

# Shearing interferometry and structured illumination for the depth-resolved detection of fluorescent light

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## Motivation

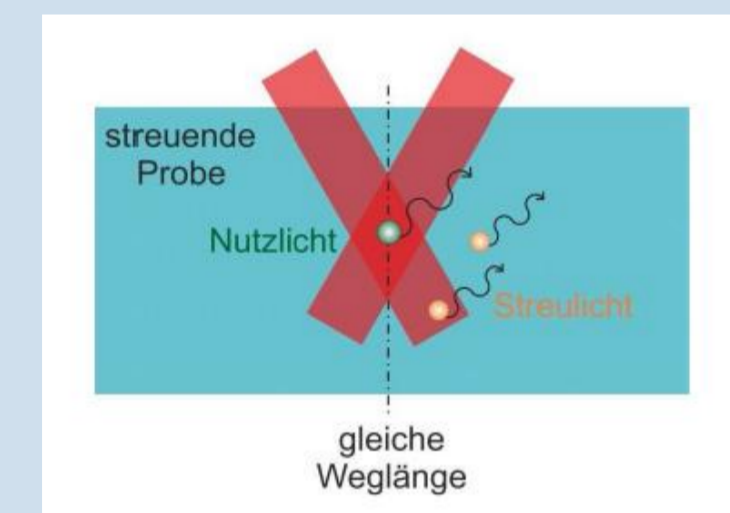
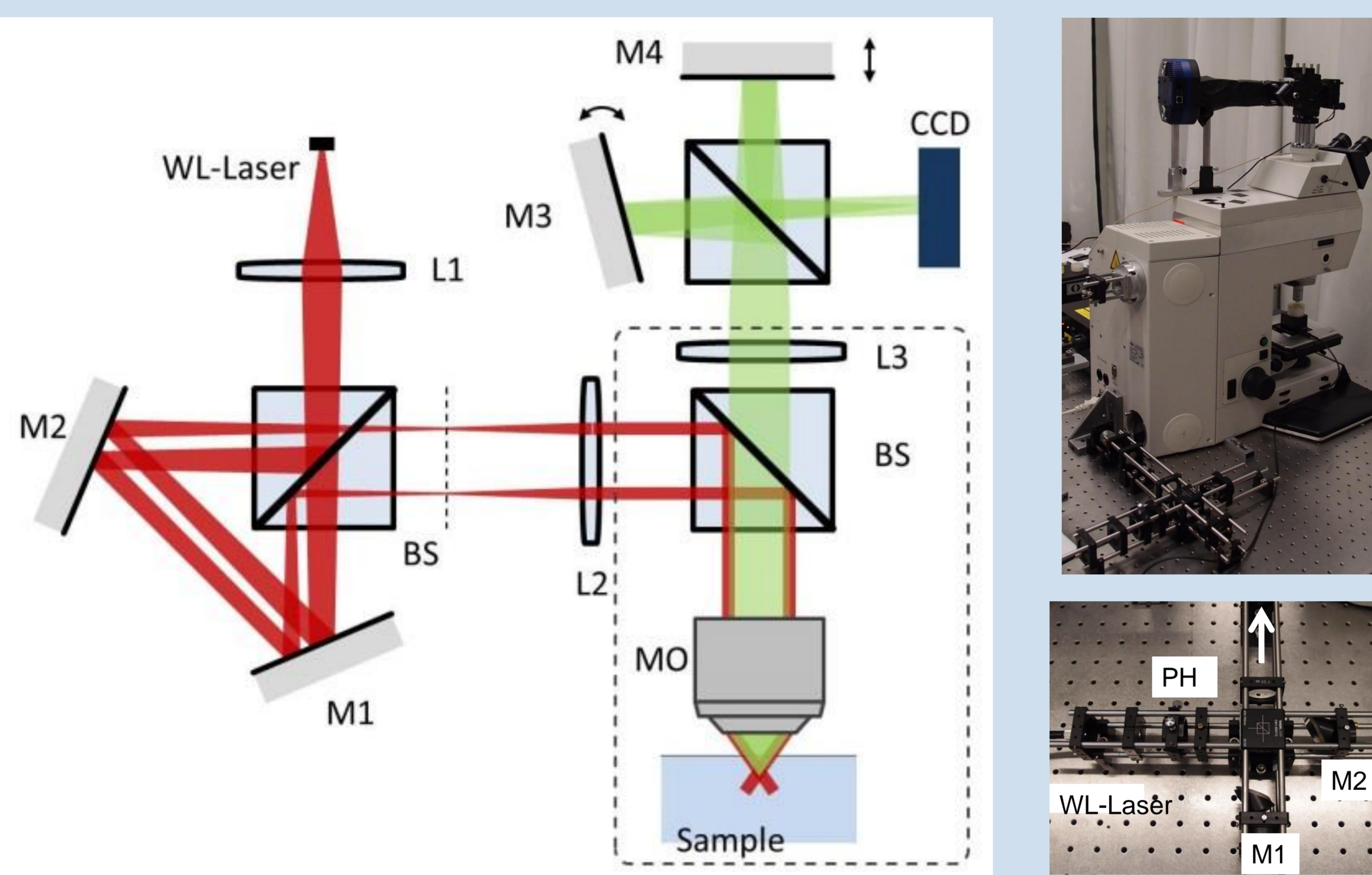
**Context:** Non-invasive methods for imaging in scattering media for e.g. in-vivo diagnostics of skin cancer

