

## **Fixation and Sample Processing Issues for Super-Resolution Microscopy**

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Paraformaldehyde (PFA) is the most commonly used fixative for immunostaining of cells, but has been associated with various problems, ranging from loss of antigenicity to changes in morphology during fixation. Numerous other fixatives have been employed over the last decades, ranging from dialdehydes (such as glutaraldehyde) to protein-unfolding reagents such as methanol. All fixatives need to address two problems: to maintain the sample morphology in an in vivo-like condition, and to enable the penetration of imaging probes, such as antibodies. Here I discuss potential optimization procedures, including the use of a small dialdehyde, glyoxal, that can successfully replace PFA. Glyoxal acts faster than PFA, cross-links proteins more effectively, and improves the preservation of cellular morphology. Interestingly, it also enables better antibody penetration into samples. At the same time, the use of smaller probes, such as affibodies or nanobodies, also provides further improvements of sample imaging, for multiple super-resolution techniques. I also present recent progress in this respect, comparing classical antibody labeling to small probe-labeling.