Turn Your Microscope into a Robot
(with PARDUS)

Dr Paul J. Tadrous, *TadPath Diagnostics*, London.

What's the point?

Despite the growing importance of digital image analysis in histology[1], the specialist tools needed for automated image capture at high magnifications and quality are not widely available or affordable.
This work aimed to develop an open source (OS) hardware and software standard to address this.

Aren't you re-inventing the wheel? You've heard of high throughput whole slide imaging scanners (WSI) and OS analysis software, right?

Sure, but WSI are designed for speed and data compression. They have difficulties with autofocus at high magnification for subcellular detail[2,3,4,5] and can't deal with polarisation, oil and wet preps making them unsuited to applications such as routine diagnosis, AAFB detection, cytology, live cell imaging and 3D deconvolution. They're also expensive (>£100K[4,6]) and difficult to customise for research due to trade secret closed source software and hardware.

So what have you done about it?

I developed a hardware standard and software (PARDUS[7]) to enable almost any professional microscope to be automated using off-the-shelf and 3D printed components costing about £800 (fig. 1).
The software uses open source tools (pigpio, v4l2 and ZeroMQ) to provide stepper motor and camera remote control over a standard network. It also defines a script language making it easy to do custom complex tasks like Z-stacks and WSI with auto focus and tissue detection built in.
All hardware and software source code are due for release later in 2020 as open source for scientific transparency and to make the system easily customisable for all who wish to use it.

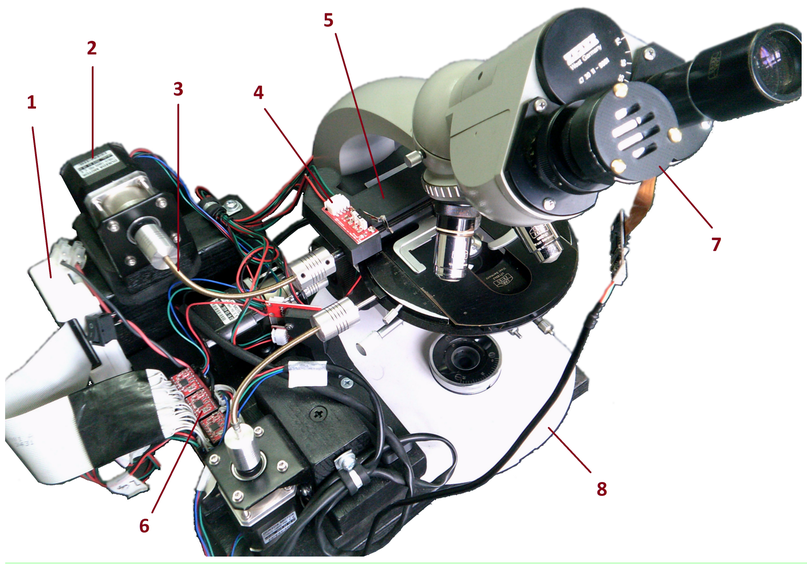
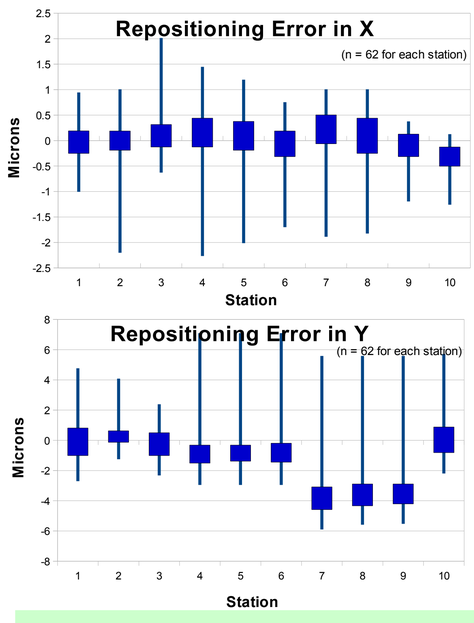
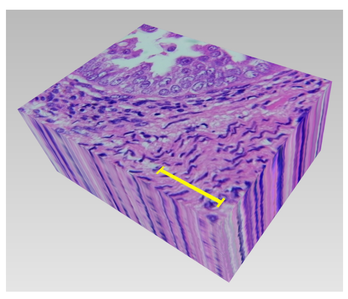


Fig. 1. PARDUS on an old Zeiss scope
1. Raspberry Pi, 2. Nema17 geared stepper motor (1 of 3), 3. Flexible coupling to stage, 4. Limit switch (1 of 6), 5. 3D printed switch mount, 6. Motherboard with 3x A4988 stepper drivers, 7. USB camera, 8. Scope base.





(Above) A stack of 62 images from station 2 illustrates the repositioning accuracy in visual form.
The vertical streaks down the side of the stack show the same feature imaged at each pass. Dark streaks indicate nuclei (scale bar = 50 microns). The images were captured with a x40 objective but, due to the small camera chip, the field of view is more like that seen with a x63 objective.

Fig. 2. Repositioning Accuracy
The scope was sent to 10 different locations ('stations') 62 times and the variation in positioning was (mean) -0.02 microns in X and -1.06 microns in Y (n = 620).

