

Indirectly Exploring the Inner Structure of Spherulites

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A variety of diseases are caused by the misfolding of proteins into amyloid fibrils and their secondary structures. One such structure is the spherulite. Spherulites have been found in many diseased organisms and are thought to be a cause of such diseases. Spherulites exhibit birefringence and create useful images when viewed between crossed polarizers. These images change according to the inner structure of the spherulite. To better understand these inner structures, several types of possible spherulites were modeled in 3 dimensions using Mathematica. The images these various models would create when viewed between crossed polarizers were constructed in order to be compared to experiment. Various challenges with the way polarization was simulated led to images that were not necessarily useful for this comparison. More work needs to be done to more realistically model the way light interacts with spherulites in 3 dimensions.

I. INTRODUCTION

All things are made of matter, but all known living things are made of proteins. Proteins are integral to organism structure and function. However, when certain proteins misfold, it can be disastrous. Many debilitating diseases, affecting both the mind and the body, can be caused by the folding of proteins into amyloid fibrils [1]. Alzheimer's disease [2], Parkinson's disease [3], Huntington's disease [4] and Type 2 diabetes [5] are all thought to be caused by the abnormal accumulation of amyloid fibrils and are hence referred to as amyloidoses. The scientific community is still lacking detailed knowledge of the inner structure of these amyloid aggregates, but more information may lead to the cures of some of these diseases.

Especially interesting are amyloid aggregates displaying spherulitic structure. These aggregates are highly ordered and bend light in interesting ways [1]. When placed between crossed polarizers, these aggregates exhibit different patterns and colors which can be interpreted to extract information. The orientation of the fibrils within the spherulite can be inferred, shining light on the inner structure of the mysterious conglomerate.

Recently, several physicists at the College of Wooster used Mathematica to model 2-dimensional cross sections of these structures and what they look like when placed between crossed polarizers [1]. These models proved to be very similar to experimental photos of spherulites, implying that the inner structures of the simulated spherulites are similar to physical spherulites present in living organisms. The closer the simulated photo is to experimental photos, the closer the simulated structure is thought to be to the physical structure.

This project builds on the research of these scientists by extending the modeling of spherulites into the third dimension in an effort to even better understand their inner structure.

II. THEORY

A. Experimental Background

Spherulites are semi-crystalline structures that form when a synthetic liquid is slowly cooled [6]. They consist of a nucleation point from which fibrils radiate. These structures have a high enough degree of order that they bend the polarization of light in an ordered, predictable way [1]. Spherulitic structures have also been observed in many organic compounds. The spherulites simulated in this experiment are organic spherulites composed of amyloid fibrils. Amyloid fibrils come together at a nucleation point, forming a spherical structure not unlike that of a koosh ball. When viewed through crossed polarizers or a polarizing light microscope, these spherulites exhibit patterns that can be interpreted to determine their inner structure.

Light is an electromagnetic wave that is composed of both an oscillating electric field and an oscillating magnetic field which are perpendicular to each other. The orientation of the electric field of a light wave is known as its polarization. The polarization of a lightwave can be altered by many means, such as reflecting it off several mirrors or having it pass through polarizers. Polarizers only allow the portion of light waves through that have an electric field component in the same direction as the polarization vector [7]. The intensity of a light wave, a scalar, is the magnitude of the electric field squared. The intensity is what is recorded in two-dimensional images such as photographs.

When light passes through a polarizer, both the magnitude and direction of the electric field change. The magnitude of the electric field after it exits a polarizer is given by the dot product of the electric field vector and the polarization vector, so that

$$|\vec{E}_f| = \vec{E}_i \cdot \vec{P}, \quad (1)$$

where \vec{E} is the electric field vector and \vec{P} is the normalized polarization vector. The direction of the electric field as it leaves the polarizer is the same as that of the

