Aberration correction for laser scanning microscopy by 'closed-loop accumulation of single scattering (CLASS)' algorithm

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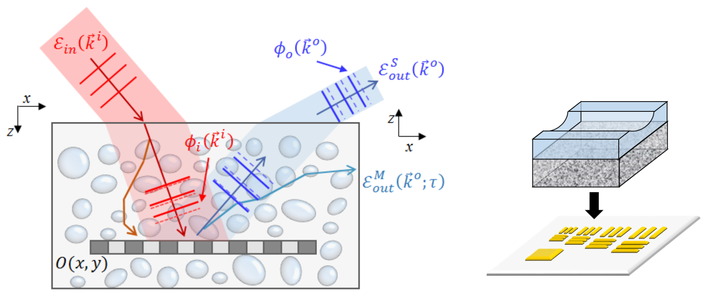
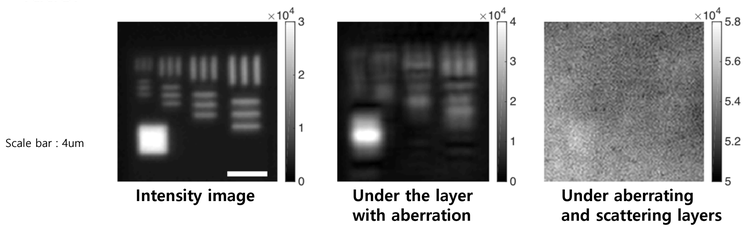
Abstract

'Collective accumulation of single scattering (CASS)' and 'Closed-loop accumulation of single scattering (CLASS)' microscopy was introduced recently. These methods provide novel solutions to the problems of light scattering and aberration in optical imaging, and they provided increased imaging depth while maintaining diffraction limited resolution. Until now these methods demonstrated monochromatic contrast by utilizing back scattered illumination light. Here, I introduce a novel imaging method to correct aberration for the laser scanning microscopy by adopting CLASS algorithm. This method can be applied to any type of laser scanning microscopy, and it is expected that aberration correction will enhance the imaging depth and resolution while it is still providing full spectral information of regular confocal/multiphoton microscopy. The strength and weakness of the introduced method over the current adaptive optical microscopy will be discussed.

Introduction



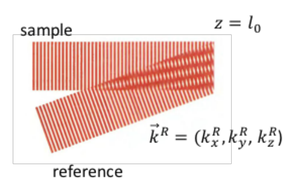
- Multiple light scattering attenuates the total intensity of the single-scattered waves which carry the information of the imaging object.
- Aberration broadens the width of the point spread function and degrade spatial resolving power.
- Both scattering and aberration cause the reduction in the signal to noise ratio.



In this presentation, the principle and method to implement 'collective accumulation of single scattering (CASS)' microscopy which enables overcoming multiple scattering by utilizing spatial input-output correlation. Algorithm to correct aberration caused by an imaging sample will be presented with applications in biological imaging.

Measuring electric field, not intensity

Off-axis Digital Holographic Microscopy



$$E(x,y,z=l_0) = E_S(x,y) + E_R e^{-i\vec{k}^R\cdot\vec{x}}$$

$$I(x,y) = |E_S|^2 + |E_R|^2 + E_S \cdot E_R\{e^{-i\vec{k}^R\cdot\vec{x}} + e^{i\vec{k}^R\cdot\vec{x}}\}$$

$$\tilde{I}(k_x,k_y) = \tilde{E_S}\otimes\tilde{E_S} + |E_R|^2\delta(k_x,k_y) + E_R\{\tilde{E_S}(k_x-k_x^R,k_y-k_y^R) + \tilde{E_S}(k_x+k_x^R,k_y+k_y^R)\}$$

Hilbert Transform

$I_{tot}(\vec{x}) = I_s(\vec{x}) + I_r(\vec{x}) + 2Re\{E_r^*(\vec{x})E_s(\vec{x})\exp(i\vec{k}_0\cdot\vec{x})\}$ $\quad \vec{k}_0$: relative vector between sample and reference

