→**What is residual registration mismatch ?**

→Refers to the small remaining misalignments between two or more images after an image registration process has been applied

→**What is whole mount sectioning ?**

Whole-mount sectioning is a technique in histopathology where a full slice of tissue, such as a transversal cross-section of a prostate specimen, is prepared on a large microscope slide without further sectioning into smaller fragments.

→**Advantages of whole mount sectioning slides ?**

→improved correlation with pre-operative imaging

→reduction in tissue cutting artifacts

→the preservation of tissue context

→the availability of WMS is indispensable for research studies (e.g. radiology-pathology correlation studies), as evidenced by the multitude of imaging studies employing WMS as a reference standard

→preliminary evidence suggests that artificial WMS can reduce pathologist reading time

→**Disadvantages of whole mount sectioning slides ?**

→technical difficulty

→associated cost

→ cumbersome integration in (digital) pathology workflows

→Firstly, WMS require larger microscope glass slides due to the larger tissue area

→These larger microscopy glass slides are generally double the width of regular slides and may not always be available in pathology labs since these tend to increase the complexity of archival slide storage

→Additionally, even when these larger glass slides are available, they may still be slightly undersized for some larger prostates, necessitating further sectioning on a case-by-case basis

→Furthermore, the pathology labs that have switched to digital pathology encounter the practical limitation that most whole-slide scanners are limited to regularly sized microscopy slides and cannot scan the larger slides resulting from WMS.

→Lastly, there is some evidence that the preparation of WMS requires more expertise and technical proficiency than quartered sectioning

→The substantial impact of these practical limitations was corroborated by a working group aimed at establishing consensus on radical prostatectomy sampling, which demonstrated that only 16% of 148 respondents reported using WMS for sectioning of radical prostatectomy specimens

→**What does pythostitcher provide?**

→reconstructs artificial whole-mount sections from digitized tissue fragments, thereby bringing the benefits of whole-mount sections to pathology labs currently unable to employ this technique.

→**Overall steps of pythostitcher ?**

→automatically determines how fragments need to be reassembled (Maybe there is no metadata involved)
→iteratively optimizes the stitch using a genetic algorithm
→efficiently reconstructs the final artificial whole-mount section on full resolution (0.25 µm/pixel)

→The validation of pythostitcher ?

→PythoStitcher was validated on a total of 198 cases
→spanning five datasets with a varying number of tissue fragments
→originating from different organs from multiple centers

→residual registration mismatch of 0.65–2.76 mm on automatically selected landmarks.

→**Resolution Ranges ?**

→Firstly, the image size of a WSI scanned at the highest resolution, usually 0.25–0.50 µm/pixel, can easily surpass 100,000 pixels in both width and height,

→**Where is WMS applicable ?**

→WMS technique is applicable to various organs
→it is most prevalent in the histopathological evaluation of prostate cancer

→**The advantages of WMS are not currently accessible to all of the labs**

→the advent of digital pathology presents a potential remedy. In digital pathology, physical slides are scanned with dedicated scanners to obtain a high resolution (0.25 µm/pixel) digital image of the slide

→With the growing adoption of digital pathology and with increasing amounts of tissue fragments being digitized, there is an avenue for researchers to develop algorithms that digitally stitch tissue fragments into an artificial WMS resembling the original cross-section of the specimen.

→These artificial WMS are expected to provide similar benefits as actual WMS, while being more accessible to labs where routine processing of WMS is infeasible.

→**Challenges of fragment stitching**
→the image size of a WSI scanned at the highest resolution, usually 0.25–0.50 µm/pixel, can easily surpass 100,000 pixels in both width and height
→which is prohibitively large for even trivial image processing tasks such as rotating the image by several degrees.
→Secondly, in contrast to image stitching in panoramic photos, WSI do not exhibit any degree of overlap, rendering stitching based on overlapping landmarks infeasible.

→Limitations of Algorithms developed for reconstructing WMS from individual tissue fragments

    →HistoStitcher

        →specifically developed for prostate WMS
        →four prostate quadrants must be stitched to emulate an artificial WMS
        →required a pathologist to annotate corresponding landmarks on adjacent fragments which were subsequently used to compute the coordinate transform to stitch the fragments.
        →manual annotation of landmarks was cumbersome and introduced interobserver variability.

    →AutoStitcher

        →employed a genetic algorithm with a domain-inspired cost function to stitch adjacent fragments, thereby eliminating the need for manual landmark annotation.
        →still required manual labeling of the fragment's location (i.e. upper-left or lower-right quadrant) in the original cross-section.

        →This hinders the use of AutoStitcher for larger datasets where pathology reports with the sectioning reference may not be available and manual labeling is too time-consuming.

