## **Supporting Information**

## Making colourful sense of Raman images of single cells

Lorna Ashton\*1,2, Katherine A. Hollywood<sup>1,3</sup> and Royston Goodacre<sup>1</sup>

<sup>1</sup>Manchester Institute of Biotechnology, University of Manchester, 131, Princess Street, Manchester, M1 7DN, UK.

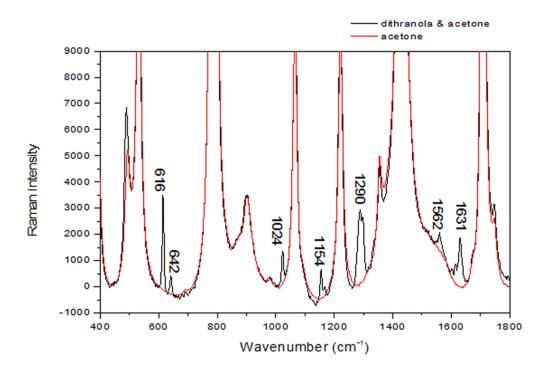
<sup>2</sup>Department of Chemistry, Faraday Building, Lancaster University, Lancaster, LA1 4YB, UK <sup>3</sup>Faculty of Life Science, University of Manchester, Manchester, M13 9PT, UK.

\*Corresponding author

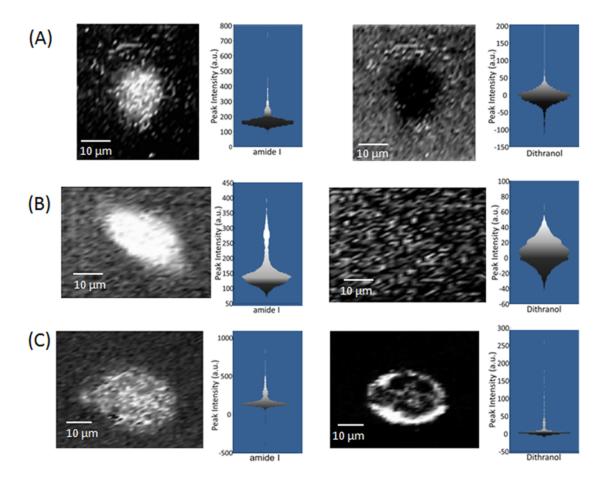
E-mail: l.ashton@lancaster.ac.uk

## **Identification of Dithranol Features**

Figure S1 displays the Raman spectrum of dithranol dissolved in acetone (black spectrum), at a concentration of 1 mg/mL, compared to acetone alone (red spectrum). Several drug-specific peaks arising from dithranol alone can be observed at ~616, 642, 1024, 1154, 1290, 1562 and 1631 cm<sup>-1</sup>. As strong Raman features arising from the cells themselves are observed in the region 800-1750 cm<sup>-1</sup> it was decided to identify the presence of dithranol from the region 598-653 cm<sup>-1</sup> therefore incorporating any Raman intensity from the two dithranol peaks at ~616 and 642 cm<sup>-1</sup>. Figure S2 also supports this choice of region 598-653 cm<sup>-1</sup> as no features are observed in Raman images constructed from Raman maps of untreated cell (Figure S2A) or cells treated with acetone only (Figure S2B) for the peak area. It is only after treatment with dithranol and acetone that clear features can be observed in the Raman image for the peak area (Figure S2C).



**Figure S1.** Raman spectra of dithranol mixed with acetone (black spectrum) and acetone only (red spectrum).



**Figure S2**. A comparison of Raman images constructed from Raman maps of: (A) an untreated HaCaT cell, (B) a HaCat cell treated with acetone, and (C) a HaCaT cell treated with dithranol and acetone mix. The shading represents the peak area intensity at 1600-1700 cm<sup>-1</sup> (amide 1) and 598-653 cm<sup>-1</sup> (Dithranol). Distribution plots overlaid with the colour ramps used to construct the images are also shown.