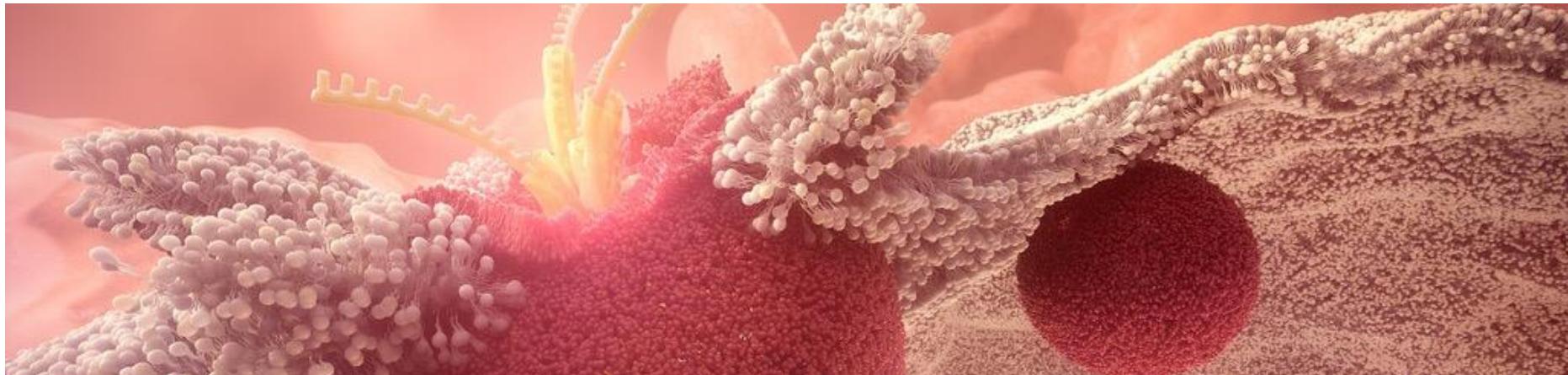


# Hackathon adipocyte data set reference material

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Principal scientist, Advanced Drug Delivery, Pharmaceutical Sciences, AstraZeneca R&D

Nov 2020



# Contents

Cell origins

Why are these cells interesting

How do we normally measure them?

About the dataset

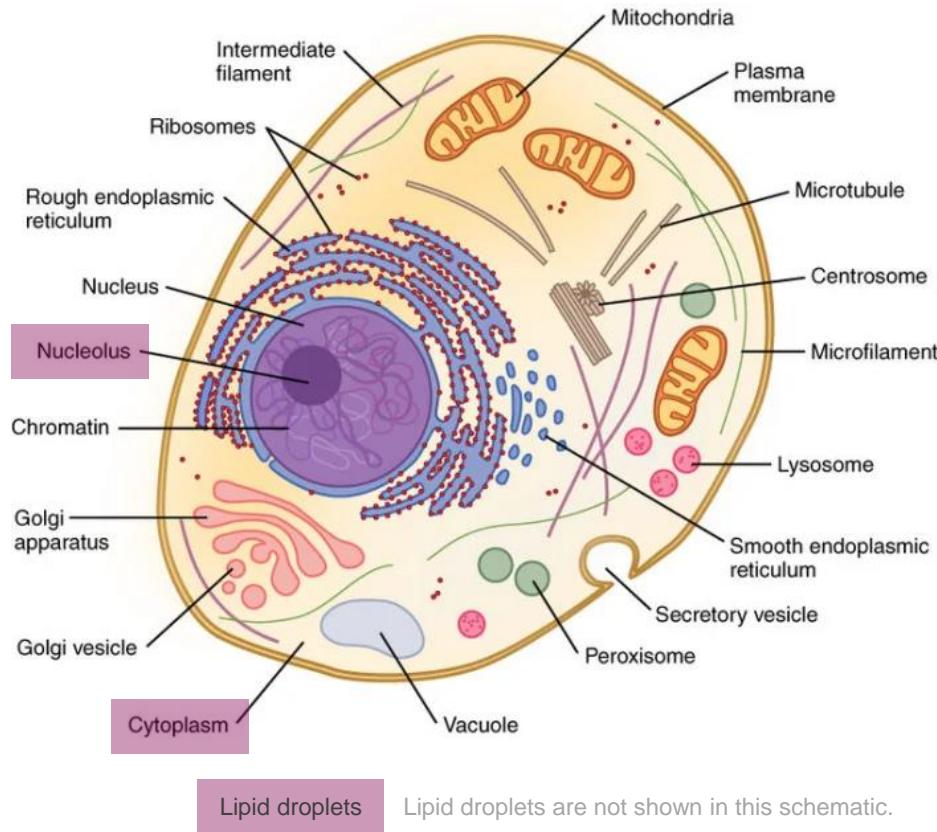
CellProfiler parameter description

Additional technical detail

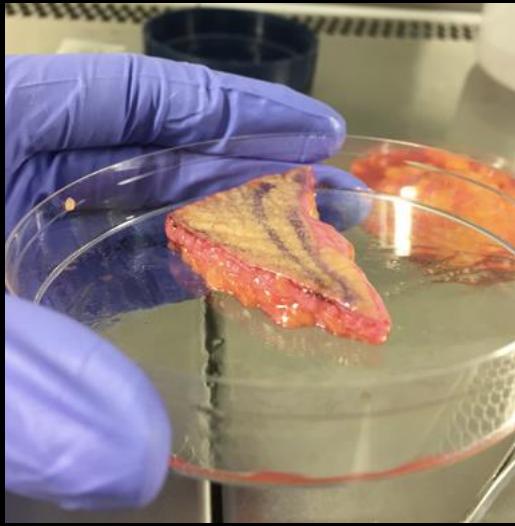


**Where do these cells come from?**

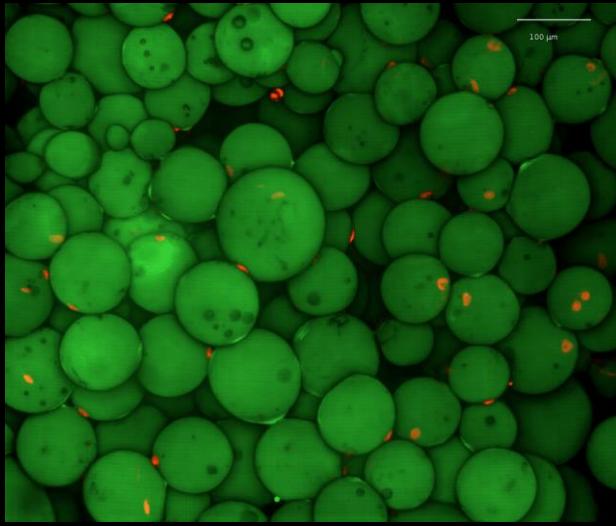
Here is a textbook cell, just for review



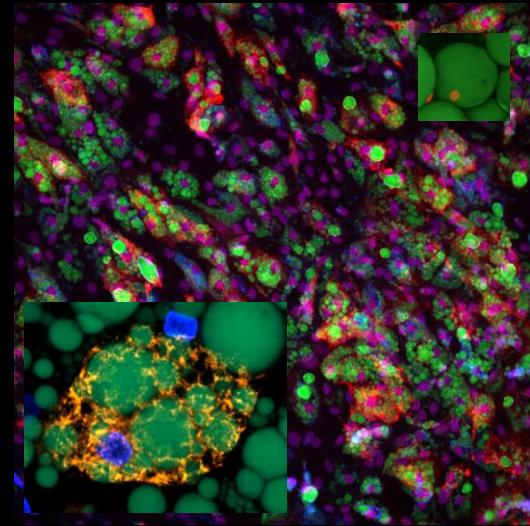
Fat cells obtained from patients



Mature fat cells (adipocytes)

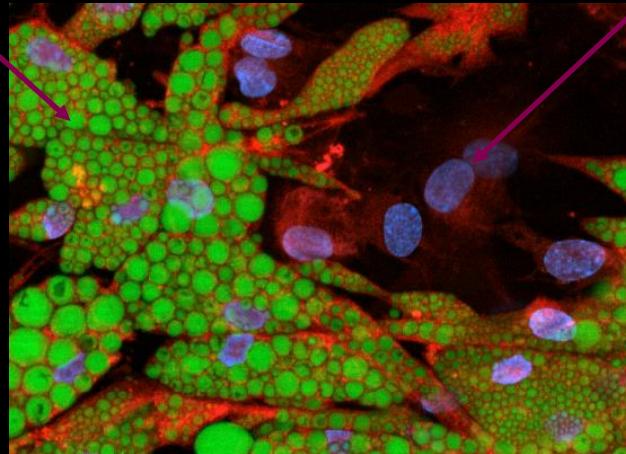


Stem cell-derived adipocytes

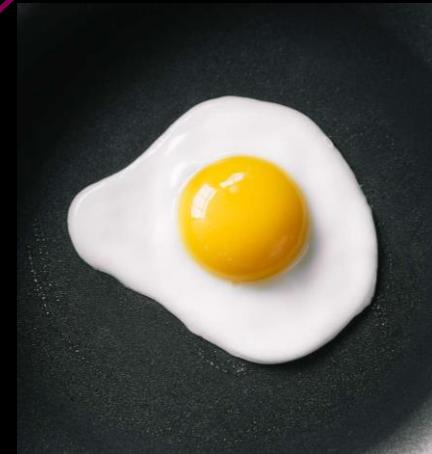


We start from stem cells, obtained from fat, and differentiate the cells into adipocytes. This allows us to make many cells cheaply without having to harvest continually from patients.

Adipocytes look like bunches of grapes

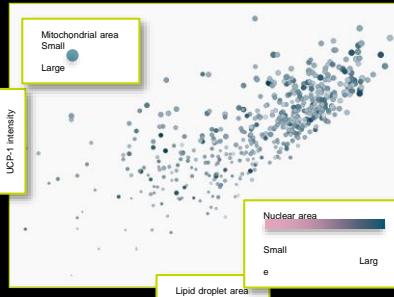
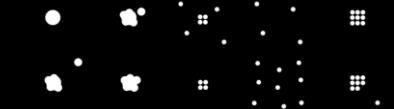
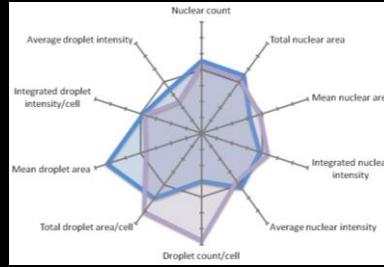
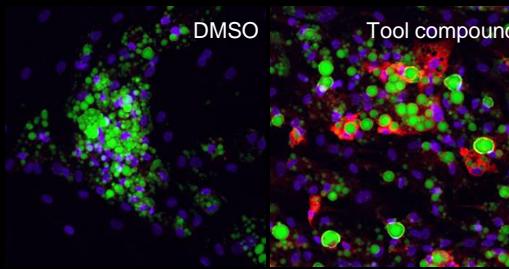
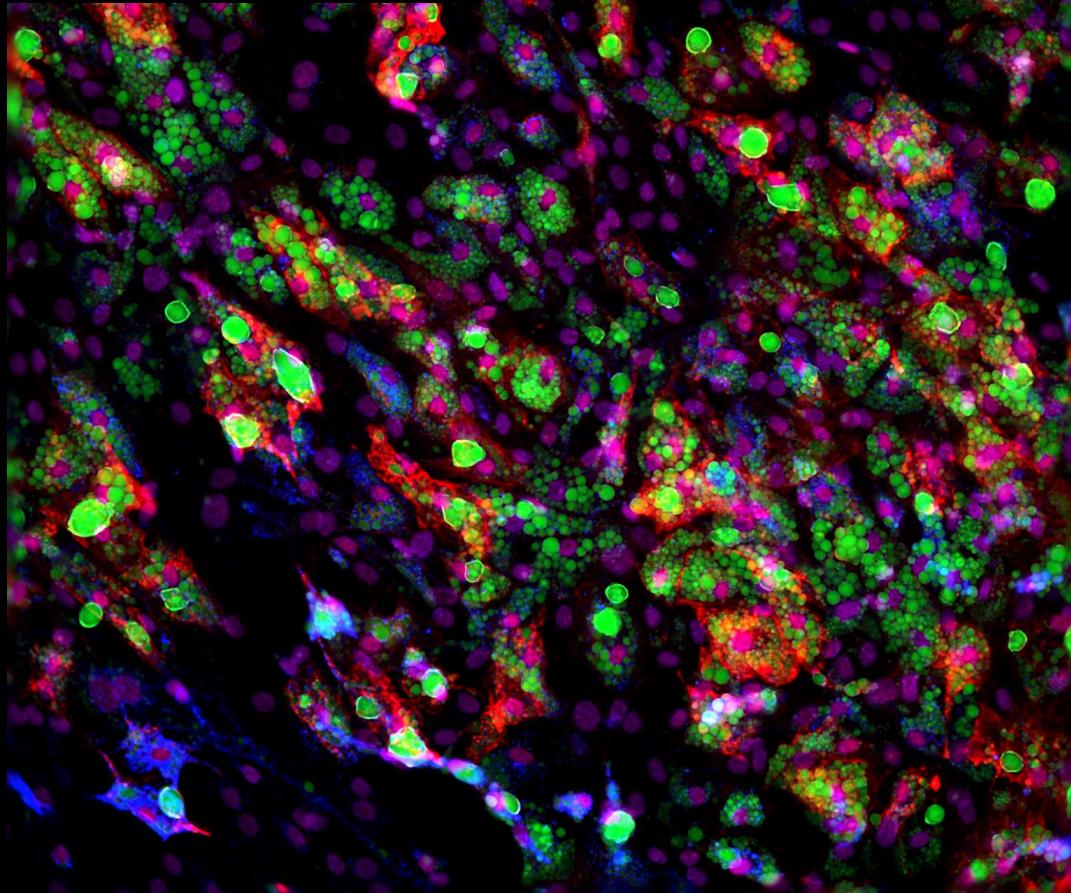


