# Internet-Connected Virtual Microscope for Large-Scale Automated Histology

Introduction

Knife Edge Scanning Microscopy (KESM) is a high-throughput and high-resolution physical sectioning microscopy technique. KESM instruments enable to scan and analyze multiple tissue samples like the mammalian brain, parts of spinal cord, and kidneys. Ideally, it is capable of producing high contrast digital images of at a voxel resolution of 0.6µm × 0.7µm × 1.0µm in a span of ≈ 50 hours for a specimen tissue block with 1cm^3 volume. A prototype of the KESM has played an important role in neuroscience study via providing whole brain explorations of neurons, vasculature and cell structures of a mouse brain. The current project was to develop the second generation of the KESM that is named as the Internet-Enabled RObotic Microscope (IEROM) with an open-source image processing pipeline. The IEROM has enhanced the first prototype by reducing the manufacturing cost in several folds through making components modular and changing them with a cheaper but comparable parts. We expect the IEROM be used to image multiple datasets and analyze them with in-house image processing software that is being developed in parallel.
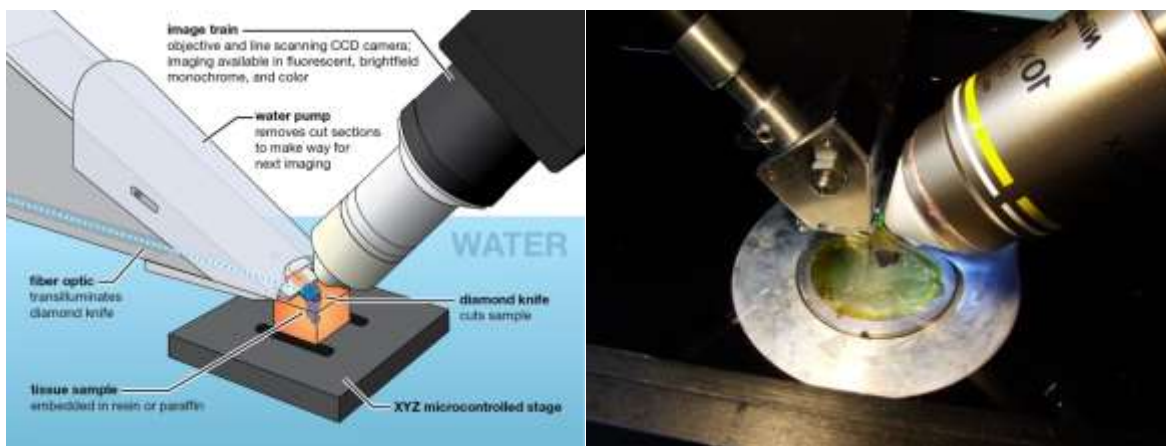
The next section will describe hardware components of the IEROM. The outline of software infrastructure supporting the IEROM will be followed.

