MitoBrilliant™ Product Guide

Next-generation fluorescent stains for the localization and tracking of mitochondria

bio-techne®

MitoBrilliant™ Technology Overview

Fluorescent probes that accumulate specifically in cellular organelles are valuable components of the 'toolkit' for fluorescence imaging and flow cytometry. Selectively labeling mitochondria with fluorescent probes is of particular interest due to the important role of mitochondria in many cellular processes and in overall cell health.

Functioning mitochondria underpin many critical cellular processes, while mitochondrial dysfunction is linked to numerous diseases. The mitochondrial membrane potential ($\Delta\psi_m$) is an indicator of mitochondrial activity and health because it relates directly to the cells' ability to generate ATP and Reactive Oxygen Species (ROS). Mild depolarization of the inner mitochondrial membrane is thought to be the antioxidant mechanism in mouse embryos. On the other hand, mitochondrial permeability transition (MPT) induced depolarization in cells during nutrient deprivation triggers selective mitophagy. Fluorescent probes that accumulate in the mitochondria specifically as a result of the transmembrane potential can therefore provide insights into mitochondrial and cell health.

Sensitive, selective and dynamic labeling of mitochondria with fluorescent probes is desirable, yet existing probes often have limitations such as toxicity/phototoxicity or interference with mitochondrial functions. Data validating the use of the probes in different experimental protocols is also often incomplete. MitoBrilliant probes have been developed to overcome some common limitations encountered with standard mitochondrial trackers, offering clearer answers to scientific questions. They are next-generation fluorescent stains for the localization and tracking of mitochondria in both live and fixed cells. The range harnesses Janelia Fluor® dye technology, conferring some of the properties that make these widely used dyes, into mitochondrial stains. Two types of MitoBrilliant probes are available.

The MitoBrilliant™ Live dyes accumulate in the mitochondria of live cells in a mitochondrial membrane potential ($\Delta\psi_m$) dependent manner. Upon loss of the mitochondrial membrane potential, the dyes disperse, and so provide a dynamic assessment of $\Delta\psi_m$ in live cells. Two dyes are available: MitoBrilliant™ Live 646 (red emission) and MitoBrilliant™ Live 549 (yellow/orange emission).

MitoBrilliant™ 646 is a universal stain for mitochondria in both live and fixed cells. It is retained in mitochondria following fixation with exceptionally clear staining and is retained in mitochondria following loss of the mitochondrial membrane potential. Additionally, MitoBrilliant™ 646 can be used to stain pre-fixed cells and tissue, making this dye suitable for IHC and ICC.

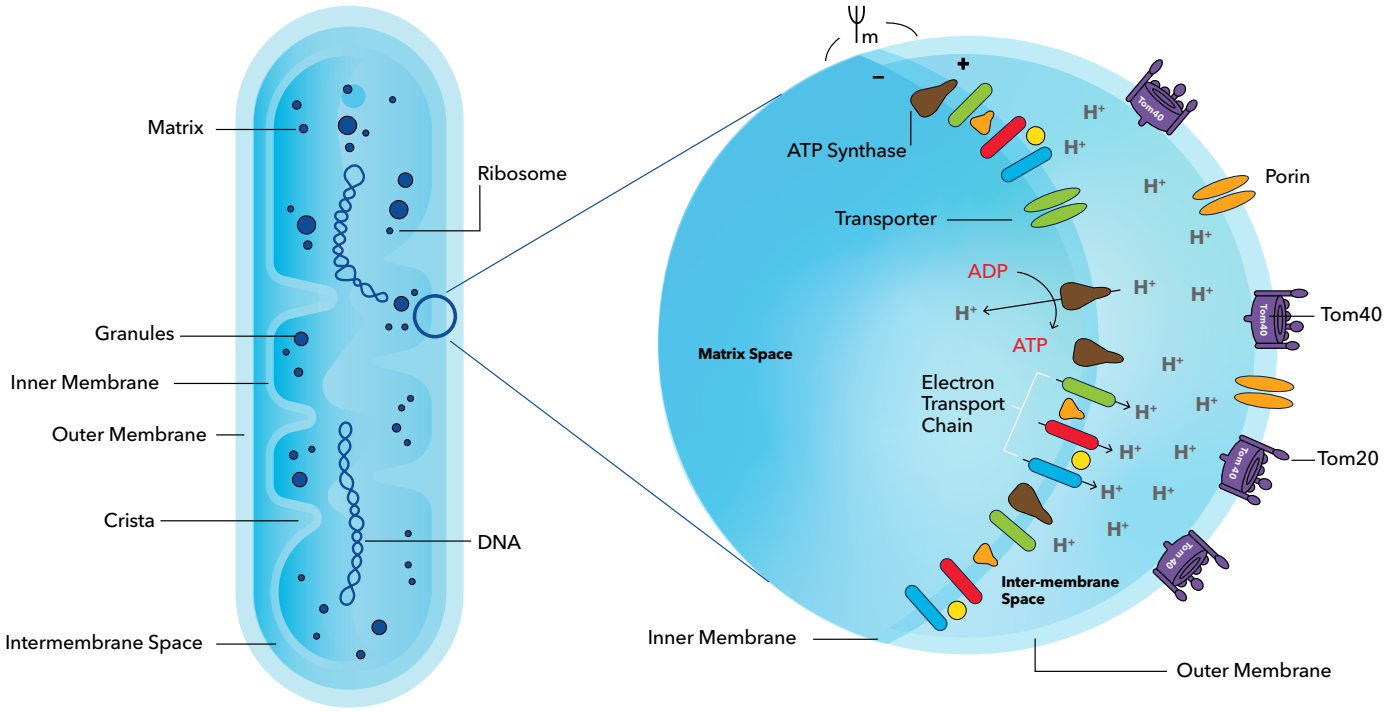


FIGURE 1. Mitochondrial structure. Illustration of basic mitochondrial structure.

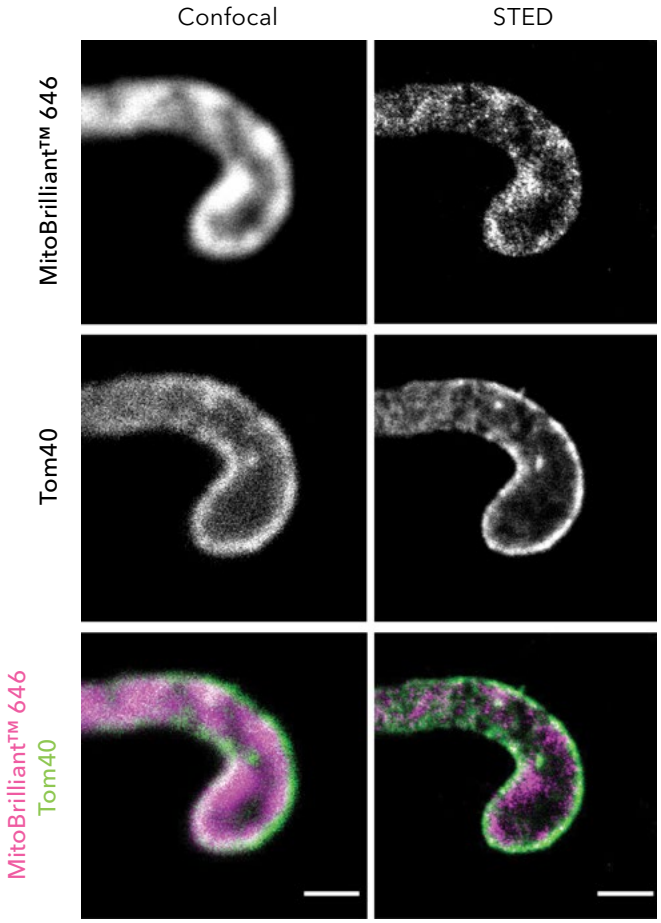


FIGURE 2: Confocal and STED microscopy images of COS-7 cells stained with MitoBrilliant™ 646 (100 nM, magenta) for 30 minutes followed by immediate fixation with 6% PFA for 20 minutes at 37°C. Samples were further processed for immunofluorescent antibody staining (outer mitochondrial membrane protein Tom40, green). Scale bar = 1 µm.

Janelia Fluor® Technology

Developed by Professor Luke Lavis and his team at the Janelia Research Campus, Janelia Fluor® (JF) dyes provide scientists with an exceptional palette of bright, photostable fluorophores for a broad range of applications.

