Order parameters from image analysis: a honeycomb example

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Abstract

Honeybee combs have aroused interest in the ability of honeybees to form regular hexagonal geometric constructs since ancient times. Here we use a real space technique based on the pair distribution function (PDF) and radial distribution function (RDF), and a reciprocal space method utilizing the Debye-Waller Factor (DWF) to quantify the order for a range of honeycombs made by *Apis mellifera ligustica*. The PDFs and RDFs are fit with a series of Gaussian curves. We characterize the order in the honeycomb using a real space order parameter, OP_3, to describe the order in the combs and a two-dimensional Fourier transform from which a Debye-Waller order parameter, u, is derived. Both OP_3 and u take values from [0, 1] where the value one represents perfect order. The analyzed combs have values of OP_3 from 0.33 to 0.60 and values of u from 0.59 to 0.69. RDF fits of honeycomb histograms show that naturally made comb can be crystalline in a 2D ordered structural sense, yet is more 'liquid-like' than cells made on 'foundation' wax. We show that with the assistance of man-made foundation wax, honeybees can manufacture highly ordered arrays of hexagonal cells. This is the first description of honeycomb utilizing the Debye-Waller Factor, and provides a complete analysis of the order in comb from a real-space order parameter and a reciprocal space order parameter. It is noted that the techniques used are general in nature and could be applied to any digital photograph of an ordered array.

Keywords: bee honeycomb, radial distribution function, pair distribution function, Debye-Waller Factor, order parameters.

Introduction

The ancient civilizations of Egypt, Greece, and Rome all recorded (Crane 2004) an interest in honeybee colonies and in the regular structure of honeycomb. In particular, Greek mathematicians studied the regular hexagonal structure of honeycomb, and Zenodorus of Sicily (Betts 1921) in the 100s BC showed that, of the three regular figures that will completely fill a plane surface (namely, the equilateral triangle, the square, and the regular hexagon), the hexagon has the greatest content for a given circumference. The classical honeycomb conjecture, which asserts that any partition of the plane into regions of equal area has perimeter at least that of the regular hexagonal honeycomb tiling, was recently proven (Hales 2001). It has commonly been assumed that honeybees construct comb so as to maximize the cell volume for storing honey with a parsimonious use of wax. It can be shown (Toth 1964) that a more economical construction than that used by honeybees can be made. However, Toth admits that the more economical construction has minimal savings of wax and is slightly more complicated to form.

Honeybees manufacture comb in a communal manner (von Frisch 1974). That is, one bee can pick up where another has left off, with little discernible loss of regularity in the comb. Bees have epidermal glands that secrete wax, which they masticate, and fashion into the cell structure. Recently, it has been suggested (Pirk, et al 2004) that honeycomb could be constructed through a liquid equilibrium process, where the body temperature of the honeybees heats the wax and a hexagonal structure results from two-dimensional packing of liquid cells. In nature bees will form colonies in locations that support a measure of defense, but under man's domestication, a more advanced hive is constructed. Rectangular, movable hives were developed (von Frisch 1974) in the nineteenth century, and about the same time machines for making 'foundation' wax were invented (Coggshall and Morse 1984). Foundation wax is manufactured in a press and provides a substrate upon which bees can build honeycomb.

Honeycomb is an ordered hexagonal array and has analogies to 2D modeling of a granular fluid (Reis et al 2006). We have previously introduced (Kaatz 2006) methods that describe how to quantify the order in porous arrays. We use the pair distribution function (PDF) and radial distribution function (RDF) to describe the crystallinity of the arrays. The RDF is commonly used to study liquids (2D or 3D) and the PDF has become increasingly used to model crystalline and non-crystalline materials (Egami and Billinge 2003). In this paper, we analyze several honeycombs and describe techniques to measure the regularity of the structures.

Materials and methods

Four honeycombs (worker comb) that varied in structural regularity and methods of fabrication were purchased from a local apiary. These combs came from different colonies and consecutive years. One, labeled 1062, was supported only by a flat ribbon, but was planar in nature. This was obtained early in the spring, while the rest came from the previous fall. A second, 1049, was made in a rectangular frame, while the remaining two, 1022 and 1060, were made from comb on foundation wax. To enhance contrast in digital photographs, liquid vanilla was inserted into the cells with an eyedropper. Photoshop was used to maximize contrast and to crop the photos for further analysis. Image SXM (Barrett 2008), a digital microscopy software package, was used to identify pores in the comb. Image SXM uses a best-fit ellipse to calculate the center and area of particles (cells) in the arrays. We define a cell as the center of the pore as determined by Image SXM. Subsequently, Excel macros were written that use the center coordinates to generate two-dimensional pair distribution functions (PDF) and radial distribution functions (RDF). Also, reciprocal space maps of the honeycombs were developed using MATLAB and two-dimensional Fast Fourier transforms (FFT). MATLAB uses the FFTW3 routine (Frigo and Johnson 2005). Order parameters from the real space and reciprocal space analysis were created to describe the order in the porous arrays.

