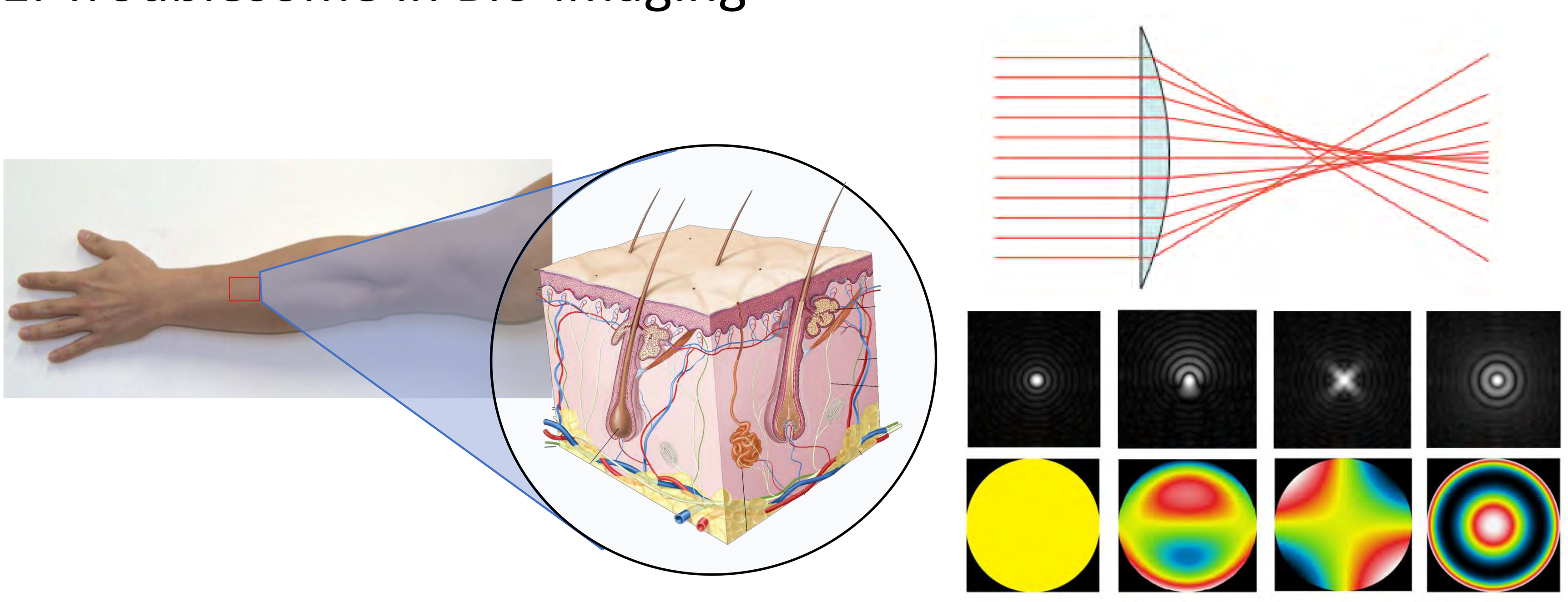
High-resolution adaptive optical imaging within scattering media using point-illumination based closed-loop accumulation of single scattering microscopy

