# Modern Microscopy with the Web of Things: The OpenFlexure Microscope Software Stack

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January 25, 2021

#### Abstract

Automated and computerised control of scientific instrumentation is almost ubiquitous in the modern laboratory. Most instrumentation is controlled over decades old communication busses or is accessed via proprietary system libraries. This limits which languages and operating systems can be used to control instruments, and poses a significant problem when interfacing multiple instruments into the same experiment. Here we present the OpenFlexure Microscope software stack as an example of how a scientific instrument can be controlled using existing, cross-platform, language-independent, industry-supported standards. We split the control code into client and server applications interfaced via a web API that conforms to the W3C Web of Things standard. This enables simple control of the microscope from multiple languages, provides a modern graphical control interface, and minimises duplicated code. Network control also makes the software stack more robust, allows multiple microscopes to be controlled by one computer, and facilitates sharing of equipment between local or remote users. Using a Web of Things approach in research laboratories has the potential to solve many of the key challenges of experiment integration, using technology that is already well established.

# 1 Introduction

Microscopists are increasingly required to write complicated experiment automation code, in multiple languages, to bind together inconsistent interfaces and proprietary software to run their experiments. Currently, a wide mix of often proprietary or "legacy" connectors and protocols are commonplace even in modern microscopes. This has led to many research labs relying on outdated hardware and software because of the requirement for interfaces that are largely obsolete in every other field of technology. Common practice is to make use of third-party solutions to retrofit interoperability, often maintained predominantly by microscope users rather than manufacturers. Since not all hardware is supported by these solutions, time must often be spent developing compatibility layers in the project's programming language of choice to enable even the most basic control.

In recent years, open web technologies have been widely adopted for controlling domestic hardware, in the "Web of Things" (WoT) [1]. These network and web technologies have already addressed many of the problems faced by laboratories and have been proven robust, fast, and secure by their long-standing ubiquity throughout modern life. Nowadays, support staff deeply familiar with networking and web technologies are already in place at most research laboratories and teaching institutions.

While prior work has introduced web technology into laboratories [2, 3, 4, 5, 6], these have lacked the comprehensive standardisation required for true interoperability. Recently however W3C, the primary international standards organization for the open web, have moved to standardise the Web of Things, with solid industry and community support [7].

Here, we introduce the software stack developed for the OpenFlexure Microscope [8, 9], an open-source, 3D-printed, and fully-automated laboratory microscope. The microscope has been deployed around the world in a wide range of operating environments, posing unique challenges as it is used in almost equal measure by novice and expert microscopists. While most commercial microscopes use direct connections to a single, physical computer, we use a web API (application programming interface) to enable local and remote control through internet protocol (IP) networks. The software stack makes use of modern networking technologies and Web of Things standards for microscope control and data management. The presented architecture for network-connected microscopy has allowed the OpenFlexure Microscope to be used in a diverse range of settings without re-implementation or code duplication. Additionally, the extensibility and interoperability has allowed users to develop additional functionality and entirely new imaging modes without having to re-implement the more complex instrument control code.

By designing our software stack around the W3C WoT Architecture [7], we have avoided introducing yet another competing device control system, and paved the way for comprehensive integration with existing microscopy software solutions such as µManager [10, 11, 12, 13] and Microscope Cockpit [14, 15]. Most modern programming languages have well-developed libraries for handling web requests. This means that WoT extensions can be developed for various microscopy control platforms to enable networked interaction with not just the OpenFlexure Microscope, but any device using the W3C WoT API model. This architecture is not limited to microscopy, and the same approach would work for most instrumentation. Client applications controlling multiple instruments for larger experiments can then be written in any modern language that supports web requests.

# 2 Architecture

## 2.1 Client-Server Model

The microscope's software stack includes low-level device control code, logic to integrate the hard-ware components into a useful instrument, automation for common tasks, a graphical interface for interactive control, and APIs for scripting automated experiments from various languages. Treating the microscope as an IoT device naturally splits these functions into client applications (the graphical interface and scripting APIs) and a server handling the bulk of the logic and hardware control.

This split between the server application and clients has several important advantages. First, it enables multiple client applications to connect to a microscope simultaneously. This allows, amongst other things, a graphical interface to display a real-time camera feed, while a script controls sample manipulation, data acquisition, and analysis. Conversely, a single client can manage multiple microscopes simultaneously. This has allowed clinicians to image sample slides from several microscopes concurrently, dramatically increasing data acquisition throughput.

