

# Redox Derivations

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## 1 Converting 410/470 intensity ratio to the pre-portion of oxidized sensor molecules

The *roGFP*<sub>12</sub> sensor is genetically encoded into a tissue. The intensity values emitted from the *roGFP*<sub>12</sub>-containing tissue are recorded after being stimulated 410nm and 470nm light. The ratios between these values describe relative levels of tissue oxidation. For example, a 410/470 ratio of 2.0 indicates that a tissue is more oxidized than a ratio of 1.0. In this brief summary, we algebraically convert the 410/470 intensity ratio into the more biologically-meaningful measure of redox potential ( $E$ ) via the description of ratio of of *roGFP*<sub>12</sub> molecules in an oxidized (as opposed to reduced) state ( $OxD$ ).

Assume a fully reduced state. Then, the intensities observed at a wavelength  $\lambda$  are equal to the product of  $N_T$ , the total number of roGFP molecules, and  $I_{\lambda,R}$ , the intensity of each roGFP molecule at a given wavelength in the reduced state.

$$I_{\lambda,R} = N_T * I_{\lambda,R} \quad (1)$$

The same is true for the fully oxidized state:

$$I_{\lambda,Ox} = N_T * I_{\lambda,Ox} \quad (2)$$

At a redox state between maximally reduced and maximally oxidized, the intensity at a given wavelength is a weighted sum of the molecules found at either discretely oxidized or reduced state. We therefore can rewrite any state in terms of equations (1) and (2)

$$I_{\lambda} = \frac{N_{Ox}}{N_T} * I_{\lambda,Ox} + \frac{N_{Red}}{N_T} * I_{\lambda,Red} \quad (3)$$

Because all sensor molecules must be in either an oxidized or reduced state,  $N_{Red} = N_T - N_{Ox}$ . So we can rewrite equation (3) :

$$I_{\lambda} = \frac{N_{Ox}}{N_T} * I_{\lambda,Ox} + (1 - \frac{N_{Ox}}{N_T}) * I_{\lambda,Red} \quad (4)$$

Using equation (4), consider the intensity ratio of 410 nm / 470 nm:

$$\frac{I_{410}}{I_{470}} = \frac{\frac{N_{Ox}}{N_T} * I_{410,Ox} + (1 - \frac{N_{Ox}}{N_T}) * I_{410,Red}}{\frac{N_{Ox}}{N_T} * I_{470,Ox} + (1 - \frac{N_{Ox}}{N_T}) * I_{470,Red}} =$$

For brevity, let  $OxD = \frac{N_{Ox}}{N_T}$ . Then cross-multiply:

$$I_{410} * OxD * (I_{470,Ox} + (1 - OxD) * I_{470,Red}) =$$

$$I_{470} * OxD * (I_{410,Ox} + (1 - OxD) * I_{410,Red})$$

Simplify and express  $OxD$  in terms of known quantities:

$$OxD = \frac{I_{470}I_{410,R} - I_{410}I_{470,R}}{I_{410}I_{470,Ox} - I_{410}I_{470,R} - I_{470}I_{410,Ox} + I_{470}I_{410,R}} \quad (5)$$

To simplify, let:

$$R_{Red} = \frac{I_{410,R}}{I_{470,R}} \quad (6)$$

$$R_{Ox} = \frac{I_{410,Ox}}{I_{470,Ox}} \quad (7)$$

$$\frac{I_{410}}{I_{470}} = \frac{I_{410}}{I_{470}} \quad (8)$$

$$\delta_{470} = \frac{I_{470,Ox}}{I_{470,Red}} \quad (9)$$

We can now re-derive the definition of  $OxD$  in terms of ratio values.

Step: Re-arrange terms, multiply by  $\frac{-1}{-1}$ :

$$OxD = \frac{I_{410}I_{470,R} - I_{470}I_{410,R}}{I_{410}I_{470,R} - I_{470}I_{410,R} + I_{470}I_{410,Ox} - I_{470,Ox}I_{410}}$$

Step: Work to factor out  $I_{470,R}$  from the numerator and denominator write some in terms of ratio values:

$$OxD = \frac{I_{470,R}I_{470}(\frac{I_{410}}{I_{470}} - R_{Red})}{I_{470,R}I_{470}(\frac{I_{410}}{I_{470}} - R_{Red} + \delta_{470}(R_{Ox} - \frac{I_{410}}{I_{470}}))}$$

And simplify:

$$OxD = \frac{\frac{I_{410}}{I_{470}} - R_{Red}}{\frac{I_{410}}{I_{470}} - R_{Red} + \delta_{470}(R_{Ox} - \frac{I_{410}}{I_{470}})} \quad (10)$$

Where  $\delta_{470}$  describes the sensor-specific dynamic range 470nm.

- 2 Analyzing the conversion between intensity ratio and ratio of oxidized molecules
- 3 Converting fraction of oxidized sensors into an electric potential
- 4 Reparameterizing the intensity ratio