### State of the art:

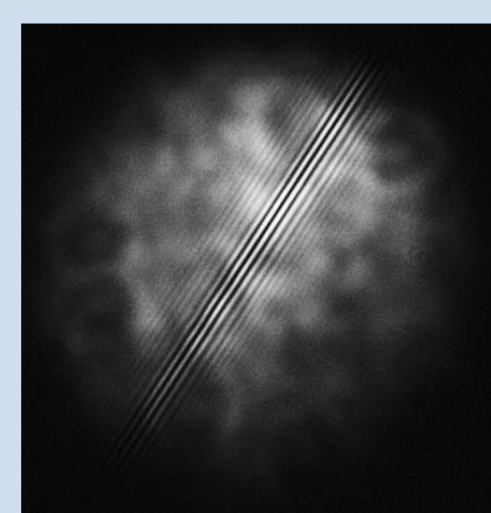
1. Optical Coherence Tomography (OCT) is established for imaging in biological tissues, but cannot be combined with fluorescent light
2. The use of fluorescent marker materials allows the separation of measurement signal and scattering background by appropriate bandpass filters
3. Confocal fluorescence microscopy allows depth-resolved imaging, but without interferometric depth detection

**Goal:** Development of a microscopy technique for the depth-resolved detection of fluorescent light in scattering media with high axial resolution and penetration depth

