Journal of Neuroscience Methods 186 (2010) 209–214

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

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

Kaeley Anderson, Erin Yamamoto, Joshua Kaplan, Markus Hannan, Bob Jacobs *

Laboratory of Quantitative Neuromorphology, Psychology, The Colorado College, 14 E. Cache La Poudre, Colorado Springs, CO 80903, United States

ARTICLE INFO

Article history:
Received 10 September 2009
Received in revised form
16 November 2009
Accepted 30 November 2009

Keywords:
Tracing
Reconstruction
Neuromorphology
Reliability
Dendrites
Spines

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

* Corresponding author. Tel.: +1 719 389 6594; fax: +1 719 389 6284.
E-mail address: BJacobs@ColoradoCollege.edu (B. Jacobs).

0165-0270/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2009.11.024

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-

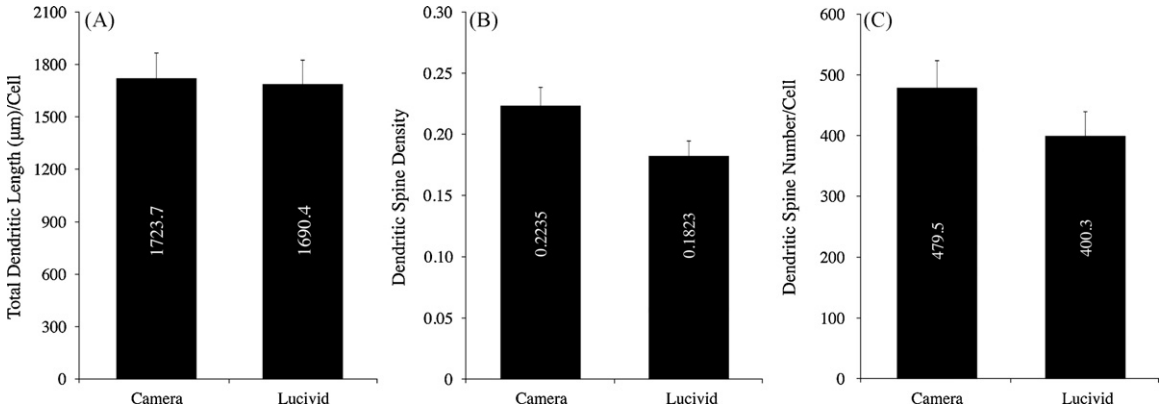

Fig. 2. Bar graphs comparing the camera and Lucivid. (A) For TDL (μm/cell), no significant difference was found between tracing types. (B) Camera traces had a significantly greater DSD (spines/μm) than Lucivid tracings. (C) Traces completed on the camera had a significantly greater DSN (spines/cell) than those of the Lucivid. Error bars represent SEM.

sures of dendritic complexity (Jacobs et al., 2001). Briefly, total dendritic length (TDL, μm) is the linear sum of all basilar dendrites. Mean segment length (MSL, μm) is the average length of each segment, and dendritic segment count (DSC) is the number of segments per cell. Dendritic spine number (DSN) is the sum of spines per cell, and dendritic spine density (DSD) is the average number of spines per micron.

2.5. Independent variables and analysis

Three individuals (E.Y., K.A., B.J.) each traced 10 different cells twice (once by each hardware setup), for a total of 60 tracings. The data were grouped by system type (camera or Lucivid) and analyzed by paired samples *t*-test (SPSS version 17 for Mac) to test the effects of each tracing method on the dependent measures. The three tracers each represented a different level of tracing experience: one was a novice (E.Y.), one an intermediate (K.A.), and the third an experienced (B.J.) tracer. Tracers were initially tested for both intra- and inter-rater reliability by tracing the same dendritic arbor 10 times. For intra-rater reliability, the average coefficients of variation for all three raters were 5% for soma size, 2% for TDL, and 5% for DSN. A split-plot design further tested the intra-rater reliability by comparing the first five tracings to the final five tracings ($\alpha = 0.05$), and no significant differences were found. For inter-rater reliability, tracers were normed by comparing ten of their tracings to the same ten tracings completed by the primary investigator (B.J.). The averages of the correlations for all tracers were 97% (soma size), 96% (TDL), and 96% (DSN).

