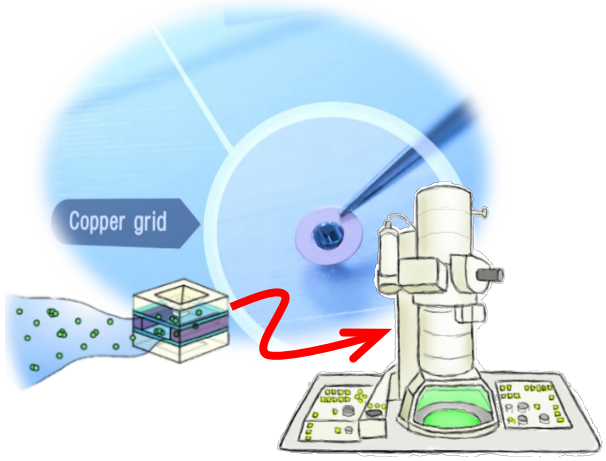
K-kit for EM-based imaging of Nanopharmaceuticals

Outline

- K-kit for nano pharma applications
- What is K-kit
- K-kit process
- Conclusions



K-kit for nano pharma applications

EM-based imaging with K-kit for nano pharma applications

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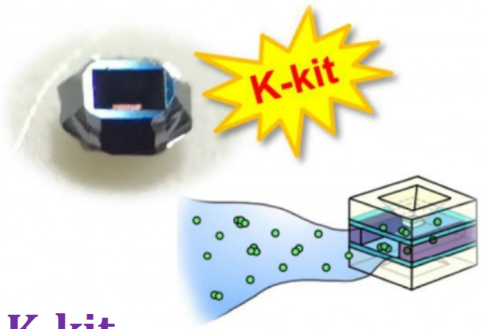
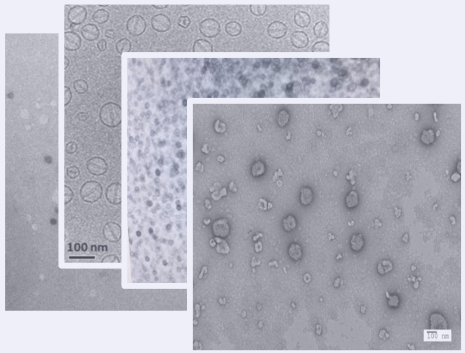
1. Electron microscope

Sample preparation and imaging

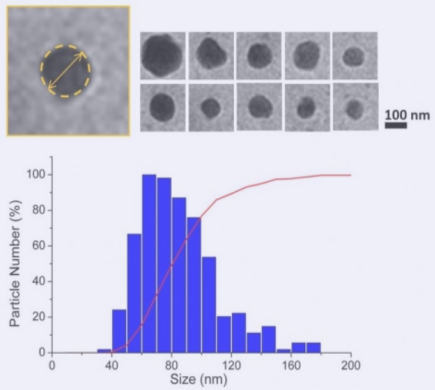


2. K-kit

Direct TEM observation in liquid



TEM/ SEM Images



Statistic Results

Applications

- Drug discovery and development
- Drug Manufacture (CMC)
- Pharmacokinetic studies
- Early-phase clinical studies

Valuable Analysis

- Nanoparticles in bio-liquid (like blood, serum or drug)
- Drug carriers (exosomes or liposomes) imaging analyses
- AAV imaging analysis for gene therapy
- Others

Example of EM-based imaging with K-kit

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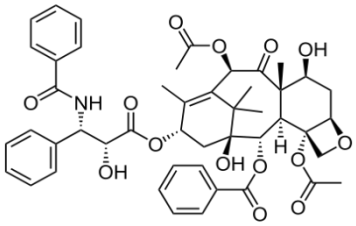
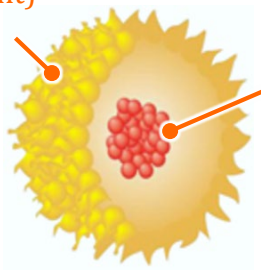
☐ Protein particles (Paclitaxel @ Albumin) in Abraxane®

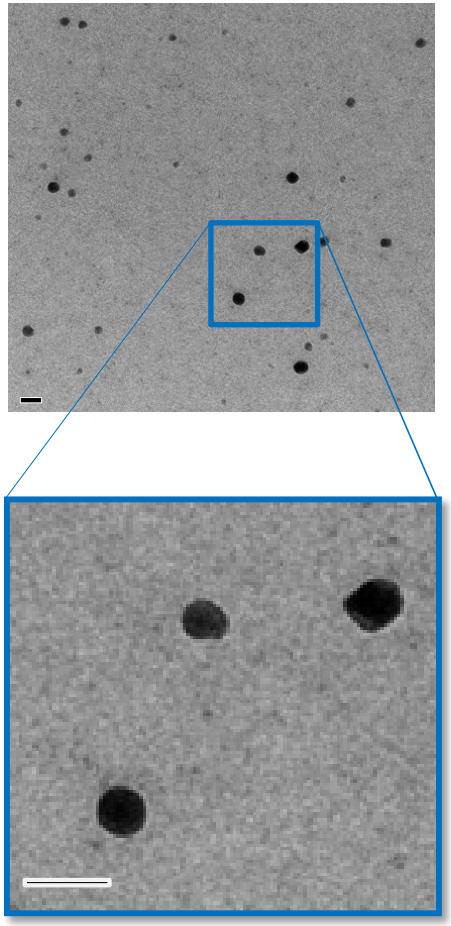
Abraxis BioScience

** US FDA approved 2005*



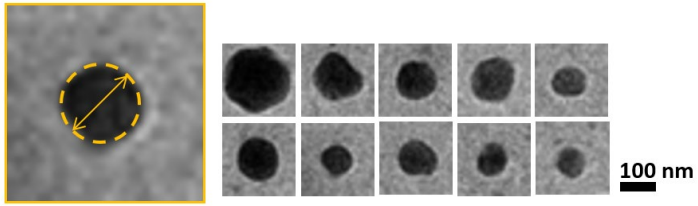
- Hydrophobic
- M.W. 854 Da

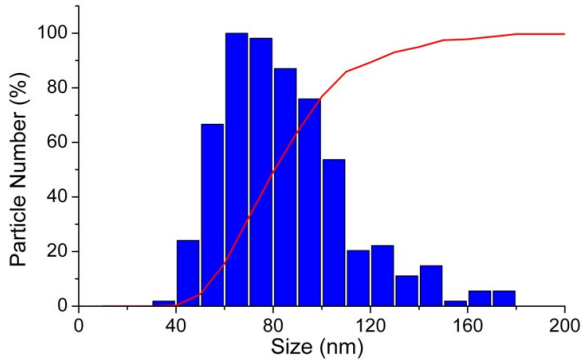




** Scale bar: 200 nm*

◆ Abraxane in saline; size & size distribution (D10/ D50/ D90)





- Total calculated particle #: 319
- Average size: 85.1 nm
- Standard deviation: 27.0 nm

Parameter	Size (nm)
D10	55.6
D50	80.1
D90	122.2
Span: (D90 - D10) / D50	0.831

Liquid-TEM observation in Nanopharmaceuticals

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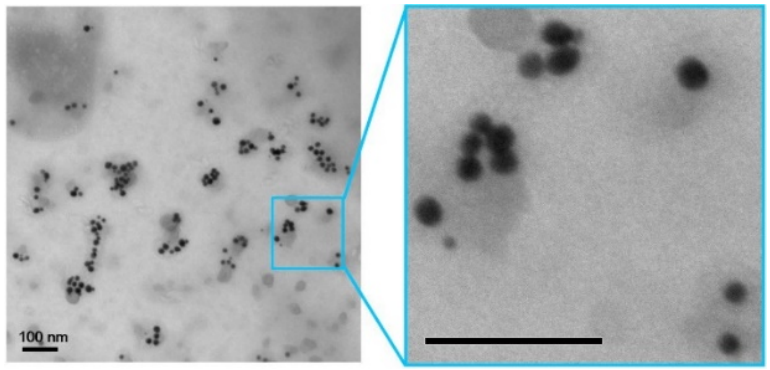
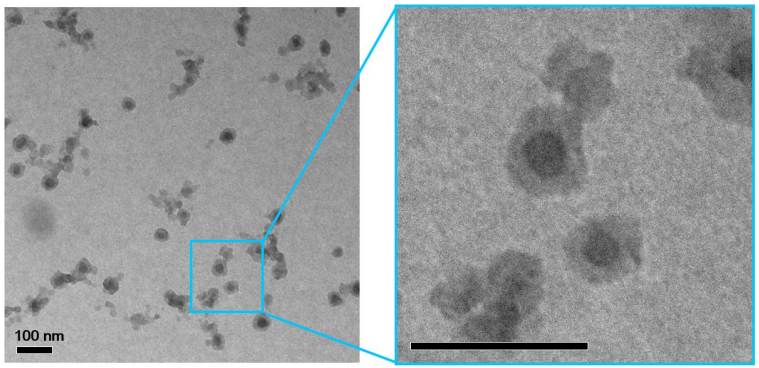
- **Applicable particle concentration for K-kit: 10^{11}~10^{14} particles/ml**



AuroVist® solution was directly loaded and sealed in a K-kit in liquid form.



