

NET4mPLASTIC Project

Work Package 4.4

Deliverable 4.4.1

Supplementary Materials

DETERMINATION OF THE FATE OF MICROPLASTICS
WITHIN CELLS

June, 27, 2022 – Version 1.0

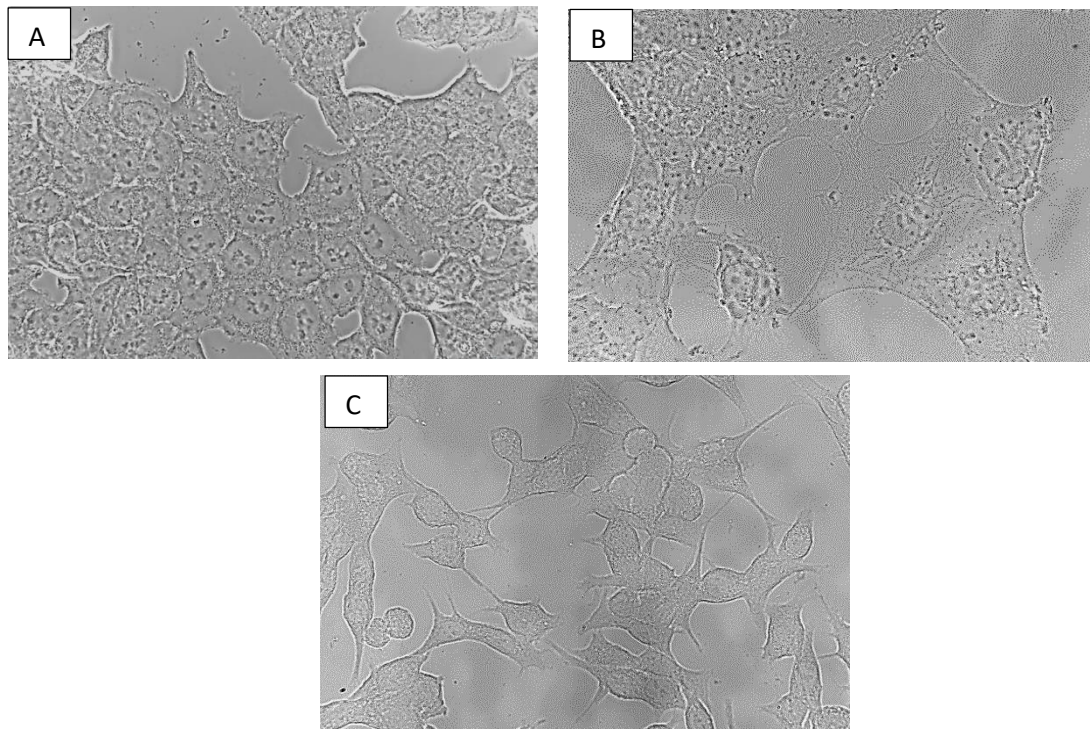


Figure 1. Phase contrast pictures of the human cell lines used for the in vitro experiments: a) HCT-116, b) Mahlavu, c) A549. Magnification 60x.

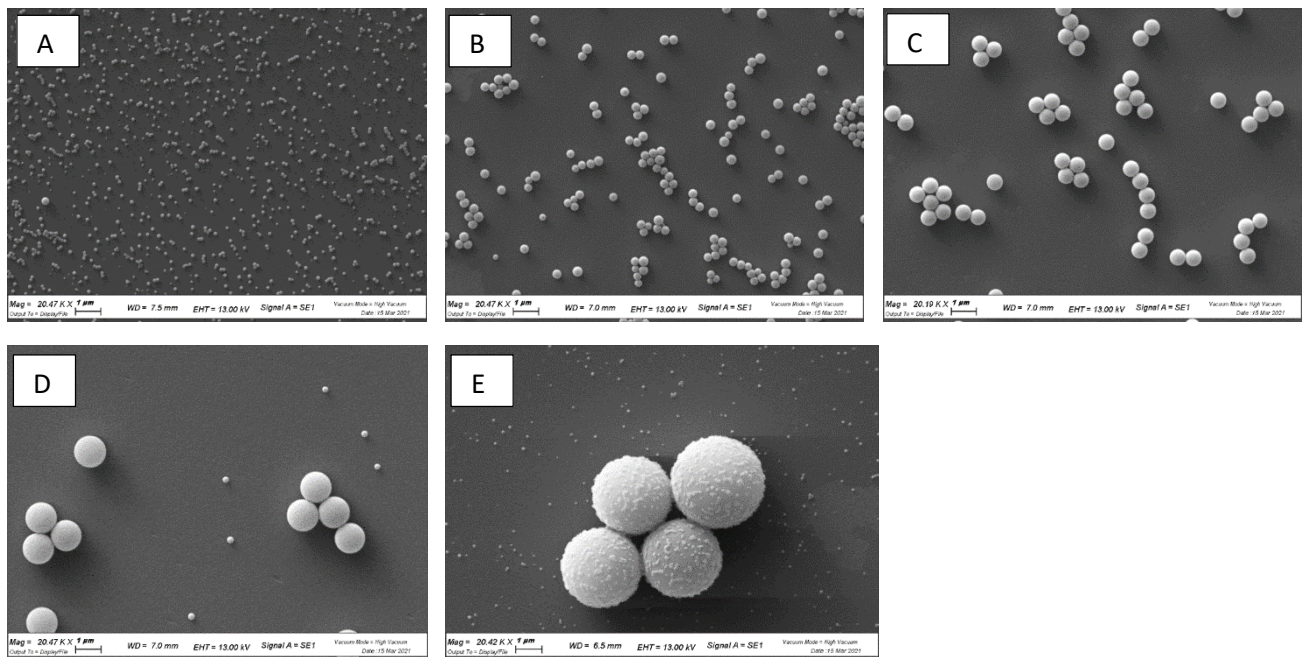


Figure 2 Characterization of PS-MPS with SEM: a) 0.1 μm , b) 0.3 μm , c) 0.6 μm , d) 1.1 μm and e) 3 μm . Smaller particles than the expected size were observed in d and e. Images were captured at the same magnification. Magnification: 20.000x.

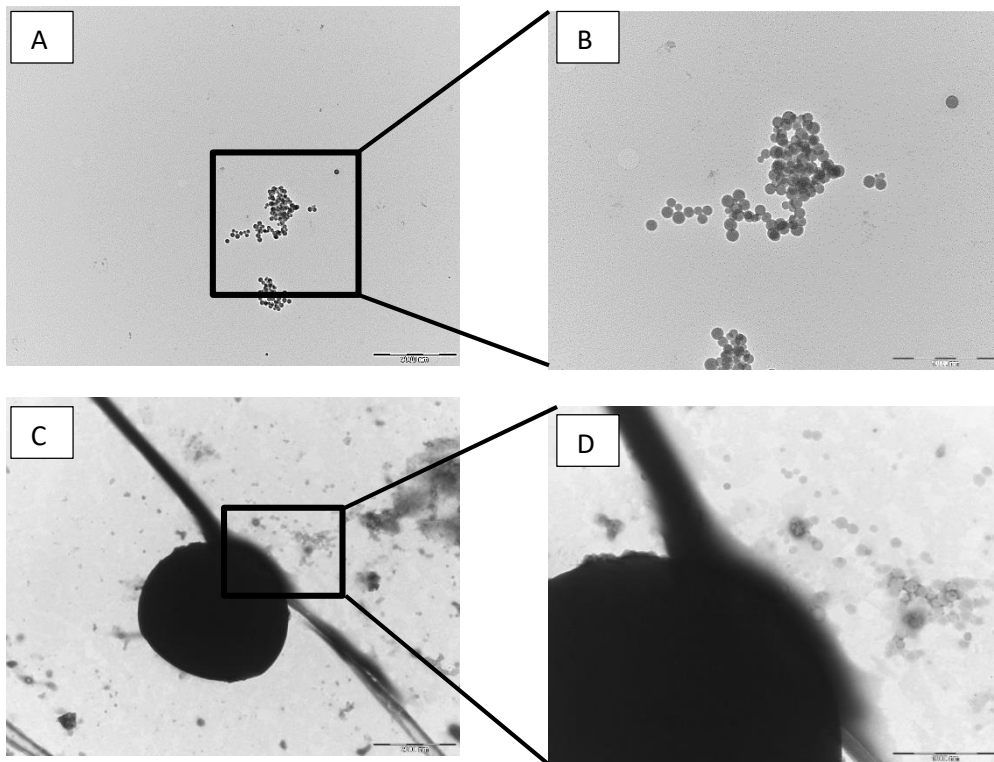


Figure 3 Examples of PS-MPs characterized by TEM: a) 0.1 μm , b) enlargement of 0.1 μm , c) 3 μm , d) enlargement of 3 μm . Smaller particles than the expected size were observed in d. Magnification: 8,000x (a and c) and 20,000x (b and d).

