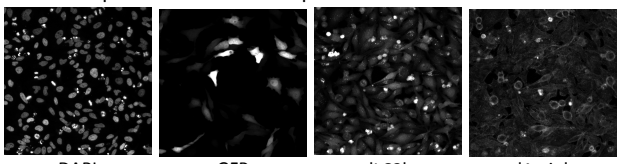
Example: Set 3 Part 1 Rep 2 D04 Site 3

Import images

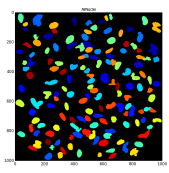


Filter image files based upon name

Images grouped into respective wells, and separated by channel (DAPI, GFP, 'LC3', and 'Actin')

'LC3' and 'Actin' refer to mCherry and phalloidin detection, respectively.

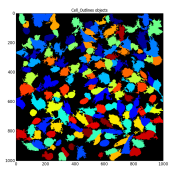
Identify Nuclei



Nuclei identified by signal intensity from the DAPI channel and highlighted in a range of colours.

Identify outer limits of cells

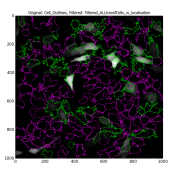
Nuclei identified above were used as 'seeds' from which to identify the outer limits of cells from the actin channel



Cells identified by signal intensity from the 'actin' channel and highlighted in a range of colours.

Measure the amount of GFP signal within each identified cell

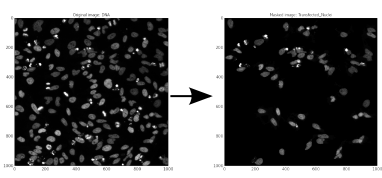
Cell 'objects' from above were individually measured for GFP content, identifying transfected cells



Cells outlines of transfected cells highlighted in green. Untransfected cells are excluded and are highlighted in fuchsia.

Mask nuclei to leave transfected nuclei

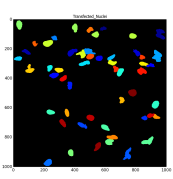
Use identified transfected cells 'as a mask' so that only transfected nuclei remain



Only masked nuclei remain, representing those of transfected cells.

Identify transfected nuclei

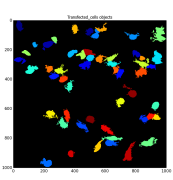
Signal intensity and size used to identify transfected nuclei as 'objects'



Transfected cell nuclei identified by signal intensity from masked DAPI channel and highlighted in a range of colours.

Identify transfected cells

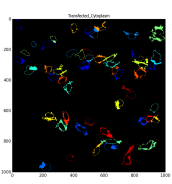
Transfected nuclei used as seeds to identify transfected cells as 'objects'



Transfected cells are identified by signal intensity from the 'Actin' channel using transfected nuclei as seeds, and then highlighted in a range of colours.

Identify cell cytoplasm minus nuclei

Transfected nuclei objects removed from transfected cell objects to leave transfected cytoplasm



Transfected cytoplasm for each transfected cell, highlighted using a range of colours.

Measure objects

Transfected nuclei and cells measured

Object	Feature	Mean	Median	STD
Transfected_Nuclei	Eccentricity	0.76	0.80	0.13
Transfected_Nuclei	MajorAxisLength	59.11	57.88	12.12
Transfected_Nuclei	MinorAxisLength	35.89	33.51	7.59
Transfected_Nuclei	Orientation	4.48	6.05	44.46
Transfected_Nuclei	Compactness	1.22	1.19	0.16
Transfected_Nuclei	Area	1595.36	1480.00	505.14
Transfected_Nuclei	Center_X	536.91	539.00	261.59

Example of some measurements obtained from the object measurement module.

Measure correlations

Correlation between transfected cell GFP-actin, and GFP-DAPI measured

Measure radial distribution

Measurement of the mean fractional intensity of GFP in each of 4 bins

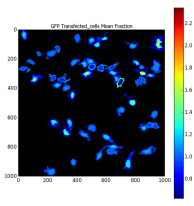
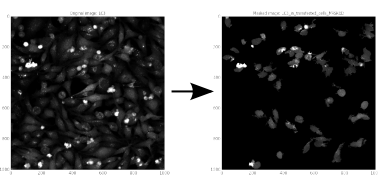


Image	Objects	Bin	Fraction	Intensity	COV
GFP	Transfected_cells	1	0.0331	1.1558	0.0406
GFP	Transfected_cells	2	0.1318	1.1332	0.0722
GFP	Transfected_cells	3	0.2828	1.0867	0.115
GFP	Transfected_cells	4	0.5523	0.9414	0.233

Intensity of GFP in each given fraction is shown in a heat map: a colour closer to red is displayed when GFP is localised towards the nuclear region. The table shows average fractional intensity within each bin for all transfected cells.

Mask LC3 in transfected cells

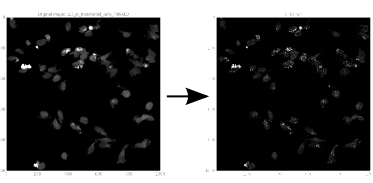
Transfected cell objects used to mask LC3 channel



LC3 channel masked so that only transfected cell LC3 remains.

Morph transfected LC3 with Tophat function

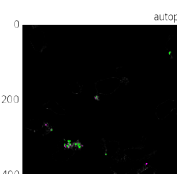
Transfected LC3 masked image treated with Tophat filter



Transfected cell LC3 subjected to a Tophat transformation, highlighting puncta in cells.

Identify autophagosomes from LC3 channel

Transfected cell LC3 image used to identify suspected autophagosomes using intensity and size



Autophagosomes identified by signal intensity from LC3 channel and highlighted in a range of colours. Due to their size, a zoom of the CellProfiler output is shown.

Measure autophagosomes

Based on the morphed LC3 image, the area and perimeter of detectable autophagosomes was calculated

Maths module calculation

Number of autophagosomes are divided by the number of transfected cells identified

Measure correlation

Correlation between transfected cell GFP-LC3 measured

Export to database

All data exported to a SQLite database for analysis in CellProfiler Analyst