LINE-SCANNING OPTICAL TOMOGRAPHIC MICROSCOPE

Laszlo Dudas[1], Miklos Erdelyi[1,2,3], Jozsef Sinko[1], Gabor Gajdatsy[4] and Gabor Szabo[1]

[1.] Department of Optics and Quantum Electronics, University of Szeged, Szeged, Dom ter 9, Hungary

[2.] Analytical Science Division, National Physical Laboratory, Teddington, Middlesex, TW11 0LW, UK

[3.] Department of Chemical Engineering and Biotechnology, University of Cambridge, New Museums Site, Pembroke Street, Cambridge CB2 3RA, UK

[4.] Furukawa Electric Institute of Technology, 1158, Vasgolyo u 2-4. Budapest, Hungary

Introduction

Optical tomography methods apply a special illumination and image formation geometry and reconstruct the image by means of computer algorithms. Optical projection tomography works in the same way as computed tomography, but a laser beam is applied instead of an X-ray. The sample is illuminated and scanned with a laser beam, and longitudinal projections are captured by a CCD detector. Projections are captured from different directions and the final image is reconstructed with a computer tomography algorithm. The line-scanning tomographic optical microscope (LSTOM) applies a diffraction limited line as illumination and performs tomographic data acquisition.

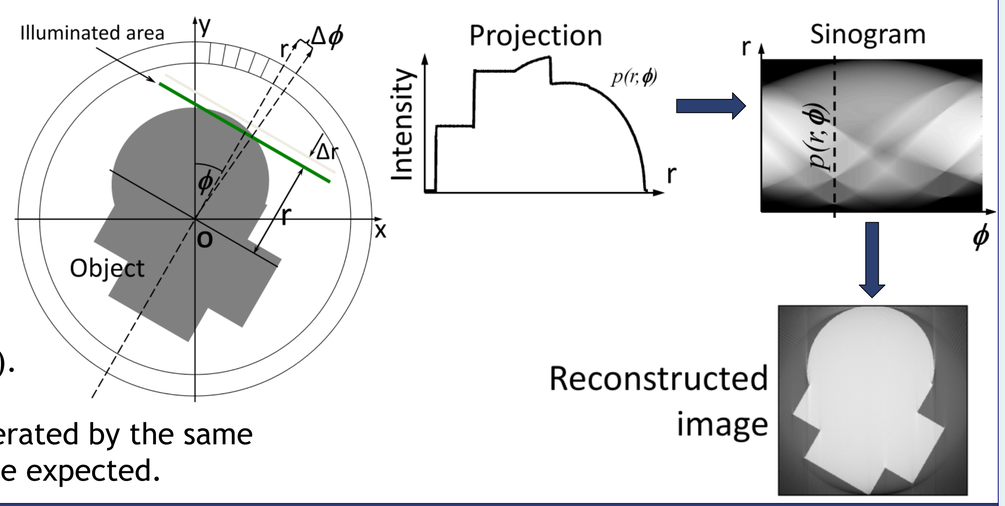
Theoretical background

A line-scanning tomographic optical microscope (LSTOM) makes the resulting transfer function of the imaging system isotropic. In this method:

•The sample is scanned by a diffraction limited line, and the reflected total intensity is detected. One such scan is equivalent to a **transverse projection** in tomography.

•Projections are captured from different directions by rotating either the sample or the illumination line. The stack of the projections captured in this way is referred to as a **sinogram**.

•The final image is reconstructed with a **computer tomography algorithm** (Filtered Back Projection, FBP).



Theoretically, the line spread function (LSF) is ***~15% narrower*** than the point spread function (PSF) generated by the same objective (numerical aperture (NA), wavelength); therefore, even a slight ***resolution enhancement*** can be expected.