    →could not reconstruct the artificial WMS in full resolution due to memory constraints,
    →and the common use-case of stitching two halves instead of four quadrants was not supported.

→Some Results :
    →Automatic fragment configuration

        →A consideration :

            →We consider a reconstruction to be successful once the correct configuration is present in any of the suggested reconstructions.

            →For all test set cases with four fragments we observed a median of four and maximum of six suggested reconstructions.

→**How is accuracy measured ?**

→The accuracy is measured in two folds

→once for the fully automatic approach

→PythoStitcher obtains the correct reconstruction in 68.2–82.7% across the different test sets.

→**Reasons of failure :**
→a wrong prediction of the fragment classifier
→a fragment was horizontally flipped during slide preparation

→failure to accurately detect the stitch edge
→failure to identify the correct solution as a feasible solution.
→The first two of these categories represent the majority of all failure cases and can be easily solved with a slight manual correction

→manually specifying the location of the stitch edge
→horizontally flipping the culprit fragment before running PythoStitcher
→once when taking small manual adjustments into account.

→Taking these manual corrections for initial failure cases into account, this notably improves the configuration accuracy to 86.4–100% across all test sets.

| Dataset	Accuracy without manual correction	Manual corrections	Accuracy with manual correction	Fragment configuration time (min)	Stitching time (min)
Development set	83.3%	4 (7%)	90.0%	2.41 (0.46)	1.80 (0.52)
Internal test set 4	82.7%	9 (9%)	90.8%	2.47 (0.83)	1.93 (0.86)
Internal test set 2	81.8%	6 (18%)	100%	0.03 (0.01)	1.93 (0.52)
External test set	68.2%	4 (18%)	86.4%	2.07 (0.51)	1.64 (0.56)
Pancreas test set	n.a.*	n.a.*	100%	n.a.*	3.32 (0.98)
Artificial test set	73.3%	8 (27%)	100%	1.63 (0.24)	1.52 (0.44)

→PythoStitcher efficiently narrows down the initial solution space of 64 configurations by not suggesting more than six feasible fragment configurations per case.

→Based on the available computational resources, the end user can then choose to generate the full-resolution end result of either a selection or all of these solutions.

→these feasible solutions are ranked in likelihood

→**Problem :**

  →automatically selecting the correct solution, as swapping two adjacent fragments may lead to a seemingly correct solution with a very similar mean squared error, such as in solution one and two for the case in the upper row.
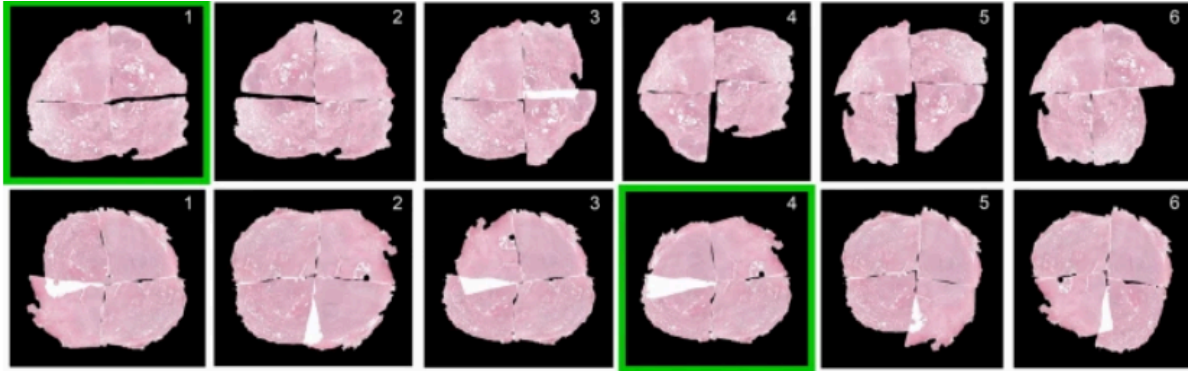
  →Nevertheless, the figure demonstrates that the **solution likelihood ranking by PythoStitcher** is able to identify most correct solutions and can be used to notably reduce computational overhead at the expense of a slightly lower configuration accuracy.
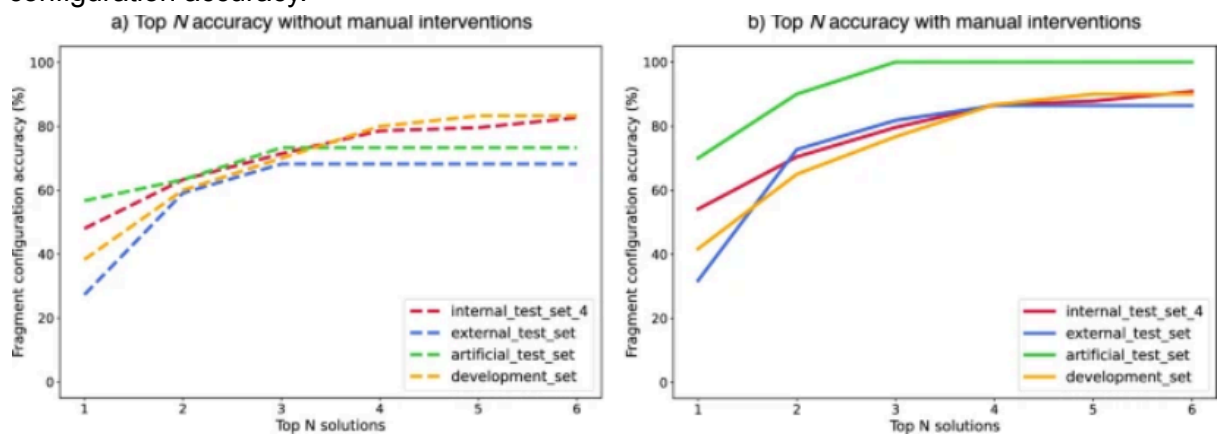

