计算光学成像与光信息处理技术前沿

第十二周课（ lecture 8 ）

差分相衬成像

左 超

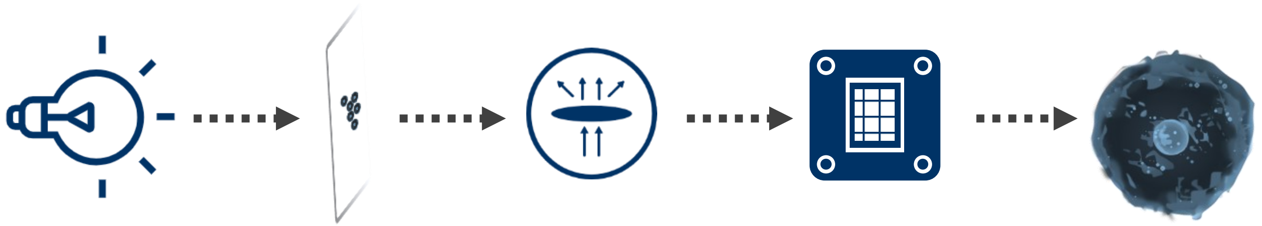
南京理工大学电光学院光电技术系

Jiangsu Key Laboratory of Spectral Imaging & Intelligent Sense (SIIS)
Nanjing University of Science and Technology,
Nanjing, Jiangsu Province 210094, China

电子工程与光电技术学院
School of Electronic and Optical Engineering

江苏省光谱成像与智能感知重点实验室
Jiangsu Key Laboratory of Spectral Imaging & Intelligent Sense

传统显微成像局限性

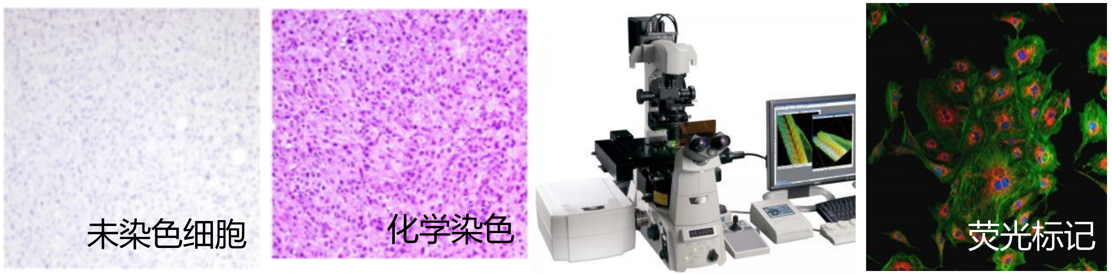


光源 物体 成像系统 传感器 图像

传统透镜式光学成像系统

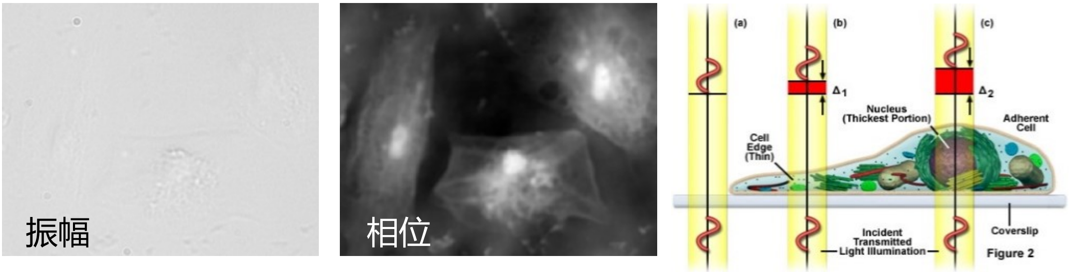
传统透镜式成像
点对点光强信号探测

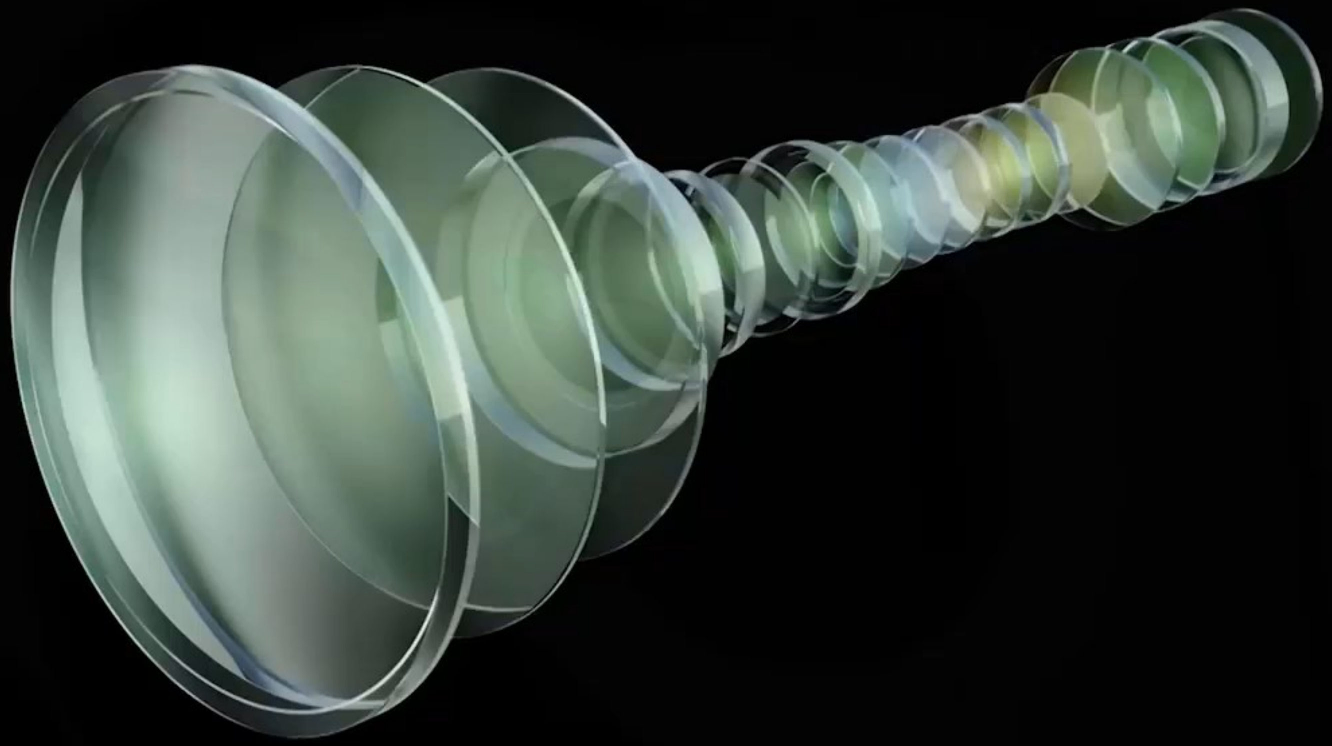
- **成像依赖染色标记**
- **无法定量探测相位**

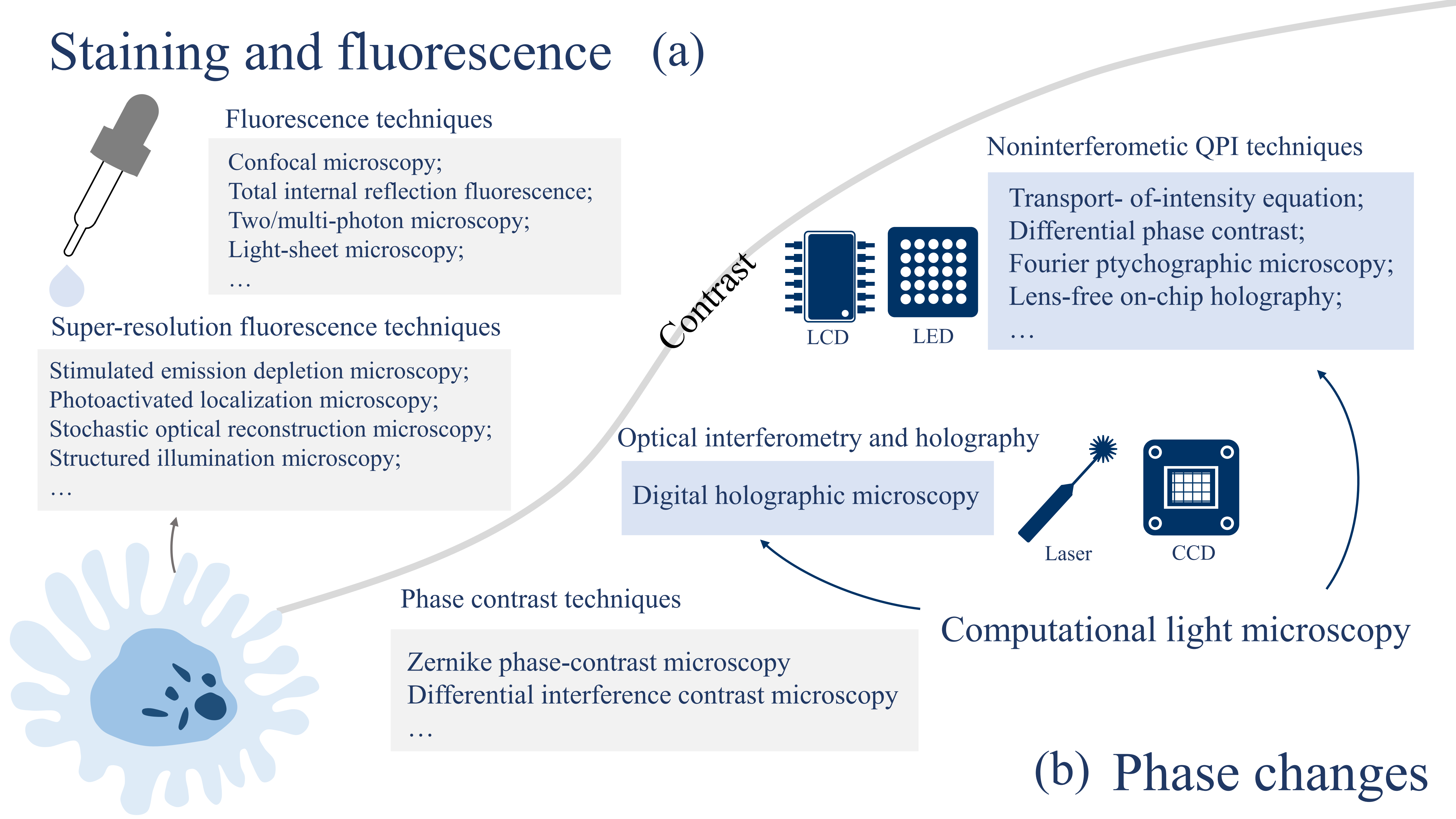


侵入式成像
(Invasive)

无标记成像
(Label-free)

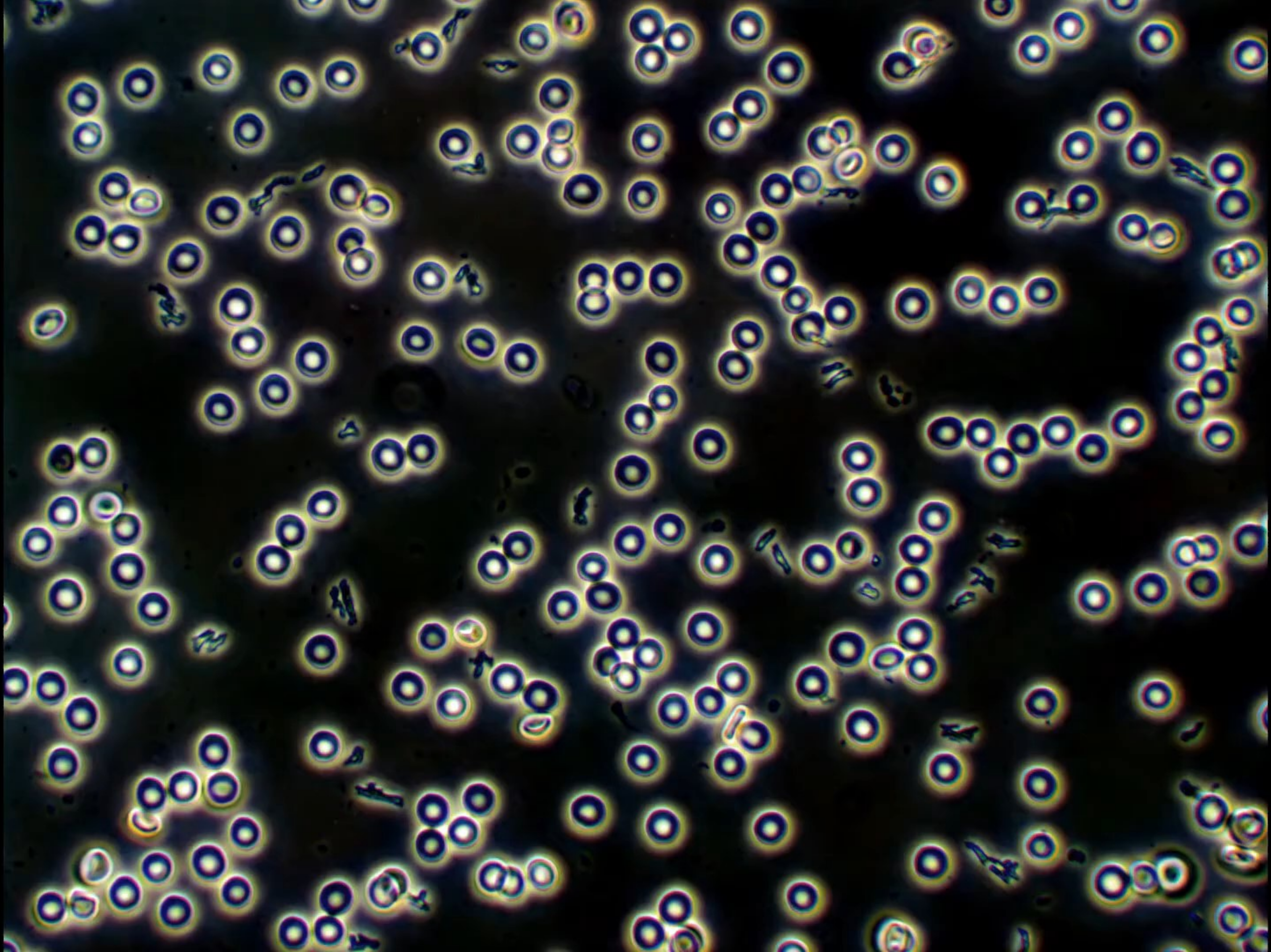




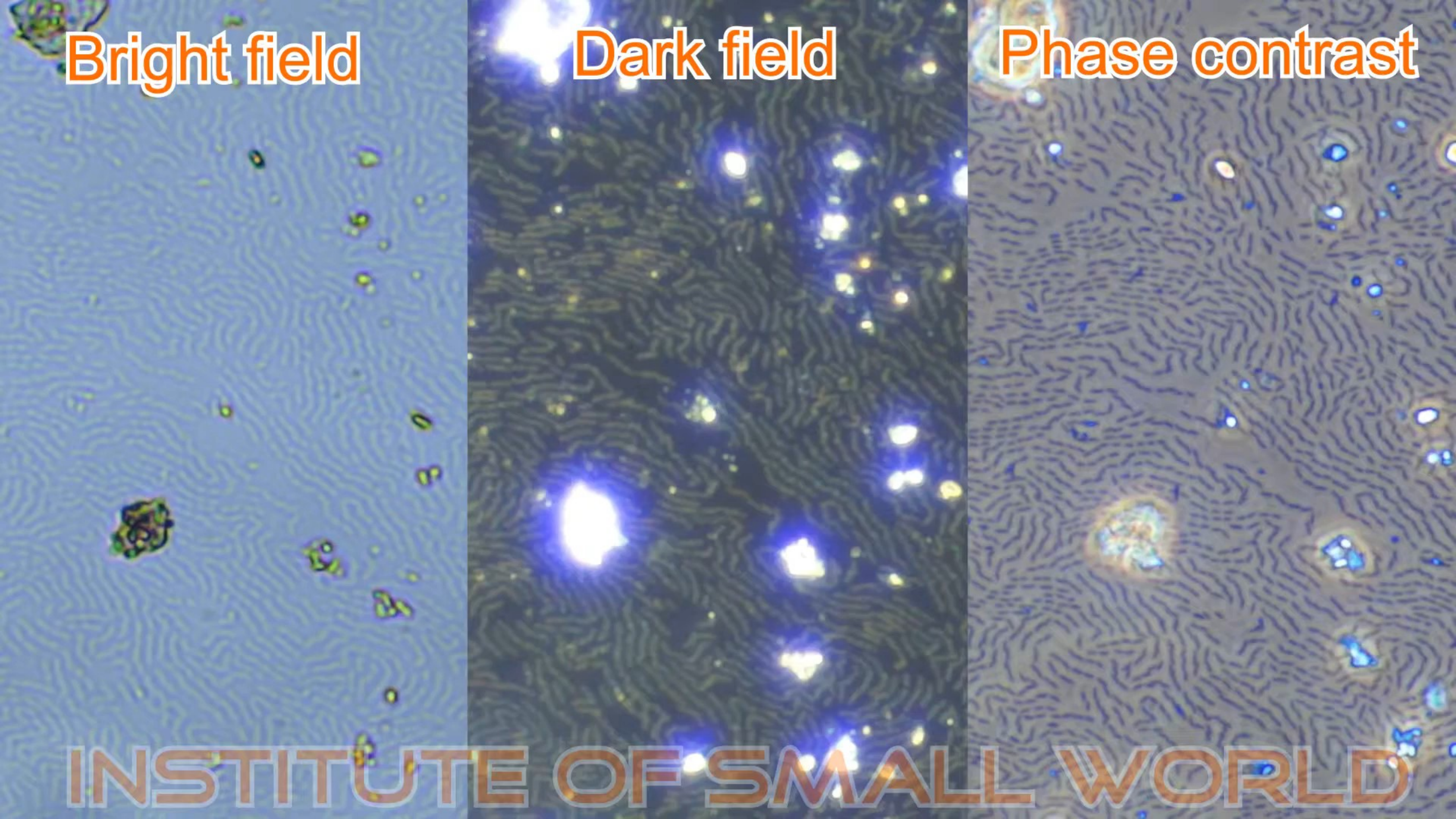

Staining and fluorescence (a)
Fluorescence techniques
Confocal microscopy;
Total internal reflection fluorescence;
Two/multi-photon microscopy;
Light-sheet microscopy;
…
Super-resolution fluorescence techniques
Stimulated emission depletion microscopy;
Photoactivated localization microscopy;
Stochastic optical reconstruction microscopy;
Structured illumination microscopy;
…
Contrast
LCD
LED
Noninterferometic QPI techniques
Transport- of-intensity equation;
Differential phase contrast;
Fourier ptychographic microscopy;
Lens-free on-chip holography;
…
Optical interferometry and holography
Digital holographic microscopy
Laser
CCD
Phase contrast techniques
Zernike phase-contrast microscopy
Differential interference contrast microscopy
…
Computational light microscopy
(b) Phase changes