Hojun Lee, Seokchan Yoon, Jin Hee Hong and Wonshik Choi

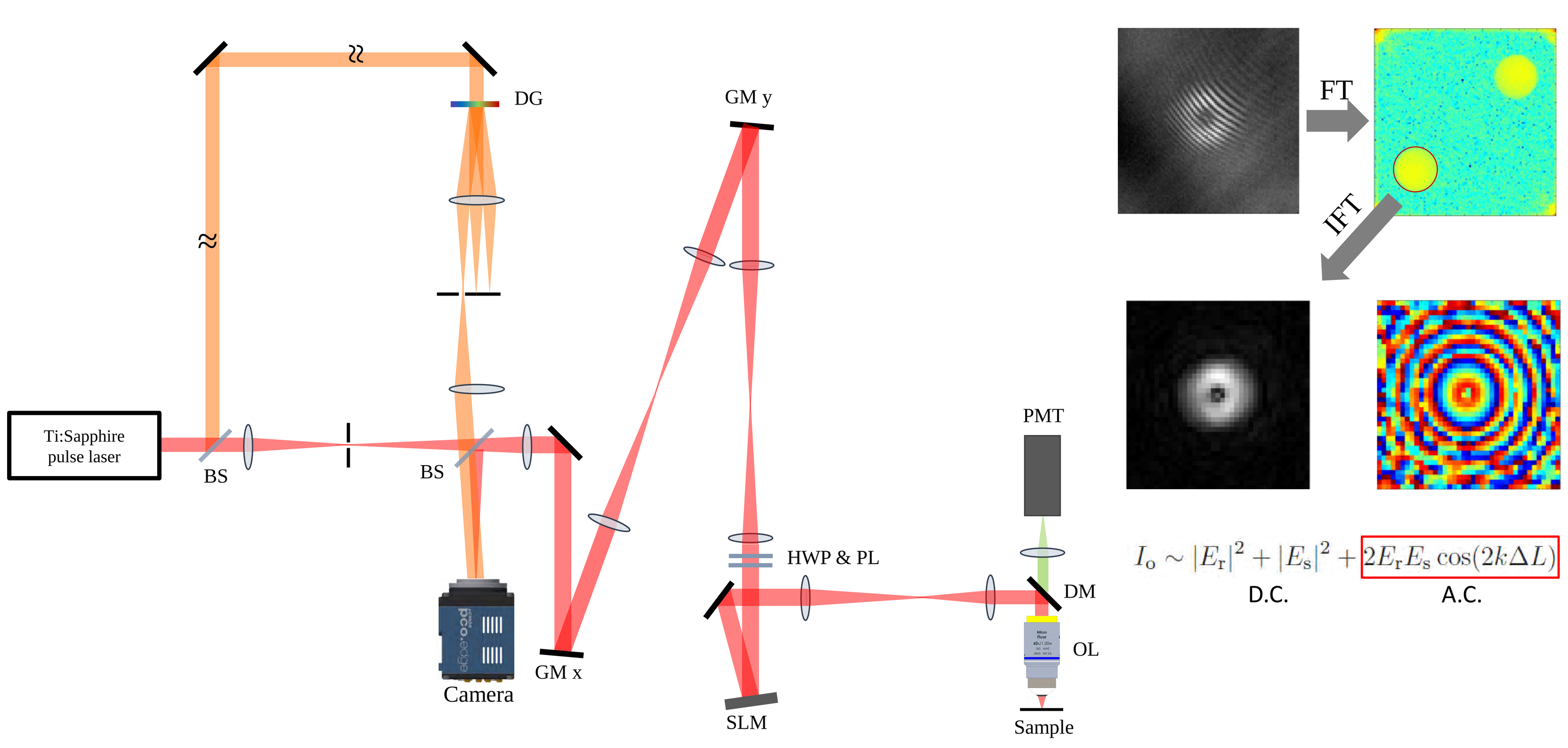
Center for Molecular Spectroscopy and Dynamics, Institute of Basic Science (IBS)
Department of Physics, Korea University