3. Results

Both quantitative and qualitative differences were noted between tracings with the camera and the Lucivid systems. Quantitatively, analyses revealed significant differences for DSN, $t(29) = 6.7$, $p < 0.001$, whereby cells traced with the camera system ($M = 480 \pm 44$ spines/cell) exhibited approximately 17% more spines (see Fig. 2) than those traced with the Lucivid system ($M = 400 \pm 40$ spines). In terms of DSD, there was a significant difference between cells traced with the camera system ($M = 0.22 \pm .02$ spines/μm) and the Lucivid system ($M = 0.18 \pm .01$ spines/μm), $t(29) = 7.4$, $p < 0.001$, by approximately 18% (see Fig. 2). The difference in tracing time was small, but significant, $t(29) = 3.1$, $p < 0.01$, with camera tracings ($M = 28.5 \pm 2.2$ min) requiring an average of two and a half minutes more than the Lucivid tracings ($M = 26.0 \pm 2.2$ min). There were no significant differences between the two systems for any of the other dependent measures.

Because the significant differences in spine measures suggested potential magnification issues between the Lucivid and the camera setups, we explored the issue further by comparing the DSD of ten 50 μm dendritic segments traced (by B.J.) with both hardware setups (i.e., camera versus Lucivid) under different magnification levels (i.e., 40× and 100×-oil, with an Olympus UVFL100, 1.3 NA objective). Order of tracing was counterbalanced to avoid practice effects. Results indicated a stepwise increase in DSD from the Lucivid-40× tracings to the Camera-100× tracings (see Fig. 3). A 2×2 doubly repeated measures ANOVA indicated that there were significant differences, $F(1,9), = 27.7$, $p < 0.001$, between the two the hardware setups, and between the two levels of magnification, $F(1,9), = 14.9$, $p < 0.0014$.

Qualitatively, several notable differences existed between tracings made with the Lucivid versus the camera systems. As noted above, the first involves overall magnification, with the camera system providing greater magnification than the Lucivid. Secondly, the two systems differ in their requirements for background illumination through the microscope. The Lucivid system, because it utilizes a luminescent CRT overlay, functions best with relatively

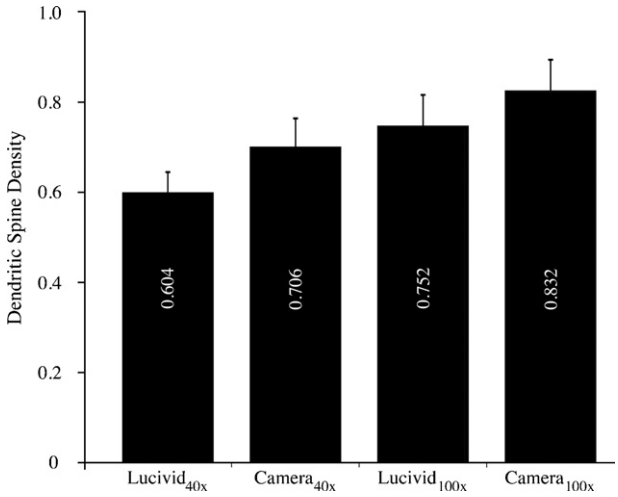

Fig. 3. Bar graph comparing the average spine density (DSD, spines/μm) of ten 50 μm dendritic segments traced with the camera and the Lucivid under 40× and 100×-oil objectives. Traces completed on the camera had a significantly greater DSD than those on the Lucivid for each of the magnifications. Moreover, DSD estimates increased from 40× to 100×-oil. Note that this 50 μm segment exhibits greater DSD values those presented in Fig. 2B because the whole-cell DSD values in Fig. 2B also include first and second order branches, which generally have fewer spines. Error bars represent SEM.

