

1 **SUPPLEMENTARY INFORMATION**

2 **Spatial microfluidic holographic integrated platform for**  
3 **label-free and high-dimensional analysis of cancer**  
4 **heterogeneity**

5 **Jiayi Shi<sup>1</sup>, Huijing Teng<sup>1</sup>, Ziyi Zhang<sup>1</sup>, Yanping Liu<sup>1</sup>, Dan Gao<sup>1</sup>,**  
6 **Jianglei Di<sup>2</sup>, Zijian Yang<sup>3</sup>, Ping Su<sup>1,\*</sup>, Ying Tan<sup>1,\*</sup>, Jianshe Ma<sup>1</sup>**

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7 <sup>1</sup> Shenzhen International Graduate School, Tsinghua University, Shenzhen, 518055, PR  
8 China

9 <sup>2</sup> Institute of Advanced Photonics Technology, School of Information Engineering,  
10 Guangdong University of Technology, Guangzhou, 510006, PR China

11 <sup>3</sup> Department of Breast and Thyroid Surgery, Peking University Shenzhen Hospital,  
12 Shenzhen, 518034, PR China

13 Correspondence to: Ying Tan, tan.ying@sz.tsinghua.edu.cn;

14 Ping Su, su.ping@sz.tsinghua.edu.cn

15 The supplementary information contains notes, figures and tables in support of the main  
16 body. It discusses specific topics:

17 **Supplementary Note 1. Theoretical simulation and experimental validation of**  
18 **spatial hydrodynamic focusing**

19 **Supplementary Note 2. Design and construction of digital holographic**  
20 **microscopy (DHM) setup**

21 **Supplementary Note 3. Reconstruction process of quantitative phase**  
22 **reconstruction process of DHM**

23 **Supplementary Note 4. List of the hierarchical QPM phase features**

24 **Supplementary Note 5. Feature engineering for machine learning**

25 **Supplementary Note 6. Evaluation of the classification performance of machine**  
26 **learning to predictively classify multiple cancer cell types**

27 **Supplementary Note 7. Evaluation of the classification performance of machine**  
28 **learning to predictively classify breast cancer subtypes**

29 **Supplementary Note 8. Identification and enumeration of breast cancer cells in**  
30 **human blood**

31 **Supplementary Note 9. Comparison of the state-of-art QPM-IFCs**

## **Supplementary Note 1. Theoretical simulation and experimental validation of spatial hydrodynamic focusing**

We first validated the efficacy of spatial hydrodynamic focusing within a microfluidic chip designed and fabricated by ourselves. Specifically, the sample flow rate was fixed at 1  $\mu\text{L}/\text{min}$ , and the width or height of the focused sample flow was measured by varying the sheath-to-sample flow rate ratio. To begin with, numerical simulations were conducted using COMSOL Multiphysics. The results (see Supplementary Table 1 and 2) indicated that as the sheath-to-sample flow rate ratio increased, the height and width of the central sample flow decreased, which represented an enhancement in focusing intensity. The variation in focused flow width or height with the flow rate ratio was found to conform to a one phase exponential decay function fit.

Then, the actual focusing effect within the chip was experimentally assessed using the fluorescent dye Rhodamine B. Rhodamine B was dissolved in deionized water to serve as the sample flow, with deionized water also used as the sheath flow. The focusing of the sample flow was observed under an inverted fluorescence microscope, and fluorescence images were captured (see Supplementary Fig. 1 and 2). Obviously, there was a proportional relationship between the sheath-to-sample flow rate ratio and the focusing effect. The fluorescence intensity was quantified using the grayscale values of the pixel points in the images, and the full width at half maximum (FWHM) was employed as a metric for the width and height of the focused fluorescent sample flow. The measured values of width and height were found to be in good agreement with the simulation values, also fitting a one phase exponential decay relationship (see Supplementary Fig.1 and 2).

Furthermore, the stability of the focusing was experimentally verified, ensuring that the focusing state remained consistent within a wide injection flow rate range under the same flow rate ratio. This was done by fixing the sheath-to-sample flow rate ratio and varying the sample injection flow rate, then measuring the width and height of the focused sample. The results demonstrated that over a broad flow rate range, the focusing state (characterized by the size and the intensity distribution of the fluorescent sample flow) was maintained consistently under the same flow rate ratio ( $R_v = 0.25$ ,  $R_h = 2$ ) (see Supplementary Fig. 3, Table 3 and 4).

**Supplementary Table 1. Results of the efficacy validation of vertical hydrodynamic focusing through simulations and experiments**

| $R_v$ | $H_s(\text{exp}) / \mu\text{m} (V_{\text{sample}} = 1 \mu\text{L} \cdot \text{min}^{-1})$ |      |      |      |      |      |           | $H_s(\text{sim})$ |
|-------|-------------------------------------------------------------------------------------------|------|------|------|------|------|-----------|-------------------|
|       | E1                                                                                        | E2   | E3   | E4   | E5   | Mean | Deviation | $/\mu\text{m}$    |
| 0     | 35.0                                                                                      | 35.0 | 35.1 | 35.0 | 34.9 | 35.0 | 0.07      | 35.0              |
| 0.125 | 21.4                                                                                      | 22.1 | 21.0 | 20.9 | 21.3 | 21.3 | 0.47      | 21.3              |
| 0.25  | 16.2                                                                                      | 16.1 | 16.2 | 17.3 | 16.9 | 16.5 | 0.53      | 16.9              |
| 0.50  | 12.5                                                                                      | 11.8 | 11.8 | 13.3 | 12.5 | 12.4 | 0.62      | 12.3              |
| 0.75  | 10.3                                                                                      | 10.3 | 10.3 | 10.1 | 9.9  | 10.2 | 0.18      | 9.7               |
| 1.00  | 7.4                                                                                       | 7.4  | 7.1  | 8.0  | 8.0  | 7.6  | 0.40      | 8.0               |
| 1.25  | 7.7                                                                                       | 7.4  | 6.6  | 7.2  | 6.8  | 7.1  | 0.44      | 6.7               |
| 1.50  | 6.0                                                                                       | 6.0  | 5.6  | 5.6  | 5.4  | 5.7  | 0.27      | 5.9               |

$R_v$  is the velocity ratio of the vertical sheath flows to the sample flow,  $H_s(\text{exp})$  is the experimental focused sample flow's height,  $H_s(\text{sim})$  is the simulated focused sample flow's height,  $V_{\text{sample}}$  is the velocity ratio of the sample flow, E1-5 represent 5 independent repeated experiments.

**Supplementary Table 2. Results of the efficacy validation of horizontal hydrodynamic focusing through simulations and experiments**

| $R_h$ | $W_s(\text{exp}) / \mu\text{m} (V_{\text{sample}} = 1 \mu\text{L} \cdot \text{min}^{-1})$ |      |      |       |       |       |           | $W_s(\text{sim})$ |
|-------|-------------------------------------------------------------------------------------------|------|------|-------|-------|-------|-----------|-------------------|
|       | E1                                                                                        | E2   | E3   | E4    | E5    | Mean  | Deviation | $/\mu\text{m}$    |
| 0     | 100.0                                                                                     | 99.8 | 99.9 | 100.3 | 100.1 | 100.0 | 0.19      | 100.0             |
| 0.50  | 35.6                                                                                      | 35.7 | 34.2 | 35.7  | 34.9  | 35.2  | 0.66      | 35.0              |
| 1.00  | 23.5                                                                                      | 23.3 | 22.3 | 23.5  | 23.3  | 23.2  | 0.50      | 22.7              |
| 1.50  | 16.3                                                                                      | 17.4 | 16.3 | 16.3  | 16.5  | 16.6  | 0.48      | 16.9              |
| 2.00  | 14.3                                                                                      | 14.3 | 13.3 | 13.3  | 12.6  | 13.6  | 0.73      | 13.5              |
| 2.50  | 11.2                                                                                      | 11.2 | 11.2 | 10.2  | 11.6  | 11.1  | 0.52      | 11.2              |
| 3.00  | 9.2                                                                                       | 9.2  | 9.2  | 9.2   | 9.7   | 9.3   | 0.22      | 9.6               |
| 3.50  | 9.2                                                                                       | 8.7  | 8.7  | 8.2   | 8.7   | 8.7   | 0.35      | 8.4               |
| 4.00  | 8.2                                                                                       | 7.1  | 8.2  | 7.6   | 7.8   | 7.8   | 0.46      | 7.5               |
| 4.50  | 6.8                                                                                       | 6.8  | 6.8  | 6.8   | 6.8   | 6.8   | 0.00      | 6.7               |
| 5.00  | 5.8                                                                                       | 5.8  | 5.8  | 5.8   | 5.8   | 5.8   | 0.00      | 6.1               |

$R_h$  is the velocity ratio of the horizontal sheath flows to the sample flow,  $W_s(\text{exp})$  is the experimental focused sample flow's width,  $W_s(\text{sim})$  is the simulated focused sample flow's width,  $V_{\text{sample}}$  is the velocity ratio of the sample flow, E1-5 represent 5 independent repeated experiments.

**Supplementary Table 3. Results of the stability validation of vertical hydrodynamic focusing through simulations and experiments**

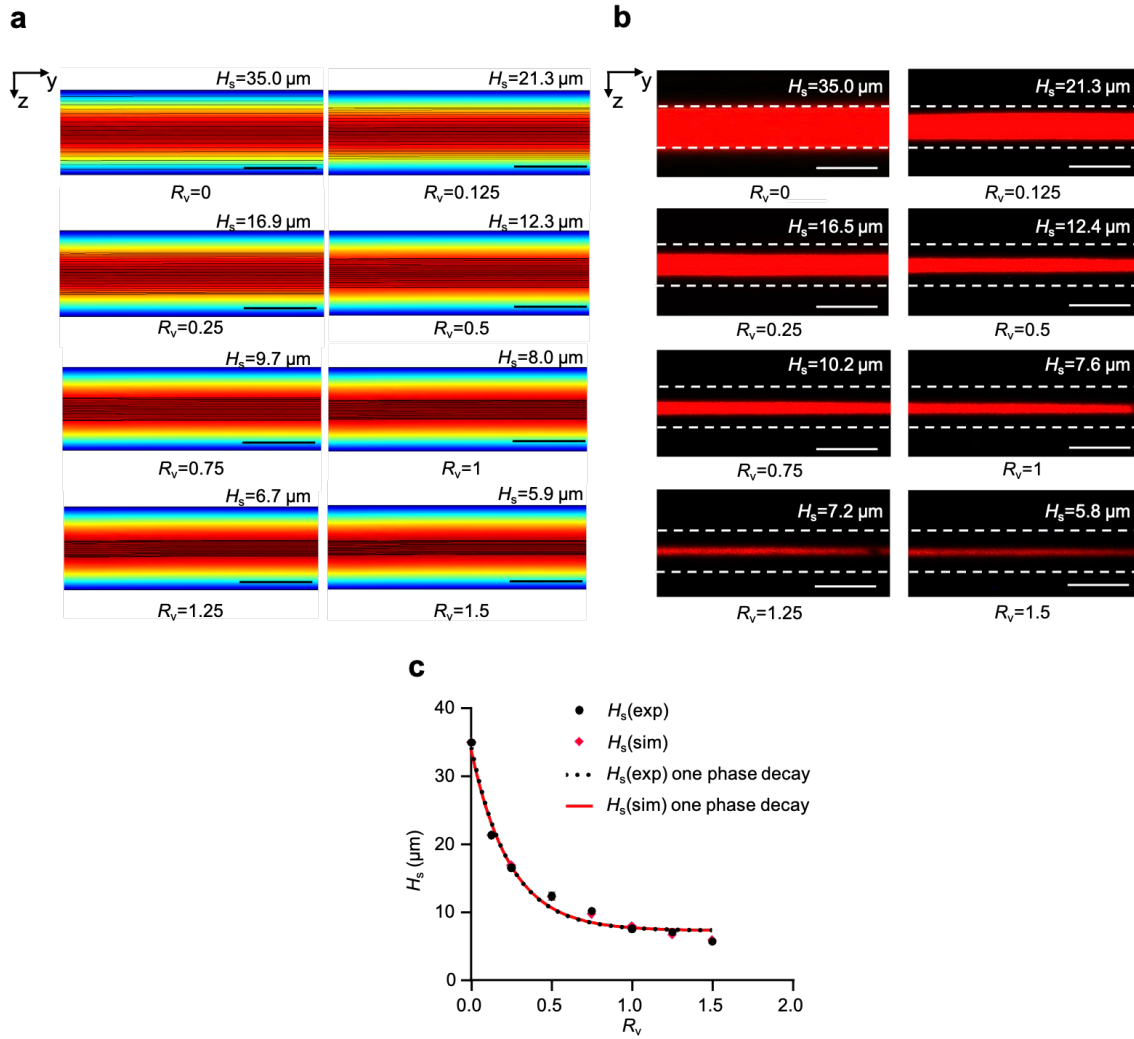
| $V_{\text{(sample)}}$<br>/ $\mu\text{L} \cdot \text{min}^{-1}$ | $H_s(\text{exp}) / \mu\text{m} (R_v = 0.25, R_h = 2)$ |      |      |      |      |      |           | $H_s(\text{sim})$<br>/ $\mu\text{m}$ |
|----------------------------------------------------------------|-------------------------------------------------------|------|------|------|------|------|-----------|--------------------------------------|
|                                                                | E1                                                    | E2   | E3   | E4   | E5   | Mean | Deviation |                                      |
| 1.0                                                            | 16.1                                                  | 17.6 | 16.9 | 17.7 | 17.9 | 17.2 | 0.74      | 16.9                                 |
| 2.5                                                            | 15.5                                                  | 17.6 | 16.2 | 17.3 | 17.5 | 16.8 | 0.93      | 16.9                                 |
| 5.0                                                            | 15.8                                                  | 17.6 | 16.9 | 16.1 | 16.1 | 16.5 | 0.74      | 16.9                                 |
| 7.5                                                            | 16.1                                                  | 17.3 | 16.1 | 17.7 | 17.9 | 17.0 | 0.87      | 16.9                                 |
| 10.0                                                           | 16.2                                                  | 16.1 | 16.5 | 16.9 | 17.7 | 16.7 | 0.65      | 16.9                                 |
| 12.5                                                           | 16.9                                                  | 16.2 | 16.9 | 16.9 | 16.1 | 16.6 | 0.41      | 16.9                                 |
| 15.0                                                           | 16.2                                                  | 17.7 | 16.9 | 16.5 | 16.1 | 16.7 | 0.65      | 16.9                                 |
| 17.5                                                           | 15.5                                                  | 16.9 | 16.9 | 16.7 | 16.5 | 16.5 | 0.58      | 16.9                                 |
| 20.0                                                           | 15.4                                                  | 16.9 | 17.7 | 16.5 | 16.1 | 16.5 | 0.86      | 16.9                                 |

$R_v$  is the velocity ratio of the vertical sheath flows to the sample flow,  $H_s(\text{exp})$  is the experimental focused sample flow's height,  $H_s(\text{sim})$  is the simulated focused sample flow's height,  $V_{\text{(sample)}}$  is the velocity ratio of the sample flow, E1-5 represent 5 independent repeated experiments.

