



The Way Ahead.™

# **“The Future of Medical Laboratory Practice”**

## **A Manufacturer’s Perspective on Regulations, Standards, and Guidelines in Advanced Diagnostics Microarray Product Development**

*CLIAC Advisory Meeting, CDC, Atlanta, Georgia*

*September 20-21, 2006*

# Agenda

- Manufacturability of microarray platform technology
  - process reproducibility,
  - manufacturing controls
  - verification
- Standardization
  - MACQ
  - ERCC
- Role of the CLIA Laboratory in translating complex assays to clinical practice
  - CLIA lab role for Affymetrix
  - Impact on microarray assay quality assurance and standardization in the laboratory

# Affymetrix Today

Bedford, MA



London, UK



Tokyo



Singapore



Shanghai



Emeryville, CA



Sacramento, CA



Santa Clara, CA

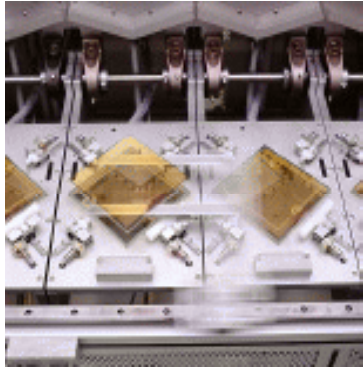


S. San Francisco, CA



- Headquartered in Santa Clara, CA
- >1,000 employees worldwide
- R&D in Santa Clara, S. San Francisco, Bedford & Emeryville
- Array manufacturing in Sacramento & Singapore
- Instrument manufacturing in Bedford
- Commercial offices in Santa Clara, London, Tokyo, and Shanghai

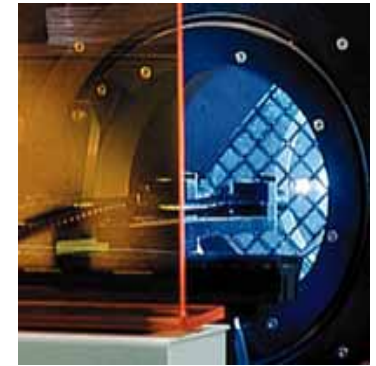
# Semi-Conductor Manufacturing and Combinatorial Chemistry



**Wafer Prep**



**2005: Currently approaching  $\sim 10^{10}$  oligonucleotides per day!**



**Photolithography**



**Cartridge Assembly**



**Finished GeneChip® Probe Array**

# Industrial Manufacturing Capability



**Sacramento  
Expansion**



**New Site:  
Singapore**

- Producing commercial research products since 1996
- >500,000 commercial arrays per year
- Added ~60% to array manufacturing capacity since September 2005
- Broke ground on Sacramento array and reagent manufacturing facility expansion in October 2005
- Initiating commercial manufacturing operations in Singapore in 2H 2006
- Doubling our installed wafer capacity in 2006 - should meet our needs for years to come

