Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

Luminescent nanomaterials for droplet tracking in a microfluidic trapping array

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Development of microfluidic devices: Soft Lithography and PDMS Replication

The microfluidic devices were developed by a combination of soft lithography and PDMS replication. The geometry of the device was designed with AutoCAD software prior to fabrication. A two-step soft-lithography was used to fabricate the silicon master: starting with generating the bottom fluidic layer using a negative photoresist polymer, SU-8 2025 (Microchem). The SU-8 was deposited on a clean 4" silicon wafer and baked at 65 °C for 10 min followed by a second bake at 95 °C for 20 min. After cooling down, the wafer was exposed to UV light with 1.2 mW/cm^2 power intensity for 40 s using an iron oxide/chrome photo mask (Front Range) to create the fluidic channels. The wafer was baked again at 65 °C for 15 min and at 95 °C for 30 min, post UV exposure. These steps were repeated to generate the top trapping layer. The silicon wafer was developed with an SU-8 developer solution (Microchem) to remove the uncrosslinked SU-8 to produce the microfluidic patterns. The wafer was hard baked at 150 °C for 30 min to increase wafer durability.

PDMS replicas (Slygard 184, Ellsworth Adhesives) were generated by mixing the base agent in a 10:1 ratio with the curing agent, followed by degassing in a vacuum chamber to create a bubble-free mixture. This PDMS was poured on the silicon master and was cured for at least 6 h at 65 °C. Once cured, the PDMS was removed from the wafer, and the inlet and outlet ports were punched using a blunted 18-gauge needle. The PDMS replicas were permanently bonded to 25X75 mm glass slides (Corning) using a O_2 Harrick Plasma PDC-32G basic plasma cleaner with a 30 s exposure to plasma. The devices were left overnight to ensure proper bonding between the PDMS and the glass. The fluidic channels in the microfluidic device were made hydrophobic by Aquapel treatment. Aquapel was manually injected into the device using a filtered syringe with excess Aquapel flushed out using Novec 7500 oil (3M). The channels were dried by blowing nitrogen and the resultant device was ready to be used for on-chip experiments.

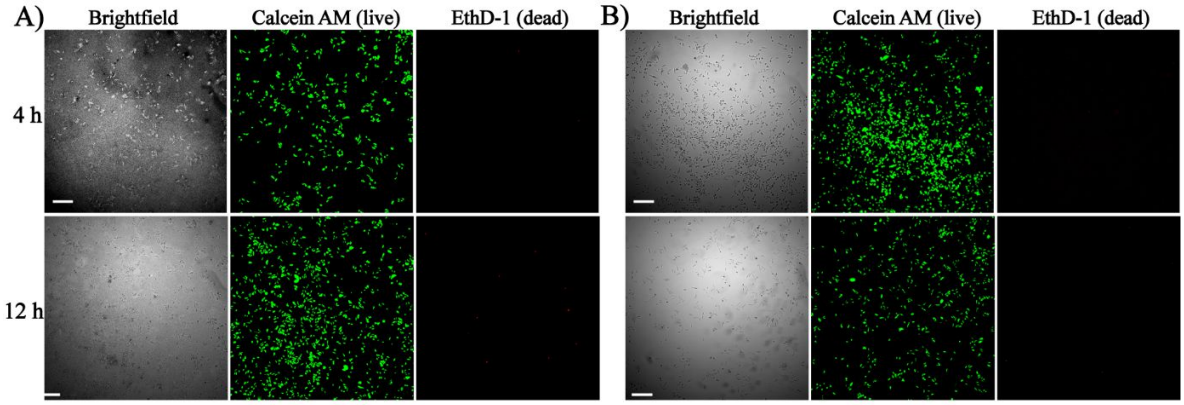

Fig. S1 (A) Determination of Tb^{3+}-doped luminescent NPs on cellular viability. MDA-MB-231 cells were incubated with Tb^{3+}-doped $NaYF_4$ NPs at 37°C for 4 h (top row) and 12 h (bottom row) followed by live/dead staining using 2.5 µM Calcein AM (green, middle column) and 4 µM EthD-1 (red, right column). (B) Observation of cell viability in no-NP control experiment for 4 h and 12 h. Scale bar is 300 µm

