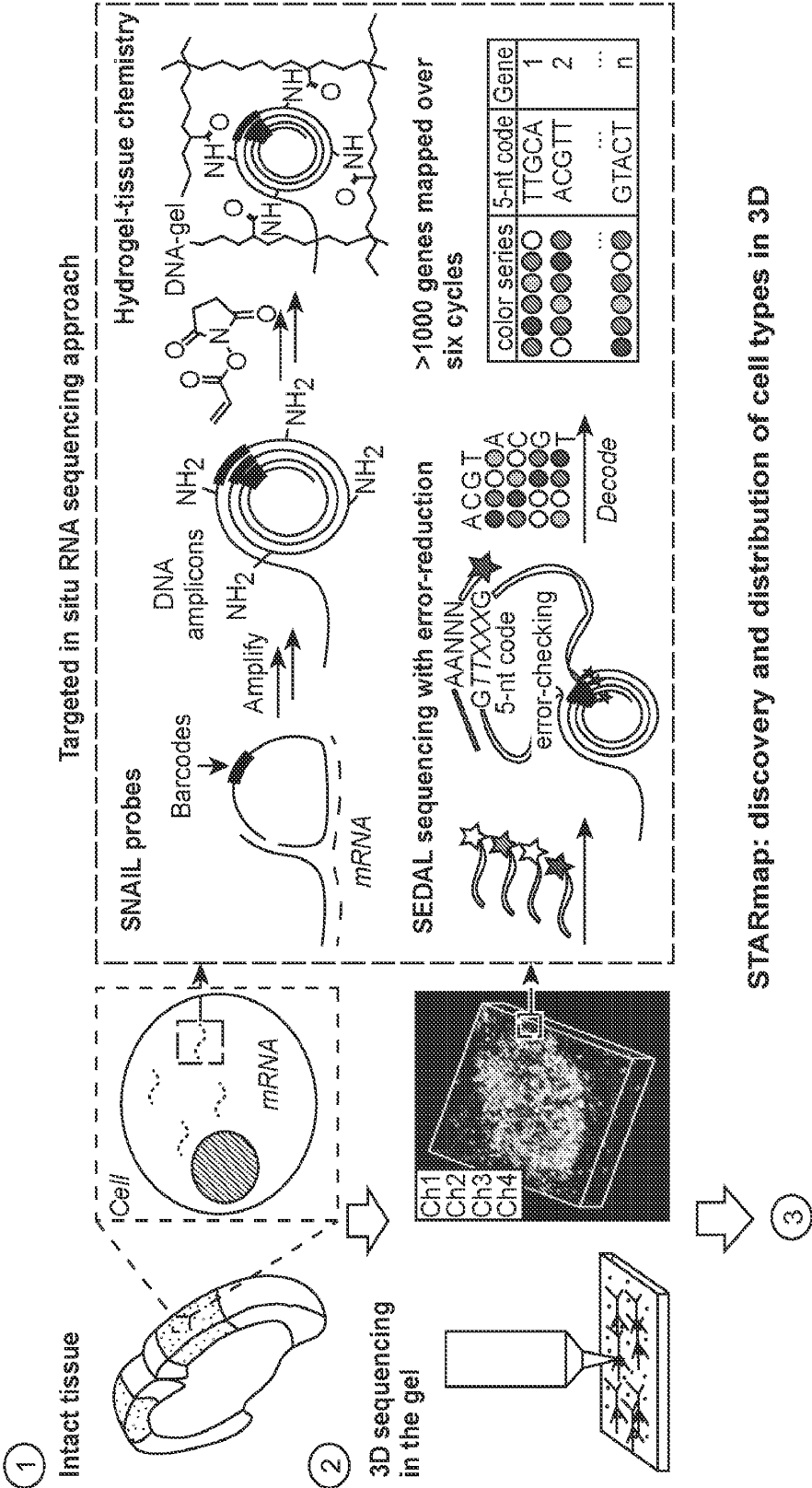


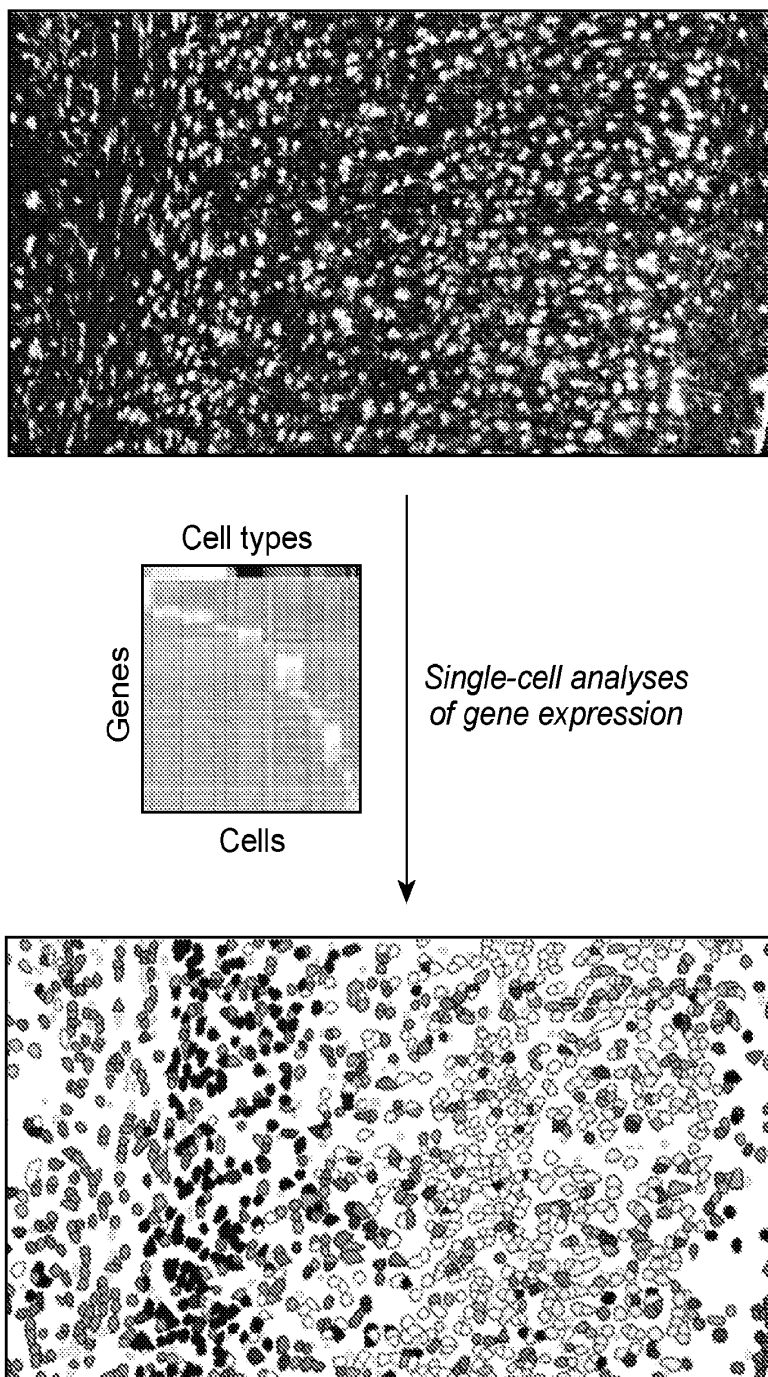
FIG. 1



2/10

FIG. 1 (Cont.)

③

**The STARmap process for in-situ RNA sequencing**

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3/10

FIG. 2

Challenges in intact tissue sequencing

- Tissue processing throughput
 - Round-to-round chemistry time
 - Imaging time
 - Extensive manual labor
 - Parallel preparation of samples but sequential sequencing
- Process robustness – > sample loss
- Limited round numbers for sequential sequencing