Hardware Components

The picture below shows the overall shape of the system. Four main parts consist of the IEROM system: (1) the controller PC, (2) the stage control box, (3) the tissue scanner, and (4) the server rack.
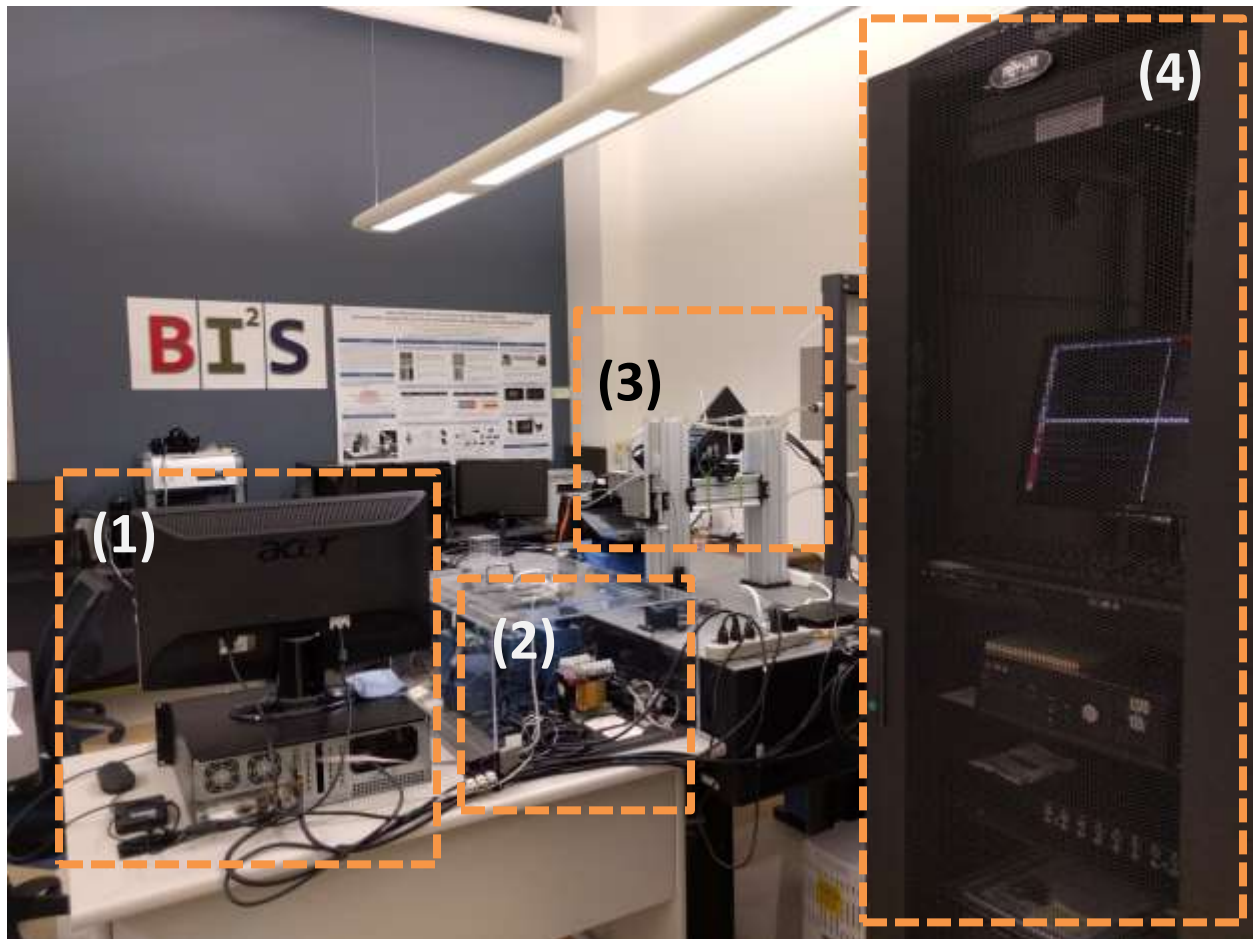

(1)   Controller PC

This is a regular PC that hosts the main device drivers of the stage controller and the line-scan camera. Also, this controller PC runs the IEROM controller software package named as **scope**. The Web-based Graphical User Interface (GUI) for operating the stage can be run at any PC in the network but this controller PC is a good place to host it as well.

