# Multiparametric physicochemical analysis of a type 1 collagen 3D cell culture model using light and electron microscopy and mass spectrometry imaging: supplementary information

Camilla Dondi[1], Dimitrios Tsikritsis[1], Jean-Luc Vorng[1], Gina Greenidge[1], Ibolya Kepiro[1], Natalie A. Belsey[1,2], Greg McMahon[1], Ian S. Gilmore[1], Maxim G. Ryadnov[1], & Michael Shaw[1,3]*

[1]National Physical Laboratory, Hampton Road, Teddington, TW11 0LW, UK
[2]School of Chemistry & Chemical Engineering, University of Surrey, Guildford GU2 7XH, UK
[3]Department of Computer Science, University College London, Gower Street, London, WC1E 6BT, UK
*mike.shaw@npl.co.uk

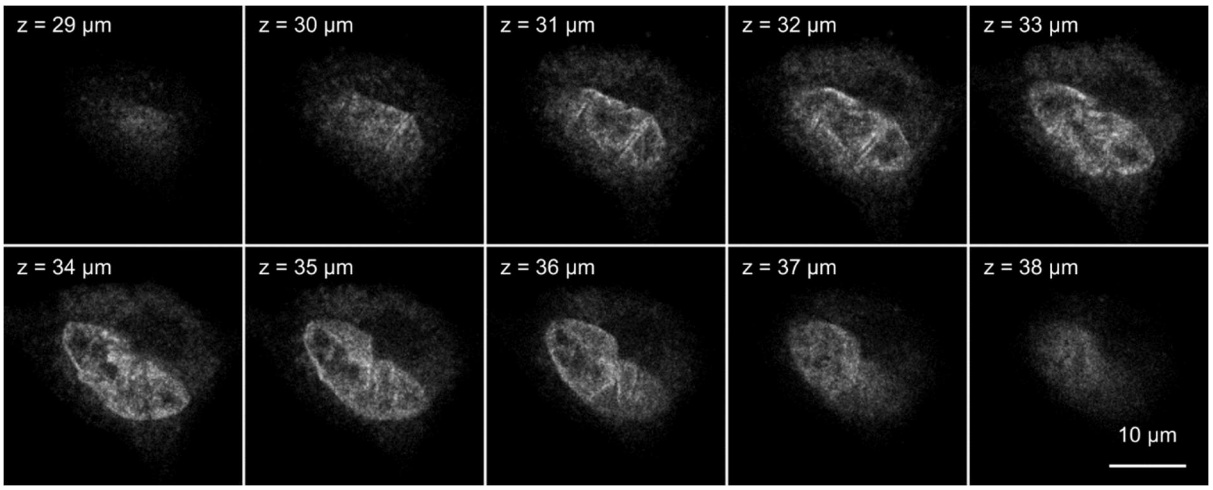


***Figure S1. Sequential slices from a CLSFM z-stack showing the nucleus of the cell highlighted in Fig. 4(b) of the main manuscript.***

