The title:

A glimpse into chromatin organization by HD-DNA FISH~

The data analysis pipeline for HD-DNA FISH~

Outline

- Introduction
- Explain the general analysis pipeline
  - The SpotterUI
- Interleaved 4-loci spotting
- Combinatorial labeling spotting
- Measuring and correcting the shift
  - projective transform as a first approach
  - a more refined approach
- What would be next

What is HD DNA-FISH?

- A methodology that allow us to visualize genomic regions between 3 kbps and 10 kbps
- It uses hybridization of ~4 times labeled 200nt amplicons
- Similar to smRNA-FISH, this regions are detected as small fluorescent spots
- Adds some sophistication to smRNA FISH
- Requires higher precision for spot identification
- Exact 3D position matters

The spot identification pipeline

1. Segmentation of the ROIs
2. Correct for the shift between channels
3. Increase S/N ratio (filter for particle size - LoG)
4. Select for the intensity threshold
5. Count, assign, and compute spot features
    - Precise position
    - Intensity, volume, FWHM

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    - **Precise position**
    - **Intensity, volume, FWHM**

The SpotterUI is a cub from ImageM

The shift is corrected by a linear transformation

The applied transformation seems to work

The spots are counted after selecting a threshold value

The 4 loci experiment

The 4 loci experiment

- Interleaved probe sets in both A594 and Cy5 are separated each 10 Mbp
- Single chromosomes are observed to be separated in a large fraction of cells
- 1 distance between spots of the same color
- 4 distances between spots of different colors