4/10

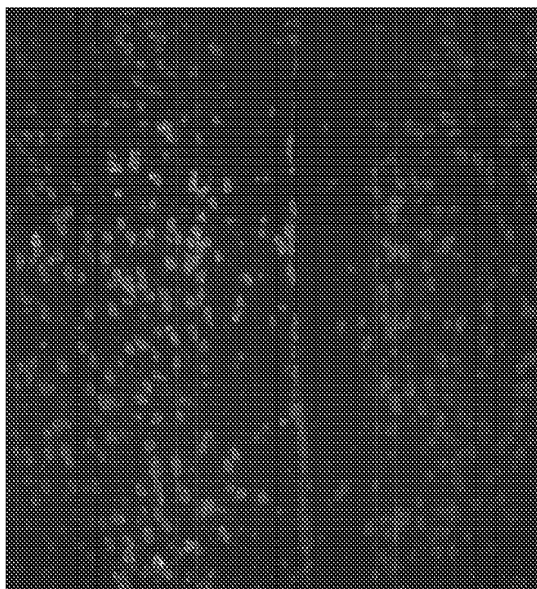
FIG. 3

CISITS method advantages

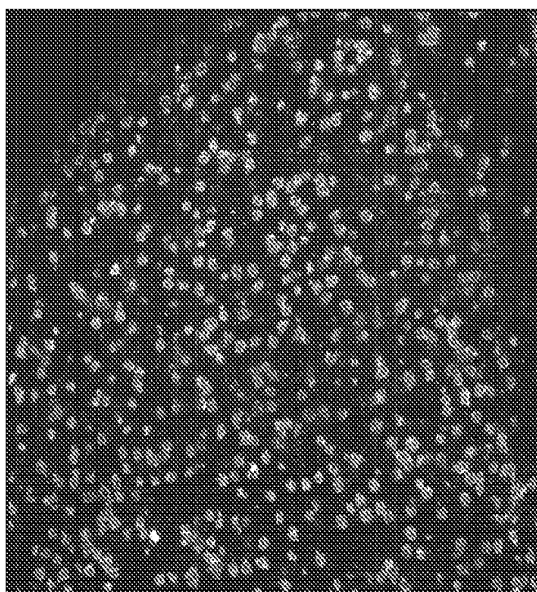
- Tissue processing throughput
 - Round-to-round chemistry time – Reduced for thick section tissues by ~2 hrs per round
 - Image time
 - Dual-camera, dual excitation imaging >2x increase in imaging throughput
 - Spinning disk confocal microscope accelerates acquisition when optical sectioning necessary
 - Other imaging modalities possible, such as wide field microscopy, depending on sample type and detail needed
 - Antifade in imaging buffer enables increased laser power, decreased bleaching, and reduced exposure time
 - Extensive manual labor
 - Automated CISTIS fluidic and acquisition system performs sequencing rounds beginning-to-end
 - Non-pressurized sample well-plate chambers facilitate parallel manual preparation of sample and ease of coupling to automation component
 - Parallel preparation of samples but sequential sequencings
 - Automation parallelization capacity of up to 8 samples by parallelizing reagent delivery to samples
- Process robustness –> sample loss
 - Robustness features (vibration damping, leak detection, fluid flow monitoring, setup validation, software robustness) for automation
 - Thiol-linkage / cleavage of fluors reduces round to round signal contamination
 - Antifade prevents combinatorial sequencing read out signal from falling below noise floor, preventing sequencing loss
 - Sample collection ring
 - Chemistry robustness – reagent freshness requirements, better reaction buffering

