K-kit Applications for Biotechnology & Nanopharmaceuticals

MA-tek
The Best R&D Partner

www.bioma-tek.com
chrischen@ma-tek.com

OUTLINE

- EM-based imaging for pharma & CRO
- K-kit for biotechnology & pharmaceuticals
- What is K-kit
- Conclusion

EM-based imaging for pharma & CRO

Drug discovery and development

- Candidate drug screening
- Structures and sizes
- Stability test
- Pre-clinical study

Drug Manufacture (CMC)

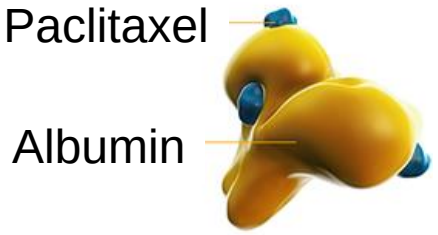
- Raw material IQC
- Drug homogeneity
- Uniformity between batches
- Process monitoring

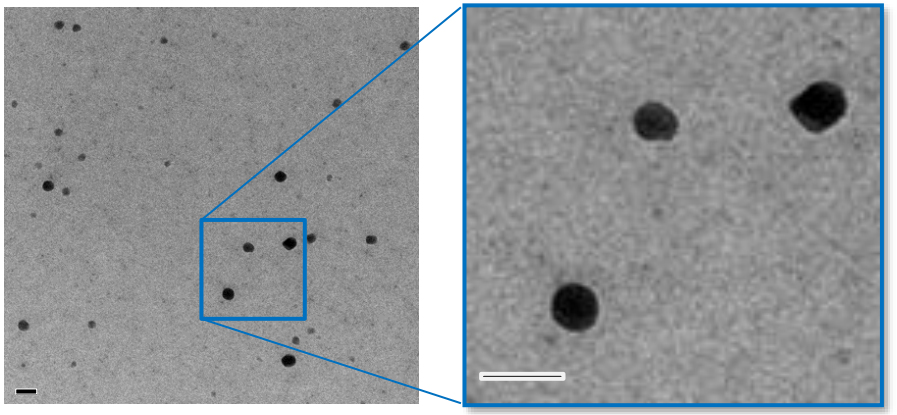
Pharmacokinetic studies (PK)

- In vitro stability
- Bioavailability
- Sizes and concentrations
- Drug Interaction
- Excipient effect

Early-phase clinical studies

Protein particles in Abraxane®

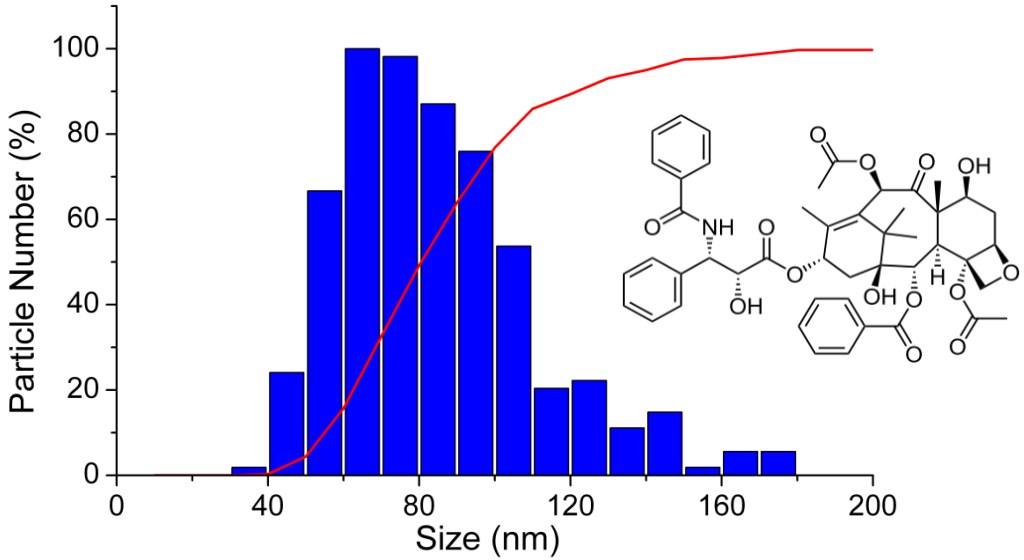




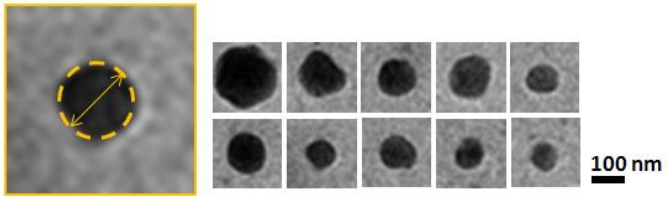
(TEM images by K-kit)

** Scale bar: 200 nm*

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



Size/size distribution (D10 , D50 , D90)

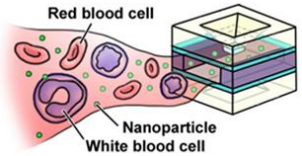


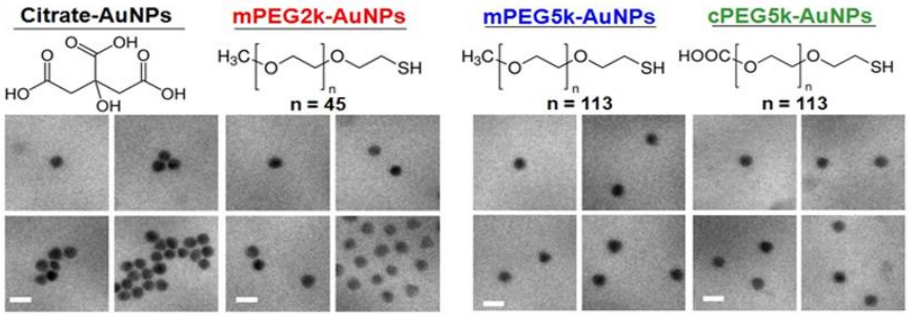
Parameter	Size (nm)
D 10	55.6
D 50	80.1
D 90	122.2
Span: $(D_{90} - D_{10}) / D_{50}$	0.831

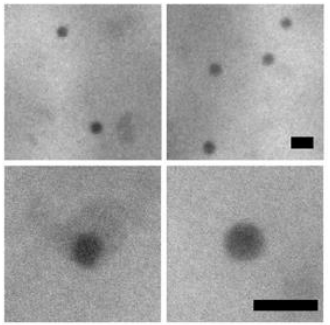
NOAAs of Au Nanoparticles (NPs) in Blood

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

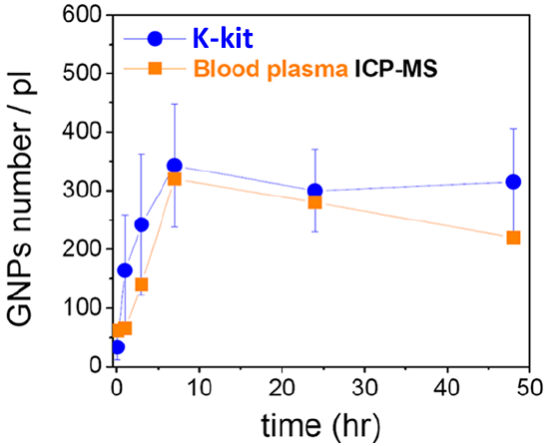
(Tai etc. Anal. Chem. 2012, 84: 6312-6316)

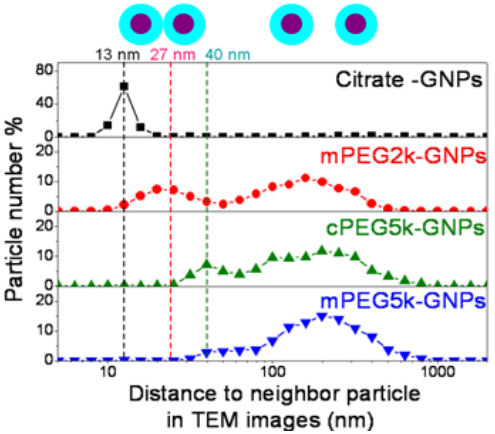


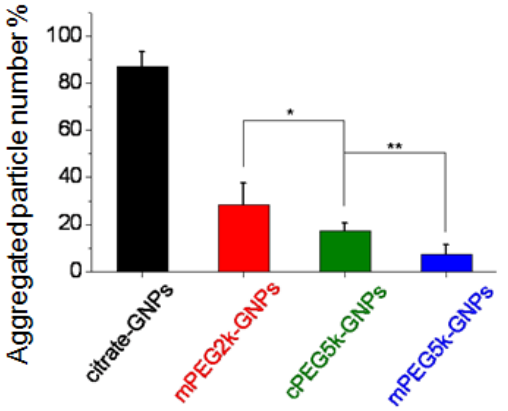




* Scale bar: 20nm







Statistic analysis of Aggregation and agglomeration of Au NPs in blood

K-kit can be used to analyze physicochemical characteristics of NPs in blood by TEM.

K-kit for biotechnology & pharmaceuticals

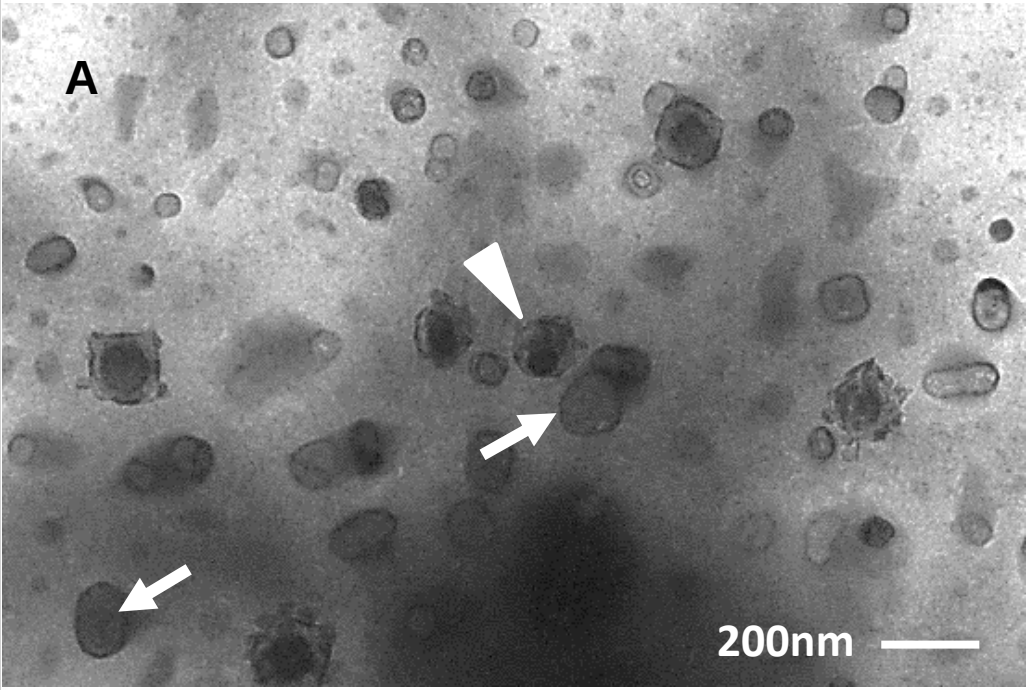
Does ANDA ≈ RLD?