Cells that differentiate along the fibroblast lineage look more like fried eggs



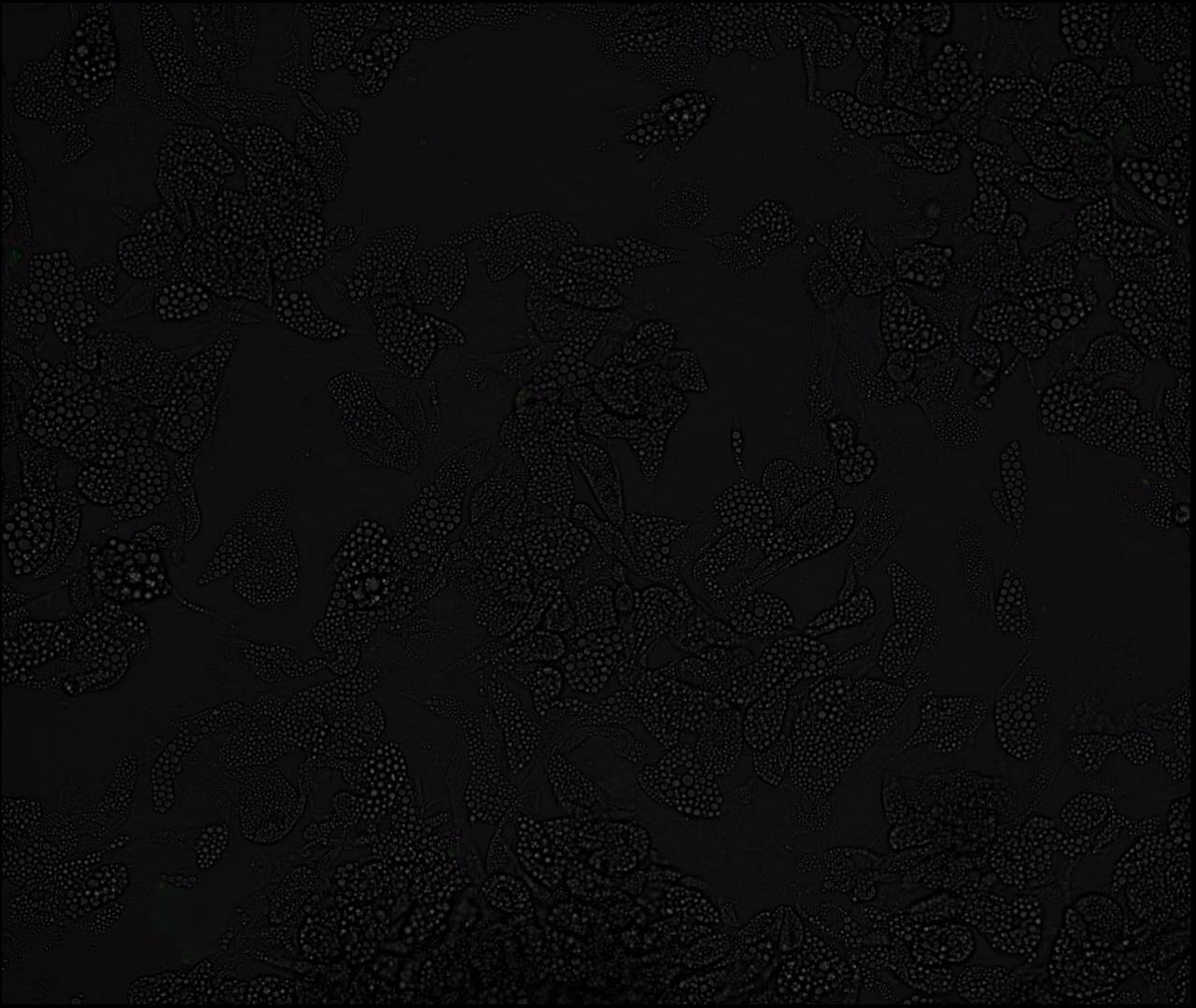
**Why are these cells interesting?**

We can use these cells to study adipocytes respond to therapies that change their metabolic profile. This is important for diabetes for example.



## MC3 high dose time-lapse

We can use these cells to study how nanomedicines behave in the skin following injections.



**How do you measure them?**

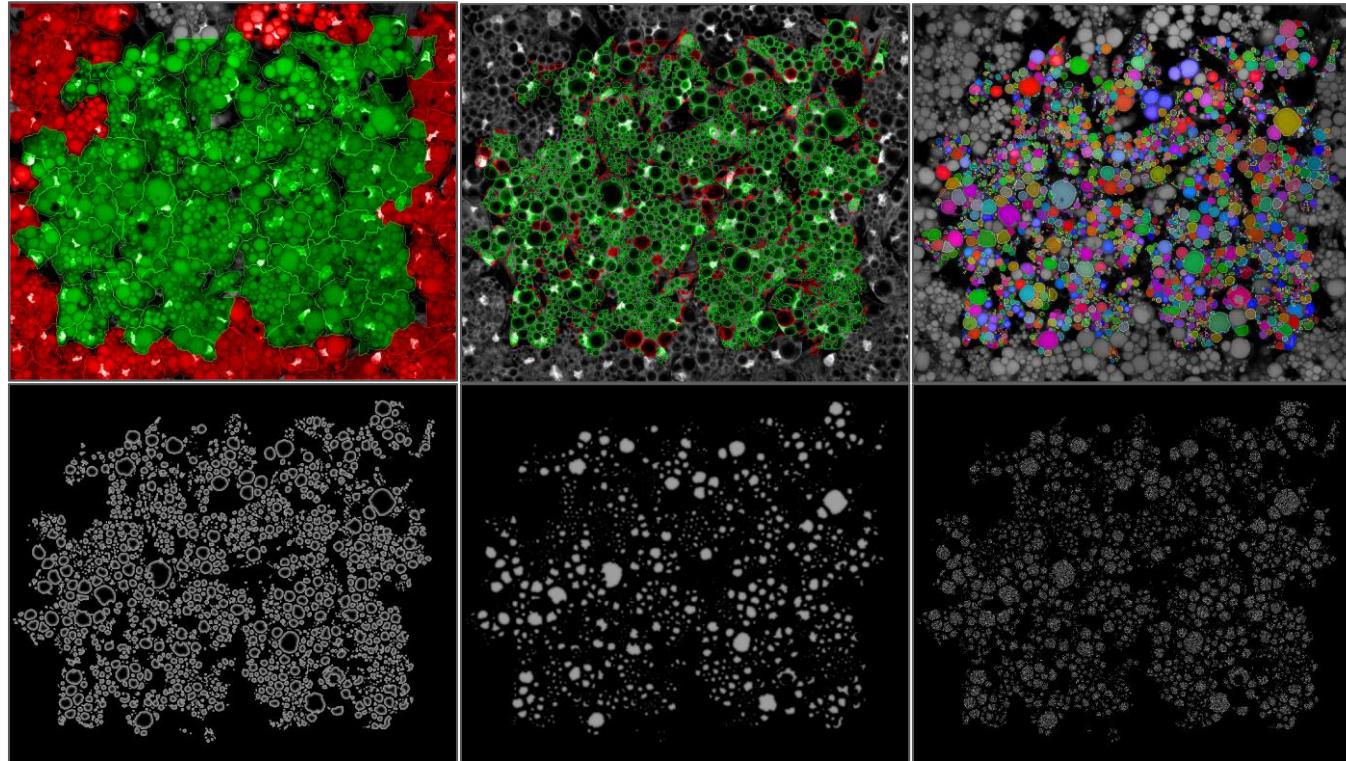
For the Nov 2020 hackathon we are providing CellProfiler analytical pipelines.

These are open source.

For internal work at AstraZeneca we use similar software to extract image data to compare the effects of various potential therapeutics on cell physiology prior to testing in animals or people.



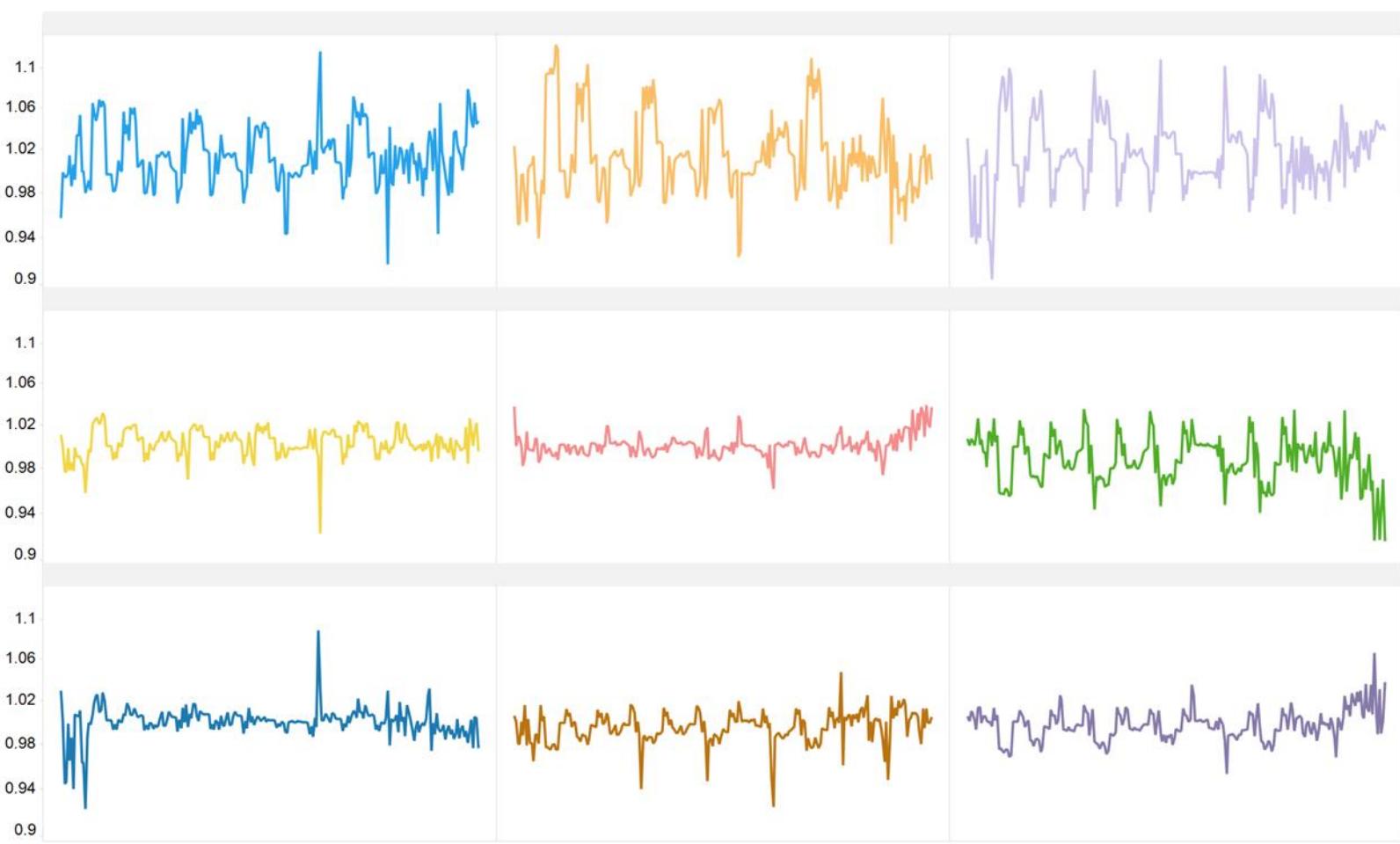
## Textural data extraction from lipid vesicles



Columbus image analysis, **241** parameters per cell  
Focused on lipid phenotypes

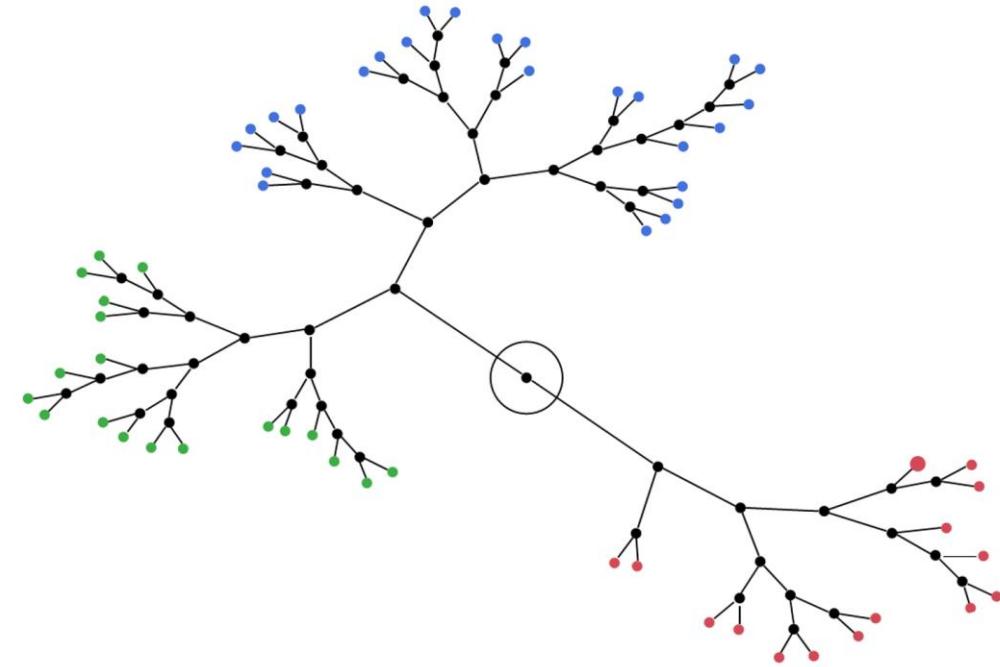
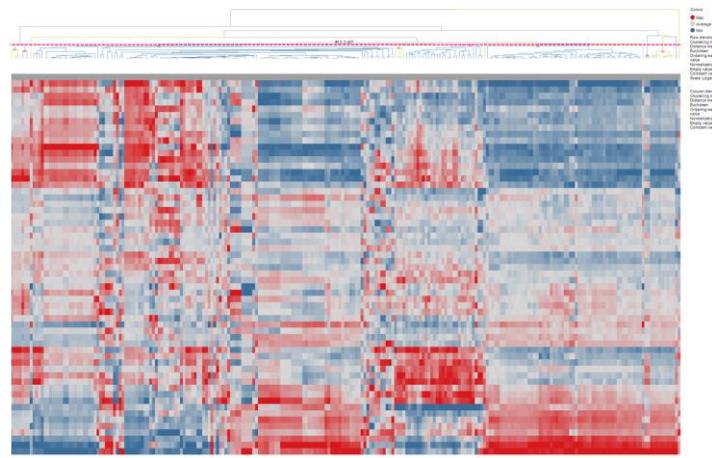
Number  
Size  
Shape  
Intensity  
Texture  
Organisation (symmetries, compactness etc)  
Intracellular distance metrics  
Bisected objects excluded





The various cationic lipids do create distinct phenotypes.