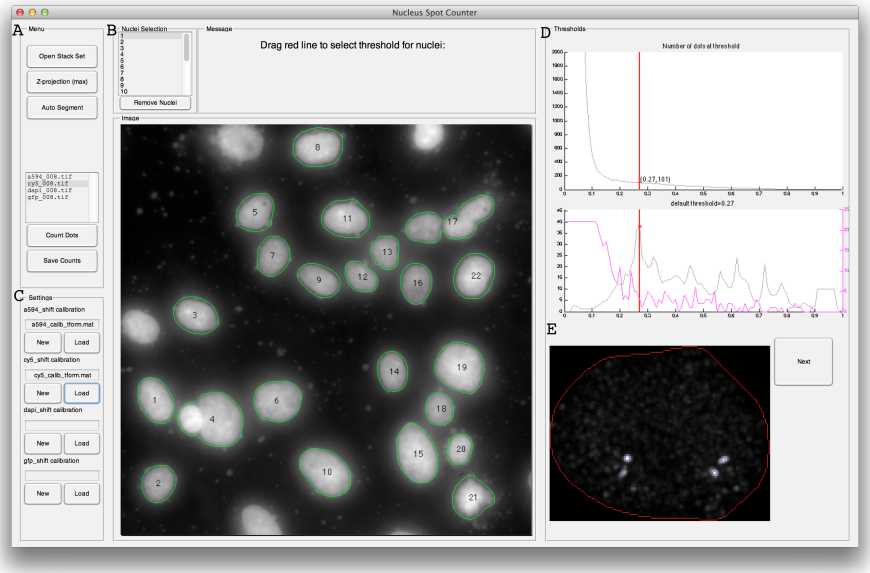

Figure 1: SpotterUI

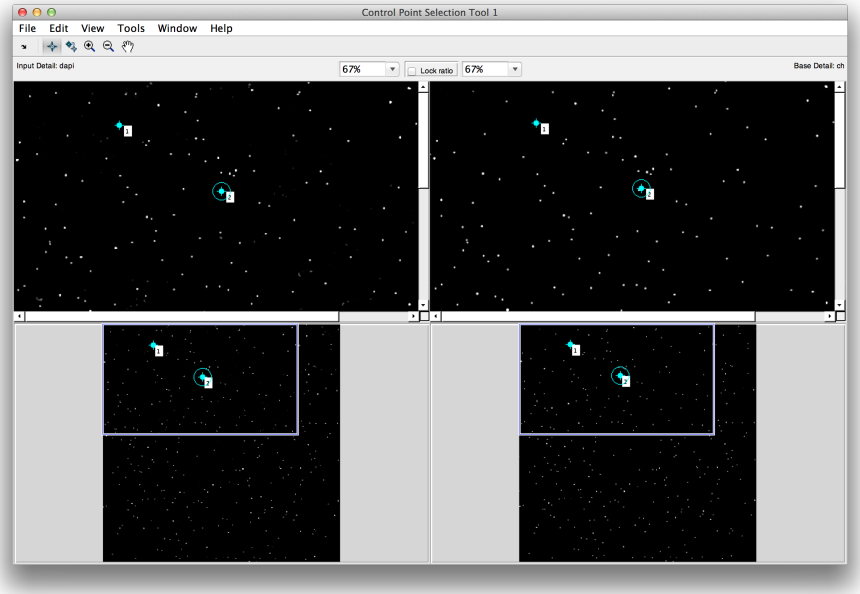

Figure 2: bead selection tool

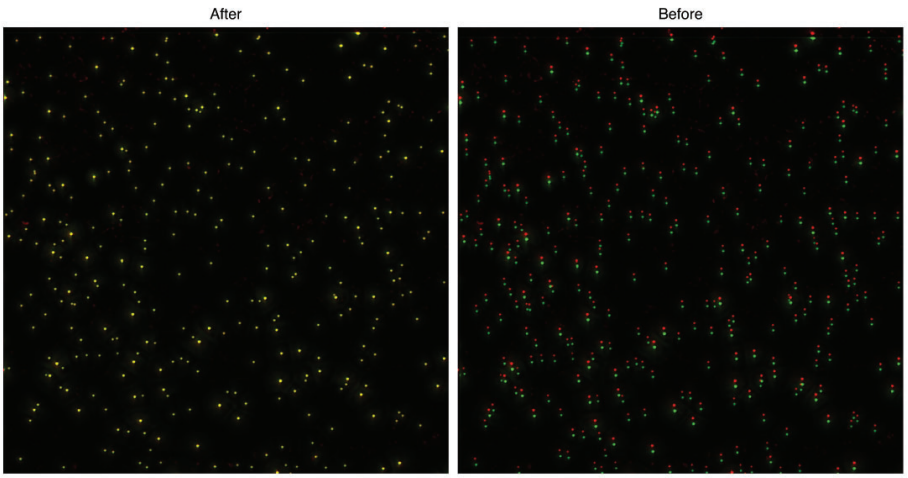

Figure 3: TMR shift

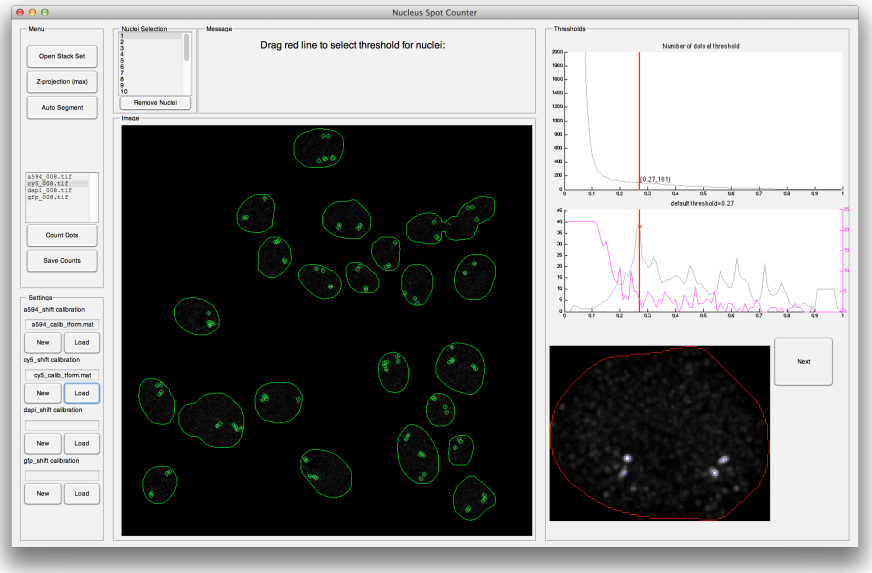

Figure 4: SpotterUI2