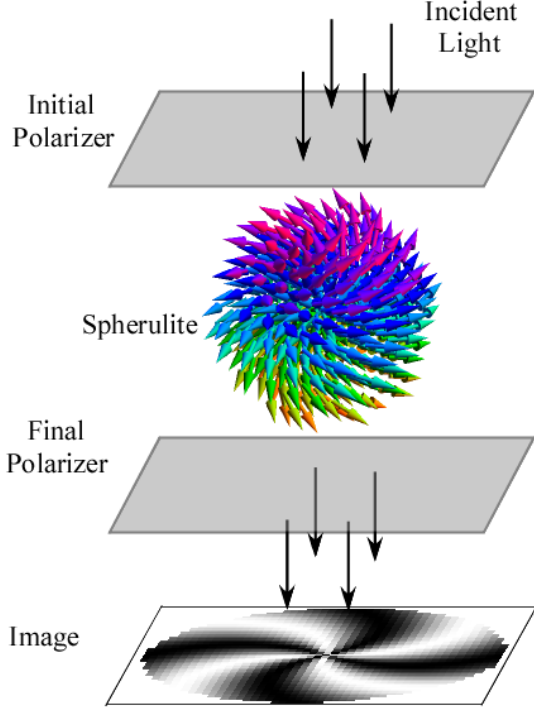


FIG. 1: Schematic view of experimental set up that is modeled in this project. Light passes through an initial polarizer, interacts with the spherulite, and passes through a final polarizer, creating an image.

polarization vector. To calculate the electric field vector after it leaves the polarizer, the magnitude of the electric field as it leaves the polarizer is projected onto, or multiplied by, the polarization vector. Combining these two calculations results in the equation

$$\vec{E}_f = (\vec{E}_i \cdot \vec{P})\vec{P}. \quad (2)$$

Light passing through a polarizer, a spherulite, and a final polarizer goes through this process occur many times. A certain amount of light is let through in the direction of the first polarization vector, then only a certain amount of this light is let through the fibrils, and finally a small amount of light is let through the final polarizer in the direction of the final polarization vector. A schematic diagram of the process can be found in Fig. 1.

This process can be modeled using the computer program Mathematica. By modeling different structures of spherulites and what images they would produce when placed between crossed polarizers, experimental photos taken under these circumstances can be better interpreted.

A spherulite in which all fibrils point directly radially outward produces a pattern known as the Maltese-cross pattern when viewed between crossed polarizers. This is the most common pattern produced, but some other

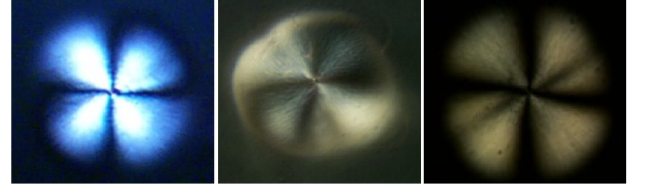


FIG. 2: Experimental photographs of spherulites displaying different inner structures. These images have previously been prescribed inner structures based on the results of a 2 dimensional modeling project. The image on the left shows a spherulite with radial fibrils, the center image is thought to be of a spherulite with fibrils curling opposite ways on each hemisphere, and the image on the right is of a spherulite with two nucleation points. These images are taken from Hardin's paper describing the two dimensional model of spherulites [1].

photos taken experimentally do not display this pattern. They can be interpreted to suggest irregular spherulite structure, which can include the curling of the fibrils or multiple nucleation points. Fig. 2 shows some example experimental photographs taken with polarizing light microscopes, that imply radial fibrils, curled fibrils, and a spherulite with multiple nucleation points.

This project models many types of typical and atypical structures of biological spherulites as well as the images they would create when viewed between crossed polarizers.

III. PROCEDURE

A. Simulating Spherulites with Mathematica

This simulation built off the work of K. Domike, E. Hardin, D. Armstead, and A. M. Donald, who recently modeled the structure and polarization of 2-dimensional cross-sections of spherulites composed of the protein β -lactoglobulin [1]. The simulation process began with looking at and improving their methods.

Modeling a 2-dimensional spherulite cross-section is relatively easy when working with polar coordinates. An overview of the polar coordinate system can be seen in Fig. 3; any point in the polar coordinate system can be mapped using the two variables R , the distance from the origin, and φ , the angle that a line drawn from the point to the origin makes with the x-axis. Using the unit vectors \hat{r} , and $\hat{\varphi}$, a unit vector pointing in any direction can be created. A perfectly radial spherulitic cross-section is simply a map of \hat{r} at every (x, y) point when $\sqrt{x^2 + y^2} < 1$. The same idea is used to model a 3-dimensional spherulite, except that it is a map of \hat{r} at every (x, y, z) point when $\sqrt{x^2 + y^2 + z^2} < 1$. Screenshots of the simulations of these perfectly radial spherulites can be seen in Fig. 4.