Oil emulsion in water was loaded and sealed in a K-kit in liquid form.

Brand Name of Pharmaceuticals	**Doxil ® (1995 approved)**	**Abraxane ® (2005 approved)**	**Aurimune ® (Phase II)**	**Resovist ®**	**Rexin-G ® (Phase II)**
Particle Size	80-100 nm	~ 130 nm	~ 27 nm (AuNPs core), ~ 30-40 nm as hydrated	~ 45-60 nm (Hydradynamic diameter)	~ 100 nm
Particle Concentrations	1.0×10^{14} liposome /ml	4.3×10^{13} albumin particles /ml	$\leqq 1.7 \times 10^{12}$ gold particles /ml	1×10^{14} particles /ml	$1\text{-}4 \times 10^{11}$ cfu

Liposomes can be clearly observed by K-kit

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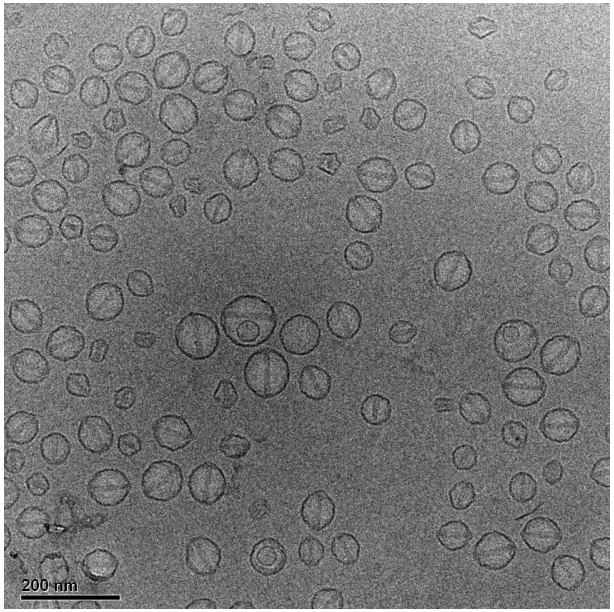
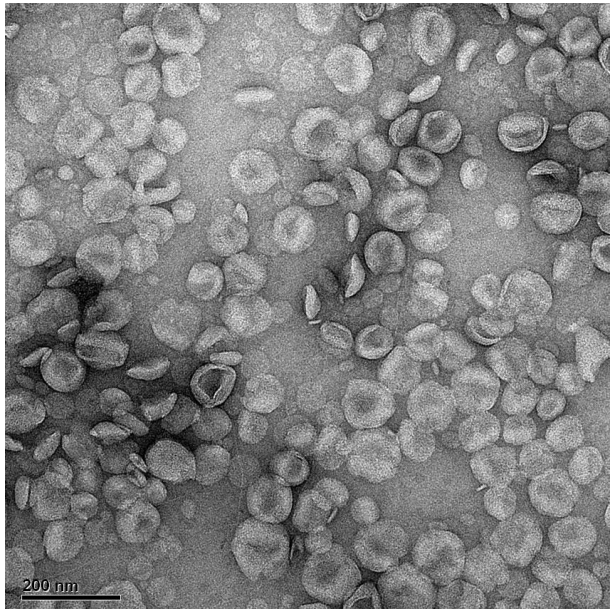
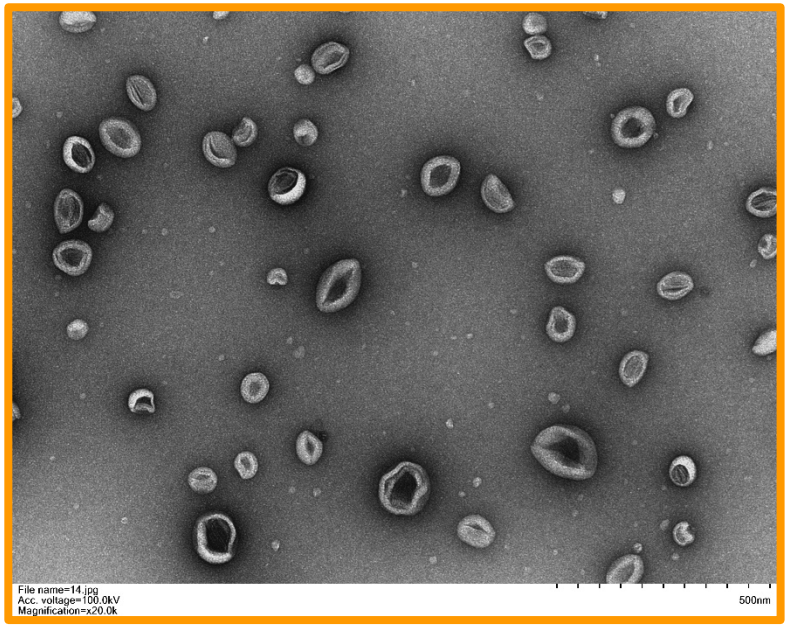
☐ Liposomes in Doxil®



Cryo-TEM (In formula)

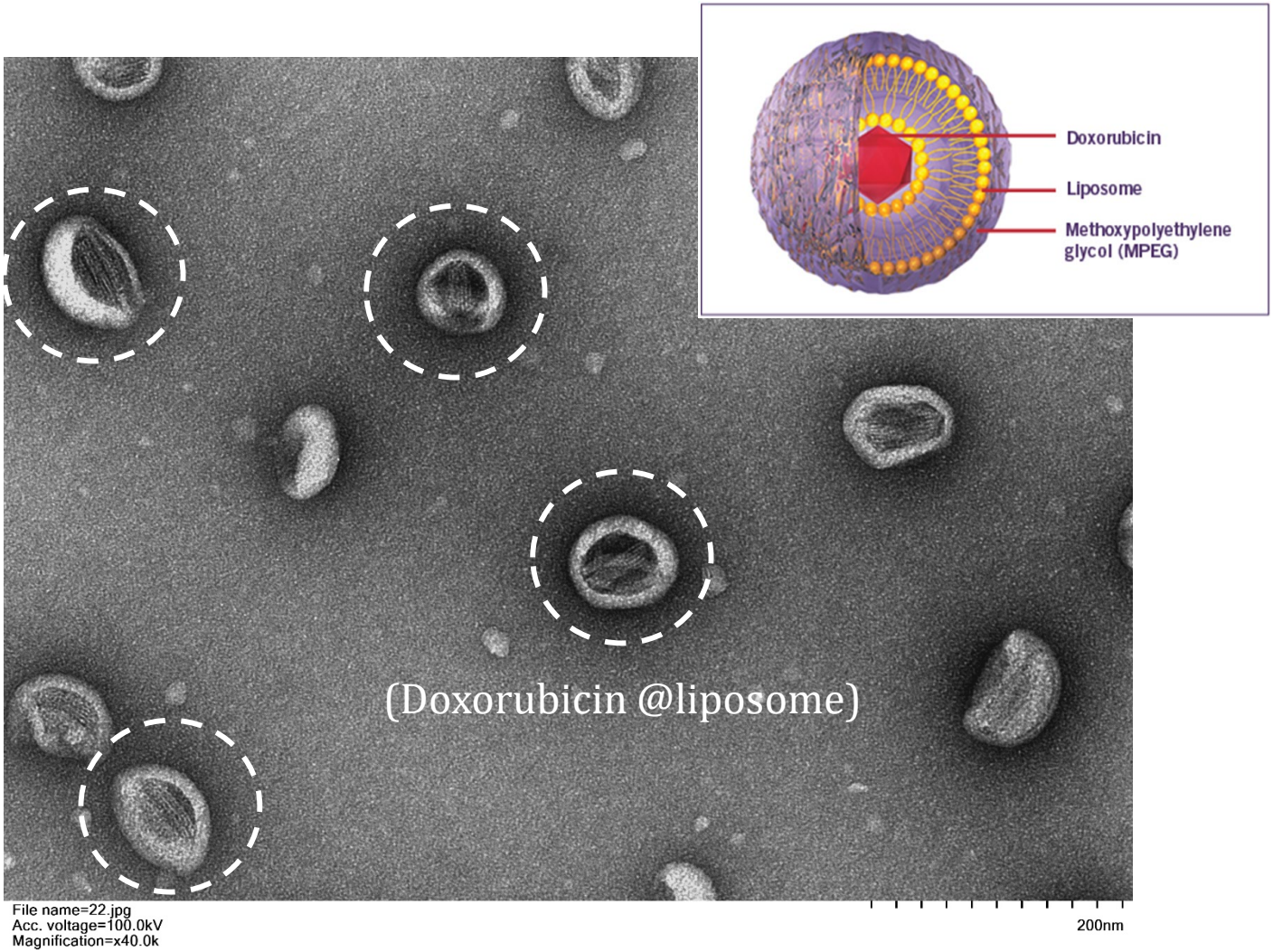


On Cu grid (Negative stain)



In K-kit (Negative stain)

The original look of particle distribution in liquid can be observed by K-kit and Cryo-TEM, whereas Cryo-TEM is very expensive and difficult to be done well.



The enclosed drug crystals in liposomes can be well identified with K-kit.

