

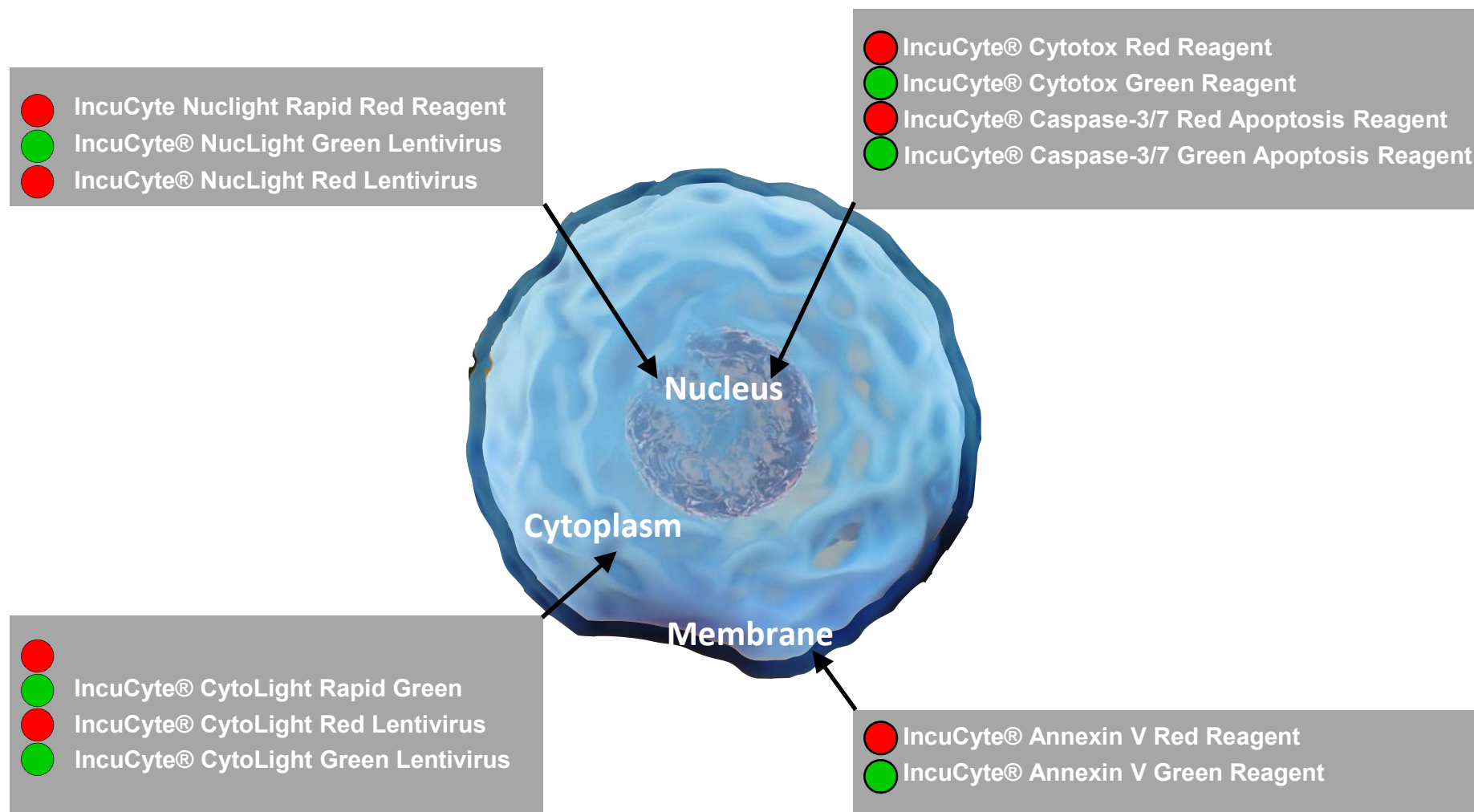
IncuCyte® S3 Training



Applications and Reagents Discussion










Cell Health and Viability






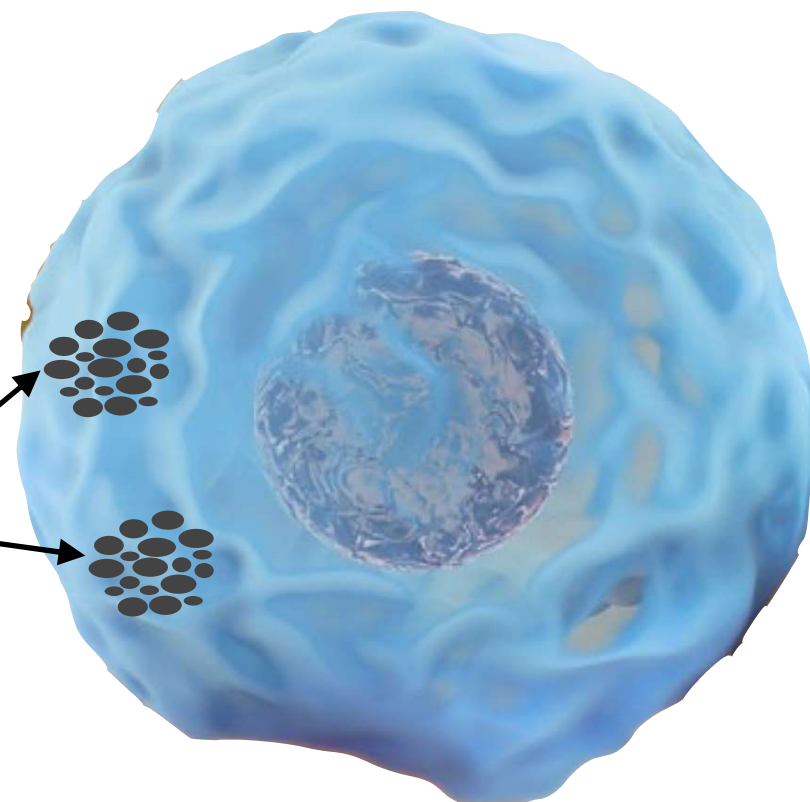
Live Cell Assays

Phagocytosis

-  IncuCyte® pHrodo® Red Cell Labeling Kit
-  IncuCyte® pHrodo® Red E. coli Bioparticles®
-  IncuCyte® pHrodo® Green E. coli Bioparticles®
-  IncuCyte® pHrodo® Red Zymosan Bioparticles®
-  IncuCyte® pHrodo® Green Zymosan Bioparticles®
-  IncuCyte® pHrodo® Red S. aureus Bioparticles®
-  IncuCyte® pHrodo® Green S. aureus Bioparticles®

Antibody Internalization

-  IncuCyte® Human FabFluor Red Antibody Labeling Reagent
-  IncuCyte® Mouse IgG1 FabFluor Red Antibody Labeling Reagent
-  IncuCyte® Rat FabFluor Red Antibody Labeling Reagent



IncuCyte Overview and Best Practices



Overview of IncuCyte

- **Image Channels**
 - HD Phase (brightfield with spheroid scan)
 - Green
 - Ex: 440-480nm
 - Em: 504-544nm
 - Red
 - Ex: 565-605nm
 - Em: 625-705nm
- **Objectives**
 - 4x, 10x, 20x on automated turret
- **Compatible with most tissue culture vessels**
- **Software**
 - Remote Access
 - Guided interface
 - Unlimited user license



Live Cell Experimental Optimization

Condition	Things to consider
Cell Seeding	<ul style="list-style-type: none">• Density may need to be optimized (always seed cells at least 10% confluence)• Allow cells to settle at ambient temperature for 15 minutes before placing into the incubator for optimal cell distribution• Non-adherent may require a coating for optimal cell distribution

IncuCyte functions Inside your Incubator

- IncuCyte is NOT an incubator and will not regulate the environment
- **Operating Environmental Conditions**
 - 0°C to 42°C
 - 5% to 95% Relative Humidity Non-Condensing
- **Best Practices**
 - Set Incubator Temp 0.5°C below desired temp (e.g. if 37°C is desired, then set incubator to 36.5°C)
 - Check water pan and humidity settings 2-3 times per week
 - Always wear gloves and practice sterile technique
 - Read the manufacturer's recommendations for routine incubator maintenance

Automated Image Acquisition and Auto-Focus

Manual Focusing Analogy

- Image based auto-focus
- Performs a series of wide (coarse and fine) sweeps to assess several image planes
- Finds plane of highest contrast and collects phase, green, and red image at the same plane
- Auto-focuses on every well, every plate, and every time point



The image consists of three panels, each with a title above it. The first panel, titled 'Fingerprints', shows a close-up of a textured surface with distinct, parallel ridges and valleys. The second panel, titled 'Condensation', shows a surface covered in numerous small, irregular droplets of liquid. The third panel, titled 'The Bubble Buster', shows four white plastic bottles with colored caps (red, yellow, blue, green) and curved spouts, arranged in a row.

Rules to remember

- Hold the pipette vertically, when aspirating
- Immerse the tip only 2-3 mm
- Pre-rinse 3-5 times before pipetting (forward)
- Pause consistently after aspiration
- Dispense at an 30 – 45° angle
- Pipette against the inside wall of the receiving vessel
- Operate with smooth and consistent thumb action



Ultrapure Water for Multiple Applications

Technique is Key for Accurate Results

arium® pro



arium® mini



- Adapt an ultrapure water system for different applications
- Ensure water for cell culture applications is free of impurities

Cell Culture should be Free of Contaminants

Technique is Key for Accurate Results

Microsart® AMP Mycoplasma Kit



MD8 Air Samplers



MYCAP™



- Check cell culture routinely for mycoplasma contamination
- Ensure cell culture environment has the lowest concentration of airborne contaminants
- Aseptically remove samples and close containers without contaminating contents

Breaking Point to go into Lab

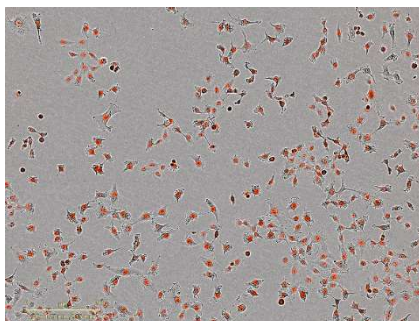
Live Cell Analysis Software Training

4 Steps to Image Analysis

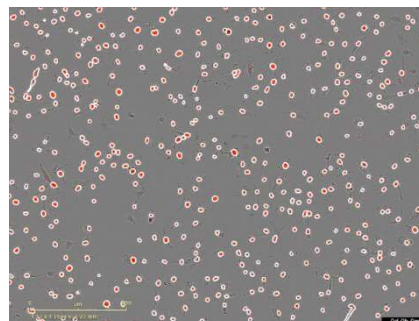
Acquire Images

Scan Properties	
Vessel Type	24-well Corning Falcon
Scan Type	Standard
Image Channels	<input checked="" type="checkbox"/> Phase <input checked="" type="checkbox"/> Green
	Acquisition Time (ms) 300
Objective	20x
Scan Duration	2 min (estimated)
Number of Daily Scans	24

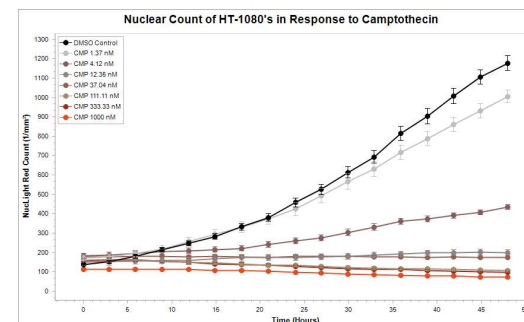
View Images



Process Images

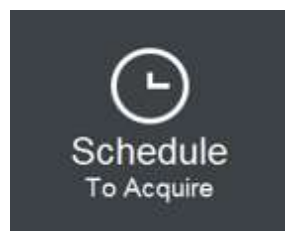


Analyze Images

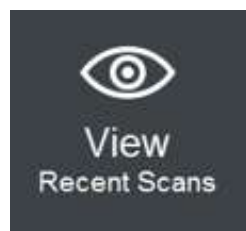


IncuCyte: A Guided Interface

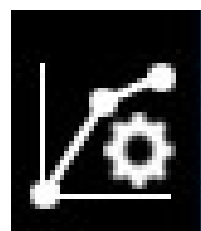
Schedule Scans



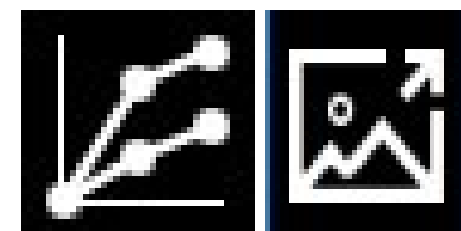
Vessel View



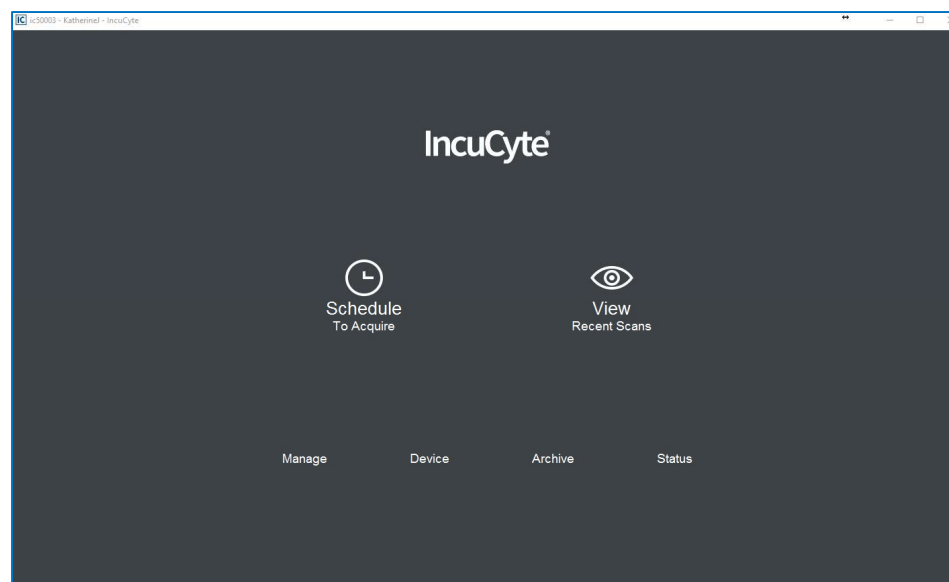
Analysis Definition



Graphing and Exporting



Logging into IncuCyte® S3



- IncuCyte: Instrument identifier assigned by your FSE at installation (e.g. IC50014 or an IP address)
- User Name and Passwords can be set up by the Admin
- Main Window opens up to guide you through the interface

User Accounts

Function	Admin	Standard	Limited	Guest
Add/Remove/Edit Scheduled Scans	Any	Any	No	No
View Vessels	Any	Any	Any	Any
Create Analysis Definitions	Any	Any	Any	No
View Analysis Definitions	Any	Any	Any	Any
Edit/Delete Analysis Definitions	Any	User Owned	User Owned	No
Edit Vessel Documentation	Any	Any	Any	No
Delete Vessels	Any	User Owned	No	No
Archive Vessels	Any	Any	Any	No

*Limits are placed on user accounts to prevent mistakes, while also providing a collaborative environment for colleagues/peers to view and interact with data. Additional scheduling scans functions and log files help restore or diagnose mistakes if they happen to occur, which is rare.

