**Multimodal super-resolution optical microscopy using a transition metal-based probe provides unprecedented capabilities for imaging both nuclear chromatin and mitochondria.**

Sreejesh Sreedharan[1], Martin R Gill[2,3], Esther Garcia[4], Hiwa K Saeed[1], Darren Robinson[2], Aisling Byrne[6], Ashley Cadby[5], Tia E. Keyes[6], Carl Smythe[2], Patrina Pellett*[7], Jorge Bernardino de la Serna*[4,8], and Jim. A. Thomas*[1]

[1] Department of Chemistry, University of Sheffield, Sheffield, UK

[2] Department of Biomedical Science, University of Sheffield, Sheffield, UK

[3] Current address: CRUK/MRC Oxford Institute for Radiation Oncology, Department of Oncology, University of Oxford, Oxford, UK

[4] Central Laser Facility, Science and Technology Facilities Council, Rutherford Appleton Laboratory, Research Complex at Harwell, Harwell-Oxford, UK

[5] The Department of Physics and Astronomy, University of Sheffield, Sheffield, UK

[6] School of Chemical Sciences, National Centre for Sensor Research, Dublin City University, Dublin 9, Ireland

[7] GE Healthcare Bio-Sciences Corp, 800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327 USA

[8] Department of Physics, King's College London, London, UK.

## Supporting Information

**SS 1: Cellular uptake of $1^{4+}$ (RRP) at different concentration**

The concentration-dependent uptake experiments were carried out over MCF7 cells (breast cancer cell line). The broad spectral characteristics shown my RRP is due to the MLCT. RRP is excited either at 405 or 488 nm and the emission was collected at far red region (> 650 nm). Due the MLCT the emission exhibited by RRP is a broad one from 600 nm to 750 nm with an emission maxima at 660 nm SIM experiment imaging conditions needed to be optimised to compromise the challenges created due to long stoke's shift shown by RRP. The concentration dependent uptake experiments were performed at a wide range of concentration from 250 nM to 300 μM. The experiment was performed by using the conventional Wide field mode of the OMX-SIM. The post processing was carried out offline by FIJI free software. As expected there was greater nuclear uptake of RRP observed at 300 μM exhibited by the brightness. Apart from the nuclear uptake observed there were signals observed coming from the cytosol region for low concentration from 250 nM to 10 μM.

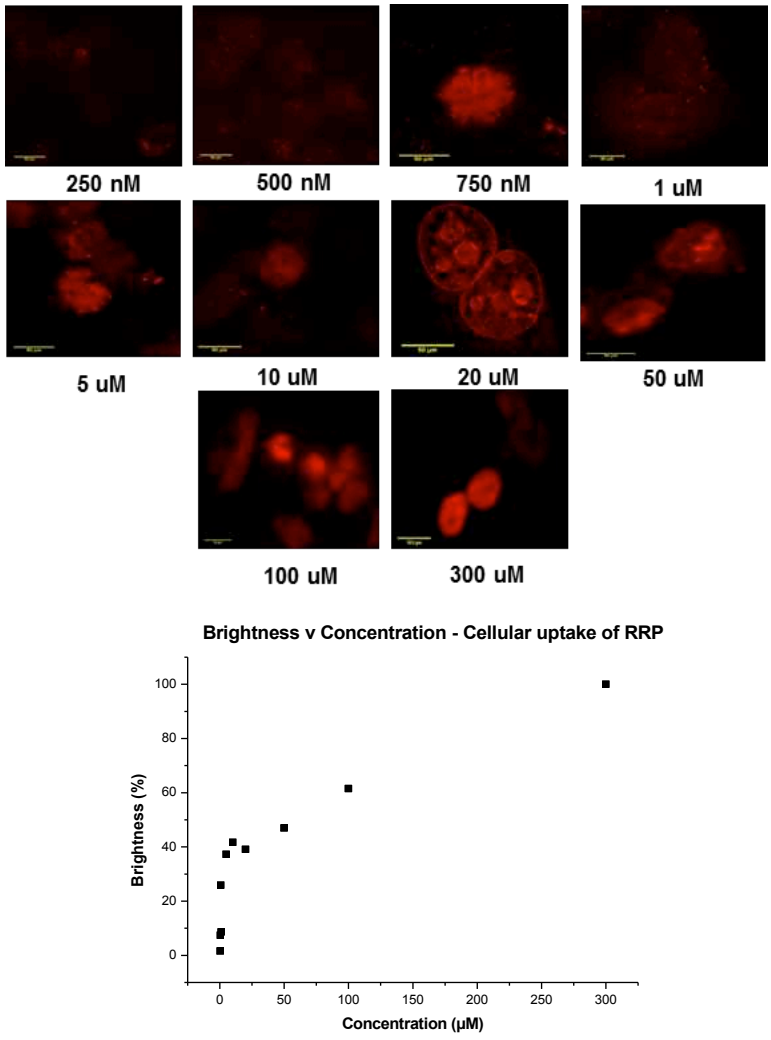



**Fig. SS 1:** Cellular uptake of $\mathbf{1}^{4+}$ (RRP) shown by Wide field deconvoluted microscopy (Pseudo colour has been employed in all the images)

## SS 2: Colocalisation experiments of $1^{4+}$ (RRP) with Standard Trackers

### $1^{4+}$ (RRP) 4 µM, Co-stanined with Mito Tracker Green

The live cell concentration dependent uptake experiments for determining the localization of RRP over mitochondria was confirmed by using Mito Tracker Green with Wide field fluorescence microscopy. The Mito T Green was excited at 488 nm and the emission was collected at the FITC channel (500 nm to 550 nm) and RRP was excited at 405 nm and emission collected > 650 nm

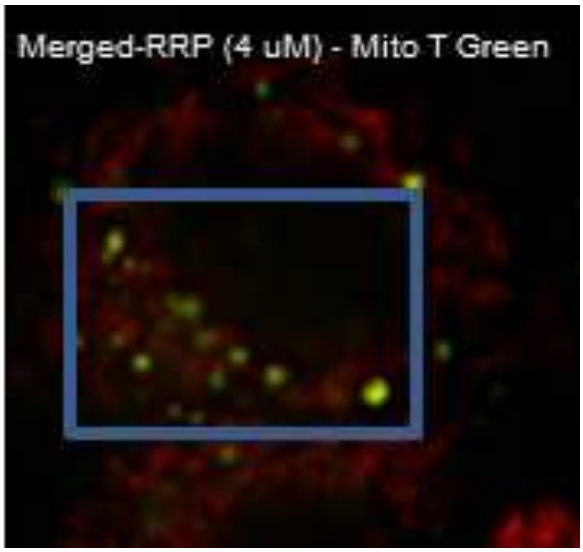



**Fig SS 2(I)** Localization over mitochondria at $\mathbf{1}^{4+}$ at 4 µM , Figure details: $\mathbf{1}^{4+}$ RRP (Green), MitoTracker Green (Red) (Pseudo colour has been employed in all the images)

**$1^{4+}$ 4 µM (RRP), costained with Mito Tracker Deep Red**

The live cell concentration dependent uptake experiments for determining the localization of $\mathbf{1}^{4+}$ over mitochondria was again confirmed by using Mito Tracker Deep Red with Wide field fluorescence microscopy. The Mito T Deep Red was excited at 644 nm and the emission was collected at the Far Red channel (> 650) and $\mathbf{1}^{4+}$ was excited at 405 nm or 488 nm and emission collected > 650 nm. Although the spectral range of emission channel for both the dyes fall in the same channel (Far red) $\mathbf{1}^{4+}$ could never be excited at 644 nm.

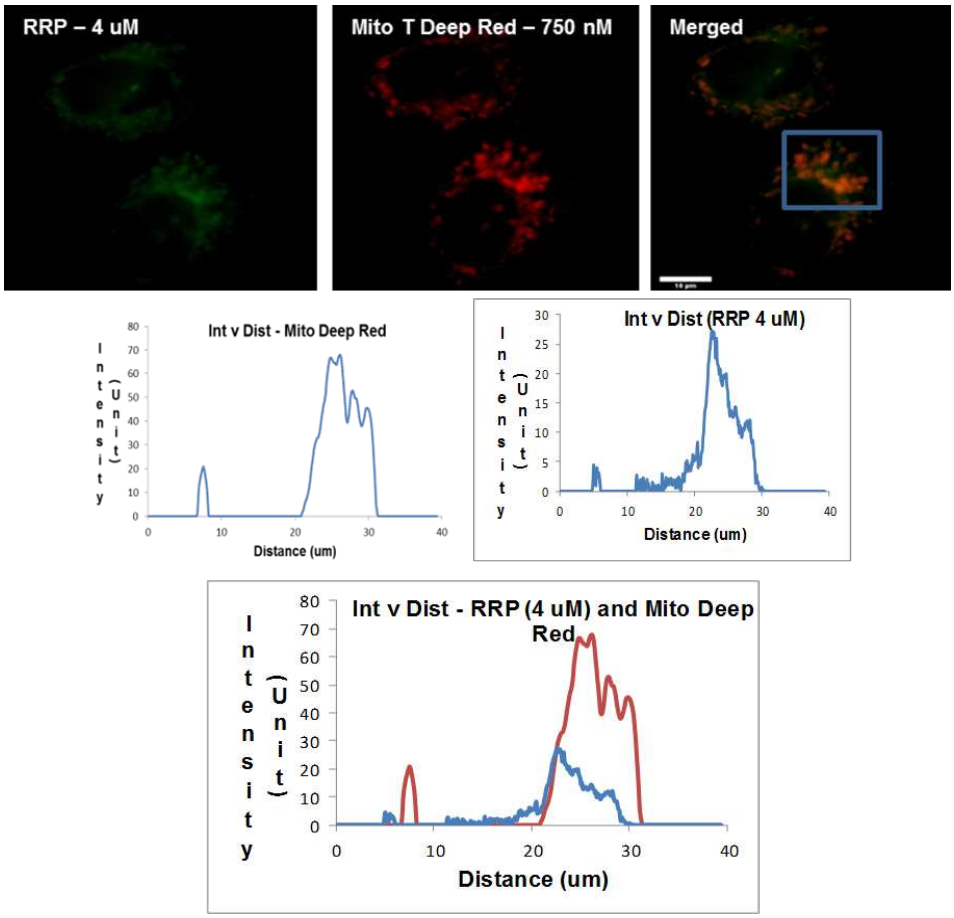



**Fig SS 2(II):** $\mathbf{1}^{4+}$ localization over mitochondria at 4 µM. $\mathbf{1}^{4+}$ (colocalisation with Mito T Deep Red), Inset: Mito colocalisation (Pseudo colour has been employed in all the images)

**SS 3: Concentration-dependent Nuclear and Mito uptake at different $1^{4+}$ (RRP) concentration (Mito Tracker Red)**

The concentration dependent nuclear and mito uptake was carried out at different concentrations of $\mathbf{1}^{4+}$ starting from 4 μM to 25 μM. The standard which was used to track mito localization of $\mathbf{1}^{4+}$ is Mito Tracker Red this is because Mito Tracker Red gave nice Wide field and SIM images compared to Mito Tracker Deep Red and Mito Tracker Green. Mito Tracker Red withstood SIM conditions better than Mito Tracker Deep Red and Mito Tracker Green. The Imaging data was processed by using FIJI offline software. Pearson's coefficient for $\mathbf{1}^{4+}$ localization over mitochondria was calculated by using Volocity offline software.