Janelia Fluor® dye key features and applications:

- Exceptionally bright
- Cell permeable
- Highly photostable
- Supplied with a choice of reactive groups for simple biomolecule conjugation
- Can be converted to relevant substrate for use in self-labeling tag systems, e.g. HaloTag® and SNAP-tag®
- Suitable for use in confocal microscopy, IHC, ICC and flow cytometry
- Especially well-suited to live-cell imaging
- Ideal for super-resolution techniques including STED and dSTORM
- Photoactivatable Janelia Fluor® dyes compatible with PALM microscopy

TABLE 1. Key features of MitoBrilliant™ dyes

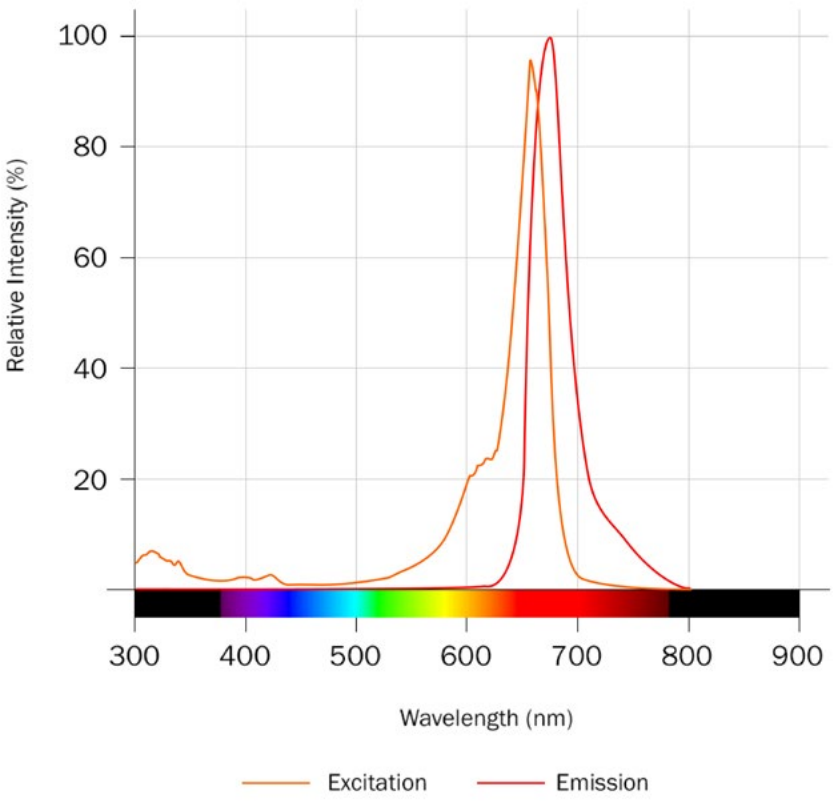
Product Name	Core Dye Structure	Abs/Em (nm)	$\Delta\psi_m$ Dependent	Live/ Fixed Cell Use?	Stains Pre-Fixed Cells?	Image Without Wash Step?	Demonstrated Applications
MitoBrilliant™ 646 Cat. No. 7700	Janelia Fluor® technology	655/668	No*	Suitable for live and fixed cell work	Yes	Yes, but replacing media recommended	Fixed-cell imaging, Live-cell imaging Flow cytometry, IHC/ICC, Super-resolution, microscopy – STED, High-content screening
MitoBrilliant™ Live 646 Cat. No. 7417	Janelia Fluor® technology	648/662	Yes	Live-cell work only	No	Yes, but replacing media recommended	Live-cell imaging, Flow cytometry, High-content screening
MitoBrilliant™ Live 549 Cat. No. 7693	Janelia Fluor® technology	550/568	Yes	Live-cell work only	No	Yes, but replacing media recommended	Live-cell imaging, Flow cytometry, High-content screening

* In live-cell staining, $\Delta\psi_m$ drives initial recruitment of the dye into mitochondria. After staining, localization of the dyes becomes insensitive to $\Delta\psi_m$ changes. In pre-fixed cell staining, MitoBrilliant™ 646 can localize and stay in mitochondria without $\Delta\psi_m$ (see **FIGURE 6**).

MitoBrilliant™ 646 (Cat. No. 7700)

Highly versatile ‘universal stain’ for both live and fixed cell applications

- Microscopy/Imaging
- Flow cytometry
- IHC/ICC
- Super-resolution microscopy – STED
- High-content imaging/screening



Emission Color	Red
λ_{abs}	655 nm
λ_{em}	668 nm
Extinction Coefficient (ε)	125,000 M^{-1} cm^{-1}
Closest Laser Line	640 nm

FIGURE 3. Fluorescence excitation and emission spectra for MitoBrilliant™ 646.

MitoBrilliant™ 646 (Cat. No. 7700)

Application Data: Confocal Live-Cell Imaging

MitoBrilliant™ 646 is highly cell permeable, staining is stable and it is retained in mitochondria after a washout step. These properties make it an ideal choice for confocal live-cell imaging (see **FIGURE 4**).

In addition, after staining, MitoBrilliant™ 646 is insensitive to changes in mitochondrial membrane potential (see **FIGURE 7**).

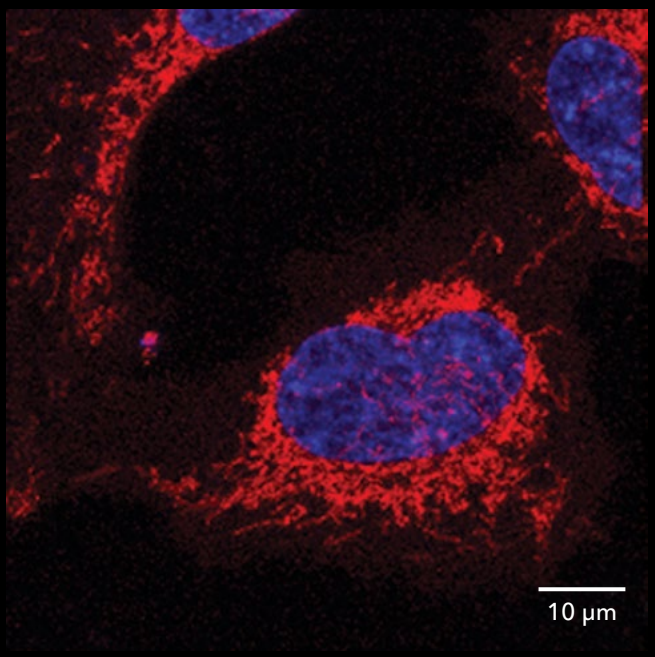


FIGURE 4. Mitochondria morphological examination at higher resolution in live cells. HeLa cells were incubated with MitoBrilliant™ 646 (100 nM) for 40 minutes. Cells were counterstained with DAPI (Cat. No. 5748). Image was taken using an LSM880 Confocal using a 63x objective. Scale bar = 10 µm.

Property: Performance and Staining Fidelity Post-Fixation

MitoBrilliant™ 646 staining is retained after fixation with PFA and provides significantly improved staining resolution than a leading competitor dye.

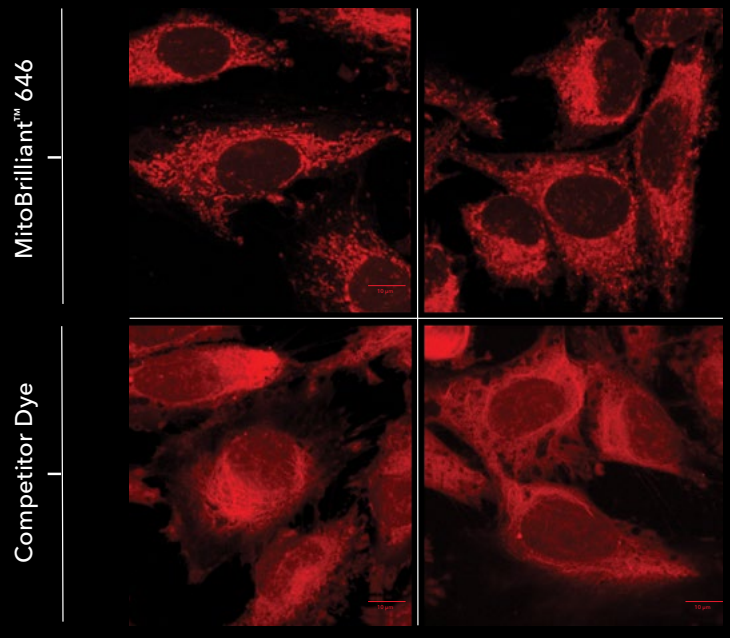


FIGURE 5. Performance of MitoBrilliant™ 646 after fixation. HeLa cells were incubated with MitoBrilliant™ 646 (100 nM) for 40 minutes. Cells were then fixed for 10 minutes in 4% PFA. All images were taken using the LSM880 Confocal using a 63x oil objective. Laser power and gain were set to each individual probe. Scale bar = 10 µm.

MitoBrilliant™ 646 (Cat. No. 7700)

Property: Suitability for Staining Pre-Fixed Cells

The data in **FIGURE 6** demonstrates that MitoBrilliant™ 646 is able to target and stain mitochondria in PFA fixed cells, and the staining is as specific as a leading competitor dye (applied before fixation).

