Supporting Information

# Development of a Label-free Raman Imaging Technique for Differentiation of Malaria Parasite Infected from Non-Infected Tissue

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**S1:** Synthetic route for the preparation of β-hematin (a synthetic form of hemozoin).

*All chemicals were purchased from Sigma-Aldrich, unless otherwise stated.*

- 75 mg of hemin chloride was dissolved in 3.0 mL of 0.5 M sodium hydroxide and then neutralised with 1.5 mL 1.0 M hydrochloric acid.
- 10 mL 3.9 M sodium acetate (pH 5.0) was added and the solution stirred in a water bath at 60°C for 3 hours.
- Solution was then diluted in 40 mL deionised $H_20$ ($d.H_20$) and filtered through a Buchner funnel.
- The residue was washed with $d.H_20$ to get rid of any excess salts. It was then resuspended in $d.H_20$ and freeze dried overnight to yield powder and gain an estimate of the weight of synthetic hemozoin produced.
- Final product was resuspended, at a concentration of 2.5 mg/mL, in PBS (10mM, pH 7.2) and stored at -20°C. Before use, the thawed aliquots were sonicated for five minutes to break up the larger particles.

**S2.** Image shows white light images of both tissue sections, with the grey box indicating the area that was mapped, along with peak intensity ratio maps for selected Raman peaks. Intensity ratio maps were created using the peak intensities for: 745 $cm^{-1}$, 1130 $cm^{-1}$, 1170$cm^{-1}$, and 1307 $cm^{-1}$ ($I_{745}$ / $I_{1307}$; $I_{1130}$ / $I_{1307}$; $I_{1170}$ / $I_{1307}$). These ratio maps were created from a different control and infected sample area and again visually show overall relative changes in the intensity of these Raman peaks within the area of *P. berghei*-infected tissue that was mapped compared to the uninfected area. The overall intensity of the $I_{1130}$ / $I_{1307}$ ratio for the control was comparable to that of the infected for these samples; however, a marked intensity ratio difference was seen between the $I_{745}$ / $I_{1307}$ and $I_{1170}$ / $I_{1307}$ maps.

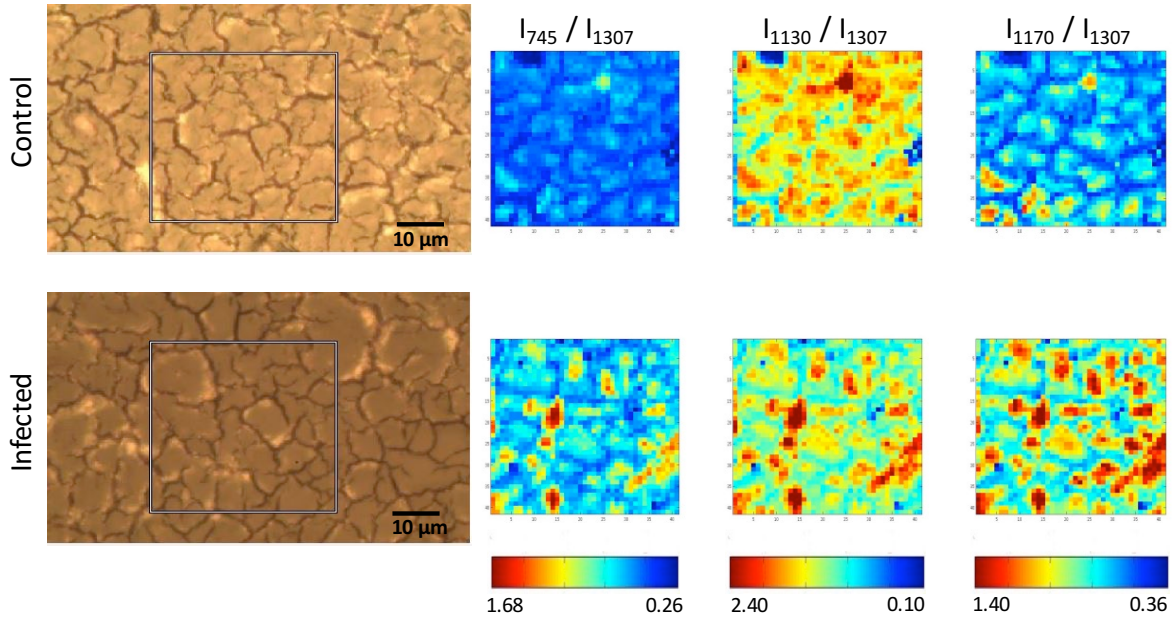



Figure S2. White light image for different control and infected tissue section area, with the corresponding peak intensity ratio maps.