(2)   Stage Control Box

We use the Aerotech Ensemble Stage Driver. The picture below shows three controller in the box for x, y, and z axis.
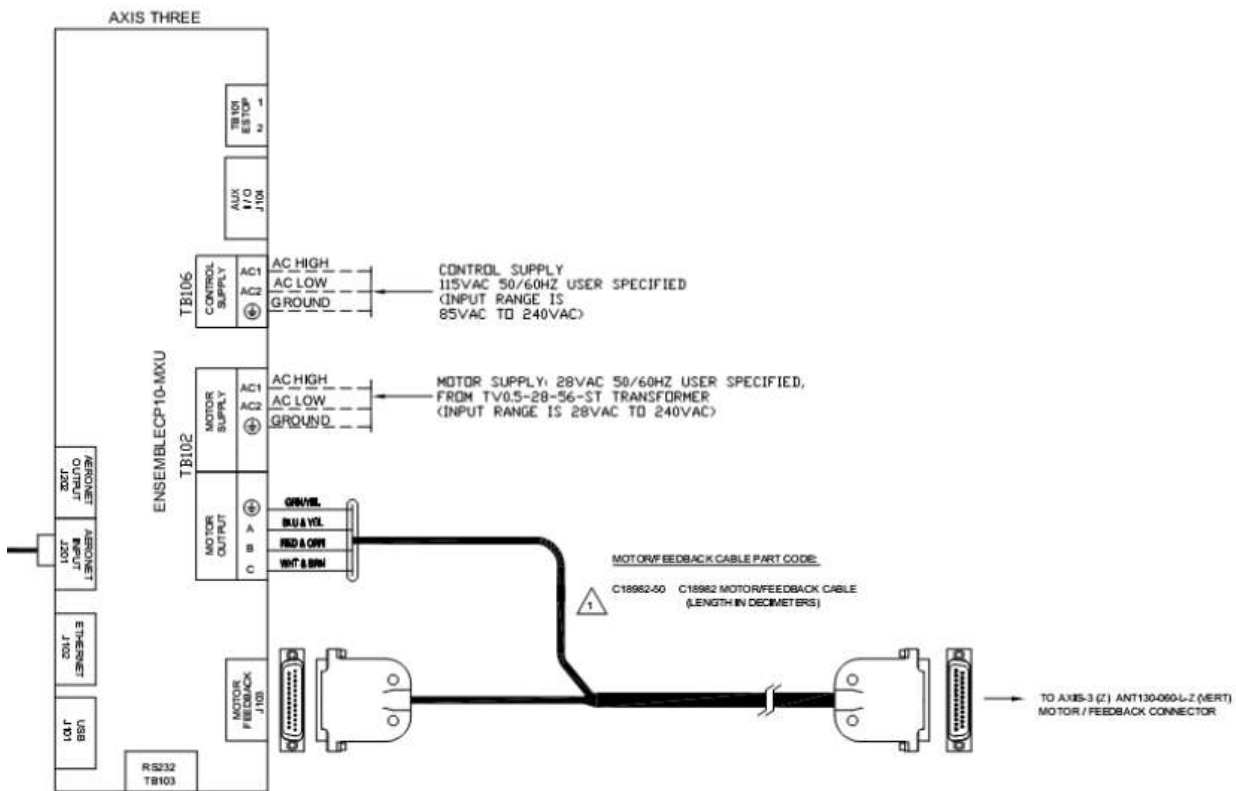
The wiring above show only axis-three, but the other axis wiring is the same. They are daisy-chained form the first to the second and to the third.
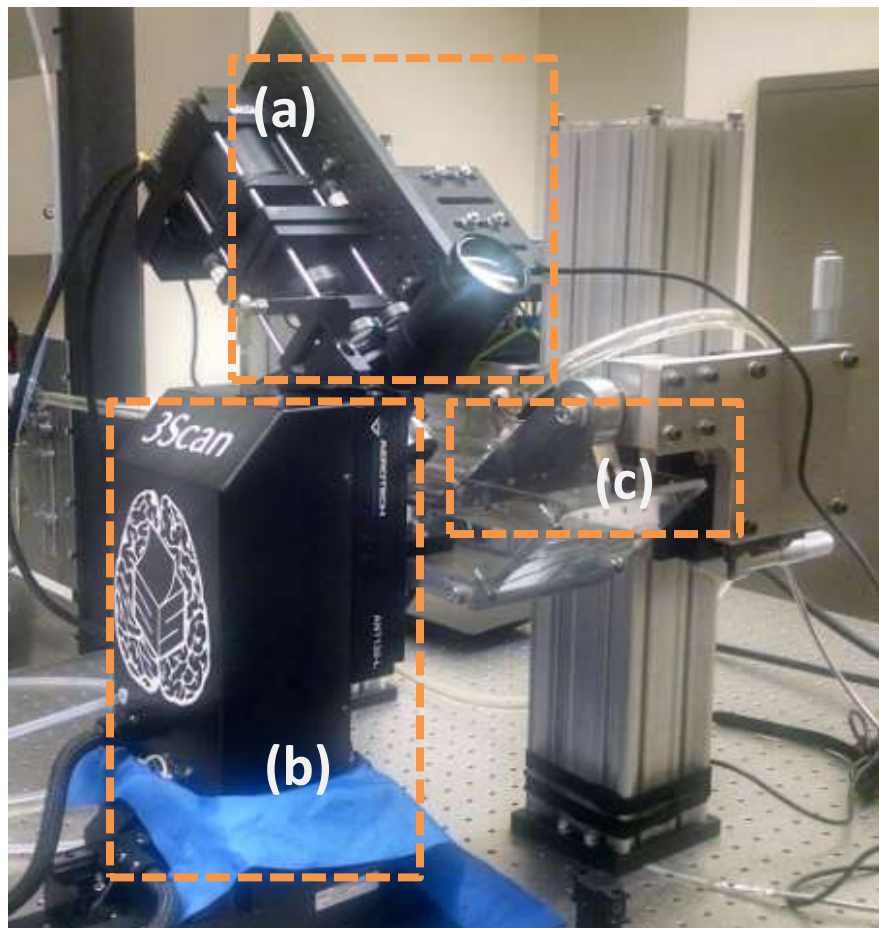
- Axis 1 - J202 -> Axis 2 - J201

