Electronic Supplementary Material (ESI) for RSC Advances.


# Supplementary Information

## Profiling Single-Cell Level Phagocytic Activity Distribution with Blood Lactate Levels

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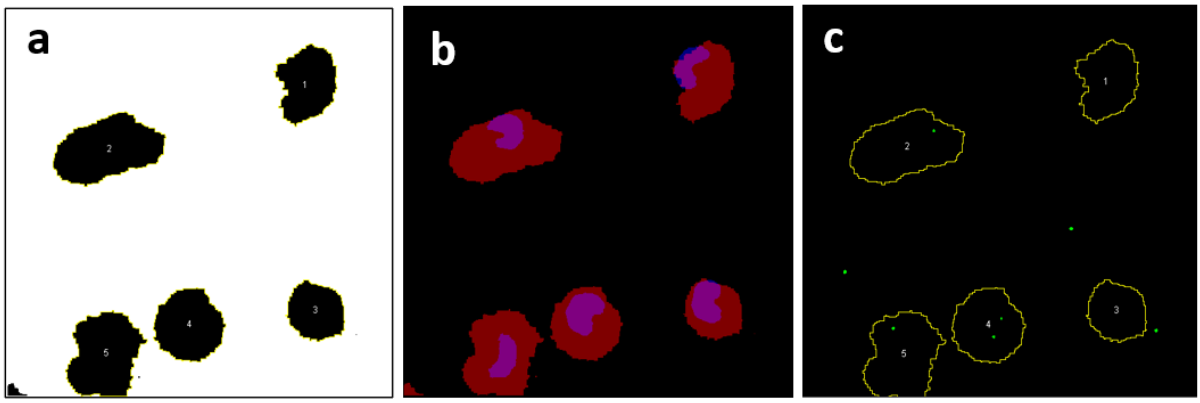


Fig S1. (a) Determines regions of interest (ROI) for future steps. (b) Each ROI is checked for presence of nuclei, to exclude membrane debris. (c) Beads in each region of interest are counted, results are exported to spreadsheet.

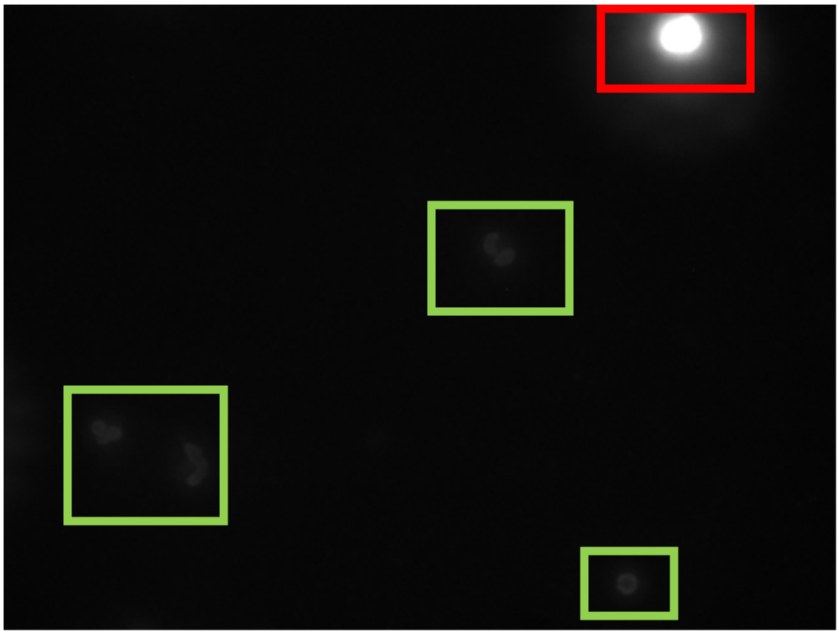

Fig S2. Representative image of excluding dead cells using Hoescht nuclear stain. Nuclei in red box excluded, due to excess fluorescence. Nuclei in green boxes were included in data.

