Error Analysis and Tolerance Allocation for Confocal Scanning Microscopy, using Monte Carlo Method

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ABSTRACT

The errors can cause the serious loss of the performance of a precision machine system. In this paper, we propose the method of allocating the alignment tolerances of the components and apply this method to Confocal Scanning Microscopy (CSM) to get the optimal tolerances. CSM uses confocal aperture, which blocks the out-of-focus information. Thus, it provides images with superior resolution and has unique property of optical sectioning. Recently, due to these properties, it has been widely used for measurement in biological field, medical science, material science and semiconductor industry. In general, tight tolerances are required to maintain the performance of a system, but a high cost of manufacturing and assembling is required to preserve the tight tolerances. The purpose of allocating the optimal tolerances is minimizing the cost while keeping the performance of the system. In the optimal problem, we set the performance requirements as constraints and maximized the tolerances. The Monte Carlo Method, a statistical simulation method, is used in tolerance analysis. Alignment tolerances of optical components of the confocal scanning microscopy are optimized, to minimize the cost and to maintain the observation performance of the microscopy. We can also apply this method to the other precision machine system.

Keywords: Confocal, microscopy, Monte Carlo, error analysis, tolerance analysis, tolerance allocation

1. INTRODUCTION

CSM(Confocal Scanning Microscopy) has higher resolution than conventional optical microscopy both in axial and transverse directions. This property is due to the confocal aperture located in front of the photo detector. Because of the aperture block, the out-of-focus signal, CSM has high depth discrimination and optical slicing property. It can inspect the inside of the biological specimen and it can reconstruct the three-dimensional configuration of the object. [1] Due to these superior properties, CSM has been widely used in the field of biology, medical science, inspection, and metrology. The measuring performance will be evaluated by resolution and contrast of the acquired images. Unsurprisingly, The performance is mainly decided by the design itself. However the manufacturing and assembling errors will degrade the system performance. Thus we should manage the errors within the proper level, allocating the adequate tolerances.

The precision machine system should be built up precisely. Even though it is designed to perform the best ability, it will not be realized without precise manufacture and assembly. In fact it is impossible to make any component exactly same as it is designed. Thus, the designer should allocate the proper tolerances to the components of the precision machine system. If they are too tight, the manufacturing cost will be very expensive or even sometimes it is impossible to make the precise components with any present manufacturing processes. Otherwise, if the tolerances are too loosely set, the system cannot work properly, even though the manufacturing process is easy and cheap. Using the optimal tolerances, the system works properly with minimal cost.

In the case of precision optical systems, they are not usually mass-produced, thus we can use precision stages to assemble and align the parts, which should be placed at the exact position. However, precision stages make the alignment process more complex and time-consuming. Some high procession stages make the total system weak and unreliable because they have relatively low stiffness. [2] Thus, we need to minimize the number of the stages. Through

242

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tolerance analysis and optimal tolerance allocation, we can determine weather the precision stage is needed or not. The optimal tolerance tells us the preciseness of the manufacturing as well as the need of stages.

The previous studies about the optimal tolerance allocation based on the assembly-reliability have been conducted. [3][4] In this study, we propose the method of error analysis and tolerance allocation for the optical system. Then we apply this method to the CSM to allocate the optimal tolerances.

2. METHOD OF ERROR ANALYSIS

2.1 Basic Principle of Confocal Scanning Microscopy

The performance of the microscopy can be evaluated with the resolving power, the clearness, or the contrast of the captured images. In the case of CSM, spot size and FWHM (Full Width Half Maximum) are main features because CSM is a scanning type. [5] The smaller the spot size is, the more detailed object can be pictured by this microscopy. The FWHM is a criterion to evaluate an axial resolving power. It is measured by scanning the point or plane object in the axial direction. The CSM, which has narrow FWHM, can optically slice the object thinly.

The errors degrade the functioning of the CSM. Due to theses errors, the spot size gets to be bigger, the peak intensity becomes weaker, and the FWHM becomes wider. This leads the image to be blurred, thus it will be impossible to distinguish the minute structure of the object. In this study, we will use the peak intensity and the FWHM to evaluate the performance of the CSM according to the errors.

