XXI IMEKO World Congress "Measurement in Research and Industry"
August 30 – September 4, 2015, Prague, Czech Republic

DEVELOPMENT OF A COMPACT SPECTROMETER FOR FLUORESCENCE ANALYSIS

Maik Rosenberger[1], *Eduard Muslimov*[2], *Railya Akhmetgaleeva*[3],
[1]Ilmenau University of Technology, Ilmenau, Germany, maik.rosenberger@tu-ilmenau.de
[2] Kazan National Research Technical University - KAI, Kazan, Russian Federation, eduard.r.muslimov@gmail.com
[3] Kazan National Research Technical University - KAI, Kazan, Russian Federation, railushechka@mail.ru

Abstract – In the present paper an optical scheme of compact two-element spectrometer for detection of fluorescence spectra is presented. It consists of a concave holographic grating and a spherical projection mirror. The working spectral range is 500-1000 nm and the spectral resolution reaches 0.91 nm. The scheme is extremely compact and has F/# equal to 2.4.The design procedure is described in details with special emphasis on stray light suppression. The developed scheme can be used in a small-sized affordable diagnostic device, which is of a special interest for cancer diagnostics.

Keywords: Concave holographic grating, fluorescence analysis, numerical optimization, aberration correction, stray light.

1. INTRODUCTION

Fluorescence spectral analysis is a powerful method used in different areas. Particularly, it became a very valuable diagnostic tool for the fields of medicine and biology, because it allows to perform a non-invasive study of polymers, inorganic materials, cells, and tissues [1].

As the field of application of fluorescence analysis grows, a demand on compact, simple and affordable spectrometer specialized for fluorescence measurements becomes evident. Such instrument should be suitable for *in situ* measurements, so it should be compact, light-weight and have a simple and robust mechanical design. The optical scheme should have F/# no less than 3 to detect relatively weak fluorescence signal and provide a spectral resolution about 0.5-1 nm [2]. Finally, we should draw special attention to the spectral working range of such instruments. In a considerable quantity of cases the analytical features of fluorescence spectra located in long-wave visible and near infrared region between 500 and 1000 nm [2, 3].

The above-listed requirements can be met in an optical scheme of a flat-field spectrograph with an aberration-corrected concave holographic grating. Such spectrograph provides relatively high spectral resolution in an extremely compact and simple device with just one optical element. In the present papers we consider an ordinary scheme of flat-field spectrograph for fluorescence analysis. We use computer modeling to define its image quality and demonstrate shortcomings. Further we propose an improvement of this scheme and investigate it in detail to indicate its advantages and special features.

2. SINGLE-GRATING SPECTROGRAPH

According to the aforesaid, we used the following initial data to develop the single-grating flat-field spectrograph scheme: working spectral range is 500-1000 nm and F/# is 3. In addition we limited the typical size of the spectrograph, which is the distance from the entrance slit to the grating, by value of 61 mm. Finally, we assume that the spectrum length is equal to 27.8 mm, which is length of a commercial detector sensitive area.

We used a known design technique to develop the spectrograph optical scheme [4]. This technique is based on analytical minimization of separate terms of the grating aberration function. It allows to obtain tangential defocusing correction along the spectrum together with correction of coma and astigmatism at the central wavelength. General view of the obtained scheme is presented on Fig. 1. The overall dimensions of the spectrograph are 61x60x20 mm.

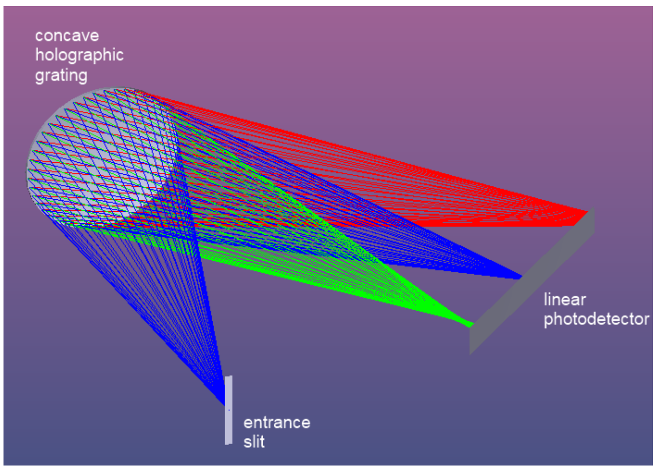

Fig.1. Optical scheme of the single-grating spectrometer

The scheme parameters are as follows: the distance between entrance slit and grating is 61mm and the distance between grating and detector is 60.78 mm; the angle of incidence (AOI) is 6°30' and the spectrum length is 28 mm. The grating is recorded holographically on a concave surface with radius of 60 mm by HeCd laser (λ=441.6 nm) and has grooves frequency of 700 mm^{-1}. The recording point sources polar coordinates are (32°56'; 58.54mm) and (13°35'; 57.93mm).

