



VisionArray SingleScan Software

REF E-4301-1



For the analysis of hybridization signals on VisionArray Chips

4250380SWSDM



In vitro diagnostic medical device
according to IVDR (EU) 2017/746

1. Intended purpose

The *VisionArray* SingleScan Software is intended to be used for the detection and analysis of hybridization signals on compatible microarray chips like *VisionArray* Chips in combination with the corresponding chip file.

The product is intended for professional use only. All tests using the product should be performed in a certified, licensed anatomic pathology laboratory under the supervision of a pathologist/human geneticist by qualified personnel.

2. Test principle

DNA-fragments with a specific sequence are detected from a pool of DNA-fragments on a glass chip with the help of immobilized DNA capture sequences by DNA/DNA-hybridization. For this detection system DNA-samples from formalin-fixed, paraffin-embedded tissue or cell samples can be used as raw material. As a first step, the target sequences in these samples have to be amplified and biotinylated by PCR. The hybridization between the amplified sequences and the complementary DNA capture sequences is performed subsequently. After the hybridization, the unspecifically bound DNA is washed away by short stringent wash steps. The specifically bound biotinylated sequences are secondary labelled with a streptavidin-peroxidase-conjugate afterwards and visualized by tetramethylbenzidine (TMB) staining.

3. Reagents provided

Not applicable.

4. Materials required but not provided

The *VisionArray* SingleScan Software should only be used for the analysis of compatible microarray chips like *VisionArray* Chips. The corresponding chip file information needs to be present in the *VisionArray* SingleScan Software in order to be able to scan the *VisionArray* Chip. New chip files can be imported if required.

For a simple data entry, it is recommended to use a hand-held QR scanner.

5. Storage and handling

Not applicable.

6. Warnings and precautions

- Read the instructions for use prior to use!
- Report any serious incident that has occurred in relation to the product to the manufacturer and the competent authority according to local regulations!
- Each new *VisionArray* chip type has its own characteristic chip file that has to be installed prior to use!
- Make sure to have sufficient disc space for image acquisition

Hazard and precautionary statements:

Not applicable.

7. Limitations

- For *in vitro* diagnostic use.
- For professional use only.
- For non-automated use only.
- Interpretation of results must be made within the context of the patient's clinical history with respect to further clinical and pathologic data by a qualified pathologist/human geneticist.
- In addition to the initial amount of the target sequences, further factors can influence the system. It is therefore not possible to access quantitative data on the basis of signal intensities.
- The performance was validated using the procedures described in these instructions for use. Modifications to these procedures might alter the performance and have to be validated by the user. This IVD is only certified as CE when used as described in this instruction for use within the scope of the intended use.
- Depending on the *VisionArray* chip type, the specific capture sequence for every dot is stored in the corresponding chip file and allows a qualitative evaluation of the sample. For other microarray chips, only signal intensity can be evaluated.
- The software needs Windows 11 Version 21H2 or higher and at least 8 GB RAM with at least 1 GB disk space for installation.
- The software runs best with a resolution of 1920x1080 pixel
- Only use the Scanner Plustek OptiFilm 8100 or a similar slide scanner that supports a WIA scanning interface with a 16-bit Grayscale resolution of 7200x7200 dpi and a Region of Interest of 2300x2280 pixel to acquire the images.

8. Interfering substances

Not applicable.

9. Preparation of specimens

Only use hybridized *VisionArray* Chips or comparable chips. Please refer to the instructions for use of the respective chip.

10. Preparatory treatment of the device

Installation routine:

Connect the provided dongle to a free USB port on your PC. The software will only function when the dongle is present. Do not remove the dongle during operating the software.

The installation of the VisionArray SingleScan Software on your Windows System automatically starts after double clicking the VisionArray Installer. The Installer automatically installs all required functions and tools for a successful run of the software.

When reinstalling the software, perform a backup of your data prior to starting the installation routine.

Do not use hardware or software differing from the ones described in 4. Materials required but not provided.

Whenever unexpected problems occur, please contact helptech@zytovision.com or your local distributor.


11. Assay procedure

11.1 Terminological Conventions and Symbols

The following terminological conventions and symbols are used in the instruction for use:

Italic specific terms (e.g. *Wizard*);
terms that occur in the software itself (e.g. *Save Changes*);
and trade names (e.g. *VisionArray*)

Framed, bold buttons that occur in the software (e.g. **Scan**)

