

MEASURING BROOD DIGITALLY

An easy way to measure and
record brood production in
your colony.

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INTRODUCTION

In apicultural research and honey bee management, it can be important to measure the reproductive output of the colony (i.e. worker production). Fast Spring build-up is desirable for honey production and pollination, and stress on colonies is often manifest by reductions in brood production. The usual way of measuring comb use has been by a threaded grid reference frame (1 in x 1 in squares delineated by thread) and counting the squares with capped brood, with uncapped brood, drone cells, honey and pollen. Brood production can be measured most efficiently and quickly through estimates of areas of capped brood in the hive. Even so, the grid and counting method is time consuming and results only in estimations.

Digital photography and computer-aided analyses of the images captured eases the task of calculating brood area (capped-brood) for management and research. This method can be equally well applied to measuring areas of capped honey, pollen and drone brood by simple adaptation of the methods described below.

METHODS

Digital Camera

Any digital camera may be used and the important issue to keep in mind is consistency. The consistency of lighting in the photographs increases the consistency of the analysis. The same lighting in each photo-

graph can be achieved by always using the camera's flash, regardless of lighting conditions. It is a good idea to take photos from a consistent distance, to ensure that the brightness of the flash is the same for every image. We designed and built a camera and frame stand that lends itself well to the task of capturing hive frame images (Figure 1). Images can be stored in any format (.TIFF, .JPEG, etc.), however it is suggested that the same format be used throughout the analysis so all the images can be measured consistently.

Building the Camera Stand and Frame Mounting Apparatus

The objective of making the stand is to allow the frame to be photographed and measured to remain in line with the camera, held steady, and at a constant distance from the camera while the camera is securely mounted and oriented square with the frame. The actual dimensions of the components may be varied depending on the camera to be used. Portability and convenience should also be considered. The stand should secure the camera in a position that allows the hive frame to fill the whole area of the photo. We provide more details on building the stand at www.uoguelph.ca/~inesp.

Procedures for Image Capture and Analysis

Step 1: Mount the camera firmly onto the stand so that it is oriented towards the frame holder. Place the frame to be measured into the frame holder and assure that the holder is at right angles (normal) to the camera and its lens. Allow for a border (5 cm or 2 in) around the frame within the photo to ensure the comb is fully within the picture. A removeable black backdrop (sheet of plastic) that fits into a slot behind frame allows any holes in the comb to appear uniform in colour for ease of analysis in later steps (see below *Steps 2 and 3*).

Focus the camera and take the picture. Rotate the frame holder so that the other side of the frame can be photographed, move the black backdrop to the back of the frame and take the picture. Remove that frame, replace it in the hive, and continue to the next frame. Figure 2 presents an uncropped picture as would be stored in the memory of the camera to be returned to the computer for manipulation and analysis.

Step 2: Copy the images from the digital camera onto the computer's hard drive and open the images in Adobe Photoshop®. Any version of Adobe Photoshop® will work. We have used Adobe Photoshop 6.0®.

Crop the image so that only the comb or area of interest (inside the wooden end, top, and bottom bars, without any wood showing) is left in the image. Cropping can be done even if the image is slightly askew in the picture. The location of the *cropping tool* and the cropped image are shown Figure 3. With the *cropping tool*, click in the top left corner of the image, hold down the left mouse button and drag to the bottom right of the image. Release the mouse button and press enter. The resulting cropped image can be enlarged with the top-right-corner icon (little square with a black bar across the bottom).

Step 3: The image must be edited to remove superfluous components (i.e. non-brood; holes, honey,

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pollen, and bees) and the colors in the image must be standardized to make all of the capped cells appear solid white and all of the uncapped cells solid black. The aim of the following manipulation is to provide two numbers: the number of pixels (the tiny dots that make up the image) that represent capped brood (A), and the number of pixels that are enclosed by the entire image (B). With this information the amount of capped brood as a percentage (C) of the entire frame ($C = A/B \times 100\%$), or as the area, D (cm^2, in^2) ($D = C \times \text{area of frame within the top, end, and bottom bars}$).