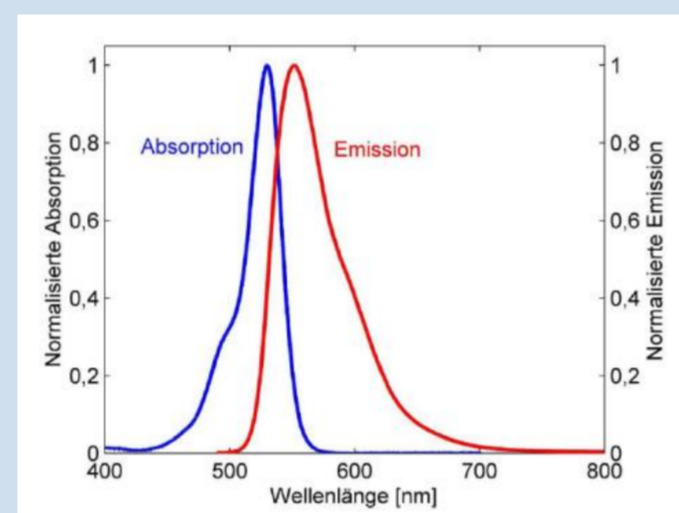
## Experimental setup



Coarse restriction of excitation



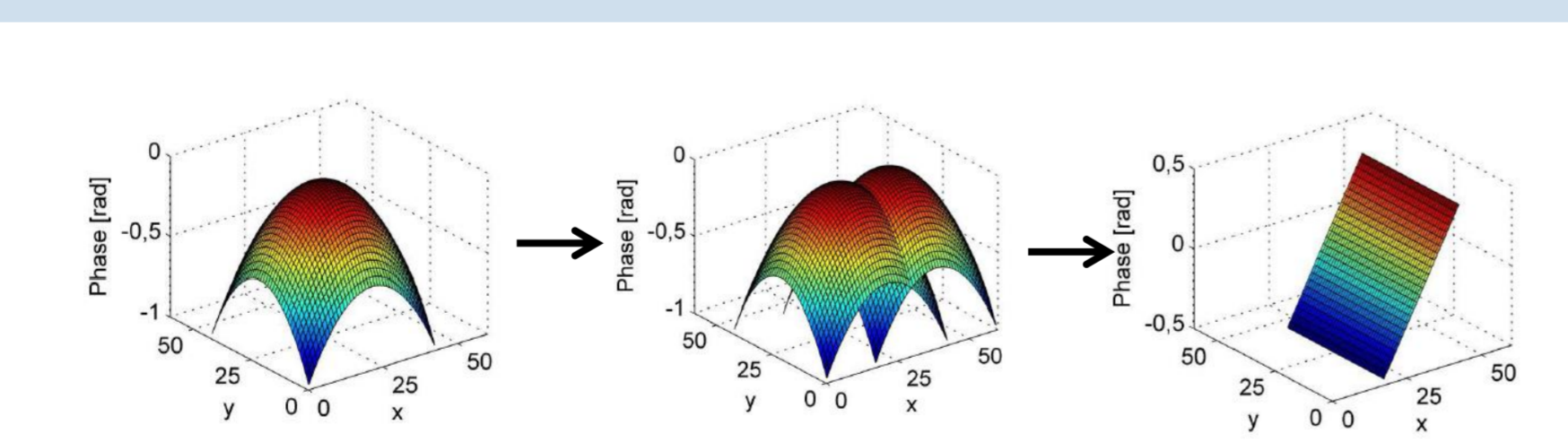
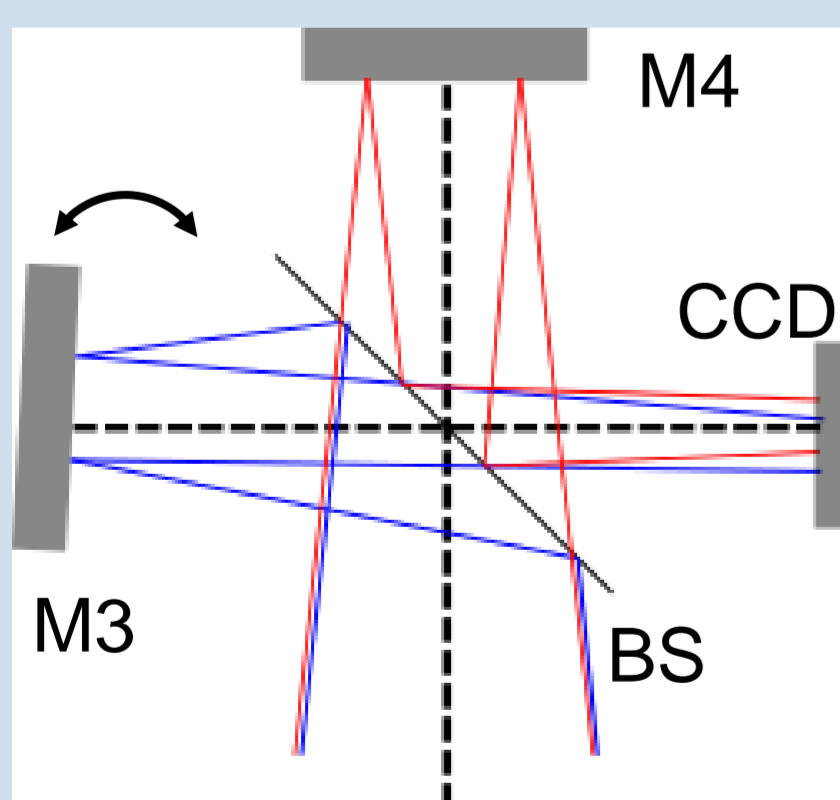
White-light interferogram of structured illumination