Experimental alignments and results

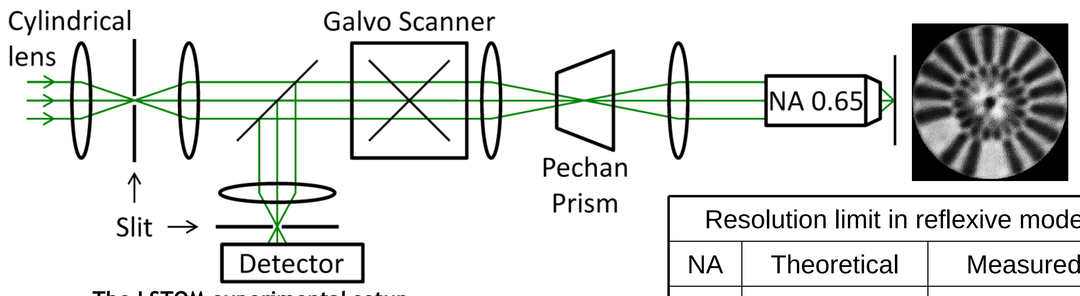
To prove the resolution enhancement, the LSF was measured by knife edge method and the Full Width at Half Maximum (FWHM) was compared to the theoretical value. The suitability for image processing was tested by means of Richardson Star Pattern (RSP). The imaging performance of LSTOM system in fluorescent mode was studied on the Convallaria Majalis test sample provided by Ziess Inc. and the resolution limit estimated using Fluorescent Microspheres. Two experimental alignments have been presented.

Line-scanning tomographic optical microscope

•The imaging system is illuminated with cylindrical wave generated by the cylindrical lens and spatially filtered by a slit.

•The scanning was accomplished by an X/Y galvo scanner, the rotation of the beam was performed by a Pechan prism, mounted on a rotation stage.

•The axis of the prism and the rotation axis of the stage could be aligned parallel to the optical axis of the system. The remaining inaccuracy of this alignment introduced a motion error, which was corrected by a mapping procedure.

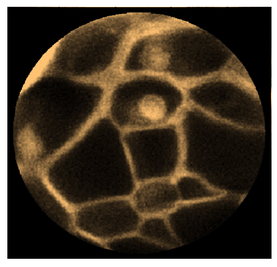


The LSTOM experimental setup

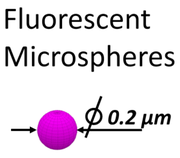
Resolution limit in reflexive mode		
NA	Theoretical	Measured
0.65	0.409 µm	0.412 µm

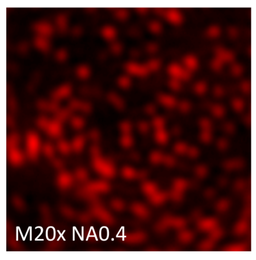
•The detection was carried out in confocal mode.

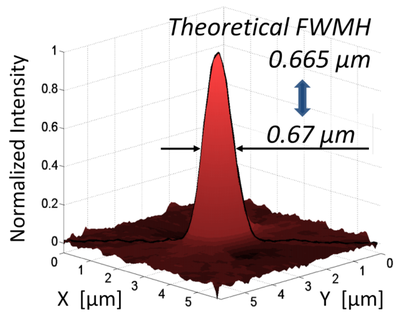
Convallaria Majalis



Fluorescent Microspheres



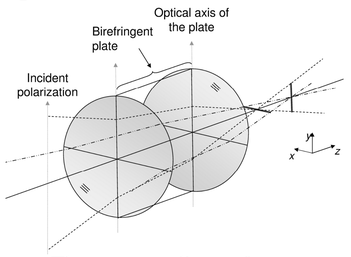




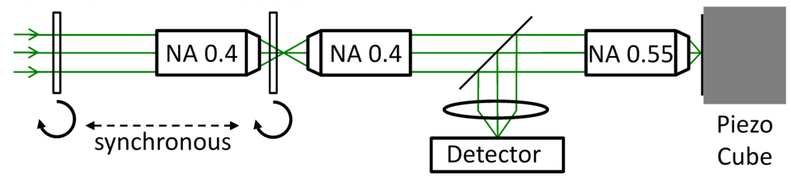
Astigmatic line-scanning tomographic optical microscope

•The rotation of the illumination line with high precision (and therefore the mapping procedure is avoidable) was carried out by means of translation invariant optical elements (polarizer and birefringent crystal plate, optical axis directed parallel to the surface)

•By the birefringence caused astigmatism during focusing through the plate generates two lines in the extraordinary focus.

