



(43) International Publication Date
23 February 2017 (23.02.2017)

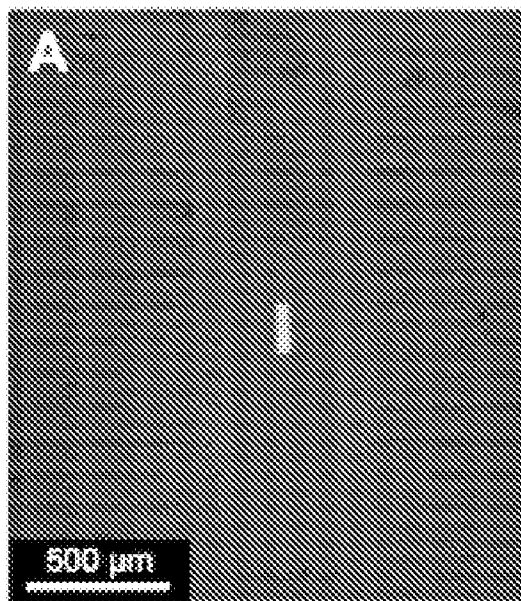
- (51) International Patent Classification:
H01J 37/20 (2006.01) *H01J 37/285* (2006.01)
H01J 37/26 (2006.01)
- (21) International Application Number:
PCT/US2016/047541
- (22) International Filing Date:
18 August 2016 (18.08.2016)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
62/206,690 18 August 2015 (18.08.2015) US
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- (81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.
- (84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU,

[Continued on next page]

(54) Title: NANOPARTICLE DETECTION

FIG. 1A



(57) Abstract: There are provided methods for detecting one or more nanoparticles within one or more liquid droplets using transmission electron microscopy.



TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

NANOPARTICLE DETECTION

CROSS-REFERENCES TO RELATED APPLICATIONS

- 5 **[0001]** This application claims the benefit of U.S. Provisional Appl. No. 62/206,690, filed August 18, 2015, the content of which is incorporated herein by reference in its entirety and for all purposes.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

- 10 **[0002]** This invention was made with government support under W911NF-15-1-0800 awarded by the U.S. Army Research Office, and under FA9550-11-1-0105 and FA9550-12-1-0414 awarded by the U.S. Air Force Office of Scientific Research. The government has certain rights in this invention.

BACKGROUND

- 15 **[0003]** Liquid cell transmission electron microscopy (LCTEM) is beginning to be recognized as useful in the field of nanomaterial analysis. Without wishing to be bound by any theory, it is believed that the development of LCTEM methods requires the continued development of devices, e.g., liquid cell TEM holders, and sample application methods. For example, loading and analysis of multiple samples can be problematic due to the quick evaporation of liquid
20 samples in small volume, especially in view of the associated time and efforts required for such preparation of samples for LCTEM.

[0004] Thus, there are provided herein solutions to these and other problems in the art of nanoparticle detection.

SUMMARY

- 25 **[0005]** In a first aspect, there is provided a method for detecting a nanoparticle in a liquid. The method includes depositing a liquid droplet containing a nanoparticle onto a transmission electron microscope sample holder under humidifying conditions, where the volume of the liquid droplet is less than about 1000 pL. The method further includes covering the transmission electron microscope sample holder with a transmission electron microscope sample holder cover.

The method further includes detecting the nanoparticle within the liquid droplet using a transmission electron microscope.

[0006] In another aspect, there is provided method for detecting a plurality of nanoparticles and an interaction thereof in a liquid. The method includes depositing a plurality of liquid droplets each containing a nanoparticle onto a transmission electron microscope sample holder under humidifying conditions, where the volume of each liquid droplet is less than about 1000 pL. The method further includes covering the transmission electron microscope sample holder with a transmission electron microscope sample holder cover. The method further includes detecting the plurality of nanoparticles and interaction thereof upon diffusion of the plurality of liquid droplets using a transmission electron microscope.

[0007] In another aspect, there is provided method of analyzing a sample using transmission electron microscope. The method includes automatically depositing a liquid droplet of the sample onto a transmission electron microscope sample holder, where the volume of said liquid droplet is less than about 1000 pL and analyzing the liquid sample using transmission electron microscope.

BRIEF DESCRIPTION OF THE DRAWINGS

[0008] FIGS. 1A-1F. The figures depict optical images of Hummingbird Scientific 200 nm spacer chips with a single 50 nm thick, 200 x 50 micron window (FIGS. 1A-1C) and a Norcada 10 nm thick 100 x 100 micron, 9 window array (FIGS. 1D-1F), showing the windows pre-deposition (FIG. 1A and FIG. 1D), post-deposition of pure water to create a high humidity environment (FIG. 1B and FIG. 1E), and post deposition of 20 nm Au NPs (white dashed circles) and 200 nm UiO-66 (dark dashed circles) (FIG. 1C and FIG. 1F).

[0009] FIGS. 2A-2G. Figure depict TEM images of 200 nm UiO-66 (FIGS. 2A-2C) and 20 nm Au NPs (FIG. 2E-2G) dispensed onto a single window on a liquid cell and allowed to dry (chip from FIGS. 1A-1C). FIG. 2D: The tall and narrow center image is a low magnification image of the entire window area of the chip from FIG. 1C after the deposited liquid droplets have completely dried at ambient conditions. The images in the left (FIG. 2A-2C) and right (FIG. 2E-2G) columns show clear separation between the UiO-66 and Au NPs, and solvent edges for each solution are indicated by lines. The structures of each NP are also confirmed by selected area electron diffraction. Dashed-line boxes indicate the regions on where the top row images were recorded on the window. The small circles indicate the regions where the center row images and

corresponding diffraction patterns were acquired. Note the difference in scale bars for SAED patterns which is indicative of the large difference in Au and MOF lattice spacing.

[0010] FIGS. 3A-3G. The figures depict TEM images of a liquid cell sealed immediately after dispensing 200 nm UiO-66 and 20 nm Au NPs onto opposite ends of the a single window. The images show clear separation of the UiO66 (FIGS. 3A-3C) with Au (FIGS. 3E-3F) as well as an initial mixing front (FIG. 3D). “t =” refers to the time after sealing the liquid cell. The tall narrow graphic in the center (FIG. 3G) is a qualitative scheme of the liquid composition within the window region as observed over the first 20 minutes after sealing the cell. Observed are pure Au NPs, pure UiO-66 MOF and a mixed composition of Au and MOF. Labeled squares in FIG. 3G indicate the position on the window area where the respective TEM images (FIGS. 3A-3F) were acquired.

[0011] FIGS. 4A-4G. The figures depict TEM images of the same liquid cell in FIGS. 3A-3G, after full diffusion and mixing of the UiO-66 and Au NPs had occurred. The images show significant diffusion of Au into the UiO66 end of the cell (FIGS. 4A-4E) and little or no diffusion of the MOFs into the Au end of the cell (FIG. 4F). “t =” refers to the time after sealing the liquid cell. The tall narrow graphic in the center (FIG. 4G) is a qualitative scheme of the liquid composition within the window region as observed 30 minutes after sealing the cell. Observed are pure Au NPs, pure UiO-66 MOF and a mixed composition of Au and MOF (now the primary composition). Labeled squares in FIG. 4G indicate the position on the window area where the respective TEM images (FIGS. 4A-4F) were acquired. Image FIG. 4C was acquired at the same location as FIG. 4B, 90 seconds later. Circles in FIG. 4C indicate Au nanoparticles that have diffused into the field of view and stuck to the top or bottom window during the 90 second time period from image FIG. 4B.

[0012] FIGS. 5A-5C. The figures depict optical images of (FIG. 5A) Piezo Dispensing Capillary - Uncoated 80um diameter orifice Sample = Gold NP (wt%) Volume = 316 pL, (FIG. 5B) Piezo Dispensing Capillary – Coated 80um diameter orifice Sample = MOF (wt%) Volume = 327 pL and (FIG. 5C) Sample dispense pattern of 6 drops/spot to opposite edges of the liquid cell TEM window. The surrounding water droplets totaled 450 nL.

[0013] FIGS. 6A-6B. FIG. 6A depicts a photograph of the SCI-TEM Liquid Cell sample prep system, including enclosing chamber and Cartesian robot [element 1]. The chamber provides a local environment of near-saturated solvent vapor to control drying of volatile samples. FIG. 6B depicts a photograph of the interior of the SCI-TEM Liquid Cell sample prep system, including

location of imaging systems (Global camera, Head Camera, Drop Camera) [FIG. 6B, element 2], low volume non-contact dispensing nozzles [FIG. 6B, element 3], vacuum nozzle [FIG. 6B, element 4], Microarray chamber (i.e., humidifier chamber) loaded with LC TEM stage and grids [FIG. 6B, element 5].

5 [0014] FIGS. 7A-7B. FIG. 7A: The figure depicts a schematic of the SCI-TEM Liquid Cell sample prep system and the processes (e.g., convection, diffusion) involved in preparation of a sample. A Controllable Heating Sleeve is depicted surrounding the enclosure holding the solvent. FIG. 7B: The figure depicts an alternative method for controlling the temperature of the enclosure, where the enclosure sits on a Controllable Heating Plate.

10 [0015] FIGS. 8A-8B. FIG. 8A schematically depicts the robotic working area of the SCI-TEM Liquid Cell sample prep system. A photographic inset (FIG. 8B) depicts the humidifier chamber (the white cassette) and the chip positioning.

[0016] FIGS. 9A-9C. FIG. 9A: Photograph depicting relative positioning of Top Chip and Bottom Chip, also depicting 30 nm thick SiN_x “windows” and 50 μm x 50 μm lateral area. FIG. 15 9B: Photograph depicting *in situ* Liquid-Cel TEM (LC-TEM) grids, including the sample holder, the sample holder cover (i.e., over clamp) and the side-clamp. The area indicated in dashed rectangle is enlarged in FIG. 9C, which depicts liquid flow lines, S/TEM electron beam, silicon support chip, and further inset depicting top SiN_x, liquid sample, and bottom SiN_x regions.

DETAILED DESCRIPTION

20 [0017] Absent express indication otherwise, definitions used herein assume the usual and customary meaning in the art.

[0018] The term “transmission electron microscope (TEM) grid” refers, in the usual and customary sense, to a sample holding device for use in a TEM, as known in the art. The TEM grid may include equally spaced score lines (grid lines) to aid in material deposition and 25 identification.

[0019] The term “piezo dispensing device” and the like refer, in the usual and customary sense, to a device capable of dispensing small amounts of solution using pressure waves, typically transduced via piezoelectric methodology. In embodiments, piezo dispensing devices disclosed herein and embodiments thereof can dispense sample aliquots on the picoliter scale, e.g., about 30 10 pL, 20 pL, 30 pL, 40 pL, 50 pL, 60 pL, 70 pL, 80 pL, 90 pL or even 100 pL or greater.

[0020] The term “drop on demand” and the like refers to the methods disclosed herein which allow accurate and reproducible placement of droplets of solution for analysis in the LCTEM.

[0021] The term “multiplex liquid cell TEM,” “multiple LCTEM” and the like as used herein refer to the capability of manipulating and analyzing a plurality of aliquots of solution for

5 LCTEM, using the method and apparatus disclosed herein.

[0022] The term “nanoparticle” and the like refer, in the usual and customary sense, to particles having a largest dimension of between about 1 and 100 nm, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 60, 70, 80, 90 or even 100 nm.

[0023] Absent express indication otherwise, the term “about” in the context of a numerical
10 value means the nominal value $\pm 10\%$ thereof.

Methods

[0024] The invention described herein relates, in part, to methods for detecting nanoparticles, in particularly detecting mixing and reaction of nanoparticles using Liquid Cell Transmission Electron Microscopy (TEM) or any TEM that involves depositing a small liquid droplet sample
15 during the sample preparation stage. Controlling the addition of multiple solutions to the liquid cell remains a hurdle (for example, due to the quick evaporation of liquid samples in small volume) in the ability to increase throughput and to study processes dependent on solution mixing, e.g., chemical reactions. Previous methods directed to related problems include mixing into wells of a TEM holder tip, or premixing solutions and flowing the solution into a TEM cell.
20 These methods, however, fail to allow the imaging of multiple samples which mix directly within the viewing area of the TEM, or the analysis of multiple isolated samples with respect to, e.g., diffusion and mixing. These prior methods, therefore, are not able to detect the initial stage of the reactions (e.g., the initial stage within the first 15 seconds).

[0025] In embodiments, the methods described herein utilize novel approaches of depositing
25 multiple samples in small (e.g., nanoliter or picoliter) droplets on one TEM support (e.g., TEM grid) under humidifying conditions, which significantly increases the number of samples that can be analyzed within a single measurement cycle and provides new experimental procedures, including, e.g., the ability the monitor diffusion and mixing of nanoparticle-containing solutions on the TEM support.

30 [0026] The methods described herein can be used for analyzing a sample using TEM and/or detecting nanoscale molecules using TEM, for example, chemistry manifests on the nanoscale

using TEM, such as, nanoparticles formation through block copolymer assembly, formation of a ZIF-8 metal organic-framework system, nanocrystal formation, and chemical recognition of molecules conjugated to a nanoparticle.

[0027] In a first aspect, there is provided a method for detecting a nanoparticle in a liquid. The method includes depositing a liquid droplet containing a nanoparticle onto a transmission electron microscope sample holder under humidifying conditions, where the volume of the liquid droplet is less than about 1000 pL. The method further includes covering the transmission electron microscope sample holder with a transmission electron microscope sample holder cover. The method further includes detecting the nanoparticle within the liquid droplet using a transmission electron microscope.

[0028] In another aspect, there is provided method for detecting a plurality of nanoparticles and an interaction thereof in a liquid. The method includes depositing a plurality of liquid droplets each including a nanoparticle onto a transmission electron microscope sample holder under humidifying conditions, where the volume of each liquid droplet is less than about 1000 pL. The method further includes covering the transmission electron microscope sample holder with a transmission electron microscope sample holder cover. The method further includes detecting the plurality of nanoparticles and interaction thereof upon diffusion of the plurality of liquid droplets using a transmission electron microscope. In embodiments, a plurality of liquid droplets includes at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96 or more liquid droplets, where each liquid droplet includes at least one nanoparticle.

[0029] In another aspect, there is provided method of analyzing a sample (e.g., a liquid sample) using transmission electron microscope (TEM). The method includes automatically depositing a liquid droplet of the sample onto a TEM sample holder (e.g., TEM compatible substrate), where the volume of said liquid droplet is less than about 1000 pL and analyzing the liquid sample using TEM. In embodiments, the automatically depositing is depositing within 5 microns (e.g., about 5, 4, 3, 2, or 1 micron) of a predetermined location on the TEM sample holder. In embodiments, each liquid sample may contain one type of nanoparticles. In embodiments, each liquid droplet of the sample may include one type of nanoparticle. In embodiments, each liquid sample may contain one type of chemicals (e.g., reactants that have size of smaller than 1nm). In

embodiments, each liquid droplet may include one type of chemicals. In embodiments, the liquid sample includes a plurality of nanoparticles or a plurality of chemicals. In embodiments, the analyzing includes detecting this plurality of nanoparticles or this plurality of chemicals. In embodiments, the method includes covering the TEM sample holder with a TEM sample holder cover prior to the analyzing. In embodiments, this covering is automated. In embodiments, the automatically depositing is performed under humidifying conditions.

[0030] In embodiments, at the time of detecting or analyzing the liquid sample with TEM according to any method described herein, the liquid sample may not be a liquid but rather a solid resulting from evaporation of said liquid.

[0031] In embodiments, the volume of each liquid droplet being deposited in any method described herein is about 1 pL to about 1000 pL. In embodiments, the volume of each liquid droplet being deposited is about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, 229, 230, 231, 232, 233, 234, 235, 236, 237, 238, 239, 240, 241, 242, 243, 244, 245, 246, 247, 248, 249, 250, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 264, 265, 266, 267, 268, 269, 270, 271, 272, 273, 274, 275, 276, 277, 278, 279, 280, 281, 282, 283, 284, 285, 286, 287, 288, 289, 290, 291, 292, 293, 294, 295, 296, 297, 298, 299, 300, 301, 302, 303, 304, 305, 306, 307, 308, 309, 310, 311, 312, 313, 314, 315, 316, 317, 318, 319, 320, 321, 322, 323, 324, 325, 326, 327, 328, 329, 330, 331, 332, 333, 334, 335, 336, 337, 338, 339, 340, 341, 342, 343, 344, 345, 346, 347, 348, 349, 350, 351, 352, 353, 354, 355, 356, 357, 358, 359, 360, 361, 362, 363, 364, 365, 366, 367, 368, 369, 370, 371, 372, 373, 374, 375, 376, 377, 378, 379, 380, 381, 382, 383, 384, 385, 386, 387, 388, 389, 390, 391, 392, 393, 394, 395, 396, 397, 398, 399, 400, 401, 402, 403, 404, 405, 406, 407, 408, 409, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440,

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30 992, 993, 994, 995, 996, 997, 998, 999, 1000 pL.

[0032] In embodiments, the volume of each liquid droplet being deposited in any method described herein is about 10 pL to about 1000 pL. In embodiments, the volume of each liquid droplet being deposited is about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26,

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958, 959, 960, 961, 962, 963, 964, 965, 966, 967, 968, 969, 970, 971, 972, 973, 974, 975, 976,
977, 978, 979, 980, 981, 982, 983, 984, 985, 986, 987, 988, 989, 990, 991, 992, 993, 994, 995,
996, 997, 998, 999, 1000 pL.

[0033] The term “humidifying conditions” refers to conditions providing sufficient amounts of
20 solvent vapor or gas (e.g., water vapor) to prevent and/or decrease evaporation of solvent during
deposition and subsequent preparation of the liquid droplet (i.e., sample liquid droplet) for TEM
analysis.

