GelMap User Guide

visualise bio

Unpacking

Open the coverslip holder carefully! Inside are the GelMap coverslips. The protein-coated (top) side is always facing the front (green sticker).

Storage and shelf-life

We suggest to transfer the coverslips to well plates to label and keep track of the protein and conjugate for each coverslip.

The coverslips are stable – we have imaged with GelMap coverslips that have been left dry at room temperature up to 3 months after manufacture, with no noticeable loss in function. However, please let us know your experiences with stability and shelf-life.

Checking coverslip quality

It is recommended to check the coverslips upon receipt. It can be that due to manufacture and shipping, that the quality of the pattern is not homogenous. Also due to shipping, the pattern at the edges of the coverslip can be scuffed/poor quality. It is worth screening the coverslips to become familiar with both the pattern design, and the quality per coverslip.

For routine screening, we typically image through a well plate at 10X magnification on an EVOS Cell Imaging System (ThermoFisher).

Washing and sterilising

Before using GelMap coverslips with expansion hydrogels, we recommend washing the coverslips three times in 1XPBS to remove any debris from patterning.

If using with biological samples, we routinely sterilise the coverslips after washing by dipping in 70% ethanol, followed by washing again in 1X PBS. We have not observed any significant signal or quality loss from this.

Sample preparation

Cells can be cultured directly on the GelMap grids. If desired, a top-coating of choice can be applied as with any standard coverslip. Some cells preferentially adhere to laminin, so a top-coating may be desired for laminin patterned coverslips. For neuron culture, in our lab we use 37.5 µg ml−1 poly-L-lysine and 1.25 µg ml−1 laminin in 0.1 M borate buffer, pH 8.5.

For tissue slices, we typically prepare a gelation chamber by sandwiching a sample between a glass slide and a coverslip, with square coverslips stacked up as walls of the chamber. The glass slide, top coverslip, or both can be replaced with GelMap coverslips, with patterned side facing the gel.

Amplification

We primarily supply GelMap coverslips patterned using protein pre-conjugated to a fluorophore. For expansion factors beyond 4X, this labelling may be weak or not visible in the fully expanded gel (particularly when looking through the eyepiece). We therefore recommend to amplify the GelMap grid with antibody labelling.

The GelMap grid can be labelled in both pre- and post-expansion protocols, and all fixation/anchoring/gelation methods and recipes that we have tested are compatible. For the best results, post-expansion labelling protocols are recommended.

- R2-myc-his can be amplified using the myc-tag, we prefer rabbit anti-myc-tag (Cell Signaling Technology, 2272) at 1:100 dilution (pre-expansion).

It is possible to use any colour secondary antibody for labelling regardless of the conjugated fluorophore used to pattern the coverslips.

We recently received a report that amplifying with another NHS-conjugated fluorophore after FA/AA anchoring works more efficiently than antibody amplification pre-ExM, however we are currently in the process of confirming this.

Deformation correction and expansion factor determination

When you have imaged your sample, the underlying GelMap grid then gives you information about anisotropy and deformation of your sample, as well as a precise readout of the local expansion factor. We currently use two methods to quantify this:

Squareness
Squareness is a measurement of deformation without requiring any correlation with ground-truth. As the GelMap grid is comprised of repeated squares, analysing how close to a perfect square each of these squares are, can give you an estimate of how deformed that region is.

To measure squareness, we first apply a gaussian blur to the image and then find maxima using a manually-adjusted threshold for each image. With a well-sampled image, this should be sufficient to detect all intersections of the GelMap grid. Some manual adjustment may be required to move mis-localised points to the intersections. For each square, we then determine the length of the square diagonals as well as the intersection angles of these diagonals.

We define squareness as the average of:

(1) the ratio between the shorter and the longer diagonal length
(2) the ratio between the smaller and the larger intersection angles of the diagonals.

This takes into account both stretching and skewness and will yield a value between 0 and 1, with 1 corresponding to a perfect square and 0 to a completely flattened square, where two of the opposite corners are overlapping.

Local expansion factor can be determined by dividing the measured area of each square using the detected intersection points, by the known pre-expansion area.

Landmark-based correction
If correction of deformations is desired in addition to quantification, we provide a full workflow of this process in extended data figure 3 of the paper: https://www.nature.com/articles/s41592-023-02001-y/figures/7.

Most correction will be performed using a virtual reference grid instead of a pre-expansion image. Example code to generate virtual reference grid images is on the GelMap website: https://visualise.bio/gelmap-vrg/.

We look forward to hearing back about your experiences with GelMap! Any user feedback is greatly appreciated in order to improve the product.