

MAX-PLANCK-INSTITUT FÜR MARINE MIKROBIOLOGIE  
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## **Overview Image Acquisition of Microscopic Slides via Web Camera**

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# 1 Introduction

For automated microscopy it is essential that the system knows the location of the samples of interest. One possibility is to place them exactly in a predefined positioning pattern, but it is often quite challenging to position the samples with the needed accuracy by hand, especially if they are of small dimension.

In the publication of Zeder et al., 2010 <sup>[1]</sup> the automated detection of samples on microscopic glass slides via image analyses is featured. The described software tool SamLoc needs an overview image of the whole slide the samples are placed on. Microscopes are made for high magnification; this makes it impossible to image a whole microscopic slide with a build in microscope camera in a single shot. The publication of Zeder et al. showed that even with a low magnification objective of factor 1x,  $3 \times 11 = 33$  tile images are needed to cover the full slide area. This leads into a time consuming process, as multiple slides have to be imaged (usually 8).

Nevertheless the most important reason for alternative imaging methods is not time, but cost. Low magnification objectives are rarely used. This might be the reason for a relatively high price up to more than 1500 Euro. In addition, a specific bright field condenser may be required.

Here we would like to present an alternative faster and cheaper method. The idea is to equip the microscope with an additional side mounted camera, able to image the whole slide area at once. In this setup we feature the use of a standard USB web camera.

Build for consumer mass-market even high quality web cameras are available for a relatively low price. The camera we use here was 75 Euro and the setup images 8 slides in less than 2 minutes.

Corresponding software and documentation can be found on our web page [http://www.mpi-bremen.de/automated\\_microscopy](http://www.mpi-bremen.de/automated_microscopy).

## 2 Hardware

A microscopic stand with at least a motorized xy-stage is needed. In this study a Nikon ECLIPSE 90i microscopic stand <sup>[2]</sup> with a Märzhäuser scanning stage <sup>[3]</sup> was used. The stage can hold 8 standard microscopic glass slides.

The webcam should provide at least a mega-pixel resolution in order to capture images of high quality. In addition the camera must be able to focus on the relative small distance between its mounting point at the microscope stand and the slides surface on the stage, which is usually in the range of just 10 to 15 cm. Most web cameras are made for the 'monitor to user distance' of around 50 cm, which makes it necessary to test the small distance focus capabilities of a certain camera model beforehand. Further more it is important to get images without any distortion. To achieve this it is first important to mount the camera exactly parallel to the slide surface with the slide in the center of the camera view and second to buy a camera with high quality optics, such as glass lenses. In your setup we use a Logitech 905 C webcam with two mega-pixel resolution and build in Carl Zeiss glass optics. The original notebook design has a small housing, which contributes to an easier mounting at the space-limited environment of a microscopic stand. We removed the original monitor clamp and screwed the camera housing directly onto an aluminum rail, which has been mounted under the Nikon 90i filter rotor. The Nikon 90i has already some screws at this position available (originally used to hold a light shield) so that no modification at the microscope stand was necessary. Alternatively, it might be possible to glue the lightweight web camera at its final position if no mounting screws are available. The whole setup is shown in Figure 1.