 critical steps that should be performed with particular care

11.2 Starting Screen

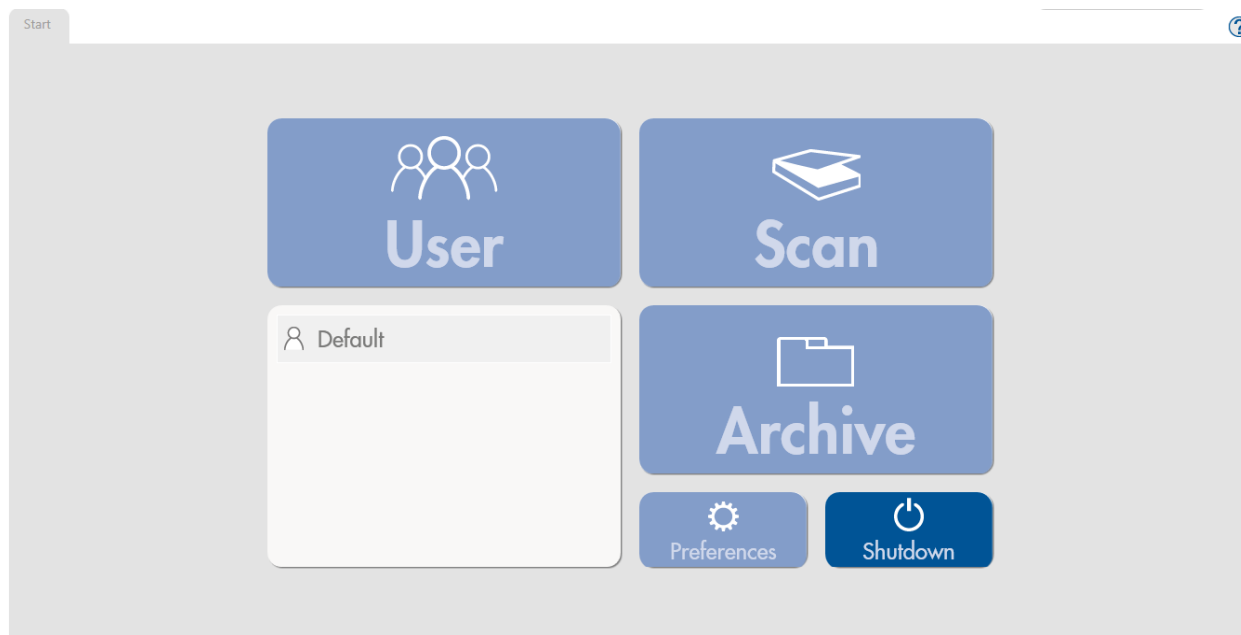



Figure 1: Starting Screen, no user logged in

The starting screen (Figure 1) of the VisionArray SingleScan Software gives access to all main control elements of the software. A user has to be logged in to be able to activate all control elements. Under **Preferences** a new user can be created.

Regardless of the login status the button **Shutdown**, which closes the program, and the Help-Function  can always be selected.

For the first setup, select the preinstalled default user by double clicking.

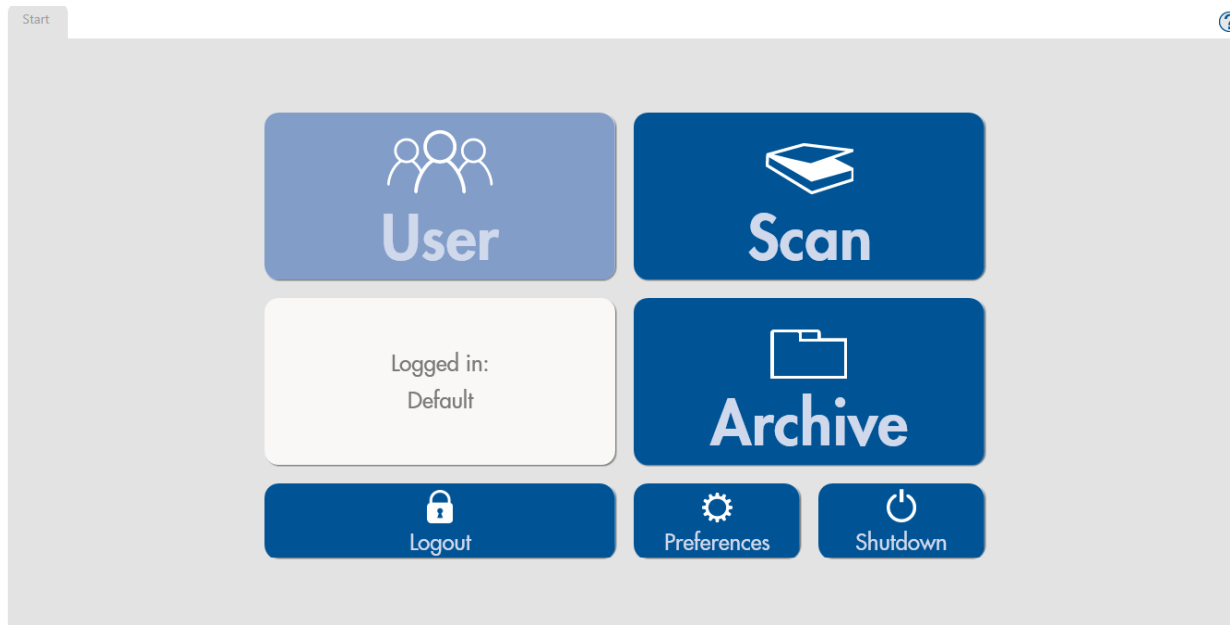


Figure 2: Starting Screen, user logged in

When a user has logged in, all functions of the starting screen are accessible (Figure 2). All subsequent steps are saved under the logged in user.

Scan enables the scanning of a *VisionArray*Chip or comparable chips. The data are stored automatically into a searchable **Archive** in the storage folder and can be opened or edited if necessary.

Logout logs out the active user, but does not close the program.

Preferences leads to all program information of the installed software version. Additionally, new chip files can be imported, new users can be edited, and a backup can be produced and restored under this tab.

Shutdown closes the program.

