**9. Dissociation Kinetics Image Acquisition**

The dissociation kinetics experiment requires three parameters for analysis:

* Quantity of solubilized DNA in each chamber
* Intensities of surface bound transcription factor
* Time-resolved intensities of dissociating transcription factor bound DNA

Although the first three images of the series capture the equilibrium state (t=0) of the wells with the standalone Micro-Manager as outlined in Section 7 Gridded Image Acquisition, the time series component is acquired via a Matlab script that interfaces with Micro-Manager. Refer to strategies listed in Section 6 Imaging Suggestions. All images are taken after the DNA has solubilized in MITOMI.

 **Solubilized DNA Image**

The solubilized DNA image is captured identically to the settings outlined in Section 8. Image the solubilized DNA before washing out the chambers.

Typical settings (vary as needed):

* Binning: 3x3
* Channel: Cy5
* Exposure: { Equilibrium : 500ms , Affinity : 10ms }

**Surface Bound Transcription Factor Image**

The surface bound TF image is captured identically to the settings outlined in Section 8. Image the TF and bound DNA at the same time.

Typical settings (vary as needed):

* Binning: 3x3
* Channel: GFP
* Exposure: 1000ms

**Transcription Factor Bound DNA Image (t=0)**

The TF bound DNA image is captured identically to the settings outlined in Section 8. Image the TF and bound DNA at the same time.

Typical settings (vary as needed):

* Binning: 3x3
* Channel: Cy5
* Exposure: { Equilibrium : 2000ms , Affinity : 500ms }

 **Transcription Factor Bound DNA Image (t>0)**

1. Record valve states and save Micro-Manager position list
2. Close Matlab and Micro-Manager
3. Change valve states in ValveNumbers.txt and save
4. Run Matlab control program with a call too Micro-Manager
[scr, chip, scope, camera, mfcs] = chipAutomation\_RRP('pc1kv1p0', 'wago', 'ValveNumbers.txt', false, **true**, false, 'Cameras\_LBL.txt', false)
5. Set up Micro-Manager for a Muli-D. Acq. AS IF you were going to acquire another t=0 Cy5 image
6. Load saved position list file
7. When ready to begin dissociation image series, navigate to the scripts folder with the Matlab GUI and select the proper MeasureDissociationCurves\_...m file. NOTE: Setup 1 has switched valve polarities and Setup 2 does not.
8. Run script

Typical settings:

Use settings identical to t=0