Towards a Rapid Malaria Detection & Quantification System Using Attenuated Total Reflectance Infrared (ATR-FTIR) Spectroscopy

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The detection of the rings and gametocytes at low parasitemia in peripheral blood is critical for early diagnosis and treatment of malaria. Here we show for the first time that ATR-FTIR spectroscopy in combination with a partial least squares regression models has the required sensitivity and ease of sample preparation to become a laboratory standard for malaria detection and most importantly quantification.

The absolute quantification limit was found to be 0.001% (50 parasites/uL of blood) for cultured ring stage and gametocyte parasites in a suspension of normal erythrocytes. The absolute detection limit was found to be 0.00001%. Results are compared with resonance Raman and synchrotron imaging FTIR spectroscopy along with conventional microscopy and immunohistological approaches.

In a second study we have investigated live malaria infected cells using synchrotron FTIR and compared the different stages of parasite throughout the erythrocytic life cycle. We report for the first time the detection of malaria parasite DNA as evinced by the important phosphodiester marker bands including the asymmetric phosphate stretch at 1242 cm^{-1} and the symmetric phosphate stretch at 1082 cm-1 along with the base-stacking mode at 1715 cm^{-1}. The appearance of the asymmetric phosphate stretch at 1242 cm 1 is particularly interesting as this is consistent with the A-DNA conformation normally only observed in dehydrated cells.

More work is required to verify this assignment. Second derivative spectra of the different stages of the parasite are presented and the important bands assigned in Figure 1. The band at 1208 cm^{-1} is assigned to the C-O stretching vibration of the propionate group from hemozoin. This band, along with the DNA marker bands, appear more intense in spectra from trophozoite and gametocyte infected cells compared to the ring stage parasites. None of these bands are observed in the uninfected cells. The results are compared with the analogous spectra recorded of fixed cells. The extremely high throughput and sensitivity of the IR beamline at the Australian Synchrotron has enabled us for the first time to record spectra of living malaria parasites and detect the important DNA and hemozoin marker bands.

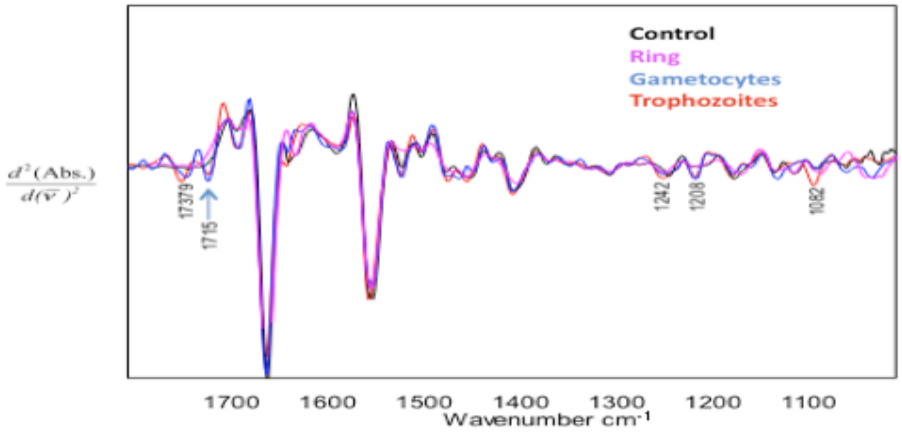

Figure 1. Second derivative spectra of living malaria parasites at different stages of the erythrocytic lifecycle highlighting the important haemozoin and DNA marker bands.