**S3.** Empirical analysis results of peak intensity ratio for ~1170 and 1585 $cm^{-1}$ peaks ($I_{1170}$ / $I_{1585}$). (A) Scatter plot of the peak intensity ratios ($I_{1170}$/ $I_{1585}$) for the 43 average infected and 43 uninfected Raman spectra. A clear separation of the two data sets was observed, with little intra-sample variability. (B) Average intensity ratio plots for the control (0.183 ± 0.005) and infected (0.255 ± 0.004) data sets. This difference in mean ratios was shown to be statistically relevant (unpaired Student's t-test, $p < 0.0001$). Error bars highlight the standard deviation between the control and infected datasets, respectively.

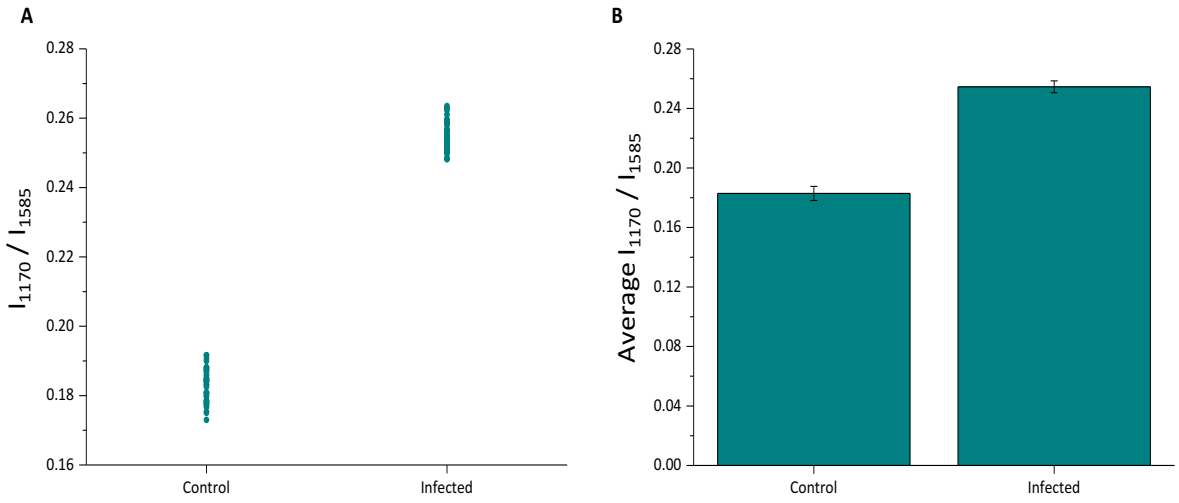
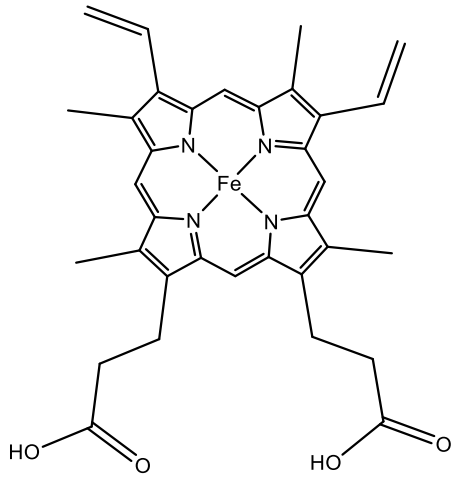
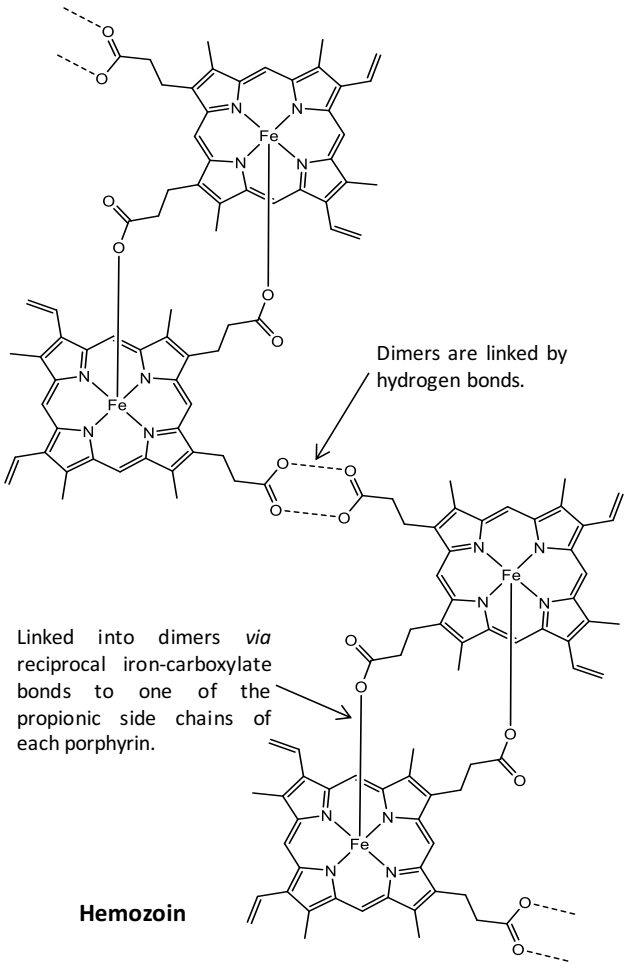



Figure S3. Empirical analysis results using two different peak intensity ratios. (A) Scatter plot of peak intensity ratios ($I_{1170}$/ $I_{1585}$) for the 43 average Raman spectra from all the control and malaria infected mice spleen tissue that were mapped. As with Figure 2, clear separation is also observed using these peaks. (B) Average intensity ratio plots for the control (0.183 ± 0.005) and infected (0.255 ± 0.004) data sets. This difference in mean ratios was also shown to be statistically relevant (unpaired Student's t-test, $p < 0.0001$).

