

Woolverton Papers

Bedside Device and Clinical Application

Lyotropic liquid crystal as a real-time detector of microbial immune complexes

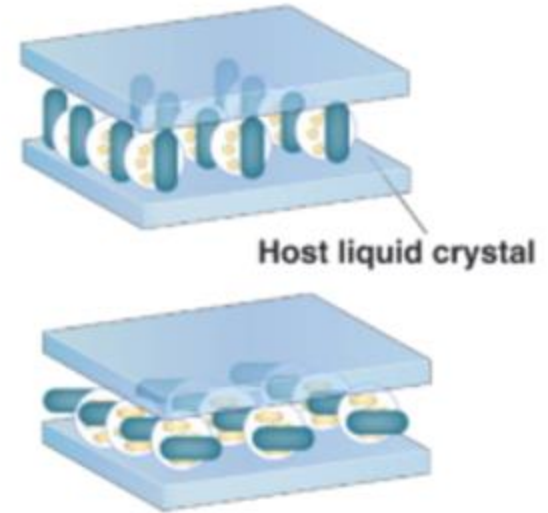
L. Helfinstine¹ , O.D. Lavrentovich² and C.J. Woolverton¹

Purpose

- Amplify the capture of *bacillus atrophesus* through antibody-antigen complexes
- Ability to provide a physiologic environment for microbes
- Aggregation of immune complexes using liquid crystal detection techniques

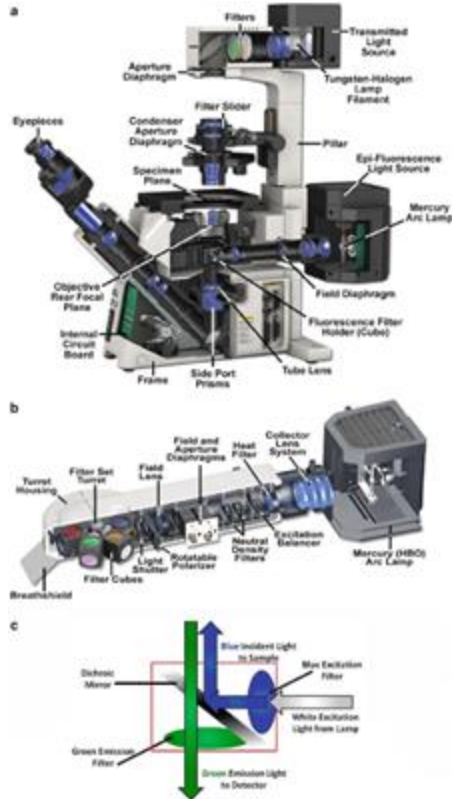
Glass Cassettes and Liquid Crystal Preparation

- Liquid Crystal was obtained from Spectrum Chemical
- Glass cassettes were prepared for evaluating optical events
 - Polyimide surfaces were treated to create an alignment layer for the liquid crystal
 - Two treated slides were placed together to create the cassette
 - Liquid Crystal immune aggregate mixture was allowed to flow into the cassette



Imaging

- Microscope used for imaging: Olympus IX81 Confocal Microscope
- Could image the same region in both polarized and fluorescent confocal modes
- View through cross-polarizers is similar to how we view laptop screens