lower levels of microscopic illumination. In contrast, the camera system allows higher illumination levels. Thirdly, differences exist in how each hardware setup responds to movement of the microscope stage in the *x*-, *y*-, and *z*-planes. The Lucivid system transitions relatively smoothly in response to stage adjustments. By comparison, with the camera, if the exposure settings are too long, the system occasionally lags and blurs in changing the image to match the stage. Finally, ergonomic differences (e.g., viewing through oculars versus on a large computer screen, or differences in sitting position) may influence the tracer, particularly a novice tracer with little microscope experience. The effects of these differences are addressed below.

4. Discussion

The present study compared MBF's Lucivid and camera systems to determine if these two setups had a significant effect on neuronal quantification. Previous research suggests that, due to the inherent subjectivity of neuronal reconstruction (Ascoli et al., 2001) individual tracers may create distinct reproductions of the same cell (Jaeger, 2001). Moreover, comparisons across laboratories of the reconstructions of one cellular anatomical class revealed significant differences that were greater than those of each individual laboratory's experimental parameters (Scorcioni et al., 2004). As such, the current study did not focus on comparisons across the three investigators; instead, we examined differences between hardware setups (although our setup is, admittedly, unique to our laboratory). Similarly, we did not attempt to compare the absolute accuracy of the two hardware systems. It is well known that spine counts typically underestimate actual spine numbers (Jacobs et al., 1997, 2001). Although techniques such as obverse–reverse computer microscopy (Glaser and Van der Loos, 1981) or serial reconstructions using electron microscopy (White et al., 2004) may provide more accurate spine estimates than a Neurolucida tracing, these methods are not practical for extensive neuromorphological research, where multiple neurons are quantified in their entirety.

In general, significant differences between Lucivid and camera tracings were found. Camera tracings showed a greater number of spines despite no significant difference in dendritic length. We also report that camera tracings took slightly longer to complete than did Lucivid tracings. Several operational and ergonomic differences between the two hardware setups may have contributed to the current findings.

In terms of overall magnification, the camera's exceeded that of the Lucivid, resulting in a 39% smaller field of view with the camera. Greater magnification aided in spine detection, presumably resulting in the significant differences reported above. Using the camera, tracers were able to identify spine elements that were not detected with the Lucivid system under the same level of magnification. Moreover, the higher magnification on the camera facilitated assigning the thickness of a dendritic branch, a detail that should be particularly relevant to investigators who are interested in volume measurements. A tradeoff exists, however, insofar as the camera's small field of view lessens a tracer's ability to determine the directionality of dendrites, which may require frequent, time-consuming referrals to the microscope to ensure the accuracy of the trace. This may explain the documented time differences in the present study. Given the relative tradeoff between magnification and field of view size, some laboratories may find a 60–63× oil-immersion objective to be an acceptable middle ground.

As with magnification, differences in background illumination between the Lucivid and the camera may have contributed to the observed variation in spines. When tracing with the Lucivid, the microscope illumination must be kept relatively low to allow one to see the phosphor Lucivid overlay. As a result, small or weakly-stained spines may be easier to identify when using the more brightly-lit camera image. Indeed, the camera setup functions well with greater levels of illumination. The added brightness serves to enhance the contrast between the impregnated Golgi-stained elements and the yellow background, although increased illumination may enhance the fading of Golgi-stained tissue (Overdijk et al., 1978). The amount of brightness for camera images can be controlled by increasing the microscope illumination and/or by adjusting the camera's exposure settings (*note*: exposure settings of between 75 ms and 100 ms were optimal for the present study). There is a tradeoff, however, insofar as longer exposure times permit a brighter background image when substage illumination of the microscope is kept constant, but can cause a slight lag in focusing.