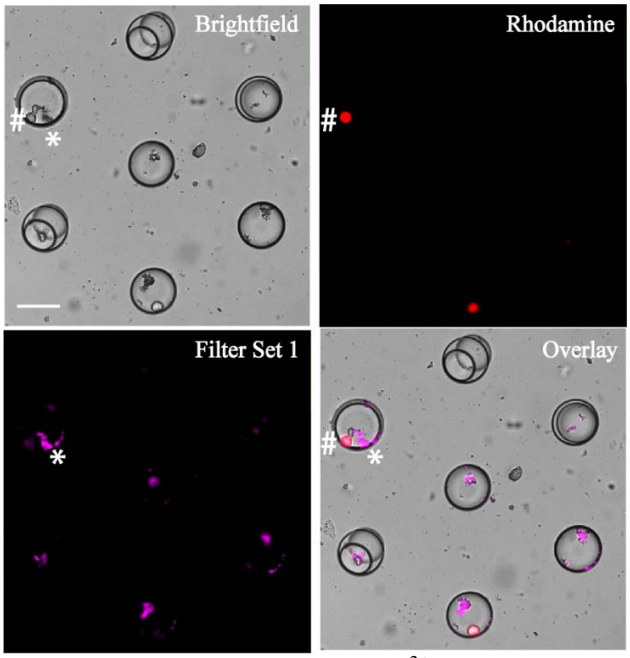

Fig. S2 Visualization of no spectral overlap between Eu^{3+}-doped NPs and red fluorescent protein (RFP). Eu^{3+}-doped $NaYF_4$ NPs (magenta) co-encapsulated with RFP-expressing MDA-MB-231 cells (red). Eu^{3+}-doped NPs are depicted in magenta to distinguish from the RFP-MDA-MB-231 cells (red). * denotes NPs and # denotes cells in the droplet. Scale bar is 70 µm