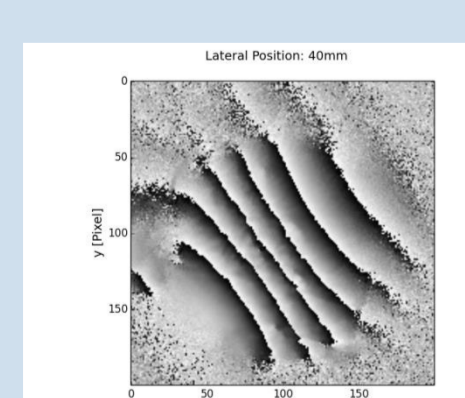
Absorption and emission spectrum of Rhodamin 6G [2]

- Fluorescence microscope in epi-illumination, fluorophor: Rhodamin 6G
- Structured illumination with broadband laser source:  
**Locally addressed fluorescence excitation**
- Realization: Sagnac interferometer (common path)
- Precise depth detection: lateral shearing in Michelson interferometer

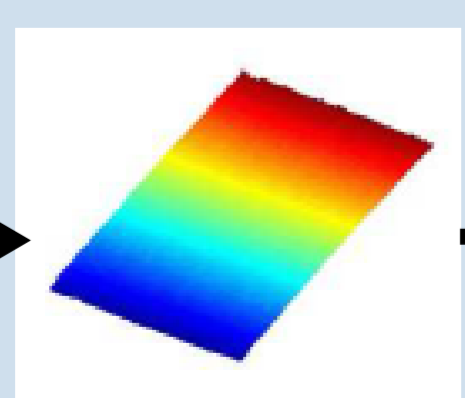
## Depth reconstruction: Shearing interferometry



- Object wave as self-reference
- Low requirement of temporal coherence
- No requirement of spatial coherence



