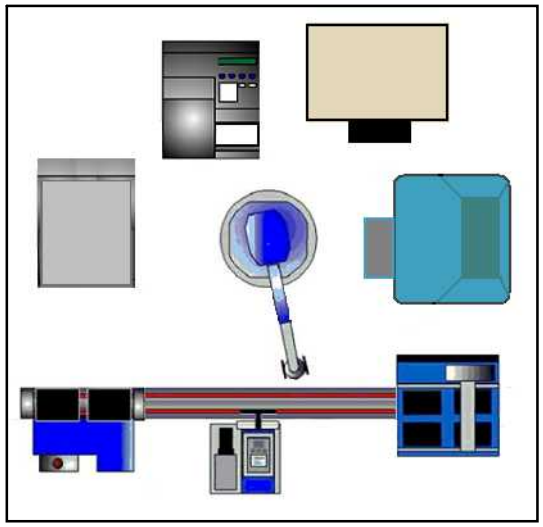
The Hudson VaryScreen I

An Assay Development System

Alan H. Katz, CSO

Hudson Control Group

The VaryScreen I
Automated Assay System



Includes 6 general pre-written protocols
- Fluorescence Intensity
- Fluorescence Polarization
- FRET (LANCE, VIPER)
- Luminescence (Reporters)
- ELISA
- Alpha Screen

Model, optimize and run in-house Assays
- Evaluate subtle changes in conditions
  - solvents, stoichiometry, pH, time
- Determine importance of individual steps
  - incubation, agitation, filtration
- Introduce intelligence into protocols
  - complex control structures
  - e-mail alerts, milestone response

Easily customized
- Add new features
- Duplicate heavily used features
- Omit unrequired features
- Use alternate 3rd party tools

Fully software controlled

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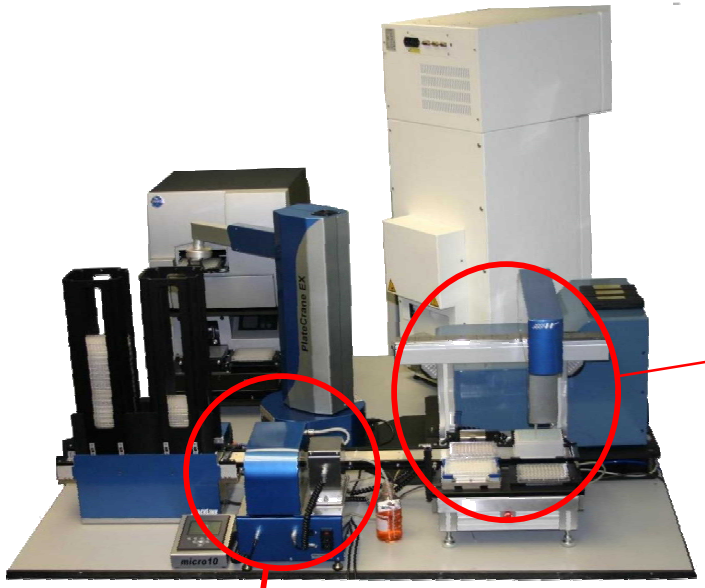
Liquid Dispensing Tools



Ivek Syringe

Hudson SOLO

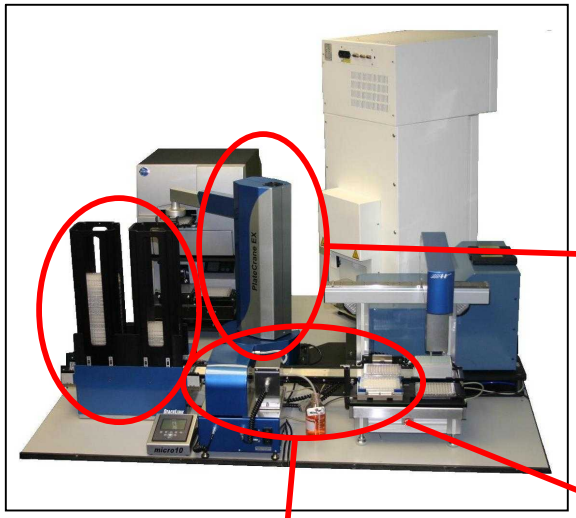
- Single Channel Pipettor
- 125 or 250 uL Ivek Pump
  and/or
- Hamilton Syringe (1-11 liquid sources)
- 1 microliter and up
- Works with cell cultures
- Supports Microplate Cherry-Picking

Hamilton Syringe

Hudson Micro10

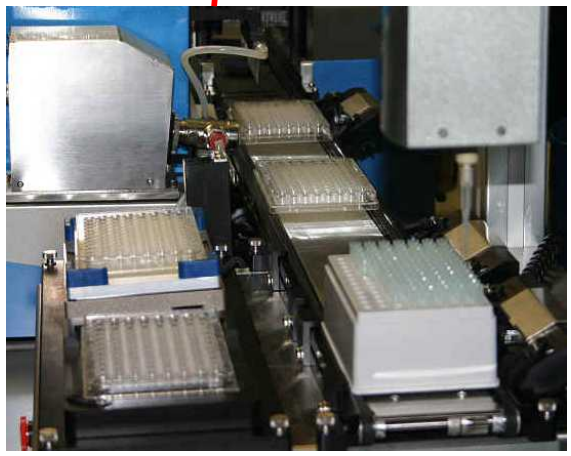
- 12 Channel Dispenser
- Feeds 1-4 liquids
- Low micromolar and up
- Works with cell cultures

