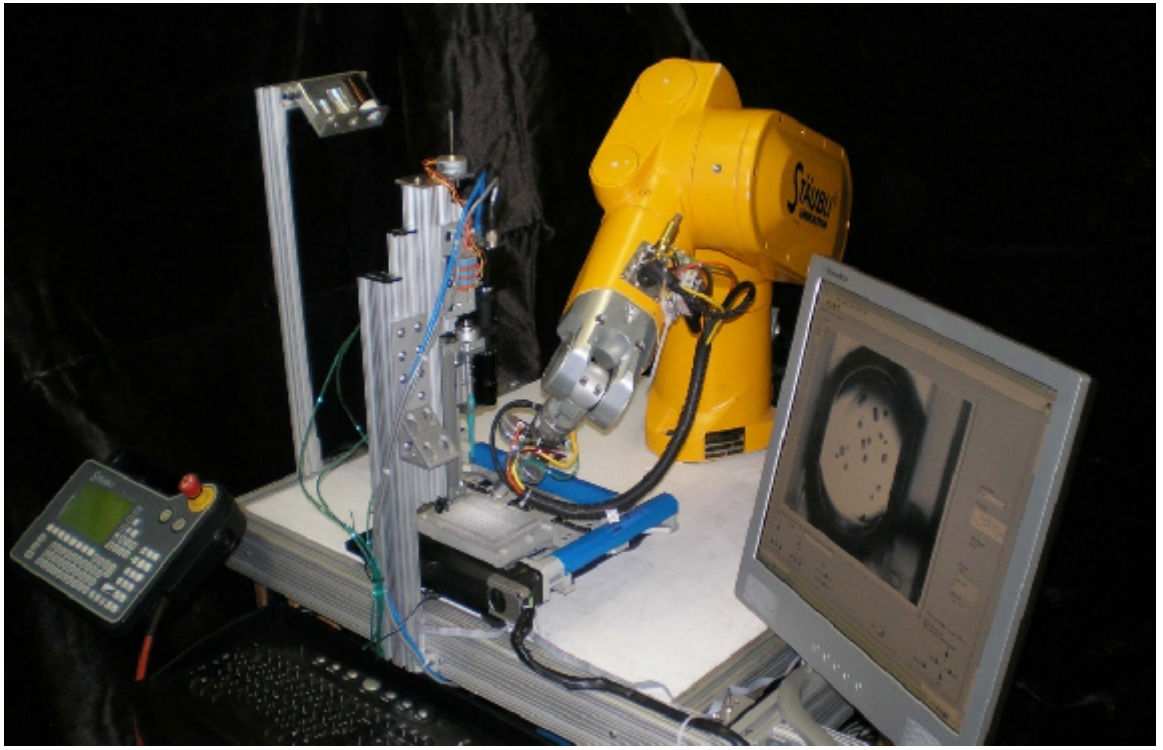
Robotic crystal harvesting: A progress report



NIH STTR Phase II No. R42 GM073278-02A1

Square One Systems Design
Jackson, Wyoming

Reminder: Why automate?

* **Harvesting (and cryo-protection) are the last remaining manual steps in an otherwise (arguably) fully-automated pipeline**

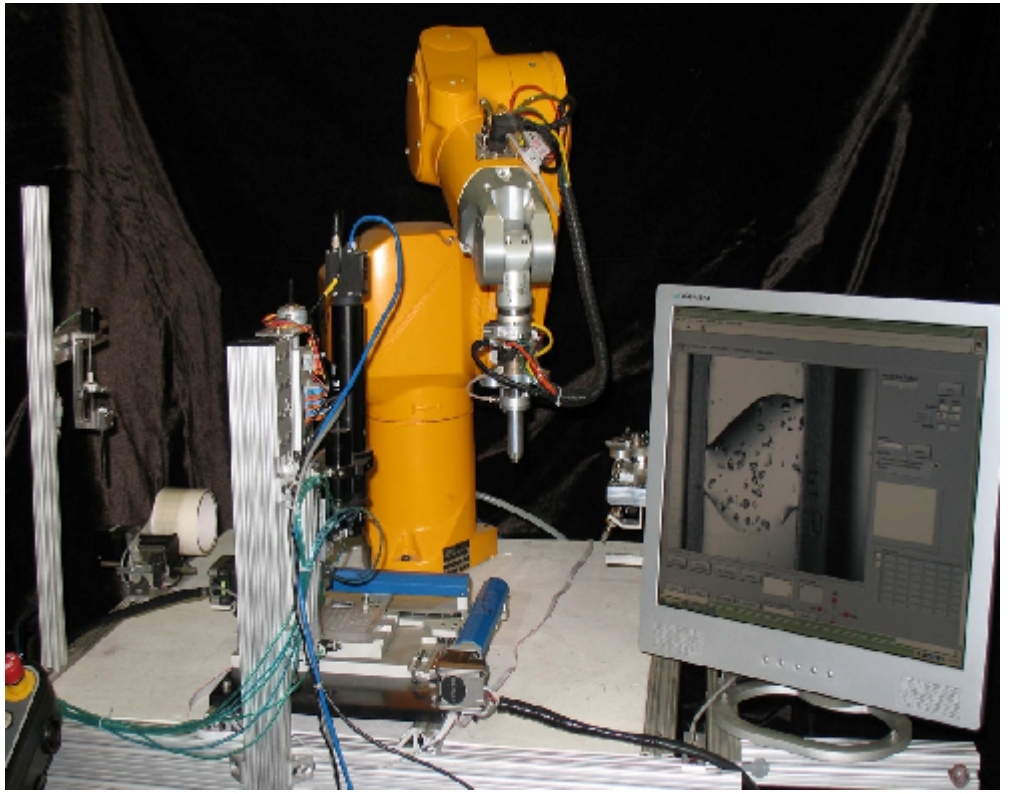
* **Automated crystallization has reached a point where harvesting presents a bottleneck, particularly for drug discovery (same protein- many ligands) and HTPX facilities**