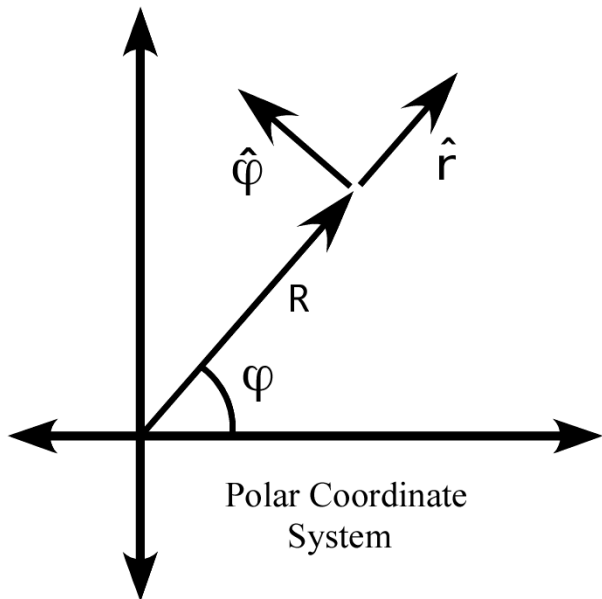


FIG. 3: Diagram of the Polar Coordinate system. Any point can be represented by a distance, R , from the origin, and the angle, φ , that a line drawn from the point to the origin makes with the x-axis. This coordinate system is especially useful when graphing objects that exhibit circular or cylindrical symmetry. Generalized to three dimensions, this coordinate is useful for modeling or graphing objects displaying spherical symmetry.

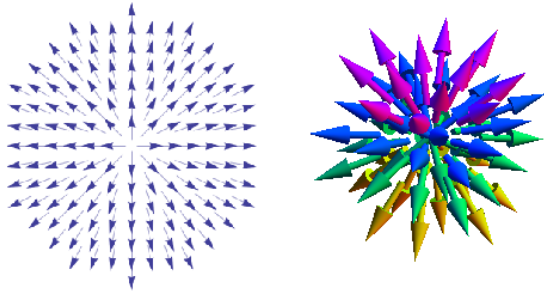


FIG. 4: 2- and 3-dimensional models of a perfectly radial spherulite. These models are simply plots of the unit vector \hat{r} at different points in space with parameters in place so that they form a sphere.

B. Irregular Spherulites and their Parameters: Tilt, Curl, and Nucleation Points

However, as previously discussed, not all spherulites are perfectly radial. There are several different kinds of possible spherulites, and this project modeled two: spherulites with curled fibrils and spherulites with multiple (two) nucleation points. Furthermore, a method was developed in which the spherulites could be tilted and therefore viewed at different angles. This becomes important when the simulated polarizers are put in place.

The first and easiest to model variation of the radial

spherulite is the curled spherulite. Instead of being a map of \hat{r} s, it combines both the polar unit vectors in a function so that the fibrils begin radially at the center and then curl more and more towards the edges. This is accomplished through creating a “curl” parameter. Also, the value $R = \sqrt{x^2 + y^2}$ was used in the equation so that the curling happens stronger as the fibrils radiate outwards from the center. The direction of the vectors used to model these “single curled” spherulites is given by the equation

$$\text{Vector}[x, y, z] = (1 - cR)\hat{r} + cR\hat{\varphi} \quad (3)$$

where c is the curl parameter and R governs the strength of the curl through space. When R is zero, the $\hat{\varphi}$ component is eliminated, resulting in a perfectly radial middle. When R is one, the \hat{r} component is minimized, resulting in maximum curl around the edges. Subtracting $cR\hat{\varphi}$ instead of adding it results in a curl in the opposite direction. This vector is then divided by its length in order to insure that the vector field is composed entirely of unit vectors. These equations were generalized to 3 dimensions by adding the z -component to the R value. This type of spherulite has fibrils that curl one way around the spherulite.

Also modeled were spherulites with opposite curls on top and bottom. These were created due to the thinking that it would be less likely to find a spherulite in nature that only curled one way than it would be to find one that curled two. A twist at one end would create a double curled spherulite, but what, physically, would cause a spherulite with only one curl direction?

