Utilizing Near Infra-Red to Eliminate Cross Channel Contaminate for Cell Painting Assays

Kevin Chambers, Wenjun Zhou, Daniel Beacham, Edward Parkin, and Stephen Full
Thermo Fisher Scientific, Protein & Cell Analysis, 29851 Willow Creek Rd, Eugene, OR 97402

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Abstract and introduction

Since the release of Carpenter et al's 2016 paper in the journal Nature Protocols titled "Cell Painting, a high-content image-based assay for morphological profiling using multiplexed fluorescent dyes", the cell painting assay has been an important tool for academia and industry to interrogate phenotypic changes in cell culture models resulting from factors such as drug treatment and environmental changes ([1]). The use of this assay has grown significantly since it was first published, however the spectral overlap of some of the dyes used in this assay have limited the ability to accurately quantify the targets of some of the dyes used in this assay on many high-content analysis platforms. Specifically, the overlap of Syto 14™ and Concanavalin A, Alexa Fluor™ 488 disrupt the quantification of RNA, nucleoli, RNA, and ER in the green channel and the overlap of Wheat Germ Agglutin, Alexa Fluor™ 555 and Phalloidin, Alexa Fluor™ 568 for plasma membrane, golgi, and actin in the orange channel. Here within, we show that advances in near infra-red reagents and imaging platforms, paired with platform specific reagent optimization can eliminate or significantly reduce the spectral interference of these targets.

These reagents are research use only and not for diagnostic purposes.

Materials and methods

Cell Culture

U-2 OS were used in these experiments and cultured in standard conditions.

Fluorescence imaging

High Content Analysis (HCA) imaging was carried out on the Thermo Fisher™ CellInsight™ CX7 LZR Pro High Content Screening platform.

Dye preparation and loading

Invitrogen™ Image-iT™ Cell Painting Kit was used for the original cell painting assay and all of the dyes used in the original cell painting assay were prepared and loaded in accordance with the manufacturer's recommended protocol with the exception of MitoTracker™ Deep Red. MitoTracker Deep Red was used at 100ng/mL concentration. Concanavalin A, Alexa Fluor™ 750 Plus was labeled at 100µg/mL and Phalloidin, Alexa Fluor 594 at 50ng/mL for the optimized version of cell painting.

Spectral overlays

All spectral overlays were generated in Fluorescence SpectraViewer from Thermo Fisher Scientific

PROTOCOL

Cell Painting, a high-content image-based assay for morphological profiling using multiplexed fluorescent dyes

Mark-Anthony Bray[1], Shantanu Singh[1], Han Han[2], Chadwick T Davis[2], Blake Borgeson[2], Cathy Hartland[3], Maria Kost-Alimova[3], Sigrun M Gustafsdottir[3], Christopher C Gibson[2] & Anne E Carpenter[1]

[1]Imaging Platform, Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA. [2]Recursion Pharmaceuticals, Salt Lake City, Utah, USA. [3]Center for the Science of Therapeutics, Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA. Correspondence should be addressed to C.C.G. (chris.gibson@recursionpharma.com) or A.E.C. (anne@broadinstitute.org).

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Results

Original cell painting in the green channel with SYTO 14 and Concanavalin A, Alexa Fluor 488

