

A Universal Micromanipulation Robot (UMR) for Crystallography

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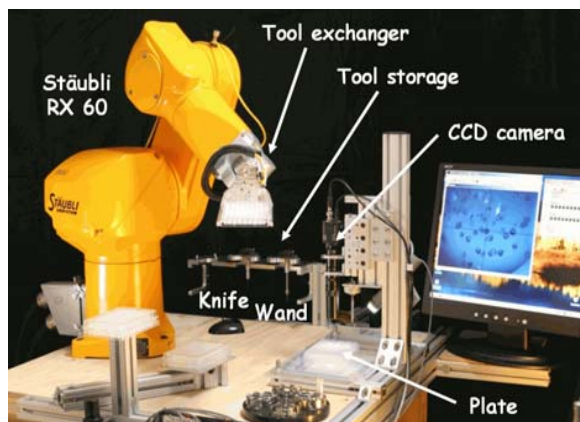
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Abstract

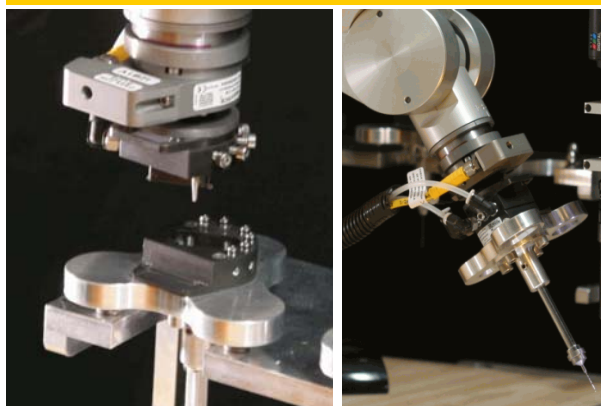
High throughput protein crystallography has reached a level of automation, where complete computer-assisted robotic crystallization pipelines are capable of cocktail preparation, crystallization plate setup, and inspection and interpretation of results. Mounting of crystal pins, data collection and structure solution are also highly automated. Remaining challenges towards full automation are crystal harvesting and cryo-cooling. These critical operations occur late in the crystallization process and are a major source of loss of valuable crystals. To pursue the final frontier in achieving fully automated high throughput crystallography, we have designed and tested an anthropomorphic, 6-axis Universal Micromanipulation Robot (UMR) capable of reliably harvesting and cryo-quenching protein crystals as small as 10µm from a variety of 96 well plates. The UMR arm is equipped with a versatile tool exchanger. Tape cutters, harvesting pins with MiTeGen MicroLoops, cryo-liquid exchange and custom tools provide additional operational flexibility. The current user interface is a simple game-style keyboard layout allowing intuitive manual control. Semi-automated process steps such as tool location, loop approach to crystals, as well as fully automated tape cutting and quenching are presently implemented in the prototype. Integrated machine vision and object tracking are under development to achieve minimally supervised, automated crystal harvesting.

Overview

The UMR is based on an anthropomorphic Staubli RX60 6-axis robot. The arm is equipped with a pneumatically operated tool exchanger. The exchange adapter includes liquid feed-throughs allowing cryo-liquids to be added or exchanged. The operations are viewed through a CCD video camera, and the robot is controlled via a game-style keyboard layout.

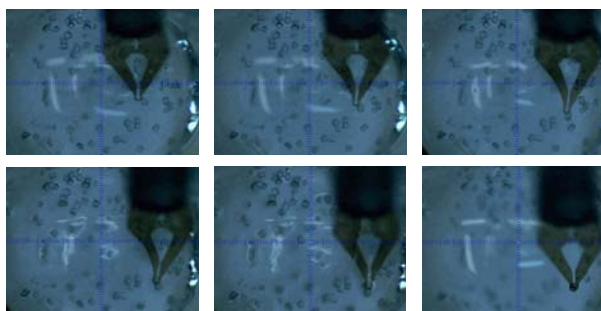


Tool Exchanger



The left figure shows the pneumatically operated tool exchange adapter with liquid feed-throughs. The tools rest on kinematic supports and can be precisely and reproducibly mounted. Right picture shows the robotic arm with mounted magnetic harvesting wand, carrying a standard Hampton base with a MiTeGen loop (next column).

Crystal Capture Sequence



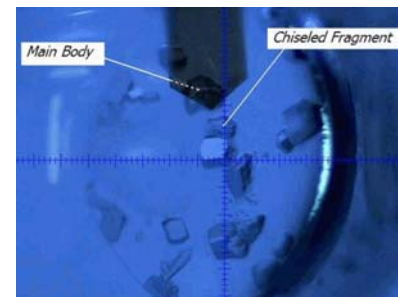
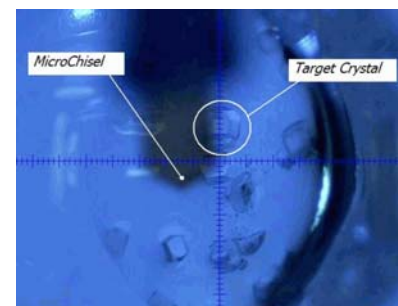
Sequence of snap shots from a live capture video. A MiTeGen 30µm loop is used to harvest a small lysozyme crystal. Note the flexible loop being used to move the crystal and then to scoop it up. Semi-automated, supervised harvesting includes pre-positioning of the loops and automated quenching once captured. Further automation using real time machine vision and image tracking will be developed during Phase II of the UMR project.

MiTeGen Harvesting loops

Crucial for the ability to harvest crystals robotically is the availability of reproducibly machined harvesting loops. The MiTeGen Kapton loops are stiff and allow cutting and prying manipulations while being flexible enough not to break when stressed.



Crystal manipulation and dissection



Cleavage of a lysozyme crystal. A MiTeGen micro-chisel is used to split a crystal. The top picture shows the intact target crystal. In the lower panel, the crystal is already split in two fragments. A variety of Kapton etched micro-tools and bi-stable devices mounted on exchangeable tool adapters allow great flexibility of micro-manipulations exceeding the capabilities of manual tools use. Liquid exchange for cryo-protection and ligand soaking are also possible.

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