Lastly, by separating the more complex server application from comparatively simple client applications, it becomes significantly easier to write client libraries in a broader set of languages. This means that microscope users can script experiments without having to re-implement the hardware control code in their language of choice, interface with a binary library, or learn a new language. It also ensures consistency between different languages and avoids duplicated effort, as most of the complexity is in the server application.

### 2.2 Hardware Architecture

Our server runs on a Raspberry Pi computer embedded in the microscope (Figure 1). This application handles communication with the sample translation stage, imaging camera, and any additional

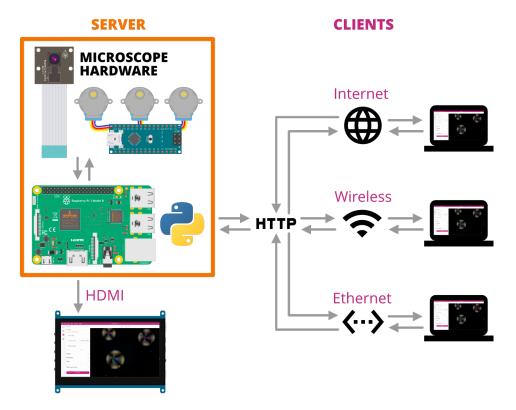


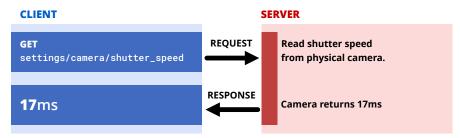
Figure 1: OpenFlexure Microscope implementation of the client—server architecture. A Raspberry Pi runs Python code to control physical hardware (camera, Arduino-based motor controller), and set up an HTTP web server and mDNS discovery records. Users can either control the microscope from the Raspberry Pi itself using HDMI output and USB peripherals, or connect over any IP network.

hardware, as well as logic for data management and additional functions such as tiled scans and autofocus. Running the server on an embedded computer ensures the hardware control code is running in a very well controlled environment. We automatically build and distribute an SD card image with a correctly configured operating system and our server and client applications pre-installed [16]. This eliminates the most troublesome aspect of distributing instrument control software, which is correctly installing and configuring the low-level drivers and libraries on an unknown computer, often clashing with system-level changes made to support other instruments connected to the same machine.

Client applications can run on the embedded computer, making the microscope a stand-alone system to which keyboard, monitor, and mouse may be attached. More usually, client applications will run on other devices connected via a wired or wireless IP network, using the Raspberry Pi's ethernet and WiFi interfaces. By using IP networking for instrument control we enable control of multiple instruments with any external router or switch. Replacing different (often proprietary and expensive) connectors and adaptors [17] with commodity hardware makes experimental science more efficient and more accessible to resource-constrained scientists.

The internet protocol itself allows for high-speed, low-latency plug-and play communication. Fully remote control can be enabled using existing, well-established secure protocols such as SSH forwarding and VPN connections. Clients can automatically detect the server's IP address and capabilities via mDNS (multicast Domain Name System) [18], which is already used extensively by consumer WoT devices.

### a) READ PROPERTY



#### b) WRITE PROPERTY

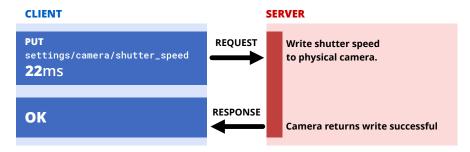


Figure 2: Simplified example of an HTTP request flow for reading and writing a "property" resource. a) Reading a property requires a GET request sent to the URL corresponding to the property to be read (camera shutter speed). The server then sends a response containing the properties value (17 milliseconds) in the response body. b) Writing a property requires a PUT request sent to the same URL, containing the new value in the request body (22 milliseconds). The server then sends an "OK" response confirming the operation's success.

# 2.3 Server application and Web API

The server application is written in Python, with much of the back-end code released as a separate library (Python-LabThings) [19]. This library uses the "Flask" [20] web application framework, and includes various utilities to simplify thread-based concurrency, mDNS discovery, hardware synchronisation, and documentation generation. We also make use of the standard scientific python libraries [21, 22, 23, 24]. Client–server interactions are governed by a web API based W3C Web of Things interaction model. This model defines properties, actions, and events that map onto HTTP URLs. The HTTP method then describes the *type* of operation to be handled. For example, an HTTP GET request will read the value of a property, whereas an HTTP PUT request will write a new value to the property (Figure 2).

