Program a FRAP sequence for a more quantitative measurement, as follows.

1) In live mode, close the field diaphragm to a small size (~30 µm diameter). Adjust focus. Stop illumination and move to a nearby, unbleached area without changing focus. Image 5 frames at low laser power (10-20 µW, or 15-30 nW/µm^2) to record the pre-bleach intensity. Open the shutter for illumination only during the time of acquisition or bleaching.

2) Expose the area to 70-100 times higher laser power (limited by the highest power available) until most of the fluorophores are bleached in the exposed area. The bleaching time will depend on the maximum laser power available. With the equipment and parameters listed in this protocol, 1-4 s is sufficient.

3) Record the fluorescence recovery at low laser power, as in step 1. Because most of the recovery occurs during the initial 10-20 s, record the initial recovery at higher time resolution, e.g. at 2 Hz for 10 frames. Monitor the slower, asymptotic approach to the final fluorescence value using a lower acquisition rate of 0.25 Hz for 2-4 min to minimize bleaching.