SUPPLEMENTARY INFORMATION

Differential labelling of human sub-cellular compartments with fluorescent dye esters and expansion microscopy

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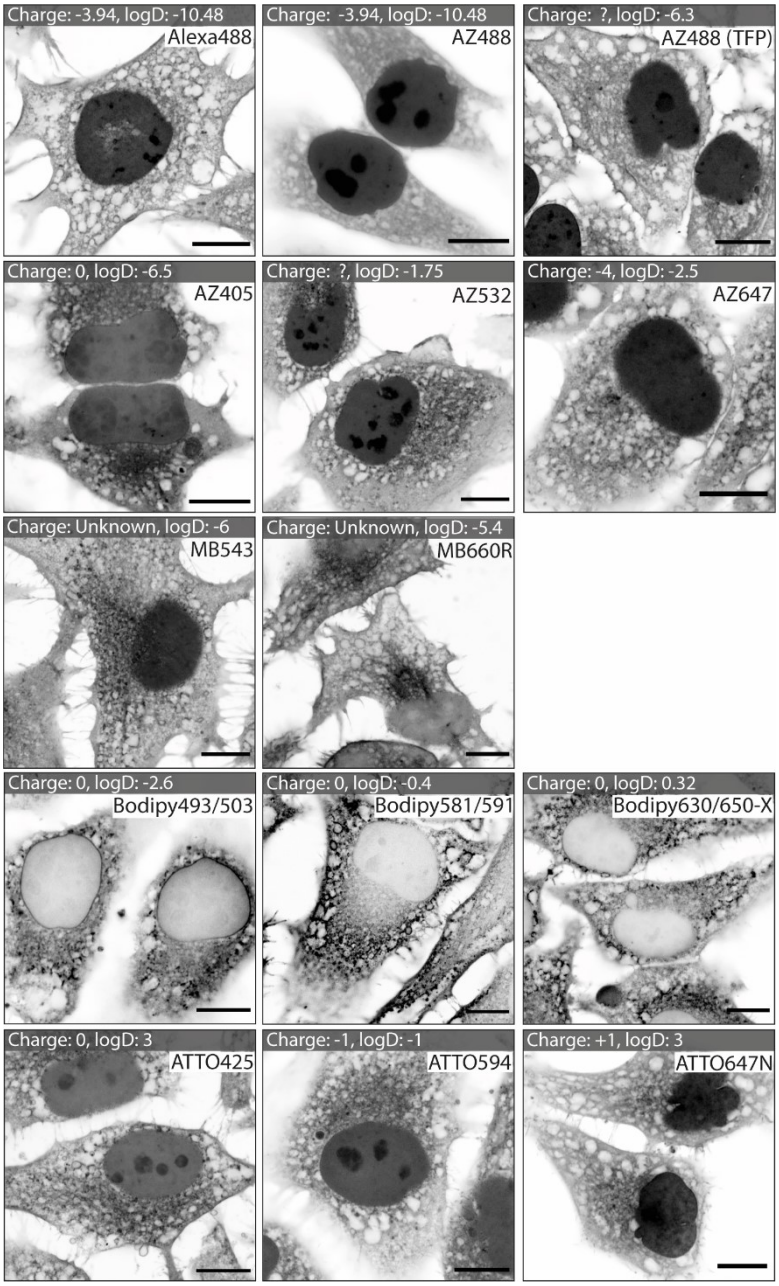
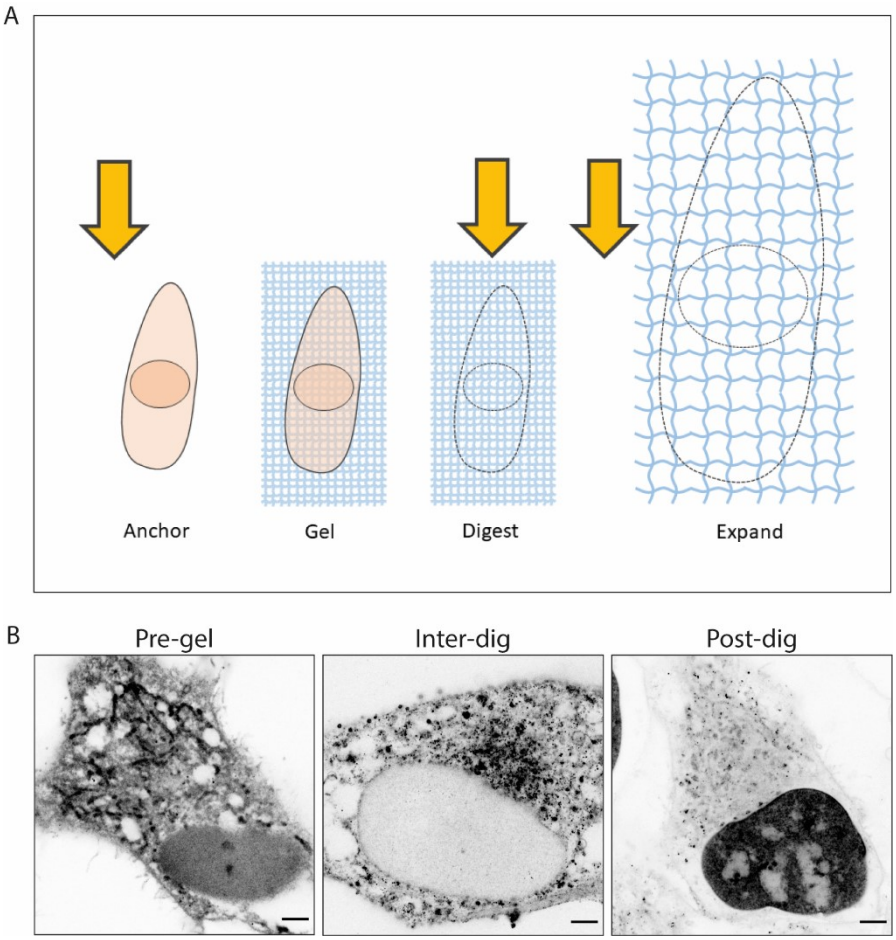
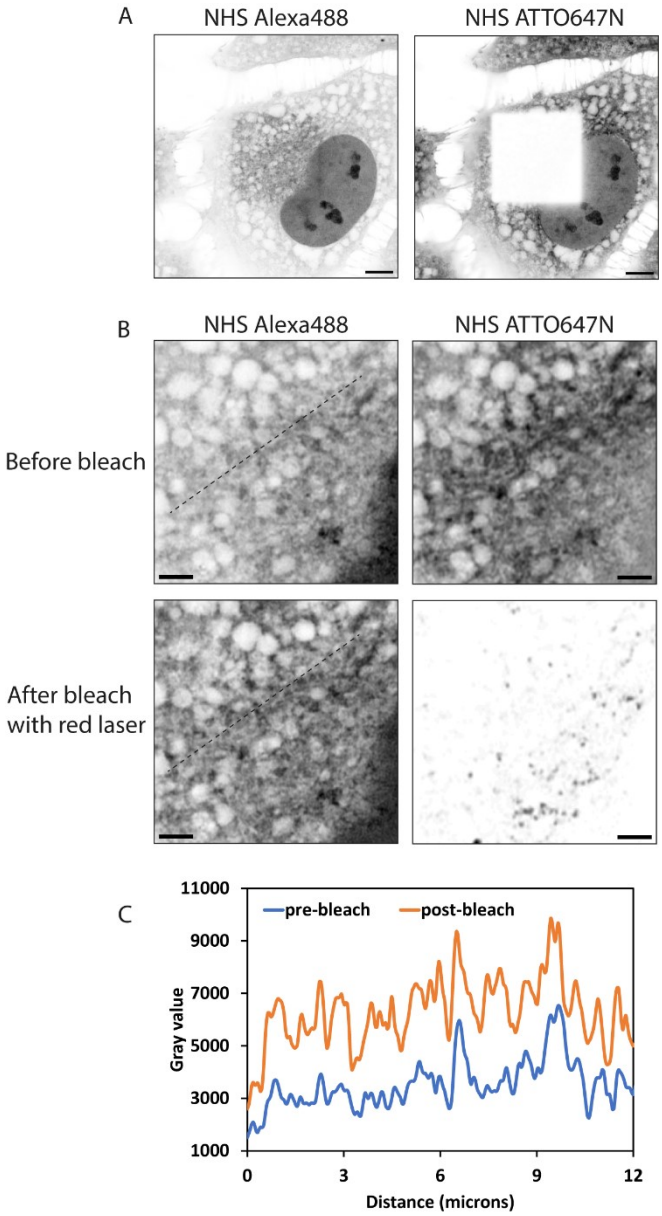
Supplementary figures



