Visualize microtubule dynamics in live cells
With the highly photostable Tubulin Tracker Deep Red reagent.

Fluorescence imaging of live cells allows researchers to visualize dynamic cell processes such as those associated with the microtubule component of the cytoskeleton. Generally, microtubules are imaged in live cells after transfection or transduction with genes encoding fusion proteins of a fluorescent protein (e.g., GFP) and the tubulin monomer. There are a number of challenges inherent to this approach. Transfection or transduction can induce cellular stress and cytotoxicity, and expression of the transgene via plasmid or viral delivery requires significant time before enough protein is synthesized to generate a fluorescent signal suitable for live-cell imaging. Additionally, fluorescent proteins are largely limited to green and red detection channels, which can limit the ability to multiplex with commonly used markers. Most notably, transgene expression tends to produce vastly heterogeneous protein levels, yielding uneven labeling across the sample and making quantitative characterization of the microtubule cytoskeleton intractable by fluorescence imaging.

Tubulin Tracker Deep Red
To address these challenges, we have developed Invitrogen™ Tubulin Tracker™ Deep Red, a novel membrane-permeant fluorescent molecule that selectively stains microtubules in live cells and exhibits deep-red emission. Eliminating the need for genetic modification, Tubulin Tracker Deep Red provides uniform labeling of the microtubule cytoskeleton in live cells with a rapid and simple staining protocol.

The far-red spectral properties of Tubulin Tracker Deep Red—with excitation/emission maxima of 652/669 nm—result in minimal photo-toxicity to the cells, allow the signal to be detected using common Cy®5/deep red filter sets, and make it amenable to multiplexing with blue, green, orange, red, and near-IR fluorophores. Moreover, Tubulin Tracker Deep Red shows superior photostability, as we show using HeLa cells labeled with this deep-red fluorescent stain and then imaged continuously for 60 seconds on the Invitrogen™ EVOS™ FL Auto 2 Imaging System (Figure 1A).

Our data demonstrate that Tubulin Tracker Deep Red can be used to label microtubules in live cells in as little as 30 minutes, with no measurable cytotoxicity or disruption of normal cellular function during a 72-hour incubation. Furthermore, Tubulin Tracker Deep Red is compatible with a no-wash protocol. With its advanced photostability and the inherent low phototoxicity of far-red–emitting labels, Tubulin
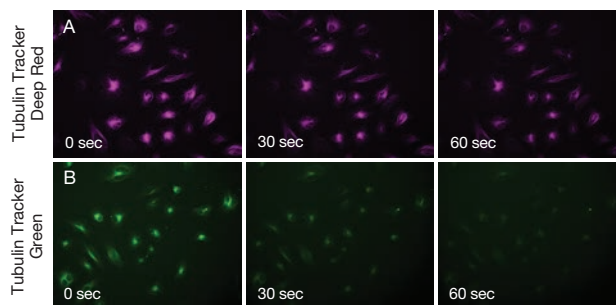

Figure 1. Superior photostability of Tubulin Tracker Deep Red in live cells. HeLa cells were labeled with Invitrogen™ Tubulin Tracker™ Deep Red (Cat. No. T34077) and Invitrogen™ Tubulin Tracker™ Green (Cat. No. T34078) according to the recommended protocol and then imaged continuously for 60 sec on the Invitrogen™ EVOS™ FL Auto 2 Imaging System using a 20x/0.75NA objective. Whereas Tubulin Tracker Green was largely photobleached after 30 sec, Tubulin Tracker Deep Red retained >85% of its initial signal, even after a full 60 sec of continuous illumination.

Tracker Deep Red is ideal for long-term time-lapse imaging of live cells, as well as for rapid tubulin counterstaining to facilitate analysis in endpoint assays.

Invitrogen™ Tubulin Tracker™ Green has long been used to visualize microtubules in live cells. However, its short-wavelength excitation produces rapid photobleaching of the signal (Figure 1B) and increases phototoxicity to the cells during extended time-lapse imaging. Additionally, Tubulin Tracker Green cannot be multiplexed with commonly used GFP–tubulin monomers and related fluorescent protein fusions. Given its more photostable deep-red fluorescence, Tubulin Tracker Deep Red resolves many of these limitations without introducing any measurable cytotoxic side effects.

Tubulin labeling in live cells with minimal cytotoxicity
One of the primary concerns with using fluorescent taxanes to label microtubules is the possibility of cytotoxicity upon continuous incubation. The microtubule cytoskeleton is an essential regulator of the cell cycle, and taxane-mediated inhibition of microtubule depolymerization has been used as a common chemotherapeutic strategy in oncology for decades.

To assess the cytotoxicity of Tubulin Tracker Deep Red and Tubulin Tracker Green compared with that of their parent taxane molecules docetaxel and paclitaxel, respectively, HCASM (human cardiac arterial smooth muscle) cells and HeLa cells were incubated with 1 µM of

either the unconjugated taxanes or Tubulin Tracker reagents for up to 24 hours, and cell viability was assessed using Invitrogen™ PrestoBlue™ Cell Viability Reagent and the Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader. Our results indicate that after 2 hours of continuous incubation, cell viability was not significantly affected by any of the treatments (Figure 2B). After 24 hours, however, cells incubated with the unlabeled taxanes showed decreased viability whereas cells incubated with the Tubulin Tracker reagents did not show a significant change in viability (Figures 2A and 2B). These results were confirmed by a qualitative assessment of cell number and morphology. Additionally, Tubulin Tracker Deep Red remained in cells for the entire duration of treatment, thus demonstrating exceptional retention of the probe over extended periods.