2.2 Error effects on the system performance

In general, the manufacturing and assembling errors make the system performance degrade. The effect of misalignments of the optical components has been studied by computer-simulations and experimental results. [6][7][8] Generally, the system performance has a relationship with the errors, as shown in Fig 1. Some errors, like e_1 , affect the performance considerably, some errors have a linear property with the performance, and another errors can be insensitive to some extent. We should assign the optimal tolerance to the error, such as e1 in Fig 1., which affects the performance seriously.

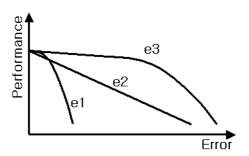


Fig 1. Relationship between errors and performance

In CSM, tilt errors, defocus errors, and transverse offset errors make FWHM bigger or peak intensity weaker. If this misalignment occurs, the axial and the transverse resolution of CSM will degrade. Thus, the deterioration of captured 2D or 3D image is predicted. In this case, tilt errors, defocus errors, and transverse offset errors are alignment $error(e_i)$ variables, and FWHM and peak intensity are performance errors indices(errors). They have a functional relationship as following expression

$$\delta S_k = f(e_1, e_2, \dots, e_N) \tag{1}$$

2.3 The relationship between allocated tolerance and occurred error: Monte Carlo Method

The effects of errors are determined by the designed system because they have a functional relationship with the system performance. If we know the system errors exactly, we can calculate the performance degradation. However, as a matter of fact, the system errors can never be measured exactly. The errors, unavoidably occurred in the manufacturing and assembling processes, cannot be predicted perfectly. However, to manage the quality of the system we should manage the processes. To maintain the errors in the specific ranges, the engineer allocates the tolerances to the components in the manufacturing processes. In optical system, because the assembling errors as well as the manufacturing errors can affect a system performance seriously, the proper tolerances need to be allocated in both processes.

If the engineer allocates the tolerances, the manufacturer manages the process to make errors inside the tolerances. Because the errors occur randomly to some extent, if we assume that the error has normal distribution statistically, the probability, at which the specific error occur, can be defined by the allocated tolerance (t_i) mathematically as following expression. [9][10][11]

$$p(e_i) = \frac{1}{\sqrt{2\pi\sigma}} \exp\left[-\frac{(e_i)^2}{2\sigma^2}\right]$$
 (2)

If we assume that the error is managed within the 3σ of allocated tolerance at the manufacturing process, 3 times of standard deviation (3σ) has a same value with the allocated tolerance (t_i). Thus, the predicted error has a probability graph as Fig 2. Because $\pm 3\sigma$ include 99.74%, we can say that the error occurs statistically within the allocated tolerance with 99.74% if the tolerance is managed with 3σ .

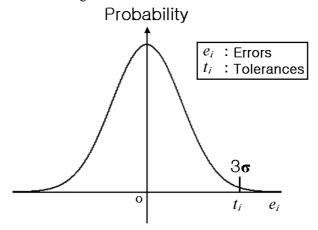


Fig 2. Distribution of Errors according to the allocated Tolerance

As mentioned before, the error is rather random variable, thus the statistical approach is required. Monte Carlo Method is a statistical method to solve the complex and nonlinear problem, which is difficult to be solved by analytical method. [12] The statistical random numbers, distributed within the specific range, are used to solve the complex problem.

Fig 3(a). is an example of randomly generated numbers, whose probabilities have a nominal distribution. At this simulation, Matlab 6.1 is used. The nominal target value is 0.0, the allocated tolerance is 0.1, and the number of generation is 10000. In this case, the mean value and the standard deviation is 0.0002 and 0.0334, respectively. 3 times of the standard deviation is well matched with the tolerance. With these randomly generated values, we can predict the system errors and the deterioration of the system performance.