NOAAs of Au Nanoparticles (NPs) in Blood

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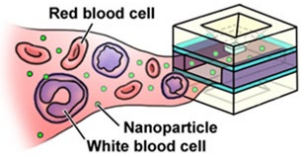
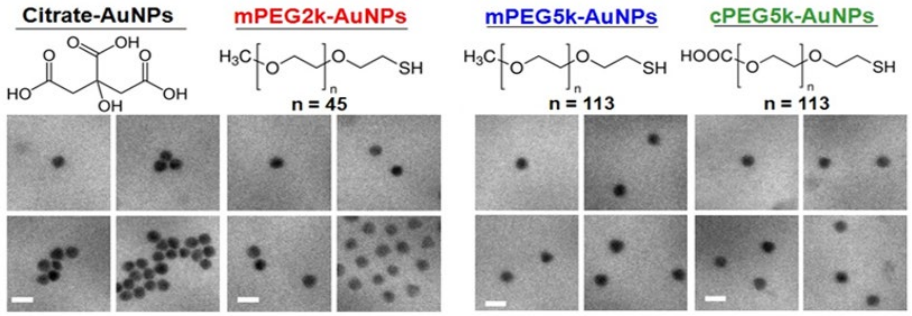
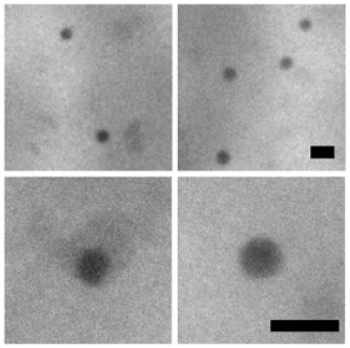
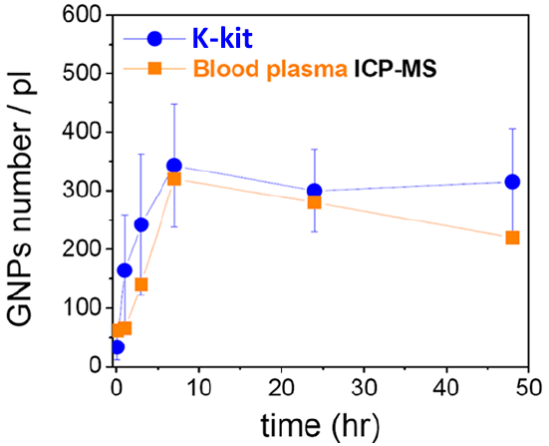
- Image-based statistic analysis of particle concentration (K-kit vs. ICP-MS)

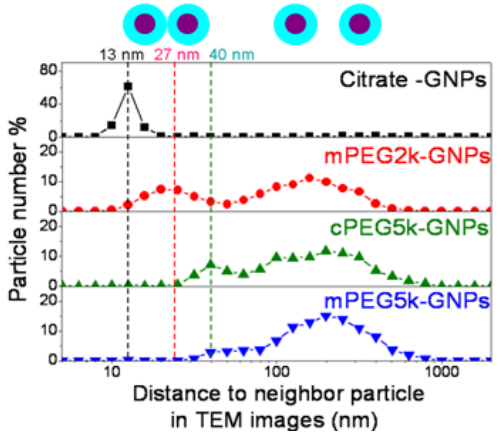


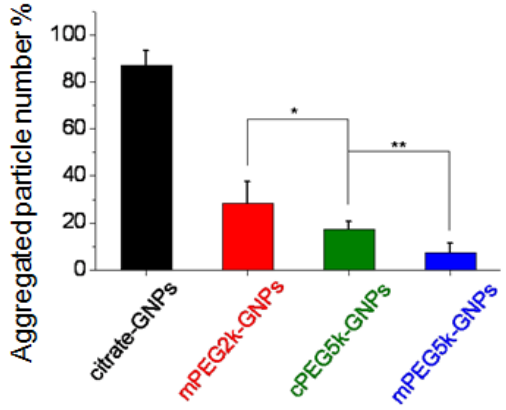




* Scale bar: 20nm





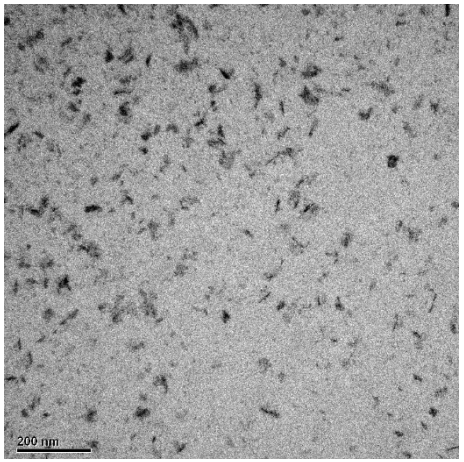


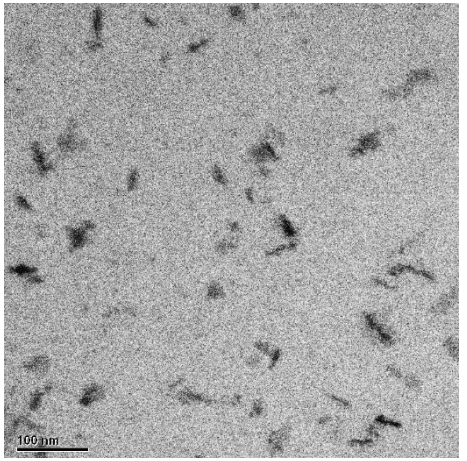
- Statistic analysis of Aggregation and agglomeration of Au NPs in blood

→ K-kit can be used to perform physicochemical characteristics of NPs in blood.

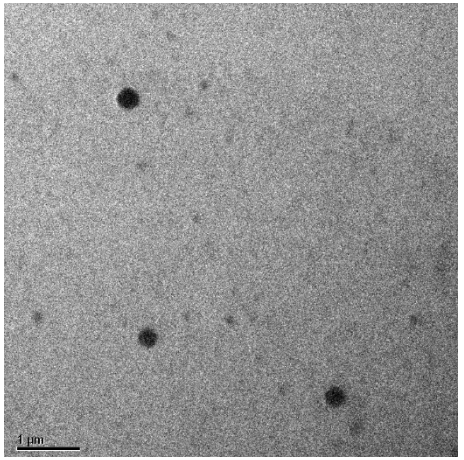
Other K-kit examples in Nanopharmaceuticals

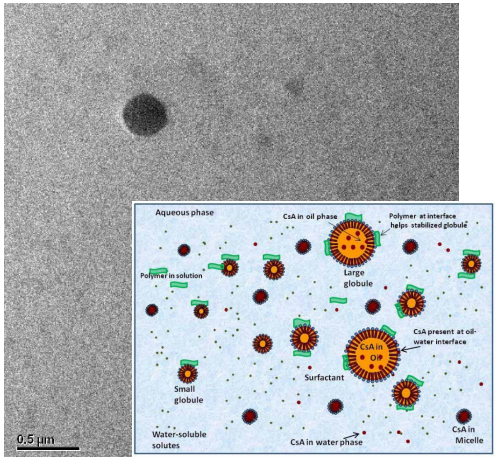
(Example) Resovist® solution, which a human used MRI T2 contrast agent with iron oxide nanoparticles in the solution, was directly loaded into K-kit and sealed for TEM observation in wet condition.





(Example) Restasis®, cyclosporine ophthalmic emulsion





What is K-kit

An innovative specimen holder for liquid analysis in TEM

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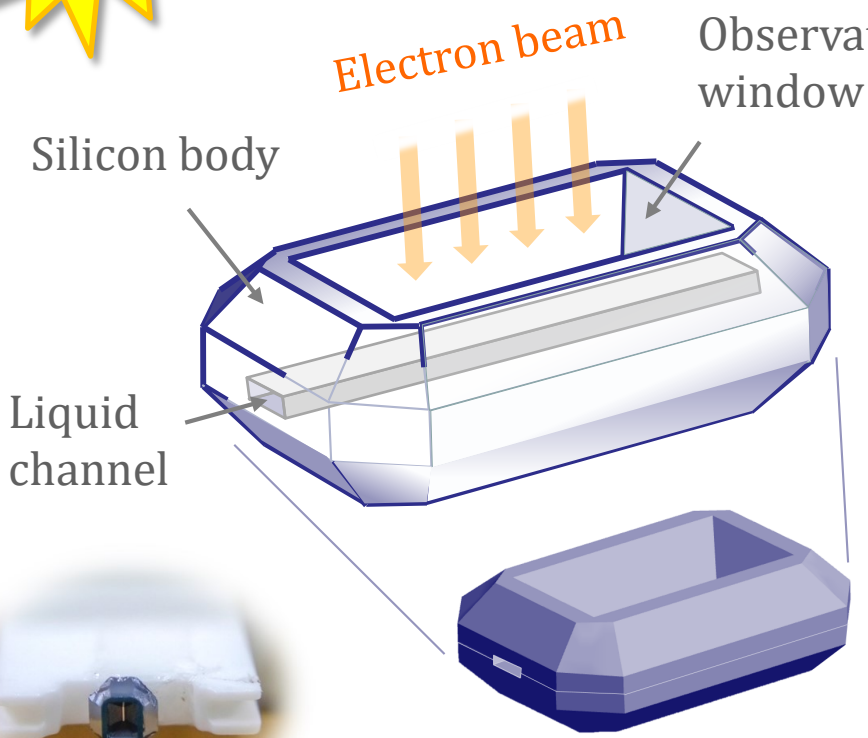
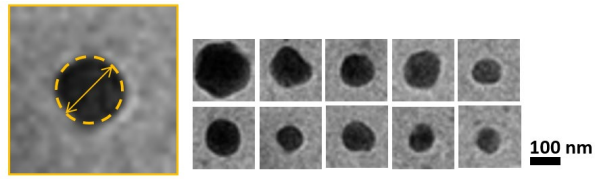
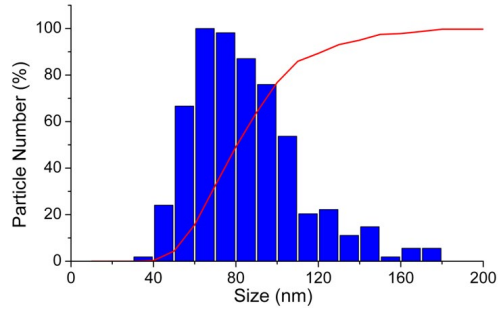
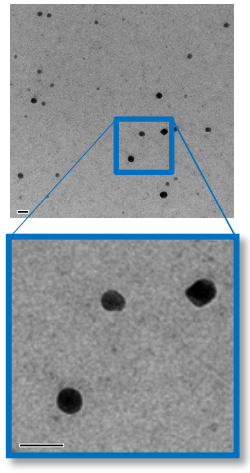
K-kit