* **Lack of reproducibility in manual methods and skill-dependence are contributing to losses and inconsistent data**

*** Cryo-techniques are still anthropomorphic, poorly standardized and poorly explored; hyper-quenching can be readily applied**

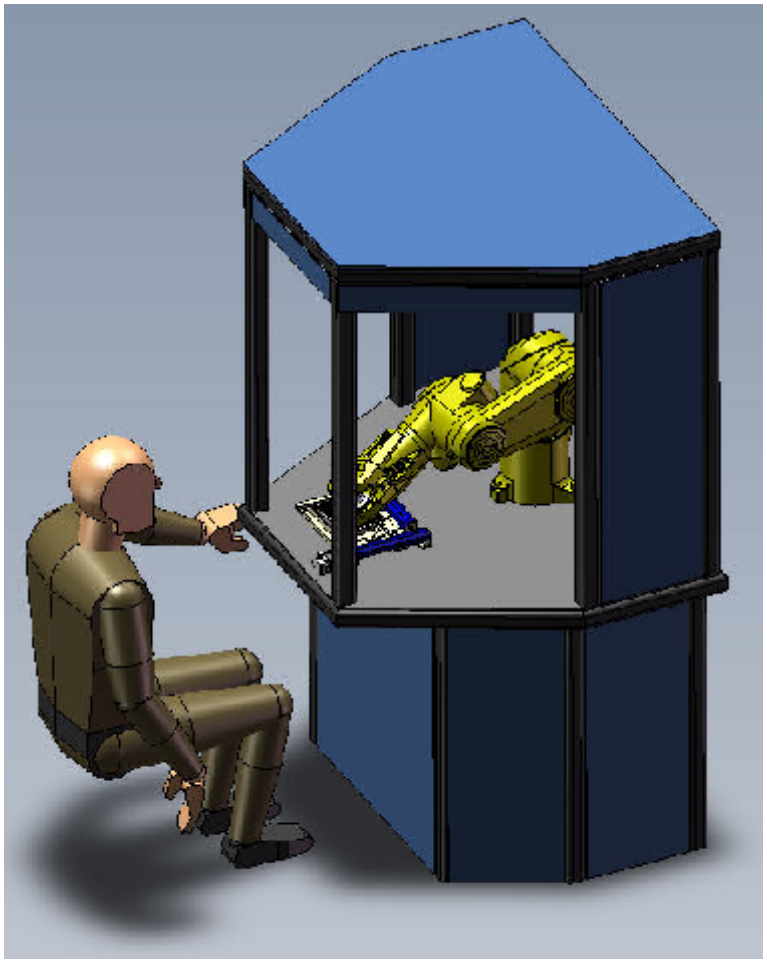
*** Room-temperature diffraction analysis possible; simple RT mounting and quenching allows systematic exploration**

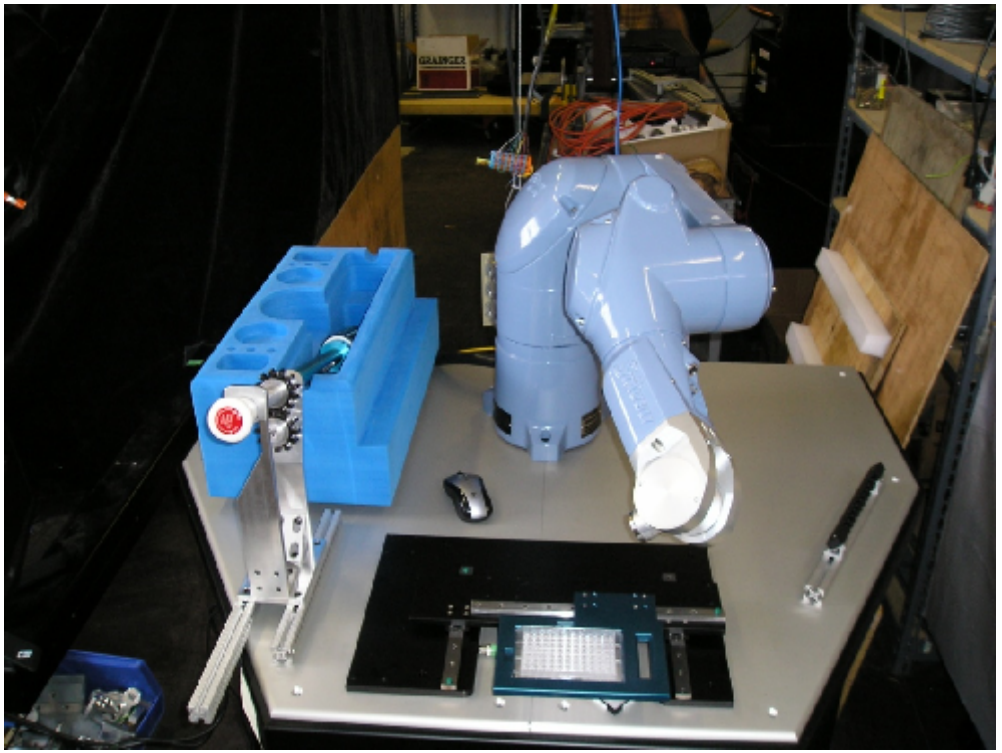
From development platform…



- Key Components
  - Plate Handling
  - Camera, Optics, & Lighting
  - Machine Vision
  - Sample Access
  - Crystal Harvesting
  - Cryo-Protecting
  - Cryo-Cooling
  - User Interface

…to operator-assisted module





- Semiautonomous modular and flexible UMR unit with the capability of linking with upstream and downstream equipment.

