

# **K-kit Meets All EM-based Imaging Needs in Nanopharmaceuticals**

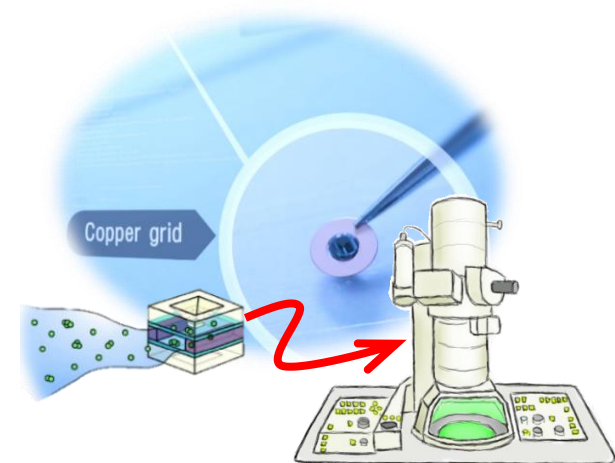


The Best R&D Partner

[www.bioma-tek.com](http://www.bioma-tek.com)  
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## OUTLINE

- ❑ K-kit for CRO Application
- ❑ What is K-kit
- ❑ Product Feature
- ❑ Conclusion



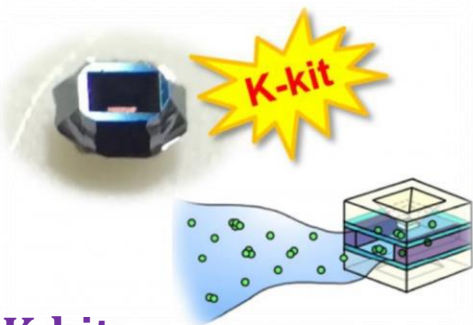
# K-kit for CRO Application

# EM-based Imaging Analysis with K-kit for CRO Application



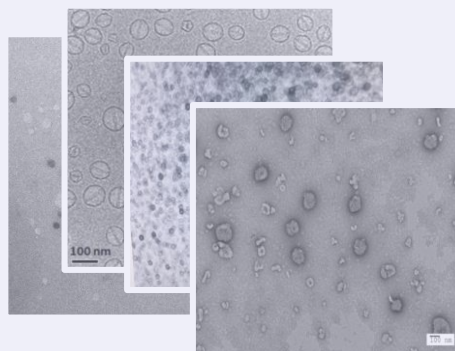
## 1. Electron Microscope

Sample preparation and imaging

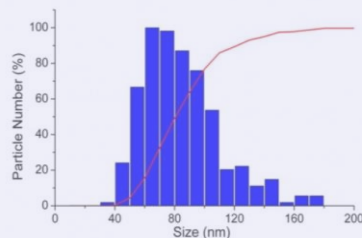
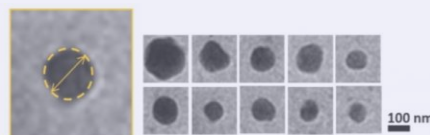


## 2. K-kit

Direct TEM observation in liquid



TEM/ SEM Images



Statistic Results

## Possible CRO Application

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

## Valuable Analysis

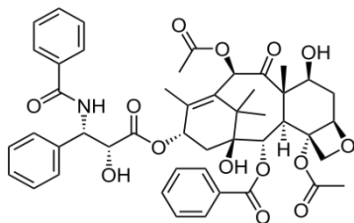
- Nanoparticles in bio-liquid (like blood, serum or drug)
- Drug carriers (exosomes or liposomes) imaging analyses
- AAV imaging analysis for gene therapy
- Others

# Example of EM-based Imaging Analysis with K-kit

## □ Protein particles (Paclitaxel @ Albumin) in Abraxane®

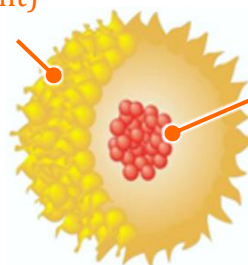


\* US FDA approved 2005

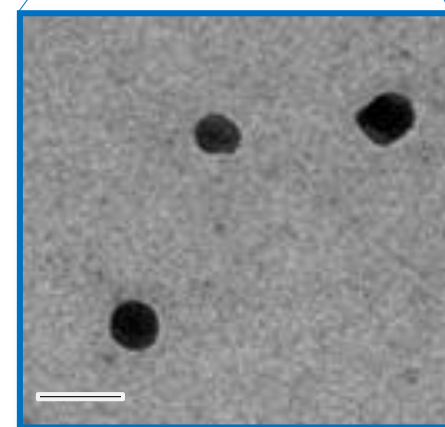
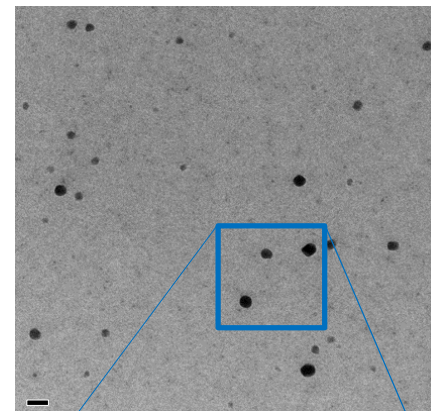


- Hydrophobic
- M.W. 854 Da

Albumin Shell  
(Excipient)

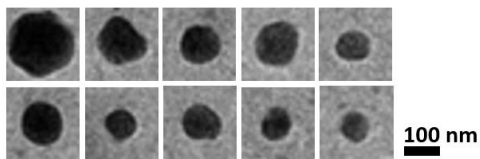
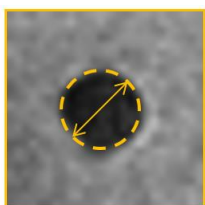


Paclitaxel  
(Drug)

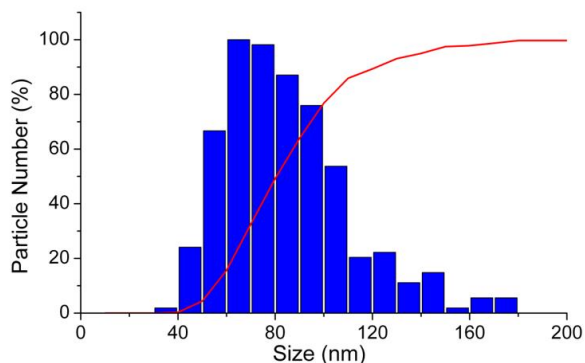


\* Scale bar: 200 nm

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



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

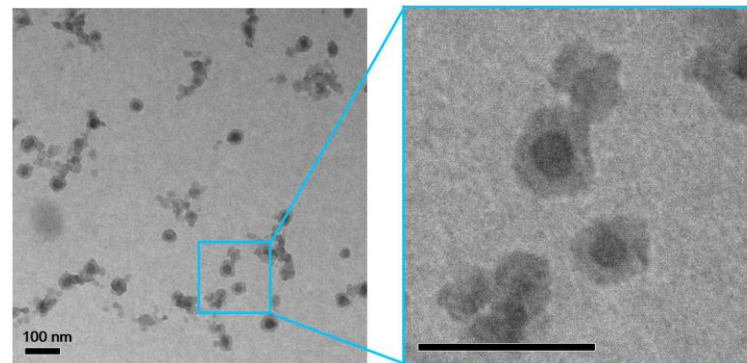
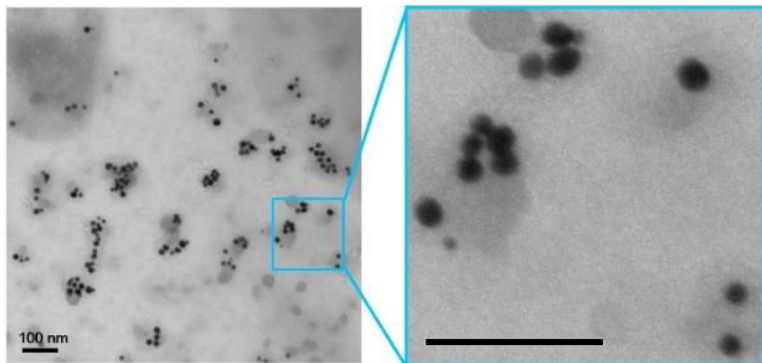


Parameter	Size (nm)
D10	55.6
D50	80.1
D90	122.2
Span: (D90 - D10) / D50	0.831



# Liquid-TEM Observation in Nanopharmaceuticals

- Applicable particle concentration for K-kit:  $10^{11} \sim 10^{14}$  particles/ml