Unwrapping



Wave front reconstruction



Zernike-Fit

$$D = \frac{1}{4c_0^2 + 2\sqrt{(c_2^2)^2 + (c_2^-)^2}}$$

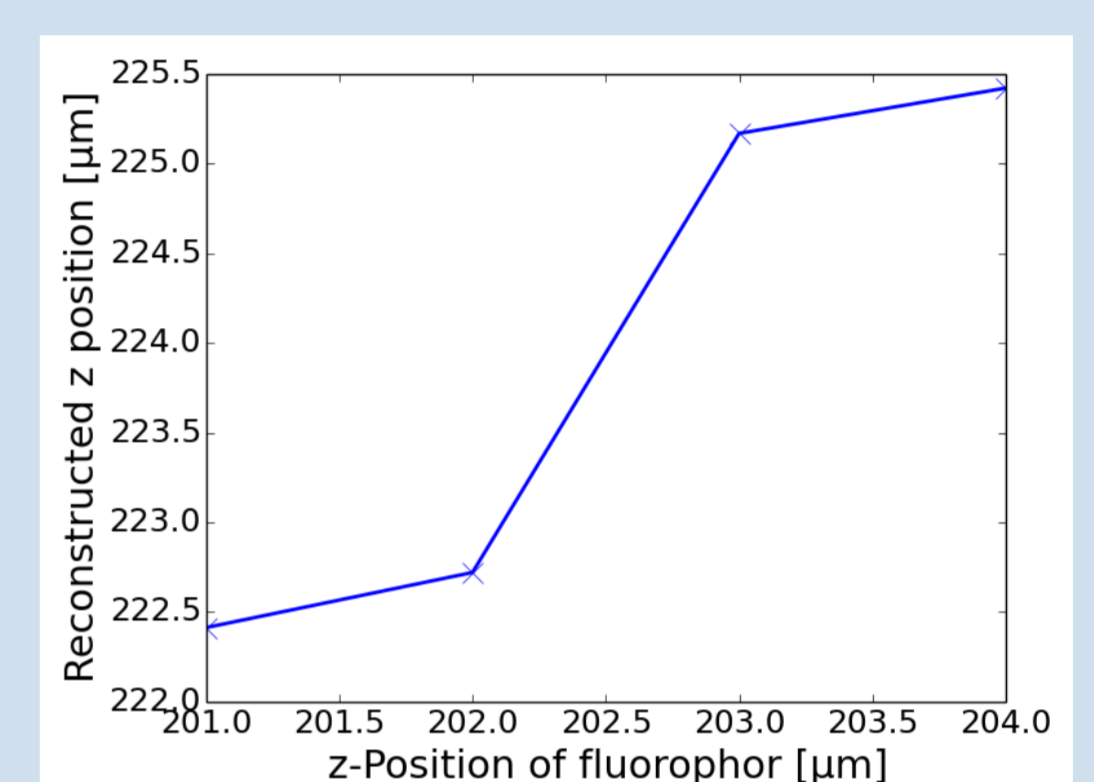
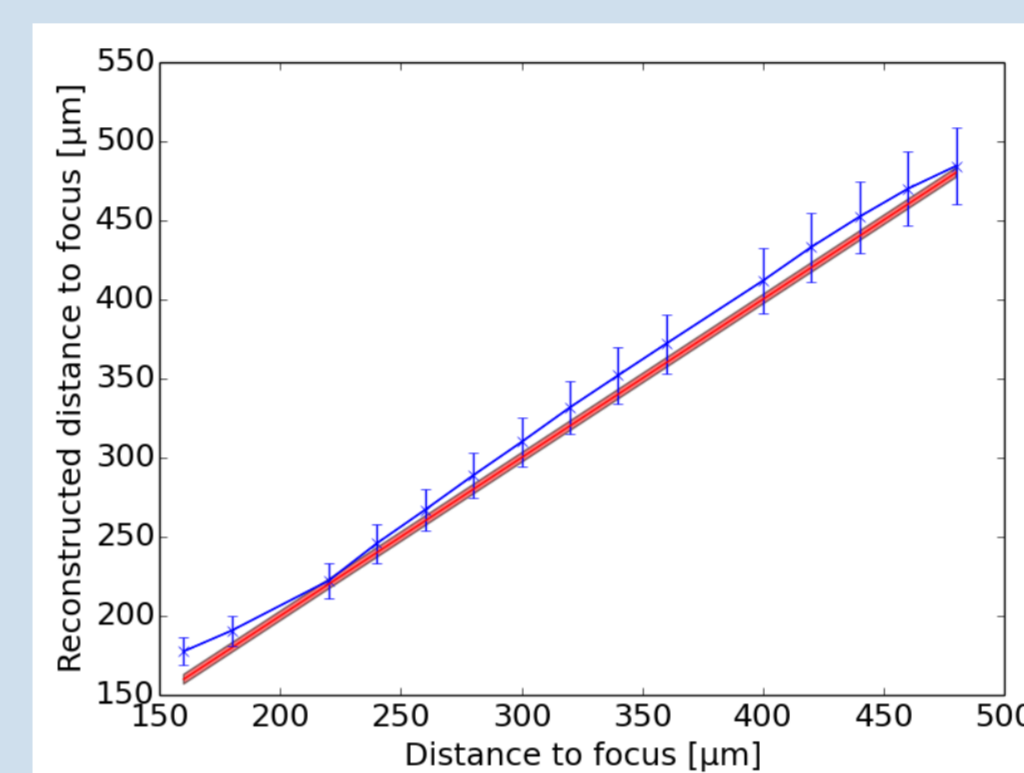
**Measurement principle:** Curvature of wave front is directly related to distance from focus

## References

- [1] Schindler et al., „Resolving the depth of fluorescent light by structured illumination and shearing interferometry“, *Proc. SPIE* 9718, Quantitative Phase Imaging II, 97182H, 2016
- [2] Kubin and Fletcher, „Fluorescence quantum yields of some rhodamine dyes,“ *Journal of Luminescence*, 27, Nr. 4, pp. 455-462, 1983
- [3] Krauter et al. „Optical phantoms with adjustable subdiffusive scattering parameters“, *J. Biomed. Optics*, 2015, 20, 105008

## Validation

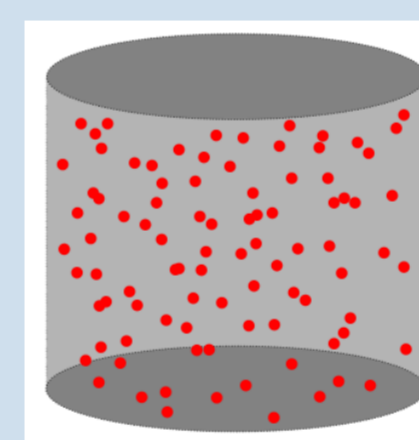
Specimen: Rhodamin 6G crystals on sample holder



