The Characterization of an Aggregated Three-Dimensional Cell Culture Model by Multimodal Mass Spectrometry Imaging

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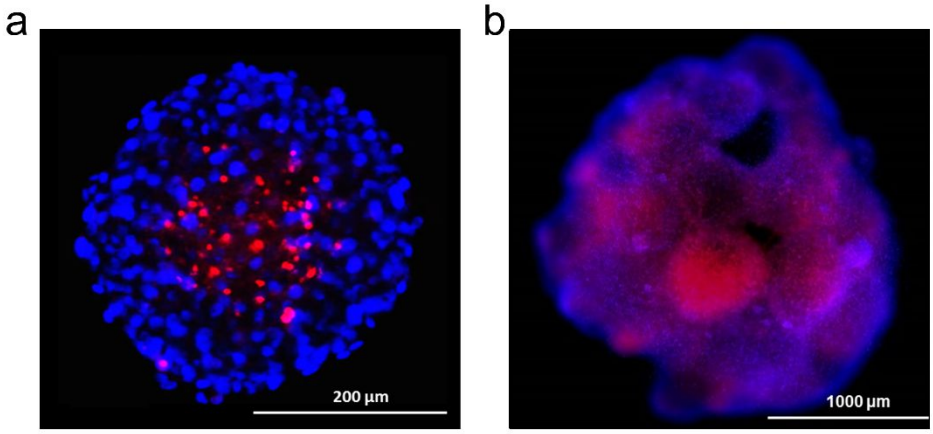
Supporting Information

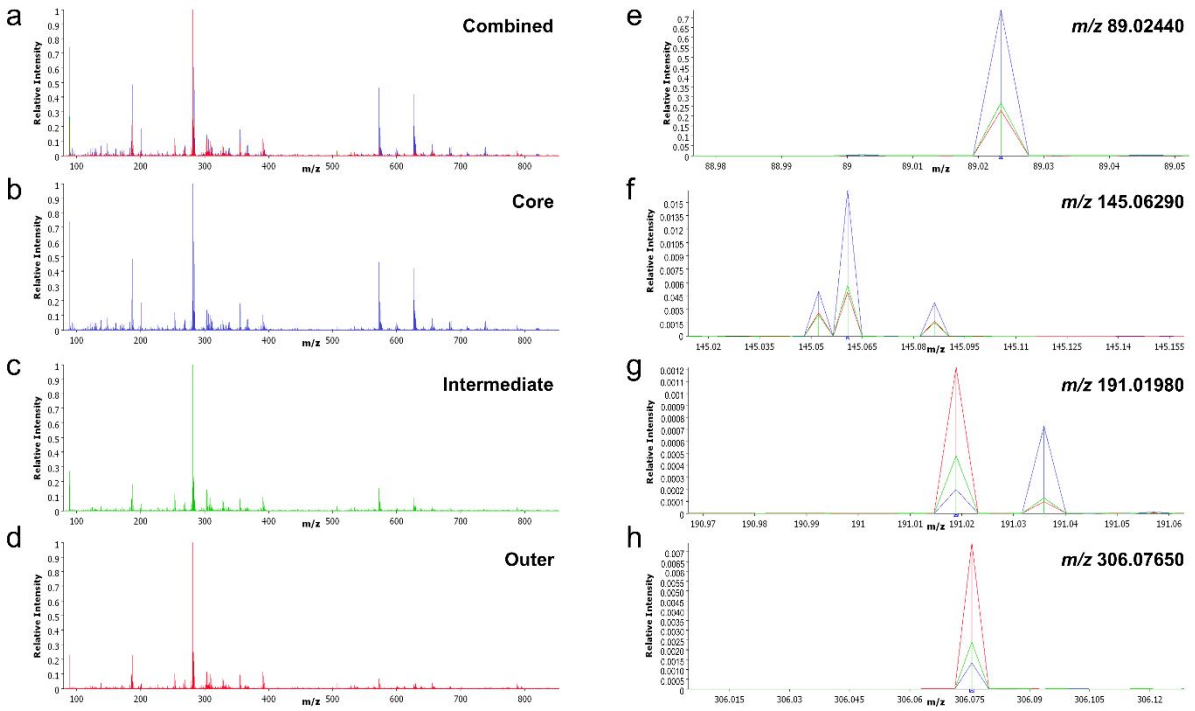
Supplementary Figure 1. Fluorescently stained image of 3D cell culture. Viable and necrotic regions identified by Hoechst 33342 and propidium iodide staining. **(a)** Spheroid grown in alginate. Scale 200 µm. **(b)** Aggregated spheroid "aggregoid". Scale 1000 µm.



Supplementary Table 1. Assignments and errors for [M-H]$^-$ adducts, and discriminatory analysis of metabolites between the core and outer from SCiLS Lab software. Mass accuracy of identified metabolites with the measured *m/z* values and calculated *m/z* values (< 2.5 ppm). Area under the curve (AUC) determined by receiver operated characteristics (ROC) analysis, represents the discrimination power of *m/z* signal. A perfect discrimination would yield an AUC equal to 0 or 1. An AUC closer to 0.5 defines the m/z value less suitable as a univariate criterion. In this case an AUC equal to 0 discriminates the *m/z* value to the core, 1 to the outer.

Compound name	Ion cluster	*m/z* Measured	*m/z* Calculated	Mass error (ppm)	AUC
Pyruvate	[M-H]-	87.00880	87.00877	0.4	0.240
Lactate	[M-H]-	89.02440	89.02442	0.2	0.138
Succinate	[M-H]-	117.01940	117.01930	0.9	0.640
Malate	[M-H]-	133.01430	133.01425	0.4	0.700
Glutamine	[M-H]-	145.06190	145.06187	0.2	0.114
Glutamate	[M-H]-	146.04590	146.04588	0.1	0.396
Citrate	[M-H]-	191.01980	191.01973	0.4	0.903
FA (18:2)	[M-H]-	279.23280	279.2330	0.7	0.639
FA (20:4)	[M-H]-	303.23300	303.23295	0.2	0.564
Glutathione (GSH)	[M-H]-	306.07650	306.07653	0.1	0.991

Supplementary Figure 2. Average mean spectra of metabolites within the aggregoid regions extracted from SCiLS Lab software. (a) Combined spectra of core, intermediate and outer region classified from segmentation analysis. (b) Core spectrum. (C) Intermediate spectrum. (d) Outer spectrum. Relative intensity of metabolites for each aggregoid region: (e) Lactate, *m/z* 89.02440; (f) Glutamine, *m/z* 145.06290; (g) Citrate, *m/z* 191.01980; (h) GSH, *m/z* 306.07650.



Supplementary Figure 3. IMC classification and spatial segmentation using HALO™ software. (a) Regions of core, outer and background were classified from the IMC image analysis of aggregoid. Classification of aggregoid was objective to Glut1 distribution which is localised within the core. **(b)** Spatial segmentation of each protein marker to determine percentage positive cells. From top left to bottom right: Pan-CK, E-Cadherin, Glut1, Ki-67, TNC, pS6, γH2AX, pHH3, DNA.