W3C WoT compatibility is introduced by an automatically generating a description the microscope's web API functionality in a standardised format [25]. This allows clients to traverse the API with minimal prior knowledge, aligning closely with the principles of representational state transfer (REST) [26, 27], an API architectural style widely used across web service and WoT device APIs [27]. The widespread existing use of REST APIs means that users can interact with the OpenFlexure Microscope using existing standard libraries. We make use of the OpenAPI standard [28] for automatic generation of interactive documentation [29].

Many long-running tasks, such as acquiring large tile scans, must run in the background without blocking new API requests. Each request and action is therefore handled by its own thread to allow concurrency. Access to physical hardware is carefully managed to avoid conflicting instructions by use of re-entrant locks (Rlocks). In the simplest case, the lock prevents requests from sending instructions to a device already in use. For example, if the translation stage is currently in the middle of a long

movement, a request to move elsewhere will be denied until the initial move has completed, releasing the stage's lock. Clients can continue to interact with the microscope while an action is running, as long as they do not require a locked piece of hardware. This, for example, allows the live camera stream to be monitored during long-running experiments without users having to manually manage multiple threads.

Background tasks are handled automatically by the server. Whenever an "action" is requested, the server will start the function in a new thread and immediately send a "created" response back to the client, including the URL of an "action resource" the client can poll to check its progress or final return value. In the future, we can improve efficiency by allowing the server to asynchronously *push* updates to clients without polling, for example by utilising Server-Sent Events [30].

Most automation and integration can be done using client-side code, but for modifications that are best done on the server (e.g. adding new hardware, or low-latency integration) we use an extension system. The main server application handles only the most basic microscope functionality: capturing images, moving the stage, and managing device settings. All functionality beyond this is provided by extensions. This allows functionality to be customised on each microscope. Functions deemed broadly useful have been included and enabled by default in the server application, however all can be disabled as required. Extensions are written as Python scripts that have direct access to physical components comprising the microscope (e.g. camera and translation stage) through Python objects. Extensions can provide HTTP API endpoints and HTML interfaces that are displayed as part of the microscope's web app.

### 2.4 Clients

Our primary client for the OpenFlexure Microscope is a web application included in the microscope's internal API server. This application provides a comprehensive graphical interface for the microscope, including a live stream of the camera, capture functionality, basic data management, and full extension support. By developing the client as a browser-accessible web application, we are able to support many different operating systems without any additional code, while simultaneously drawing on the expertise brought by a large community of existing libraries.

The web application is accompanied by a desktop application (OpenFlexure Connect) handling device discovery and connection. The application finds and displays discovered microscopes using mDNS, as well as allowing manual connections and saving a list of commonly accessed microscopes. Upon connecting, the application finds and loads the microscope's graphical user interface (Figure 3).

Using a modular interface served by the microscope allows the client to only render user interface elements for enabled functionality. Server extensions are able to define new graphical interface components to be rendered within the main client application. For example, the interface to manage where images are stored is defined in the relevant extension.

For experiment scripting, we have created a Python client for the microscope [31, 32] that converts the web API into native Python functions. Extensions functionality is also mapped to Python functions automatically. This enables both scripted experiments as well as interactive sessions using, for example, iPython/Jupyter notebooks [33, 34]. This lowers the barrier to entry for scripting microscopy experiments since many students and lab users are familiar with iPython notebooks, especially for data analysis. The ability to run the graphical client at the same time, e.g. to view the live video feed, further simplifies setting up and monitoring automated experiments. We have also created a MATLAB client [35] with similar features. As the scripting clients are lightweight wrappers for the HTTP API, this involves a minimal amount of duplicated code.

The flexibility of the client–server architecture allows task or experiment specific interfaces to be created quickly. For example, a client for controlling the microscope with a USB game pad was developed[36]. This client is useful in remote field applications where a keyboard and mouse are not practical.

