Hochschule Niederrhein
University of Applied Sciences

REAL-TIME IMAGING FOR MICROFLUIDIC APPLICATIONS

BACHELOR THESIS

In the bachelor's degree course in Engineering in Industrial Technologies at the Public University of Navarre. Project developed within the Erasmus program at the Department 03 Electrical Engineering / Computer Science of the Hochschule Niederrhein by:

ASIER GARCIANDIA LÓPEZ

Matriculation number: 1150030

Tutor: Professor Dr. Jost Göttert

Supervisor: Dipl. –Ing. Georg Toszkowski.

STATUTORY DECLARATION

I hereby declare that the project entitled REAL-TIME IMAGING FOR MICROFLUIDIC APPLICATIONS is an original work done by me as an Independent Research Project and only with the aid of the specified means and sources.

I also declare that the project is the result of my own effort and dedication.

Krefeld, 28.07.2017

Asier Gaciandia López

ACKNOWLEDGMENT AND DEDICATIONS

I would like to thank both the mentors Professor Dr. Jost Göttert and Dipl. -Ing. Georg Toszkowski for their patience and dedication even in busy times and helping me with the realization of the project.

I would like to finish with an Albert Einstein's quote I learnt and found identified with during the realisation of the project: "If we knew what we were doing, it would not be called research, would it?".

ABSTRACT

Outline
Demand of visualization in real-time for research in microfluidics.
Examples – cell investigations, droplet chemistry, PIV (Particle Image Velocimetry).

Flexible microscopic hardware for different requirements demand a software solution easily adapting to the hardware configuration and producing images at maximum frame rate of the camera (typ. 20f/s). Customer can select and store any image along with set-up information for documentation and further analysis. Graphical user interface allows easy and guided (self-guided) operation.

Keywords: Imaging, Camera, Microfluidics, Optics, IMAQ Vision LabVIEW.

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1. INTRODUCTION

Microfluidic solutions are of great interest for biological, chemical and medical applications. The term Lab-on-Chip (LoC) illustrates the overall goal for these applications implying that typical lab operations such as mixing, diluting, delivery and observations are done within the confinement of a chip. The basic configuration for LoC experiments is shown in **Figure 1.1**:

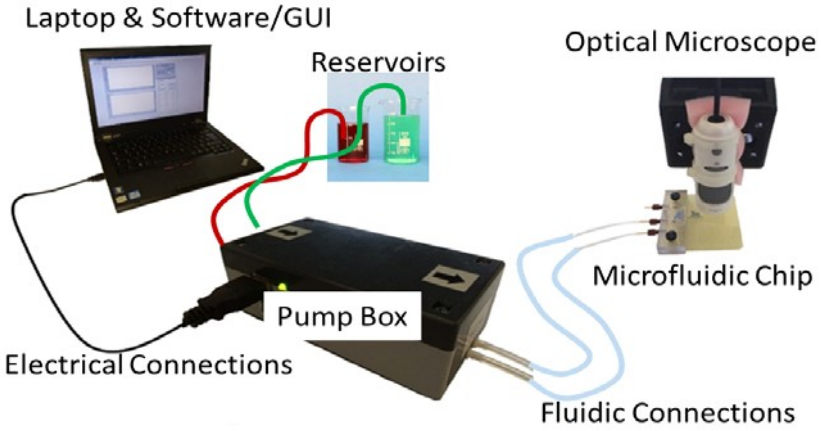
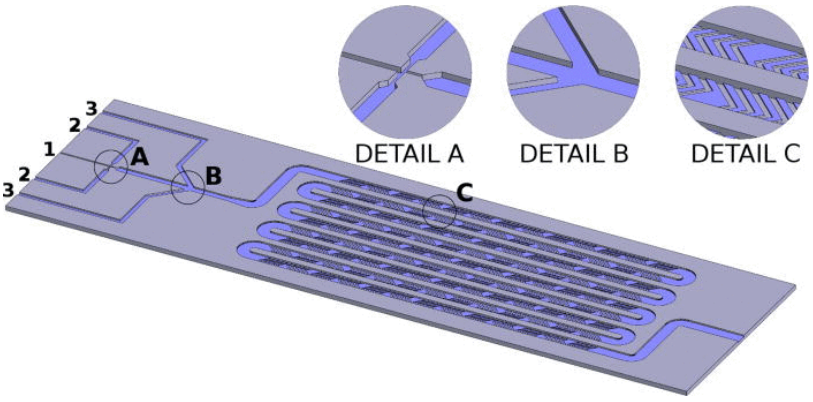


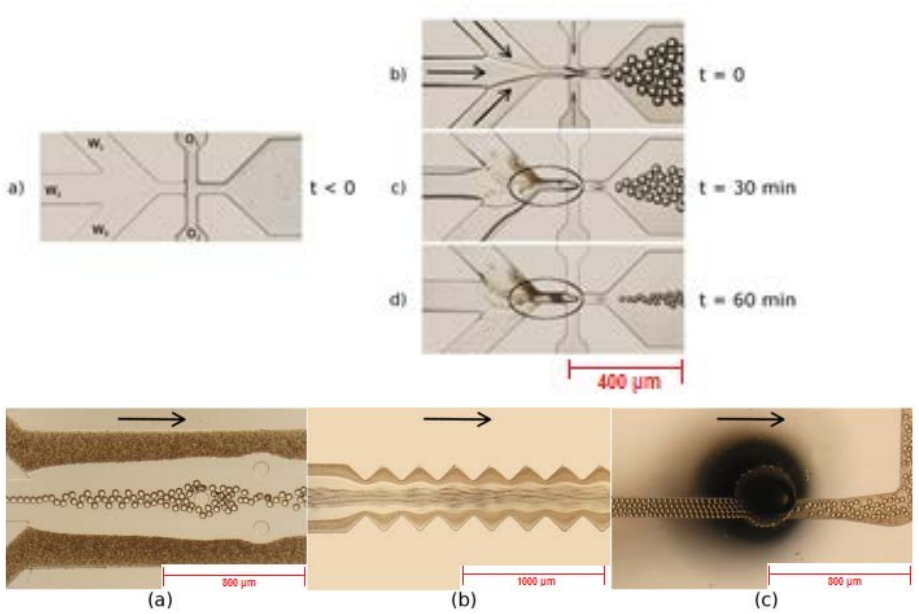
Figure 1.1- Basic configuration for LoC experiments.

The pump box delivers chemicals from the reservoirs to the microfluidic chip, which performs the desired lab operations. The experimental set-up and execution are controlled with a user interface from a laptop or tablet: sensors and actuators ensure proper execution and chip performance and help with evaluation and analysis of results.

An optical microscope is a required and compulsory hardware for understanding and evaluating LoC microfluidic experiments, due to the scale and the relatively small environment in which the scientist is working and the detail demanded in those applications. This fact is plainly illustrated in the images from **Figure 1.2** (for further information about microfluidic experiments, read the highly recommended article “Microfluidic channel structures speed up mixing of multiple emulsions by a factor of ten” written by Kevin John Land, Mesuli Mbaniw and Jan Gerrit Korvink [1]):



1) Inspection quality assurance: metrology and defect.



2) Intelligent documentation of experiments: Video and time-lapsed imaging.

3) In depth analysis: Image processing

Figure 1.2- Typical microfluidic structures [1].
1) Design of a microfluidic circuit showing the positioning of structures in the serpentine channel. Structure testing was done in straight channels, while comparative tests without structures were done in the serpentine channel shown. 2) Series of photographs showing the problem experienced when introducing reagents in continuous form. (a) shows the channels before the experiment starts, with w1, w2, w3 representing water phase and o1 and o2 representing oil phase, (b) shows the start of the experiment, with arrows indicating the direction of fluid flow, while (c) and (d) show various stages of blocking in the channel. As blockage gets worse ((c) and (d)), droplet formation (frequency and size) decreases dramatically. Ellipse shows the region of worst blockage. 3) Various experiments tried to force mixing of the pre-emulsion and the larger droplets (a) introduction of structures into the main channel, (b) constrictive side wall structures, and (c) introduction of the pre-emulsion from the top of the channel.

A simple visualization of the sample within the confinement of the microfluidic chip is often desired (for example, for monitoring cell growth and flow signals). **Figure 1.2** illustrates that depending on the type of research, users have many different requirements for information, such as documentation of the type of microscope, acquisition of image details, possibility to zoom in and out, projection of a scale bar or comments, storing an image or a video and many more… .The implementation of those features in a user-guided software interface will enable the researcher to properly document his work and allows easy sharing of information with others.

The following information requirements of the software for real-time imaging are derived from **Figure 1.2**:

1) Inspection quality assurance: metrology and defect. Necessity to include tools in the software solution so that the user can perform operations of high precision inspection (for instance, particle and defect diameter measurements and set-ups for different microscopes) and storing (necessity of retrieving the information when needed).

2) Intelligent documentation of experiments: Video and time-lapsed imaging. Possibility to record and play videos with an arbitrary frame-rate or time span, so that real-time microfluidic experiments can be visualized and analyzed in

depth and without reproduction problems due to the speed.

3) In depth analysis: Image processing. Possibility to zoom-in and -out and include text/comments in images. Distance measurements with both machine-vision and a user-guided drawing tool.

From those three bullet points, the necessity of a database to store all the information (paths of saved images and videos, type of saved images and videos, classification of the analysis information as set-up or in-depth…) is clear.

This project has been developed in room F-305 of the Hochschule Niederrhein: Technische Informatik Kompetenzzentrum ISA, Mikroprozessortechnik Labor. The software tools used to realize the project were fully accessible in the computers of the laboratory.

1.1. GOAL/OBJECTIVE

In order to standardize optical inspection and monitoring of LoC based experiments, it is the goal of this research project to develop a software that guides a user in setting up his imaging equipment and supports the research efforts.

LIST OF INDIVIDUAL TASKS

1) Hardware setups- Library including typical fluidic chips and examples of images.

2) Software concept for in-situ chip inspection as well as image analysis.

3) Implementation of the software with LabVIEW.

4) Tests & Documentation.

2. BACKGROUND OF THE PROJECT

For the decision making of which functionalities (documentation of type of microscope, projection of scale bar…) should be included in the user interface, initial tests were conducted. This chapter summarizes the main ideas of this work and connects them to the specifications of the final software solution.

2.1. MICROFLUIDICS

Microfluidics is the name of the field where the real-time imaging software is focused, being highly conditioned by its rules. Therefore, an intense literature research of this field was required.

Microfluidics is defined as the science and technology of systems that process or manipulate small (10^{-9} to 10^{-18} liters) amounts of fluids [2]. To fabricate a microfluidic device, a patterning process utilizing lithography and molding is applied as illustrated in **Figure 2.1**. The various process steps are described more in detail in **Table 2.1**.

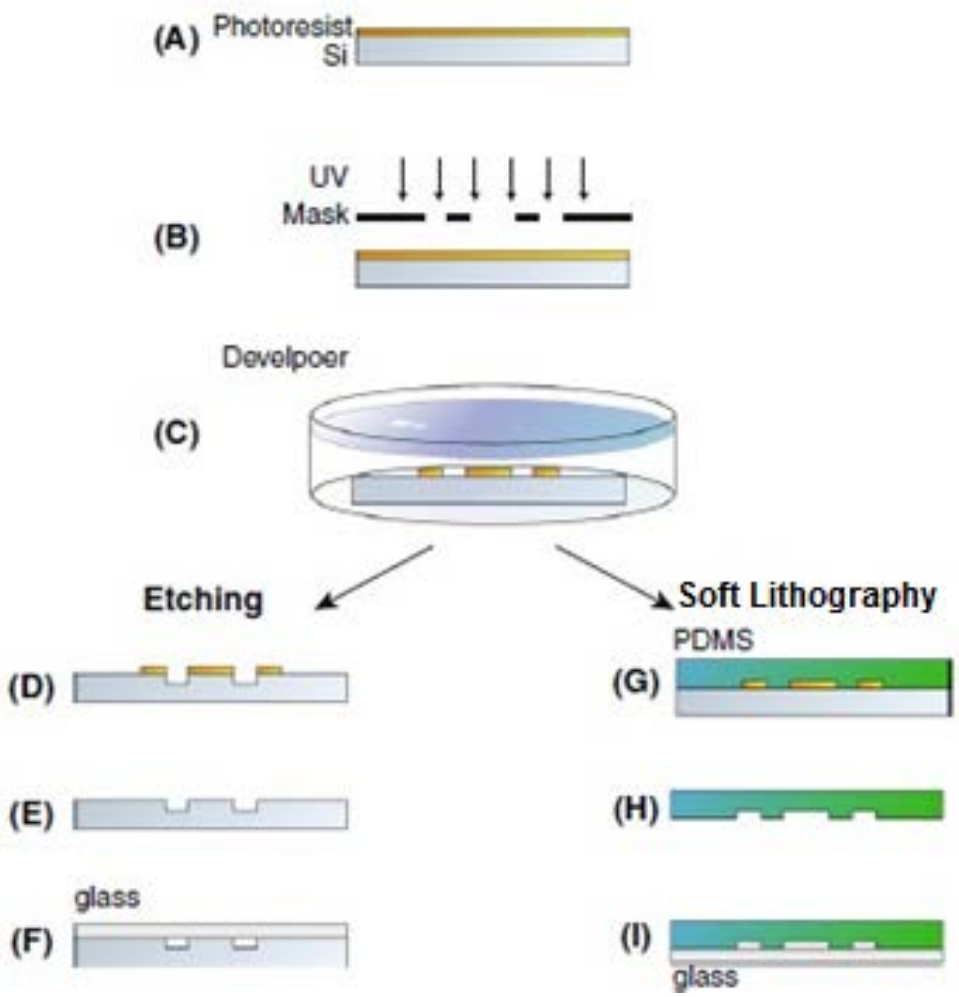


Figure 2.1- Patterning process for microfluidic applications [2].

STEP NUMBER	NAME	DESCRIPTION
A	Preparing the substrate.	Silicon with several types of doping is usually used.
B	Spin coating the photoresist.	Use libraries to choose the adequate photoresist.
C	Photolithography.	Transfer of micrometre-sized features on the photo-mask to photoresists coated on top of the substrate.
D, E, F	Etching.	Transfer the pattern to the substrate with HF, KOH, etc.
G, H, I	Soft Lithography.	Use polydimethylsiloxane (PDMS) to cast against the photoresist mould. PDMS is softer and more transparent than silicon and it is permeable to gasses and biocompatible. Patterned PDMS can directly be used as a substrate for cell culture or be used as a stencil to pattern the inoculation of cells onto other substances.
J	Repetition.	Repetition of steps A-I to fabricate more layers of features. Finally, a 3D hierarchy structure with different chemical compositions is obtained

Table 2.1- Patterning process for microfluidic applications [2].

Finally, the substrate is sealed to ensure that the fluidic channel can sustain a desired amount of pressure. When macro-to-micro adapters are attached, the chip is ready for operation. Microfluidic devices are usually combined with optical characterization techniques such as fluorescent microscopy.

With the new technologies that allow detailed investigation of individual cells, it is clear that such a single-cell approach has become compulsory for the understanding of cellular heterogeneity and its biomedical importance. The ability to isolate subpopulations of cells resistant to certain drugs in cancer treatment and microbial pathogenesis has shown up that cells comprising less than 1% of the total population can be the most important cells to extinguish during treatment [5]. The imaging, measuring, documenting and counting of those separate cells communities are features of high interest for the software which is being considered.

The goal of the high throughput technologies required is to increase the understanding of the biological processes, as well as to develop improved clinical diagnostics and more effective therapeutics that can target those rare cell populations. Those technologies range from photolithographically patterned 3D microcell technologies and 2D adhesive substrates, to continuous glow technologies and miniaturization of conventional techniques to an automated, on-chip format. In relation to that, the real-time imaging software considered in this project could be seen as a first step or prerequisite of all those techniques.

In analysing single cells, sample heterogeneity in specific cell types shows the need for high-throughput and quantitative measurement of cellular

parameters. Recently, high throughput single-cell analysis platforms have revealed rare genetic subpopulations in growing tumours, begun to uncover the mechanisms of antibiotic resistance in bacteria, and described the cell-to-cell variations in stem cell differentiation and immune cell response activation by pathogens [6]. The next sub-points survey those recent technologies and tries to relate them to the particular needs of the real-time imaging for microfluidic applications solution proposed.

In the last five years, the world of microfluidics has become very important due to its applications in stem cell biology [2], cancer diagnosis [5] and cell culture systems [4]:

2.1.1. STEM CELL BIOLOGY

Stem cells are cells that have the capacity to self-renew and give rise to descendants, which can commit themselves in a number of distinct directions. This process is named cell proliferation. Stem cell research can benefit from the advances of microfluidics technology, since controls of fluids help to reconstruct the physiological environment and high-throughput along with miniaturization and parallel processing has reached a high degree of automation.

They are very influenced and regulated by microenvironment, so to direct their differentiation, it is necessary to know how biochemical cues like glucose and oxygen act in the decision-making process of stem cells. Microfluidic devices are used to study microenvironment from two major aspects [2]:

ASPECT	REASONS FOR BEING IMPORTANT
High-Throughput Screening.	Advantages respect conventional tissue culture dishes: much lower amounts of starting cells, precise control of inoculation number and dynamic adjustment of culture conditions.
Reconstructing the Physiological Environment of Stem Cells.	Possibility to tune the microenvironment around stem cells in a variety of ways, the composition of the local population and cell-cell interactions.

Table 2.2-Aspects for studying microenvironments [2].

2.1.2. CANCER DIAGNOSIS

Microfluidics and spectroscopic imaging can be a complementary predictive method for all types of cancers: the biomarker approach[1] is effective for predicting how a concrete patient could respond to a treatment, but only very specific cancer subtypes have associated biomarkers (for example, the BRCA mutation for breast cancer).

In the microfluidics' approach, small amounts of tissues are sectioned into spheroid-sized samples. Then, the samples are put into a microsystem. Mélina Astolfi *et al.* proposed a novel approach for testing drugs simultaneously on tissue from a specific patient (see **Figure 2.2**) [5].

[1] Statistical method that associates drug-response rates with specific patient characteristics.

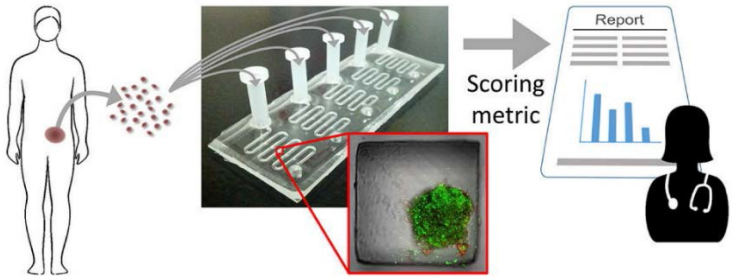


Figure 2.2- Personalized approach for the selection of an optimal anticancer treatment. Small amounts of tissue from a patient are sectioned into spheroid-sized samples. These samples are then introduced into a microsystem. Inset shows a sample trapped inside a well, being labelled with fluorescent probes marking cells that are viable (green) and dead (red). The imaging used is confocal fluorescence microscopy (see Table 2.3). The necessity of a software to analyse and save information emerges with the objective of helping medical specialists choose the most effective treatment for each patient [5].

The microfluidic chip used in that experiment was highly specific, making it possible to trap and incubate several microtissues and expose them to different cancer drugs. The proposed tissue format is about 100 times smaller than the common ones: greater number of independent assays while maintaining high viability due to facilitating access to nutrients. A highly automated and precise software solution would enable to make a more effective report of those experiments, for instance, being able to store time-lapse pictures of the incubation in a database.

Microtissues (formed from mouse xenograft tumours and used as a cancer tissue model), remained alive for more than a week when cultured under non-treated conditions in those microsystems (**Figure 2.3**).

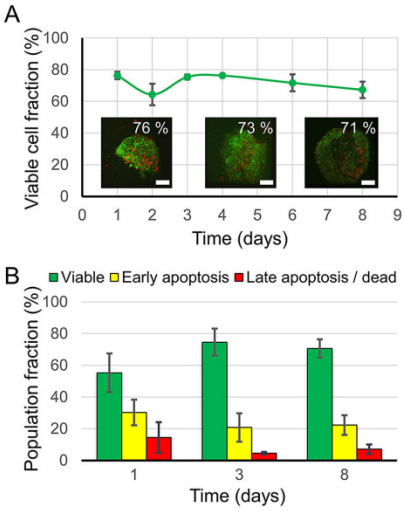


Figure 2.3- Viability results of the micro-dissected tumour tissue samples produced from mouse xenografts (formed using the OV90 ovarian cancer cell line) and cultured in a microfluidic chip. A) Confocal microscopy results: relative area of live cells divided by total area of both viable and dead cells. The live cells are labelled in green and the dead cells in red in the shown confocal images. The scale bars, important feature provided in the software developed in this project for acquired images, indicate 100μm. B) Flow cytometry results of the samples categorized into three cell groups. Error bars show the standard error of the mean from at least three independent experiments [5].

2.1.3. CELL CULTURE SYSTEMS

Microfluidic cell culture platforms combine advantages of miniaturization and real-time microscopic observation with the ability to pattern cell culture substrates, to vary the composition of culture medium over space using gradient generators, and to create cell culture conditions that are more physiological than those found in other *in vitro* systems.

A good example for this application is the cell culture screening system based on a microfluidic chip which creates arbitrary culture media formulations in 96 independent culture chambers and maintains cell viability for weeks that was built in 2007 by Rafael Gómez-Sjöberg *et al.* [4]. **Figure 2.4** illustrates the concept.

In conclusion, some features that the set-up (software for image acquisition + hardware) should provide are:

- In relation to the patterning process for microfluidic applications, it is clear that the microfluidic chips are delicate material to work with. It follows that handling of microfluidic chips should be minimized.

- **Figure 2.1**: it follows that a microscope with bottom illumination gives better results, since the sharp edges created in the 'Etching' or 'Soft lithography' processes produce shadows due to light reflection in case of up illumination. However, such a microscope was not available for the work conducted in this thesis.

- In relation to the three examples of application, high-resolution microscopes with very high magnifications are required to work in this field, since stereo optical microscopes are limited. The software should be compatible and provide support for this kind of microscope, in spite of not having them in the lab. It follows that the software has to support any microscope set-up with any possible magnification, so that the scientist can work in different scales and areas.

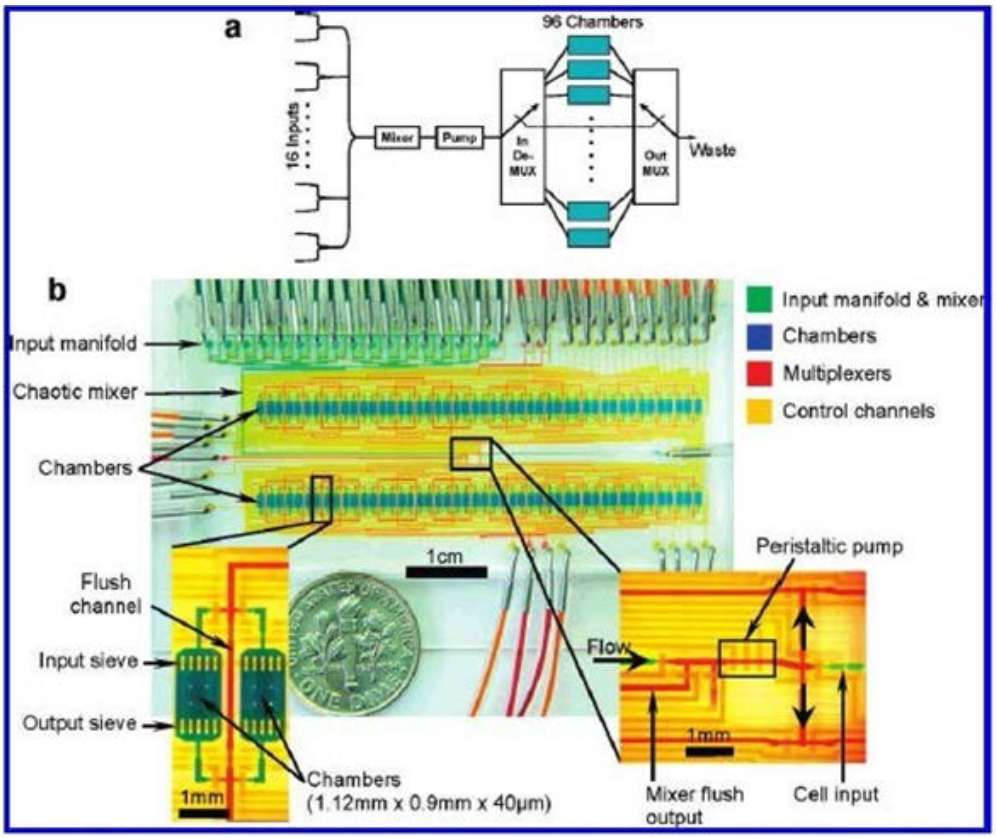


Figure 2.4- Design of the cell culture chip [4].
a) Simplified schematic diagram of the fluidic path in the chip (MUX, multiplexer). b) Annotated photograph of a chip with the channels filled with coloured water to indicate different parts of the device. The left insert gives a closer view of two culture chambers, with the multiplexer flush channel in between them. The right insert shows the root of the input multiplexer with the peristaltic pump, a waste output for flushing the mixer, and the cell input line.

2.2. ANALYTICAL DETECTION TECHNIQUES FOR DROPLET MICROFLUIDICS

Microdroplet technology has recently been exploited to provide new and diverse applications via microfluidic functionality. Formation, mixing, and analysis of minute sample volumes in droplet form is a promising technique in different fields of biology and chemistry. One of the challenges lies in the ability to analyze droplet content qualitatively and quantitatively [7]. The analytical detection techniques for droplets play critical roles in the development and application of droplet-based microfluidic systems [9]. **Table 2.3** gives general information about analytical detection techniques and **Table 2.4** summarizes potentially relevant techniques for the designs in question:

Analytical method	Sensitivity	Analysis speed	Labeling required	Advantages	Disadvantages	Main applications
Bright-field microscopy	Poor	Good with high-speed camera	No	Convenient; Imaging the shape, size, color, and trajectory of droplets, and the interaction between droplets; Analysis of heterogeneous reactions inside droplets	Low sensitivity	Droplet generation and manipulation [6,41,44,45]; Mixing inside droplet [47]; Droplet encoding [49]; Protein crystallization [28,50]; Clotting and agglutination reaction [52,53]; Cell and organs [11,43]
Colorimetry	Good	Good	Yes	Good sensitivity; Simple setup	Limited applications	Detection of aluminum ion [116]
Fluorescence microscopy	Good	Fair	Yes	Convenient; Good sensitivity; Quantification	Low analysis speed; Need derivatization	Single cell and single molecule analysis [11,21–23,54]; Enzyme kinetics [16,55,56]; Digital PCR [57,58]; Protein absorption on water–oil interface [59,60]
Laser-induced fluorescence	Excellent	Excellent	Yes	High sensitivity; High analysis speed; Quantification; Large dynamic range	Need derivatization	Single molecule detection [68,69]; Digital PCR [19,20,71,72]; Single cell analysis [25,30]; High throughput sorting and screening [12,32]; Enzyme inhibition assay [73]
Laser Raman spectroscopy	Poor/Good with SERS	Good	No	Label free; Good sensitivity with SERS; Good analysis speed	Matrix effect	Studying mixing and reaction process [82–84]; High throughput detection [86]; Bacteria identification [90]
Electrochemical analysis	Good	Good	Yes	Low cost; Simple structure; Small size; Good sensitivity	Need derivatization; Poor reproducibility	Measuring size, frequency, velocity of droplets [91–93]; Enzyme kinetics [94]
Capillary electrophoresis	–	Fair	Yes	Separation of multiple analytes	Need derivatization with fluorescence detection; Low analysis speed	Separation of amino acids; [95,96] In vivo monitoring of neurotransmitter [97]; Enzyme assay [98]
Mass spectrometry	Good	Good	No	Label free; Simultaneous detection of multiple analytes; Good sensitivity Capability of elucidating chemical structures	Matrix effect; Requiring sample pretreatment	Screening and optimizing reaction conditions [99]; Peptides [103–106]; Monitoring of alkylation reaction [105]; Enzyme inhibitor screening [107]; Off-line LC-ESI-MS [108]
Nuclear magnetic resonance spectroscopy	Poor	Poor	No	Label free; Capability of elucidating chemical structures	Requiring sample pretreatment; Low analysis speed	Rapid identification of compound libraries [111,112]
Absorption spectroscopy	Poor	Good	No	Label free; Good analysis speed; Quantification	Low sensitivity	Detection of aqueous K_2IrCl_6 [114]

Table 2.3- Analytical detection methods for droplet microfluidics [9].

For confection the real-time imaging software, bright field microscopy is used as a first step, since it is the simplest one to work with and that is really helpful when developing and programming a software. However, all the other analytical methods will be necessary in future software versions, following the priority order established in **Table 2.3**.

2.3. MATERIALS IN MICROFLUIDICS

The materials used in microfluidic experiments have become a challenge to confront for real-time imaging and image acquisition over the years, mainly due to the reflectivity and shadowing linked to them. Therefore, it becomes essential to know the properties of those materials for a correct and precise visualisation of the samples.

In microfluidics, a vast variety of materials is used. All materials have their pros and cons, looking e.g. at cost or geometrical freedom polymers are dominating. Each material has different optical characteristics along with unique patterning technologies. **Table 2.4** provides an overview of the different fabrication technologies. It should be noted that especially polymers along with cost-effective mass-production technologies offer the option for disposable chip production suitable for medical applications [10].

Material	Technology	Comment
Metal	• Precision mechanical machining. • Laster machining. • Electro Discharge machining.	
Silicon	• Wet chemical etching. • Dry etching (DRIE).	
Glass	• Wet chemical etching. • Powder or sandblasting. • Photostructuring.	
Elastomers	• Casting.	
Thermoplastic polymers	• Injection molding. • Hot embossing. • Laser machining. • Precision mechanics.	Injection molding as replicative technology allows for the most cost-efficient fabrication of microstructured devices.

Table 2.4- Technologies for microstructuring of different materials [11].
Usually at least a cover lid needs to be placed on the microstructures too. For glass and silicon, established processes are available. Those processes exceed easily the 100ºC temperatures, even the ones known as "cold". Silicone can be mounted onto itself or glass and silicon, but the joint can be released. For thermoplastic polymers, several technologies are at hand permitting to put together parts without damaging microstructures and working without elevated temperatures.

Comparing two main materials in microfluidic applications, namely glass and polymers, their pros and cons can be mentioned. It is important to note that those two materials are highly optically transparent, which is a thing to be considered when visualizing the samples via an optical system:

Optics	Standard thermoplasts	Glass
Transparency	• Good (bad for visualization).	• Good (bad for visualization).
Autofluorescence	• Low (right polymer choice important).	• Low.
Application in UV region	• In near UV special polymers available.	• Quartz glass needs to be chosen.
Surface roughness	• Depending on mold insert quality. • Can be optically smooth.	• Smooth for wet etched devices, rough surface after powder blasting. Afterward chemical polishing possible.
Thermal stability	• Depending on the polymer choice. Standard polymers used for PCR application withstand 100ºC and slightly higher temperatures.	• Usually transfers to liquid phase around 600ºC for many glasses.
Stability against organic solvents	• Limited	• High
Stability against standard solvents in life sciences (acetone, alcohol)	• Polymers available	• High
Stability against acidic solutions	• High	• High
Stability against basic solutions	• High	• Medium
Unspecific binding of biological components	• Polymers with low unspecific binding available. Surface functionalization to avoid this problem available.	• High. Surface functionalization to avoid this problem available.
Part design	**Standard thermoplasts**	**Glass**
Design freedom	• High	• Low.
Combination of different structural depths in one device	• Easy	• Difficult and more than one depth directly increases the price.
Direct integration of fluidic interfaces	• Easy-directly in the injection molded part	• Difficult, usually an afterwards assembling process of a non-glass-component.

Direct integration of e.g. reservoirs	• Easy-directly in the injection molded part	• Limited. Large structures cannot be integrated as glass part due to cost issues.
Additional functionalities	**Standard thermoplasts**	**Glass**
Integration of liquid and dry reagents in the chip	• Easy	• Limited to impossible. For bioreagents like enzymes with limited thermal stability impossible.
Integration of hybrid components like filters	• Easy	• Limited to impossible.
Integration of valves on chip	• Easy	• Limited to passive and elastomeric membrane valves
Fabrication	**Standard thermoplasts**	**Glass**
Material cost	• Low to medium, 2-20€/kg	• High
Highest price impact	• Replication (micro-structuring) has a negligible impact! • Assembly.	• Footprint of the device. E.g. already the material price for a microfluidic chip in the format of a microscopy slide is a few € (depending on material choice). • Microstructuring. • Assembly.

Table 2.5- Characteristics of glass and polymers.
Looking at the different characteristics and the price, we can deduce the reason why polymers are always used when glass is not required: they are cheaper devices. Glass is more used if high temperatures, much above 100ºC, are involved in the application, which is usually not the case in life sciences. It can also be required with specific organic solvents. If bioreagents need to be stored on-chip, complex fluidics, hybrid components are necessary, valves are used etc. polymers are the material of choice. Moreover, interfaces, reservoirs and different structural depths do not have an impact on the price of devices in case of polymers, yet they are partly impossible to be implemented in glass devices due to a massive increase of the cost [11].

For the software solution in question, the most important aspects about all those materials and their characteristics can be summarized in three points:

1. Visible light and shadows.

2. Reflective indexes.

3. Sharpness of edges.

In the proposed software solution, the user does the set-up of the microscope, so that the image gives good information. An automatic set-up would imply autofocus problems due to those three points, not being all the edges detected. That is why it is left as a future challenge to implement in the software an automatic focusing of the sample.

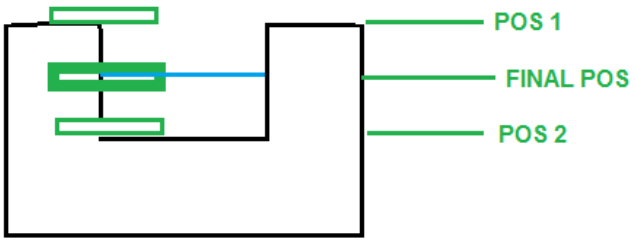


Figure 2.5- Autofocus problem in microfluidic chips. The challenge is to identify the plane of interest. The reflective indexes are similar in all points of the material, so three pictures are necessary for focusing: position 1 (front plane), position 2 (background plane) and final position. The final position is deducted from positions 1 and 2.

2.4. CHIP HARDWARE: SIZE AND ACCESIBILITY

Each microfluidic chip has its own features, such as different sizes, shapes and thicknesses, which creates a challenge to automatically setup the imaging microscope. This is an important reason for being suggested a manual solution where the user/operator sets up the hardware until being pleased and informs the software that the set-up is ready for experimenting. Microfluidic chips' forms and materials are very detailed in order to reduce uncertainties and errors when performing microfluidic experiments in those small scales. In addition, there are cases where the microfluidic chips' structure is very particular comparing to the simple ones (**Figure 2.6**), making this problem even bigger (**Figure 2.7**).

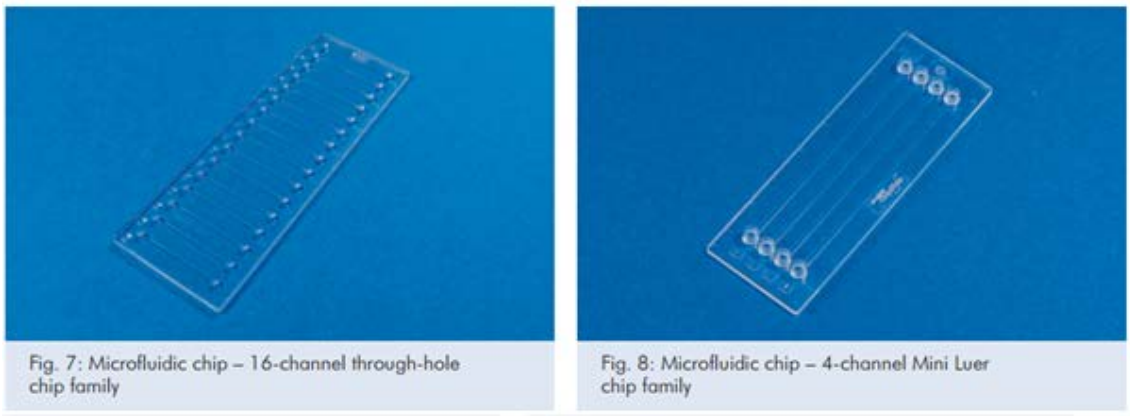


Figure 2.6- Examples of microfluidic chips-Simple structures [11].

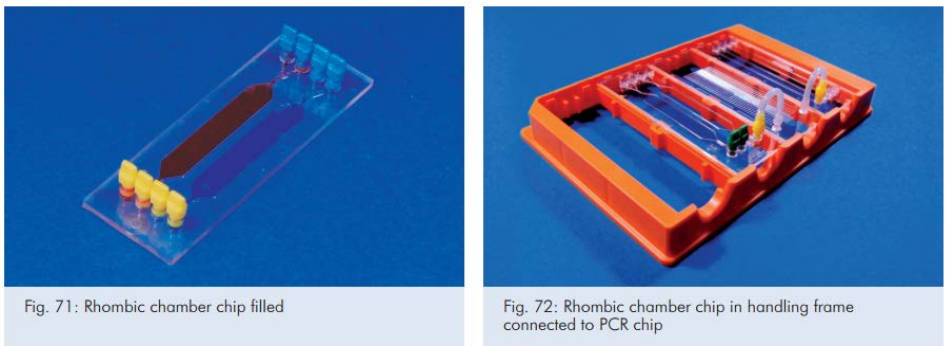


Figure 2.7- Examples of microfluidic chips-Complex structures[11].

Complex designs in microfluidic chips become important in metrology, since overview and detail pictures are required (**Figure 2.8**). That is why it is considered important to include the possibility of working with both type of images in the software. However, complex topology as in varying heights is a challenge due to the low depth of focus (especially with high magnification). This aspect shows certain limitations of the software, because it is possible to try to do these detailed measurements as precise as possible, but there is really nothing to do to avoid problems related to depth of focus.

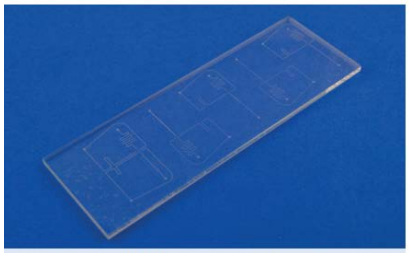


Fig. 249: Glass droplet generator chip Fluidic 441

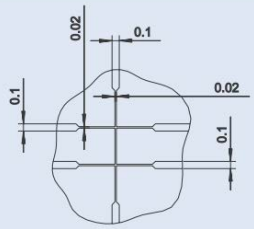


Fig. 250: Glass droplet generator chip Fluidic 441 – detail of cross element

Product Code	Description	Channel Depth [µm]	Material	Price [€/chip] 1+	3+	5+	10+
13-1300-0441-20	Droplet generator chip	20	Glass	143.70	109.75	99.78	89.70

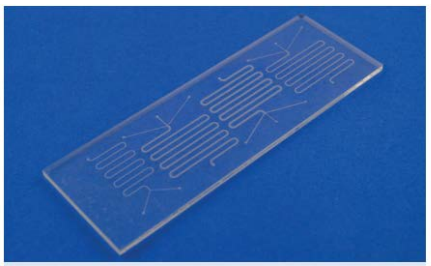


Fig. 251: Meander chip Fluidic 442

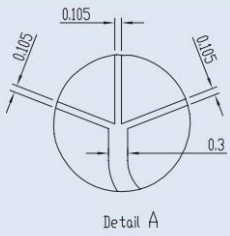


Fig. 252: Meander chip Fluidic 442 – channel details

Product Code	Description	Channel Depth [µm]	Material	Price [€/chip] 1+	3+	5+	10+
14-1301-0442-20	Meander chip	20	Glass	143.70	109.75	99.78	89.70

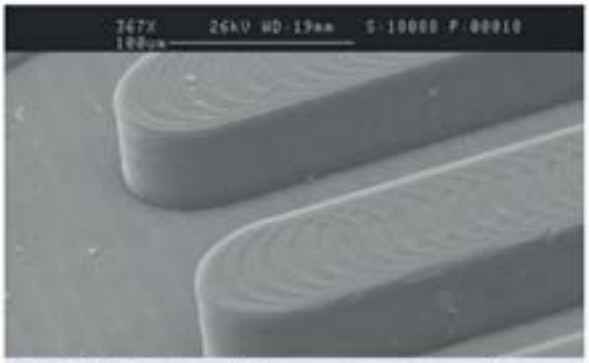


Fig. 560: Microchannel with nanostructured channel floor. The nanostructures have a 1.2 µm period and 200 nm height

Figure 2.8- Examples of microfluidic chips- Overview and detail pictures [11].

2.5. OPTICAL IMAGING TECHNIQUES IN MICROFLUIDICS AND THEIR APPLICATIONS

Optical imaging techniques are necessary in microfluidics for observing and extracting information from samples. That is why an optical microscope implementation is important for the set-up in **Figure 1.1** [12].

Traditionally, bench-top conventional microscopes and bulky imaging systems were used. However, nowadays a lot of compact microscopic techniques have been developed with the objective of providing low-cost and portable solutions:

Methods	Cost	Size	Resolution	Field-of-view
Conventional Microscope	High	Large	High	Small
Optical coherence tomography (OCT)	High	Large	Low, usually used to detect flow speed	Large
Digital in-line holography	Low	Compact	Moderate	Large
Optofluidic microscopy (OFM)	Low	Compact	Moderate	Moderate, depends on applications
Shadow imaging	Low	Compact	Low	Large
Subpixel perspective sweeping microscopy (SPSM)	Low	Compact	Moderate	Large
Compact lens-imaging systems	Low	Moderate	Moderate	Small

Table 2.6- Optical imaging methods in microfluidics [12].

Again, conventional microscopes are used for developing the real-time imaging system, but the other types of microscopy could also be used with the software solution.

2.6. BASICS IN MICROSCOPY AND MICROSCOPE SELECTION

The basics of microscopy have evolved over the years, giving rise to precise and advanced set ups.

2.6.1. MICROSCOPE SELECTION

The things to consider when selecting a microscope to work with are type of specimen, details wanted to resolve, size of the sample, type of illumination, resolution, working distance… Of course, the cost is also an essential aspect to care for. In this application, an upright microscope is enough. In case of working with cells, an inverted microscope would be better, since it keeps a good accessibility to the cells and it facilitates the essential free space and the required proximity. The microscope needs to have a place (trinocular port) where the filming camera can be placed, in order to be able to visualize the information in the computer.

Figure 2.9- Upright microscope (left) and inverted microscope (right).

While the ultimate goal is to create a software implementable in any modular hardware or microscope (**Figure 1.1**), work in this thesis was performed

with a Zeiss Stemi 2000-C Stereo Microscope (**Figure 3.7**) and an Olympus microscope (**Figure 3.8)**, microscopes already available at the university and fully suitable for microfluidic chip inspection (the instruction manual of the Zeiss Stemi 2000-C Stereo Microscope is attached in **APPENDIX B: STEMI 1000/2000/2000-C STEREO MICROSCOPES MANUAL**).

2.6.2. THINGS TO CONSIDER: MICROSCOPE RESOLUTION

The resolution of a microscope is the ability to distinguish detail, that is, the minimum distance at which two distinct points of a specimen can be seen as separate things. It is linked both to the numerical aperture (NA) and to the wavelength of light:

FACTOR	DESCRIPTION	RELATION WITH RESOLUTION
Numerical aperture (NA)	-Related to the refractive index (n) of the medium and the angular aperture (α) of the objective: NA= n x sin α. -Whole system is considered; not only the objective.	High NA→High resolution
WAVELENGTH	-Wavelength of light	Short λ→High resolution

Table 2.7-Resolution factors.

Abbe's Diffraction Limit, Airy Discs and The Rayleigh Criterion are taken into account when dealing with resolution too [16]:

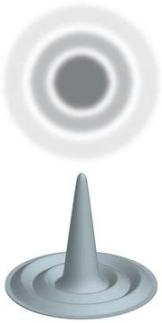


Figure 2.10- Airy Disc Phenomenon [16].

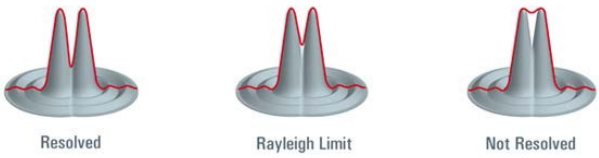


Figure 2.11- The Rayleigh Criterion [16].

2.7. HARDWARE SET-UPS USED IN LABS

There are some microscope and software setups which have already been used for real-time imaging for microfluidic applications. They are really helpful to give a closer approach to the real scope and the individual tasks (see **1.1. GOAL/OBJECTIVE**) of the project.

2.7.1. DOLOMITE EXPERIMENTS

Most of the cameras used for these experiments are colour high speed cameras with video frame rates over 1000 per second and still image capture with exposure time down to 0.04ms. Due to the high velocity requirements needed to analyse fluidic and particle movements, the resolution becomes an aspect to sacrifice, being the typical values of around 1,3MP (**Figure 2.12**).

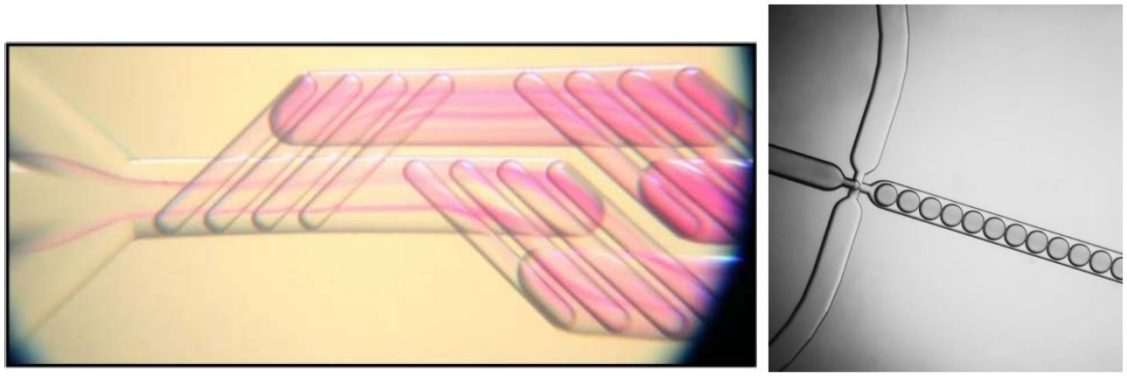


Figure 2.12- Typical microscope images of the micromixer chip (left) and of the droplet junction chip (right) of Dolomite [13].

The cameras are screwed into C-mount attachments on microscopes and they link to a desktop PC via FireWire. Free software is available for image capture most of the times. However, those software solutions only provide control over parameters such as region of interest, exposure time, gamma, gain and frame rate. The lack of elemental operations for scientists such as documentation via database storage, text overlay in images and automated distance measurements becomes clear. The possibility of recording videos is usually offered by those tools, but the frame rate is always an aspect to analyse in this particular application regarding those tools, since it has to be near the maximum frame rate offered by the camera to capture high quality fluidic movement (mixing, droplet generation…) images (**Figure 2.13**).

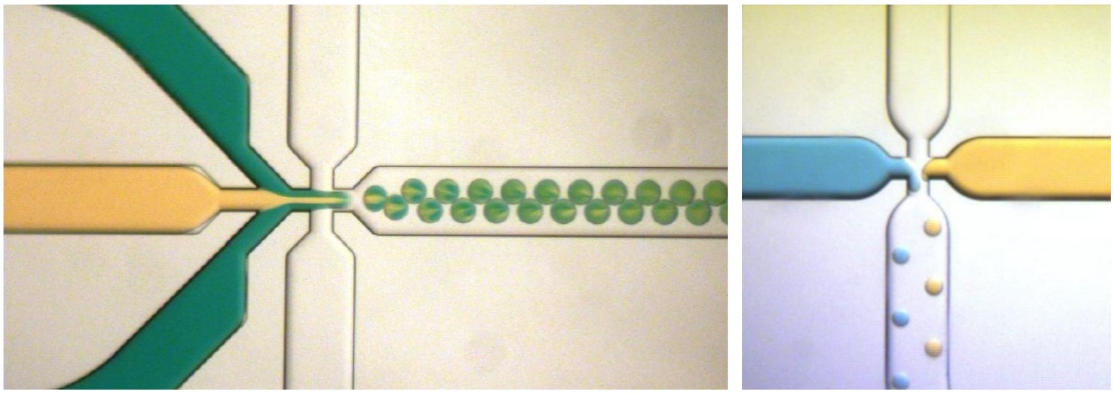


Figure 2.13- Typical camera images of the Droplet Generation Chip (left) and the Droplet Junction Chip, hydrophobic (right). Dolomite [13].

2.7.2. LABSMITH MICROFLUIDICS LIVELAB

This lab experiment was performed by LabSmith [14] and it is a clear reflection of which types of experiments are done regarding microfluidics. The objectives of the experiments were to introduce experimental parameters that are important for successful microfluidic experiments and to provide practical experience using the SVM340 to image experiments.

The hardware used in the experiment was an integration of breadboard prototypes with the LabSmith SVM340 synchronized video microscope and imaging tools (**Figure 2.14**).

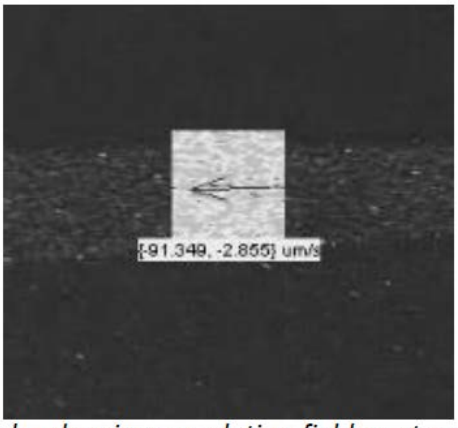
Top stage with steel plate

Integration stage with LS-600-CH breadboard

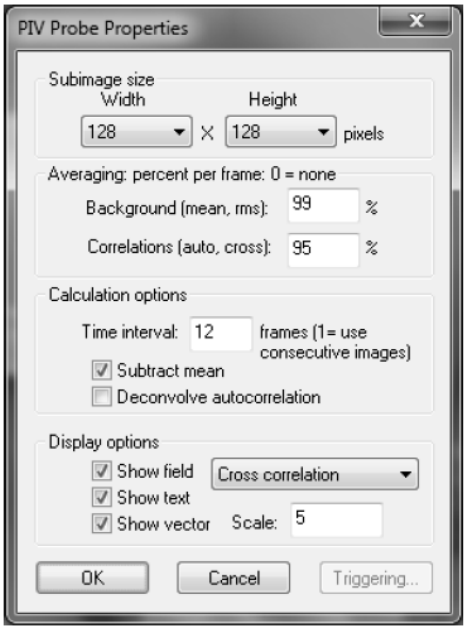
Integration stage with integrated breadboard (iBB) and uProcess™ flow automation components

Figure 2.14- The three possible ways to integrate the imaging experiment with the SVM340 [14].

The LabSmith SVM340 offered the possibility to perform flow and intensity measurements, as it can be seen in **Figure 2.15** and **Figure 2.16**.

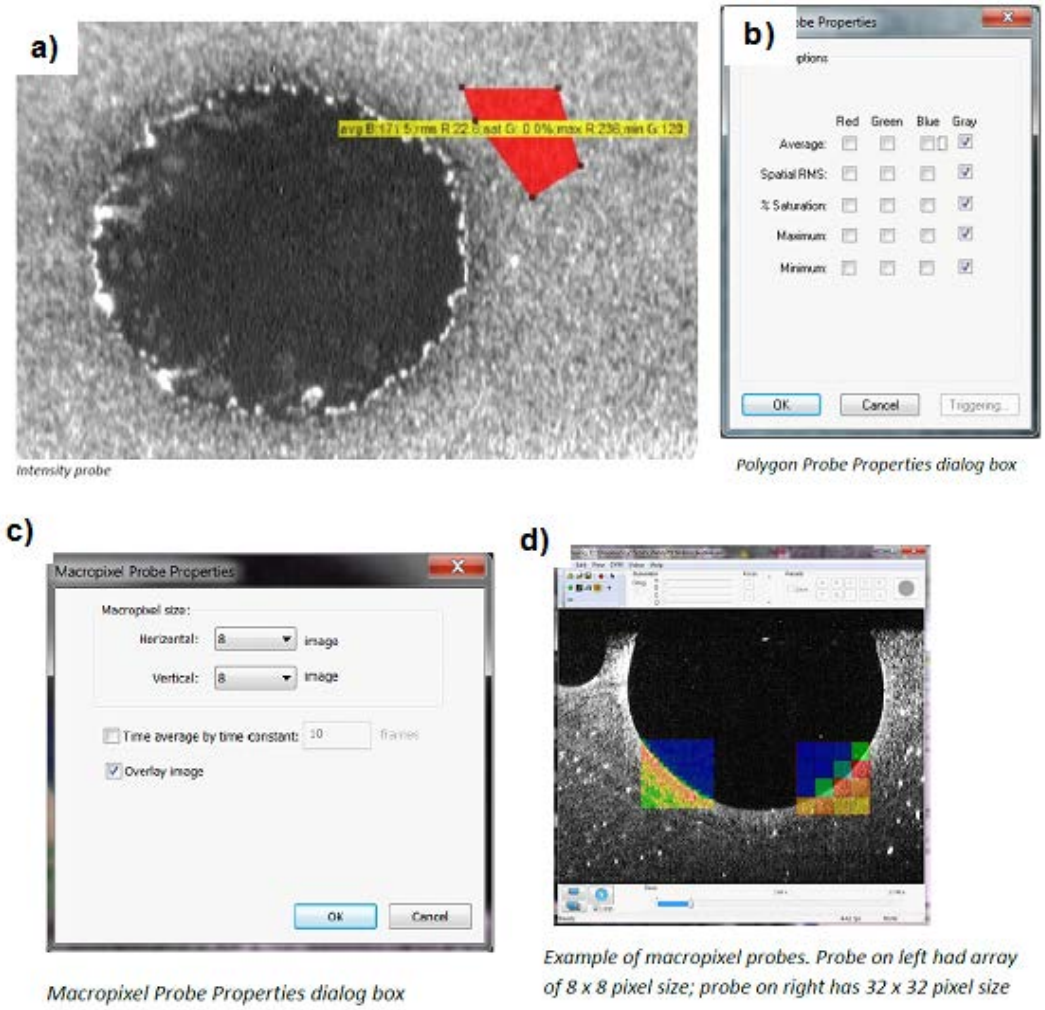


New probe showing correlation field, vector arrow and real time velocity as text



PIV Probe Properties dialog box

Figure 2.15- Flow measurement experiment [14].
Left image: flow measurement result; right image: configuration window displayed in the interface in order to set and control different parameters related to the visualization of the experiment.



Intensity probe

Polygon Probe Properties dialog box

Macropixel Probe Properties dialog box

Example of macropixel probes. Probe on left had array of 8 x 8 pixel size; probe on right has 32 x 32 pixel size

Figure 2.16- Intensity measurement experiment [14].
a) Tool for outlining the probe of interest. When the shape is defined, it is enough to do a double click with the mouse to close the polygon. It is possible to create as many probes as desired and they can be dragged to the desired location. Their shape can also be modified by dragging the polygon points. b) Intensity probe data displayed and recorded can be selected with the Polygon Probe Properties dialog box. c)-d) Micropixel intensity probes. They are used when spatially-resolved intensity data is needed. The probe is divided into an array of sub-probe sections based on the input pixel size.

3. EXPERIMENTAL SET-UPS

The real-time imaging for microfluidic applications software to be developed needs to interact with the CCD Chip of the microscope, so that it gets the information that the user wants to analyse from the microfluidic chip (**Figure 3.1**). The optical instrument used for each microfluidic application is determined by the object or chip to be imaged as well as the purpose of imaging: it is not the same to acquire a picture for overview analysis or for detail analysis. That is why the software needs to be independent of the instrument. In other words, it has to give support to the user regardless of which optical instrument (optics and CCD Chip) is being used.

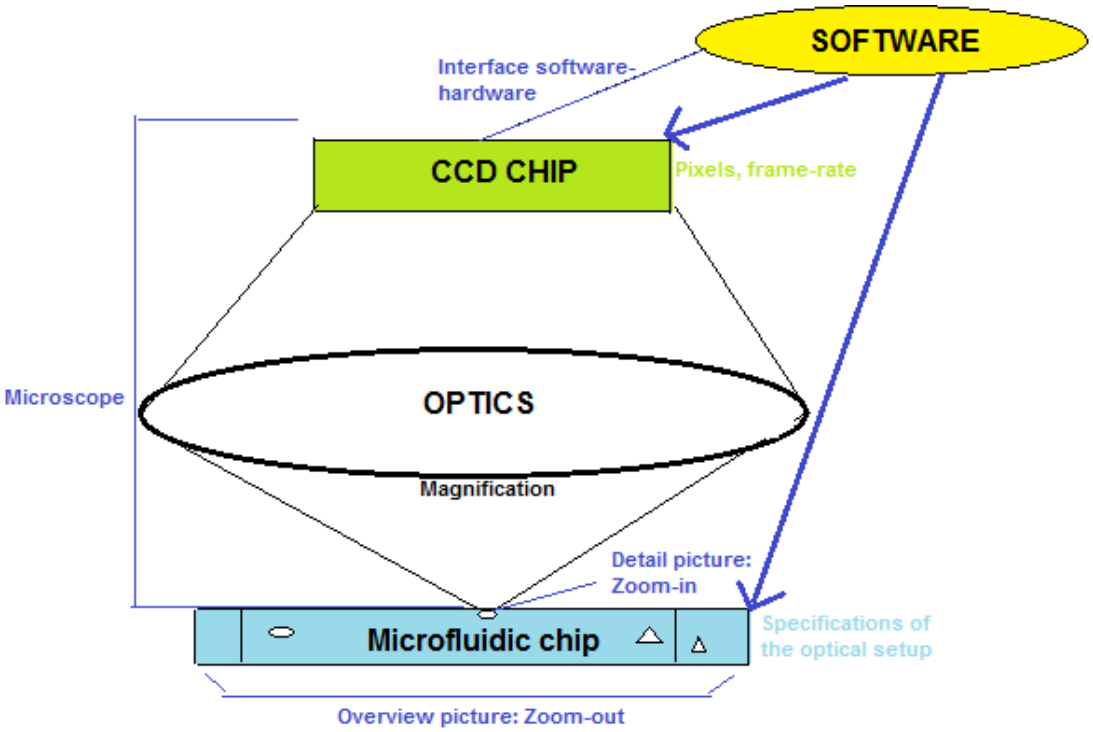


Figure 3.1- Experimental set-ups: Software-Hardware interface.

Figure 3.1 illustrates that it is not a straight step to process and to work with images from microfluidic chips using the software solution presented in **4.OVERALL SOFTWARE CONCEPT** and **5.DETAILS OF SOFTWARE SOLUTION**, but that an optical instrument or microscope is needed in between. The steps followed to get the image in the software are presented below:

1. Depending on the microfluidic chip and the purpose of the imaging, an adequate optical instrument is chosen.

2. The overview or detail picture is magnified in the optics system with an appropriate magnification. If the desired image is a detail picture, a higher magnification is needed than for an overview one.

