20.109 MOD1
Measuring Genomic Instability

Fall 2023
Day 5

Bevin P. Engelward, *Sc.D*.
Professor of Biological Engineering

A Look at Your Data

How the γH2AX Assay has Shaped History

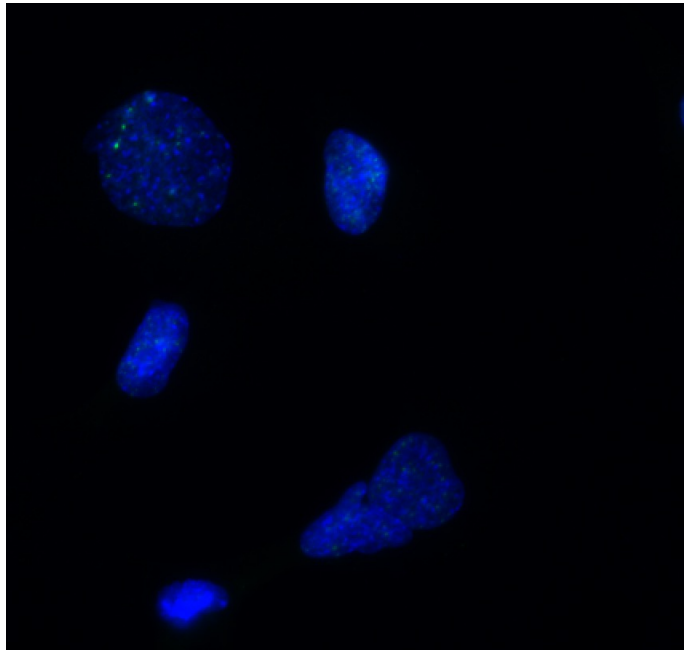
Rationale for Creating the CometChip
How the Comet Assay Works
Development of the CometChip
Why Commercialize?
Example of a CometChip Application

Images taken at 20x

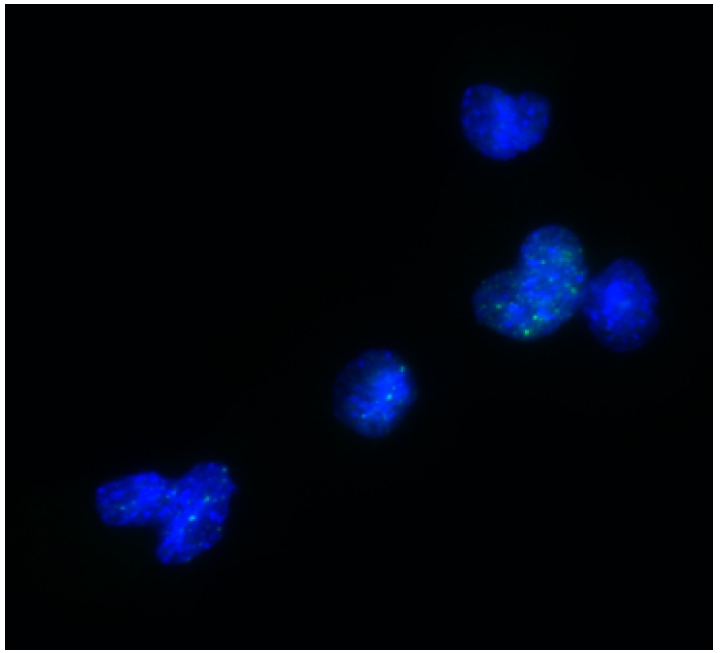
Zoomed in on nuclei

Blue= DAPI
Green=yH2AX

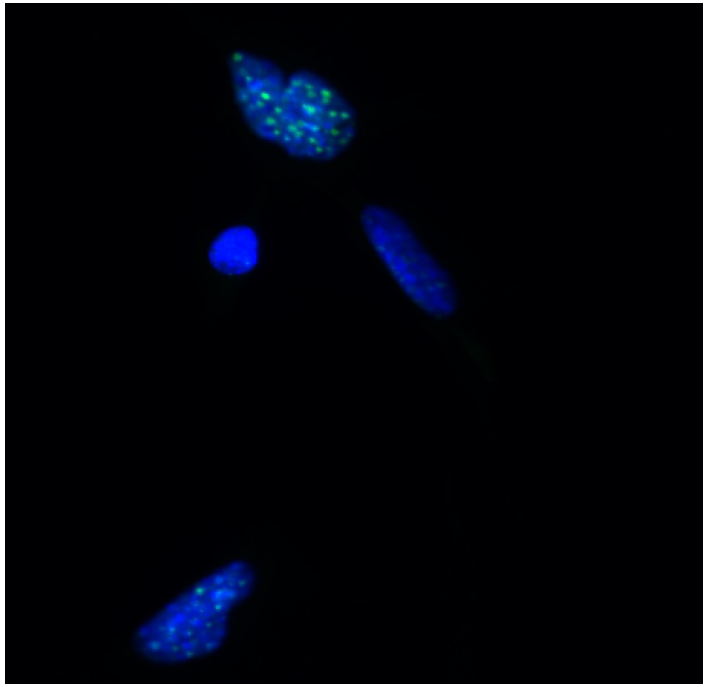
0uM As
0uM H2O2



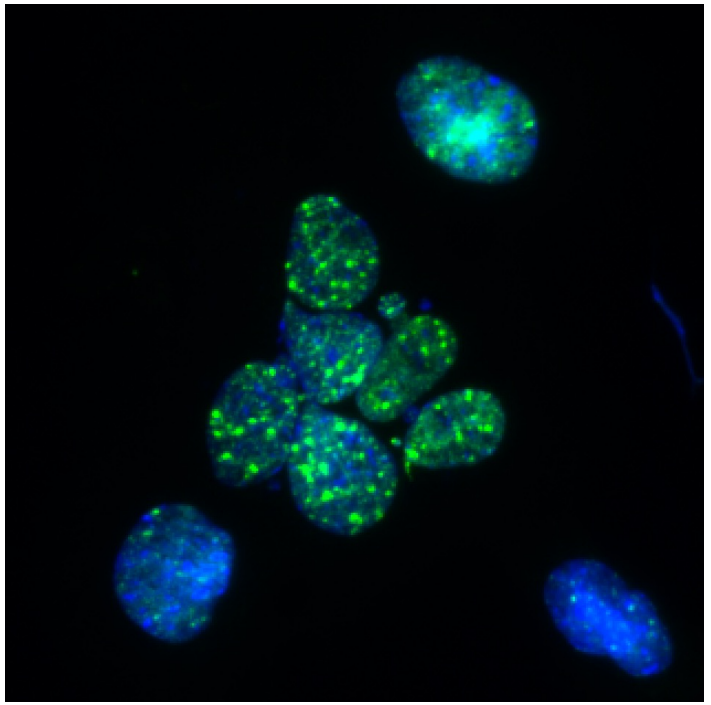
40uM As
0uM H2O2



0uM As
10uM H2O2



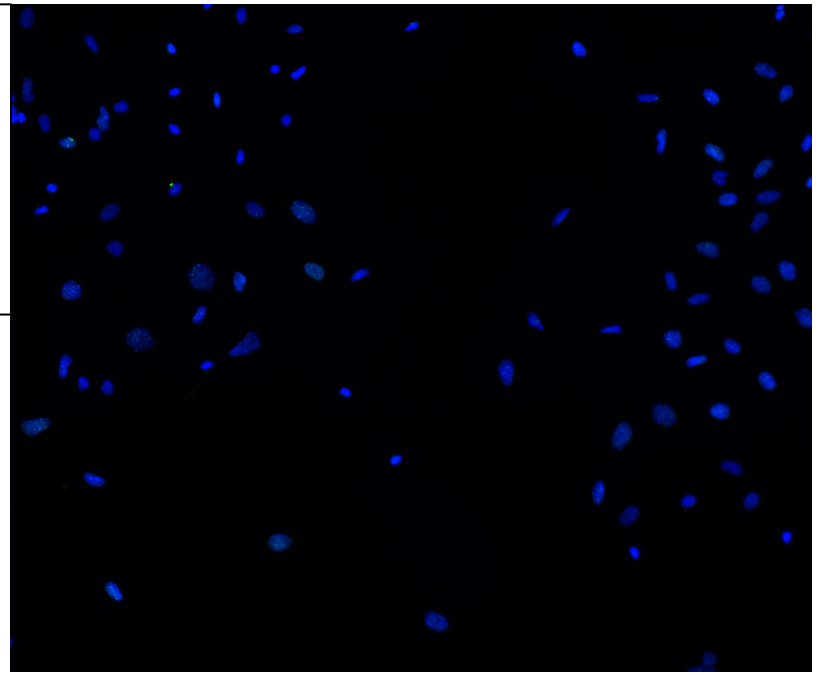
40uM As
10uM H2O2



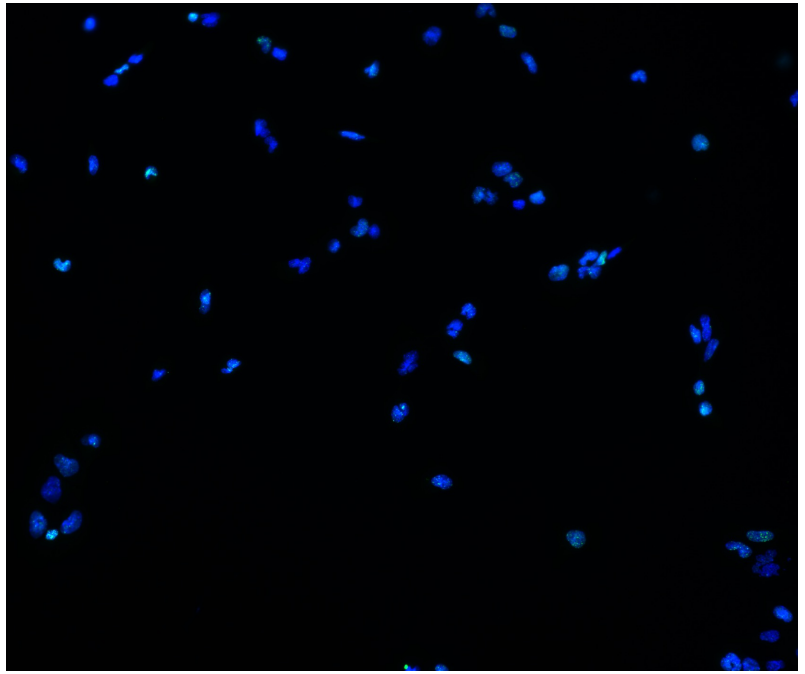
Images taken at 20x

Full field view

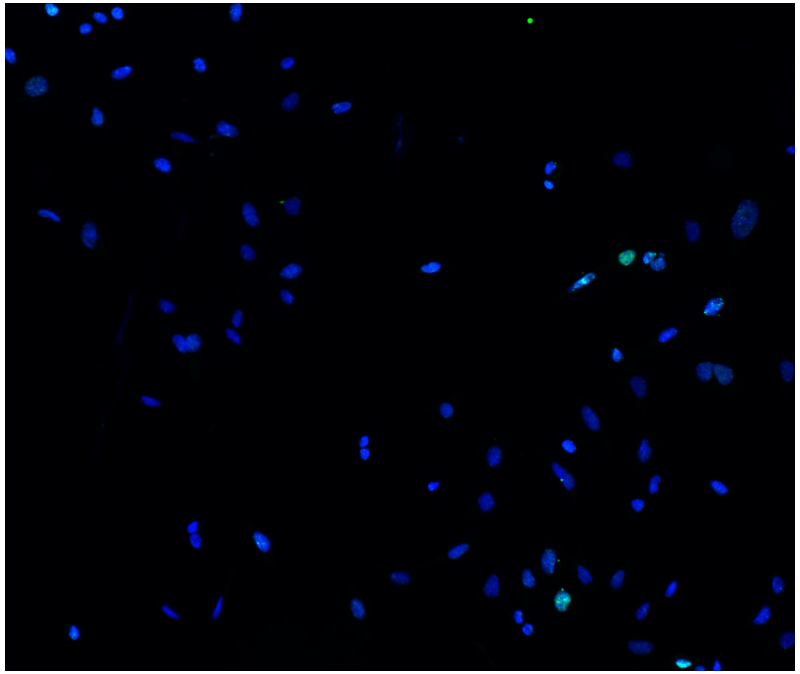
Blue= DAPI
Green=yH2AX



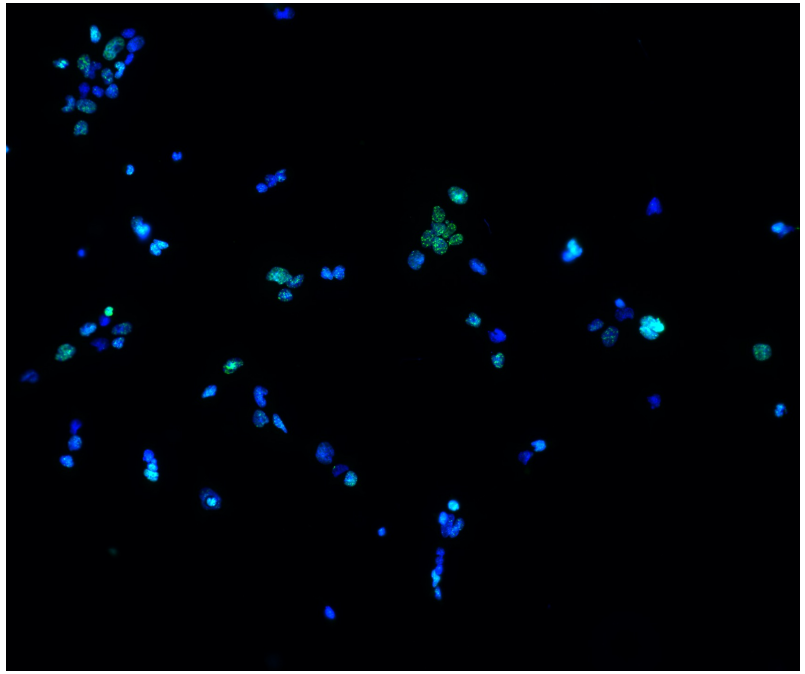
0uM As
0uM H2O2



40uM As
0uM H2O2



0uM As
10uM H2O2



40uM As
10uM H2O2

Combine qualitative and quantitative results,
whenever possible.

Be sure to show clear pictures
both zoomed out and zoomed in.

Perform Validation

Need to check results from software
to what you can see by eye (gold standard).
How could you do this for your experiment?

Combine qualitative and quantitative results, whenever possible.

Be sure to show clear pictures
both zoomed out and zoomed in.