[0034] In embodiments, the humidifying conditions are generated by depositing a volatile
solvent droplet onto the sample holder. In embodiments, the volatile solvent droplet is a plurality
25 of volatile solvent droplets. In embodiments, the volatile solvent droplet or a plurality of volatile
solvent droplets is being deposited prior to depositing the sample liquid droplet. In
embodiments, the volatile solvent droplet or a plurality of volatile solvent droplets is being
deposited simultaneously with the sample liquid droplet or the first batch of sample liquid
droplets.

[0035] In embodiments, the volatile solvent droplet or a plurality of volatile solvent droplets
30 are sufficiently proximal to the sample(s) to keep the sample(s) from drying out. In
embodiments, the volatile solvent droplet or a plurality of volatile solvent droplets are about 50

to about 300 μm (e.g., about 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300 μm) from at least one of the samples of the interest on the sample holder.

[0036] In embodiments, the volatile solvent is compatible with the material of the sample holder and its components (e.g., the substrate material). In embodiments, the volatile solvent is water, alcohol, toluene, tetrahydrofuran (THF), dimethylformamide (DMF), acetone, acetonitrile or cyclohexanone. Exemplary alcohol includes, but is not limited to, ethanol, isopropyl alcohol, methanol, butanol, isobutanol, and glycerol. In embodiments, the volatile solvent is water.

[0037] In embodiments, the humidifying conditions are generated by a humidifier chamber. In embodiments, the humidifier chamber surrounds the sample holder as illustrated in FIG. 8B. The humidifier chamber can be made from any suitable materials, for example, acetal (Delrin®), aluminum, stainless steel, glass. In embodiments, the humidifier chamber is made from acetal (Delrin®). In embodiments, the humidifier chamber is made from aluminum. In embodiments, the humidifier chamber includes a volatile solvent reservoir that provides sufficient vapor to prevent the samples from drying out. In embodiments, the volatile solvent reservoir is underneath the transmission electron microscope sample holder. In embodiments, the volatile solvent is compatible with the material of the humidifier chamber. In embodiments, the volatile solvent is water, alcohol, toluene, tetrahydrofuran (THF), dimethylformamide (DMF), acetone, acetonitrile or cyclohexanone. Exemplary alcohol includes, but is not limited to, ethanol, isopropyl alcohol, methanol, butanol, isobutanol, and glycerol. In embodiments, the volatile solvent is water.

[0038] In embodiments, the volatile solvents compatible for glass include, but are not limited to, the following in Table 1 below. A-Excellent is defined as no effect, no detectable corrosion or discoloration.

Table 1. Volatile solvents compatible for glass			
Chemical	Compatibility	Chemical	Compatibility
Acetic Acid	A-Excellent	Oils:Aniline	A-Excellent
Acetic Acid 20%	A-Excellent	Oils:Anise	A-Excellent
Acetic Acid 80%	A-Excellent	Oils:Bay	A-Excellent
Acetic Acid, Glacial	A-Excellent	Oils:Bone	A-Excellent
Antifreeze	A-Excellent	Oils:Castor	A-Excellent
Asphalt	A-Excellent	Oils:Cinnamon	A-Excellent
Beer	A-Excellent	Oils:Citric	A-Excellent
Beet Sugar Liquids	A-Excellent	Oils:Clove	A-Excellent

Benzene	A-Excellent	Oils:Coconut	A-Excellent
Boric Acid	A-Excellent	Oils:Cod Liver	A-Excellent
Brewery Slop	A-Excellent	Oils:Corn	A-Excellent
Butter	A-Excellent	Oils:Cottonseed	A-Excellent
Buttermilk	A-Excellent	Oils:Creosote	A-Excellent
Calcium Chloride	A-Excellent	Oils:Diesel Fuel (20, 30, 40, 50)	A-Excellent
Calcium Hypochlorite	A-Excellent	Oils:Fuel (1, 2, 3, 5A, 5B, 6)	A-Excellent
Carbonated Water	A-Excellent	Oils:Ginger	A-Excellent
Chocolate Syrup	A-Excellent	Oils:Hydraulic Oil (Petro)	A-Excellent
Chromic Acid 10%	A-Excellent	Oils:Hydraulic Oil (Synthetic)	A-Excellent
Chromic Acid 30%	A-Excellent	Oils:Lemon	A-Excellent
Chromic Acid 5%	A-Excellent	Oils:Linseed	A-Excellent
Cider	A-Excellent	Oils:Mineral	A-Excellent
Citric Acid	A-Excellent	Oils:Olive	A-Excellent
Citric Oils	A-Excellent	Oils:Orange	A-Excellent
Coffee	A-Excellent	Oils:Palm	A-Excellent
Cream	A-Excellent	Oils:Peanut	A-Excellent
Detergents	A-Excellent	Oils:Peppermint	A-Excellent
Diesel Fuel	A-Excellent	Oils:Pine	A-Excellent
Dyes	A-Excellent	Oils:Rapeseed	A-Excellent
Epsom Salts (Magnesium Sulfate)	A-Excellent	Oils:Rosin	A-Excellent
Formic Acid	A-Excellent	Oils:Sesame Seed	A-Excellent
Fruit Juice	A-Excellent	Oils:Silicone	A-Excellent
Fuel Oils	A-Excellent	Oils:Soybean	A-Excellent
Furan Resin	A-Excellent	Oils:Sperm (whale)	A-Excellent
Gasoline (high-aromatic)	A-Excellent	Oils:Tanning	A-Excellent
Gasoline, leaded, ref.	A-Excellent	Oils:Transformer	A-Excellent
Gasoline, unleaded	A-Excellent	Oils:Turbine	A-Excellent
Gelatin	A-Excellent	Paraffin	A-Excellent
Glucose	A-Excellent	Pentane	A-Excellent
Glue, P.V.A.	A-Excellent	Petroleum	A-Excellent
Glycerin	A-Excellent	Phosphoric Acid (<40%)	A-Excellent
Grape Juice	A-Excellent	Phosphoric Acid (crude)	A-Excellent
Grease	A-Excellent	Phosphoric Acid (molten)	A-Excellent
Heptane	A-Excellent	Potassium	A-Excellent

		Sulfate	
Hexane	A-Excellent	Propane (liquefied)	A-Excellent
Honey	A-Excellent	Rum	A-Excellent
Hydraulic Oil (Petro)	A-Excellent	Rust Inhibitors	A-Excellent
Hydraulic Oil (Synthetic)	A-Excellent	Salad Dressings	A-Excellent
Hydrazine	A-Excellent	Salt Brine (NaCl saturated)	A-Excellent
Hydrochloric Acid 20%	A-Excellent	Sea Water	A-Excellent
Hydrochloric Acid 37%	A-Excellent	Shellac (Bleached)	A-Excellent
Hydrogen Peroxide 10%	A-Excellent	Shellac (Orange)	A-Excellent
Ink	A-Excellent	Silicone	A-Excellent
Iodine	A-Excellent	Silver Bromide	A-Excellent
Iodine (in alcohol)	A-Excellent	Silver Nitrate	A-Excellent
Iodoform	A-Excellent	Soap Solutions	A-Excellent
Jet Fuel (JP3, JP4, JP5)	A-Excellent	Soda Ash (see Sodium Carbonate)	A-Excellent
Kerosene	A-Excellent	Sodium Acetate	A-Excellent
Lacquer Thinners	A-Excellent	Sodium Borate (Borax)	A-Excellent
Lacquers	A-Excellent	Sodium Chloride	A-Excellent
Lactic Acid	A-Excellent	Sodium Hydroxide (20%)	A-Excellent
Lard	A-Excellent	Sodium Hydroxide (50%)	A-Excellent
Latex	A-Excellent	Sodium Nitrate	A-Excellent
Lime	A-Excellent	Soy Sauce	A-Excellent
Lye: Ca(OH) ₂ Calcium Hydroxide	A-Excellent	Starch	A-Excellent
Lye: KOH Potassium Hydroxide	A-Excellent	Sugar (Liquids)	A-Excellent
Magnesium Chloride	A-Excellent	Sulfuric Acid (<10%)	A-Excellent
Mash	A-Excellent	Sulfuric Acid (10-75%)	A-Excellent
Mayonnaise	A-Excellent	Sulfuric Acid (hot concentrated)	A-Excellent
Melamine	A-Excellent	Tallow	A-Excellent
Milk	A-Excellent	Tanning Liquors	A-Excellent
Mineral Spirits	A-Excellent	Tomato Juice	A-Excellent
Molasses	A-Excellent	Turpentine	A-Excellent
Motor oil	A-Excellent	Urea	A-Excellent

Mustard	A-Excellent	Uric Acid	A-Excellent
Naphtha	A-Excellent	Urine	A-Excellent
Naphthalene	A-Excellent	Varnish	A-Excellent
Natural Gas	A-Excellent	Vegetable Juice	A-Excellent
Nickel Chloride	A-Excellent	Vinegar	A-Excellent
Nitric Acid (20%)	A-Excellent	Water, Acid, Mine	A-Excellent
Nitric Acid (50%)	A-Excellent	Water, Deionized	A-Excellent
Nitric Acid (5-10%)	A-Excellent	Water, Distilled	A-Excellent
Nitric Acid (Concentrated)	A-Excellent	Water, Fresh	A-Excellent
Nitrogen Fertilizer	A-Excellent	Water, Salt	A-Excellent
		Weed Killers	A-Excellent
		Whey	A-Excellent
		Whiskey & Wines	A-Excellent
		White Liquor (Pulp Mill)	A-Excellent
		White Water (Paper Mill)	A-Excellent

[0039] In embodiments, the volatile solvents compatible for stainless steel (e.g., 316 Stainless Steel) include, but are not limited to, the following in Table 2 below. A-Excellent is defined as no effect, no detectable corrosion or discoloration.

Table 2. Volatile solvents compatible for 316 stainless steel

Chemical	Compatibility	Chemical	Compatibility
Acetaldehyde	A-Excellent	Mash	A-Excellent
Acetamide	A-Excellent	Mayonnaise	A-Excellent
Acetate Solvent	A-Excellent	Mercurous Nitrate	A-Excellent
Acetic Acid 20%	A-Excellent	Mercury	A-Excellent
Acetic Acid, Glacial	A-Excellent	Methane	A-Excellent
Acetic Anhydride	A-Excellent	Methanol (Methyl Alcohol)	A-Excellent
Acetone	A-Excellent	Methyl Acetone	A-Excellent
Acetyl Chloride (dry)	A-Excellent	Methyl Alcohol 10%	A-Excellent
Acetylene	A-Excellent	Methyl Bromide	A-Excellent
Acrylonitrile	A-Excellent	Methyl Butyl Ketone	A-Excellent
Adipic Acid	A-Excellent	Methyl Chloride	A-Excellent
Alcohols: Amyl	A-Excellent	Methyl Ethyl Ketone	A-Excellent
Alcohols: Butyl	A-Excellent	Methyl Isopropyl Ketone	A-Excellent
Alcohols: Diacetone	A-Excellent	Methylamine	A-Excellent

Alcohols: Ethyl	A-Excellent	Milk	A-Excellent
Alcohols: Hexyl	A-Excellent	Mineral Spirits	A-Excellent
Alcohols: Isobutyl	A-Excellent	Molasses	A-Excellent
Alcohols: Methyl	A-Excellent	Monochloroacetic Acid	A-Excellent
Alcohols: Octyl	A-Excellent	Monoethanolamine	A-Excellent
Alcohols: Propyl	A-Excellent	Morpholine	A-Excellent
Allyl Chloride	A-Excellent	Motor Oil	A-Excellent
Aluminum Acetate (saturated)	A-Excellent	Mustard	A-Excellent
Aluminum Nitrate	A-Excellent	Naphtha	A-Excellent
Aluminum Potassium Sulfate 10%	A-Excellent	Naphthalene	A-Excellent
Alums	A-Excellent	Natural Gas	A-Excellent
Amines	A-Excellent	Nitrating Acid (<1% Acid)	A-Excellent
Ammonia 10%	A-Excellent	Nitric Acid (20%)	A-Excellent
Ammonia Nitrate	A-Excellent	Nitric Acid (5 to10%)	A-Excellent
Ammonia, anhydrous	A-Excellent	Nitric Acid (50%)	A-Excellent
Ammonia, liquid	A-Excellent	Nitric Acid (Concentrated)	A-Excellent
Ammonium Acetate	A-Excellent	Nitromethane	A-Excellent
Ammonium Caseinate	A-Excellent	Oils: Aniline	A-Excellent
Ammonium Hydroxide	A -Excellent	Oils: Anise	A-Excellent
Ammonium Nitrate	A-Excellent	Oils: Bay	A-Excellent
Ammonium Oxalate	A-Excellent	Oils: Bone	A-Excellent
Ammonium Thiosulfate	A-Excellent	Oils: Castor	A-Excellent
Amyl Acetate	A-Excellent	Oils: Cinnamon	A-Excellent
Amyl Alcohol	A-Excellent	Oils: Citric	A-Excellent
Amyl Chloride	A-Excellent	Oils: Clove	A-Excellent
Antifreeze (glycol-based)	A-Excellent	Oils: Coconut	A-Excellent
Arsenic Acid	A-Excellent	Oils: Cod Liver	A-Excellent
Asphalt	A-Excellent	Oils: Corn	A-Excellent
Barium Chloride	A-Excellent	Oils: Cottonseed	A-Excellent
Barium Cyanide	A-Excellent	Oils: Crude Oil	A-Excellent
Beer	A-Excellent	Oils: Diesel Fuel (20, 30, 40, 50)	A-Excellent
Beet Sugar Liquids	A-Excellent	Oils: Fuel (1, 2, 3, 5A, 5B, 6)	A-Excellent
Benzol	A-Excellent	Oils: Hydraulic Oil (Petro)	A-Excellent
Bleach	A-Excellent	Oils: Hydraulic Oil (Synthetic)	A-Excellent
Borax (Sodium Borate)	A-Excellent	Oils: Lemon	A-Excellent

Boric Acid	A-Excellent	Oils: Linseed	A-Excellent
Brewery Slop	A-Excellent	Oils: Mineral	A-Excellent
Butadiene	A-Excellent	Oils: Olive	A-Excellent
Butane	A-Excellent	Oils: Orange	A-Excellent
Butanol (Butyl Alcohol)	A-Excellent	Oils: Palm	A-Excellent
Butter	A-Excellent	Oils: Peanut	A-Excellent
Buttermilk	A-Excellent	Oils: Peppermint	A-Excellent
Butyl Amine	A-Excellent	Oils: Pine	A-Excellent
Butyl Ether	A-Excellent	Oils: Rapeseed	A-Excellent
Butylacetate	A-Excellent	Oils: Rosin	A-Excellent
Butylene	A-Excellent	Oils: Sesame Seed	A-Excellent
Calcium Bisulfate	A-Excellent	Oils: Silicone	A-Excellent
Calcium Bisulfite	A-Excellent	Oils: Soybean	A-Excellent
Calcium Oxide	A-Excellent	Oils: Sperm (whale)	A-Excellent
Calgon	A-Excellent	Oils: Tanning	A-Excellent
Cane Juice	A-Excellent	Oils: Transformer	A-Excellent
Carbon Dioxide (dry)	A-Excellent	Oils: Turbine	A-Excellent
Carbon Dioxide (wet)	A-Excellent	Oleic Acid	A-Excellent
Carbon Monoxide	A-Excellent	Oleum 100%	A-Excellent
Carbon Tetrachloride (wet)	A-Excellent	Oxalic Acid (cold)	A-Excellent
Carbonated Water	A-Excellent	Ozone	A-Excellent
Carbonic Acid	A-Excellent	Palmitic Acid	A-Excellent
Catsup	A-Excellent	Paraffin	A-Excellent
Chlorinated Glue	A-Excellent	Pentane	A-Excellent
Chloroacetic Acid	A-Excellent	Perchloroethylene	A-Excellent
Chloroform	A-Excellent	Petrolatum	A-Excellent
Chocolate Syrup	A-Excellent	Petroleum	A-Excellent
Chromic Acid 5%	A-Excellent	Phosphorus	A-Excellent
Cider	A-Excellent	Phosphorus Trichloride	A-Excellent
Citric Acid	A-Excellent	Photographic Developer	A-Excellent
Citric Oils	A-Excellent	Phthalic Acid	A-Excellent
Coffee	A-Excellent	Phthalic Anhydride	A-Excellent
Copper Nitrate	A-Excellent	Plating Solutions: Antimony Plating 130°F	A-Excellent
Cream	A-Excellent	Plating Solutions: Arsenic Plating 110°F	A-Excellent
Cresols	A-Excellent	Plating Solutions: Brass: High-Speed Brass Bath 110°F	A-Excellent
Cresylic Acid	A-Excellent	Plating Solutions: Brass: Regular Brass Bath 100°F	A-Excellent

