Visual control of correct cell placement on AmpliGrid slides prior to molecular analysis

A key feature of the AmpliGrid slide is the ability to verify visually that cells are deposited correctly prior to molecular analysis. This application note describes a simple procedure for investigators to optically verify correct deposition of single cells on the AmpliGrid slide – no matter whether cells were deposited via flow cytometry, LCM, or micromanipulation.

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One of the frustrations of single cell molecular analysis in conventional reaction vessels such as tubes or MTPs is that it is nearly impossible to verify correct single cell placement. E.g., when sorting cells via flow cytometry into MTPs, placement success is typically in the 30-70% range, in part due to MTPs electric charge causing cells to be deflected before reaching the well bottoms. Similarly, when capturing single cells via LCM or micro-manipulation, some cells fail to transfer correctly into the bottom of a tube or MTP well.

Since there is no straight-forward optical quality control (QC) option in tubes or MTPs, investigators are left to guess whether a failed PCR reaction was due to PCR failure, failed cell deposition, or whether there is a valid biological reason.

AmpliGrid puts an end to this frustrating guess work. Following cell placement via flow cytometry, LCM, or micromanipulation, this application report describes a simple procedure to visually inspect cells to verify correct cell placement before moving on to molecular analysis. When the molecular analysis results are in, investigators interpret results with the confidence of knowing that cells were present. When optimizing new assays, or for ongo-ing QC in clinical research, or forensics, this presents a major advance in single cell molecular analysis.

1. Microscope system requirements

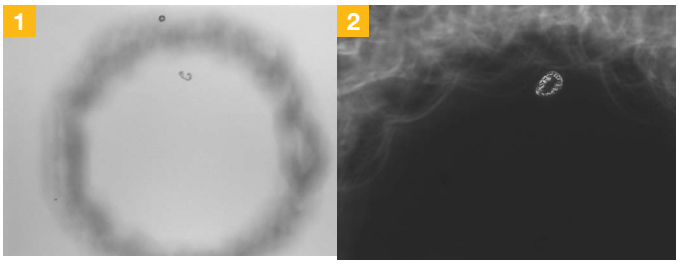
- Fluorescent bulb: 50W min
- Fluorescent lamp: HBO
- Magnification lens: 4x and 10x
- Filter: DAPI filter (e.g. for Olympus microscope IX61 use: Brightline HC 377/50, make sure filter wavelength matches wavelength of Hoechst stain used for cell staining)

2. Cell staining

Because uncolored cells are difficult to see under a microscope, this procedure requires staining of cells prior to deposition. We recommend a DNA stain, such as Hoechst Bisbenzimid H 33342 (Sigma, Cat. # 14533) with a concentration of 1mg/ml (100*staining solution). We diluted the cell suspension 1:100 with Hoechst and incubated for 5-10min at room temperature. Then cells are ready for deposition via flow cytometry, LCM, or micromanipulation.

3. Optical detection workflow

- Switch on microscope, fluorescent light source and computer software plus camera, if available
- Place AmpliGrid on slide stage of microscope
- Use 4x or 10x lens and bright field to detect crystals of dried buffer on A1 reaction site (figure 1,2)
- Switch to fluorescent light source, adjust brightness and sharpness
- Cell is now visible as bright spot. Depending on your filter set either as blue spot on black background or if digitally processed as white spot (figure 3–6).



Brightfield – 4x magnification (figure 1), 10x magnification (figure 2)

Cell as white spot – 4x magnification (figure 3), 10x magnification figure 4)

Cell as blue spot – 4x magnification (figure 5), 10x magnification (figure 6)

1. Useful hints and precautions

- Prevent exposure of sorted slides to fluorescent light as this will bleach the dye. Sorted slides should be stored in the dark.
- For long term storage, keep slides at RT
- Camera and software can be used to digitally enhance signal intensity (f. e. F-View and Cell^P from Olympus), press “auto” for searching automatically the ideal exposure time. Typical exposure times are under 1s. The intensity of the light should be maximum for low signals and minimum for bright signals, and gray-scale should be adjusted for the fluorescence intensity of the sample.

Trouble shooting:

- minimize light interference by working under dark room conditions
- workarounds if scope does not meet specifications: start with 20x objective and extended search (field of vision only 1/20 vs. 4x objective) or look for cells in phase contrast and then turn on fluorescence
- check to ensure the correct filter is being used (DAPI)
- if problems are encountered with the microscope, please consult the manufacturing company for technical support. Use of earlier model microscopes may compromise resolution, signal to noise ratio and autofluorescence.

In case of questions, please contact the Advalytix application specialist
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