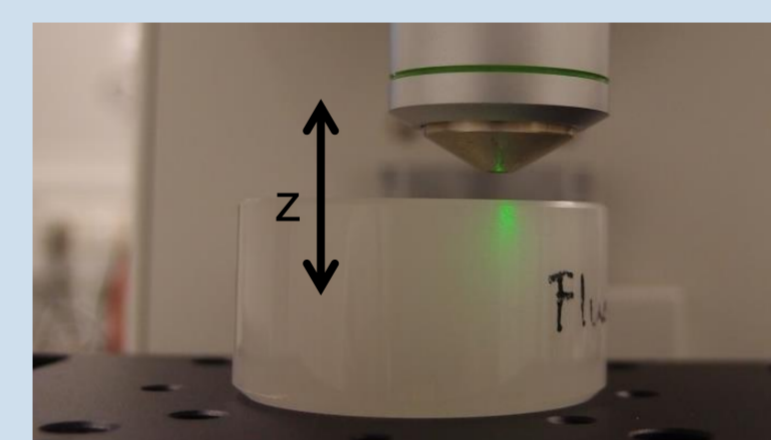
- Depth reconstruction: Measurement range of several hundred microns with deviations from nominal values of less than 10 %.
- Axial resolution: displacements in the range of 1 µm are resolved.

## Results: Phantom measurements

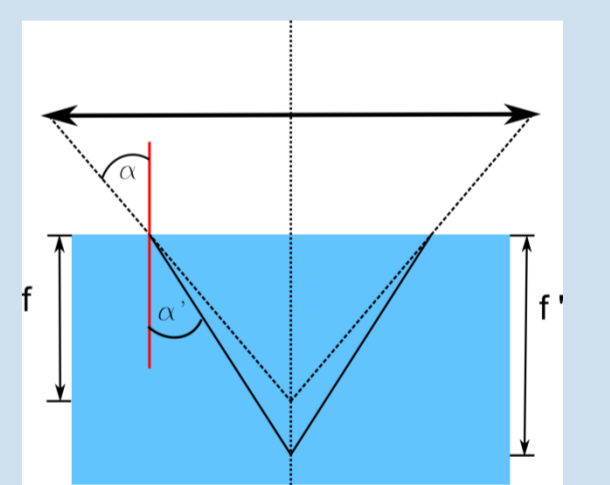
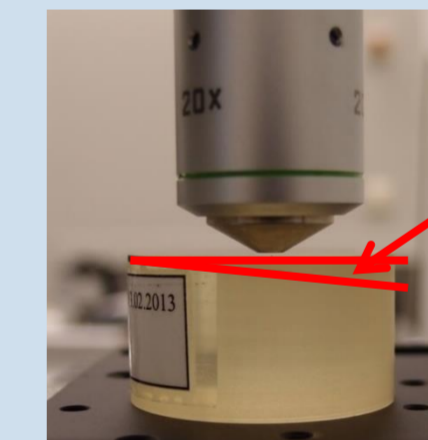
- Phantoms with characterized refractive index, absorption and scattering
- Scattering coefficient: 2/mm [3]



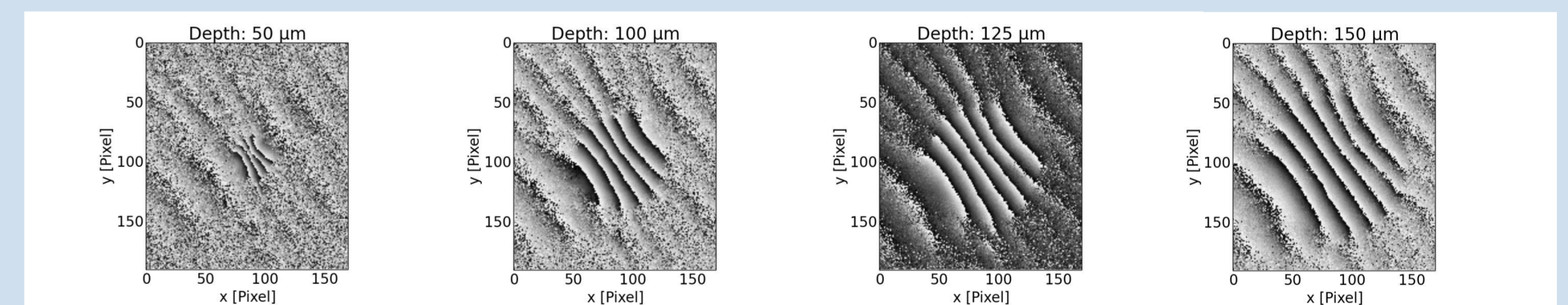
Phantom with isolated fluorescent inclusions