11.3 Preferences

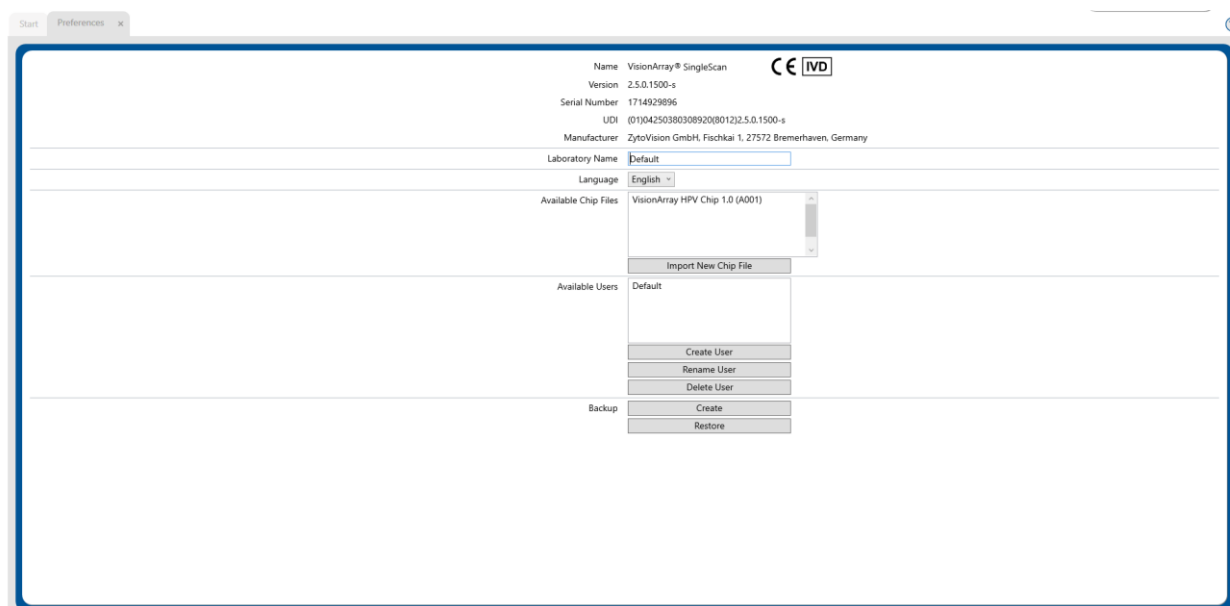


Figure 3: Preferences tab


The Preferences tab (Figure 3) gives an overview of the implemented program information such as version number, installed chip files and users. It is also possible to import new chip files, to create new users, to change the name of the laboratory, or to change the language. The default language is English.

Chip files

Import New Chip File imports the *VisionArray*chip file of a new type of chip. The button opens an explorer window in which the chip file can be selected and imported from an external device.

New chip files can be downloaded as a .zip file on the ZytoVision homepage:

<https://www.zytovision.com/products/visionarray>

 The chip file and its corresponding signature file have to be unpacked before it can be imported. After the import, the new chip file appears in the list and can be used immediately.


User

Below the section with the installed chip files the users created for the program are listed. The buttons **Create User**, **Rename User** and **Delete User** are also located in this section.

Backup

Furthermore, it is possible in the Preferences tab to backup all saved data via **Create** or to **Restore** saved data from an external device. The recovery process overwrites the database entirely with the recovery file.

All data in the database that are not saved will be lost during this process. We recommend creating backup files on an external device periodically in order to minimize the risk and amount of data loss due to computer problems.

 Changes will be saved by closing the Preferences tab.

11.4 Scan of an Array

When using the Plustek OptiFilm 8100 proceed as described below.

For other slide scanners that match the requirements stated in 7. Limitations please refer to the respective instruction for use.

The slide holder of the Plustek OptiFilm 8100 includes 2 slide slots that both can be used for scanning of an array. The VisionArrayChip is held in place by plastic rails that help with the insertion and ensure a successful scan.

The upper rim of the slide holder is marked with the word *Top*, the lower rim with the word *Bottom*. The VisionArrayChip has to be placed in the holder with the labelling on the top and the label field on the right.

In order to insert a VisionArrayChip, push the spring mechanism at the bottom of the slide holder with the lower edge of the slide down and fix it to the top of the holder. The chip should be flush with the plastic edges of the holder. Poor positioning in the scanner may result in irregular scans, or the scanner may not recognize the array field and the scan may have to be repeated.

The slide holder can be inserted into the Plustek OptiFilm 8100 via the two slide holder slots on both sides of the scanner. The slide holder has to be inserted into the scanner until the chip is fully comprised by the scanner and the holder clicks into place. Do not alter this position, since small deviations from this position lead to irregular scans and the array field might not be scanned properly.

In order to scan a chip in the second slot of the chip holder, push the holder further into the scanner until it clicks into place.

11.5 Enter Data and Start a Scan

Scan on the starting screen opens a tab for data entry (Figure 4). All individual data about the case/patient and the chip can be entered here.