3. The intermediate image acquired by the optics is projected to the CCD chip via a C-mount with a given magnification, demagnification or 1X factor (see **APPENDIX D: C-MOUNT POSSIBLE SOLUTIONS**).

4. The image acquired in the CCD chip is transmitted to the computer software via a USB port or an Ethernet port.

5. The user is able to perform and document microfluidic analyses using the software.

It makes sense to explain the reader the different fluidic chips that can be of interest for scientific research and the hardware (both optical microscopes and microfluidic chips) used during the realisation of this particular project before presenting the software solution in **4.OVERALL SOFTWARE CONCEPT**, following the scheme presented in **Figure 3.1**.

3.1. EXAMPLES OF FLUIDIC CHIPS

Once already discussed the background information regarding microfluidics, it is of interest to initiate the reader in typical microfluidic chip designs and droplet producing designs before explaining the software solution, so that he/she can understand why the software includes some features and no others. This chapter serve also as motivation for the 3 experiments (images of fluidic chips, life images of fluids in microfluidic chip and video of droplet formation) carried out after the development of the software, which are later explained in **6. FLUIDIC RESULTS**.

3.1.1. REASONS FOR USING MICROFLUIDIC CHIPS

The first 2 experiments performed with the software in **6. FLUIDIC RESULTS** need to be done working with “microfluidic chips” or “Lab-on-a-chip” (LoC) devices. Those devices are used for research in life sciences and diagnostics and represent a very fast-moving field. LoC devices are designed, prototyped and assembled using a lot of strategies and materials, but in general they typically need to be (1) sealed, (2) supplied with liquids, reagents and samples, and (3) often interconnected with electrical or microelectronic components. Closing and connecting to the outside world of these miniature labs is a challenge irrespectively of the type of application pursued.

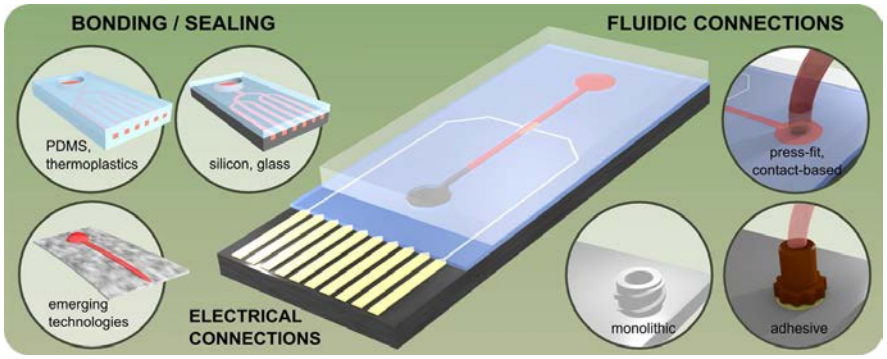


Figure 3.2- Microfluidic chip/LoC device: design features [23].

Over the last 10-20 years, LoC devices have demonstrated their strengths and benefits for many applications in different fields, such as point-of-care diagnostics, genomic and proteomic research, analytical chemistry,

environmental monitoring and the detection of biohazards. They offer many advantages compared to bulkier and "historical" analytical instruments: they support precise control of liquids flowing usually under laminar regime, minimize consumption of reagents and samples, support short reaction times, enable highly parallel and multiplexed analysis, require little or less power to operate, are portable, and, potentially, have low cost of production.

Nowadays, structures can be built with sub-micrometre precision, flows of liquids can be sustained and precisely controlled using integrated or external pumps and valves or capillary forces, and quantitative detection of different analytes can be done in high sensitivity using optical-, electrical- or magnetic-based techniques.

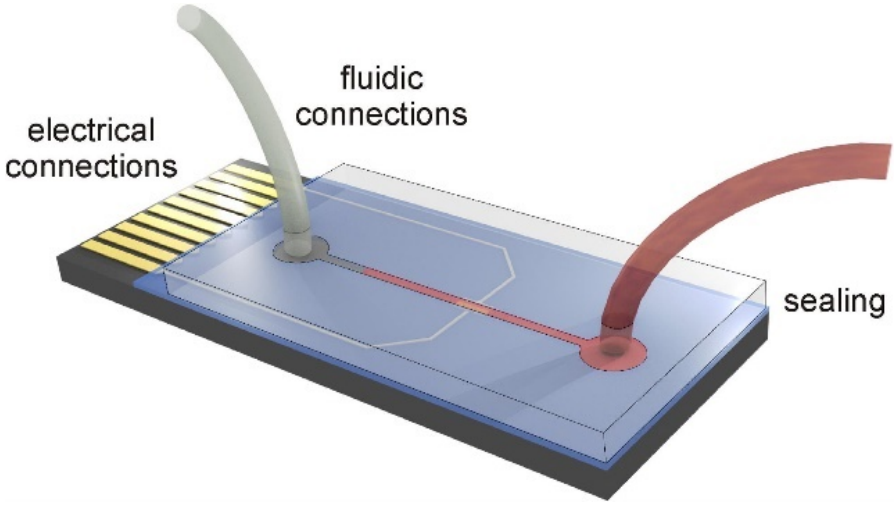


Figure 3.3- Microfluidic chips: connections.
LoC devices cover a broad range of applications and research areas, yet these microfluidic devices need to be sealed, connected to pumping peripherals, and often bear electrical connections. "Plugging" and "closing" these small labs is a challenge [23].

The real-time imaging software, objective of this project, offers a powerful tool to work with optical images of LoC devices. It offers the possibility to both analyse defects (e.g. due to fabrication processes) or life imaging and documenting fluid related experiments in relation to them (see **6. FLUIDIC RESULTS**).

3.1.2. DROPLET PRODUCING DESIGNS

Droplet producing designs are of high interest for experiments related to microfluidics. That is why the last experiment performed in the **8. FLUIDIC RESULTS** section is related to droplet production and evolution. The first thing that comes the one's mind when talking about droplet producing designs is a syringe tip.

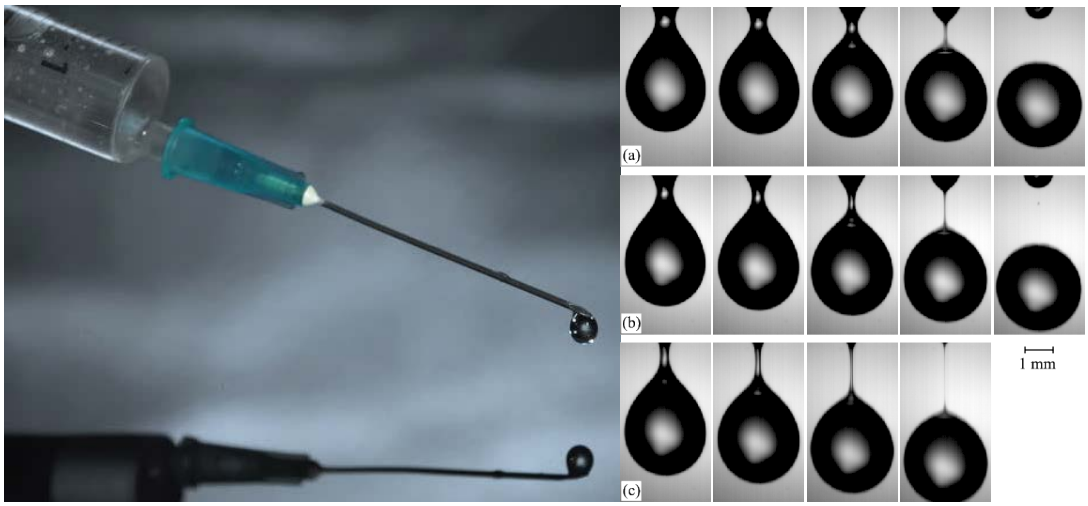


Figure 3.4- Evolution of a droplet produced with a syringe tip.
The purpose of the third experiment performed in the project is something similar to this illustration, but done with a microfluidic tube instead of a syringe tip: capturing a movie and showing 'individual shots' from the movie' so that it is possible to see how the droplet evolves.

Of course, there are much more developed methods to create droplets (see **Figure 1.2**), yet this method is enough to accomplish with the purpose of the experiment: see if the software works as specified.

3.2. OPTICAL HARDWARE

In this part, general background information and detailed information of hardware available in the lab is included. This implies that all electronic and microfluidic devices used are described. The purpose is to offer the reader a better view of the material that was needed in relation to **Figure 3.1**, and that somehow, directs the development of the software solution. This topic has already been partially introduced in **2.BACKGROUND OF THE PROJECT,** with the microscope selection, but this chapter goes deeper into the subject.

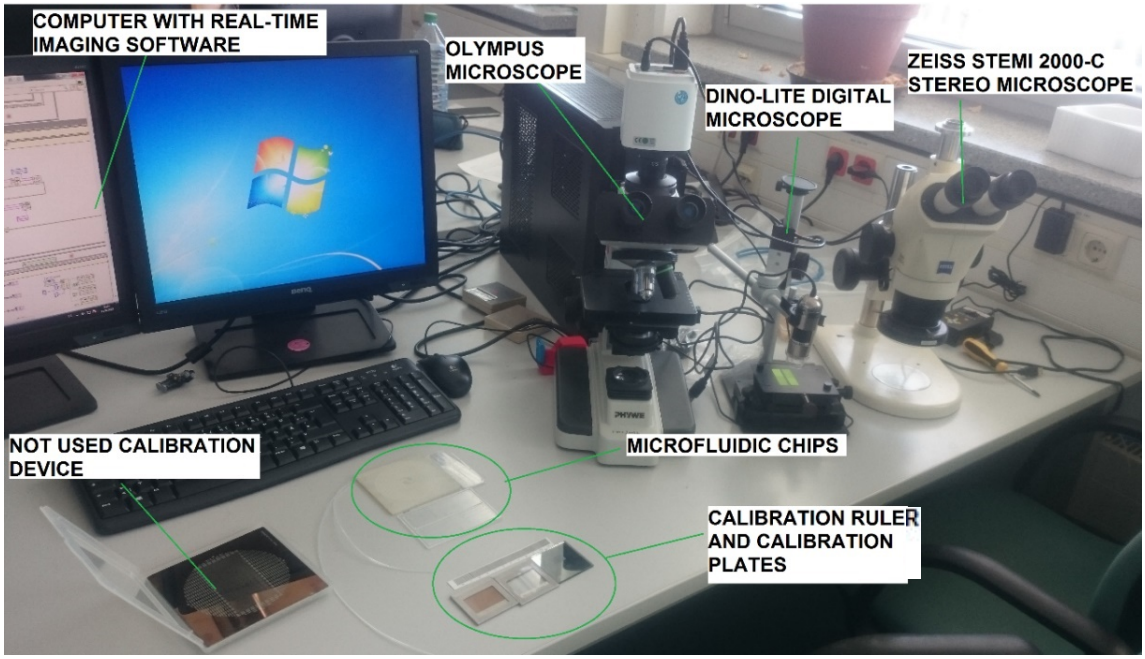


Figure 3.5- Hardware available/used in the lab: General picture.

The general picture of the hardware used in the lab is present in **Figure 3.5**. There are 3 different microscope set-ups presented in that picture, consisting each one of a different type of solution for specific tasks (**Table 3.1**):

SET-UP	EXPERIMENTS WHERE WAS USED
Dino-Lite Digital Microscope Camera	Edge detection.
Zeiss Stemi 2000-C Stereo Microscope	Magnification input and scale bar experiments.
Olympus Microscope	Rest.

Table 3.1- Set-ups used in each experiment of the software development.

According to the other elements, the real-time imaging software refers to the program in LabVIEW, the microfluidic chips refer to chips used for performing the experiments described in **6. FLUIDIC RESULTS** and the calibration ruler and calibration plates refer to material used for calibration purposes

3.2.1. GENERAL BACKGROUND INFORMATION AND DETAILED INFORMATION OF HARDWARE AVAILABLE IN THE LAB

- **DINO-LITE DIGITAL MICROSCOPE CAMERA**

The Dino-Lite Premier AM7013MZT (**Figure 3.6**) digital handheld microscope has a 5 Megapixels sensor with adjustable polarizer abilities, allowing to observe things in high resolution: it offers a magnification range of [20X,50X], polarization and 2592x1944 pixels. The AM7013MZT is appropriately incased with an aluminum alloy housing robust protection. It also includes the Microtouch II sensor for on microscope picture taking and built-in bright white LEDs to instantly illuminate objects.

It is a suitable microscope for quick, fast and flexible set-ups, with moderate resolution. It can be easier connected to the computer via USB than the other set-ups coming next. The microscope and the camera are assembled together in the same piece.

However, its relatively low camera speed (less than 20 frames per second) values when capturing real-time images in LabVIEW make this microscope useless for working with microfluidics in a real-time application, although its resolution of 2592x1944 pixels and magnification range of [20X,50X] values are more than enough for that purpose. Precision is also a factor that is affected due to the small ruggedness of the setup when compared with common microscopes (see ZEISS STEMI 2000-C STEREO MICROSCOPE and OLYMPUS MICROSCOPE), as well as the imprecise method of setting the magnification with a small wheel which is difficult to control.

It is built to work together with an already developed software called *DinoCapture 2.0*, not offering good support for new software developers. It is important to remember that the real-time imaging software to be developed in this project has to offer support for all types of set-ups, and not only a specific model: that is why LabVIEW is being used.

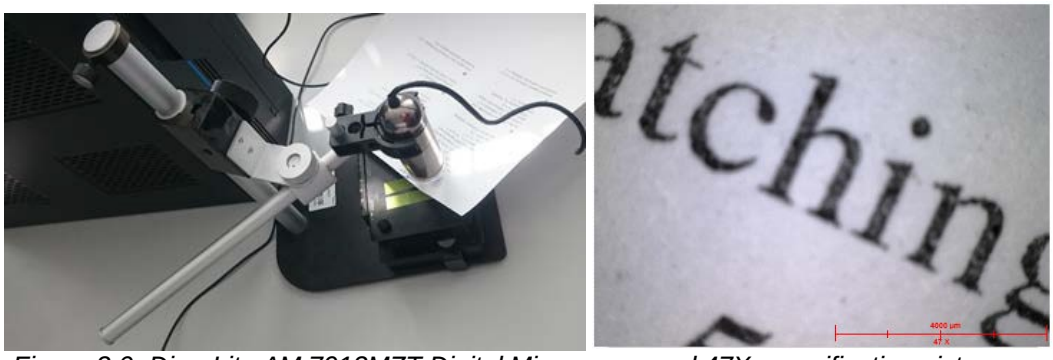


Figure 3.6- Dino-Lite AM 7013MZT Digital Microscope and 47X magnification picture.

Specifications of the set-up:

- Included comes the DinoCapture 2.0 software that allows taking pictures, recording videos and annotating on images.
- 5 Megapixels (2592x1944 pixels of resolution).
- 20X-50X magnifications.
- Adjustable polarizer abilities.
- Sensor: Color CMOS.
- Lighting: 8 white LED lights switched on/off by software.
- A C-Mount is not needed, as the camera is a microscope by itself.
- Slow focusing of the microscope and low f/s rate: not valid for real-time imaging.
- Unit weight: 140g.
- Unit dimension: 10,5cm (H) x 3,2cm (D).
- Connection to PC: USB 2.0 Interface. Compatible with PC or MAC.
- The fixture of samples is done on a mechanical stage which is movable, as in a common microscope set-up.

For more information, see **APPENDIX A: DINO-LITE DIGITAL MICROSCOPE. USER MANUAL**.

- **ZEISS STEMI 2000-C STEREO MICROSCOPE**

The Zeiss Stemi 2000-C Stereo Microscope (**Figure 3.7**) was used for certain other tests after the Dino-Lite Premier AM7013MZT, since its specifications were much better regarding magnification ranges (6,5X-50X), ruggedness of set-up (big compared to the Dino), method of magnification setting (focus and magnification selection with precise wheels) and field of view.

It had already connected a camera of higher speed (more than 20 frames per seconds) and higher quality than the Dino-Lite Premier AM7013MZT. It was a *Mikroskopkamera 16:9 HDMI* [17] attached with a C-Mount fixed support (not magnification) and a light source. The pixel resolution, however, was worse than in the Dino-Lite Premier AM7013MZT (smaller pixel array: 2 Megapixels, 1920 x 1080 pixels of resolution).

Figure 3.7- Zeiss Stemi 2000-C Stereo Microscope

Specifications of the set-up:

- Mikroskopkamera 16:9 HDMI (2 Megapixels 1920 x 1080 pixels of resolution): suitable for biology and stereo microscopes[2]. Connection to PC: USB 2.0 Interface. Compatible with PC or MAC. The camera has an independent power supply than the stereo microscope.

- 6,5X-50X magnifications with basic microscope equipment. Eyepiece: W-PL 10X/23 expectable focusing. 1,95X-250X magnifications could be reached with a replaceable optical system (not available in the lab).

- Built-in 6V, 10W halogen vertical illumination (230V).

- Unit weight (with power supply, mains cable and lamp): 4,8kg.

- Unit dimension (width x depth x height): 65 x 336 x 371mm.

- The fixture of samples is done on a fixed stage: not movable stage.

For more information, see **APPENDIX B: STEMI 1000/2000/2000-C STEREO MICROSCOPES MANUAL**.

[2] More information about the camera Mikrosckopkamera in http://www.loetdampf.de/kameras.html.

- **OLYMPUS MICROSCOPE**

The Zeiss Stemi 2000-C presented some problems related to the appearance of shadows in the images that were been acquired:

- The illumination method was not good at all: it was done with a ring attached to the objective, called in the specifications as "halogen vertical illumination", of the microscope

- The material of the microfluidic chips (see **2.3. MATERIALS IN MICROFLUIDICS**) was translucent, making it difficult to illuminate the borders of the channels without creating shadows.

- The edges in the microfluidic channels were not sharp at all, but rounded.

Due to those problems, the edge detection feature of the software (see **5.7.EDGE DETECTION TOOL**) was not able to detect the edges easily and the captured images were not so clear. The best possible solution would have been to use collimated coaxial illumination (**Figure 3.8**) [22], but there were no means at all for it so the final decision was to use the Olympus microscope with backlighting illumination and the same camera as the one of the Zeiss 2000-C (Mikroskopkamera 16:9 HDMI). Nevertheless, a different C-tube with a 0,5X magnification was used (**Figure 3.9**).

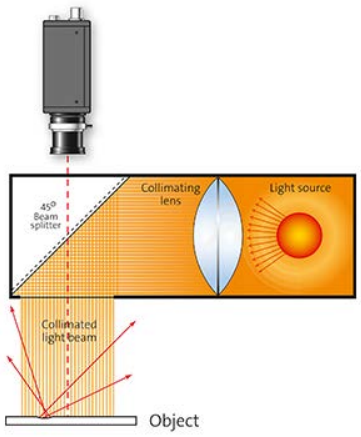


Figure 3.8- Collimated coaxial illumination [22].

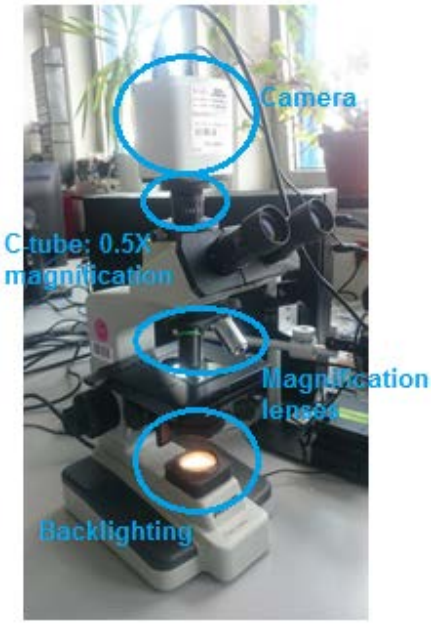


Figure 3.9- OLYMPUS microscope

The Olympus microscope had different microscope objectives. The ones used in the lab offered 10X, 20X and 40X magnifications (**Figure 3.10**): total magnifications of 50X, 100X and 200X for the image being acquired taking into account all the microscope (complete image, but with approximately half of the maximum magnification intensity visualized directly with the lenses, due to the C-Mount of 0,5X [3]).

[3] For more information, see **APPENDIX E: C-MOUNT POSSIBLE SOLUTIONS.**

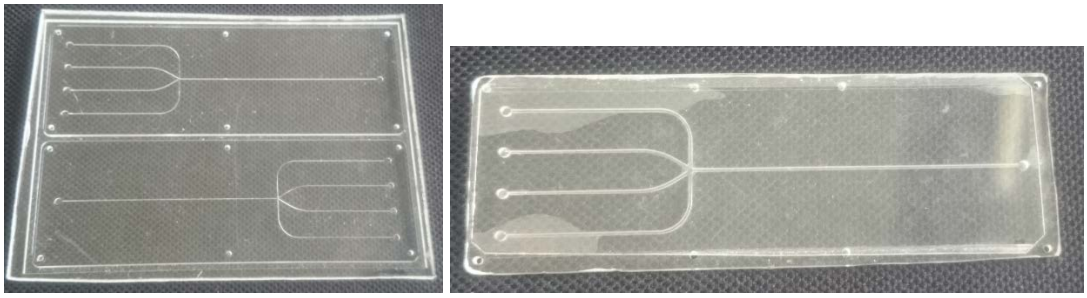
Figure 3.10- Microscope objectives used in the OLYMPUS microscope

The name of the exact model was unknown, since it was an old microscope which is not included in the actual Olympus models' library. That is why there are not specifications included for this microscope. However, it is possible to mention that the magnification is not the only feature that was known, the ruggedness of set-up was similar to the one of the Zeiss Stemi 2000-C and the magnification setting was done combining the microscope objectives (**Figure 3.10**) with a precise focus wheel. The stage for the fixture of the samples was mechanical. The camera used was the same as the one of the Zeiss Stemi 2000-C stereo microscope.

Those features, together with the possibility of using different microscope objectives and backlighting illumination, made this stereo microscope more suitable to work with microfluidic chip experiments than the others (see **6.FLUIDIC RESULTS)**. In fact, this was the last microscope used to complete and finish the programming of the software. Nevertheless, the objective of the software solution is to be able to work with any type of set-up depending on the requirements, so it is logical that several types of microscopes/cameras were used during its development (see **Table 3.1**).

- **MICROFLUIDIC CHIPS**

The microfluidic chips (**Figure 3.11** and **Table 3.2**) are used to perform different lab operations and experiments, for example the ones performed for the final testing of the real-time imaging software described in **6. FLUIDIC RESULTS**. In fact, the objective of the project was from the beginning to work with this kind of chips.



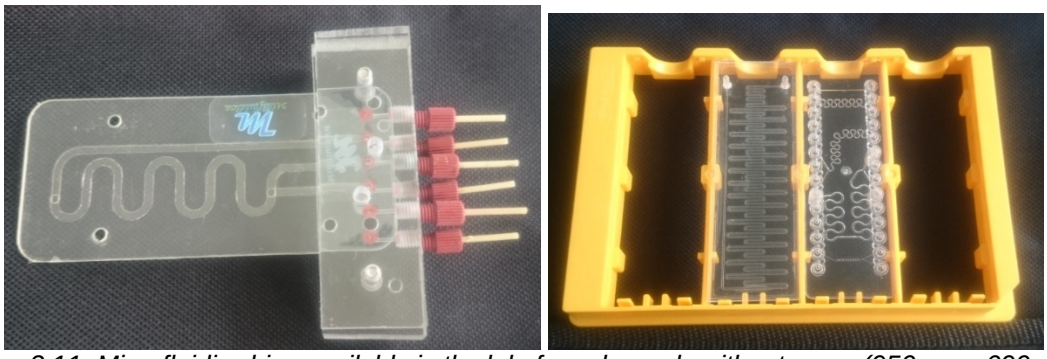


Figure 3.11- Microfluidic chips available in the lab: four channels without cover (950mm x 690mm, top left), final version of four channels with cover (890mm x 290mm, top right), two channels (950mm x 430mm, bottom left) and structure where different chips can be put (750mm x 250mm each chip, bottom right). Material: polymer.

PRODUCT CODE	CHANNEL			Cover Lid Thickness [µm]	Material	Price [€/chip]		
	Width [µm]	Depth [µm]	Length [mm]			1+	10+	30+
01-0152-0143-01	20	20	58.5	175	PMMA	42.50	31.20	23.50
01-0153-0143-02	20	20	58.5	140	Topas	42.50	31.20	23.50
01-0154-0145-01	50	50	58.5	175	PMMA	42.50	31.20	23.50
01-0155-0145-02	50	50	58.5	140	Topas	42.50	31.20	23.50
01-0156-0144-01	100	100	58.5	175	PMMA	42.50	31.20	23.50
01-0157-0144-02	100	100	58.5	140	Topas	42.50	31.20	23.50
01-0158-0156-01	200	200	58.5	175	PMMA	36.20	24.30	18.10
01-0159-0156-02	200	200	58.5	140	Topas	36.20	24.30	18.10
01-0203-0180-01	800	20	58.5	175	PMMA	36.20	24.30	18.10
01-0204-0180-02	800	20	58.5	140	Topas	36.20	24.30	18.10
01-0160-0138-01	1,000	200	58.5	175	PMMA	36.20	24.30	18.10
01-0161-0138-02	1,000	200	58.5	140	Topas	36.20	24.30	18.10

Table 3.2- Typical chip dimensions [11].

3.2.2. CALIBRATION OF MICROSCOPES

When capturing the different images in the computer (real-time image of the probe, taken pictures, zoomed pictures…) with the camera and the software, calibration information is needed in order to work in real world distance units (µm and mm). In other words, the number of px/µm related to the image is needed. This relation is linear with respect to magnification if the set-up corresponding to the microscope and the lenses in use remains the same. That is, if the magnification is multiplied by a factor, the px/µm information is multiplied by the same factor.

Nevertheless, it is common to need different type of lenses in the same microscope (see the case of the OLYMPUS microscope in **Figure 3.9** and **Figure 3.10**) or even different microscopes depending on the requirements of the experiment in question. In those cases, a new calibration process is needed every time the set-up changes. That is why the software solution proposed for real-time imaging includes this feature of asking the user for calibration every time a new set-up is chosen.

To make this essential process as precise as possible, scale rulers for calibration are used: the user is prompted to take a photo of the appropriate scale in order to calibrate the device. It is important to highlight the fact that this process has to be performed as precisely as possible, since the measurement errors at the scale of microfluidics (manipulation of 10^{-9} to 10^{-18} litres of fluids) are easily maximized with an incorrect calibration. That is why the software offers the user the possibility of using different calibration rulers depending on the magnification he/she is working with.

Without the software, the process of measuring inside the field of view is complex, tedious and can result vague. A stage scale or a transparent scale is needed to be placed over the microscope stage, such that the readings on the scale are visible. The tape of the microscope is used to secure the scale at the two ends of the stage, so that they do not move. Then the sample is placed over the ruler and brought it to a focus. Looking through the eye piece and moving the slide slowly, it is possible to make the sample come over the readings on the scale. For more accurately calculating the size, a scale (called reticule) directly built into one eyepiece would be needed [25]. With the real-time imaging software solution, those problems disappear.

In the lab, there were two types of calibration devices available: a calibration ruler from the book STEMMER IMAGING GmbH "The Imaging & Vision Handbook" [21] (**Figure 3.12**) and 3 different calibration plates (**Figure 3.14**). The first one was used only in the beginning for certain experiments and tests regarding the proper functioning of the Calibration Sub VI in the software (see **4.4.SUB-VI ARCHITECTURE**). However, it was substituted by the calibration plates due to the better accuracy and pattern quality they offer (see **Figure 3.13** and **Figure 3.15**). In fact, the calibration ruler is not even included as a possibility to calibrate the microscope inside the software.

Figure 3.12- Calibration ruler from the book STEMMER IMAGING GmbH "The Imaging & Vision Handbook"[21].

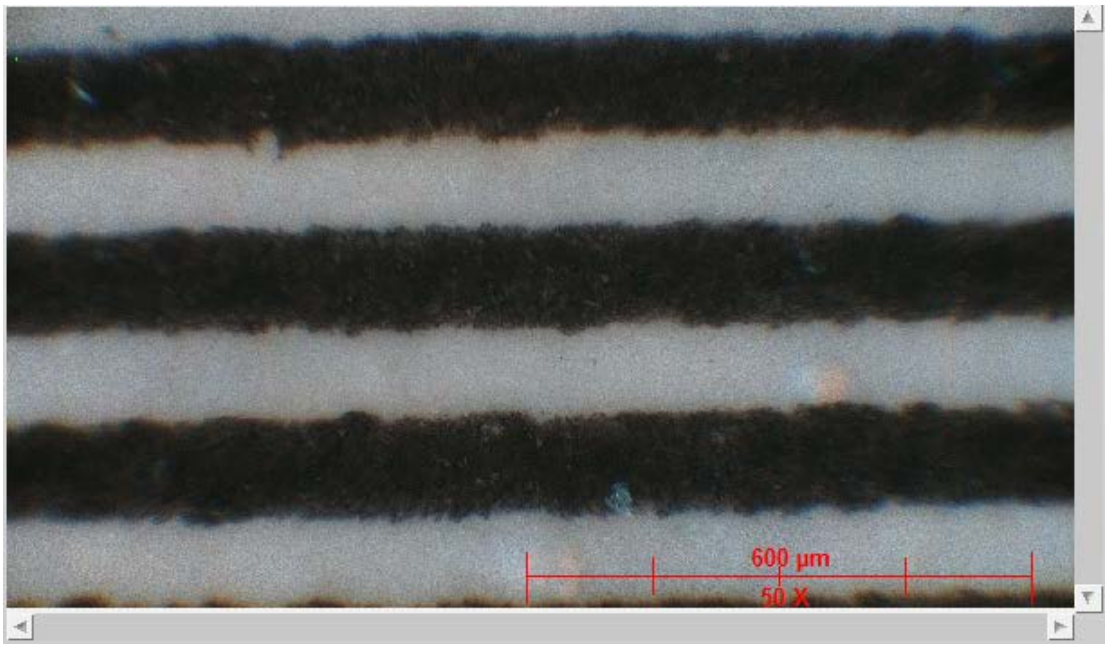


Figure 3.13- Calibration ruler from the book STEMMER IMAGING GmbH "The Imaging & Vision Handbook" [21]: close-up pictures taken with the developed software solution using the Olympus microscope and a 50X total magnification. The poor line quality becomes clear.

The calibration plates are more precise according to the lines shape than the calibration ruler is, since they are made with an etching process.

Figure 3.14- Calibration plates. From left to right: (a)100lines/cm, (b)500lines/cm and (c)1000lines/cm.

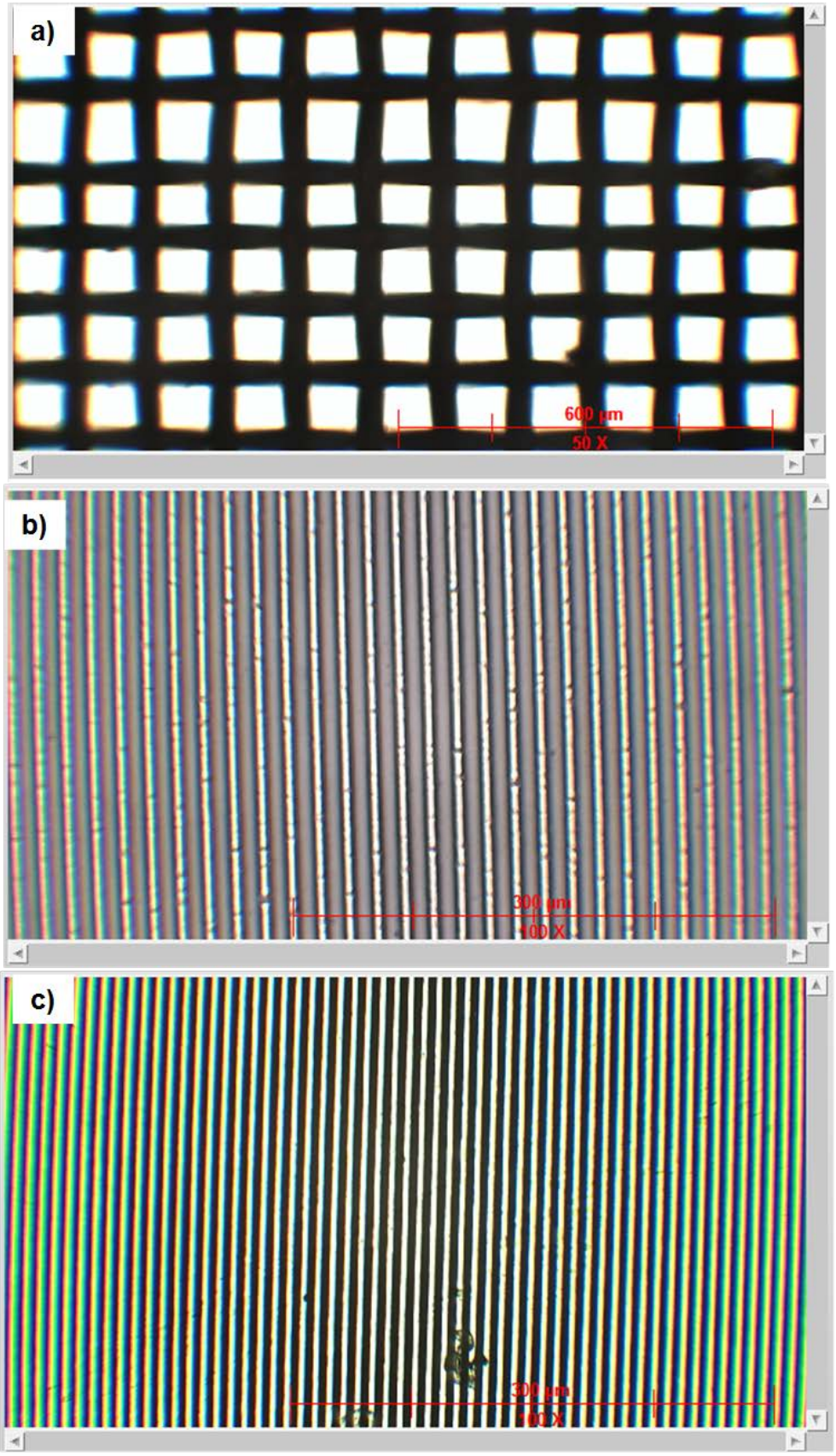


Figure 3.15- Calibration plates: close-up pictures taken with the developed software solution using the Olympus Microscope. (a)100lines/cm, (b)500lines/cm and (c)1000lines/cm.

The software is able to measure distances in px, so it is enough to prompt the user to draw a line taking a given number of calibration lines, since the distance containing that number of lines can be calculated from the information of the calibration plates. It was considered that it was fine to ask about taking 5 lines, given that the user is able to select a proper calibration plate according to the configuration.

Summarizing, a given calibration plate picture is taken and projected onto the CCD chip using a chosen magnification. Then the known scale of the ruler is compared with the physical distance of pixels on the CCD chip. The number of pixels per ruler distance at the setting being in used in that moment can be derived from there (see **Table 3.3**).

Figure 4.15	**CALIBRATION PLATE**	**CALCULATION**	**px/µm** px/µm=nº px/(5 lines·1000µm /n)
a	n=100 lines/cm	$5\ \text{lines} \cdot \frac{1\ \text{cm}}{100\ \text{lines}} \cdot \frac{10000\mu\text{m}}{1\ \text{cm}} = 500\mu\text{m}$	px/µm=nº px/500µm
b	n=500 lines/cm	$5\ \text{lines} \cdot \frac{1\ \text{cm}}{500\ \text{lines}} \cdot \frac{10000\mu\text{m}}{1\ \text{cm}} = 100\mu\text{m}$	px/µm=nº px/100µm
c	n=1000 lines/cm	$5\ \text{lines} \cdot \frac{1\ \text{cm}}{1000\ \text{lines}} \cdot \frac{10000\mu\text{m}}{1\ \text{cm}} = 50\mu\text{m}$	px/µm=nº px/50µm

Table 3.3- Calibration references. How the px/µm information is related to each specific calibration plate used in the lab.

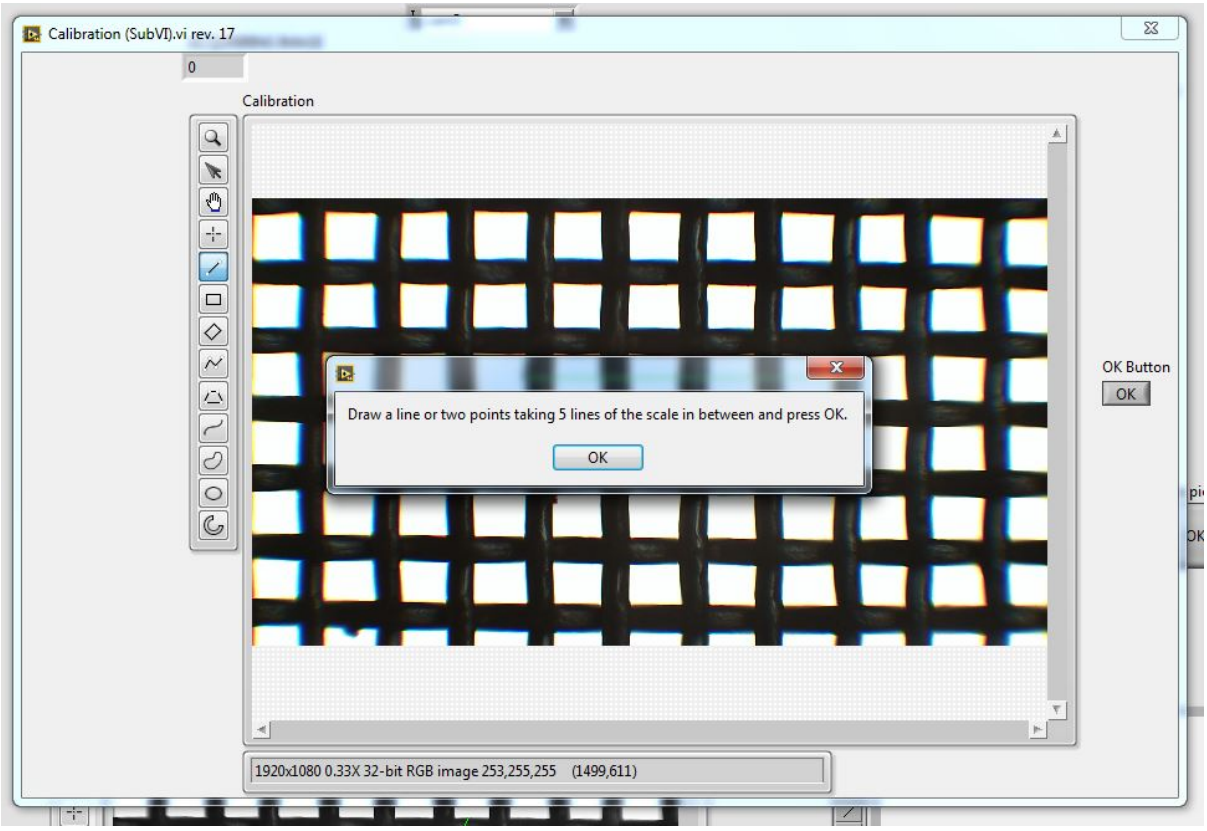


Figure 3.16- Calibration procedure in the software using the n=100 calibration plate and a total magnification of 50X.

4. OVERALL SOFTWARE CONCEPT

The software solution interacts both with the optical system and the user to offer a proper analysis tool for overview and detail inspection in microfluidic devices (see **Figure 3.1**). The challenge was to choose a proper software platform to both develop the program solution and to design a proper graphical user interface (GUI) solution (the interface where the user interacts with the imaging system). **Figure 4.1** illustrates how the logic of a GUI based on a windowing systems looks like.

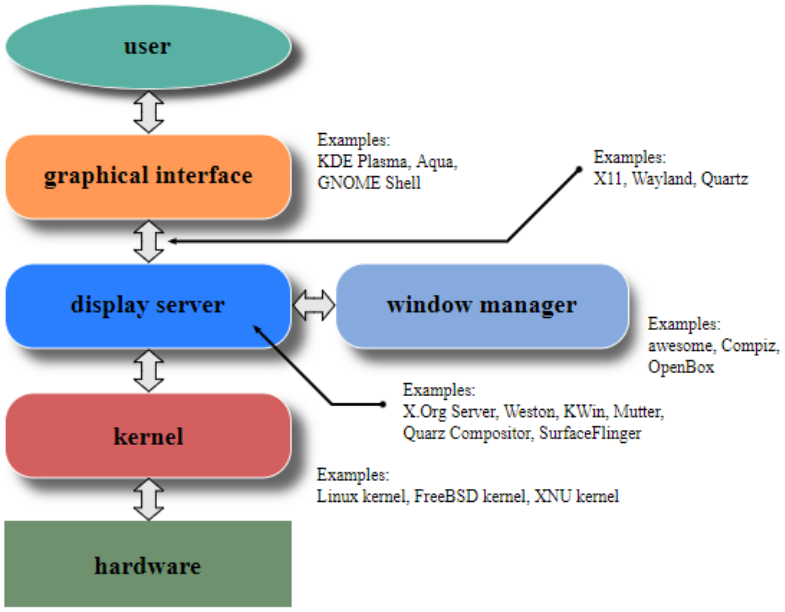


Figure 4.1- Layers of a GUI based on a windowing system.

As it can be seen from **Figure 4.1**, the structure of a GUI is quite complex, without even including the program and its relation to it. This complexity is very simplified in the LabVIEW programming software of National Instruments. It integrates the possibility to program and to create a GUI all together: while the program is developed in the window called Block Diagram the user GUI is organized in another window called Front Panel. The user only sees the Front Panel while running the program, becoming invisible for him/her the programming aspects of the Block Diagram. In addition, the possibilities to customize the Front Panel appearance are high, so a user-friendly GUI can be developed without excessive effort.

Even though the easiness for the GUI implementation is the most important reason while LabVIEW was the chosen platform to develop the program, it offers other tools and extensions that facilitate the task of developing this particular real-time imaging for microfluidic applications software too. For example, the IMAQ Vision for LabVIEW extension offers the possibility to direct image acquisition and processing, together with offering a high variety of easy and handy tools for that purpose. In addition, different Sub VI can be used as

windows that pop-ups when a certain part of the program is executed. The version used was LabVIEW 2014.

Once the programming platform was chosen, the specifications that the program should include were determined. Those specifications, together with the solution found for each of them, are presented in **Table 4.1**.

Later in this chapter, why each solution was chosen is explained and an overview of each of them is given, corresponding each subheading to one solution. In **5.DETAILS OF SOFTWARE SOLUTION**, the info given in this chapter is completed, explaining more in detail the different tasks that the real-time imaging for microfluidic applications program is able to perform.

Needed specifications	Description	Solution
Graphical user interface.	The user is not a programming expert. An adequate and user-friendly interface needs to be programmed.	LabVIEW Front Panel with a Tab Control.
The program structure needs to be able to do simultaneous operations.	A real-time image needs to be continuously acquired with a frames per second rate that is as high as possible. There also has to be a support for the other tools, such as zoom-in and –out, distance measurements, text inclusion, save picture... .	Producer/Consumer model.
Necessity for documentation.	Depending on the type of research, users have many different requirements for information, such as documentation of the type of microscope, acquisition of image details, possibility to zoom-in and -out, projection of a scale bar or comments, storing an image or a video… .The implementation of those features in a user-guided software interface enables the researcher to properly document his work and allows easy sharing of information with others, so a platform for storing information is needed.	Database in phpMyAdmin with XAMPP.
Modularity.	The program requires high amount of completely different operations: use of modules or smaller parts for the overall objective.	Sub VI-s

Table 4.1- Specifications the program needs to include and solutions proposed for each of them.

4.1. ORGANISATIONAL/VISUAL ASPECTS IN THE FRONT PANEL

The front panel represents what the user is seeing when the program is executing, in order words, it corresponds to the graphical user interface (GUI). The GUI has to be visually helpful and easy to use, since it is oriented to non-expert users or users with no knowledge in programming at all.

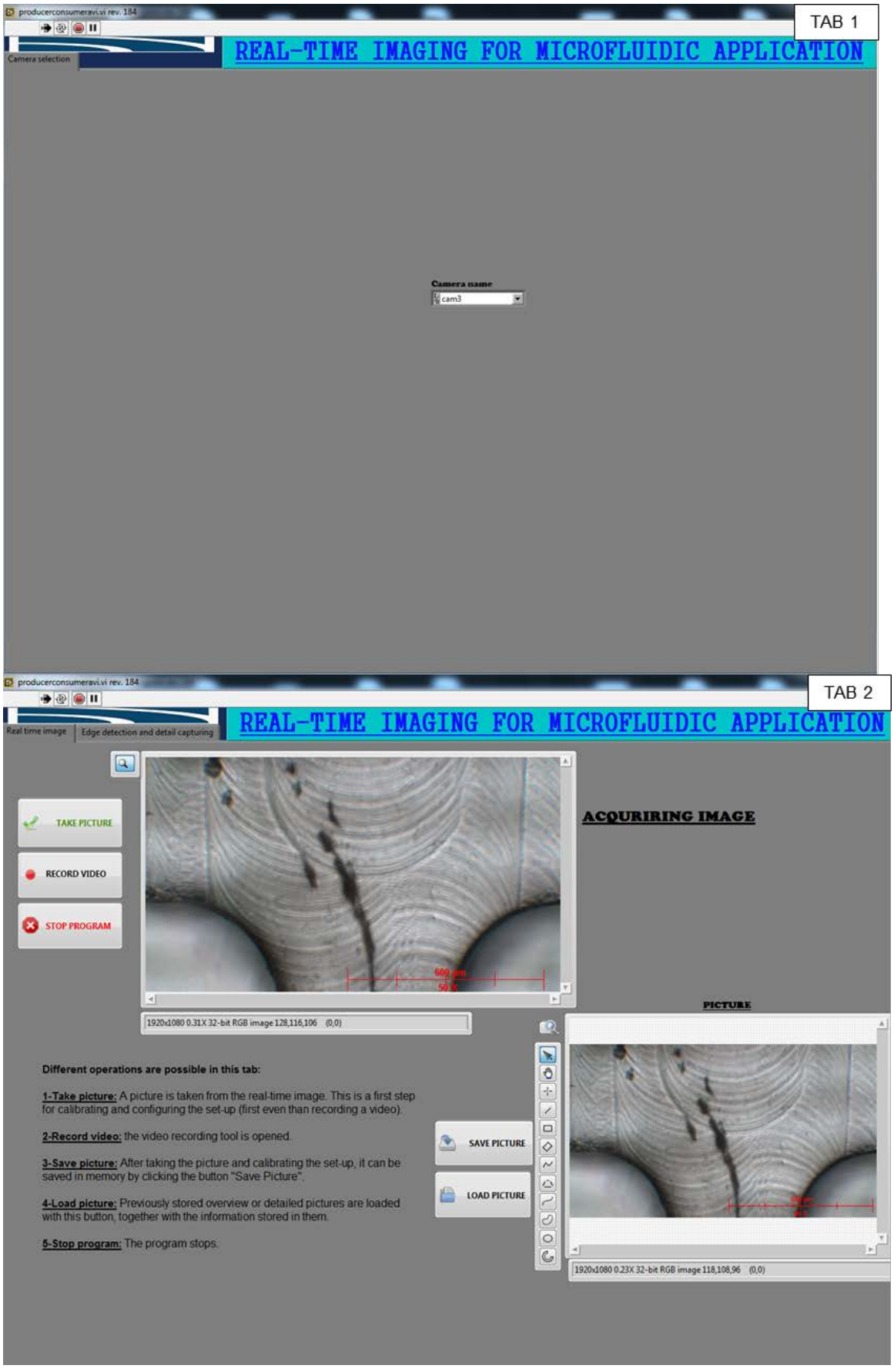
It was organised with a Tab Control (see **Figure 4.2**), in order to have space for all the tools and image/picture indicators. The image/picture indicators, precisely, occupy a high space, so a proper organization and distribution in the available space of them becomes challenging.

The used Tab Control has 4 pages, organised programmatically as follows:

Tab page name	Description	When is the tab visible?
1- Camera selection	It contains a control called camera name where the name of the camera wanted to open is specified.	In the beginning of the program, when the user has to select a port. It becomes invisible after this and only after a new execution is possible to interact in this page again.
2- Real-time image	It contains the image indicators corresponding to “Real-time image” (real-time visualisation of the sample) and “Picture taken image” (overview picture taken by the user). The controls available in this tab are “Take picture”, “Record video”, “Stop program”, “Save picture” and “Load picture”. The user can also zoom-in and –out in “Real-time image” and “Picture taken image”.	It is visible after the user has selected the camera and during normal execution of the program. It is made invisible when the tab “Record video tool” is being used or while the user interacts with certain controls of the tab “Edge detection and detail capturing”.
3- Edge detection and detail capturing	It contains the image indicators corresponding to “Picture taken image 2” (overview picture taken by the user), “Distance measured” (edge detection tool output) “Zoom/Manual distance measuring” (zoom tool with drawing rectangles, detail picture). The controls available in this tab are “Settings” (for adjusting the parameters for the edge detection), “Zoom tool”, “Zoom out”, “Finished zooming”, “Text tool” and “Save zoomed image”. The user can also zoom-in and –out in a normal way (not using the zoom tool) and draw ROI-s for edge detection in “Picture taken image 2” and draw lines in “Zoom/Manual distance measuring” for distance measurements.	It is visible after the calibration and the projection of scale bars in “Real-time image”, “Picture taken image” and “Picture taken image 2” take place and during normal execution of the program. It is made invisible when the tab “Record video tool” is being used or while the user interacts with certain controls of the tab “Real-time image”.
4- Record video tool	It contains the image indicator corresponding to “Video”, where a video can be recorded. The controls available in this tab are “Start recording”, “Load video, “Play”, “Exit video tool” and “milliseconds between images in play mode”.	It is visible only when the “Record video” button from the tab “Real-time image” is pressed and it becomes invisible when clicking the button “Exit video tool”.

Table 4.2-Front Panel (GUI) organisation: Tab Control.

The pages described in **Table 4.2** look in the GUI as separate tabs, being the user able change from one to other (Figure 4.2).


TAB 1
producerconsumeravi.vi rev. 184
Camera selection
REAL-TIME IMAGING FOR MICROFLUIDIC APPLICATION
Camera name
cam3
TAB 2
producerconsumeravi.vi rev. 184
Real time image
Edge detection and detail capturing
REAL-TIME IMAGING FOR MICROFLUIDIC APPLICATION
TAKE PICTURE
RECORD VIDEO
STOP PROGRAM
ACQUIRING IMAGE
1920x1080 0.31X 32-bit RGB image 128,116,106 (0,0)
PICTURE
1920x1080 0.23X 32-bit RGB image 118,108,96 (0,0)
Different operations are possible in this tab:
1-Take picture: A picture is taken from the real-time image. This is a first step for calibrating and configuring the set-up (first even than recording a video).
2-Record video: the video recording tool is opened.
3-Save picture: After taking the picture and calibrating the set-up, it can be saved in memory by clicking the button "Save Picture".
4-Load picture: Previously stored overview or detailed pictures are loaded with this button, together with the information stored in them.
5-Stop program: The program stops.
SAVE PICTURE
LOAD PICTURE

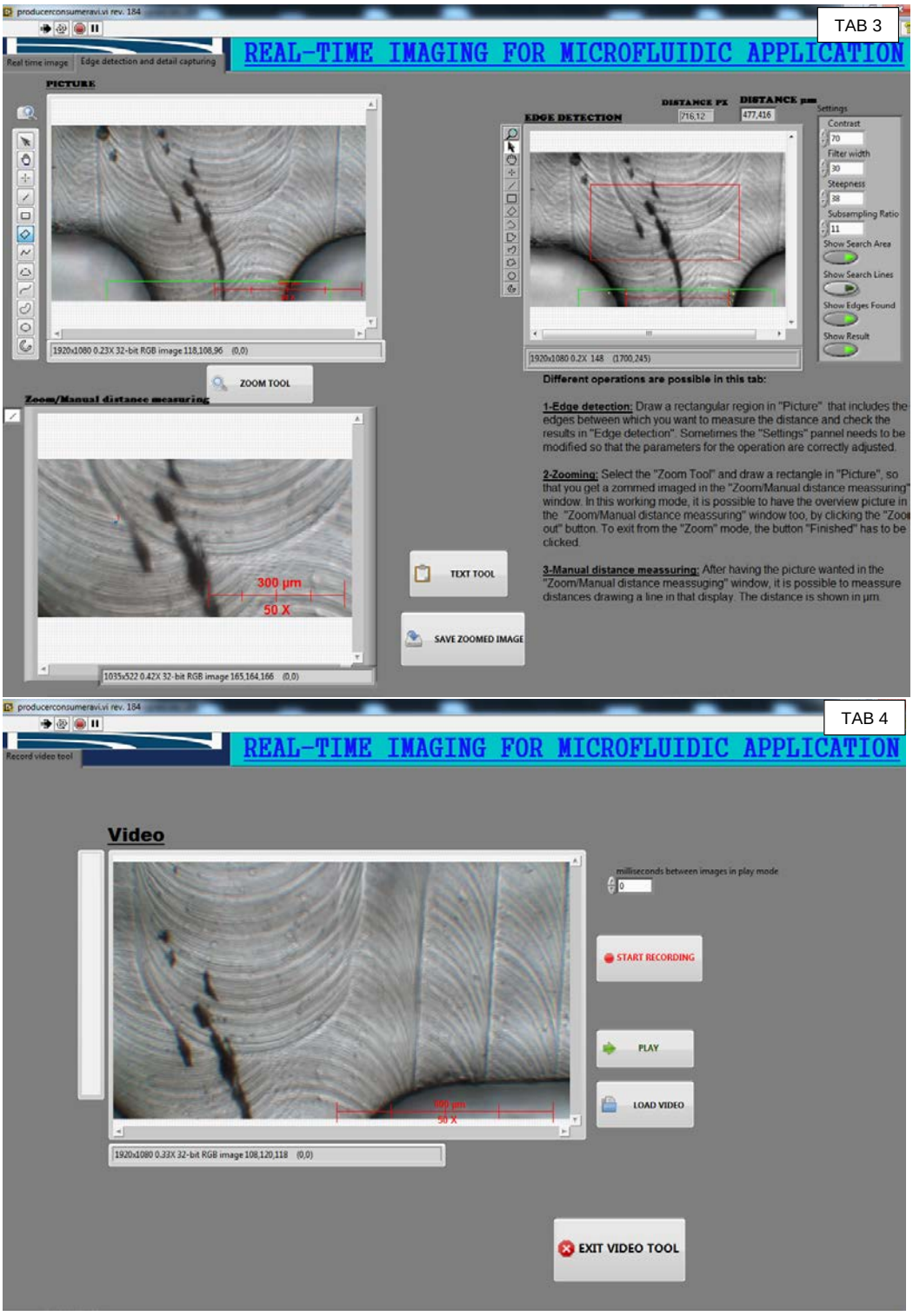


Figure 4.2- Front panel organisation: Tab Control.

4.2. SOFTWARE GENERAL ARCHITECTURE

The challenge about the software is that a real-time image needs to be acquired. The frames per second being acquired need to be as high as possible (typ. 20f/s). Taking that into account, it is not a promising idea to use a single *while loop* for all the operations, since it would make the real-time image acquisition really slow: a lot of time span until the next frame is taken is needed

because all operations are performed in a row order (linear software structure). **Figure 4.3** illustrates this concept.

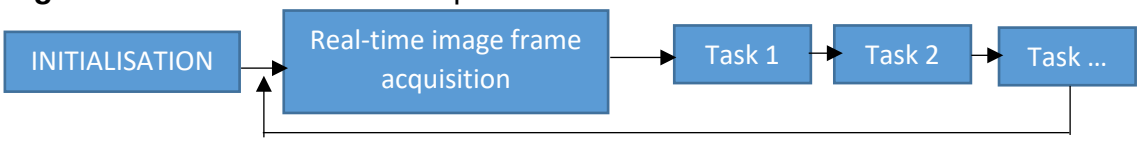


Figure 4.3- How a linear software structure would look like. As it can be seen, all the tasks need to be completed before the next frame of the real-time image can be acquired. This is unacceptable for this application.

Instead of that, the Producer/Consumer design pattern was used as general structure of the software, so that a unique *while loop* could be continuously running only acquiring the real-time image. This design pattern is based on the Master/Slave pattern and it enhances data sharing between multiple loops running at different rates. It is used to decouple processes that produce and consume data at different rates (**Figure 4.4**).

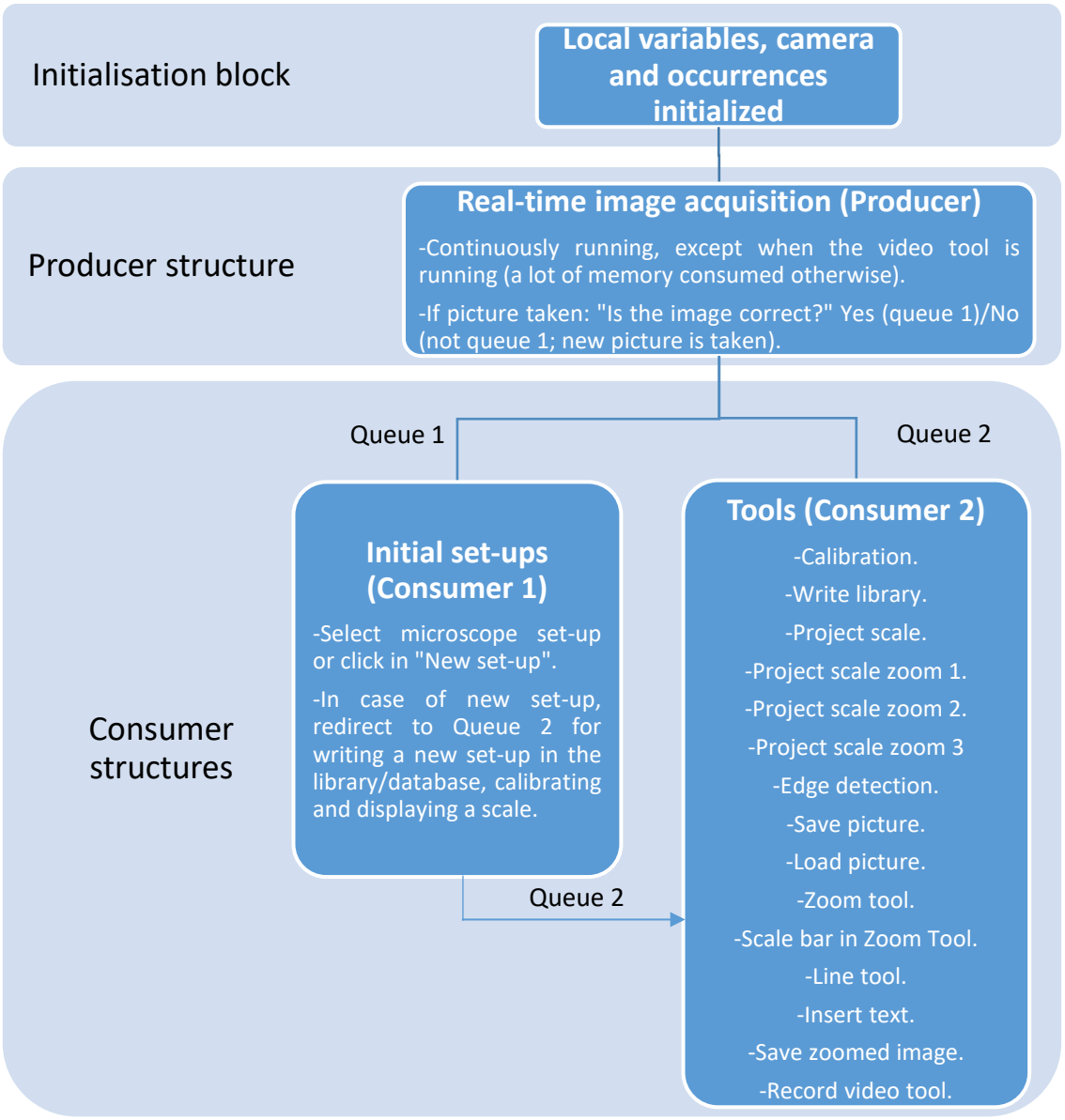


Figure 4.4- Overall software structure: Producer/Consumer. Three while loops: Producer, Consumer 1 and Consumer 2.