To eliminate any unwanted parts of the image (bees, honeycomb, pollen cells, etc.) go to the tool bar and into the *Paint Bucket Tool* (this tool looks like a paint bucket in the tool bar). With the cursor, click within the areas that need removing. This process may require numerous clicks to select all unwanted areas and will result in converting the unwanted areas into black pixels. There are a number of simple steps that must be taken to leave only capped brood for consideration versus the total area of the comb on the frame.

To standardize the colors in the image, the next step is to make the image black and white. Go to *Image > Adjust > Threshold* function in the top menu of Photoshop® (shown in Figure 4). The *Threshold* function leaves the image with only black and white pixels, and no grey tones. The holes in the frame will remain black due to the use of the black backdrop while taking the photo. The proper *Threshold* setting must be determined by the user and should be adjusted to show the highest amount of white comb outlines surrounding the black uncapped cells, without compromising the size of any black cells themselves. We find that threshold settings between 50 and 80 serve well, but user practice is required to get the best results for each set of personal equipment. For example, too much emphasis on contrast can result in counts that include the areas of rims of vacant cells. An example of the desired image is shown with the *Image* menu item indicated by the arrow. Once the threshold setting has been determined, it should remain consistent in future analyses and should not need to be reset.



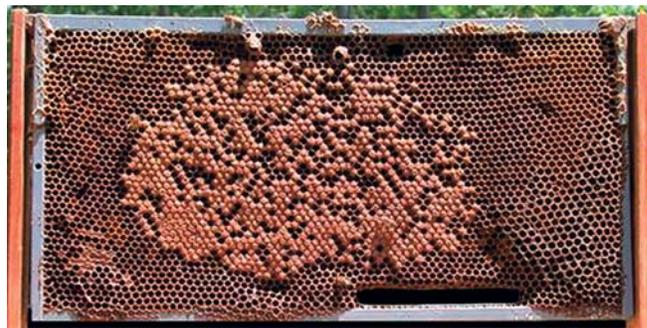
Stand for holding the digital camera and hive frame, placed on top of a super.

Step 4: Select the *magic wand tool* (shown by the bottom black arrow in Figure 5) and click inside one of the black cells that are fully enclosed with white comb. This results in a *marching ants selection line* around a single black uncapped cell. The *marching ants* indicate that all pixel information within the confined area has been selected. With the *marching ants selection* still selected, type the number “135” into the *Tolerance window* at the top of the Photoshop® screen (just below the *Select menu item*). This value of “135” is independent of previous threshold adjustment value and works well for this application. Once this setting has been entered, it does not have to be adjusted again. Next, go to the *Select > Similar* function in the top menu (shown by the top black arrow in Figure 5). The result of using this function is that all the black pixels, representing all uncapped cells, are now selected within the marching ants.

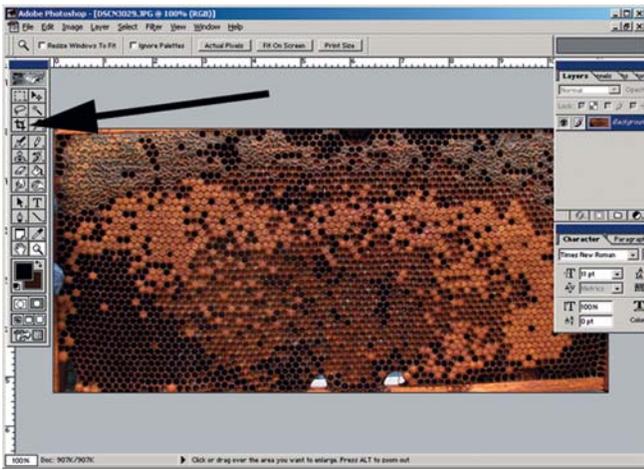
Step 5: Now that all of the pixels which represent the capped cells are isolated, the number of pixels selected can be calculated. Figure 6 presents the elements needed to complete the area measurements. Go to *Image > Histogram* in the top menu. This activates a pop-up window, with the word *Luminosity* in the pull-down menu at the top of the window and a variety of numbers across the bottom. The number we are interested in is the bottom left number beside the word *Pixels*. In this particular example, the number was 197299 pixels. This number represents the number of pixels selected inside of the *marching ants*, and therefore the number of pixels that the uncapped brood area covers. Record this number.

