A Comparison between Real and DLA Simulated Liver Lobules using a Population Density Analysis

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A liver lobule is comprised of networks of sinusoids and hepatocytes. Here, a liver lobule was computationally constructed by using diffusion-limited aggregation (DLA) method. A population density analysis of the sinusoids and hepatocytes was performed and then compared with a real lobule image. The resulting images were compared using a histogram to interpret the ratio of hepatocytes to sinusoids.

Background

The liver is a complex chemical factory that operates 24 hours a day to remove chemical compounds, including drug molecules, from the blood (the detoxification process). The average human liver weighs about one kilogram, making it the second largest human organ with a typical volume of 1500 cm^3 (only the skin is heavier and larger). The liver performs over 500 separate functions related to digestion, metabolism, immunity, and storage of nutrients (Rezania et al., 2013a).

As vital as it is, the detoxification process generally damages liver tissue and may cause liver failure. Therefore, a quantitative understanding of drug distribution and metabolism in the liver is essential for the ability to predict both liver performance and structural integrity. This would also be of great utility in the toxicology assessment of newly developed drugs (Rezania et al., 2013a & 2013b).

Unfortunately, there are not many images from real lobules available in the literature and performing experiments would be difficult and costly. As a result, in order to get a better understanding on how structural

variations affect drug distribution in the liver lobule, a computational algorithm was designed.

Liver Architecture

At the macroscopic level, the liver consists of three vascular trees, two supply trees that originate from the portal vein and hepatic artery, and one collecting tree that drains into the portal artery (Arias, 2001). The vessels bifurcate down to the terminal arterioles and venules, which are organized into portal tracts along with a terminal bile duct. Liver cells, called hepatocytes, radiate outward from the terminal vessels. These plates of hepatocytes are interspersed by sinusoids, which play the role of the capillary in the liver, and the spaces of Disse, which are the extravascular space of the liver (Goresky, 1980). Finally, the blood is collected and removed by the hepatic venules.

Functional Unit

The functional unit of an organ is the smallest structural unit that can independently serve all of the organ's functions (Saxena, Theise, and Crawford, 1999). The liver is organized into roughly hexagonal cylinders, called lobules that are commonly accepted as the functional unit of the liver (King, 2010). Each lobule is centered around a hepatic venule and with portal tracts situated at the corners (King, 2010). The typical volume of a lobule is about 1 mm^3, so a typical human liver is approximately composed of 1.5 million lobules. From the hepatic vein span chords of hepatocytes (liver cells) which are separated by networks of sinusoids as seen in Figure 1 (King, 2010). Hepatocytes are responsible for the metabolic, endocrine, and secretory functions in the liver (Bowen, 1998). The hepatocytes are separated by low pressure sinusoid channels which receive blood from the periphery of the lobules and drains through the central vein (Bowen, 2003). The focus of this research was to compare the population density of hepatocytes and sinusoids in a simulated image with those of a real image.

Some anatomical values for a typical human liver are listed in Table 1.

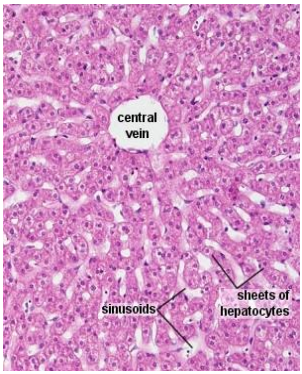

Figure 1: Labeled liver lobule (http://www.lab.anhb.uwa.edu.au/mb140/CorePages/Liver/Images/liv11he.jpg)

Table 1 *Anatomical parameter values for the liver* (Roberts, Donaldson, and Rowland, 1989 & Arias, 2001)

Parameter	Value
Hepatocyte diameter	12-24 µm
Diameter of liver cell sheets	25 µm
Lobule diameter	1 - 2.5 mm
Mean sinusoid diameter	7.3 µm
Vascular tissue component	28 - 30 %

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Specific gravity of liver	1.05
Liver volume	1071 ± 228 cm^3

Methodology

To capture a realistic structure, we adopt the diffusion-limited aggregation (DLA) method.