Plate Handling Tools



Hudson PlateCrane EX

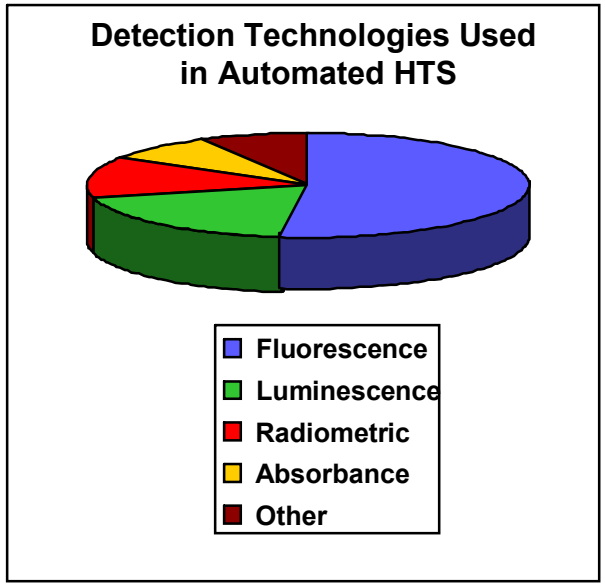
Hudson StackLink



Hudson TrackLink

Multi-Mode Readers in HTS
Fluorescence, Luminescence, Absorbance

BioTek Synergy 4



BMG LabTech PolarStar Omega

Perkin Elmer Envision

Beckman Coulter DTX 800

Turner Modulus

Other Commonly Used Tools

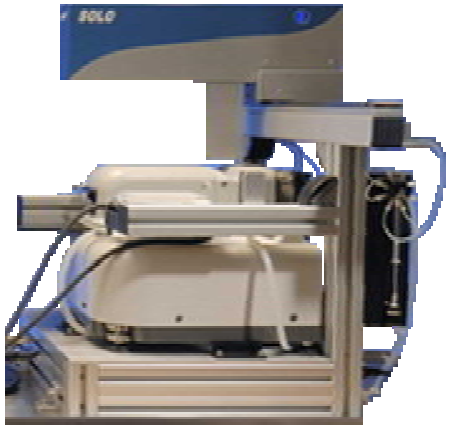
Liconic STX40
Incubator

BioTek ELx405
Plate Washer

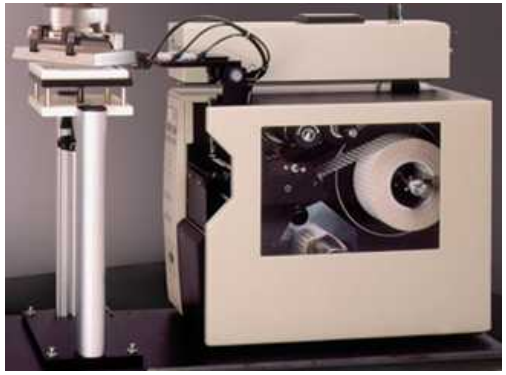
Variomag TeleShake
Plate Shaker

Hudson VaryVac
Plate Filter

Examples of Other Add-Ons with software interfaces

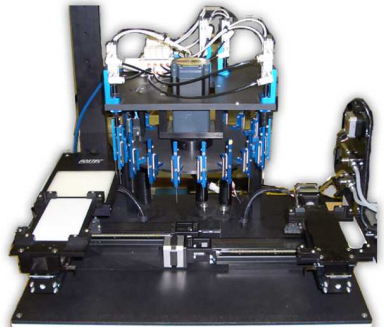


Hudson AutoReact
Mini-Bioreactor



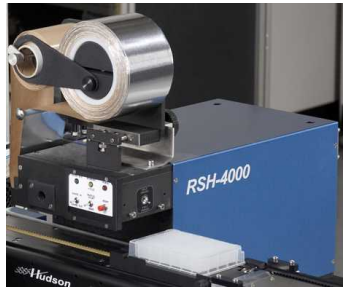
Hudson Print & Apply
Bar Code Reader/Writer

Guava EasyCyte
Flow Cytometer



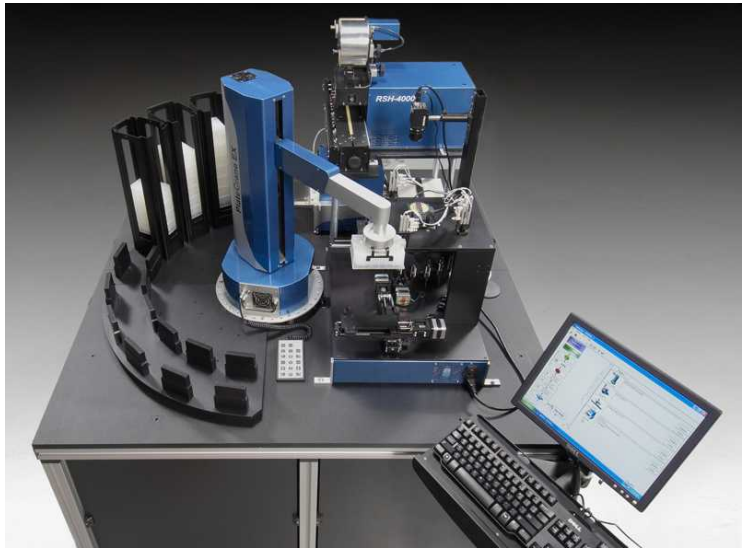
Norgren 7200
Colony Picker

Mettler-Toledo WSX
Automated Balance



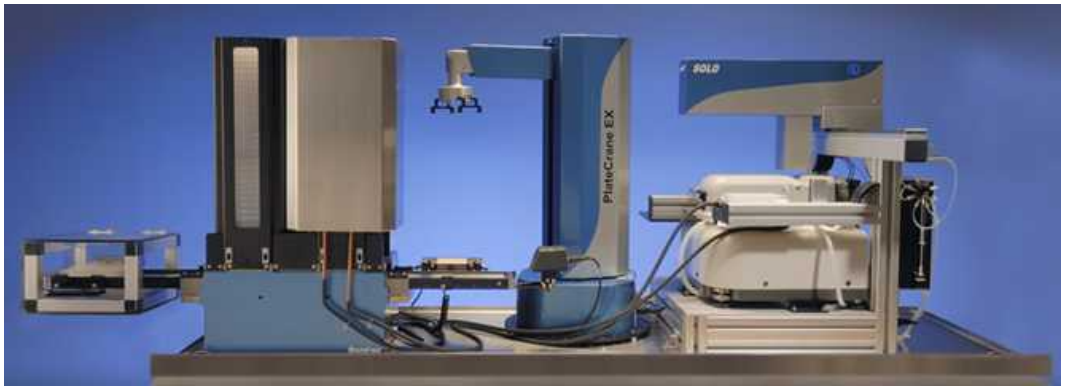
Hudson RSH-4000
Non-Thermal Plate Sealer

Other Automated WorkCells
Designed at Hudson Control Group



Hudson RapidPick
Colony Picking WorkCell

Hudson Prolink Express
Proteomics WorkCell



Hudson AutoReact Mini-Bioreactor

Designing a Typical High Throughput Screen

Sample Questions

1. How must the materials be prepared?
    1. Purifications
    2. Centrifugation
    3. Filtrations
    4. Washings
    5. Incubations

- What are the conditions of the experiment?
    - How are they optimized
    - How many factors effect results

- How do known standards perform?