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

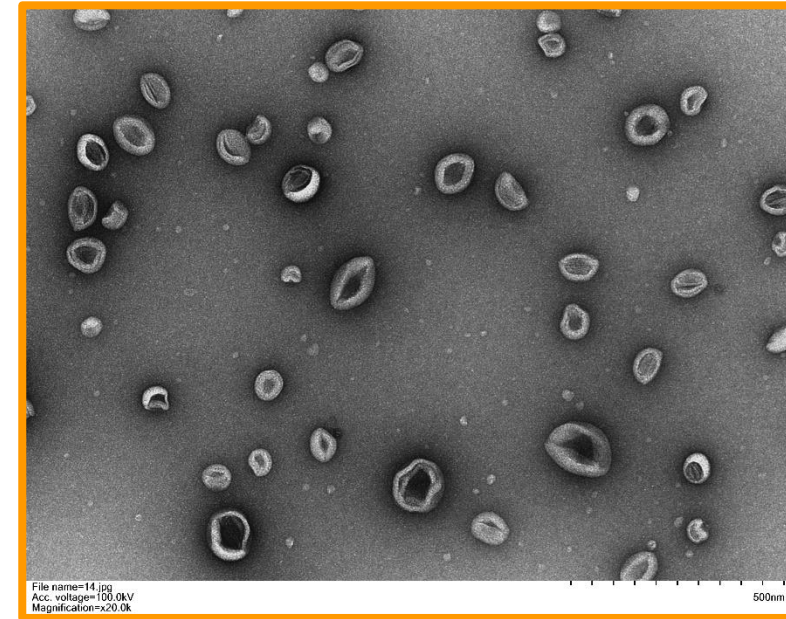
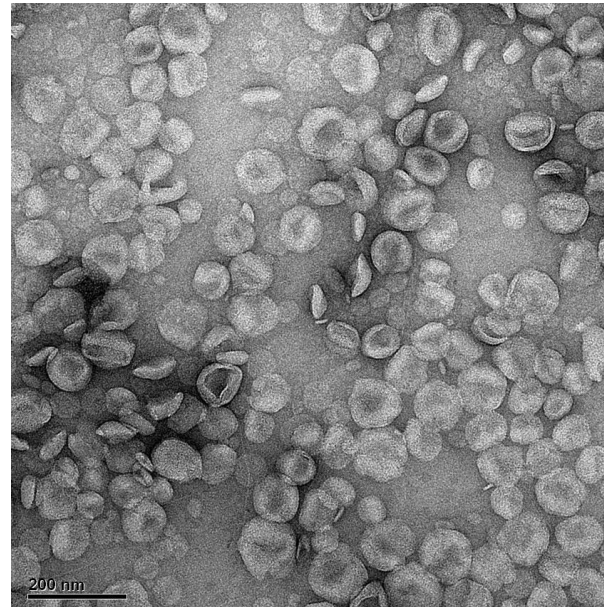
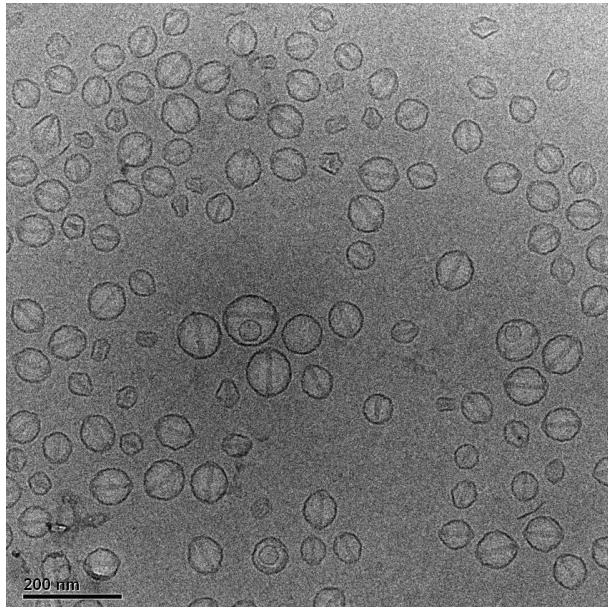
Oil emulsion in water was loaded and sealed in a K-kit in liquid form.

Brand Name of Pharmaceuticals	<b>Doxil ® (1995 approved)</b>	<b>Abraxane ® (2005 approved)</b>	<b>Aurimune ® (Phase II)</b>	<b>Resovist ®</b>	<b>Rexin-G ® (Phase II)</b>
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	$1.0 \times 10^{14}$ liposome /ml	$4.3 \times 10^{13}$ albumin particles /ml	$\leq 1.7 \times 10^{12}$ gold particles /ml	$1 \times 10^{14}$ particles /ml	$1-4 \times 10^{11}$ cfu

# Liposomes Can Be Clearly Observed by K-kit



## □ Liposomes in Doxil®

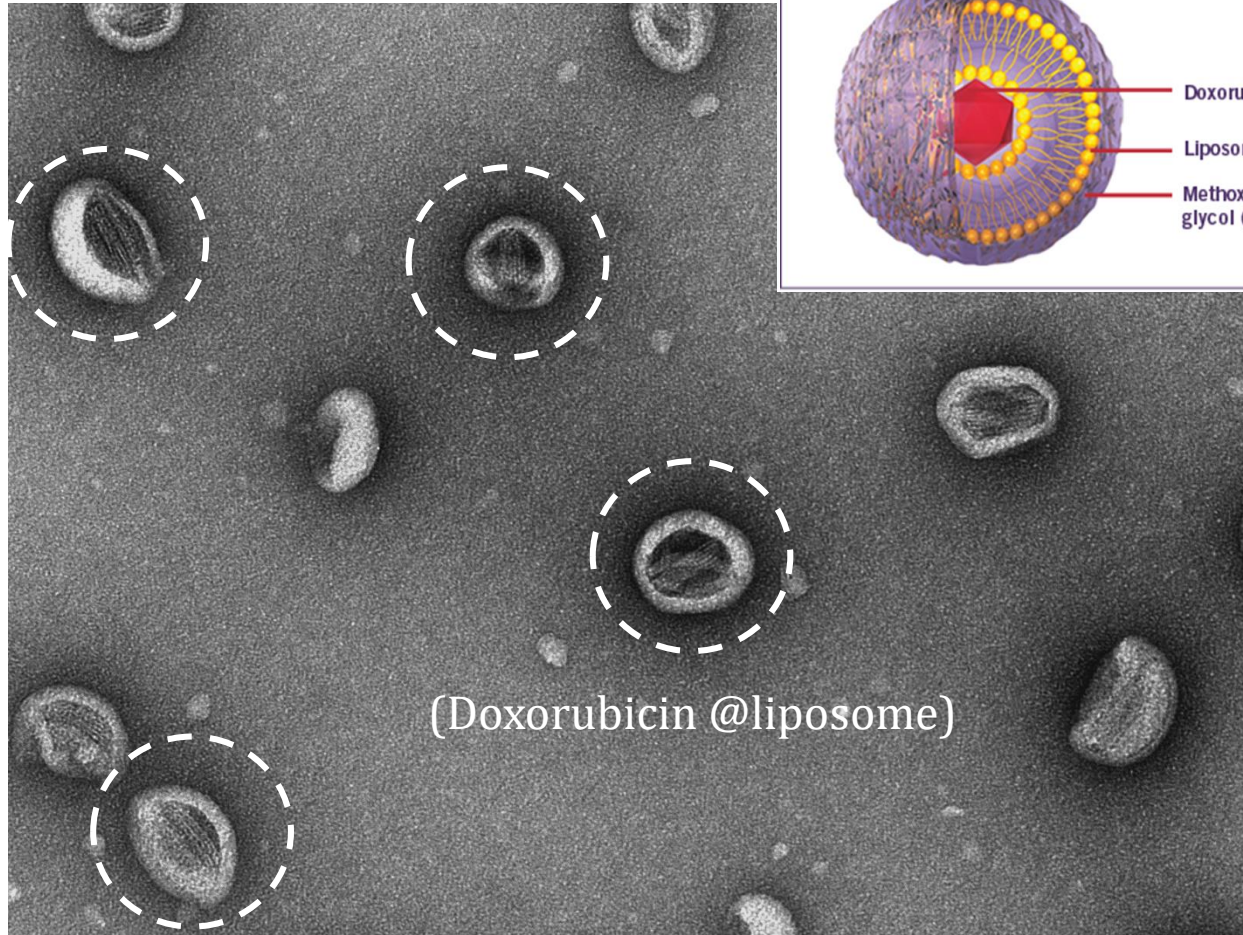


Cryo-TEM (In formula)

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 Cyro-TEM, whereas Cyro-TEM is very expensive and difficult to be done well.