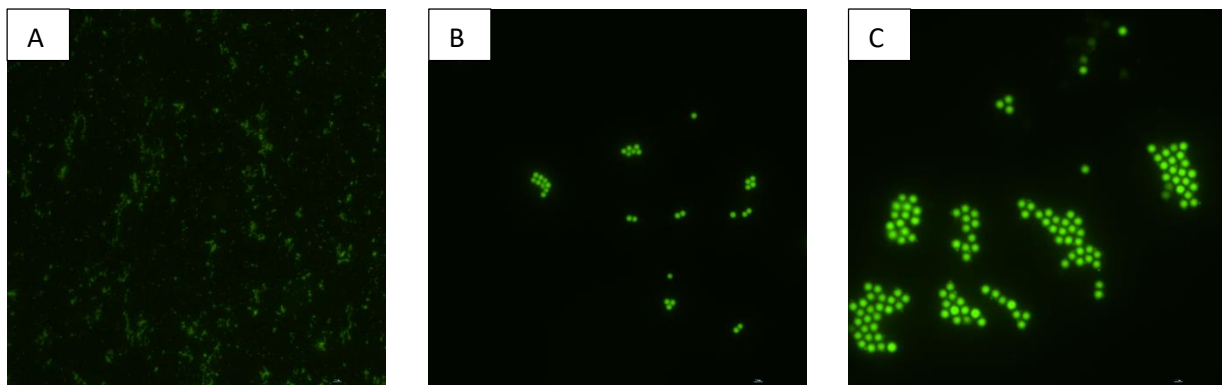


Figure 4. Characterization of PS-MPS with light fluorescence microscopy: a) 0.1 μm , b) 1 μm and c) 3 μm . Images were captured at the same magnification. Magnification: 100x.

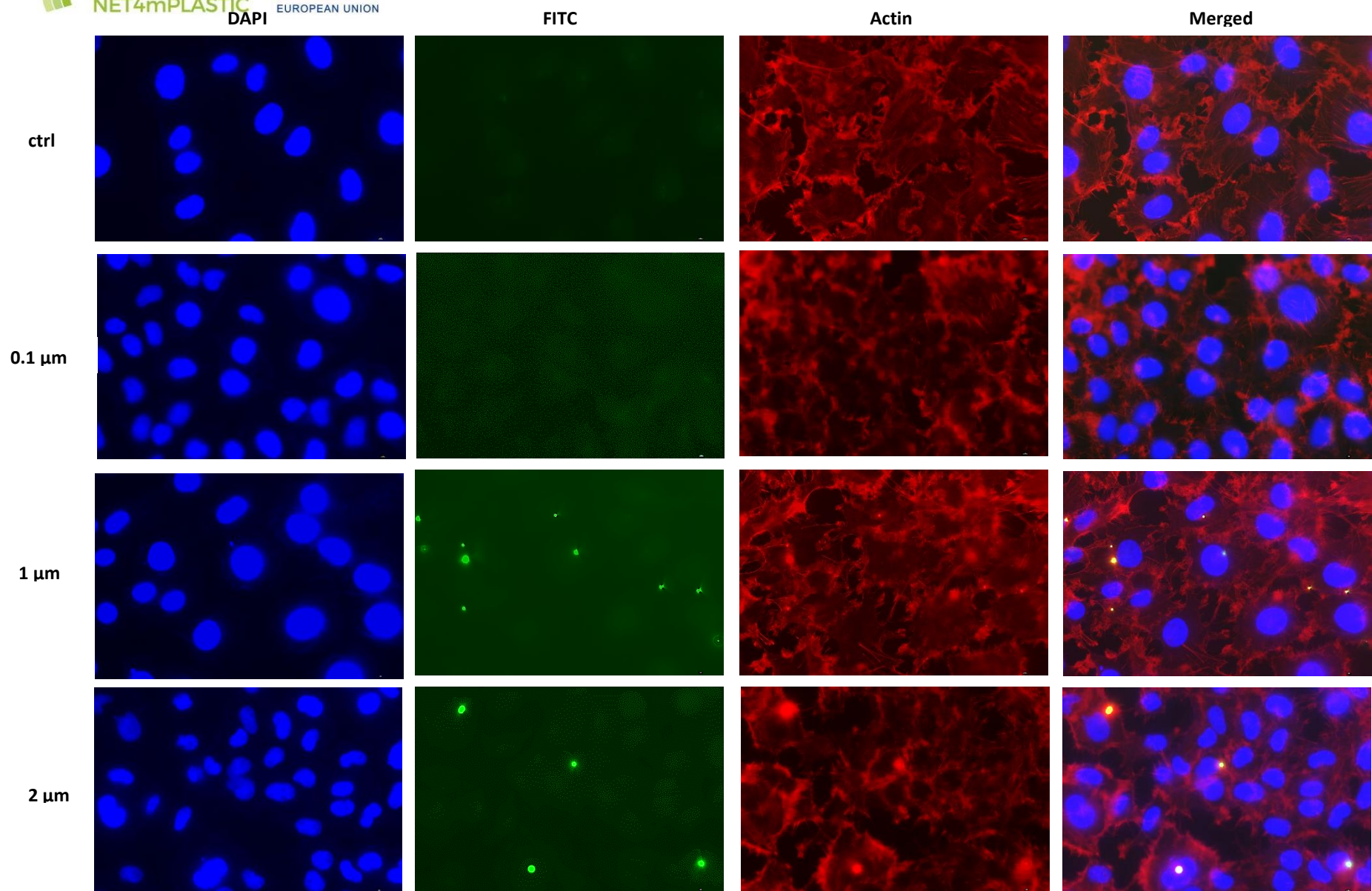


Figure 5. Analysis of the interaction of PS-MPs (1,000 beads/ mm^2) with Mahlavu cells by light fluorescence microscopy: ctrl, 0.1 μm , 1 μm and 2 μm . After 48 h of treatment, cells were fixed and stained with DAPI (blue, nucleus) and phalloidin (red, cytoskeleton); PS-MPs were fluorescent labelled (green). Magnification: 60x.

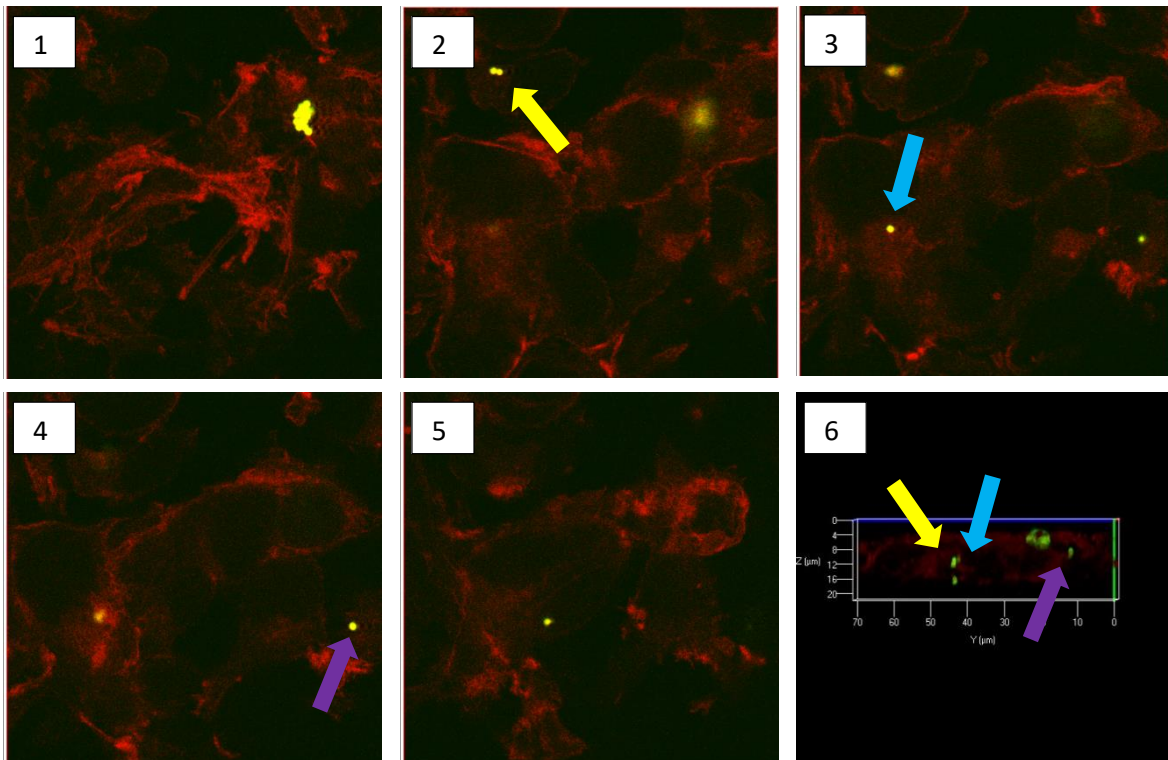


Figure 6. Confocal microscopy pictures of Mahlavu cells treated with 1 μm PS-MPs (1,000 beads/ mm^2) for 48 h. 1÷5) five consecutive Z-sections (layer distance: 0.3 μm); .6) 3D reconstruction of the five Z-sections. Internalized PS-MPs from the cells are pointed by yellow / light-blue / purple arrows. Red: cell cytoskeleton, green/yellow: 1 μm PS-MPs. Magnification: 63x.