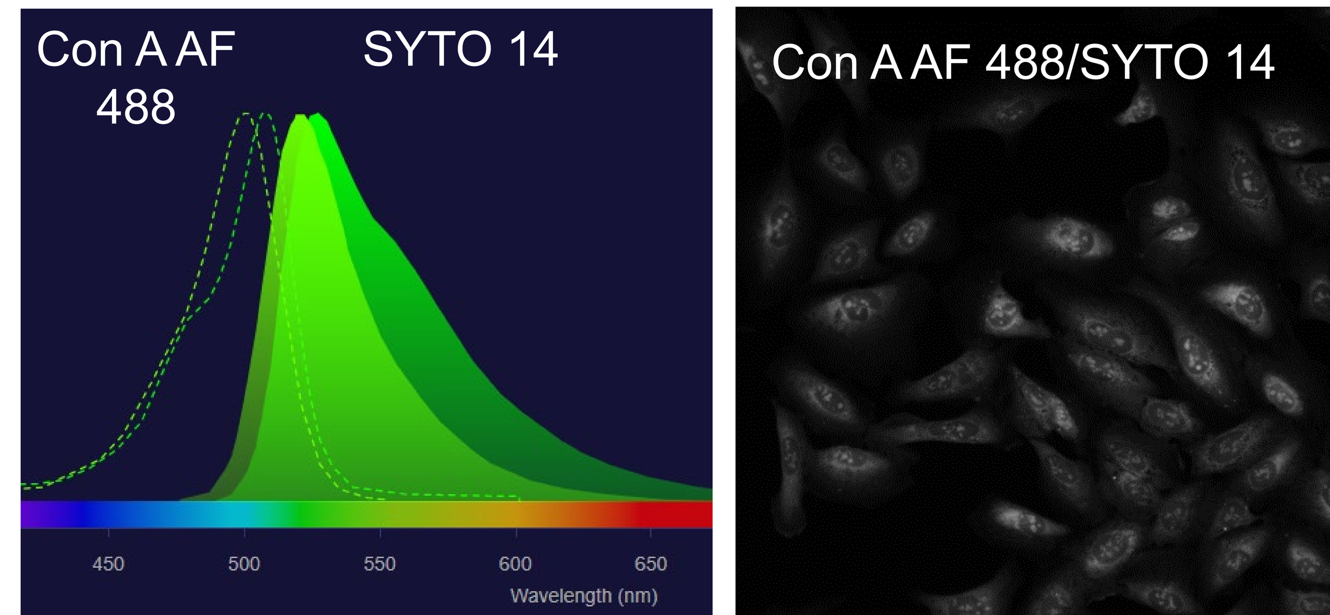
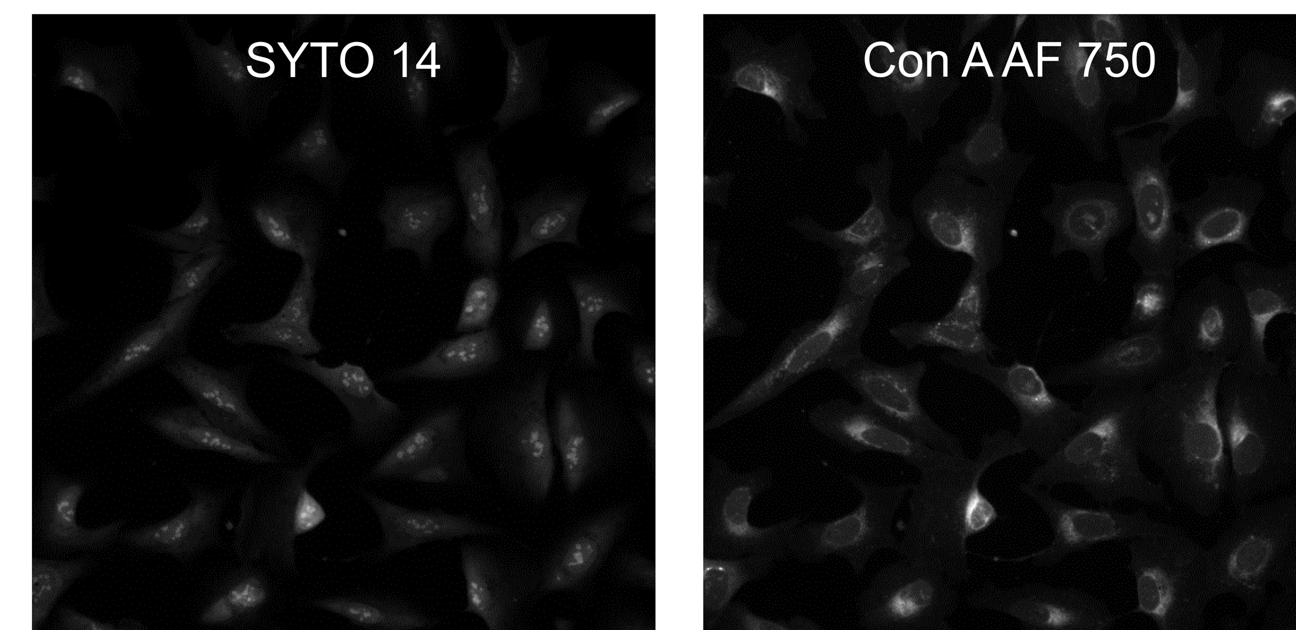


Figure 1. For the original Cell Painting reagent formulation, SYTO 14 for studying nucleoli and cytoplasmic RNA and Concanavalin A, Alexa Fluor 488 for studying endoplasmic reticulum, both have very similar excitation and emission spectral and are both imaged in the green channel.