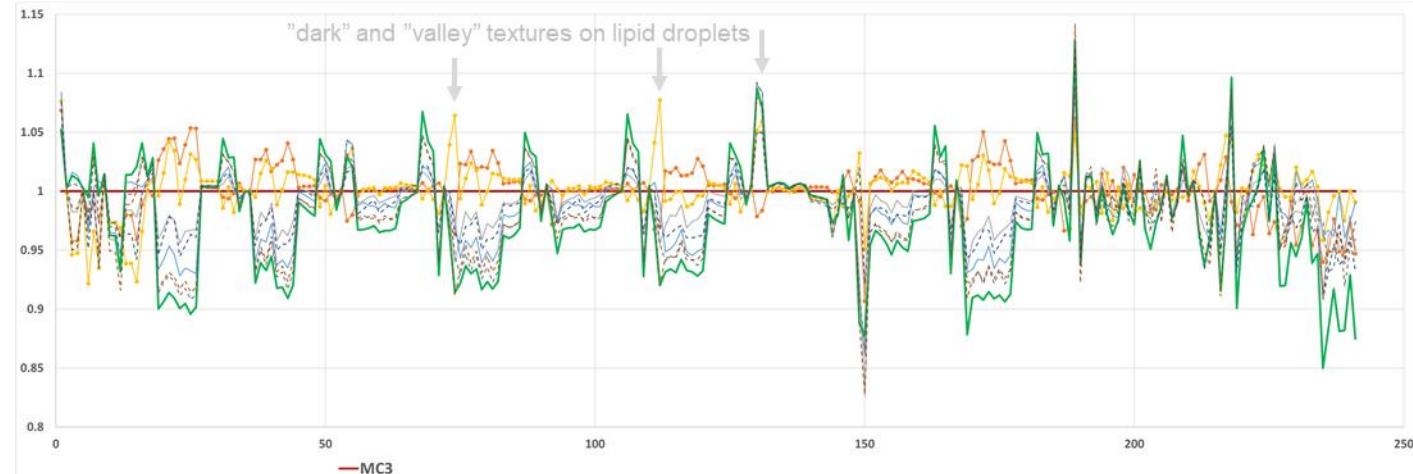




All parameters  
normalised to global  
median/parameter



All parameters  
normalised to MC3



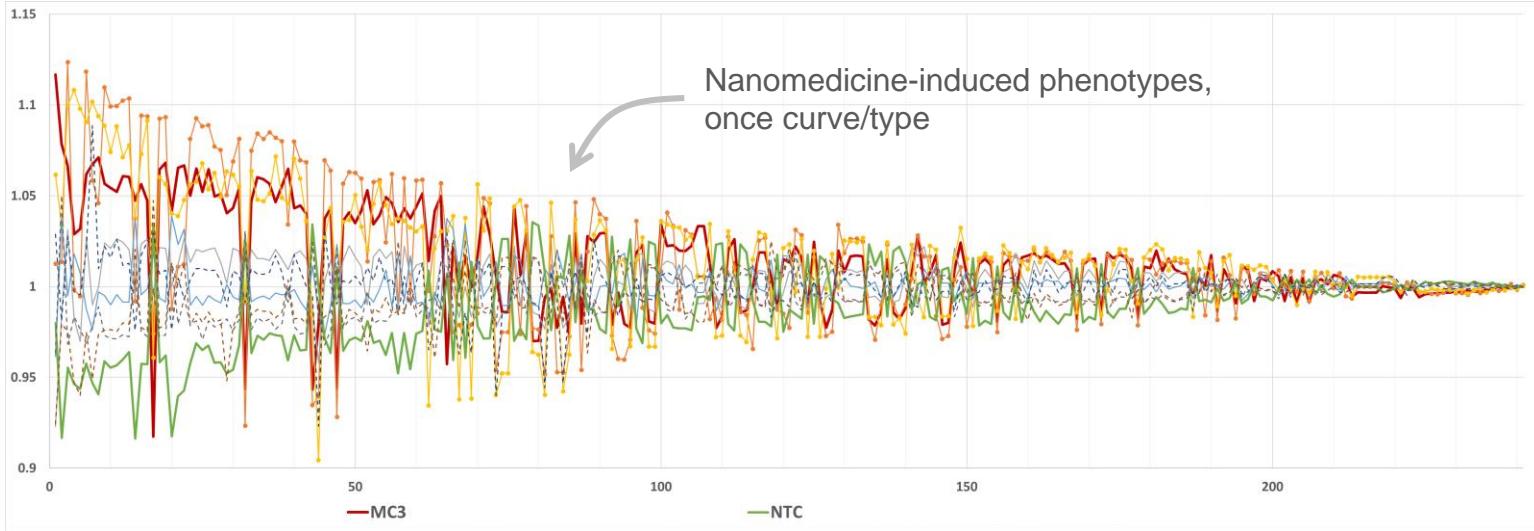
Data overlays shown relative to global parameter medians (top) and to MC3 (bottom)



Focusing on parameters with high info content can reduce computational load.  
Multivariate linear classifiers can be created.

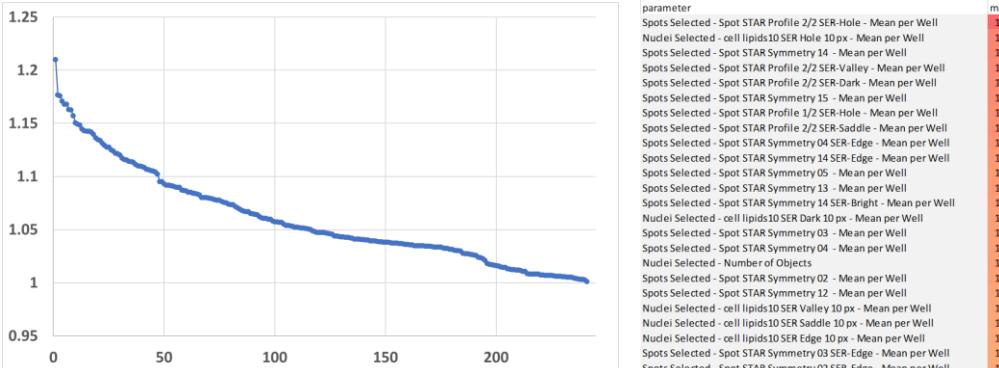
All parameters  
normalised to  
global  
median/parameter

Ranked by  
max/min ratio



All parameters  
normalised to  
global  
median/parameter

Ranked by  
max/min ratio

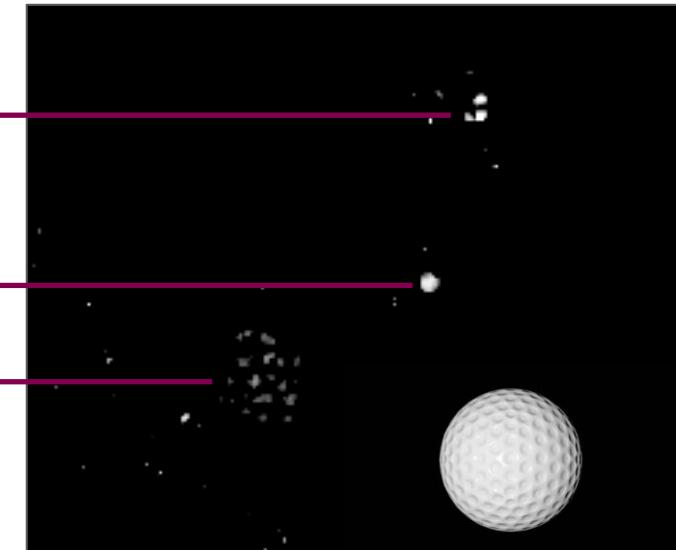
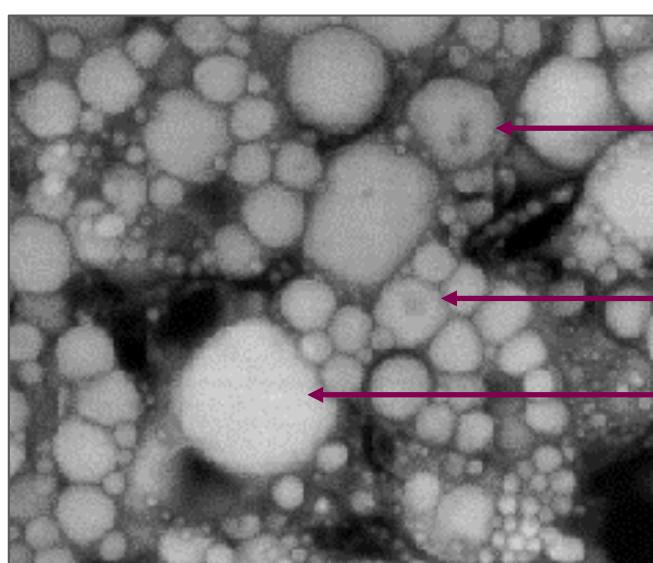
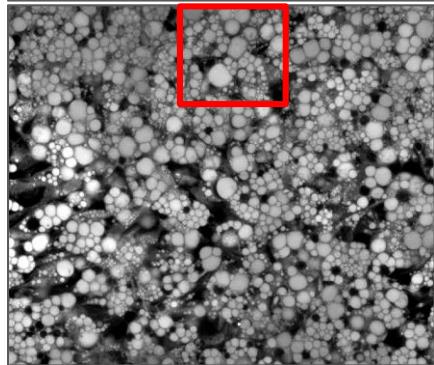


Information content  
Top 10%

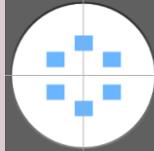
Almost all textural



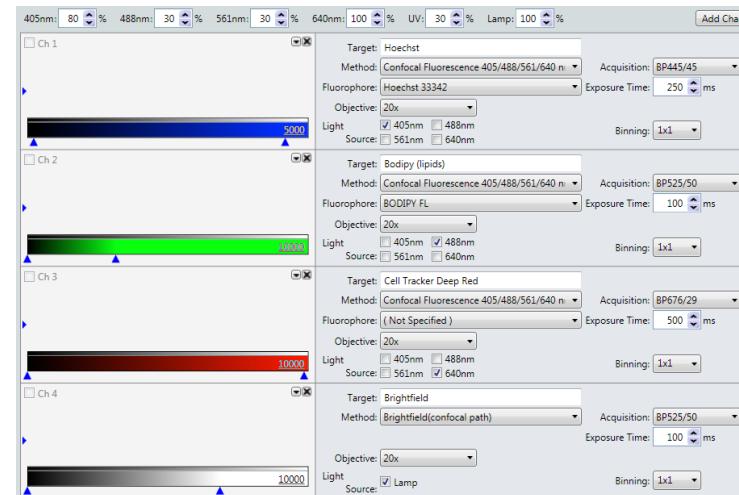
Many phenotypic characteristics are difficult to see.



