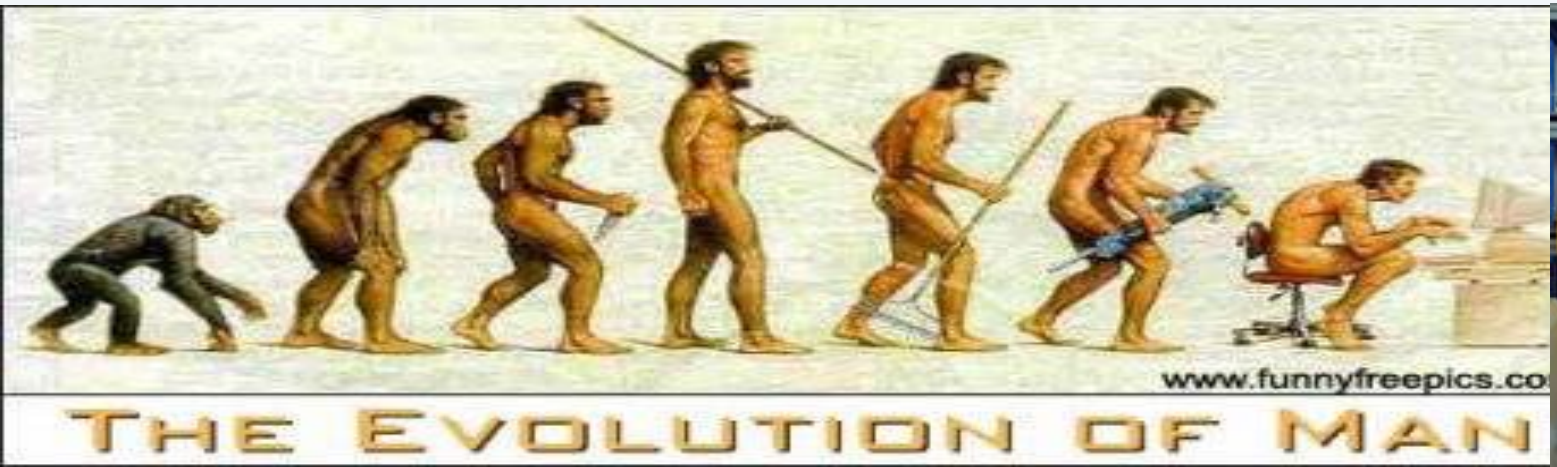
The Evolution of Automation for Pharmaceutical Lead Discovery

Andy Pope, Platform Technology & Science, GlaxoSmithKline

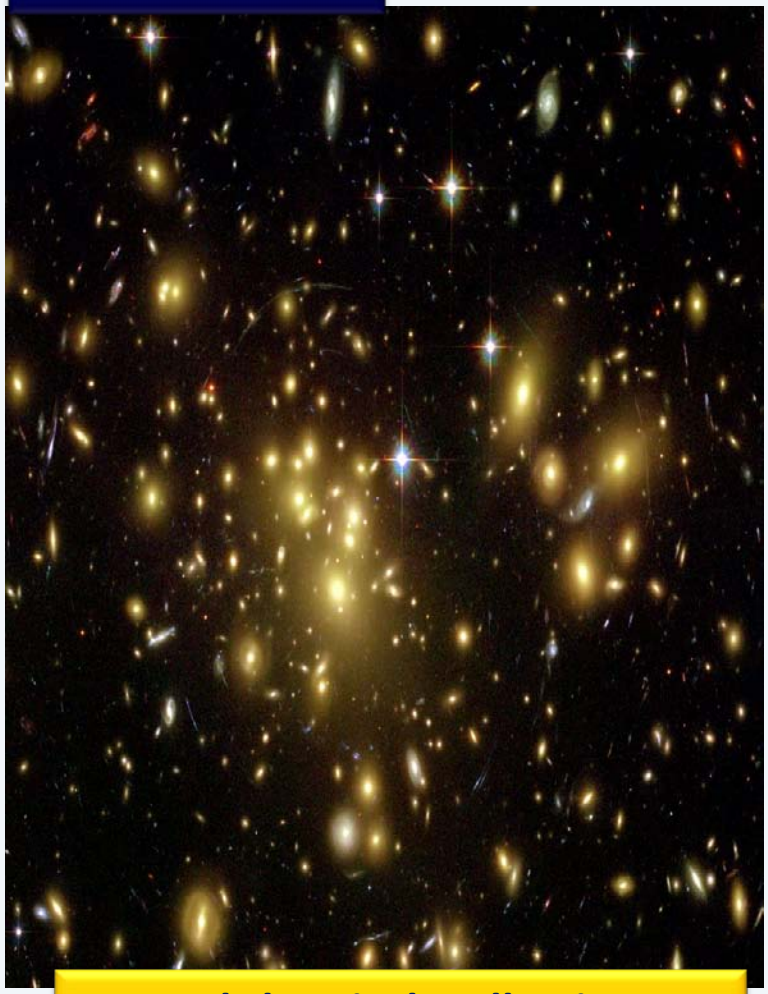
Some themes

- The role of Hit identification/cpd profiling in Pharma R&D
- The physical tasks required
- How automation is used to perform those tasks
- Process/technology evolution in the last decade
- How have our thinking, emphasis and capabilities evolved?
- Prospects for the future



Screening: a central component of drug discovery

Potential drugs



Curated chemical Collection
- Sampling drug-like space

New medicine

Safety and efficacy

Drug candidate

Medicinal chemistry

Validated drug target & lead molecule

Medicinal chemistry

Selectivity, specificity, MoA assays
- validation of disease intervention concept

Biological screening assay

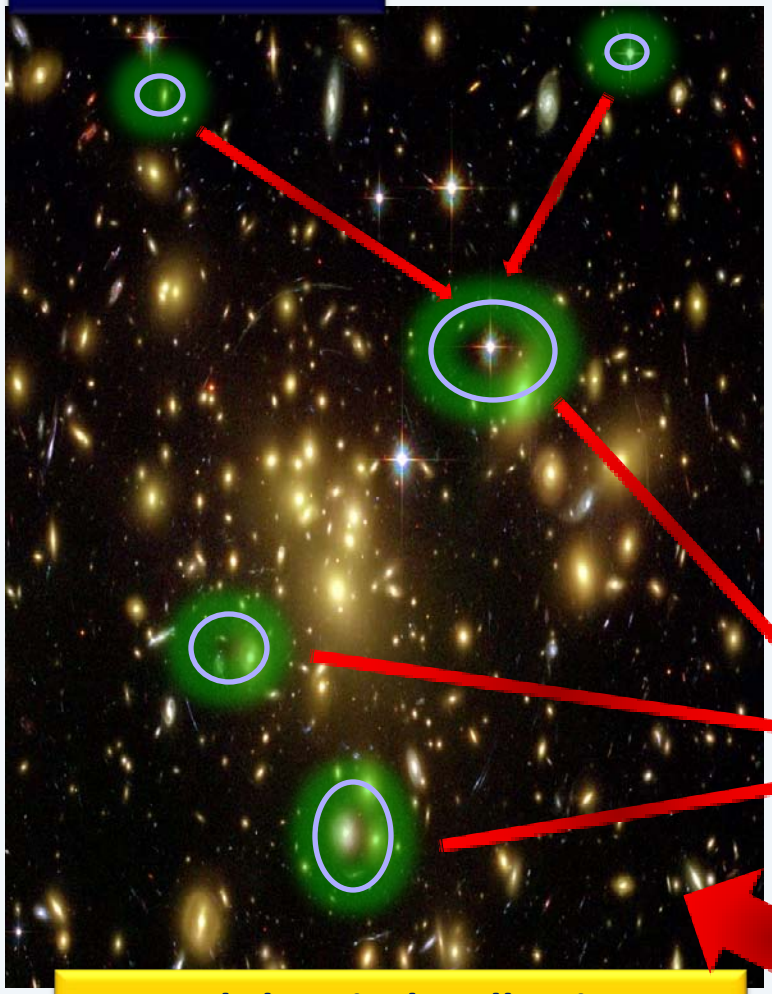
Potential targets

Disease modification hypothesis

Biological reagents (cells, proteins etc.)

Compound Screening: a central component of drug discovery

Potential drugs



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- *Sampling drug-like space*

Biological screening assay

Selectivity, specificity, MoA assays
- *validation of disease intervention concept*

Medicinal chemistry

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Potential targets

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Biological reagents (cells, proteins etc.)

Compound Screening in The Drug Discovery Process

Test compounds

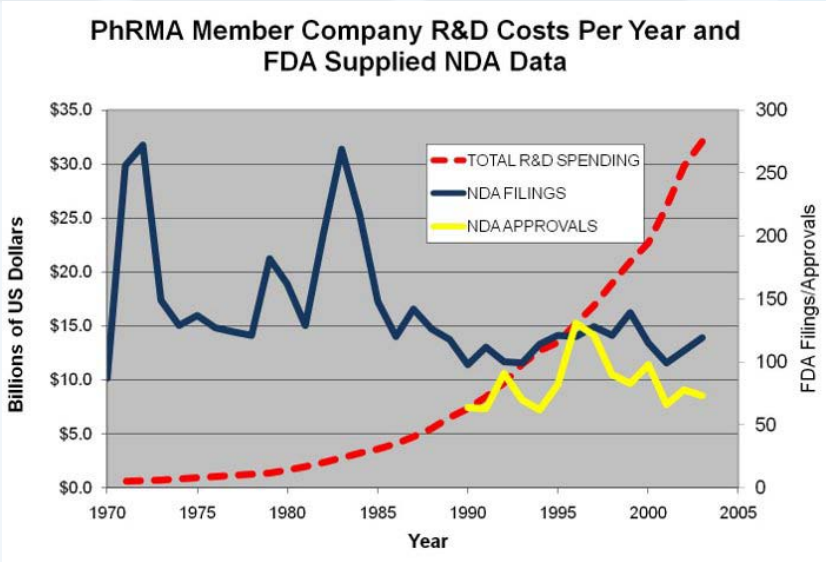
~10^6 ~10^6 ~1000's ~30 10's few

Drug targets

1000's 100's ~100 ~30 10's few

Target Identification & Validation → Lead Identification → Lead Optimization → Candidate selection to POC → Commit to medicine development → File and Launch

- Chemical Probe discovery
- Hit identification
- Compound optimization profiling
- Safety & attrition profiling
- Compound Mechanism of action
- Compound Characterization profiling



Overall ~15 years, ~1.5B dollars/NCE

~1 yr ~ 3 yrs

Compound Screening: The Physical process

- Liquid dispense

Cpd Stock Solution
(e.g. 1 mM DMSO)

Biological reagent
(e.g. purified enzyme)

Biological co-factor
(e.g. enzyme substrates

Detection reagents
(e.g. fluorescent)

~1/100th final vol.

~1/3rd final vol.

~1/3rd final vol.

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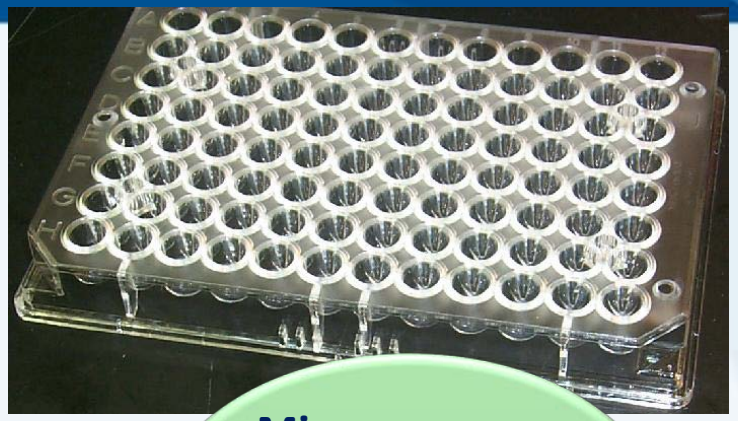
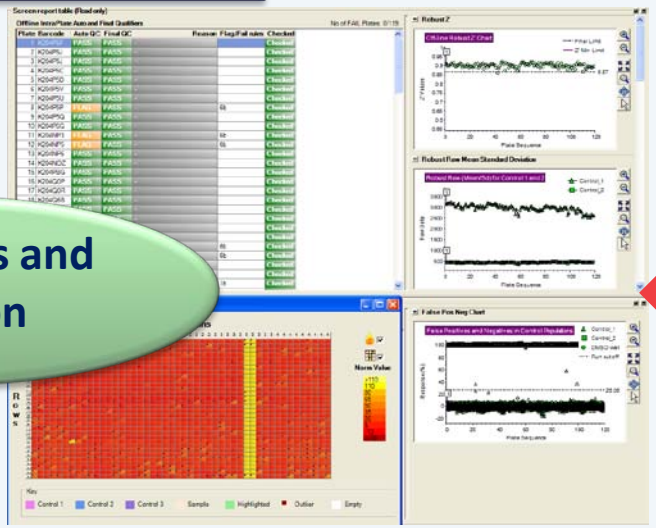