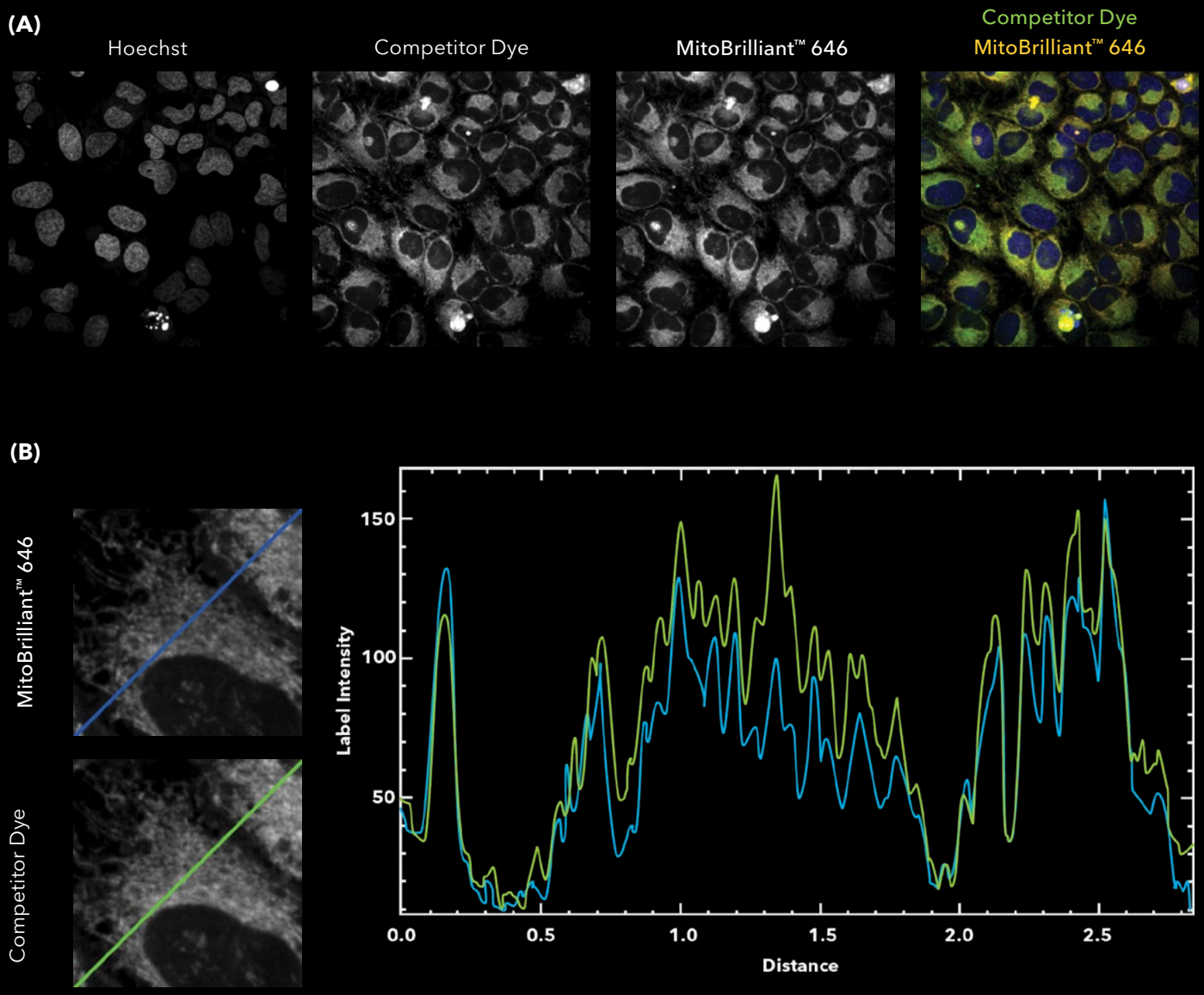


FIGURE 6. Validation of MitoBrilliant™ 646 for staining pre-fixed cells. (A) Competitor dye (250 nM) was added to HeLa cells 45 minutes prior to fixation. Cells were fixed in 4% PFA for 20 minutes cells were not permeabilized. MitoBrilliant™ 646 (75 nM) was then applied and the cells were imaged. The merged image shows the colocalization of the competitor dye (λ_{abs} = 578 nm, λ_{em} = 598 nm. Imaging setup: Ex: 561 nm, Em: BP 617/73 nm) and MitoBrilliant™ 646 (λ_{abs} = 655 nm, λ_{em} = 668 nm. Imaging setup: Ex: 640 nm, Em: BP 685/40 nm). **(B)** The intensity profiles of competitor dye (green line, applied to live cells prior to fixation) and MitoBrilliant™ 646 (blue line, applied to the same sample after fixation) are shown. Intensity profiles were taken across a set frame (left) on a single cell and compared (right). The data shows very high similarity on staining profile, demonstrates that MitoBrilliant™ 646 can be applied post-fixation. Experiments and analysis were performed by PhenoVista.

MitoBrilliant™ 646 (Cat. No. 7700)

Property: $\Delta\psi_m$ Dependency

For live-cell staining, the mitochondrial membrane potential ($\Delta\psi_m$) is only required for initial recruitment of MitoBrilliant™ 646 to the mitochondria. Subsequent retention is insensitive to loss of $\Delta\psi_m$. This is demonstrated in **FIGURE 7** by using carbonyl cyanide m-chlorophenyl hydrazone (CCCP, Cat. No. 0452) to disrupt $\Delta\psi_m$ before or following staining. MitoBrilliantTM 646 is therefore an ideal dye for stable labeling of mitochondria during cellular events that might affect $\Delta\psi_m$.

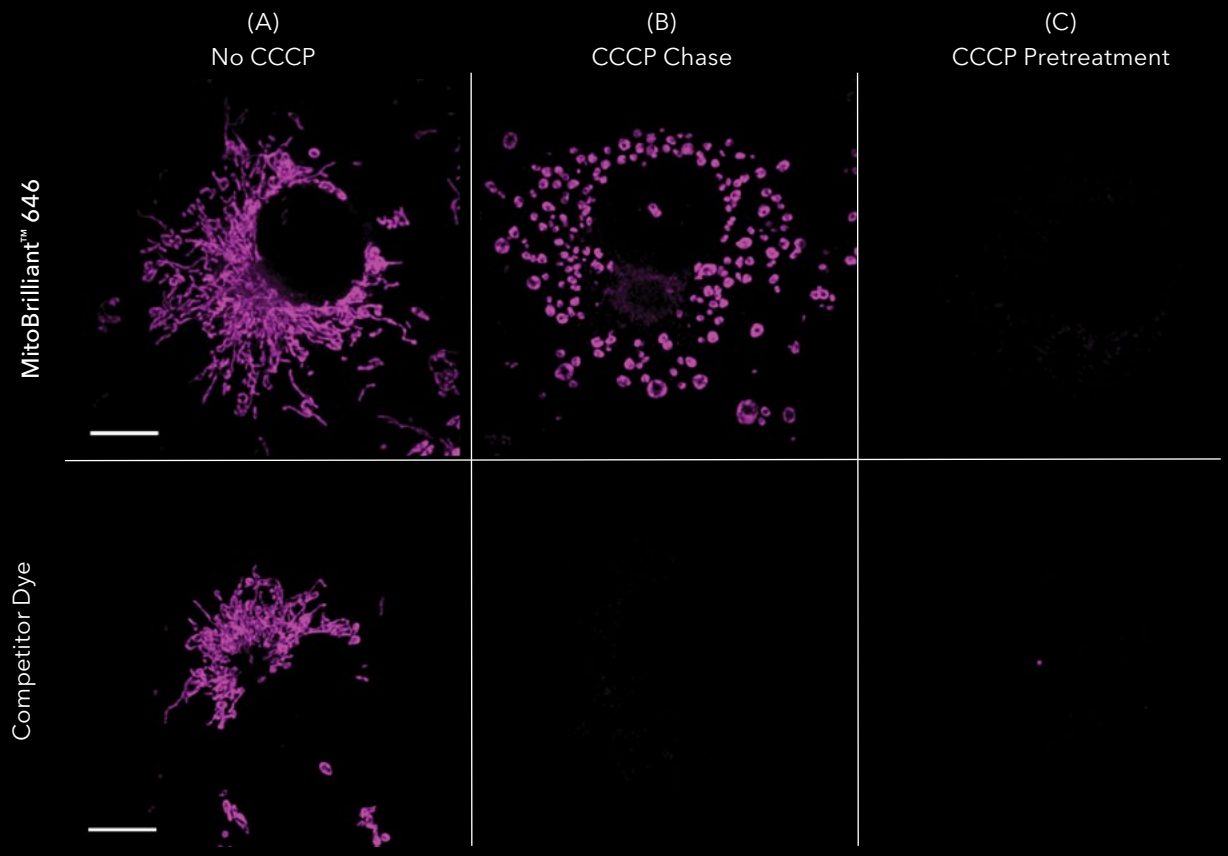


FIGURE 7. $\Delta\psi_m$ Dependency of MitoBrilliant™ 646. COS-7 cells were stained with MitoBrilliant™ 646 (100 nM) or a leading competitor dye (50 nM) for 60 minutes. Cells were treated with 20 µM CCCP at different stages of staining. **Column A**: No CCCP treatment. **Column B**: CCCP treatment applied only after the mitochondrial staining step, for 30 minutes. Only the MitoBrilliant 646 signal remained, suggesting that this dye was retained in mitochondria even after the loss of the membrane potential, whereas the accumulation of the competitor dye was reversible. **Column C**: CCCP was added 30 minutes before the staining step. Excessive dye was then washed out with media containing CCCP for 30 minutes. Mitochondrial accumulation of both MitoBrilliant™ 646 and the competitor dye were abolished. Scale bar = 10 µm.

MitoBrilliant™ 646 (Cat. No. 7700)

Application Data: High-Content Imager Data in Different Cell Types

MitoBrilliant™ 646 reliably produces high quality data in different cell types as shown in **FIGURE 8**.

