Detecting Small Genomic Targets and Transgenes for Cell and Gene Therapy

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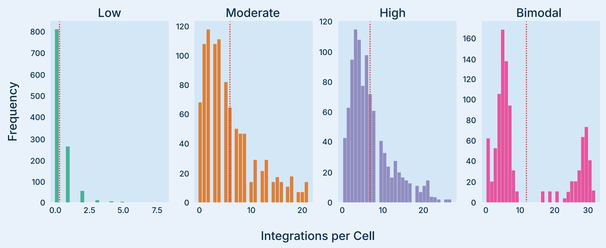
Genome editing systems utilize nuclease-based editing in combination with insertion of one or more transgenes.

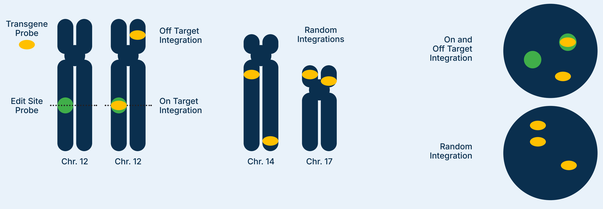


The average number (red dashed line) and the count distribution of on/off-target insertions can vary across different editing systems, methods, and cell lines.

Tracking Targeted Integration Events:
InSite probes to span and bracket the genomic target for integration and map transgenic integrations

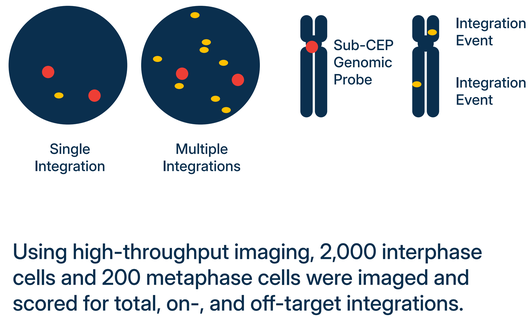
Tracking Random Integration Events:
InSite probes designed to track random transgenic integrations.

Tracking Integration Events in Nuclei:
PFF probes designed to enumerate random and targeted transgenic integrations.

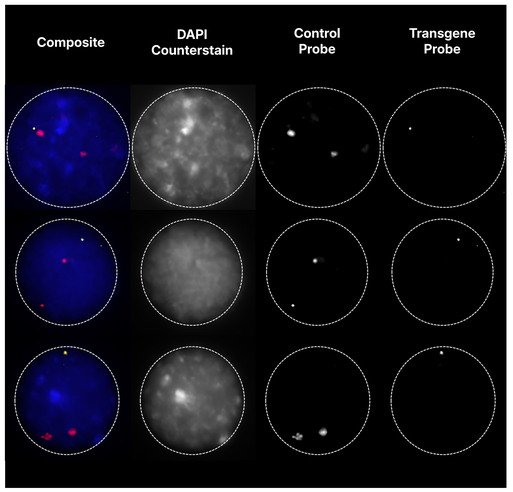


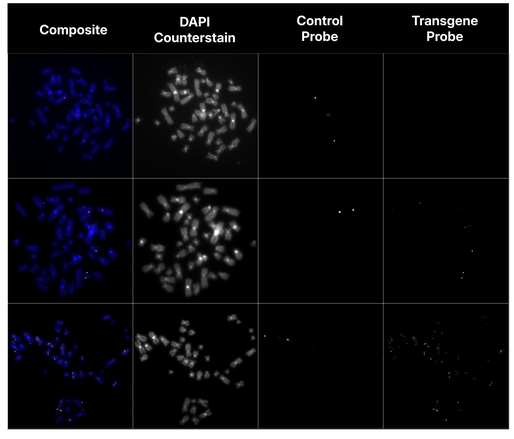
KROMASURE InSite and PinPoint FISH Solutions offer a variety of custom assay configurations that allow for single-cell analysis of both on- and off-target integrations.

This custom KROMASURE Solution targets a 2.5 Kb insert (yellow) paired with a control sub-centromere probe (red) and can be used for simultaneous single-cell interphase and metaphase analysis.