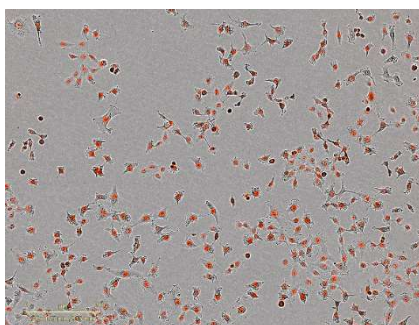
Scheduling Scans

Acquire Images

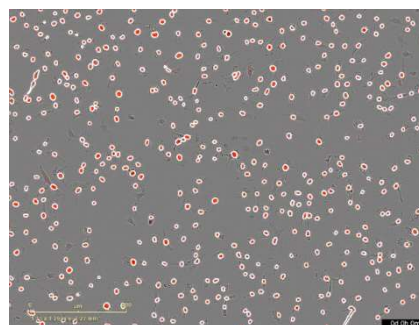
Scan Properties

Vessel Type 24-well Corning Falcon
 Scan Type Standard
 Image Channels ☒ Phase ☒ Green
 Acquisition Time (ms) 300
 Objective 20x
 Scan Duration 2 min (estimated)
 Number of Daily Scans 24

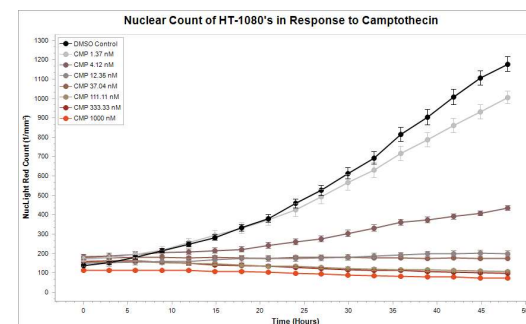
View Images



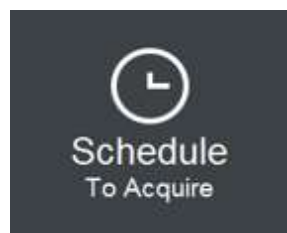
Process Images



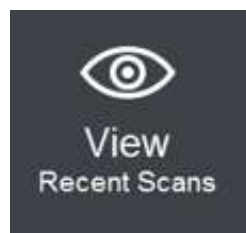
Analyze Images



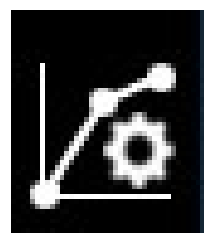
Schedule Scans



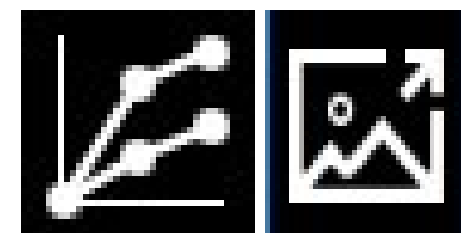
Vessel View



Analysis Definition



Graphing and Exporting



Schedule Scans – Guided Interface

ADD Vessel

Scan Type


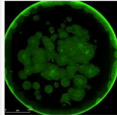
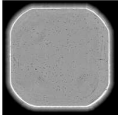
Based on your assay and application, specify the way in which the vessel should be scanned.

Standard

Image Lock

Scratch Wound

Whole Well



Whole Well

Acquire whole-well images of select multi-well and 35mm dishes to enable monitoring and analysis of rare events within a cell population.

Dilution Cloning

Chemotaxis

Spheroid

Back

Next

Pre-set scan types will automatically select objectives, plates, scan patterns downstream. Most experiments = standard

[illegible]

Select image channels and objective.
Default acquisition times work for most applications.

Add Vessel

Vessel Selection

Select the type of vessel to scan.

Enter text to search...

Manufacturer	Category	Wells	Area	Catalog Numbers	Vessel Name	Tray Name
Agilent Seahorse	Plate	24	N/A	101037-004	24-well Seahorse XF24 VPET	Microplates
Agilent Seahorse	Plate	24	N/A	100777-004	24-well Seahorse XF24 VP-PG	Microplates
Aurora	384	N/A		ABE1-11010A, ABE1-11100A, ABE1-11101A, ABE1-11010A, ABE1-11100A, ABE1-11101A,	384-well Aurora X-E8	Microplates
				ACD1-11010A, ACD1-11100A, ACD1-11101A, ABV1-11010A, ABV1-11100A, ABV1-11101A,		
				AWC1-11010A, AWC1-11100A, AWC1-11101A, ABE2-11010A, ABE2-11100A, ABE2-11101A,		
				P12-1104H		
				P96-1104H		
Aurora	Plate	384	N/A	3336, 3471, 3504, 3526	384-well Aurora X-E8 Ultra Low Base	Microplates
Celtek	Plate	12	N/A		12-well Celtek	Microplates
Celtek	Plate	96	N/A		96-well Celtek	Microplates
Corning	Plate	6	N/A		6-well Corning	Microplates
Corning	Plate	6	N/A	35304, 35322A, 35384B, 35393A, 354400, 354402, 35440A, 354411, 354417, 35462B, 354631, 354632, 354610, 354615, 354690, 354693, 354694, 354698, 354699, 354611, 354615, 354692	6-well Corning Falcon	Microplates
				35306, 35312, 35313		
Corning	Plate	12	N/A	35304S, 35322S, 354470, 354500, 354501, 354502, 354503, 354570	12-well Corning	Microplates
Corning	Plate	24	N/A	35371, 3474, 3524, 3526, 3527	24-well Corning Falcon	Microplates
Corning	Plate	24	N/A	35304T, 35322S, 35384T, 35393S, 354408, 354411, 354412, 354414, 354433, 354605, 354619, 354835, 354609, 354608, 354614	24-well Corning Falcon	Microplates
Corning	Plate	48	N/A	3338, 3548	48-well Corning	Microplates

Back

Next

Search for plate by catalog # to ensure proper focusing and alignment of images

Vessel Location

Specify the location in the drawer for the vessel.

Selected the cabinet where the vessel will be scanned.

The drawing to the left depicts the IncoCyle drawer as viewed from above.

If schedule currently contains another vessel or tray in the selected location, then a list of vessels that will be removed from the schedule will appear below.

☐ wish to remove the following vessel(s) from the schedule:

Vessel ID	Vessel Name	Owner	First Scan
192C...	dskd	AdminUser	184

Replace the middle tray.

Physically place plate in instrument before selecting cutout in software. Warning will appear if someone else's plate is occupying your location of choice.

Add Visual **Print Scan**

Scan Pattern

Specify the scan pattern to use for image acquisition.

Select Wells

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Images per Well: 2

Scan Duration: 10 min (estimated)

[View Sample Patterns](#)

[Back](#) [Save](#)

Do not scan empty wells. Consider the health of sensitive cells when deciding # of images per well and frequency of scanning.

Notebook

Provide information about the vessel

Name

Cell type

Plate Map + 📄 🗑️ ⌂

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

No Plate Map Specified

Notes

Include details about experiment including a platemap which can be mapped to data

Set the Scan Frequency

Set interval by right clicking in black timeline

IC Add Vessel

Scan Schedule

Review and modify the daily scan times here.

Scan Group

Scan

12 2a 4a 6a 8a 10a 12

12 2p 4p 6p 8p 10p 12

+ - ↕ ↻

Vessel ID	Name	Owner	Scan duration h:mm
8 Scans Per Day - 0h 2m per Scan			
experiment 1			02

Delete All Scans
 -5 Min
 -1 Min
 +1 Min
 +5 Min
 Add Scans at Intervals...

IC Set Timeline Scan Intervals

Starting At 12:00 PM

Add Scans Every 2 Hours

For a Total of 24 Hours

Schedule Repeats Daily

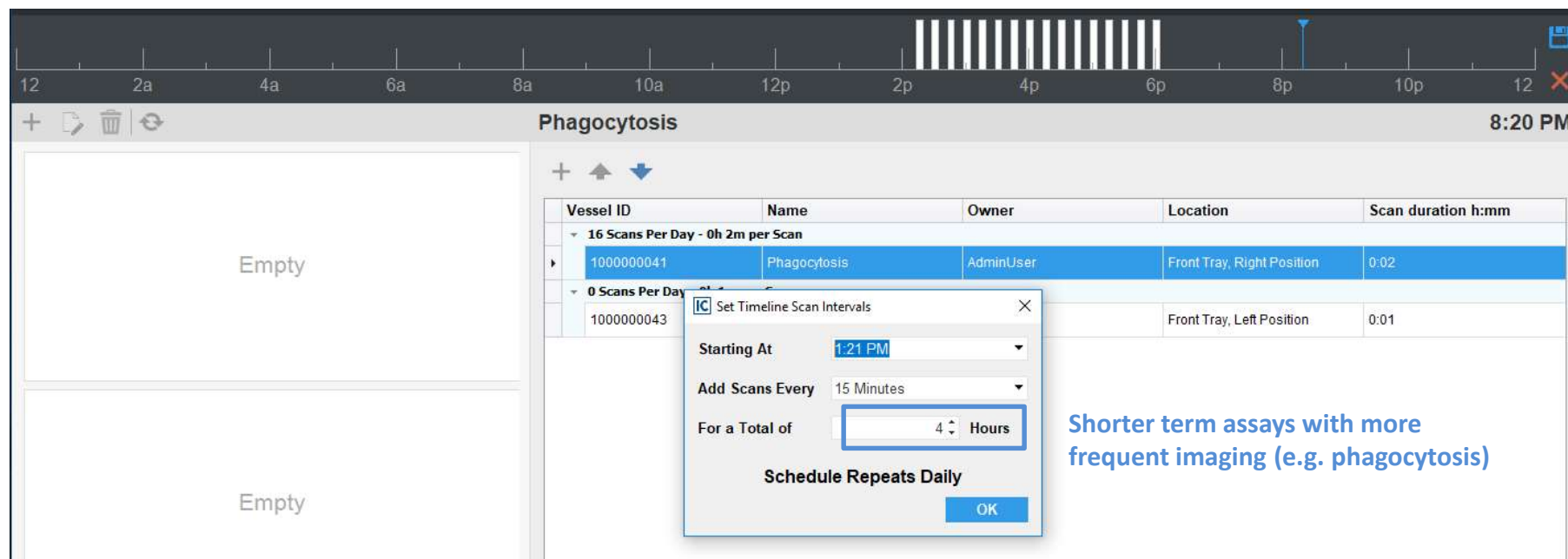
OK

Most Assays

Add and modify scan times for the current scan group in the timeline above.
Right click rows in the grid for vessel grouping and ordering options.

Back Next

Schedule Repeats Daily



Empty

Empty

Phagocytosis 8:20 PM

Vessel ID	Name	Owner	Location	Scan duration h:mm
16 Scans Per Day - 0h 2m per Scan				
1000000041	Phagocytosis	AdminUser	Front Tray, Right Position	0:02
0 Scans Per Day				
1000000043			Front Tray, Left Position	0:01

IC Set Timeline Scan Intervals

Starting At 1:21 PM

Add Scans Every 15 Minutes

For a Total of 4 Hours

Schedule Repeats Daily

OK

Shorter term assays with more frequent imaging (e.g. phagocytosis)


Adding Multiple Vessels

Additional Vessels Added

Set interval by right clicking in black area

Scan Schedule

Review and modify the daily scan times here.



Vessel ID	Name	Owner	Location	Scan duration h:mm
12 Scans Per Day - 0h 1m per Scan				
1000000000	Slide	AdminUser	Middle Tray, Center-right Pos...	0:01
4 Scans Per Day - 0h 22m per Scan				
	Whole Well		Front Tray, Left Position	0:22


Add and modify scan times for the current scan group in the timeline above.
Right click rows in the grid for vessel grouping and ordering options.

Back Next

Add to another person's scan group by using the arrows

Scan Schedule

Review and modify the daily scan times here.



Vessel ID	Name	Owner	Location	Scan duration h:mm
12 Scans Per Day - 0h 23m per Scan				
1000000000	Slide	AdminUser	Middle Tray, Center-right Pos...	0:01
	Whole Well		Front Tray, Left Position	0:22

Add and modify scan times for the current scan group in the timeline above.
Right click rows in the grid for vessel grouping and ordering options.

Back Next


2 Heat Threshold Warnings

Maintain Cell Health with Optimal Temperature Conditions

For every 1 minute of scanning, there should be at least 1 minute of non-scanning

Scan Schedule

Review and modify the daily scan times here.



+
↑
↓
↺

Vessel ID	Name	Owner	Location	Scan duration h:mm
48 Scans Per Day - 0h 23m per Scan				
1000000000	Slide	AdminUser	Middle Tray, Center-right Pos...	0:01
	Whole Well		Front Tray, Left Position	0:22

Warning - For optimal system performance, there should be no more than 12 hours of scanning in a 24 hour period.

Reason - You are currently exceeding this recommendation.

How to Fix


- Reduce the scanning frequency for one or more scheduled vessels, or
- Reduce the number of vessels, or
- Reduce the number of locations imaged in a given vessel.

Back
Next

Scan groups should not exceed 45 minutes

Scan Schedule

Review and modify the daily scan times here.



+
↑
↓
↺

Vessel ID	Name	Owner	Location	Scan duration h:mm
8 Scans Per Day - 1h 14m per Scan				
1000000003	experiment 1	AdminUser	Rear Tray, Right Position	0:02
1000000004	Whole well	AdminUser	Rear Tray, Left Position	0:36
	whole well 2	AdminUser	Middle Tray, Left Position	0:36

Warning - For optimal system performance, the total duration of any one scan group should not exceed 45 minutes.

Reason - You are currently exceeding this recommendation.

How to Fix - Split these scan groups into multiple scan groups.