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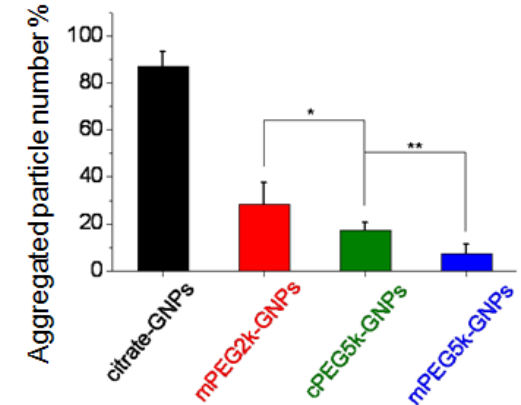
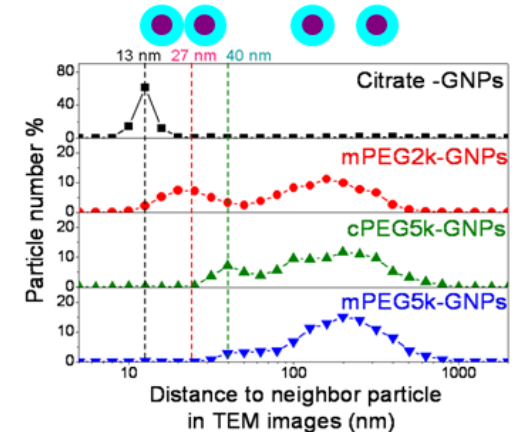
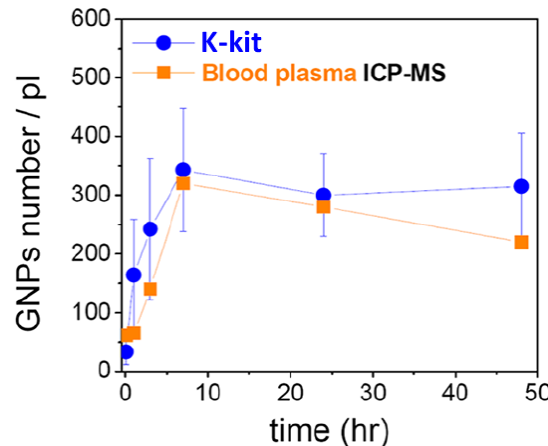
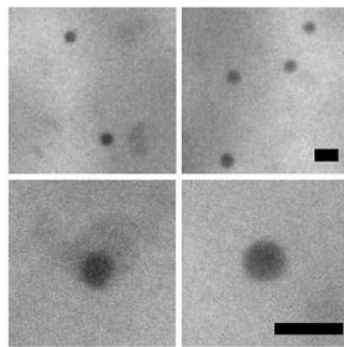
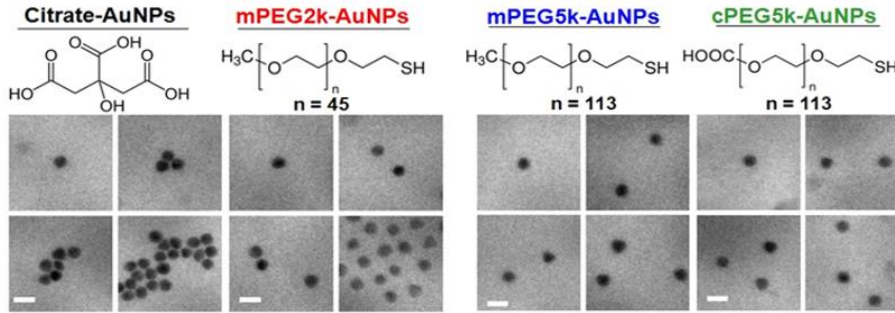
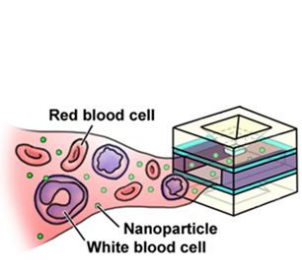
200nm

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



# NOAAs of Au Nanoparticles (NPs) in Blood

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



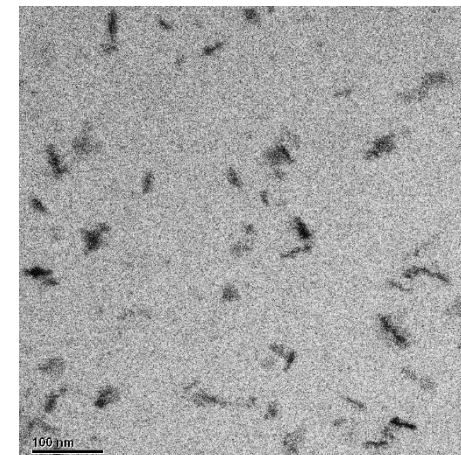
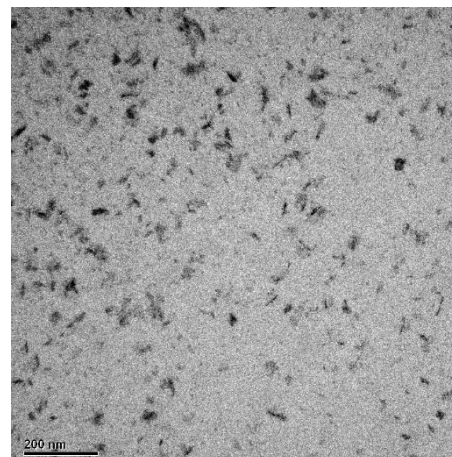
## Statistic analysis of Aggregation and agglomeration of Au NPs in blood

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

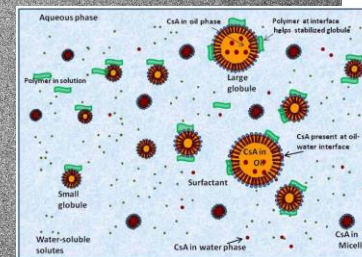
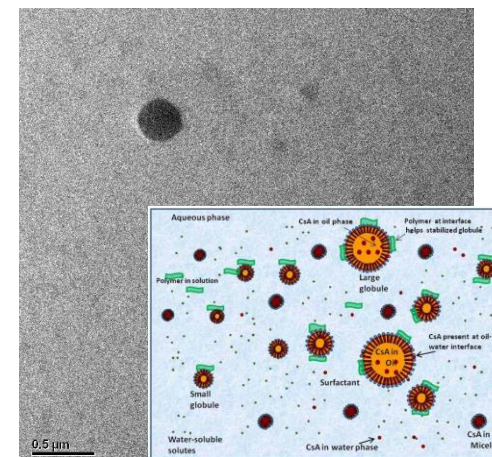
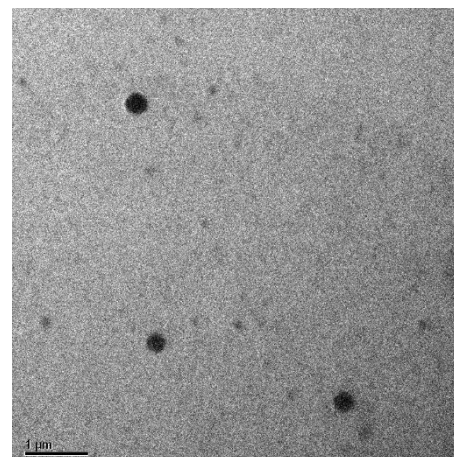
## Other K-kit Examples in Nanopharmaceuticals



(Example) Resovist<sup>®</sup> 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.