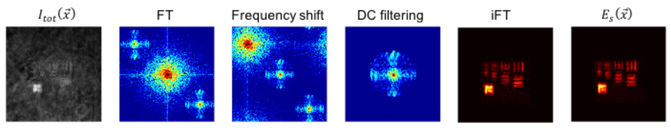
FT ➡ $\mathcal{I}_S(\vec{k}) + \mathcal{I}_R(\vec{k}) + 2Re\{FT\,[E_r^*(\vec{x})E_s(\vec{x})](\vec{k}-\vec{k}_0)\}$

Frequency shift ➡ $\mathcal{I}_S(\vec{k}+\vec{k}_0) + \mathcal{I}_R(\vec{k}+\vec{k}_0) + 2Re\{FT\,[E_r^*(\vec{x})E_s(\vec{x})](\vec{k})\}$

DC filtering ➡ $FT\,[E_r^*(\vec{x})E_s(\vec{x})](\vec{k})$

iFT ➡ $E_r^*(\vec{x})E_s(\vec{x})$

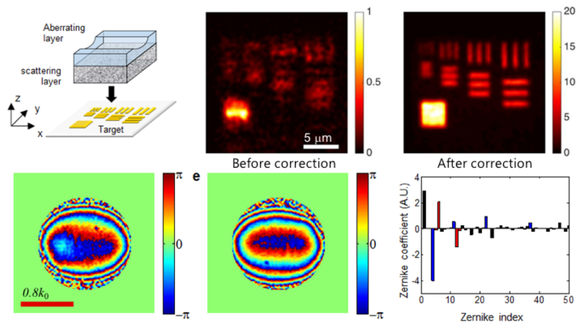
Normalize reference ➡ $E_s(\vec{x})$ ← Assuming that $E_r(\vec{x}) = \sqrt{I_r(\vec{x})}$



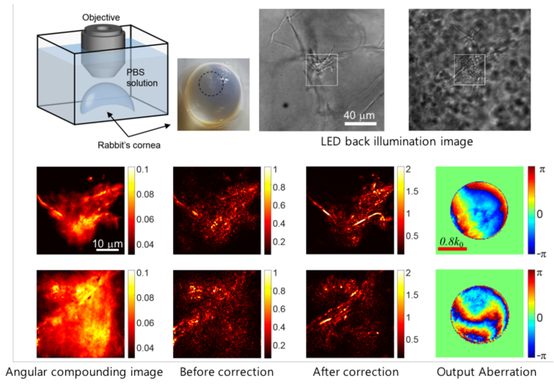
Electric field (amplitude and phase) can be acquired by off-axis digital holographic microscopy and Hilbert transformation.

Application of CLASS microscopy

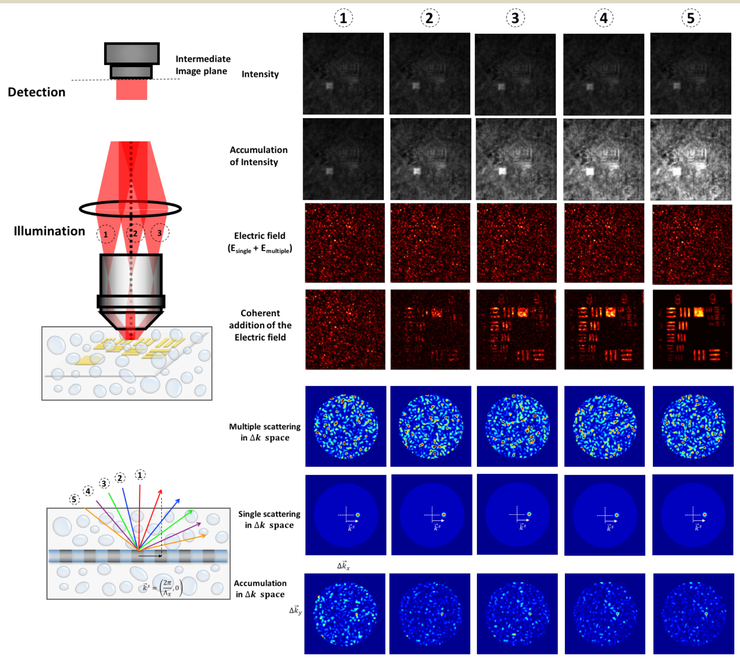
Aberration correction for tissue phantom



