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# Biosensing II

Manijeh Razeghi Hooman Mohseni Editors

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# Introduction

The rapidly growing field of biosensing is a multi-disciplinary area where biology, nanotechnology, physics, chemistry, and electronics are truly merged. It is not only delightful and fascinating, but also an emerging field with great promises to improve the quality of our life. The term biosensing is interpreted by the community mainly as "sensing biological signals", but also as "sensing inspired by biological sensors," or biomimetics. This conference covers both areas.

This year's conference has been very successful in achieving its original goal of bringing together researchers and engineers who work on different aspects of this intriguing field, and to provide an interdisciplinary review of innovations in biomimetics, biosensors, nano-engineered, and bio-inspired sensors and systems, as well as theoretical and experimental tools that support and enable these innovations.

This volume features contributions from scientists and engineers in the general areas of biosensors, bio-inspired devices and systems, advances in surface plasmon resonance sensors, biomimitation, and DNA sensors.

This year, several important advances in the field were reported at the Biosensing conference. Some of the most exciting examples were the following. In the area of biological sensing, achieving the "ultimate" sensitivity detection level of a single molecule was reported. In the area of plasmonics, controlled release of multiple species from gold nanorods using laser activation was presented. In the area of bioinspired sensors, polarization sensitive imager with enhanced identification was demonstrated. In the area of sensing systems, several unique "wireless health" system approaches, including a functional contact lens, were presented. In the area of integrated (lab-on-chip) sensors, several novel devices including a label-free siliconon insulator biosensor chip, an optofluidic microscope, and a magnetic lab-on-chip were reported.

Although this volume cannot include all recent improvements in the emerging field of biosensing, it does cover an important cross-section of the most significant discoveries and advances. Hopefully, it provides a roadmap and inspires visions for the future of this field.

Although the Biosensing conference is only in its second year, we are quite excited about the rapid improvement of the quality and quantity of the submitted papers. We would like to thank members of the technical program committee for their help in selection of the most significant research activities in the field and organizing the conference. We would also like to thank the invited and contributing speakers, as well as all of the attendees for their contribution and participation in the conference. They made the conference a success.

Manijeh Razeghi Hooman Mohseni

# **Sub-Nanometer Resolution for the Inspection of Reflective Surfaces Using White Light**

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#### **ABSTRACT**

The quality control of highly reflective surfaces requires a measurement method which is able to resolve the surface shape in the nanometer range. Different methods have been developed in the past, e.g. based on interferometry or by tactile coordinate measurement machines. However, most of them do not match the industrial need for a fast method which is insensitive to environmental disturbance.

The newly developed method using the reflection of fringe pattern by the surface under test, and therefore called "Fringe Reflection Technique (FRT)", overcomes the difficulties of known measurement methods. In this method a pattern of straight fringes is generated by a monitor. The mirrored pattern is observed by a camera via the object surface under test. Any deviation of the surface against the ideal, i.e. the mathematically accurate surface will yield a distortion of the pattern. This distortion is analyzed by an image processing system, called the Fringe Processor. The surface topology is delivered by local surface gradients which can be integrated to object shape or differentiated to local curvature. The resolution of the system can be adapted to the measurement requirements in a wide range from micrometer down to subnanometer. Anyhow, the system is stable against environmental disturbances. It works without vibration isolation in rooms without any climate control. It is possible to measure freeform surfaces with no constraints on object geometry.

The measurement of a silicon mirror surface produced by diamond turning in a high precision tool machine serves as one example. The surface shape could be determined with a resolution below one nanometer. The measurements match the results of an interferometer and are better in certain areas.

**Keywords:** Shape, reflectometry, white light, nanometer resolution, topometry, freeform

### 1. INTRODUCTION

Shape measurements with a resolution down into the nanometer range are getting an increasingly importance [1], not only for the micro- and nano-system production but for standard products like coatings on cars or mirrors. Different metrology technologies have been developed in the past like phase sensitive microscopy, confocal microscopy, atomic force microscopy, or scanning near field optical microscopy [2]. The majority of these high resolution technologies are based on a point wise acquisition of the data and a scanning system for the determination of the whole surface topology. Furthermore, the methods are mostly sensitive to environmental disturbance.

