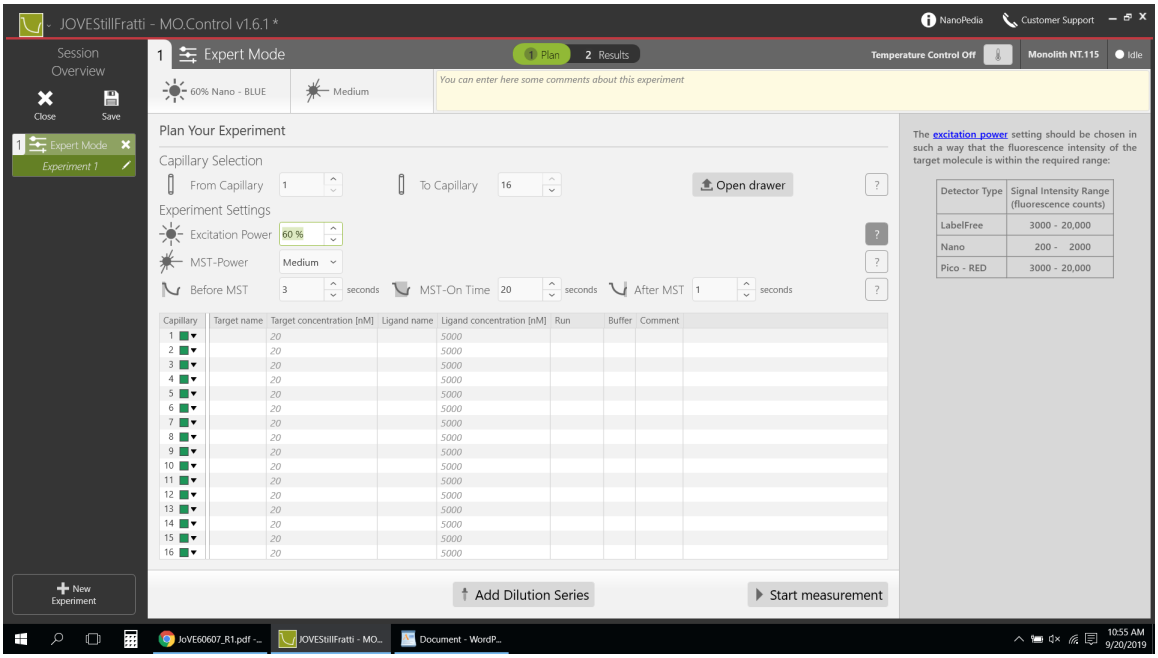
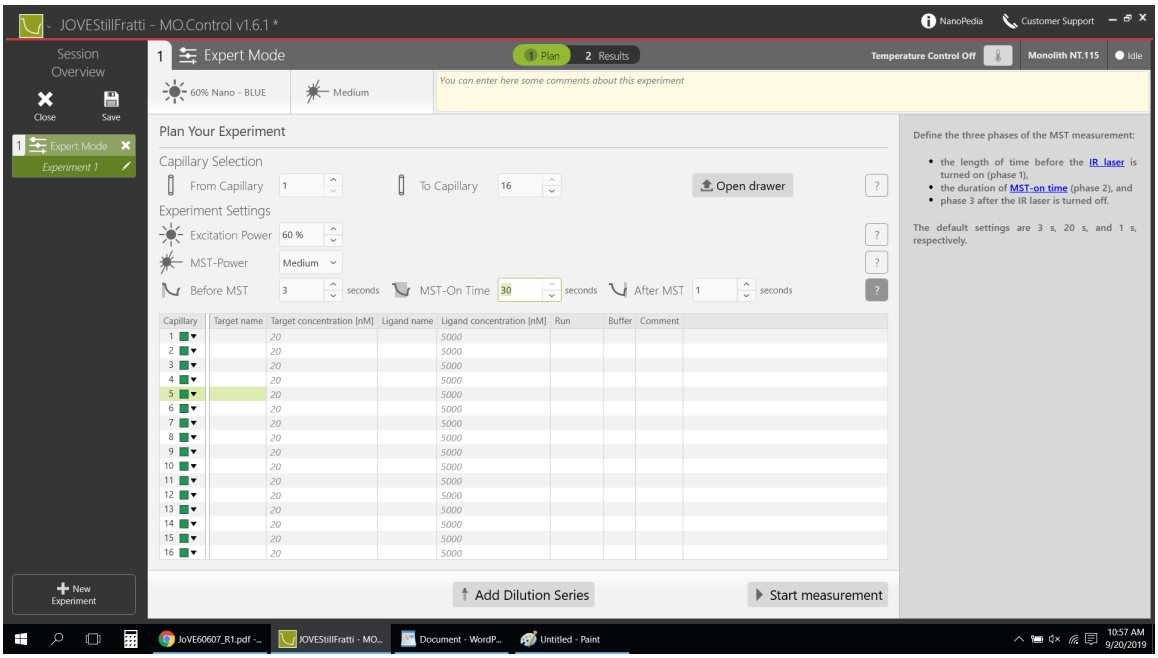
2.3.-2.6.