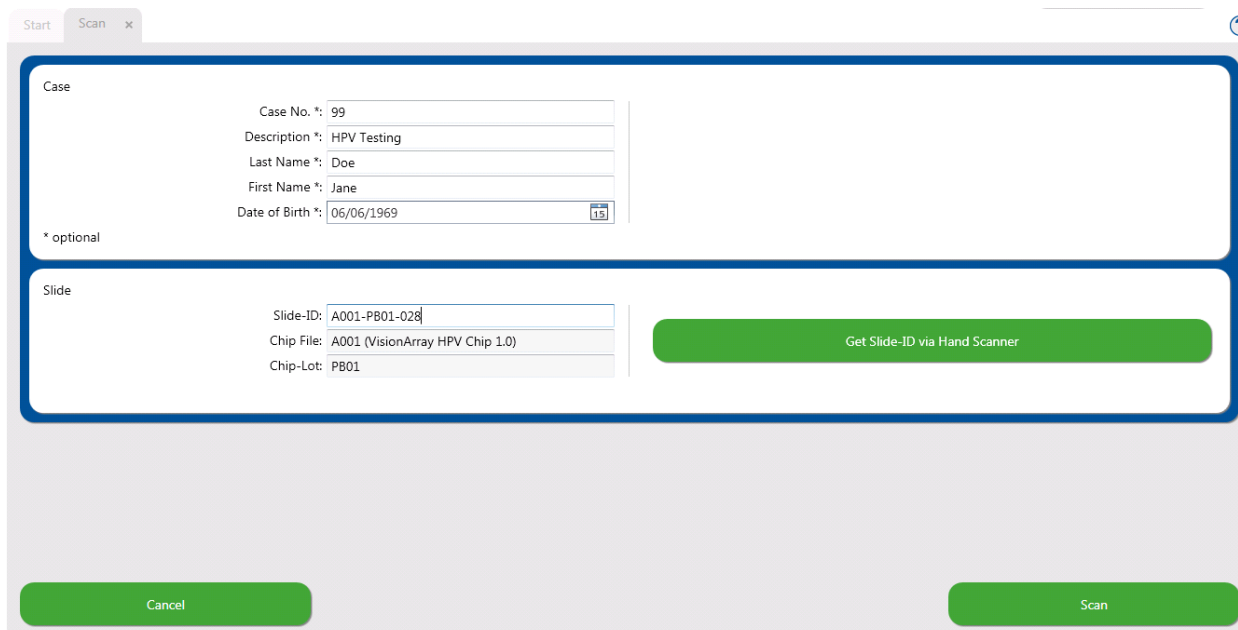


Figure 4: Scan tab

Case (sample and/or patient data)

In the upper section of the Scan tab case/patient data may be entered. The completion of the field is optional and not necessary for conducting a scan. The entered data are stored in the archive. Editing or completion of the data is not possible at a later time. We recommend a detailed entry of all essential data in order to make the assignment as easy as possible.

Slide (chip-information)

In the lower section of the Scan tab all relevant data for the chip type are entered. The information can be entered manually or quick and easy by clicking **Get Slide-ID via Hand Scanner** and scanning the QR-Code on the *VisionArray*Chip with the suggested hand scanner. This hand scanner should be held a few centimetres above the QR-code. By pressing the switch on the scanner it is activated and a light indicates the scanning area. When the data are captured, an acoustic signal sounds and the chip information is collected automatically.



Figure 5: Exemplary chip label with unique identifier

11.6 Preview of a scan

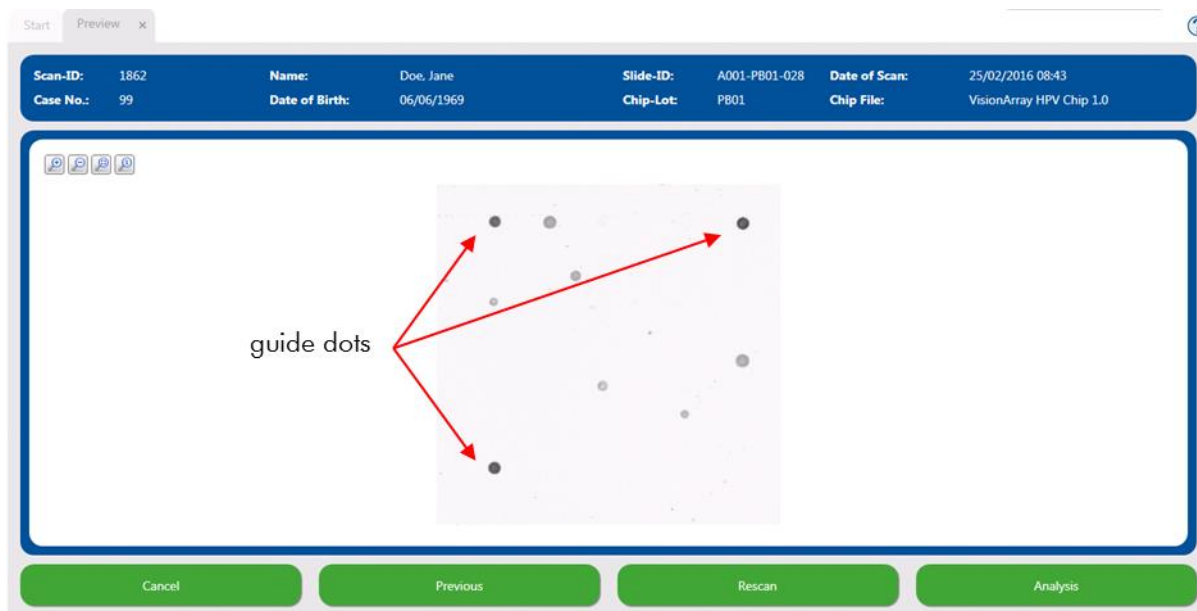



Figure 6: Preview of a scan

In the Preview tab the performed scan is displayed as a greyscale image (Figure 6). This tab can be used to check whether the chip is inserted correctly (using the 3 guide dots on each *VisionArray* chip) or whether there is major pollution on the array field. If necessary, the chip holder can be removed and corrections can be made by adjusting the orientation of the chip or removing the pollution. A new scan can then be performed by clicking **Rescan**.

Click **Previous** to return to the Scan tab and correct spelling mistakes, for example. The changes are applied automatically and the chip is scanned again by clicking **Scan**.

 **Cancel** closes the view and all entered data about patient/sample and chip are lost.

If the preview of the scan is correct, the automated evaluation of the scan can be started by pressing **Analysis**.