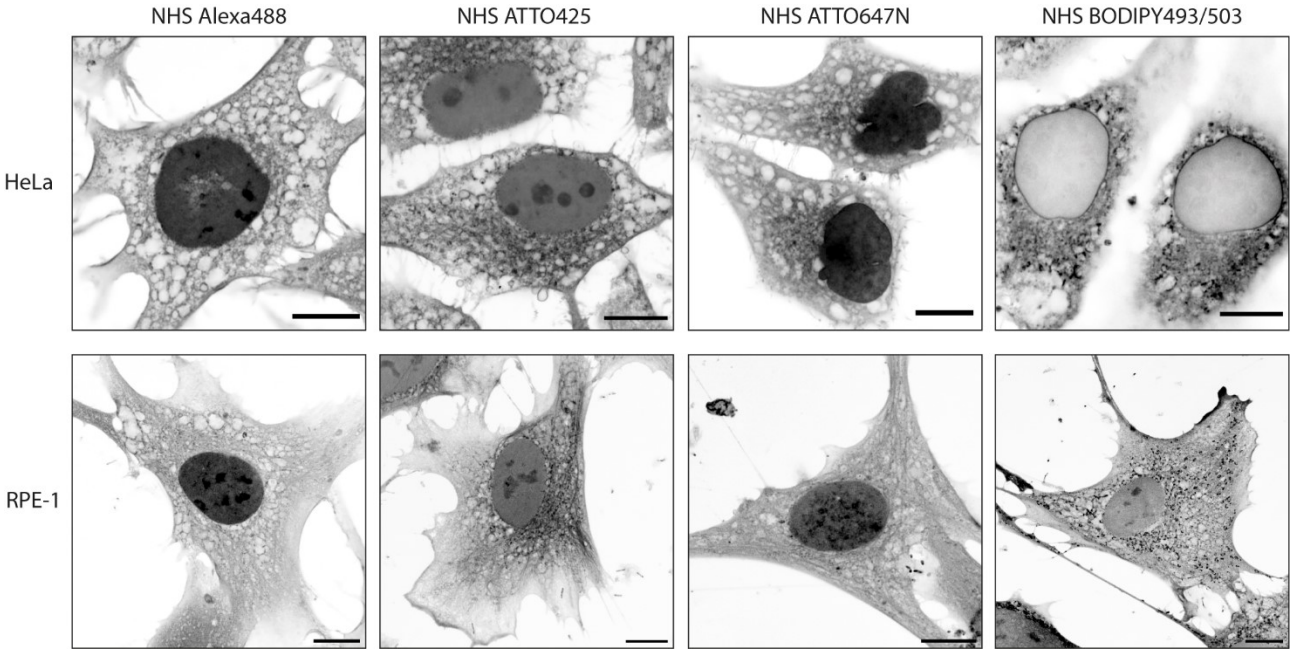
Supplementary figure 1. Catalogue of unexpanded dye ester images. (A) Chart displaying the ester dyes, distributed based on their excitation wavelength and hydrophobicity (determined from logD values). **(B)** A gallery of unexpanded images of each of the esters in HeLa cells. Between different dye families there are substantial differences, and within a single dye family there are similarities in patterns. Scale bars: 10 µm.



