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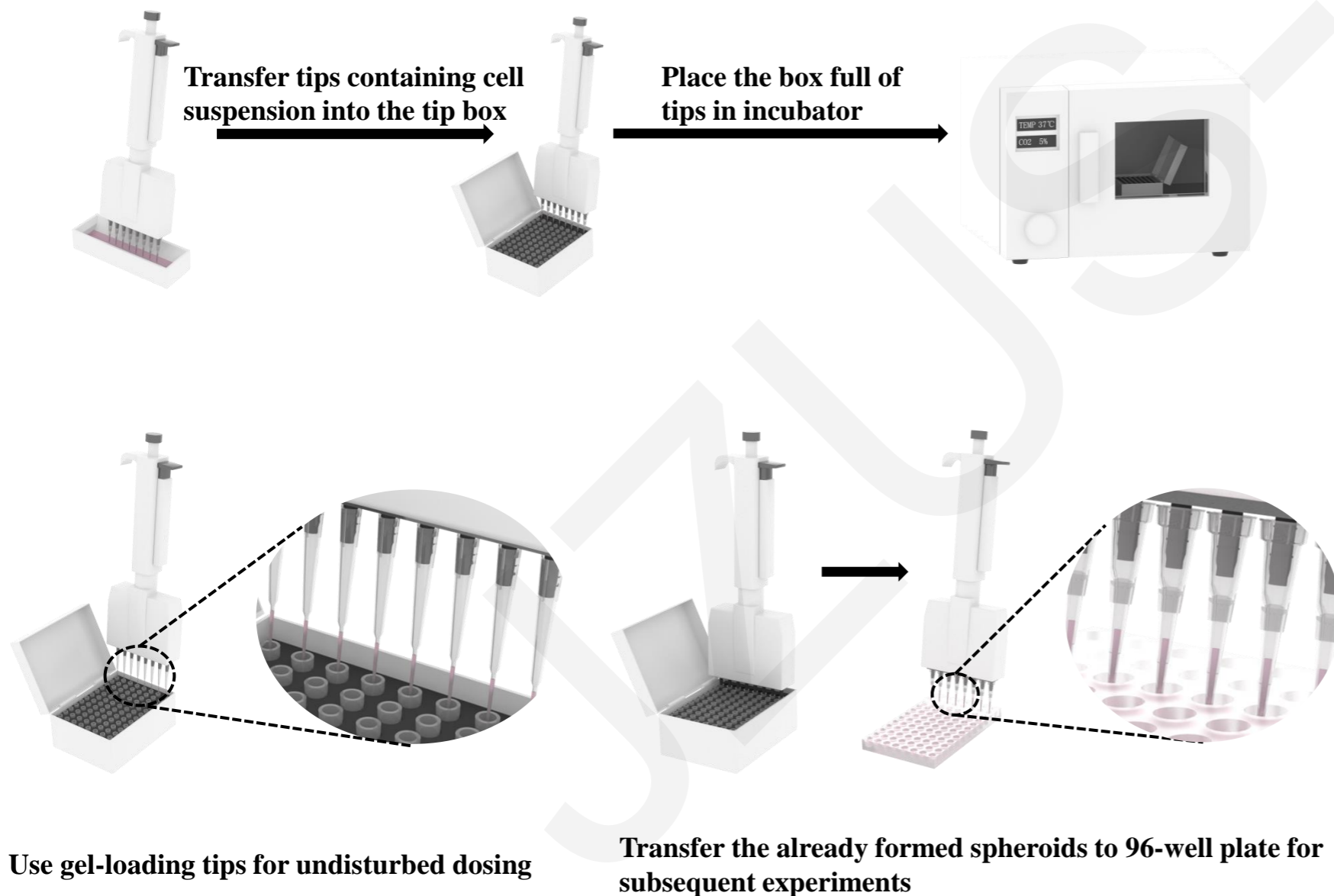
Using pipette tips to readily generate spheroids comprising single or multiple cell types

Key words:

Pipette tip; 3D cell culture; Tumor spheroids; Co-culture; In-situ observation

Innovation point

Using pipette tips as micro vessels for generating three-dimensional (3D) cell spheroids just with a simple aspiration and incubation procedure.