The Producer/Consumer concept or model is broken down into two categories: those that produce data and those that consume the data produced. The communication and synchronisation between the different loops is done using a 'Queue'. **Table 4.3** illustrates how this structure is implemented in this particular software. The software is divided into 4 main blocks:

BLOCK	NAME OF THE BLOCK	HOW IT IS SYNCRONIZED	DESCRIPTION
1	Initialisation block	Using occurrences, first this block is executed. When its execution is finished, the occurrence is set and blocks 2, 3 and 4 start working. This block has a 'Stacked Sequence Structure', that is, it is executed only once.	Stack sequence (one or more subdiagrams, or frames, that execute once sequentially) with 3 frames: 1-Initalization of Booleans, DBL Numeric Constants and string variables. Tab Control Pages visibility initialisation. Creation of temporary memory location for the images used (4 images; all RGB U32 Type). Set-up of the visualisation state of the different tools (for example, ROI tools) and buttons. Initialisation of the Basic Functional Global Variable "FGV Sub VI" ('Selected microscope', 'Selected magnification' and 'px/µm'). Initialisation of image area sizes and zoom factors. 2-Camera port selection and its configuration. Rearrangement of pages in the Tab Control. 3-The occurrence is set up and blocks 2, 3 and 4 start working.
2	Real-time image acquisition block (producer)	Waits for the occurrence.	*While loop* that is continuously running, so that a real-time visualization is acquired (each frame corresponds to one execution of the loop). The *while loop* is contained in page number 0 of a stacked sequence structure, being page number 1 the actions to be performed for resetting the system when the user clicks the 'Stop Acquisition' button (close camera, flush queues in use and destroy the images created to free the space occupied in memory). While the image acquisition is taking place, an event structure is used to detect different actions that the user can perform. Those actions are related to blocks number 3 (initialize set-up if an image is taken) and 4 (tools) with queues (see picture **Figure 4.4**).
3	Initial set-up of the system (consumer 1)	Waits for the occurrence + Queue of images (queue 1).	It is a *while loop* continuously running. It only performs the operations necessary for taking an image if there is an element in the queue. Otherwise, there is a time out of 5ms and the *while loop* executes again without performing any action. This distinction is done with a *Case of* structure. The operations include reading the different microscope set-ups available in the database and directing to some given frames or tools in block number 4 depending on the fact whether a new configuration is needed (3 actions are necessary: writing in the database, calibration process and projection of the scale bar) or not (only projection of the scale bar is necessary).

BLOCK	NAME OF THE BLOCK	HOW IT IS SYNCRONIZED	DESCRIPTION
4	Different tools for image and video processing (consumer 2)	Waits for the occurrence + Queue of strings (queue 2).	It is *a case* of structure enclosed in a *while loop* as in *Block 3*. There is a time out of 5ms again. In the FALSE case (element in the queue waiting to be processed) of the *Case of*, another *Case of* structure is included with the different tools in its frames. Those tools are related to user actions detected in *Block 1 and Block 2* and their name coincides with different strings inserted in queue 2 depending on those actions: that is how synchronisation between the different blocks is possible.

Table 4.3-Overall software structure: blocks.

For realizing, the different tasks in the 4 blocks present in **Table 4.3**, numerous VI-s and programming structures were needed, so the organisation of the program becomes a really important part concerning future modifications and easiness of understanding. To ensure that organisation, a database and some Sub VI-s were created. The database gives the opportunity to store and document the information the user works with, while Sub VI-s make possible to use modularity in LabVIEW.

Figure 4.5 illustrates the organisation of the database and the Sub VI-s in the program (where in the program they are located), in relation to the structure present in **Table 4.3**:

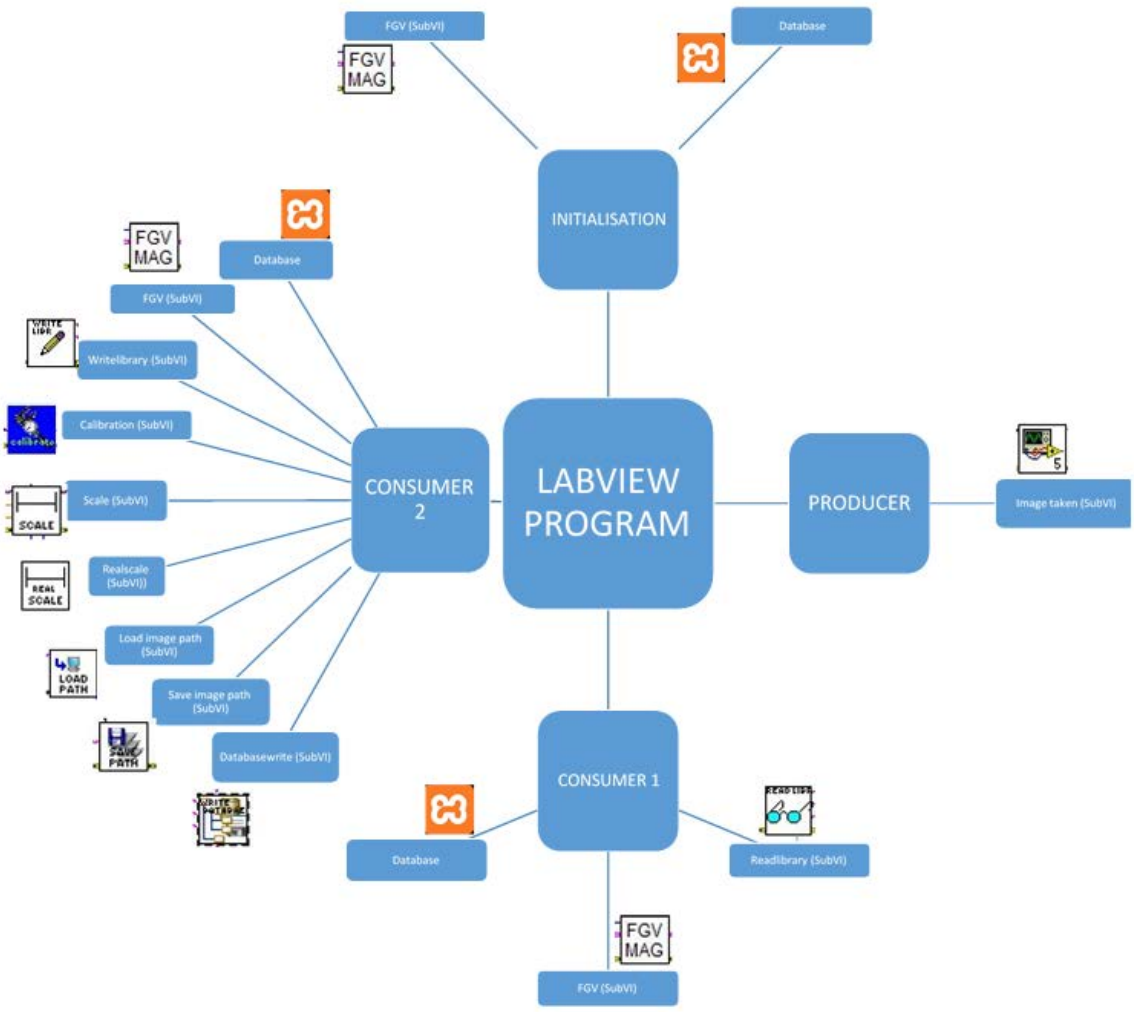


Figure 4.5- Interactions of the program with the SubVI-s and the database.

Taking into account the relations of the program with the database and the Sub VI-s presented in **Figure 4.5**, it is considered necessary to explain more broadly how the database is organized and which are the functions of the different Sub VI-s.

4.3. DATABASE ARCHITECTURE

The storage of the information processed during the execution of the program becomes a necessity for the researches to ensure proper documentation and sharing of information with others. To create a database to store all the information becomes meaningful, since the storing functionalities offered by databases are much broader than those of a normal table of file: they support processes requiring information, such as queries.

LabVIEW offers support to database communication with VI functions that can be found in the palette *Connectivity->Database*. Therefore, it is not any problem to use a database in relation to the programming platform.

The database was created in phpMyAdmin with XAMPP, mainly because of the easiness of installation and because nothing more complex is needed to

store the data wanted to be saved. XAMPP is a free and open source cross-platform web server solution stack package developed by Apache Friends, consisting mainly of the Apache HTTP Server, MariaDB database, and interpreters for scripts written in the PHP and Perl programming languages. XAMPP stands for Cross-Platform (X), Apache (A), MariaDB (M), PHP (P) and Perl (P).

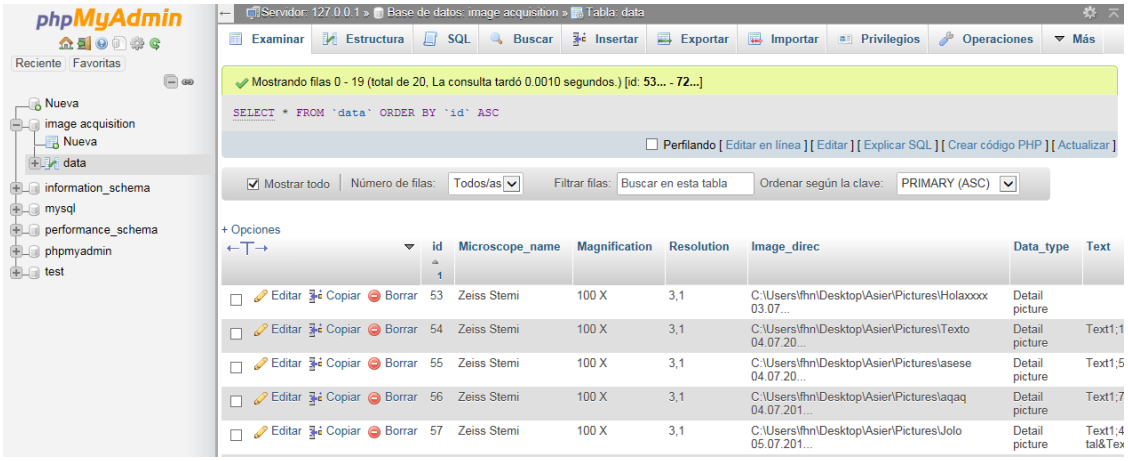
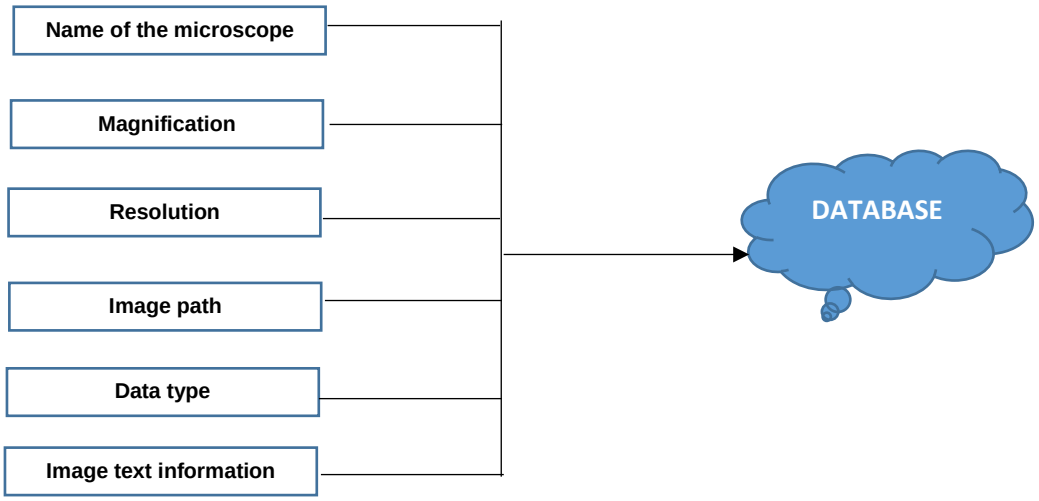


Figure 4.6- Appearance of the database.

The database created was called “image acquisition”, being “data” the table used to save all the information needed. “data” is organized in rows. Those rows represent the different inputs saved directly from LabVIEW to the database (see **Figure 4.6**). There are 7 columns to classify that information:



COLUMN	MEANING OF THE INFORMATION IN THE COLUMN
id	This is the unique key of the database: there are no two distinct tuples (rows) that have the same values for the attributes in this set.
Microscope_name	Name of the microscope being used.
Magnification	Magnification being used.
Resolution	It contains the px/µm information of each particular microscope+magnification set-up. This information is retrieved from FGV(SubVI), together with the Microscope_name and Magnification information, at the end of the calibration step.
Image_direc	It contains the path where an image or a video is saved. This information is necessary to load images or videos in the program. The

	images/videos are stored in the computer system and the path contains the information to recover them in the program.
Data_type	It can contain four different texts depending on the information saved. This column helps to identify which kind of information has been stored in each row: • Calibration: calibration information about a particular setting up process; no picture or video. • Overview picture: general picture with scale bar. • Detail picture: zoomed picture with scale bar. It can contain text information. • Video: recorded *.avi* video.
Text	It contains the text information in the case of a detail picture. It is stored as follows: *Group 1*; *PositionX 1; PositionY 1; Text 1* $ *Group* 2; *PositionX 2; PositionY 2; Text 2*… • Group: Name to identify the inserted text. The names used are *Text 1*, *Text 2*, *Text 3*… • Position X and Y: mouse coordinates of the inserted text in the image. • Text: information the user has written. All that information has to be saved in order to retrieve the text information correctly when a detailed picture is loaded by the user.

Table 4.4-Columns used to organize the information in the database.

4.4. SUB-VI ARCHITECTURE

Modularity, by definition, means to use modules or smaller parts for the overall objective. Within LabVIEW, program modularity means creating smaller sections of code known as Sub VI-s. Sub VI-s are the same as VI-s. They contain front panels and block diagrams, but it is possible to call them from within a VI. A Sub VI is similar to a subroutine in text-based programming languages.

When creating a Sub VI and using it, it appears an icon within the Block Diagram representing the Sub VI. That icon can be customized (see the different icons of Sub VI-s for this program in **Figure 4.5**), especially for program organisation purposes [26].

It is necessary to explain the function of each Sub VI before describing the details of the software solution in **5. DETAILS OF SOFTWARE SOLUTION**, since these Sub VI-s were used to solve a lot of different particular tasks there.

SUB VI	FUNCTION
FGV	Functional Global Variable. It is used in the program to store in a cluster of elements the information corresponding to the “Selected microscope” (string data), “Selected magnification” (double data) and “px/µm” (double data). This Sub VI allows to read/write from/in the database those 3 data corresponding to calibration information, since they are used a lot of times for different tasks. It is not handy to read/write the data from/to the database for performing each task: it is better to perform the necessary operation with FGV and interact as less as possible with the database. It is important to mention that in the interaction FGV-Database the “Selected magnification” and the “px/µm” data have to be handled correctly, since the

	database works with string type information and the FGV with double type information for those cases.
Image taken	Sub VI used for asking the user if the image or picture taken by the him/her was correct or not.
Readlibrary	It is used to read from the database the information with Datatype='Calibration'. It gives the user the opportunity to work with already stored set-ups without needing a new calibration process. There is an option of 'New configuration' too, for which a calibration process has to be done.
Writelibrary	It is used in the calibration process: it asks the user for the name of the microscope and the magnification in use.
Calibration	It is used in the calibration process: it asks the user to input the number of lines/cm of the calibration plate being in used and to draw a line or two points taking 5 lines in between.
Scale	It draws a scale bar in an image using overlay information.
Realscale	It draws a scale bar in a real-time image using picture indicators. With real-time images, it is not possible to use overlay information, since every time a new frame is captured that information is lost[4].
Load image path	This SubVI displays a table where the user can choose a row of the database he is interested in. All the information of the chosen row (6 columns) is recovered in the FGV and 3 other indicators. The path part ("Image_direc" column in the database) plays an important role in this Sub VI, since it is used to retrieve the image or the video wanted.
Save image path	Sub VI used to save an image in a given path and to store all the information concerning that image in the database via the SubVI Databasewrite.
Databasewrite	Sub VI used to write in the database.

Table 4.5-Sub VI-s and their functions.

[4] For more information, see **REAL-TIME IMAGE PROBLEM** in **APPENDIX D: FURTHER DETAILS ABOUT THE LABVIEW SOFTWARE**.

5. DETAILS OF SOFTWARE SOLUTION

Once explained the overall software solution, it follows to clarify the exact details and how the different tasks were solved. The different tasks have already been mentioned in **4.OVERALL SOFTWARE CONCEPT**. In that previous point, on the one hand, how they are organized and coordinated between them using queues (see **Figure 4.4** and **Table 4.3**) has been explain. In this point, on the other hand, how each task is programmatically solved is explained. The tasks are summarized in the followings:

1. Initialisation.
2. Real-time image acquisition.
3. Initial set-ups and calibration.
4. Projection of scales.
5. Save picture tool.
6. Load picture tool.
7. Edge detection tool.
8. Zoom tool.
9. Scale bar projection in zoom tool.
10. Line tool.
11. Insert text tool.
12. Save zoom image tool.
13. Record video tool.

When developing this software, it was very important to think about future lines. The software is never completely finished: there are always new features to add and things to improve. That is why the program has to be structured in an easy and understandable way from the beginning, so that the next person working with it can make further developments and modifications out of it (see **7.3.OUTLOOK: FUTURE LINES**).

The differentiation and organization of the software in tasks facilitates this model. In fact, splitting the software in tasks was the first step to come up with all the overall software concept presented in **4.OVERALL SOFTWARE CONCEPT.** It is remarkable that in this chapter only detailed overviews about the given approach are introduce, presenting only the highlights. More details like specific

VI-s or specific software functionality information are documented in **APPENDIX D: FURTHER DETAILS ABOUT THE LABVIEW SOFTWARE**.

The VI-s used are the ones integrated in LabVIEW and in the IMAQ Vision and Motion extension. The first ones are used to perform common operations, measurements and functions and the last ones for creating, dealing with, programing and overlaying images and for acquiring information from the camera.

5.1. INITIALISATION

The initialisation part is the first part of the program. It only executes once. Although it is not compulsory to have this part separated from the others as a complete task, it is in fact convenient, since every time that a new element (button, variable…) is created needs to be initialised. Having a definite place in the program for this makes it easy to know where to initialise each new element, saving time and ordering effort.

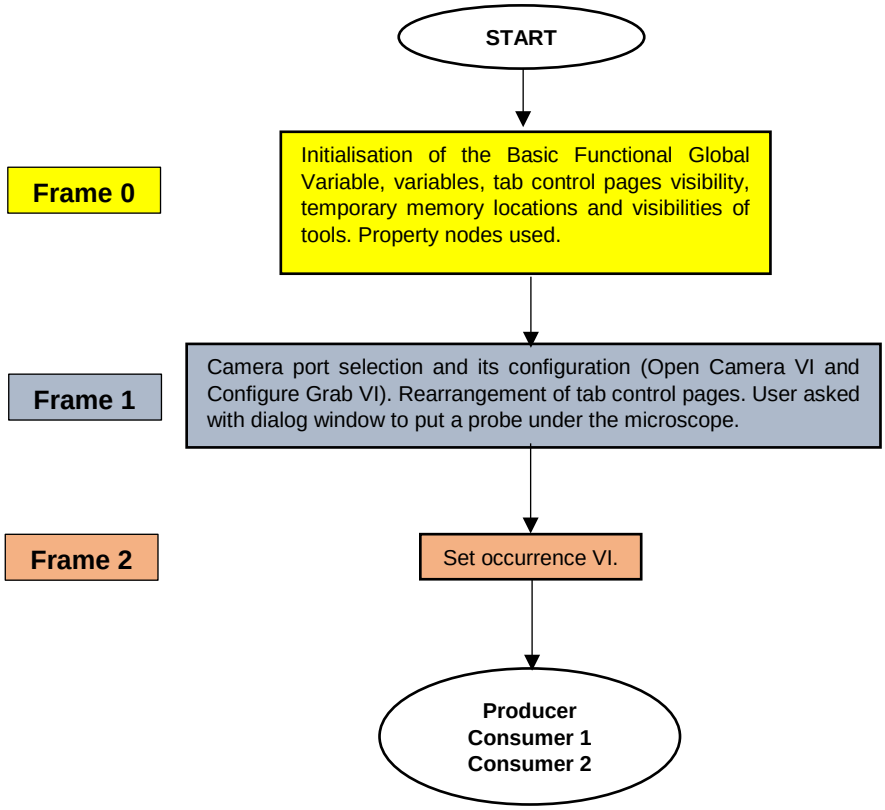


Figure 5.1- Initialisation flowchart. The frames refer to the stacked sequence structure used.

There is not much left to explain about this task in detail, since it is a really simple part of the program and it has already been explained in **4.OVERALL SOFTWARE CONCEPT.** The information concerning important VI-s used in this part (as the VI-s corresponding to occurrences) is further developed in **APPENDIX C: FURTHER DETAILS ABOUT THE LABVIEW SOFTWARE**.

5.2. REAL-TIME IMAGE ACQUISITION

This is the essential part of the program, since it is even present in the name of the project: “Real-time Imaging for Microfluidic Applications”. It is essential to make use of the highest possible frame rate that the camera can offer (typ. 20f/s), without having any major delays due to program operation and iterations. That is why the Producer/Consumer design pattern is used: it gives very good results concerning this. The producer part of the program fulfils this basic task of “Real-time image acquisition”, constituting the core of the software, and the consumer part, the forthcoming ones:

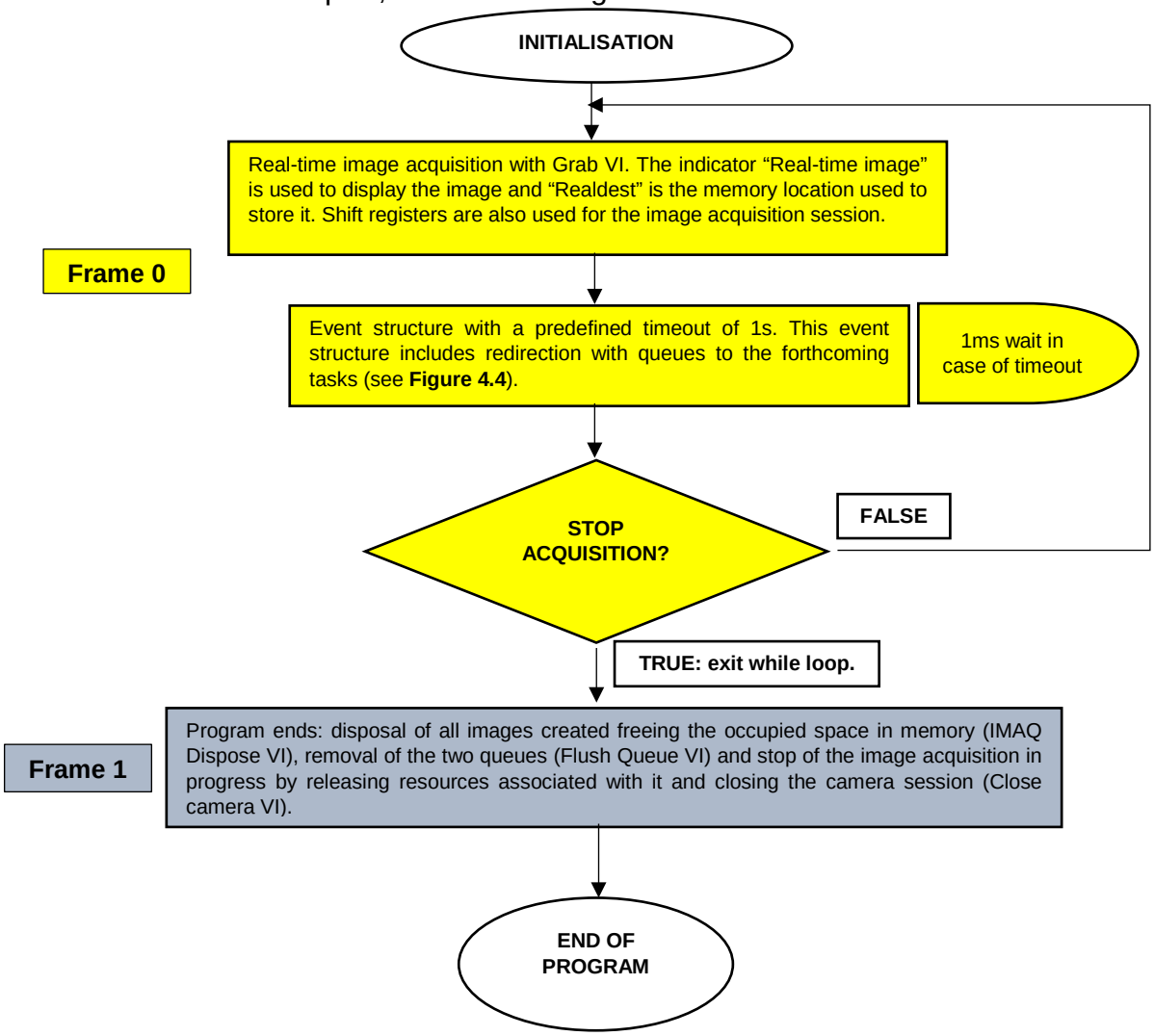


Figure 5.2- Real-time image acquisition flowchart. The frames refer to the stacked sequence structure used.

The event structure mentioned in **Figure 5.2** has 14 entries and each one is organised as follows:

1. “Take picture”: Value Change. User clicks “Take picture” button. Image taken Sub VI executes and the user is asked if the image is correct. If TRUE (*Case of* structure), the image is inserted in queue 1 and sent to the block consumer 1. If FALSE, nothing happens.

2. "Zoom tool1": Value Change. User clicks "Zoom tool1" button next to the image indicator "Picture taken image". The Zoom Tool is selected with a property node, the string "Project scale zoom1" is introduced in queue 2 and sent to the block consumer 2.

3. "Zoom tool2": Value Change. User clicks "Zoom tool2" button next to the image indicator "Picture taken image 2". The Zoom Tool is selected with a property node, the string "Project scale zoom2" is introduced in queue 2 and sent to the block consumer 2.

4. "Realscale": Mouse Down. User click with the mouse the "Realscale" control, wanting to zoom in in the "Real-time image" (for more information, see **REAL-TIME IMAGE PROBLEM** in **APPENDIX C: FURTHER DETAILS ABOUT THE LABVIEW SOFTWARE**). The "Realscale" picture control becomes invisible (F=Visible property node).

5. "Real-time image": Mouse Down. User click with the mouse the "Real-time image" control (second click, counting the "Realscale" click). The string "Project scale zoom3" is introduced in queue 2 and sent to the block consumer 2.

6. "Save picture": Value Change. User clicks "Save picture" button next to "Picture taken image" (he/she want to save an overview picture). The string "Save picture" is introduced in queue 2 and sent to the block consumer 2.

7. "Load picture": Value Change. User clicks "Load picture" button next to "Picture taken image" (he/she want to load a picture). The string "Load picture" is introduced in queue 2 and sent to the block consumer 2.

8. "Picture taken image 2": Mouse up. User clicks in the control "Picture taken image 2". If the action is to draw a ROI different to a previous drawn one (comparison+*case of* structure), the string "Edge detection" is introduced in queue 2 and sent to block consumer 2.

9. "Zoom tool": Value Change. User clicks in the control button "Zoom tool". The strings "Zoom tool", first, and "Scale bar in Zoom tool", second, are introduced in queue 2 and sent to block consumer 2.

10. "Zoom/Manual distance measuring": Mouse up. User clicks in the control "Zoom Manual distance measuring", made visible after the "Zoom tool" has been used. The string "Line tool" is introduced in queue 2 and sent to block consumer 2.

11. "Insert text": Value Change. User clicks in the control button "Insert text", made visible after the "Zoom tool" has been used. The string "Insert text" is introduced in queue 2 and sent to block consumer 2.

12. "Save zoom": Value Change. User clicks in the control button "Save zoom" (he/she want to save an overview picture), made visible after the "Zoom tool" has been used. The string "Save zoomed image" is introduced in queue 2 and sent to block consumer 2.

13. "Record video": Value Change. User clicks in the control button "Record video", made visible after **5.3. INITIAL SET-UPS AND CALIBRATION** has taken place. The string "Record video tool" is introduced in queue 2 and sent to block consumer 2.

This is the only case of the "Event structure" where the time out is change to -1: never time out. This is done to stop executing the *Real-time image acquisition block* while loop, since a new real-time image acquisition needs to be done with the record video tool and a lot of CPU memory is consumed if the process in the *Real-time image acquisition block* continues executing without offering any functionality (the tab "Real-time image" is invisible for the user when the record video tool is being executed, so the functionality is nonsense).

14. Timeout. When the user does not perform any actions of the ones described before, this case executes. Nothing is done: continuation of error line and next iteration of while loop (new frame acquired for the real-time image). The time out time is 1ms (except when case 13 is executed).

5.3. INITIAL SET-UPS AND CALIBRATION

This task is distributed in both consumer 1 and consumer 2 and it takes place when a new image is taken (event number 1 of **7.2. REAL-TIME IMAGE ACQUISITION** happens and user says that the image is correct).

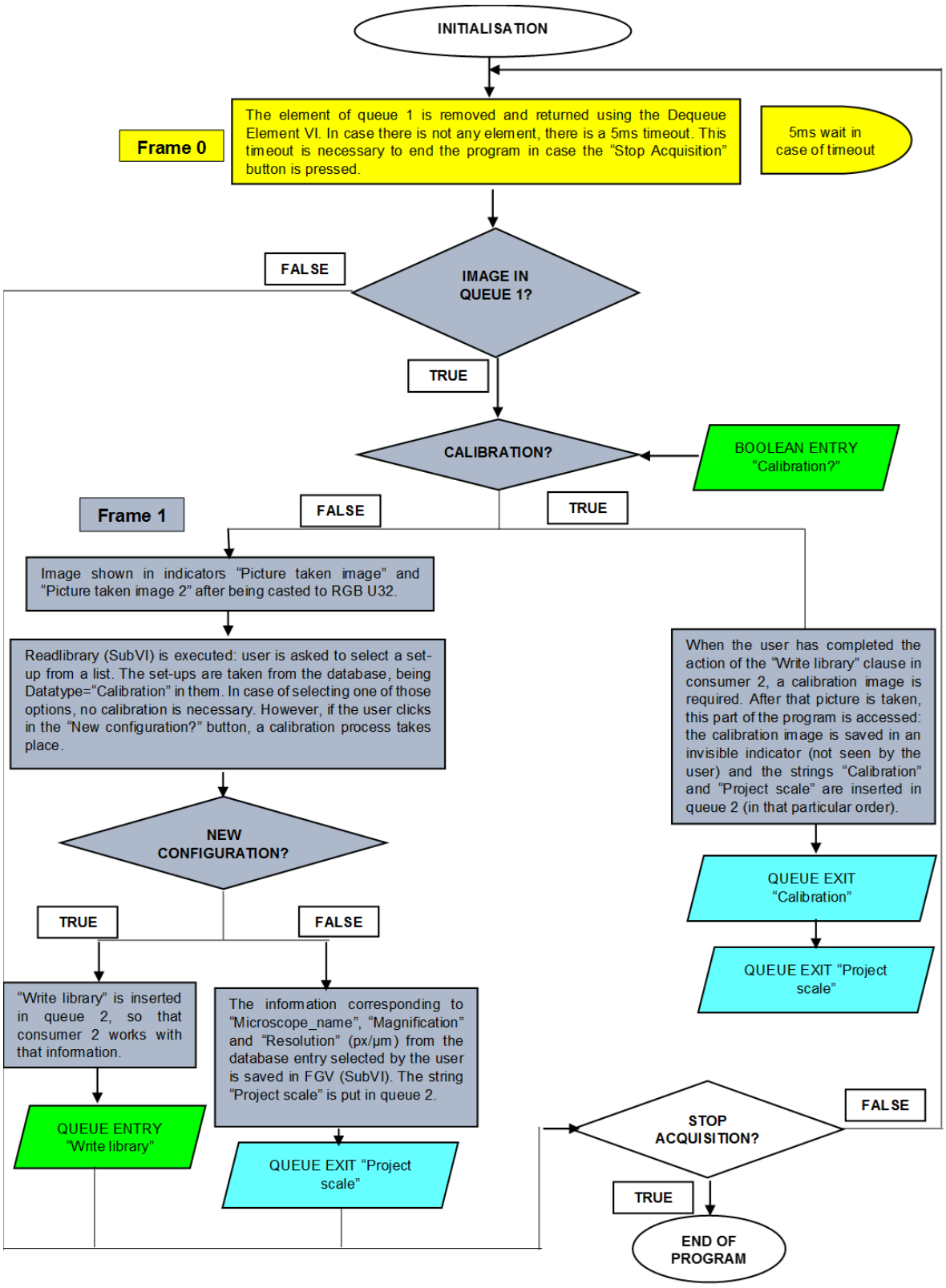


Figure 5.3- Initial set-ups and calibration flowchart 1. The frames refer to the flat sequence structure used.

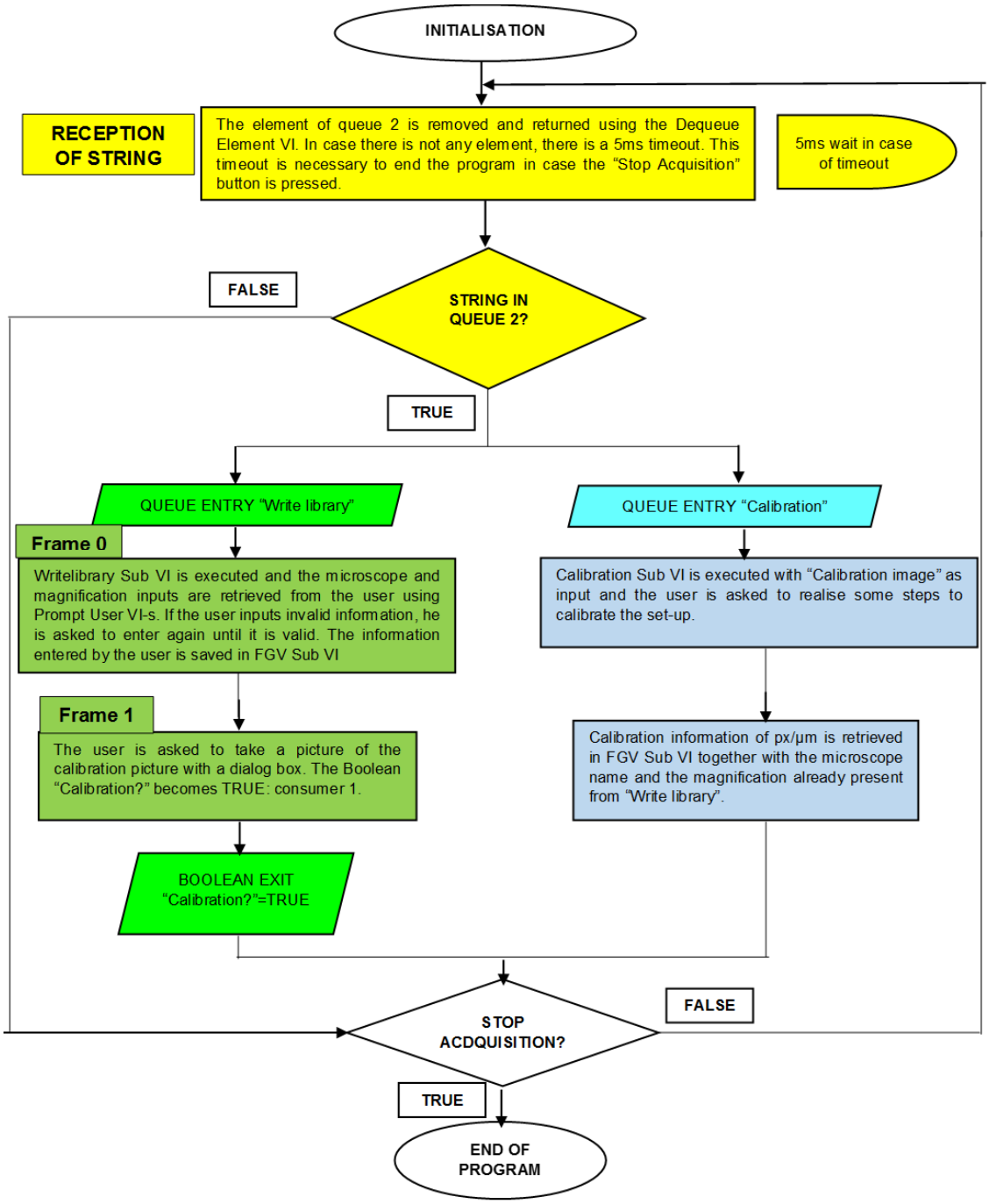


Figure 5.4- Initial set-ups and calibration flowchart 2. The frames refer to the stacked sequence structure used.

Summarizing, after an image is received, in consumer 1, the possible configurations available in the database with Datatype='Calibration' are read with Readlibrary Sub VI. If the user decides to enter a new configuration clicking the "New configuration?" button, the input of microscope name and magnification and the calibration process take place in consumer 2 structure, after being redirected from consumer 1.

The project scale tool redirected from consumer 1 is also processed in consumer 2, being the logic for it the one described in the next task.

5.4. PROJECTION OF SCALES

After writing the information in the FGV Sub VI, in the case of selecting an already existing set-up in Readlibrary Sub VI, or after the calibration process takes place, in the case of selecting the "New configuration?" option in Readlibrary Sub VI, different scale bars are projected in the image/picture indicators "Picture taken image" (image indicator), "Picture taken image 2" (image indicator) and "Realscale" (2D picture indicator placed in front of "Real-time image").

It is also necessary to delete the previous scale and project new scales when event numbers 2, 3 or 5 of **7.2. REAL-TIME IMAGE ACQUISITION** happen, in other words, when the users zooms in one of the image controls.

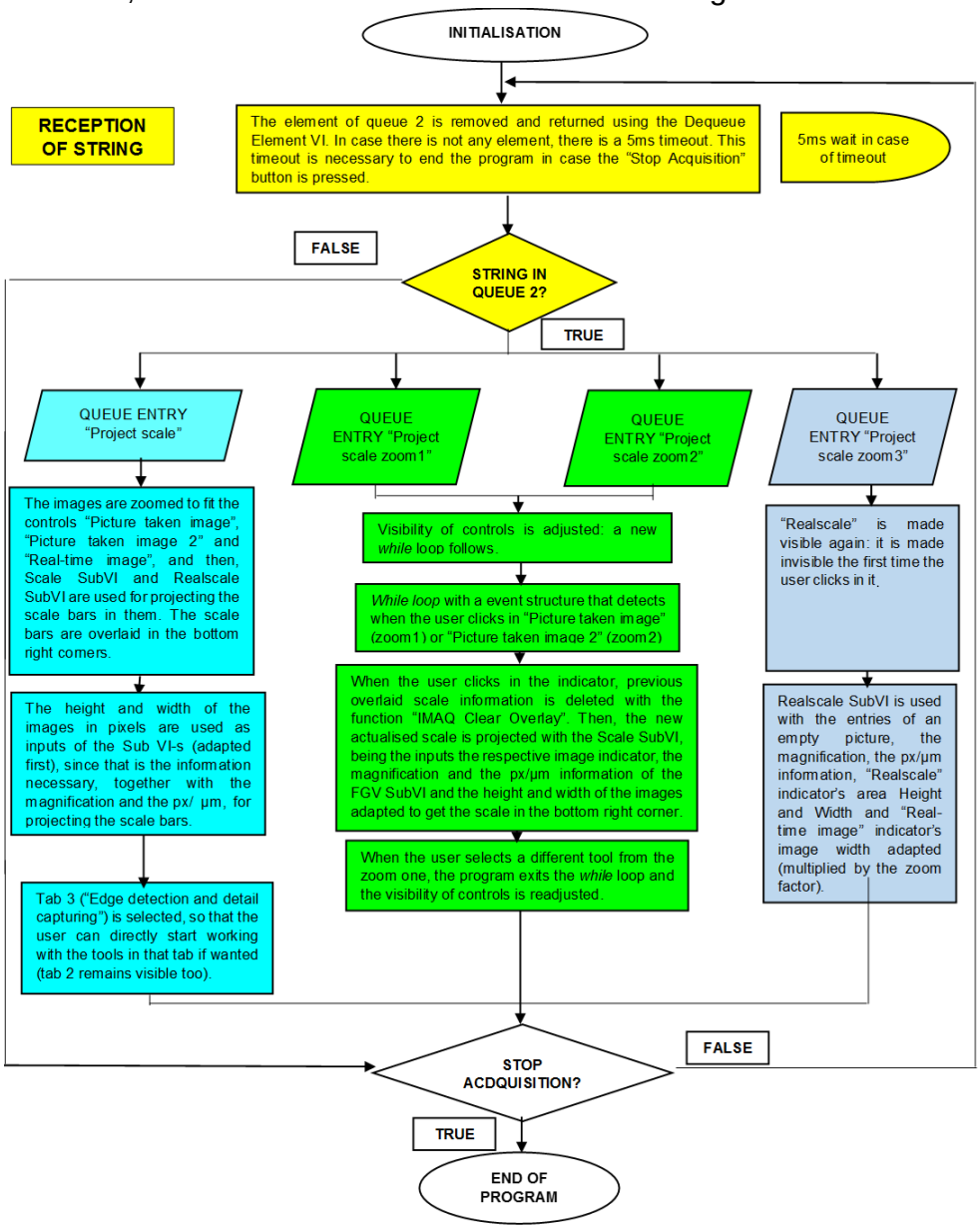


Figure 5.5- Projection of scales flowchart.

It is of interest to explain how the length of the scale bar to project is calculated programmatically in Scale SubVI and Realscale SubVI:

<table>
<tr><td>Step 1</td><td>Step 2</td><td>Step 3</td><td>Step 4</td><td>Step 5</td><td>Step 6</td><td>Step 7</td><td>Step 8</td><td>Step 9</td></tr>
<tr><td>Width of acquired image
/2</td><td>Result 1
/px/μm</td><td>Result 2
>10?</td><td>Result 3
If Result 3 =Yes: /10</td><td>Result 4: Return to step 3 with Result 4 in the place of Result 2.</td><td></td><td></td><td></td><td></td></tr>
<tr><td></td><td></td><td></td><td colspan="2">If Result 3=No: Finish iteration</td><td>Result 5: number of iterations
$10^{\text{Result 5}}$</td><td>Result 6
x [Result 4]</td><td>Result 7
x px/μm</td><td>Final result: scale bar length in pixels</td></tr>
</table>

Table 5.1-Scale bar calculation (in pixels).

- **Step 1:** The width of the acquired image is divided by 2, since the scale bar should occupy approximately that space.

- **Step 2:** The result of the division is divided by the px/μm, in order to get the measurement in μm.

- **Steps 3, 4, 5 and 6:**The μm information is rounded to the first significant figure of the number. For doing that, the number is divided by 10 until the result is lower than 10. Then, the number is multiplied by 10 to the power of the number of times the μm first number was divided by 10, and finally, the result is rounded to the nearest integer.

- **Step 8**: The number obtained from the previous steps is multiplied by the px/μm information. That is how it is get the pixel information representing the wanted number of μm-s.

- **Step 9**: Final result in pixels.

It is also remarkable to mention that the *width of acquired image*, written in **Table 5.1**, is programmatically multiplied by the inverse of the zoom factor before being inserted into the Scale or the Realscale SubVI-s in any of the scale events. This is necessary because LabVIEW multiplies such value internally by the zoom factor. This operation is also necessary for the image height input ("Picture taken image" and "Picture taken image 2") and the area height and area width of the "Realscale" picture indicator ("Real-time image").

In the events "Project scale zoom1" and "Project scale zoom2", it is also considered that if the complete image does not fit in the control area, the image height and width used for positioning the scale (not the width measure used for calculating the length of the scale bar: this is a different entry for the Sub VI) need to be adapted taking into account the image center position: the position is not any more the bottom right corner of the image, but the one of the indicator.

As an additional feature, the zoom-out operation is limited until the position where the complete image fits in the indicator both horizontally and vertically, since it does not help in anything the user to be able to zoom-out more from that position on.

5.5. SAVE PICTURE TOOL

It takes place in consumer 2 when event number 6 of **7.2. REAL-TIME IMAGE ACQUISITION** happens.

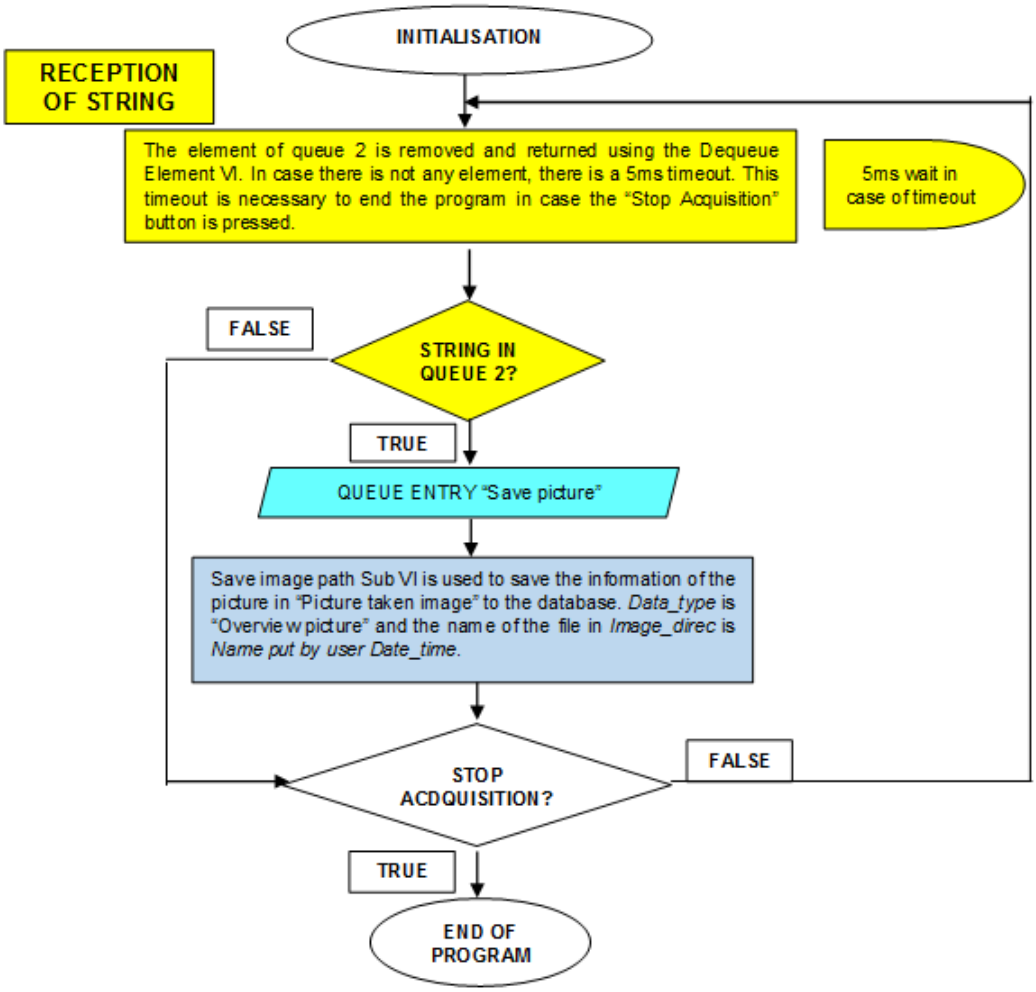


Figure 5.6- Save picture tool flowchart.

5.6. LOAD PICTURE TOOL

It takes place in consumer 2 when event number 7 of **7.2. REAL-TIME IMAGE ACQUISITION** happens.

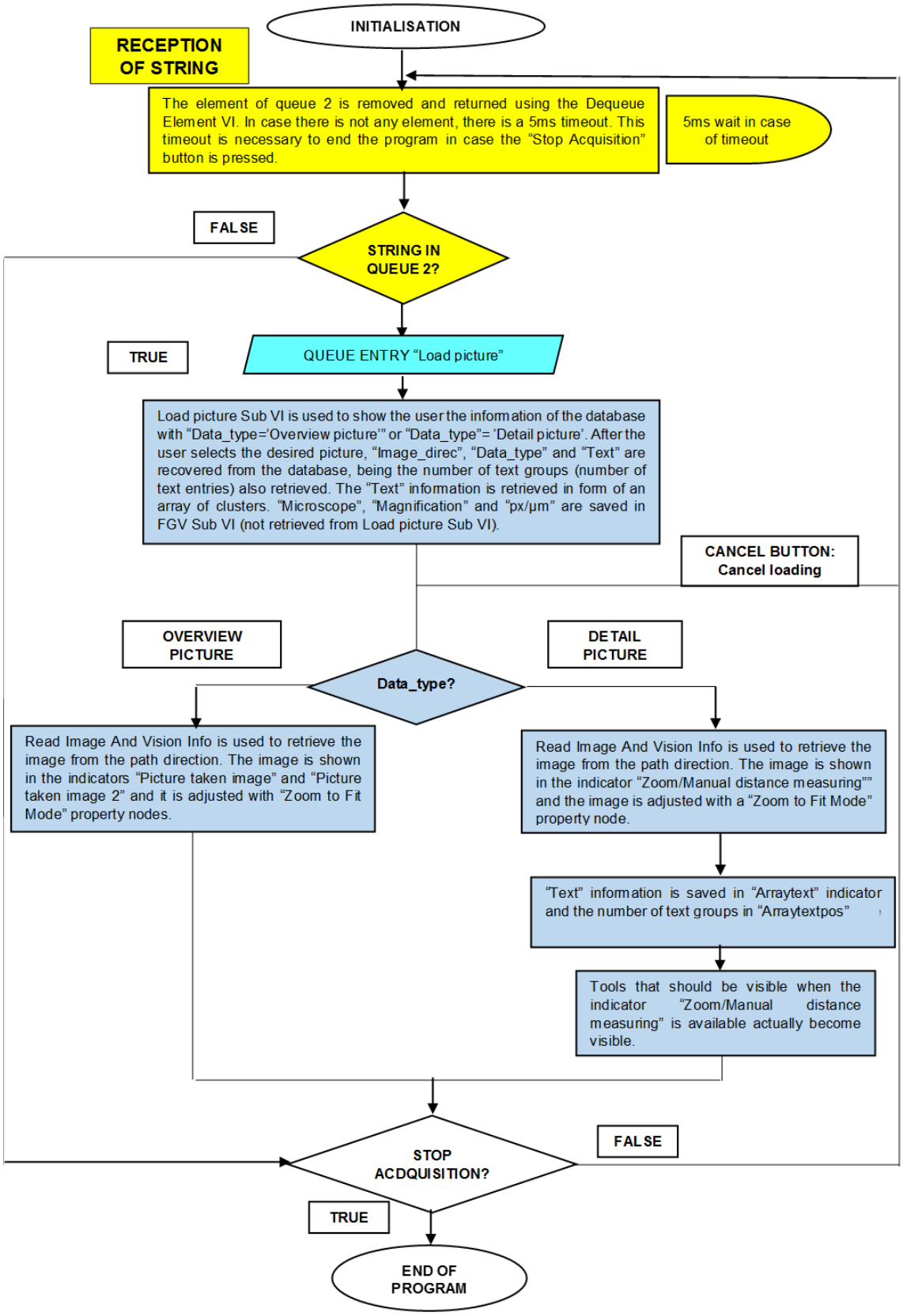


Figure 5.7- Load picture tool flowchart.

5.7. EDGE DETECTION TOOL

It takes place in consumer 2 when event number 8 of **7.2. REAL-TIME IMAGE ACQUISITION** happens.

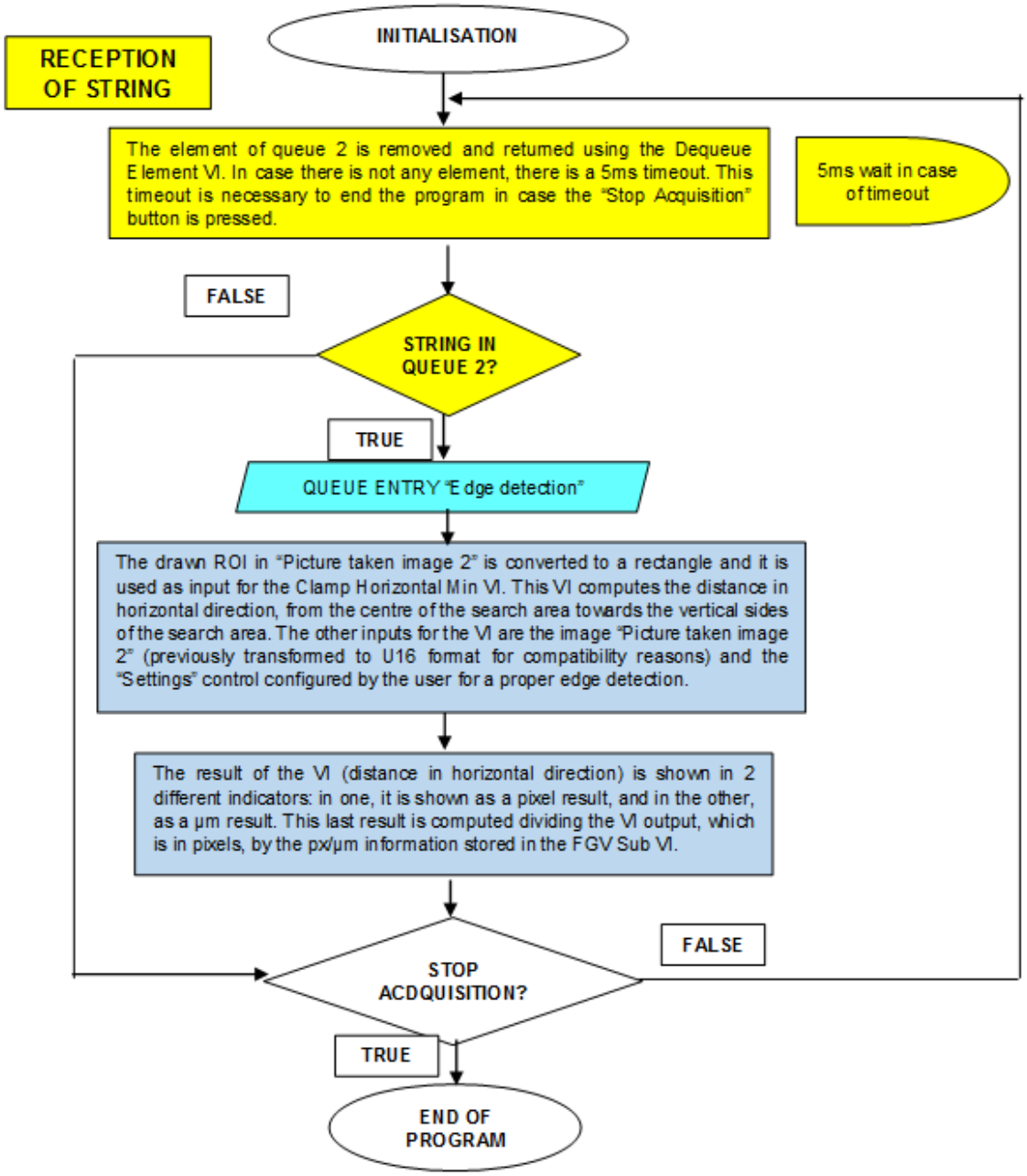


Figure 5.8- Edge detection tool flowchart.

5.8. ZOOM TOOL

It takes place in consumer 2 when event number 9 of **7.2. REAL-TIME IMAGE ACQUISITION** happens.

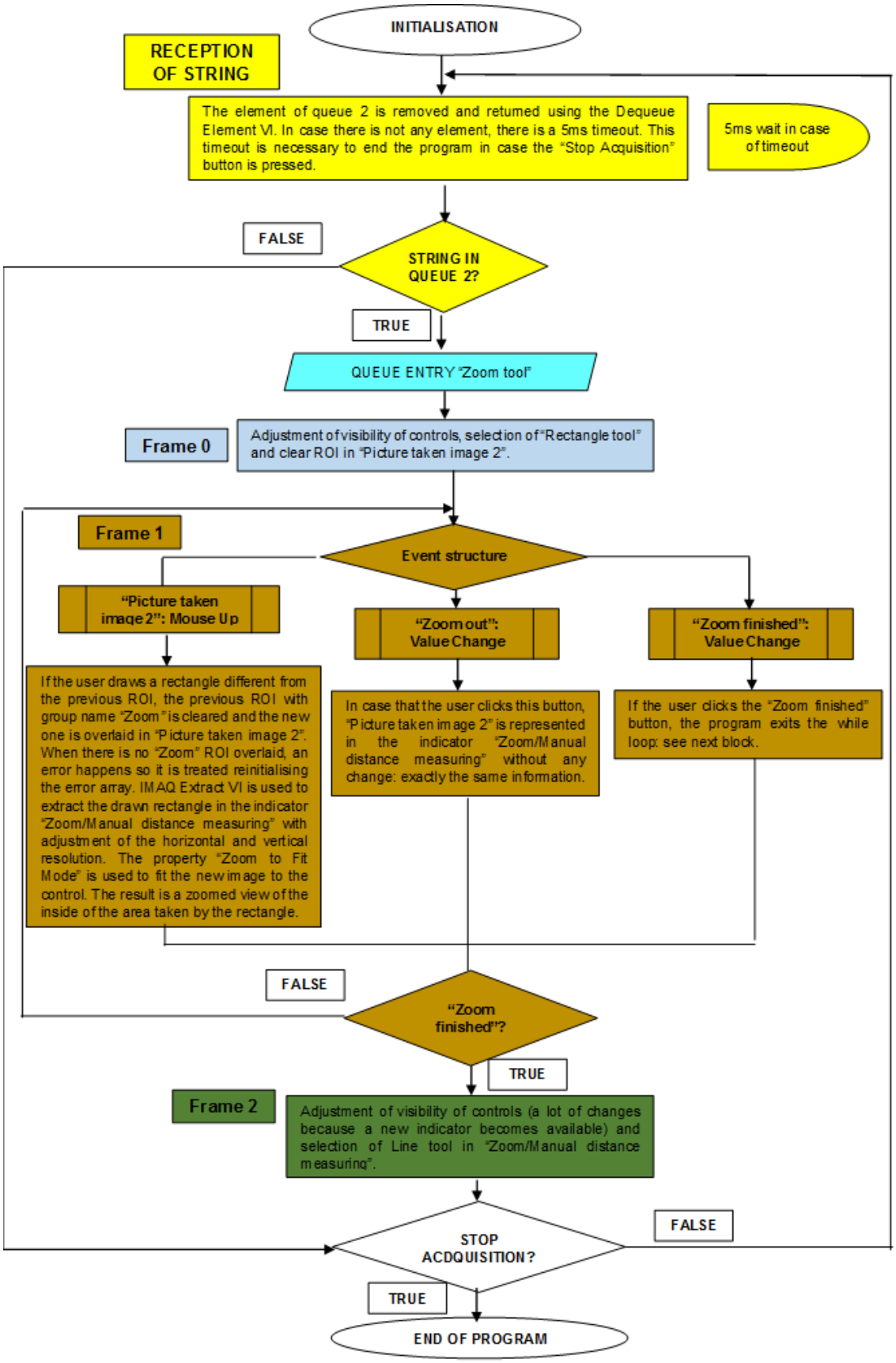


Figure 5.9- Zoom tool flowchart. The frames refer to the stacked sequence structure used.

