Query Details

Back to Main Page

1. Please check if “medium form the culture” should be changed to “medium from the culture”.

yes, it should be changed to "medium from the culture"

Laser-Assisted Single-Cell Labeling and Capture

Nicolas Desjardins-Lecavalier Affiliationids : Aff1

Graziana Modica Affiliationids : Aff1

Santiago Costantino✉

Email : Santiago.costantino@umontreal.ca

Affiliationids : Aff1 Aff2, Correspondingaffiliationid : Aff2

Aff1 Maisonneuve-Rosemont Hospital Research Center, Montreal, QC, Canada

Aff2 Department of Ophthalmology, University of Montreal, Montreal, QC, Canada

Abstract

Single-cell technologies have become critical tools to understand and characterize the complex dynamics that govern biological systems, from embryonic development to cancer heterogeneity. In this context, identification and capture of live individual cells in heterogenous ensembles typically rely on genetic manipulations that encode fluorescent probes. However, a precise understanding of how several molecular components interact to yield the phenotype of interest is a prerequisite to distinguishing and isolating such target cells based on fluorescence alone. Indeed, cellular phenotypes associated with migration, shape, location, or intracellular protein distribution play critical and well-understood roles in cancer biology, but the technologies to tag and isolate cells based on information obtained from imaging are not readily available.

Cell labeling via photobleaching (CLaP) and single-cell magneto-optical capture (scMOCa) represent convenient and cost-effective systems for labeling, capturing, and expanding single cells from a heterogenous population, without altering cellular physiology and therefore enabling not only transcriptomic profiling but also biological characterization of target cells. Both techniques allow capturing cells after observation and permit researchers to choose target cells based on information obtained from images. The implementation of these technologies only needs the lasers of a confocal microscope and low-cost, commercially available chemical reagents. Here, we describe a detailed protocol to set up and perform CLaP and scMOCa and highlight critical points for optimal performance.

Key words

Single-cell capture

Photobleaching

Single-cell tag

Laser scanning microscope

CLaP

scMOCa

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/978-1-0716-2914-7_21.