Close this *Histogram* window. Press *Control + D* to deselect the marching ants and go again to *Image > Histogram*. This time, the number of *Pixels* displayed represents all the pixels in the image (equivalent to the total area of the frame). In this example, the number was 254935 pixels. Record this number.

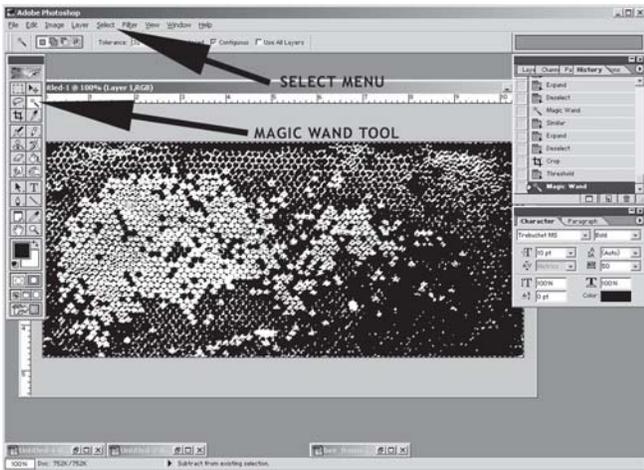
Step 6: Depending on the number of frames to be analyzed, one can either use a calculator or set up a Microsoft Excel® file to do the calculations. Take the number of black pixels calculated (from within the



Frame image captured by the digital camera. This image demonstrates excellent lighting conditions, and good squaring of the hive frame within the digital image.



Cropped digital image in Adobe Photoshop® 6.0. The black arrow shows the cropping tool on the main tool bar, as viewed on the computer screen.



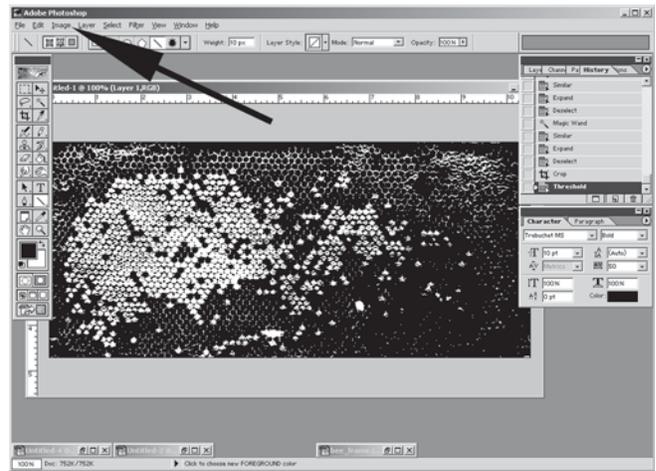
Black areas contained within the marching ants selection area. (The marching ants do not show up in this image as they are too small.) The black arrows indicate the location of the Image menu (top arrow) and the magic wand tool (bottom arrow).

marching ants selection) and divide that number by the total number of pixels in the photo. For this example $197299 \text{ pixels} / 254935 \text{ pixels}$ gives a value of 77.4% uncapped cells in this frame, which in turn gives us a value of 22.6% capped cells in this frame.