5/10

FIG. 4

Regular imaging buffer

3x SNR increase

Antifade modified imaging buffer

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FIG. 5A

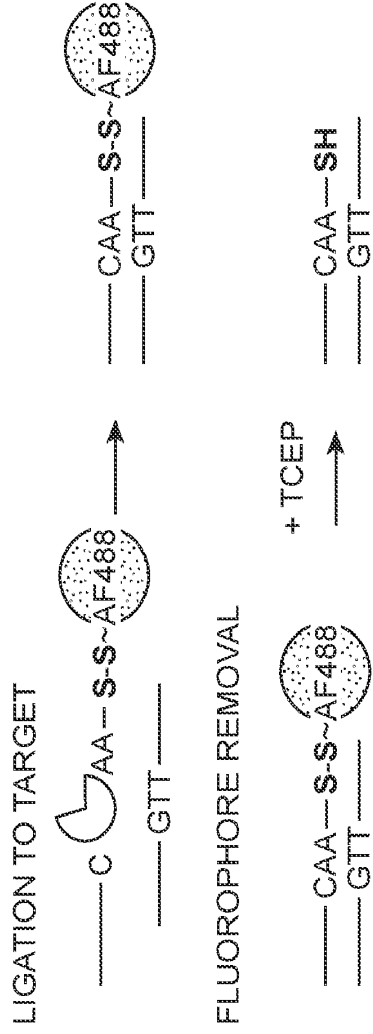


FIG. 5B

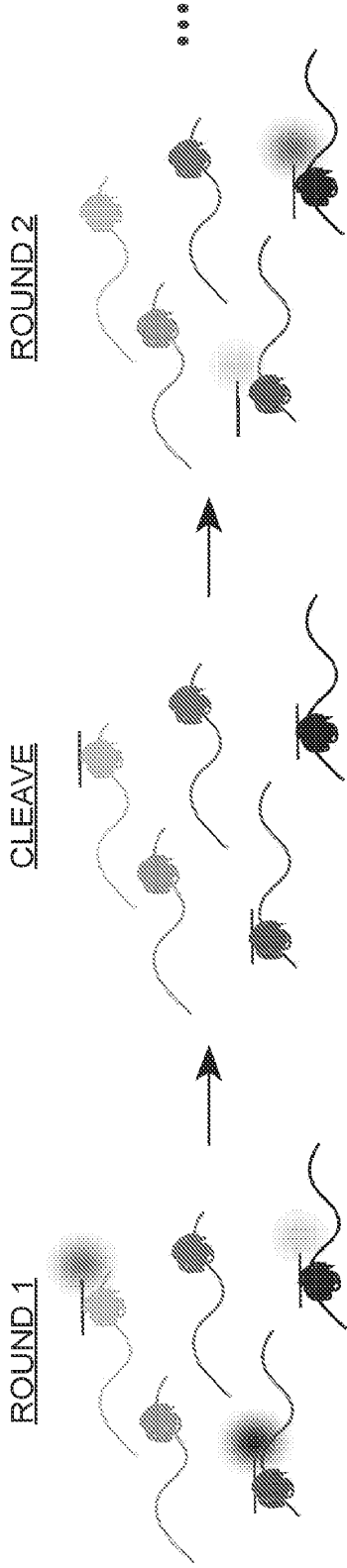


FIG. 5C

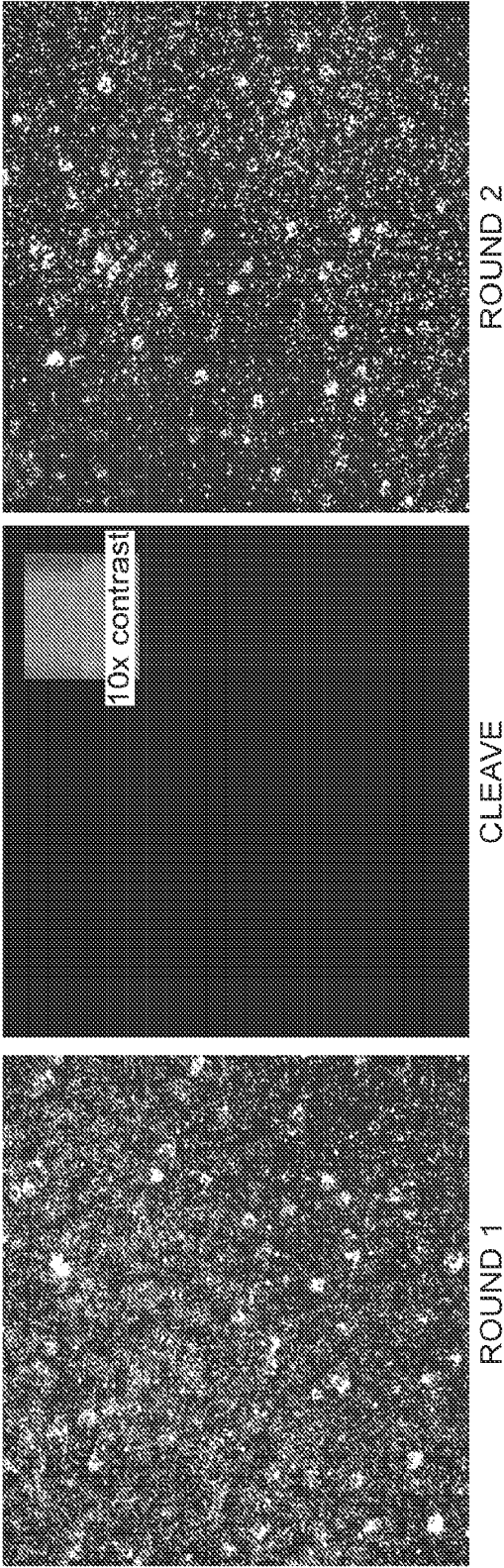


FIG. 6

Combinatorial sequencing in thick tissues

- New amplification method
 - Pre-incubation in RCA polymerase buffer and polymerase without dNTPs to allow uniform enzyme diffusion
 - Pulse RCA solution with dNTPs for uniform and controlled amplification
- Linkage of endogenous RNAs into hydrogel before subsequent chemistry steps to allow aggressive sample clearing and enhanced enzyme diffusion

