Supplementary Information

Label-free analysis of membrane protein binding kinetics and cell adhesions using evanescent scattering microscopy

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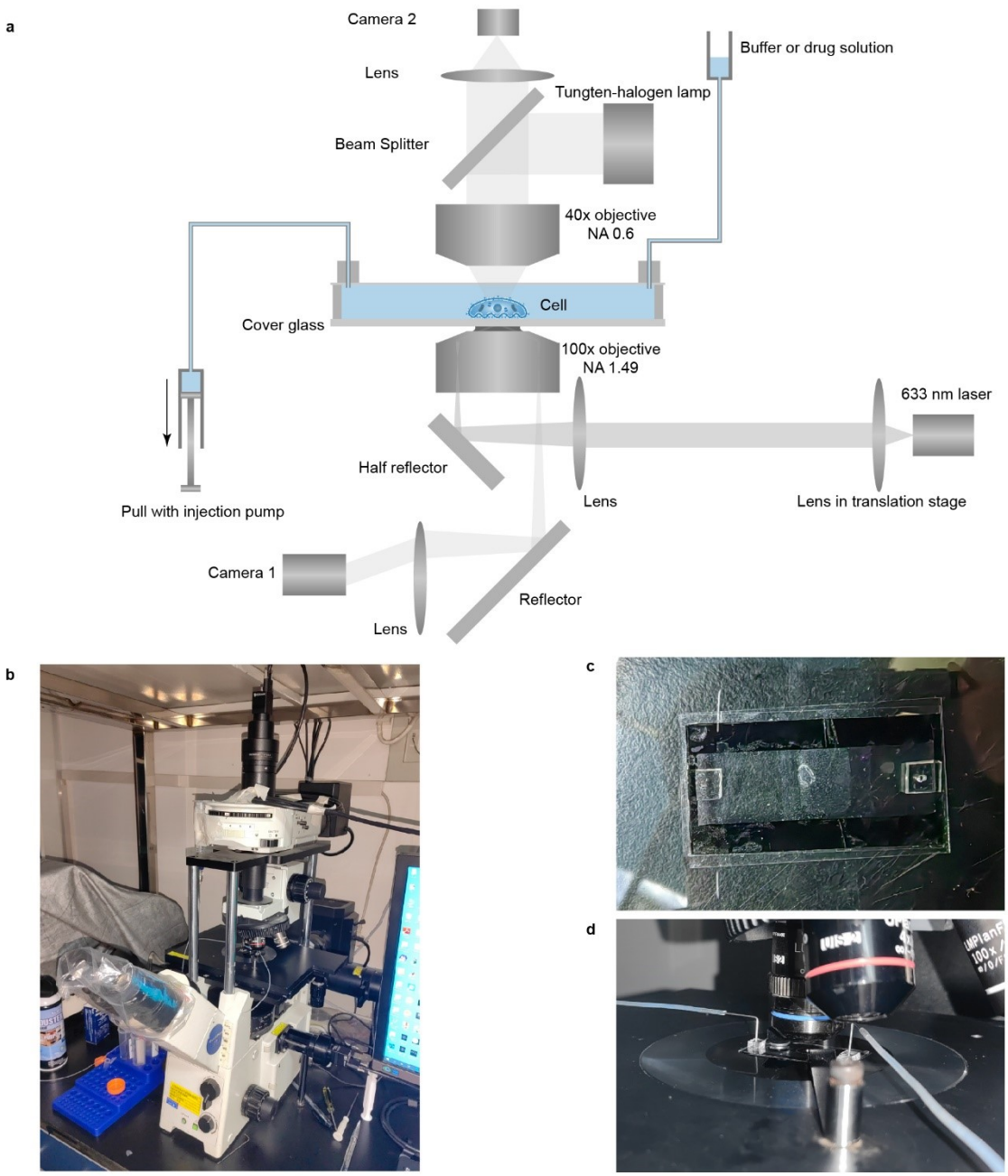

Figure S1 Schematic representation and photographs of the system.