**S4.** Hemoglobin is composed of 2 α-polypeptide chains plus 2 β-polypeptide chains, with inorganic heme structure. The iron core can be in either +2 or +3 oxidation state. Shown below is the core heme structure of hemoglobin which is the basic building block for hemozoin. Hemozoin consists of an array of hematin units (oxidised form of heme), linked into dimers through reciprocal iron-carboxylate bonds between central iron atoms of one heme and the propionic acid side chain of another. These dimers are then connected by hydrogen bonding to build a large regular network.




***ferrous [$Fe^{II}$] protoporphyrin IX***
**Heme**




**Hemozoin**

Structure S4. Core heme-structure of hemoglobin and hemozoin units, highlighting the structural similarities of the two compounds.

**S5.** Photographs of an uninfected spleen section and *P. berghei* infected section stained with a Prussian blue stain. A Prussian blue stain was chosen as this can differentiate between the malaria pigment, hemozoin, from hemosiderin (a normal by-product of hemoglobin degradation). With a typical Giemsa stain, both will stain brown. However, with Prussian blue hemozoin stains brown and hemosiderin stains blue, therefore, allowing differentiation.[1] The brown hemozoin deposits within the *P. berghei* infected tissue are indicated by the black arrows.

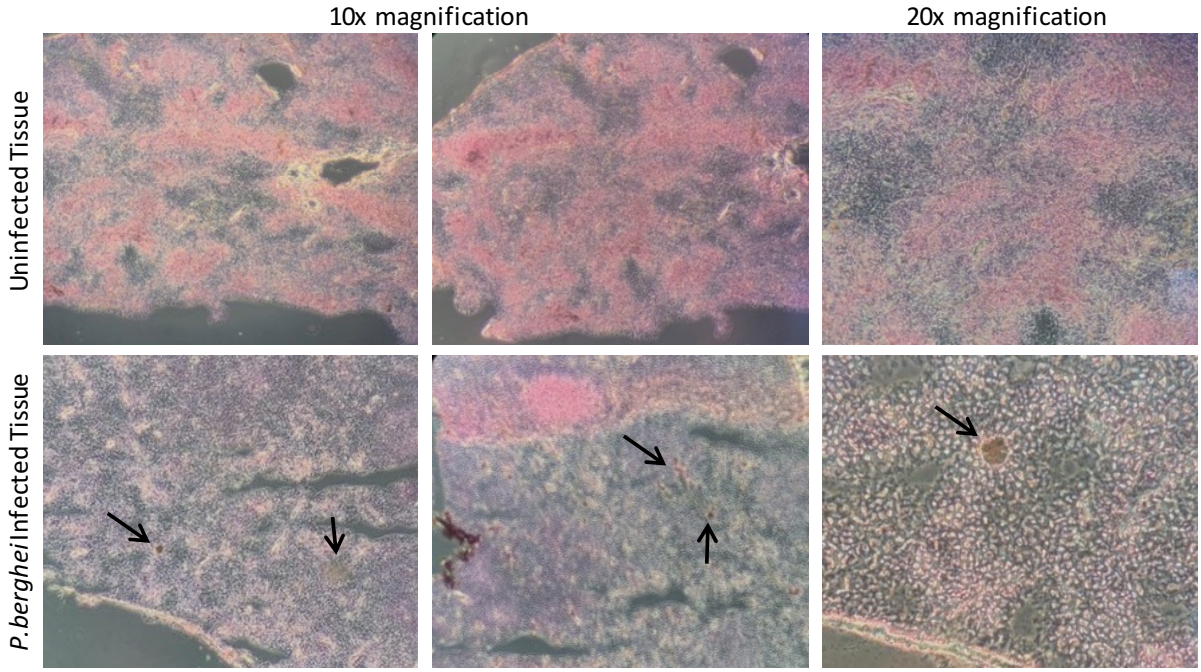



Figure S5. Images of uninfected & *P. berghei* infected spleen sections, stained using a Prussian blue stain. Brown hemozoin deposits within the infected sample are indicated by the black arrows. Other results: Iron (hemosiderin) – blue; Nuclei – red; Background – pink.

1 Parekh, F. K.; Davison, B. B.; Gamboa, D.; Hernandez, J.; Branch, O. H., *Am. J. Trop. Med. Hyg.*, 2010, **83**, 973-980.