Tools of the trade

AI-driven detection and analysis of label-free protein aggregates

Misfolded protein aggregation is closely associated with incurable neurodegenerative diseases such as Alzheimer, Parkinson, and Huntington diseases. To advance treatments and diagnostics, a mechanistic understanding of aggregate formation and its role in disease pathogenesis is essential. One of the prevailing challenges lies in the lack of suitable tools to monitor the different phases of aggregation. Fluorescence microscopy offers high specificity and spatiotemporal resolution in living samples, but fluorescent labelling has been shown to cause drastic modifications of the biochemical and biophysical properties of the formed aggregates and may not accurately reflect their physiological behaviour. These limitations underscore the need for alternative label-free techniques that can provide a more comprehensive understanding of the dynamics of protein aggregation without introducing unwanted perturbations. Current label-free methods such as Raman and autofluorescence microscopy are useful, but they are limited in specificity, contrast and temporal resolution.

We have therefore developed 'label-free identification of neurodegenerative disease-associated aggregates' (LINA), which achieves 96% accuracy in identifying and analysing protein aggregates in cells, while avoiding the drawbacks of fluorescent labelling and overcoming the limitations of other label-free techniques. LINA is based on training a convolutional neural network to map between label-free (bright-field or quantitative phase) and fluorescent images, such that after training, only a label-free input image is needed for the model to determine the presence or absence of aggregates.

Using LINA, we were able to measure and compare the dry mass and area of different Huntingtin aggregates, which vary in their composition (different polyglutamine repeat lengths or the absence of certain domains). We found that Huntingtin proteins with shorter polyglutamine repeat lengths have a higher average dry mass and area than those with longer repeats, illustrating the importance of their different ultrastructures and aggregation mechanisms. LINA facilitated the dynamic identification of label-free aggregates from live-cell images, and enabled the precise measurement of their dry mass and area fluctuations during growth. Hence, LINA replicated the benefits of fluorescence microscopy, while avoiding alterations and phototoxicity. LINA demonstrated high generalizability and robustness across various imaging conditions, aggregate types and cell lines. With its potential as a simple, fast, gentle, highly-specific and automated method, LINA promises accurate identification and analysis of protein aggregates, offering high-fidelity results for neurodegenerative disease research.

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Competing interests
The authors declare no competing interests.

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Tools of the trade

Intrinsic calibration and deformation mapping for expansion microscopy using GelMap

Expansion microscopy (ExM) is an emerging technique that increases the resolution of light microscopy by physically expanding biological specimens. In ExM, specimens are embedded into swellable hydrogels, followed by homogenization of the samples to ensure even expansion. ExM has seen rapid adoption by many research groups contributing to its development, in part due to the great potential of ExM for volumetric imaging and visualization of biological ultrastructures, and its ability to increase the achievable resolution of conventional microscopes. Despite this, widescale adoption has been hampered by a lack of standardized practices for quantitative determination of expansion factors and assessment of sample quality and potential deformations induced by anisotropic expansion.

The current best practice for validating an ExM experiment is to image a structure of interest before and after expansion and compare the two datasets. However, this is time-consuming because of the doubled imaging time and the need to locate the same cellular structure after expansion. As the gel volume increases 1,000-fold at 10-fold expansion, challenges with sample navigation after expansion preclude routine evaluation of reproducibility and variability across different samples.

To overcome these challenges, we have developed GelMap, a flexible workflow for introducing a scalable fluorescent reference grid into the ExM hydrogel. The GelMap grid expands together with the sample (for example, cells or tissue) and thereby intrinsically calibrates the gel. Consequently, if expansion is uneven or if the sample is deformed, for example, due to incomplete homogenization or poor sample mounting, the grid will provide a visual readout that can be used to correct for this deformation.

The GelMap workflow is easily accessible and does not require extensive specialized equipment — standard micropatterning techniques can be used to pattern a fluorescent grid onto glass coverslips. With the inclusion of GelMap grids in the ExM workflow, scaling calibration and correction for deformations no longer require imaging before expansion. In cases in which sample imaging before expansion is still desired, for example, for live-cell imaging, the inclusion of numbers and letters in the grid drastically reduces the time spent finding the same cell after expansion.

GelMap therefore contributes a vital quality-control step to the ExM workflow. This is an important step towards standardization of this method, which is crucial for quantitative accuracy and reproducibility, in basic research and in clinical and diagnostic settings.

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Competing interests
J.B.P. and H.G.J.D. are inventors on a filed patent application covering the presented method.

Related article: Damstra, H. G. J. et al. GelMap: intrinsic calibration and deformation mapping for expansion microscopy. *Nat. Methods* **20**, 1573–1580 (2023)