# GMP-compliant, ISO-certified Array Manufacturing



**Photolithography**



**Chemistry**



The Way Ahead.™

# Manufacturing Overview

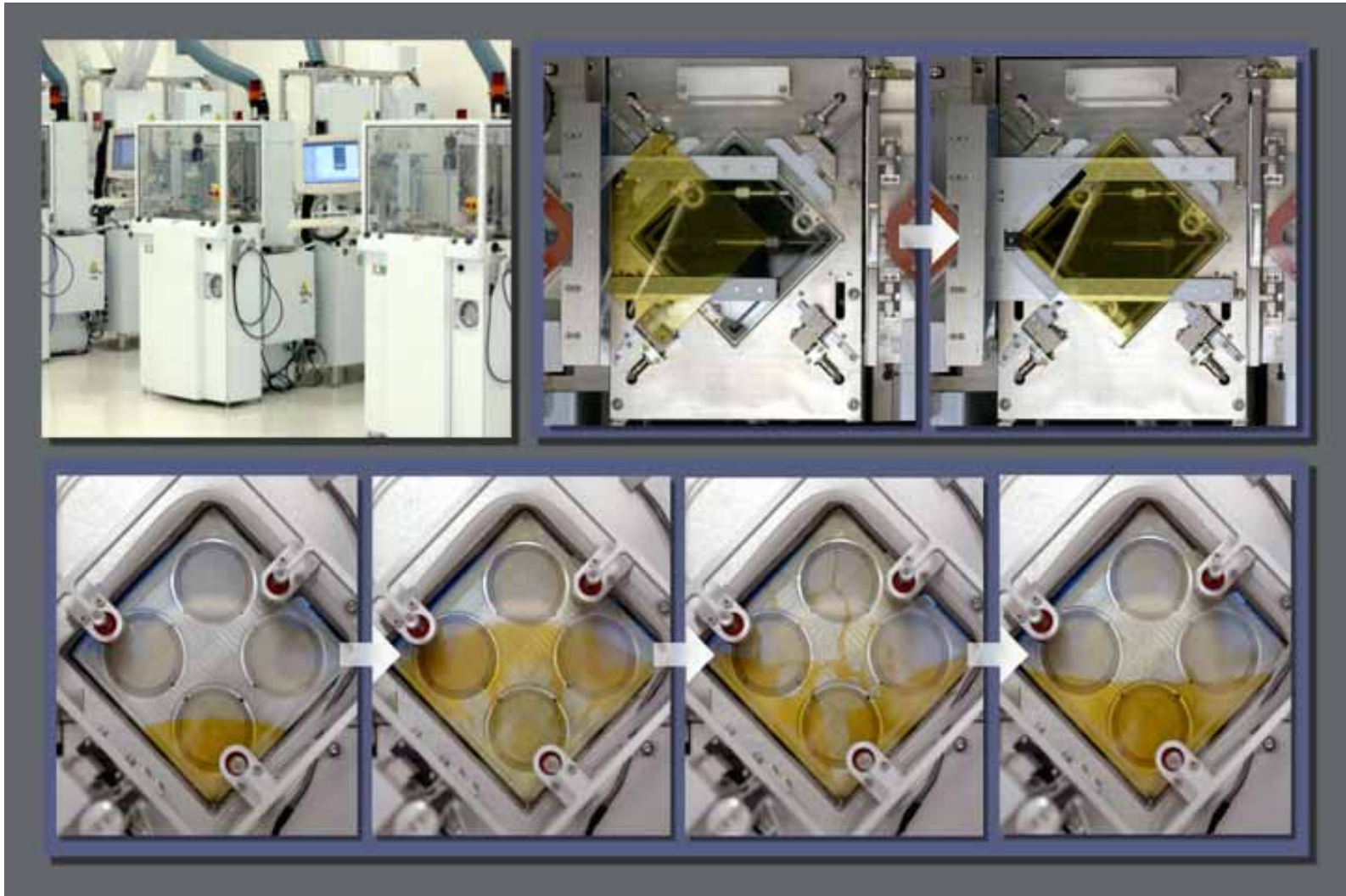
*West Sacramento Array Manufacturing*

# GeneChip Array Production Process

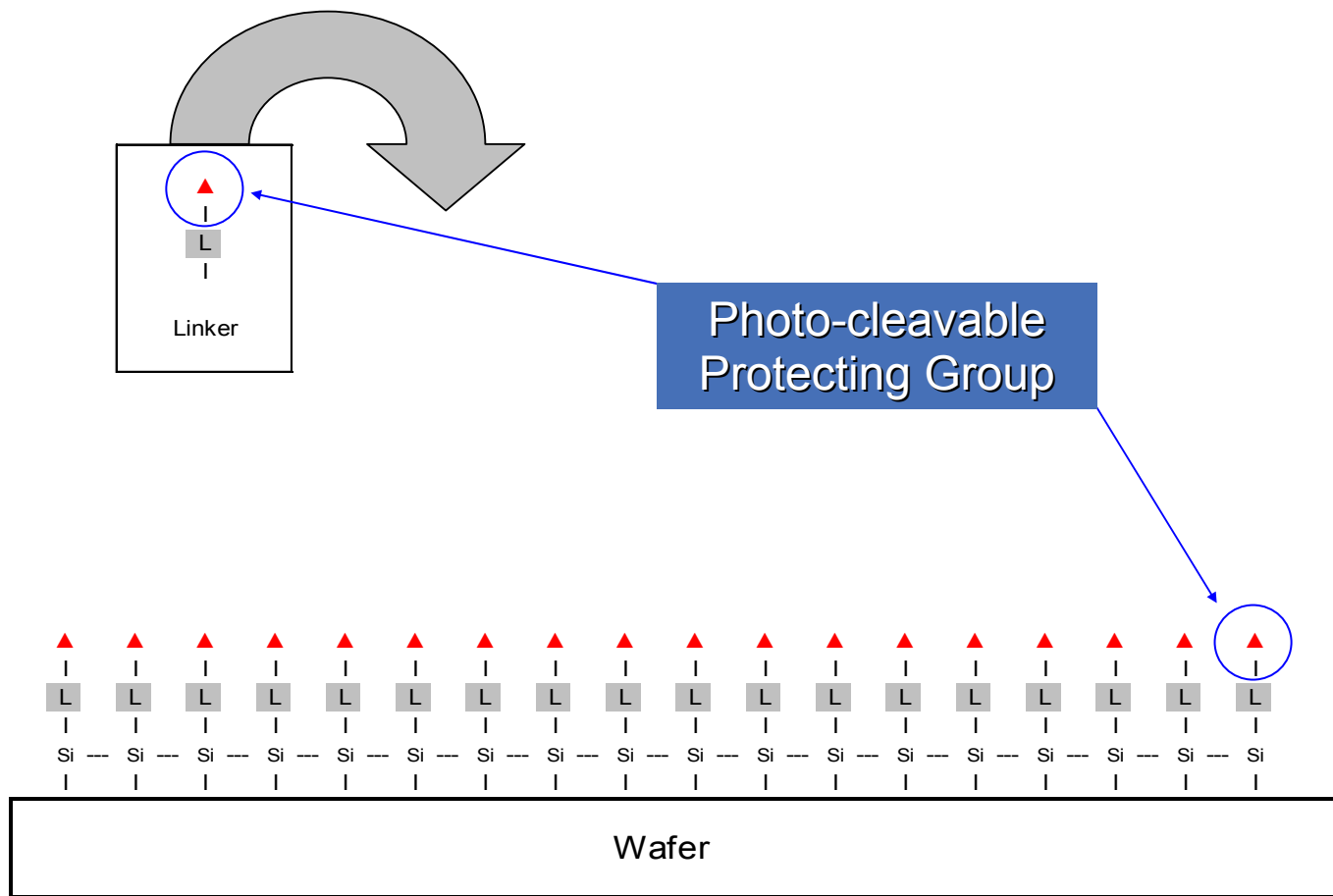
- Design verification
- Synthesis verification
- Signal intensity
- Validation of functional performance