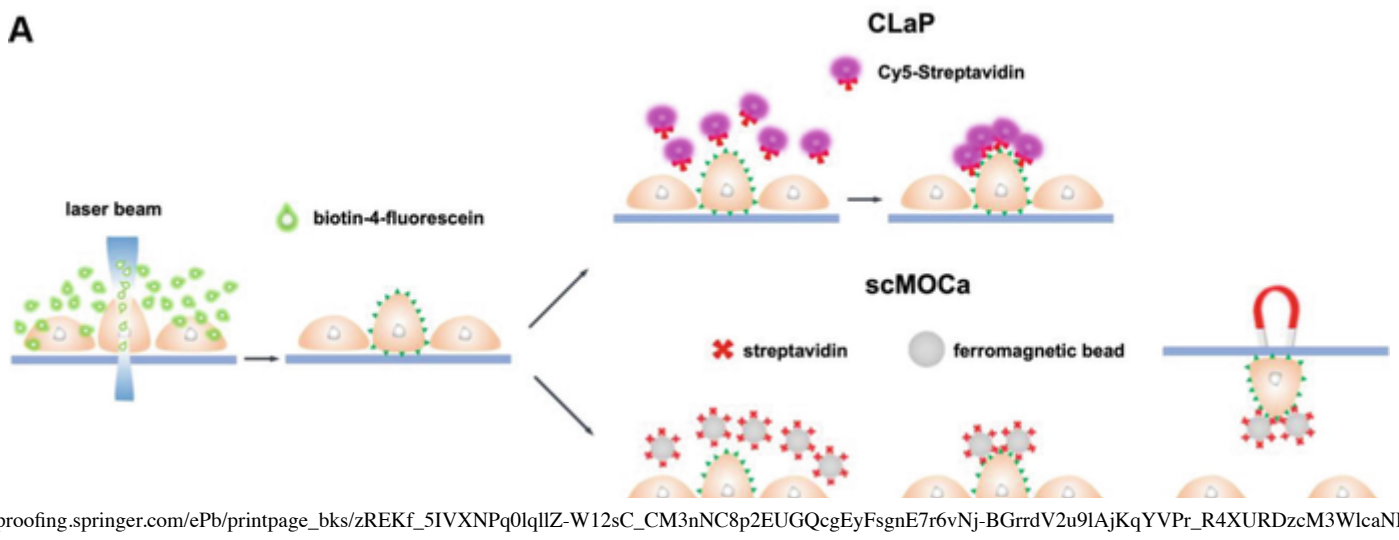
1. Introduction

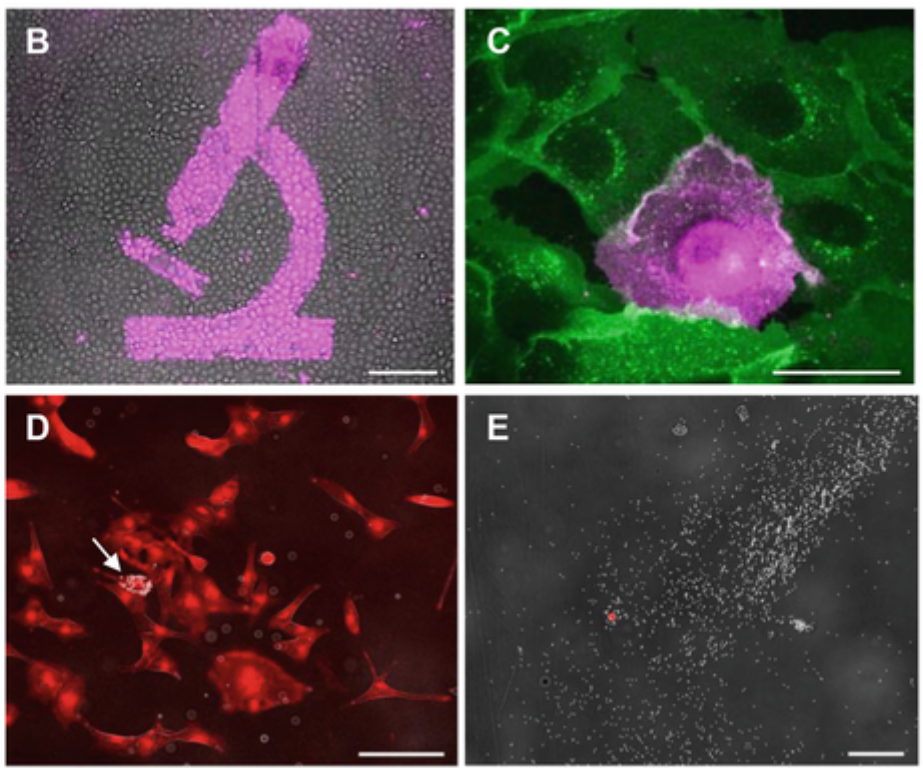
Genotypic and phenotypic heterogeneity is a fundamental characteristic of biological systems which becomes especially critical in the tumor microenvironment [1]. Indeed, the success of cancer therapies is often undermined by the differential response of individual cell types within complex tumors, whereby single cells with an innate or acquired ability to override therapeutic response can lead to the insurgence of resistant clones with a poor prognosis for the patient [2]. Hence, dissecting the tumor characteristics, at the single cell level, is of paramount importance to choose the most appropriate therapeutic strategy. In the last decade, an increasing number of sequencing approaches have been developed, allowing for single-cell profiling of cancers and unravelling of the clonal dynamics underlying tumor biology [3, 4]. Moreover, the development of spatially resolved transcriptomic approaches enables researchers to correlate the molecular profile of single cells with tissue location, offering a depiction of tumor organization at high resolution [5, 6, 7]. However, these techniques are associated with the lysis of analyzed cells, hampering the possibility to further investigate the biological relevance of the identified molecular profile. The ability to label and isolate single cells preserving their integrity is therefore key to linking genetic and epigenetic data with biological phenotypes.

Cell labeling via photobleaching (CLaP) [8] and single-cell magneto-optical Capture (scMOCa) (Fig. 1) [9] allow image-based labeling or capture, respectively, of single cells from large ensembles according solely to visual characteristics in an efficient yet cost-effective way. In CLaP, the sample is incubated with high concentration of biotin-4-fluorescein and inspected via microscopy. Selected cells within the field of view are illuminated with a low-power laser to photobleach fluorescein, thereby inducing its covalent binding to cell membranes via photogenerated radicals [10, 11]. Since fluorescein is conjugated with biotin, this approach results in the plasma membrane biotinylation of illuminated cells. Thus, streptavidin constructs can be used to reveal irradiated cells, either fluorescently or otherwise. Importantly, the low power required to photobleach fluorescein, together with the extracellular localization of the chemical reactions, makes this labeling procedure non-toxic, minimally invasive, and with negligible impact on both cell viability and transcriptomic profiles [8].

Fig. 1

(a) Outline of CLaP and scMOCa. The sample is incubated with biotin-4-fluorescein and a low-power laser is used to illuminate the cell(s) of interest to attach biotin to their membranes. Biotinylated cells can either be revealed using fluorescent streptavidin (top) or isolated with streptavidin-coated ferromagnetic beads and a magnet (bottom). (**b**) Wide field image of MDCK cells biotinylated with CLaP and labeled with Alexa-647 conjugated streptavidin (magenta). Scale bar 200 µm. (**c**) Confocal image of a single MDCK cell labeled with Alexa-647-streptavidin (magenta). Wheat Germ Agglutinin-Alexa 488 is used to label all cell membranes. Scale bar 10 µm. (**d**) One U2OS cell is biotinylated and labeled with streptavidin-coated ferromagnetic beads (arrows), and Wheat Germ Agglutinin-Alexa 594 is used to label the cell membrane. Scale bar 50 µm. (**e**) The same cell after the magnetic sorting. Scale bar 50 µm. (**b** and **c**) Adapted from Ref. [8]. Creative Commons Attribution License. (**d** and **e**) Adapted from Ref. [9]. Creative Commons Attribution License