→ To be used for Liquid-TEM applications

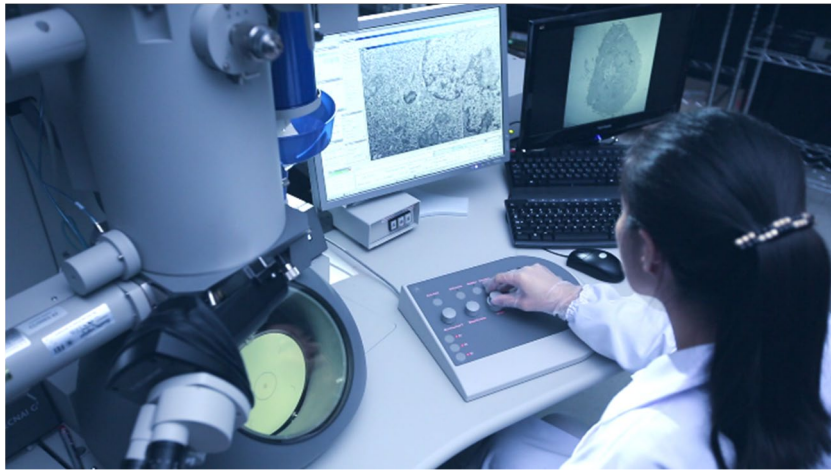




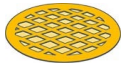


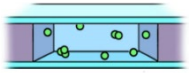
** Scale bar: 200 nm*

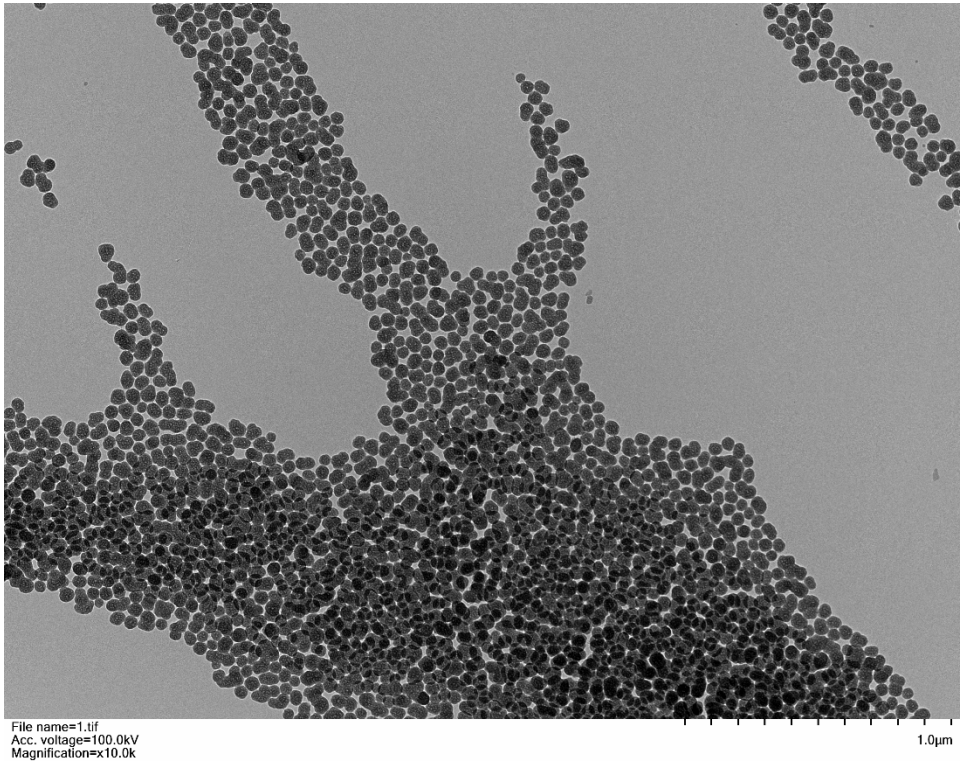
Quantitative analysis of nanoparticles in liquid

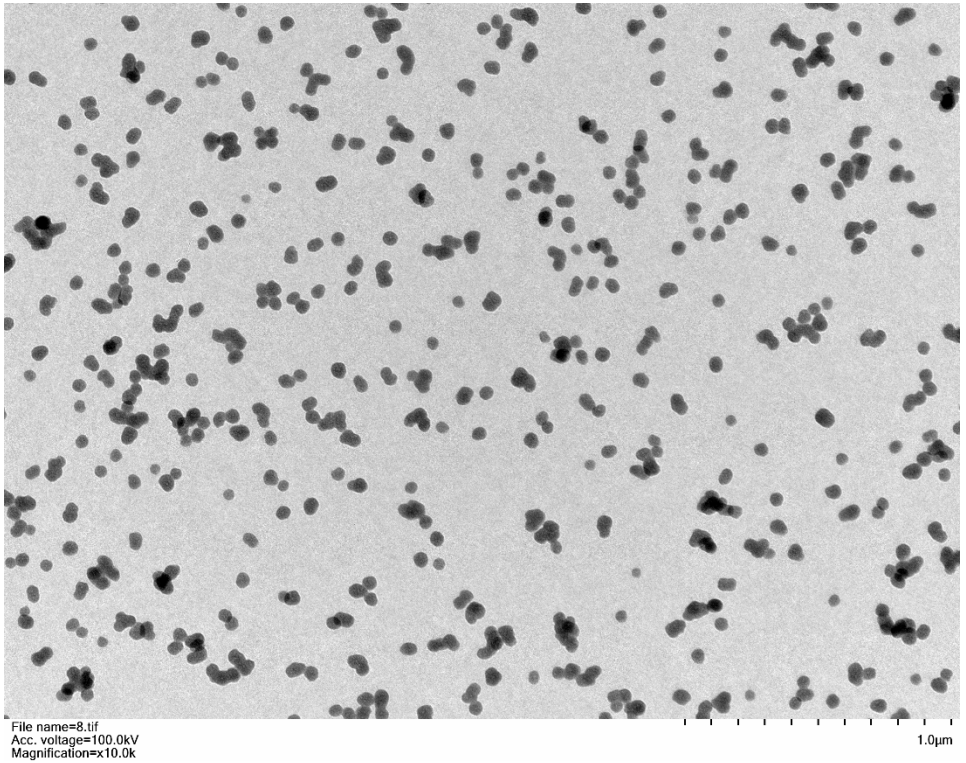


Nanoparticles of CMP slurry in K-kit and on Cu grid

✖  Aggregated as drying on Cu grid

✔  Nanoparticles in liquid by K-kit





(Wet Mode/ gap 0.2 µm K-kit)