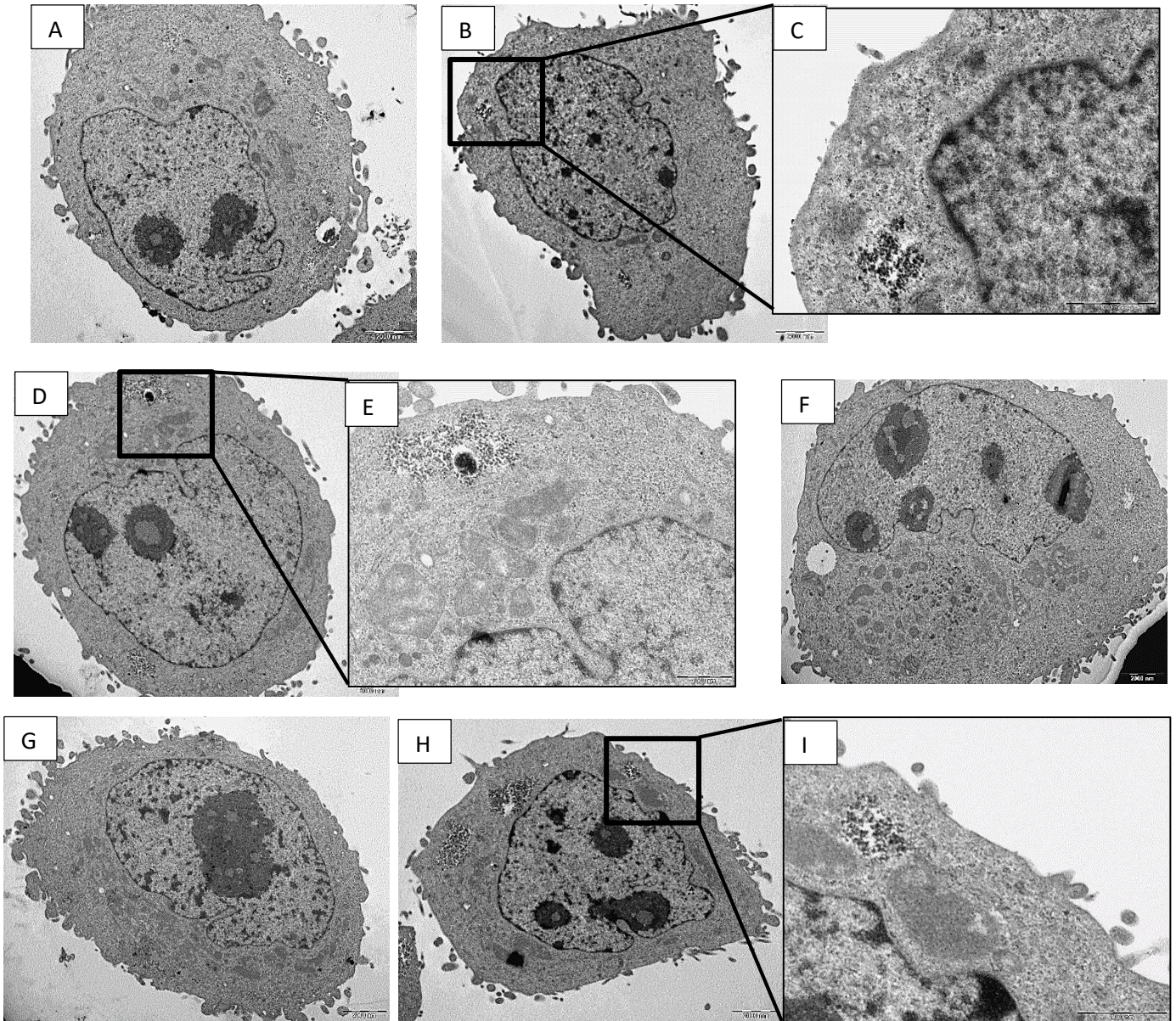
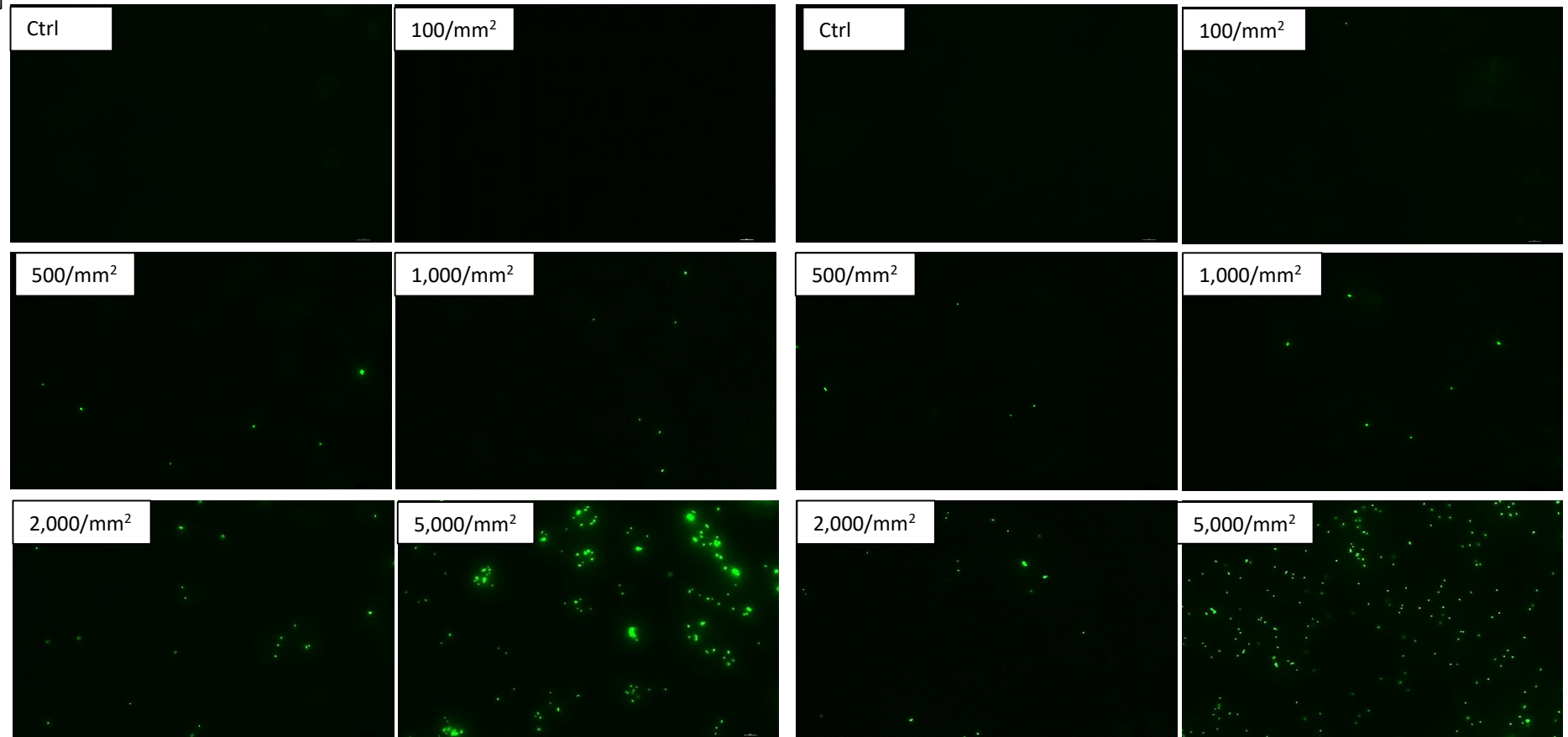


Figure 7. TEM images of Mahlavu treated with unlabelled PS-MPs for 48 h (10,000 beads/mm²): a) ctrl, b) 0.1 μ m, c) enlargement of the 0.1 μ m treated cell, d) 0.3 μ m, e) enlargement of the 0.3 μ m treated cell, f) 0.6 μ m, g) 1.1 μ m, h) 3 μ m, i) enlargement of the 3 μ m treated cell. Magnification: 5,000x (a, b, d, f, g and h) and 20,000x (c, e, and i).

