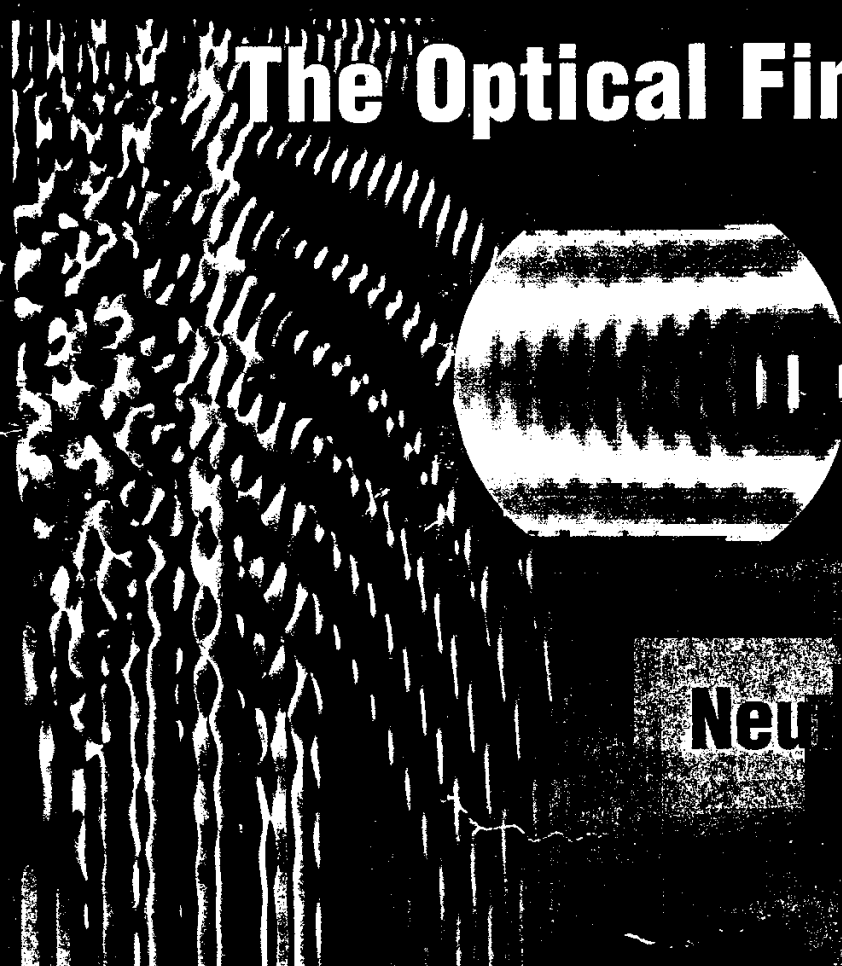


IEEE

COMPUTATIONAL SCIENCE & ENGINEERING

Spring 1996

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IEEE SOCIETY FOR COMPUTATIONAL SCIENCE & ENGINEERING

1063-4303/96/0000-0000\$05.00

The Optical "Fingerprints" of Cells: Catching the Bad Guys

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When a particle is illuminated by a beam of light, it scatters part of the incident light in all directions. The intensity and polarization of the scattered light all around the particle can be viewed as the particle's "fingerprint." In the spirit of famous detectives, our team at the University of Amsterdam is devising a method to match these fingerprints with the particles that created them. We do not try to solve the inverse problem,

that is, calculating backwards from the optical fingerprint to its creator [see *IEEE CS&E*, Winter '95]. Instead we are trying to apply to one important class of particles, namely biological cells, a method much like that a police detective uses to identify criminals by their fingerprints. The aim of our research is to develop a laboratory-based system that can recognize subsets of human white blood cells and bone marrow cells using a very limited set of light-scattering signals (analogous to a limited set of whorls and loops in an actual fingerprint). One application of such work is as a new way to distinguish diseased cells from healthy ones in real time.

For a useful system we need

- ◆ a database of the scattering "fingerprints" of the types of cells we want to distinguish;
- ◆ a good experimental method of gathering scattering data from the particular cells we want to identify, that lets us use a small set of scattering signals; and
- ◆ an effective way to match new scattering signatures to the database.

The heart of the system we are devising is a flow cytometer, where cells are sent one by one through a highly focused laser beam. Light-scattering signals from each cell are measured in forward, sideward, and backward directions. We can then adapt the polarization of the incident laser beam and measure carefully chosen polarization states of the scattered light.

Although the four main types of white blood cells can already be recognized via correlation of forward- and sideward-scattered intensities, sensitive and reliable detection of subsets of cell types

requires carefully designed laboratory experiments, exploiting the information present in the polarization states of the scattered light. For instance, the group of Bart de Groot and Jan Greve (University of Twente, the Netherlands) showed that by measuring the depolarization of scattered light it is possible to discriminate between eosinophilic and neutrophilic granulocytes (both subsets of this kind of white blood cell are shown in Figure 1), which is impossible to do with scattered intensities alone.

Our approach to devising the best experiment is through simulation. First we solve the forward problem: starting with a broad and fairly realistic range of simulated particles we compute the light-scattering matrices of cells—their optical fingerprints. We do this for a large number of particle models, slowly varying a morphological parameter such as the position or roughness of a nucleus in a blood cell. From these data we try to extract experimental settings which are most sensitive to the morphological characteristic that we are trying to measure. Unfortunately, the size, morphology, and optical parameters of real cells (for a typical white blood cell, see Figure 1) are such that we cannot rely on exact light-scattering theories, such as the Mie theory for scattering by spheres, nor on low- or high-frequency approximations, such as the Rayleigh-Debye-Gans theory or physical optics methods.