Imaging the hyphae of Aspergillus cells in a rabbit's cornea

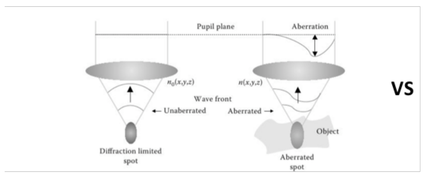


Collected accumulation of single scattering (CASS) and closed-loop accumulation of single scattering (CLASS)

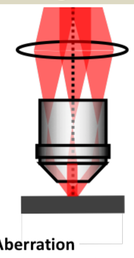


- Coherent addition of Electric field reflected from the sample object increases proportional to N^2 (N: number of illumination angle), and this increased SNR as N increases and enables deep tissue imaging under the condition of high scattering.
- Aberration can be acquired by assigning additional phase to the complex electric field from each illumination angle in such a way as to maximize the total intensity of the reconstructed images.

Aberration dealt with by CLASS



VS



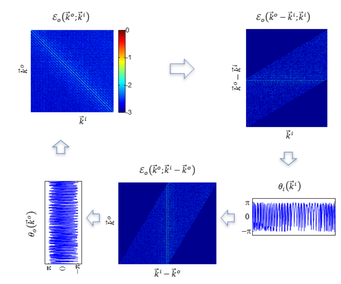
Aberration

$$\Psi(x,y) = \int_F^{P(x,y,z_0)} \frac{2\pi}{\lambda_c}(n(\tilde{x},\tilde{y},\tilde{z}) - n_0(\tilde{x},\tilde{y},\tilde{z}))\mathrm{d}w$$

Aberration
Angle-dependent phase retardation of the single scattered waves

While traditional adaptive optics optimized the phase for a single point (guide star), CLASS correct aberration of the full field of view.

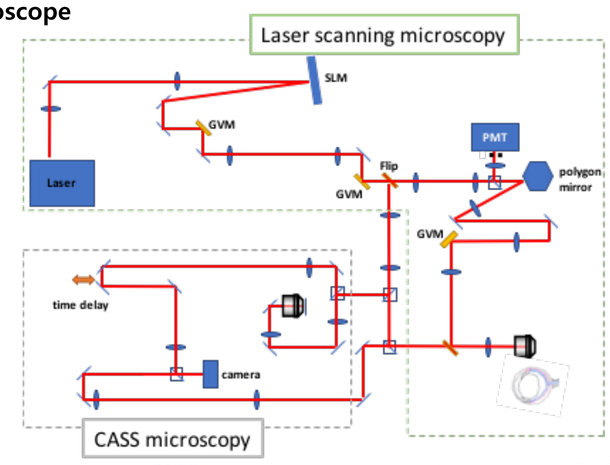
Flowchart of CLASS algorithm



- Time-resolved reflection matrix in k-domain is made.
- E-field (in k-domain) is aligned so that single scattering can be readily added.
- Aberration is acquired at each illumination angle.
- Aberration correction in the phase-conjugation process id made

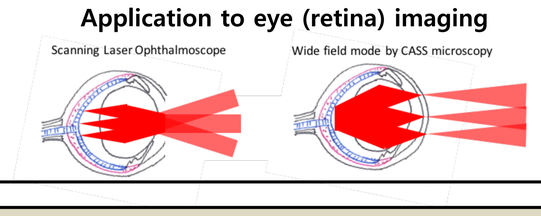
Aberration correction for laser scanning microscopy

Schematics of Laser scanning microscope combined with CASS microscope



- Laser scanning and CASS microscopy can be acquired sequentially from the same sample.
- 'CASS microscopy' can either directly acquire deep tissue aberration corrected image or play a role of sensor to measure the phase to correct aberration for laser scanning microscope.

Application to eye (retina) imaging



Summary

- The concept and principle of CASS and CLASS microscopy were reviewed.
- Aberration correction by CLASS method was compared with the one of traditional adaptive optics.
- CLASS microscopy can measure the phase to correct the aberration for laser scanning microscope.

Acknowledgement

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Reference

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