Internet-Enabled High-Resolution Brain Mapping and Virtual Microscopy

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Abstract

Virtual microscopy is a new technology involving the conversion of histological sections mounted on glass microscope slides to high resolution digital sections. Virtual microscopy offers several advantages over traditional microscopy, including remote viewing and data-sharing, data-management and annotation, and data-mining.

Here we describe a novel method that utilizes virtual microscopy for the generation of internet-enabled, high-resolution brain maps and atlases. Virtual microscopy-based digital brain atlases have resolutions approaching 100,000 dpi, which exceeds by several orders of magnitude resolutions obtainable in conventional print atlases, MRI, and flat-bed scanning. We find that virtual microscopy-based digital brain atlases are superior to conventional print atlases in five respects: 1) resolution, 2) annotation, 3) interaction, 4) data integration, and 5) data-mining.

We demonstrate the implementation of virtual microscopy-based digital brain atlases at BrainMaps.org, which is based on more than 10 million megapixels (30 terabytes) of scanned images of serial sections of primate and non-primate brains with a resolution of 0.46 microns/pixel (55,000 dpi).

Our method will be straightforward to replicate by other labs seeking to increase accessibility and sharing of their neuroanatomical data. In addition, by offering the possibility of data-mining completely digitized sections of brains at the sub-neuronal level, our online tools will prove useful for very large-scale histochemical and stereological analyses.

1 Introduction

Internet technologies have revolutionized the way we access and share data (Martone, Gupta, & Ellisman, 2004). In recent years, the merging of internet and digital technologies with conventional microscopy has created revolutionary new capabilities for online viewing and navigation through high-resolution digitized "virtual" slides (Ferreira, Moon, Humphries, Sussman, Saltz, Miller, & Demarzo, 1997; Afework, 1998; Felten, Strauss, Okada, & Marchevsky, 1999; Romer & Suster, 2003). These new digital capabilities are commonly referred to as "virtual microscopy", in contradistinction to "real microscopy".

Virtual slides facilitate data sharing by transmission over computer networks and offer considerable advantages over conventional glass microscope slides in terms of ease and speed of navigation and inspection of large numbers of brain sections. Virtual slides also facilitate data mining and analysis. Earlier,

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methods of digitizing slides via flatbed scanners or film scanners could acquire images at resolutions up to 5000 dpi at best. Nowadays, it is possible to achieve more than 100,000 dpi, enabling the creation of brain atlases at microscopic resolution that far exceeds the resolutions obtainable through the use of other digital scanning technologies, MRI, and conventional print media. The immense size of high-resolution neuroanatomical image datasets, however, presents an obstacle to visualization, manipulation, and analysis, especially when used online.

Here we report on the methodology involved in the implementation at BrainMaps.org, of an interactive zoomable high-resolution digital brain atlas and virtual microscope that is based on more than 10 million megapixels of scanned images of serial sections of primate and non-primate brains and that is integrated with a high-speed database for querying and retrieving data about brain structure and function over the internet.

The resolution of the images, 0.46 micron per pixel, is considerably higher than any histological atlases currently available, either in print or in digital form, and is more than three orders of magnitude greater than the resolution of MRI acquired brain images. The atlas permits a viewer to zoom in from the gross sectional outline to the sub-neuronal level, exactly as if viewing the sections through a microscope. Manipulation and navigation in 3-D space is also possible, thereby providing a more complete understanding of the geometric and topographic relationships of regions of interest.

2 Materials and Methods

A summary of the high-throughput virtual microscopy and web accessibility steps is shown in Figure 1. This diagram outlines the major steps in going from immunocytochemically or histochemically processed slide-mounted tissue to a web-accessible image pyramid. Each of the individual steps are considered below.

2.1 Acquisition of Glass Slide Datasets

Various datasets were available, made up of serial sections of brains stained by the Nissl stain or by histochemistry or immunocytochemistry. Species included were *Macaca mulatta*, *Macaca fascicularis*, *Chlorocebus aethiops*, *Homo sapiens*, *Felis catus*, *Mus musculus*, and *Tyto alba* (Table 1). Brains were sectioned frozen or after embedding in celloidin in the frontal, horizontal, or sagittal planes. Three of the *Macaca mulatta* brains contained fiduciary marks made by inserting electrodes at fixed stereotaxic coordinates prior to perfusion with fixative.