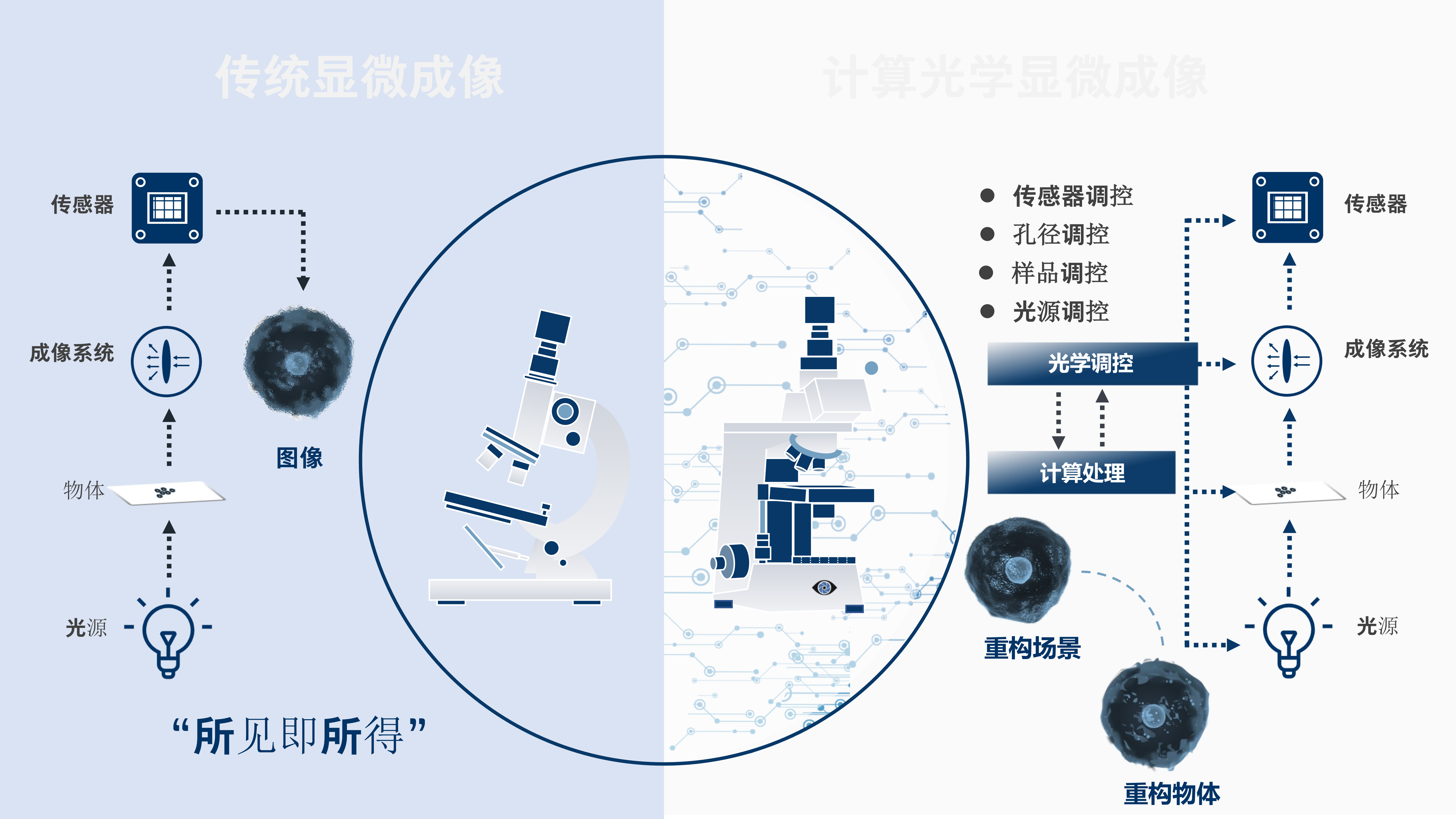
“无标记 ” 相衬成像技术

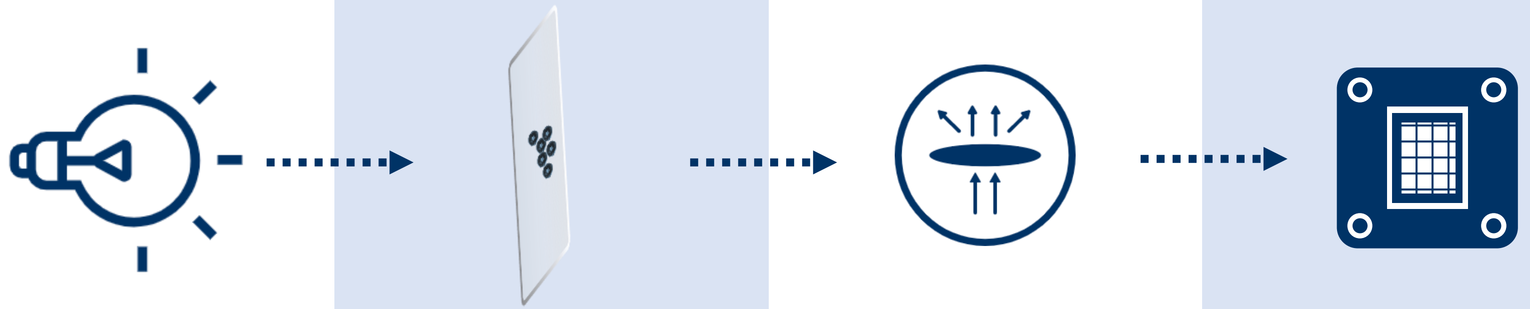
暗场显微镜



Dark field and phase contrast microscopes

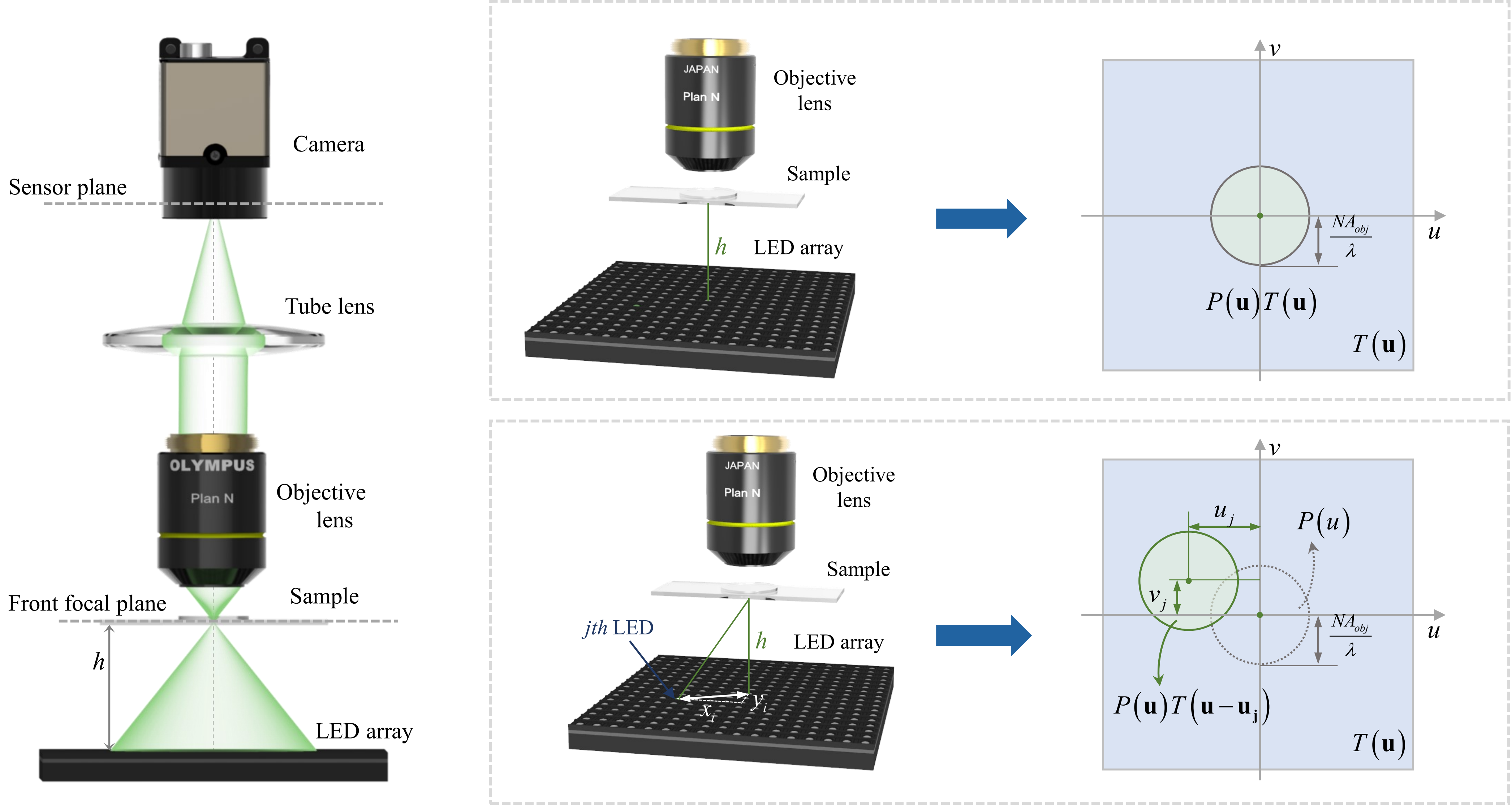

Bright field
Dark field
Phase contrast
INSTITUTE OF SMALL WORLD


传统显微成像
计算光学显微成像
传感器
成像系统
物体
光源
图像
“所见即所得”
传感器调控
孔径调控
样品调控
光源调控
光学调控
计算处理
传感器
成像系统
物体
光源
重构场景
重构物体



	Source	Sample	Imaging system	Detector
Principle	Linear decomposition	Iteraction Diffraction theory	Propagation or modulation	Intensity detection and sampling
Solution	Source points coherent modes	Scalar diffraction theory; Vector diffraction theory;	Linear systems theory	Amplitude square Non-coherent superposition
Analysis	Coherent properties	Superposition under plane-wave illumination	Linear spatially invariant system;	Time average
Model	Complex intensity; Wavelengh; Coherence function; Spectrum distribution; Azimuth; Polarization	Complex transmittance function; Absorption function; Phase function; Refractive index; Polarization characteristics;	2D/3D coherent spread function 2D/3D point spread function; Coherent transfer function; Optical transfer function; 4D phase-space model;	Spectral response function; Quantum efficiency; Single Photon; Noise statistics; Modulation transfer function; Nonlinearity;
Modulation	Intensity; Phase; Angle; Aperture; Wavelength; Spatial coherence; Temporal coherence; Polarization; Paraxial approximation; etc.	Pure phase/weak object/ slowly varying approximations; Born or Rytvo approximation; Multi-slice propagation model; Non-negative refractive index approximation; etc.	Imaging aperture; Out-of-focus; Light deflection; Light intensity masks; Chromatic dispersion etc.	Controlled displacement: lateral and axial; Spectrum integration: spectrum response color cross-talk martix; Pixelation effects: binning, downsampling, pixel response functon, Bayer filter; Polarization detection; etc.

◻ 倾斜照明


Camera
Sensor plane
Tube lens
OLYMPUS
Plan N
Objective lens
Front focal plane
Sample
h
LED array
JAPAN
Plan N
Objective lens
Sample
h
LED array
v
u
$\frac{NA_{obj}}{\lambda}$
$P(\mathbf{u})T(\mathbf{u})$
$T(\mathbf{u})$
JAPAN
Plan N
Objective lens
Sample
jth LED
h
LED array
x_i
y_i
v
u_j
v_j
$P(u)$
u
$\frac{NA_{obj}}{\lambda}$
$P(\mathbf{u})T(\mathbf{u}-\mathbf{u_j})$
$T(\mathbf{u})$