- Overall harvesting module design

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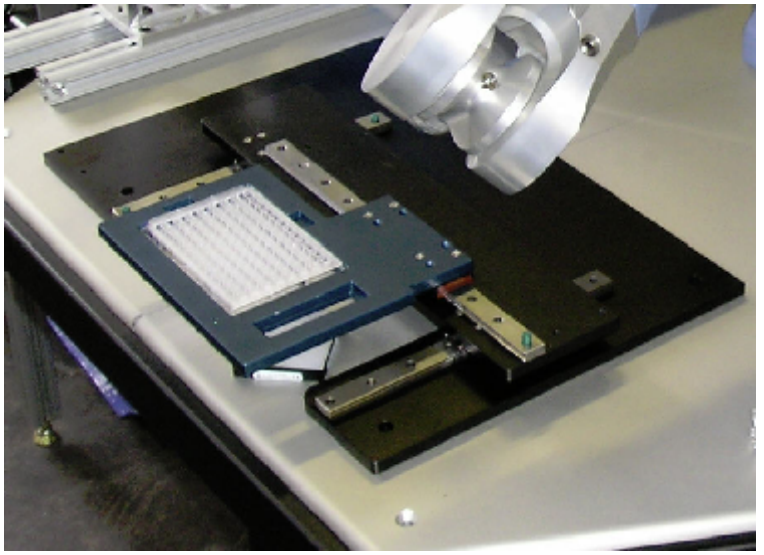
Plate handling

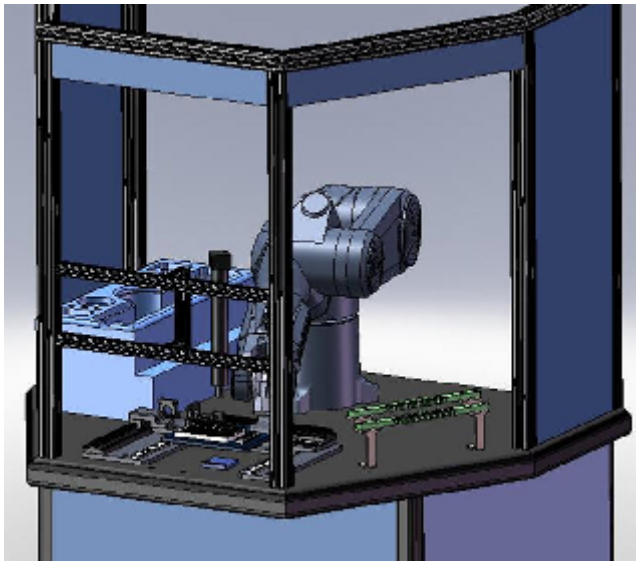
- Initially Art Robbins stage; now custom design. Operator can either
  - systematically view each individual well
  - enter the address of a well of interest or
  - pick up location from visualization data (silo)

  and the stage will center it under the imaging system.

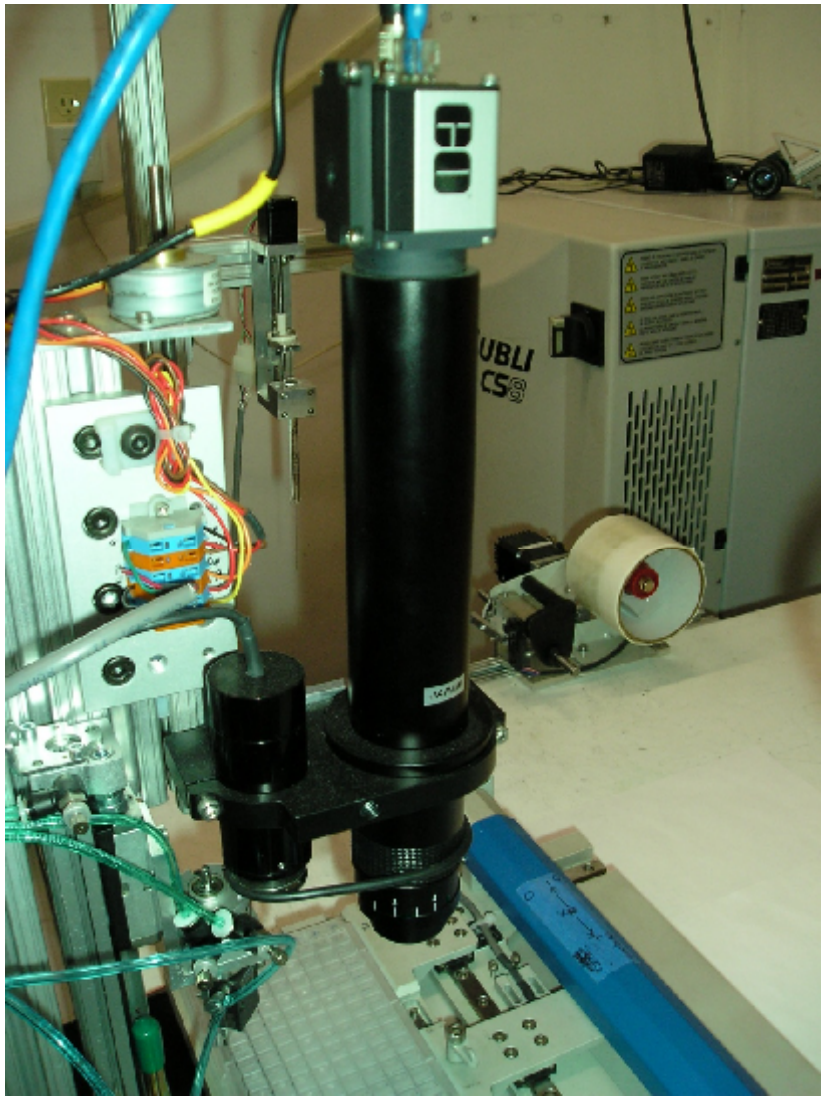
- An integrated LED light under the tray provides backlighting.

