Supplementary Information

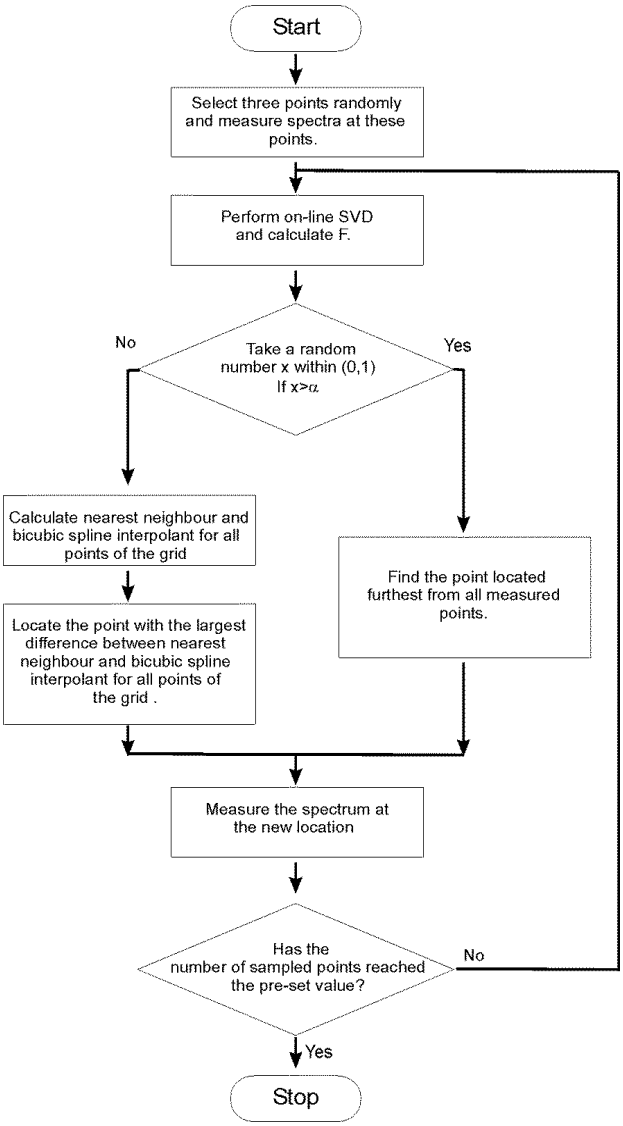

Fig. S1: The selective scanning algorithm for acquisition of Raman spectra of the cells. The singular value decomposition (SVD) was used to reduce the noise during the Raman measurements and allow a more accurate calculation of the scalar value F. The SVD was calculated by adding the measured Raman spectra to a database of 500 Raman spectra previously measured from HBMECs. The scatter probability in this study was α=0.5.

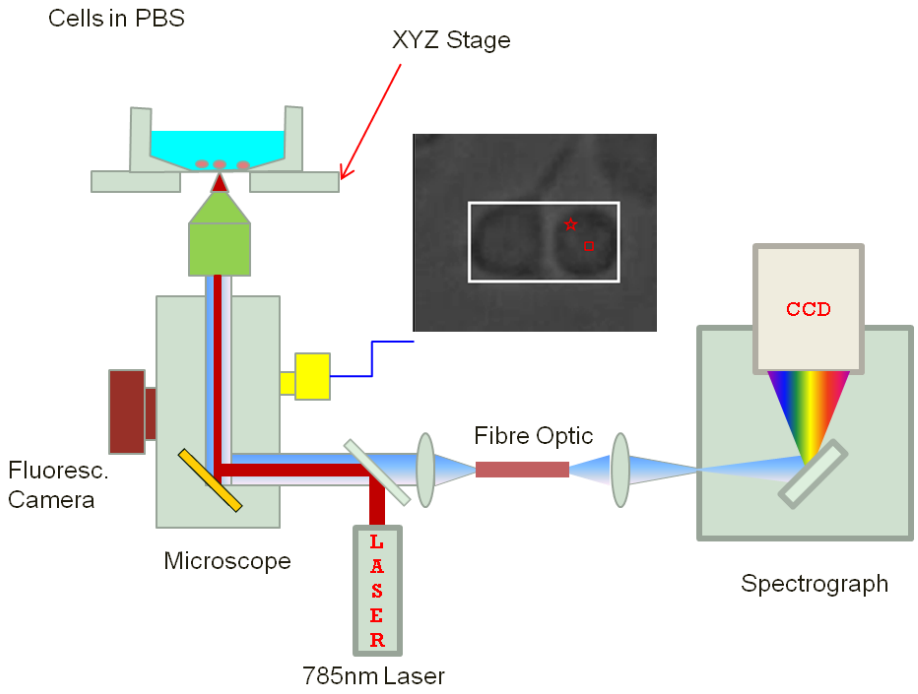

Fig. S2: Schematic description of the Raman micro-spectrometer: inverted optical microscope (Eclipse-Ti, Nikon), automated sample stage (H107 controlled by Proscan II controller, Prior Scientific), 785 nm laser (Starbright XM, Torsana), 60×/0.9 NA water immersion objective (Olympus), spectrograph (77200, Oriel) onto a CCD (DU401-A-BR-DD, Andor Technology), control software was written in-house using LabVIEW 8.5.

Growth conditions for the HBMECs and *N. caninum*

Human brain microvascular endothelial cells (HBMECs) were grown in T75 flasks (Corning) in tissue-culture medium composed of RPMI-1640 medium containing L-glutamine and sodium bicarbonate, and supplemented with 20% (v/v) heat inactivated FBS, 2mM L-glutamine, 1mM Sodium Pyruvate, 1% MEM non-essential amino acids, 1% MEM vitamins and 5ml penicillin/streptomycin. Cells were maintained in an incubator in a humidified atmosphere at 37°C and 5% CO_2. Once confluent (~ 3-5 days), cells were trypsinized using trypsin-EDTA (Invitrogen, GIBCO, UK). Cells were considered confluent when their expansion had reached a point where cells touched each other on all sides and no intercellular gaps were present. To exclude if cell viability could be regarded as a factor affecting parasite interaction with host cells and therefore any subsequent measurements, viability of cells was assessed on a minimum of 200 cells using trypan blue exclusion assay prior to inoculation onto culture chambers or flasks.

Neospora caninum (Nc-Liverpool) strain was a gift from Professor Sandy Trees (University of Liverpool) and was propagated in HBME cells. Parasites were harvested from their feeder cell culture and purified as described previously (Elsheikha et al., 2006). The number of tachyzoites was

estimated using a haemocytometer. The final volume of suspension was adjusted with culture medium to achieve a ratio of 1:1 parasite/host cell. All experiments were conducted at least in triplicate.

Elsheikha HM, Rosenthal BM, Murphy AJ, Dunams DB, Neelis DA, Mansfield LS (2006) Generally applicable methods to purify intracellular coccidia from cell cultures and to quantify purification efficacy using quantitative PCR. Vet. Parasitol. 135:223–234.