The cell structure may be modeled by a discrete pair distribution (PDF) (Egami and Billinge

2003) approach in two dimensions. Suppose there are N_T total cells (about 250 in a typical photo), and N centers from which the RDF and PDF are calculated ($N = 19$ in our examples), and let r_{ij} be the distance between the center of cell i and cell j. N is less than from N_T to minimize edge effects in the calculated PDF and RDF, for a center near the edge would have only three or so neighbors. To make the analysis scale independent, we use the average distance σ of two neighboring cells as a unit and we shall in the rest of this paper assume that this rescaling has been done. Thus the distance between the centers of two neighboring cells will be approximately 1. This σ is called the lattice constant of the honeycomb. Then the RDF is defined by

$$R(r) = \frac{1}{N} \sum_{i=1}^{N_T} \sum_{j=1}^{N_T} \delta(r - r_{ij}) \tag{1}$$

where the $\delta(x)$ is a delta function. There will be a peak around 1, the average distance between two neighboring cells, another peak around the average distance between the center of a cell and the centers of the next-to-closest ones, which is around √3, and subsequent peaks around 2, √7, 3, √12, √13, and 4. The position of these peaks is characteristic for the hexagonal structure of the comb. We shall consider eight of these characteristic distances since that will be sufficient to compute the regularity of the comb. The PDF is then obtained by:

$$\rho(r) = \frac{1}{2\pi r} R(r) \tag{2}$$

where $\rho(r)$ is the PDF.

   In review, a delta function is not a function at all, but is defined by the limiting behavior underneath an integral sign. Originally, P.A.M. Dirac defined it in the study of quantum mechanics. We discuss two cases of $\delta(x)$ which have the proper limiting behavior as shown in Figure 1 (Byron and Fuller 1970). The simplest set of functions are the histograms found for the PDF and RDF. The function $\delta_c(x)$ is defined for c > 0 as:

$$\delta_c(x) \equiv \begin{cases} \dfrac{1}{c} & \text{for } |x| \leq \dfrac{c}{2} \\ 0 & \text{for } |x| > \dfrac{c}{2}. \end{cases} \tag{3}$$

Figure 1: Delta functions represented by a column and by Gaussian curves.

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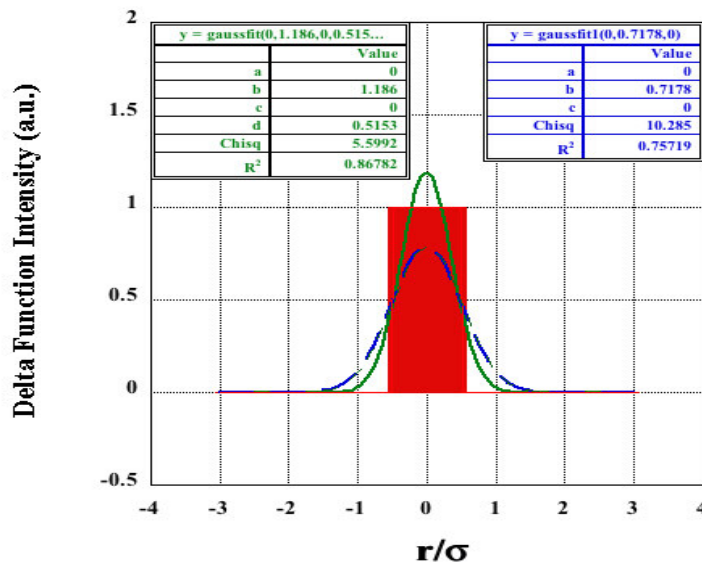
In Figure 1, we use $c = 1$ for the column of data. We have

$$\lim_{c \to 0} \int_{-\infty}^{\infty} \delta_c(x)dx = 1$$

and$\hspace{10cm}$(4)

$$\lim_{c \to 0} \int_{-\infty}^{\infty} f(x)\delta_c(x)dx = f(0)$$

so the definition is satisfied. We may also use a Gaussian distribution as shown in Figure 1 by the dashed line, where

$$\delta_a(x) \equiv \frac{1}{a\sqrt{\pi}} e^{-x^2/a^2} \hspace{5cm} (5)$$

provides another representation of the δ – function. Note that

$$\lim_{a \to 0} \delta_a(x) = 0 \quad \text{for all } x \neq 0;$$

$$\int_{-\infty}^{\infty} \delta_a(x)dx = 1 \quad \text{independent of } a; \hspace{3cm} (6)$$

$$\lim_{a \to 0} \int_{-\infty}^{\infty} f(x)\delta_a(x)dx = f(0).$$

Since computed values of $\rho(r)$ have Gaussian-like distributions around the characteristic distances, we shall approximate the PDF and RDF by a sum of Gaussians.