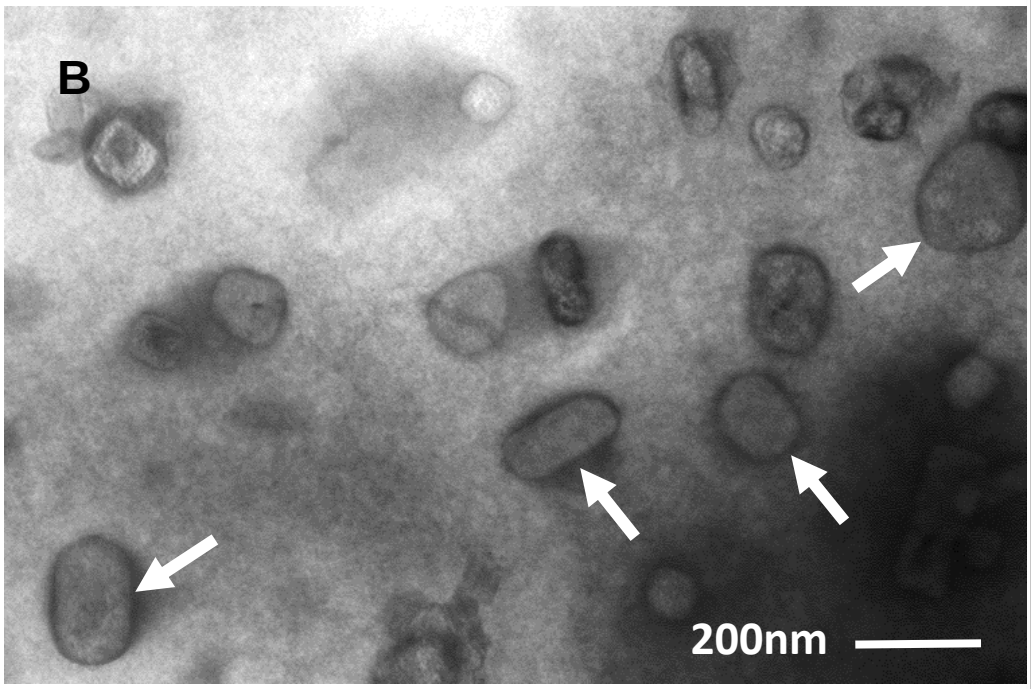
Observing the morphology and size distribution by electron microscopy

What we can do by using K-kit:

- In-situ EM Imaging of biological specimens and Nanopharmaceuticals in aqueous solutions
- Immunoelectron microscopy to identify nanogranules in liquid
- Image-based statistic analysis allowing nanoobjects, aggregates, and agglomerates (NOAAs) in aqueous condition to be characterized

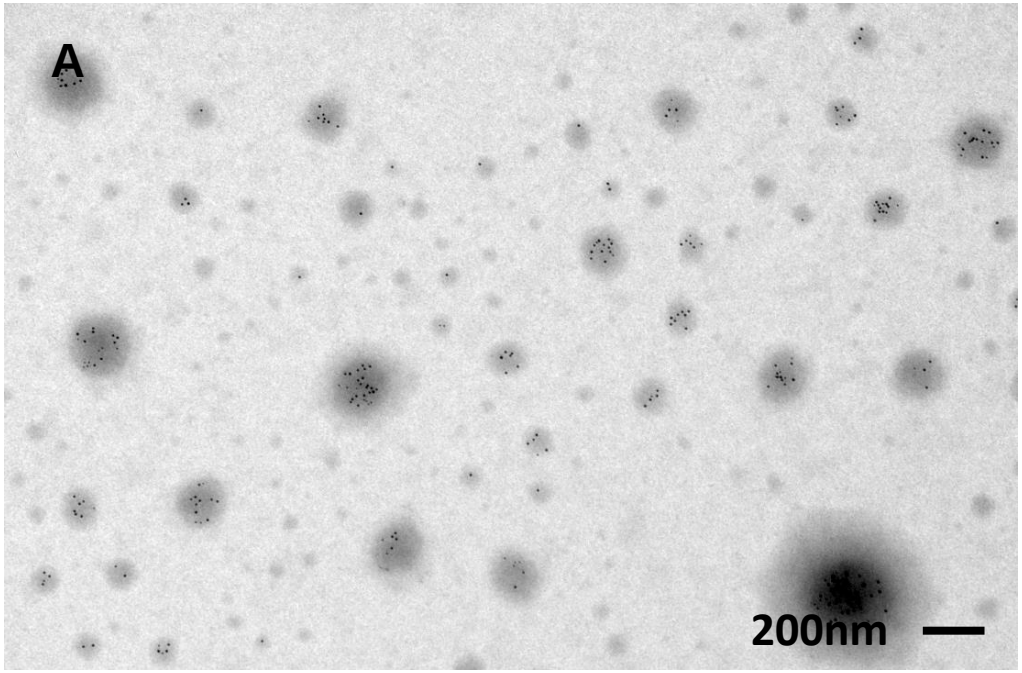
Isolated platelet granules in K-kit

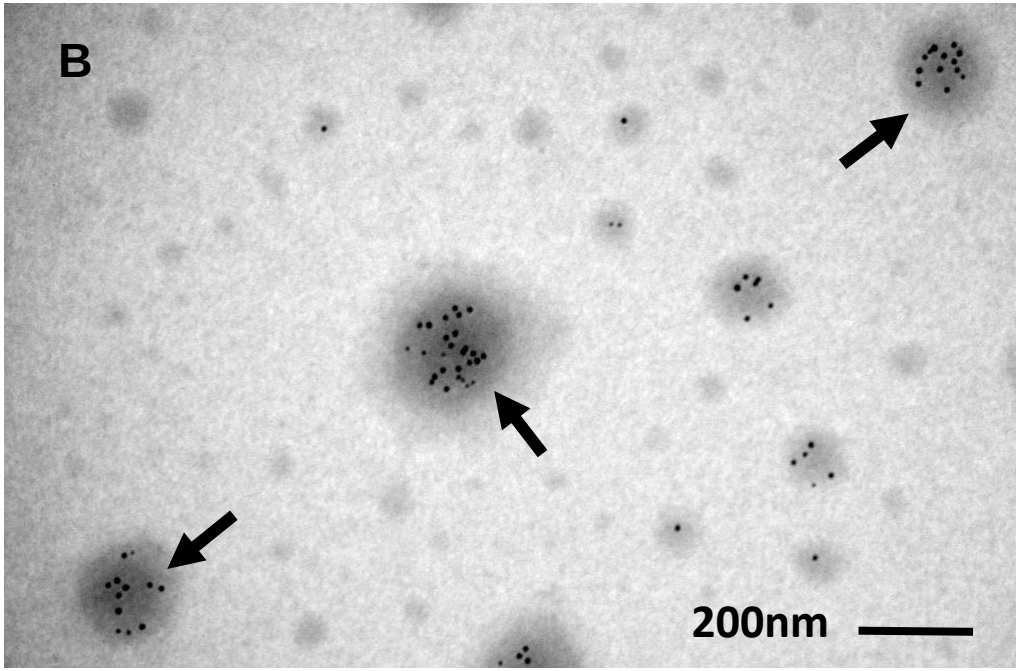




(A) α-granules are large and round or oval in shape (indicated by white arrows). A dense granule with a high-density core was also identified (by a white arrowhead). The small circular vesicles with low electron density are lysosomes or exosomes. Some broken granules could also be observed. (B) The α-granules (by white arrows) could be identified with a long axis length of about 200 nm.

