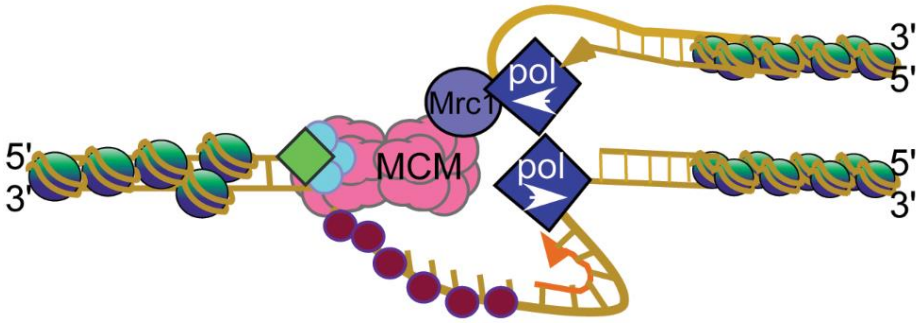
INTRODUCTION

The **replication fork** is a structure that is formed by DNA helicase during replication between the areas of unreplicated and replicated DNA.

Fork initiation, structure, and progression has been inferred by genetic and molecular methods such as DNA combing, chip-ChIP, and sequencing, but there is a critical gap in knowledge as to what the fork actually looks like.

Replication protein co-localization can be used to model fork structures and dissociation.



model of replication fork in Schizosaccharomyces pombe

OBJECTIVE: Develop a tool to systematically correlate DNA synthesis with protein location

CHROMATIN FIBERS

Also called fiber-spreads, retain protein components and epigenetic domains, which can then be visualized

Preparation Method:

1. Grow and treat cultures (if applicable)
2. Add nucleoside analogue to label replicating DNA
3. Dry cells on coverslip and lyse with salt/detergent buffer
4. Tip vertically to make fiber
5. Probe with antibodies (against protein/BrdU)
6. Add DAPI (labels DNA)

BrdU

- *5'-bromo-2'-deoxyuridine*
- Thymidine analog
- Phosphorylated by thymidine kinase to be incorporated into DNA

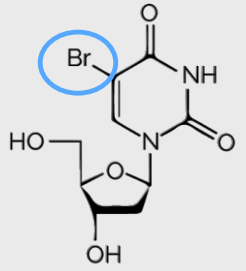
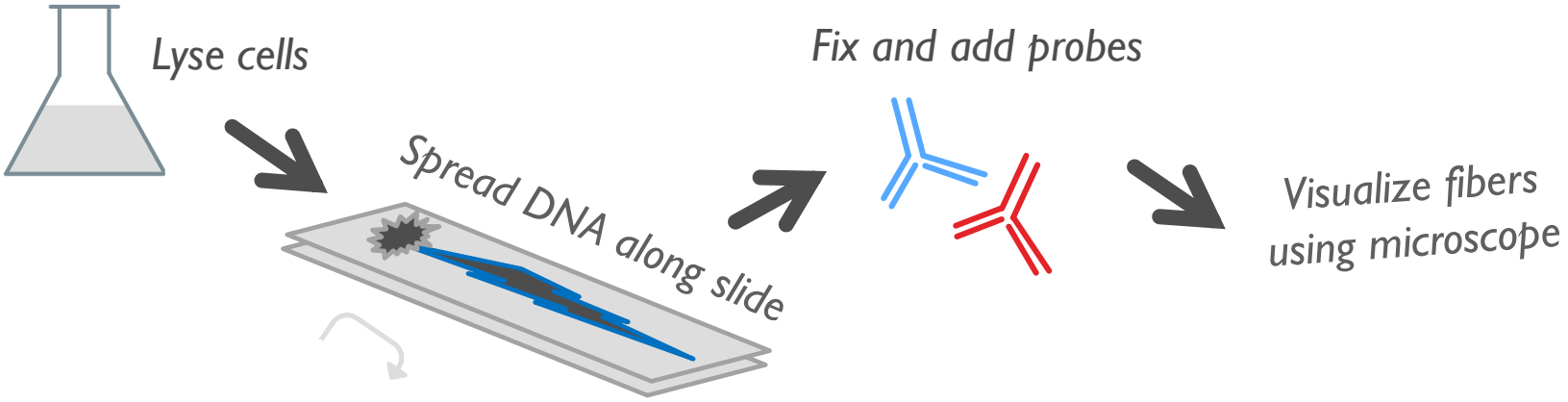
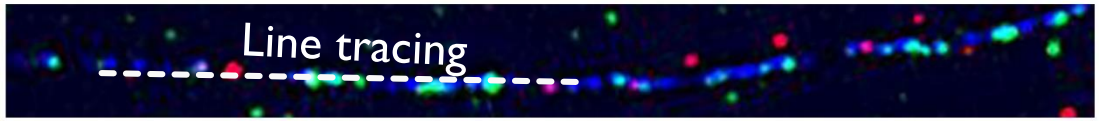