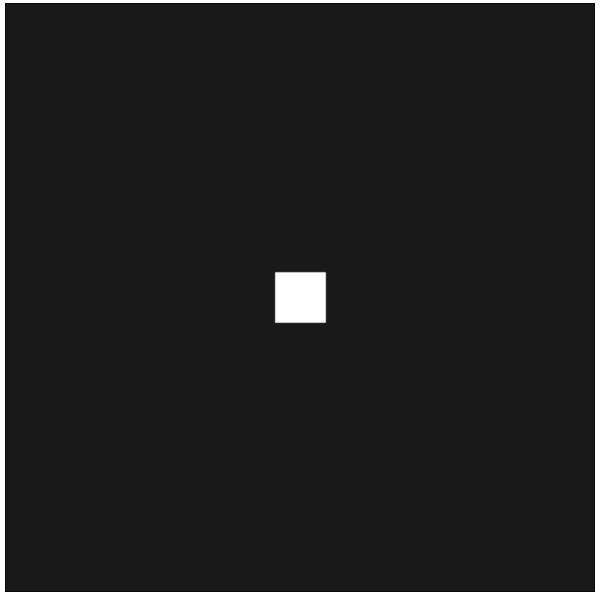
倾斜照明

$$F\{w\} = F\{ae^{i\varphi}\} = W$$

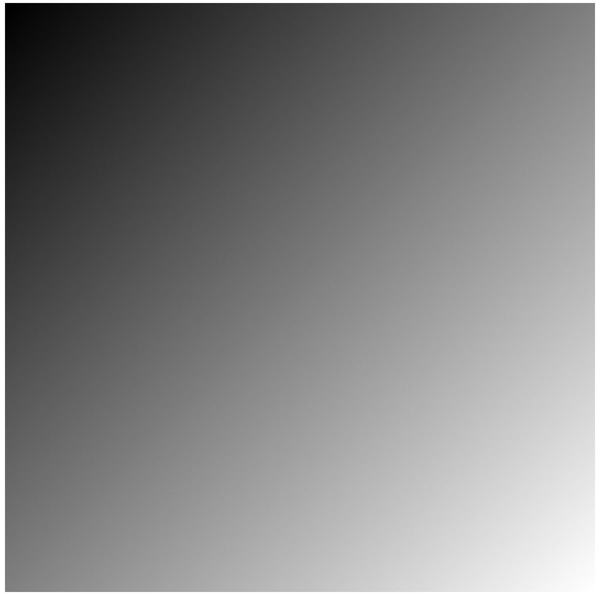
傅立叶变换对

$$F\{we^{i\theta}\} = F\{ae^{i\varphi}e^{i\theta}\} = W(u-k_x, v-k_y)$$

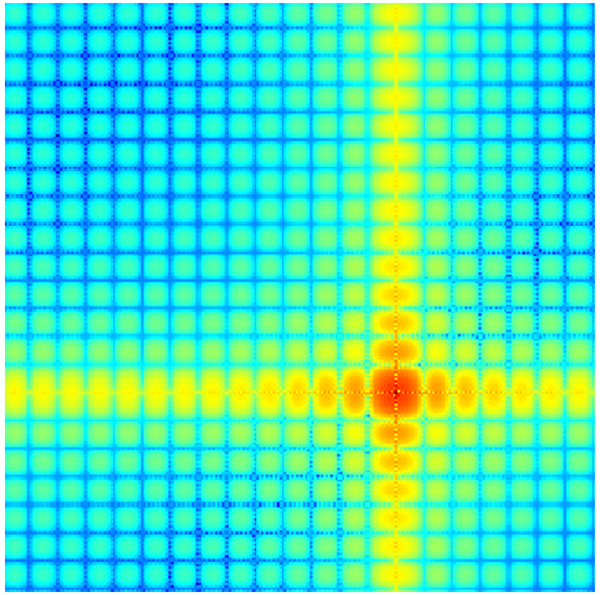
a



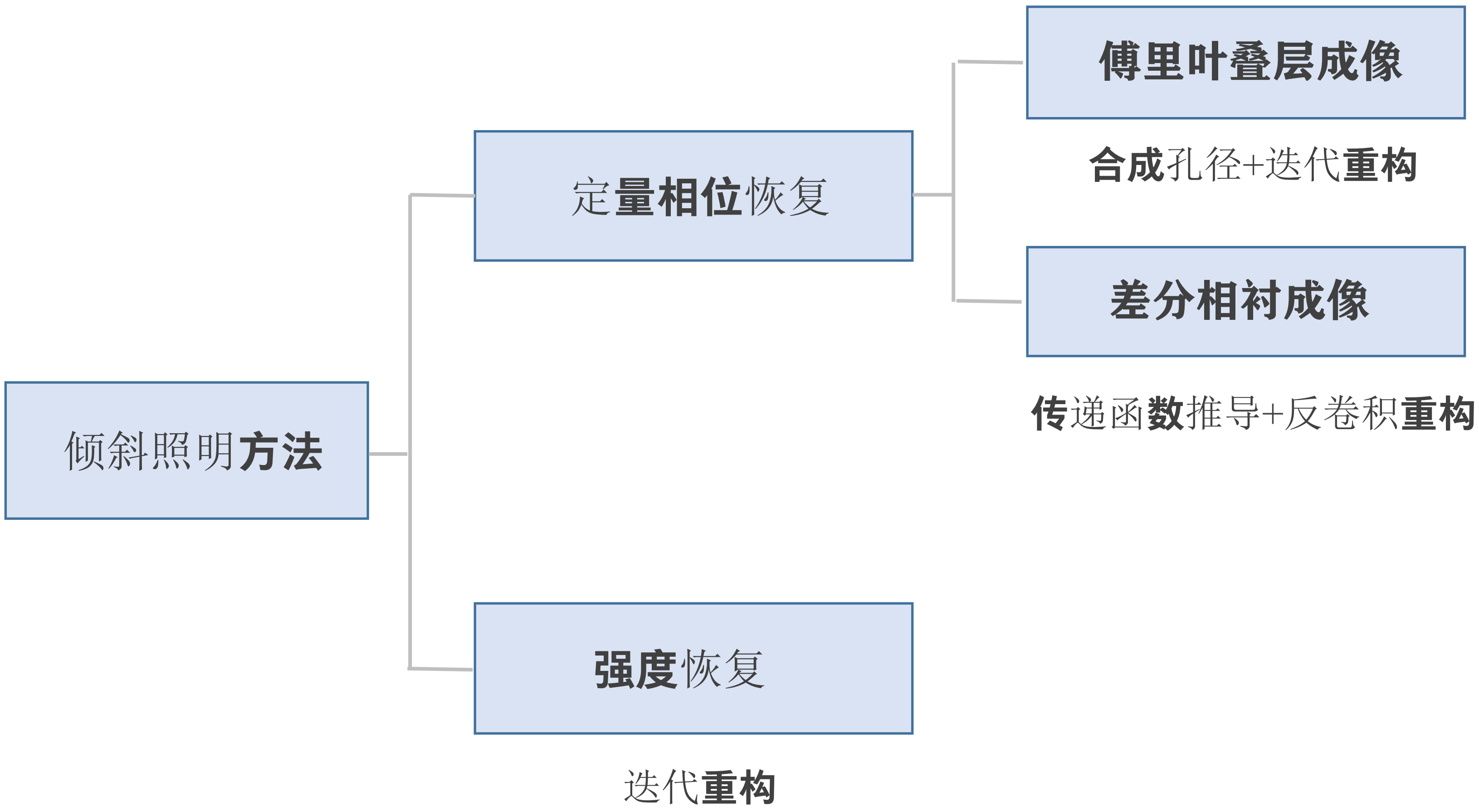
$\varphi + \theta$



$W(u-k_x, v-k_y)$



□ 倾斜照明


倾斜照明方法
定量相位恢复
傅里叶叠层成像
合成孔径+迭代重构
差分相衬成像
传递函数推导+反卷积重构
强度恢复
迭代重构

□ 部分相干成像系统图像生成模型

强度分布:

$$I(\mathbf{x}) = \iiint S(\mathbf{u})\hat{T}(\mathbf{u}_1)\hat{T}^*(\mathbf{u}_2)H(\mathbf{u}+\mathbf{u}_1)H^*(\mathbf{u}+\mathbf{u}_2)e^{j2\pi\mathbf{x}(\mathbf{u}_1-\mathbf{u}_2)}d\mathbf{u}_1 d\mathbf{u}_2 d\mathbf{u}$$

分离与样品**无关的系统**项**:**

$$I(\mathbf{x}) = \iint \hat{T}(\mathbf{u}_1)\hat{T}^*(\mathbf{u}_2)TCC\left(\mathbf{u}_1,\mathbf{u}_2\right)e^{j2\pi\mathbf{x}(\mathbf{u}_1-\mathbf{u}_2)}d\mathbf{u}_1 d\mathbf{u}_2$$

部分相干**传**递函**数**/交叉**传**递**系数**

$$C\left(\mathbf{u}_1,\mathbf{u}_2\right) = \iint S(\mathbf{u})H(\mathbf{u}+\mathbf{u}_1)H^*(\mathbf{u}+\mathbf{u}_2)d\mathbf{u}$$

□ 部分相干成像系统图像生成模型

$$I(\mathbf{x}) = \iiint S(\mathbf{u})\hat{T}(\mathbf{u}_1)\hat{T}^*(\mathbf{u}_2)H(\mathbf{u}+\mathbf{u}_1)H^*(\mathbf{u}+\mathbf{u}_2)e^{j2\pi\mathbf{x}(\mathbf{u}_1-\mathbf{u}_2)}d\mathbf{u}_1 d\mathbf{u}_2 d\mathbf{u}$$

1. 弱物体**近**似

$$t(x) = 1 + a(x) + i\phi(x)$$

$$I(x) = C(0,0) + 2\,\mathrm{Re}\left\{\int C(\mathbf{u},0)\left[a(\mathbf{u}) + j\phi(\mathbf{u})\right]e^{j2\pi\mathbf{x}\mathbf{u}}d\mathbf{u}\right\}$$

$$I(x) = C(0,0) + 2\,\mathrm{Re}\left\{\mathcal{F}^{-1}\left[A(\mathbf{u})a(\mathbf{u}) + jP(\mathbf{u})\phi(\mathbf{u})\right]\right\}$$

$A(\mathbf{u})$ 振**幅传**递函**数**　　$P(\mathbf{u})$ **相位传**递函**数**

2. 缓**变**物体**近**似

$$t(x) = e^{j\phi'(x)}$$

$$I(\mathbf{x}) = a^2 C\left(\frac{\phi'}{2\pi}, \frac{\phi'}{2\pi}\right)$$

$C(\mathbf{u},\mathbf{u})$ **相位**梯**度传**递函**数**

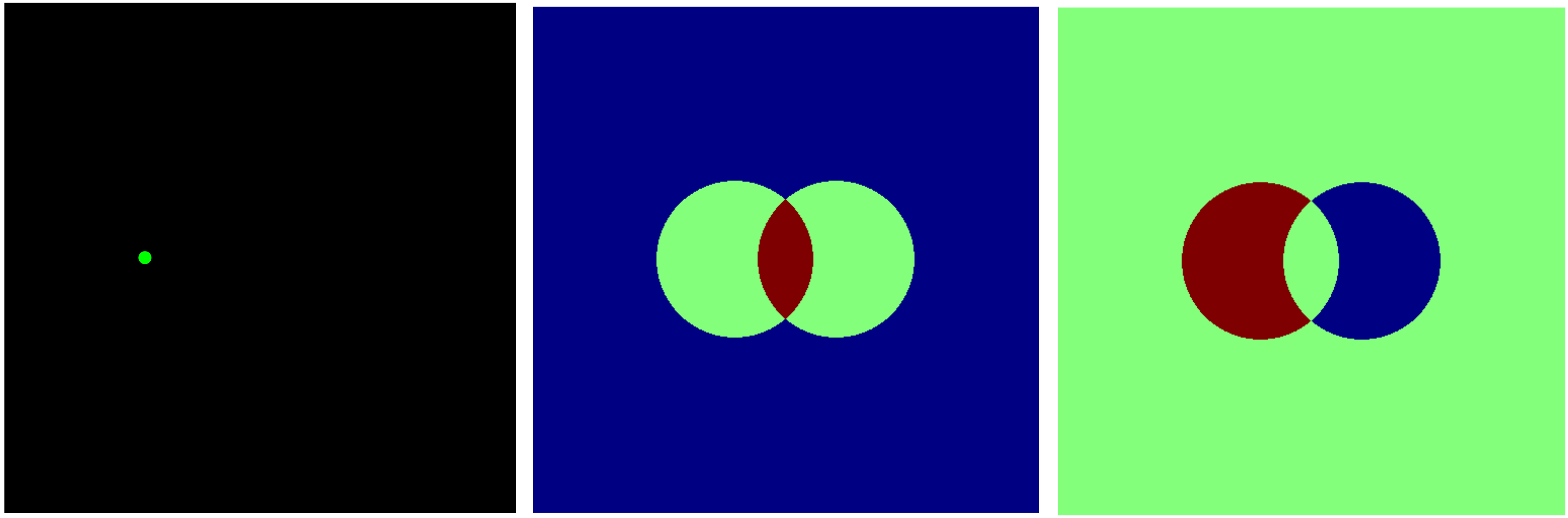
Hamilton, D. K., C. J. R. Sheppard, and T. Wilson. "Improved imaging of phase gradients in scanning optical microscopy." Journal of microscopy 135.3 (1984): 275-286.

□ 弱物体近似

$$A(\mathbf{u}) = \iint S(\mathbf{u}')P^*(\mathbf{u}')P(\mathbf{u}+\mathbf{u}')d\mathbf{u}' + \iint S(\mathbf{u}')P(\mathbf{u}')P^*(\mathbf{u}-\mathbf{u}')d\mathbf{u}'$$

$$P(\mathbf{u}) = \iint S(\mathbf{u}')P^*(\mathbf{u}')P(\mathbf{u}+\mathbf{u}')d\mathbf{u}' - \iint S(\mathbf{u}')P(\mathbf{u}')P^*(\mathbf{u}-\mathbf{u}')d\mathbf{u}'$$

单点倾斜照明 振幅传递函数 相位传递函数



差分相衬显微成像基础知识

□ 弱物体近似下的定量相位求解

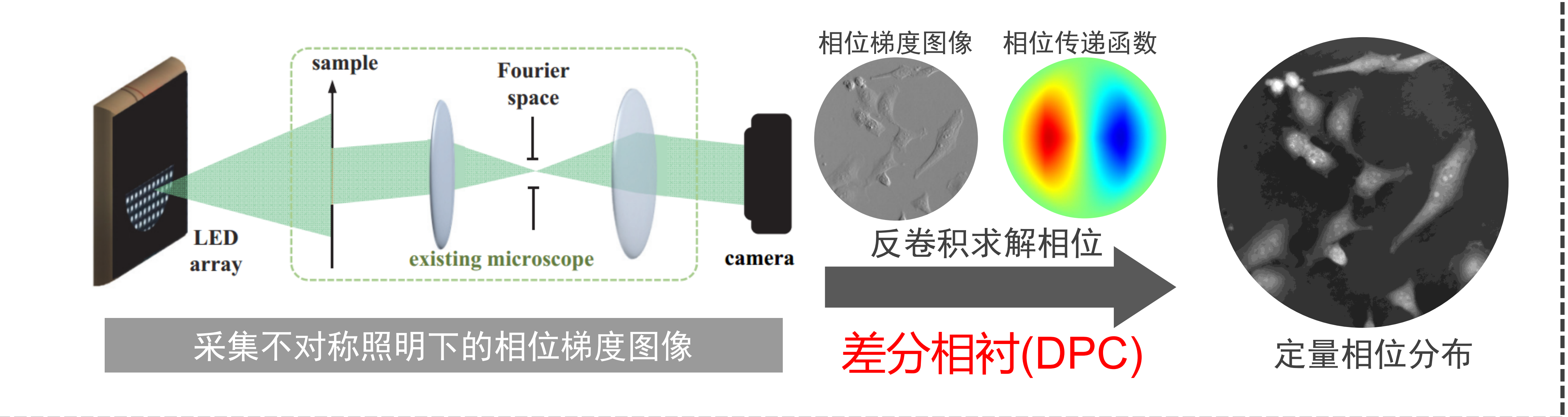
$$I(x) = B\delta(\mathbf{u}) + A(\mathbf{u})a(\mathbf{u}) + jP(\mathbf{u})\phi(\mathbf{u})$$

消去背景项和吸收项

$$I_{DPC} = \frac{I_L(x) - I_R(x)}{I_L(x) + I_R(x)}$$

反卷积**求解**:

$$\phi = \mathcal{F}^{-1}\left\{\frac{\sum P_j^*(\mathbf{u}) I_{DPC,j}(\mathbf{u})}{\sum |P_j(\mathbf{u})|^2 + \alpha}\right\}$$



Hamilton, D. K., & Sheppard, C. J. R. (1984). Journal of microscopy, 133(1), 27-39

缓变物体近似

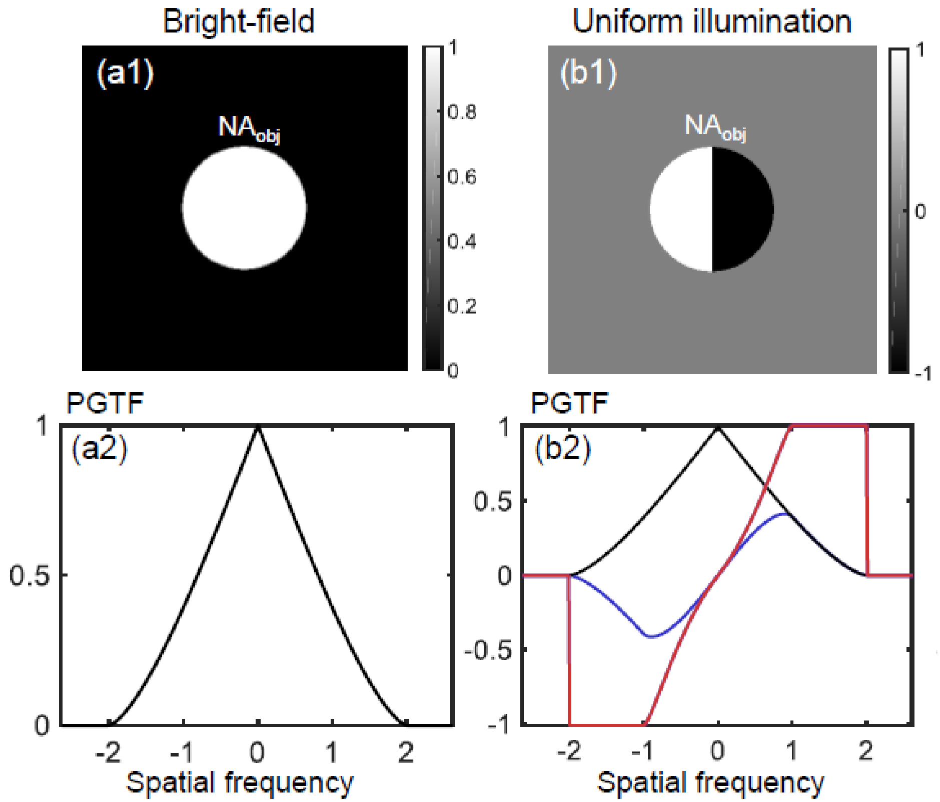
$$I(\mathbf{x}) = a^2 C\left(\frac{\phi'}{2\pi}, \frac{\phi'}{2\pi}\right)$$

相位梯度传递函数

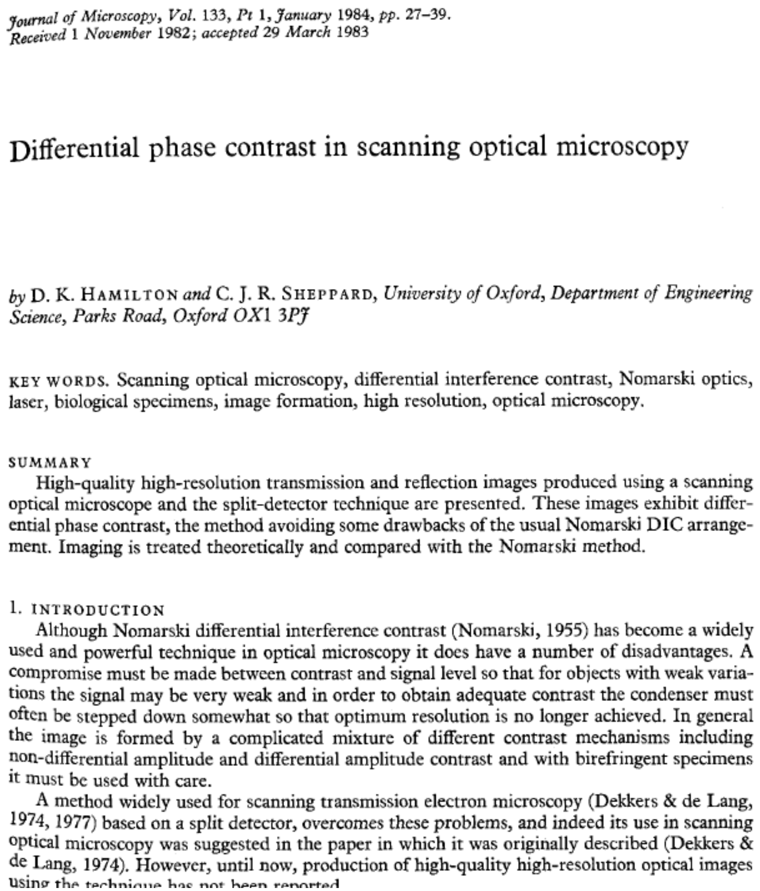
$$PGTH = C(\mathbf{u},\mathbf{u}) = \int S(\mathbf{u}'-\mathbf{u})\left|P(\mathbf{u}')\right|^2 d\mathbf{u}'$$