Zinc oxide Nanoparticles in sunscreen lotion

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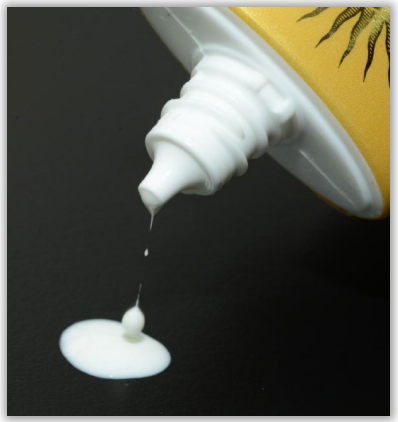
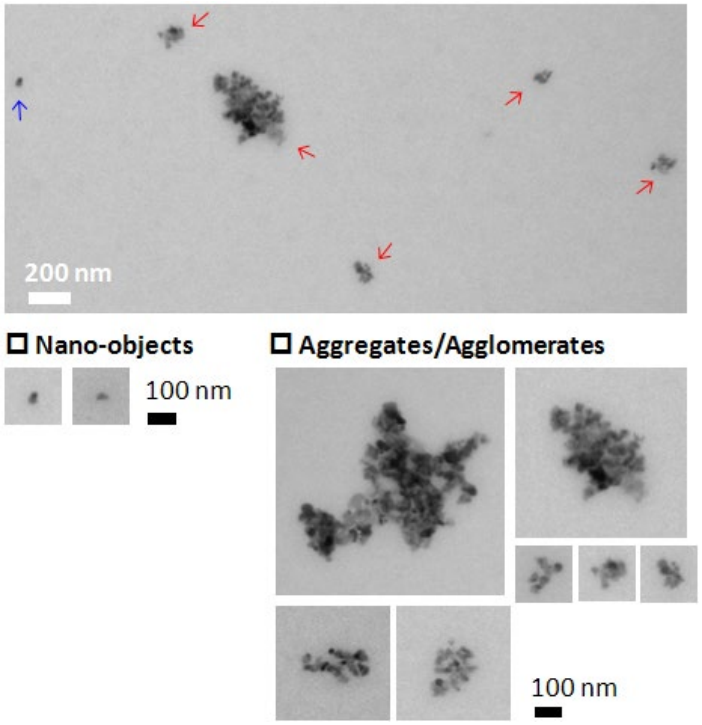
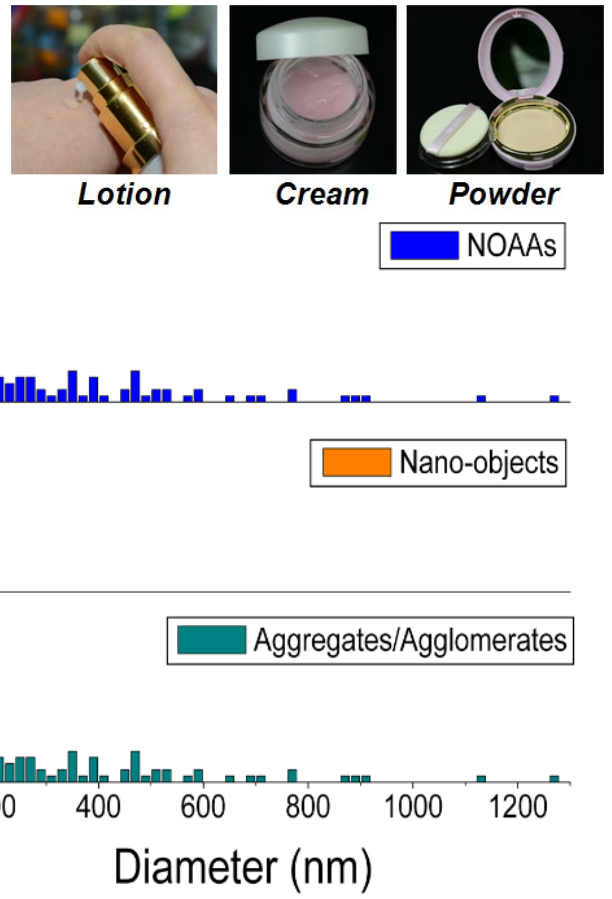
- K-kit can be used for characterizing NOAAs in cosmetics in final product forms.





TEM images



Size and size distribution

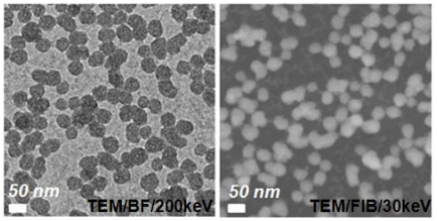
→ To assess the safety risks of nanomaterials in cosmetic ingredients.

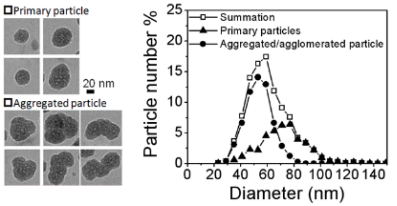
K-kit application

- Disposable
- Free of cross contamination
- Easy use

Slurry

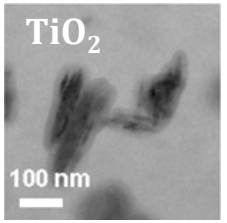
- SiO_2 Nanoparticles in CMP Slurry

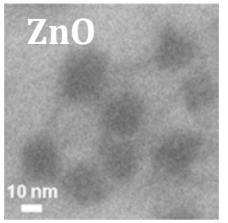


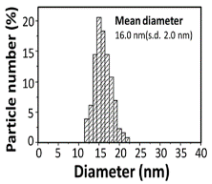


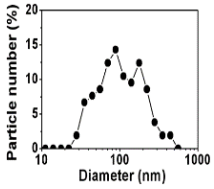
Lotion

- TiO_2 and ZnO Nanoparticles in Sunscreen







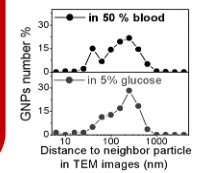


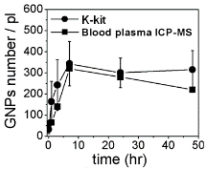
Electronics

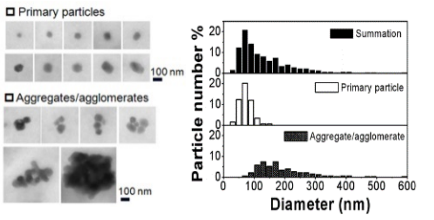
Cosmetics

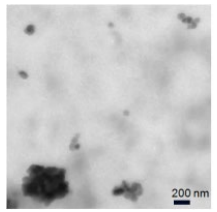
Food

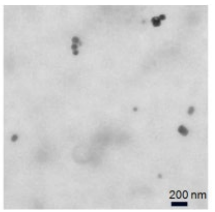
Bio-Med





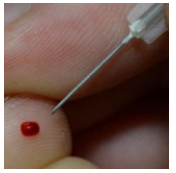


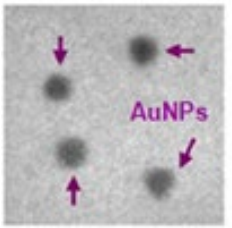


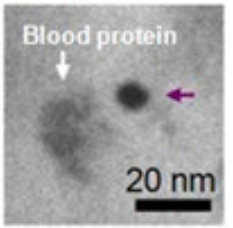


Beverage

- $CaCO_3$ Nanoparticles in milk





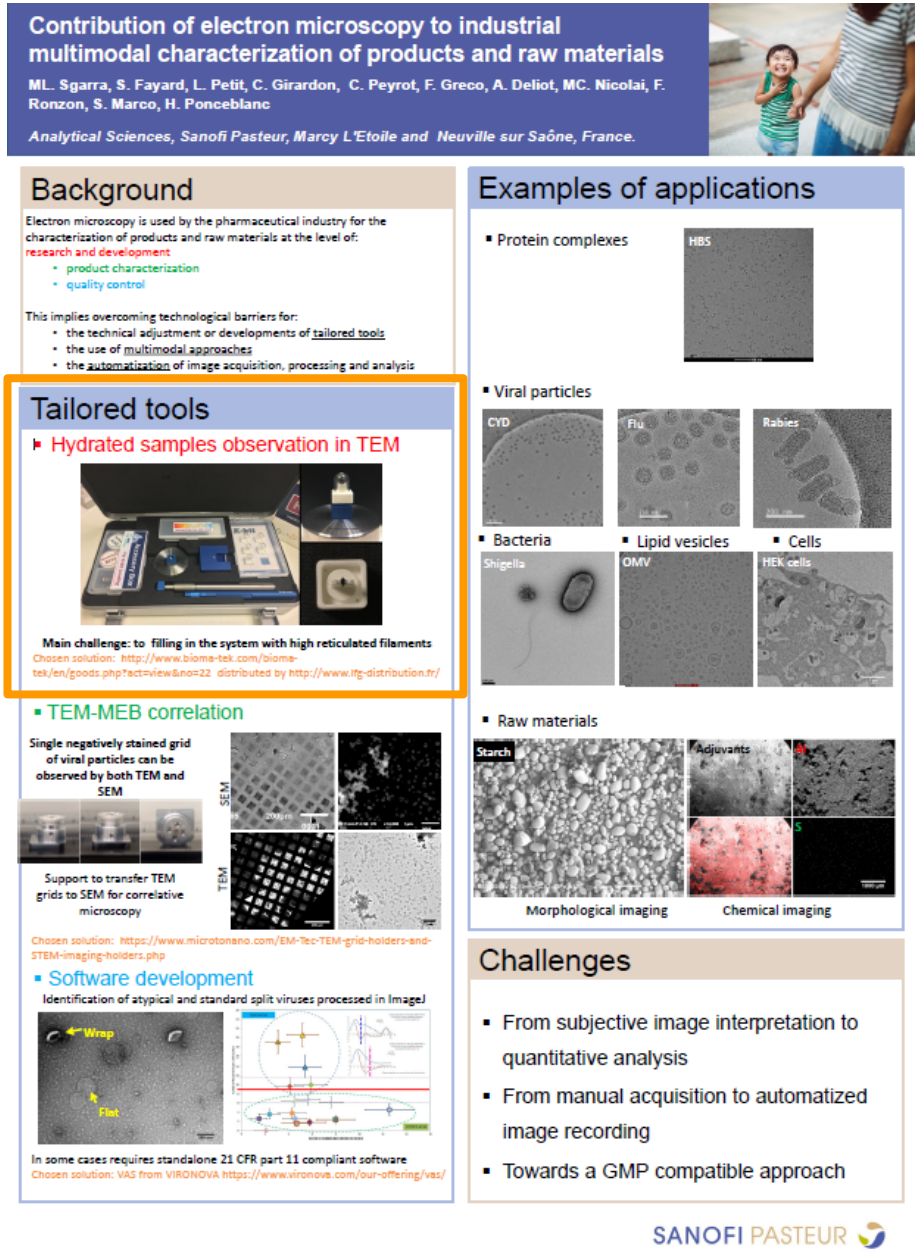


Bio Sample

- Au nanoparticles in blood

K-kit in the news

The poster on right side was published in June at RCCM Banyuls 2019 meeting in France; K-kit has been considered as an EM-based imaging solution on new drug development by the pharmaceutical company **SANOFI**