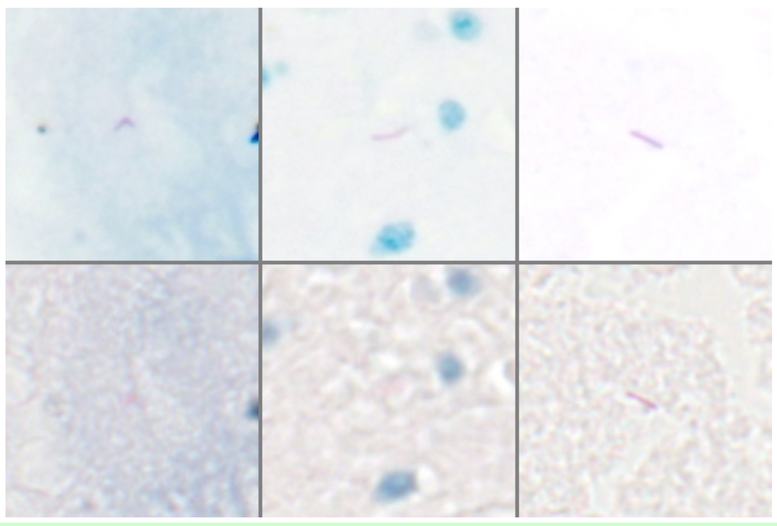


Fig. 3. AAFB: PARDUS vs. WSI Scanner
Examples of AAFB located using PARDUS (top) show good autofocus, contrast and definition on very different backgrounds: intracellular (left), intercellular (mid) and in pale bland necrosis (right). A total of 20 definite or probable bacilli were found in one section using PARDUS (only 2 were seen on initial manual diagnostic screening). Examining the same areas on a Hamamatsu S210 WSI scan (bottom) shows either such poor 'auto' focus as to make the images unusable for AAFB detection or abnormal contrast due to lack of true x40 optics without optimal Kohler illumination (as with similar WSI like the Aperio AT2[5] or CS2). Of course, ***by far*** the highest quality images of all – with zero digitisation artefacts and allowing real-time focus control with rapid application of lighting effects such as polarisation – are obtained by simply looking down the microscope. All forms of digital imagery are an inferior seeing modality compared to this.

That's all very nice in theory – but does it actually work?

Results from PARDUS fitted to a 1975 Zeiss Standard microscope show a stage repositioning accuracy of better than 5 microns in X and Y (fig. 2). A medium resolution whole section scan took just 3.5 minutes.
Twelve high resolution scans of a ZN-stained lymph node with autofocus (no user help) and a x40 objective captured AAFB in good focus on all 12 occasions despite the bacilli being very sparse (fig.3, top). This indicates the system can be an input for rare event detection by machine vision AI algorithms[8].
For comparison the same slide was scanned with a commercial WSI scanner[9] (fig 3, bottom). This required user interaction to select 'auto'-focus points and uses pseudo x40 (its actual objective lens is x20). Despite user-assistance, the Hamamatsu WSI still had difficulty achieving focus and the images showed obvious digital and illumination artefacts at the highest levels of detail. Many of the bacilli were so poorly imaged that they would be missed on routine 'digital pathology' diagnosis (let alone image analysis).

Mmm. So I suppose WSI scanners aren't the answer to all our histology imaging needs after all?

I would rather the take home points be:

- PARDUS provides affordable high quality critical micro-imaging for research and for automated machine vision algorithms in histology – particularly for high resolution and rare event detection. Current WSI scanners are just not optimised for such tasks.
- Its lack of commercial constraints and low cost means it is readily available to a wide range of users.
- Its lack of 'black box' trade secrets in both hardware and software make it ideal to customise for reproducible innovative open scientific research.

Contact: ptadrous@tadpath.co.uk

References

[1] Komura D, Ishikawa S. Machine learning approaches for pathologic diagnosis. *Virchows Arch*. 2019 Aug; **475**(2):131-138.
[2] Madabhushi A, Lee G. Image analysis and machine learning in digital pathology: Challenges and opportunities. *Med Image Anal*. 2016 October; **33**:170-175.
[3] Senaras C, Niazi MKK, Lozanski G, Gurcan MN. DeepFocus: Detection of out-of focus regions in whole slide digital images using deep learning. *PLoS ONE.* 2018; **13**(10): e0205387
[4] Zarella MD, Bowman D, Aeffner F, et al. A Practical Guide to Whole Slide Imaging: A White Paper From the Digital Pathology Association. *Arch Pathol Lab Med*. 2019; **143**:222-234
[5] Kohlberger T, Liu Y, Moran M, Chen PC, Brown T, Hipp JD, *et al*. Whole-slide image focus quality: Automatic assessment and impact on AI cancer detection. *J Pathol Inform* 2019;**10**:39
[6] David McClintock, Overview of Digital Pathllogy's Current State. https://www.executivewarcollege.com/wp-content/uploads/McClintock.THUR_.8.10am.FINAL_.pdf [last accessed December 2019].
[7] TadPath's **P**rogrammable, **A**ffordable, **R**emote-controlled **D**rive **U**tility **S**tandard (in preparation, due for public release mid 2020)
[8] Tadrous PJ. Computer-assisted screening of Ziehl-Neelsen-stained tissue for mycobacteria. Algorithm design and preliminary studies on 2,000 images. *Am J Clin Pathol*. 2010 Jun; **133**(6):849-58.
[9] Hamamatsu Photonics. NanoZoomer S210 Digital slide scanner. C13239-01