- Axis 2 - J202 -> Axis 3 - J201

Axis 1 is connected to the host computer with the provided USB cord.

(3)   Tissue Scanner

The tissue scanner consists of three main parts: (a) the optics train, (b) 3-axis precision motion stage, and (c) knife assembly.

(a)   Optics Train

The optics train is to image of tissue samples. LED light is delivered to the back of the diamond knife via a custom terminated multimode fiber bundle. The diamond knife is in (c) the knife assembly. The light passes through the knife and the slices of tissue as they are sectioned. This light is
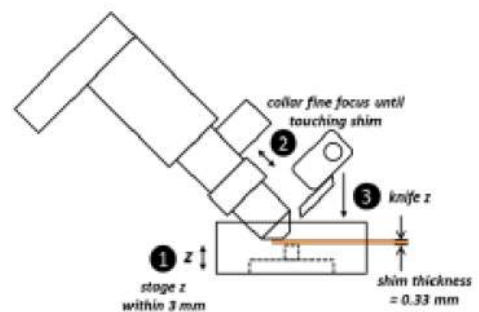
collected by an infinity-corrected microscope objective placed with its focal plane right on the sliced tissue. The light slit that is a line of the tissue image will be captured by the sensor of the line-scan camera at the terminal of the optics train.

Dalsa HA-80-04K40, a monochrome TDI line scan camera with a camera link interface is used in conjunction with a high resolution water immersion objective. The knife assembly is aligned with the optics and its position is registered by the control software. The light from the LED passes through the diamond knife that acts as a collimator and helps in imaging the tissue as it is being sectioned. The tissue is imaged just above the knife edge.

(b)   3-Axis Precision Motion Stage

This system consists of ANT130-060-XY-25DU-XYZ precision motion stage and the Ensemble controllers for interfacing to the stage from Aerotech Inc. The water bath is mounted on this stage. On the bottom of the water bath, a tissue block is placed. The control software moves the motion stage. This movement will move the water bath holding the tissue block. This causes the tissue to be sliced. The stage has a minimum incremental step of 2nm. It operates using an air bearing mechanism which causes lesser wear and tear than mechanical parts. This ensures longer runtime and life for the stage. The compressed air required for the stage counterbalance inlet is obtained from a compressor that is continuously on, via a SMC filter regulator for maintaining air purity requirement. The inlet air pressure to stage is 75-90psi.

The image capturing in the line-scan camera is triggered by the stage movements, and this synchronizes the stage movements with the collecting lines of images.

