

Quantitative phase imaging of mitotic cells using d'Biomager

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ABSTRACT

The d'Biomager from d'Optron Pte.Ltd uses the Transport of Intensity Equations to extract phase from three or more images at different focus. The novel optics and algorithm allows for near real-time measurement of phase which can add clarity to the various mitotic stages in cells to govern cell fate. The various phenotypes such as cell blebbing, slippage and cell death in mitotic cell lines were recorded and investigated under the effect of certain drugs. The height variations at different stages were obtained quantitatively and imaged over time. Elevated peaks with noticeable increase in height during the various stages were noticed which may account to characteristic cell behaviour such as multinucleation, slippage and cell death. The average area and optical volume of the cells also show significant changes when compared with control cells. Knowledge of how a cell behaves under the effect of certain drugs could provide insight into providing more quantised and localised cancer treatments and improved drug delivery systems in tumour cells. In this presentation, the basics of the d'Biomager and various applications in medical imaging will be exemplified.

On the use of Artificial Intelligence for solving computational imaging problems

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ABSTRACT

Computational Imaging systems consist of two parts: the physical part where light propagates through free space or optical elements such as lenses, prisms, etc. finally forming a raw intensity image on the digital camera; and the computational part, where algorithms try to restore the image quality or extract other type of information from the images. A broad spectrum of computational imaging approaches exist: in one extreme, computer vision, the physical part typically comprises standard imaging optics; at the other extreme, in lens-less imaging the burden of forming images or extracting other types of information from the optical field falls entirely on the computation.

In this talk I will discuss the emerging trend in computational imaging to train deep neural networks (DNNs) to perform image extraction and restoration tasks. In a lens-less imaging experiment carried out by our group, the objects were “invisible”—technically, phase objects where electromagnetic scattering consists mostly of path delay imparted on the field. The DNN was trained from examples consisting of pairs of known phase objects and their intensity diffraction patterns after free-space propagation of the light. The objects were drawn from databases of faces and natural images, with the brightness converted to phase through a liquid-crystal spatial phase modulator. After training, the DNN was capable of recovering the phase from the intensity diffraction patterns even of unknown objects and outside the database that it was trained on (e.g. handwritten digits for a DNN trained on faces) and recovery was robust to disturbances such as axial or lateral displacements and rotation. This suggests that DNNs may form robust internal models of the physics of light propagation and detection and generalize priors from the training set to more general inverse problems.

About the speaker

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George Barbastathis received the Diploma in Electrical and Computer Engineering in 1993 from the National Technical University of Athens (Πολυτεχνείο) and the MSc and PhD degrees in Electrical Engineering in 1994 and 1997, respectively, from the California Institute of Technology (Caltech.) After post-doctoral work at the University of Illinois at Urbana-Champaign, he joined the faculty at MIT in 1999, where he is now Professor of Mechanical Engineering. He has worked or held visiting appointments at Harvard University, the Singapore-MIT Alliance

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Beam characterization for flash X-ray imaging experiments

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ABSTRACT

Flash X-ray imaging (FXI) is a method holding the potential to determine the 3D structure of non-crystalline biological samples at high resolution using bright and short X-ray pulses [1, 2]. The method still faces some key experimental and computational challenges and its development would benefit from a comprehensive description of the beam conditions used at FXI experiments. Based on experimental data taken at the Linac Coherent Light Source, we used mixed-state ptychography [4] and single-particle diffraction analysis [3] to characterize the beam profile and its shot-to-shot variations at the AMO and CXI end station. We extracted the properties which are most relevant for FXI and discussed their implications for high-throughput data collection and orientation recovery. We hope that our findings aid interpretation of data from past and future FXI experiments and will support the ongoing development of this technique towards high-resolution structural imaging of single molecules.

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A python API for rapid development of EMC-like algorithms

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ABSTRACT

Free-electron lasers provide X-ray pulses shorter than 100 fs long and with a peak brilliance that is more than a billion times higher than that of synchrotrons. This opens up for the possibility to image single biomolecules and other biological systems at atomic resolution and with a time-resolution that is orders of magnitude faster than what is possible with other methods. Data analysis has been a major bottleneck towards this type of experiment. The most successful approach is the Expand, Maximize and Compress algorithm which has been used successfully for both simulated data[1] and diffraction from synthetic samples[2] and large viruses[3]. Many challenges do however remain for making these algorithms more versatile, efficient and robust.