(a) Optical setup for ESM. The ESM are constructed based on an inverted microscopy (Olympus IX71). Light from a laser diode with a center wavelength of 633 nm is conditioned and directed via a 100x objective (NA=1.49) onto a cover glass mounted on the objective via refractive index match oil. Light reflected from the cover glass is detected by camera 1 (MQ042MG-CM, XIMEA), which is used to help adjust the incident angle for total internal reflection. Simultaneously, light scattered from glass surface is collected by a 40x objective (NA=0.60) and detected by camera 2 (MC023MG-SY, XIMEA) placed on top of the sensor surface. The incident light intensity is 7 W cm^{-2}. Camera 2 records the ESM images. A tungsten-halogen lamp was employed as the illumination for bright field imaging. The flow cells were constructed as described before (Angewandte Chemie International Edition 2022, 61, e202209469). (b) The photograph of the optical setup. (c) The photograph of the flow cell. (d) The photograph of the flow cell placed in the optical setup.

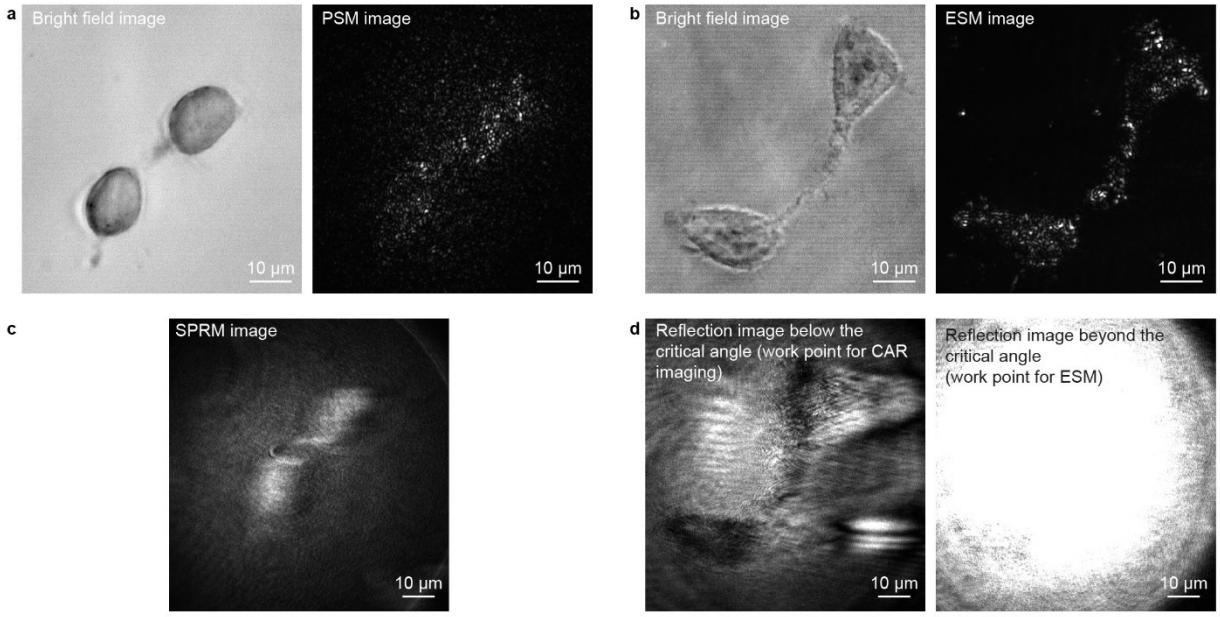

Fig. S2 Bright field and PSM images (a) and SPRM image (c) of live A431 cells on gold-coated cover glasses. Bright field and ESM images (b) and reflection image (d) of live A431 cells on the cover glasses.

By simultaneously recording the reflection and scattering light, the SPRM and PSM images can be achieved to image the live cells at the same time (a and c). Similarly, the reflection image of the cells on the cover glass can be achieved to compare with the ESM images (b and d). These results clearly show that both the ESM and PSM can provide higher spatial resolution for observing the cell adhesions clearly than the traditional SPRM and critical angle reflection (CAR) microscopy detecting the reflected light. Also, the ESM also presents higher image contrast than PSM because the cover glass has much smaller surface roughness than the gold film.

Note S1

Data analysis

The ESM images of the cells were recorded with exposure time of 30 ms. The plot z-axis profile plugin of ImageJ Fiji was employed to achieve the image intensity variation against the time. Scrubber v.2.0a was used to determine the association and dissociation rate constants by fitting the curves with the first-order binding kinetics model.

