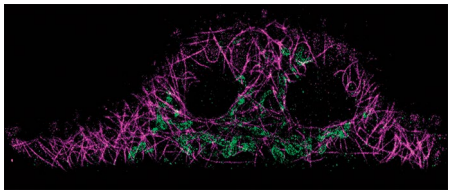
Single-objective light sheet microscopy

Single-objective light sheet fluorescence microscopes are driving innovation in volumetric imaging.

Light sheet fluorescence microscopy has made a name for itself by providing fast imaging of relatively large samples, like developing embryos, with excellent optical sectioning. Because the sample is illuminated by a thin sheet of light, total light doses are also relatively low, which can improve longitudinal imaging.

Geometry, however, has presented a challenge for light sheet fluorescence microscopy. Most conventional light sheet microscopes have two objectives arranged perpendicularly to one another, one for illuminating the sample and one for collecting emitted light. The size and orientation of these objectives restricts the types of sample that can be mounted between the objectives. This has led to creative strategies for mounting diverse samples and alternative instrument designs, including light sheet microscopes that use the same objective for illumination and detection.



obSTORM image of microtubules (magenta) and mitochondria (green) in a mammalian cell. Adapted with permission from Kim et al. *Nat. Methods* **16**, 853–857, 2019, Springer Nature.

Single-objective light sheet methods like oblique plane microscopy (OPM) (*Opt. Express* **16**, 20306-20316, 2008) and swept, confocally aligned planar excitation (SCAPE) microscopy (*Nat. Photonics* **9**, 113–119, 2015; *Nat. Methods* **16**, 1054–1062, 2019) bypass these restrictions using remote focusing and have been used to image diverse sample types, from adherent cells grown in multiwell plates to freely moving organisms. An unrelated approach, single-objective selective plane illumination microscopy (soSPIM), uses micromirror devices to create light sheets perpendicular to the optical axis that such that fluorescence emissions are collected through the same objective as the illumination, and have been used for 3D localization microscopy in cells (*Nat. Methods* **12**, 641–644, 2015).

More recently, researchers have been building on OPM to develop single-objective light sheet microscopes that are increasingly versatile in terms of performance and applicability. These include single-molecule oblique-plane microscopy (obSTORM); a microscopy technique that can be used for super-resolution microscopy in thick tissues (*Nat. Methods* **16**, 853–857, 2019); epi-illumination SPIM (eSPIM) for high-resolution and high-throughput volumetric imaging (*Nat. Methods* **16**, 501–504, 2019); and high numerical aperture OPM microscopy using custom objective lenses that offer high-quality imaging of challenging samples in standard mounting positions (*bioRxiv* https://doi.org/10.1101/2020.09.22.309229; *eLife* https://doi.org/10.7554/eLife.57681, 2020). We think these next-generation single-objective light sheet microscopes will prove essential for biological discovery and further propel light sheet microscopy into the mainstream. ❐

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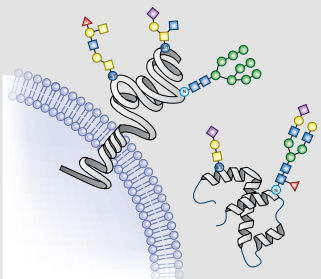
Glycoproteomics

Glycoproteomics is coming of age, thanks to advances in instrumentation, experimental methodologies and computational search algorithms.

Glycosylation is one of the most common post-translational modifications, and glycoproteins play crucial roles in important biological processes like cell signaling, host–pathogen interaction, immune response and disease, including cancer and even the ongoing COVID-19 pandemic (*Science* **369**, 330–333, 2020). Glycoproteomics aims to determine the positions and identities of the complete repertoire of glycans and glycosylated proteins in a given cell or tissue.

Mass spectrometry (MS)-based approaches allow large-scale global analysis; however, the structural diversity of glycans and the heterogeneous nature of glycosylation sites make comprehensive analysis particularly challenging. Glycans obstruct complete fragmentation of the protein backbone, and they were traditionally removed for simplicity at the cost of losing glycan information.



Glycans are everywhere. High-throughput glycoproteomics approaches offer insights. Credit: Katherine Vicari, Springer Nature

The MS spectra tend to be complicated due to the presence of isomers, often requiring manual interpretation. Furthermore, database searching for spectral matches can quickly become a combinatorial problem and requires innovative bioinformatics solutions.

Recent developments in MS instrumentation, fragmentation strategies (*J. Proteome Res.* **19**, 3286–3301, 2020) and high-throughput workflows have made analyzing intact glycoproteins a possibility. Several specific enrichment strategies have made even low-abundance glycans and glycopeptides detectable (*Mol. Cell. Proteomics* https://doi.org/10.1074/mcp.R120.002277, 2020). A variety of experimental workflows tailored for either N-linked glycans, which are found at consensus sites on the proteins, or O-linked glycans, which have no recognizable consensus sequence, have been developed (*Nature* **549**, 538–542, 2017; *Nat. Commun.* **11**, 5268, 2020; *Nat. Methods* **16**, 902–910, 2019). New software packages based on fragment-ion indexing strategies offer substantial increases in speed for glycopeptide and site assignments (*Nat. Methods* **17**, 1125–1132, 2020; *Nat. Methods* **17**, 1133–1138, 2020).

With other -omics fields taking the lion's share of attention in recent years, it is now time for glycoproteomics to shine. Comprehensive understanding of glycosylation at different levels of granularity is bound to serve both basic and translational research. ❐

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