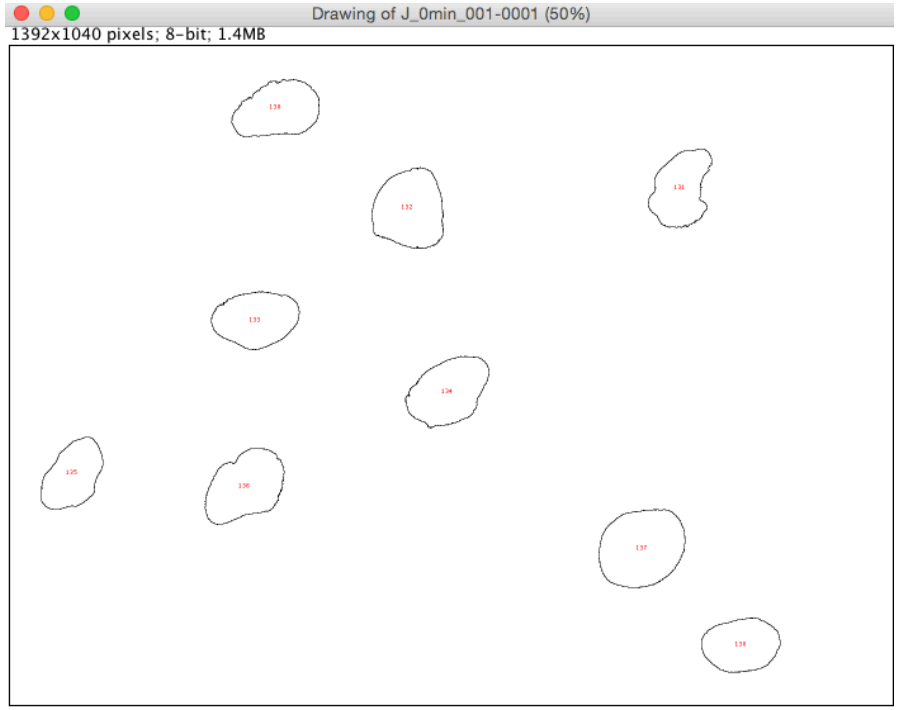
M1D5: Analyze Gamma-H2AX images and CometChip electrophoresis

09/27/19

Perform analysis on resultant excel spreadsheet

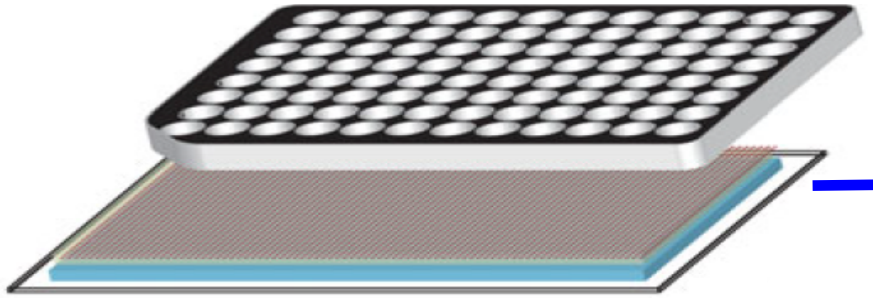


	A	B	C	D	E	F	G	H	I	J	K	L
1		Label	Area	Mean	Min	Max	Circ.	IntDen	RawIntDen	AR	Round	Solidity
2	1	J_H2O2_001-0002	10213	487.021	371	1062	0.768	4973943	4973943	1.382	0.723	0.951
3	2	J_H2O2_001-0002	10249	591.748	391	1005	0.862	6064824	6064824	1.077	0.929	0.979
4	3	J_H2O2_001-0002	8482	459.955	381	549	0.811	3901340	3901340	1.519	0.658	0.97
5	4	J_H2O2_001-0002	11661	496.568	380	865	0.838	5790485	5790485	1.386	0.721	0.978
6	5	J_H2O2_001-0002	10959	659.783	439	2451	0.729	7230565	7230565	2.062	0.485	0.96
7	6	J_H2O2_001-0002	38645	595.773	398	1937	0.456	23023658	23023658	2.432	0.411	0.837

Compare:

Overview of CometChip Assay: Treating cells and visualization

Treat captured cells in comet chip with MMS then As

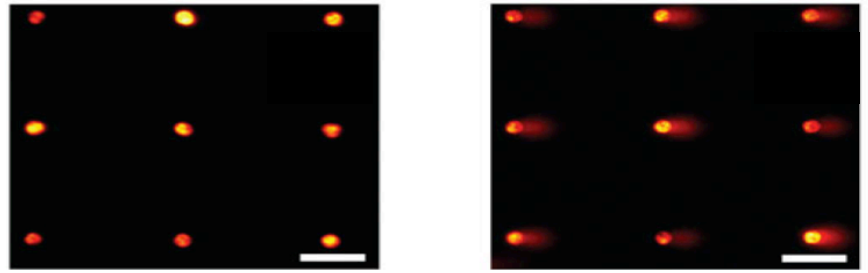


Last class

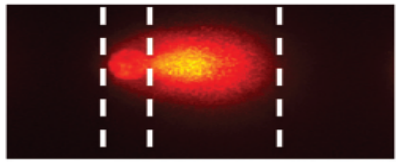
Lyse cells & unwind DNA
(DNA still captured agarose in overlay)

