Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

Two Fluorinated Thulium Complexes as Molecular Temperature Sensors in MR Applications

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Experimental section

All chemicals were purchased from commercial sources. Water and oxygen-sensitive reactions were performed in an in vacuo dried flask under a nitrogen atmosphere. Dry solvents were stored for at least one week over molecular sieves (3 or 4 Å) and dry DCM was directly taken from a solvent purification system SPS-800 constructed by M. Braun. TLC controls were performed on ALUGRAM® Xtra SIL G/UV_{254} purchased from Macherey-Nagel and were visualized through staining with $KMnO_4$.

Variable-temperature NMR spectra were recorded with a Bruker Avance NEO spectrometer in the temperature range of 298.15 K (25 °C) to 323.15 K (50 °C) in 0.5 mL solvent (D_2O) and 3-5 mg of probe. The relaxation delay d1 was 2.5 s and the number of scans ns was 16.

Cell viability tests

The cell culture was performed using materials from Gibco®, including Dubecco's Modified Eagle Medium (DMEM) and fetal calf serum (FCS) for medium preparation, and Hank's balanced salt solution (HBSS) and a 0.5% EDTA-trypsin mixture for releasing the cells from the culture flasks (250 ml, Greiner Bio-One GmbH). Ciprofloxacin antibiotic was obtained from Fresenius Kabi AG, and tryptan blue solution (0.5%) for staining the cells for viability testing was obtained from Carl Roth® GmbH and Co. KG. The tryptan blue solution was prepared by dissolving 50 mg of the substance in 10 ml of isotonic saline solution. The complexes were used in concentrations of 0.19 mM for **TmL1** and 0.24 mM for **TmL2**.

To assess the toxicity of the synthesized complexes, their effect on fibroblasts of the L929 cell line is tested. The cells are first cultivated in cell culture flasks. After cultivation, the cells are divided into several 12-well plates and mixed with the respective complexes. Then, the cells are incubated for 24 and 48 hours. After the incubation times, the cells are freed from the medium and detached from the plate. The cells are then stained with a tryptan blue solution. These are quickly transferred to a counting chamber. An evaluation of the cell viability is performed using the Countess™ II automated cell counter from ThermoFischer Scientific©. The cell counter distinguishes between live and dead (stained) cells. The resulting viability is graphically represented.

Synthesis

N-(2,5-Bis(trifluoromethyl)phenyl)-2-bromoacetamide (2):

2,5-Bis(trifluoromethyl)aniline (1 g, 0.68 mL, 4.36 mmol), and K_2CO_3 are dissolved at 0 °C in 43.6 ml of DCM. Bromoacetyl bromide (0.75 mL, 8.72 mmol) is slowly added in 8.72 mL of DCM. The reaction is stirred for an additional 30 min at 0 °C and then for 1.5 h at room temperature. Water is added and the phases are separated. The organic phase is washed with saturated $NaHCO_3$ and brine and dried over $MgSO_4$. The solvent is removed by rotary evaporator. The product is obtained as white solid (1.52 g, 4.36 mmol, quant.).

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) = 8.76 (s_{br}, 1H, NH), 8.64 (s, 1H, Ar-H), 7.81 (d, 1H, Ar-H), 7.57 (d, 1H, Ar-H), 4.11 (s, 2H, CH_2).

^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) = 163.85, 135.40, 127.01, 124.05, 123.86, 122.24, 122.05, 121.75, 121.73, 120.54, 120.52, 29.12.
^{19}F NMR (564 MHz, $CDCl_3$): δ (ppm) = –61.22, –63.38.

HRMS (ESI, MeOH, positive): found: *m/z* = 371.94282 $[M+Na]^+$, calc.: *m/z* = 371.94292 $[M+Na]^+$.

Tri-tert-butyl-2,2',2''-(10-(2-((3,5-bis(trifluoromethyl)phenyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (3):

DO3A-tBu (108.9 mg, 0.207 mmol) and Na_2CO_3 (51.4 mg, 0.48 mmol) are dissolved in 11 mL $CHCl_3$ and the appropriate acetamide **2** (78.75 mg, 0.22 mmol) is added dropwise in 2.2 mL $CHCl_3$. The reaction is stirred for 7 days at 55 °C, then the solid is filtered and the organic phase is washed with water and brine. After column chromatography (silica gel, $CHCl_3$/MeOH 9+1, R_F = 0.41), the product is received as white solid (149.9 mg. 0.191 mmol, 92 %).

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm) = 10.17 (s, 1H, NH), 7.78 (s, 1H, Ar-H), 7.67 (d, 1H, Ar-H), 7.46 (d, 1H, Ar-H), 3.77 – 2.05 (m_{br}, 24H, CH_2), 1.40 – 1.10 (m, 27H, CH_3).

^{13}C NMR (151 MHz, $CDCl_3$): δ (ppm) = 172.86, 172.45, 172.30, 136.57, 134.02 (q, *J* = 33.0 Hz), 129.72 (q, *J* = 30.8 Hz), 127.22 (q, *J* = 3.8 Hz), 126.90 (q, *J* = 5.0 Hz), 125.44 – 119.99 (q), 125.76 – 120.34 (q), 122.66 (d), 81.92 (d), 56.46 (d), 55.60 (d), 52.33 (d), 48.38, 27.92 – 27.34 (m).

^{19}F NMR (564 MHz, $CDCl_3$): δ (ppm) = –62.73, –64.40.

HRMS (ESI, MeOH, positive): found: *m/z* = 806.38831 $[M+Na]^+$, calc.: *m/z* = 806.38979 $[M+Na]^+$, found: *m/z* = 784.40650 $[M+H]^+$, calc.: *m/z* = 784.40784 $[M+H]^+$.

2,2',2''-(10-(2-((2,5-Bis(trifluoromethyl)phenyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (L1):

The DOTA derivative **3** (40 mg, 0.05 mmol) is solved in 0.4 mL formic acid and heated to reflux for 48 h. Then the reaction is cooled to room temperature and the solvent is removed in vacuo. The residue is solved in water, then the water is removed. This procedure is repeated two times. The residue is then solved in water and freeze-dried. The free ligand is received as white solid (30 mg, 0.049 mmol, quant.).

^{1}H NMR (600 MHz, D_2O) δ (ppm) = 7.90 (s_{br}, 1H, Ar-H), 7.77 (s_{br}, 2H, Ar-H), 3.92 – 3.00 (m, 24H, CH_2).

^{13}C NMR (151 MHz, D_2O): δ (ppm) = 170.11, 170.10, 170.07, 166.83, 134.15, 133.89, 127.87, 127.22, 127.20, 124.90, 123.84, 122.04, 59.59, 56.53, 53.97, 53.01, 51.35, 51.05, 48.04.

^{19}F NMR (564 MHz, D_2O): δ (ppm) = –61.66, –63.18.

HRMS (ESI, MeOH, positive): found: *m/z* = 638.20162 $[M+Na]^+$ calc.: *m/z* = 638.20199, found: *m/z* = 616.21986 $[M+H]^+$ calc.: *m/z* = 616.22004 $[M+H]^+$

Di-*tert*-butyl 2,2'-(4,10-bis(2-((3,5-bis(trifluoromethyl)-phenyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate (4):

DO2A-tBu (45 mg, 0.11 mmol) and Na_2CO_3 (58 mg, 0.55 mmol) are dissolved in 10 mL $CHCl_3$ and the appropriate acetamide **2** (92 mg, 0.26 mmol) is added dropwise in 2 mL $CHCl_3$. The reaction is stirred for 7 days at 55 °C, then the solid is filtered and the organic phase is washed with water and brine. After column chromatography (silica gel, $CHCl_3$/MeOH 9+1, R_F = 0.14), the product is received as white solid (35 mg, 0.038 mmol, 35 %).

^{1}H NMR (600 MHz, MeOD-d_4, 297 K): δ (ppm) = 7.98 (d, *J* = 8.3 Hz, 2H), 7.92 (d, *J* = 1.6 Hz, 2H), 7.87 (s, 1H), 7.78 (d, *J* = 8.7 Hz, 2H), 3.78 (s, 2H), 3.37 (s, 2H), 3.30 – 3.01 (m, 6H), 2.75 (s, 4H), 2.43 – 1.99 (m, 7H), 1.22 (s, 18H).

^{13}C NMR (151 MHz, MeOD-d_4, 297 K): δ (ppm) = 176.78, 176.35, 140.09, 138.49, 138.30, 133.12, 132.92, 131.30, 130.75, 129.62, 127.81, 127.35, 126.05, 126.00, 124.20, 85.09, 82.01, 60.86, 60.01, 59.93, 58.96, 56.54, 52.38, 52.10, 30.68, 30.63.

^{19}F NMR (554 MHz, MeOD-d_4, 297 K): δ (ppm) = –62.79, –64.64.

HRMS (ESI, MeOH, positive): found: *m/z* = 961.34806 $[M+Na]^+$ calc.: *m/z* = 961.34924, found: *m/z* = 939.36730 $[M+H]^+$ calc.: *m/z* = 393.36730 $[M+H]^+$.

2,2'-(4,10-Bis(2-((2,5-bis(trifluoromethyl)phenyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (L2):

The DOTA derivative **4** (270 mg, 0.288 mmol) is dissolved in 4 mL formic acid and heated to reflux for 48 h. Then the reaction is cooled to room temperature and the solvent is removed in vacuo. The residue is solved in water, then the water is removed. This procedure is repeated two times. The residue is then solved in water and freeze-dried. The free ligand is received as white solid (197.3 mg, 0.239 mmol, 83 %).

^{1}H NMR (600 MHz, MeOD-d_4) δ (ppm) = 8.10 (s, 2H, Ar-H), 7.93 (d, 2H, Ar-H), 7.75 (d, 2H, Ar-H), 3.81 (m, 8H, CH_2), 3.49 (m, 8H, CH_2), 3.19 (m, 8H, CH_2).

^{13}C NMR (151 MHz, MeOD-d_4): δ (ppm) = 171.00, 170.93, 163.85, 135.64, 134.39, 134.17, 132.66, 127.30, 127.26, 126.42, 126.40, 125.86, 124.05, 123.87, 123.21, 122.24, 122.06, 120.25, 96.83, 92.57, 76.73, 76.64, 74.91, 73.48, 72.48, 71.57, 70.49, 70.44, 70.36, 69.86, 68.00, 64.51, 63.14, 63.09, 61.49, 61.38, 56.43, 54.34, 51.50, 48.44, 48.16.

^{19}F NMR (564 MHz, MeOD-d_4): δ (ppm) = –62.59, –64.67.

HRMS (ESI, MeOH, positive): found: *m/z* = 827.24106 $[M+H]^+$ calc.: *m/z* = 827.24210 $[M+H]^+$.

Complexation

TmL1

The free Ligand (20 mg, 0.032 mmol) is dissolved in 5 mL water and 1.1 eq of the $TmCl_3$*6 H_2O (13.5 mg, 0.035 mmol) is added. The pH is adjusted to 7 and the mixture is stirred for 1 day at

45 °C. Then the pH is adjusted to 10 using 0.1 M NaOH to precipitate unchelated Ln-salt as their hydroxy salt. After filtration, the pH is neutralized using 1M HCl and the solvent is evaporated. The NaCl is removed through centrifugation in EtOH. After evaporating the solvent, the residue is dissolved in water and dried in a freeze dryer. The complex is received as white solid (22.9 mg, 0.029 mmol, 92%).

^{19}F NMR (554 MHz, D_2O): δ (ppm) = –34.73, –78.97.

HRMS (ESI, MeOH, positive): found: *m/z* = 804.11120 $[M+Na]^+$, calc.: *m/z* = 804.11273 $[M+Na]^+$.

TmL2

A solution of **L2** (17 mg, 0.020 mmol) in MeOH (3 mL) is mixed with $TmCl_3*6H_2O$ (8.6 mg, 0.022 mmol) and stirred for 3 d at room temperature at a pH of 7. The solvent is then removed under reduced pressure, and the residue is dissolved in 3 mL H_2O. The pH is adjusted to 10 with a 0.1M NaOH solution and filtered. The filtrate is neutralized with 1M HCl and the solvent is removed under reduced pressure. The residue is mixed with EtOH and the solution is centrifuged. The solvent is removed by rotary evaporation; the residue is mixed again with water and dried in a freeze dryer. The complex was obtained as colorless solid (17 mg, 17 µmol, 85%).

^{19}F NMR (554 MHz, D_2O): δ (ppm) = –37.07, –67.91.

HRMS (ESI, MeOH, positive): found: *m/z* = 993.15181 $[M]^+$, calc.: *m/z* = 993.15284 $[M]^{+\cdot}$

N-(2,5-Bis(trifluoromethyl)phenyl)-2-bromoacetamide (2):

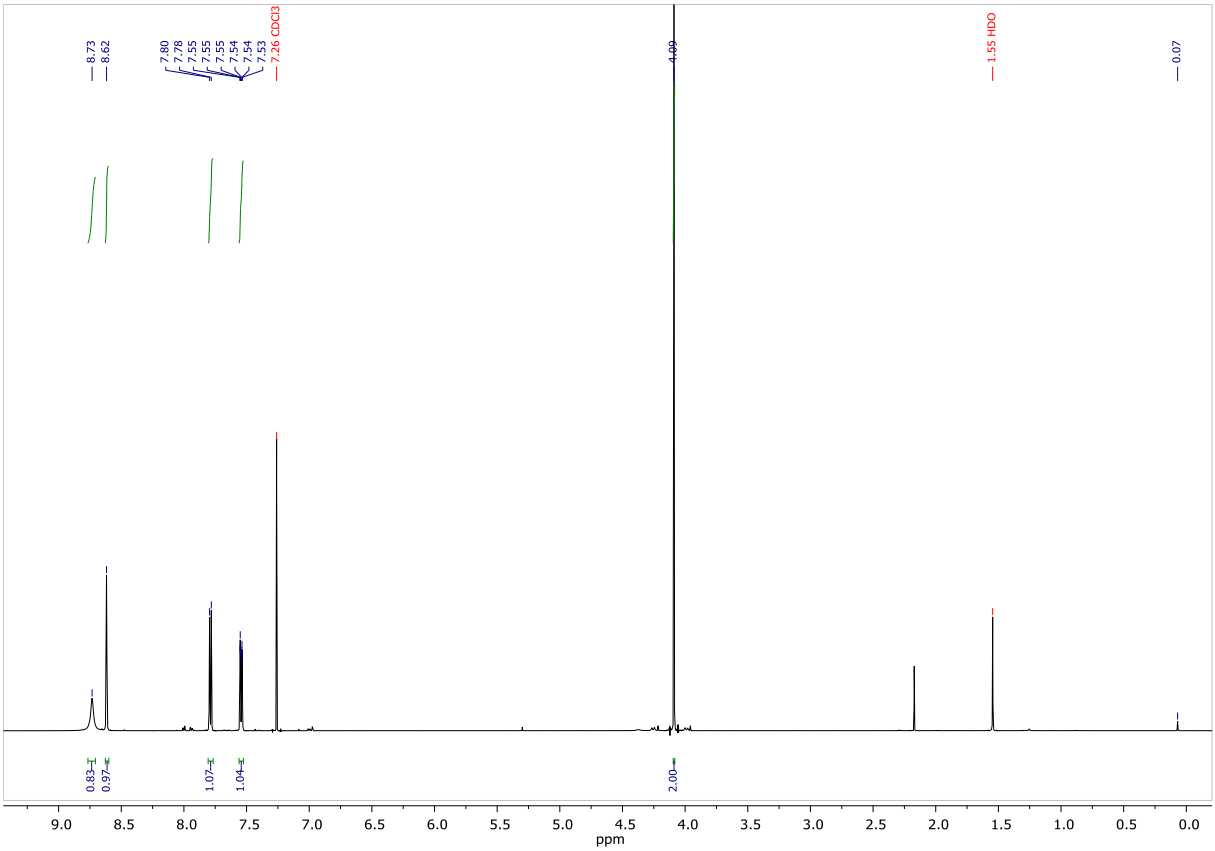

Figure 1: ^{1}H NMR spectra of **2** in $CDCl_3$.

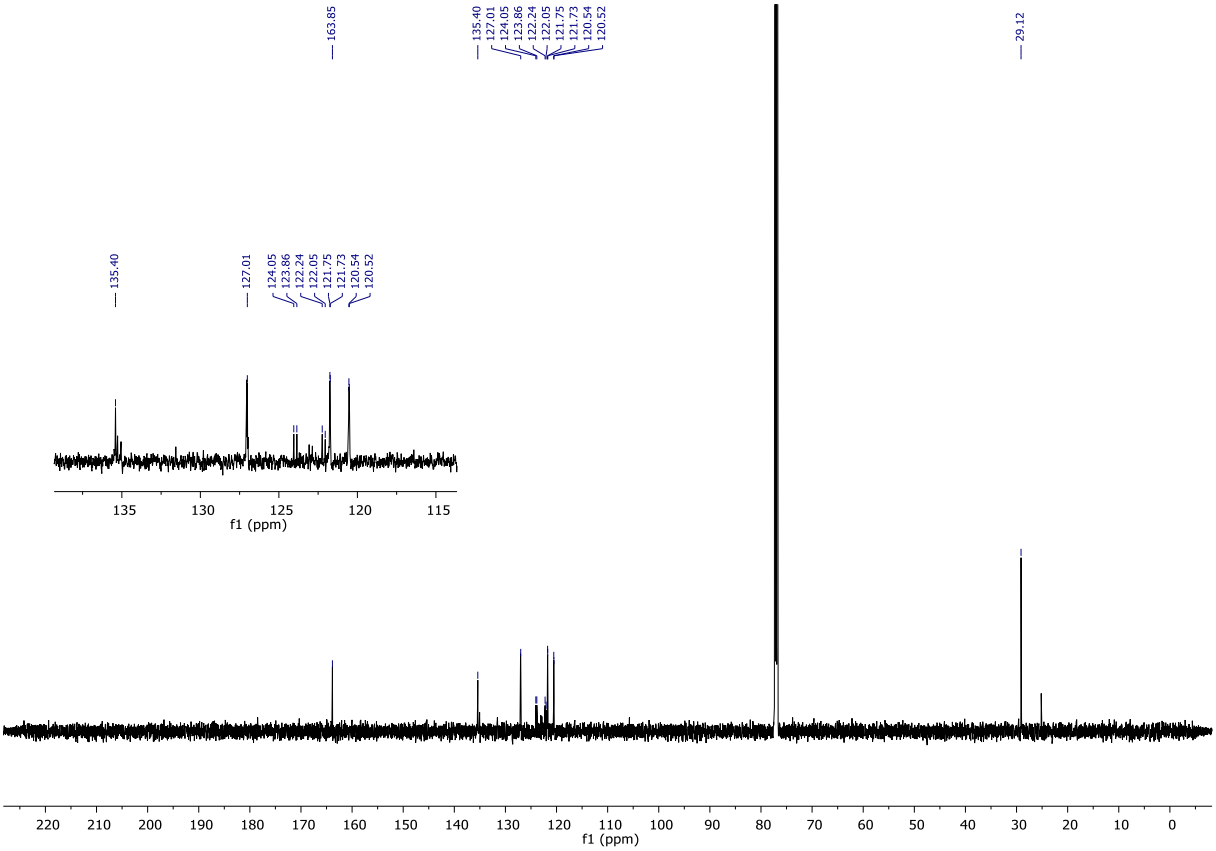

Figure 2: ^{13}C NMR spectra of **2** in $CDCl_3$.

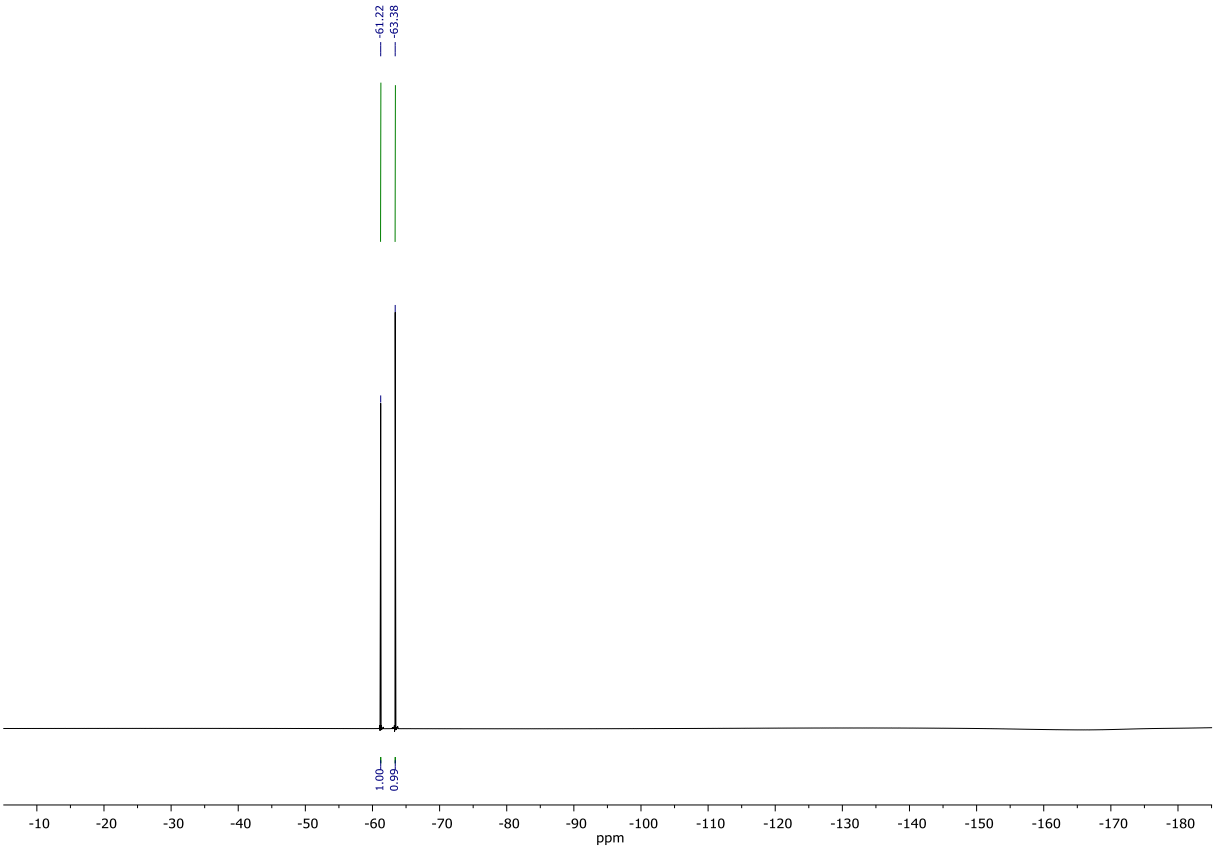

Figure 3: ^{19}F NMR spectra of **2** in $CDCl_3$.

Tri-tert-butyl-2,2',2''-(10-(2-((3,5-bis(trifluoromethyl)phenyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (3):

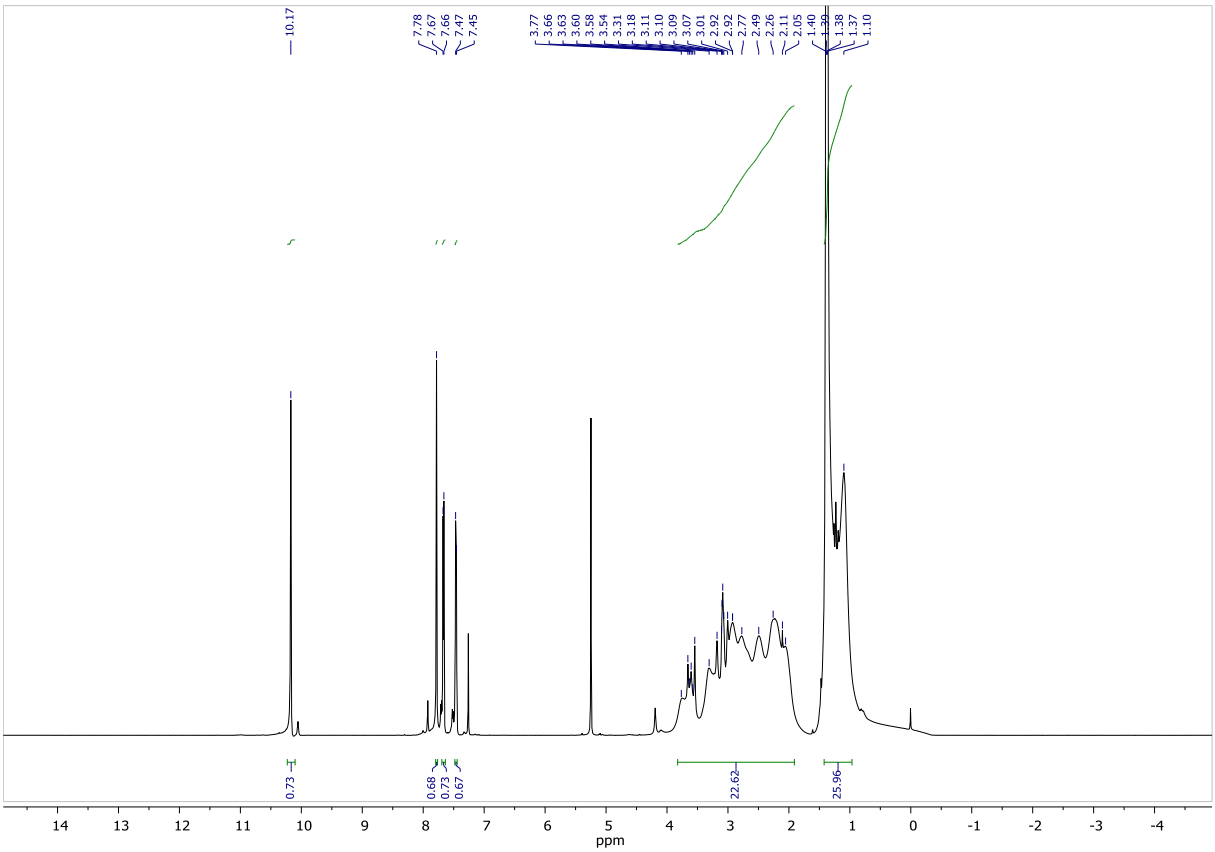

Figure 4: ^{1}H NMR spectra of **3** in $CDCl_3$.