Here, we present a python API that implements the EMC algorithm and that allows for rapid prototyping and testing of new algorithms while maintaining a fast execution time. The main building blocks of the algorithm are implemented to run on GPUs with custom CUDA kernels for fast execution. The API interfaces with the `afnumpy` library which allows for the user to do quick development in python while the execution will be done on the GPU.

I will demonstrate the use of this framework in three settings. First, a variant of EMC that can separate diffraction data from multiple conformations. Second, a variant that handles the effects of a known background. And last, a variant that uses any type of a-priori orientation information to aid the reconstruction.

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50 years of phase retrieval in 50 minutes

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ABSTRACT

The current wave of activity in the applied math community on the phase retrieval problem has so far not produced a single algorithm that can solve even routine crystal structures. Clearly the appeal of the new methods is based more on ideas than results! This talk reviews the development of phase retrieval before the “modern era”, with special emphasis on the wealth of ideas that went into the creation of practical algorithms. As results are always going to be the decisive criterion, the talk concludes with a new definition of phase retrieval hardness and a crafted set of benchmark instances for comparing algorithms.

Development and Application of Decoherence Models in Ptychographic Diffraction Imaging

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ABSTRACT

In the past decade, ptychography has emerged as a disruptive tool for microscopy. In its simplest variant, the method scans the specimen through a coherent illumination and images the diffraction contrast for each transversal shift. As it samples the specimen in both real and reciprocal space simultaneously, ptychography creates high-dimensional and redundant images of phase space. In contrast to conventional microscopy, ptychographic imaging does not require any objective lensing system but relies on iterative phase-retrieval algorithms. They utilize the redundancy in the data to reconstruct the wave field of the illumination and the complex-valued transmission of the specimen at diffraction limited resolution - a feature sought after particularly for X-ray microscopy at synchrotrons.

Recent developments indicate that the high-dimensional phase space of ptychography is suited to compensate for coherence reducing effects caused by a certain degree of randomness in the measurement procedure. However, these partially coherent conditions require substantial alterations to the diffraction model of numerical phase-retrieval and also to the reconstruction algorithms employed.

This presentation will begin with a stronger emphasis on theory, reviewing the basics of ptychographic imaging and building a comprehensive forward scattering model condenses encompassing many of the current trends, including partial coherence.

Moving on towards application, this presentation reports about a selection of experiments and simulations in the field of partially coherent ptychography in the visible and X-ray regime of light, ranging from high-flux conditions to fast fluctuations of the specimen. Special emphasis is given to broad-bandwidth conditions.

Furthermore, this presentation covers current ptychographic challenging imaging activities at the ALS and upcoming research with the newly

funded STROBE, a new NSF Science and Technology Center on Real-Time Functional Imaging (<http://strobe.colorado.edu>).

If time permits a flexible computational framework developed in particular for non-ideal experimental conditions will be presented and discussed. This framework, called "PtyPy", is available online to any researcher in the field of ptychography and has already found a user base at synchrotrons.

Experimental 3D Coherent Diffractive Imaging from photon-sparse random projections

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ABSTRACT

Single Particle Imaging (SPI) at X-ray Free Electron Laser Sources requires collection of hundreds of thousands of diffraction patterns from a continuous stream of reproducible particles [1]. As the particles arrive at the interaction region in random orientations, the 3D intensity has to be assembled in Fourier space from the typically very noisy patterns, before the phasing step can be performed to obtain the real-space structure. In order to advance XFEL-based SPI to the resolution level required to solve the structure of biological macromolecules, a great variety of experimental and analysis challenges have to be overcome [2]. Despite recent advances [3], an experimental demonstration for orientation determination of SPI data in the very relevant weak scattering limit ($\lesssim 100$ scattered photons per pattern) is still outstanding. We report here on an experiment at a synchrotron source in which a small ($< 1\mu\text{m}$) lithographically produced particle has been illuminated with a coherent synchrotron beam. This way, hundreds of thousands of very weak diffraction patterns in hundreds of particle orientations have been collected. We will show how these data, without explicit knowledge of the individual frame orientations and with around 50 scattered photons per pattern, can be used to reconstruct a 3D diffraction volume in Fourier space using the Expansion-Maximization-Compression algorithm [4]. This diffraction volume is then used to reconstruct the real-space density of the particle by conventional iterative phase retrieval as used in classical plane-wave Coherent Diffractive Imaging.

