Live-cell imaging

All Amytracker variants cross the cell membrane of living cells without permeabilization. Due to their low background fluorescence and minimal interference with biological autofluorescence, we recommend **Amytracker 630** or **Amytracker 680** for live-cell imaging. As Amytracker do not bleach easily, they are excellently suited for repeated illumination during time-lapse imaging. If possible, use serum-free medium during incubation.

Perform imaging using the filter settings listed in the table below.

Solutions and Reagents:

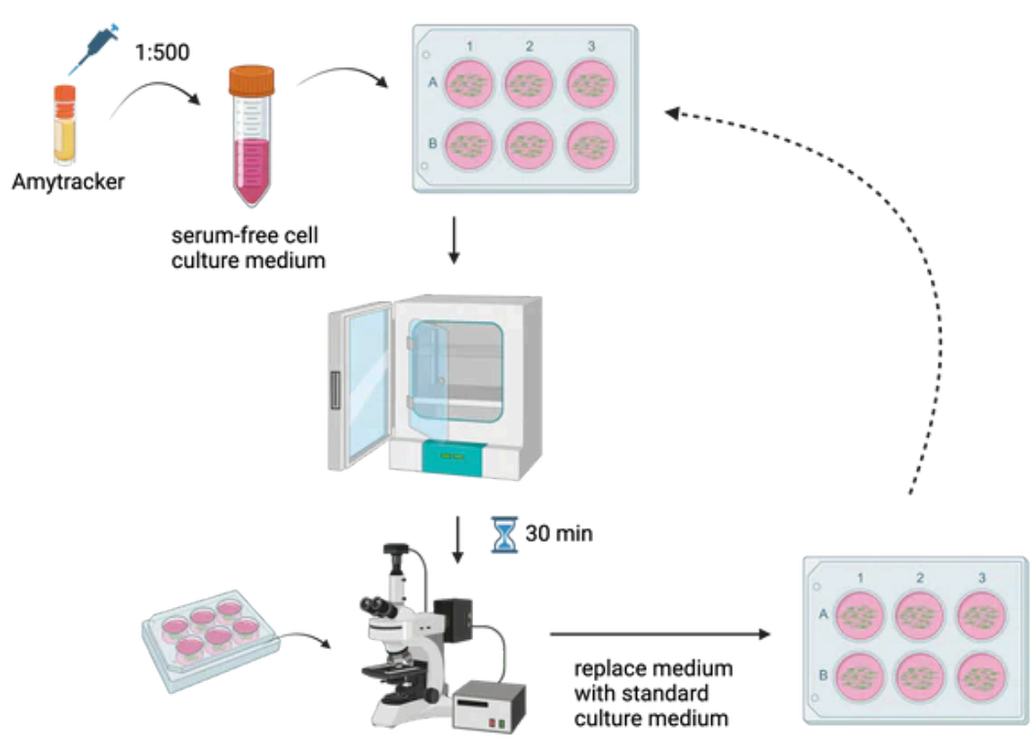
- **Amytracker - Aqueous** or **Amytracker - DMSO**
- Imaging medium: Serum- and Phenol Red free cell culture medium

Assay Procedure:

- Dilute Amytracker in Imaging medium 1:500.
- Incubate your cells in Imaging medium for 30 min under normal culture conditions.
- Image cells
- Replace Imaging medium with standard cell culture medium.

Readout:

Perform imaging using the filter settings listed in the table below.



Multi-Laser / Multi-Detector Imaging with Amytracker

Amytracker are optotracers with structure-dependent photo-physical properties. All Amytracker variants are designed to bind to the Congo red binding pocket on the amyloid fibril and require a theoretical minimum of eight in-register parallel-β-strands for binding. Therefore, Amytracker reliably labels amyloids derived from a variety of amyloidogenic proteins or peptides from different species. Due to their structure-dependent photo physical properties, the Amytracker variants are only fluorescent when binding to a target and different targets can produce a difference in the molecules fluorescence spectrum. To investigate different targets, we recommend to perform imaging by exciting the sample with different wavelengths collecting fluorescence intensity in multiple emission ranges (see the table below for reference). Excitation- and emission spectra for all Amytracker variants can be accessed here.

Table: Multi-laser / Multi-detector imaging protocol performed on a Zeiss LSM800 CLSM.

Channel	Excitation	Emission range	Amytracker variant
CH1	405 nm	400-490 nm	Amytracker 480 Amytracker 680
CH2	405 nm	490-600 nm	Amytracker 480 Amytracker 680
CH3	405 nm	600-660 nm	Amytracker 480 Amytracker 680
CH4	405 nm	660-700 nm	Amytracker 480 Amytracker 680
CH5	488 nm	500-580 nm	Amytracker 520 Amytracker 540 Amytracker 630
CH6	488 nm	580-650 nm	Amytracker 520 Amytracker 540 Amytracker 630
CH7	488 nm	650-700 nm	Amytracker 520 Amytracker 540 Amytracker 630
CH8	561 nm	600-650 nm	Amytracker 630 Amytracker 680
CH9	561 nm	650-700 nm	Amytracker 630 Amytracker 680
CH10	Brightfield		

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