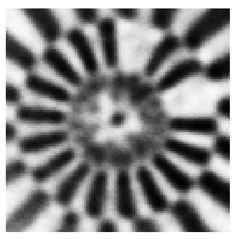


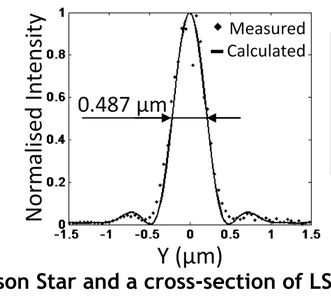
The extraordinary focus



The aLSTOM experimental setup

•One of these lines was used as illumination line in a LSTOM system. The rotation of the line can be achieved by rotation of the polarizer and the birefringent plate, synchronously. During the scanning process the pattern was moved perpendicular to the actual position of scanning line.





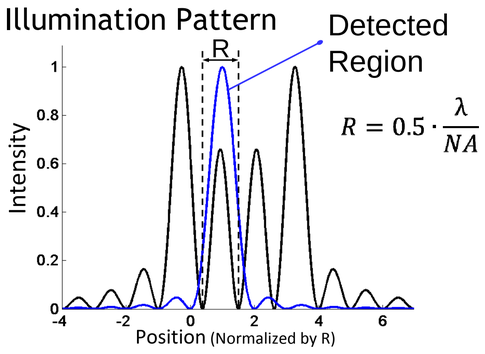
Reconstructed Richardson Star and a cross-section of LSF

NA	Theoretical FWHM	Measured FWHM
0.55	0.487 µm	0.487 µm

Line-scanning tomographic optical microscope using Phase manipulation

Illumination applied in LSTOM

Cross Section of the Illumination Pattern

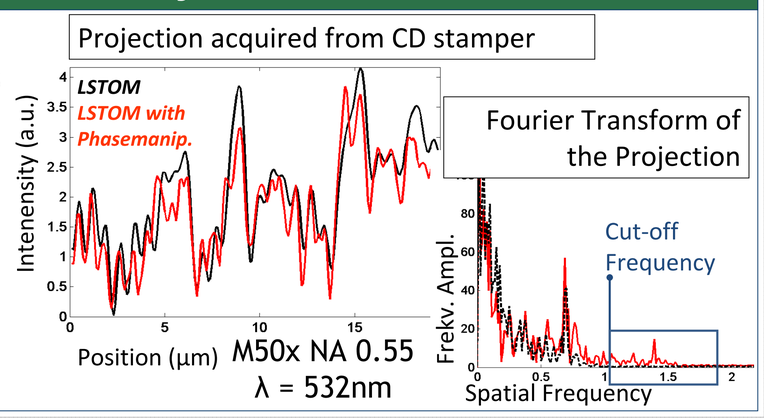


The illumination pattern is generated using phase manipulation.
The 2nd and 3rd peak is 35% narrower than the non-manipulated scanning line.
The region, illuminated by the 2nd peak, is detected, using confocal detection.

CCD image of the Illumination Pattern

Preliminary Results

Single projection was acquired using CD stamper as sample.
The projection shows finer details if phase manipulation is applied. Higher frequency components are emerging.
The blue line shows the cut-off frequency of the objective.



References

[1] G. Gajdátsy, L. Dudás, M. Erdélyi, G. Szabó, Line-scanning tomographic optical microscope with isotropic transfer function, *Journal of Optics* **12**(11) 115505 (2010).

[2] J. Sinkó, L. Dudás, G. Gajdátsy, M. Erdélyi, G. Szabó, Map-free line-scanning tomographic optical microscope, *Optics Letters*, **36** (20), 4011-4013 (2011)

Information

If you have any question, please contact Miklos Erdelyi: meerdelyi@gmail.com

Acknowledgment

The project has been supported by the Hungarian Scientific Research Fund (OTKA-NKTH CNK 78549).

The project is supported by the European Union and co-funded by the European Social Fund. Project title: “Broadening the knowledge base and supporting the long term professional sustainability of the Research University Centre of Excellence at the University of Szeged by ensuring the rising generation of excellent scientists.”

Project number: TÁMOP-4.2.2/B-10/1-2010-0012