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Single-particle tracking and sizing in focused aerosol beams

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ABSTRACT

Ultrabright femtosecond X-ray pulses generated by free-electron lasers can be used to image structures at room temperature and without the need to crystalize them [5, 8]. Delivering the sample as an aerosol without any container avoids parasitic scattering from solid supports and allows high-speed data collection. The 'Uppsala aerosol injector' has been the pioneering sample injector for this imaging method and demonstrated successful operation for relatively large particles (100-2000 nm) [8, 3, 7, 2, 6]. Imaging smaller particles has proven difficult with this set-up because of aggregation of sample contaminants and lower particle densities [4, 1]. Advancing the method towards higher resolution and to smaller particle sizes requires improvements in sample injection. Here we demonstrate a lab-based microscopy technique allowing us to track and size the aerosolized particles as they exit the sample injector. Analysis is carried out with an open-source software package that extracts particle position, velocity, and size for individual particles from the image frames. We used this technique to characterize the Uppsala sample injector including its particle beam focus, particle transmission, peak number density, and particle velocities at various operating conditions and for a large range of particle sizes. We identify optimized focussing conditions and we demonstrate that replacing the regular gas dynamic virtual nozzle nebuliser on the injector with a specially-adapted electrospray nebuliser reduces the problem of contamination significantly. This development paves the way for studies of sample particles as small as single protein molecules.

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Coherent phase retrieval in serial femtosecond nanocrystallography

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ABSTRACT

Since its advent in 2009, serial femtosecond crystallography [1] has become a very popular technique for solving protein structures because it is possible to perform ultrafast time-resolved studies, mitigate the problem of radiation damage, collect data at room temperature, and utilize tiny crystals down to sub-micrometer sizes. This is all possible because of the unique properties of x-ray free-electron lasers [2], which generate laser-like pulses of angstrom-wavelength x-rays, with about 10^{12} photons per pulse of a few-femtoseconds in duration. These pulses are also highly coherent, and when protein nanocrystals are smaller than the micro-focused x-ray beam, the features of coherent nanocrystal diffraction are striking. In particular, measurable “shape-transform” diffraction intensities can be found in regions outside of the usual Bragg condition, which is a source of new information that can be used to solve the crystallographic phase problem [3, 4]. Here we consider some of the challenges in applying so-called “shape-transform” phasing to protein nanocrystal targets.

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A fluctuation x-ray scattering approach for biological imaging

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ABSTRACT

Fluctuation x-ray scattering (FXS) proposes to measure cross-correlation functions from scattered intensity fluctuations to facilitate structure determination of a single particle in solution [1]. Due to its intrinsic capability to treat multiple-particle scattering data, the FXS approach is advantageous for structural determination of weakly-scattering objects, or particles that cannot be crystallized [1, 2]. With the emergence of new generation x-ray sources, such as x-ray free electron lasers (XFELs) and diffraction limited storage rings (DLRSs), the FXS approach offers an alternative technique for biological structure determination [3, 4].

During the past decade, significant progress in the development and application of the FXS approach has been demonstrated for various types of model systems and real materials. Recovery of the structure of two-dimensional (2D) nanostructures from disordered mixtures of particles at a synchrotron radiation source, and 3D structure determination of viruses in “diffraction-before-destruction” experiments at an XFEL are two vivid examples of this progress. In this talk, an overview of recent theoretical and experimental achievements in the field of structure determination by FXS will be presented [2].

