

## **Intensity Benchmarking Fluorescence Microscope Performance with Fluorescent Glass**

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Summary: A fluorescent glass and a non-fluorescent glass sample are used to benchmark the relative lamp intensity and proper operation of a fluorescence microscope. The data generated with this procedure can be used to compare day-to-day and hour-to-hour operation of a fluorescence microscope.

NOTE1: GG475 fluorescent Schott glass disks and identically sized KG-1 glass (non-fluorescent) disks (Edmund Scientific) are used as intensity benchmarks. Work in our lab suggest the GG475 is relatively photostable under the broad illumination conditions used with optical microscopy and has a broad emission that can be used to benchmark DAPI and TxRed filter sets on the same sample.

NOTE2: The incident lamp intensity should be measured with a photometer and this value recorded before obtaining measurements of the fluorescent glass.

1. A 2 cm hole is prepared at the bottom of a single well in a 6-well plate.
2. The GG475 Schott glass is placed over the hole.
3. Phase imaging is used to focus onto the bottom of the Schott glass disk.
4. The filters are set appropriately and the exposure time is determined so that an image of the fluorescent glass uses approximately  $\frac{1}{2}$  of the dynamic range of the camera.
5. An image of the GG475 glass is collected and stored. The imaging conditions are also recorded.
6. An image of the KG-1 blank glass is collected under the same settings and stored.
7. Steps 2-6 are repeated for each filter condition to benchmark.
8. Mean value of the intensity of the glass images are determined with image analysis software and the value from the blank glass is subtracted from the value of the fluorescent glass. This value along with the incident lamp intensity (see note 2) can be used to ensure reproducible day-to-day performance of a fluorescence microscope.

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