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# Birefringence analysis of hemozoin with surface plasmon microscopy towards malarial species distinction

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## I. Introduction

Female Anopheles mosquitoes carrying the Plasmodium parasite are the primary vectors of malaria mostly prevalent in countries exhibiting tropical climate [1]. The parasite species Plasmodium falciparum, Plasmodium vivax, Plasmodium malaria, Plasmodium ovale, and Plasmodium knowlesi are the five that can infect humans. Plasmodium falciparum is responsible for 70% of the infections, whereas Plasmodium vivax is responsible for 20% of them [1]. WHO reports that 249 million cases were recorded in 2022, of which 233 million (about 94%) were reported in Africa. Nearly half of all cases were reported in Nigeria (27%), the Democratic Republic of the Congo (12%), Uganda (5%), and Mozambique (4%). Malaria is expected to have caused 608,000 fatalities worldwide in 2022. Just four nations accounted for more than half of all deaths: Tanzania (4%), Niger (6%), the Democratic Republic of the Congo (12%), and Nigeria (31%). Eleven countries namely Burkina Faso, Cameroon, the Democratic Republic of the Congo, Ghana, India, Mali, Mozambique, Niger, Nigeria, Uganda, and Tanzania account for almost 70% of the world's malaria cases [2].

There exist many techniques for detection of malarial species. Conventional light microscopy involves examining blood smears with Giemsa stain has been the golden standard, while other techniques including rapid diagnostic test and polymerase chain reaction (PCR) have also been utilized. Most of these techniques require a long processing time and are not quantitative in general. Further, label free techniques are scarce in malarial detection.
This work is based on the anisotropic characterization of hemozoin utilizing surface plasmons. We explicitly studied the difference in the birefringence of whole blood with and without hemozoin.

## II. Methods

Focused surface plasmon microscopy [3] is a technique that measures light-matter interaction at sub-wavelength level to investigate local optical properties of the sample at the metal-dielectric interface. It relies on optical near-field effects to map the refractive index profile of the samples with high spatial resolution. Numerous samples (i.e., thin films and liquids) have been probed (probe size:180 nm) by this technique to reveal circular absorption pattern at the exit pupil of the high numerical aperture microscope objective. By correlating the radius of the absorption pattern one can determine the refractive index of the sample. Further, anisotropic characteristics of the sample (if present) can be extracted from the elliptical absorption pattern to reveal the magnitude of birefringence and orientation of its fast axis respectively [4].

NLAF21 coverslips (refractive index: 1.78) were cleaned with ethanol, acetone, and isopropanol for 5 mins each. Then the coverslips were subjected to oxygen plasma for 5 mins. Then a chromium layer (~2 nm, r.i.: 3.316+i3.312) followed by gold layer (~ 47 nm, r.i.: 0.18377+i3.4313) were deposited by E-beam vapor deposition. The chromium layer was added for a better adhesion of the gold layer. The thickness of the subsequent layers was correlated with X-ray diffraction analysis. Artificial blood (~1 µl) was added on top of the substrate. Later various concentrations of hemozoin were mixed in the artificial blood and pipetted on top of the substrate.

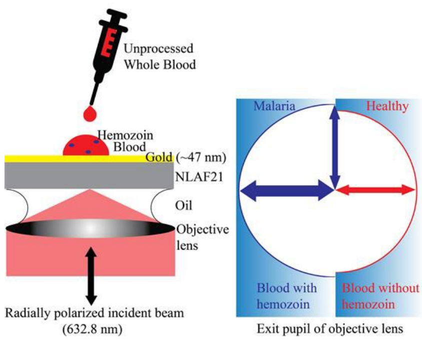
III. Results

The reflected spatial frequency distribution at the exit pupil of the high numerical aperture objective lens was recorded. When artificial blood was used as sample, we observed a circular absorption pattern at the exit pupil plane indicating the absence of any anisotropic components. The refractive index was recorded from the radius of the circular absorption pattern with a semi-automated custom developed software. When hemozoin was mixed in artificial blood, we observed an elliptical absorption pattern at the exit pupil plane. The birefringence was extracted from these images with the developed software as stated before.

IV. Conclusion

In this proof-of-concept study, we conclude that refractive index and more so birefringence can be used as a label-free estimator differentiating healthy blood and malarial blood. Further this approach can be used to distinguish between various malarial species as reported previously based on the refractive index [5]. We believe the quantitative characterization of the hemozoin crystals from various plasmodium species could be used in future to easily distinguish the species.

**Graphic:**




**Keywords:**

Label-free
Birefringence
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