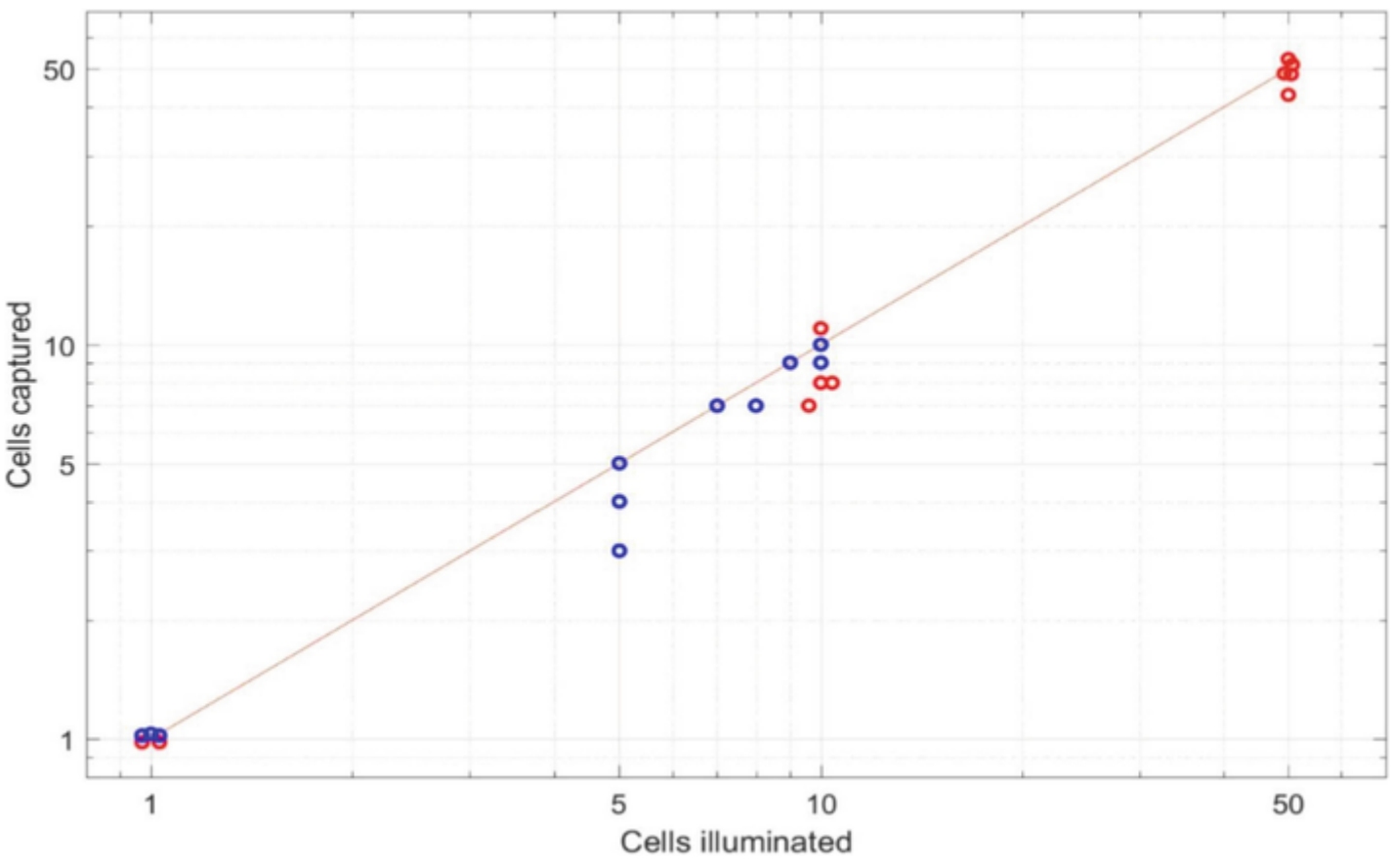




Building on CLaP, we scMOCa to isolate and eventually expand single cells, biotinylated with CLaP, by exploiting ferromagnetic streptavidin-coated beads and a magnetic field [9]. After laser illumination, cells are rinsed of excess biotin and incubated with beads. A rare earth magnet is then used to attract and transfer the target cells to a separate chamber where they can be expanded and further analyzed. The technique allows researchers to capture single cells with high sensitivity and specificity (Fig. 2) and also enables high-throughput analyses when automated.

Fig. 2

scMOCa efficiency. A total of 27 independent captures of 1, 5, 10, and 50 selected cells. The x-axis displays the number of laser-targeted cells, and the y-axis counts the captured cells. The line corresponds to a 100% success rate. Red dots represent experiments performed on glass substrate and blue dots, on plastic. (Adapted from Ref. [9]. Creative Commons Attribution License)



In this chapter, we provide a step-by-step protocol to set up both CLaP and scMOCa using a laser scanning microscope and custom-made 6-mm polydimethylsiloxane (PDMS) chambers. We describe variations to the main protocol related to both alternative illumination systems and the culture setup, as well as suggestions to scale up throughput. Finally, we emphasize critical points that need special consideration regarding experimental design, according to the particular sample to be processed.

2. Materials

2.1. PDMS Stock Plate

1. Sylgard 184 silicone encapsulant base and curing agents: In a 50-mL Falcon tube, mix Sylgard 184 silicone encapsulant base and curing agents in a 10:1 ratio. Vortex until the solution is homogeneous. Pour 10 mL of the solution into a 10-cm dish to obtain a gel of ~1.5 mm thick, and degas the dish in a vacuum chamber overnight using a bull's eye level to ensure a uniform thickness of the PDMS. Wait two days for the polymer to be cured. Dishes can be stored at room temperature for several months.

2.2. Cell Culture Medium

1. Trypsin-EDTA. Trypsin concentration depends on the cell type used in the experiment.
2. 1× phosphate buffer saline (PBS) pH 7.4: 137 mM of NaCl, 2.7 mM of KCl, 8 mM of Na_2HPO_4, 2 mM of KH_2PO_4.
3. Biotin-4-fluorescein (B4F) stock solution: Re-suspend 2 mg/mL (B4F) in PBS. Store at 4 °C in the dark.
4. Cy5-Streptavidin stock solution: Re-suspend 2 mg/mL of Cy5-streptavidin in PBS. Store at 4 °C in the dark.
5. 10 mg/mL of 2.8-µm streptavidin-coated ferromagnetic beads.