Light Passing Through Crossed Polarizers

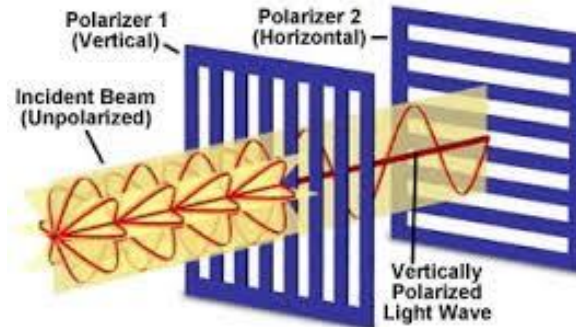
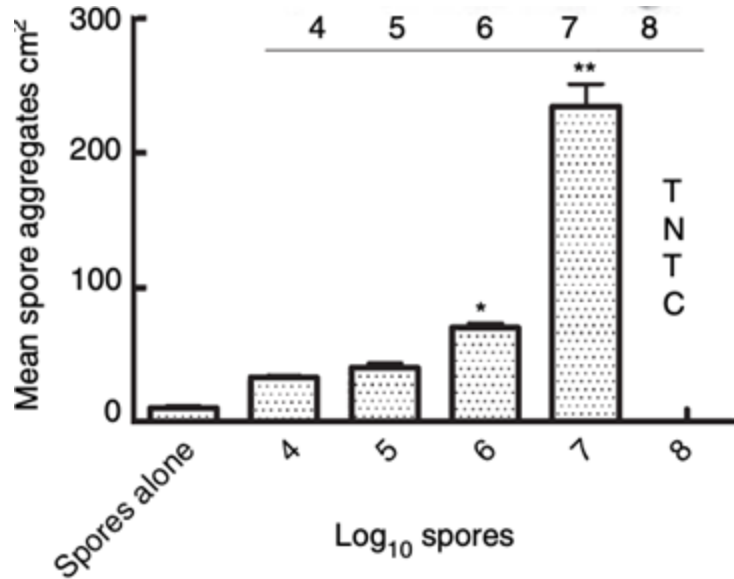


Figure 1

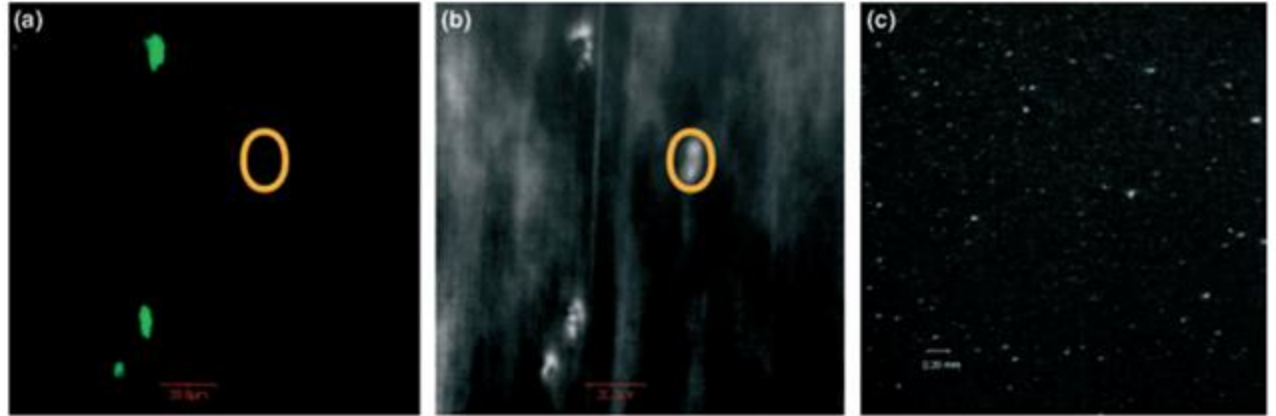
Imaging Results



- Polarized light transmittance
- Detection of aggregates at different spore concentrations

Imaging Results

- Image A:
Fluorescent imaging
of AB-complexes
- Image B:
Birefringent imaging
of AB-complexes
- Image C: Low
magnification view
of immune
complexes between
cross polarizers