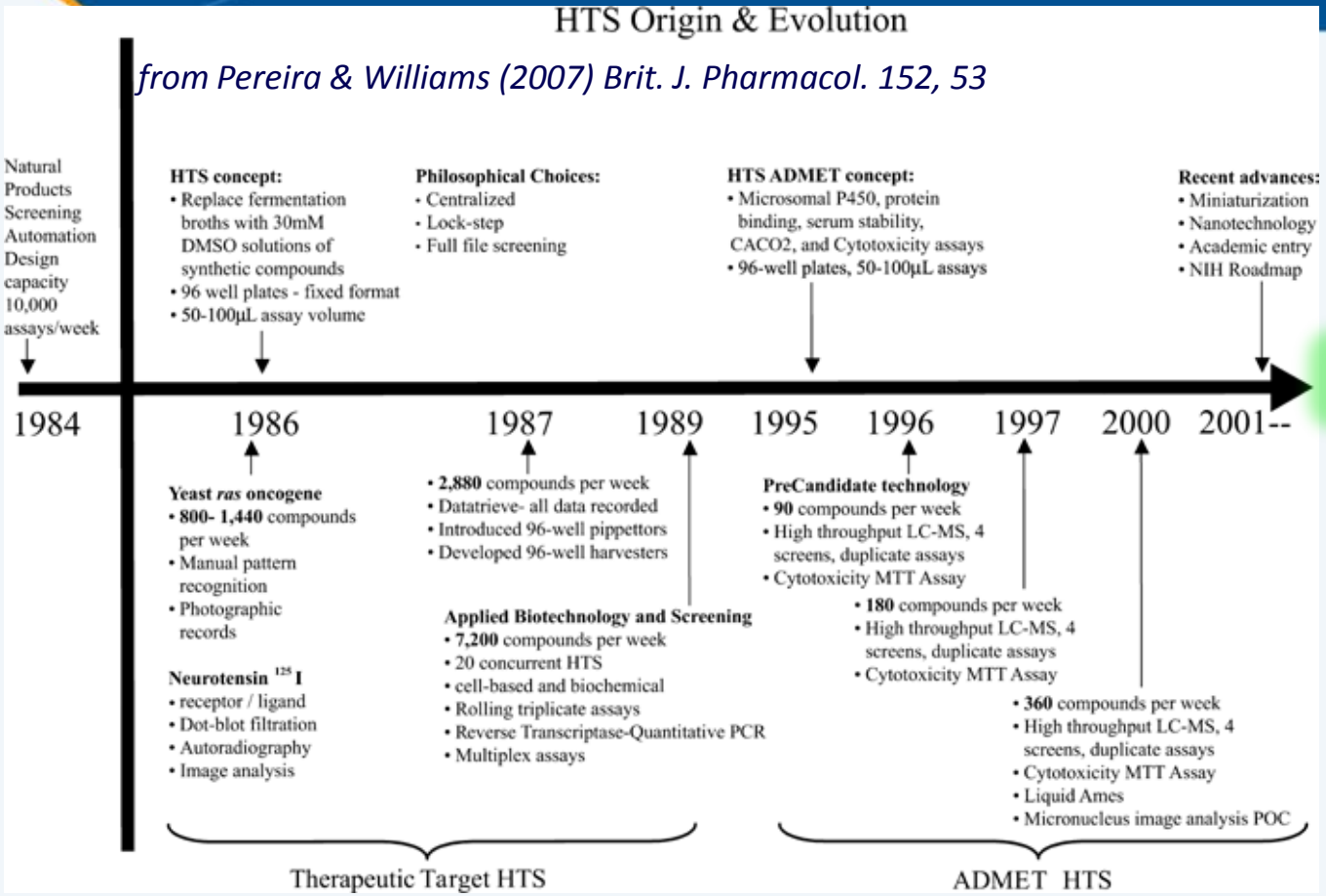
- Mix
- Incubate
- Centrifuge
- (not!) Separate



Data analysis and interpretation

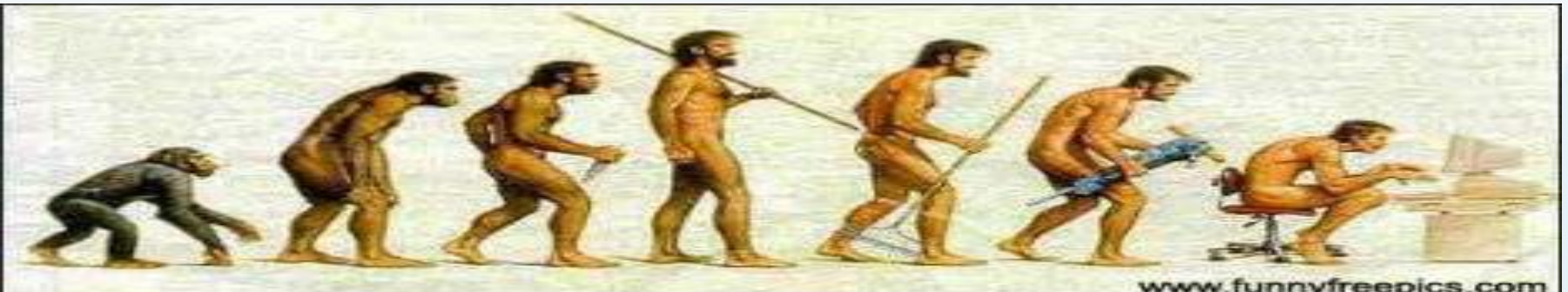
Signal detection
- Photon-based

The Origins of Compound Screening

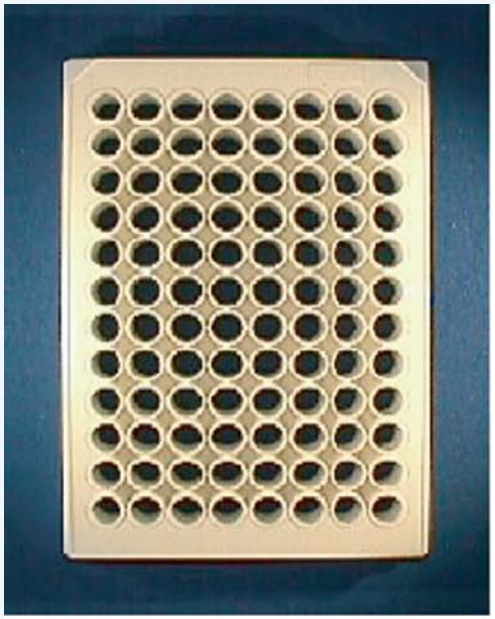


Today's focus

The "modern" era 2001-2010

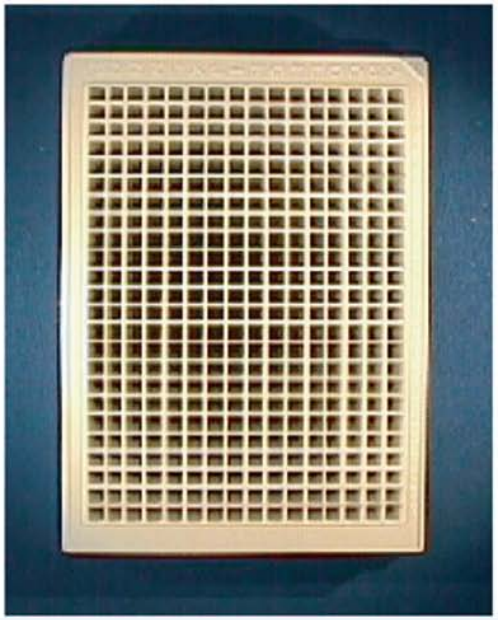


Another view – the microplate



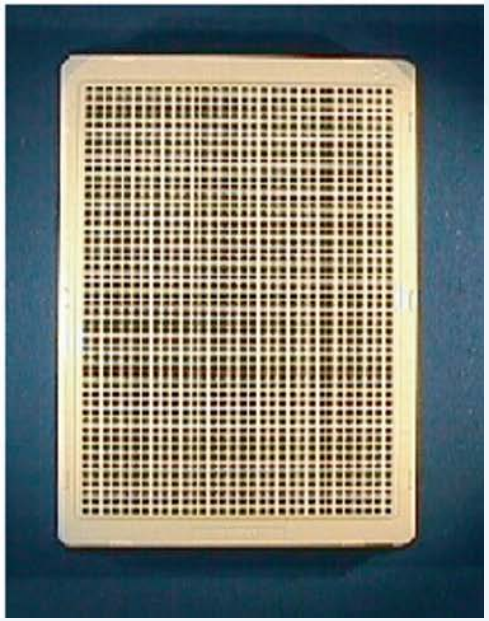
1980's

- 96-well plate
~100 uL volume



1990's

384-well plate
~ 50 uL volume



2000's

***1536*-well plate**
2-5 uL volume

Looking back on the last 20 years..

1990 – Screening (as opposed to design) becomes the method of choice to discover drug starting points.

1995 – Excitement builds around genome sequencing and combi-chem

2000 - Major investments to “industrialize” drug discovery

2005 - Major focus on time, cost, efficiency, quality (i.e. real data manufacturing)

2008 - Focus on “re-personalizing” drug discovery, solving drug attrition, maximizing success on difficult targets, “new” biology

2010 - Integration, flexibility and return on investment in a cost-contrained environment

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Pre-2000 flavor – “How do I get this to work?”

- Techniques for performing miniaturized homogeneous biological assays in microplates nascent
- Hardware for detection just emerging
- Reagent dispense in the sub uL volume emerging
- Compound dispense hardware for nL not commercially available – limiting assay miniaturization
- Automation systems relatively rudimentary, unreliable and limited by v. slow single motion devices
  - *corporate deals aimed yield unique capability and competitive advantage via large bespoke integration (e.g. Evotec, Aurora)*
- IT (and statistics) for handling large data volumes emerging

REVIEWS research focus

Homogeneous fluorescence readouts for miniaturized high-throughput screening: theory and practice

Andrew J. Pope, Ulrich M. Haupts and Keith J. Moore

JOURNAL OF BIOMOLECULAR SCREENING

Real Experiences of uHTS: A Prototypic 1536-Well Fluorescence Anisotropy-Based uHTS Screen and Application of Well-Level Quality Control Procedures

Single-Molecule Detection Technologies in Miniaturized High-Throughput Screening: Fluorescence Intensity Distribution Analysis

Drug Discovery Today: HTS supplement | reviews

Macroscopic versus microscopic fluorescence techniques in (ultra)-high-throughput screening

Ulrich Haupts, Martin Rüdiger and Andrew J. Pope

Typical Pre-2000 HTS Screening Lab

Compound store

Compound dispense

Screening robot

- **100-250K cpds, screened as mixtures**
  - *the only way to manage cost and throughput*
- **96/384-well plates only**
- **<1M assay data points per year**

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Year 2000 flavor - “industrialization of drug discovery”

Inspired by Human Genome and promise of combinatorial chemistry, vision was to solve R&D productivity via increases in scale and automation of drug discovery activities

- *Specialized facilities with large flexible lab spaces*
- *Infrastructure and manpower to manage compound logistics*
- *Large (impressive, expensive) Integrated Screening Robots*
- *Big budgets*

The coffee mug said;

“we will marry genes & chemistry to create a small molecule ligand for every potential drug target”

Typical High Throughput Screen Process

Primary Screen
(10 uM – singlicate)

Statistical separation from null effect population

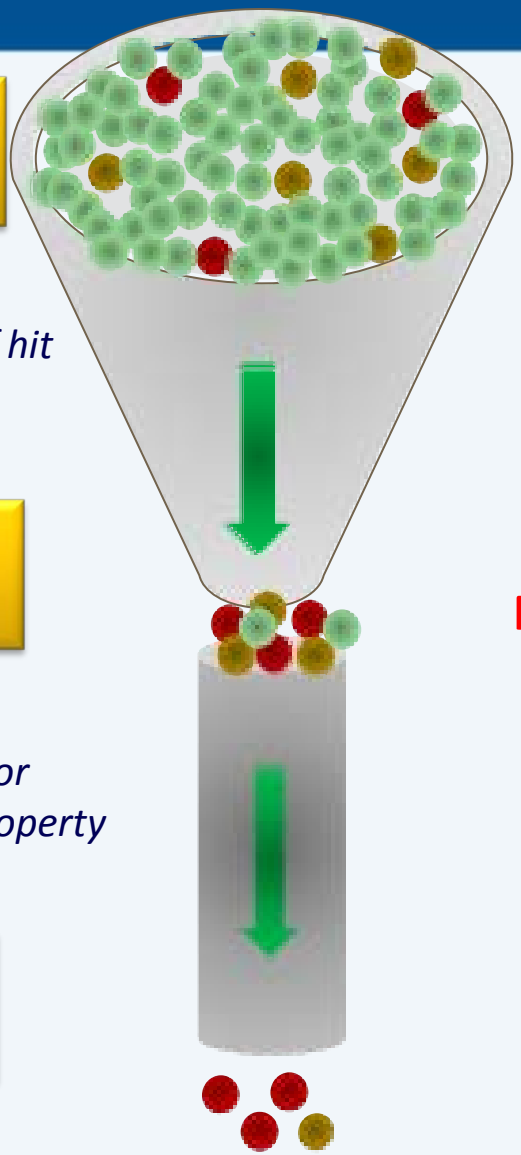
Chemical clustering if hit rate >1%

Confirmation
(10 uM – duplicate)