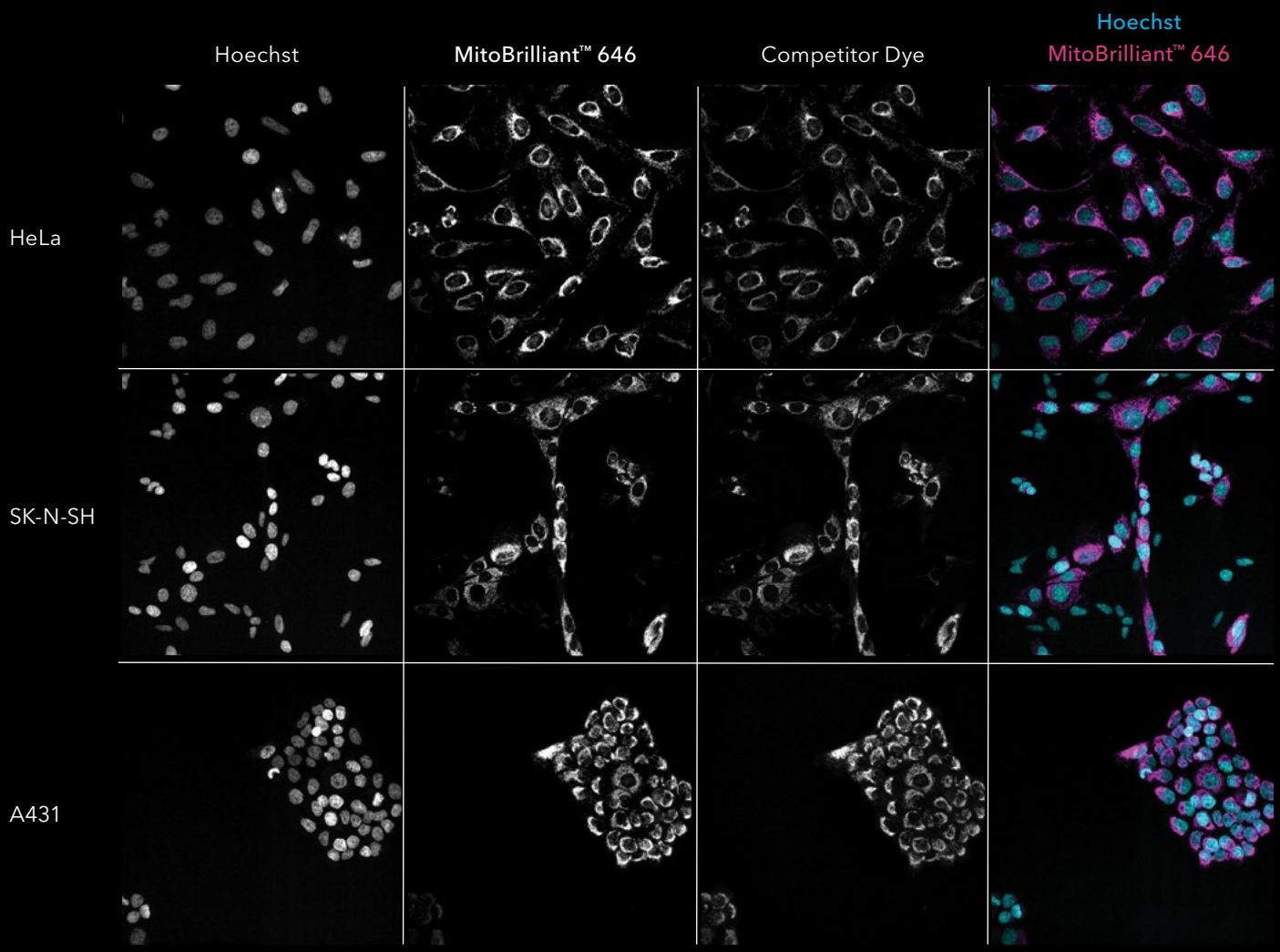


FIGURE 8. High-content imager data of mitochondria in different cell types. Cells were incubated with either MitoBrilliant™ 646 (75 nM) or a leading Competitor dye (250 nM) for 45 minutes and washed in DMEM complete media prior to live imaging. Cells were counterstained with DAPI (Cat. No. 5748). Images taken using a Yokogawa CQ1 high-content imager using a 60x objective. Experiments and analysis performed by PhenoVista.

MitoBrilliant™ 646 (Cat. No. 7700)

Application Data: Flow Cytometry

For live-cell staining, MitoBrilliant™ 646 is highly cell permeable with unique properties relating to $\Delta\psi_m$ sensitivity, it is therefore a valuable tool for various flow cytometry experiments.

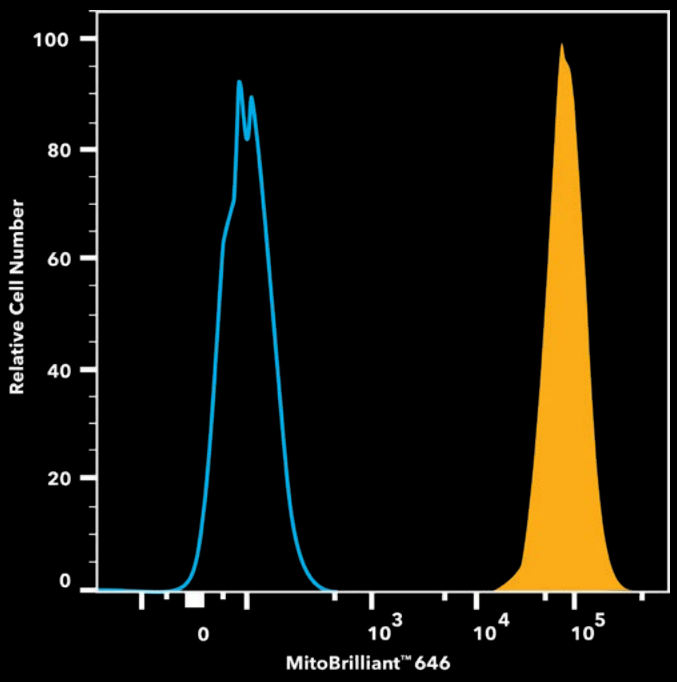
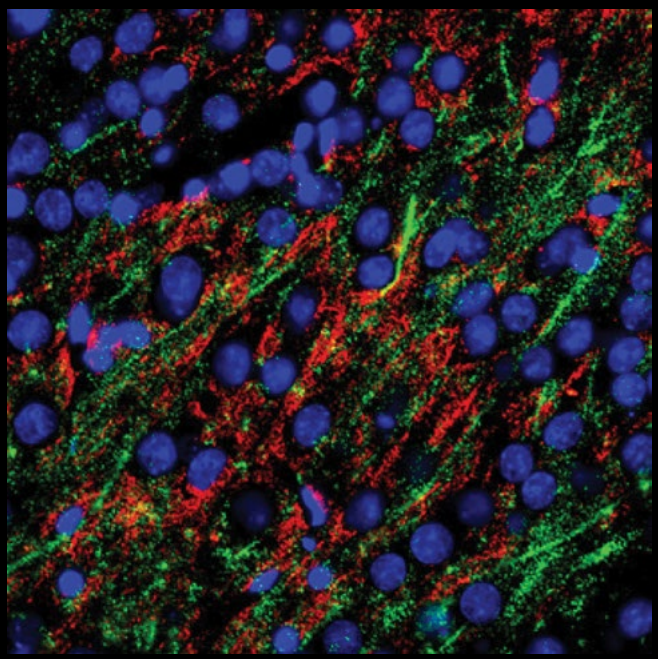


FIGURE 9. Flow cytometry histogram of cell populations following MitoBrilliant™ 646 staining. K562 cells were stained with MitoBrilliant™ 646 for 30 minutes at 37°C. Cells were then washed, and the data were acquired on BD Fortessa Flow Cytometer using 640 nm laser for excitation and 670/14 nm emission filter. Data shows overlay of stained (orange) and unstained cells (blue).

Application Data: Immunohistochemistry (IHC)

MitoBrilliant™ 646 can stain mitochondria either at the pre-fixation or post-fixation stages of sample preparation for IHC as shown in **FIGURE 10**.

Piriform Cortex



Hypothalamus

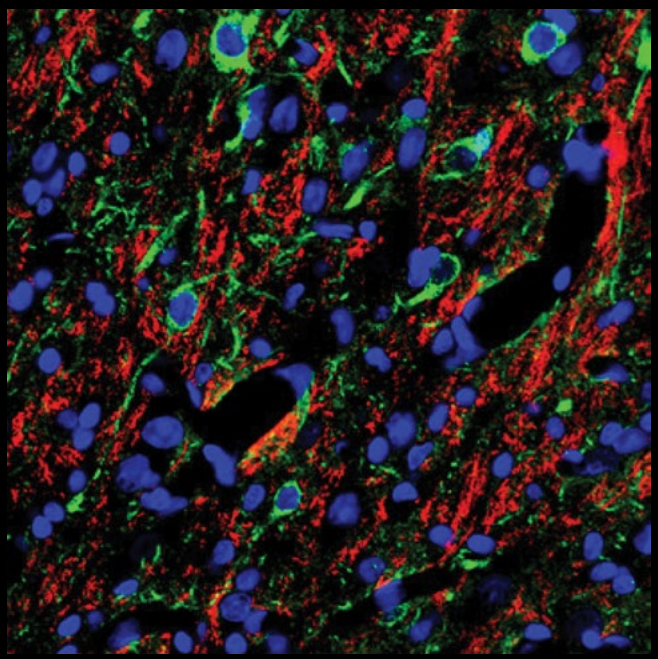


FIGURE 10. Fluorescence IHC was performed on 10 µm thick rat brain tissue sections followed by staining with MitoBrilliant™ 646. Labeling for phospho-ERK1 (T202/Y204)/ERK2 (T185/Y187) (pseudo-colored green) combined with MitoBrilliant™ 646 (pseudo-colored red). **Hypothalamus**: labeling for nNOS (pseudo-colored green) combined with MitoBrilliant™ 646 (pseudo-colored red). Tissue sections were stained with DAPI (Cat. No. 5748) nuclear counterstain (blue).

MitoBrilliant™ 646 (Cat. No. 7700)

Application Data: Super-Resolution Microscopy (STED)

Super-resolution microscopy (SRM) enables image generation at resolutions beyond the optical diffraction limit (~200 nm). Stimulated Emission Depletion (STED) microscopy is an SRM technique that can provide single molecule resolution data with appropriate fluorophores.

