# Broadening Application Spectrum of iDPC-STEM Imaging from Beam Sensitive Solid Materials to Biological and Cryo Nano-particles Using Single Particle Analysis

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**Meeting-report**

# Broadening Application Spectrum of iDPC-STEM Imaging from Beam Sensitive Solid Materials to Biological and Cryo Nano-particles Using Single Particle Analysis

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In the recent years, scanning transmission electron microscopy (STEM) applying focused electron illumination, has been shown to have the capacity to image extremely electron beam sensitive materials [1, 2], including single molecules trapped within porous Zeolite channels [3, 4]. This has been achieved through integrated differential phase contrast STEM mode, known as iDPC-STEM [5–7], which is a dose efficient imaging technique, with directly interpretable contrast transfer function (CTF) and high signal to noise ratio (SNR), as main advantages over the conventional (S)TEM techniques. Recently, biological nano particle structures at near atomic resolution have been also revealed using iDPC-STEM in combination with single particle analysis (SPA) [8, 9]. Previously, SPA has been almost solely used with conventional TEM (CTEM).

In iDPC-STEM [5, 6] images the contrast is linear to electrostatic potential field of the thin sample and therefore the atomic numbers. This gives iDPC-STEM a strong capability to image light next to heavy elements at atomic resolution [6, 7, 10], including sensitivity to the lightest elements such as hydrogen [11]. Experimentally, ability to image metal organic frameworks (MOF-s) [1] and zeolites including single molecules [3, 4] has been proven. These crystalline structures cannot withstand electron doses larger than typical biological cryo dose levels (~50 $e^-/Å^2$) without suffering substantial damage of their fine structure. Fig. 1a shows an example of the iDPC-STEM image of a MOF, MIL-101 structure with resolution of 2 Å using total electron dose of 42 $e^-/Å^2$, while Fig. 1b, shows an image of MOF, UiO-66 structure with resolution of 1.4 Å obtained using electron dose of 54 $e^-/Å^2$.

The above results strongly erase the borders between material and life science (LS) with respect to sample electron beam sensitivity. Therefore, STEM investigation in biology [2] and more specific the imaging of cryo nano particles at near-atomic resolution [8, 9] is now strongly encouraged. Fig. 2 shows examples of iDPC-STEM micrographs of various vitrified single-particles cryo-EM samples. It includes typical 3D reconstructions from iDPC-STEM micrographs of keyhole limpet hemocyanin (KLH) protein and tobacco mosaic virus (TMV), well-known and in-depth studied specimens [12]. The iDPC-STEM micrographs show complete signal transfer to high resolution, enabling 3D structure volume SPA reconstruction at 3.5 Å [8].

Here, we present the specifics, requirements, and optimization of STEM parameters to enable imaging of various extremely beam sensitive organic and inorganic materials (as in Fig. 1) at atomic, as well as LS cryo-EM specimens (as in Fig. 2) at near atomic resolution.