Immunoelectron micrographs of isolated platelet granules

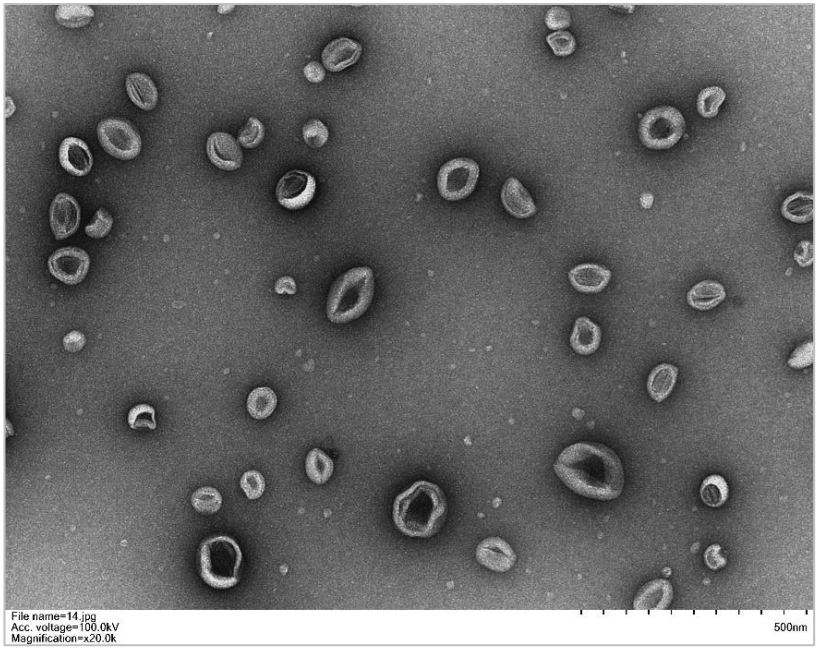




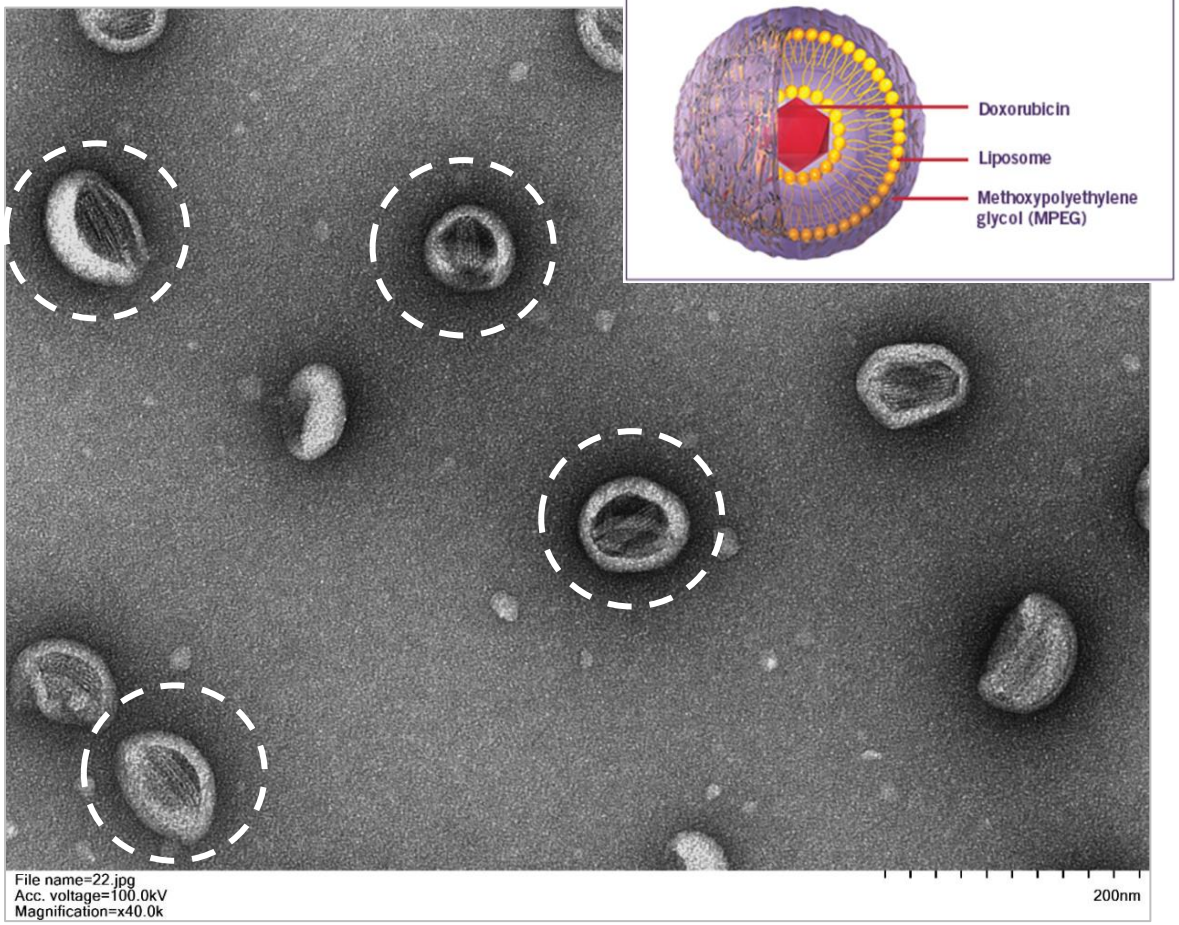
(A) This picture shows that the 6-nm gold particle-labeled granules are α-granules. The other vesicles not significantly labeled by gold particles might be lysosomes or exosomes. (B) There are abundant gold particles located on the surface of α-granules (indicated by arrows).

(Appl. Sci. 2020, 10, 4946; doi:10.3390/app10144946)

Drug crystals of Doxorubicin in liposomes

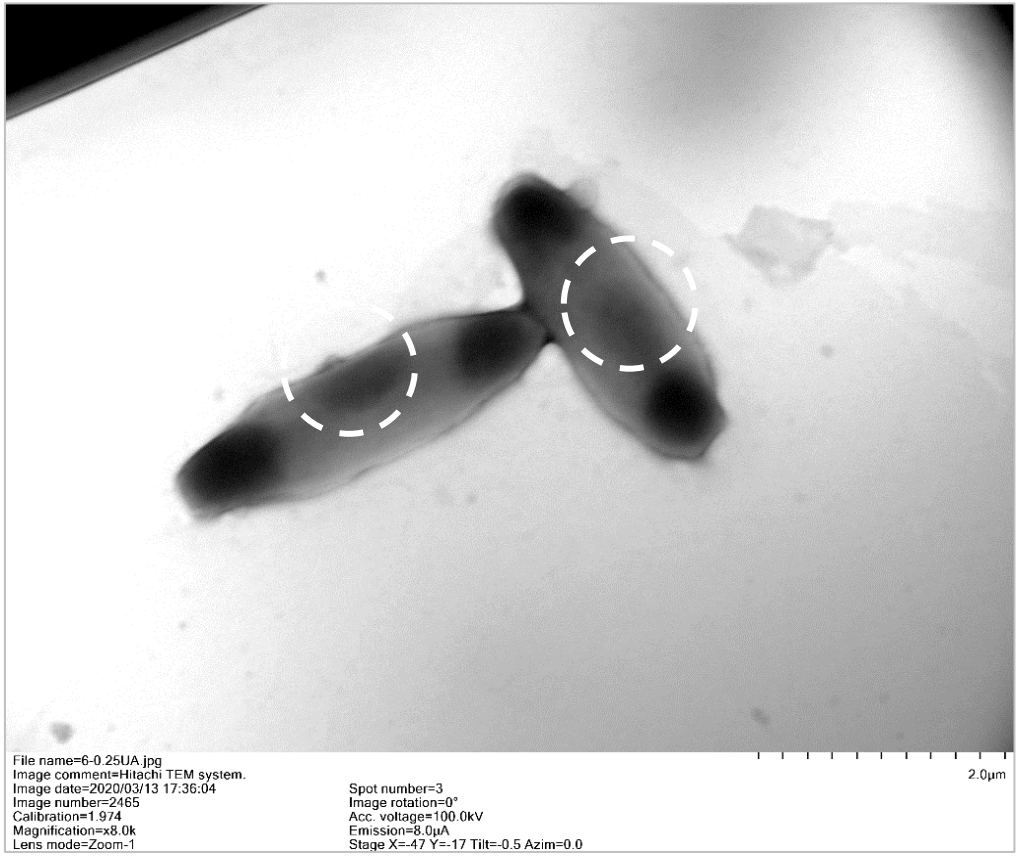


(with negative staining)

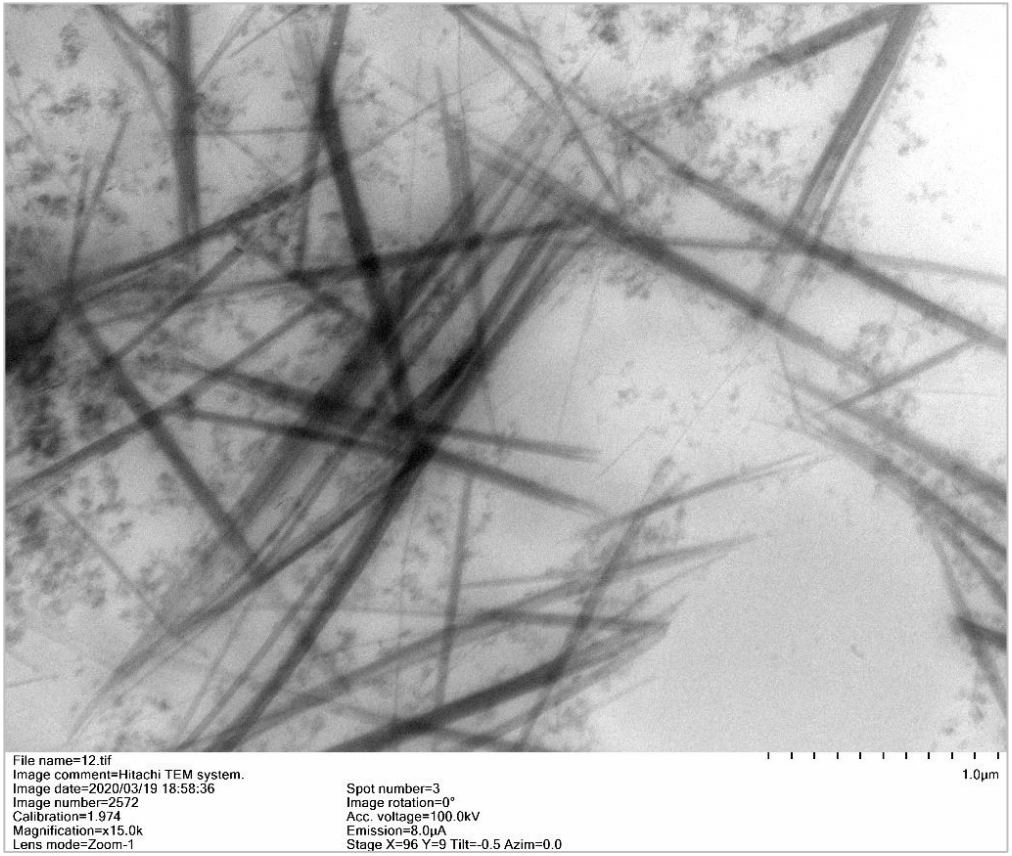


The enclosed drug crystals in liposomes can be well identified with K-kit.