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Reconstructing 3D protein crystal intensity from unoriented sparse diffraction patterns

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ABSTRACT

The femtoseconds long pulses of an X-ray free electron laser (XFEL) enable the measurement to outrun the irreversible radiation damage. This concept of ‘diffract before destroy’ inspires new methods such as serial crystallography, which determines 3D protein structures by merging 2D snapshots of microcrystals collected at random orientations. In recent years, there has been a growing interest to apply this technique to the synchrotron storage ring sources because of their wider availability. For very small crystals, however, radiation damage occurs before sufficient numbers of photons are diffracted to determine the orientation of the crystal. The challenge is to merge data from a large number of such “sparse” frames in order to measure the full reciprocal space intensity.

In this talk I will discuss our effort to develop an analysis method to analyze the unoriented sparse crystal diffraction patterns. Using the EMC algorithm (Loh & Elser, 2009), we reconstruct the 3D crystal intensity by iteratively maximizing the likelihood function of the crystal orientations. I will demonstrate the ability of our method to analyze sparse patterns through several proof-of-concept experiments with increasing complexity. Finally I will present our recent progress in applying our approach to a serial crystallography dataset taken at a synchrotron storage ring source.

X-ray scattering image classification and assembly

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ABSTRACT

X-ray Free Electron Lasers produce ultrashort and bright X-ray pulses to allow scattering from single particles. By assembling many scattering data into 3D volume, it is possible to obtain 3D density maps after an additional phase retrieval procedure. I would like to share some progress in image classification using machine learning approach and how we can possibly speed up the orientation recovery using a correlation function derived from raw images.

Using convolutional neural network trained with some manually classified images, it is possible to fast screen large number of scattering images. The selected images can be further merged to a 3D scattering volume.

The orientation recovery for each particle remains computational challenging. Orientation determination can be solved using Bayesian approach, through which a model can be constructed to best match a whole set of experimental projections at their 'correct orientations'. Without considering the centering of each particle projection, there are three degrees of freedoms (three Euler angles) that need to be fixed, usually resulting a computational complexity $O(n^3)$ where n is the number of discretization for each rotation angle. Here, we propose a method based on Maximum Likelihood approach with angular auto-correlation function of each projection, which is utilized to decouple the determination of three Euler angles to stepwise determination of two Euler angles and the subsequent third angle, the in-plane rotation. This approach reduces computational complexity from $O(n^3)$ to $O(n^2)$. Using simulation data, the accuracy and speed of the method is compared with the original maximum likelihood approach. We also investigated the impact of noise to the performance of this proposed method. This approach can be potentially applied in the analysis of both XFEL single particle scattering data and CryoEM single particle imaging data.

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Single Particle Imaging Open Discussion

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ABSTRACT

In X-ray based Single Particle Imaging (SPI), a stream of ultrafast and bright X-ray pulses randomly illuminates individual particles in a prepared ensemble. This random series of illumination events produces many noisy and incomplete diffraction patterns each arising from only single particles (hence the term SPI). Subsequently, through a complex and inter-dependent workflow of computational data processing (e.g. photon extraction, hit-finding, classification by orientation and/or structure, intensity reconstruction, phase retrieval), we then infer the structural classes persistent within this particle ensemble.

While the SPI technique may be enticingly promising for resolving heterogeneous ensembles, it is admittedly a highly data-driven and data-hungry procedure fraught with pitfalls for computation and interpretation errors. Notably, two key properties of this workflow make it impractical for humans to ‘manually’ validate the conclusions that they report. First, SPI reconstructions typically require very large number of noisy patterns. Second, the steps within current workflows rely heavily on heuristically designed algorithms that in turn rests on *ad hoc* parameters. Consequently, it is nearly impossible for any independent assessor to determine if the inferred results overfit the data, are artifacts of inappropriate parameter choices, or simply due to subtle errors hidden in thousands of lines of computer code.

This difficulty in validating SPI reconstructions will be exacerbated as openly available SPI data accumulates, likely at an accelerating pace as faster X-ray laser sources come online. And while the SPI community has eagerly defined a roadmap for its experimental goals, we must not fall prey to confirmation biases. The habitual use of widely cited but poorly-validated/understood techniques encourages a glut of bad practices from which the SPI community may find difficult to extricate itself in the future.

In this open discussion, I seek the help of members of this workshop to define the necessary checks and good practices in the SPI workflow. For the checks that already exist, their fair use will be discussed; those that are as yet uninvented, we will define their necessary functions and possible options, hoping to spur their timely creations. The results here will be jointly published as an open letter to the wider imaging community.