To model the PDF and RDF we use a more generalized form of a Gaussian function, i.e. a sum of eight Gaussians:

$$\rho(r) = \sum_{i=1}^{8} a_i \exp\left[\frac{-(r-b_i)^2}{c_i^2}\right] \hspace{3cm} (7)$$

where a_i, b_i, and c_i are constants determined by the fit. We use eight since that is the limit we can use in the best-fit model. In Figure 1, this is represented by the solid curve and provides better least-square statistics to a fit of the histogram. The coordination number of a general peak is derived from the RDF as:

$$N_C = \sum_{i=r_1}^{r_2} R(r_i) \hspace{4cm} (8)$$

where $R(r)$ is the RDF, N_C is the coordination number of a nearest neighbor site and r_1 and r_2 define the limits of the peak in the RDF. In our examples the coordination number of the nearest neighbor to the central sites are all six-fold coordinated, or $N_C = 6$ for the first peak, and see the discussion in the Results section for the other peaks. We find that the RDF drops to zero away from the center, in agreement with analysis of samples with finite sizes (Mason 1968 and Kodama et al 2006).

Now R(r) as we have defined it (and hence also $\rho(r)$) is only different from zero in a finite number of r-values, which can also conveniently be represented by a bin diagram. Suppose the bin size is 0.02 and that we consider 205 bins, which is enough to include the 8 first peaks (the eighth one being at $r = 4$), and includes some extra to get the right side of the eighth peak. We then define a disorder parameter as:

$$\Delta G = (0.02) \sum_{\xi=0.02}^{4.1} \rho(r_\xi) - (0.06) \sum_{\xi=n(r_\xi)} \rho(r_\xi). \hspace{2cm} (9)$$

Note that this disorder parameter is simply the sum of the product of the width of the bins (0.02) multiplied by the height of the PDF (the amount in the bin) and subtracting the amount in an ideal location. For very narrow bins these sums are integrals. Therefore, if all the contribution comes from the PDF in the ideal state $\Delta G = 0$. In general, ξ is stepped by the bin width, 0.02. In the sum

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of the subtracted term, $\xi = n(r_\xi) = 1, \sqrt{3}, 2, \sqrt{7}, 3, \sqrt{12}, \sqrt{13}, 4$, is the sum over the ideal eight scaled nearest neighbors positions, and includes a bin above and below the (rounded) nearest neighbor location. The subtracted term is the amount in an ideal location and it is distributed over three bins. Three bins are used since simulations show that it is difficult to generate a PDF of an ordered hexagonal array with $\Delta G = 0$ in a computer model unless one takes a bin above and below the nearest neighbor locations, $n(r_\xi)$, some of which are irrational. This results in a rather sensitive order parameter

$$OP_3 = 1 - \frac{\Delta G}{(0.02) \sum_{\xi=0.02}^{4.1} \rho(r_\xi)} \tag{10}$$

where OP_3 takes values from [0, 1] and equals one when there is no disorder.

It is possible to get quantitative results from reciprocal space via a FFT through the Debye-Waller Factor (DWF). Traditionally, in diffraction analysis, the DWF is associated with thermal decay of the diffraction intensity and does not exist (Chaikin and Lubensky 1995) for two-dimensional crystals. Nevertheless, the definition of the DWF as the term W in the expression exp $(-2W)$ allows us to calculate the disorder in a 2D lattice, without using the thermal approximation. In 2D, the DWF is defined (Kittel 1976) through the equations:

$$I(k_x, k_y) = I_0 \exp(-K^2 \cdot u^2 \cdot \langle cos^2\theta \rangle)$$
$$\|K\|^2 = k_x^2 + k_y^2 \tag{11}$$

where the DWF is defined by the exponential term, K is a reciprocal space vector, u is the mean displacement, $u = r - <r>$, and $<r>$ the average position. The geometrical average of $<cos^2\theta> = 1/2$ in 2D, and is 1/3 in 3D. The FFT and DWF as determined by MATLAB, are sensitive to cell position and number of cells. In order to characterize the data, we use a number of cells as determined by Image SXM, a software package for the analysis of microscope images. The image coordinates (91 sites) are imported into KaleidaGraph, a commercial graphing program. The MATLAB routine calculates the displacement from:

$$\ln\left(I(k_x, k_y)\right) = \ln(I_0) - \frac{1}{2}K^2 \cdot u^2 \tag{12}$$

and a linear fit (Derlet et al. 2005) is obtained from the logarithm of the data versus K^2. A normalized value of K is used, so that in each case the real space distance between cells is one unit.