The Fringe Reflection Technology (FRT) [3] is a whole field measurement technique using the imaging of fringes via the object surface: A fringe system displayed by a monitor is observed after the reflection by the object surface. Any tilt of the object surface changes the angle of reflection. This yields in a displacement of the fringe system to be measured by means of an image processing system. The displacement is strongly related to the angle of the surface against the bisector of the angle between camera normal and monitor normal. If this is the angle of a local part of the surface then it can be indicated as the surface gradient at this point, i.e. the FRT provides primarily the gradient field of the surface shape. By integration, the shape can be evaluated down to nanometers [4]. The FRT enables the characterization of reflective – not even specular – surfaces which cannot be measured by means of standard optical or tactile methods.

The basics of the FRT are described in the first part of this paper. The method can be used in a number of adapted setups. But in principle a monitor with software generated sinusoidal fringes and a CCD camera with an image processing system is needed. The nest point is the description of the data generation and the resolution that can be achieved depending on the parameters of the system.

Three examples of application will be described in order to demonstrate the wide variety of possible measurements with high resolution.

# 2. FUNDAMENTALS OF THE FRINGE REFLECTION TECHNIQUE

# 2.1 Principle of the FRT and the Set-up

The basic set-up of a fringe reflection set-up comprises three hardware components, Fig. 1:

- a monitor to display parallel sinusoidal fringes,
- a CCD camera as a part of an image processing system, and
- the object under investigation.

The camera on top of the monitor, Fig.1a, observes the fringe system via the object under investigation via the test object, in this case a car front window, Fig.1b left. The object is reflective by condition of the method, i.e. the fringe system is visible even when the surface is only partly specular. The camera is focused onto the surface of the object, Fig.1c. This results in a blurred image of the fringe system. However, a sinusoidal fringe remains sinusoidal under blurred conditions, too. A flat mirror would cause an undistorted image of the fringe system. Any tilt of the surface or a part of it displaces the related fringe system. A curved object will deform the former straight parallel fringe system according to the local angle of the surface against the normal or in other terms according to the surface gradient. The reflected fringe system is measured using a phase shifting method [5] and evaluated by means of an image processing system [6].

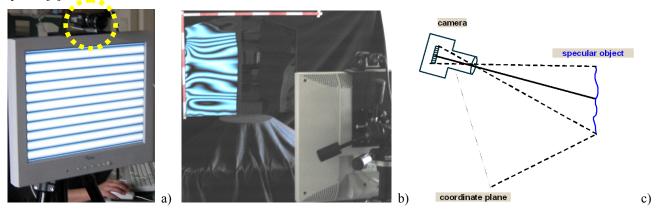


Fig. 1. System components: a) monitor displaying the fringe system and a CCD camera fixed on top of the monitor (dash line circle), b) car window (test object) at the left and monitor from back side at the right, c) schematic sketch

## 2.2 Geometrical Model and Evaluation

The evaluation will be derived on a geometrical model neglecting the scattered part of the light coming from the object, Fig.2. A surface angle of  $\Delta\alpha/2$  against the normal yields a deflection of the reflected image by an angle  $\alpha$  according to the laws of optics and by this a displacement of  $\Delta s$  of the imaged fringe system on the target in the distance l from the

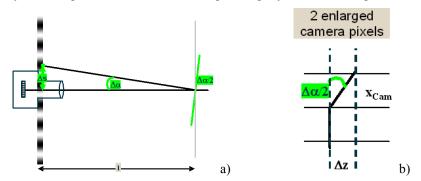


Fig. 2. Evaluation model: a) geometrical relations of fringe displacement, b) gradient and displacement angle

object. The deflection is caused by a local gradient of the surface  $\Delta \alpha/2$  – the same as above – which is given by the change of height  $\Delta z$  between two neighbored points of the surface in a distance of  $x_{\text{cam}}$ . The relations between the geometrical quantities are given by:

$$\tan \alpha = \frac{\Delta s}{l} \tag{1}$$

$$\tan\frac{\alpha}{2} = \frac{\Delta z}{x_{cam}} \tag{2}$$

and:

$$\Delta z \approx \frac{\Delta s}{2.1} x_{cam} \tag{3}$$

In this equation  $x_{cam}$ , - the size of an "object point" on the target - is given by the linear dimension of the object divided by the pixel number. The distance l is a measurable parameter of the set-up. The displacement of the fringe system - or the regarded part of it - has to be determined by image processing. Since the displacement shifts the fringe system by parts of the period the local phase of the fringes must be measured. For this, phase shifting technique is the state of the art [5]. In order to evaluate the minimum phase shift to be resolved in a given set-up one has to take into account the imaging system, Fig.3. The distance l from the camera to the object is the same as the distance from the monitor to the object assuming that the camera is fixed on the monitor or close to it. It can be derived by the geometrical relations:

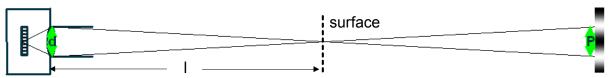


Fig. 3. Geometrical model of the imaging: the circle of confusion has identical aperture size in double focus distance

$$\frac{\Delta s}{P} = \frac{\Delta \varphi}{2 \pi} \qquad or \qquad \Delta s = P \frac{\Delta \varphi}{2 \pi} \tag{4}$$

with  $\Delta s$ : smallest displacement to be measured  $\Delta \varphi$ : smallest phase change to be measured

*P*: smallest possible period of the fringe system  $2 \pi$ : full fringe angle

The geometry of the set-up yields the equality d = P, Fig. 3. With the focal length f and the f-number k the size of d is

$$d = \frac{f}{k} \tag{5}$$

Equ.(5) together with Equ.(4) leads to

$$\Delta s = \frac{f}{k} \frac{\Delta \varphi}{2 \pi} \tag{6}$$

Combining Equ.(6) with Equ.(3) results in

$$\Delta z = x_{cam} \frac{f}{l} \frac{1}{2k} \frac{\Delta \varphi}{2 \pi} \tag{7}$$

 $x_{\rm cam}$  is the distance between two neighbored object points; f/l is the reduction of the object size by the imaging. The product of  $x_{\rm cam}$  and the reduction is the distance of two neighbored points on the target which is the size of a camera pixel.  $\Delta \phi/2\pi$  is the smallest resolvable phase change. Taking these relations into account results in equation for the height resolution  $\Delta z$  as a function of the camera pixel size pixel, the aperture k and the phase resolution  $\Delta \phi/2\pi$ :

$$\Delta z = pixel \frac{1}{2k} \frac{\Delta \varphi}{2 \pi} \tag{8}$$

There are three parameters in this equation that influence the resolution:

- The camera pixel size *pixel* is given by the available targets and is approximately 10 μm for a common camera.
- The aperture *k* of the imaging objective which is close to 10. This value cannot be extended very much since a smaller aperture yields less light and by this more noise in the camera signal.
- The resolvable phase change which is normally expected to be 1/100 in phase shifting. However, this value can be improved up to about 1/1000.

Inserting the following values:  $\Delta z=10 \text{ µm}$ , k=16,  $\Delta \varphi=2\pi/1000 \text{ Equ.}(8)$  results in the height resolution of  $\Delta z=0.63 \text{ nm}$ 

## 2.3 Hierarchical Phase Shifting

Phase shifting is the state-of-the-art method to measure the phase of a fringe with high resolution. The method applies sequential fringe systems with different periods or spatial frequencies. The largest period is adapted to the requirement of unambiguity; the smallest period is given to the possible resolution of the imaging system. In common the period from one fringe system is divided by a factor of 2 by simplicity. However, this is not the optimum as shown by Osten [8]: The next smaller period must be chosen with regard to the noise. Taking this into account the spatial frequency may be increased by a factor *a* between two sequential fringe systems, Fig.4. The evaluation of the phase by fringe systems of