To demonstrate the obtained image quality we provide the spectrometer instrument functions and spot diagrams on Fig. 2. The entrance slit width is assumed to be 50 μm.

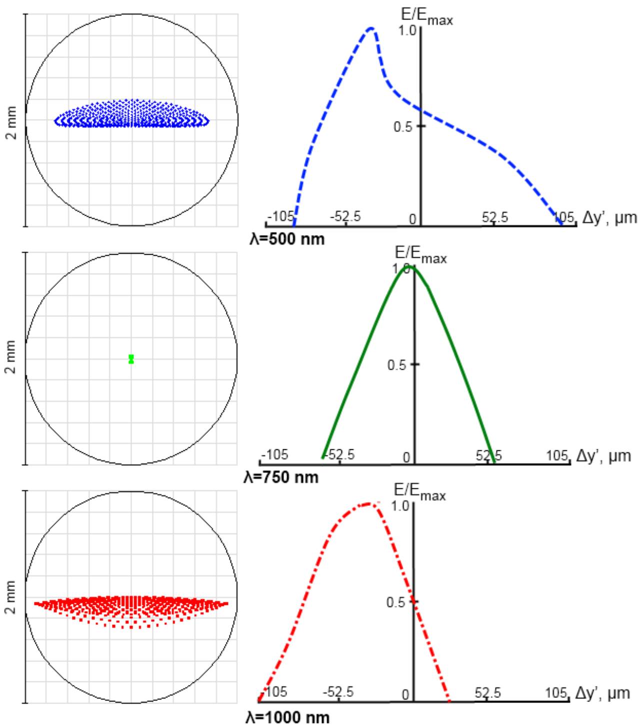

Fig.2. Instrument functions of the single-grating spectrometer

The spectral resolution of the scheme is equal to 1.16 nm in the spectrum center and 1.50 and 1.27 nm on its edges. Hereafter we calculate the spectral resolution as product of the instrument function FWHM by the spectrometer's reciprocal linear dispersion. One can see an obvious tangential coma on the spectrum edges. In addition, we should note here that the astigmatic extension of spot image on the edges is 0.90 and 0.71mm, while in the center it's completely corrected. This large astigmatism will decrease the image illumination and, therefore, reduce the instrument sensitivity.

Thus, in spite of being very simple and compact, the single-grating scheme can't be used with the required F/# because of its significant aberrations. Nevertheless a high-aperture compact spectrometer with a high resolution can be based on concave holographic grating. We describe such design in the next paragraph.

3. S-SHAPED SPECTROGRAPH

3.1 The optical design

It's clear that further increase of the spectrometer functional capacities requires better aberration correction, i.e. larger number of free correction parameters. The simplest way to introduce such parameters is to add to the scheme another optical element - concave projection mirror. As soon as the working beam is folded twice in this case, we'll refer to this two-element scheme as to S-shaped one.

The analytical procedure of the initial parameters computation is almost identical to that used before. The only difference is the grating linear magnification, which should be about 2 instead of 1.

At the first stage the concave mirror has low optical power and works with the smallest possible AOI.

Further design procedure consists of numerical optimization of the optical scheme with presence of obvious boundary conditions. The latter describe free propagation of the working beams through the system, conservation of the spectrum length and limitations on the overall size. After the optimization we obtain the scheme shown on Fig. 3.