Results

Images of the various honeycombs and their associated PDFs are shown (Fig 2).

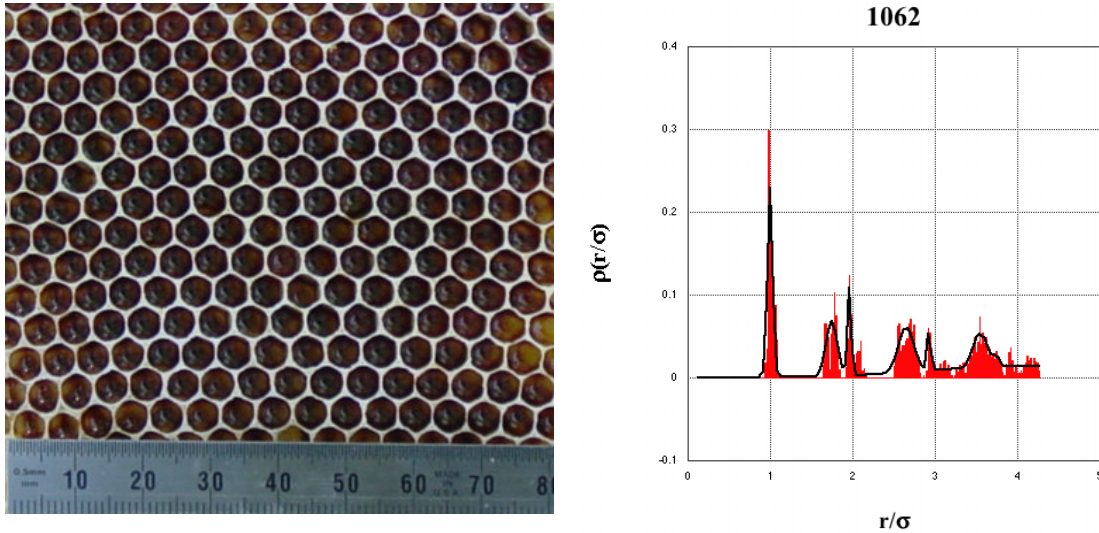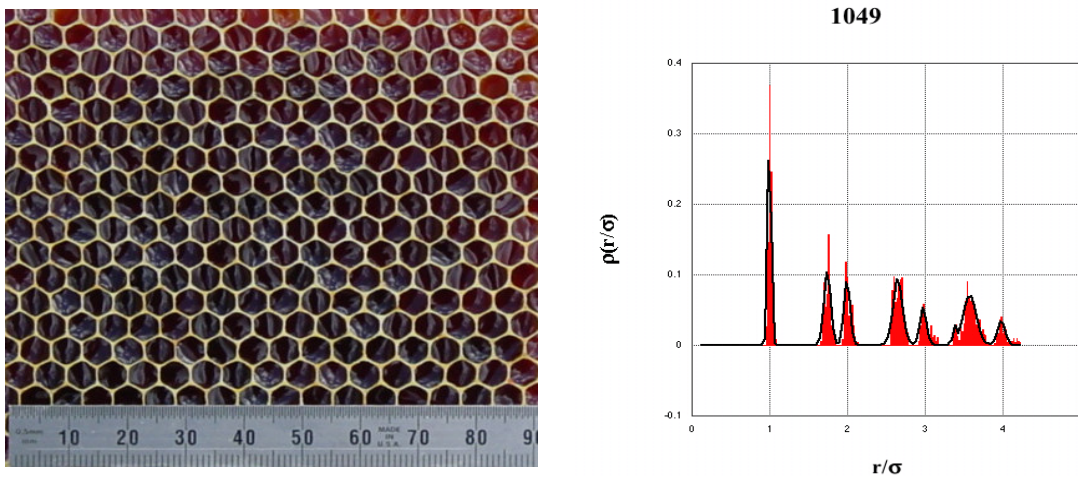**Figure 2A**. Honeycomb 1062 and its' PDF.

Figure 2B. Honeycomb 1049 and its' PDF.

