



**Michael E. Calhoun, Ph.D.**

<http://www.sinq-systems.com>  
mcalhoun@sinq-systems.com

## **PROPOSAL**

### **Stereological analysis of dendritic structure**

*(from preparations with neuronal tree populations such as Golgi & tg-xFP)*

**Objective:** To provide robust quantitative data on dendritic trees and spines without sampling bias.

**Rationale:** Traditional analysis of dendritic structure from such preparations has relied upon preselection of neurons with features most amenable to analysis. Typically this involves selection of neurons oriented most parallel to the plane of section, excludes neurons that neighbor other labeled neurons, and preferentially excludes dendritic segments traveling perpendicular to the plane of section. Taken together, these selection criteria have multiple possible types of influence on the population data / variance, which may differentially affect group or individual comparisons.

**Strategy:** Apply stereological sampling techniques to the labeled population such that all neurons and dendrites have the same probability of being sampled. Provided data will be total values for a region, and will be representative of the underlying population. Analyzed parameters:

1. Total number of labeled neurons sampled with the optical disector.
2. Total length of dendrites sampled with isotropic virtual spheres.
3. Total number of dendritic branch points sampled with the optical disector.
4. At each location sampled in #2, measurement of spine parameters such as: spine # / segment length; spine length & subtype.

Derived values include average dendritic length per neuron ( $\#2 / \#1$ ), and average # of dendritic branches ( $(\#3 + 1) / 1$ ). Spine parameters in #4 are appropriately sampled based on dendritic length.

**Efficiency:** Time estimates for the above analyses combined should be similar to the time necessary for evaluation of the same parameters on 4-6 reconstructed neurons.

**Summary:** Although no analytical technique can overcome selection bias in the visualization method, the proposed techniques substantially improve the ability of the researcher to assess whether labeling differs between treatment groups (for instance by comparing total # of labeled neurons), and eliminates the sampling bias inherent in traditional reconstruction-based methods.