Nanopositioning of Microscopy Components for DNA-Origami-, DNA-Paint- and TIRF techniques

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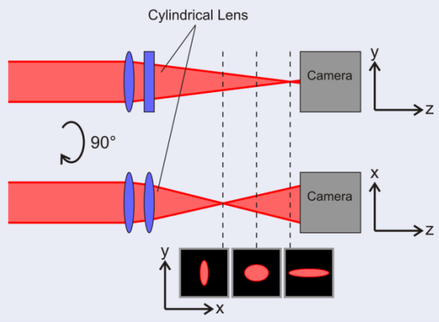
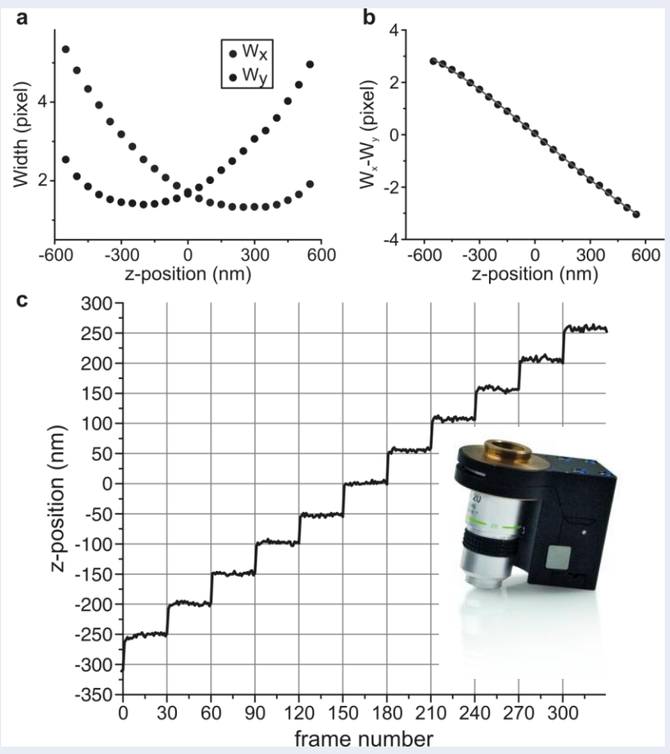
Calibration of astigmatism based 3D localization microscopy with a PIFOC

- Super-resolution by transient localization of stochastically blinking or transient binding fluorescent molecules
- z-depending PSF shape due to a cylindrical lens in the detection beam path
- 3D localization by the comparison of the PSF width in x and y

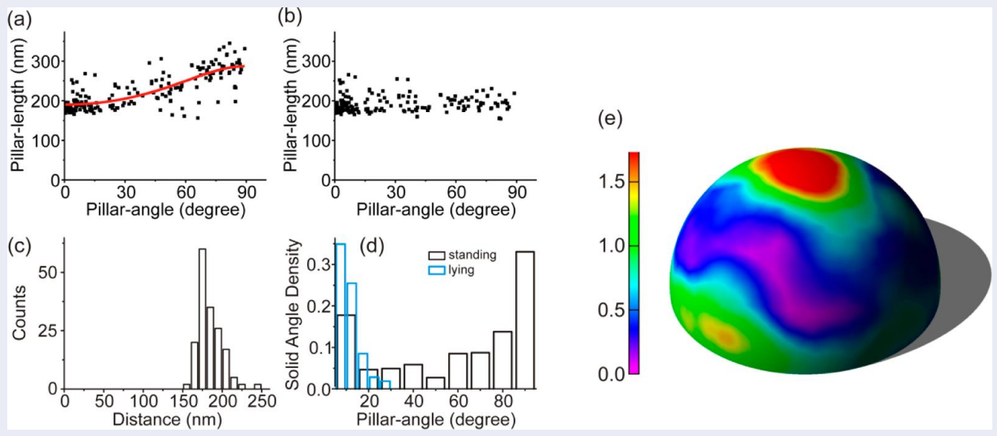




Calibration

- Measured width of the PSF in x- and y direction generated by a bead depending on the z-position of the objective (a)
- Difference of W_x and W_y fitted with a polynomial function (b)
- Test of this localization calibration with a fluorescent bead by moving the objective in regular steps of 50nm in z-direction every 30 frames with an objective PIFOC P-725.4CD from PI (c)

Results of 3D super-resolution measurements with GATTAquant nanorulers



- Correlation between measured pillar length and pillar angle with respect to the surface. Because of the refractive index mismatch, the nanopillar length appears to correlate with the z-component. The fit describes a theoretical relation assuming a constant magnification factor for the z-direction (a)
- For z-magnification corrected distribution (b)
- Histogram of measured distances between the two dyes on the nanopillar corrected for the refractive index mismatch between glass and solution (c)
- Angular distribution of DNA origami nanopillars presented as solid angle density (occurrence normalized by 1/cos(Θ)) (d)
- Solid angle distribution of the nanopillar tips with the bottom of the nanopillar placed in the center of the sphere. The scale is normalized to the density of a uniform distribution (e)
- The recording of the calibration curve and the test of this localization calibration had been done with an objective PIFOC P-725.4CD from PI but could have been done with a P-737 z-stage positioner PIFOC from PI, too.