1. Troublesome in Bio-imaging



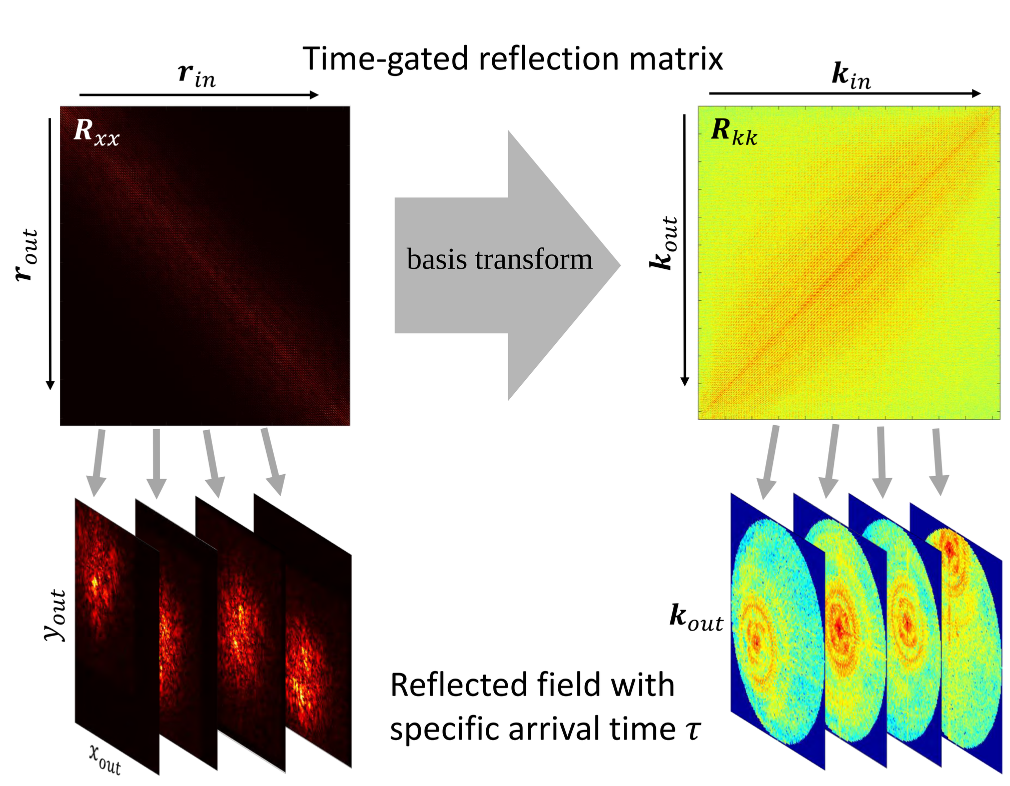
- ✓ *Biological tissues are an inhomogeneous medium that induces optical aberration*
- ✓ Due to optical aberration, image information is severely distorted.
- ✓ *We calibrate these optical aberrations through computational and physical methods*

2. Imaging Setup



- ✓ *Time-gated detection* by using low-coherence light source (Ti:Sa ; pulse width ~ 50 fs)
- ✓ Point spread function (PSF) detection by off-axis holographic microscopy
- ✓ *Wave front shaping* to correct the aberration induced by sample
- ✓ Reflection measurement