Eliminate false positives from primary

Chemical clustering for diversity property sampling

Dose response
(11 pt 3-fold dilution

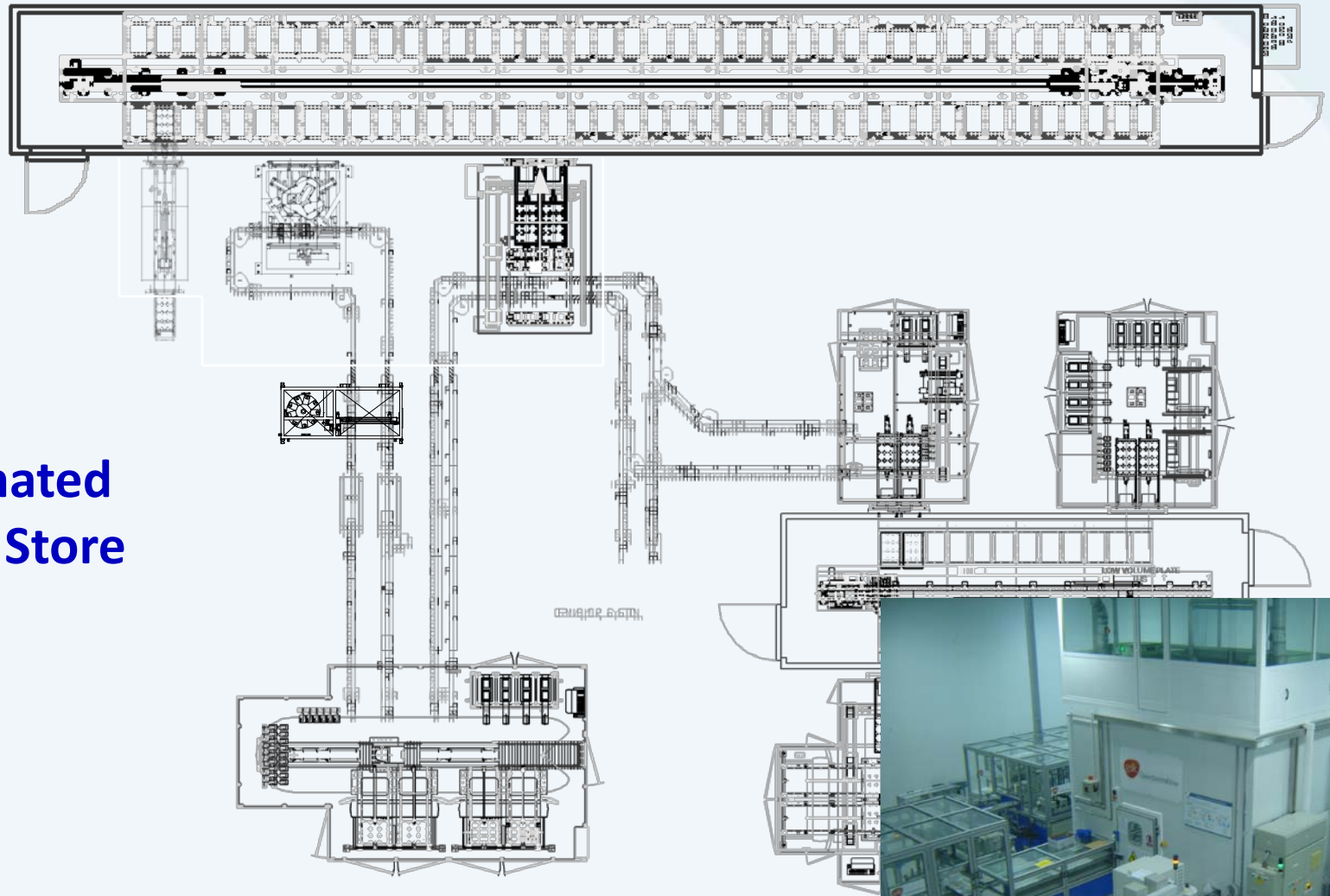


Entire collection (100%)

Potential actives (<1%)

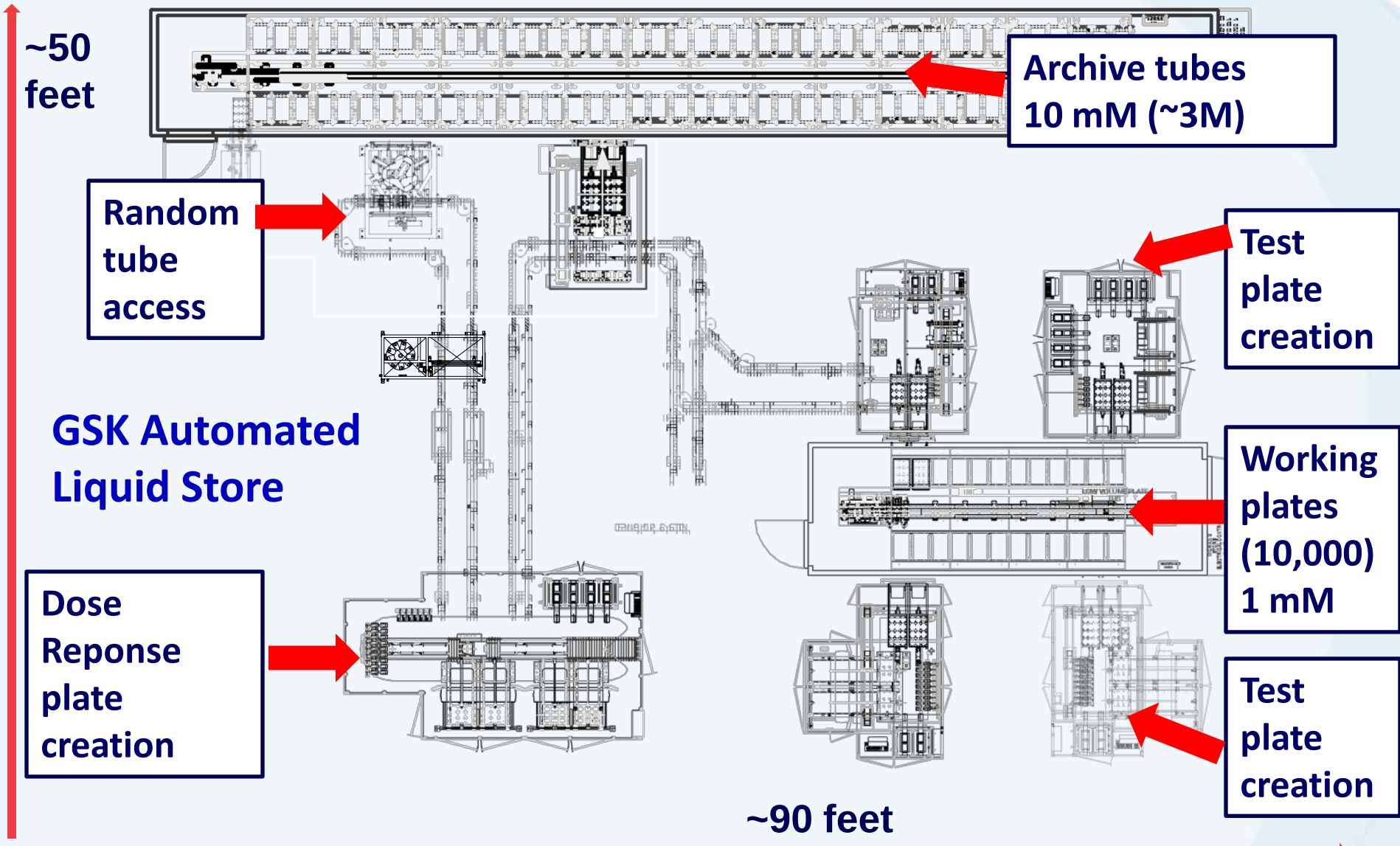
Real hits (<0.1%)

Compound management and supply

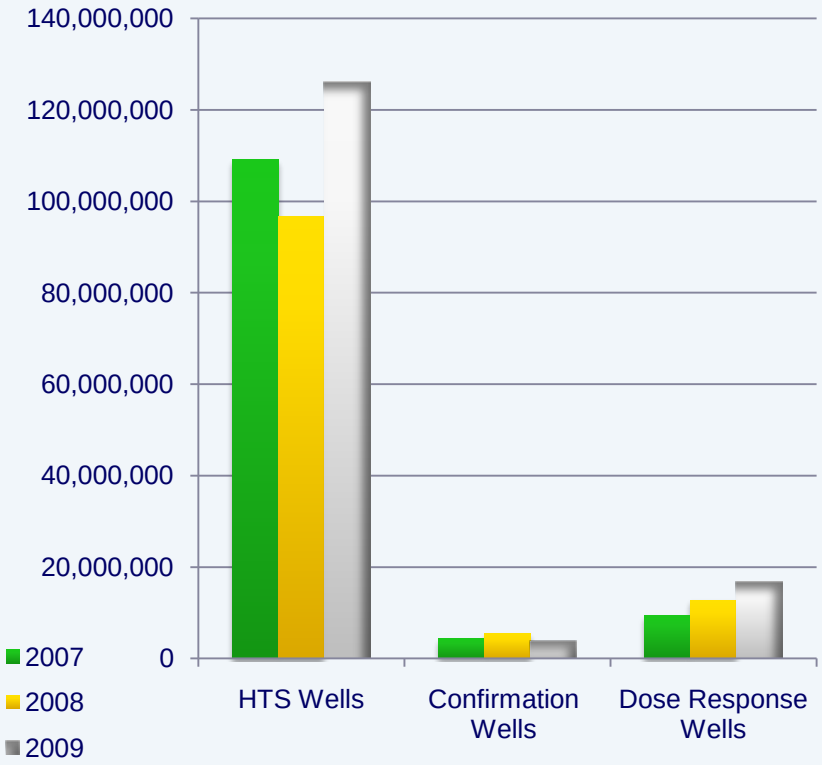


Automated Liquid Store

Compound management and supply – large Scale automation



Large robotics, large output…



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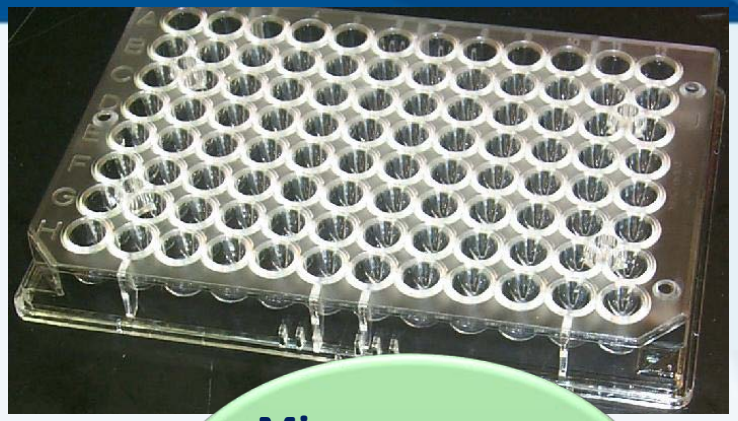

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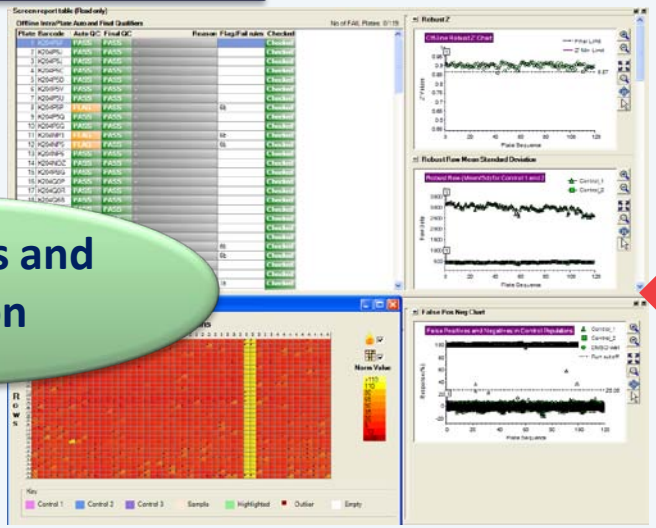

Data analysis and interpretation

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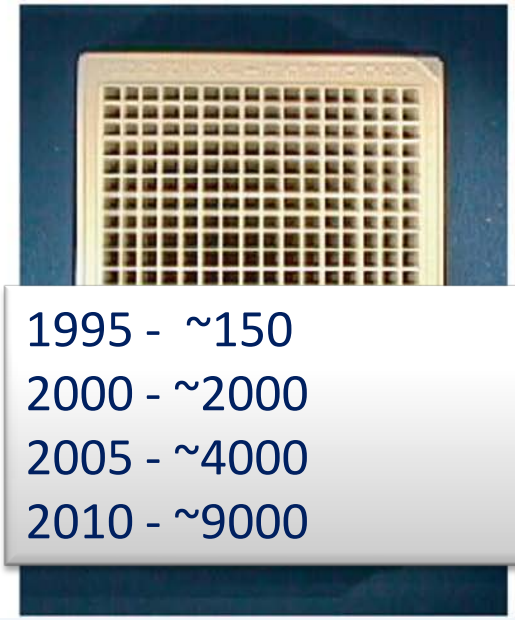
Automating compound handling versus biological assays

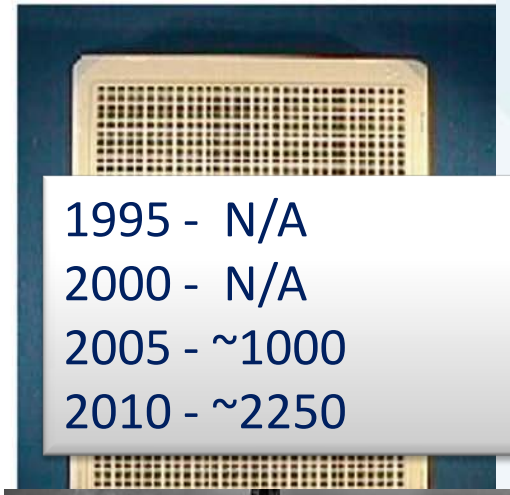
	Compounds	Biological assays
No. of operations per test	few (1-2)	More (4-8)
Typical liquid handling range - liquids dispensed	50 nL – 500 nL DMSO, H20	1- 5 uL Buffers, proteins, detergents, cells
Process variability	None	As biology demands - incubations etc.
Batch size	Optimal for system	Determined by system and signal stability
Instrumentation used	Constant – liquid handlers bar code readers, transport, holding devices, lid/de-lid	Variable – liquid handlers, incubators, centrifuges, multiple readers, lid/de-lid,

A lot of plates to handle!