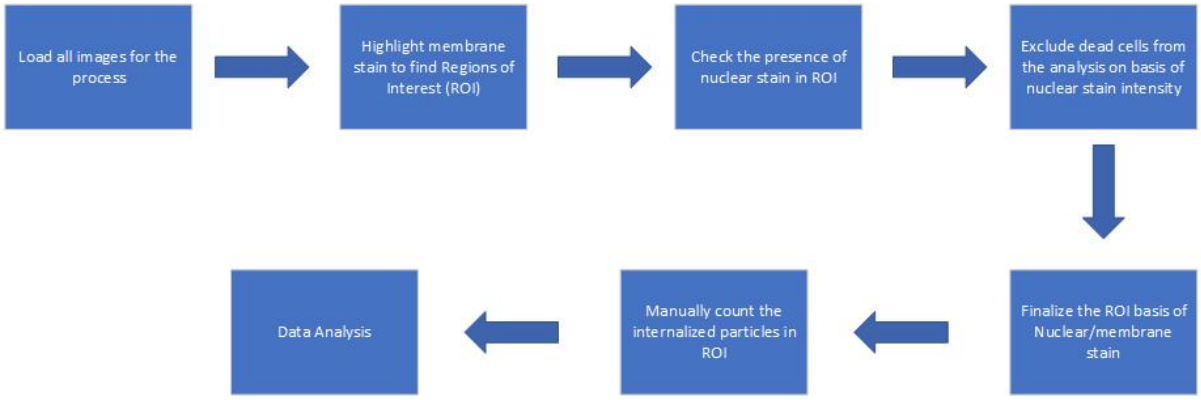


Fig S3. Flowchart listing the steps invloved in processing of the images obtained from the phagocytosis assay for data collection and analysis.

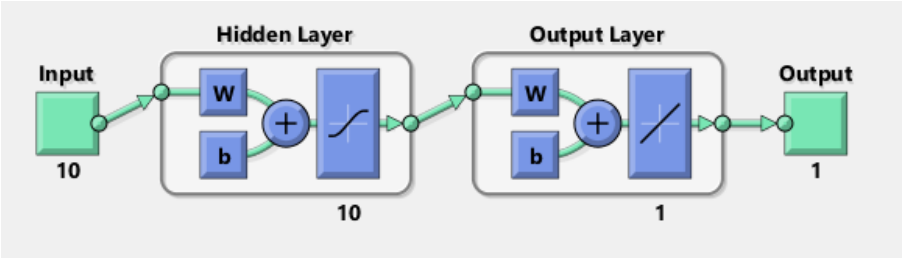


Fig S4. A two layer feed forward network used for differentiating between the phagocytic activity distribution of the neutrophils from the two groups.

| **Protocol Step** | **Approximate time requirement** |
|---|---|
| Neutrophil Isolation | Approximately 90 minutes |
| Neutrophil seeding in 24 well plate | Approximately 15 minutes |
| Incubation with IgG beads and membrane stain | Approximately 40 minutes |
| Washing of excess beads and membrane stain | Approximately 10 minutes |
| Incubation with nuclear stain | Approximately 10 minutes |
| Placement on ice | Approximately 10 minutes |
| Washing of excess nuclear stain | Approximately 10 minutes |
| Fluorescent imaging of the neutrophils | Approximately 30 minutes |
| Merging captured images using image processing for final image | Approximately 15 minutes per merged image |
| Data analysis of the final merged image | Approximately 20 minutes per image |

Table S1: Approximate time required for each step involved in the protocol used for quantification of neutrophil phagocytic activity.

