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Lipid ROS measurements via high-throughput imaging

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We use this protocol and it's working

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Abstract

Lipid ROS measurements via high throughput imaging on neurons using C11-BODIPY dye



- 1 Cells cultured in PLO and laminin in 96-well microplate. From day 14 after neuronal induction, cells are ready to be imaged.
- 2 Stain cells with [M] 2 micromolar (μM) C11-BODIPY 581/591 in HBSS for ⌚ 00:15:00 at 🌡️ 37 °C 15m
- 3 Remove probe by washing with HBSS and treat with [M] 20 micromolar (μM) RSL3+/-
- 4 Immediately after treatment, image cells on High Content Imaging system (Operetta), confocal setting with 20x objective. A minimum of 9 fields of view and 6 z-slices per well are acquired for a total of ⌚ 06:00:00 (acquisition every ⌚ 00:30:00 for each well).
- 5 An image analysis pipeline was used to detect cells with reduced (red) or oxidised (green) signal. Lipid ROS levels are determined by calculating ratio of oxidised over reduced.