# Template ZEISS optical microscopes

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# Introduction

This template explain what I think is important to report and how to report this information for images acquired with any of the ZEISS optical microscopes (stereo-, light, digital or confocal microscopes), using either ZEN core, ZEN blue or Smartzoom software packages.  
The template is meant as an **easy and quick way to report extensive information (metadata) about microscope images**.

This template is split into two parts:

1. What to report in the method section of a paper
2. How to report all necessary metadata together with the data

In the first part, I provide a “fill-in-the-blanks” text that can be pasted into the method section of a paper. In the second, I will show that extensive metadata can be reported and shared without any effort, when the data are shared in appropriate formats.

While this template is specifically targeted at images from the ZEISS light microscopes, I believe that it can be adapted quite easily for images acquired with other optical microscopes.

This template is available as a markdown file (this file) as well as a [DOCX file](/Guidelines/ZEISS_Optical-microscopes.docx).

# What and how to report

## Method section of a paper

I suggest to use the following text snippets. Parts in square brackets must be adjusted using the text within the brackets as examples or list to choose from. The rest of the text should of course also be adapted to the study.

Settings and their values can alternatively be presented as tables, either in the main text (recommended) or as supplementary material. The report(s), or parts of it (them), from the Shiny App [imaging-reports](https://github.com/ivan-paleo/imaging-reports) can be used for this.

### Widefield documentation

“[*Objects* or *Features*] were documented with a [*stereo-*, *light*, *digital* or *confocal*] microscope at the IMPALA, using the [objective(s) manufacturer and name(s) including nominal magnification(s) and numerical aperture(s)] and the [*Axiocam 105 color*, *Axiocam 305 color* or *built-in*] camera. [*EDF*, *stitched*, etc.] [*brightfield*, *darkfield*, *polarized*, etc.] images were acquired.”

### Confocal topography

“Height maps were acquired with an upright light microscope Axio Imager.Z2 Vario coupled to laser-scanning confocal microscope (LSCM) LSM 800 MAT (Carl Zeiss Microscopy GmbH). The system was turned on at least one hour before starting acquisition, so that all components were warmed up to limit thermic drift. The LSCM was equipped with an [objective(s) manufacturer and name(s) including nominal magnification(s) and numerical aperture(s)] objective. A violet laser (405 nm) was used for acquisition. Z-stacks were acquired with [e.g. *3000 x 3000*] pixels for [field of view, e.g. *255.6 x 255.6*] µm; the Z-step size was set to [e.g. *0.25*] µm and the pinhole to 1 Airy Unit.”

See <https://doi.org/10.1038/s41598-019-42713-w> for an example.

### Correlative microscopy

“The coordinate system was calibrated with the [objective(s) manufacturer and name(s) including nominal magnification(s) and numerical aperture(s)] objective on the [*digital microscope* and/or *light/confocal microscope*], and at 150x magnification on the scanning electron microscope.”

### Commons

“All data in original formats, together with their metadata (acquisition and analysis settings), can be found on Zenodo ([*DOI*]).”

Also add details about image processing: e.g. about EDF/stitching (widefield), topography application, and alignment (correlative microscopy). See recommendations in the repo’s [README](/README.md#processing).

## Data & Metadata

### General

The data should be uploaded on an open repository (e.g. Zenodo) in original formats to preserve the metadata as well as in open formats for reusability. Add a README file to the upload.  
Even though many settings are included in the files as metadata, some of these settings should also be listed in the main text (see [Method section of a paper](#method-section-of-a-paper)).

### Widefield images

* Upload the full-resolution, uncompressed images in CZI format (Zeiss’ original and open format). It is recommended to share the pre-processed (e.g. EDF, stitched) images because of the size of the raw images (especially z-stacks).
* Also upload the overview/preivew images showing the location of images on the object. Alternatively, share the CZI files of the images calibrated with Shuttle-and-Find (correlative microscopy).
* Specify in the README: “[*Raw* or *Pre-processed*] images were acquired with the software [*ZEN core*, *ZEN blue*, or *Smartzoom*] [version number] from Zeiss. All metadata (acquisition settings) are included in the CZI-files and can be retrieved using e.g. the Bio-Formats plugin for ImageJ/Fiji (<https://docs.openmicroscopy.org/bio-formats/5.8.2/users/imagej/installing.html>) or Zeiss ZEN software (<https://www.zeiss.com/microscopy/en/products/software/zeiss-zen.html>).”

### Topography & surface texture analysis data

* Upload MNT files of the surface texture analysis with Mountains.
* Alternatively or complementarily, upload the SUR files resulting from the topography analysis in ZEN.
* Export the MNT files to PDF and upload them too.
* Specify in the README: “Height maps were acquired with the software ZEN blue [version number] from Zeiss. Each surface has been processed in batch with a template in ConfoMap v. [8.2.10044] (a derivative of MountainsMap by Digital Surf, <https://www.digitalsurf.com/software-solutions/profilometry/>). The result of the analysis on each surface is saved in MNT format (including all original and processed surfaces, their metadata, as well as results) and exported to a PDF file. Ultimately, the results are collated into CSV file(s).”

See <https://doi.org/10.5281/zenodo.6645445> for an example (although the information was added as a description rather than a README file).

### Correlative microscopy

* Upload ZEN Connect project(s) (project-name.a5proj).
* Upload images as CZI (all microscopes, see [Widefield images](#widefield-images)) and/or TIF (SEM only, see [SEM images](/Guidelines/ZEISS_EVO25.md#sem-images)), ideally in the ZEN connect data folder (project-name\_data).
* Optionally, upload the image export(s) as PNG.
* Specify in the README: “ZEN Connect projects were created with Zeiss’ ZEN desk v. [*3.5*] (blue edition, <https://www.zeiss.com/microscopy/en/products/software/zeiss-zen-desk.html>) software with the module ZEN Connect (<https://www.zeiss.com/microscopy/en/products/software/zeiss-zen/zen-connect-toolkit.html>).”