Electronic Supplementary Material (ESI) for Analyst.
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Mapping Sub-Cellular Protein Aggregates and Lipid Inclusions Using Synchrotron ATR-FTIR Microspectroscopy

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ELECTRONIC SUPPLEMENTARY INFORMATION

Method of optimising ATR-crystal contact with sample

Brain tissue sections (or bacteria) that were adhered to glass microscope glass were directly mounted onto aluminium disc using double-sided polyimide (Kapton®) tape. The aluminium disc was then transferred onto the sample stage of the ATR-FTIR unit, which was enclosed within a Perspex casing. The Perspex encased ATR-FTIR-unit was allowed to purge with dry nitrogen, until the humidity had dropped to ~20%. Following purging a background spectrum was recorded. The sample was then brought into contact with the Ge ATR crystal, monitoring spectral signal in real time. Once characteristic spectral features were observed. E.g., Amide I band, ν(C-H) (as ATR crystal was bought into contact with the sample), a rapid coarse spatial resolution overview SR-ATR-FTIR map was collected to confirm sample location and identify the region of interest to map. Following identification of the region of interest, the ATR to sample contact was subtly adjusted to optimise baseline signal, and the fine spatial resolution map was collected.

ELECTRONIC SUPPLEMENTARY FIGURES

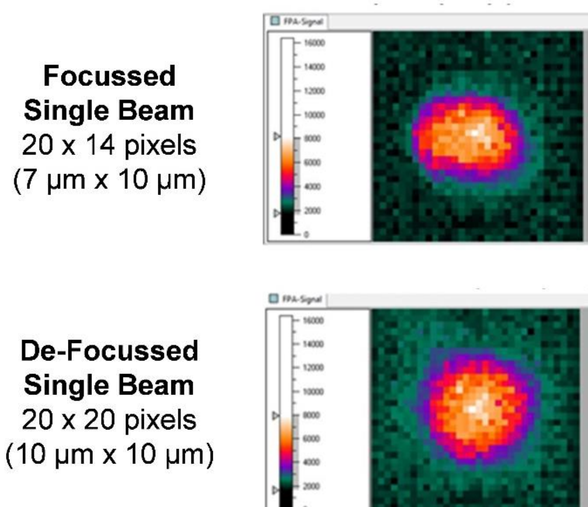

Figure S1: Representative examples of the foot print of the focussed and de-focussed single synchrotron IR beam, as recorded on the 32 × 32 active pixels of the FPA imaging detector.