Using NIR to eliminate the spectral overlap of Syto 14 and Concanavalin A



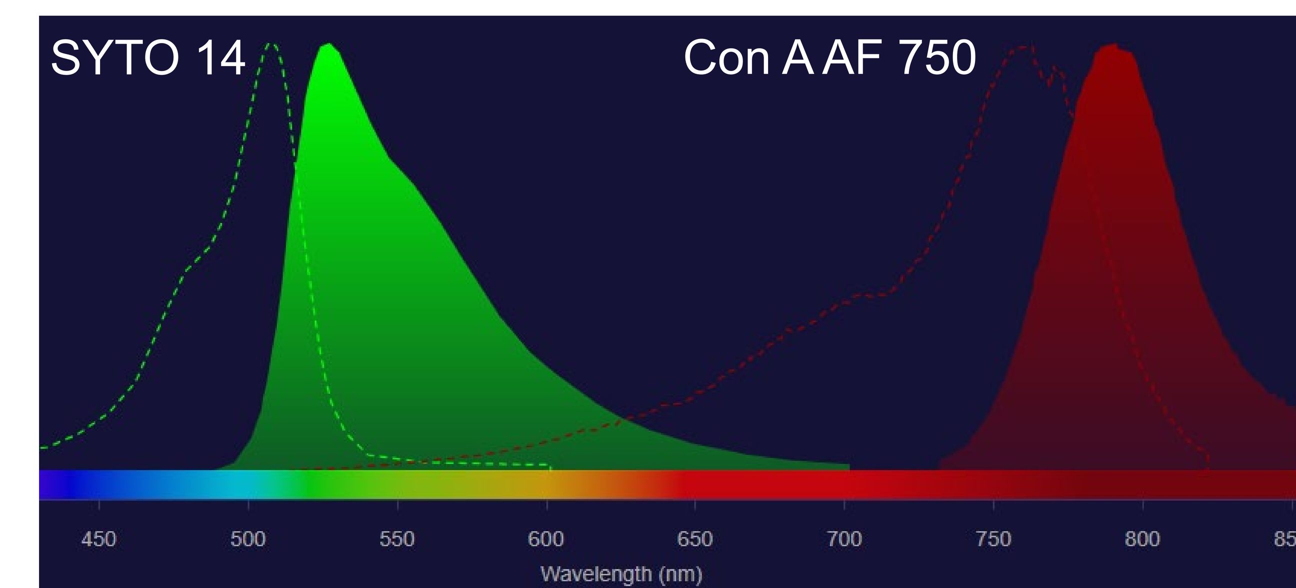


Figure 2. For instruments that have NIR capabilities, using Concanavalin A, Alexa Fluor 750 Plus instead of Alexa Fluor 488 removes the ER from the green channel, leaving clearer staining from SYTO 14 for cytoplasmic RNA. In the NIR channel, the ER staining from Concanavalin A is no longer being interfered with by cytoplasmic RNA staining from SYTO 14 (B).

Original cell painting in the orange channel with Wheat Germ Agglutin, Alexa Fluor 555 and Phalloidin, Alexa Fluor 488