K-kit process

Quick and easy sample preparation

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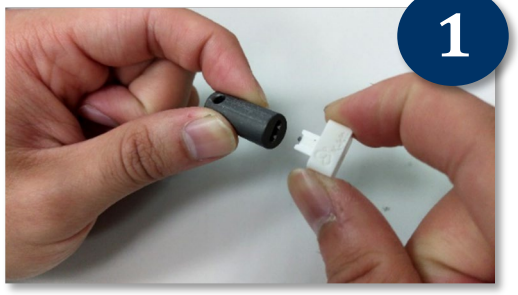
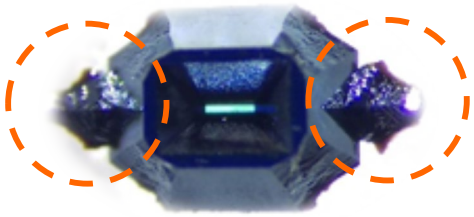
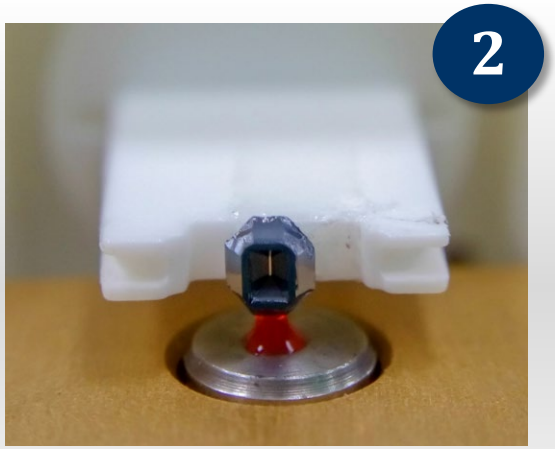


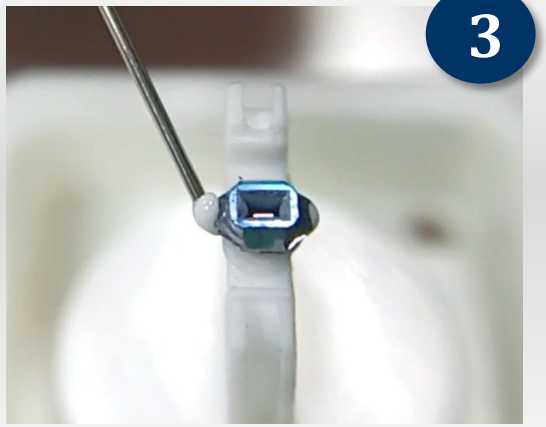
Remove the channel tips

Channel tips

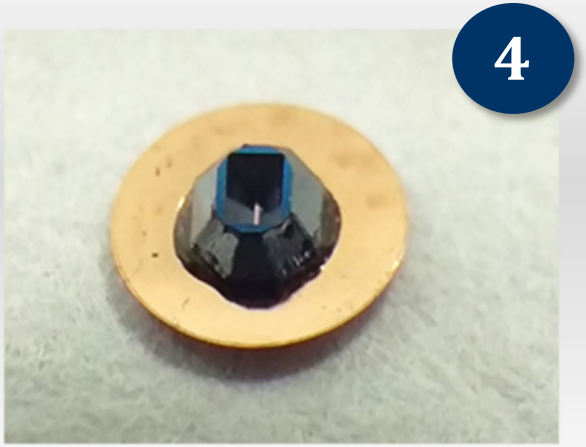




Liquid loading



Gluing



Copper grid

The fastest speed for liquid-TEM observation

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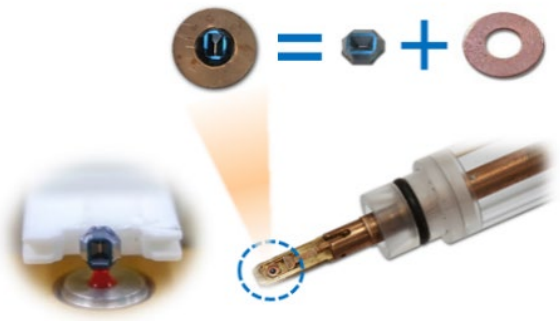
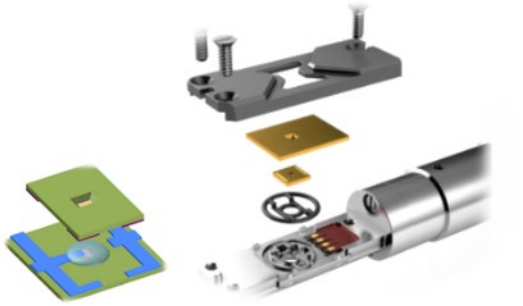


K-kit

Less than 1/5 of the time required, as compared with the others

90min required for 10 samples

Liquid loading and gluing for 10 K-kits (70min) + vacuum pumping (20min)



Other Solutions

450min at least for 10 samples

One by one; it needs the steps including surface treatment, assembly, leakage detection, and post-cleaning etc. for each.

Functional features

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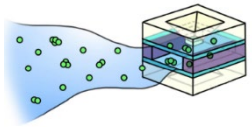
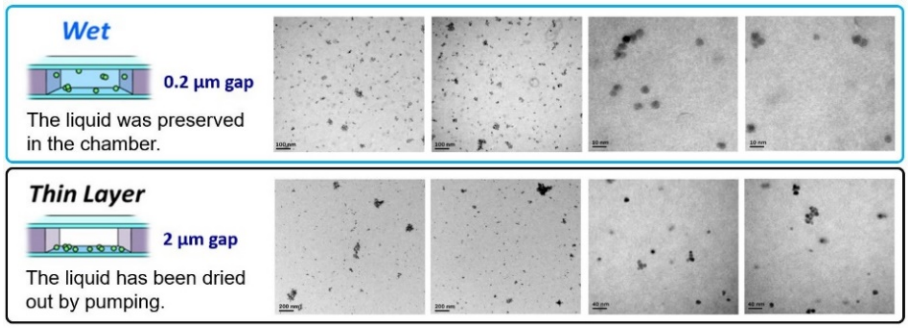
1. Native state in liquid

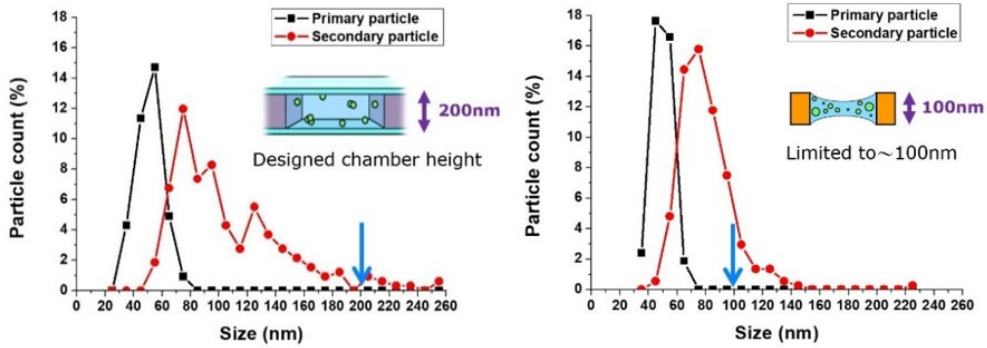
- QDs particles in chloroform





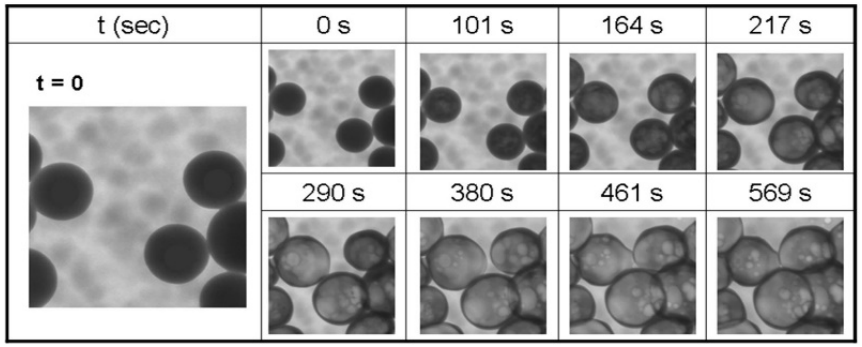
2. Quantitative analysis

- Abrasives in CMP slurry (K-kit vs. Cyro)