5.9. SCALE BAR PROJECTION IN ZOOM TOOL

It takes place in consumer 2 when event number 9 of **7.2. REAL-TIME IMAGE ACQUISITION** happens, after using **5.8. ZOOM TOOL**.

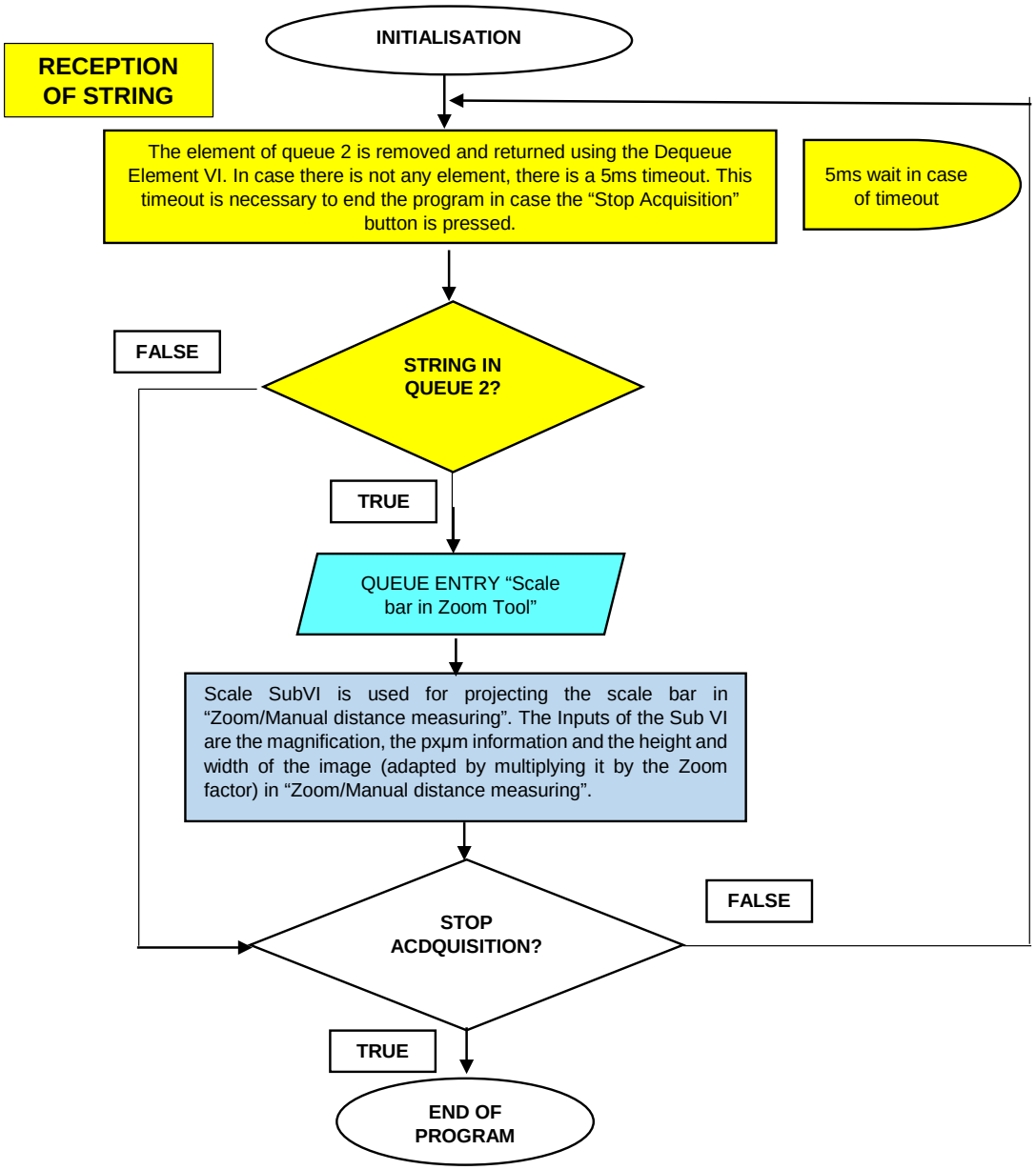


Figure 5.10- Scale bar projection in zoom tool flowchart.

5.10. LINE TOOL

It takes place in consumer 2 when event number 10 of **7.2. REAL-TIME IMAGE ACQUISITION** happens.

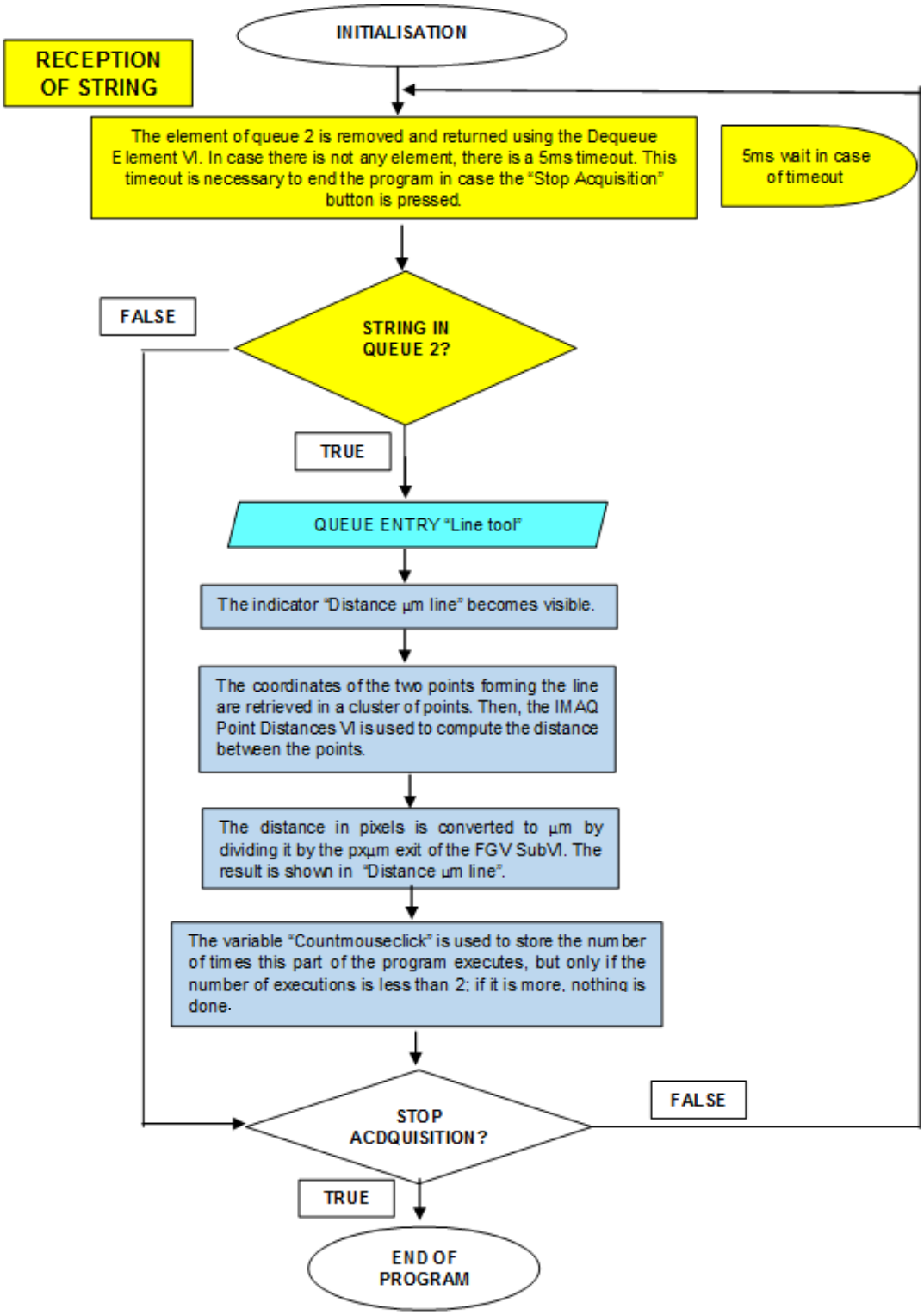


Figure 5.11- Save picture tool flowchart.

5.11. INSERT TEXT TOOL

It takes place in consumer 2 when event number 11 of **7.2. REAL-TIME IMAGE ACQUISITION** happens.

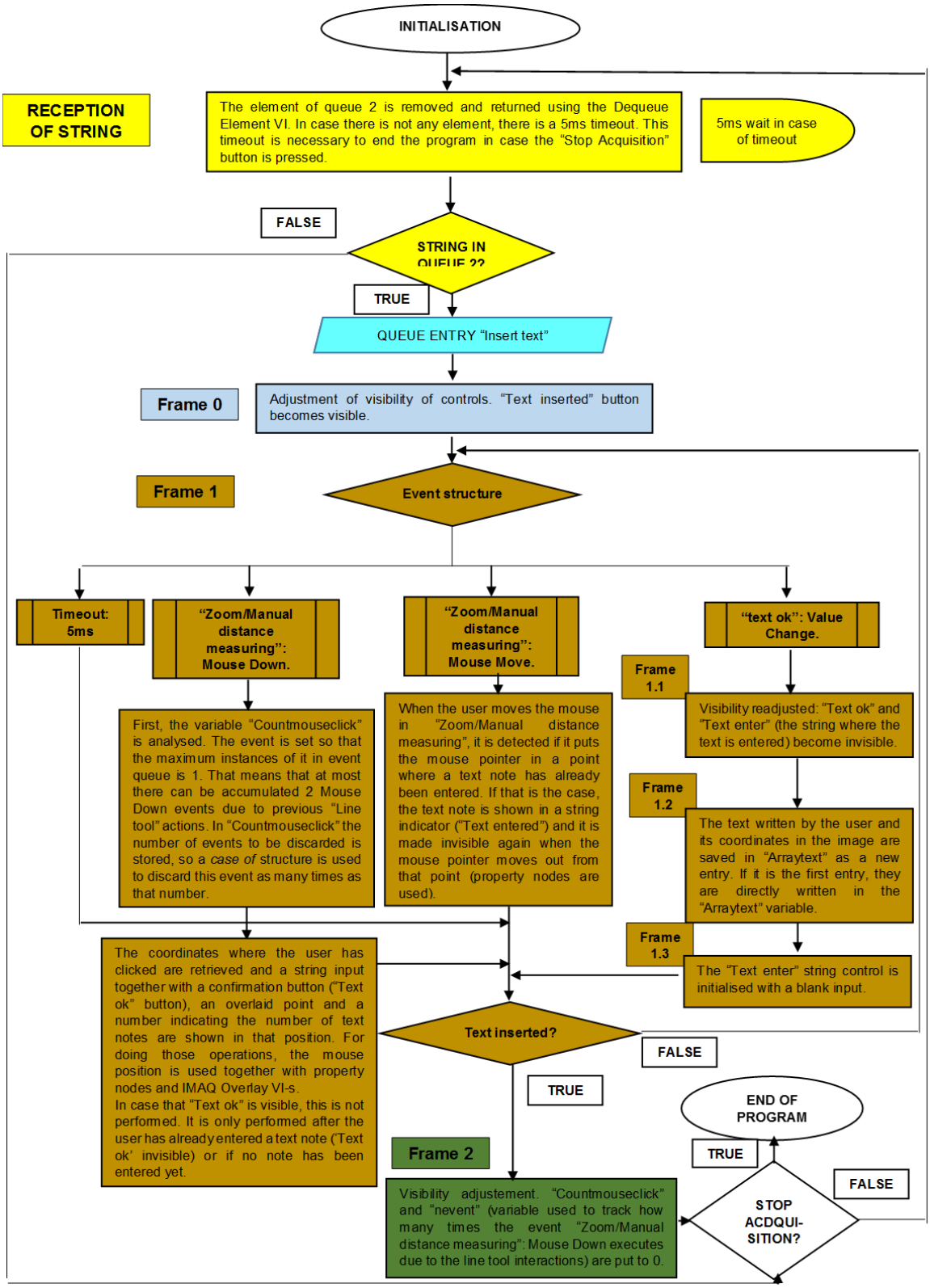


Figure 5.12- Insert text tool flowchart. The frames refer to the stacked and flat sequence structures used.

To keep track of the texts the user inputs and the positions in the image of them, a 1D array of clusters named "Arraytext" is used. The clusters have 4 elements:

- **Group (string):** number of text notes. The first text note is called *Text 1*, the second *Text 2*… and so on. This number is also used to display the overlaid number for each note in the image (see **Figure 5.13**).

- **X (unsigned word 16-bit integer):** X coordinate in the image representing the position where the text note has been entered.

- **Y (unsigned word 16-bit integer):** Y coordinate in the image representing the position where the text note has been entered.

- **Text (string):** Text information input by the user for each note.

Figure 5.13- Text tool in Front Panel.

5.12. SAVE ZOOM IMAGE TOOL

It takes place in consumer 2 when event number 12 of **7.2. REAL-TIME IMAGE ACQUISITION** happens.

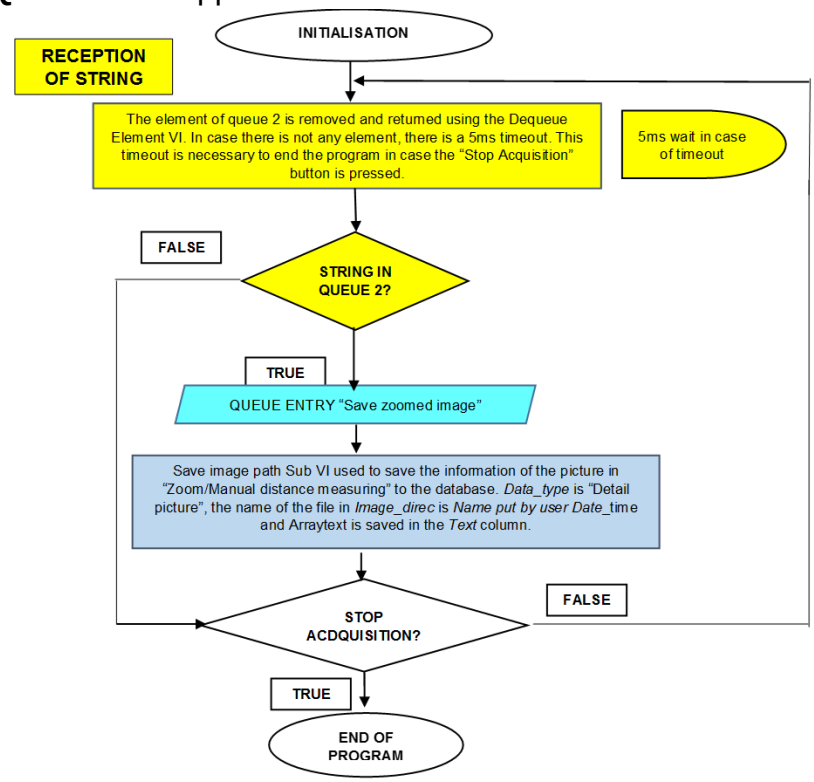


Figure 5.14- Save zoom image tool flowchart.

The text information contained by “Arraytext” is saved in the database “Text” column as follows: $Group_1;X_1;Y_1;Text_1\&Group_2;X_2;Y_2;Text_2\&Group_3;X_3;Y_3;Text_3$… and so on. The delimiters “;” and “&” allow to recover the information in “Arraytext” when an image is loaded in **5.6.LOAD PICTURE TOOL**.

	id	Microscope_name	Magnification	Resolution	Image_direc	Data_type	Text
Copiar Borrar	85	Zeiss Stemi 2000-C	50 X	1,5	C:\Users\fhn\Desktop\Asier\Videos\Video 1 13.07.20...	Video	
Copiar Borrar	86	Zeiss Stemi 2000-C	50 X	1,5	C:\Users\fhn\Desktop\Asier\Videos\kjubnkiuj 13.07....	Video	
Copiar Borrar	87	Dino microscope	47 X	0,3	Set up	Calibration	
Copiar Borrar	88	Dino microscope	47 X	0,3	C:\Users\fhn\Desktop\Asier\Pictures\Dino 17.07.201...	Overview picture	Nodetail;0;0;
Copiar Borrar	89	Dino microscope	47 X	0,3	C:\Users\fhn\Desktop\Asier\Pictures\Dino2 17.07.20...	Overview picture	Nodetail;0;0;
Copiar Borrar	90	Dino microscope	47 X	0,3	C:\Users\fhn\Desktop\Asier\Pictures\dsfasd 17.07.2...	Overview picture	Nodetail;0;0;
Copiar Borrar	91	Zeiss Stemi 2000-C	50 X	1,5	C:\Users\fhn\Desktop\Asier\Pictures\drfsvg 17.07.2...	Overview picture	Nodetail;0;0;
Copiar Borrar	92	Olympus microscope	100 X	3,1	Set up	Calibration	
Copiar Borrar	93	Zeiss Stemi 2000-C	50 X	1,5	C:\Users\fhn\Desktop\Asier\Pictures\Detail1 19.07....	Detail picture	Text1;606;108;Imperfection 1&Text2;423;250;Dot1

Figure 5.15- How the “Text” column is organised in the database.

5.13. RECORD VIDEO TOOL

It takes place in consumer 2 when event number 13 of **7.2. REAL-TIME IMAGE ACQUISITION** happens.

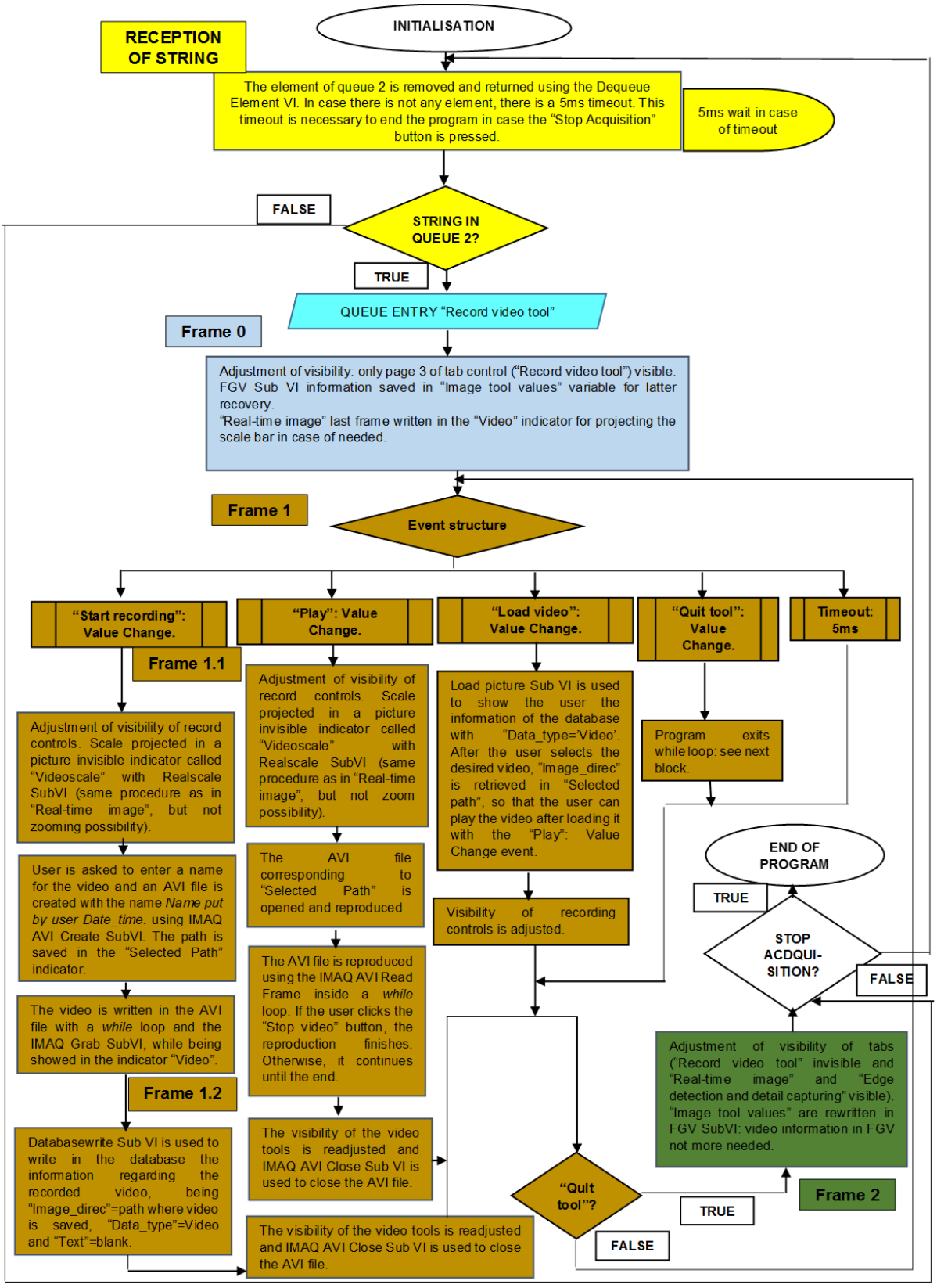


Figure 5.16- Record video tool flowchart.

6. FLUIDIC RESULTS

After the complete software was developed, it was necessary to verify that it fulfils the tests and documentation features required by the user. Three experiments related to microfluidic chips were performed for testing purposes (see **Figure 6.1** and **Table 6.1**). The objective of those experiments was to see how far the program satisfies the different microfluidic requirements introduced in **1. INTRODUCTION**.

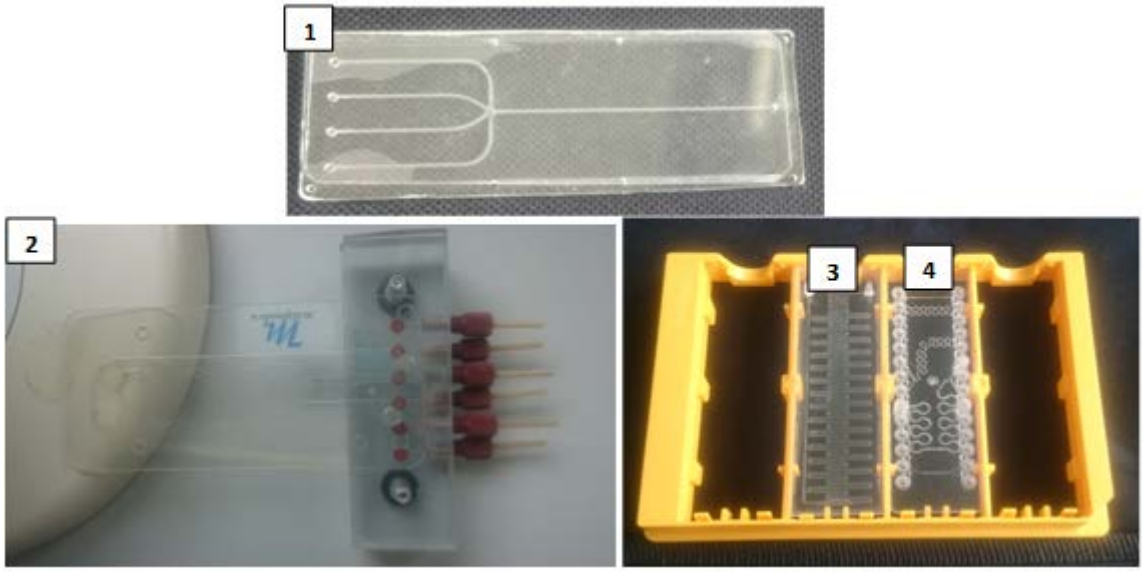


Figure 6.1- Microfluidic chips used for the experiments. They have already been presented in 3.2.1. GENERAL BACKGROUND INFORMATION AND DETAILED INFORMATION OF HARDWARE AVAILABLE IN THE LAB. Chip 3 was not used.

Experiment	Chip(s) used	Objective
1. Images of fluidic chips.	1, 2 and 4.	Documenting typical structures along with defects and how the software helps the user to properly document his/her observations.
2- Life images of fluids in microfluidic chip (mixer, hydrodynamic focusing).	4.	Seeing fluids flowing through the channels and their mixing in real-time. How the software is able to capture that in a video.
3- Video of droplet formation	-	Recording video with the evolution of droplets in the tip of a microfluidic tube.

Table 6.1-Performed fluidic experiments, chip(s) used and objectives.

6.1. IMAGES OF FLUIDIC CHIPS

Two of the requirements that the software should fulfil are the inspection and quality assurance (metrology and defect) and the in-depth analysis (image processing). The user has to be able to perform operations of high precision inspection, such as particle and defect diameter measurements, zoom-in and –out, include text/comments in images and make distance measurements with both machine-vision and a user-guided drawing tool. This experiment is intended to test those features. Four different tests were done.

The first test was done with chip number 1 and the Olympus microscope with a 50X magnification:

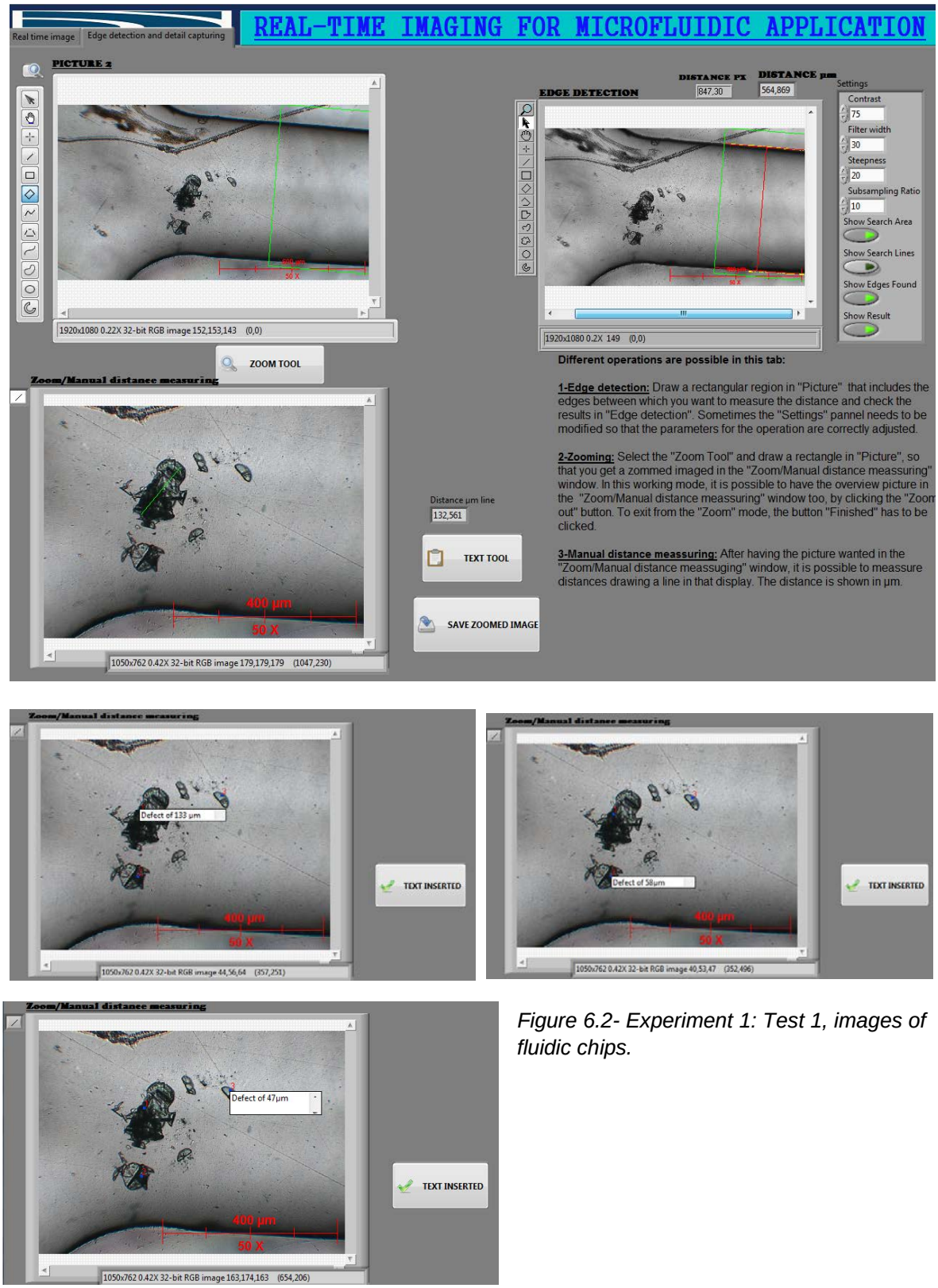


Figure 6.2- Experiment 1: Test 1, images of fluidic chips.

Figure 6.2 illustrates the software operations done in this first test. The image taken was in the region where the four channels of the microfluidic chip emerge in one unique channel:

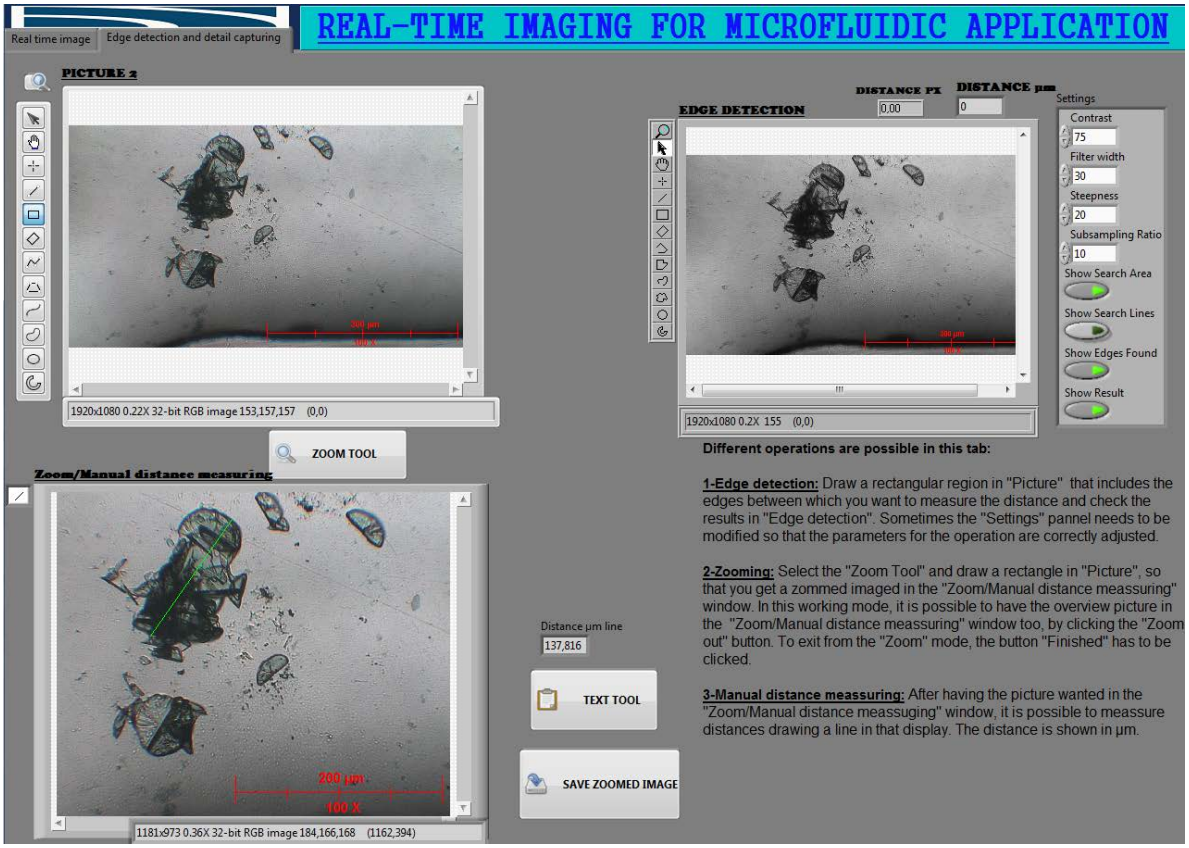
- The microfluidic chip's channel width was measured using "Edge detection". The result was 564,869µm.

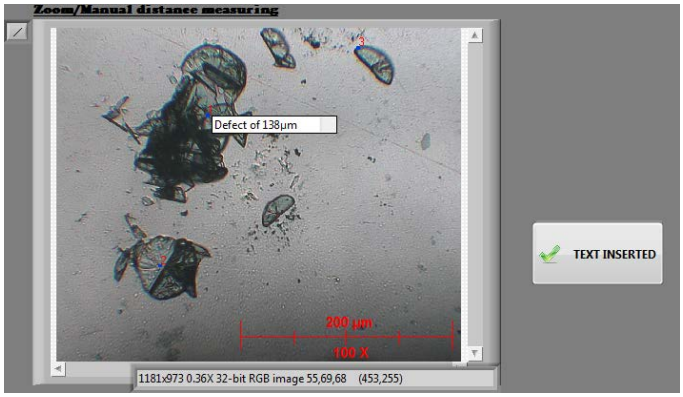
- The length of the biggest defect was measured manually with the line tool after zooming the overview picture. The result was 132,561µm.

- 3 text notes were inserted in the detail picture documenting the size of some defects: "Defect of 133µm", "Defect of 58µm" and "Defect of 47µm"

- Finally, the detailed picture was saved so that it could be retrieved later with the calibration information, scale bar and the text notes.

The second test was done with chip number 1 and the Olympus microscope too, but with a 100X magnification:





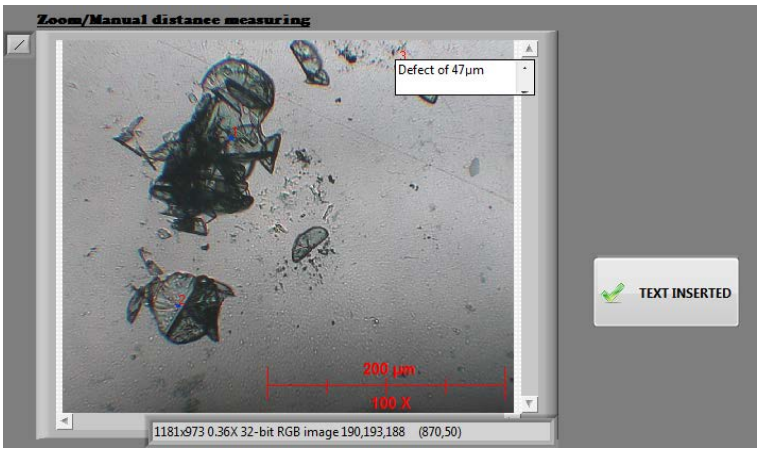


Figure 6.3: Experiment 1: Test 2, images of fluidic chips.

Figure 6.3 illustrates the software operations done in this second test. They were the same as in the first test: except from the chip's channel measurement, since it was impossible to see the whole channel with a 100X magnification:

- The length of the biggest defect was measured manually with the line tool after zooming the overview picture. The result was 137,816µm.

- 3 text notes were inserted in the detail picture documenting the size of some defects: "Defect of 138µm", "Defect of 58µm" and "Defect of 47µm"

- Finally, the detailed picture was saved so that it could be retrieved later with the calibration information, scale bar and the text notes.

The results are very similar to the ones obtained with the 50X magnification (biggest defect length with 50X: 132,561µm; biggest defect length with 100X: 137,816µm), but the small defects can be measured in higher precision with this magnification.

The third test was done with chip number 2. The Olympus microscope needed to be changed, since the dimensions in that chip cannot be correctly seen with magnifications equal or higher than 50X (that is what the three lenses available for that microscope in the lab were able to offer). The Zeiss Stemi 2000-C was used in its place, with a 6,5X magnification:

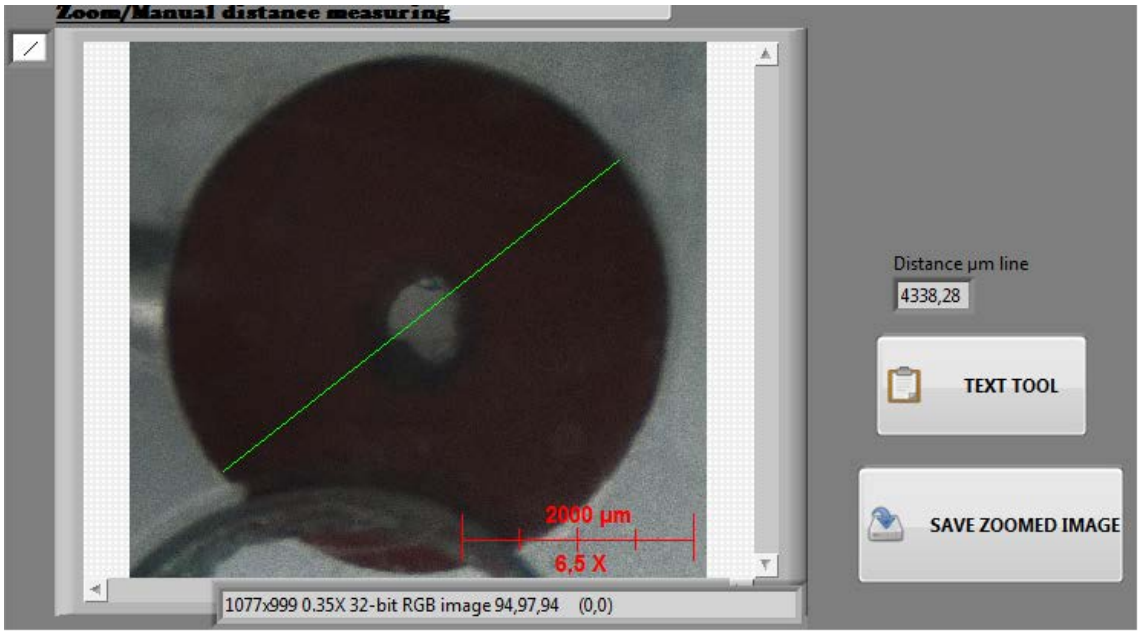


Figure 6.4- Experiment 1: Test 3, images of fluidic chips.

The out diameter of one of the channel entrance tube was measured in this test using the line tool. The result was of 4338,28µm, a much higher length value than the ones obtained in tests numbers 1 and 2, since the channel dimensions in this chip were much bigger.

The fourth and last test was done with chip number 4. The Olympus microscope was used again, with a 50X magnification.

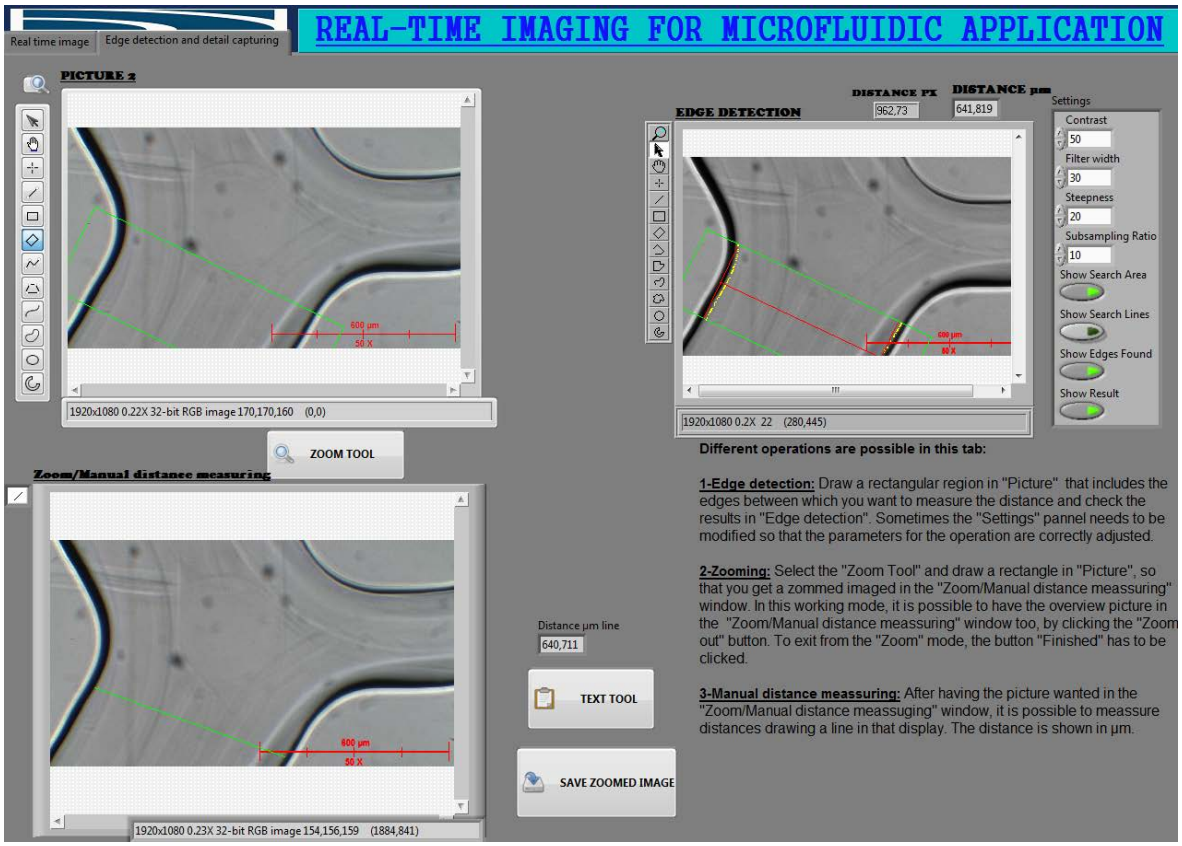


Figure 6.5- Experiment 1: Test 4, Images of fluidic chips.

Figure 6.5 illustrates the software operations done in this fourth test. The image taken was in the region where two channels of the microfluidic chip emerge in one unique channel. One of those channel's width was measured, both with machine vision or "Edge detection" and manually or with the line tool. As it can be seen in the image, the results are close (machine vision: 641,819µm; Manual: 640,711µm).

It can be also seen in the image that there is a shadow present in the channel borders due to the illumination, since the edges are not completely sharp. In these cases, the user may prefer to use the manual distance measuring feature instead of the machine vision one, because the machine vision feature do not work so well if there are shadowed areas that are not of interest. The ideal working environment that gives the best results for the machine vision "Edge detection" feature is to have two edges where the contrast in the edges is high and precise, without big shadowed areas.

If this channel width is compared to the result obtained for chip number 1 in the first text, on the other hand, it is possible to conclude that in this case the channel is about 80µm wider (in chip number one, the result was approximately 565µm).

6.2. LIFE IMAGES OF FLUIDS IN MICROFLUIDIC CHIP

Another requirement for the software was intelligent documentation of experiments (video and time-lapsed imaging). The user has to be able to record and play videos with arbitrary frame-rate or time span.

This experiment was done with chip number 4 and the Olympus microscope with a 50X magnification.

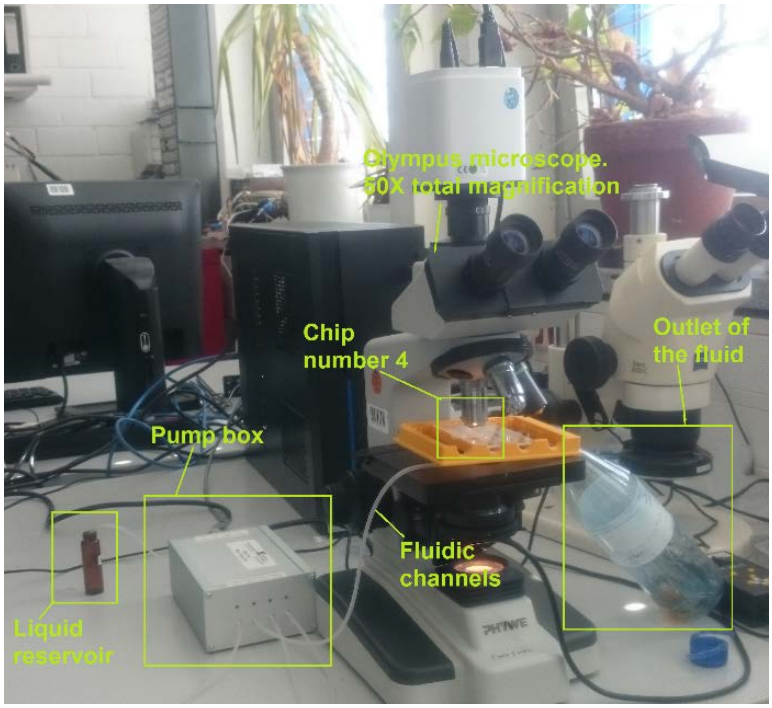


Figure 6.6- Set-up for experiment 2: Test 1.

Figure 6.7- Set-up for experiment 2: Test 2.

Figure 6.6 illustrates how the set-up was organized for performing the first test. It consisted in introducing some water inside a microfluidic channel with a pumping box and to see the changes in real-time due to this process. A video was recorded about the process and it was tested with the control "milliseconds between images" in "Play mode" that the time-lapse reproduction of the video was possible with the specified time in that control between images (**Figure 6.8** shows some photograms of the time-lapse video in chronologic order):

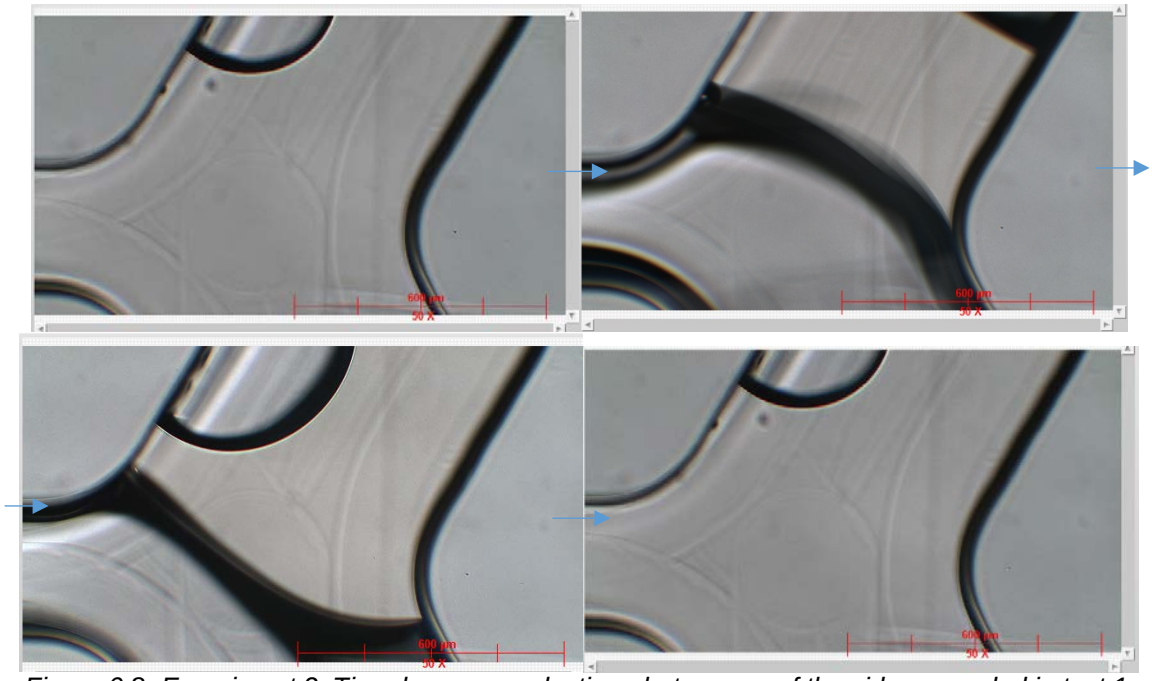


Figure 6.8- Experiment 2: Time-lapse reproduction photograms of the video recorded in test 1.

Figure 6.9 shows that when the pumping of the liquid stopped from the pump box, some droplets could be visualised inside the channel:

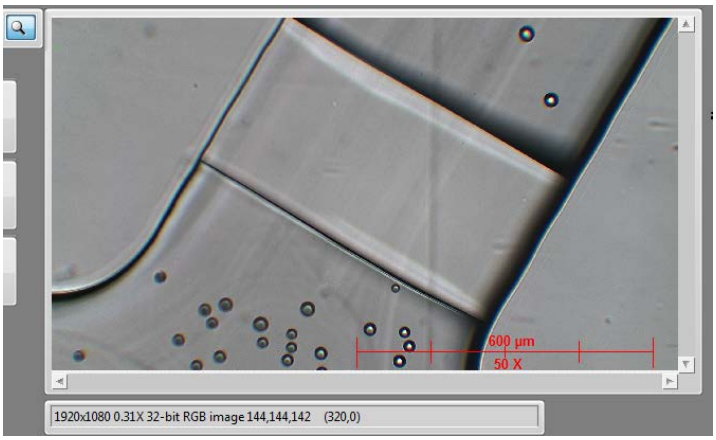


Figure 6.9- Experiment 2: Remaining droplets in the channel in test 1.

Figure 6.7 illustrates how the set-up was organized for performing the second test, which consisted in mixing in chip number 4 two liquids with different colours: red and blue. The coloured liquids are obtained diluting ink in water. Again, a video was record and a time-lapse reproduction was performed (**Figure 6.10**). It was observed that when the pump box's pumping amplitude of one channel was bigger than the other, the liquid inside the dominating channel took more space after mixing (**Figure 6.11** and **Figure 6.12**).

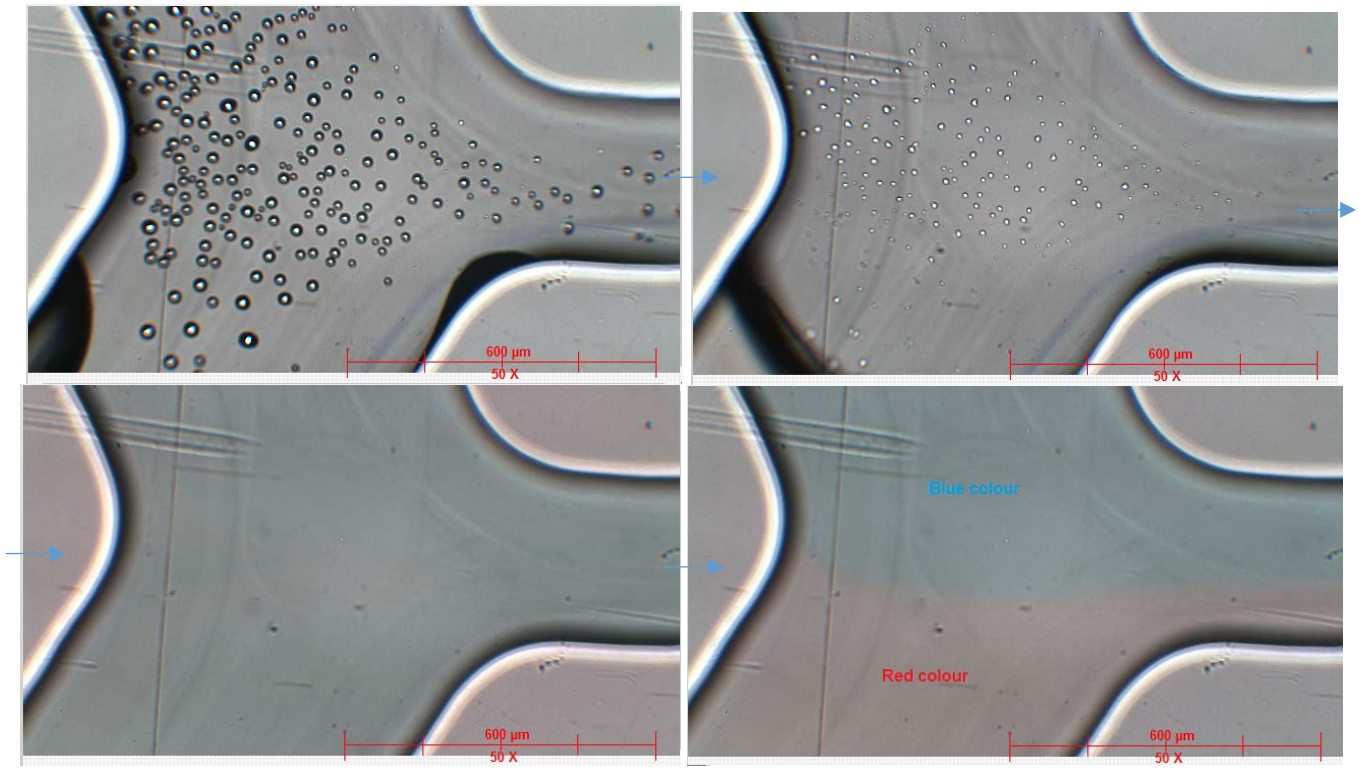


Figure 6.10- Experiment 2: Time-lapse reproduction photograms of the video recorded in test 2.

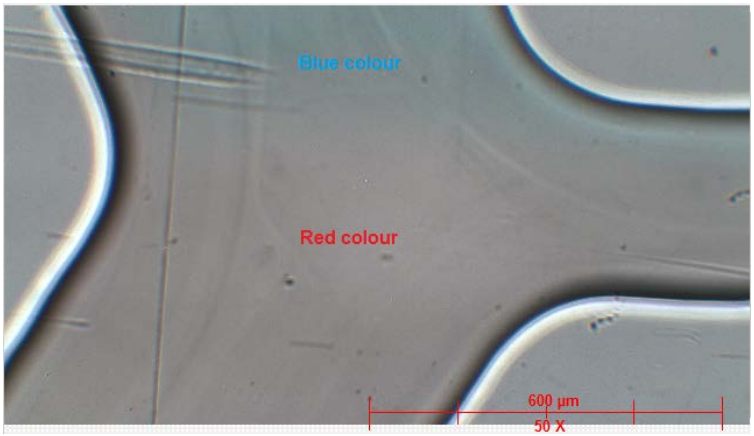


Figure 6.11- Experiment 2: Red colour dominating over blue colour in test 2.

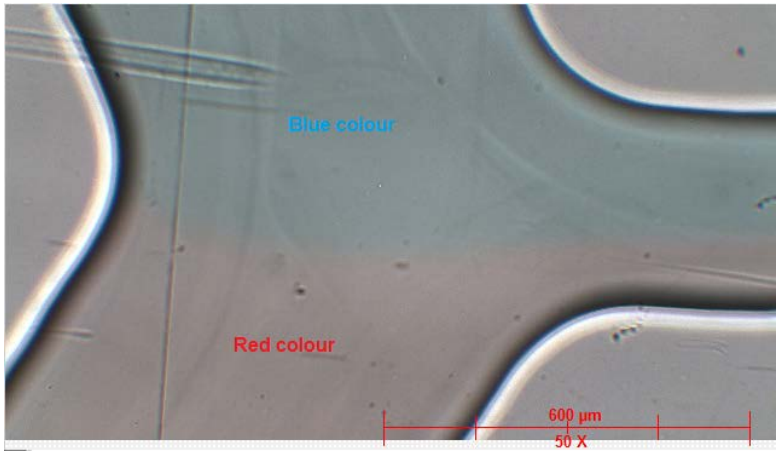


Figure 6.12- Experiment 2: Blue colour dominating over red colour in test 2.

6.3. VIDEO OF DROPLET FORMATION

In this last experiment, the requirement checked was once again the video feature. The Zeiss 2000-C microscope was used with a 6,5X magnification. The evolution of a droplet in the tip of a microfluidic tube was recorded and a time-lapse reproduction was made. One of the photos illustrating the time-lapse is **Figure 6.13**

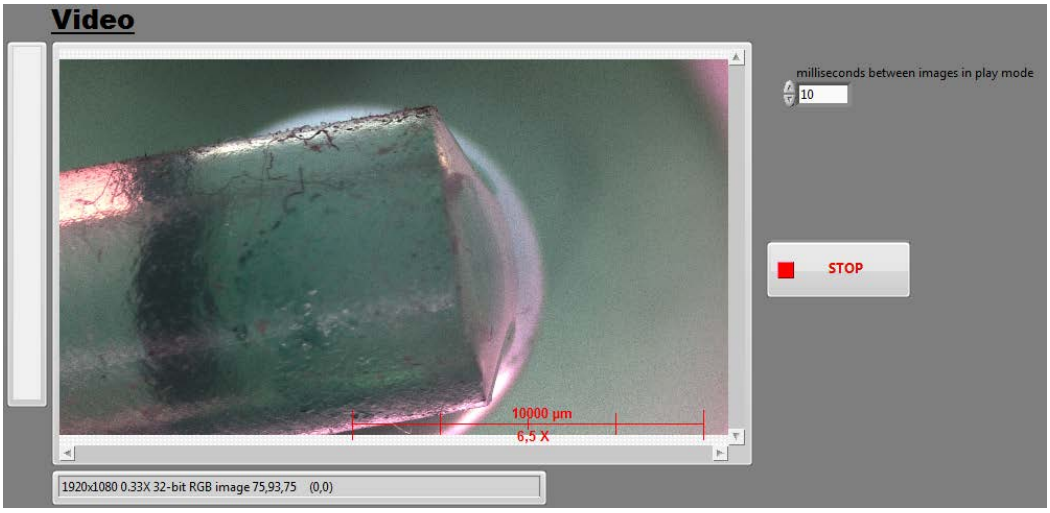


Figure 6.13- Experiment 3: Droplet evolution in the tip of a microfluidic tube.

With this experiment, the speed of the video recording tool was also probed. The real-time image acquisition feature gives as a result a higher frames/second ratio than the video feature. This is an aspect to improve in future lines. Nevertheless, apart from that, the droplet forming video was quite clear and representative: the video feature was able to record in a higher ratio than 20fps, fulfilling what is specified in **ABSTRACT**.

7. SUMMARY, CONCLUSIONS AND OUTLOOK

7.1. SUMMARY

The goal of this project was the conception and development of a real-time imaging software for microfluidic applications that could guide a user in setting up this imaging equipment and supported the research efforts. The list of individual tasks were hardware setups, software concept for in-situ chip inspection as well as image analysis, implementation of the software with LabVIEW and tests & documentation.