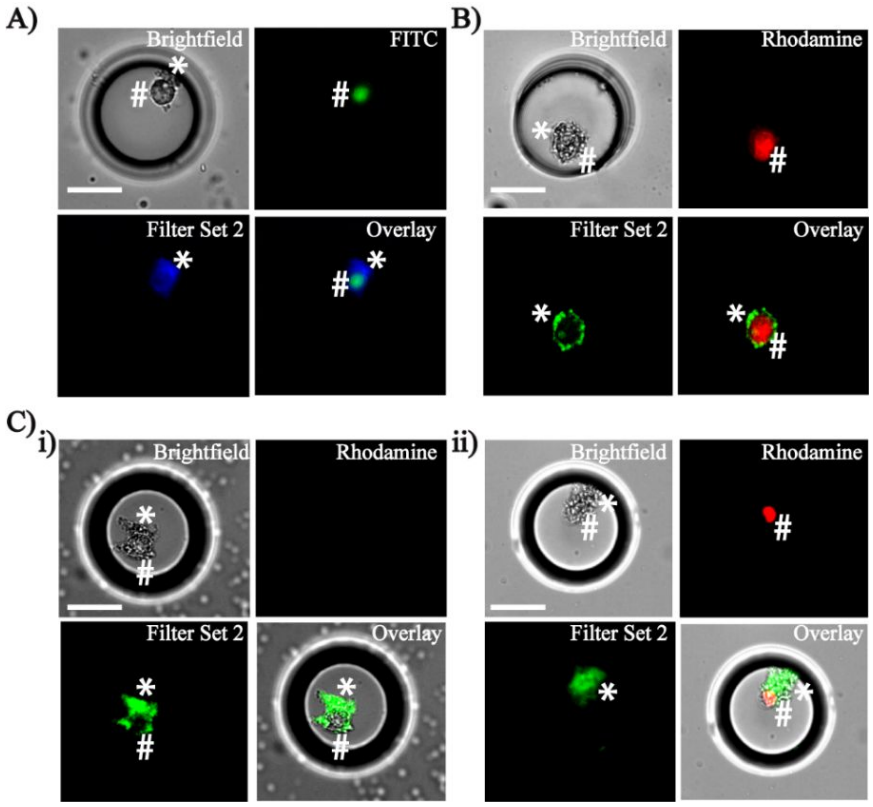

Fig. S3 Visualization of no spectral overlap between Tb^{3+}-doped NPs and fluorescent cells. (A) Tb^{3+}-doped $NaYF_4$ NPs (blue) co-encapsulated with GFP-expressing HeLa cells (green). Tb^{3+}-doped NPs are depicted in blue to distinguish from the GFP-HeLa cells (green). (B) Tb^{3+}-doped NaY_4 NPs (green) co-encapsulated with RFP-MDA-MB-231 cells (red). (C) Spectral independence of EthD-1-stained dead cells with Tb^{3+}-doped NPs using filter set 2. Live cells remained colorless in (C-i, rhodamine) and dead cells are shown in red due to EthD-1 (C-ii). * denotes NPs and # denotes cells in the droplet. Scale bar is 35 μm

