

# Scanning and analysis of microarray slides

This protocol is specific for the Axon GenePixPro 3.0 and 4.0 software.

1. Once scanner is turned on, software is started, and the slide to be scanned is inserted into the scanner, the PMTs are set to 600 in both 635nm (Cy5) and 532nm (Cy3) channels.
2. A low resolution Preview Scan is performed in order to determine the location of spots to set the Scan Area around, and the initial fluorescence intensities.
3. A visual inspection of the fluorescence intensities allows for initial adjustments to PMTs. Since these are dual-labeled, global gene-expression hybridizations, the ratio for the whole scan area is preferably near 1.0.
4. Before the actual data scan is performed, the scanner is set so that it will scan each pixel twice and average these data counts. This reduces any background noise that may be present.
5. A high-resolution scan is performed. During the scan, the PMTs are fine-tuned, not just to achieve the aforementioned normalized balance of 1.0 but also to have pixels represented across a broad dynamic range.
6. Once the PMT levels have been set, a final Data Scan is performed.