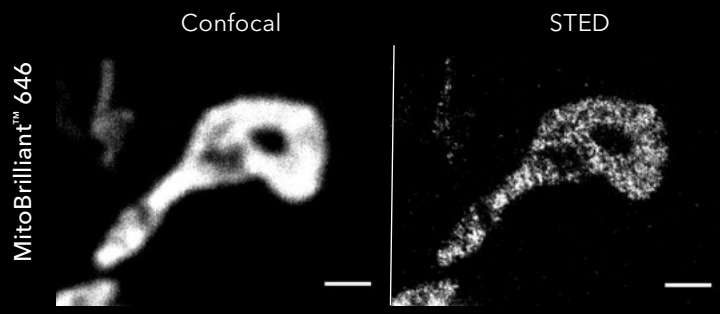


FIGURE 11. Comparison of confocal and STED data for MitoBrilliant™ 646. COS-7 cells stained with MitoBrilliant™ 646 (100 nM) for 30 minutes followed by immediate fixation with 6% PFA for 20 minutes at 37 °C. Shown are confocal (left) and super-resolution (right, 20 nm pixel size) images of mitochondria. Scale bar = 1 µm.

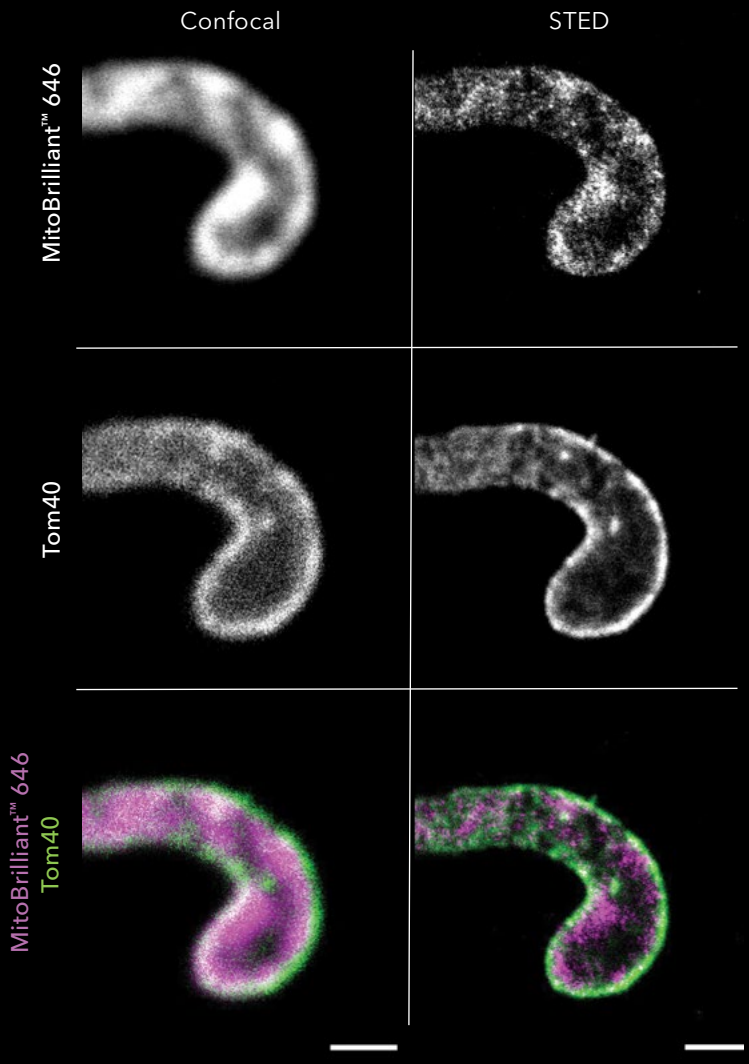
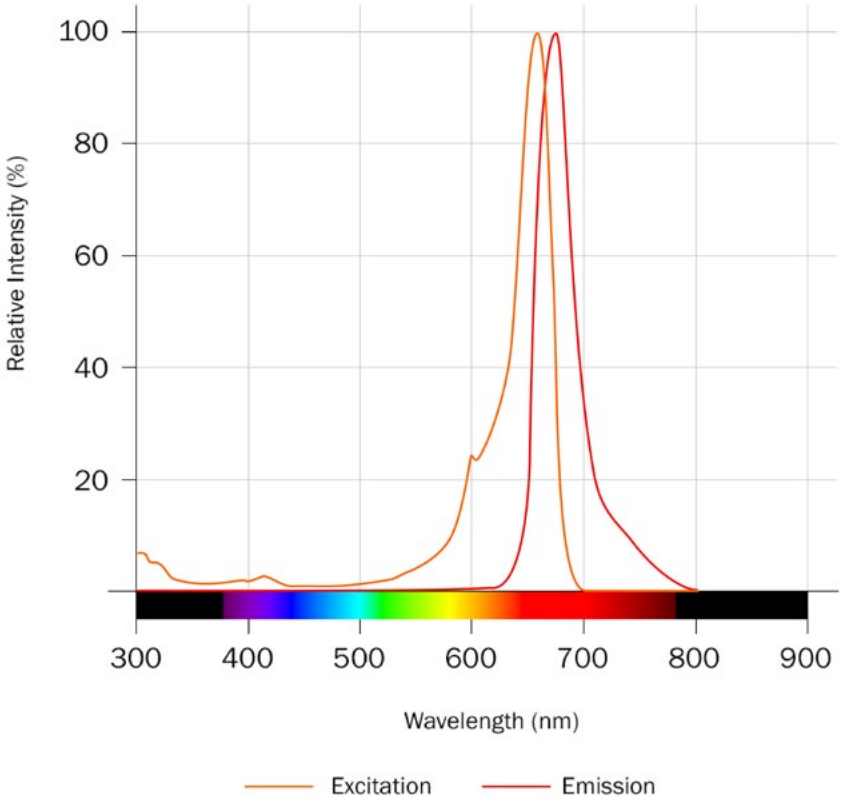


FIGURE 12. Colocalization of MitoBrilliant™ 646 with Tom40 using STED microscopy. COS-7 cells dual-stained with MitoBrilliant™ 646 (100 nM, magenta) for 30 minutes followed by immediate fixation with 6% PFA for 20 minutes at 37 °C. Samples were further processed for immunofluorescent antibody staining (outer mitochondrial membrane protein Tom40, green). Shown are confocal (left) and super-resolution (right), 20 nm pixel size images of mitochondria. Scale bar = 1 µm.

MitoBrilliant™ Live 646 (Cat. No. 7417)

Photostable, $\Delta\psi_m$ dependent mitochondrial dye for live-cell applications

- Microscopy/Imaging
- Flow cytometry
- High-content imaging/screening



Emission Color	Red
λ_{abs}	648 nm
λ_{em}	662 nm
Extinction Coefficient (ε)	127,800 M^{-1} cm^{-1}
Closest Laser Line	640 nm

FIGURE 13. Fluorescence excitation and emission spectra for MitoBrilliant™ Live 646.

MitoBrilliant™ Live 646 (Cat. No. 7417)

Property: Mitochondrial Specificity

The following dataset provides experimental data showcasing the key properties of MitoBrilliant™ Live 646 and its utility in different applications.

Using mitochondrial outer membrane protein Tom20 as the localization marker, **FIGURE 14** shows that MitoBrilliant™ Live 646 specifically accumulates in mitochondria in live cells without any detectable off-target staining.