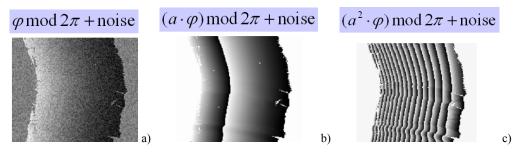


Fig. 4. Sequential fringes system: a) fundamental spatial frequency, b) a times larger spatial frequency than a), c) a \* a larger spatial than a)

according to Fig.4a and Fig.4b results in

$$\varphi \mod 2\pi + noise \implies \varphi \mod 2\pi + \frac{noise}{a}$$
 (9)

Combining this result with the fringe system according to Fig.4c results in

$$\varphi \mod 2\pi + noise \implies \varphi \mod 2\pi + \frac{noise}{a^2}$$
 (9)

This means that any additional fringe system in this sequence reduces the noise by a factor of a. Therefore highly resolved phase measurements can be achieved.

### 2.4 Resolution of the FRT Applied to a Coated Mirror

The surface of a coated mirror has been investigated in comparison with the FRT, with a commercial grating interferometer, and with a tactile coordinate measurement system, Fig.5a. The first step was to determine the shape of the whole

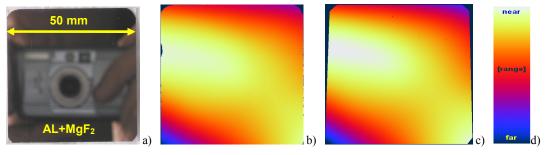


Fig. 5. Flat Al-mirror, MgF<sub>2</sub> coated: a) Photo, shape measured a) by prating interferometer with range 21.4 μm, c) by FRT with range 18.8 μm, d) color scale

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mirror by means of a commercial grating interferometer (by Zygo), Fig.5b) and by the fringe reflection technique, Fig.5c. The measurements showed a nearly cylindrical shape with a diagonal "hill" in the middle. The peak-to-valley value of the contour is about  $20 \mu m$ . In a next step the first and second order components of the shape were removed by subtracting fitted polynomials. This yields the fine structure with peak-to-valley values of 15 nm for the FRT results, Fig. 6b. The grating interferometer is not able to reach the same resolution by physical reasons of the method, Fig 6a.

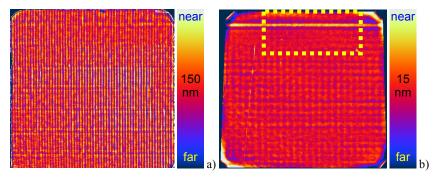


Fig. 6. Flat Al-mirror, MgF<sub>2</sub> coated, third order shape components: a) grating interferometer, b) FRT

The results of the FRT should be compared to the results achieved with the tactile coordinate measurement machine Tencor P15 in the marked part of the mirror, Fig. 6b. The marked part of the mirror was investigated with the tactile method (Fig. 7a) and compared to the FRT result, Fig. 7b. The whole range of the surface structure is 11 nm. This proofs that the resolution is below 1 nm. It shall be pointed out that the FRT measurement was achieved with the original data for whole the mirror area. In conclusion: FRT is a robust shape measurement method with a resolution of better than 1 nm and a dynamic range of more than 1:10,000.

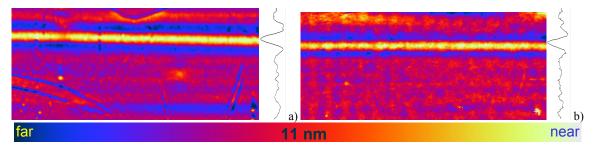


Fig. 7. Flat Al-mirror, MgF<sub>2</sub> coated, fine structure of the shape: a) tactile, b) FRT