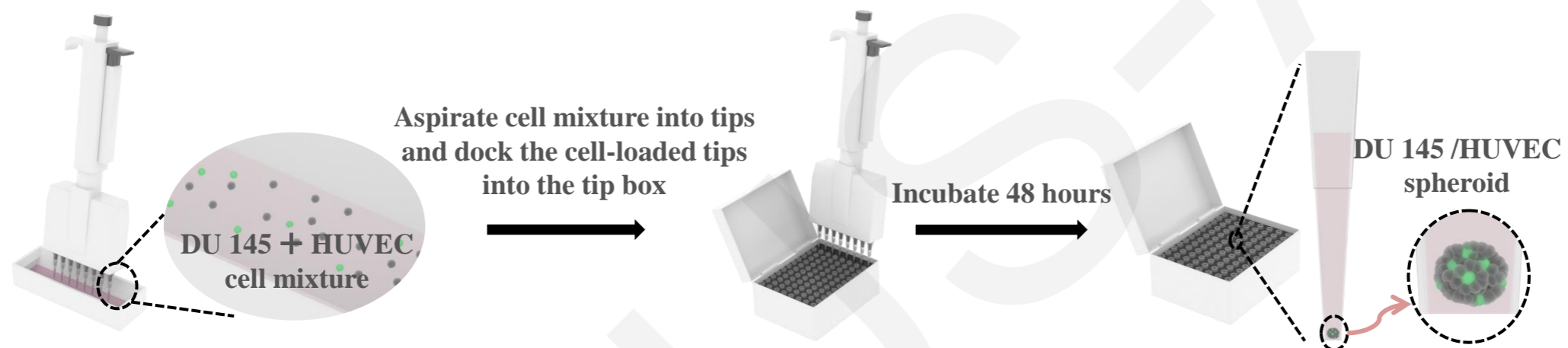
- Without any chemical or physical modifications
- High-throughput
- Fast spheroids formation
- In-situ observation
- Multiple experiments can be performed on the platform

