The Zeiss confocal internal calibration is correct - the calibration factor is stored with every image.

The calibration scale has been applied using Zeiss freeware LSM Image Browser v4.2 [Overlay, Scale].

40x objective 2.5x zoom [512x512] 40x objective 1.5x zoom [512x512]

The internal Zeiss calibration is correct - this information is saved with every image you capture on the Zeiss LSM-510 confocal.

Use the Zeiss LMS Image Browser software to recover the calibration [um/pixel] data.

You can obtain the calibration factors using the i - Info button in Zeiss LMS Image Browser, see screenshot below.

Zeiss LSM Image Browser v4.2
Image scaling information obtained using the [i - Info button] - um/pixel & image size in um

This image is 2,048 x 2,048 pixels
X 142.86 X 0.07 um
Y 142.86 Y 0.07 um
This is the frame size in um This is the um/pixel calibration value

The internal Zeiss calibration is correct for [i.e. image X & Y length] all confocal objectives and any LSM optical zoom.

Note: The 0.07 value is a little inaccurate due to rounding up errors - I would advise dividing the image width in um (142.86um) by the image pixel width [2,048 pixels in this image]. Thus 142.86/2,048 = 0.0698 um/pixel, granted not far from 0.07 in this case.

The latest Zeiss LSM Image Browser can be downloaded from the Microscopy Core web pages.

Zeiss Image Browser is freeware and runs on any XP/2000 PC - use the Image Browser commands 'export' or 'copy' to convert images to TIFF/JPG.

Note that 'copy' will retain the original LUT [blue/green: cyan] for DAPI, whereas export will convert the DAPI LUT to the RGB blue channel.

LUT = Look Up Table: the colour overlay on the Zeiss B&W confocal images, e.g. DAPI/CY5 = blue, FITC/GFP = Green, TRITC/Rhodamine = red.

Dr Keith J Morris
Molecular Cytogenetics and Microscopy Core Tel: +44 (0)1865 287568
The Wellcome Trust Centre for Human Genetics, Roosevelt Drive, Oxford OX3 7BN, United Kingdom
Email: kjmorris@well.ox.ac.uk HomePage: http://www.well.ox.ac.uk/cytogenetics