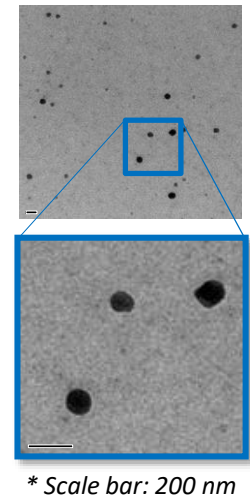
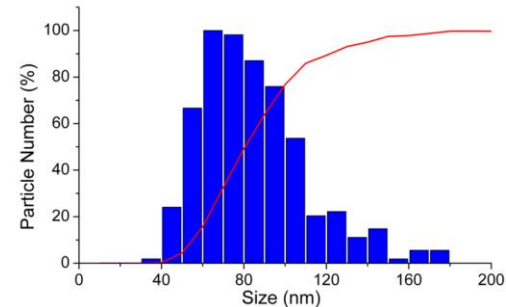
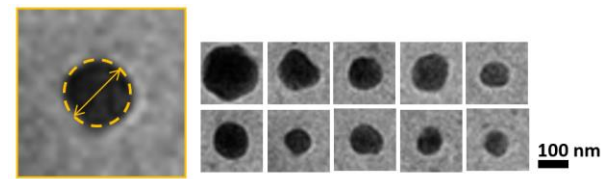
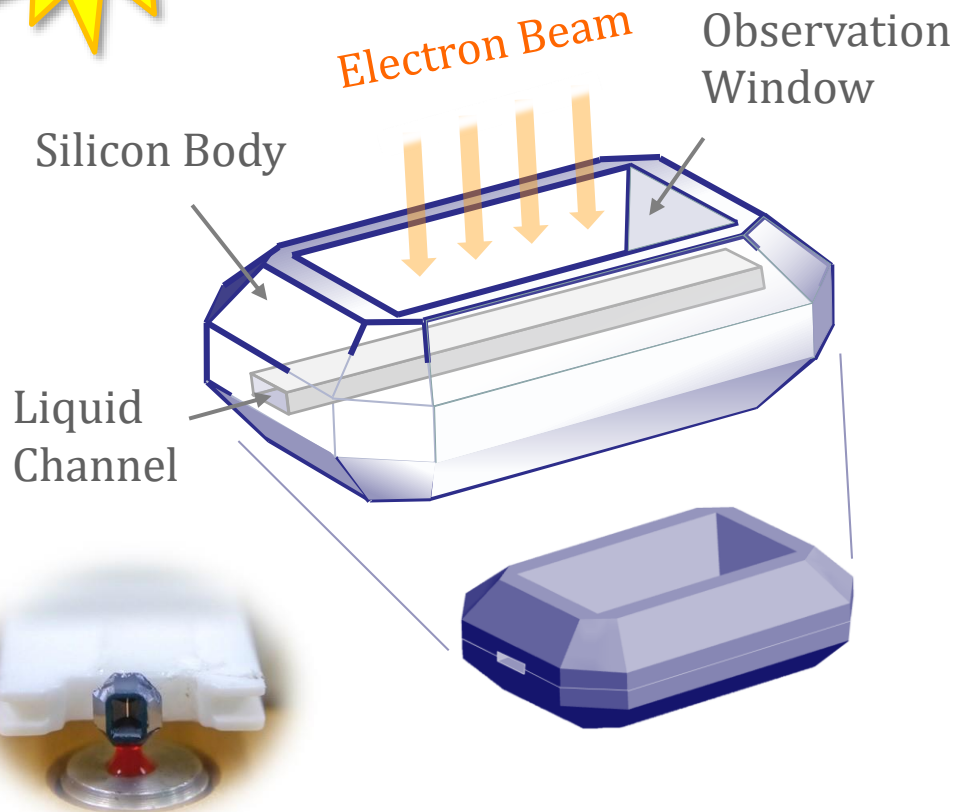


(Example) Restasis<sup>®</sup>, cyclosporine ophthalmic emulsion



# What is K-kit

# An Innovative Specimen Holder for Liquid Analysis in TEM



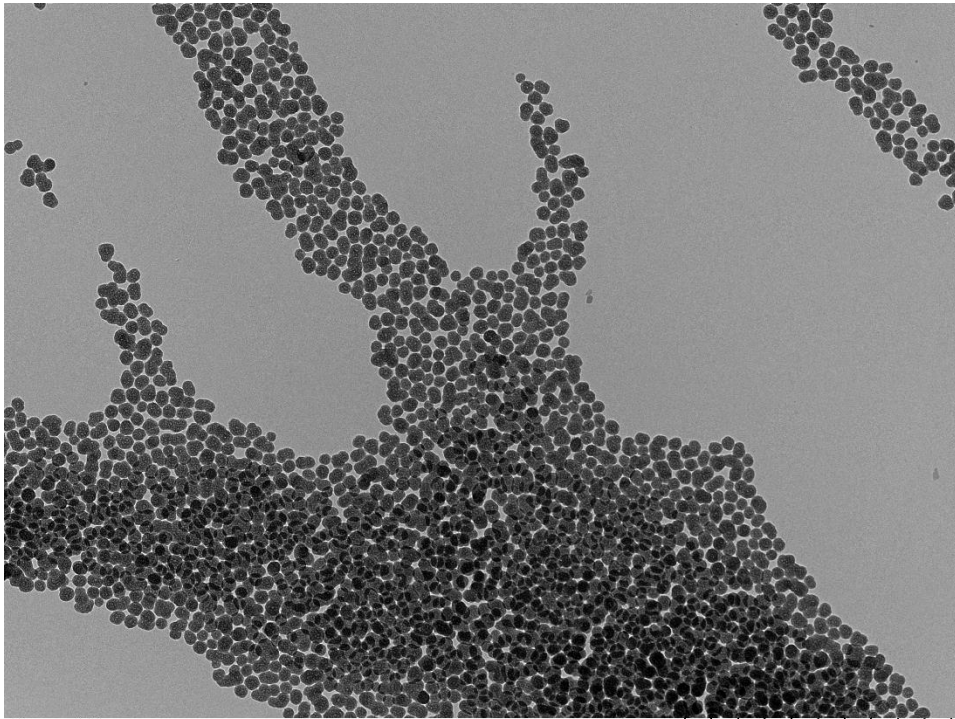
Quantitative analysis of nanoparticles in liquid



→ To be used for Liquid-TEM applications

# Nanoparticles of CMP Slurry in K-kit and on Cu grid

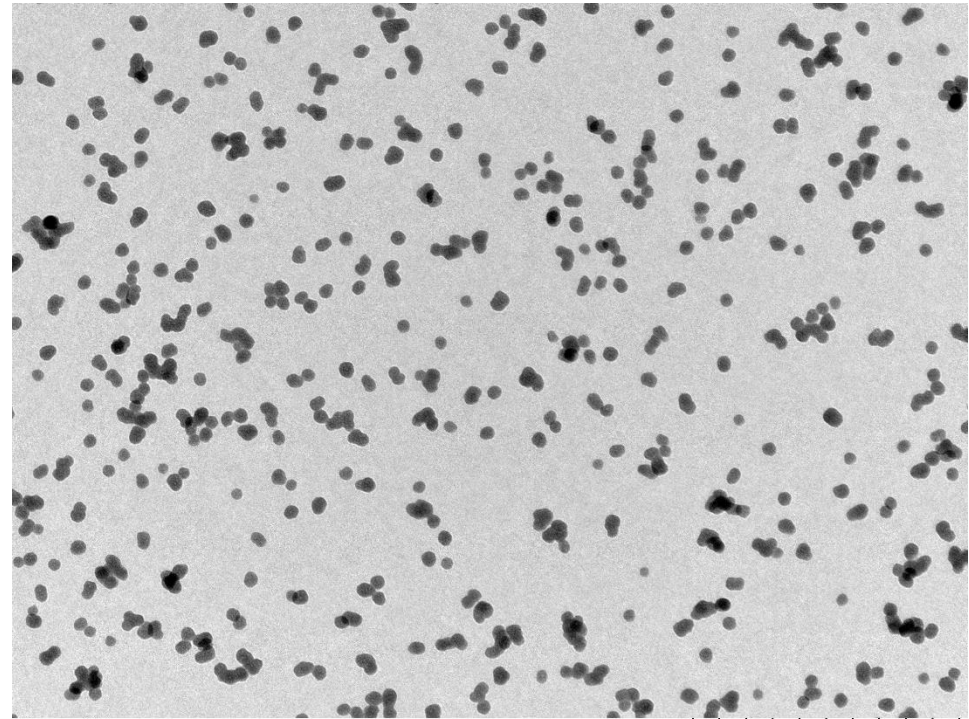
✘  Aggregated as drying on Cu grid



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Magnification=x10.0k

1.0µm

✔  Nanoparticles in liquid by K-kit



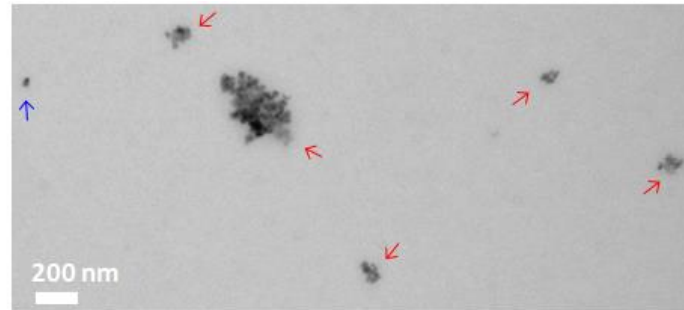
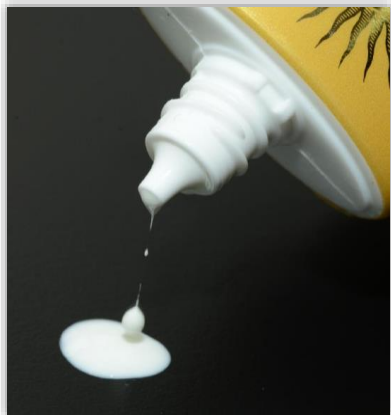
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1.0µm

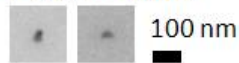
(Wet Mode/ gap 0.2 µm K-kit)

# Zinc Oxide Nanoparticles in Sunscreen Lotion

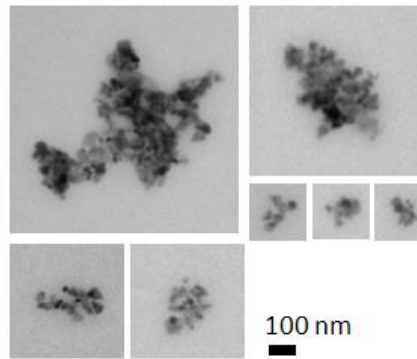
- K-kit can be used for characterizing NOAAs in cosmetics in final product forms.