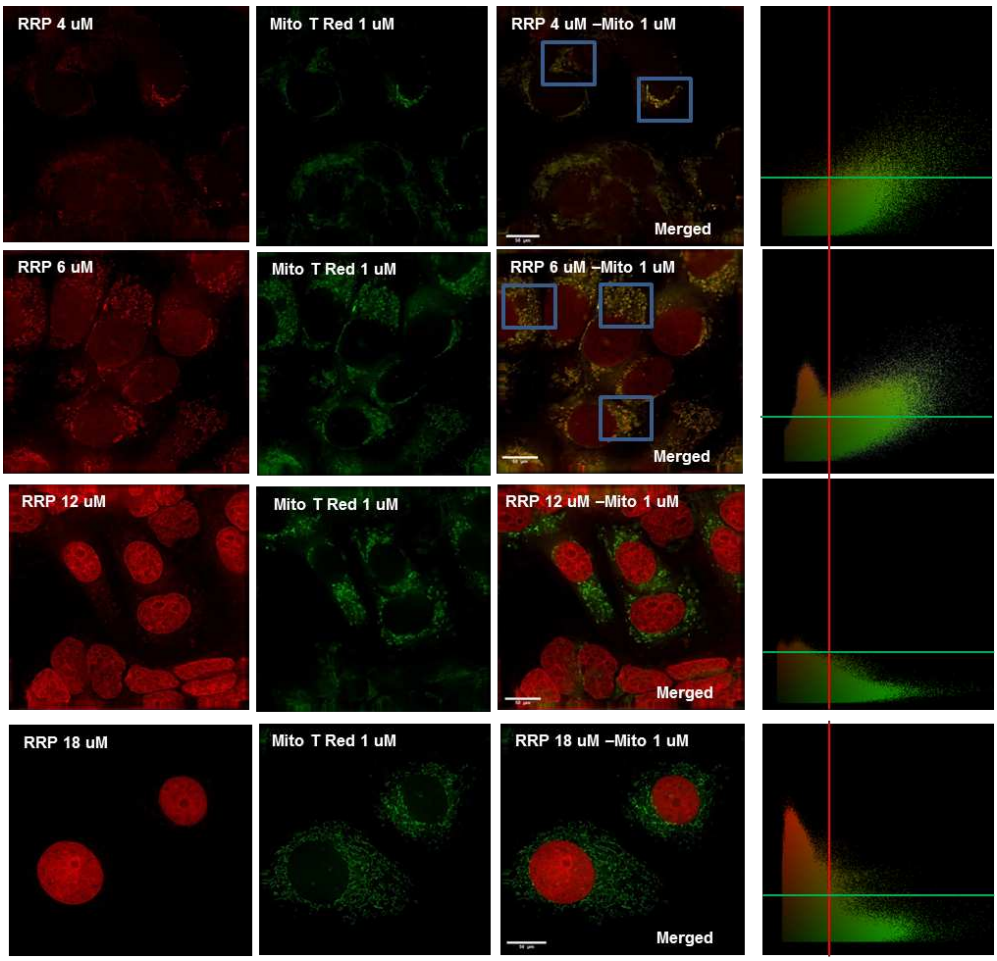



**Fig SS 3(I):** Nuclear and mitochondrial localization at different $\mathbf{1}^{4+}$concentration, Deconvoluted wide field HR images and Pearson's profiles showing change in localization (Pseudo colour has been employed in all the images)

**Data analysis for Concentration dependent uptake at 6 μM**

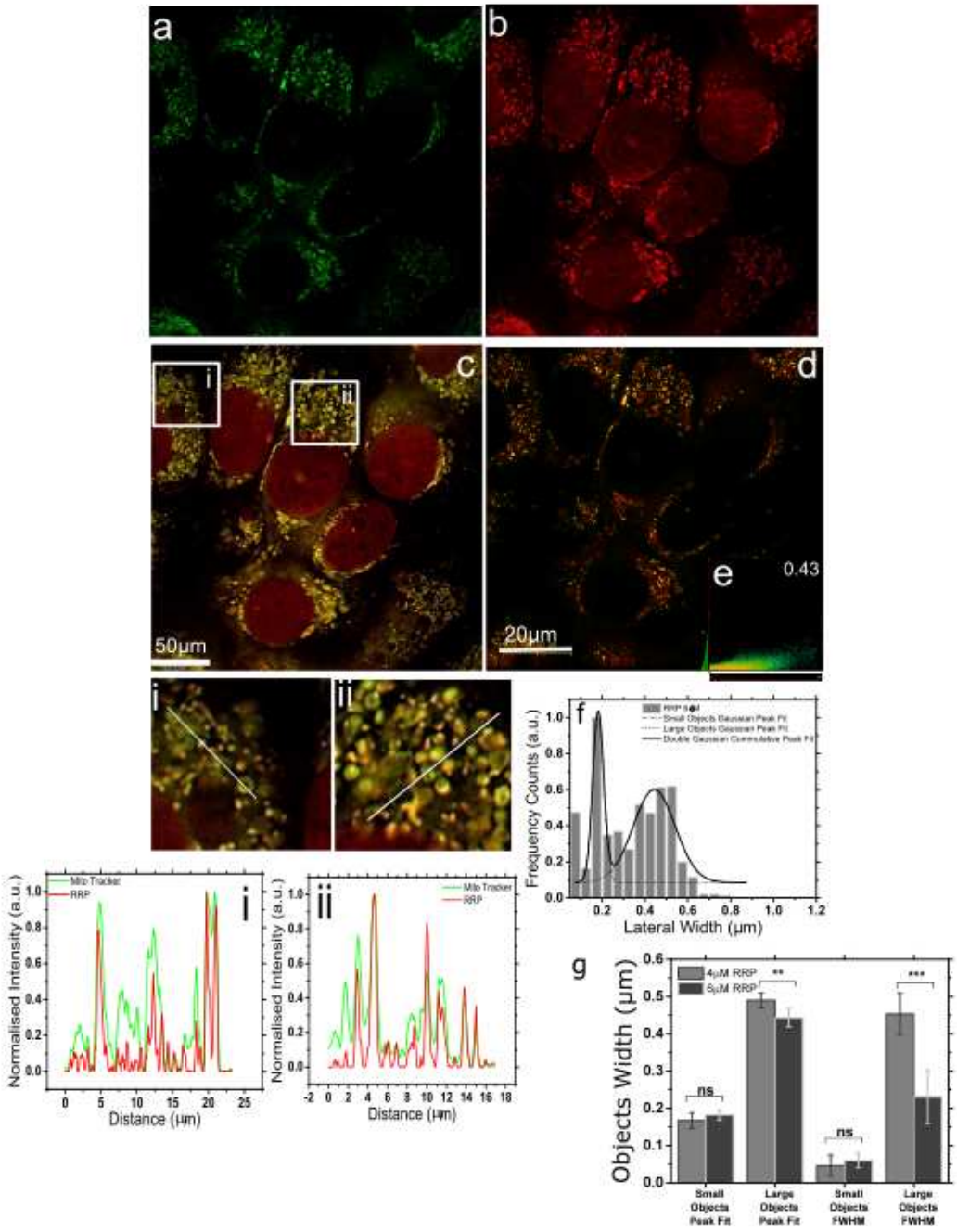



**Fig SS 3(II)**: Wide field deconvoluted images of MTR (showed in green) and $\mathbf{1}^{4+}$ (in red**)** stained MCF7 over concentration, 6 μM **(a,b)**. Combined images of MTR and [**1**]$Cl_4$ **(c)** and colocalisation maps, displaying only the object that colocalise **(d)**; graphs at the bottom right corner show the colocalization profiles with the calculated Pearson's coefficients **(e)**, which gradually decrease with the concentration of $\mathbf{1}^{4+}$**.** The images inside the squared insets of the merged images **(c)**, numbered as **(i, ii)** are zoomed-in for detailed visualisation and quantification purposes. The intensity profiles drawn inside the insets graphed below, show and $\mathbf{1}^{4+}$ in red simultaneously MTR in green and the complex $\mathbf{1}^{4+}$ (displayed as RRP) in red. Population distribution analysis of lateral width **(f)**, obtained from the colocalization map **(e)** The structures resolved 6 μM could be fitted to a double gaussian distribution. Comparison of the gaussian peak fit and the full width half max (FWHM) **(g)** obtained from the analysis of n=3 colocalisation maps of the small and large colocalised structures at 6 μM $\mathbf{1}^{4+}$Whilst the smaller structures show no significant differences, the large ones do, which indicates that with increasing concentrations $\mathbf{1}^{4+}$ is gradually disappearing from the mitochondria with largest width.

**S 4: High Resolution Microscopy (Airyscan) data for $1^{4+}$ (RRP) stained cells**

The mito uptake observed above using Wide field deconvolved microscopy was resolved by High resolution Airy scan microscopy which is the newest High resolution microscopy technique available which is based on confocal microscopy with improved resolution through the newly developed Airy disc technology[18,19]. High resolution Airy scan provides improved resolution of lateral: 140 nm, Axial: 400 nm. The mito and nuclear uptake of RRP was imaged at High resolution. A range of $\mathbf{1}^{4+}$ concentration were scanned ranging from 8 μM to 18 μM. Maximum colocalisation was observed at 8 μM, minimum colocalisation was observed at 18 uM and complete nuclear uptake was observed at 50 μM.

