Dual Centrifugation for Liposome- and LNP-preparation

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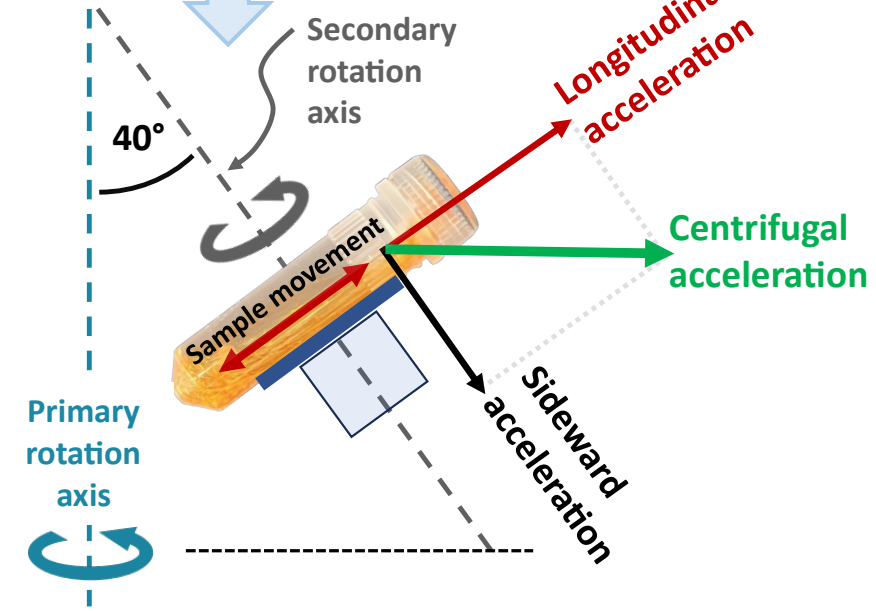
Mixing, Extraction,
Nanomilling,
Homogenization
(Lipid-nanoparticles)

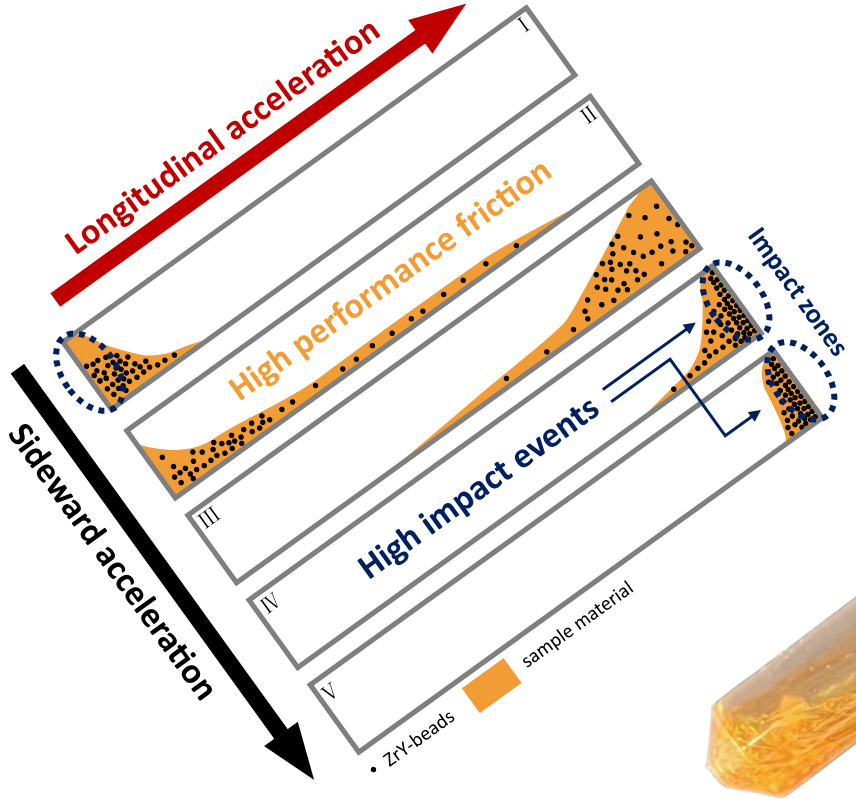
Dual centrifugation enables “**in-vial**”-homogenization

