Design of an Epifluorescent Microarray Scanning Microscope

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Background

Microarray printing for:

- High throughput toxicology assays
- Enzyme inhibition assays
- Gene expression, etc.

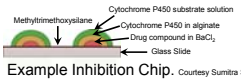
For toxicity assays:

Fluorescence microscopy of fluorophore stained slides is used to quantify cell viability.

Epiflurorescent scanning microscope design for rapid, precise scanning of toxicity assay slides at high sensitivity

Toxicity Assay Slide Preparation

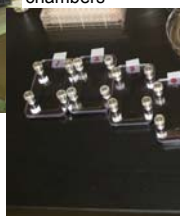
1. Clean & sterilize slides
2. Spin coat slides
3. Print scaffolding on cell slide and drug slide
4. Print live cells on scaffolding; freeze
5. Print drug on scaffolding
6. Mate cell slide to drug slide; incubate
7. Separate cell/drug slides
8. Stain cell slide
9. **Image cell slide – analyze cell death.** ← Microscope design



Example Inhibition Chip. Courtesy Sumitra Sukumaran

Microarrayer (Genomic Solutions MicroSys 4100 XL)

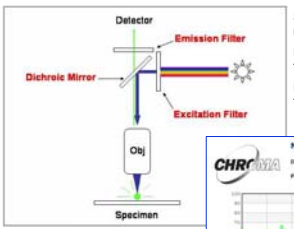
Microarray slide hybridization chambers



Microarray Slide

Photo courtesy Sumitra Meena Sukumaran; Rensselaer

Epifluorescent Microscopy

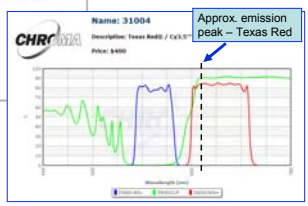


http://en.wikipedia.org/wiki/Fluorescence_microscope

Specimen is stained with a fluorescent molecule (fluorophore)

Excitation Filter used to excite fluorophore

Emission Filter passes desired fluorescence signal into camera



http://www.chroma.com/index.php?option=com_products&Itemid=56&task=printView&productType=set&id=34

Weak fluorescent signal requires high sensitivity and high s/n

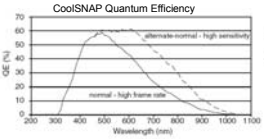
Camera and Lighting

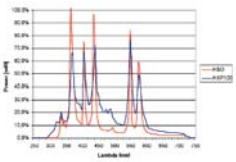
Photometrics CoolSNAP K4

- 2048 x 2048
- monochrome
- 7.4 µm x 7.4 µm pixel
- 10 MHz & 20 MHz

Leica EL6000 Metal Halide Fluoresecence Light Source

CoolSNAP Quantum Efficiency





Prototype System

Goal: *Demonstrate feasibility using off-the-shelf optics*

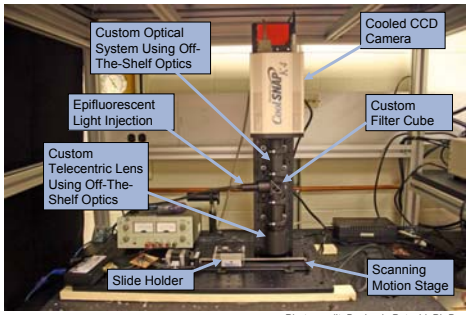
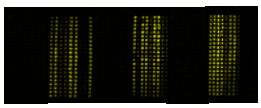


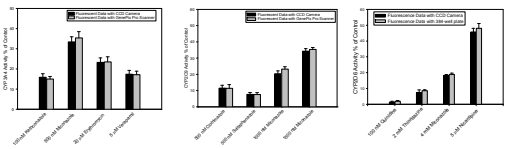
Photo credit: Benjamin Potsaid, Ph.D.