| | | Input | | | | | | | | | | Target |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Training Data | Sample 1 | 0.38717 | 0.561795 | 0.59679 | 0.396147 | 0.41549 | 0.703447 | 0 | 0.374074 | 0.824074 | 0 | 0 |
| | Sample 2 | 0.053195 | 0 | 0.689191 | 0.669302 | 0.519734 | 0.163684 | 0.07086 | 0.128662 | 0 | 0 | 0 |
| | Sample 3 | 0.234294 | 0.261332 | 0.632158 | 0.508699 | 0.460902 | 0.440004 | 0.209906 | 0.285849 | 0 | 0 | 0 |
| | Sample 4 | 0.343015 | 0.447686 | 0.459527 | 0.616768 | 0.770177 | 0.259615 | 0.390351 | 0.177193 | 0 | 0 | 0 |
| | Sample 5 | 0.814085 | 0.673964 | 0.214015 | 0.609903 | 0.556113 | 0.658402 | 1 | 0 | 1 | 0 | 0 |
| | Sample 6 | 0.25108 | 0.207431 | 0.565163 | 0.60309 | 0.544811 | 0.441405 | 0.110697 | 0.301493 | 0 | 0 | 0 |
| | Sample 7 | 0.432087 | 0.57666 | 0.507983 | 0.625195 | 0.448688 | 0.302067 | 0.460345 | 0.557241 | 0 | 0.348276 | 0 |
| | Sample 8 | 0.460827 | 0.59076 | 0.442445 | 0.889803 | 0.219538 | 0.396377 | 0.393805 | 0.715044 | 0.393805 | 0 | 1 |
| | Sample 9 | 0.616471 | 1 | 0.559671 | 0.421688 | 0.224852 | 0.437912 | 0.495668 | 1 | 0 | 1 | 1 |
| | Sample 10 | 0.034269 | 0.189964 | 0.764805 | 0.703711 | 0.252568 | 0.19514 | 0.171154 | 0 | 0 | 0.388462 | 1 |
| | Sample 11 | 1 | 0.583481 | 0 | 0.609378 | 0.850268 | 1 | 0.628024 | 0.325806 | 0.358871 | 0.407258 | 1 |
| | Sample 12 | 0.484942 | 0.606506 | 0.448497 | 0.878604 | 0.147666 | 0.297035 | 0.75282 | 0.759398 | 0 | 0 | 1 |
| | Sample 13 | 0.856533 | 0.772866 | 0.122608 | 0.932161 | 0.540519 | 0.414394 | 0.722403 | 0.787013 | 0.577922 | 0.327922 | 1 |
| | Sample 14 | 0.754604 | 0.366946 | 0.154362 | 0.511307 | 1 | 0.624451 | 0.728423 | 0.240476 | 0 | 0 | 1 |
| | Sample 15 | 0.371917 | 0.183717 | 0.362663 | 1 | 0.345474 | 0.617988 | 0.404545 | 0 | 0 | 0 | 1 |
| Test | Sample 1 | 0 | 0.444159 | 1 | 0.372388 | 0 | 0.010779 | 0.526974 | 0.318947 | 0 | 0.265789 | 0 |
| | Sample 2 | 0.905046 | 0.668509 | 0.266133 | 0 | 0.938111 | 0.873288 | 0.953571 | 0.192381 | 0 | 0.480952 | 1 |
| | Sample 3 | 0.081015 | 0.588928 | 0.909807 | 0.376173 | 0.270656 | 0 | 0.196903 | 0.357522 | 0 | 0.893805 | 1 |
| | Sample 4 | 0.662136 | 0.507851 | 0.233745 | 0.777219 | 0.634291 | 0.527502 | 0.533775 | 0.510101 | 0.337121 | 0 | 1 |

Table S2: Input and target matrices used with the  fro the purpose of training nad testing to evaluate its performance.