相位梯度积分

$$\phi = \mathrm{Im}\left[\mathcal{F}^{-1}\begin{cases}\dfrac{\mathcal{F}\{\phi'_x+\phi'_y\}}{2\pi(u+iv)}, & |\mathbf{u}|\neq 0 \\ C, & |\mathbf{u}|=0\end{cases}\right]$$



扫描式差分相衬成像系统


Journal of Microscopy, Vol. 133, Pt 1, January 1984, pp. 27–39.
Received 1 November 1982; accepted 29 March 1983

Differential phase contrast in scanning optical microscopy

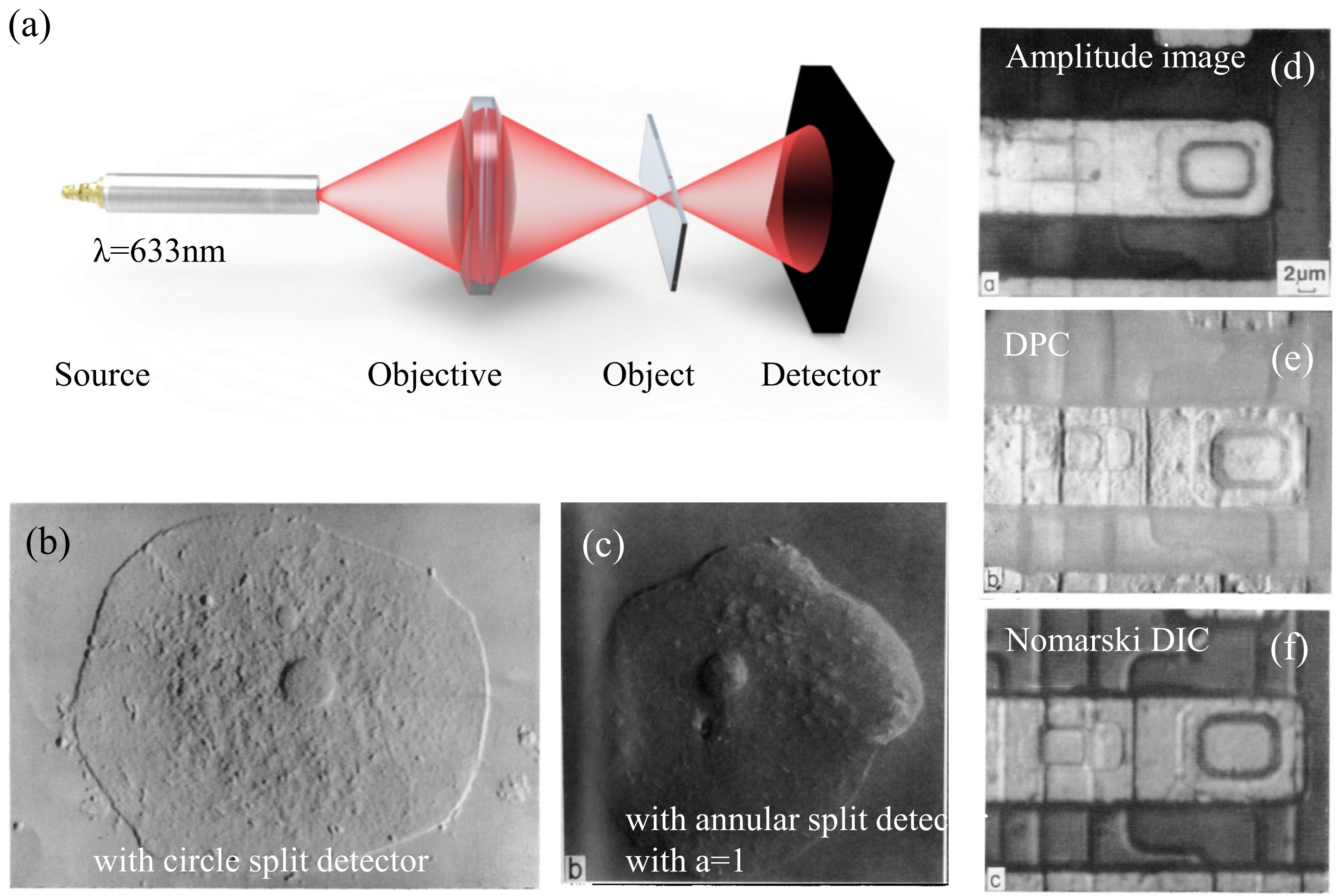
by D. K. Hamilton and C. J. R. Sheppard, University of Oxford, Department of Engineering Science, Parks Road, Oxford OX1 3PJ

KEY WORDS. Scanning optical microscopy, differential interference contrast, Nomarski optics, laser, biological specimens, image formation, high resolution, optical microscopy.

SUMMARY
High-quality high-resolution transmission and reflection images produced using a scanning optical microscope and the split-detector technique are presented. These images exhibit differential phase contrast, the method avoiding some drawbacks of the usual Nomarski DIC arrangement. Imaging is treated theoretically and compared with the Nomarski method.

1. INTRODUCTION
Although Nomarski differential interference contrast (Nomarski, 1955) has become a widely used and powerful technique in optical microscopy it does have a number of disadvantages. A compromise must be made between contrast and signal level so that for objects with weak variations the signal may be very weak and in order to obtain adequate contrast the condenser must often be stepped down somewhat so that optimum resolution is no longer achieved. In general the image is formed by a complicated mixture of different contrast mechanisms including non-differential amplitude and differential amplitude contrast and with birefringent specimens it must be used with care.
A method widely used for scanning transmission electron microscopy (Dekkers & de Lang, 1974, 1977) based on a split detector, overcomes these problems, and indeed its use in scanning optical microscopy was suggested in the paper in which it was originally described (Dekkers & de Lang, 1974). However, until now, production of high-quality high-resolution optical images using the technique has not been reported

Hamilton, D. K., & Sheppard, C. J. R. (1984).



全场差分相衬成像系统

1924 OPTICS LETTERS / Vol. 34, No. 13 / July 1, 2009

Quantitative phase-gradient imaging at high resolution with asymmetric illumination-based differential phase contrast

Shalin B. Mehta[1,2,4,*] and Colin J. R. Sheppard[1,2,3]
[1]Optical Bioimaging Lab, Division of Bioengineering, National University of Singapore, Block-E3A, #7-10, 7 Engineering Drive 1, Singapore 117574
[2]NUS Graduate School for Integrative Sciences and Engineering, 28 Medical Drive, Singapore 117456
[3]Department of Biological Sciences, National University of Singapore, 14 Science Drive, Singapore 117543
[4] shalin@nus.edu.sg
*Corresponding author: shalin@nus.edu.sg

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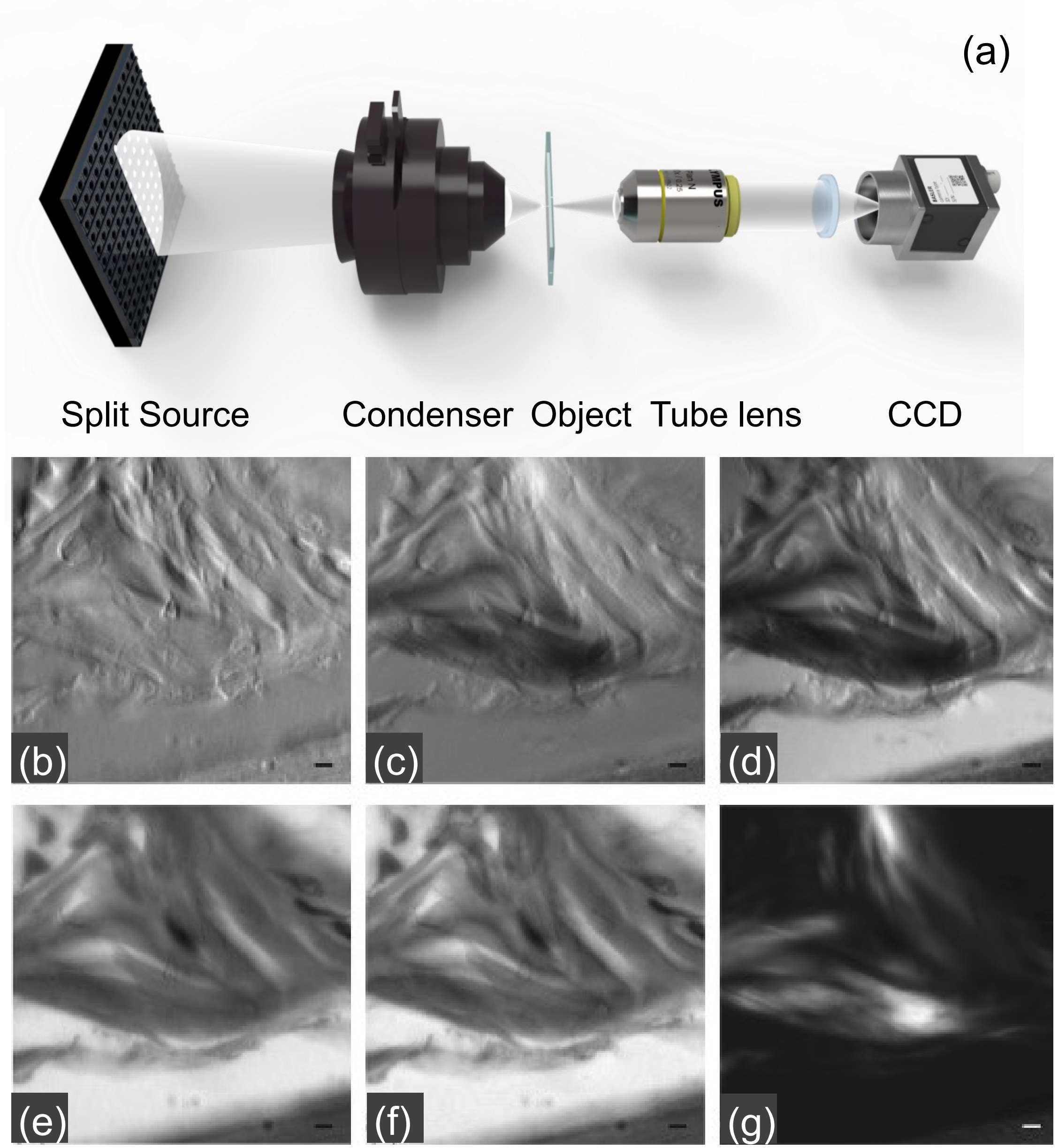
We describe a full-field phase-gradient imaging method: asymmetric illumination-based differential phase contrast (AIDPC). Imaging properties of AIDPC are evaluated using the phase-gradient transfer-function approach and elucidated with experimental images of an optical fiber and a histochemical preparation of a skeletal muscle section. In comparison with full-field differential interference contrast, AIDPC does not require phase shifting for quantitative imaging of phase gradient, provides artifact-free images of birefringent specimens, requires shorter camera exposure, and has larger depth of focus. It is amenable to transfer-function engineering, simultaneous fluorescence imaging, and automated live cell imaging. © 2009 Optical Society of America

OCIS codes: 110.0180, 110.4850, 100.5070, 110.4980.

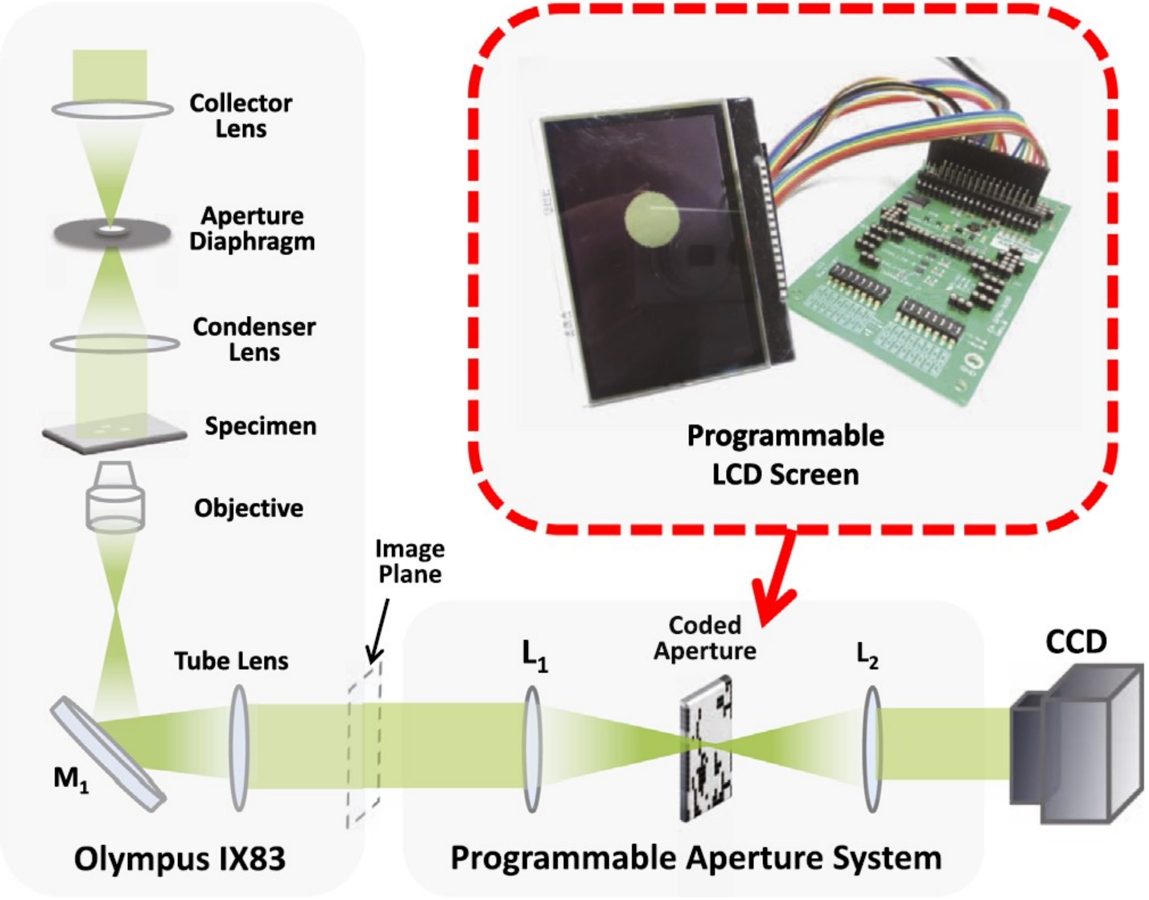
Imaging of biological specimens requires special optical processing to translate the optical thickness (i.e., phase) information to image intensity. Direct quantitative measurement of phase requires use of coherent illumination, leading to limited spatial resolution, lack of optical sectioning, and speckle from imperfections in the optical train. Phase-gradient imaging methods such as Nomarski's differential interference contrast (DIC) can accommodate large illumination apertures (i.e., partially coherent illumination), alleviating the above problems. In scanning optical microscopy, an intrinsically linear phase-gradient contrast method, termed differential phase contrast (DPC) has been evaluated [1–3]. In contrast to DPC, DIC images a complex mix of amplitude and phase-gradient information, necessitating approaches such as phase shifting (PSDIC) to establish a linear rela-

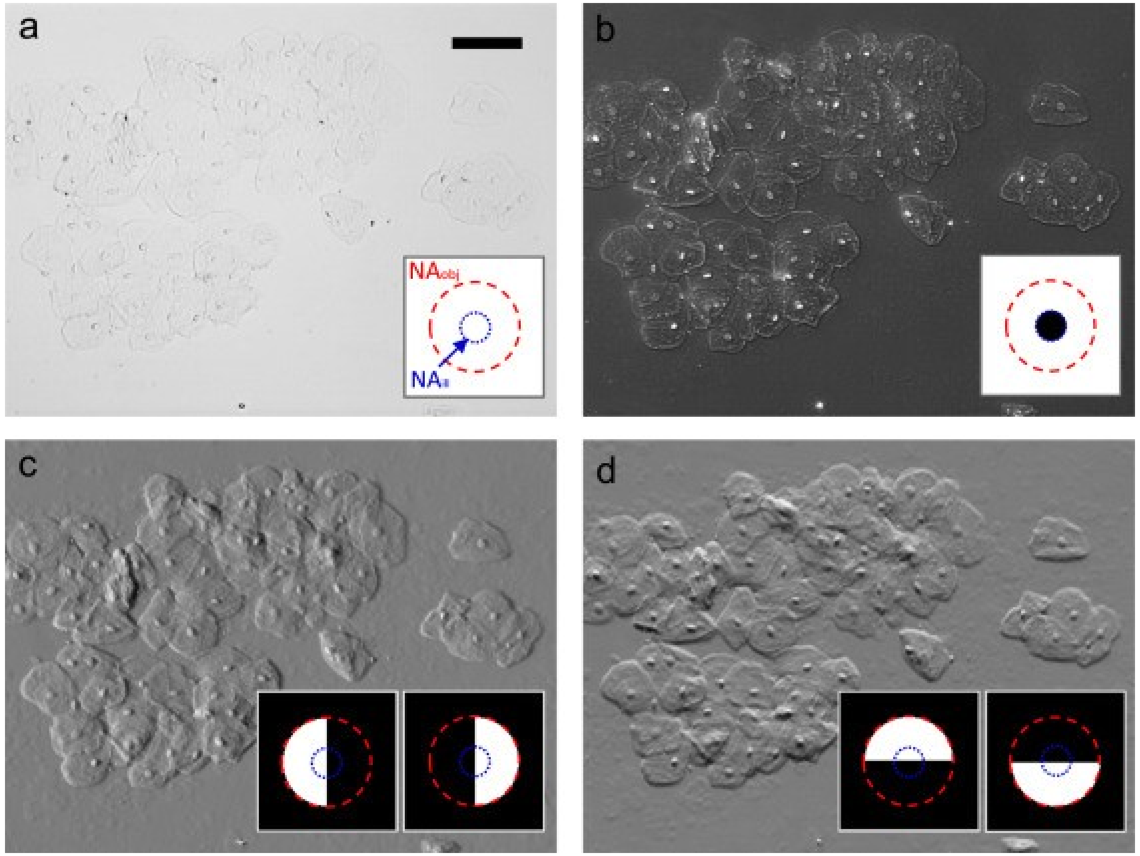
tical to those of the full-field system with an incoherent source if two conditions are met [11]: (1) each system has the same objective apertures (i.e., pupil amplitude), $P_o(\xi,\eta)$ and (2) the sensitivity distribution of the detector in the scanning system is the same as the intensity distribution of the condenser aperture, $|P_c(\xi,\eta)|^2$, in the full-field system. In scanning DPC, a split-detector or a quadrant diode is placed in the Fourier plane of the collector, and the image is formed by subtracting intensities recorded by two halves of the detector. A reciprocal wide-field DPC system has an antisymmetric condenser aperture with half of the aperture having negative effective intensity. We synthesize negative condenser intensity by subtracting two images acquired with semicircular condenser apertures in the direction of differentiation

Mehta S. B. & Sheppard, C. J. R. (2009).



