

Motivation and Objective

Single-cell manipulation based on image-derived selection criteria is a prerequisite for morphology-based screening [1]. This requires a fast and robust **"pick-and-place" technology** to single-out the identified hits from the population.

Current limitation: Low throughput by moving only one hit per cycle in single-cell picking technologies. High effort and consumption of reagents and materials by isolating hits through stochastic confinement [2].

Objective: Developing a droplet-based microfluidic system that enables the sequential translocation of multiple objects per cycle, by temporarily compartmentalizing them in individual droplets.

The workflow consists of three main steps (Fig. 1):

- 1) Loading of a petri dish into the robotic system and identification of hits based on morphological characteristics such as color, size or shape.
- 2) Automated pick-up and encapsulation of selected objects in individual droplets. Droplet storage in tubing.
- 3) Sequential deposition of the droplets into individual wells for post-processing.

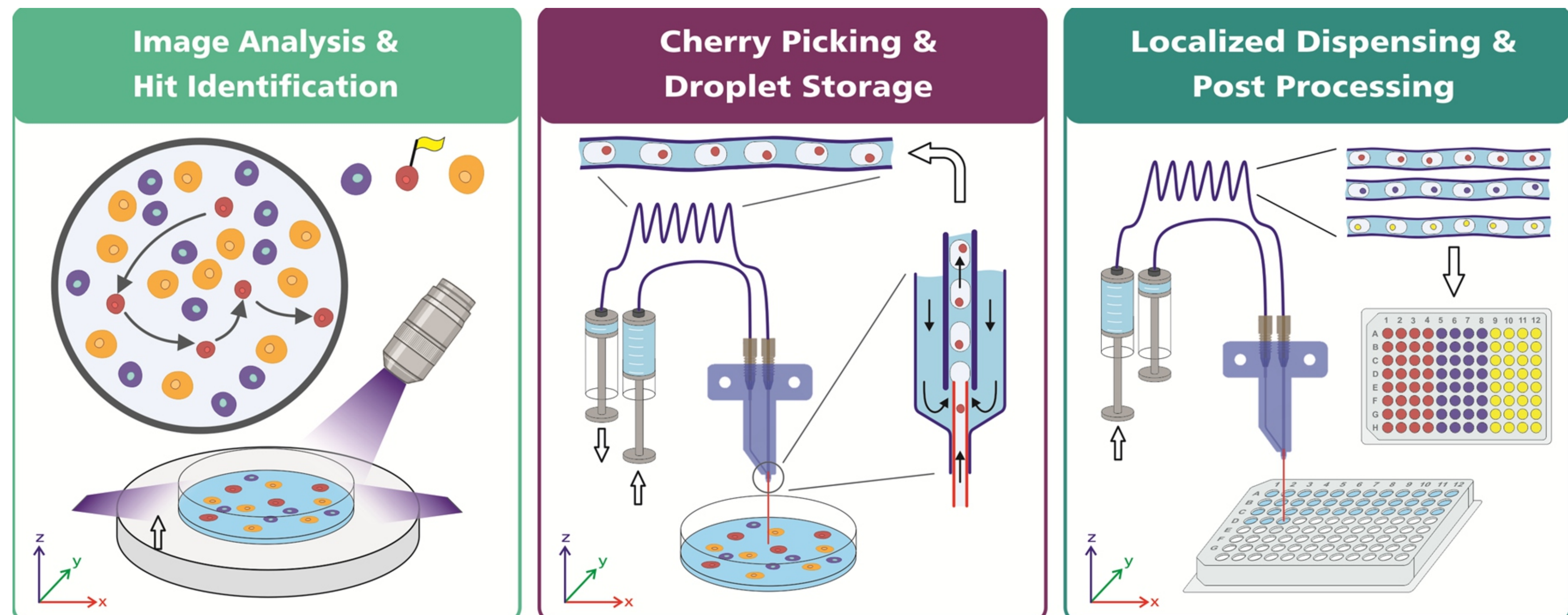


Fig. 1: Graphical illustration of the microfluidic system's workflow to transfer targeted single microscopic objects out of a mixed bulk into individual wells. 1) Image analysis of the reservoir containing the sample and identification of possible hits. 2) Automated picking process of the identified hits and storage in droplets inside the tubing. 3) Sequential droplet deposition into individual wells for post processing.