| Sample Number | Lactate Level | Total cells imaged* | Avg. beads per cell | S. Dev. beads per cell | % cells with 0 bead | % cells with 1 bead | % cells with 2 bead | % cells with 3 bead | % cells with 4 bead | % cells with 5 bead | % cells with 6 bead | % cells with 7 bead |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.6 | 54 | 1.2 | 1.35 | 38.89 | 27.78 | 16.67 | 12.96 | 0 | 1.85 | 1.85 | 0 |
| 2 | 0.7 | 157 | 0.92 | 1 | 42.04 | 32.48 | 18.47 | 5.73 | 0.64 | 0.64 | 0 | 0 |
| 3 | 1 | 212 | 1.08 | 1.16 | 40.09 | 29.72 | 17.45 | 9.43 | 1.89 | 1.42 | 0 | 0 |
| 4 | 1.3 | 114 | 1.17 | 1.28 | 34.21 | 31.58 | 22.81 | 7.02 | 3.51 | 0.88 | 0 | 0 |
| 5 | 1.4 | 89 | 1.56 | 1.42 | 25.84 | 31.46 | 19.1 | 12.36 | 8.99 | 0 | 2.25 | 0 |
| 6 | 1.5 | 201 | 1.09 | 1.13 | 37.81 | 31.34 | 18.91 | 9.45 | 1 | 1.49 | 0 | 0 |
| 7 | 1.5 | 145 | 1.24 | 1.36 | 35.86 | 31.72 | 17.24 | 7.59 | 4.14 | 2.76 | 0 | 0.69 |
| 8 | 1.9 | 190 | 0.88 | 1.28 | 52.63 | 27.37 | 9.47 | 3.68 | 4.74 | 1.58 | 0 | 0.53 |
| 9 | 2.2 | 113 | 1.27 | 1.37 | 33.63 | 36.28 | 13.27 | 8.85 | 3.54 | 3.54 | 0.88 | 0 |
| 10 | 2.3 | 202 | 1.4 | 1.63 | 37.62 | 28.22 | 13.37 | 9.41 | 4.46 | 4.95 | 0 | 1.98 |
| 11 | 2.4 | 130 | 0.91 | 1.12 | 44.62 | 33.08 | 13.85 | 6.15 | 1.54 | 0 | 0 | 0.77 |
| 12 | 2.4 | 124 | 1.72 | 1.36 | 18.55 | 31.45 | 24.19 | 16.94 | 5.65 | 1.61 | 0.81 | 0.81 |
| 13 | 2.8 | 210 | 1.64 | 1.42 | 27.62 | 20.95 | 25.71 | 15.24 | 8.57 | 0.95 | 0 | 0.95 |
| 14 | 3.9 | 133 | 1.29 | 1.38 | 33.83 | 36.09 | 12.03 | 7.52 | 6.77 | 3.76 | 0 | 0 |
| 15 | 4.4 | 113 | 0.95 | 1.37 | 49.56 | 27.43 | 14.16 | 3.54 | 1.77 | 1.77 | 0 | 1.77 |
| 16 | 4.5 | 154 | 1.6 | 1.48 | 22.73 | 37.01 | 18.83 | 9.09 | 6.49 | 3.9 | 1.3 | 0.65 |
| 17 | 6.7 | 396 | 1.43 | 1.32 | 26.52 | 34.34 | 20.45 | 10.61 | 4.8 | 2.53 | 0.76 | 0 |
| 18 | 10.7 | 169 | 1.51 | 1.23 | 23.81 | 29.76 | 26.79 | 11.9 | 6.55 | 1.19 | 0 | 0 |
| 19 | 22 | 110 | 1.19 | 1.11 | 30.91 | 38.18 | 15.45 | 11.82 | 3.64 | 0 | 0 | 0 |

Table S3: Table showing the phagocytic activity distributions for individual samples along with their lactate levels.