- How will Positive Results be measured?
    1. False Positives
    2. False Negatives
    3. Sensitivities
        1. Condition Dependence
        2. Instrument Sensitivity

1. How will Results be Obtained?
    1. Raw from instrument
    2. Additional processing required
    3. Format of final output

Available Steps

Liquid Handling

Micro10
- Prime
- Dispense
- Run Program

SOLO
- Grab/Shuck Tips
- Dispense
- Aspirate
- Cherry-Pick

ELx405
- Wash Cycles

Plate Manipulation
- Storage
- Track Movement
- Crane Movement

Reaction Conditions
- Heating
- Agitation
- Incubation
- Filtration

Plate Reading

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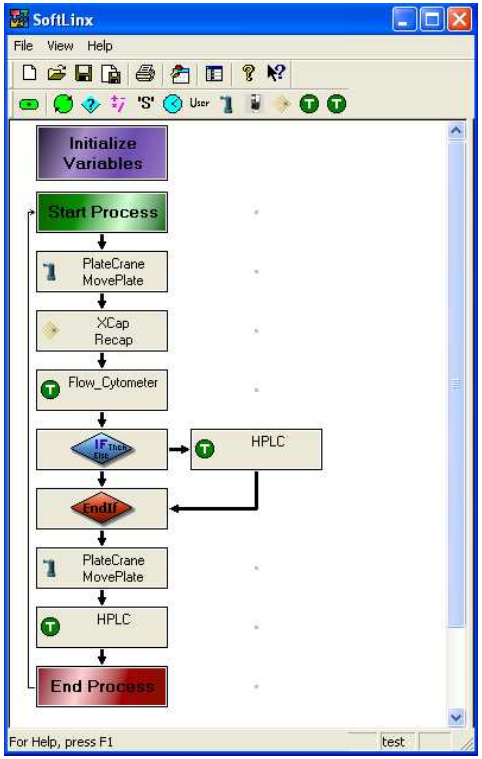
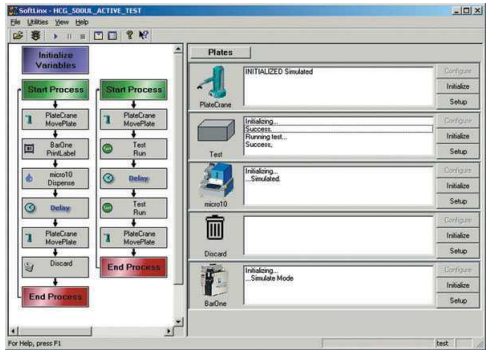
SoftLinx Assay Management System

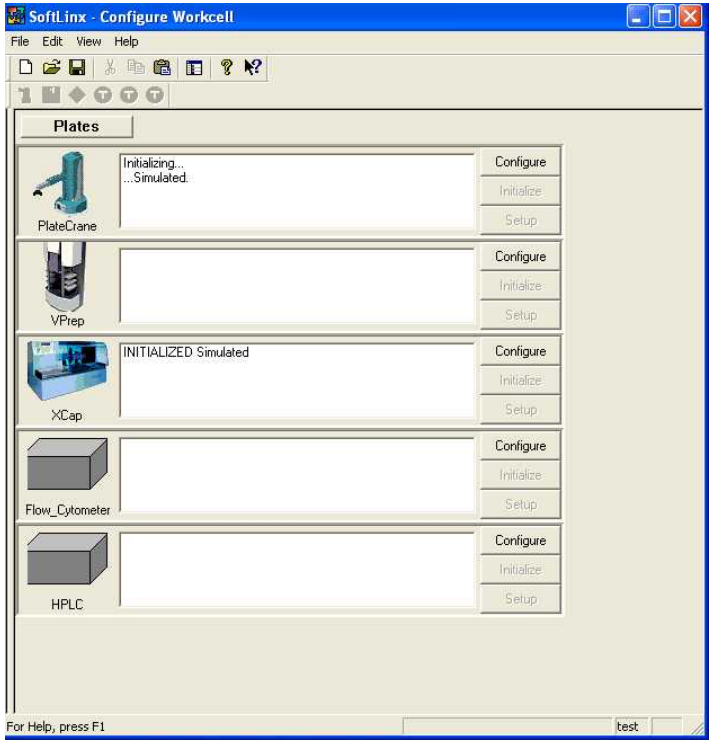
Method Editor



Operating Screen



WorkCell Configuration



- Develop Complete Assay Protocols
- Add individual steps from a library of instrument functions
- Include user-defined functions with Visual Basic for Applications
- Design complex conditional control structures
- Design processes that change dynamically based on results

- Add instruments as needed
- Configure instruments
- Constantly check instrument status/availability
- Simulate unavailable instrumentation