The double curled spherulites were modeled using the Unit Step function in Mathematica, which allowed the two halves of the spherulite to be governed by different equations. The top half of the spherulite was governed by the same equation that was used to model the single curled spherulite, and the bottom half was governed by its sister equation, where $cR\hat{\varphi}$ is subtracted from $(1 - cR)\hat{r}$ instead of added. This resulted in a spherulite with an opposite curl on the top than is present on the bottom. Screenshots of spherulites with both types of curls can be seen in Fig. 5.

Another irregular type of spherulite that was modeled was one with multiple nucleation points. This was done by creating a spherulite that has an origin offset by certain values in the x , y , and z directions, and then superimposing it on a spherulite with the origin centered at zero. For unknown reasons, the resulting spherulites were asymmetrical, but this was not resolved due to time constraints. An image of a spherulite produced using this method can be found in Fig. 6.

The way the simulation is set up, the spherulites can only be viewed through the polarizers in one direction; however, in reality one cannot always guarantee that a spherulite will be in a certain orientation, so a method was developed to tilt the spherulites in different ways so that they can be viewed from different angles. This was done using the Mathematica function Rotation Trans-

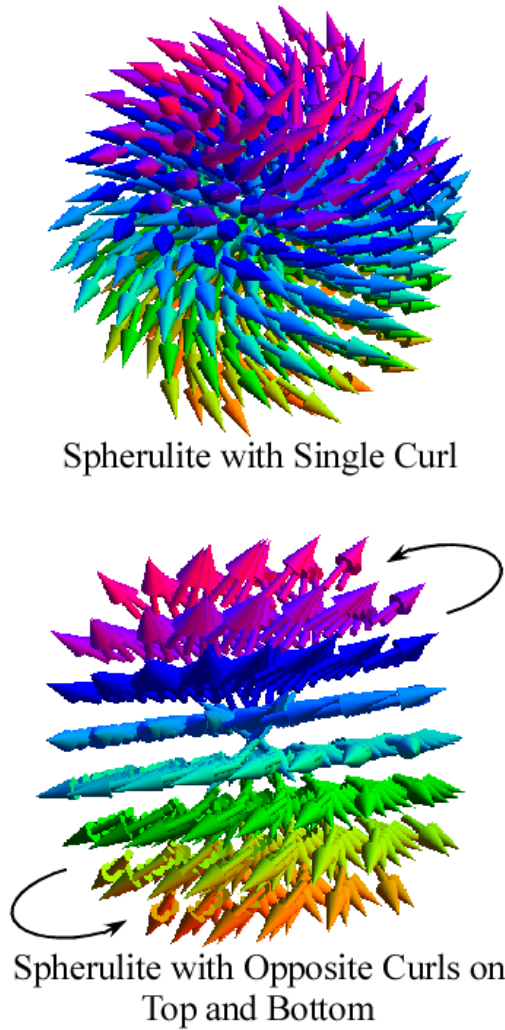


FIG. 5: 3-dimensional models of spherulites curled two different ways. On the left is a spherulite with one curl, and on the right is a spherulite that is curled opposite directions on the top and bottom half. Both have curl strengths of 0.5 on a scale of 0 to 1.

form. This function rotates a function or vector about the origin by the angle θ in the direction of a given vector. The simulation can then be performed at several different angles to see if certain structures correspond better to experimental pictures at different angles. Fig. 7 shows a double curled spherulite tilted at an angle of $\pi/6$ along the x axis. Notice that the division between the hemispheres of the spherulite is not parallel to any of the axes— this is due to the tilt.

C. Adding the Polarization Element

The purpose of modeling the spherulites is so that we can place them between simulated polarizers and create images which can be compared to those of experiment.

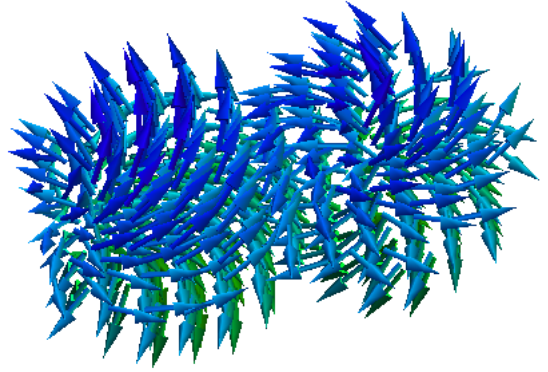


FIG. 6: Computer simulation of a spherulite with multiple nucleation points. Actual spherulites would not have such a large distance between the nucleation points, but this image illustrates the method of simulation nicely. The spherulite was duplicated, translated, and superimposed on the first. There is a lack of symmetry for unknown reasons.