Experimental advances in Flash X-ray Imaging and the importance of structure validation

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ABSTRACT

Since the first demonstration of diffraction-before-destruction in Hamburg at the TESLA Test Facility 2 (which was promptly renamed to FLASH) in 2006, there has been an impressive development of the method by large worldwide collaborations. The end goal of imaging single macromolecules at atomic resolution, the driving force being most of this work, is still somewhat in the future but a lot of development work has been done. Much of this work relates to the development of new image reconstruction algorithms and new ways to analyse the data.

In this talk I will present the evolution of experiments in Flash X-ray Imaging up to the latest ones at the European XFEL and demonstrate the importance of developing robust structure validation procedures for the long term success and wider adoption of the method.

Real-space local 3D structure of disordered matter from fluctuation diffraction

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ABSTRACT

Coherent diffraction from a disordered material is a seemingly random, continuous signal, which fluctuates as the beam and sample are scanned relative to each other. Statistical measures of these diffraction fluctuations contain significant information about local structure in the material. The widely used small-angle and wide-angle x-rays scattering methods (SAXS/WAXS) measure the mean signal that informs us about two-body real-space correlations. This is one-dimensional data that provides limited information about 3D structure. The emerging intensity cross-correlation methods aim to measure higher order statistics that contain multi-atom information [1, 2], but are difficult to interpret structurally. We have discovered a method of transforming intensity cross-correlation data into a 3D real-space angular distribution function [3], which greatly facilitates direct structural interpretation and contains symmetrised bond-angle distributions. For proteins and biological matter, the angular functions can provide greater sensitivity to protein conformation and inter-particle correlations than SAXS. It also has applications to amorphous materials, liquids and soft matter. Here we will present the background theory behind the technique and present some preliminary experimental results. We will discuss practical issues of experimental design such as resolution, the number of measurements required and expected signal-to-noise.

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EMDB and EMPIAR: Public archiving of cryo-EM data

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ABSTRACT

The Electron Microscopy Data Bank (EMDB; emdb-empiar.org) is a public repository for 3D electron microscopy reconstructions of macromolecular complexes and subcellular structures. The use of electron microscopy in structural biology has increased dramatically due to recent advances such as the introduction of the direct electron detector. The growth in EMDB has mirrored the growth in the field and the EMDB represents an important and valuable resource for the structural community for a wide range of purposes such as data-mining, comparative analysis and as references in further structural work.

The Electron Microscopy Public Image Archive (EMPIAR; empiar.org) is a public resource for raw, 2D electron microscopy images. EMPIAR is now expanding to include other imaging modalities including serial block face scanning electron microscopy (SBF-SEM), focused ion beam SEM (FIB-SEM), soft X-ray tomography (SXT). In this talk I will introduce the resources available for these archives and discuss the challenges in making big imaging data usable and useful for data integration purposes and by a wider audience.

Teaching Optical Systems by Example

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ABSTRACT

An optical imaging system generally measures a property of an object as a function of its spatial coordinates and makes this information available for further use. Conventionally, the design of the system relies on our understanding of the physical properties of the optical system that transfers the light scattered by the object to the detector. For example, since a lens undoes the blurring that occurs when a light field propagates through free space it is used to focus a distant object onto a 2D sensor directly forming an image. In other cases the situation is more complex and it is not possible to present to the detector directly a clear image of the unknown object. In such cases the detected signal must be processed further in order to extract the image. In this presentation we describe how we can perform imaging in such complex systems through the presentation of examples. We describe two cases. First we consider a multi-mode fiber as an imaging element and we show that we can learn to transmit or interpret arbitrary images sent through the fiber by training the fiber with a set of basis functions. Secondly we show that we can learn the shape of an object from examples formed by reconfiguring the optical system in a predictable way. We demonstrate this second modality by constructing a neural network that models the optical system and training the network to reproduce the experimentally measured data. The adaptable parameters of the trained network yield the image of the unknown object.