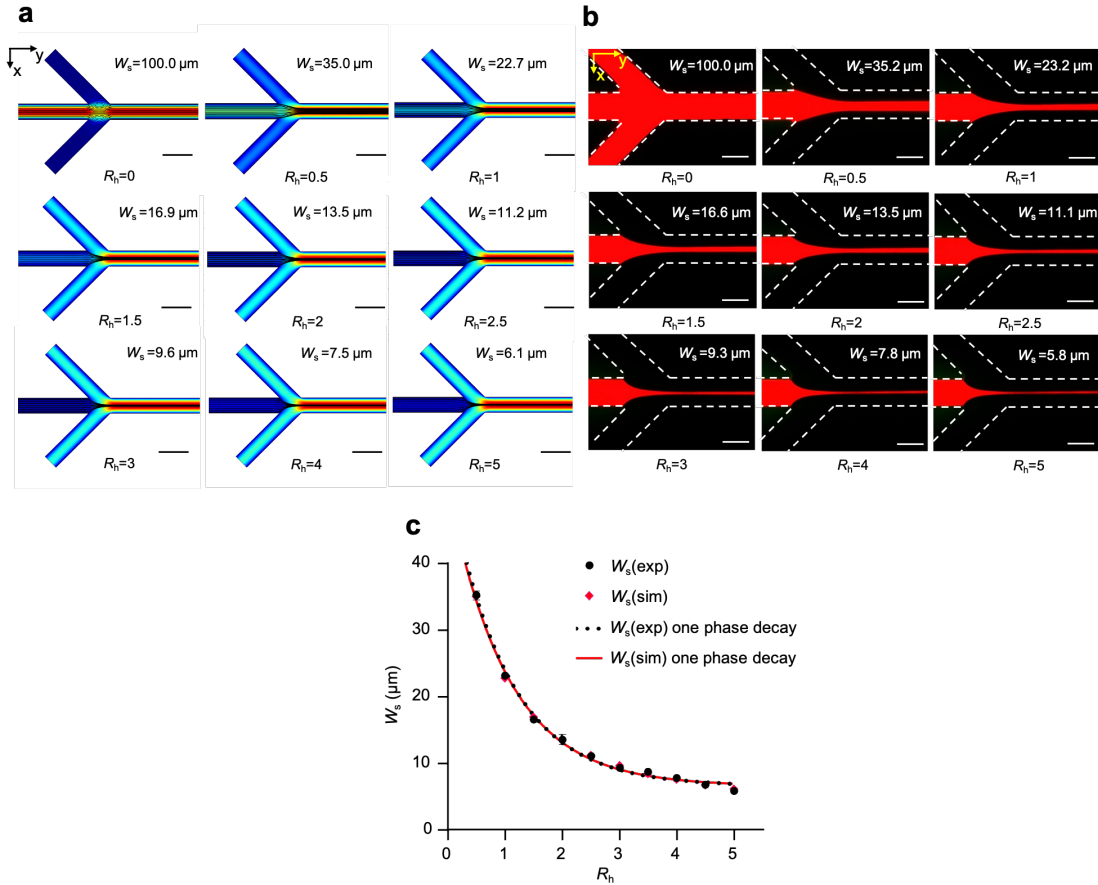
**Supplementary Table 4. Results of the stability validation of horizontal hydrodynamic focusing through simulations and experiments**

| $V_{\text{(sample)}}$<br>/ $\mu\text{L} \cdot \text{min}^{-1}$ | $W_s(\text{exp}) / \mu\text{m} (R_v = 0.25, R_h = 2)$ |      |      |      |      |      |           | $W_s(\text{sim})$<br>/ $\mu\text{m}$ |
|----------------------------------------------------------------|-------------------------------------------------------|------|------|------|------|------|-----------|--------------------------------------|
|                                                                | E1                                                    | E2   | E3   | E4   | E5   | Mean | Deviation |                                      |
| 1.0                                                            | 14.7                                                  | 13.7 | 15.1 | 14.6 | 13.7 | 14.4 | 0.63      | 13.5                                 |
| 2.5                                                            | 15.1                                                  | 14.7 | 13.7 | 14.6 | 14.2 | 14.5 | 0.53      | 13.5                                 |
| 5.0                                                            | 14.6                                                  | 14.6 | 12.8 | 13.7 | 14.6 | 14.1 | 0.80      | 13.5                                 |
| 7.5                                                            | 14.6                                                  | 13.7 | 12.8 | 13.7 | 12.9 | 13.5 | 0.73      | 13.5                                 |
| 10.0                                                           | 14.7                                                  | 13.8 | 12.8 | 13.7 | 14.6 | 13.9 | 0.77      | 13.5                                 |
| 12.5                                                           | 12.8                                                  | 12.8 | 13.7 | 14.7 | 13.7 | 13.5 | 0.79      | 13.5                                 |
| 15.0                                                           | 12.8                                                  | 13.7 | 12.8 | 11.9 | 11.9 | 12.6 | 0.75      | 13.5                                 |
| 17.5                                                           | 13.7                                                  | 12.8 | 11.9 | 12.8 | 12.8 | 12.8 | 0.64      | 13.5                                 |
| 20.0                                                           | 11.9                                                  | 13.7 | 12.8 | 12.8 | 11.9 | 12.6 | 0.75      | 13.5                                 |

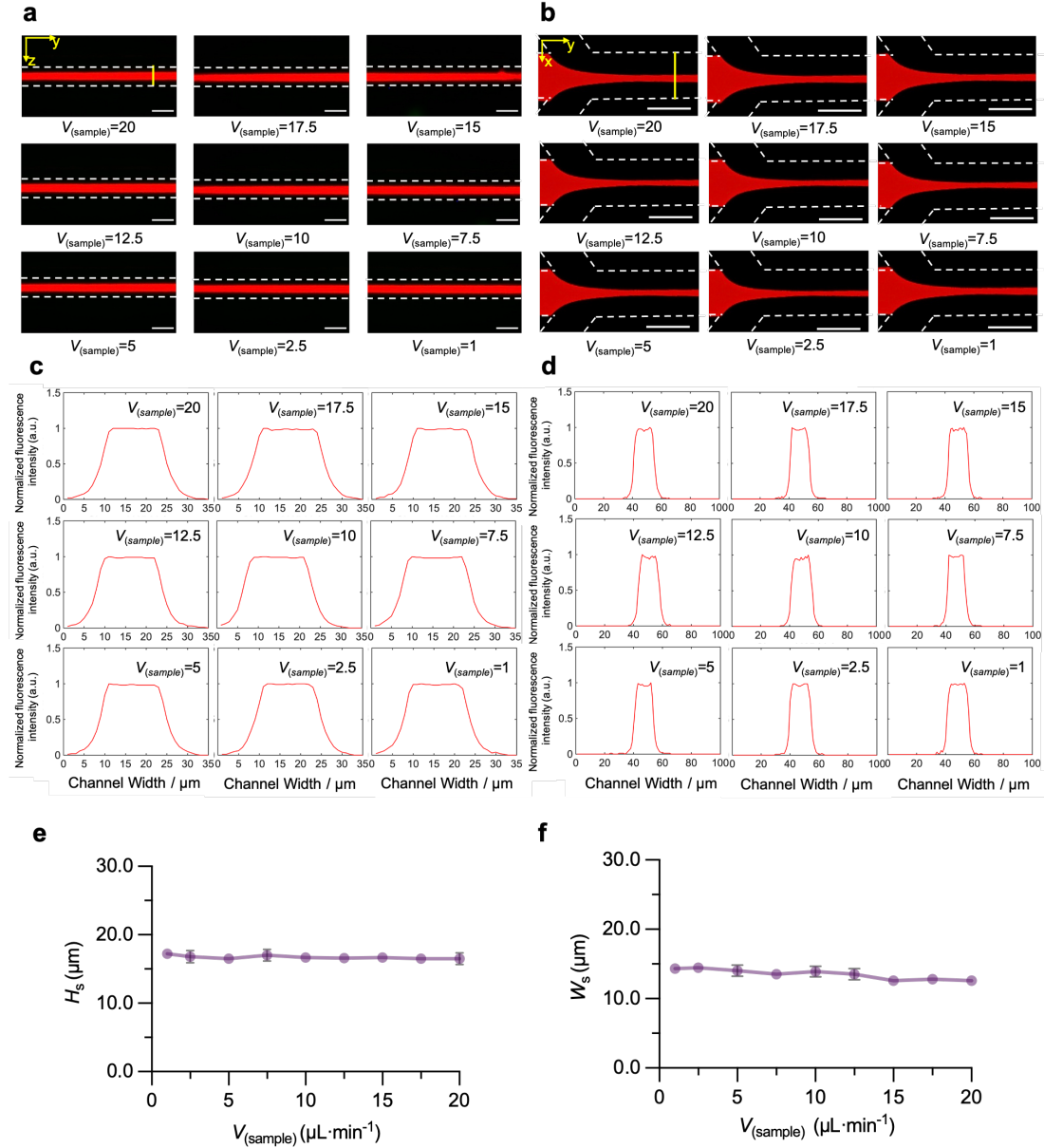
$R_h$  is the velocity ratio of the horizontal sheath flows to the sample flow,  $W_s(\text{exp})$  is the experimental focused sample flow's width,  $W_s(\text{sim})$  is the simulated focused sample flow's width,  $V_{\text{(sample)}}$  is the velocity ratio of the sample flow, E1-5 represent 5 independent repeated experiments.



86 **Supplementary Fig. 1 Visualization results of the efficacy validation of vertical**  
87 **hydrodynamic focusing through simulations and experiments.** The sample injection speed  
88 is 1  $\mu\text{L}/\text{min}$  for both simulation and experiment. **a** CFD simulation of vertical focusing ( $y$ - $z$   
89 plane).  $R_v$  represents the velocity ratio of vertical sheath flow to sample flow. The focused  
90 sample flow is visualized by velocity streamlines (black), of which the width is measured in  
91 COMSOL and numerically annotated. **b** Experimental results of vertical focusing ( $y$ - $z$  plane).  
92 The red band represents the sample flow containing fluorescent dye Rhodamine B. The dashed  
93 lines represent the upper and lower boundaries of microchannel, which are actually 35  $\mu\text{m}$  apart.  
94 Each  $H_s$  is the mean of 5 independent repeated experiments under the corresponding  $R_v$   
95 (recorded on Supplementary Table 1). **c** Statistic conclusion of vertical focusing. It shows the  
96 change in  $H_s$  with  $R_v$ .  $H_s(\text{sim})$  represents the CFD simulation data, and  $H_s(\text{exp})$  is the experiment  
97 value. Data are means from 5 independent repeated experiments; error bars show SD. Scale  
98 bars: 30  $\mu\text{m}$  (**a**), 50  $\mu\text{m}$  (**b**). Source data are provided as a Source Data file.



**Supplementary Fig. 2 Visualization results of the efficacy validation of horizontal hydrodynamic focusing through simulations and experiments.** The sample injection speed is 1  $\mu\text{L}/\text{min}$  for both simulation and experiment. For horizontal focusing test,  $R_v$  is preliminarily fixed at 0.25. **a** CFD simulation of horizontal focusing ( $x$ - $y$  plane).  $R_h$  represents the velocity ratio of horizontal sheath flow to the combined flow of two vertical sheath flows and sample flow. The focused sample flow is visualized by velocity streamlines (black), of which the width is measured in COMSOL and numerically annotated. **b** Experimental results of horizontal focusing ( $x$ - $y$  plane). The red band represents the sample flow containing fluorescent dye Rhodamine B. The dashed lines represent the lateral boundaries of microchannel, which are actually 100  $\mu\text{m}$  apart. Each  $W_s$  is the mean of 5 independent repeated experiments under the corresponding  $R_h$  (recorded on Supplementary Table 2). **c** Statistic conclusion of horizontal focusing. It shows the change in  $W_s$  with  $R_h$ .  $W_s(\text{sim})$  represents the CFD simulation data, and  $W_s(\text{exp})$  is the experiment value. Data are means from 5 independent repeated experiments; error bars show SD. Scale bars: 200  $\mu\text{m}$  (**a**), 100  $\mu\text{m}$  (**b**). Source data are provided as a Source Data file.

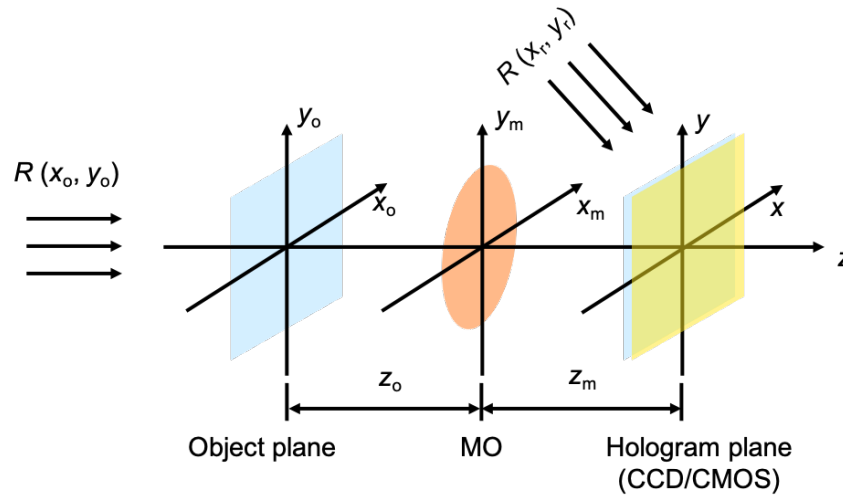


**Supplementary Fig. 3 Experimental results of the stability validation of spatial hydrodynamic focusing.**  $R_v$  is fixed at 0.25 and  $R_h$  is 2. The sample injection speed varies from 1 to 20  $\mu\text{L}/\text{min}$ . (a) and (b) are the visible results of vertical (a) and horizontal (b) focusing. The red band represents the sample flow containing fluorescent dye Rhodamine B. The white dashed lines represent boundaries of microchannel. The yellow dash lines indicate the following analysis positions. (c) and (d) show the corresponding normalized fluorescence distributions from which the full width at half maximum (FWHM) was estimated to quantify  $H_s$  and  $W_s$ . The statistic result of the  $H_s$  and  $W_s$  variation patterns with different sample injection rate  $V_{\text{sample}}$  are demonstrated in (e) and (f) respectively. Data are means from 5 independent repeated experiments; error bars show SD. Scale bars: 50  $\mu\text{m}$  (a), 100  $\mu\text{m}$  (b). Source data are provided as a Source Data file.

## Supplementary Note 2. Design and construction of digital holographic microscopy (DHM) setup

### 2.1 Principle of DHM measurement

Digital holographic microscopy (DHM) emerges from the combination of digital holography and optical microscopy<sup>1</sup>. This technology utilizes photoelectric detectors such as charge-coupled devices (CCDs) and complementary metal-oxide-semiconductor (CMOS) sensors to record the interferogram formed between the magnified object wave and a reference wave. This interferogram, also known as a hologram, is subsequently numerically reconstructed on a computer to extract the wavefront information of tiny objects. To further simplify the holographic reconstruction, the photoelectric detector can be positioned directly at the image plane<sup>2</sup>. This strategy eliminates the need for the computation of optical diffraction process within the computer, thereby accelerating the reconstruction procedure. The theory underlying this method is demonstrated in Supplementary Fig. 4.



**Supplementary Fig. 4 Recording principle of pre-amplified off-axis image-plane DHM.**

Object plane ( $x_o$ - $y_o$  plane) is where the object is located. MO ( $x_m$ - $y_m$  plane) is where the optical system is located. Hologram plane ( $x$ - $y$  plane) is where the hologram is recorded using a CCD or CMOS sensor.  $z_o$  is the distance between object plane and MO, while  $z_m$  is between MO and hologram plane.  $O(x_o, y_o)$  is the object beam.  $R(x_r, y_r)$  is the reference beam.

In the typical DHM measurement<sup>3</sup>, the object is placed in the object plane ( $x_o$ - $y_o$  plane), of which the transmission light is then collected by the optical system. The lens, with a focal length  $f$ , forms an image of the object in the MO plane ( $x_m$ - $y_m$  plane). CCD or CMOS sensor captures the off-axis hologram generated by the object beam and a

reference beam. The distance between the object and MO plane ( $z_0$ ) satisfies the object-image conjugate relationship with the distance between the MO and hologram plane ( $z_m$ ), described as the Supplementary Equation (1):

$$\frac{1}{z_0} + \frac{1}{z_m} = \frac{1}{f} \quad (1)$$

Under the paraxial approximation, after free-space propagation and the phase transformation imparted by the lens, the complex amplitude distribution of the object wavefront on the CCD detection plane  $O(x, y)$  is described as follows:

$$O(x, y) = \exp \left[ \frac{j\pi}{\lambda} \left( \frac{z_0 + z_m}{z_m^2} \right) (x^2 + y^2) \right] \cdot O_0 \left( -\frac{x}{M}, -\frac{y}{M} \right) \quad (2)$$

where  $O_0(x, y)$  is the the complex amplitude distribution of the geometric image of the object optical field.  $\lambda$  denotes illumination wavelength,  $M = z_m / z_0$  is the magnification.

The reference beam originates from a point source ( $x_r, y_r, z_r$ ). Upon reaching the CCD, the complex amplitude distribution of this wavefront  $R(x, y)$  is established as follows<sup>4</sup>:

$$R(x, y) = \exp \left\{ \frac{j\pi}{\lambda z_r} [(x - x_r)^2 + (y - y_r)^2] \right\} \quad (3)$$

After the interference of reference and object wave, the intensity distribution of the hologram  $I(x, y)$  is

$$I(x, y) = |O(x, y)|^2 + |R(x, y)|^2 + O(x, y)R^*(x, y) + R(x, y)O^*(x, y) \quad (4)$$

where  $*$  stands for the complex conjugate.