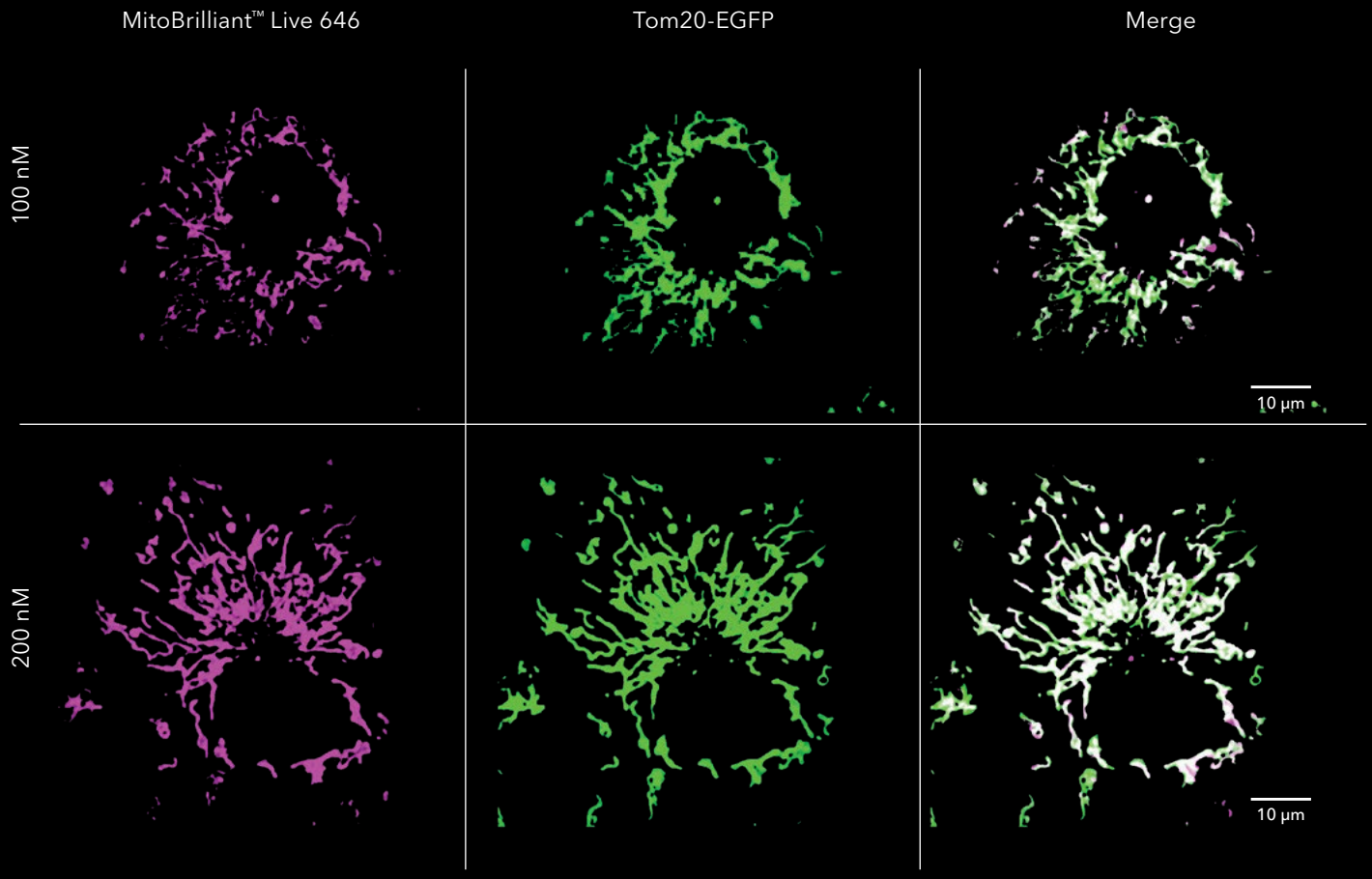


FIGURE 14. Colocalization of MitoBrilliant™ Live 646 with Tom20. COS-7 cells stably expressing human Tom20-EGFP were stained with 100 nM (top panel) or 200 nM (lower panel) MitoBrilliant™ Live 646 for 30 minutes followed by a washout step with fresh media. MitoBrilliant™ Live 646 localization matched closely with Tom20-EGFP indicating specific staining of the mitochondria. Scale bar = 10 µm.

MitoBrilliant™ Live 646 (Cat. No. 7417)

Property: $\Delta\psi_m$ Dependency

Mitochondrial membrane potential ($\Delta\psi_m$) is required for both initial recruitment and subsequent retention of MitoBrilliant™ Live 646 in the mitochondria of live cells.

This is demonstrated in **FIGURE 15** by using CCCP (Cat. No. 0452) to disrupt $\Delta\psi_m$ before and following treatment with MitoBrilliant™ Live 646.

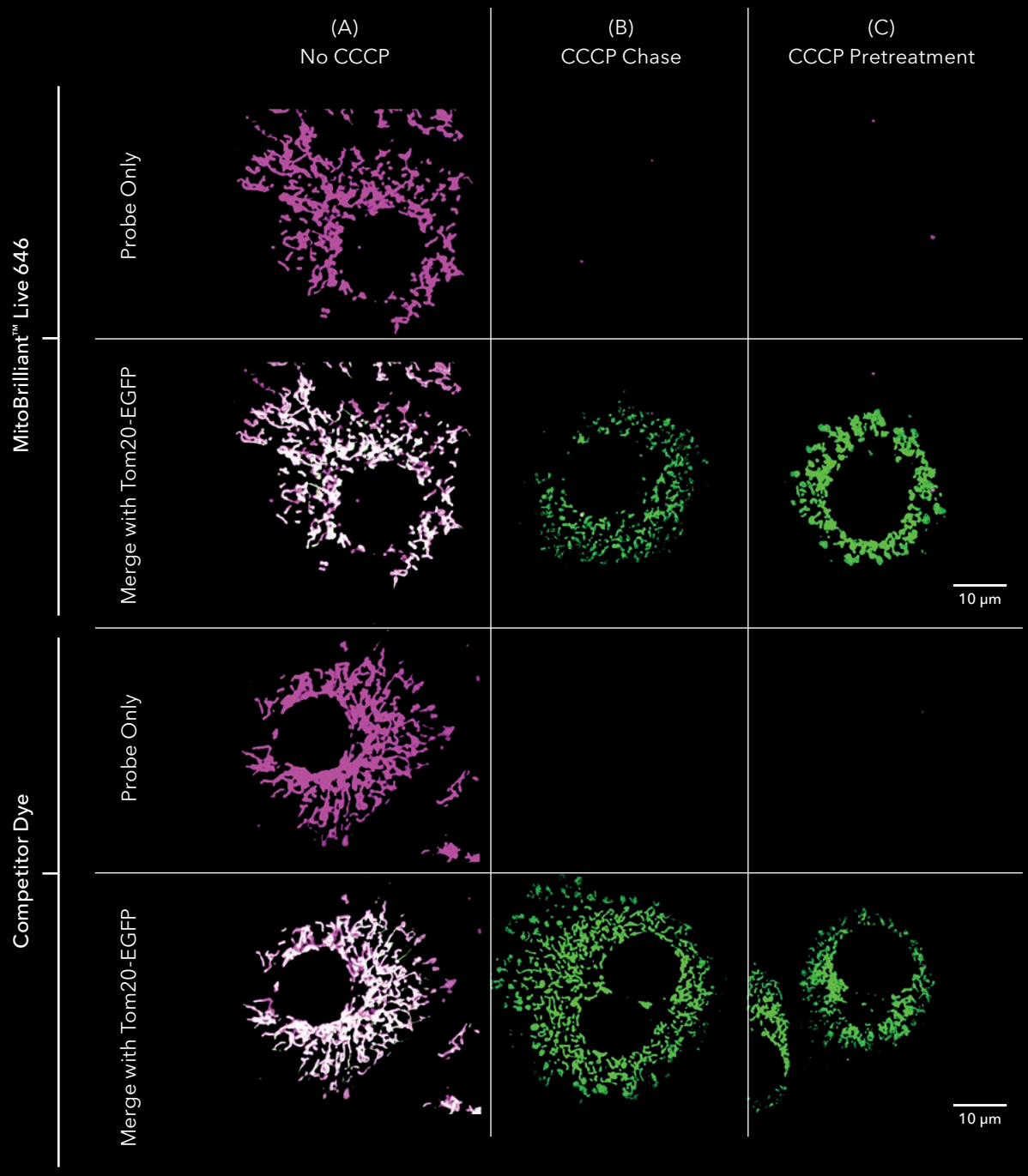


FIGURE 15. $\Delta\psi_m$ dependency. COS-7 cells expressing Tom20-EGFP were stained with MitoBrilliant™ Live 646 (100 nM) or a leading competitor dye (50 nM) for 60 minutes. Cells were treated with 20 µM CCCP (Cat. No. 0452) to disrupt $\Delta\psi_m$ at different stages of staining **Column A**: No CCCP treatment. **Column B**: CCCP treatment applied only after the mitochondrial staining step, for 30 minutes causing the loss of MitoBrilliant Live 646 and competitor dye signal. **Column C**: CCCP was added 30 minutes before the staining step. Excessive dye was then washed out with media containing CCCP for 30 minutes. No staining observed for either dye.

MitoBrilliant™ Live 646 (Cat. No. 7417)

Property: Photostability

In live-cell time lapse imaging, MitoBrilliant™ Live 646 produced a more stable, longer-lasting fluorescent signal than a leading competitor dye.

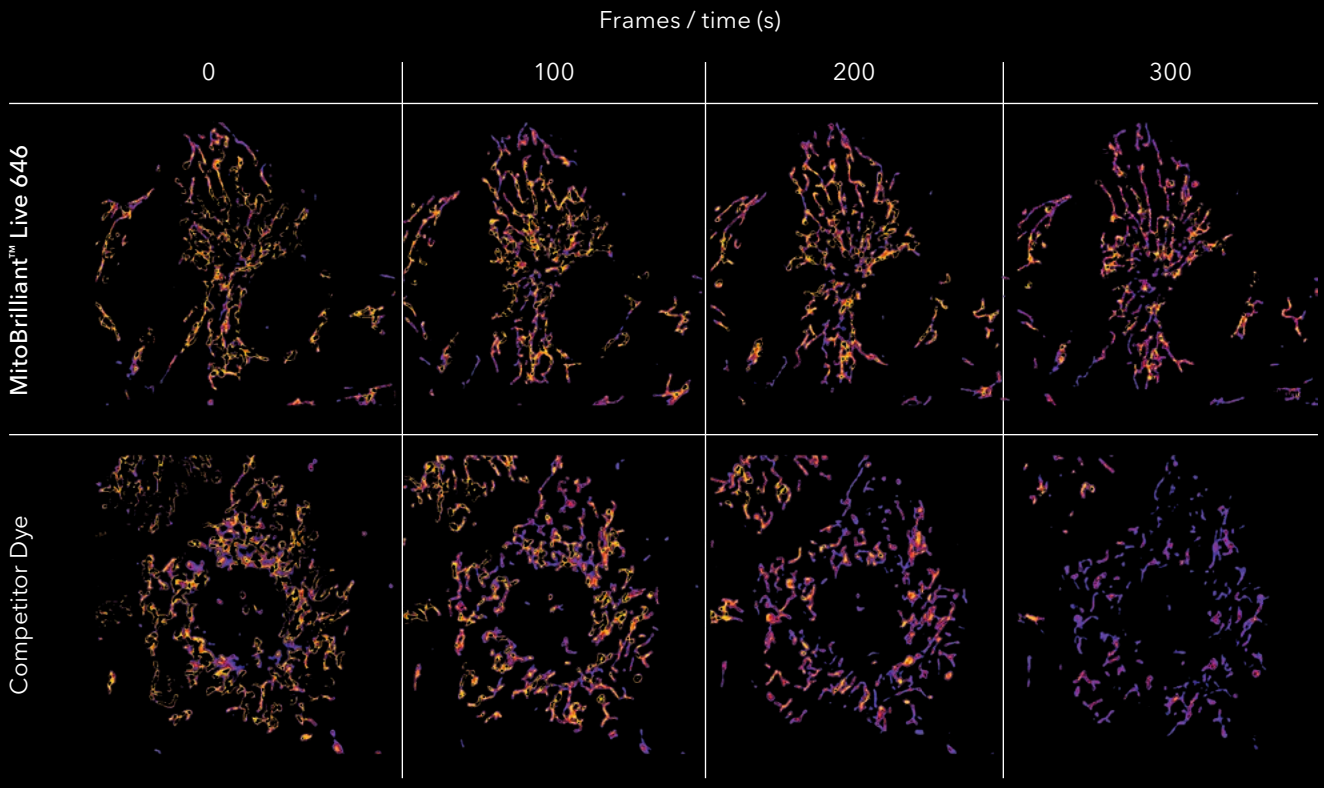


FIGURE 16. Fluorescence intensity changes during live-cell imaging. COS-7 cells were stained with MitoBrilliant™ Live 646 (100 nM) or a leading competitor dye (50 nM) for 60 minutes followed by the washout of excess dye with fresh media. Images were acquired every second over a time frame of 5 minutes. Shown are representative images after 0, 100, 200 and 300 seconds.