# Chemical Synthesis Station



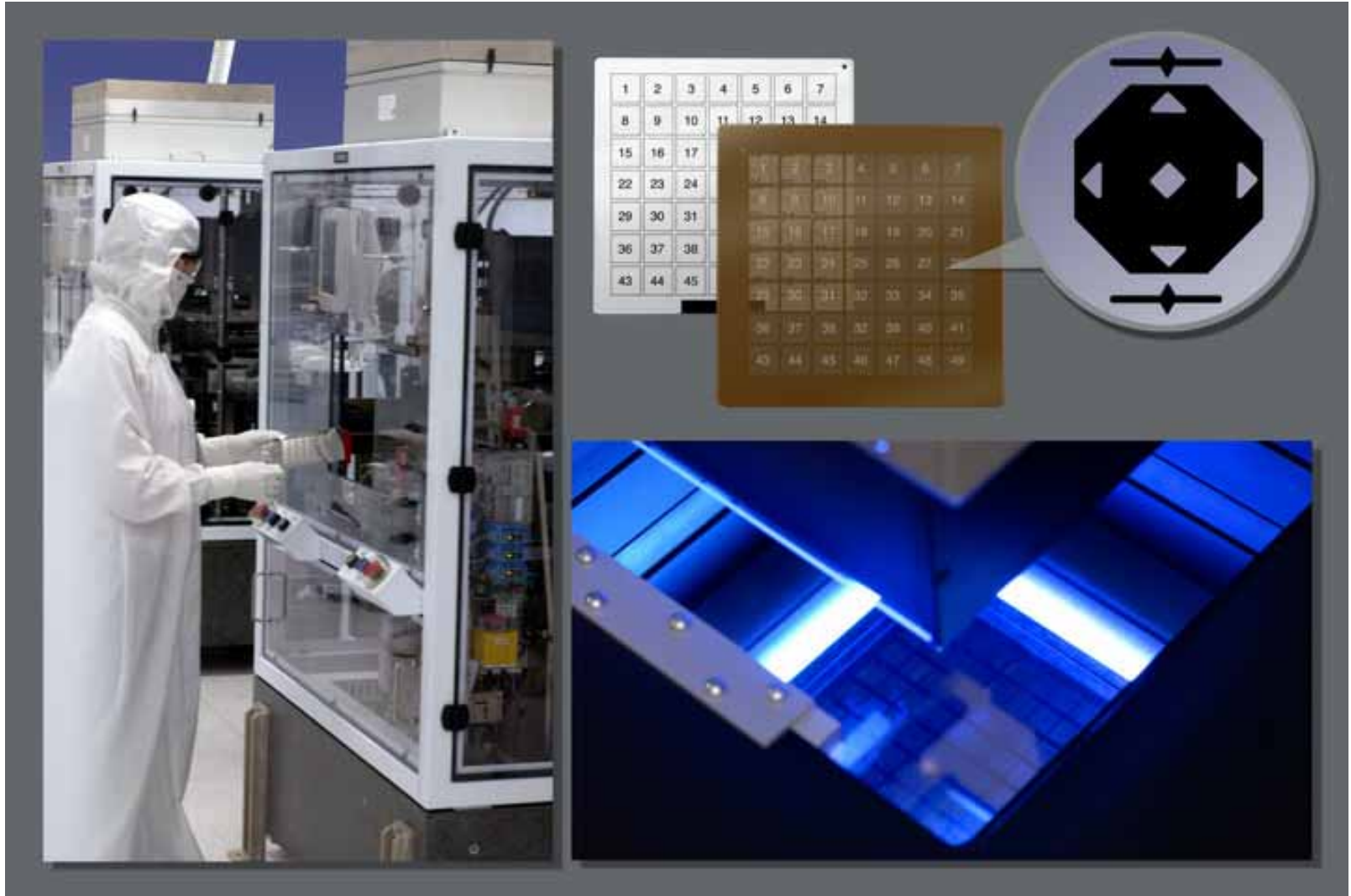
# Linker Addition



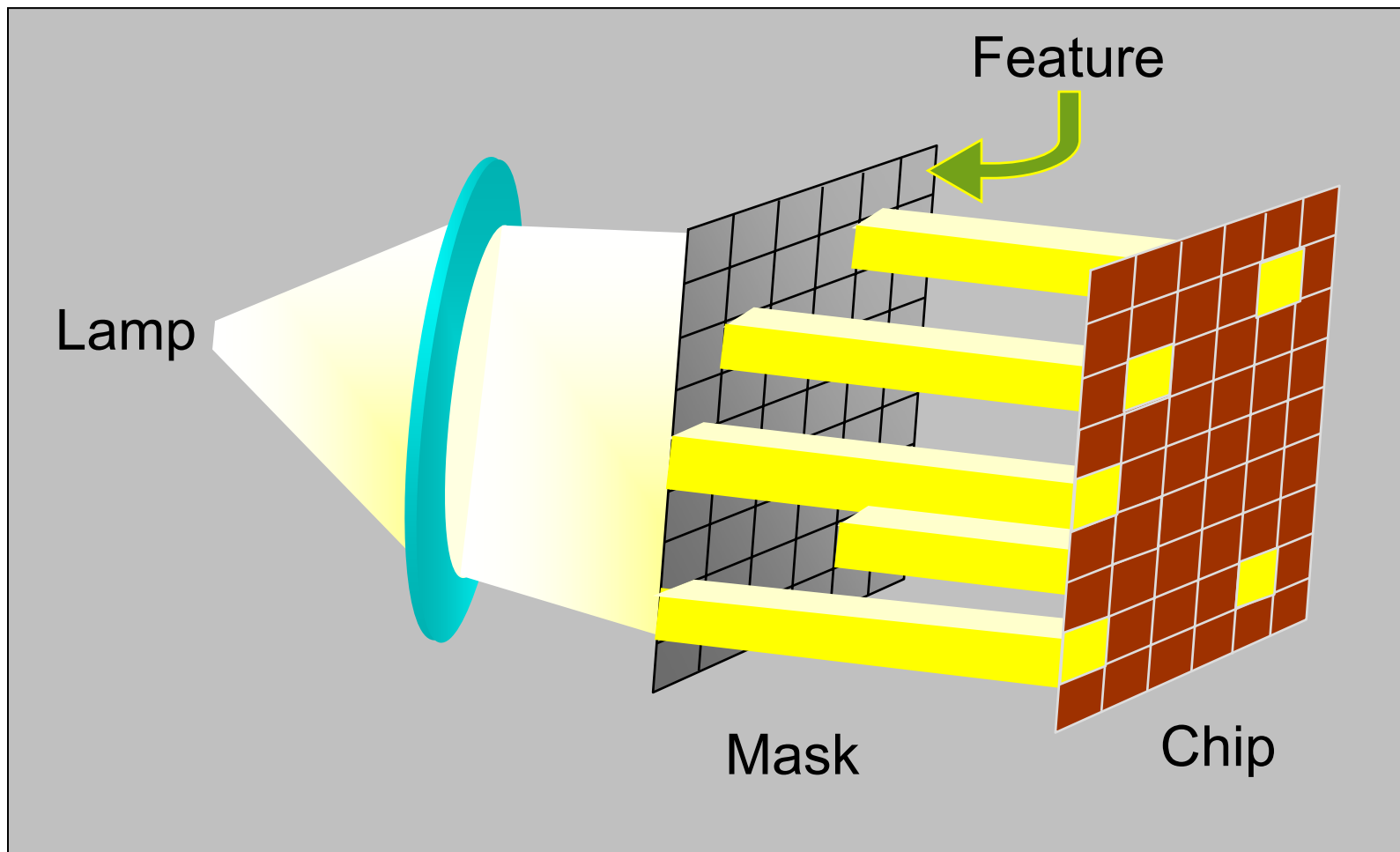


The Way Ahead.™

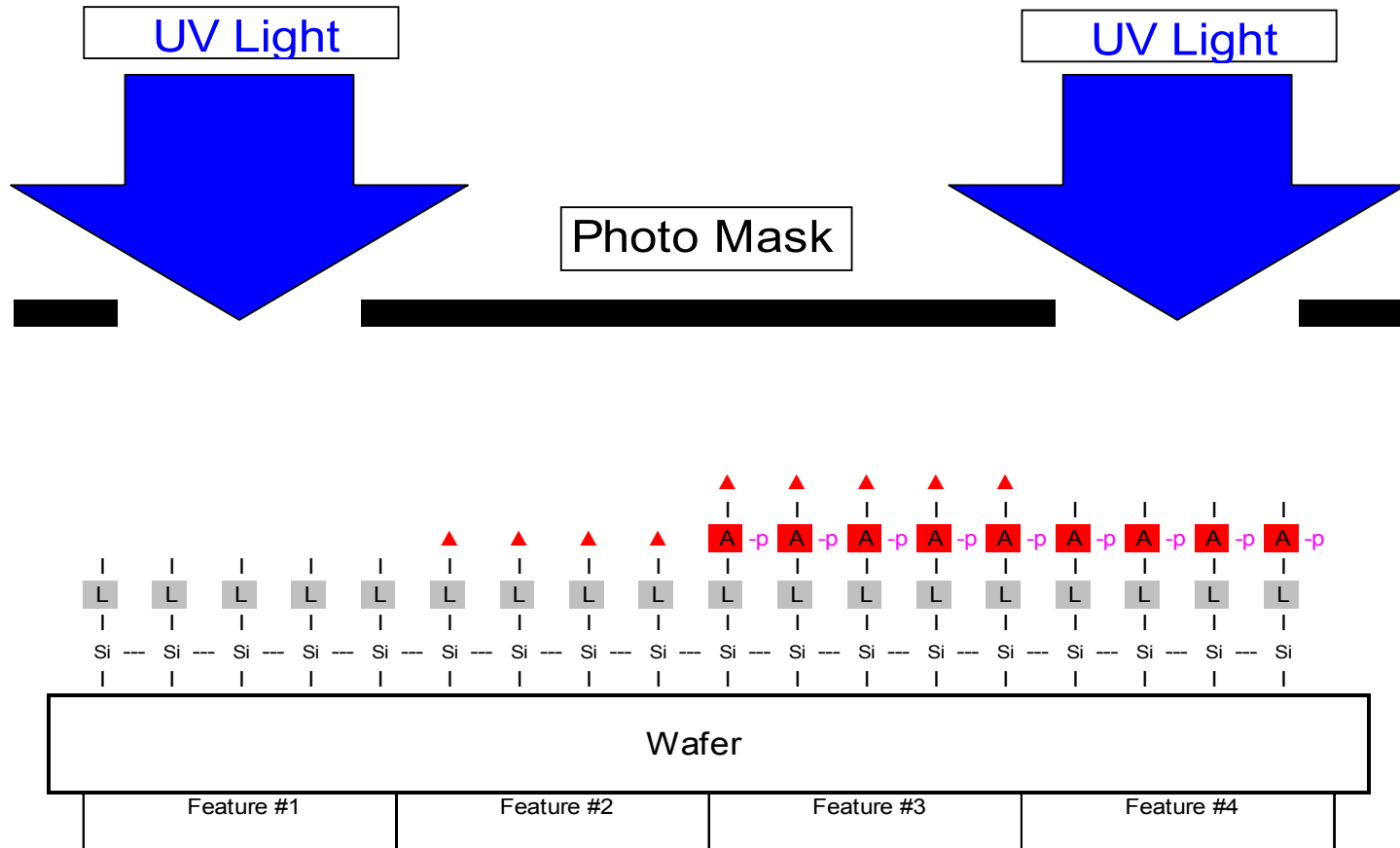
# Photo Aligner



# Photolithographic Synthesis



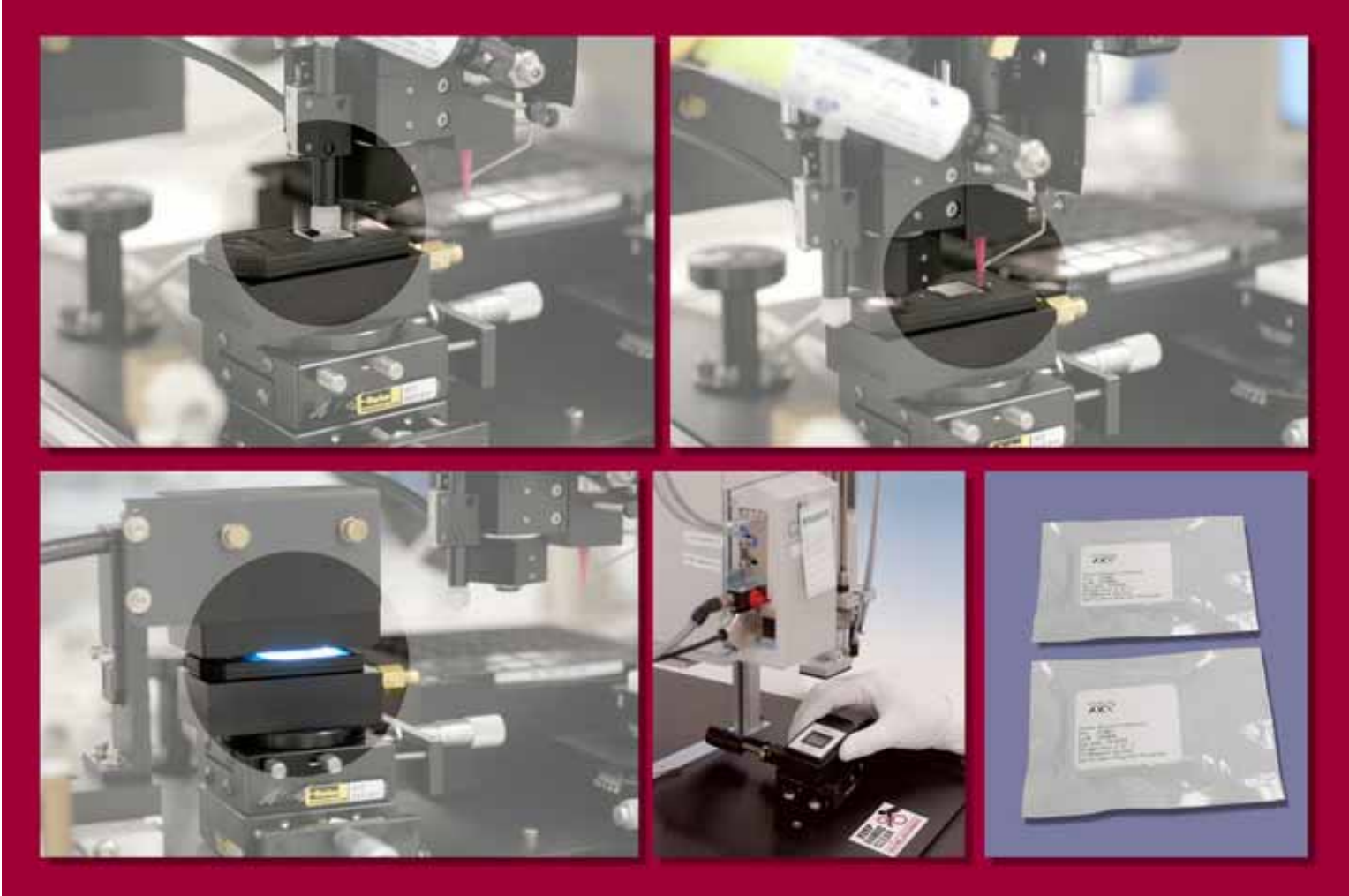
# Spatial Photo-Activation





The Way Ahead.™

# Cartridge Assembly Station





The Way Ahead.™

# GeneChip® Quality Assurance

**Quality Systems**

**Documentation**