Perform Validation

Need to check results from software
to what you can see by eye (gold standard).
Be Very Careful to avoid Bias!

How the γH2AX Assay has Shaped History

Rationale for Creating the CometChip

How the Comet Assay Works

Development of the CometChip

Why Commercialize?

RESEARCH ARTICLE | BIOCHEMISTRY |

Evidence for a lack of DNA double-strand break repair in human cells exposed to very low x-ray doses

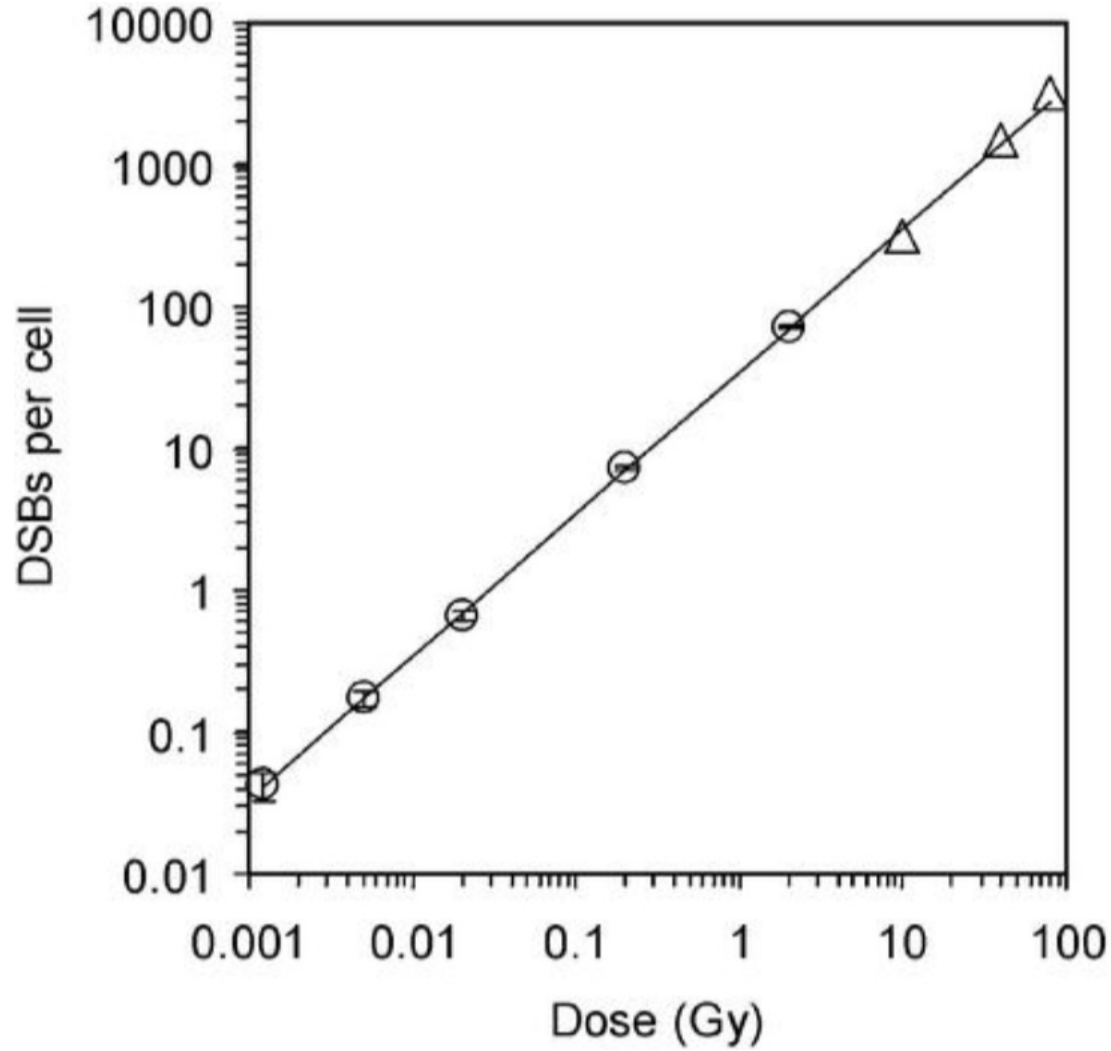
Kai Rothkamm and Markus Löbrich Authors Info & Affiliations

Analysis of Radiation-Induced DSBs

Linear no-threshold model

Claim that damage induced by low-dose radiation is not effectively repaired

This led some to conclude that no level of radiation is safe.

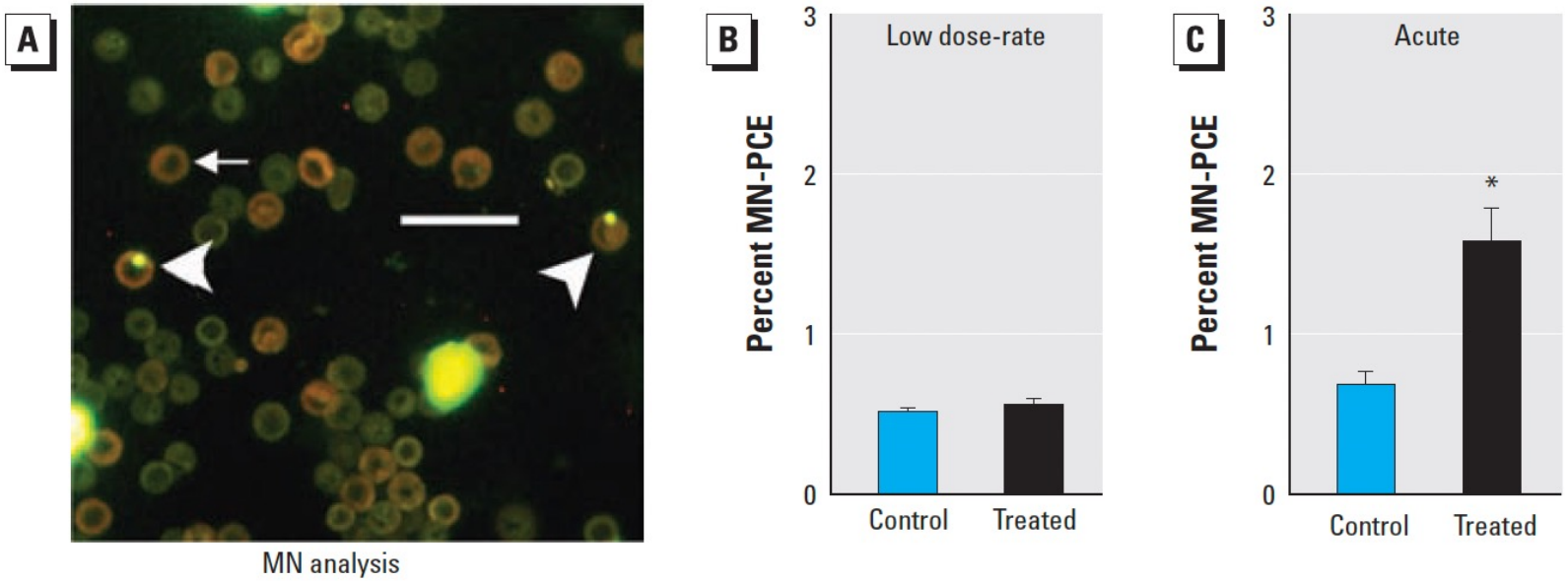


Integrated Molecular Analysis Indicates Undetectable Change in DNA Damage in Mice after Continuous Irradiation at ~400-fold Natural Background Radiation

Werner Olipitz,[1] Dominika Wiktor-Brown,[1] Joe Shuga,[1] Bo Pang,[1] Jose McFaline,[1] Pallavi Lonkar,[1] Aline Thomas,[1] James T. Mutamba,[1] Joel S. Greenberger,[2] Leona D. Samson,[1] Peter C. Dedon,[1] Jacquelyn C. Yanch,[3] and Bevin P. Engelward[1]*

The same dose delivered either chronically or acute.

Model: Repair keeps up if dose is chronic.



Deaths from Nuclear Power Plant Disaster: Chernobyl Example

- 31 people died as an immediate result of Chernobyl while the
- UN estimates a further 4,000 might eventually die as a result of the radiation exposure.
- Union of Concerned Scientists estimates 12,000 to 57,000 cancer fatalities.

Deaths from Fossil Fuel Combustion Emissions:

Deaths from Global Warming in Europe:

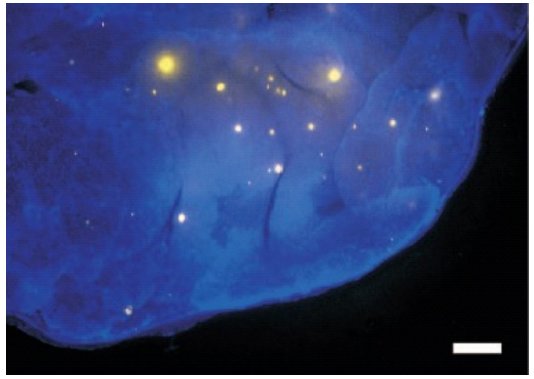
Rationale for Creating the CometChip

How the Comet Assay Works

Development of the CometChip

Why Commercialize?

Goal: High-throughput Assay for DNA damage



Need High Throughput for Safety Testing, the Clinic & Epidemiology

Extremely Low Throughput

Rapid and Sensitive Toxicity testing is Critical

>100,000 synthetic chemicals currently in use

~2,000 added every year

Pharmaceuticals

Industrial chemicals

Household chemicals

Occupational Exposures

Food

Pollutants

Cosmetics

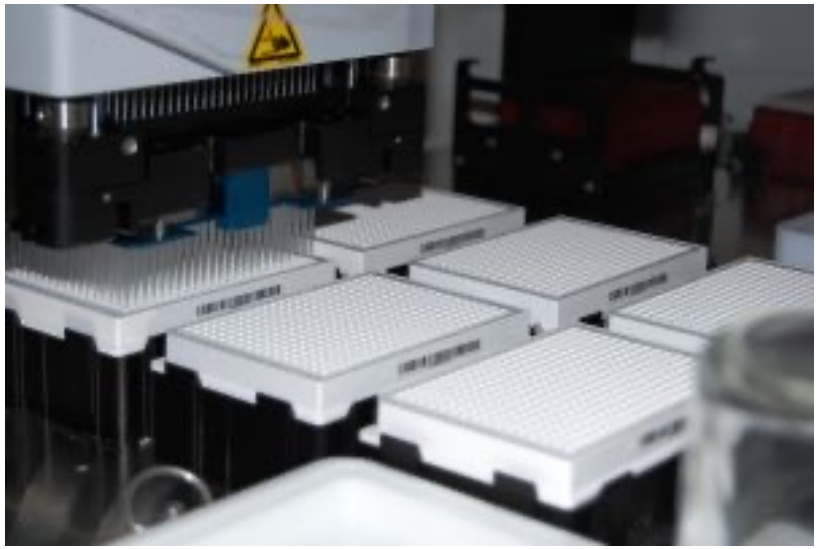
Home Renovation Chemicals

Paradigm shift in toxicity testing in the 21st century

Traditional approach

Costly, slow, laborious
Concerns about human relevance

21st century approach



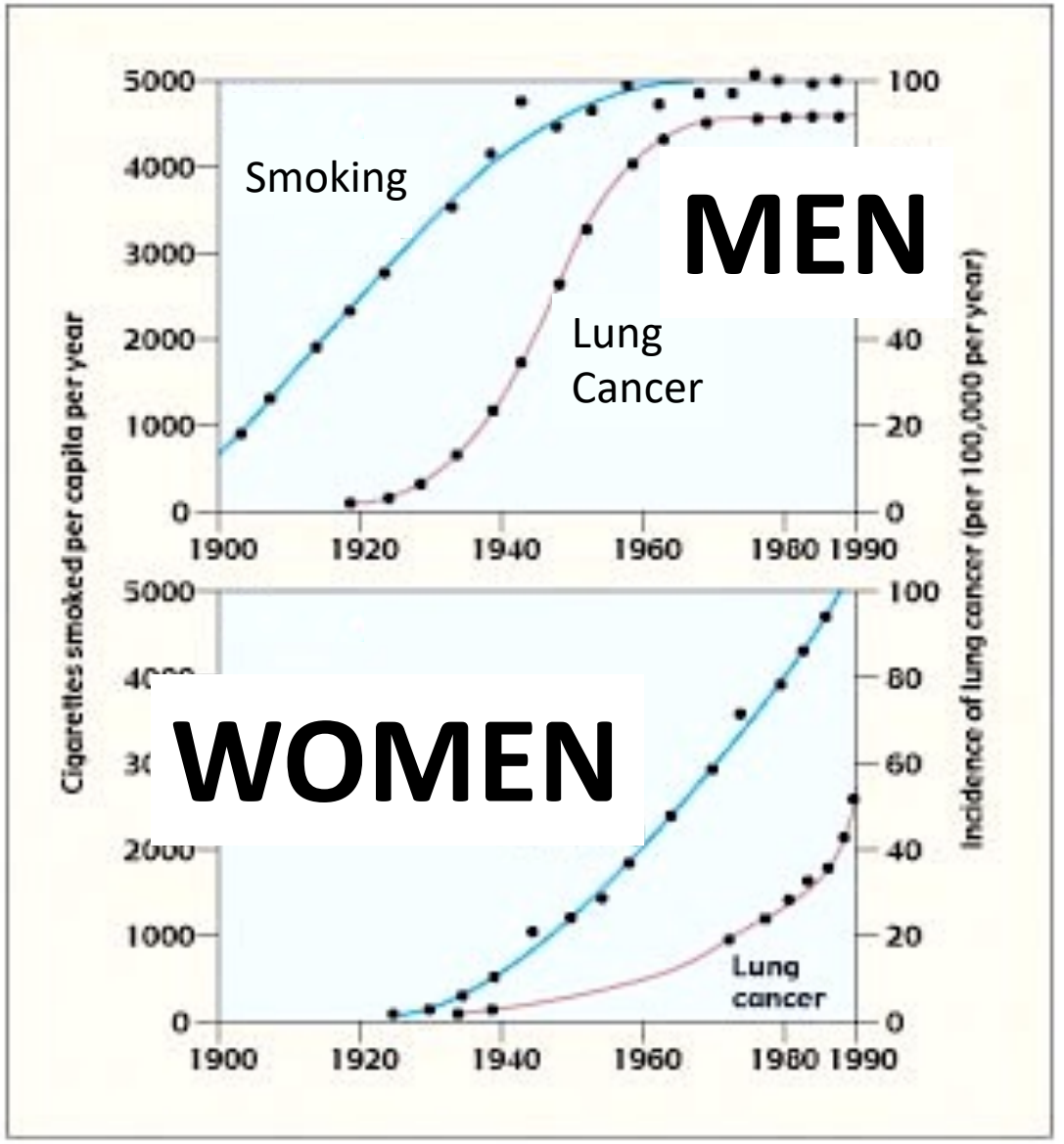
Goals:

- Less expensive
- Higher throughput
- Potentially more predictive using human cell/organoid models

Concept: Need *predictive* biomarkers.