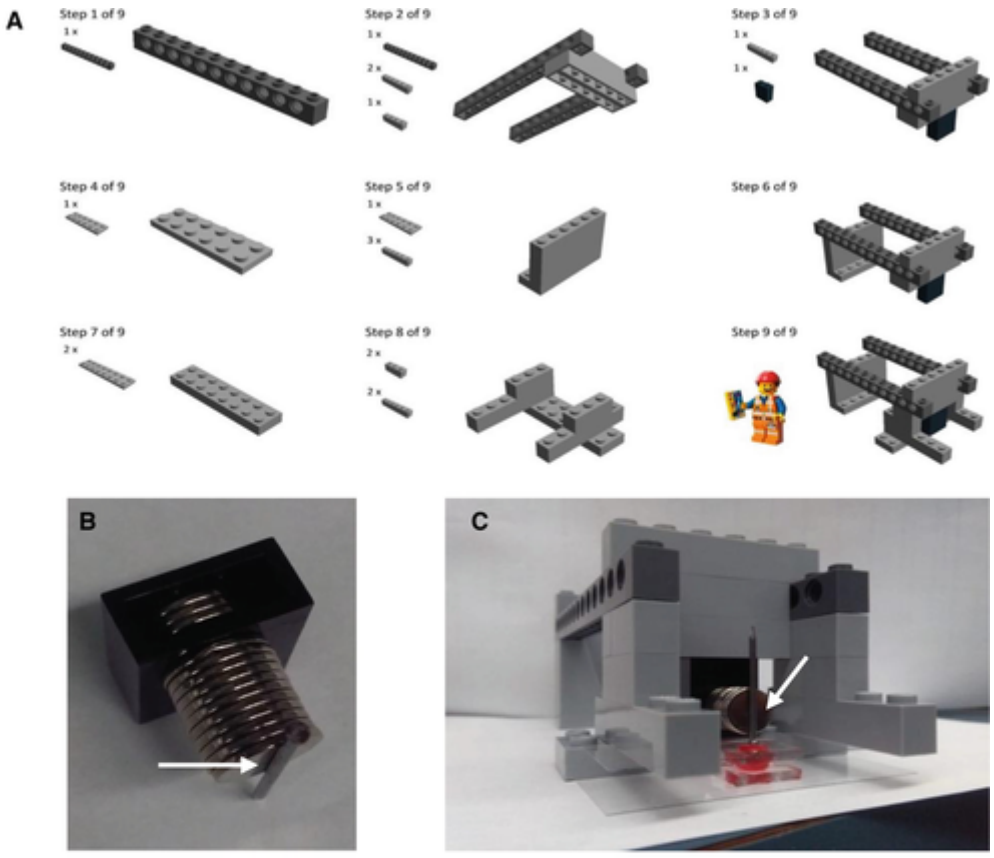
2.3. Microscopy and Illumination System

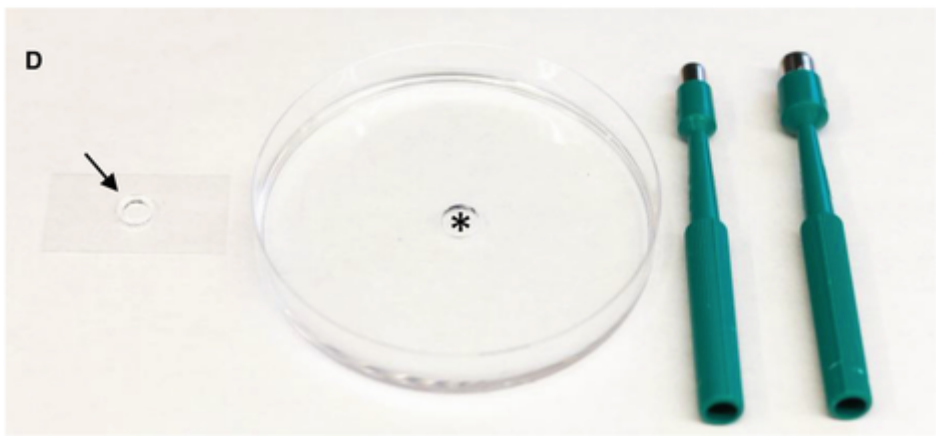
1. Inverted laser scanning microscope equipped with a 475–488-nm low-powered laser, 10×, 0.3NA microscope objective, power meter (*see* **Note 1**). The microscope needs to be equipped with fluorescence recovery after photobleaching (FRAP) or laser stimulation module or the equivalent.
2. 22 × 40 mm^2 plasma-treated glass coverslips thickness 1.5 (0.17 mm).
3. 35-mm plastic bottom imaging dish, height 12 mm.
4. 6- and 8-mm biopsy punches.
5. Ten (10) N35 magnets.
6. 1-mm diameter head iron nail.
7. Magnet holder. List of suggested Lego bricks (Fig. 3a):
   (a) 2× 4211428
   (b) 8× 4211393
   (c) 2× 4211406
   (d) 1× 324526
   (e) 2× 4210963
   (f) 1× 4211394
   (g) 2× 4211452

Fig. 3

Lego bricks chamber holder. (**a**) List of suggested Lego bricks. (**b**) Magnet disposition. (**c**) Final setup holding a collection-donor chamber pair. (**d**) Six-mm PDMS chamber. Six-mm and 8-mm biopsy punches

were used to carve a PDMS ring in a PDMS stock plate (star). The PDMS ring is placed on a 22 ° 40 mm^2 glass coverslip (arrow). (**a–c**) Adapted from Ref. [9]. Creative Commons Attribution License





Follow the instructions to build the magnet holder with Lego bricks as described in Fig. 3. Place three magnets inside the designated Lego brick and seven outside the block (Fig. 3b). Place the nail on the magnets, head to the floor (Fig. 3b–c, arrow) (*see* **Note 2**).

