$DiBAC_4(3)$ for early *Xenopus* embryos, Dany Spencer Adams, version: 12/2/14

$DiBAC_4(3)$ is one of the oxonol family of slow-response probes. It is a small, negatively charged fluorophore that's brightness increases significantly when it is associated with a membrane inside a cell. As resting potential becomes more positive (depolarized), more dye enters the cell and the brightness (a.k.a. intensity, quantified as pixel value) increases. As the cell becomes more negative/polarized, the intensity goes down.

This protocol will yield information about the *relative* resting potential of different cells *within the same image*. It is useful for imaging early *Xenopus* embryos that have relatively small (not very negative) resting potentials. It may not work on cells with more negative resting potentials. You must test it on your own specimens; this is a limited liability protocol.

BEFORE:

1. Store powder at 4°C
2. Stock is 1mg/mL (1.9 mM) in DMSO, store at room temp for up to 3 months.

DURING

1. Dilute stock 1:1000 in your regular medium.
2. Put your specimens in, making sure you don't add any loose embryo gunk (it makes the dye clump up and sparkle VERY brightly).
3. Wait 20 minutes.
4. Leaving it in the DiBAC, image with a FITC (GFP) cube, making sure to take as good an image as you can, using the same exposure for all images. *Do not rely at all* on post-processing, because the pixel values are the data - any processing other than the two corrections described below is cheating.
5. Close the shutter and take an image of the blackness using the same exposure that you have been using to image specimens. This is your Darkfield image; it is a picture of the noise introduced by the electronics and the camera.
6. Open the shutter then take a picture of just DiBAC in medium; make it as out-of-focus as possible (I find that raising the stage as far as you can is the most reliable). This is your Flatfield image; it is a picture of the uneven-ness of the illumination.

AFTER:

There are two corrections that *absolutely must* be done before you can believe the images, darkfield and flatfield, (or background and shading, or whatever, pick your own vocabulary):

1. Darkfield correction: subtract the darkfield image from each image, including the flatfield image.
2. Flatfield correction: divide each darkfield-corrected image by the darkfield-corrected flatfield image.
3. Measure region statistics on the dfcor (darkfield-flatfield-corrected) image. Make sure the region used is all in focus and excludes any bright spots that are due to dye clumping.