2.3 Enter Fluorescence and MST settings for this experiment.

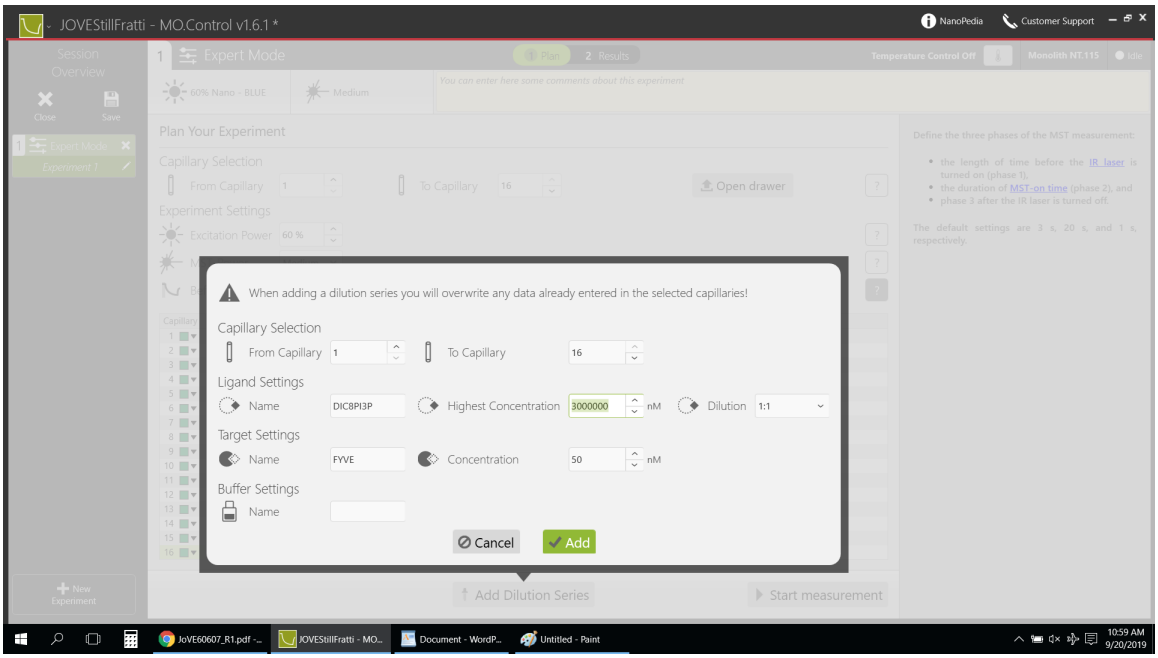


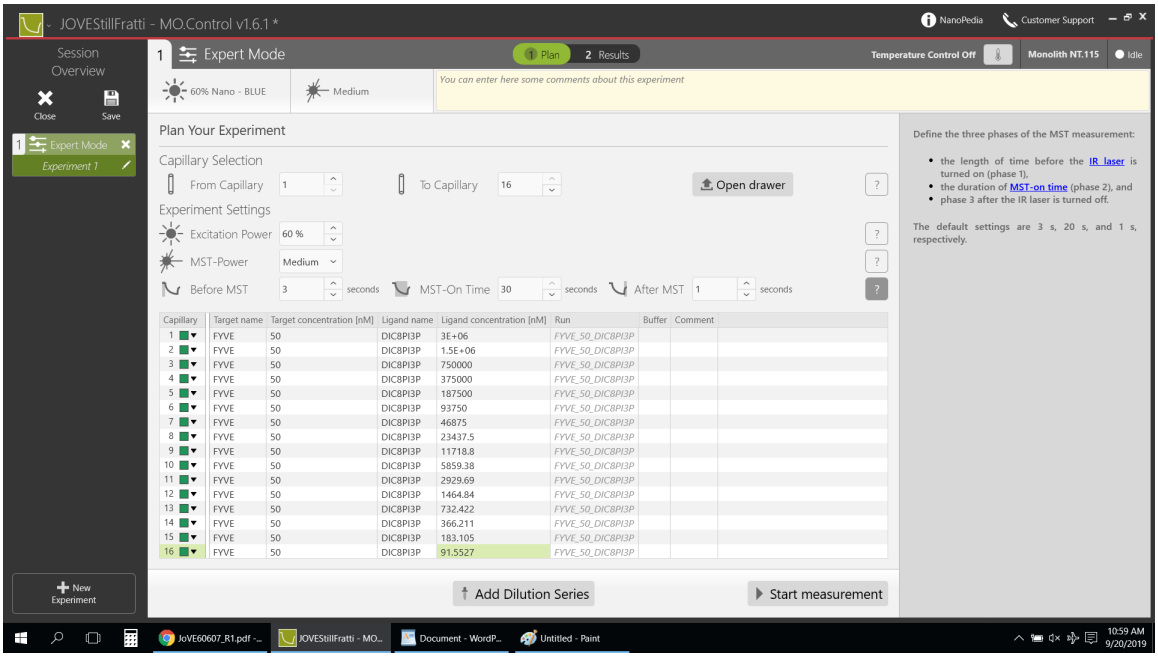
2.4 Set MST Before to 3 s, MST on 30 s, and Fluorescence recovery (Fluo.) after 1 s (Before measures initial fluorescence and does not require long times, MST is the actual