Photoconversion of Dendra2 with the Laser Scanning Microscope LSM 710 from Carl Zeiss

A startup guide

Dendra2 is photoconvertible either by use of a 488nm-laser or a 405nm-laser. To prevent photobleaching of the initially green Dendra2 use of 405nm is preferable, if available. To minimize photobleaching during the acquisition of the green channel over time, use as little 488nm-laserpower as possible and fastest available scanspeed.

Set up a fast sequential red/green-acquisition with linewise switching [see Fig. 2]:

1. Create two tracks in the ‘Imaging Setup’
2. Define the tracks in the ‘Light Path’-dialog (‘pro’-Mode):
   a. Track1 ‘Dendra2-green’: one detector with a detection range from 490nm to 560nm
   b. Set 488nm laser active for Track1; power < 1% (even 0.3% should work)
   c. Track2 ‘Dendra2-red’: another detector with a detection range from 570nm to >700nm
   d. Set 561nm laser active for Track2; power < 1% (0.7% should work)
   e. Choose ‘MBS 488/561’ for both
3. ‘Acquisition’ Dialog (‘pro’-Mode): Set ‘scanspeed’ to ‘Max’ and switch ‘Direction’ to bidirectional ‘<->’
4. Find an appropriate value for the ‘Master Gain’ while scanning with ‘Fast’

Set up the photoconversion parameters [see Fig. 2]:

These parameters will vary depending on your particular cells/application. The following parameters work well for the conversion of freely diffusing Dendra2 in COS-cells.

1. If in use, switch off the attenuation of the 405nm-Laser (‘Laser’-Dialog)
2. Draw the ROI(s) for bleaching (i.e. photoconversion) using the tools in ‘Regions’
3. Define the number of pre-bleach scans: ‘Start Bleaching after # of scans’ = 10
4. Photoconversion setup using the ‘Bleaching’-Dialog:
   a. Lower the scan speed for conversion to a pixel dwell time > 10µs (e.g. speed 4)
   b. Set the laser power during bleaching to 100%
   c. Use 5 iterations of bleaching every 5 scans:
      i. ‘Repeat Bleach after # scans’ = 5
      ii. ‘Iterations’ = 5
5. Define the ‘Time Series’: set ‘Interval’ to 0.0 to obtain continous acquisition.
   The number of cycles should exceed the expected duration of the experiment. You can stop the acquisition then at any timepoint
Fig.2: ZEN 2008-Interface of the LSM 710; Settings for fast acquisition and photoconversion of Dendra2

**Sources of supply:**

**LSM 710:**
Carl Zeiss MicroImaging GmbH
Königsallee 9-21
37081 Göttingen
http://www.zeiss.de

**Dendra2:**
BioCat GmbH
Im Neuenheimer Feld 584
69120 Heidelberg
http://www.biocat.com

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