



iFLUOR: Bridging the Gap from Macro to Micro

Ultra-wide Field Imaging of Neuronal Development using Neurosetta Micropatterned Arrays

Ultra-wide field acquisition of hundreds of human iPSC-derived neural rosettes on micropatterned supports

At D-Tails, we are dedicated to making ultra-wide field imaging accessible to all researchers, empowering them to push their scientific potential and breakthroughs to the next level.

iFLUOR is a relay lens-based ultra-wide field imaging system capable of capturing multicellular structures across a $10 \times 10 \text{ mm}^2$ field of view and is compatible with various sample holders.

A suite of standout features makes iFLUOR a versatile and adaptable system, including capabilities for transmission and dual-channel fluorescence imaging, the option to integrate up to four microfluidic pumps, a micro-incubator adapter, and all-in-one software for timelapse design, programmable fluidic delivery (timed and/or ramped), acquisition control, and data analysis.

In this Application Note, we demonstrate that iFLUOR can acquire a $10 \times 10 \text{ mm}^2$ surface containing 900 experimental replicates at $2.5 \mu\text{m}$ pixel resolution. The system enables detailed inspection of individual replicates directly from the acquired full-field image.



iFLUOR Imaging of Neurosetta Plates to Streamline Human Neurodevelopmental Screening

We utilized Rosette Array assays provided by NEUROSETTA®, specialized custom plates featuring a goldcoated micropatterned culture surface designed to induce the formation of arrays of 900 brain rosettes, which genetically and morphologically model human neural tube development in a reproducible, costefficient, and high-throughput manner. Human iPSCs were seeded on this gold-coated micropatterned surface, allowing cells to aggregate into 900 individual rosettes. After 12 days, the rosettes were fixed with 4% PFA and stained for the phalloidin marker (Sigma Aldrich, 49409).

iFLUOR was used to excite the stained cells using a 470 nm LED source and acquire an ultra-wide field image (10 × 10 mm²) at 2.5 μm pixel resolution, enabling detailed visualization of individual regions within a single dataset. As shown in Figure 1, iFLUOR enables 1:1 acquisition of the entire well across a 10 × 10 mm² field of view, capturing hundreds of 250 μm microenvironments and facilitating evaluation of variability between replicates.

The native image sampling preserves structural detail, allowing clear visualization of rosette morphology when inspecting selected regions of interest.

This application demonstrates that iFLUOR enables consistent imaging analysis spanning from populationlevel overviews to detailed structural inspection within the same dataset. Dedicated, tailored one-to-one application support is available upon request to assist users in implementing this workflow.

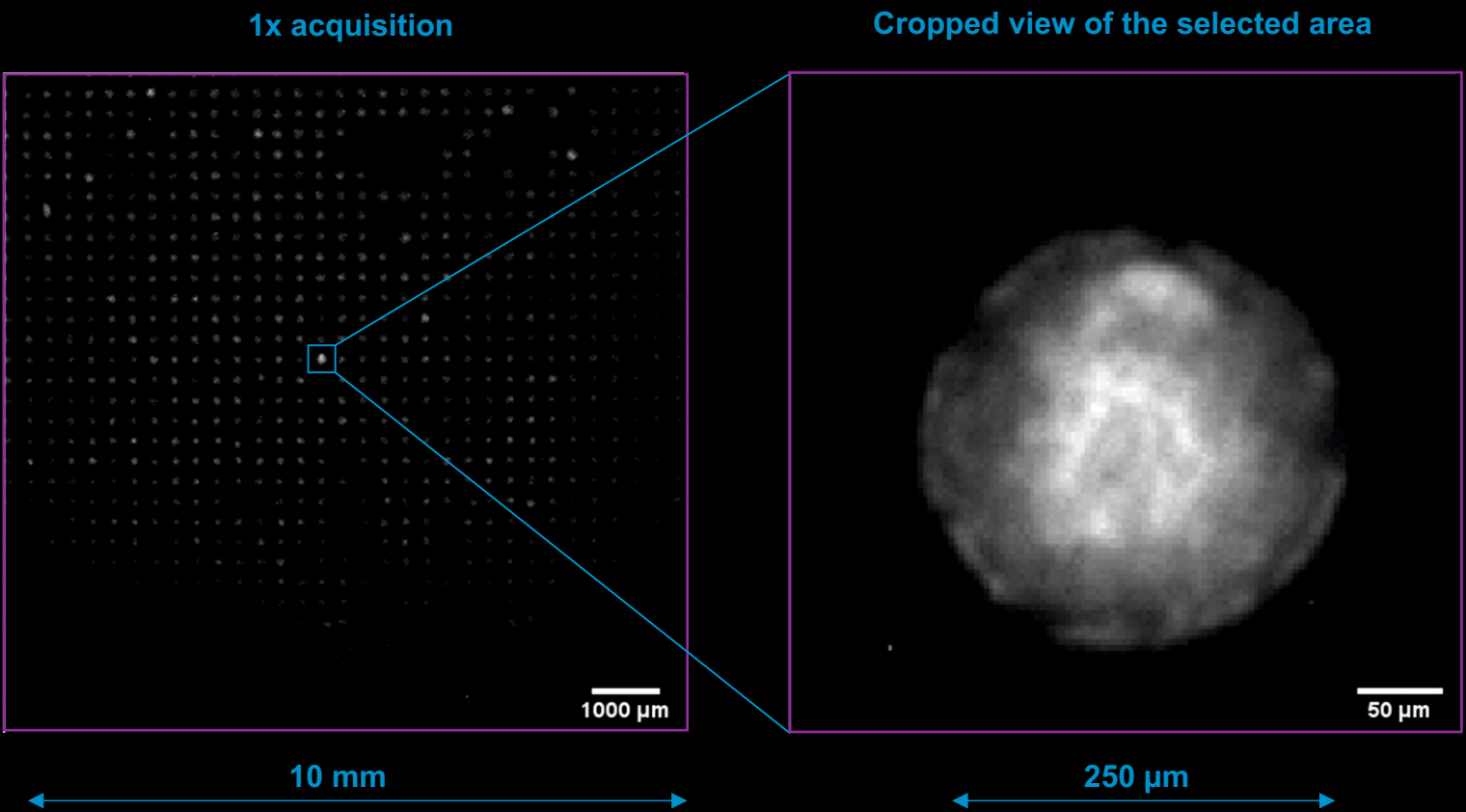


Figure 1. iFLUOR enables full-field 1:1 acquisition across a 10 × 10 mm² field of view (left). Structural details are preserved when inspecting a single brain rosette from the native image (right).