□ Nano-objects



□ Aggregates/Agglomerates



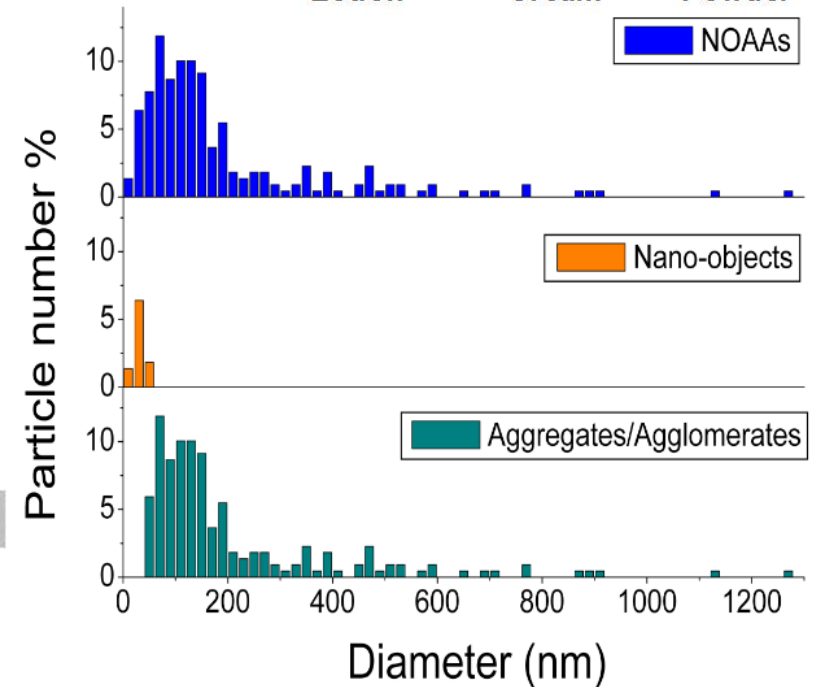
TEM images



Lotion

Cream

Powder



Size and size distribution

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

# K-kit Application



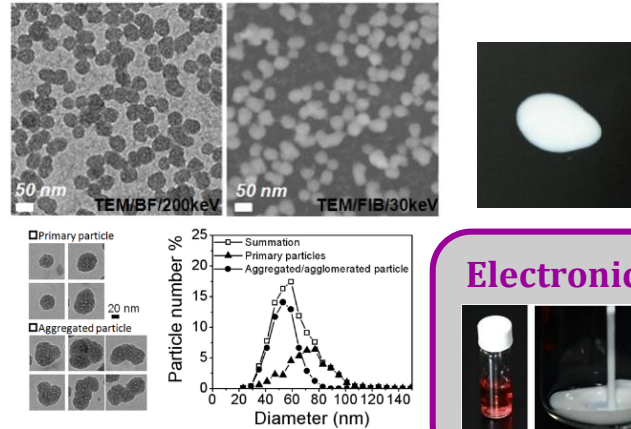
Disposable

Free of Cross Contamination

Easy Use

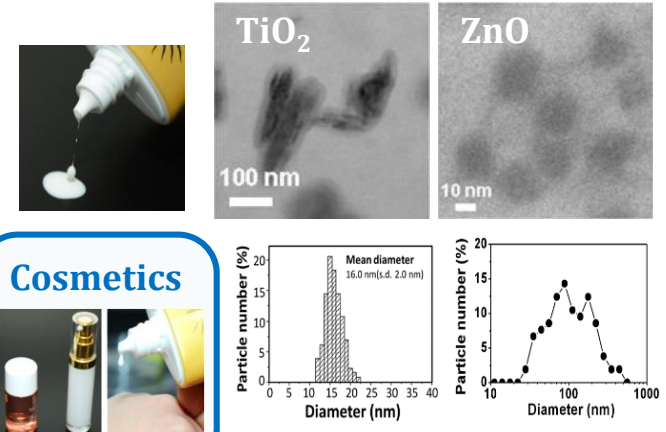
## Slurry

- SiO<sub>2</sub> Nanoparticles in CMP Slurry

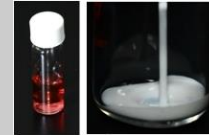


## Lotion

- TiO<sub>2</sub> and ZnO Nanoparticles in Sunscreen



## Electronics



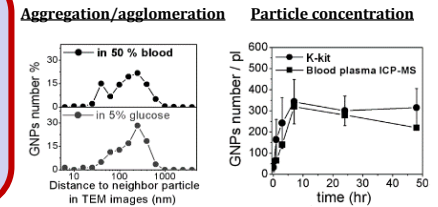
## Cosmetics



## Food

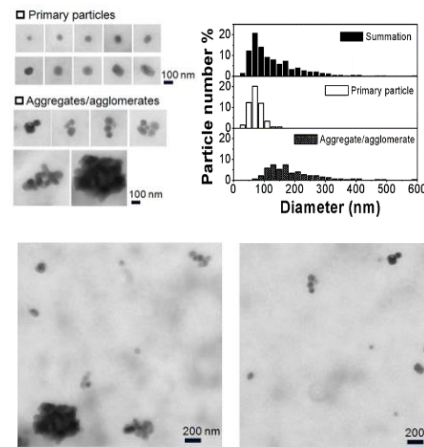


## Bio-Med



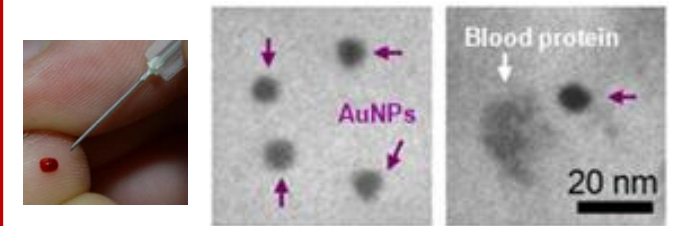
## Beverage

- CaCO<sub>3</sub> Nanoparticles in milk



## Bio Sample

- Au nanoparticles in blood



# The Latest News about K-kit in the Market

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 famous pharmaceutical company **SANOFI**

## Contribution of electron microscopy to industrial multimodal characterization of products and raw materials

M.L. Sgarra, S. Fayard, L. Petit, C. Girardon, C. Peyrot, F. Greco, A. Deliot, MC. Nicolai, F. Ronzon, S. Marco, H. Ponceblanc

Analytical Sciences, Sanofi Pasteur, Marcy L'Etoile and Neuville sur Saône, France.



### Background

Electron microscopy is used by the pharmaceutical industry for the characterization of products and raw materials at the level of:

- research and development
- product characterization
- quality control

This implies overcoming technological barriers for:

- the technical adjustment or developments of tailored tools
- the use of multimodal approaches
- the automatization of image acquisition, processing and analysis

### Tailored tools

#### Hydrated samples observation in TEM



Main challenge: to filling in the system with high reticulated filaments

Chosen solution: <http://www.biome-tek.com/oioms-tek/en/goods.php?act=view&no=22> distributed by <http://www.ifg-distribution.fr/>

#### TEM-MEB correlation

Single negatively stained grid of viral particles can be observed by both TEM and SEM

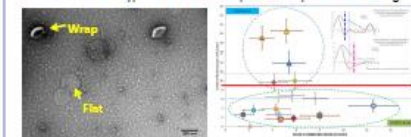


Support to transfer TEM grids to SEM for correlative microscopy

Chosen solution: <https://www.microtonano.com/EM-90-TEM-grid-holders-and-STEM-imaging-holders.php>

#### Software development

Identification of atypical and standard split viruses processed in ImageJ



In some cases requires standalone 21 CFR part 11 compliant software

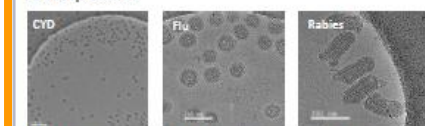
Chosen solution: VAS from VIRIONOVA <https://www.vironova.com/our-offering/vas/>