The first two terms of the Supplementary Equation (4) do not provide spatial information about the object optical field and represent the so-called zero-order diffraction. The last two terms provide the spatial frequency of the recorded hologram and are recognized as being responsible for the twin-images. Spectral filtering technique can be utilized to eliminate the first two zero-order images and the fourth twin image. Finally, subsequent calculations are performed solely on the third term,

169 corresponding to the original image:

$$\begin{aligned}
 OR^*(x, y) = & \exp \left[ \frac{j\pi}{\lambda} \left( \frac{z_0 + z_m}{z_m^2} - \frac{1}{z_r} \right) (x^2 + y^2) \right] \\
 & \cdot \exp \left[ \frac{j\pi}{\lambda} (xx_r + yy_r) \right] O_0 \left( -\frac{x}{M}, -\frac{y}{M} \right)
 \end{aligned} \tag{5}$$

170 Specifically, to isolate the original image, a Fourier transform<sup>5</sup> ( $FT$ ) is applied to  
 171 the hologram  $I(x, y)$ , yielding its spectrum. The spectral components of the original  
 172 image are extracted and translated to the coordinate center. An inverse Fourier  
 173 transform ( $FT^{-1}$ ) is then applied to finally recover the complex amplitude distribution  
 174 of the object optical field  $O'(x, y)$ .

$$\begin{aligned}
 O'(x, y) = & FT^{-1} \{ \{ FT[I(x, y)] \} \}_{filter} \\
 = & \exp \left[ \frac{j\pi}{\lambda} \left( \frac{z_0 + z_m}{z_m^2} - \frac{1}{z_r} \right) (x^2 + y^2) \right] \\
 & \cdot \exp \left[ \frac{j\pi}{\lambda} (xx_r + yy_r) \right] O_0 \left( -\frac{x}{M}, -\frac{y}{M} \right)
 \end{aligned} \tag{6}$$

175 The intensity distribution of  $O'(x, y)$  is calculated as:

$$I_i(x, y) = |O'(x, y)|^2 = |O(x, y)|^2 \tag{7}$$

176 The corresponding phase  $\varphi(x, y)$  is demonstrated as:

$$\varphi(x, y) = \arctan \left\{ \frac{Im[O'(x, y)]}{Re[O'(x, y)]} \right\} \tag{8}$$

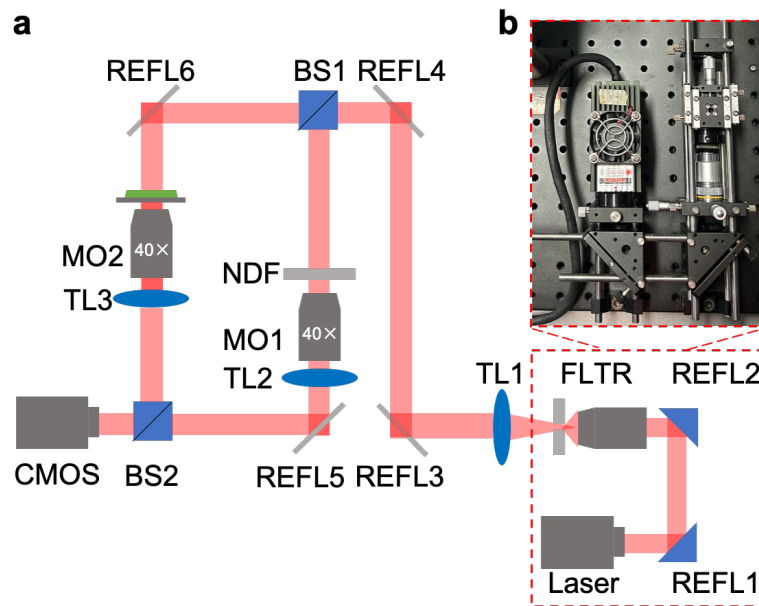
177 where  $Re[\bullet]$  and  $Im[\bullet]$  represents the real and imaginary parts of the complex  
 178 amplitude distribution, respectively.

## 179 2.2 Demonstration of DHM setup

180 An inverted, pre-amplified off-axis image-plane DHM system has been designed  
 181 and constructed, the structure of which is shown in Supplementary Fig. 5. The system  
 182 utilizes a laser with an emission wavelength of 632.8 nm, which is first directed through  
 183 a beam path alignment mirror assembly consisting of reflector 1 (REFL1) and 2  
 184 (REFL2), to ensure central incidence along the optical axis. Next, the laser passes  
 185 through a spatial filter composed of a microscope objective and a pinhole (FLTR),  
 186 followed by a tube lens (TL1), to generate a uniform parallel light spot. This light is

then adjusted to an inverted microscope light source direction, from above downward, after reflection by reflector 3 (REFL3) and 4 (REFL4).

Afterward, the laser light is divided by beam splitter 1 (BS1) into an object beam and a reference beam. The object beam, after passing through reflector 6 (REFL6), traverses the sample. Once magnified by the microscope objective (MO2), this object wave interferes with the reference beam on the CCD recording plane, as combined by beam splitter 2 (BS2), resulting in the hologram. The reference optical path employs an identical microscope objective (MO1) to the object path, and the distances from MO1 and MO2 to the CCD are adjusted to be approximately equal. The tube lenses (TL2 and TL3) positioned behind the microscope objectives are utilized to fine-tune any discrepancies in magnification between the two optical paths, thereby minimizing the impact of secondary phase distortions and balancing the intensities of the beams. This setting reduces the complexity of the optical setup and the computational load for subsequent digital compensation processing in the computer. Additionally, to compensate for the attenuation of light intensity in the object path by the sample, a neutral density filter (NDF) is introduced into the reference path.



**Supplementary Fig. 5 Design of the pre-amplified off-axis image-plane DHM system.** **a** DHM light path. REFL: reflective mirror. FLTR: filter. TL: tube lens. BS: beam splitter. NDF: neutral density filter. MO: microscope objective. **b** Schematic diagram of the laser incident unit.

### 2.3 Resolution of DHM system

In a pre-amplified DHM imaging system, the resolution is jointly determined by

208 the parameters of the CCD (or CMOS) and the microscope objective. The resolution of  
209 our DHM was finally determined by the numerical aperture (NA) of the microscope  
210 objective, conforming to the Rayleigh criterion<sup>6</sup> as follows:

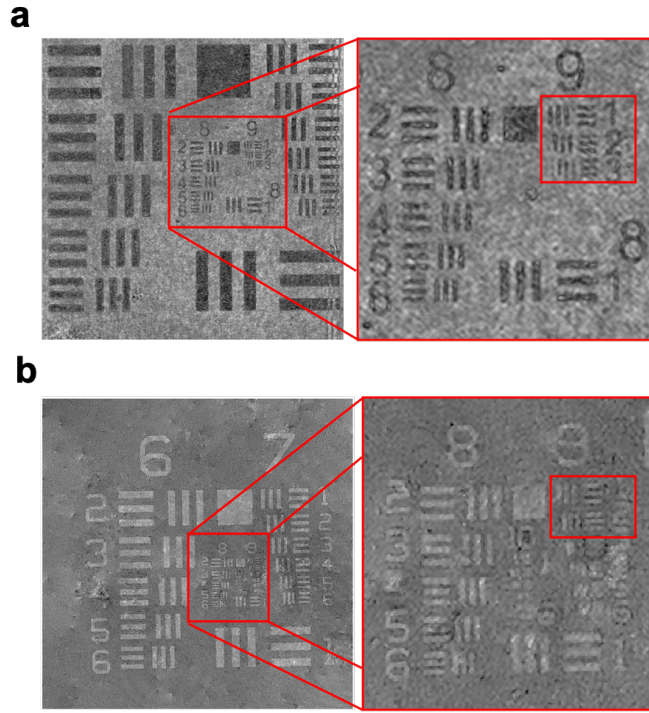
$$\delta_{xy} = \frac{0.61\lambda}{NA} \quad (9)$$

$$\delta_z = \frac{2\lambda n}{NA^2} \quad (10)$$

211 where  $\lambda$  is the illumination wavelength,  $n$  is the medium's refractive index in which  
212 the objective lens is located.  $\delta_{xy}$  and  $\delta_z$  represent the lateral resolution and axial  
213 resolution respectively.

214 We utilized a 40× objective lens (Olympus) with a numerical aperture (NA) of  
215 0.75 in the air. A single-frequency solid-state laser was employed as the light source,  
216 with a wavelength of 632.8 nm. The resolution of this DHM, as calculated, is 0.52  $\mu\text{m}$   
217 and 2.25  $\mu\text{m}$  in the lateral and axial directions. Theoretically, by substituting a light  
218 source with a shorter wavelength or an objective lens with a higher NA, we can further  
219 improve the resolution.

220 We used the USAF 1951 resolution target as the object for imaging  
221 (Supplementary Fig. 6). The experimentally-measured resolution of reconstructed  
222 amplitude and phase image was 0.78 and 0.87  $\mu\text{m}$  respectively, which has reached the  
223 sub-micrometer level and is very close to the theoretical value. Considering that the  
224 dimensions of the target cells and microparticles all fall within the range of 10 to 30  
225  $\mu\text{m}$ , the current resolution is deemed sufficient to meet the requirements for particle  
226 analysis.



**Supplementary Fig. 6 Spatial resolution measurement of the DHM system. a** Reconstructed amplitude of the USAF 1951 resolution target. The smallest resolvable information corresponds to the third line pair of the ninth group of the resolution target, of which the actual width is  $0.78 \mu\text{m}$ . **b** Reconstructed phase of the USAF 1951 resolution target. The smallest resolvable information corresponds to the second line pair of the ninth group of the resolution target, of which the actual width is  $0.87 \mu\text{m}$ .

#### 2.4 Noise measurement of DHM system

Phase noise, which is intrinsically linked to phase fluctuations, is a critical parameter in the assessment of image quality and stability of DHM measurement. It is quantified by determining the standard deviation of phase fluctuations in the phase map without any sample<sup>7</sup>.

The spatiotemporal phase fluctuation  $\delta\varphi(x, y, t)$  is defined as

$$\delta\varphi(x, y, t) = \varphi(x, y, t) - \varphi_0(x, y, t) \quad (11)$$

where  $\varphi(x, y, t)$  is the phase of the background at certain time being,  $\varphi_0(x, y, t)$  is the initial phase background for the same scene.

Further, the spatiotemporal phase fluctuation can be characterized both spatially and temporally<sup>8</sup> as

$$\delta\varphi(x, y) = \varphi_t(x, y) - \varphi_t(x_0, y_0) \quad (12)$$

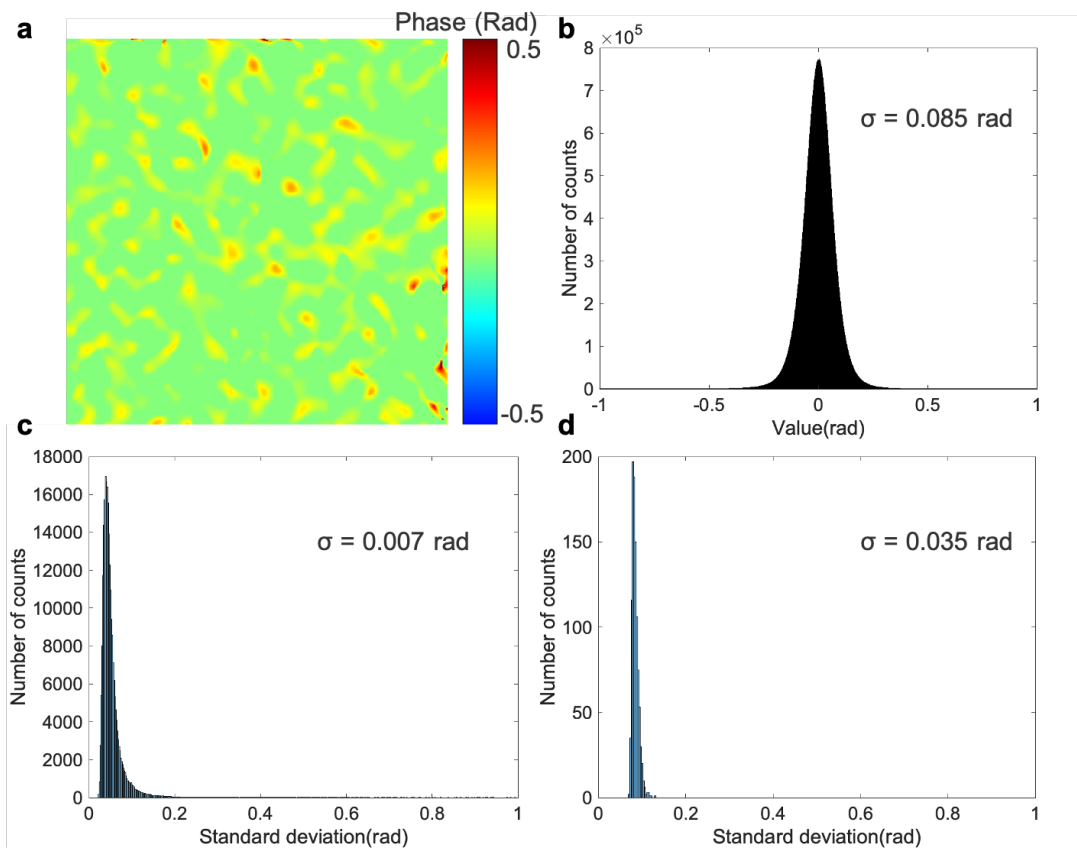
$$\delta\varphi(t) = \varphi_{xy}(t) - \varphi_{xy}(t_0) \quad (13)$$

where  $\delta\varphi(x, y)$  represents the spatial phase fluctuation at a fixed time, defined as the difference between a point's phase value and that of a reference point within the same phase map.  $\delta\varphi(t)$  represents the temporal phase fluctuation at a fixed spatial location, defined as the change in phase over time for that point.

To quantify the noise of our DHM system, our approach to measuring the spatiotemporal phase noise involved capturing a series of background holograms (496 pixels  $\times$  496 pixels) at 10-second intervals over a period of approximately 2.8 hours, resulting in a total of 1000 images. Using the first image of the sequence as a reference, we determined the phase fluctuation for each subsequent image. Supplementary Fig. 7a corresponds to the phase fluctuation in the first frame of this series. Supplementary Fig. 7b shows the histogram distribution of the overall spatiotemporal phase fluctuation which had a standard deviation of 0.085 rad. This value represents the overall spatiotemporal noise in our system.

We began by analyzing the spatial noise. Supplementary Fig. 7c shows the histogram distribution of spatial noise, with a mean of 0.085 rad and a standard deviation of 0.007 rad. Given that the average phase height of typical cells we measure is around 3-5 rad, we believe this level of noise is significantly smaller than the cell's phase height, and thus, the impact of this noise on our measurements should be minimal. Next, we analyzed the temporal noise. Supplementary Fig. 7d illustrates the histogram distribution of temporal noise. The results show a mean of 0.053 rad, with a standard deviation of 0.035 rad. This is considerably lower than the 0.2 rad standard deviation reported for typical Mach-Zehnder configurations in DHM<sup>9, 10</sup>, demonstrating the high stability of our measurement system.

In conclusion, our measurements indicate that our DHM system is capable of achieving high stability and sensitivity in phase distribution, with the noise levels being well below what would significantly affect our ability to accurately measure cell phase.