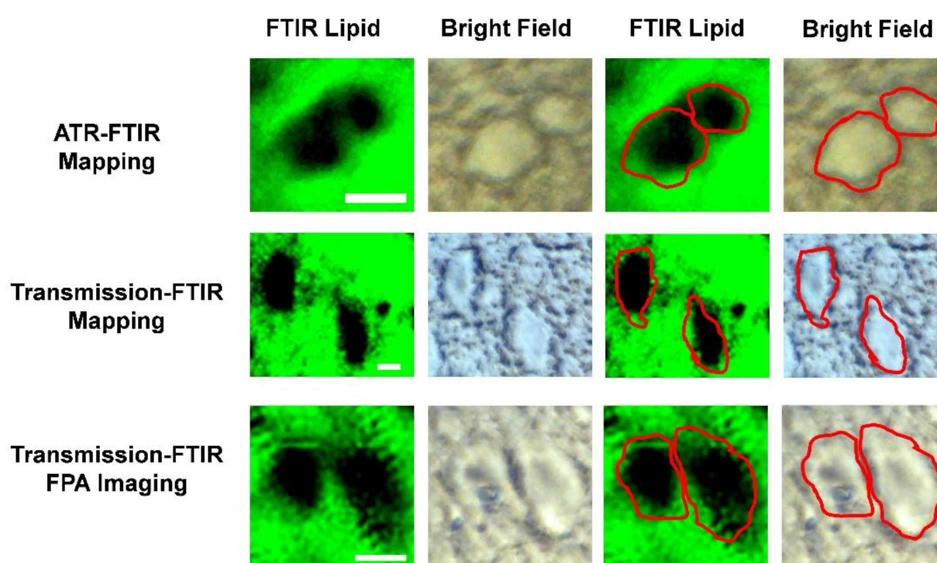

Figure S2: Representative examples of using false-colour FTIR images based on integrated areas under the carbonyl stretching band of esters in lipids (ν(C=O): 1755 – 1715 cm^{-1}) to reveal the location of cell bodies in healthy (sham) brain tissue. Note that neurons have relatively lower lipid contents than those of the surrounding neuropil tissue. Min-Max scale ranges from 0 to 0.2 in each image. Scale bars = 10 µm.

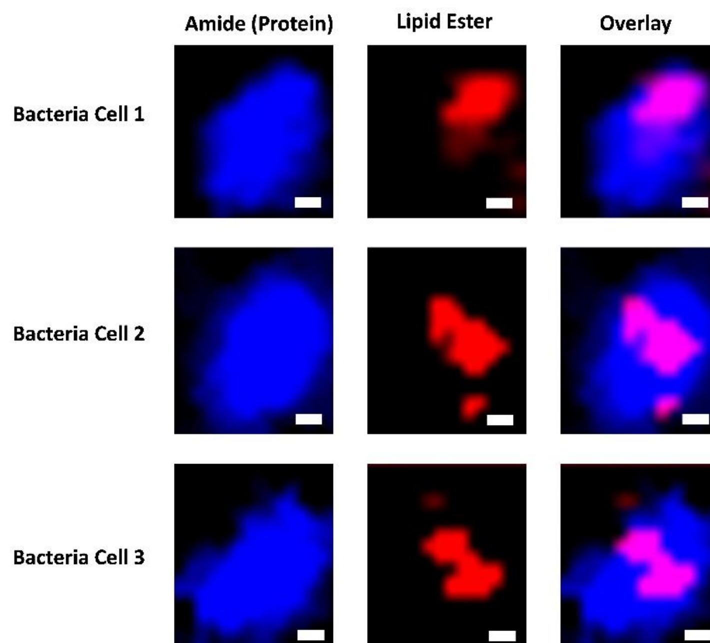

Figure S3: SR-ATR-FTIR maps of protein and lipid distribution acquired from triplicates of individual bacterial cells at 1 µm pixel size, to reveal the location of lipophilic inclusions within bacterial cells. Scale bar = 2 µm.

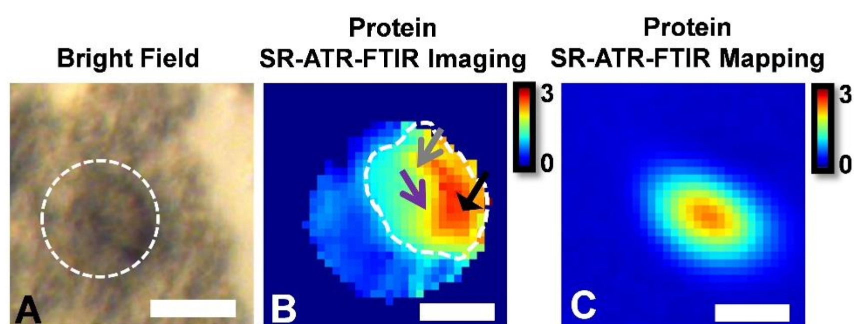

Figure S4: Comparison of the amide I protein images of the same brain cell imaged with single-beam SR-ATR-FTIR-FPA imaging, and SR-ATR-FTIR mapping. (**A**) Bright field optical image of a degenerating cell in ischemic brain tissue. (**B**) False colour functional group image of total protein (amide I band), showing location of degenerating neuron, recorded with single-beam SR-ATR-FTIR-FPA imaging. (**C**) False colour functional group image of total protein (amide I band), showing location of degenerating neuron, recorded with SR- ATR-FTIR mapping. Scale bar = 5 µm.