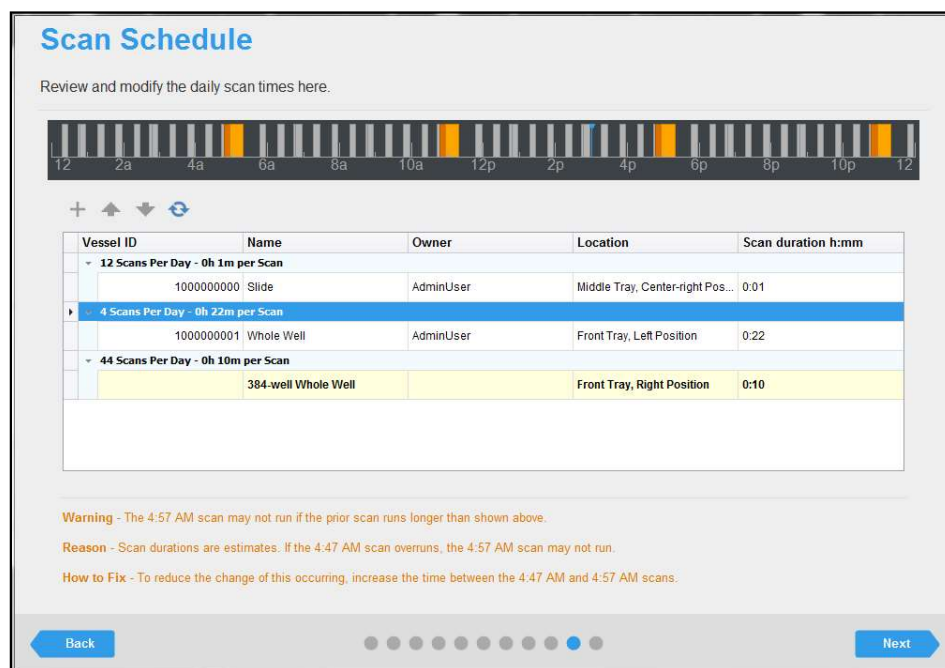
Back
Next

**Recommendations in Orange
Do NOT Ignore**

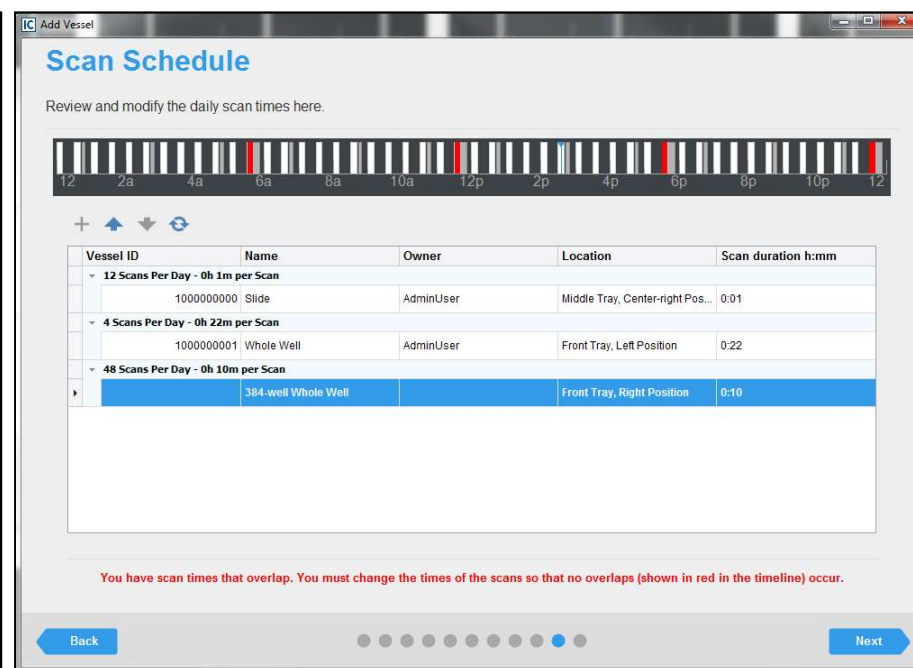
2 Scan Overlap/Duration Warnings

Avoid Conflicts in Scheduling

Scan Durations are Estimates



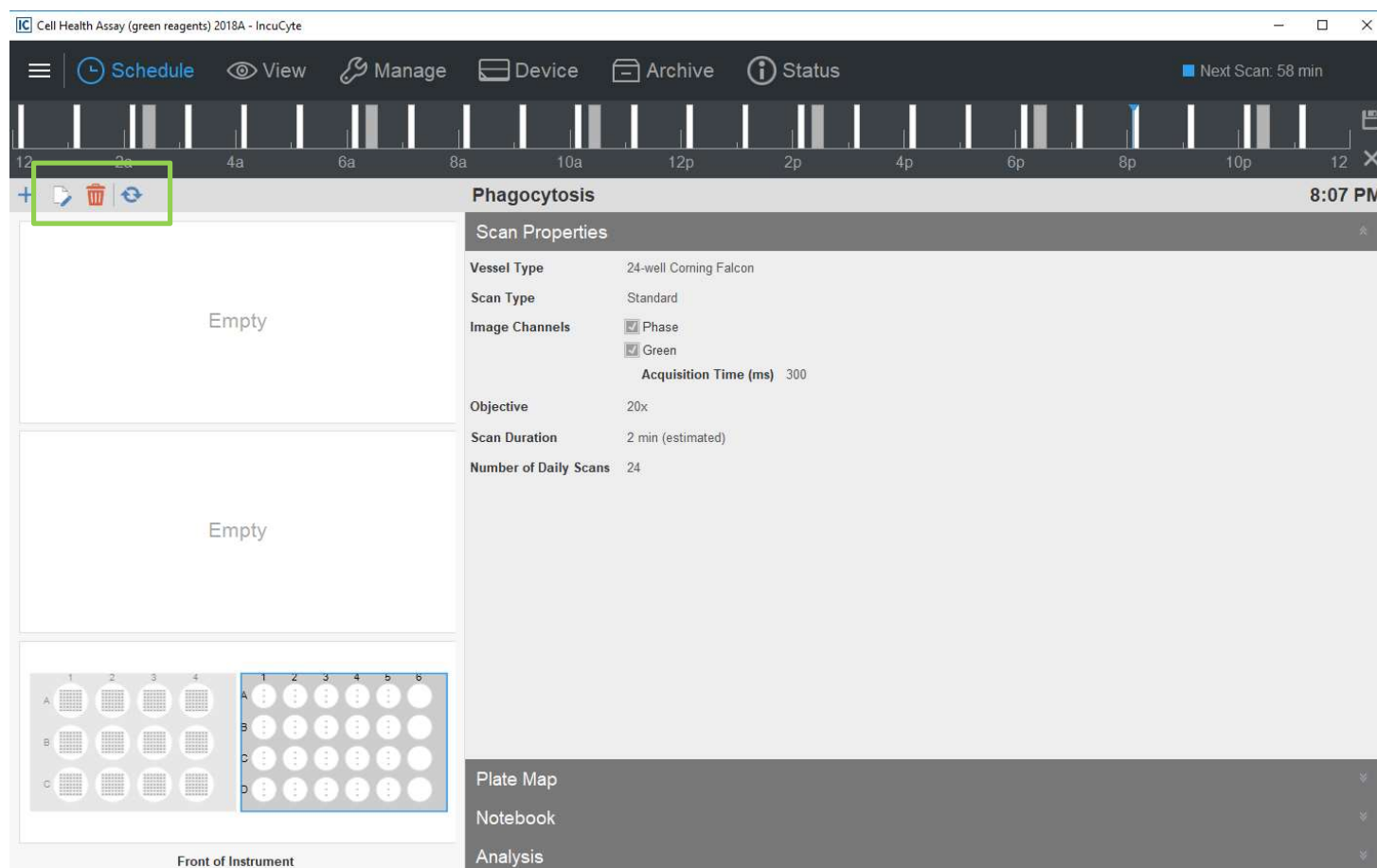
Scans Overlap
May occur with multiple scan intervals



Drag 'red/orange' bars to add more time in between scans or right click on individual scans to delete some time points

Will not let you ignore

Drawer Layout Summary



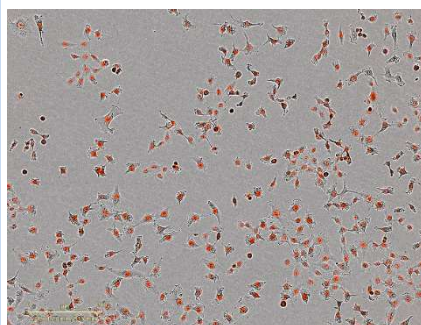
- Vessel selected in 'blue'
 - View Scan Properties/Notebook/Analysis
 - Schedule is white (e.g. every hour; other plate in drawer is gray at every 4h)
- Edit or Remove Vessels from the schedule

Vessel View

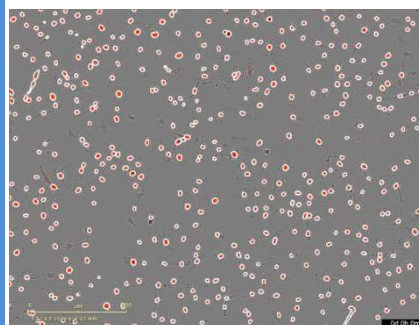
Acquire Images

Scan Properties	
Vessel Type	24-well Corning Falcon
Scan Type	Standard
Image Channels	<input checked="" type="checkbox"/> Phase <input checked="" type="checkbox"/> Green
	Acquisition Time (ms) 300
Objective	20x
Scan Duration	2 min (estimated)
Number of Daily Scans	24

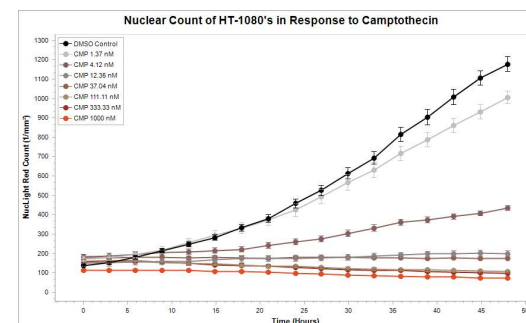
View Images



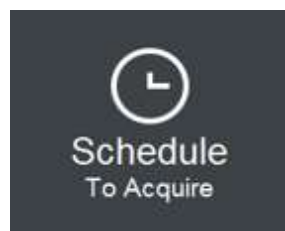
Process Images



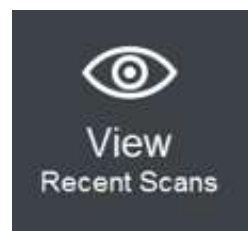
Analyze Images



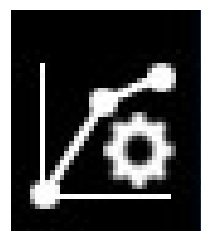
Schedule Scans



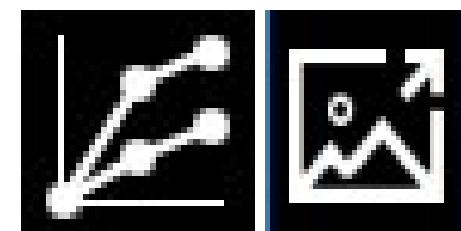
Vessel View



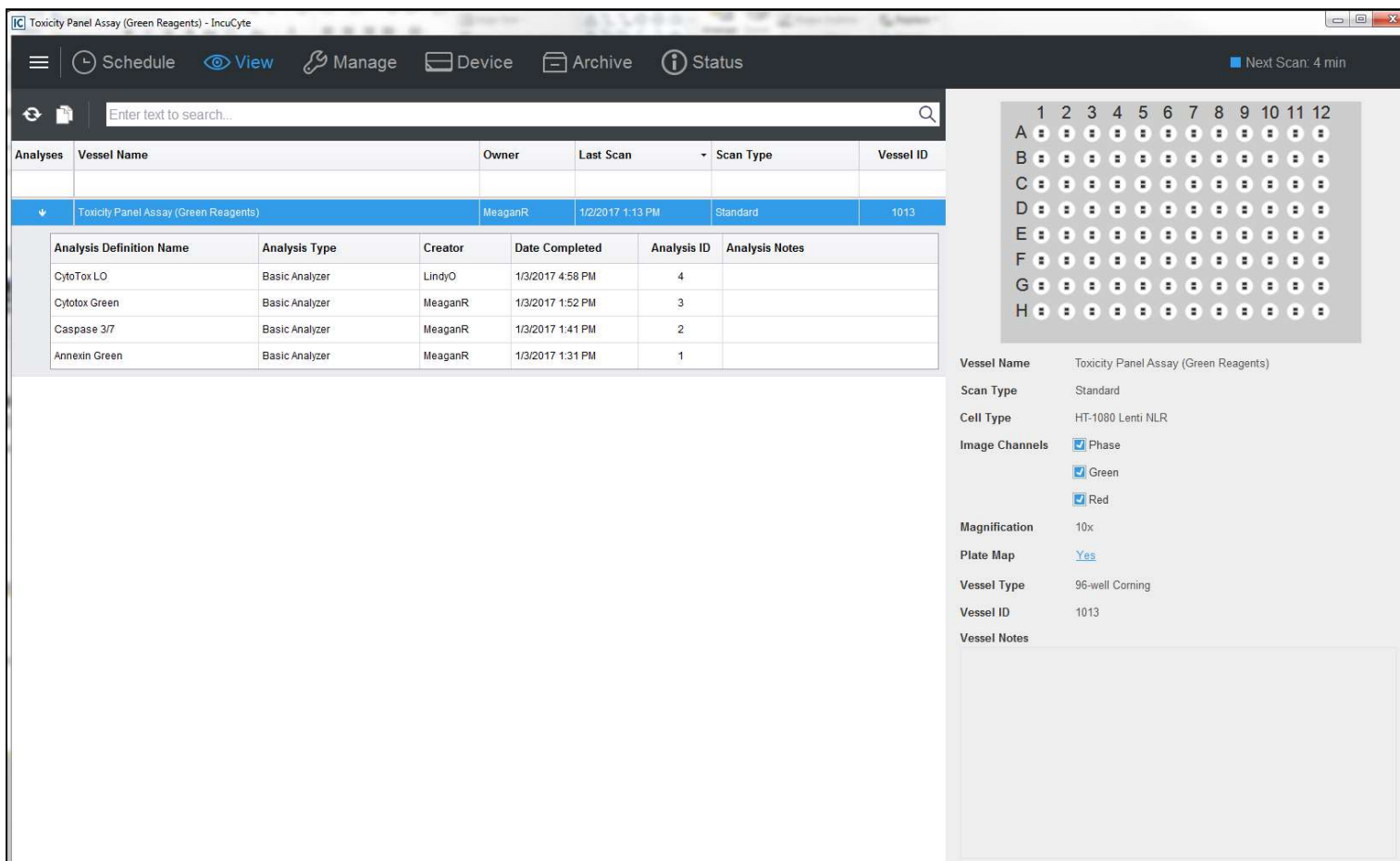
Analysis Definition



Graphing and Exporting



Search for Experiments by Label/user/etc.



