**Supplemental Material: Matlab Programs**

**Batch Script for analyzing PM targeting for a folder of images:**

Note: currently setup for parallel processing (toolbox required), replace parfor with for loop for linear processing

%%%% Copy write Daniel J Anderson 12/2010 Genentech %%%%

clc

clear

files = dir('\*.tif');

parfor l = 1:numel(files)

 I = imread(files(l).name);

 handle = @adaptiveRadialScan;

 [meanRatios nucCount] = handle(I);

 PMRations(l,1) = meanRatios;

 TotNuclei(l,1) = nucCount;

 l

end

data(:,1) = PMRations(:);

data(:,2) = TotNuclei(:);

csvwrite('meanRatios.csv', data)

**Function to measure PM targeting for each image:**

%%%% Copy write Daniel J Anderson 12/2010 Genentech %%%%

function [meanRatios nucCount] = adaptiveRadialScan(I)

nucDiameter = 80;

nucIntensity = 0.07;

lineScans = 0;

lowerArea = 1500;

updderArea = 3000;

R = I(:,:,3);

G = I(:,:,2);

G = im2double(G);

%%Ras Membrane Masking

h = fspecial('gaussian',8,8);

Rttt = imfilter(R,h);

Rtot = zeros(size(R,1),size(R,1));

for thRtotsh = 1:49

 Rt = im2bw(Rttt,thRtotsh/80);

 RtotdDisk = strel('disk',5);

 RtErode = imerode(Rt,RtotdDisk);

 RtErode = imdilate(RtErode,RtotdDisk);

 Rt = Rt-RtErode;

 Rt = bwareaopen(Rt,10);

 Rtot = Rtot+Rt;

 Rtot(Rtot<0) = 0;

 Rtot(Rtot>1) = 1;

end

Rtot = bwareaopen(Rtot,100);

Rtot = imdilate(Rtot,RtotdDisk);

RtotdDisk = strel('disk',6);

Rtot = imerode(Rtot,RtotdDisk);

Rtot = bwareaopen(Rtot,600);

PM = R;

neg = ~Rtot;

PM(neg) = 0;

%nuclear centroid finder

h = fspecial('gaussian',2,2);

Gnuc= imfilter(G,h);

BtTot = im2bw(Gnuc,1);

for NucThreshold = 1:60;

 Bt = im2bw(Gnuc,NucThreshold/100);

 Bt=~Bt;

 Bt = bwareaopen(Bt,lowerArea);

 BtBig = Bt;

 BtBig = bwareaopen(BtBig,updderArea);

 Bt = Bt-BtBig;

 BtTot = BtTot + Bt;

end

L = bwlabel(BtTot);

s = regionprops(L, 'centroid');

centroids = cat(1, s.Centroid);

centroids = round(centroids(:,:));

nucCount = bwconncomp(BtTot);

nucCount = nucCount.NumObjects;

Rtot(Rtot<0)=0;

Rtot(Rtot>1)=1;

%Radial scanner

if centroids>0

 nucCount = length(centroids(:,1));

else

 lineScans = 0;

 return

end

sizeX = length(R(1,:));

sizeY = length(R(:,1));

d=1;

stoRtotX=1;

stoRtotY=1;

hitCounter=1;

hitCountNorm=1;

totalScans=1;

hitCount=0;

xx=0;

for rA = 1:nucCount

 jstart = centroids(rA,1);

 istart = centroids(rA,2);

 j = jstart;

 i = istart;

 degPM = 0;

 countPM = 0;

 rDeg = 0;

 for rPM = 1:12

 for rC = 1:nucDiameter

 j = j+sind(degPM);

 i = i-cosd(degPM);

 if round(i)<1

 break

 end

 if round(j)<1

 break

 end

 if round(i)>(sizeY)

 break

 end

 if round(j)>(sizeX)

 break

 end

 if Rtot(round(i),round(j),1) == 1 && rC>(nucDiameter/3);

 countPM = countPM+1;

 break

 break

 end

 %I(round(i),round(j),3) = 255;

 rC = rC+1;

 end

 j = jstart;

 i = istart;

 degPM = degPM+30;

 rPM = rPM+1;

 if countPM == 6

 for rD = 1:180

 iA = i;

 iAA = i;

 iB = i;

 iC = i;

 iD=i;

 iE=i;

 jA = j;

 jAA = j;

 jB = j;

 jC = j;

 jD=j;

 jE=j;

 for rC = 1:round(nucDiameter)

 j = j+sind(rDeg);

 i = i-cosd(rDeg);