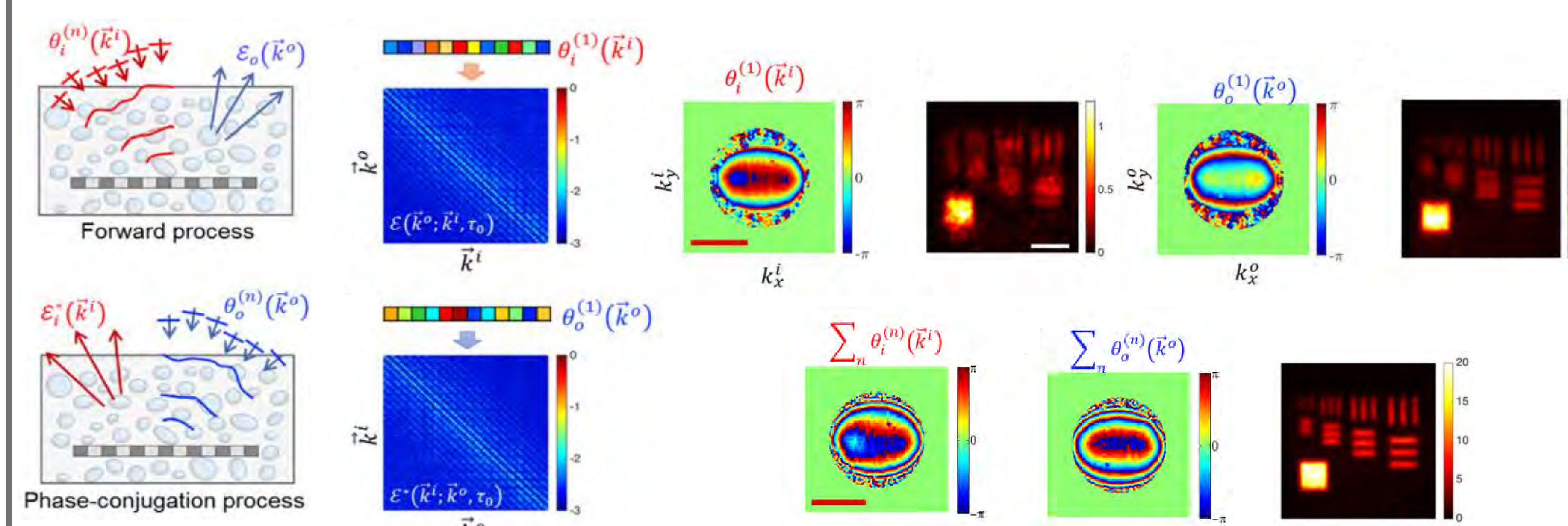
3. Time-resolved reflection matrix



- ✓ Time-gating was matched to the depth of target object.
- ✓ We used 10,000 illumination pattern within 1.0 NA to cover orthogonal basis for $40 \times 40\ \mu m^2$ FOV.
- ✓ We measured reflected image as each input illumination positions.
- ✓ Time-gated reflection matrix was matrix multiplication,

$$T_{xx} = A_{xx}O_{xx}A_{xx} \quad \Rightarrow \quad T_{kk} = R_{kx}A_{xx}O_{xx}A_{xx}R_{xk}$$
$$= [R_{kx}A_{xx}R_{xk}][R_{kx}O_{xx}R_{xk}][R_{kx}A_{xx}R_{xk}]$$
$$= A_{kk}O_{kk}A_{kk}$$

4. Closed-loop accumulation of single scattering (CLASS) microscopy



$$\mathcal{E}_o(\vec{k}^i + \Delta\vec{k}) = \sqrt{\alpha}\mathcal{O}(\Delta\vec{k}) \cdot P_i^a(\vec{k}^i)P_o^a(\vec{k}^i + \Delta\vec{k}) + \sqrt{\beta}\mathcal{E}_o^M(\vec{k}^i + \Delta\vec{k}; \tau_0).$$

$$P_i^a(\vec{k}^i) = P(\vec{k}^i) \cdot exp[-i\phi_i(\vec{k}^i)]$$
$$P_o^a(\vec{k}) = P(\vec{k}^o)\exp[-i\phi_o(\vec{k}^o)]$$

