Appendx I. Attributes calculated for segmented cells and sub-cellular structures

- This prefix marks geometrical attributes calculated in pixel units, with DelPix[3] is initially set to 1.
Upon convertion to physical units (microns for Perimeters, square microns for areas, etc) DelPix[3] is set to image pixel X,Y,Z dimensions (microns).

% - This prefix mark Fluorescence intensity-derived attributes, calculated in digital camera image counts as read from the detector, with Inorm[w] set to 1.
Upon normalization of intensities Inorm[w] is reset to the normalization factor for each color component w.
Normalization is typically derived from controls, calibration of light source intensity in fluorophore molecule number etc.

Run-Length encoding† : Typical loop to scan all pixels of the segmented mask for object number n (n=1,N) in image IMG:
RLSum[IMG]==for (k=0; k<Ns[n];k++){ y=yy[n,k]; y0=y*nx; for (x=x0[n,k]; x<=x1[n,k], x++){ … IMG[y0+x] … } }
Ns[n] are the number of y-lines, where for each line y=yy[n,k] the line segment starts at x=x0[n,k] and ends at x=x1[n,k].
e.g.: RLSum[IMG]==total object intensity, RLSum[1]==object area, etc.

Angles†† are with +x axis positive: anti clock-wise

Global attributes for an image or set of images (e.g. montage of images covering an area in sample larger than a single imaged field)

Attribute Name	Description
nx,ny,nz,nc	Image dimensions in X,Y,Z and number of colors. (x-coordinates run fastest, y-next, z-next and color last)
x00, y00,z00	Absolute coordinates of image center, (typically stage XY and focus positions in microns).
N	Number of primary objects (e.g. cells) within an image [or within a montage of contiguous images] Objects may also be cell islands, spheroids, etc.
FracArea	Fraction of objects mask area (or volume) within the image Important for quality control.
% In[w],Im[w],Imn[w]	Minimum, Maximum and Mean intensity for each image color component, w=1,nc Important for quality control.
DelPix[3]	Physical Pixel scaling (initially=1, after conversion to physical units = pixel X,Y,Z sizes in microns) All geometrical attributes must be either in pixels or microns
Inorm[w]	Intensity normalization factor for each color (initially=1, after conversion from image counts to normalized intensity: Inorm[w]= normalization factor)
% Bkgnd[w]	Image-averaged background intensity (out of objects mask pixels) Important for quality control.

Morphological attributes for each of the primary objects [for (n=0; n<N; n++)]

Attribute Name	Description
Flag[n]	Initially=1; excluded object (e.g. outliers) have negative Flag (-1, -2 etc. indicating exclusion criterion). Image border objects may take fractional flag values. Objects statistics use the flags.
SN[n]	Serial object number (typically sorted e.g. by area, largest first)
ID[n]	Object identification number (e.g. time-tracked identification number) In " NEXT" tracking mode ID[n]=SN in previous time. In "CONT" mode same ID for all times.
# Cx[n],Cy[n]],Cz[n]	Object geometric "center of mass" XYZ in image pixels
xs[n], xe[n]	Object rectangular x-extent (s-start and e-end extreme x coordinates in pixels)
ys[n], ye[n]	Object rectangular y-extent (s-start and e-end extreme y coordinates in pixels)
zs[n], ze[n]	Object rectangular z-extent (s-start and e-end extreme y coordinates in pixels)
Ns[n]	Number of **Run-Length**† y-line segments [for (k=0; k<Ns[n]; k++)] See remarks.
yy[n,k], x0[n,k], x1[n,k]	Object mask **Run-Length encoding**† yy[n,k]: y-line and x0[n,k],x1[n,k]: x-start,stop See remarks.
# Area[n] or Volume[n]	Object area (volume for 3D) in pixels if DelPix = 1 , or in microns^2 after geometrical scaling
# Perim[n]	Perimeter = length of boundary in 8-neighbors connectivity (incrementing 1 along ±XY, $\sqrt{2}$ along diagonals) or number of boundary pixels in 8-neighbors connectivity
# RadGyr[n]	Geomertical Radius of Gyration == RLSum[(x- Cx[n])^2+(y- Cy[n])^2+(z- Cz[n])^2]/ RLSum[1]
# Lax[n], Sax[n]	Ellipsoid Long and Short axes (half diameters) of from Eigevalues of geometrical second moments: Mx^2== RLSum[(x- Cx[n])^2]/ RLSum[1]; Mxy== RLSum[(x- Cx[n])(y- Cy[n])]/ RLSum[1] etc
Orient [n]	Orientation=**Angle**†† of long axis with respect to image coordinate system. From Eigenvector for the largest Eigenvalue of second moment matrix
AxRatio[n]	Axial ratio= Lax[n]/Sax[n]==1/R EPerim[n]==Ellipsoid Perim~π Lax[n]{3(1+R)-sqrt[(1+3R)*(3+R)]}
ShapFact[n]	Shape factor = Perim/$\sqrt{(\pi$Lax[n]) >1 if boundary is dispersed from best fitted Ellipse, EArea[n]==Ellipsoid Area= 4π Lax[n]*Sax[n]
Solid[n]	Solidity = $\sqrt{(}$CellArea/π)/(CellPerim/π) Evaluates closeness to a circle
Round[n]	Roundness = Area[n]/EArea[n]
SmFact [n]	Smooth factor = Perim[n]/EPerim[n] >1 if divert from ellipsoid
Dispers[n]	Disperssion[after Graham Dunn] = log(4π^2□□□□□□□□□□^{2} –($\sqrt{[}$(Mxx-Myy)^2 +4Mxy^2])^2}/{2log(2)}
Eccent[n]	Eccentricity= $\sqrt{(}$1-R^2) =0 for a sphere, ->1 for elongated ellipse
Compact[n]	Compactness= $\sqrt{(}$Area/4π)/Lax[n]
FormFac[n]	Form Factor= π Area/Perim^2
EulerNo[n], HArea[n]	Euler number (number of holes) and total holes area
# AreaCH[n]	Convex Hull area (pixels)