The screenshot displays the IncuCyte software interface for a 'Toxicity Panel Assay (Green Reagents)'. The top navigation bar includes icons for Schedule, View, Manage, Device, Archive, and Status, along with a 'Next Scan: 4 min' indicator. A search bar is present with the placeholder text 'Enter text to search...'. Below the search bar, a table lists analyses with columns for Vessel Name, Owner, Last Scan, Scan Type, and Vessel ID. The selected analysis is 'Toxicity Panel Assay (Green Reagents)' by 'MeaganR', last scanned on '1/2/2017 1:13 PM', with a 'Standard' scan type and Vessel ID '1013'. A detailed table below shows analysis definitions with columns for Analysis Definition Name, Analysis Type, Creator, Date Completed, Analysis ID, and Analysis Notes. The detailed table lists four analysis types: CytoTox LO, Cytotox Green, Caspase 3/7, and Annexin Green, all created by 'MeaganR'. To the right of the tables is a 12x8 grid representing a plate map, with columns numbered 1-12 and rows lettered A-H. Below the grid, a panel displays assay parameters: Vessel Name (Toxicity Panel Assay (Green Reagents)), Scan Type (Standard), Cell Type (HT-1080 Lenti NLR), Image Channels (Phase, Green, Red), Magnification (10x), Plate Map (Yes), Vessel Type (96-well Corning), Vessel ID (1013), and Vessel Notes.

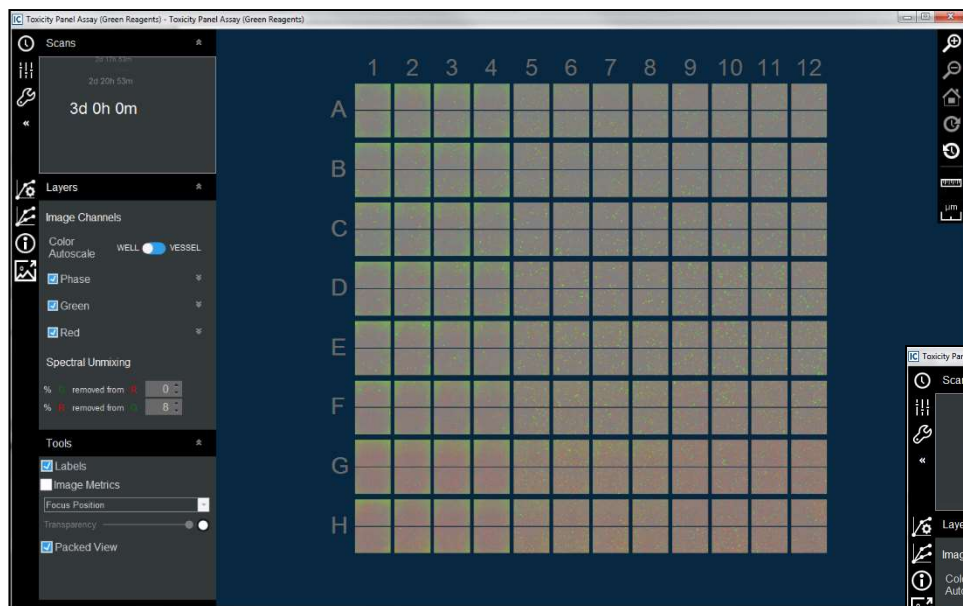
Analyses	Vessel Name	Owner	Last Scan	Scan Type	Vessel ID
▼	Toxicity Panel Assay (Green Reagents)	MeaganR	1/2/2017 1:13 PM	Standard	1013

Analysis Definition Name	Analysis Type	Creator	Date Completed	Analysis ID	Analysis Notes
CytoTox LO	Basic Analyzer	LindyO	1/3/2017 4:58 PM	4	
Cytotox Green	Basic Analyzer	MeaganR	1/3/2017 1:52 PM	3	
Caspase 3/7	Basic Analyzer	MeaganR	1/3/2017 1:41 PM	2	
Annexin Green	Basic Analyzer	MeaganR	1/3/2017 1:31 PM	1	

Vessel Name: Toxicity Panel Assay (Green Reagents)
Scan Type: Standard
Cell Type: HT-1080 Lenti NLR
Image Channels: ☒ Phase, ☒ Green, ☒ Red
Magnification: 10x
Plate Map: [Yes](#)
Vessel Type: 96-well Corning
Vessel ID: 1013
Vessel Notes

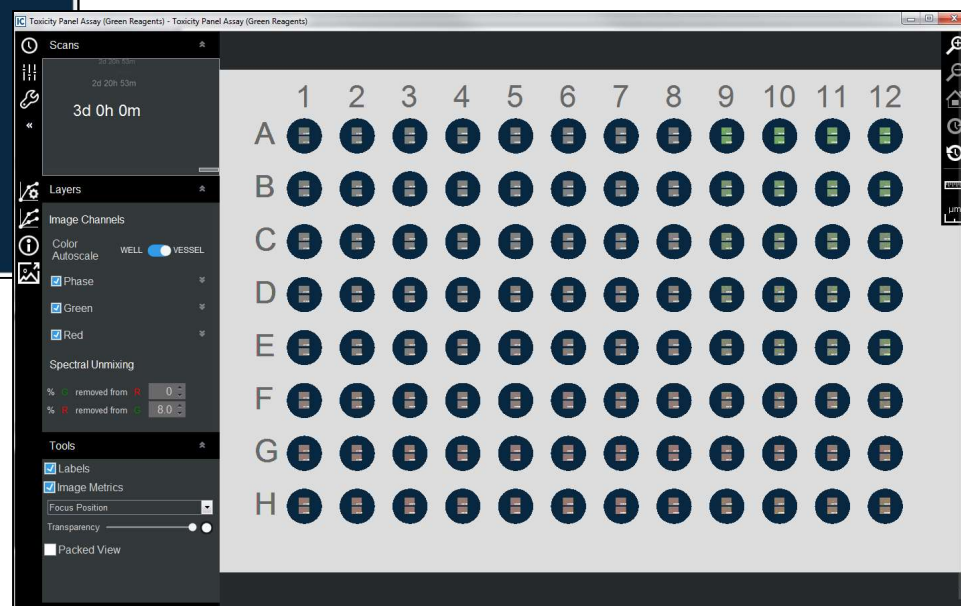
Vessel (Image) Display

Packed View



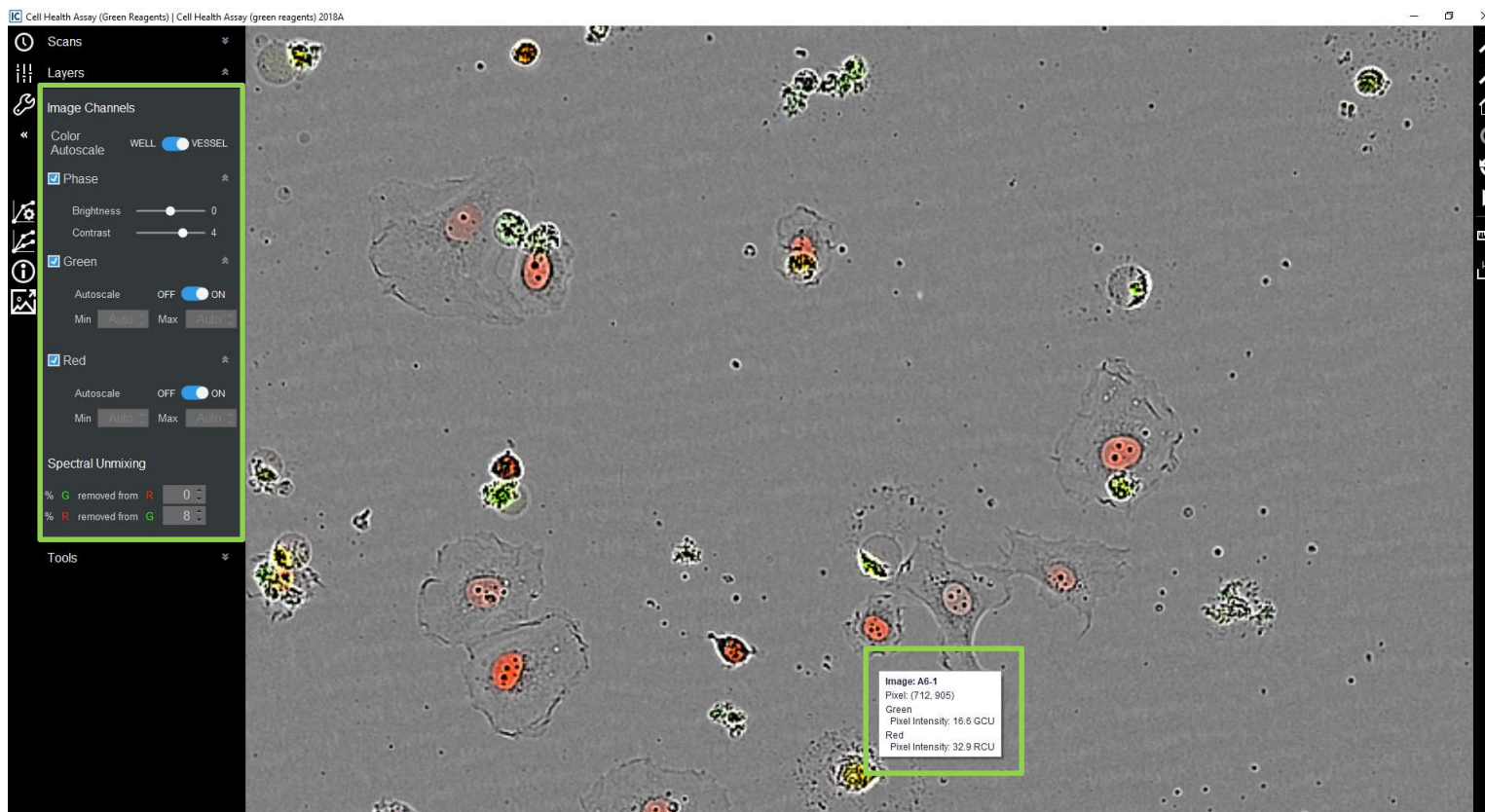
Quickly assess trends in data in default 'Packed View'

Unpacked View



Visualize location of images in the well

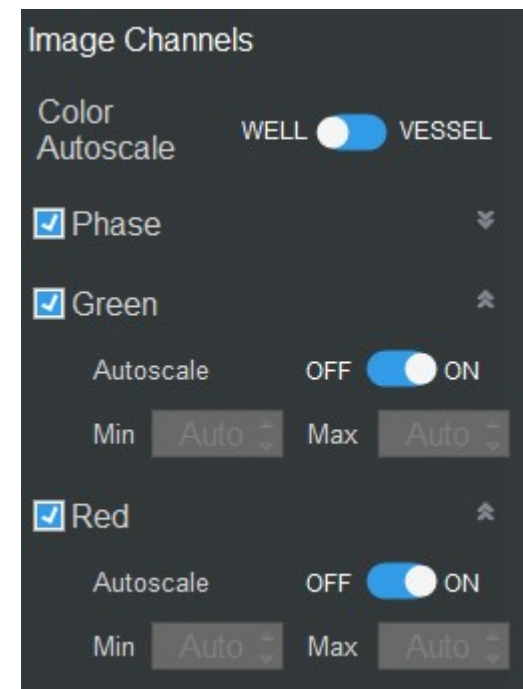
View and Interact with Images



- **Calibrated Images**
 - Compare images over time and between instruments
 - Images made up of pixels with values used to define analysis
 - Fluorescent pixel intensities are reported in GCU (Green Calibrated Unit) and RCU (Red Calibrated Unit). Hover over any pixel to find these values.
- **Visualize images**
 - Adjust Brightness/Contrast for phase-contrast
 - Adjust min/max intensity settings for fluorescence
 - Adjusting these values will not change the pixel values of the image

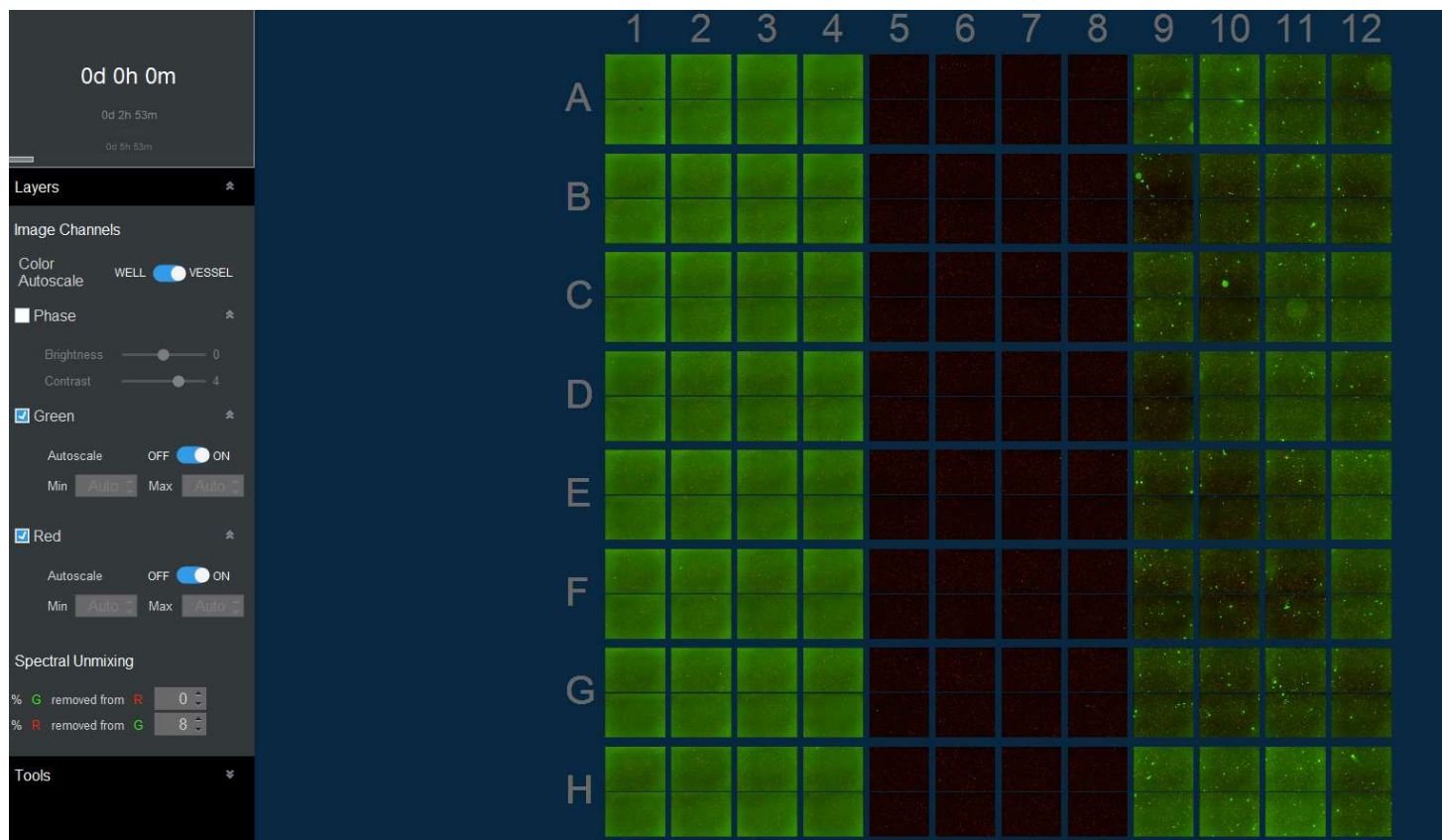
Autoscale

- **What is it?**
 - Sets a range of min/max intensities to account for changes in biological samples across different samples and time
 - A 'halo' or 'cross' artifact might appear when imaging samples with dim signal



