

## **Fluorescent Proteins**

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In the fifteen years since the development of Green Fluorescent Protein as an expressible fluorescent marker, the field of fluorescent protein biology has exploded. Fluorescent proteins are now available that excite and emit across the entire visible spectrum; red fluorescent proteins such as DsRed, mCherry and mKate2 are now in common use in cell biology, and longer red fluorescent proteins are in development. The use of fluorescent proteins as FRET donors and acceptors is now widespread, and is a critical tool in molecular biology that can be accessed by flow cytometry. Fluorescent proteins can be generated that are sensitive to environmental conditions, can be photoactivated after expression, and can be used to detect biochemical activities such as enzyme activity and protein conformation. In this tutorial, we will review the use of fluorescent proteins in flow cytometry, with particular attention to the newest class of red fluorescent proteins. Topics to be covered will include selecting the correct fluorescent protein for a particular application, transfection and expression of fluorescent proteins, and detection using modern multilaser flow cytometric technology.