Application Data: Confocal Live-Cell Imaging

MitoBrilliant™ Live 646 is highly cell permeable, stable and is retained in mitochondria due to the membrane potential after a washout step. These properties make MitoBrilliant™ Live 646 an ideal choice for confocal live-cell imaging.

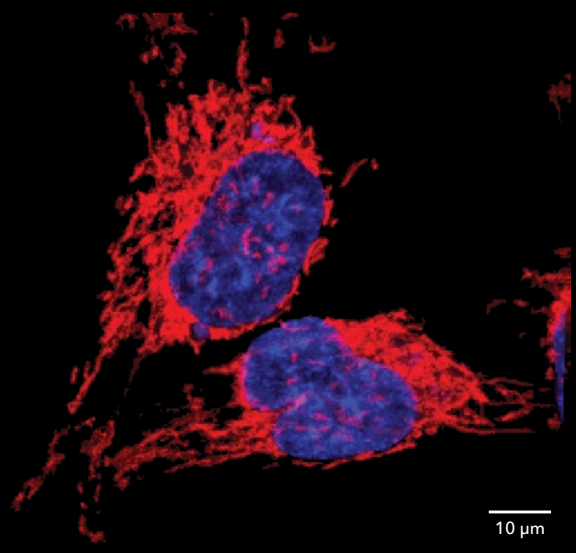


FIGURE 17. Mitochondria morphological examination at higher resolution in live cells. HeLa cells incubated with MitoBrilliant™ Live 646 (100 nM) for 40 minutes. Cells were counter stained with DAPI (Cat. No. 5748). Image taken using an LSM880 Confocal using a 63X objective. Scale bar = 10 µm.

MitoBrilliant™ Live 646 (Cat. No. 7417)

Application Data: High-Content Imager Data with Different Cell Types

MitoBrilliant™ Live 646 reliably produces high quality data in different cell types as shown in **FIGURE 18**.

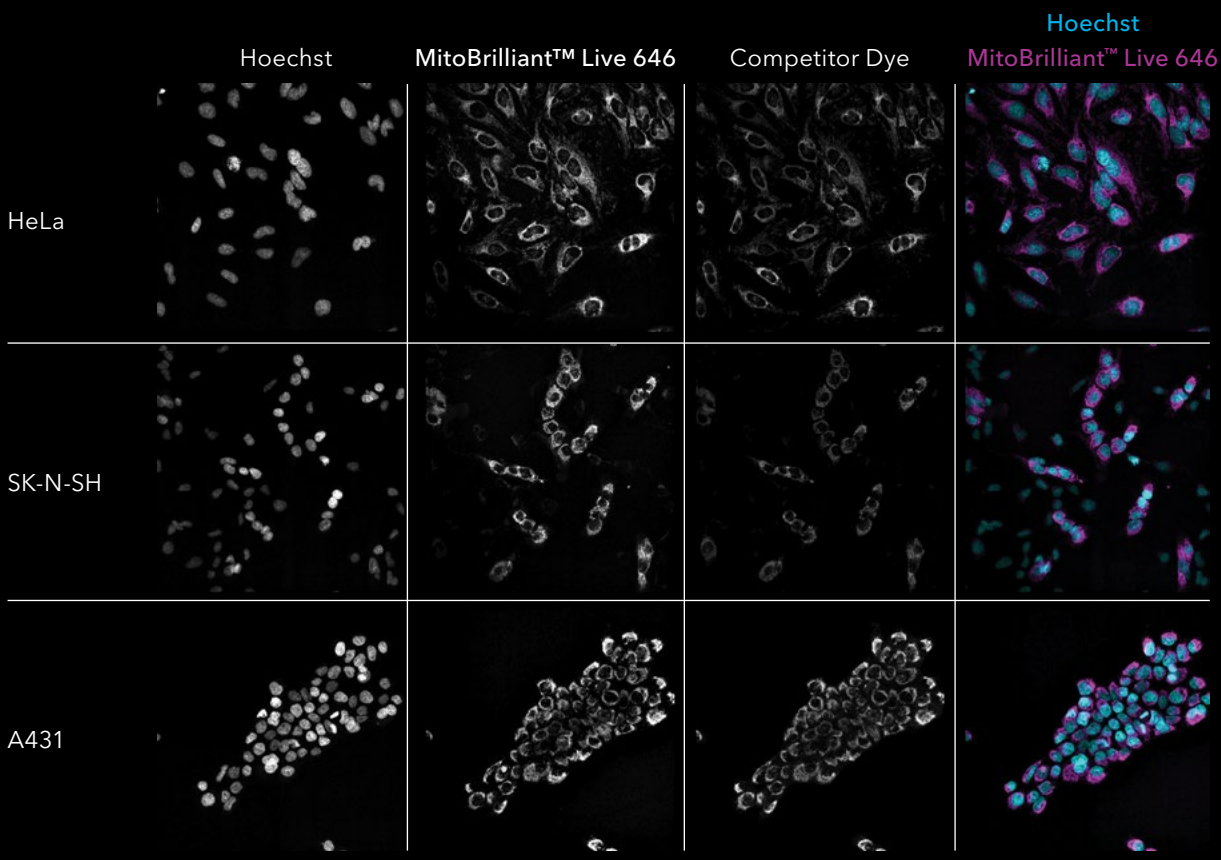


FIGURE 18. High-content imager data of mitochondrial staining in different cell types. Cells were incubated with either MitoBrilliant™ Live 646 (75 nM) or a leading competitor dye (250 nM) for 45 minutes and washed in DMEM complete media prior to live imaging. Cells were counter-stained with Hoechst. Images taken using a Yokogawa CQ1 high-content imager using a 60X objective. Experiments and analysis performed by PhenoVista.

Application Data: Flow Cytometry

MitoBrilliant™ Live 646 is highly cell permeable, staining is stable and it is retained in mitochondria after a washout step. It can be used to stain cells in culture flasks or in suspension solution. These properties make MitoBrilliant™ Live 646 an ideal choice for flow cytometry.

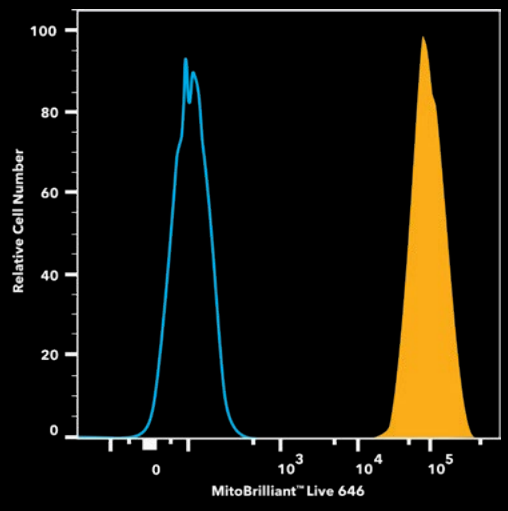
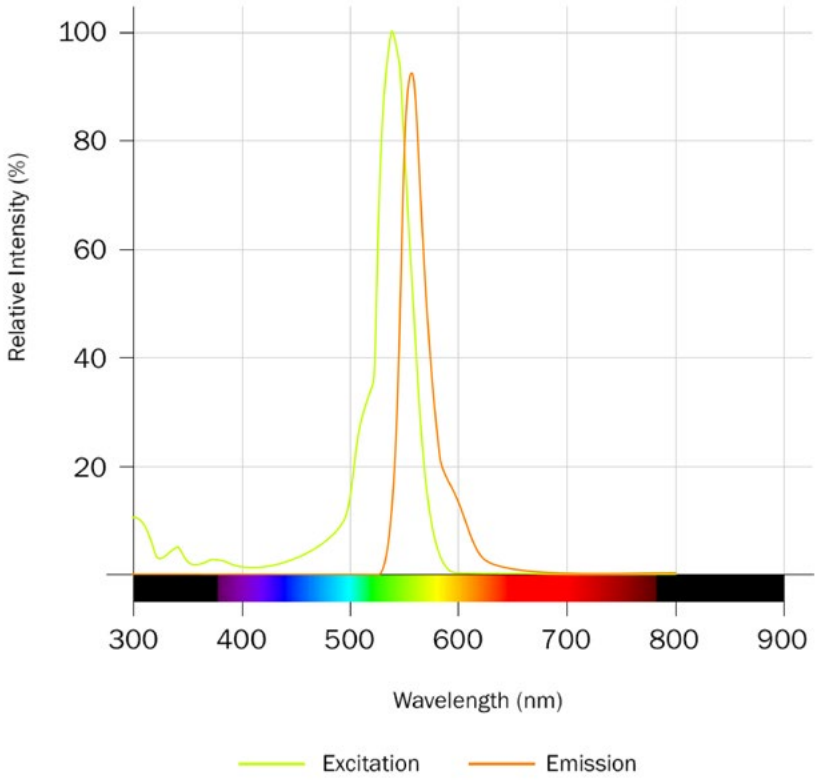


FIGURE 19. Flow cytometry histogram of cell populations following MitoBrilliant™ Live 646 staining. K562 cells were stained with MitoBrilliant™ Live 646 for 30 minutes at 37°C. Cells were then washed and data were acquired on BD Fortessa Flow Cytometer using 640 nm laser for excitation and 670/14 nm emission filter. Data show overlay of stained (orange) and unstained cells (blue).