1995 - ~500
2010 - N/A (40,000!)



1995 - ~150
2000 - ~2000
2005 - ~4000
2010 - ~9000



1995 - N/A
2000 - N/A
2005 - ~1000
2010 - ~2250

Number of microplates required for a single HTS campaign

1995 - 200K cpds (mixtures of 10/well)
2000 - 500K cpds (discretes)
2005 - 1M cpds (discretes)
2010 - 2.5M cpds (discretes)

Integrated High Throughput Screening Assay Systems

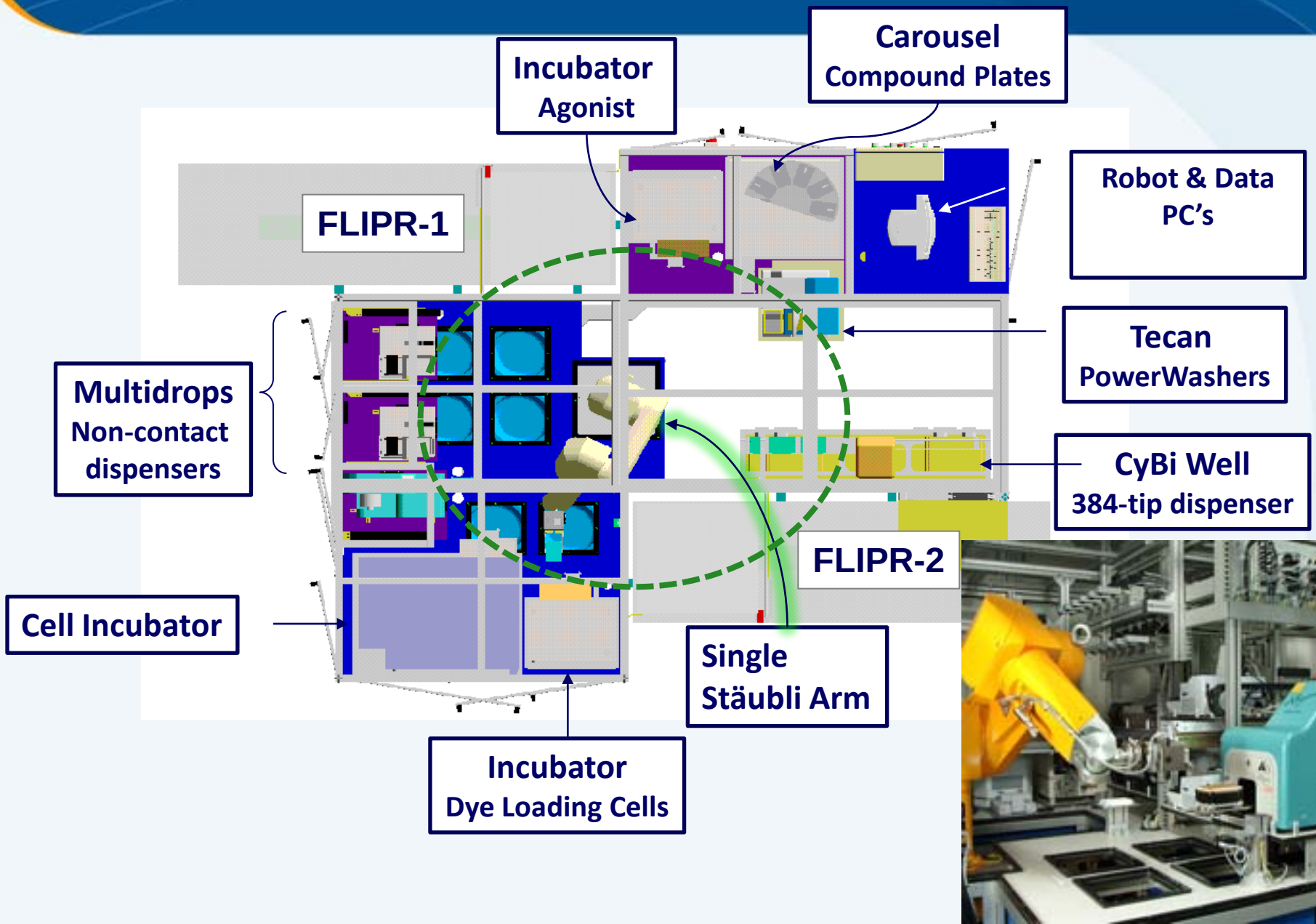
Integration of existing stand-along lab instruments

- anthropmorphic arm used to transport microplates between third party components
  plate readers, dispensers, incubators etc.
  e.g. Thermo CRS, RTS etc.

Total solutions

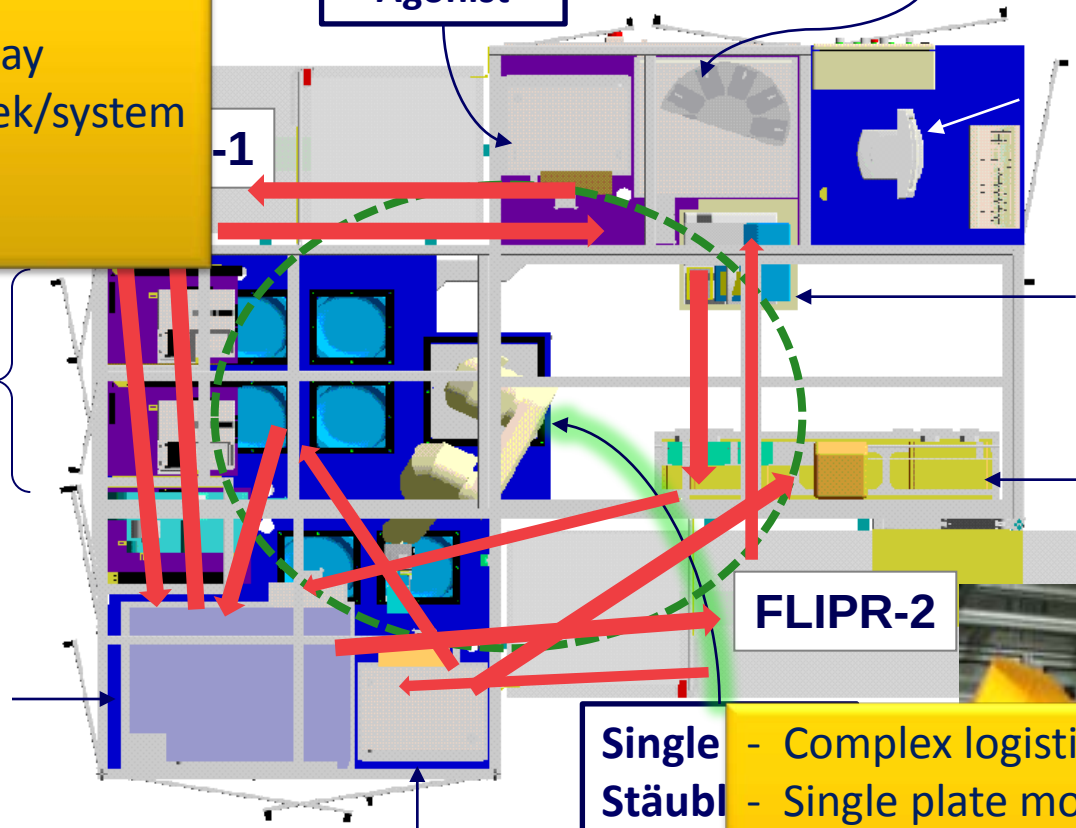
-"Soup to nuts" including specialized devices from vendor
- Variety of motion types
  e.g Evotec, TAP, Proteodyne

RTS Cellular Assay Platform (~year 2000)



RTS Cellular Assay Platform (~year 2000)

~ 189 x384 well plates/run
~ 20 hr (if perfect!)
~ 72,500 tests per day
~ 220,000 tests/week/system
~ 1-3 systems



Incubator
Agonist

Carousel
Compound Plates

Robot & Data
PC's

Tecan
PowerWashers

CyBi Well
384-tip dispenser

Multidrops
Non-contact
dispensers

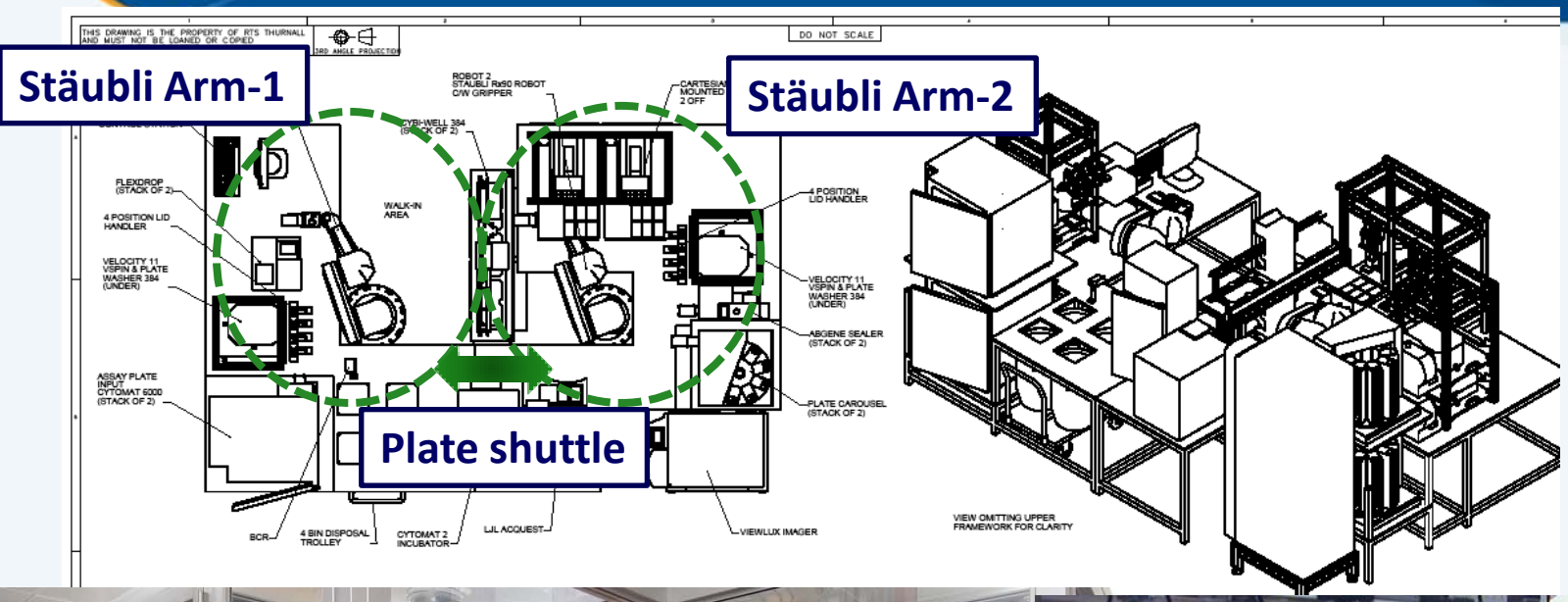
Cell Incubator

Single
Stäubl

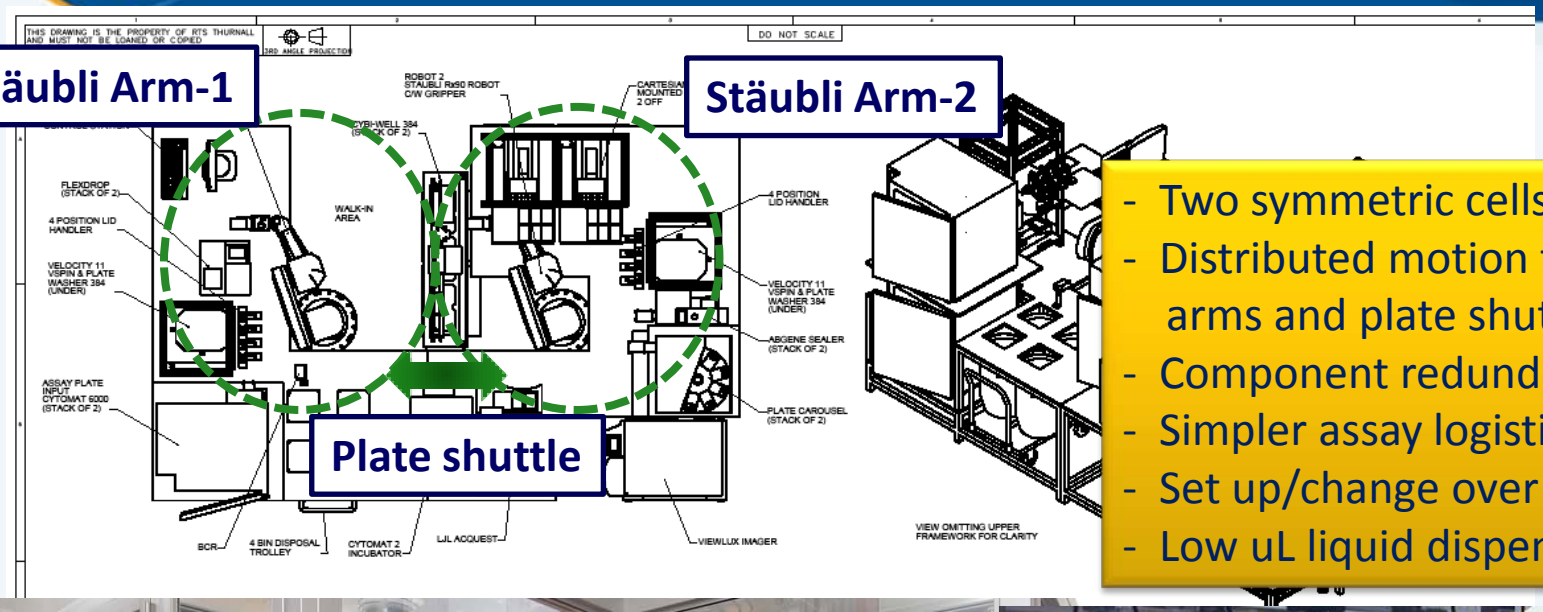
Incubator
Dye Loaded Cells

- Complex logistics and motions
- Single plate moving arm
- Fixed scheduling
- Component redundancy
- Plate motions limiting
- Error recovery difficult