A

Mahlavu

HCT-116



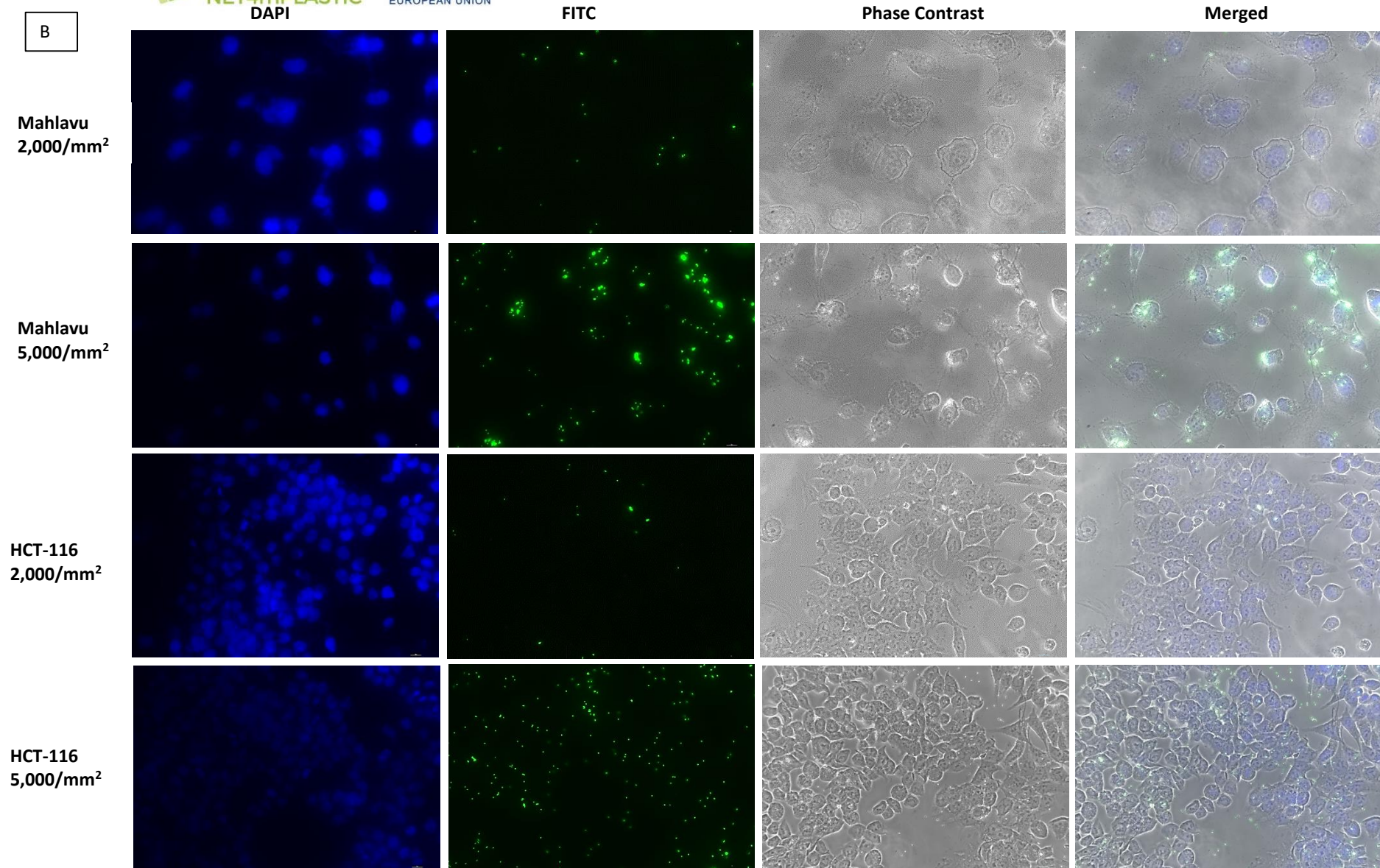


Figure 8. Light fluorescence microscopy images of Mahlavu and HCT-116 cell lines exposed to 1 μ m PS-MPs (100 – 500 – 1,000 – 2,000 – 5,000 beads/mm²) for 48 h: A) FITC images of PS-MPs at the different concentrations, B) examples of distribution of PS-MPs at concentrations of 2,000 and 5,000 beads/mm² and cells. Nucleus: blue (DAPI); cells: phase contrast images; PS-MPs: green. Magnification: 40x.

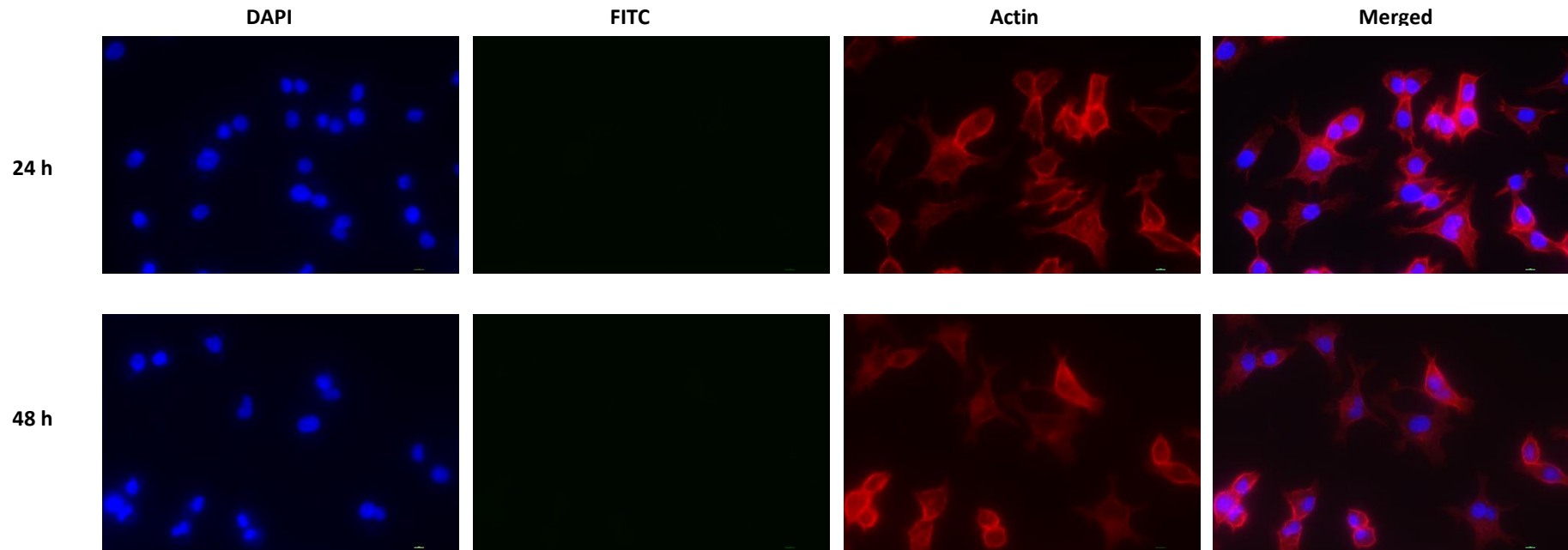
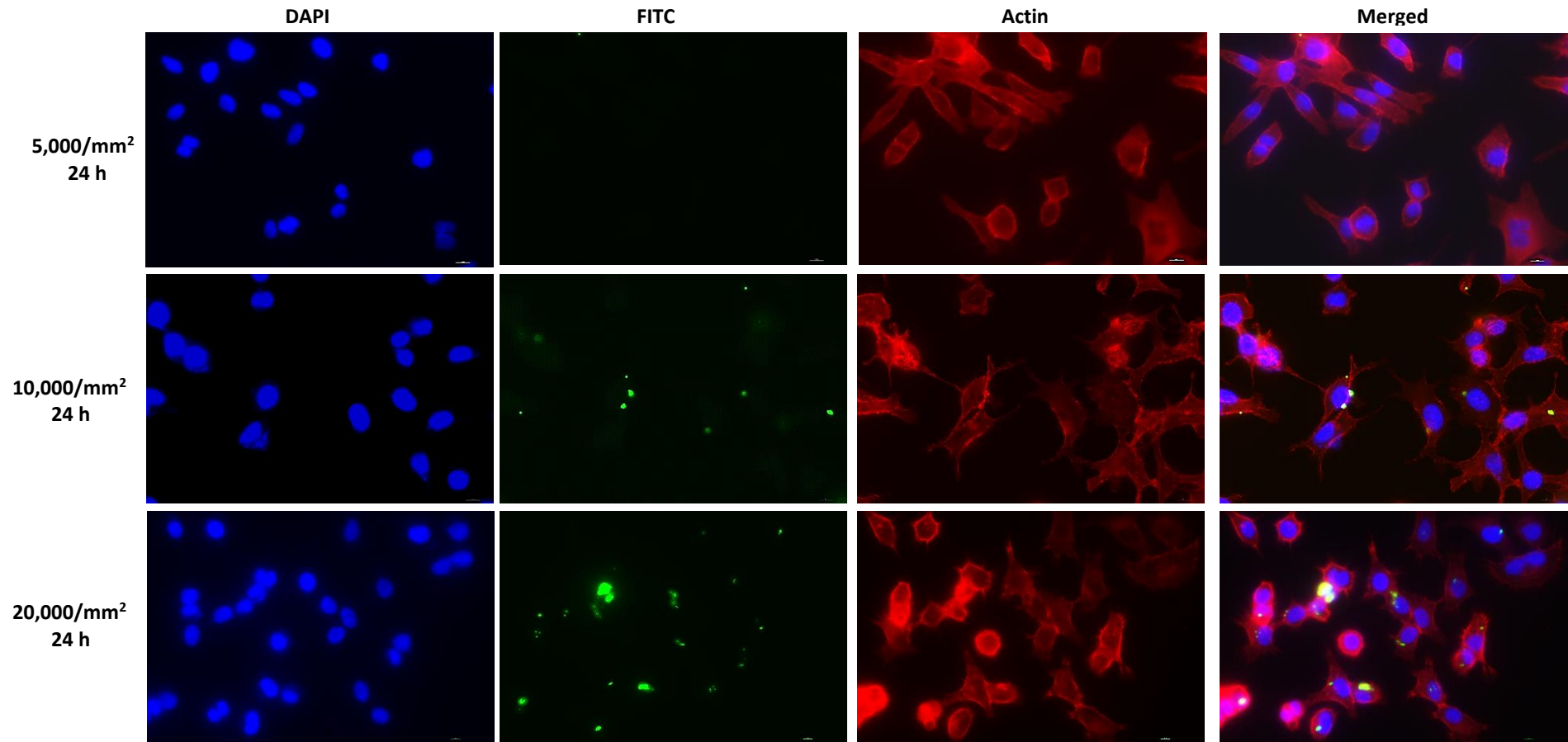


Figure 9. Light fluorescence microscopy images of untreated A549 cells (ctrl) at 24 and 48 h. Nucleus: blue (DAPI); cytoskeleton: red (phalloidin iFluor-555). Magnification: 60x.



