Application Note

IMMUNOFLUORESCENCE STAINING OF ACTIN, TUBULIN AND NUCLEI USING PANC-1 AND HEK293 CELLS GROWN ON LIFEGEL

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PROCEDURE

LifeGel on 15 well µ-slide from IBIDI

This procedure was optimized for cells growing on Lifegel on 15 well µ-slide from IBIDI.

1. Fix cells using 4% formaldehyde (10% formalin solution) – 40 µL per well and incubate for 15 minutes at room temperature.
2. Wash cells 3x with 40 µL of PBS.
3. Perforate cells using 0,1% Triton X-100 in PBS with 10% FBS. Use 40 µL of solution per well and incubate for 15 minutes at room temperature.
4. Wash cells twice with PBS.
5. Change PBS for 40 µL of working solution of primary antibody (1:1000 dilution) and incubate plate overnight at 4ºC.
6. Next day wash cells 3 times with PBS (wait 5 minutes between each washing).
7. Prepare mix of working solution containing secondary antibody (1:500), phalloidin (1:500) and Hoechst (10 µg/mL) in PBS. Add 30 µL per well and incubate plate for 3 hours at room temperature in darkness.
8. Wash cells with PBS, 3 times (wait 5 minutes between each washing).
9. Then take images of brightfield and fluorescence using fluorescence microscope and objectives characterized by working distance not less than 1,5 mm – objectives with higher magnifications like 10x and 20x are preferable for cytoskeleton training.

LifeGel on 96 well plate

1. Fix cells using 4% formaldehyde (10% formalin solution) – 100 µL per well and incubate for 15-30 minutes at room temperature (the bigger 3D structures, the longer time of incubation is recommended).
2. Wash cells 3x with 150 µL of PBS.
3. Perforate cells using 0,1% Triton X-100 in PBS with 10% FBS. Use 100 µL of solution per well and incubate for 15-30 minutes at room temperature (time similar as for cell fixation).
4. Wash cells twice with PBS.
5. Change PBS for 100 µL of working solution of primary antibody (1:500 dilution) and incubate plate overnight at 4ºC.
6. Next day wash cells 3 times with PBS (wait 5 minutes between each washing).
7. Prepare mix of working solution containing secondary antibody (1:250), phalloidin (1:500) and Hoechst (10 µg/mL) in PBS. Add 100 µL per well and incubate plate for 3 hours at room temperature in darkness.
8. Wash cells with PBS, 3 times (wait 5 minutes between each washing).
9. Then take images of brightfield and fluorescence using fluorescence microscope and objectives characterized by working distance not less than 2 mm – objectives with higher magnifications like 10x and 20x are preferable for cytoskeleton imaging.

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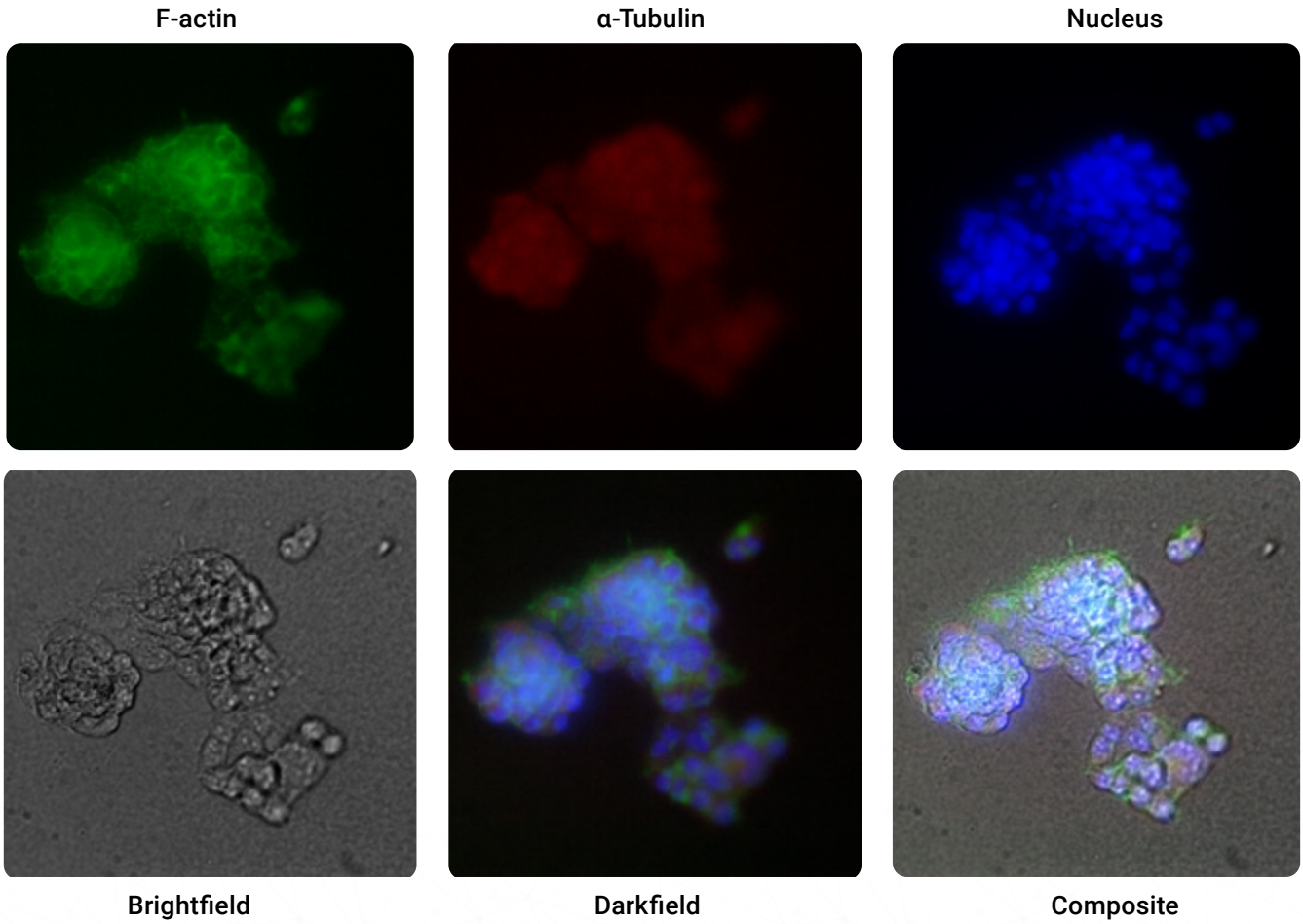