Experimental Setup

Experimental Robotic Environment

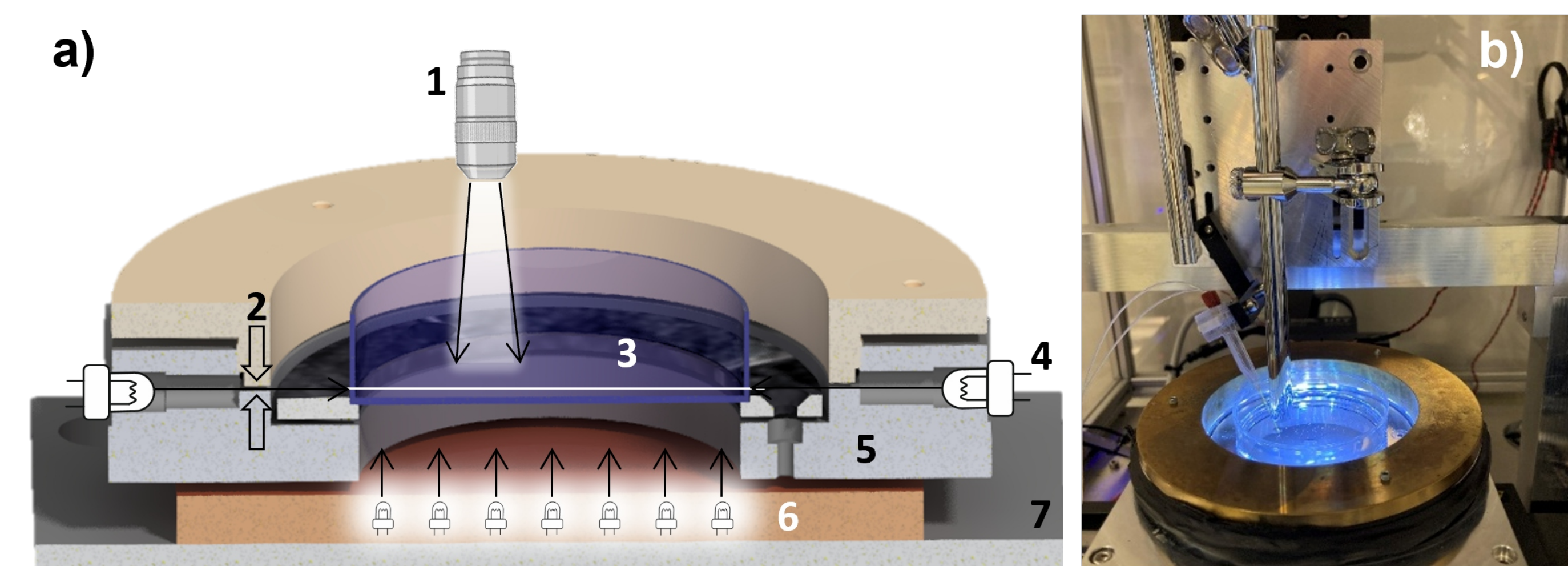


Fig. 2: Experimental robotic setup. a) Cross-sectional scheme with: (1) Pen-Camera with integrated epi-illumination LEDs, (2) adjustable brass ring controlling the ring excitation gap, petri dish (3) and ring excitation LEDs (4) on leveling frame (5), (6) LED-array for trans-illumination and (7) robotic xy-stage. b) Image of the fully equipped xyz-robot in ring-gap excitation mode using blue light LEDs.