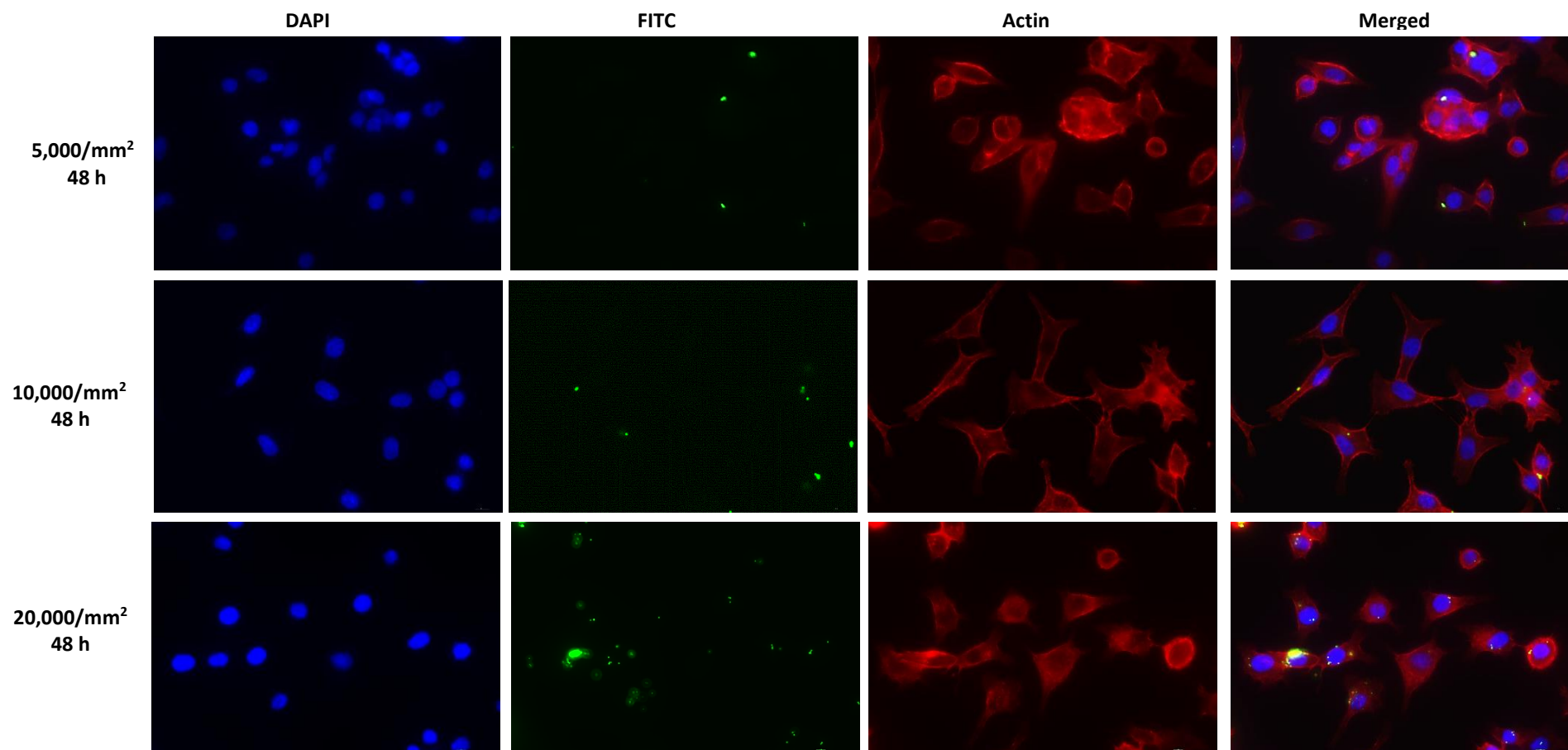
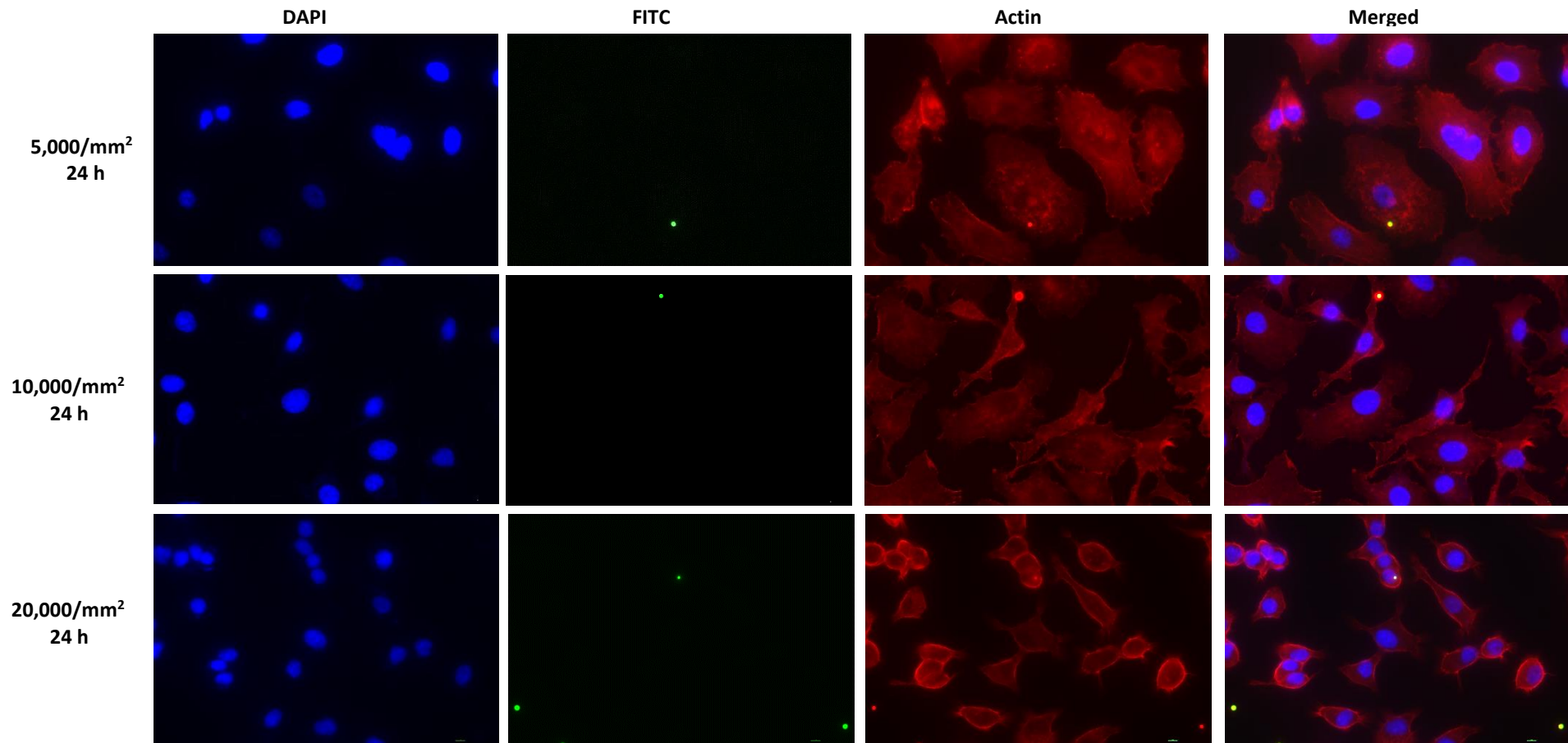


Figure 10. Light fluorescence microscopy images of A549 cells treated with 1 μm PS-MPs (5,000 – 10,000 – 20,000 beads/mm²) for 24 and 48 h. Nucleus: blue (DAPI); cytoskeleton: red (phalloidin iFluor-555), PS-MPs: green. Magnification: 60x.