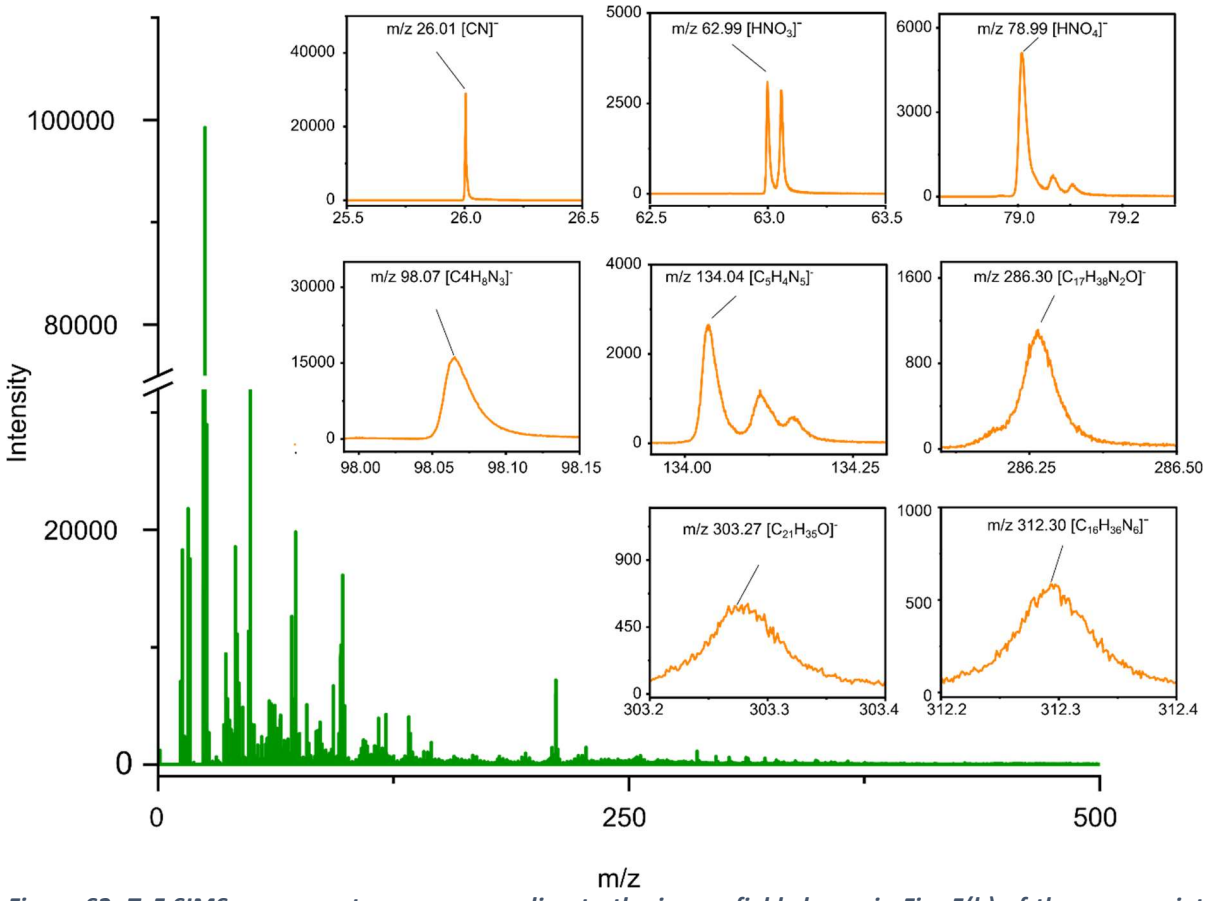


***Figure S2. ToF-SIMS mass spectrum corresponding to the image field shown in Fig. 5(b) of the manuscript.*** *Insets show the spectra corresponding to the individual ion images shown the figure.*

| Experimental mass | Theoretical mass | Assignment | Mass deviation (ppm) |
|---|---|---|---|
| 26.01 | 26.00 | $[CN]^-$ | 76.91 |
| 62.99 | 63.00 | $[HNO_3]^-$ | -79.37 |
| 78.99 | 78.99 | $[HNO_4]^-$ | 12.65 |
| 98.07 | 98.07 | $[C_4H_8N_3]^-$ | -20.39 |
| 134.04 | 134.05 | $[C_5H_4N_5]^-$ | -52.22 |
| 286.30 | 286.30 | $[C_{17}H_{38}N_2O]^-$ | 6.98 |
| 303.27 | 303.27 | $[C_{21}H_{35}O]^-$ | 3.29 |
| 312.30 | 312.30 | $[C_{16}H_{36}N_6]^-$ | -3.20 |

***Table S1. Measured peak positions, theoretical masses of the assigned ions and mass deviation corresponding to the ToF-SIMS image channels shown in Fig. 5(b) of the manuscript.***

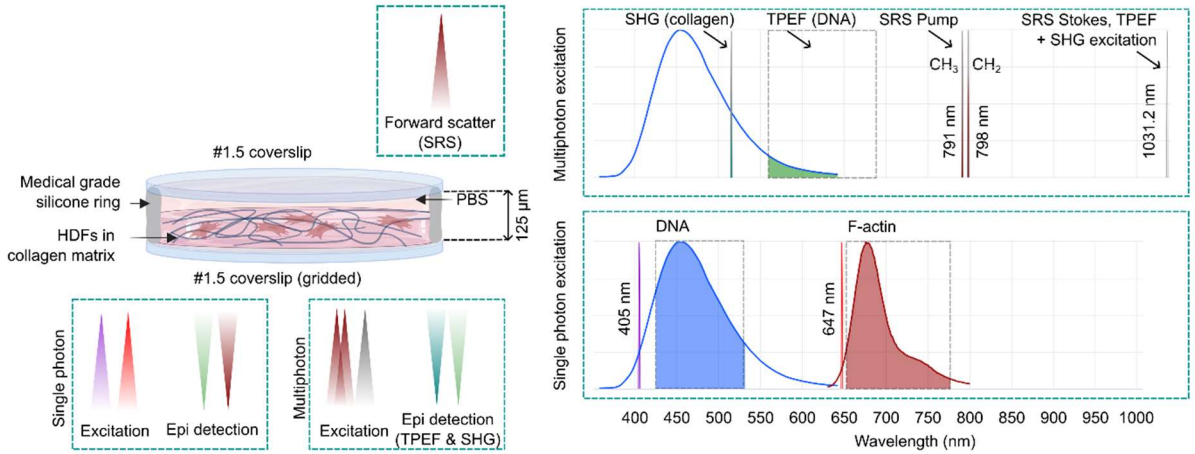


***Figure S3. Sample mounting and imaging geometry for optical microscopy techniques used in the correlative imaging workflow.*** *Type I collagen scaffolds seeded with HDFs were mounted between two #1.5 coverslips separated by a silicone spacer ring to allow illumination and light collection using oil immersion objective and condenser lenses for SRS imaging and reduce evaporation and maintain sample structure during multimodal optical imaging.*