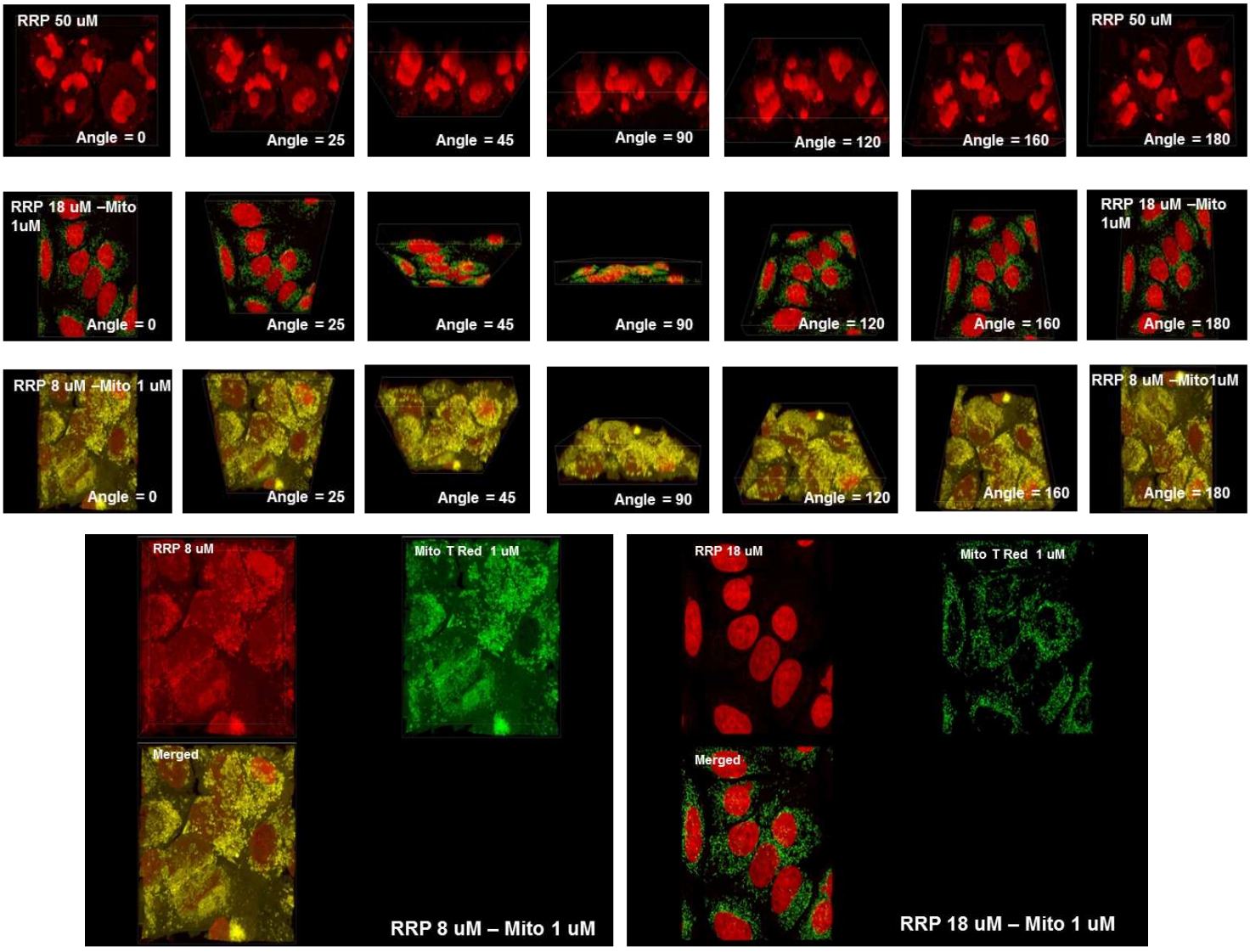
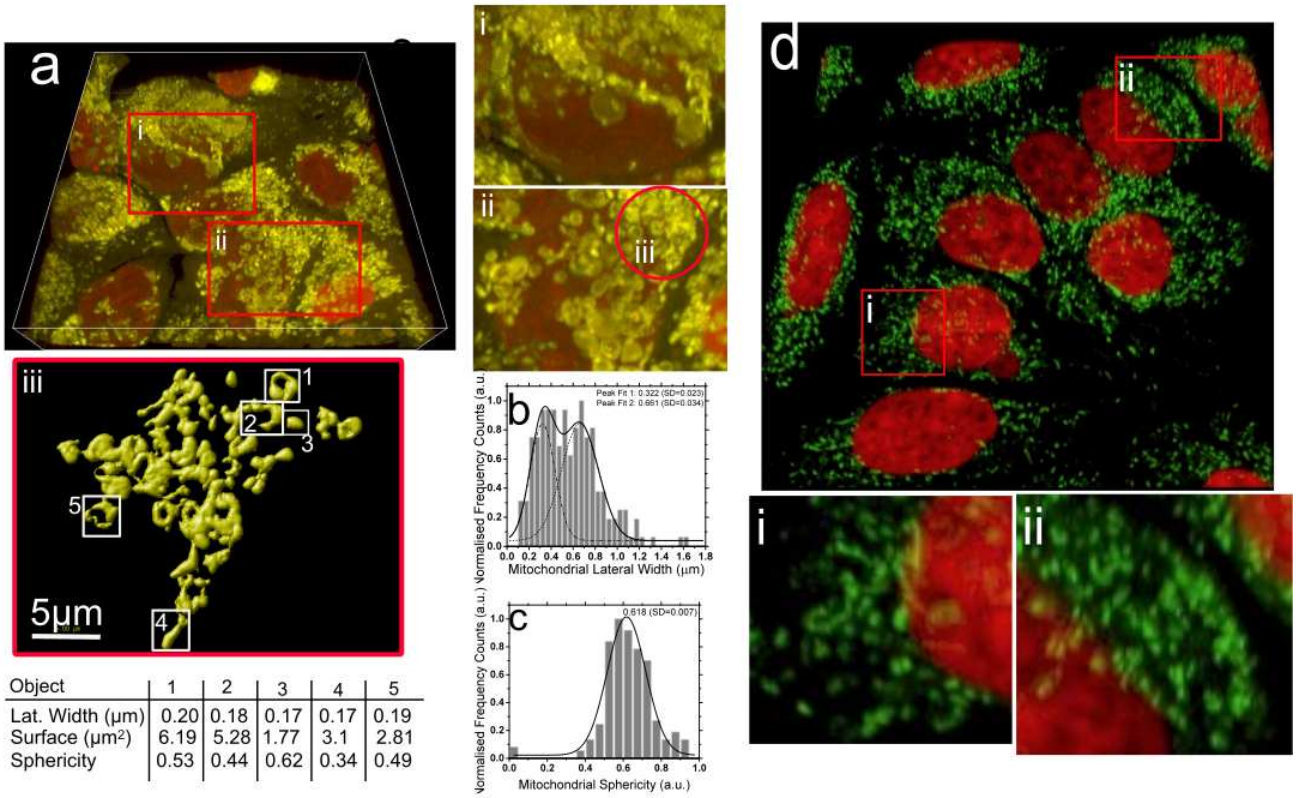



**Fig SS 4(I):** High Resolution Airyscan images for $\mathbf{1}^{4+}$ localization over nucleus and mitochondria (Pseudo colour has been employed in all the images)

**Data analysis for High Resolution Airy scan for $1^{4+}$ (RRP), 8 µM and 18 µM staining MCF7**




| Object | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Lat. Width (µm) | 0.20 | 0.18 | 0.17 | 0.17 | 0.19 |
| Surface (µm²) | 6.19 | 5.28 | 1.77 | 3.1 | 2.81 |
| Sphericity | 0.53 | 0.44 | 0.62 | 0.34 | 0.49 |

**Fig SS 4(II):** Airyscan HR Microscopy dual colour-3D reconstitution **(a)** and single plane projection **(d)** showing colocalisation with MTR for concentration 8 µM and 18 µM of $\mathbf{1}^{4+}$ respectively, Magnified images of the red-squared insets in (a), and (d) are shown in (i,ii). Airy Scan 3D surface-rendered reconstitution image of the mitochondrial structures (iii) as shown in the red-circled inset (iii) Quantification of the structures highlighted by white squares and numbered is shown in the table below. Values for mitochondrial width, surface, and sphericity are given. The population distribution analysis of the mitochondrial width and sphericity obtained from the surface rendered structures in (a) are graphed in (b) and (c). Data revealed that mostly mitochondria are not highly spherical and had a smaller and larger width distribution, as observed with wide field microscopy.

**SS 5: Mitochondrial health: Membrane potential studies using $1^{4+}$ (RRP)**

$1^{4+}$ (RRP) does not impact mitochondrial membrane potential or impact mitochondrial function. This is shown from the flow cytometry experiments. The flow cytometry was performed with working standard Valinomycin; a big increase in green emission was observed for Valinomycin but for $1^{4+}$ (RRP), no such change was observed . Western blot experiments which were performed involved activation of JNK and p38 pathways; JNK was activated (phosphorylated) by ROS-mediated stress and mitochondrial dysfunction. p38 is downstream of JNK and also activated by ROS, along with lots of other analogues. It could be seen clearly that no activation of either pathways in response to $1^{4+}$ (RRP) was observed, therefore ROS stress as part of the mechanism could be discounted. In this case, it was infered that no catastrophic mitochondrial damage was happening during specific organelle $1^{4+}$ (RRP) uptake.

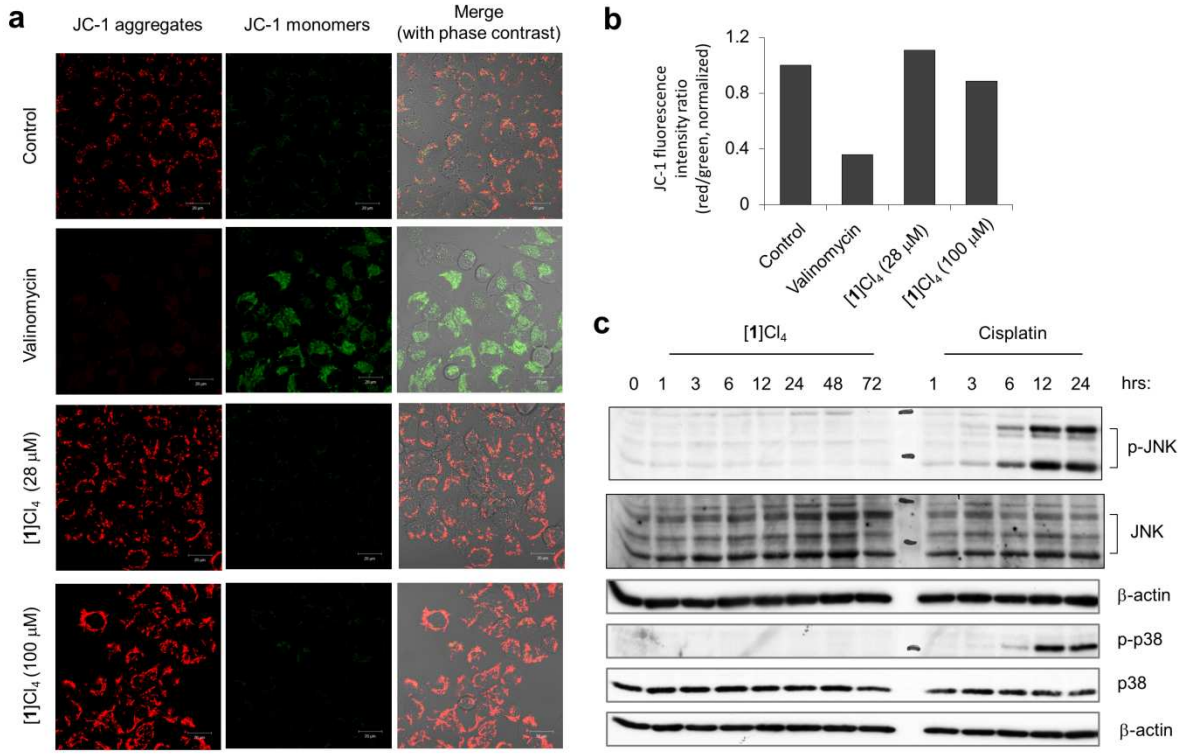



**Fig SS 5** (a) CLSM images of $JC^{-1}$ stained CP70 cells. Cells were incubated with $1^{4+}$ for 24 h at the stated concentrations and stained with JC-1 dye. JC-1 aggregates (red) and JC-1 monomers (green) were visualized to provide indication of changes in mitochondrial membrane potential. Valinomycin (1 μg/ml, 20 mins) was employed as a positive control for mitochondrial gradient dissipation. (b) Quantitative analysis of JC-1 red/green fluorescence ratio in untreated and treated cells, as determined by flow cytometry. The values are expressed as a ratio of median fluorescence intensities and normalized to data obtained in untreated cells. (c) Lack of activation of JNK and p38 signalling pathways in lysates of CP70 cells treated with $1^{4+}$ (100 μM), as determined by Western blotting using phospho-specific antibodies. Cisplatin treatment was employed as a positive control for pathway activation. β-actin was employed as a loading control.

