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Neurolucida Lucivid versus Neurolucida camera: A quantitative and qualitative comparison of three-dimensional neuronal reconstructions

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ABSTRACT

A critical issue in quantitative neuromorphology is the accuracy and subsequent reliability of the tracing techniques employed to characterize neuronal components. Historically, the camera lucida was the only option for such investigations. In 1987, MBF Bioscience, Inc. (Williston, VT) developed the integrative Neurolucida computer-microscope system, replacing the camera lucida drawing tube with a Lucivid cathode ray tube, thereby allowing computer overlays directly on the view through microscope oculars. Subsequent advances in digital cameras have allowed the Lucivid system to be replaced so that microscope images can be traced by viewing the digital image on a computer monitor. Indeed, the camera systems now outsell Lucivid systems 9 to 1 (J. Glaser, personal communication, 08/2008). Nevertheless, researchers seldom note which of these configurations are being used (which may confound the accuracy of data sharing), and there have been no published comparisons of the Lucivid and camera configurations. The present study thus assesses the relative accuracy of these two hardware configurations by examining reconstructions of human pyramidal neurons. We report significant differences with respect to dendritic spines, with the camera estimates of spine counts being greater than those obtained with the Lucivid system. Potential underlying reasons (e.g., magnification, illumination, and resolution, as well as observer ergonomic differences between the two systems) for these quantitative findings are explored here, along with qualitative observations on the relative strengths of each configuration.

1. Introduction

Accurate reconstruction and analysis of neuronal elements is the goal of neuromorphology. Before the advent of computer microscopy, quantitative reconstructions of neuronal elements (e.g., dendritic lengths and arborizations) required a camera lucida setup that allowed the investigator to trace a cell by hand onto paper. Two-dimensional reconstructions of a dendritic arbor could then be roughly extrapolated to three dimensions using computer programs (Jacobs and Scheibel, 1993). Glaser and Van der Loos (1965) were the first to accomplish accurate 3D reconstructions (tracings) using a computing light microscope and analog techniques. In 1980, Glaser and Van der Loos patented the image superimposition technique: a cathode ray tube (CRT) mounted to the microscope projected a computerized overlay onto the image as seen through the oculars. The overlay depicts a tracing cursor and a control panel with several different tools. This system became known as the ICCM (image combining computer microscope) and was Unix based (Glaser et al., 1983). It evolved several years later into the commercial Neurolucida system (henceforth referred to as Lucivid) that became adapted to the PC (Glaser and Glaser, 1990).

Early tracing systems had been proposed whereby the microscope image was viewed on a computer or television monitor (Paldino, 1979; Yelnik et al., 1981); however, these systems were not optimal until camera technology transitioned from low-resolution (640×480) to high-resolution (1600×1200) digital cameras. Other semi-automatic systems not utilizing a CRT or a camera were also developed. These allowed for neurons to be reconstructed by direct observation through the microscope (Wann et al., 1973; Overdijk et al., 1978). Although these systems were arguably more accurate and less time-consuming than early manual tracings, the reconstructions they produced were not ideal, lacking the varicosities, taperings, and contours of the dendrite (Wann et al., 1973). Until recently, the Lucivid system remained one of a limited number of neuromorphological methodologies. Presently, however, the digital camera setup outsells the Lucivid system 9 to 1 (J. Glaser, personal communication, 08/2008).

To our knowledge, tracings made by both the Lucivid and camera systems have never been quantitatively compared. In fact, many authors fail to indicate which hardware setup they use (Bruno et al., 2009; Hauser et al., 2009), our laboratory included (Jacobs et al., 2001). Because neuronal reconstruction depends

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Fig. 1. Photograph of the Neurolucida tracing workstation. From left to right: (a) the Dell LCD monitor that displays the Lucivid (image plus CRT overlay, not in use); (b) stage-controlling joystick; (c) microscope with Lucivid CRT mounted behind the trinocular head and the camera mounted above the head; (d) the Dell LCD monitor that displays the camera images; a trace is in progress.

largely on the abilities of the tracer, and distinct laboratories and investigators possess tracing idiosyncrasies that reduce reliability (Ascoli, 2006), more detailed information regarding tracing procedures could reduce potential data inconsistencies. The reliance of researchers on past studies as well as the growing trend of data sharing (e.g., www.NeuroMorpho.Org) urge careful methodological descriptions.

The present study addresses both quantitative and qualitative differences between Lucivid and camera tracings of pyramidal neurons. Despite system-related differences in magnification, resolution, and illumination, there was no *a priori* reason to suspect quantitative tracing differences in the two hardware setups. However, qualitative, ergonomic differences were immediately apparent, and are discussed below along with suggestions on minimizing differences between the two tracing methods.

2. Materials and methods

2.1. Subjects

Brain samples from three separate cortical areas (insula, superior parietal lobule, and Wernicke's area) were removed from five human subjects. Tissue samples were donated by Dr. R. Bux of the El Paso County coroner's office. Autopsy records indicated that the tissue was neurologically normal and that autolysis time was under 24 h. This research was approved by The Colorado College Human Subjects Review Board (#H94-004).

2.2. Tissue processing

Tissue blocks, which remained in 10% buffered formalin for two weeks prior to staining, were processed according to a modified rapid Golgi technique (Scheibel and Scheibel, 1978) and subsequently vibratome-sectioned at 120 μm perpendicular to the long axis of the gyrus.

2.3. Cell selection and dendritic quantification

All traced neurons ($N = 30$) were supragranular pyramidal cells that met previously detailed criteria (Jacobs et al., 1997, 2001); in general, neurons were relatively isolated, fully impregnated, and as complete as possible (i.e., not overly sectioned or broken).

Cells were traced in three dimensions (the *x*-, *y*-, and *z*-planes) using an Olympus BH-2 microscope under an Olympus planachromat 40× (0.70 numerical aperture, NA) dry objective interfaced with a Neurolucida system (MBF Bioscience, Williston, VT). The Neurolucida Lucivid system utilizes a green phosphor CRT overlay (Model MR1-103, MBF Bioscience), which is mounted directly beneath the super-wide-field trinocular head (model 1-L0229, Olympus) with SWHK 10XL eyepieces (Olympus). The overlay is simultaneously viewed on a Dell E248WFP 24-in. LCD monitor (see Fig. 1), which is set at a resolution of 800 × 600. The Neurolucida camera system utilizes a MicroFire Digital CCD 2-Megapixel camera (Optronics, Goleta, CA), which is mounted on the trinocular head. The camera image is viewed on a separate Dell E248WFP 24-in. LCD monitor (see Fig. 1) set at a higher resolution (1920 × 1200) than the Lucivid monitor. The microscope stage is motorized and controlled by a joystick (MAC 2000, Ludl Electronics Products, Hawthorn, NY).

Selected cells were traced once using each setup. The order in which cells were traced (i.e., Lucivid first or camera first) was counterbalanced to reduce practice effects. Tracings always began at the soma and continued with each subsequent basilar dendrite until the dendritic arbor, including all visible spines, was fully traced. In keeping with previous protocols (Anderson et al., 2009), neither dendritic thickness nor apical dendrites were traced, and spine subtypes (e.g., stubby, mushroom, or thin; Horner, 1993) were not differentiated.

2.4. Dependent measures

Dendritic data were automatically compiled according to centrifugal nomenclature (Uylings et al., 1986a) by the Neurolucida software. Data were analyzed using five previously described mea-