Although the developed software is implementable in any modular hardware or microscope, work in the thesis was performed with the help of an Olympus microscope and a Zeiss Stemi 2000-C Stereo Microscope. Several microfluidic chips and devices were borrowed for helping in the development of the software solution too.

The real-time imaging software solution developed tries to give answers to different requirements that users may have depending on the type of research. Those requirements are inspection quality assurance (metrology and defect), intelligent documentation of experiments (video and time-lapsed imaging) and in-depth analysis (image processing). Different tools and controls has been incorporated to the software for dealing with all those requirements. Moreover, the software has been structured accordingly to those requirements, offering a user-friendly GUI, making use of the producer-consumer software architecture and using VI-s such as the ones dealing with queues to coordinate all tools and controls.

Finally, it is remarkable that without the microfluidic and modular hardware equipment borrowed from Hoschschule Niederrhein, the development of this real-time imaging software would not have been possible.

7.2. CONCLUSIONS

The main conclusion is that the real-time imaging for microfluidic applications software has been successfully developed as a result of an independent study project. This can be concluded after verifying in the microfluidic experiments performed in **6. FLUIDIC RESULTS** that the software requirements specified in **1. INTRODUCTION** are fulfilled.

With the first experiment, it is proved that the inspection and quality assurance (metrology and defect) and the in-depth analysis (image processing) requirements are fulfilled, since manual and machine vision distance measurements, zoom-in and –out operations and text insertions were successfully made.

With the second experiment, it is proved that the software supports intelligent documentation of experiments, via video and time-lapse imaging. Experiments in microfluidic chips, such as mixing, can be satisfactory visualized, recorded and time-lapsed played.

With the third experiment, the video recording speed is tested, getting a satisfactory result of more than 20fps.

7.3. OUTLOOK: FUTURE LINES

As mentioned, the requirements of the software system have been achieved, and also some more functions have been developed in order to improve it even more. However, in spite of having developed a software system whose reliability is good, providing numerous tools and fulfilling the requirements scientists need for microfluidic documentation and testing, it would be required to further improve the software in order to achieve a better performance and to support more functions.

For example, it could be interesting to add a feature where droplets could be counted with machine vision. They are also left as future lines to make the software compatible with other analytical microscopy methods in addition to bright field microscopy, such as colorimetry and fluorescence microscopy, and to have an automatic set-up of the microscope (automatic focusing of the sample).

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9. ANNEX

This thesis is accompanied by the following appendices:

- **APPENDIX A: DINO-LITE DIGITAL MICROSCOPE. USER MANUAL**
- **APPENDIX B: STEMI 1000/2000/2000-C STEREO MICROSCOPES MANUAL.**
- **APPENDIX C: FURTHER DETAILS ABOUT THE LABVIEW SOFTWARE.**
- **APPENDIX D: C-MOUNT POSSIBLE SOLUTIONS.**
- **APPENDIX E: OPERATOR'S MANUAL.**

APPENDIX A

ideal-tek

Switzerland

Dino-Lite
digital microscope
User Manual

EN - User Manual

CONRAD ARTICLE CODE	MODEL	CONRAD ARTICLE CODE	MODEL
1284397	AD4113ZT	1284413	AM4116ZT
1284406	AM2111	1284414	AM4023X
1284407	AM3113T	1284415	AM4515ZT
1284408	AM4112PZT	1284416	AM4815ZT
1284409	AM4113T5	1284417	AM7013MZT
1284410	AM4113TL	1284418	AM7023
1284411	AM4113ZT	1284419	MS35B
1284412	AM4115ZT		

ENGLISH

Thank you for purchasing a Dino-Lite microscope. The DinoCapture software is designed to give you the best possible digital microscopy experience by the inventors of the handheld digital microscope.

The DinoCapture software runs on computers with a Windows XP, Windows Vista or Windows 7/8 operating system.

The DinoXcope software is designed to work with Apple Macintosh OS.

IMPORTANT SAFETY INFORMATION

- Avoid touching the lens with finger to protect the product from electrostatic damage
- Do not drop
- Keep dry

INDEX

SOFTWARE INSTALLATION

The DinoCapture and DinoXcope software is licensed from Anmo Electronics Corporation and is subject to an End User License Agreement (EULA) that users will have to accept during the installation process.

⚠ **Important notice: DO NOT connect the USB cable of the Dino-Lite to the PC before installing the software.**

1. Use the CD delivered with your Dino-Lite to install the DinoCapture and DinoXcope software and drivers. Alternatively, download and run the latest version of the software from the download section of the website: www.dino-lite.com.
2. Click 'Next' and the Installshield wizard will start. (An 'Open File-security warning' may appear on some systems. Select 'Run' or 'YES'). Choose the language you want for the DinoCapture 2.0 interface.
3. Read the User License Agreement. If you agree, press 'Yes' to continue or press 'No' to stop the installation.
4. Select a destination folder for the DinoCapture software. When done, press 'Next'. Click 'Install' to start installing the software. If the Windows security warning message appears, click 'Install this driver software anyway'.
5. When the installation is complete, selecting 'Finish' completes the software installation.
6. The DinoCapture software has an auto-update feature that will check for software updates when you start DinoCapture.
7. A full manual can be found in the help function of DinoCapture, as a pdf on the CD.

HARDWARE INSTALLATION

1. After full installation of the DinoCapture software and driver package, connect the Dino-Lite to one of the USB ports of your computer.

2. Please use a USB 2.0 port that is fully powered. Some USB ports on portable computers do not supply sufficient power.
3. The driver will be automatically installed. Please WAIT until the notification appears: 'Device driver software installed successfully'.
4. Now start DinoCapture 2.0 by double clicking on the desktop icon.
5. The LED lights should go on and an image should appear in DinoCapture.

HARDWARE FEATURES

1. At the center of the device, the adjustable dial is used to set the focus. The focus of the image depends on the distance to the object. Once you have focused on the object, you can read the magnification rate achieved from the number next to the △symbol.
2. The models in the AM/AD 411X series feature a magnification lock. The magnification lock is particularly useful for repetitive inspection at a given level of magnification.
3. Dino-Lite models with the letter T in their product name have a Microtouch function at the cable end of the device. Touching this sensor will capture the current image or start/stop video recording (for usb-devices). On the Dino-Lite high speed real time models the Microtouch sensor can be used to switch the LEDs on/off (all models) or freeze the image (AM5116 models only).
4. Models with the letter Z in their product name have a polarization function, that can be controlled by turning the adjustable cap.
5. Models with the letter L operate at a longer working distance. These models only achieve focus at some distance from the object.
6. The AD and Edge models have exchangeable front covers/caps. Align the red lines on cap and frontend of the Dino-Lite to remove or place the cap, then turn the cap 180 degrees. Some caps are designed to be clicked onto the microscope. In this case, there is no red line on the cap.
7. The DinoEye models are designed as a replacement of the existing ocular of a traditional microscope. The U model is intended to be placed over

an existing ocular and the C model is intended to be connected to a C-mount adapter on a suitable microscope or optical device.

8. Models with the letters TW have the macro zoom function.

SOFTWARE INTERFACE DINOCAPTURE / WINDOWS

There are five key sections in the DinoCapture software:

1. MENU BAR
2. IMAGE LIST TOOLS
3. PREVIEW WINDOW MANAGEMENT BAR
4. TOOL BAR
5. PREVIEW WINDOW TOOLS

MENU BAR

Folder – create folders or access existing folders.

Files – manage files, such as open, copy, save, print or delete as well as send as e-mail or create slide show.

Settings – customize the way the software operates such as:

- Showing or hiding elements on the screen
- The properties for measurements
- The way the Microtouch or foot pedal (if attached) work
- Accessing the Motorized focus accessory settings
- Connecting over IP
- Bar code recognition function
- External GPS
- Auto update function

Help – access the full user guide on screen, or read DinoCapture 2.0 information or the license agreement.

IMAGE LIST TOOLS

Open – open a picture selected from the image thumbnail gallery below the image list tool bar.

Copy – copy a selected picture to the windows clipboard.

Save as – save selected picture(s) to the desired file format or folder.

E-mail – attach the selected picture(s) to a new message opened in your default e-mail client.

Print – print the selected picture.

Slideshow – run a slideshow of the selected pictures.

Delete – clean up the image thumbnail gallery by deleting the selected picture(s).

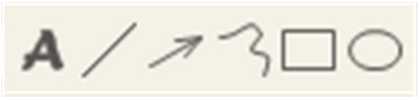
PREVIEW WINDOW MANAGEMENT BAR

This window enables you to switch between windows if two or more Dino-Lite's are connected at the same time or multiple pictures are opened.

TOOL BAR

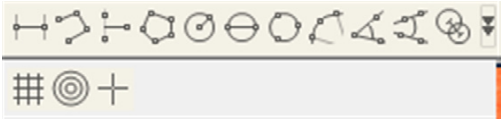
The tool bar is divided into four parts:

Draw tools – The draw tool set allows you to write text and draw on the images. The bar is located below the preview window management bar.



Measurement tools – Many different measurement types can be done with the measurement tools. The double flash icon gives you access to the grid and ruler settings.

Notice: The measurement and calibration feature is only available for certain models. For the DinoEye models, it is recommended to make calibrated measurements only.



LINE	Left click and drag to the desired length, and click again to finish.
CONTINUOUS LINE	Click and drag to form one section of distance, click again to start another section. Continue until the total desired distance is measured. Double click to finish.
POINT TO LINE	Create a line to represent the base by clicking once to start, drag, and then click again to set the end-point. Branch off from the base line to start measuring the line that is 90 degrees from the base line and a final click to finish the measurement.
POLYGON	Click and drag to form the desired length, and click again to start the next section. When finished, double click to finish the polygon measurement.
RADIUS CIRCLE	Click and extend out to the desired radius.
DIAMETER	Click and extend out to the desired dia-meter.
THREE POINT CIRCLE	Click on any three points on the circle you wish to measure.
THREE POINT ARC	Click on three consecutive points on an arc to measure.

THREE POINT ANGLE	Start at the pivot point and extend out to start measuring an angle.
FOUR POINTS ANGLE	Select two points from one line segment and another two points from another line segment to complete the angle measurement.
CENTER DISTANCE	After drawing at least two circles, select the icon and the mouse pointer will change to a pointing finger for selectable circles. Click on each circle and the software will measure the distance.
GRIDLINES	The pitch will match the magnification inputted.
CIRCLE GRID	The pitch will match the magnification inputted.
CROSSHAIR ON CAMERA	The crosshair XY position can be moved when you select it. The cursor position is the location of the mouse pointer and location 0,0 is the center coordinates of the crosshair.
SCALE CROSSHAIR	The scale crosshair increments can be compared to the scale on the bottom left corner. The centre of the crosshair can be moved. (Magnification value needs to be entered for this feature to work).

Text and line tools

LINE FORMAT	Select line color, style and width
FONT	Select font, font style, size and color

Measurement options

MEASUREMENT PROPERTIES	Organize and show the status of all the measurements as well as the ability to adjust how the results are shown on the image.
MAGNIFIER	Digitally enlarge the area around your mouse pointer for more details and accurate measurements.
MAGNIFICATION	Input the magnification in the blue box. The magnification can be read from the dial on the microscope.
UNITS	Select the unit of measurement, inch, mil, m or um.
CALIBRATION MENU	Select calibration, create new calibration profile or open the calibration folder.
BARCODE READER	Click on the icon to enable and disable barcode detection.

PREVIEW WINDOW TOOLS

Microtouch - This is activated when you touch the Microtouch sensor. Activate or deactivate the Microtouch. Some Dino-Lite models do not have this feature.

Auto Exposure – This allows you to change the exposure or turn off auto exposure. Sliding the bar to the right increases the exposure time and vice-versa.

White Balance - Select the required White Balance Mode (available on certain models only)

LED control - Lets you turn ON/OFF or switch LED's on the Dino-Lite (most models).

Setting - The setting window allows you to control the camera settings.

Maximize - Lets you see the live video or picture in full screen. To exit full screen mode, click anywhere on the screen or press "ESC".

Close window - Allows you to close the current window

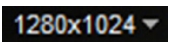
Snapshot - Take a picture.

Video recorder - Start/stop recording of a video

Time-Lapsed Video - Create a series of images or videos taken at a regular time interval.

EDR - Save a picture using Extended Dynamic Range (EDR)

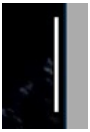
EDOF - Save a picture using EDOF (Extended Depth of Field)



Choose the resolution for the image. Please note that an additional Codec may be required to display 5 megapixel resolution.

Choose the folder where images/videos will be stored

Click on the grey bar on the bottom of the preview window and the annotation box will expand. Write text within this box and press ENTER to start a new line. When done, left click anywhere in the preview window to save the annotation and exit the annotation box.

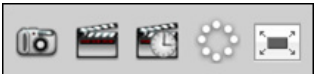


Focus strength indicator - Indicates the level of focus achieved (available on certain models only)

SOFTWARE INTERFACE DINOXCOPE / MAC

There are four main sections in the DinoXcope program window:

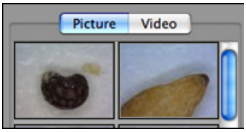
1. Action bar



Make picture. If you want to make measurements on the picture later, open the picture by double clicking and add the input magnification that you read from the focus dial.

- Make video. Choose frame rate, recording time, quality and compression. To stop recording manually, use the ESC key
- Make time lapse video. Choose capture interval, number of frames and playback frame rate, as well as quality and compression.
- Switch LED's on/off
- Go to full screen mode, to end full screen mode use the ESC key

2. Image list

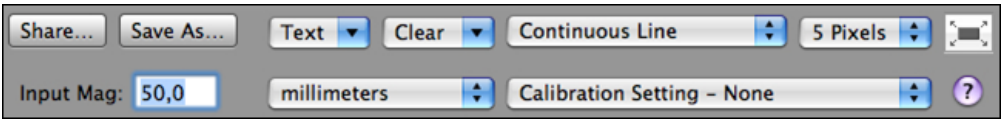


- Choose between pictures or videos
- Look at an image with mouse-over, or double-click to open in new window

3. Live image

- Use the action bar for the action that you want to perform
- Use the Controls menu (the menu bar is on top of the screen) to change settings, use (digital) zoom, freeze the image, change the resolution or change the functioning of the Microtouch button.
- Use the Live measurement function (in the Tools menu) to open a special selection window for drawing and measurement tools. After selection, click okay and use the tool on the live image.

4. Open image



- If you open a stored image, you will see a menu bar above it, that allows you to choose saving, drawing and measurement tools.

CLEANING AND MAINTENANCE

Avoid touching the lens with any substance. Clean the lens periodically with compressed air. Clean the body of the microscope regularly with a soft cloth dampened with disinfectant. Clean the detachable caps with a 50%-70% alcohol solution, do not use diethyl ether, 100% alcohol or alcohol gel.

WARRANTY

The DinoCapture software is provided for use with a Dino-Lite digital microscope supplied by AnMo Electronics or one of its distributors or resellers. The use of the DinoCapture software is guided by the End User License Agreement. The Dino-Lite product is delivered with a two year warranty from the date of purchase by the end customer. Please note that accessories (i.e. detachable caps/extensions) are not covered under warranty. For warranty issues please contact the reseller or store where you bought the product.

SUPPORT

If you have any problem or issue with your Dino-Lite or the DinoCapture software, please contact your reseller. An extensive user manual for DinoCapture can be found within the software.

APPENDIX B

Stemi 1000/2000/2000-C
Stereo Microscopes

Operating Manual

Fisher Bioblock Scientific
Parc d'innovation - BP 50111 - F67403 illkirch cedex

France
tél 03 88 67 14 14
fax 03 88 67 11 68
email infos@bioblock.fr
www.bioblock.com

Belgique / België
tél 056 260 260
fax 056 260 270
email belgium@bioblock.com
www.bioblock.be

España
tfno 91 515 92 34
fax 91 515 92 35
email ventas@bioblock.com
www.es.fishersci.com

Knowledge of this manual is required for the operation of the instrument. Would you therefore please make yourself familiar with the contents of this manual and pay special attention to hints concerning the safe operation of the instrument.

The specifications are subject to change; the manual is not covered by an update service.

Issued by:

Carl Zeiss
Mikroskopie
D-07740 Jena
Telephone (**49) 03641 / 64-1616
Telefax: (**49) 03641 / 64-3144
Internet: mikro@zeiss.de
http://www.zeiss.de

Number of this manual: B 40-002 e

Date of issue: 12/98

TABLE OF CONTENTS

4 CARE AND TROUBLESHOOTING

Page

ANNEX

LIST OF FIGURES

Page

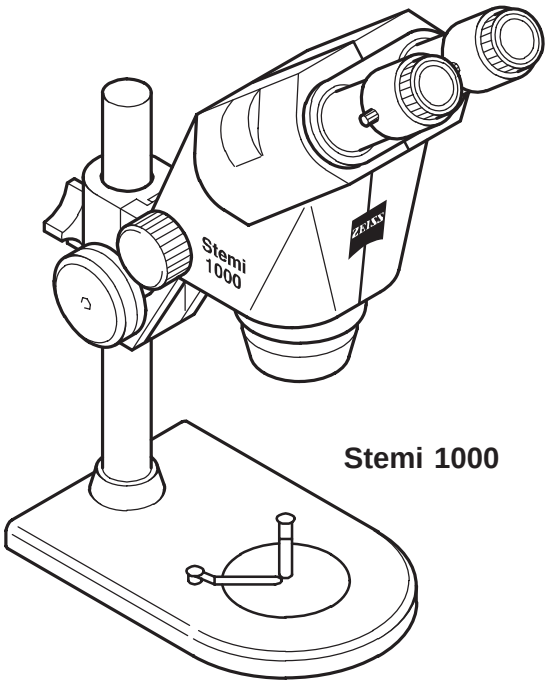
NOTE

- The figures integrated in the text each have a figure number and a caption, e.g. ”Fig. 2-8” signifies: the figure in Section 2 with the serial number 8. In each figure, details discussed in the text are assigned a reference line marking and an item number. In the running text, ”T2 adapter (2-8/3)”: in Fig. 8 of Section 2, the T2 adapter is marked with the item number 3.

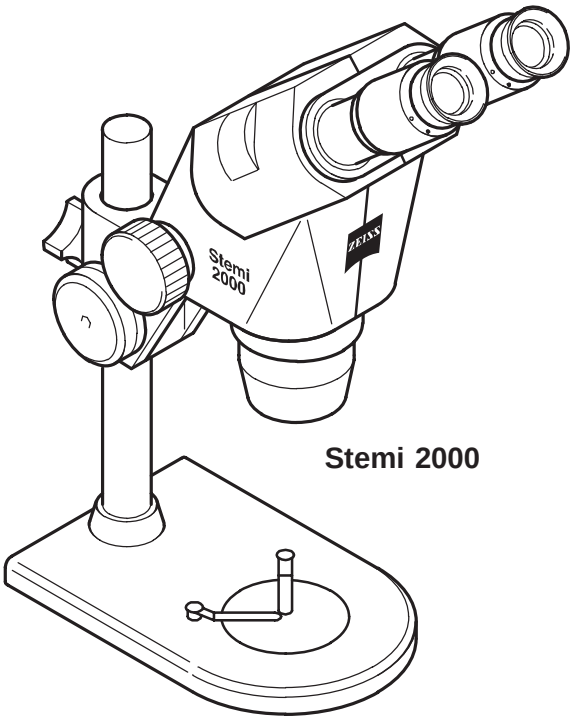
- Refer to the annex for explanations of the abbreviations.

- This instruction manual refers to the Stemi 1000, Stemi 2000 or the Stemi 2000-C microscope versions (see Page 1-9) and their options.
  Apply this manual analogously when working with other variants of the unit.

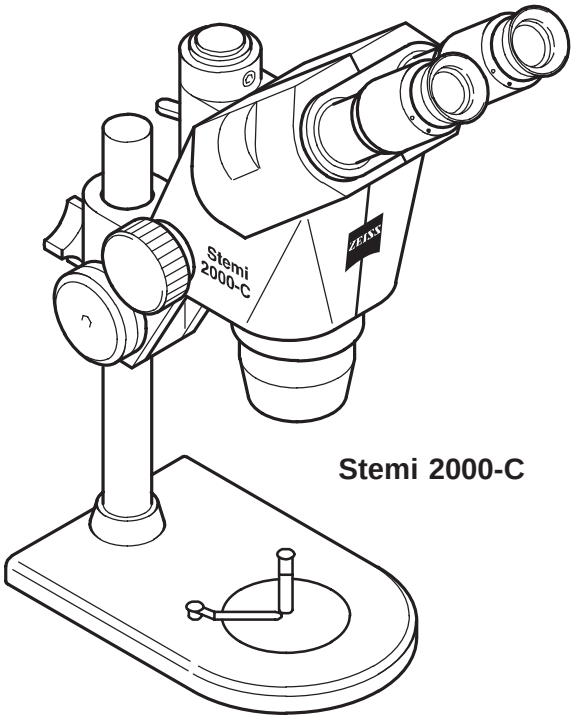
GENERAL VIEW
(Basic unit)



Stemi 1000



Stemi 2000



Stemi 2000-C

NEW MODEL

We have included a further Stemi model in our line of Greenough-type stereo microscopes.

In response to many requests received from customers, the

Stemi 2000-CS 45 50 55

is now also available as a brand-new item in addition to the Greenough-type stereo microscopes Stemi 1000, Stemi 2000 and Stemi 2000-C.

Unlike the Stemi 2000-C, the Stemi 2000-CS (”C” for ”Camera” and ”S” for ”Splitter”) features a

fixed camera port

with a 50/50 % beam splitting ratio, i. e. the camera port has no switching lever.

Unlike the Stemi 2000-C, the Stemi 2000-CS therefore provides the benefit of

simultaneous stereoscopic viewing

while taking photographs or working in the monitor mode.

All configurations or accessory components are identical to those of the other Stemi’s.

NOTES OF DEVICE SAFETY

The Stemi 1000, Stemi 2000 or the Stemi 2000-C stereo microscope including original accessories or options must only be used for the microscopy applications described in this operating manual.

The manufacturer cannot assume any liability for any other applications, possibly also involving individual modules or single parts. This also applies to all service or repair work that is not carried out by authorised service personnel. All guarantee/warranty claims also expire for all those parts that were not directly affected by repair.

Particular attention must be paid to the following warning notes:

- ☞ The Stemi was designed and tested in conformity with the IEC Publication 1010-1 ”Safety requirements for electrical measuring devices, control equipment and laboratory apparatus” and was delivered in a safe state. This operating manual contains information and warnings which must be followed by the operator.

- ☞ The Stemi is a light microscope conceived in accordance with the latest scientific and technical knowledge for visual, microphotographic and video analysis of microscopic specimens. The unit must only be used for the intended purpose. It is not intended for continuous unsupervised operation!

- ☞ The Stemi has no special facilities to protect against samples that have caustic, toxic, radioactive or other effects that are damaging to health.

- ☞ Users must check whether the available power supply agrees with the value specified on the rating plate of the respective power supply unit.

- ☞ The power supply unit belonging to the Stemi is a protective class II unit.

- ☞ Internal parts can carry hazardous voltages when a power supply unit is connected to the mains. The power supply unit must therefore be disconnected from the mains before opening it.

- ☞ Make sure that only those fuses are used as spares for the respective power supply unit that are actually intended for the prescribed rated current and the specified version. It is forbidden to use provisional fuses or to short-circuit the fuse holders.

☞ Modifications to the unit to keep in line with technical progress are always reserved.

1 Description

1.1 Designation, purpose

Manufacturer's designation: stereo microscope Stemi 1000
stereo microscope Stemi 2000
stereo microscope Stemi 2000-C
stereo microscope Stemi 2000-CS

Brief designation: Stemi 1000
Stemi 2000
Stemi 2000-C
Stemi 2000-CS

Within the product family of stereo microscopes from Carl Zeiss, these units fit in as follows:

- Greenough type: Stemi D
  Stemi DRC

  Stemi 1000
  Stemi 2000
  Stemi 2000-C
  Stemi 2000-CS

- Telescopic type: Stemi SV 6
  Stemi SV 11
  Stemi SV 11 Apo

Stemi 1000 and Stemi 2000 are universally applicable Greenough type stereo microscopes that unite a high imaging quality, compact ergonomic design and internationally standardised connection and coupling points.

While the Stemi 1000 and Stemi 2000 are units for visual stereoscopic observation, with its trinocular tube the Stemi 2000-C offers all possibilities of micrographic and video image documentation.

Unlike the Stemi 2000-C, the Stemi 2000-CS features a fixed camera port with a 50/50 % beam splitting ratio, i. e. the camera port has no switching lever. Unlike the Stemi 2000-C, the Stemi 2000-CS therefore provides the benefit of simultaneous stereoscopic viewing while taking photographs or working in the monitor mode.

Both stereo microscopes' areas of use cover the following focal areas, among other things:

- Materials technology and sciences
- Biotechnology and medical technology
- Semiconductors industry and research
- Hobby and leisure

IMPORTANT The units may only be used for the work and applications described in this manual. Refer also to the product liability declaration (Page VIII).

1.2 Description of the units

The Stemi 1000, Stemi 2000 and Stemi 2000-C are highly powerful stereo microscopes that are distinguished by compact and ergonomic design.

Figure 1-1 shows the principal assemblies of the Stemi 2000-C.

- Stand S with 260 mm column (1-1/4)
- Stemi mount with drive for column 32 (1-1/6)
- Stemi 2000-C microscope body (1-1/2)
- Eyepieces W-PL 10x/23 Spec. foc. (1-1/1) with eye cup
- Built-in 6 V, 10 W halogen vertical illumination (1-1/5) with 115/230 V – 6 V 50 VA power supply (1-1/3)

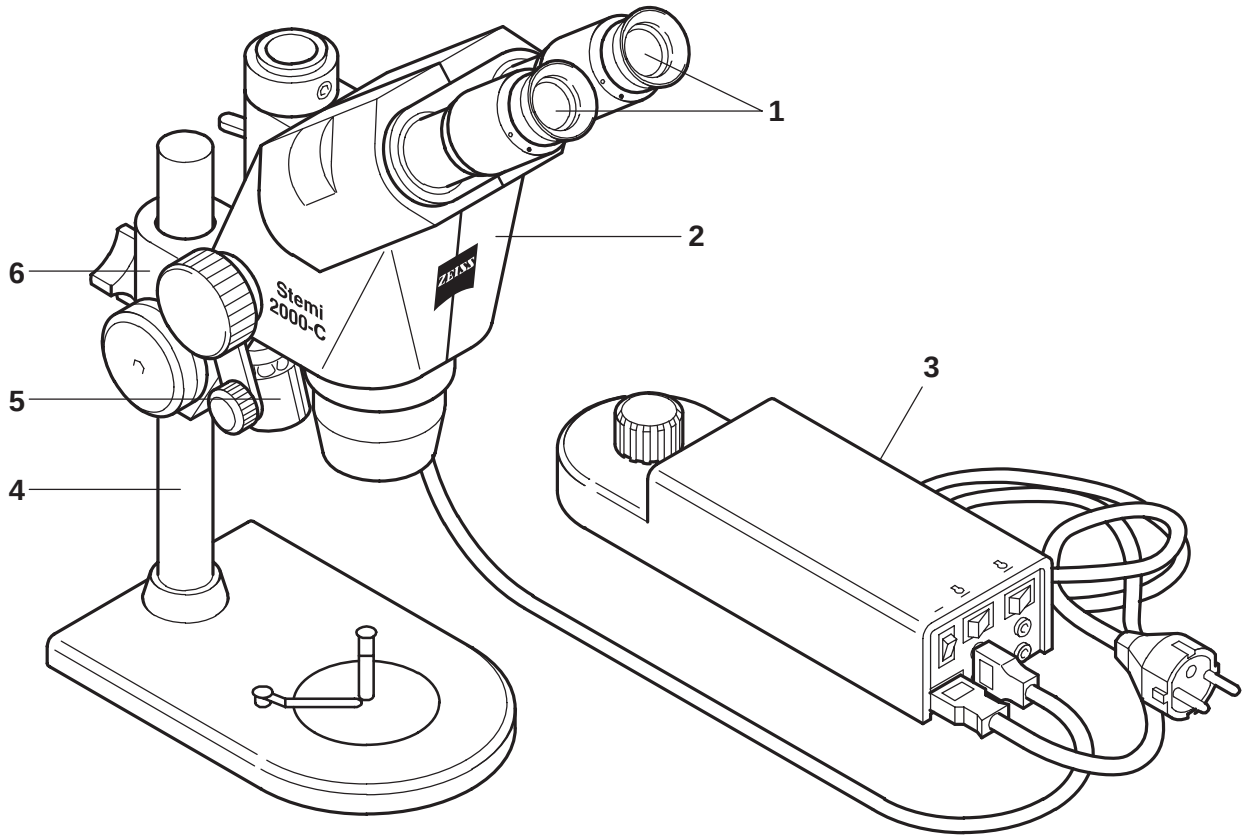


Figure 1-1 Principal assemblies of the Stemi 2000-C

Figure 1-2 schematically shows the optical beam path in the Stemi 2000-C.
The unit is switched over between the right tube connector and the camera output with the aid of an adjustable reflector group located under the tube system that features 100/0 % and 0/100 % intensities.

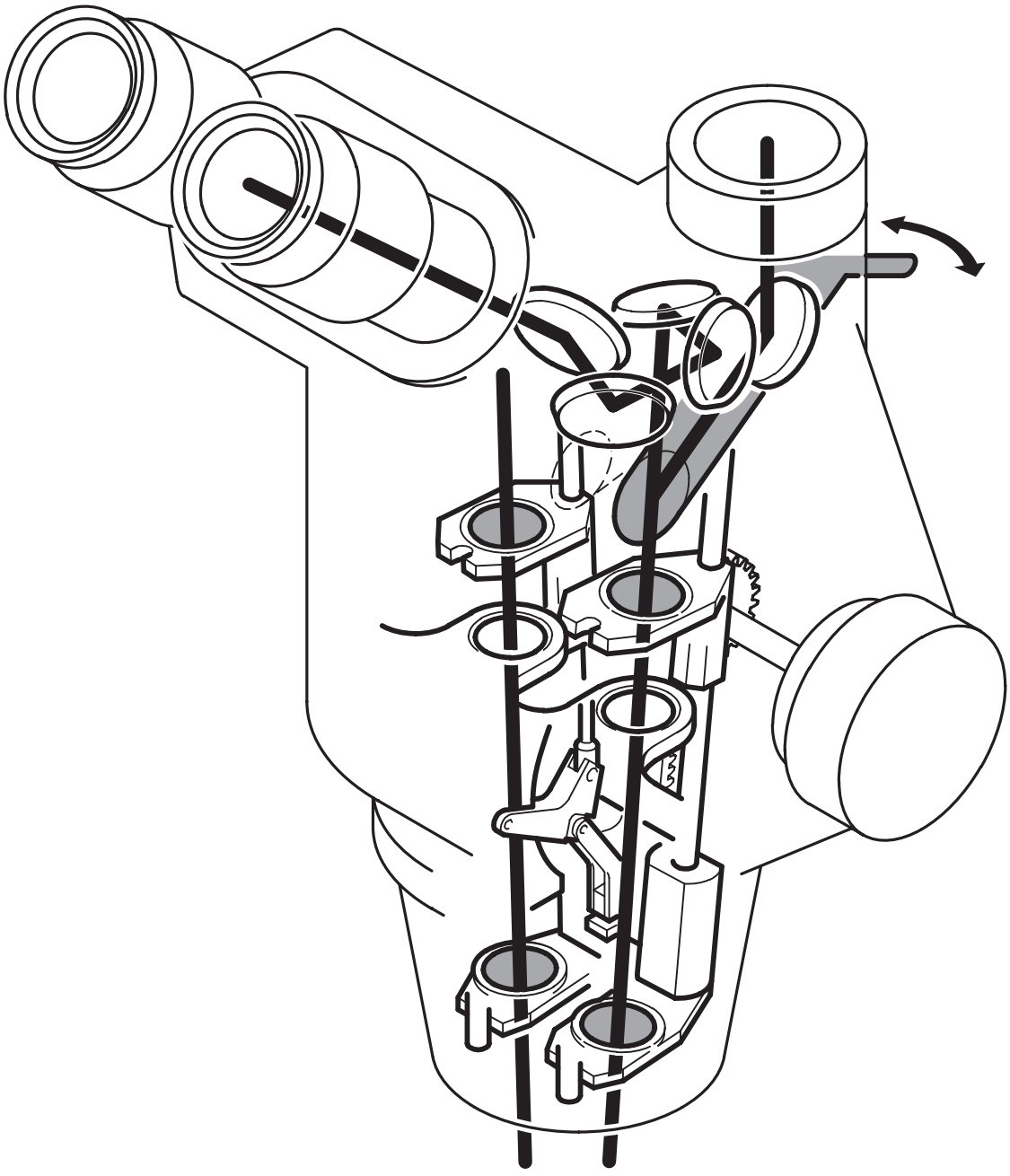


Figure 1-2 Optical schematic of the Stemi 2000-C

1.3 Technical data

(1) Dimensions and weight

Mikroscope

- Stemi 1000

  Dimensions (width x depth x height) . . . . . 65 x 336 x 371 mm
  Weight (with power supply, mains cable and lamp) . . . . . 4.33 kg

- Stemi 2000

  Dimensions (width x depth x height) . . . . . 65 x 336 x 371 mm
  Weight (with power supply, mains cable and lamp) . . . . . 4.53 kg

- Stemi 2000-C

  Dimensions (width x depth x height) . . . . . 65 x 336 x 371 mm
  Weight (with power supply, mains cable and lamp) . . . . . 4.8 kg

- Stand S

  Support area (width x depth) . . . . . 180 x 270 mm
  Weight . . . . . 1.3 kg

- Stand S and power supply unit 230 V - 6 V 50 VA

  Support area (width x depth) . . . . . 263 x 270 mm
  Weight . . . . . 1.96 kg

City Box

Dimensions (closed) (width x depth x height) . . . . . 560 x 450 x 240 mm
Weight (unequipped) . . . . . 4.75 kg

(2) Ambient conditions

Storage and transport (in packaging)

Permissible ambient temperature . . . . . -40 to +70 °C
Permissible relative humidity . . . . . ≤ 100 %
Permissible atmospheric pressure . . . . . 500 to 1060 mbar
Permissible sinusoidal oscillations . . . . . 10 to 150 Hz; 0.5 g
Permissible impact (handling impact) . . . . . 30 g; 6 ms

Operation

Permissible ambient temperature . . . . . +15 to +35 °C

Permissible relative humidity . . . . . ≤ 85 %

Permissible atmospheric pressure . . . . . 800 to 1060 mbar

Permissible impact (handling impact without unit packaging) . . . . . 10 g; 6 ms; 100 impacts in operating position

(3) Operating data

230 V – 6 V 50 VA power supply (Order No. 458420)
120 V – 6 V 50 VA power supply (Order No. 458421)

Mains voltage . . . . . 120 V or 230 V

Permissible mains voltage fluctuation . . . . . +6/-10 %

Mains frequency . . . . . 50/60 Hz

Power consumption . . . . . max. 50 VA

Light source . . . . . halogen 6 V 10 W

Variation range . . . . . 2.4 ... 5.8 V continuously

Class of protection II

Electrical degree of protection . . . . . IP 20

Electric safety . . . . . in acc. IEC 1010-1

RFI suppression . . . . . in acc. EN 55011 Class B

Power supply unit, stabilised 6 V 20 W, 115 V – 230 V, variable 1.5 ... 6 V, 50 ... 60 Hz, 40 VA (Order No. 458415)

Mains voltage . . . . . 100 V, 115 V, 220 V, 240 V, switchable

Permissible mains voltage fluctuation . . . . . ±10 %

Mains frequency . . . . . 50 ... 60 Hz

Power consumption . . . . . 40 VA

Light source . . . . . halogen 6 V 20 W

Variation range . . . . . 1.5 ... 6.0 V continuously

Class of protection I

Electrical degree of protection . . . . . IP 40

Electric safety . . . . . in acc. IEC 1010-1

RFI suppression . . . . . in acc. EN 55011 Class B

Specimen stages, adjustment ranges

Sliding stage ..... ±20 mm, rotatable by 360°
Spherical stage ..... ±30° horizontal tilt angle, rotatable by 360°
Rotary stage ..... rotatable by 360°
Specimen guide D ..... 28 x 75 mm
Expandable stage with specimen guide and holding frame ..... 76 x 50 mm

Stage inlay plates

Outer diameter ..... 84 mm
Outer diameter (rotary stage) ..... 72 mm

Stemi mount with drive for column 32

Focusing by means of focusing drive ..... 46 mm
Mount for microscope body ..... ∅ 76 mm

(4) Optical and mechanical data

Stemi 1000 microscope body

Effective principle ..... according to GREENOUGH with optical compensation
Stereo angle ..... 11°
Zoom range of the pancratic lens ..... 0.7x ... 3.5x
Free working distance (without dust glass) ..... 112 mm
Viewing angle ..... 35°
Adjustable eye distance ..... 55 to 75 mm
Mount diameter ..... 76 mm

Stemi 2000/2000-C microscope body

Effective principle ..... according to GREENOUGH with mechanical compensation
Stereo angle ..... 11°
Zoom range of the pancratic lens ..... 0.65x ... 5.0x
Free working distance (without dust glass) ..... 92 mm
Viewing angle ..... 35°

Adjustable eye distance . . . . . 55 to 75 mm
Mount diameter . . . . . 76 mm

Replaceable optical systems

Eyepieces . . . . . W-PL 10x/23 Br. spect. foc.
W 10x/21 foc.
W-PL 16x/16 Br. spect. foc.
W 25x/10 foc.

Attachment systems, fixed . . . . . Γ' = 0.3x FOD = 285 mm
Γ' = 0.4x FOD = 210 mm
Γ' = 0.63x FOD = 130 mm
Γ' = 2.0x FOD = 31 mm

Stemi 1000 visual viewing magnification ranges

Basic microscope equipment . . . . . 7x ... 35x
with replaceable optical system . . . . . 2,1x ... 175x

Stemi 2000/2000-C visual viewing magnification ranges

Basic microscope equipment . . . . . 6.5x ... 50x
with replaceable optical system . . . . . 1.95x ... 250x

VSS		Okular							
		W 10x/21 foc.		W-PL 10x/23 spect. foc.		W-PL 16x/16 spect. foc.		W 25x/10 foc.	
		Total magnification	Specimen field (mm)	Total magnification	Specimen field (mm)	Total magnification	Specimen field (mm)	Total magnification	Specimen field (mm)
0.3x	285	2.1x...10.5x	100.0...20.0	2.1x...10.5x	109.5...21.9	3.4x...16.8x	76.2...15.2	5.3x...26.3x	47.6...9.5
		1.95x ... 15.0x	107.7...14	1.95x... 15.0x	118...15.3	3.1x...24.0x	82.1...10.7	4.9x...37.5x	51.3...6.7
0.4x	210	2.8x...14.0x	75.0...15.0	2.8x...14.0x	82.1...16.4	4.5x...22.4x	57.1...11.4	7.0x...35.0x	35.7...7.1
		2.6x...20.0x	80.8...10.5	2.6x...20.0x	88.5...11.5	4.2x...32.0x	61.5...8.0	6.5x...50.0x	38.5...5.0
0.63x	130	4.4x...22.1x	47.7x...9.5	4.4x...22.1x	52.2...10.4	7.1x...35.3x	36.3...7.3	11.0x...55.1x	22.7...4.5
		4.1x...31.5x	51.3...6.7	4.1x...31.5x	56.2...7.3	6.6x...50.4x	39.1...5.1	10.2x...78.8x	24.4...3.2
1x	112 92	7.0x...35.0x	30.0...6.0	7.0x...35.0x	32.9...6.6	11.2x...56.0x	22.9...4.6	17.5x...87.5x	14.2...2.9
		6.5x...50x	32.3...4.2	6.5x...50x	35.4...4.6	10.4x...80.0x	24.6...3.2	16.3x...125.0x	15.4...2.0
2x	31	14.0x...70.0x	15.0...3.0	14.0x...70.0x	16.4...3.3	22.4x...112.0x	11.4...2.3	35.0x...175.0x	7.1...1.4
		13x...100.0x	16.2...2.1	13x...100.0x	17.7...2.3	20.8x... 60x	12.3...1.6	32.5x...250x	7.7...1.0

Stemi 2000 Stemi 1000

Microphotographic magnification scales for MC 80 (24 x 36 mm miniature format)

Basic microscope equipment . . . . . from 1.62:1 to 12.5:1
with replaceable optical system . . . . . from 0.49:1 to 25:1

1.4 Overview of modules

Basic microscope equipment

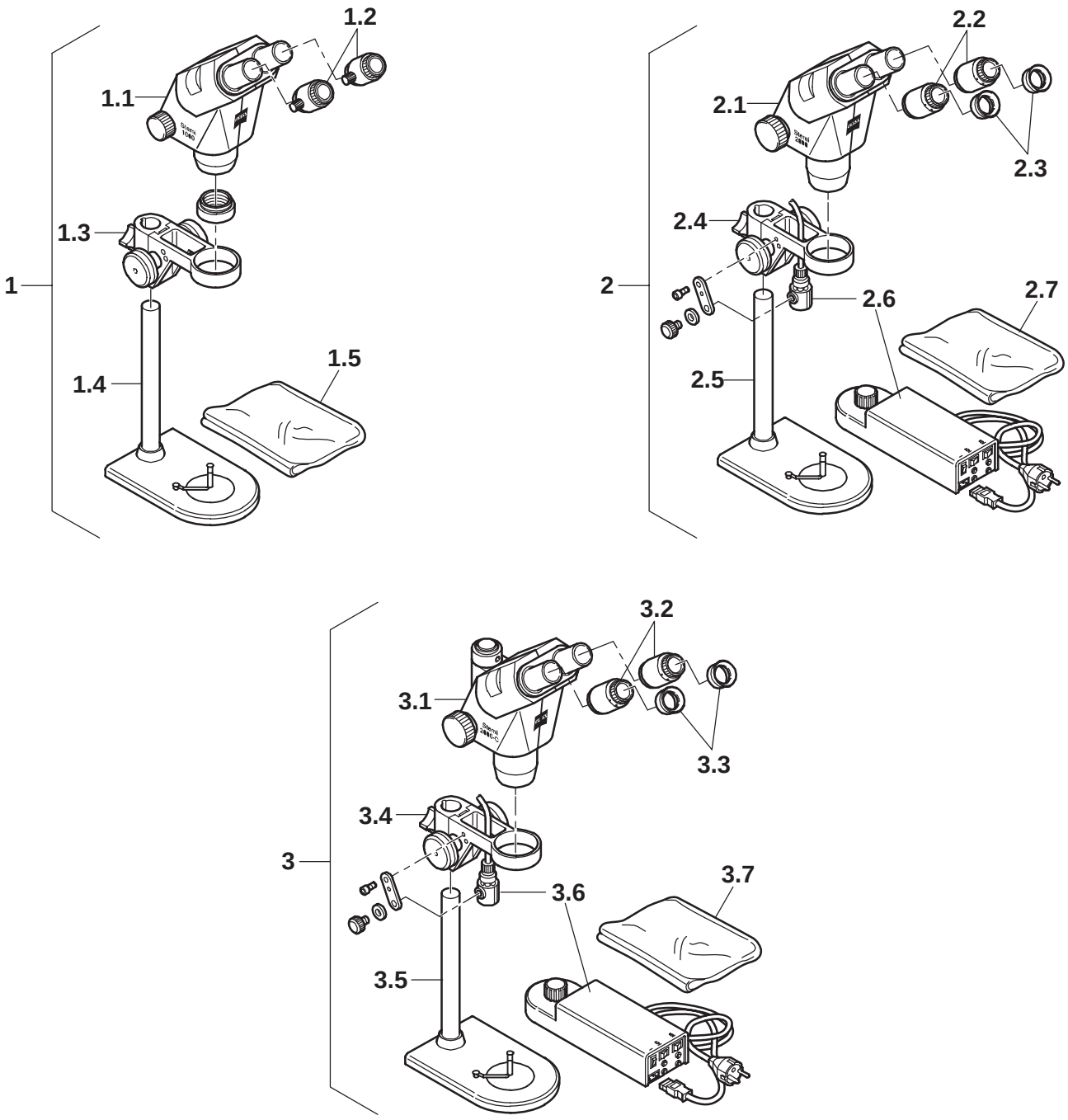


Figure 1-3 Basic microscope equipment

	Microscope equipment		Order No.	Stemi 1000	Stemi 2000	Stemi 2000-C
1	**Stereo microscope Stemi 1000** consisting of			●		
1.1	• Stemi 1000 microscope body		455050	●		
1.2	• Eyepiece W 10x/21 foc.	2x	455042	●	●	●
-	• Attachment systems adapter (contained in 455050)		-	●		
1.3	• Stemi mount with drive for column 32		455094	●	●	●
1.4	• Stand S, with column 32, 260 mm long		455104	●	●	●
1.5	• Dust cover K		459300	●	●	●
2	**Stereo microscope Stemi 2000 with built-in 6 V, 10 W halogen vertical illumination (230 V)** consisting of		495102 9804		●	
2.1	• Stemi 2000 microscope body		455052		●	
2.2	• Eyepiece W-PL 10x/23 spectacle focusing	2x	455043	●	●	●
2.3	• Eyepiece cup	2x	444801	●	●	●
2.4	• Stemi mount with drive for column 32		455094	●	●	●
2.5	• Stand S with column 32, 260 mm long		455104	●	●	●
2.6	• Built-in 6 V, 10 W halogen vertical illumination (230 V)		485001 9804		●	●
2.7	• Dust cover K		459300	●	●	●
not illus.	**Stereo microscope Stemi 2000 with built-in 6 V, 10 W halogen vertical illumination (120 V)**		495102 9904		●	
3	**Stereo microscope Stemi 2000-C with built-in 6 V, 10 W halogen vertical illumination (230 V)** consisting of		495104 9804			●
3.1	• Stemi 2000-C microscope body		455053			●
3.2	• Eyepiece W-PL 10x/23 spectacle focusing	2x	455043	●	●	●
3.3	• Eyepiece cup	2x	444801	●	●	●
3.4	• Stemi mount with drive for column 32		455094	●	●	●
3.5	• Stand S with column 32, 260 mm long		455104	●	●	●
3.6	• Built-in 6 V, 10 W halogen vertical illumination (230 V)		485001 9804		●	●
3.7	• Dust cover K		459300	●	●	●
not illus.	**Stereo microscope Stemi 2000-C with built-in 6 V, 10 W halogen vertical illumination (120 V)** but with:		495104 9904			●
	• 120 V – 6 V 50 VA power supply with mains cable and American flat plug		458421		●	●
not illus.	**Stereo microscope Stemi 2000-CS with stand N and universal vertical illumination**					
	(230 V) (115 V)		000000 1006 130 000000 1006 132			

● Part of the microscope equipment
▲ Unit capable of functioning in conjunction with the microscope equipment

Accessories

(1) Replaceable optical systems

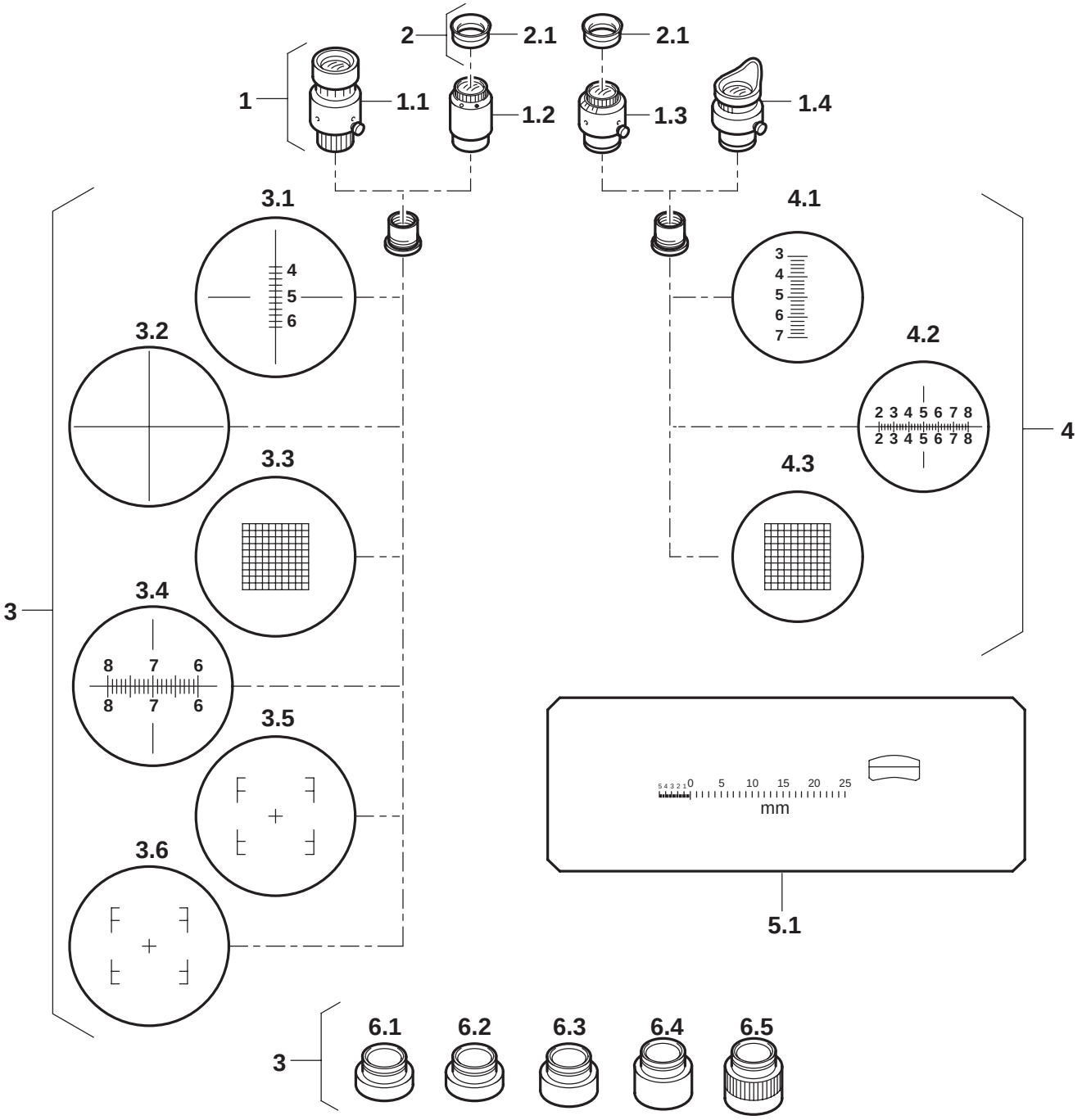


Figure 1-4 Eyepieces/eyepiece cups/reticules/attachment systems/specimen measuring plate

	Replaceable optical systems		Order No.	Stemi 1000	Stemi 2000	Stemi 2000-C
1	**Eyepieces**					
1.1	W 10x/21 foc. with eyepiece cup	2x	455042	▲	▲	▲
1.2	W-PL 10x/23 spectacle focusing	2x	455043	▲	▲	▲
1.3	W-PL 16x/16 spectacle focusing	2x	455048	▲	▲	▲
1.4	W 25x/10 foc. with eyepiece cup	2x	455046	▲	▲	▲
2	**Eyepiece cups**					
2.1	• Cup for eyepieces W-PL 10x/23 and W-PL 16x/16	2x each	444801	+	+	+
3	**Reticules for eyepieces W 10x/21 and W-PL 10x/23**					
	• Reticule holder			+	+	+
3.1	• Cross hair micrometer 10:100, ∅ 26		474066 9901	+	+	+
3.2	• Eyepiece cross hair reticule, ∅ 26		474064	+	+	+
3.3	• Reticulated micrometer 12.5 x 12.5/5; 10 ∅ 26		474068	+	+	+
3.4	• Cross hair micrometer 14:140, ∅ 26		454060	+	+	+
3.5	• Format reticule MC 10x, ∅ 26		454075			+
3.6	• Format reticule MC 12.5x, ∅ 26		454076			+
4	**Reticules for eyepieces W-PL 16x/16 and W 25x/10**					
4.1	• Eyepiece micrometer 10:100, ∅ 21		434011	+	+	+
4.2	• Cross hair reticule 10:100, ∅ 21		434013	+	+	+
4.3	• Reticulated micrometer 10x10/5: 10, ∅ 21		454020	+	+	+
5	**Specimen measuring plates**					
5.1	• Specimen micrometer 25+50/10 mm (Stemi)		474025	▲	▲	▲
6	**Attachment systems**					
6.1	• Attachment system 0,3 x		455025	▲	▲	▲
6.2	• Attachment system 0,4 x		455026	▲	▲	▲
6.3	• Attachment system 0,63 x		455027	▲	▲	▲
6.4	• Attachment system 2,0 x		455028	▲	▲	▲
6.5	• Zoom attachment system 0.3 x ... 0.5 x		455029	▲	▲	▲

▲ Unit capable of functioning in conjunction with the microscope equipment
\+ Functions in conjunction with further additional equipment

(2) Stands

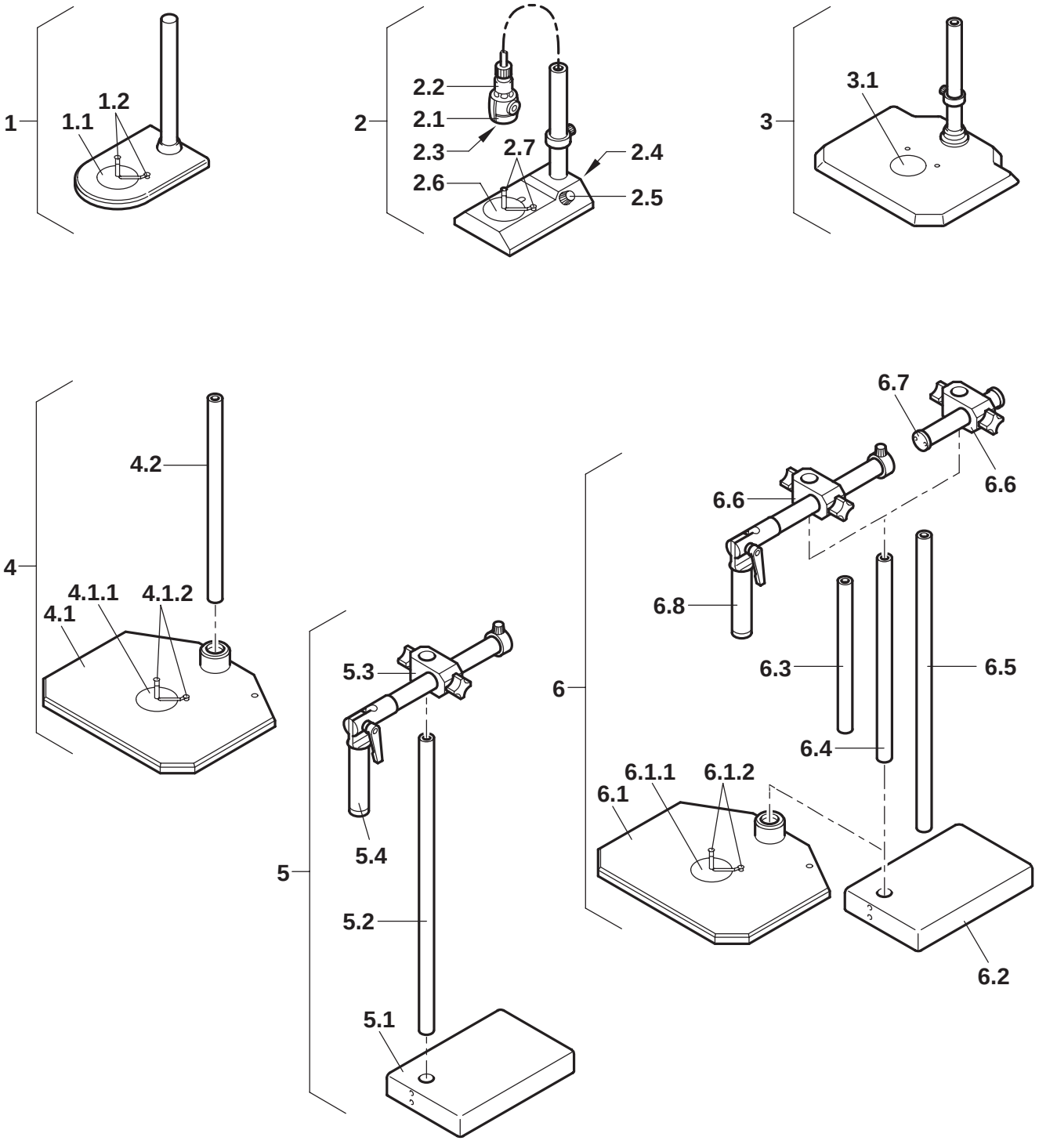


Figure 1-5 Stands

	Stands		Order No.	Stemi 1000	Stemi 2000	Stemi 2000-C
1	**Stand S, assy.** containing:		455104	▲	▲	▲
1.1	• Plastic plate, b/w		475290 9901	+	+	+
1.2	• Stage spring	2x	473371 9902	+	+	+
2	**Stand L, assy. (230 V)** containing:		455110	▲	▲	▲
2.1	• Lamp 10		467253 9902	+	+	+
2.2	• Lamp socket			+	+	+
2.3	• Halogen lamp 6 V 10 W		386108	+	+	+
2.4	• Built-in transformer					
2.5	• Brightness control			+	+	+
2.6	• Plastic plate, b/w		475290 9901	+	+	+
2.7	• Stage spring	2x	473371 9902	+	+	+
2.8	• Safety-ring		455105 8031	+	+	+
not illus.	**Stand L, assy. (120 V)**		455108	▲	▲	▲
3	**Stand N, assy.** containing		455107	▲	▲	▲
3.1	• Plastic plate, b/w		475290 9901	+	+	+
3.2	• Safety-ring		455105 8031	+	+	+
4	**Large stand plate with column 32/450 mm, assy.** consisting of:			▲	▲	▲
4.1	• Large stand plate 32 (260 x 360 mm) containing:		455101	+	+	+
4.1.1	- Plastic plate, b/w		475290 9901	+	+	+
4.1.2	- Stage spring	2x	473371 9902	+	+	+
4.1.3	- Safety-ring		455105 8031	+	+	+
4.2	• Column 32/450 mm		475120	+	+	+
5	**Workplace stand with swivel arm, assy.** consisting of:			▲	▲	▲
5.1	• Stage foot 32		455113	+	+	+
5.2	• Column 32/650 mm		475119	+	+	+
5.3	• Cross element 32		455125	+	+	+
5.4	• Tilt head		455117	+	+	+
6	**Single components of expandable stands**					
6.1	• Large stand plate 32 (260 x 360 mm) containing:		455101	+	+	+
6.1.1	- Plastic plate, b/w		475290 9901	+	+	+
6.1.2	- Stage spring	2x	473371 9902	+	+	+
6.1.3	- Safety-ring		455105 8031	+	+	+
6.2	• Stage foot 32		455113	+	+	+
6.3	• Column 32/350 mm		475123	+	+	+
6.4	• Column 32/450 mm		475120	+	+	+
6.5	• Column 32/650 mm		475119	+	+	+
	Required to connect the columns:					
6.6	• Cross element 32		455125	+	+	+
	Required to hold the Stemi with swivel arm:					
6.7	• Column 32/210 mm with end pieces or		475122	+	+	+
6.8	• Tilt head		475117	+	+	+