Biological specimens observed with K-kit



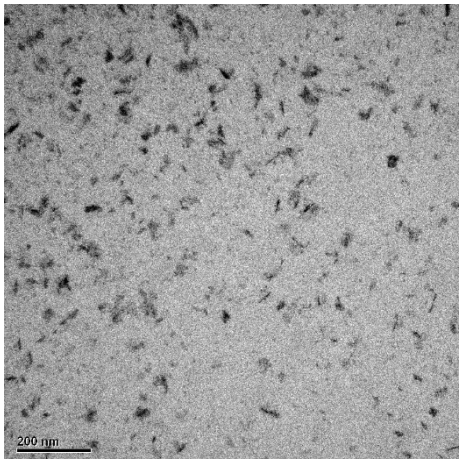
The nucleoid of E.coli

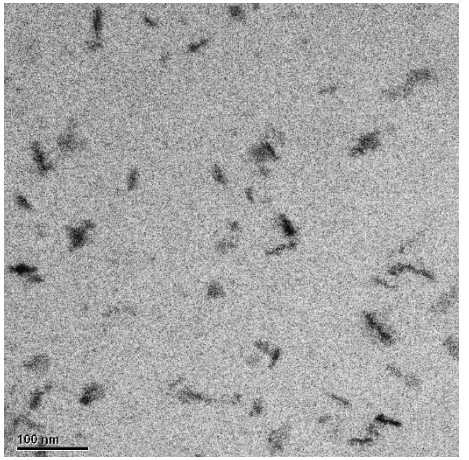


The nanofiber structures of collagen

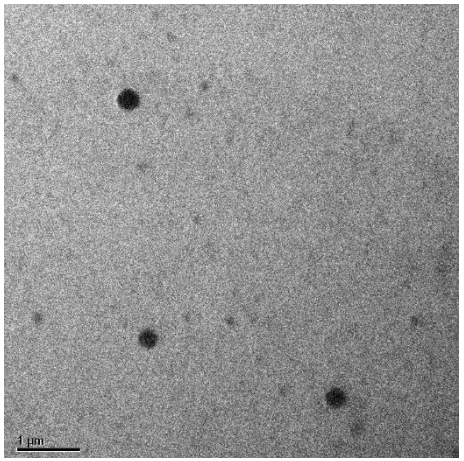
Nanopharmaceuticals observed with K-kit

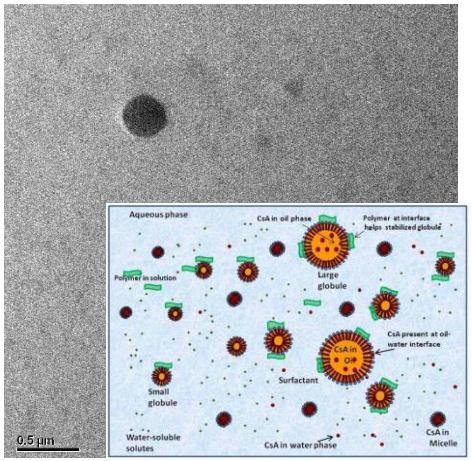
Resovist®, which a human used MRI T2 contrast agent with iron oxide nanoparticles in the solution





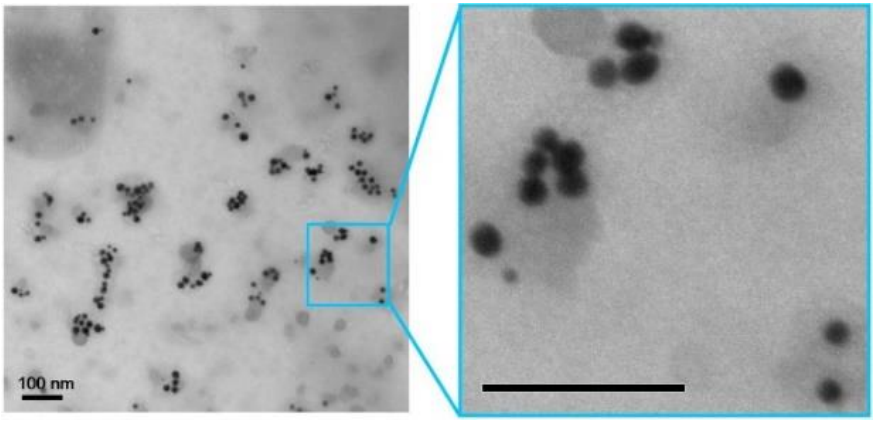
Restasis®, cyclosporine ophthalmic emulsion



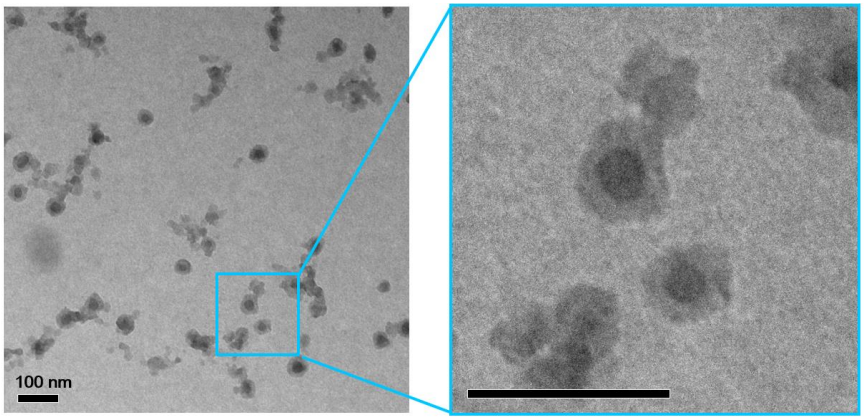


Suitable for most Nanopharmaceuticals

- 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.

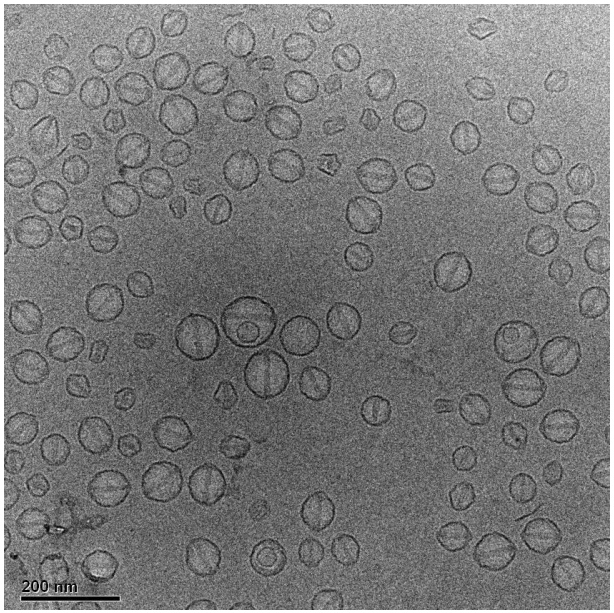


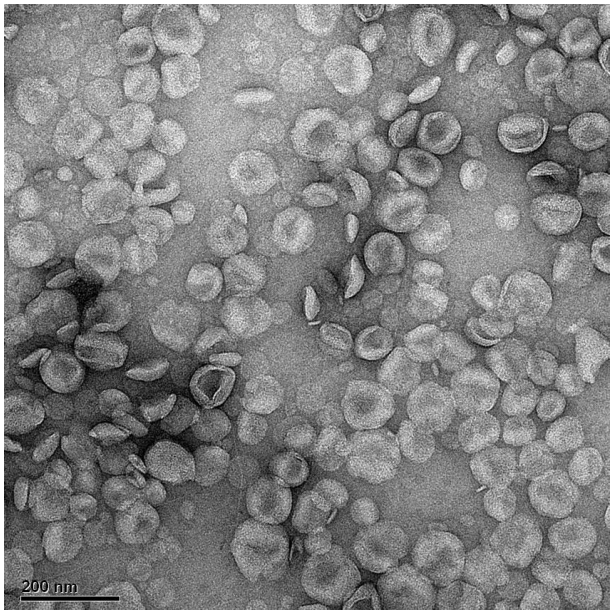
Oil emulsion in water was loaded and sealed in a K-kit in liquid form.

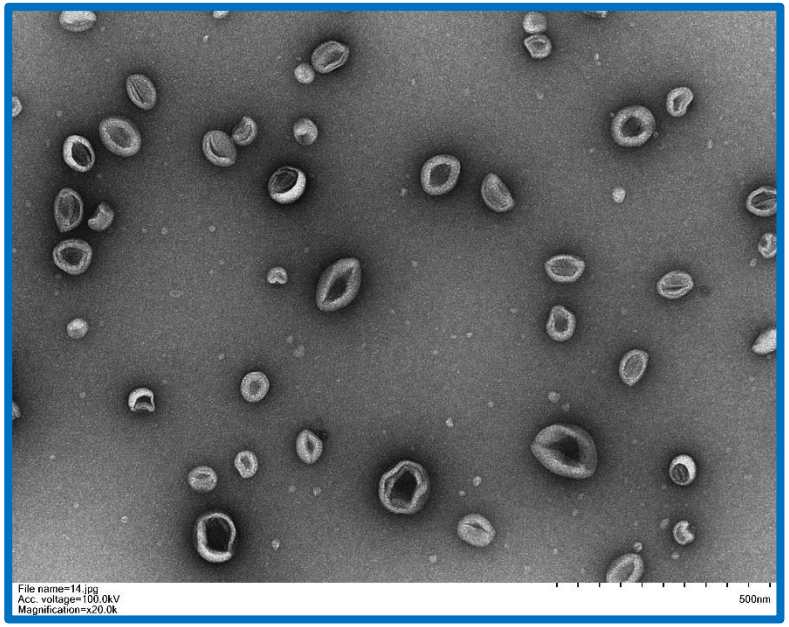
Brand Name of Pharmaceuticals	**Doxil ® (1995 approved)**	**Abraxane ® (2005 approved)**	**Aurimune ® (Phase II)**	**Resovist ®**	**Rexin-G ® (Phase II)**
Particle Size	80-100 nm	~ 130 nm	~ 27 nm (AuNPs core), ~ 30-40 nm as hydrated	~ 45-60 nm (Hydradynamic diameter)	~ 100 nm
Particle Concentrations	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

Available for low-contrast sample observation

TEM images of liposomes in Doxil®







Cryo-TEM (In formula)

- **High cost & difficult**
- **Cryo-holder required**
- **Low success rate**

On Cu grid (With N.S.)