(c)   Knife Assembly

Kinematic principles employed in the new knife design allow for quick swapping of knives without the need for recalibration. The knife is required to have two ports, one for fluid-flow-based tissue removal and the other for light. The fluid flow is achieved using a channel milled between the knife shank and the knife arm, which has a hole leading to the top of the knife arm with a tubing connection to the water pump and filtration subsystem. The light port is behind the knife, which can accommodate

the fiber optics used to transmit light from the source to the knife. Ball lenses used to be the coupling method for light from the illumination source to the diamond knife. However, this was replaced with a better strategy by coupling the optic fiber directly to the rear-end of the knife module.

The picture left shows illumination through the edge of the diamond knife. The light goes through the objective in the optics train and reaches to the line-scan camera at the terminal of the optics train to be imaged.

(4)   Server Racks

The server racks host Linux backend systems with high capacity storages.

A Keyboard-Video-Mouse (KVM) interface is installed to support multiple servers and storages in the racks. More details of the functions will be discussed in the software section.

Software Components

The control software consists of many different parts as detailed in the following sections.

Overview of Control Software
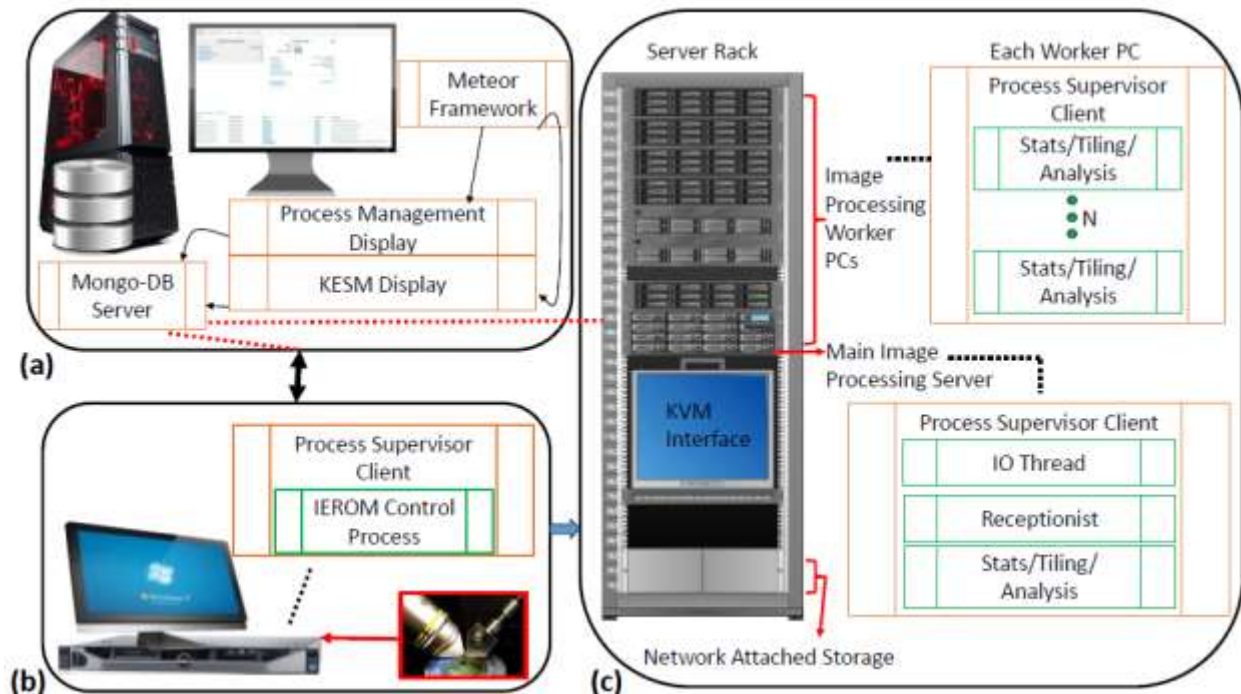
(a) IEROM Instrument Control Module. The stage controller and the line scan camera of the IEROM prototype are connected to a PC running an IEROM control client. (b) Management and User Interface Module. A server hosts the MongoDB database and runs the database process and system display management process for the user interface. (c) Processing and Storage Module. A server rack has processing servers to analyze images from the scope for statistics, tile them for display, and compress them before saving. The main processing server receives the images from the microscope and distributes the load to available worker servers. It also has a Keyboard-Video-Mouse (KVM) unit for direct debug if necessary, and houses the NAS to store the digital tissue samples for access in the future.

