

Additional file 1: Table S1. Feulgen image analysis densitometry protocol with automatic measurement program wizard.

STEP	Operation	Comments	Window	
1	Image enhancement	Alignment - Exposure – White balance -	Optical enhancement including brightness, contrast, and gamma correction	LIVE
2	Multidimensional image acquisition	Exposure (the same as step 1 - Set all channel into the dynamic range – start – measure – save as xxx in folders – start taking picture	Camera set up and saving parameters. When you capture picture always use the window 6D and never the Live window for DNA quantification. It is important to organize al images in separate folders	6D acquisition
3	Set Automatic Measurement Program Wizard	Open an image – Measure – Automatic multidimensional measurement – new program. Select: Sigma filter NO – Shading Correction NO – Edge Enhancement NO. Select the object of interest with the pick up tools and then open “advanced” options: refine selection with the histogram and then set up as follow:	Generate the program that allows you to analyses images. Brightness, contrast, and gamma correction. Segmentation is the most important part: you separate the nuclei you want to measure from the background	Wizard Segmentation

Deletion of artifacts: remove - Interactive processing
 Fill holes YES - Automatic of the measurement
 object separation YES - Mode mask
 Erosion/Dilation YES -
 Watersheds NO
 Set measurement properties: Set measurement Definition of a
 Region feature (for a nucleus) properties Measurement
 - Field feature (for all area) - Condition
 Draw features
 Measures Features: Chose Measures Measurement
 Densitometric SUM, Features and check
 Densitometric AREA selected objects for
 Densitometric SD artefacts /
 overlapping.

4 Run Automatic Measure menu - Run Execute a program Run Automatic
 Measurement Program Automatic Multidimensional you have generated Measurement
 program -Execute for any number of Program
 images saved.