SIM Specimen Preparation Guide

Coverslips:

Only #1.5 coverslips (170 µm) should be used.

Mounting media:

The choice of mounting medium can severely affect the imaging results.

- We recommend as mounting medium the non-setting form of VectaShield: **VectaShield H-1000.**
- Avoid mounting media that solidify (e.g., hard-setting VectaShield, Mowiol® 488 and ProLong Gold), as these media tend to shrink and flatten the sample, resulting in a loss of volume information. Also avoid Citifluor or DAKO mounting media.
- Avoid mounting media that contain DAPI. This creates background in the image that can easily be avoided by staining for DAPI during the IF labelling and washing off.
- Other mounting media can be used, these include:
  - 90 % glycerol with some anti-fade agent
  - Murray Antifade Mounting Media
  - nPG (n-propyl gallate) Antifade Mounting Media
  - PPD (P-phenylenediamine) Antifade Mounting Media
  - Antifade 1: 1,4-phenylene-diamine
  - DABCO (1,4 diazobizyclo[2,2,2]octane) Antifade

The specimen:

- SIM works best close to the coverslip and best results are typically within the first 18 µm. Imaging deeper is possible but after 25 µm image quality deteriorates rapidly. Imaging in tissue usually gives poor results unless the tissue has been thoroughly cleared.
- Imaging plated cells and bacteria often yields great results. With good labelling and bright secondaries (e.g. Alexa Fluor 488, 568, 647), one should expect excellent images.
- Imaging in transparent organisms has been successful but more challenging. SIM in *C. elegans* has given good results.

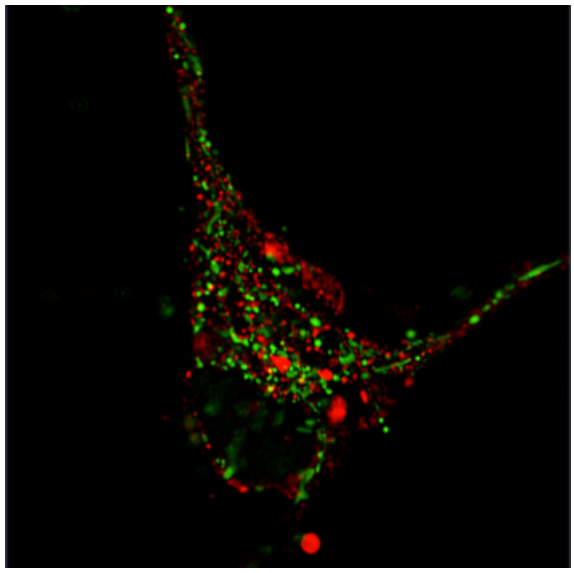

Figure 1. Endoplasmic reticulum (green) and mitochondria (red), two important organelles regulating cellular life and death. A. Grimm (Goetz Lab)