2.2 Generating Virtual Slides

Glass-mounted sections were scanned in at 20x (0.46 um/pixel) on an Aperio ScanScope T3 scanner (Aperio Technologies, Vista, CA), specially adapted to accommodate 3"x2" slides, to generate virtual slides. The image file format we used for the virtual slides is JPEG-compressed TIFF, which results in 1/15 compression ratio (and corresponding reduction in file size) with no perceptible loss of image quality. The raw size of each virtual slide is typically about 25 gigabytes uncompressed, but as a JPEG-compressed TIFF, is reduced down to 1.5 to 2.5 gigabytes.

Since the width of each virtual slide commonly exceeds 100,000 pixels, the slides cannot be saved solely as JPEG since JPEG files cannot exceed 30,000 pixels in either width or height. The TIFF file format has a maximum size of 4 gigabytes, making the use of TIFF alone not an option for images greater than this maximum. However, as JPEG-compressed TIFFs, it is possible to have 25 gigabyte images of 100,000 pixel width, thereby making JPEG-compressed TIFF the sole option for use with the very large images used in constructing the database. The compression schemes offered by DJVU and JPEG2000 present viable alternatives to JPEG but were not employed in this study due to incompatibility with tiling software used to generate image pyramids.
2.3 Uploading to Server

Following virtual slide generation, transfer to the image hard drives on the server (see Figure 4) is done via the File Transfer Protocol (FTP) or other network protocols.

2.4 Generating Image Pyramids

In the final step of Figure 1, virtual slides are converted to a digital format that permits rapid web-based navigation and visualization using software customized for working with very large images, tiled to multiple small (256x256 pixels) JPEG images to generate composite hierarchically-organized, multiresolution image pyramids (Figure 2), and finally, integrated into a relational database.

It should be noted that, strictly speaking, the image pyramid is still considered a "virtual slide", albeit consisting of multiple files (image tiles) instead of just monolithic image file.

Image Tile Generation and Placement

The Zoomifier EZ tool (Zoomify Inc., Santa Cruz, CA), which is used to produce the tiled image pyramid, generates the image tiles at multiple resolutions (each resolution differs from the preceding one by a multiple of two), places them into a canonical directory structure, and writes an information file (ImageProperties.xml) at the top of the directory structure. The meaning of the terms used in ImageProperties.xml are shown in Table 2, along with the term as used in this paper.

Image tiles are named using the pattern http://baseName/TileGroupG/T-C-R.jpg in which baseName represents the path to the directory containing ImageProperties.xml, G is an integer such that 0 ≤ G ≤ [N/256], T is the tier number of the desired image such that 0 ≤ T ≤ \[\log_2(\max(W,H)/T)\], and C and R are the column and row (each numbered from 0) of the tile in the image at tier T.

Image Tile Retrieval

The data in ImageProperties.xml, along with the knowledge of data file placement, are key to retrieving the image tiles necessary to reconstruct the desired image. It is also helpful to know that logically the tile image files are created and stored in row-order starting with Tier 0 in TileGroup0, and that each TileGroup directory is completely filled with 256 entries before another is started.

As a brief example, consider the image pyramid of one of the monkey images, RH4-0801: From ImageProperties.xml, we make the following assignments: W=93707, H=70262, N=134841, and T=256. There are 10 Tiers, numbered 0 to 9 (\[\log_2(\max(93707,70262)/256)\] + 1 = 9). The directory containing ImageProperties.xml itself contains directories TileGroup0 to TileGroup526. TileGroup0 contains files 0-0-0.jpg, 1-0-0.jpg to 1-1-1.jpg, 2-0-0.jpg to 2-2-2.jpg, 3-0-0.jpg to 3-5-4.jpg, 4-0-0.jpg to 4-11-8.jpg, and 5-0-0.jpg to 5-22-3.jpg. The remainder of the Tier 5 files are contained in TileGroup1 (256 files) and TileGroup2, which contains the remainder of Tier 5. The remainder of TileGroup2 contains 6-0-0.jpg up to 6-45-3.jpg for a total of 256 files. All of the TileGroup directories contain exactly 256 files with the possible exception of the last (TileGroup526 in this case), which will contain N mod 256 files, if this value is greater than zero; otherwise, it will contain the full 256 files. In this example, it contains 134841 mod 256 = 185 files.