**About the dataset....**

	<b>20x</b>	<b>40x</b>	<b>60x</b>
Wells	9	9	9
No. of image sites per well	6	8	12
Site orientation			
Z-planes brightfield	7	7	7
Slice interval, um	2	1	1
Stack depth, um	12	7	7
Pixel scale um/pixel	0.3250	0.1625	0.1083
Objective numerical aperture	0.75	0.95	1.2
Blue exposure, ms	250	300	300
Green exposure	100	200	300
Red exposure	500	500	500
Brightfield exposure	100	100	150

Dye	organelle	color
Hoechst 33342	nucleus	blue
Bodipy	Lipids	Green
Cell Tracker Deep Red	Cytoplasm	Red



# Data set file-name reference

File name: AssayPlate\_Greiner\_#655090\_B02\_T0001F001L01A01Z01C01

B02	well (row B, column 02)
T0001	timepoint (irrelevant for this dataset)
F001	field of view = site
L01	timeline (irrelevant for this dataset)
A01	action list number (3 fluorescent + 1 brightfield action)
Z01	3D z-number (slice number or vertical position)
C01	imaging channel (1 nuclear, 2 lipids, 3 cytoplasm, 4 brightfield)



# Example images

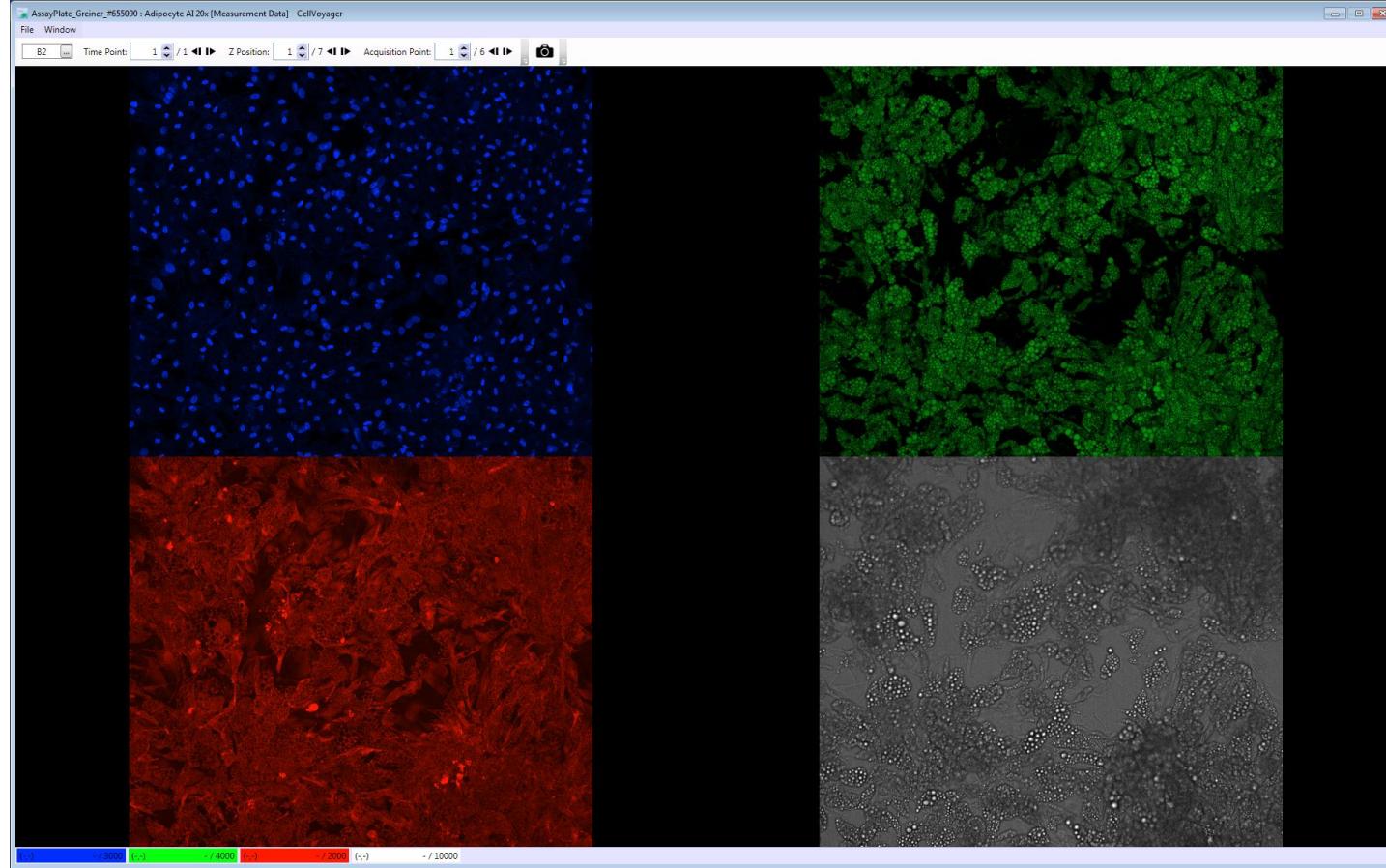
The following slides show sample images from 20, 40 and 60x imaging.

Contrast settings are the same for all channels and magnifications.

Each slide shows, in clockwise order: nuclei, lipids, brightfield, cytoplasm

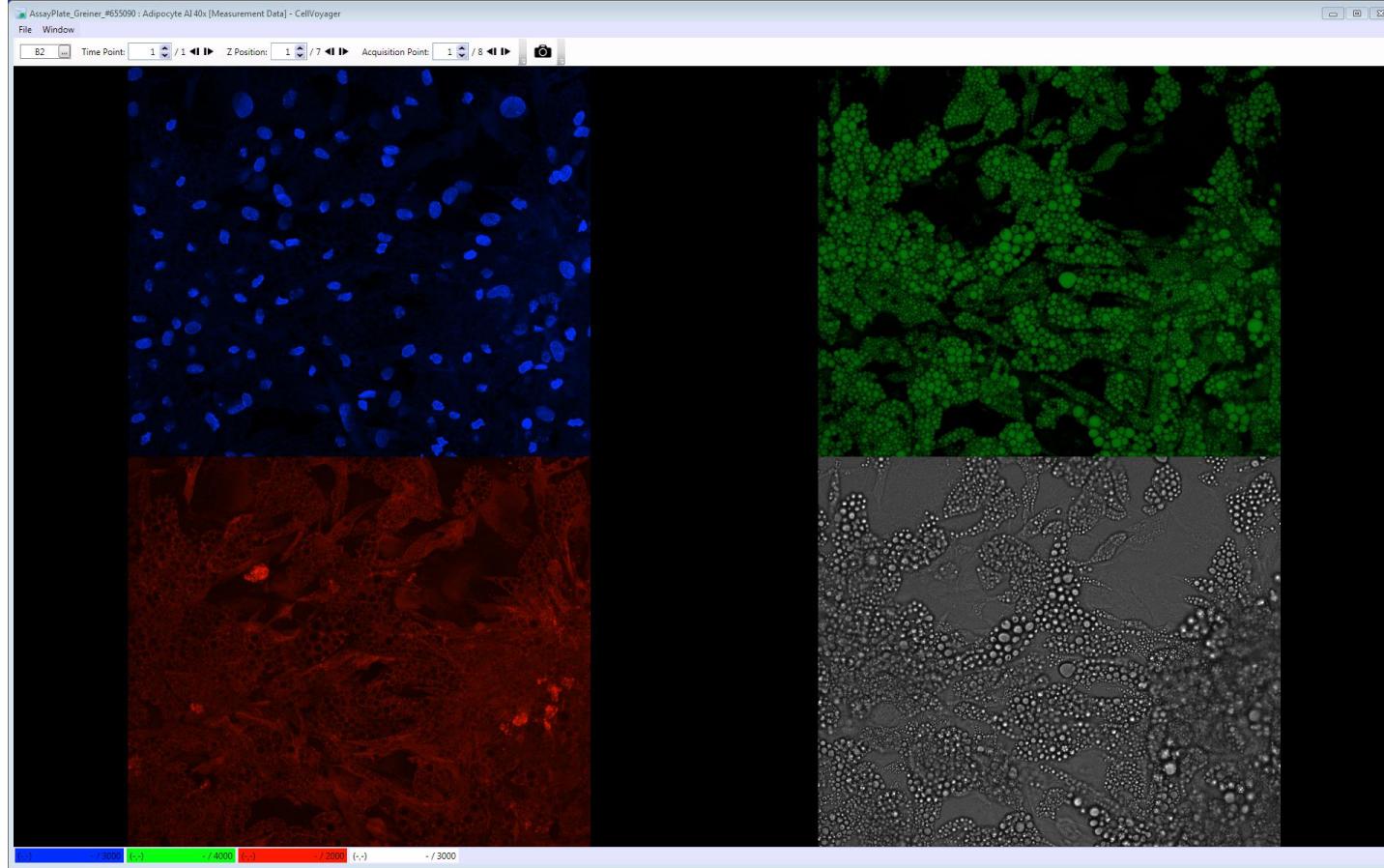
For this dataset the same 9 wells were imaged at all magnifications, but different sites (fields of view, FOVs) and different numbers of sites were imaged at each magnification. There is some site overlap between magnifications.





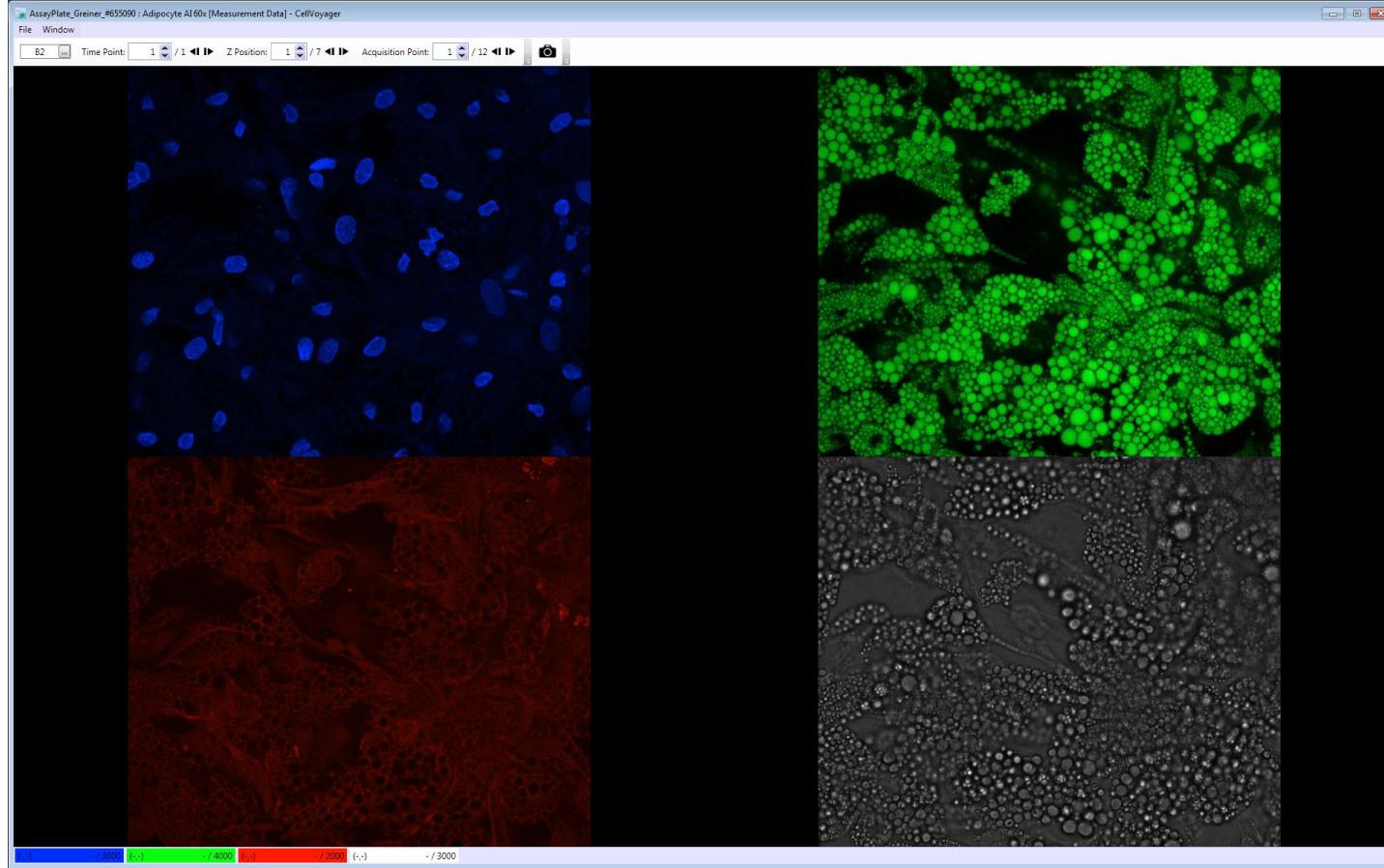
20x plane 1, clockwise: nuclei, lipids, brightfield, cytoplasm





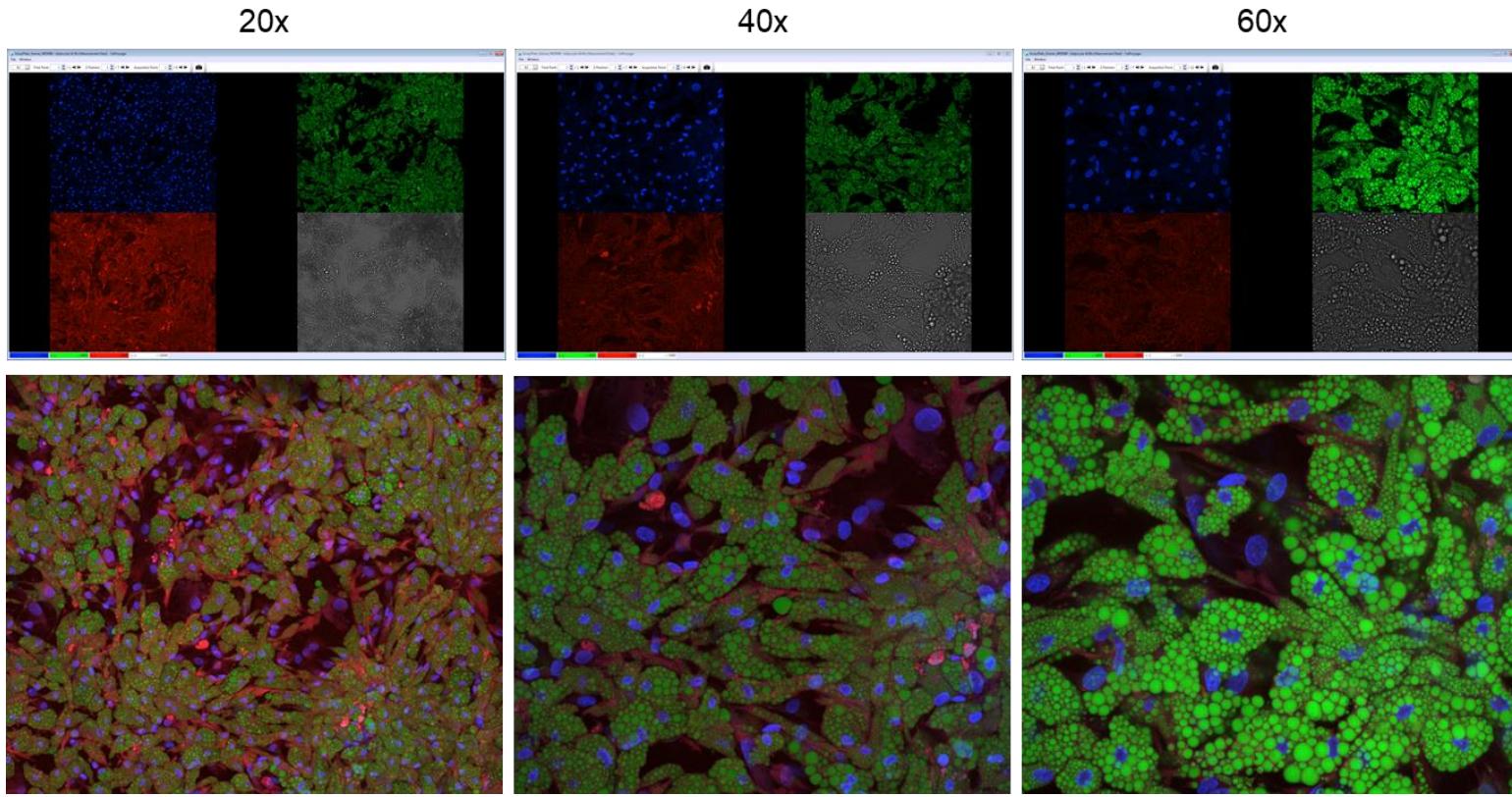
40x plane 1, clockwise: nuclei, lipids, brightfield, cytoplasm