Administrative Control Interface

An administrative meteor server running a front end web interface helps manage the entire system. It is running on an Ubuntu server PC called "cerebellum." This interface can be used to control different KESM machines connected to the framework. In our case, a single KESM, named "Black" is connected to this framework and its status and history is listed on the main page as shown.

It consists of links to connected machines, process monitor page, logs from different machines and a link to the KESM control interface. It also displays most recent log messages from the control software system. A snapshot of this framework with all systems up and running is as shown below.

Logs tab takes us to detailed system logs displayed from the database to see the events in the system. Clicking on KESM Control gives a drop down list of all the KESMs connected to the control software backbone. In this case, only a link to the Black KESM control interface is shown. .

Processes tab is used to view and modify the processes running on each system. Process can be added and stopped in every connected system which makes it very interactive and easy to use. This tab is shown in the screenshot below.
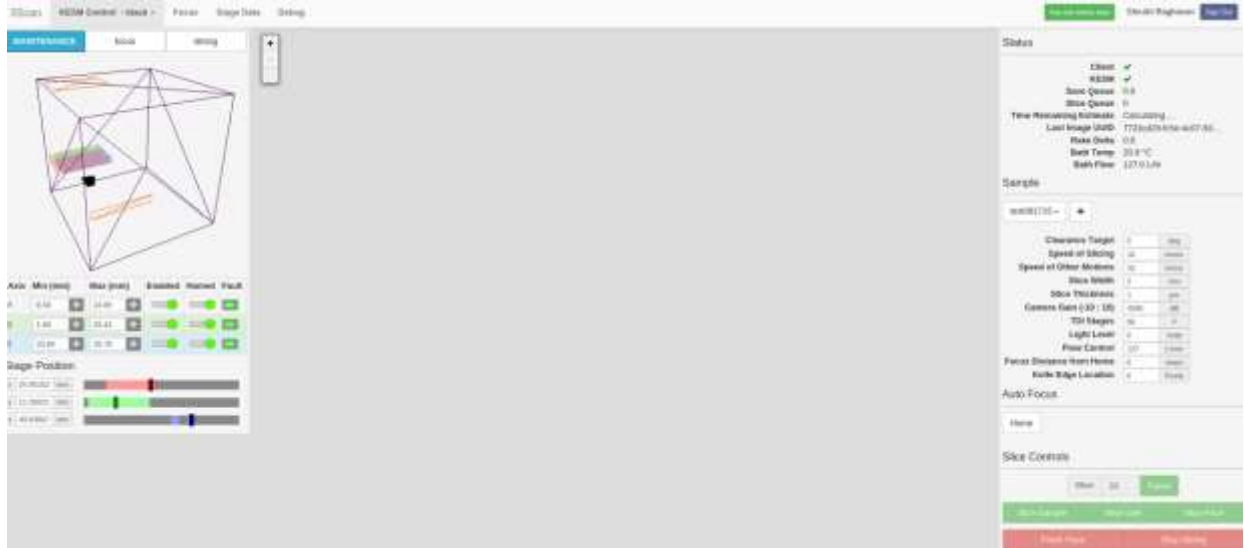
Control Interface

A meteor server for each KESM instrument, which in our case is only Black KESM is also available. This provides an interface to control the current state and settings of the KESM. There are 3 different control modes in the KESM – Maintenance, Focus and Slicing, each described below.

Maintenance

This tab allows the user to configure the 3-axis high precision positioning stage. We can enable or disable each axis of the stage. Initial configuration of controller after reset involves homing the stage. Followed by this, the sample start position is registered by filling the min and max value fields of each

axis in their respective field. These values can be measured by disabling just the axis to be measured and moving it to note the position at each end of the tissue block to be sliced. A black cursor indicates on the screen, the virtual knife position with respect to the tissue in the figure shown.