Proc. of SPIE Vol. 5324

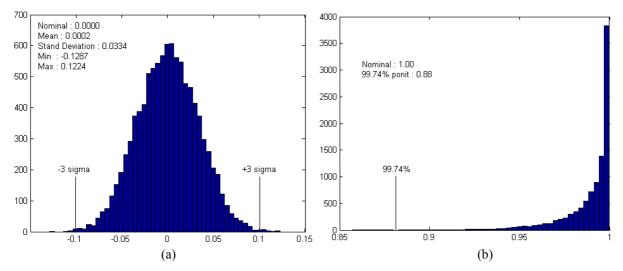


Fig 3. (a) Sample error generation with Monte Carlo Method (b) Sample Performance index calculation with Monte Carlo Method

2.4 The relationship between allocated tolerance and system performance

As eq(1), performance index(S_k) is calculated by errors(e_i) and the error has a probability relationship with the allocated tolerance(t_i) as eq(2). Thus, the performance index is statistically expressed by the tolerance. When we assign the tolerance, we can predict the error according to the tolerance. From the distribution of the error, the performance index can be calculated with each generated error using eq(1). If this calculation is finished, we can get the distribution of system index such as Fig 3(b).

Fig 3(b). is an example of change of performance index according to the generated numbers, shown in Fig 3(a). In this calculation, it is assumed that the performance index of the system is expressed as eq(3).

$$S_1 = 1 - 10 \times e_1^2 \tag{3}$$

In fact, in the real optical system, this error-performance function is quite complex, thus sometimes it is almost impossible to express the relationship such an equation. Some commercial simulation tools like CODE V, OSLO, or ZEMAX are used to calculate the performance index. In this study, Matlab 6.1 is used to simulate the system and Monte Carlo Method. Using the result of the Monte Carlo Method, the system performance index is predicted as 0.88. Because the tolerance is assumed to be managed with $\pm 3\sigma$, the error is distributed within the $\pm 3\sigma$ range with the 99.74% probability. Thus, the deterioration of the system performance is predicted by this probability in the Fig 3(b).

3. METHOD OF TOLERANCE ALLOCATION

In Chap.2, the method of tolerance analysis is proposed. With this method, the effect of tolerances to the system can be predicted. In this chapter, the method of tolerance synthesis will be proposed to allocate the optimal tolerances. This method uses the analysis method suggested in Chap. 2 and the SQP optimization algorithm. The flow chart to allocate the optimal tolerances is shown below.

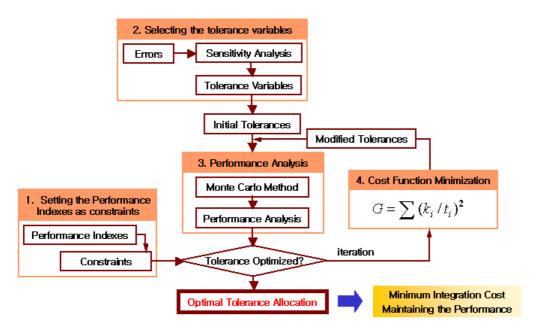


Fig 4. Flow diagram of optimal tolerance allocation

At first, performance indices are selected according to the system. In optical system, they can be spot size, peak intensity, S/N ratio, RMS wave-front aberration, FWHM (Full-Width-Half-Maximum), transverse resolution, axial resolution, and so on. Other properties that are not listed here also can be performance indices, if they can be represent the quality of the system. In this stage, the quality, which the system should maintain to work properly, is determined as the performance index.

Secondly, the sensitive errors are selected as tolerance variables. The optimal tolerances should be allocated to the errors sensitive to the system performance, while the other insensitive errors do not need to be optimally allocated.

Then, using the method of performance analysis with Monte Carlo method, the performance index is predicted according to the initial tolerances. This result is used in optimization algorithm. The cost function is expressed as eq(4).

$$G(t_1, t_2, \dots, t_n) = \sum_{i=1}^{n} (k_i / t_i)^2$$
(4)

where, $k_i = scale \ factor$, $t_i = tolerances$

The bigger tolerances are preferred, because it is easier to manufacture. Thus, the cost function should be minimized in the optimization process, while the constraints are limits of the loss on the performance indices(S_k).

4. SIMULATION RESULTS FOR CONFOCAL SCANNING MICROSCOPY

4.1 Performance Index Selection for CSM

With the proposed method, the optimal tolerances are allocated for the CSM(confocal scanning microscopy). The system configuration is shown below.