 if round(i)<1

 break

 end

 if j<1

 break

 end

 if round(i)>(sizeY)

 break

 end

 if round(j)>(sizeX)

 break

 end

 jE=jD;

 jD=jC;

 jC=jB;

 jB=jA;

 jA=jAA;

 jAA=j;

 iE=iD;

 iD=iC;

 iC=iB;

 iB=iA;

 iA=iAA;

 iAA=i;

 if Rtot(round(iB),round(jB)) == 1 && rC>(nucDiameter/2) && G(round(iE),round(jE))<0.95 && G(round(iE),round(jE))>0.001 && G(round(iAA),round(jAA))>0.001

 cytoRatio=((G(round(iAA),round(jAA)))/((G(round(iE),round(jE)))));

 lineScans(stoRtotY)=cytoRatio;

 stoRtotY=stoRtotY+1;

 totalScans = totalScans+1;

 break

 end

 rC = rC+1;

 end

 j = jstart;

 i = istart;

 rDeg = rDeg+2;

 rD = rD+1;

 end

 break

 end

 end

end

meanRatios = mean(lineScans);

**Batch script for measuring nuclear/cytoplasmic Erk distribution**

clc

clear

files = dir('\*.tif');

%for l = 1:4

for l = 1:numel(files)

 I = imread(files(l).name);

 handle = @nuclearErk;

 [meanRatio] = handle(I);

 totErk(l) = meanRatio;

meanRatio

end

csvwrite('meanRatios.csv', totErk)

**Sub-batch script for measuring nuclear/cytoplasmic Erk distribution**

Note: currently setup for parallel processing (toolbox required), replace parfor with for loop for linear processing

clc

clear

files = dir('\*.tif');

%for l = 1:4

for l = 1:numel(files)

 I = imread(files(l).name);

 handle = @nuclearErk;

 [meanRatio] = handle(I);

 totErk(l) = meanRatio;

meanRatio

end

csvwrite('meanRatios.csv', totErk)

function [meanRatio] = nuclearErk(I)

lowerArea = 200;

updderArea = 700;

blurPixels = 2;

B = I(:,:,3);

B = im2double(B);

h = fspecial('gaussian',blurPixels,blurPixels);

B= imfilter(B,h);

BtTot = im2bw(B,1);

R = I(:,:,2);

G = I(:,:,2);

Gnuc = B;

h = fspecial('gaussian',3,3);

Gnuc= imfilter(Gnuc,h);

BtTot = im2bw(Gnuc,1);

for NucThreshold = 2:50;

 Bt = im2bw(Gnuc,NucThreshold/200);

 Bt=~Bt;

 Bt = bwareaopen(Bt,lowerArea);

 BtBig = Bt;

 BtBig = bwareaopen(BtBig,updderArea);

 Bt = Bt-BtBig;

 L = bwlabel(Bt);

 s = regionprops(L,'Perimeter', 'Area');

 Perm\_values = [s.Perimeter];

 Perm\_values = double(Perm\_values);

 Area\_values = [s.Area];

 Area\_values = double(Area\_values);

 Area\_values = sqrt(Area\_values);

 Ratio\_values = Perm\_values./Area\_values;

 idx = find(6 > Ratio\_values);

 Bt = ismember(L, idx);

 BtTot = BtTot + Bt;

end

BtTot = bwareaopen(~BtTot,150);

BtTot = ~BtTot;

nucCount = bwconncomp(BtTot);

nucCount = nucCount.NumObjects;

cc = bwconncomp(BtTot);

totRatio = 0;

parfor A = 1:nucCount

 [ErkRatio] = nuclearErksub(BtTot,R,A,I)

 totRatio(A) = ErkRatio;

end

meanRatio = mean(totRatio);

**Function to measure nuclear/cytoplasmic Erk distribution**

function [ErkRatio] = nuclearErksub(BtTot,R,A,I)

 cc = bwconncomp(BtTot);

 subI = false(size(BtTot));

 subI(cc.PixelIdxList{A}) = true;

 CC = bwconncomp(subI);

 L = regionprops(CC,R,'MeanIntensity');

 intensity = cat(1,L.MeanIntensity);

 nuclearmCherry = mean(intensity);

 GeDisk = strel('disk',3);

 Ring = imdilate(subI,GeDisk);

 Ring = Ring-subI;

 CC = bwconncomp(Ring);

 L = regionprops(CC,R,'MeanIntensity');

 intensity = cat(1,L.MeanIntensity);

 cytomCherry = mean(intensity);

 ErkRatio = nuclearmCherry/cytomCherry;