### Examples of applications

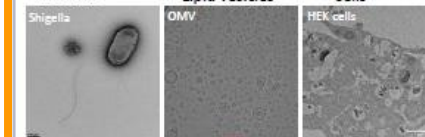
#### Protein complexes



#### Viral particles

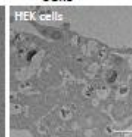


#### Bacteria

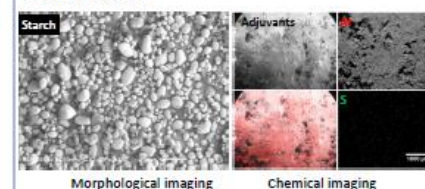


#### Lipid vesicles

#### Cells



#### Raw materials



### Challenges

- From subjective image interpretation to quantitative analysis
- From manual acquisition to automatized image recording
- Towards a GMP compatible approach

SANOFI PASTEUR



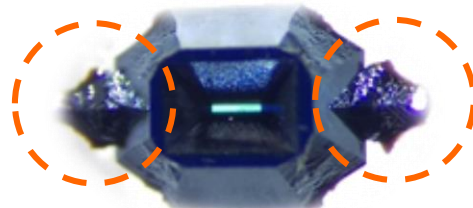
# Product Feature

# Quick and Easy Sample Preparation

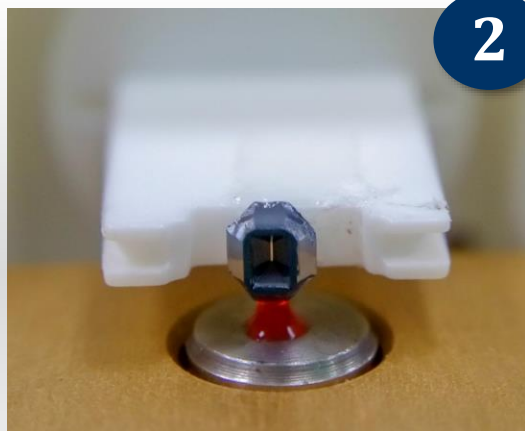


1

Channel tips

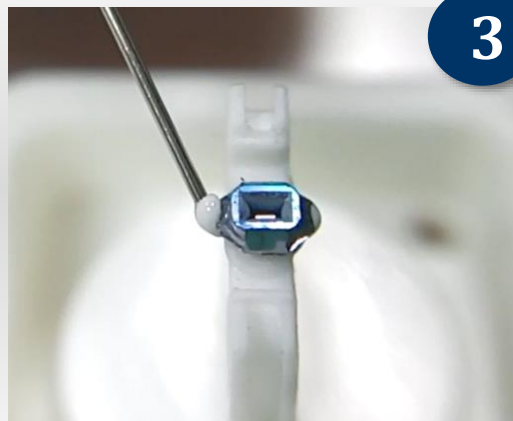


Remove the channel tips



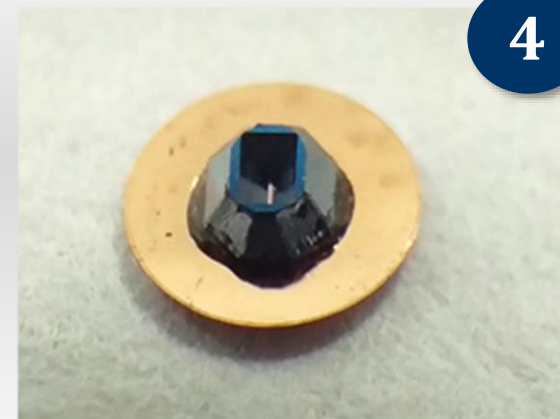
2

Liquid loading



3

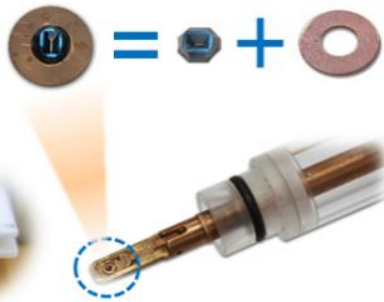
Gluing



4

Copper grid

# The Fastest Speed for Liquid-TEM Observation



**K-kit**



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

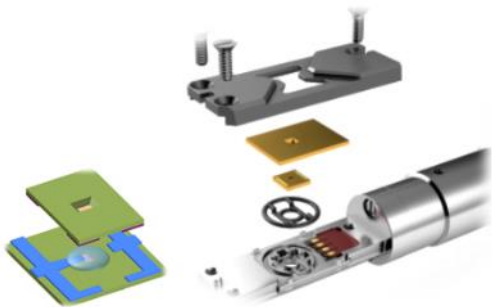
**90min required for 10 samples**

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



**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.

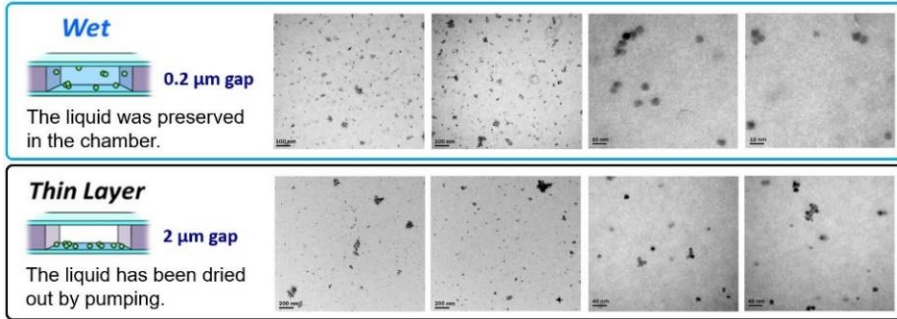
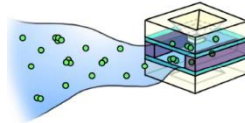


