Surgical success depends on the accuracy with which disease and vital tissue can be intraoperatively detected. However, the dominant visualization approach, i.e., human vision, does not see under the tissue surface and operates on low contrast between sites of disease, such as cancer, and the surrounding tissue. Intraoperative fluorescence imaging is emerging as a highly effective method to improve surgical vision and offers the potential to be integrated seamlessly into the normal workflow of the operating room without causing disruption or undue delay. We review and compare two critical fluorescence imaging directions: one that uses nonspecific fluorescence dyes, addressing tissue perfusion and viability, and one that uses targeted agents, interrogating pathophysiological features of disease. These two approaches present detection sensitivity challenges that may differ by orders of magnitude and require different detection strategies. Nevertheless, fluorescence imaging provides the surgeon with previously unavailable real-time feedback that improves surgical precision and can become essential for interventional decision-making.