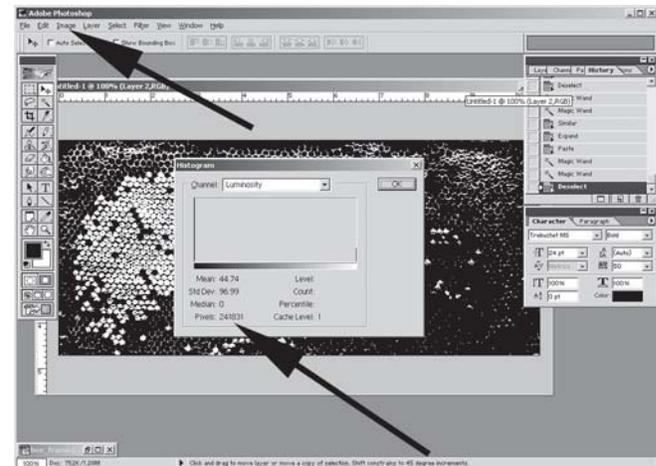
DISCUSSION

The stand is quite simple in design, but the dimensions need to be tailored to the kind of camera to be used. The stand is easily portable to the bee yard, accepts the frames easily, allows the frame to be rotated for both sides to be photographed, holds the camera steady, and is generally sturdy for field use and transport.

This method of using digital photos and computer analysis of the photos to determine brood area does not take long to learn, and once practiced several times can result in the analysis of the area of a frame cover of capped brood cells versus uncapped cells in a matter of minutes per frame. The main inconvenience is



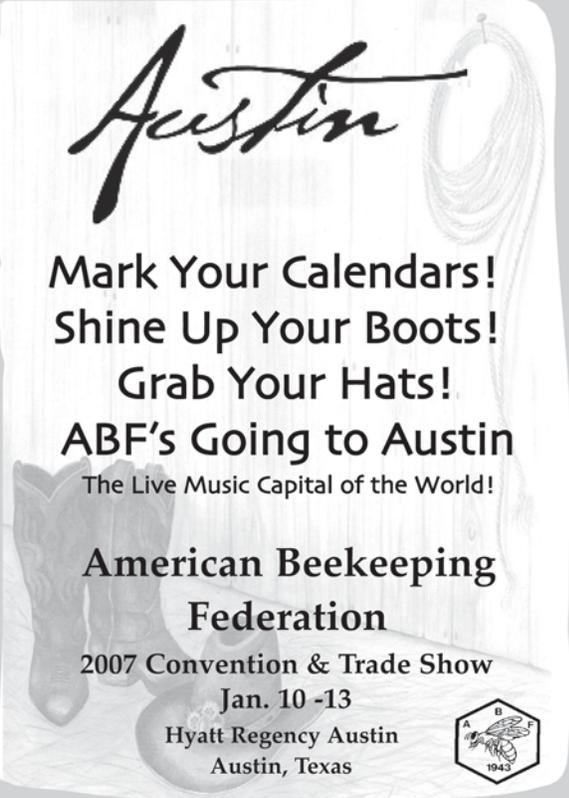
Adjusted black and white image and the desired effect of standardizing the cell colors by using the Threshold command and values. The outline of the cells and capped brood should appear white, while the empty or uncapped brood and unanalyzed areas of the hive frame should appear black.



Computer screen with the black and white image in the background and the Histogram window open. In the Histogram window, the pixel count to be recorded is shown (bottom black arrow). The top black arrow shows the location of the drop down menu.

the present necessity of transposing by hand the sets of two numbers of pixels to a spread sheet for analyses of large experiments. We have test driven the method using over 600 frames from experiments on honey bee nutrition and have found the method to be effective and efficient.

Comparisons have not yet been completed between the results obtained by estimations of capped brood areas using threaded grids and measurements by digital photography. Taking pictures using the stand requires less manipulation and time that the frames are out of the hives. In turn, this results in much less disruption to the hive. Moreover, if the colony being studied is rather defensive, the semi-automated recording for the frames by digital photography allows for less distraction and fewer mistakes in the apiary. Although some of the image processing with the computer requires some user judgment (subjectivity), these are minimal and consistency of analysis on the part of the user heightens as experience is acquired. This



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method is accurate and precise, and is more efficient and less invasive to the hive estimates from threaded grids. **BC**

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