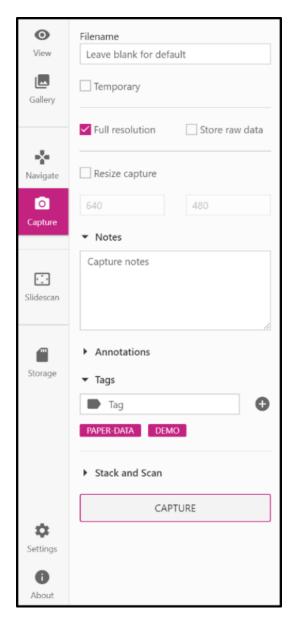


Figure 3: An example side pane from the OpenFlexure Connect microscope client. Using a web API for instrument communication allows the client to be written in any programming language. Using javascript we were able to create a cross-platform, responsive, and modern user interface using general purpose user interface frameworks.

# 3 Implementation of microscope functionality

## 3.1 Live stream

Central to the microscope interface is the integrated live video display. The Raspberry Pi camera is supported by GPU firmware that provides accelerated JPEG compression of each video frame, allowing us to serve a real-time Motion JPEG (MJPEG) live stream of the camera. The server starts a background thread on startup that records JPEG frames from the camera into a buffer. When clients

connect to the stream, the server will begin sending a multi-part stream of frames taken from that buffer. A synchronisation event is created for each client thread ensuring that clients never receive the same frame twice. As a new frame is read from the camera, the event for each client is set, at which point the response handler for each client will pass the frame onto the client, unset the event, and then wait for the event to be set again, dropping frames if neccesary to ensure latency is minimised for clients not able to receive frames at the full rate. This system is based on the work of Miguel Grinberg [37], and is included in the Python-LabThings library. We use MJPEG in preference to more sophisticated video formats in order to minimise latency in the stream. The MJPEG format also makes it easy to extract or drop individual frames, and enables our fast auto-focus algorithm.

## 3.2 Data collection

The Raspberry Pi camera can capture JPEG images and the raw 8 megapixel Bayer data from the camera sensor [38, 39]. This raw Bayer data can be used for more advanced analysis, avoiding artefacts from gamma correction, demosaicing, and compression. The server records metadata about the state of the microscope at the time of capture (camera settings, stage position, calibration data, and custom metadata added by the user), stored as a JavaScript Object Notation (JSON) formatted string in the "UserComment" EXIF field. Captures are stored locally on the Raspberry Pi, either on the SD card or an available USB storage device, and can be listed (including metadata) and downloaded through the HTTP API. Separating the actions of capturing images and downloading them avoids the need to transfer large amounts of data over the network during time-critical phases of an experiment. The standard graphical client provides an gallery interface to view captured images and view their metadata

As previously mentioned, multiple microscopes can be run in parallel to increase data throughput for a single operator. The greatest time saving can be achieved by setting microscopes to automatically scan over a large area, building a composite image of hundreds of overlapping fields of view (FOVs). The server has the option to perform such scans with movements, paths and capture types chosen by the user. Capture options and metadata are the same as individual captures, and individual images are saved, with metadata, as the scan runs [9]. Scans run as background tasks, so the microscope's video feed and position can be monitored as they run, and scans can be aborted without losing or corrupting images that are already acquired.

### 3.3 Auto-focus

Due to the parallelogram-based mechanisms controlling the motion of the OFM translation stage, changes to the x-y position move the sample over a sphere cap relative to the optics rather than a plane [8]. This necessitates an auto-focus procedure which can be run reliably at each x-y location in an automatic scan. As a typical diagnostic scan may require over  $100 \ x$ -y sites to be imaged, the software must focus rapidly while still being sufficiently reliable to not invalidate a large scan with any out of focus images.

A basic auto-focus procedure captures a z-stack of images regularly spaced between points expected to be far above and below the focal point. At each height, an image is captured and converted to grey-scale. A Laplacian convolution is applied to the whole image, assigning higher values to areas of greater spatial brightness variance. These values are raised to the fourth power and summed over the image to provide a sharpness value. The translation stage is then returned to the z-position with the highest sharpness. This procedure is based on methods used previously to detect focused areas in an out of focus image [40], and while highly reliable, typically takes 10–20 seconds to complete, limited by capturing and image processing time.