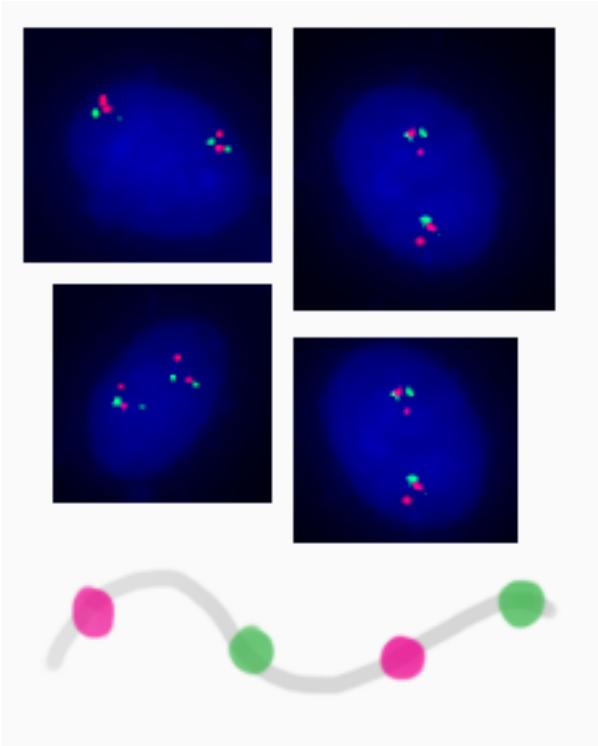

Figure 5: 4 loci

The numbers after counting

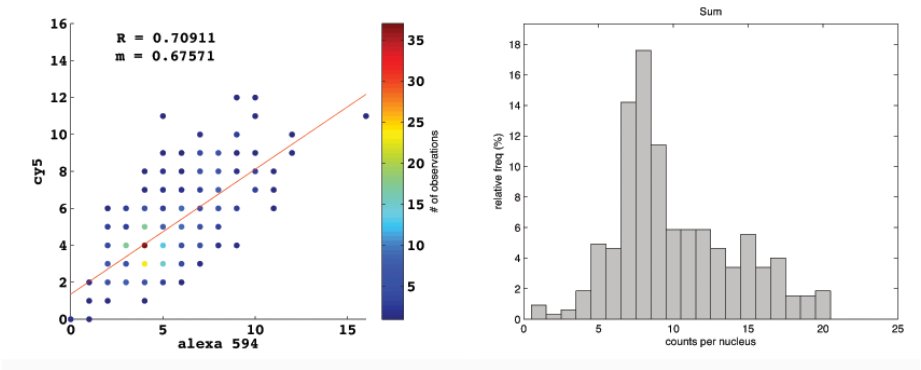

Figure 6: 4 loci counts

- Counts for both channels are expected to be *almost* the same
- The total spot counts resembles the cell-cycle-phase distribution