**SS 6: Single colour and Dual colour Structured Illumination Microscopy for $1^{4+}$ (RRP) stained A2780 cells**

**Single colour SIM and 3D-SIM:**

Structured Illumination Microscopy (SIM) was carried out for $\mathbf{1}^{4+}$ (50 μM) using OMX-SIM. The SIM Images of nucleus (chromatin) was obtained using particular imaging conditions to suit the long stoke's shift and interesting DNA binding characteristics of the RRP probe. $\mathbf{1}^{4+}$ was excited at 405 nm or 488 nm and the emission was collected greater than 650 nm

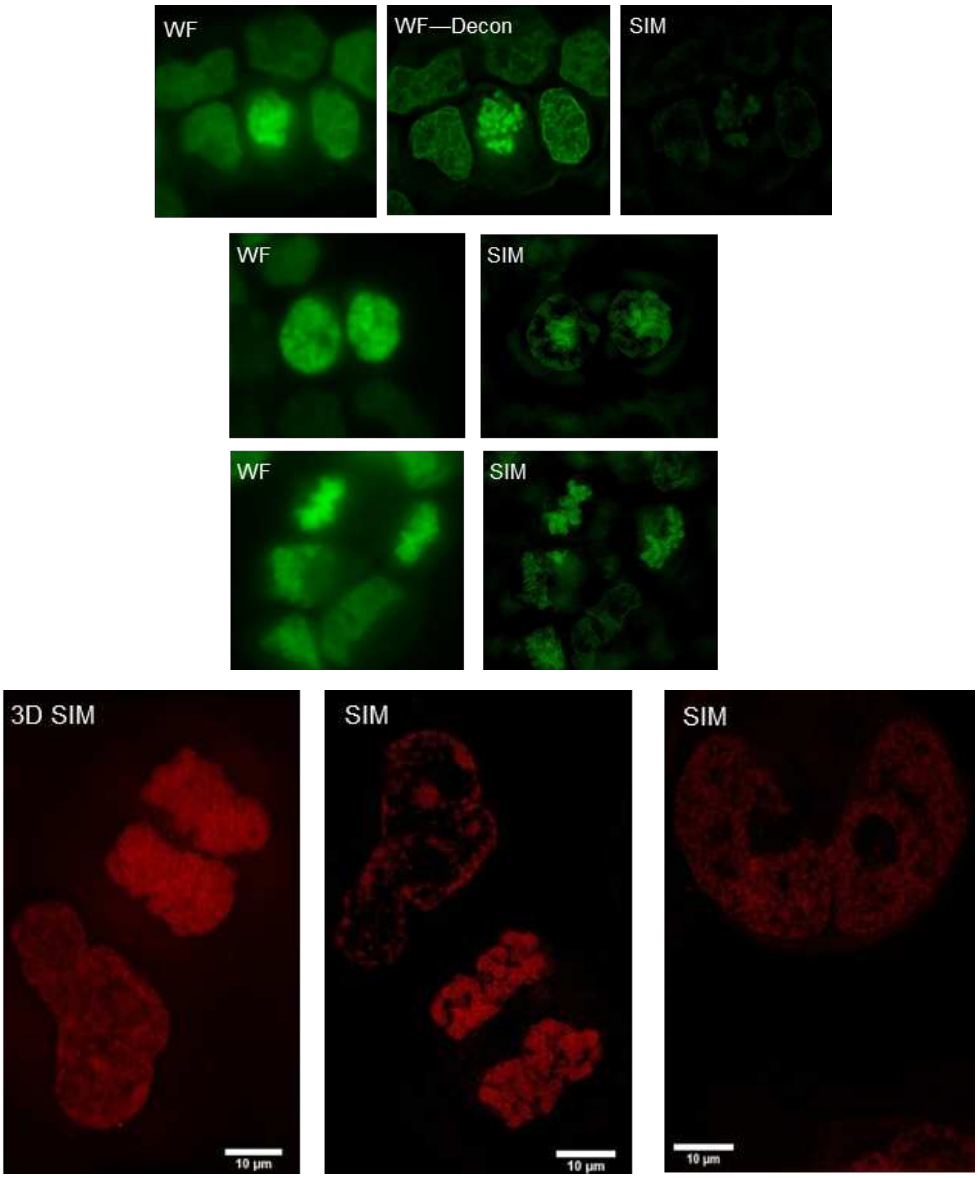



**Fig SS 6(I):** Single colour SIM and 3D-SIM Projections (for $\mathbf{1}^{4+}$ concentration > 50 μM**)** (Pseudo colour has been employed in all the images)

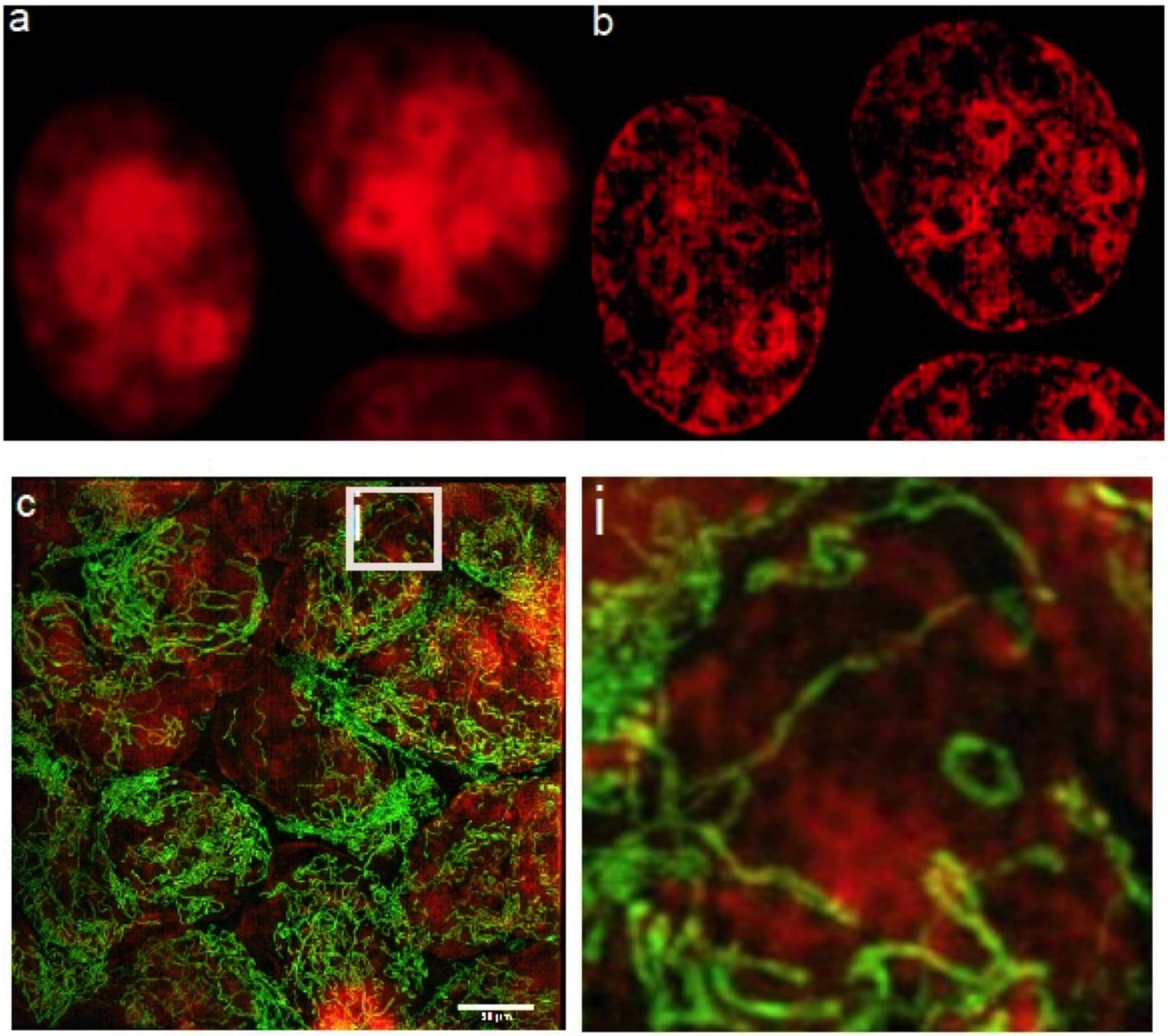



**Fig SS 6(II):** Single colour (b) and dual colour SIM (c) images for $\mathbf{1}^{4+}$ at concentration > 20 μM, WF and SIM comparison (a and b), inset (box) magnification.

**Dual Colour SIM: $1^{4+}$ (RRP) and Tracker Channels**

At high concentration $\mathbf{1}^{4+}$ specifically stain the nucleus and this enabled us to utilize $\mathbf{1}^{4+}$ as a dual colour probe. Here we have employed $\mathbf{1}^{4+}$ (20 μM) and it is clearly seen that there is no localization of $\mathbf{1}^{4+}$ observed on to the mitochondria and hence clearly could be employed as a dual colour.

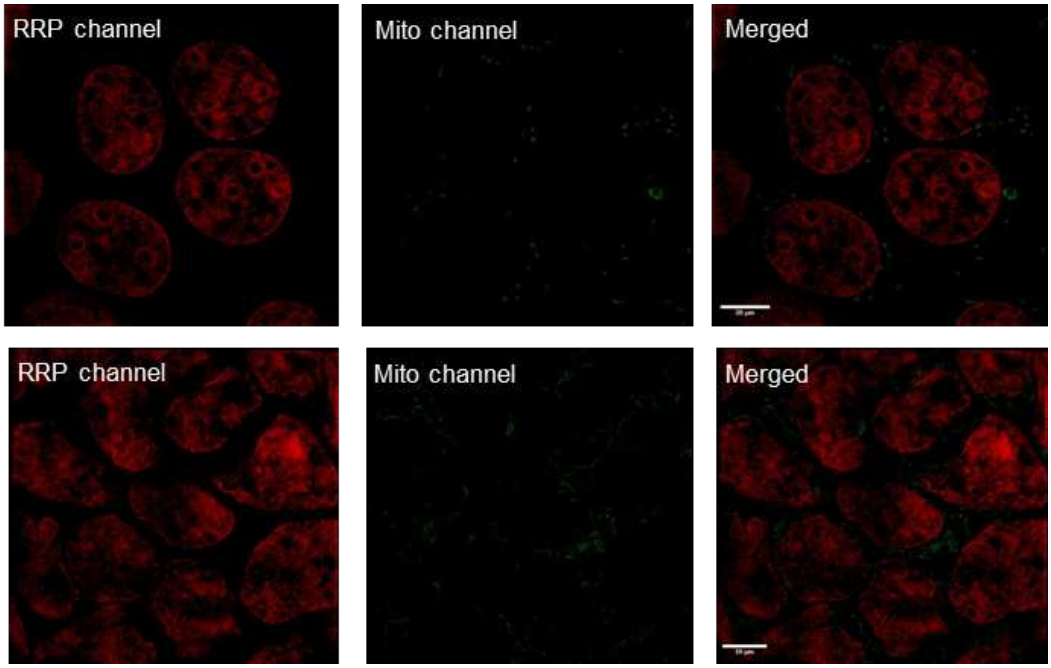



**Fig SS 6(III):** Dual colour SIM for $\mathbf{1}^{4+}$ at concentration > 20 μM (Both Mito and $\mathbf{1}^{4+}$ channel are shown separately (Pseudo colour has been employed in all the images)