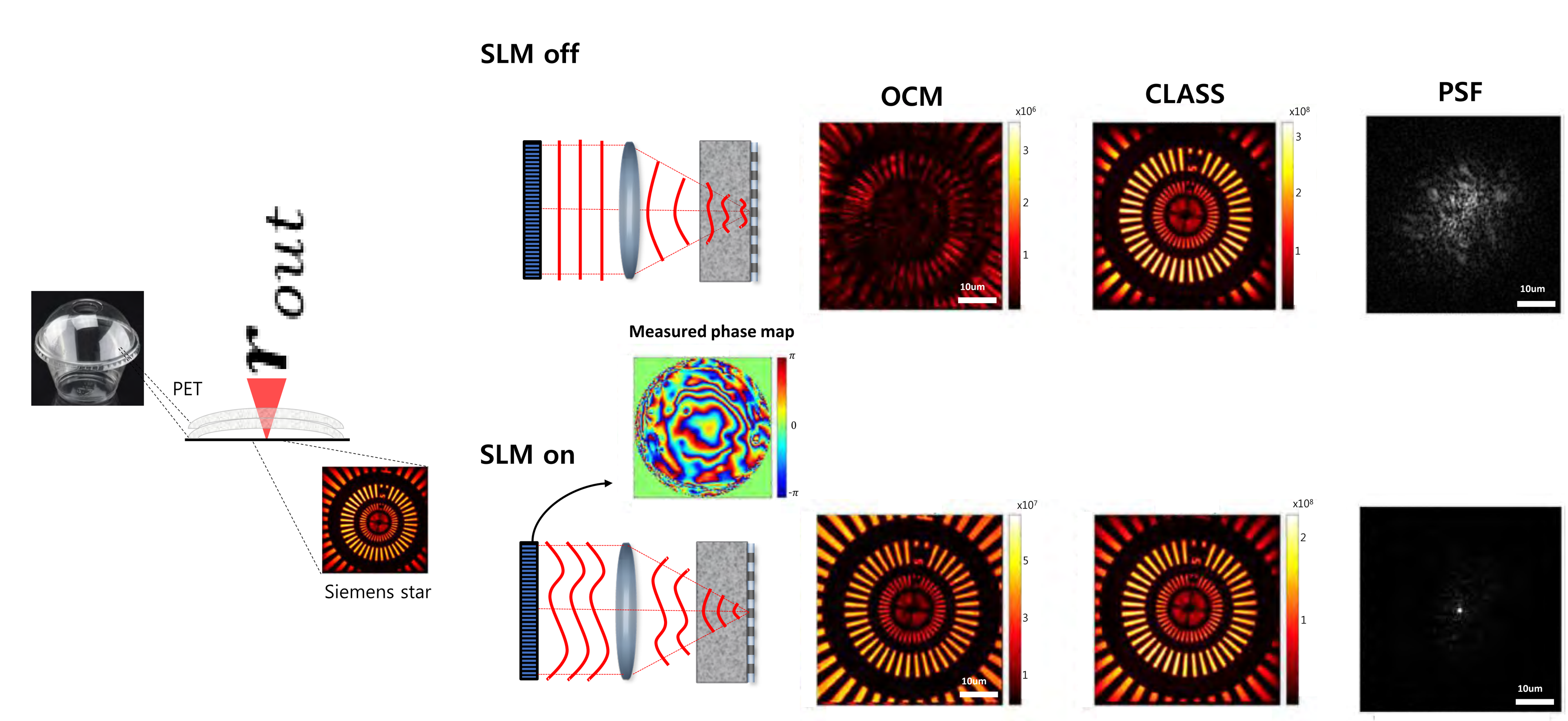
$$\mathcal{E}_{CASS}(\Delta\vec{k}) = \sum_{\vec{k}^i} \mathcal{E}_o(\vec{k}^i + \Delta\vec{k}) = \sqrt{\alpha}\mathcal{O}(\Delta\vec{k}) \cdot \sum_{\vec{k}^i} P_i^a(\vec{k}^i)P_o^a(\vec{k}^i + \Delta\vec{k}) + \sqrt{\beta}\sum_{\vec{k}^i} \mathcal{E}_o^M(\vec{k}^i + \Delta\vec{k}).$$

$$\left|\sum_{\vec{k}^i} P_i^a(\vec{k}^i)P_o^a(\vec{k}^i + \Delta\vec{k})\right| \le \left|\sum_{\vec{k}^i} P(\vec{k}^i)P(\vec{k}^i + \Delta\vec{k})\right|. \quad \Rightarrow \quad \max_{\theta_i^{(1)}(\vec{k}^i)} \sum_{\Delta\vec{k}} \left|\mathcal{E}_{CASS}^{(1)}(\Delta\vec{k})\right|^2.$$

$$\mathcal{E}_{CASS}^{pc}(\Delta\vec{k}) = \sqrt{\alpha}\mathcal{O}^*(\Delta\vec{k}) \cdot \sum_{\vec{k}^o} P_o^a(\vec{k}^o)^* P_i^a(\vec{k}^o - \Delta\vec{k})^* + \sqrt{\beta}\sum_{\vec{k}^o} \mathcal{E}_o^M(\vec{k}^o - \Delta\vec{k})^*.$$