Electrophoresis

This class



Stain DNA and image via fluorescence microscopy

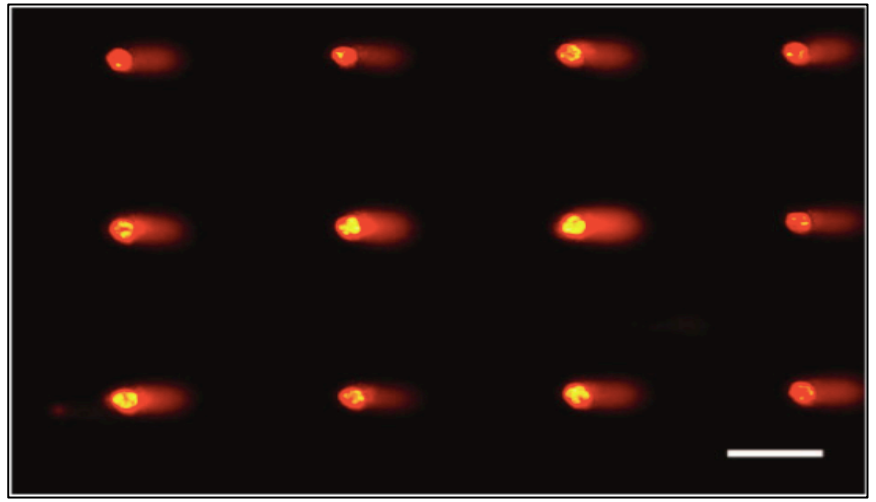


Analysis via Matlab

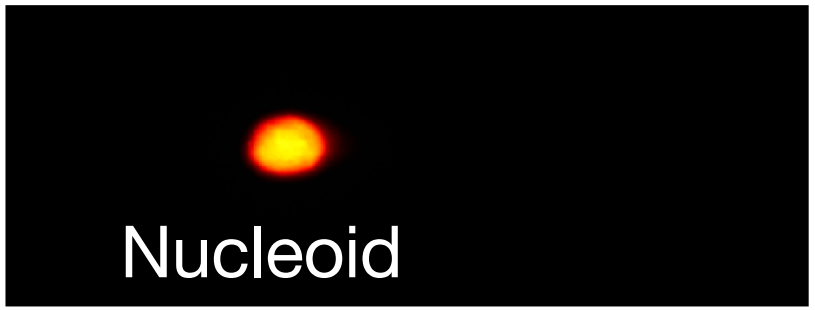
Next class

Lysis, electrophoresis & staining CometChips

- ❑ Alkaline lysis solution (pH 10)
  - 2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris
  - Triton X-100
- ❑ Unwinding/ electrophoresis buffer (pH 13.5)
  - 0.3M NaOH, 1mM Na_2EDTA
- ❑ Neutralize (pH 7.5)
  - 0.4M Tris
- ❑Florescent stain for DNA (dye)
  - SYBR Gold in PBS

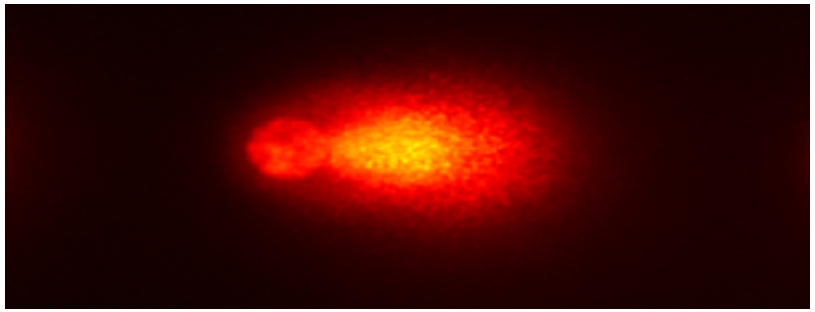


Output of Alkaline CometChip Assay



No Damage

- Supercoiled nucleoid
- Little or no migration



High Damage

- SSBs, abasic sites, alkali labile sites
- forms a “Comet tail”

Genomic damage from direct strand breaks and REPAIR INTERMEDIATES

HW M1D6:

1. Revise methods homework (group) M1D1-M1D5
2. Mini Presentation Outline (individual)
3. Prepare for paper discussion
    a) Discussion guidelines on wiki

REPORT

Cell Cycle 12:6, 907–915; March 15, 2013; © 2013 Landes Bioscience

Single-cell microarray enables high-throughput evaluation of DNA double-strand breaks and DNA repair inhibitors

David M. Weingeist,[1,†] Jing Ge,[1,†] David K. Wood,[2] James T. Mutamba,[1] Qiuying Huang,[3] Elizabeth A. Rowland,[1] Michael B. Yaffe,[1,3,4,5] Scott Floyd[4,6] and Bevin P. Engelward[1,*]

Notes on Mini-presentation…

- Bullet / outline format
- Follow time and content guidelines:
  - Introduce yourself and your research project
  - Clearly state hypothesis to identify main question
  - Be quantitative when stating results (NOT “this was more/less than...”)
  - For now, use placeholder statements for key findings
- Logistics:
  - Submission should not be edited / spliced
  - Ensure that you can be clearly heard in the recording
  - Be mindful of background distractions

Grading rubric for Mini-presentation

Category	Elements of a strong presentation	Weight
Introduction	• Introduce yourself and the research • Summarize the background information necessary to understand the research • Provide a clear and concise description of the central question / hypothesis	25%
Methods & Data	• Provide ONLY the method information necessary to understand the results • Give complete and concise explanations of the results • Relate the results to the central question	25%
Summary & Conclusions	• Highlight the key finding(s) relevant to the central question / hypothesis	25%
Organization	• Give a logical, easy-to-follow narrative • Include transition statements	15%
Delivery	• Show confidence / enthusiasm and speak clearly • Use appropriate language (technical or informal, as appropriate) • Be mindful of the time limit (3 minutes +/- 15 seconds!)	10%

The mini-presentation will be graded by Dr. Noreen Lyell with input from Dr. Leslie McClain, and Dr. Becky Meyer.