Cyanic Acid	A-Excellent	Plating Solutions: Bronze: Cu-Cd Bronze Bath R.T.	A-Excellent
Cyclohexane	A-Excellent	Plating Solutions: Bronze: Cu-Sn Bronze Bath 160°F	A-Excellent
Cyclohexanone	A-Excellent	Plating Solutions: Bronze: Cu-Zn Bronze Bath 100°F	A-Excellent
Detergents	A-Excellent	Plating Solutions: Cadmium: Cyanide Bath 90°F	A-Excellent
Diesel Fuel	A-Excellent	Plating Solutions: Cadmium: Fluoborate Bath 100°F	A-Excellent
Diethylamine	A-Excellent	Plating Solutions: Copper (Cyanide): Copper Strike Bath 120°F	A-Excellent
Diethylene Glycol	A-Excellent	Plating Solutions: Copper (Cyanide): High-Speed Bath 180°F	A-Excellent
Diphenyl Oxide	A-Excellent	Plating Solutions: Copper (Cyanide): Rochelle Salt Bath 150°F	A-Excellent
Disodium Phosphate	A-Excellent	Plating Solutions: Copper (Misc): Copper Pyrophosphate	A-Excellent
Dyes	A-Excellent	Plating Solutions: Gold: Cyanide 150°F	A-Excellent
Ethane	A-Excellent	Plating Solutions: Silver Plating 80-120°F	A-Excellent
Ethanol	A-Excellent	Plating Solutions: Zinc: Alkaline Cyanide Bath R.T.	A-Excellent
Ethanolamine	A-Excellent	Potassium Chloride	A-Excellent
Ether	A-Excellent	Potassium Hydroxide (Caustic Potash)	A-Excellent
Ethyl Chloride	A-Excellent	Potassium Iodide	A-Excellent
Ethylene Bromide	A-Excellent	Potassium Sulfate	A-Excellent
Fatty Acids	A-Excellent	Propane (liquefied)	A-Excellent
Ferric Sulfate	A-Excellent	Propylene	A-Excellent
Fluorine	A-Excellent	Pyridine	A-Excellent
Formaldehyde 100%	A-Excellent	Rosins	A-Excellent
Formaldehyde 40%	A-Excellent	Rum	A-Excellent
Formic Acid	A-Excellent	Rust Inhibitors	A-Excellent
Freon® 11	A-Excellent	Salad Dressings	A-Excellent

Freon® 22	A-Excellent	Salt Brine (NaCl saturated)	A-Excellent
Freon® TF	A-Excellent	Shellac (Bleached)	A-Excellent
Fruit Juice	A-Excellent	Shellac (Orange)	A-Excellent
Fuel Oils	A-Excellent	Silicone	A-Excellent
Furan Resin	A-Excellent	Soap Solutions	A-Excellent
Gasoline (high–aromatic)	A-Excellent	Soda Ash (see Sodium Carbonate)	A-Excellent
Gasoline, leaded, ref.	A-Excellent	Sodium Aluminate	A-Excellent
Gasoline, unleaded	A-Excellent	Sodium Bicarbonate	A-Excellent
Gelatin	A-Excellent	Sodium Carbonate	A-Excellent
Glucose	A-Excellent	Sodium Hyposulfate	A-Excellent
Glue, P.V.A.	A-Excellent	Sodium Metaphosphate	A-Excellent
Glycerin	A-Excellent	Sodium Metasilicate	A-Excellent
Glycolic Acid	A-Excellent	Sodium Peroxide	A-Excellent
Gold Monocyanide	A-Excellent	Sodium Sulfite	A-Excellent
Grape Juice	A-Excellent	Sodium Tetraborate	A-Excellent
Grease	A-Excellent	Sorghum	A-Excellent
Heptane	A-Excellent	Soy Sauce	A-Excellent
Hexane	A-Excellent	Stannic Fluoborate	A-Excellent
Honey	A-Excellent	Stannous Chloride	A-Excellent
Hydraulic Oil (Petro)	A-Excellent	Starch	A-Excellent
Hydraulic Oil (Synthetic)	A-Excellent	Stearic Acid	A-Excellent
Hydrazine	A-Excellent	Stoddard Solvent	A-Excellent
Hydrocyanic Acid	A-Excellent	Styrene	A-Excellent
Hydrogen Gas	A-Excellent	Sugar (Liquids)	A-Excellent
Hydrogen Peroxide 100%	A-Excellent	Sulfur Dioxide	A-Excellent
Hydrogen Peroxide 50%	A-Excellent	Sulfur Dioxide (dry)	A-Excellent
Hydrogen Sulfide (aqua)	A-Excellent	Sulfur Trioxide (dry)	A-Excellent
Hydrogen Sulfide (dry)	A-Excellent	Tallow	A-Excellent
Iodoform	A-Excellent	Tannic Acid	A-Excellent
Isooctane	A-Excellent	Tanning Liquors	A-Excellent
Isopropyl Acetate	A-Excellent	Tetrachloroethane	A-Excellent
Isopropyl Ether	A-Excellent	Tetrachloroethylene	A-Excellent
Jet Fuel (JP3, JP4, JP5, JP8)	A-Excellent	Tetrahydrofuran	A-Excellent
Kerosene	A-Excellent	Toluene (Toluol)	A-Excellent
Ketones	A-Excellent	Tomato Juice	A-Excellent
Lacquer Thinners	A-Excellent	Trichloropropane	A-Excellent
Lacquers	A-Excellent	Triethylamine	A-Excellent

Lard	A-Excellent	Turpentine	A-Excellent
Latex	A-Excellent	Urine	A-Excellent
Ligroin	A-Excellent	Varnish	A-Excellent
Lime	A-Excellent	Vegetable Juice	A-Excellent
Linoleic Acid	A-Excellent	Vinegar	A-Excellent
Lithium Chloride	A-Excellent	Vinyl Chloride	A-Excellent
Lubricants	A-Excellent	Water, Deionized	A-Excellent
Lye: KOH Potassium Hydroxide	A-Excellent	Water, Distilled	A-Excellent
Magnesium Bisulfate	A-Excellent	Water, Fresh	A-Excellent
Magnesium Hydroxide	A-Excellent	Weed Killers	A-Excellent
Magnesium Oxide	A-Excellent	Whey	A-Excellent
Maleic Anhydride	A-Excellent	Whiskey and Wines	A-Excellent
Malic Acid	A-Excellent	White Liquor (Pulp Mill)	A-Excellent
		White Water (Paper Mill)	A-Excellent
		Zinc Hydrosulfite	A-Excellent
		Zinc Sulfate	A-Excellent

[0040] In embodiments, the volatile solvents compatible for acetal (Delrin®) include, but are not limited to the ones in the Table 3 below. A-Excellent is defined as no effect, no detectable corrosion or discoloration.

Table 3. Volatile solvents compatible for acetal (Delrin®)			
Acetaldehyde	A-Excellent	Magnesium Hydroxide	A-Excellent
Acetamide	A-Excellent	Magnesium Nitrate	A-Excellent
Acetone	A-Excellent	Magnesium Oxide	A-Excellent
Acetylene	A-Excellent	Maleic Acid	A-Excellent
Alcohols: Amyl	A-Excellent	Malic Acid	A-Excellent
Alcohols: Benzyl	A-Excellent	Manganese Sulfate	A-Excellent
Alcohols: Butyl	A-Excellent	Mash	A-Excellent
Alcohols: Diacetone	A-Excellent	Mayonnaise	A-Excellent
Alcohols: Ethyl	A-Excellent	Melamine	A-Excellent
Alcohols: Hexyl	A-Excellent	Mercury	A-Excellent
Alcohols: Isobutyl	A-Excellent	Methane	A-Excellent
Alcohols: Isopropyl	A-Excellent	Methanol (Methyl Alcohol)	A-Excellent
Alcohols: Methyl	A-Excellent	Methyl Alcohol 10%	A-Excellent

Alcohols: Octyl	A-Excellent	Milk	A-Excellent
Alcohols: Propyl	A-Excellent	Mineral Spirits	A-Excellent
Aluminum Hydroxide	A-Excellent	Molasses	A-Excellent
Ammonium Nitrate	A-Excellent	Naphtha	A-Excellent
Amyl Alcohol	A-Excellent	Naphthalene	A-Excellent
Amyl Chloride	A-Excellent	Nickel Chloride	A-Excellent
Aniline	A-Excellent	Nickel Sulfate	A-Excellent
Aromatic Hydrocarbons	A-Excellent	Nitromethane	A-Excellent
Barium Carbonate	A-Excellent	Oils: Castor	A-Excellent
Barium Chloride	A-Excellent	Oils: Citric	A-Excellent
Barium Sulfide	A-Excellent	Oils: Coconut	A-Excellent
Beer	A-Excellent	Oils: Corn	A-Excellent
Benzaldehyde	A-Excellent	Oils: Cottonseed	A-Excellent
Benzene	A-Excellent	Oils: Crude Oil	A-Excellent
Benzol	A-Excellent	Oils: Ginger	A-Excellent
Benzyl Chloride	A-Excellent	Oils: Linseed	A-Excellent
Boric Acid	A-Excellent	Oils: Mineral	A-Excellent
Butadiene	A-Excellent	Oils: Olive	A-Excellent
Butane	A-Excellent	Oils: Palm	A-Excellent
Butanol (Butyl Alcohol)	A-Excellent	Oils: Peanut	A-Excellent
Butter	A-Excellent	Oils: Pine	A-Excellent
Buttermilk	A-Excellent	Oils: Rapeseed	A-Excellent
Butylacetate	A-Excellent	Oils: Silicone	A-Excellent
Butylene	A-Excellent	Oils: Soybean	A-Excellent
Butyric Acid	A-Excellent	Oils: Transformer	A-Excellent
Calcium Carbonate	A-Excellent	Oils: Turbine	A-Excellent
Calcium Chlorate	A-Excellent	Oleic Acid	A-Excellent
Calcium Hydroxide 10%	A-Excellent	Palmitic Acid	A-Excellent
Calcium Oxide	A-Excellent	Paraffin	A-Excellent
Calgon	A-Excellent	Picric Acid	A-Excellent
Cane Juice	A-Excellent	Plating Solutions: Antimony Plating 130°F	A-Excellent
Carbon Bisulfide	A-Excellent	Plating Solutions: Arsenic Plating 110°F	A-Excellent
Carbon Dioxide (dry)	A-Excellent	Plating Solutions: Brass: High-Speed Brass Bath 110°F	A-Excellent
Carbon Dioxide (wet)	A-Excellent	Plating Solutions: Brass: Regular Brass Bath	A-Excellent

		100°F	
Carbon Disulfide	A-Excellent	Plating Solutions: Bronze: Cu-Cd Bronze Bath R.T.	A-Excellent
Carbon Monoxide	A-Excellent	Plating Solutions: Bronze: Cu-Zn Bronze Bath 100°F	A-Excellent
Carbon Tetrachloride (wet)	A-Excellent	Plating Solutions: Cadmium: Cyanide Bath 90°F	A-Excellent
Carbonated Water	A-Excellent	Plating Solutions: Copper (Acid): Copper Sulfate Bath R.T.	A-Excellent
Chlorine, Anhydrous Liquid	A-Excellent	Plating Solutions: Copper (Cyanide): Copper Strike Bath 120°F	A-Excellent
Chloroform	A-Excellent	Plating Solutions: Copper (Misc): Copper Pyrophosphate	A-Excellent
Chocolate Syrup	A-Excellent	Potassium Bromide	A-Excellent
Cider	A-Excellent	Potassium Chloride	A-Excellent
Coffee	A-Excellent	Potassium Dichromate	A-Excellent
Copper Chloride	A-Excellent	Potassium Hydroxide (Caustic Potash)	A-Excellent
Copper Cyanide	A-Excellent	Potassium Nitrate	A-Excellent
Copper Nitrate	A-Excellent	Potassium Permanganate	A-Excellent
Cream	A-Excellent	Propane (liquefied)	A-Excellent
Cyclohexane	A-Excellent	Rum	A-Excellent
Cyclohexanone	A-Excellent	Rust Inhibitors	A-Excellent
Detergents	A-Excellent	Salad Dressings	A-Excellent
Dichloroethane	A-Excellent	Sea Water	A-Excellent
Diesel Fuel	A-Excellent	Shellac (Bleached)	A-Excellent
Diethylene Glycol	A-Excellent	Shellac (Orange)	A-Excellent
Ethane	A-Excellent	Silicone	A-Excellent
Ethanol	A-Excellent	Silver Nitrate	A-Excellent
Ether	A-Excellent	Soap Solutions	A-Excellent
Ethyl Acetate	A-Excellent	Soda Ash (see Sodium Carbonate)	A-Excellent
Ethyl Chloride	A-Excellent	Sodium Bicarbonate	A-Excellent
Ethyl Ether	A-Excellent	Sodium Bromide	A-Excellent
Ethylene Chloride	A-Excellent	Sodium Carbonate	A-Excellent
Fatty Acids	A-Excellent	Sodium Chlorate	A-Excellent
Fluoboric Acid	A-Excellent	Sodium Chloride	A-Excellent
Fluosilicic Acid	A-Excellent	Sodium Cyanide	A-Excellent
Formaldehyde 100%	A-Excellent	Sodium Ferrocyanide	A-Excellent

Formaldehyde 40%	A-Excellent	Sodium Hydroxide (20%)	A-Excellent
Formic Acid	A-Excellent	Sodium Hydroxide (50%)	A-Excellent
Freon® 113	A-Excellent	Sodium Nitrate	A-Excellent
Freon® 22	A-Excellent	Sorghum	A-Excellent
Freon® TF	A-Excellent	Soy Sauce	A-Excellent
Fuel Oils	A-Excellent	Starch	A-Excellent
Furfural	A-Excellent	Stearic Acid	A-Excellent
Gasoline, leaded, ref.	A-Excellent	Stoddard Solvent	A-Excellent
Gasoline, unleaded	A-Excellent	Styrene	A-Excellent
Glucose	A-Excellent	Sugar (Liquids)	A-Excellent
Glue, P.V.A.	A-Excellent	Sulfuryl Chloride	A-Excellent
Glycerin	A-Excellent	Tallow	A-Excellent
Glycolic Acid	A-Excellent	Tetrachloroethane	A-Excellent
Gold Monocyanide	A-Excellent	Tetrachloroethylene	A-Excellent
Grape Juice	A-Excellent	Tetrahydrofuran	A-Excellent
Heptane	A-Excellent	Trichloroethane	A-Excellent
Hexane	A-Excellent	Trichloropropane	A-Excellent
Honey	A-Excellent	Trisodium Phosphate	A-Excellent
Hydrofluosilicic Acid 100%	A-Excellent	Turpentine	A-Excellent
Hydroquinone	A-Excellent	Urea	A-Excellent
Hydroxyacetic Acid 70%	A-Excellent	Urine	A-Excellent
Jet Fuel (JP3, JP4, JP5, JP8)	A-Excellent	Varnish	A-Excellent
Kerosene	A-Excellent	Vegetable Juice	A-Excellent
Lard	A-Excellent	Water, Acid, Mine	A-Excellent
Lead Sulfamate	A-Excellent	Water, Fresh	A-Excellent
Lithium Chloride	A-Excellent	Water, Salt	A-Excellent
Lubricants	A-Excellent	Weed Killers	A-Excellent
Lye: KOH Potassium Hydroxide	A-Excellent	Whey	A-Excellent
Magnesium Carbonate	A-Excellent	Whiskey and Wines	A-Excellent
		Xylene	A-Excellent

[0041] In embodiments, the volatile solvents compatible for aluminum include, but are not limited to the ones in the Table 4 below. A-Excellent is defined as no effect, no detectable corrosion or discoloration.

Table 4. Volatile solvents compatible for aluminum

Chemical	Compatibility	Chemical	Compatibility
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Acetamide	A-Excellent	Mash	A-Excellent
Acetate Solvent	A-Excellent	Mayonnaise	A-Excellent
Acetic Anhydride	A-Excellent	Methane	A-Excellent
Acetone	A-Excellent	Methanol (Methyl Alcohol)	A-Excellent
Acetylene	A-Excellent	Methyl Acetate	A-Excellent
Adipic Acid	A-Excellent	Methyl Acetone	A-Excellent
Alcohols: Diacetone	A-Excellent	Methyl Alcohol 10%	A-Excellent
Alcohols: Hexyl	A-Excellent	Methyl Isopropyl Ketone	A-Excellent
Alcohols: Methyl	A-Excellent	Methylamine	A-Excellent
Alcohols: Octyl	A-Excellent	Milk	A-Excellent
Alcohols: Propyl	A-Excellent	Mineral Spirits	A-Excellent
Alums	A-Excellent	Molasses	A-Excellent
Ammonia 10%	A-Excellent	Morpholine	A-Excellent
Ammonia, anhydrous	A-Excellent	Motor Oil	A-Excellent
Ammonia, liquid	A-Excellent	Naphtha	A-Excellent
Ammonium Acetate	A-Excellent	Natural Gas	A-Excellent
Ammonium Sulfate	A-Excellent	Nitric Acid (5 to10%)	A-Excellent
Amyl Acetate	A-Excellent	Nitromethane	A-Excellent
Amyl Chloride	A-Excellent	Oils: Castor	A-Excellent
Antifreeze (glycol-based)	A-Excellent	Oils: Citric	A-Excellent
Arochlor 1248	A-Excellent	Oils: Coconut	A-Excellent
Aromatic Hydrocarbons	A-Excellent	Oils: Cod Liver	A-Excellent
Asphalt	A-Excellent	Oils: Corn	A-Excellent
Beer	A-Excellent	Oils: Cottonseed	A-Excellent
Beet Sugar Liquids	A-Excellent	Oils: Crude Oil	A-Excellent
Bleach	A-Excellent	Oils: Diesel Fuel (20, 30, 40, 50)	A-Excellent
Butadiene	A-Excellent	Oils: Hydraulic Oil (Petro)	A-Excellent
Butane	A-Excellent	Oils: Hydraulic Oil (Synthetic)	A-Excellent
Butter	A-Excellent	Oils: Lemon	A-Excellent
Buttermilk	A-Excellent	Oils: Mineral	A-Excellent