Given that the *z*-plane lacks the resolution of the '*xy*' planes (Ascoli et al., 2001), it becomes necessary to maximize the resolution provided by the hardware settings. For example, our joystick has a focus rate control switch for high, medium and low speed depth of vision (or field) focusing. When tracing through the Lucivid, one may use the stepping motor's fastest setting because there is no blurring of the microscope image. However, when tracing through the camera, precise focusing is more difficult. The medium setting provided the smoothest and most controlled changes with the camera setup. As shown in Fig. 4, the camera setup occasionally produced traces with increased fluctuations in depth of vision, reflecting inaccuracies of possible under- and over-focusing. Inter-individual variation in depth of vision is well documented (Uylings et al., 1986b; Guillery, 2002). In the present study, we note that more pronounced focusing variations were produced by the less experienced tracers. If focusing in the *z*-plane is erratic, the experimental measures may be altered, particularly with regards to dendritic length (compare Fig. 4C with A). Although our *overall* results indicated no significant differences in TDL with all three tracers combined, the novice and intermediate tracers both exhibited a greater TDL for camera traces than for Lucivid traces (by an average of 4.5%). Conversely, the expert tracer, with many years of microscope experience, reported a slightly greater TDL (by 2%) when using the Lucivid. It appears that the less experienced tracers inadvertently increased their TDL by hyper-focusing in the *z*-plane, as noted in Fig. 4. Thus, the level of tracing experience for the morphologist, which is seldom if ever reported in the literature, is another relevant factor when interpreting quantitative results.

In general, the camera setup provided a different tracing experience than did the Lucivid. For example, on the Lucivid, the Neurolucida software's tool bars (which are projected onto the microscope image via the CRT) are poorly resolved, resulting in icons and menus that are somewhat difficult to distinguish. Thus, the investigator is often forced to look up from the oculars to the computer monitor and back again when selecting a new function. When using the camera, however, the toolbars are clearly displayed on the monitor and tracing is not interrupted. On the whole, tracing with the camera is less ergonomically strenuous than tracing with the Lucivid (Paldino, 1979). Those with little microscope experience may be especially sensitive to the eye-strain afforded by tracing with the Lucivid and consequently may prefer the camera system. The opposite may hold true for those who are more accustomed to microscopy. Also, because the camera image is displayed on a monitor, one may observe another investigator as s/he traces, which facilitates the training process, and may help increase reliability among tracers.

In terms of which system is more accurate, our results only provide the basis for conjecture. We may reasonably assume that the 'extra' spines found in camera tracings represent spines that were "missed" in Lucivid tracings. The same obtains for 100× spine estimates over 40× spine estimates. Although one could quantify spines by examining dendritic segments at 100× magnification, this is not always a practical option for Golgi-stained