A Gaussian fit is applied to the PDF histograms. Since they are normalized, the peaks appear at the hexagonal nearest neighbors locations, i.e., 1, $\sqrt{3}$, 2, $\sqrt{7}$, 3, etc. The honeycomb of photo 1062 (Fig 2a) is an example of what bees can do naturally, as only a flat ribbon in the hive supported it, and they produced a planar comb. The measured value of OP_3 is 0.33. A summary of the real space and reciprocal space data is given in Table 1.

Honeycomb	Pore Diam (mm)	σ (mm)	RDF R^2	OP_3	u
1062	4.65	5.19	0.7660	0.33	0.59
1049	4.92	5.28	0.8813	0.49	0.63
1022	4.98	5.42	0.8955	0.50	0.66
1060	4.62	5.03	0.9301	0.60	0.69

Table 1: A summary of the real space and reciprocal space parameters is given here. The least-square statistics of the RDF fits are included.

The honeycomb of photo 1049 (Fig. 2b) is one where the bees created the comb in a rectangular frame. The Gaussian fit is somewhat better than 1062 and the order parameter is 0.49. Two honeycombs are shown, 1022 and 1060, (Figs. 2c & 2d) where foundation wax was used as a substrate for the bees to build cells on. The order parameters are 0.50 and 0.60, respectively. The last honeycomb, 1060, (Fig.2d) is the best we have measured to date.

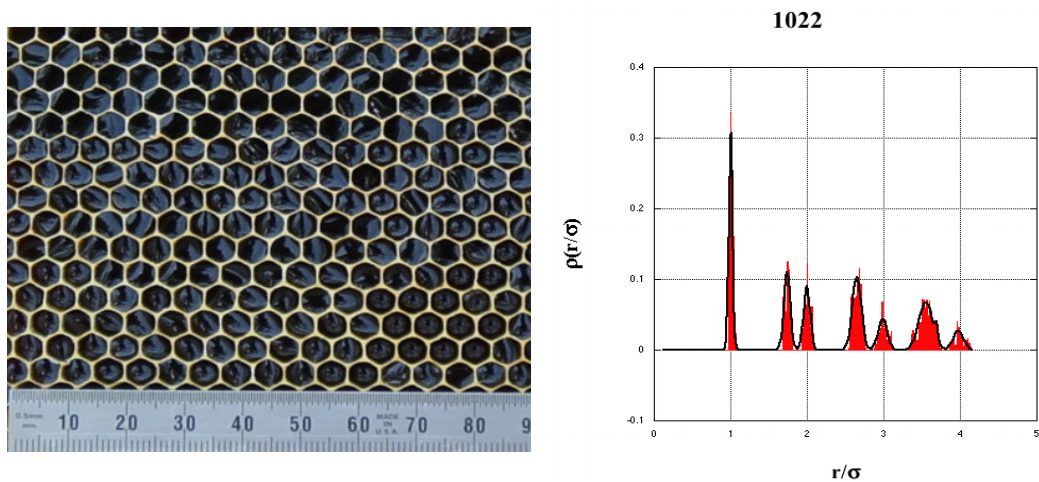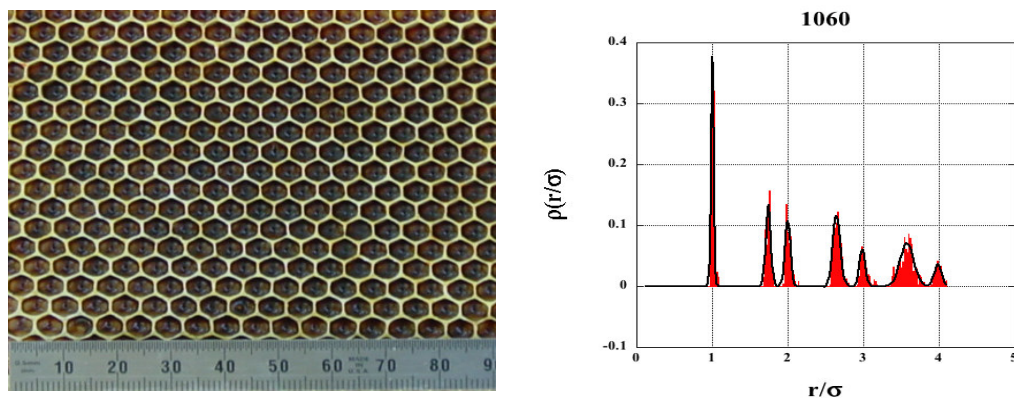**Figure 2C**.: Honeycomb 1022 and its' PDF.

Figure 2D.: Honeycomb 1060 and its' PDF.