RTS Biochemical Assay Platform (~year 2003)



RTS Biochemical Assay Platform (~year 2003)



- Two symmetric cells (1 or 2 robots)
- Distributed motion to two arms and plate shuttle
- Component redundancy
- Simpler assay logistics
- Set up/change over time
- Low uL liquid dispense critical

- 378 x 1536-well plates/run
- >500,000 data points/run
- 20h = 3 min click time /plate
- Reagent stability often limitation
-- 1-2 systems

Integrated High Throughput Screening Assay Systems – Issues/learning's

- Third party devices often not robust enough (product refresh cycles)
- Locked out of product cycles/technology advances by monolithic integrations
- Large systems (and redundancy) tie up devices even when not used
- True process bottlenecks were not foreseen; particularly around motion and plate holding
- Biology is unique each time – transfer/adaptation to automation can take as long as screening
- Change over time relatively slow
- When screening lab output = Output of a few big robot(s); long lead times and uncertainty from scheduling limited automation resource result

And ……Biology does not arrive in a predictable fashion or matched to hardware resource!

Looking back on the “Screening Factory” era

- Many investments were essential and pivotal to success; others didn’t stand the test of time
- Throughput and volume of work increased greatly, but so initially did lead times and duration
- Quality
- The extent to which senior management or investors were impressed just by the scale of the operation (as opposed to value created) decreased over time
- Initial thinking the industrialization would lead to de-skilling (robot operators) was naïve **– more data needs more interpretation and higher scientific skills**

Quote from 1999 management presentation “We will miniaturize, homogenize, de-humanize”

If there is a better way…don’t be a slave to the machine

2008-10

- Outdated (and/or unreliable) peripherals
- Slow change-over's, lead in times
- Logistics can’t keep up with process
- People find a better way

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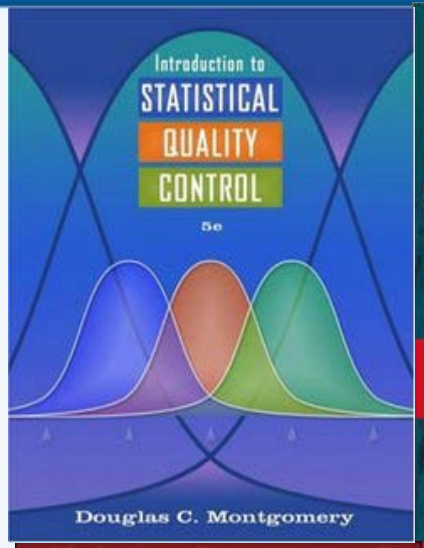
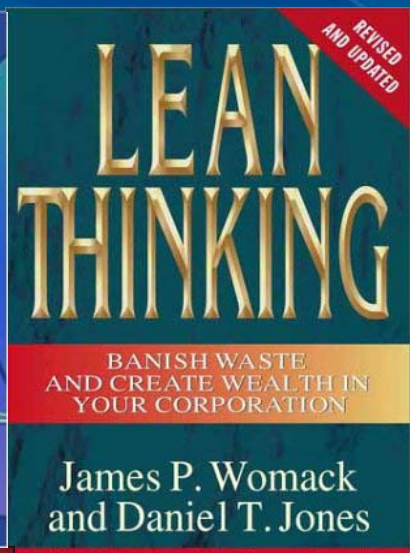
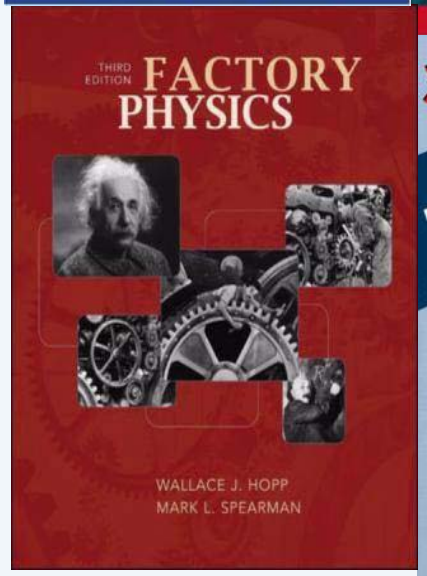
Learning to see (2005)

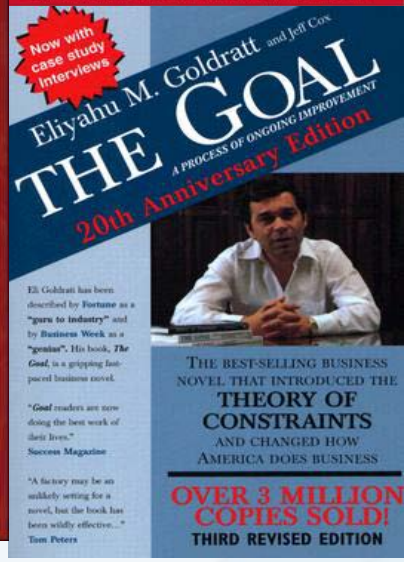
"If we're calling it a Screening Factory, maybe we sshould learn something about manufacturing!"

- *Discovery teams as customers of a product*
- *Importance of delivery speed*
- *Customer demand/satisfaction*
- *Work in progress*
- *Quality*
- *Policy deployment*
- *Identification of bottleneck*
- *Waste elimination/workspace organization*
- *Human and machine resource deployment*



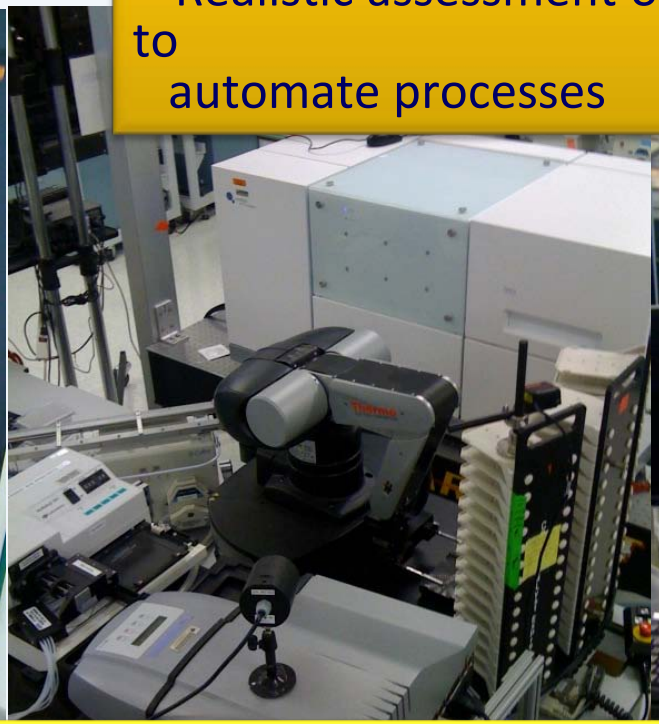






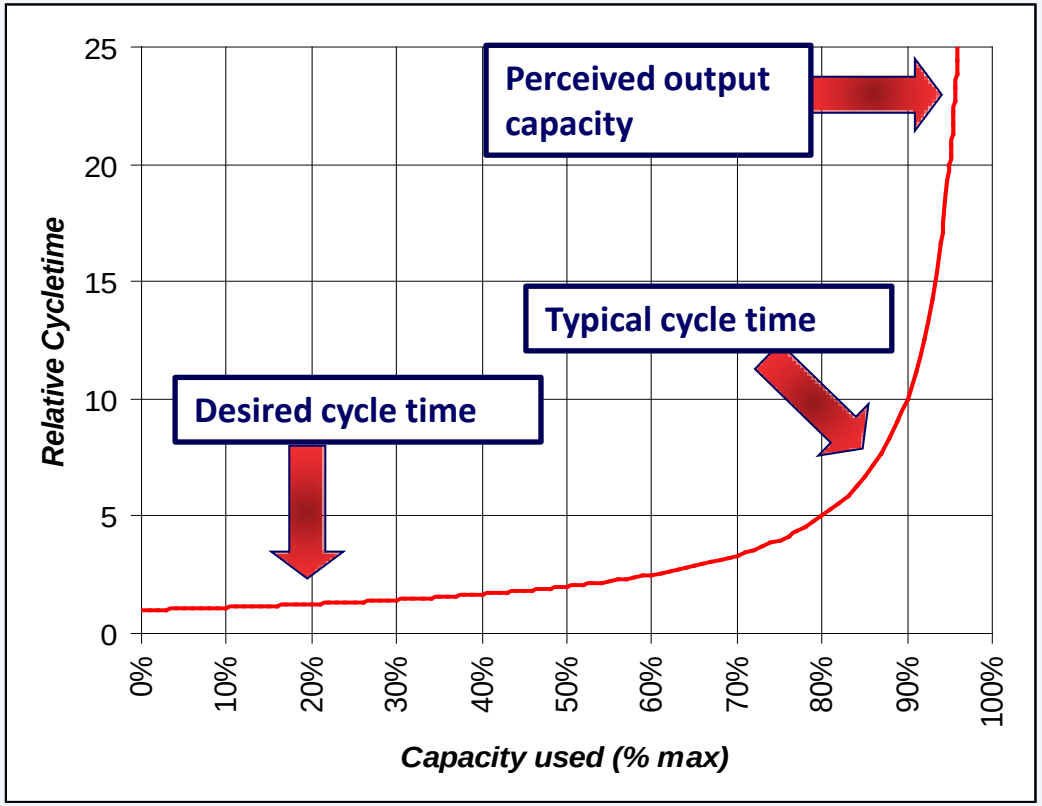
“Right sizing” automation of lab processes

- Timeliness and Quality critical
- Constraining work in progress is critical to cycle time (which helps lead time)
- Realistic assessment of how, whether and when to automate processes



- Light, flexible integrations of parts of a process
- Rapid changeovers
- Low costs = high useable redundancy (low utilization)

Cycle-time versus capacity



Limited number of “monument” resources resulted in long scheduled lead times

Total integrations tied up devices and fixed stoichiometry

Large uncontrolled fluctuations in number of screens resulted in extended cycle time

Perception that increasing speed decreased quality was false
- the opposite was true

Policy deployment examples

CONWIP (Constrained **W**ork **i**n **P**rogress**)**

Max one primary screen at a time per group of ~10 scientists

- between 0-4 running (median 2), previously 0-11!

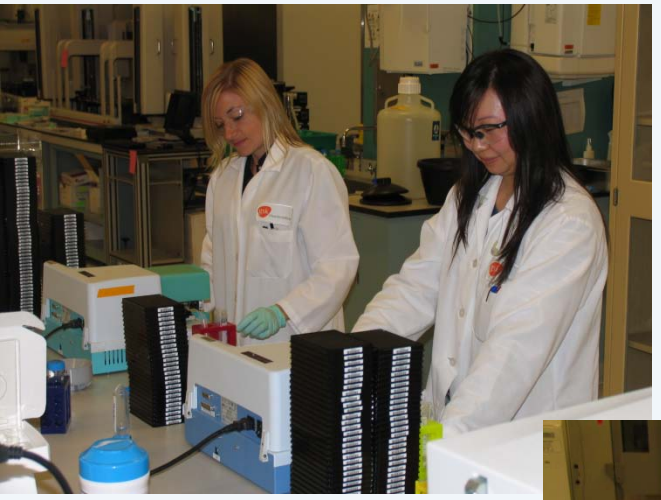
ACP (**A**bsolute **C**ompletion **P**riority)

Priority always given to effort at latest stage in the overall process
(i.e. Dose response screen X >> Confirm screen Y >>> Primary screen Z)

….fit of previous automation paradigms?

