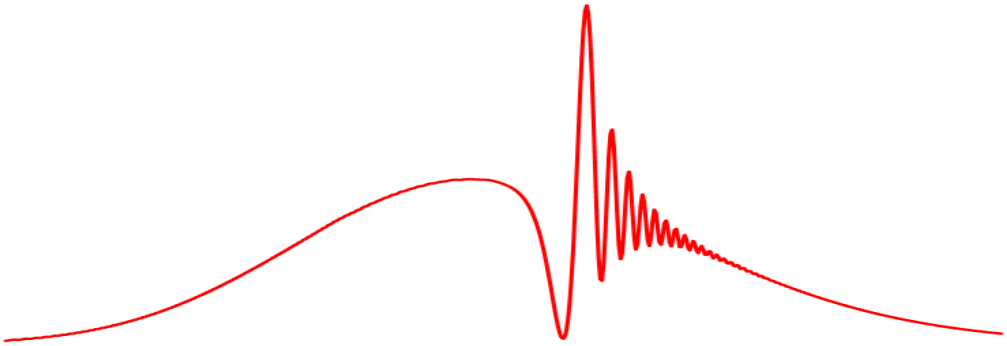

Biosensor EVA 3.0

(imaging)

A label-free biosensor with 2D spatial selectivity based on Photonic Crystal Surface Modes.

http://pcbiosensors.com/

Operations Manual for the Biosensor EVA 3.0 *(imaging)*

Photonic Crystal Surface Modes (PC SM)-based Biosensor EVA 3.0 is a label-free biosensor with 2D spatial selectivity.

Before operating the system, you must read the manual. Follow the instructions provided to avoid personal injuries or damage of the equipment.

WARNING!

Biosensor EVA 3.0 is powered by 15 V DC.

Use only a stabilized 15 V power supply.

Do not open cover before disconnecting mains cord.

Do not insert any objects into the Biosensor's optical unit.

Never expose the Biosensor EVA 3.0 to water or liquids.

Avoid direct sun.

Do not use near open flame or heat.

Connect the Biosensor only to a properly measured electrical supply.

Use only those cords, which are provided with the Biosensor EVA 3.0.

1. Getting Started

What is the Biosensor EVA 3.0 used for?

The Biosensor EVA 3.0 is a system for real-time interaction analysis based on photonic crystal surface waves. The Biosensor EVA 3.0 monitors in real time both the formation and dissociation of biomolecular complexes on a photonic crystal (PC) surface as the interaction occurs, and can do this simultaneously in several spatially different interaction spots.

The solutions under investigation are brought up to the flow cell by a peristaltic pump. The flow cell forms by the PC sensor chip pressing against a flow cell made from quartz glass or plastic plate with two holes.

One sensor chip can be repeatedly used for a huge number of measurements – because the last deposited layer of the PC sensor chip is SiO_2, it is easy to clean the sample after a previous measurement.

Installation

The Biosensor EVA 3.0 consists of the optical unit, the peristaltic pump and the computer (**Figure 1**).

Place the Biosensor EVA 3.0 on a table or bench that is free of strong electrical and magnetic fields. Do not place vibrating equipment on the same table. There should be at least 15 cm in front of the device (where you can place the holder for small tanks) and 30 cm above the device (for air circulation).