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The RDF is related to the PDF and the fits are shown in Fig 3. The R^2 least-square statistics of the fits are listed in Table 1. The histogram of an ideal hexagonal computer generated array is plotted with the Gaussian curve fits. Note that the coordination numbers N_C of the eight peaks are 6, 6, 6, 12, 6, 6, 12, and 6 in agreement with the ideal model. The height of the computer generated peaks are lower than the ideal since they are distributed over three bins. RDFs are used in the modeling of 2D granular fluids (Reis et al. 2006). They find that a granular fluid crystallizes near a packing density of $\phi = 0.719$. Using Image SXM, we find that the packing densities in the honeycomb photos are 0.729, 0.764, 0.792 and 0.792 for photos 1062, 1049, 1022 and 1060, respectively. We thus expect all the honeycombs to be crystalline in a 2D sense, and this agrees with the RDF plots (Fig. 3). It is seen that the RDF plot of 1062 is rising with increasing r and has wider peaks, indicating less structural order. In a 2D liquid, the model shows that the RDF goes to one for large r. The value of ϕ for image 1062 is also closer to the transition from liquid to crystal. In this sense, 1062 is more liquid-like than the other honeycombs.

Figure 3: RDF plots of three of the honeycombs. Also shown is the histogram of an ideal hexagonal array.
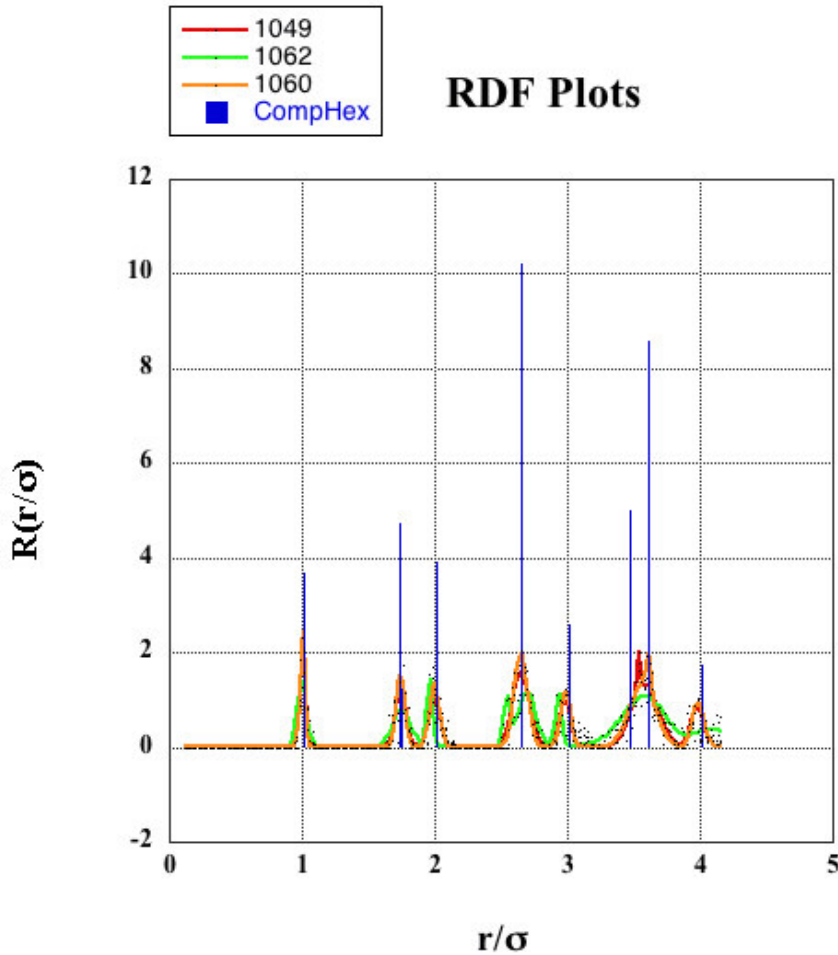
Figure 4: Left: Plot of honeycomb 1060 sites with Kaliedagraph marker size 15. Center: Top view of the FFT showing the hexagonal symmetry. Right: Side view of the intensity versus K space. The other combs have similar plots.
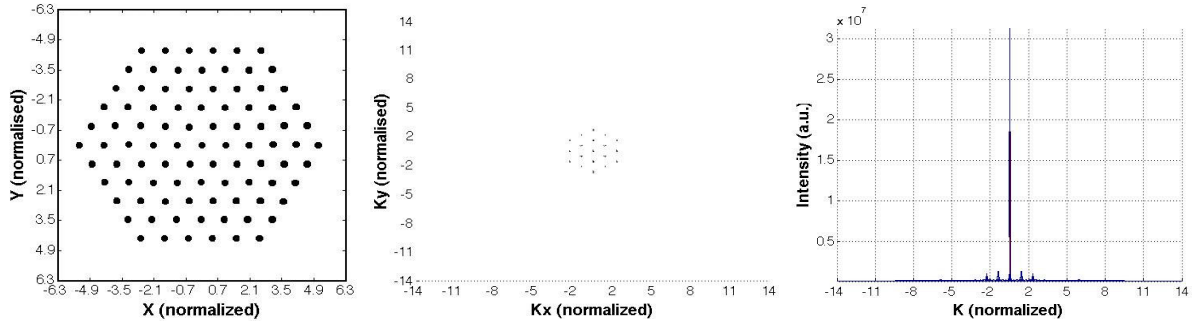