amount of time for equilibrium to be reached after heat induction).



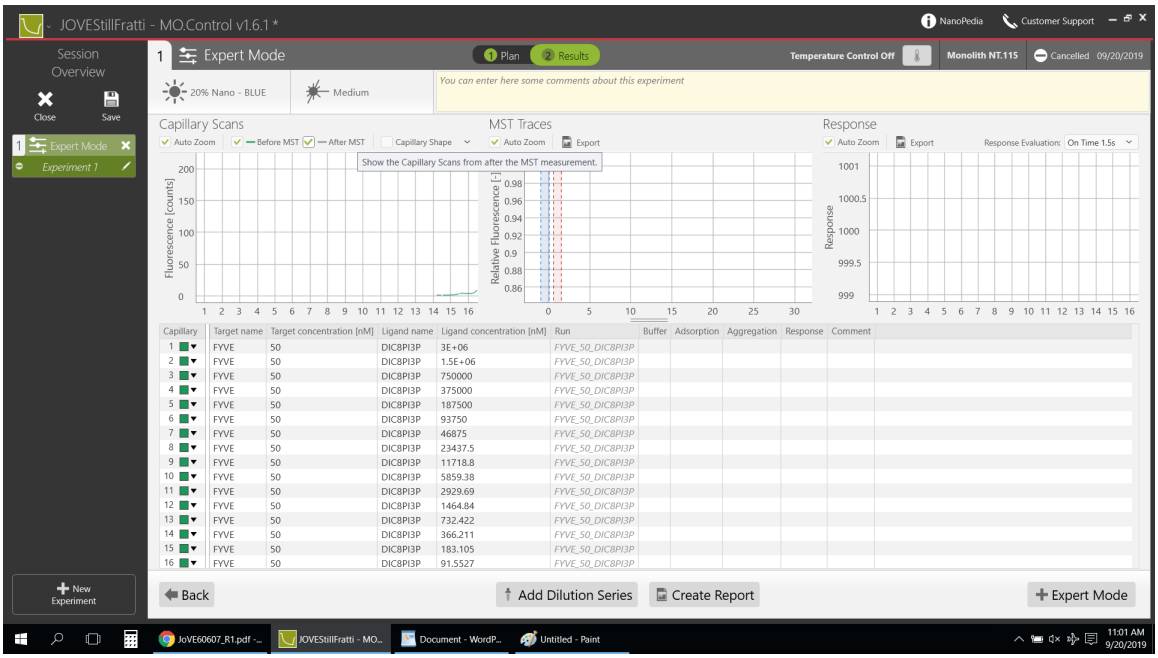
2.5 Table of Capillaries for each capillary tube enter name of target (ligand), name of ligand(analyte), concentration of target, and highest titration concentration and use autofill titration ratio. For example,