- Additional lighting scenarios are customized to application – example Rigaku's UV detection.





Camera, optics, and lighting

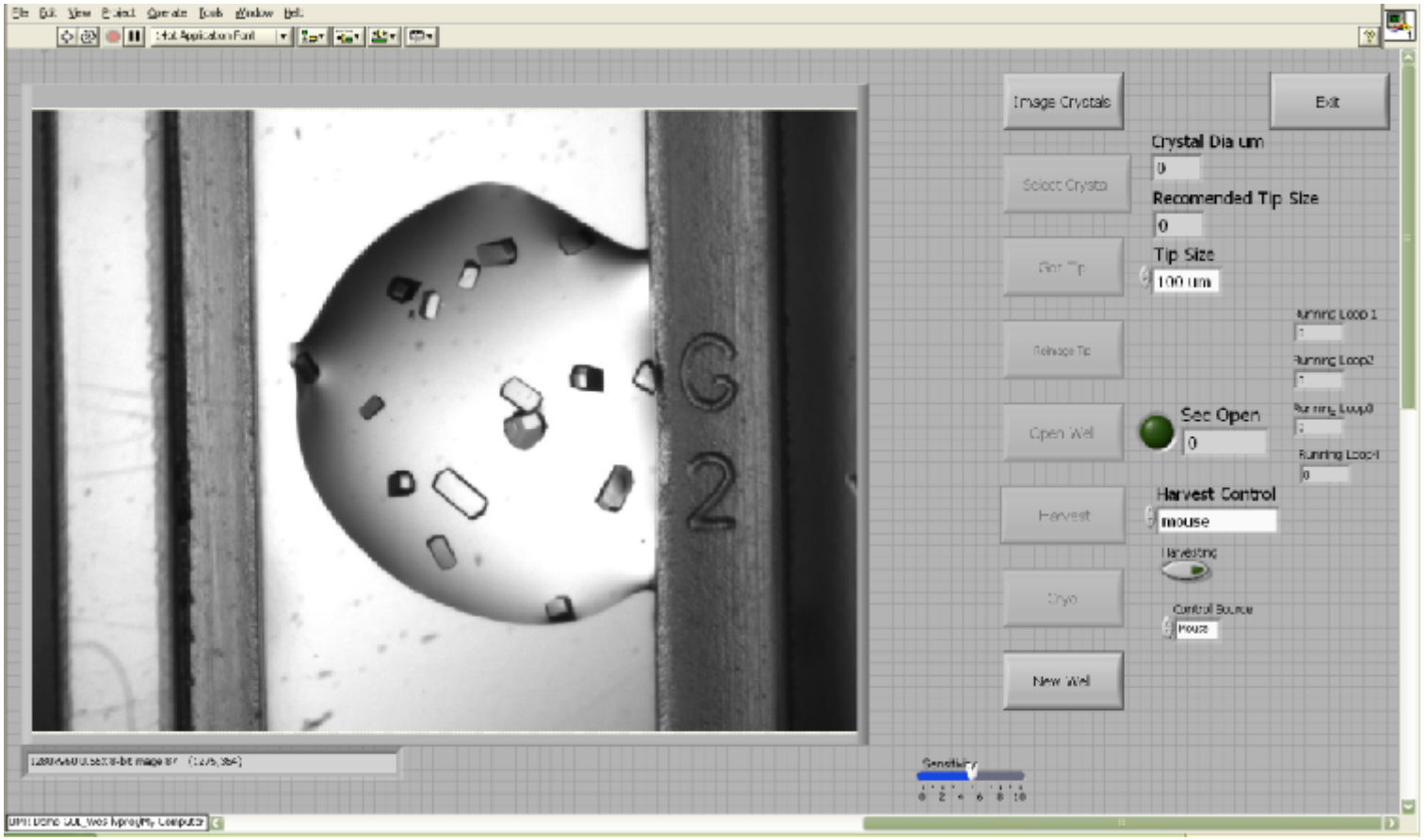


- Current System
  - **Camera:** 5 Mpix GigE CMOS
  - **Optics:** Edmund Optics VXM 450

    0.7X – 4.5X motorized zoom
  - **Lighting:** White LED backlight, with custom illumination, dark field lighting, as applicable