Butyl Amine	A-Excellent	Oils: Olive	A-Excellent
Butyl Ether	A-Excellent	Oils: Orange	A-Excellent
Butylacetate	A-Excellent	Oils: Peanut	A-Excellent
Butylene	A-Excellent	Oils: Pine	A-Excellent
Carbolic Acid (Phenol)	A-Excellent	Oils: Silicone	A-Excellent
Carbon Dioxide (wet)	A-Excellent	Oils: Soybean	A-Excellent
Carbon Disulfide	A-Excellent	Oils: Transformer	A-Excellent
Carbon Monoxide	A-Excellent	Oils: Turbine	A-Excellent
Carbonated Water	A-Excellent	Oleic Acid	A-Excellent
Cellulose Acetate	A-Excellent	Oxalic Acid (cold)	A-Excellent
Chlorobenzene (Mono)	A-Excellent	Paraffin	A-Excellent
Chocolate Syrup	A-Excellent	Pentane	A-Excellent
Coffee	A-Excellent	Phenol (10%)	A-Excellent
Cream	A-Excellent	Phenol (Carbolic Acid)	A-Excellent
Cresols	A-Excellent	Phthalic Anhydride	A-Excellent
Cyclohexane	A-Excellent	Plating Solutions: Antimony Plating 130°F	A-Excellent
Cyclohexanone	A-Excellent	Plating Solutions: Arsenic Plating 110°F	A-Excellent
Diacetone Alcohol	A-Excellent	Plating Solutions: Brass: High-Speed Brass Bath 110°F	A-Excellent
Diesel Fuel	A-Excellent	Plating Solutions: Brass: Regular Brass Bath 100°F	A-Excellent
Dimethyl Aniline	A-Excellent	Plating Solutions: Bronze: Cu-Cd Bronze Bath R.T.	A-Excellent
Dimethyl Formamide	A-Excellent	Plating Solutions: Bronze: Cu-Sn Bronze Bath 160°F	A-Excellent
Ethyl Acetate	A-Excellent	Plating Solutions: Bronze: Cu-Zn Bronze Bath 100°F	A-Excellent
Ethylene Dichloride	A-Excellent	Plating Solutions: Cadmium: Cyanide Bath 90°F	A-Excellent
Ethylene Glycol	A-Excellent	Plating Solutions: Cadmium: Fluoborate Bath 100°F	A-Excellent
Fatty Acids	A-Excellent	Plating Solutions: Chromium: Barrel Chrome Bath 95°F	A-Excellent
Fluorine	A-Excellent	Plating Solutions: Chromium: Black Chrome Bath 115°F	A-Excellent
Formaldehyde 100%	A-Excellent	Plating Solutions: Chromium: Chromic-	A-Excellent

		Sulfuric Bath 130°F	
Formic Acid	A-Excellent	Plating Solutions: Chromium: Fluoride Bath 130°F	A-Excellent
Fruit Juice	A-Excellent	Plating Solutions: Chromium: Fluosilicate Bath 95°F	A-Excellent
Furan Resin	A-Excellent	Plating Solutions: Copper (Acid): Copper Fluoborate Bath 120°F	A-Excellent
Furfural	A-Excellent	Plating Solutions: Copper (Acid): Copper Sulfate Bath R.T.	A-Excellent
Gasoline, leaded, ref.	A-Excellent	Plating Solutions: Copper (Cyanide): High-Speed Bath 180°F	A-Excellent
Gasoline, unleaded	A-Excellent	Plating Solutions: Copper (Cyanide): Rochelle Salt Bath 150°F	A-Excellent
Gelatin	A-Excellent	Plating Solutions: Copper (Misc): Copper (Electroless)	A-Excellent
Glucose	A-Excellent	Plating Solutions: Copper (Misc): Copper Pyrophosphate	A-Excellent
Glue, P. V. A.	A-Excellent	Propane (liquefied)	A-Excellent
Glycerin	A-Excellent	Propylene	A-Excellent
Heptane	A-Excellent	Shellac (Bleached)	A-Excellent
Hexane	A-Excellent	Shellac (Orange)	A-Excellent
Honey	A-Excellent	Silicone	A-Excellent
Hydraulic Oil (Petro)	A-Excellent	Sodium Benzoate	A-Excellent
Hydraulic Oil (Synthetic)	A-Excellent	Sodium Ferrocyanide	A-Excellent
Hydrocyanic Acid	A-Excellent	Sodium Hydrosulfite	A-Excellent
Hydrogen Gas	A-Excellent	Sodium Silicate	A-Excellent
Hydrogen Peroxide 10%	A-Excellent	Sodium Sulfate	A-Excellent
Hydrogen Peroxide 100%	A-Excellent	Sodium Thiosulfate (hypo)	A-Excellent
Hydrogen Peroxide 30%	A-Excellent	Soy Sauce	A-Excellent
Hydrogen Peroxide 50%	A-Excellent	Starch	A-Excellent
Iodine	A-Excellent	Stoddard Solvent	A-Excellent
Isooctane	A-Excellent	Styrene	A-Excellent
Isopropyl Ether	A-Excellent	Sugar (Liquids)	A-Excellent

Jet Fuel (JP3, JP4, JP5, JP8)	A-Excellent	Sulfur Trioxide	A-Excellent
Kerosene	A-Excellent	Sulfur Trioxide (dry)	A-Excellent
Lacquer Thinners	A-Excellent	Tallow	A-Excellent
Lacquers	A-Excellent	Tanning Liquors	A-Excellent
Lard	A-Excellent	Toluene (Toluol)	A-Excellent
Latex	A-Excellent	Tomato Juice	A-Excellent
Lime	A-Excellent	Turpentine	A-Excellent
Linoleic Acid	A-Excellent	Varnish	A-Excellent
Lubricants	A-Excellent	Vinyl Acetate	A-Excellent
Magnesium Carbonate	A-Excellent	Water, Deionized	A-Excellent
Maleic Anhydride	A-Excellent	Water, Distilled	A-Excellent
		Xylene	A-Excellent

[0042] In embodiments, the humidifier chamber includes a lid. In embodiments, the humidifier chamber includes a controllable heating device (e.g., a controllable heating sleeve, see FIG. 7A; controllable heating plate, see FIG. 7B). In embodiments, the heating element may be a base plate connected to a recirculating water bath.

[0043] Further to any method set forth above and embodiments thereof, in embodiments sample holder includes a first chip, where the liquid droplet is being deposited onto. In embodiments, the method further includes placing a second chip onto the first chip after the liquid droplet and optionally the volatile solvent droplet(s) are being deposited onto the first chip, and thereby forming a chip-sample-chip sandwich.

[0044] In embodiments, the chip is a silicon supporting chip or includes silicon nitride as substrate material. In embodiments, the chip includes silicon, silica, graphene, graphene oxide, cellulose, Gold-coated silicon nitride, Titanium-coated silicon nitride, nucleic acid-coated silicon nitride, peptide-coated silicon nitride, or protein-coated silicon nitride as a substrate material.

[0045] In embodiments, the sample holder/cover and the chips (e.g., the first chip and the second chip) are together referred as TEM grids. In embodiments, the TEM grids are liquid flow grids (e.g., for Liquid Cell TEM (LCTEM)). In embodiments, the TEM grids are electrochemical grids. In embodiments, the TEM grids are heating grids. In embodiments, the TEM grids are gas flow grids. Various commercial TEM grids can be purchased, for example, from Hummingbirds Scientific.

[0046] In embodiments, the methods described herein include one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12) steps of the following steps: (1) placing a first chip onto the TEM sample holder; (2) conducting Fiducial recognition of the first chip; (3) taking a volatile solvent by a depositing device; (4) depositing one or more volatile solvent droplets onto the sample holder/first chip; (5) taking a liquid sample or a plurality of liquid samples by a depositing device; (6) conducting a drop calibration for the depositing device; (7) depositing a liquid droplet or a plurality of liquid droplets onto the TEM sample holder/first chip; (8) taking images of deposited grids (i.e., sample holder/first chip); (9) placing a second chip onto the sample holder/first chip, thereby forming a sandwich assembly (i.e., first chip-sample-second chip); (10) aligning the windows of the first chip and the second chip; (11) covering the TEM sample holder with a TEM sample holder cover (i.e., over clamp); and (12) inserting side-clamp into holder to seal the assembly, thereby providing a sealed TEM holder assembly. In embodiments, each liquid sample may contain one type of nanoparticles. In embodiments, each liquid droplet may include one type of nanoparticle. In embodiments, each liquid sample may contain one type of chemicals (e.g., reactants that have size of smaller than 1nm). In embodiments, each liquid droplet may include one type of chemicals.

[0047] In embodiments, the methods described herein include one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10) steps of the following steps: (1) placing a first chip onto the TEM sample holder; (2) conducting Fiducial recognition of the first chip; (3) taking a liquid sample or a plurality of liquid samples by a depositing device; (4) conducting a drop calibration for the depositing device; (5) depositing a liquid droplet or a plurality of liquid droplets onto the TEM sample holder/first chip; (6) taking images of deposited grids (i.e., sample holder/first chip); (7) placing a second chip onto the sample holder/first chip, thereby forming a sandwich assembly (i.e., first chip-sample-second chip); (8) aligning the windows of the first chip and the second chip; (9) covering the TEM sample holder with a TEM sample holder cover (i.e., over clamp); and (10) inserting side-clamp into holder to seal the assembly, thereby providing a sealed TEM holder assembly. In embodiments, each liquid sample may contain one type of nanoparticles. In embodiments, each liquid droplet may include one type of nanoparticle. In embodiments, each liquid sample may contain one type of chemicals (e.g., reactants that have size of smaller than 1nm). In embodiments, each liquid droplet may include one type of chemicals.

[0048] In embodiments, one or more of the steps of the methods derided herein are automated. For example, in embodiments, one or more or all (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12) steps of the following steps are automated: (1) placing a first chip onto the TEM sample holder; (2)

conducting Fiducial recognition of the first chip; (3) taking a volatile solvent by a depositing device; (4) depositing one or more volatile solvent droplets onto the sample holder/first chip (as above); (5) taking a liquid sample or a plurality of liquid samples by a depositing device; (6) conducting a drop calibration for the depositing device; (7) depositing a liquid droplet or a plurality of liquid droplets onto the TEM sample holder/first chip; (8) taking images of deposited grids (i.e., sample holder/first chip); (9) placing a second chip onto the sample holder/first chip, thereby forming a sandwich assembly (i.e., first chip-sample-second chip); (10) aligning the windows of the first chip and the second chip; (11) covering the TEM sample holder with a TEM sample holder cover (i.e., over clamp); and (12) inserting side-clamp into holder to seal the assembly, thereby providing a sealed TEM holder assembly. In embodiments, each liquid sample may contain one type of nanoparticles. In embodiments, each liquid droplet may include one type of nanoparticle. In embodiments, each liquid sample may contain one type of chemicals (e.g., reactants that have size of smaller than 1nm). In embodiments, each liquid droplet may include one type of chemicals.

[0049] In embodiments, one or more of the steps of the methods derided herein are automated. For example, in embodiments, one or more or all (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11) steps of the following steps are automated: (1) conducting Fiducial recognition of the first chip; (2) taking a volatile solvent by a depositing device; (3) depositing one or more volatile solvent droplets onto the sample holder/first chip; (4) taking a liquid sample or a plurality of liquid samples by a depositing device; (5) conducting a drop calibration for the depositing device; (6) depositing a liquid droplet or a plurality of liquid droplets onto the TEM sample holder/first chip; (7) taking images of deposited grids (i.e., sample holder/first chip); (8) placing a second chip onto the sample holder/first chip, thereby forming a sandwich assembly (i.e., first chip-sample-second chip); (9) aligning the windows of the first chip and the second chip; (10) covering the TEM sample holder with a TEM sample holder cover (i.e., over clamp); and (11) inserting side-clamp into holder to seal the assembly, thereby providing a sealed TEM holder assembly. In embodiments, each liquid sample may contain one type of nanoparticles. In embodiments, each liquid droplet may include one type of nanoparticle. In embodiments, each liquid sample may contain one type of chemicals (e.g., reactants that have size of smaller than 1nm). In embodiments, each liquid droplet may include one type of chemicals.

[0050] In embodiments, one or more of the steps of the methods derided herein are automated. For example, in embodiments, one or more or all (e.g., 1, 2, 3, 4, 5, 6, 7, 8, or 9) steps of the following steps are automated: (1) conducting Fiducial recognition of the first chip; (2) taking a

liquid sample or a plurality of liquid samples by a depositing device; (3) conducting a drop calibration for the depositing device; (4) depositing a liquid droplet or a plurality of liquid droplets onto the TEM sample holder/first chip; (5) taking images of deposited grids (i.e., sample holder/first chip); (6) placing a second chip onto the sample holder/first chip, thereby forming a sandwich assembly (i.e., first chip-sample-second chip); (7) aligning the windows of the first chip and the second chip; (8) covering the TEM sample holder with a TEM sample holder cover (i.e., over clamp); and (9) inserting side-clamp into holder to seal the assembly, thereby providing a sealed TEM holder assembly. In embodiments, each liquid sample may contain one type of nanoparticles. In embodiments, each liquid droplet may include one type of nanoparticle.

10 In embodiments, each liquid sample may contain one type of chemicals (e.g., reactants that have size of smaller than 1nm). In embodiments, each liquid droplet may include one type of chemicals.

[0051] Further to any method set forth above and embodiments thereof, in embodiments the depositing device is a piezo dispensing device.

15 **[0052]** Further to any method set forth above and embodiments thereof, in embodiments automated step or steps are performed by a machine using a computer controller. In embodiments automated step or steps are carried out by a robot (e.g., a Catesian robot) that is controlled by a computer.

[0053] Automated steps (placements and loadings) may be used for high throughput TEM analysis. In embodiments, a plurality of liquid droplets each including a nanoparticle (i.e., a plurality of samples) is being deposited onto the sample holder at the same time. In

20 embodiments, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96 or more liquid droplets

25 samples are being deposited onto the sample holder at the same time.

[0054] In embodiments, every 1000 samples can be deposited and assembled in 15 hours, 10 hours, 9 hours, 8 hours, 7 hours, 6 hours, 5 hours, 4 hours, 3 hours, 2 hours, 1 hour, 30 minutes, 15 minutes or less.

30 **[0055]** In embodiments, the position of each liquid droplet sample can be predetermined. The term “predetermined” in this context means that the location of the deposition of the liquid droplet on the TEM sample holder (or grid) is at a desired location (e.g., the location is

determined prior to deposition and then deposited at the location). In embodiments, the deposited position is within 5 μm (e.g., about 5 μm , 4 μm , 3 μm , 2 μm , 1 μm) of the desired location. In embodiments, a desired location can be adjacent to, or distal from, the predetermined position of another droplet. In embodiments, different liquid droplets are deposited at predetermined positions adjacent one another. In embodiments, liquid droplets diffuse from the initial predetermined position. In embodiments, different liquid droplets are deposited at predetermined positions which are close enough to afford diffusion of the solutions and mixing of the liquid droplets. In embodiments, placement of liquid droplets at a predetermined position can be reproducibly achieved with an accuracy and precision of $\pm 5 \mu\text{m}$, e.g., about 5 μm , 6 μm , 7 μm , 8 μm , 9 μm , 10 μm , 15 μm , 20 μm , 25 μm , 30 μm , 35 μm , 40 μm , 45 μm , 50 μm , 60 μm , 70 μm , 80 μm , 90 μm , 100 μm , or even greater. In embodiments, placement of two liquid droplets can be as close as 5 μm , 6 μm , 7 μm , 8 μm , 9 μm , 10 μm , 15 μm , 20 μm , 25 μm , 30 μm , 35 μm , 40 μm , 45 μm , 50 μm , 60 μm , 70 μm , 80 μm , 90 μm , 100 μm , or even greater. In embodiments, the position of the deposition of the liquid droplet on the TEM sample holder is predetermined by headcam Fiducial recognition of chips (e.g., z-calibration, target recognition of chip features).

[0056] Further to the method and any embodiment thereof, in embodiments the transmission electron microscope is a liquid cell transmission electron microscope (LCTEM).

EXAMPLES

[0057] *Example 1 - Picoliter Drop-on-Demand Dispensing for Multiplex Liquid Cell TEM*

[0058] Abstract: Liquid Cell Transmission Electron Microscopy (LCTEM) provides a unique insight into the dynamics of nanomaterials in solution. Controlling the addition of multiple solutions to the liquid cell remains a key hurdle in our ability to increase throughput and to study processes dependent on solution mixing including chemical reactions. Here, we report that a piezo dispensing technique allows for the mixing of multiple solutions directly within the viewing area. This technique permits the deposition of 50 pL droplets of various aqueous solutions onto the liquid cell window, prior to assembly of the cell in a fully controlled manner. This study demonstrates the great success of picoliter dispensing in combination with LCTEM for observing nanoparticle mixing in the solution phase and the creation of chemical gradients.

[0059] Introduction.

[0060] Liquid Cell Transmission Electron Microscopy (LCTEM) is bringing about a paradigm shift in the analysis of nanomaterials in solution. For the first time, we can use electron microscopy to not only characterize critical features including particle size and morphology, but to observe liquid phase dynamics, in real-time with nanometer resolution (de & Ross, 2011; Williamson, et al., 2003). LCTEM has shown great potential for advancing our understanding of nanoparticle growth (Evans, et al., 2011; Liao & Zheng, 2013; Liao, et al., 2014; Patterson, et al., 2015a; Smeets, et al., 2015; Woehl, et al., 2012; Woehl, et al., 2014; Zheng, et al., 2009b) and particle-particle interactions. (Chen, et al., 2015a). There has been a particular focus on inorganic nanocrystalline materials such as Au, Pt, and Pd because these materials provide high contrast and the ability to undergo electron beam induced growth via radiolysis and metal complex reduction of precursor solutions during the imaging experiments (Evans, et al., 2011; Liao & Zheng, 2013; Liao, et al., 2014; Woehl, et al., 2014). However, increasingly other materials, including ones containing organic components (Proetto, et al., 2014a) and having low contrast, are being studied.