It can take a long time before a carcinogen exposure presents as cancer.

Rapid Screening of Chemicals is Critical: Long Lag Time



Concept: Need *predictive* biomarkers.

It can take a long time before a carcinogen exposure presents as cancer.

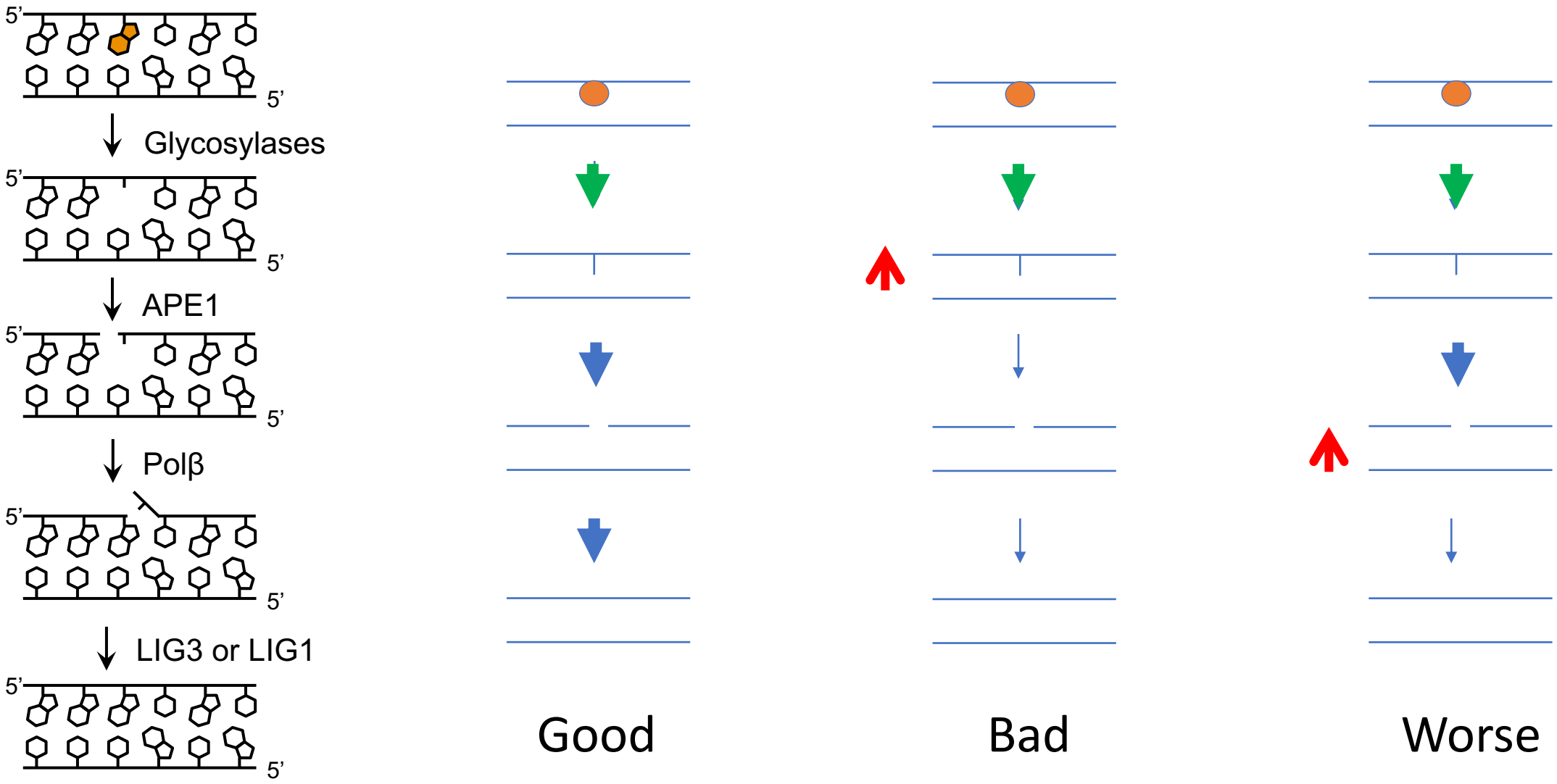
Delay can be 20 years (*e.g.*, cigarette smoke)

Biochemical Assays are a Proxy for What is Happening in the Cell

Why not test the cell's response more directly?

Analyze the efficiency of the *Pathway*

Increased Levels of a DNA Glycosylase may be Good or Bad Depending on Downstream Kinetics



Concept: Analyzing *cellular responses* reflects the *integrated* effect of multiple steps in a pathway.

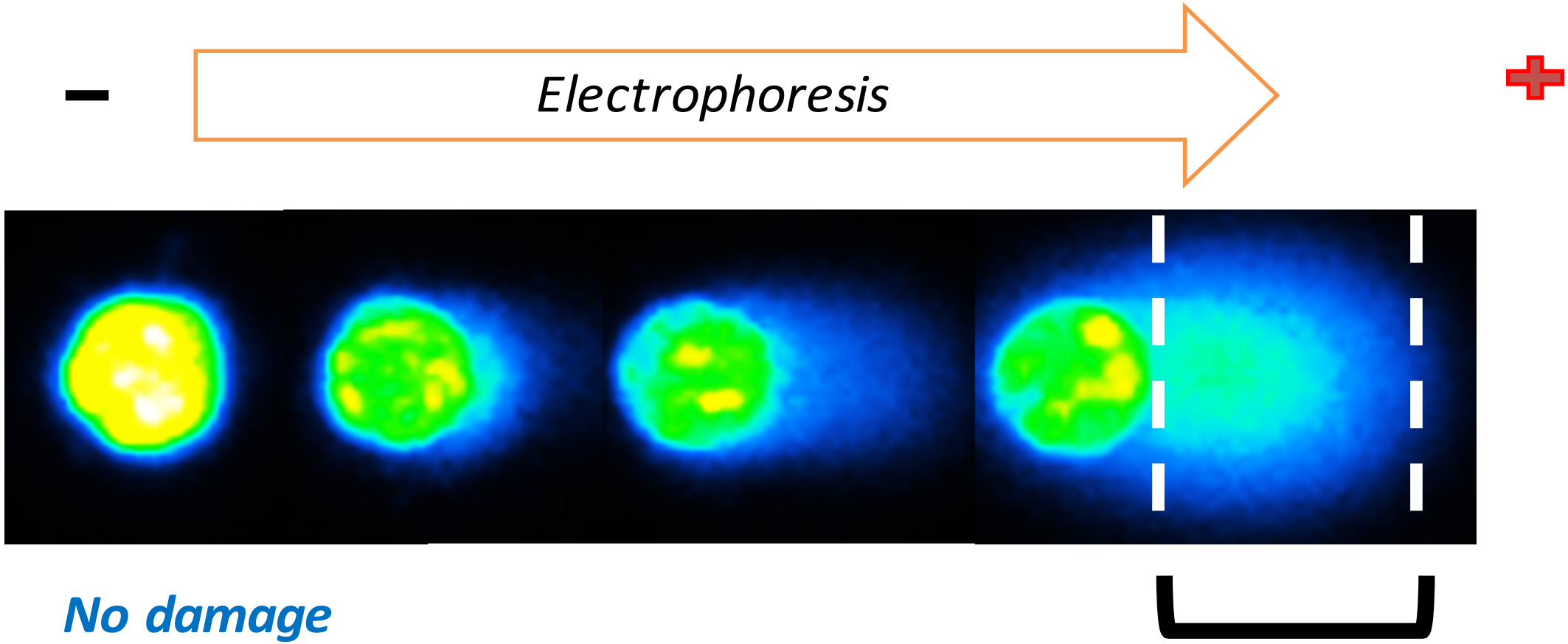
Rationale for Creating the CometChip

How the Comet Assay Works

Development of the CometChip

Why Commercialize?

Example of a CometChip Application



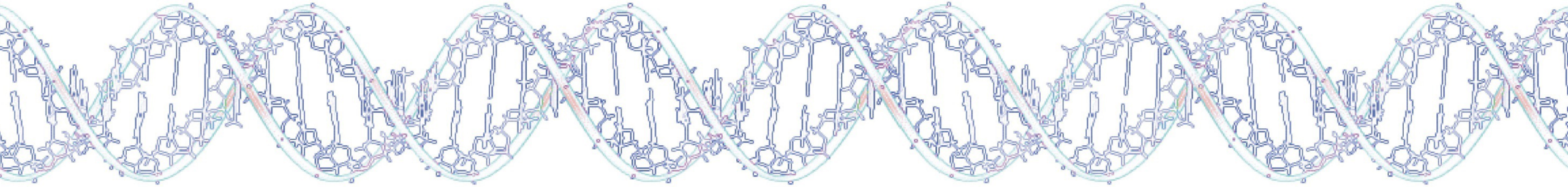
No damage

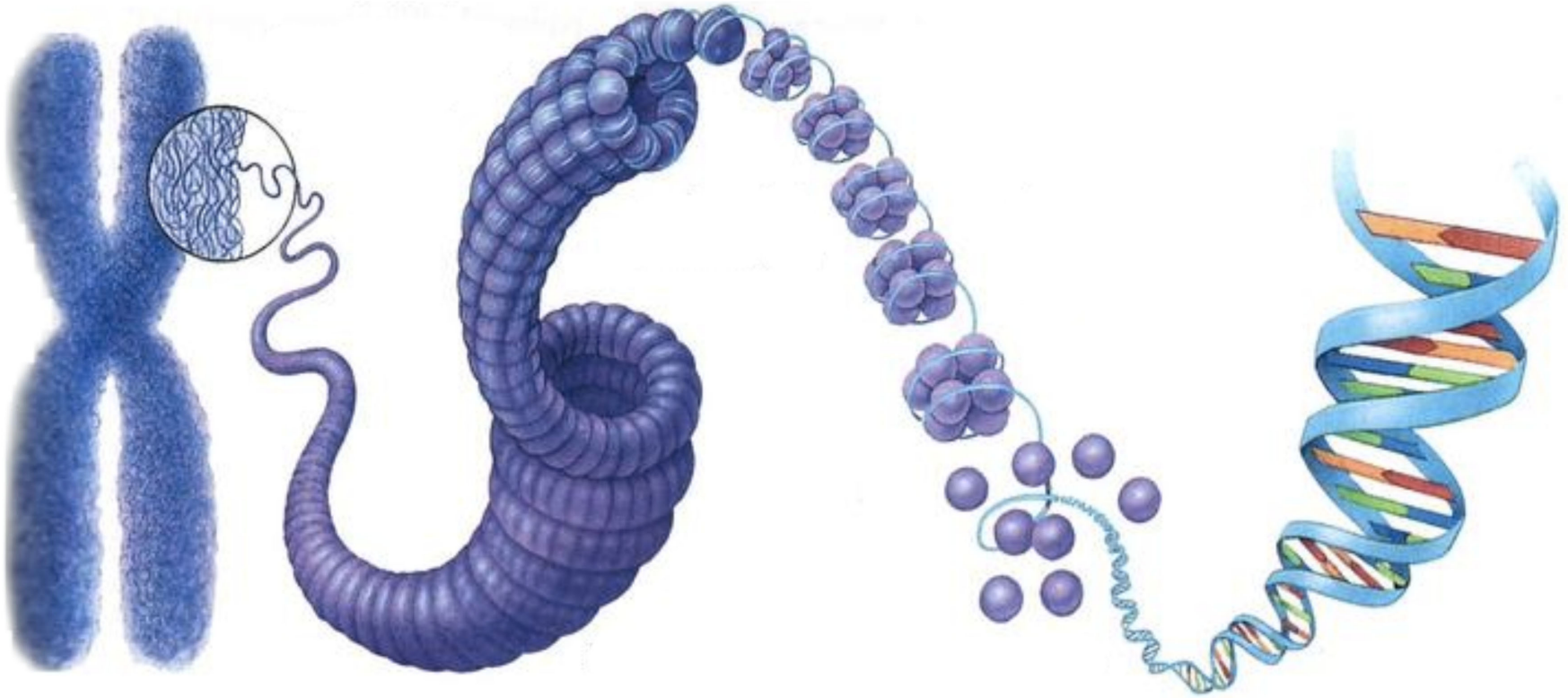
DNA is highly supercoiled, doesn't migrate in a gel

High damage

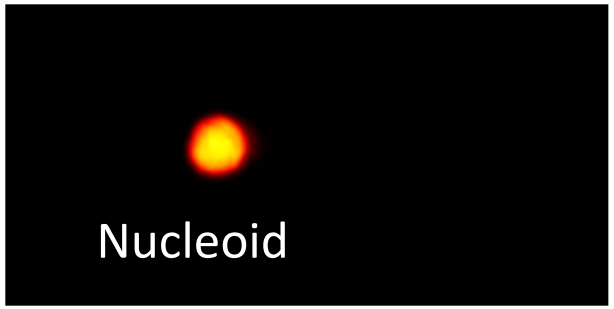
Damaged DNA migrates during electrophoresis