Vessel – Autoscale (default)

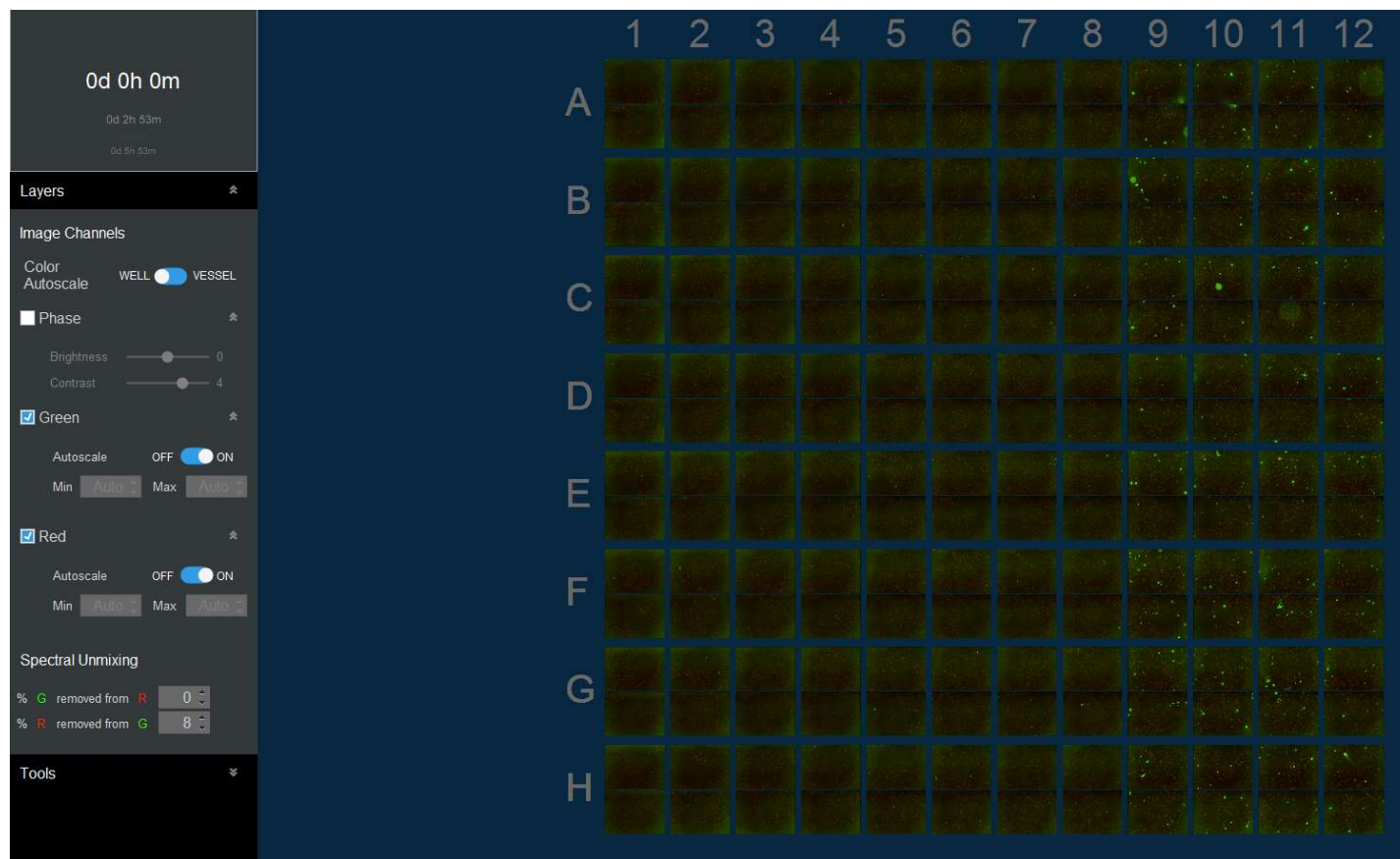
Applies an autoscale across entire vessel based on min/max brightness across entire plate



Ideal for experiments with the same reagent. In this experiment, 3 sections of the plate were treated with different reagents of different fluorescent intensity; therefore, vessel autoscale is not recommended

Well – Autoscale

Applies an autoscale for each individual well based on min/max brightness in each individual well

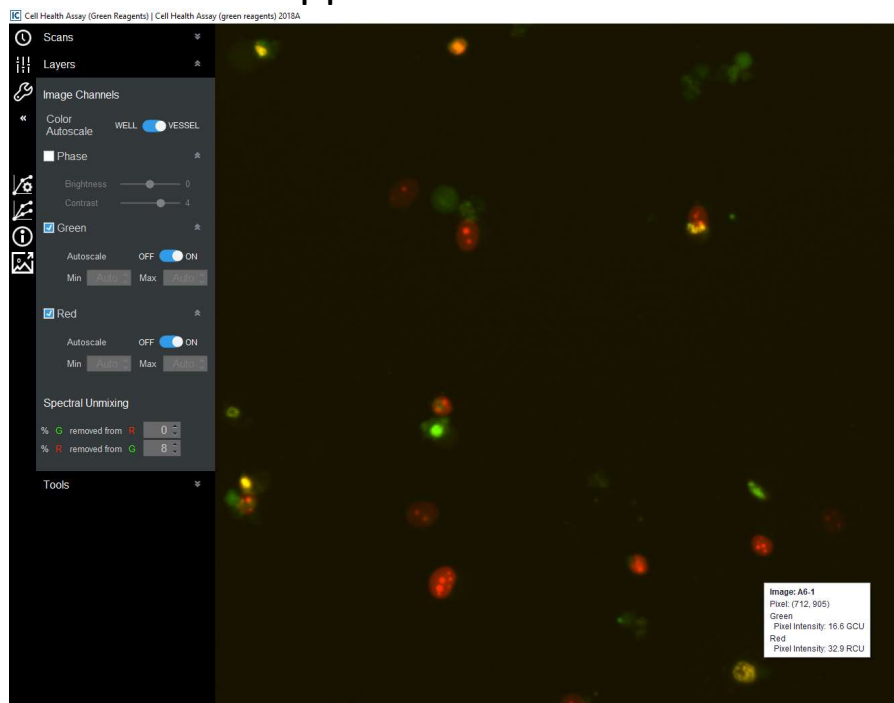


Ideal for experiments with different reagents and to determine the baseline intensities of individual wells. In this example, well autoscale is recommended

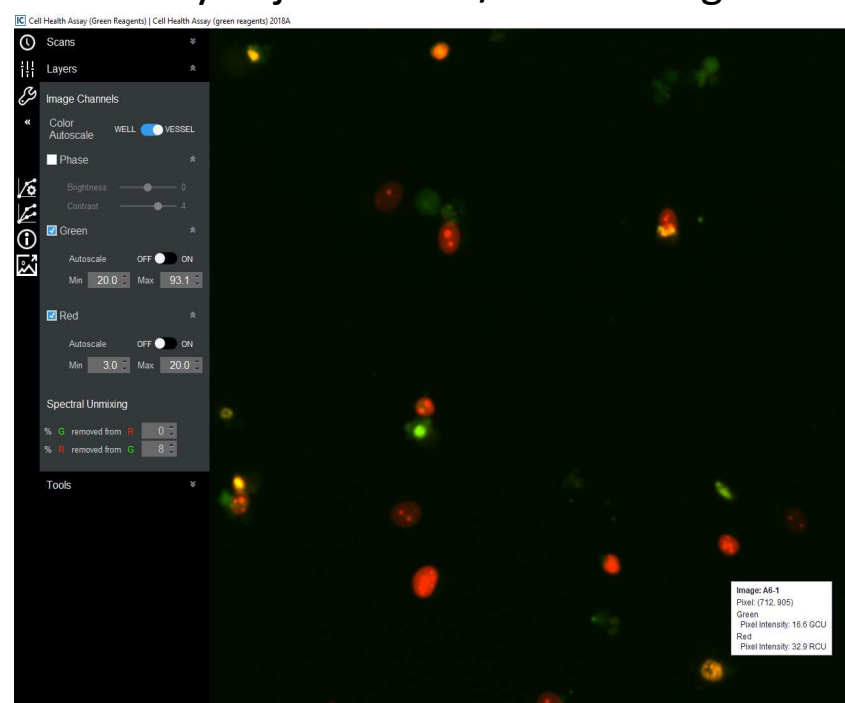
Turn off Autoscale (recommended)

Use the default autoscale settings to find a base line by turning off autoscale. Adjust min and max intensity values for optimal visualization (does not alter pixel intensity)

Auto-scale applied



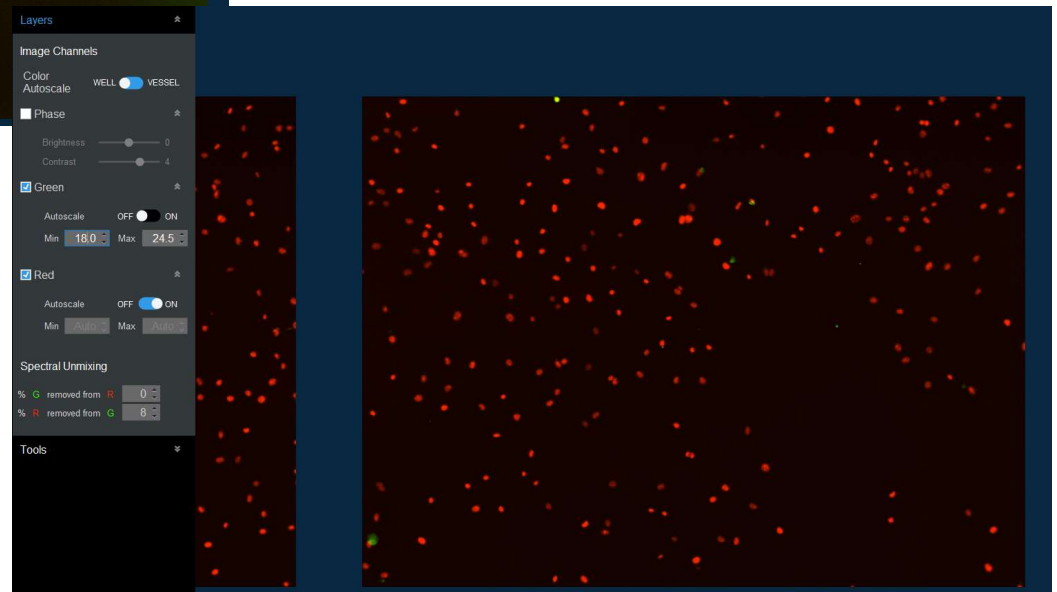
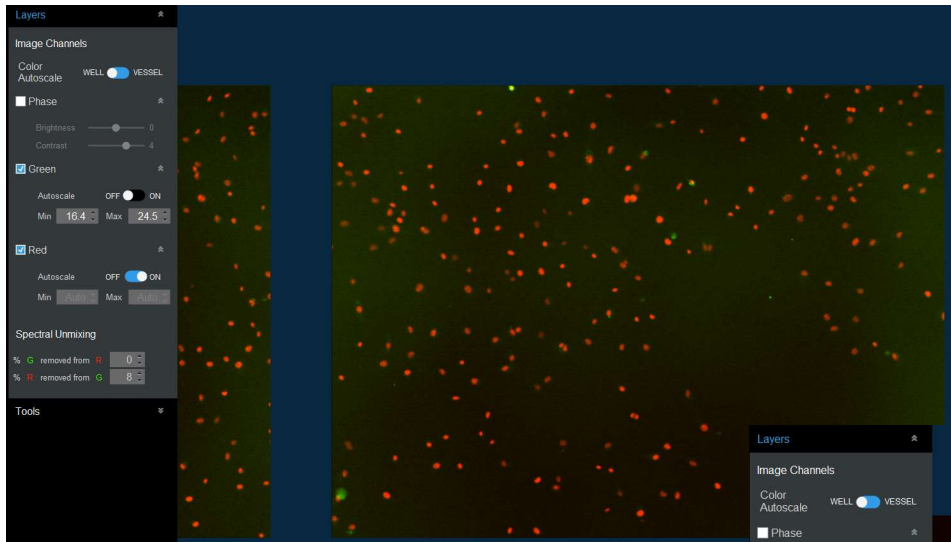
Manually adjusted min/max settings



Example of same image (1 with auto-scale, 1 with manual adjustment). Pixel intensity remains the same despite changing the visualization range.

Analogy: Think of playing the same song at different volumes.

How would you adjust the min or max settings in this image for the green channel to remove background?