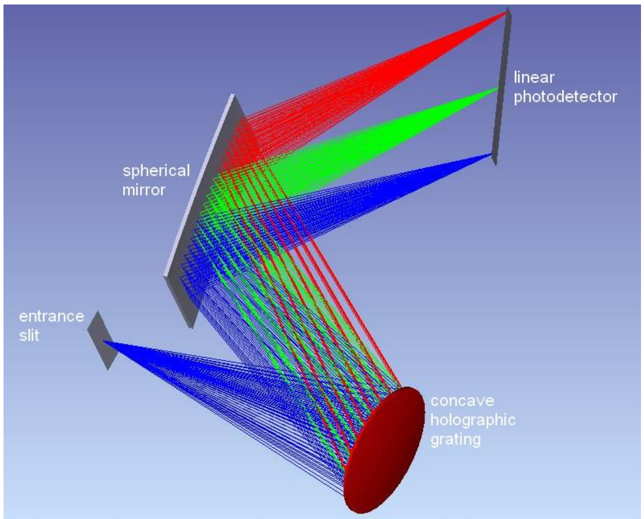

Fig. 3. Optical scheme of the S-shaped spectrometer

The scheme parameters are: the distance between entrance slit and grating – 58 mm, the distance between grating and mirror – 60 mm, the distance between mirror and detector – 55 mm, AOI for the grating is 4º34'; AOI for the mirror is 39º. The grating radius is 79.65 mm; its grooves frequency is 459mm^{-1} and the recording sources coordinates for the same wavelength are (28°18'; 121.08mm) and (15°455'; 114.72mm). Let us note that in comparison with the single-grating case the recording interferometer arms are significantly longer and thus more practically feasible. The mirror radius is 504 mm. It's also notable that the spectrometer has larger aperture - the f-number reaches 2.4.

Similarly to the previous case we use instrument functions and spot diagrams to estimate the image quality (see Fig. 4.). The entrance slit width remains the same.

The spectral resolution found from the instrument function is 0.89 nm for the central wavelength and 1.14nm and 0.95 nm for the edges.

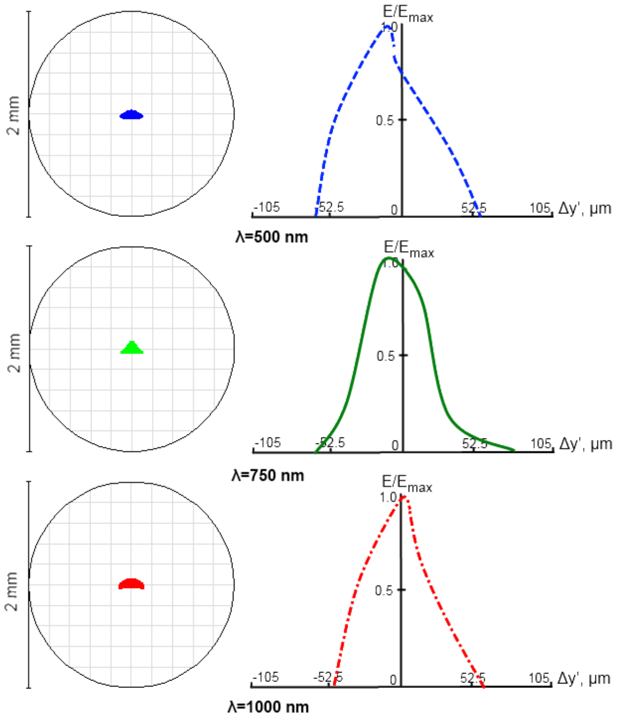

Fig.4. Instrument functions of the S-shaped spectrometer

Finally, we should emphasize the astigmatism correction achieved in the scheme. The astigmatic extension doesn't exceed 112 μm. So it's possible to increase the spectrum illuminance for a few times, thus obtaining higher sensitivity.

Thus the S-shaped design has higher spectral resolution with a higher aperture and smaller size. It fulfills all the requirements to a compact specialized spectrometer for fluorescence analysis. However, this decision isn't free of disadvantages. Probably the main of them is stray light, which occurs in a two-mirror optical system. Further we estimate the stray light and discuss means for its suppression.

3.2. Stray light

The primary source of the stray light in the S-shaped spectrometer is the non-working diffraction orders. They can incidence to the detector either directly after reflection on the mirror, or after second diffraction on the grating. To account them for we should model the system using ray splitting algorithms.

The grooves profile of such holographic gratings is usually quasy-sinusoidal. So we can easily model the diffraction efficiency distribution using Bessel function [5]. We define the groove depth corresponding to the maximum of diffraction efficiency in the 1^{st} order for the central wavelength of the working range. Fig. 5 shows theoretical diffraction efficiency for the 0^{th}, 1^{st} and 2^{nd} orders when the grooves depth is 0.97 μm. As soon as the groove profile is almost sinusoidal and the AOI is small, we assume that the efficiency distribution is identical for the symmetric orders.