The Genome is Immense – Need to Fit DNA into a Nucleus

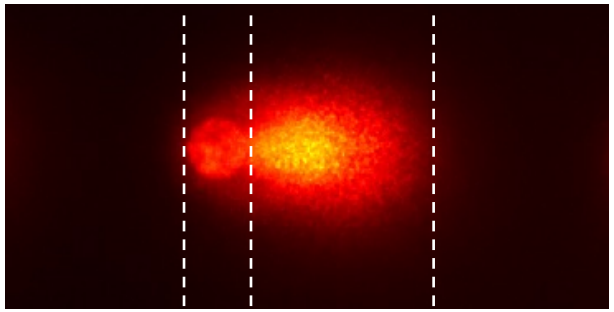




No Damage

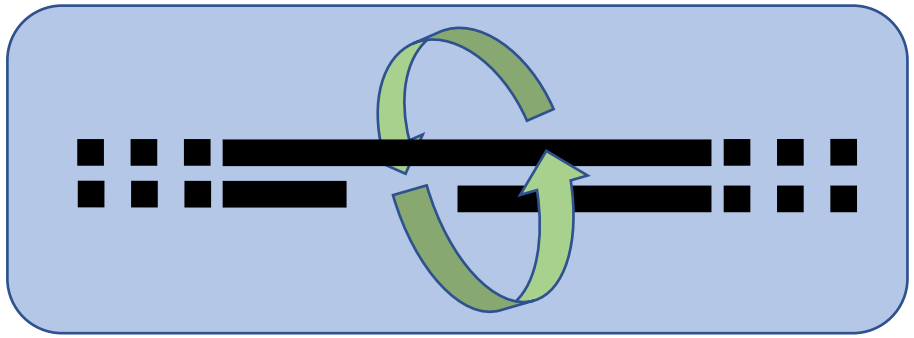


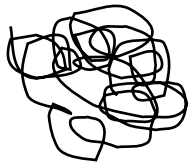
High Damage



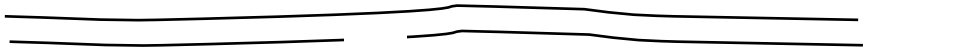
Head Tail

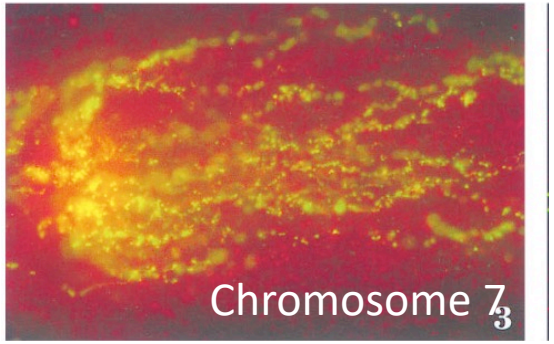
Electrophoretic Migration
Reveals DNA Strand-Breaks





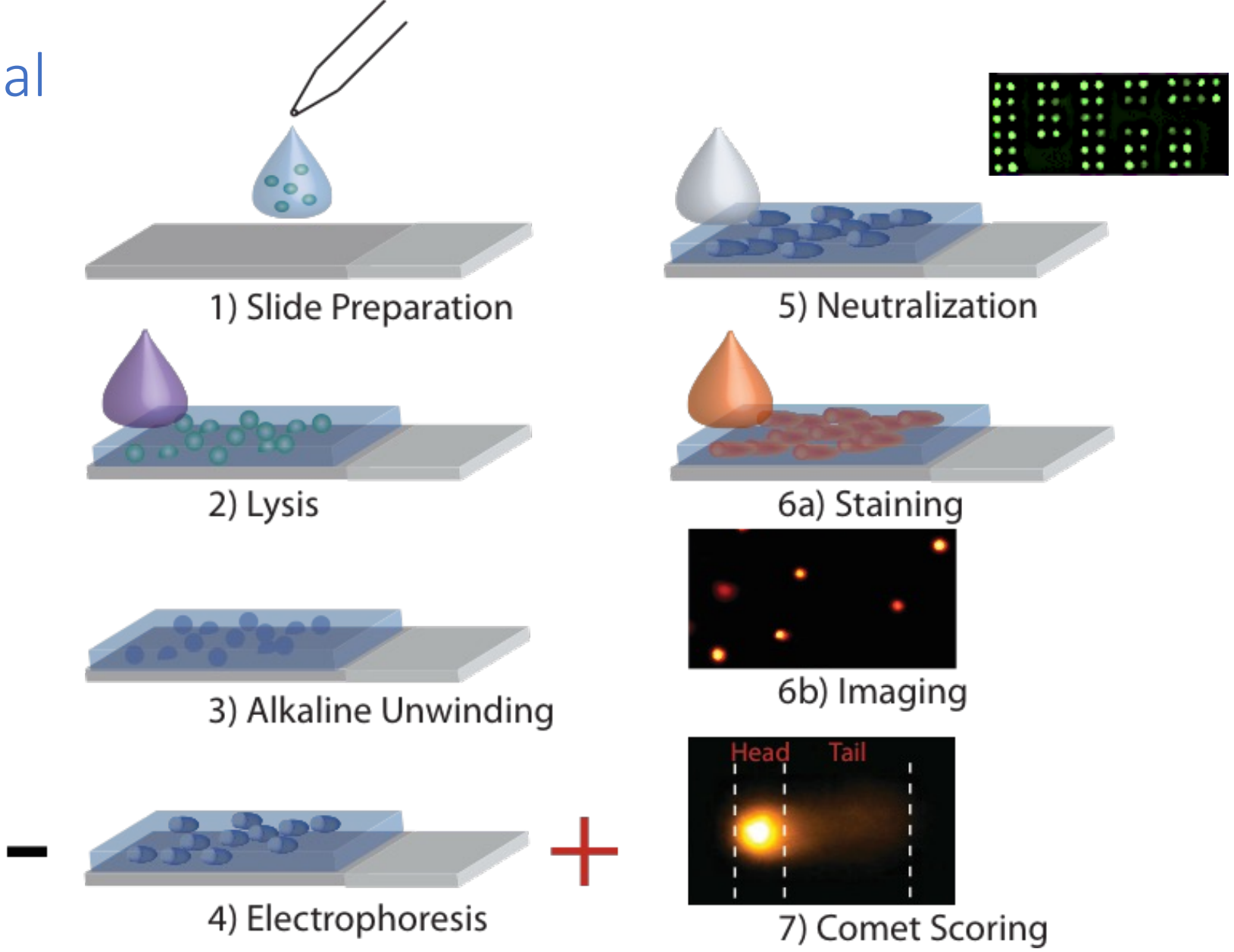
Single Strand Break





Santos, Singh and Natarajan, Experimental Cancer Research 232, 411 (1997)

The Traditional Comet Assay



The Traditional Comet Assay was Developed by Ostling & Johanson, and Singh in 1980s

Problems with noise in the system
(slide-to-slide variation)

Inconsistency among experiments

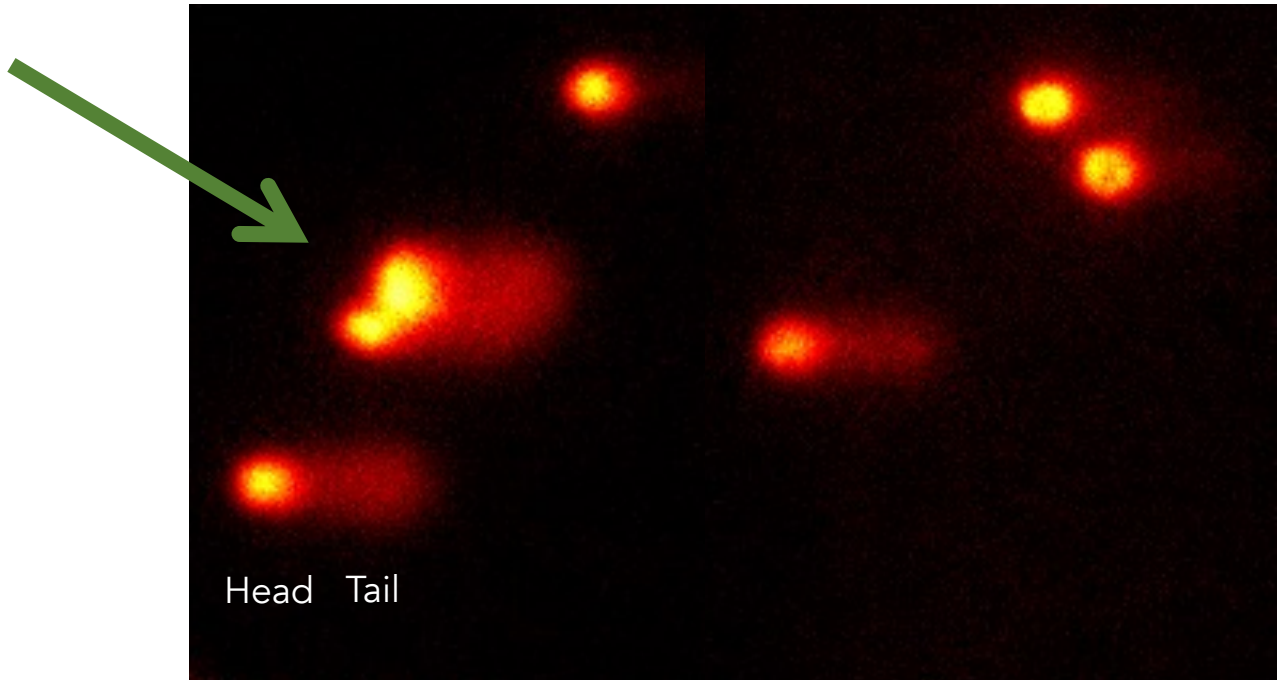
Inconsistency among people

Inconsistency among laboratories

Overlapping comets

Overlapping Comets

Overlapping Comets



Noisy Data

Bias

Rationale for Creating the CometChip

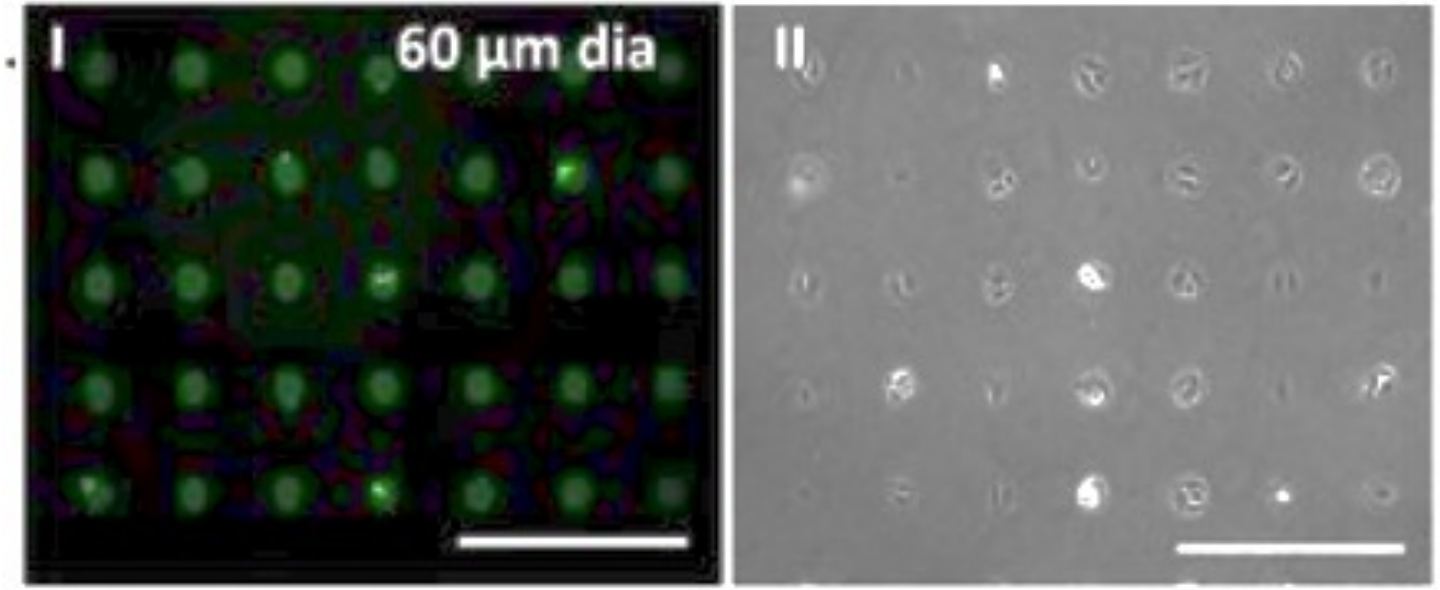
How the Comet Assay Works

Development of the CometChip

Why Commercialize?

Lots and lots of failed attempts….

Collagen Islands



Patterning of C2C12 myoblasts on FITC-conjugated collagen I islands. Protein was printed using a stamp of 60 um diameter fabricated with soft lithography

Low Cost and Lithography-free Stamp fabrication for Microcontact Printing

Akshada J. Khadpekar, Moin Khan, Abhishek Sose & Abhijit Majumder

Microfluidics

Advice:

Don’t give up if your premise is strong.