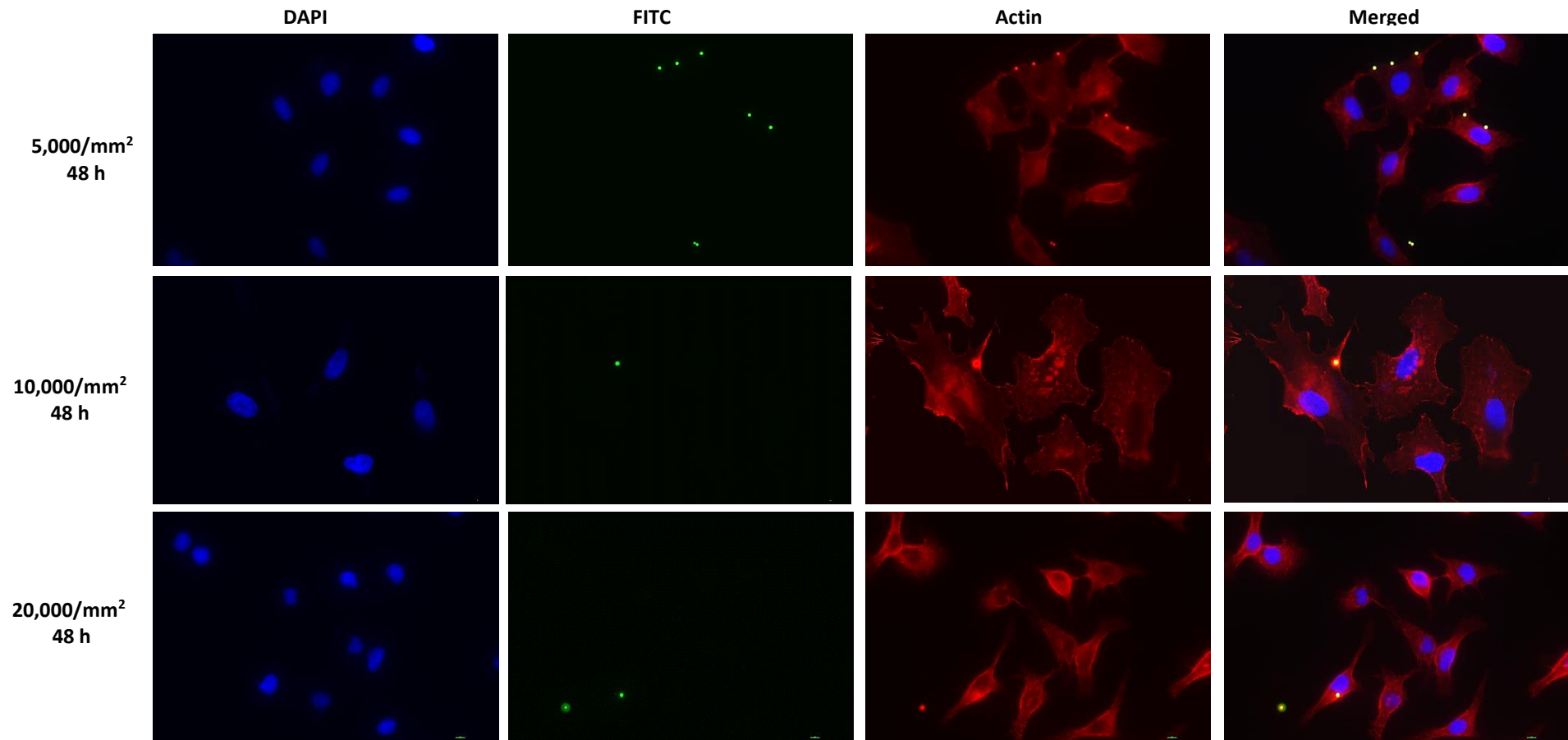


Figure 11. Light fluorescence microscopy images of A549 cells treated with 2 μm PS-MPs (5,000 – 10,000 – 20,000 beads/mm²) for 24 and 48 h. Nucleus: blue (DAPI); cytoskeleton: red (phalloidin iFluor-555), PS-MPs: green. Magnification: 60x.

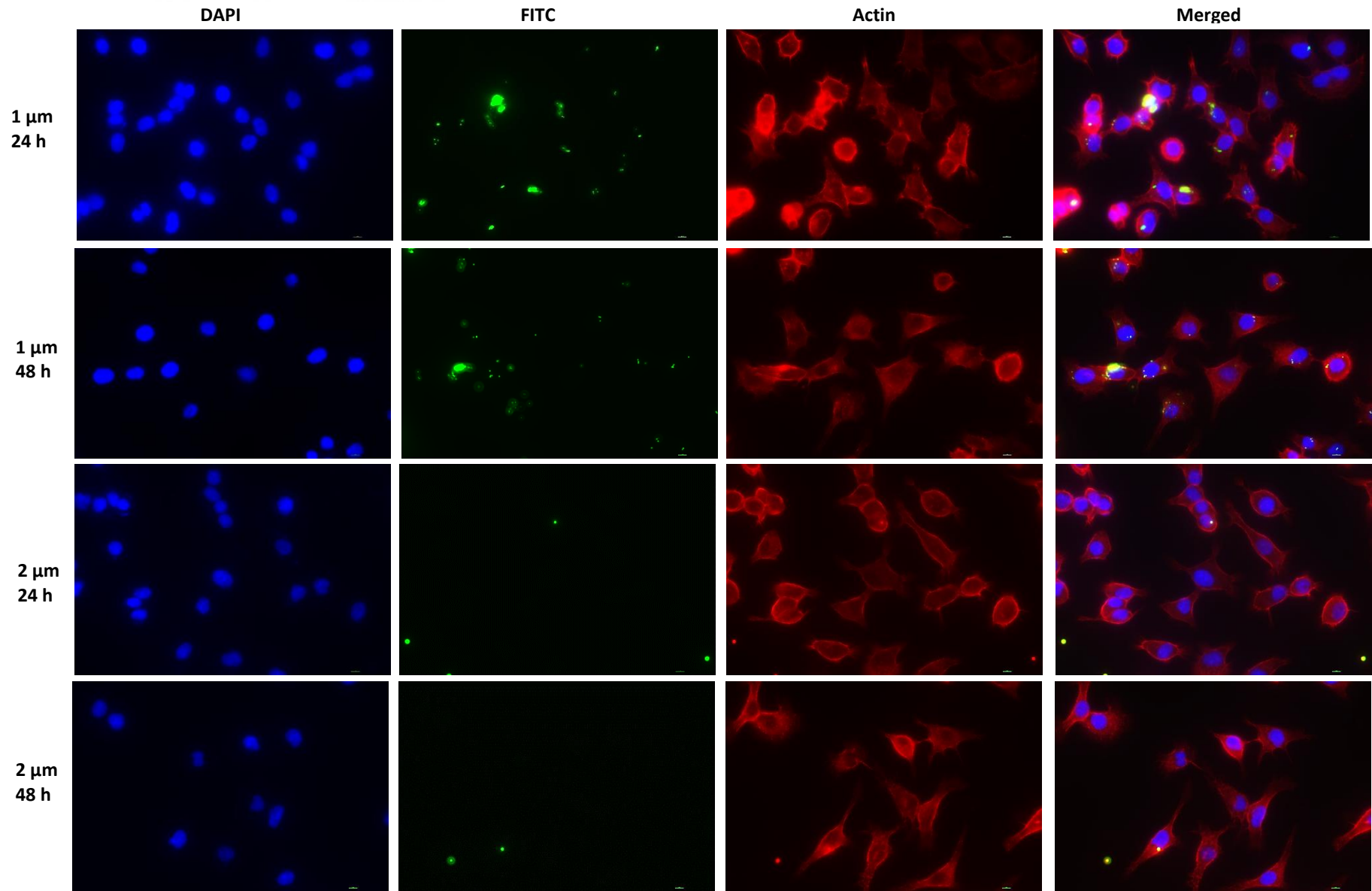
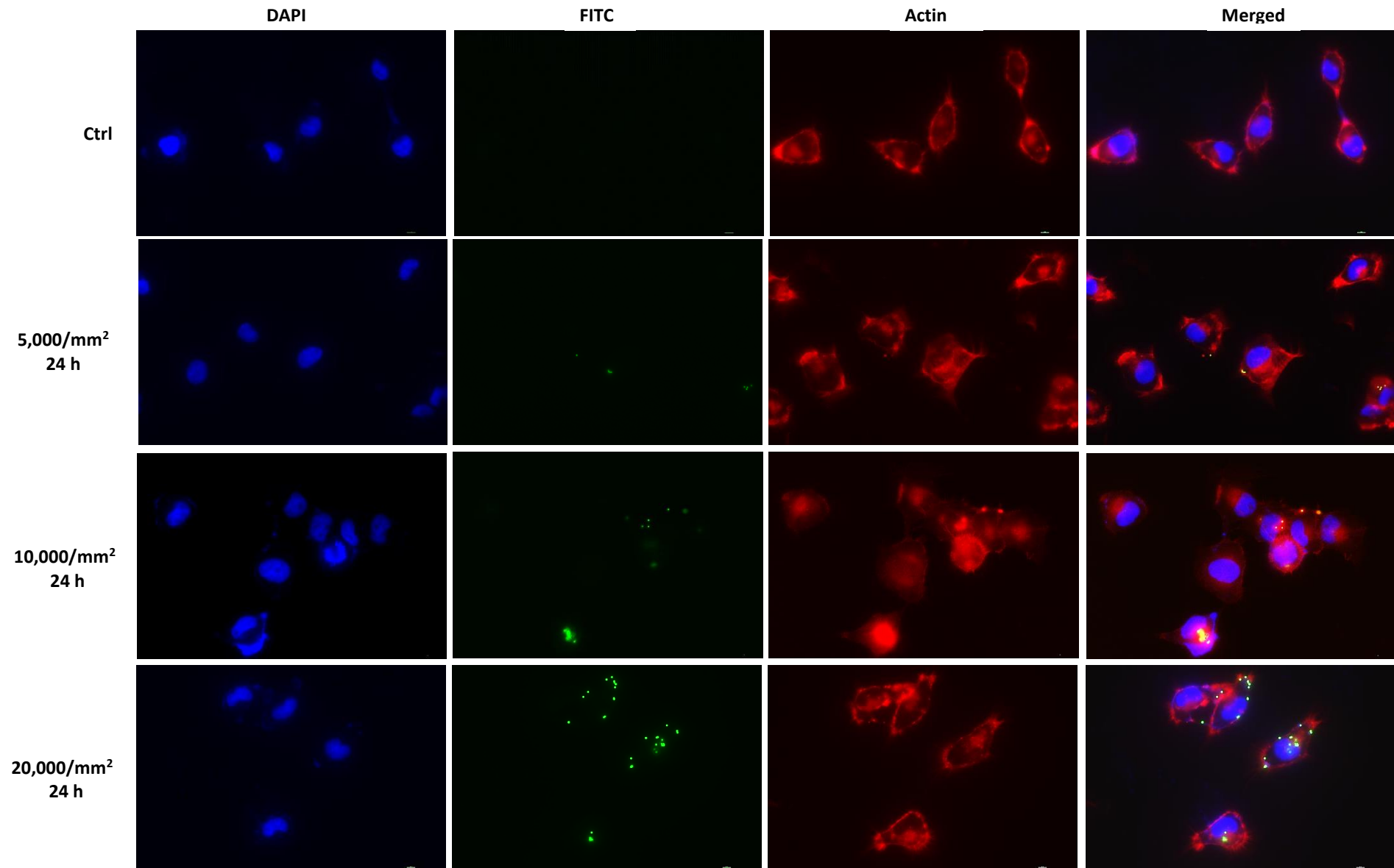