3. Methods

Except for the laser-assisted membrane biotinylation (*see* **step 3.4**), all procedures must be performed in a biological safety cabinet to prevent sample contamination.

3.1. PDMS 6-mm Chamber Preparation

Using the 8-mm biopsy punch, carve a disk in the PDMS stock plate, and then carefully place the 6-mm punch at the center of the disk to chisel out the chamber. Transfer the PDMS ring onto a plasma-treated plastic coverslip, and press until it adheres to the surface (Fig. 3d). Ensure that no air bubbles remain between the PDMS and coverslip (*see* **Notes 2** and **3**). Coat the chamber according to sample requirements.

3.2. Sample Preparation

The day prior to the sorting, plate the cells in the 6-mm PDMS chamber. Cell confluency is strictly dependent on the desired experimental output. Given the small volume used, we suggest placing the 6-mm chamber inside a 100-mm dish together with a 35-mm plate (without a lid) that is filled with water to prevent medium evaporation and sample to dry.

3.3. Laser Calibration

Place the power meter probe close to the microscope objective, and adjust the laser power to 100 µW (*see* **Note 6**).

3.4. Cell Selection and Laser-Assisted Membrane Biotinylation

1. Incubate the sample with B4F at a concentration of 40 µg/mL (i.e., 1:50 dilution in cell culture medium) (*see* **Note 7**).
2. Place the culture on the microscope stage and select the cells of interest (*see* **Note 4**). Illumination intensity and exposure time must be kept as low as possible during acquisition to avoid unspecific photobleaching and labeling.
3. Illuminate only the cells of interest with the 475–488-nm laser for 2 s with 100 µW (*see* **Notes 5** and **8**).
4. Rinse the culture five times with 1× PBS (*see* **Note 6**).
5. After completing **step 4**, proceed to single cell labelling (*see* Subheading 3.5) if the aim of the experiment is labeling the cells of interest. To capture illuminated cells, skip the Cy5-streptavidin labeling and move to magnetic capture (*see* Subheading 3.6).

3.5. Single Cell Labeling

1. Incubate the sample for 10 min at 37 °C with 50 µg/mL of Cy5-Streptavidin (i.e., 1:50 of stock solution in cell culture medium) (*see* **Note 9**).
2. Rinse the culture five times with 1× PBS.
3. Image the cells using the appropriate filter set.

3.6. Magnetic Capture

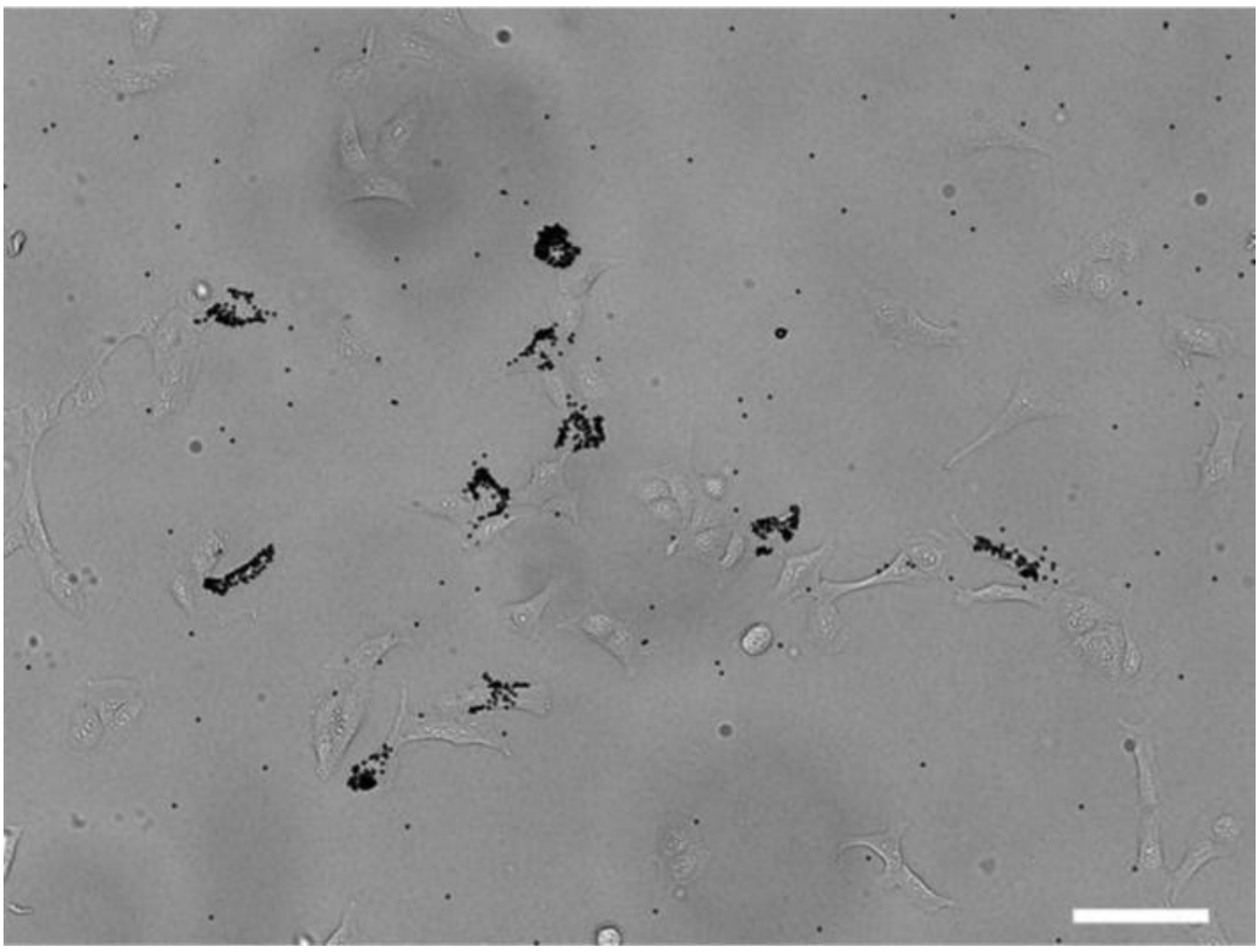
1. Add 3 µL of streptavidin-coated ferromagnetic beads to the 6-mm PDMS chamber. Put a N35 magnet under the well to attract the beads down toward the cells. Use the magnet to move the beads across the whole chamber area to maximize the binding.
2. Resuspend the beads by pipetting up and down, and immediately place three (3) N35 magnets approximately 1 mm above the chamber to bring the beads to the surface and quickly aspirate them. Repeat to remove as many of the unbound beads as possible.
3. Rinse the chamber three times with 1× PBS.
4. Observe the sample under a bright field microscope to verify the attachment of the beads (Fig. 4).
5. Incubate the cells in 80 µL of trypsin-EDTA for 5 min or until the cells have become round. Gently pipet to completely detach the cells, avoiding air bubble formation.
6. Fill the collection chamber with 80 µL of trypsin-EDTA, place it on the magnetic holder, and rapidly flip it symmetrically on top of the donor chamber to bring the two trypsin drops to merge (*see* **Note 10**).
7. Wait for 4 min to allow positive cells to attract to the magnets, while the negative cells are retained in the donor chamber by gravity (*see* **Note 11**).
8. Flip the holder and inspect the sample under the microscope. If negative cells remain, repeat **steps 6** and **7** (up to four times) to increase the specificity of the capture. At the beginning of each sorting repeat, the collection chamber becomes the donor chamber for the next isolation. The last isolation must be