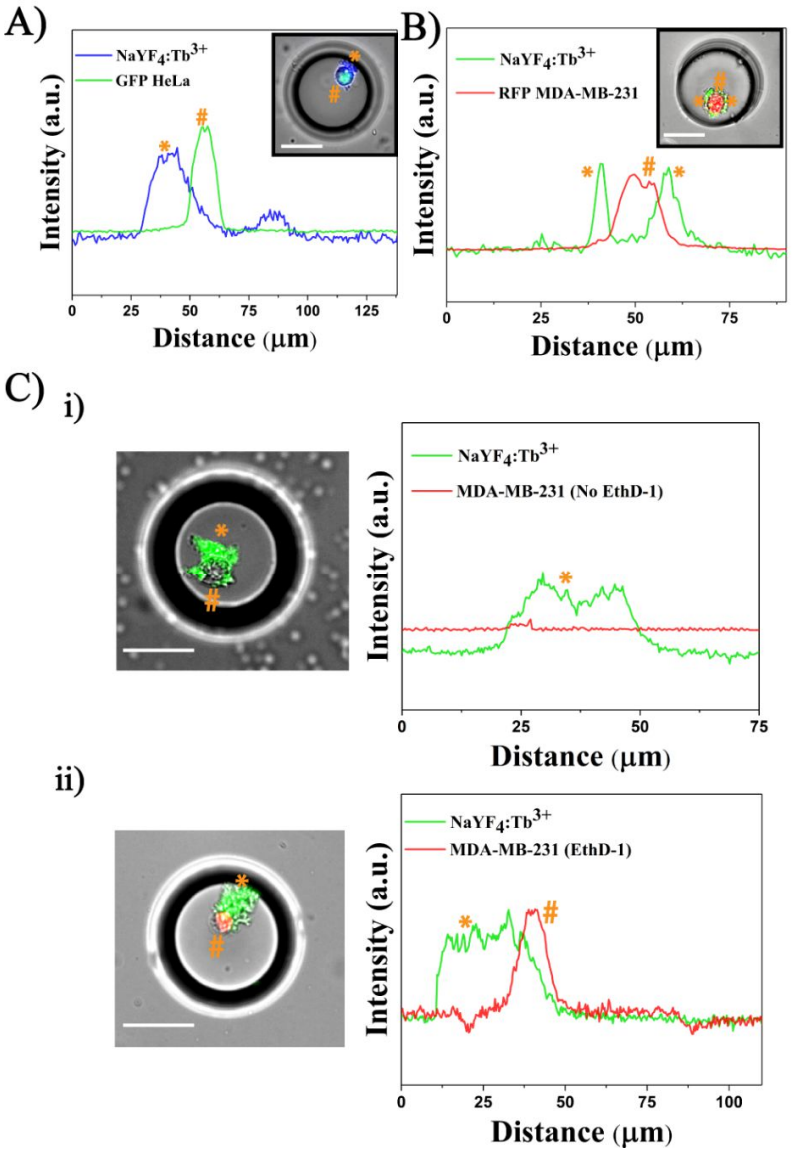

Fig. S4 Quantification of the spectral independence of Tb^{3+}-doped NPs with fluorescent cells. (A) Line scan across the droplet shows independent peak for Tb^{3+}-doped NPs (blue), a GFP-HeLa cell (green). Note: Tb^{3+}-doped NPs are depicted in blue to distinguish from GFP (green). (B) Spectral independence of Tb^{3+}-doped NPs (green) with an RFP-MDA-MB-231 cell (red). (C) Live (i) and dead (ii) MDA-MB-231 cells co-encapsulated with Tb^{3+}-doped NPs. * denotes NP and # denotes cell and their corresponding signal intensities across the droplet. Scale bar is 35 μm

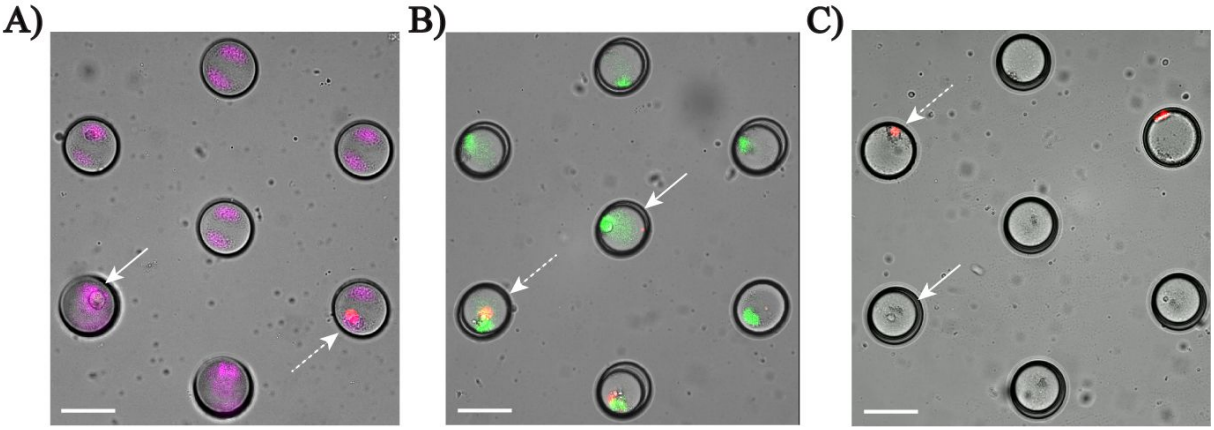

Fig. S5 Tracking dose-dependent response of single MDA-MB-231 cells to different doses of PTX. Cells were treated with three doses of PTX, off-chip; stained for viability with EthD-1 and co-encapsulated with three corresponding NPs in individual experiments on the single-input droplet microfluidic platform. A) 10 µM PTX treatment tracked by Eu^{3+}-doped NP, B) 50 µM PTX treatment tracked by Tb^{3+}-doped NP and C) 100 µM PTX treatment tracked by undoped NP. Full and dashed white arrowheads pointing at live and dead cells. Scale bar is 70 µm. Note: Eu^{3+}-doped NPs in A) are depicted in magenta to distinguish from the dead cells (red)