**Other Solutions**

# Functional Features

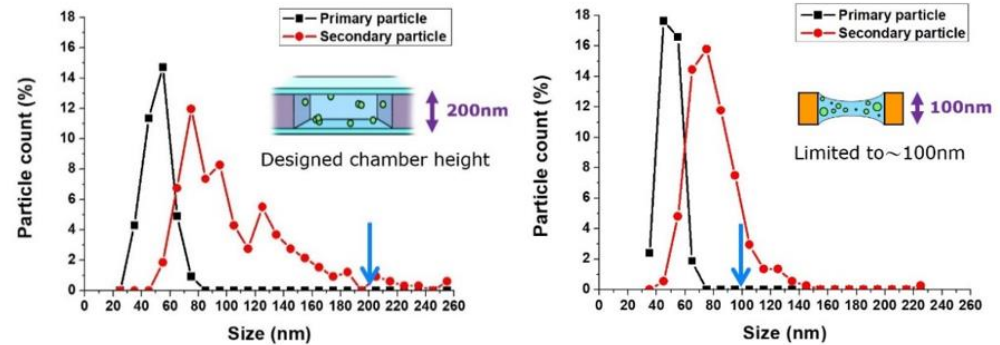
## 1. Native State in Liquid

- QDs particles in chloroform



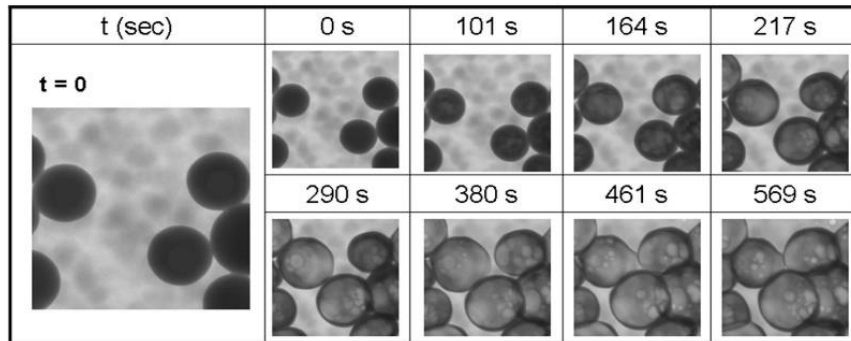
## 2. Quantitative Analysis

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



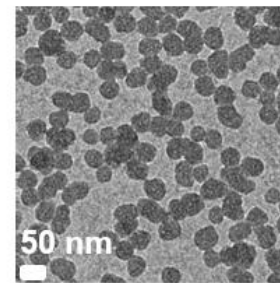
## 3. In-situ Observation

- Dynamic observation of silicate particles

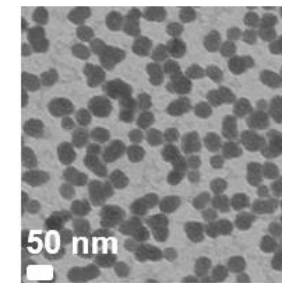


## 4. Compatible to Versatile Microscopy Analyses

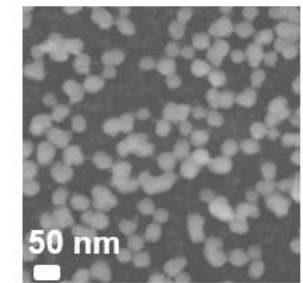
- Applicable to TEM, FIB, and STEM



FEI-TEM @200Kev

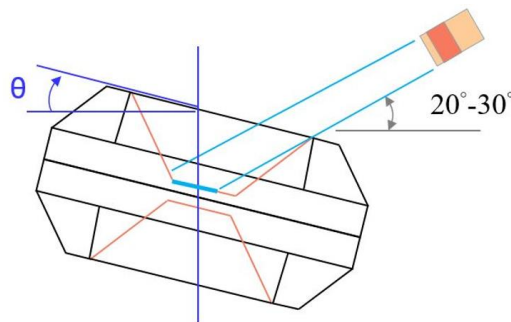


Hitachi-TEM @100Kev



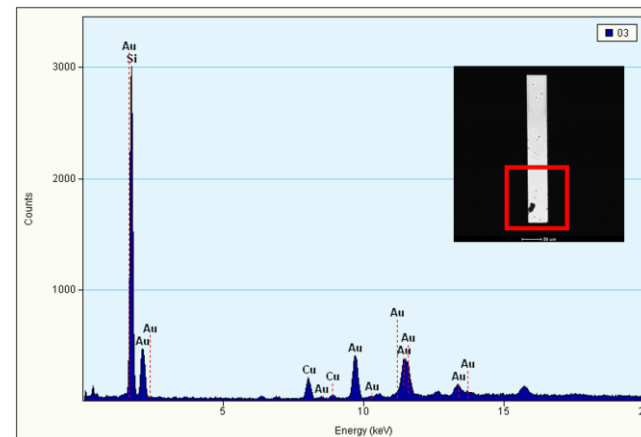
FEI-STEM @30Kev

# Available for SEM & EDX Analyses

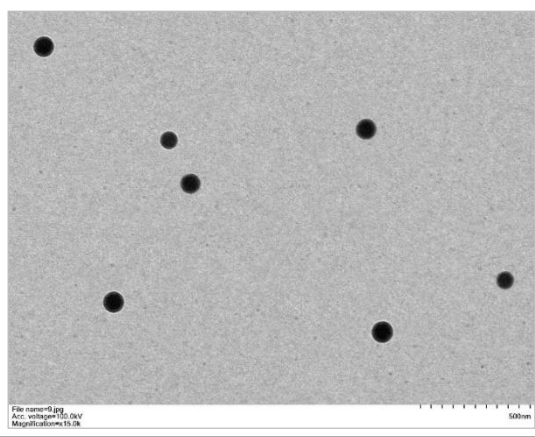


For EDX analysis, it needs to give a tilt toward the detector

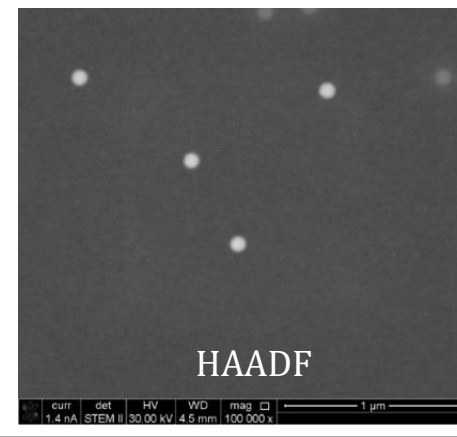
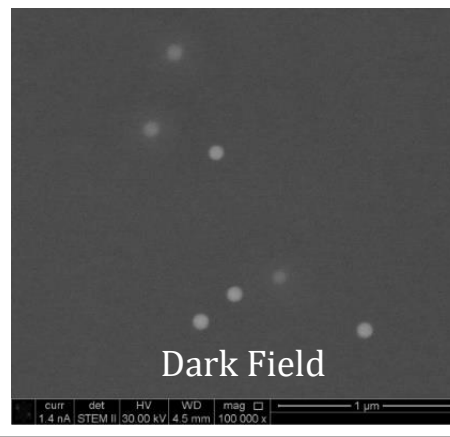
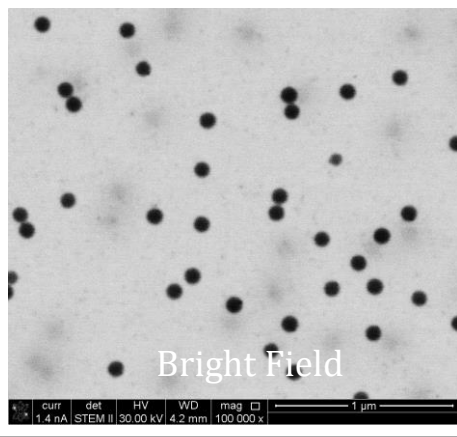
## K-kit with Au particles



### TEM by Hitachi HT7700



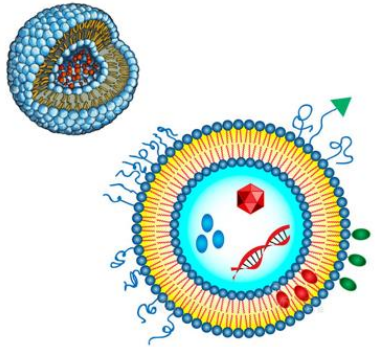
### SEM/ STEM by FEI Helios 400



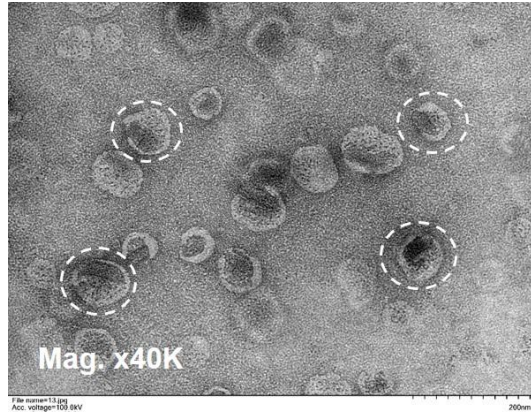
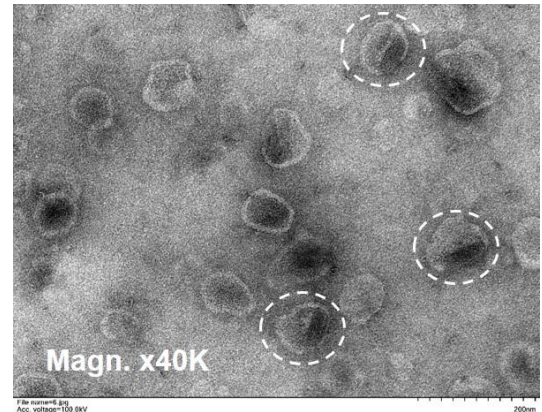
# Example Concept for K-kit Application (1)

## □ The applications by multiple loadings of K-kit

1

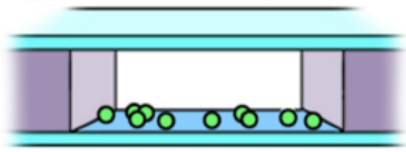


1<sup>st</sup> loading with liquid A  
(Such as liposomes/ LDL)



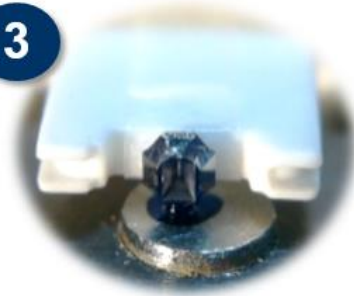
(ex. Liposomes with negative staining treated)

2



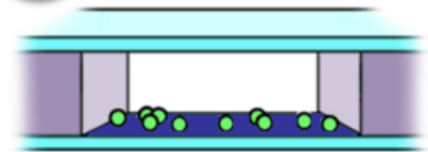
The K-kit prepared in  
dried mode for liquid A

3



2<sup>nd</sup> loading with liquid B  
(Such like staining solution)

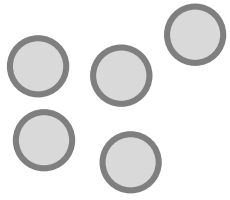
4



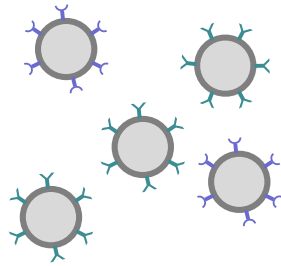
The K-kit in dried mode  
again for liquid B