**Validation**

# Affymetrix Quality Policy

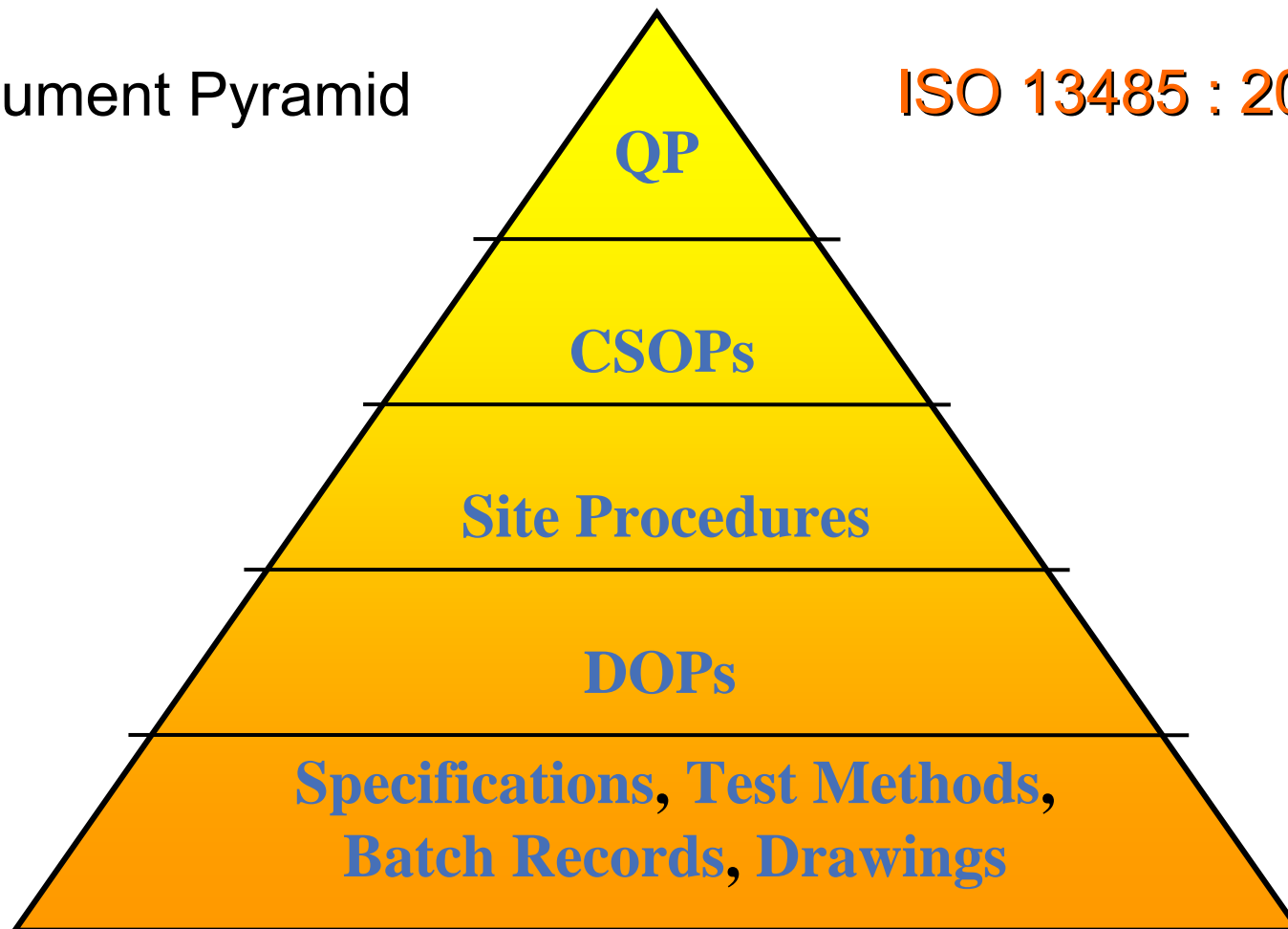
**Affymetrix is committed to our customers.** We are dedicated to consistently providing the **highest quality genetic information products, systems, software, and services.** Our goal is to understand and then **meet or exceed our customer requirements.** We will accomplish that goal through an environment of **innovation and continuous improvement throughout the organization.**