[0061] In a typical LCTEM experiment, a liquid sample is deposited onto the surface of a flat silicon nitride chip (ca. 2.5 x 2.5 mm square) and subsequently sealed off to prevent exposure to the internal vacuum of the electron microscope. The seal is made by placing a second chip on top of the solution and enclosing the chips within a liquid cell TEM holder (de & Ross, 2011). Therefore, the liquid thickness of the cell is set by the size of nanoparticles within the sample, which physically hold the chips apart. If a thicker cell is required, so-called ‘spacer-chips’, which have raised columns on the silicon nitride surface, can be used to physically separate the two silicon nitride surfaces. These spacer-chips allow for the cells to be assembled “in air” (no liquid initially between the chips), and then flow external liquid into the window region of the cell after insertion in the microscope. While many reports discuss the great potential of LCTEM for the study of nanoparticle systems formed through the mixing of solutions containing various required components or conditions (Chen, et al., 2015b; de & Ross, 2011), there has been no demonstration whereby two solutions have been mixed within the viewing windows of the liquid cell, which is believed to be largely due to technical limitations in dispensing and circulating separate liquids within the confined nanoliter volumes typical of the cells. Furthermore, while several holder designs allow the flow of multiple liquids into the tip region of the LCTEM holder using inlet/outlet lines, mixing of the liquids by this method occurs in the collection wells prior to the mixture reaching the window region of the cell (Nielsen, et al., 2014). This inability to

control the location and extent of liquid mixing significantly limits the ability to study how chemistry manifests itself on the nanoscale using LCTEM. For example, nanoparticles formed through block copolymer assembly are typically synthesized through a solvent mixing process (Barnhill, et al., 2015; Choucair & Eisenberg, 2003; Mai & Eisenberg, 2012; Proetto, et al., 2014b), whereby particle assembly occurs within seconds of reaching a certain mixing ratio, which is followed by a period of particle relaxation (Barnhill, et al., 2015). Unless solution mixing is initiated directly in the window region and in the TEM field of view, initial particle assembly cannot be observed. Furthermore, we recently studied the formation of a ZIF-8 metal organic-framework system, which again occurs through solution mixing (Patterson, et al., 2015a). Due to the inability to mix the components directly in the viewing region of the cell, the initial stages of particle formation which can occur within the first 15 seconds (Cravillon, et al., 2011) cannot be observed.

[0062] We demonstrate herein that picoliter (pL) drop-on-demand dispensing can be used to controllably load multiple samples onto a single liquid cell window or a cell with an array of windows under humidifying conditions, which can provide the ability to pattern samples for both high throughput liquid cell experiments, as well as to perform mixing of multiple different solutions inside the cell. We demonstrate herein methods of dispensing and imaging solutions of two types of crystalline particles amenable to selected area diffraction for verification of structure during LCTEM. The two particles can also be chosen to be morphologically distinguishable by TEM imaging. Therefore, we employed gold nanoparticles (AuNPs) and nanocrystals of the metal-organic-framework (MOF) UiO-66 (Patterson, et al., 2015a). These two particle types were dispensed onto a single liquid cell window where initial mixing occurs upon assembly of the cell and continues through diffusion of the components during *in situ* LCTEM imaging. Without wishing to be bound by theory, we believe that the LCTEM technique disclosed herein can facilitate *in situ* nanomaterial synthesis studies that require the mixing of precursor solutions directly in the viewing area, and for studies of responsive nanomaterials that undergo dynamic transformation upon changes in solution conditions and/or constituents.

[0063] Material and Methods.

[0064] Transmission electron microscopy was performed on a FEI Sphera microscope operated at 200 keV. Micrographs were recorded on a 2K X 2K Gatan CCD. Movies were recorded at dose rates between $15.0 \text{ e.nm}^{-2}.\text{s}^{-1}$ ($6.7 \times 10^5 \text{ Gy.s}^{-1}$) and $0.2 \text{ e.nm}^{-2}.\text{s}^{-1}$ ($1.2 \times 10^5 \text{ Gy.s}^{-1}$). UiO66 was synthesized and characterized as previously reported (Patterson, et al., 2015a), and dispersed

in water at 5 mg/mL. 20 nm gold nanoparticles were purchased from BBI International, EM.GC20, and used as received. LCTEM experiments were carried out with a Hummingbird Scientific Liquid Flow TEM Holder.. Drop-On-Demand dispensing was performed on a SCI-TEM (SCIENION AG, Berlin, Germany). The SCI-TEM is a liquid handling platform designed for dispensing volumes as low as 35 pL with a lateral precision of $\pm 2 \mu\text{m}$. These capabilities enable the user to spot up to 10 x 10 individual spots on a single TEM grid (dimension 1 mm²). The spot positions can be easily positioned using a multi-point touch screen and optical camera system which is specifically designed to recognize TEM grids. All steps are captured and recorded by three independent cameras: the DropCam controls drop formation, stability, and volume; the GlobalCam enables intuitive selection of the target grids; and the high resolution HeadCam allows precise fiducial-aided drop positioning. Unlike other picoliter printing systems, the SCI-TEM's unique nozzle design, which is made out of medical-grade borosilicate glass, exhibits a round orifice with no edges. This eliminates the onset of crystallization, minimizing the chance for drop deviation. The nozzles have an orifice ranging between 50 – 80 μm , depending on the nozzle type. This allows the Sci-TEM to dispense both nanomaterials and microstructures up to 20 μm in diameter without clogging (i.e. carbon nanotubes, catalysts, and quantum dots). In contrast to other dispense technologies, SCIENION's dispensers do not impose a strong shock wave on the sample and thus create minimal shear forces: e.g. living Eukaryotic cells can be dispensed without altering their viability. All liquid handling parts are composed of inert materials: PEEK®, TEFLON® and glass. The liquid phase can be freely changed from aqueous to organic solvents to allow printing of aqueous and organic samples respectively. Four coatings have been developed to modify the nozzle's surface energy to enable stable drop formation of various sample types without the need to change the sample properties. The Sci-TEM can dispense solutions within a viscosity range of 0.4 – 6 mPa.

[0065] Results and Discussion.

[0066] Dispensing of low volume liquids can be used for many scientific applications including high-throughput mass spectrometry analysis (Aerni, et al., 2006), low volume synthesis (Wixforth, et al., 2004), the preparation of materials through inkjet printing of polymer/inorganic materials (Calvert, 2001; de Gans, et al., 2004; Tekin, et al., 2008), and biosensing applications (Li, et al., 2015). The development of these approaches is of interest to the field of LCTEM for their ability to fully automate the dispensing of nano- or picoliter volumes of liquid in a controlled manner. The SCI-TEM (SCIENION AG, Berlin, Germany) used herein is an automated picoliter drop-on-demand dispensing instrument capable of recognizing and aligning

TEM grids to be decorated with multiple and varied samples by dispensing liquid droplets down to 35 pL and allowing them to dry directly on the grid. Depending on the surface hydrophobicity of the substrate, droplets will either spread over large areas or remain as high contact angle ellipsoids. Typically, an array of such low volume droplets on a hydrophobic surface (e.g., silicon nitride) will evaporate within a few seconds (and even faster for hydrophilic surfaces), much too quickly to allow sealing of the liquid cell after deposition without complete or significant dehydration of the samples. However, if an array of water droplets is first dispensed around the sample area, the evaporation of these water droplets creates a local high humidity microenvironment, greatly reducing evaporation rates of the sample droplets (FIGS. 1A-1F). This method of controlled water array dispensing to create stable droplets of sample-solution, which persist for approximately 1 minute, provides sufficient time to seal the liquid cell and ensure that the samples remain hydrated. Various array designs can be imagined, utilizing different TEM grids, in order to dispense multiple picoliter sample volumes onto a single channel for controlled mixing (FIGS. 1A-1C) or, to dispense multiple separate samples onto a single grid for high-throughput analysis (FIGS. 1D-1F). In a proof-of-concept demonstration, we used this method to observe progressive diffusion mixing of a 20 nm Au NP solution and a MOF UiO-66 solution (both in H₂O). For the initial test, after dispensing the array (FIG. 1C), the solution droplets were left to dry, and the window was imaged under vacuum to show full separation of the Au NPs and MOF nanoparticles (FIGS. 2A-2G) that match very well the optical images of the dispensed droplets (FIG. 1C). Particle type was confirmed both by imaging the morphology and size of the particles and via selected area diffraction (FIGS. 2A-2G). This demonstrates that even with conventional, commercially available liquid-cell chips, two different liquid samples can both be dispensed over the imaging window (200 μm x 50 μm) while still being isolated from each other prior to sealing the holder, at which point mixing would be initiated due to the lateral spreading of each droplet.

[0067] To create a hydrated liquid cell for *in situ* TEM observation, we dispensed picoliter droplets of the two samples onto a single chip using the same two sample array and 200 μm x 50 μm imaging window (FIG. 1C), intending to quickly seal the cell prior to sample evaporation. The sample and water droplets were all dispensed within a 2 minute time period, and the cell was sealed in the liquid stage tip approximately 20 seconds after the two sample droplets were dispensed. At the moment of sealing the sample droplets under the top chip, both droplets were visually still hydrated on the bottom chip surface. For this experiment, the droplets were dispensed onto a 200 nm height spacer-chip, and the cell was sealed with a flat chip (windows

aligned parallel) in order to set the liquid thickness to roughly the same as the MOF nanoparticles (200 nm). The spacer chip was not subjected to plasma treatment to avoid the droplets spreading immediately after dispensing. However, the flat chip was plasma treated to help the spreading of the liquid upon sealing the cell and to create a mixing front within the window. Due to the surrounding water droplets, which create the high humidity environment, sealing the cell is believed possibly to create some dilution in the NP solutions. However, for this experiment, the demonstration of mixing was the aim, rather than loading particles of a specific concentration. The liquid cell was then loaded into the microscope to start imaging within 7 minutes of sealing the cell. The imaging was performed intermittently at electron doses between $15.0 \text{ e.nm}^2\text{s}^{-1}$ ($6.7 \times 10^5 \text{ Gy.s}^{-1}$) and $0.2 \text{ e.nm}^2\text{s}^{-1}$ ($1.2 \times 10^5 \text{ Gy.s}^{-1}$) in order to limit beam damage. We have previously shown these dose rates are low enough to prevent beam damage to the MOFs (Patterson, et al., 2015a), which are the more beam sensitive materials in the experiment. Without wishing to be bound by theory, it is believed that the beam has no effect on particle size or morphology, although some charging of the particles can be envisaged to occur.

[0068] Initial imaging of the cell (FIGS. 3A-3G) showed discrete areas of MOFs nanocrystals at the ‘top’ of the cell (FIGS. 3A-3C), AuNPs at the ‘bottom’ of the cell (FIGS. 3E-3F), and an area with initial mixing of the two components at the center of the cell (FIG. 3D). After approximately 30 minutes following cell assembly, we observed AuNPs (based on their distinctive size, morphology and contrast) at the top of the cell mixed with the MOF nanoparticles (FIGS. 4A-4G) indicating AuNP migration across the cell. The number of detected AuNPs at the top of the cell continued to increase over time during our observation. This observed movement / mixing is much slower than would be expected based on bulk diffusion of these nanomaterials, but is consistent with other recent work studying AuNP dynamics in a similar LCTEM assembly (Verch, et al., 2015; Woehl & Prozorov, 2015). It is well known that near surface effects in the liquid cell can dramatically reduce diffusion rates (Proetto, et al., 2014b; Zheng, et al., 2009a). Motion of individual Au NPs was observed, as well as their adherence to the surface of the MOFs, which can be clear indicators of a fully hydrated cell. No migration of MOF particles was observed to the bottom end (i.e. the location initially patterned with AuNPs) of the liquid cell. We recently reported that the assembly of liquid cell immediately after plasma treatment (as in these experiments) resulted in the observation of MOF particles stuck to the liquid cell windows; however, assembly of the cell 1-2 hours post plasma treatment resulted in insignificant motion of particles inside the cell (Patterson, et al., 2015a). Therefore the lack of motion observed here can be likely due to either their adherence to the plasma treated

SiN_x membrane and/or to their large size (roughly equal to the spacer height). Controlling particle motion inside the cell is of paramount importance for the observation of dynamics. Particles moving too quickly will not be able to be observed, either due to their motion exceeding the maximum framerate of the CCD camera, or due to decreased signal-to-noise when using high framerates. However, if particle motion is too slow, then the time scales over which mixing or dynamic process (e.g., particle collisions or interactions) will occur can exceed the threshold timescales related to beam damage. Further work in controlling particle and small molecule motion in the cell is clearly needed (Patterson, et al., 2015b). This can involve not only differences in plasma coating times, intensities and surface aging, but also more permanent surface coatings and functionalization. Furthermore, detailed experiments involved controlled liquid thickness and beam doses can be conducted. However, it is believed that controlled motion combined with the mixing procedures outlined here can enable much more detailed experiments involving solution mixing and chemistry inside the cell. The ability to spot two different liquids at μm -scale separation distances on LCTEM chips greatly improves the probability for solution mixing over the viewing window for directly observing chemical reactions by LCTEM, and can help overcome the limitations associated with the sub-diffusion motion of nanomaterials inside liquid cells, shorting the imaging times (radiolysis doses) required to observe particle migration and mixing.

[0069] Conclusion. These experiments demonstrate that automated picoliter drop-on-demand dispensing, combined with automated grid recognition and alignment in the Sci-TEM instrument can be used to mix multiple solutions within the liquid cell, allowing assembly of liquid cells to observe nanoparticle migration and interaction, and potentially chemical reactions, while imaging in the microscope. Furthermore, using this dispensing technology and imaging strategy, one can conceive of many array designs where the mixing time of multiple different solutions could occur within one experiment. From a fundamental standpoint, technology for controlling the dispensing of low liquid volumes is essential for the advancement of LCTEM and this study shows the great success to perform direct solution mixing and/or high-throughput *in situ* experiments. This study also demonstrated a new approach that enables researchers to study processes involving mixing of materials and/or reagents.

[0070] References (Example 1).

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[0100] *Example 2 - Sci-TEM Liquid Cell Sample Preparation System.*

[0101] Overview. As depicted in FIGS. 6A-6B, the SCI-TEM Liquid Cell sample prep system includes a Cartesian robot [FIGS. 6A-6B, element 1] equipped with imaging systems (Global camera, Head Camera, Drop Camera) [FIG. 6B, element 2], low volume non-contact dispensing
5 nozzles [FIG. 6B, element 3], vacuum nozzle [FIG. 6B, element 4], Microarray chamber loaded with LC TEM stage and grids [FIG. 6B, element 5].

[0102] Instrument. The depicted chamber (FIG. 6A) provides a local environment of near-saturated solvent vapor to control drying of volatile samples. As depicting in FIGS. 7A-7B, liquid sample arrays can be loaded on a variety of supports including liquid cell stages inserted
10 through a stage port located in the front of the chamber. Liquids are introduced through a small opening in the top of the chamber with liquid dispensing nozzles. This vertical opening is also used as line of sight for targeting the grid, analyzing deposited droplets, and placement of the top chip. Access to the liquid cell stages can be through a hole large enough for imaging and printing (e.g., deposition) to the whole grid with a single nozzle. Physiochemical processes
15 depicted in FIGS. 7A-7B include e.g., diffusion and convection of solvent. A controllable heating sleeve can be employed as indicated in FIG. 7A. A controllable heating plate can be employed as indicated in FIG. 7B.

[0103] Instrument Setup. Setup times for 2.5 nL drops of H₂O were determined for the SciTEM Liquid Cell sample prep system as depicted in FIGS. 6A-6B and 7. The drying after
20 droplet deposition is tabulated in Table 5 following.

Table 5. Setup drying times for SciTEM Liquid Cell sample prep system

Setup conditions	Drying time
No lid, dry	< 10 sec
No lid, room temperature H ₂ O	15 sec
With lid, room temperature H ₂ O	5 min
With lid, heated H ₂ O	> 10 min

[0104] Loading the SciTEM Liquid Cell sample prep system.

[0105] The processing can begin when all necessary components are loaded into the robotic
25 working area. This includes liquid samples in open container on Plate Holder; Microarray chamber reservoir filled with solvent; LC stage loaded with bottom chip in the Microarray

chamber; top chip located face down in the chip cradle. FIG. 8A schematically depicts the robotic working area. A photographic inset (FIG. 8B) depicts the humidifying chamber and the chip positioning.

[0106] The process of loading takes the following steps. Steps 2-6 can be done a variety of orders based on the needs.

[0107] 1. The Headcam Fiducial recognition of chips – z-calibration, target recognition of chip features.

[0108] 2. Aspiration of samples into nozzles.

[0109] 3. Drop calibration using recognition and actuation software.

[0110] 4. Deposition of droplets onto grid with each nozzle.

[0111] 5. Optionally, acquisition of images from Headcam of printed guid.

[0112] 6. Moving pick-up tool to top-chip position and activation of vacuum pump to pick up top-chip.

[0113] 7. Moving top chip to location above bottom chip and encapsulating droplets in patterned thin liquid films.

[0114] 8. Removal of LC-Stage from microarray chamber, finishing assembly of the LC stage, insertion into a TEM device.

[0115] Using these steps, it has been found that the total robot time can be about 2-min, and the total time from robot to TEM imaging can be about 1-5 min.

[0116] *Example 3 - In situ Liquid-Cell TEM (LC-TEM)*

[0117] FIG. 9A provides a photographic depiction of relative positioning of Top Chip and Bottom Chip, showing 30 nm thick SiN_x “windows” and 50 μm x 50 μm lateral area. FIG. 9B provides a photographic depiction of probe for *in situ* Liquid-Cel TEM (LC-TEM) grids (including the sample holder, the over clamp and the side clamp). The area indicated in dashed rectangle is enlarged in FIG. 9C, which depicts liquid flow lines, S/TEM electron beam, silicon support chip. The further inset to FIG. 9C depicts relative positioning of top SiN_x, liquid sample, and bottom SiN_x regions.

