ANALYSIS DESCRIPTION

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# Cropcode

## Intro

This code prepares raw microscopy images for later processing, plus general cell contour analysis.

Developers/main researchers F.Wu, A. Japaridze ,J. Kerssemakers

## step-by-step

1. -open your own user file
2. -add a new experiment
3. -set 'autorun' to 1 and maxcelperframe to 2
4. trial: run the 'X0000\_AutodataTestShell' with all on 'if 1'. It should run without errors
5. full run + manual crop:
6. -set 'autorun' to 0 and maxcelperframe to 10E6 ('infinite')

# Donutcode

## Intro

Code used for analysis donut-shaped chromosomes plus ori-ter positions. Developers/main researchers F. Wu, A. Japaridze ,J. Kerssemakers

## step-by-step

1. Cell circular chromosome analysis, step by step quicksheet
2. Starting data: take a look in \TESTdata\OriData; naming of the mat-files is crucial for proper analysis. Next, open/use the programs in the following order:
3. A000\_WF\_Init: Copy the line ‘initval= A000\_\_WF\_Get\_JacobPathsandExperiments’ ; change ‘Jacob’ to your own user name.
4. A000\_\_WF\_Get\_JacobPathsandExperiments : User-specif experiment settings. Open it and save a copy under the name used above. This function contains general and experiment-specific settings. First, try the following steps on the ‘VersionTest’ lalbel, then set up your new data. To add a new experiment:
   1. copy line: initval.expi='VersionTest' into a new label (and comment out the others…)
   2. Go to the ‘switch’ section ; copy the ‘VersionTest’ case and give it the new label; check the settings carefully (read the descriptive comments) and change settings (such as paths) accordingly.
   3. Important: when the ‘alignment’ settings (line 23-40) are changed, ‘A020’ (see below) should always be re-run for changes have effect in the A030 and higher programs
5. A010: Just run it. This program combines channels, tracks ori/ter, determines chromosome contour data and saves results&thumbnails.
6. A015: Just run it. This program performs automatic screening on proper cell size, ori-ter positions etc. and reports the results to the command line. Check the screening filters from line 77 downwards. Check on the command line if screening is balanced (in terms of number of rejects). If not, adapt filter settings in code.
7. A020. Just run it. This program aligns density curves, determines ‘distance’ and ‘genomic’ axes and saves the results for later use. Always re-run this one after changing alignment settings (in A000).
8. Next programs process the data but will not change the stored data.
   1. A030. Just run it. This program plots density curves and related plots
   2. A040 Just run it. This program analyses minima and maxima in density curves and collects the results
   3. A050 (Development version) Just run it. This program performs ‘blob analysis’ independent of the ‘circular analysis’ that is the basis of A020-A040
   4. A070\_WF\_GetContourLengths: This program just collects the ‘peaks’-and outer contour lengths of the chromosome
   5. A080\_WF\_BuildDistanceDensityMaps: This program plots 2D maps of spatial distance between genomic positions, as measured along the circular contour; thus it present a ‘live’ Hi-C map.
   6. A090\_WF\_BuildCorrelationCurves: This program measures correlations between following density curves. For movies, this yields a timescale of the dynamics in the chromosome.
   7. A010000\_WF\_2DLoadTemplate: this is just a template to build new ‘A0xx’ Processing programs.

# Replicode

## Intro

Developers/main researchers A. Japaridze ,J. Kerssemakers

Short analysis description:

A001 is just creating directories

A005 is just making image lists

A010 is analyzing the shape of the cell

A013 is detecting the rfp and cfp(ter) spots. Both allow for multiple spot analysis via the 'spot peeler' protocol. For cfp, smoothing is applied first (20 pixels).

A055 is analyzing the chromosome pattern by the same 'spot peeler' protocol. It saves pictures of clusters and the properties of these (each cluster is described as a compact group of single-spot Gaussians spots and can therefore have any shape)

## Step by Step

Cropcode should have been used; creates a directory 'X0…density data' that is used by the repli code

1. Replicode: Example used is experiment ‘VersionTest’
2. Open excel 'Overview\_testruns\_replidata'; Add new entry row (typically, just copy a line; some columns are only important for the donut code). Here, We use ‘VersionTest’.
3. If you didn’t do so, copy the config file ‘’A\_User\_config\_Jacob.m’’ to a file having your own name; add your name to the top of ‘LazyShell’’ accordingly.
4. Add a new experiment to the list following naming template; ; name should match col A in the Excel table. Nb check that the excel table is named correctly (line 33)
5. -Open A00\_\_LazyShell. Different programs can be run by setting them to ‘if 1’ (they may need each other outputs). Once run, you can uncheck them to save time with re-runs
   1. If you want to run a quick check if the new experiment is set up correctly, open your config file and set ‘initval.shortset=10;’ ; this will crop the maximum number of cells to be analysed. For ful runs, set this number to 10E6 or so, i.e. exceeding the real number of cells.
   2. Run A001. Note: it sometimes crashes, refusing to overwrite. In that case, try a few times or you may first delete old directories with explorer.
   3. Run A010; A013 , A60. Note: A60 can be run directly after A001.