- Lipids
- Buffer
- Beads (ZrO)
- (Drug)

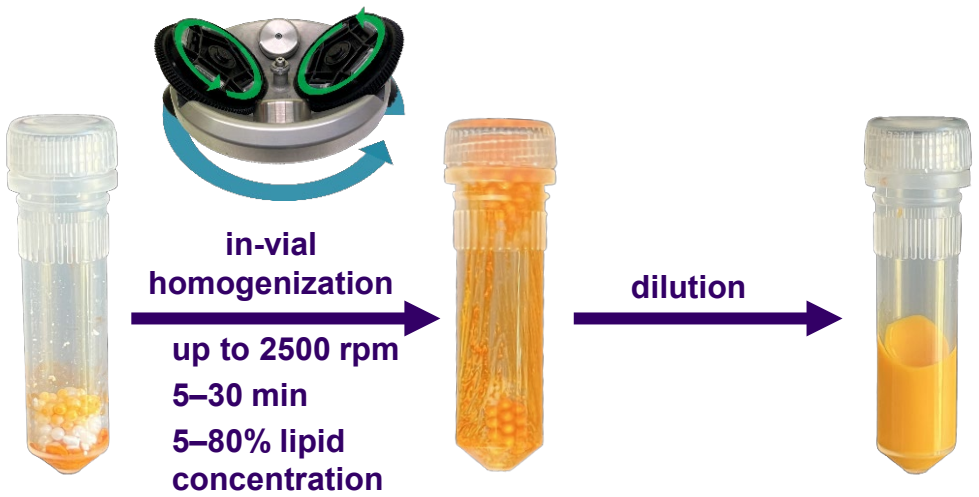
- High performance friction
- High impact events
- High number of homogenization cycles

→ **Efficient and gentle** homogenization





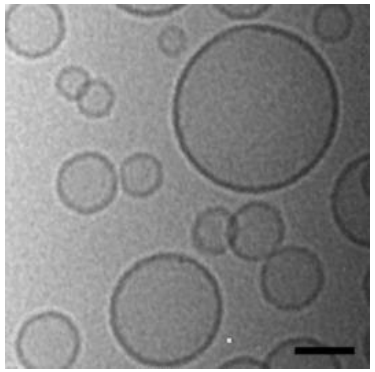
Example – preparation of liposomes by in-vial homogenization (DC)



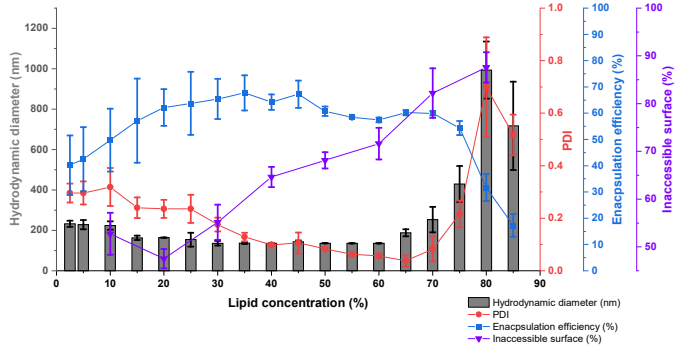
- **Beads**
- **Buffer**
- **(Drug)**
- **Lipids**

Vesicular phospholipid gel (VPG)

