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High Angle Liquid Cell TEM Tomography for In Situ Observation and 3D Reconstruction in Liquid

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Liquid-Cell (LC) electron microscopy (EM) is a rapidly emerging field in EM [1]. While 2-D LC observations are very common for inorganic materials and biological systems, studies of molecular moieties in 3-D in the liquid environment are scarce [2, 3] mainly due to very limited available tilt range of the current LC holders (e.g. usually not more than ± 35 º). To perform electron tomography, LCs need to reach high angular tilting ranges, preferably more than 140º (± 70 º). This high angular range without excessive missing wedge artefacts is important to obtain a reliable 3D model. Since such LC do not exist currently, we have developed a prototype "tomographic" LC (coined Tomochip) by modifying a commercial monolithic LC (K-Kit from Bio MA-TEK), whose original total inclination angular range is about 60º (±30º), to extend its available angular range to about 120º (± 60 º) [4].

We have designed an elaborate precise process to reduce the original thickness of these LCs from 800 µm to just 80 µm, preserving the integrity of the very fragile ultra-thin 30 nm thick SiN membranes. Once the LC has been thinned to 80 µm (using Leica TXP precision micromachining device), it is possible to obtain a high tilt range without the silicon body casting shadow on the observation area. The central part of the LC viewing window could be tilted more than ±70º (e.g. 140º total range) (Figure 1) showing an effective observation area of approximately 100µm x 25µm. The obtained LC-Tomochip was glued to a standard grid (3 mm grid with 1 mm central hole) for TEM observation. The liquid (that includes the particles/samples to be studied) can be introduced into the LC microchannels by capillary forces. Once the microchannel has been filled with liquid, both LC openings are sealed with a specific epoxy resin. We found that when LC spacer is between 100-200 nm, it is very probable that samples (e.g. crystals) may stay inside the liquid for long periods of times (e.g. several minutes), which is adequate in order to perform 3D tomography experiments within the liquid.

Flufenamic acid (FFA) shows two different morphologies (two polymorphic forms, Form I and Form III) in the dry medium [5]. The aim of the experiment we present here was to observe (beam-induced) crystal growth in our tomographic LC. For our first experiment, Form III was used. 50 mM solution of (FFA) was prepared using absolute ethanol as the solvent and then the liquid was introduced into the Tomochip. Beam induced crystal growth was performed at 200 kV (Jeol F200 CF TEM), while image tomography data collection was performed at 120 kV. During our experiment we have observed that

crystals formed at lower voltage have longer lifetime, which makes it suitable for 3D imaging study.

Image data were collected with a Gatan Orius SC200 CCD camera at maximum resolution (2048x2048 pixels) with an exposure time of 0.9s. We minimized the total tomography acquisition time by performing 10° tilt steps from -50° to 50° (Figure 2a, 2b), while crystals were maintained still inside the liquid during the tilt. The image tilt series was downscaled to 512x512 pixels for image processing and aligned using the TomoJ (v2.6) plugin for tomographic reconstruction [6] of the ImageJ (v1.53e) image processing program [7]. The 3D image tomography was reconstructed using Total Variation Minimization reconstruction algorithm [8]. Indicative views of the 3D volume are shown in Figure 2 (c-g). [9]

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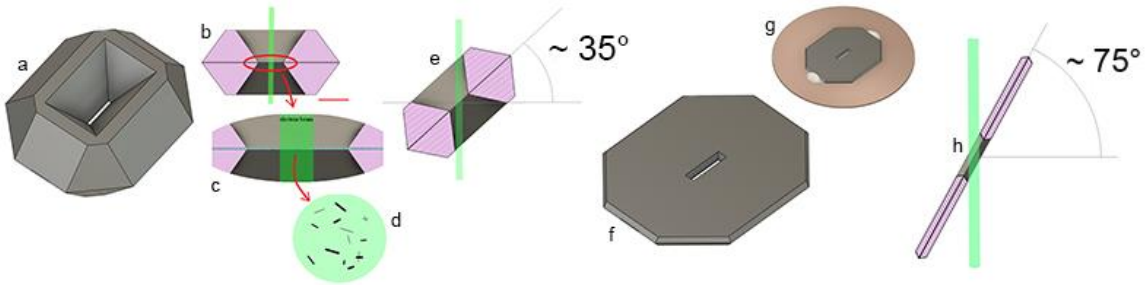

Figure 1. (a) K-kit relevant dimensions 300x25µm window, 800µm thickness, (b, c, d) Schematic process for TEM observation, (e) Maximum tilting range limited to ±35°, (f, g) Tomochip thinned to approx. 80 µm with standard 1mm diameter single hole grid that supports it, (h) The Tomochip tomographic LC can be tilted more than ±70°