Diagram of generating cell spheroids in pipette tips and dosing / transforming

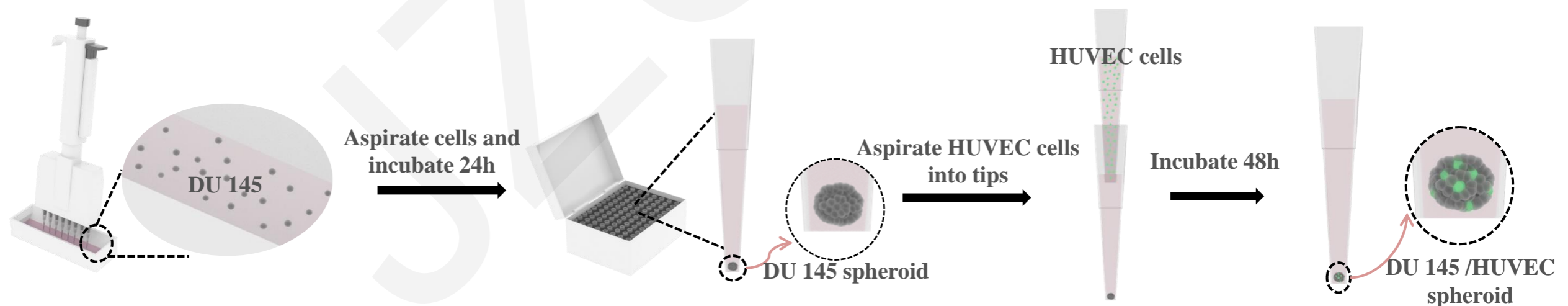
Experimental methods

1. Generating spheroids with multiple cell types in pipette tips

(i) One-step co-culture method



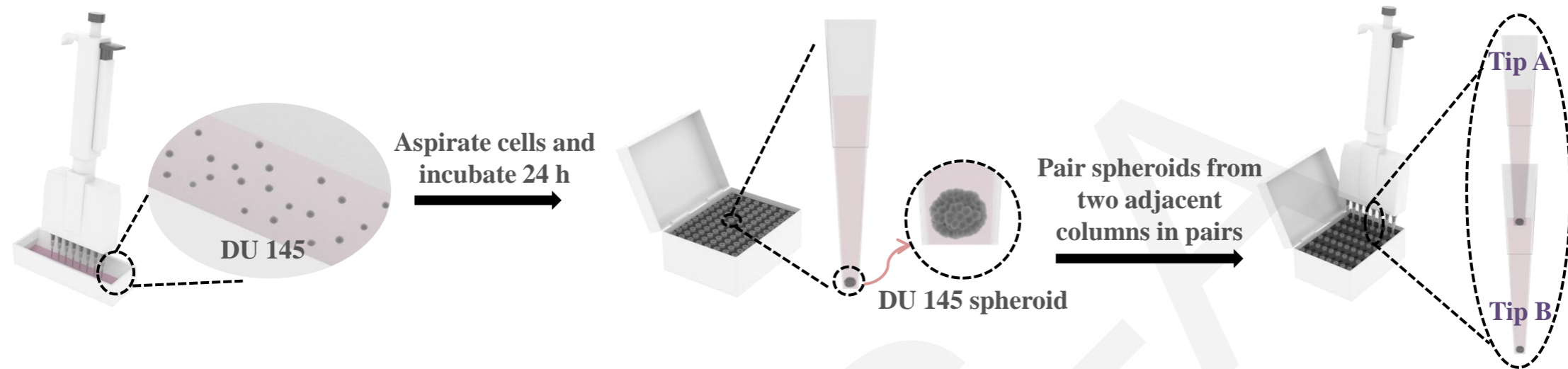
(ii) Two-step co-culture method



✓ A higher density of HUVEC cells (labeled with green fluorescence) were observed in the inner core of the tumor spheroid.

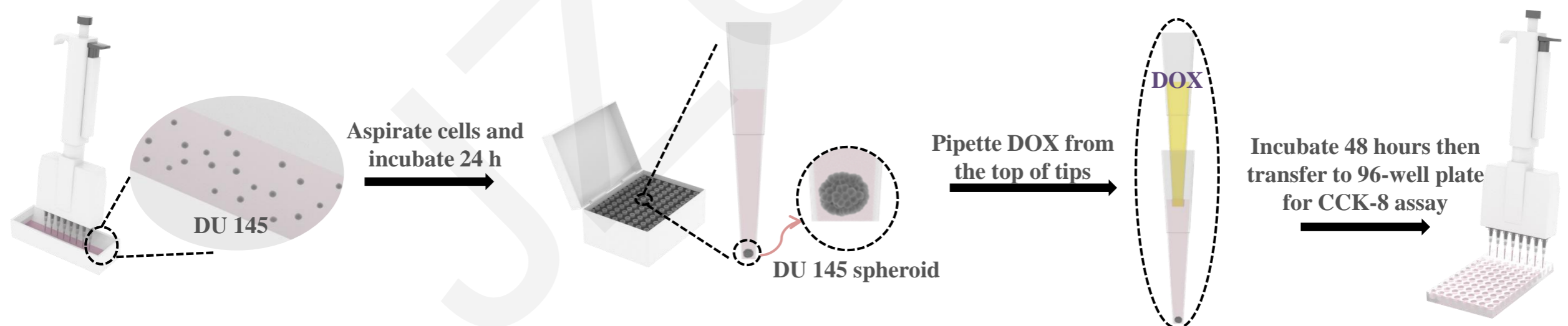
✓ Both methods proved that co-culture of different cell types could be achieved in the pipette tips.

2. Assembling and pairing of tumor spheroids



- ✓ Two tumor spheroids could be paired by pipetting, the two spheroids merged and eventually formed a single bigger tumor spheroid.
- ✓ Although occupied by two spheroids, there was still space available in the tip which can support more spheroids fusion

3. Evaluating drug efficacy using tumor spheroids in pipette tips



- ✓ The morphology of the spheroids was impacted by the drug treatment.
- ✓ With increasing DOX concentration, the percentage of live cells within the spheroids decreased.
- ✓ Cells in 2D culture were more sensitive to DOX treatment compared to pipette tip culture (3D).

Significance and application

Significance:

- **Providing a new platform for 3D cell culture with low cost and high throughput.**
- **Cell spheroids can be observed in-situ, avoiding the disturbance of cells caused by transferring.**
- **The culture platform supported a variety of experiments.**

Three-dimensional (3D) cell culture methods have been validated that can replicate the tumor environment in vivo to a large extent, providing an effective tool for studying tumors. In this study, we demonstrated the use of standard laboratory pipette tips as micro vessels for generating 3D cell spheroids. No microfabrication or wet-chemistry surface modifications were involved in the procedure. A simple aspiration and incubation procedure was applied to generate spheroids consisting of a single or multiple cell types. In addition, pairing and fusion, drug screening also successfully implement. Since biological laboratories are commonly equipped with pipettes and pipette tips, the proposed pipette tip-based spheroid formation platform can eliminate the equipment barrier for resource-limited laboratories to conduct 3D cell culture and research.