▲ Unit capable of functioning in conjunction with the microscope equipment
\+ Functions in conjunction with further additional equipment

(3) Specimen stages

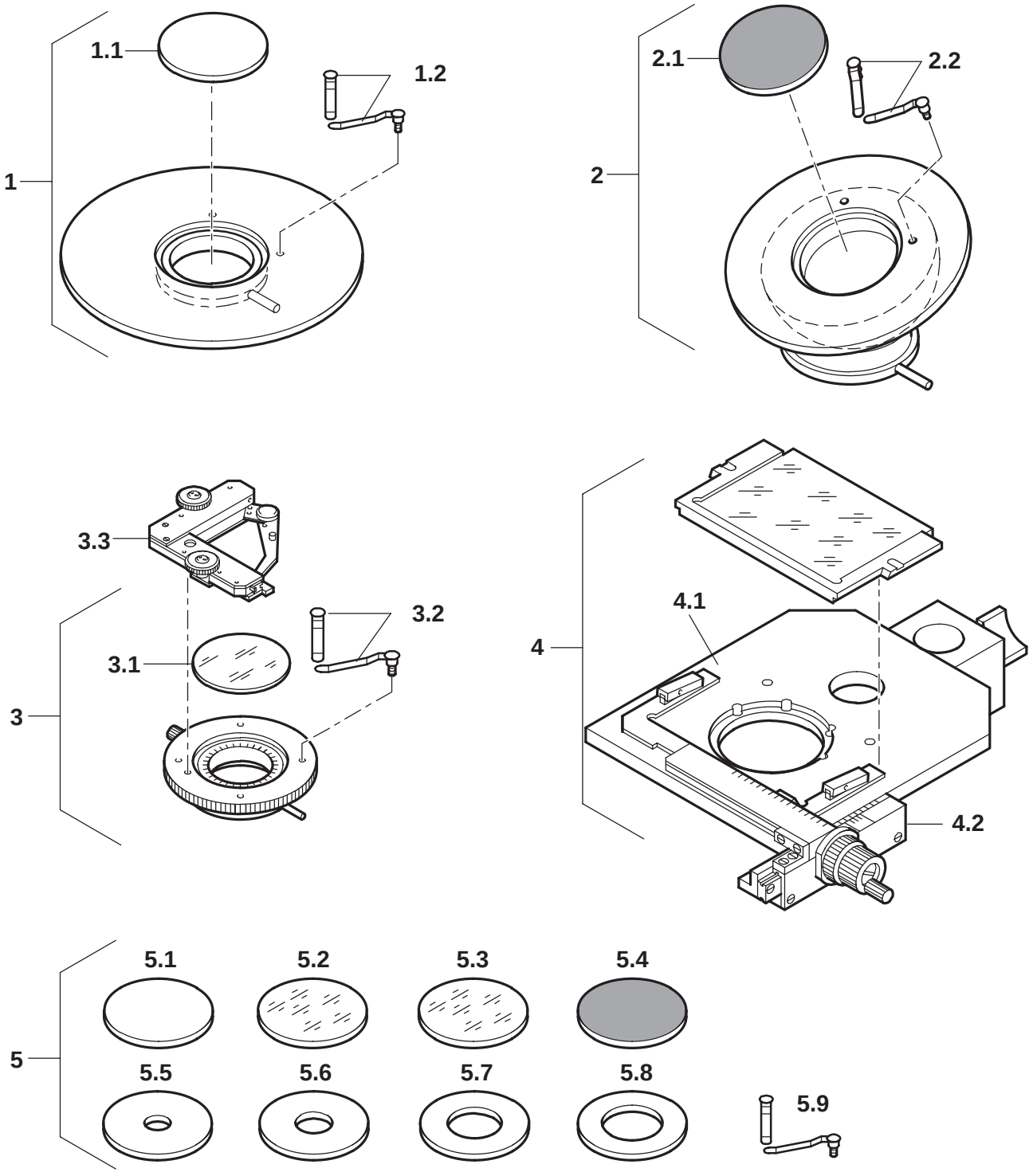


Figure 1-6 Specimen stages

	Specimen stages	Order No.	Stemi 1000	Stemi 2000	Stemi 2000-C
1	**Sliding stage** requiring:	455122	▲	▲	▲
1.1	• Plastic plate, b/w ∅ 84	475290 9901	+	+	+
1.2	• Stage spring 2x	473371 9902	+	+	+
2	**Ball stage** containing	455123	▲	▲	▲
2.1	• Non-slip plate	475288	+	+	+
2.2	• Stage spring 2x	473371 9902	+	+	+
3	**Rotary stage for transmitted and vertical illumination** containing:	455120	▲	▲	▲
3.1	• Glass plate ∅ 72	473378	+	+	+
3.2	• Stage spring 2x	473371 9902	+	+	+
	additionally:				
3.3	• Specimen guide D (28 x 75 mm)	413455	+	+	+
4	**Compound table stage for transmitted and vertical illumination, assy.** consisting of:		▲	▲	▲
4.1	• Expandable stage with mount 32	413458	+	+	+
4.2	• Specimen guide with holding frame and glass plate (76 x 50 mm shift range)	413458 9001	+	+	+
	Refer to the ”Axiovert” price list for details of further holding frames		+	+	+
5	**Inlay plates and stage springs**				
5.1	• Plastic plate, b/w ∅ 84	475290 9901	+	+	+
5.2	• Glass plate, mat coating ∅ 84	475291	+	+	+
5.3	• Glass plate, transparent ∅ 84	475265 0001	+	+	+
5.4	• Non-slip plate ∅ 84	475288	+	+	+
5.5	• Perforated plate ∅ 15	475269-110	+	+	+
5.6	• Perforated plate ∅ 25	455140-110	+	+	+
5.7	• Perforated plate ∅ 40	455140-111	+	+	+
5.8	• Perforated plate ∅ 43	475297	+	+	+
5.9	• Stage springs	473371 9902	+	+	+
5.10	• Stage spring 2x	473371 9902	+	+	+

▲ Unit capable of functioning in conjunction with the microscope equipment
\+ Functions in conjunction with further additional equipment

(4) Halogen lighting systems for vertical illumination

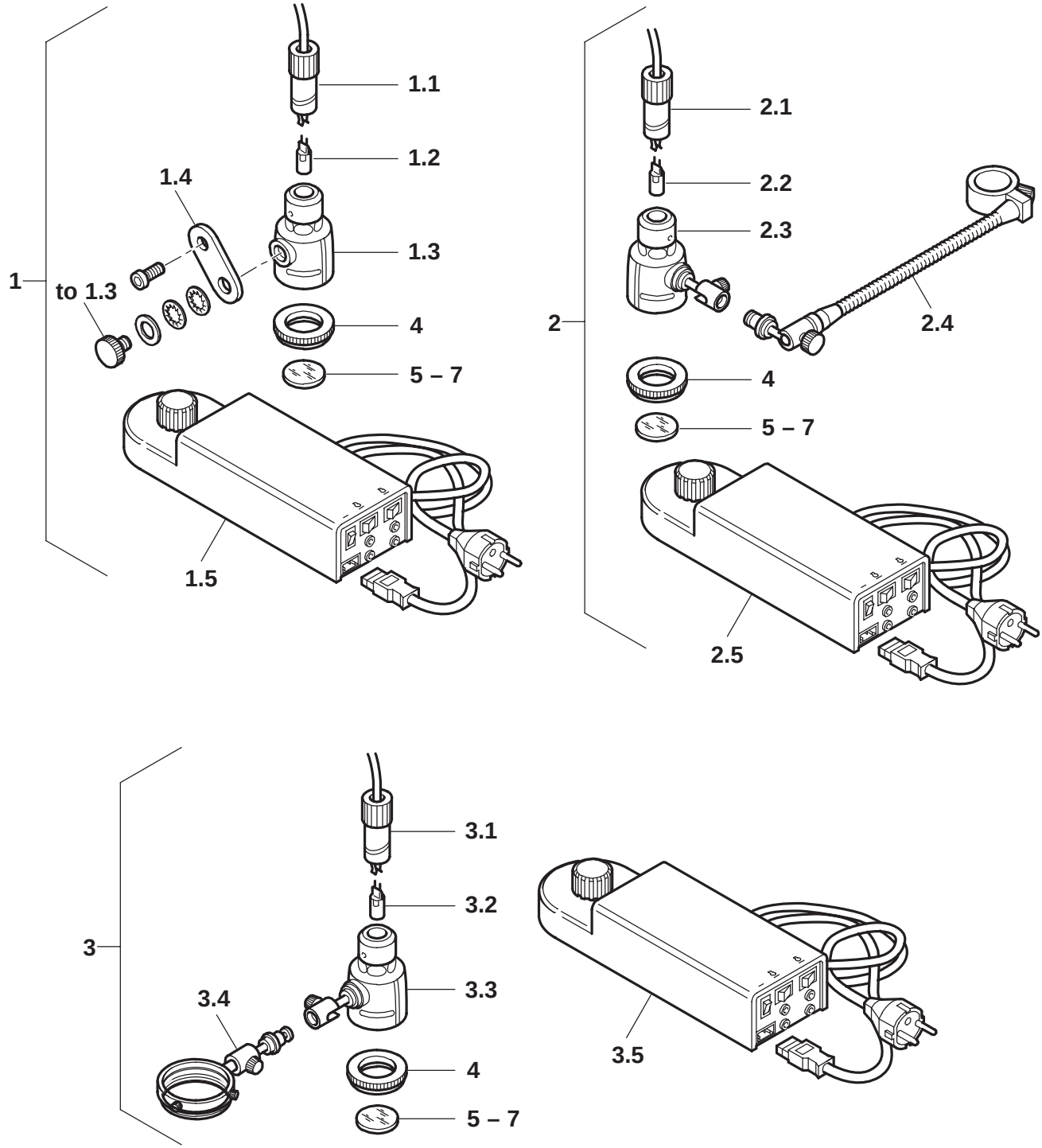


Figure 1-7 Halogen lighting systems for vertical illumination

	Halogen lighting systems for vertical illumination	Order No.	Stemi 1000	Stemi 2000	Stemi 2000-C
	Halogen lighting systems				
1	**Built-in vertical illumination system 6 V 10 W halogen (230 V)** consisting of:	485001 9804	▲	▲	▲
1.1	• HAL 10 W socket for lamp 10	468043 9901	+	+	+
1.2	• Halogen lamp 6 V 10 W	386108	+	+	+
1.3	• Lamp 10	467253 9902	+	+	+
1.4	• Adapter for built-in lamp	455149	+	+	+
1.5	• 230 V – 6 V 50 VA power supply unit with mains cable and Euro-plug	458420	+	+	+
not illus.	**Built-in vertical illumination system 6 V 10 W halogen (120 V)** consisting of:	485001 9904	▲	▲	▲
	• Parts 1.1 ... 1.4		+	+	+
	• 120 V – 6 V 50 VA power supply unit with mains cable and American flat plug	458421	+	+	+
2	**Fit-on vertical illumination system 6 V 10 W halogen (230 V)** consisting of:	485002 9804	▲	▲	▲
2.1	• HAL 10 W socket for lamp 10	468043 9901	+	+	+
2.2	• Halogen lamp 6 V 10 W	386108	+	+	+
2.3	• Lamp 10 for lamp mount	455153	+	+	+
2.4	• Lamp mount for column 32	455150	+	+	+
2.5	• 230 V – 6 V 50 VA power supply unit with mains cable and Euro-plug	458420	+	+	+
not illus.	**Fit-on vertical illumination system 6 V 10 W halogen (120 V)** consisting of:	485002 9904	▲	▲	▲
	• Parts 2.1 ... 2.3		+	+	+
	• 120 V – 6 V 50 VA power supply unit with mains cable and American flat plug	458421	+	+	+
3	**Fit-on vertical illumination system 6 V 10 W halogen (230 V) on the front optics** consisting of:		▲	▲	▲
3.1	• HAL 10 W socket for lamp 10	468043 9901	+	+	+
3.2	• Halogen lamp 6 V 10 W	386108	+	+	+
3.3	• Lamp 10 for lamp mount	455153	+	+	+
3.4	• Lamp mount for column 32	455151	+	+	+
3.5	• 230 V – 6 V 50 VA power supply unit with mains cable and Euro-plug	458420	+	+	+
	Accessories				
4	• Filter holder ∅ 32	466051	+	+	+
5	• Thermal protection filter KG1 ∅ 32 x 2	467830	+	+	+
6	• Wide band interference filter, green, ∅ 32 x 4	467803	+	+	+
7	• Conversion filter CB12 ∅ 32 x 2	467850 9901	+	+	+

▲ Unit capable of functioning in conjunction with the microscope equipment
\+ Functions in conjunction with further additional equipment

(5) Cold light sources for vertical illumination

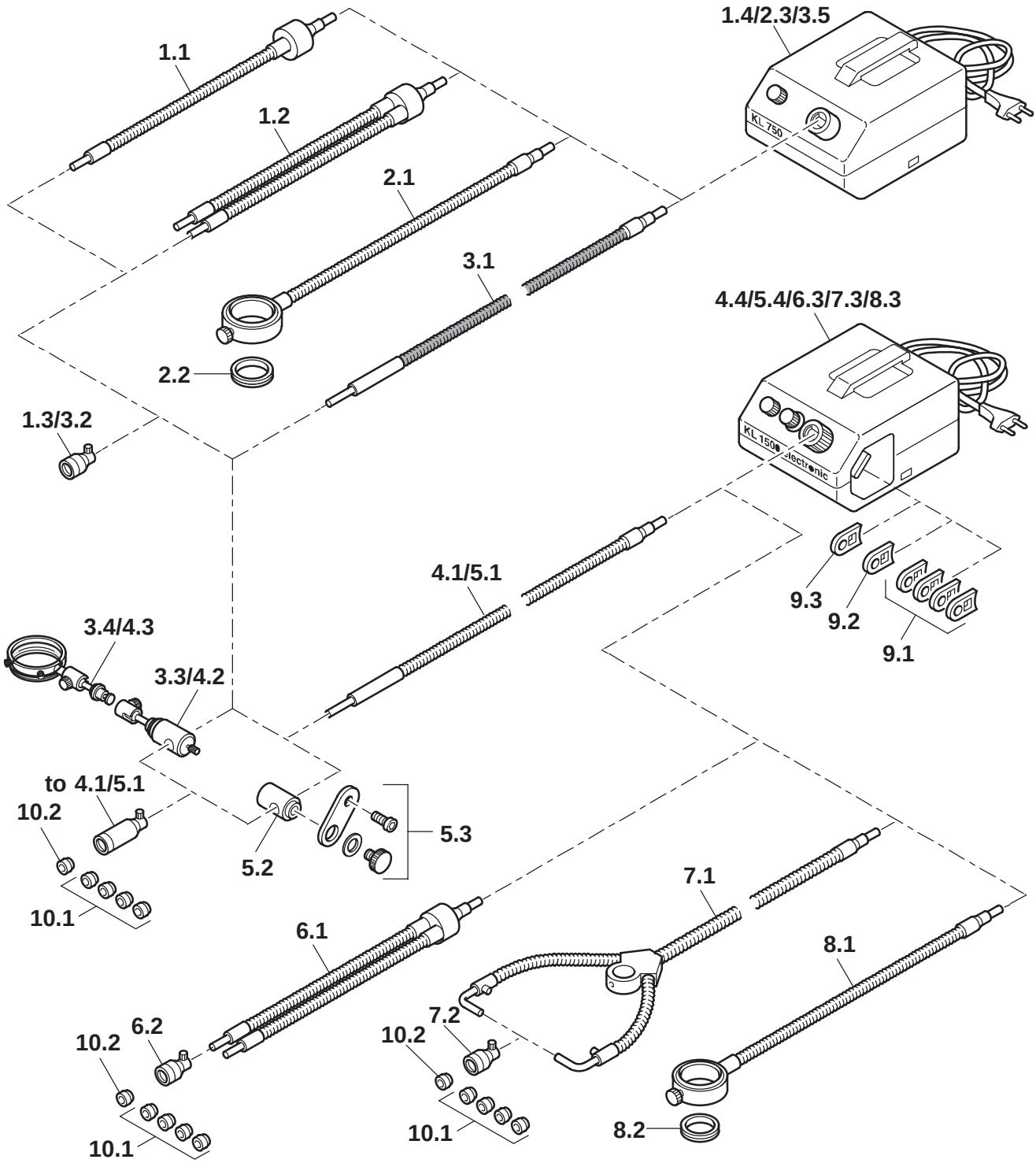


Figure 1-8 Cold light sources for vertical illumination

Cold light sources for vertical illumination (75 W)		Order No.	Stemi 1000	Stemi 2000	Stemi 2000-C
1	**Vertical illumination system with swan-neck fibre-optic conductor** consisting of:		▲	▲	▲
1.1	• Swan-neck fibre-optic conductor, one-arm, 4.5 mm fibre bundle, 500 mm long for KL 750 (D)	417080 9001	+	+	+
	or				
1.2	• Swan-neck fibre-optic conductor, two-arm, 3.5 mm fibre bundle, 500 mm long for KL 750 (D)	417080 9002	+	+	+
1.3	• Focusing attachment without filter (D) 2x	417059 9901	+	+	+
1.4	• Schott cold light source KL 750 (230 V)	417080	+	+	+
	or				
-	• Schott cold light source KL 750 (120 V)	417081	+	+	+
2	**Vertical illumination system with ring lamp** consisting of:		▲	▲	▲
2.1	• 6-point ring lamp d = 58 mm (D)	417080 9008	+	+	+
2.2	• Retaining ring for ring lamp	455184	+	+	+
2.3	• Schott cold light source KL 750 (230 V)	417080	+	+	+
	or				
-	• Schott cold light source KL 750 (120 V)	417081	+	+	+
3	**Fit-on lighting system with fibre-optic conductor** consisting of:				
3.1	• Flexible fibre-optic conductor, one-arm, 4.5 mm fibre bundle, 1000 mm long for KL 750 (D)	417080 9005	+	+	+
3.2	• Focusing attachment without filter (D)	417059 9901	+	+	+
3.3	• Holder for built-in fibre-optic conductor for lamp mount	455143	+	+	+
3.4	• Lamp mount for front optics	455151	+	+	+
3.5	• Schott cold light source KL 750 (230 V)	417080	+	+	+
	or				
-	• Schott cold light source KL 750 electronic (120 V)	417081	+	+	+

Accessories		Order No.	Stemi 1000	Stemi 2000	Stemi 2000-C
9	**Inlay filter for Schott cold light source KL 1500 electronic** consisting of:				
9.1	• Inlay filter set (D) (blue/red/green/orange filters)	417075 9005	+	+	+
9.2	• Neutralisation filter 0.25 (D)	417075 9006	+	+	+
9.3	• Conversion filter (D)	417075 9007	+	+	+
10	**Filter set for focusing attachment**				
10.1	• Threaded filter set (D) (blue/red/green/orange filters)	on request	+	+	+
10.2	• Polarisation filter	417065	+	+	+

▲ Unit capable of functioning in conjunction with the microscope equipment
\+ Functions in conjunction with further additional equipment

	Cold light sources for vertical illumination (150 W)	Order No.	Stemi 1000	Stemi 2000	Stemi 2000-C
4	**Fit-on lighting system with fibre-optic conductor** consisting of:		▲	▲	▲
4.1	• Flexible fibre-optic conductor with focusing attachment	455145	+	+	+
4.2	• Holder for built-in fibre-optic conductor for lamp mount	455143	+	+	+
4.3	• Lamp mount for front optics	455151	+	+	+
4.4	• Schott cold light source KL 1500 electronic (230 V)	495054 9801	+	+	+
	or				
-	• Schott cold light source KL 1500 electronic (120 V)	495054 9901	+	+	+
5	**Built-in lighting system with fibre-optic conductor** consisting of:		▲	▲	▲
5.1	• Flexible fibre-optic conductor with focusing attachment	455145	+	+	+
5.2	• Holder for built-in lamp	455144	+	+	+
5.3	• Built-in lamp adapter	455149	+	+	+
5.4	• Schott cold light source KL 1500 electronic (230 V)	495054 9801	+	+	+
	or				
-	• Schott cold light source KL 1500 electronic (120 V)	495054 9901	+	+	+
6	**Vertical illumination system with swan-neck fibre-optic conductor** consisting of:		▲	▲	▲
-	• Swan-neck fibre-optic conductor, one-arm, 4.5 mm fibre bundle, 750 mm long, self-supporting (D)	417052 9001	+	+	+
	or				
6.1	• Swan-neck fibre-optic conductor, two-arm, 4.5 mm fibre bundle, 750 mm long, self-supporting (D)	417075 9001	+	+	+
	or				
-	• Swan-neck fibre-optic conductor, three-arm, 4.5 mm fibre bundle, 750 mm long, self-supporting (D)	417075 9003	+	+	+
6.2	• Focusing attachment without filter (D) 3x	417059 9901	+	+	+
6.3	• Schott cold light source KL 1500 electronic (230 V)	495054 9801	+	+	+
	or				
-	• Schott cold light source KL 1500 electronic (120 V)	495054 9901	+	+	+
7	**Universal lighting system for vertical illumination with fibre-optic conductor** consisting of:		▲	▲	▲
7.1	• Universal lighting system for vertical illumination with two-arm fibre-optic conductor	455146	+	+	+
7.2	• Focusing attachment without filter (D) 2x	417059 9901	+	+	+
7.3	• Schott cold light source KL 1500 electronic (230 V)	495054 9801	+	+	+
	or				
-	• Schott cold light source KL 1500 electronic (120 V)	495054 9901	+	+	+
8	**Lighting system for vertical illumination with split ring lamp** consisting of:		▲	▲	▲
8.1	• Split ring lamp (D)	417068	+	+	+
8.2	• Retaining ring for ring lamp	455184	+	+	+
8.3	• Schott cold light source KL 1500 electronic (230 V)	495054 9801	+	+	+
	or				
-	• Schott cold light source KL 1500 electronic (120 V)	495054 9901	+	+	+

(6) Halogen lighting systems for transmitted illumination

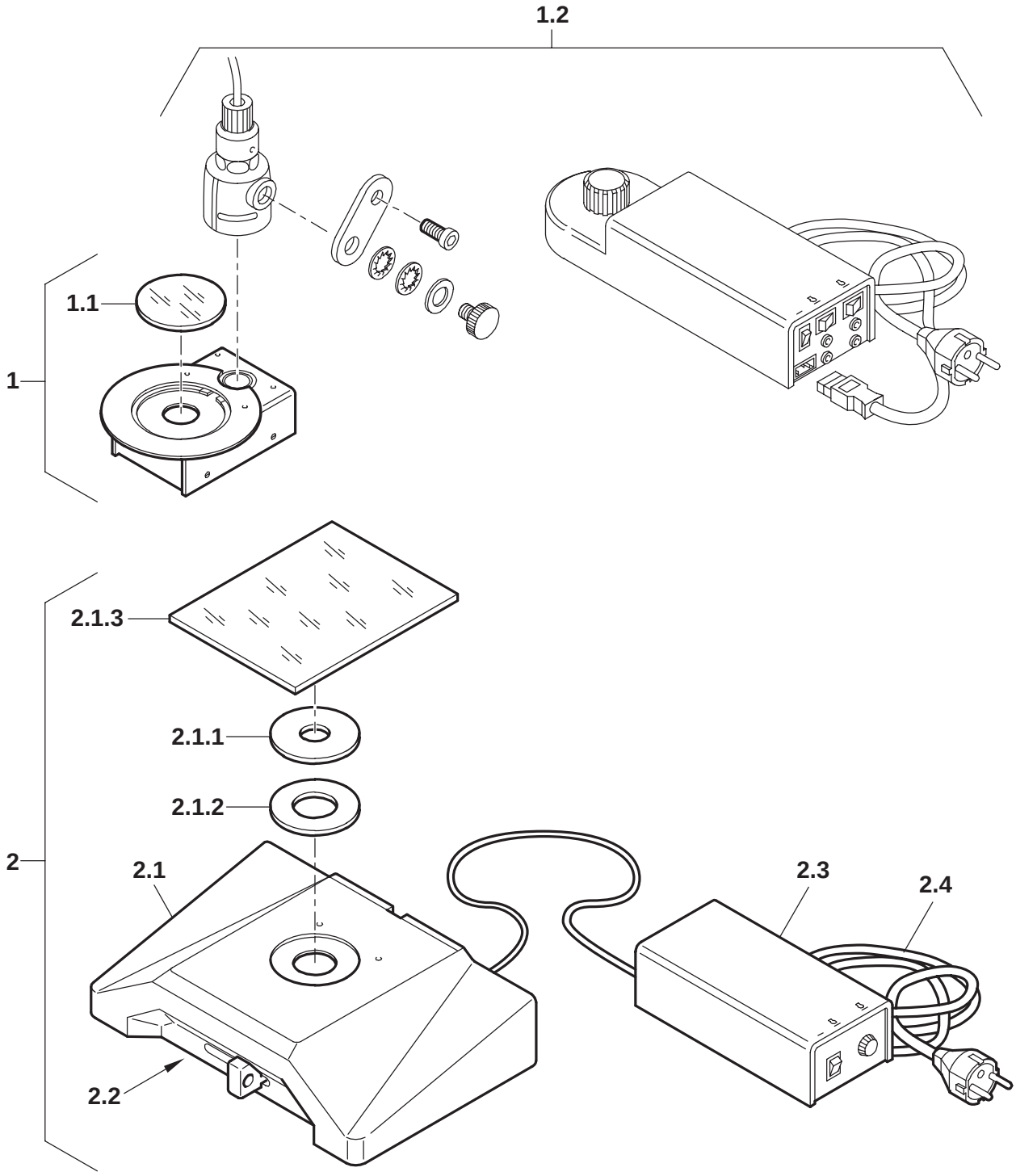


Figure 1-9 Halogen lighting systems for transmitted illumination

	Lighting systems for transmitted illumination (halogen)	**Order No.**	**Stemi 1000**	**Stemi 2000**	**Stemi 2000-C**
1	**Option for transmitted illumination with plate ∅ 140** containing:	455137	▲	▲	▲
1.1	• Glass plate, transparent ∅ 84	475265 0001	+	+	+
	Additionally required:				
1.2	• Built-in vertical illumination system 6 V 10 W halogen (230 V)	485001 9804	+	+	+
	or				
1.3	• Built-in vertical illumination system 6 V 10 W halogen (120 V)	485001 9904	+	+	+
2	**Universal transillumination system (for stand N and stand plate 32)** consisting of:	495052 9801	▲	▲	▲
2.1	• Universal transillumination system with swivel reflector with:	455140	+	+	+
2.1.1	– Perforated plate ∅ 25				
2.1.2	– Perforated plate ∅ 40	455140-111	+	+	+
2.1.3	– Glass plate		+	+	+
2.2	• Halogen lamp 6 V 20 W 2x	380143 1350	+	+	+
2.3	• Power supply unit, stabilised 6 V 20 W (115 V – 230 V)	458415	+	+	+
2.4	• Mains cable with Euro-plug	380138 5810	+	+	+
3	**Universal transillumination system (for stand N and stand plate 32)** consisting of:	495052 9901	▲	▲	▲
	• Parts 2.1 ... 2.3		+	+	+
not illus.	• Mains cable with American flat plug		+	+	+

▲ Unit capable of functioning in conjunction with the microscope equipment
\+ Functions in conjunction with further additional equipment

(7) Cold light sources for transmitted illumination

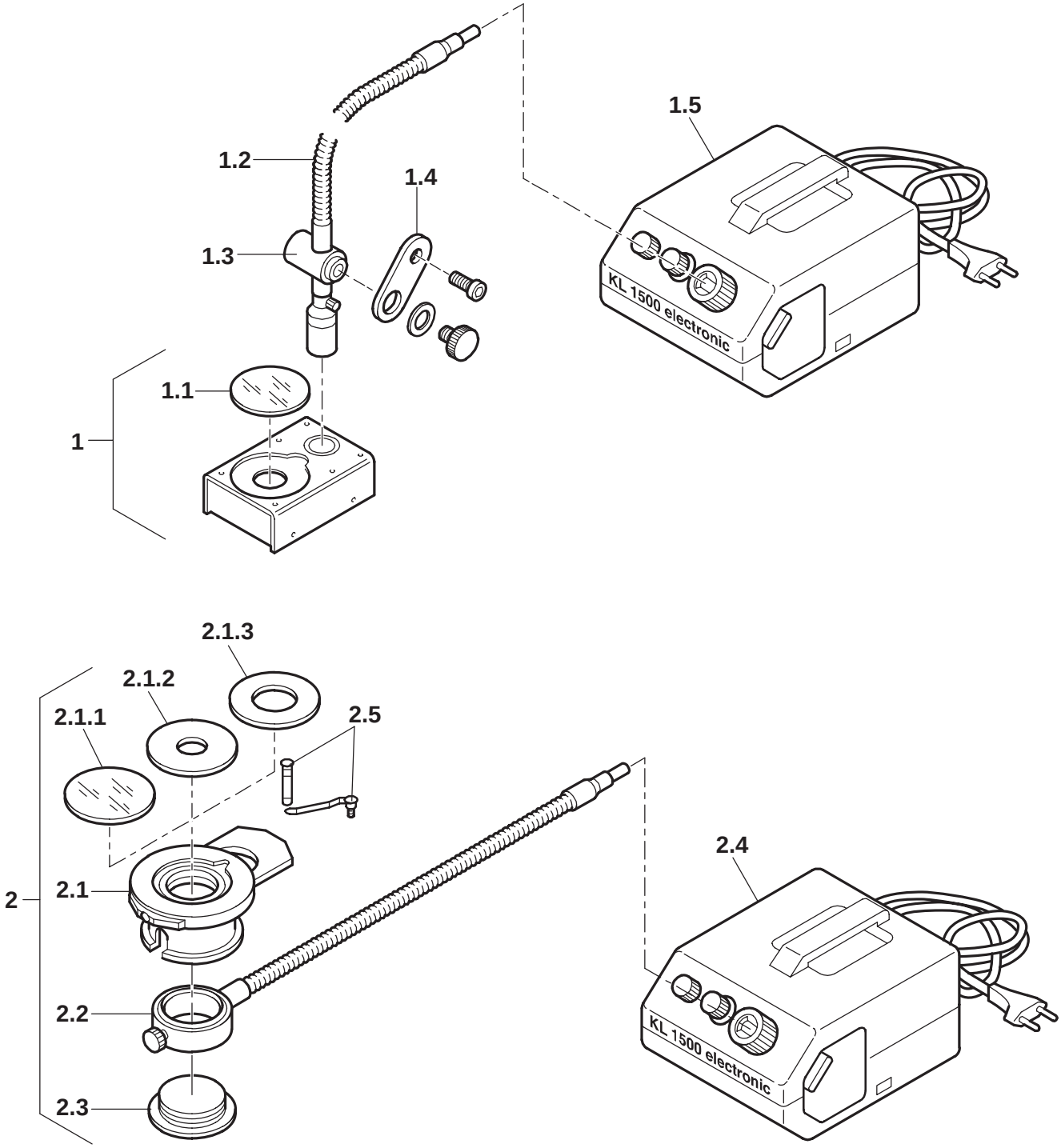


Figure 1-10 Cold light sources for transmitted illumination

	Cold light sources for transmitted illumination	Order No.	Stemi 1000	Stemi 2000	Stemi 2000-C
1	**Option for transmitted illumination** containing:	455136	▲	▲	▲
1.1	• Glass plate, transparent ∅ 84	475265 0001	+	+	+
	Additionally required:				
1.3	• Built-in fibre-optic conductor with focusing attachment	455145	+	+	+
1.4	• Holder for fibre-optic conductor	455144	+	+	+
1.5	• Built-in lamp adapter	455149	+	+	+
1.6	• Schott cold light source KL 1500 electronic (230 V)	495054 9801	+	+	+
	or				
1.6	• Schott cold light source KL 1500 electronic (120 V)	495054 9901	+	+	+
2	**Transillumination system with fibre-optic conductor for dark field on all sides, switchable to bright field** consisting of:	495052 9801	▲	▲	▲
2.1	• Option for bright-dark field transillumination with:	475269	+	+	+
2.1.1	– Glass plate, transparent ∅ 84	475265 0001	+	+	+
2.1.2	– Perforated plate ∅ 25	455140-110	+	+	+
2.1.3	– Perforated plate ∅ 40	455140-111	+	+	+
2.2	• Split ring lamp (D)	417068	+	+	+
2.3	• Transmitted light dark field illumination with specimen support for contact lenses	475278	+	+	+
2.4	• Schott cold light source KL 1500 electronic (230 V)	495054 9801	+	+	+
	or				
2.4	• Schott cold light source KL 1500 electronic (120 V)	495054 9901	+	+	+
2.5	• Stage spring 2x	4733719902	+	+	+

▲ Unit capable of functioning in conjunction with the microscope equipment
\+ Functions in conjunction with further additional equipment

(8) Polarisation systems

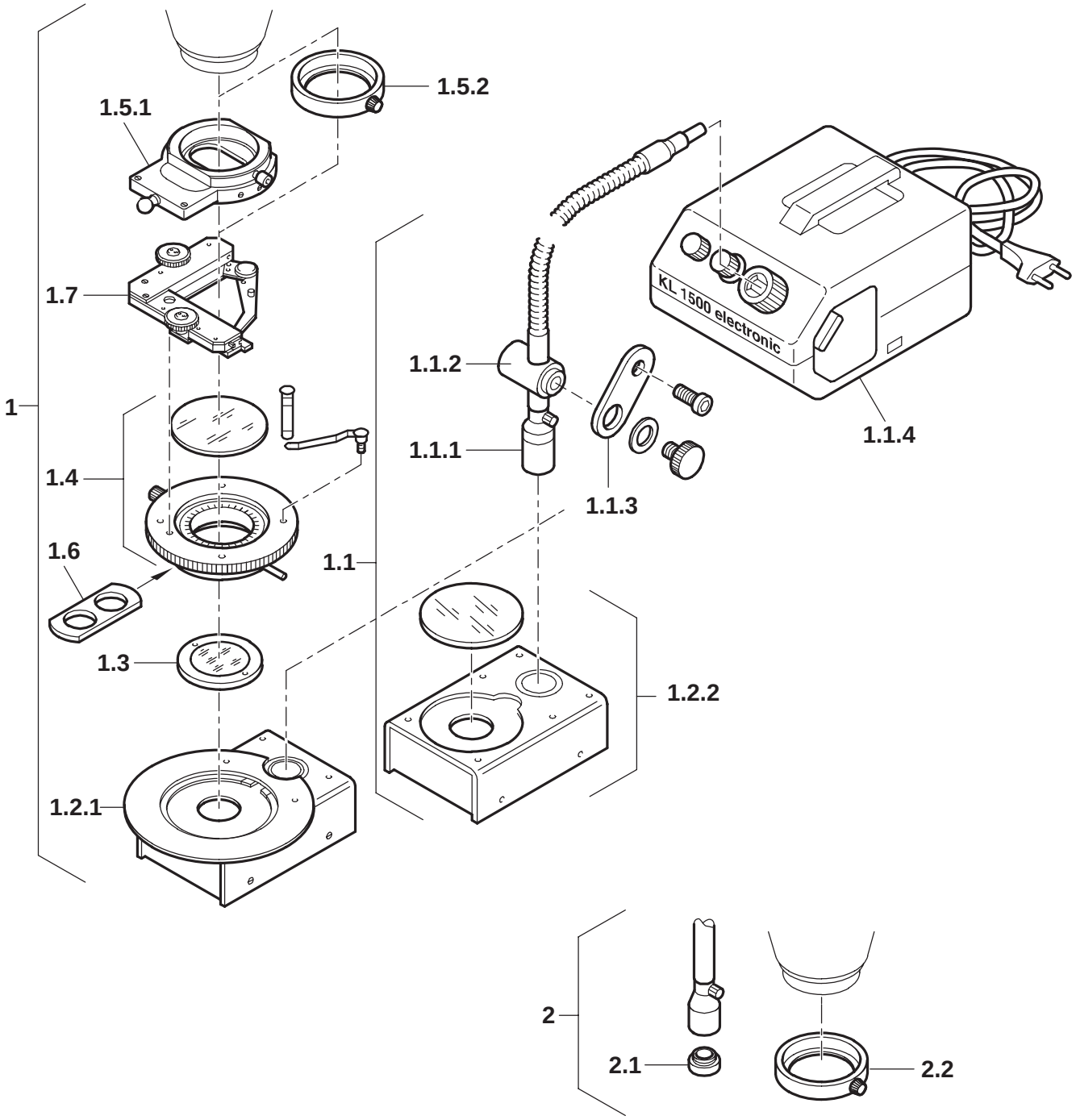


Figure 1-11 Polarisation systems

	Polarisation systems	**Order No.**	**Stemi 1000**	**Stemi 2000**	**Stemi 2000-C**
1	**Polarisation contrast in transmitted light** consisting of:		▲	▲	▲
1.1	• Built-in vertical illumination system with fibre-optic conductor		+	+	+
1.1.1	– Built-in fibre-optic conductor with focusing attachment	455145	+	+	+
1.1.2	– Holder for built-in fibre-optic conductor	455144	+	+	+
1.1.3	– Built-in lamp adapter	455149	+	+	+
1.1.4	– Schott cold light source KL 1500 electronic (230 V) or	495054 9801	+	+	+
not illus.	– Schott cold light source KL 1500 electronic (120 V)	495054 9901	+	+	+
1.2.1	• Option for transmitted illumination with plate ∅ 140 or	455137	+	+	+
1.2.2	• Option for transmitted illumination	455136	+	+	+
1.3	• Polariser S	455174	+	+	+
1.4	• Rotary stage for stereo microscopes with stage springs, mount for polariser and glass plate ∅ 72	455120 9901	+	+	+
1.5.1	• Analyser slide	455171 9901	+	+	+
1.5.2	• Analyser (A53)	455170	+	+	+
	Accessories				
1.6	• λ slide plate	455172	+	+	+
1.7	• Specimen guide D 28 x 75 mm	413455	+	+	+
2	**Additional option for suppressing surface reflections in vertical illumination** consisting of:		▲	▲	▲
2.1	• Polarisation filter for focusing attachment (D)	417065	+	+	+
2.2	• Analyser (A53)	455170	+	+	+
	Only applicable in conjunction with the following fibre-optic conductor systems:				
	– Built-in lighting with fibre-optic conductor	(Fig. 1-8/6)			
	– Universal vertical illumination with fibre-optic conductor	(Fig. 1-8/1 or 5)			
	– Universal vertical illumination with swan-neck fibre-optic conductor	(Fig. 1-8/4)			

▲ Unit capable of functioning in conjunction with the microscope equipment
\+ Functions in conjunction with further additional equipment

(9) Documentation systems using photo cameras

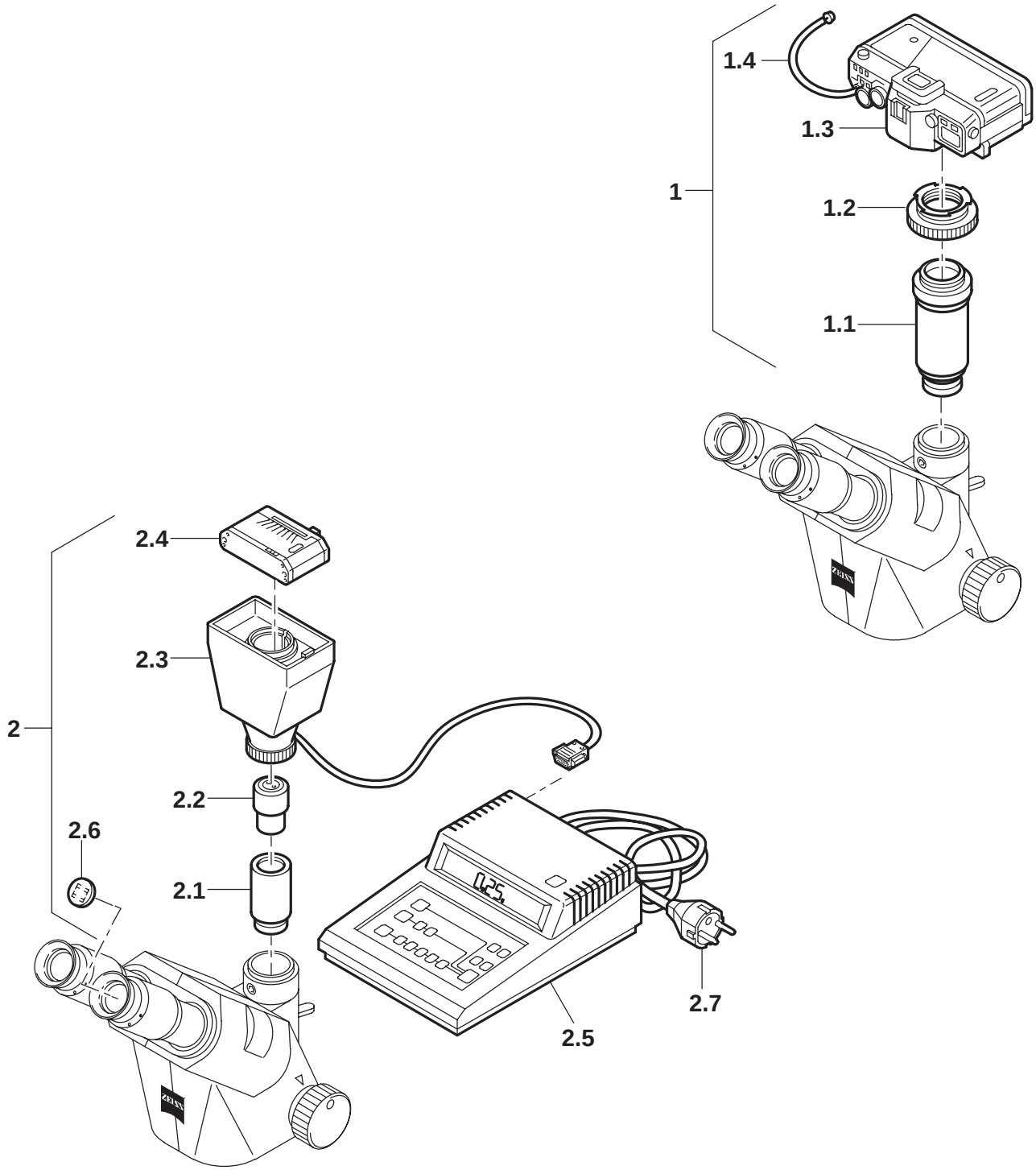


Figure 1-12 Documentation systems using photo cameras

	Documentation systems using photo cameras	Order No.	Stemi 1000	Stemi 2000	Stemi 2000-C
1	**CONTAX 167 MT reflex camera** consisting of:		–	–	▲
1.1	• Reflex camera connection 2.5x for T2	456005			+
1.2	• Special adapter T2 for CONTAX	416010			+
1.3	• Reflex camera housing CONTAX 167 MT	416181			+
1.4	• Cable release	416167			+
	Accessories				
not illus.	• T2 adapter for OLYMPUS OM (OM bayonet)	416002			+
not illus.	• T2 adapter for MINOLTA (SR bayonet)	416003			+
not illus.	• T2 adapter for CANON (FD bayonet)	416004			+
not illus.	• T2 adapter for NIKON F (F bayonet)	416009			+
not illus.	• T2 adapter for PENTAX (KA bayonet)	416011			+
not illus.	• Format reticule MC 10x/∅ 26 if focusing through the viewfinder is not required	454075			+
2	**Microscope camera MC 80 DX for 24 x 36 mm** consisting of:		–	–	▲
2.1	• Microscope camera connection ∅ 30	456006			+
2.2	• Projection lens P 2.5x for MC 80 / MC 80 DX	456021			+
2.3	• Base MC 80 DX	456031			+
2.4	• Film cartridge 35 mm Mot DX	456071			+
2.5	• Control panel MC 80 DX	456048			+
2.6	• Format reticule MC 2,5x/∅ 26	454075			+
2.7	• Mains cable with Euro-plug	380137 6750			+
	or				
not illus.	• Mains cable with American flat plug				+

▲ Unit capable of functioning in conjunction with the microscope equipment
+ Functions in conjunction with further additional equipment
- Application not possible

(10) Adapters for video cameras

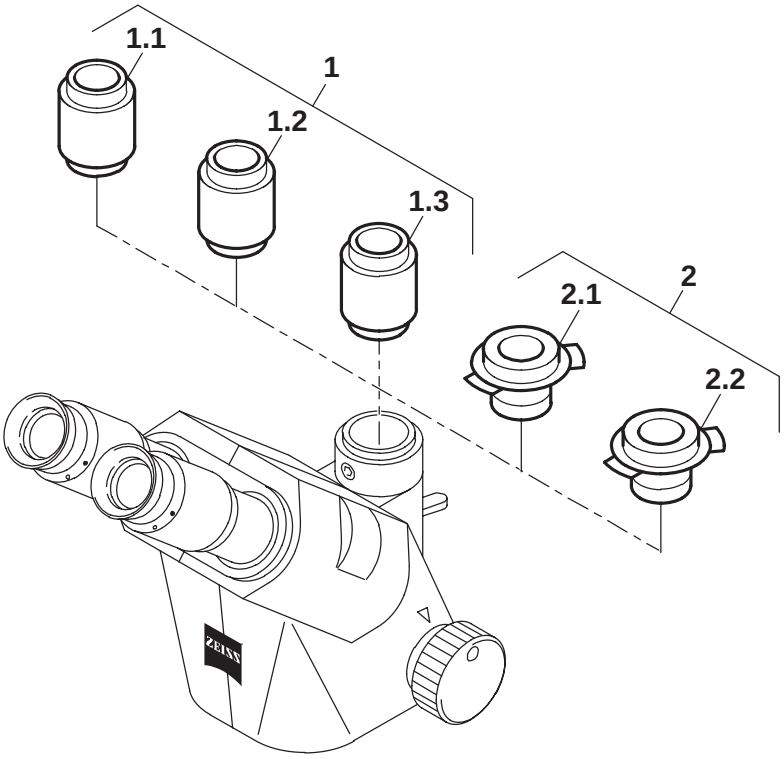


Figure 1-13 Adapters for video cameras

Documentation systems for video cameras		Order No.	Stemi 1000	Stemi 2000	Stemi 2000-C
1	**Standard C connection thread**				
1.1	• TV camera 2/3” standard C connection (factor 1x)	456105	-	-	+
1.2	• TV camera 1/2” standard C connection (factor 0.5x)	456106	-	-	+
1.3	• TV camera 2/3” standard C connection (factor 0.63x)	456107	-	-	+
2	**ENG bayonet connection**				
2.1	• TV camera 2/3” ENG mount connection (factor 1x)	456115	-	-	+
2.2	• TV camera 2/3” ENG mount connection (factor 0.8x)	456117	-	-	+
	Video cameras and periphery device on request				

\+ Functions in conjunction with further additional equipment
\- Application not possible

1.5 Function elements

Item No.	Designation	Purpose/description
1	Beam changeover switch (Stemi 2000-C only)	Enables switchover from the right beam to the camera output
2	Camera output (Stemi 2000-C only)	Enables connection of photographic or video cameras
3	Clamping screw (Stemi 2000-C only)	For clamping the camera adapter
4	Binocular tube	Accommodates the two eyepieces; adjustable to the user's eye distance
5	Eyepiece	Enables eye error correction Every marking in the ± range: 1 diopter
6	Hexagon socket screw	Optional adjustment of continuous zooming and click stop
7	Clamping screw	Fixes the body of the microscope in the Stemi mount
8	Adjustment control	Alters the lamp voltage (brightness)
9	Power switch	Switches the power supply on/off (0 visible: Off)
10	Lamp switch (2x)	Switches the lamp connected the affiliated lamp connector On/Off (red bar visible: On)
11	Mains cable	Mains connection: 230 V - Euro-plug, 120 V - American flat plug
12	Lamp connector, 2-pol (2x)	Connection for lamp
13	Plastic plate b/w	Specimen support (detachable)
14	Stage spring (2x)	Fixes the specimen
15	Dust glass	Prevents dust ingress and serves as a mount for a ring lamp and analysers; when using attachment systems with the Stemi 1000, it must be replaced by the so-called "attachment system adapter"
16	Lamp	Illuminates the specimen
17	Built-in lamp adapter	Secures the lamp on the Stemi mount
18	Securing ring	Secures the Stemi mount

Item No.	Designation	Purpose/description
19	Focusing drive	Enables adjustment of the optical system to the specimen Max. adjustment range: 46 mm The left/right focusing drives are coupled mechanically; their ease of movement can be adjusted by means of the hexagon socket screw
20	Zoom adjustment	Enables continuous adjustment of the zoom range
21	Locking screw	Fixes the Stemi mount at working height

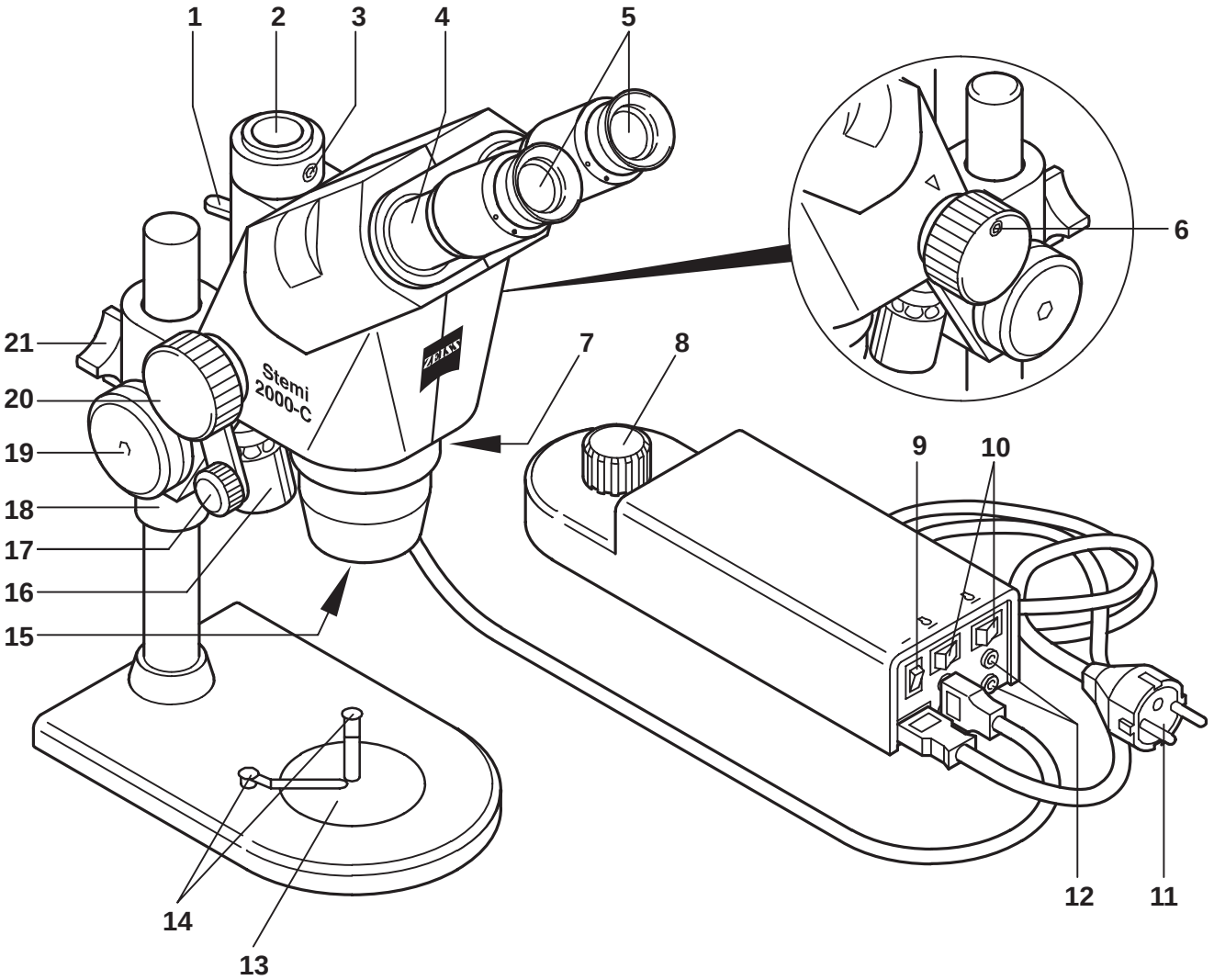
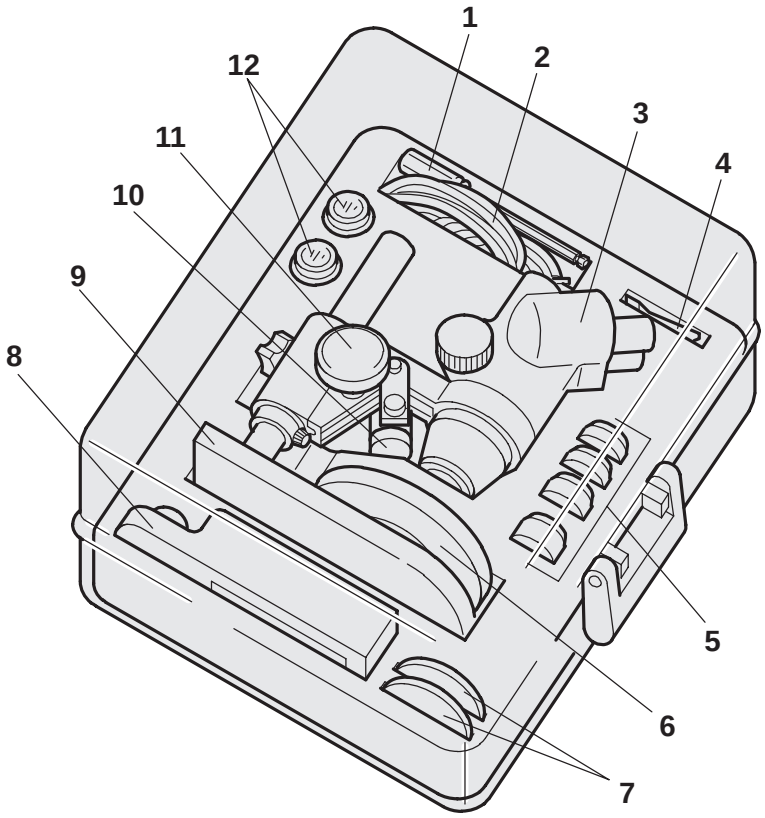


Figure 1-14 Function elements of the Stemi 2000-C

2 Operation

2.1 Unpacking and setting up

The components belonging to the microscope are optionally supplied in transport containers or in a city box.



1 3 mm hexagon socket screw key
2 Cable
3 Microscope body
4 Stage springs (2x)
5 Attachment systems
6 Sliding stage
7 Stage inlay plates (2x)
8 Power supply
9 Stand S
10 Lamp 10
11 Stemi drive
12 Eyepieces

Figure 2-1 Stemi 2000-C packing unit in city box

NOTE Components depend on the equipment of the microscope.
Accessories are supplied in commercially available packaging.

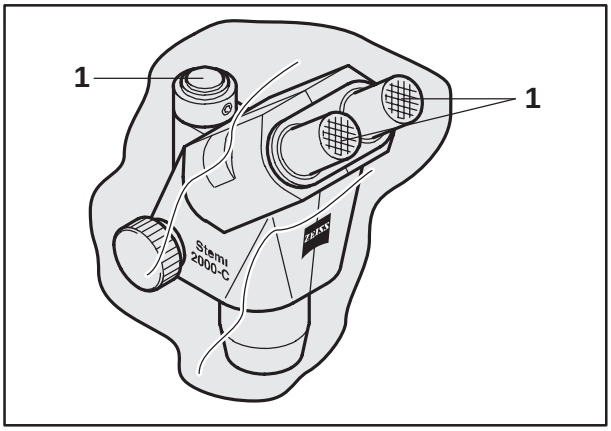


Figure 2-2 Unpacking

(1) Preparations

- Remove the transport sleeve from the body of the microscope.
- Remove the dust caps (2-2/1) from the tube openings.

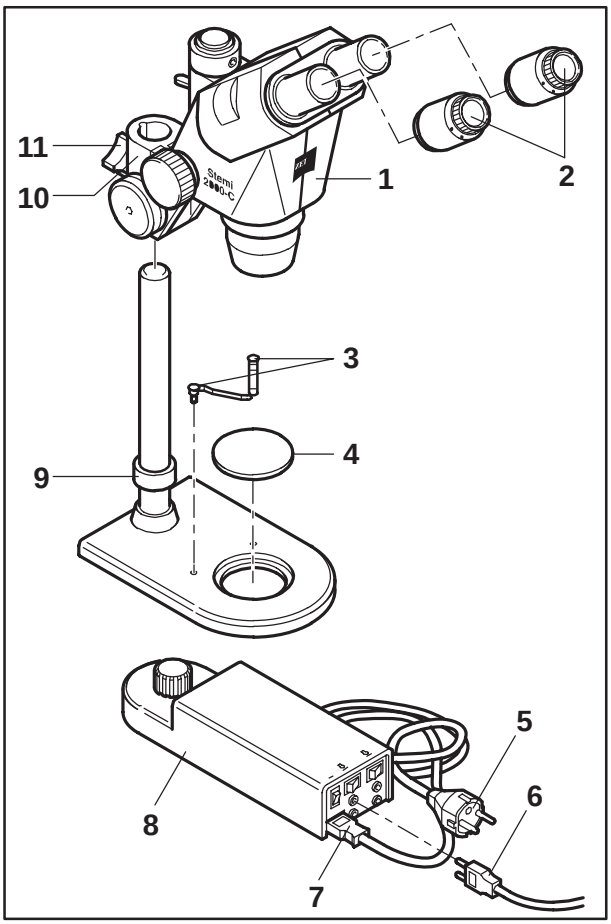


Figure 2-3 Setting up the Stemi 2000-C

(2) Setting up the stereo microscope

- Place the microscope (2-3/1) on your workbench.
- With the locking screw (2-3/11), fix the Stemi mount (2-3/10) at working height.
- If necessary, secure the Stemi mount with the securing ring (2-3/9).
- Insert the b/w plastic plate (2-3/4) in the mount and insert stage springs (2-3/3).
- Insert the eyepieces (2-3/2) in the tube connection. Turn the eyepieces until their reticules are oriented.
- Place the power supply (2-3/8) next to the stand S. Insert the plug of the lamp cable (2-3/6) in the required lamp connection.

IMPORTANT The unit's voltage must correspond to the mains voltage.

- Connect the mains cable (2-3/7) to the power supply.
  Plug in the mains plug (2-3/5).

2.2 Commissioning

Carry out the following work when commissioning for the first time.

- Unpack and set up the unit as described in Section 2.1 .
- The following adjustments have to be carried out before commissioning or as required.

(1) Adjusting the Stemi 2000 (applies to 1000/2000/2000-C)

- Adapt the binocular tube (2-4/7) to the user's eye spacing.
- Adjust the "0" mark on the eyepieces (2-4/1). without reticule: to the white point (2-4/2) with reticule: to the red point (2-4/3).
- Place a specimen in position and illuminate it if necessary.
- With the zoom adjuster (2-4/5), adjust the maximum magnification and focus on the specimen with the focusing drive (2-4/6).
- With the zoom adjuster (2-4/5), adjust the minimum magnification and, if necessary adjust the focus with the respective eyepiece (eye error correction).
- After adjustment, identical specimen details remain focused over the complete zoom range.