Auto-focussing for depth (Z coordinate) determination

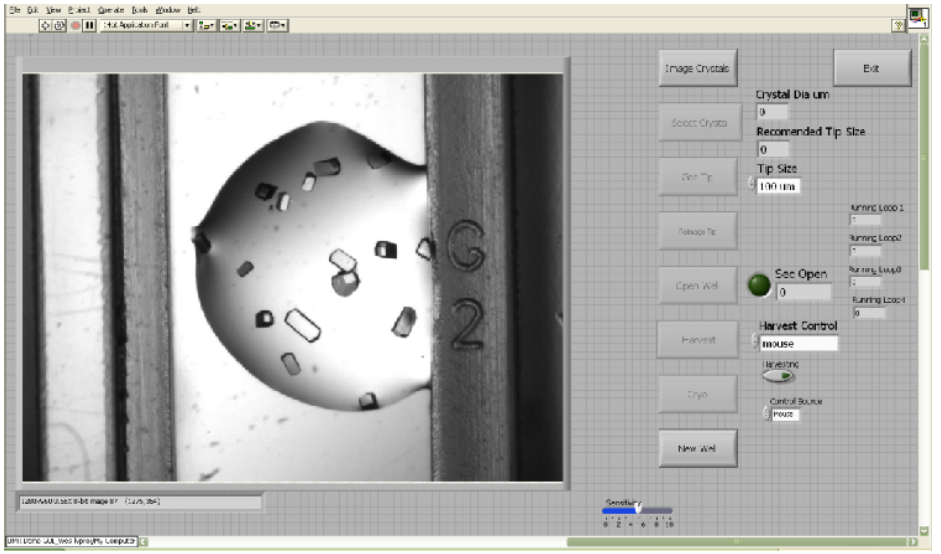
User interface and machine vision

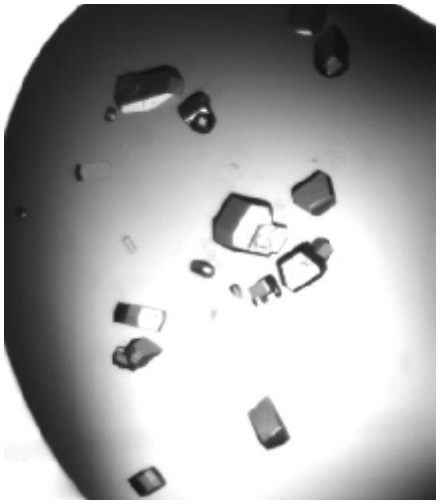


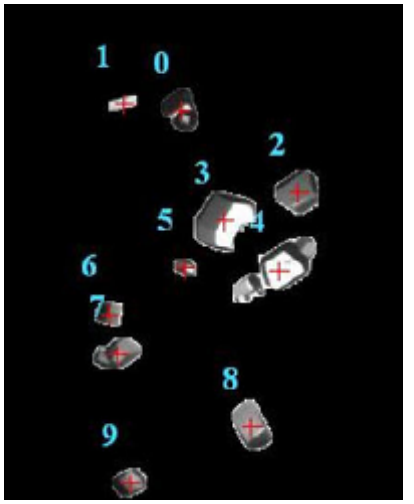
- Intuitive, process-oriented user interface integrated with machine vision system allows viewing, selection and execution of procedures as well as manual override with multi-DoF mouse. Grand vision: a harvester's toolbox

User interface and machine vision

- The images are analyzed and crystals located and evaluated. The result of this analysis are X, Y location (Z by auto-focusing), and size and shape factors for each crystal identified.
- If a crystal of interest is not identified automatically, the operator can use a drawing tool to outline the crystal and re-analyze the well.
- The software (based on automated ranking) or the operator selects which crystal to harvest, auto-tool selection
- Either automated or operator-controlled harvest is initiated and verified. The harvested crystal is then cooled and quenched.

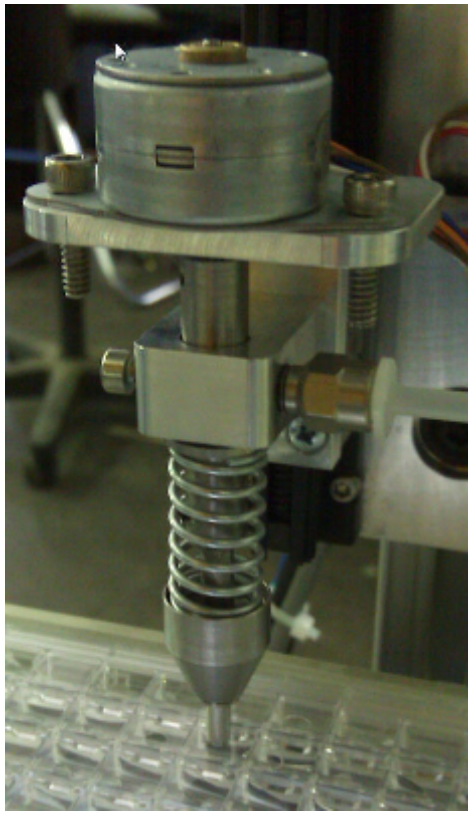


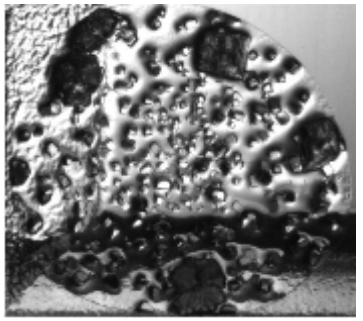




Crystal access

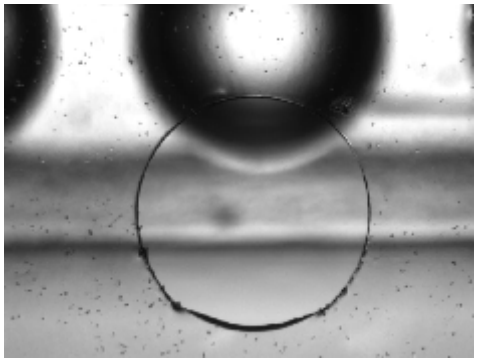
- To provide access to the well, electrically-powered razor punch. Slight pressure is applied to the tape while the punch is rotating, vacuum removes/ejects tape disc. The punch cuts a clean, circular hole—2 to 8 mm in diameter determined by the blade size—in the tape.
- Small hole diameter greatly reduces evaporative losses, increasing total open working time.
- The offset of the access hole from the imaging area allows for consistent images to be used for crystal evaluation and harvesting.