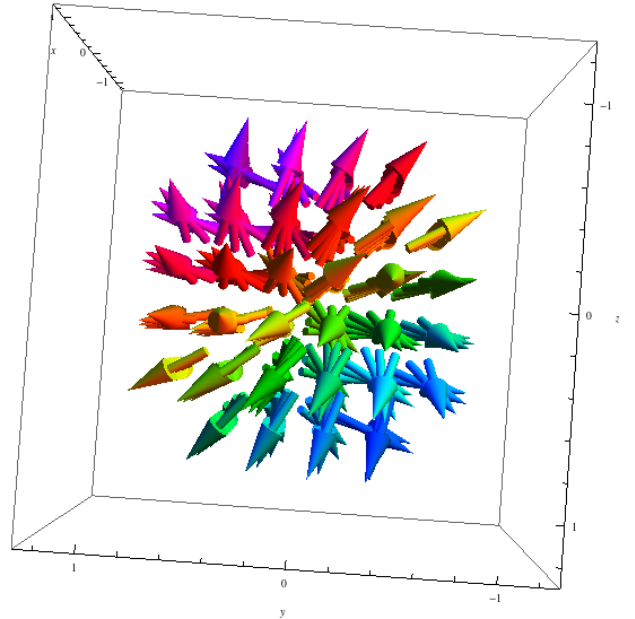


FIG. 7: Image of a tilted spherulite. This spherulite was tilted $\pi/6$ degrees using the Mathematica function Rotation Transform. The division between the hemispheres is not parallel to any of the axis; theoretically this spherulite should produce a different image than one viewed straight down the z axis.

In order to simulate the way light passes through the system, we modeled both the way light acts when it is passed through polarizers and the way it interacts with the spherulite.

Instead of having simulated light pass through a simulated polarizer, the experiment begins with light polarized as if it had just passed through the first polarizer. So, the “polarizers” are actually simulations of the way the light acts directly after it passes through each polar-

izer. The angle at which these “polarizers” are crossed can be changed in order to produce different images.

Similarly, we did not simulate light interacting directly with the spherulite fibrils, but instead simulated what the magnitude and the direction of the light would be after passing through the spherulite.

In order to do this, the spherulite was divided into cross sections which were vertically flattened so that they had no z -components and the cross sections are then rotated 90° in order to simulate how the light would exit the fibrils.

The spherulites are divided into cross sections because it is easier to calculate the polarization in parts than to compute how the light interacts with the entire spherulite at once. They were divided in this manner to best model what is happening physically. The light is propagating in the z -direction, and has so electric field component in the z -direction. So, theoretically, the light should not be affected in the z -direction. The light is rotated 90° to the fibrils because all light with electric fields parallel to the fibrils is absorbed by them in this model.

All these components, the first polarizer, the spherulite cross sections, and the final polarizer, are dotted with and projected onto the next plane in order. For example, in the two dimensional model, there is only one cross section of spherulite, so the equation used to calculate the final image is

$$\vec{E} = (\vec{P1} \cdot \vec{SCS})(\vec{SCS} \cdot \vec{P2})\vec{P2} \quad (4)$$

where $P1$ and $P2$ are the first and second polarizers and SCS is the spherulitic cross section. Each dot product between layers creates a scalar at each point, which are the magnitudes of light let through. Each multiplication by a layer projects the scalars into the directions of the vectors in the next cross section, modeling the magnitude and orientation of the light after exiting the layer.

In order to perform this process in 3 dimensions, a product was inserted in the equation, resulting in the equation

$$\vec{E} = \vec{P1} \cdot \vec{SCS} \left(\prod_1^{\Delta z} (\vec{SCS}_z \cdot \vec{SCS}_{z-\Delta z}) \right) \cdot \vec{P2} * \vec{P2}. \quad (5)$$

The product allows for a variable, Δz , which controls the thickness and number of cross sections the spherulite is divided into.

The intensity of the light as it leaves the final polarizer is the magnitude of the electric field squared. It is this intensity that is used in order to create the final image of what a modeled spherulite would look like when viewed through crossed polarizers.