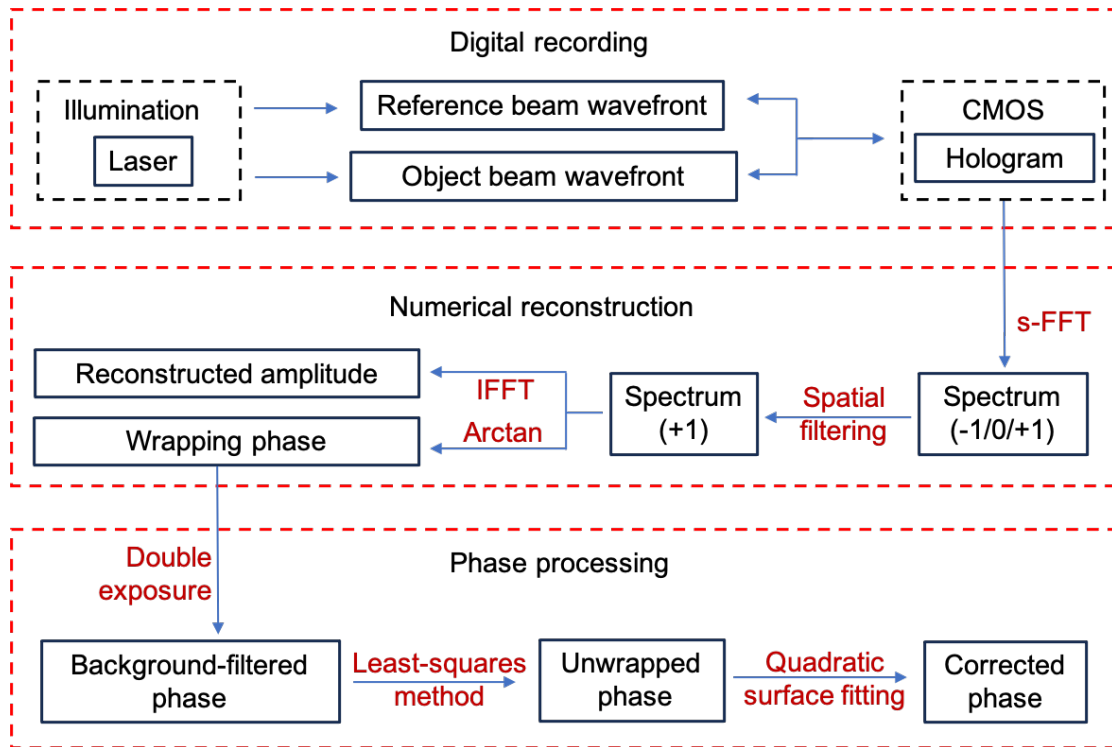
**Supplementary Fig. 7 Noise measurement of our DHM.** **a** Spatial noise distribution of background at first frame. **b** Spatiotemporal noise histogram of 1,000 frames in approximately 2.8 h time-series. **c** Histogram of the standard deviation of spatial noise. **d** Histogram of the standard deviation of temporal noise. Source data are provided as a Source Data file.

## **Supplementary Note 3. Reconstruction process of quantitative phase reconstruction process of DHM**

### **3.1 Demonstration of QPM reconstruction process**

As shown in Supplementary Fig. 8, the process of digital holographic quantitative phase reconstruction can be divided into three main parts: digital recording, numerical reconstruction and phase processing. Digital recording refers to using our off-axis DHM system to record the hologram of target object in focus. Then, in our study based on the image plane holography, the numerical reconstruction only requires a single fast Fourier transform of the hologram to distribute spectra, and successively using spatial filtering to extract the spectrum of the target image (i.e. +1<sup>st</sup> spectrum). Finally, the intensity and phase of the object optical wavefront can be calculated using an inverse Fourier transform followed by an arctangent operation.

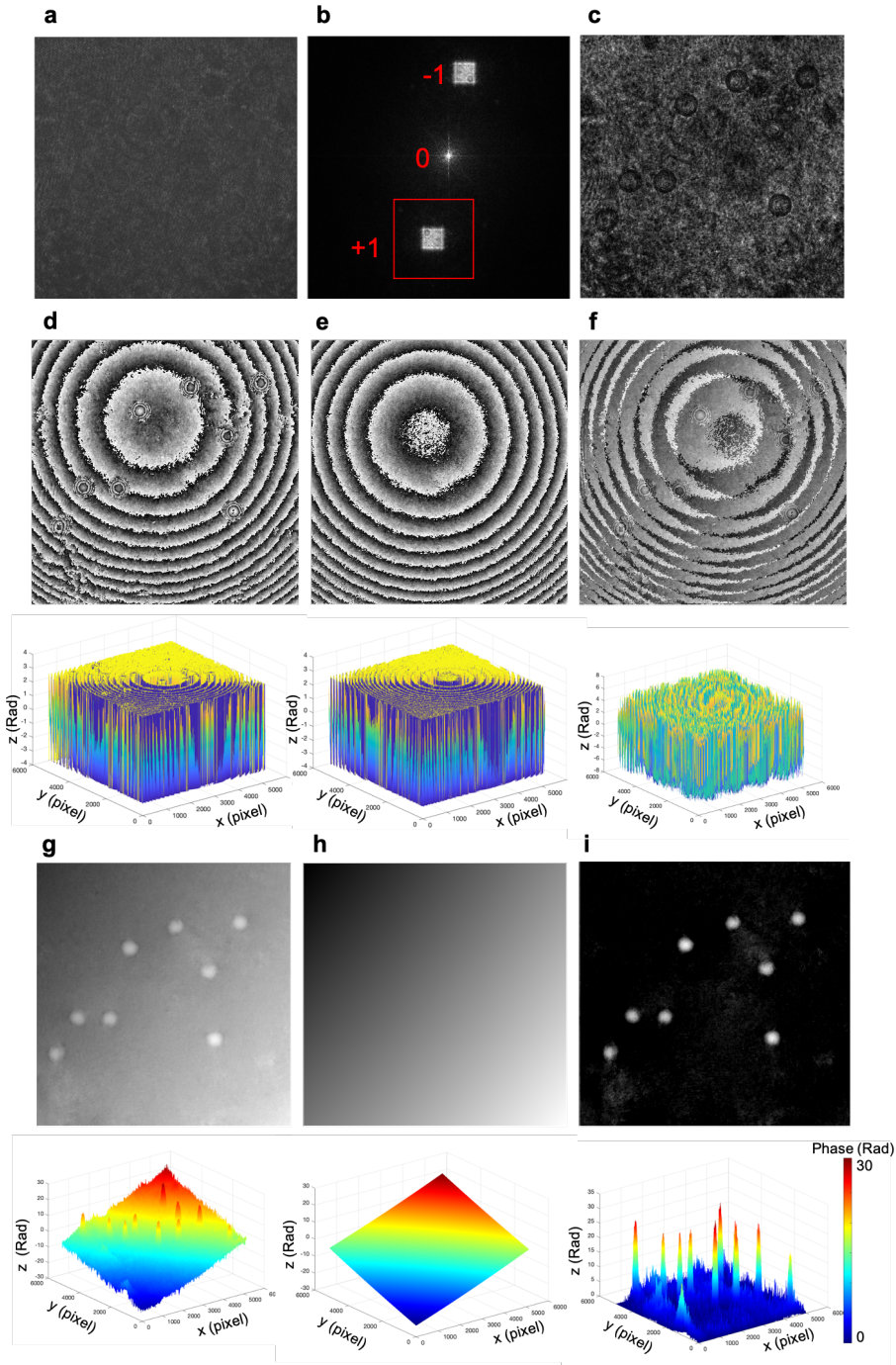
We next use the double exposure method<sup>11, 12</sup> for phase filtering to reduce noise. This method involves recording holograms for both the sample and the background, and subsequently eliminates background interference by subtracting the background phase from the reconstructed sample phase. However, the holographic phase obtained currently is still wrapped in the range  $[-\pi, \pi]$ , which can't be used to characterize the distribution of the surface shape of an object. To obtain the true, continuously varying phase distribution, we employ the least-squares phase unwrapping algorithm<sup>13</sup>. This algorithm addresses the discrete Poisson equation by incorporating the discrete cosine transform (DCT), thereby yielding the unwrapped phase data in the least-squares sense, which is both rapid and robust. Additionally, we notice that the spherical curvature mismatch between the object and the reference beam, coupled with inherent optical aberrations, results in significant tilt, bend, and higher-order phase aberrations, especially the second-order phase aberrations. Therefore, a method of quadratic surface fitting is applied to the continuous phase distribution to eliminate the second-order phase aberrations, and finally recover the object's phase distribution accurately.



**Supplementary Fig. 8 Process of digital holographic quantitative phase reconstruction.** s-FFT: a single fast Fourier transform. IFFT: inverse fast Fourier transform. Arctan: arctangent operation.

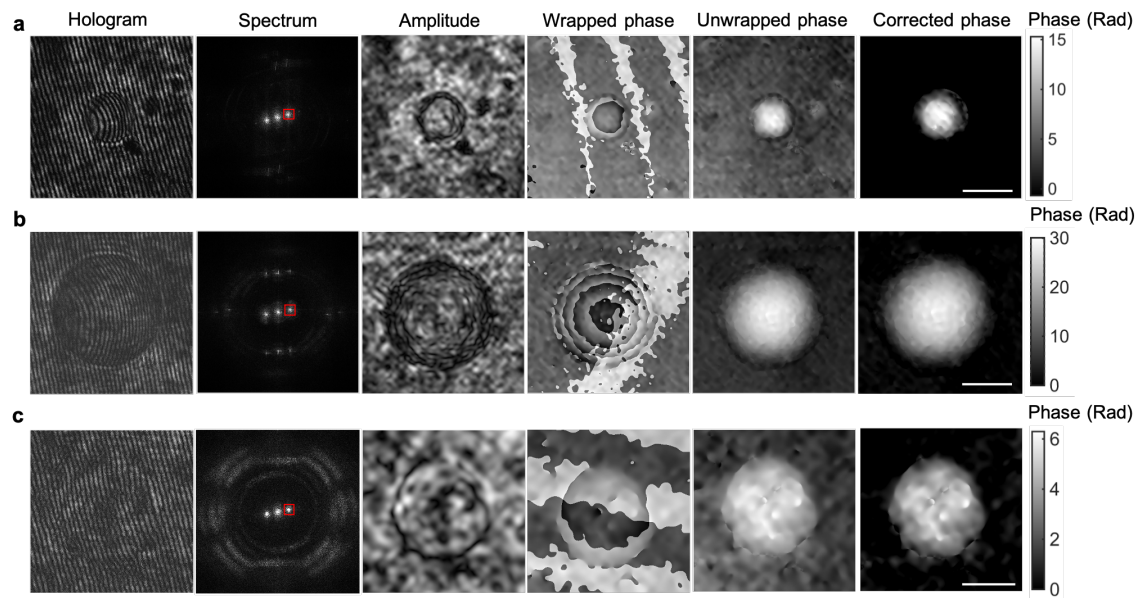
### 3.2 Processing results of microspheres and cells

We first used on-chip, static polystyrene microspheres as samples to verify the entire process of amplitude and phase image reconstruction, thereby preliminarily confirming the feasibility of the algorithm. Polystyrene microspheres are widely used in the field of biochemistry. They are considered to be objects with a mixed amplitude and phase profile, characterized by a spherical shape and a uniform refractive index across their surface. It should be specifically noted that in this case, the CMOS sensor's resolution was  $5120 \text{ pixels} \times 5120 \text{ pixels}$ , allowing for the simultaneous visualization of multiple microspheres within the field of view, as shown in Supplementary Fig. 9.



**Supplementary Fig. 9 Results of digital holographic quantitative phase reconstruction.** **a** Holographic interferogram of the polystyrene microspheres. **b** Spectrum after Fourier transform, where the -1st, +1st, and 0th orders are separated. The red box indicates the window of the spatial filtering. **c** Reconstructed amplitude image obtained through inverse Fourier transform. **d** Wrapped sample phase obtained after arctangent operation. **e** Wrapped background phase. **f** Wrapped sample phase after background subtraction. **g** Unwrapped sample phase obtained through least-squares method. **h** Distortion phase obtained from quadratic surface fitting. **i** Corrected sample phase after distortion calibration.

We also present the reconstruction process and results of the microspheres and cells in the spatial hydrodynamic focusing state (see Supplementary Fig. 10). Optimal focusing for the 10  $\mu\text{m}$  microspheres was achieved with an  $R_v$  of 0.75, resulting in an  $H_s$  of 9.7  $\mu\text{m}$ , and an  $R_h$  of 3, yielding a  $W_s$  of 9.6  $\mu\text{m}$ . For the 20  $\mu\text{m}$  microspheres, the  $R_v$  was 0.125 with an  $H_s$  of 21.3  $\mu\text{m}$ , and the  $R_h$  was 1.25 with a  $W_s$  of 19.5  $\mu\text{m}$ . The hologram was captured by CMOS operating at a resolution of 496 pixels  $\times$  496 pixels and a frame rate of 30 fps. Following frequency domain filtering, numerical reconstruction, we obtained amplitude reconstruction images of microspheres or cells. After phase extraction, unwrapping, and distortion compensation, the final QPM phase images of microspheres or cells were successfully obtained.



**Supplementary Fig. 10 QPM reconstruction of in-focus microspheres and cells.** **a** The data processing to determine the phase of a 10  $\mu\text{m}$  polystyrene microsphere, from hologram to the final corrected phase (the same below). **b** The data processing to determine the phase of a 20  $\mu\text{m}$  polystyrene microsphere. **c** The data processing to determine the phase of an MCF-10A cell. The color bar denotes the scale of phase. Scale bars: 20  $\mu\text{m}$  (**a-c**).

### 3.3 QPM phase reconstruction speed

In terms of phase reconstruction speed, the reconstruction of a single-cell hologram (496 pixels  $\times$  496 pixels) can be completed within about 0.34 seconds using lab-written MATLAB codes on our current laptop equipped with a 2.8 GHz Intel Core i7 CPU and 16 GB of RAM. Here, we have listed the duration of each phase reconstruction step (see Supplementary Table 5), which is the average time obtained by

measuring the reconstruction time of 20 single cell holograms. Our phase reconstruction steps include: frequency filtering at 0.084 seconds, numerical reconstruction at 0.009 seconds, phase extraction at 0.004 seconds, phase unwrapping at 0.056 seconds, and phase correction at 0.189 seconds, totaling approximately 0.34 seconds. We would like to emphasize that this reconstruction time is potentially advantageous compared to the similar study<sup>14</sup>. Upon reviewing the literature, the authors listed the duration for a complete digital holographic image reconstruction (see Supplementary Table 5). They also used a digital holographic microscope in off-axis configuration employing a Mach-Zehnder interferometric configuration, making their study comparable to ours. In their traditional holographic reconstruction process, the phase reconstruction of a single-cell hologram (384 pixels  $\times$  384 pixels) took 7.71 seconds (their computer configuration was a 64Gb RAM Intel® Core™ i9-9900K CPU), including: holographic demodulation at 0.06 seconds, numerical refocusing at 4.46 seconds, phase extraction at 0.01 seconds, aberration compensation at 0.42 seconds, and denoising & unwrapping at 2.76 seconds. It is evident that numerical refocusing is the most time-consuming step, and our study's innovative integration help us to omit this step, thereby gaining a significant advantage in the reconstruction time of phase maps.

**Supplementary Table 5. Comparison of QPM phase reconstruction workflow and speed**

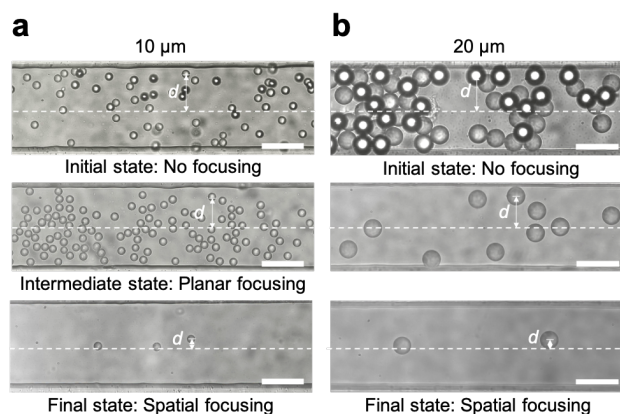
| QPM phase reconstruction workflow in our research                 |                          |                  |                         |                        |         |
|-------------------------------------------------------------------|--------------------------|------------------|-------------------------|------------------------|---------|
| Frequency filtering                                               | Numerical reconstruction | Phase extraction | Phase unwrapping        | Phase correction       | Total   |
| 0.084 s                                                           | 0.009 s                  | 0.004 s          | 0.056 s                 | 0.189 s                | 0.343 s |
| QPM phase reconstruction workflow in other research <sup>14</sup> |                          |                  |                         |                        |         |
| Holographic demodulation                                          | Numerical refocusing     | Phase extraction | Aberration compensation | Denoising & Unwrapping | Total   |
| 0.06 s                                                            | 4.46 s                   | 0.01 s           | 0.42 s                  | 2.76 s                 | 7.71 s  |

### 3.4 Enhanced spatial localization precision by spatial focusing

The final state, spatial focusing, is achieved with further constraining the sample flow in the horizontal direction. This step accomplishes the second goal of strictly localizing the single-cell flow to the center of the microchannel. As illustrated in Fig. 2d and e, planar focusing still leaves particles dispersed distributed within the plane. To

extract information from individual particles, extensive particle localization and image segmentation are required over a larger field-of-view, which will increase computational complexity and introduce potential errors. In contrast, under spatial focusing, particles are concentrated in a streamlined region at the channel center, enabling direct capture of single-particle images within a fixed central acquisition window ( $496 \text{ pixels} \times 496 \text{ pixels}$ ,  $31 \text{ } \mu\text{m} \times 31 \text{ } \mu\text{m}$ ).