→**The Added advantage of the Multi-resolution approach**

  →This figure displays how the mean residual registration error for internal test set 4 steadily decreases after optimization at different resolution levels.

  →lthough the genetic algorithm was run to convergence at each resolution level, it appears that a single resolution level is insufficient to fully optimize the stitch between fragments.

→the lowest resolution is used to quickly close the gap between adjacent stitch edges

→the higher resolutions are used to finetune the exact fit between fragments.
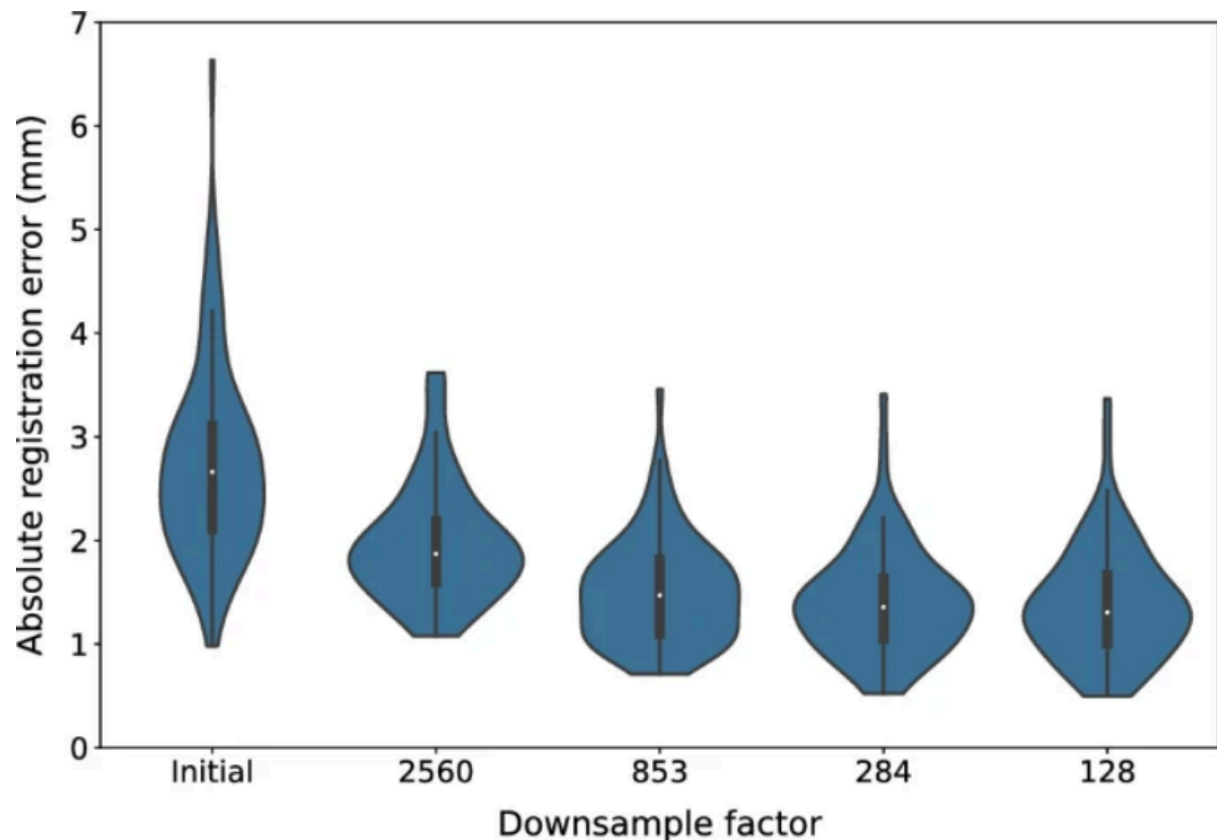
→Stitching results

→The quantitative stitching accuracy was computed for all cases where PythoStitcher identified the correct solution and is summarized in Table.

