4PI MICROSCOPY OF NUCLEAR STRUCTURES

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Many new techniques are developed to reach higher resolutions than the limitations predicted by Ernst Abbe. To reach these higher resolutions different tricks are used. The 4Pi microscope invented and developed by Stefan Hell [1], is one of the very few techniques that increases the resolution in the axial (Z) direction instead of the lateral (XY) direction. The resolution of the 4Pi microscope is in our biological samples about 120nm. This resolution is reached by sending the excitation light in 2 parallel beams trough two opposing lenses. The sample that can have a thickness of about 50 µm is fitted between 2 cover slips between these two lenses.

The incredible high axial resolution makes this microscope ideal for the studies of small cellular structures that are lying on top of each other, which is often the case in nuclear structures.

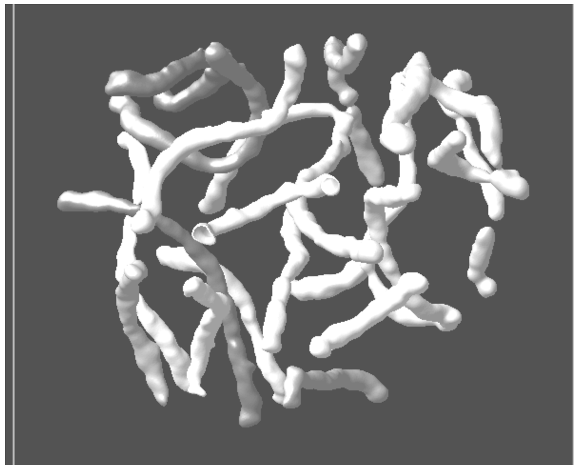

Figure 1. 3D XZ surface rendered SYCP3 axis in a mouse spermatocytes imaged with 4Pi

During meiosis the number of chromosomes is halved during the gamete formation. and parts of the paternal and maternal chromotids are exchanged. In the spermatocytes of mice (and humans) the paternal and maternal chromosomes are paired and aligned along the synaptonemal complex [2]. After this alignment breaks are made in both the paternal and maternal chromatid and parts of the DNA between the breaks are exchanged. Very little is known about the processes that control the alignment, the formation of the cross-overs and the exchange of the DNA. One of the problems is the difficulty to culture and transfect spermatocytes with fluorescent proteins which limits our studies to fixed samples.

Testicular seminiferous tubules were dissected cut into small pieces and put into culture for one or 2 days. After culture the samples were fixed with anti-SYCP3-alexa488 and propidium iodide. 4Pi-imaging was used to visualize the SYCP3-axis. With the confocal microscope we were not able to separate the individual synaptonemal axis.

[1] Hell SW, Schrader M and van der Voort HT, Far-field fluorescence microscopy with three-dimensional resolution in the 100-nm range, *J Microsc,* 187, 1-7 (1997).
[2] Zickler, D, From early homologue recognition to synaptonemal complex formation, *Chromosoma* 115, 158–174 (2006).