D. Mimicking Realistic Polarizers

The model above assumes perfect polarizers. However, even the best physical polarizers aren’t perfect, allowing

a small amount of unpolarized light through. In order to achieve more realistic results, an efficiency parameter was added to the simulation. With this parameter in place, each polarizer and cross section was only the percent efficient that the parameter allowed. Each layer let through x amount of light that it was expected to and $x - 1$ amount of the component of the lightwaves perpendicular in direction. For instance, if a polarizer with a 80% efficiency parameter was meant to let through light oriented in the horizontal direction, it would really let through 80% of the horizontal light and 20% of the vertically oriented light. This parameter could be altered to produce different images.

IV. RESULTS

A. Polarization Problems

Initial images produced using low resolution and a small number of cross sections within the spherulite were promising, crudely matching the experimental photos available. However, as the the number of cross sections the spherulite was divided into was increased, more and more light was let through, and the images strayed farther and farther from experimental images. Theoretically, as the cross sections become so thin that the system approaches a continuum, the images should become more realistic, not less.

It was thought that perhaps this was due to the angles of the vectors in the adjacent cross sections becoming more and more similar. If there is not a great change in the angle between cross sections, almost all the light is let through. It was for this reason that we decided to try to implement an efficiency coefficient. However, images of similar resolution using and not using the efficiency coefficient did not show much difference. If anything, the images produced using the efficiency factor were less similar to experimental photos than images produced without the efficiency coefficient. Due to time constraints, this problem could not be solved, and it was decided that dividing the spherulites into two cross sections, or cutting them in half, produced the most realistic images. A continuum of images with different numbers of cross sections can be seen in Fig. 8. These images were created using a single curled spherulite with a curl factor of 0.5 (on a scale of 0 to 1) and polarizers crossed at an angle of $\pi/2$. An image produced using the efficiency factor and same parameters can be found in Fig. 9. It was hard-wired into the efficiency programming that the number of cross sections is proportional to the number of pixels in the resulting image, so that this image was created using significantly more cross sections than those created using the original programming. This may or may not be why the images created using the efficiency factor are so different than experimental images.

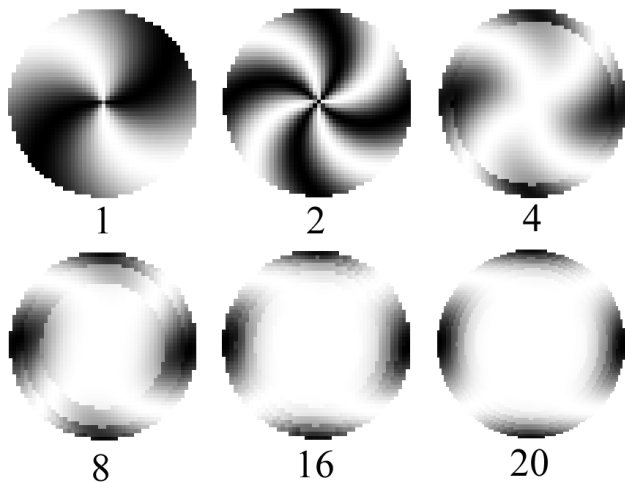


FIG. 8: Images produced by computer simulation. For all images, the spherulites have a curl value of 0.5 and the polarizers are crossed by $\pi/2$. The numbers underneath each image indicate the number of cross sections the spherulite was divided into. As more cross sections are taken, more light is let through, and the image produced correlates less to experimental photos. The image correlating most to available experimental images is the top center, when the spherulite is divided into two cross sections.

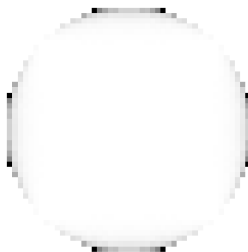


FIG. 9: Image produced with a polarizer efficiency factor of 90% taken into account. In order to better mimic physical polarizers, the simulated polarizers did not completely polarize the light. How efficient the polarizers were was governed by an efficiency parameter. Notice that almost all of the light is let through. This does not correspond with experimental photos available.

B. Radial and Curled Spherulites

With this knowledge, many simulations were performed on spherulites with different parameters using two cross sections. The images produced by the completely radial and single curled spherulites appeared as expected— they matched images produced using 2-dimensional modeling and were very similar to experimental photos. What was unexpected was the similarity of the images produced using the double curled model and the images using the single curled model. When created using the same parameters and compared side, the images using the double curled model and the images using the single curled model were exactly the same.