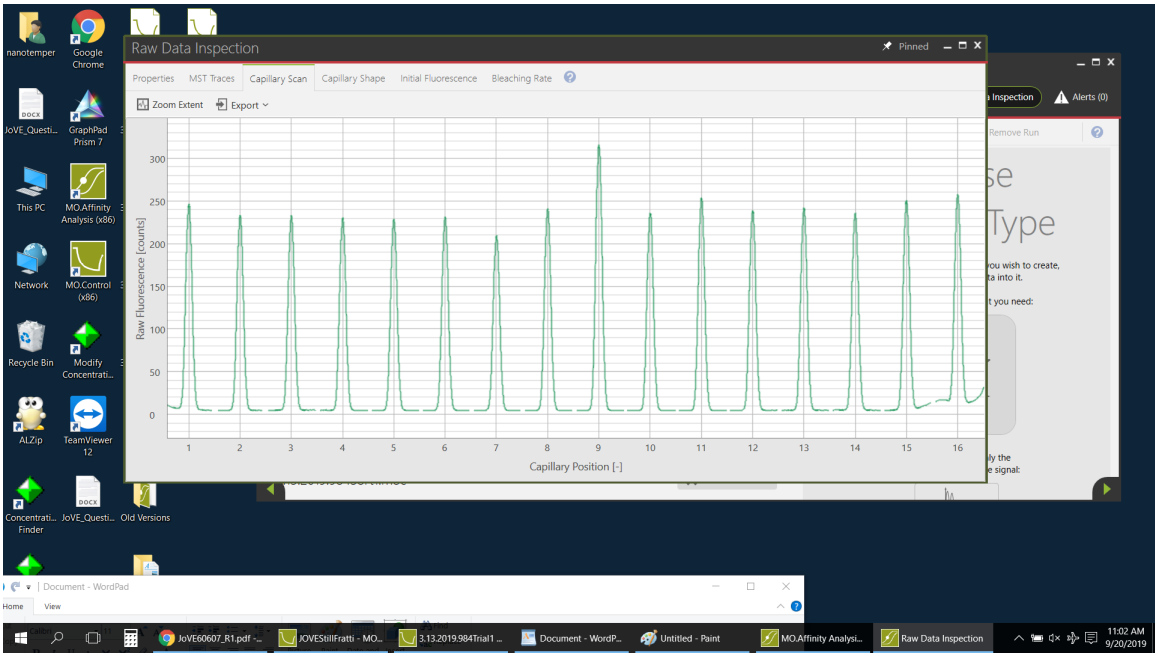
here we enter 50 nM for the target concentration of FYVE domain, FYVE domain for target name, Di-C8 PI3P for ligand name, and the highest concentration of 25000 nM selecting 1:1 and dragging down to autofill slots 2-16.

