# NON-INVASIVE, LABEL-FREE BIOCHEMICAL IMAGING OF INTACT CEREBRAL ORGANOIDS VIA DEEP LEARNING-ENHANCED RAMAN MICROSPECTROSCOPY

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## 1 MOTIVATION

Advances in stem cell biology have enabled the *in vitro* generation of three-dimensional, self-assembling brain tissues, termed cerebral organoids, that recapitulate the early foetal development of the human brain (Lancaster et al., 2013; Lancaster & Knoblich, 2014b; Pașca, 2018; Lancaster & Knoblich, 2014a). Compared to traditional two-dimensional cell culture models, cerebral organoids exhibit enhanced cellular complexity and functionality (Chiaradia & Lancaster, 2020), providing a more advanced model system for studying neurodevelopmental biology and disease (Qian et al., 2019; Di Lullo & Kriegstein, 2017; Eichmüller & Knoblich, 2022; Yildirim et al., 2024).

Immunofluorescence staining combined with confocal microscopy is the gold standard technique for high-resolution imaging and analysis of cellular models, including organoids (Brémond Martin et al., 2021). In this approach, fluorescently labelled antibodies are used to bind and visualise specific target molecules. However, this technique has several inherent limitations. First, it requires multiple washing and permeabilisation steps, which can disrupt cellular integrity and function, and displace small molecules such as drugs and metabolites. Sample sectioning is also often required to improve antibody penetration and signal quality. Furthermore, while protein labelling is often straightforward, detecting other molecular classes – such as carbohydrates, lipids and various small molecules (e.g. drugs and metabolites) – requires more specialised techniques, making them more challenging to visualise. Lastly, the spectral overlap of fluorescent markers constrains multiplexing, typically to five targets in standard fluorescence microscopy (Shi et al., 2022).

## 2 DEEP LEARNING-ENHANCED RAMAN MICROSPECTROSCOPY

To overcome these limitations, we developed a technique for non-invasive, label-free imaging of cerebral organoids based on Raman microspectroscopy and physics-constrained autoencoder representation learning (Figure 1). First, data are acquired from organoid samples by performing point-wise Raman imaging scans, generating hyperspectral images with pixels representing the measured Raman scattering. Data are then unfolded along their spatial dimensions, and preprocessed to remove non-specific signals and artefacts, such as cosmic spikes, autofluorescence baselines and dark noise, using the RamanSPy toolbox (Georgiev et al., 2024b). After preprocessing, hyperspectral

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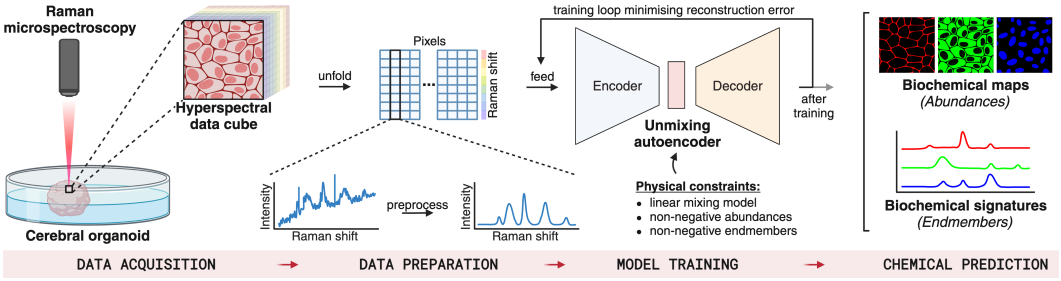


Figure 1: Schematic diagram of our organoid imaging pipeline based on Raman microspectroscopy combined with physics-constrained autoencoder representation learning.

unmixing is performed to extract biochemical components (called endmembers) and quantify their relative concentrations (called abundances) (Keshava & Mustard, 2002; Li et al., 2017). This is achieved using an autoencoder neural network model designed for hyperspectral unmixing following the approach in Georgiev et al. (2024a). The autoencoder architecture comprised: (i) a deep fully-connected encoder responsible for abundance estimation, (ii) and a one-layer decoder responsible for endmember identification. To guide the learning of interpretable biochemical features, we incorporated relevant physical constraints in the representation learning process. This includes constraining the learnt endmembers and abundances to be non-negative through the use of appropriate activation functions and weight clipping, and constraining the mixing process to linear mixing through the use of a single-layer linear decoder. The model was trained in an unsupervised manner by minimising a reconstruction loss based on the spectral angle divergence (Kruse et al., 1993).