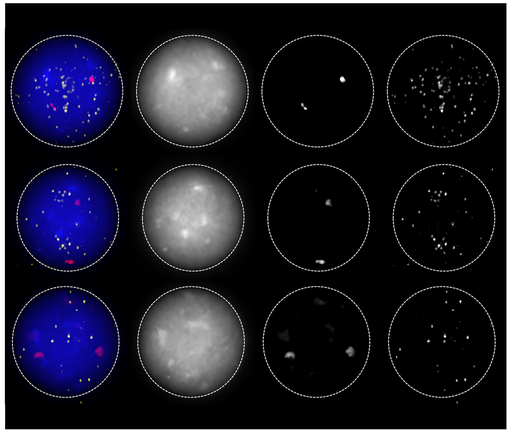


Using high-throughput imaging, 2,000 interphase cells and 200 metaphase cells were imaged and scored for total, on-, and off-target integrations.

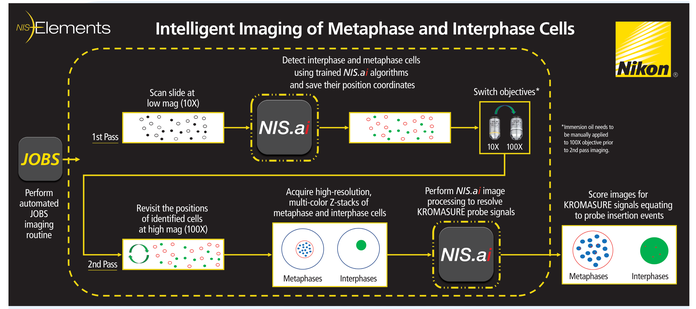


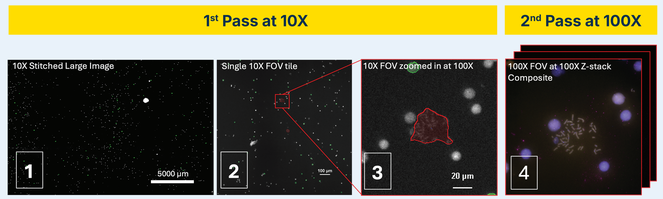


These example metaphase cells exhibit different patterns of on- and off- target integration.



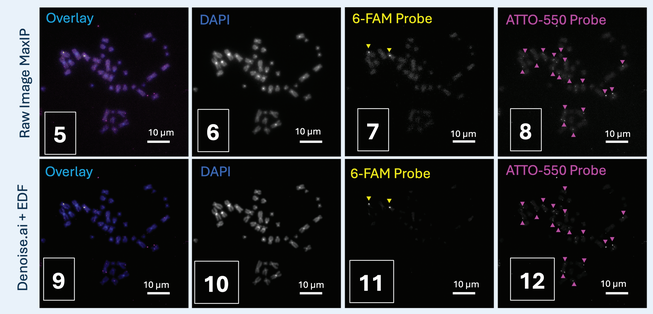
For this edited batch, integrations exhibited bimodal behavior. While most cells had a mean integration count of 1, a second hidden distribution of cells showed significantly higher counts with a mean >20.





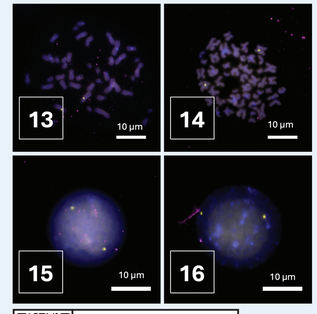
Nikon BioImaging Lab developed an AI-enhanced “two-pass” approach to achieve high-throughput, single-nuclei imaging on KROMASURE inSite slides. This work demonstrates a scalable, robust imaging-based cytogenetics assay for small target detection.

NIS.ai + EDF Image Processing Augments Detection of KROMASURE Probes

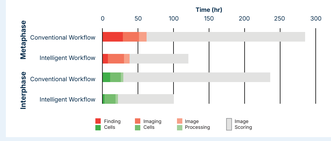


Detection of KROMASURE probes for very small genomic targets requires sensitive cameras. Here we demonstrate how a small series of image processing steps enhances these inherently weak signals further, allowing for more accurate quantification.

Intelligent Imaging Improves Detection of KROMASURE Probe Integration



Workflow Improvements



Above: NBIL’s two-pass workflow leads to considerable time savings for downstream image scoring, leading to higher throughput and faster data delivery times.

Left: Example images show a high number of genomic integrations into the chromosomes. Some cells display a high background of punctate FISH signal outside of the chromosomes, indicating non-integrated exogenous DNA.

Learn more about Nikon Bioimaging Lab
Contact: Brenna.Fearey@Nikon.com