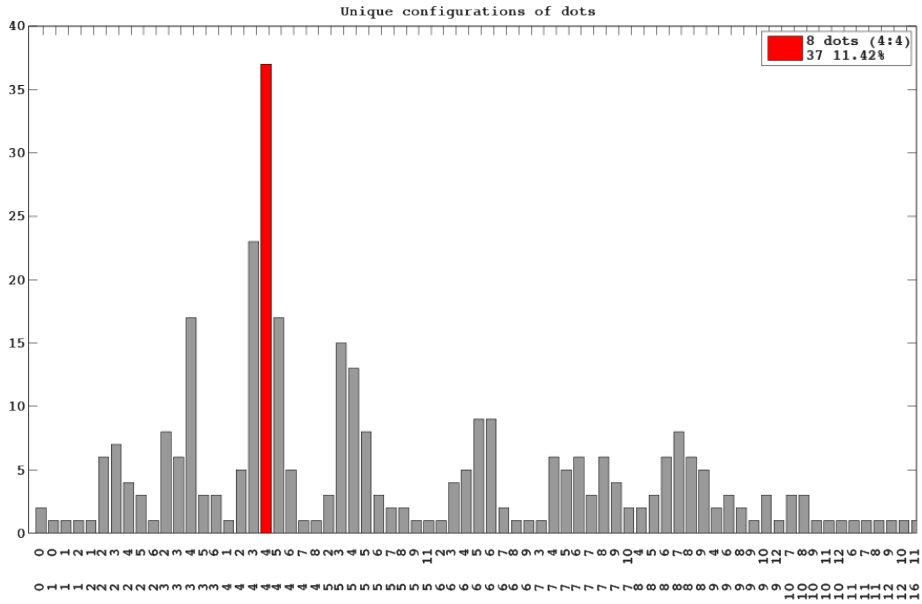

Figure 7: 4 loci counts

37 out of 324 nuclei have a 4:4 spots configuration

K-means clustering is applied and certain configuration selected

61 out of 324 nuclei have 4:4,3:4,4:3,4:5,5:4 spots configurations

A leveling-off of physical distances > 20 Mbp is observed

Combinatorial labeling setup

- When observing to the data, I found no major co-localization of the probes

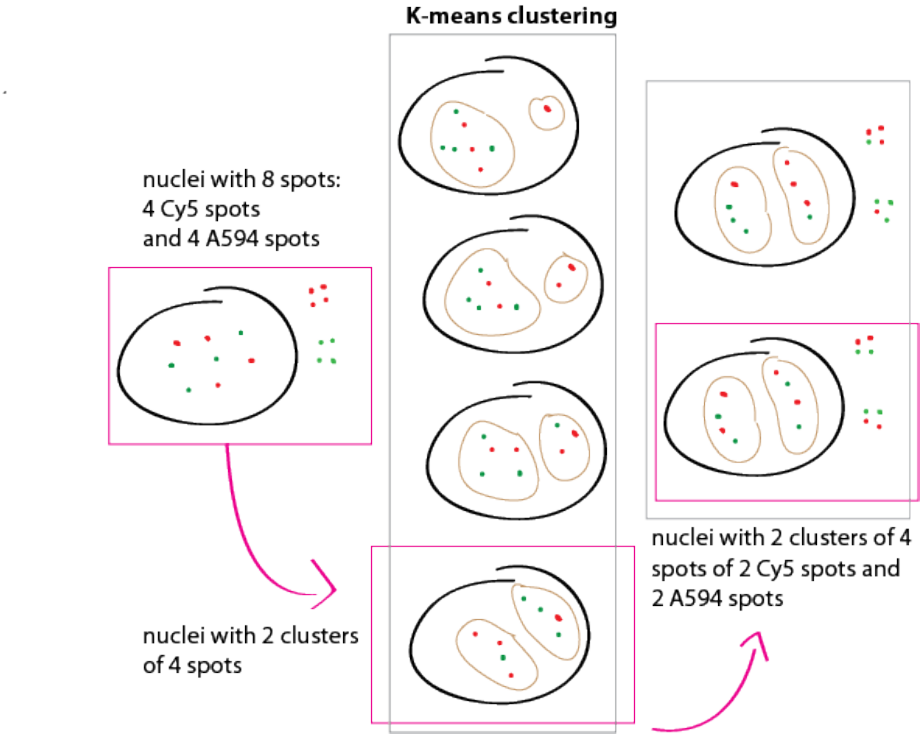

Figure 8: 4 loci conf

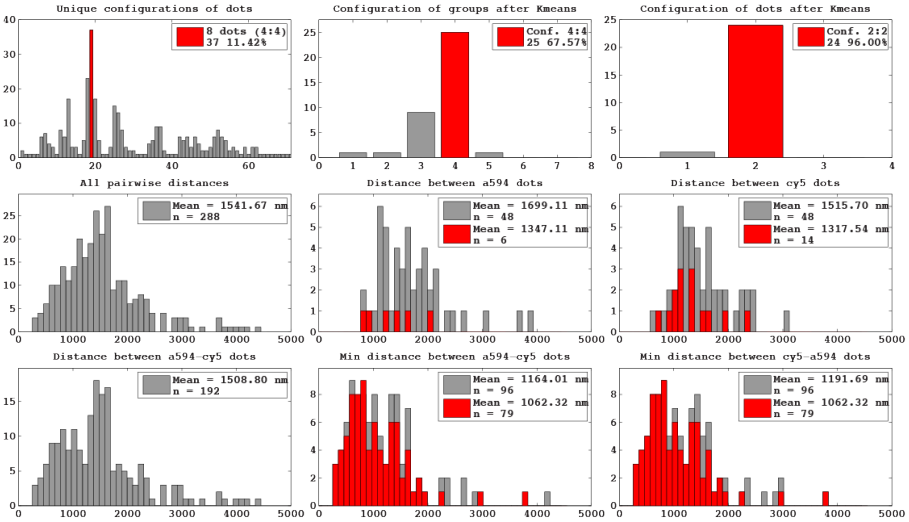

Figure 9: 4 loci counts

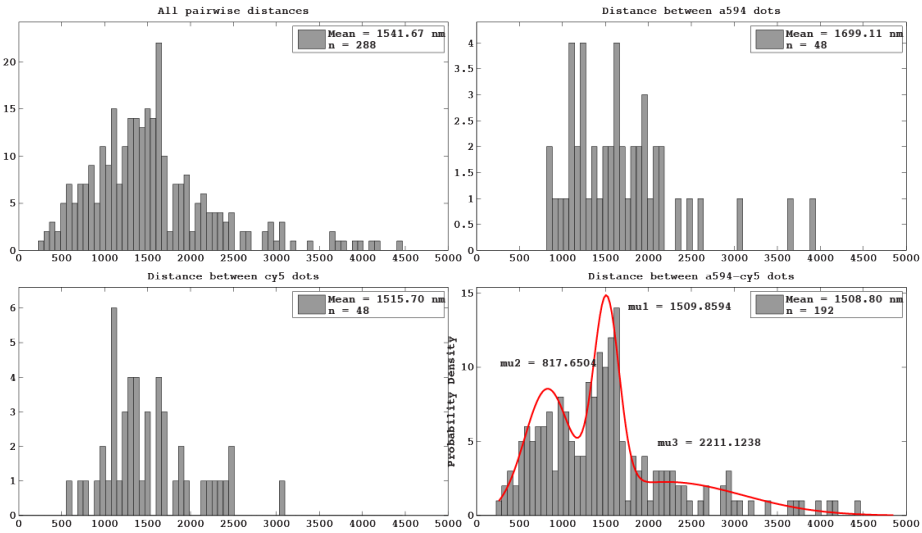

Figure 10: 4 loci counts

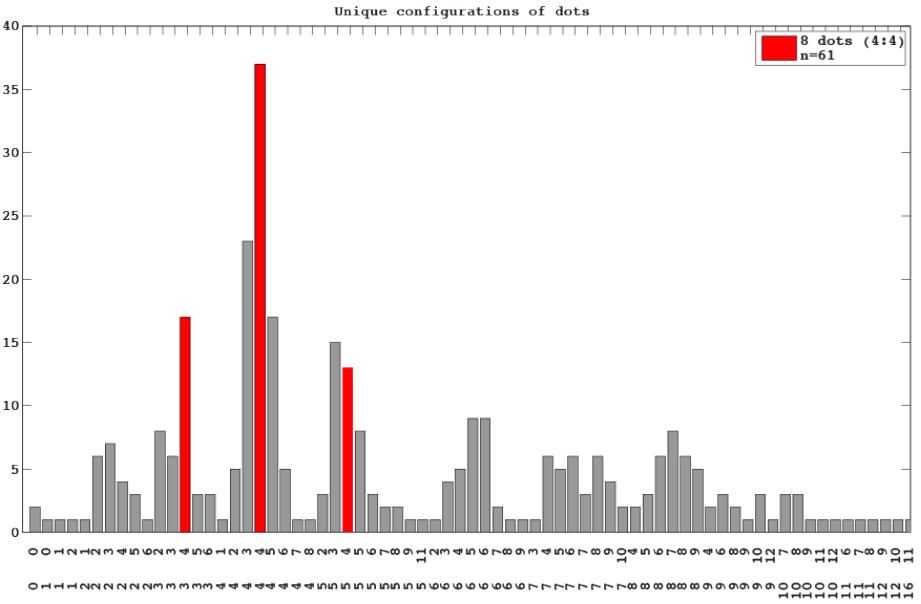

Figure 11: 4 loci counts

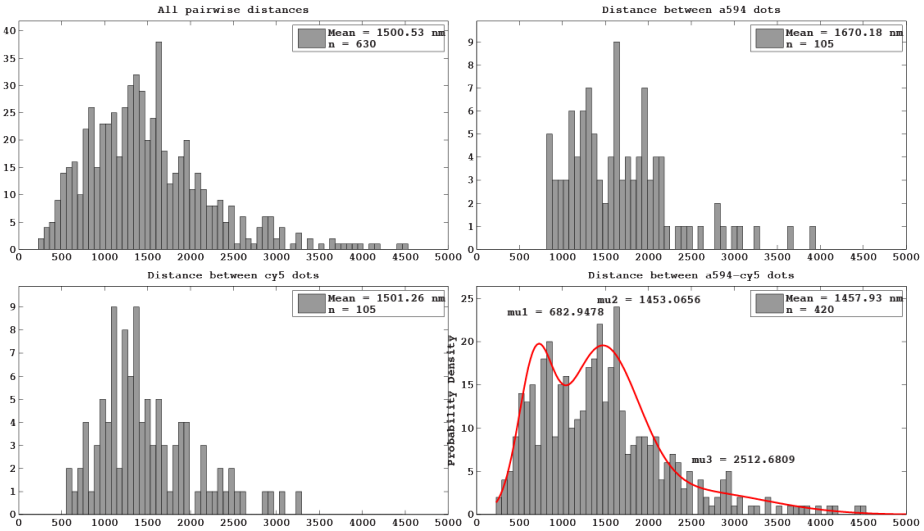

Figure 12: 4 loci counts

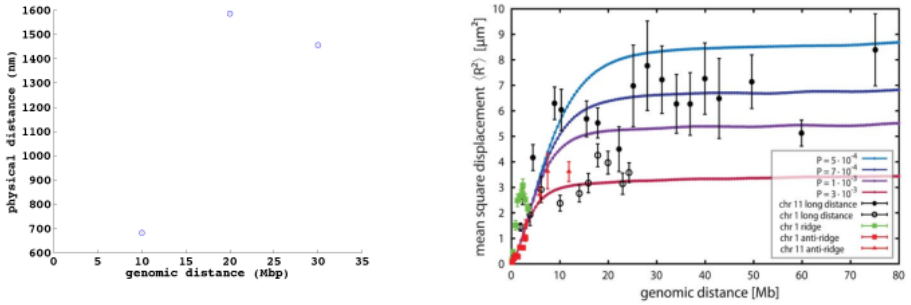

Figure 13: 4 loci counts

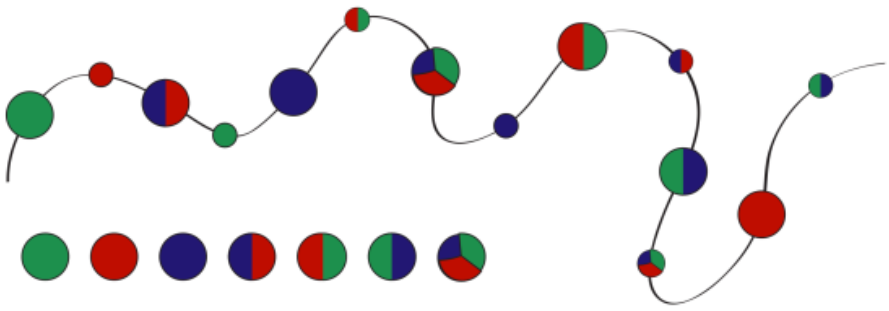

Figure 14: Combinatorial labeling

- I then went back to do a more detailed assessment of the shift calibration

Summary I

- Some improvements to ImageM were made: auto-segmentation, interaction with single nuclei, etc.