Microfluidic Transfer Tool (MTT)

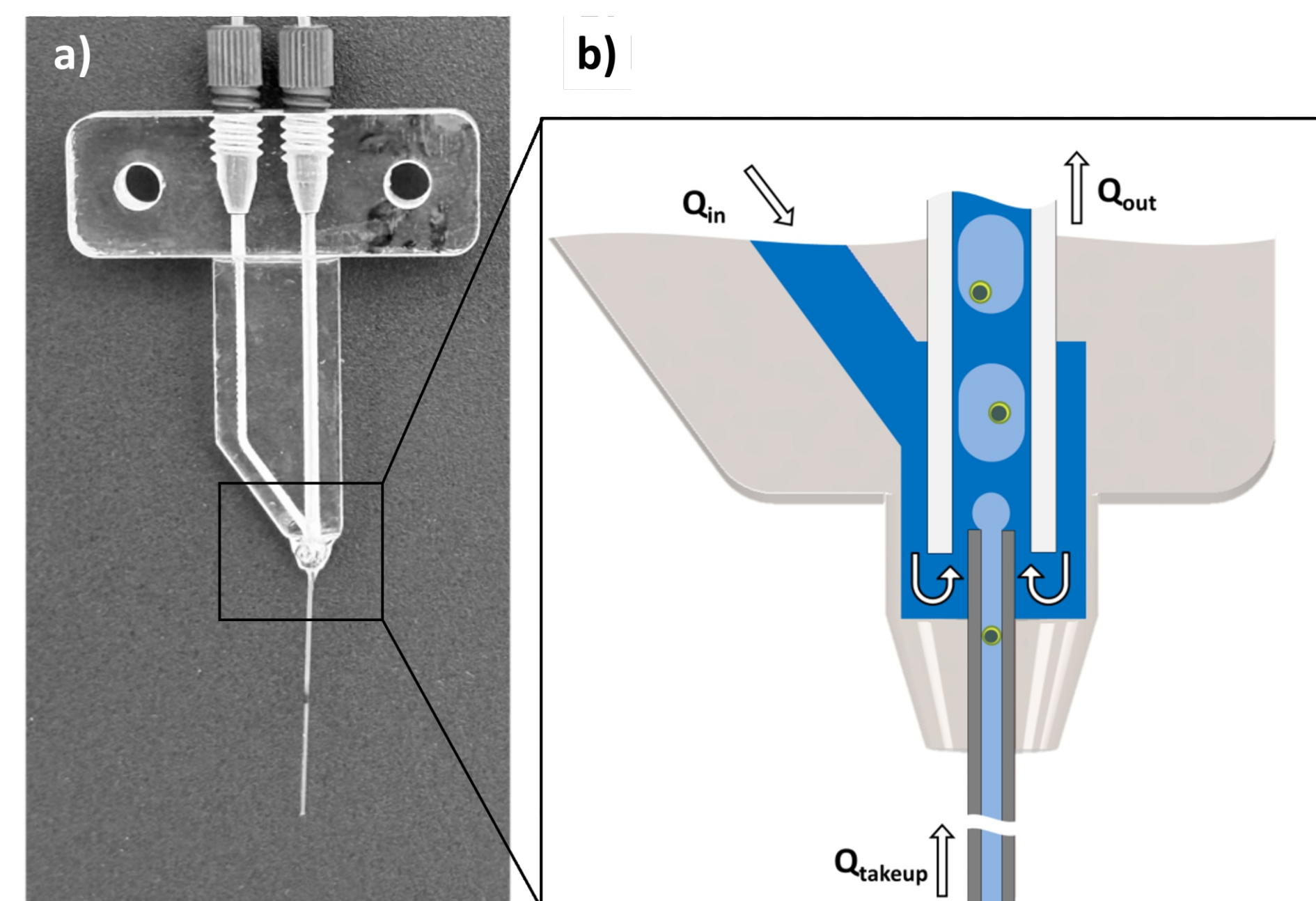


Fig. 3: Microfluidic Transfer Tool (MTT). a) 3D-printed main body with fused silica capillary (ID = 100 μm) as nozzle tip. b) Illustration of droplet formation mechanism. $Q_{\text{takeup}} = Q_{\text{out}} - Q_{\text{in}}$

- ❖ The microfluidic transfer tool (MTT) consists of two parts: **3D-printed microfluidic main body** and a **fused silica capillary** as aspiration tip.
- ❖ **Co-flow mechanism** is used for droplet generation. An applied higher flowrate at the outlet Q_{out} than at the inlet Q_{in} leads to an additional flowrate Q_{takeup} at the nozzle tip.
- ❖ MTT is mounted to the z-linear table of the xyz-stage.
- ❖ USB camera used to analyze the position of the MTT tip and the objects for single object picking.
- ❖ **Ring-slit excitation** provides high contrast imaging as illumination takes place only in the object's plane.