performed in a chamber coated according to sample requirements and filled with cell culture medium (*see* **Note 12**).

9. Incubate the cells as described in **step 3.1**. When the culture reaches approximately 60% confluency, transfer to an appropriate culture dish.

Fig. 4

Bright field image of U2OS labelled with ferromagnetic beads. CLaP was used to biotinylate 10 cells in the microscope field of view, followed by incubation with streptavidin-coated ferromagnetic beads. Scale bar 100 µm



4. Notes

1. If required, the objective magnification can be changed. In this case, the illumination power (P_x) must be adjusted in relation to the numerical aperture of the objective (NA_x) according to the following relation:

$$\frac{P_1}{NA_1^2} = \frac{P_2}{NA_2^2}$$

2. If a greater throughput is required, cells can be isolated from a larger imaging dish. In this scenario, a 3D-printed capture tool (*see* **Note 4** for detailed instructions) is suggested for the first isolation (Fig. 5), while the Lego bricks setup can be used for subsequent rounds (*see* Subheading 3.6, **step 8**). The volume of the ferromagnetic beads must be scaled up according to the size of the chamber used.

3. In CLaP, the chamber size is not crucial and can be easily modified. However, in scMOCA the chamber size must be optimized to generate a surface tension to prevent liquid from falling during the sorting steps; hence, it is critical to keep the diameter of the chamber at 6mm.

4. 3D-printed capture tool.

   Materials: 3D printer, ten N35 magnets, 1-mm head iron nail, four (4) 6′ long round-head carriage bolts, nine (9) ¼′ wing nuts, ten (10) washers, two (2) hexagonal ¼′ nuts, one (1) 1′ bolt, 14-mm diameter round glass coverslip, 4 × 6 × 0.25 inch plexiglass sheet, a drill with ¼″ and 11/64″ drill bit, sandpaper, and shoe glue.

1. Print the 3D model (Fig. 5a). The required files are available in supplementary materials. This step takes approximately 2 h.
2. Drill a ¼″ hole at the top and a 11/64″ hole at the bottom of the printed piece (Fig. 5a, arrows). Sand the inside of the holes and one side of the 14-mm glass coverslip. Glue the coverslip on the round part of the holder, sanded side in contact with the printed part (Fig. 5b, arrow). Let it dry for 24 h.
3. Place 5 N35 magnets in the hollow side of the piece and 5 on the outside (Fig. 5c, d). Arrange the nail as show in (Fig. 5c, d arrows): The head of the nail must go through the hole and touch the coverslip. Position a 1′ long bolt inside the other hole, and tighten it in place with two hexagonal nuts.
4. Drill one hole at the center of the plexiglass sheet and four at the corners (leave approximately10 mm from the edges to avoid cracks). Screw a ¼′ wing nut on each of the four 6′ long round-head carriage bolts at approximately 3′ of the end, wings in the direction of the round head. Place a washer on every screwed wingnut, and insert a bolt assembly at every corner of the plexiglass sheet. Tighten the plexiglass with a wing nut/washer pair per bolt. Add a washer to the bolt of the capturing tool, and insert it in the center hole of the plexiglass. Add a second washer and tighten it with a wing nut (Fig. 5e, arrow). Use the wing nuts to adjust the height of the capture tool to keep 6 mm distance from the donor chamber (Fig. 5f, g).
5. Cells of interest can be selected manually after a visual inspection or automatically with a software-guided analysis.
6. When choosing the illumination parameters, labeling efficiency and phototoxicity must be considered according to the sample type. The optimal setup allows efficient photobleaching and minimal cell damage. For high-throughput experiments, laser power can be increased to reduce illumination time (Fig. 6). Cell viability assays, e.g., calcein-AM and propidium iodide staining, may help in choosing the appropriate illumination parameters.
7. Fluorescein can be easily photobleached. To avoid nonspecific labeling, keep the sample in the dark.
8. This step can be performed by any FRAP or laser stimulation module or the equivalent. Another approach consists of reducing the laser scanning area from the confocal to the cell of interest.
9. The final concentration of streptavidin can be adjusted according to the sample being analyzed.
10. Cell detachment during the rinsing steps can occur more often when cells are cultured in small wells. On the other hand, proper rinsing of free B4F is key for the success of the labeling and most importantly of the sorting. When cell detachment is a concern, we suggest to gently replace approximately 90% of the medium of the 6-mm chamber for 10 times. Alternatively, the chamber can be immersed in a large volume of PBS (up to 30 mL) for 5 min.
11. During the isolation step, abrupt movements should be avoided to reduce vibrations of the workspace that could favor negative cell transfer to the collection chamber.
12. For the success of the isolation, the distance between the chambers must be 6mm to maximize the movement of positive cells toward the receiving chamber while minimizing the transfer of the negative ones.
13. To favor the growth of sorted cells, it is possible to incubate them in conditioned medium [12]. When plating the cells the day prior to the sorting, collect the medium form the culture AQ1 and filter using a