- **Low cost & easy**
- **At dry state**
- **Serious aggregates**

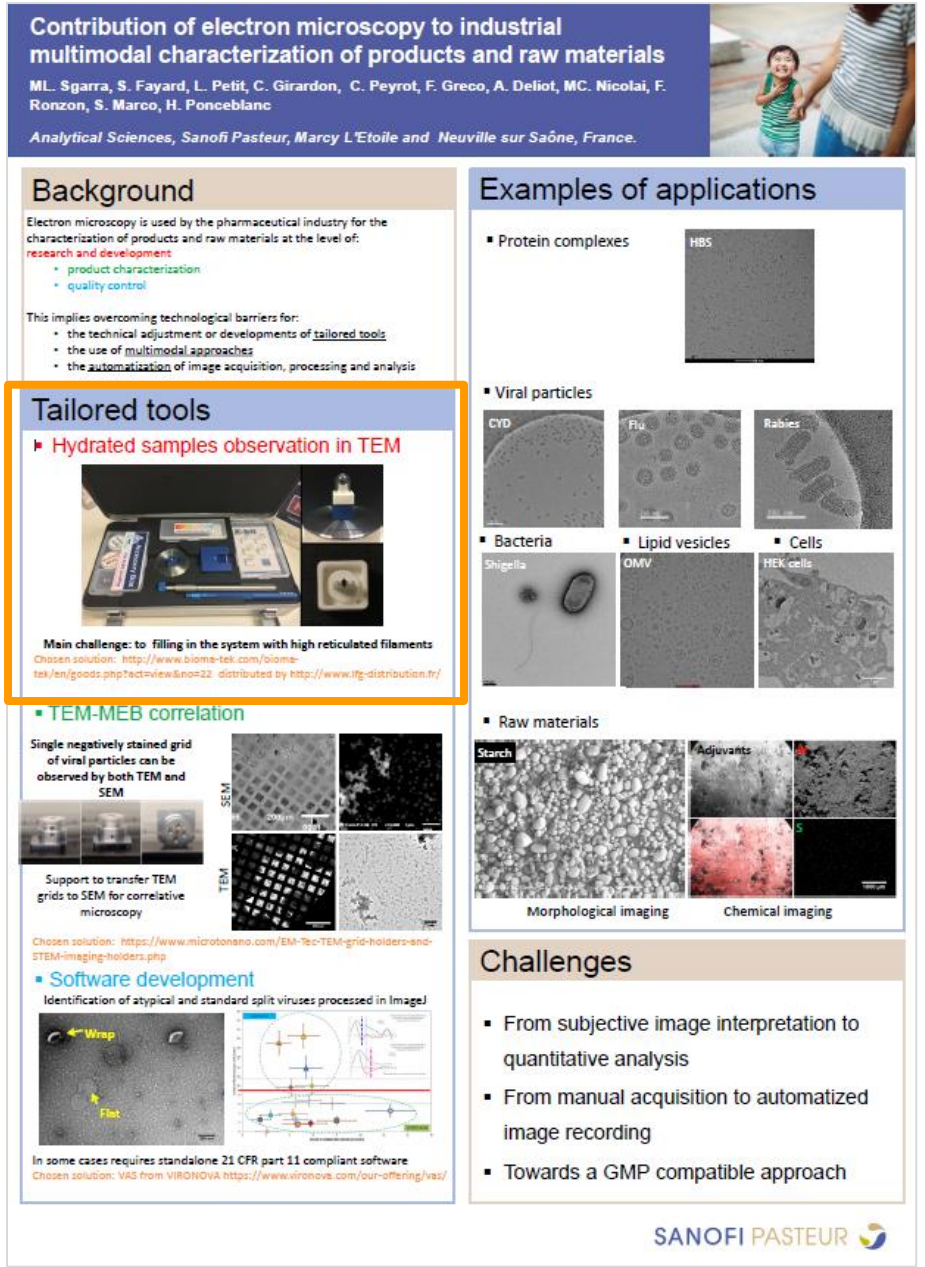
In K-kit (With N.S.)

- **Low cost & easy**
- **In-situ liquid state**

(N.S. - Negative Staining)

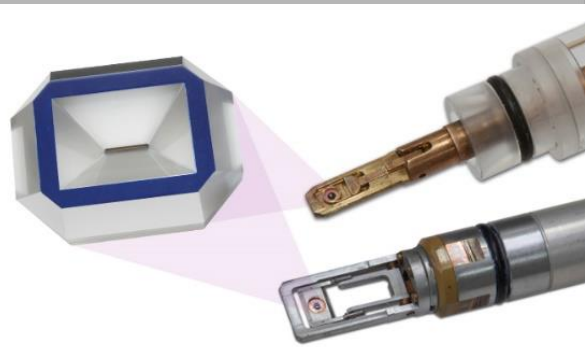
The latest news about K-kit

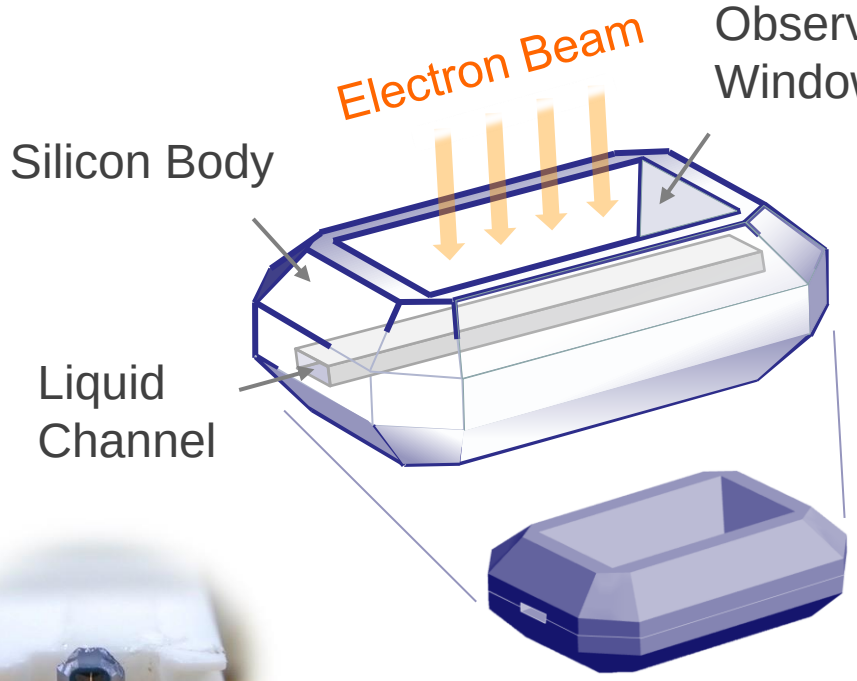
The poster on right side was published in June at RCCM Banyuls 2019 meeting in France; K-kit has been considered as an EM-based imaging solution for new drug development by the famous pharmaceutical company **SANOFI.**

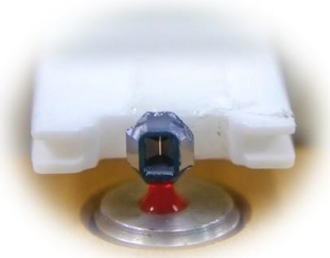


What is K-kit ?

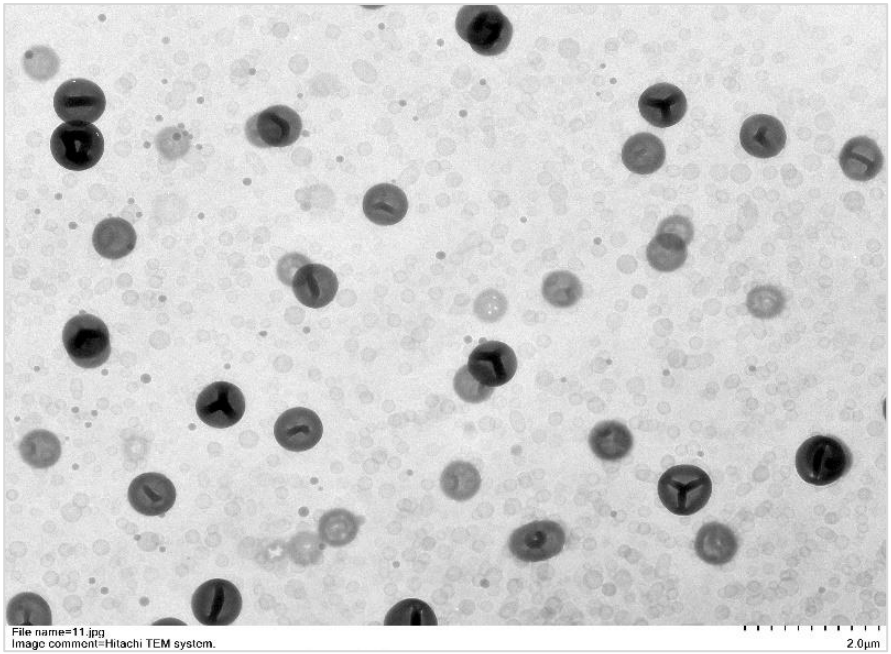
A disposable device for liquid sample analysis in TEM, SEM and FIB, free of cross contamination issue.