The size of the simulated image was M x M where M is 256 particles. Then we ran simulations for 37*M (9472), 36*M (9216), and 38*M (9728) particles. In this method, each particle starts with a random location in the field of study. The particle diffuses (performing a random walk) towards the chosen center point. If the particle hits another one in its path, it will stick to it and stop. Then a new particle at a new position starts the journey until all the particles are done. The general DLA structure will grow from the central point towards boundaries. See Figure 2 for detail. This is somewhat similar to the real lobule where the sinusoidal network is outwardly distributed from the central vein towards portal veins.

Similar to a real lobule, we also implemented a hexagonal shape as the boundaries with six outlets (representing portal veins) and one inlet (representing the central vein). The only difference is that in the real lobule,

the blood flows out from portal veins and flows into the central vein.

Simulated Lobule

MATLAB was used to code and generate the sinusoid network using a DLA method. We used a standard DLA algorithm that randomly clusters a specified number of particles (here N_{par}) with particle size (dp x dp – pixel^2).

Here, in order to create a hexagonal lobule comparable to a realistic liver lobule, five steps were taken:

A zero M_{dim} X M_{dim} matrix (field) was created and the DLA algorithm was called to create a DLA pattern with specified N_{par} and dp. A particle, valued 1, was located at a random position in the field by the DLA algorithm and then performed a random walk (with a desired step size, here is dp) toward the center of the field. The newer particle will do the same until it hits another (older) particle, sticks to it and stops. This will continue for all N_{par} = 40M_{dim} particles. See Figure 2a. Here M_{dim} = 256 and dp = 1.

As shown in Figure 3a, the central region is very crowded. We overwrote the central region (here we chose half-the original field) using the DLA algorithm and with a smaller number of zero-valued particles (blue dots). Here we use N_{cen1_par} = 4M_{dim}, M_{cen1_dim} = M_{dim}/2 and dp = 1. See figure 2b.

To fill the central region with sinusoids again (red dots), the DLA algorithm was called for the third time with fewer particles, i.e. N_{cen2_par} = 3M_{dim}. Here M_{cen2_dim} = M_{dim}/2 and dp = 1. See Figure 2c.

In this step, we determine the six corners of the hexagon to create the out-flowing wells at these locations. Again the DLA algorithm was used to create a DLA pattern around each corner. See Figure 2d. Here we use the domain size of 1/3 of the original domain, i.e. M_{dim}/3, and fewer particles, N_{cor_par} = 2M_{dim}. Here dp = 1.

At the final stage, any point out of the hexagon was eliminated. Figure 2e shows the final result of the DLA

hexagonal lobule. As shown, hepatocytes are blue islands that are encompassed by red sinusoids.
Note. Blue dots have value of zero representing no particle is on that site (to be used as hepatocytes) and red dots have value of 1 representing a particle is on the site (to be used as sinusoids).

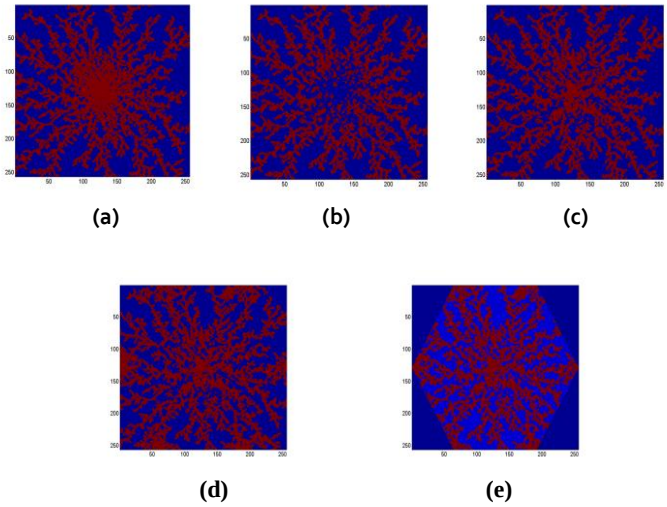
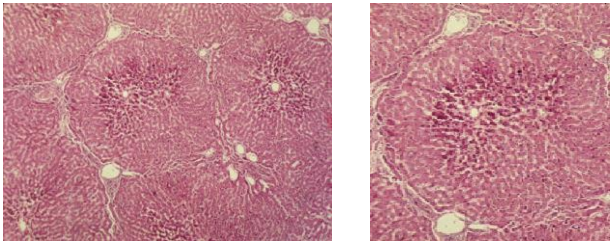


