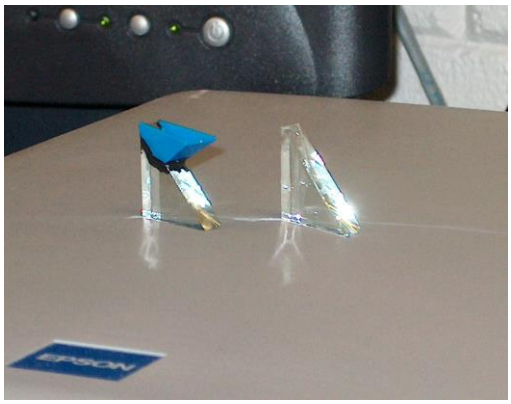
**Imaging Technique: Anatomic profiles of vesicles, synapses at nerve terminals, from 3-D reconstructions.**

The stereology of the serial sections will be carefully considered with error measures incorporated into our analysis. This is not commonly tackled in the 3-D rendering of synaptic structures but it is essential to consider in order to quantify

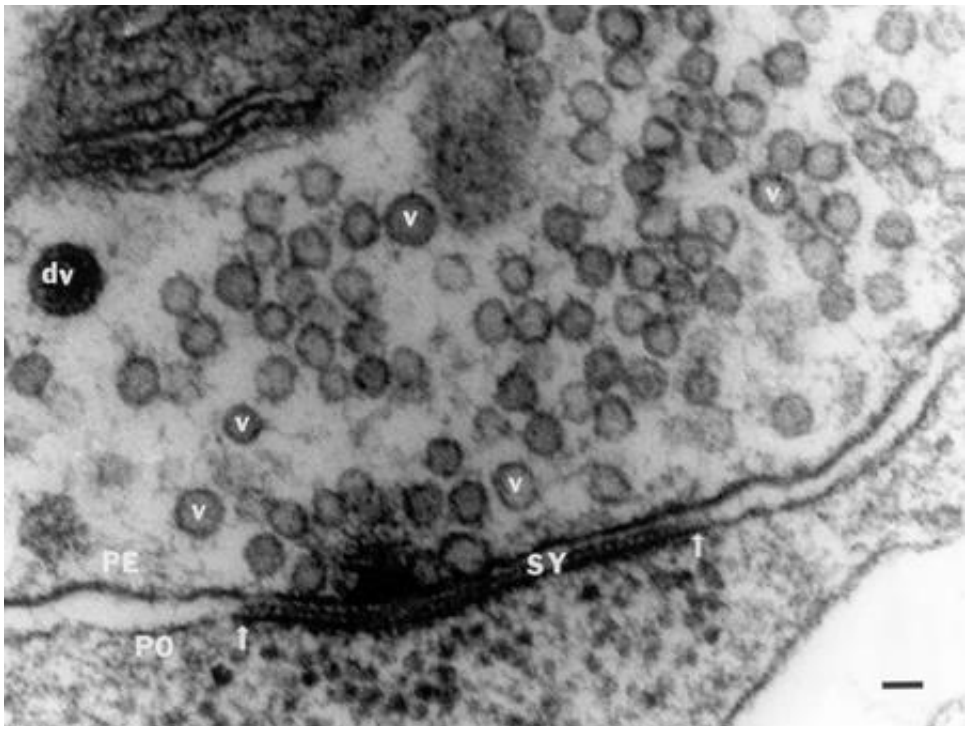
changes that are taking place in order to assess the significance of an alteration. The error in measure must be considered in rendering structures from 2D planes into 3D space. Unfortunately there are many erroneous reports of comparative studies and of synaptic structural differences due to experimental conditions in which the investigators did not consider the degree of error in the measurements (Govind et al., 1994; see- Atwood and Cooper, 1996a; Kim et al., 2000).