246 Proc. of SPIE Vol. 5324

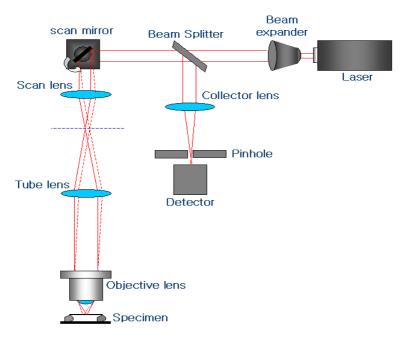


Fig 5. Configuration of confocal scanning microscopy (Objective lens NA: 0.25)

This CSM has only one scanning-optics for the simplicity. The main advantageous property of CSM is it's optical-sectioning ability. Due to this property, it has a power of depth discrimination and it can reconstruct the 3D image of the object's external shape and even internal feature. Thus, FWHM is selected as a performance index for CSM. FWHM is the with at which the intensity falls half of the maximum, in the axial response graph. Fig 6. is a FWHM of this CSM. It is $13.26\mu m$.

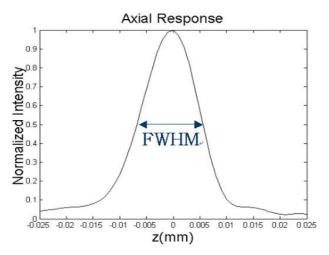


Fig 6. Axial response of CSM for plane specimen (z : axial displacement of specimen)

Axial response is measured by detecting the intensity, scanning the sample up and down near the focal point. A confocal pinhole blocks the out-of-focus light from the sample, thus the intensity has a sharp peak at the focal point. The CSM that has narrow FWHM has strong power of depth discrimination, thus sharp images can be obtained.[5]

4.2 Effect of errors on FWHM

As mentioned above, smaller FWHM is better in CSM. However obtaining the desired FWHM is quite difficult because the errors occur in the manufacturing process of optical components and assembling process of system. The change of FWHM according to the tilt and displacement error of collector lens is shown in Fig 7.

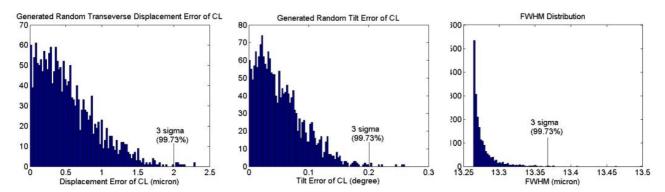


Fig 7. Histogram of Generated Random Errors of Collector Lens (a) Transverse Offset Error (b) Tilt Error (c) FWHM of CSM

For this simulation, transverse offset tolerance is assigned as $2\mu\text{m}$ and tilt tolerance is assigned 0.2° . As shown in Fig 7(a). and Fig 7(b)., the 3σ value is well matched with the tolerance. FHWM distribution is calculated for these error sets.(Fig 7(c).) The FWHM that matches with 99.74%(3σ) is $13.37\mu\text{m}$. It means that if the tolerance for transverse offset error and tilt error is allocated as $2\mu\text{m}$ and 0.2° , respectively, and other errors are negligible, the FWHM will not exceed $13.37\mu\text{m}$ (nominal= $13.26\mu\text{m}$) with the probability of 99.74%. With this procedure, performance indices can be predicted according to the tolerances.

4.3 Optimal tolerances

Using SQP function of Matlab 6.1, the tolerances of sensitive errors are optimized. The components of detection part of CSM are more sensitive than scanning part. The cost function is express as eq(5). In the optimization process, the tolerances are maximized, minimizing the cost function. The constraint is that FWHM is smaller than $13.51\mu\text{m}(5\%)$ increase of nominal value).

$$f = (\frac{k_1}{t_1})^2 + (\frac{k_2}{t_2})^2 + (\frac{k_3}{t_3})^2 + (\frac{k_4}{t_4})^2$$

$$k_1, k_4 : linear \ scale \ factor = 1$$

$$k_2, k_3 : angular \ scale \ factor = 2.86$$
(5)