**SS 7: Fixed and Live cell STED:**

**Fixed Cell STED:**
STED resolution improvement was achieved for $\mathbf{1}^{4+}$ (RRP) stained A2780 cells

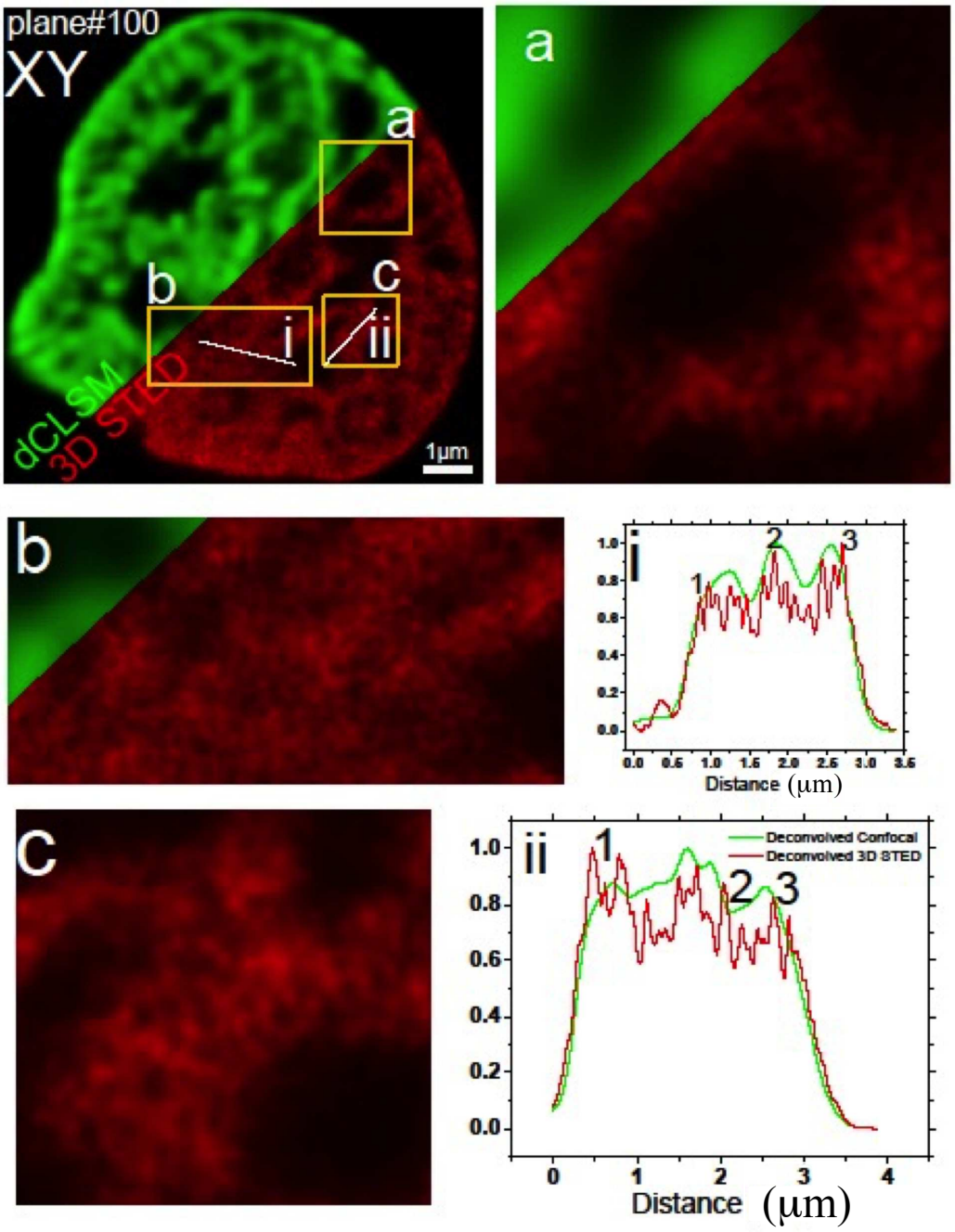



Table 1. Dimentions of the resolved structures

| (nm) | i | ii |
|---|---|---|
| FWHM Peak 1 | 67 | 71 |
| FWHM Peak 2 | 73 | 81 |
| FWHM Peak 3 | 44 | 37 |

**Fig SS 7(I):** Comparative Hyvolution and STED images showing improvement in resolution across XY planes, Comparative Hyvolution and STED images of individual plane from the STED Image volume (a-c) and Intensity profiles.

**3D-STED sectioning of Individual plane of 3D-STED volume stack $1^{4+}$ (RRP) stained A2780 cells:**

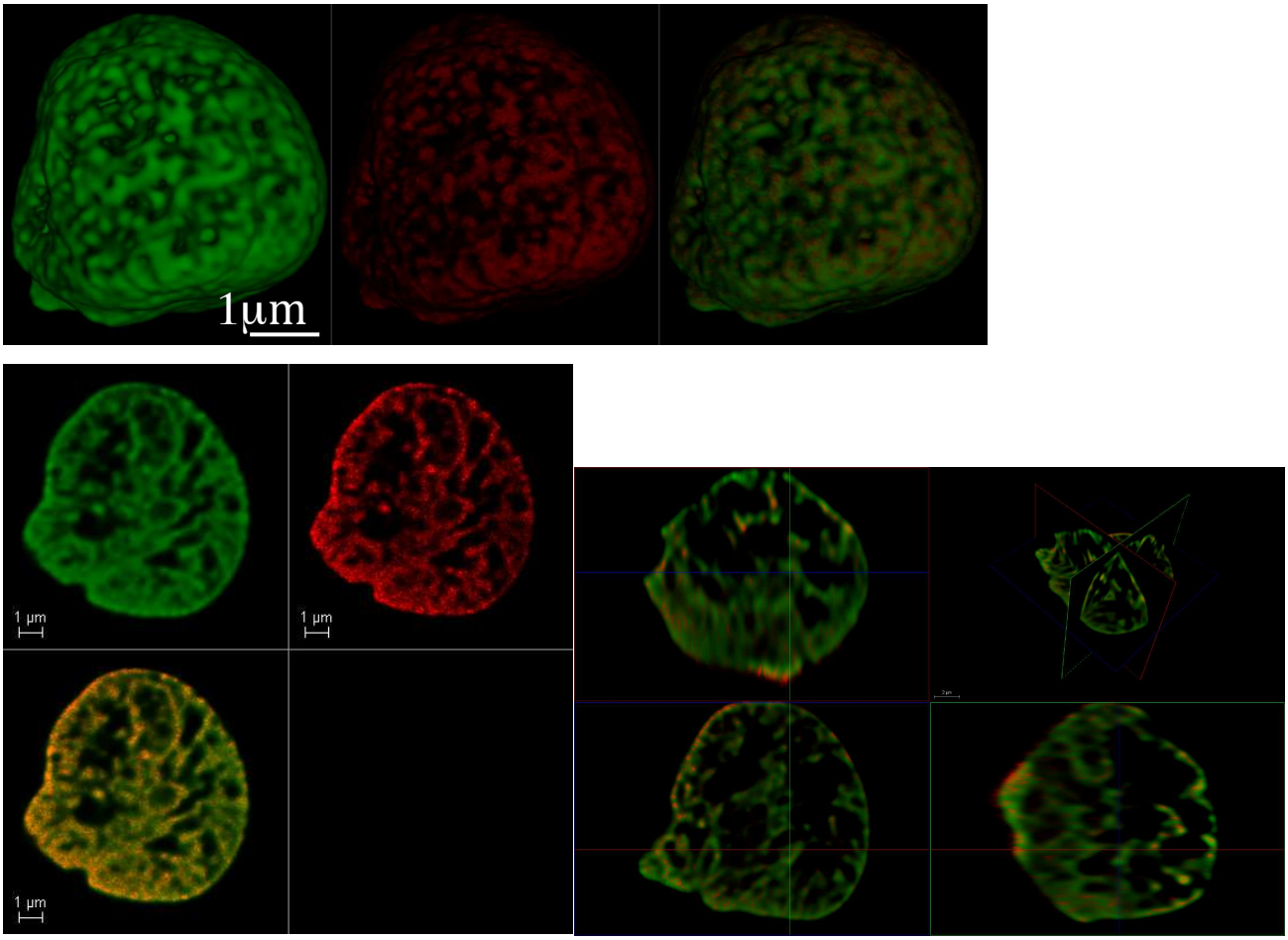



**Fig SS 7(II):** Whole 3D-STED volume, comparison (3D-Hyvolution (dCLSM), 3D-STED and Merged Images) (Top), Individual plane (XY) of 3D-STED sectioned Hyvolution (dCLSM), STED and Merged Images, Orthogonal slicing of 3D-STED Quadrant (Bottom)

**Live cell STED Images:**

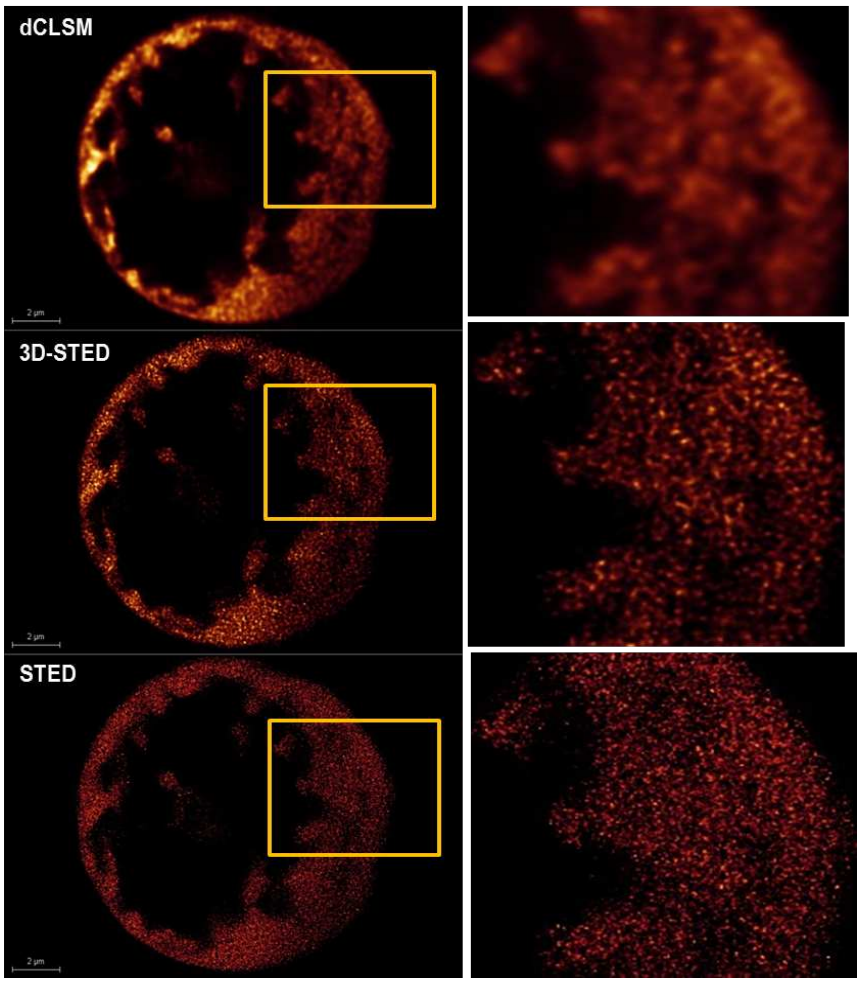



**Fig SS 7(III):** Comparative Live cell Images: Hyvolution (dCLSM), 3D-STED and STED , Orange boxes are magnified to the right.

**SS 8: Time Lapse Uptake of [1]$Cl_4$ (RRP) inside the Cells (MCF7)**

The time lapse RRP uptake experiments were performed to determine the rate of uptake of $\mathbf{1}^{4+}$ on to mitochondria and the nucleus. It was found that the rate of uptake of $\mathbf{1}^{4+}$ to mito and nucleus is happening very quickly within 2 minutes for concentration as low as 10 μM. 2 minutes incubation time is the minimum time required for $\mathbf{1}^{4+}$ uptake for both mitochondria and nucleus. The rate of $\mathbf{1}^{4+}$ uptake to Mito is followed by using regular co-staining dyes like Mito Tracker Deep Red, Mito Tracker Red and Mito Tracker Green. $\mathbf{1}^{4+}$ was excited at 405 nm and emission collected > 650 nm, Mito Tracker Deep Red was excited at 644 nm and collected > 650 nm, Mito Tracker Red was excited at 568 nm and collected between 570 to 620 nm, Mito Tracker Green was excited at 488 nm and collected between 500 to 550 nm.