**Fix tissue:**
Transmission Electron Microscopy- processing tissue
Gluteraldehyde fixation
$OsO_4$ (post stain)
EtOH dehydration
Embedding
Trimming

Breaking Glass Knives For Thick/Thin Sectioning Thick: 1~2 µM
Thin: 60~100 nM




View specimen on Electron Microscope




**EM of a synapse (SY) with vesicles (V). PE- presynaptic (nerve terminal); PO-postsynaptic**

**Size of vesicles: What Are the Real Sizes of Synaptic Vesicles in Nerve Terminals?**

Many investigators report means diameters of vesicles measured from electron micrographs for obtaining an index of vesicle dimensions and comparisons among experimental samples. The mistake in reporting a mean diameter as such is that it is very misleading and in error since it does not reflect the true diameters of vesicles. This mean value is obviously not the mean diameter of the vesicles present, but a mean of the distribution of sectioned vesicles projected from 3-D to a 2-D plan (i.e., a photomicrograph). Obtaining true diameters of vesicles difficult since they are small and can only be visualized for measurements on micrographs obtained by electron microscopy. To obtain images on electron micrographs, the tissue needs to be sectioned within a range of 50 to 100 nm in thickness. Since sectioning of vesicles is random, will be sectioned at various planes while other vesicles may reside between the sectioning planes depending on the section thickness and vesicle dimensions. The stereological complexity arises when the spherical vesicles are sectioned with less than half remaining within a section or just the caps (i.e., ends). These less than half sections of the vesicles will give rise to various sized projected circular images. When half or more than half of the spherical vesicle resides within the sectioned slab, the true diameter of the sphere will be represented in the projected image. If the projected 2-D circles are measured as representing vesicle diameters than the data is degraded by the less than true sized projections and will obscure results in mean diameter to be less than the true dimensions. If the vesicles were all of a given size than the true diameters would be the largest values obtained. This would be represented in a plotted distribution of random sections as the predominate values of the largest diameters. But as already mentioned, vesicles appear to be truly of mixed sizes within nerve terminals which results in even greater misleading information by reporting mean values of degraded distributions obtained by projects parts of the different sized vesicles. One further

biological problem is the fact that when the very ends of vesicles are sectioned, the small projected circles may not be resolved in electron micrographs from background staining of the cell's cytoplasm and other organelles, thus are not able to be resolved as parts of vesicles.

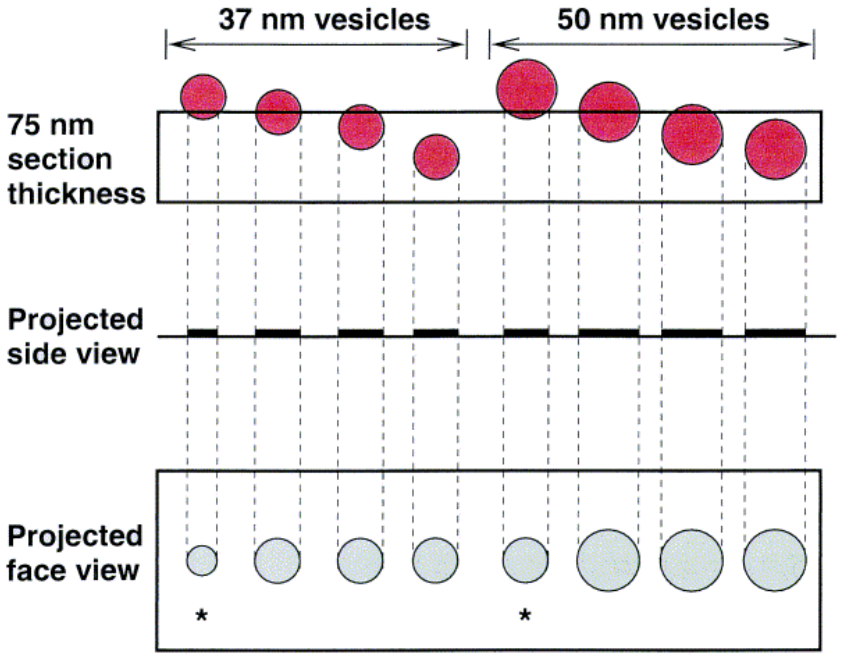



**Figure A. Spheres of two different sizes (37 nm and 50 nm) are placed randomly in space. A section of 75 nm in width is shown through this space. The projected images of the sectioned spheres are shown in 2-D surface. A range in projected circle diameters from the section are shown in side and face view.**