Prototype System: Results*



Six snapshots with different filters were taken of the slide, false-colored in green, and reconstituted to give a single image

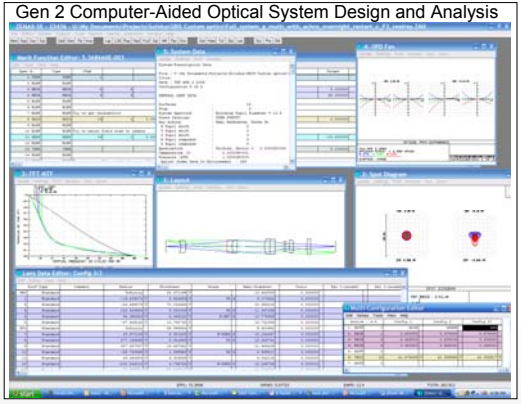
Photo courtesy Sumitra Meena Sukumaran; Rensselaer



Correlation of inhibition data between the CCD Imaging System and the GenePix Pro Scanner system for CYP3A4(a) and CYP2C9 (b); and between the CCD Imaging System and 384-well plate reader for CYP2D6 (c)

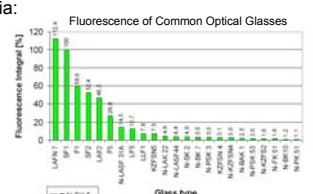
* Publication in progress: Sumitra Meena Sukumaran, and BenjaminPotsaid, Ph.D.

Gen 2 Computer-Aided Optical System Design and Analysis

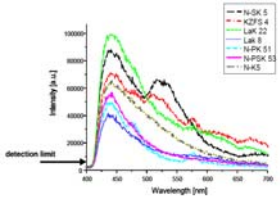


Glass Selection Criteria:

- Refractive Index
- Fluorescence
- Cost/Availability



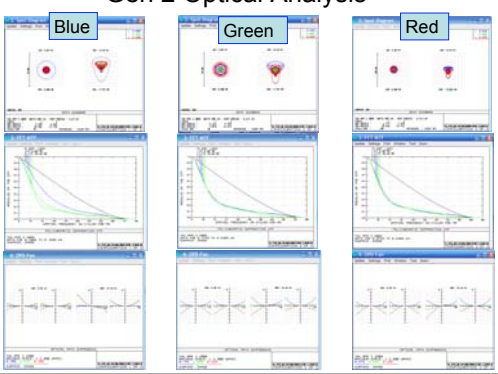
Both images: "TIE-36: Fluorescence of Optical Glass", Technical Information, Advanced Optics, Schott GmbH, September 2004



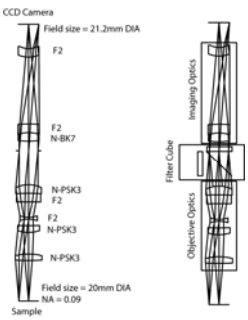
Use of lead-free glasses reduces fluorescence

(Schott N- prefix)

Gen 2 Optical Analysis

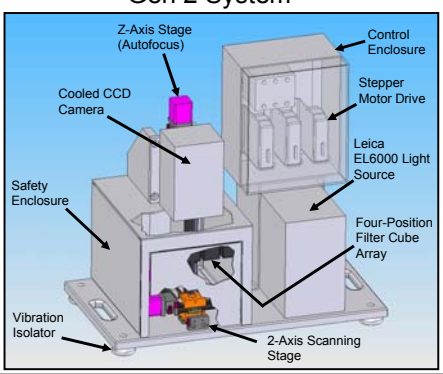


Gen 2 Final Optical Design



1. Improved sensitivity:
   - Smaller field of view (requiring 2 axes of motion)
   - Higher Numerical Aperture
2. Improved resolution: Variable focus
3. Improved ease-of-use: Four-Position filter cube array
4. Improved safety: Interlocked safety enclosure

Gen 2 System



Rensselaer

CATS Center for Automation Technologies and Systems

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