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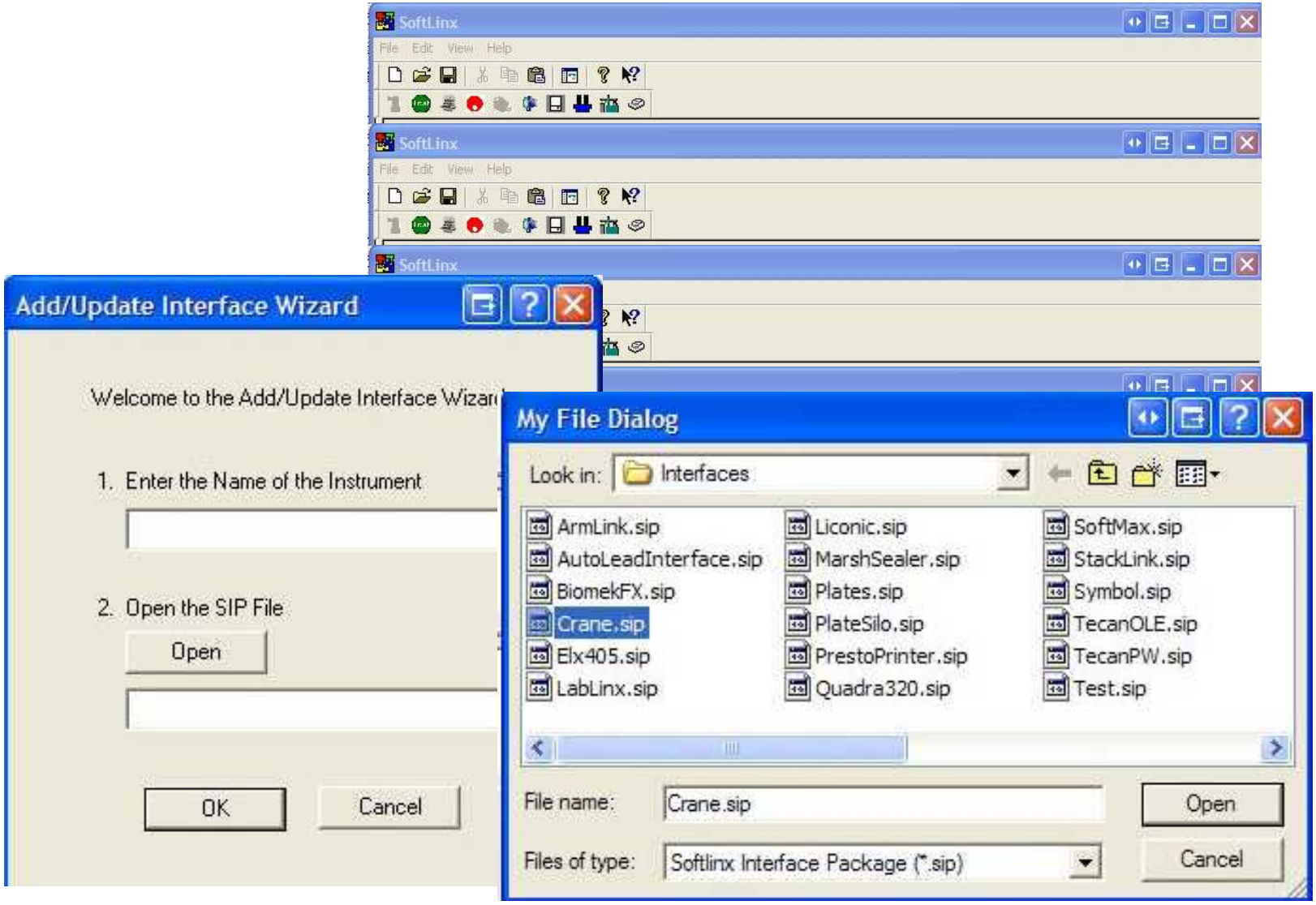
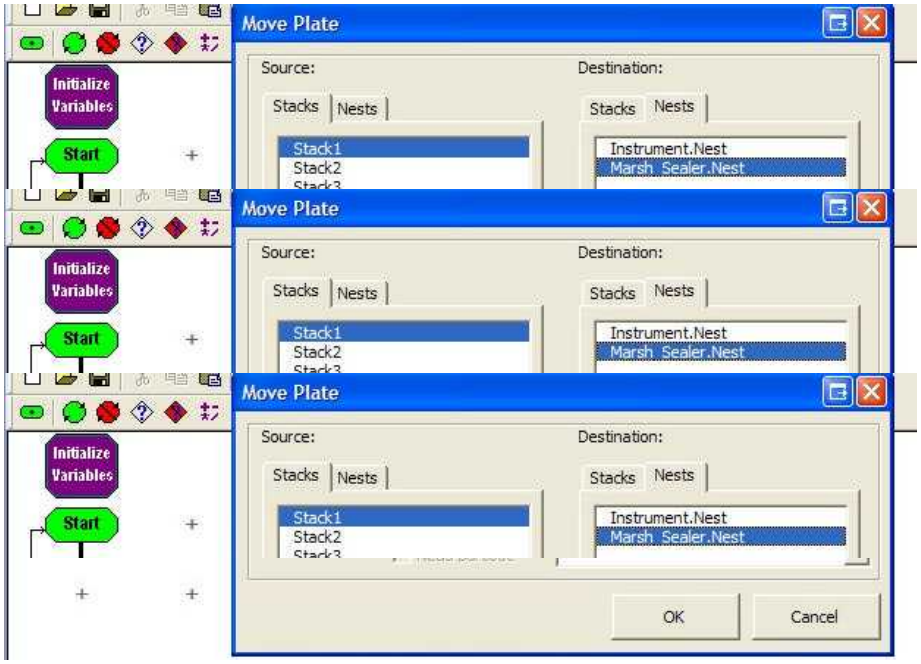
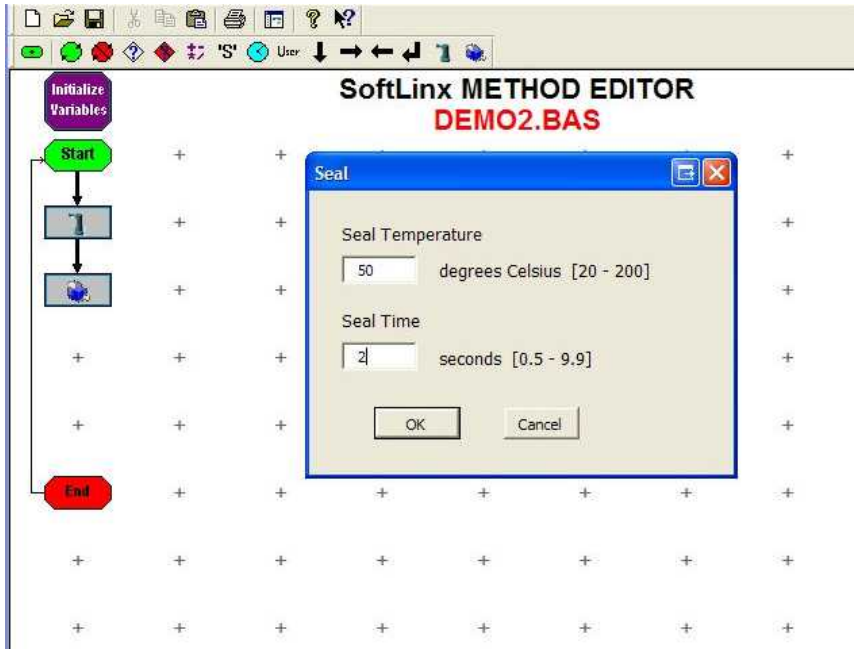
Adding an Instrument to an Assay



Individual Steps can be Modified in Many Ways



Defining a Robot Movement



Running an Instrument

- Many 3rd party instruments have their own software that follows specificscripts
- Softlinx will submit these scripts to the instrument
- Users can write Visual Basic methods to create these scripts on the fly