Figure 12. Light fluorescence microscopy images of A549 cells treated with 1 - 2 μm PS-MPs (20,000 beads/ mm^2) for 24 and 48 h. Nucleus: blue (DAPI); cytoskeleton: red (phalloidin iFluor-555), PS-MPs: green. Magnification: 60x.



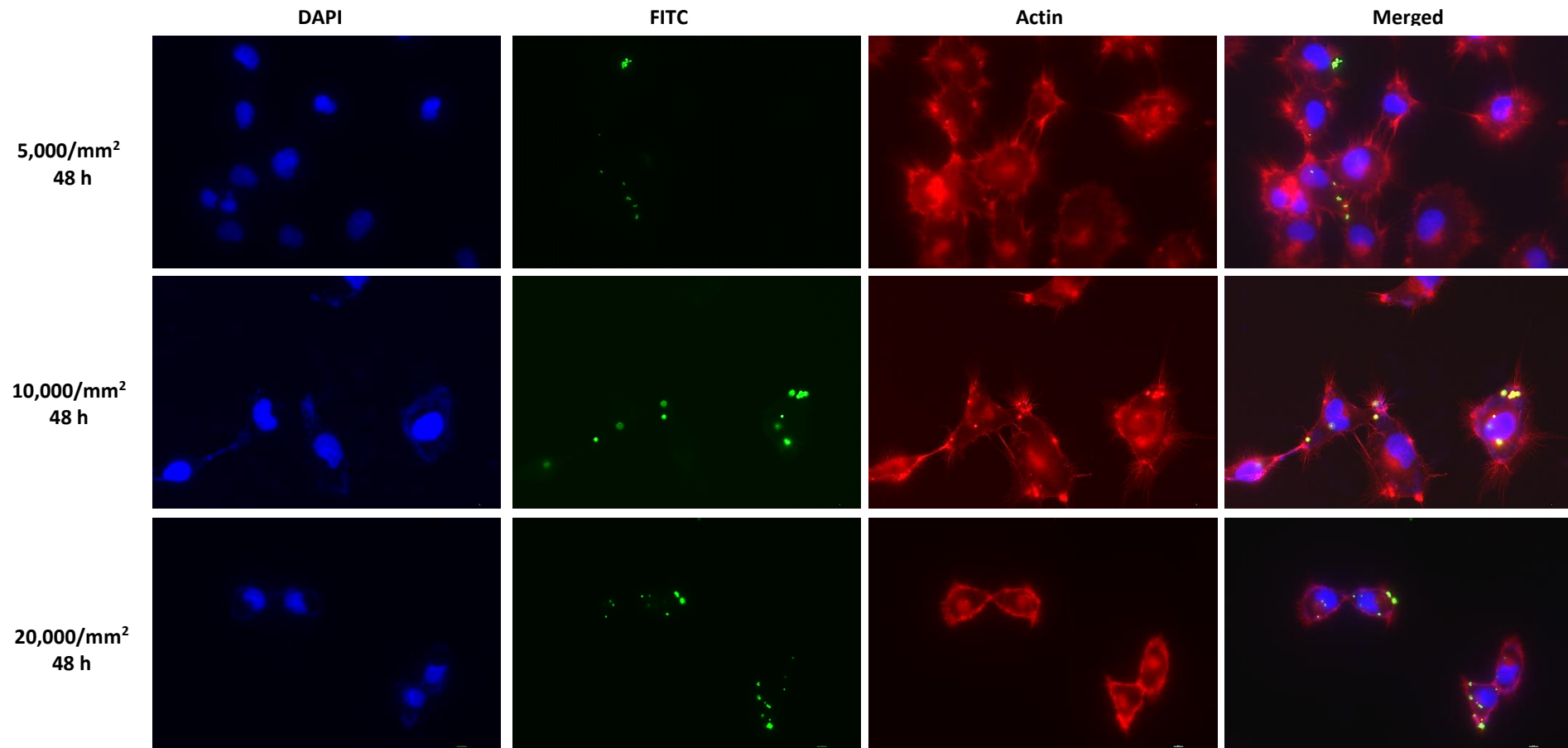
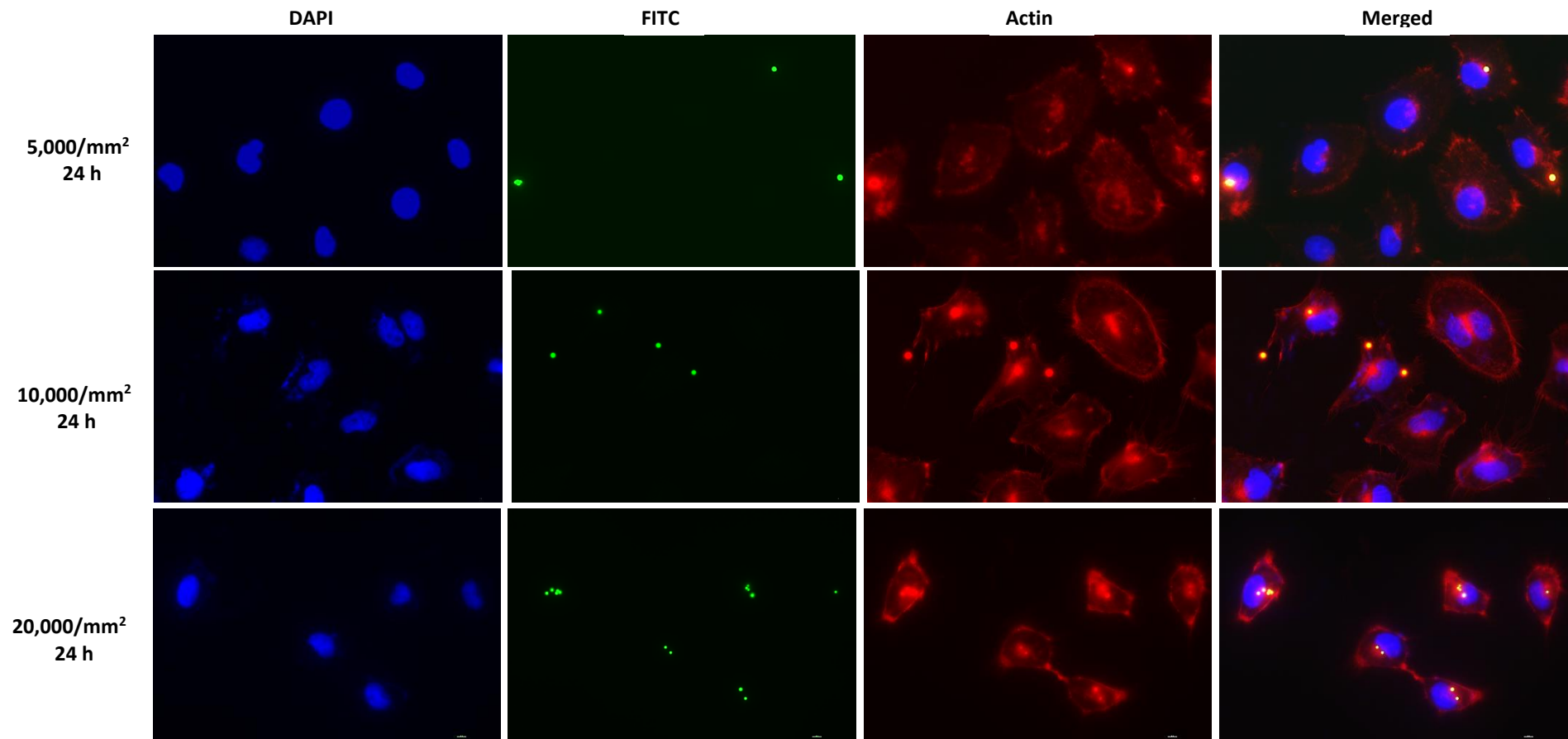


Figure 13. Light fluorescence microscopy images of Mahlavu cells untreated (an example) and treated with 1 μm PS-MPs (5,000 – 10,000 – 20,000 beads/mm²) for 24 and 48 h. Nucleus: blue (DAPI); cytoskeleton: red (phalloidin iFluor-555), PS-MPs: green. Magnification: 60x.