→The stitching accuracy on the artificial test set is hereby of particular importance since this registration error was computed using ground truth landmarks and serves as a fully objective evaluation.

→To put the absolute error into context, Table 2 also displays the relative registration error which was computed by dividing the absolute error by the mean WMS image dimensions.

Dataset	WMS size (mm)	Absolute registration error (mm)	Relative registration error (mm)
Development set	36 × 49 (27 × 47, 39 × 62)	1.11 (0.77)	2.46% (1.54)
Internal test set 4	39 × 45 (30 × 40, 50 × 56)	1.31 (0.66)	3.07% (1.47)
Internal test set 2	28 × 38 (25 × 28, 36 × 44)	1.23 (0.91)	3.76% (2.70)
External test set	33 × 45 (28 × 32, 38 × 54)	1.60 (0.71)	4.16% (1.00)
Pancreas test set	34 × 48 (29 × 36, 44 × 51)	2.76 (2.57)	6.94% (7.12)
Artificial test set	29 × 42 (20 × 36, 36 × 55)	0.83 (0.83)	2.16% (1.75)

→Computational efficiency

→When assessing computational overhead for the correctly stitched cases, we demonstrate that both the fragment configuration and the optimization with the genetic algorithm are achieved in a few minutes

→It should be noted that the stitching time refers to the stitching time per suggested solution and the total stitching time is therefore approximately a multitude of this number depending on the number of suggested solutions that were propagated from the automatic fragment configuration.

→For example, the total computational overhead for a case with three suggested solutions amounts to the fragment configuration time plus three times the stitching time.

→In addition, there is some computational overhead in writing the full-resolution stitched WMS to disk,

→but this greatly depends on external factors such as network speed and computer usage

→Empirically, we found that saving a 5GB stitched WMS at 0.**25 µm/pixel** resolution takes approximately **one hour** and saving a **35MB** stitched WMS at **4.0 µm/pixel** resolution takes a **few minutes**.

→**Methods**

→the automatic fragment configuration
→multi-resolution stitching
→generating the full-resolution WMS

→Algorithm inputs

→As algorithm input, PythoStitcher requires either two or four tissue digitized tissue fragments

→their corresponding tissue segmentation mask

→These tissue segmentation masks can be obtained through image processing operations such as Otsu thresholding

→advanced deep learning based methods

→**Automatic fragment configuration:**

→This automatic fragment configuration in PythoStitcher is mainly powered by a convolutional neural network (EfficientNet-B0)

→hich classifies where the stitch edges in a fragment are located (i.e. the upper/lower border and right/left border)

→PythoStitcher then uses this information to compute the exact coordinates of the stitch edges of all fragments.

→**Multi-resolution stitching**

→For each of the top N configurations from the automatic fragment configuration, PythoStitcher aims to optimize the stitch through a multi-resolution stitching approach with increasingly higher resolution versions of the fragments.

→An additional feature of the full-resolution reconstruction is that PythoStitcher blends overlapping areas of adjacent fragments, leading to a more visually appealing stitch edge between fragments.

→Full resolution construction

→To enable WMS reconstruction at a resolution as high as 0.25 µm/pixel, PythoStitcher leverages a tile-based approach using the PyVips

→where the final reconstruction is computed in a tile-based manner.

→This approach entails that the WSI is split into a large number of small tiles (e.g. images of size 64 × 64) after which the optimal rotation and translation for that fragment obtained from the multi-resolution stitching component are applied individually to all these tiles.

→ In essence, this splits up the computationally infeasible task to rotate and translate the full WSI into multiple smaller transformations that are computationally feasible while obtaining the exact same transformed WSI.

→With this approach, the required computational overhead only depends on the tile size and not the image size of the WSI.

→In principle, this allows PythoStitcher to reconstruct the artificial WMS at arbitrarily high resolutions and image sizes without any memory bottlenecks.

→Hence, this enables PythoStitcher to be employed on regular clinical workstations rather than requiring dedicated compute infrastructure.