3 Results

3.1 Total Quantity of Virtual Slides

A total of 2447 sections of primate and nonprimate brains were scanned at high-resolution (0.46 microns per pixel), digitized, and uploaded to the server at BrainMaps.org. The total quantity of image data currently directly accessible online is 8,222,674 Megapixels (or 24.668 TeraBytes, uncompressed). Figure 3 shows a histogram of image sizes as of Aug 2, 2006.
3.2 Server Architecture

The server architecture (Figure 4) can be divided into four main components: 1) the web server (Microsoft IIS), 2) the relational database management system (MySQL), 3) the internal hard disk drives, denoted HDD (PHP), for holding web server PHP scripts and MySQL database files, and 4) the external image data hard disk drives, labeled HDD (1) and HDD (2), for holding image data (LaCie, 2TB LaCie Biggest, RAID 5). The first three components are straightforward to implement and require no additional explanation except to note that the first component, the IIS web server, utilizes the PHP active scripting language to dynamically generate HTML and embed the graphical user interface. Regarding the fourth component, multiple external image data HDD's can be added as additional image data is added to the server. With each LaCie (LaCie USA, Hillsboro, OR) external HDD holding 2 terabytes, it is realistic to have over 30 terabytes on a single server (the upper limit is over 100 terabytes but space restrictions make this unfeasible). The external HDDs are configured as a RAID 5 array, which has redundancy spread over disks so that, in the event of hard disk failure, no data will be lost.

3.3 Online Navigation and Graphical User Interfaces

Clients interact with the web server (Figure 4) to access image data or retrieve database information. Clients can access the image data either through a web browser (Client A in Figure 4) or a different desktop application (Client B in Figure 4). If accessed through a web browser, then the graphical user interface (GUI) to the image data is coded through Flash, AJAX, or Java. If accessed through a different desktop application, then the GUI can be written in other programming languages, such as C.

We have coded GUI's for the brainmaps.org image data in Flash, Java, and C. The brainmaps.org GUI may also be coded in AJAX, though this has not been done yet. Each programming language has its own strengths and weaknesses for the GUI. For example, Java is relatively slow for a GUI and requires a separate browser plugin, but it has the advantage of more sophisticated programming. The C programming language is fast and versatile but is operating system-dependent and driver-dependent. Flash is fast but requires a browser plugin (which, however, is present on over 90% of browsers). AJAX is fast, but may have browser compatibility issues and requires that Javascript be enabled (about 10% of users disable Javascript).

Figure 5 shows an example of navigation through virtual slides at brainmaps.org using the Chlorocebus aethiops (African Green Monkey) Nissl dataset. All images are actual screenshots from a web browser and are what a visitor to brainmaps.org would see. (A) An array of virtual slides for the Chlorocebus aethiops dataset, shown as clickable thumbnails that, when clicked on, launch a new browser window allowing navigation through the high-resolution image (B). The image in (B) is 95,040 x 74,711 pixels and 20 gigabytes in size. The thumbnail in the upper left is for navigation purposes. Shown also are overlaying labels of brain areas that may be toggled on and off. (C) Zooming in on the slide in (B). The red box in (B) corresponds to (C). (D) Zooming in to full resolution in (C), showing details of individual neurons in the insula. The red box in (C) corresponds to (D).

Figure 6 shows an example of a Flash-based GUI for viewing high-resolution neuroanatomical images at BrainMaps.org. Note the label-specific context menu in the upper right that enables the user to retrieve related information, including the position of the label in the labeling hierarchy, connectivity, and gene expression.

3.4 Image Labeling

The terminology employed for online labeling of virtual slides is derived from that of Berman and Jones (Berman & Jones, 1982), Olszewski (Olszewski, 1952), and Jones (Jones, 1985, 2006). It incorporates many of the terms found in NeuroNames (Bowden & Martin, 1995) and other brain atlases, including the atlases of Swanson (Swanson, 1998), Paxinos (Paxinos, Toga, & Feng, 2000), and Emmers and Akert (Emmers & Akert, 1963). A total of 19,702 labels were made. An example of some labels overlaying a nissl section is shown in Figure 6 for the labels, "Pa", "MD", and "Pc".
3.5 Speed of Online Image Accessibility

Figure 7 shows a 2D scatter plot with marginal histograms indicating tile fetch times (in ms) on the Y-axis and tile sizes (in kb) on the X-axis. The number of data points is 1000 and corresponds to a random selection of tiles in an image pyramid for a single monkey section of size 95,040 x 74,061 pixels. The mean image tile fetch time is 84.4 ms and the mean image tile size is 4.11 kb. From the tile size marginal histogram, there are two prominent peaks, with the one centered at 1-2 kb corresponding to non-tissue containing image tiles (which tend to be predominantly white and contain low frequency components), and the one centered at 12 kb corresponding to tissue-containing image tiles (which tend to contain high frequency components, such as cells). Note that tile fetch times are not related to tile size.