# PerimCH[n]	Convex Hull Perimeter (pixels)
RoughCH[n]	Roughness = Perim[n]/PerimCH[n]
SolidCH[n]	Solidity from Convex Hull= Area[n]/AreaCH[n]
ConvxCH[n]	Convexity= PerimCH[n]/Perim[n]
CellZernk[n,i]	Zernike moment (for mask)

Multi-color Fluorescent intensity-derived attributes for primary objects and each color component, w

#Cx1, Cy1[n,w]	Color w intensity weighted center of mass in image x&y pixels
# Dx1, Dy1[n,w]	Distance between geometrical and intensity weighted centers
# RadGyr1[n]	Intensity-weighted Radius of Gyration
# Lax1, Sax1[n,w]	Long and short axes from intensity-weighted second moments
Orient1[n,w]	Angle of long axis with +x coordinate
AxRatio1[n,w]	Axial ratio = Lax1/Sax1
%MinInt1,MaxInt1[n,w]	Minimum and Maximum intensities in the mask area
% TotInt1[n,w]	Total (integrated) intensity for each labeled color
% AvgInt1[n,w]	Average intensity for each labeled color
% Bcgnd1[n.w]	Local background intensity (e.g. calculated in dilated mask, or in extent out of object masks)
% Tot_b1[n,w]	Background-subtracted total intensity
% Avg_b1[n,w]	Background-subtracted average intensity
% Txr1[n,w,l]	Total Textural energies within the object (e.g. Variances) [l=0 … Lt-1 for Lt texture kernels]
% TxrOArea1[n,w,l]	Textural energy per unit area
TxrOint1[n,w,l]	Textural energy per unit intensity
Zernk1[n,w,l]	Intensity-weighted Zernike moments [l=0 … Lz-1 for Lz moments]
Haralick1[n,w,l]	Intensity-weighted Haralisk textural coefficients, [l=0 … Lh*Lr-1 , Lh=14 coeff. for each resolution Lr resolutions]

Fluorescent intensity-derived primary (e.g. 1-cytoplasm) to secondary (e.g. 2-nucleus) objects intensity ratios

TotNucOCyt[n,w]	Total fluorescence intensity Nucleus over Cytoplasm ratio = TotInt2[n,w,0]/(TotInt1[n,w]-TotInt2[n,w,0])
TotNucOCyt_b[n,w]	Background-subtracted Total fluorescence intensity Nucleus over Cytoplasm ratio
AvgNucOCyt[n,w]	Average fluorescence intensity Nucleus over Cytoplasm ratio
AvgNucOCyt_b[n,w]	Background-subtracted Average fluorescence intensity Nucleus over Cytoplasm ratio
Colocal[n,w1,w2]	Colocalization factors between two colors (depends on method)

