Fast acquisition of 3D-data with Structured Illumination Microscopy

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We will discuss the efficiency of Structured-Illumination Microscopy (SIM) and the relation between measurement uncertainty and measuring time. Beyond that we provide a new measurement procedure which drives the sensor up to 20 times faster.

1 Introduction

Structured-Illumination Microscopy (SIM) is commonly used for biological purposes on dilute volume samples [1]. We use it as a method to measure full-field 3D-data of rough and smooth *technical surfaces* [2, 3]. SIM is characterised by a high dynamical depth range. However, to operate the system competitively compared to other 3D sensors, it must be tuned much faster than it is presently. Therefore we want to understand the interrelation between measurement time and measurement accuracy. Finally, we will describe how to make the measurement much faster by introducing a new data acquisition approach based on a continous depth scan (instead of stop & go).

2 Sensor principle

A sinusoidal fringe pattern is projected into the focal plane of a microscope. While the object is scanned axially, the contrast C(z) of the observed pattern is recorded in each pixel (Fig. 1). The z-position of the contrast peak corresponds to the local object height [4].

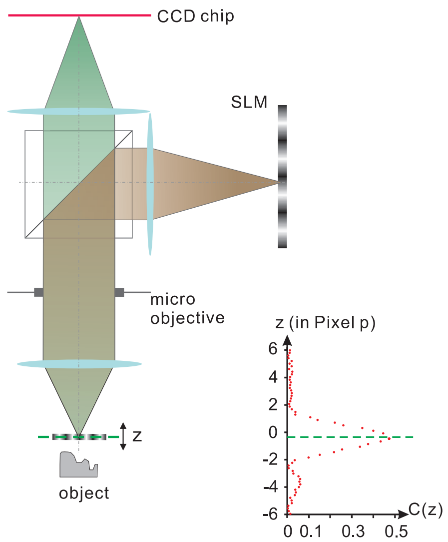

***Fig. 1** Setup and contrast curve C(z) of SIM.*

3 Measurement with 'stop and go'

In 'classical' SIM, the contrast is determined by at least three phase shifted exposures at equidistant measurement positions (M).

To obtain a precise measurement, the contrast curve (C(z)) has to be finely sampled. If the contrast noise can be regarded as spatially uncorrelated (which is true for the case of low speckle contrast), the measurement uncertainty decreases by $\frac{1}{\sqrt{M}}$. The corresponding high number of stops naturally entails a long measurement time.
Certainly, a fast measurement can be obtained by choosing larger sampling distances (few M). However, with decreasing M, additionally the curve itself has to be broadened to ensure at least three points under each curve width. For the minimum of three measurement points, the curve width has to be streched over the whole measurement distance. The curve width can be adjusted by choosing a lower pattern frequency. This means that for the 'fast' case, the MTF of the system cannot be fully exploited anymore - resulting in a accuracy loss that is considerably worse than just $\sqrt{\frac{M_{accurate}}{M_{fast}}}$.

Results:
We achieved a 15 times faster measurement with a loss of 10 times measurement uncertainty.

Bringing everything together, Table 1 shows an example of the achievable statistical measurement uncertainty for a z-range of 60μm for different chosen parameters $\left(\sqrt{\frac{M_{accurate}}{M_{fast}}} = 5\right)$.

	accurate	fast	factor
time	270s	11s	$\frac{1}{25}$
M	75	3	
σ_z	45nm	460nm	≈ 10

***Tab. 1** Accuracy vs measuring time (example measurement with 20x, 0.5 and $\Delta z = 60\mu m$).*

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4 Measurement 'on the fly'

Beyond the optimization of the system parameters, our goal is to further reduce measurement time. This leads us to a measurement without 'stop and go'.
To achieve this, we developed a method grabbing only one sinusoidal intensity pattern per measurement position [4]. Between two measurement points, the screen is triggered to shift the pattern by $\pi/2$ (Fig. 2).

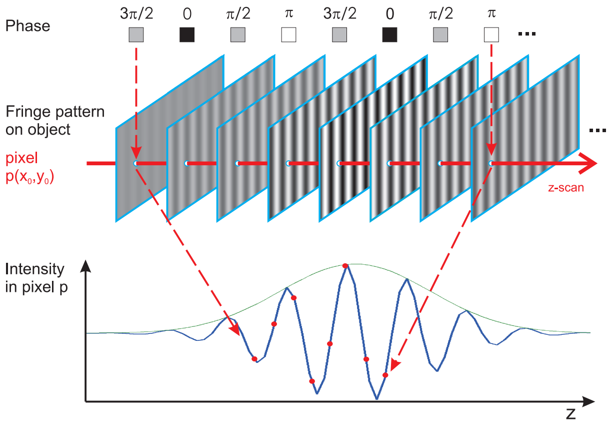

__Fig. 2__ Principle of measurement 'on the fly'.

This measurement provides a pattern similar to the WLI correlogram [5]. The envelope is equivalent to C(z) (Fig. 3).
Analog to 'stop and go', its maximum is the height value in each pixel.

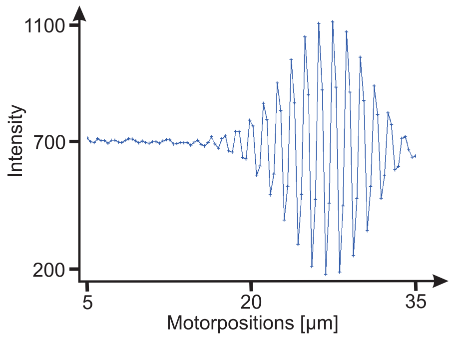

__Fig. 3__ Measurement 'on the fly'.

To obtain the envelope we use methods known for WLI like Single Side Band (SSB) demodulation and Quadrature demodulation.
For SSB demodulation the carrier frequency is cut out in the Fourier domain. Certainly the Nyquist sampling condition must be met which still results in a large amount of data.
In contrast to WLI we control the width and carrier frequency of the correlogram. This allows the alternative of a considerable sub-sampling with quadrature demodulation. Therefore we use two consecutive measurement positons shifted by 90 degrees. The second position must be approximated by few iterations. Only three measurement pairs are needed under each measurement peak.

Results:
We achieved a measurement 20 times faster compared to the conventional measurement with this approach. The measurement noise is increased only by a factor of two.

5 Conclusion

With this new knowlege it is possible to satisfy the requests of the user. By using the optimal parameter, the system can be driven very acurate for precise single measurements. By contrast it also can be tuned very fast for usage at production lines. An application might be solder paste inspection in electronics.

6 Acknowledgement

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