Supplementary figure 2. (A) Schematic showing the generalised ExM pipeline. Ester labelling can be performed at different timepoints (highlighted by yellow arrows) either pre-gelation, inter-digestion (4 hours of digestion, ester labelling, another 4 hours digestion), or post-digestion. **(B)** 4x EExM images of NHS ATTO647N in HeLa cells applied either pre-gelation, inter-digestion (4 hours of digestion, ester labelling, another 4 hours of digestion), or post-digestion, demonstrate the variety of intracellular structures which can be targeted. Scale bars (expansion factor rescaled): 2.5 µm.



Supplementary figure 3. Observation of fluorescence resonance energy transfer (FRET) interaction between multiple esters in unexpanded samples. A HeLa cell sample was labelled with NHS Alexa488 and NHS ATTO647N. **(A)** After bleaching a square in the NHS ATTO647N channel using a 639 nm laser, by zooming out it is possible to see that the intensity of NHS Alexa488 in the same region is increased in intensity **(B)**. Images of the region before and after bleaching with a 639 nm laser. **(C)** Line profile demonstrates the increase in pixel intensity post-bleach. Scale bars: (A) 5 µm; (B) 2 µm.



Supplementary figure 4. Unexpanded images of four NHS esters in HeLa and RPE1 cells. Scale bars: 10 µm.

Supplementary dataset

Additional images of unexpanded HeLa cells labelled with each ester are provided in the PowerPoint file, to demonstrate the consistency of labelling patterns across a wider selection of cells. The images were acquired with the LSM 880 Airyscan microscope (Carl Zeiss, Jena), using a 40x oil immersion 1.3 NA objective.