To simulate light scattering from complex particles such as white blood cells, we use the discrete dipole approximation (DDA), which is closely related to the method-of-moments solution of the volume integral equation formulation

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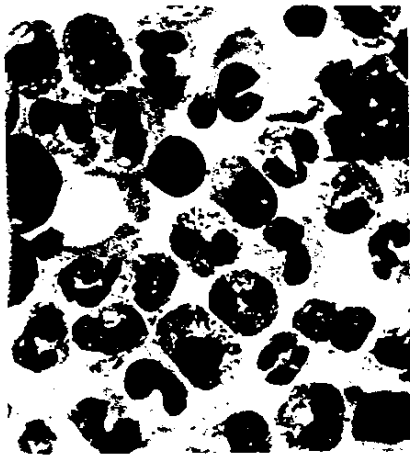


Figure 1. The nuclei of granulocytes exhibit a rather complex shape. The cytoplasm contains many small vesicles.

of frequency-domain computational electromagnetics. (For an overview of these methods, see the article by Volakis and Kempel, *IEEE CS&E*, Spring '95, pp. 42-57.) The DDA, much like the method of moments, discretizes the volume of a particle in equal cubical volume elements. An accurate sampling of the wave phenomena that we are trying to simulate requires that the sides of the cubes should be small compared to the wavelength of the incident light. In our experience, a spatial resolution of $\lambda/15$ (where λ denotes wavelength) gives accurate results.

The DDA requires that we compute the polarization of each volume element. Using a conjugate gradient method we solve a system of N linear equations with N unknowns, where N is the number of volume elements in the model. The matrix-vector products in the iterations can be identified as discrete convolutions and can therefore be computed efficiently using 3D fast Fourier transformations, resulting in an $O(N \log N)$ method to solve the DDA equations.

Typical white blood cells have diameters ranging from 7 to 11 μm . Normally in our experiments we employ HeNe lasers, whose wavelength is 632.8 nm. Combining all the numbers shows that our models will contain two to nine million dipoles. Unlike the efficient numerical procedure we use to solve the equations, such enormous models pose serious computational problems in terms of memory requirements and execution times. DDA models with about 10^4 dipoles consume 2 to 20 Mbytes of RAM and are computed in a few minutes on a

PC or a workstation. Larger models, with about 10^5 dipoles, need much more memory and execution time. For instance, a model containing 500,000 dipoles would need 120 Mbytes of RAM, and the execution time on a Sun Sparcstation 5 would be on the order of 5 to 10 hours.

Still larger models, with millions of dipoles, grow beyond the capabilities of current workstation technology. We have therefore developed a parallel version of the code, allowing us to run models with several million dipoles using workstation clusters or massively parallel computers.

Figures 2 and 3 show the results of DDA simulations of spherical particles. The figures illustrate the intensity of the internal electric field in a slice through the sphere. In Figure 2 the sphere is illuminated by a plane wave, and in Figure 3 by a focused laser beam. To determine our model's accuracy we compared these results with Mie theory and found that DDA produces a very accurate model. Once the internal fields are known, scattered fields can be calculated.

The large rectangular image on the cover is a visualization of a light-scattering data set that we computed. Here we have simulated scattering by concentric spheres, continuously varying the diameter of the inner sphere from 0 to the diameter of the outer sphere. On the short axis we have the scattering angle from 0 to 180 degrees, on the long axis the diameter of the inner sphere. We have simulated 3,000 slightly different particles, each one represented by a line on the short axis. The colors code for the intensity of a specific element from the scattering matrix.

In the near future we plan to generate such data sets for realistic models of human white blood cells, and then devise laboratory experiments capable of discriminating between cell types. ♦

Acknowledgments

We thank Michel Grimminck for his inspiring contribution to this work, and for his visualizations. We also thank Carl Figdor, University of Nijmegen, for providing detailed information on the morphology of blood cells, and Francis W. Ruscetti of the National Cancer Institute for providing the image in Figure 1.

This article appears on *CS&E's* Web page.

For further reading

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- C.R. Bohren and D.R. Huffman, *Absorption and Scattering of Light by Small Particles*, John Wiley & Sons, New York, 1983.

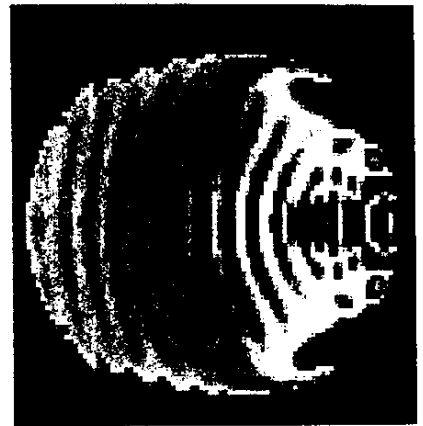


Figure 2. The intensity of the internal field in a plane through a sphere illuminated by a plane wave. Dark blue is low intensity, red-brown is high intensity.

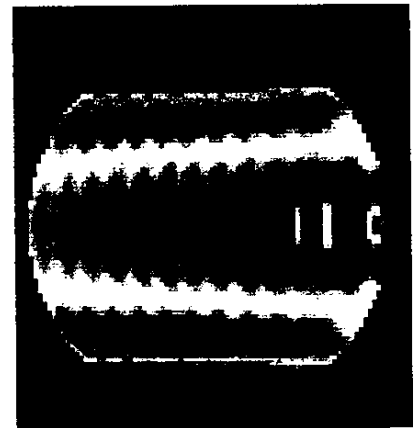


Figure 3. A plane in the sphere is now illuminated by a focused laser beam, using the same color scale as in Figure 2.

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