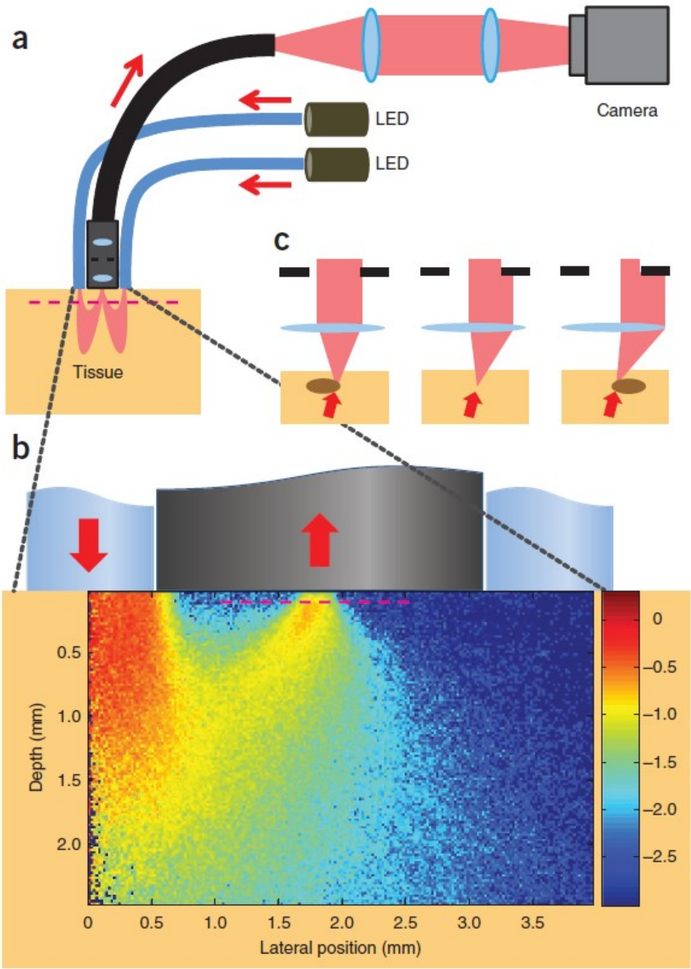
□ 基于LCD的可编程孔径差分相衬成像系统

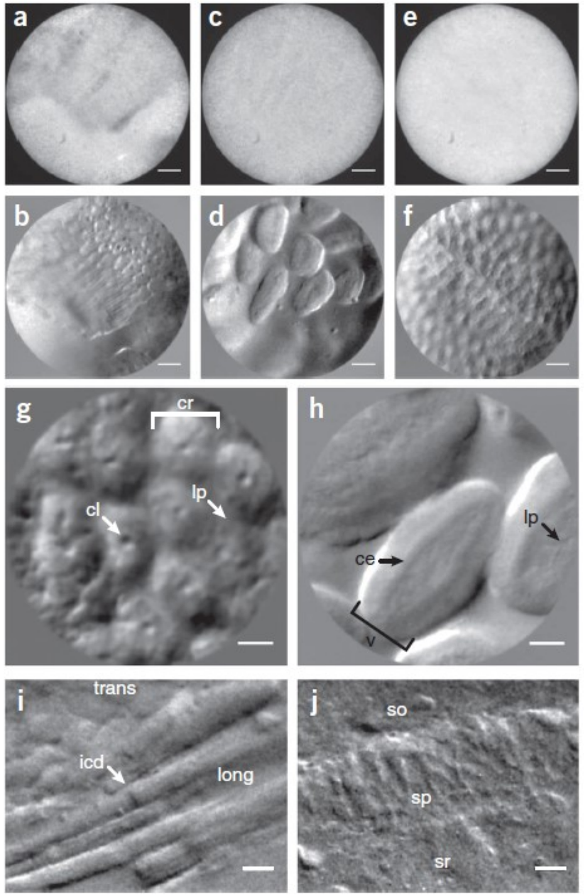


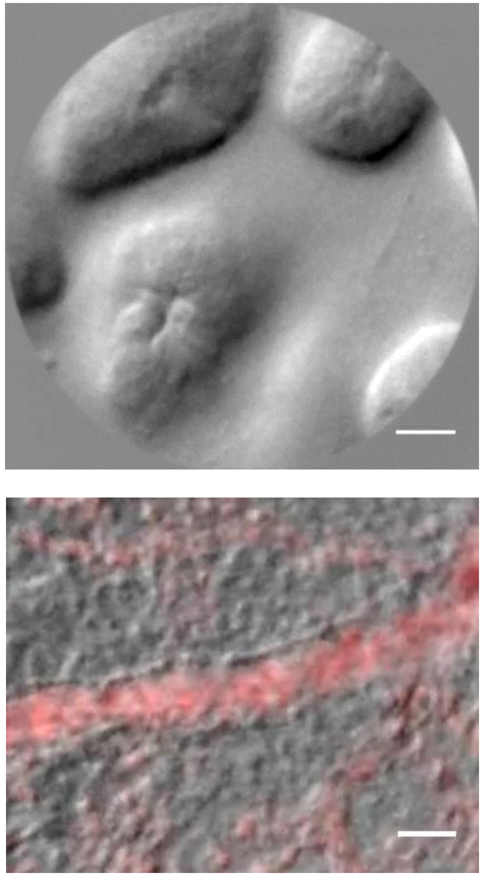


差分相衬成像技术发展

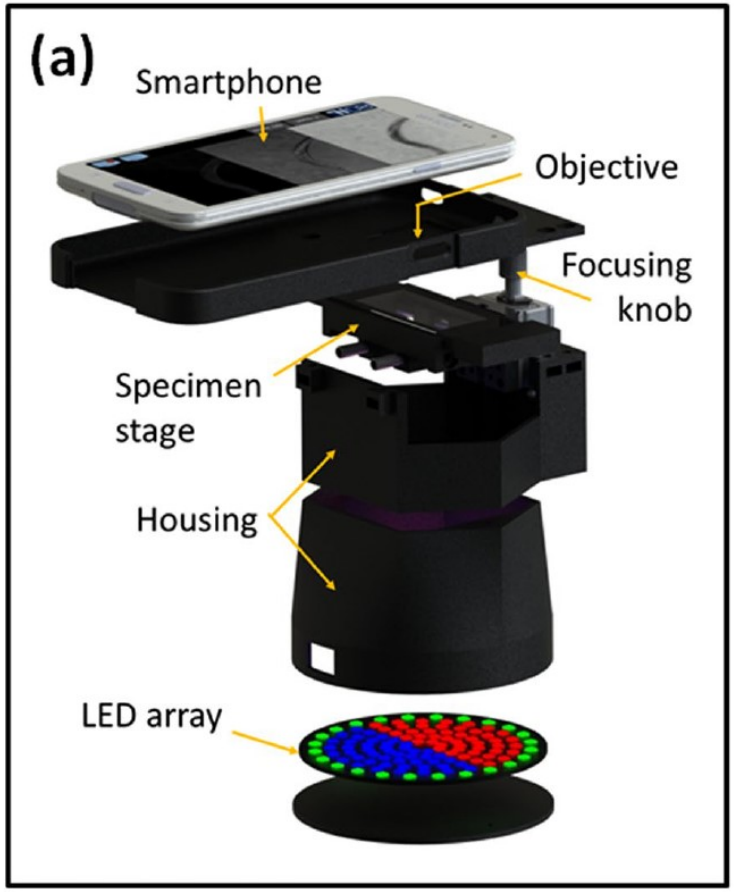
□ 和内窥镜相结合，用于手术过程中成像

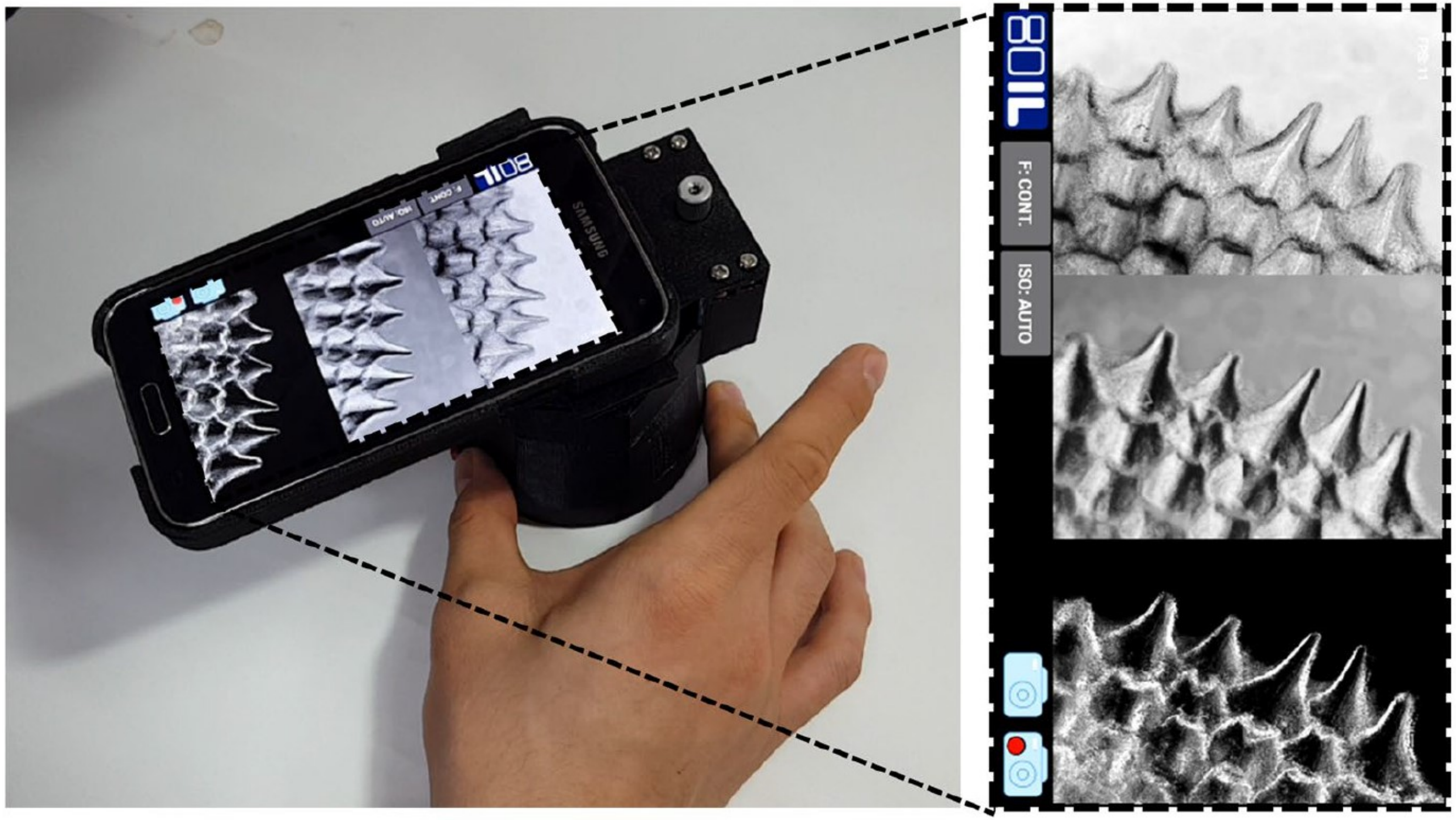




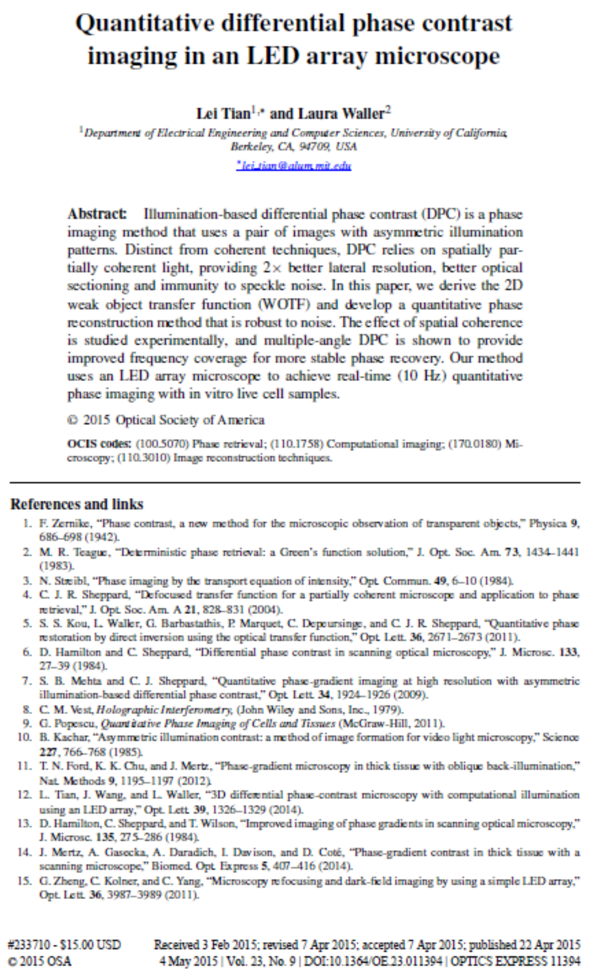


□ 结合智能手机，便携化差分相衬成像系统





差分相衬成像定量相位恢复算法



Quantitative differential phase contrast imaging in an LED array microscope

Lei Tian[1,*] and Laura Waller[2]

[1] *Department of Electrical Engineering and Computer Sciences, University of California, Berkeley, CA, 94709, USA*

[*] lei_tian@alum.mit.edu

Abstract: Illumination-based differential phase contrast (DPC) is a phase imaging method that uses a pair of images with asymmetric illumination patterns. Distinct from coherent techniques, DPC relies on spatially partially coherent light, providing 2× better lateral resolution, better optical sectioning and immunity to speckle noise. In this paper, we derive the 2D weak object transfer function (WOTF) and develop a quantitative phase reconstruction method that is robust to noise. The effect of spatial coherence is studied experimentally, and multiple-angle DPC is shown to provide improved frequency coverage for more stable phase recovery. Our method uses an LED array microscope to achieve real-time (10 Hz) quantitative phase imaging with in vitro live cell samples.

OCIS codes: (100.5070) Phase retrieval; (110.1758) Computational imaging; (170.0180) Microscopy; (110.3010) Image reconstruction techniques.

