# Cell culture, transfection, and imaging

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## Abstract

This protocol describes general procedures for culturing HeLa cells, transient transfection, and imaging using an Andor Dragonfly spinning disk confocal system.

### Keywords

Cell culture, transfection, spinning-disk confocal, fluorescent microscopy.

## Solutions to prepare

**DMEM** containing 10% FBS, 100 U/ml penicillin, 100 mg/ml streptomycin, and 2 mM L-glutamine (all from Gibco).

## A. General preparation

1. HeLa-M cells were cultured at 37°C in 5% $CO_2$ and DMEM containing 10% FBS, 100 U/ml penicillin, 100 mg/ml streptomycin, and 2 mM L-glutamine (all from Gibco).

**Note**: For general maintenance, when cells reached 80-90% confluency, they were deattached from the dish with Trypsin and diluted 1:20 in a new dish.

2. For live-cell imaging experiments, cells were seeded on glass-bottomed dishes (MatTek) at a concentration of 35,000 cells per dish and transfected after 24h using FuGene HD (Promega) in Opti-MEM (Gibco).
3. Cell were imaged 24 hours after transfection
4. Just before imaging, the growth medium was removed and replaced with pre-warmed live-cell imaging solution (Life Technologies).
5. For lysotracker experiments, cells were incubated in 50 nM LysoTracker Red DND-99 (ThermoFisher) in complete DMEM for 30 minutes, washed twice with media, then imaged in live-cell imaging solution.
6. All live-cell imaging was performed at 37°C and 5% $CO_2$.
7. Spinning-disk confocal microscopy was performed using an Andor Dragonfly system equipped with a plan apochromat objective (63×, 1.4 NA, oil) and a Zyla scientific CMOS camera.
8. For any given experiment, the same exposure time, laser power, and gain were used for image acquisition to allow for quantitative comparison.

## B. Imaging of cells stably expressing STING-GFP

1. Cells stably expressing STING-GFP were generated as described elsewhere.
2. Stable STING-GFP HeLa-M cells were cultured at 37°C in 5% $CO_2$ and DMEM containing 10% FBS, 100 U/ml penicillin, 100 mg/ml streptomycin, and 2 mM L-glutamine (all from Gibco).
3. For experiments using siRNA, 60 pmols of the indicated siRNA was transfected using 6 µL Lipofectamine RNAiMax (ThermoFisher) in Opti-MEM (Gibco) per dish according to manufacturer protocol. Cells were imaged 72 hours after siRNA transfection.
4. For experiments using cGAMP, 50 µg/L of cGAMP was transfected using using 18 µL Lipofectamine RNAiMax (ThermoFisher) in Opti-MEM (Gibco) per dish according to manufacturer protocol. Cells were imaged 14 hours after transfection.