Integrated beam splitters for parallel microscopy in life science

D. Wohlfeld, E. Slogsnat, K.-H. Brenner

Chair for optoelectronics, ziti, Universität Heidelberg

mailto:denis.wohlfeld@ziti.uni-heidelberg.de

Parallel microscopy is currently investigated to speed up high content screens in life science using wield field microscopy. The parallelism can be further increased by miniaturization using micro lens or GRIN-rod arrays. We present approaches for miniaturized parallel microscopy and introduce an integrated beam splitter and describe its fabrication technology.

1 Introduction

High-throughput fluorescence-microscopy is used by biologists to define and model gene functions on a genome-wide scale [1]. Besides a high spatial resolution, the imaging speed, especially in time-lapse imaging [2], is an important factor.

A significant increase in scan speed can be achieved by parallelization. The parallelism of microscopy is limited by the size of one imaging system and the ratio between lens diameter (D) and field of view (FoV) of the objectives. Thus miniaturization offers the possibility of a higher degree of parallelism. Furthermore, the aberrations scale down with the lens size [4].

In the following section parallel microscopy is investigated and macro objectives, micro lenses and GRIN-rod lens systems are compared. The latter will be further described in the third section. Since in fluorescence microscopy the excitation light has to be separated from the imaging light, a beam splitter is necessary. Therefore section 4 deals with the fabrication and replication of integrated beam splitters.

2 Parallel microscopy

It is clear that the speed of microscopy can be increased by reducing the number of mechanical scans over the substrate. If we assume, that the mechanical scans occur along the y-axis, we distinguish between a parallelization in the x-direction and in the y-direction. For the beginning, we concentrate on a parallelization in the x-direction, since the y-direction requires only a replication of the hardware.

An important factor for parallel microscopy is the ratio between FoV and D. The maximum degree of parallelism can be achieved ideally, if the ratio of FoV/D is one. The FoV is limited geometrically by the desired magnification and in quality by aberrations of the lens system. Table 1 shows a comparison of a classic fluorescence objective, an approach with micro lens arrays (one freeform and one planar surface) and a GRIN-rod system.

	Classic parallel	Micro lens system	GRIN-rod lens system
Magnification	10	6.1	4.6
NA	0.4	0.35	0.35
Object Resolution	~ 1 µm	~ 1 µm	~ 1 µm
Chromatic aberration correction	yes	no	no
Lens diameter	28 mm	1.2 mm	2 mm
Field of View	2600 µm	120 µm	400 µm
Scans for total plane	12	10	5
Scans to cover spots	**12**	**8**	**3**

***Tab. 1** Comparison of classic and miniaturized parallel microscopy assuming a substrate with a spot diameter of 400 µm and a pitch of 750 µm*

While classic parallel objectives and GRIN-rod lenses are products off the shelf, micro lenses have to be custom designed and fabricated. Also GRIN-rod lenses show the highest possible degree of parallelism while minimizing the number of layers. Hence we investigate in the next section a system based on GRIN-rod lenses.

3 GRIN-rod lens system

Figure 1 shows the concept of a GRIN-system consisting of 2 GRIN-rod layers and one beam splitter.

A GRIN-rod lens is a cylinder with a radial symmetric sech gradient-index profile which can be approximated by a Taylor series

$$y = n_0 + n_2 r^2 + n_4 r^4 + \ldots \qquad (1).$$

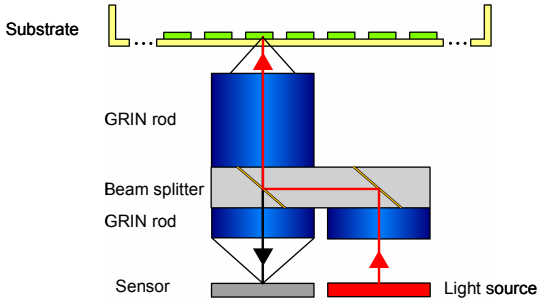

Fig. 1 *Concept of a miniaturized GRIN-rod lens system*

The GRIN-rod lens system described in table 1 and in figure 2 was achieved by optimizing the length of available GRIN-rod lenses and the parameter n_4 of the index distribution.