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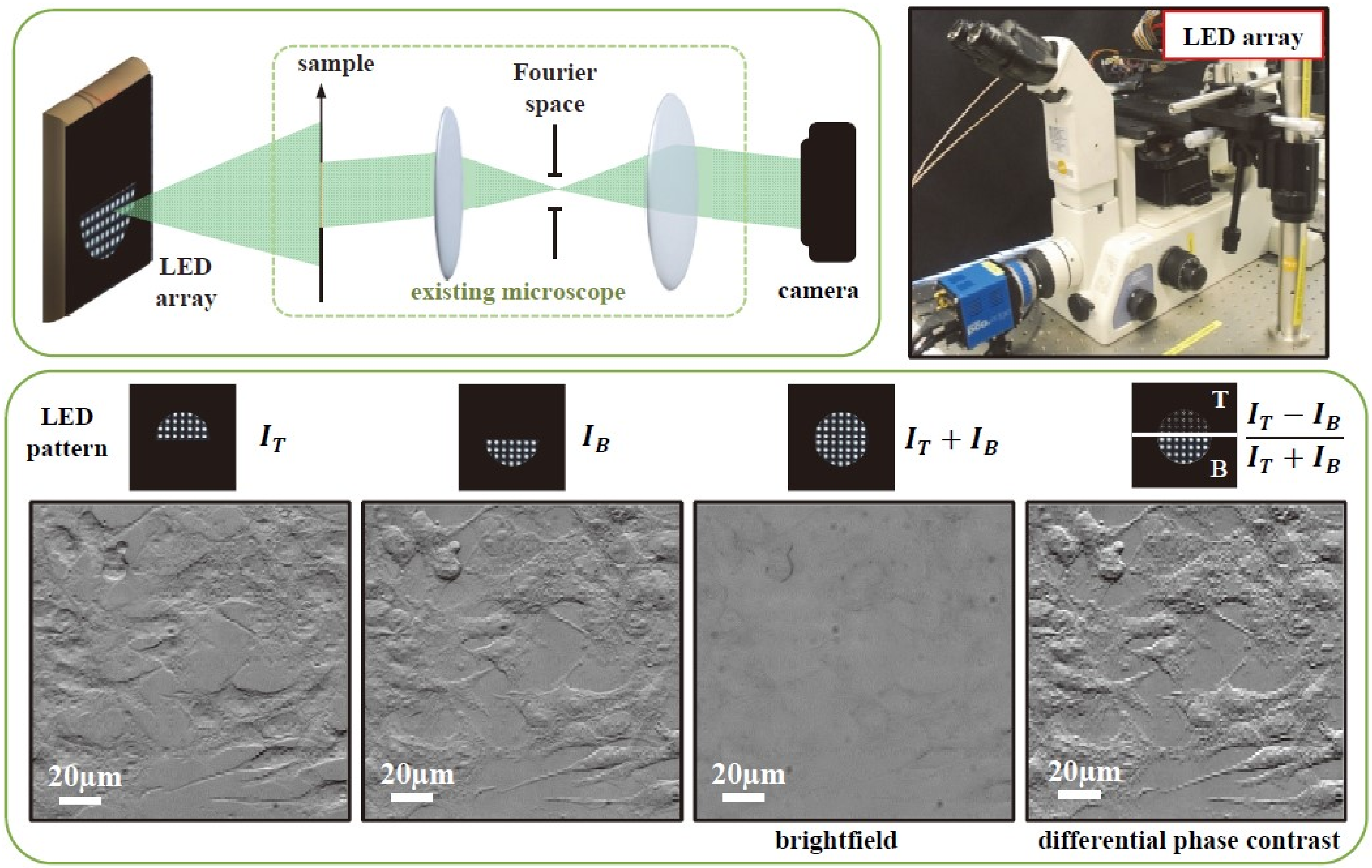
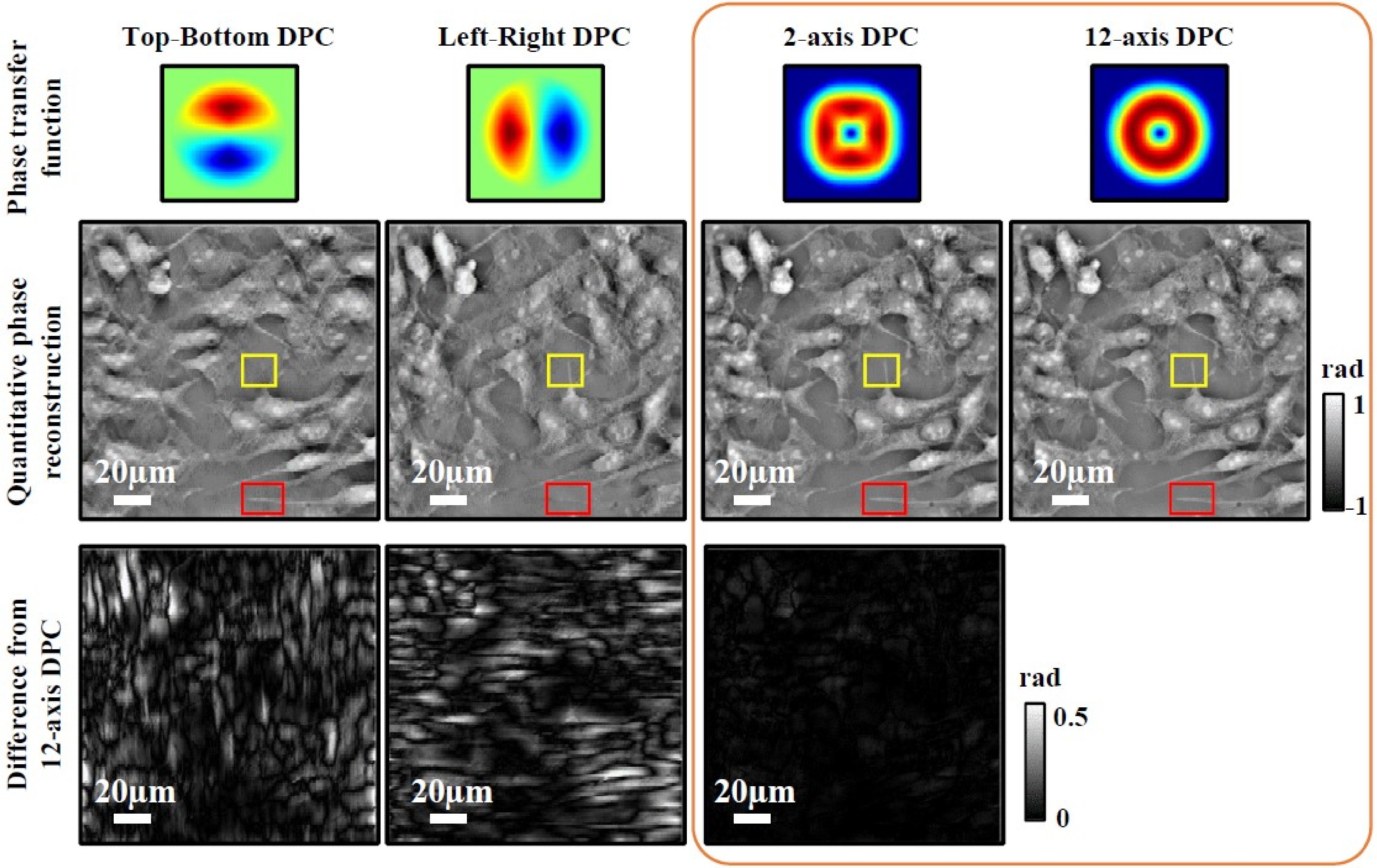
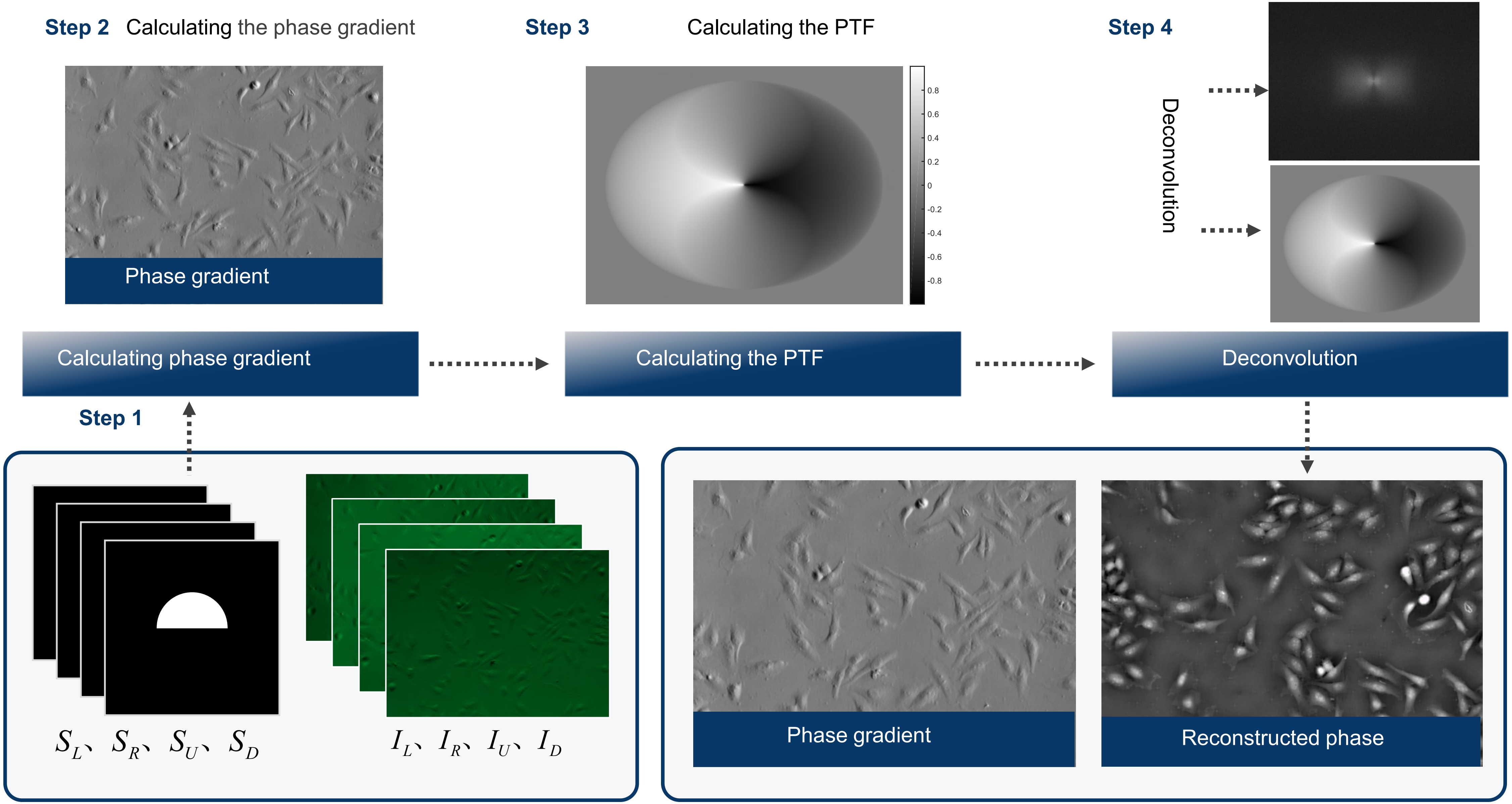


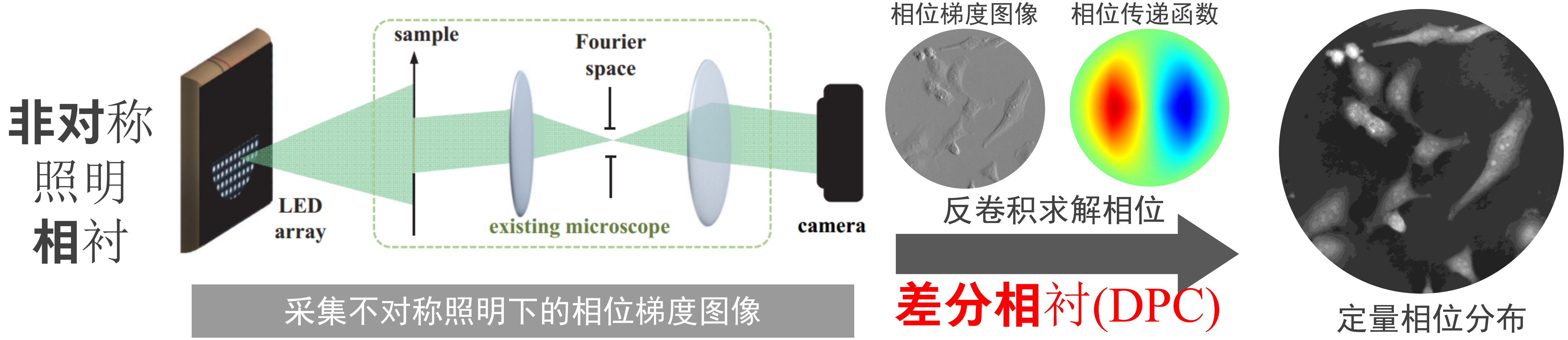
差分相衬成像定量相位恢复算法



差分相衬成像定量相位恢复算法



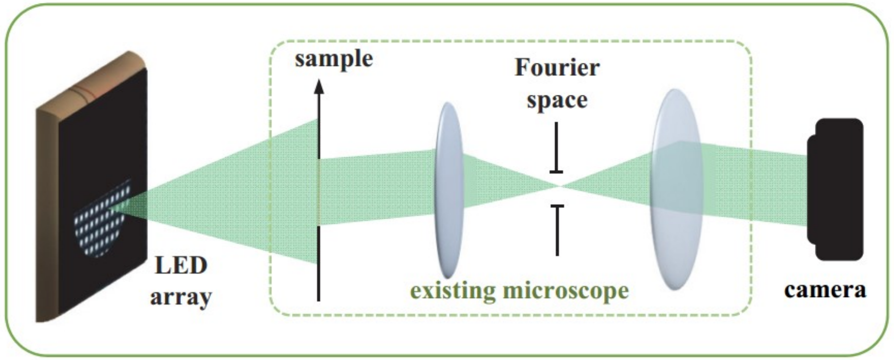
差分相衬成像定量相位恢复算法

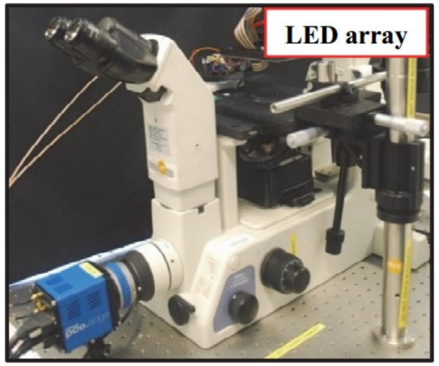


Hamilton, D. K., & Sheppard, C. J. R. (1984). Journal of microscopy, 133(1), 27-39.

1. 探究成像机理，完善成像模型与算法
2. 提升成像分辨率，实现高分辨率成像
3. 发展快速定量相位成像技术，实现活细胞实时动态成像
4. 构建定量相位成像系统，针对专业化应用进行系统设计和开发

差分相衬定量相位成像



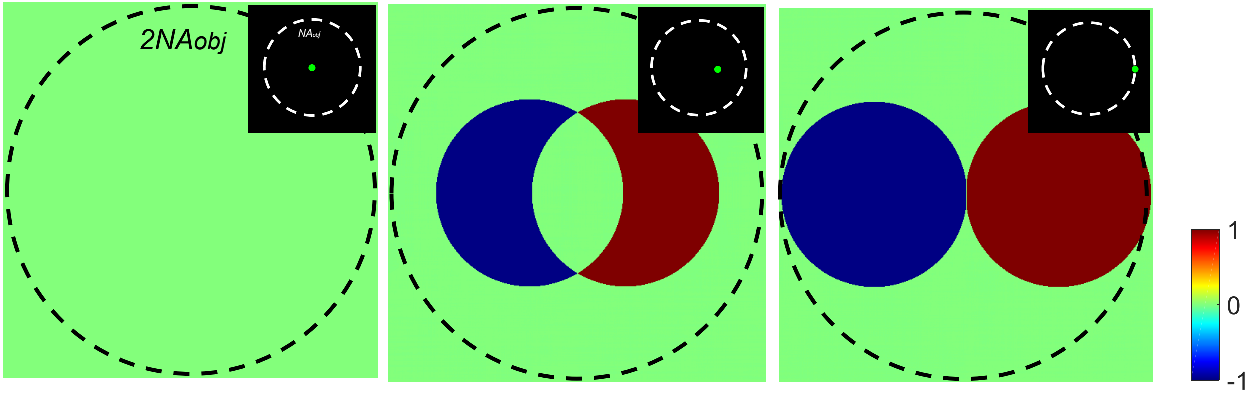


$$PTF(\mathbf{u}) = \frac{\iint S(\mathbf{u}_j)[P^*(\mathbf{u}_j)P(\mathbf{u}+\mathbf{u}_j) - P(\mathbf{u}_j)P^*(\mathbf{u}-\mathbf{u}_j)]d^2\mathbf{u}_j}{\iint S_l(\mathbf{u}_j)\left|P(\mathbf{u}_j)\right|^2 d^2\mathbf{u}_j}$$