60x plane 1, clockwise: nuclei, lipids, brightfield, cytoplasm



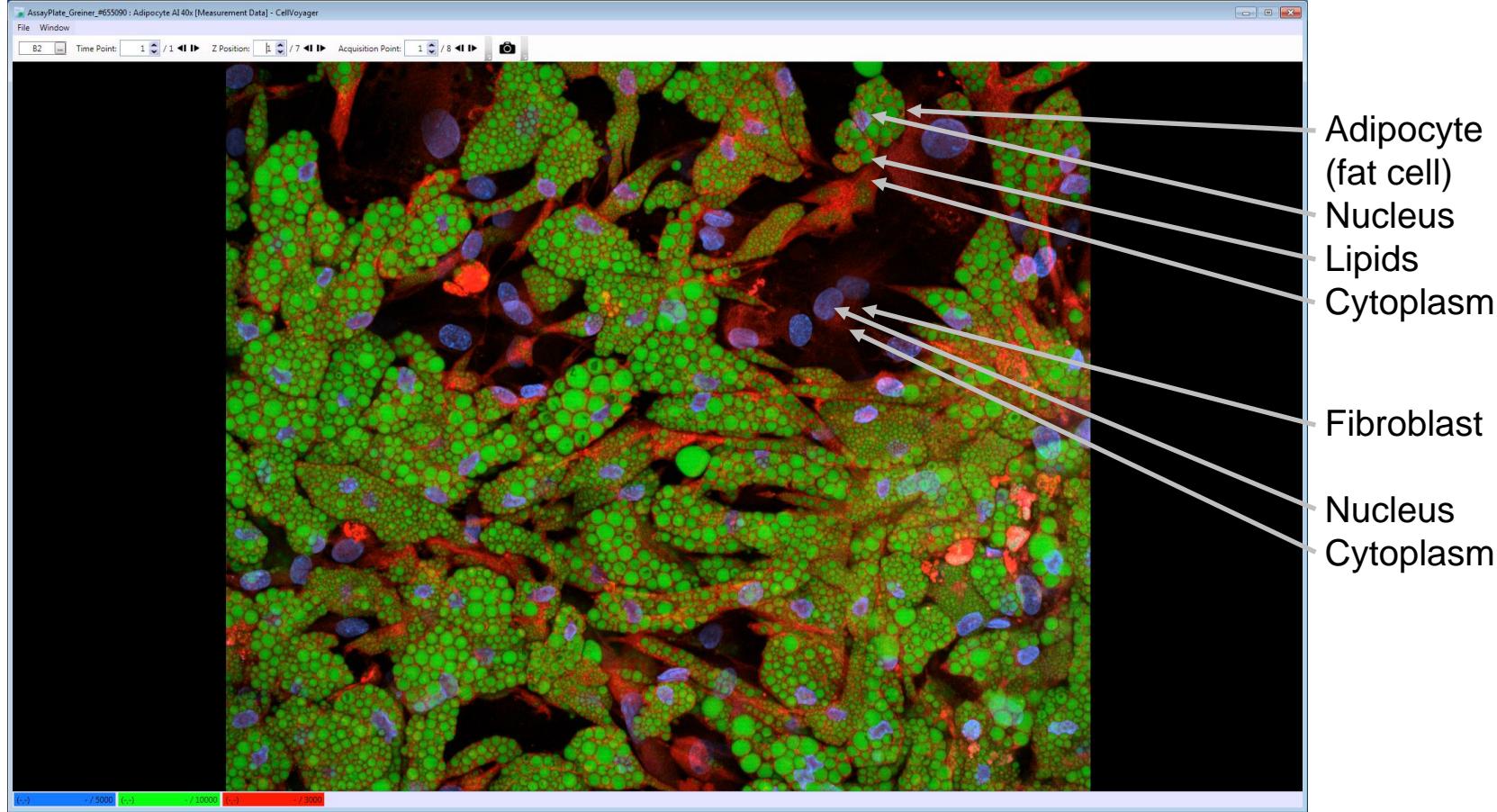


20, 40 and 60x (left to right) images from site one, well B2.

Here the three magnifications are shown together with the same contrast/brightness.

Upper panels show individual channels. Lower panels show an overlay of fluorescence channels (brightfield not shown).



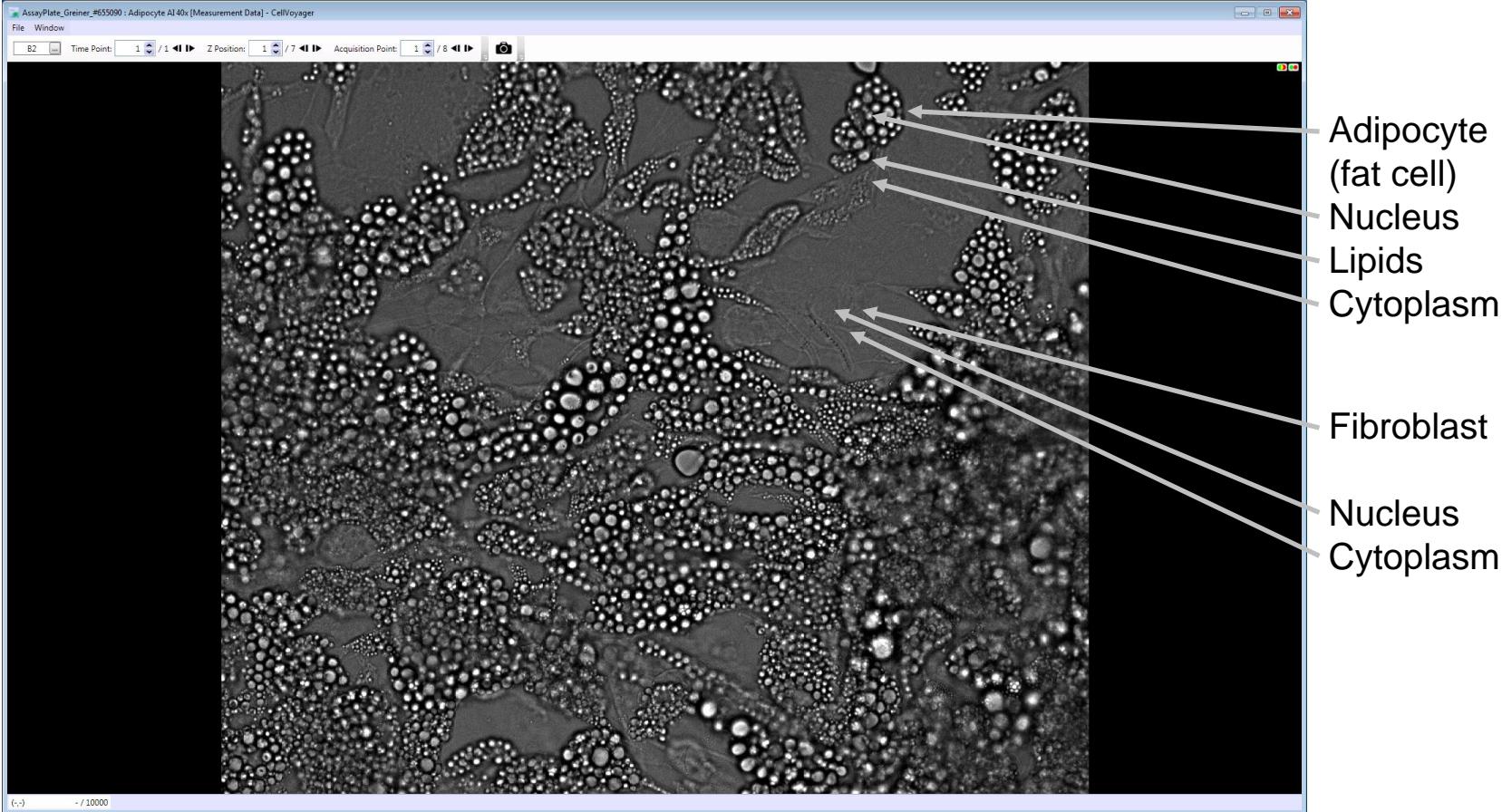


Representative maximum intensity projections of fluorescence channels, 40x.

Fibroblast-like cells are relatively flat and do not contain droplets.

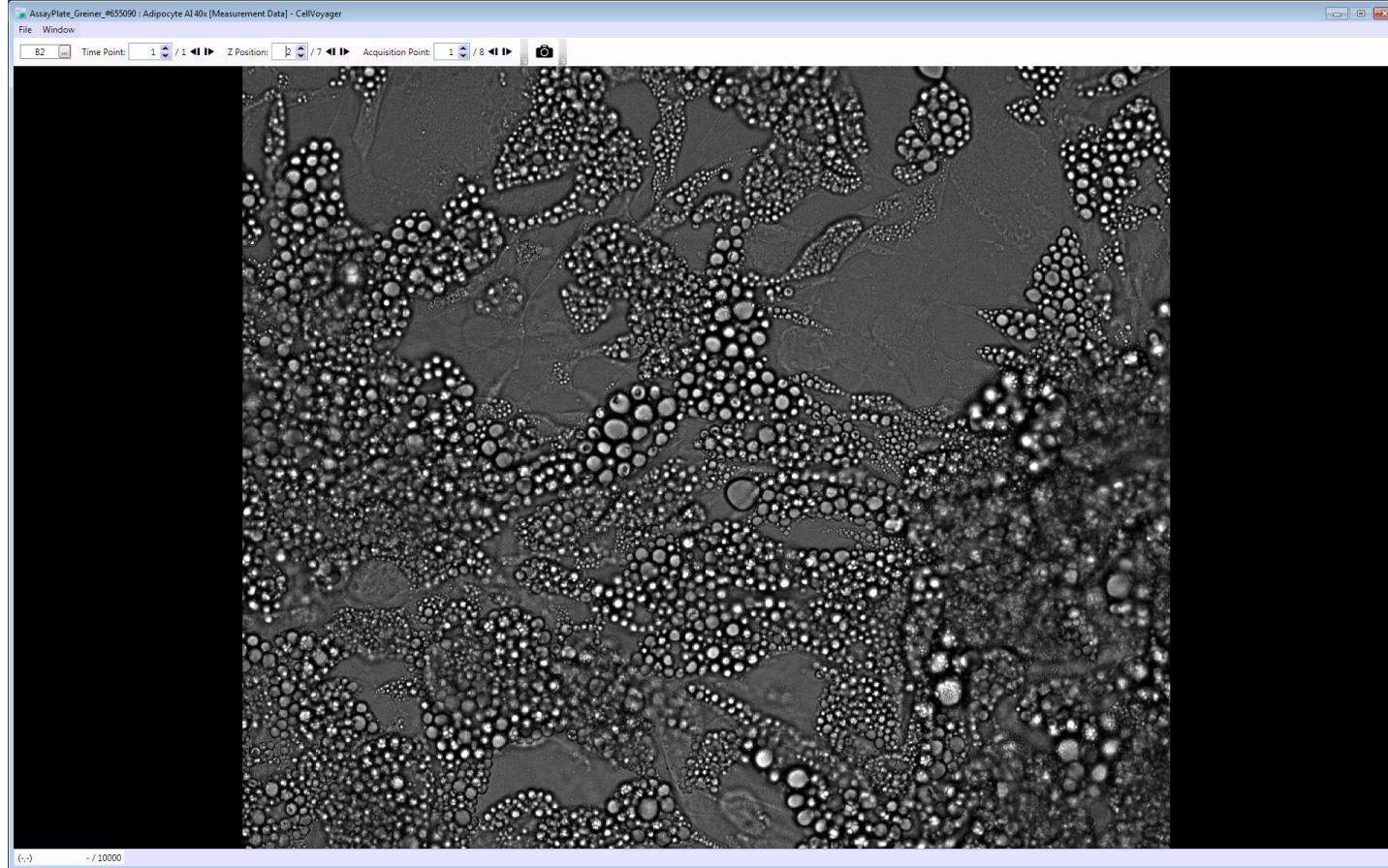
The following slides show representative brightfield images taken at 7 different z positions