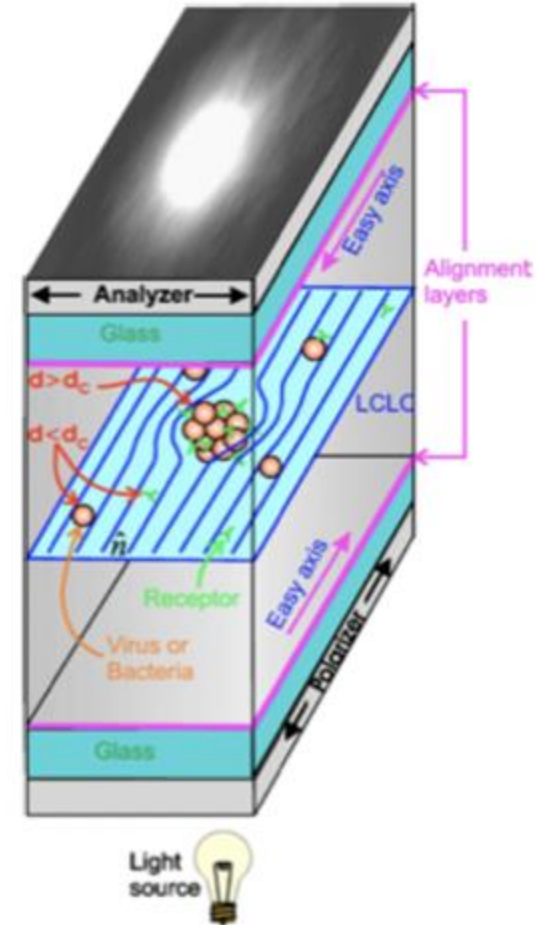
What Did Woolverton Find?

- When targeted spores were added to LCLC-AB complex, spore-AB complexes formed
- Decreasing spore concentration resulted in decreased fluorescent and birefringent signals
- Aggregates were visualized through director distortions
 - Based on elastic properties of liquid crystal
 - Distortions allow for optical events that are larger than the aggregates themselves
 - Small immune aggregates can be detected
- Biosensor function in real time because aggregates form within seconds



Real-time microbe detection based on director distortions around growing immune complexes in lyotropic chromonic liquid crystals

S. V. Shiyanovskii, T. Schneider, I. I. Smalyukh, T. Ishikawa, G. D. Niehaus, K. J. Doane, C. J. Woolverton,³ and O. D. Lavrentovich^{1,2,*}