We have now supplemented our analysis with quantitative data on spatial localization by measuring the average distance ( $d$ ) of the particles' centers from the microchannel centerline. As shown in Supplementary Fig. 11 and Table 6, compared with planar focusing, we find that spatial focusing significantly reduces this distance and tightens the distribution. Specifically, for  $10 \text{ } \mu\text{m}$  and  $20 \text{ } \mu\text{m}$  microparticles, the average distances of their centers from the channel centerline are  $(1.5 \pm 0.8) \text{ } \mu\text{m}$  and  $(1.6 \pm 1.0) \text{ } \mu\text{m}$ , respectively. The highly efficient spatial focusing not only proves spatial localization precision but also provides a stable and efficient foundation for automated single-cell image acquisition. Additionally, because we can directly capture single-cell holograms at fixed position, localization and segmentation algorithms are unnecessary, further streamlining our phase reconstruction process.



**Supplementary Fig. 11 Distance distribution of microparticles under different focusing states.** (a) and (b) show the observation of  $10 \text{ } \mu\text{m}$  (a) and  $20 \text{ } \mu\text{m}$  (b) polystyrene microspheres in microfluidic channels under difference focusing conditions in white light illumination.  $d$  measures the distance of single particle centers from the microchannel centerline. Scale bars:  $50 \text{ } \mu\text{m}$ .

**Supplementary Table 6. Distance distribution of microparticles under different focusing states**

| Focusing state                  | No focusing                   | Planar focusing               | Spatial focusing            |
|---------------------------------|-------------------------------|-------------------------------|-----------------------------|
| 10 $\mu\text{m}$ microparticles | $(27.1 \pm 13.1) \mu\text{m}$ | $(21.2 \pm 12.8) \mu\text{m}$ | $(1.5 \pm 0.8) \mu\text{m}$ |
| 20 $\mu\text{m}$ microparticles | $(27.9 \pm 13.0) \mu\text{m}$ | $(22.1 \pm 12.1) \mu\text{m}$ | $(1.6 \pm 1.0) \mu\text{m}$ |

### 3.5 Enhanced measurement consistency by spatial focusing

Spatial focusing also improves the consistency of amplitude and phase measurements. As illustrated in Fig. 2h-k, while planar focusing improves accuracy, it still results in higher variability in measurement values. Spatial focusing, through its dual constraints in both vertical and horizontal directions, reduces this variability. We introduced the Coefficient of Variation (CV), the ratio of the standard deviation to the mean, as an important metric for comprehensively evaluating the precision (accuracy) and stability (consistency) of a measurement system in IFC. Our calculations indicated that the CVs for amplitude and phase measurements reached 3.3%/4.3% (10  $\mu\text{m}$  microspheres) and 1.9%/4.7% (20  $\mu\text{m}$  microspheres) under spatial focusing. These values align with previous studies on the measurement consistency in flow cytometry where CVs are typically  $<5\%$ <sup>15</sup>, demonstrating the effectiveness of spatial focusing in achieving reliable and reproducible single-cell analysis. Thus, the quantification of CV values further highlights the necessity of spatial focusing.

**Supplementary Table 7. Measurement CV values for microparticles under different focusing states**

| Focusing state                            | No focusing | Planar focusing | Spatial focusing |
|-------------------------------------------|-------------|-----------------|------------------|
| 10 $\mu\text{m}$ microparticles amplitude | 5.9%        | 3.7%            | 3.3%             |
| 20 $\mu\text{m}$ microparticles amplitude | 7.9%        | 2.2%            | 1.9%             |
| 10 $\mu\text{m}$ microparticles phase     | 25.6%       | 6.2%            | 4.4%             |
| 20 $\mu\text{m}$ microparticles phase     | 16.1%       | 6.5%            | 4.7%             |

#### 410 **Supplementary Note 4. List of the hierarchical QPM phase features**

411       Totally 81 QPM phase features are extracted, including **14 bulk features**, **17 first-**  
412 **order histogram features** and **50 higher-order texture features**, detailed as follows:

413     1. Bulk features: These describe the geometric properties of the ROI and cell, including  
414 metrics such as volume, surface area, and sphericity. The phase information is also  
415 leveraged to estimate the cell's dry mass.

416     2. First-order histogram features: These capture the statistical attributes of different  
417 gray levels (voxel intensities) and their probability distributions within the ROI and cell,  
418 without accounting for spatial relationships. Calculated from the global gray-level  
419 histogram, these features include the maximum, minimum, mean, standard deviation,  
420 variance, skewness, kurtosis, entropy, and energy, among others. These characteristics  
421 reflect the symmetry, uniformity, and variations in local intensity distribution of the  
422 voxels.

423     3. Higher-order texture features: These describe the spatial relationships of gray-level  
424 values within the ROI and cell. They include matrices such as the Gray-Level Co-  
425 occurrence Matrix (GLCM), Gray-Level Gradient Co-occurrence Matrix (GGCM), and  
426 Gray-Level Run-Length Matrix (GLRLM). For this study, relevant features from these  
427 three matrices were selected.

**Supplementary Table 8. QPM phase features and their hierarchical categories**

|                                          |                             |                                            |                                          |                                        |                  |                        |                       |                                          |
|------------------------------------------|-----------------------------|--------------------------------------------|------------------------------------------|----------------------------------------|------------------|------------------------|-----------------------|------------------------------------------|
| Bulk features                            | 1. Area                     | 1 <sup>st</sup> order histogram features   |                                          | 28. Mean Absolute Deviation            | Texture Features | 55. Gradient Asymmetry |                       |                                          |
|                                          | 2. Perimeter                |                                            |                                          | 29. Robust Mean Absolute Deviation     |                  | 56. Energy             |                       |                                          |
|                                          | 3. Major Axis Length        |                                            |                                          | 30. Root Mean Squared                  |                  | 57. Gray Mean          |                       |                                          |
|                                          | 4. Minor Axis Length        |                                            |                                          | 31. Energy                             |                  | 58. Gradient Mean      |                       |                                          |
|                                          | 5. Elongation               |                                            |                                          | 32. Autocorrelation                    |                  | GGCM features          | 59. Gray Variance     |                                          |
|                                          | 6. Circularity              |                                            |                                          | 33. Contrast                           |                  |                        | 60. Gradient Variance |                                          |
|                                          | 7. Eccentricity             |                                            |                                          | 34. Correlation                        |                  |                        | 61. Correlation       |                                          |
|                                          | 8. Smoothness               |                                            |                                          | 35. Cluster Prominence                 |                  |                        | 62. Gray Entropy      |                                          |
|                                          | 9. Volume                   |                                            |                                          | 36. Cluster Shade                      |                  |                        | 63. Gradient Entropy  |                                          |
|                                          | 10. Surface                 |                                            |                                          | 37. Dissimilarity                      |                  |                        | 64. Mixing Entropy    |                                          |
|                                          | 11. Dry Mass                |                                            |                                          | 38. Energy                             |                  |                        | 65. Inertia           |                                          |
|                                          | 12. Dry Mass Density        |                                            |                                          | 39. Entropy                            |                  |                        | 66. Homogeneity       |                                          |
|                                          | 13. Surface to Volume Ratio |                                            |                                          | 40. Homogeneity                        |                  |                        |                       | 67. Short Run Emphasis                   |
|                                          | 14. Sphericity              |                                            |                                          | 41. Maximum Probability                |                  |                        |                       | 68. Long Run Emphasis                    |
| 1 <sup>st</sup> order histogram features | 15. Mean                    |                                            |                                          | 42. Sum of Squares                     |                  |                        |                       | 69. Gray Level Non-Uniformity            |
|                                          | 16. Median                  |                                            |                                          | 43. Sum Average                        |                  |                        |                       | 70. Gray Level Non-Uniformity Normalized |
|                                          | 17. Range                   |                                            |                                          | 44. Sum Variance                       |                  |                        |                       | 71. Run Percentage                       |
|                                          | 18. Minimum                 |                                            |                                          | 45. Sum Entropy                        |                  |                        |                       | 72. Run Length Non-Uniformity            |
|                                          | 19. Maximum                 | 46. Difference Variance                    | 73. Run Length Non-Uniformity Normalized |                                        |                  |                        |                       |                                          |
|                                          | 20. Variance                | 47. Difference Entropy                     | 74. Run Variance                         |                                        |                  |                        |                       |                                          |
|                                          | 21. Skewness                | 48. Informational Measure of Correlation 1 | GLRLM features                           | 75. Gray Level Variance                |                  |                        |                       |                                          |
|                                          | 22. Kurtosis                | 49. Informational Measure of Correlation 2 |                                          | 76. Low Gray Level Run Emphasis        |                  |                        |                       |                                          |
|                                          | 23. Entropy                 | 50. Inverse Difference Normalized          |                                          | 77. High Gray Level Run Emphasis       |                  |                        |                       |                                          |
|                                          | 24. Uniformity              | 51. Inverse Difference Moment Normalized   |                                          | 78. Short Run Low Gray Level Emphasis  |                  |                        |                       |                                          |
|                                          | 25. 10th Percentile         | 52. Small Gradient Dominance               |                                          | 79. Short Run High Gray Level Emphasis |                  |                        |                       |                                          |
|                                          | 26. 90th Percentile         | 53. Large Gradient Dominance               |                                          | 80. Long Run Low Gray Level Emphasis   |                  |                        |                       |                                          |
|                                          | 27. Interquartile Range     | 54. Gray Asymmetry                         |                                          | 81. Long Run High Gray Level Emphasis  |                  |                        |                       |                                          |

## Supplementary Note 5. Feature engineering for machine learning

In machine learning, feature engineering is a critical step that involves transforming raw data into informative features that better represent the underlying problem to the predictive models, thus improving their performance. The steps of feature engineering can vary depending on the specific problem, but a general process includes not only feature extraction and preprocessing (such as data cleaning, missing values padding, feature scaling, etc.), but also feature analysis and selection<sup>16</sup>. Additionally, based on the machine learning model's performance and the feedback from the validation set, it might need to go back and refine features, so it is an iterative process.

In our research, we harnessed expertise from the domain of feature engineering to propel machine learning algorithms toward peak performance. Our analysis was predicated on a dataset including 81 distinct features. We implemented an initial Min-Max normalization step, which scaled numerical data uniformly within a specific range between 0 and 1, thereby ensuring an equitable contribution of each feature to the analysis, and preventing any single variable from dominating the model's performance.

Then, to gain a deeper comprehension of the derived features, an analytical framework was constructed, encompassing one-way analysis of variance (ANOVA) and the correlation analysis<sup>17</sup>. One-way ANOVA determines if there are any statistically significant differences between the means of three or more independent (unrelated) groups. If the ANOVA test result is statistically significant (usually at a p-value less than 0.05), it suggests that at least one group's mean is different from the others. Multiple comparison tests are successively used to identify which specific groups are different. We choose the commonly used Tukey's HSD (Honestly Significant Difference) test as the multiple comparison method. Specifically, in our multiple cancer cell types' predictive classification problem, there were five cell types, resulting in ten group combinations; in the classification of multiple breast cancer subtypes, eight cell types lead to twenty-eight group combinations. For each pair comparison, the Tukey's HSD method calculated the mean difference and standard error, ultimately providing an adjusted p-value. A p-value below the predetermined significance level (we set it at 0.05) led to the rejection of the null hypothesis, indicating a significant difference between the means of the two groups. Conversely, a higher p-value suggested that the

feature variable's differences were insufficient, and thus it might not be a suitable feature to characterize different cell populations.

In addition to assessing the classificatory significance of each feature, we also conducted a correlation analysis to examine the degree of inter-feature correlation. The Pearson correlation coefficient<sup>18</sup> was the metric of choice for this purpose, quantifying the linear association between pairs of continuous variables on a scale from -1 to 1, where 1 denotes perfect positive correlation, -1 perfect negative correlation, and 0 the absence of correlation. A high linear relationship is suggestive of feature redundancy, which can exacerbate model complexity without providing additional informational value. The results of our feature analysis correspond to Fig. 3 and Fig. 5 in the main text, visualized through appropriate formats such as violin plots and heatmaps.

Feature analysis provides a basis for feature selection. Feature selection is the process of identifying the most critical feature subset from the original dataset that are predictive of the target variable. This process aims to reduce the dimensionality of the data as well as the risk of overfitting, enhance the model's generalizability, and potentially improve the model's interpretability. Our hierarchical feature extraction strategy from fundamental bulk features to texture features has naturally resulted in five distinct subsets. Therefore, we first independently utilized each hierarchical level of features for building model and predicting with classifiers to demonstrate the significance of different feature types (see Supplementary Table 9 and 10, Supplementary Fig. 12).

After that, we reverted to the comprehensive feature set to construct an optimal feature subset tailored to the classification model. Clearly, the key question here was how to evaluate the importance of features to enable their ranking and selection. We determined which important features to include by using different feature ranking algorithms. The feature ranking algorithm displayed a plot of the sorted feature importance scores, where larger scores (including Infs) indicate greater feature importance. Different feature ranking algorithms are distinguished by the criteria they consider. We selected and compared four representative feature ranking algorithms, each predicated on distinct evaluative criteria. Supplementary Fig. 13 presents the importance scores (normalized values) assigned to the 81 features by four distinct feature ranking algorithms in a heatmap format.

ANOVA performed one-way ANOVA for each predictor variable, grouped by class, and then ranked features using the p-values. Chi2<sup>19</sup> examined whether each predictor variable is independent of the response variable by using individual chi-square tests, and then ranked features using the p-values of the chi-square test statistics. ReliefF<sup>20</sup> evaluated the importance of features by calculating the similarity and difference in the values among ten nearest neighbor samples, thereby identifying the features that are most discriminative and informative. MRMR<sup>21</sup> ranked features sequentially using the Maximun-Relevance and Minimun-Redundancy (MRMR) algorithm based on the concept of mutual information which aims to maximize the relevance between features and the target variable while maintaining a minimum of redundancy among the features themselves. We next integrated these four feature ranking algorithms with an incremental feature selection strategy into three machine learning models-LDA, KNN, and Naive Bayes-for both training and testing purposes. The selection of these three machine learning models was strategic, reflecting a range of overall classification accuracies from high to moderate to low, thus providing a representative sample. The results, as illustrated in Supplementary Fig. 14, indicated that the feature combination constructed using the MRMR algorithm yielded the most optimal outcome, achieving the highest classification accuracy with the minimal number of features needed. Consequently, we have elected to employ the MRMR algorithm for the optimized training and predictive modeling of other machine learning models.