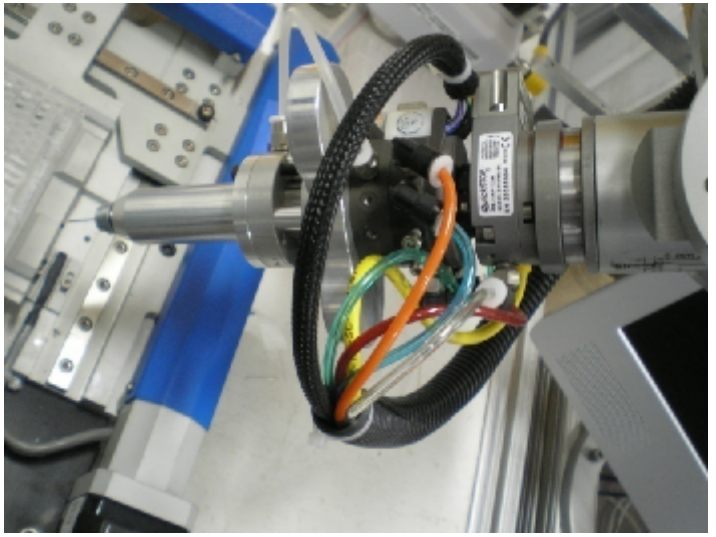


Well fully open (15 min)

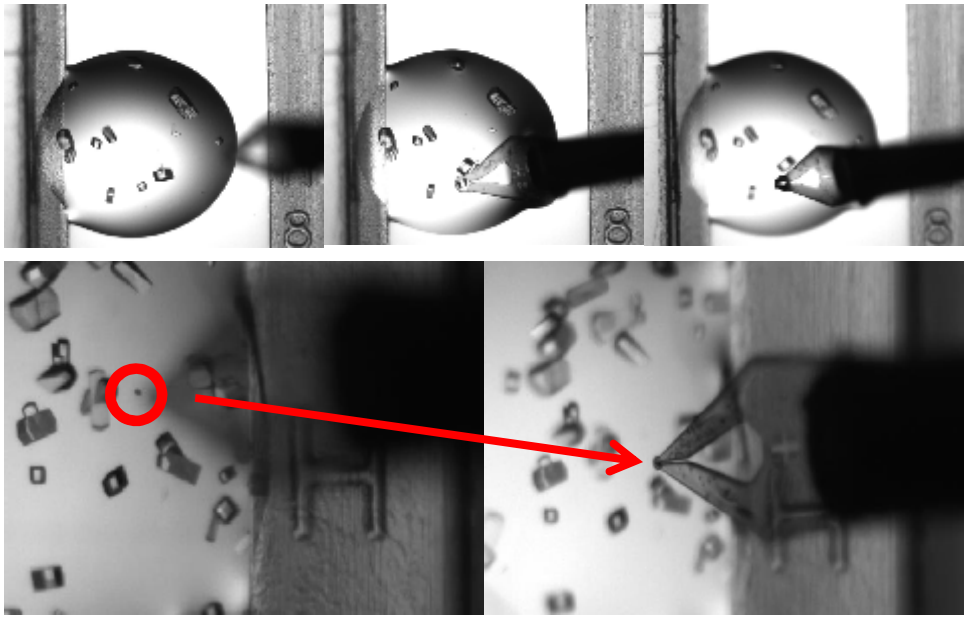
Well punched (15 min)



Crystal harvesting



- Machine vision subsystem communicates size and shape factors to the system.
- The robot then selects the correctly-sized tool and positions it in a nominal location next to the well, confirmed and adjusted by machine vision.
- The robot attempts the autonomous harvest step, verifies, cryo-protects and quenches. If it fails, the operator can take control of the robot, directing the harvesting tool in the desired fashion to harvest the crystal.
- Capable of moving in 1 micron steps, the UMR has successfully harvested crystals smaller than 10 microns in diameter.



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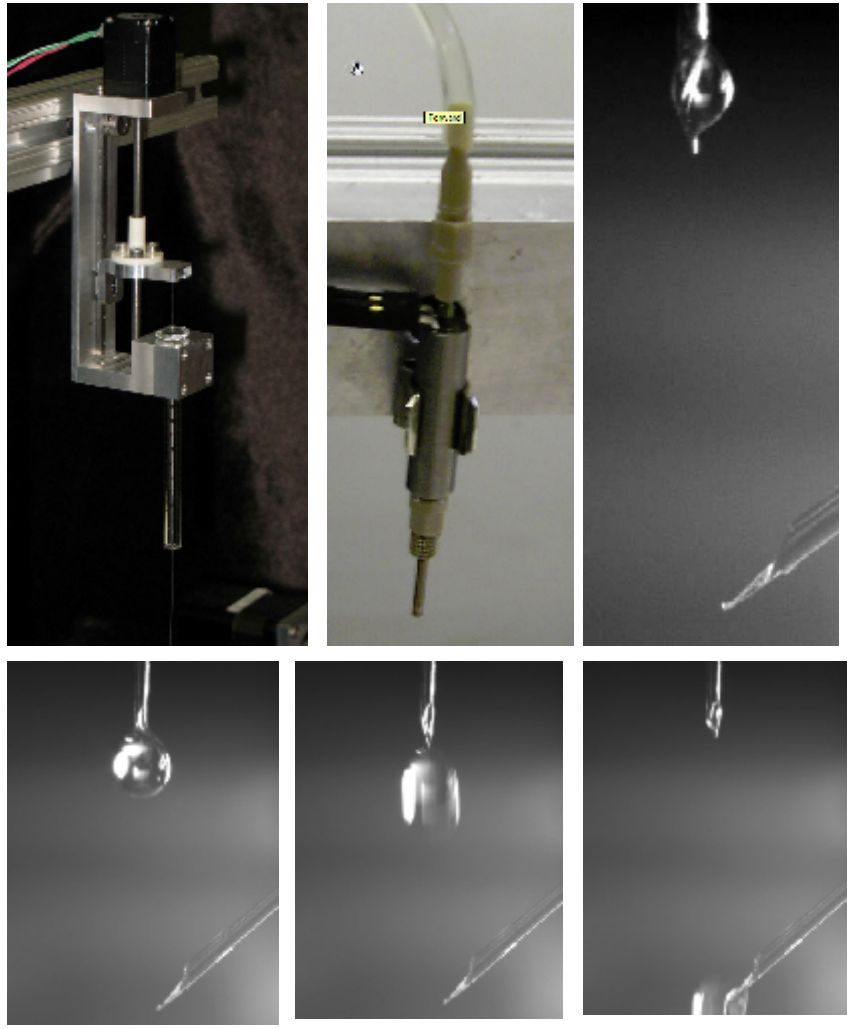
Cryo-protecting