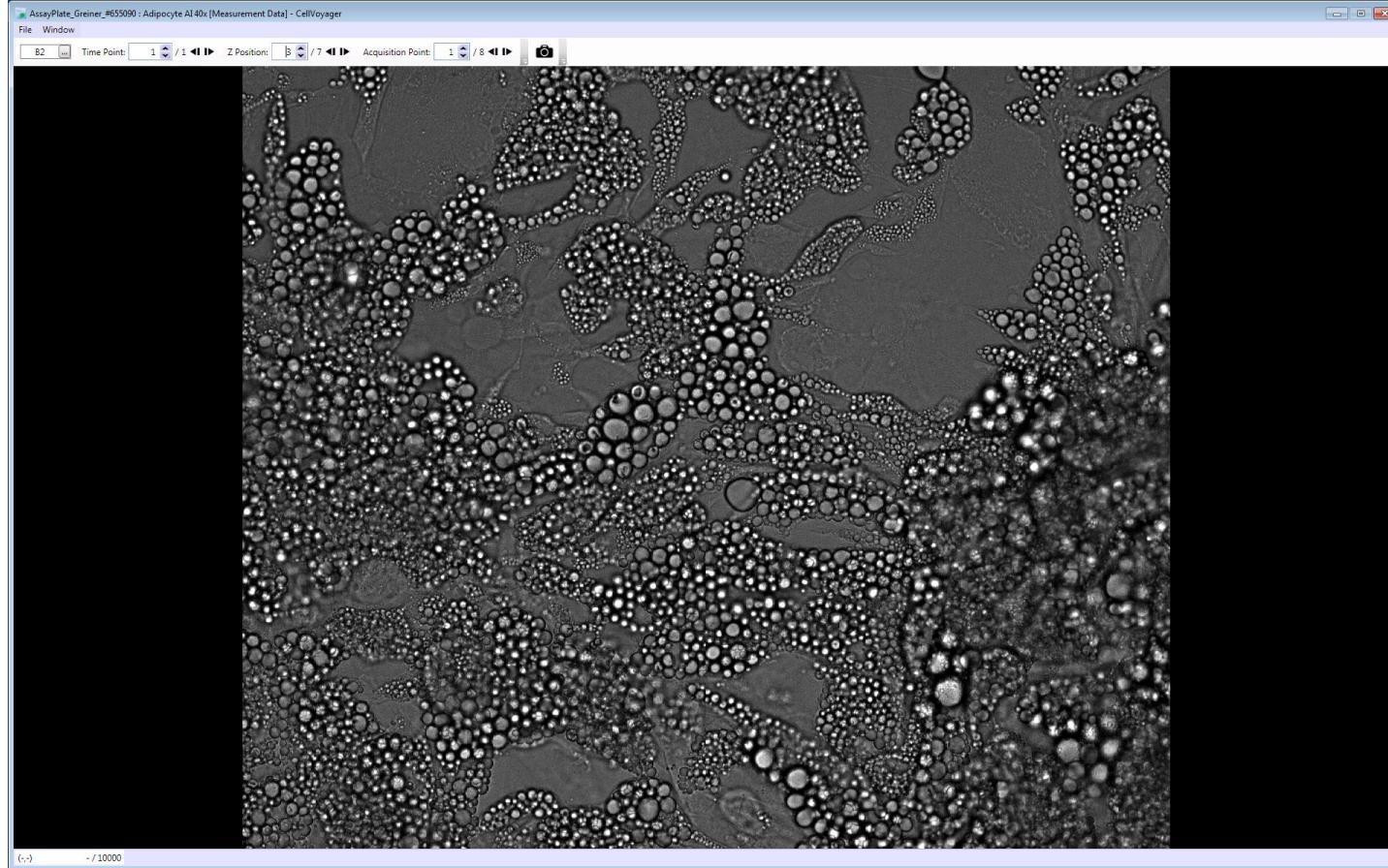
Z planes 1 um slices, slice 1





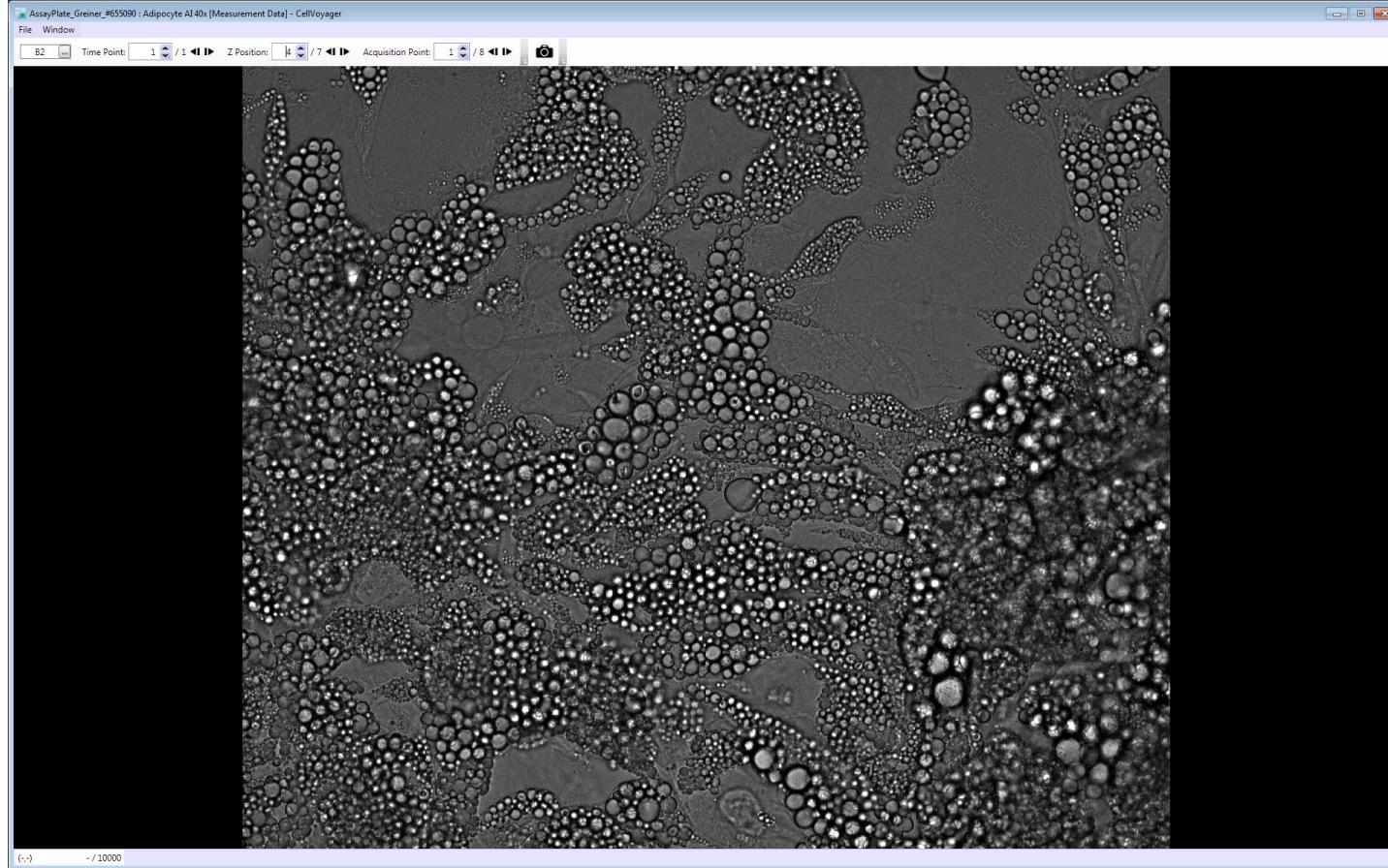
Z planes 1 um slices, slice 2





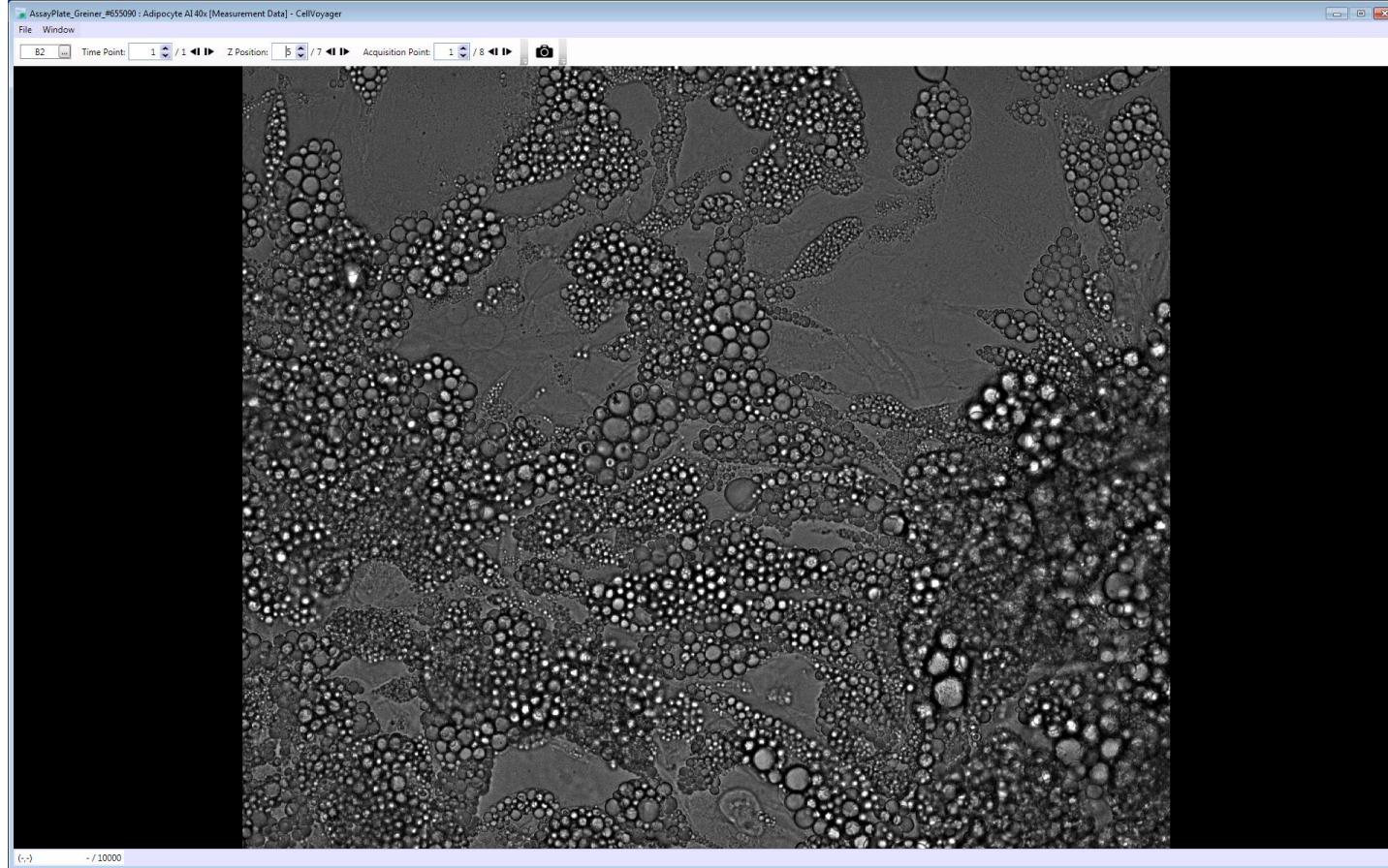
Z planes 1 um slices, slice 3





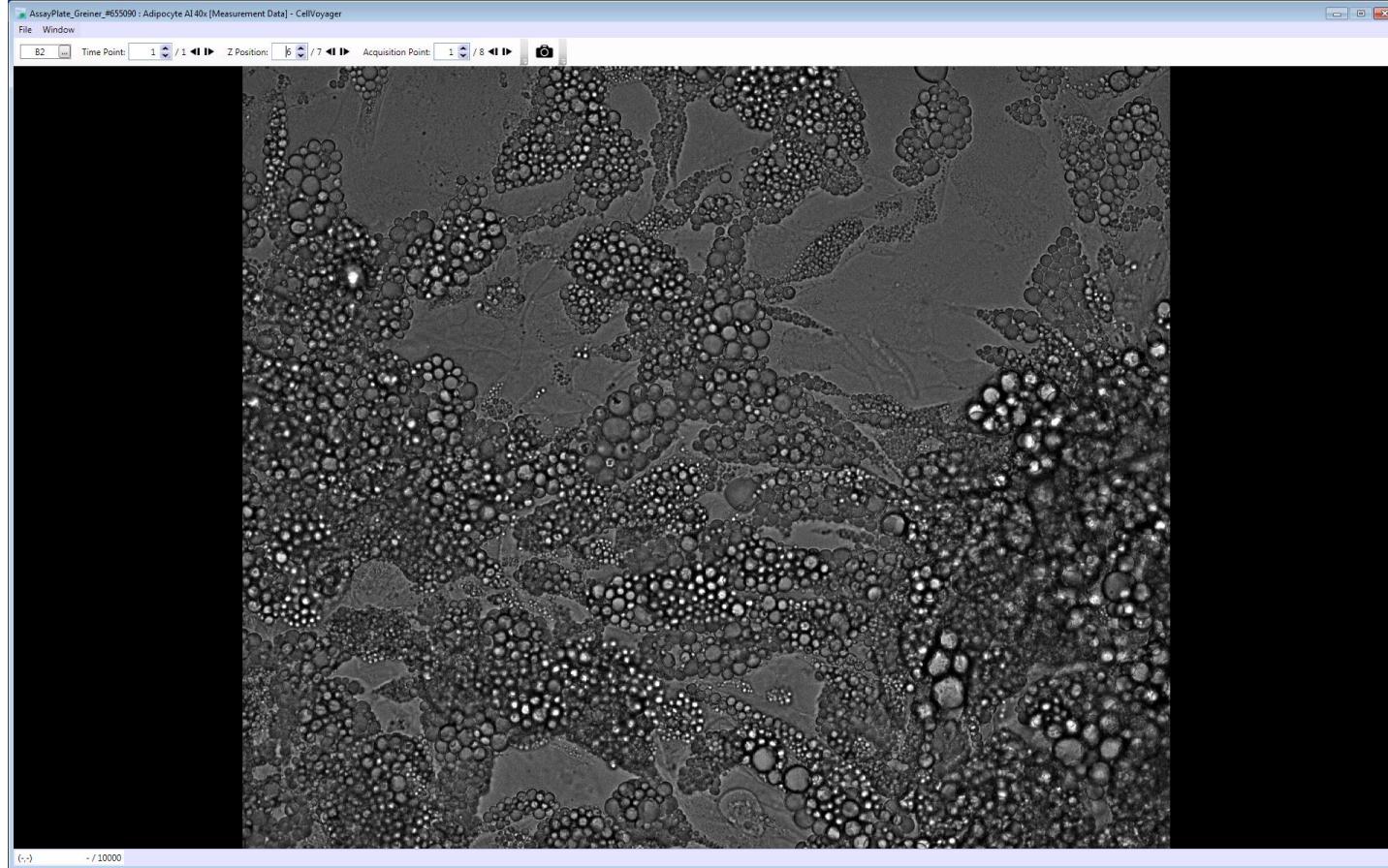
Z planes 1 um slices, slice 4





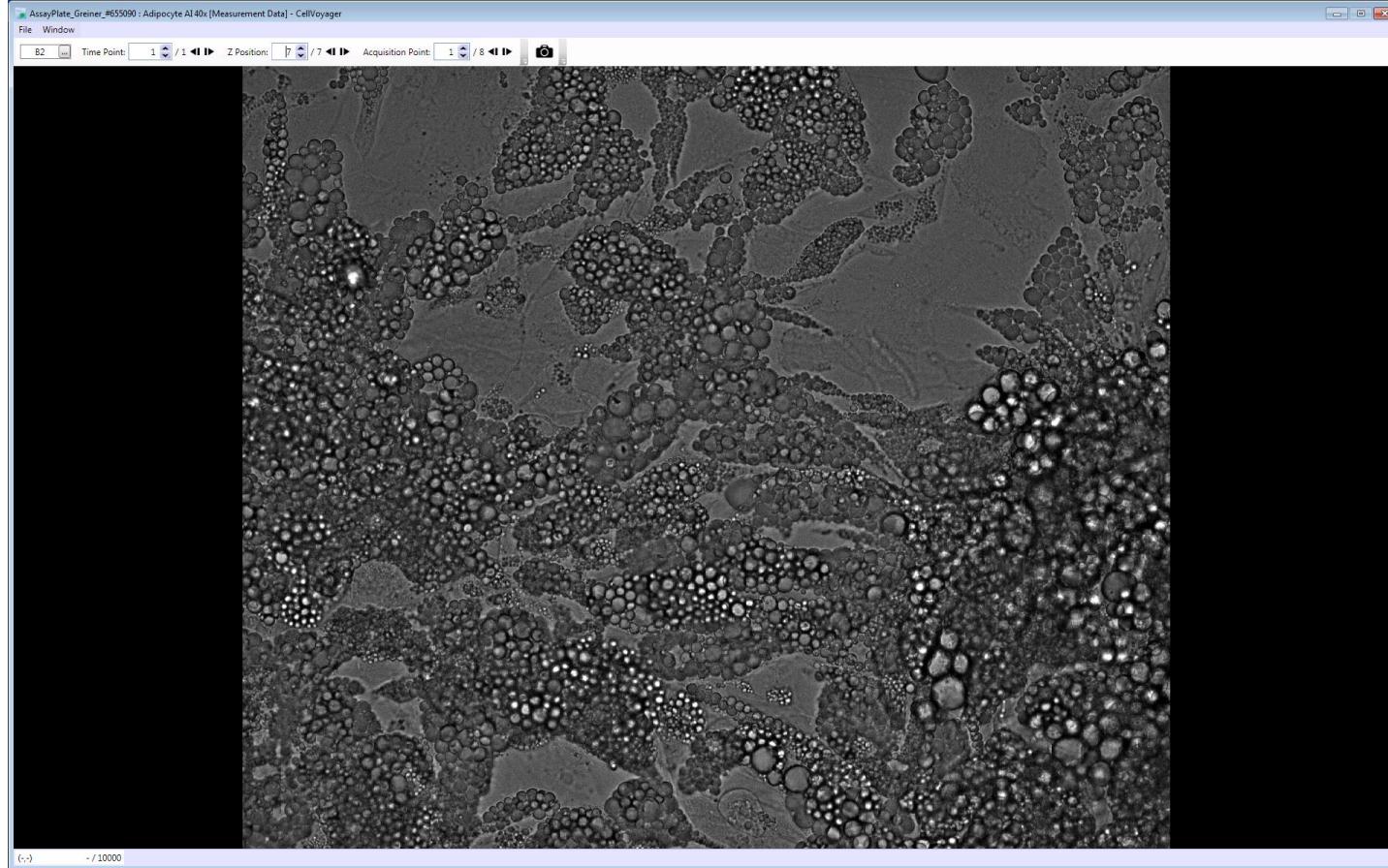
Z planes 1 um slices, slice 5





Z planes 1 um slices, slice 6





Z planes 1 um slices, slice 7



# **CellProfiler parameter description**

# Notes about the pipelines

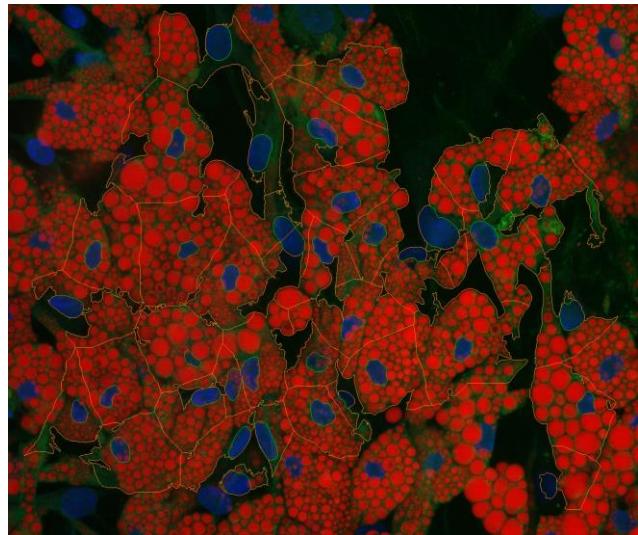
- Designed to emulate the types of analysis typically done (AZ uses proprietary software for this)
- Not designed to be extremely accurate, although they are pretty good
- Allow data patterns to be created for each image, facilitating comparisons using relevant types of analysis
- The lower the magnification, the more objects and the longer the processing time
- Pipelines can be dissected to improve speed (analyse only nuclei for example)
- You will need to adjust the output folder
- There may be errors in these pipelines – updates may occur



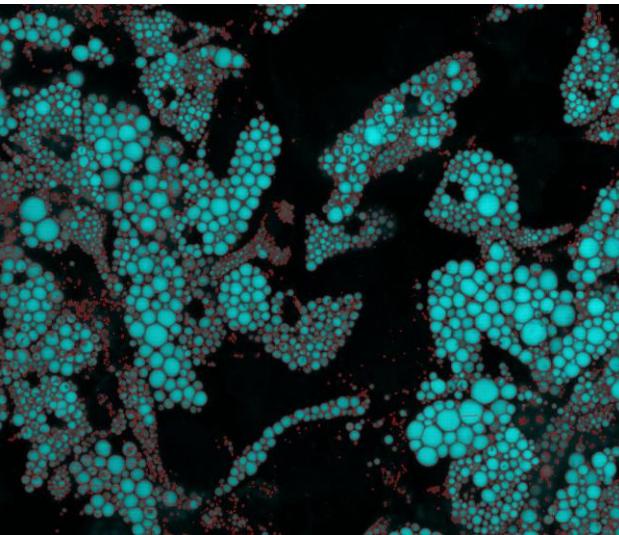
Parameter	Description (from CellProfiler documentation)
Area	The number of pixels in the region.
Compactness	The mean squared distance of the object's pixels from the centroid divided by the area. A filled circle will have a compactness of 1, with irregular objects or objects with holes having a value greater than 1.
Formfactor	Calculated as $4\pi \cdot \text{Area} / (\text{Perimeter})^2$ . Equals 1 for a perfectly circular object.
Mean radius	The mean distance of any pixel in the object to the closest pixel outside of the object.
Perimeter	The total number of pixels around the boundary of each region in the image.
Solidity	The proportion of the pixels in the convex hull that are also in the object, i.e., $\text{ObjectArea} / \text{ConvexHullArea}$ .
Integrated intensity	The sum of the pixel intensities within an object.
Mean intensity	The average edge pixel intensity of an object.
Standard deviation of intensity	The standard deviation of the pixel intensities within an object.
Granularity	The module returns one measurement for each instance of the <b>granularity</b> spectrum.



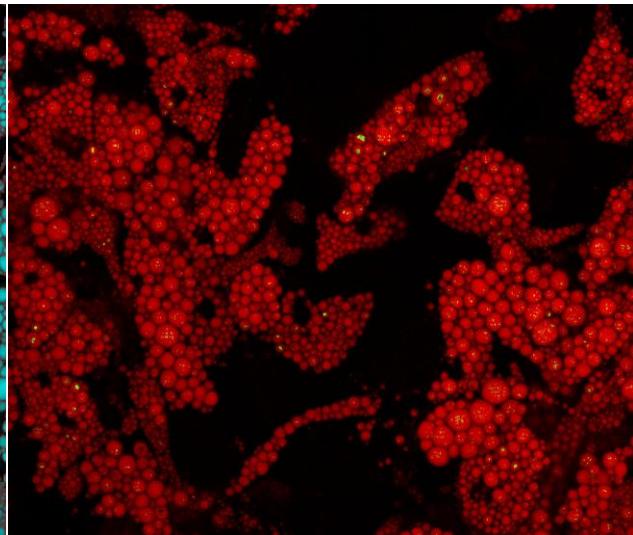
Cell segmentation



Lipid segmentation



Droplet defects



Consistently accurate segmentation is difficult

The idea is to create data for comparisons using analysis close to what will be used



Count_Defective_lipid_droplets
Count_Lipids_no_edge
Count_cells_no_edge
Count_nuclei
Count_nuclei_no_edge
Granularity_10_Bodipy
Granularity_11_Bodipy
Granularity_12_Bodipy
Granularity_13_Bodipy
Granularity_14_Bodipy
Granularity_15_Bodipy