Image: https://www.compoundmag.com/article/1049



FIBER ANALYSIS – INPUT AND OUTPUT



blue- BrdU, green-protein 1, red-protein 2

fibers imaged on a Deltavision Spectris microscope (1.4 NA, 63x oil immersion lens)

deconvolved using "Arbitrary Line Profile" tool (SoftWorx)

	A	B	C	D	E	F	G	H
2	Tue Dec 21 17:25:03 2010							
3	Segment	Z Section	Time Point	Time (second	Angle	Segment Len	Pixel Size (m	Known Bias
4	1	1	1	0	24.8553	16.3673	0.1092	0
5								
6	Channel 452	Channel 523	Channel 594	Channel 676	X (pixel)	Y (pixel)	X (microns)	Y (microns)
7	136	0	481	0	52	403	5.6784	44.0076
8	130	0	669	0	53	403	5.7876	44.0076
9	146	0	877	0	54	404	5.8968	44.1168
10	137	0	1315	0	55	404	6.006	44.1168
11	127	0	3199	0	56	405	6.1152	44.226
12	127	135	1352	0	57	405	6.2244	44.226
13	128	0	629	0	58	406	6.3336	44.3352
14	134	0	442	0	59	406	6.4428	44.3352
15	129	137	523	0	60	407	6.552	44.4444
16	127	0	409	0	61	407	6.6612	44.4444
17	134	0	412	0	62	408	6.7704	44.5536
18	130	0	383	0	63	408	6.8796	44.5536
19	137	0	0	0	64	409	6.9888	44.6628
20	131	0	0	0	65	409	7.098	44.6628
21	132	0	0	0	66	409	7.2072	44.6628
22	134	0	277	0	67	410	7.3164	44.772
23	140	0	324	0	68	410	7.4256	44.772
24	130	0	315	0	69	411	7.5348	44.8812
25	125	0	239	0	70	411	7.644	44.8812
26	131	0	0	0	71	412	7.7532	44.9904
27	133	0	0	0	72	412	7.8624	44.9904
28	131	0	0	187	73	413	7.9716	45.0996
29	137	0	0	241	74	413	8.0808	45.0996
30	137	0	0	385	75	414	8.19	45.2088
31	132	0	238	237	76	414	8.2992	45.2088
32	125	139	0	158	77	415	8.4084	45.318
33	130	0	0	0	78	415	8.5176	45.318

Pixel intensities for 4 channels:

- DAPI (DNA)
- BrdU (Synthesis)
- Proteins (1&2)