4 Discussion

Prior to recent advances in virtual microscopy, slides were commonly digitized by various forms of film scanner and image resolutions rarely exceeded 5000 dpi. Nowadays, it is possible to achieve more than 100,000 dpi and thus resolutions approaching that visible under the optical microscope. This increase in scanning resolution comes at a price; whereas a typical flatbed or film scanner ranges in cost from $200 to $600, a 100,000 dpi slide scanner will range from $80,000 to $100,000.

Virtual microscopy has been characterized as potentially a "disruptive technology". A disruptive technology is a new technological innovation, product, or service that eventually overturns the existing dominant technology in the market, which in this case would be real (i.e., conventional) microscopy. Our experience with virtual microscopy suggests that it is unlikely to replace real microscopy any time soon, but for the time being, it nicely complements and extends the capabilities of real microscopes. Specifically, we find a three-fold extension of virtual microscopy over real microscopy in the following areas: 1) data-sharing and remote access, 2) data-management and annotation, and 3) data-mining. Data management and data-mining of virtual (digitized) slides are capabilities that cannot be directly applied to real slides. In addition, the online distribution and sharing of virtual slides with anyone with an internet connection ensures the rapid dissemination of neuroanatomical data that otherwise would not be possible. While largely emphasizing the pros of virtual slides in this article, it is worthwhile to point out the cons. Namely, it is not possible to change focus in a virtual slide as it is in a real slide. Normally, this is not a problem since virtual slides tend to be completely in focus. However, the inability to change the plane of focus in a virtual slide rules out their use in unbiased stereological estimation methods that require optical dissectors. Nonetheless, biased stereological estimation methods, or unbiased methods not using optical dissectors, are still possible. Another drawback is that the resolution of the virtual slide is limited to the optical lens used in the scanner. For example, if we generate a virtual slide at 20x and subsequently want to examine part of the slide at 40x, then it is necessary to rescan the entire slide using the higher objective, which in some cases, is not possible due to file size restrictions or hardware issues. Finally, at the time of writing, virtual microscopy does not deal well with fluorescence, and is only recommended for light microscopy.

Virtual microscopy-based digital brain atlases are superior to conventional print atlases in five respects: 1) resolution, 2) annotation, 3) interaction, 4) data integration, and 5) data-mining. The resolution of conventional print brain atlases typically does not exceed 7200 dpi, whereas virtual microscopy-based digital brain atlases attain 100,000 dpi and offer the ability to zoom in and out. Annotation can be more complete in virtual microscopy-based digital brain atlases, with options to display some types of annotations and make the rest invisible. Greater interactivity means that the user can zoom in/out and pan through brain image data, which is not possible in print-based atlases. Data-integration capabilities, including the integration of connectivity and gene expression data, are superior for the virtual microscopy-based digital brain atlases. And finally, the ability to data-mine virtual microscopy-based digital brain atlases is a feature not available for print-based atlases. While we do not foresee virtual microscopy-based digital brain atlases completely replacing conventional print-based brain atlases, we expect that they will be progressively more commonly used in place of print-based atlases.

How does the BrainMaps.org database, containing over 10 million megapixels (or 10 terapixels), compare with other image databases? The Allen Brain Atlas (brain-map.org), featuring in situ hybridizations
of over 100,000 serial sections of the mouse brain, is estimated to have 10 - 50 million megapixels. Perhaps the best known and arguably most massive is Google Maps (maps.google.com), which provides high resolution satellite image data covering the entire earth, and which is estimated to have 50-100 million megapixels (with an upper bound of 460 million megapixels). Both of these image databases are recent, appearing only in the last few years. If the trend towards increasingly massive online image databases continues, we expect not only increasing numbers of online image databases to appear, but also the size of said databases to increase as well, in line with technological advances. The largest image databases in existence today, including brainmaps.org, brain-map.org, and maps.google.com, are terabyte-size databases. We have yet to penetrate into petabyte territory. However, given the pace of technology, we can expect the appearance of petabyte-size image databases within the next 5-10 years. Neuroanatomical image databases of this size would contain more data than any individual lab could accumulate, and would necessitate the formation of a community of data contributors. The end-users would be the entire public. The ramifications of this massive sharing of neuroanatomical data have yet to be borne out, particularly when additional tools are provided for data-mining.