Granularity_16_Bodipy
Granularity_1_Bodipy
Granularity_2_Bodipy
Granularity_3_Bodipy
Granularity_4_Bodipy
Granularity_5_Bodipy
Granularity_6_Bodipy
Granularity_7_Bodipy
Granularity_8_Bodipy
Granularity_9_Bodipy
ImageNumber
Mean_Lipids_no_edge_AreaShape_Area
Mean_Lipids_no_edge_AreaShape_Compactness
Mean_Lipids_no_edge_AreaShape_FormFactor
Mean_Lipids_no_edge_AreaShape_MeanRadius
Mean_Lipids_no_edge_AreaShape_Perimeter
Mean_Lipids_no_edge_AreaShape_Solidity
Mean_Lipids_no_edge_Intensity_IntegratedIntensity_Bodipy
Mean_Lipids_no_edge_Intensity_IntegratedIntensity_Defect_image_rescaled
Mean_Lipids_no_edge_Intensity_MeanIntensity_Bodipy
Mean_Lipids_no_edge_Intensity_MeanIntensity_Defect_image_rescaled
Mean_Lipids_no_edge_Intensity_StdIntensity_Bodipy
Mean_Lipids_no_edge_Intensity_StdIntensity_Defect_image_rescaled
Mean_cells_no_edge_AreaShape_Area
Mean_cells_no_edge_AreaShape_Compactness
Mean_cells_no_edge_AreaShape_FormFactor
Mean_cells_no_edge_AreaShape_MeanRadius
Mean_cells_no_edge_AreaShape_Perimeter
Mean_cells_no_edge_AreaShape_Solidity
Mean_cells_no_edge_Intensity_IntegratedIntensity_Bodipy
Mean_cells_no_edge_Intensity_IntegratedIntensity_CellTrackerRed
Mean_cells_no_edge_Intensity_MeanIntensity_Bodipy
Mean_cells_no_edge_Intensity_MeanIntensity_CellTrackerRed
Mean_cells_no_edge_Intensity_StdIntensity_Bodipy
Mean_cells_no_edge_Intensity_StdIntensity_CellTrackerRed
Mean_nuclei_no_edge_AreaShape_Area
Mean_nuclei_no_edge_AreaShape_Compactness
Mean_nuclei_no_edge_AreaShape_FormFactor
Mean_nuclei_no_edge_AreaShape_MeanRadius
Mean_nuclei_no_edge_AreaShape_Perimeter
Mean_nuclei_no_edge_AreaShape_Solidity
Mean_nuclei_no_edge_Intensity_IntegratedIntensity_Bodipy
Mean_nuclei_no_edge_Intensity_IntegratedIntensity_CellTrackerRed
Mean_nuclei_no_edge_Intensity_IntegratedIntensity_Hoechst
Mean_nuclei_no_edge_Intensity_MeanIntensity_Bodipy
Mean_nuclei_no_edge_Intensity_MeanIntensity_CellTrackerRed
Mean_nuclei_no_edge_Intensity_MeanIntensity_Hoechst
Mean_nuclei_no_edge_Intensity_StdIntensity_Bodipy
Mean_nuclei_no_edge_Intensity_StdIntensity_CellTrackerRed
Mean_nuclei_no_edge_Intensity_StdIntensity_Hoechst
Metadata_FoV
Metadata_Well

Two types of data:

Morphological (area, shape etc.)  
Intensity

Yellow: Object counts

Orange: Lipid droplet granularity

Green: lipid droplet parameters (bodipy dye)

Red: cytoplasmic parameters (CellTracker dye)

Blue: nuclear parameters (Hoechst dye)

Gray: area measures for cells

Pink: meta data

Standard deviation is also measured for many parameters



**Additional technical detail.**

There are CellProfiler pipelines for each magnification that extract nuclear, lipid and cytoplasmic data from the grayscale 16-bit fluorescence images.