11.7 Analysis Screen

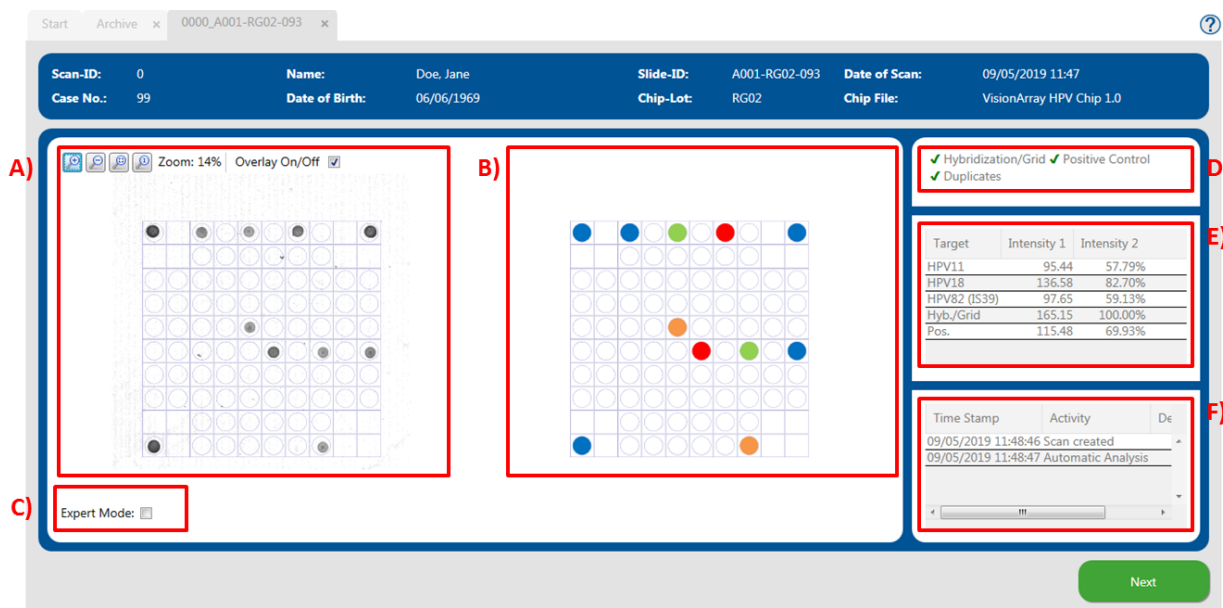


Figure 7: Analysis screen; A) Original Scan; B) Schematic view of the detected dots; C) Expert mode; D) Control mechanisms; E) Signal intensities; F) Analysis Protocol

11.7.1 Overview of the Analysis Screen

The analysis screen is divided into 3 parts (Figure 7):

On the left side the original greyscale scan is displayed (Figure 7 A). A grid can be toggled on/off by checking the box *Overlay On/Off*. The grid is toggled on per default. The visualization of the signals of the chip can be adjusted with the and buttons (see chapter 11.7.4). The automated evaluation of the array is performed based on the preview. The scan and all corresponding data are now saved in the database and cannot be edited anymore.

The centre of the analysis screen (Figure 7 B) shows a schematic view of the dots that have been detected by the software. The signals depend on the chip type. The associated information is stored in the corresponding chip file, if applicable. The signals are colour-coded depending on the chip type (see manual of the *VisionArrayChip*).

On the right side of the screen the control mechanisms, the signal intensities, and the analysis protocol are located (Figure 7 D,E,F).

11.7.2 Control mechanisms

The *VisionArraySingleScan* Software has 3 different control mechanisms (Figure 7 D).

Hybridization/Grid-control:

This control mechanism is based on the 3 guide dots in the corners of the array field that the software uses for orientation. The software spans the grid based on these 3 dots and allocates the positions to the signals. Additionally, the presence of the guide dots shows successful hybridization, labelling and staining. If the guide dots are too weak (intensity 1 < 150), a warning appears at this point. This may indicate poor hybridization.

Positive control

The positive control on the *VisionArray* Chips is used for assessment of the quality of the used PCR-template and of the PCR.

Duplicates

The third control mechanism implements a check by duplicates. This control is based on the fact that all catcher molecules are applied in duplicates at different positions on the chip. A positive signal is therefore always visualized by 2 dots.

A green checkmark indicates the correct sequence of checks for each test. Irregularities are displayed by a red cross. Weak guide dots are highlighted by a yellow exclamation mark. The software evaluates the data even if the positive control or duplicates fail. The user has to assess the validity of the data by himself.

11.7.3 Signal intensities and analysis protocol

The signals with corresponding intensities are listed below the control mechanism tab (Figure 7 E). Since all dots are applied as duplicates (triplicates for the guide dots) the intensity value of the dots is the result of the mean.

Intensity 1 shows the arithmetic mean of the grey value of the dots.

Intensity 2 shows the percentage signal of the guide dots. The guide dots are set as 100% intensity for an optimal hybridization result.

Below is a list of all actions that were performed after saving in the analysis protocol (Figure 7 F).

11.7.4 Zoom Level

The original scan and the schematic view are originally shown as an overview of the chip (Figure 7 A). A section of the chip can be enlarged by pressing the or by clicking into the overview and scrolling the mouse wheel up (Figure 8). In order to zoom out it is possible to either use the or to scroll the mouse wheel down after clicking on the scan.