Attributes for cytoskeleton fibers morphology and associated fluorescence intensity

# AreaFib[n]	Fibers area (defined by fiber mask)
# LenFib[w]	Total fibers length
% TotFib[w]	Fiber-associated total intensity
% TotFib_b[w]	Background-subtracted Fiber-associated total intensity
% AvgFib_b[w]	Background-subtracted Fiber-associated average intensity
#% FibOLen[w]	Background-subtracted Fiber-associated Intensity per unit length
PolFib[w]	Fiber Polarization Factor = <cos^2(2α)+sin^(2 α)> [averaged over fiber pixels] α =fiber angle at pixel [i,j]

Attributes for secondary objects (organelles , m=0; m<M) within primary object (cell number n) color w
(e.g. Nucleus, Focal Adhesions, Golgi, Mitochondria, Endoplasmic Reticulum, Lysozomes and other organelles)
Some attributes are calculated for each secondary object relative to the parimary object (e.g. Dx21,Dy21,rAng2,pAng2,Dp2,Dc2,NDE2)
Others are independent on primary objects, (e.g. Cx2,Cy2,xs2,xe2,ys2,ye2,Area2,Perim2,Lax2,Sax2,Orient2,AxRatio2)
And others are sums on all secondary objects within each primary object, [marked as *21]

M[n]	Number of secondary objects per primary object#n
# Cx2,Cy2[n,w,m]	Geometric "centers of mass" x&y coordinates for a secondary object
# Dx21,Dy21[n,w,m]	Distance between intensity weighted centers of secondary and primary objects
xs2,xe2,ys2,ye2[n,w,m]	Rectangular extent (start and end extreme x&y coordinates in pixels)
# Area2[n,w,m]	Secondary Objects area
# Perim2[n,w,m]	Secondary Objects Perimeter
# Lax2, Sax2[n,w,m]	Long (A2) and short (B2) axes (half diameters) of best fitted ellipsoid from geometrical second moments
Orient2[n,w,m]	Angle of long axis with respect to +x coordinate
AxRatio2[n,w,m]	Axial ratio = Lax2/Sax2
rAng2[n,w,m]	Angle between long axis and line connecting secondary object center to primary center
pAng2 [n,w,m]	Angle with respect to cell polarity axis
# Dp2[n,w,m]	Shortest distance to primary object boundary
# Dc2[n,w,m]	Distance to primary object center
NDE2[n,w,m]	Normalized distance between cell edge and cell center = Dp2/(Dp2+Dc2)
# Area21[n]	Total area of secondary objects within each primary objects
# Perim21[n]	Total perimeter for secondary objects within each primary objects
# RadGyr21[n]	Intensity-weighted Radius of Gyration for all secondary object in a primary object [indicating if secondary objects are spread or concentrated]
% MinInt21, MaxInt21[n,w]	Minimum and Maxumum intensities of all secondary objects within primary object
% TotInt21[n,w]	Total (integrated) fluorescent intensity for each labeled color for all secondary objects within primary object
% AvgInt21[n,w]	Average fluorescent intensity for each labeled color for all secondary objects within primary object
% Bcgnd21[n.w]	Local background intensity [from dilated masks, extent out of segment etc] for all secondary objects within primary object
% Tot_b21[n,w]	Background-subtracted total intensity for all secondary objects within primary object
% Avg_b21[n,w]	Background-subtracted average intensity for all secondary objects within primary object
% Txr21[n,w,l]	Total Textural energies for all secondary objects [l=0 … Lt-1 for Lt texture kernels] within the primary object
#% TxrOArea21[n,w,l]	Textural energy per unit area for all secondary objects within primary object

TxrOInt21[n,w,l]	Textural energy per unit intensity for all secondary objects within primary object
Zernk21[n,w,l]	Zernike moments [l=0 … Lz-1 for Lz moments] for all secondary objects within primary object
Haralick21[n,w,l]	Haralisk textural coefficients, [l=0 … Lh*Lr-1 , Lh=14 coeff. for each resolution Lr resolutions] for all secondary objects within primary object