Visualization

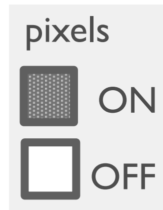
Modelling

Analysis

ODD BLOBS LOGIC

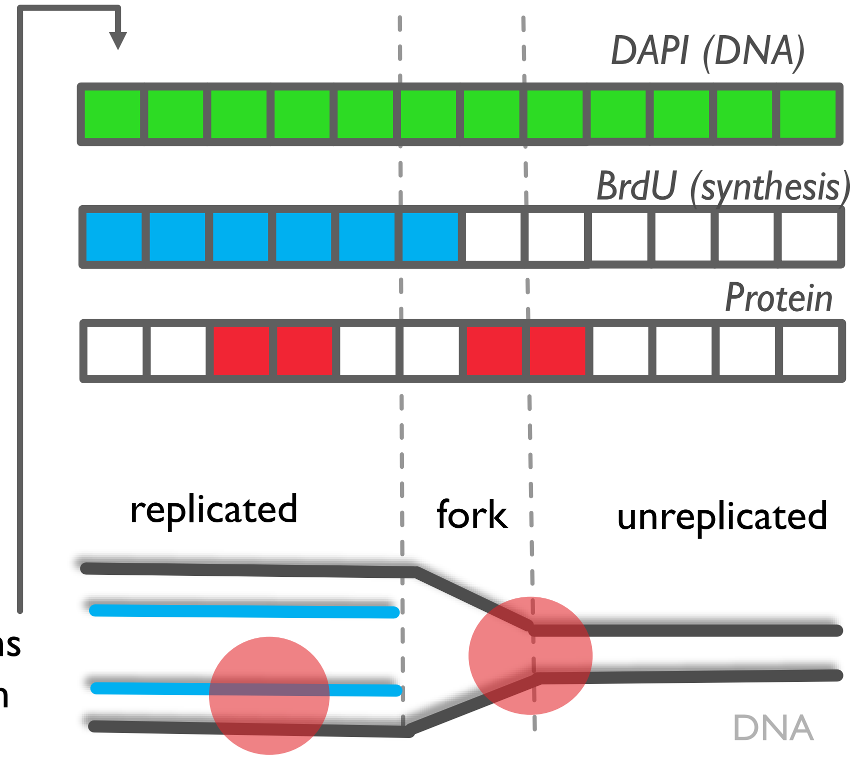
One Dimensional Data Boolean Logic Binning System (Sabatinos & Green, 2017)

INPUT – pixel intensity data
– thresholds
– smooth it
– tip window



ODD-BLOBS:

1. Applies thresholds to pixel intensities:
   if intensity > threshold = ON, else OFF
2. Finds fork, replicated, and unreplicated regions
3. Finds localization of protein relative to region

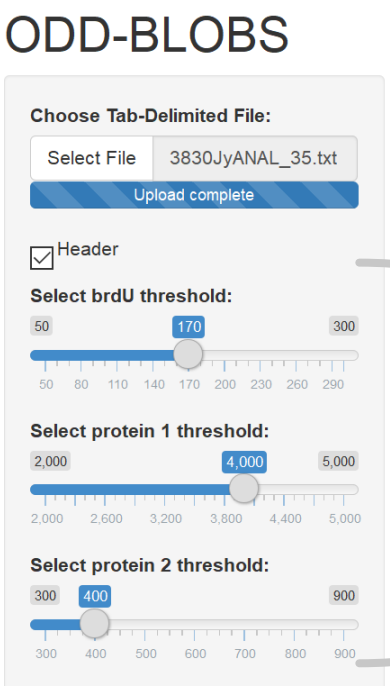


OUTPUT (current)

