

## Accelerating Scientific Progress through Open Science: Sharing Documentation for Continuous Development of Open-Source Microscopy in Live-Cell and Single-Molecule Fluorescence Imaging

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### Abstract

Imaging the dynamics and interactions of biomolecules at the single-molecule level in live cells and reconstituted systems has generated unprecedented knowledge about the biomolecular processes underlying many cellular functions. To achieve the speed and sensitivity needed to detect and follow individual molecules, these experiments typically require custom-built microscopes or custom modifications of commercial systems. The costs of such single-molecule microscopes, their technical complexity and the lack of open-source documentation on how to build custom setups therefore limit the accessibility of single-molecule imaging techniques. To advance the adaptation of dynamic single-molecule imaging by a wider community, we present the "K2": an open-source, simultaneous triple-color total internal reflection fluorescence (TIRF) microscope specifically designed for live-cell and single-molecule imaging. The modular design of this TIRF microscope allows users to customize it to their scientific and financial needs, or to re-use parts of our design to improve the capabilities of their existing setups without necessarily having to build a full copy of the K2 microscope, and enables users to easily upgrade and incorporate new technologies as they become available, thereby promoting continuous development and improvement.