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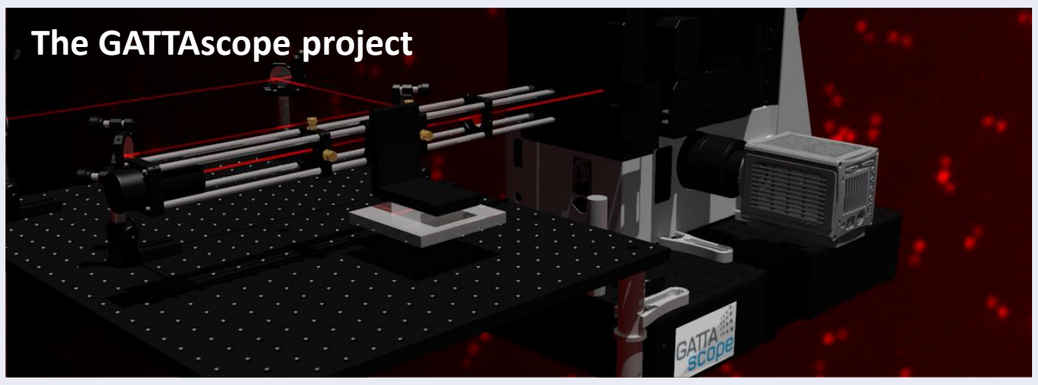
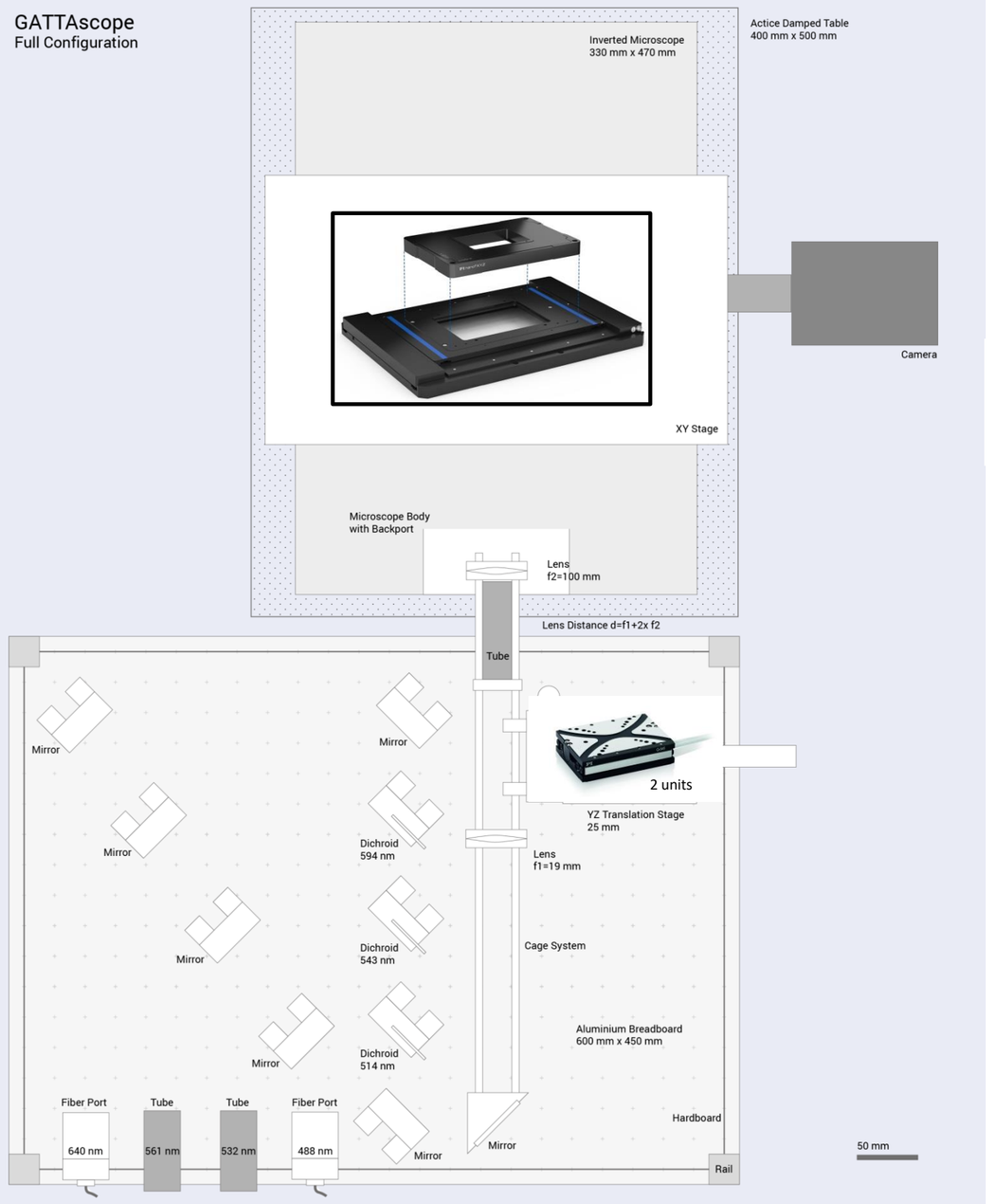
The GATTAscope project



The GATTAscope detects fluorophores on the single molecule level to produce super-resolution microscopy results by methods as TIRF and dSTORM. For these techniques positioning precisions for specimen and optical elements in the range of a few nanometer are mandatory.

For these tasks piezo-based nano positioning solutions from Physik Instrumente (PI) can be used: objective PIFOC P-725 for objective positioning up to 400µm range, specimen z-positioning PIFOC stage P-737 with z-travel range up to 500µm, PILine x-y piezo ultrasound stages U-780.DOS for coarse specimen positioning ±250nm and on top PInano x-y piezo-flexure stages P-545.3R8S for fine specimen positioning ±5nm at 200µmx200µmx200µm ranges and for the TIRF method the 2 axis laser coupling unit with 2pcs Q-545 Q-Motion linear drives with precision of ±18nm at a range of 26mm per axis.



http://gattascope.com/

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