## Example Concept for K-kit Application (2)

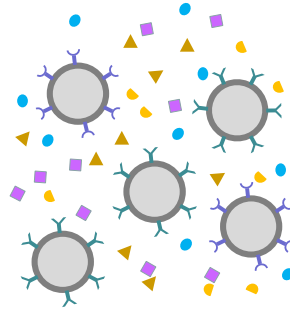
### □ Rapid and selective detection of pathogens by K-kit



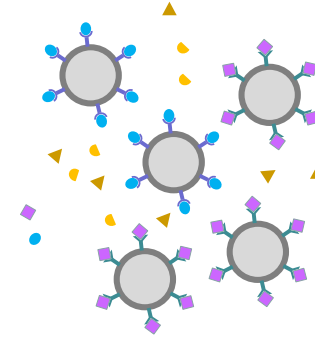
1. Nanoparticles  
(Polystyrene or Au)



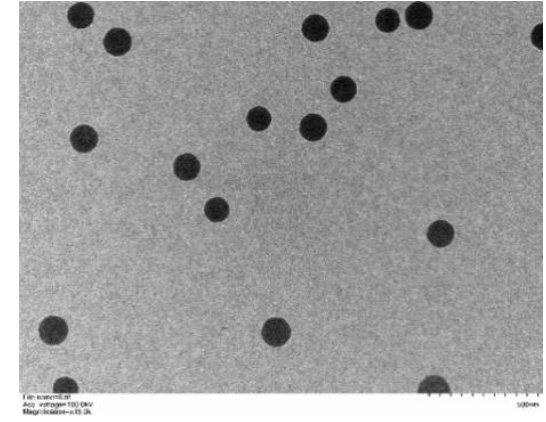
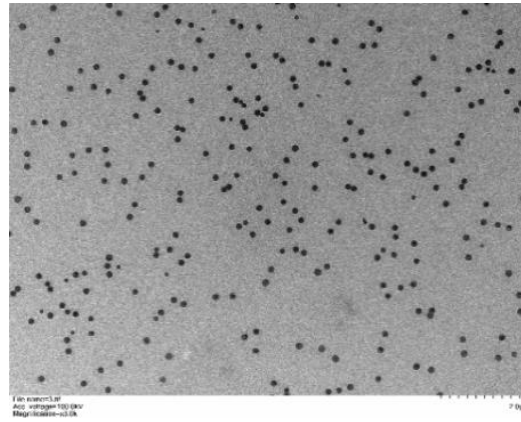
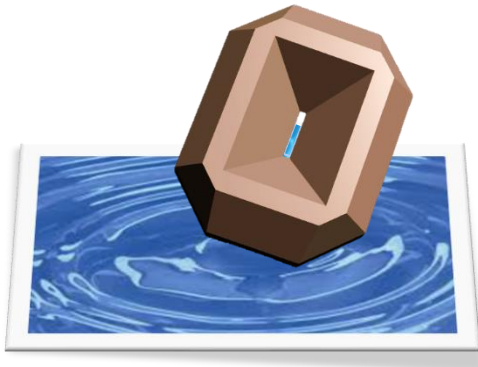
2. Coating with  
different antibodies



3. Mixed with  
multiple antigens

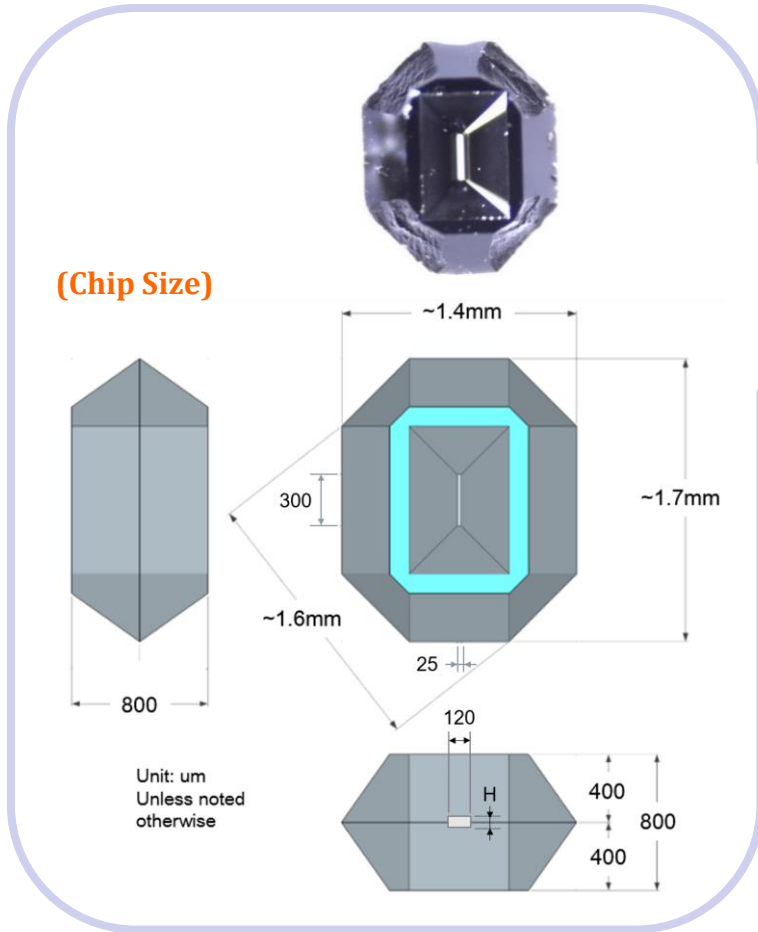


4. To observe the captured  
antigens on the beads by K-kit

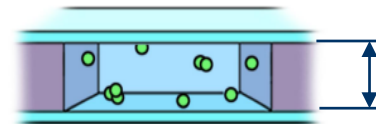


The polystyrene beads in K-kit can be clearly observed by TEM.

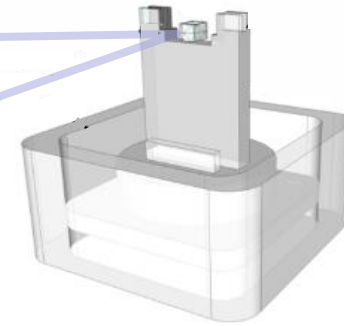
# K-kit Size and Shipping Package



- Window Length  $300 \mu\text{m}$ , Width  $25 \mu\text{m}$
- Channel Height (H):  
**0.2 and 2.0 standard;** 0.1, 0.5, 1.0 and 5.0 available



$H = 0.2, 0.5, 1, 2, 5 (\mu\text{m})$



**K-kit**



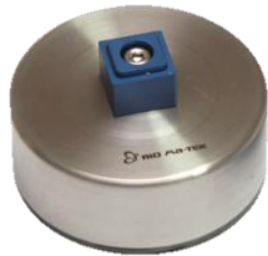
**K-kit Carrier**



**Shipping Packages**



# K-kit Tool Box for Sample Preparation



Gluing stand



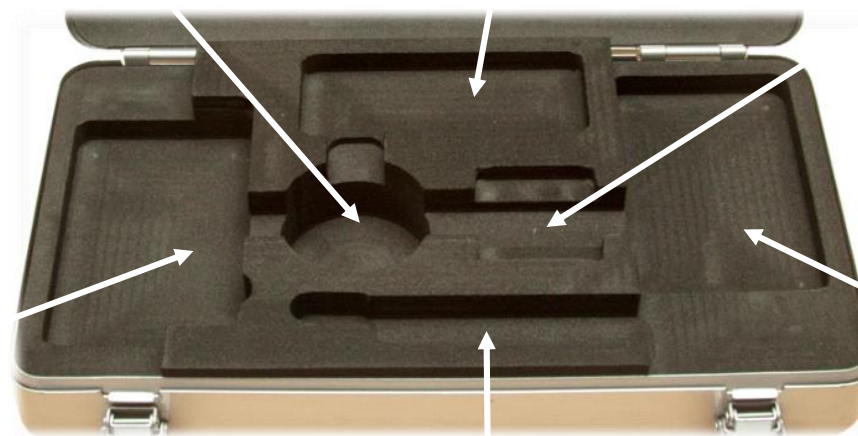
Glass-slide pack



Sample-loading stage



Accessory box



K-kit holder & needle pen



Shipping package

# K-kit Distributors Worldwide



# Conclusion

1. K-kit is a sample holder designed to facilitate convenient TEM observation of liquid samples, allowing nanoobjects, 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 of the brand names such like FEI, JEOL and Hitachi etc.
3. K-kit can be the fastest and easiest solution on the market for liquid-TEM application. It's suitable for the nanomaterial researches that with multiple test conditions and industrial applications such like IQC for CMP slurry or CMC in Nanopharmaceuticals etc. especially.
4. We have a full product plan of K-kit for the future; many exciting possibilities ahead!

**Thank you!**

