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Near-Infrared Fluorescence of the NBT/BCIP Chromogenic Stain[1] M. D. MCCUTCHEN, L. A. BUMM, Homer L. Dodge Department of Physics and Astronomy, University of Oklahoma, Norman, OK, D. W. MCCAULEY, Department of Zoology, University of Oklahoma, Norman, OK, L. A. TRINH, M. BONNER-FRASER, S. E. FRASER, Division of Biology, California Institute of Technology, Pasadena, CA — We demonstrate the previously unreported near infrared (NIR) fluorescence of the dark purple stain formed from 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitro blue tetrazolium (NBT). Although the product is a solid with strong optical absorption, its fluorescence enables high cellular resolution imaging of gene expression. We use spectrofluorometry to identify NBT diformazan as the component of the stain that is the fluorophore exhibiting the strong fluorescence signal. The fluorescence shows an intense emission signal (780-910 nm) that is well separated from excitation (645-685 nm). The NBT diformazan fluorescence is also photostable. Because NBT/BCIP is a widely used chromogenic stain, existing staining protocols can also be applied to fluorescence imaging techniques to increase the resolution of gene expression patterns.

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