Table S1 Spectral independence of Eu^{3+}-doped NPs with GFP, RFP, EthD-1: average S:N ratio values of Eu^{3+}-doped NPs, GFP-HeLa cells, RFP-MDA-MB-231 cells, dead cells and their spectral independence within their detection filters and other commonly used filter sets. N/A denotes not applicable

Example Case	Category	ROI	FITC Filter	Rhodamine Filter	Filter Set 1	Filter Set 2
Fig. 7 (A)-i	GFP HeLa	The only ROI	**6.54**	1.21	1.01	1.69
	Europium NP	N/A	N/A	N/A	N/A	N/A
Fig. 7 (A)-ii	GFP HeLa	Distinct ROI	**4.78**	1.09	0.99	1.28
	Europium NP		1.18	1.05	**5.56**	1.15
Fig. 7 (B)-i	RFP MDA-MB-231	The only ROI	1.10	**5.32**	1.23	1.11
	Europium NP	N/A	N/A	N/A	N/A	N/A
Fig. 7 (B)-ii	RFP MDA-MB-231	Distinct ROI	1.14	**3.97**	1.36	1.11
	Europium NP		1.02	1.34	**5.21**	1.09
Fig. 7 (C)-i	Live MDA-MB-231	Distinct ROI	1.02	1.14	1.07	1.19
	Europium NP		1.03	1.12	**4.57**	1.01
Fig. 7 (C)-ii	Dead MDA-MB-231	Distinct ROI	1.16	**7.89**	1.54	1.07
	Europium NP		1.12	1.17	**3.28**	1.16

Table S2 Spectral independence of Tb^{3+}-doped NPs with GFP, RFP, EthD-1: average S:N ratio value of Tb^{3+}-doped NPs, GFP-HeLa cells, RFP-MDA-MB-231 cells, dead cells and their spectral independence within their detection filters and other commonly used filter sets

Example Case	Category	ROI	FITC Filter	Rhodamine Filter	Filter Set 1	Filter Set 2
Fig. S3 - (A)	GFP HeLa	Distinct ROI	**5.02**	0.98	1.03	1.27
	Terbium NP		0.99	1.09	1.01	**3.52**
Fig. S3 - (B)	RFP MDA-MB-231	Distinct ROI	0.90	**4.03**	1.12	1.08
	Terbium NP		0.92	1.21	1.04	**3.36**
Fig. S3 (C)-i	Live MDA-MB-231	Distinct ROI	1.06	0.84	0.97	1.02
	Terbium NP		0.99	1.05	1.13	**3.59**
Fig. S3 (C)-ii	Dead MDA-MB-231	Distinct ROI	0.96	**6.97**	1.32	1.15
	Terbium NP		1.09	0.97	1.07	**3.28**

Table S3 Single/two-tailed hypotheses statistics for the analysis of threshold values of average S:N ratio values of Eu^{3+}-doped NPs and GFP-HeLa cells. '$\mu_{(S:N)}$' denotes mean S:N ratio values Null hypotheses: $3<\mu_{(S:N)}<7.5$; $3<\mu_{(S:N)}<10$; $\mu_{(S:N)}<1.5$ for respective cases (i.e. the average S:N ratio values fall within the range). Alternate hypotheses: the average S:N ratio values do not fall within the given range. *P value* <0.05 rejects the null hypothesis in favor of the alternate, whereas larger *p values* fail to reject the null hypotheses

Cases	Average S:N ratio value (AU)	S:N ratio value - Standard Deviation (AU)	Null Hypothesis	p-value
Eu^{3+} doped NP	4.34 (Filter Set 1) 1.06 (FITC filter)	1.26 0.09	$3<\mu_{(S:N)}<7.5$ $\mu_{(S:N)}<1.5$	>0.90 >0.90
GFP - HeLa	5.86 (FITC filter) 1.12 (Filter Set 1)	2.22 0.19	$3<\mu_{(S:N)}<10$ $\mu_{(S:N)}<1.5$	>0.90 >0.90
Other filters				
Filter Set 2	1.04	0.15	$\mu_{(S:N)}<1.5$	>0.90
Rhodamine filter	0.98	0.45	$\mu_{(S:N)}<1.5$	>0.90