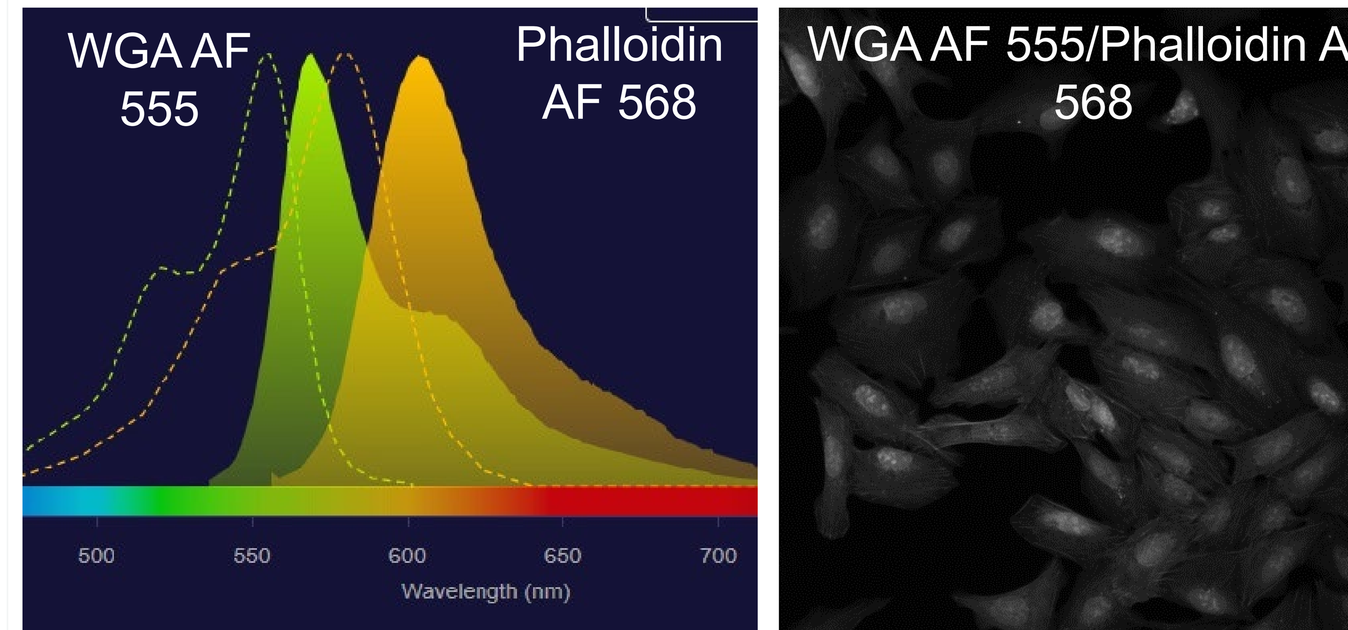


Figure 3. For the original Cell Painting reagent formulation, Wheat Germ Agglutin, Alexa Fluor 555 for plasma membrane and Phalloidin, Alexa Fluor 568 for actin, both have very similar excitation and emission spectral, and they are both visible in the orange channel.

Using Phalloidin, Alexa Fluor 594 removes the bleed through of the phalloidin into the orange channel and doesn't bleedthrough into the deep red channel