Fig. 1. PANC-1 spheroids immunostaining on LifeGel. Objective 10x, optovar lenx 2,5x.

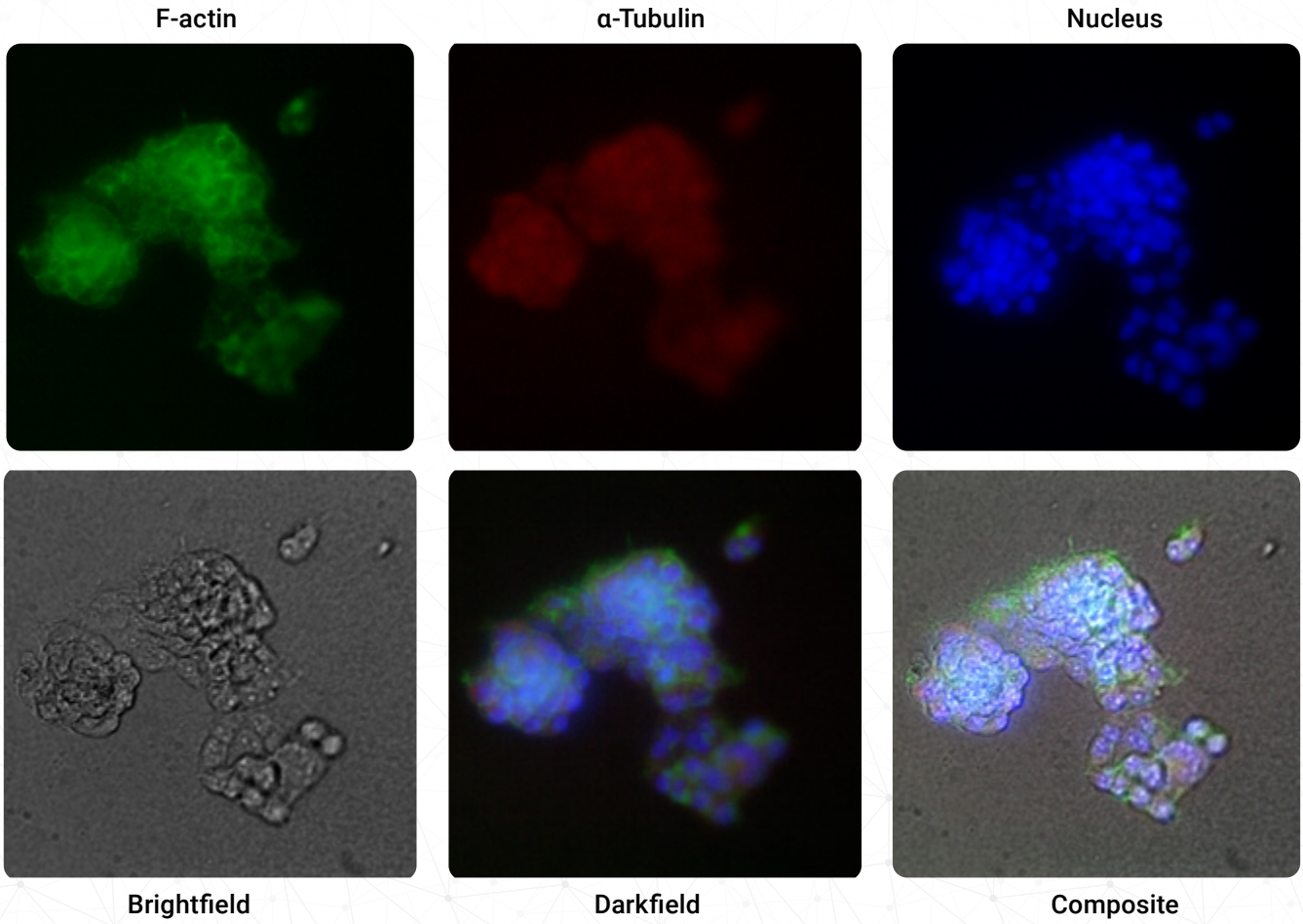

Fig. 2. HEK293 cells immunostaining on LifeGel. Objective 10x, optovar lens 2,5x.

Find out more on LifeGel and another application notes at
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