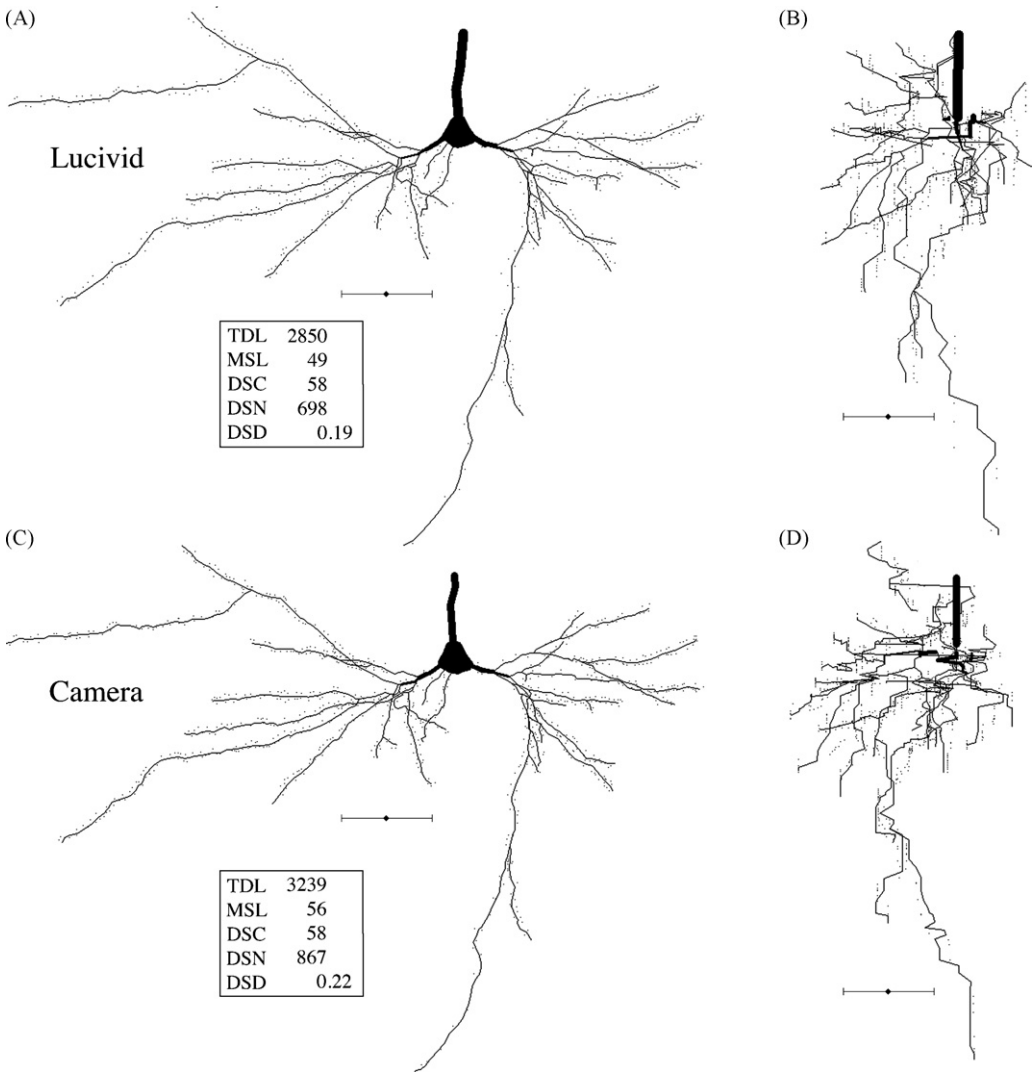

Fig. 4. Sample tracings of the same cell as completed by the novice tracer (E.Y.) on the Lucivid (A) and the camera (C) with inset of dependent measures. Images (A) and (C) appear nearly identical, with virtually no differences in branch placement, number, and order. As expected from the present results, the camera tracing exhibits greater spine density/number than does the Lucivid tracing. However, this particular tracing also illustrates a relatively large difference in dendritic length, with the camera tracing TDL and MSL being larger than the Lucivid tracing. The reason for these length differences becomes clear when examining the *z*-plane of the cell traced with the Lucivid (B) and the camera (D). The dendritic profile in image (D) appears more jagged and erratic than in image (B), suggesting more inaccurate *z*-plane focusing for the inexperienced morphologist. Scale bars represent 50 μm.

tissue sectioned at 120 μm (thicker sections, when appropriate, are advisable because they encompass more of the neuronal structures within one physical section). Unfortunately, there is a tradeoff between higher magnifications, which provide higher resolutions but a shorter working distance, and lower magnifications, which provide lower resolution but a longer working distance. High-powered objectives that offer an extra-long working distance do exist, although they tend to be more expensive. It should be noted, however, that the objective employed may depend on the goal of the study: higher power objectives might be better if the emphasis is on estimating spine numbers in a limited sample; lower power objectives might be adequate, and more efficient, if estimating dendritic extent across a large number of neurons. Here, too, a 60–63× oil-immersion objective might be an acceptable option for spine tracings across the entire dendritic array.