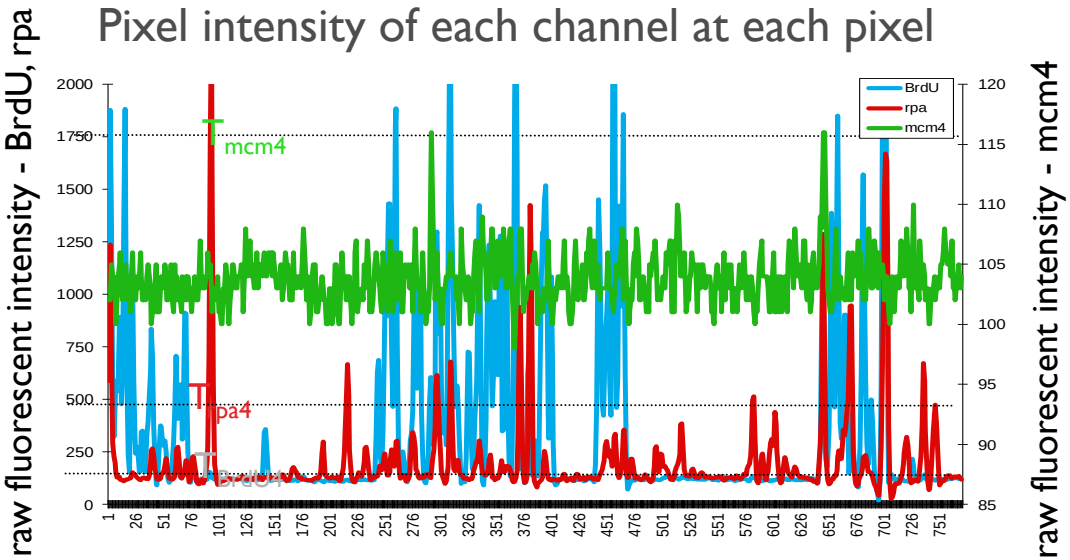
```
> print(tracts_df)
   Tract No. Starts At Ends At Length                                   Prot1 in Tract                       Prot2 in Tract Fork 1 Starts Fork 1 Ends Fork 2 Starts Fork 2 Ends
1          1         1       3      3                                                3                                  2 3             1           2             2           4
2          2        15      17      3                                                                                                  14          16            16          18
3          3        32      34      3                                                                              32 33 34            31          33            33          35
4          4        64      68      5                                                                                                  63          65            67          69
5          5        74      78      5                                            75 76                                                 73          75            77          79
6          6        84      89      6                                                                                 84 85            83          85            88          90
7          7       107     109      3                                          108 109                                  109           106         108           108         110
8          8       124     128      5                                                                       124 125 126 127           123         125           127         129
9          9       143     151      9                                          150 151                      148 149 150 151           142         144           150         152
10        10       154     156      3                                                                                   156           153         155           155         157
11        11       158     187     30 163 164 175 176 177 180 181 182 186 187 169 170 171 176 177 185 186 187           157         159           186         188
12        12       199     202      4                                          200 201                                                198         200           201         203
13        13       205     211      7                                                                                                 204         206           210         212
14        14       225     228      4                                          227 228                      225 226 227 228           224         226           227         229
> paste("Mean tract length is ", mean_tract_length, "+/-", std_tract_length, "pixels" )
[1] "Mean tract length is  6.42857142857143 +/- 7.01333110488969 pixels"
>
```

I) THRESHOLD

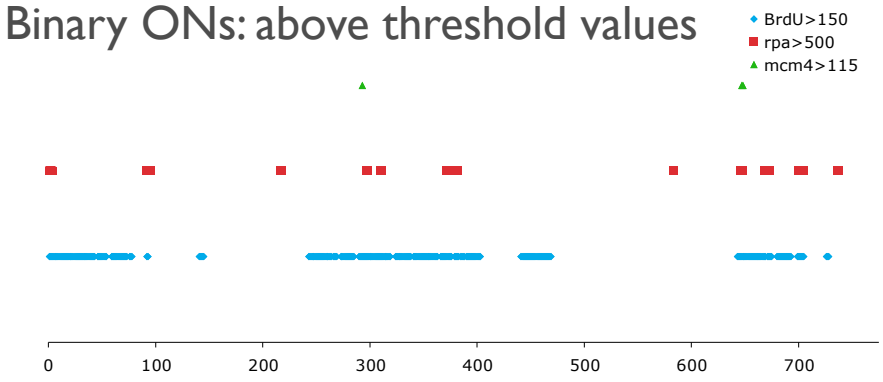
Threshold: value that allows desgination of ON (above) or OFF (below) signal at pixel