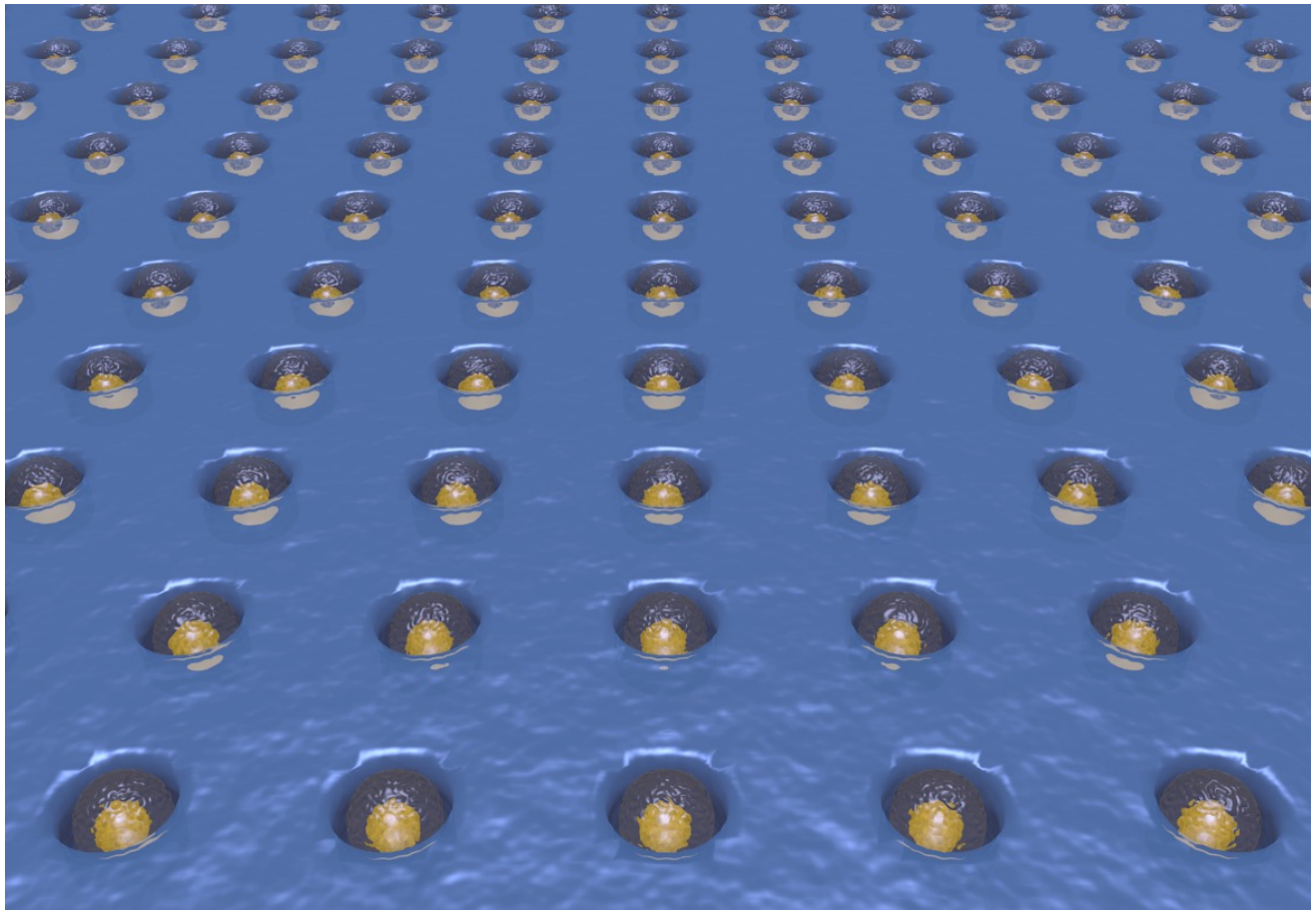
CometChip Team

David Weingeist, David Wood, Jing Ge and Sangeeta Bhatia

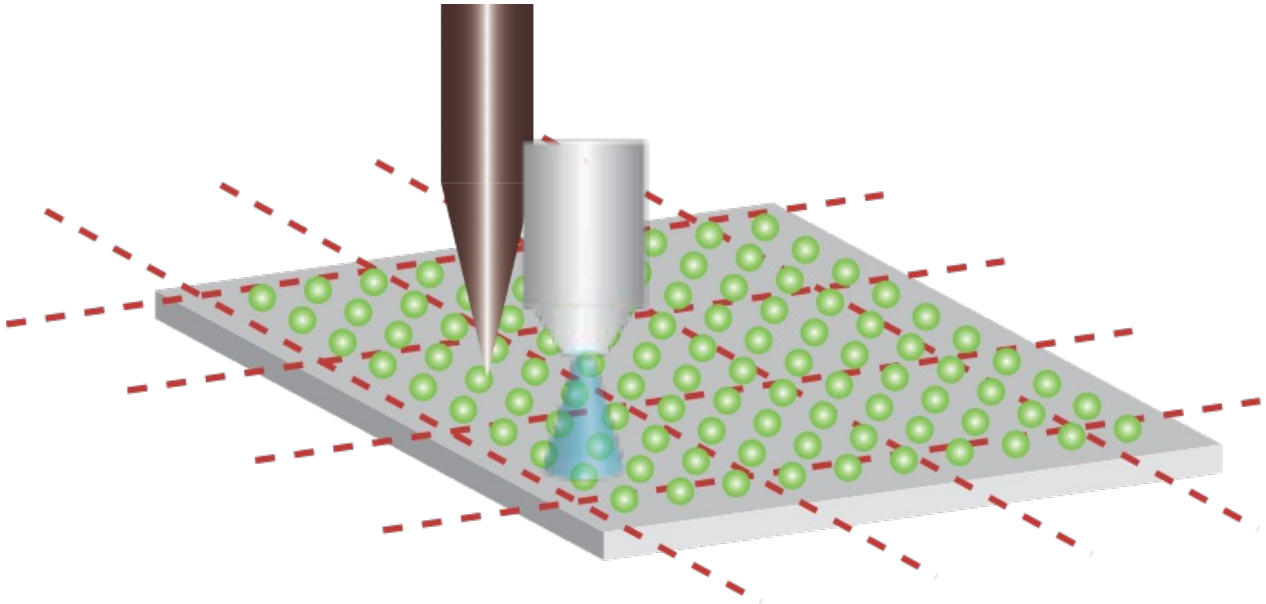
Jing Ge

Patented and Licensed to Trevigen

New Concept: Cell Trapping



From Comet to CometChip



Spatial encoding

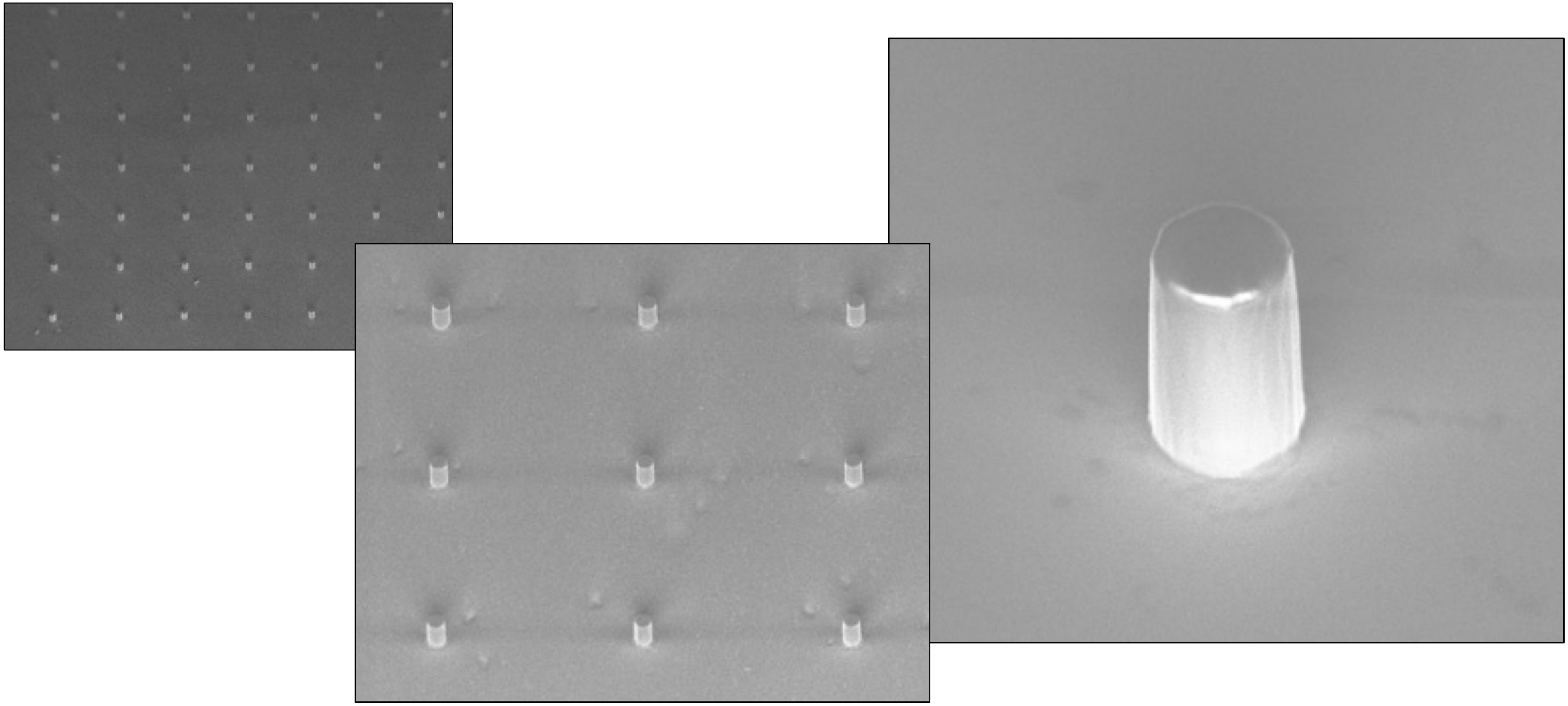
Real estate maximization

Automated imaging

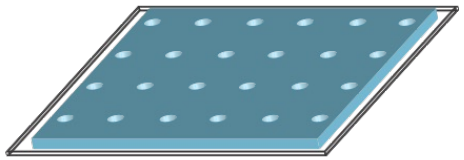
Automated analysis

Scalable to 96 well plate

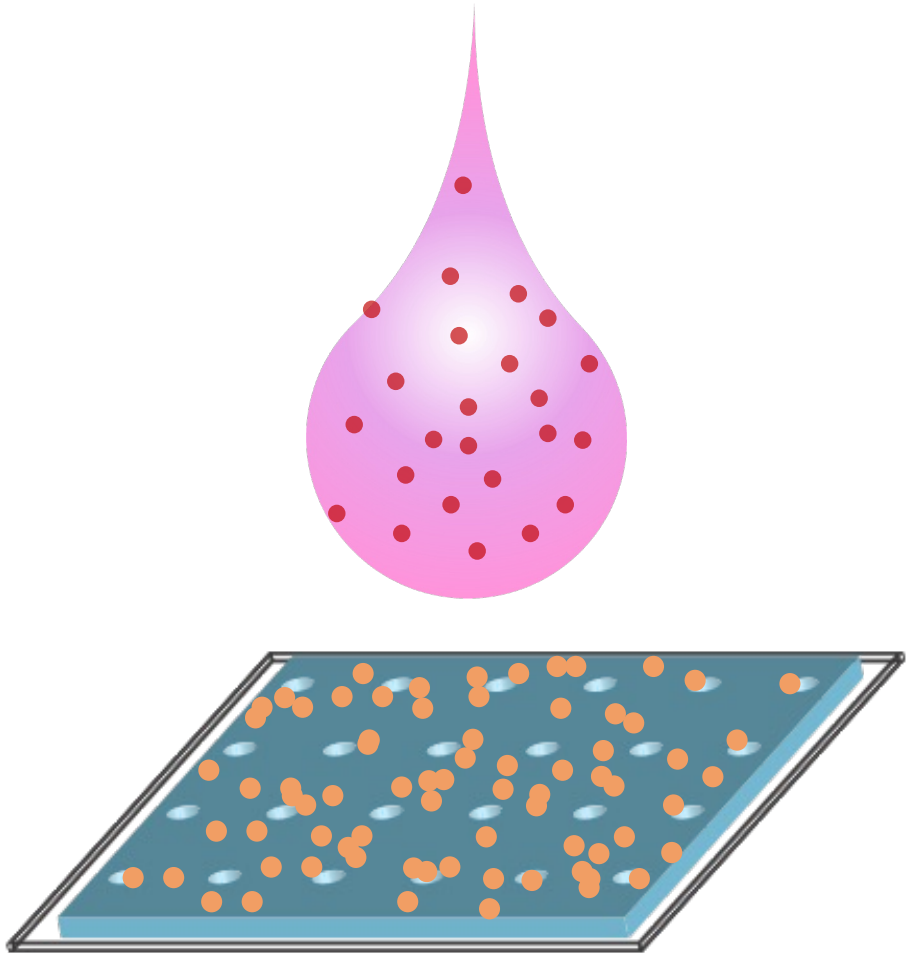
Use of Photolithography to Create Microposts