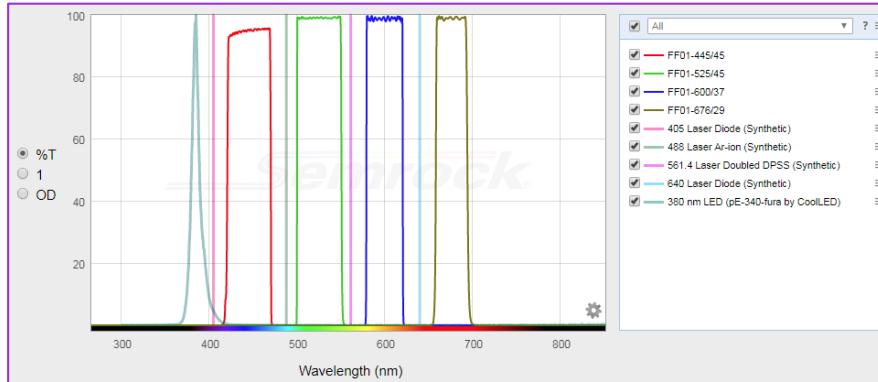
Illumination correction is applied during acquisition.

The dyes, and therefore the images, are relatively consistent in terms of intensities.



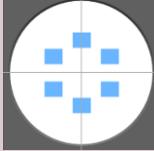
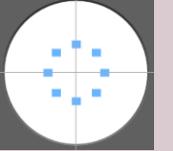
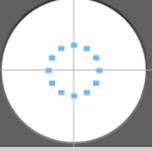
# AstraZeneca Gothenburg

## Yokogawa CV7000 laser lines, emission filters, dyes used



Lasers and lines	Optimal dyes	Emission /width	Color	
UV diode	Hoechst 33342	445/45	blue	Not used
405	Hoechst 33342	445/45	blue	
488	bodipy	525/50	green	
561	AF555, AF568, Propidium iodide	600/37	orange	Not used
640	cell tracker red	676/29	red	



	<b>20x</b>	<b>40x</b>	<b>60x</b>
Wells	9	9	9
No. of image sites per well	6	8	12
Site orientation			
Z-planes brightfield	7	7	7
Slice interval, um	2	1	1
Stack depth, um	12	7	7
Pixel scale um/pixel	0.3250	0.1625	0.1083
Objective numerical aperature	0.75	0.95	1.2
Blue exposure, ms	250	300	300
Green exposure	100	200	300
Red exposure	500	500	500
Brightfield exposure	100	100	150



# AstraZeneca Gothenburg

## Yokogawa CV7000 robotic confocal microscope, optical specifications

Objective	Not used	Not used	Not used	Not used	Not used	Not used
	UPLSAPO 4x	UPLSAPO 10x2	UPLSAPO 20x	LUCPLFLN 20x	UPLSAPO 40x2	UPLSAPO 60xw
Magnification	4x	10x	20x	20x	40x	60x
Air	+	+	+	+	+	-
Water	-	-	-	-	-	+
Phase Contrast	-	-	-	+	-	-
Numerical Aperture	0.16	0.4	0.75	0.45	0.95	1.2
Working distance (mm)	13	3.1	0.6	6.6	0.18	0.28
Field number (mm)	26.5	26.5	26.5	22	26.5	26.5
Pixel size (um)* no binning	1.6250	0.6500	0.3250	0.3250	0.1625	0.1083
Pixel size (um)* 2x2 binning	3.2500	1.3000	0.6500	0.6500	0.3250	0.2167
Image field of view*	4.16 mm x 3.51 mm Area 14.60 mm <sup>2</sup>	1.66 mm x 1.40 mm Area 2.34 mm <sup>2</sup>	0.83 mm x 0.70 mm Area 0.58 mm <sup>2</sup>	0.83 mm x 0.70 mm Area 0.58 mm <sup>2</sup>	0.42 mm x 0.35 mm Area 0.15 mm <sup>2</sup>	0.28 mm x 0.23 mm Area 0.06 mm <sup>2</sup>

\* Andor Zyla cameras have a pixel size of 6.5 um. At 1x1 binning, and 40x, the pixel size is therefore  $6.5/40 = 0.1625$  um. Full resolution: 2560x2160, pixel aspect = 1.

Not used



# Zyla sCMOS

Dynamically Image Cells with  
Breakthrough Precision and Clarity



## ZYLA 4.2 PLUS

- 4.2 megapixel sCMOS
- 82% QE, optimized for all fluorophores
- 0.9 e<sup>-</sup> read noise
- 100 fps (53 fps USB 3.0)
- 33,000:1 dynamic range

## ZYLA 5.5

- 5.5 megapixel sCMOS
- Rolling & True Global Shutter
- 0.9 e<sup>-</sup> read noise
- 100 fps (40 fps USB 3.0)
- 33,000:1 dynamic range

- ✓ QE Boosted to 82%
- ✓ Industry fastest USB 3.0 speeds
- ✓ >99.8 % Quantitative Linearity

Click for  
brochure



## TECHNICAL SPECIFICATIONS

### MODEL SPECIFIC SPECIFICATIONS<sup>a</sup>

Model	Zyla 5.5	Zyla 4.2 PLUS
Sensor type	Front Illuminated Scientific CMOS	Front Illuminated Scientific CMOS
Active pixels (W x H)	2560 x 2160 (5.5 Megapixel)	2048 x 2048 (4.2 Megapixel)
Sensor size	16.6 x 14.0 mm 21.8 mm diagonal	13.3 x 13.3 mm 18.8 mm diagonal
Pixel readout rate (MHz)	200 (100 MHz x 2 sensor halves) 560 (280 MHz x 2 sensor halves)	Slow Read 216 (108 MHz x 2 sensor halves) Fast Read 540 (270 MHz x 2 sensor halves)
Read noise (e <sup>-</sup> ) Median [rms] <sup>a</sup>	@ 200 MHz 0.9 [1.2] @ 560 MHz 1.2 [1.6]	@ 216 MHz 0.90 [1.1] @ 540 MHz 1.10 [1.3]
Maximum Quantum Efficiency <sup>a</sup>	64%	82%
Sensor Operating Temperature		
Air cooled	0°C (up to 30°C ambient)	0°C (up to 27°C ambient)
Water cooled	-10°C*	-10°C*
Dark current, e <sup>-</sup> /pixel/sec @ min temp <sup>a</sup>		
Air cooled	0.10	0.10
Water cooled	0.019	0.019
Readout modes	Rolling Shutter and True Global Shutter (Snapshot)	Rolling Shutter and Global Clear <sup>a</sup>
Maximum dynamic range	33,000:1	33,000:1
Photon Response Non-Uniformity (PRNU)		
Half-light range	< 0.01%	< 0.1%
Low light range	< 0.1%	< 0.1%
Pre-defined Region of Interest (ROI)	2048 x 2048, 1920 x 1080, 1392 x 1040, 512 x 512, 128 x 128	1920 x 1080, 1392 x 1040, 512 x 512, 128 x 128
User defined ROI (Granularity)	Yes (1 pixel) **	
Data range	12-bit (fastest USB 3.0 speeds) and 16-bit (maximum dynamic range)	
Interface options	USB 3.0 <sup>a</sup> Camera Link 10-tap	

\* Cooling temperature must be above the dew point

\*\* Minimum ROI size 4 x 8 (W x H) possible for 12- or 16-bit modes and for both Camera Link 10-tap and USB 3.0 models

### GENERAL SPECIFICATIONS<sup>a</sup>

Pixel size (W x H)	6.5 µm
Pixel well depth (e <sup>-</sup> )	30,000
Linearity (%), maximum <sup>a</sup>	
Full light range	Better than 99.8%
Low light range (< 1000 electrons signal)	Better than 99.9%
MTF (Nyquist @ 555 nm)	45%
Pixel binning	Hardware binning: 2 x 2, 3 x 3, 4 x 4, 8 x 8
Anti-blooming factor	x 10,000
I/O	External Trigger, Fire, Fire n, Fire All, Fire Any, Arm
Trigger Modes	Internal, External, External Start, External Exposure, Software Trigger
Software Exposure Events <sup>a</sup>	Start exposure - End exposure (row 1), Start exposure - End exposure (row n)
Hardware timestamp accuracy	25 ns
Internal memory	1 GB

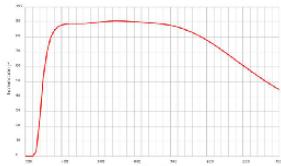


## Data Sheet

# UPLSAPO 20X

### UPLSAPO Series

Thanks to the application of Olympus' original UW multicoating, these Super Apochromat objectives fully compensate for both spherical and chromatic aberrations from the UV to the near infrared region. Their sensitivity to fluorescence emissions ensures the acquisition of sharp, clear images, without color shift, even in brightfield and Nomarski DIC observations.



W.D.	MAG	F.N.	NA	IM	
BF	DF	FL	DIC	IR	TIRF
0.6	20x	26.5	0.75	-	
MPE	PH	PO	RC	UV	CY

Numerical Aperture (NA)	0.75
Working distance (W.D.) (mm)	0.6
Field Number (F.N.)	26.5
Cover Glass Thickness (mm)	0.17
Immersion	-
Spring	Yes
Correction Collar	-
Iris Diaphragm	-
Waterproof & Oil proof function	-
Ultra Wide anti-reflection coating	Yes
Super apochromatic	Yes
Brightfield	Excellent
Darkfield	Good
DIC	Excellent
Phase Contrast	N/A
Relief Contrast	N/A
Polarized Light	Good
Fluorescence (B, G Excitation)	Excellent
UV Fluorescence (at 365nm)	Excellent
IR DIC	Good
Multi photon excitation	Limitation

Specifications, design and accessories are subject to change without any notice or obligation on the part of the manufacturer.

**OLYMPUS**

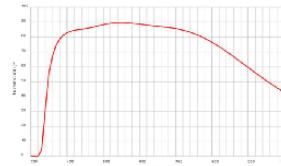
05/12 DEH

## Data Sheet

# UPLSAPO 40X2

### UPLSAPO Series

Thanks to the application of Olympus' original UW multicoating, these Super Apochromat objectives fully compensate for both spherical and chromatic aberrations from the UV to the near infrared region. Their sensitivity to fluorescence emissions ensures the acquisition of sharp, clear images, without color shift, even in brightfield and Nomarski DIC observations.



W.D.	MAG	F.N.	NA	IM	
BF	DF	FL	DIC	IR	TIRF
0.18	40x	26.5	0.95	-	
MPE	PH	PO	RC	UV	CY

Numerical Aperture (NA)	0.95
Working distance (W.D.) (mm)	0.18
Field Number (F.N.)	26.5
Cover Glass Thickness (mm)	0.11 - 0.23
Immersion	-
Spring	Yes
Correction Collar	Yes
Iris Diaphragm	-
Waterproof & Oil proof function	-
Ultra Wide anti-reflection coating	Yes
Super apochromatic	Yes
Brightfield	Excellent
Darkfield	N/A
DIC	Excellent
Phase Contrast	N/A
Relief Contrast	N/A
Polarized Light	Good
Fluorescence (B, G Excitation)	Excellent
UV Fluorescence (at 365nm)	Excellent
IR DIC	Good
Multi photon excitation	Limitation

Specifications, design and accessories are subject to change without any notice or obligation on the part of the manufacturer.

**OLYMPUS**

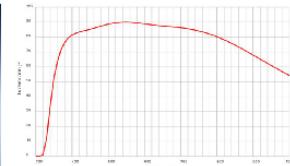
05/12 DEH

## Data Sheet

# UPLSAPO 60XW

### UPLSAPO Series

Thanks to the application of Olympus' original UW multicoating, these Super Apochromat objectives fully compensate for both spherical and chromatic aberrations from the UV to the near infrared region. Their sensitivity to fluorescence emissions ensures the acquisition of sharp, clear images, without color shift, even in brightfield and Nomarski DIC observations.



W.D.	MAG	F.N.	NA	IM	
BF	DF	FL	DIC	IR	TIRF
0.28	60x	26.5	1.2	Water	
MPE	PH	PO	RC	UV	CY

Numerical Aperture (NA)	1.2
Working distance (W.D.) (mm)	0.28
Field Number (F.N.)	26.5
Cover Glass Thickness (mm)	0.13 - 0.21
Immersion	Water
Spring	Yes
Correction Collar	Yes
Iris Diaphragm	-
Waterproof & Oil proof function	Yes
Ultra Wide anti-reflection coating	Yes
Super apochromatic	Yes
Brightfield	Excellent
Darkfield	N/A
DIC	Excellent
Phase Contrast	N/A
Relief Contrast	N/A
Polarized Light	Good
Fluorescence (B, G Excitation)	Excellent
UV Fluorescence (at 365nm)	Excellent
IR DIC	Good
Multi photon excitation	Good

Specifications, design and accessories are subject to change without any notice or obligation on the part of the manufacturer.

**OLYMPUS**