# Affymetrix Quality Structure

Document Pyramid

ISO 13485 : 2003



# Array Manufacturing Validation

- Facilities Validation
  - HVAC, DI Water, Compressed Gasses, etc.
- Equipment Qualification
  - IQ
  - OQ
  - PQ
- Sub-Process Qualification
  - PPQ for substrate, synthesis, and packaging
- Process Validations (2000 & 2003)
  - From incoming materials to released inventory

# PROMIS Wafer Tracking

- Manufacturing Execution System
- Used by leading semiconductor companies since the 1960's
- Shop floor control system which is integrated into the individual instruments that process the arrays
- Allows full tracking and tractability throughout the manufacturing process

# Wafer Tracking

- PROMIS interactively tracks each step of the synthesis process
- Assures Quality within Process
- Use of Optical Character Recognition ensures correct wafer chemistry and exposure sequence
- Synthesis master instructions maintained in a central storage location

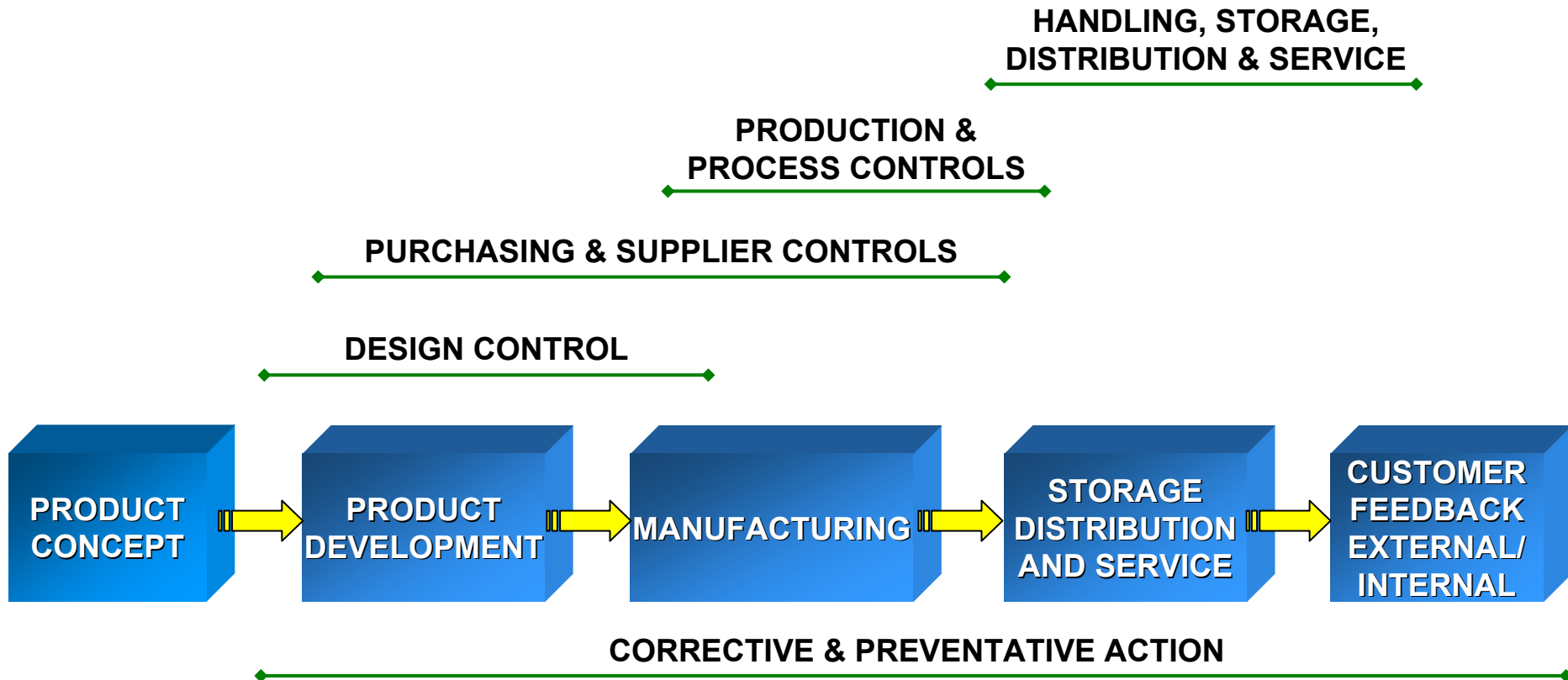
# Each Synthesis Step Is Tracked and Verified by PROMIS

```

11/15/01 110520 1007697e10 17:23:19 17:40:43 O7ODO EXPOSE_63-010163-71 DONE IDLE
11/15/01 110520 1007697e11 17:40:44 __:__:__ O7MOS1 ADDBASE_C
PROCESSING
11/15/01 110520 1007697e11 17:40:44 17:50:55 O7MOS1 ADDBASE_C      DONE IDLE
11/15/01 110520 1007697e12 17:51:15 __:__:__ O7ODO EXPOSE_63-010163-70
PROCESSING
11/15/01 110520 1007697e12 17:51:15 17:55:59 O7ODO EXPOSE_63-010163-70 DONE IDLE
11/15/01 110520 1007697e13 17:56:00 __:__:__ O7MOS1 ADDBASE_G
PROCESSING
11/15/01 110520 1007697e13 17:56:00 18:03:08 O7MOS1 ADDBASE_G      DONE IDLE
11/15/01 110520 1007697e14 18:09:01 __:__:__ O7ODO EXPOSE_63-010163-69
PROCESSING
11/15/01 110520 1007697e14 18:09:01 18:14:33 O7ODO EXPOSE_63-010163-69 DONE IDLE
11/15/01 110520 1007697e15 18:14:34 __:__:__ O7MOS1 ADDBASE_T
PROCESSING
11/15/01 110520 1007697e15 18:14:34 18:20:51 O7MOS1 ADDBASE_T      DONE IDLE
11/15/01 110520 1007697e16 18:25:48 __:__:__ O7ODO EXPOSE_63-010163-68
PROCESSING
11/15/01 110520 1007697e16 18:25:48 18:32:21 O7ODO EXPOSE_63-010163-68 DONE IDLE
11/15/01 110520 1007697e17 18:32:22 __:__:__ O7MOS1 ADDBASE_A
PROCESSING
    
```