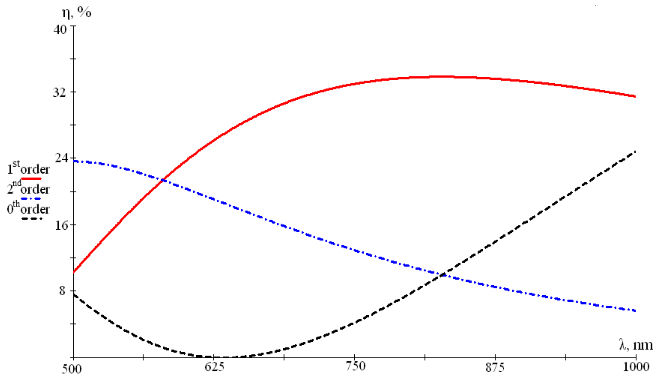

Fig.5. Diffraction efficiency distribution model

Using this data for splitting of rays into orders we calculate the stray light on the detector. We used 6 equally spaced reference wavelengths, which cover all the working range. The modeling results are shown on the Fig.s 6-7. The Fig. 6 represents the split ray traces.

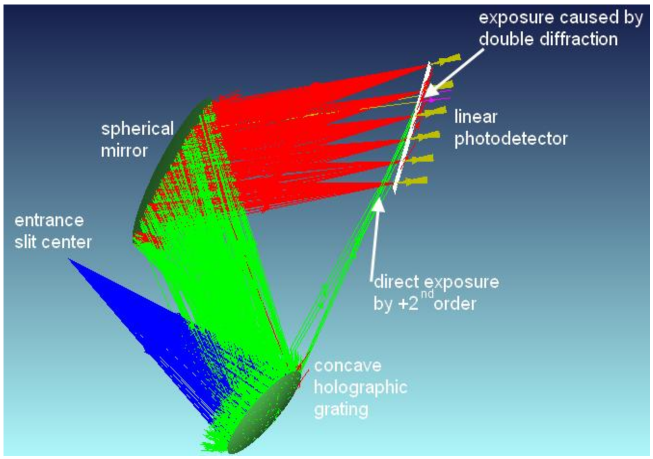

Fig.6. Analysis of the spectrometer stray light

One can see that the stray light has two origins: the first one is direct exposure by the $+2^{nd}$ order; the second one is double diffraction. The 2^{nd} order exposure can be easily obscured by a simple screen or inner wall of the spectrometer body. The double diffraction occurs when light, which was diffracted to the -1^{st}, $+2^{nd}$ and 0^{th} orders, is reflected back by the mirror and then re-diffracted into the $+1^{st}$ order. This stray light corresponds to the spectral region 920-1000 nm and it isn't separated from the main signal in space. So it can't be removed by means of a filter.

Fig. 7 represents detector signal: on the plot 7a both origins of the stray light are accounted for, and on 7b direct exposure is removed.