0.22-μm filter. The day of the sorting, fill the final collection chamber with the same conditioned media. The enrichment of secreted factors will help cells grow.

Fig. 5

3D-printed capturing tool. (**a** and **b**) 3D-printed piece. (**c**) Magnets and nail arrangement. (**d**) Assembly of the 6′ long round-head carriage bolts, the wing nuts, and the washer. (**e**) Final assembly of the holder

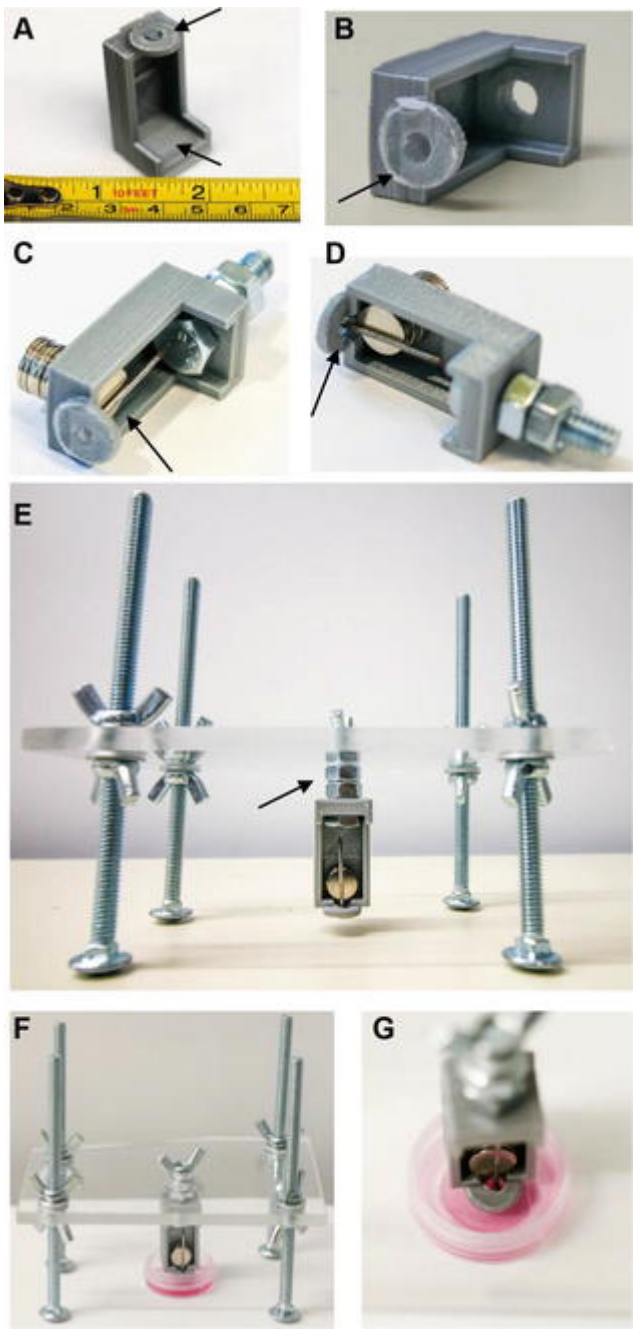
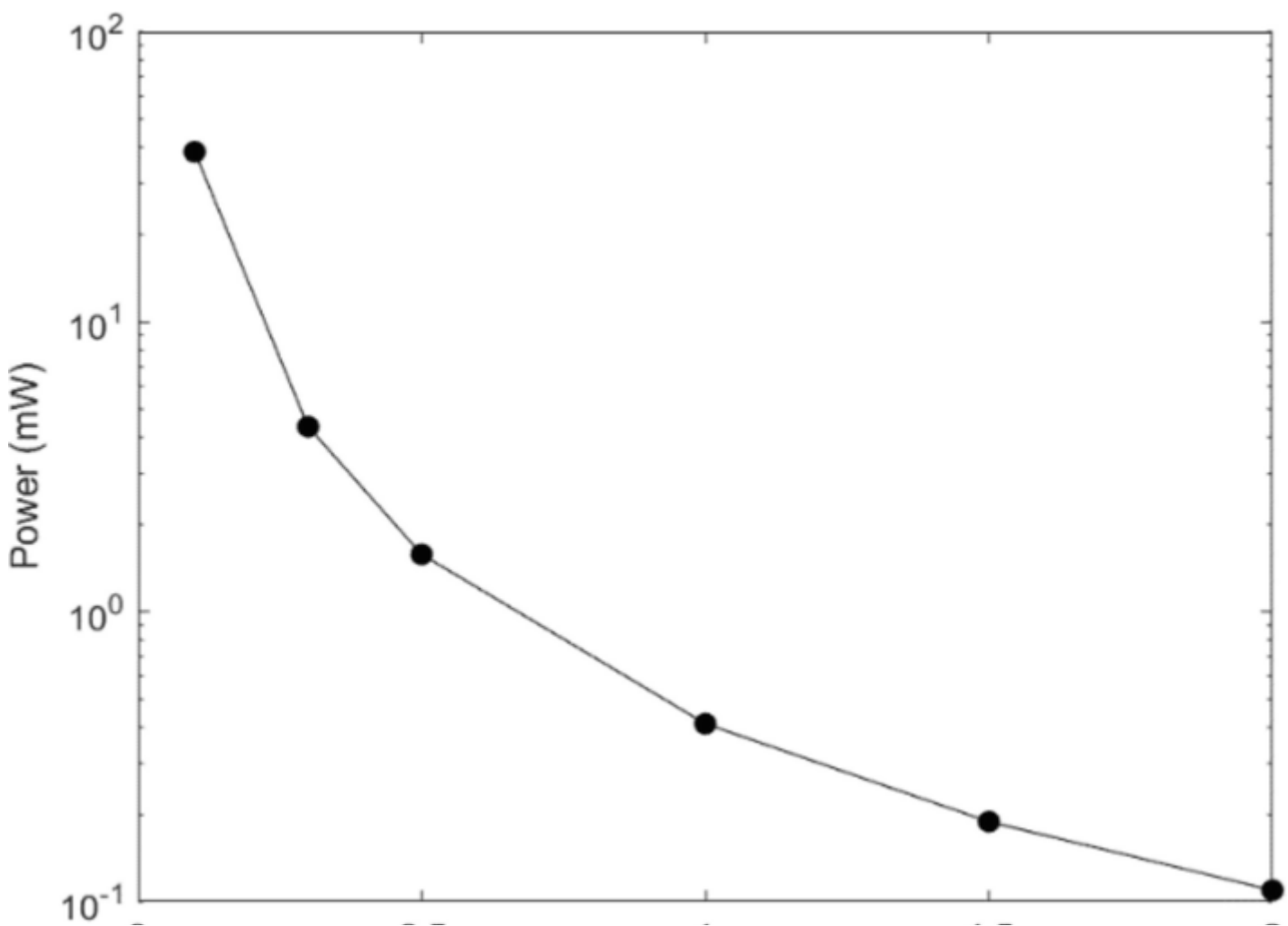
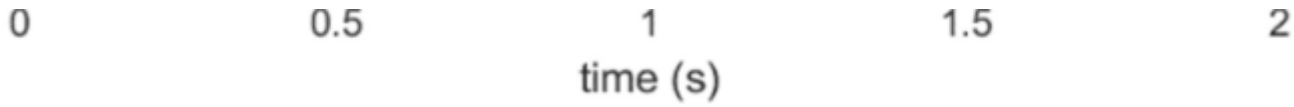