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Advantages of Automated Assay Optimization

Identify Subtle Effects that can Invalidate an Assay

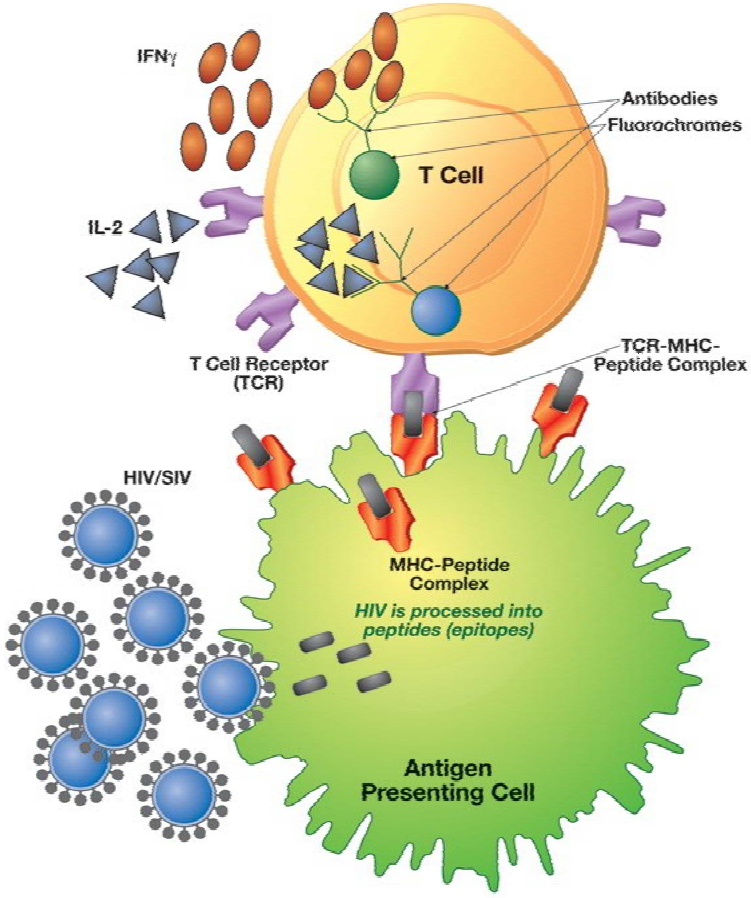
- Involvement of small polar entities – eg. DMSO
  - Introduce false positives/negatives

- Occlusion of binding sites
  - Multiple protein crystal forms

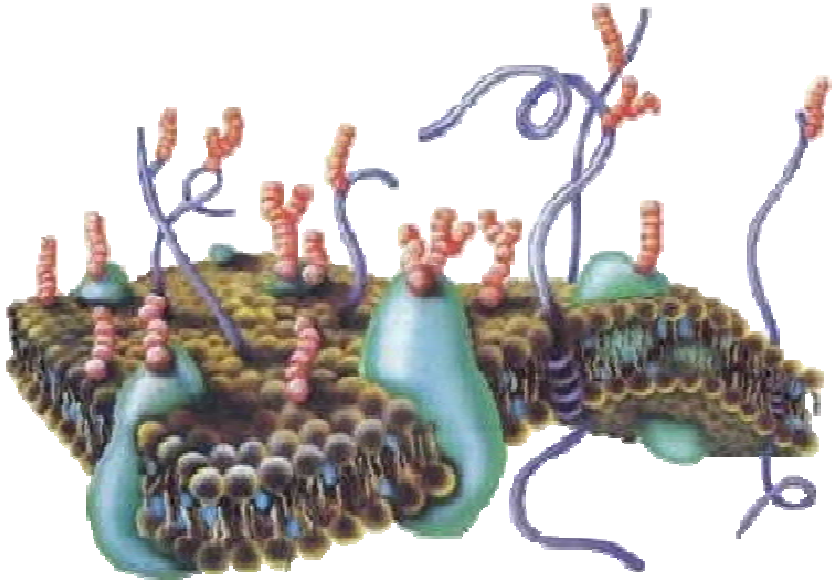
- Changes in protein conformation
  - Loss of normally available binding site
  - Appearance of non-physiological binding site
  - Interference of protein-protein interactions
  - Altered antibody affinity

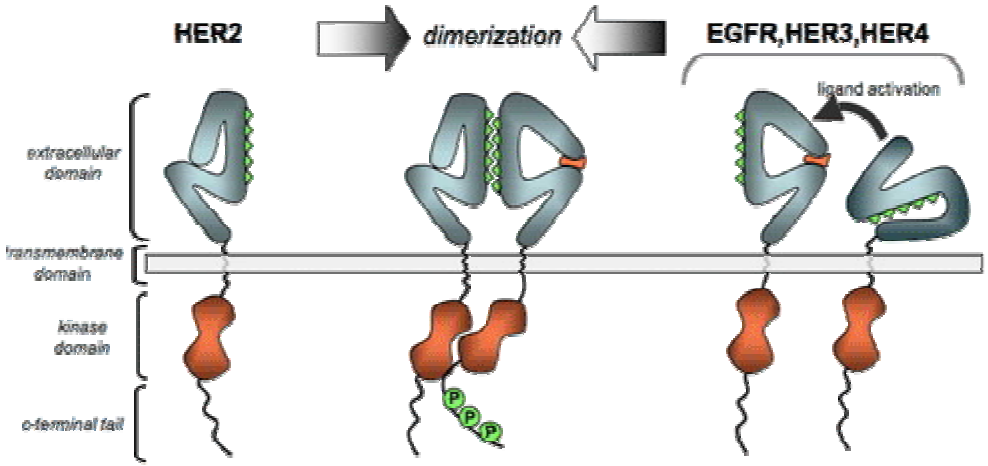
- Changes in chemical reactivity
  - Altered solvation of small molecules/functional groups
  - Altered chemical properties of reactants