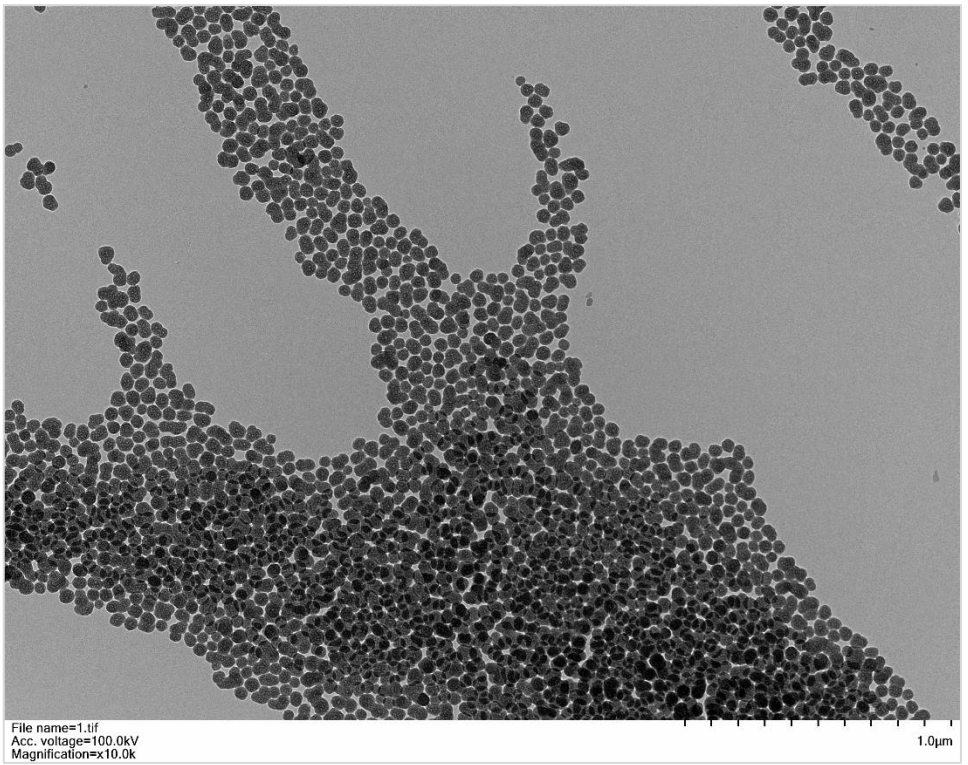
Liquid loading by capillary action



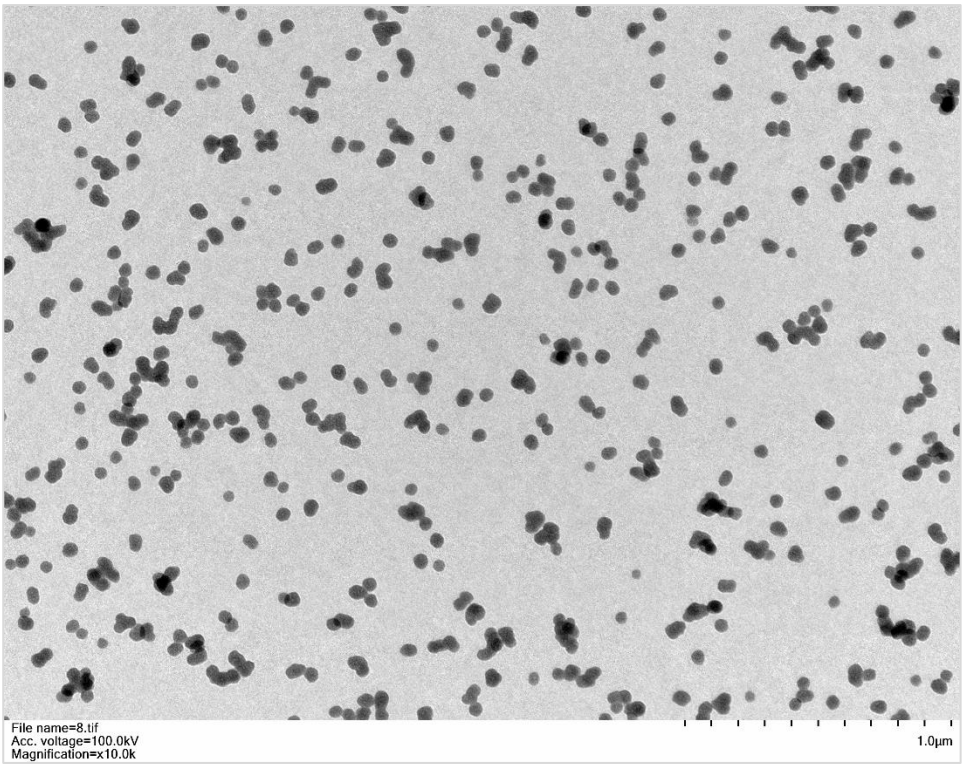
Extracellular vesicles of platelets (With negative staining in K-kit)

Imaging at original liquid state

✖ Aggregated as drying on Cu grid



✔ Nanoparticles in liquid by K-kit

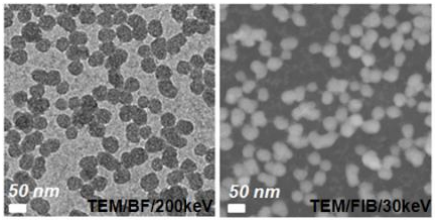


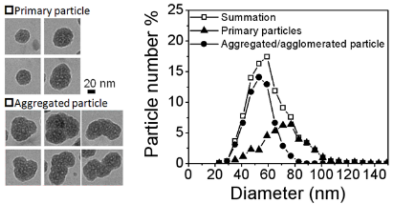
By using K-kit, one can observe the particles that distributed uniformly in liquid, preventing the nano particles from dry-induced aggregation and agglomeration.

K-kit Application

Slurry

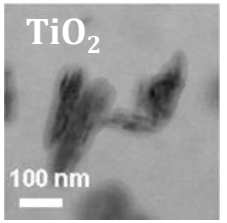
- SiO_2 Nanoparticles in CMP Slurry

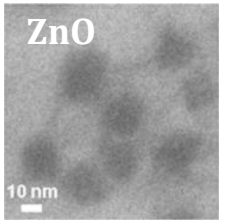


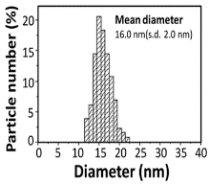


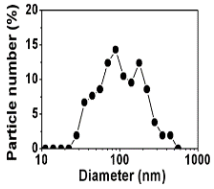
Lotion

- TiO_2 and ZnO Nanoparticles in Sunscreen







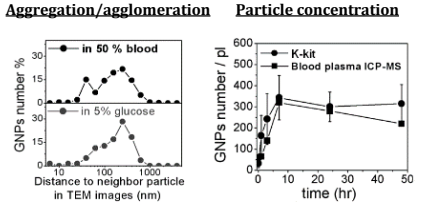


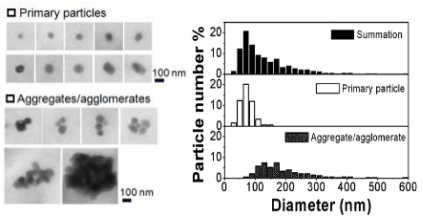
Electronics

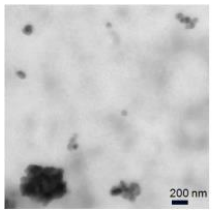
Cosmetics

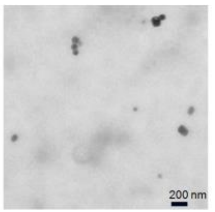
Food

Bio-Med



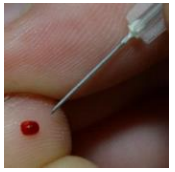


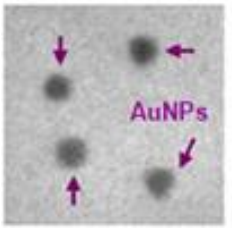


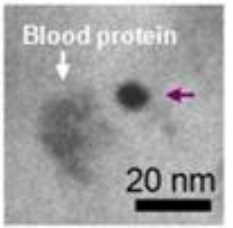


Beverage

- $CaCO_3$ Nanoparticles in Milk







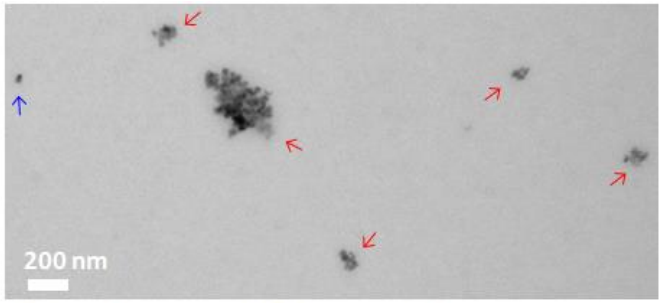
Bio Sample

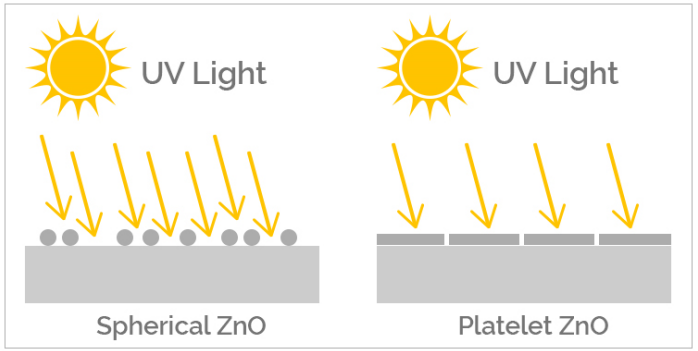
- Au Nanoparticles in Blood

(Example) NOAAs of ZnO NPs in sunscreen

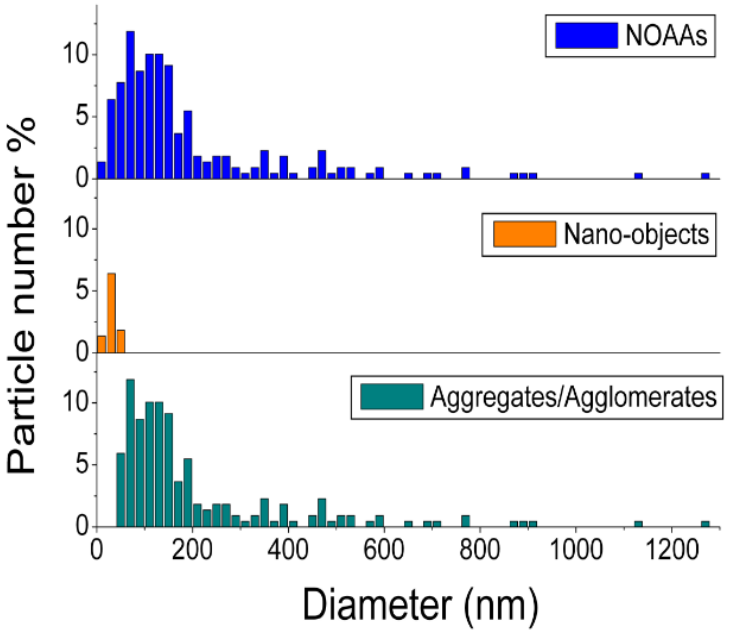
Lotion

ZnO NOAAs in sunscreen





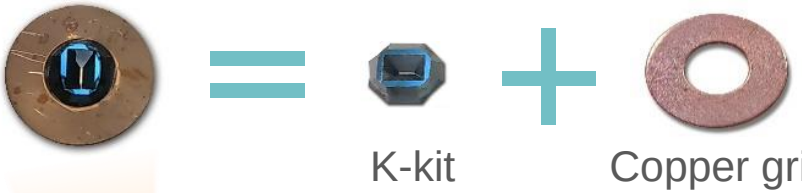
TEM images



Size and size distribution

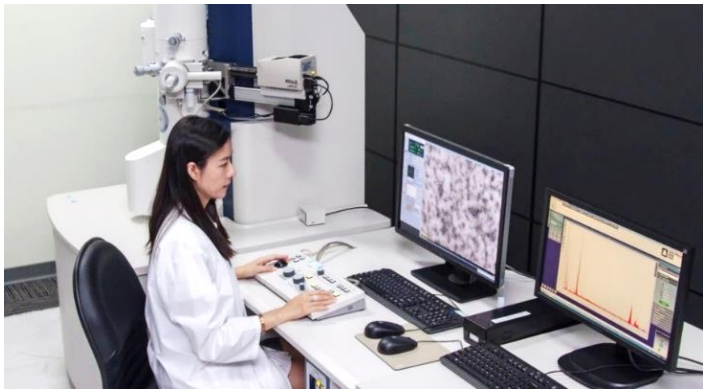
K-kit can be used for characterizing NOAAs of cosmetics in final product forms, to assess the safety risks of nanomaterials in cosmetic ingredients.

