Supplementary Information for

Contrast-Enhanced Fluorescence Microscope by LED Integrated Excitation Cubes

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Supplementary Figure S1. The LEC module for Nexcope NE900 fluorescence microscope

We have tried U-MF2-based LECs in Nexcope NE900 and Olympus IX71 fluorescence microscope, which can also be used for Olympus BX41/ BX51/ BX61/ IX81 fluorescence microscopes and replace U-MNUA2, U-MWBV2, U-MWIB3, U-MNIBA3 filter cubes.

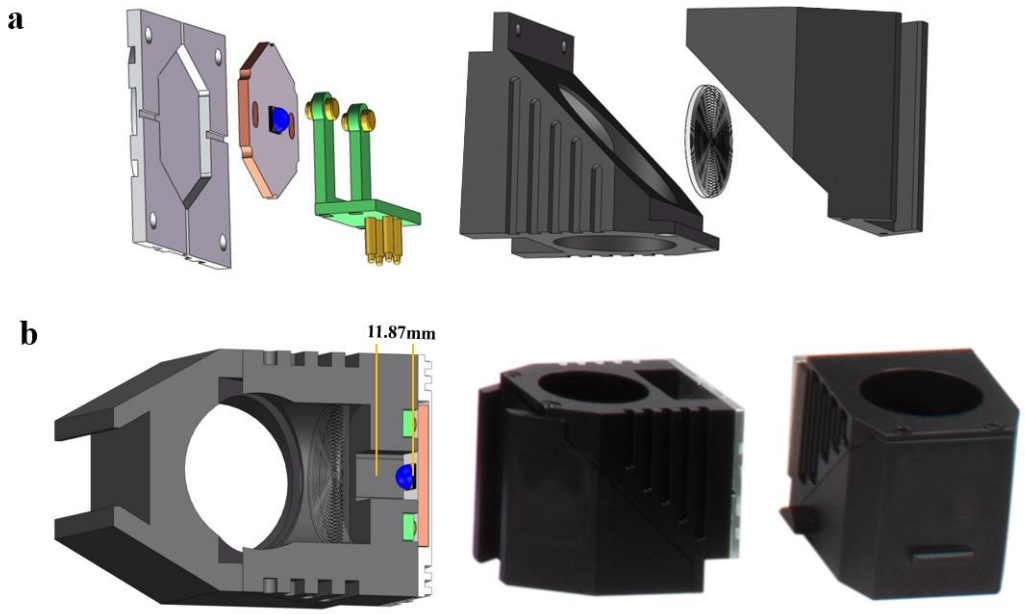

Figure S1. The LEC module was designed regarding U-MF2 series fluorescence filter cubes, so it can be perfectly installed in Nexcope NE900 fluorescence microscope.

Supplementary Figure S2: Schematic illustration of time-gated luminescence technology

The lifetime of DAPI and autofluorescence is nanosecond scale and that of long-lived lanthanide probe is in the range of tens of microseconds to a few milliseconds. When the LED is switched off, the chopper blade will block the fluorescence signal enter the detector during a delay time. The short-lived signal decays rapidly, leaving only long-lived fluorescence when the chopper blade leaves the pinhole. Thus, during the imaging window, only long-lived signal can be detected. The related mechanism is illustrated in Figure S2.