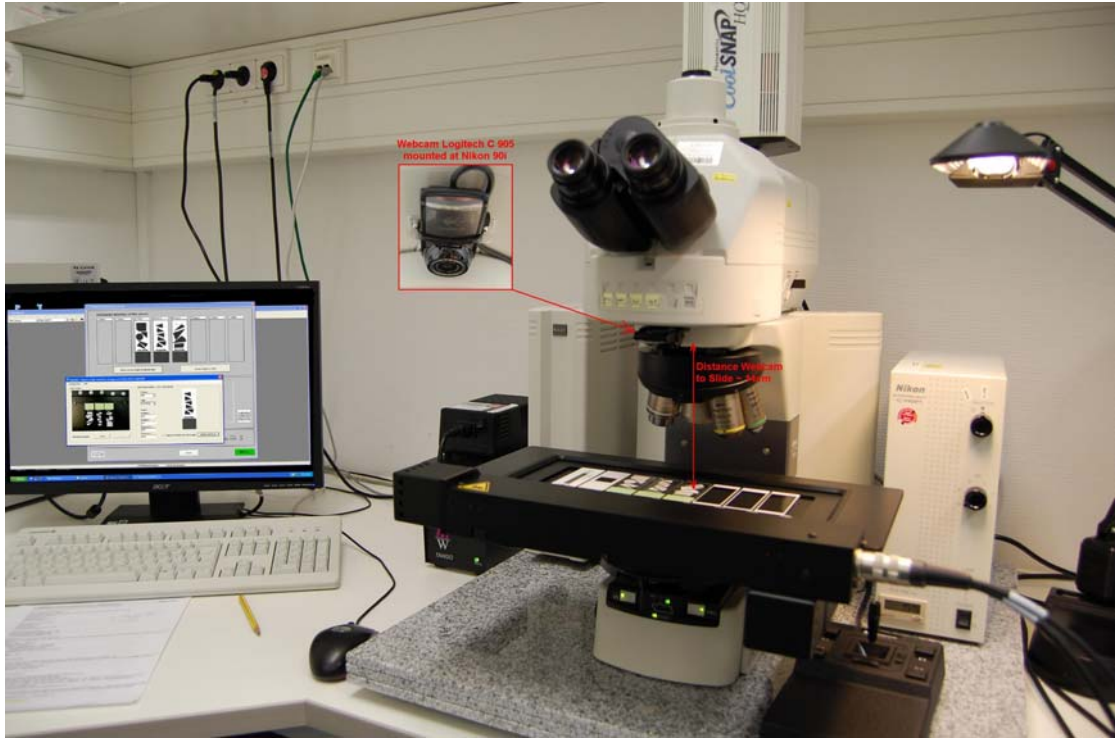


Figure 1: Nikon 90i microscope with Logitech 905 C web camera

### 3 Software

First of all a software tool able to capture a high quality still image from a web camera is needed. Of course most web camera manufactures provide such software together with the camera for free, but unfortunately it is usually not possible to integrate this software in an automated system. What we need is a software tool which, ones called, automatically captures and save a high quality image, without any user interaction. In general it is easy to program such software by using the 'capCreateCaptureWindowA' function of the 'avicap32.dll' with just few lines of code. Unfortunately this easy method is limited to a maximum supported resolution of 640x480 pixel, which is too less for a high quality overview image. To get full camera resolution it is necessary to implement the methods of Microsoft's DirectShow and DirectX components. We created a program called 'CapCam' in Microsoft Visual Studio 2010 using the programming language VisualBasic.net. Figure 2 shows the main window of CapCam.

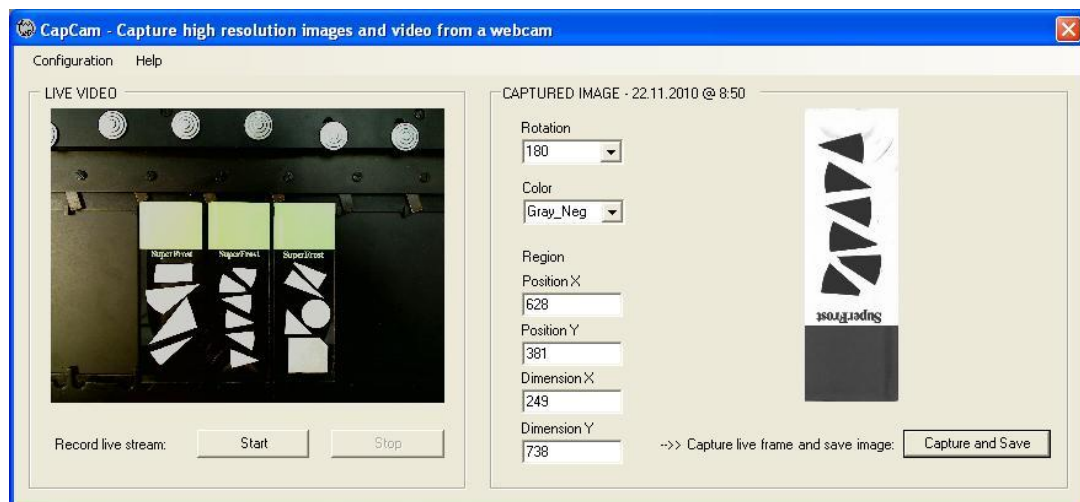


Figure 2: Main window of the software tool CapCam. Left: Full area live view of the webcam. Right: Captured part of the live image.

The settings are chosen in that way, that only the region of the central slide is captured, rotated, inverted and saved as negative gray-scale image. The software CapCam can be called with several command line arguments to hand over these settings. By doing so the software will open, capture the

slide, saves the image under the given path and name and closes itself when finished automatically without any user interaction. A description of all command line arguments can be found at the help menu of the software.