FIG. 7

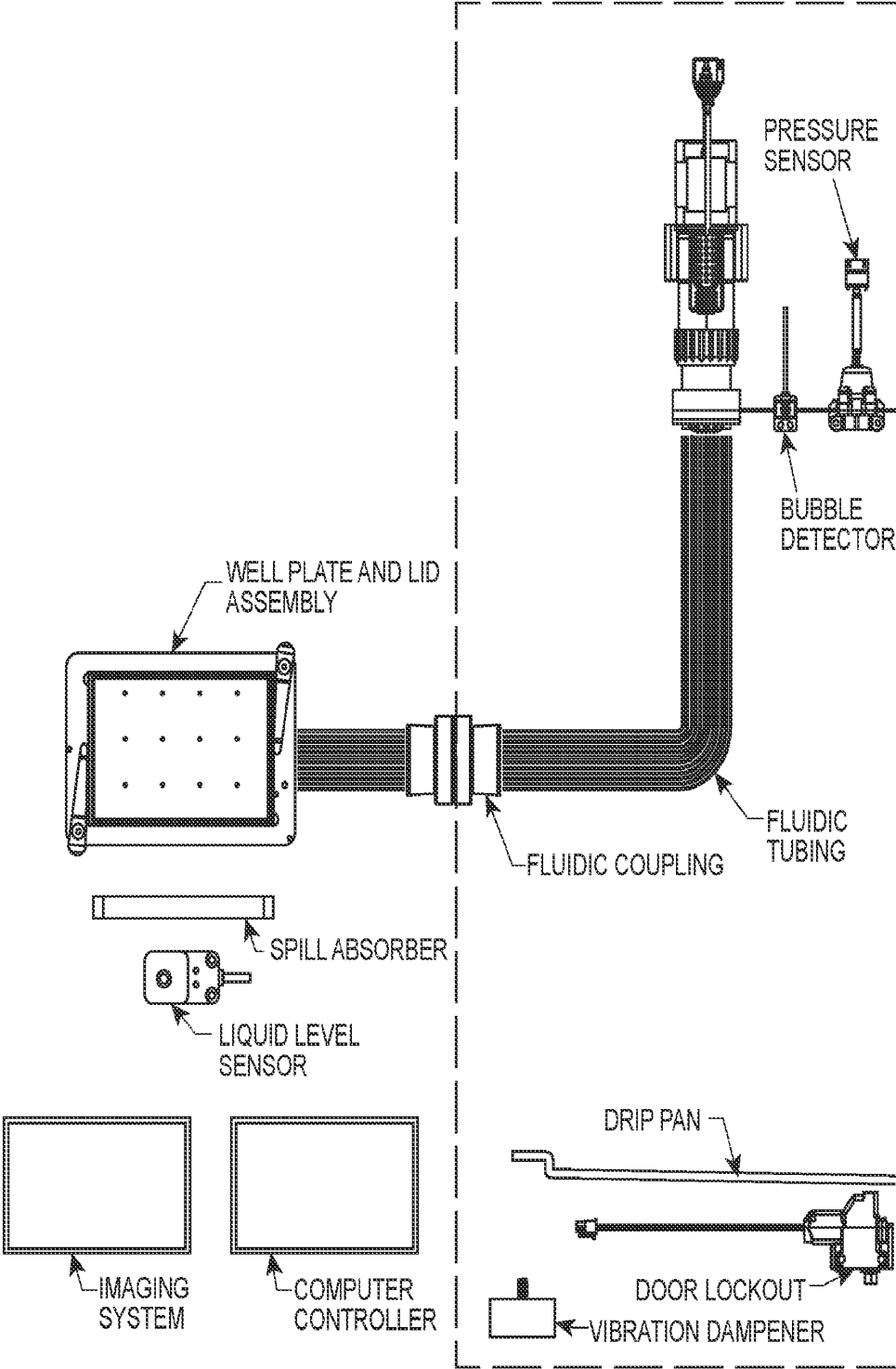


FIG. 7 (Cont.)