Bucket brigade plus light automation

- For simple logistics, perhaps the fastest method (~750K data points/day)
- People move individual plates for rapid steps – stacks for extended steps (e.g. signal read)
- No inherent fixed device stoichiometry
- Quality equivalent or higher



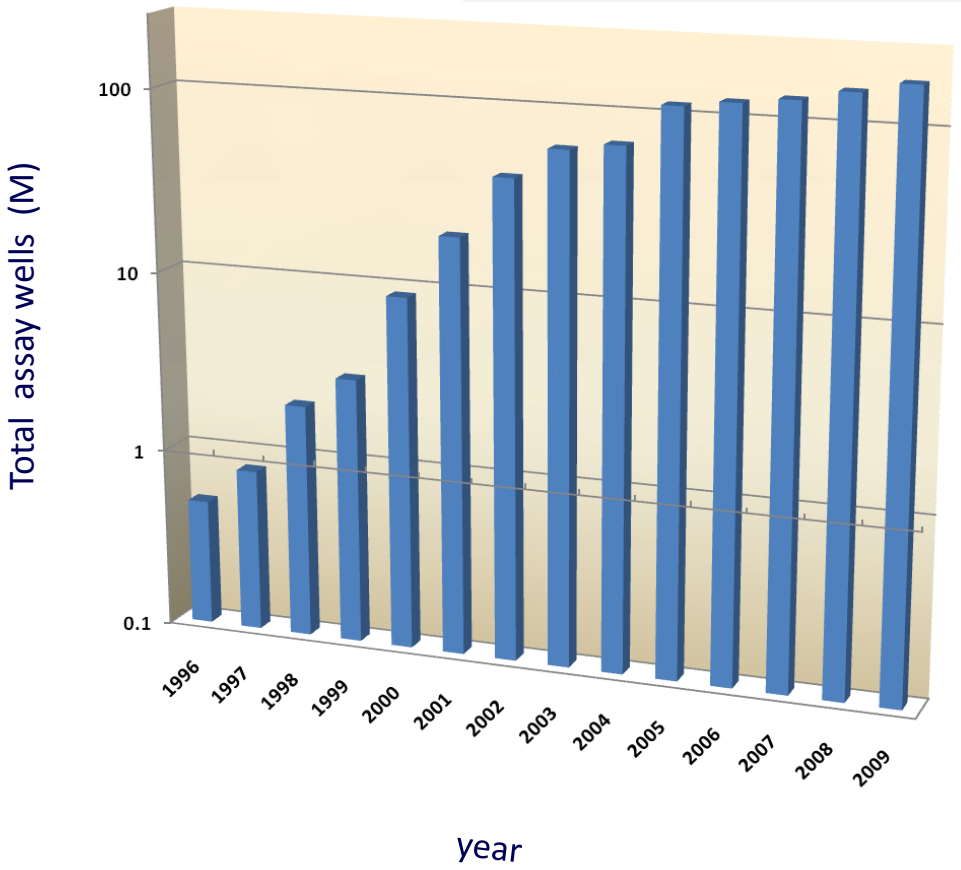
Implications of “Learning to see”

- Hardware redundancy as opposed to utilization
- Policy deployment for speed, lead time, quality, productivity
- “Right-size” the automation process, including de-coupling tasks
- Recognize and respect bottlenecks/monuments (e.g. carefully manage order priority and Utilization of large cpd supply robots)
- Get quality and process right at the start – the fun part is before and after the (boring uneventful) screen
- Organizational structure
  - Team based working, hands (and brains) to solve problems if needed

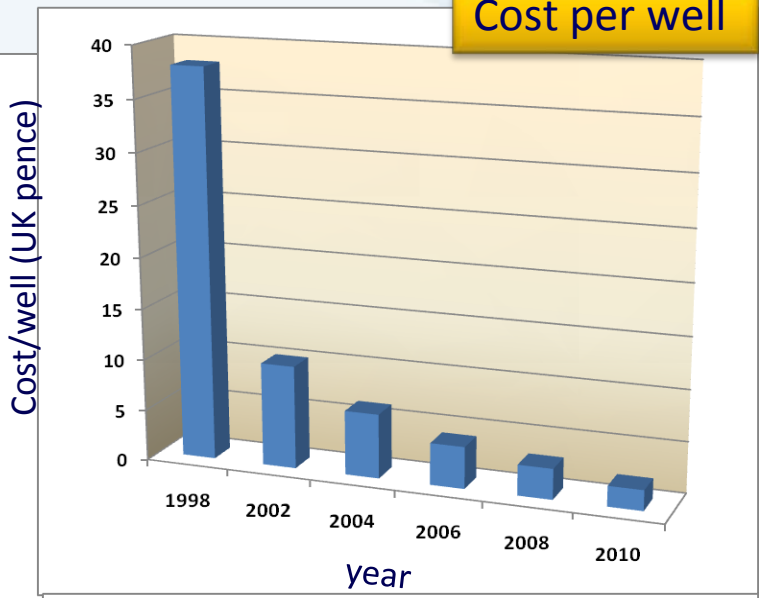
Screening history through beverage containers

Cost and volume

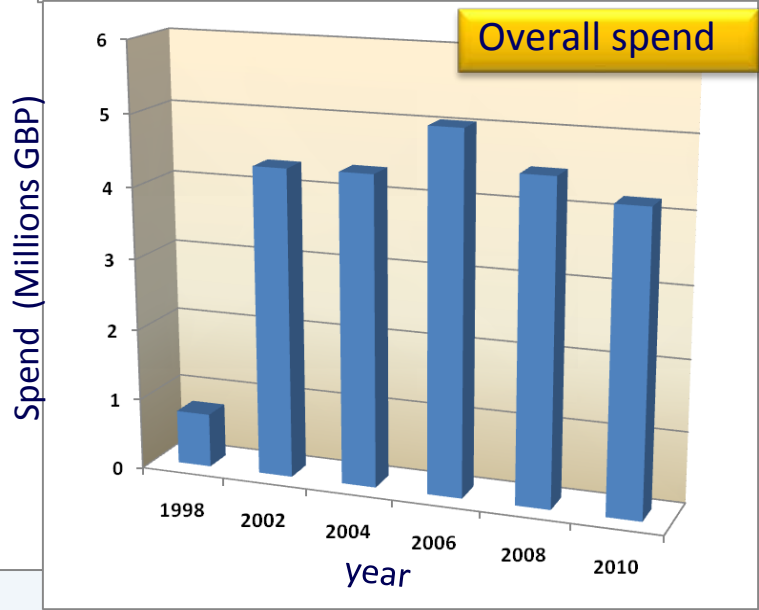
Total HTS wells



Cost per well

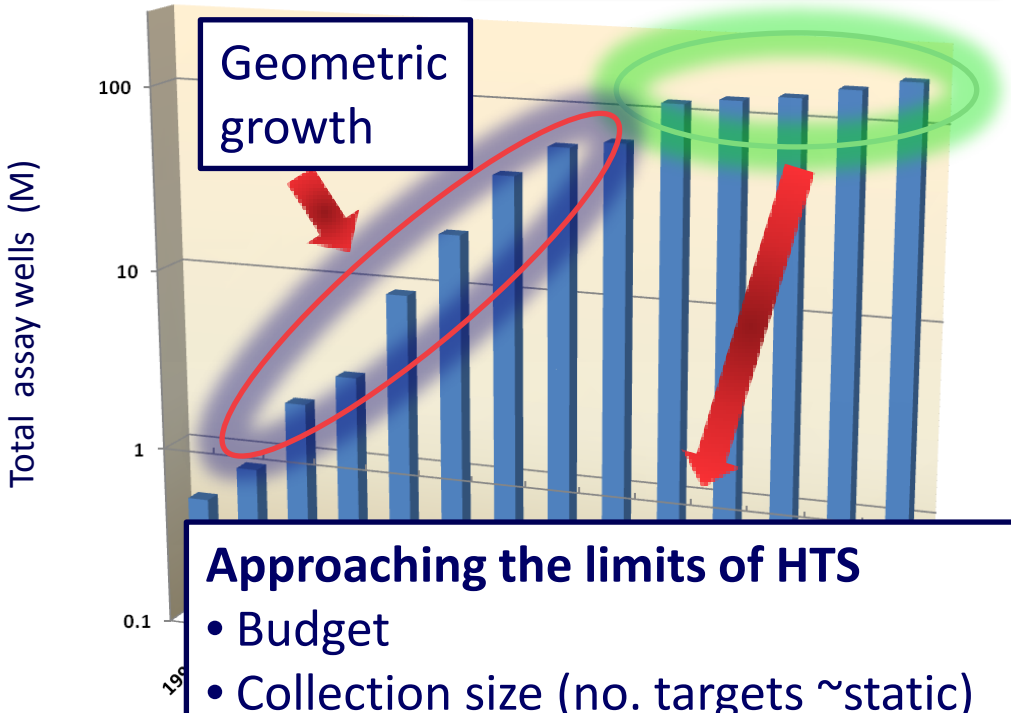


Overall spend



Cost and volume

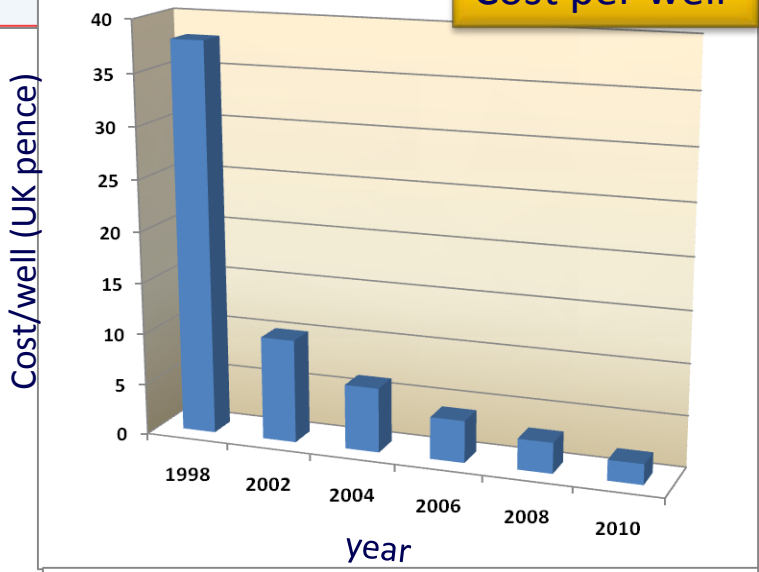
Total HTS wells



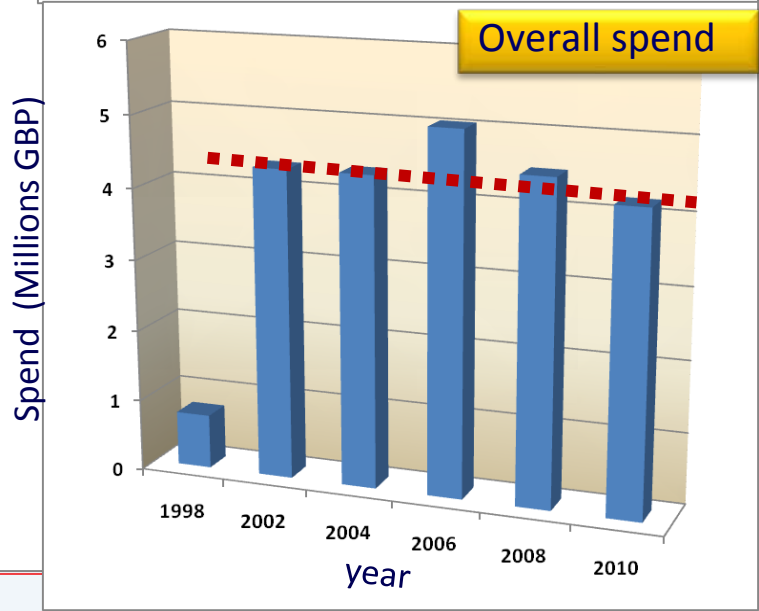
Approaching the limits of HTS

- Budget
- Collection size (no. targets ~static)
- Infrastructure (cpd store capacity)
- Emphasize quality of cpds, assays and targets as opposed to quantity