[0118] Embodiments.

[0119] Embodiments disclosed herein include embodiments P1 to P7 following.

[0120] Embodiment P1. A method for depositing a solution directly on a transmission electron microscope (TEM) grid, the method including: 1) loading a solution into a piezo dispensing device; and 2) depositing an aliquot of the solution from the piezo dispensing device directly onto the TEM grid.

[0121] Embodiment P2. The method of embodiment P1, wherein step 1) is repeated for each of a plurality of solutions.

[0122] Embodiment P3. The method of embodiment P2, wherein step 2) is repeated for each of the plurality of solutions.

[0123] Embodiment P4. The method of embodiment P3, wherein each depositing for each of the plurality of solutions is at a predetermined position on the TEM grid.

[0124] Embodiment P5. The method of embodiment P4, wherein the predetermined position is suitable to afford mixing of a plurality of solutions on the TEM grid.

[0125] Embodiment P6. The method of embodiment P1, wherein the solution includes a nanoparticle.

[0126] Embodiment P7. A method for liquid cell transmission electron microscopy (LCTEM), the method including: 1) preparing a TEM grid as set forth in any one of embodiments P1 to P6; and 2) conducting LCTEM on the TEM grid in a LCTEM instrument.

[0127] Further embodiments includes embodiments 1 to 40 following.

[0128] Embodiment 1. A method for detecting a nanoparticle in a liquid, the method including: a. depositing a liquid droplet including a nanoparticle onto a transmission electron microscope sample holder under humidifying conditions, wherein the volume of the liquid droplet is less than about 1000 pL; b. covering the transmission electron microscope sample holder with a transmission electron microscope sample holder cover; and c. detecting the nanoparticle within the liquid droplet using a transmission electron microscope.

[0129] Embodiment 2. The method of embodiment 1, wherein the depositing and the covering are automated.

[0130] Embodiment 3. The method of embodiment 1, wherein the humidifying conditions are generated by depositing a volatile solvent droplet onto the sample holder.

[0131] Embodiment 4. The method of embodiment 3, wherein the volatile solvent is water, alcohol, toluene, tetrahydrofuran (THF), dimethylformamide (DMF), acetone, acetonitrile or cyclohexanone.

[0132] Embodiment 5. The method of embodiment 3, wherein the volatile solvent is water.

[0133] Embodiment 6. The method of embodiment 3, wherein the volatile solvent droplet is a plurality of volatile solvent droplets.

[0134] Embodiment 7. The method of embodiment 1, wherein the humidifying conditions are generated by a humidifier chamber.

[0135] Embodiment 8. The method of embodiment 7, wherein the humidifier chamber is surrounding the sample holder.

[0136] Embodiment 9. The method of embodiment 7, wherein the humidifier chamber includes a volatile solvent reservoir.

[0137] Embodiment 10. The method of embodiment 9, wherein the volatile solvent reservoir is underneath the transmission electron microscope sample holder.

[0138] Embodiment 11. The method of embodiment 10, wherein the volatile solvent is water, alcohol, toluene, tetrahydrofuran (THF), dimethylformamide (DMF), acetone, acetonitrile or cyclohexanone.

[0139] Embodiment 12. The method of embodiment 10, wherein the volatile solvent is water.

[0140] Embodiment 13. The method of embodiment 1, wherein the sample holder includes a first silicon support chip.

[0141] Embodiment 14. The method of embodiment 13, further including placing a second silicon support chip onto the first silicon support chip.

[0142] Embodiment 15. The method of embodiment 14, wherein the placing a second silicon support chip is automated.

[0143] Embodiment 16. The method of embodiment 1, wherein the transmission electron microscope is a liquid cell transmission electron microscope (LCTEM).

[0144] Embodiment 17. A method for detecting a plurality of nanoparticles and an interaction thereof in a liquid, the method including: a. depositing a plurality of liquid droplets each including a nanoparticle onto a transmission electron microscope sample holder under humidifying conditions, wherein the volume of each liquid droplet is less than about 1000 pL; b. covering the transmission electron microscope sample holder with a transmission electron microscope sample holder cover; and c. detecting the plurality of nanoparticles and interaction thereof upon diffusion of the plurality of liquid droplets using a transmission electron microscope.

[0145] Embodiment 18. The method of embodiment 17, wherein the depositing and the covering are automated.

[0146] Embodiment 19. The method of embodiment 17, wherein the humidifying conditions are generated by depositing a volatile solvent droplet onto the sample holder.

[0147] Embodiment 20. The method of embodiment 19, wherein the volatile solvent is water, alcohol, toluene, tetrahydrofuran (THF), dimethylformamide (DMF), acetone, acetonitrile or cyclohexanone.

[0148] Embodiment 21. The method of embodiment 19, wherein the volatile solvent is water.

[0149] Embodiment 22. The method of embodiment 17, wherein the volatile solvent droplet is a plurality of volatile solvent droplets.

[0150] Embodiment 23. The method of embodiment 17, wherein the humidifying conditions are generated by a humidifier chamber.

[0151] Embodiment 24. The method of embodiment 23, wherein the humidifier chamber is surrounding the sample holder.

[0152] Embodiment 25. The method of embodiment 23, wherein the humidifier chamber includes a volatile solvent reservoir.

[0153] Embodiment 26. The method of embodiment 25, wherein the volatile solvent reservoir is underneath the transmission electron microscope sample holder.

[0154] Embodiment 27. The method of embodiment 26, wherein the volatile solvent is water, alcohol, toluene, tetrahydrofuran (THF), dimethylformamide (DMF), acetone, acetonitrile or cyclohexanone

[0155] Embodiment 28. The method of embodiment 26, wherein the volatile solvent is water.

[0156] Embodiment 29. The method of embodiment 17, wherein the sample holder includes a first silicon support chip.

5 [0157] Embodiment 30. The method of embodiment 29, further including placing a second silicon support chip onto the first silicon support chip.

[0158] Embodiment 31. The method of embodiment 30, wherein the placing a second silicon support chip is automated.

[0159] Embodiment 32. The method of embodiment 17, wherein the transmission electron microscope is a liquid cell transmission electron microscope (LCTEM).

10 [0160] Embodiment 33. A method of analyzing a sample using transmission electron microscope, the method including: a. automatically depositing a liquid droplet of the sample onto a transmission electron microscope sample holder, wherein the volume of the liquid droplet is less than about 1000 pL; b. analyzing the liquid sample using transmission electron microscope.

15 [0161] Embodiment 34. The method of embodiment 33, wherein the automatically depositing is depositing within 10 microns of a predetermined location on the transmission electron microscope sample holder.

[0162] Embodiment 35. The method of embodiment 33, wherein the liquid sample includes a plurality of nanoparticles.

20 [0163] Embodiment 36. The method of embodiment 35, wherein the analyzing including detecting the plurality of nanoparticles.

[0164] Embodiment 37. The method of embodiment 33, further including covering the transmission electron microscope sample holder with a transmission electron microscope sample holder cover prior to the analyzing.

25 [0165] Embodiment 38. The method of embodiment 37, wherein the covering is automated.

[0166] Embodiment 39. The method of embodiment 33, wherein the automatically depositing is performed under humidifying conditions.

[0167] Embodiment 40. The method of embodiment 39, wherein the humidifying conditions are generated by a humidifier chamber.

WHAT IS CLAIMED IS:

- 1 1. A method for detecting a nanoparticle in a liquid, the method comprising:
 - 2 a. depositing a liquid droplet comprising a nanoparticle onto a transmission
3 electron microscope sample holder under humidifying conditions, wherein the volume of said
4 liquid droplet is less than about 1000 pL;
 - 5 b. covering said transmission electron microscope sample holder with a
6 transmission electron microscope sample holder cover; and
 - 7 c. detecting said nanoparticle within said liquid droplet using a transmission
8 electron microscope.
- 1 2. The method of claim 1, wherein said depositing and said covering are
2 automated.
- 1 3. The method of claim 1, wherein said humidifying conditions are generated
2 by depositing a volatile solvent droplet onto said sample holder.
- 1 4. The method of claim 3, wherein said volatile solvent is water, alcohol,
2 toluene, tetrahydrofuran (THF), dimethylformamide (DMF), acetone, acetonitrile or
3 cyclohexanone.
- 1 5. The method of claim 3, wherein said volatile solvent is water.
- 1 6. The method of claim 3, wherein said volatile solvent droplet is a plurality
2 of volatile solvent droplets.
- 1 7. The method of claim 1, wherein said humidifying conditions are generated
2 by a humidifier chamber.
- 1 8. The method of claim 7, wherein said humidifier chamber is surrounding
2 said sample holder.
- 1 9. The method of claim 7, wherein said humidifier chamber comprises a
2 volatile solvent reservoir.
- 1 10. The method of claim 9, wherein said volatile solvent reservoir is
2 underneath said transmission electron microscope sample holder.

1 11. The method of claim 10, wherein said volatile solvent is water, alcohol,
2 toluene, tetrahydrofuran (THF), dimethylformamide (DMF), acetone, acetonitrile or
3 cyclohexanone.

1 12. The method of claim 10, wherein said volatile solvent is water.

1 13. The method of claim 1, wherein said sample holder comprises a first
2 silicon support chip.

1 14. The method of claim 13, further comprising placing a second silicon
2 support chip onto said first silicon support chip.

1 15. The method of claim 14, wherein said placing a second silicon support
2 chip is automated.

1 16. The method of claim 1, wherein said transmission electron microscope is a
2 liquid cell transmission electron microscope (LCTEM).

1 17. A method for detecting a plurality of nanoparticles and an interaction
2 thereof in a liquid, the method comprising:

3 a. depositing a plurality of liquid droplets each comprising a nanoparticle
4 onto a transmission electron microscope sample holder under humidifying conditions, wherein
5 the volume of each liquid droplet is less than about 1000 pL;

6 b. covering said transmission electron microscope sample holder with a
7 transmission electron microscope sample holder cover; and

8 c. detecting said plurality of nanoparticles and interaction thereof upon
9 diffusion of said plurality of liquid droplets using a transmission electron microscope.

1 18. The method of claim 17, wherein said depositing and said covering are
2 automated.

1 19. The method of claim 17, wherein said humidifying conditions are
2 generated by depositing a volatile solvent droplet onto said sample holder.

1 20. The method of claim 19, wherein said volatile solvent is water, alcohol,
2 toluene, tetrahydrofuran (THF), dimethylformamide (DMF), acetone, acetonitrile or
3 cyclohexanone.

1 21. The method of claim 19, wherein said volatile solvent is water.

1 22. The method of claim 17, wherein said volatile solvent droplet is a plurality
2 of volatile solvent droplets.

1 23. The method of claim 17, wherein said humidifying conditions are
2 generated by a humidifier chamber.

1 24. The method of claim 23, wherein said humidifier chamber is surrounding
2 said sample holder.

1 25. The method of claim 23, wherein said humidifier chamber comprises a
2 volatile solvent reservoir.

1 26. The method of claim 25, wherein said volatile solvent reservoir is
2 underneath said transmission electron microscope sample holder.

1 27. The method of claim 26, wherein said volatile solvent is water, alcohol,
2 toluene, tetrahydrofuran (THF), dimethylformamide (DMF), acetone, acetonitrile or
3 cyclohexanone

1 28. The method of claim 26, wherein said volatile solvent is water.

1 29. The method of claim 17, wherein said sample holder comprises a first
2 silicon support chip.

1 30. The method of claim 29, further comprising placing a second silicon
2 support chip onto said first silicon support chip.

1 31. The method of claim 30, wherein said placing a second silicon support
2 chip is automated.

1 32. The method of claim 17, wherein said transmission electron microscope is
2 a liquid cell transmission electron microscope (LCTEM).

1 33. A method of analyzing a sample using transmission electron microscope,
2 the method comprising:

- 3 a. automatically depositing a liquid droplet of said sample onto a transmission
4 electron microscope sample holder, wherein the volume of said liquid droplet
5 is less than about 1000 pL;
6 b. analyzing the liquid sample using transmission electron microscope.

1 34. The method of claim 33, wherein said automatically depositing is
2 depositing within 10 microns of a predetermined location on said transmission electron
3 microscope sample holder.

1 35. The method of claim 33, wherein said liquid sample comprises a plurality
2 of nanoparticles.

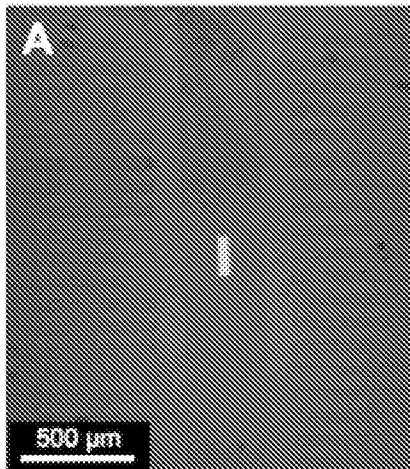
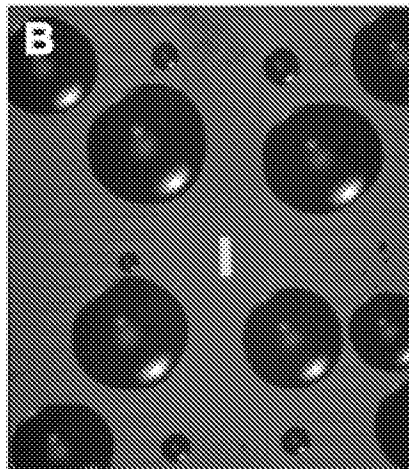
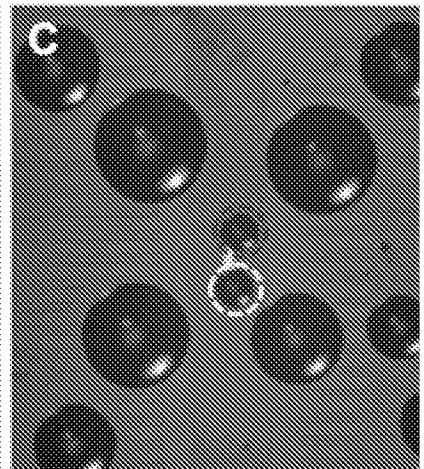
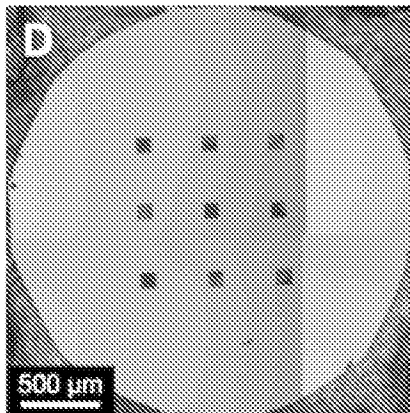
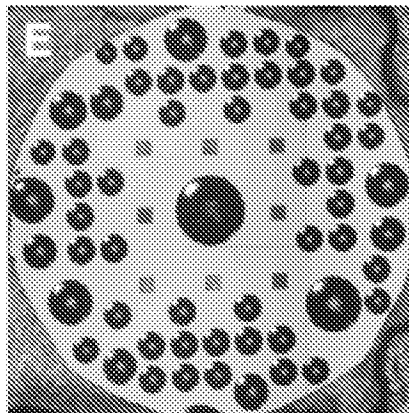
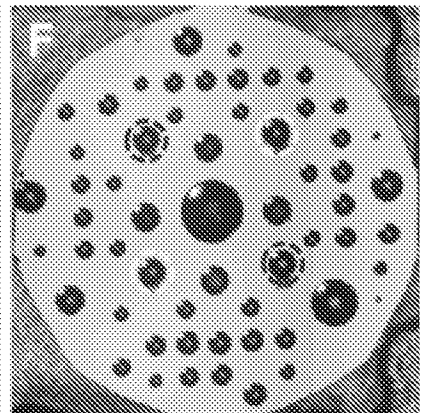
1 36. The method of claim 35, wherein said analyzing comprises detecting said
2 plurality of nanoparticles.

1 37. The method of claim 33, further comprising covering said transmission
2 electron microscope sample holder with a transmission electron microscope sample holder cover
3 prior to said analyzing.

1 38. The method of claim 37, wherein said covering is automated.

1 39. The method of claim 33, wherein said automatically depositing is
2 performed under humidifying conditions.

1 40. The method of claim 39, wherein said humidifying conditions are
2 generated by a humidifier chamber.