Liposomal dispersion

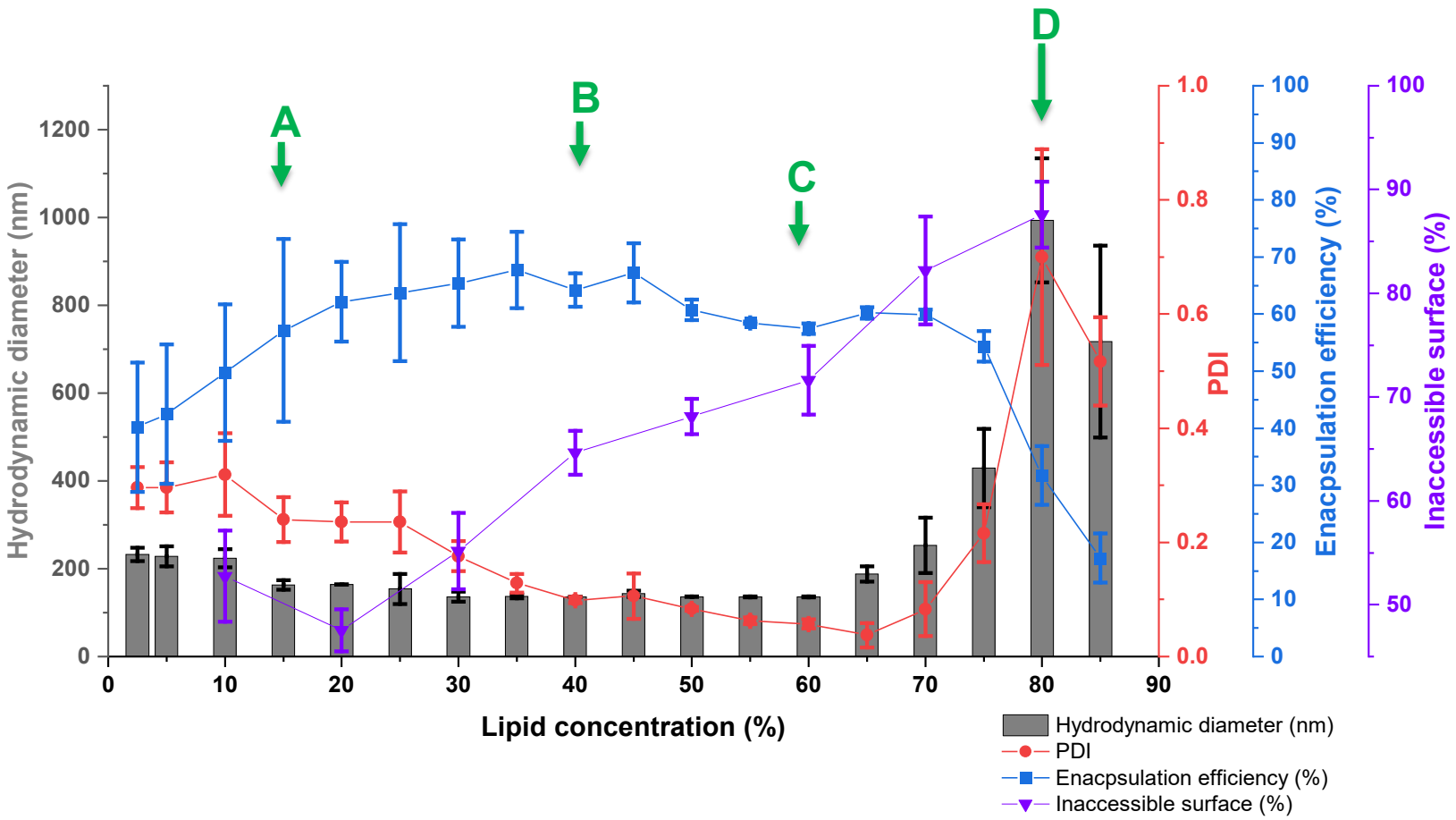


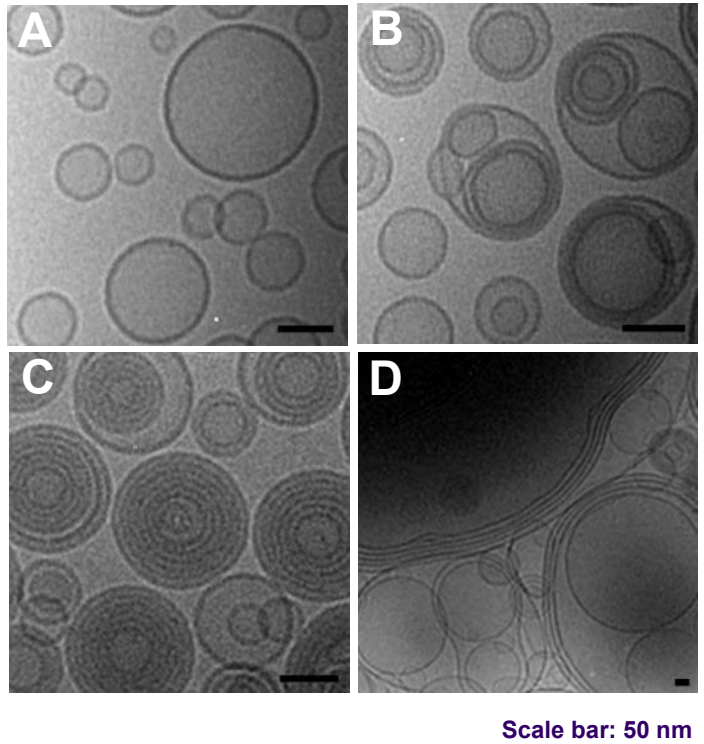
- Fast & cheap
- Disposable vials (no cleaning)
- Sterile (bedside prep. possible)
- Small to medium sized batches (ca. 1 mg up to 2 g lipid)
- High passive liposomal entrapping efficiencies (up to 70%)
- Lipid concentration determines the liposome characteristics
- ….



Lipid concentration during in-vial homogenization determines the liposome characteristics

HEPC/Chol 55/45 mol%

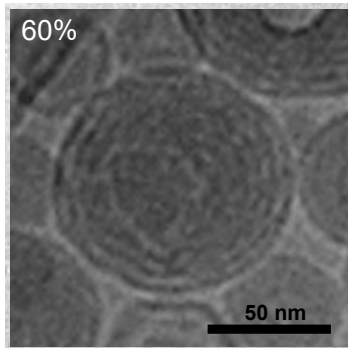




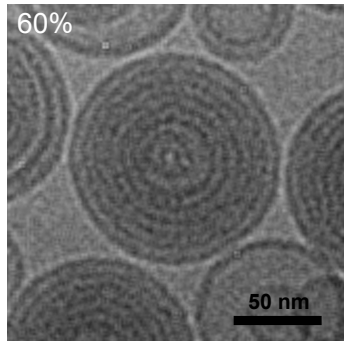
Small Multilamellar Vesicles (SMVs)

- Homogenization under a shortage of water, only possible using in-vial homogenization
- Possible with all lipid mixtures
- Very interesting new carrier for hydrophilic as well as lipophilic compounds