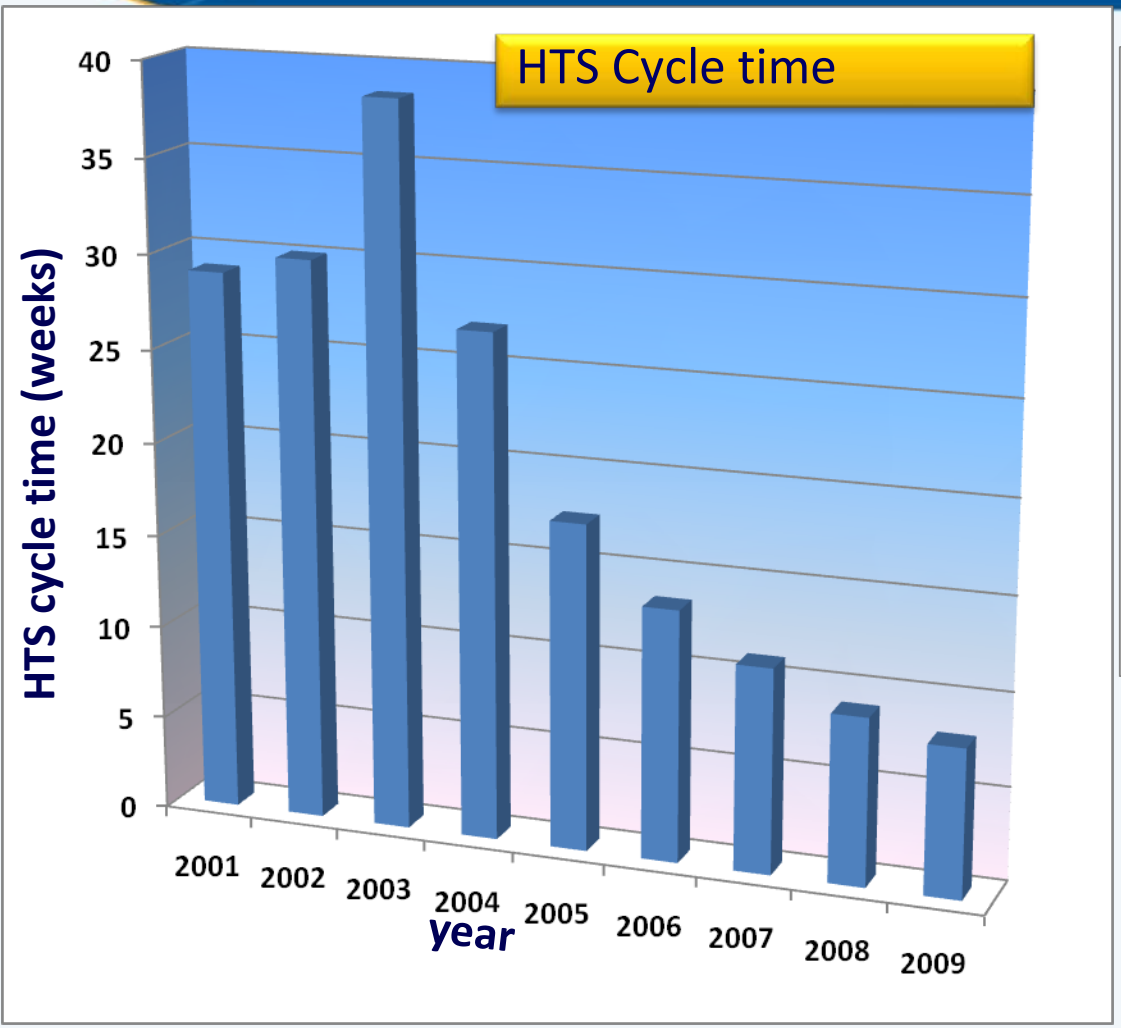
Cost per well

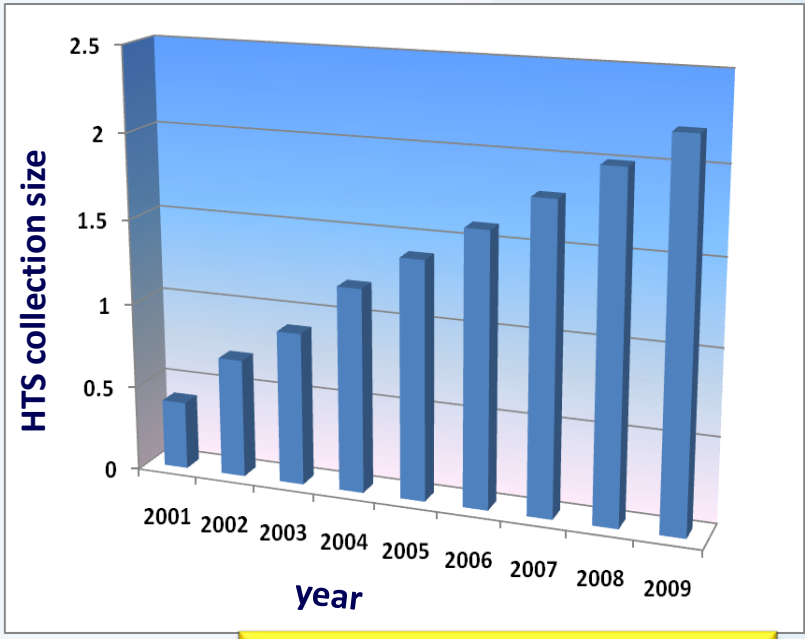


Overall spend

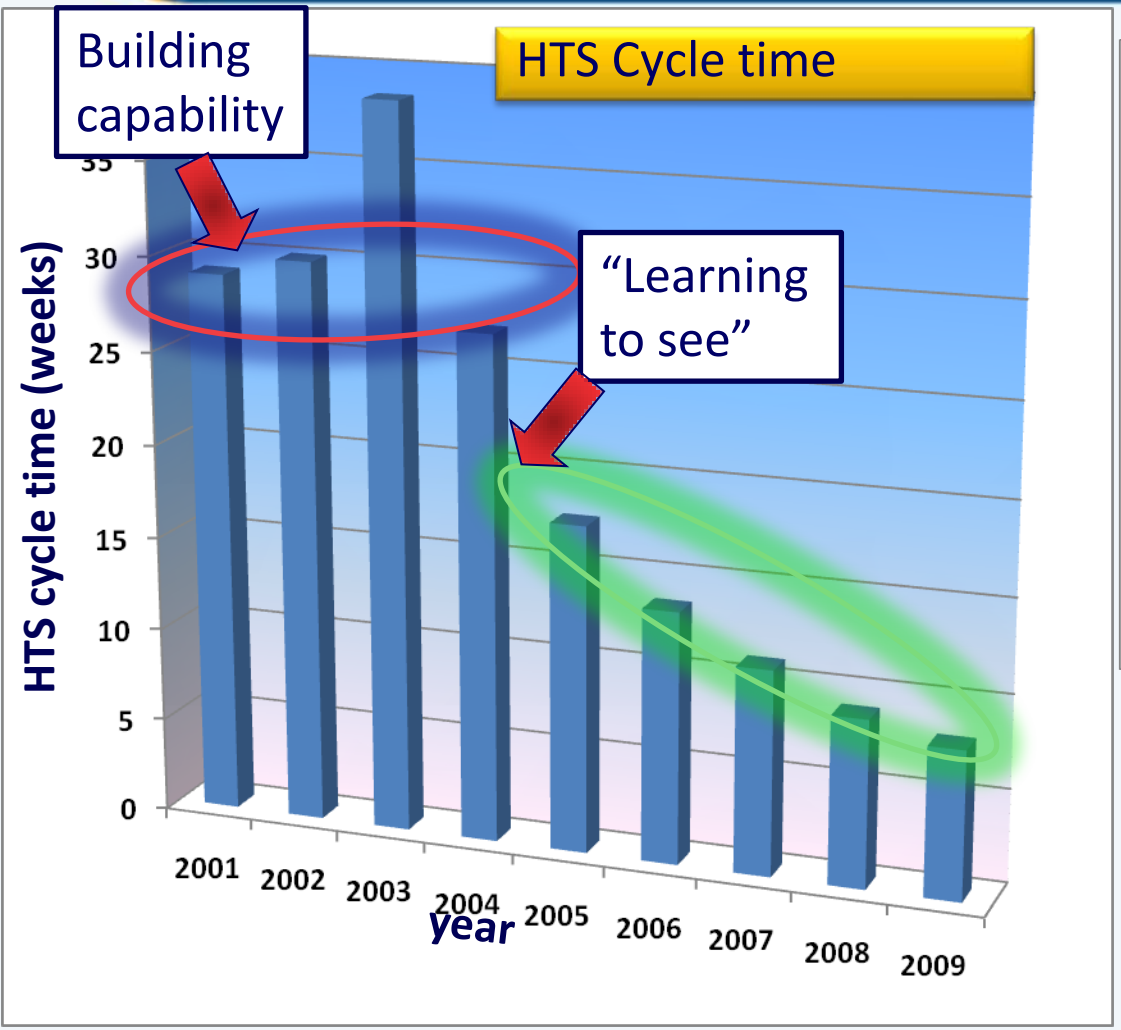


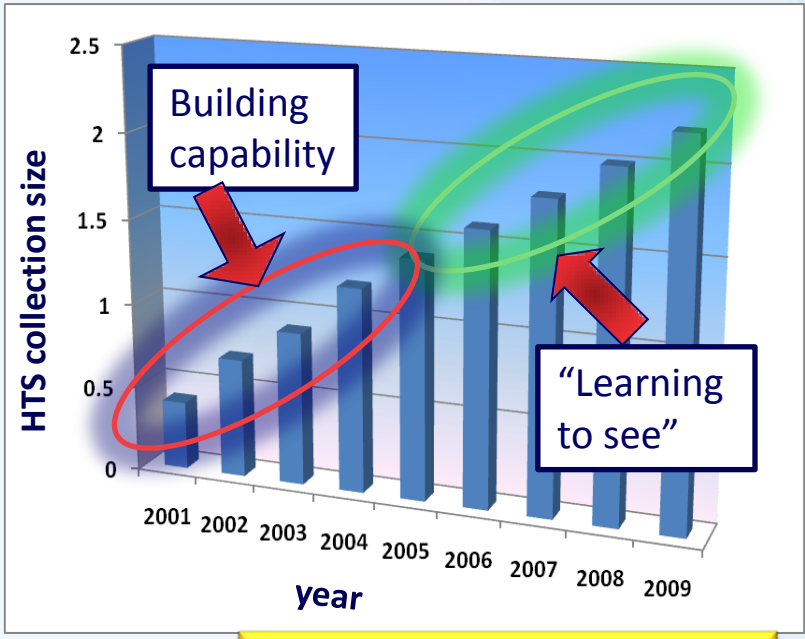
Cycle times and scale for HTS in the last decade





Cycle times and scale for HTS in the last decade





Looking back on the last 20 years..

1990 – Screening (as opposed to design) becomes the method of choice to discover drug starting points.

1995 – Excitement builds around genome sequencing and combi-chem

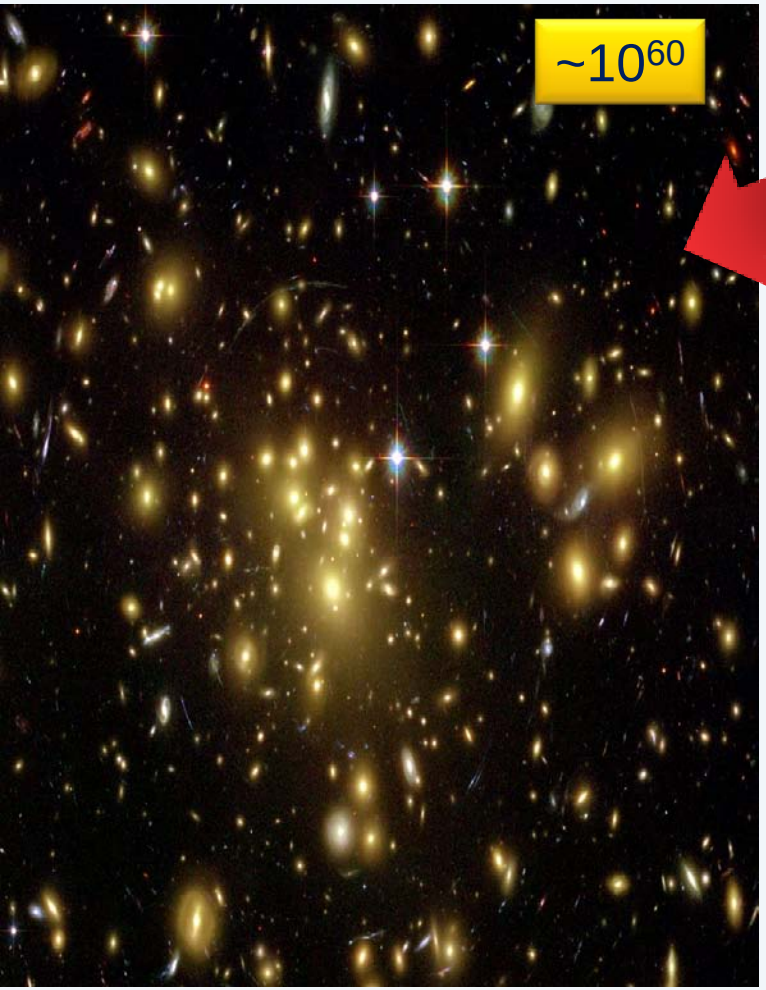
2000 - Major investments to “industrialize” drug discovery

2005 - Major focus on time, cost, efficiency, quality (i.e. real data manufacturing)

2008 - Focus on “re-personalizing” drug discovery, solving drug attrition, maximizing success on difficult targets, “new” biology

2010 - Integration, flexibility and return on investment in a cost-constrained environment

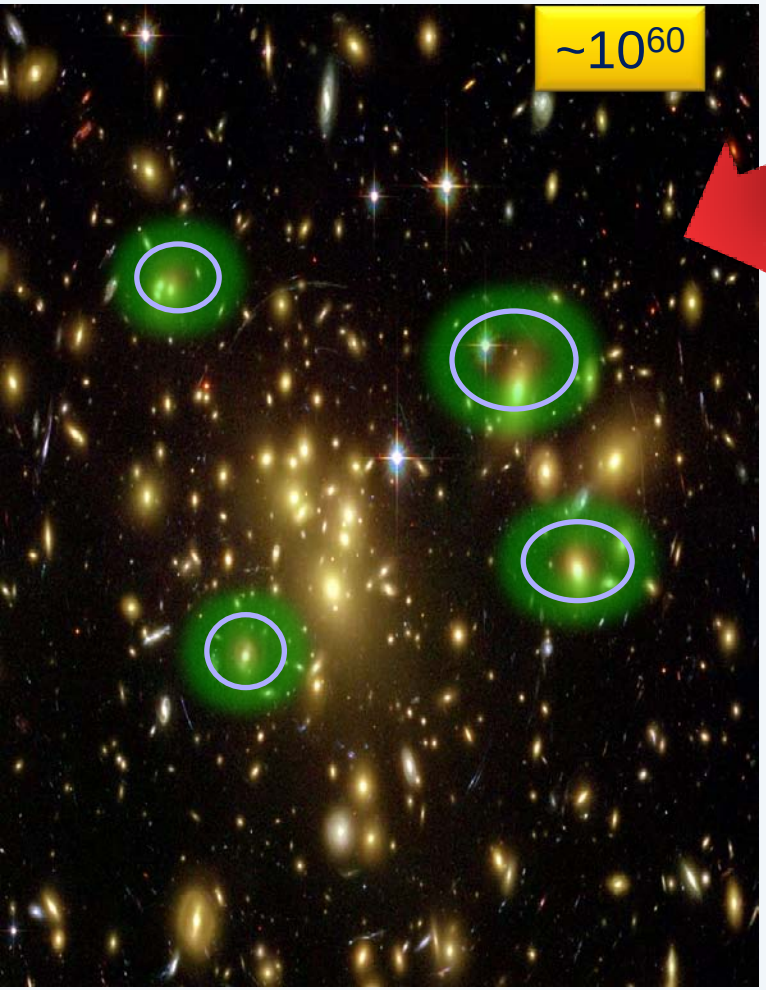
Drug-like space might be huge…..