For the spring constant calculations, the relation between evanescent field intensity I_E and z-displacement can be given by

$$I_E = I_{E0} e^{-z/l}, \quad (1.1)$$

where I_{E0} is the evanescent field intensity at $z = 0$, and l is the decay length of the evanescent field, which is 100 nm for this system. Under scattering conditions, the z-displacement of the analyte can be estimated by

$$\Delta z = z(t + \Delta t) - z(t) = l \times \ln\left(\frac{I_{E0}}{I_E(t + \Delta t)}\right) - l \times \ln\left(\frac{I_{E0}}{I_E(t)}\right) = l \times \ln\left(\frac{I_E(t)}{I_E(t + \Delta t)}\right), \quad (1.2)$$

The statistical distribution of fluctuating z-displacement amplitude can be used for evaluating the free energy profiles of one binding pair according to

$$P(z) = A \exp\left[-\frac{G(z)}{k_B T}\right], \quad (1.3)$$

where A is a constant that can be determined by normalization of $P(z)$, k_B is the Boltzmann constant, and T is the temperature. The effective spring constant k can be determined from the simplified expression of free energy profile near equilibrium shown as

$$G(z) = G(0) + \frac{1}{2} k z^2, \quad (1.4)$$

where $G(0)$ is the free energy at equilibrium.

For estimating the positions of cell adhesion sites, the TrackMate plugin of ImageJ Fiji was used to achieve the locations of all bright spots generated by adhesion sites, and the deformation and cell center movement trace were achieved using the MATLAB code shown in Section 6.

Note S2

MATLAB codes for deformation and migration tracking

```
frame=A(:,4);positionx=A(:,1);positiony=A(:,2);
uniqframe=unique(frame);sizesize=size(uniqframe);sizes=sizesize(1);
for i=1:sizes
   afra=i-1;
   afram=find(frame==afra);
   sizesize1=size(afram);
   sizes1=sizesize1(1);
   for j=1:sizes1
      a1=afram(j);
      positionx1(j)=positionx(a1);
      positiony1(j)=positiony(a1);
   end
   meanpositionx1=mean(positionx1);
   Acenterpositionx1(i)=meanpositionx1;
   meanpositiony1=mean(positiony1);
   Acenterpositiony1(i)=meanpositiony1;

   for k=1:sizes1
      b1=afram(k);
     distance12=((positionx(b1)-Acenterpositionx1(i))^2)+((positiony(b1)-Acenterpositiony1(i))^2);
     distance12x=(positionx(b1)-Acenterpositionx1(i))^2;
     distance12y=(positiony(b1)-Acenterpositiony1(i))^2;
     distance1(k)=sqrt(distance12);
     distance1x(k)=sqrt(distance12x);
     distance1y(k)=sqrt(distance12y);
   end
   Ashape(i)=sum(distance1);
   Ashapex(i)=sum(distance1x);
   Ashapey(i)=sum(distance1y);

    afram=1;
    positionx1=1;
    positiony1=1;
    distance1=1;
end
Ashapex=Ashapex';
Ashapey=Ashapey';
Ashape=Ashape';
Acenterpositionx1=Acenterpositionx1';
Acenterpositiony1=Acenterpositiony1';

siz=size(Acenterpositionx1);
period=100;
siz1=siz(1)/period;
```

```
Raws(:,1)=Acenterpositionx1';Raws(:,2)=Acenterpositiony1';

for i1=1:siz1
   Raw(:,1)=Raws(((i1-1)*period+1):(i1*period),1);
   Raw(:,2)=Raws(((i1-1)*period+1):(i1*period),2);
   Atracex(i1)=mean(Raw(:,1));
Atracey(i1)=mean(Raw(:,2));
end

siz2=siz1-1;
for i2=1:siz2
   a1=(Atracex(i2+1)-Atracex(i2))^2;A2(i2)=sqrt(a1);
   a2=(Atracey(i2+1)-Atracey(i2))^2;A3(i2)=sqrt(a2);
   a=a1+a2;A1(i2)=sqrt(a);
end

Atrace(:,1)=Atracex';
Atrace(:,2)=Atracey';
A1=A1';
A2=A2';
A3=A3';
Aratemean=mean(Arate);
```