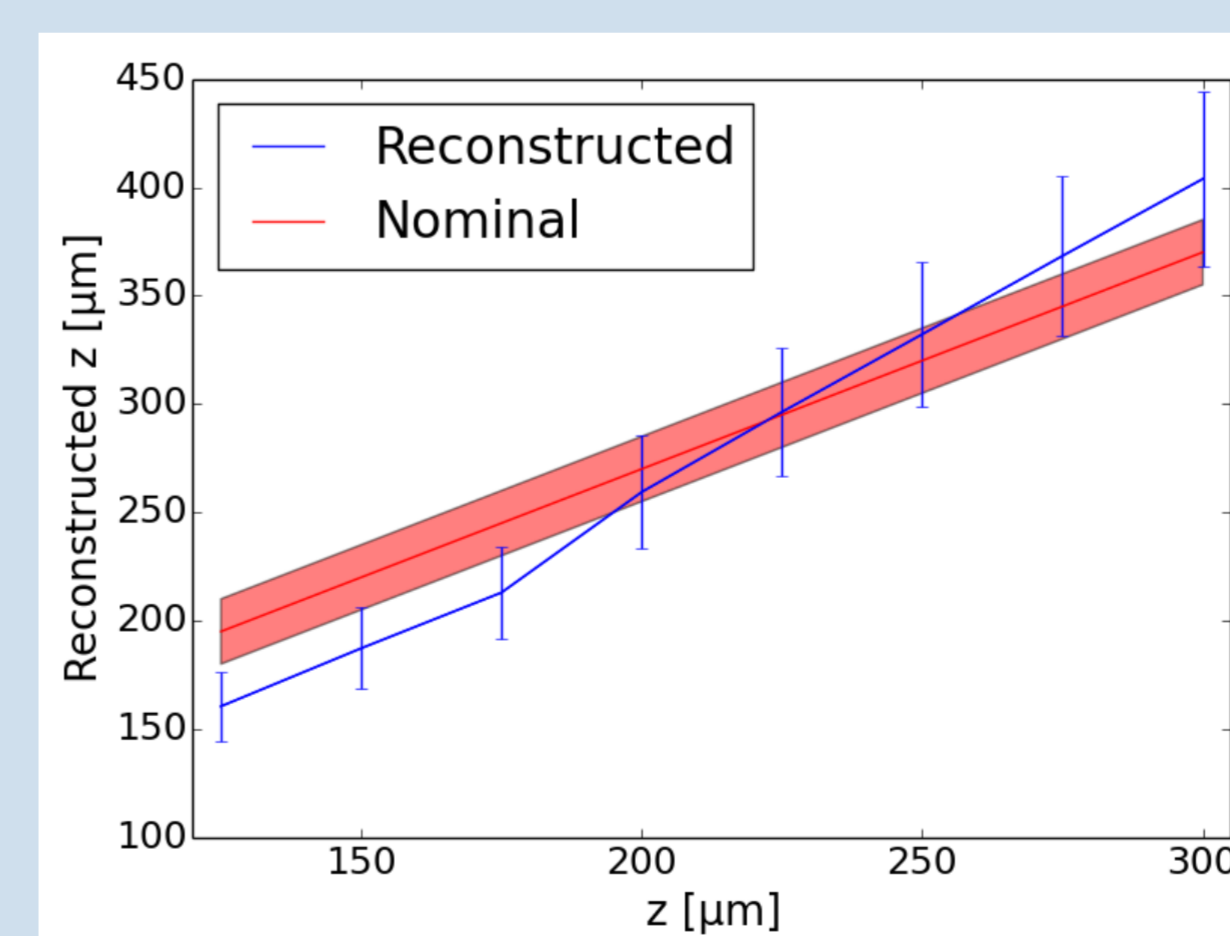
Extended layer of fluorescent material (200 µm thickness)