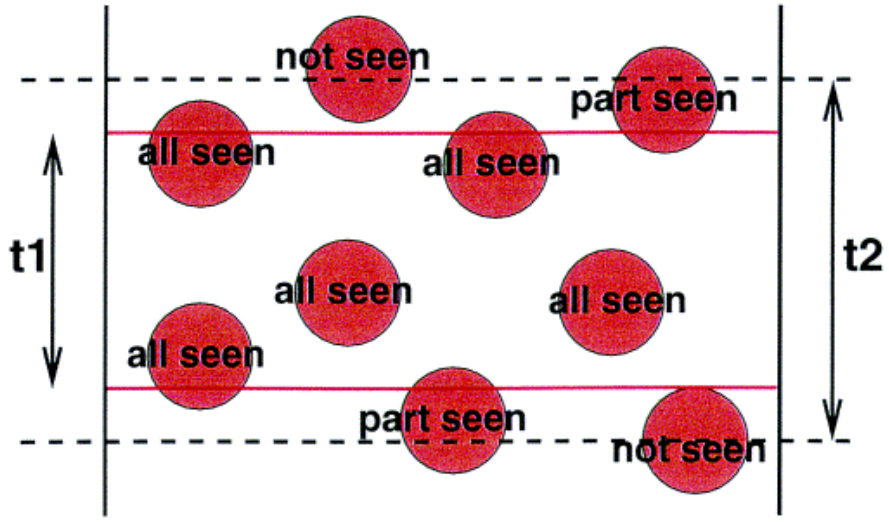



**Figure B. It is important to determine optimal section thickness, in order to prevent overlap of projected images. This can readily be determined by section thickness close to the size of the thickness of the objects of interest. For synaptic vesicles at the crayfish neuromuscular junction, 75 nm is sufficient. The lengths *t*1 and *t*2 denote the thickness of the sections.**

If one plotted the distribution of the 2-D projections of spheres as shown in Fig. A for a single-sized vesicle population in a large number of sections, then a (synthetic) graphical representation would be as indicated in Fig.C. This theoretical distribution of random sectioning of a sphere for one given diameter would provide the true diameter of the sphere as the largest values for diameter, i.e., on the right end of the distribution. The fragmented pieces give rise to the left end of the distribution, which drops in the number of occurrences for the smaller fragments. This indicates that the smallest fragments would occur less in frequency than the larger fragments, in particular when half or more of a sphere is contained within a section.

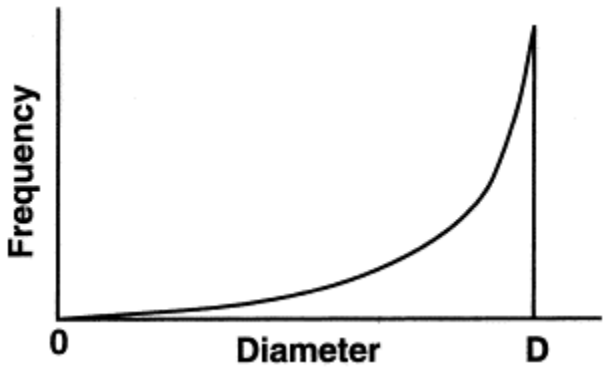



**Fig. C. The theoretical distribution of random sectioning of a sphere for one set diameter would result in a probability histogram with the true diameter of the vesicles being the values furthest to the right (i.e. *D*).**

Combining two populations of spheres used for the illustrative problem into the same space would result in an additive distribution, with the overall probability larger for the peak of the smaller spheres because of the addition of the fragmented projections for the larger spheres