# Affymetrix's Quality System

## MANAGEMENT RESPONSIBILITY





The Way Ahead.™

# GeneChip® Quality Control

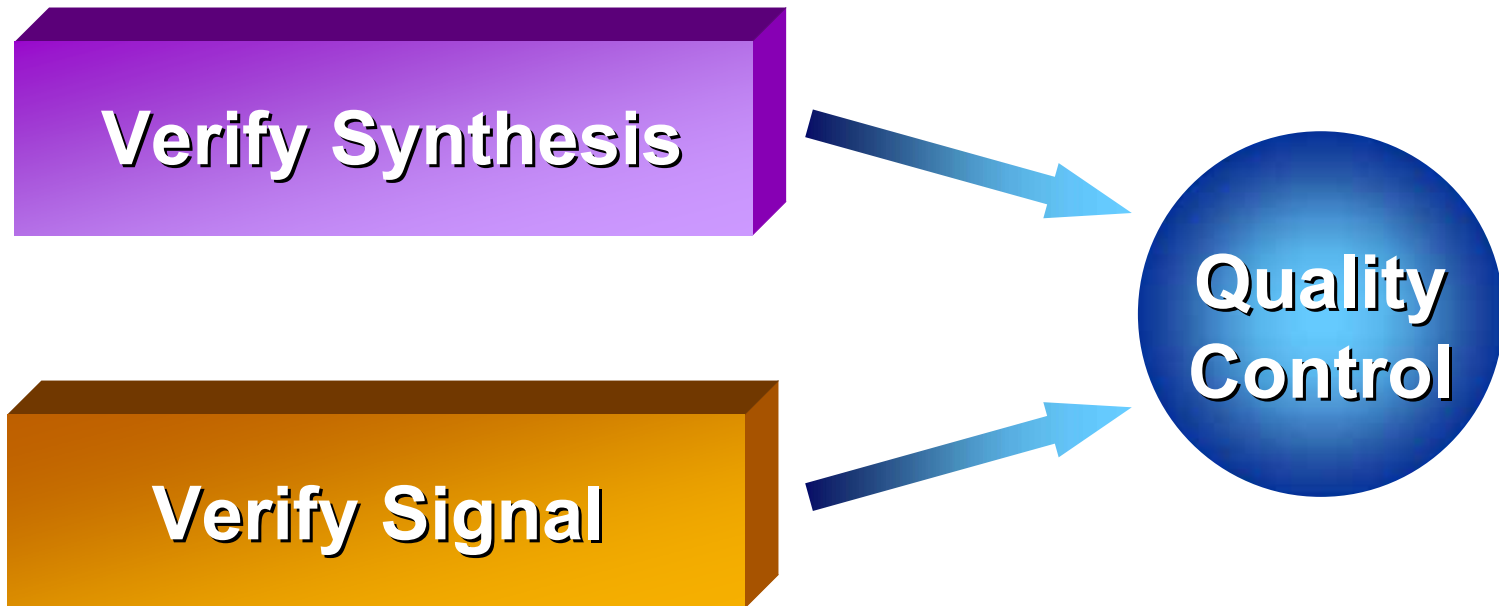
**Verify Design**

**Verify Synthesis**

**Verify Signal**

# Quality Control Testing for Gene Expression Arrays

Goal: Ensure the delivery of acceptable arrays  
(> 1,000,000 probes / array)





# Synthesis Confirmation

	1	2	3	4	5	6	7	8	Probes Synthesized
	A	C	G	T	A	C	G	T	
<b>Probe1</b>	<b>A</b>	<b>C</b>	-	<b>T</b>	-	<b>C</b>	-	-	ACTC
<b>Probe2</b>	-	-	<b>G</b>	-	<b>A</b>	-	<b>G</b>	<b>T</b>	GAGT
<b>Probe3</b>	<b>A</b>	-	<b>G</b>	-	<b>A</b>	-	<b>G</b>	-	AGAG
<b>Probe4</b>	-	<b>C</b>	-	<b>T</b>	-	<b>C</b>	-	<b>T</b>	CTCT

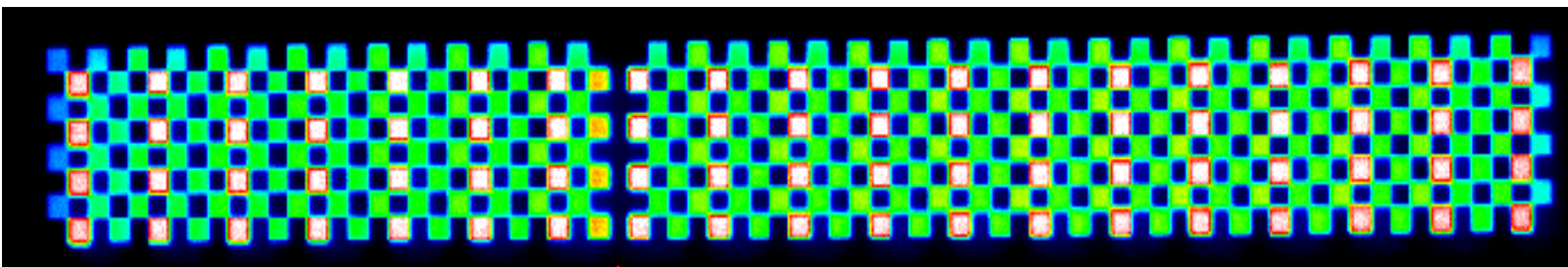
Verifying synthesis of a few probes confirms acceptable synthesis of all probes due to the power of combinatorial synthesis

# Verification of Synthesis via Synthesis Control Probes

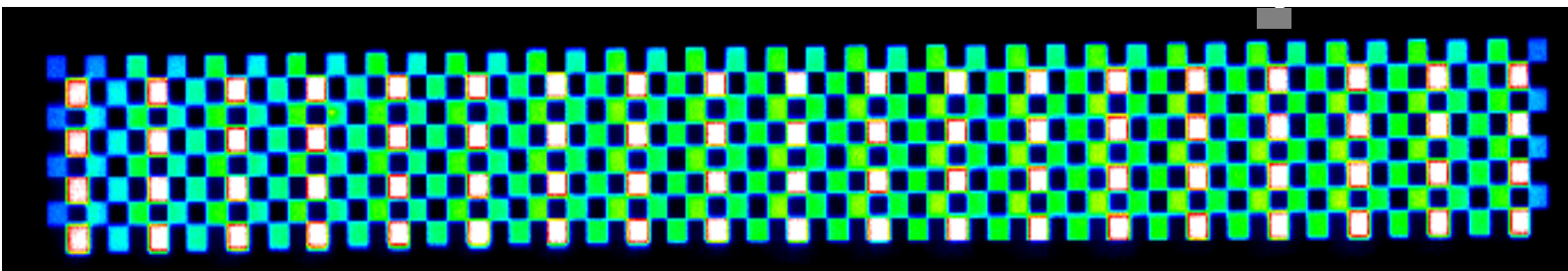
- Control probes provide synthesis confirmation
- Unique group of probe cells represent each chemistry cycle
- Analysis of probe location identifies errant photolithography and chemistry cycles