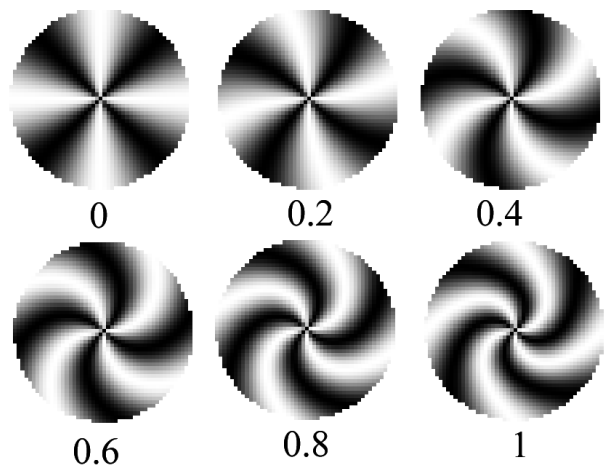


FIG. 10: A series of images created using progressively stronger curl values. As expected, the curl becomes stronger as the curl value is increased. The curl values are on a scale from 0 to 1, with 0 being completely radial and 1 resulting in the edges being completely curled.

So, how curl affects the images was explored further using the single curl model. While the single and double curl models produce identical images, the single curl model results in a faster simulation. Various curl values were used, and, as expected, the curl in the images became stronger as the curl value increased. A sampling of images created with different curl values can be found in Fig. 10.

How the tilt of the spherulite affects the images produced was not able to be explored due to time constraints. The first simulations to be performed would have been tilted double curled spherulites; it is unknown what would happen at the hemisphere line of tilted double curled spherulites. However, although there was one successful modeling of a double curled spherulite tilted at the angle $\pi/6$ along the x axis, the tilt simulation promptly stopped working when a new angle was tried, and was unable to be restored to full function.

C. Multiple Nucleation Points

Modeling the image of a spherulite with multiple nucleation points as it would be seen when viewed between crossed polarizers presented a challenge. The circular shape of the images produced by the other spherulites is due to the limitation in the programming that light only interacts with the region where $\sqrt{x^2 + y^2 + z^2} < 1$. However, spherulites with multiple nucleation points do not have a spherical shape, and therefore running the simulation results in parts of the image missing. Due to time constraints, this problem was not resolved, and no complete images of the multiple-nucleation-point spherulites between crossed polarizers were created.

V. CONCLUSION AND FUTURE WORK

After modeling many different types of regular and irregular spherulites and calculating the image they would produce when viewed between crossed polarizers, it has been concluded that our version of 3 dimensional modeling does not produce images that match experimental photos much better than images produced using 2 dimensional modeling. In fact, as the number of spherulitic cross sections goes up and their thickness goes down, the images produced stray farther and farther from reality. This was thought to be because the magnitude of light let through is governed by the dot product of two vectors, which is proportional to the cosine of the angle between them. The cosine function is not a linear function, and when the cosine of the angle is reduced enough, nearly all the light is let through.

The images were not improved by adding an efficiency factor to the polarization process.

Much knowledge was gained from this project, but there is still much that can be done. Although modeling the spherulites themselves was generally successful, there are a few points at which it can be strengthened. First, the spherulites with multiple nucleation points were asymmetrical when they shouldn't be. Why this is needs to be figured out, and a way to make them symmetrical needs to be found. Also, that the spherulites

with opposite curls produce the same images as single curled spherulites is troubling. A new model for double curled spherulites, with different curl parameters for each side, could be modeled and analyzed. One last task that could be performed is the mending of the tilt parameter.

The modeling of how light is polarized as it passes through the spherulites needs to be improved. More cross sections should result in more realistic images. Future projects could involve perfecting the method of which the light is affected as it goes through each layer; making this process both more realistic and more efficient. Also, the model needs to be improved so that we can look at non-spherical objects, such as spherulites with multiple nucleation points, through the crossed polarizers in their entirety.

Furthermore, in order to find the best models for spherulitic structure, a qualitative method must be developed to relate the computer simulated images to experimental photos. This project focused on the modeling of spherulites and polarizers, not on the comparison to experimental photos. Whether a certain model was considered realistic was determined by visually comparing the images to those taken experimentally. Qualitatively analyzing these images and those of experiment may result in better simulations of the spherulite's inner structure.

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