Table S4 Single/two-tailed hypotheses statistics for analysis of threshold values of average S:N ratio values of Eu^{3+}-doped NPs and RFP-MDA-MB-231 cells. '$\mu_{(S:N)}$' denotes mean S:N ratio values. Null hypotheses: $3<\mu_{(S:N)}<7.5$; $3<\mu_{(S:N)}<10$; $\mu_{(S:N)}<1.5$ for respective cases (i.e. the average S:N ratio values fall within the range). Alternate hypotheses: the average S:N ratio values do not fall within the given range. *P value* <0.05 rejects the null hypothesis in favor of the alternate, whereas larger *p values* fail to reject the null hypotheses

Cases	**Average S:N ratio value (AU)**	**S:N ratio value - Standard Deviation (AU)**	**Null Hypothesis**	**p-value**
Eu^{3+} doped NP	4.61 (Filter Set 1) 1.24 (Rhodamine filter)	1.12 0.08	$3<\mu_{(S:N)}<7.5$ $\mu_{(S:N)}<1.5$	>0.90 >0.90
RFP-MDA-MB-231	5.41 (Rhodamine filter) 1.19 (Filter Set 1)	1.89 0.17	$3< \mu_{(S:N)}<10$ $\mu_{(S:N)}<1.5$	>0.90 >0.90
Other filters				
Filter Set 2	1.06	0.09	$\mu_{(S:N)}<1.5$	>0.90
FITC filter	1.11	0.08	$\mu_{(S:N)}<1.5$	>0.90

Table S5 Single/two-tailed hypotheses statistics for analysis of threshold values of average S:N ratio values of Eu^{3+}-doped NPs and dead MDA-MB-231 cells (EthD-1-stained). '$\mu_{(S:N)}$' denotes mean S:N ratio values. Null hypotheses: $3<\mu_{(S:N)}<7.5$; $3<\mu_{(S:N)}<10$; $\mu_{(S:N)}<1.5$ for respective cases (i.e. the average S:N ratio values fall within the range). Alternate hypotheses: the average S:N ratio values do not fall within the given range. *P value* <0.05 rejects the null hypothesis in favor of the alternate, whereas larger *p values* fail to reject the null hypotheses

Cases	**Average S:N ratio value (AU)**	**S:N ratio value - Standard Deviation (AU)**	**Null Hypothesis**	**p-value**
Eu^{3+} doped NP	4.28 (Filter Set 1) 1.01 (Rhodamine filter)	1.63 0.21	$3<\mu_{(S:N)}<7.5$ $\mu_{(S:N)}<1.5$	>0.90 >0.90
EthD-1	6.41 (Rhodamine filter) 1.28 (Filter Set 1)	2.47 0.21	$3< \mu_{(S:N)}<10$ $\mu_{(S:N)}<1.5$	>0.90 >0.90
Other filters				
Filter Set 2	1.10	0.07	$\mu_{(S:N)}<1.5$	>0.90
FITC filter	1.03	0.13	$\mu_{(S:N)}<1.5$	>0.90

Table S6 Single/two-tailed hypotheses statistics for analysis of threshold values of average S:N ratio values of Tb^{3+}-doped NP and GFP-HeLa cells. '$\mu_{(S:N)}$' denotes mean S:N ratio values. Null hypotheses: $2.5<\mu_{(S:N)}<6$; $3<\mu_{(S:N)}<10$; $\mu_{(S:N)}<1.5$ for respective cases (i.e. the average S:N ratio values fall within the range). Alternate hypotheses: the average S:N ratio values do not fall within the given range. *P value* <0.05 rejects the null hypothesis in favor of the alternate, whereas larger *p values* fail to reject the null hypotheses