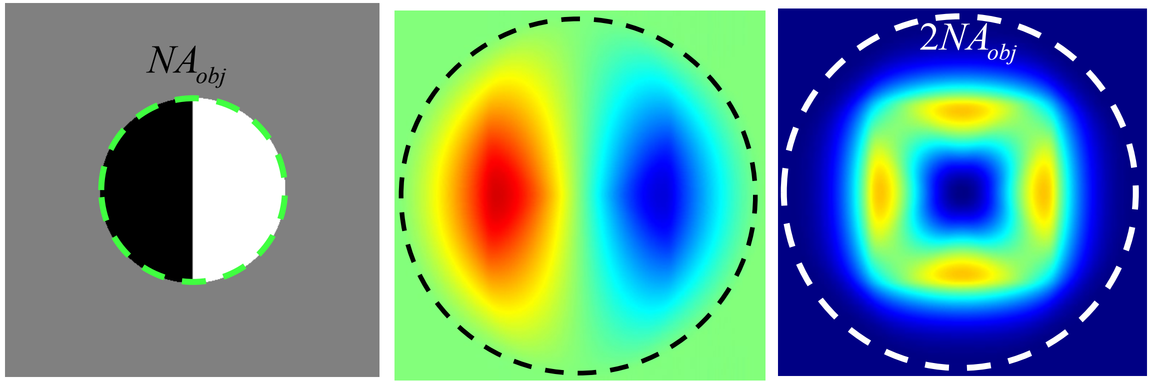
$PTF(\mathbf{u})$ 相位传递函数

$S(\mathbf{u})$ 照明函数　　$P(\mathbf{u})$ 光瞳函数

单点非对称照明的相位传递函数



传统半圆形照明　　左右PTF　　合成PTF



- ☐ **频谱带宽受限，低频对比度差，无法恢复高分辨率细节**
- ☐ **传递响应各向异性，成像分辨率各向异性**

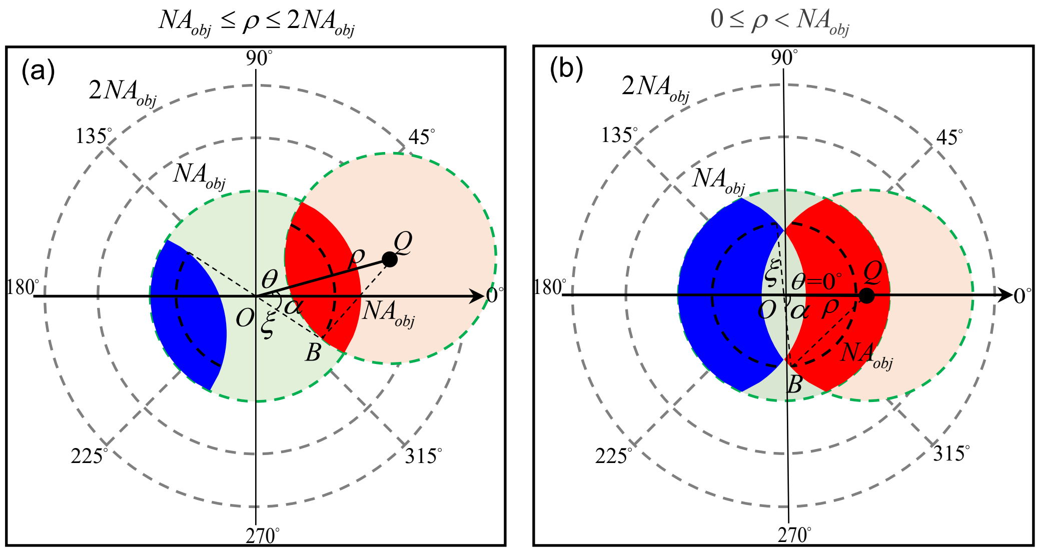
Tian, L., & Waller, L. (2015). Optics express, 23(9), 11394-11403.

基于最优照明的差分相衬定量相位成像

$$PTF(\mathbf{u}) = \frac{\iint S(\mathbf{u}_j)[P^*(\mathbf{u}_j)P(\mathbf{u}+\mathbf{u}_j) - P(\mathbf{u}_j)P^*(\mathbf{u}-\mathbf{u}_j)]d^2\mathbf{u}_j}{\iint S_l(\mathbf{u}_j)\left|P(\mathbf{u}_j)\right|^2 d^2\mathbf{u}_j}$$

$PTF(\mathbf{u})$ 相位传递函数

$S(\mathbf{u})$ 照明函数 $P(\mathbf{u})$ 光瞳函数



极坐标相位传递函数建模

$$PTF_{lr}(\rho,\theta) = \begin{cases} \dfrac{2\int_{\rho-NA_{obj}}^{NA_{obj}}\int_{\theta-\alpha}^{\theta+\alpha} S_{lr}(\xi,\varepsilon)d\varepsilon d\xi}{\int_0^{NA_{obj}}\int_0^{2\pi}\left|S_{lr}(\xi,\varepsilon)\right|d\varepsilon d\xi} & NA_{obj} \le \rho \le 2NA_{obj} \\[2ex] \dfrac{2\int_{NA_{obj}-\rho}^{NA_{obj}}\int_{\theta-\alpha}^{\theta+\alpha} S_{lr}(\xi,\varepsilon)d\varepsilon d\xi}{\int_0^{NA_{obj}}\int_0^{2\pi}\left|S_{lr}(\xi,\varepsilon)\right|d\varepsilon d\xi} & 0 \le \rho < NA_{obj} \end{cases}$$

广义各向同性照明函数

$$S_{lr}(\rho,\theta) = L(\rho)\cos(n\theta)$$
$$S_{ud}(\rho,\theta) = L(\rho)\sin(n\theta)$$

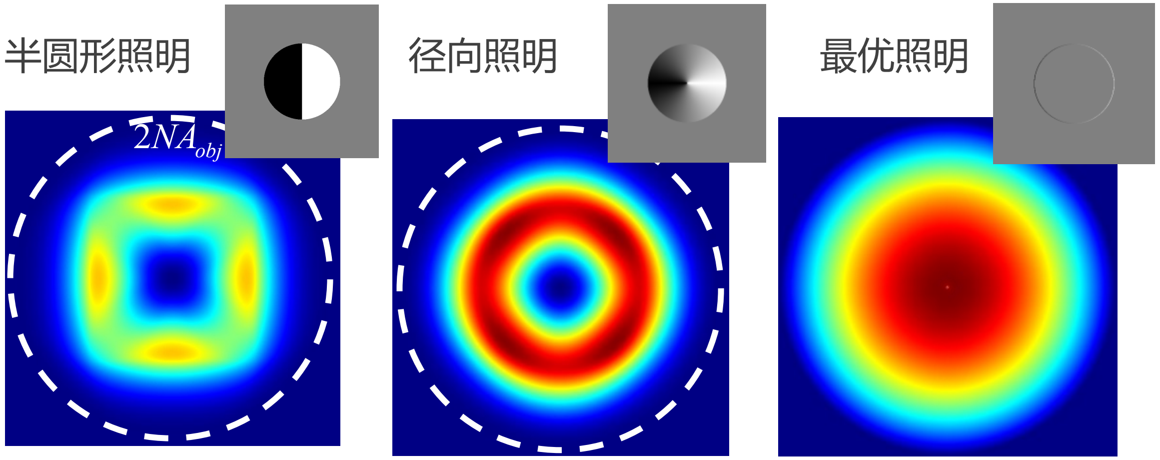
基于最优照明的差分相衬定量相位成像

广义各向同性照明函数 →

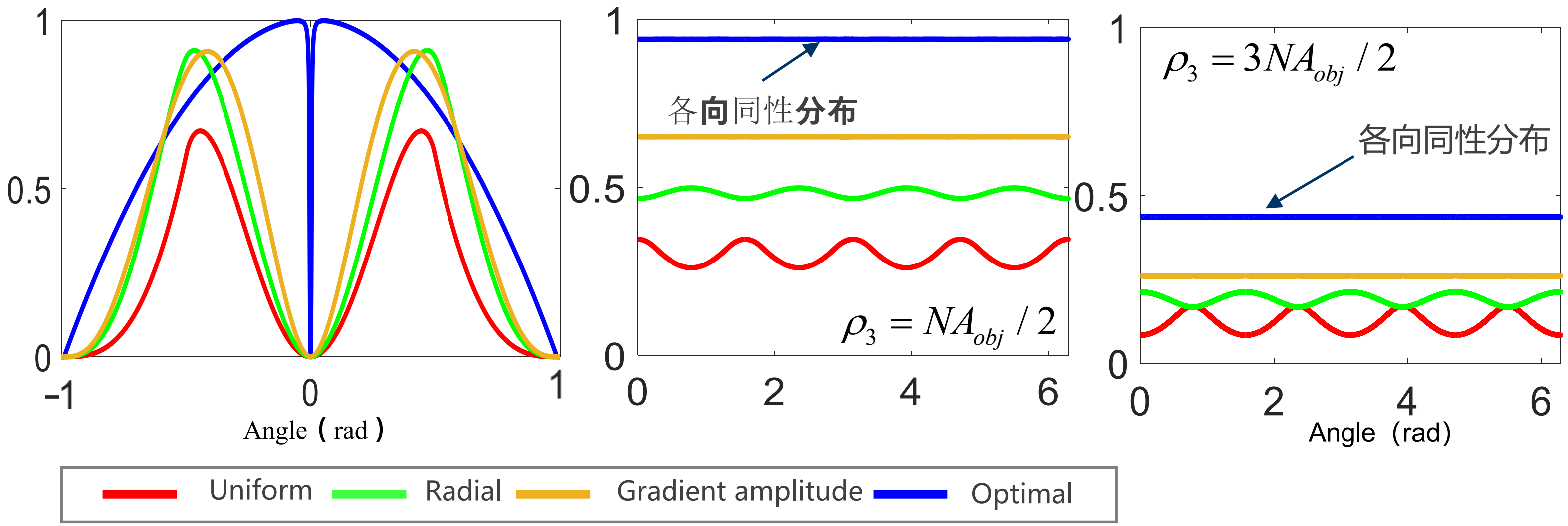
$$S_{lr}(\rho,\theta)=M(\rho)\cos(n\theta)$$
$$S_{ud}(\rho,\theta)=N(\rho)\sin(n\theta)$$

最优照明函数 →

$$S_{lr}(\rho,\theta)=\delta(\rho-NA_{obj})\cos(\theta)$$
$$S_{ud}(\rho,\theta)=\delta(\rho-NA_{obj})\sin(\theta)$$