MitoBrilliant™ Live 549 (Cat. No. 7693)

Exceptionally bright mitochondrial dye for live-cell applications

- Microscopy/Imaging
- Flow cytometry
- High-content imaging/screening



Emission Color	Yellow/ Orange
λ_{abs}	550 nm
λ_{em}	568 nm
Extinction Coefficient (ε)	100,000 M^{-1} cm^{-1}
Closest Laser Line	532 nm

FIGURE 20. Fluorescence excitation and emission spectra for MitoBrilliant™ Live 549.

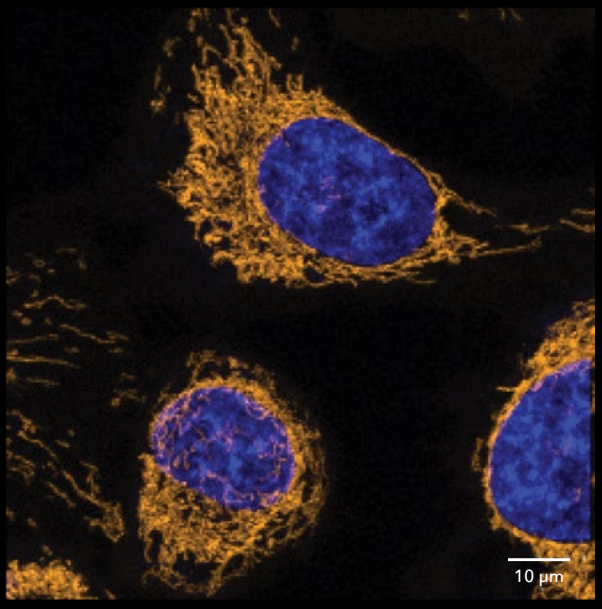
MitoBrilliant™ Live 549 (Cat. No. 7693)

Property: Brightness

The following dataset provides experimental data showcasing the key properties of MitoBrilliant™ Live 549 and its utility in different applications.

Derived from Janelia Fluor® technology, MitoBrilliant™ Live 549 is significantly brighter than a leading competitor dye with similar maximum absorption and emission wavelength (see **FIGURE 21**). It provides exceptional data in both live-cell imaging and flow cytometry.

MitoBrilliant™ Live 549



Leading Competitor Dye

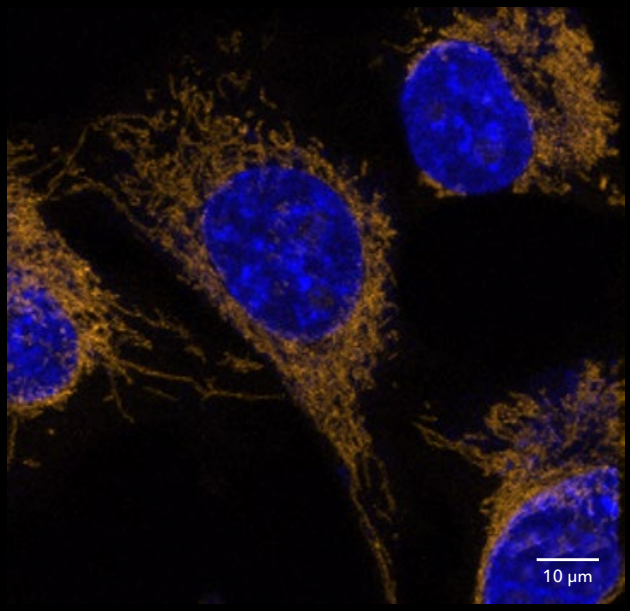


FIGURE 21. Mitochondrial probe brightness comparison. HeLa cells where incubated with MitoBrilliant™ Live 549 (100 nM, left) or a leading competitor dye (100 nM, right) for 40 minutes. Cells were counterstained with DAPI (Cat. No. 5748). Image taken using an LSM880 Confocal using a 63X objective with the same setting for both images. Scale bar = 10 µm.

Application: Flow Cytometry

MitoBrilliant™ Live 549 is highly cell permeable, staining is stable and it is retained in mitochondria due to the membrane potential after a washout step. It can be used to stain cells in culture flasks or in suspension solution. These properties make MitoBrilliant™ Live 549 an ideal choice for flow cytometry.

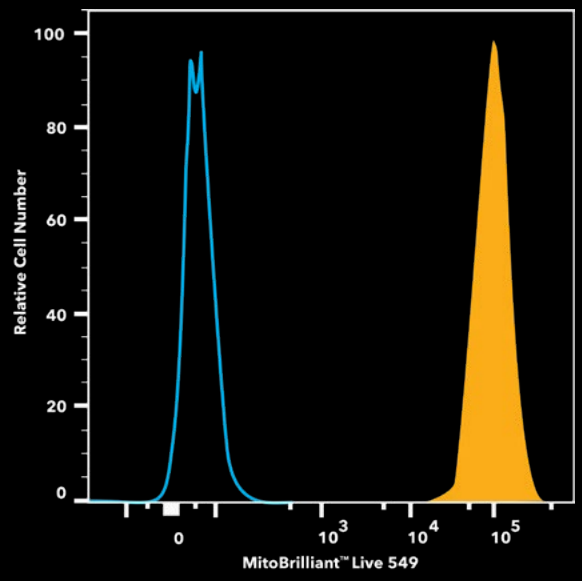


FIGURE 22. Flow cytometry histogram of cell populations following MitoBrilliant™ Live 549 staining. K562 cells were stained with MitoBrilliant™ Live 549 for 30 minutes at 37°C. Cells were then washed, and data were acquired on BD Fortessa Flow Cytometer using 561 nm laser for excitation and 586/15 nm emission filter. Data show overlay of stained (orange) and unstained cells (blue).

Staining Live Cells

Applicable to

- MitoBrilliant™ 646 (Cat. No. 7700)
- MitoBrilliant™ Live 646 (Cat. No. 7417)
- MitoBrilliant™ Live 549 (Cat. No. 7693)

Prepare a 1 mM MitoBrilliant™ stock solution with DMSO (see the table below for recommended reconstitution). We recommend that stock solutions, once prepared, are stored aliquoted in tightly sealed vials at -20°C or below and used within 1 month.

- Dilute the DMSO stock solution using a warm (37°C) buffer or growth medium and apply to live cells at a final working concentration between 50-200 nM. We recommend optimizing the final concentration used for individual experiments. Aqueous working solutions should be prepared and used on the same day.
- Incubate for 30-60 minutes at 37°C prior to imaging (longer incubation times may give brighter staining). A washing step prior to imaging is not required but it is recommended: rinse the cells with 1x PBS and apply fresh media prior to imaging.

Staining cells in suspensions for flow cytometry: After gentle centrifugation, carefully resuspend the cell pellet in a prewarmed (37°C) buffer or growth medium containing 50-200 nM MitoBrilliant™ at a cell concentration of 1 x 10^6 per mL. After 30-60 minutes incubation at 37°C in the dark, re-pellet the cells by gentle centrifugation, then resuspend cells in fresh pre-warmed medium ready for flow cytometry.

Staining Fixed Cells

Applicable to MitoBrilliant™ 646 (Cat. No. 7700), which can be applied at either pre-fixation **(FIGURE 5)** or post-fixation **(FIGURE 6)** stages of sample preparation.

- Dilute the 1 mM DMSO stock solution to a final working concentration between 50 to 200 nM. We recommend optimizing the final concentration used for individual experiments. Aqueous working solutions should be prepared and used on the same day.
- Appropriate fixation methods are critical for preserving cellular structure and achieving optimal staining. For HeLa cells, we recommend fixing with freshly prepared pre-warmed buffer or growth medium containing 4% paraformaldehyde at 37°C for 10 to 20 minutes. Optimization might be required for different model systems. After fixation, rinse the cells several times in PBS.
- Permeabilization step (optional). Incubate fixed cells for 10 minutes in PBS containing 0.05% Triton® X-100. Staining cells before permeabilization is recommended for getting the best results (see **FIGURE 10** and **FIGURE 12**).

Product Name	Molecular Weight	Quantity Per Vial	Recommended Reconstitution
MitoBrilliant™ 646 Cat. No. 7700	493.6	50 µg (101 nmoles)	Add 101 µL DMSO for a 1 mM stock solution
MitoBrilliant™ Live 646 Cat. No. 7417	445.1	50 µg (112 nmoles)	Add 112 µL DMSO for a 1 mM stock solution
MitoBrilliant™ Live 549 Cat. No. 7693	417.0	50 µg (120 nmoles)	Add 120 µL DMSO for a 1 mM stock solution

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