Results and Discussion

Object Detection and Encapsulation in Individual Droplets

- ❖ Successful single-cell picking requires sufficient spacing between the individual objects. Spacing of less than 200 μm increases the probability of parallel uptake.

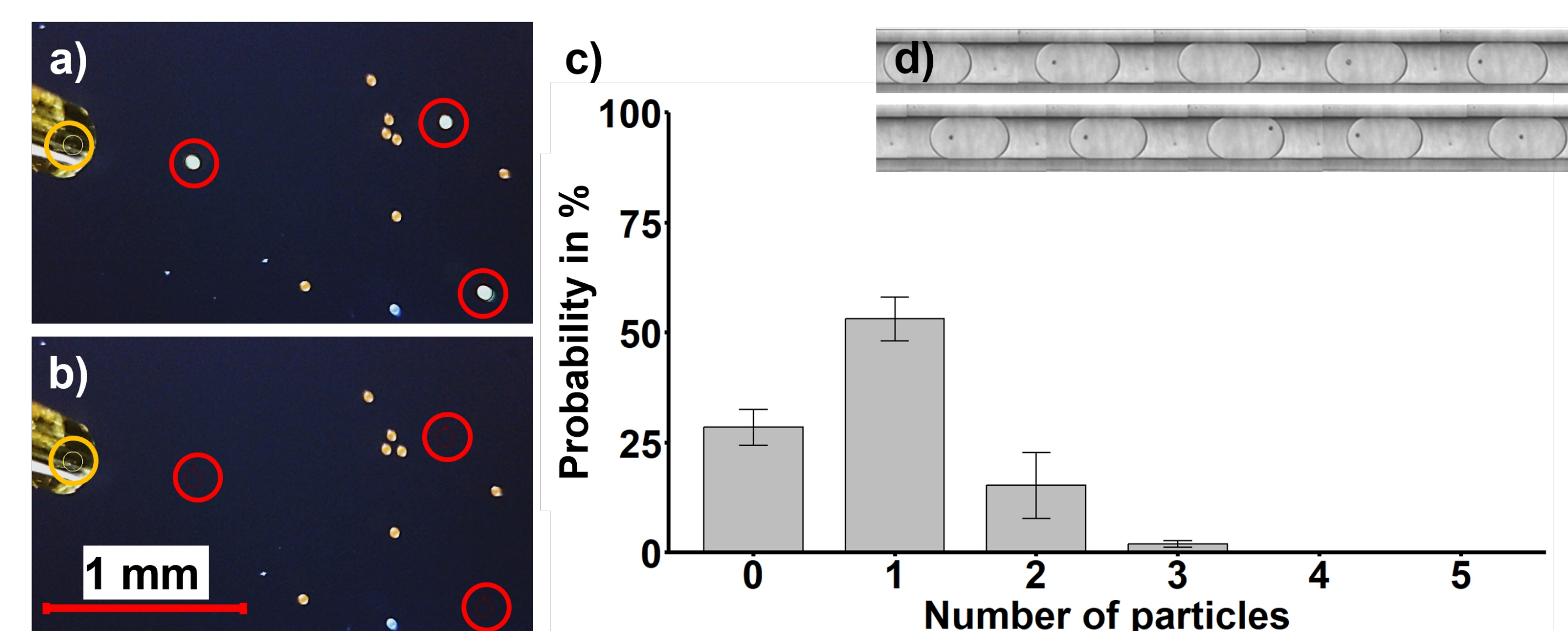


Fig. 4: a+b) Image comparison before and after picking process. Particle field with color-selected particles highlighted by red circles. Yellow circle marks the position of the MTT capillary orifice. c) Distribution of the number of particles per droplet. d) Microscope images of the storage tubing with droplet-encapsulated objects (arrayed from video stream).

