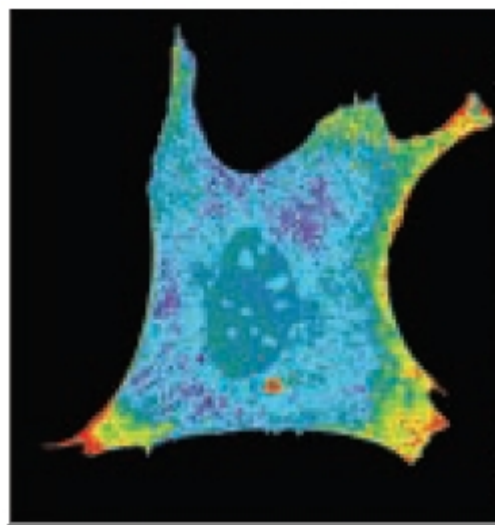


# Introduction

## Overview and Purpose

The Mero-CBD Biosensor was recently created to examine the spatial and temporal distribution of activated Cdc42 in living cells (Nalbant et al. 2004). This document's intent is to describe the image processing techniques to employ when using our biosensor. Common problems and solutions to ratiometric imaging will be discussed. Image analysis scripts (for Metamorph and Matlab software) and documents describing their use are available for downloading. A methods paper (Hodgson et al., 2005), expanding upon the topics points covered here, will be made available shortly.



## Imaging The Mero-CBD Biosensor in Living Cells

The Mero-CBD Biosensor is an example of a single-chain probe as both of its fluorophores, I-SO (a merocyanine dye; Touthkine et al., 2003) and EGFP (Enhanced Green Fluorescent Protein) reside on the same molecule. Ratio imaging of the Mero-CBD probe is used to detect the activity of Cdc42 in living cells. Sample preparation (cell care, labeling, microinjection) is discussed elsewhere (Nalbant et al., 2004; Hodgson et al., 2005).

And although Fluorescence Microscopy techniques lie outside the scope of this article - - a few pertinent points should be noted. I-SO illumination is at a much longer wavelength than EGFP; this results in the focal point for I-SO being approximately 200 nm away from the EGFP focal point in the Z plane. A standard offset (Z) should be measured and the Image Acquisition Software set to move the objective appropriately while collecting images. Remember Cell health is important during image acquisition, so maintain physiological conditions (temperature, CO<sub>2</sub> concentrations, and media). A final point: it is better to expose cells to longer levels of dimmer light than to expose them to a shorter duration of much brighter light.

## Additional Imaging Considerations:

Damage to cells and fluorophores can be minimized by:

- Using Neutral Density Filters
- Minimizing Exposure to Fluorescent Illumination during Field Selection
- Minimizing Integration Time per image by setting Gain on Camera to 2X
- Minimizing Integration Time per image by Binning images (2X2)
- Use of Oxygen Radical Scavengers (i.e Oxyrase)

Improve Signal to Noise for Digital Image Analysis by:

- Targeting Maximal Cell Pixel Intensity values from 1500-3000 (12 bit images) during image acquisition
- Targeting Peripheral Cell Values at least 100-150 over background intensity
- Using higher End Sensitive CCD Cameras
- Selection of appropriate Excitation, Dichroic and Emission filters to maximize appropriate fluorescence throughput

Typical Settings for imaging Mero-CBD Biosensor

- 2 X 2 Binning
- 40X Oil Objective/NA 1.3
- Exposure Settings: 0.1 - 1 sec (EGFP) and 0.3 - 3 sec (I-SO)
- 30-60 sec intervals between time points
- I-SO can be imaged using commercially available filter sets (see Hodgson et al., 2005).

Inspect Digitized Images Before Processing

- Inspect for appropriate Signal to Noise (see above)
- Ensure that Images are in Focus
- Ensure that Images are not Saturated (i.e. for 12-bit images having values > 4095)
- Check that no undesirable major event (focal shift, stage repositioning, cell death) occurred during Image Acquisition.
- Backup all Raw Images

### **Publications cited:**

Hodgson L, Perihan, Nalbant P, Shen F, Hahn K. 2005. Methods in Enzymology. Imaging and photobleach correction of MeroCBD, sensor of endogenous Cdc42 activation. In press Meth. Enzymol.

Nalbant P, Hodgson L, Kraynov V, Touthkine A, Hahn KM. Activation of endogenous Cdc42 visualized in living cells. Science. 2004 Sep 10;305(5690):1615-9.

Touthkine A, Kraynov V, Hahn K. Solvent-sensitive dyes to report protein conformational changes in living cells. J Am Chem Soc. 2003 Apr 9;125(14):4132-45.