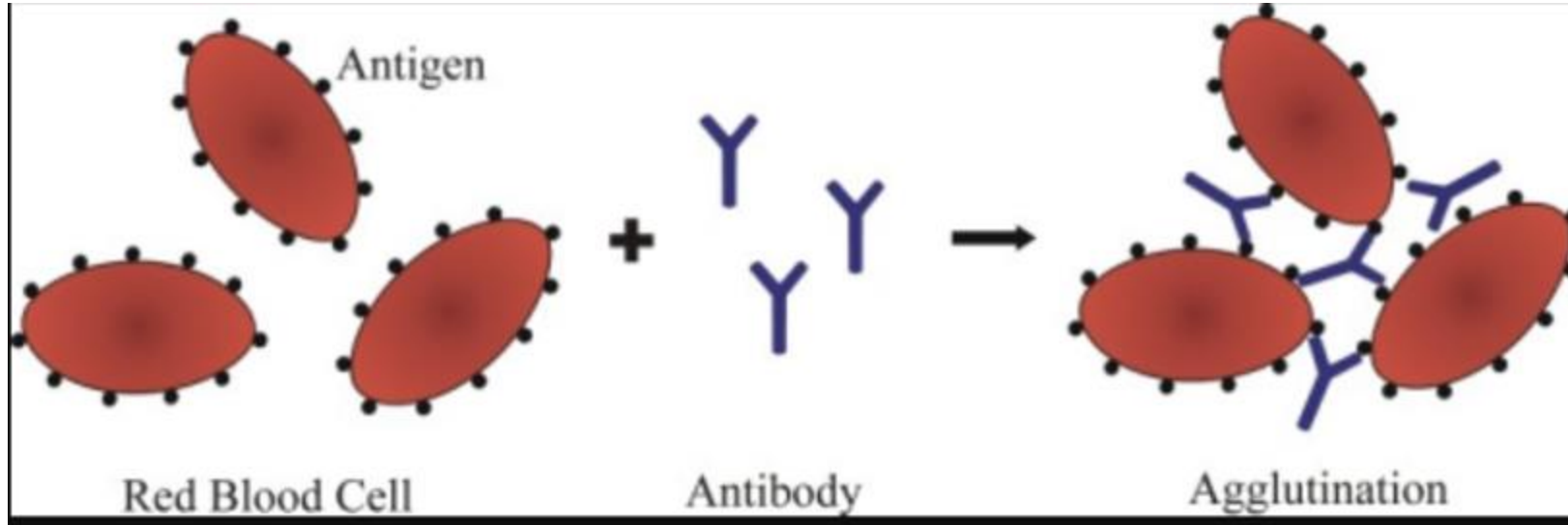
Shade on Abbott

- **LC Alignment:** Difficult to align liquid crystals with antigen receptors.
- **Water Incompatibility:** Thermotropic LCs don't mix with water, needed for biology.
- **Toxicity:** Thermotropic LCs are toxic, limiting biological use.

Solutions

- **LC Alignment:** “surface alignment of LCLC is achieved in a standard fashion, say, by rubbed polyimide films”
- **Water-Based: LCLCs** are water-compatible.
- **Non-Toxic: LCLCs** are safe for biological applications.

Agglutinate



Each Antibody has 2 binding sites so it makes sense that multiple antigen bearing particles can end up glued together.

Agglutination is crucial to forming a large enough object to distort a LC.

Let's see how to connect agglutinate diameter (d) to light transmittance

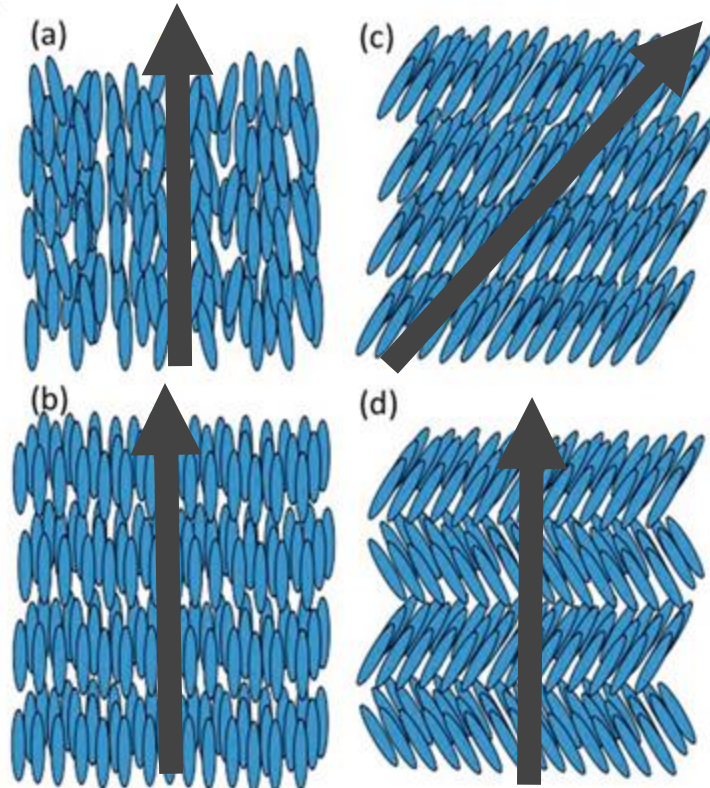
Why?

- agglutinate size: directly correlated to the quantity of antigen, like # of bacteria
- light transmittance can then be connected to quantity of antigen (i.e. how contaminated is a food sample)

How do we define the state of a LC?

A vector ' n ' is denoted to define the average orientation of LC's in a region.

We will call this vector the "director"



https://www.researchgate.net/figure/Molecular-arrangement-of-liquid-crystal-phases-a-nematic-b-SmA-c-SmC-and-d-fiq1_347306904