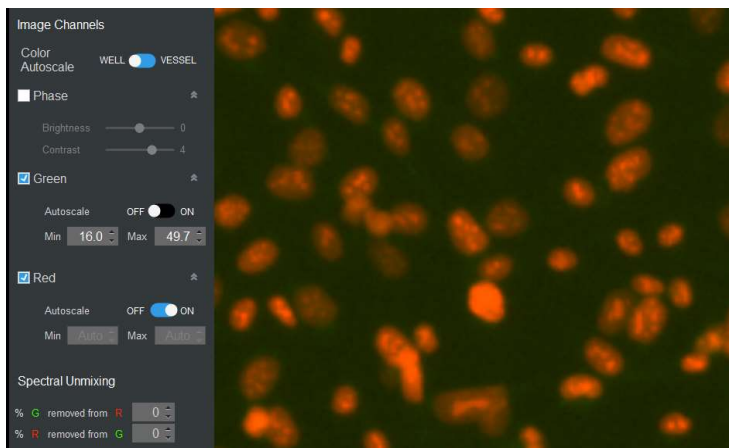


Spectral Unmixing

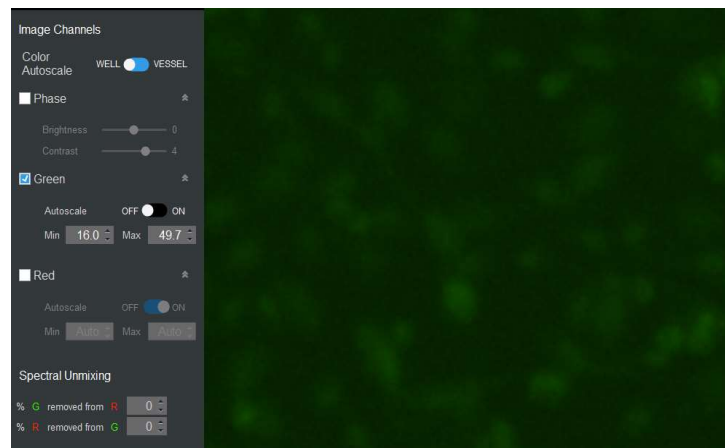
- Some fluorophores may be excited by the opposite channel's LED (e.g. a red fluorophore may show up with a dim green signal)
- Determine spectral unmixing for your fluorophore only the first time you run the experiment and apply to all future experiments
 - Set-up an experiment with only one fluorophore per well
 - Image the well in both channels

Example of how to adjust spectral unmixing

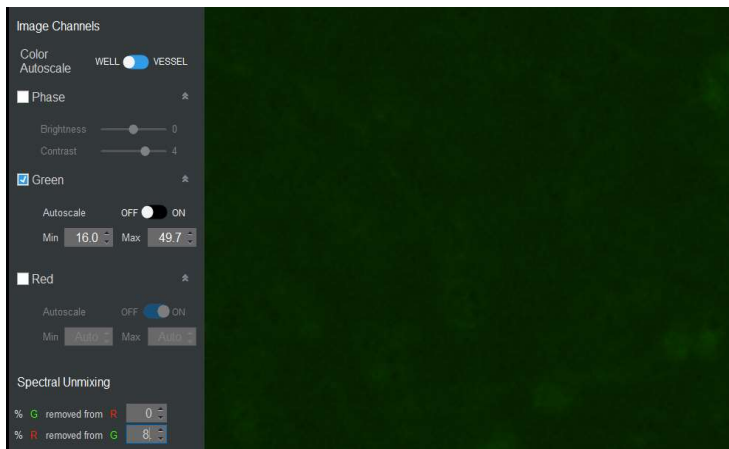
Red only fluorophore – imaged in both red and green channel



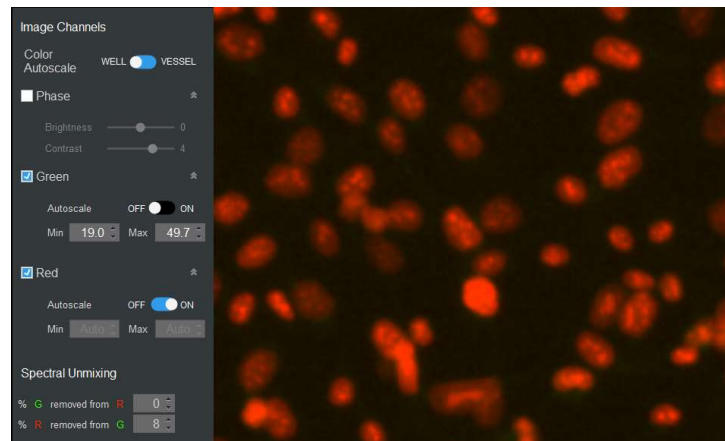
Turn off the Red channel and determine if there is any green signal



Increase the %R removed from G until there is little/no green signal left



Adjust the min/max intensities to remove remaining green background

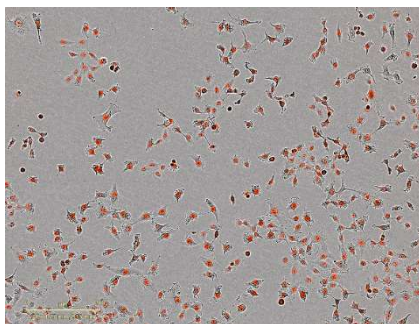


Create an Analysis Definition

Acquire Images

Scan Properties	
Vessel Type	24-well Corning Falcon
Scan Type	Standard
Image Channels	<input checked="" type="checkbox"/> Phase <input checked="" type="checkbox"/> Green
	Acquisition Time (ms) 300
Objective	20x
Scan Duration	2 min (estimated)
Number of Daily Scans	24

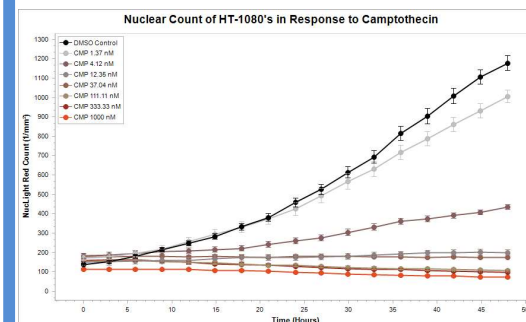
View Images



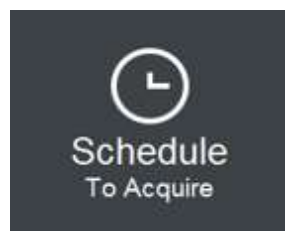
Process Images



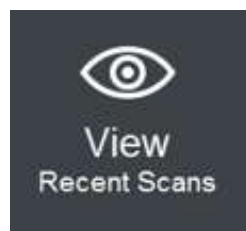
Analyze Images



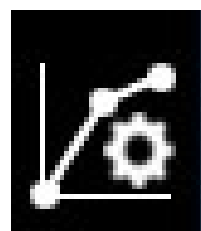
Schedule Scans



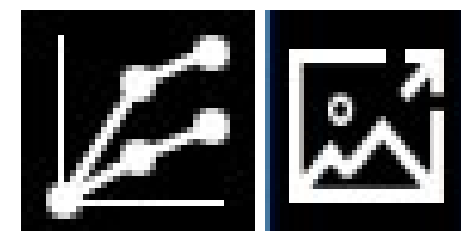
Vessel View



Analysis Definition

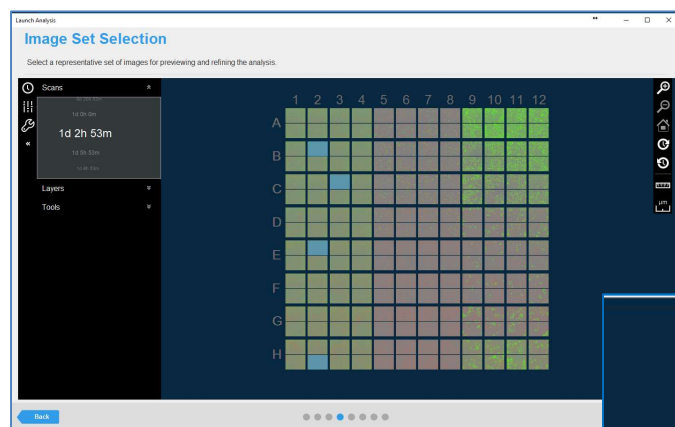


Graphing and Exporting



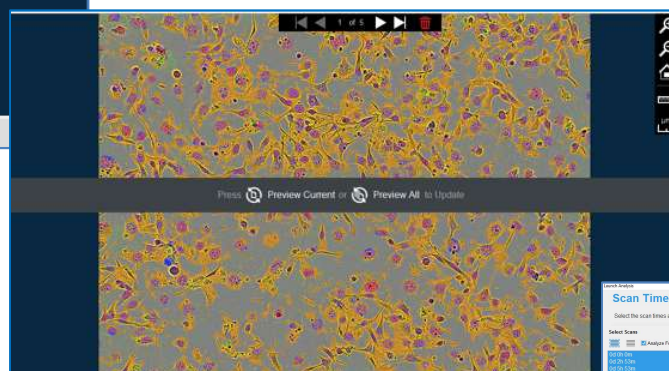
3 steps to run an Analysis on the IncuCyte S3

1. Select representative images



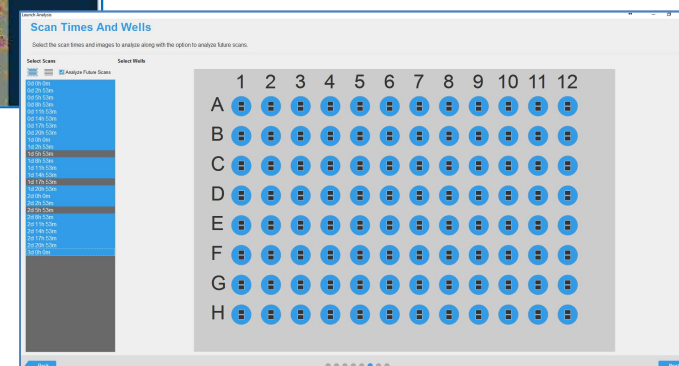
*Include images from beginning/end,
untreated/treated, dim/bright*

2. Preview Analysis Definition



Adjust parameters to create mask

3. Select Time Points/Wells



Run analysis definition on all images/time points of choice

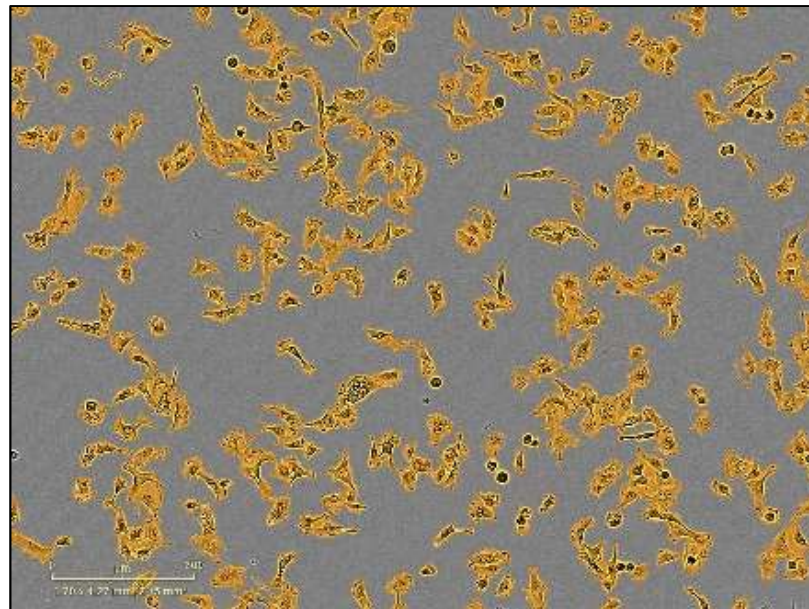
Selection of representative images

- Images representing the variability inside an experiment (cell morphology, density, fluorescence intensity, background)
- HD-phase
 - Different confluency level (10-80%range)
 - Different cell morphology (differentiation, spreading, death)
 - Images with debris/scratches if representative
- **Brightfield (spheroid)**
 - Different size and density
 - Different background
- **Fluorescence**
 - Different intensity after correct setting of Min/Max
 - Different background

Define Masks and use them for analyses

What is a mask?

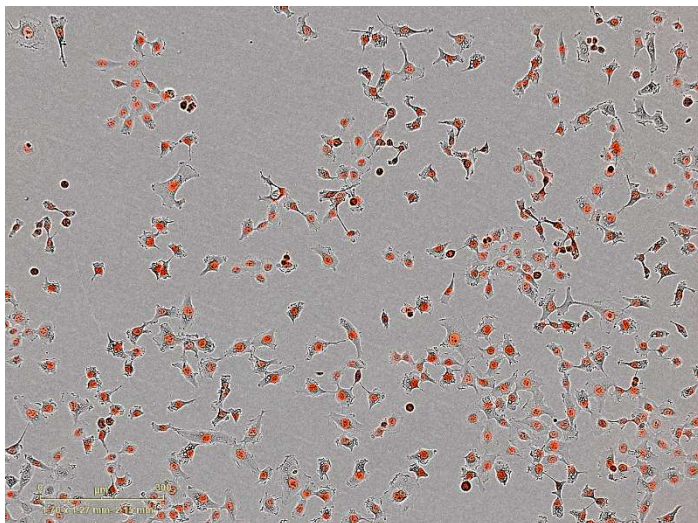
- Indication of areas of interest (cells) within an image
- Define which pixels in image correspond to cells (by eye)



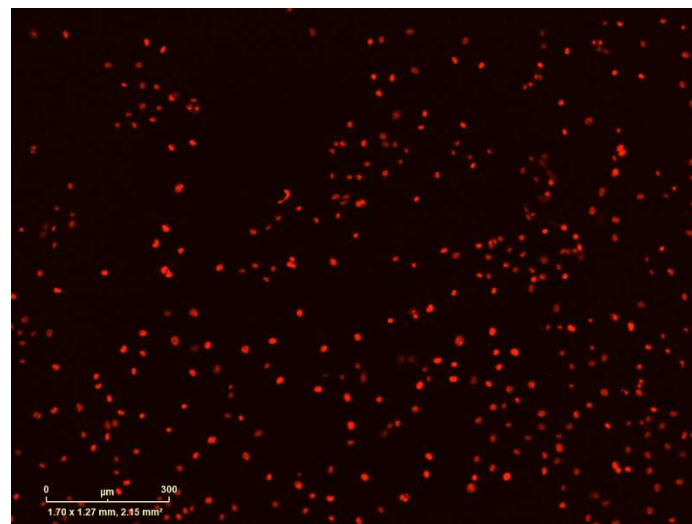
The pseudo yellow color in this image indicates what phase areas of the image are of interest (cells) and should be quantified in the data analysis.