- The linear transformation for the correction of the shift was fine as a first approach, however not enough for the multi-labeling scenario
- A refined method for correcting the shift is necessary

Chromatic Aberrations

Dispersion: When different colors of light propagate at different speeds in a medium, the refractive index is **wavelength dependent**. Chromatic aberrations are those **departures** from **perfect imaging** that are due to dispersion.

Axial chromatic aberration

![Axial chromatic aberration][axial]

the shift is in the z direction

Transverse chromatic aberration

![Transverse chromatic aberration][transverse]

the shift is in the xy plane

First approach to correct for the shift

The projective transformation

Drawbacks

- although is position based (XY), it stills linear
- based on manual selection of the beads (variability)
- only performs correction on the XY plane

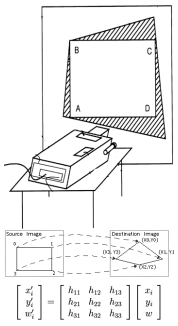

Figure 15: Projective transformation

Second approach to correct the shift

1. Automatic identification of the coarse position for all beads in the different channels
2. Refinement of the coarse position by fitting a 2D gaussian for the XY plane and a 1D gaussian for the Z position along the x,y coordinates gotten from the 2D gaussian
3. Fit a plane for each Z-position and interpolate. (Before talking to Stefan)

1. Beads are identified by (close) distance relation

- a **clique** is a complete subgraph (all nodes are conected to each other)
- **Automatic identification of fluorescent beads in different channels**
  - how to know that a given bead position in one channel corresponds to same bead in the other channel?
    1. use of distances to build an adjacency matrix
    2. from the adjacency matrix find the cliques of size(number of channels)
  - possible problems:
    1. misidentification of debris as beads
    2. movement of beads from one channel to another
    3. beads closer than corresponding shift

Beads identification

A.beads image B. identification of single cliques C. zoom of the identified cliques