Finally, given that there is no absolute or uniform standard in tracing equipment, a critical issue for neuromorphological research is accurate methodological reporting of hardware setups. As previously mentioned, most neuromorphological papers—including our own—do not provide a detailed account of their equipment and refer simply to the generalized Neurolucida system. Numerous microscope-related factors are well documented (e.g., the influence of Snell's law on dry lens *z*-axis measurements, Glaser, 1982; dry versus oil-immersion objectives, Uylings et al., 1986a; stepping motor calibration, Guillery, 2002), and also require detailed reporting. Specifically, with regards to quantification of spines, it is important to communicate the NA of the objective lens as it may affect not only the resolving power of the microscope, but also the depth of vision. Both of these can influence the number of spines counted. For example, a 40× objective with a 0.65NA will be able to resolve to 0.42 μm whereas one with a 0.95NA will be able to resolve to 0.29 μm and will also have a larger depth of vision, allowing more unambiguous identification of spines.

We are certainly not the first to stress the importance of detailed methodological reporting (Guillery, 2002). Reporting failures increase the heterogeneity of published data (Ascoli, 2006) and complicate accurate interpretations of results. For instance, our previous morphological studies, which were completed on the Lucivid, may have underestimated spine counts. Specifically, we reported an average of 1342 spines/cell in the secondary insular gyrus brevis (Anderson et al., 2009). Recalculating this average by the 17% difference obtained in the present study to simu-

late a camera tracing at 40× results in 1570 spines/cell. Thus, equipment-dependent results are not trivial, especially in the face of widespread data sharing (e.g., www.NeuroMorpho.Org; Brown et al., 2008). As bioinformatics gains popularity among neuroscientists, accurate secondary discoveries, particularly those that make computationally-derived hypotheses based on certain morphological parameters (Wilt et al., 2009), will require careful methodological reporting, including the relative experience levels of the morphologists quantifying the cells, and levels of intra- and intra-rater reliability for these morphologists (Jacobs et al., 2001). Doing so will lighten the task of accurately determining the applicability of shared data (Ascoli, 2007). At a minimum, neuromorphological studies should specify the details (e.g., Lucivid versus camera, NA of the objective) of the tracing system utilized.

Acknowledgements

Several individuals have provided assistance throughout this project. We would like to thank Dr. Robert Bux for his continuous generosity. Also, Dr. Mark Saviano assisted with the data analysis. Jack Glaser, Dr. Ed Glaser, and Dr. Susan Hendricks provided continuous support, feedback, and technical assistance.

References

Anderson K, Bones B, Robinson B, Hass C, Lee H, Ford K, et al. The morphology of supragranular pyramidal neurons in the human insular cortex: a quantitative Golgi study. Cereb Cortex 2009;19:2131–44.

Ascoli GA. Mobilizing the base of neuroscience data: the case of neuronal morphologies. Nat Rev Neurosci 2006;7:318–24.

Ascoli GA. Successes and rewards in sharing digital reconstructions of neuronal morphology. Neuroinformatics 2007;5:154–60.

Ascoli GA, Krichmar JL, Nasuto SJ, Senft SL. Generation, description, and storage of dendritic morphology data. Philos Trans R Soc Lond B Biol Sci 2001;356:1131–45.

Brown KM, Gillette TA, Ascoli GA. Quantifying neuronal size: summing up trees and splitting the branch difference. Semin Cell Dev Biol 2008;19:485–93.

Bruno RM, Hahn TT, Wallace DJ, de Kock CPJ, Sakmann B. Sensory experience alters specific branches of individual corticocortical axons during development. J Neurosci 2009;29:3172–81.

Glaser EM. Snell's law: the bane of computer microscopists. J Neurosci Methods 1982;5:201–2.

Glaser JR, Glaser EM. Neuron imaging with Neurolucida—a PC-based system for image combining microscopy. Comput Med Imaging Graph 1990;14:307–17.