Most beautiful
Lead-like molecules

Practical/economic
limits of scale

We need a map…(and maybe a new ship)



Most beautiful Lead-like molecules

Drug (lead)-like cpd knowledge

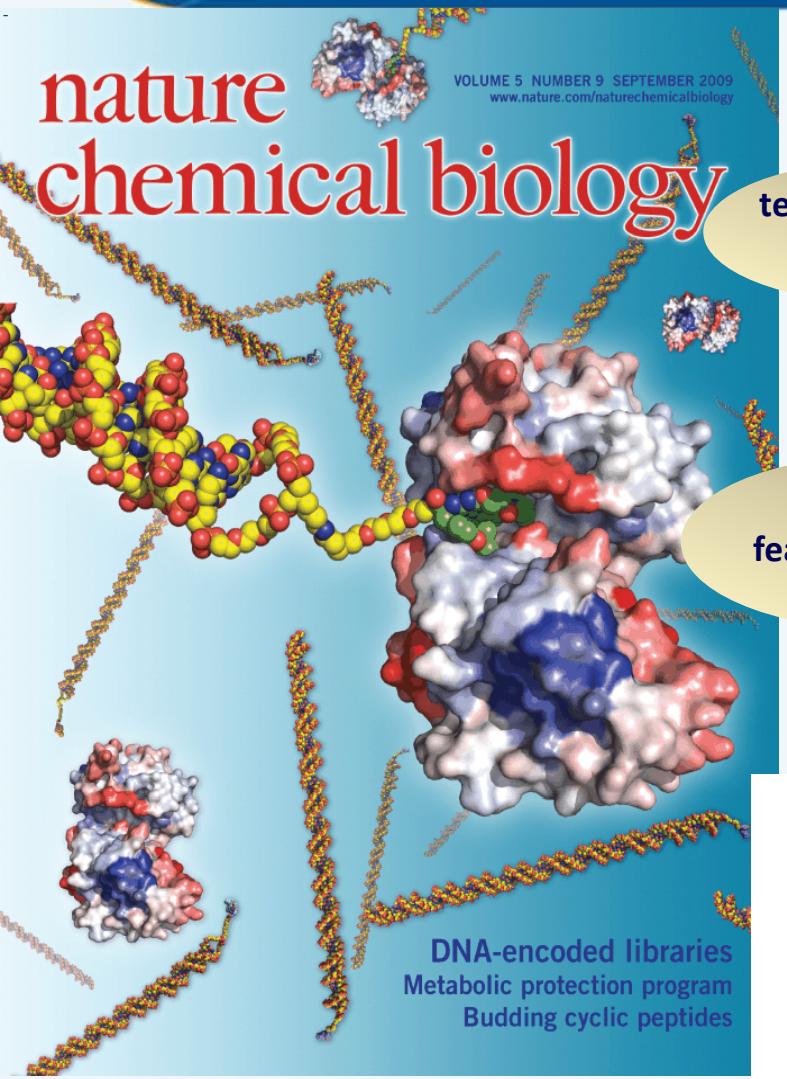
Target/chemistry knowledge

New screening methodologies

Practical/economic limits of scale

~2.5 x10^7

A different screening paradigm….DNA Encoded Libraries

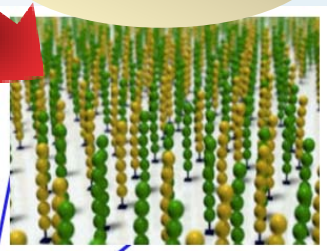


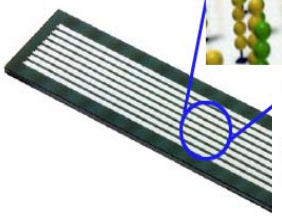
Library size ~10^{10} compounds

µg target protein + µL library pool

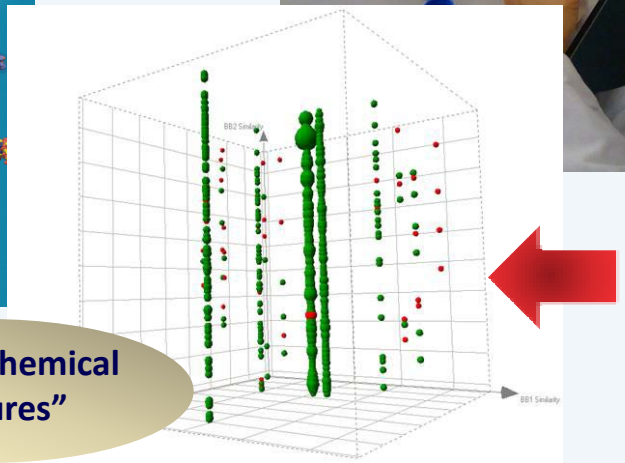
affinity-based selection

Sequence DNA tags





illumina®



Identify chemical "features"

test in biological assay

synthesize feature cpds off-DNA

ELT removes the infrastructure cost ceiling

HTS cpd store
~ 25,000 sq ft
~ 2.5 x10^6 compounds
~ 11 full time staff, incl. engineer
~ max capacity ~3.5M

ELT cpd store
-2 small freezers
- ready to use aliquots
>10^{10} compounds
-1 part time person to maintain libraries
- No limit to capacity

..But, all chemical diversity needs to be synthesized in house..

..and all hits need off-DNA synthesis

Looking back on the last 20 years..

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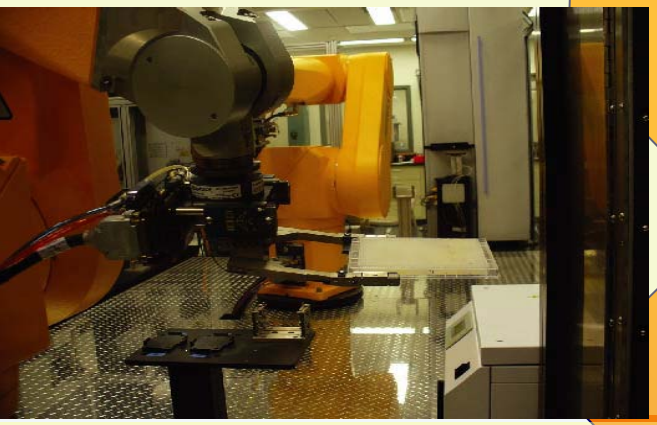
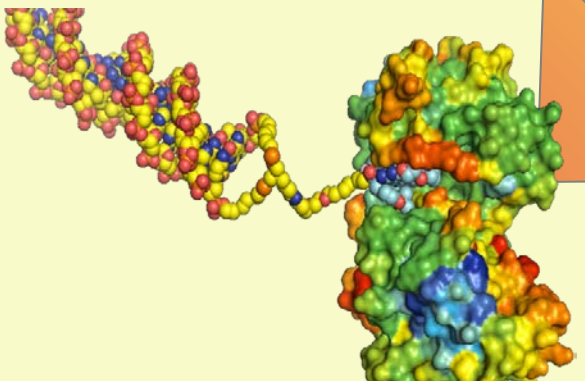
2010 - Integration, flexibility and return on investment in a cost-constrained environment

Integration, choice & knowledge incorporation
- Key to increasing success and investment return

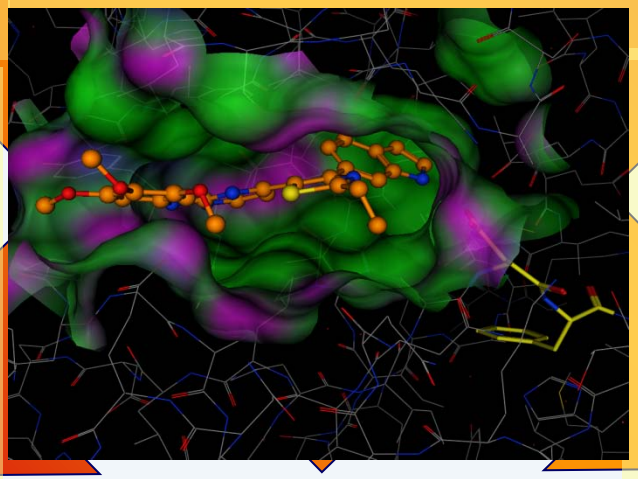
HTS
- *maximize diversity, "lead likeness"*
- *big scale process (3M wells)*
- *cpds handled conventionally*



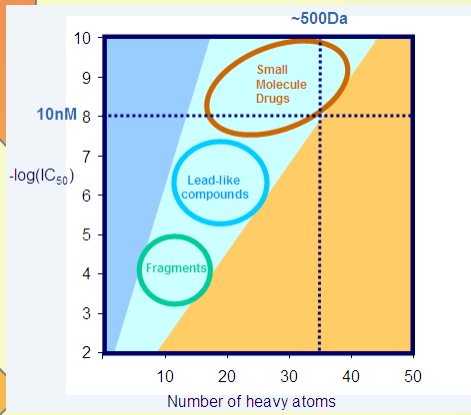
ELT
- *maximize diversity, "lead-like cores"*
- *small flexible process*
- *cpds need to be made to follow up*



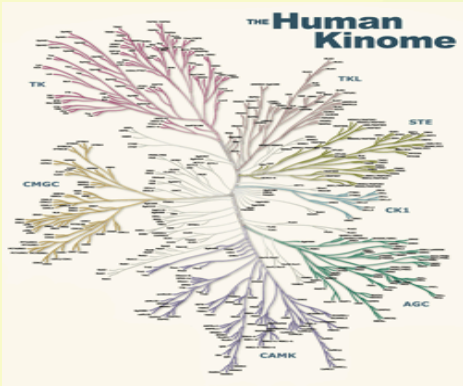
Structure-based design
- *takes starting points from all other methods*
- *Xstallography of protein bound cpds*
- *Ab initio design methods*



Fragment screening
- *smallest, most efficient starting points for lead optimization*

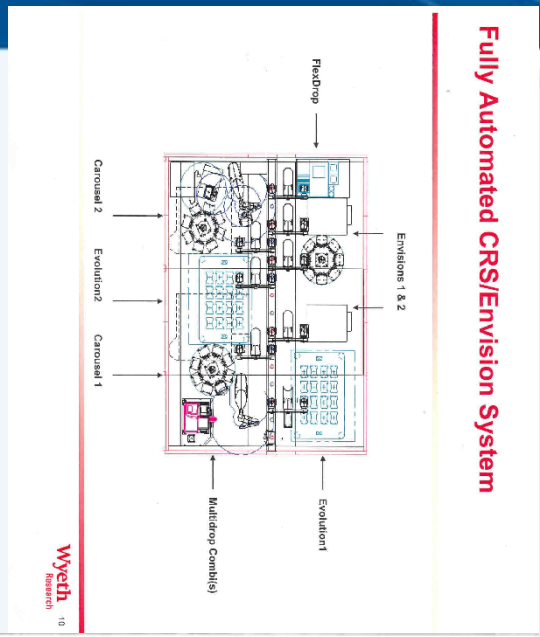


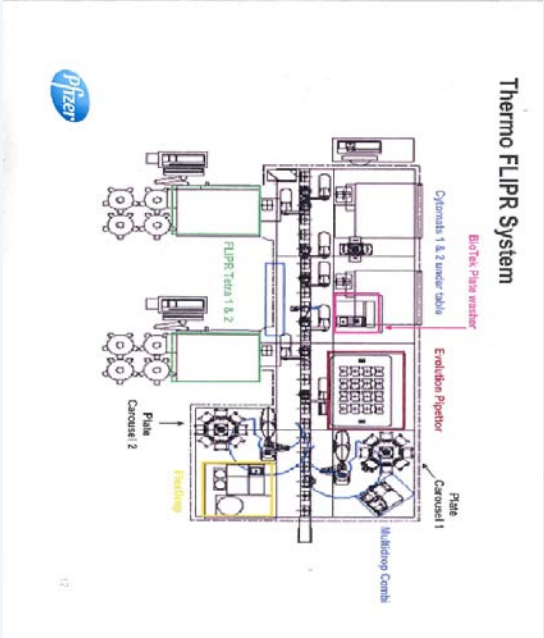
Focussed screening
- *exploit chemical/biological connectivity of therapeutic targets*
- *biased to certain target types*
- *small scale process (quick, early)*



2010 – Another visit to integrated automation

- Easy integration of components
- Plate motions not limiting
- Flexible design/re-tool
- Bucket brigade-like throughput





Summary

- **Automation has played a key role in the evolution of methods to discover new medicines**

- **Within the existing paradigm, the HTS physical process is close to optimal, given sustainable investment levels**
  - *Focus on best possible chemical libraries*
  - *Understanding chemical: biological relationships in data*
  - *Screening novel disease biology in new ways*
  - *Integration (or intelligent choice) of methods*

- **We will continue to need and to develop automation technology to help discover drugs…**
  - *Compound biological profiles*
  - *Drug safety, efficacy and attrition-risk addressed early*

- **Timely discovery of chemical starting points (chemical probe or potential drug) are now well addressed**
  - *but there is a lot more involved in making a new medicine!*

Acknowledgements

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Ricardo Macarron, Sue Crimmin and colleagues (cpd supply)

Barry Morgan, Jeff Messer and colleagues (ELT)

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