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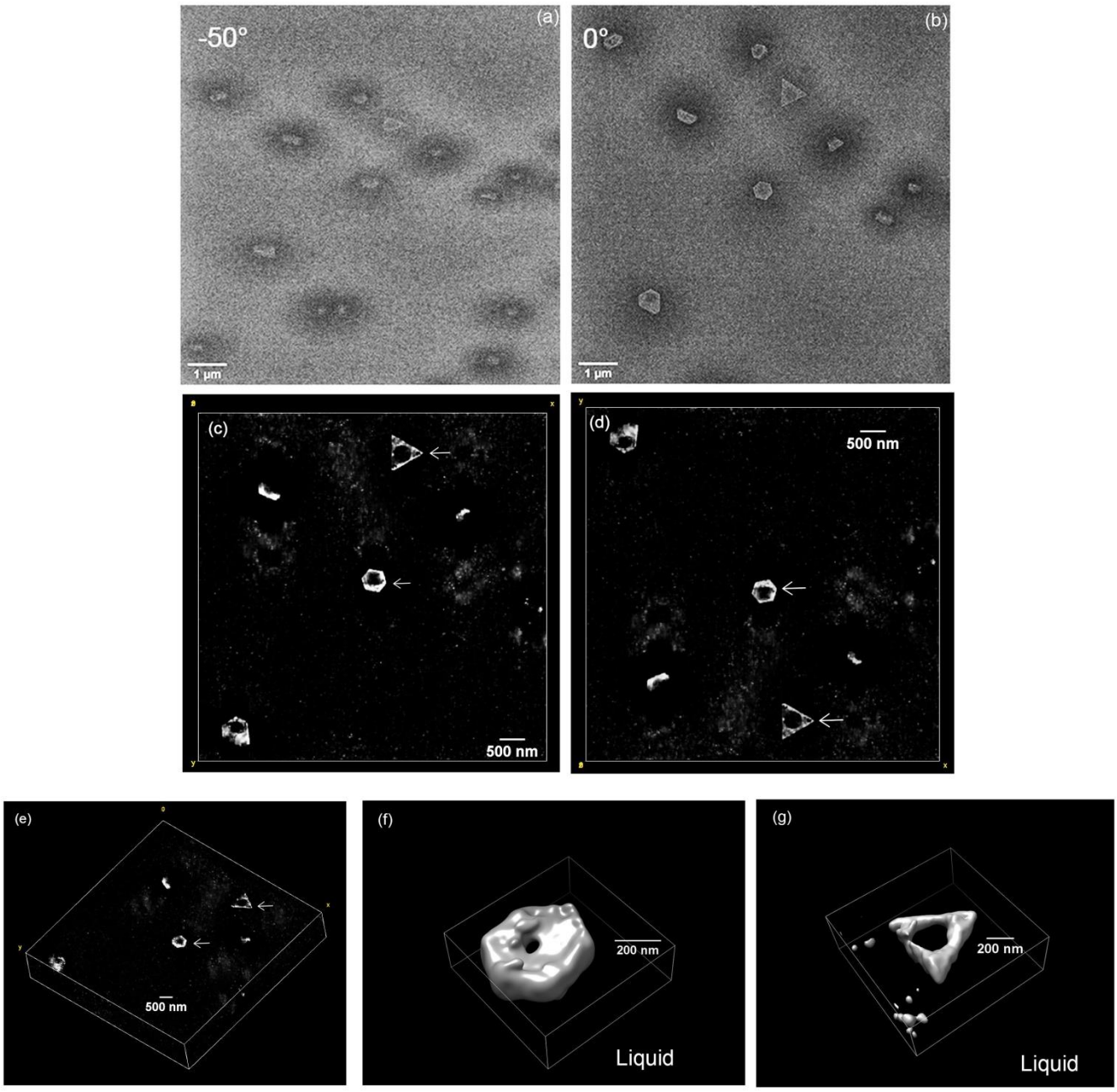

Figure 2. **(a, b) Image tilt series captured every 10° from -50° to 50° for 3D image tomography reconstructions in liquid (images at -50° and 0° shown here), Reconstructed 3D tomography views: (c) top view with axes rotation: (x,y,z)=(0°,0°,0°), (d) bottom view with axes rotation: (x,y,z)=(180°,0°,0°), (e) tilt view with axes rotation: (x,y,z)=(-30°,30°,30°), (f) view focused on the hexagonal crystallite feature (g) view focused on the trigonal crystallite feature**

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