Object Transfer into Individual Wells

- ❖ Object transfer was carried out on a paper-based target with 4.5 mm spacing (see Fig. 5a).
- ❖ Analysis of 80 paper spots revealed particle occupancy similar to that found in the storage droplets.
- ❖ Droplets were stained with Cochineal Red A to calibrate deposition process on MTP wells with an accuracy of 98 %.

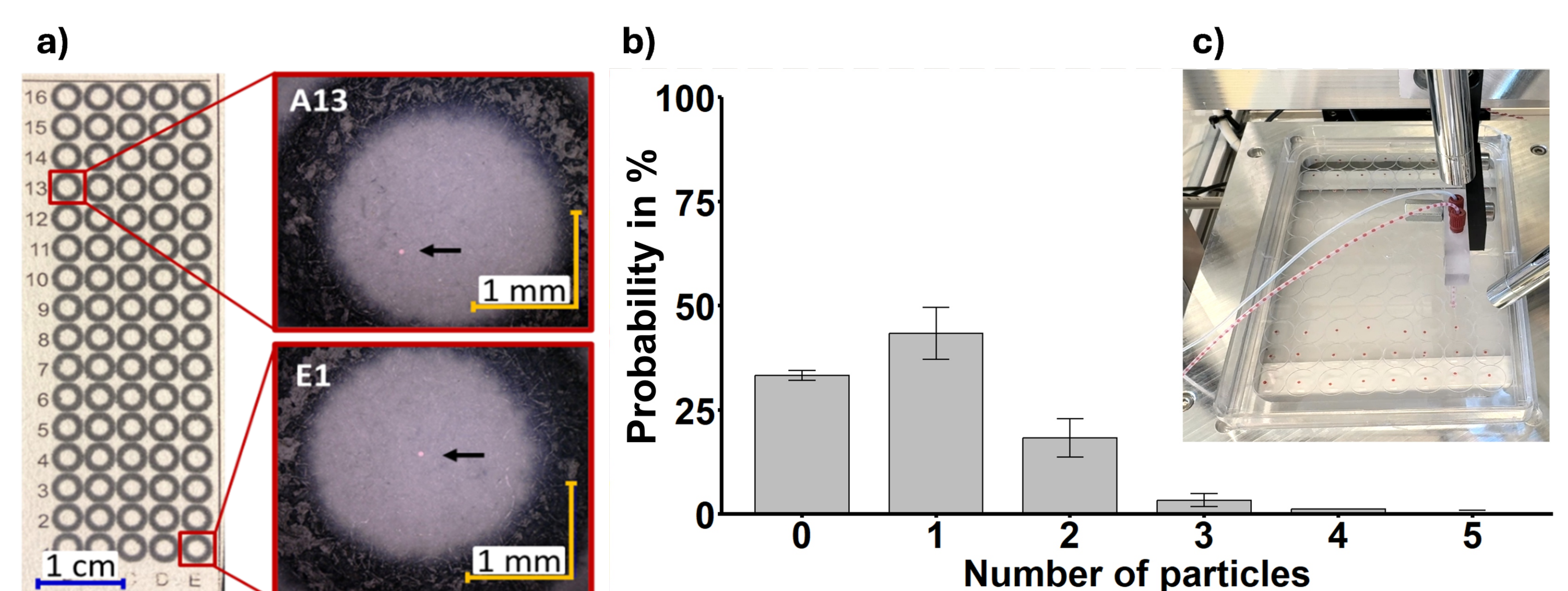


Fig. 5: a) Particle deposition onto paper-based target with 80 spots. b) Distribution of the number of particles per spot. c) Particle deposition into individual MTP wells.

Conclusions

- ❖ Aim of the work was to demonstrate how incorporating droplet-based intermediate object storage could enhance the efficiency and performance of image-based pick-and-place operations.
- ❖ We developed a MTT which allows a selective pick-up of dispersed objects with a diameter of about 50 μm and their encapsulation into nanoliter-scale droplets.
- ❖ An experimental robotic environment with a multipurpose excitation stage allows high contrast imaging, which is necessary for identifying μm-objects using a low-cost camera.
- ❖ With an automatic object recognition and classification algorithm based on size and color, about 300 classified objects were singled out from a mixture within 10 min.