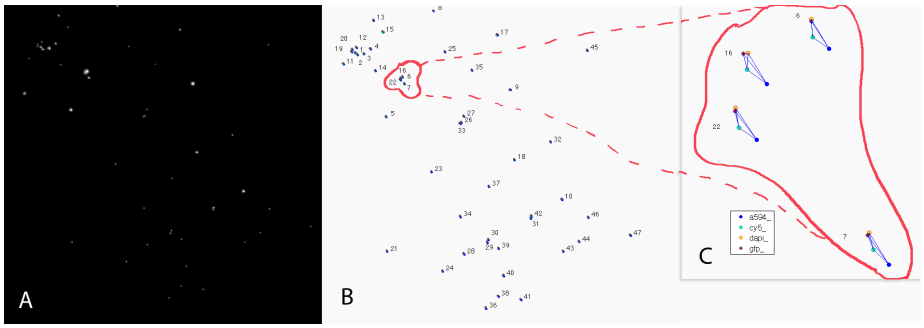

Figure 16: Beads identification

Dense distribution of beads

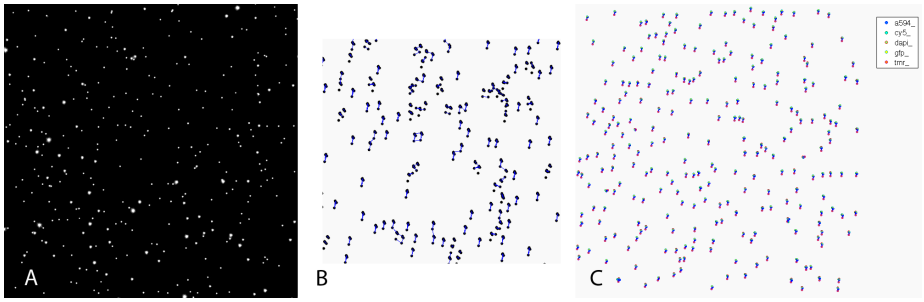

Figure 17: Beads dense

A.beads image B.identification of nearest beads in other channels C.identification of beads' cliques