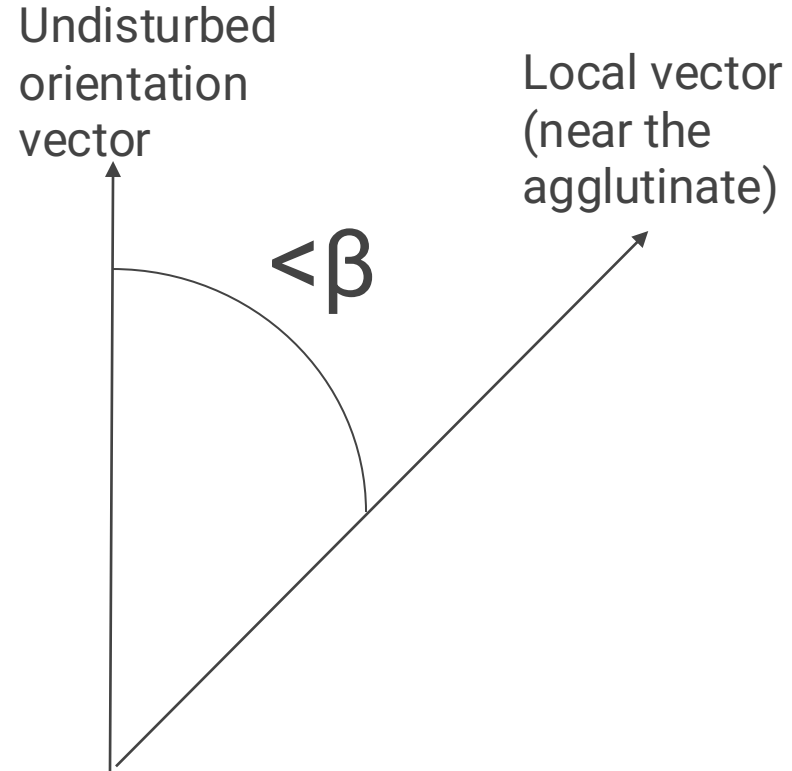
Agglutination inducing LC distortions

The mesogens want to lie flat (tangentially) to an agglutinate.

This distortion can be mathematically described by referencing the vector at the distortion to the resting LC vector.

In this paper, the distortion is described as the angle β

β is the **angle** between the local director and the director where the liquid crystal is undisturbed



Solving for β

The equation that describes β is:

$$\nabla^2 \beta - \frac{\sin 2\beta}{2r^2 \sin \theta} = 0.$$

This equation arises from basic thermodynamics: minimizing the total free energy!

It captures how the director distortions propagate around an immune complex in the liquid crystal. You can define a radial position and the size of the agglutinate to solve for β .

Equations are derived from this to model the LC director (vector) in different cases.

Small particles are easy to model and calculating β is simple.

$$\beta = \beta_0 \left(\frac{R}{r} \right)^3 \sin 2\theta,$$

Solving for β -Large Agglutinate

$$\beta = \arctan \frac{g(r)\sin 2\theta}{1 + g(r)\cos 2\theta},$$

r: The radial distance from the center of the immune complex (assuming spherical particle)

θ : This is the angle between the vector to the point you are interested in and the director where the liquid crystal is undisturbed

$g(r)$: A function of the radial distance **r**, which describes how the director distortion behaves in space due to strong anchoring.

The authors note that:

- these equations are not perfect models
- the first equation is best for modeling small agglutinates
- the second equation (this one) is better for large agglutinations

Okay, last equation I'll cover

$$F_{\text{tot}} = \frac{K}{2} \int_V \left(\left(\frac{\partial \beta}{\partial r} \right)^2 + \frac{1}{r^2} \left(\frac{\partial \beta}{\partial \theta} \right)^2 + \frac{\sin^2 \beta}{r^2 \sin^2 \theta} \right) dV + \frac{1}{2} \int_S W \cos^2(\beta + \theta) dS$$

The total free energy (F_{tot}) of the system is calculated with this equation. Like we said before, the Laws of Thermodynamics wants F_{tot} to be as small as possible.

K: elastic constant that defines the "stiffness" of the liquid crystal

W: surface anchoring energy coefficient, defines how strongly the liquid crystal aligns at the surface of the particle.