Focus

Clicking on the focus tab next to maintenance will switch the mode of the controller to focus mode. The focus tab above on the main toolbar will open an interface page for viewing the images from the microscope and to adjust the focus. We can see the image at the knife-edge at any point in this tab. An example of such a view is as shown below.
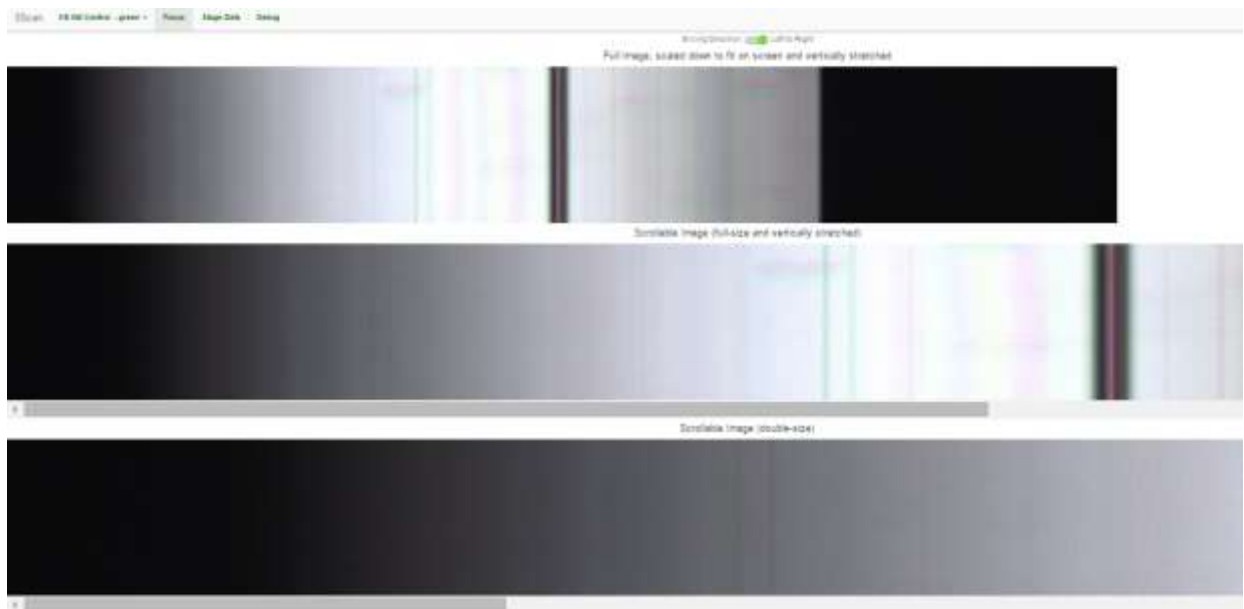
Slicing

Clicking on the slicing tab will switch KESM to slicing mode. The slicing mode enables the user to take one or multiple samples for testing imaging. We can also start slicing the entire sample. The slicing is done according to the settings for the particular sample. Sample Settings are shown on the right of the window. We can add more samples by using the "+" button on screen with the settings we want. We can also use "default settings" which will auto-fill standard values for parameters or clone a different sample's settings. The layout of the slicing tab is same as the maintenance tab & switching to slicing mode causes knife to go to the start position of tissue or to the previous knife position. Slicing is done by moving the controller. The motion of the controller in turn automatically triggers the image capture via the camera link cables connected on the same PC bus as the controller.

