

Microscope Specifications to process dGH in-Site, SCREEN and Pinpoint FISH assays. dGH SCREEN will use all the filters but the other assays may use less filters depending on experimental design or number of probes and colors.

The critical components for the imaging portion of the assay methods:

- The light source

Light source: broad spectrum white light source or multiple LED and/or laser sources

- The filter set (microscope turret needs a minimum of six filter slots)

Channel	Excitation	Dichroic	Emission
DAPI	376/30	425	460/50
Aqua	436/20	455	480/30
Green	490/20	505	525/20
Gold	546/10	556	572/23
Red	599/13	612	632/28
Cy5	640/30	660	690/50

- The objectives

High NA, high magnification oil immersion objective (60X or greater magnification, recommended 1.4 NA). We use 100X objectives.

- The camera

Monochrome CMOS or sCMOS. Six separate B&W images are falsely colored and combined to give the images we distribute. The false colors are defined to match the wavelength of light

passing through the filters so that the processed data is an accurate representation of what you would see if you used a multi-pass filter and looked down the barrel of the scope.

- An automated high-precision stage and an automated filter turret if the method is to be run without human control.

The method is multistep and each step is repeated for each cell (so 50 to 200+ times per sample) An overview image is taken of the slide at low magnification and then the metaphases are located and rescanned at high magnification to provide our data stream. Each channel is imaged separately and then combined in silico to form the single cell composite images for analysis. Manual processing is possible, but as that takes multiple steps per cell, automation is highly desirable and efficient analysis of hundreds of cells per sample is only possible with an optimized and automated method.

There are two large suppliers of automated microscopy systems for metaphase analysis, ASI and Metasystems both of whom provide the software layer which replaces the standard software that comes with the scopes. Either of these suppliers' standard configurations will work as long as the filter set and light source match our recommendations and either system would work for automated imaging of dGH SCREEN, we would just have to translate and qualify our ASI method to run on a Metasystems instrument.

ASI scopes are built on Zeiss Axios systems and our Nikon is a standard Nikon fluorescence scope running the manufacturers software. KromaTiD has the automated methods running well on the ASI, the Nikon is semi-automated, so more labor, but equivalent imaging results. Olympus, Leica and Generic scopes will also all work if properly configured, but none of them would provide the optimized metaphase automation, so more appropriate for R&D than routine analysis.