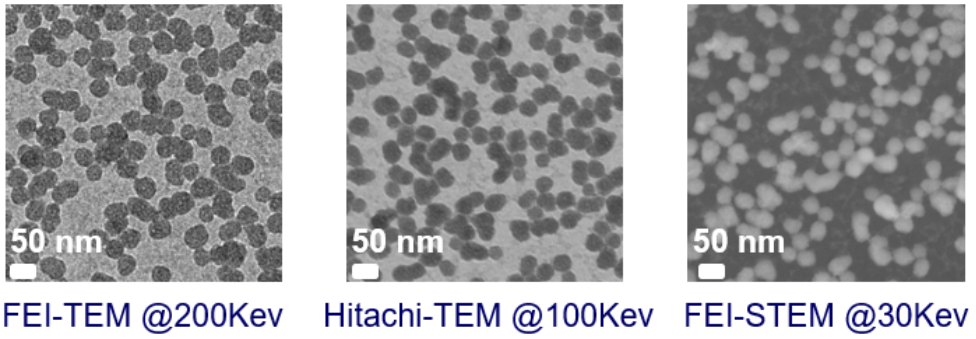
3. In-situ observation

- Dynamic observation of silicate particles



4. Compatible to versatile microscopy analyses

- Applicable to TEM, FIB, and STEM



FEI-TEM @200Kev | Hitachi-TEM @100Kev | FEI-STEM @30Kev

Available for SEM & EDX analyses

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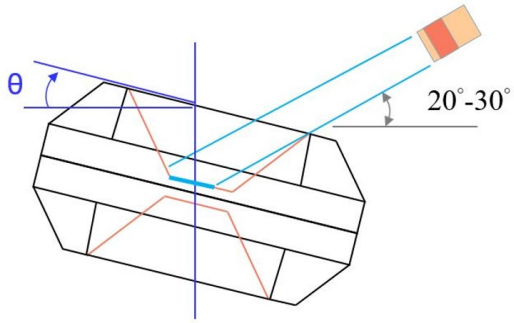
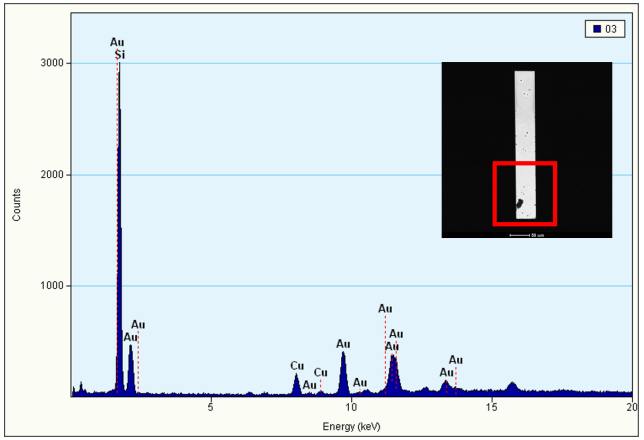
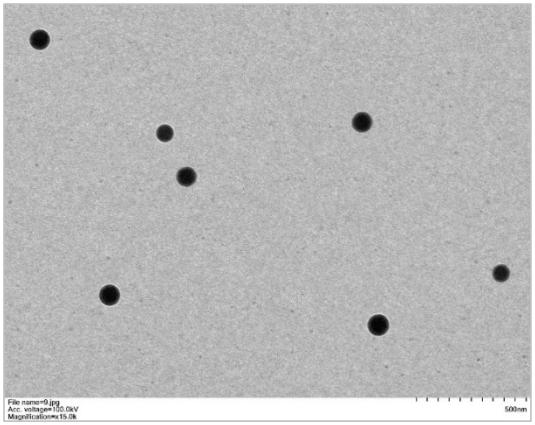
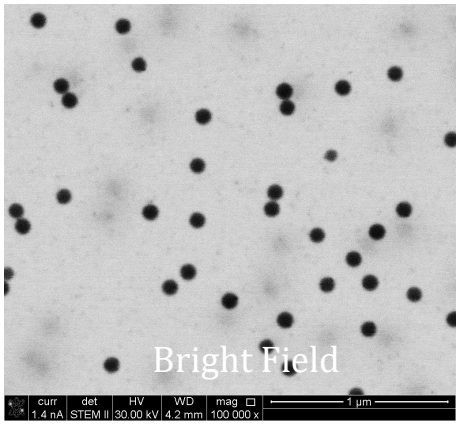
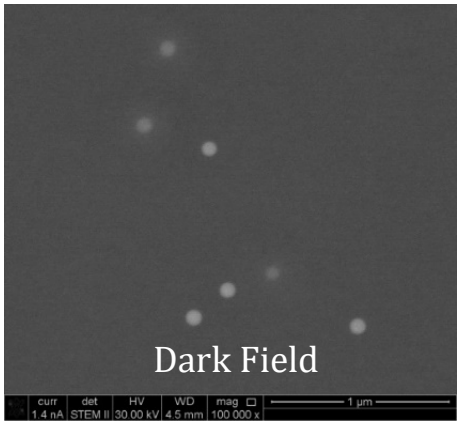
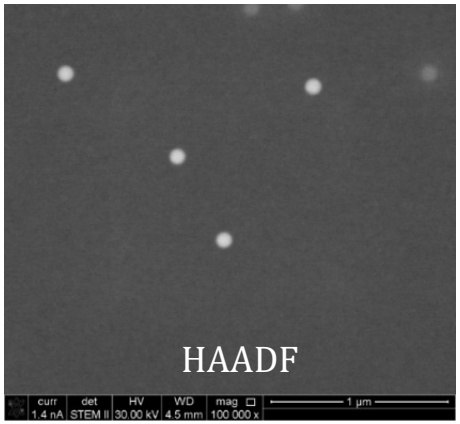


For EDX analysis, it needs to give a tilt toward the detector

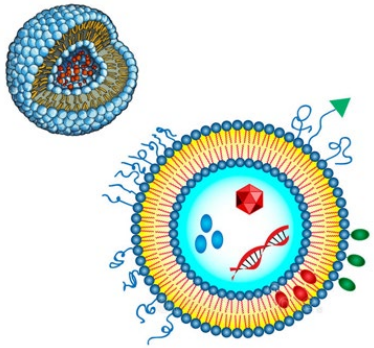
K-kit with Au particles

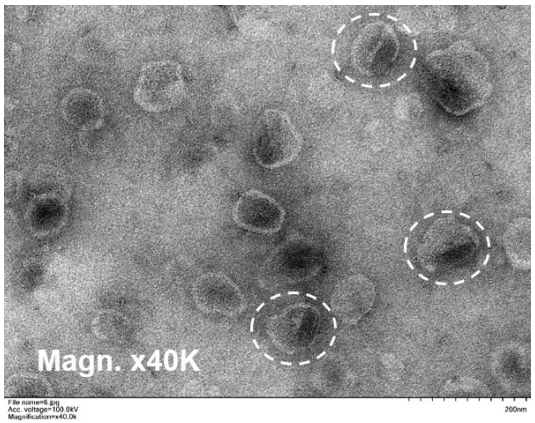


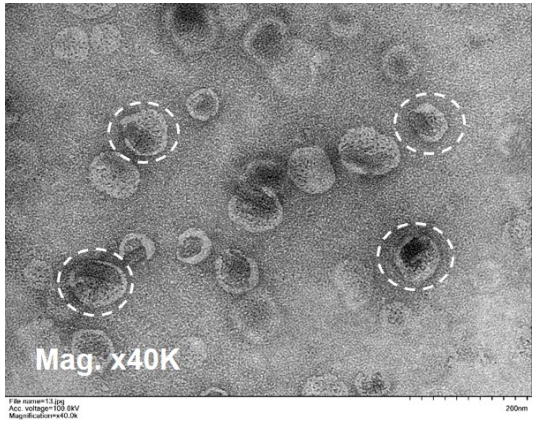
TEM by Hitachi HT7700	**SEM/ STEM** by FEI Helios 400		
	 Bright Field	 Dark Field	 HAADF

☐ The applications by multiple loadings of K-kit

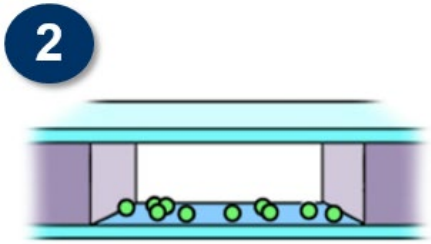
1st loading with liquid A
(Such as liposomes/ LDL)