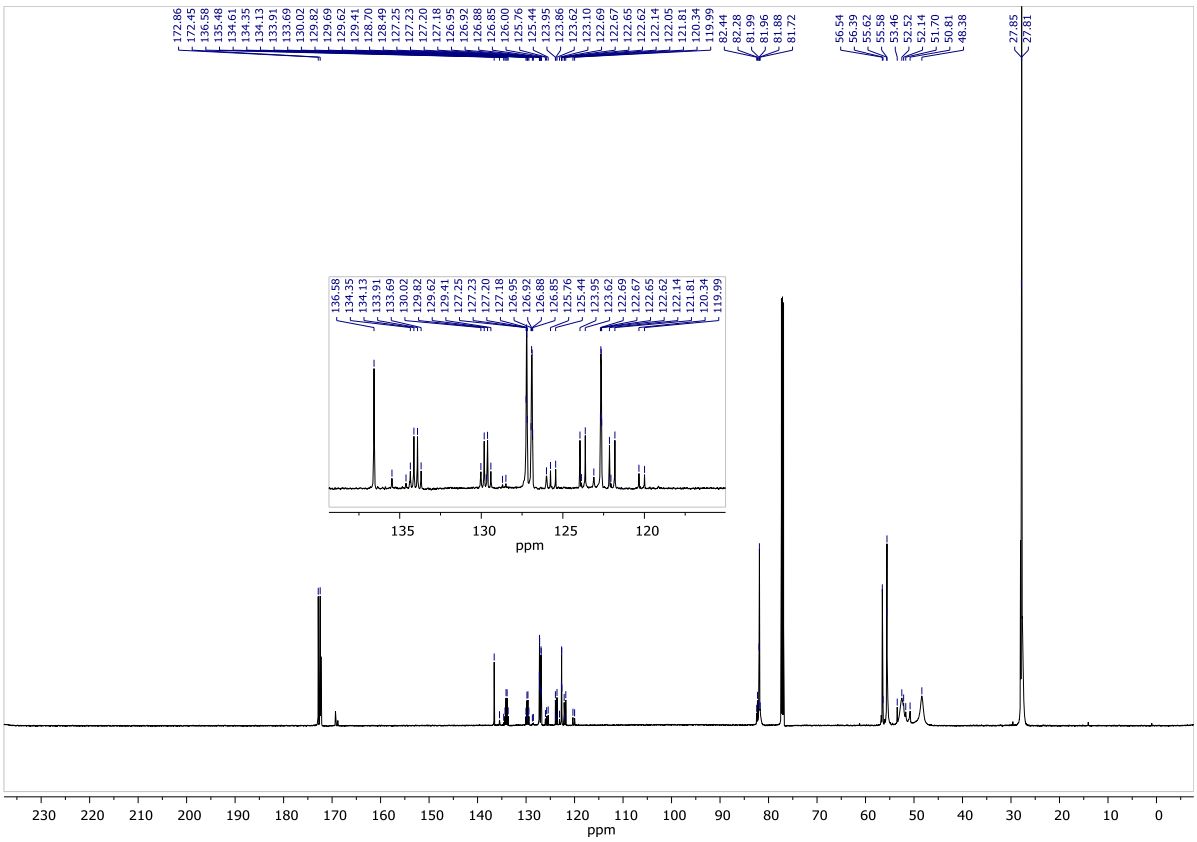

Figure 5: ^{13}C NMR spectra of **3** in $CDCl_3$.

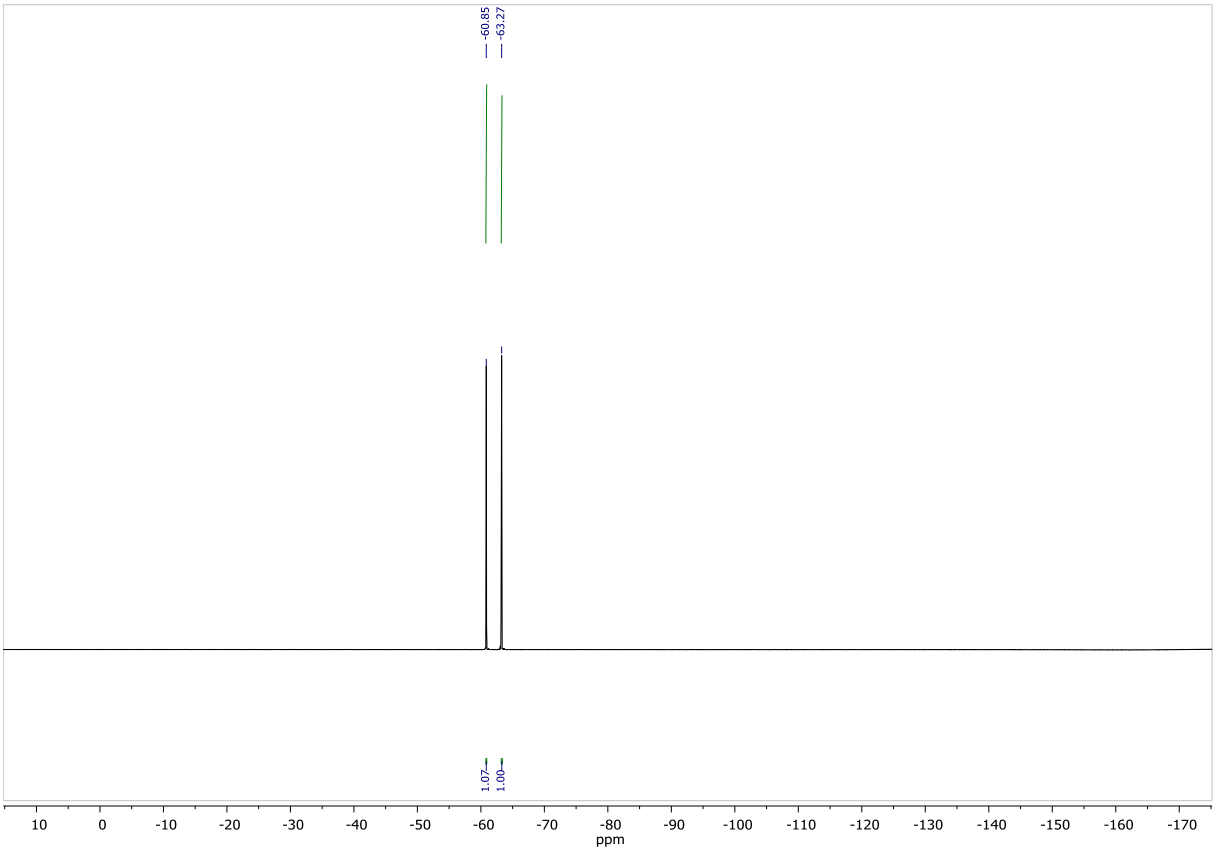

Figure 6: ^{19}F NMR spectra of **3** in $CDCl_3$.

2,2',2''-(10-(2-((2,5-Bis(trifluoromethyl)phenyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (L1):

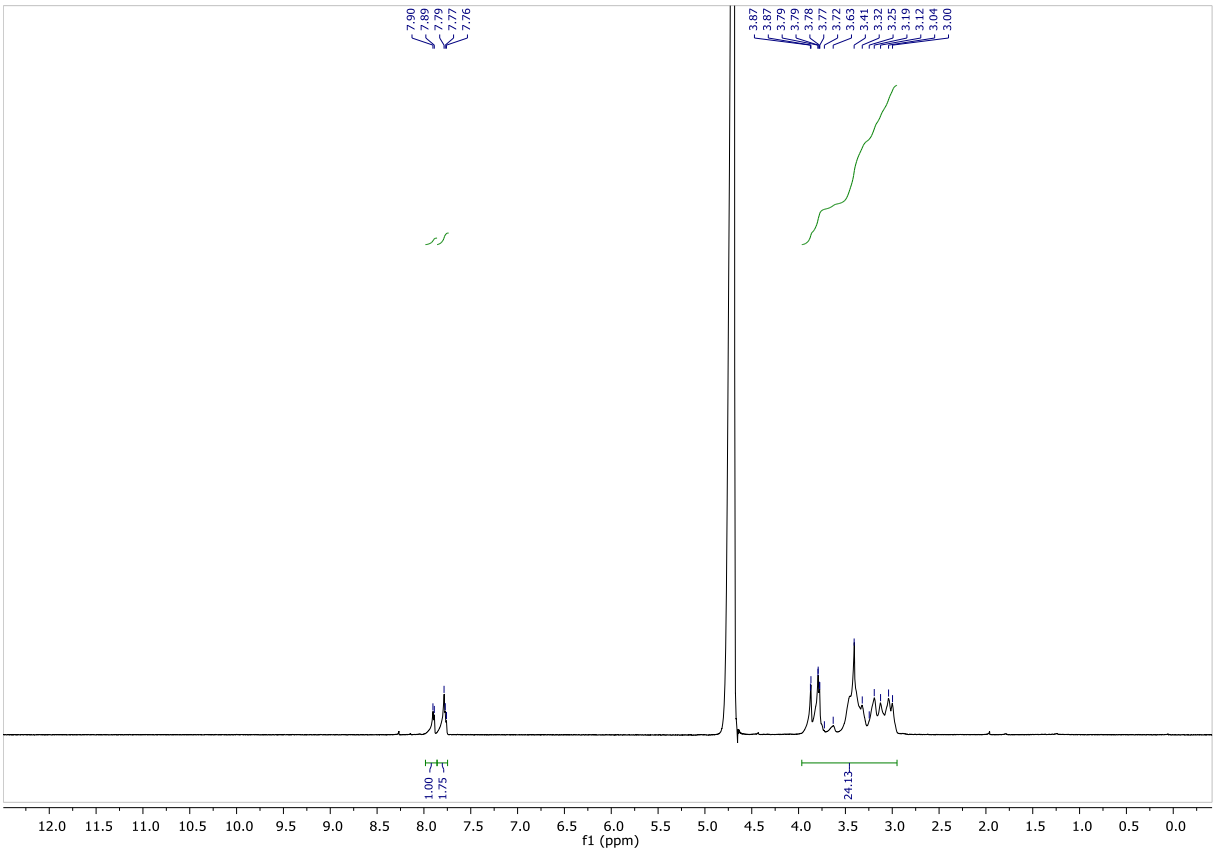

Figure 7: ^{1}H NMR spectra of **L1** in D_2O.

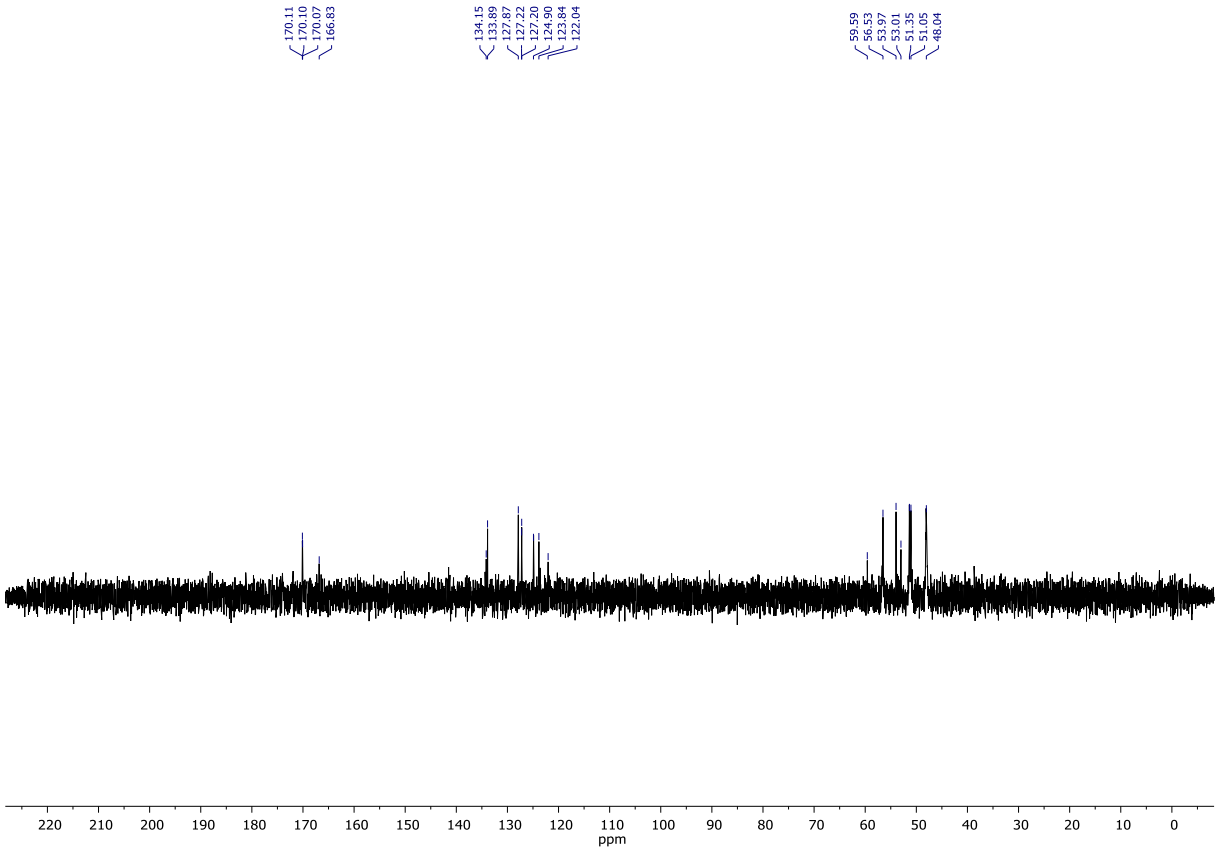

Figure 8: ^{13}C NMR spectra of **L1** in D_2O.

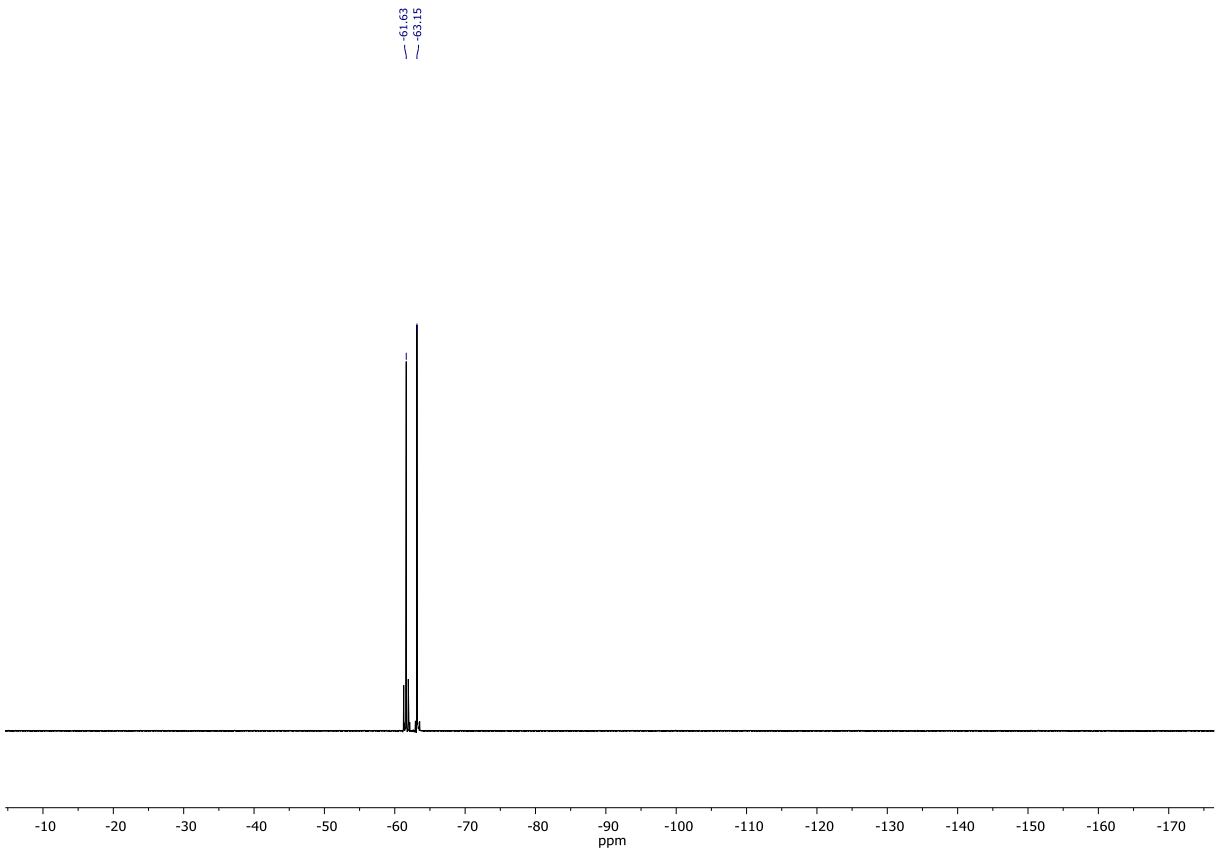

Figure 9: ^{19}F NMR spectra of **L1** in D_2O.

Di-*tert*-butyl 2,2'-(4,10-bis(2-((3,5-bis(trifluoromethyl)-phenyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetate (4):

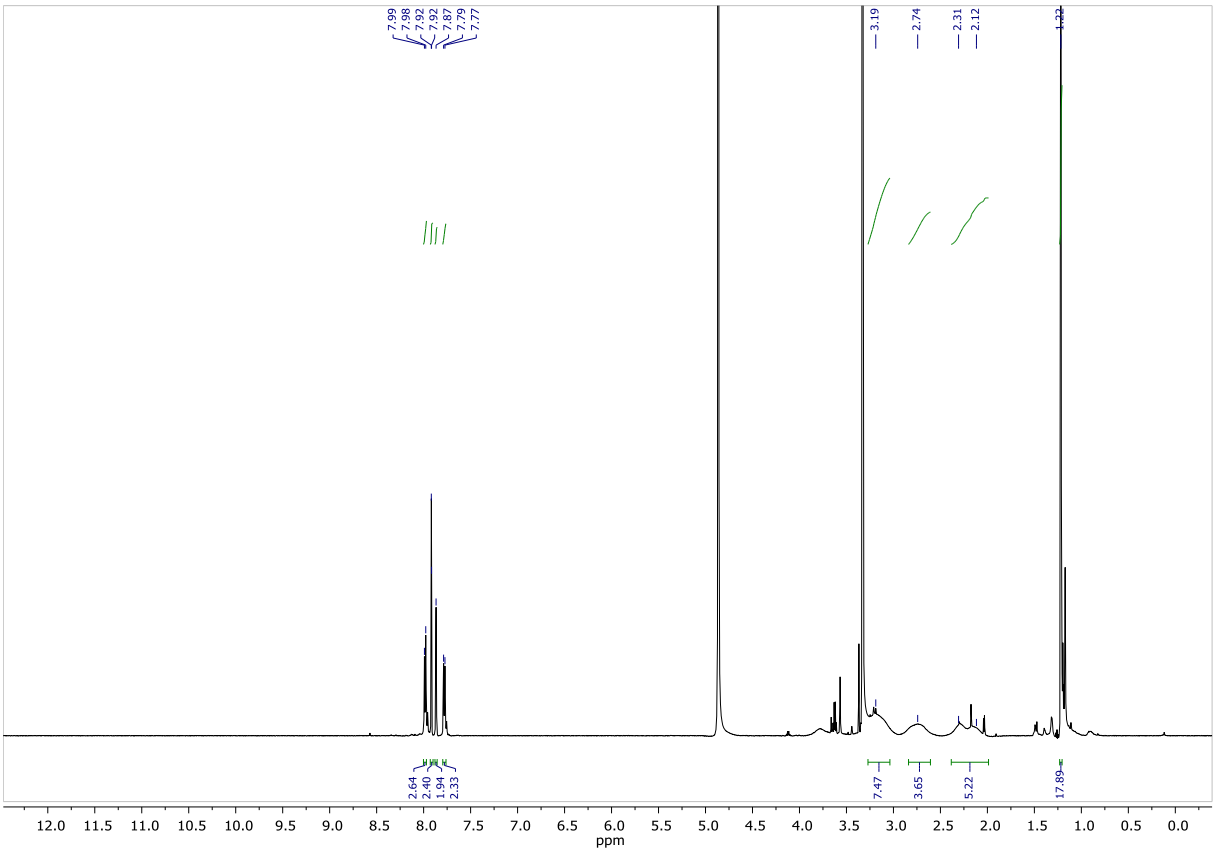

Figure 10: ^{1}H NMR spectra of **4** in $CDCl_3$.

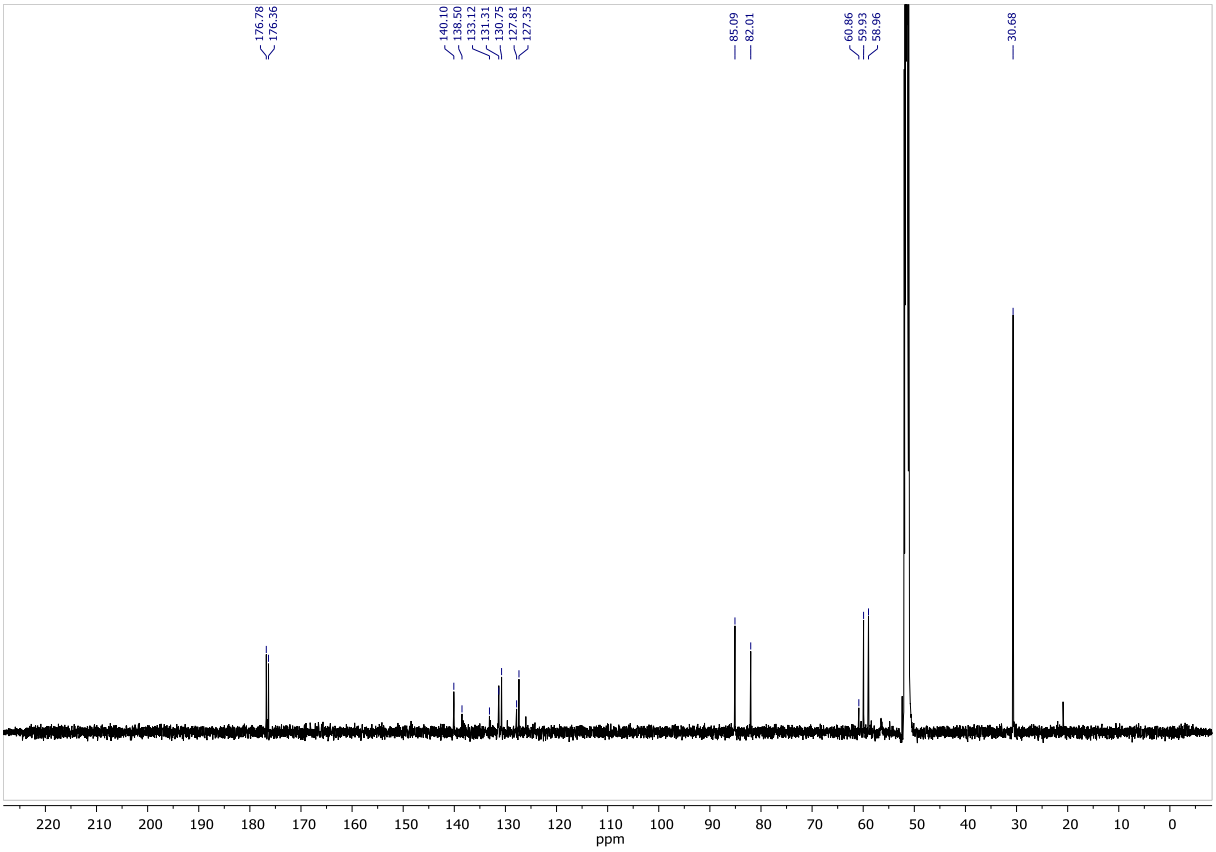

Figure 11: ^{13}C NMR spectra of **4** in $CDCl_3$.

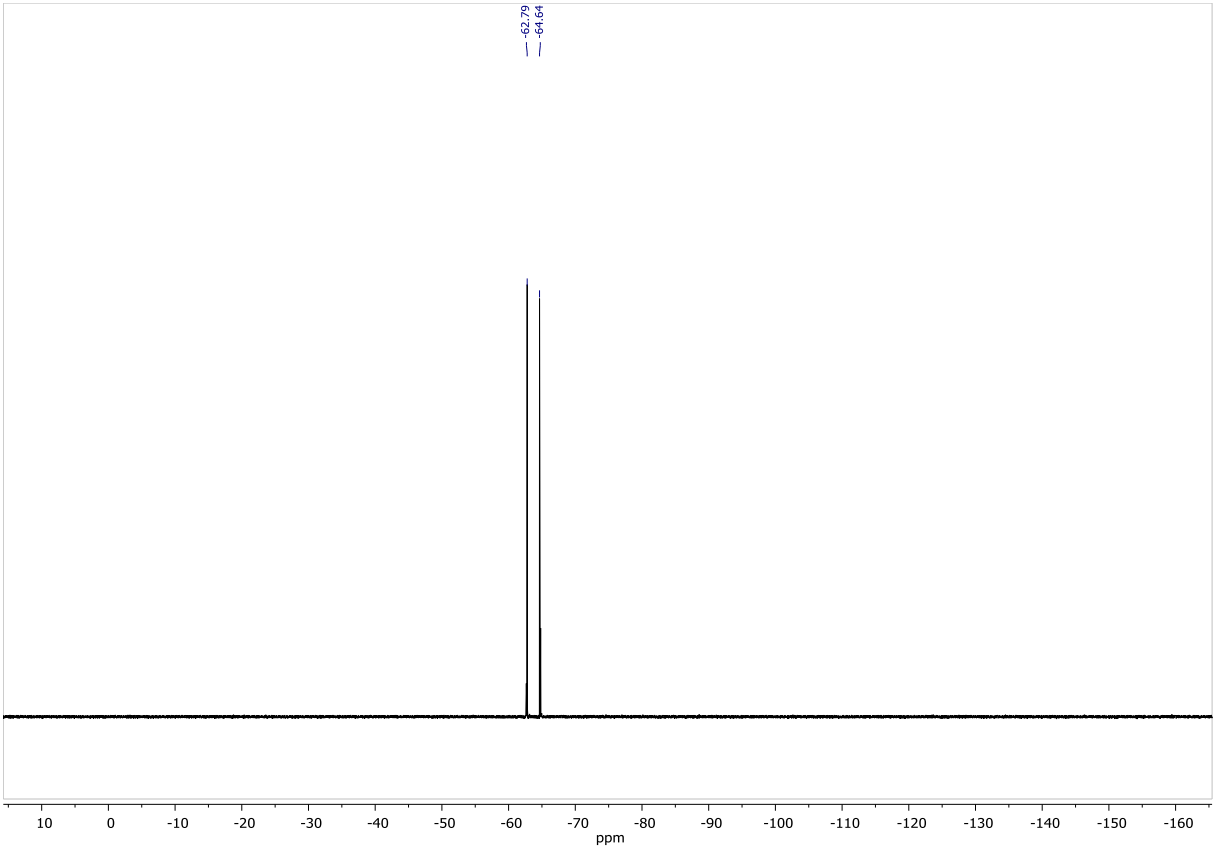

Figure 12: ^{19}F NMR spectra of **4** in $CDCl_3$.

2,2'-(4,10-Bis(2-((2,5-bis(trifluoromethyl)phenyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid (L2):

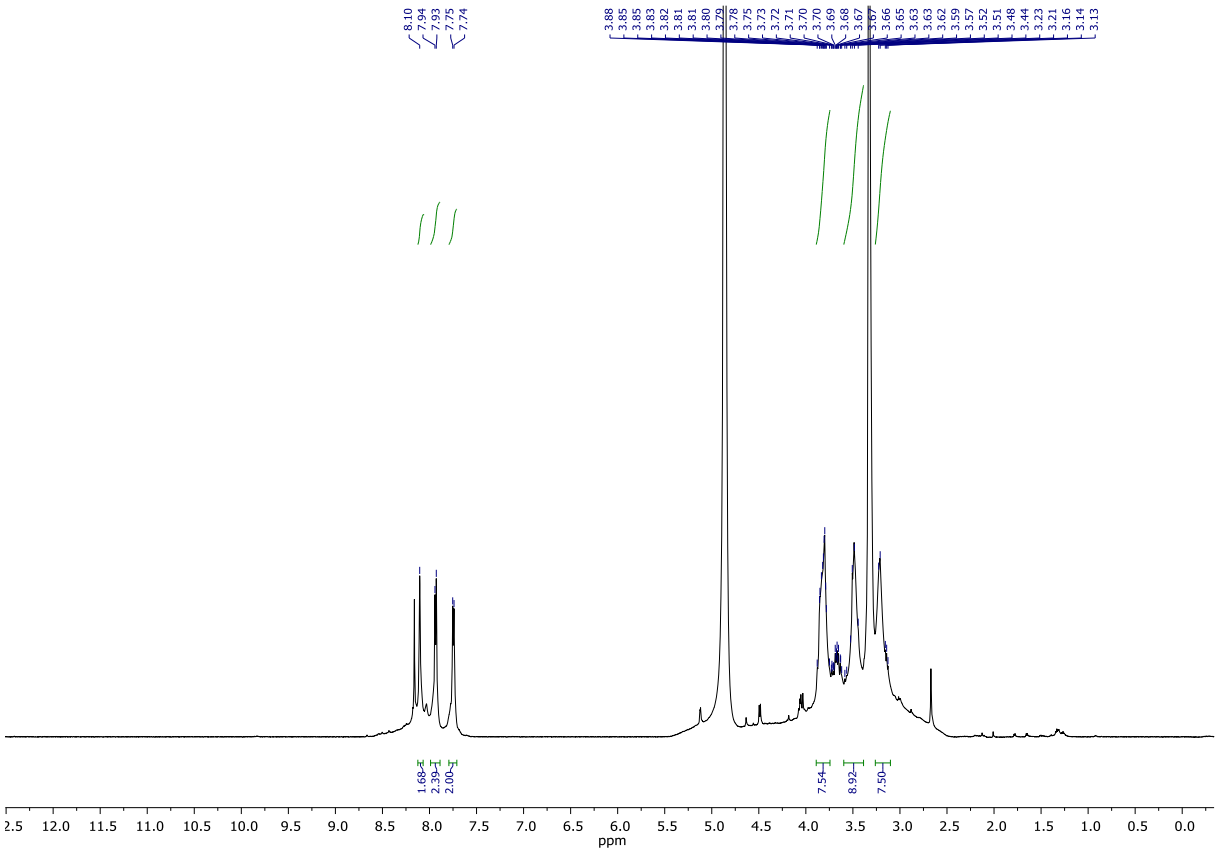

Figure 13: ^{1}H NMR spectra of **L2** in MeOD-d_4.

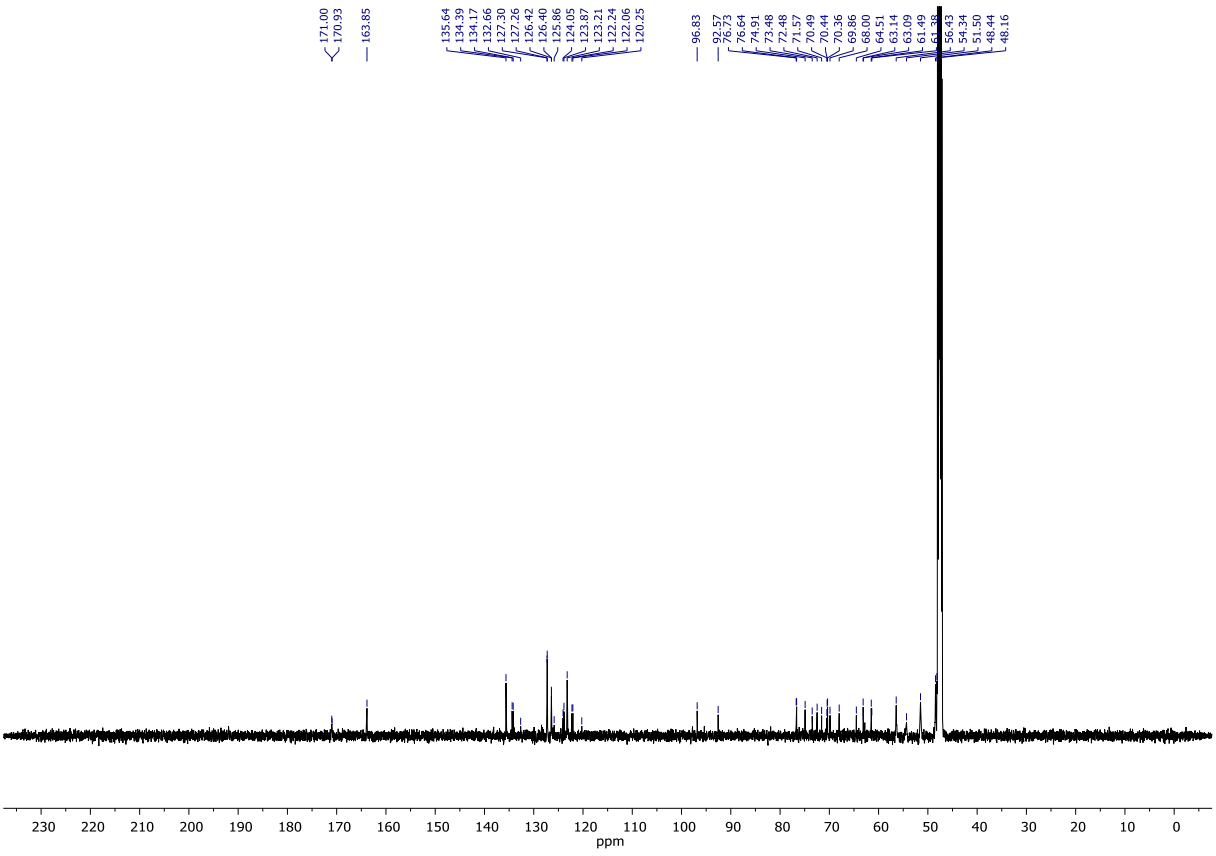

Figure 14: ^{13}C NMR spectra of **L2** in MeOD-d_4.

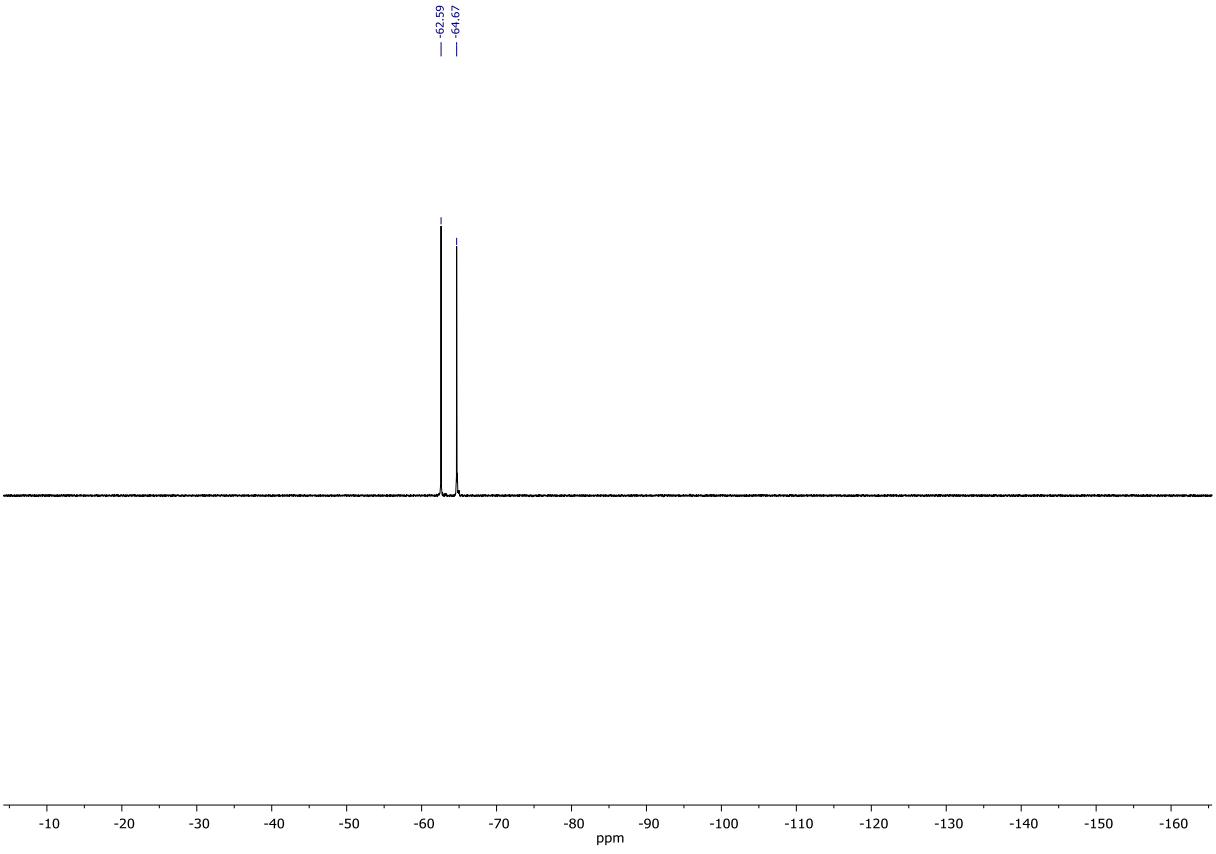

Figure 15: ^{19}F NMR spectra of **L2** in MeOD-d_4.

TmL1

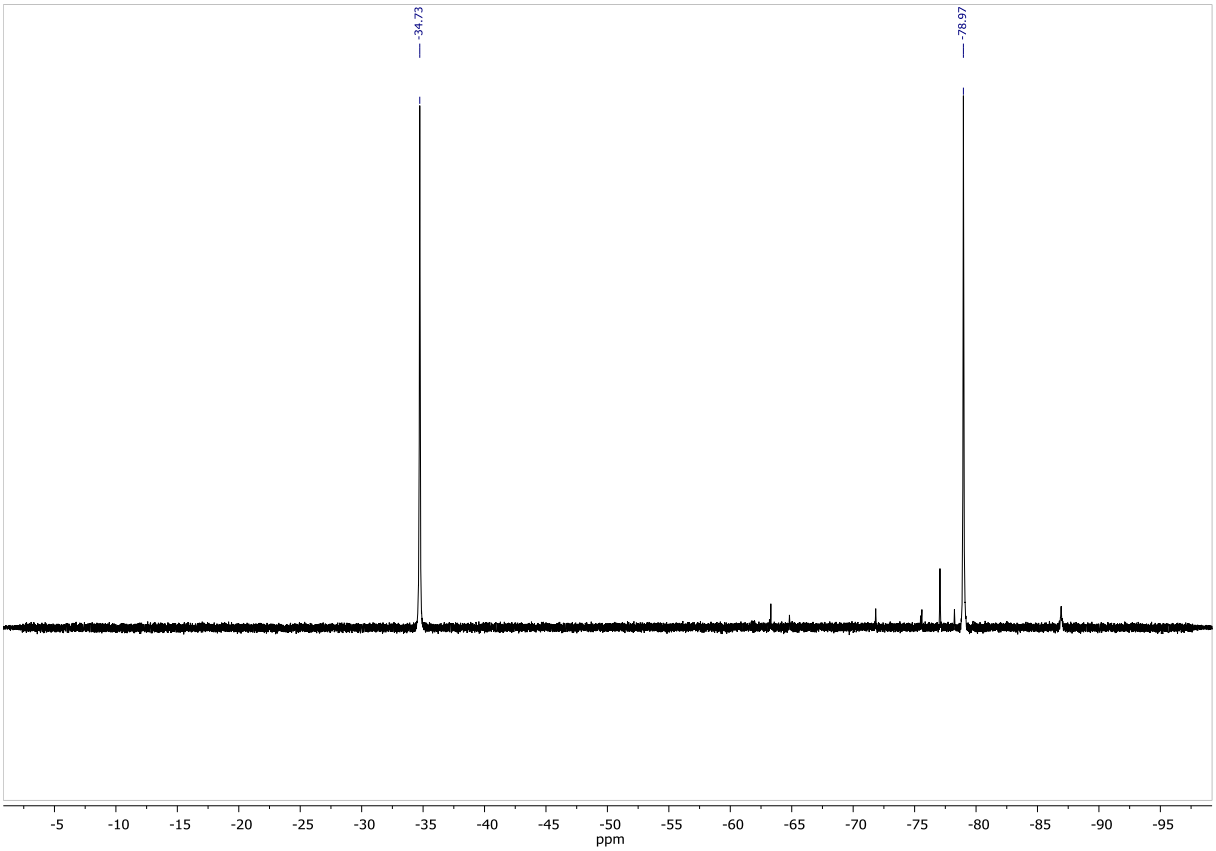

Figure 16: ^{19}F NMR spectra of **TmL1** in D_2O.

TmL2

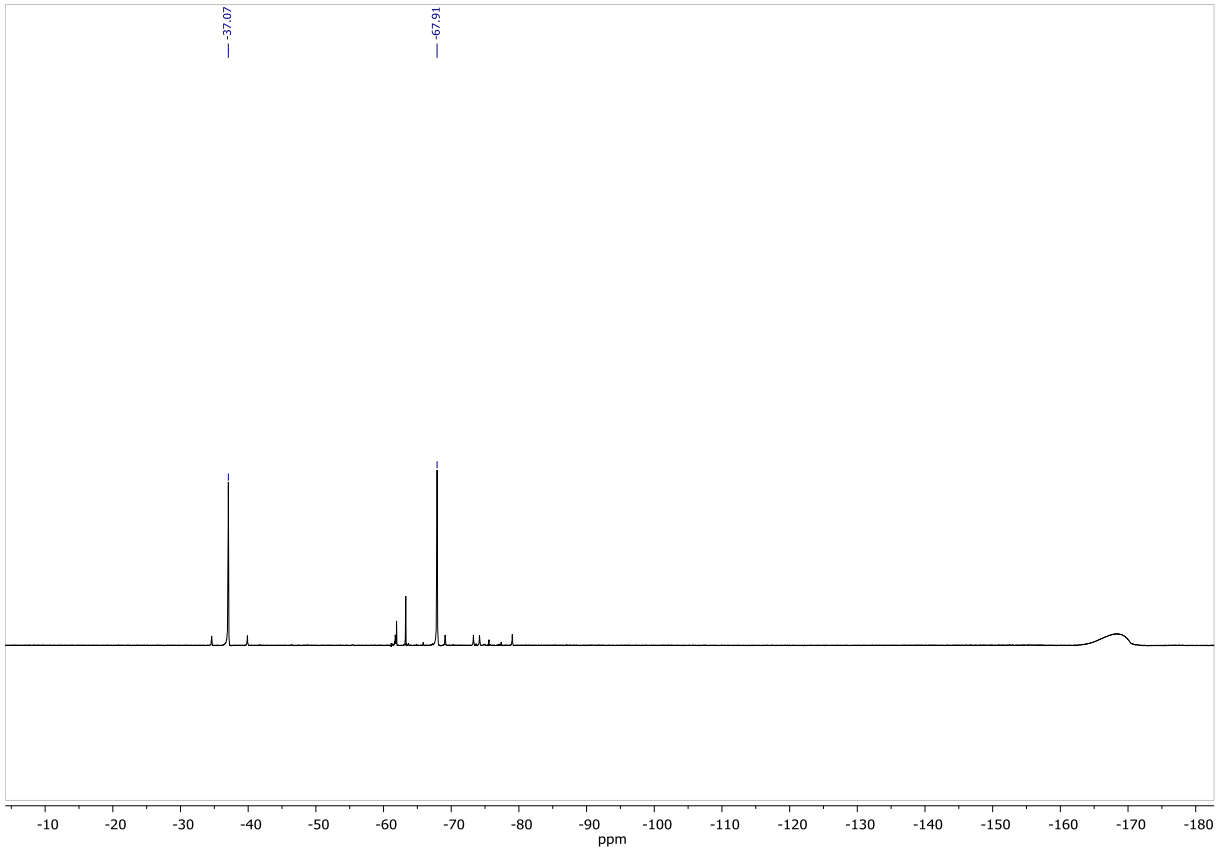

Figure 17: ^{19}F NMR spectra of **TmL2** in D_2O.

VT NMR

^{19}F VT NMR of TmL2

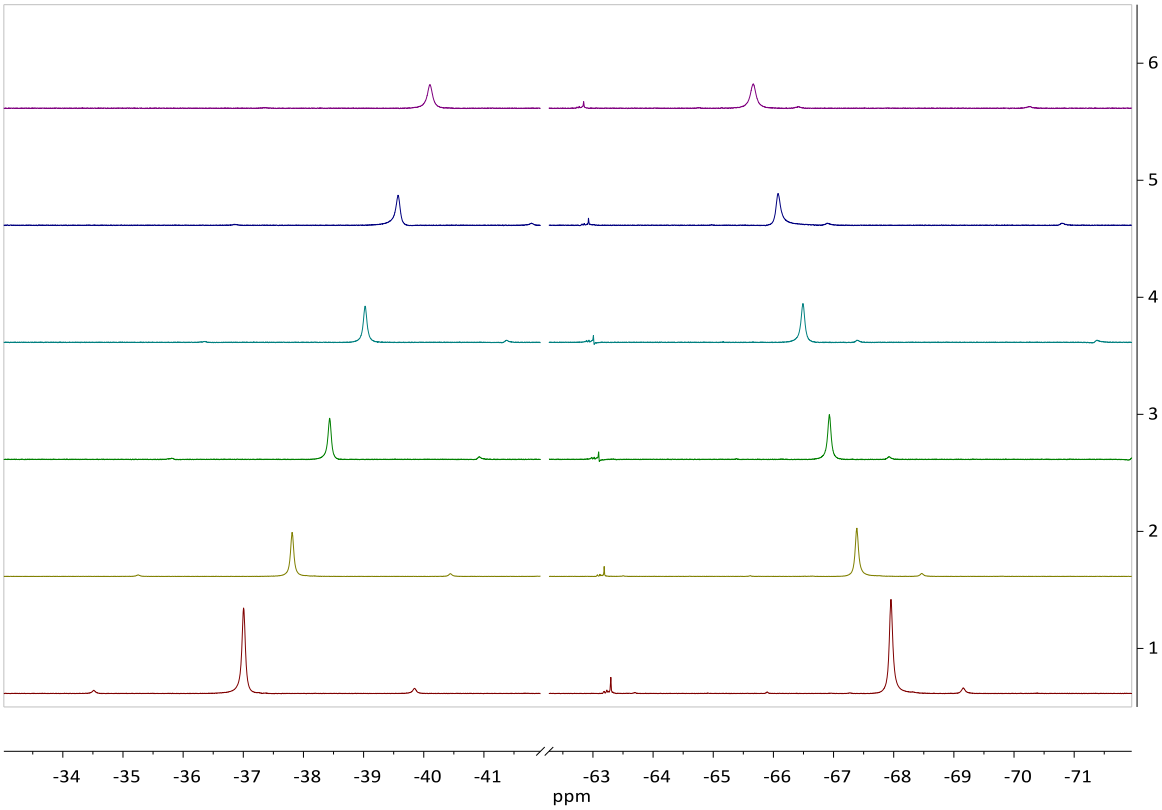

Figure 18: Variable-temperature ^{19}F NMR spectra of **TmL2** in D_2O. The temperature range was chosen to 298 K to 323 K. 1: T = 298 K, 2: T = 303 K, 3: T = 308 K, 4: T = 313 K, 5: T = 318 K and 6: T = 323 K.

^{19}F VT NMR of TmL2

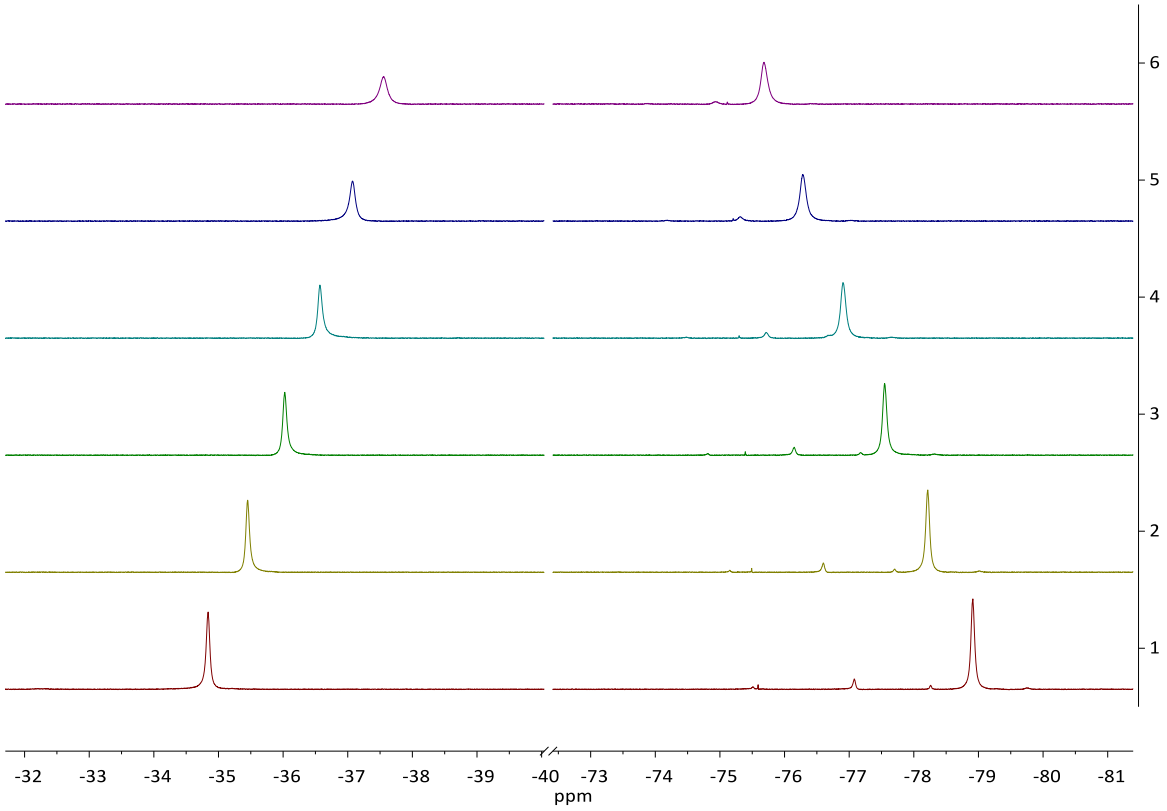

Figure 19: Variable-temperature ^{19}F NMR spectra of **TmL1** after addition of two equivalents of $ZnCl_2$ in D_2O. The temperature range was chosen to 298 K to 323 K. 1: T = 298 K, 2: T = 303 K, 3: T = 308 K, 4: T = 313 K, 5: T = 318 K and 6: T = 323 K.

Graphical depiction of ^{19}F VT NMR of TmL1

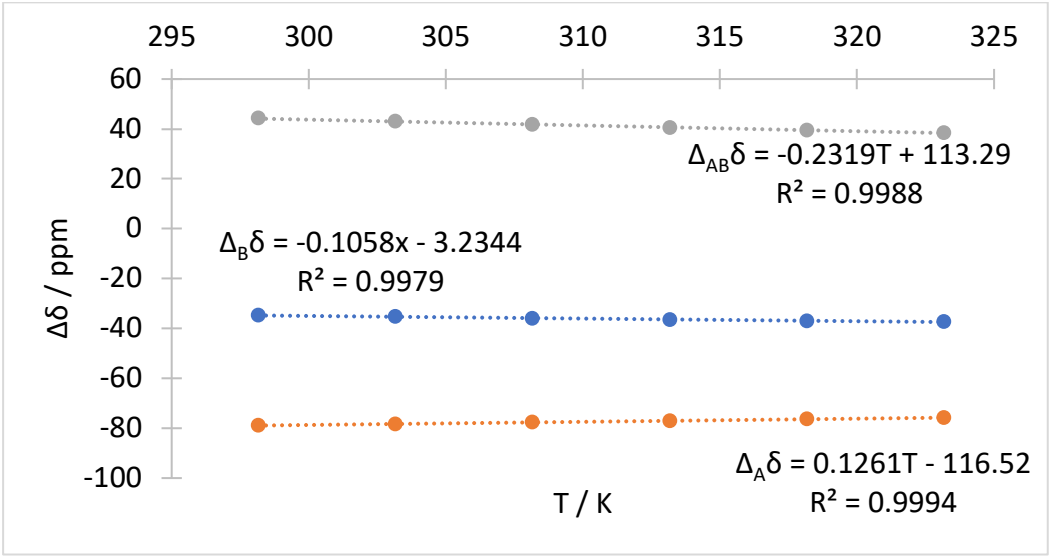

Diagram 1: Graphical plot of ^{19}F VT NMR of **TmL1** in D_2O *and* δ in ppm with orange: linear regression of VT NMR from one CF_3 signal; blue: linear regression of VT NMR from the other CF_3 group; grey: linear regression of the difference between the two signals.

Graphical depiction of ^{19}F VT NMR of TmL1

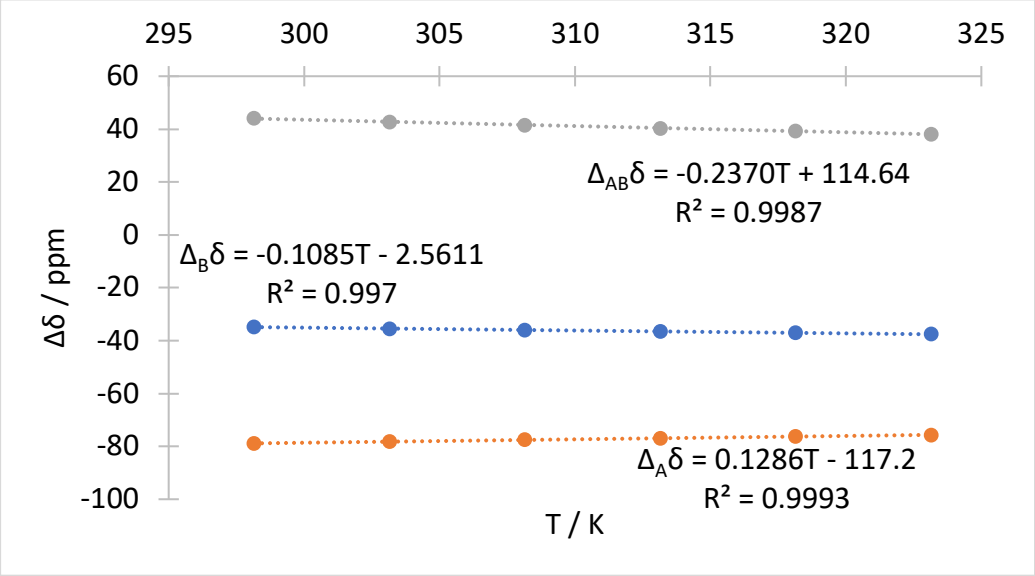

Diagram 2: Graphical plot of ^{19}F VT NMR of **TmL1** after addition of two equivalents of $ZnCl_2$ in D_2O *and* δ in ppm with orange: linear regression of VT NMR from one CF_3 signal; blue: linear regression of VT NMR from the other CF_3 group; grey: linear regression of the difference between the two signals.

Graphical depiction of ^{19}F VT NMR of TmL2

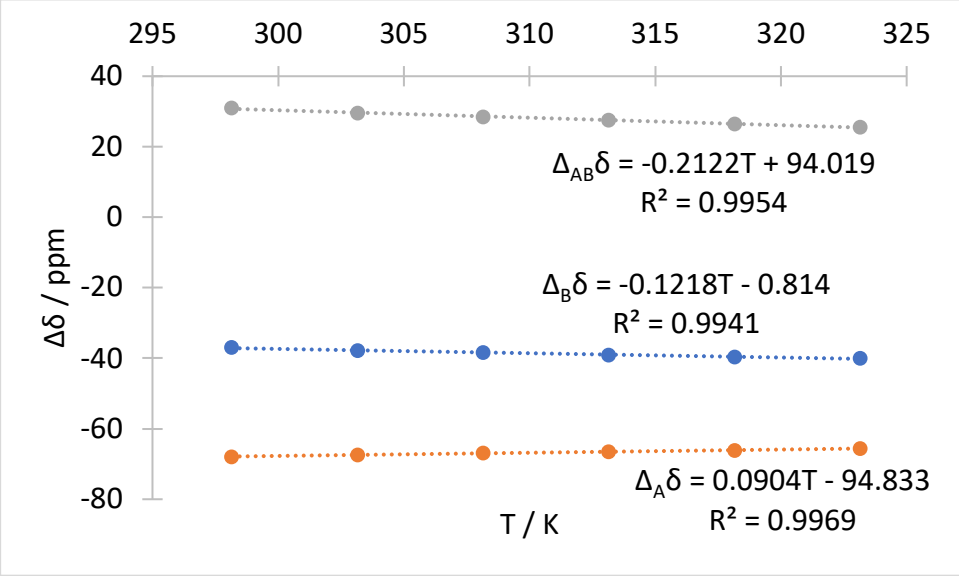

Diagram 3: Graphical plot of ^{19}F VT NMR of **TmL2** in D_2O *and* δ in ppm with orange: linear regression of VT NMR from one CF_3 signal; blue: linear regression of VT NMR from the other CF_3 group; grey: linear regression of the difference between the two signals.

Cell viability tests

Results of TmL1

Table 1: Results of the viability tests of **TmL1** after 24 and 48 h.

Incubation time [h]	
24	48
Viability [%]	
90	88
89	84
91	86
92	90
87	89
82	85
88	86
87	88
∑ 88.25	87

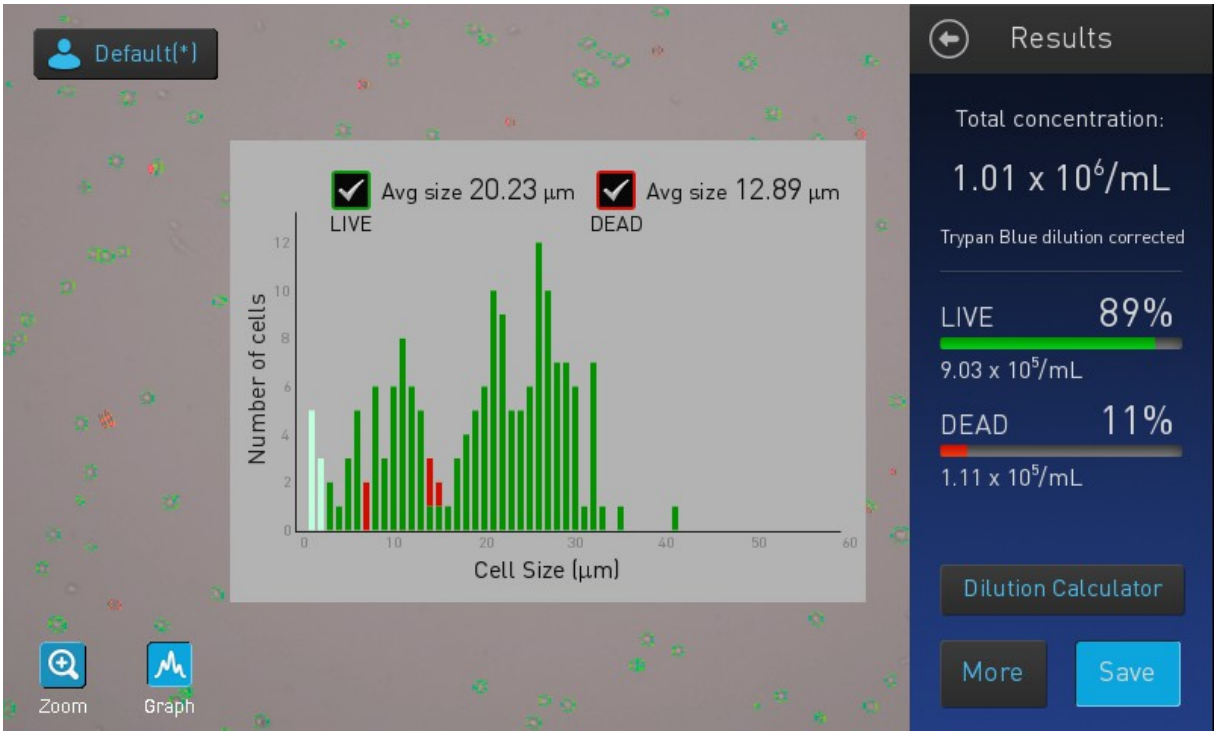

Figure 20: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of **TmL1**.

Figure 21: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of **TmL1**.

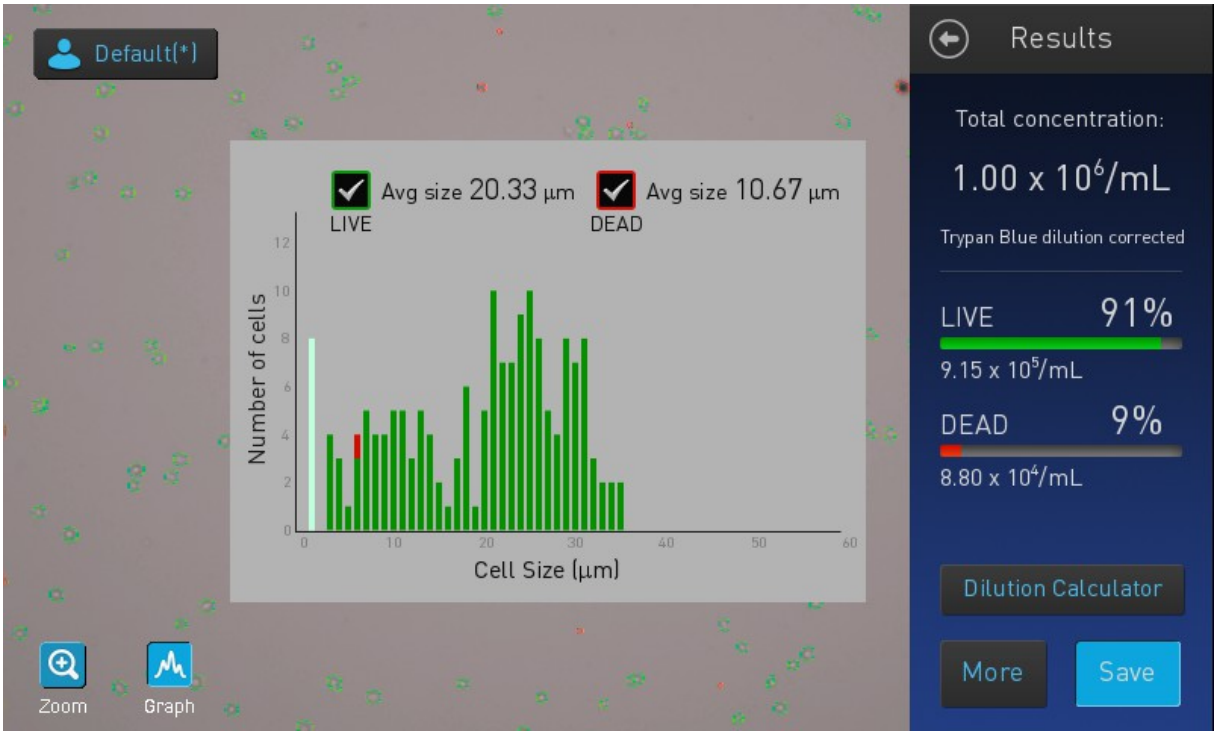

Figure 22: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of **TmL1**.

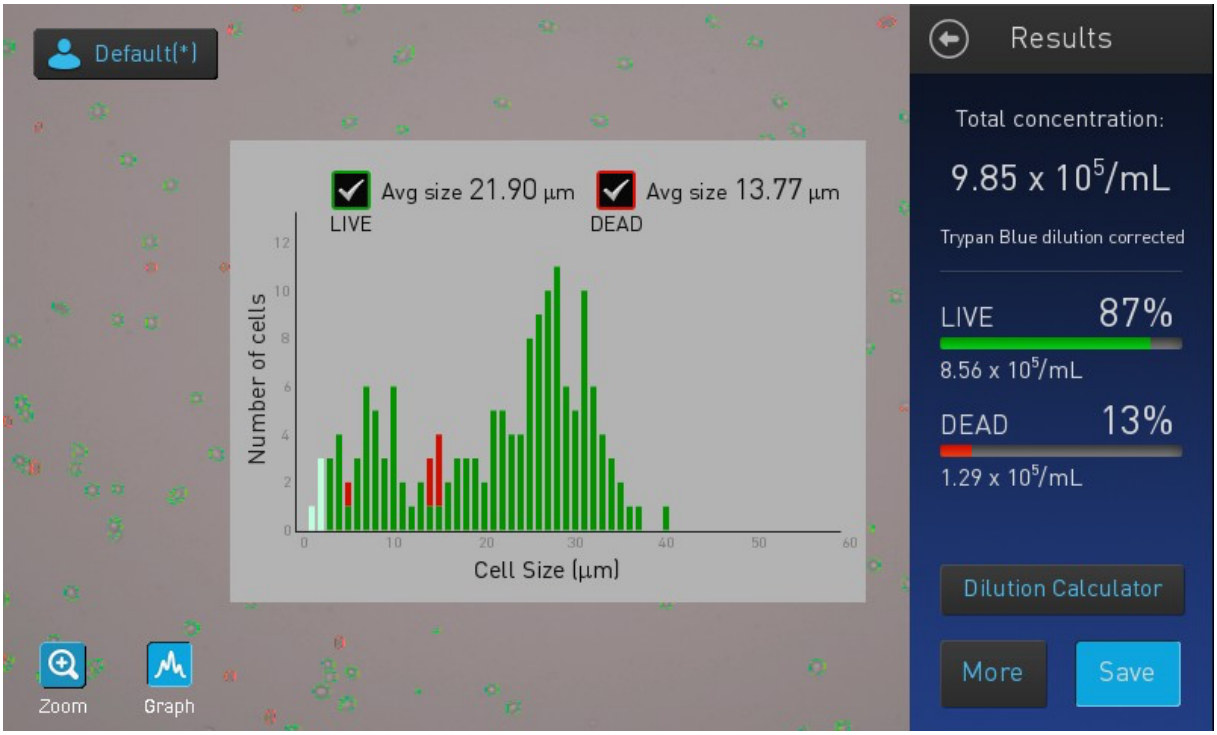

Figure 23: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of **TmL1**.

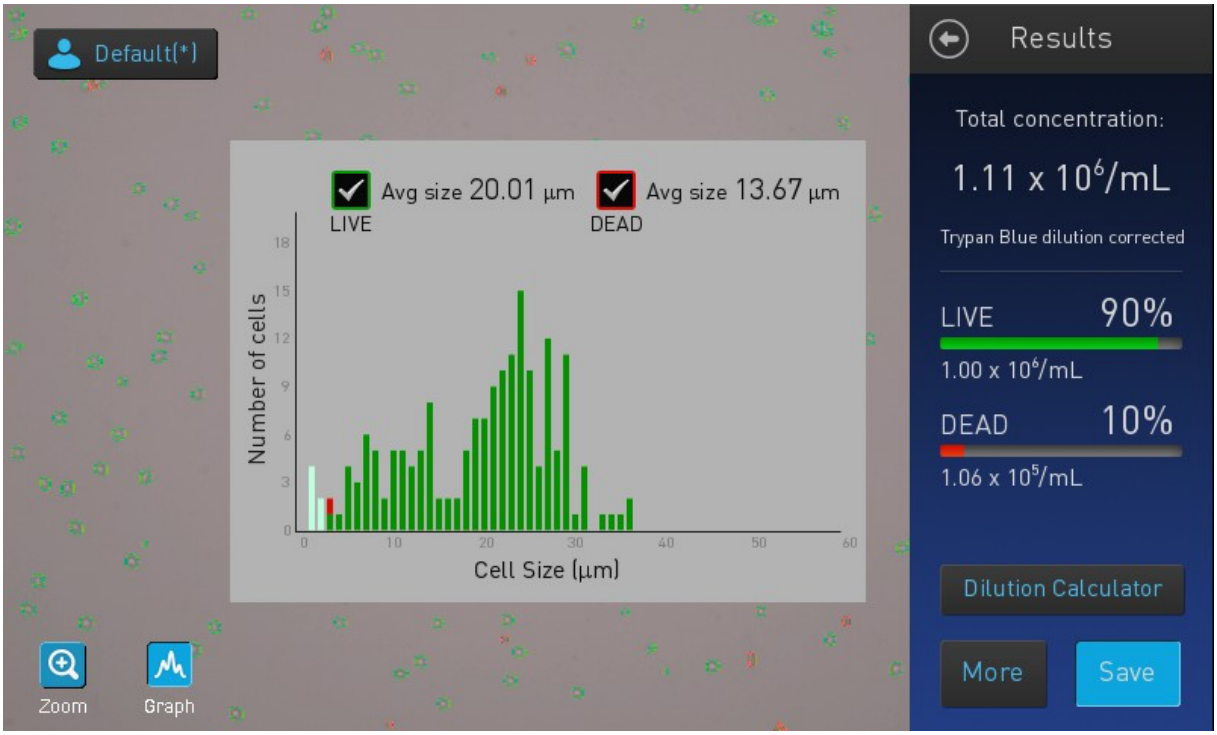

Figure 24: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of **TmL1**.

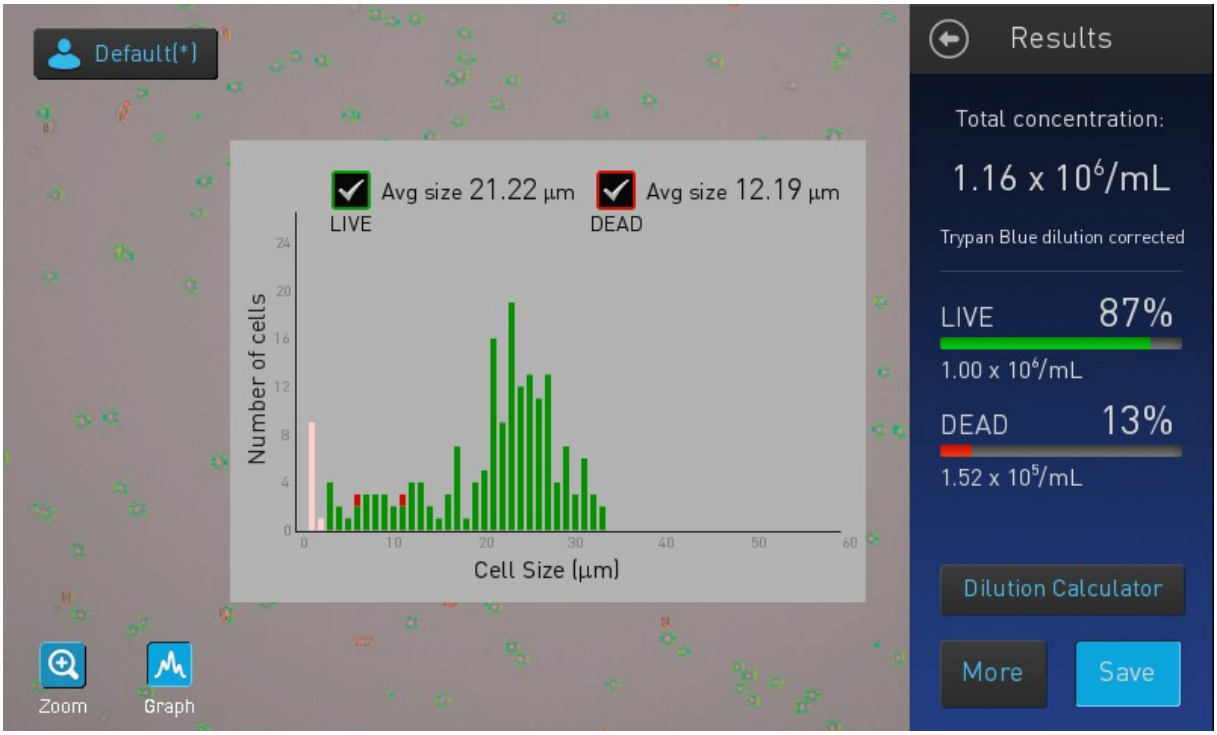

Figure 25: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of **TmL1**.

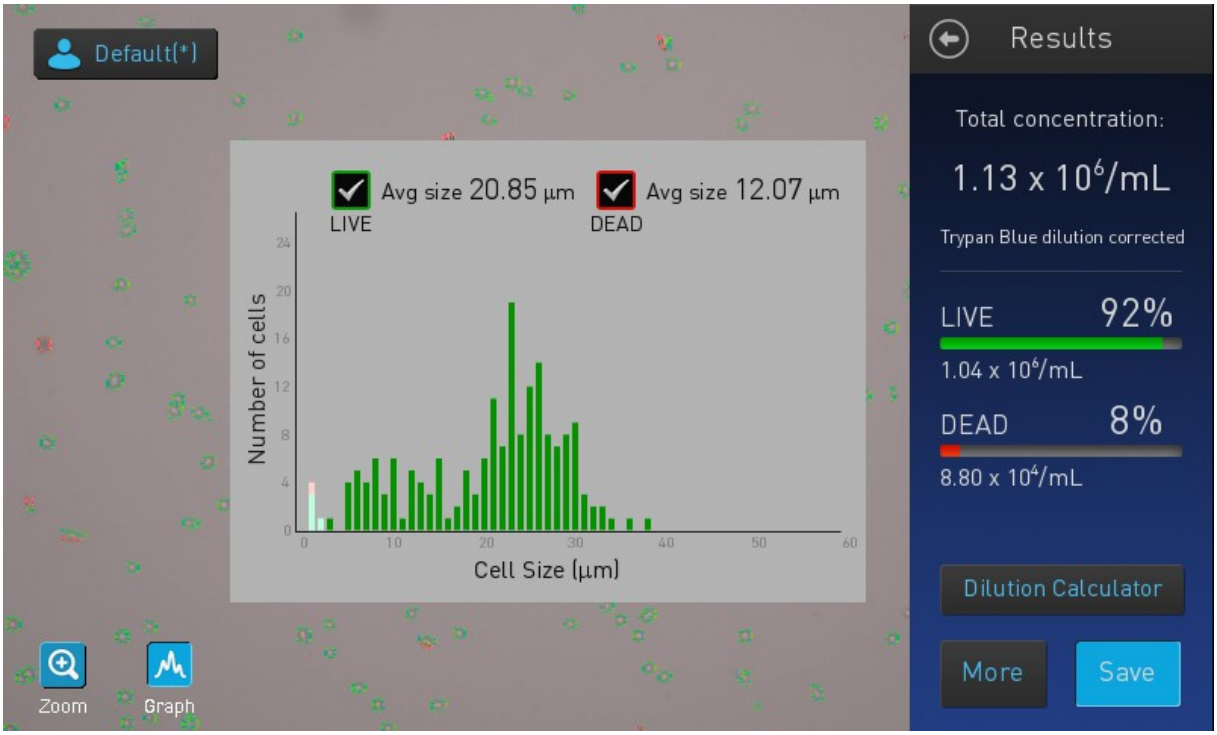

Figure 26: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of **TmL1**.

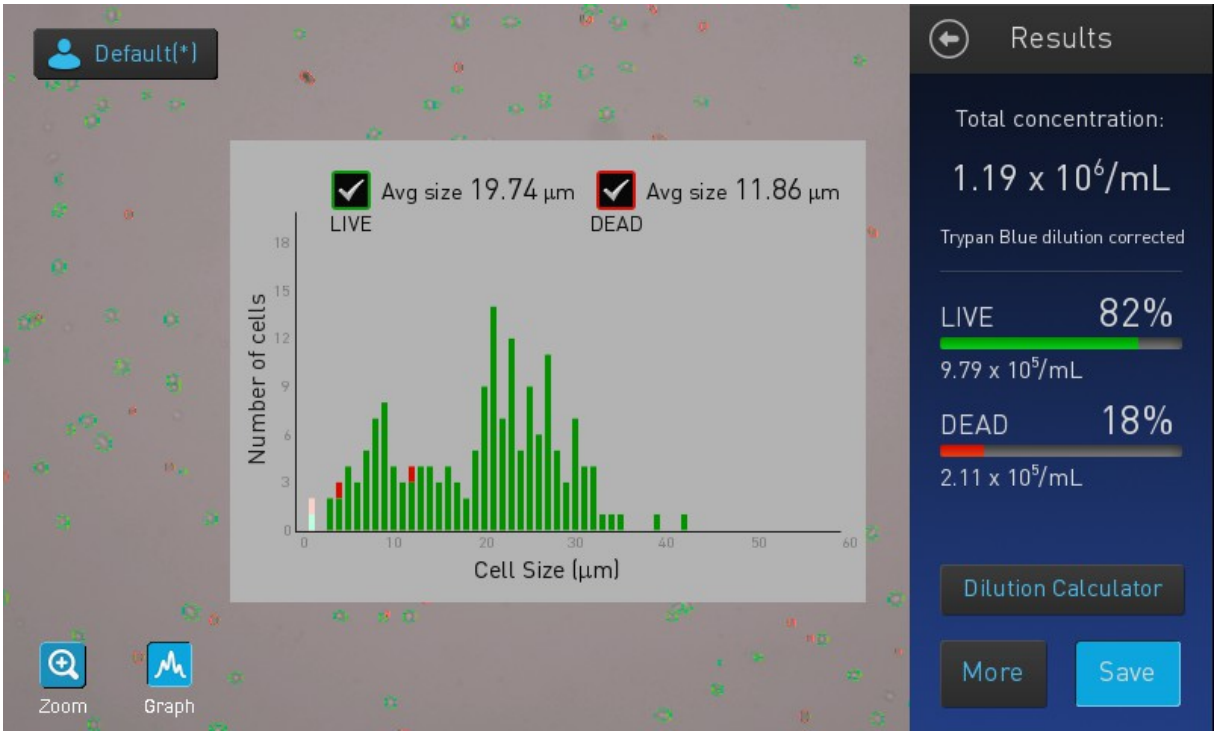

Figure 27: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of **TmL1**.

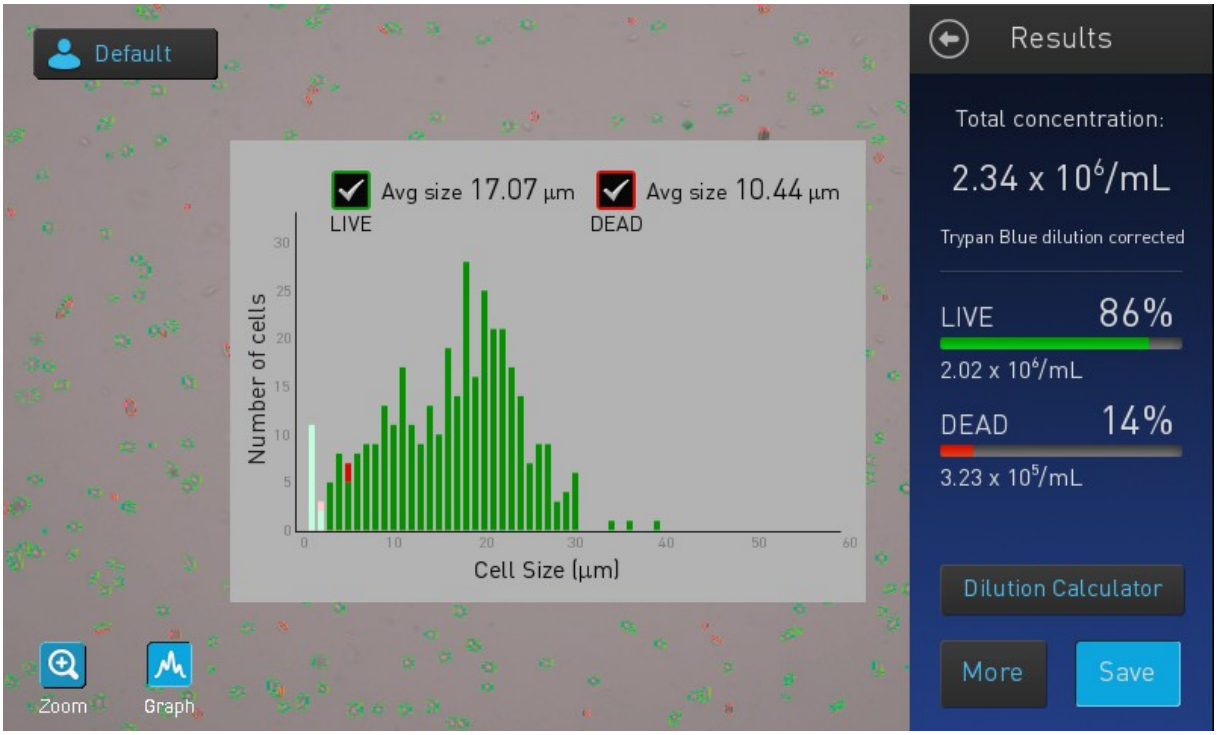

Figure 28: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 48 h of **TmL1**.

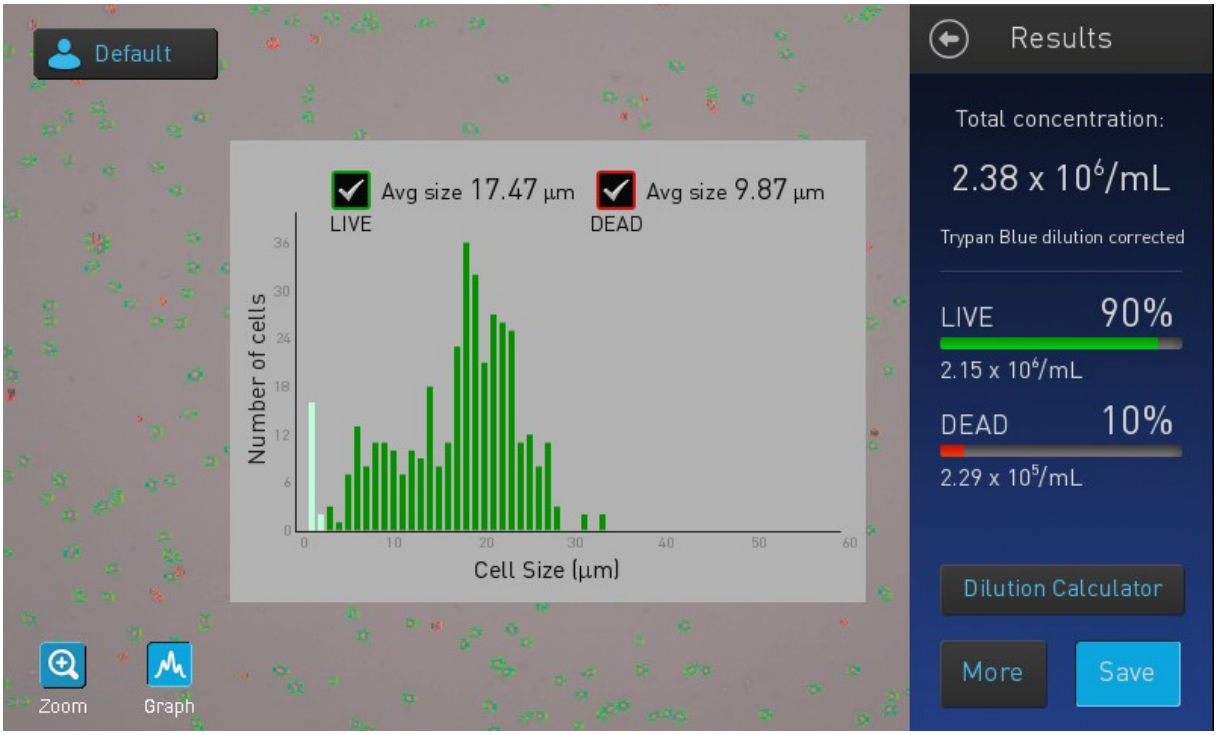

Figure 29: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 48 h of **TmL1**.

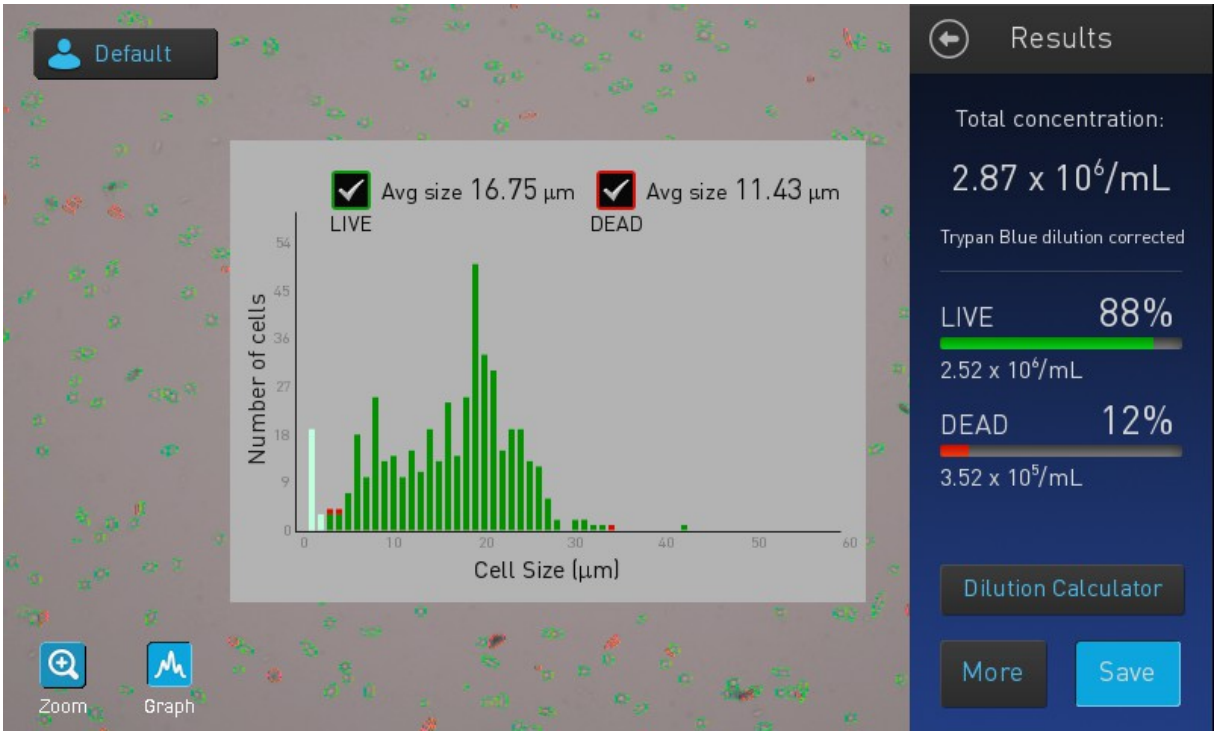

Figure 30: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 48 h of **TmL1**.

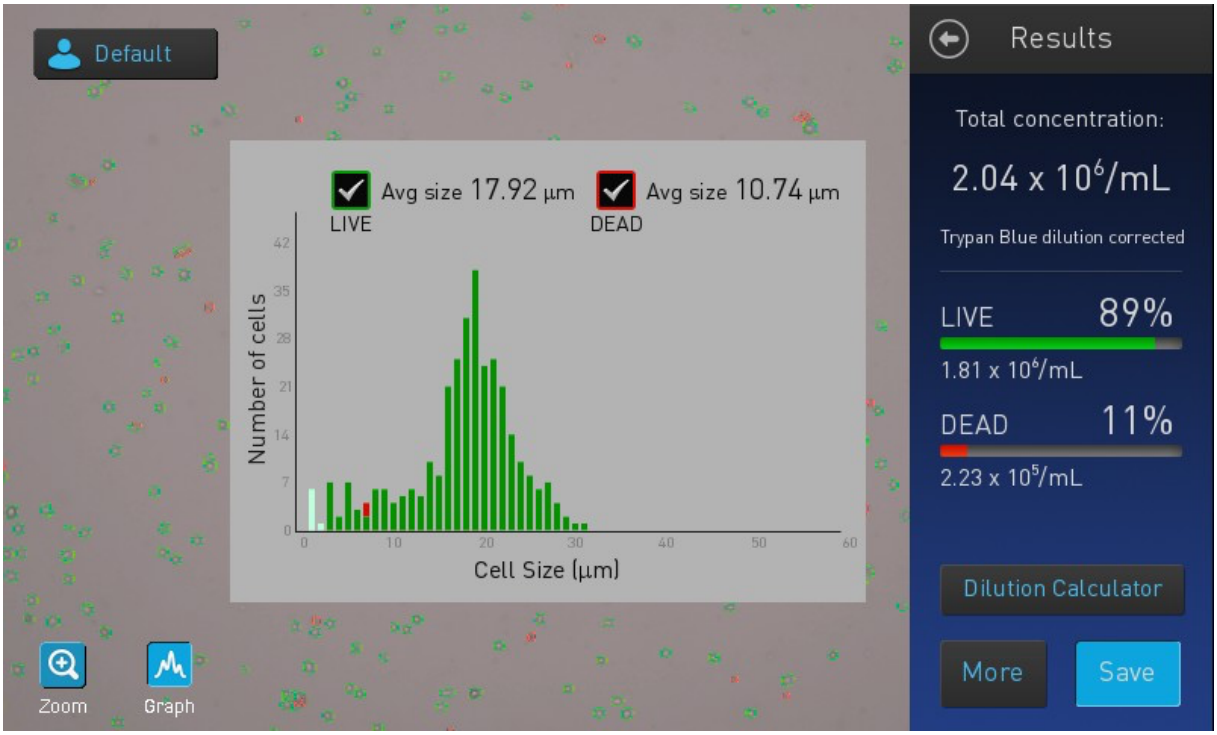

Figure 31: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 48 h of **TmL1**.

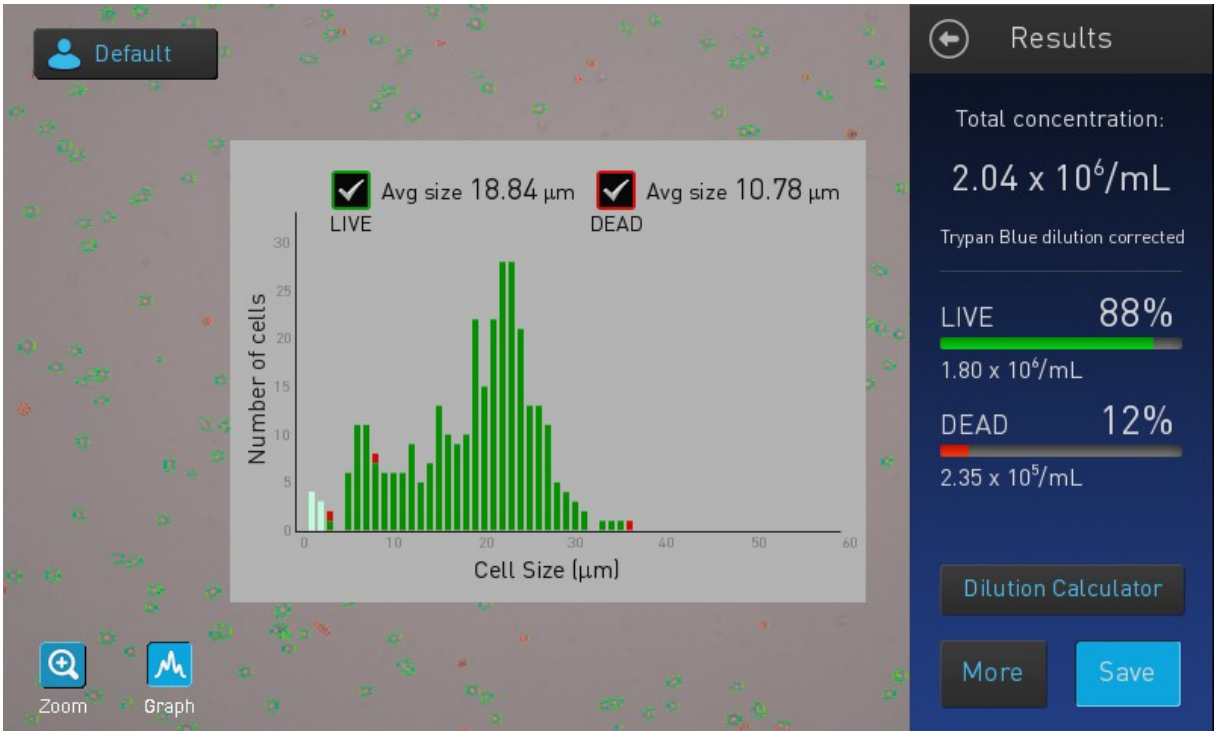

Figure 32: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 48 h of **TmL1**.

Figure 33: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 48 h of **TmL1**.

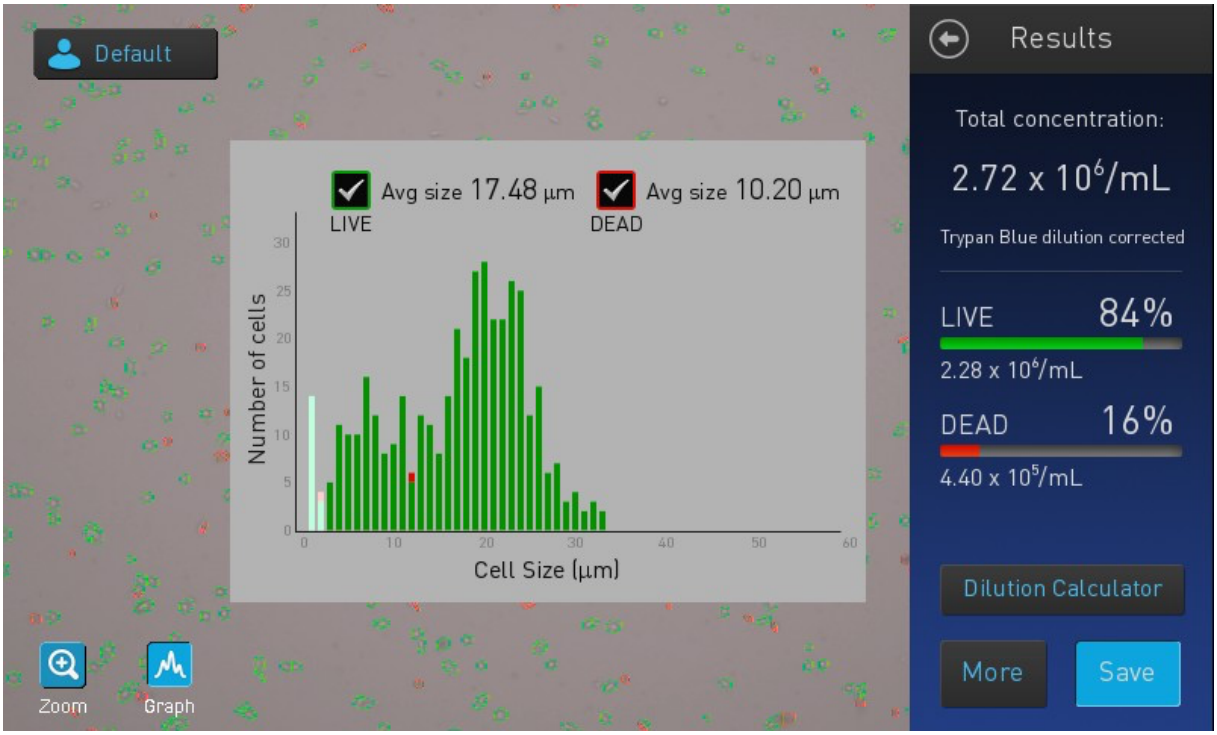

Figure 34: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 48 h of **TmL1**.

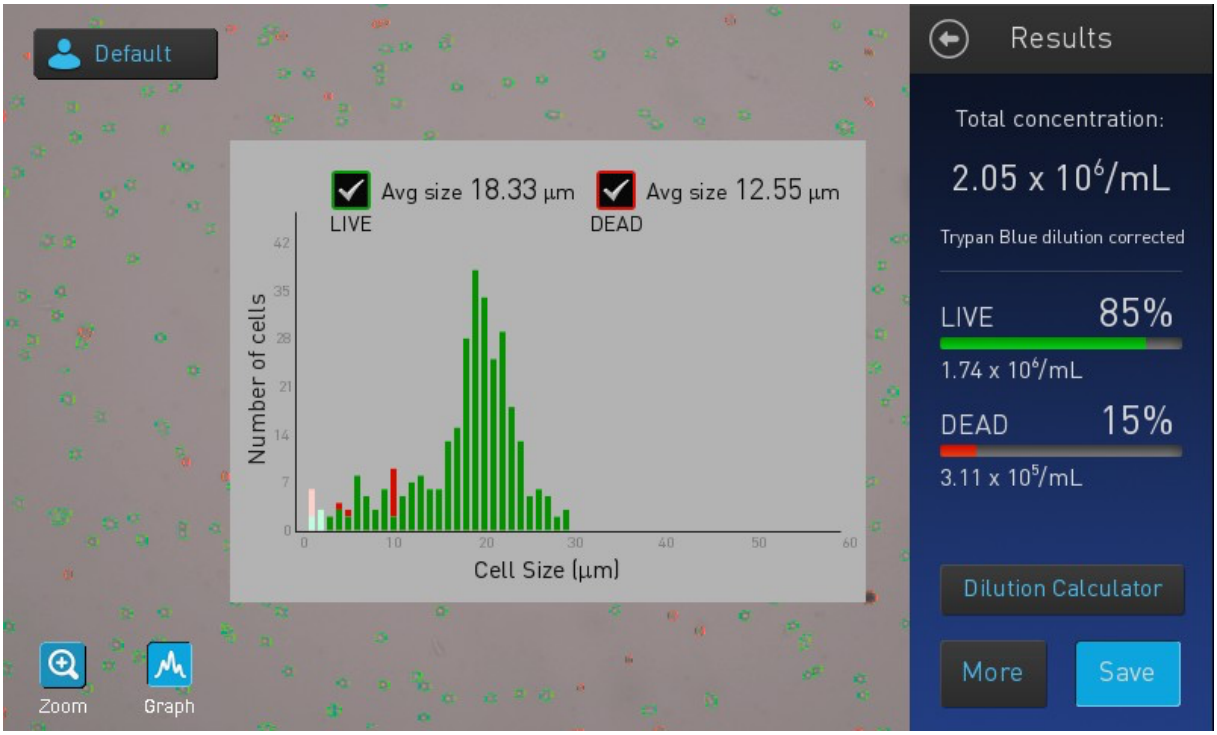

Figure 35: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 48 h of **TmL1**.

Results of TmL2

Table 2: Results of the viability tests of **TmL2** after 24 and 48 h.

Incubation time [h]	
24	48
Viability [%]	
84	75
85	77
82	83
77	83
95	
90	
∑ 85.5	79.5

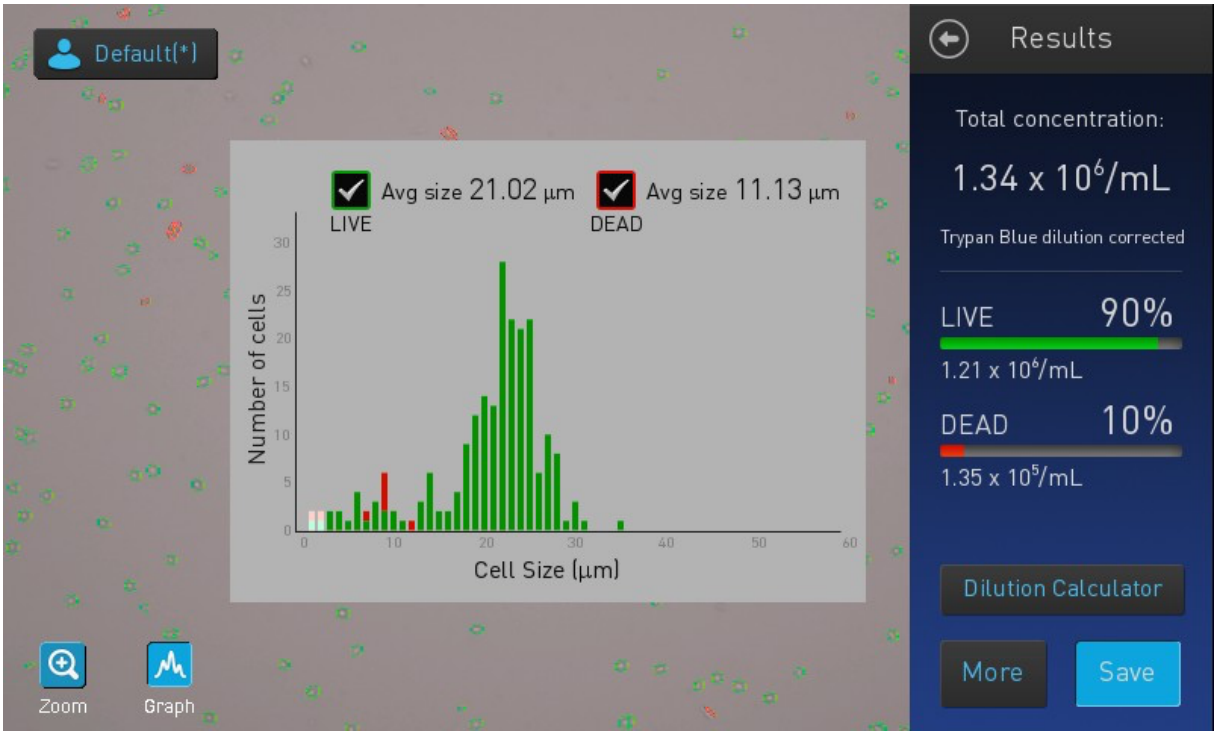

Figure 36: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of **TmL2**.

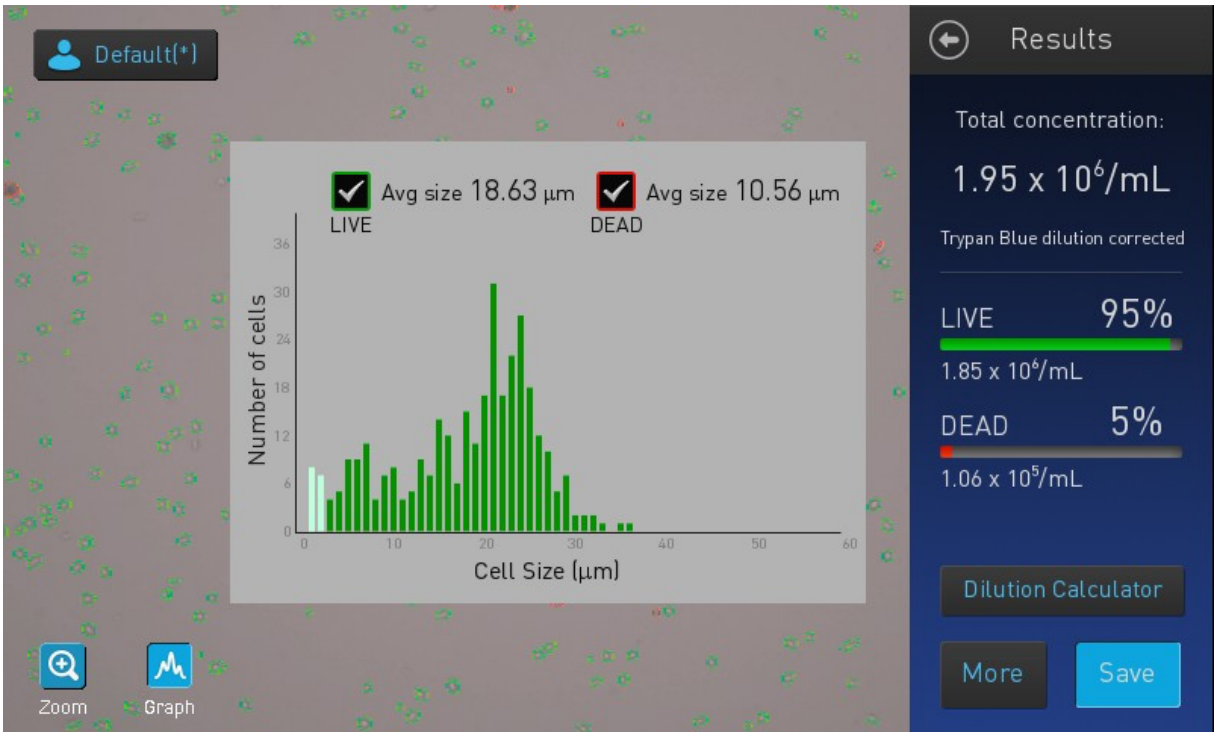

Figure 37: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of **TmL2**.

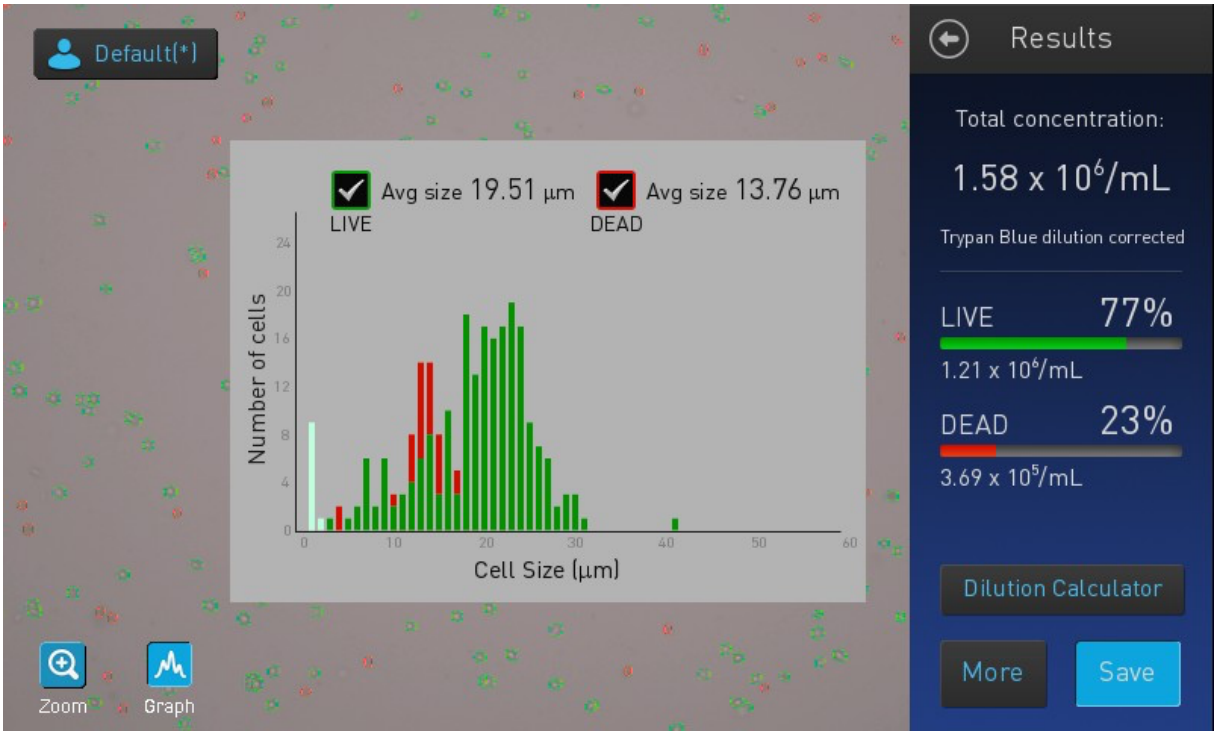

Figure 38: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of **TmL2**.

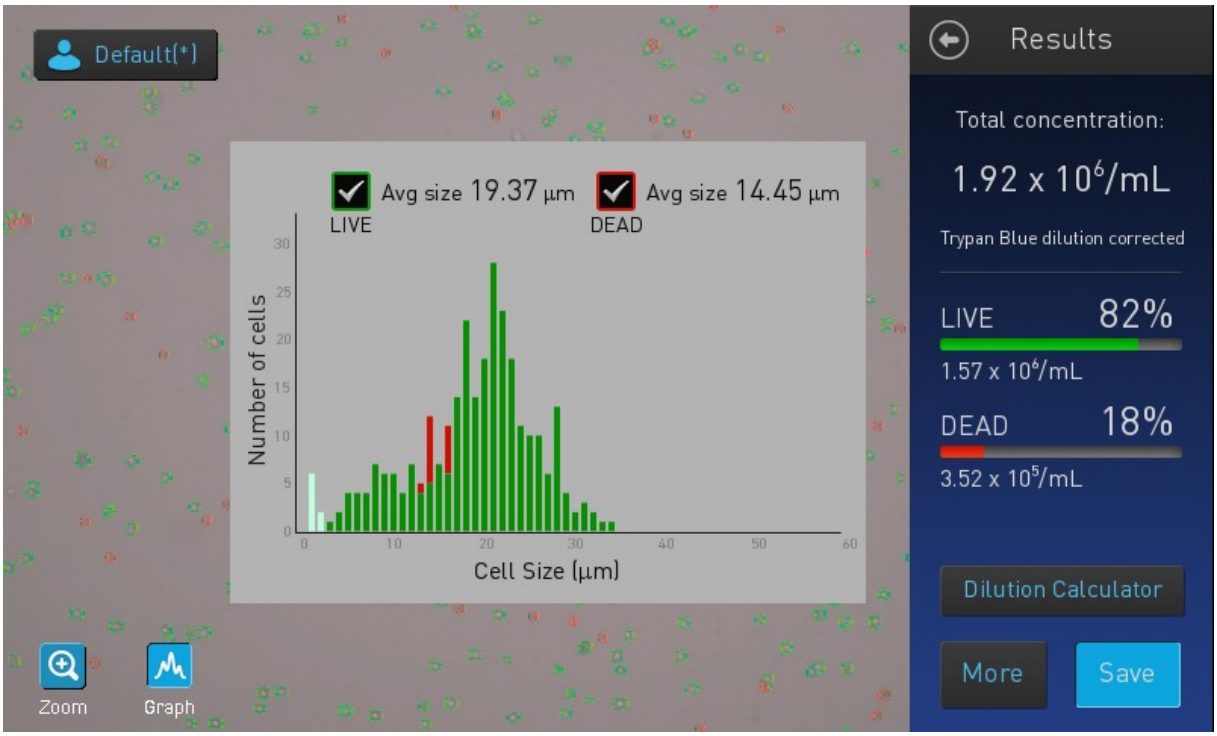

Figure 39: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of **TmL2**.

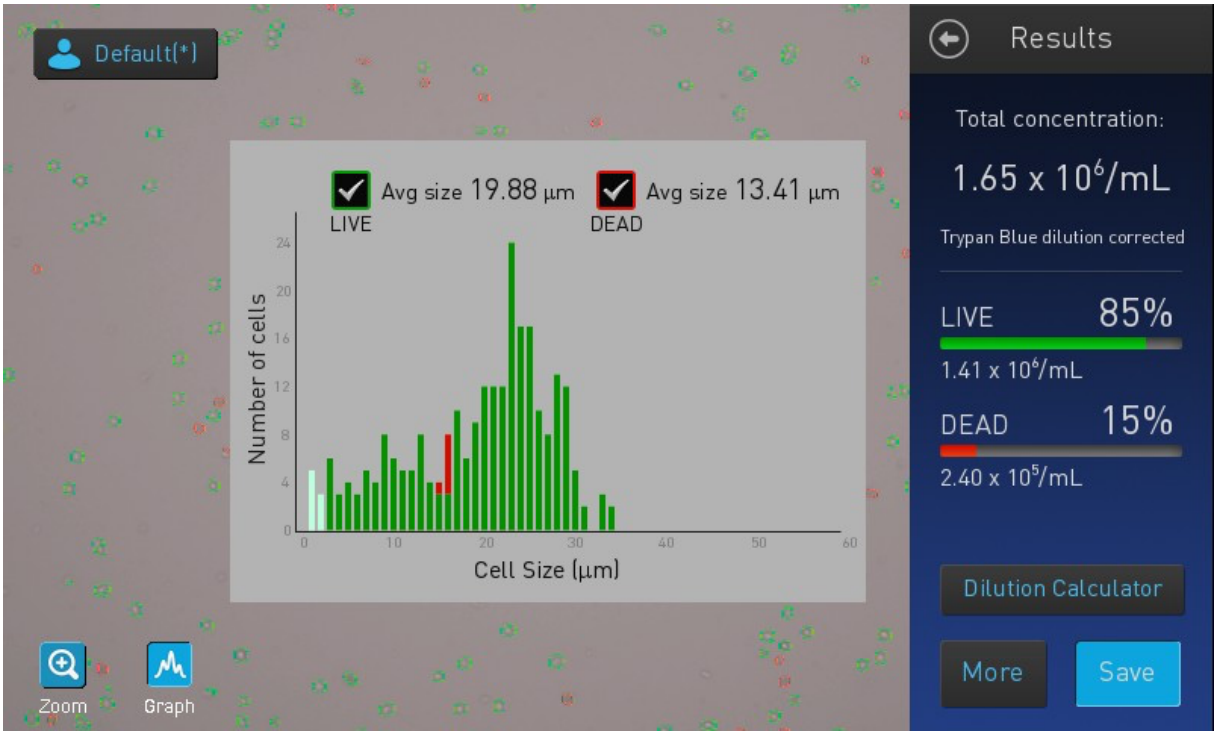

Figure 40: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of **TmL2**.

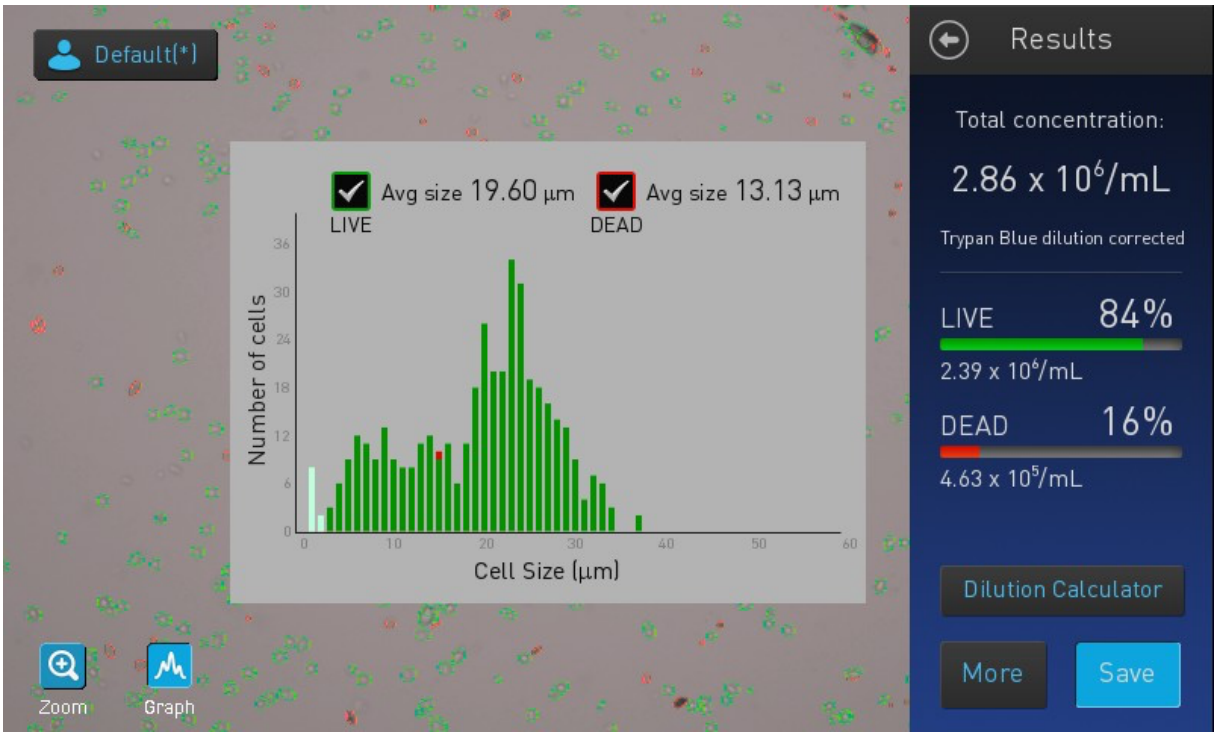

Figure 41: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of **TmL2**.

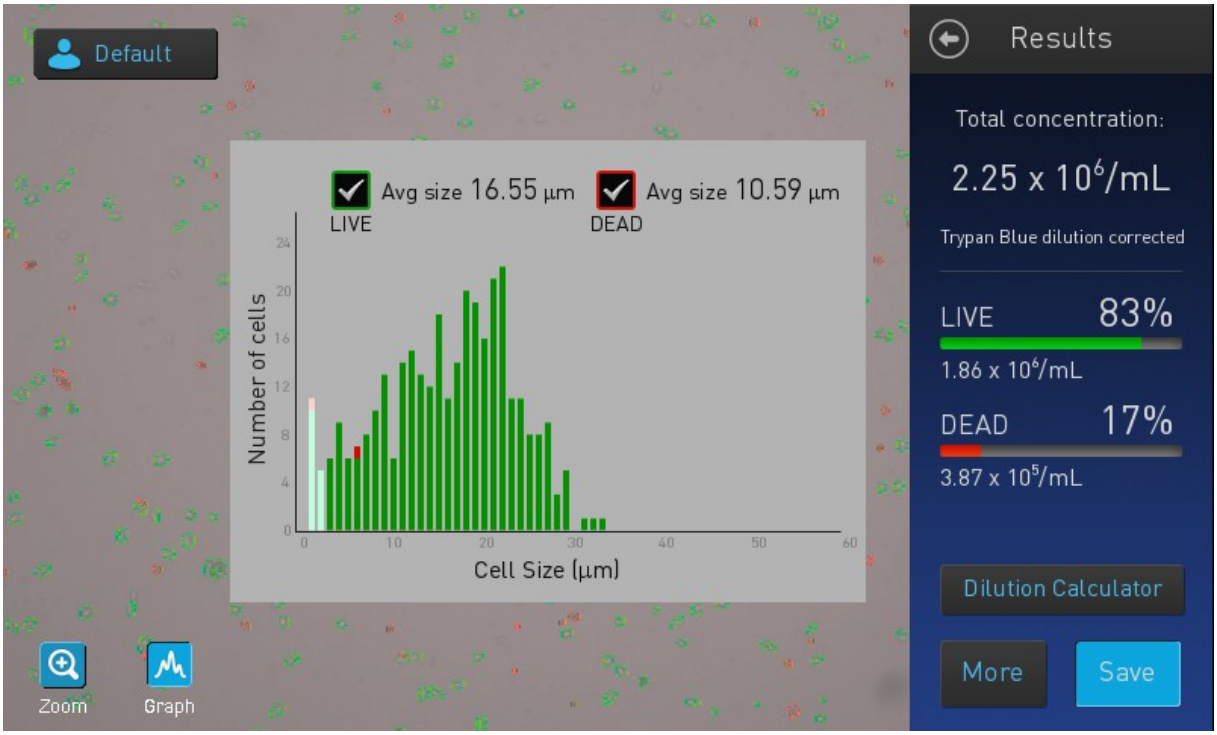

Figure 42: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 48 h of **TmL2**.

Figure 43: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 48 h of **TmL2**.

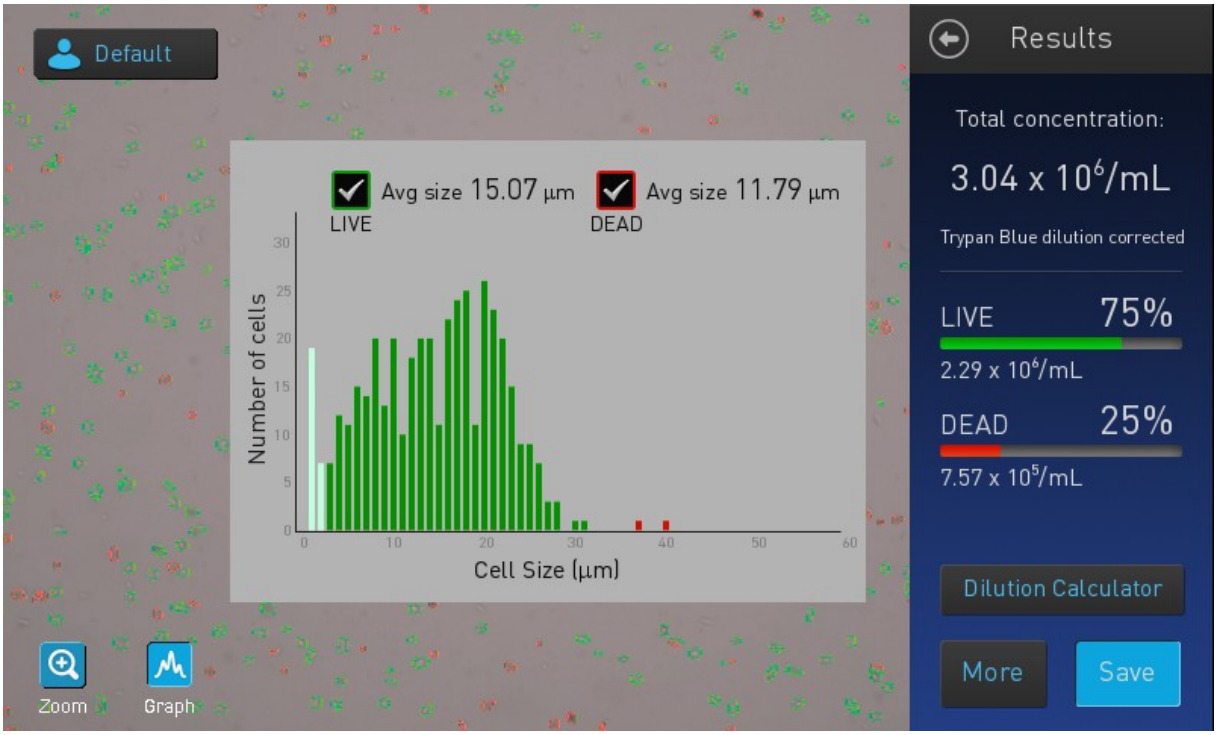

Figure 44: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 48 h of **TmL2**.

Figure 45: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 48 h of **TmL2**.

Results of reference probe

Table 3: Results of the viability tests of a reference probe after 24 and 48 h.

Incubation time [h]	
24	48
Viability [%]	
96	86
86	83
90	85
89	89
94	89
89	86
95	87
91	82
	83
∑ 91.25	85.56

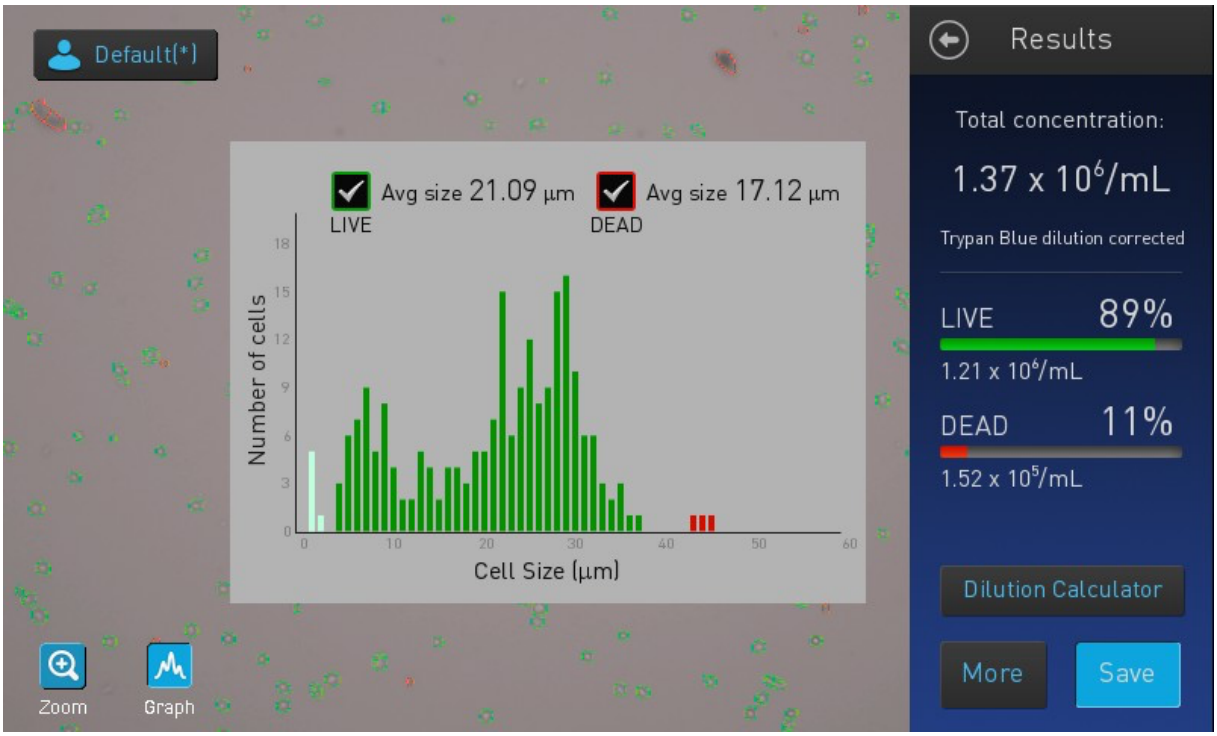

Figure 46: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of a reference probe.

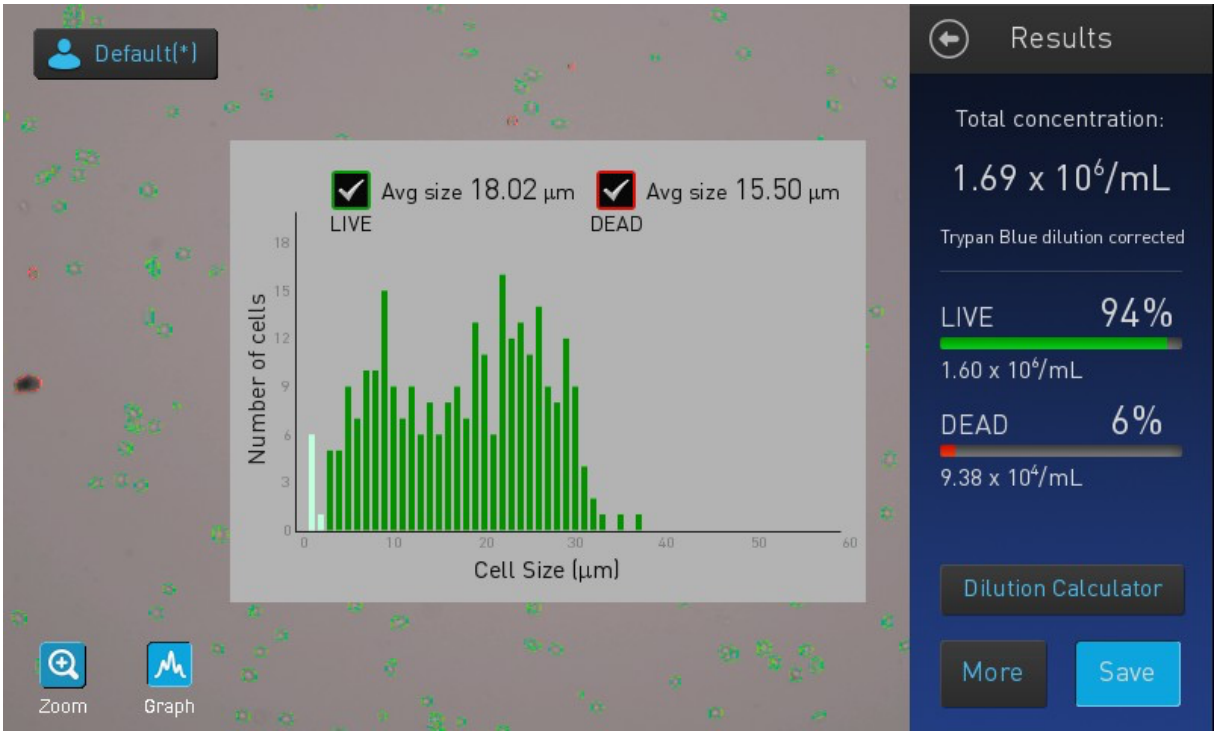

Figure 47: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of a reference probe.

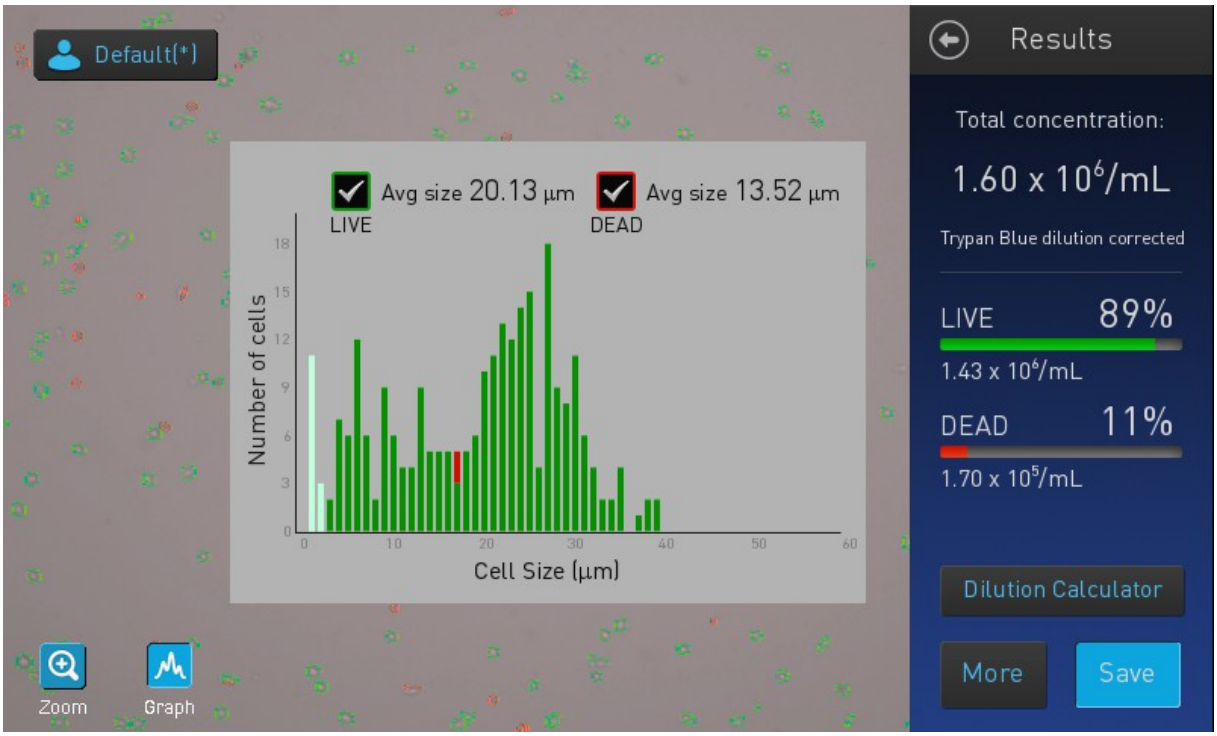

Figure 48: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of a reference probe.

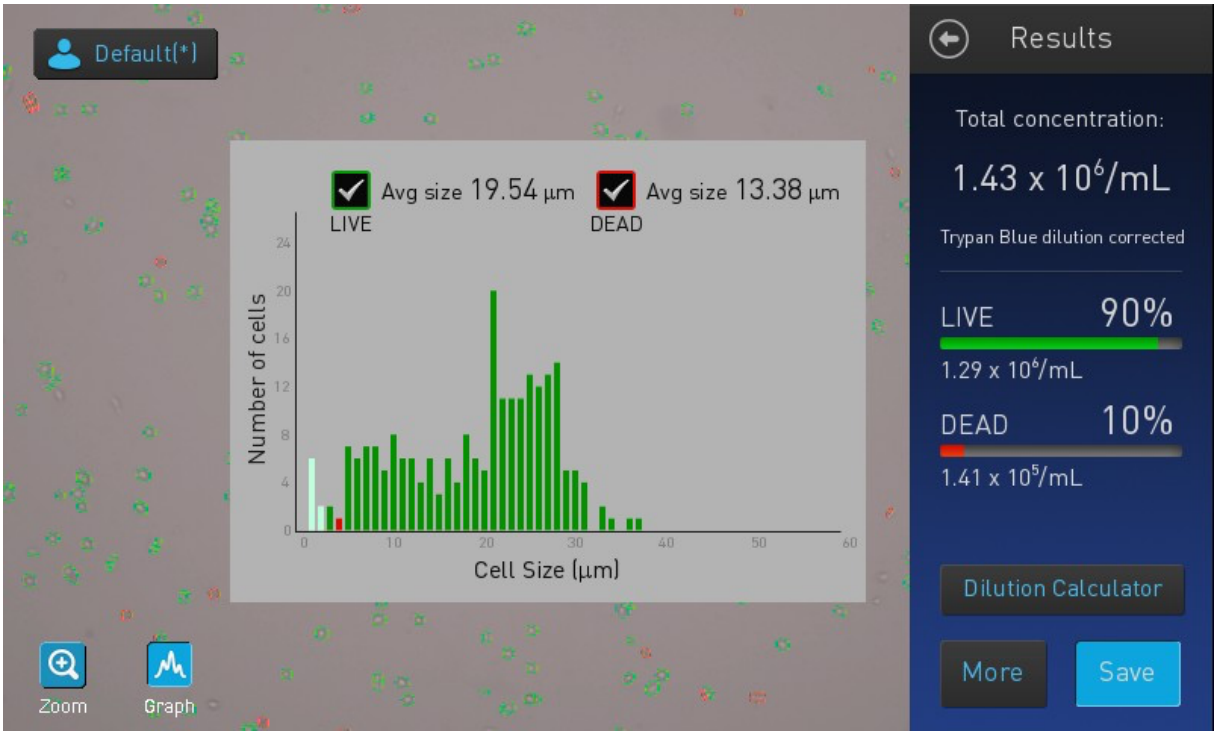

Figure 49: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of a reference probe.

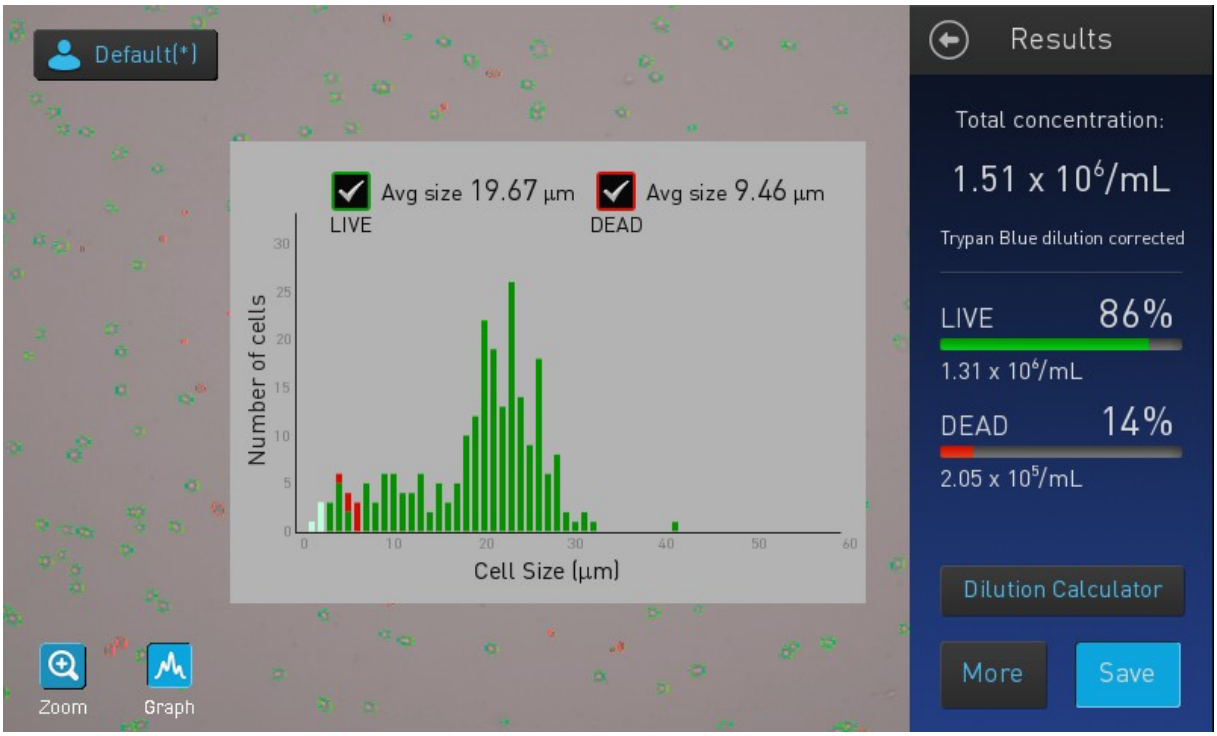

Figure 50: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of a reference probe.

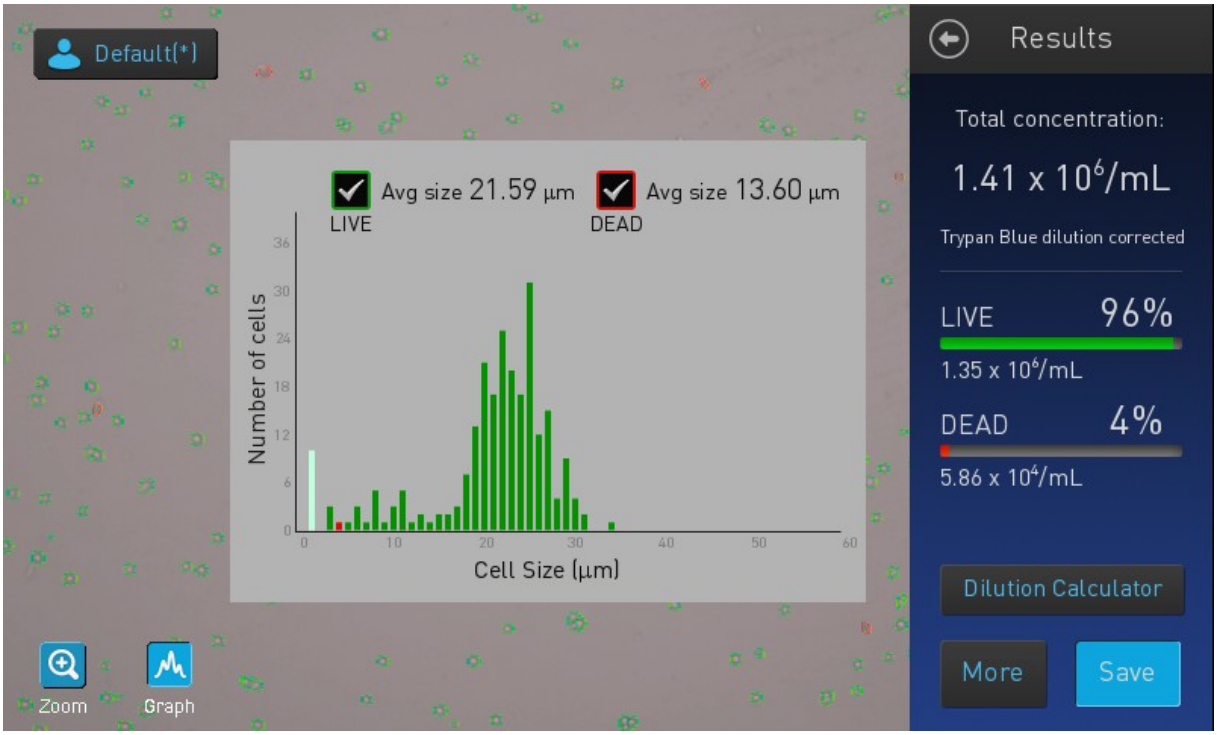

Figure 51: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of a reference probe.

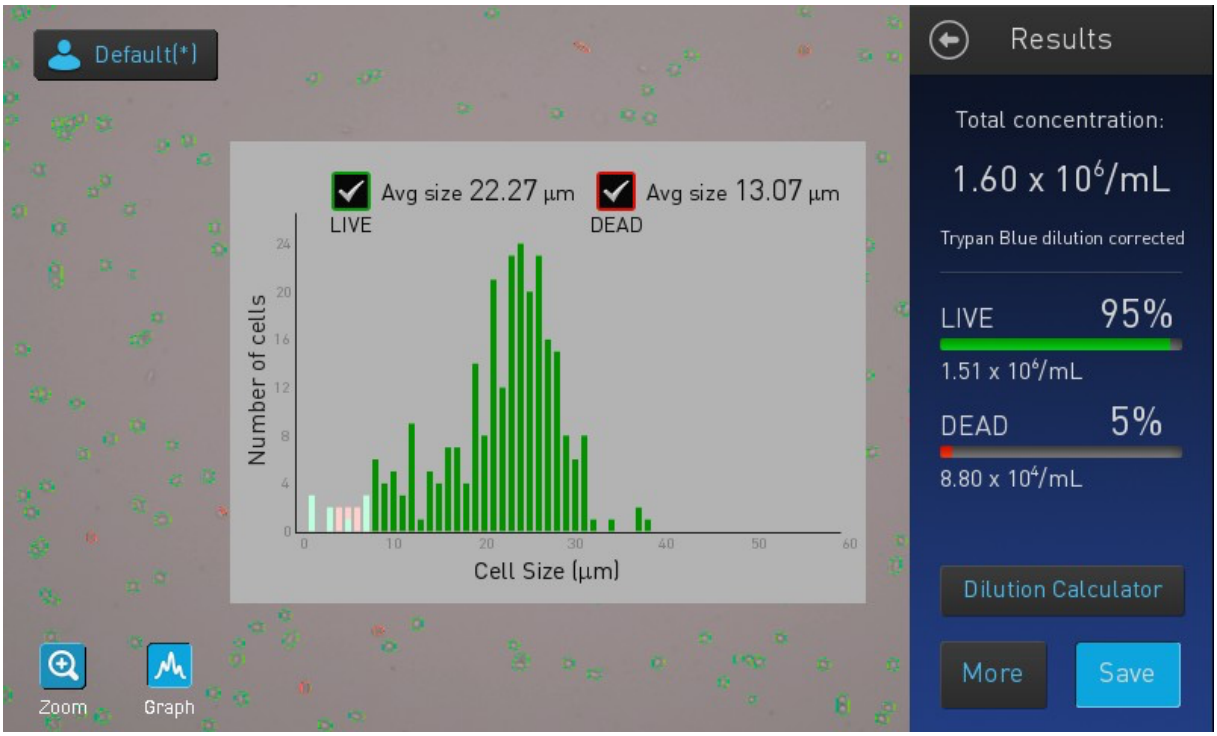

Figure 52: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of a reference probe.

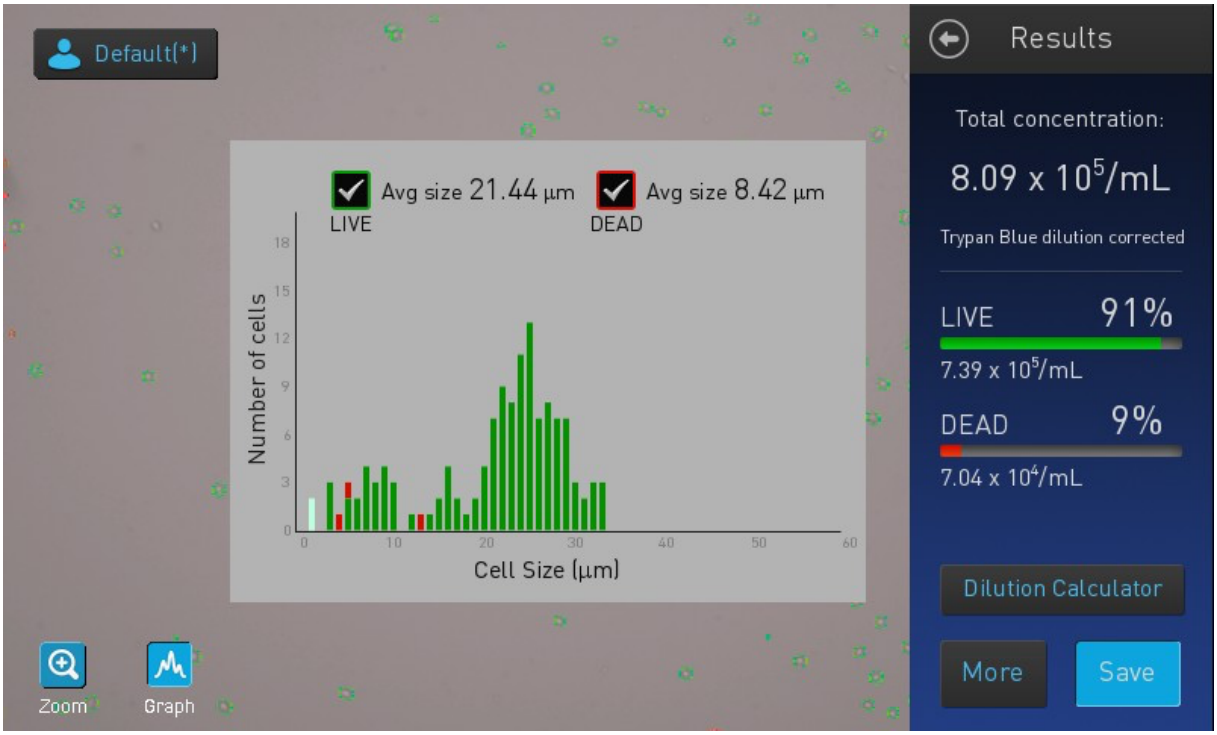

Figure 53: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of a reference probe.

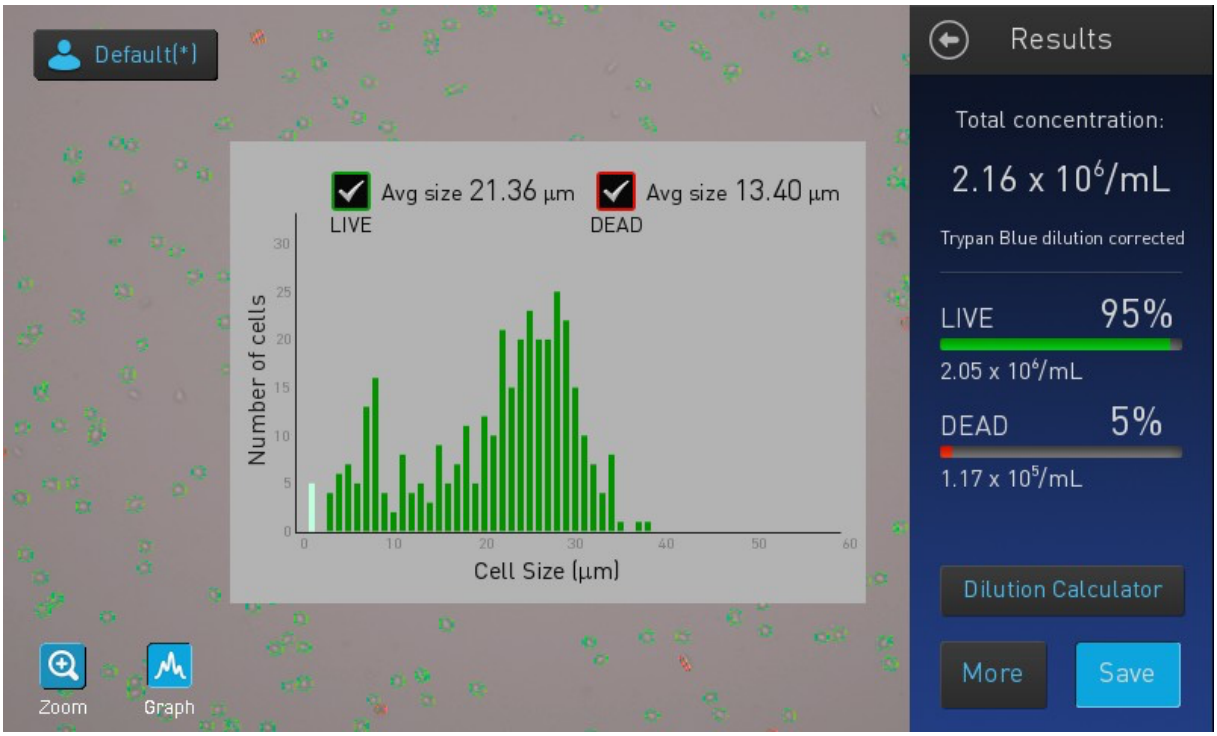

Figure 54: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 24 h of a reference probe.

Figure 55: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 48 h of a reference probe.

Figure 56: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 48 h of a reference probe.

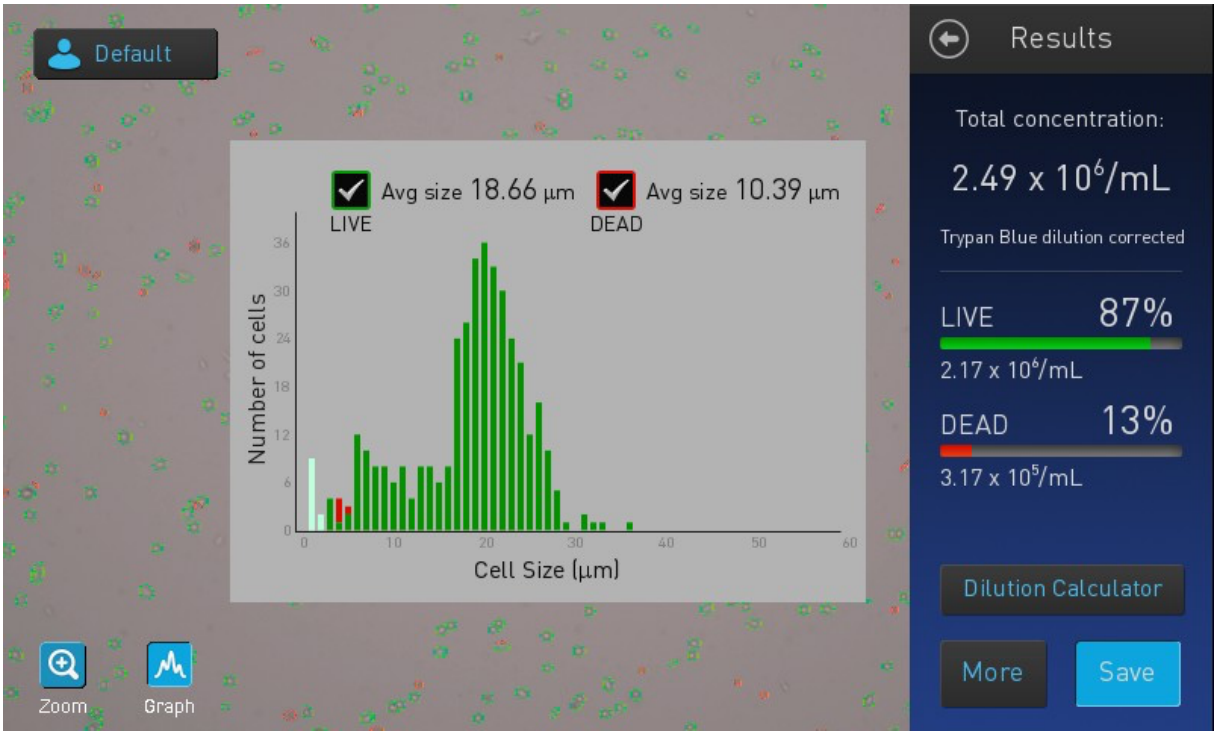

Figure 57: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 48 h of a reference probe.

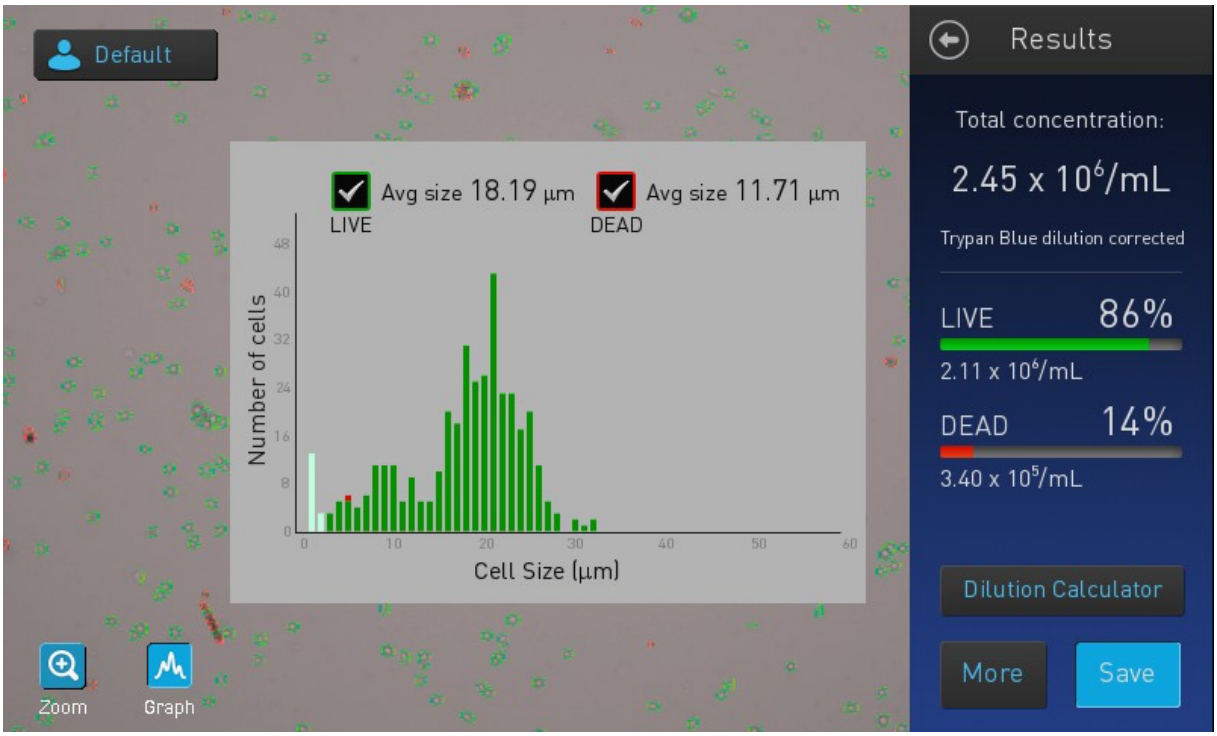

Figure 58: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 48 h of a reference probe.

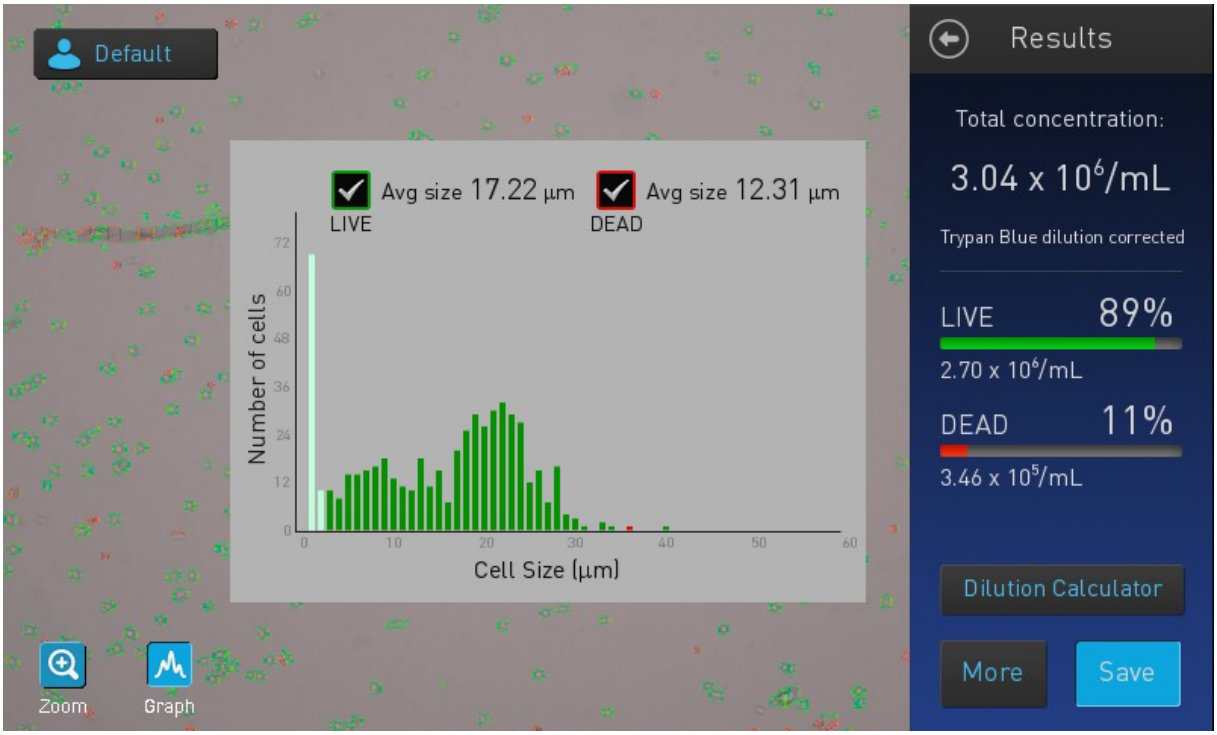

Figure 59: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 48 h of a reference probe.

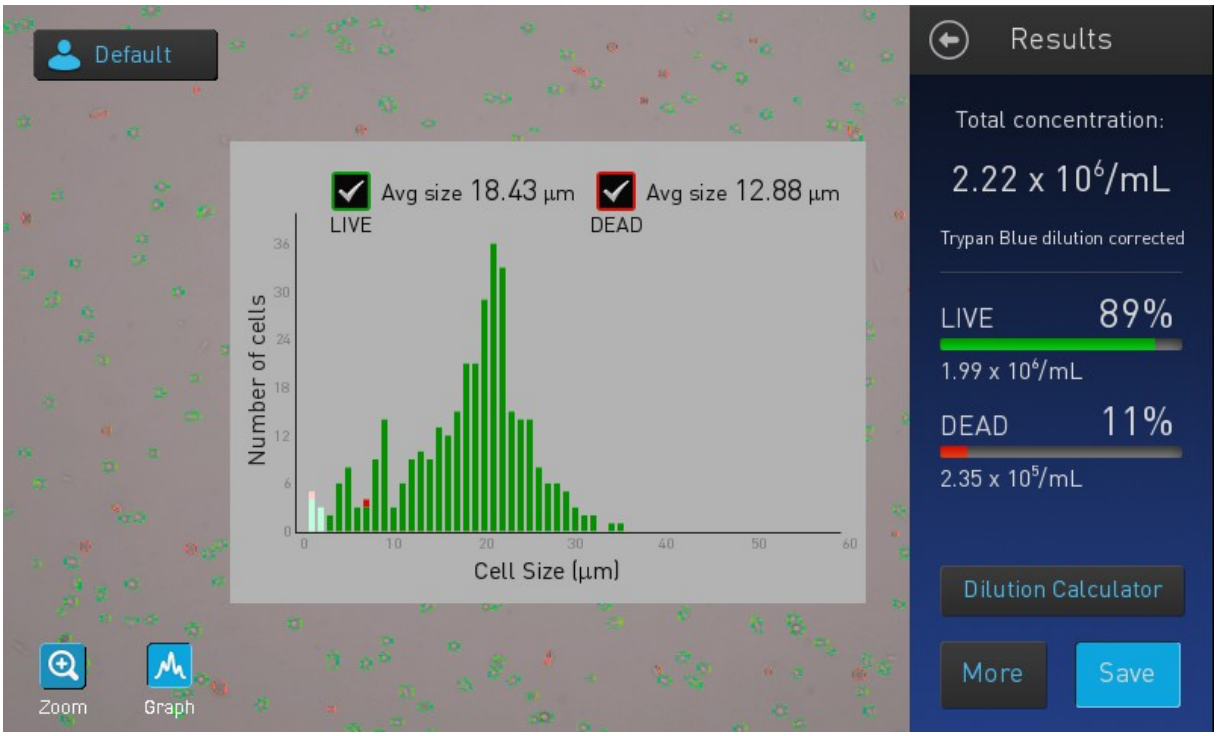

Figure 60: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 48 h of a reference probe.

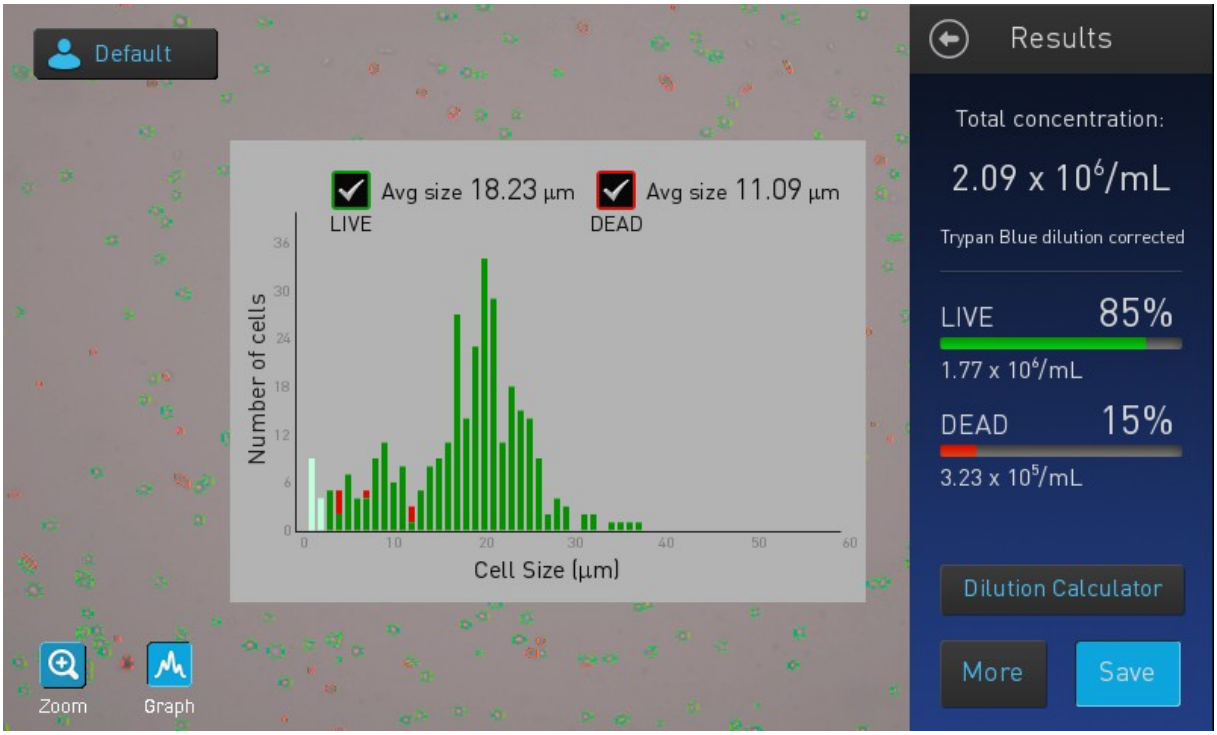

Figure 61: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 48 h of a reference probe.

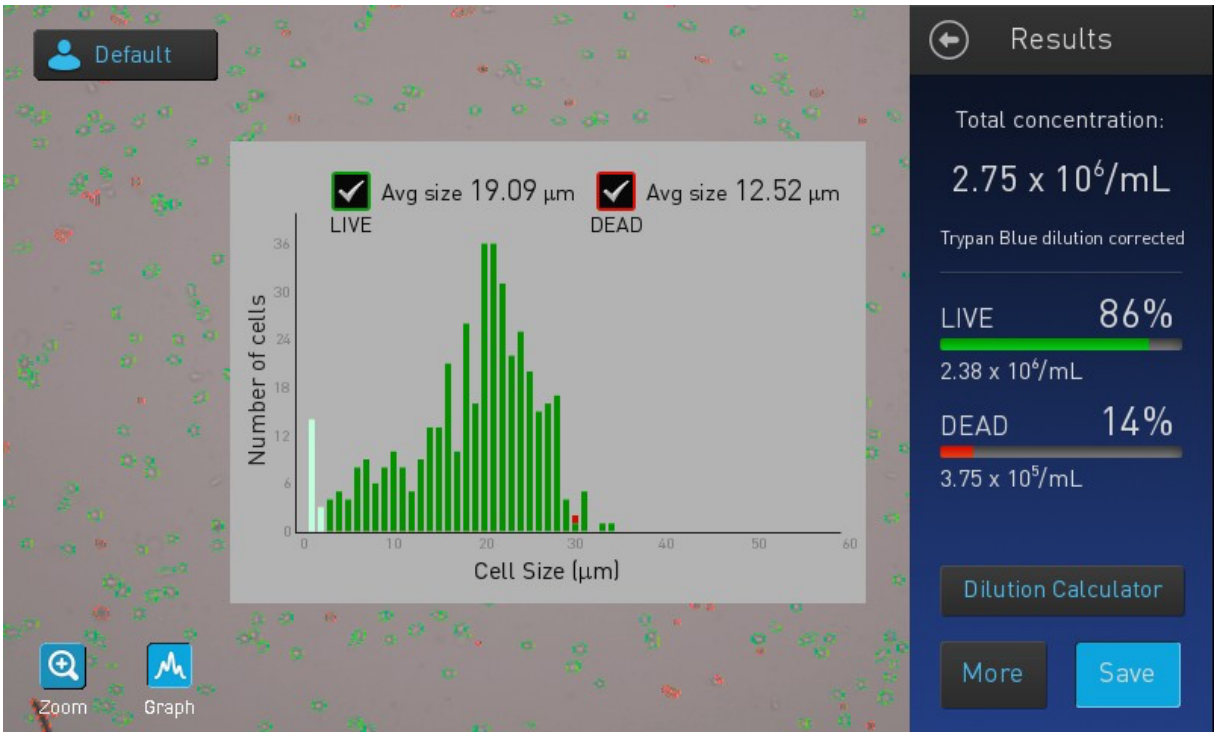

Figure 62: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 48 h of a reference probe.

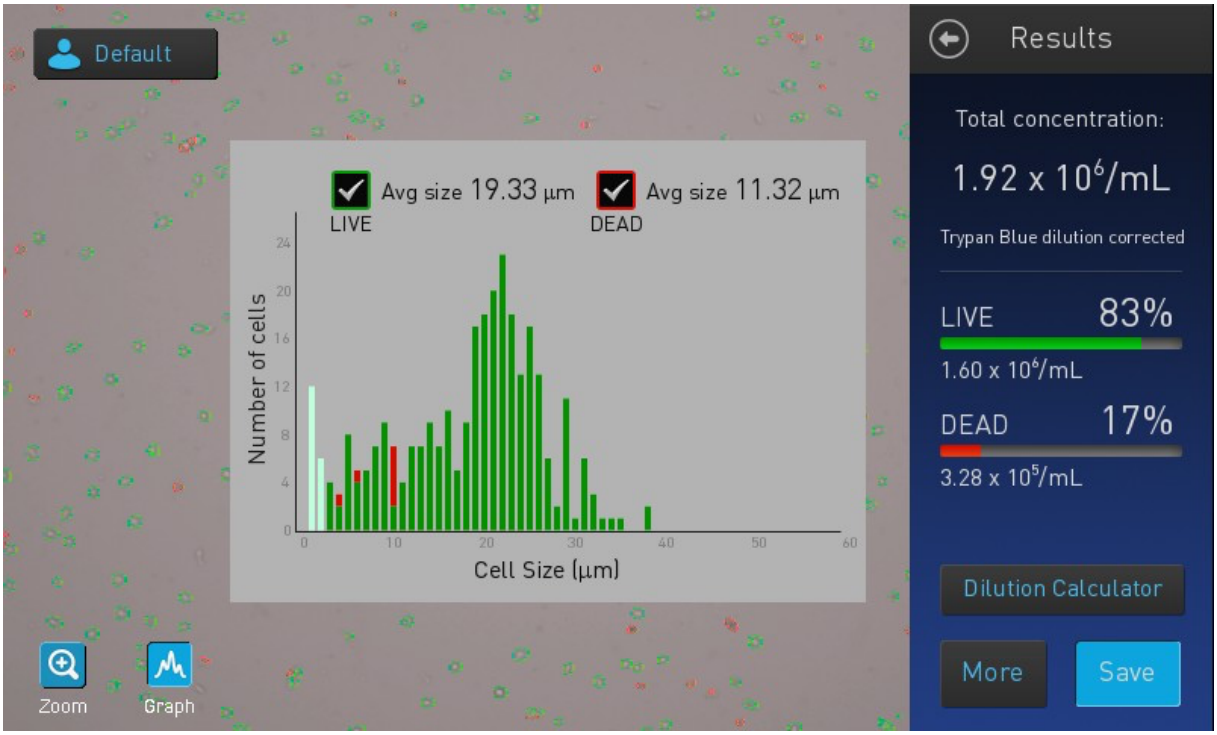

Figure 63: Results of living and dead cells using the Countess™ II automated cell counter from ThermoFischer Scientific© after incubation of 48 h of a reference probe.