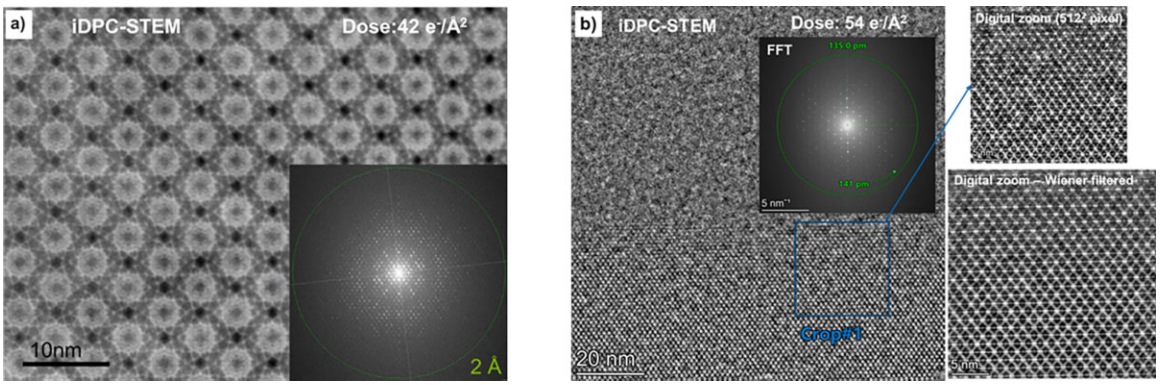


**Fig. 1.** 200kV iDPC-STEM imaging, convergence semi-angle (CSA) 10 mrad (Sample courtesy: Prof. Y. Han, KAUST Catalysis Center). **(a)** Image of metal organic framework MOF, MIL-101 acquired with total electron dose of 42 $e^-/Å^2$, showing resolution of 2 Å. **(b)** Image of MOF, UiO-66 acquired with total electron dose of 54 $e^-/Å^2$, showing resolution of 1.41 Å.

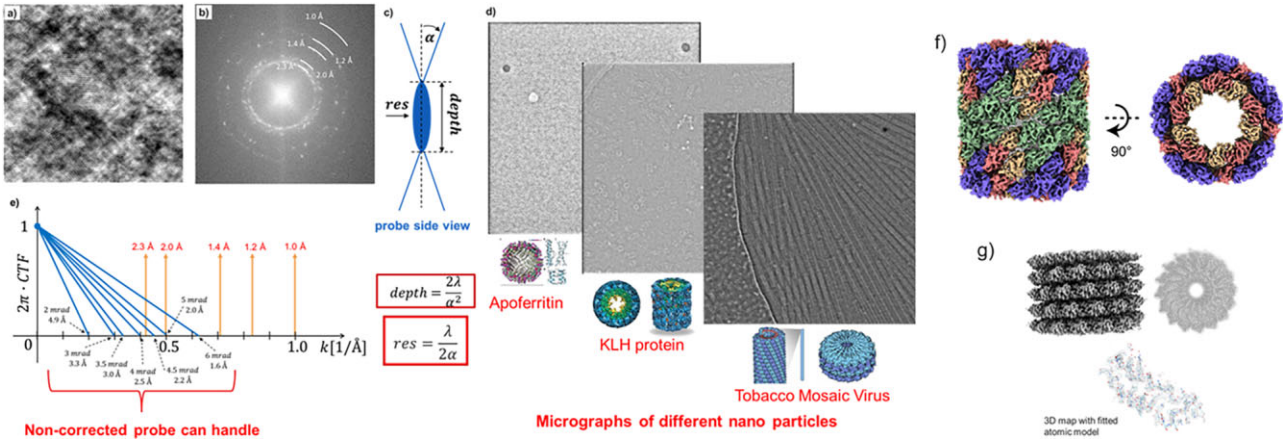


**Fig. 2.** 300kV iDPC-STEM cryo single particle analysis (SPA) examples. (**a)** Reference iDPC-STEM image of Au on C sample using a convergence semi-angle (CSA) beam 20 mrad, Cs-corrected probe and dose $10^4$ $e^-/Å^2$ [5–8] (**b)** Power spectrum of iDPC-STEM image showing gold rings of corresponding gold planes (distances indicated). (**c)** Schematic illustration of the side view of the probe (electron beam), indicating the CSA (α), effective resolution (width) and depth of focus (length) of the beam waist and their mathematical relations [2, 8]. (**d)** Examples of three different vitrified single-particles cryo-EM iDPC-STEM micrographs: apoferritin, keyhole limpet hemocyanin (KLH) and tobacco mosaic virus (TMV). (**e)** Simplified iDPC-STEM azimuthally averaged CTF's (illustrated as straight lines) of several CSA beams with respect to gold ring positions in reciprocal space (yellow arrows). (**f)** Typical reconstructed 3D volume (side and top view) of KLH at 6.5 Å resolution [8]. (**g)** Reconstructed 3D volume (side and top view) and molecular model fit of TMV at 3.5 Å resolution [8].

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