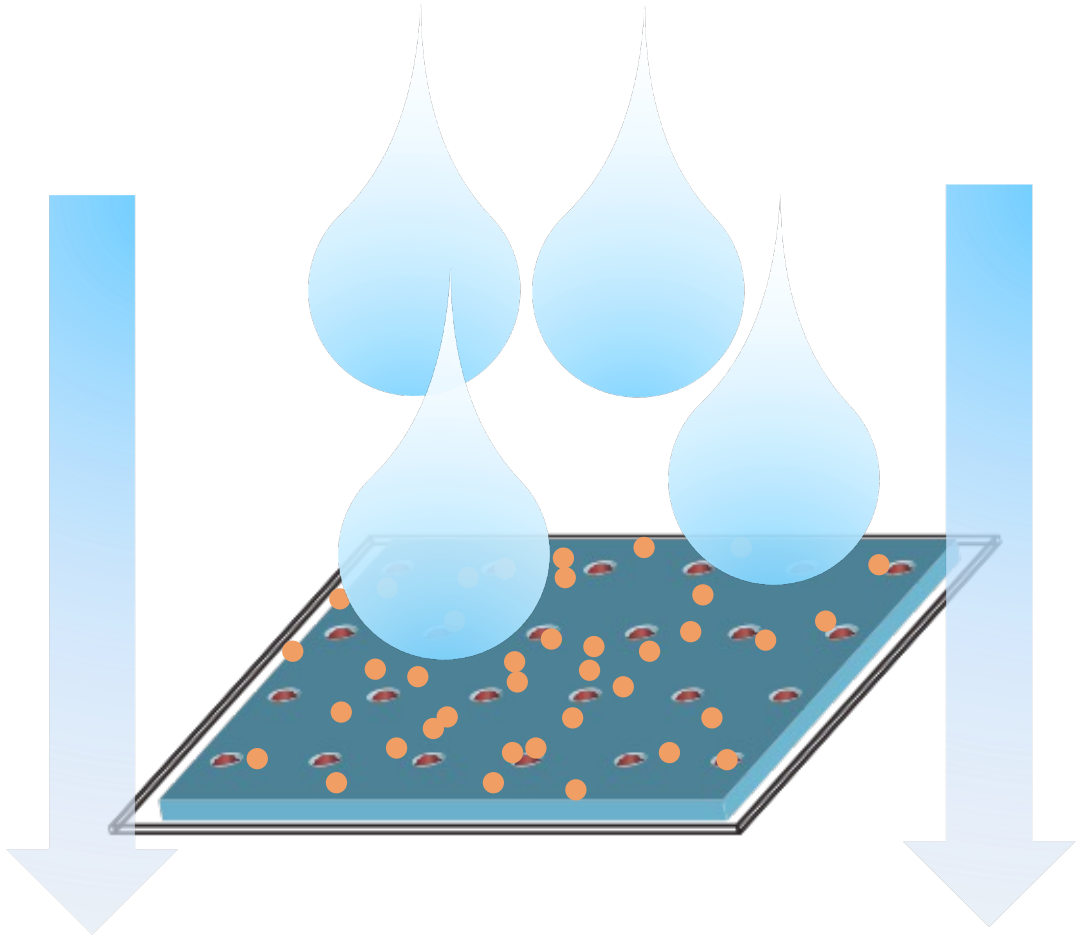
The CometChip



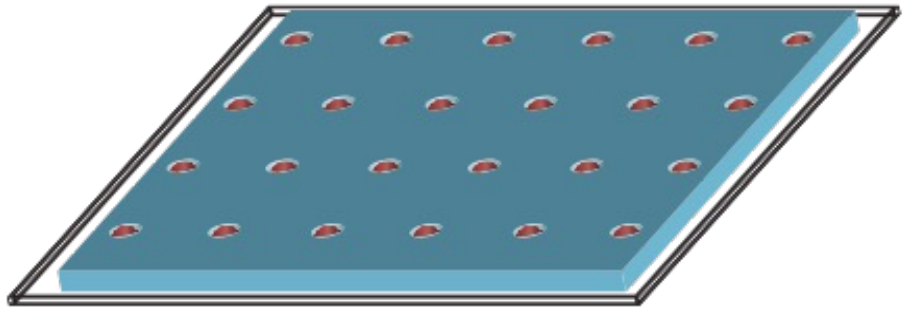
The CometChip



The CometChip

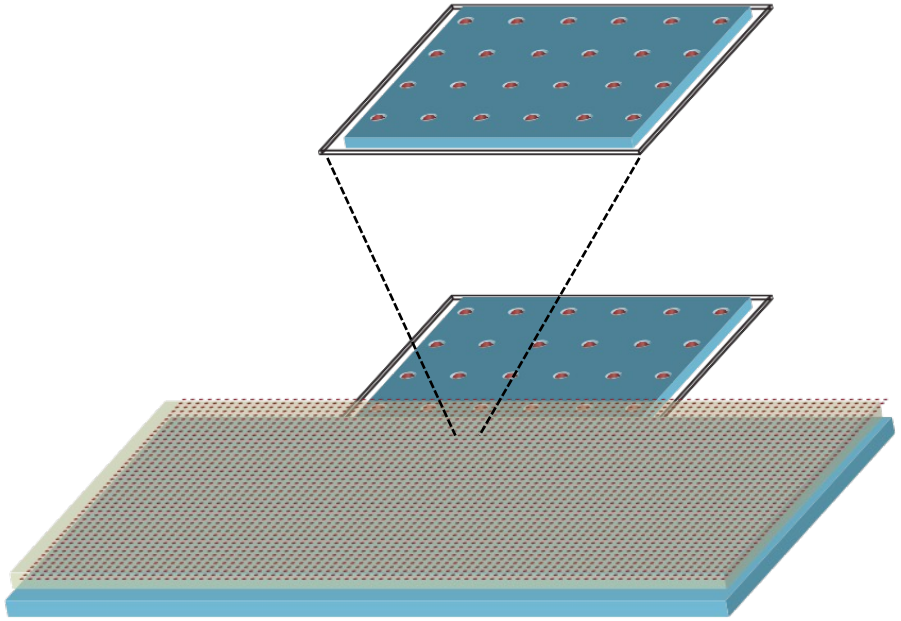


The CometChip

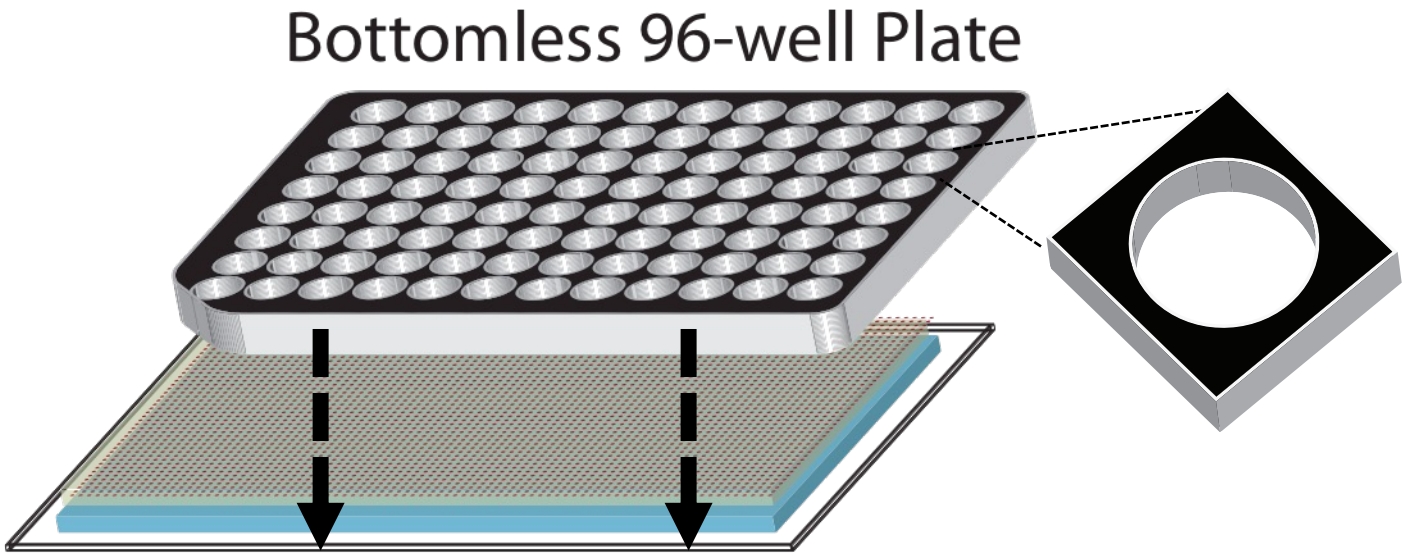


Patterned Cells

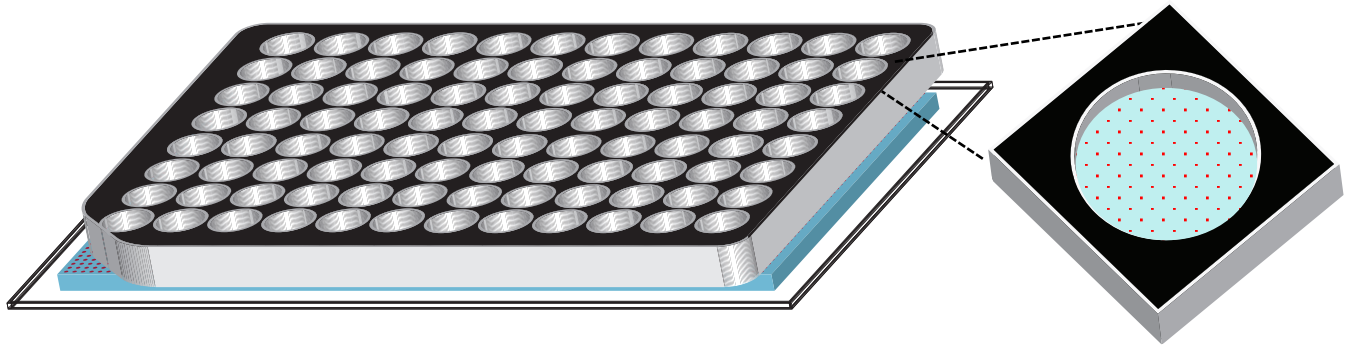
The CometChip



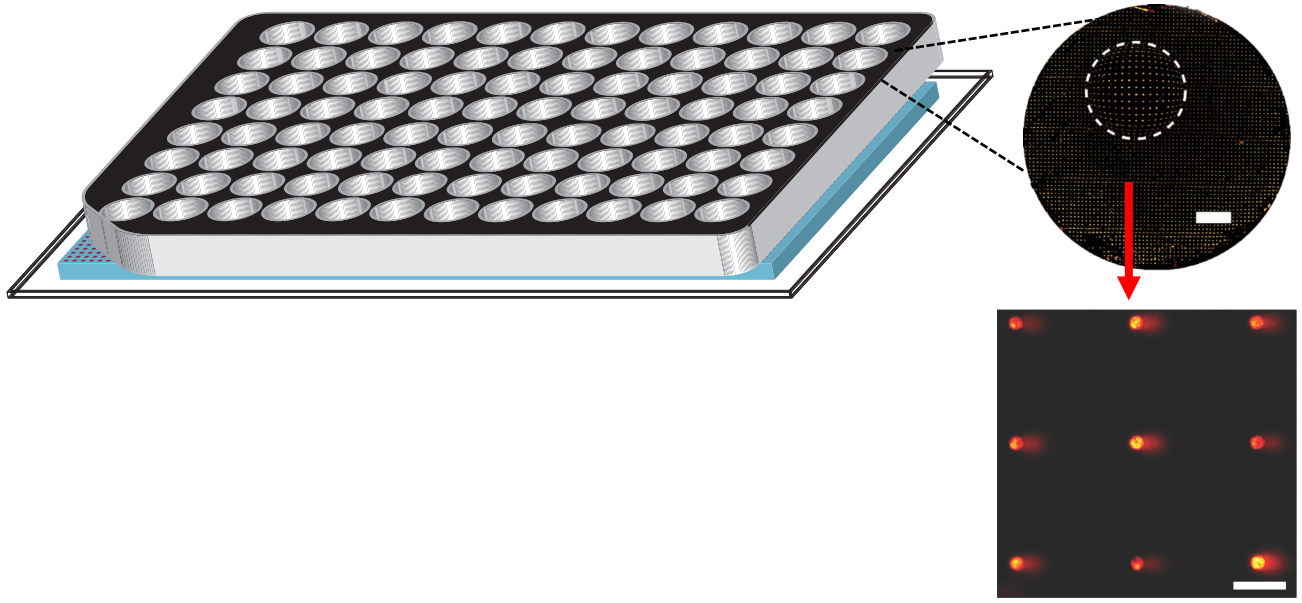
The CometChip



The CometChip

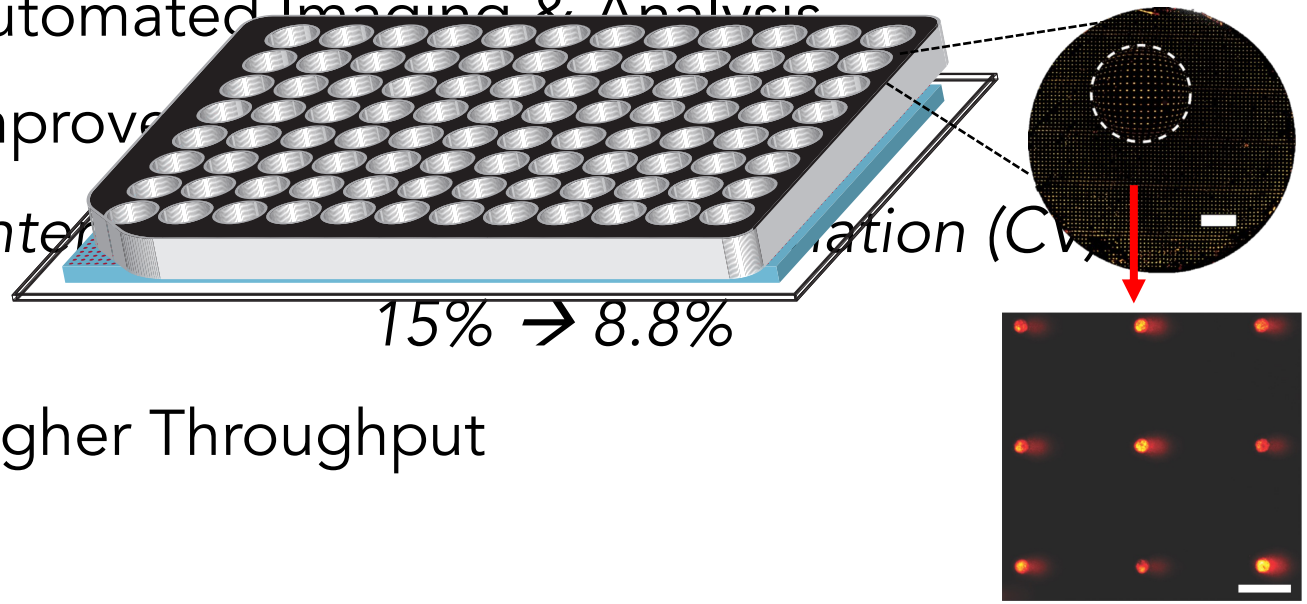


The CometChip

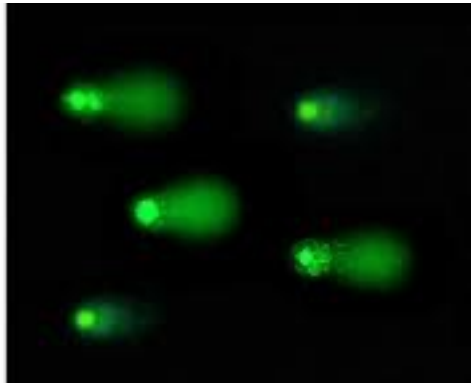


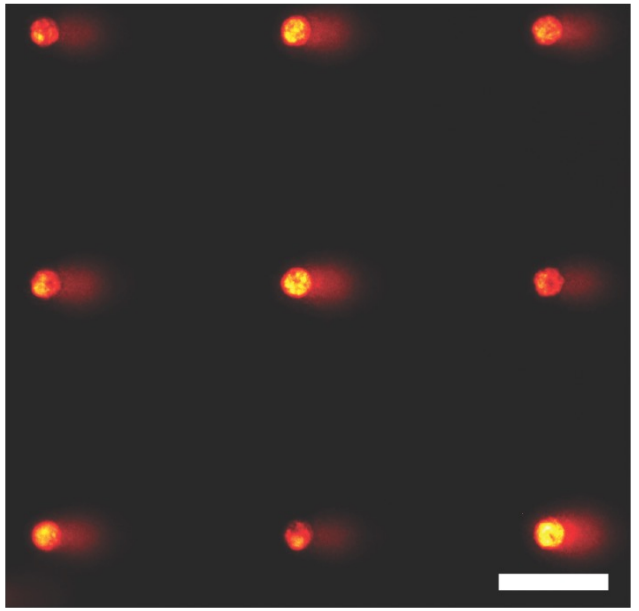
The CometChip

- Comparable sensitivity
- Automated Imaging & Analysis
- Improve
  Inter ation (CV)
  15% → 8.8%
- Higher Throughput