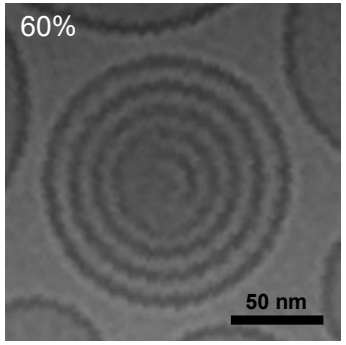
DPPC 100 mol%



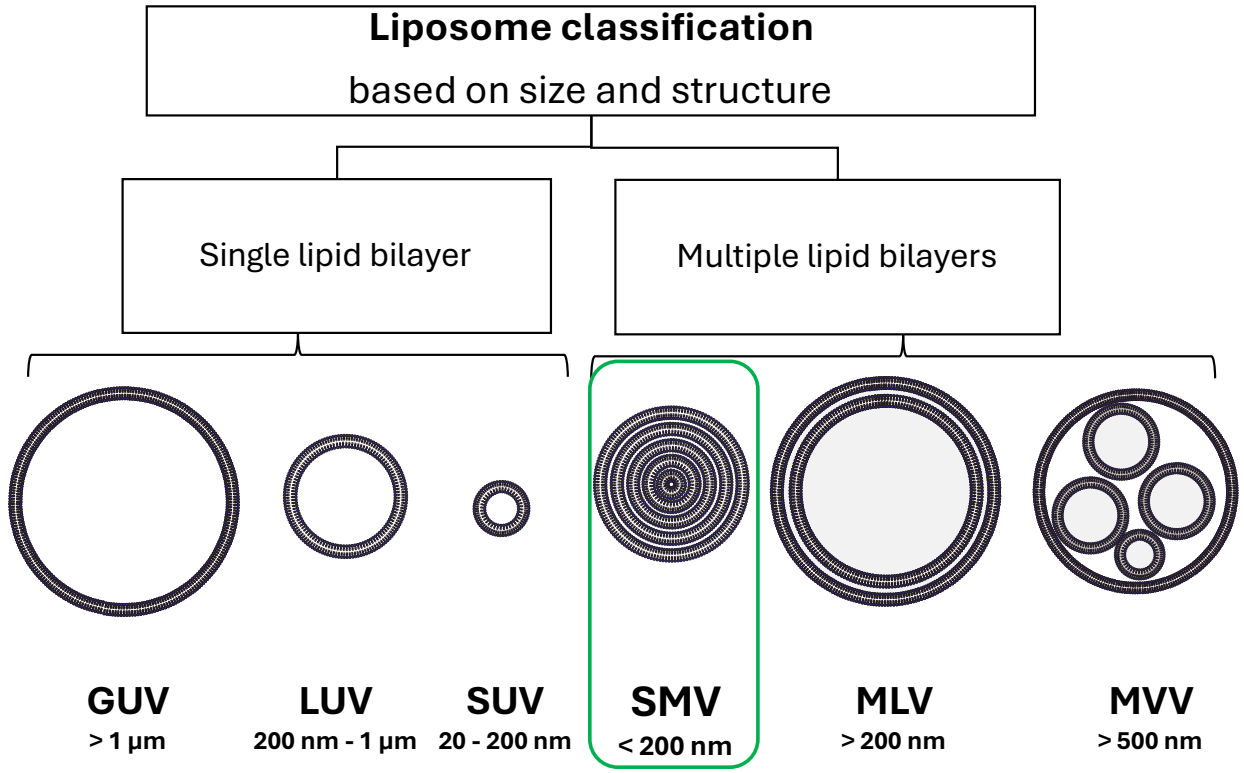
HEPC/Chol.
55:45 mol%



HEPC/Chol./
DSPE-PEG$_{2000}$
50:45:5 mol%



Adopted and adjusted from Barba et al., *Pharmaceutics*, 2019

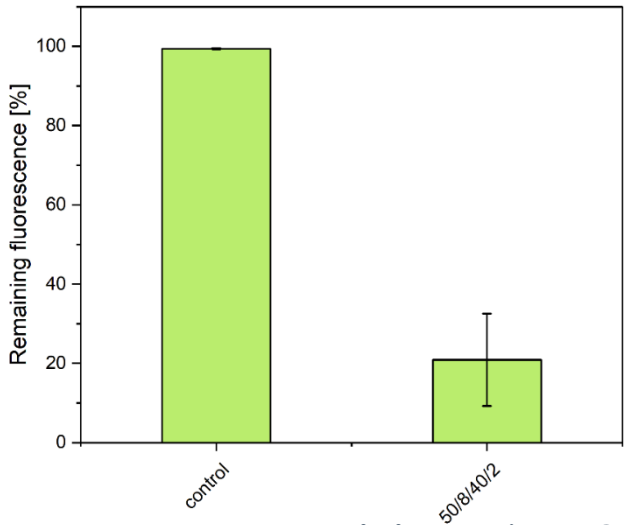


Preparation of LNPs by in-vial homogenization

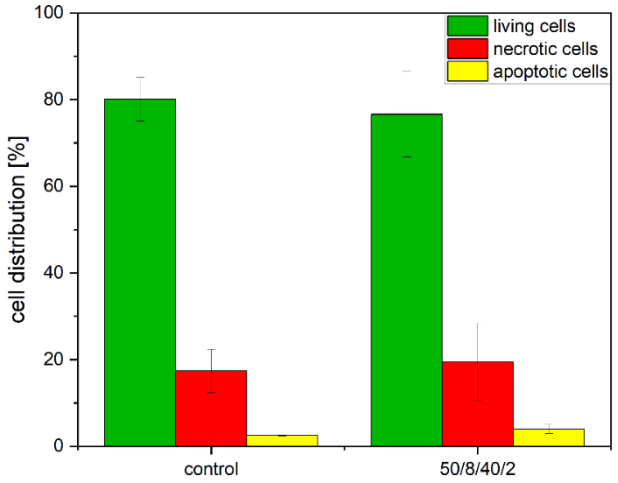
DC-conditions:

- *1 mg SM102/Chol/DSPC/ DMG-PEG2000 (50/40/8/2)*
- *miRNA-100-5p (ZS-Green kockdown)*
- *Acidic buffer*
- *DC: 5 min. (RT)*
- *Dilution with neutral buffer*

Formulation	Z-average [nm]	PDI	Encapsulation efficiency [%]	Zeta potential [mV]
SM102/CHOL/DSPC/ DMG-PEG2000 (50/40/8/2)	115.8 ± 4.0	0.175 ± 0.021	91.3 ± 1.0	-1.43 ± 0.97



Knockdown efficiency *in ZsGreen-expressing HEK293A cells. The cells were treated with 75 nM miRNA-100-5p for 2 h, followed by 72 h incubation prior to analysis by flow cytometry (n=3).*



Cytotoxicity *was determined with the CellEvent™ Caspase-3/7 Green Flow Cytometry Assay Kit after the same treatment scheme as the Knockdown measurements (n=3).*

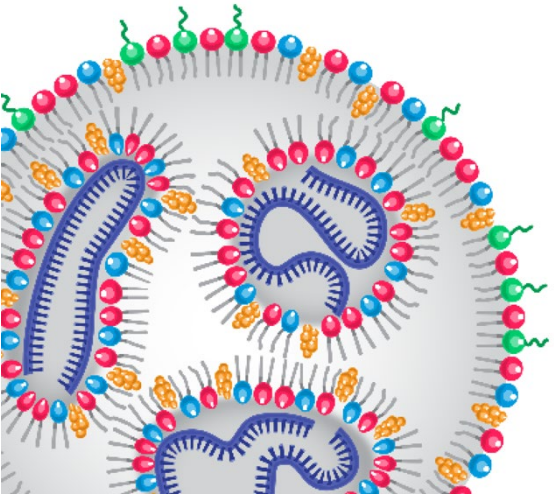


Fig. adapted from **Kularatne et al.** *Pharmaceuticals* **2022**, *15*(7), 897

Preparation of LNPs by in-vial homogenization

- In-vial homogenization is gentle enough to keep RNA intact but efficient enough to form LNPs
- Very low lipid- and RNA-amounts can be processed
- Alternative to LNP-preparation techniques which bases on alcoholic lipid solutions (faster, no alcohol, no buffer exchange)
- Allow a fast formulation screening

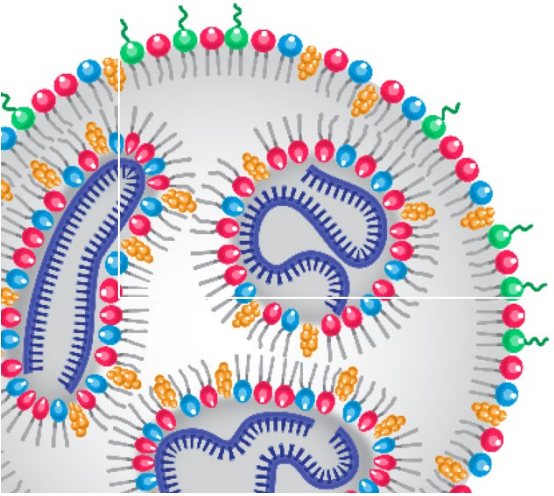


Fig. adapted from **Kularatne et al.** *Pharmaceuticals* **2022**, *15*(7), 897

Take home

- In-vial homogenization based on DC is a highly efficient but at the same time very gentle method for liposome and LNP preparation in closed and sterile vials (unique combination of impact and friction).
- The lipid concentration during in-vial homogenization strongly influences the morphology of the resulting particles or vesicles.
  - Unilamellar liposomes
  - Small multilamellar vesicles (SMVs; accessible for the first time).
  - Unusual high EE-values can be achieved
- LNP-preparation is possible in small quantities without the use of alcohol or buffer exchange, thus supplementing the existing technology.

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