Fig. 6

Calculated curve of equivalent amount of photobleached fluorescein molecules at a concentration of 40 μg/mL of B4F for a given combination of laser power (ordinate) and illumination time (abscissa)





Electronic Supplementary Material

Supplementary Materials (STEP 322 kb)

References

1. Lawson DA, Kessenbrock K, Davis RT et al (2018) Tumour heterogeneity and metastasis at single-cell resolution. Nat Cell Biol 20:1349–1360

2. Gatenby RA, Brown JS (2020) Integrating evolutionary dynamics into cancer therapy. Nat Rev Clin Oncol 17:675–686

3. Suva ML, Tirosh I (2019) Single-cell RNA sequencing in cancer: lessons learned and emerging challenges. Mol Cell 75:7–12

4. Kolodziejczyk AA, Kim JK, Svensson V et al (2015) The technology and biology of single-cell RNA sequencing. Mol Cell 58:610–620

5. Vickovic S, Eraslan G, Salmen F et al (2019) High-definition spatial transcriptomics for in situ tissue profiling. Nat Methods 16:987–990

6. Stahl PL, Salmen F, Vickovic S et al (2016) Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. Science 353:78–82

7. Srivatsan SR, Regier MC, Barkan E et al (2021) Embryo-scale, single-cell spatial transcriptomics. Science 373:111–117

8. Binan L, Mazzaferri J, Choquet K et al (2016) Live single-cell laser tag. Nat Commun 7:11636

9. Binan L, Belanger F, Uriarte M et al (2019) Opto-magnetic capture of individual cells based on visual phenotypes. Elife 8:e25439

10. Belisle JM, Mazzaferri J, Costantino S (2014) Laser-assisted adsorption by photobleaching. Methods Cell Biol 119:125–140

11. Holden MA, Cremer PS (2003) Light activated patterning of dye-labeled molecules on surfaces. J Am Chem Soc 125:8074–8075

12. Huang B, Bird S, Kemble R et al (1990) Effects of culture density, conditioned medium and feeder cultures on microspore embryogenesis in Brassica napus L. cv. Topas. Plant Cell Rep 8:594–597