Figure 4 shows the array 1060 created from the honeycomb cells. The left panel is the 91 sites in real space followed by the top view shown in the middle image, which shows the FFT with six-fold symmetry in reciprocal space. A side view of the intensity of the FFT is shown in the third panel. These plots are normalized so that the real space distance between cells is one unit. The FFT intensity is different when the cells change, so in order to compare the deviation from position for the arrays, we use the same marker size (15) in KaleidaGraph. In Figure 5, we show the 3D plot of intensity versus k_x and k_y for the data from honeycomb 1060. The other combs have FFTs that generate similar figures.

Figure 5: 3D image of the intensity versus k_x and k_y for honeycomb 1060.
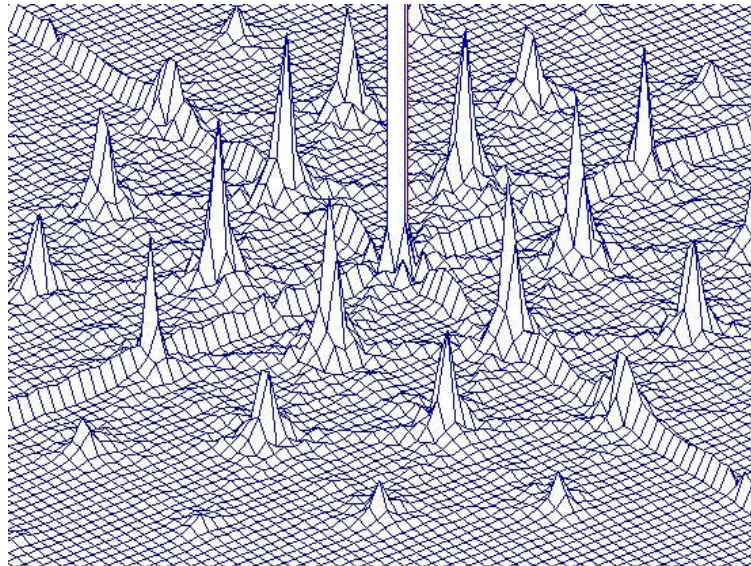
The MATLAB routine sums the intensity in reciprocal space and plots the logarithm of the intensity versus K^2. The code uses a mesh of 180 concentric circles in the k_x, k_y plane over a plot of about 360x360 pixels. Figure 6 shows the plots of the ln(I) vs. K^2, the gradient of which gives -1/2 u^2. A normalized value of K is used in each case so that the real space distance between pores is one unit. We note that since a normalized value of K^2 is used the peaks should appear at the values of the hexagonal nearest neighbors positions squared, i.e., 1, 3, 4, 7, 9, etc. This is reflected in the plots of the data. From this we generate the plots (Fig. 6) of the DWF, u,

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which shows the linear fit (Derlet et al. 2005) to the natural logarithm of intensity vs. K^2. The error bars represent +/- 1% of the data within +/- 1% of the standard deviation. The measured values of u are 0.59, 0.63, 0.66, and 0.69 for photos 1062, 1049, 1022, and 1060 respectively.