Database System

A Mongo Database management system that logs system messages and retains system state for recovery is run on "cerebellum." It is used to store image metadata records as well as to communicate the states of various processes into a centralized location. The visualization for the system operations and data storage, retrieval and communication is as given below. The control software ensures the smooth operation of system by retaining multiple properties even if the system is stopped, as long as the database is running and stable. Since the database is the heart of this system, we are running it on a powerful 8GB RAM desktop PC with AMD 64 bit processor and NVIDIA graphics card to support minimal GUI operations for the web interface. The flow of data and properties in the system is shown in the figure below. This configuration is described in the KESM design documents.
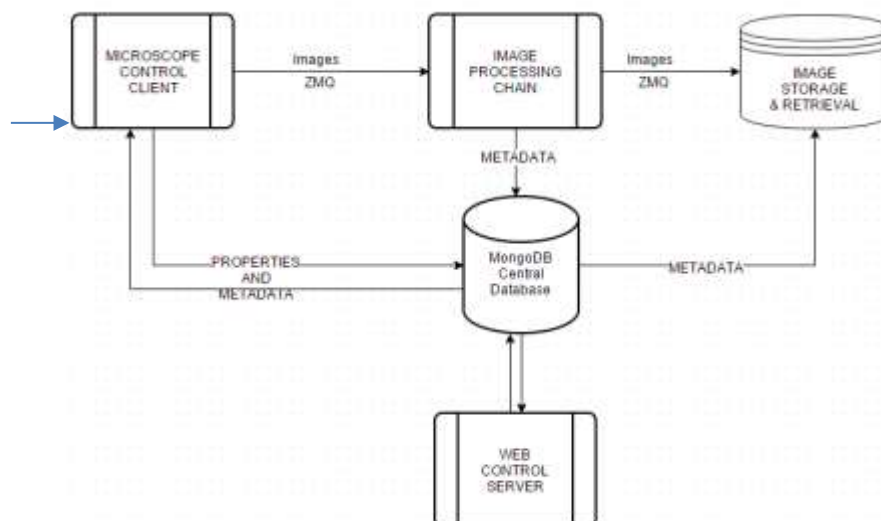
Microscope Control Client

A main client process runs on a client PC connected to the microscope camera and controller. This process handles the state and actions of the KESM. It communicates with MongoDB to write into it the current system properties as well as to read and respond to flagged requests from other parts of the system. It is also responsible for the hardware device management which includes the driver API in C for the components, interfaced to python via a special framework called CFFI (C Foreign Function Interface). It has processes that monitor the system state as well as response times. It flags an error into MongoDB if a process takes too long or other faults occur in the system. MongoDB in turn communicates them to the web interface for alerting the user.

Image Processing Chain

Receptionist and Image processing functions are available on every Image worker PC. Receptionist pulls the images from the scope computer. Images then go through analyze, compress and tile stages before they are sent back to the server. Currently the system setup has a single worker PC. But we will expand this by adding more machines as the slicing of tissue starts and KESM is fully operational. An IO-thread process running on an Image Server machine that receives all KESM images after processing, stores them to the preconfigured destination memory.

Although this is a basic processing chain, it is only for temporary visualization of results and does not completely do justice to the amount of data collected. So we are building a software pipeline that can handle KESM and any light microscopy image stacks, starting from preprocessing, up until visualization of data on an interactive web framework.

Data Processing Pipeline Overview
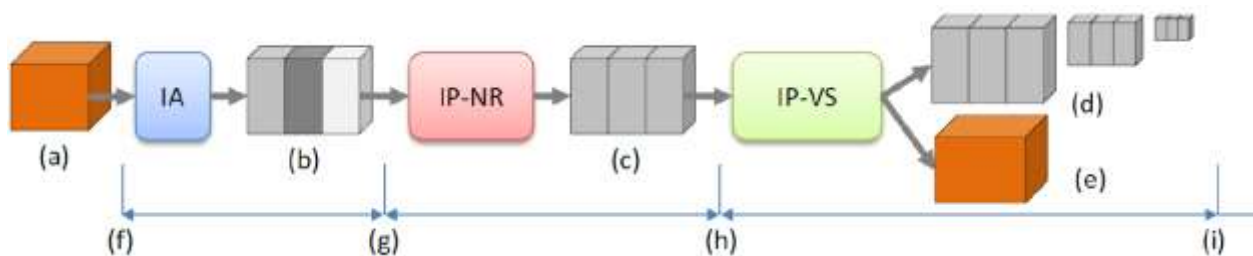
IA: Image Acquisition System. IPNR: Image Processing System for Noise Removal. IP-VS: Image Processing System for Visualization. (a) tissue block (b) scanned raw image stacks (c) processed image

stacks (d) multi-scale image volumes (e) mesh data for 3D visualization. (f)-(g): image scanner. (g)-(h) image processing. (h)-(i) data processing for visualization.