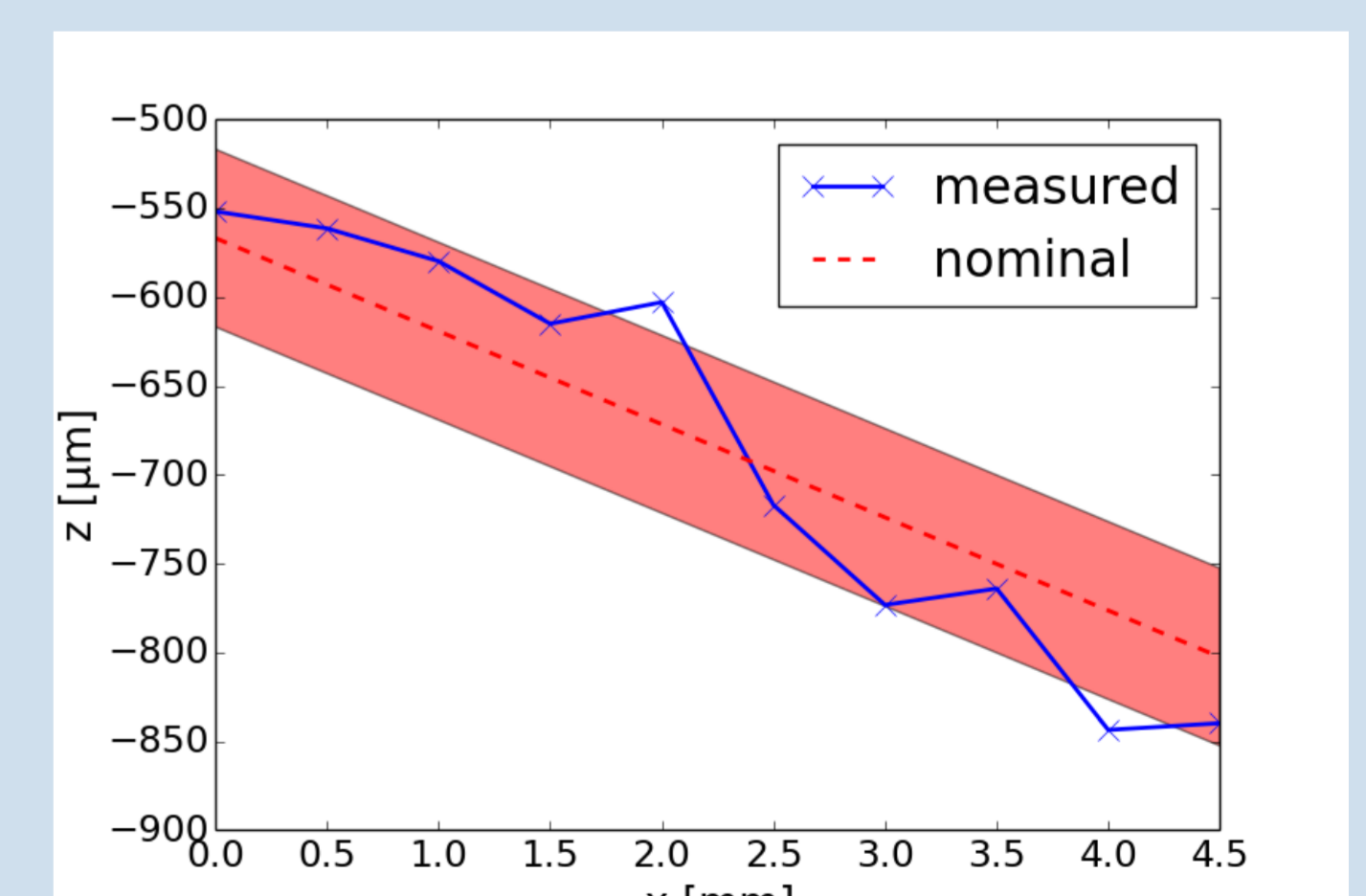
Shift of focus position



Raw signals of particles in 110 µm depth and different distances to focus



Depth reconstruction: isolated fluorophors in scattering medium



Depth reconstruction: fluorescent layer in weakly scattering phantom

## Conclusion

- Demonstration of interferometric depth detection for fluorescent sources
- Penetration depth: Several hundred microns for scattering coefficient of 2/mm with axial resolution in the range of 1 µm

## Acknowledgements

We thank our colleagues at the Institut für Lasertechnologien in der Medizin und Messtechnik at the University of Ulm for the productions and characterization of the phantoms and the Baden-Württemberg Stiftung for financing the project "FluoTis".