Allows user to test different thresholds



apply threshold



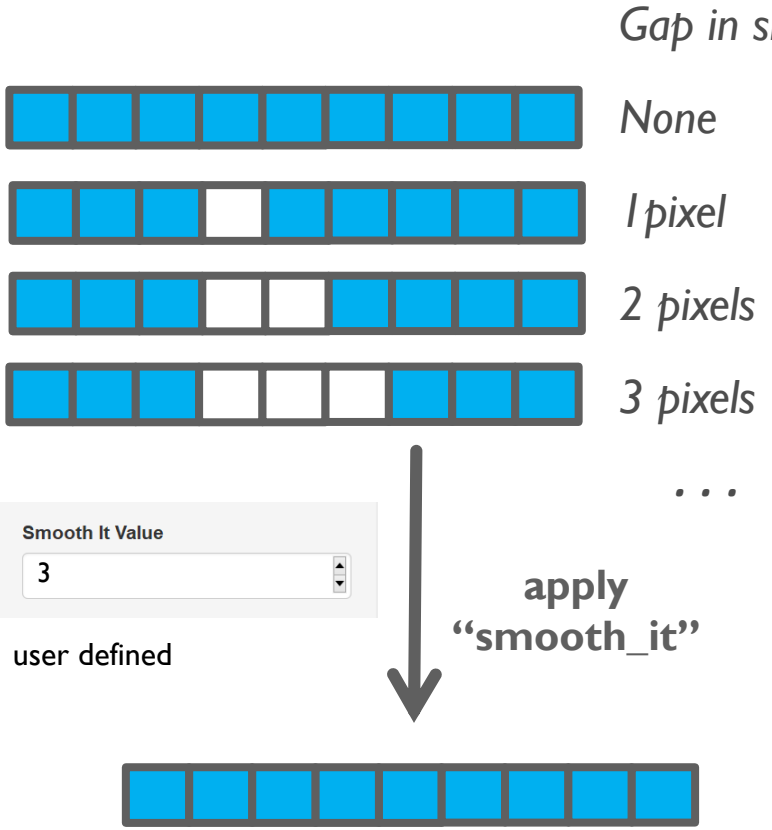
Issue: How does user define threshold?

Need to make automatic

2) "SMOOTH IT"

Smooth it: value that accounts for gaps in signal when processing images

"Smooth it" gets rid of gap by filling OFFs with ONs! i.e,



Abbe Raleigh theorem

Resolution (r) = 0.61 λ /NA

+

system pixel length = 0.1092 µm

average limit of detection is ~ 2 pixels (0.2184 µm)

Wavelength (λ, nm)	r (nm)	pixels
350	152.5	1.4
488	212.6	1.9
546	237.9	2.2
647	281.9	2.6

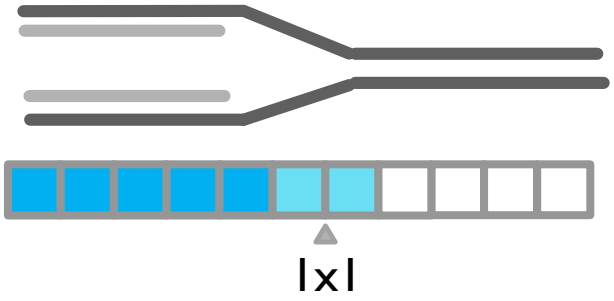
3) TIP WINDOW

ODD-BLOBS allows user to choose and change the "size" of a forks (i.e, the ends of a synthesizing tract (extent into replicated and unreplicated areas)