A fast auto-focus procedure utilises the MJPEG preview stream as a metric of focus. By disabling bit rate control, the stream becomes a simple series of independent JPEG images each with identical compression settings. This JPEG compression uses the discrete cosine transform to describe blocks of the image [41], where each block is described using a superposition of the fewest discrete cosine

functions possible, minimising the storage space required. As focused images typically have sharper feature boundaries, the storage size of an MJPEG frame will peak when the sample is in focus. By tracking frame size and z-position as the objective moves through the focal point without stopping, the position of peak sharpness can be identified and returned to. Monitoring the frame size while moving back to the focus and comparing to the initial data can be used to correct for imperfections such as backlash. Far from focus, sample features can overflow from one JPEG block to its neighbours. On a sparse, predominantly dark sample, this can introduce information into blocks which would otherwise be empty. If this increase in the number of blocks containing some non-zero information outweighs the reduction of blocks containing a large amount of information, the JPEG size may maximize away from focus. However, for feature-dense samples the size of a JPEG image can generally be used as a reliable measure of focus. This auto-focus method has a greater positional resolution than the discrete steps of the simpler auto-focus as MJPEG frame size can be tracked on-the-fly, reducing the time taken to less than 5 seconds.

### 3.4 Automatic Calibration

## 3.4.1 Lens Shading Table

Due to chief ray angle compensation on the Raspberry Pi camera module's Sony IMX219 image sensor, the raw images captured by the microscope suffer from vignetting even when the sample is uniformly illuminated. However, flat-field correction allows us to recover uniform images in software [42]. We use a forked version [39] of the "picamera" library [38], to access the lens shading table in the camera's GPU-based image processing pipeline, enabling us to correct for vignetting in both captured images and the real-time preview. A reduction in saturation at the edges of the image remains, but this can be corrected by post processing at the expense of higher noise in the image [42].

### 3.4.2 Camera-stage Mapping

It is often convenient to move the microscope stage by a given displacement in pixels on the camera, but the axes and step size of the translation stage rarely align perfectly. We calibrate this relationship by moving back and forth along the stage's x and y axes in turn, analysing the resulting displacement in the image from the camera [43]. We combine the calibrations into a  $2 \times 2$  affine transformation matrix that maps stage to camera coordinates. This is a similar approach to  $\mu$ Manager's Pixel Calibrator plugin [13], but treating the camera's coordinate system as ground truth rather than the stage. We avoid hard-coded step sizes by moving the stage in gradually increasing steps, and measure mechanical backlash by comparing motion in opposite directions. This allows an intuitive click-to-move feature of the microscope's graphical interface, and will in the future be used when scanning and tiling images.

The same image analysis used for stage calibration is used as a 2D displacement encoder, allowing for closed-loop sample translation and closed-loop scanning. This significantly reduces the error in each individual move, as well as ensuring that errors do not accumulate over the course of a large scan. Going forward, we will extend this functionality to include simultaneous location and mapping (SLAM) [44]. This will enable the creation of a map of the sample by comparing predictions based on commands sent to the motors to observations from the camera. This will enable accurate movements to features or areas of interest using the camera and estimated motor position.

## 4 Conclusions

In this manuscript we have demonstrated the benefits of adopting a client–server architecture for instrument control, that is built upon standardised web APIs. This architecture allows for local and remote control of an instrument, allows for multiple devices to safely access an instrument simultaneously, or for a device to control multiple instruments. Due to the ubiquity of web requests this allows experiment to be scripted in almost any modern language on any operating system using only standard libraries. We show how this architecture has allowed a highly extensible user interface for the OpenFlexure Microscope, from simple, cross-platform graphical clients to robust scripting libraries.

We have created a desktop client that provides a graphical interface for the OpenFlexure microscope suitable for scientific, educational, and clinical use. This can be extended via server plug-ins to allow the interface to be adapted for new purposes. We enable remote scripting of experiments with our Python and MATLAB clients, which can run alongside the graphical interface and integrate well with notebook-based programming.

The core architecture of our software is written as a stand-alone libraries which are not specific to the microscope itself [19]. This allows other instruments to adopt our standards compliant client–server architecture. We hope that this will enable greater standardisation of instrument control software built upon open protocols, and reduce the number of instruments that are tied to specific programming languages, proprietary software libraries, and legacy communication busses.

# 5 Acknowledgements

We would like to acknowledge financial support from EPSRC (EP/R013969/1, EP/R011443/1) and the Royal Society (URF\R1\180153, RGF\EA\181034)

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