Visualizing microtubule dynamics in live cells using a novel, deep red cytoskeletal label

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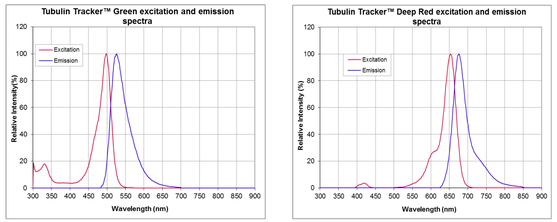
ABSTRACT

The microtubule cytoskeleton is of interest to researchers studying numerous cellular functions with live cell fluorescence microscopy. Currently, microtubules are visualized in live cells by way of fluorescent proteins (FPs) fused to tubulin monomers, which are subsequently transfected and overexpressed thus providing a number of challenges inherent to this approach. For instance, transfection can induce cellular stress and cytotoxicity, while transgene expression from a plasmid or viral delivery requires significant time before sufficient protein is expressed to generate signal that is suitable for live cell imaging. Additionally, fluorescent proteins are largely limited to the green and red channels, thus preventing the use of these commonly used channels for other labels of interest. Most notably, overexpression results in vastly heterogeneous expression levels, yielding uneven labeling across the sample and making quantitative characterization of the microtubule cytoskeleton intractable by fluorescence imaging.

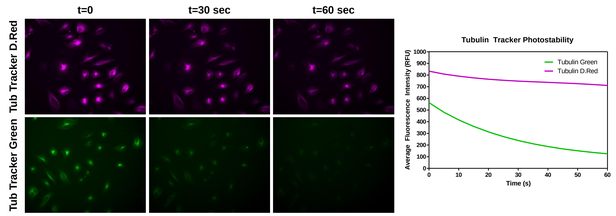
To answer these and other needs, here we describe Tubulin Tracker™ Deep Red; a novel, membrane permeable fluorescent molecule with deep red emission and specificity for microtubules in live cells. Tubulin Tracker Deep Red mitigates many of the challenges associated with FP-based microtubule labeling, bypassing the need for transfection and thus providing uniform labeling of microtubule cytoskeleton in live cells with a rapid workflow. Our data demonstrates that Tubulin Tracker Deep Red can be used to label microtubules in live cells in as little as 30 minutes, with no measurable cytotoxicity up to 24 hour incubation. We also demonstrate the ability to use the label in a 'no wash' protocol, enabling quantitative analysis of neurite outgrowth in a primary neuronal culture model as well as assessment of pharmacological effects of microtubule destabilizing drugs. Combined with advanced photobleach protection properties, plus the enhanced depth permeation in 3D spheroid models and low phototoxicity of deep red emitting labels, Tubulin Tracker Deep Red is suitable for long term time lapse imaging as well as rapid labeling of tubulin for endpoint assay analysis.

Existing Tubulin Tracker Green | **New - Tubulin Tracker Deep Red**



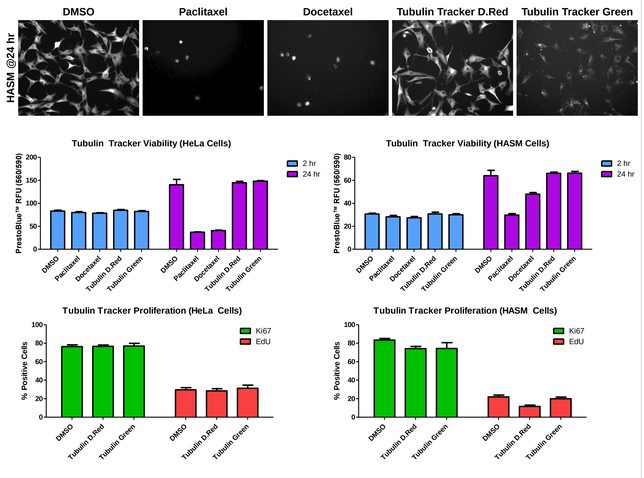
RESULTS

Figure 1. Tubulin Tracker Deep Red demonstrates superior photostability in live cells



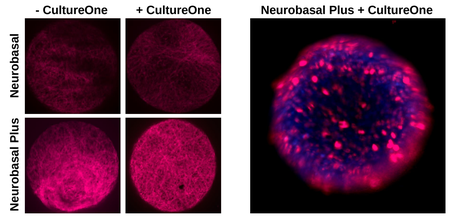
HeLa cells were labeled with each Tubulin Tracker as recommended, and imaged continuously for 60 seconds on the EVOS FL Auto 2 using a 20x/0.75 NA Olympus SApo objective.