Second a software tool to move the microscopic stage from slide to slide position is needed. This task can be usually performed with the software provided by the microscope manufacture or with software coming along with the motorized stage. There are also tools available, which support nearly any microscopic hardware, e.g., the open source ImageJ<sup>[4]</sup> based  $\mu$ Manger<sup>[5]</sup> or the commercial MetaMorph<sup>[6]</sup> software package. For our system we programmed the stage movement and call for image capture as VisualBasic6.dll to feature a nice graphical user interface, which is then loaded within MetaMorph. The needed core functionality of that code could be also done with a simple script or journal of following structure.

```
*** Move the focus drive to get the needed distance between camera and slides
MoveStageZ to WebcamImagingDistance

*** Move the slides to a centered vertical position of the camera view
MoveStageY to SlideYposition

*** Loop via all slides to capture
for i = 0 to (numberSlidesToCapture - 1)

    *** Move the slides one by one to a centered horizontal position of the camera view
    MoveStageX to FirstSlideXposition + (i x DistanceSlideToSlide)

    *** Call capture of images with needed command line arguments
    *** Note: You need to pause the program loop until the slide has been captured!
    *** This can be achieved by a modal application call or with a fixed delay time after the call.
    ShellModal C:\CapCam.exe 1 1600x1200 C:\img Test 1000 180 Gray_Neg 628,381-249,738

next position
```

Figure 3: Pseudo-code example for stage movement and capture call

In a third step the taken images are used for automated detection of samples on the slides by using the software tool SamLoc<sup>[1]</sup>.

## 4 Setup and Calibration

Given the hardware is set like described previously and the needed software has been installed.

Place a microscopic slide on the first slide position of the stage.

Start the program CapCam without command line parameters and set the web camera 'frame size' to the highest supported value. A list of all supported resolutions of your camera can be viewed via the option 'video capture pin'. On the left hand side you will see a live video of your camera view.

Move your microscopic stage in xy-direction until the first slide is in the middle of the camera view. Additionally you might need to adjust the stage z-position to fit the whole slide in the viewing area of the camera. Depending on the camera model you might need to adjust the focus of the webcam as well to get a clear image. Write down the xy-coordinates of the stage and the z-position of the microscope stands focus drive. These positions are needed to program the stage movement like shown in Figure 3.

Use CapCam now to capture an image at full resolution of the total camera view. Open this image in an external viewer. Get the pixel coordinates of the left, upper corner of your slide in the image. These are the values for Region Position X and Position Y in CapCam. Get the width and height of the slide in your image and transfer these pixel values to Region Dimension X and Y at CapCam.

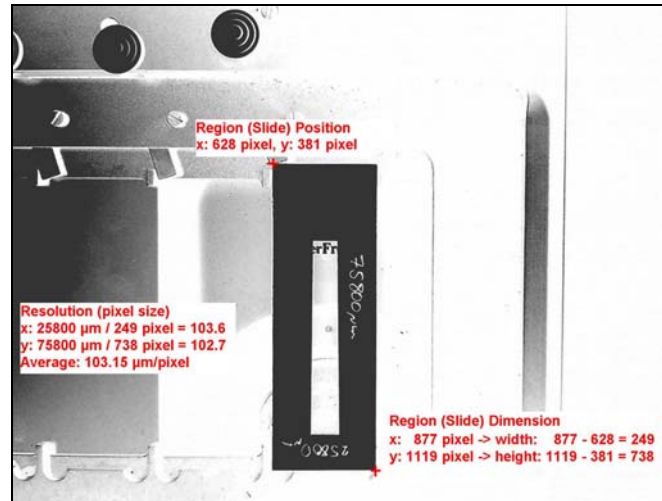


Figure 4: Full size webcam view with slide positioning calculations

If you now capture with CapCam again you should get just the region of your slide imaged. Depending on your camera mounting it might be required to rotate the image. For later on automated sample location the samples of interest should be the black regions in the image, so it might be needed to go for a negative color palette as well. Remember all this settings, they are needed to hand over as command line arguments when CapCam is called in an automated manner like shown in Figure 3.

Now all slide positions can be captured automatically.

Before generating a coordinate list by loading the images in the SamLoc program it is necessary to edit the 'current.txt' file of SamLoc.

In the first line you need to calculate the pixel size in  $\mu\text{m}$  of your overview images as shown in Figure 4. Second and third line values should be 1 because taken via webcam, there is just one image per slide. The fourth value is the width in pixel of your slide image. The fifth value is the height. The sixth value is the number of slides imaged followed by a list of xy-coordinates for each slide position. This coordinates represent the absolute stage positions of the left, upper corner from each corresponding slide. Easiest is to view the slides through the microscope at low magnification, move the stage to a



positions there the left, upper slide corners are exactly in the center of the field of view and write down the stage position values.

To assess the accuracy of such a setup, a test slide is imaged and loaded into SamLoc. The coordinate points are placed at distinct locations, e.g., exactly at the corners of a triangular shaped object. Generate a coordinate list with SamLoc and move the stage to these calculated positions. By observing the test slide through the microscope, the difference from the calculated position can be seen. Theoretically the position calculated in SamLoc is exactly in the middle of the field of view. The difference from this position reflects the accuracy of the setup. If the divergence is too large, the settings in the 'current.txt' of SamLoc might need to be adjusted.

The following diagram and table show the accuracy we could achieve with our setup. Note: To make it easier we usually assume that the zero point of the stage is the upper, left corner, so the first slide position is on the left hand side. Our stage has the zero point on the right, front position. For a direct comparison the coordinates have been converted accordingly.