# 3. EXAMPLES OF APPLICATION

# 3.1 Polished Freeform Lens

Spherical lenses are tested by standard interferometry available as commercial systems with outstanding performance. However, a problem arises when the surface has a freeform shape like those in varifocal lenses [9], Fig. 8a. The use of adapted reference waves for the interferometric comparison is nearly impossible, especially in an industrial environment. An additional difficulty is the required high dynamic range of the measurement since over a height difference of some millimeter a resolution of less than one micrometer is demanded. The fringe reflection technique (FRT) can be adapted to this problem in an elegant way. The set-up is a standard one with a monitor displaying sinusoidal fringes, a CCD camera mounted close to the monitor, and with a computer for the image processing. As mentioned above, the first results out of the measurement are the surface gradients. The first derivative of the gradient is the curvature which is equal to the optical power, which is changing for a varifocal lens over the surface, Fig. 8b. The gradient field can be integrated to achieve the surface shape. Interesting is again the fine structure of the shape which can be evaluated by subtracting fitted polynomials, Fig. 8c. In this case the peak-to valley values are in the higher nanometer range. However, the measured fine structure clearly demonstrates some properties of the polishing process: One can see the relief of the polishing tool and by this any deviation from the symmetry. More interesting is the effect of the process to build a "mountain"-like structure in the middle of the lens. Furthermore, the counter-measures to this effect deliver a smaller mountain with a grove around. The achieved resolution of some ten nanometers is sufficient in this case.

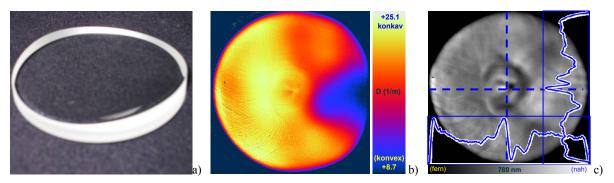


Fig. 8. Freeform lens, plastic: a) photo, b) curvature = optical power, c) scale

#### 3.2 Flat Silicon Mirror

High precision metal mirrors with specular surfaces are manufactured today by fly cutting diamond turning [10]. This production method allows to generate flat or spherical surfaces with deviations of a few nanometer compared to the ideal (mathematical) surface. FRT matches the control requirements of such surfaces, too. A flat silicon mirror shall be an example for testing such objects, Fig. 9a. The set-up is comparable to the one described above. It was possible to measure the fine structure directly in this case. The results of the commercial Fizeau interferometer showed a fine structure with peak-to-valley values of 30 nm, Fig. 9b. This is less than the FRT determined, Fig. 9c. The difference can be explained by the limited lateral resolution of the Fizeau interferometer functioning as a low pass filter. This cuts the high peaks and the deep groves of the structure. However, the FRT measurement delivers much more information if the different components are extracted, Fig. 10. One component are the traces of the cutting diamond with peak-to-valley values up to 20 nm, the other significant component is the vibration of the diamond after hitting the substrate with values up to 10 nm. Then there is a random component in the range of 16 nm.

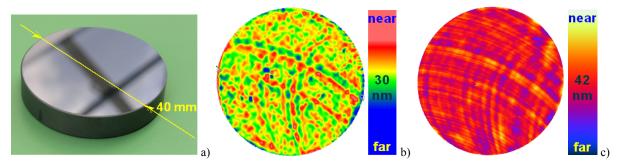


Fig. 9. Flat mirror (Si): a) photo, fine structure measured by b) commercial Fizeau interferometer, c) FRT, both with scale

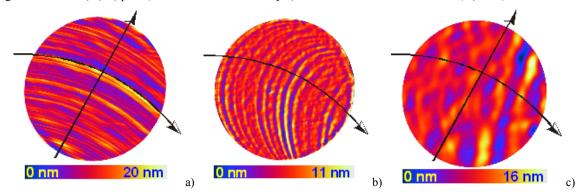


Fig. 10. Flat mirror (Si), fine structure: a) cutting traces b) vibrations of the tool, c) remaining random structure

# 3.3 Internal disturbance of a Lacquer Coating

The investigation of a lacquer coating on a car door is an example for non specular reflecting surfaces with scattered light components, Fig. 11a. The reflection of the room ceiling light is influenced by different components of the surface structure: overall shape, orange peel effect, local defects, micro structure. FRT was applied to investigate the contribution of the each layer of the coating onto the final structure of the surface. The measurements were performed with a set-up comparable to the one that was used for the car window. The results at the final layer – visible for the customer – are an excellent demonstration for the high dynamic range of the FRT in one acquisition of the data: The shape of the shown area of the door has a height range of 1.8 mm, Fig. 11b. The processing of the data by means of subtracting fitted polynomials unfolded an interesting detail: Before the first layer someone has marked a certain point, Fig. 11c. This marking could not be recognized by bare eye since the peak-to-valley height was less than 10 µm and thus below the values of the orange peel effect. Taking into account that the resolution is better than 1/10 of the range the dynamic range of the FRT can be calculated to be better than 1/10,000 of the original captured data.