Figure 2: *Various steps to create a DLA hexagonal lobule*. (a) Main DLA pattern with $N_{par} = 40M_{dim}$ particles and size $dp = 1$ on a domain $M_{dim} = 256$, (b) de-crowding the central region using a DLA pattern, (c) filling the central region again with a DLA pattern, (d) creating corner wells using a DLA pattern, (e) eliminating exterior points of the hexagon.

Real Lobule Image
In order to study and compare the population densities between the simulated image and the actual images, several steps were taken.

MATLAB interprets a jpeg image as an array with three dimensions, red, green, and blue (RGB). Each numerical value for a row, column, and dimension in the array corresponds to the intensity. Building on the concept of an array, we were able to crop an image, isolating a specific lobule. This was done by changing the intensity value for all the rows for a specified number of columns for each dimension to the empty set in order to "crop" the image. This process was repeated until we were able to isolate a rectangular region the same size as the simulated lobule, see Figure 3. It was also important to make sure that the real image and simulated image were the same size in order to be able to compare the number of particles.



(a) **(b)**

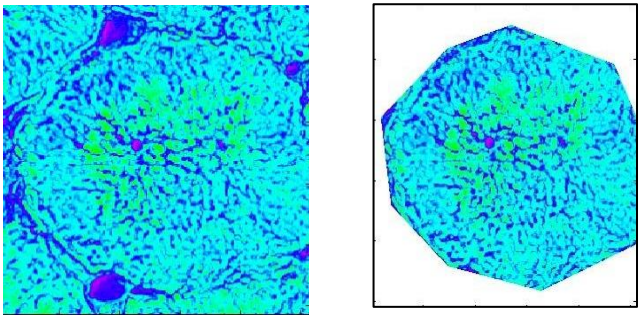
Figure 3: (a) Real liver lobule (http://www.meddean.luc.edu/lumen/MedEd/orfpath/images/fig02x.jpg) (b) Cropped lobule

Once the real image was cropped to the same size as the simulated image, the next step was to increase the contrast between the hepatocytes and sinusoids. The function *dither* was used to convert the RGB (red, green, and blue) image to an indexed image approximation and increase the apparent color resolution. Then the color

map HSV (hue, saturation, and value) was used to make the separation between sinusoids and hepatocytes clear, see Figure 4a.

Isolating the Hexagonal Shape of the Lobule

In order to isolate the lobule, we overlaid a polygon on to the unwanted areas of the liver segment. By repeating this procedure, we were able to isolate the polygonal region of the lobule and making it more comparable to the simulated one, see Figure 4b.



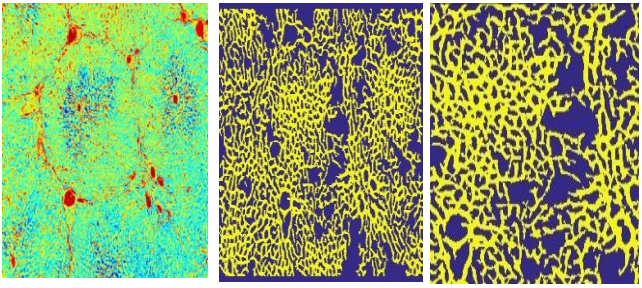
(a) **(b)**

Figure 4: (a) Dithered lobule (b) Isolated lobule

However, there are several issues that arise with this method of image manipulation. One issue is the selected region would not be directly comparable with the simulated image. This is because the polygonal region of the real image would be a different size than the simulated one. Another issue is that the hepatocytes and sinusoids are not distinctly separated. There is a color gradient between the sinusoids and hepatocytes which would make it difficult to compare using a histogram graph. Furthermore, the real image is usually stored as a three-dimensional array as previously stated. This creates

complications with comparing to the simulated image, as it is interpreted as a one dimensional array.