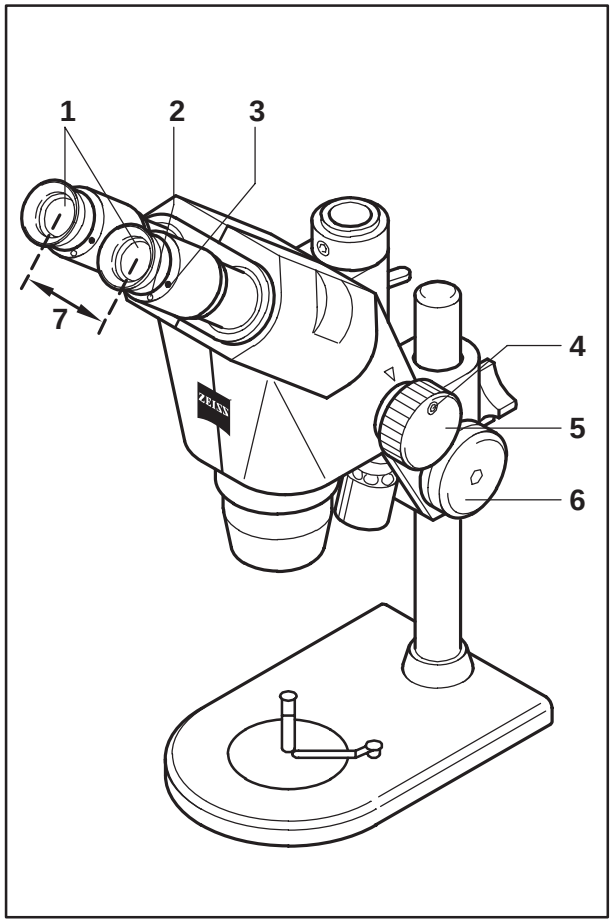


Figure 2-4 Adjusting the Stemi 2000-C

(2) Zoom adjustment

Continuous zooming or **click stop zooming** is possible with the Stemi. To do this, turn the 3 mm hexagon socket screw (2-4/4) slightly to the left or right to switch over from Continuous to Click Stop (and vice versa).

(3) Focusing the Stemi 2000

Assuming the Stemi 2000 is adjusted (Section 2.2 (1)), when focusing to different specimen levels the user merely has to readjust the Stemi mount by means of the focusing drive (if possible, with the maximum zoom factor).

2.2.1 Working with accessories

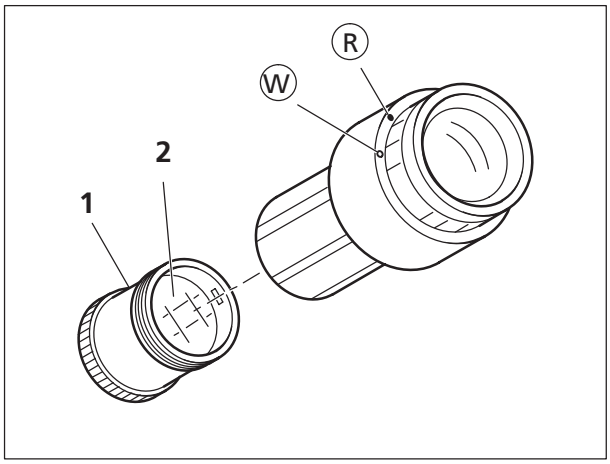


Figure 2-5 Inserting a graticule

(1) Graticules

- Eyepieces of type W-PI 10/23 Br. foc. are designed to allow the use of graticules.
- To compensate the image displacement caused by the extra glass path through the graticule, use the red dot (R) on the diopter scale as zero index instead of the white one (W).
- The graticules (2-5/2) come cemented in mounts with external screw threads (2-5/1) for easy exchange.
- To change a mounted graticule, unscrew the mount (2-5/1) and screw in the desired one instead.
- Check the focus and readjust it if necessary (Section 2.2 (1)).

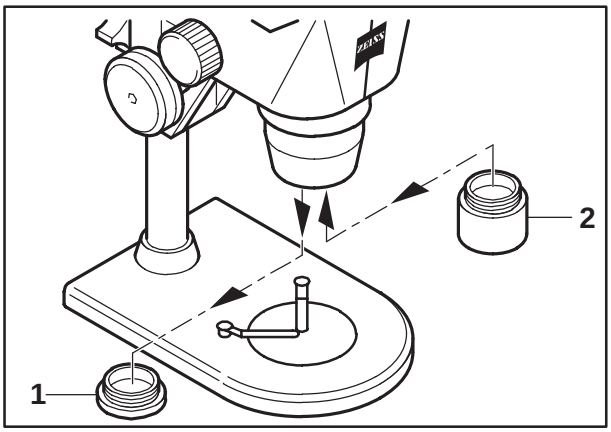


Figure 2-6 Fitting an attachment system to the Stemi 2000

(2) Attachment systems

- Unscrew the dust glass (2-6/1).
- Screw in the required attachment system (2-6/2).

IMPORTANT The free operating distance changes when using an attachment system. Pay attention to the table in Section 1.3(4)).

NOTE In the case of the Stemi 1000, in the ”attachment systems adapter” supplied, instead of the dust glass.
Then screw the required attachment system onto it.

(3) Polarisation contrast in transmitted light

- Insert the polariser (2-7/7) in the rotary stage (2-7/9) from below so that the dot marking on the polariser agrees with the line marking on the stage mount and secure it with two 2.0 mm hexagon socket screws (2-7/4).
- Take the glass plate ∅ 84 out of the option for transmitted light illumination (2-7/5). Insert the rotary stage in the mount (2-7/6) so that the dot markings of the polariser agree in the x direction.
- Fix the rotary stage with the eccentric clamp (2-7/3).
- Fit the glass plate ∅ 72 (2-7/10).
- Secure the specimen guide (2-7/11) with the 2.5 mm hexagon socket screw (2-7/12) or insert stage springs (2-7/2).
- Secure the analyser slide (2-7/14) on the dust glass (2-7/1) or on the attachment system.
- Slide in the slide (2-7/13) completely and adjust the graspball to y-direction.
- Slide in the λ-plate slide (2-7/8) with free passage in the beam path.
- In this position, the analyser and polariser are aligned to each other at 90° (dark setting).
- Fine orientation of the dark setting is possible by undoing the eccentric clamp (2-7/3) and by turning the rotary stage slightly.
- The polarisation-optical "Red I" function setting can be selected by sliding in the λ-plate slide (2-7/8).

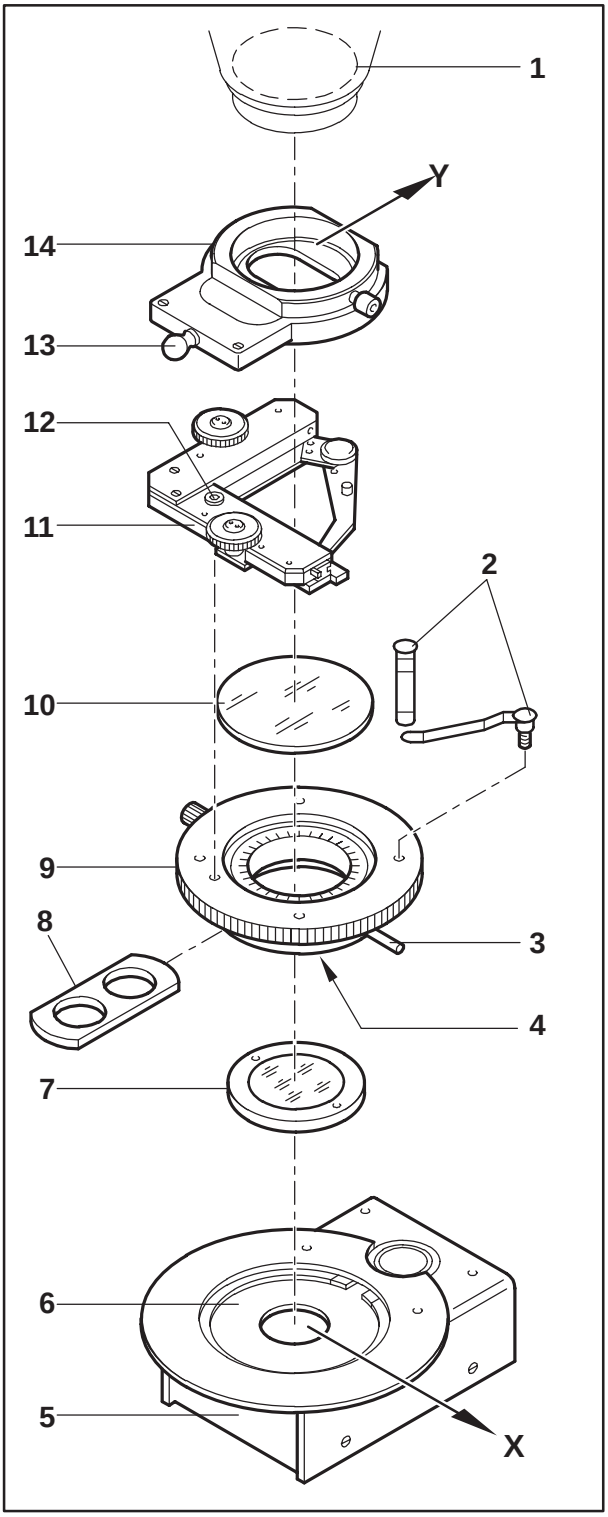


Figure 2-7 Mounting the polariser

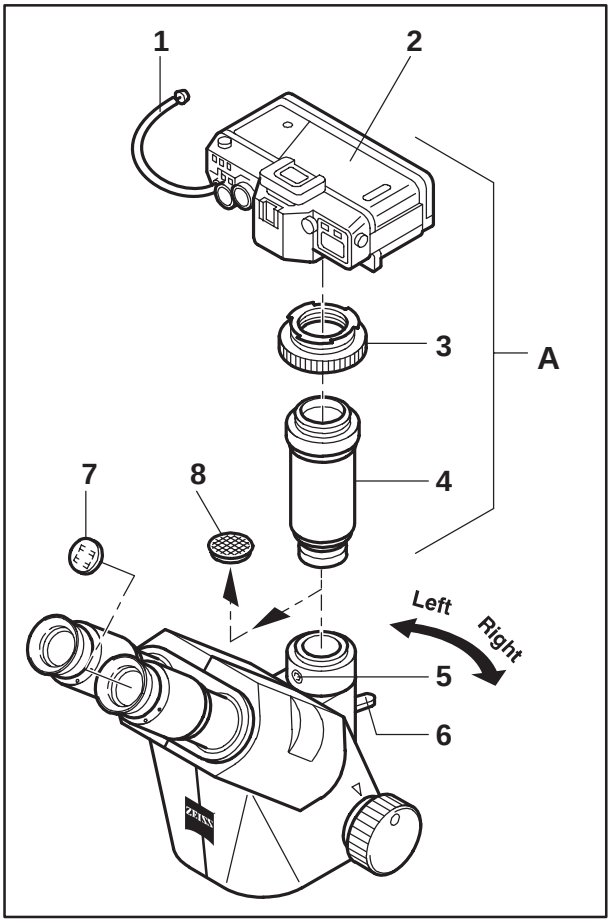


Figure 2-8 Mounting a reflex camera

(4) 35 mm photography with a reflex camera (e.g. CONTAX 167 MT)

- Select the suitable T2 adapter (2-8/3) for the camera system used and screw it onto the connection for a reflex camera 2.5x for T2 (2-8/4).
- Fit the camera (2-8/2) and, if necessary, attach the release (2-8/1).
- Insert the format reticule (2-8/7) if it is not intended to focus via the camera's viewfinder (Section 2.2.1 (1)).
- Detach the dust cap (2-8/8) from the camera tube and insert the pre-assembled unit A in the camera tube.
- Align the unit in the required position and secure it with the hexagon socket screw (2-8/5).
- Adjust the required method of viewing the specimen by means of the changeover switch (2-8/6).
  Right: camera not connected
  Left: camera connected.

NOTE Refer to operating manual G 42-406/II "35 mm reflex cameras for microscopes and stereo microscopes" for detailed information on reflex cameras.

(5) 35 mm photography with microscope camera MC 80 DX

- Detach the dust cap (2-9/6) and insert the ∅ 30 microscope camera connection (2-9/5) in the camera tube and secure it with the hexagon socket screw (3 mm) (2-9/7).
- Screw on the projecting lens P2.5x (2-9/4).
- Fit on the base (2-9/2) until it bottoms and fix it with the knurled screw (2-9/3).
- Insert film.
- Insert the film cartridge (2-9/1) (the EJECT knob springs out). To remove it, press EJECT.
- Connect the connecting cable (2-9/2) to the exposure control (2-9/10).

IMPORTANT The voltage of the exposure control must agree with the mains voltage.

- Plug in the mains plug (2-9/11).
- Use the format reticule (2-9/8) for focusing (Section 2.2.1 (1)).
- Adjust the required method of viewing the specimen by means of the changeover switch (2-9/9):
  Right: camera not connected
  Left: camera connected.

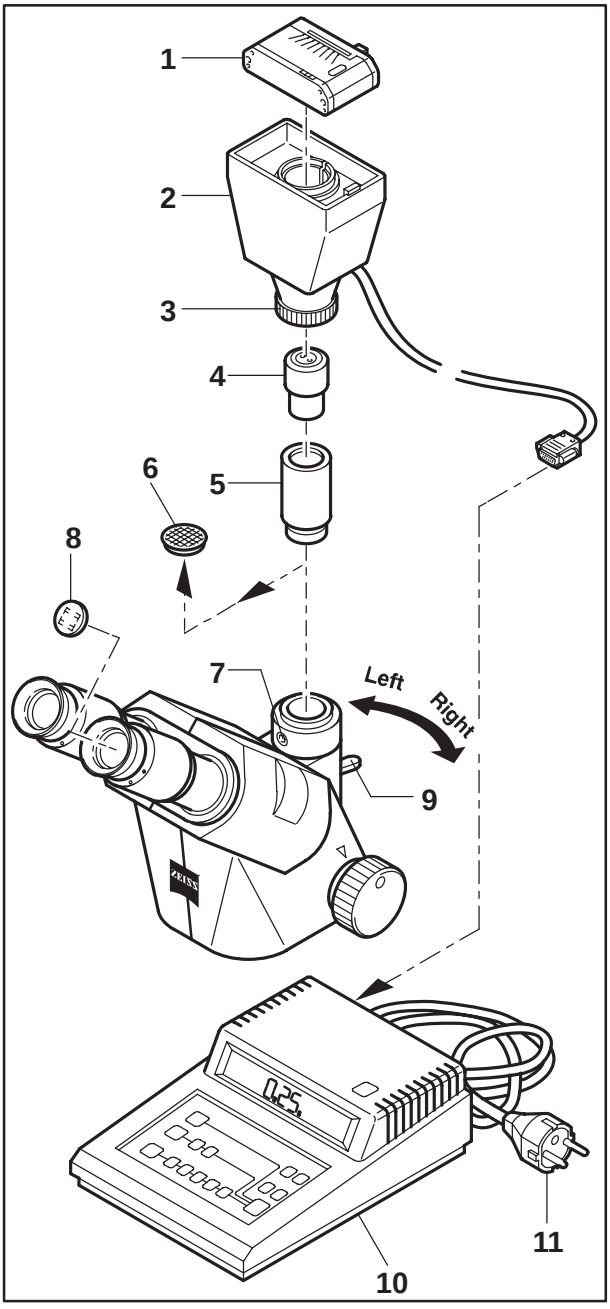


Figure 2-9 **Mounting the microscope camera MC 80 DX for 35 mm photography**

NOTE Refer to the operating manual G 42-407/I "Microscope camera MC 80 DX" for detailed information on the microscope camera MC 80 DX.

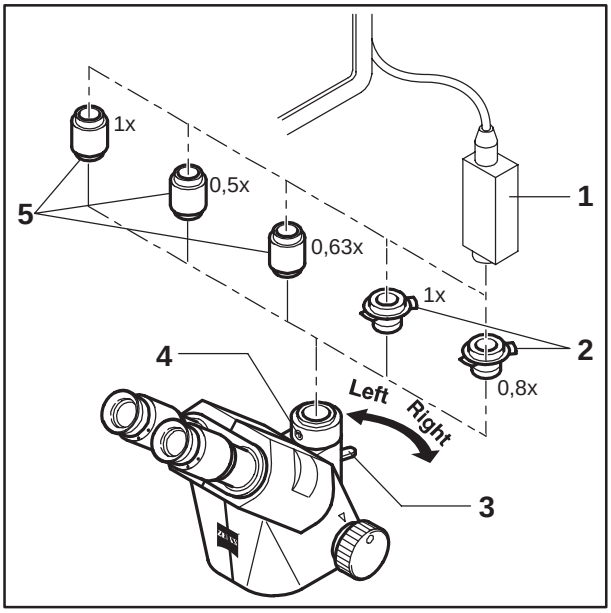


Figure 2-10 Mounting a video camera

(6) Video microscopy

- Depending on the video camera used, adapt the
  - – Standard C connection thread (2-10/5)

    or
  - – ENG-bayonet connection (2-10/2)

  to the video camera (2-10/1) used.
- Insert the pre-assembled unit in the camera tube, align it and secure it with the 3 mm hexagon socket screw (2-10/4).
- Adjust the required method of viewing the specimen by means of the changeover switch (2-10/3):
  Right: camera not connected
  Left: camera connected.

NOTE With regard to operation of video cameras, pay additional attention to camera manufacturers' instructions.

3 Fluorescence contrast with stereomicroscopes

Additional chapter for the following operating manuals:

- Stemi stereomicroscopes 1000/2000/2000-C/2000-CS and
- Stemi stereomicroscopes SV 6/SV 11/SV 11 Apo

3.1 FL S (Fluorescence for Stereomicroscope) configuration

3.1.1 Name and intended application

With the FL-S configuration, Carl Zeiss provides the possibility of performing fluorescence tasks with stereomicroscopes (regardless of their type).

The features of fluorescence contrast can thus be combined with the benefits of the various stereomicroscope models (orthoscopy, wide object fields and long working distances).

Application examples for the FL S configuration in combination with

Greenough-type stereomicroscopes (Stemi DV: Double Lens Vario) or **Telescope-type stereomicroscopes (Stemi SV: Single Lens Vario)**

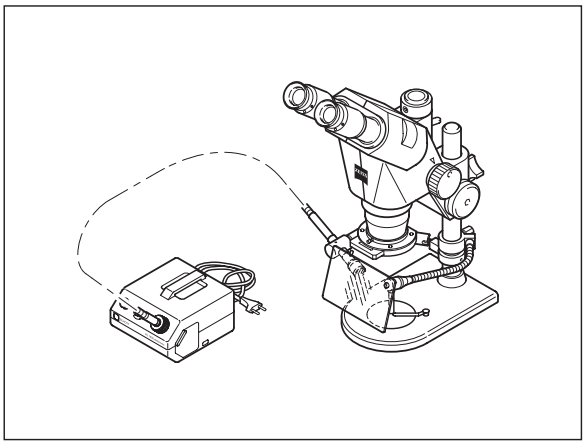


Fig. 3-1 Stemi 2000 with KL 1500 electronic

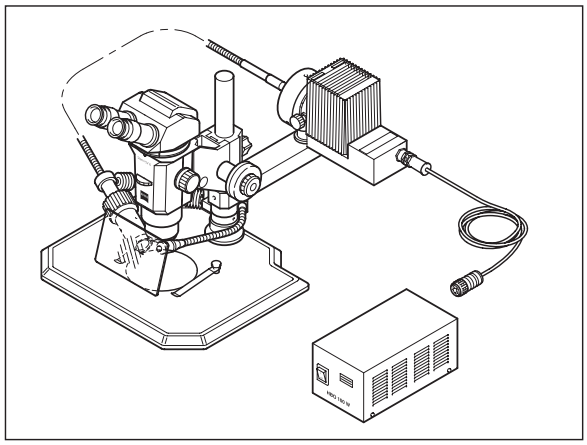


Fig. 3-2 Stemi SV 6 with HBO 100

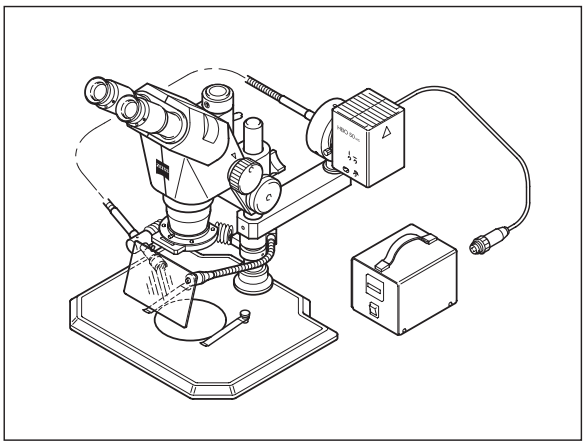


Fig. 3-3 Stemi 2000 with HBO 50

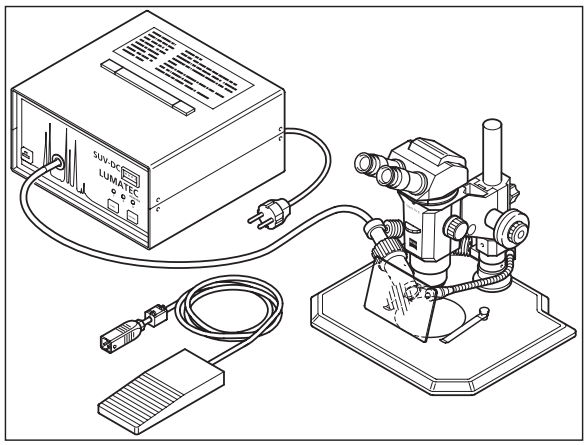


Fig. 3-4 Stemi SV 11 with LUMATEC HBO 200

3.2 Configurations

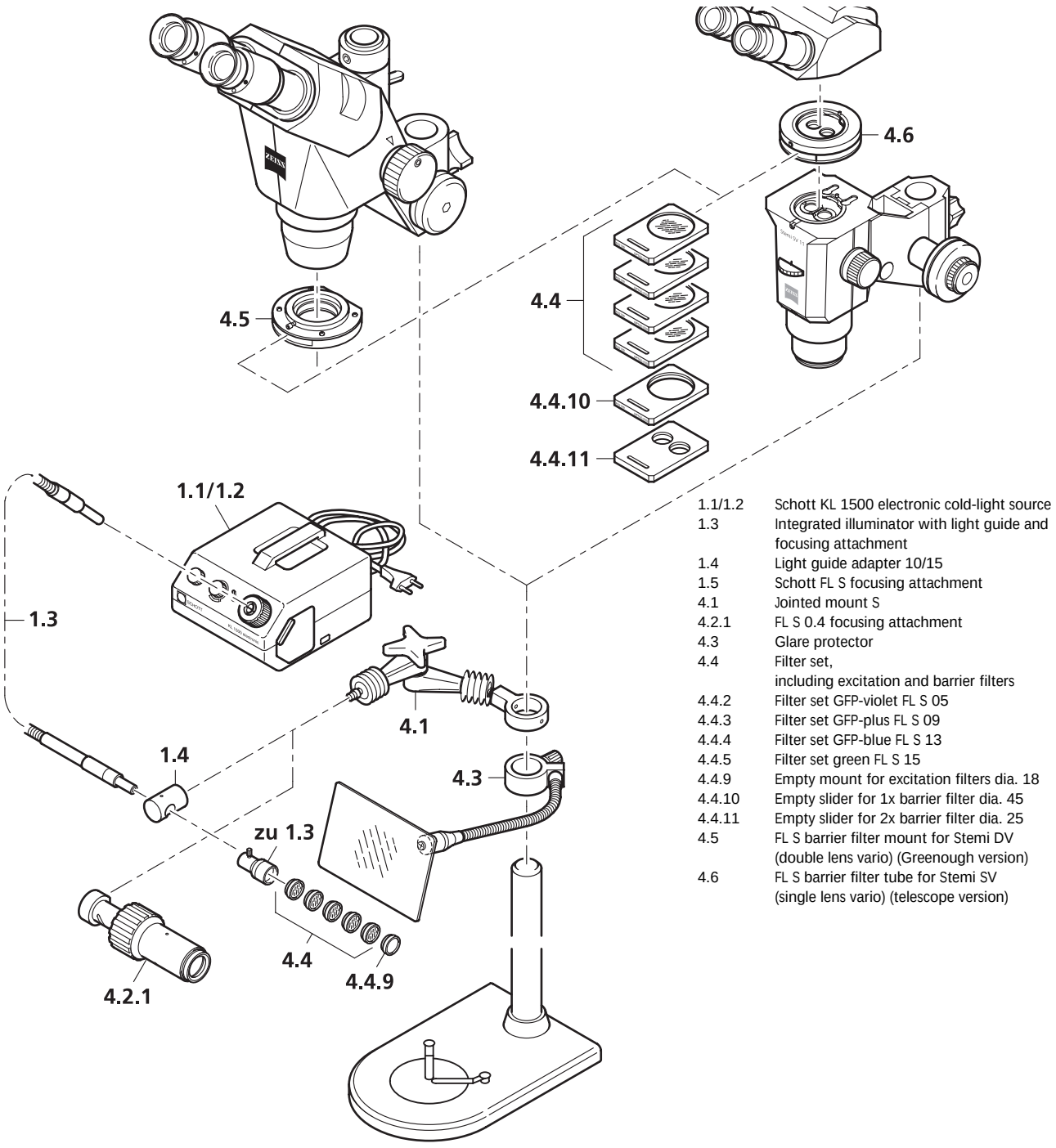
Regardless of which stereomicroscope is used, different configurations are possible:

No..	Description	Cat.No.	Comments
1	FL S configuration with KL 1500 electronic, consisting of:		
1.1	– Schott cold-light source KL 1500 electronic (230 V) or	495054-9801-000	
1.2	– Schott cold-light source KL 1500 electronic (120 V)	495054-9901-000	
1.3	– Integrated illuminator with light guide and focusing attachment	455145-1014-925	
1.4	– Light guide adapter 10/15		
1.5	– Schott FL S focusing attachment	417088-0000-000	
2	FL S configuration with HBO 50 or HBO 100, consisting of:		
2.1	– mount 32 for HBO 50/100	455188-0000-000	
2.2	– HBO 50 illuminator, consisting of:	487201-9804-000	
2.2.1	– HBO 50 lamp housing incl. lamp mount	447220-0000-000	
2.2.2	– Power unit for HBO 50, 220-240 V, 50-60 Hz, 350 VA	392642-0000-000	
2.3	HBO 100 illuminator, consisting of:	487207-9804-000	
2.3.1	– HBO 100 W/Z lamp housing, incl. lamp mount		
2.3.2	– power unit for HBO 100		
2.4	– collector for light guide	447250-0000-000	
2.5	– FL S liquid light guide, d=8 mm, l=1000 mm	417087-0000-000	
3	FL S configuration with LUMATEC HBO 200, consisting of:		
3.1	SUV-DC-P light source, LUMATEC HBO 200	000000-1023-506	
3.2	FL S liquid light guide, d=8 mm, l=1500 mm	000000-1023-507	
4	Accessories		
4.1	– jointed mount S	000000-1013-082	
4.2.1	– focusing attachment FL S 0.4	000000-1012-895	
4.3	– FL S glare protector	455177-0000-000	
4.4	– Filter sets, each consisting of excitation filter and barrier filter slider		
4.4.1	– UV FL S 02 filter set	000000-1015-034	Standard filter set
4.4.2	– GFP-violet FL S 05 filter set	000000-1015-035	Standard filter set
4.4.3	– GFP-plus FL S 09 filter set	000000-1015-036	Standard filter set
4.4.4	– GFP-blue FL S 13 filter set	000000-1015-037	Standard filter set
4.4.5	– FL S 15 green filter set	000000-1015-038	Standard filter set
4.4.6	– UV special FL S 02 HT filter set	000000-1017-341	LUMATEC HBO 200
4.4.7	– GFP-violet special FL S 05 HT filter set	000000-1017-342	LUMATEC HBO 200
4.4.8	– GFP-plus special FL S 09 HT filter set	000000-1017-343	LUMATEC HBO 200
4.4.9	– Empty mount for excitation filter dia. 18	000000-1013-083	customer's choice
4.4.10	– Empty mount for 1x barrier filter dia. 45	000000-1013-085	customer's choice
4.4.11	– Empty mount for 2x barrier filter dia. 25	000000-1013-084	customer's choice
4.5	– FL S barrier filter mount for Stemi DV (Double Lens Vario)	455031-0000-000	Stemi 1000/2000
4.6	– FL S barrier filter tube for Stemi SV (Single Lens Vario)	455176-0000-000	SV6/11/11 Apo

3.2.1 FL S configuration with KL 1500 electronic (not suitable for UV excitation)

Stemi 1000 / 2000 / 2000 C / 2000 CS (in Stemi mount)

Stemi SV 6 / SV 11 / SV 11 Apo (with Stemi mount)



1.1/1.2	Schott KL 1500 electronic cold-light source
1.3	Integrated illuminator with light guide and focusing attachment
1.4	Light guide adapter 10/15
1.5	Schott FL S focusing attachment
4.1	Jointed mount S
4.2.1	FL S 0.4 focusing attachment
4.3	Glare protector
4.4	Filter set, including excitation and barrier filters
4.4.2	Filter set GFP-violet FL S 05
4.4.3	Filter set GFP-plus FL S 09
4.4.4	Filter set GFP-blue FL S 13
4.4.5	Filter set green FL S 15
4.4.9	Empty mount for excitation filters dia. 18
4.4.10	Empty slider for 1x barrier filter dia. 45
4.4.11	Empty slider for 2x barrier filter dia. 25
4.5	FL S barrier filter mount for Stemi DV (double lens vario) (Greenough version)
4.6	FL S barrier filter tube for Stemi SV (single lens vario) (telescope version)

Fig. 3-5 FL S configuration with KL 1500 electronic

- Remove microscope body with Stemi carrier (6/2) and safety ring (6/3) from the stand S (6/4).
- Attach jointed mount S (6/1) and FL S glare protector (6/5) to the stand column.

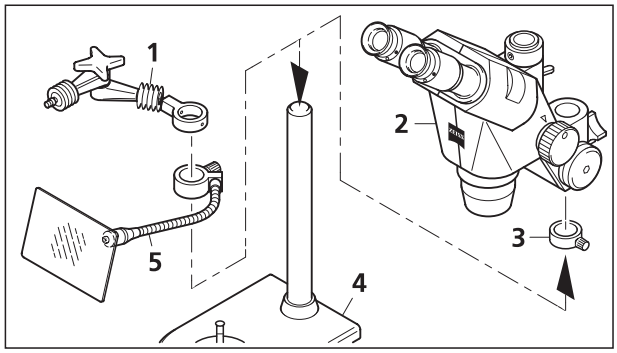


Fig. 3-6

- Place Schott KL 1500 electronic cold-light source (7/8) in position.
- Connect integrated light guide (7/1) to the cold-light source.
- Screw excitation filter (7/5) in the used focusing attachment (7/4 or 6).
- Attach end of integrated light guide (7/2) to jointed mount S (7/3) via light guide adapter 10/15 (7/7), attach focusing attachment (7/6) and clamp it.
- Attach focusing attachment (7/4) directly to jointed mount S (7/3).

NOTE
For the installation of the light guide and for the filter change please see the separate notes on page 3-10.

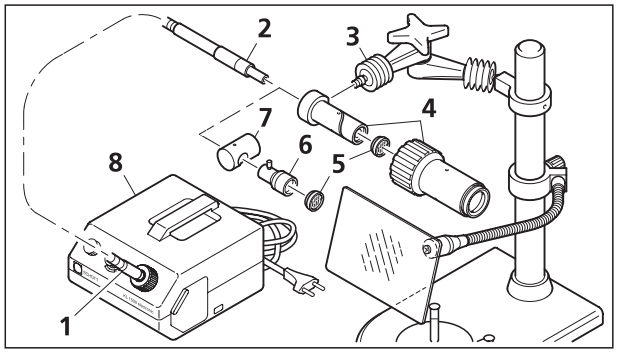


Fig. 3-7

- Attach microscope body (8/1) and, if required, safety ring (8/2) to the stand again.
- When using a Double Lens Vario microscope (Stemi 1000/ 2000), screw Allen screw (8/6) to the receptacle with dia. 53 using the screwdriver (8/5) to attach the barrier filter mount FL S (8/3).
- Push required barrier filter (8/4) into the barrier filter mount. (KL 1500 electronic not suitable for UV excitation!)
- Switch on KL 1500 electronic (9/7), align the luminous field diameter with the required object field and optimize illuminance through focusing and variations of the working distance.
- Adjust glare protector (9/6) for glare-free microscopy.

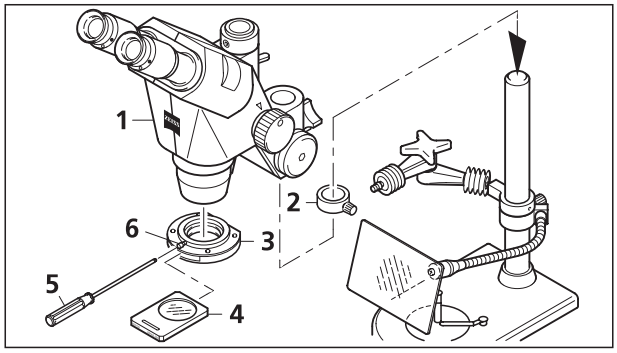


Fig. 3-8

- When using a Single Lens Vario microscope (SV 6/11), first remove the binocular tube (9/3) by unscrewing Allen screw (9/4).
- Insert the FL S barrier filter tube (9/2) between the microscope body (9/5) and the binocular tube (9/3).
- Attach the binocular tube to the barrier filter tube.
- Push required barrier filter slider (9/1) into the barrier filter tube.
- Switch on KL 1500 electronic (9/7), align the luminous field diameter with the required object field and optimize illuminance through focusing and variations of the working distance.
- Adjust glare protector (9/6) for glare-free microscopy.

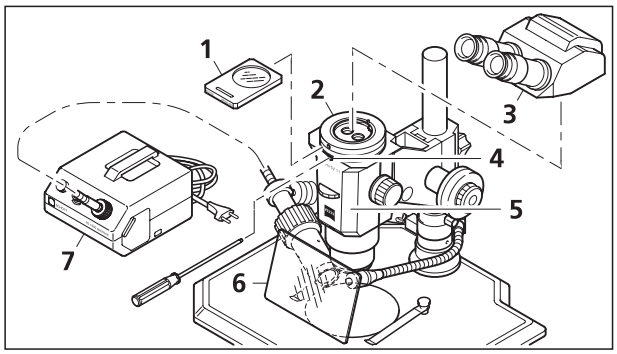


Fig. 3-9

3.2.2 FL S configuration with HBO 50 or HBO 100

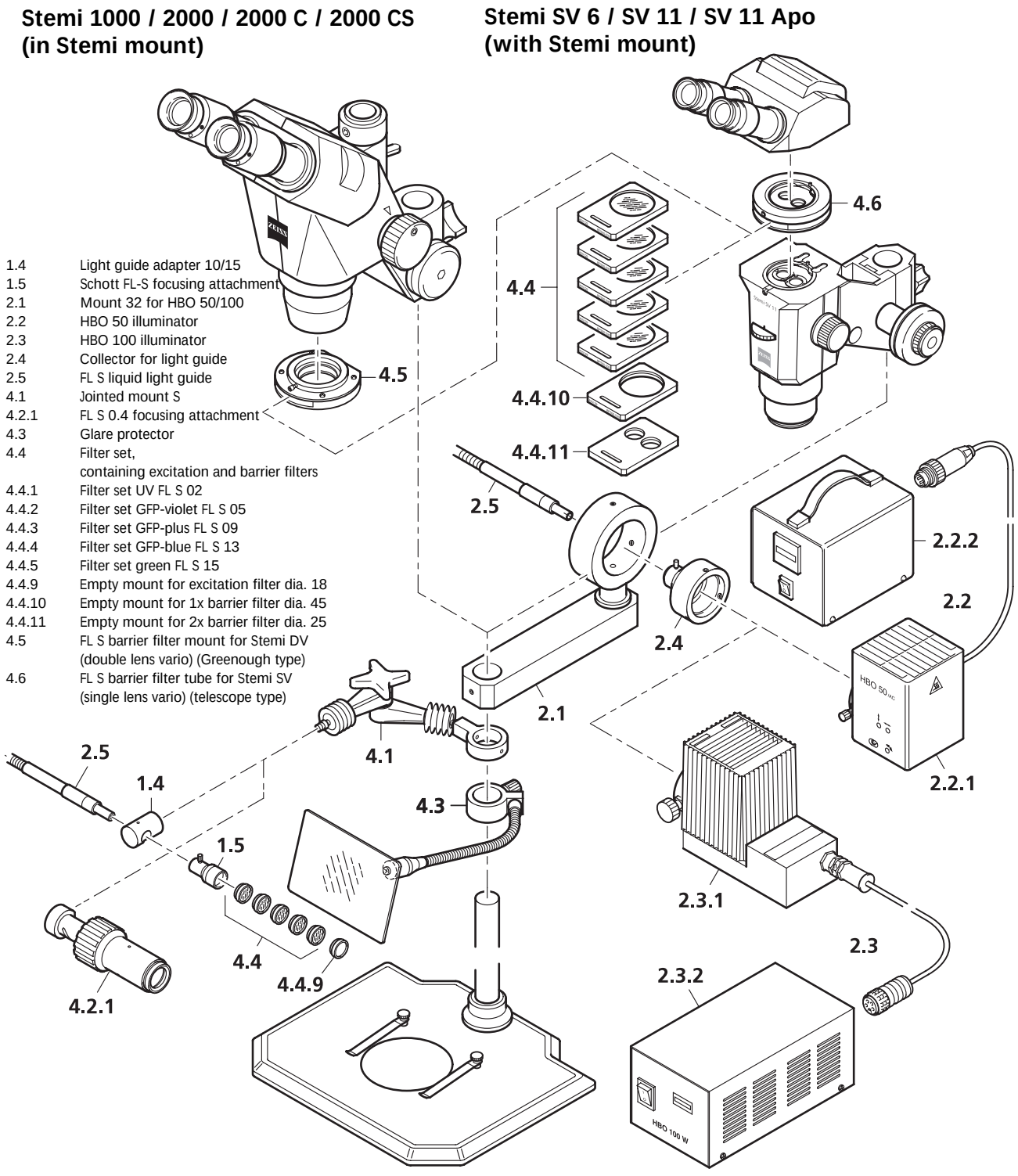


Fig. 3-10 FL S configuration with HBO 50 or HBO 100

- Remove microscope body (with Stemi carrier) (11/2) and safety ring (11/3) from stand N (11/4).
- Attach jointed mount S (11/6) and FL S glare protector (11/5) to the stand column.
- Also attach mount 32 for HBO (11/1) to the stand column.
- Adjust HBO 50/100 to ∞ in accordance with the operating instructions.
- Remove the dovetail from the HBO 50/100, then use 3 Allen screws to attach the collector for the light guide (12/4) to the HBO 50 (12/5) or HBO 100 (12/6).
- Attach collector with attached lamp housing to mount 32 for HBO (12/2) (3 Allen screws).
- Use fixation screw (12/3) to fix the liquid light guide (12/1) to the collector.

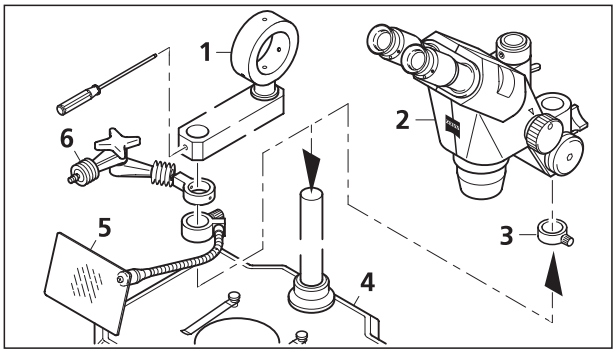


Fig. 3-11

- Screw excitation filter (12/8) into the focusing attachment used (12/7 or 9).

  NOTE
  For the installation of the light guide and for the filter change please see the separate notes on page 3-10.

- Attach liquid light guide (12/1) to the jointed mount S (12/11) via light guide adapter 10/15 (12/10), attach focusing attachment (12/9) and clamp it.
- Attach focusing attachment (12/7) directly to the jointed mount S (12/11).

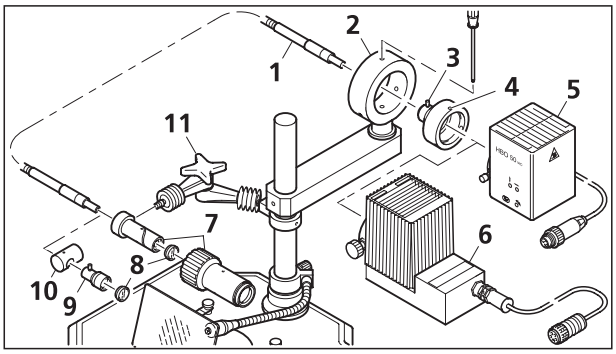


Fig. 3-12

- Attach microscope body (13/1) and, if required, safety ring (13/2) to the stand again.
- Connect lamp housing of the used illuminator to the relevant power unit (13/3 or 4) and connect the power unit to the line.

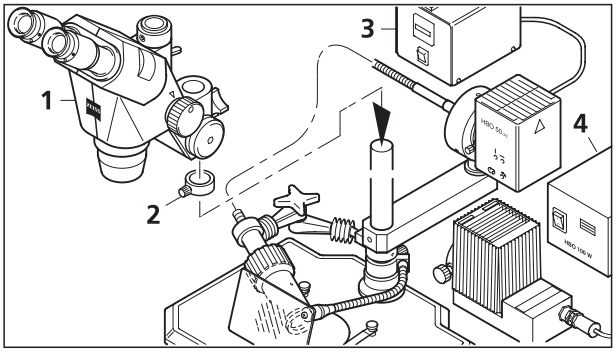


Fig. 3-13

- Depending on the used microscope version, attach FL-S barrier filter mount (14/1) for the DV-version or FL S barrier filter mount (14/2) for the SV-version together with the required barrier filter slider (14/3) (for a detailed description please see Figs 3-8 and 3-9).

  NOTE
  For UV excitation, please remember to use the barrier filter slider (protects your eyes from reflecting/scattered UV excitation light)!
- Switch on illuminator (HBO 50 or HBO 100), align the luminous field diameter with the required object field and optimize illuminance through focusing and variations of the working distance.
- Adjust glare protector (14/4) for glare-free microscopy.
  CAUTION
  Glare to the eyes and direct irradiation of the skin with excitation light must be avoided.

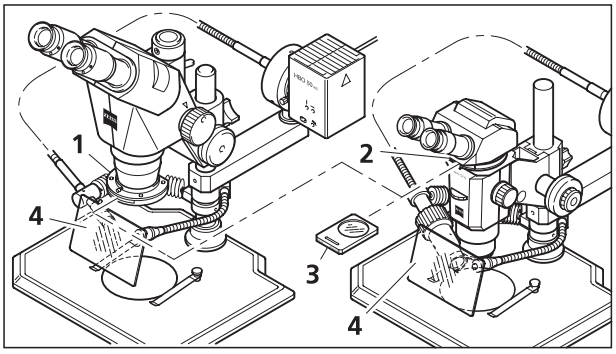


Fig. 3-14

3.2.3 FL S configuration with LUMATEC HBO 200

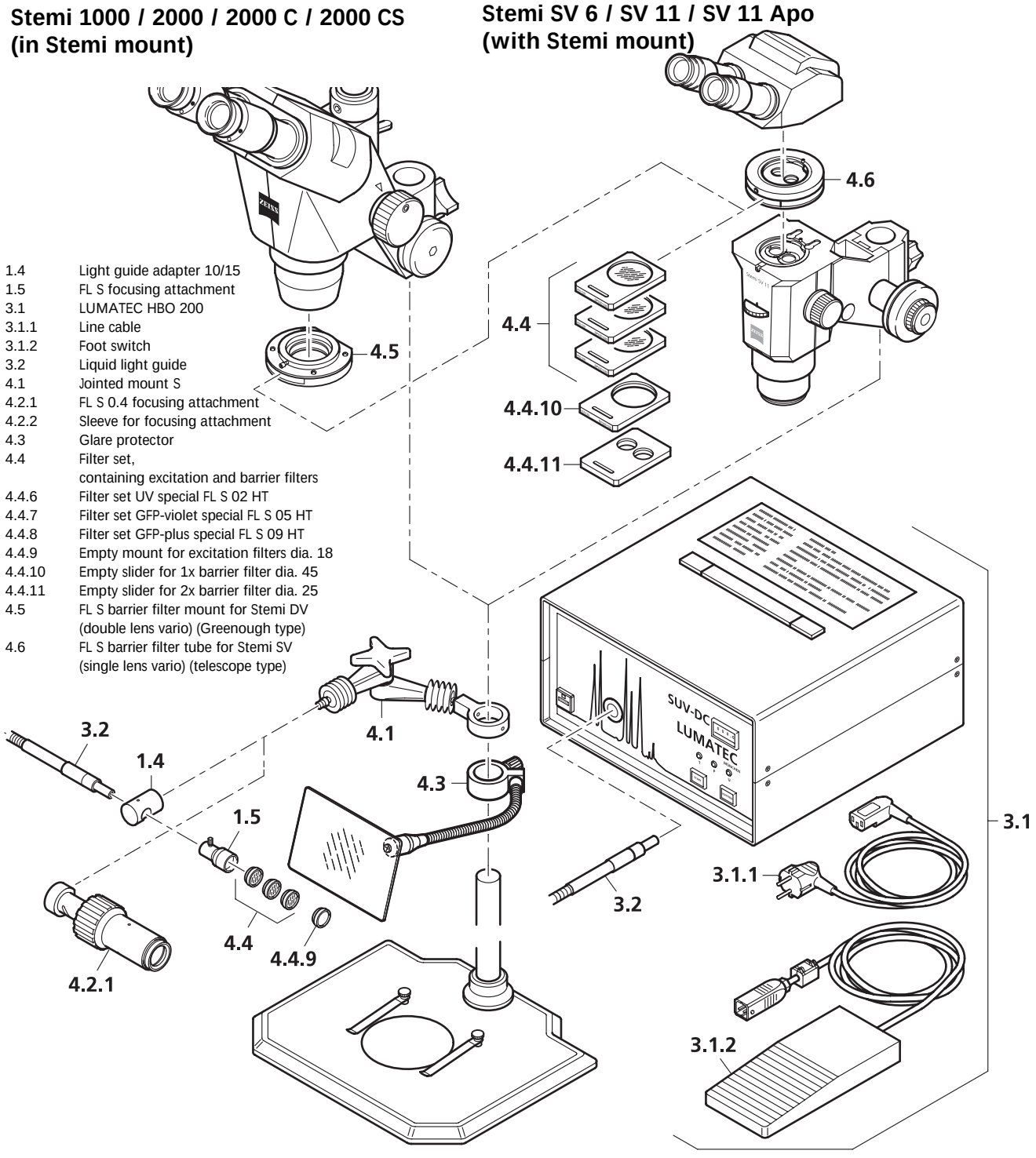


Fig. 3-15 FL S configuration with LUMATEC HBO 200

- Remove microscope body with Stemi mount (16/4) and safety ring (16/5) from stand N (16/3).
- Attach jointed mount S (16/1) and FL S glare protector (16/2) to the stand column.
- Then attach microscope body to the stand column again.

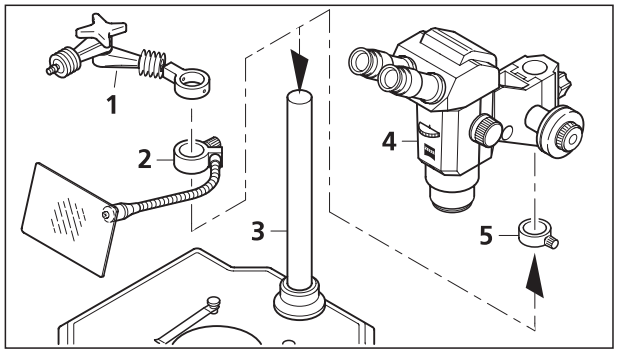


Fig. 3-16

- Place LUMATEC HBO 200 SUV-DC-P light source (17/2) in position, connect foot switch (17/4) to the instrument rear, connect the unit to the line via line cable (17/3) (also see the LUMATEC HBO 200 operation manual and the safety notes included!).
- Connect the liquid light guide (17/1) to the front of the LUMATEC HBO 200 light source (use liquid light guide of 1500 mm length with special connector for LUMATEC HBO 200).
- Screw temperature-resistant special excitation filter (18/3) into the used focusing attachment (18/2 or 4).
- Attach liquid light guide (18/6) to jointed mount S (18/5) via light guide adapter 10/15 (18/1), attach focusing attachment (18/2) and clamp it.

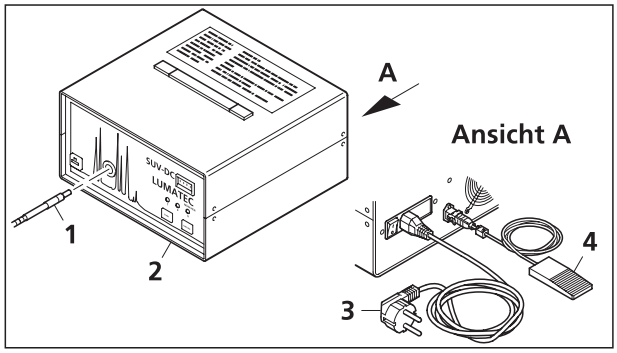


Fig. 3-17

- Attach focusing attachment (18/4) directly to the jointed mount S (18/5).

  NOTE
  For the installation of the light guide and for the filter change please see the separate notes on page 3-10.

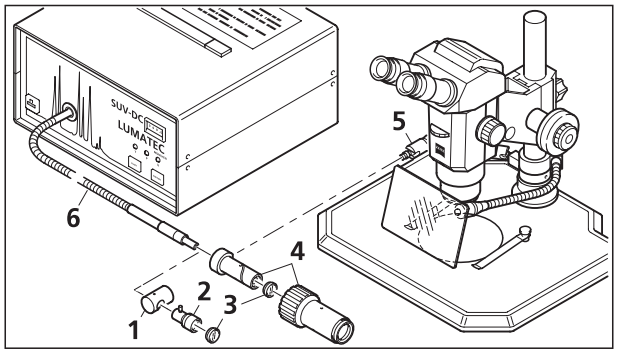


Fig. 3-18

- Depending on the used microscope version, attach FL-S barrier filter mount (19/1) for the DV-version or FL S barrier filter mount (19/2) for the SV-version together with the required barrier filter slider (14/3) (for a detailed description please see Figs 3-8 and 3-9).

  CAUTION Only use the temperature-resistant special filter sets for the LUMATEC HBO 200.
  NOTE For UV excitation, please remember to use the barrier filter slider (protects your eyes from reflecting/scattered UV excitation light)!
- Switch on LUMATEC HBO 200 illuminator in accordance with the instrument manual, align the luminous field diameter with the required object field and optimize illuminance through focusing and variations of the working distance.
- Adjust glare protector (19/4) for glare-free microscopy.
  CAUTION Glare to the eyes and direct irradiation of the skin with excitation light must be avoided. If excitation is not required, close the shutter which is integrated in the LUMATEC HBO 200.

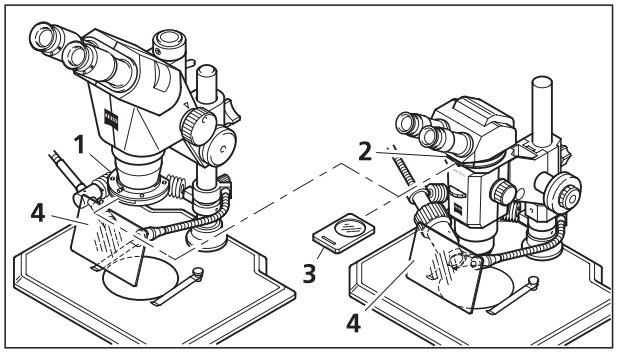


Fig. 3-19

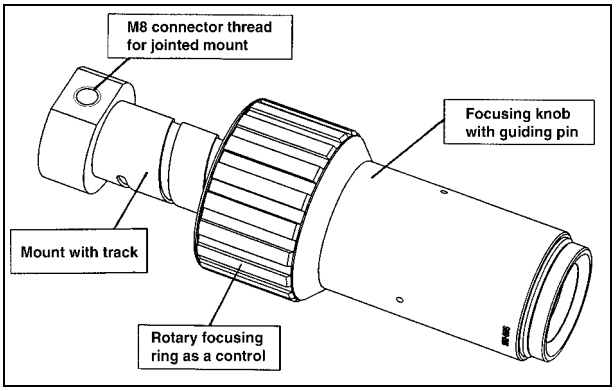


Fig. 3-20

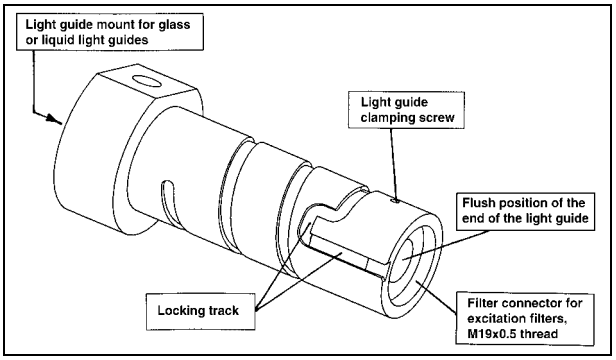


Fig. 3-21

NOTES

- For installation of the light guide and for filter change, the focusing head must be separated from the mount. To unlock the focusing attachment, push it through the track until the end. Then slightly push back the focusing knob at the bottom end of the track, slightly turn it and remove it in forward direction (also see Figs 3-20 and 3-21).

- To mount the light guide, push the end of the light guide into the light guide mount until it is flush with the inner plane surface of the excitation filter connector, and carefully clamp it using the clamping screw and the enclosed Allen key (also see Fig. 3-21).

4 Care and troubleshooting

4.1 Care

Care of the stereo microscope is limited to the following:

- Cover the unit with the dust cover after every use.
- Do not place the unit in a humid room.
- Cover open tubes with dust caps.
- Remove dust from optical surfaces with a rubber blower or with a natural hair brush. Degrease the brush in alcohol and then dry it. Remove stubborn soiling and fingerprints with a dust-free cloth or leather.
- Use commercially available optical and spectacle cleaning cloths to remove extreme soiling (e.g. fingerprints) from optical surfaces; if necessary, moisten cloths lightly with petroleum ether. If necessary, clean the front surfaces of lenses with petroleum ether, but do not use any alcohol.

Pay attention to the following notes when using the Stemi in moist and warm climates:

- Store the Stemi in bright, dry and well ventilated rooms with a humidity less than 65 %; store particularly sensitive modules and accessories such as lenses and eyepieces in dry cabinets.
- When storing the microscope or its parts in closed receptacles for longer periods of time, fungi can largely be avoided by placing absorbent substances soaked in fungicide in the receptacles.

IMPORTANT Fine mechanical and optical devices are always at a risk of fungus affection under the following conditions:

- relative humidity > 75 % for more than three days at temperatures from + 15 °C to +35 °C.
- placing them in dark rooms where there is no movement of air and
- in the event of dust deposits and fingerprints on optical surfaces.

4.2 Troubleshooting and service

Troubleshooting on the Stemis is limited to few activities:

- Checking of the power supplies.
- Checking of the illuminating devices.

(1) Checking of the power supplies

The checking of the power supplies is limited to the inspection of the fuses in:

☞ Power supply 230 V – 6 V 50 VA, order No. 458420
fuse: T 400mA/L 250 V (5 x 20) mm acc. IEC 127

☞ Power supply 120 V – 6 V 50 VA, order No. 458421
fuse: T 500mA/L 250 V (5 x 20) mm acc. IEC 127

☞ Power supply unit, establised 6 V 20 W, 115 – 230 V,
variable 1,5 ... 6 V, 50 ... 60 Hz, 40 VA, order No. 458415
fuse: T 4,0 A/H 250 V (5 x 20) mm acc. IEC 127

☞ Schott cold light source KL 750 (230 V), order No. 417080
fuse: T 630 mA/L 250 V (5 x 20) mm acc. IEC 127

☞ Schott cold light source KL 750 (120 V), order No. 417081
fuse: T 1,25 A/H 250 V (5 x 20) mm acc. IEC 127

☞ Schott cold light source KL 1500 electronic (230 V), order No. 417075
fuse; primary: T 2 A/H 250 V (5 x 20) mm acc. IEC 127
secondary: T 10 A/H 250 V (5 x 20) mm acc. IEC 127

☞ Schott cold light source KL 1500 electronic (120 V), order No. 417076
fuse; primary: T 4 A/H 250 V (5 x 20) mm acc. IEC 127
secondary: T 10 A/H 250 V (5 x 20) mm acc. IEC 127.

When checking the power supplies, also check the mains cables. Defective cables must be replaced.

(2) Checking of the illuminating devices

Check the illuminating devices including a check of the power supply units used (switch settings, mains connection) and if necessary, replace the halogen lamp.

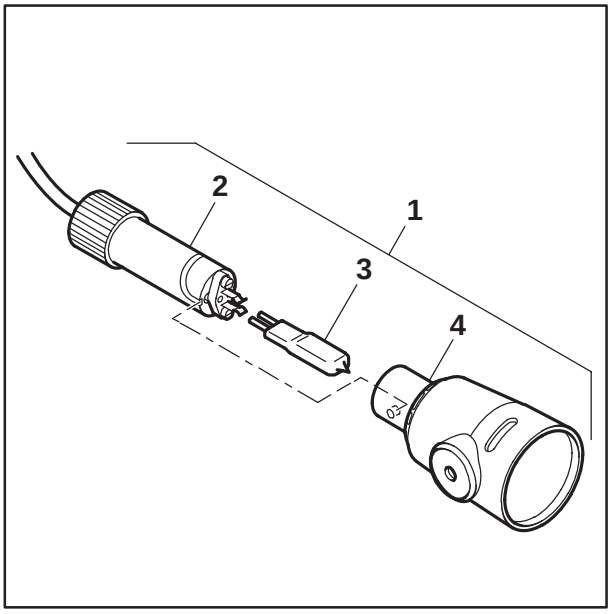


Figure 4-1 Lamp replacement

- Isolate the lighting unit (4-1/1) from the mains.
- Undo the lamp housing (4-1/4) from the lamp holder (4-1/2) by turning it lightly to the left.
- Pull the defective halogen lamp (4-1/3) out of the lamp holder and replace it by a new halogen lamp of the same type (6 V 10 W).

IMPORTANT Do not touch lamp bulbs with your bare hands; if necessary, clean the bulb **before** the first use with pure alcohol to prevent baking in of soiling.

(3) Service

All tampering on optical parts or motion elements in the interior of the unit or on the power supply may only be carried out by service specialists or specially **authorised** personnel.