| AUC Values | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 0.5 | 0.5 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 |
| 0.666667 | 1 | 1 | 1 | 1 | 0.666667 | 0.333333 | 1 | 0.666667 | 1 |
| 1 | 1 | 1 | 1 | 1 | 1 | 0.666667 | 0.5 | 1 | 1 |
| 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| 1 | 1 | 1 | 1 | 1 | 0.333333 | 1 | 1 | 1 | 0.666667 |
| 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0.333333 | 0.5 | 1 |
| 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 |
| 1 | 1 | 1 | 1 | 1 | 1 | 0.666667 | 1 | 1 | 0 |
| 1 | 1 | 1 | 1 | 0.5 | 0.5 | 0.5 | 1 | 1 | 1 |
| 0.666667 | 1 | 0.666667 | 1 | 1 | 1 | 1 | 0.5 | 1 | 0.333333 |

Table S4: Table showing the AUC values of 100 networks trained using BR algorithm.

| AUC Values | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 0.333333 | 1 | 1 | 0.333333 | 0.333333 | 1 | 0.666667 | 1 | 1 | 0 |
| 0.666667 | 0.666667 | 0.666667 | 0.666667 | 0.333333 | 0.333333 | 0.666667 | 0.666667 | 1 | 0 |
| 1 | 1 | 1 | 1 | 1 | 1 | 0.333333 | 0.666667 | 0.333333 | 0.666667 |
| 0.666667 | 0.666667 | 0.666667 | 0.666667 | 0.666667 | 0.333333 | 0 | 1 | 1 | 0.666667 |
| 0.666667 | 0.333333 | 1 | 0.333333 | 0 | 0 | 0.333333 | 1 | 0.666667 | 1 |
| 0.333333 | 0.333333 | 1 | 0.666667 | 0.666667 | 0.333333 | 1 | 0.666667 | 0.333333 | 1 |
| 0.666667 | 0.666667 | 0 | 0.333333 | 0.666667 | 0.333333 | 0 | 1 | 0.333333 | 1 |
| 0.333333 | 0.666667 | 1 | 0.666667 | 0.666667 | 1 | 0.333333 | 0.333333 | 0.333333 | 0.333333 |
| 1 | 1 | 0.666667 | 0.666667 | 1 | 0.333333 | 0.666667 | 1 | 1 | 1 |
| 0 | 0.666667 | 0.666667 | 0.333333 | 1 | 0.333333 | 0.666667 | 0 | 0.333333 | 0.666667 |

Table S5: Table showing the AUC values of 100 networks trained using LM algorithm.

| AUC Values | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.666667 | 1 | 0.333333 | 0 | 1 | 0.333333 | 1 | 0.666667 | 0.333333 |
| 0.666667 | 0 | 0.333333 | 0.666667 | 0.333333 | 0.666667 | 0.666667 | 0.666667 | 1 | 0.666667 |
| 1 | 0.333333 | 0.333333 | 1 | 0 | 0.333333 | 0.666667 | 0.333333 | 0.666667 | 0.333333 |
| 0.666667 | 0.666667 | 1 | 0.666667 | 1 | 0.666667 | 1 | 1 | 0.666667 | 1 |
| 1 | 0.666667 | 0.666667 | 0.333333 | 0.666667 | 0.333333 | 1 | 1 | 0 | 1 |
| 0.333333 | 0.333333 | 1 | 0.666667 | 1 | 0.666667 | 1 | 0.666667 | 1 | 1 |
| 0.666667 | 0.333333 | 1 | 0.333333 | 0.666667 | 1 | 0.666667 | 1 | 0.333333 | 0.333333 |
| 1 | 0 | 0.666667 | 1 | 1 | 0.666667 | 0.333333 | 0.666667 | 0.666667 | 1 |
| 0.333333 | 1 | 0.666667 | 0.666667 | 1 | 0.333333 | 0.666667 | 0.333333 | 1 | 1 |
| 0.333333 | 0.666667 | 0 | 1 | 1 | 1 | 0.666667 | 0.666667 | 0.333333 | 0.333333 |

Table S6: Table showing the AUC values of 100 networks trained using SCG algorithm.