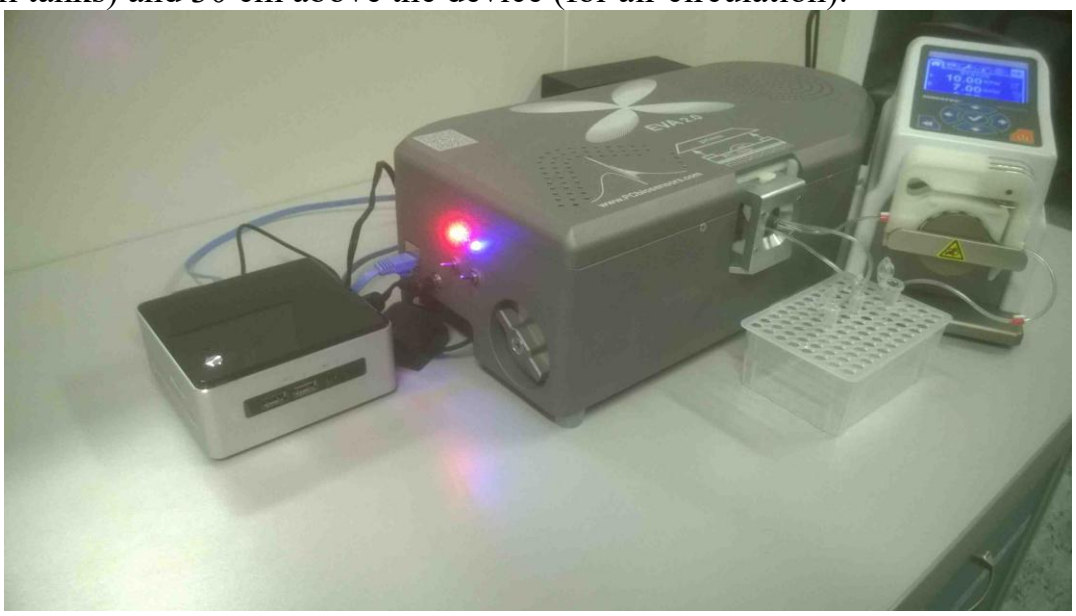

Figure 1. *Biosensor EVA 3.0 consists of the optical unit, the peristaltic pump and the computer.*

Connect the Biosensor's optical unit to the computer by a RJ-45 internet cable.

Switch on the Biosensor's optical unit (two tumblers on the left side of the device), the computer and the peristaltic pump.

PC sensor chip

The sensor chip is a glass slide (22x25 mm^2) with a photonic crystal surface on one side (**Figure 2**). On the opposite side, there are two white stripes along the longer edges of the chip. The thickness of these stripes is about 100mkm. The tip of a needle may be used to check this side of the chip (in case of any doubt).

WARNING!

Do not confuse the sides of the chip. The photonic crystal surface is pressed into contact with the flow cell, while the opposite side (with two white stripes and a drop of immersion oil between them) is pressed into contact with a glass prism in the Biosensor's optical unit.