# Synthesis Verification Results

Wafer A: unacceptable synthesis - probe cells missing



Wafer B: acceptable synthesis



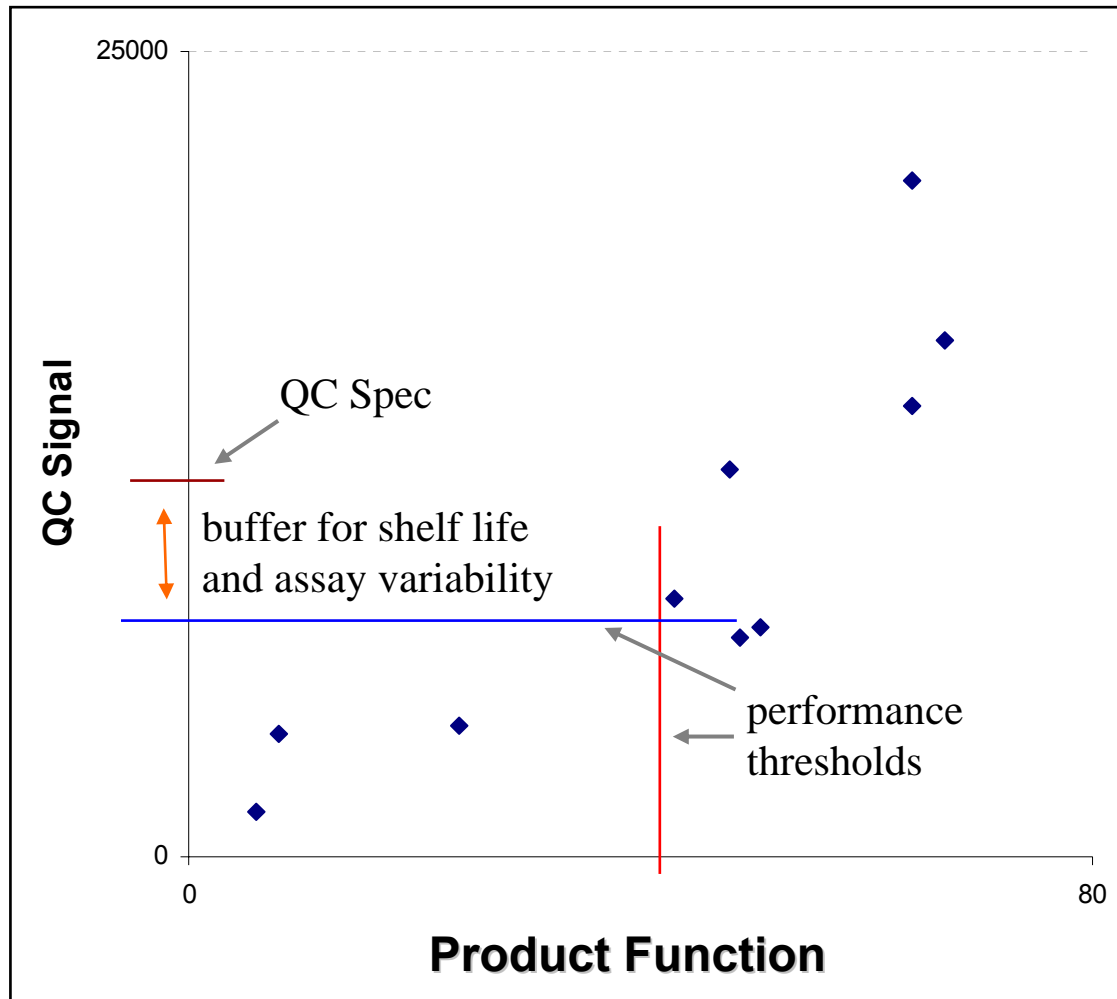
# QC Assay Mimics Product Assay

- QC Sample →
  - Synthetic oligonucleotides
  - Control transcripts (bacterial sequences)
  - No complex target background

***Controlled System***  
***Test the Arrays not the Assay***

- Analysis →
  - Based on hybridization pattern and signal

# QC Assay – Signal to Functional



Each Point represents a standard or variant lot

# GeneChip® Array Manufacturing

- Big chips – Small chips
  - Big Features – Small Features
  - DNA Analysis – RNA Analysis
- 
- Same Manufacturing Equipment
  - Same Manufacturing Process
  - Same Quality Strategy
    - Design, Signal, Synthesis



The Way Ahead.™

# Standards and Practices

*Microarray Technology 2006*

# Overview

- Standard controls are necessary tools for quality practice and results in clinical applications, these tools aid in data exchange, drive acceptance and facilitate regulatory approval
- AFFX is directly involved in moving array technology into clinical practice through the establishment of best practices, standard controls, standardized methods, accessible quality controlled kits with controls
- Dedication to quality and innovation by AFFX- leadership and participation in consortia on standards with industry, academic and national institutes



# MicroArray Quality Control (MACQ) Project 2006- Nature Biotech, 9/8/06

- Leming Shi, FDA-government, academia, industry study to aid FDA in establishing guidelines and quality control procedures for the agency
- “Gold Standard” comparisons of microarray systems
- Goal: Generate a vast reference performance data set to evaluate reliability of DNA microarrays
- Systematically investigate cross-experimental, cross-platform, and cross-laboratory variability in microarray assay format

# MACQ Project Design

- 137 participants, 51 organizations
- Gene expression levels were measured from 2 RNA samples in four titration pools, seven microarray platforms, in addition three alternative expression methodologies were analyzed, multiple sites
- Software used to generate quantitative signal and qualitative detection call provided by each platform manufacturer
- Protocol included both one-color and two-color microarray platforms, sequence information provided by each manufacturer

# Outcome

- All microarray platforms tested showed both intra- and inter-platform consistency with high degree of concordance in terms of identifying expressed genes
- Platforms detected known differences in gene abundance between defined RNA mixtures and generated comparable results to alternative gene expression methods
- Differences were noted in various performance parameters between platforms- intra-site repeatability, inter-site reproducibility consistency in the detection calls-tradeoffs in repeatability, sensitivity, specificity and ratio compression

# Additional Analysis

- Platforms with divergent approaches to measuring expression often generated comparable results- AFFX (short oligonucleotides), and ILM (plasma-etched silicon wafers with long oligo-probes)
- Quantitative assessment of actual measured quantity of differential expression identify reproducible gene lists
- Importance of probe sequence information
- Protocol, time, or other variables not addressed

# External RNA Controls Consortium (ERCC)

- Leadership position- Chair-holder, Dr. Janet Warrington, AFFX
- 175 Members, 14 countries, 92 organizations
- Goal-produce external RNA expression controls, analysis tools and protocols for assessing within-platform technical performance
- Test Plan 2005- BMC Genetics, 6:150
- Microarrays, QRT-PCR
- CLSI guideline for use
- Timeline Oct 07

# Affymetrix Standards and Guideline Development Commitments

- Best Practices Paraffin Embedded Protocol Working Group
- External RNA Controls Consortium (ERCC)
- Microarray Quality Control Project (FDA MAQC)
- International Meeting on Clinical Laboratory Genomic Standards (IMCLGS)
- Affymetrix Control Program Genotype Data
- CLSI MM-16A Subcommittee
- CLIS Genetic Standards Committee
- IQLM
- OECD

# Publications

- Tong, W. Nature Biotech 24 (9), 1132-1139, 2006
- Shippy, R et al Nature Biotech 24 (9) 1123-1131, 2006
- MACQ Nature Biotech 24 (9) 1151-1161, 2006
- The External RNA Controls Consortium, Nature Methods 2(10):731-734 2005
- The External RNA Controls Consortium. BMC Genomics 6:150 2005
- Mutter et al. BMC Genomics 5:88-93 2004
- Hoffman et al. Nature Reviews 5:229-232 2004

## -Publications Continued

- Tumor Expression Best Practices, Nat Rev Genet 5, 229-237 2004
- Debey, S., et al, Pharmacogenomics J, 2004
- Dumbkowski, AA, FEBS Lett, 560, 120-4 2004
- Kenzelmann, M Genomics 83, 550-8 2004
- Bolstad, BM Bioinformatics 19, 185-93 2003