The CometChip





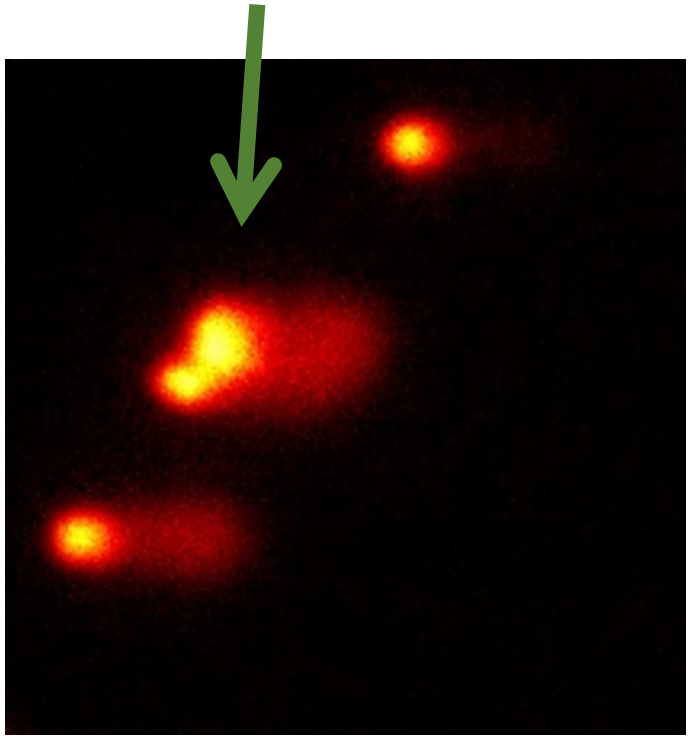
Round versus Square Wells

Round versus Square Wells

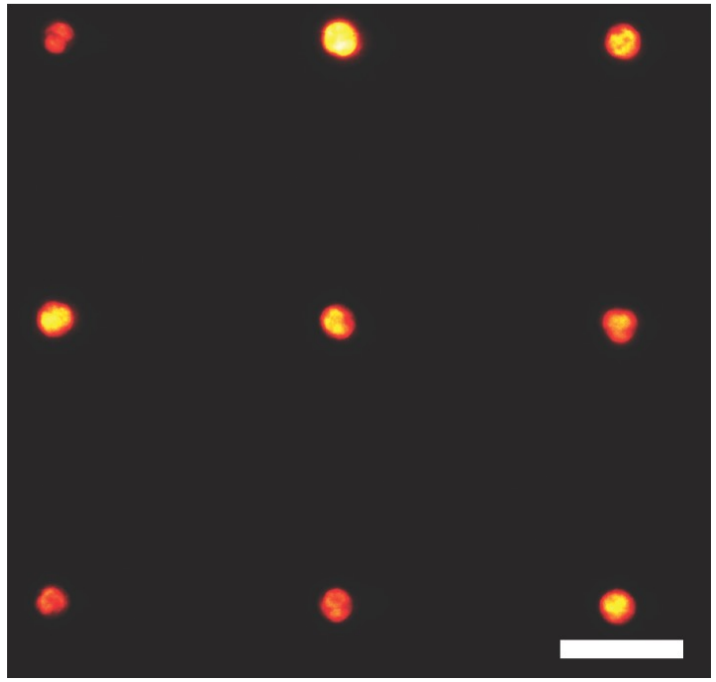
Does this really matter?

Advice: Know your Audience

Overlapping Comets

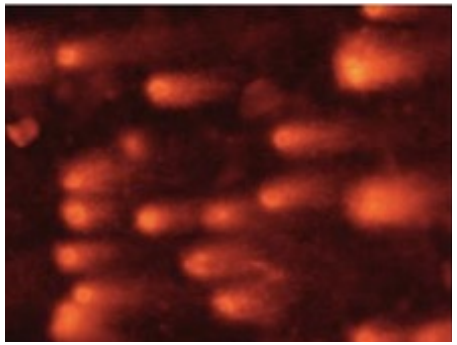


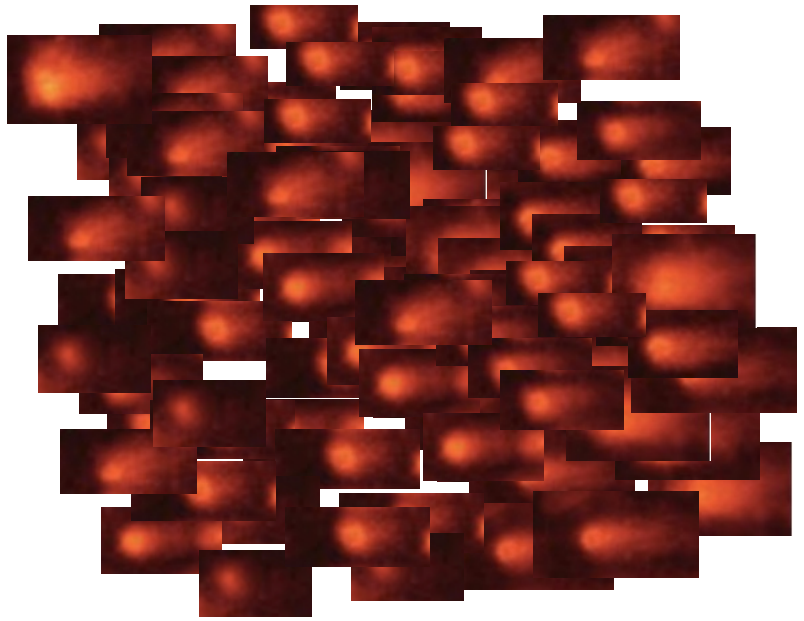
Reduces Overlapping Comets



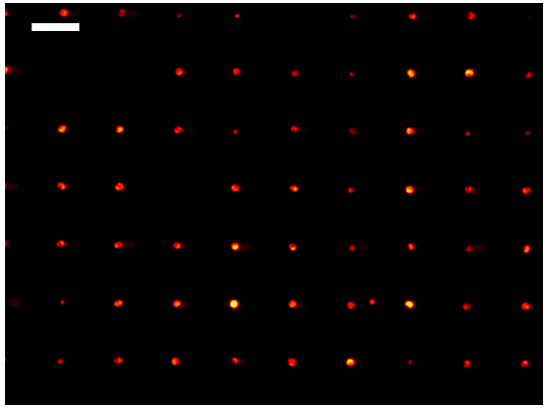
CometChip: Reduce Noise & Automate Analysis

Traditional Comet Imaging
Image one comet at a time, 100X





CometChip Imaging



1-2 images

Smaller Real Estate plus
Shared Focal Plane
= 100X Faster Imaging

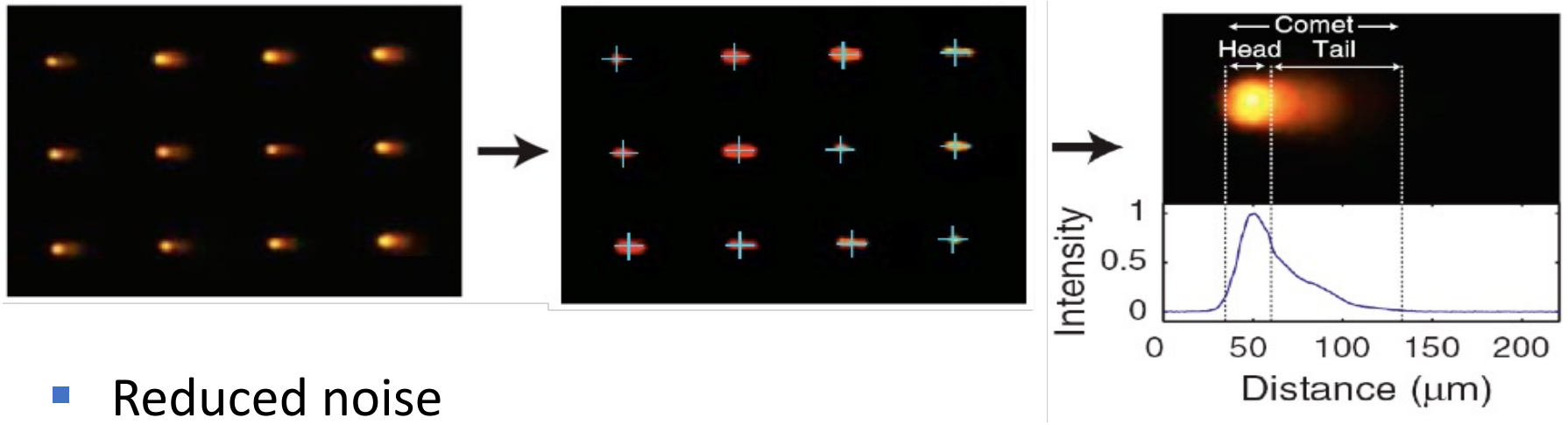
Automated Analysis =
Reduced Labor/Time
Reduced Noise

Automated Image Analysis

Automated Imaging

Comet Identification

Individual Comet Analysis



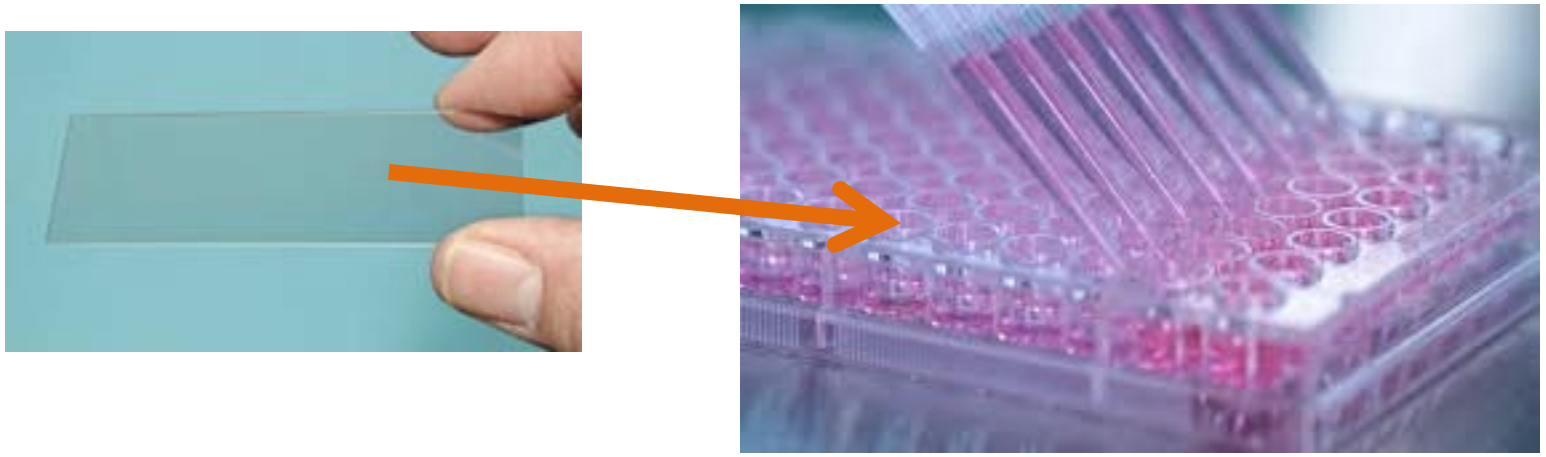
- Reduced noise
- Instant and unbiased analysis
- Lower inter-experimental variation
- Minimal analysis time required

Advice:

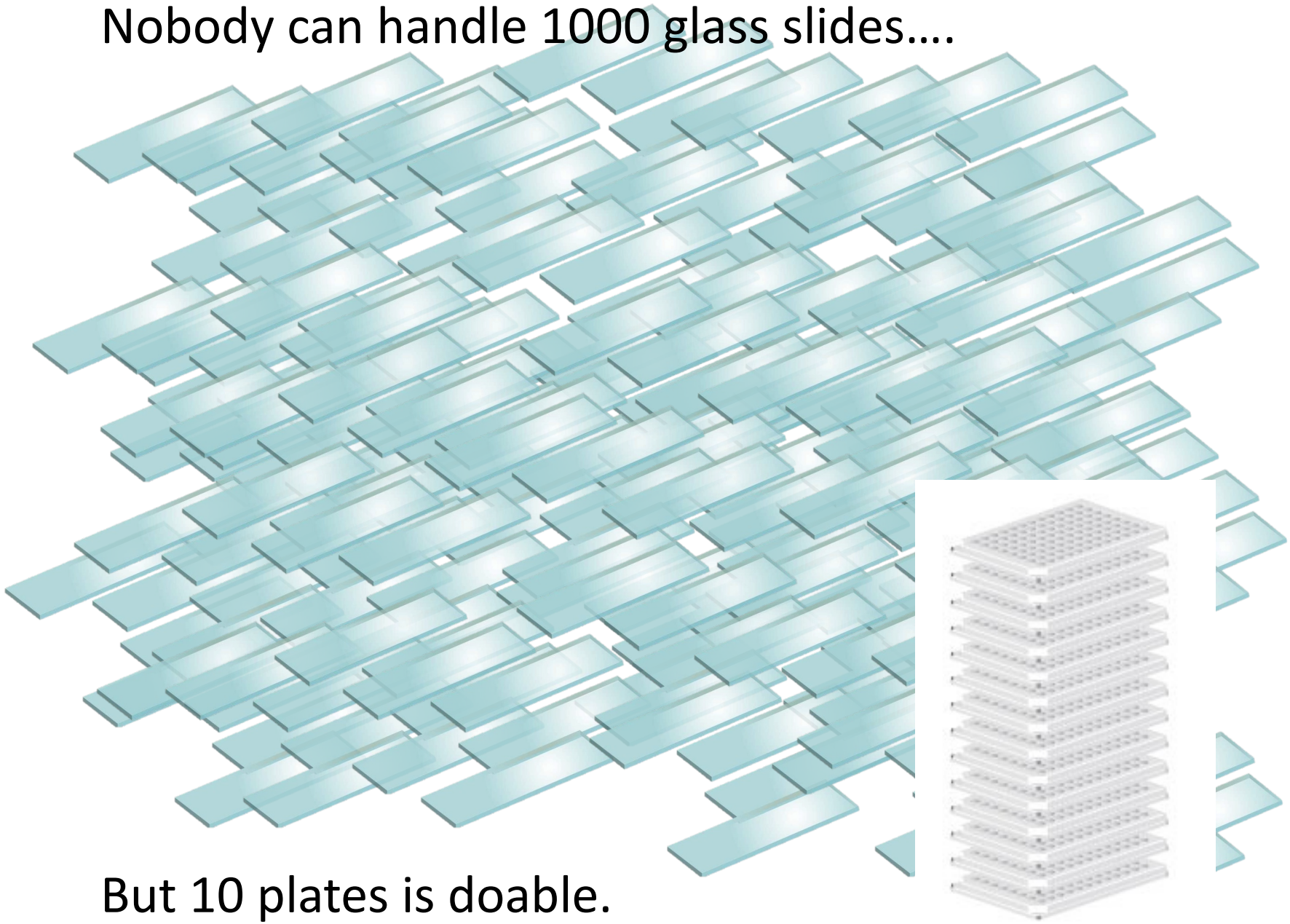
It takes a multitude of skills to generate solutions to complex problems.

Learn to communicate with and work with peers in different fields.

Better Use of Real Estate:
One Slide to One 96 Well (Macrowell)



Nobody can handle 1000 glass slides….



But 10 plates is doable.

