

## Combining Far-IR from beneath and UV-Vis from above to follow de-oxygenation and re-oxygenation of haemoglobin in blood.

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An Ocean Optics ISP-REF Integrating Sphere connected to USB4000 XRI spectrophotometer and Spectrasuite software was placed centrally above the diamond window of a GladiATR Single Reflection Attenuated Total Reflection (ATR) Accessory on the Far-IR/THz Bruker IFS125HR FTIR spectrometer with OPUS software. By placing a drop of blood on the out-of-vacuum ATR window then positioning the small Integrating Sphere, connected to a micro-spectrophotometer, the conversion of oxy-haemoglobin to deoxy-haemoglobin and reverse can be followed by observing both spectra. Oxy-haemoglobin has characteristic absorption peaks at 540 nm and 575 nm whereas, in deoxy-haemoglobin these are replaced by a single peak at 555 nm. By enclosing the combined spectrometers in a 'tent' of clear plastic that is sealed around the edge and connected to a gas flow inlet and an electric air-pump the atmosphere experienced by the blood can be altered. Initially, the Far-IR spectra is dominated by water in the blood, but when dry air is blown through the bag the water peaks decrease and the Far-IR spectrum of oxy-haemoglobin in blood is obtained. Pumping out the air in the tent aids the removal of water. Once a stable spectrum is obtained the tent can be filled with nitrogen and the transition of oxy-haemoglobin to deoxy-haemoglobin followed by the UV-Vis spectrometer. The Far-IR spectra is also observed to change. Once both spectra have stabilized the Far-IR and UV-Vis spectra can be recorded. To reverse the reaction the blood is wetted with water and air is readmitted into the tent and the conversion back to oxy-haemoglobin is followed by UV-Vis. In this way we can be sure that the spectrum we are measuring by Far-IR is in fact from deoxy-haemoglobin or oxy-haemoglobin or, in the transition stage, by a changing ratio of oxy and deoxy-haemoglobin. This process can be repeated for several cycles.

The interconversion of oxy-haemoglobin and deoxy-haemoglobin is relevant to the state of blood in cadavers during autopsy. When a person died they stop getting oxygen to their blood and the oxy-haemoglobin can loose oxygen to become deoxy-haemoglobin. However, when chilled in a mortuary fridge the cold blood has a higher affinity for oxygen and the authors [1] have shown that oxygen can be absorbed through the skin after death - unless there is a barrier such as a plaster on the skin or the skin is encased in plastic or some other impermeable material. So the oxygenated state of the blood at autopsy is a consequence of the temperature and stage of reoxygenation.

This innovation enabled the interconversion of these crucial blood components to be studied. Although this innovation has only been used for this specific investigation, that lends itself to coupling Far-IR with UV-Vis, it shows that other ranges of spectrometry could be undertaken in tandem on the ATR Far-IR at the Australian Synchrotron to investigate conversions.

[1] Watchman, H.M., Walker, G.S., Randeberg, L.L., & Langlois, N.E., 2011. Re-oxygenation of post-mortem lividity by passive diffusion through the skin at low temperature", *Forensic Science, Medicine, and Pathology*, 7(4), 333-335.

### Speakers Gender

Male

### Travel Funding

No

### Level of Expertise

Experienced Researcher

### Do you wish to take part in the poster slam

No

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