Quick, easy, and budget friendly



K-kit Copper grid

(Compatible with all kinds of TEM holders)



Hitachi
H-7501 SS holder

JEOL
EM31640 STHB holder

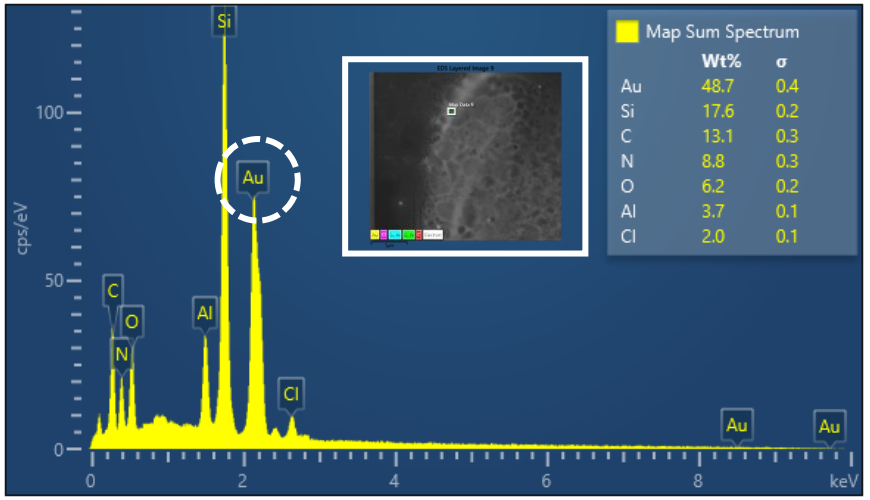
FEI
TECNAI F20 holder

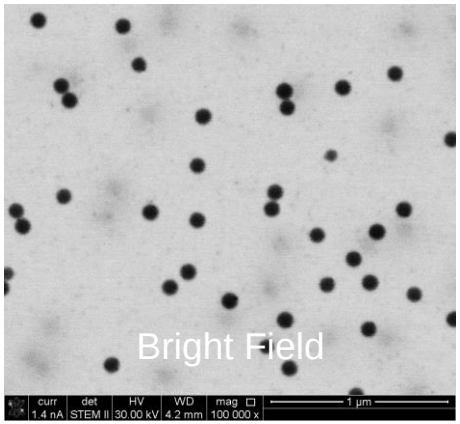
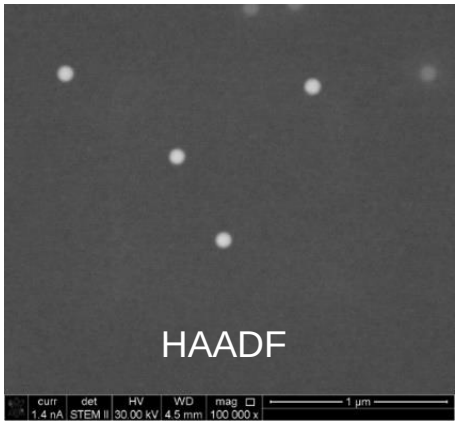
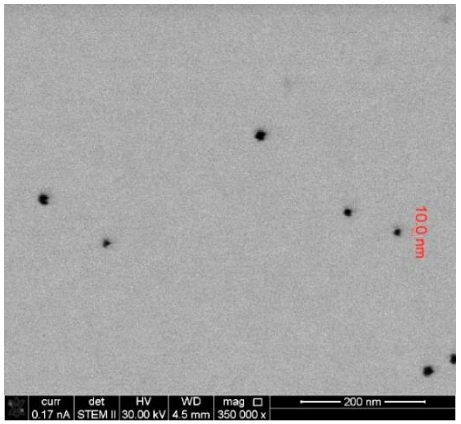
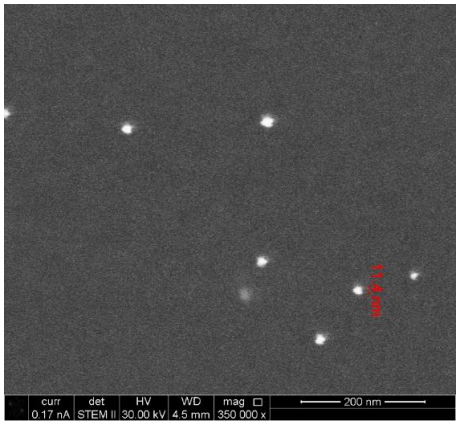
Gatan
CT3500HT holder

K-kit is disposable and with a vacuum-encapsulated channel structure so that no any cross-contamination risk and no need to do further cleaning before the use.

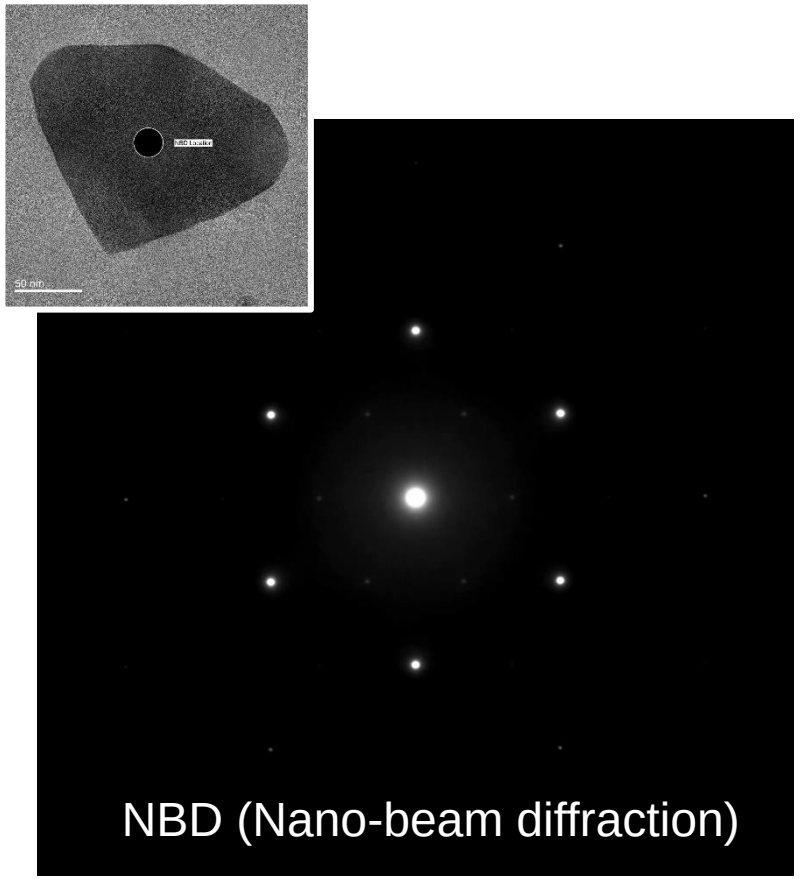
Available for SEM and EDX analysis

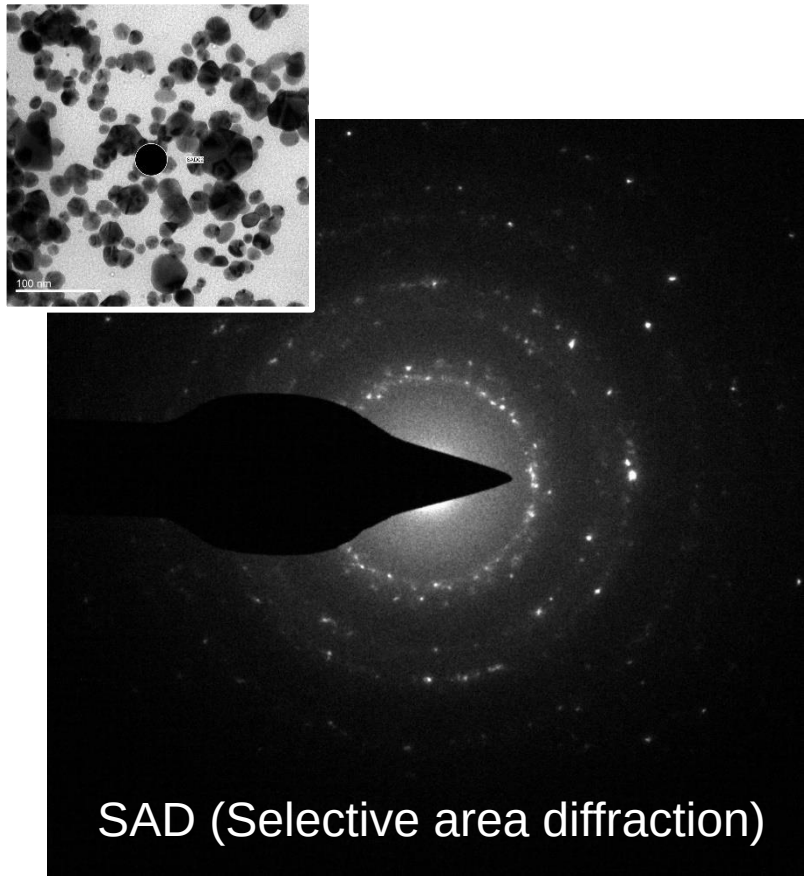
SEM/ STEM by FEI Helios 400



100nm Polystyrene beads	10nm Au nanoparticles
 	 

Available for TEM diffraction analysis

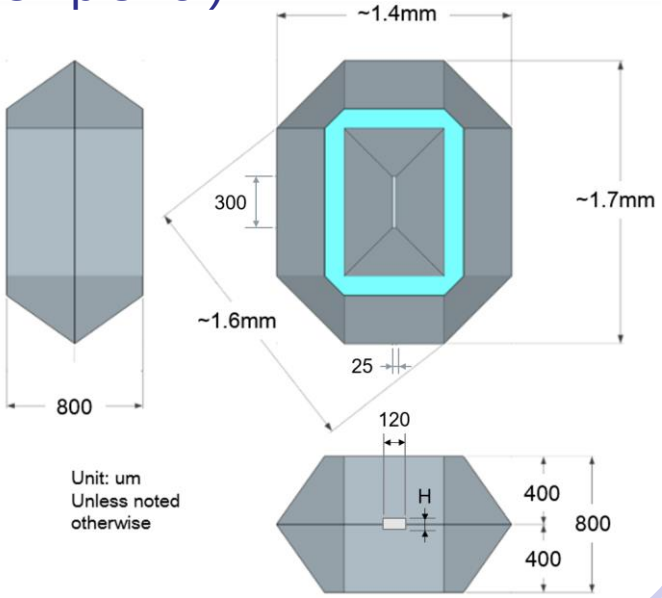


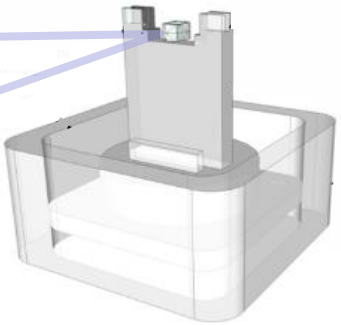


As shown the examples here, Au nanoparticles in K-kit were formed by the reduction process from $AuCl_4$ solution in TEM.