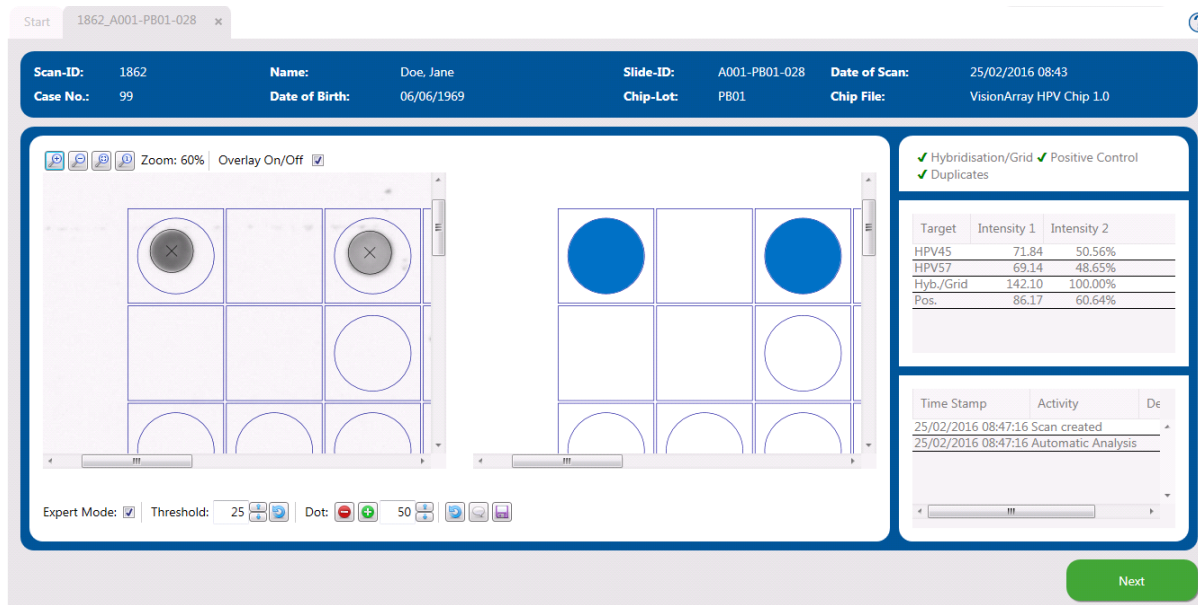


Figure 8: Analysis screen - zoomed in

11.7.5 Expert Mode

If it is necessary to make manual changes during chip analysis, the Expert Mode can be toggled on (Figure 7 C). All tools in the expert mode are visible when the box in the lower left corner is checked (Figure 9).

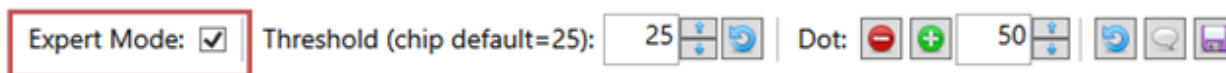


Figure 9: Active Expert Mode

11.7.5.1 Change Threshold

The threshold describes the negligible background on the chip (Figure 10). The threshold is predefined for each chip type. All dot signals that are stronger than the threshold are visualized in the schematic view and the table. Weaker signals are neglected and are not used for the evaluation. Depending on the samples and the detection process, signals or background could be extremely strong. By adjusting the threshold manually it is possible to react to the individual circumstances. By pressing the original threshold is reset.



Figure 10: Expert Mode - Threshold

11.7.5.2 Add/Delete Dots

If the software does not correctly detect a weak dot or a dot with an unusual morphology, it is possible to add it manually via Dot: (Figure 11). It is possible to either add a dot with a preset size or set the size with the radius indicator. The added dot is displayed in the scan and the schematic view, respectively.



Figure 11: Expert Mode – Add/Delete Dots

In rare cases, pollutions on the array field can be wrongly interpreted as positive dots. Via Dot: , false positive dots can be removed in the Expert Mode (Figure 11). After clicking the dot of interest can be selected and removed. The affected grid field is marked with an "X" to indicate a deleted dot. The changes are visible immediately in the schematic view, the detection status, and the listing table (Figure 12). The changes appear in the protocol only after changes have been saved by clicking the symbol.