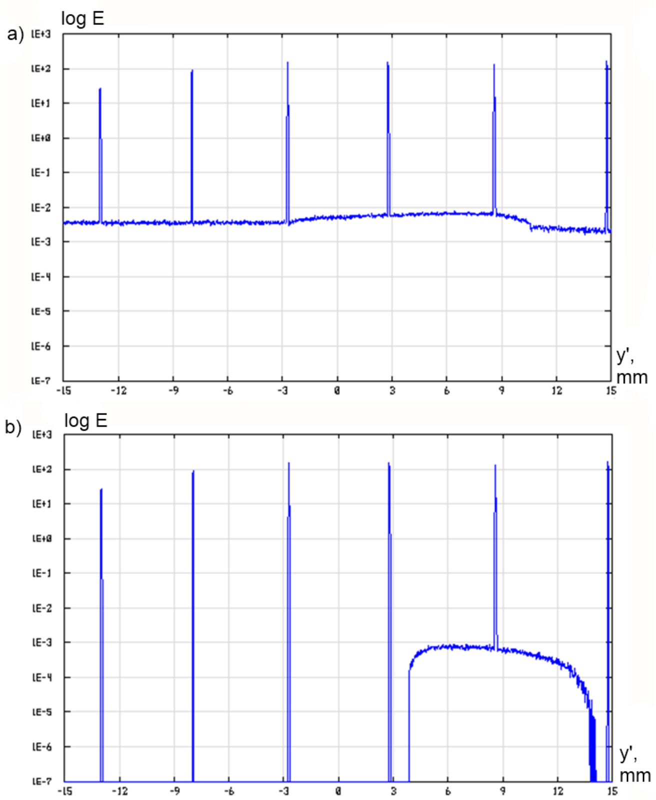

Fig.7. Detector signal simulation
a) full stray light included; b) double-diffraction stray light only.

However, it's possible to show that the coordinate of stray light spot on the detector has inverse dependence on the grating rotation angle. So we can completely eliminate the stray light by introducing boundary conditions, which limits angles of incidence and diffraction on the grating. Further we can repeat optimization of the optical system and check undesired exposures again.

Applying this iterative procedure to the optical scheme under consideration we obtain the following finalized parameters: the distance between entrance slit and grating – 58 mm, the distance between grating and mirror – 60.06 mm, the distance between mirror and detector – 58.03 mm, AOI for the grating is 2º22'; AOI for the mirror is 25º29'. The grating radius becomes 78.07 mm; its grooves frequency is 511.5 mm^{-1} and the recording sources polar coordinates are (22°27'; 120.49mm) and (8°59'; 112.65mm). The mirror radius is 517.66 mm. Overall size of the scheme is 58.2x77.2x30.7 mm instead of 58.2x85.8x36.5 mm. Other parameters remain the same.

Repeating the image quality analysis we found that the spectral resolution is 0.91 nm at the center of working range and 0.91 to 0.93 on its edges. The astigmatic extension is increased and reached 245 µm on the shortwave edge. But it still is much less than the corresponding amount for the single-grating scheme.

Thus the final design fits all the requirements to a compact spectrometer for fluorescence spectra analysis. It's notable for its simplicity and small size and free of stray light.

4. CONCLUSIONS

An optical scheme of compact spectrometer with relatively high spectral resolution and small F/# is proposed in the present paper. The scheme consists of a concave holographic grating and a spherical projection mirror. Use of two reflective elements allows to introduce an additional aberration correction, thus increasing the resolution, and to reduce the spectrometers' dimensions. On the other hand, such scheme can suffer of stray light contributed by non-working diffraction orders.

We developed a simple design algorithm, which starts with computation of the grating parameters by a known technique. Then the scheme is optimized numerically under a few geometrical constrains. On the last stage the stray light is modeled by means of a ray splitting algorithm and the scheme is iteratively optimized with boundary conditions limiting the grating rotation angle.

Modeling shows that the finalized design provides spectral resolution up to 0.91 nm across the range of 500-1000 nm, while the F/# is equal to 2.4 and the stray light is completely eliminated. A spectrometer with such parameters is of specific interest for measurements *in situ* in medicine like rapid analysis for cancer, because many endogenous fluorophores or purposefully injected markers have clear spectral features in its working region [6].

ACKNOWLEDGMENTS

The authors thank Prof. Nadezhda K. Pavlycheva for her help with the optical design concept.

REFERENCES

[1] M. Sauer, J. Hofkens and J. Enderlein *Handbook of Fluorescence Spectroscopy and Imaging: From Ensemble to Single Molecules*, Wiley-VCH, Weinheim, 2011.

[2] J.R. Lakowicz *Principles of Fluorescence Spectroscopy*, Springer, New York, 2007.

[3] K. Koenig and H. Schneckenburger, "Laser-Induced Autofluorescence for Medical Diagnosis", *Journal of Fluorescence*, Vol. 4, nº 1, pp.17-40, 1994.

[4] M. N. Nazmeev, N.K. Pavlycheva "New generation spectrographs" ,*Optical Engineering*, Vol. 33, nº 8, pp. 2777-2782, 1994.

[5] J.W. Goodmen *Introduction to Fourier optics. Third edition*, Roberts&Co, Englewood, 2004.

[6] I. Pavlova et. al., "Understanding the biological basis of autofluorescence imaging for oral cancer detection: high-resolution fluorescence microscopy in viable tissue", *Clinical Cancer Research*, Vol. 8, nº 14, pp. 2396–2404, 2008.