Different Masks of the same Image

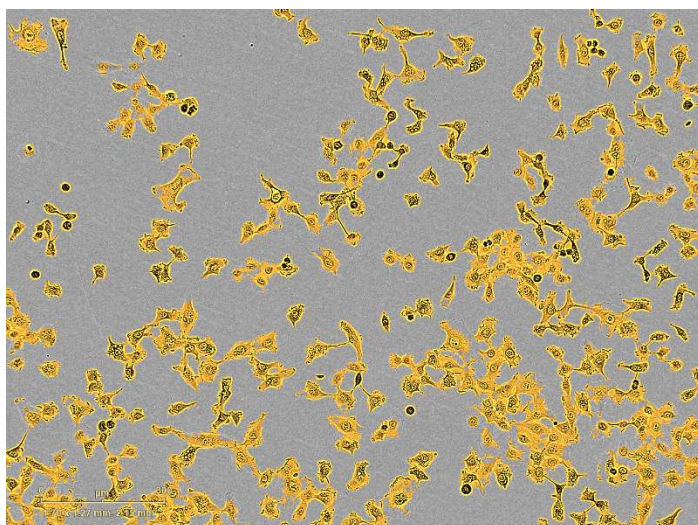
Phase and Red Blend Image – No Mask



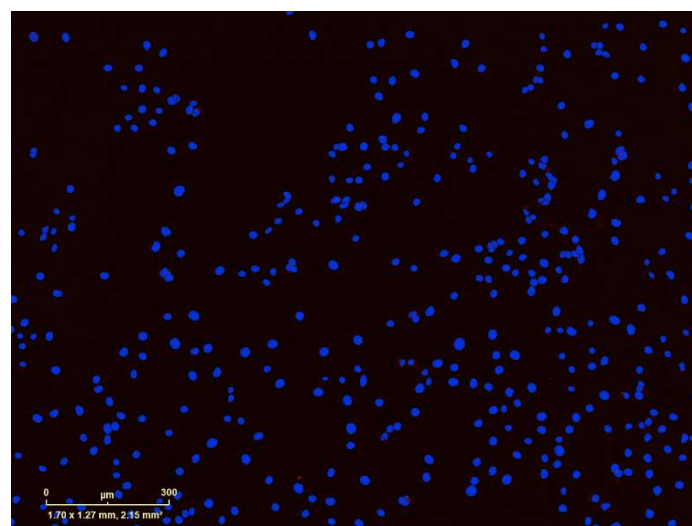
Red Image – No Mask



Phase Image – Blend Phase Mask (yellow)



Red Image – Overlay Red Mask (blue)



Analysis definition

Launch Analysis

Analysis Definition

Refine the Analysis Definition settings. Click Preview to analyze the Image Set.

Analysis Definition

Phase

Object Name

Segmentation

Segmentation Adjustment: 1

Background

Cleanup

Hole Fill (μm^2)

Adjust Size (pixels)

Filters


Area (μm^2):

☐ min ☐ max

Eccentricity:

☐ min ☐ max

Whole Well



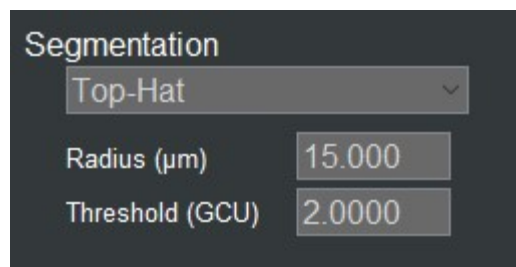
Press Preview Current or Preview All to Update

Metrics for Image H7-2 @ 0d 8h 53m <Cell Health Assay (Green Reagent)

Image Channel	Confluence (Percent)	Count (1/Image)	Avg Area (μm^2)	Avg Eccentricity
Confluence	40.038	247	3673.3	0.7180

Back Next

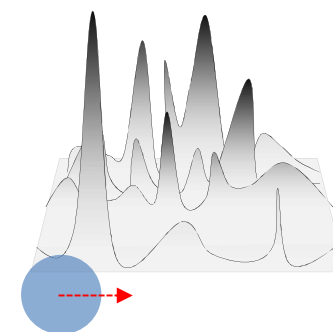
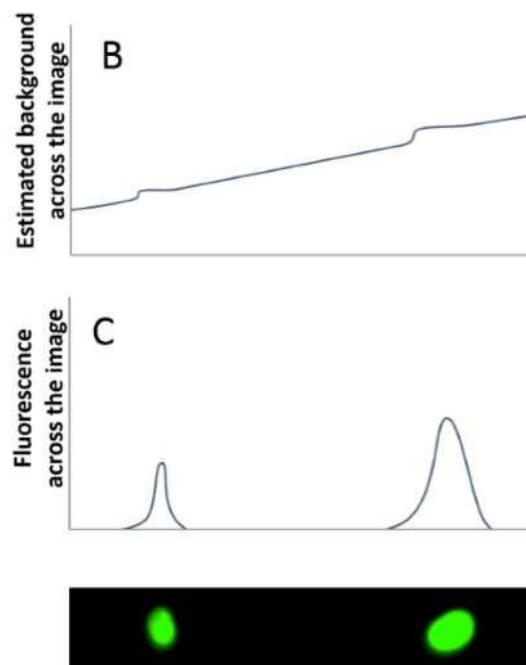
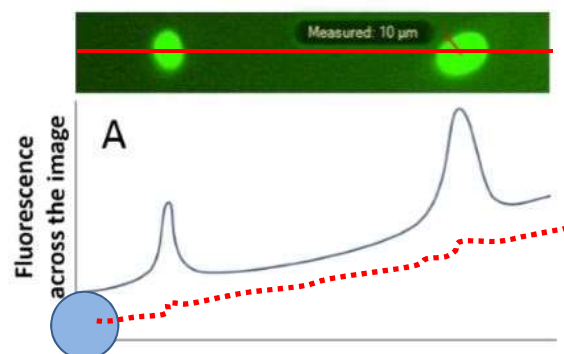
Top-Hat background subtraction for fluorescence



Top-hat:

- radius larger than the one of the largest object of interest
 - Separation into 2 components: background and real signal
- Threshold applied to include only real signal in the mask

How does it work:

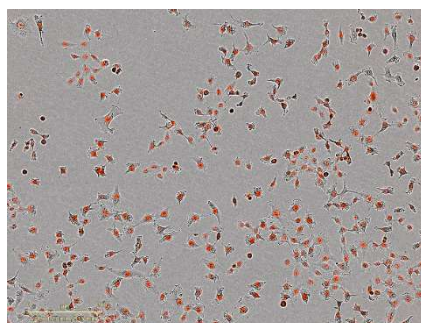


Analyze Images

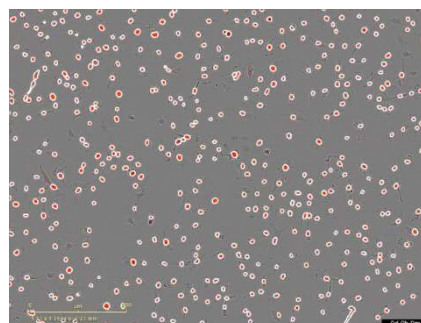
Acquire Images

Scan Properties	
Vessel Type	24-well Corning Falcon
Scan Type	Standard
Image Channels	<input checked="" type="checkbox"/> Phase <input checked="" type="checkbox"/> Green
	Acquisition Time (ms) 300
Objective	20x
Scan Duration	2 min (estimated)
Number of Daily Scans	24

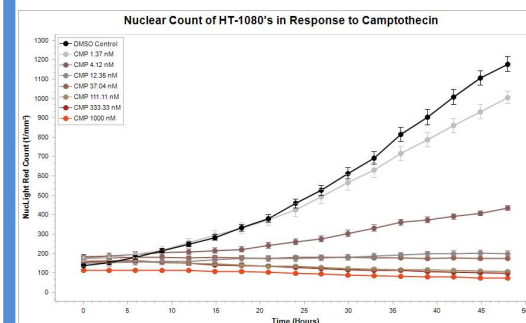
View Images



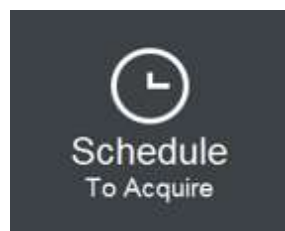
Process Images



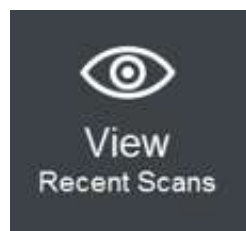
Analyze Images



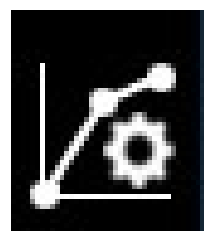
Schedule Scans



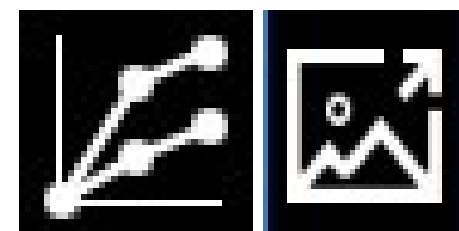
Vessel View



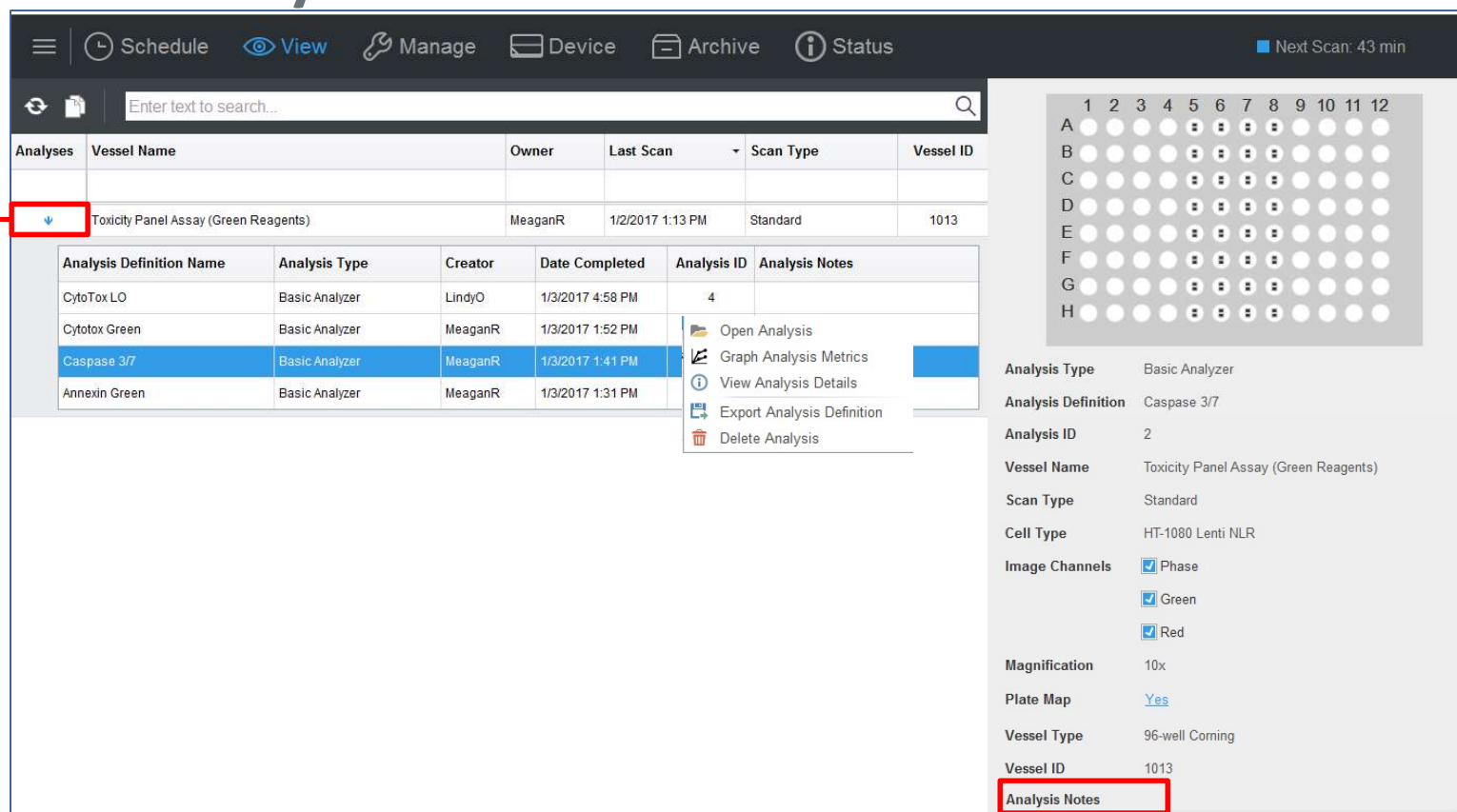
Analysis Definition



Graphing and Exporting



Search Analyses



Analyses

Vessel Name	Owner	Last Scan	Scan Type	Vessel ID
Toxicity Panel Assay (Green Reagents)	MeaganR	1/2/2017 1:13 PM	Standard	1013

Analysis Definition Name | Analysis Type | Creator | Date Completed | Analysis ID | Analysis Notes

CytoTox LO	Basic Analyzer	LindyO	1/3/2017 4:58 PM	4	
Cytotox Green	Basic Analyzer	MeaganR	1/3/2017 1:52 PM		
Caspase 3/7	Basic Analyzer	MeaganR	1/3/2017 1:41 PM		
Annexin Green	Basic Analyzer	MeaganR	1/3/2017 1:31 PM		

Open Analysis
Graph Analysis Metrics
View Analysis Details
Export Analysis Definition
Delete Analysis

Analysis Type: Basic Analyzer
Analysis Definition: Caspase 3/7
Analysis ID: 2
Vessel Name: Toxicity Panel Assay (Green Reagents)
Scan Type: Standard
Cell Type: HT-1080 Lenti NLR
Image Channels: ☒ Phase ☒ Green ☒ Red
Magnification: 10x
Plate Map: Yes
Vessel Type: 96-well Corning
Vessel ID: 1013
Analysis Notes

Analyses associated with vessel indicated by blue arrow

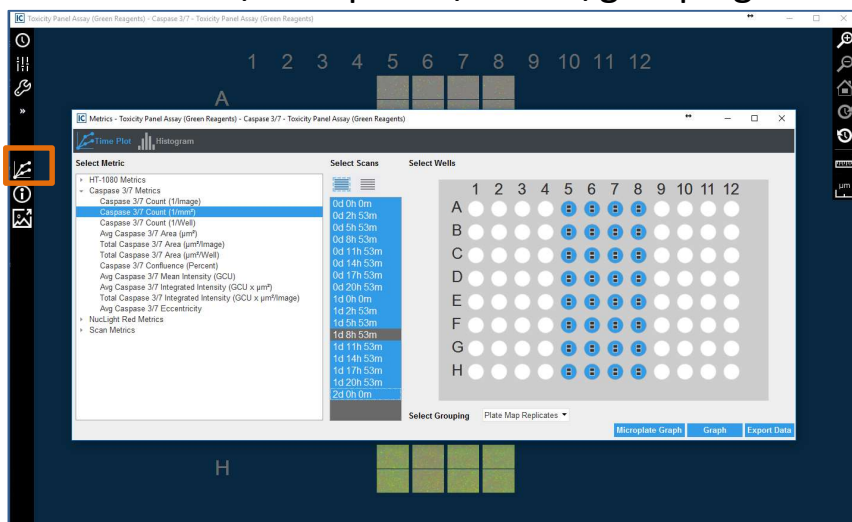
- Analyses saved by Analysis Definition Name
- Right click on Analysis Definition to view details
- Directly go to graphing window