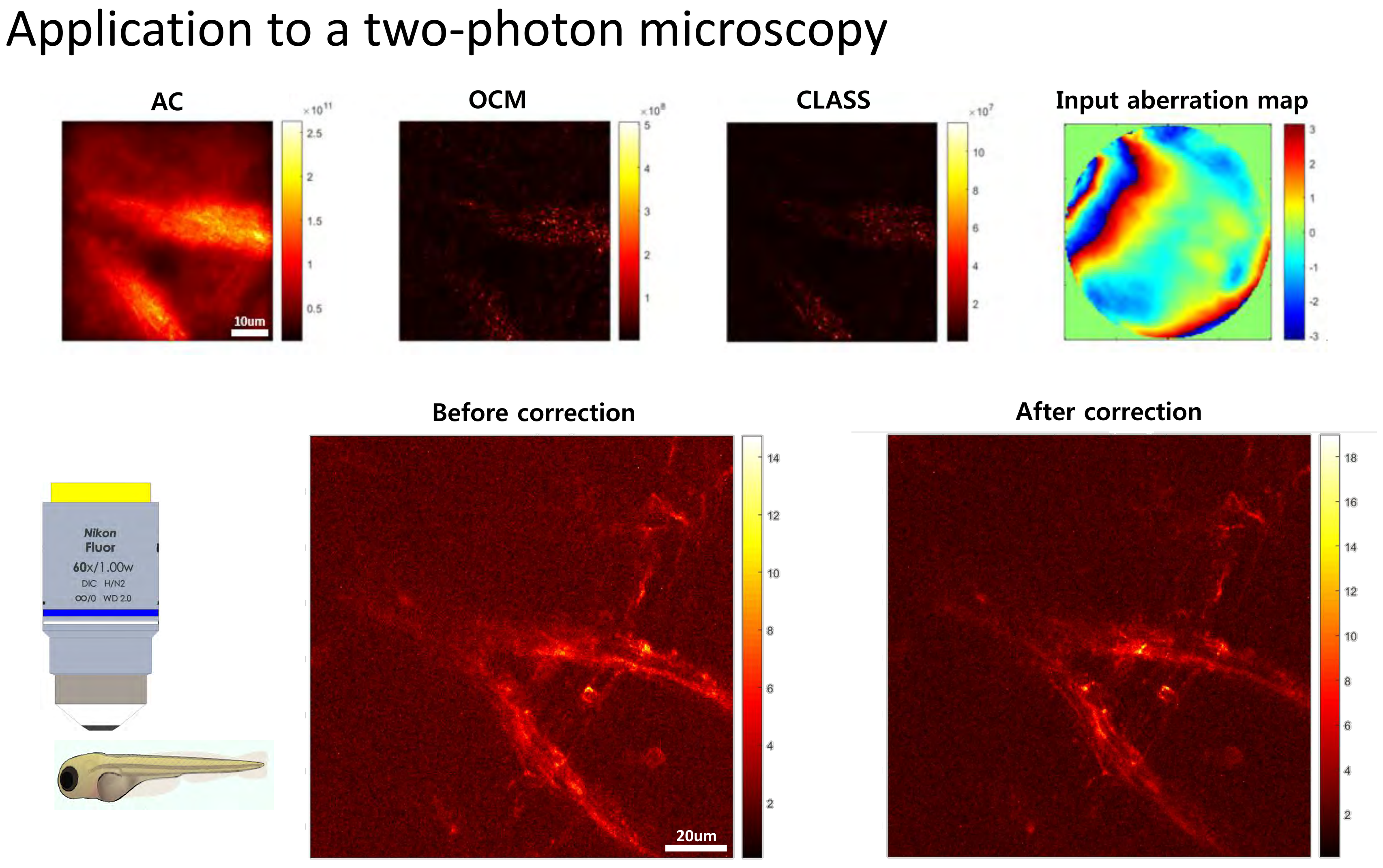
- ✓ The input/output phase map is accumulated by repeating the above process several times.
- ✓ The accumulated phase map when the intensity of the whole image converges to a constant value becomes the final input/output aberration map.

5. Physical wave-front shaping combined with CLASS microscopy



- ✓ The resolution and SBR of the reflected image have been improved because the PSF is sharpened after the physical aberration correction.

6. Application to a two-photon microscopy



- ✓ Two-photon image of nerve bundle of zebrafish stained with wtGFP.
- ✓ After the aberration correction, the SBR of the two-photon image increased and the resolution also improved.