**Title**

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**Abstract**

**Introduction**

Genetically-encoded biosensors have revolutionized our ability to measure a wide variety of cellular properties in live animals. As experimentalists, any time a new biosensor is developed, we would like to know: what is that biosensor good for? That is, what range of values of the cellular property of interest is that biosensor well-suited to measure accurately? Here, we present a theoretical framework to determine the suitability of two-state biosensors.

Two-state biosensors change conformation, and spectral properties, in response to a specific input. Existing two-state biosensors respond to various biochemical properties, including pH, ATP, and glutathione redox potential (*EGSH*)[1-3](#_ENREF_1). The development of these biosensors has enabled real-time biochemistry in live animals. However, the potential of these biosensors has not been fully realized because raw fluorescence measurements must be indirectly transformed into absolute biochemical measurements.

In our previous work with the roGFP1\_R12 biosensor in *C. elegans*, we deployed a mathematical framework that enabled us to calculate *EGSH* from fluorescence ratio measurements given knowledge of the spectral and biochemical properties of the biosensor, and the properties of our microscope[4](#_ENREF_4),[5](#_ENREF_5). That framework enables absolute quantitative *EGSH* measurement but does not account for measurement limits set by imprecise ratio measurement. Here, we extend and generalize that framework to predict the accuracy of biochemical measurements for all two-state biosensors with known spectral and biochemical properties, given fluorescence ratio precision. This new framework enables us to: (i) determine the range of values the roGFP1\_R12 biosensor is well-suited to accurately measure in live *C. elegans*; (ii) optimize the precision of our imaging and image-analysis methods to improve that biosensor’s suitability; (iii) choose optimal biosensors for the measurement of *EGSH*, pH, and other biochemical properties; (iv) reclaim underused biosensors uniquely suited for certain conditions; and (v) identify what new biosensors are needed.

To help the community find biosensors that are well-suited for their experimental needs, we developed web-based tool, the Sensor Overlord (<http://www.sensoroverlord.com/>), that implements these analyses with a user-friendly interface.

**Results**

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**Discussion**

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**Materials and Methods**

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**Competing interests**

The authors declare that no competing interests exist.

**Figure legends**

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