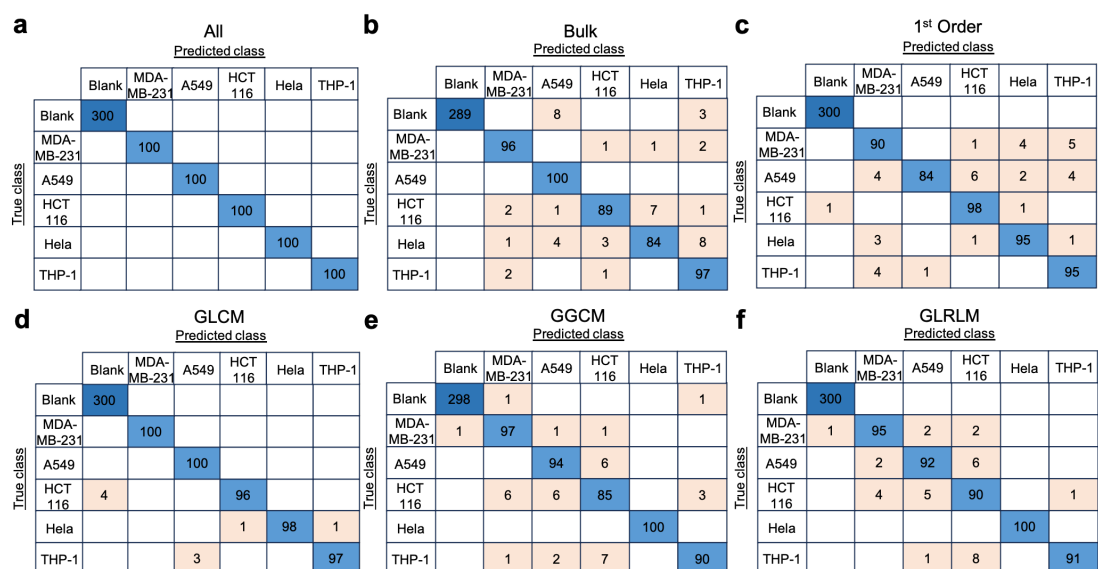
515 **Supplementary Table 9. Validation accuracy of machine learning models for multiple**  
516 **cancer cell types classification trained with features from different hierarchies**

| Classifier   | Feature type |       |                       |       |       |       |
|--------------|--------------|-------|-----------------------|-------|-------|-------|
|              | All          | Bulk  | 1 <sup>st</sup> Order | GLCM  | GGCM  | GLRLM |
| LDA          | 100.0%       | 95.5% | 95.7%                 | 99.0% | 95.4% | 95.6% |
| SVM          | 99.9%        | 98.6% | 97.6%                 | 98.5% | 96.7% | 97.4% |
| NN           | 99.9%        | 99.6% | 98.9%                 | 99.7% | 99.3% | 99.2% |
| LR           | 97.8%        | 95.5% | 92.1%                 | 87.7% | 85.0% | 81.8% |
| Subspace KNN | 98.1%        | 97.8% | 91.9%                 | 86.8% | 90.8% | 78.8% |
| Bagged Trees | 98.4%        | 98.1% | 91.2%                 | 88.0% | 91.5% | 87.2% |
| DT           | 94.6%        | 93.4% | 70.9%                 | 59.7% | 70.8% | 59.3% |
| KNN          | 90.3%        | 87.6% | 83.0%                 | 73.4% | 80.6% | 67.1% |
| Naïve Bayes  | 89.0%        | 72.8% | 40.8%                 | 46.9% | 51.2% | 40.3% |

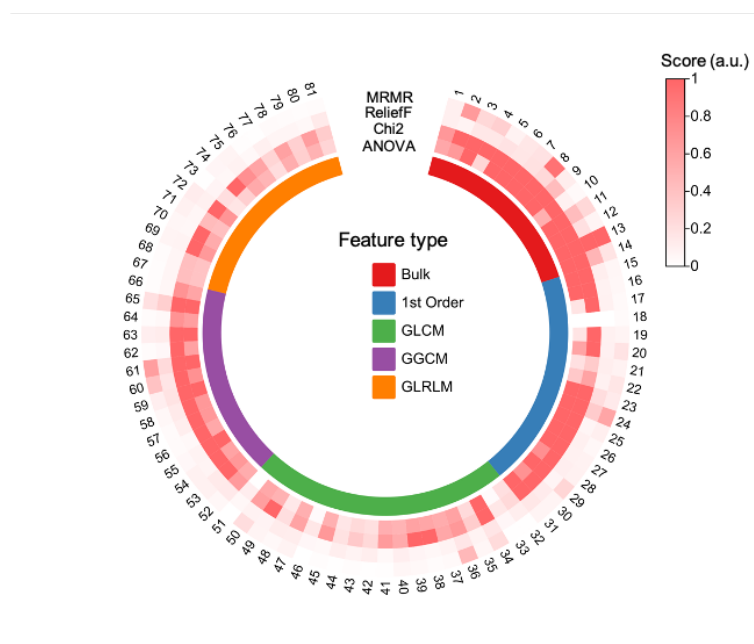
517 **Supplementary Table 10. Testing accuracy of machine learning models for multiple**  
518 **cancer cell types classification trained with features from different hierarchies**

| Classifier   | Feature type |       |                       |       |       |       |
|--------------|--------------|-------|-----------------------|-------|-------|-------|
|              | All          | Bulk  | 1 <sup>st</sup> Order | GLCM  | GGCM  | GLRLM |
| LDA          | 100.0%       | 94.4% | 95.2%                 | 98.9% | 95.5% | 96.0% |
| SVM          | 100.0%       | 98.9% | 97.6%                 | 99.1% | 96.5% | 97.2% |
| NN           | 99.8%        | 99.1% | 98.8%                 | 99.8% | 99.8% | 99.8% |
| LR           | 98.8%        | 94.8% | 92.0%                 | 89.4% | 83.8% | 80.6% |
| Subspace KNN | 98.4%        | 97.5% | 93.4%                 | 89.6% | 89.6% | 79.2% |
| Bagged Trees | 98.0%        | 98.0% | 93.4%                 | 90.2% | 90.8% | 87.4% |
| DT           | 95.8%        | 92.2% | 71.0%                 | 61.6% | 67.4% | 58.4% |
| KNN          | 90.0%        | 85.9% | 85.5%                 | 75.5% | 78.4% | 65.5% |
| Naïve Bayes  | 87.9%        | 70.4% | 41.5%                 | 45.4% | 54.5% | 41.1% |

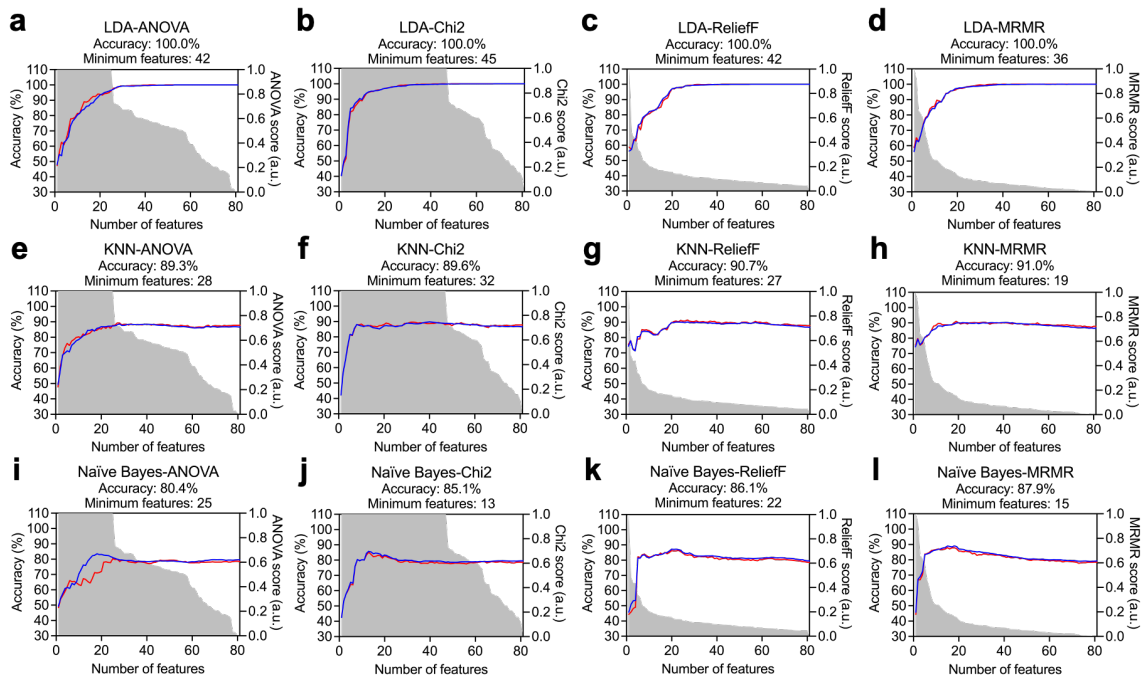
519



**Supplementary Fig. 12 Confusion matrices of LDA for multiple cancer cell types classification trained with features from different hierarchies.** The feature hierarchies include (a) All features, (b) Bulk features, (c) 1<sup>st</sup> Order histogram features, (d) GLCM features, (e) GGCM features, and (f) GLRLM features.

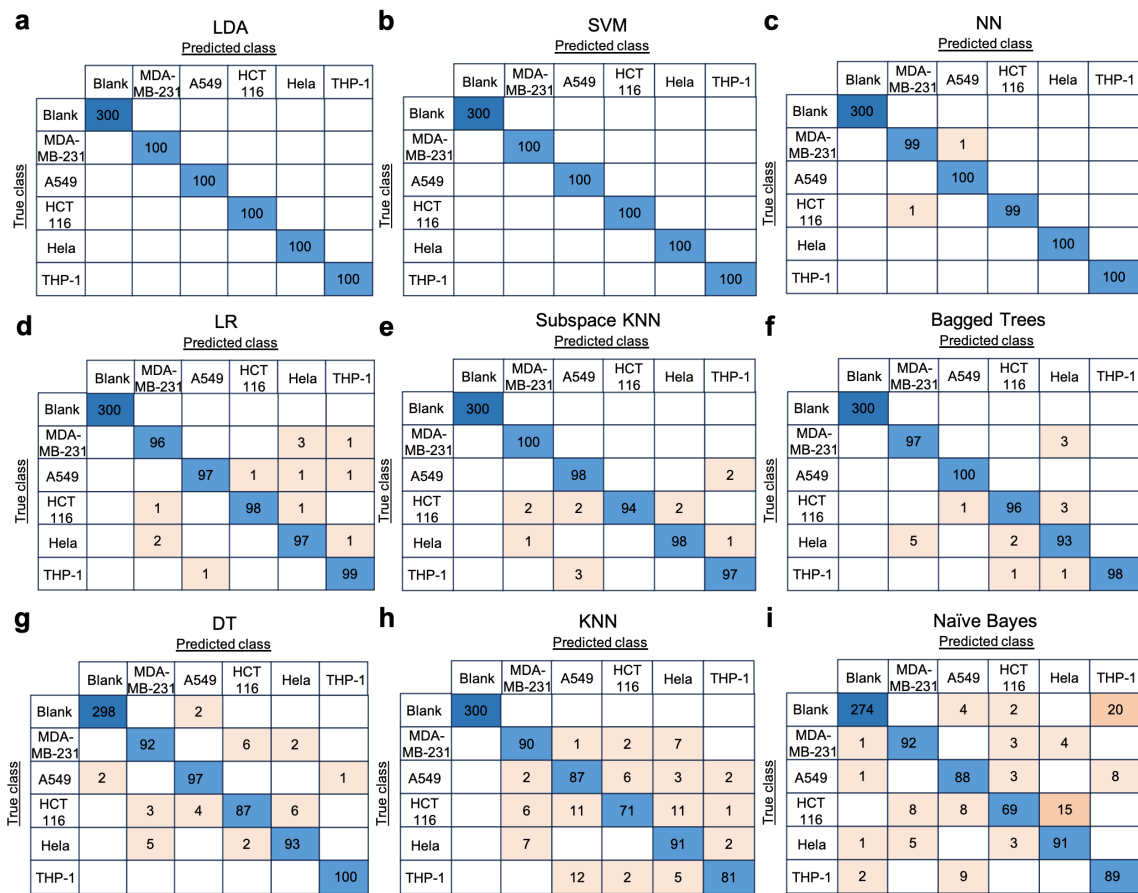


**Supplementary Fig. 13 Importance scores of 81 features for multiple cancer cell types classification given by different feature ranking algorithms.** The color bar denotes the scale of normalized values. Source data are provided as a Source Data file.

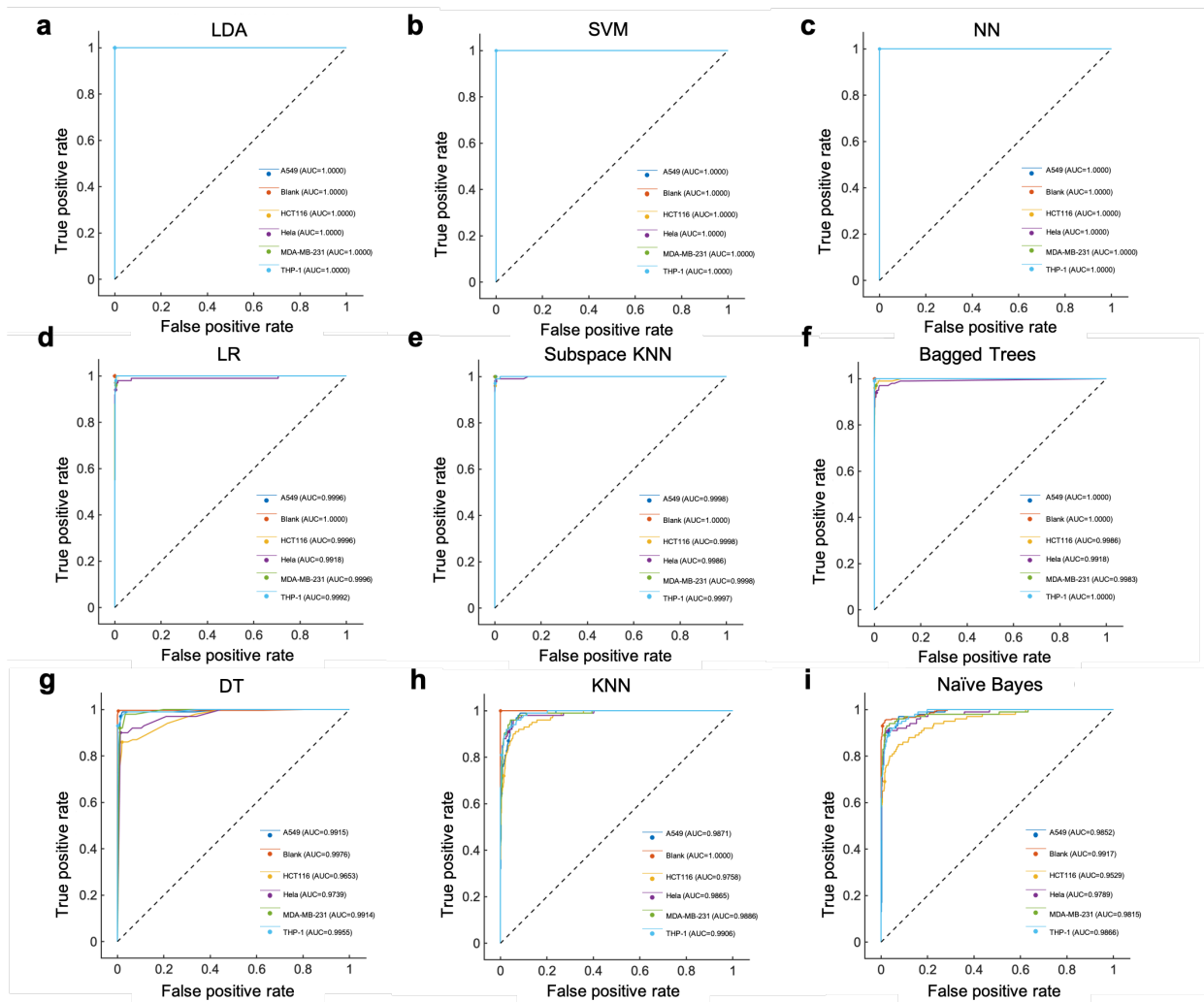


**Supplementary Fig. 14 Incremental feature selection (IFS) curves for machine learning models to conduct multiple cancer cell types' predictive classification.** Machine learning algorithms include LDA, KNN, and Naive Bayes. The gray shadow represents the importance scores of features given by different feature ranking algorithms. Note that the initial scores assigned to the top dozens of features by ANOVA and Chi2 are infinite (Infs); so, for visualization, the final scores are normalized to a value of 1 while preserving the ranking order. The blue curve illustrates the accuracy rates on the ten-fold cross-validation set, while the red curve indicates the accuracy rates on the test set. Source data are provided as a Source Data file.

**Supplementary Note 6. Evaluation of the classification performance of machine learning to predictively classify multiple cancer cell types**



**Supplementary Fig. 15 Confusion matrices of different machine learning models to predictively classify multiple cancer cell types.** Machine learning models are trained and validated including (a) LDA, (b) SVM, (c) NN, (d) LR, (e) Subspace KNN, (f) Bagged Trees, (g) DT, (h) KNN, and (i) Naïve Bayes.



541 **Supplementary Fig. 16 Receiver operating characteristic (ROC) curve of different**  
 542 **machine learning models to predictively classify multiple cancer cell types.** Machine  
 543 learning models are trained and validated including (a) LDA, (b) SVM, (c) NN, (d) LR, (e)  
 544 Subspace KNN, (f) Bagged Trees, (g) DT, (h) KNN, and (i) Naïve Bayes.

