RO-index

Quantifying fluorescent protein sensor cytGRX-roGFP2 redox state with a reduced–oxidized (RO) index.

Example image: https://nph.onlinelibrary.wiley.com/cms/asset/43d9b2d6-30c5-46de-b688-b91628468678/nph71392-fig-0004-m.jpg

Procedure

1. Load image

1.1 Read confocal microscopy CZI file.

1.2 Extract fluorescence channels:

- **E405**: 405 nm excitation channel
- **E488**: 488 nm excitation channel
- **PI**: Propidium iodide channel

2. Root region segmentation

For each channel $C \in \{E_{405}, E_{488}\}$:

2.1 Normalize intensity values of C to the range $[0, 255]$.

2.2 Apply a median filter.

2.3 Compute an Otsu threshold T_c.

2.4 Generate binary mask M_c such that

$$M_c(x, y) = \begin{cases} 1, & C(x, y) > T_c \\ 0, & \text{otherwise} \end{cases}$$

2.5 Retain the masked image

$$C' = C \cdot M_c$$

3. Protein presence mask

$$M_{\text{protein}} = M_{405} \vee M_{488}$$

where $\vee$ denotes the logical OR operator.

4. Saturated pixel removal

For each processed channel $C' \in \{E'_{405}, E'_{488}\}$:

4.1 Extract all non-zero pixels.

4.2 Compute percentile thresholds

$$T_{\text{high}} = P_{99}(C')$$

4.3 Ties handling

To avoid too many tied data, only when the ratio of unique intensity values exceeds 0.3, set:

$$C'(x,y) = 0 \quad \text{if} \quad C'(x,y) > T_{\text{high}}$$

5. Redox index calculation

5.1 Square both channels

$$E''_{405} = (E'_{405})^2$$

$$E''_{488} = \left(E'_{488} \cdot \frac{\max(E'_{405})}{\max(E'_{488})} \right)^2$$

5.2 Compute the redox index (RO)

$$RO = \frac{E''_{405} - E''_{488}}{E''_{405} + E''_{488}}$$

5.3 Replace undefined values with zero.

5.4 Restrict analysis to valid protein regions

$$RO = RO \cdot M_{\text{protein}}$$

6. Reduced–Oxidized (RO) index classification

$$-1 \leq RO \leq 1$$

- **RO > 0** indicates a more oxidized state
- **RO < 0** indicates a more reduced state