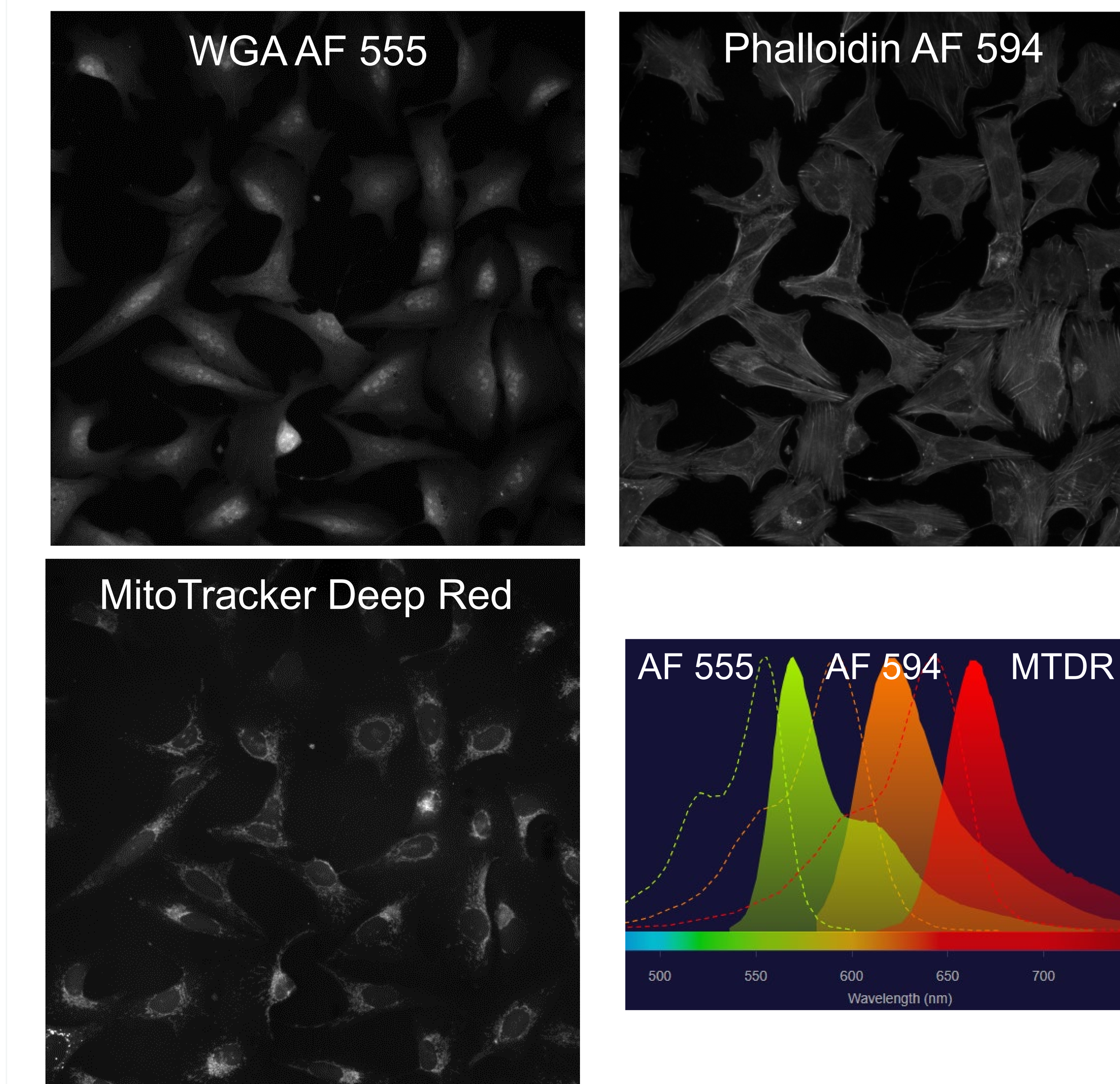


Figure 4. Switching from Phalloidin, Alexa Fluor 568 to Phalloidin, Alexa Fluor 594 removes the actin staining from the Wheat Germ Agglutin, Alexa Fluor 555 staining for plasma membrane/golgi in the orange channel while still showing good actin staining in the red channel. The Phalloidin, Alexa Fluor 594 doesn't interfere with the MitoTracker Deep Red labeling in the deep red channel.

Utilizing NIR channel with original cell painting formulation to research new targets of interest

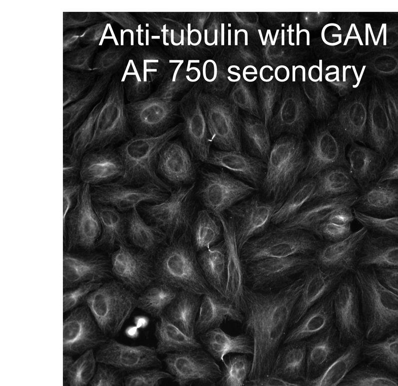


Figure 5. Using antibody labeling, such as anti-tubulin primary antibody with Alexa Fluor 750 Plus secondary antibody allows users to interrogate an additional target in addition to the data generated with the original cell painting formulation.

