Pullen Lab Protocol for Using the VersaDoc to Detect Chemiluminescence

This is an operation guide for using the Bio-Rad VersaDoc 4000 MP imaging system to detect chemiluminescence on Western Blots. This should not be followed for other VersaDoc applications.

If at any point red lights start to flash on the door of the instrument, stop and notify immediately.

Procedure:
1. If not already done: attach the shorter 50mm Nikon lens. If you don't know what this is, STOP and get someone who does.
     a. Remove the lens cap
     b. Make sure the middle shelf is in place in the instrument Chamber.
2. Turn the AC power supply and then the instrument power switches to the "On" positions.
3. Wait for a solid green light to appear above "Power" on the instrument door.
4. Open the "Quantity One" software on the PC.
5. Click the "Select Scanner" button, *i.e.* step 1 on the "Volumes Quick Guide" menu.
6. Click "VersaDoc…" in the pop-up window.
7. Under the "Channel 1" tab click "Select…"
     a. Then click "Blotting"
     b. Then click "Chemi Ultra Sensitivity"
8. In the "Channel 1" tab, under "Step II", click "Position"
     a. Use a target, like a sheet of paper with a drawing, to focus the camera by hand.
     b. A black image means you forgot to remove the lens cap.
     c. When done focusing, click "Stop" under "Step III".
9. Place your blot on the shelf, and close the instrument door.
10. Do **NOT** click "Acquire"!!!
11. Click "Optimize Exposure" under "Step III" and then in the pop-up:
     a. Set your total desired exposure time.
     b. Set the time point when you want the first image captured
     c. Set the number of images you want taken over the exposure period.
     d. Click "OK".
12. The instrument will take a minute to communicate and then start the exposure run. Images should then appear on the monitor, with increasing quality, over the exposure period. Usually the first capture looks like static.
     a. You can save any of these images, so there is no need to proceed to the "Acquire" button.
     b. If you get static in all your images, then your total exposure was not long enough: go back to optimize, and make it longer. If that doesn't work, then there is something wrong with your Western blot.
     c. If your images appear as a photonegative, it means your exposure was too long, and/or sensitivity is too high. Go back and shorten the exposure time, if that is already at the minimum then go back to Step 8 and choose a setting lower than "Ultra".
13. Save your file, place your blot in its appropriate buffer container, clean off the shelf in the instrument, replace the lens cap, shut the door, close the software, and then **TURN OFF BOTH POWER SWITCHES**!!!
     a. Leaving the camera on will cause it to overheat and malfunction.