



## Bioenno Tech, LLC

12630 Westminster Ave., Suite H  
Santa Ana, California 92706, USA  
www.bioenno.com  
contact@bioenno.com  
Phone: +1 714 234-7363  
+1 949 310-9899

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### Introduction to The Golgi Method & Bioenno's superGolgi Kit

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The Golgi method is elegant in its simplicity, staining the spines that extend from the dendrites of neurons and make synaptic connections with passing axons. The method has been fruitful to numerous scientists in the laboratory and clinic since its introduction. The Golgi method including the original and its modifications can be grouped into two main categories: the silver "rapid Golgi" and the mercuric "Golgi-Cox" methods, based on the metallic salts used and the impregnation time that took place (e.g. Rosoklija et al., 2003; de Castro et al., 2007).

In 1873, Camillo Golgi introduced the silver impregnation method in which potassium dichromate and silver nitrate were used to stain the neural cells, including their axons and dendrites. By using the silver Golgi method, Santiago Ramón y Cajal was able to describe the cellular structure of the vertebrate nervous system (Ramón y Cajal et al., 1999; López-Muñoz et al., 2006). To acknowledge their contributions to formulating the foundation of modern neuroscience, Golgi and Cajal were jointly awarded the Nobel Prize in Physiology or Medicine in 1906.

The principle of the Golgi method is based on the crystallization of silver chromate ( $\text{Ag}_2\text{CrO}_4$ ), a brown-red monoclinic crystal. However, the exact mechanism by which individual cells are selectively impregnated still remains unknown. As silver impregnation is notoriously unpredictable (Pasternak and Woolsey, 1975; Friedland et al. 2006), modifications have therefore been performed to improve the reliability of the original method. Among the modifications, the Golgi-Cox method described by Cox in 1891 is widely used, in which a mixture of potassium dichromate and mercuric chloride is used instead of silver nitrate, followed by a photographic development like procedure. The impregnation generally takes 2 to 4 weeks, and the black deposit in impregnated neurons has been suggested to be mercuric sulphide (Stean, 1974). This modified method is particularly useful for tracing dendritic branches and quantifying dendritic spines.

New techniques including intracellular labeling and transfection of fluorescent protein (e.g. GFP, YFP) have become increasingly attractive for neuroscientists to study the dendritic structure and function, but none of these methods even come close to matching the overview of whole brain regions that the Golgi method can provide. The fact remains that even after one century of its introduction, the Golgi method is still superior, not only in neuroanatomy studies, but also in studies exploring the relationships between morphology and behavior (e.g. Williams et al., 1980; Gibb and Kolb, 1998; Robinson and Kolb, 1999; Kolb et al., 2003; Bustamante et al., 2010; Pascual et al., 2010; Ivy et al., 2010). Furthermore, scientists are continuously improving the reliability and sensitivity of the method. For example, lithium hydroxide (LiOH) (Zhang et al., 2011) and autometallographic (AMG) enhancement (Orlowski and Bjarkam, 2009) have been recently employed in the development of the Golgi-Cox impregnated neurons.

The Golgi method has been treated as the primary technique to visualize dendrites and dendritic spines, because of the beautiful visualization of individual dendritic arbors and its many advantages, such as lower cost, ease of use, no need for transgenic animals or special equipment.

Based on the principles of both the original and modified Golgi methods, Bioenno Tech LLC has developed the superGolgi Kit, an enhanced and rapid Golgi-Cox impregnation and staining system. In the Kit, a refined Golgi-Cox solution and an enhanced developer of impregnated neurons are included. The

Kit has been extensively tested on brain tissues from infants to adult animals, yielding isolated dendritic arbors with a very clear background. The Kit is optimal for impregnation of dendritic spines, and excellent for ensuring the staining of entire dendritic trees. In addition, the Kit has the following features:

- Reliable labeling of dendrites and dendritic spines
- Refined Golgi-Cox solution is stable for more than 18 months
- Short impregnation time needed, only 1 to 2 weeks
- Optimal for freshly harvested brain tissues
- Sufficient for 10-12 blocks of brain tissue (size in ~1×1×2 cm)
- Streamlined staining protocol and lower costs

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