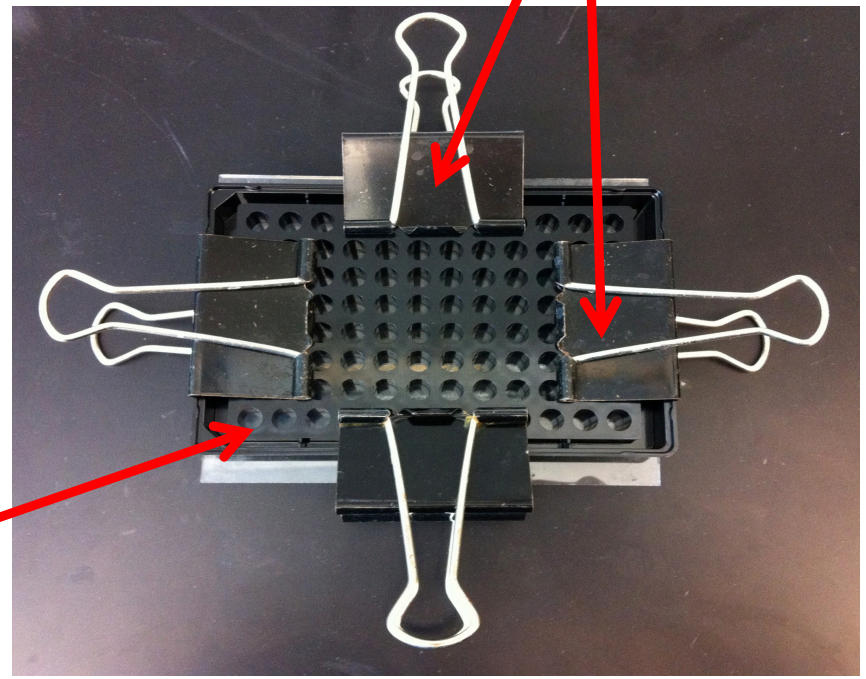
Concept:

Simple advances can be powerful.

~8,000 papers have been published in "Lab on a Chip"

CometChip

Binder clips provide clamping force



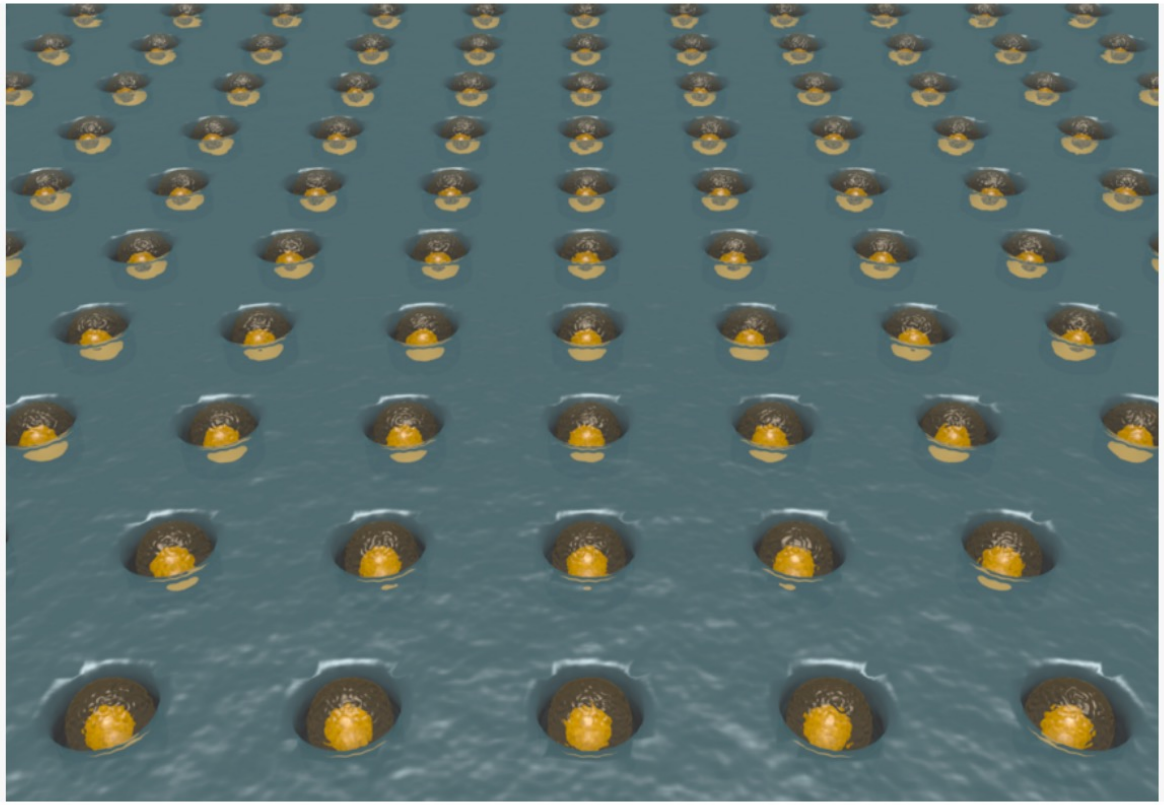
Bottomless 96well plate

MIT News
ON CAMPUS AND AROUND THE WORLD

Rapid analysis of DNA damage now possible

Technology offers a new way to test potential cancer drugs, detect effects of hazardous agents in our environment.

Anne Trafton, MIT News Office



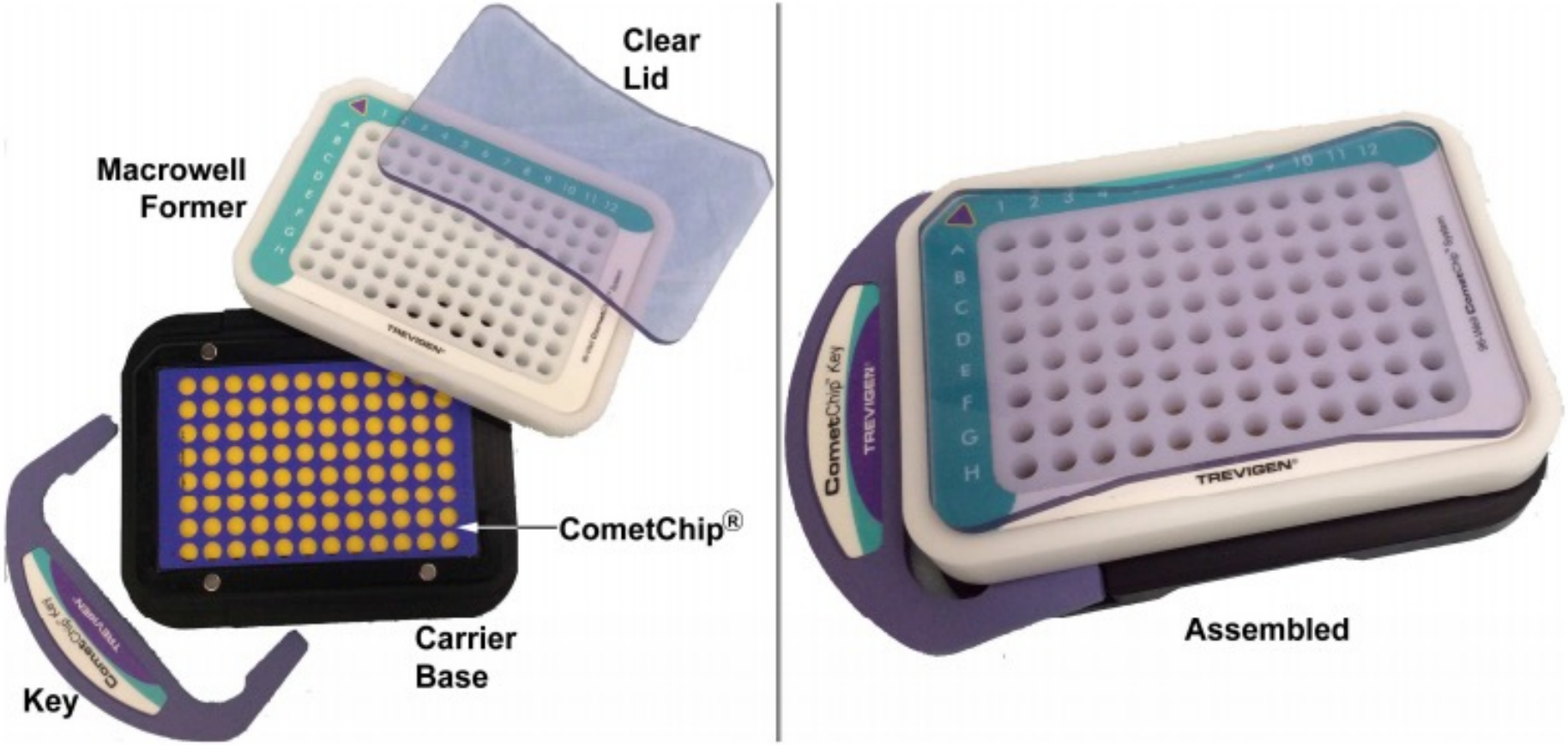
Rationale for Creating the CometChip

How the Comet Assay Works

Development of the CometChip

Why Commercialize?

How to set a Price Point for CometChip



Concept:

Price point needs to be low enough to be popular and high enough to cover costs.

96-Well CometChip System $1250

Catalog # 4260-096-CS

Catalog #	Availability	Size / Price	Qty
4260-096-CS	In Stock	96 Tests / $1250.00	0

Bulk Order | Add To Cart

Key Product Details

Features: A reusable cassette consisting of a carrier base, a macrowell former, a lid and a key. When the CometChip is inserted into the magnetically sealable cassette, 96 separate macrowells are created on the CometChip.

CometChip Kit $350

Catalog # 4260-096-K Citations (3)

Catalog #	Availability	Size / Price	Qty
4260-096-K	In Stock	96 Tests / $350.00	0

Bulk Order | Add To Cart

Key Product Details

Features: Includes one CometChip and reagents to test 96 samples.

Key Benefits:

- CometChip for single cell capture – no overlapping comets
- Ready to use low melt agarose (LMAgarose) in convenient size
- Lysis Solution suitable for either alkaline or neutral comet assay
- Optimized electrophoresis conditions

Concept:

Time Really is Money

Cost for a Graduate Student: _____

One experiment saving 2 days = $783

10 experiments = $7830

Can process 3X more samples = ~$23,000

There is no point to a patent unless it is cost effective.

You Need to Know Your Market

There is no point to a patent unless someone uses it.

Have a plan:
Either a new company or a company that wants to license the technology

Concept:

Patents are not just about making money.

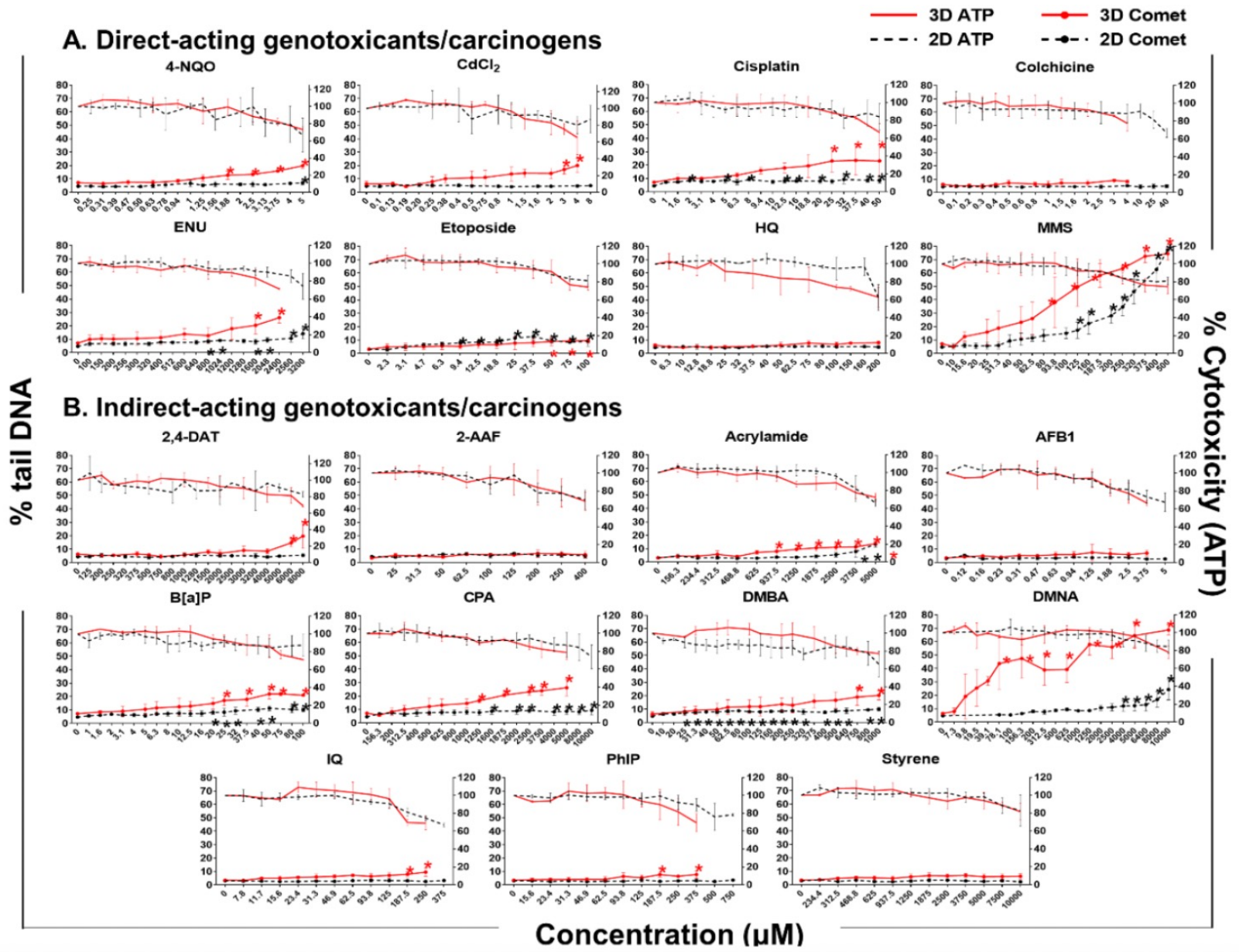
Companies need to profit from their work and so they won’t produce something that others can take freely.

Concept:

Profits from Patents
are not what you think they are.

19 Chemicals
20 Doses Each
2 Conditions (2D Black
3D Red)

3 Repeats



Work from the laboratory of Carole (Xiaoqing) Guo

CometChip is being used by the
National Center for Toxicological Research
to test for Chemical Safety

Rationale for Creating the CometChip

How the Comet Assay Works

Development of the CometChip

Why Commercialize?