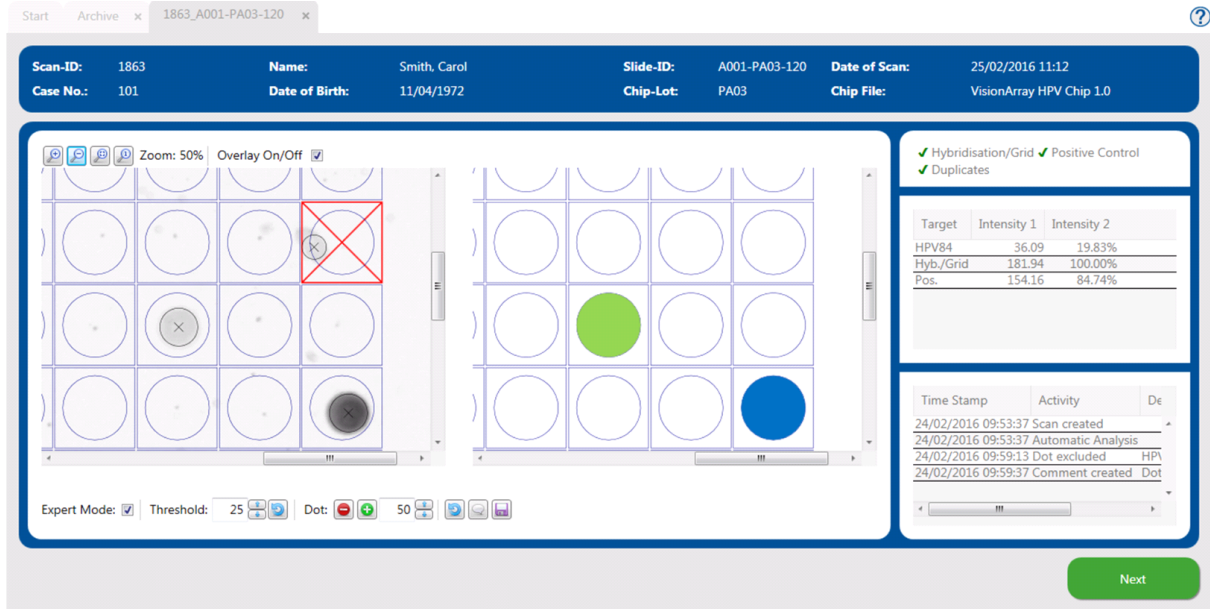


Figure 12: Analysis screen - excluding a dot

Addition or deletion of dots can be repeated infinitely. A deleted dot can be added again for the analysis by pressing and vice versa.

11.7.5.3 Reset and Saving Options

All changes that have been made manually can be reset to the state of the automated scan by pressing the button.

The changes can be saved by clicking the floppy disc symbol or by closing the Expert Mode. After saving, all changes are listed in the analysis protocol.

11.7.5.4 Comments

The speech bubble icon enables a comment field, where remarks can be made by the user. The comments will be displayed in the analysis protocol. Note that comments are also displayed in the anonymous reports. Personal data should therefore not be included in the comments.

11.8 Report

All information about a chip analysis can be printed or saved as a PDF-report (Figure 13).

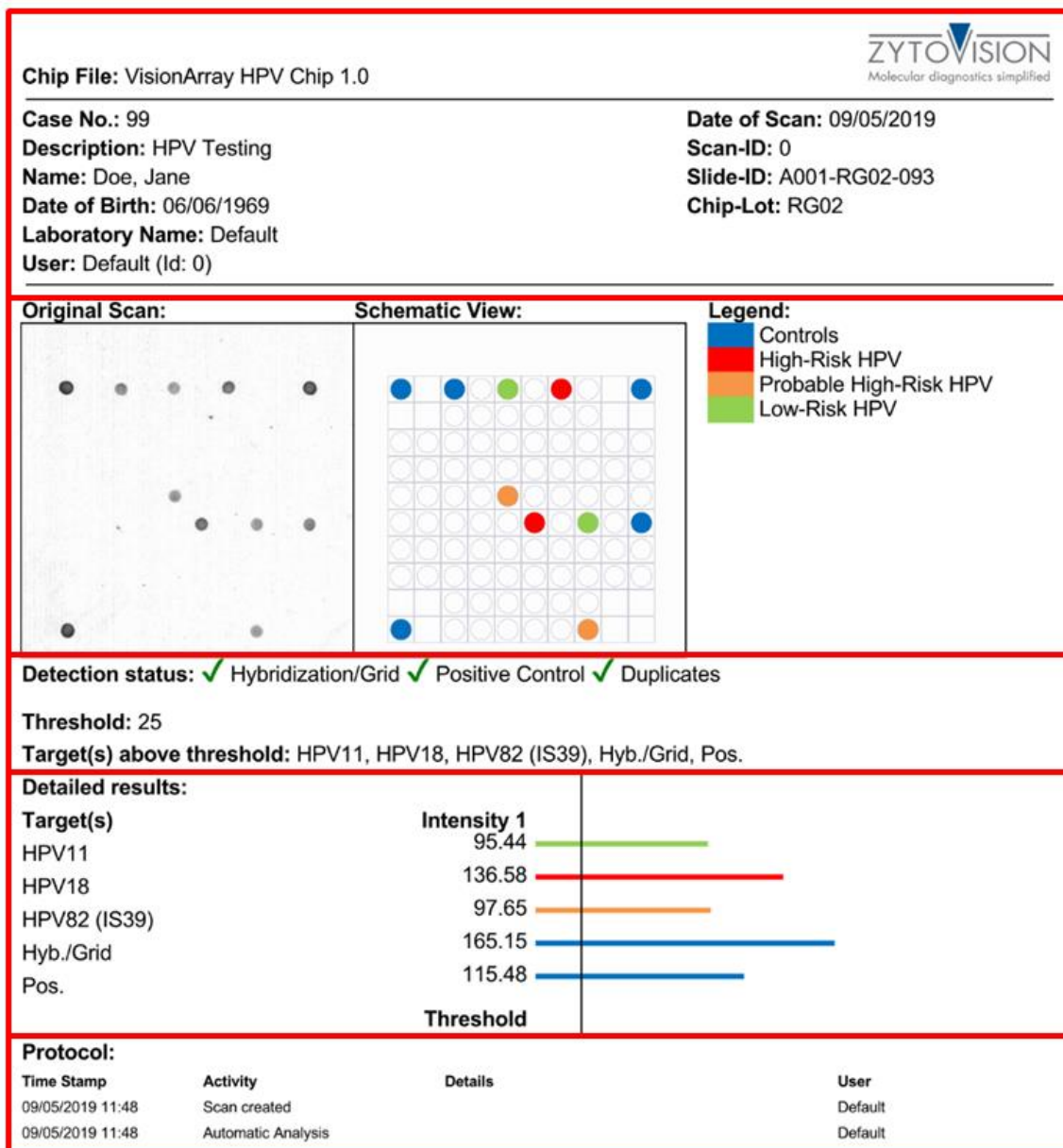


Figure 13: PDF-Report: A) Sample, patient, and chip information; B) Original scan and schematic view of the scan including legend; C) List of all controls, of the threshold, and of the detected signals; D) List and graphical display of the signal intensities; E) Protocol with detailed listing of all performed actions.