Example 1: Effect of Media on Molecular Recognition



Eg. Autoimmune disease





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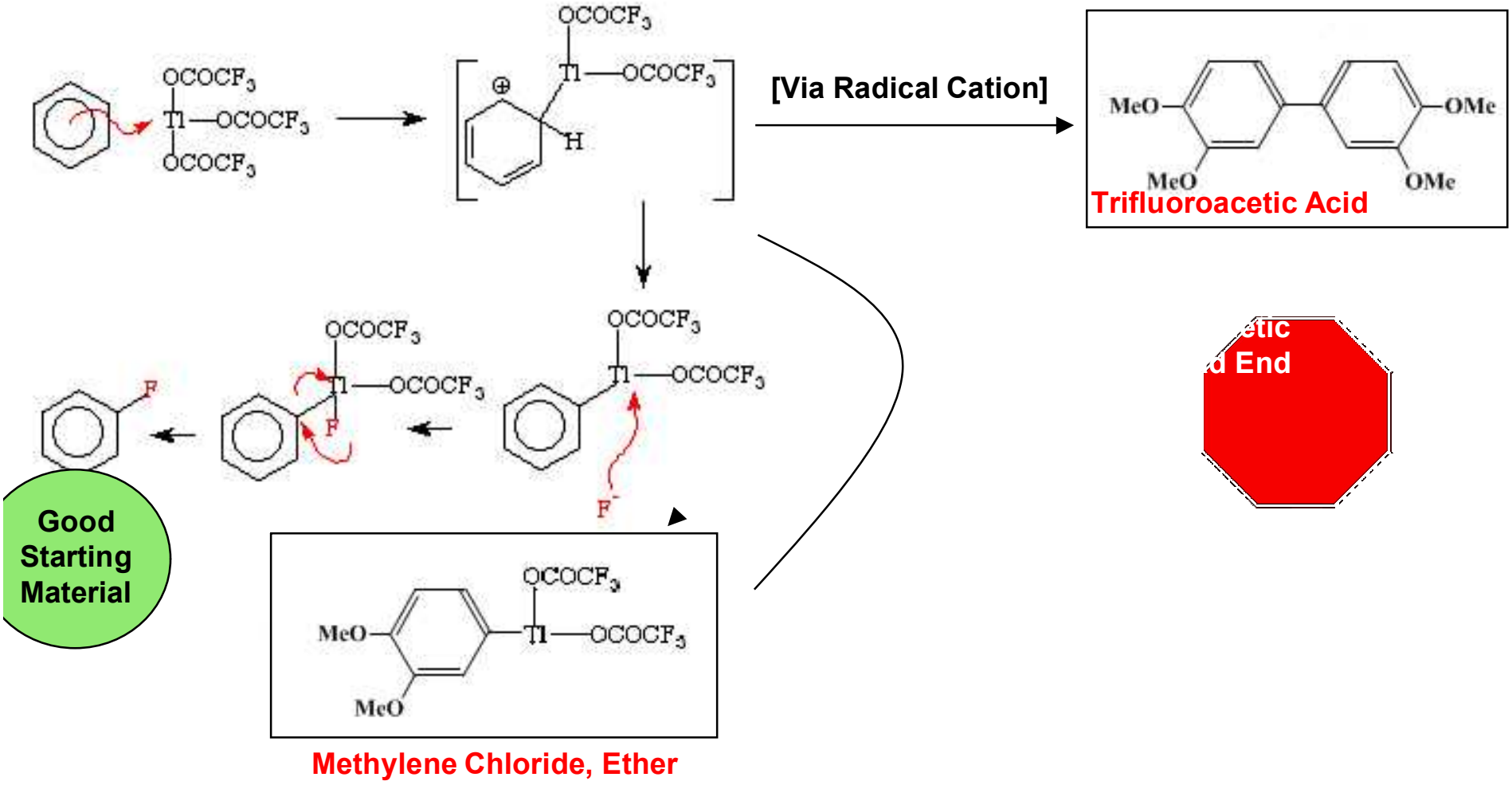
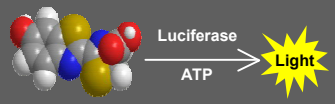
Example 2: Altered Chemical Activity

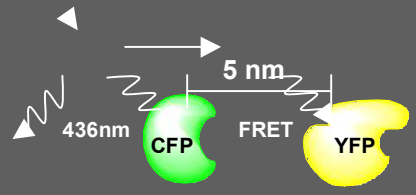
Electron-Rich Aromatic Rings:
Completely different products depending on solvent change