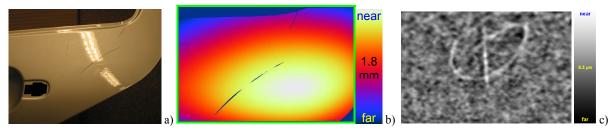


Fig. 11. Lacquer: a) photo, b) shape with scale, c) FRT, both with scale

# 4. SUMMARY AND FUTURE WORK

Shape measurements with a resolution down into the nanometer range are getting an increasing importance. The fringe reflection technique is an appropriate method to fulfill the requirements of scientific and industrial demands on a robust and fast method with a resolution in the nanometer regime and with an extraordinary large dynamic range. The method is based on the imaging of a sinusoidal fringe system via the object surface under investigation. This implies that the surface has to be partly reflective, at least. The common set-up consist of a monitor to display the sinusoidal fringe system, the object, a CCD camera mounted close to the monitor, and an image processing and control software. The evaluation of the imaged fringe system is performed using the phase shifting method. In order to speed-up the measurement the periods of the sequential fringe systems are chosen in a hierarchical way and not as most common in a binary sequence.

The gradient field of the tested surface changes the fringe system. In the simplest case of real flat mirror without any structure the change would be a deflection of the image with a displacement of the fringe system on the target. More complex surfaces deform the fringe system according to the gradient field of the shape. The evaluation of the displacement field is derived on the base of geometrical relations and optical laws. The resulting equation yielded that the resolution depends on three quantities: the pixel size of the camera, the f-number of the object and resolution of the measured fringe phase. The choice of reasonable parameters results in an achievable height resolution of less than 1 nm. This has been proven with a flat coated mirror.

Three examples demonstrated the versatility of the fringe reflection technique:

- The first example was the measurement of the shape of a freeform lens for varifocal glasses. This object is hard to be tested by standard interferometric methods since it is highly difficult to generate the reference wave. In this case a grating interferometer served as reference. The peak-to-valley values of the height were nearly 10 mm. The fine structure of the shape could be extracted out of the original shape data. This fine structure with peak-to-valley values in the nanometer regime showed properties of the polishing especially a peak in the middle with a surrounding grove.
- The second example was the measurement of the shape of a flat silicon mirror. It was produced by fly cutting diamond turning being the most precise technique for specular surfaces. The evaluation of the fine structure of the shape showed three different components: the traces of the cutting diamond, vibrations of the tool introduced by the interaction with the work piece after the first contact, and a random part due to several reasons. The range of the peak-to-valley values were below 20 nm with a resolution better than 1 nm.

- The third example was the investigation of the lacquer coating of a car door. The fringe reflection method enabled the determination of each layer of the coating onto the final orange peel effect of the surface. On the overall shape with a height of about 2 mm a fine structure could be extracted with height values in the upper and a resolution in the lower nanometer range. The evaluation of this component unfolded a mark drawn on the uncoated surface by a coworker. Although the maximum height of this mark was in the range of micrometer it could not be seen by the naked eye since the orange peel effect hired it.

The fringe reflection technique has some remarkable properties demonstrated by the examples: The resolution can be below one nanometer, the dynamic range of a measurement is higher than 1:10,000, and the resolution does not depend on the size of the object. Beside this it has to be pointed out that the technique applies white light and needs no protection against environmental disturbances.

Future work will be carried out on large spherical mirrors used e.g. in telescopes or for sun farms in solar energy plants. Another challenging task will be the application to dynamic objects, e.g. the behaviour of liquid surfaces or the interaction of biological species with a liquid environment.

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