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Intact Sample Analysis

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Chapter summary

This chapter has covered the principles and practice of three techniques that can be used for the detection of protein and nucleic acid macromolecules in intact samples, either as single cells or tissue preparations.

- The techniques for the microscopic visualization of proteins are ICC, and for nucleic acids ISH. ICC relies on the application of primary antibodies to bind to epitopes on antigens to identify proteins and provides a semi-quantitative 'readout'. ISH relies on the use of nucleic acid probes binding to target gene sequences by complimentary base pairing. The analysis for ISH may be semi-quantitative or quantitative.
- ICC and ISH may be undertaken manually or using automated platforms, and they share many procedural similarities. These include sample preparation, unmasking of target sequences, and detection of bound primary antibodies or probe/target hybrids using fluorescent or chromogenic detection methods.
- In contrast, flow cytometry (FC) is a quantitative automated technique that is used to identify cells that have bound labelled antibodies to antigens present on their cell membranes. FC may also be used as a precursor to automated cell sorting (FACS) for further analysis.
- The need to ensure that primary antibodies and nucleic acid probes are validated as specific and sensitive for use in these technologies before use together, with the incorporation of appropriate controls when these reagents are subsequently used, is emphasized.