Pre-Defined Protocols

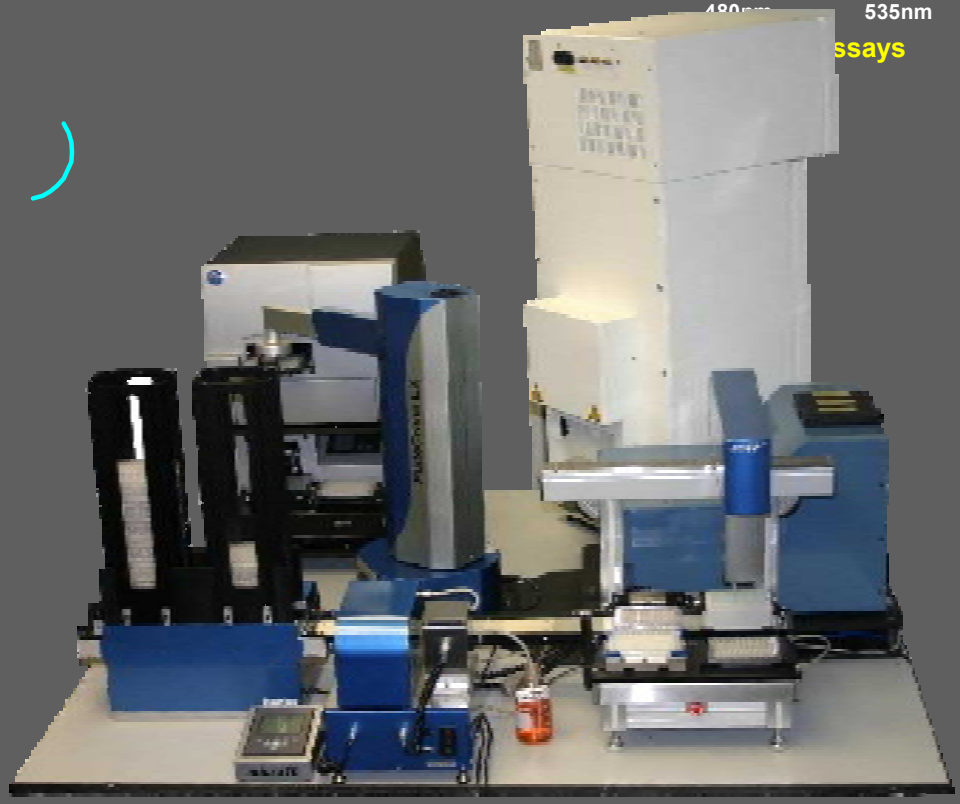


Luminescence

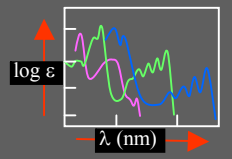


ssays

ALPHA Screen



ELISA



UV-Visible Absorbance

Fluorescence Polarization

Good Starting Points for New Complex Assays

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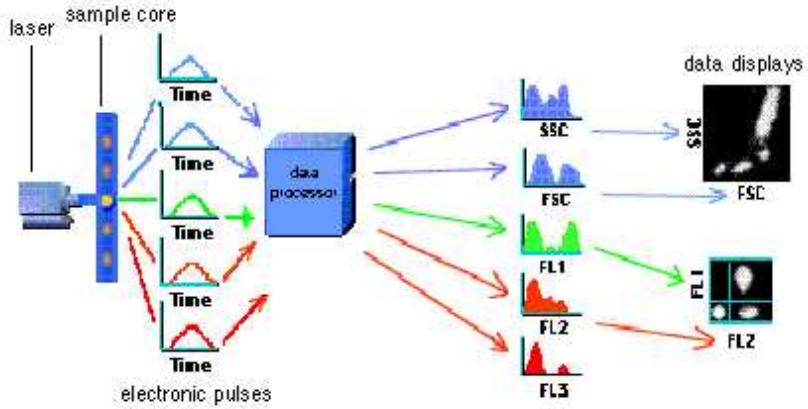
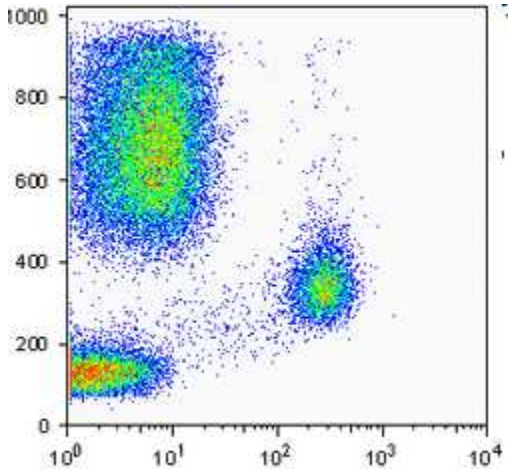
Example – Incorporate Flow Cytometry





Introduction to Flow Cytometry: A Learning Guide
http://info.med.yale.edu/immuno/cytometry/docs/pdf/introduction.pdf

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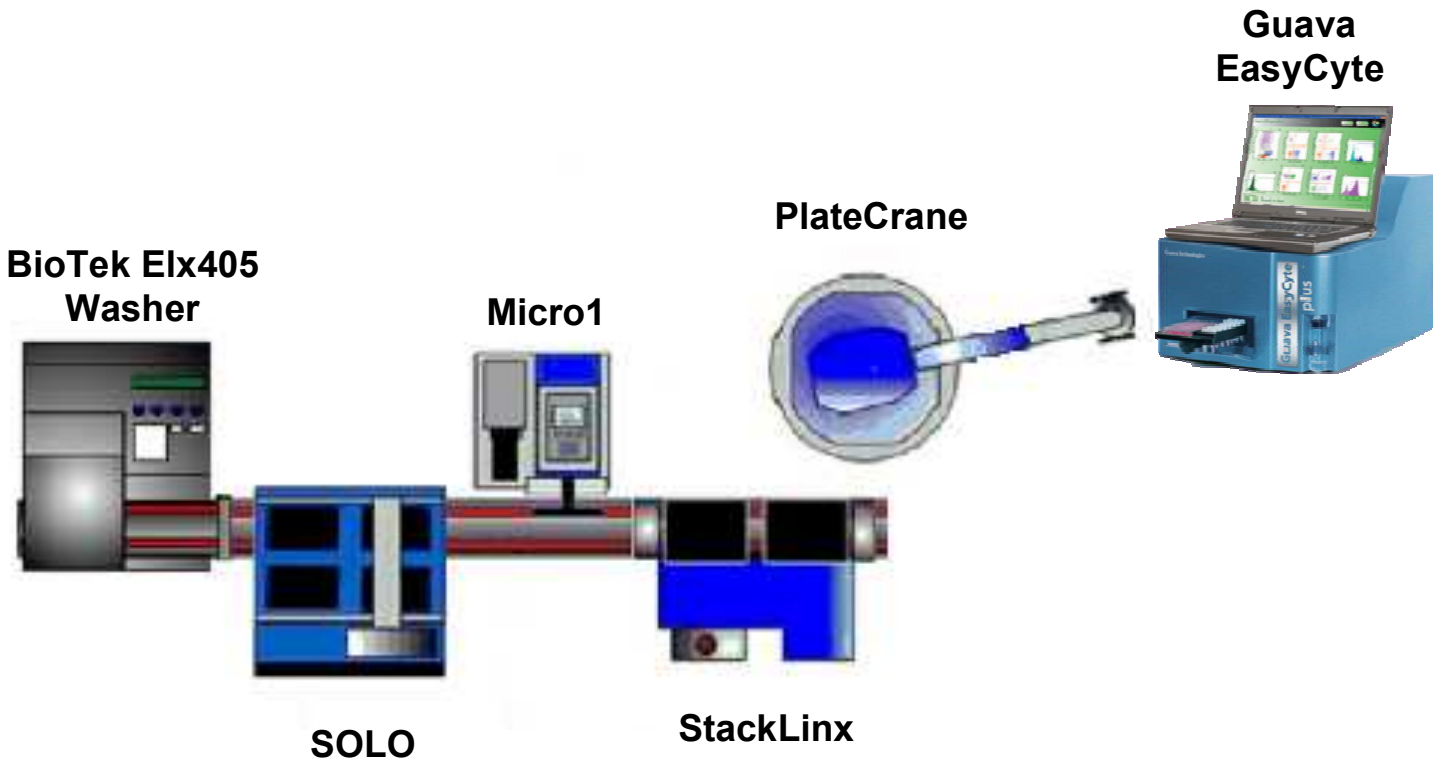
Standard Flow Cytometry WorkCell