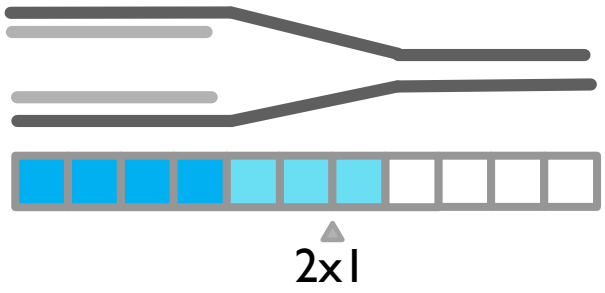
Question: How big is the fork?

Tip window: size of the fork defined by no. of "replicated" x "unreplicated" pixels

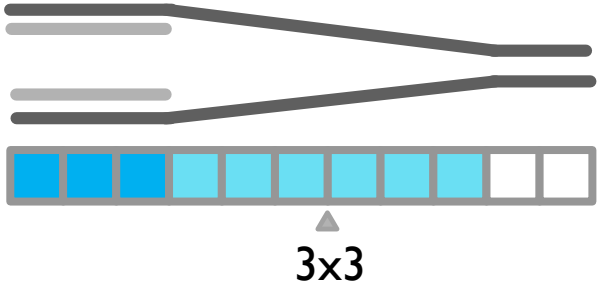
Default is 1x1 pixels

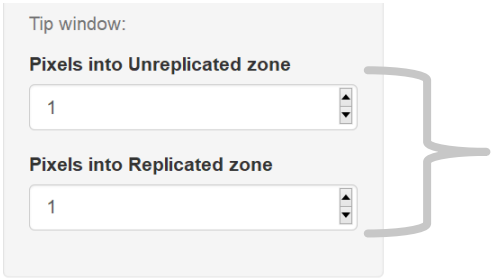


Symmetrical



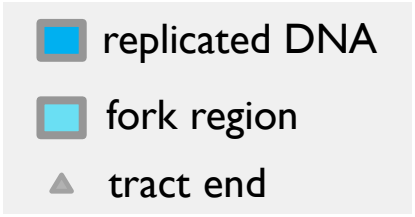
Unsymmetrical





Number of pixels into replicated area

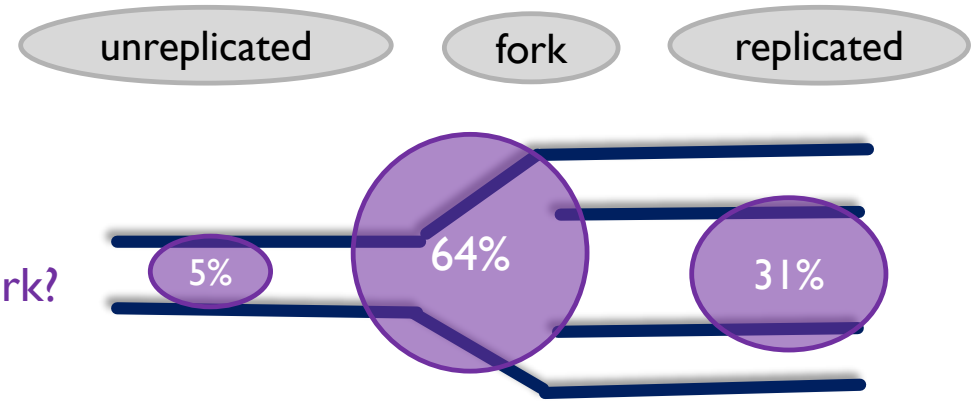
Number of pixels into unreplicated area



APPLICATIONS

Replication protein co-localization to model fork structures and dissociation

e.g. Where is Protein X located relative to fork?



Use histone antibodies specific to particular modifications and see effect

e.g. DNA damage can be associated with a certain region

Determine role of nucleotide depletion (induced by drug) in changing protein deposition along fibers