Table 1. Allocated Optimal Tolerances of Detection Parts of the Confocal Scanning Microscope

| Tolerance variables | Optimal tolerance |
|---|-------------------|
| t1 : offset tolerance of collector lens | 1.063 🙉 |
| t2 : tilt tolerance of collector lens | 0,100° |
| t3 : tilt tolerance of beam splitter | 0.002° |
| t4 : offset tolerance of pinhole | 1.045 🙉 |

The result of optimal tolerance allocation is presented in Table 1. As a matter of fact, these tolerance values are not easy to obtain. To maintain these tolerances, high precision x-y stage is needed. Actually the tilt errors induce the focal spot to move in the transverse direction, thus the errors for detection parts can be compensated by one x-y stage. Since the x-y stage is used in this part, we don't have to worry about these errors seriously. The tolerances for the scanning parts are optimized with the same processes and the result is shown in Table 2.

Proc. of SPIE Vol. 5324

248

Table 2. Allocated Optimal Tolerances of Scanning Parts of the Confocal Scanning Microscope

| Tolerance variables | Optimal tolerance |
|---|-------------------|
| t5 : offset tolerance of scan lens | 59,66μ |
| t6 : tilt tolerance of scan lens | 1,66° |
| t7: offset tolerance of tube lens | 175,09 🙉 |
| t8 : tilt tolerance of tube lens | 0,91° |
| t9 : offset tolerance of obejctive lens | 132,33 μα |

Considering these values, we can allocate the optimal tolerances. These are maximum tolerances to keep the system performance with probability. With this result we can manufacture the system with minimum cost, still maintaining the required performance.

5. CONCLUSIONS

In this paper, the method of tolerance analysis and tolerance allocation is proposed. Monte Carlo Method is used to find the effect of tolerance to system performance, and SQP algorithm is used to optimize the tolerances. With this method the optimal tolerances, which minimize the built-up cost while maintaining the quality of system, can be found. Applying this method, the optimum tolerances for the components of Confocal Scanning Microscopy are obtained. From this result it is found that the stage should be used to compensate the errors on detection part. For the scanning part, the maximum tolerances are allocated.

6. ACKNOWLEDGEMENTS

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REFERENCES

- 1. C. J. R. Sheppard, Confocal Laser Scanning Microscopy, Springer-Verlag, 1997
- 2. Jae W. Ryu, "Optimal design of a flexure hinge based XYO wafer stage", Precision Engineering, Vol. 21, pp. 18-28, 1997
- 3. Woo-Jong Lee, T. C. Woo, "Tolerances: Their Analysis and Synthesis," Journal of Engineering for Industry, Transactions of the ASME, Vol. 112, pp. 113-121, 1990
- 4. B. K. A. Ngoi and O. J. Min, "Optimum Tolerance Allocation in Assembly," International Journal of Advanced Manufacturing Technology 15, pp. 660-665, 1999
- 5. James B. Pawley, Handbook of Biological Confocal Microscopy, Plenum Press, 1995
- 6. Z.Tang, "Effects of angular misalignments of fiber-optic alignment automation", Optics Communications, Vol. 196, pp.173-180, 2001
- 7. Z.Tang, "Optimizatio of fiber-optic coupling and alignment tolerance for coupling between a laser diode and a wedged single-mode fiber", Optics Communications, Vol. 199, pp. 95-101, 2001
- 8. Chanda L. Bartlett, "Computer simulations of effects of disk tilt and lens tilt on push-pull tracking error signal in an optical disk drive, Applied Optics, Vol. 36, No. 32, pp. 8467-8473, 1997
- 9. Chun Zhang, "Robust design of assembly and machining tolerance allocations", IIE Transactions, Vol. 30, pp.17-29, 1998
- 10. Chiu-Chi Wei, "Allocating tolerances to minimize cost of nonconforming assembly", Assembly Automation, Vol. 17, No. 4, pp. 303-306, 1997
- 11. Robert C. Juvinall, "Fundamentals of machine component design", John Wiley & Sons, Inc., 1991
- 12. I. M. Sobol, The Monte Carlo Method, The University of Chicago Press, 1974