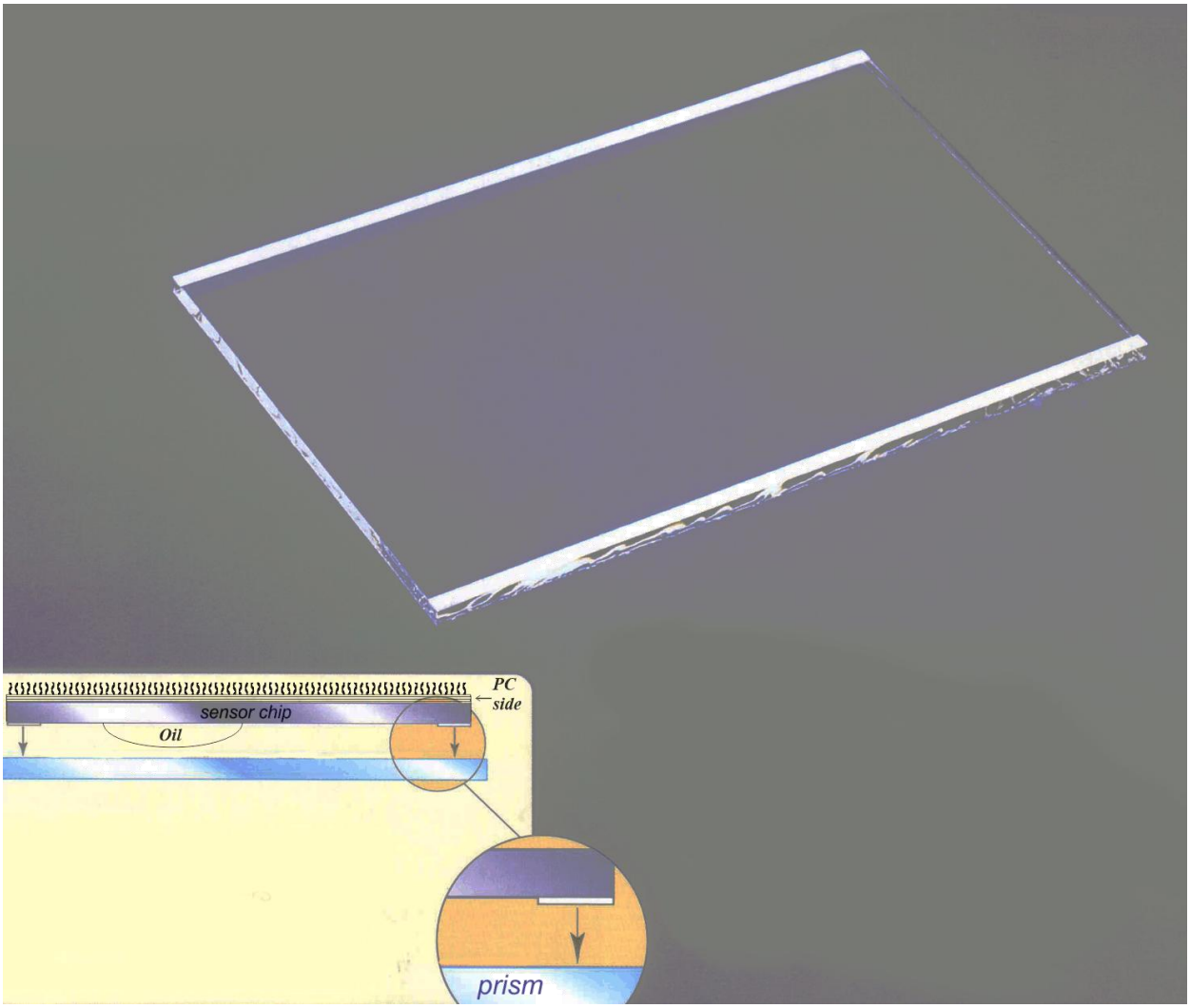

Figure 2. *The sensor chip is a glass slide coated with a photonic crystal (PC) film on one side. There are two white stripes on the other side.*

2. Brief Description of Startup Instructions

1. Clean the visible part of the prism in the Biosensor's optical unit with soft paper or cloth napkins dampened with ethanol.

2. Clean the flow cell with soft paper or cloth napkins dampened with ethanol. Place the tubes on the inlet and outlet of the flow cell. Fix the flow cell with tubes in the magnetic holder (**Figure 3**).

3. Remove any dust from the PC sensor chip (both sides of the chip should be clean), place one small drop of the matching oil on the opposite side of the sensor chip (with two white stripes), and press it into contact with the glass prism in the Biosensor's optical unit.

4. Place the magnetic holder with the flow cell on the prism and turn the lever on the left side of the Biosensor's optical unit counter-clockwise. Due to magnetic forces, the flow cell will be pressed against the sensor chip, forming a flow cell with the PC sensor chip surface as one wall.

5. Connect the outlet tube of the flow cell to the inlet tube of the peristaltic pump with the small metallic connector (enclosed). Set the inlet and outlet tubes into liquid tanks filled with water or PBS. The solutions under investigation are brought up to the flow cell by the peristaltic pump.

6. Determine the rate of the peristaltic pump about 25 to 30 and push the 'RUN' button. After the flow cell is filled with liquid, decrease the flow rate to any value not more than 15.