For servicing, contact your nearest regional representative or

Carl Zeiss Jena GmbH
Zeiss Gruppe
Unternehmensbereich Mikroskopie
Tatzendpromenade 1a
D-07745 Jena

Telefon: (03641) 64-2936
Telefax: (03641) 64-3144
Internet: micro@zeiss,de
http://www.zeiss.de

ANNEX

List of abbreviations

B & L	Bausch and Lomb
b/w	Black & white
CTV	Colour Television
EN	European standard
ENG	Electronic News Gathering
foc.	Capable of focusing
FOD	Free operating distance
HAL	Halogen
IEC	International Electrotechnical Commission
IP	International Protection
ISO	International Organization for Standardization
MC	Microscope Camera
mf	Microphotography
SK	Class of protection
SLR	Single Lens Reflex
Spect.	Spectacles
Stemi	Stereo microscope
TV	Television
W	Wide field
W-PL	Wide field, plane

APPENDIX C

This appendix contains specific information about the program, such as why specific solutions where chosen and the block diagrams of the Sub VI-s used. It is not necessary for understanding the overall solution, and that is why it is not included in Chapter 5. Nevertheless, it has been considered meaningful to include this appendix for advanced programmers and further interested professionals.

1. GENERAL CONCEPTS

1.1. TIMING OF WHILE LOOPS

Each *while loop* includes a waiting time of some milliseconds. If this is not done, the LabVIEW program uses all the capacity of the CPU, making it impossible to deal with other processes. This delay is introduced with a timeout clause (if there is an Event Structure) or with the Wait (ms) VI (if there is no Event Structure).

2. INITIALISATION BLOCK

2.1. IMAQ CREATE AND IMAQ DISPOSE

The IMAQ Create VI is used in the initialisation part to create temporary memory locations for images. All those references have to be destroyed with the IMAQ Dispose VI when the program finishes (frame 1 of the stacked sequence in the producer structure part).

2.2. OCCURRENCES

Occurrences functions are used to control separate, synchronous activities. They are used when it is wanted one VI or part of a block diagram to wait until another VI or part of a block diagram finishes a task without forcing LabVIEW to poll.

It is possible to perform the same task using global variables, with one loop polling the global variable until its value changes. However, global variables use more overhead because the loop that waits uses execution time. With occurrences, the second loop becomes idle and does not use processor time. When the first loop/structure sets the occurrence, LabVIEW activates the second loop and any other block diagrams that wait for the specified occurrence [16].

Palette object	Description
Generate occurrence	Generates an occurrence that can be passed to the Wait on Occurrence and Set Occurrence functions.
Set occurrence	Sets the specified occurrence. All nodes waiting for this occurrence stop waiting.
Wait on occurrence	Waits for the Set Occurrence function to set the given occurrence.

Figure 1- Occurrences: VI-s.

3. REAL-TIME IMAGE ACQUISITION

3.1. ZOOM TOOL1 AND ZOOM TOOL 2

Each time the user zooms in or out of “Picture taken image” or “Picture taken image 2”, the scale bar superimposed needs to be actualised. The solution thought for this was to remove the default zoom tool from the indicators and to create two new buttons (“Zoom tool1” and “Zoom tool2”) representing the zoom tools (the first thing done when clicking in those bottoms is to select the zoom tool via a property node).

For each of those button interactions, there is a Value Change event, giving queue number 2 information to enter to cases “Project scale zoom1” or “Project scale zoom2” in the Consumer 2 structure. Those cases contain a *while* loop, where there is another event structure with Mouse Down events inside a *Case of* structure: the Mouse Down events, represent a zoom in or zoom out action. When the user changes the tool, the *while* loop is left via the *Case of* Default frame.

This structure of programming is impossible if it is used the default zoom tool, since in that case the event to detect first in the Producer structure would be a Mouse Down event. After detecting the Mouse Down event, it would be necessary to distinguish that the action is a zooming one (there are more tasks related to Mouse Down events) via a *Case of* structure. It has been considered that this solution is better in order to avoid having a lot of *Case of* structures inside Mouse Down events.

4. PROJECTION OF SCALES

4.1. REAL-TIME IMAGE PROBLEM

It is a challenge to project the scale bar in the real-time image being acquired, since each time a new frame is acquired the overlay information present is deleted.

The first approach was to project the scale bar with IMAQ Overlay Functions, as in the other images. However, for that purpose the part of the program doing those operation needs to be included in the Producer structure, executing each time that a new frame is being acquired. This is very time consuming and it is unacceptable for this application, since the Producer loop has to be as fast as possible to have a high rate of frames/second being acquired.

That is the reason why the current solution was thought. A picture control (“Realscale”) is used to the easiness of making its background invisible (only drawn or displayed information in it is seen). Thanks to that feature, it is possible to put it in the front panel in front of the “Real-time image” indicator, only being

the scale bar what is seen. In the initialisation part, the position and area of "Realscale" are adjusted so that it matches the "Real-time image" indicator position and proportions. Then, Draw Multiple Lines VI and Draw Text at Point VI are used in the Real Scale Sub VI to draw the proper scale in this "Realscale" picture control.

The drawbacks of having this picture control in front is that the zoom-in and –out process is double stepped: in the first click the "Realscale" control is made invisible[1] (using a property node) and in the second one the new actualised zoomed scale is projected in "Realscale", making this last one visible again.

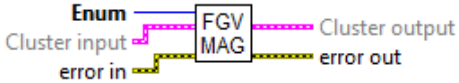
The same problem happens with the "Video" indicator when recording a video. In that case, the name of the picture control used is "Videoscale". However, in this case no zooming is possible, so the problems associated with it disappear: it is only needed to project once the scale bar in "Videoscale" using Real Scale Sub VI.

5. CUSTOMIZED SUB VI-S PROGRAMMING

The software architecture of the Sub VI-s is not presented in detail in the chapters 4 and 5 of the thesis, since it could lead to confusions regarding that the main topic is the main program software architecture. That is why this topic is included here.

5.1. FGV SUB VI

Basic Functional Global variable used to store the values of "Selected microscope" (string type), "Selected magnification" (double type) and "pxµm" (it contains the px/µm information; double type) in a cluster. The Sub VI has 3 entries and 2 exits:



Entries:

- **Enum:** unsigned word with two options: "Write" (to write new values in the FGV) and "Read" (to read the values stored in the FGV shift registers).

- **Cluster input:** connected when Enum=Write. Three elements: "Selected microscope" (string type), "Selected magnification" (double type) and "pxµm" (double type).

[1] The "Realscale" control background is already invisible for the user, but for the mouse it is in front of the "Real-time image" indicator. "Invisibility" here means to make the drawn information in the picture invisible also, making it invisible for the mouse.

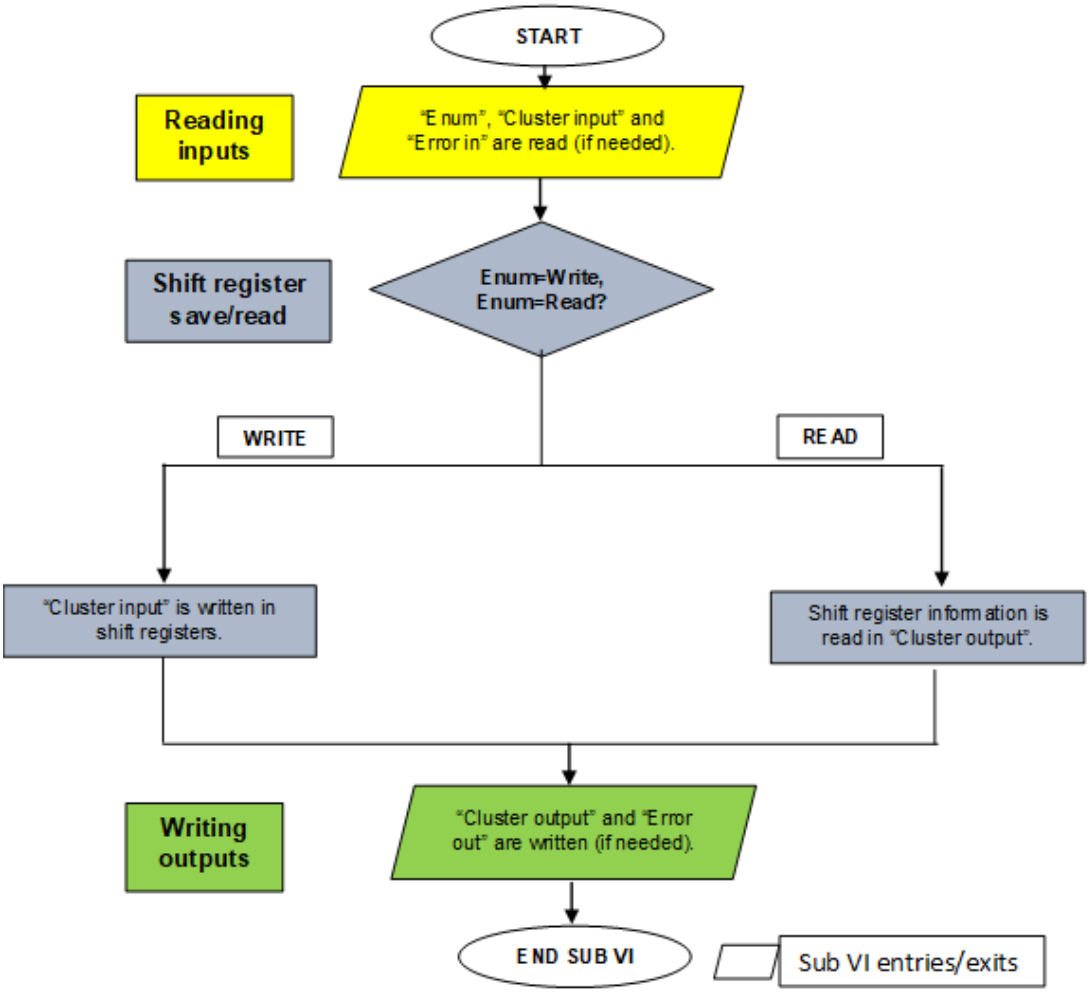
- **Error in:** error line. Gives notion of the chronology of the program and translates error codes in the case it happens one.

Exits:

- **Cluster output:** connected when Enum=Read. Three elements: “Selected microscope” (string type), “Selected magnification” (double type) and “pxµm” (double type).

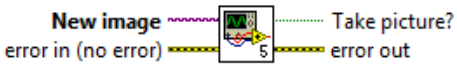
- **Error out:** error line. Gives notion of the chronology of the program and translates error codes in the case it happens one.

The flow diagram of the Sub VI is the following:



5.2. IMAGE TAKEN SUB VI

Sub VI used for asking the user if the image or picture taken by the him/her was correct or not. The Sub VI has 2 entries and 2 exits:



Entries:

- **New image:** real-time image which is being captured in the exact moment the user clicks the “Take picture” button.

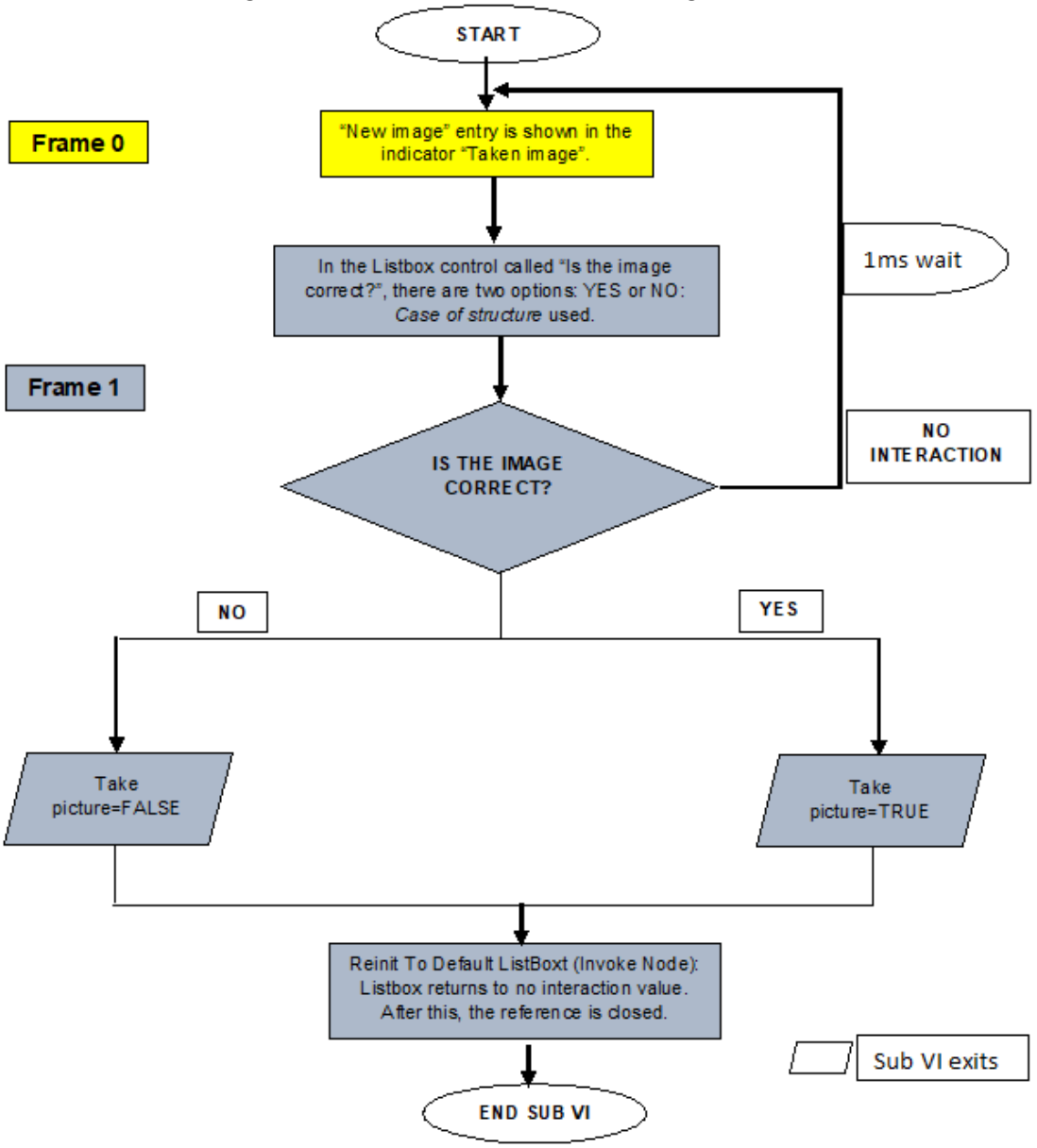
- **Error in:** error line. Gives notion of the chronology of the program and translates error codes in the case it happens one.

Exits:

- **Take picture?:** a Boolean type exit. It is TRUE if the user clicks YES in the LISTBOX. If the user clicks NO, it is FALSE.

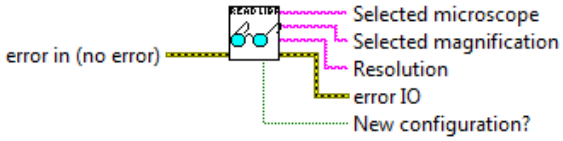
- **Error out:** error line. Gives notion of the chronology of the program and translates error codes in the case it happens one.

The flow diagram of the Sub VI is the following:



5.3. READLIBRARY SUB VI

It is used to read from the database the information with Datatype='Calibration'. The Sub VI has 1 entry and 5 exits:



Entries:

- **Error in:** error line. Gives notion of the chronology of the program and translates error codes in the case it happens one.

Exits:

- **Selected microscope:** a string type exit. It gives the name of the microscope the user selects.

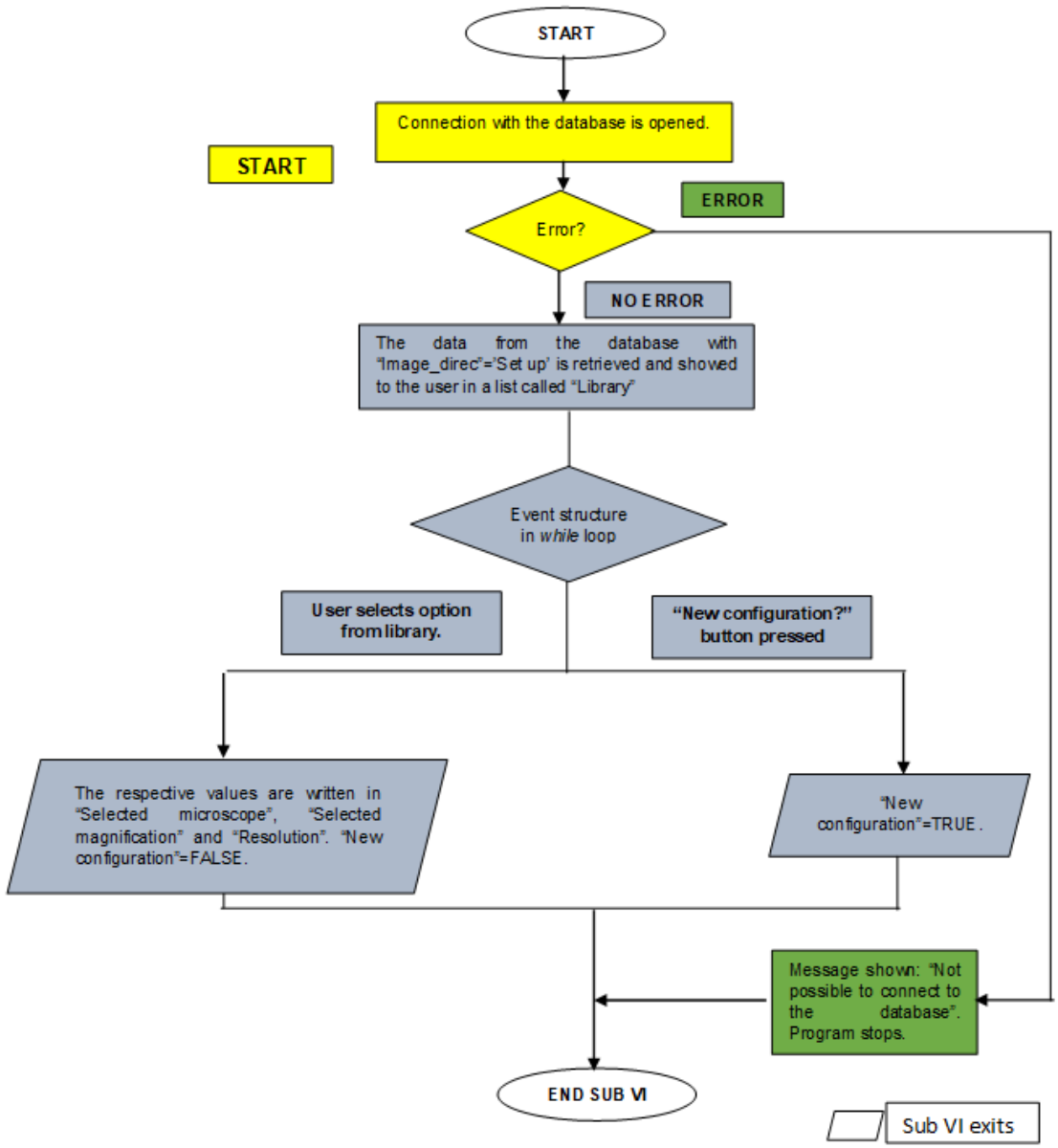
- **Selected magnification:** a string type exit. It gives the magnification value the user selects.

- **Resolution:** a string type exit. It gives the px/µm value the user selects.

- **Error out:** error line. Gives notion of the chronology of the program and translates error codes in the case it happens one.

- **New configuration?:** a Boolean type exit. It is TRUE if the user clicks the button "New configuration?". If the user selects a microscope setup already available, it is FALSE.

The flow diagram of the Sub VI is the following:



5.4. WRITELIBRARY SUB VI

It is used in the calibration process: it asks the user for the name of the microscope and the magnification in use. The Sub VI has 1 entry and 3 exits:

Entries:

- **Error in:** error line. Gives notion of the chronology of the program and translates error codes in the case it happens one.

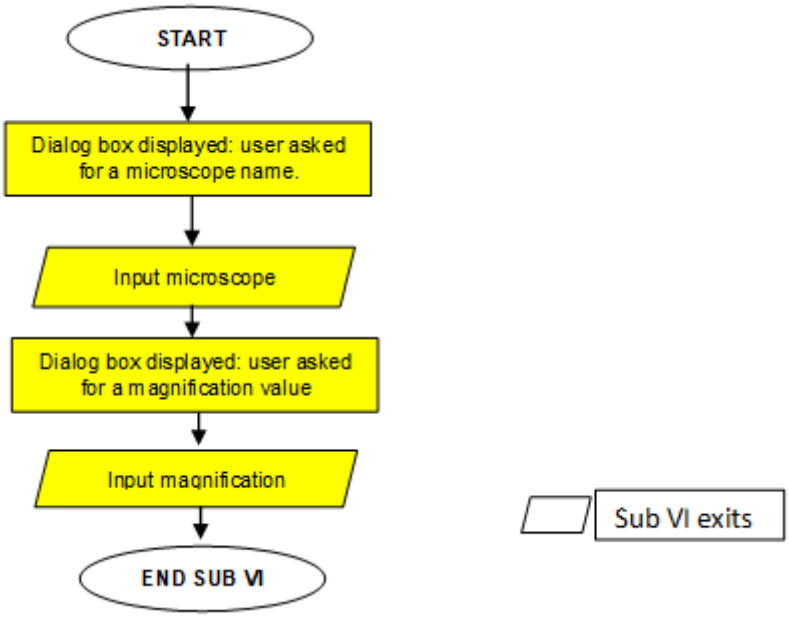
Exits:

- **Input microscope:** a string type exit. It gives the name of the microscope entered by the user.

- **Input magnification:** a string type exit. It gives the magnification entered by the user.

- **Error out:** error line. Gives notion of the chronology of the program and translates error codes in the case it happens one.

The flow diagram of the Sub VI is the following:



5.5. CALIBRATION SUB VI

It is used in the calibration process. The Sub VI has 2 entries and 3 exits:

Entries:

- **Image:** an image type entry. Image of the calibration plate.

- **Error in:** error line. Gives notion of the chronology of the program and translates error codes in the case it happens one.

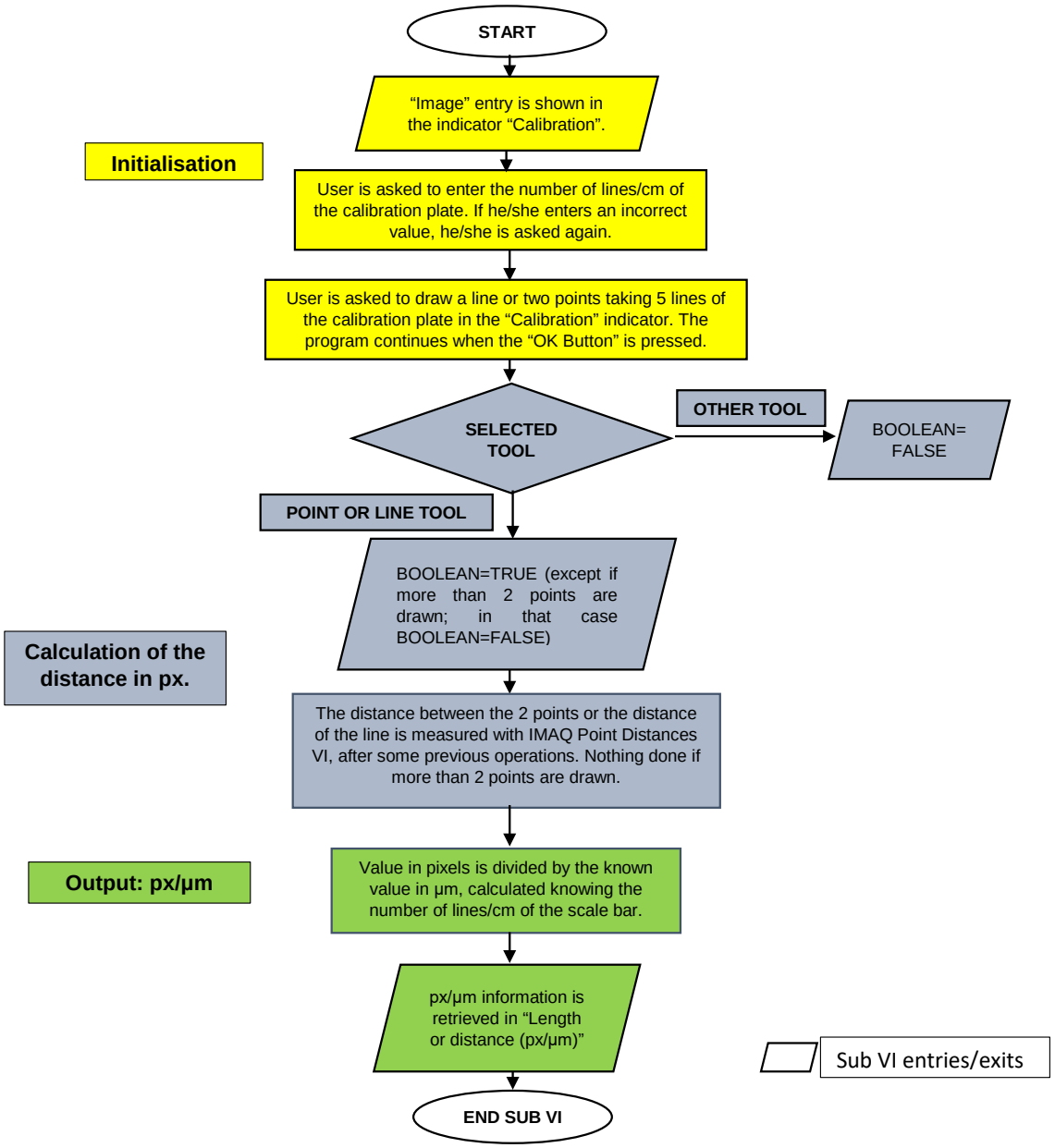
Exits:

- **Length or distance (px/µm):** a single type exit. px/µm information retrieved.

- **Boolean:** a Boolean type exit. Used for executing again the Calibration Sub VI in the case that the image tool used in “Calibration” indicator is not an appropriate one.

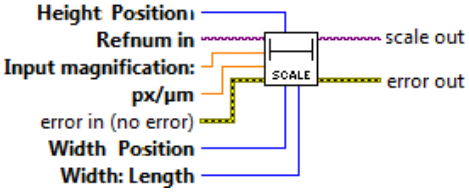
- **Error out:** error line. Gives notion of the chronology of the program and translates error codes in the case it happens one.

The flow diagram of the Sub VI is the following:



5.6. SCALE SUB VI

It draws a scale bar in an image using overlay information. The Sub VI has 7 entries and 2 exits:



Entries:

- **Height: Position:** height position in image coordinates of the bottom side.

- **Refnum in:** “Picture taken image” or “Picture taken image 2” (image where the scale is projected).

- **Input magnification:** a double type entry. Value of magnification retrieved from FGV Sub VI.

- **px/ µm:** a double type entry. Value of px/µm information retrieved from FGV Sub VI.

- **Error in:** error line. Gives notion of the chronology of the program and translates error codes in the case it happens one.

- **Width: Position:** width position in image coordinates of the right side.

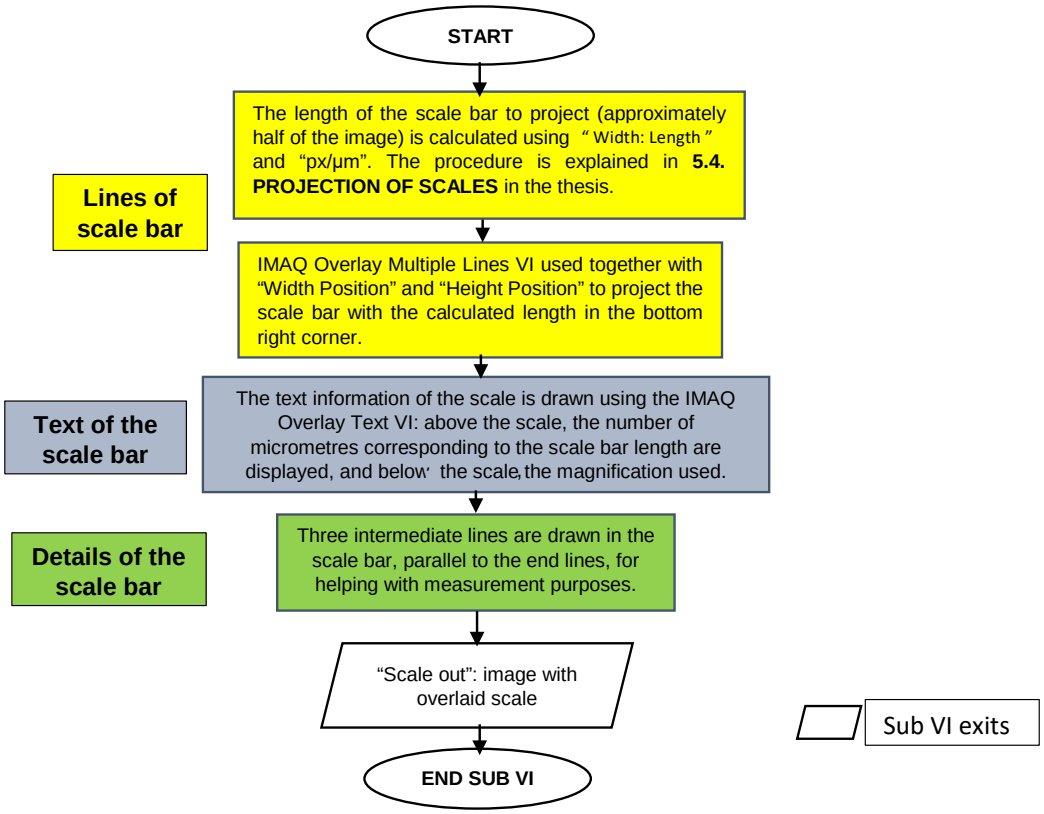
- **Width: Length:** width of the image indicator (not position, but the real length of the width: the coordinates are not the same).

Exits:

- **Scale out:** image out with projected scale.

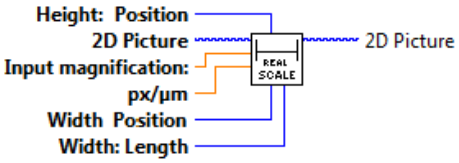
- **Error out:** error line. Gives notion of the chronology of the program and translates error codes in the case it happens one.

The flow diagram of the Sub VI is the following:



5.7. REALSCALE SUB VI

It draws a scale bar in a real-time image using picture indicators (see **4. PROJECTION OF SCALES**). The Sub VI has 6 entries and 1 exit:



Entries:

- **Height: Position:** height position in picture coordinates of the bottom side.

- **2D Picture:** empty picture used to draw there the scale bar.

- **Input magnification:** a double type entry. Value of magnification retrieved from FGV Sub VI.

- **px/ µm:** a double type entry. Value of px/µm information retrieved from FGV Sub VI.

- **Width: Position:** width position in picture coordinates of the right side.

- **Width: Length:** width of the image indicator “Real- time image” or “Video” (not position, but the real length of the width: the coordinates are not the same).

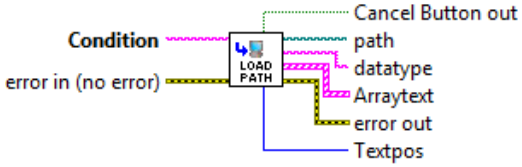
Exits:

- **2D picture:** picture out with projected scale.

The flow diagram of the Sub VI is equal to the one of **5.6. SCALE SUB VI**, but the Draw Multiple Lines VI is used instead of IMAQ Overlay Multiple Lines and Draw Text at Point VI is used instead of IMAQ Overlay Text VI.

5.8. LOAD IMAGE PATH SUB VI

This SubVI displays a table where the user can choose a row of the database he/she is interested in. The Sub VI has 2 entries and 6 exits:



Entries:

- **Condition:** condition for the SQL query used to retrieve data from the database (i.e. select * from data where “*Condition*”).

- **Error in:** error line. Gives notion of the chronology of the program and translates error codes in the case it happens one.

Exits:

- **Cancel Button out:** a boolean type exit. It is TRUE if the user clicks the "Cancel" button. If the user selects an entry of the database, on the other hand, it is FALSE.

- **path:** path of the image/video that needs to be recovered in an indicator.

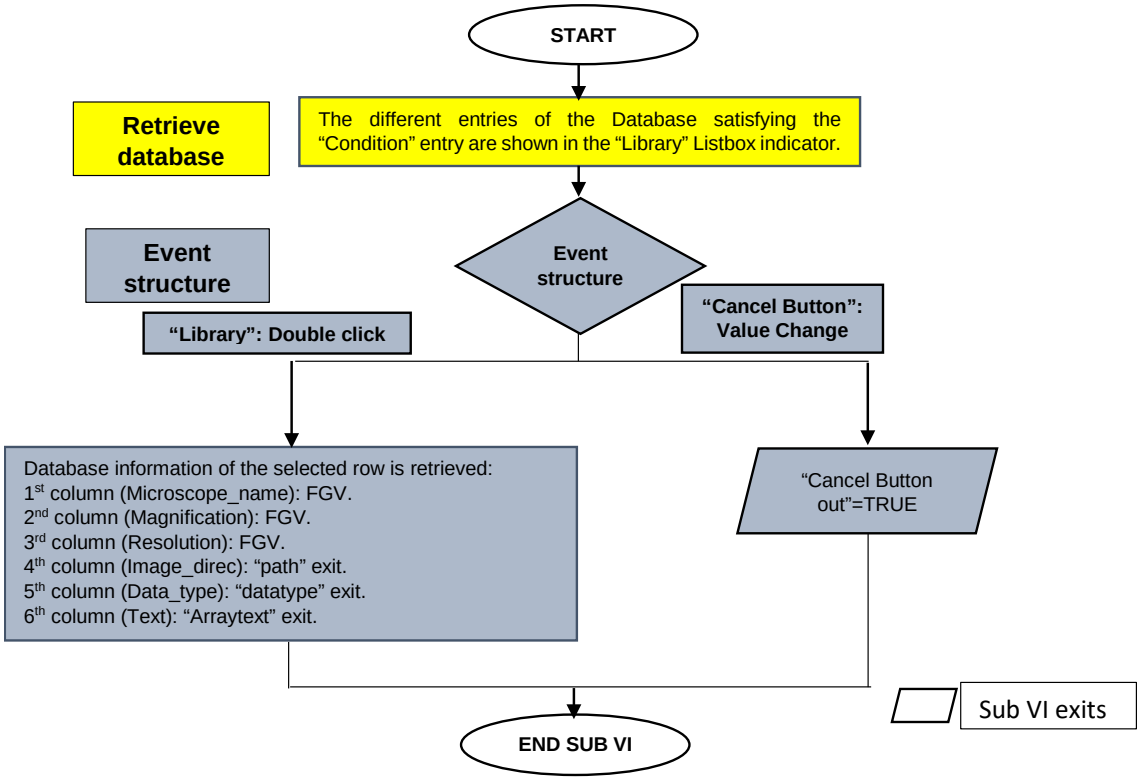
- **datatype:** a string type exit. It contains the information of the "Data_type" column of the selected entry.

- **Arraytext:** an array of clusters. It contains the text information of the column "Text", but organised in an array (see **5.11. INSERT TEXT TOOL** in the main thesis for more information).

- **Error out:** error line. Gives notion of the chronology of the program and translates error codes in the case it happens one.

- **Textpos:** a long type exit. It contains the number of different text entries present in the "Text" column. This number is necessary to continue with the numeration in the "Insert text" tool.

The flow diagram of the Sub VI is the following:



5.9. SAVE IMAGE PATH SUB VI

Sub VI used to save an image in a given path and to store all the information concerning that image in the database via the SubVI Databasewrite. The Sub VI has 4 entries and 1 exit:

Entries:

- **Picture to save:** image wanted to save by the user.

- **Type of data:** information corresponding to the column “Data_type” of the database. It can be “Calibration, “Overview picture”, “Detail picture” or “Video”.

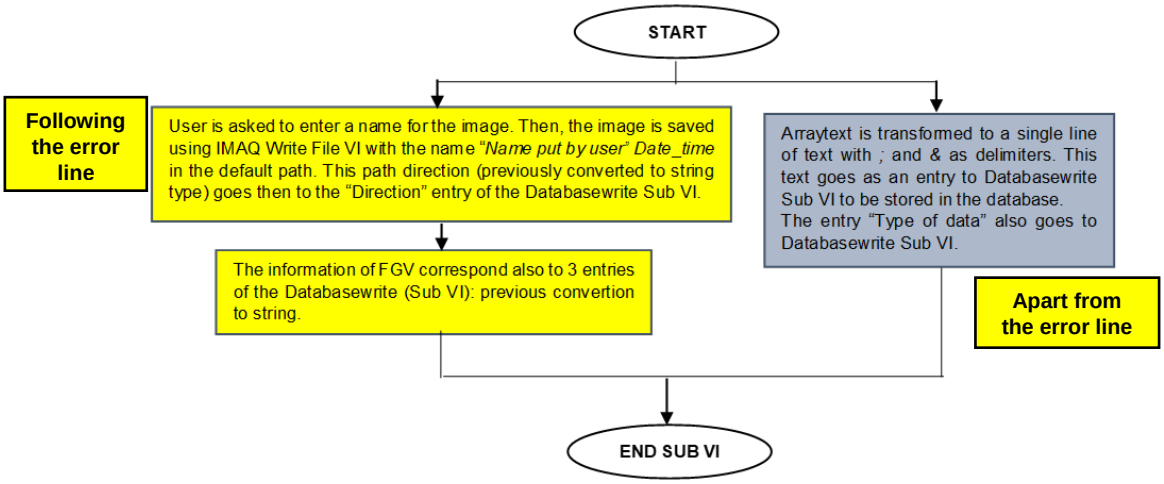
- **Arraytext:** array of clusters. It contains the information corresponding to the column “Text” of the database (see **5.11. INSERT TEXT TOOL** in the main thesis).

- **Error in:** error line. Gives notion of the chronology of the program and translates error codes in the case it happens one.

Exits:

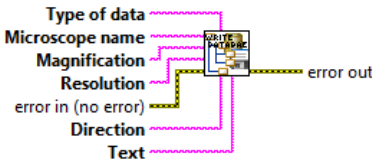
- **Error out:** error line. Gives notion of the chronology of the program and translates error codes in the case it happens one.

The flow diagram of the Sub VI is the following:



5.10. DATABASEWRITE SUB VI

Sub VI used to write in the database. The Sub VI has 7 entries and 1 exit:



Entries:

- **Type of data:** information corresponding to the column “Data_type” of the database. It can be “Calibration, “Overview picture”, “Detail picture” or “Video”.

- **Microscope name:** information corresponding to the column “Microscope_name” of the database

- **Magnification:** information corresponding to the column “Magnification” of the database.

- **Resolution:** information corresponding to the column “Resolution” of the database.

- **Error in:** error line. Gives notion of the chronology of the program and translates error codes in the case it happens one.

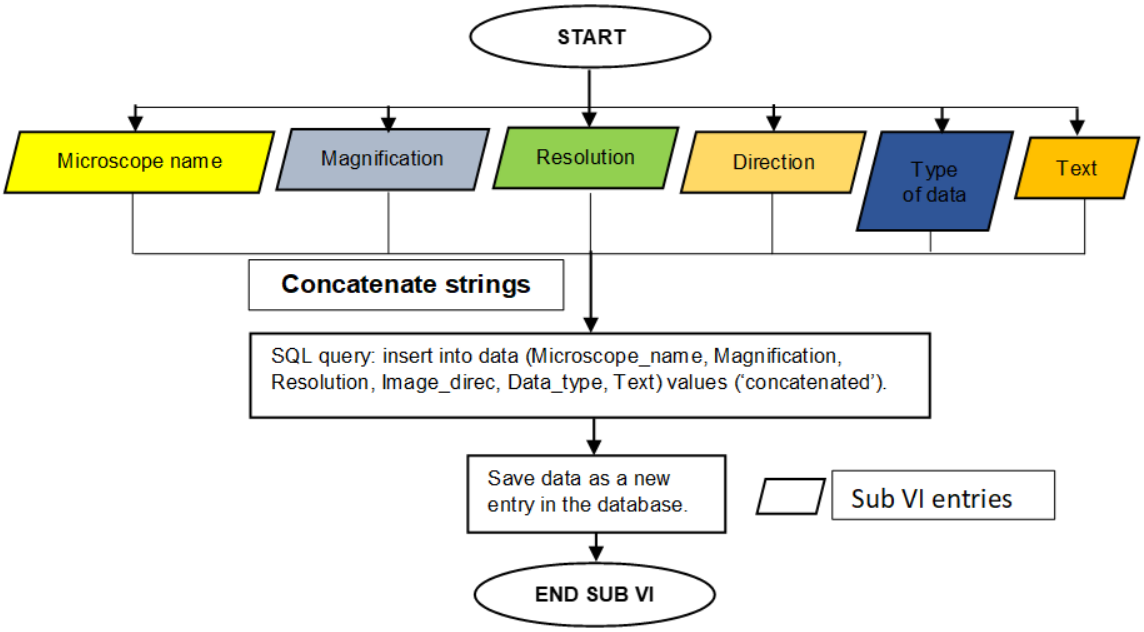
- **Direction:** information corresponding to the column “Image_direc” of the database.

- **Text:** information corresponding to the column “Text” of the database.

Exits:

- **Error out:** error line. Gives notion of the chronology of the program and translates error codes in the case it happens one.

The flow diagram of the Sub VI is the following:



The delimiter used for concatenating the strings is ‘,’.

APPENDIX D

C-MOUNT POSSIBLE SOLUTIONS

The piece that joins a trinocular microscope to a camera is called C-mount. In **Figure 1**, the most common C-mount structure is shown: one lens and demagnification factor ≤1. The intermediate image within the microscope has to be captured by a rectangular sensor, so with a magnification factor ≤1, the intermediate image is compressed and the captured area increased (**Figure 2**).

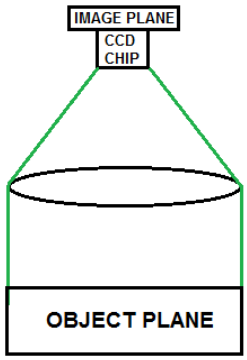


Figure 1 - C-mount with a ≤1 demagnification factor.

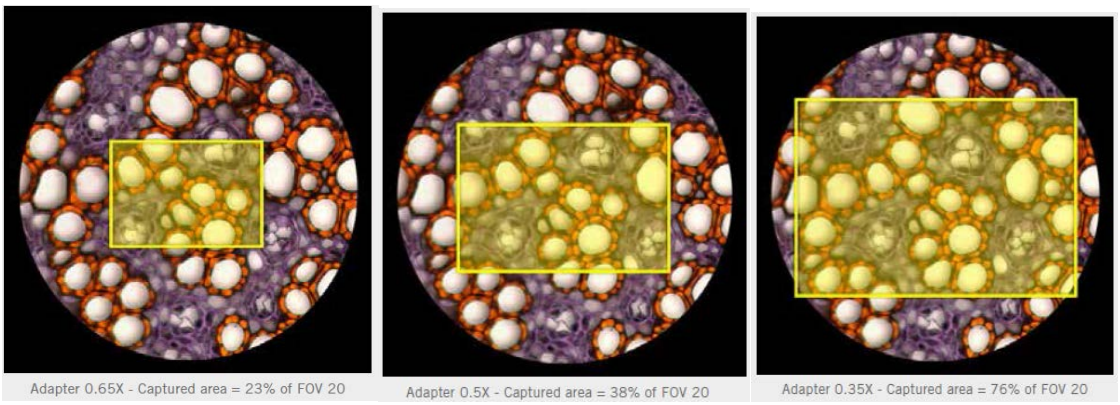


Figure 2 - C-mount captured area for demagnification factor of ≤1 [16].

However, there exist also the possibility of realizing the opposite operation for getting a close-up inspection: intermediate image magnified instead of minimized (**Figure 3**).

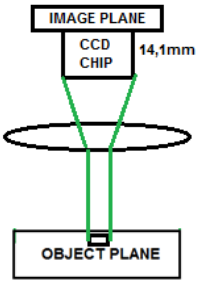


Figure 3 - C-mount with a ≥1 magnification factor.

For microfluidics applications with real-time imaging, it can be interesting to be able to vary between two different magnification factors. Here is an example with the possibility to vary between a 10X magnification and an overview mode with a 1X magnification. In this example, two lenses with adjustable distance between them are necessary for the C-Mount, in order to be able to change between the 10X and 1X magnifications (**Figure 4**).

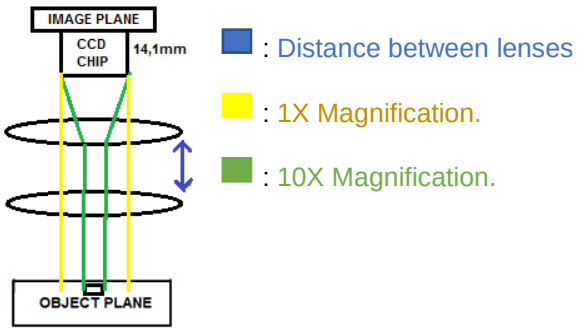


Figure 4 - C-mount with 1X and 10X magnifications changing the distance between the lenses.

It is important to remark that the set-up needs to be built such that it gives the possibility to the user to choose between 1X (overview mode) and 10X (close-up inspection) magnifications of the intermediate image of the trinocular tube in the CCD sensor of the camera.

This possibility of overview and close-up modes was considered at first (even contact was stablished with the company Motic), but finally, it was discarded due to the results obtained with the calibrations (enough μm/px for microfluidic applications) of the available microscopes: it was not necessary greater magnifications than the ones offered by the already purchased C-mounts.

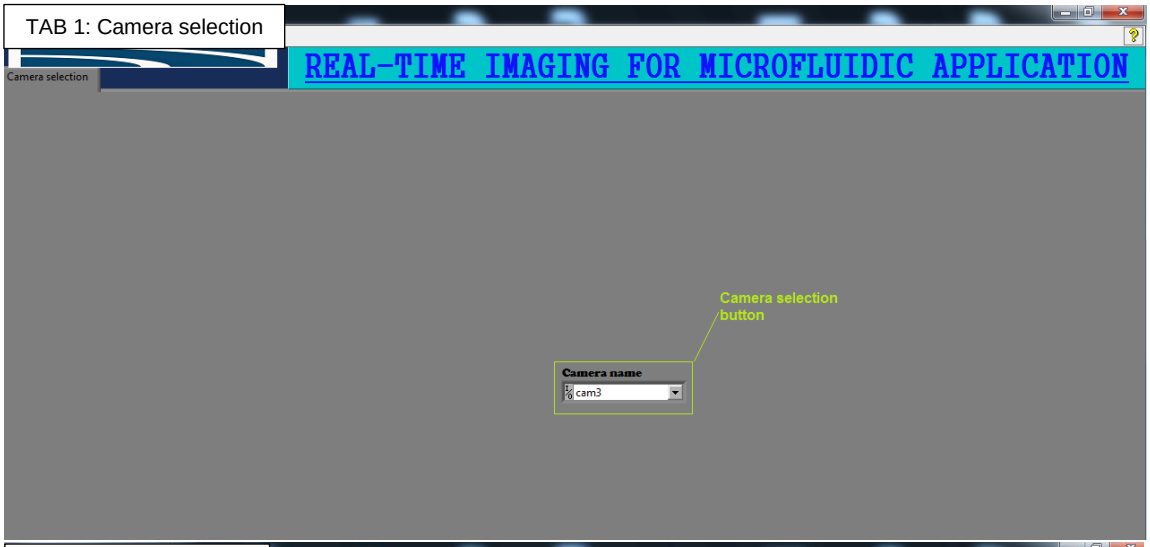
Two different C-mounts were used: one with a factor of 1X for the Zeiss Stemi 2000-C Stereo Microscope and another with a demagnification factor of 0,5X for the Olympus Stereo Microscope.

APPENDIX E

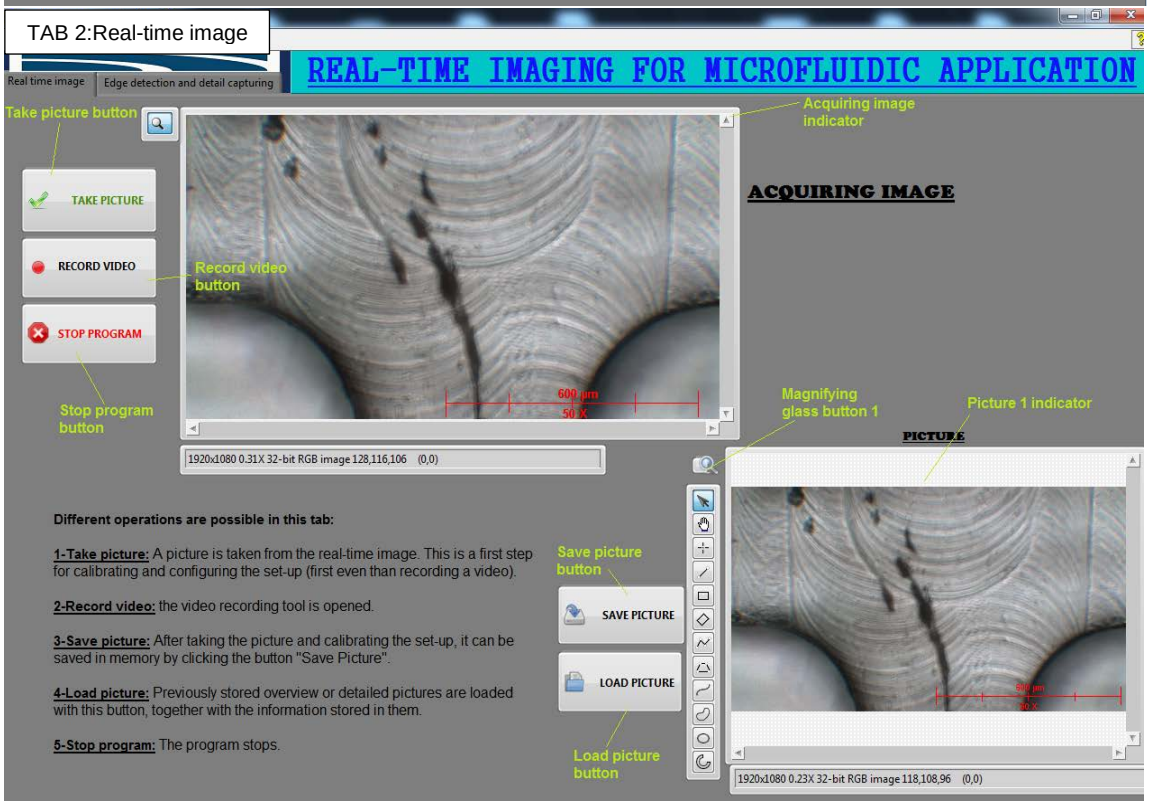
OPERATOR'S MANUAL

In this appendix, it is explained how to use the software and how to perform the different operations that it enables. It is important to mention from the very first moment that the application called XAMPP Control Panel has to be running together with the software, to enable the communication with the database.

TAB 1: Camera selection



TAB 2:Real-time image



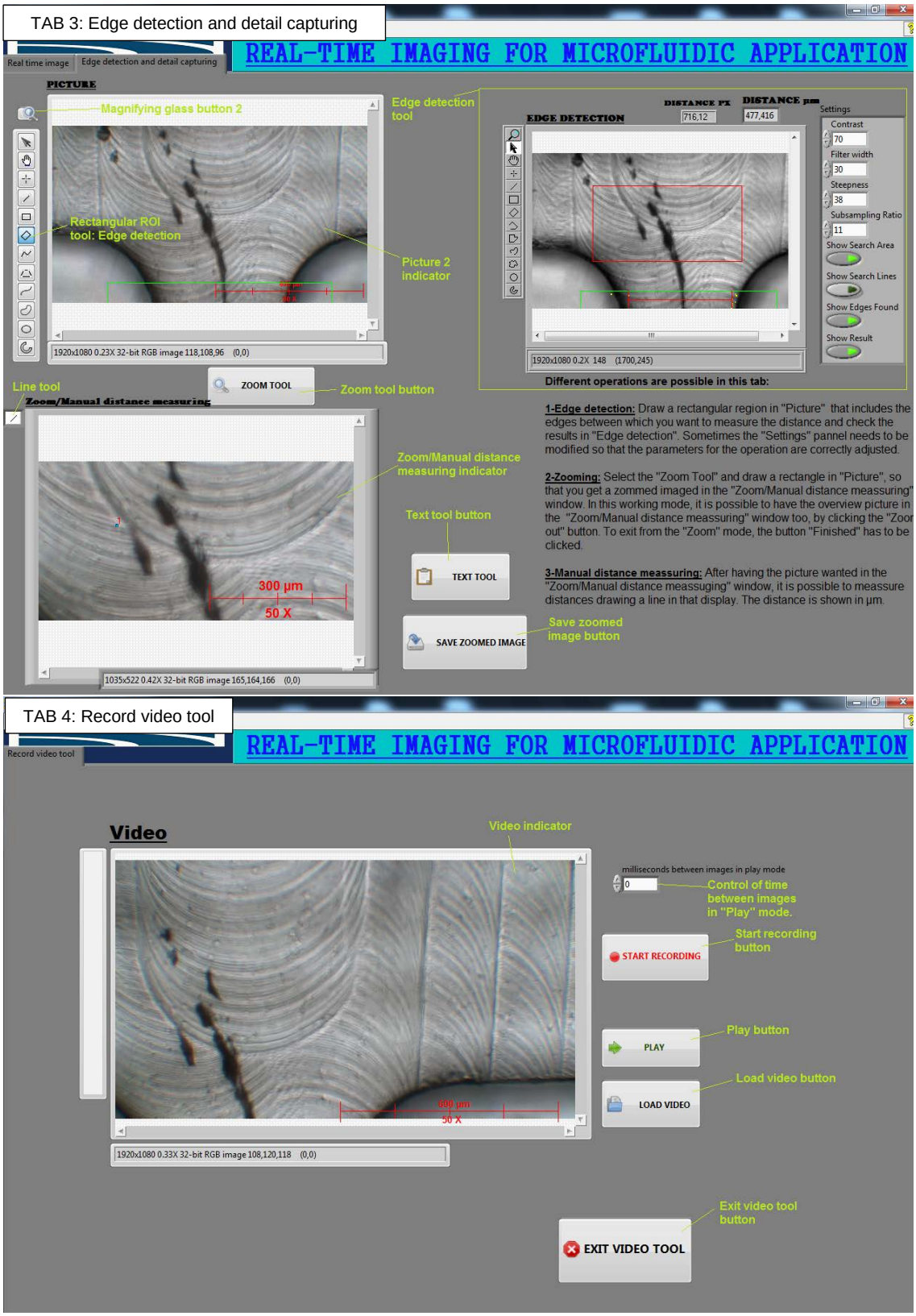


Figure 1: Front panel buttons, controls and indicators.

The program works as follows:

1. The program asks to select a camera port for capturing the real-time image. For example, *cam3*.

2. A real-time image is acquired by the software, showing the state of the probe in real-time. In this window called “Real-time image”, the “Stop program” button is available to stop the program at any time clicking there.

3. Two possibilities: “Take picture” or “Load picture”.

   Take picture button

   A new window pops-up and if the image is correct is asked. If “No” is selected, we return to the second step. If “Yes” is selected, a list with different microscope and magnification options appears.[1] If our set-up appears in that list, we select it, getting scale bars projected together with the taken image in “Picture 1” and “Picture 2”. Otherwise, we click “New configuration”.

   When clicking “New configuration”, first the microscope and then the magnification of the set-up have to be entered in two pop-up windows. Then, we are asked to take a picture of a calibration ruler. After taking it, another window asks for the number of lines/cm that the calibration ruler has and a line taking 5 lines of the scale needs to be drawn. When we press OK, the calibration of our set-up is ready and scale bars with that particular information are projected in the different images.

   Load picture button

   Different type of pictures (overview and/or detail) are shown together with the set-up information (microscope, magnification and px/µm) and the path were they are stored in a table. We can load any of those entries or press “Cancel” in case that this is not the option we wanted to select. When loading a picture, the calibration information is also loaded and its scale bar is displayed.

4. After a picture is displayed in the “Picture 1” and “Picture 2” windows, more options and tools become available. “Take picture” and “Load picture” buttons continue being available, in case we want to change the picture we have during normal operation of the program. The tab “Edge detection and detail capturing” becomes also available together with the “Real-time image” tab that was available until this point. The new options available are the followings:

[1] If the connection to the database is not made properly or if the application XAMPP Control Panel is not running, an error is obtained in this step, making the program to stop.

Save picture button

It is in the tab “Real-time image”. The picture in “Picture 1” can be saved together with its calibration information in the default folder with this option. We are asked a name for the picture.

Magnifying glass buttons

It is possible to zoom-in and –out in pictures 1 and 2 with the respective magnifying glass buttons.

Edge detection tool

It is in the tab “Edge detection and detail capturing”. A rectangular ROI in picture 2 can be drawn and the distance between 2 borders contained in that region is calculated and displayed by machine vision. The calculations made are shown in the indicator “Edge Detection”.

Zoom Tool button

It is in the tab “Edge detection and detail capturing”. After clicking on it, a rectangular ROI can be drawn in “Picture 2”. A zoomed imaged of that rectangular region is obtained in a new indicator called “Zoom/Manual distance measuring” that becomes visible.

The buttons “Zoom Out” and “Finished Zooming” appear after clicking the “Zoom Tool” button too. With “Zoom Out”, the image in “Picture 2” is copied directly to “Zoom/Manual distance measuring”. When “Finished Zooming” is clicked, we exit from this rectangle zoom option.

5. After the “Zoom Tool” button option is used, new buttons and options become available in the “Zoom/Manual distance measuring” indicator:

Line tool

A line can be drawn in “Zoom/Manual distance measuring” and the distance between the ends of the line is obtained.

Text tool button.

When clicked, text can be inserted in “Zoom/Manual distance measuring”. We only need to click the part of the image where we want to insert a text note, write the text note and click the green button to save it. When we pass the mouse cursor from a point where a text note has been inserted, that text note is displayed. This option finishes when the “Text inserted” button is clicked.

Save zoomed image button.

When clicked, we are asked for a name and the zoomed image is saved together with the calibration information, scale bar and inserted text as “Detail picture”.

6. A video can be recorded with the “Record video” button present in the tab “Real-time image”. This option is only available after a picture has been taken for the actual set-up, since calibration information is needed for the video so that a scale bar can be displayed. That is why this option is not available with a loaded picture.

   When clicking the “Record video” button, the “Record video tool” tab appears. We can interact with three different buttons here:

   Start recording button.

   When clicked, a video starts to be recorded after a proper name is given to it. When the “Stop” button is clicked, the video stops and is saved in the default directory with the name given to it.

   Load video button.

   A selected video is loaded in the program together with the respective scale bar with this option.

7. After one of those video options is used, the “Play” button can be used to reproduce the recorded/loaded video. It is possible to control the milliseconds between images during the reproduction too, to analyse with different speeds certain videos (time-lapse).

8. When clicking the “Exit video tool” button, we return to the main program (tab “Real-time image).