CISITS sequencer diagram

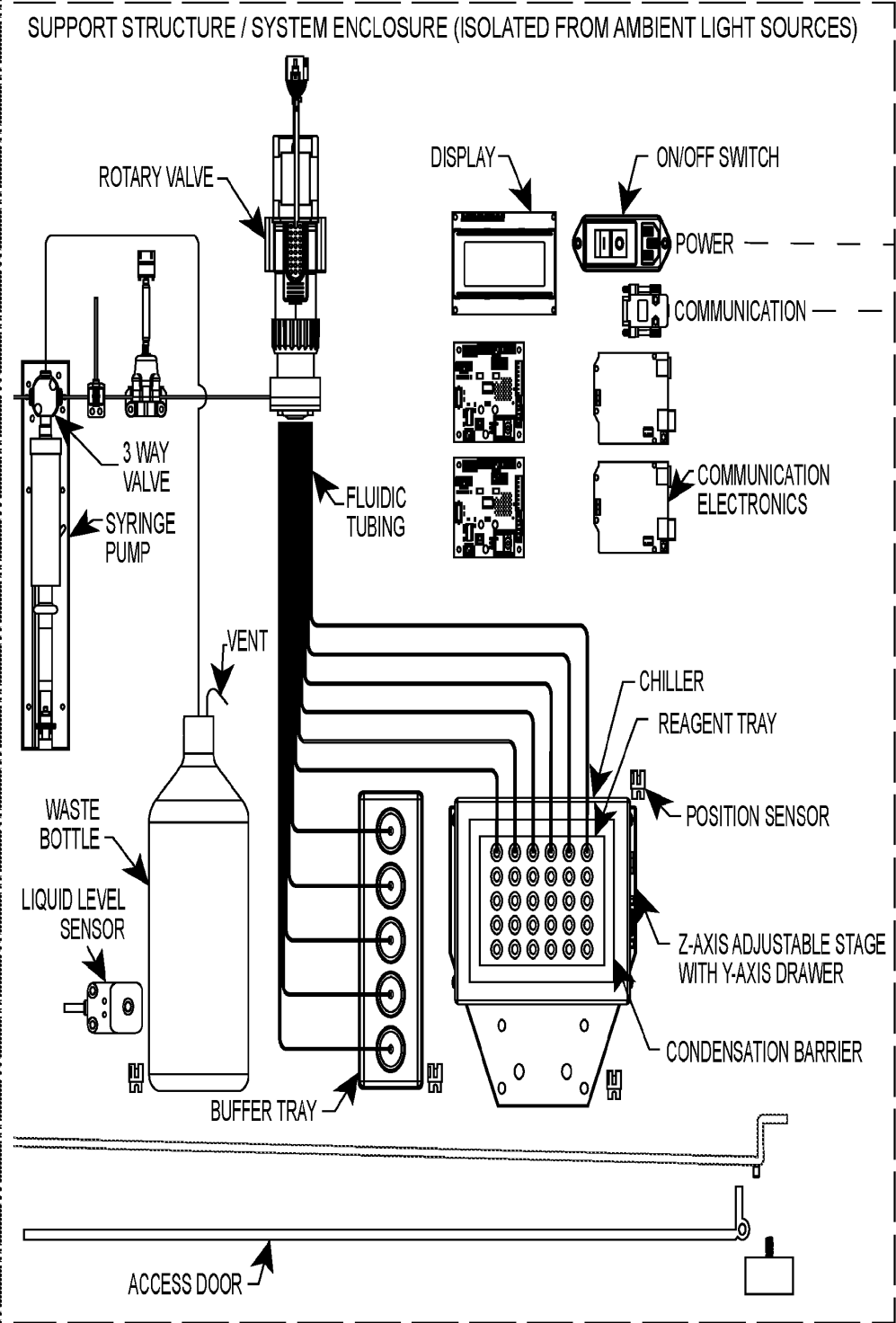


FIG. 8
CISITS software interface

