MPS-016
PHENIX – aSMA IMAGE ANALYSIS

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University of Pittsburgh Drug Discovery Institute

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A. PURPOSE AND APPLICABILITY

This protocol outlines the steps necessary to complete 2D αSMA analysis on the PerkinElmer Opera Phenix. This protocol will take you through how to get started quickly using the preconfigured analysis created for our standard models (LAMPS, vLAMPS, bpLAMPS) and how to adjust settings in Harmony for your specific image condition. This protocol assumes you acquired images using the model specific Phenix Imaging protocol.

B. SUMMARY OF METHOD

C. DEFINITIONS

Term	Explanation

D. QUALIFICATIONS

1. Laboratory personnel will read the following documents provided by PerkinElmer:
    a. Opera Phenix User Manual
        i. Section 5.1.0.0 - 5.1.8.2 (Harmony Software User Interface Section)
        ii. Section 5.2.0-5.2.7 (Analysis Building Block Reference)
2. Successfully complete training by Dillon Gavlock.

E. HEALTH AND SAFETY WARNINGS

1. All chemicals must be handled in compliance with the University of Pittsburgh's Chemical Hygiene Plan Safety Manual.
2. All biological material must be handled in compliance with the University of Pittsburgh's Biosafety Guidelines.

F. CAUTIONS

G. INTERFERENCES

H. EQUIPMENT AND SUPPLIES

- Harmony v5.2

I. PROCEDURAL STEPS

1. Turn on the Opera, open Harmony 5.0 and enter your profile information.
    a. If you do not have a profile on the Phenix please talk to Dillon Gavlock about creating one.
2. When you open Harmony, navigate directly to the "Analysis" tab.
3. On the left-hand side you will see the configuration bar that houses all the information for

setting up an analysis. You'll want to load the following analysis protocol:
   a. aSMA_Analysis_v4
4. In this protocol you will have all the preconfigured settings loaded into the configuration panel. They include the appropriate analysis building blocks to complete the αSMA quantification of our model. Overall, there are only a few building blocks that need to be tuned to gather accurate quantification of the αSMA objects.
5. Once you load the protocol, be sure to have the appropriate Measurement loaded. You can change the Measurement just below the Analysis load tab on the left-hand configuration panel.
6. With the protocol loaded and the measurement set, navigate to the "Analysis Sequence" just below. You will see the "Input Image" block will be the first.
   a. You will not need to change anything here, just be sure that the "Stack Processing" is set to "Maximum Projection".
   b. You should see an image of your chosen field in the image panel. If you don't, make sure that you have all fields selected in the stack block to the right in the navigation panel.
   c. Also, make sure that you are quantifying the correct channel (Cy3 or FITC). The protocol is defaulted for Cy3
7. Once you're ready to complete the analysis, click "Save" and then go to the "Evaluation" tab at the top and select "Start"
8. Once the analysis is completed, export the data to the desired RFE folder
9. Once the data is exported go to the RFE and merge all the desired data into a single excel sheet adding the experimental metadata for each chip/chamber.
10. Create graphs and charts appropriate for your interests, generally we report the "Normalized Area", "Normalized Intensity", and "Normalized Number of Objects". You can also report their unnormalized values which are present in the analysis files.

J. DATA AND RECORDS MANAGEMENT

K. QUALITY ASSURANCE AND QUALITY CONTROL

L. REFERENCES