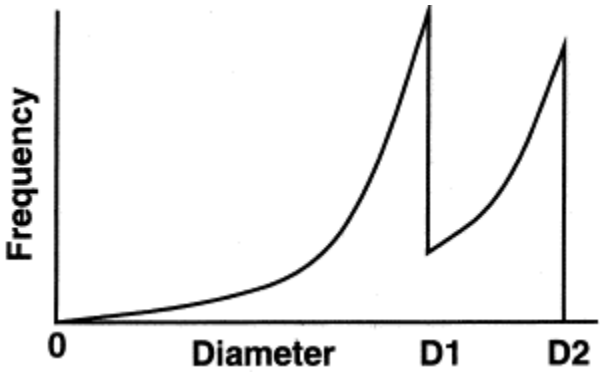



**Fig. 4. When two different vesicle populations exist within the same space, then the combined probability functions would appear as shown. The distribution of the smaller sized vesicles is offset to include a larger number of occurrence**

**Assigning the appropriate location of vesicles in 3-D space**

Location of vesicles within the nerve terminal is used to assess and correlate many aspects in the function and plasticity of synaptic transmission. A classification in the location of vesicles in relation to the inner surface of the synaptic structure is utilized for quantification purposes. In addition, the distance to dense bodies located on the cytoplasmic face of the synapse is relevant since this is the zone in which Ca2+ domains are postulated to be highest in density during terminal depolarization. Such measures of synaptic structure are made from micrographs obtained by transmission electron microscopy (TEM) of thin sections (~75 nm thick) of processed tissue. There are inherent errors is placing vesicles in three-dimensional space from two-dimensional images. The stereological issues come about because spherical vesicles may be sectioned with less than half of the vesicle remaining within a section (referred to as caps or ends). The caps then will give rise to various sized projected circular images on a 2D plane. In contrast, in the situation when half or more than half of the spherical vesicle resides within the sectioned slab, the true diameter of the sphere will be represented in the projected image. Thus, the true sized projections are in error for the caps.

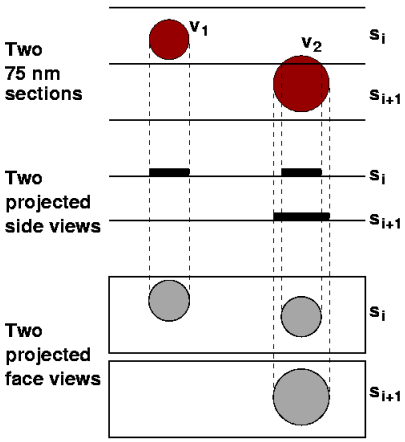



**Fig: Spheres of two different sizes can project their dimensions similarly when one is only partially sectioned. An error in assuming true dimensions needs to be considered for such problems.**

However, there remains a problem in properly placing or estimating the location of vesicles in three-dimensional space for a more complete assessment of synaptic structure and dynamics. For the purposes of this study, we have concentrated on improving methods of assessing the true dimensions of vesicles within the presynaptic terminal and their location within the nerve terminal.

We will determine if the section is parallel or perpendicular to the synaptic face or of some variation. If the synapse is sectioned at an extreme oblique angle the error in synaptic area measurements and distance of objects such as vesicles to the synaptic surface might be quite difficult. We will consider such errors in measurements. In the case of a synapse that is parallel to the sectioning face some assumptions can be made which will help to place the synapse in a more accurate plane. For example, if remnants of the synapse appear in the preceding or the following section, the synapse can be placed within the sectioning plane it is closest to. If no synaptic ruminants appear in adjoining sections, then it will be assumed that it fully resided within the width of one section. Even in this simplified situation of a synapse within a single section, a substantial error exists in assigning a distance from the vesicle surface to the synaptic surface or a dense body on the synaptic surface.

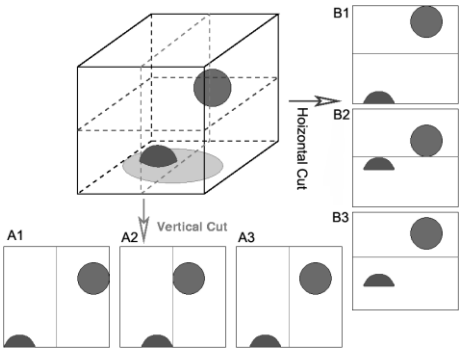



**Fig: The two extreme cases are presented for each plane of sectioning (A1-2 & B1-2) for possible placements of the synapse and its associated active zone in relation to a vesicle some distance from the active zone. An average location in controlling for errors is also shown (A3 & B3).**