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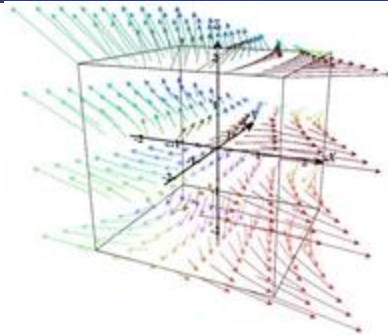
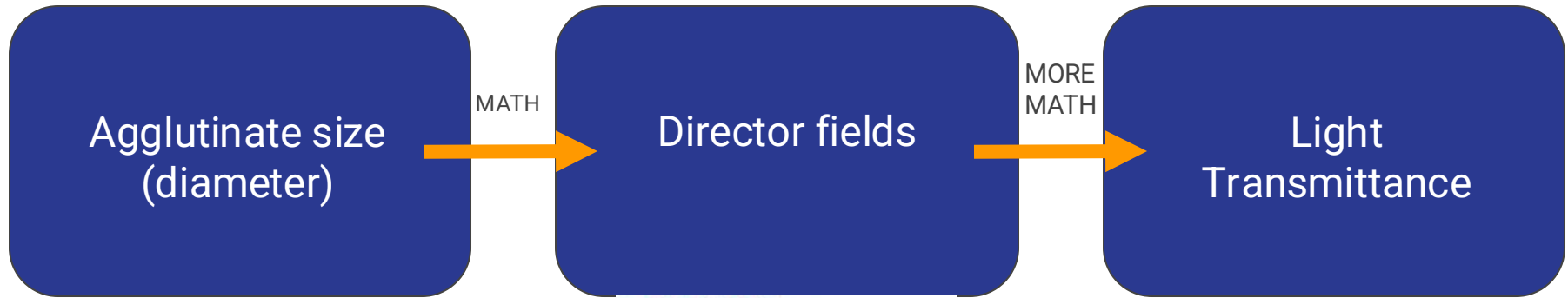
W: surface anchoring energy coefficient, defines how strongly the liquid crystal aligns at the surface of the particle.

The independent variable in equation is the **director distortion** angle $\beta(r, \theta)$. We use calculus to find the configuration of the liquid crystal director field that **minimizes the total energy**

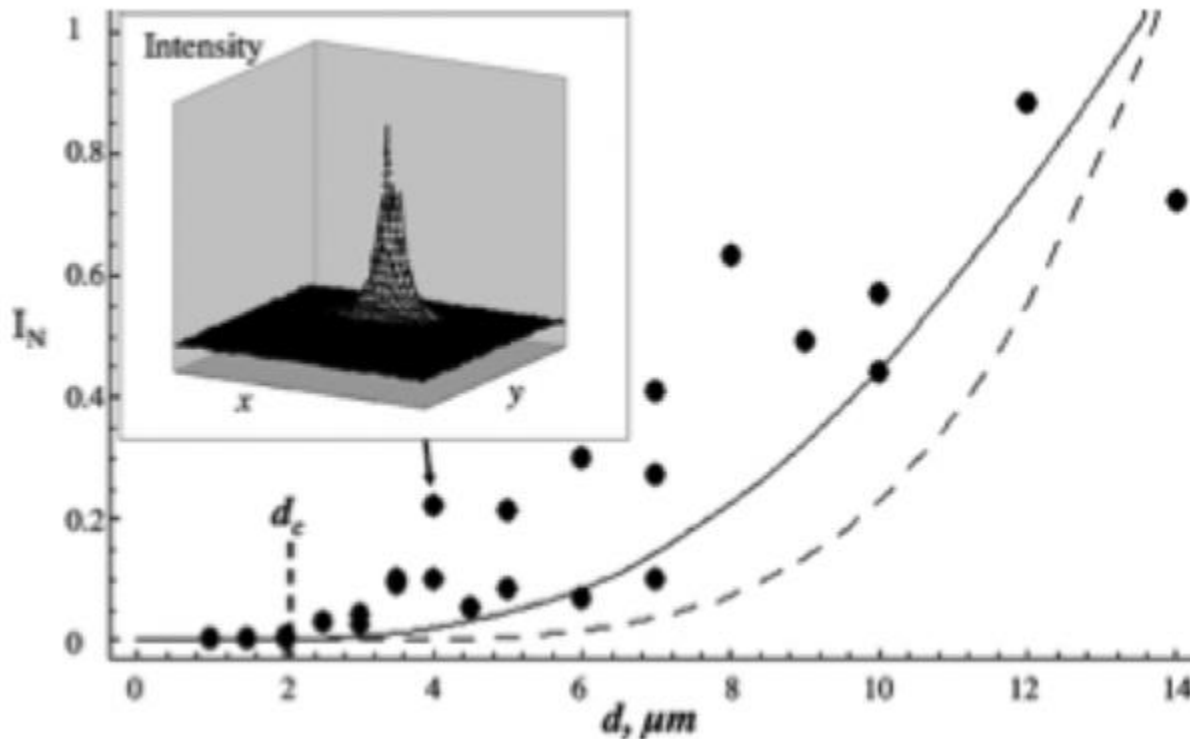
This equation reveals how the liquid crystal is distorted around the immune complex of size d , and its results are used to predict the optical output.