Product features

(Chip size)





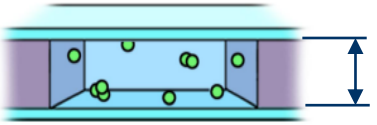
(Shipping packages)

K-kit

(K-kit carrier)

- Window length $300\ \mu m$, Width $25\ \mu m$
- Channel height (H):

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

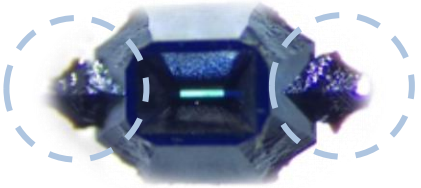


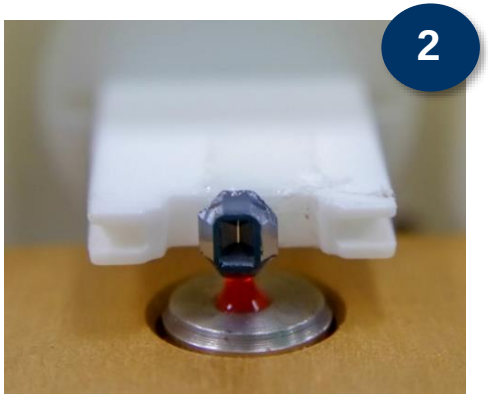
H = 0.1, 0.2, 0.5, 1, 2, 5 (μm)

Quick and easy process in sample preparation

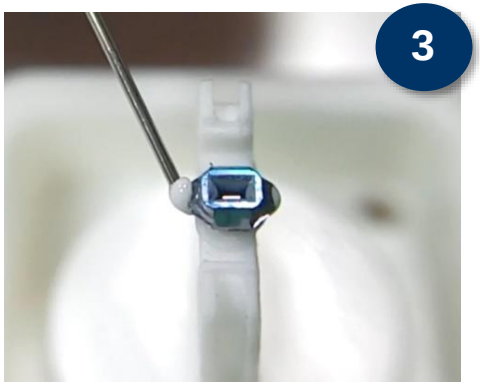
Remove the channel tips

Channel tips, to ensure the cleanness of the channel before use.

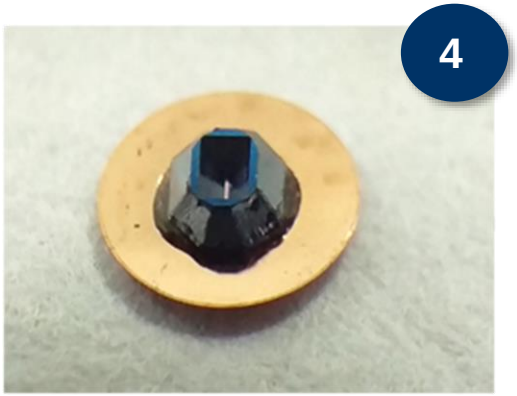




Liquid loading



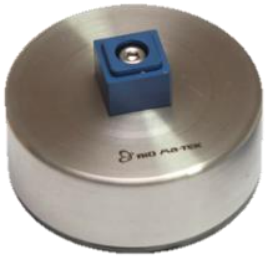
Gluing



Copper grid

K-kit Tool Box for Sample Preparation

K-kit by tools

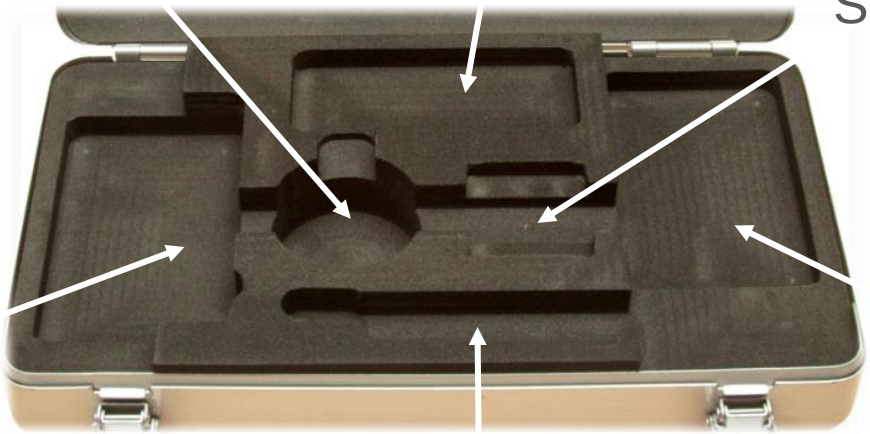


Gluing stand

Glass-slide pack

Sample-loading stage

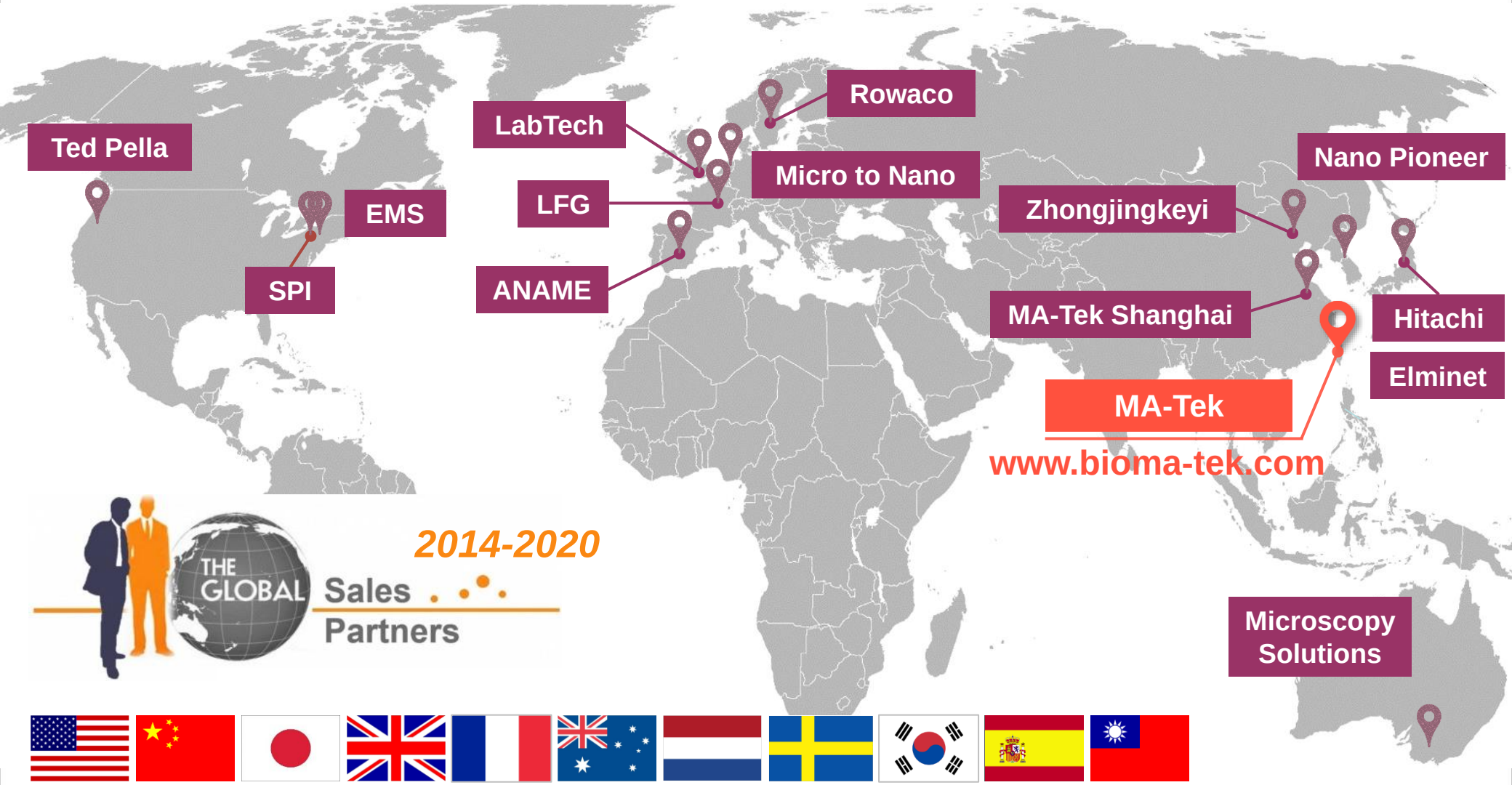
Accessory box



K-kit holder & needle pen

Shipping package

K-kit distributors worldwide



THE GLOBAL Sales Partners

2014-2020

Conclusion

1. K-kit is a liquid cell 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; it fits on a 3 mm diameter of copper grid and, hence, is compatible with most existing TEM holders such like FEI, JEOL, Hitachi and Gatan etc.; it's an affordable and the best option, bringing Liquid TEM accessible to general researchers and industries.
3. The possible applications of K-kit for pharma & CRO are basically on both fields in-situ EM imaging and immunoelectron microscopy, which the former allowing Nanopharmaceuticals to be observed the morphology and size distribution in aqueous condition and the latter providing unique insights into biological systems (e.g., to label and identify the proteins in human cells) for the development and future applications in biomedicine.


Thank you!