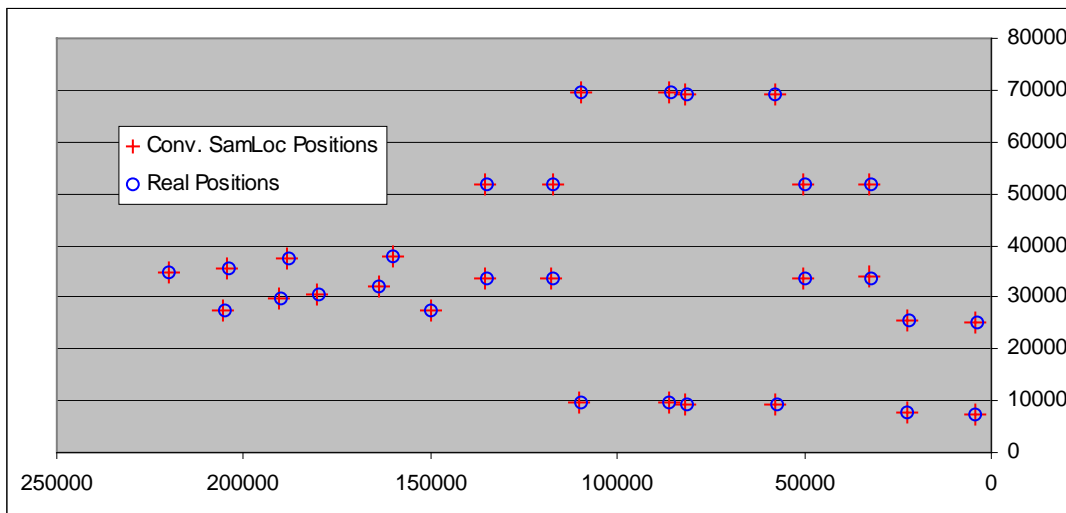


Diagram 1: Average accuracy

Slide	SamLoc coordinates conv. to stage system		Coordinates viewed with 10x Objective		Positioning difference (absolute distance)	
	x	y	x	y	x	y
1	220193.98	34920.50	219979.10	34768.70	214.88	151.80
1	204261.75	35461.88	203991.90	35365.50	269.85	96.38
1	205344.52	27495.77	204974.60	27603.00	369.92	107.23
2	180282.84	30500.07	180030.00	30364.20	252.84	135.87
2	190414.50	29881.34	190248.30	29708.50	166.20	172.84
2	188171.61	37383.41	188081.10	37325.00	90.51	58.41
3	149891.63	27394.43	149763.30	27341.10	128.33	53.33
3	163813.00	32112.22	163686.20	31932.10	126.80	180.12
3	160100.64	37912.79	159912.70	37792.20	187.94	120.59
4	135376.32	33733.38	135005.60	33619.50	370.72	113.88
4	117510.60	33733.38	117246.30	33687.30	264.30	46.08
4	135376.32	51831.16	135072.80	51685.10	303.52	146.06
4	117433.20	51831.16	117308.00	51725.80	125.20	105.36
5	110243.30	9743.70	109846.10	9820.50	397.20	76.80
5	86267.60	9666.36	86060.70	9745.70	206.90	79.34
5	109856.60	69605.57	109654.70	69636.60	201.90	31.03
5	86035.60	69605.57	85903.50	69548.70	132.10	56.87
6	81856.00	9277.66	81298.20	9293.10	557.80	15.44
6	57725.60	9277.66	57545.50	9316.40	180.10	38.74
6	81778.60	69216.86	81503.40	69046.60	275.20	170.26
6	57802.90	69371.55	57734.50	69130.00	68.40	241.54
7	50155.00	33702.38	49927.30	33581.80	227.70	120.58
7	32443.90	33857.07	32190.50	33698.80	253.40	158.27
7	50309.60	51645.47	50038.90	51630.60	270.70	14.87
7	32443.90	51877.50	32282.00	51751.70	161.90	125.80
8	22567.00	7648.49	22235.80	7632.10	331.20	16.39
8	4469.20	7416.47	4157.60	7467.80	311.60	51.33
8	22412.30	25514.25	22052.10	25392.20	360.20	122.05
8	4237.20	25282.22	3986.60	25247.00	250.60	35.22
		<b>Accuracy</b>	<b>Average</b>		243.38	98.02
			<b>StDev</b>		106.65	58.08

Table 1: Average accuracy

## 5 Acknowledgments

The program 'CapCam' is based on a the great VisualBasic.net programming example 'CapSample1' from Blas5 <sup>[7]</sup>, the 'DirectX.Capture' class of Brian Low <sup>[8]</sup> and the 'DirectShow.net' class of NETMaster <sup>[9]</sup>.

## 6 References

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