Transmittance equation

All of this work and modeling can then be plugged into more equations to predict the transmittance of light through the liquid crystal.



Models vs Experimental



four times larger than the value estimated from Eq. (11) with $\beta_0 = \pi/4$. The discrepancy is caused by a nonspherical shape of complexes and the finite h that results in $\Theta_{1,2} \neq 0$ and finite limits of integration in Eq. (9).

Y-axis: light transmitted

Dashed Line: small agglutinate model

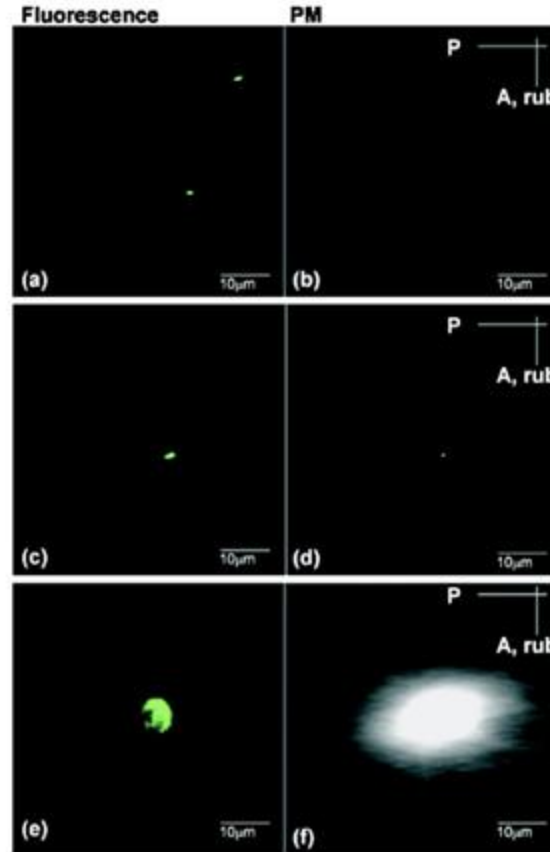
Solid Line: large agglutinate model

- Here you can obviously see how the simpler equation works just fine for small agglutinates.

- It's also obvious why a nonspherical agglutinate is distorting more LC, it has more surface area!

Optical output

A fluorescent label on the antigen was used to image the actual size of the agglutinates.



Here we see the agglutinate $d < d_c$. This could in theory be tuned by using a liquid crystal which has a higher affinity to align tangentially to the agglutinate

Here we see the optical output of light through a cross polarizer LCLC setup.

Citations

Real-time microbe detection based on director distortions around growing immune complexes in lyotropic chromonic liquid crystals. S. V. Shiyankovskii, T. Schneider, I. I. Smalyukh, T. Ishikawa, G. D. Niehaus, K. J. Doane, C. J. Woolverton,³ and O. D. Lavrentovich^{1,2,*}

Vector field picture: c3d.libretexts.org

Helfinstine, S. (2006). *Lyotropic liquid crystal as a real-time detector of microbial immune complexes*. Letters in Applied Microbiology.

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