11.9 Data Export

In addition to the report of the data, it is possible to export the scan and the calculated signal intensities (Figure 14). Two common data and graphic formats are available under *Advanced Export*. To ensure data protection, the reports can be printed or exported anonymously by activating the anonymous report checkbox.



Figure 14: Possible export options

11.10 Archive

The Archive tab can be accessed from the start screen of the program. All chip files and the collected data of the VisionArraySingleScan Software are automatically stored in the database and are accessible from this tab.

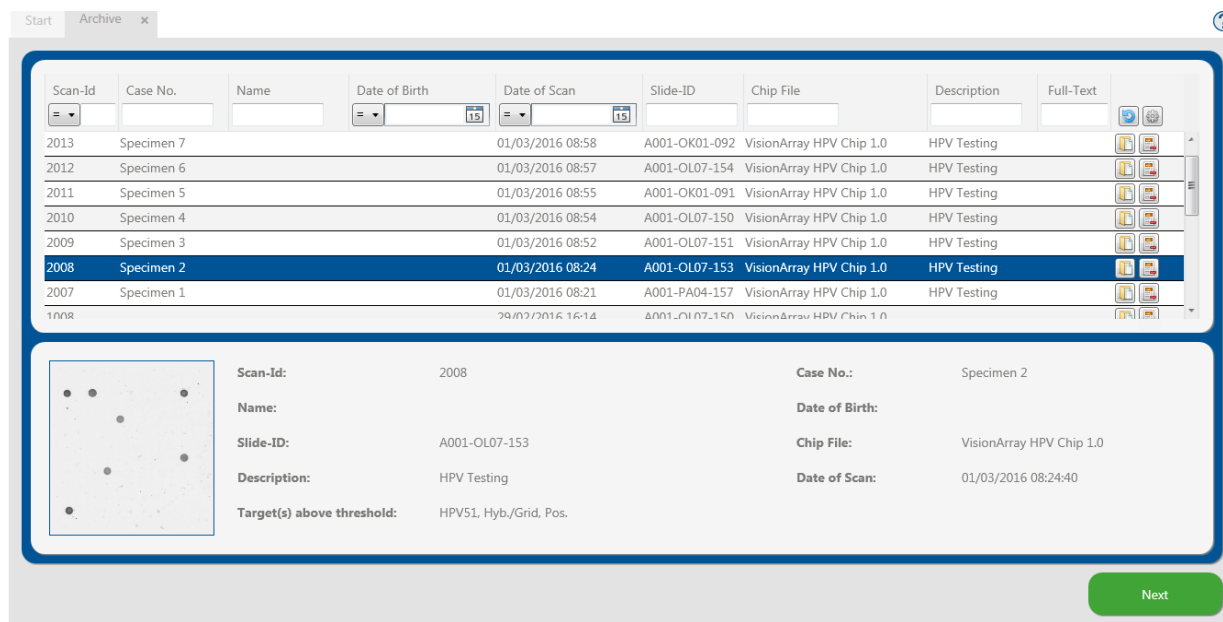







Figure 15: Archive tab

The database entries are displayed in table format (Figure 15). The information corresponds to the entered data in the Scan tab. The columns can be individually adjusted. Columns can be inserted or hidden via the setup icon , or moved into different positions by clicking and holding the column with the mouse and dragging them to the desired position. The database display is set to the default settings by selecting *reset* under  or after restarting the program.

It is possible to search the single columns or with the *Full-Text Search* the whole database for specific datasets. The button  deletes all entries in the search masks.


By selecting a single dataset, a preview of the scan is shown. The dataset of interest can be opened with a double click or by selecting it and clicking the open icon  or **Next**. The selected dataset opens in the Analysis view and can be edited or the report can be opened as described above (see chapter 11.9).

A dataset can be deleted irretrievably by clicking the delete button. 

11.11 Data Storage and Security

All scans or analyses that were produced by the VisionArray SingleScan Software are stored in an internal database. We recommend regular backups of the data on an external device in order to avoid data loss.

11.12 Help Function

The Help Function can be activated via the question mark  in the upper right corner in order to open a comprehensive guide for every tab of the VisionArray SingleScan Software.

12. Interpretation of results

With the help of the VisionArray DNA Chip it is possible to make a statement about the presence or absence of specific DNA sequences. The intensity of the signals is influenced by the frequency of the target sequences in the sample as well as by further factors of the detection system. It is not possible to use the absolute values of the signal intensity for the determination of the DNA concentration

The software works only as a display for the hybridization of VisionArray Chips or equivalent microarray chips. The interpretation of the results has to be performed by a qualified pathologist/human geneticist.

13. Recommended quality control procedures

In order to monitor correct performance of processed specimens and test reagents, each assay should be accompanied by external validated positive and negative control specimens. If internal and/or external controls fail to demonstrate appropriate staining, results with patient specimens must be considered invalid.

For internal controls refer to 11.7.2.

14. Performance characteristics

Refer to the performance characteristics of the respective VisionArray DNA Chip or compatible microarray chips.

15. Disposal

Not applicable.

16. Revision



www.zytovision.com

Please refer to www.zytovision.com for the most recent instructions for use as well as for instructions for use in different languages.

Our experts are available to answer your questions.
Please contact helptech@zytovision.com



ZytoVision GmbH
Fischkai 1
27572 Bremerhaven/ Germany
Phone: +49 471 4832-300
Fax: +49 471 4832-509
www.zytovision.com
Email: info@zytovision.com

Trademarks:

ZytoVision® and VisionArray® are a trademark of ZytoVision GmbH.