545 **Supplementary Table 11. Performance of different machine learning models used for the**  
546 **predictive classification of multiple cancer cell types**

| Classifier   | Performance (Test set) |          |         | Training time (s) | Forecasting speed (obs/sec) | Number of features used |
|--------------|------------------------|----------|---------|-------------------|-----------------------------|-------------------------|
|              | Accuracy               | F1-score | ROC AUC |                   |                             |                         |
| LDA          | 100.0%                 | 100.0%   | 1.0000  | 484.2             | 5096.8                      | 36                      |
| SVM          | 100.0%                 | 100.0%   | 1.0000  | 616.4             | 4824.1                      | 38                      |
| NN           | 99.8%                  | 99.7%    | 1.0000  | 880.7             | 3851.9                      | 59                      |
| LR           | 98.8%                  | 98.3%    | 0.9986  | 2759.8            | 1147.8                      | 44                      |
| Subspace KNN | 98.4%                  | 97.8%    | 0.9995  | 418.2             | 761.1                       | 20                      |
| Bagged Trees | 98.0%                  | 97.3%    | 0.9972  | 2403.0            | 3469.4                      | 74                      |
| DT           | 95.8%                  | 94.5%    | 0.9877  | 453.0             | 6727.4                      | 29                      |
| KNN          | 91.0%                  | 86.6%    | 0.9881  | 722.6             | 1381.1                      | 41                      |
| Naive Bayes  | 87.9%                  | 85.7%    | 0.9757  | 919.0             | 836.3                       | 18                      |

547

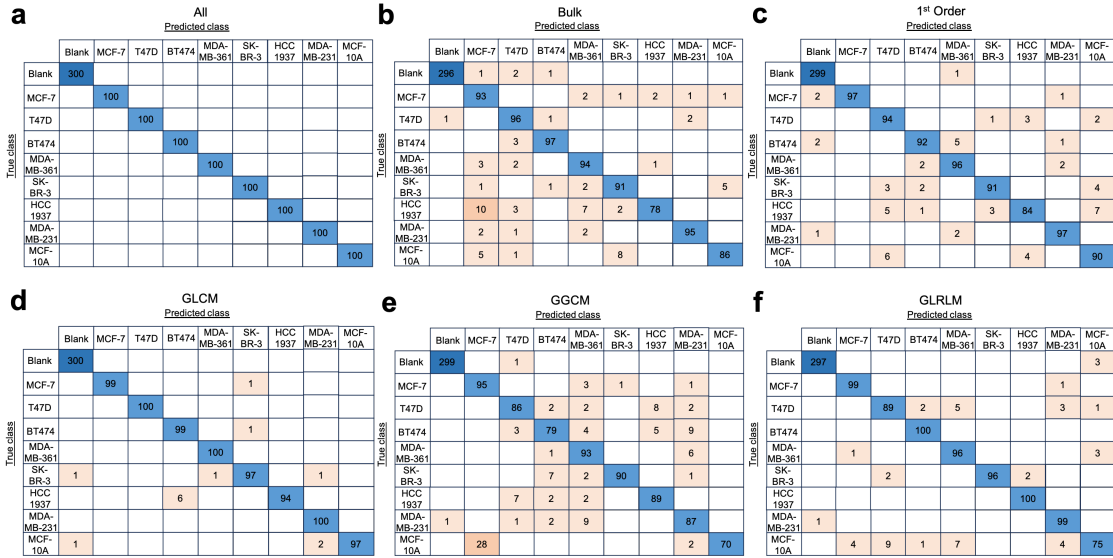
**Supplementary Note 7. Evaluation of the classification performance of machine learning to predictively classify breast cancer subtypes**

**Supplementary Table 12. Validation accuracy of machine learning models for breast cancer subtypes classification trained with features from different hierarchies**

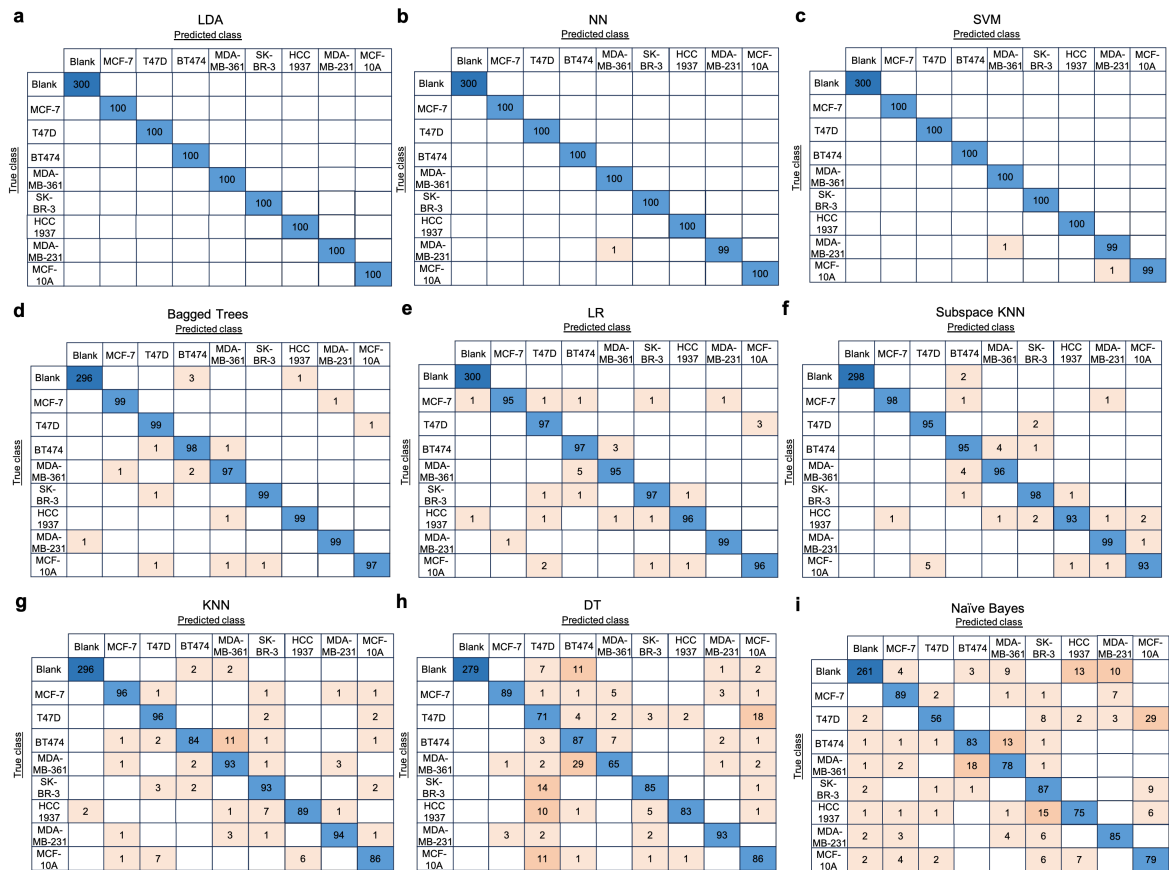
| Classifier   | Feature type |       |                       |       |       |       |
|--------------|--------------|-------|-----------------------|-------|-------|-------|
|              | All          | Bulk  | 1 <sup>st</sup> Order | GLCM  | GGCM  | GLRLM |
| LDA          | 99.9%        | 93.8% | 94.0%                 | 98.0% | 89.3% | 89.8% |
| SVM          | 99.7%        | 98.0% | 97.1%                 | 98.0% | 96.1% | 96.8% |
| NN           | 99.9%        | 99.3% | 99.1%                 | 99.6% | 99.2% | 99.6% |
| LR           | 97.4%        | 91.8% | 89.2%                 | 87.9% | 84.8% | 85.5% |
| Bagged Trees | 98.2%        | 96.6% | 91.9%                 | 88.1% | 85.1% | 84.4% |
| Subspace KNN | 97.1%        | 94.5% | 91.5%                 | 87.5% | 84.1% | 84.8% |
| KNN          | 92.5%        | 82.3% | 81.3%                 | 70.9% | 73.0% | 74.6% |
| DT           | 83.4%        | 77.0% | 67.4%                 | 50.2% | 55.1% | 54.5% |
| Naïve Bayes  | 81.0%        | 64.6% | 57.8%                 | 37.2% | 42.5% | 42.4% |

**Supplementary Table 13. Testing accuracy of machine learning models for breast cancer subtypes classification trained with features from different hierarchies**

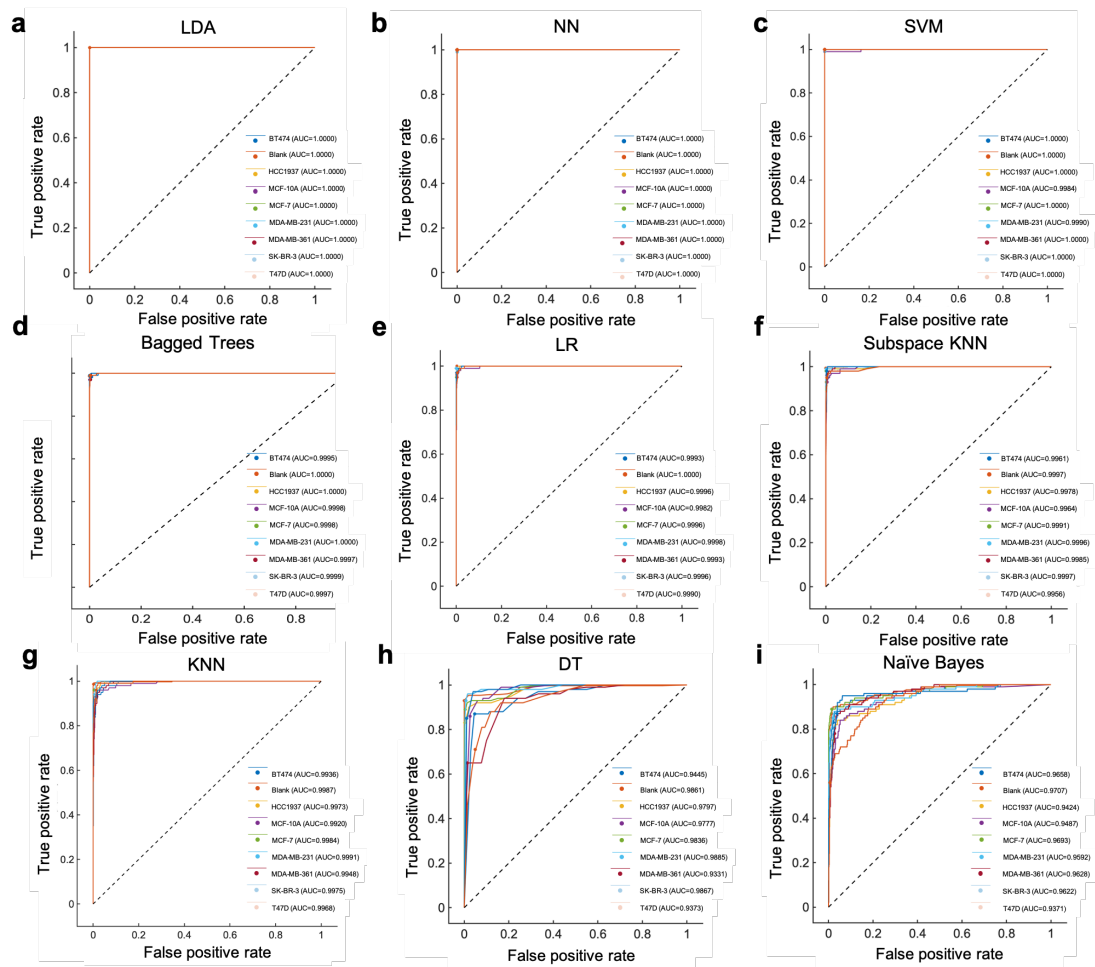
| Classifier   | Feature type |       |                       |       |       |       |
|--------------|--------------|-------|-----------------------|-------|-------|-------|
|              | All          | Bulk  | 1 <sup>st</sup> Order | GLCM  | GGCM  | GLRLM |
| LDA          | 100.0%       | 93.3% | 94.5%                 | 98.6% | 89.8% | 95.5% |
| SVM          | 99.8%        | 97.5% | 97.3%                 | 98.3% | 96.8% | 97.5% |
| NN           | 99.9%        | 99.1% | 99.0%                 | 99.7% | 99.6% | 99.5% |
| LR           | 97.5%        | 92.5% | 89.0%                 | 86.6% | 85.5% | 77.9% |
| Bagged Trees | 98.5%        | 96.6% | 92.7%                 | 87.0% | 84.4% | 85.9% |
| Subspace KNN | 96.8%        | 94.2% | 91.1%                 | 86.4% | 84.8% | 74.1% |
| KNN          | 93.4%        | 84.1% | 80.9%                 | 70.8% | 74.6% | 61.7% |
| DT           | 85.3%        | 77.0% | 66.6%                 | 49.1% | 54.5% | 51.4% |
| Naïve Bayes  | 81.2%        | 66.4% | 57.3%                 | 37.7% | 42.4% | 33.6% |



Supplementary Fig. 17 Confusion matrices of LDA for breast cancer subtypes classification trained with features from different hierarchies. The feature hierarchies include (a) All features, (b) Bulk features, (c) 1<sup>st</sup> Order histogram features, (d) GLCM features, (e) GGCM features, and (f) GLRLM features.



559 **Supplementary Fig. 18 Confusion matrices of different machine learning models to**  
 560 **predictively classify breast cancer subtypes cells.** Machine learning models are trained and  
 561 validated including (a) LDA, (b) NN, (c) SVM, (d) Bagged Trees, (e) LR, (f) Subspace KNN,  
 562 (g) KNN, (h) DT, and (i) Naive Bayes.



**Supplementary Fig. 19 Receiver operating characteristic (ROC) curve of different machine learning models to predictively classify breast cancer subtypes cells.** Machine learning models are trained and validated including (a) LDA, (b) NN, (c) SVM, (d) Bagged Trees, (e) LR, (f) Subspace KNN, (g) KNN, (h) DT, and (i) Naïve Bayes.

567 **Supplementary Table 14. Performance of different machine learning models used for the**  
568 **predictive classification of breast cancer subtypes cells**

| Classifier   | Performance (Test set) |          |         | Training time (s) | Forecasting speed (obs/sec) | Number of features used |
|--------------|------------------------|----------|---------|-------------------|-----------------------------|-------------------------|
|              | Accuracy               | F1-score | ROC AUC |                   |                             |                         |
| LDA          | 100.0%                 | 100.0%   | 1.0000  | 770.0             | 5046.4                      | 53                      |
| SVM          | 99.8%                  | 99.8%    | 0.9997  | 1434.0            | 3755.1                      | 77                      |
| NN           | 99.9%                  | 99.9%    | 1.0000  | 1160.0            | 5110.0                      | 67                      |
| LR           | 97.5%                  | 97.0%    | 0.9994  | 5064.7            | 510.7                       | 37                      |
| Bagged Trees | 98.5%                  | 98.3%    | 0.9998  | 3204.8            | 6240.0                      | 46                      |
| Subspace KNN | 96.8%                  | 96.2%    | 0.9981  | 7570.0            | 88.4                        | 64                      |
| KNN          | 93.4%                  | 92.1%    | 0.9965  | 627.7             | 1995.7                      | 29                      |
| DT           | 85.3%                  | 83.3%    | 0.9686  | 925.9             | 5410.3                      | 62                      |
| Naive Bayes  | 81.2%                  | 79.0%    | 0.9576  | 362.3             | 8270.5                      | 17                      |

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## **Supplementary Note 8. Identification and enumeration of breast cancer cells in human blood**

In real clinical applications, blood test samples from new individuals will be encountered by our model, potentially introducing variances in morphological or textual features. To assess these variances, we used the Peripheral Blood Mononuclear Cells (PBMCs) from three different donors, and three batches of MDA-MB-231 cells. We plotted feature distributions for each donor/batch and observed distribution differences for certain cell types (see Supplementary Fig. 20 and 21).

Following the approach outlined in relevant literature<sup>22, 23</sup>, we explored the impact of these differences on the generalization of our model to new donors. Each time, one donor's PBMC sample was used for training the LDA model based on the MRMR-IFS strategy (see Supplementary Fig. 22) as mentioned before, while samples from the remaining donors were used for testing. This experiment was repeated by rotating the testing donor. The same approach was applied to MDA-MB-231 batches.