Figure 6: Linear plots of showing ln(I) versus K^2 for the various honeycombs.
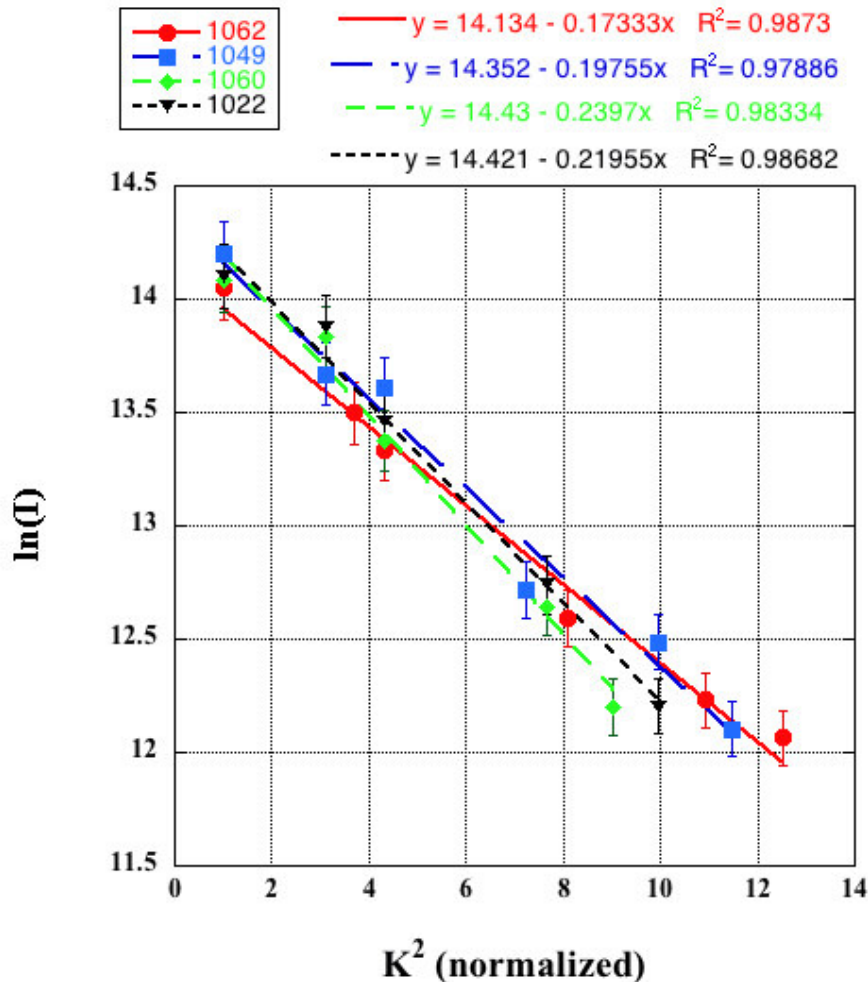
Discussion

Our image analysis indicates that the model described by Pirk et al. 2004, that honeybee comb may be constructed through a liquid equilibrium process, may have some validity. Note that the order parameters for the naturally made honeycomb are lower than any of the combs assisted by man, and that the packing density for the natural comb is very nearly liquid-like. However, a complete explanation of how bees build comb is lacking. Progress along these lines has been recently made, as a suggestion (Bergman and Ishay, 2007) that bees may use ultrasonic acoustic resonance when building comb material, but definitive evidence has not been produced.

Mathematical modeling of honeycombs and colonies has been done previously (Belic et al 1986 and Camazine et al 1990). Belic et al develop a mathematical model for numerical treatment of honeycomb construction in a beehive. The model contains essential features of the bee-bee and bee-wax interactions, and in a qualitative way captures the dynamics of parallel comb construction. Camazine et al present a mathematical model that generates the characteristic concentric pattern of brood, pollen, and honey which develops on the combs of a honeybee colony. Parameter estimates were derived from experimental observations and previously published data. Numerical solutions of the model equations exhibit patterns similar to those

observed in honeybee colonies. The model demonstrates how a colony-level pattern arises from the dynamic interactions of many bees using simple behavioral rules based on local cues. However, our results are the first comprehensive modeling of the order in the honeycomb array in both real and reciprocal space. We find that all the honeycombs investigated are crystalline in a 2D sense. It is known that honeybees can produce comb with crystalline defects (Hepburn and Whiffler 1991), such as vacancies and dislocations, although they are not seen in our examples. We used the center of the comb from a 130 mm wide frame, i.e. away from the elongated comb near the edge, which is important for foraging dances.

The use of reciprocal space techniques with respect to honeycomb is credited to digital photography and modern computing methods. With these capabilities, any photo can be used to generate a FFT and analyzed in a similar manner. While Fourier transforms have been used extensively in mathematical and physical analysis, we show that they can be applied to such diverse subjects as bee honeycomb as well.

The measured differences in the order parameters originate from the alternate ways they are defined. The order parameter OP_3 is a real space measure, essentially related to the height and width of the PDF histograms, while u is related to the reciprocal space definition of the DWF. The analyzed combs have values of OP_3 from 0.33 to 0.60 and values of u from 0.59 to 0.69. Honeybees use sophisticated equipment, feet, jaws, and sensory organs, but can be assisted in creating a well-ordered array by man-made foundation wax. We have shown that with the assistance of man-made foundation wax, honeybees can manufacture highly ordered arrays of hexagonal cells.

Acknowledgments

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