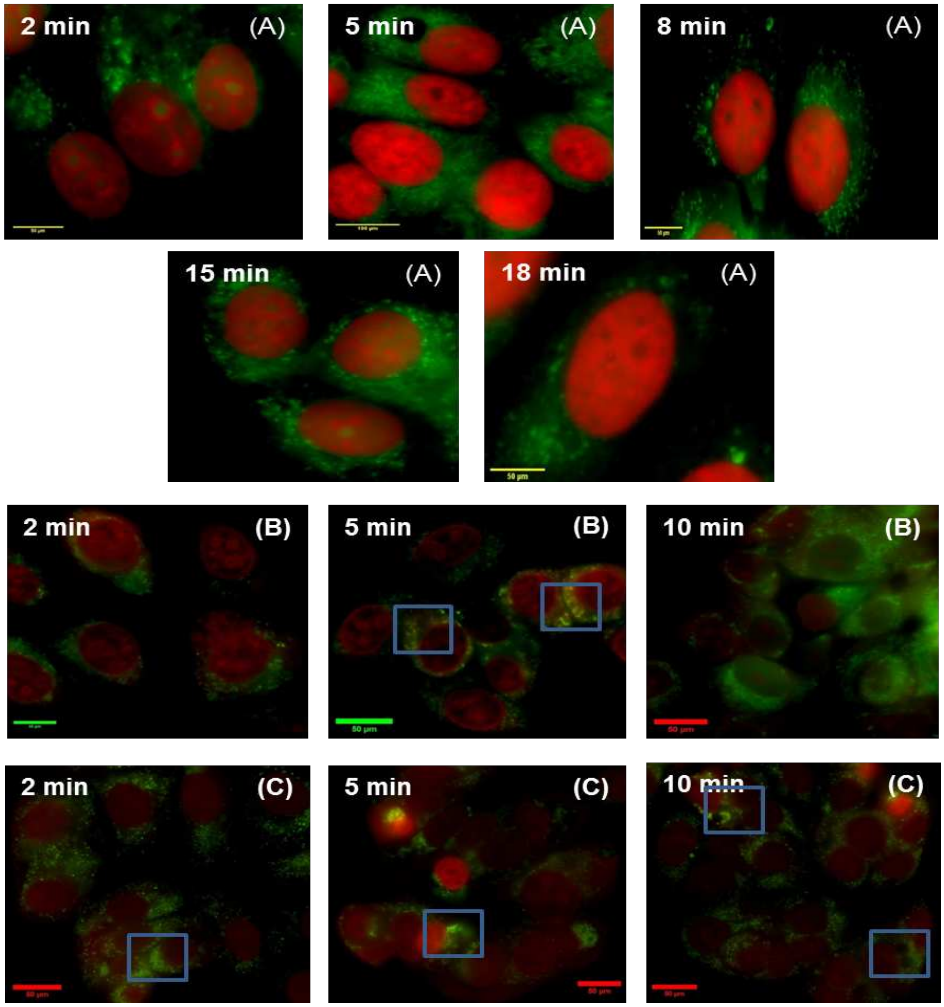



**Fig SS 8:** Time lapse uptake to nucleus and mitochondria in MCF7 cells (A) $\mathbf{1}^{4+}$ (10 μM, Red colour) and Mito T Red (1 μM, Green colour), (B) $\mathbf{1}^{4+}$ (10 μM, Red colour) and Mito T Deep Red (750 nM, Green colour), (C) $\mathbf{1}^{4+}$ (10 μM, Red colour) and Mito T Green (1 μM, Green colour), Inset (Box): Showing Mito localization (Pseudo colour has been employed in all the images).

**SS 9: Photostability of $1^{4+}$ (RRP) in comparison with co-staining dyes**

The Photostability experiments for $\mathbf{1}^{4+}$ and other dyes were performed by using verified scans of light radiations using conventional Wide field microscopy mode of the OMX-SIM. Laser power, time interval of image acquisition, number of scans per minute were all maintained similar for all the dyes. Post-processing of the imaging data set was carried out by using Fiji/Image J offline software.

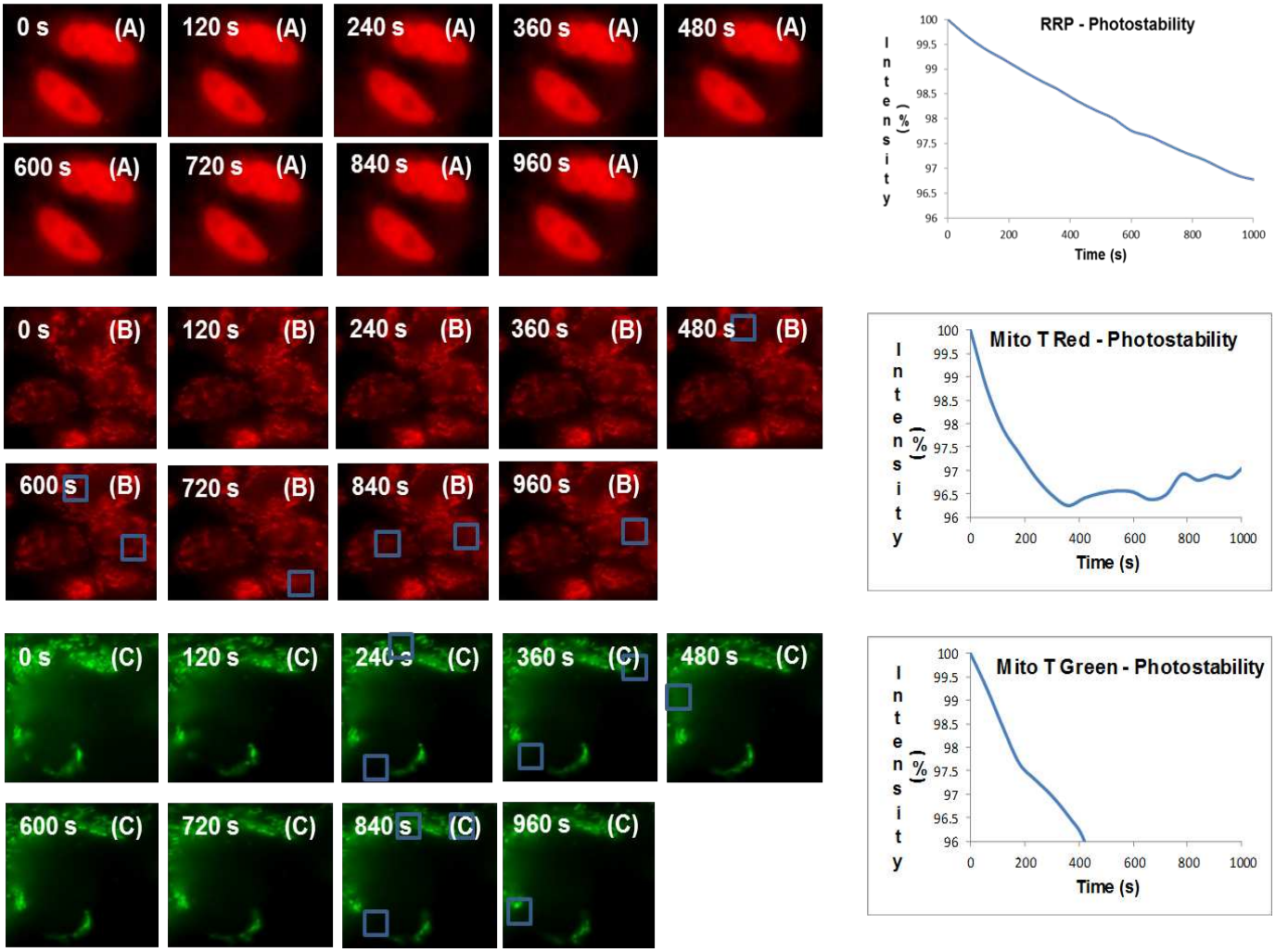



**Fig SS 9:** Photostability of $\mathbf{1}^{4+}$against standard dyes, Photostability trend of (A) $\mathbf{1}^{4+}$, (B) Mito Tracker Red and (C) Mito Tracker Green under same microscopy conditions recorded over a period of 1000 seconds, Inset: Bleached out regions after prolonged exposure of the sample containing the dye (Pseudo colour has been employed in all the images).

**SS 10: Intensity profile analysis of 3D-STED data compared to CLSM**

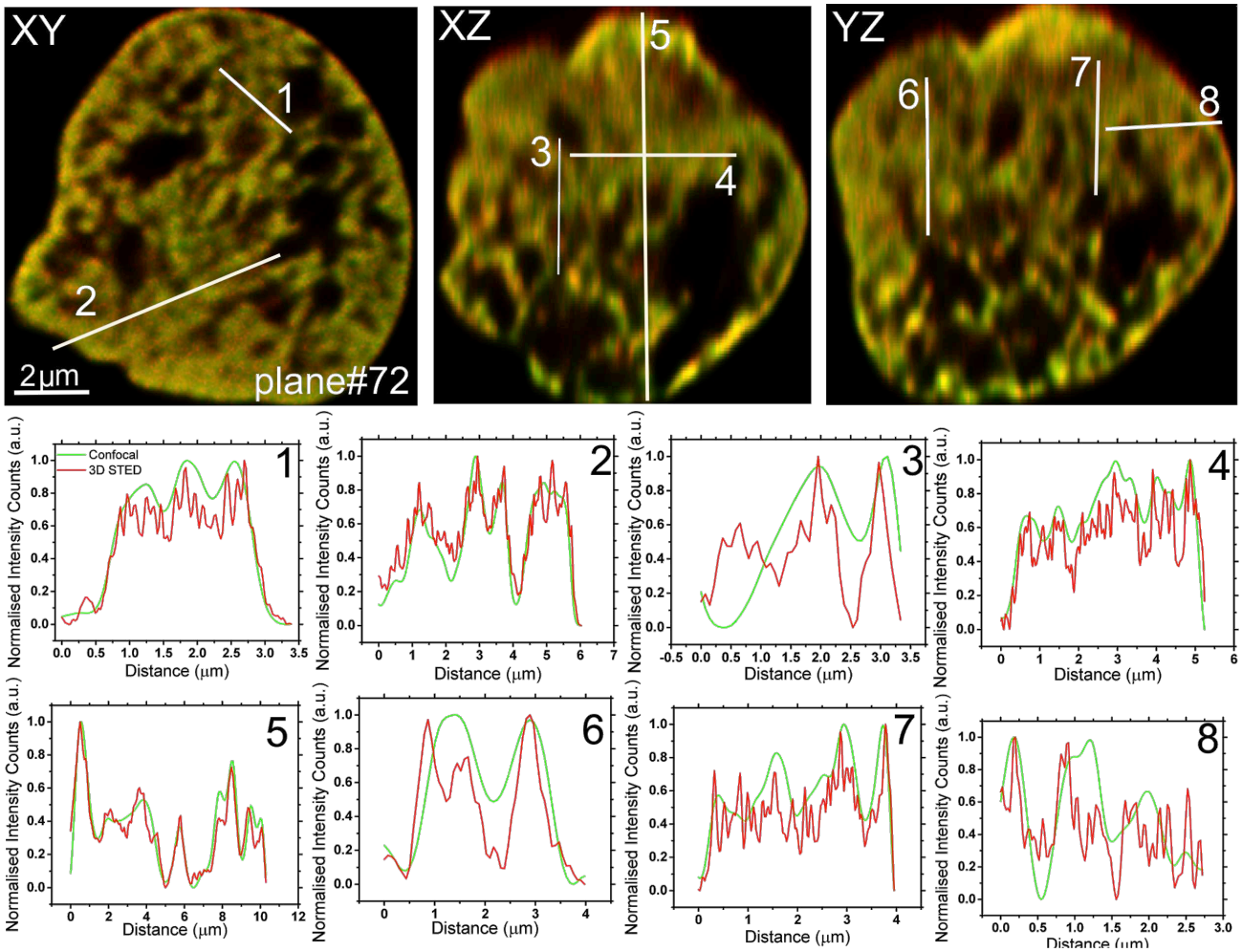



**Fig SS 10:** Top row: 3D-STED resolved planes (plane number 72), XY, XZ , YZ. Second and third row: 3D-STED Intensity profiles for the white straight lines drawn in the XY, XZ, YZ planes (1 to 8), comparing 3D-STED (red) with CLSM (green).