Analysis Notes specify details of analysis definition

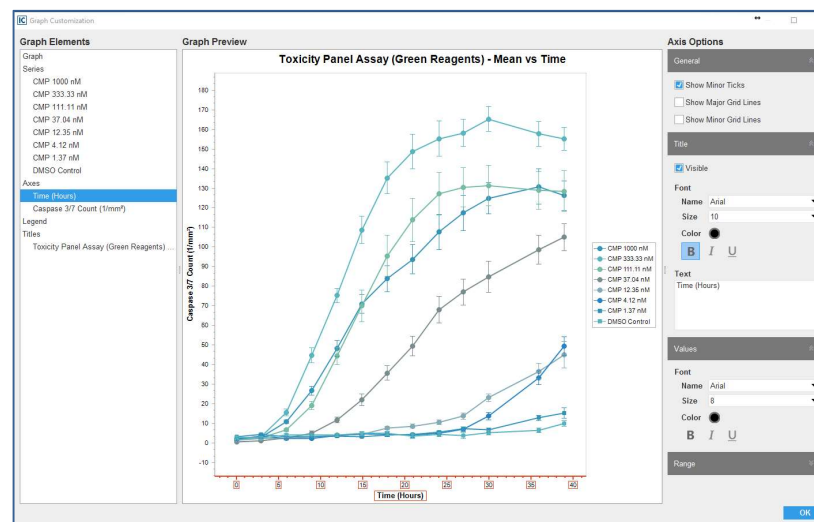
Details in the right pane show wells/image channels analyzed

Graph – View/Export

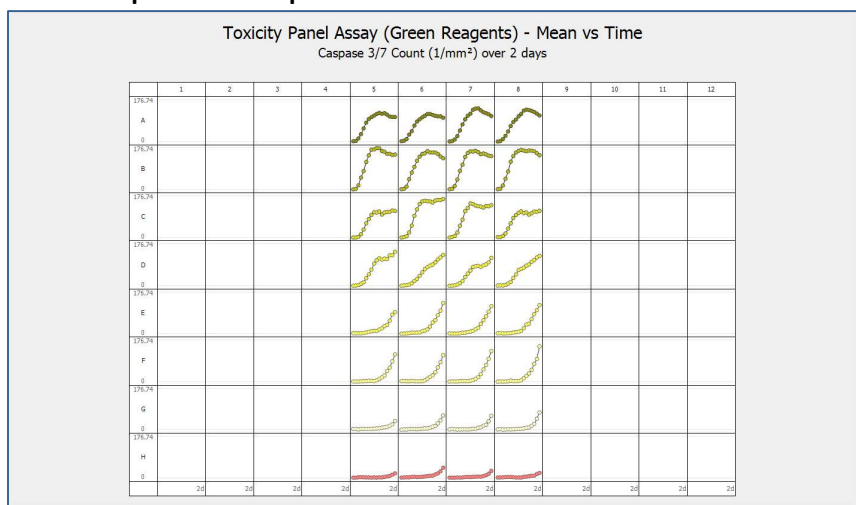
Select wells/time points/metric/grouping



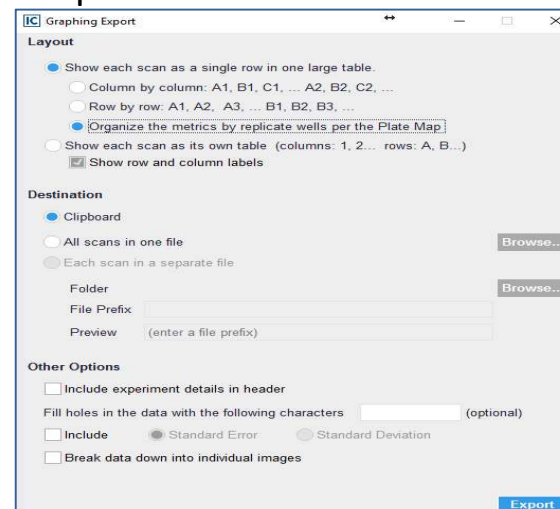
Tools – Customize Chart to edit colors/layout



Microplate Graph

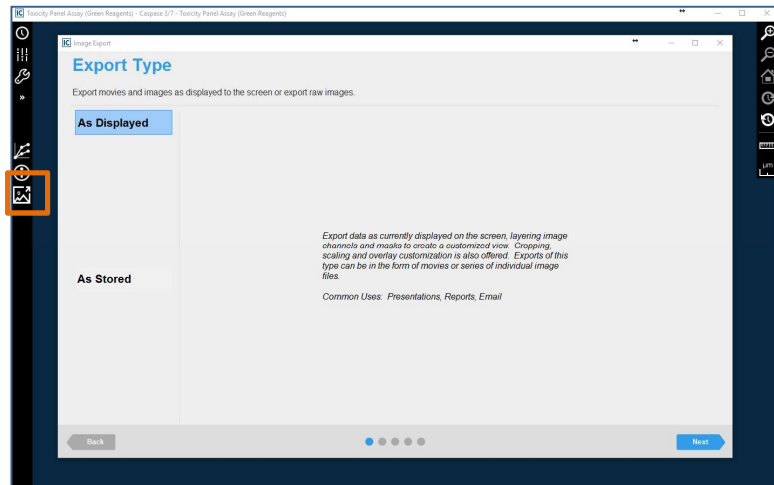


Export Data

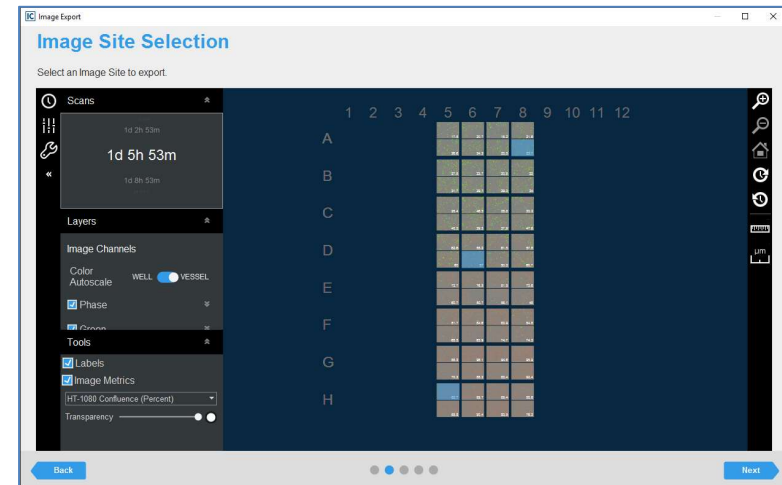


Select/Edit Images & Time Points

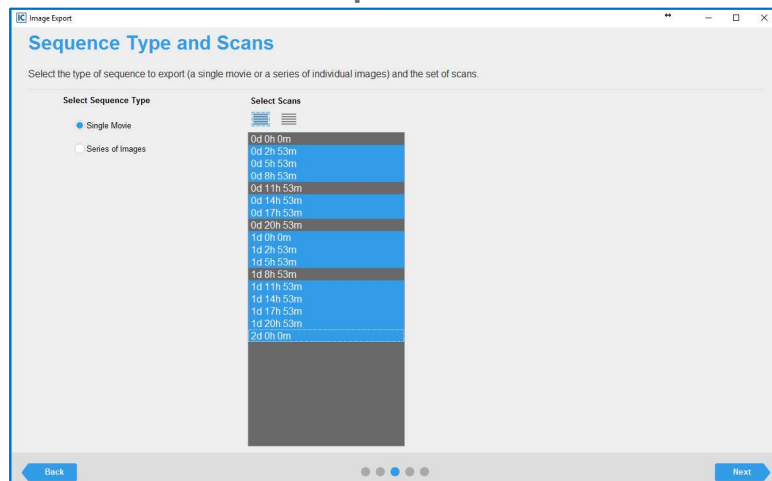
Enter guided interface from current image/time point



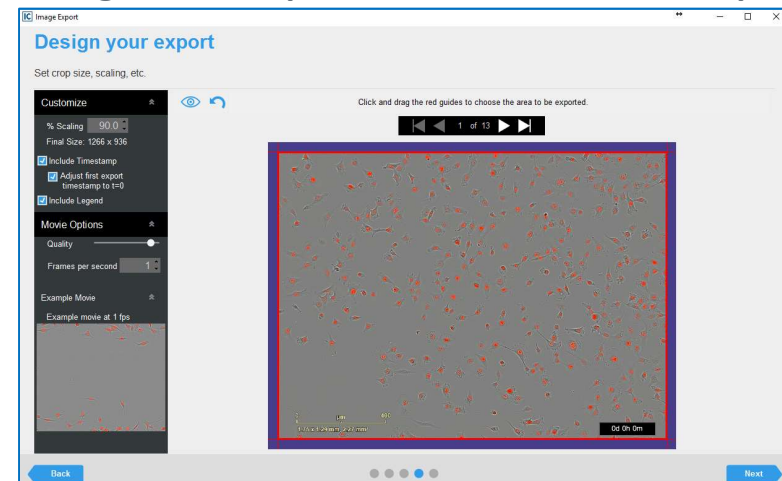
Select well/s and adjust auto-scale/image channels



Select time points of interest



Scaling/Timestamp/FPS and Preview before Export



Export Images/Movies

- **Export “As Displayed on Screen”**
 - Presentations/Publications
 - Adjust Brightness/Contrast for phase-contrast
 - Adjust min/max intensity settings for fluorescence (autoscale)
- **Export “As Stored on IncuCyte”**
 - Analyze images for 3rd Party analysis packages
 - Phase: 8-bit raw
 - Fluorescence: Uncalibrated raw 16-bit or calibrated 32-bit floating point

Software Demo – Schedule Scans

Practice Sets for Trainees

Experiment 1

- Phase/Green/Red – 4x whole well
- Corning 6-well 3335
- Front Right
- Use sample pattern (all 6 wells)
- Name: Experiment 1
- Every 6h starting at 12pm

Experiment 2

- Scratchwound
- Phase – 10x
- Wide Mode
- Front Left
- Whole Plate/1 image per well
- Name: Experiment 2
- Create a new scan group independent from Group 1: Every 1h starting at 1pm
- *How would you adjust the collisions at 12pm, 6pm, 12am, and 6am so all of the scans in the Scratchwound expt [Experiment 2] scan 10 minutes before the hour instead of on the hour?*

Experiment 3

- General
- Phase/Green – 20x
- 24-well Corning plate (3337)
- Middle Right
- Columns 1-4 (25 images per well)
- Name: Experiment 3
- Move the 24-well plate [Experiment 3] to the scan group for Experiment 1
- *How would you adjust Experiment 2 so there is enough time in between scan groups? Hint: The lab tech who setup Experiment 2 is okay with moving their 6:50 am, 12:50pm, 6:50pm, and 12:50 am scans to start at 7:00 am, 1:00 pm, 7:00 pm, and 1:00 am*
- *How would you adjust Experiment 3 to only scan every 6h but not exceed the 45 minute threshold? Hint: You prefer to start Experiment 3 at 1:30pm*

Experiment 4

- Copy Experiment 3
- Front Right (Hint: Experiment 1 is done so you can remove it)
- Add columns 5-6 to the scan pattern (i.e. scan the whole plate with 25 images per well)
- Name: Experiment 4
- Add this plate to the same interval as Experiment 2, adjust this scan group to scan only every 2h instead of every 1h starting at 2pm (Hint: you might need to delete the scan interval and set a new interval)

Software Demo - Vessel View

Practice Sets for Trainees (Cell Health Assay)

- **View in Packed View**
 - Go to the first time point
 - Turn off the phase
 - Why is there a green haze in columns 1-4 and 9-12?
 - Adjust the autoscale to each individual well
 - What are the green min/max settings of well A5?
- **View in UnPacked View**
 - Go to the last time point
 - Turn on the autoscale for each individual well
 - What are the green min/max settings of well A9
- **Additional Questions**
 - Keep the min/max settings for well A9 at the last time point
 - Return to packed view
 - Return to the first time point and notice how the haze is no longer there

Software Demo - Analysis Definition

Practice Sets for Trainees (Cell Health Assay)

- Create a new basic analyzer definition analyzing phase, green, red, and overlap
- Select 6 images from wells containing Caspase-3/7 (Columns 5-8)
 - 2 from late time point (rows C and H)
 - 2 from middle time point (rows A and F)
 - 2 from early time point (rows A and B)
- **Preview All using the default settings**
 - Adjust the phase mask to fit the cell area
 - Adjust the green mask to fit the dying cells
 - Adjust the red mask to fit all cells
 - Adjust the overlap mask to fit green/red cells
- **Select Columns 5-8 and all time points**
- **Save Analysis Definition as 'Caspase 3/7'**

Software Demo - Graph Export

Practice Sets for Trainees (Cell Health Assay – Caspase-3/7)

- **Graph Caspase 3/7 Count (1/mm²)**
 - Remove time points (1d 8h 53min and the last 3 time points)
 - Remove the 1000nM Series from the Graph
 - Keep only the compound information in the series title
 - Change the Color Palette to Green
 - Change the Title to “Caspase-3/7 Response to Camptothecin Treatment”
 - Drag and Drop into Powerpoint
- **Graph NuLight Red Count (1/mm²)**
 - Change the Color Palette to Red Orange
 - Keep only the compound information in the series title
 - Edit the color of DMSO Control to be black and circle, edit the color of CMP 1.37nM to be light gray and circle
 - Reverse the order of the legend so that DMSO is on top
 - Move the legend to the top left corner inside the graph
 - Change the Title to “Nuclear Count of HT-1080’s in Response to Camptothecin”
 - Drag and Drop into Powerpoint

Software Demo - Image Movie Export

Practice Sets for Trainees (Cell Health Assay - Caspase 3/7)

- **Export Movies from well A6**
 - Set the min setting to 1.0 and the max setting to match the auto-scale max of the last time point for well A6
 - Green only (no mask)
 - Remove time points (1d 8h 53min and the last 3 time points)
 - Include timestamp
 - 2 fps
 - Windows AVI Video (MPEG-4 compression)
- **Export Images from wells A6 and C6 (img 1 of 2)**
 - Red and Phase only + Red Object Mask (Outlines on, width = 4, mask color white)
 - Select time point 0 and 1d 14h 53m
 - Include timestamp
- **Use the right click – copy to clipboard to export the field of view of rows A-C (5-8)**
 - Make 2 fields of view: 1) Red only and 2) Phase + Blend Phase Mask
 - Show the 1) Image Metrics of the NuLight Red Count (1/Image) and 2) Confluence values from these wells
 - Select time point 0

IncuCyte Support

Field Application Scientist
Luca.Caneparo@Sartorius.com



Additional Resources:

- **Quotes, Orders:**
Claudio Bencivenga
Claudio.Bencivenga@Sartorius.com
- **Assays, Reagents, Protocols, Software:**
www.essenbioscience.com
Askascientist@Sartorius.com
- **Technical Support and Hardware:**
EUSupport.UK07@Sartorius.com