Using Matlab utilities, a function was developed that allowed us to clearly distinguish between the hepatocytes and sinusoids in a 2 dimensional array, see Figure 5a – 5b. Using a set of parameters, the blood vessels are "extracted" from the background in the image using the function rgb2gray, see Figure 5a. At this point the sinusoids are a different color than the hepatocytes (sinusoids are yellow-orange and hepatocytes are blue-green in the figure) but there is still a gradient in the color values. Our goal was to have two distinct solid colors to distinguish the hepatocytes from sinusoids. A function im2bw was used which converts a grayscale image to a binary image. This results in the figure having only two colors, blue and yellow. During the extraction process, the resulting figure is still the same size as the original image. The figure is then cropped so that it is the same size as the simulated image. However, the lobule of interest was not centered in the figure; since in the real image, the center of the image was not the centered around the lobule, see Figure 5b.



(a) **(b)** **(c)**

Figure 5: (a) Real image after blood vessel extraction (b) Real image after digitizing (c) Isolated digitized lobule, but not centered

The lobule was then centered in the figure by adjusting the corresponding coordinates, Figure 6a. Since the simulated lobule had a hexagonal shape, the surrounding area of the isolated real lobule (Figure 6a) needed to be cut. We constructed a cutting algorithm by calculating the corresponding coordinates to create a hexagonal shape. These regions represent the outside of the lobule that is not part of this study. A third color was chosen to separate the hepatocytes, sinusoids, and outer regions.

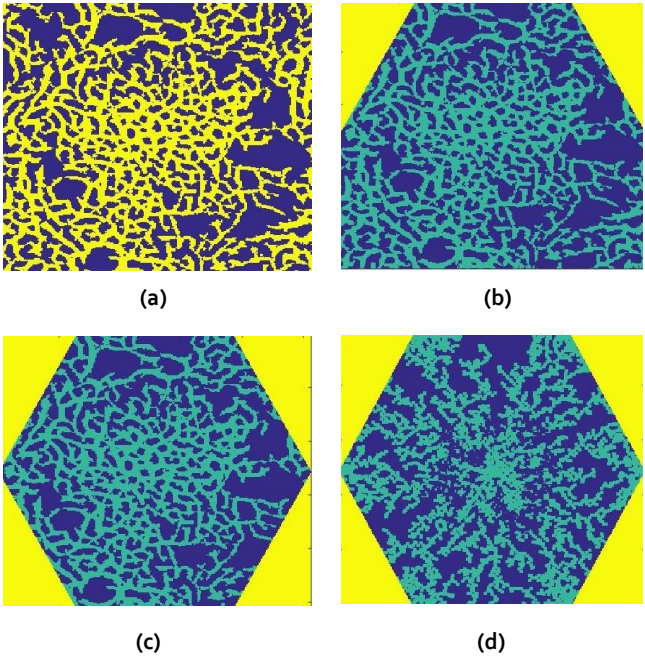


Figure 6: (a) centered real lobule (b) cutting procedure (c) lobule from a real image (d) DLA simulated lobule

Results

Population Density

In order to see whether the simulated lobule was structurally comparable with a real lobule based on the area occupied by sinusoids and hepatocytes, a population density analysis was performed.

The real image (Figure 6c) and the simulated image (Figure 6d) were compared using a histogram graph. The graph illustrates the population densities of the hepatocytes and sinusoids.

The number of sinusoid sites in the DLA function was manipulated until the histogram graph for the real image and simulated had the same shape as seen in Figures 7a-7d. In the resulting histograms, the first bar represents the hepatocyte population, and the second bar represents the sinusoid population.

Using a data curser tool, we were able to get the exact value of particles generated by the real and simulated images. The following table outlines the number of particles generated by each image.

Table 1: *Hepatocytes and sinusoids*.