Cases	**Average S:N ratio value (AU)**	**S:N ratio value - Standard Deviation (AU)**	**Null Hypothesis**	**p-value**
Tb^{3+} doped NP	3.64 (Filter Set 2) 1.06 (FITC filter)	0.96 0.04	$2.5<\mu_{(S:N)}<6$ $\mu_{(S:N)}<1.5$	>0.90 >0.90
GFP - HeLa	5.39 (FITC filter) 0.97 (Filter Set 2)	2.02 0.82	$3<\mu_{(S:N)}<10$ $\mu_{(S:N)}<1.5$	>0.90 >0.90
Other filters				
Filter Set 1	1.03	0.06	$\mu_{(S:N)}<1.5$	>0.90
Rhodamine filter	1.08	0.17	$\mu_{(S:N)}<1.5$	>0.90

Table S7 Single/two-tailed hypotheses statistics for analysis of threshold values of average S:N ratio values of Tb^{3+}-doped NPs and RFP-MDA-MB-231 cells. '$\mu_{(S:N)}$' denotes mean S:N ratio values. Null hypotheses: $2.5<\mu_{(S:N)}<6$; $3<\mu_{(S:N)}<10$; $\mu_{(S:N)}<1.5$ for respective cases (i.e. the average S:N ratio values fall within the range). Alternate hypotheses: the average S:N ratio values do not fall within the given range. *P value* <0.05 rejects the null hypothesis in favor of the alternate, whereas larger *p values* fail to reject the null hypotheses

Cases	**Average S:N ratio value (AU)**	**S:N ratio value - Standard Deviation (AU)**	**Null Hypothesis**	**p-value**
Tb^{3+} doped NP	3.59 (Filter Set 2) 0.98 (Rhodamine filter)	1.16 0.08	$2.5<\mu_{(S:N)}<6$ $\mu_{(S:N)}<1.5$	>0.90 >0.90
RFP-MDA-MB-231	4.89 (Rhodamine filter) 1.06 (Filter Set 2)	1.15 0.11	$3< \mu_{(S:N)}<10$ $\mu_{(S:N)}<1.5$	>0.90 >0.90
Other filters				
Filter Set 1	1.02	0.15	$\mu_{(S:N)}<1.5$	>0.90
FITC filter	0.96	0.26	$\mu_{(S:N)}<1.5$	>0.90

Table S8 Single/two-tailed hypotheses statistics for analysis of threshold values of average S:N ratio values of Tb^{3+}-doped NPs and dead MDA-MB-231 cells (EthD-1-stained). Null hypotheses: $2.5<\mu_{(S:N)}<6$; $3<\mu_{(S:N)}<10$; $\mu_{(S:N)}<1.5$ for respective cases. '$\mu_{(S:N)}$' denotes mean S:N ratio values (i.e. the average S:N ratio values fall within the range). Alternate hypotheses: the average S:N ratio values do not fall within the given range. *P value* <0.05 rejects the null hypothesis in favor of the alternate, whereas larger *p values* fail to reject the null hypotheses

Cases	**Average S:N ratio value (AU)**	**S:N ratio value - Standard Deviation (AU)**	**Null Hypothesis**	**p-value**
Tb^{3+} doped NP	3.44 (Filter Set 2) 0.96 (Rhodamine filter)	0.86 0.34	$2.5<\mu_{(S:N)}<6$ $\mu_{(S:N)}<1.5$	>0.90 >0.90
EthD-1	6.18 (Rhodamine filter) 1.03 (Filter Set 2)	2.31 0.15	$3< \mu_{(S:N)}<10$ $\mu_{(S:N)}<1.5$	>0.90 >0.90
Other filters				
Filter Set 1	1.19	0.25	$\mu_{(S:N)}<1.5$	>0.90
FITC filter	1.06	0.11	$\mu_{(S:N)}<1.5$	>0.90