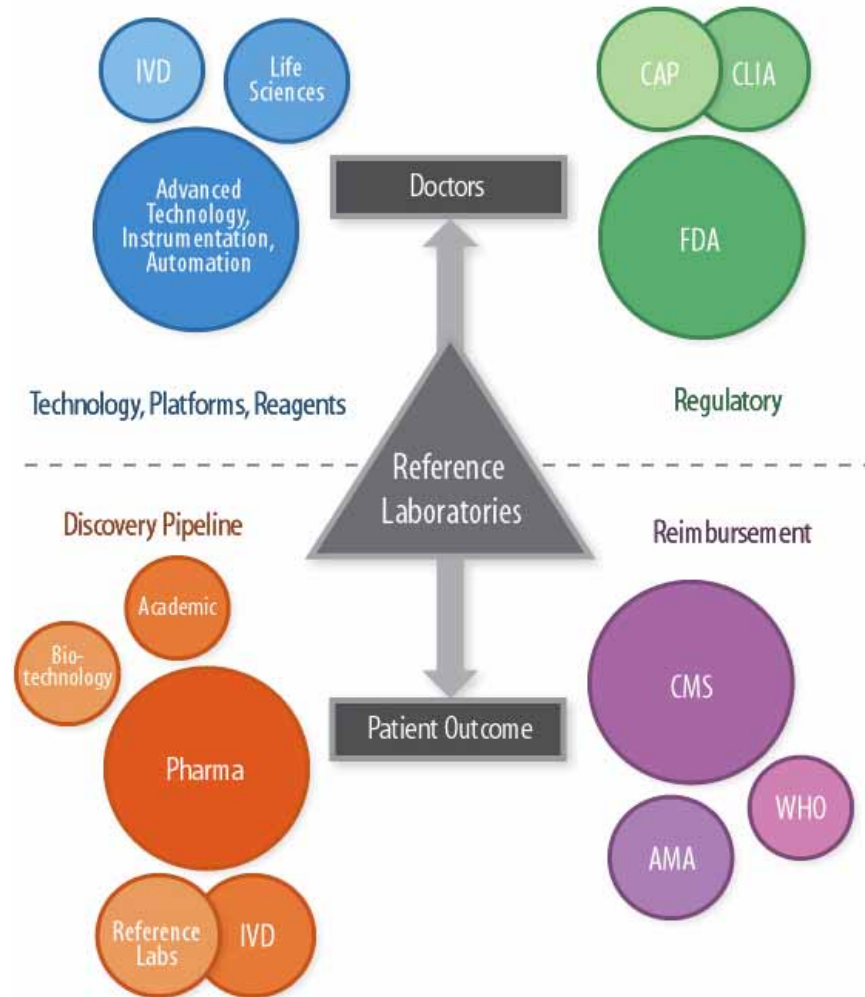




The Way Ahead.™

# **Role of the CLIA Laboratory in Advancing Emerging Technology in Clinical Practice**

# Key Stakeholders in Molecular Diagnostics



# Role of the CLIA Laboratory in Translating Complex Assays to Clinical Practice.

- Historical practice of introducing emerging technology, assays, new markers into laboratory practice by use of in-lab developed assays prior FDA regulated market release
- Esoteric laboratories specializing in advanced methods, specialized skill sets, development of clinical utility studies through collaboration
- Execute highest standard of rigorous analytical validation specifications, statistics, and workflow process development
- Allows significant data collection for emerging diagnostic applications for market and quality justification

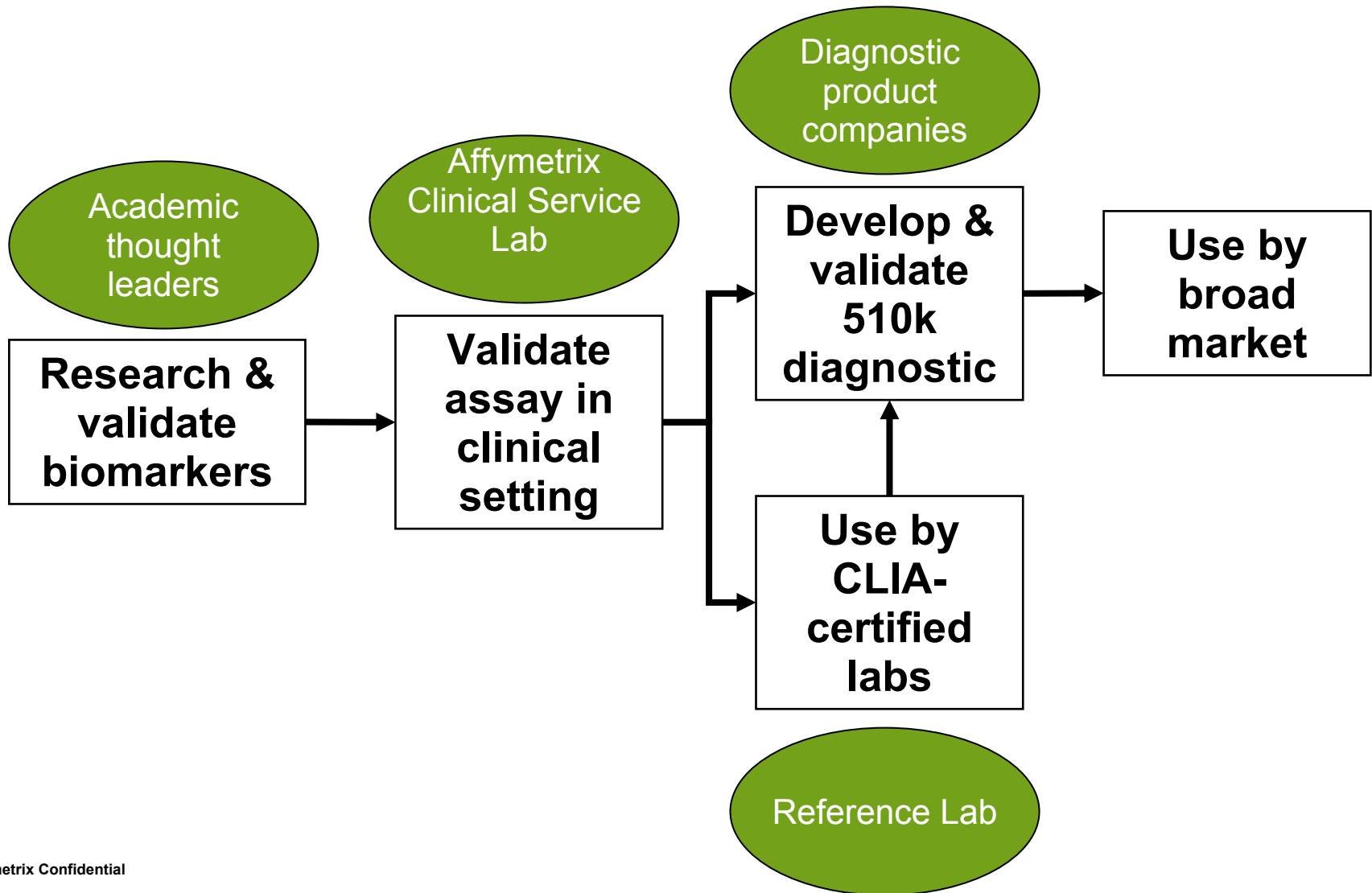
# Affymetrix CLIA Laboratory Strategy for Moving Microarray Technology to Clinical Practice

- Investment in CLIA reference laboratory for the design, implementation of workflow, quality assurance “best practices” for microarray clinical trials and patient testing
- Provide training and education programs to enable other clinical laboratories to effectively use GeneChip Services in clinical trials and patient testing
- Goal to accelerate the adoption of GeneChip technology in clinical practice through CLIA-regulated, validated, reproducible, standardized clinical laboratory operations

# Facilities and Environment

- New 10,000 sq. ft facility located in West Sacramento
- State-of-the-art molecular diagnostic laboratory specifically designed to meet optimized efficient CLIA microarray workflow requirements
- Automated high through-put nucleic acid extraction of tissue, blood- Beckman-Coulter NX Biomek systems, Agencourt and Nugen extraction chemistries optimized for automated system
- Beckman Arrayplex -dedicated automation for gene expression and genotyping assay-fully validated
- Integrated data management capabilities
- Cryostorage, dedicated clinical trials suites, security access control

# The Role Affymetrix Clinical Lab Can Play in Translating Research to Medicine



# Anticipated Outcome

- Highest level of quality over-site and continuous improvement of microarray assay technology platform in clinical laboratory practice
- Dissemination of best practices for complex testing to entire industry allowing rapid adoption of standardized practices throughout laboratories worldwide
- Continuous improvement on workflow design, data management and evaluation parameters
- Combined with manufacturing improvements and assay processing- reduced costs to healthcare providers
- Quality assurance guarantee for complex genetic, pharmacogenomics results

# Molecular Diagnostics Emergence

## Early Uses

*(increased pathogen sensitivity)*

### 1990's

Detection of Pathogens  
*(Blood Borne Viruses, STD's)*

### 2000's

Quantitative / Identification of Pathogens  
*(viral load, viral genotyping)*

### Current

Panels of Pathogens

- Respiratory
- GI
- CNS
- Single Gene Detection
- Wafer

## Future – Complex Signatures

### Current

Pharmacogenetics (*p450*)

### Oncology Screening, Staging & Therapy

- Breast
- Lung
- Leukemia
- Prostate
- Lymphoma
- Thyroid
- Colon

## Genomic / Genetic Diagnosis

### Expression Analysis

- Copy Number / inherited syndrome
- Copy Number / Cancer Dx