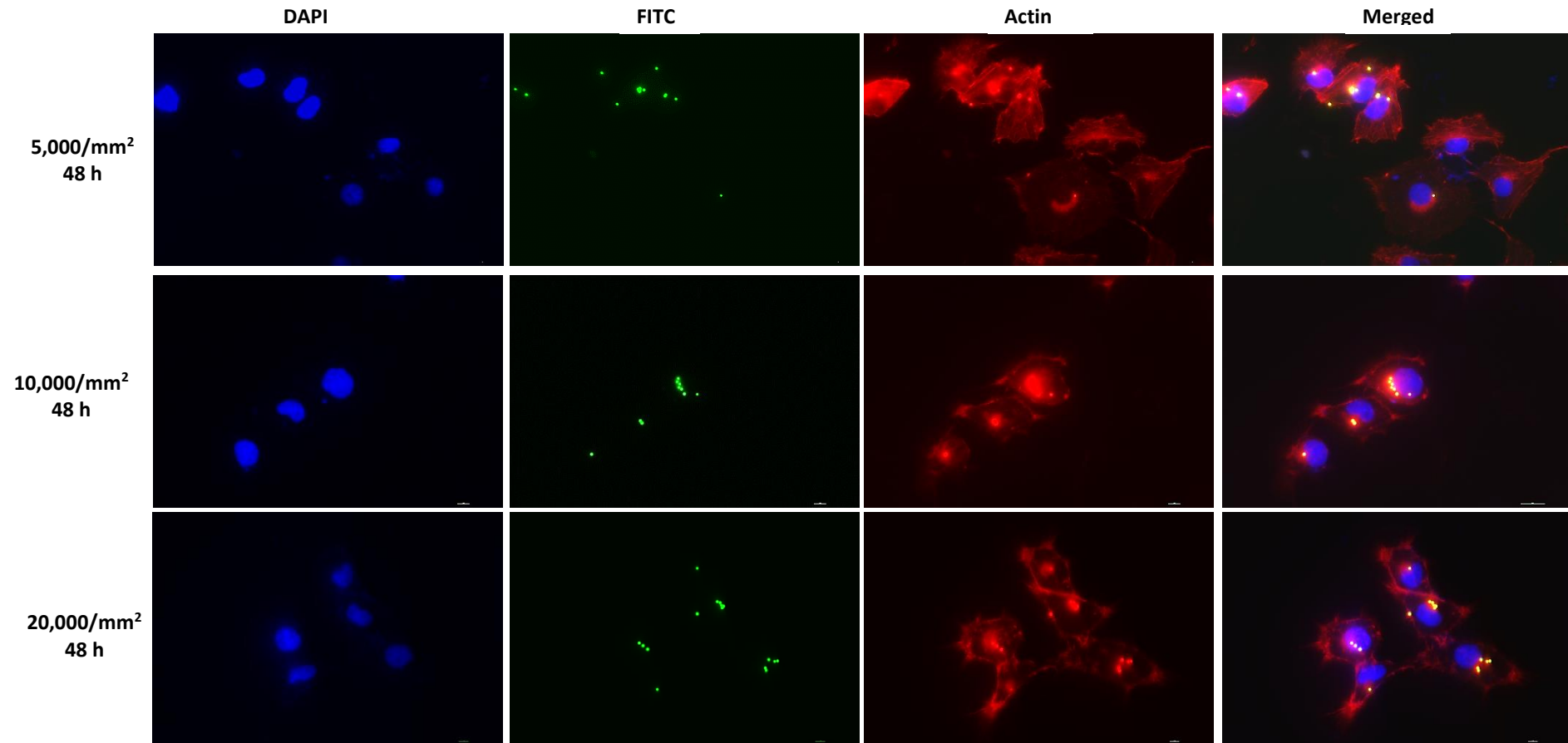


Figure 14. Light fluorescence microscopy images of Mahlavu cells treated with 2 μm PS-MPs (5,000 – 10,000 – 20,000 beads/mm²) for 24 and 48 h. Nucleus: blue (DAPI); cytoskeleton: red (phalloidin iFluor-555), PS-MPs: green. Magnification: 60x.

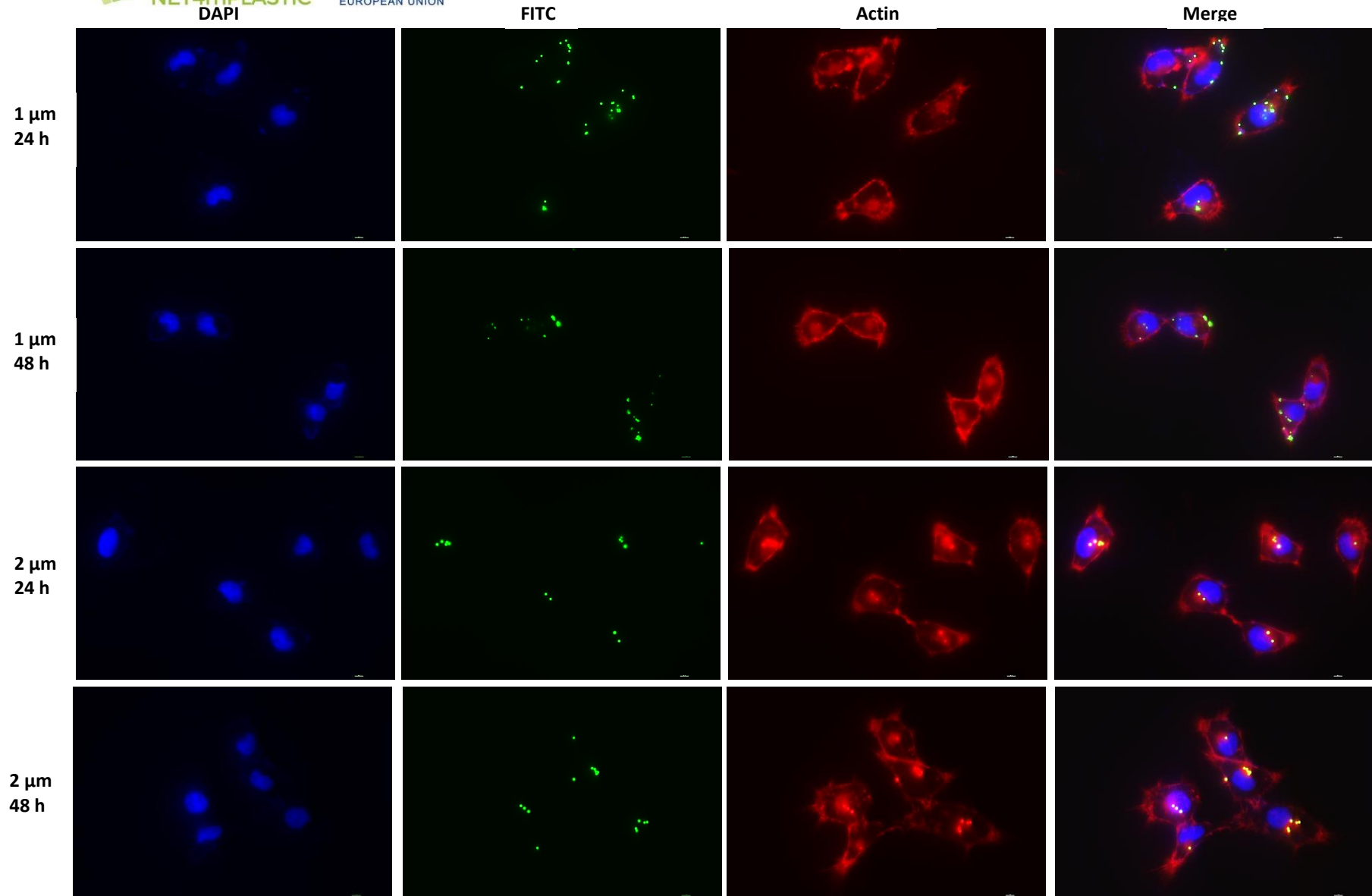


Figure 15. Light fluorescence microscopy images of Mahlavu cells treated with 1 - 2 μm PS-MPs (20,000 beads/ mm^2) for 24 and 48 h. Nucleus: blue (DAPI); cytoskeleton: red (phalloidin iFluor-555), PS-MPs: green. Magnification: 60x