Complete comparison of original verse optimized cell painting

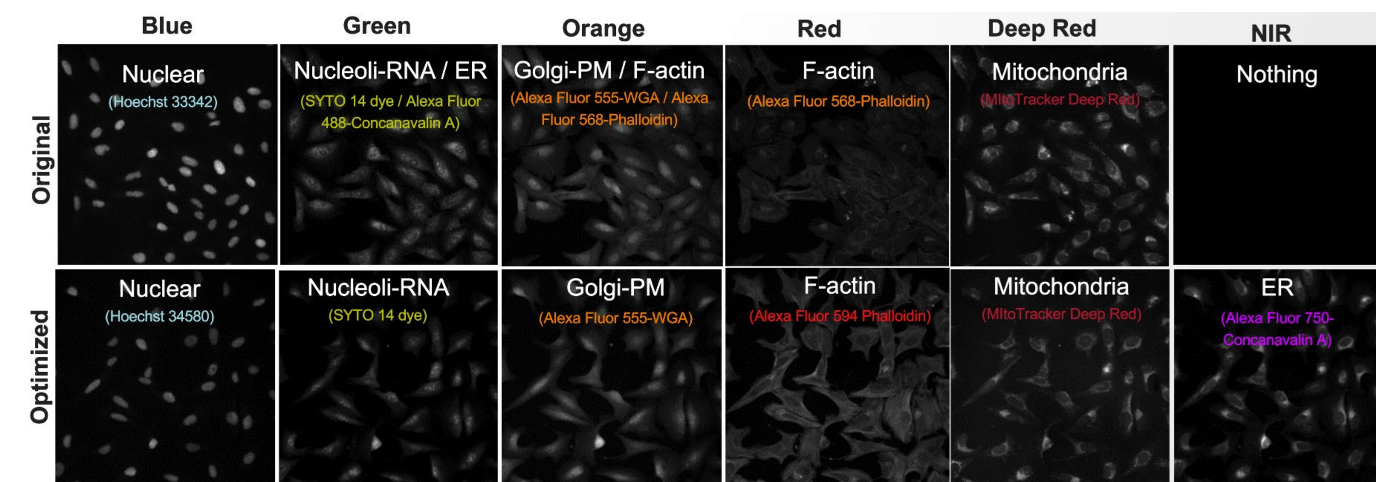


Figure 6. Comparison of all the dyes used in both the original cell painting, with 5 channels, verse the optimized dyes, with 6 channels, qualitatively shows increased spacial resolution for the cytoplasmic RNA, golgi, PM, and ER.

CellPaint Spheroids Maximum Intensity Projection from Z-Stack

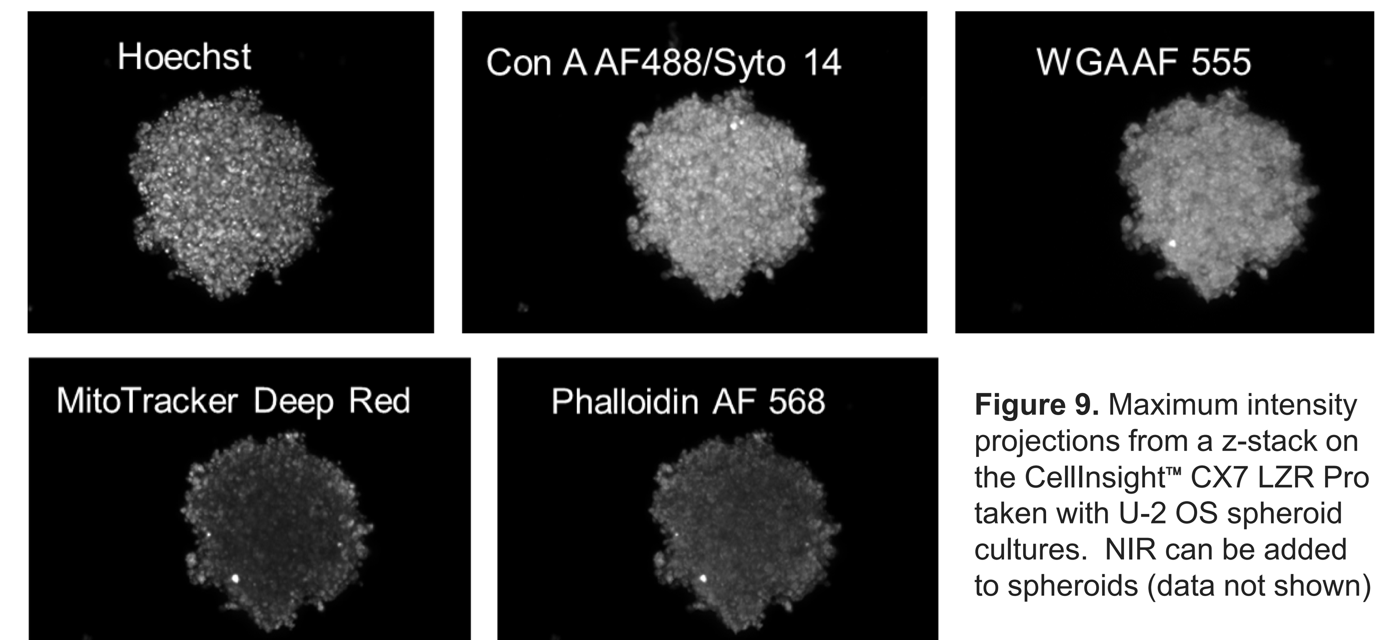


Figure 9. Maximum intensity projections from a z-stack on the CellInsight™ CX7 LZR Pro taken with U-2 OS spheroid cultures. NIR can be added to spheroids (data not shown)

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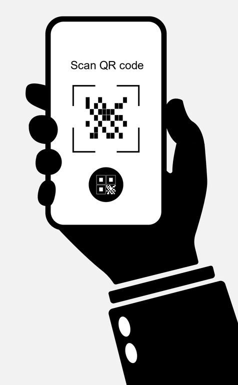

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