- Cell Counting - Viability
- Apoptosis
- Cell Proliferation
- Cytotoxicity
- Cell Cycle
- Antigen Detection
- Antibody Quantification

Flow Cytometry is Too Slow for Large HTS

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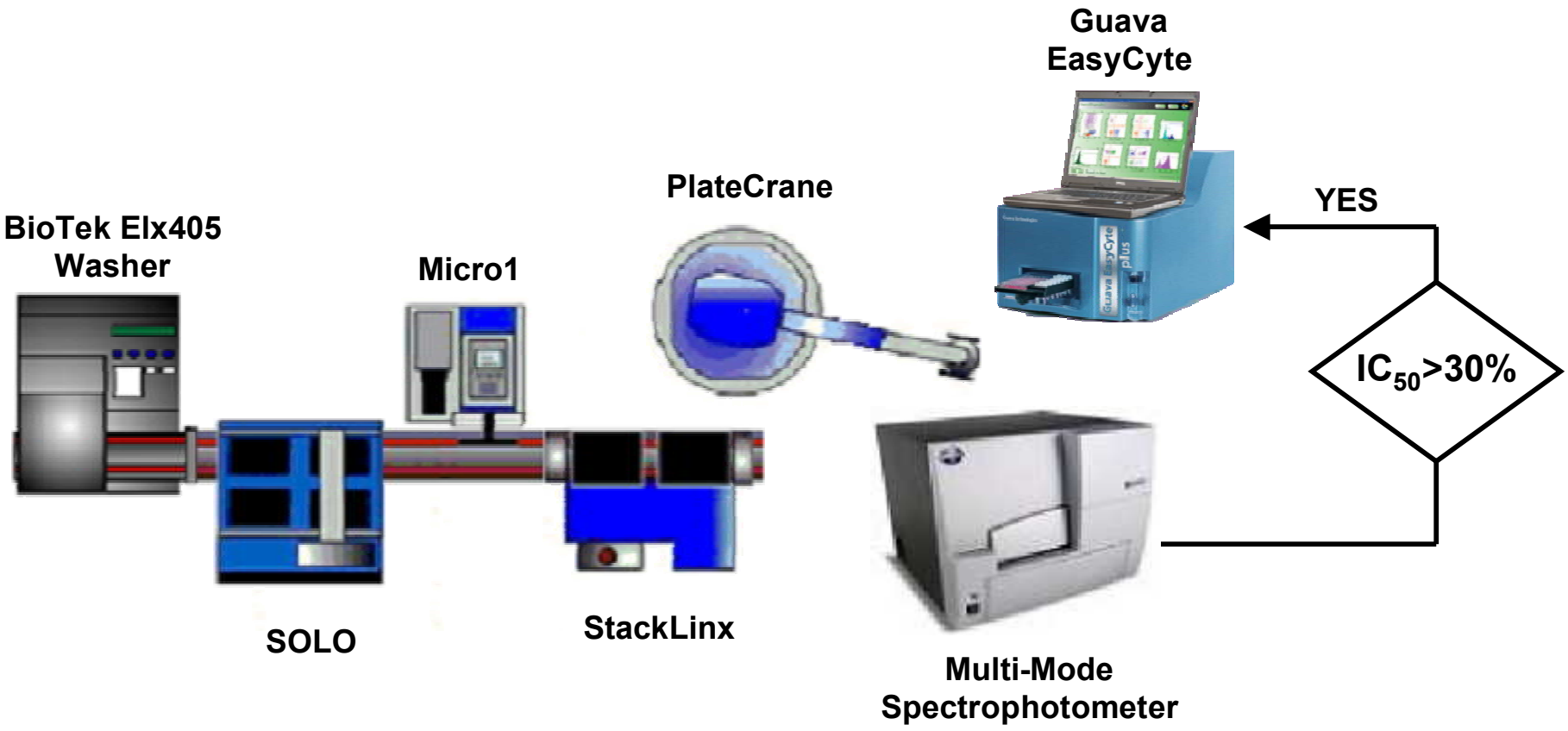
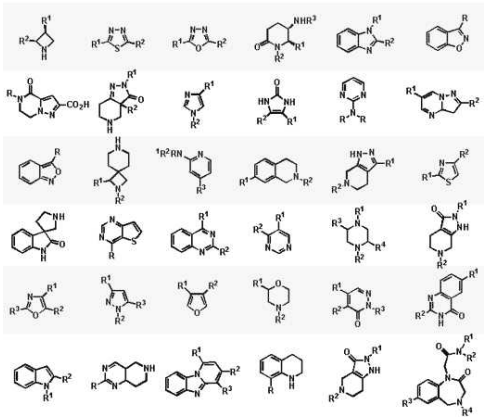
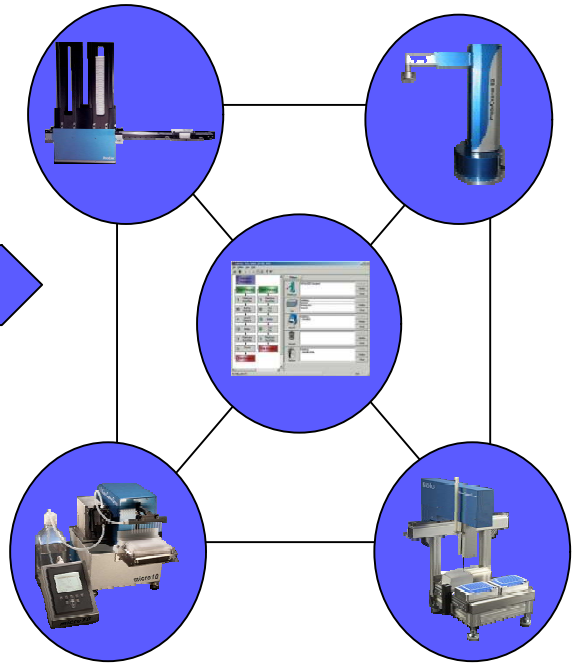
DualReader HTS System
"Interactive Cherry-Picking"



- First Check if the Cell shows Fluorescence after washing
- Fluorescence Assay Determines need to submit to the Flow Cytometer

One Step HTS with Fluorescence PreFilter





- Need compounds that cause antigen to appear on cell surface
- Flow Cytometry experiment involves Fluorescence-labeled antibody
- First check antibody's existence in spectrophotometer

Desired fluorescence?

Yes

No

Skip

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