Ideal for extended time-lapse imaging

Tubulin Tracker Deep Red can also be used to track microtubules over the course of multiple days for extended time-lapse imaging. To demonstrate this functionality, we labeled HeLa cells with 100 nM Tubulin Tracker Deep Red and placed the samples in a live-cell chamber on a laser-scanning confocal microscope for 72 hours. The dye was not removed from the medium, and images were captured at 20-minute intervals; low levels of extracellular background signal were subtracted using the offset function.

This experimental setup allowed us to track cellular movement, visualize microtubule dynamics, and observe cell proliferating throughout the entire 72-hour acquisition period; we also detected the successful completion of mitotic spindle assembly, disassembly, and cytokinesis (Figure 3).
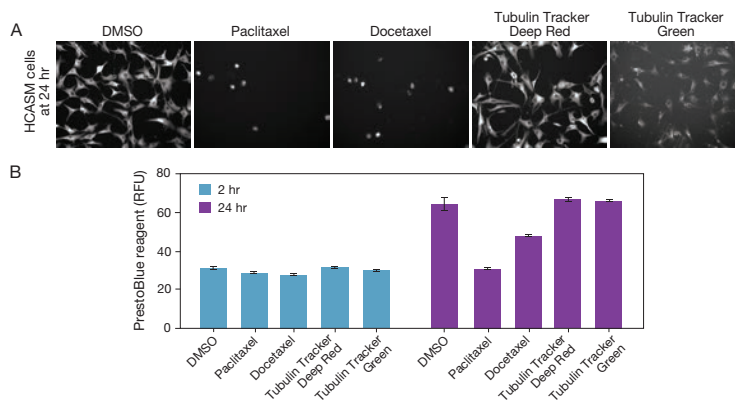

Figure 2. Minimal cytotoxicity and superior retention of Tubulin Tracker Deep Red in HCASM cells. HCASM (human cardiac arterial smooth muscle) cells were incubated with vehicle (DMSO), 1 μM unconjugated taxanes (paclitaxel or docetaxel), or 1 μM Invitrogen™ Tubulin Tracker™ reagent (Tubulin Tracker Deep Red, Cat. No. T34077; or Tubulin Tracker Green, Cat. No. T34078) for 24 hr. **(A)** After 24 hr, cells were imaged on the Invitrogen™ EVOS™ FL Auto 2 Imaging System using a 20x/0.75NA objective. **(B)** Cell viability was assessed at 2 hr and 24 hr using Invitrogen™ PrestoBlue™ Cell Viability Reagent (Cat. No. A13261) on the Thermo Scientific™ Varioskan™ LUX Multimode Microplate Reader.
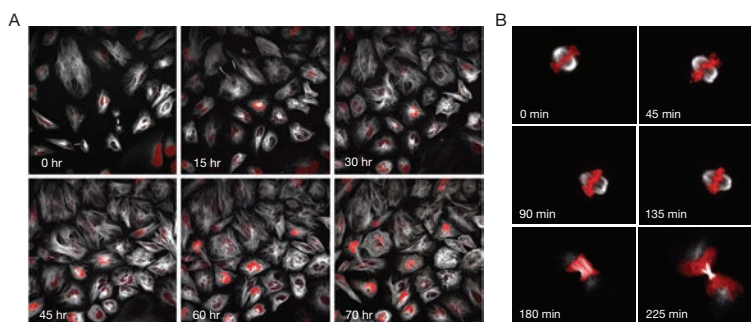

Figure 3. Extended time-lapse imaging of cells labeled with Tubulin Tracker Deep Red. Live HeLa cells were labeled with Invitrogen™ CellLight™ Histone 2B–RFP (red, Cat. No. C10595) for 24 hr and 100 nM Invitrogen™ Tubulin Tracker™ Deep Red (white, Cat. No. T34077) for 1 hr before imaging every 20 min over 72 hr on a Zeiss™ LSM 710 confocal microscope using a 40x/1.2NA water-immersion objective. **(A)** Zoomed-out view of a field of cells showing cell movement, microtubule dynamics, and cell proliferation. **(B)** Zoomed-in view of a cell undergoing mitosis.

Tubulin labeling made easy

Tubulin Tracker Deep Red can be used to visualize microtubules in a wide variety of live cell types, and its far-red spectral properties and superior photostability enable multiplexing with many common dyes and fluorescent proteins. Learn more at **thermofisher.com/tubulintracker**. ∎

Product	Quantity	Cat. No.
Tubulin Tracker™ Deep Red	60 slides	T34077
	300 slides	T34076
Tubulin Tracker™ Green (Oregon Green™ 488 Taxol™, Bis-Acetate), for live-cell imaging	60 slides	T34078
	300 slides	T34075
Tubulin Tracker™ Variety Pack	2 x 60 slides	T34079
PrestoBlue™ Cell Viability Reagent	25 mL	A13261
	100 mL	A13262
EVOS™ FL Auto 2 Imaging System	1 system	AMAFD2000
Varioskan™ LUX Multimode Microplate Reader	1 system	VLBLATD0