## 3 Proof of Concept

To demonstrate the capability of our platform, we acquired a $400 \times 346$ Raman imaging scan from a day–10 cerebral organoid sample (Figure 2A). Data were preprocessed, and analysed using our unmixing autoencoder model as described above. Twelve components were extracted and characterised via peak assignment, whereby prominent peaks were linked to chemical bonds and contributions from key biomolecular species were identified. Selected endmember signatures are provided in Figure 2C, which were linked to lipid-rich ($1303\,\mathrm{cm}^{-1}$, $1445\,\mathrm{cm}^{-1}$, $1657\,\mathrm{cm}^{-1}$), protein-rich ($1005\,\mathrm{cm}^{-1}$, $1207\,\mathrm{cm}^{-1}$, $1246\,\mathrm{cm}^{-1}$, $1272\,\mathrm{cm}^{-1}$, $1445\,\mathrm{cm}^{-1}$, $1508\,\mathrm{cm}^{-1}$, $1657\,\mathrm{cm}^{-1}$) and nucleic acid-rich species ($752\,\mathrm{cm}^{-1}$, $1331\,\mathrm{cm}^{-1}$) (Talari et al., 2015). The corresponding abundances were then visualised as chemical maps and analysed to study organoid morphology (Figure 2B). We

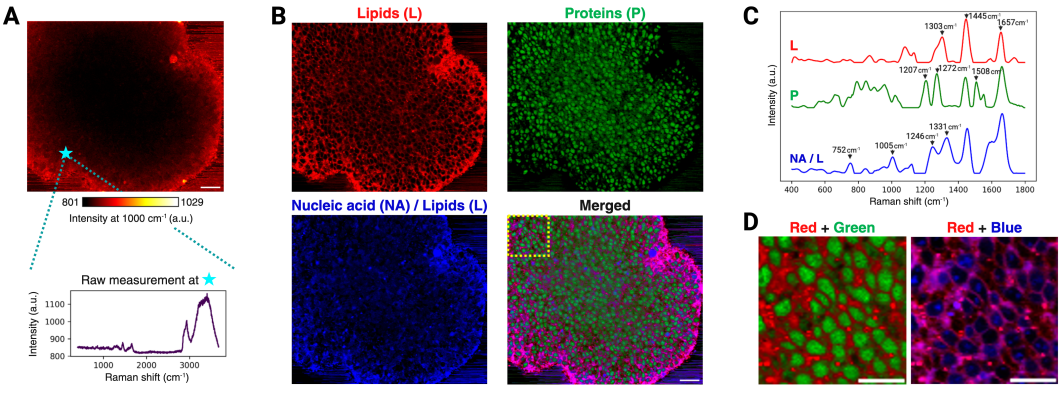


Figure 2: Raman microspectroscopy combined with autoencoder-based hyperspectral analysis enables detailed *in situ* biochemical imaging of intact cerebral organoids. A, Snapshot of the acquired Raman imaging scan (scale bar: $40\,\mu\mathrm{m}$; imaging resolution: $1\,\mu\mathrm{m}$). B-D, Selected results of hyperspectral analysis – abundance reconstructions (B; scale bar: $40\,\mu\mathrm{m}$), corresponding endmember signatures (C), and a zoomed-in area (D; scale bar: $25\,\mu\mathrm{m}$; zoomed-in area marked in yellow in B).

observed major cellular organelles, including cellular membranes, lipid bodies, cytoplasm and nuclei. Closer inspection of specific regions of interest showed that we could visualise and interrogate individual cells and subcellular organelles, highlighting the capability of our imaging platform for detailed morphological analysis (Figure 2D).

## 4 CONCLUSION

In conclusion, we have presented a technique for non-invasive, label-free organoid imaging based on Raman microspectroscopy combined with unsupervised autoencoder-based hyperspectral analysis. Our proof-of-concept experiment shows that our approach is effective at identifying key biochemical components in unlabelled Raman spectroscopy measurements, enabling detailed *in situ* visualisation of structural and morphological features in intact cerebral organoids. By eliminating the need for destructive preparation steps and external labels, our workflow offers simpler sample preparation that does not compromise organoid integrity. This opens new avenues for research into early neurodevelopment, developmental diseases and drug discovery, particularly in live organoid systems.

### MEANINGFULNESS STATEMENT

A meaningful representation of life emerges when data-driven analyses capture genuine biological signals – rather than mere numerical patterns. Our unsupervised imaging platform, based on hyperspectral unmixing autoencoders, accomplishes this by learning biochemically relevant features from Raman microspectroscopy data. This enables detailed visualisation and analysis of sample composition, as shown in our proof-of-concept demonstration. Moreover, our framework can serve as a versatile representation learning paradigm in Raman spectroscopy, offering a powerful and interpretable feature extraction method for downstream applications beyond imaging – e.g. organoid phenotyping, disease classification, drug response modelling.

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