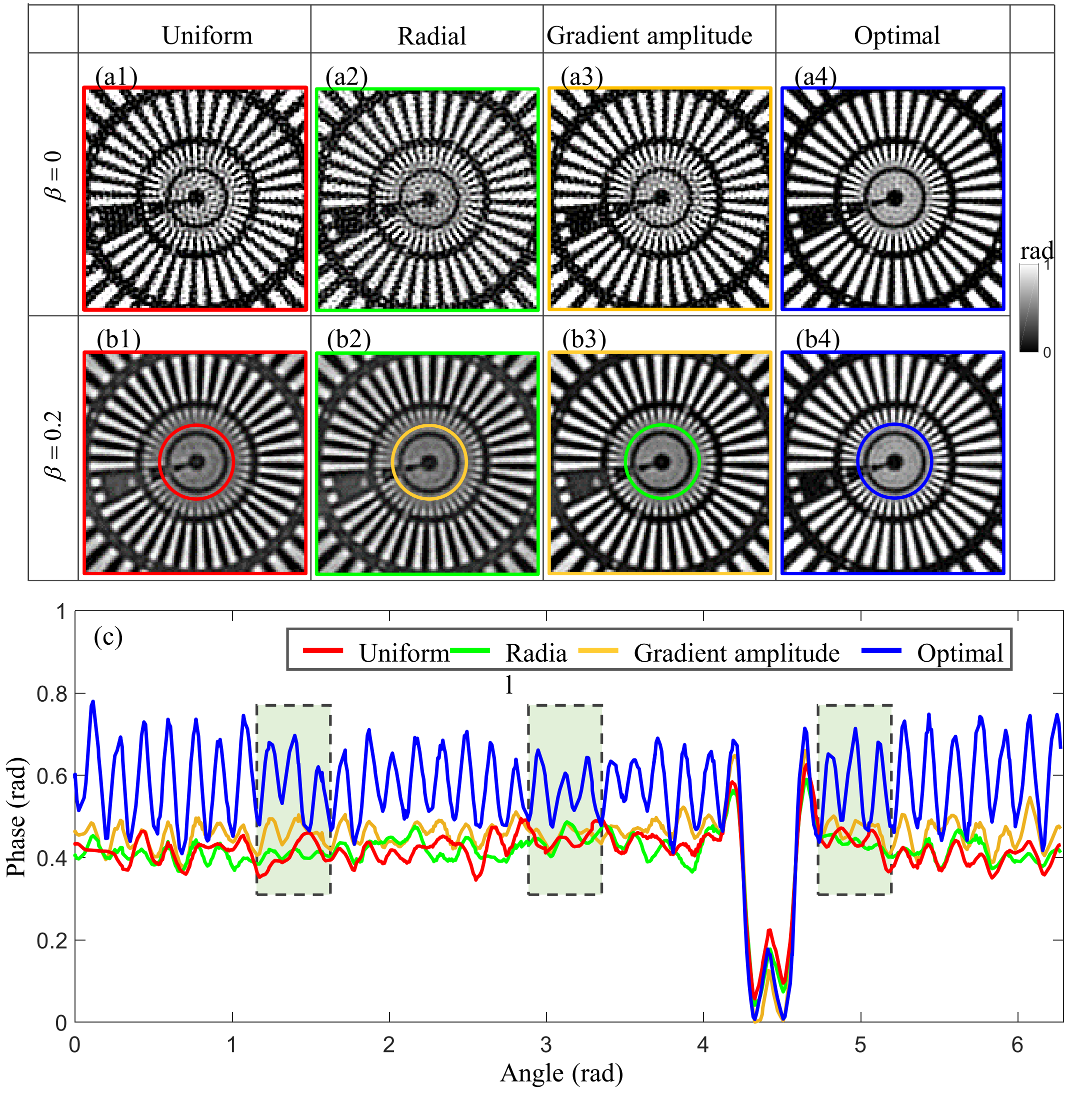


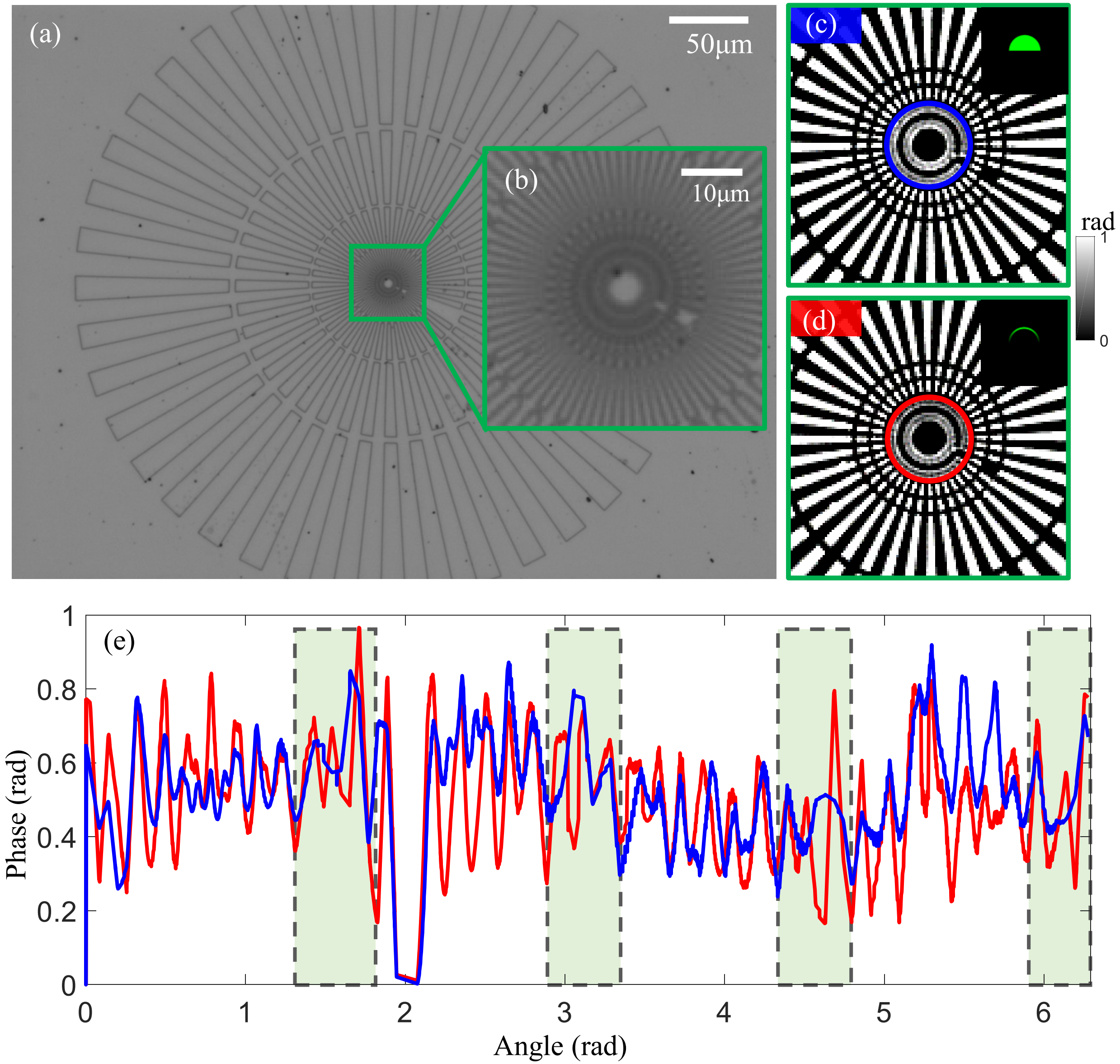
传递函数剖线对比



传递响应在
全频谱带宽内增强
传递响应
数值分布各向同性

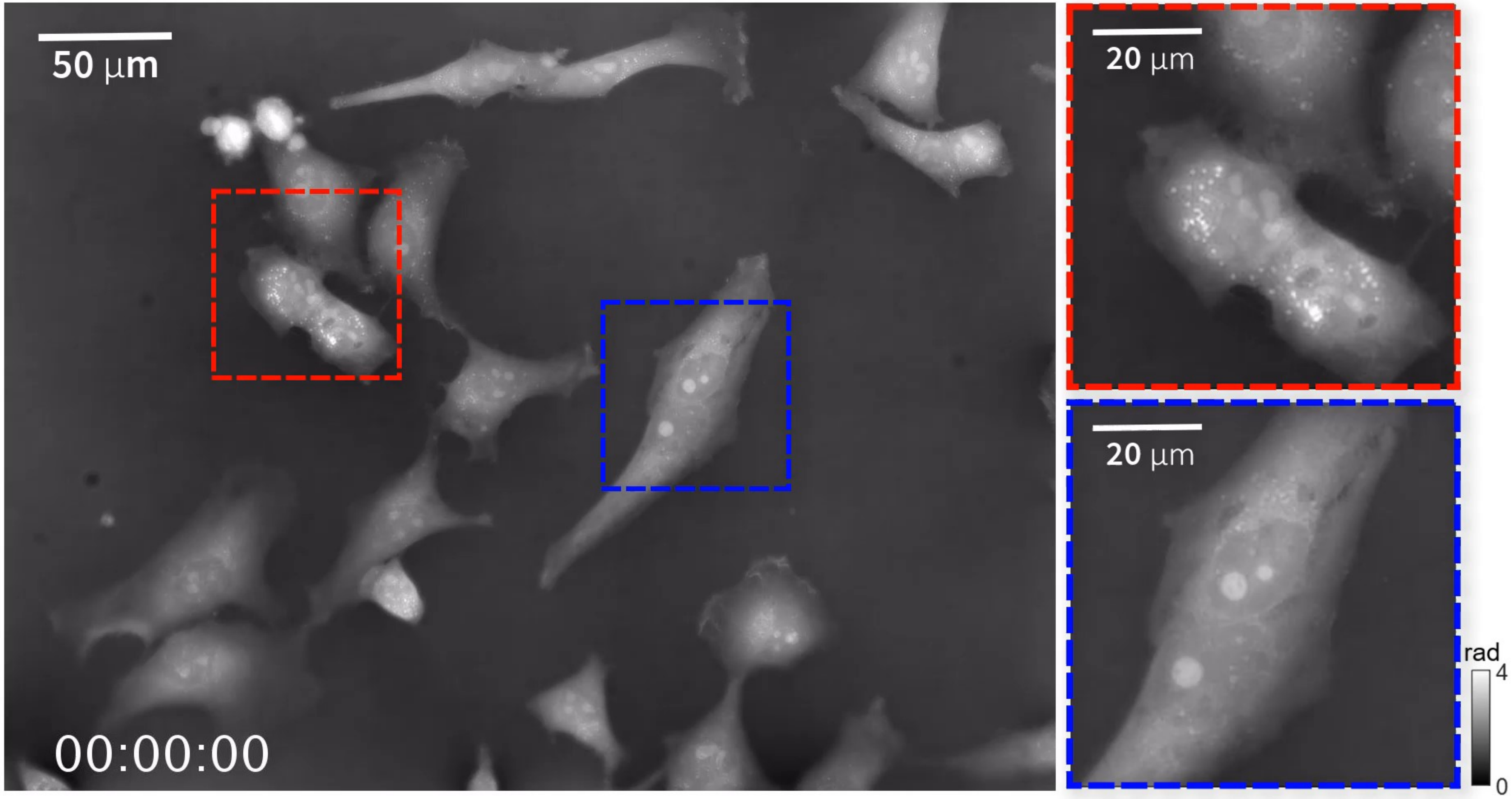
基于最优照明的差分相衬定量相位成像





基于最优照明的差分相衬定量相位成像

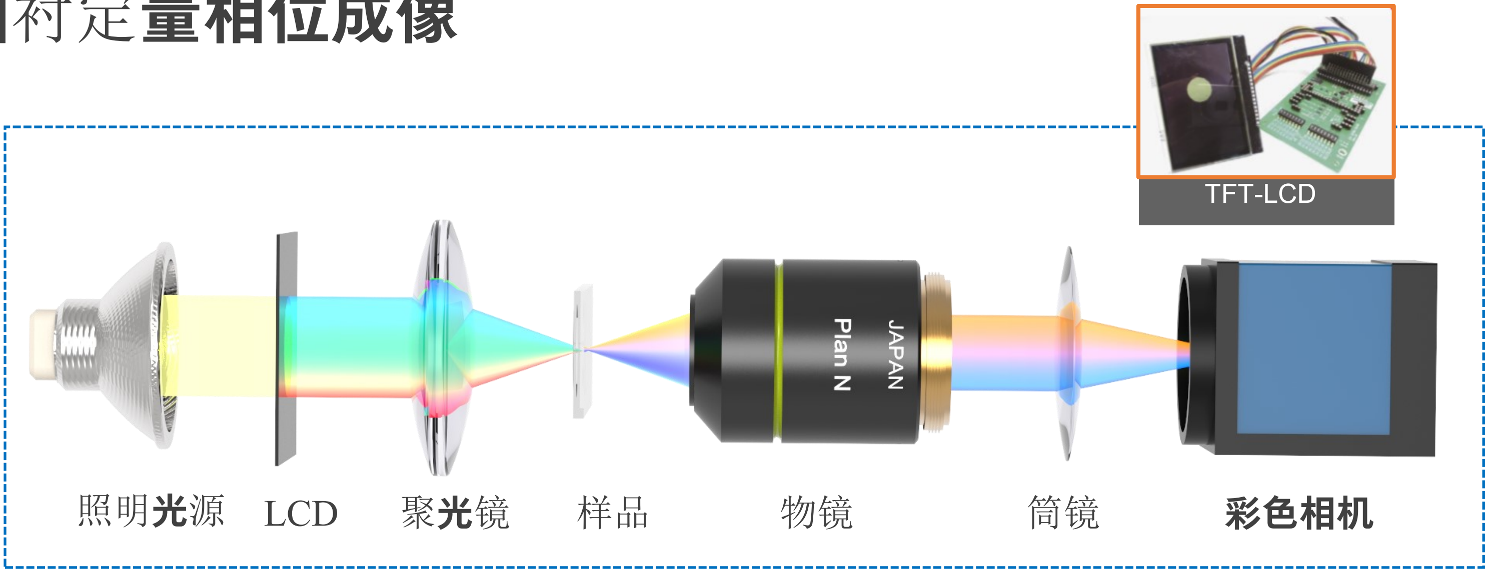
Hela细胞5小时动态定量相位结果（每幅相位由4幅采集图像恢复）

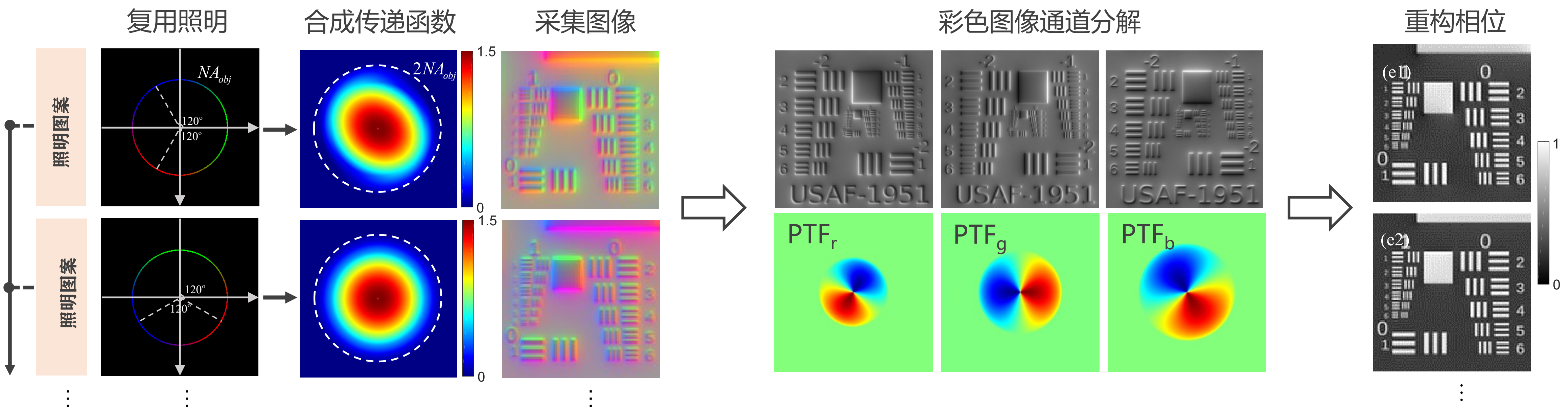


基于彩色复用照明的单帧差分相衬定量相位成像

复用照明函数分布

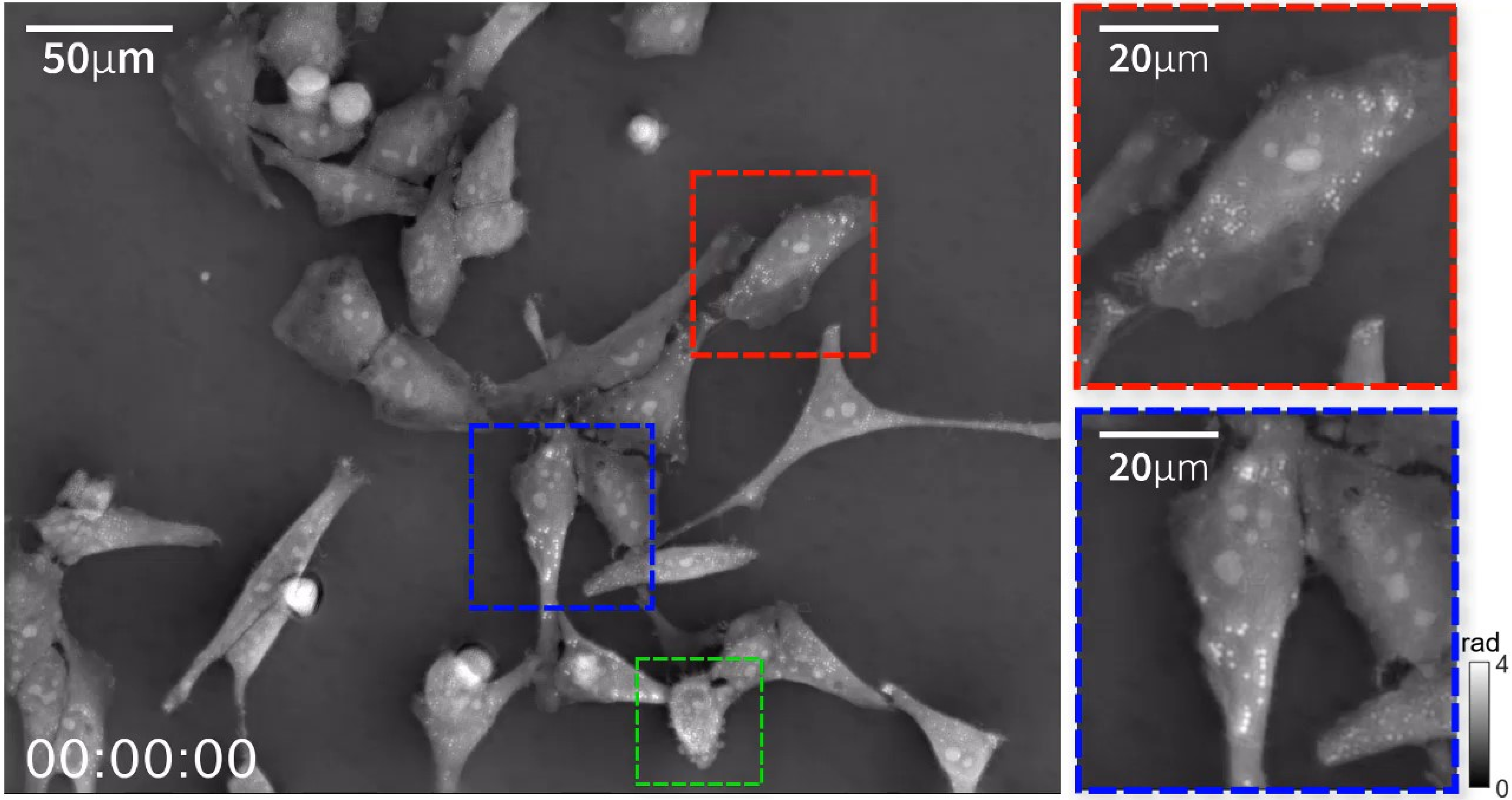
$$S_r(\rho,\theta)=\delta(\rho-NA_{obj})\sin(\theta+\theta_r)$$
$$S_g(\rho,\theta)=\delta(\rho-NA_{obj})\sin(\theta+\theta_g)$$
$$S_b(\rho,\theta)=\delta(\rho-NA_{obj})\sin(\theta+\theta_b)$$
$$(\ \theta_r=\theta_g-120^\circ,\theta_b=\theta_g+120^\circ\ )$$





基于彩色复用照明的单帧差分相衬定量相位成像

Hela细胞体外动态定量相位成像(相机帧率30Hz)

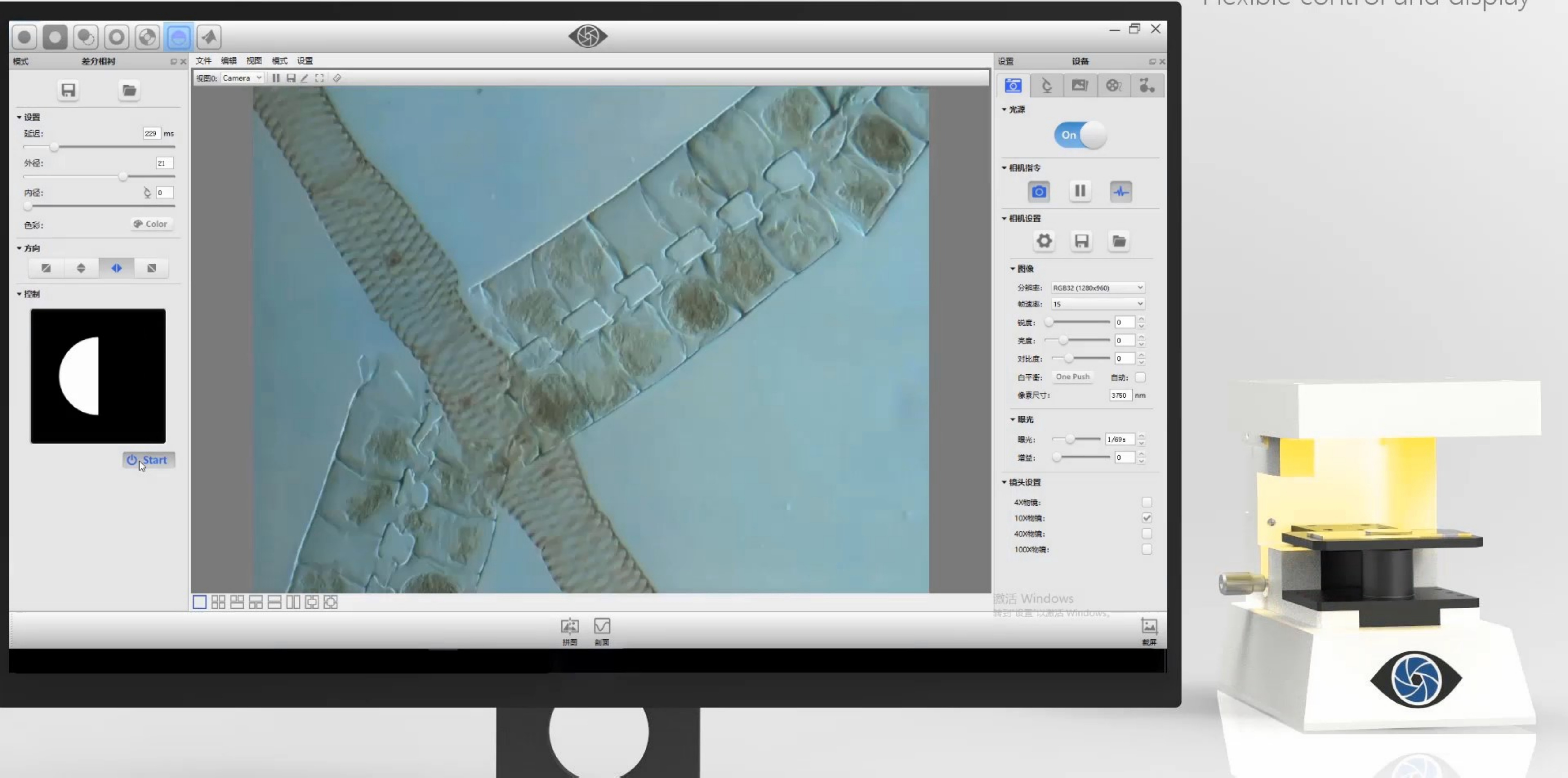


MCQ-CM

Multimode Quantitative Phase Computational Microscopy

MMC-CM frees the user's hands

Flexible control and display



Questions：

定量相位成像方法的分类

定量相位成像方法的优势和劣势