7. When the flow cell is filled with liquid, a picture analogous to that shown in **Figure 4** appears on the monitor.

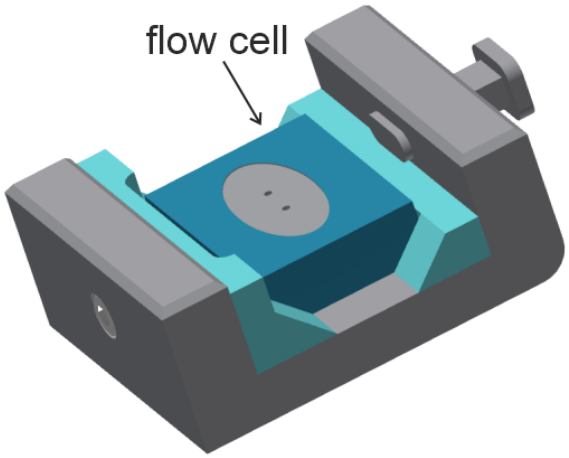

Figure 3. *The magnetic holder for the flow cell.*

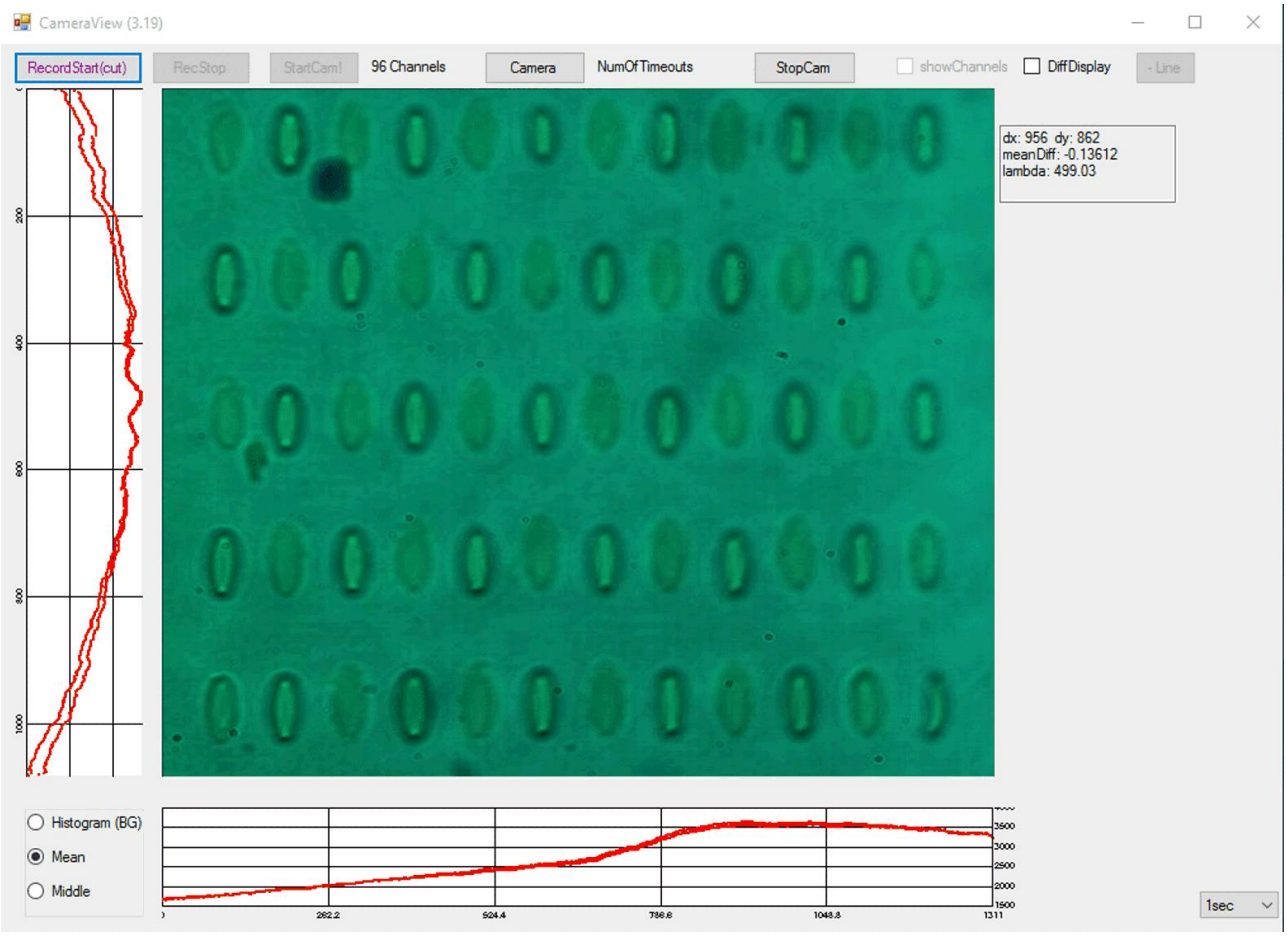

Figure 4. *Typical picture from a color camera.*

3. Detailed Description of Standard Operations

Sensor chip cleaning

The sensor chip is a glass slide with a photonic crystal surface on one side. