

Supplementary Materials

Density distribution maps: a novel tool for subcellular distribution analysis and quantitative biomedical imaging

Ilaria De Santis ^{1,2}, Michele Zanoni ³, Chiara Arienti ³, Alessandro Bevilacqua ^{4,5,*}, Anna Tesei ³

¹ Department of Medical and Surgical Sciences (DIMEC), Alma Mater Studiorum, University of Bologna, I-40138 Bologna, Italy; i.desantis@unibo.it

² Interdepartmental Centre Alma Mater Research Institute on Global Challenges and Climate Change (Alma Climate), University of Bologna, I-40126 Bologna, Italy

³ Biosciences Laboratory, IRCCS Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST) "Dino Amadori", I-47014 Meldola, Italy; michele.zanoni@irst.emr.it (M.Z.); chiara.arenti@irst.emr.it (C.A.); anna.tesei@irst.emr.it (A.T.)

⁴ Advanced Research Center on Electronic Systems (ARCES) for Information and Communication Technologies "E. De Castro", University of Bologna, I-40125 Bologna, Italy

⁵ Department of Computer Science and Engineering (DISI), University of Bologna, I-40136 Bologna, Italy

* Correspondence: alessandro.bevilacqua@unibo.it; Tel.: +39-051-20-9-5409

Table 1. Median MTs intensity in MG-63 cells.

| Optical section | Median intensity ± MAD | |
|-----------------|------------------------|-----------------------------------|
| | CTR (n=17) | PTX-Ce6@-ker _{ag} (n=14) |
| 1 | 24.0±5.00 | 782±197 |
| 2 | 36.0±8.00 | 918±233 |
| 3 | 53.0±11.0 | 991±250 |
| 4 | 76.0±17.0 | 920±236 |
| 5 | 123±27.0 | 781±193 |
| 6 | 210±46.0 | 683±163 |
| 7 | 347±76.0 | 752±195 |
| 8 | 460±105 | 717±180 |
| 9 | 525±128 | 828±216 |
| 10 | 563±143 | 857±236 |
| 11 | 555±148 | 862±237 |
| 12 | 553±147 | 789±214 |
| 13 | 524±141 | 726±198 |
| 14 | 460±124 | 663±181 |
| 15 | 377±103 | - |
| 16 | 277±72.0 | - |
| 17 | 199±50.0 | - |

Table 2. Statistical analysis of MTs LDI percentage in MG-63 cells.

| LDI | Median percentage (%) ± MAD | | |
|-----|-----------------------------|-----------------------------------|-------------------|
| | CTR (n=17) | PTX-Ce6@-ker _{ag} (n=14) | p-value (<0.05) |
| 0 | 1.40±0.96 | 0.05±0.01 | <10 ⁻⁵ |
| 1 | 2.15±1.26 | 0.10±0.02 | <10 ⁻⁵ |
| 2 | 3.01±1.53 | 0.31±0.03 | <10 ⁻⁵ |
| 3 | 4.54±1.87 | 0.96±0.06 | <10 ⁻⁵ |
| 4 | 5.94±1.05 | 3.13±0.16 | <10 ⁻⁵ |
| 5 | 8.15±1.08 | 5.88±0.38 | <10 ⁻³ |
| 6 | 9.27±0.79 | 4.07±0.16 | <10 ⁻⁵ |
| 7 | 12.4±0.79 | 5.84±0.33 | <10 ⁻³ |
| 8 | 47.1±14.2 | 79.5±0.84 | <10 ⁻⁵ |

Table 3. Statistical analysis of hybrids LDI percentage in A549 sh/p53 cells.

| LDI | Nucleus | | | Cytoplasm | | |
|-----|-----------------------------|----------------|----------------------------|-----------------------------|----------------|----------------------------|
| | Median percentage (%) ± MAD | | p-value | Median percentage (%) ± MAD | | p-value |
| | CTR (n=14) | 2 Gy (n=14) | <0.05 | CTR (n=14) | 2 Gy (n=14) | <0.05 |
| 0 | 0.32±0.13 | 1.63±0.97 | <u><10⁻⁴</u> | 0.44±0.12 | 1.86±0.60 | <u><10⁻⁵</u> |
| 1 | 0.88±0.26 | 2.39±1.65 | <u><10⁻³</u> | 0.95±0.28 | 3.02±0.87 | <u><10⁻⁵</u> |
| 2 | 1.81±0.42 | 3.84±1.74 | <u><10⁻²</u> | 2.89±0.40 | 4.79±0.93 | <u><10⁻⁵</u> |
| 3 | 4.24±0.73 | 6.07±2.45 | <u>0.02</u> | 7.85±0.98 | 9.06±1.29 | 0.05 |
| 4 | 7.50±1.11 | 10.0±3.01 | 0.08 | 13.4±1.17 | 12.1±1.42 | 0.07 |
| 5 | 9.91±1.05 | 11.5±1.83 | 0.05 | 14.9±0.89 | 13.4±0.95 | <u><10⁻²</u> |
| 6 | 10.6±1.03 | 11.0±1.37 | 0.30 | 12.2±0.38 | 12.6±1.03 | 0.73 |
| 7 | 13.6±1.02 | 13.1±1.71 | 0.80 | 15.0±0.38 | 14.2±0.99 | <u><10⁻²</u> |
| 8 | 51.1±5.05 | 41.3±12.7 | 0.01 | 32.0±3.14 | 28.0±6.29 | 0.07 |

Table 4. Median hybrids LDI percentage and blobs number in HeLa cells.

| LDI | Median percentage (%) ± MAD | | | | (Median blob number (pixel ⁻¹) ± MAD) *10 ⁻³ | | | |
|-----|-----------------------------|-------------------|-----------------------|--------------------|---|-------------------|-----------------------|--------------------|
| | CTR (n=5) | Cortical (n=4) | Intermediate (n=3) | Scattered (n=3) | CTR (n=5) | Cortical (n=4) | Intermediate (n=3) | Scattered (n=3) |
| 0 | 0.06±0.03 | 0.20±0.03 | 0.15±0.01 | 0.16±0.04 | 0.11±0.05 | 0.44±0.07 | 0.32±0.01 | 0.36±0.08 |
| 1 | 0.30±0.04 | 0.51±0.03 | 0.49±0.02 | 0.48±0.01 | 0.38±0.01 | 0.76±0.04 | 0.67±0.05 | 0.67±0.14 |
| 2 | 0.58±0.10 | 1.27±0.11 | 1.18±0.05 | 1.25±0.05 | 0.85±0.20 | 2.43±0.24 | 1.99±0.13 | 2.15±0.05 |
| 3 | 2.62±0.14 | 4.21±0.17 | 4.50±0.56 | 5.01±0.29 | 4.57±0.55 | 8.12±0.07 | 8.75±0.20 | 10.10±0.12 |
| 4 | 10.30±0.78 | 10.30±0.75 | 11.70±0.78 | 12.40±0.57 | 17.6±1.54 | 19.60±1.34 | 21.00±0.88 | 23.80±0.38 |
| 5 | 12.50±1.02 | 14.10±0.32 | 15.30±1.12 | 15.64±0.44 | 18.00±1.46 | 20.90±0.51 | 22.50±0.52 | 25.00±0.08 |
| 6 | 7.26±0.16 | 9.12±0.25 | 9.24±0.07 | 9.51±0.26 | 10.70±0.77 | 15.80±0.09 | 16.20±0.76 | 16.90±0.13 |
| 7 | 13.20±0.58 | 12.60±0.32 | 13.50±0.56 | 14.28±0.14 | 19.50±1.99 | 21.40±0.49 | 22.30±0.96 | 24.50±0.41 |
| 8 | 53.00±2.99 | 48.3±1.05 | 40.00±4.11 | 41.68±1.70 | 2.63±0.10 | 2.54±0.08 | 3.79±0.36 | 3.71±0.44 |

Table 5. Statistical analysis of hybrids LDI percentage and blob number in HeLa cells.

| LDI | p-value (<0.05) | | | | | | | |
|-----|----------------------------|------------------|----------------------|-------------------|------------------------------------|------------------|----------------------|-------------------|
| | LDI percentage (%) | | | | Blob number (pixel ⁻¹) | | | |
| | CTR- All | CTR- Cortical | CTR- Intermediate | CTR- Scattered | CTR- All | CTR- Cortical | CTR- Intermediate | CTR- Scattered |
| 0 | <u>0.01</u> | <u>0.02</u> | 0.14 | 0.14 | 0.26 | 0.19 | 0.99 | 0.32 |
| 1 | <u><10⁻²</u> | <u>0.02</u> | 0.14 | 0.07 | 0.24 | 0.22 | 0.50 | 0.68 |
| 2 | <u><10⁻²</u> | <u>0.02</u> | <u>0.04</u> | <u>0.04</u> | 0.38 | 0.76 | 0.79 | 0.25 |
| 3 | <u><10⁻²</u> | <u>0.02</u> | <u>0.04</u> | <u>0.03</u> | 0.37 | <u>0.03</u> | 0.57 | 0.39 |
| 4 | 0.31 | 0.73 | 0.25 | 0.07 | <u>0.01</u> | <u>0.02</u> | 0.07 | 0.25 |
| 5 | <u>0.03</u> | 0.41 | 0.07 | <u>0.04</u> | <u>0.01</u> | <u>0.02</u> | 0.07 | 0.25 |
| 6 | <u><10⁻²</u> | <u>0.02</u> | <u>0.04</u> | <u>0.04</u> | <u>0.03</u> | <u>0.02</u> | 0.07 | 0.79 |
| 7 | 0.31 | 0.90 | 0.57 | <u>0.04</u> | <u><10⁻²</u> | <u>0.02</u> | <u>0.04</u> | 0.07 |
| 8 | <u><10⁻²</u> | 0.06 | <u>0.04</u> | <u>0.04</u> | <u>0.01</u> | <u>0.02</u> | 0.07 | 0.29 |

Table 6. Variance of hybrids LDI percentage and blob number in HeLa cells.

| LDI | Variance | | | | Blob number (pixel ⁻¹) *10 ⁻⁷ | | | |
|-----|--------------------|-------------------|-----------------------|--------------------|--|-------------------|-----------------------|--------------------|
| | LDI percentage (%) | | | | Blob number (pixel ⁻¹) *10 ⁻⁷ | | | |
| | All (n=10) | Cortical (n=4) | Intermediate (n=3) | Scattered (n=3) | All (n=10) | Cortical (n=4) | Intermediate (n=3) | Scattered (n=3) |
| 0 | 0.003 | 0.002 | 0.002 | 0.004 | 0.15 | 0.09 | 0.10 | 0.17 |
| 1 | 0.01 | 0.002 | 0.01 | 0.01 | 0.09 | 0.05 | 0.03 | 0.22 |
| 2 | 0.03 | 0.05 | 0.04 | 0.03 | 1.23 | 1.94 | 0.24 | 0.66 |
| 3 | 0.32 | 0.14 | 0.67 | 0.10 | 11.2 | 3.16 | 14.6 | 3.02 |
| 4 | 1.44 | 0.89 | 1.02 | 0.40 | 41.1 | 29.7 | 9.36 | 16.4 |
| 5 | 1.48 | 0.35 | 2.61 | 0.50 | 35.4 | 30.9 | 21.3 | 11.5 |
| 6 | 0.12 | 0.21 | 0.05 | 0.08 | 7.38 | 6.58 | 10.5 | 1.88 |
| 7 | 0.76 | 0.75 | 0.67 | 0.04 | 21.0 | 16.2 | 10.4 | 9.06 |
| 8 | 15.8 | 9.85 | 21.4 | 4.94 | 4.42 | 2.17 | 3.19 | 2.08 |

Table 7. Abbreviations.

| | |
|-------------|---|
| Ab | Antibody |
| CT | Computed Tomography |
| CTR | Control |
| CV | Coefficient of Variation |
| DMM | Density Distribution Map |
| FG | Foreground |
| FN | False Negative |
| FNR | False Negative Rate |
| FP | False Positive |
| GUI | Graphical User Interface |
| LDI | Local Density Index |
| MAD | Median Absolute Deviation |
| MIP | Maximum Intensity Projection |
| MT | Microtubule |
| NCC | Normalized Cross Correlation |
| PTX | Paclitaxel |
| SDR | Stoichiometric Detection Rate |
| SE | Structuring Element |
| SMLM | Single-Molecule Localization Microscopy |
| TN | True Negative |
| TP | True Positive |
| TPR | True Positive Rate |
| V2R | Vasopressin Receptor 2 |