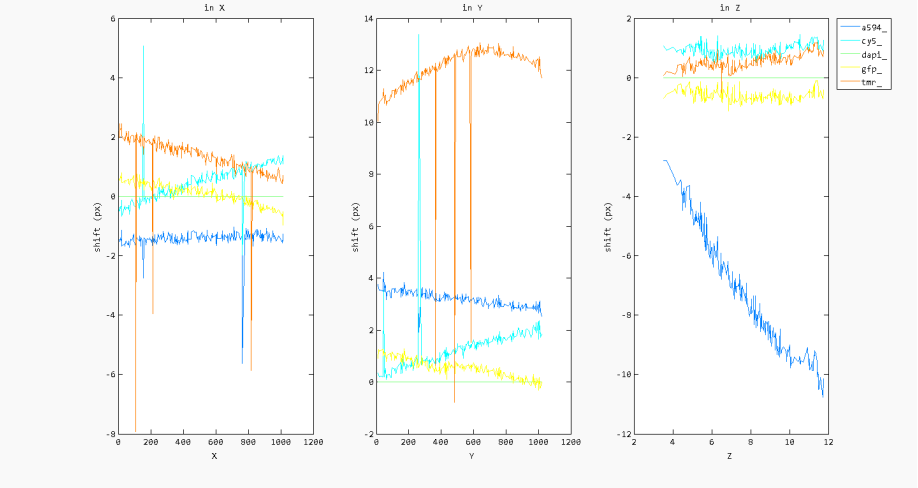

Figure 18: Shift quantification

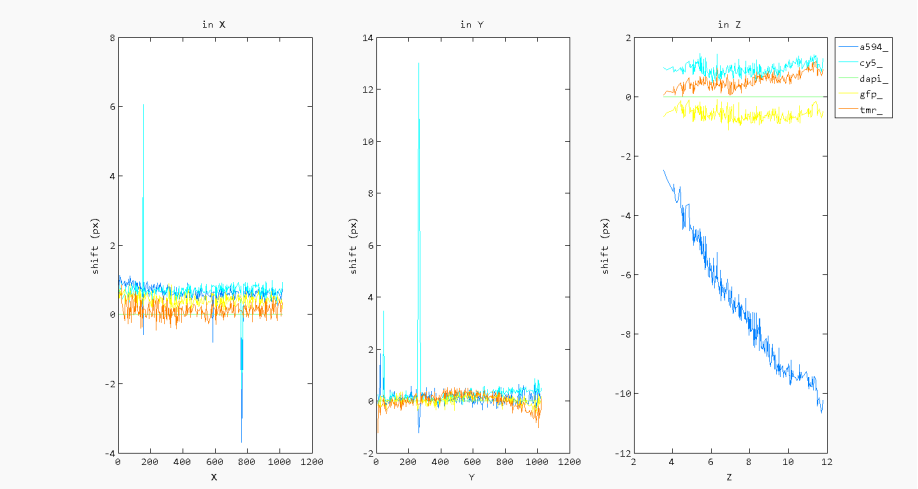

Figure 19: Shift quantification calib

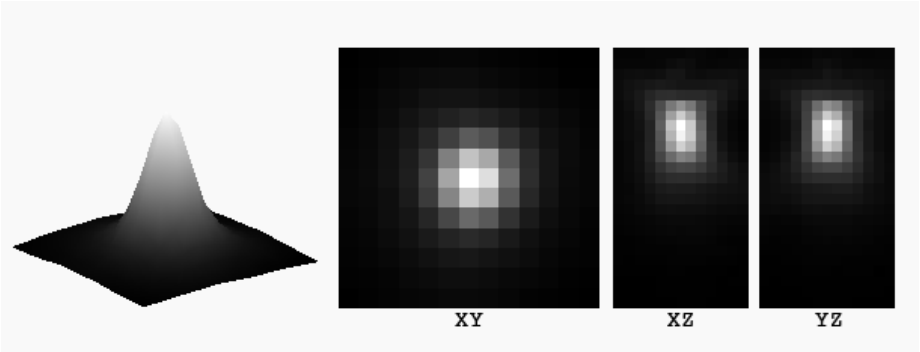

Figure 20: Dot image

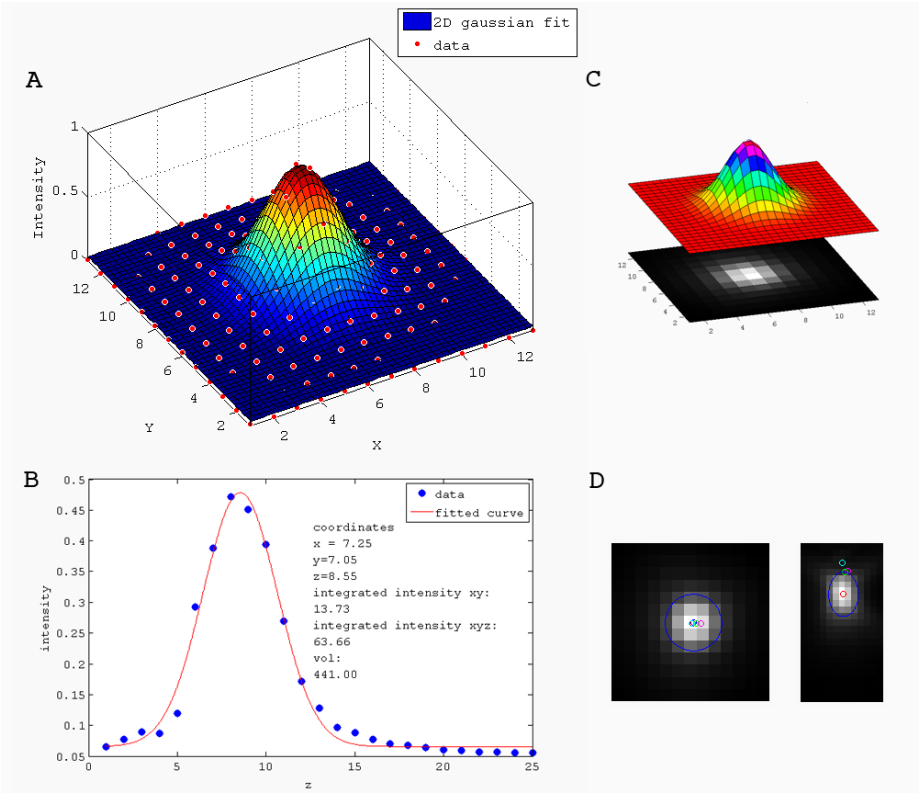

Figure 21: Gaussian Fit

Shift measurement

Shift measurement

After calibration using the linear transform

2. Beads are fitted to Gaussian functions

A bead seen from different planes

Gaussian function fitting

Outlook

Precise position estimation

- Use automatic identification of the beads together with gaussian fit correction to get the coordinate positioning of the beads and correct the shift. Use the same approach to get the HD-FISH spots features
- From there use Stefan's approach to calculate the shift as a function of the position in x,y, and z of the beads
- Experiment-wise, Magda is preparing a 2-channel single-loci DNA-FISH image acquisition which would help to calculate the shift specific for the medium in which probes are. Also to estimate the shift at different z-planes (no need of 3D experiment with beads)

After precise position estimation

- Re-analyze the 4 loci experiment
- Continue the analysis of the multi-labeling experiment

Aknowledgements

- Magda
- Stefan
- Lennart
- Mauro
- Philipp
- Alexander

Thank you!