FIG. 1A**FIG. 1B****FIG. 1C****FIG. 1D****FIG. 1E****FIG. 1F**

UiO-66 MOF

FIG. 2A

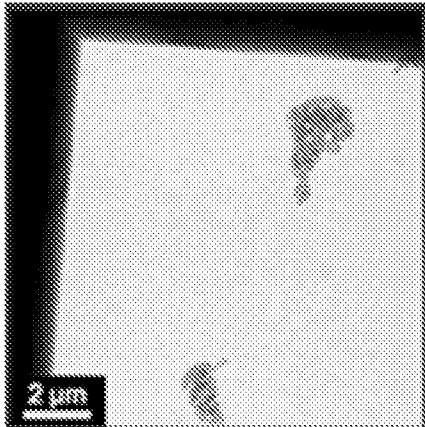


FIG. 2B

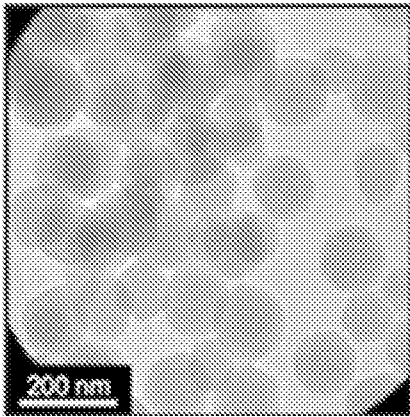


FIG. 2C

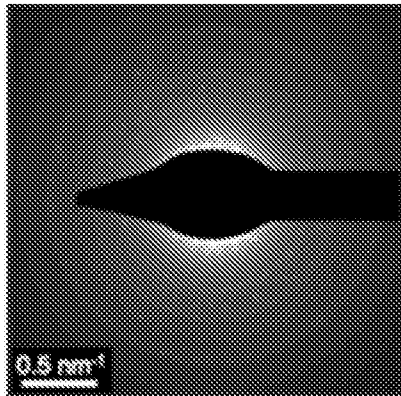
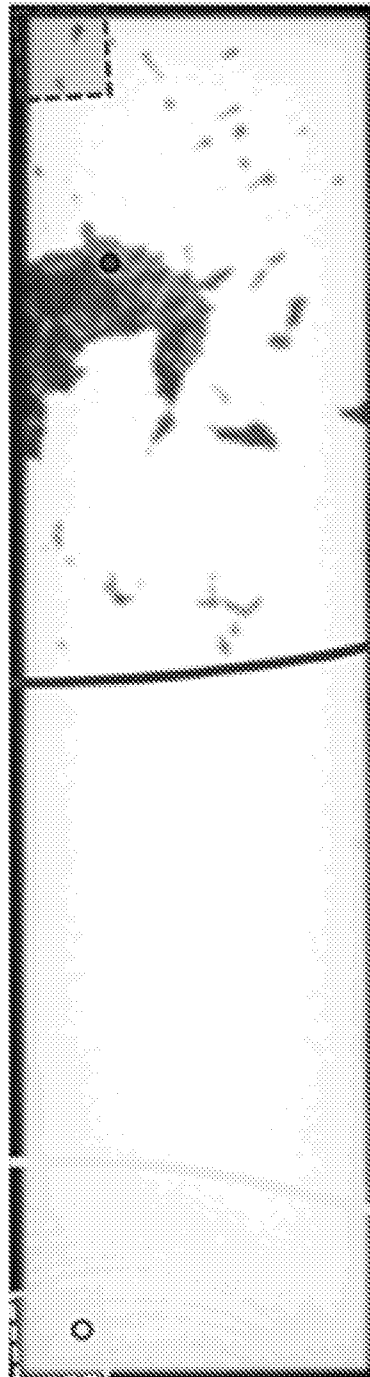


FIG. 2D



Au NPs

FIG. 2E

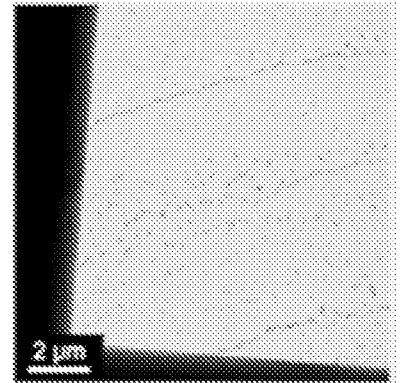


FIG. 2F

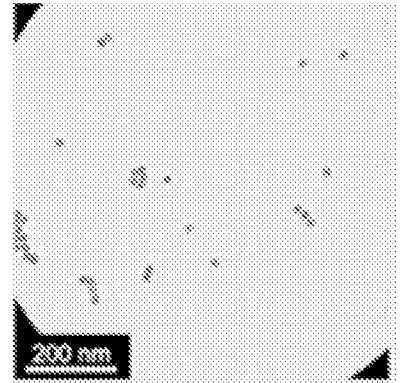


FIG. 2G

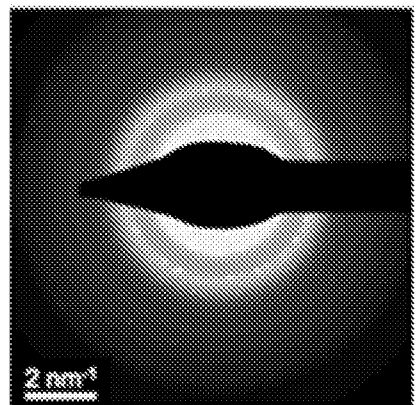


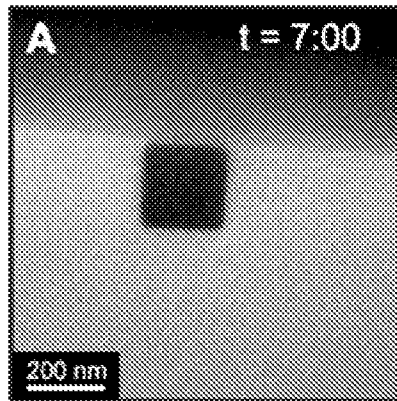
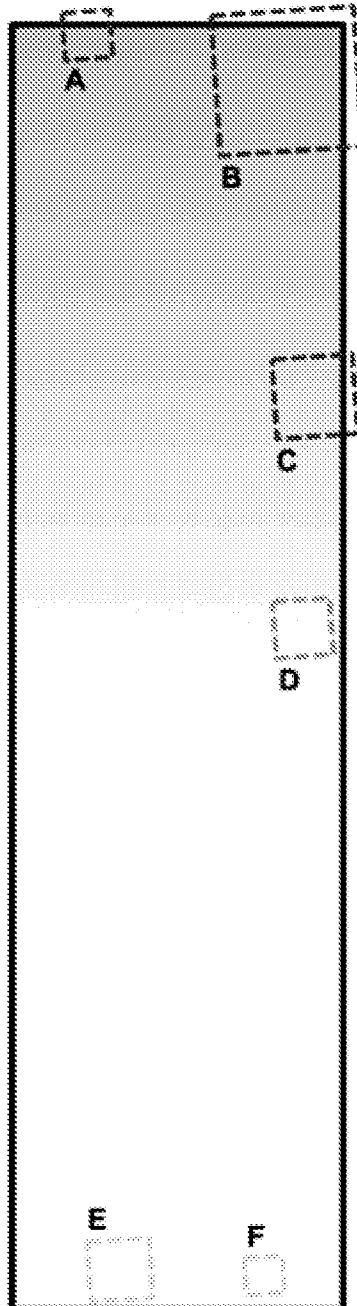
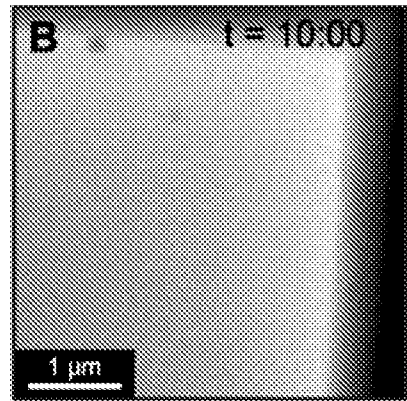
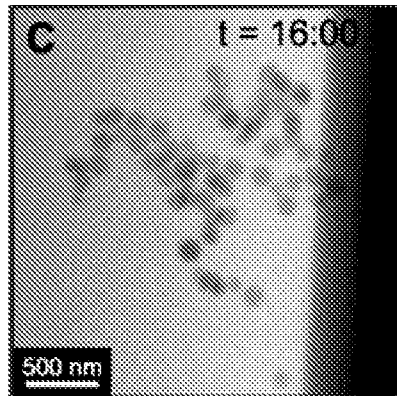
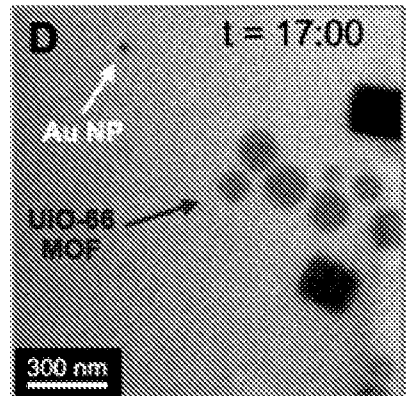
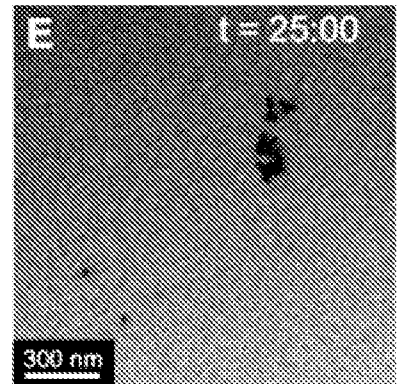
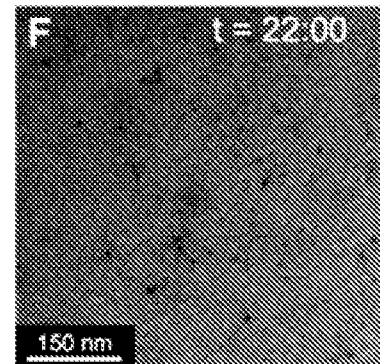
FIG. 3A**FIG. 3G****FIG. 3B****FIG. 3C****FIG. 3D****FIG. 3E****FIG. 3F**

FIG. 4A

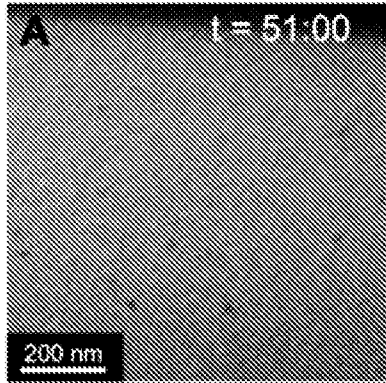


FIG. 4G

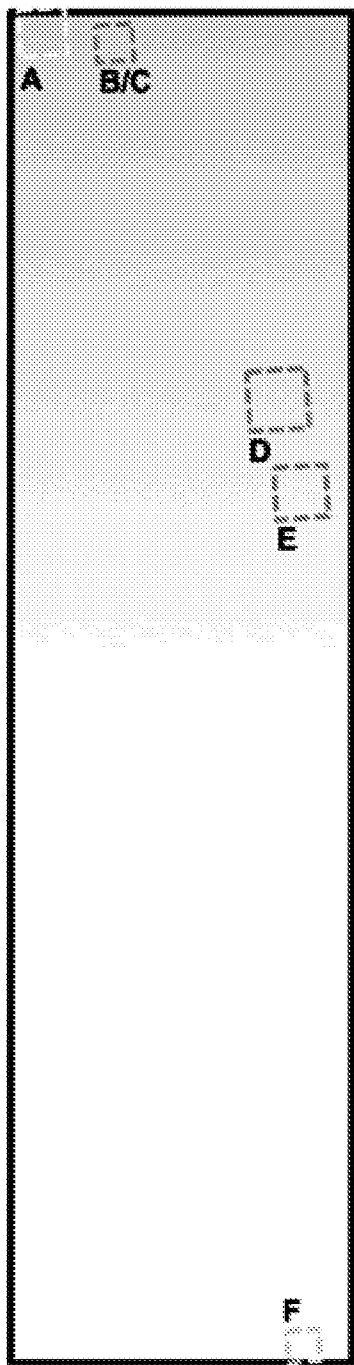


FIG. 4B

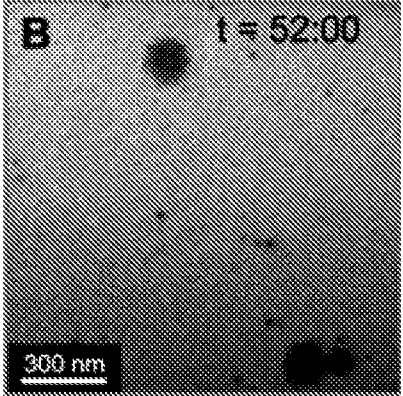


FIG. 4C

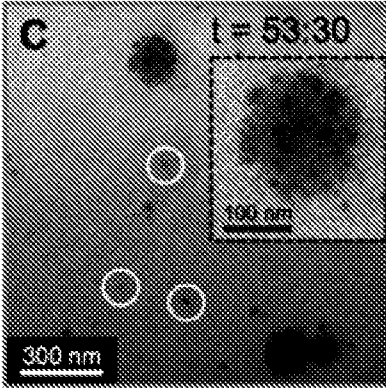


FIG. 4D

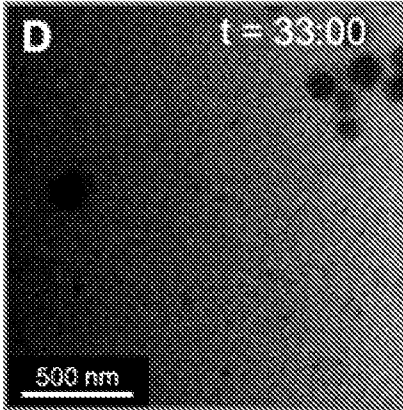


FIG. 4E

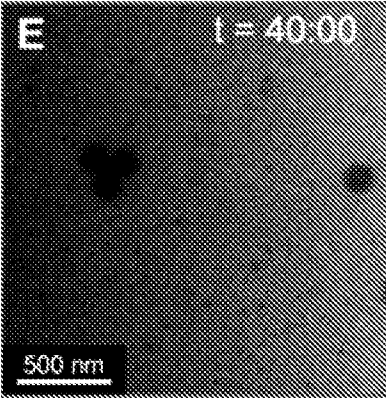


FIG. 4F

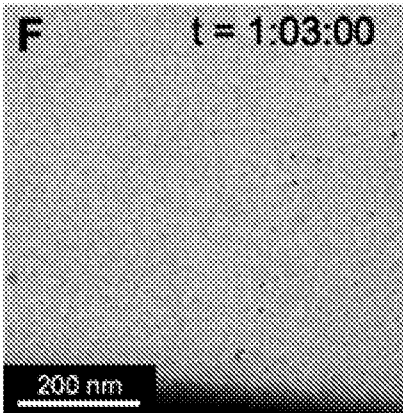


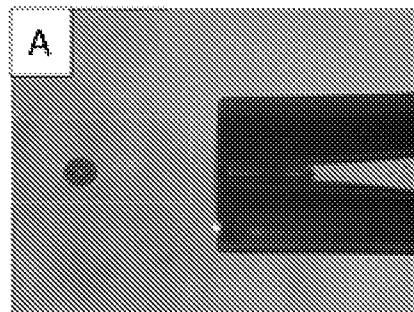
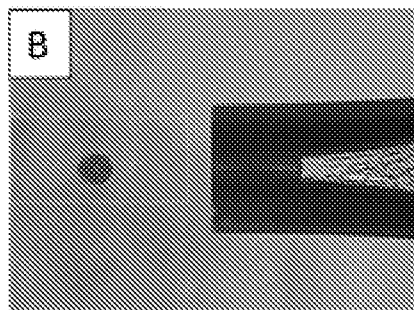
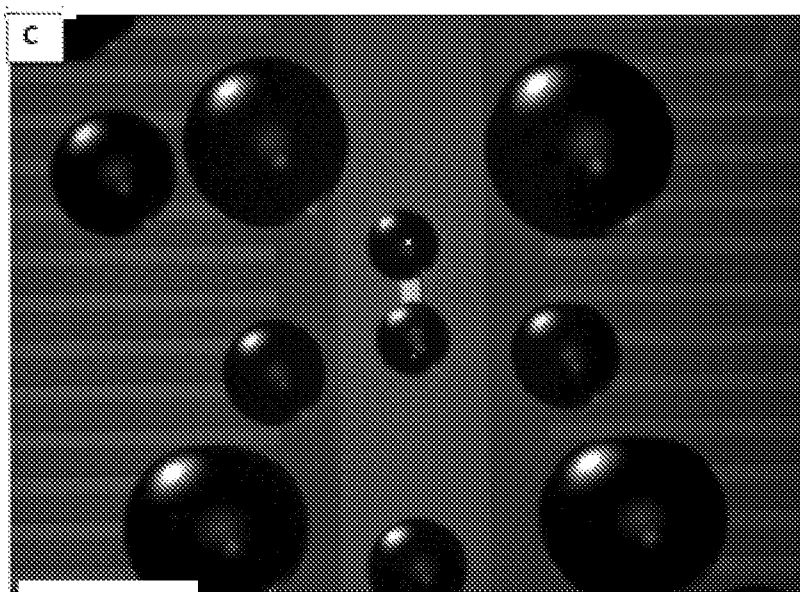
FIG. 5A**FIG. 5B****FIG. 5C**