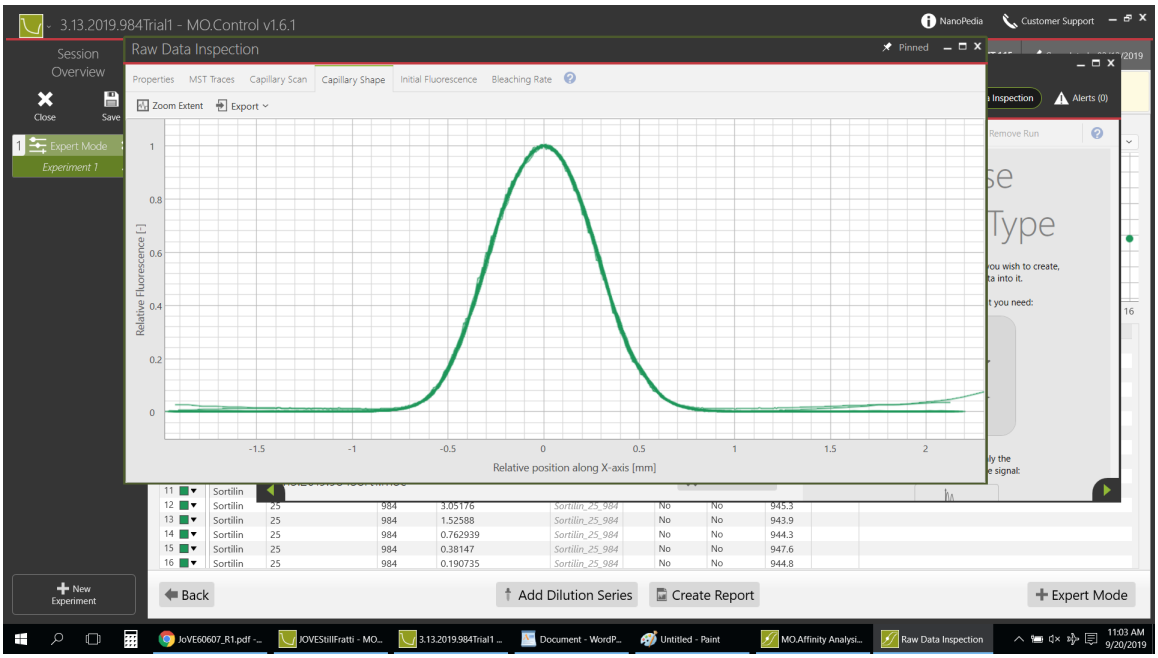




2.6 Capscan


JOVEStillFratti - MO.Control v1.6.1 *
NanoPedia
Customer Support
Session Overview
Close
Save
Expert Mode
Experiment 1
New Experiment
1
Expert Mode
Plan
Results
Temperature Control Off
Monolith NT.115
Cancelled 09/20/2019
20% Nano - BLUE
Medium
You can enter here some comments about this experiment
Capillary Scans
Auto Zoom
Before MST
After MST
Capillary Shape
Show the Capillary Scans from after the MST measurement.
Fluorescence [counts]
MST Traces
Auto Zoom
Export
Relative Fluorescence [-]
Response
Auto Zoom
Export
Response Evaluation:
On Time 1.5s
Response
Capillary
Target name
Target concentration [nM]
Ligand name
Ligand concentration [nM]
Run
Buffer
Adsorption
Aggregation
Response
Comment
1 FYVE 50 DIC8PI3P 3E+06 FYVE_50_DIC8PI3P
2 FYVE 50 DIC8PI3P 1.5E+06 FYVE_50_DIC8PI3P
3 FYVE 50 DIC8PI3P 750000 FYVE_50_DIC8PI3P
4 FYVE 50 DIC8PI3P 375000 FYVE_50_DIC8PI3P
5 FYVE 50 DIC8PI3P 187500 FYVE_50_DIC8PI3P
6 FYVE 50 DIC8PI3P 93750 FYVE_50_DIC8PI3P
7 FYVE 50 DIC8PI3P 46875 FYVE_50_DIC8PI3P
8 FYVE 50 DIC8PI3P 23437.5 FYVE_50_DIC8PI3P
9 FYVE 50 DIC8PI3P 11718.8 FYVE_50_DIC8PI3P
10 FYVE 50 DIC8PI3P 5859.38 FYVE_50_DIC8PI3P
11 FYVE 50 DIC8PI3P 2929.69 FYVE_50_DIC8PI3P
12 FYVE 50 DIC8PI3P 1464.84 FYVE_50_DIC8PI3P
13 FYVE 50 DIC8PI3P 732.422 FYVE_50_DIC8PI3P
14 FYVE 50 DIC8PI3P 366.211 FYVE_50_DIC8PI3P
15 FYVE 50 DIC8PI3P 183.105 FYVE_50_DIC8PI3P
16 FYVE 50 DIC8PI3P 91.5527 FYVE_50_DIC8PI3P
Back
Add Dilution Series
Create Report
Expert Mode
11:01 AM
9/20/2019


Raw Data Inspection
Pinned
Properties
MST Traces
Capillary Scan
Capillary Shape
Initial Fluorescence
Bleaching Rate
Zoom Extent
Export
Raw Fluorescence [counts]
Capillary Position [-]
Inspection
Alerts (0)
Remove Run
Type
nanotemper
Google Chrome
GraphPad Prism 7
This PC
MO.Affinity Analysis (x86)
Network
MO.Control (x86)
Recycle Bin
Modify Concentrati...
ALZip
TeamViewer 12
Concentrati... Finder
Old Versions
Document - WordPad
Home
View
11:02 AM
9/20/2019



4.5.-4.8.

4.5 Enter concentration, position, and name information for each capillary in the Table **as in 2.5**

4.6 Run a capillary scan by hitting Start Cap Scan at 20 % LED (preset) and adjust according between 200 and 2000 fluorescence units using either LED intensity settings or concentration of ligand (labeled protein). **as in 2.6**

4.7 Select a range of MST power. **as in**