Figure 2. Tubulin Tracker Deep Red demonstrates minimal cytotoxicity and superior retention in live cells



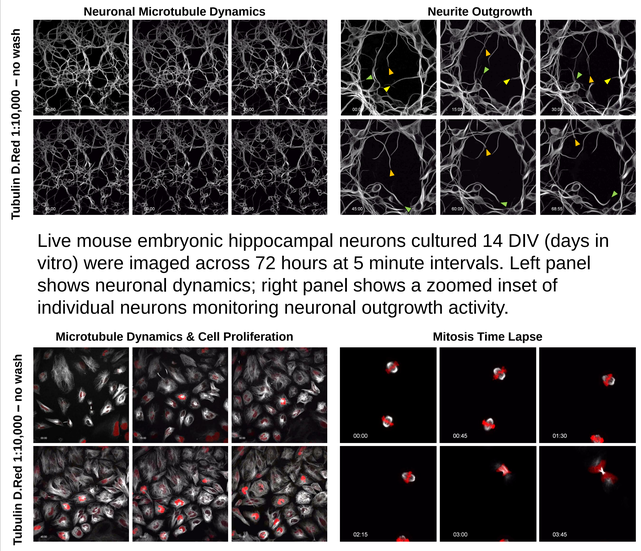
HeLa cells and HASM (Human Arterial Smooth Muscle) cells were incubated with 1 µM unconjugated taxanes and Tubulin Trackers for 24 hours, and cellular viability was assessed using PrestoBlue reagent on a Varioskan LUX multimode plate reader. Cellular proliferation was assessed using anti-Ki67 and Click-iT EdU staining on a CellInsight CX5 high content analysis platform.

Figure 3. Tubulin Tracker Deep Red enables quantitative analysis of 3D neurosphere maturation



Neural stem cell (NSC) derived neurospheres were cultured using Gibco Neurobasal and Neurobasal Plus with and without CultureOne Supplement and stained with Tubulin Tracker Deep Red to assess functional maturation of progenitors into mature neurons.

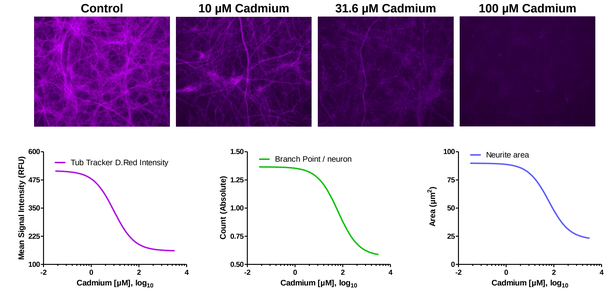
Figure 4. Tubulin Tracker Deep Red enables extended time lapse imaging in various cell types



Live mouse embryonic hippocampal neurons cultured 14 DIV (days in vitro) were imaged across 72 hours at 5 minute intervals. Left panel shows neuronal dynamics; right panel shows a zoomed inset of individual neurons monitoring neuronal outgrowth activity.

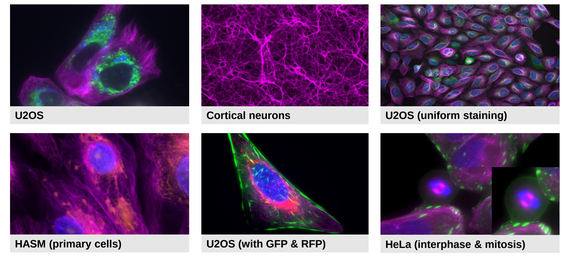
Live HeLa cells labeled with 100 nM Tubulin Tracker Deep Red and imaged across 72 hours at 20 minute intervals. Left panel shows a zoomed out view of cellular movement, microtubule dynamics, and proliferation; right panel shows a zoomed inset of two mitotic cells.

Figure 5. Tubulin Tracker Deep Red enables neurite outgrowth pharmacological response assays



Live mouse embryonic hippocampal neurons cultured 14 DIV (days in vitro) were treated in half-log intervals with Cadmium Chloride for 18 hours and stained using Tubulin Tracker Deep Red to assess neurite morphology on the CellInsight CX5 High Content Analysis platform.

CONCLUSIONS



Tubulin Tracker Deep Red can be used to visualize microtubules in a wide variety of live cell types, including 3D spheroid models, and its far-red spectra properties enable it to be multiplexed with many common fluorophores and fluorescent proteins. When compared to the existing live cell Tubulin labeling methods, Tubulin Tracker Deep Red provides uniform staining, superior photobleach resistance, and no measurable cytotoxic effects even after 24 hour incubation. This unparalleled performance enables the study of microtubule dynamics by performing extended time lapse imaging, as well as pharmacological investigations of microtubule networks.

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