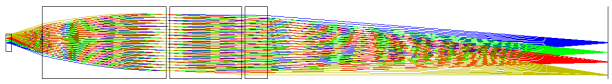

Fig. 2 *Zemax simulation of the GRIN-system, from left to right (object substrate, first GRIN-rod lens, beam splitter, second GRIN-rod lens, detector) with a D/FoV of 5*

The integrated system is shown in figure 3 for the case of complete parallelization. The y-direction shows three copies of a system with four lenses in x-direction. The GRIN-rod lenses are mounted on the glass substrates with polymeric ring structures which are fabricated lithographically in thick resist and serve for a high position accuracy of the GRIN-lenses.

4 Beam splitter

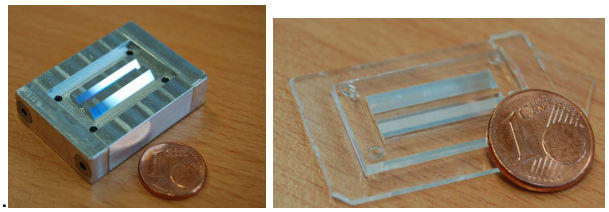

Fig. 3 *Left: Metal master for fabrication of the beam splitters. Right: Replication in transparent polymer*

The beam splitters were replicated in an UV-curable polymer [5] from a negative metal fabricated by micro-diamond turning at the LFM in Bremen. The master and the replication are shown in figure 4

The optical surface quality (RMS < 6 nm) is obtained by applying diamond milling in $AlMg_3$. The functionality of the beam splitter is obtained by coating the surfaces to be reflective for $\lambda < 400\,\mathrm{nm}$ and transparent at larger wavelengths.

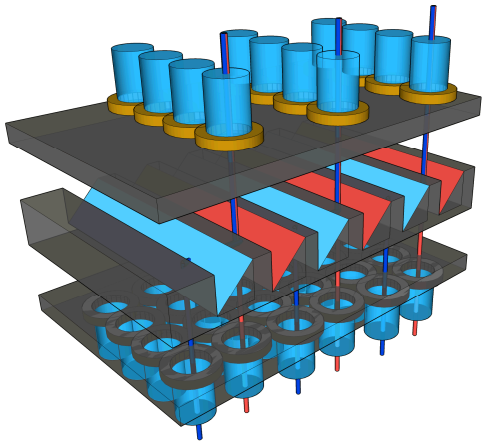

Fig. 4 *Illustration of the micro-integrated parallel microscope system. In the top layer are the front imaging lenses, the intermediate layer consists of beam splitters and in the bottom layer the collimation lenses (red) and the imaging lenses (blue) are mounted.*

5 Conclusion

Miniaturization in parallel microscopy is able to increase the scanning speed, which is essential in life science application based on high-content screens. We presented an approach using GRIN-rod lenses and replication of beam splitters for miniaturized parallel microscopy. The chromatic aberrations occurring in GRIN-rods represent a challenging problem, where further investigation is needed.

References

[1] R. Pepperkok, J. Ellenberg: “High-throughput fluorescence microscopy for systems biology” in *Nature Reviews Molecular Cell Biology* **7**, 690-696 (September 2006)

[2] B. Neumann et al: “High-throughput RNAi screening by time-lapse imaging of live human cells” in *Nature Methods* **3** , 385-390 (May 2006)

[3] E.Slogsnat et al.: “Non-stereoscopic method for deflectometric measurement of reflecting surfaces“, *DGaO-Proceedings* (Online-Zeitschrift der Deutschen Gesellschaft für angewandte Optik e. V.), ISSN: 1614-8436, 110. Jahrestagung in Brescia/Italien (2009)

[4] A.W. Lohmann: "Scaling laws for lens systems", Appl. Optics **28,** 4996-4998 (1989)

[5] D. Wohlfeld, et al.: "Doppelbelichtung und Replikation von Mikrostrukturen für die optische Informationsverarbeitung", DGaO-Proceedings (Online-Zeitschrift der Deutschen Gesellschaft für angewandte Optik e. V.), ISSN: 1614-8436, 107. Jahrestagung in Weingarten (2006)