## Experiments, Techniques and Data processing:

**Cell culture and labelling.**

**Fixed cells.**

**Single colour.**

A2780 cells or MCF7 cells were seeded on cover slips (22 mm X 22 mm, 170 ± 5 µm square cover glasses) placed in six well plates in RPMI culture medium containing (10% FBS and 1% Penicillin Streptomycin) for 24 hours at 37°C, 5% $CO_2$. After 24 hours, when 70% confluency was achieved, the cells were washed with RPMI culture medium then the cells were treated with the probe for 5 to 10 min. Cells were then washed thrice with culture medium. After that cells were washed again with Phosphate Buffer Saline (2 times PBS). After carrying out the Live cell uptake of $\mathbf{1}^{4+}$ cells were fixed with 4% PFA for 15 minutes and then washed thrice with PBS and two times and then the coverslips were mounted using Mounting medium (Vectashield h-1000). The Coverslips were then sealed using Nail varnish and the samples were then imaged by 3D-STED, STED, SIM, Wide Field Fluorescence Microscopy (Deconvolved) or Airy-scan High Resolution Microscopy.

**Multi colour.**

The colocalisation studies were carried out by using Mito Tracker Green, Mito Tracker Red and Mito Tracker Deep Red. MCF7 cells were seeded on Cover slips (22 mm X 22 mm, 170 ± 5 µm square Cover glasses) placed in six well plates in RPMI culture medium containing (10% FBS and 1% Penicillin Streptomycin) for 24 hours at 37°C, 5% $CO_2$. After 24 hours when 70% confluency was achieved the cells were washed with RPMI culture medium and then treated with $\mathbf{1}^{4+}$ for 5 to 10 minutes followed by incubation with Mito Tracker Red, Mito Tracker Green or Mito Tracker Deep Red for 30 minutes and washed with 2 times RPMI culture media. Cells were then washed thrice with culture medium. After that, cells were washed again with Phosphate Buffer Saline (2 times PBS), fixed with 4% PFA for 15 minutes and then washed thrice with PBS and then the cover slips were mounted using Mounting medium (Vectashield h-1000). The coverslips were then sealed using Nail varnish and the samples were then imaged by SIM, Wide Field Fluorescence Microscopy (Deconvolved), Airy-scan High Resolution Microscopy.

**Live cells.**

For live cell microscopy, MCF7 were grown on #1.5H glass bottom u-Slide (Ibidi) and allowed to grow for a minimum of 24 hours. Cells were thoroughly washed with RPMI 1640 and then incubated with $\mathbf{1}^{4+}$ (500 µM, 4h).for confocal, Hyvolution (dCLSM), 3d STED and STED microscopy.

**Multimodular imaging using $\mathbf{1}^{4+}$.**

**Single colour SIM.**

Single colour SIM experiments for $\mathbf{1}^{4+}$ were performed for 50 µM $\mathbf{1}^{4+}$ on A2780 cells. The uptake was carried out for 5 to10 minutes. The $\mathbf{1}^{4+}$ probe was excited at 405 or 488 nm and the emission was collected at > 650 nm. The SIM conditions maintained were: thickness of the Z-stack (sections 50 to 100), section spacing (0.125 to 0.250), thickness of the sample (8 to 11), exposure time was between 10 to 50 and the %T was in the range of 30 to 80. Concentration dependent nuclear-uptake experiments were carried by using Wide field deconvolution microscopy at diverse range of $\mathbf{1}^{4+}$ concentration ranging from 750 nm to 300 µM. The Wide Field conditions maintained were: thickness of the Z stack (sections 40 to 80), section spacing (0.250 to 0.500), thickness of the sample (8 to 11), exposure time was between 10 to 30 and the %T was in the range of 30 to 60. The Airyscan High Resolution (HR) Microscopy experiments were carried out at $\mathbf{1}^{4+}$ concentration of 50 µM.

**Dual colour SIM, Wide Field Deconvolved, Airy scan.**

Due to the broad emission spectra of $\mathbf{1}^{4+}$, colocalisation experiments were performed with a series of commercial Mito Trackers available: Mito Tracker Red, Mito Tracker Deep Red and Mito Tracker Green. Colocalisation experiments were performed using low concentrations of $\mathbf{1}^{4+}$ in to order to monitor its colocalization over mitochondria. Dual colour or multicolour imaging for $\mathbf{1}^{4+}$ with Mito Trackers was possible at high $\mathbf{1}^{4+}$ concentration.

**$\mathbf{1}^{4+}$ and Mito Tracker Red**

For the colocalisation experiments, MCF7 cells were incubated with $\mathbf{1}^{4+}$ (4 µM to 25 µM) for 5 minutes and then the cells were incubated with Mito Tracker Red (1 µM) for 30 minutes. For the Dual colour experiments, MCF7 cells were incubated with $\mathbf{1}^{4+}$ (4 µM to 25 µM) for 5 minutes and then incubated with Mito Tracker Red (1 µM) for 30 minutes. The cells were washed regularly three times with culture media and PBS (two to three times), the cells were then fixed with 4% PFA and mounted and navigated initially for proper cell morphology by using Light microscope and then imaged by using Structured Illumination Microscopy (SIM), Airyscan HR microscopy, Wide field microscopy (WF). The $\mathbf{1}^{4+}$ probe was excited at 405 or 488 nm and the emission was collected in the Alexa fluor 647 channel (> 650 nm) and the Mito Tracker Red was excited at 568 nm and the emission was collected in the Alexa fluor 568 channel (570 nm to 620 nm). The SIM imaging conditions maintained are, For $\mathbf{1}^{4+}$: Thickness of the Z stack (Sections 50 to 100), section spacing (0.125 to 0.250), thickness of the sample (8 to 11), exposure time was between 10 to 50 and the %T was in the range of 30 to 80. For Mito Tracker Red: Thickness of the Z stack (Sections 50 to100), Section spacing (0.125 to 0.250), thickness of the sample (8 to 11), exposure time was between 10 to 50 and the %T was in the range of 10 to 50. The WF imaging conditions maintained are, For $\mathbf{1}^{4+}$: Thickness of the Z stack (Sections 40 to 80), section spacing (0.250 to 0.500), thickness of the sample (8 to 11), exposure time was between 10 to 30 and the %T was in the range of 30 to 60. The WF imaging conditions maintained are, For Mito Tracker Red: Thickness of the Z stack (Sections 40 to 80), section spacing (0.250 to 0.500), thickness of the sample (8 to 11), exposure time was between 10 to 50 and the %T was in the range of 30 to 60.

**$\mathbf{1}^{4+}$ and Mito Tracker Green**

For the colocalisation experiments, MCF7 cells were incubated with $\mathbf{1}^{4+}$ (4 µM) for 5 minutes and then the cells were incubated with Mito Tracker Green (1 µM) for 30 minutes. The cells were washed regularly three times with RPMI culture media and PBS (two to three times), the Cells were then fixed with 4% PFA and mounted and navigated initially for proper cell morphology by using Light microscope and then imaged by using Wide field microscopy (Deconvolved). $\mathbf{1}^{4+}$ was excited at 405 nm and the emission was

collected in the Alexa fluor 647 channel (> 650 nm) and the Mito Tracker Green was excited at 488 nm and the emission was collected in the FITC channel (500 nm to 550 nm). The WF Imaging conditions maintained are, For $\mathbf{1}^{4+}$: Thickness of the Z stack (Sections 40 to 80), section spacing (0.250 to 0.500), thickness of the sample (8 to 11), exposure time was between 10 to 30 and the %T was in the range of 30 to 60. The WF imaging conditions maintained are, For Mito Tracker Green: Thickness of the Z stack (Sections 40 to 80), Section spacing (0.250 to 0.500), thickness of the sample (8 to 11), exposure time was between 10 to 50 and the %T was in the range of 30 to 60.

**$\mathbf{1}^{4+}$ and Mito Tracker Deep Red**

For the colocalization experiments, MCF7 cells were incubated with $\mathbf{1}^{4+}$ (4 uM) for 5 minutes and then the cells were incubated with Mito Tracker Deep Red (750 nM) for 30 minutes. The cells were washed regularly three times with RPMI culture media and PBS (two to three times), the cells were then fixed with 4% PFA and mounted and navigated initially for proper cell morphology by using Light microscope and then imaged by using Wide field microscopy (Deconvolved). The $\mathbf{1}^{4+}$ probe was excited at 405 or 488 nm and the emission was collected in the Alexa fluor 647 channel (> 650 nm) and the Mito Tracker Deep Red was excited at 644 nm and the emission was collected in the Alexa fluor channel (> 650 nm). The WF imaging conditions maintained are, For $\mathbf{1}^{4+}$ : Thickness of the Z stack (Sections 40 to 80), section spacing (0.250 to 0.500), thickness of the sample (8 to 11), exposure time was between 10 to 30 and the %T was in the range of 30 to 60. The WF imaging conditions maintained are, For Mito Tracker Deep Red: Thickness of the Z stack (Sections 40 to 80), Section spacing (0.250 to 0.500), thickness of the sample (8 to 11), exposure time was between 10 to 50 and the %T was in the range of 30 to 60.

**Time dependent Imaging (Wide Field)**

The time-dependent uptake of $\mathbf{1}^{4+}$ over mitochondria and the nucleus and concentration-dependent tracking of cellular events was carried out by using Wide field fluorescence microscopy. Images were acquired in a Nikon Dual cam Wide Field fluorescence microscope fitted with: a Spectra X LED light source (395 nm, 440 nm, 470 nm, 508 nm, 561 nm, 640 nm); single emission filter sets for DAPI, GFP, RFP, Cy5; a Quad filter for DAPI/GFP/RFP/Cy5 with matching emission filter wheel; a Dual Andor Zyla sCMOS camera 2560 x 2160; 6.5 µm pixels and inbuilt NIS software employed for Wide field deconvolution. For the time-dependent uptake experiments, 10 µM $\mathbf{1}^{4+}$ was used and the uptake was monitored for time intervals starting from 2 min to 10 min. For the concentration-dependent tracking of cellular events a range of $\mathbf{1}^{4+}$ concentration (1 µM, 5 µM and 50 µM) was used. The $\mathbf{1}^{4+}$ was excited at 470 nm and emission collected at > 650 nm (Cy 5 Channel). Mito Tracker Red was excited at 568 nm and emission collected in the RFP channel at 570 to 620 nm. Mito Tracker Green was excited at 488 nm and emission collected in the FITC channel at 500 to 550 nm. Mito Tracker Deep Red was excited at 644 nm and emission collected at > 650 nm (Cy 5 channel).

**Transmission Electron Microscopy.**

Sample preparation involved MCF7 cells being grown in 6 well plates then incubated with $\mathbf{1}^{4+}$ (500 µM, 1 h), the media is then removed and the cells fixed using 3% glutaraldehyde. For fixed cellular location TEM studies, cells were fixed with cooled 70% ethanol prior to exposure with the complex. For both methods, cell cultures were dehydrated using a series of ethanol washes (70 – 100 % ethanol) and TEM samples sectioned in Araldite resin by microtome. Samples were examined on a FEI Tecnai instrument operating at 80 kV equipped with a Gatan 1 k CCD Camera. Cells were fixed and examined using TEM. MCF7 cells strongly stained with $\mathbf{1}^{4+}$, revealing that the complex is evenly distributed throughout the cytosol of the cell. Interestingly, the TEM micrographs obtained show contrast signal from mitochondrial regions in addition to nuclear staining indicating that $\mathbf{1}^{4+}$ is located within these organelles. This suggests that $\mathbf{1}^{4+}$ is not bound to mitochondrial DNA and that the luminescence is only activated upon delivery of the molecule, and subsequent intercalation, at the DNA-rich nucleus. This could indicate the complex is bound to a non-DNA target (for example, if it were protein-bound) or located within the space that divides the inner and outer membrane of mitochondria.