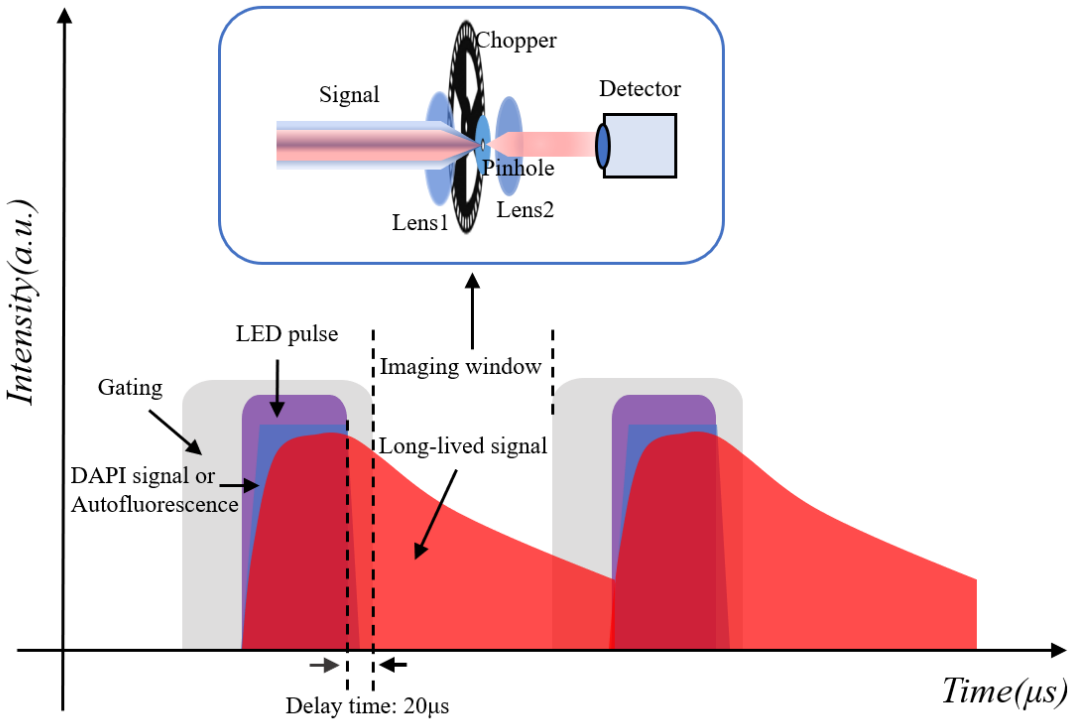

Figure S2. The schematic illustration of time-gated luminescence technology for removing short-lived fluorescence signal

Supplementary Note 1: Sample fabrication and preparation

1. Biological tissue samples preparation

All the biological samples were labeled using the standard immunofluorescence staining procedure.

- Tissue/cell fixation on slides for antigen repair:
  - Wash slides three times with PBS.
  - Use 1x Antigen repair fluid then microwave over medium-high heat until boiling. Heat to boiling over high heat, 20min, then cool to room temperature.
  - Wash slides one time with TBS.
- Enclosed
  - 10% normal goat serum (NGS stored at -20°C) diluted in PBS, 30-60 min.
- Immune response
  - Primary antibody (10% NGS dilution), 4℃ one night.
  - Wash slides two times with PBS.
  - Label the tissue/cell with fluorescent probes, RT 30-60min

- Wash slides two times with PBS.

- Stain cell nuclei with DAPI, RT 2min.
  - Wash 2 times with water, 15min.
- Coverslip sealing with glycerol.

Breast cancer cell BT474 was labeled by HER2 mRNA smFISH procedure.

- cell fixation

  Cells were incubated on slides 37°C through overnight incubation;Fixation was performed with PFA for 30min
- Pretreatment of cell slides for hybridization

  Wash slides two times with PBS;

  Permeabilization or protease treatment for 10-30min
- RNA single molecule hybridization

  Add 2ulRNA probe and 18ul hybridization solution

  The sections were sealed and hybridized overnight at 37 ℃ or for 3 hours at 40 ℃
- Wash after hybridization

  The slides were washed at SSC for 15min at room temperature and at 65 ℃ for 15min
- The samples were counterstained and sealed

  The slides were dried and counterstained with DBCO-BHHBCB-Eu and DAPI.

Breast cancer cell SKBR3 was labeled by chrosome 17 centromeres DNA FISH procedure.

- cell fixation

  Cell suspensions were fixed with 3:1 methanol and glacial acetic acid
- Prepare cell drops and pretreatment of cell slides for hybridization

  Cell drops are incubated with 2XSSC (0.5%Trition) at 37℃。
- Dna in situ hybridization

  1uL chrosome 17 centromeres DNA probe and 9 ul hybridization solution

  The cell were sealed and denaturation at 85℃, hybridized overnight at 37 ℃.
- Wash after hybridization

  The slides were washed at SSC for 15min at room temperature and at 65 ℃ for 15min
- The samples were counterstained and sealed.

  The slides were dried and counterstained with streptavidin-terbium and DAPI.