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

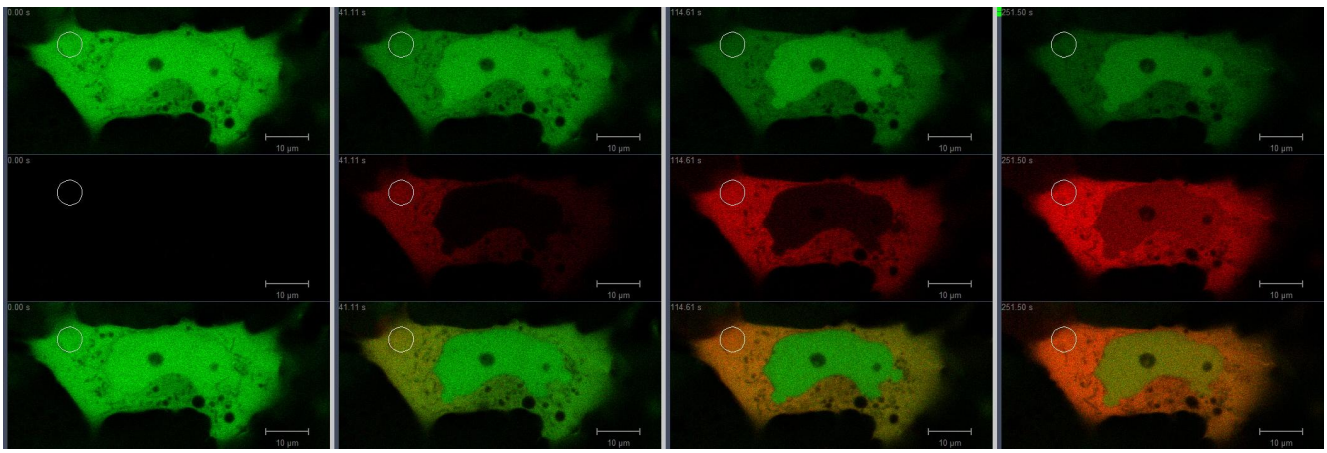

Fig.1: COS-cells expressing Dendra2; different points in time during photoconversion of cytoplasmatic Dendra2. Cells kindly provided by M. DIGEL, Medical University Hospital and Policlinic Heidelberg, Internal Med. IV

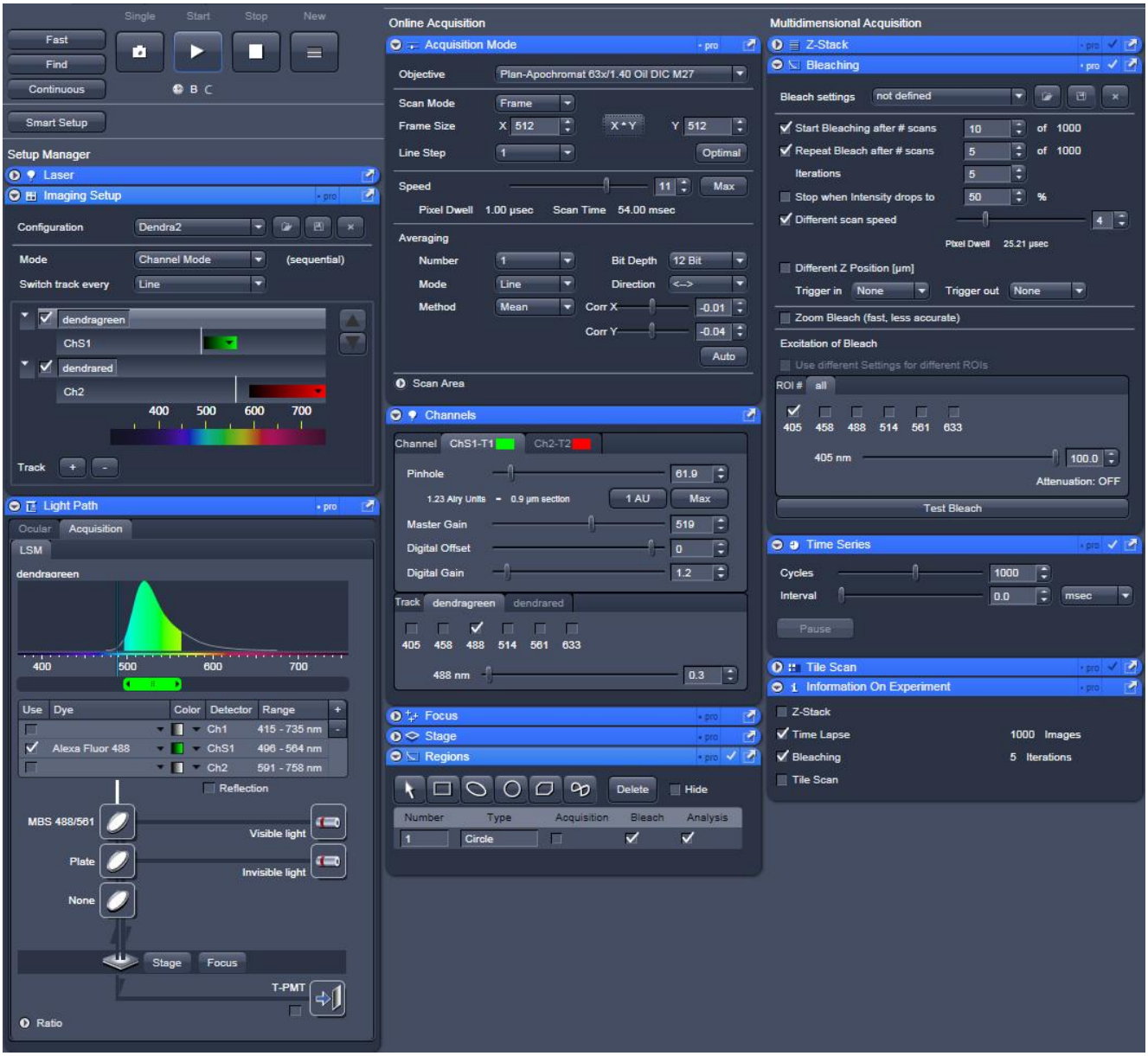

Fig.2: ZEN 2008-Interface of the LSM 710; Settings for fast acquisition and photoconversion of Dendra2

Sources of supply:

LSM 710:

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

We make it visible.

Dendra2:

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