ESI Table 1. Key resources.

	SOURCE	IDENTIFIER
Experimental models: Cell lines		
Human: HeLa cell	Sigma	Cat# 93021013
Human: hTERT retinal pigment epithelial cell	ATCC	Cat# CRL-4000
Chemicals		
Dulbecco's Modified Eagle Medium	Thermo Fisher Scientific	Cat# 11584496
Dulbecco's modified Eagle's medium nutrient mixture F12 Ham	Sigma-Aldrich	Cat# D8062
Non-essential amino acids 100x	Thermo Fisher Scientific	Cat# 11140050
Foetal bovine serum	Labtech	Cat# SKU: FB-1001/100-500
Penicillin-streptomycin	Thermo Fisher Scientific	Cat# 11548876
Poly-D-lysine	Cultrex	Cat# 3439-100-01
Paraformaldehyde	Sigma-Aldrich	Cat# P6148-500G
Bovine serum albumin	Thermo Fisher Scientific	Cat# 30063-572
Sodium azide	Sigma-Aldrich	Cat# S8032-25G
DMSO	Biotium	Cat# 90082-BT
Triton X-100	Sigma-Aldrich	Cat# T9284-100ML
Normal goat serum	Thermo Fisher Scientific	Cat# 10000C
Acryloyl-X	Thermo Fisher Scientific	Cat# A20770
Acrylamide	Sigma-Aldrich	Cat# A9099-25G
N,N'-Methylenebisacrylamide	Sigma-Aldrich	Cat# M7279-25G
Ammonium persulfate	Sigma-Aldrich	Cat# A3678-25G
N,N,N',N'-Tetramethylethylenediamine	Sigma-Aldrich	Cat# T7024-25ML
Proteinase K	New England Biolabs	Cat# P8107S
Ethylenediaminetetraacetic acid	Sigma-Aldrich	Cat# EDS-100G

Guanidine HCl	Sigma-Aldrich	Cat# G3272-25G
Tris pH 8.0	Invitrogen	Cat# P8920-100ML
Poly-L-lysine	Sigma-Aldrich	Cat# 10259194
Dye-esters		
NHS Alexa488	Thermo Fisher Scientific	Cat# a20000
NHS AZ488	Fluoroprobes	Cat# 1013-1
TFP AZ488	Fluoroprobes	Cat# 1026
NHS AZ405	Fluoroprobes	Cat# 1061-1
NHS AZ532	Fluoroprobes	Cat# 1041-1
NHS AZ647	Fluoroprobes	Cat# 1121-1
NHS BODIPY493/503	Thermo Fisher Scientific	Cat# D2191
NHS BODIPY581/591	Thermo Fisher Scientific	Cat# D2228
NHS BODIPY630/650-X	Thermo Fisher Scientific	Cat# D10000
NHS ATTO425	Sigma-Aldrich	Cat# 16805
NHS ATTO594	Sigma-Aldrich	Cat# 8741
NHS ATTO647N	Sigma-Aldrich	Cat# 18373
NHS MB543	Fluoroprobes	Cat# 1661-1
NHS MB660R	Fluoroprobes	Cat# 1661-1
Antibodies		
Rabbit polyclonal KDEL	Thermo Fisher Scientific	Cat# PA1-013
Rabbit monoclonal GM130	Abcam	Cat# ab52649
Mouse monoclonal ATP5A1	Thermo Fisher Scientific	Cat# 43-9800
Alexa Fluor 488 goat anti-mouse IgG	Thermo Fisher Scientific	Cat# A11001
Alexa Fluor 488 goat anti-rabbit IgG	Thermo Fisher Scientific	Cat# A11008
Alexa Fluor 594 goat anti-mouse IgG	Thermo Fisher Scientific	Cat# A11005
Alexa Fluor 594 goat anti-rabbit IgG	Thermo Fisher Scientific	Cat# A11012

Atto 647N goat anti-rabbit IgG	Sigma-Aldrich	Cat# 40839-1ML-F
Atto 647N goat anti-mouse IgG	Sigma-Aldrich	Cat# 50185-1ML-F
Items		
Glass coverslips, #1.5, 22x22 mm	Menzel-Glaser	Cat# 631-0851
Chamberslides	Razorlab	Custom-made
Software and algorithms		
Fiji	ImageJ	https://imagej.net/software/fiji/
Chemaxon calculator plugin	Chemaxon	https://disco.chemaxon.com/calculators/demo/plugins/logd/
Excel	Microsoft	