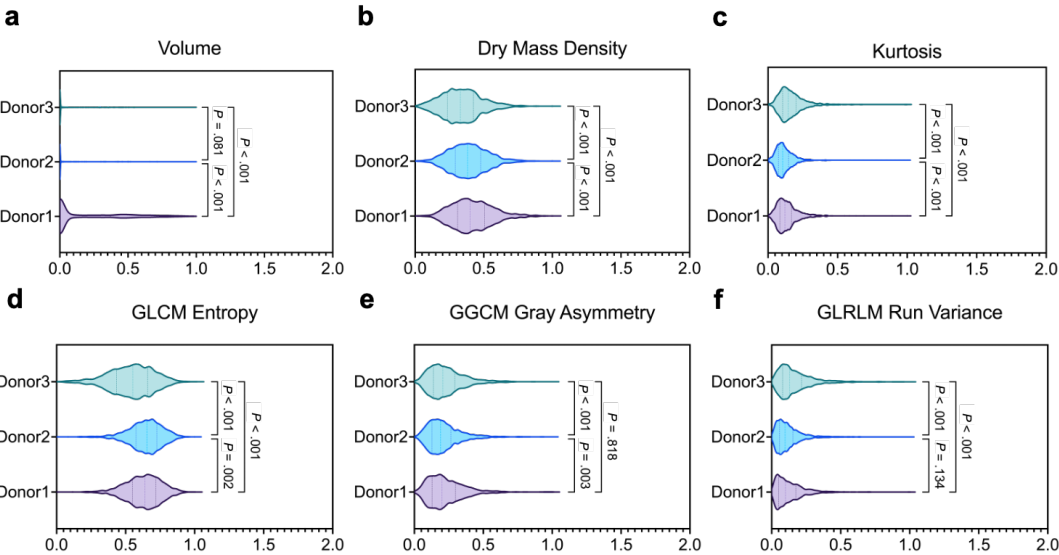
Our results (see Supplementary Table 15, Supplementary Fig. 23) demonstrated high accuracy rates in cross-validation, with discrimination accuracies above 0.90. For instance, modeling with Donor 1's PBMC data and Batch 1's MDA-MB-231 data achieved 99.6% and 98.2% discrimination accuracies for Donor 2 and Donor 3's PBMCs, and 91.0% and 98.2% for Batch 2 and Batch 3's MDA-MB-231 cells, respectively. These high cross-validation accuracies suggest that our model, trained with our strategy, has good generalization and universal applicability, showing potential in practical applications.

We attribute these high cross-validation accuracies to several key factors: 1) Fine-grained feature extraction: The high accuracy of our imaging system ensures that the extracted features are detailed and precisely represent cell features, which is crucial for the model's ability to distinguish between different cell types and states. 2) MRMR-IFS learning strategy: The use of Maximum Relevance Minimum Redundancy (MRMR) score ranking for feature selection ensures that the most relevant and non-redundant features are chosen for model training. By incrementally adding new data to the model, the learning process can adapt to new information without forgetting previously learned patterns, thus improving the model's ability to generalize to new, unseen data. 3)

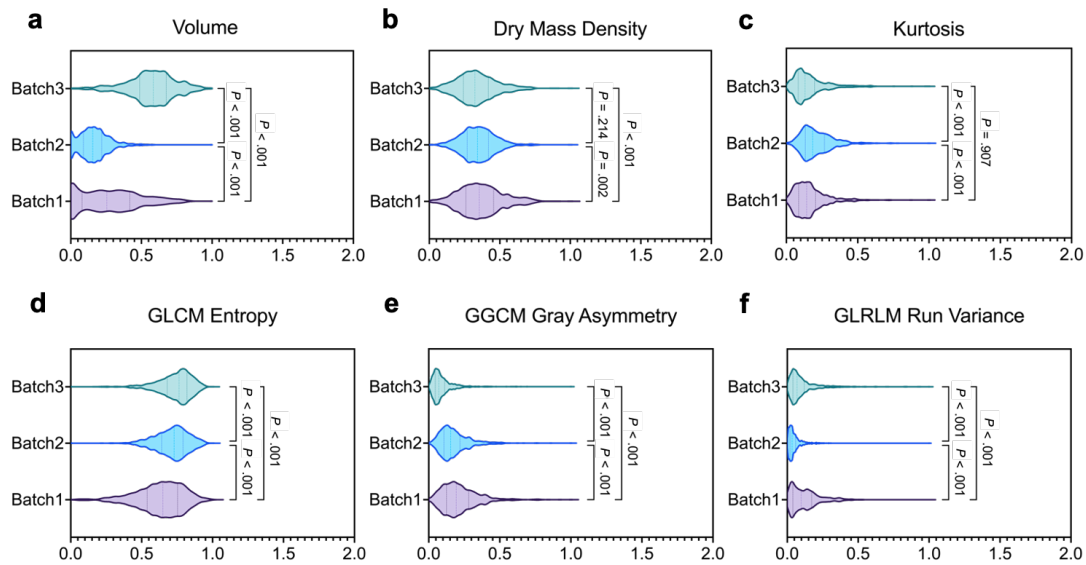
601 Substantial number of cells per type: This ensures that the model is trained on a diverse  
602 set of samples, which helps in capturing the variability within each cell type.

**Supplementary Table 15. Cross-validation results with LDA models**

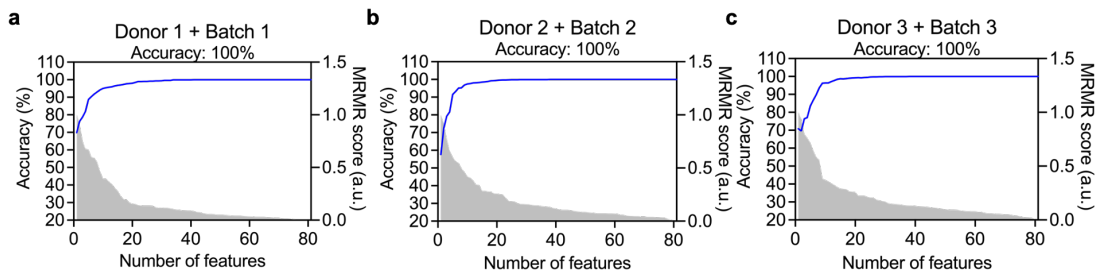
| Training dataset |         |            | Test dataset |         |         | Test dataset |         |         |
|------------------|---------|------------|--------------|---------|---------|--------------|---------|---------|
|                  |         |            | PBMC         |         |         | MDA-MB-231   |         |         |
| Blank            | PBMC    | MDA-MB-231 | Donor 1      | Donor 2 | Donor 3 | Batch 1      | Batch 2 | Batch 3 |
| Blank            | Donor 1 | Batch 1    | NA           | 99.6%   | 97.3%   | NA           | 98.2%   | 91.0%   |
| Blank            | Donor 2 | Batch 2    | 99.8%        | NA      | 99.7%   | 98.3%        | NA      | 95.3%   |
| Blank            | Donor 3 | Batch 3    | 99.3%        | 100.0%  | NA      | 97.7%        | 98.4%   | NA      |



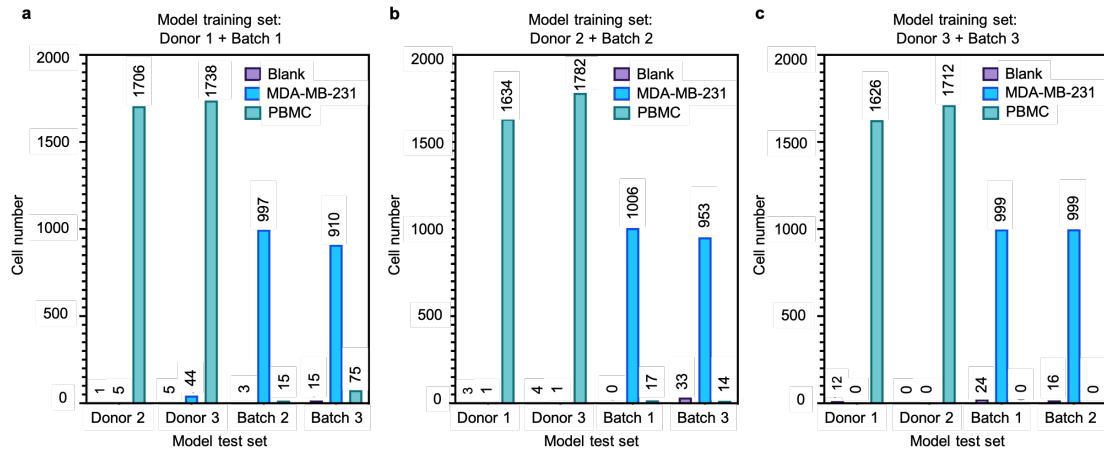
**Supplementary Fig. 20 Violin plots of 6 QPM phase features of PBMC ( $n = 1,638$  cells for Donor 1,  $n = 1,712$  cells for Donor 2,  $n = 1,787$  cells for Donor 3), including (a) volume (Bulk), (b) dry mass density (Bulk), (c) Kurtosis (First-order histogram), (d) entropy (GLCM), (e) gray asymmetry (GGCM), and (f) run variance (GLRLM). The violins are drawn with the ends at the quartiles and the median as a horizontal line in the violin, where the curve of the violin extends to the minimum and maximum values in the data set. The p-value  $< 0.05$  indicates a significant difference decided by one-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file.**



**Supplementary Fig. 21 Violin plots of 6 QPM phase features of MDA-MB-231 ( $n = 1,023$  cells for Batch 1,  $n = 1,015$  cells for Batch 2,  $n = 1,000$  cells for Batch 3), including (a) volume (Bulk), (b) dry mass density (Bulk), (c) Kurtosis (First-order histogram), (d) entropy (GLCM), (e) gray asymmetry (GGCM), and (f) run variance (GLRLM). The violins are drawn with the ends at the quartiles and the median as a horizontal line in the violin, where the curve of the violin extends to the minimum and maximum values in the data set. The  $p$ -value  $< 0.05$  indicates a significant difference decided by one-way ANOVA followed by Tukey's multiple comparisons test. Source data are provided as a Source Data file.**



**Supplementary Fig. 22 Incremental feature selection (IFS) curves for LDA machine learning algorithms, with the highest predictive accuracy of each model labeled accordingly. The gray shadow represents the MRMR scores of features. The blue curve illustrates the training accuracy rates. Source data are provided as a Source Data file.**



**Supplementary Fig. 23 Cross-validation results with LDA models.** Models are trained on (a) Donor 1's PBMC ( $n = 1,638$  cells) with Batch 1's MDA-MB-231 ( $n = 1,023$  cells), (b) Donor 2's PBMC ( $n = 1,712$  cells) with Batch 2's MDA-MB-231 ( $n = 1,015$  cells), and (c) Donor 3's PBMC ( $n = 1,787$  cells) with Batch 3's MDA-MB-231 ( $n = 1,000$  cells), with remaining batches and donors serving as the test set in a rotating fashion. The bars represent the predicted outcomes for different cell types within the test set, including Blank (purple bars), MDA-MB-231 (blue bars), and PBMC (green bars). Source data are provided as a Source Data file.

634 **Supplementary Note 9. Comparison of the state-of-art QPM-IFCs**

635 **Supplementary Table 16. Comparison of the state-of-art QPM-IFCs**

| Comparison of the state-of-art QPM-IFCs |                                                                                           |                                                   |                                                 |                  |                                                                                                                                                    |                                                                 |                                                                                                                                                                                                                             |          |                 |
|-----------------------------------------|-------------------------------------------------------------------------------------------|---------------------------------------------------|-------------------------------------------------|------------------|----------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------|-----------------|
| References                              | Imaging instrument                                                                        | Limited resolution                                | IFC technique                                   | Image defocusing | Learning type                                                                                                                                      | Features/ Phenotypes                                            | Cell types                                                                                                                                                                                                                  | Scale    | Accuracy        |
| Ours                                    | Digital holographic microscopy (DHM)                                                      | 520 nm ( $\lambda=632$ nm, 40 $\times$ , 0.75 NA) | Spatial hydrodynamic focusing microfluidics     | No               | Feature-engineering-enhanced machine learning (9 models-LR, KNN, LDA, SVM, NN, DT, Naive Bayes, Subspace KNN, Bagged Trees)                        | 81 QPM phase features                                           | Multi-cancer (5): MDA-MB-231 (Breast), A549 (Lung), HCT116 (Colon), Hela (Cervix), THP-1 (Leukemia) Breast (cancer & normal, 8): MCF-7, T47D, BT474, MDA-MB-361, SK-BR-3, HCC1937, MDA-MB-231, MCF-10A Blood (2): RBC, PBMC | >10,000  | >99.9%          |
| [23, 24]                                | Multiplexed asymmetric optical time-stretch detection (multi-ATOM)                        | 1.3 $\mu$ m                                       | Inertial microfluidics                          | Yes              | ROC analysis                                                                                                                                       | 12 biophysical phenotypes                                       | Blood & Leukemia (4): THP-1, Kasumi-1, K-562, PBMC                                                                                                                                                                          | >100,000 | 92–97%          |
| [25, 26]                                | Multimodal Free-space angular-chirp-enhanced delay quantitative phase imaging (FACED-QPI) | Sub-cellular resolution                           | Inertial microfluidics                          | Yes              | Deep neural network & transfer learning                                                                                                            | ~80 optophysical features                                       | Lung cancer (7): H358, H1975, HCC827, H520, H2170, H526, H69                                                                                                                                                                | >100,000 | 87-95%          |
| [27]                                    | Point source digital inline holographic microscopy (PSDIHM)                               | Subpixel resolution (pixel size: 5.5 $\mu$ m)     | 2D hydrodynamic focusing microfluidics          | Yes              | Deep neural network                                                                                                                                | 51 biophysical features                                         | Breast cancer (2): MDA-MB-231, MCF-7                                                                                                                                                                                        | ~8,700   | 90%             |
| [28]                                    | 3D holographic tomography                                                                 | 650 nm ( $\lambda=532$ nm, 20 $\times$ , 0.5 NA)  | Microfluidics (no focusing)                     | Yes              | Feature selection and machine learning (9 models-Nearest Neighbors, SVM Linear/RBF, Decision Tree, Random Forest, AdaBoost, Naive Bayes, LDA, QDA) | 60 optical and morphological features                           | Leukocyte (3): monocyte, granulocytes, lymphocytes                                                                                                                                                                          | ~1,900   | 95-99%          |
| [29, 30]                                | Diffraction phase microscopy (DPM) and Raman microscopy (RM)                              | 590 nm (DPM)                                      | Commercial flow cytometry (Miltenyi Germany)    | Unknown          | Machine learning (7 models-Trees, Naive Bayes, KNN, SVM, LR, LDA, Subspace-LDA)                                                                    | 54 2D QPM features 67 3D RI features                            | Endometrial cancer (2): ISK (sensitive), ISK-CisR (resistant)                                                                                                                                                               | ~180     | 2D: 88% 3D: 96% |
| [31]                                    | Interferometric phase microscopy (IPM)                                                    | 470 nm ( $\lambda=650$ nm, 40 $\times$ , 0.85 NA) | Microfluidics (no focusing)                     | Yes              | Machine learning (SVM)                                                                                                                             | 8 2D morphological features & 12 IPM-based topological features | Colorectal cancer (2): SW-480, SW-620 Blood (4): lymphocytes, granulocytes, monocytes, erythrocytes                                                                                                                         | ~4,000   | 93%             |
| [32]                                    | Digital holographic microscopy (DHM)                                                      | 590 nm ( $\lambda=528$ nm, 40 $\times$ , 0.55 NA) | 2D hydrodynamic focusing microfluidics          | Yes              | Principal component analysis (PCA) Machine learning (SVM)                                                                                          | 29 morphological parameters                                     | Blood (5): basophils, eosinophils, lymphocytes, monocytes, neutrophils                                                                                                                                                      | ~66,000  | 92%             |
| [33]                                    | Time stretch quantitative phase imaging (TS-QPI)                                          | 2.5 $\mu$ m                                       | Hydrodynamic focusing microfluidics             | Yes              | Machine learning (Neural networks)                                                                                                                 | 16 biophysical features                                         | Blood & Colorectal cancer (2): WBC (OT-II), SW-480                                                                                                                                                                          | ~850     | 85-95%          |
| [34, 35]                                | Actively stabilized phase-shifting reflection QPM                                         | 860 nm ( $\lambda=632$ nm, 20 $\times$ , 0.45 NA) | Loosely hydrodynamically focusing microfluidics | Yes              | Machine learning (SVM)                                                                                                                             | Histogram of orientation gradients (HOGs) features              | Blood & Colorectal cancer (2): WBC, HCT116                                                                                                                                                                                  | ~40,000  | 79%             |

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