	Hepatocytes ($\times 10^4$)	Sinusoids ($\times 10^4$)	Deviation ($\times 10^4$)
Real	2.694	2.222	0
$36M_{dim}$	2.747	2.169	0.053
$37M_{dim}$	2.712	2.204	0.018
$38M_{dim}$	2.667	2.249	0.027

Here, we can see that the value of $N_{par} \approx 37\ M_{dim}$ for population gives us the smallest deviation from the number of particles generated from the real image. Furthermore, by comparing the ratio of the sinusoid area to the lobule area results in an average of 45.2 % for real

ones and an average of 44.8 % for simulated ones. By taking into account the thickness of sinusoids and the tissues, the volume of sinusoid to the volume of the lobule would be about 10 % that is comparable to the experimental studies (Hoehme et al., 2010). Using this information, we were able to determine the appropriate liver density population of our simulated image. Further research can be done in modelling diffusion through the liver lobule using the generated simulated image.

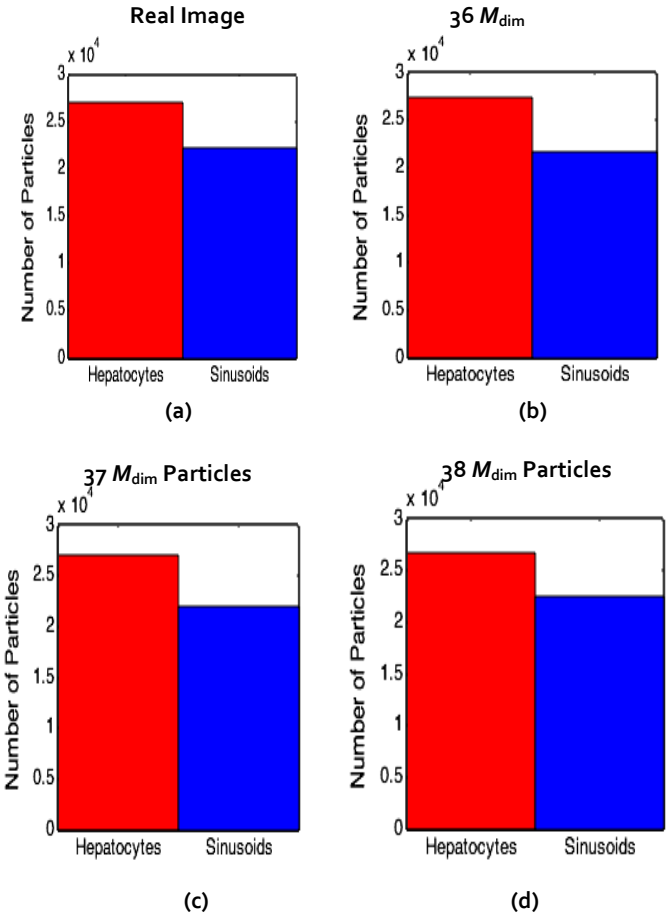


Figure 7: Histogram graphs for (a) Real image (b, c, and d) are calculated by taking the average of 10 simulations.

Conclusion

The goal of this research was to develop a way to compare the image of a real liver lobule to an image of a simulated liver lobule with the focus on the population densities of hepatocytes to sinusoids.

The simulated image was generated by using a diffusion-limited aggregation (DLA) method. In this method we started with 37*M (9472), 36*M (9216), and 38*M (9728) particles. The algorithm starts with a particle at a random location in the field (256 x 256 matrix) and diffuses towards the center making a path. The paths are non-intersecting and the algorithm stops when it has cycled through all of the particles. The resulting paths represent the sinusoidal network in the liver lobule surrounded by sheets of hepatocytes.

In order to compare the real image, we had to first extract the blood vessels in the image using a function rgb2gray. We then had to use the function im2bw to isolate the image into two distinct colors, effectively making a binary image. Once the real image was cropped and centered, it was comparable to the simulated image.

We compared the real and simulated images by using a histogram graph. By manipulating the ratio of hepatocytes to sinusoids in the simulated image, we were able to obtain a histogram graph with the same shape as the real image. The value of $N_{par} \approx 37\ M_{dim}$ (9472 particles) for population in the simulated image resulted in the closest population density to the real image.

Further research can be done using the code for the simulated lobule in the modelling of drug distribution and metabolism.

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