In summary, we have devised and implemented a method for digitizing histological, histochemical, and immunocytochemical section data and making the content easily and conveniently accessible online. We have shown that web-accessible virtual microscopes and brain atlases can be developed using existing computer and internet technologies that offer universal data-sharing, and that rapid and seamless navigation through vast image datasets can be achieved using hierarchically-organized, multisresolution images in conjunction with a graphical user interface. By offering the possibility of data-mining completely digitized brains at the sub-neuronal level, our online tools will prove useful for very large-scale histochemical, gene expression, and stereological analyses. Our method will be straightforward to replicate by other labs seeking to increase accessibility and facilitate sharing of their neuroanatomical data.

5 Acknowledgements

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References


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<tr>
<th>Species</th>
<th>Datasets</th>
<th>Sections</th>
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<td><em>Homo sapiens</em></td>
<td>5</td>
<td>45</td>
</tr>
<tr>
<td><em>Macaca mulatta</em></td>
<td>15</td>
<td>630</td>
</tr>
<tr>
<td><em>Macaca fascicularis</em></td>
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<td>6</td>
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<td>195</td>
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<td><em>Mus musculus</em></td>
<td>13</td>
<td>994</td>
</tr>
<tr>
<td><em>Tyto alba</em></td>
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<td>8</td>
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Table 1: Datasets from various species utilized in this study.

<table>
<thead>
<tr>
<th>Constant</th>
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<th>Meaning</th>
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<tr>
<td>TILESIZE</td>
<td>T</td>
<td>Dimensions of square image tile in pixels</td>
</tr>
<tr>
<td>WIDTH</td>
<td>W</td>
<td>Width of full-sized image in pixels</td>
</tr>
<tr>
<td>HEIGHT</td>
<td>H</td>
<td>Height of full-sized image in pixels</td>
</tr>
<tr>
<td>NUMTILES</td>
<td>N</td>
<td>Total number of all images tiles in this image pyramid</td>
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Table 2: Terms used in *ImageProperties.xml*. The first column is the variable as shown in the file, the second column is the term used in this paper, and the third column describes the meaning of the term.
Figure 1: High-throughput virtual microscopy. Block diagram outlining the major steps in going from immunocytochemically or histochemically processed slide-mounted tissue to a web-accessible image pyramid.
Figure 2: The concept behind an image pyramid. Each virtual slide, representing a single monolithic image file, is chopped up (i.e., tiled) to generate a multi-resolution image pyramid composed of small image tiles with a maximum size of 256 x 256 pixels. Image pyramids allow for rapid online navigation through very large images through the loading of only the image tiles that are currently being viewed.
Figure 3: Distribution of image sizes at BrainMaps.org as of 08-02-2006. The total size of the brain images is 8,222,674 MegaPixels, or 24.668 TeraBytes. The total number of images is 2447, with an average size of 3360.31 MegaPixels/image (or 10.08 GigaBytes/image).
Figure 4: Server organization. The client interacts with the server through a Flash or Java based frontend that interacts with the image file system and relational database. The active scripting language, PHP, is used as a 'glue' to tie all the components together.
Figure 5: An example of navigation through virtual slides at brainmaps.org using the *Chlorocebus aethiops* (African Green Monkey) Nissl dataset. All images are actual screenshots from a web browser and are what a visitor to brainmaps.org would see. (A) An array of virtual slides for the *Chlorocebus aethiops* dataset, shown as clickable thumbnails that, when clicked on, launch a new browser window allowing navigation through the high-resolution image (B). The image in (B) is 95,040 x 74,711 pixels and 20 gigabytes in size. The thumbnail in the upper left is for navigation purposes. Shown also are overlying labels of brain areas that may be toggled on and off. (C) Zooming in on the slide in (B). The red box in (B) corresponds to (C). (D) Zooming in to full resolution in (C), showing details of individual neurons in the insula. The red box in (C) corresponds to (D).
Figure 6: An example of a Flash-based graphical user interface for viewing high-resolution neuroanatomical images at BrainMaps.org. Note the overlaying labels ("Pa", "MD", and "Pc") and the label-specific context menu in the upper right that enables the user to retrieve related information, including the position of the label in the labeling hierarchy, connectivity, and gene expression.
Figure 7: 2D scatter plot with marginal histograms showing tiles fetch times (in ms) on the Y-axis and tile sizes (in kb) on the X-axis. The number of data points is 1000 and corresponds to a random selection of tiles in an image pyramid for a single primate section of size 95,040 x 74,061 pixels. The mean image tile fetch time is 84.4 ms and the mean image tile size is 4.11 kb.