Glaser EM, Tagaments M, McMullen NT, Van der Loos H. The image-combining computer microscope—an interactive instrument for morphometry of the nervous system. J Neurosci Methods 1983;8:17–32.

Glaser EM, Van der Loos H. A semi-automatic computer-microscope for the analysis of neuronal morphology. IEEE Trans Biomed Eng 1965;BME-12:22–31.

Glaser EM, Van der Loos H. Analysis of thick brain sections by obverse-reverse computer microsopy: application of a new, high clarity Golgi-Nissl stain. J Neurosci Methods 1981;4:117–25.

Guillery RW. On counting and counting errors. J Comp Neurol 2002;447:1–7.

Hauser KR, Hahn YK, Adjan VV, Zou S, Buch SK, Nath A, et al. HIV-1 tat and morphine have interactive effects on oligodendrocyte survival and morphology. Glia 2009;57:194–206.

Horner CH. Plasticity of the dendritic spine. Prog Neurobiol 1993;41:281–321.

Jacobs B, Driscoll L, Schall M. Lifespan dendritic and spine changes in areas 10 and 18 of human cortex: a quantitative Golgi study. J Comp Neurol 1997;386:661–80.

Jacobs B, Scheibel AB. A quantitative dendritic analysis of Wernicke's area in humans. I. Lifespan changes. J Comp Neurol 1993;327:83–96.

Jacobs B, Schall M, Prather M, Kapler L, Driscoll L, Baca S, et al. Regional dendritic and spine variation in human cerebral cortex: a quantitative Golgi study. Cereb Cortex 2001;11:558–71.

Jaeger D. Accurate reconstruction of neuronal morphology. In: de Schutter E, editor. Computational neuroscience: realistic modeling for experimentalists. Boca Raton: CRC Press; 2001. p. 159–78.

Overdijk J, Uylings HBM, Kuypers K, Kamstra AW. An economical, semi-automatic system for measuring cellular tree structures in three dimensions, with special emphasis on Golgi-impregnated neurons. J Microsc 1978;114:271–84.

Paldino AM. A novel version of the computer microscope for the quantitative analysis of biological structures: application to neuronal morphology. Comput Biomed Res 1979;12:413–31.

Scheibel ME, Scheibel AB. The methods of Golgi. In: Robertson RT, editor. Neuroanatomical research techniques. New York: Academic Press; 1978. p. 89–114.

Scorcioni R, Lazarewicz MT, Ascoli GA. Quantitative morphometry of hippocampal pyramidal cells: differences between anatomical classes and reconstructing laboratories. J Comp Neurol 2004;473:177–93.

Uylings HBM, Ruiz-Marcos A, van Pelt J. The metric analysis of three-dimensional dendritic tree patterns: a methodological review. J Neurosci Methods 1986a;18:127–51.

Uylings HBM, van Eden CG, Hofman MA. Morphometry of size/volume variables and comparison of their bivariate relations in the nervous system under different conditions. J Neurosci Methods 1986b;18:19–37.

Wann DF, Woolsey TA, Dierker ML, Cowan WM. An on-line digital-computer system for the semiautomatic analysis of Golgi-impregnated neurons. IEEE Trans Biomed Eng 1973;BME-20:233–47.

Wilt BA, Burns LD, Wei Ho ET, Ghosh KK, Mukamel EA, Schnitzer MJ. Advances in light microscopy for neuroscience. Annu Rev Neurosci 2009;32:435–506.

White EL, Winefield E, Lev DL. Qualitative analysis of synaptic distribution along thalamocortical axons in adult mouse barrels. J Comp Neurol 2004;479:56–69.

Yelnik J, Percheron G, Perbos J, Francios C. A computer-aided method for the quantitative analysis of dendritic arborizations reconstructed from serial sections. J Neurosci Methods 1981;4:347–64.