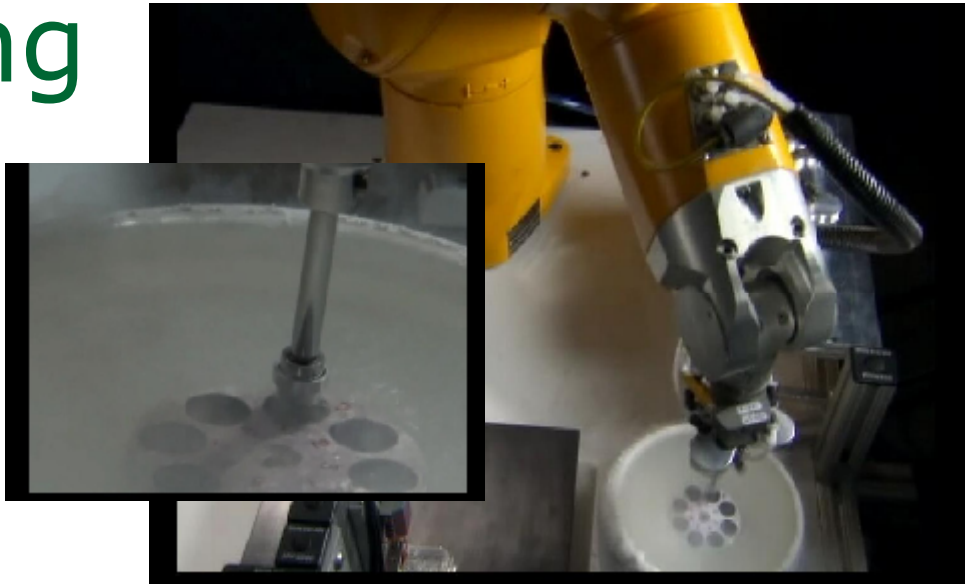
- New drip technique: drop the protectant on crystal, no swishing.
- Initially a basic liquid handling station with a Hamilton syringe. Still useful for ligand applications etc.
- Improved performance with Lee-valve, full control over drop size and speed. Optimized for low viscosity perfluoropolyether to be applied to a crystal in the loop immediately after harvesting.
- Crystals stay almost always! Even small ones – probably a universal cooling method



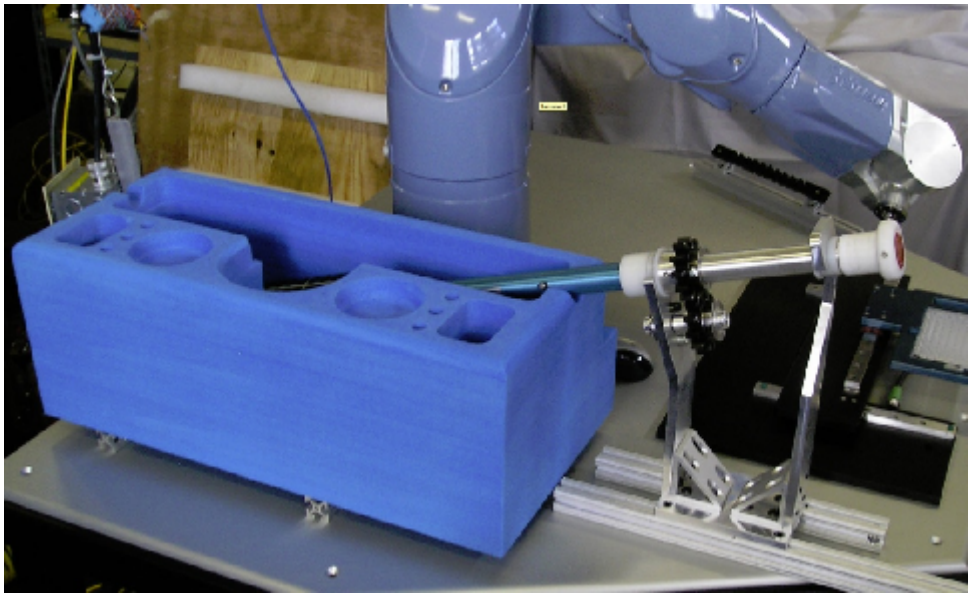
Movie

Cryo-quenching

- After the appropriate cryo-protectant is applied to the crystal, the robot transfers the harvesting tool and mounted crystal into a storage puck in a liquid nitrogen Dewar, cryo-quenching the crystal.



- The robot can be configured to any user selected puck.

