Minimum Fringe Illumination and Aberration-Free Image Shift on the JEOL CRYO ARM™

The advent of Direct Detector Device technology capable of generating movies rather than still images of biomacromolecules in the frozen-hydrated state was one of the key factors that led to a revolution in structural biology culminating in the award of the Nobel prize for Chemistry in 2017 to Dubochet, Henderson and Frank[1]. However, owing to the singularly low signal-to-noise ratio of cryoEM images, many thousands of images are required for a successful analysis. The throughput measured in images/hour is an important parameter that every researcher has been trying to optimize. Moving the stage to target holes in a Quantifoil sample is a time-consuming step as sample drift needs to be managed since this adversely affects the resolution. Because the DDDs capture a small field of view, the desire to increase the throughput organically leads to reducing the beam diameter and steering it using deflectors to multiple unique locations within a hole of a Quantifoil sample. Steering that beam farther, i.e. between target holes of the sample, is an obvious step to further increase the throughput.

JEOL has implemented Köhler-type illumination on the CRYO ARM™s to greatly minimize the fringes visible in a narrow beam (Fig. 1A and B), known as Minimum Fringe Illumination on the CRYO ARM™ 200 and Zero Fringe Illumination on the CRYO ARM™ 300. Owing to JEOL's unique alpha control, a parallel beam with a diameter of less than 500nm can readily be obtained, which can be set to match the DDDs field of view (Fig. 1B). This narrow beam is steered using beam shift and image shift deflectors with or without a correction for off-axis coma, known as Aberration Free Image Shift, to yield multiple unique images from a single Quantifoil hole (Fig. 1C) and from multiple holes (Fig. 1D). These multi-record schemes as implemented in JADAS and SerialEM have been successful in reaching a hitherto unprecedented milestone of 20,000 images/day on a CRYO ARM™ 300 II[1]. A typical multi-record scheme employing a typical 5x5x4 or a 7x7x8 pattern as in Fig. 1D would yield between 100 to nearly 300 images per stage move, meaning that under the most ideal circumstances only 68 specimen locations need to be visited to achieve this throughput.

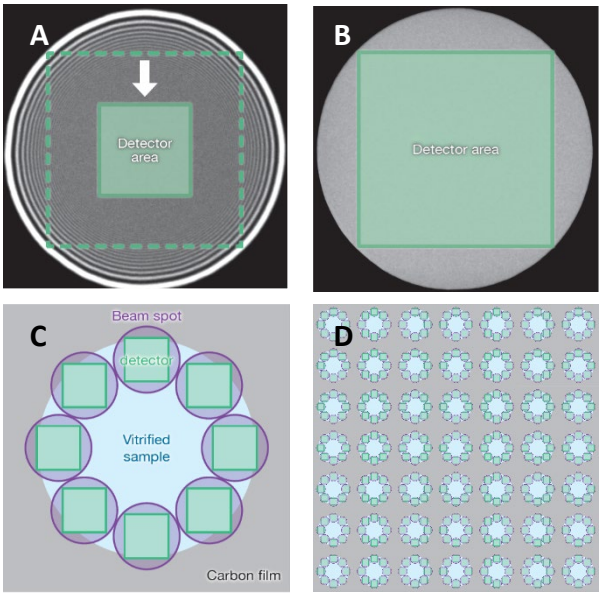

Fig. 1: Typical DDD field of view relative to the electron beam revealing Fresnel fringes (A). Using JEOL's Minimal Fringe Illumination those fringes are minimized thus optimizing the beam diameter relative to the DDD's field of view (B). Using deflectors the beam is steered within a hole (C) or between holes (D) in multi-record schemes using Coma-Free Image Shift.

Using Minimal Fringe Illumination and Coma-Free Image Shift an unprecedented throughput is possible on a JEOL CRYO ARM™. Given that a typical structure as published on EMPIAR requires 4-5000 images, the potential therefore exists of solving roughly 4-5 structures per day using a JEOL CRYO ARM™.

References:

1. Kinoshita, M., personal communication.