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Hard X-ray Ptychography, Fluorescence and SAXS for High-Sensitivity Imaging with Chemical Contrast

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ABSTRACT

In recent years, the scanning coherent imaging technique – ptychography – has been established providing high spatial resolution and high sensitivity [1]. For ptychographic imaging the sample is scanned by a confined coherent beam, recording a far-field diffraction pattern at each scan point. A certain overlap (>60%) between adjacent scan points creates redundant information in the data set allowing for the high spatial resolution reconstruction of the complex transmission function of the object (phase and amplitude) as well as the complex probe function [2].

In general, the spatial resolution and the sensitivity in ptychographic imaging are limited by the signal that is detected over noise and over background scattering in high scattering angles as well as by the stability of the microscope. However, using (resonant) hard X-ray ptychography as a tool for quantitative imaging requires not only high-resolution images but also a reliable reconstruction results. This presentation addresses the challenges in quantitative analysis of ptychographic datasets, especially due to the limitations in sensitivity to weakly scattering features. In this framework, a beamstop-based double-exposure scheme to improve the sensitivity and the spatial resolution will be presented [3].

Furthermore, an overview of current capabilities using ptychography for different applications as well as the progress in combining ptychography with other complementary techniques such as resonant scattering, fluorescence microscopy or SAXS will be given. The multi-modal approach is beneficial in order to reveal the morphology and chemical speciation of various samples on different length scales in one experiment [4].

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2d and 3d image reconstruction for full field x-ray imaging of cells and tissues

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ABSTRACT

X-rays can provide information about the functional (interior) architecture of unstained biological cells and tissues. However, this potential of hard x-rays in view of penetration power, high spatial resolution, quantitative contrast, and compatibility with environmental conditions has to date not been fully developed, mainly due to significant challenges in x-ray optics. With the advent of highly brilliant radiation, coherent focusing, and lens-less diffractive imaging this situation has changed. We show how nano-focused coherent x-ray synchrotron beams can be used for high resolution full field holographic x-ray imaging in 2d and 3d. We discuss image formation and reconstruction, for given parameters of partial coherence, optical constants and object constraints [1, 2]. We also compare reconstruction in the optical far- and near-field [3, 4], and describe combined approaches of phase retrieval and tomographic reconstruction. Example of iterative algorithms for the optical near-field and reconstruction codes will be included [5].

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Imaging with illumination and detection arrays

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ABSTRACT

Images can be formed in two basically different ways: either by directly imaging and recording the image on a detector array, or by projecting a patterned illumination (for example a scanning spot) and detecting a signal with a single-pixel camera. A more general case is to combine these two basic approaches. Then effectively we can record a four-dimensional (4D) signal from a 2D object. The 2D image of the object must then be extracted from the 4D signal. The illumination and detection arrays can be positioned in either image planes or in Fourier planes. Placing the detection array in the back-Fourier plane gives imaging by differential phase contrast, or by ptychography. Placing the illumination array in the front-Fourier plane gives so-called Fourier ptychography. In ptychography, the resolution is determined by the apertures of both illumination and detection arrays. The 4D image signal also contains sufficient capacity to reconstruct 3D image information. These approaches can be used for a scattering object in reflection or transmission. In fact, it is known that 4D scattering data reduces in some special cases to 3D. If both illumination and detection arrays are situated in image planes, fluorescent objects can also be imaged. An example is in confocal microscopy, where the illumination and detection are both scanning spots, or arrays of spots. A second example is structured illumination microscopy (SIM), where a series of structured illumination patterns are used to generate a series of images. A third example is image scanning microscopy (ISM), where a scanning spot is combined with a detector array, and the image is reconstructed by pixel reassignment. This has the advantage over confocal microscopy that the detection efficiency is much greater. The programmable array microscope (PAM) is a more general arrangement, using a patterned or stochastic illumination array.

Computational imaging: when optics meets deep learning

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ABSTRACT

It is well known that neural network techniques including deep learning [1, 2] have been widely employed to solve the problems in recognition and classification. It was not until recently that people started to use them to solve imaging problems [3, 4, 5, 6, 7, 9, 10, 11]. In this lecture, I will first introduce the basic concept of deep learning, and then discuss how it can be adopted to solve computational imaging problems. In particular, I will talk about three use cases: computational ghost imaging, imaging through scattering media, and digital holography.

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