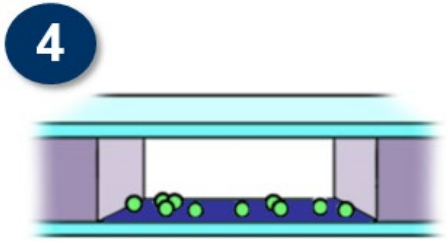


(ex. Liposomes with negative staining treated)



The K-kit prepared in dried mode for liquid A

2nd loading with liquid B
(Such like staining solution)



The K-kit in dried mode again for liquid B

Example K-kit application (2)

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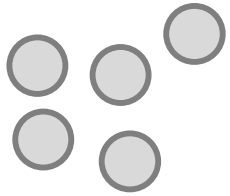
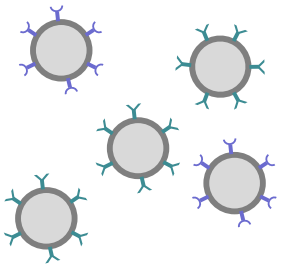
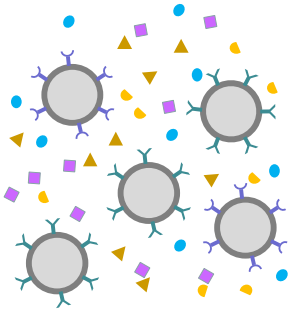
☐ Rapid and selective detection of pathogens by K-kit



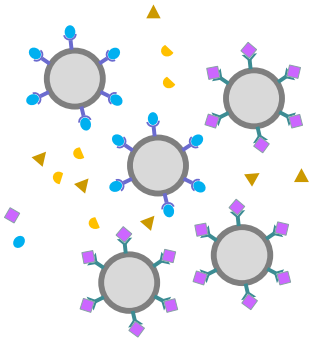
1. Nanoparticles (Polystyrene or Au)



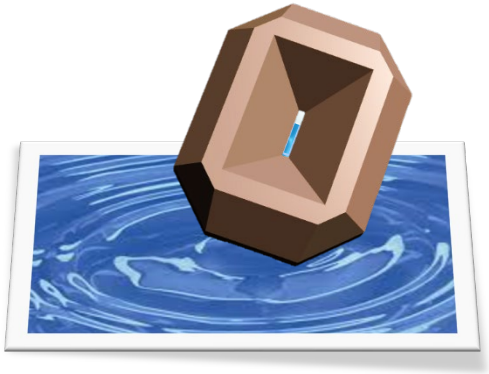
2. Coating with different antibodies

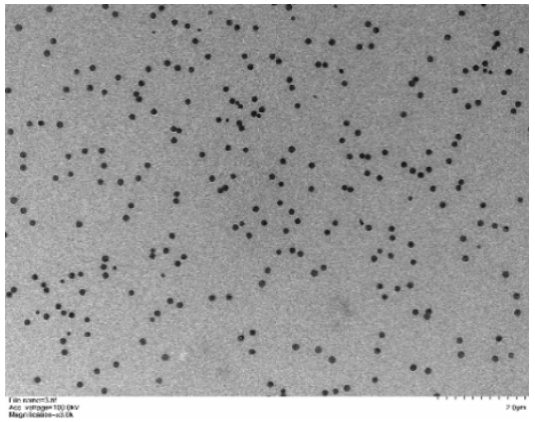


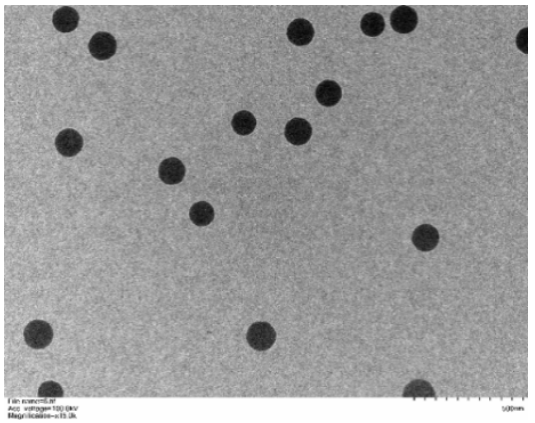
3. Mixed with multiple antigens



4. To observe the captured antigens on the beads by K-kit







The polystyrene beads in K-kit can be clearly observed by TEM.

K-kit specifications

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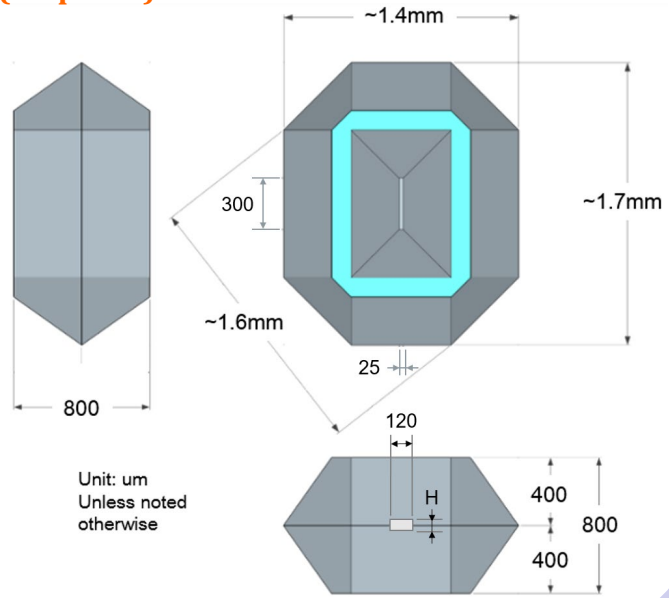
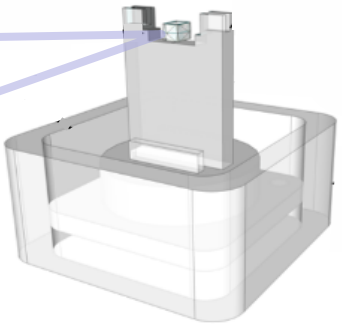
(Chip Size)





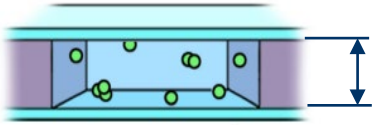
Shipping packages

K-kit

K-kit carrier

- Window length 300 μm, width 25 μm
- Channel height (H):

  0.2 and 2.0 standard; 0.1, 0.5, 1.0 and 5.0 available



H = 0.2, 0.5, 1, 2, 5 (μm)

K-kit tool box for sample preparation

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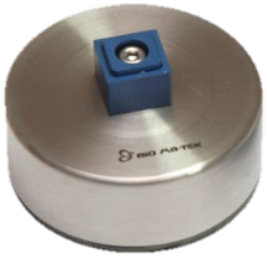
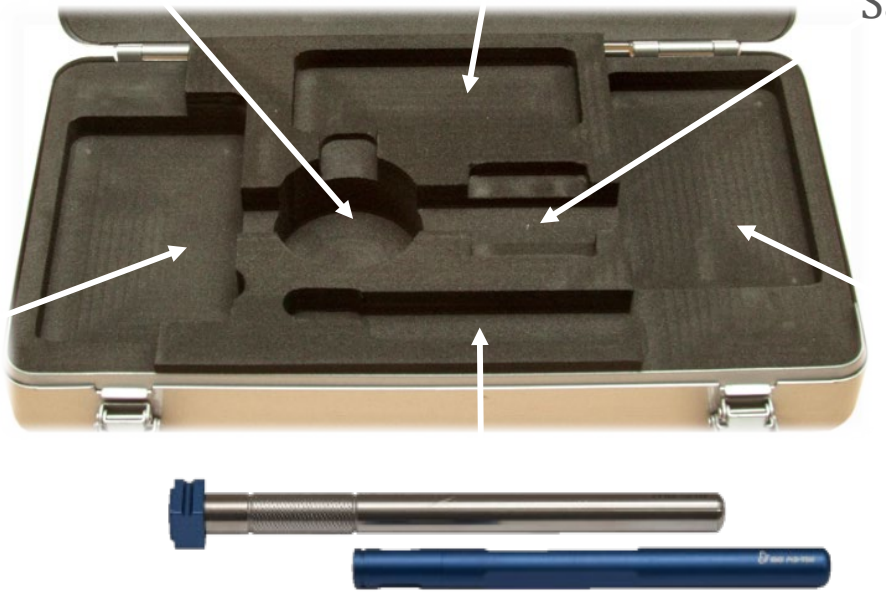


Gluing stand

Glass-slide pack

Sample-loading stage

Accessory box



K-kit holder & needle pen

Shipping package

1. K-kit is a sample holder designed to facilitate convenient TEM observation of liquid samples, allowing nano objects, aggregates, and agglomerates (NOAAs) in liquid samples to be characterized.
2. K-kit is a silicon chip made by MEMS; it fits on a 3 mm diameter of copper grid and, hence, is compatible with most existing TEM holders from FEI, JEOL and Hitachi etc.
3. K-kit can be the fastest and easiest solution for liquid-TEM applications. It is suitable for the nanomaterial research with multiple test conditions and industrial applications such like IQC for CMP slurry or CMC in Nanopharmaceuticals etc.
4. We have a full product plan of K-kit for the future; many exciting possibilities ahead!