FIG. 6A



FIG. 6B

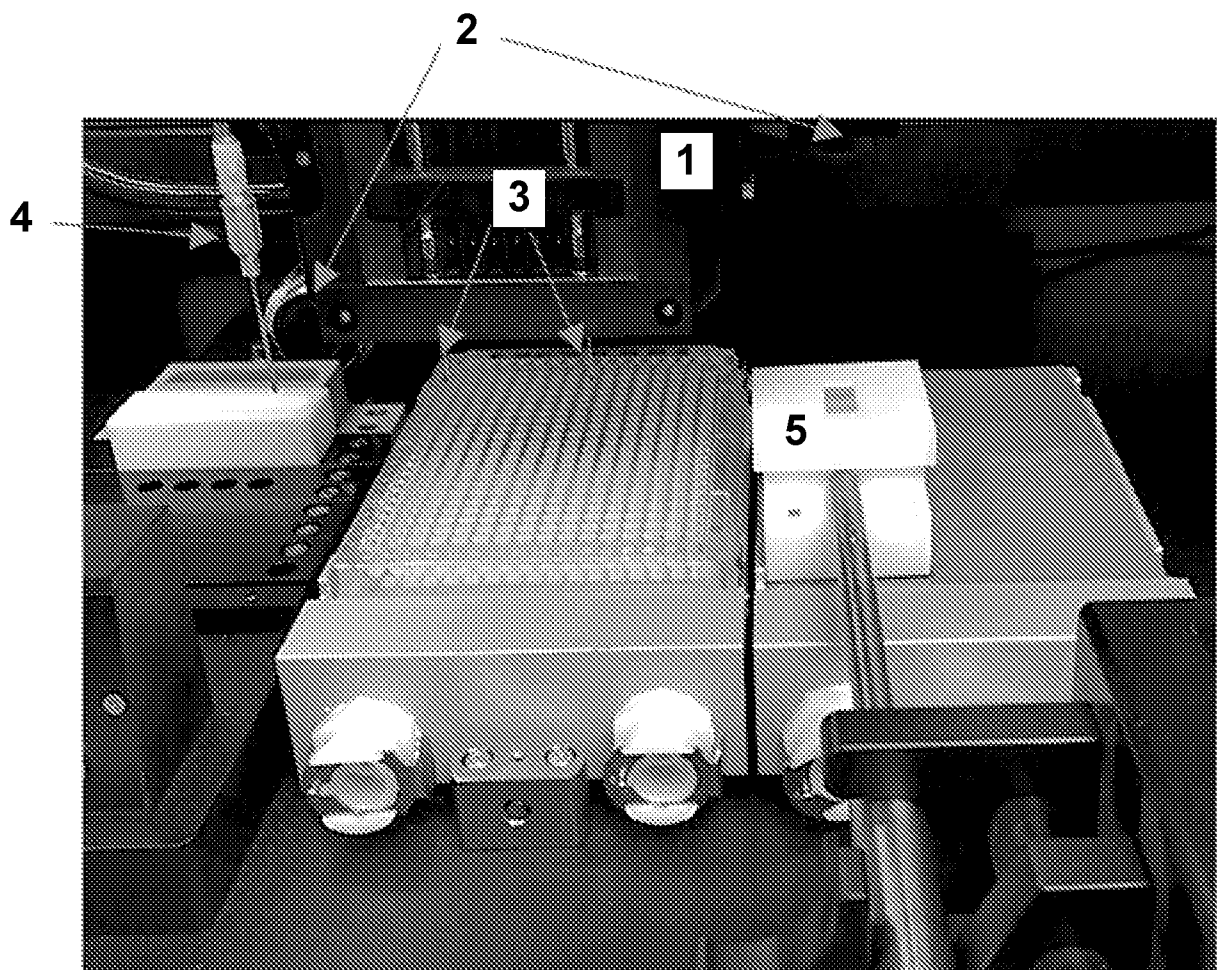


FIG. 7A

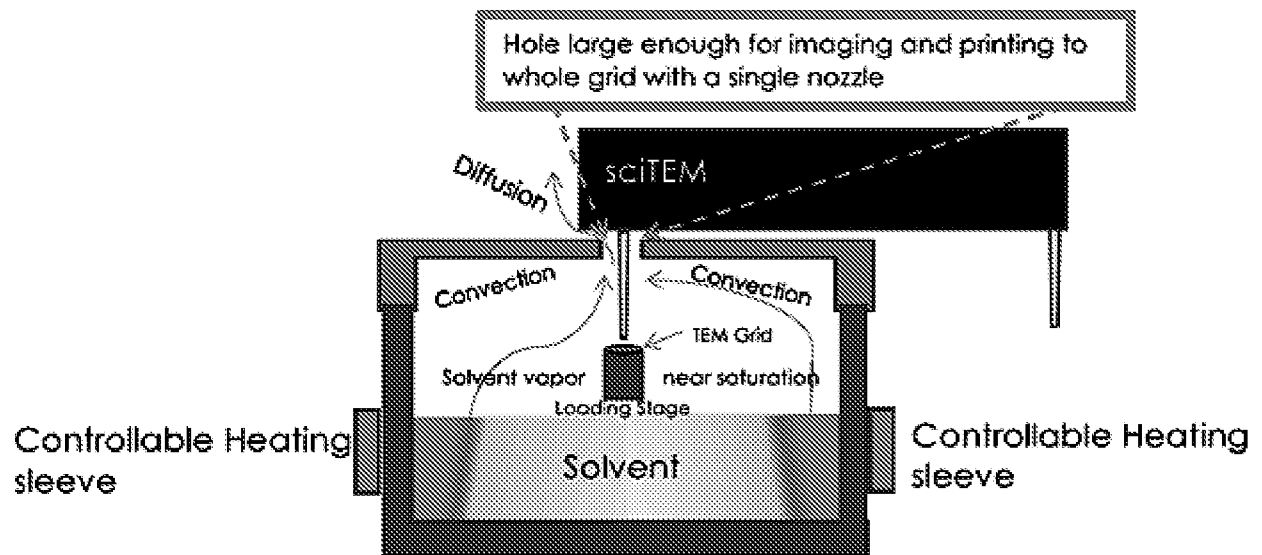


FIG. 7B

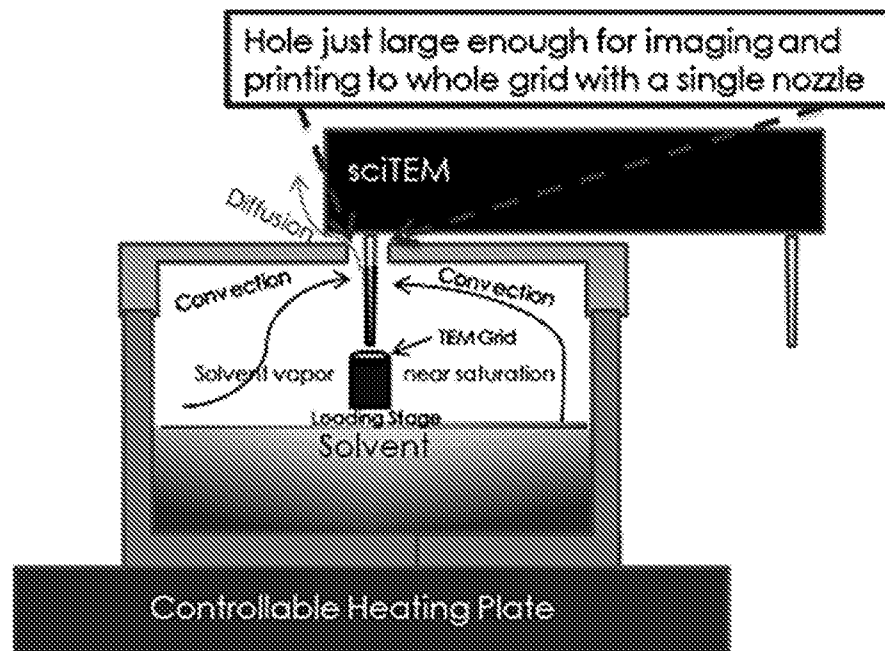


FIG. 8B

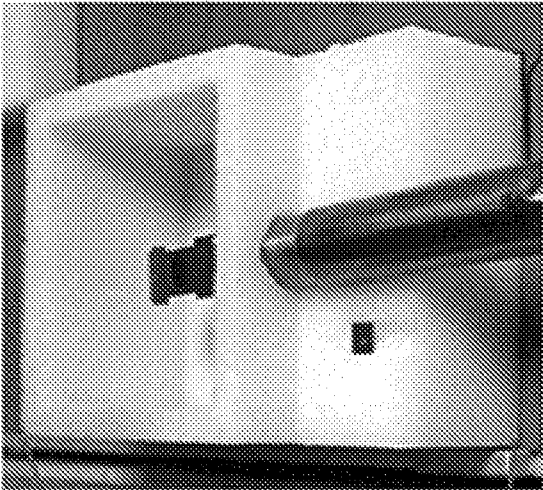


FIG. 8A

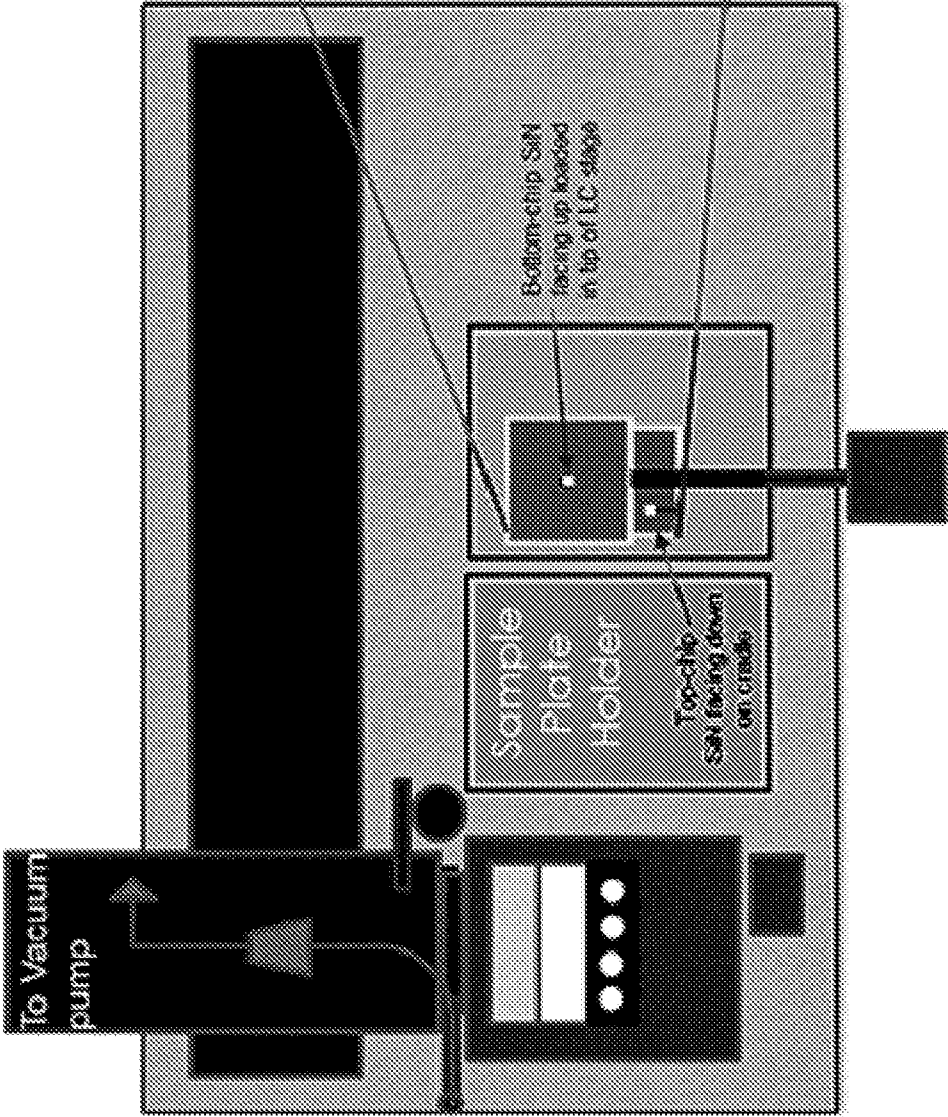


FIG. 9A

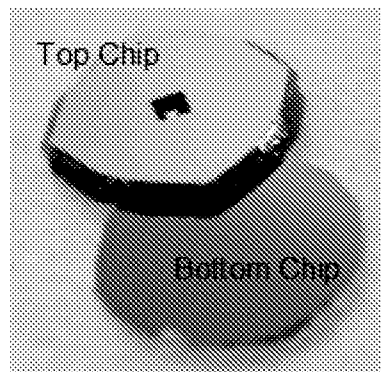


FIG. 9B

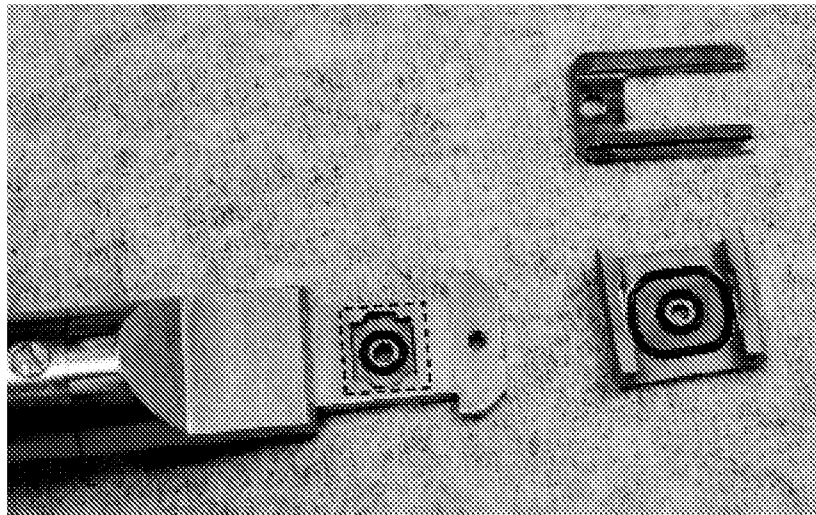
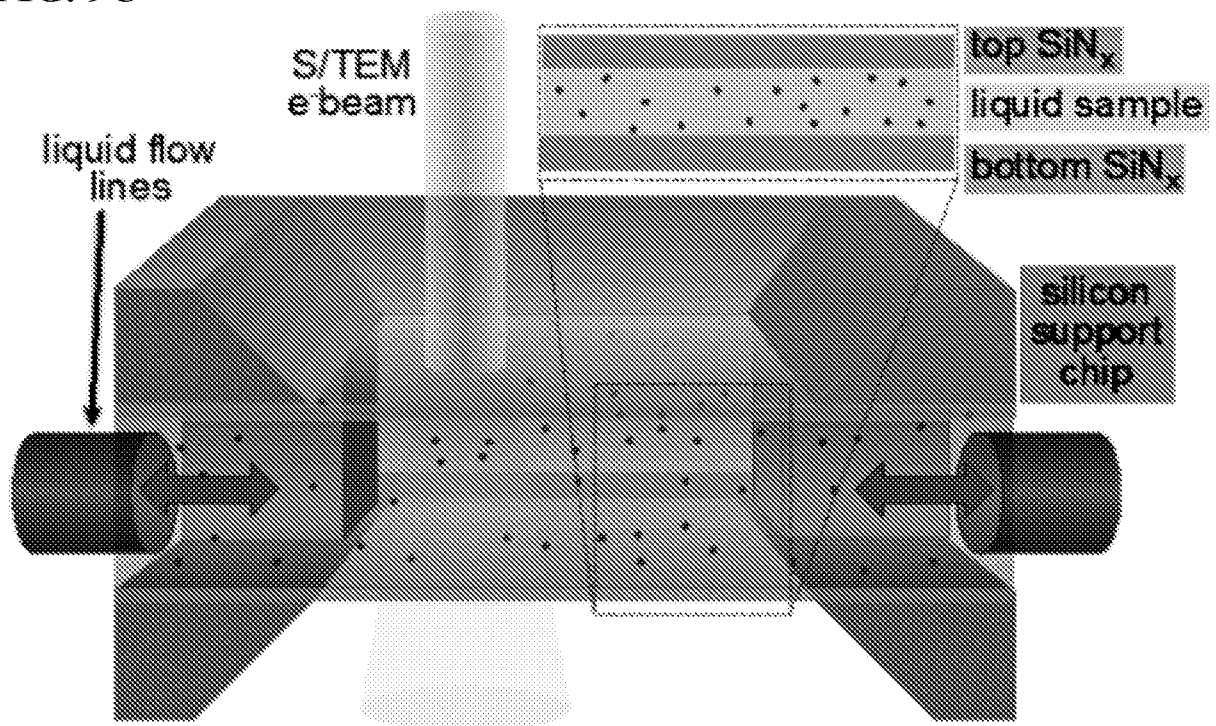


FIG. 9C



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2016/047541

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - H01J 37/20; H01J 37/26; H01J 37/285 (2016.01)

CPC - G01N 2001/4027; H01J 37/02; H01J 37/20; H01J 37/261; H01J 37/285; H01J 2237/2802 (2016.08)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - H01J 37/20; H01J 37/26; H01J 37/285 (2016.01)

CPC - G01N 2001/4027; H01J 37/02; H01J 37/20; H01J 37/261; H01J 37/285; H01J 2237/2802 (2016.08)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

USPC - 250/307; 250/311; 250/440.11; IPC(8) - H01J 37/20; H01J 37/26; H01J 37/285; CPC - G01N 2001/4027; H01J 37/02; H01J 37/20; H01J 37/261; H01J 37/285; H01J 2237/2802 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Orbit, Google Patents, Google

Search terms used: TEM, transmission electron microscope, detect, imaging, holder, support, humidifying, nanoparticle, liquid, automatic, robot, picoliter, pl, liquid cell transmission electron microscope

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CASTRO-HARTMANN et al., The Arraygrid: A Methodology for Applying Multiple Samples to a Single TEM Specimen Grid, Ultramicroscopy, Vol. 135, 26 July 2013 [retrieved on 01 October 2016]. Retrieved from the Internet <URL: http://dx.doi.org/10.1016/j.ultramicro.2013.07.014 >, Pgs. 105-112	33, 34 ----- 35-37
Y	KLEIN et al., Transmission Electron Microscopy With a Liquid Flow Cell, Journal of Microscopy, Vol. 242, Pt 2 2011 [retrieved on 01 October 2016]. Retrieved from the Internet <URL: http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2818.2010.03484.x/epdf >, Pgs 117-123	35, 36
Y	US 2006/0261270 A1 (BURKHARDT et al) 23 November 2006 (23.11.2006) entire document	37
A	US 2014/0360286 A1 (THE SCRIPPS RESEARCH INSTITUTE et al) 11 December 2014 (11.12.2014) entire document	1-40
A	US 2012/0120226 A1 (DE JONGE) 17 May 2012 (17.05.2012) entire document	1-40
A	US 2015/0194288 A1 (NATIONAL HEALTH RESEARCH INSTITUTES et al) 09 July 2015 (09.07.2015) entire document	1-40

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

03 October 2016

Date of mailing of the international search report

24 OCT 2016

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