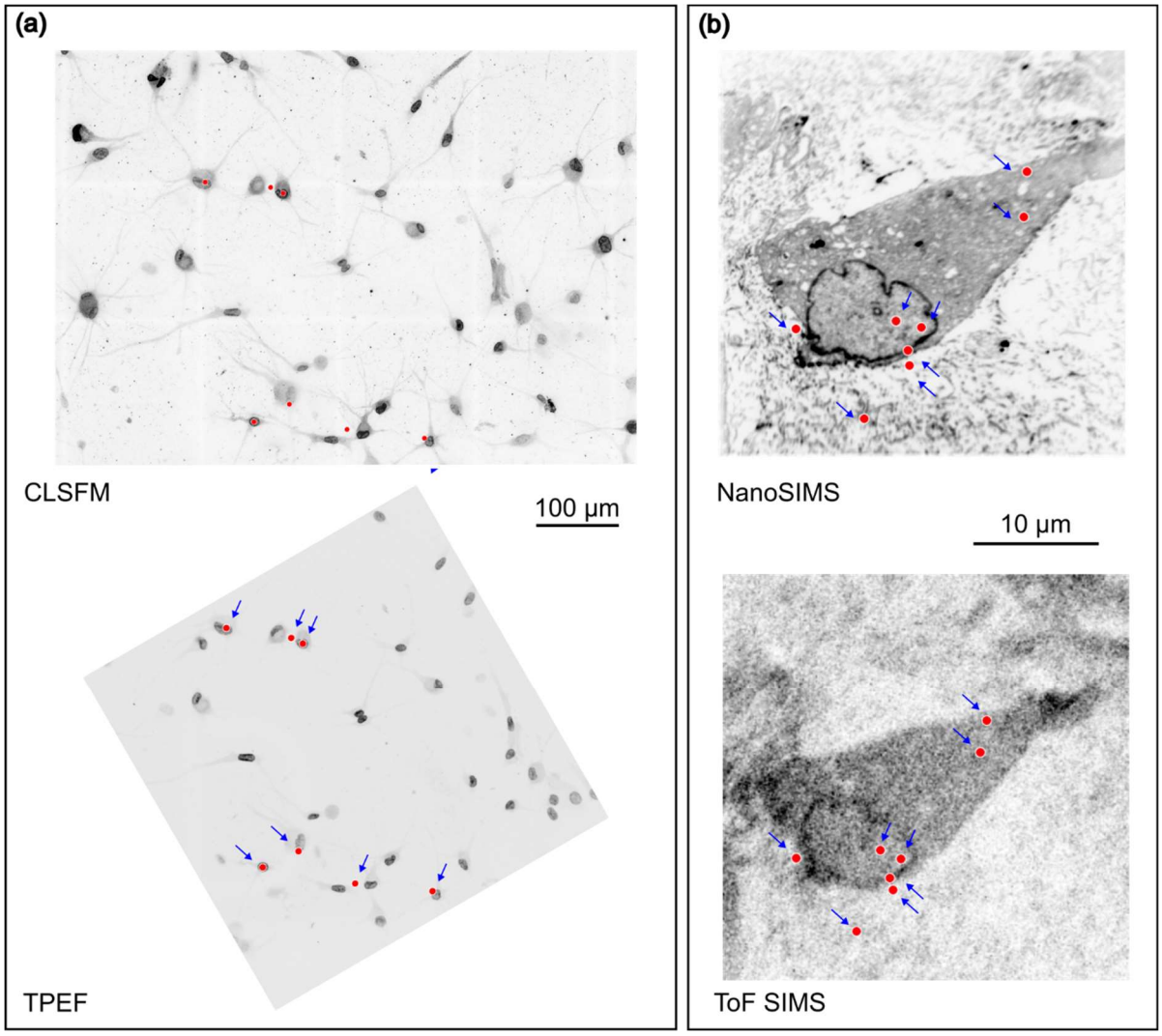


***Figure S4. Keypoints (red dots highlighted by blue arrows) used to register the images shown in figures 3(a) and 5 of the manuscript****. (a) CLSFM (top) and TPEF (bottom) images of cell nuclei stained with Hoechst. (b) Cyanide ion channel images captured using NanoSIMS (top) and ToF-SIMS (bottom).*