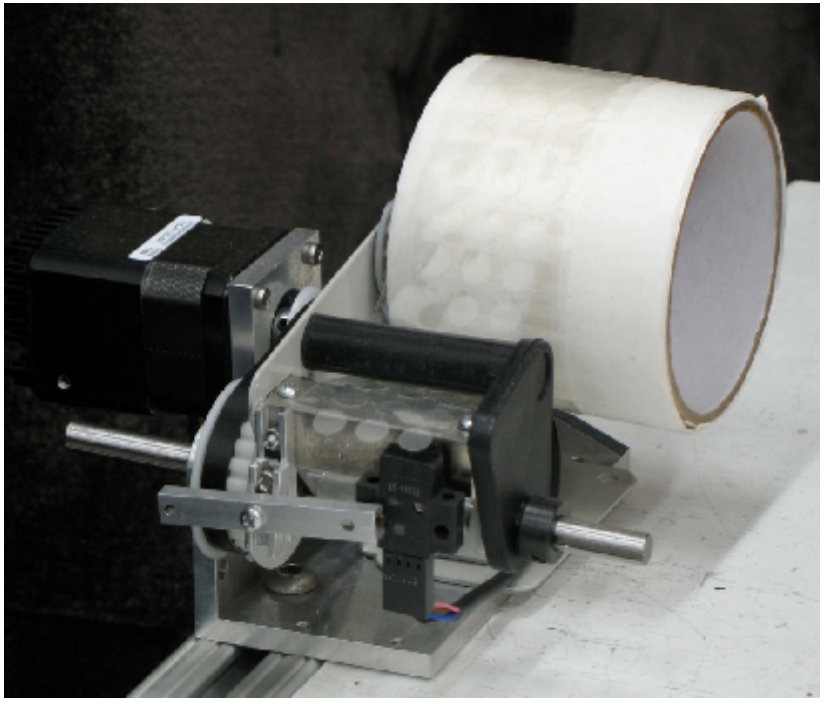


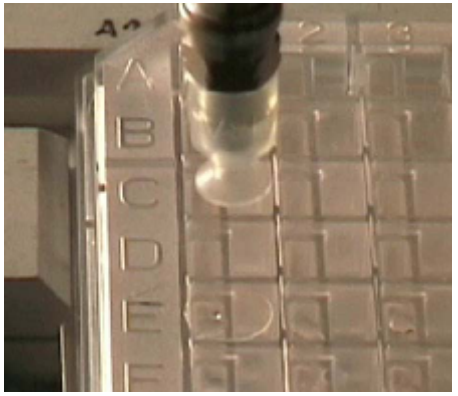
- Hyper-quenching using a stream of dry nitrogen is currently in development.

Resealing

- As an optional modular unit, tape resealing is accomplished using small tape dots acquired by the robot using a specific end effector. The dots are presented to the robot, allowing the robot to pick up and place them over the open well.

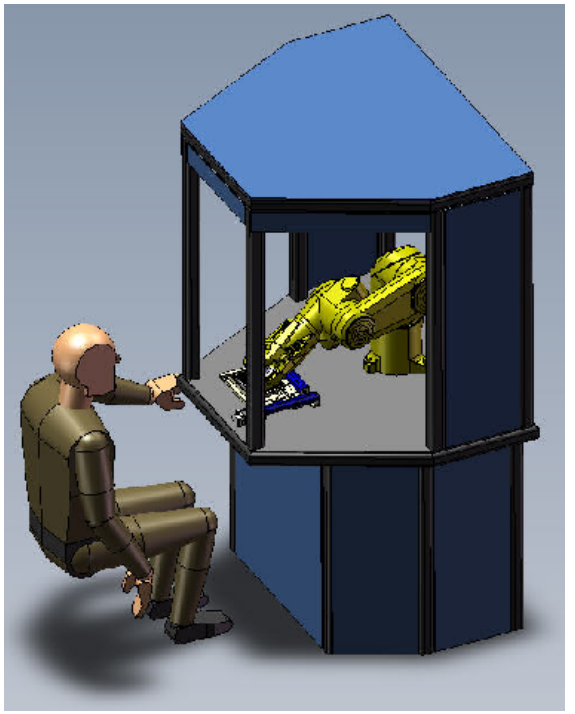
- The tape dot can be applied after the application of ligands or to preserve a well of interest after crystal harvesting.



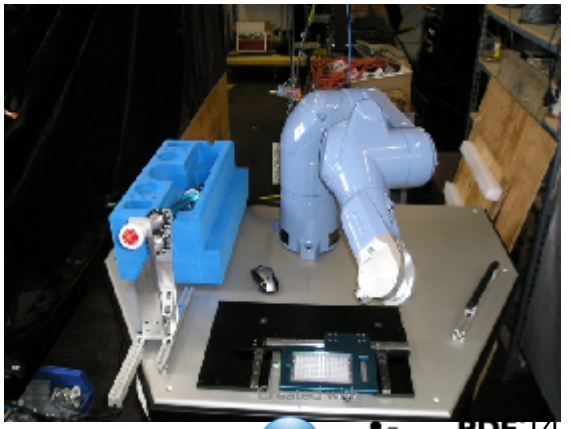


From semi-autonomous UMR unit to full auto

- The semiautonomous UMR is modular and flexible with the capability of linking to downstream equipment (most likely silo) or diffractometer
- Remaining challenges: real time tracking and error recovery – machine vision interface to process control – embedded autonomous system – field of mechatronics.



- RT mounting and diffraction analysis – straight forward, funded. Interface with basic diffractometer software. New insight into cryo expected.



Demo movie

The robotnik team

- Square One Systems Design, Wyoming: Alex Melka, Jace Wash, Bob Viola – systems design and integration
- Johns Hopkins Applied Physics Lab, Baltimore: Sean Murphy – machine vision systems
- q.e.d. life sciences (Hofkristallamt): Bernhard Rupp – crystallography and methods development
- Sponsor: NIH-MIGMS STTR program, R42 GM073278-02A1 and supplement.