**Description of Multimodal techniques employed.**

**Airyscan High Resolution Microscopy.**

The Airyscan HR Microscope, LSM 880 works by imaging the full Airy disk onto a 32-channel array detector. The Fast Module takes a similar approach by shaping the illumination beam into a short line, instead of a round spot, and then using only the centre detectors to image the beam. Airyscan delivered 1.7x higher resolution in all three spatial dimensions and increases SNR by a factor of 4 – 8x, compared to traditional confocal LSM images acquired with a 1 AU pinhole. Intensities were normed to the value obtained at 1 AU. Image conditions using a Plan Apochromat 63x / oil 1.4 objective lens and 470 nm excitation line (for $\mathbf{1}^{4+}$) and 568 nm (for Mito Tracker Red) at 0.4% were as follows: resolution 512 x 512, pixel (px) size 0.051 µm, sampling rate 1.52 µs/px. This creates simultaneous, parallel illumination and detection of a line within the sample, but still with an improvement in resolution. The final image has 1.5 times improved resolution although it is non-isometric in XY (Lateral: 140 nm, Axial: 400 nm), and a 4 times improvement in signal-to-noise ratio. All that and it's 4 times faster scanning compared to CSLM and Wide Field Microscopy.

**Wide Field Deconvolution Microscopy.**

The Wide field fluorescence microscopy involved collection of greater quantity of light (Including out of focus light) compared the confocal microscopy technique which involves loss of more than 30% of light as out of focus light is discarded during image acquisition as it being a pointilistic technique[3]. The OMX-SIM Wide Field mode utilises high-speed image acquisition. The Wide field imaging using $\mathbf{1}^{4+}$ involved images with 512X512 pixels, 95 MHz power, 8.3 ms, 120 frame per second image acquisition speed. The Wide field microscopy technique involves improvement in resolution only after post processing the Z-stacks acquired[4,5]. Processing of acquired data is done by using Fiji software. The deconvolution procedure involves the processing of the raw Wide field images obtained from the OMX-SIM (Conventional Wide field microscopy mode). This Image processing was carried out by using the Soft Worx software which is used for carrying out the post processing of the Raw Wide Field data in the OMX-SIM. Deconvolution is a computationally intensive image processing technique which helps in improving the contrast. During the deconvolution procedure the Raw Wide field images were processed by the software which removes the out of focus blur from stack of acquired images called Z-Stack.

**HyVolution.**

This methodology developed by Leica microsystems utilises for the image acquisition a reduced size of pinhole right in front of the HyD detectors, and then employs a deconvolution software developed by SVI (Hyugens) in Netherlands. The deconvolution software

uses the raw data information from the Leica acquisition files for an optimal deconvolution. This software can use either a theoretical PSF or a calculated one. For 3D objects, we used the theoretical one, as it automatically counteract for the possible aberrations caused for in depth imaging. The single plane images were deconvolved with a theoretical calculated PSF provided by the software and by an experimentally calculated PSF from fluorescent beads, in both case no apparent difference was observed.

**Image analysis and deconvolution**. gSTED nanoscopy resolved the structure of the mitochondria and DNA with high detail, but as expected the images had low intensity counts, especially when employing the continuous wavelength depletion laser at 660nm. To maximise signal-to-noise we deconvolved the images using a commercially available software (Huygens package software, SVI, Netherlands)[7]. To quantify the background level of noise we used either an automated quantification provided by the software or a manual by means of computing the averaged background intensity from regions outside the cell. We obtained better results with the manual process. For the deconvolution we used 40 iterations, a signal to noise ratio of 15, and the classical maximum likelihood estimation method provided by the software. More insight into the deconvolution algorithms and process can be consulted in Schoonderwoert, V., *et al.*[7] Colocalisation analysis was perform using the Huygens software package and the Pearson colocalisation coefficient was quantified. The software allows obtaining the colocalisation map of the regions that colocalise. This map images, can be surface rendered and the digital image can be employed to obtain quantitative values of the objects that colocalise, such as width, and sphericity. Quantification of the data from the images was obtain by using the Huygens Professional Software Package (Huygens; Scientific Volume Imaging, Hilversum, Netherlands)[7]. For the colocalisation maps and object analysis of the maps we employed the colocalisation software provided with the package. The quantification was based in the pearsson coefficient. Quantified objects were exported in ASCII and opened and further anlaysed using Origin Pro (OriginLab Corporation, Northampton, MA, U.S.A.) for data plotting and analysis. The data was represented in form of normalized distribution population. The distribution of the events and the characteristic width was graph employing a significant histogram binning and the resulting distributions where fitted to a double Gaussian. The main peak fit and the Full Width Half Maximum of the Gaussian distributions was extracted and used to build the graph. The general protocol for the analysis of any image related to wide field, Ayry Scan, Hyvolution and STED (both single plane XY, and 3D STED, i.e., XY and Z) included several steps: object localization, quantification and characterization, plotting, and image representation. As a general rule every single confocal plane of a 3D imaging stack was first deconvolved, the resulting 3D image was surface-rendered, and the 3D iso-surface was used for quantitative analysis; the advance object analysis module of Huygens was used to localize, quantify, and characterize the geometry of each individual granule; and the resulting data was exported into graphing and statistics software. US). The values represented in the population distribution graphs were obtained from the Huygens software. This software identified the center of mass of each object, the definition of each parameter used in this paper such as lateral width and sphericity can be found in the following webpage: https://svi.nl/HomePage. The figures in this paper has been done employing the free open source software Inkscape. The surface rendered images obtained by Huygens Professional, LAX software (Leica SP8) to generate the surface rendered 3D STED images, and the orthogonal views, the rest of the images has been post procesed with Fiji (ImageJ; NIH)

**Structured Illumination Microscopy (SIM)** carried out by using the Delta Vision OMX-SIM. SIM is basically a Wide Field Microscopy technique based on Moire's effect[1,3]. The Resolution improvement is achieved based on the reconstruction of the acquired image by using the inbuilt software namely Soft Worx. The Z stacks acquired during the Imaging are post-processed by using the Reconstruction option of Soft Worx. SIM acquisition is dependent mainly on the Imaging parameters and Acquisition parameters and this varies depending on the sample and in particularly on the nature of the probe. The $\mathbf{1}^{4+}$ probe was Excited at 405 or 488 nm and the emission was collected at > 650 nm (Alexa flour 647 channel of the Delta Vision OMX-SIM). In the case of $\mathbf{1}^{4+}$ the Structured Illumination (SI) experimental condition employed for running the SI experiment for Single Colour Experiments were mainly dependent on the thickness of the Z stack (Sections 80 to 100), Section spacing (0.125 to 0.250), thickness of the sample (8 to 10). As $\mathbf{1}^{4+}$ probe is a very bright probe with a large stokes shift therefore the image volume (Z-stack) should be adjusted in such a way that artefacts arising during the imaging could be minimized during the SIM image acquisition in addition to that %T and exposure time needed to be adjusted during SIM imaging of $\mathbf{1}^{4+}$, a DNA-binding probe. The 3D-SIM image acquisition mode used using $\mathbf{1}^{4+}$ probe include, z piezo step resolution of 5 nm, 800 ms scan speed in a 120 frame per cycle. The image to image interval during a complete z stack is 8 ms. The power is 300 Hz with 60% quantum efficient CMOS camera with 6.5 um pixel size. Each image in the 3D SIM z-stack is 512X512 pixels, 1 ms exposure per frame, 125 nm step, 8 z-slices, 15 image per slice, 120 images in total for single colour imaging, for colocalization and dual colour the conditions are slightly different but essentially similar. The SIM reconstruction conditions employed include essentially same conditions as z-stack image acquisition but the exposure time is 5 ms for 3D SIM reconstruction. The pixel size for 60X and 100X objective in SIM were 80 and 48 respectively. In all our Single colour experiments the exposure time was between 30 to 60 and the %T was in the range of 30 to 50. The Colocalization experiments were performed with Mito Tracker Red, Mito Tracker Green and Mito Tracker Deep Red. The Dual colour experiments were performed with Mito Tracker Red exploiting the concentration dependent preferential nuclear uptake of $\mathbf{1}^{4+}$ at high concentration. In both of these experiments the SIM conditions of these Co-staining agents were maintained in accordance to the $\mathbf{1}^{4+}$probe.

**STED Microscopy:**

STED images were taken in a commercial LEICA SP8 3X gSTED SMD confocal microscope (Leica Microsystems, Manheim, Germany). The microscope is equipped with 3 depletion lines and it is also equipped with a 3D STED additional vortex to obtain higher spatially resolved images in XY and Z. The excitation laser beam consisted of a pulsed (80MHz) super-continuum white light laser (WLL). For a cleaner emission the excitation lines had a clean-up notch filter (NF) in the optical pathway. The gSTED imaging was taken with a 660 nm depletion laser, and the pulsed STED images with a pulsed 775 nm Laser, again in every case the respective NF were in place. The objective employed was a Leica 100x/1.4 NA oil objective. The pinhole was set at one Airy unit. The gated HyD detectors were set with the gated option on and the temporal gated selected was from 2 to 6.5ns when depleting at 660nm. For the 3D STED images the depletion lasers were split in two, the second vortex was set at 50%. Images of live MCF7 cells were taken at 37°C and 5% $CO_2$. The dye was excited with a WLL at 470 nm, and STED depleted at 775 nm; the emission was collected from 620 to 710 nm and a gating between 2 and 6.5 ns was used.

**Single colour and Dual colour STED Microscopy.**
The sample preparation of Single colour STED and 3D STED Microscopy was carried out in a similar manner for Structured Illumination Microscopy[2,6]. The STED and 3D STED experiments were performed using A2780 cells; $\mathbf{1}^{4+}$ was excited at 470 nm and STED depleted at 775 nm; the emission was collected between 660 to 700 nm. Dual colour STED experiments were carried out using MCF 7 cells as was in the case for Structured Illumination Microscopy, Airyscan and Wide field deconvolution Microscopy. $\mathbf{1}^{4+}$ was excited, depleted and collected as mentioned above. The Mito Tracker Red was excited at 570 nm, STED depleted at 660 nm and collected between 600 to 650 nm. The imaging conditions employed are mentioned in the following sections. Imaging, processing, analysis and graphing has been done following the previously described protocols (see section above)

**Transmission Electron Microscopy.**
In TEM regions of high electronic density are able to scatter the electron beam while unscattered electrons pass through the sample and are detected by a fluorescent screen. This effect allows a contrast image of the specimen to be constructed. For cellular imaging using $\mathbf{1}^{4+}$, ultra-thin cross-sections of cells were prepared and then stained with various staining agents, which usually contain a heavy metal capable of deflecting the electron beam. This enhances contrast and is the phenomenon exploited in the use of reagents such as immunogold labels, ruthenium red and osmium tetroxide. In the absence of any contrast agent, no intracellular detail is observable. Since Ru(II) complexes incorporate a second row transition metal with a high electron density, these complexes can scatter the TEM electron beam; hence the localisation of the $\mathbf{1}^{4+}$ within cells was determined through TEM with high spatial resolution. The Optimised TEM imaging conditions are as follows: A 3.8-kV operating voltage and a spot size of 3.5 (FEI Quanta microscope setting). Optimum balance between the speed of image acquisition and quality of image. To balance charging, we used a vacuum setting of 0.3–0.4 Torr. ×4,900 indicated magnification collecting 3,000-x pixels, 3,000-y pixels at 10 µs per spot dwell time . These settings provide a pixel resolution of 10 nm, which is sufficient resolution to clearly identify intracellular structures. The scan time is approximately numX × numY × dwell time, where numX and numY are the number of pixels used to make up the images, and the dwell time here is 10 µs. Therefore, each scan takes ~1.5 min. The Image processing was carried out by using Image J or FIJI.

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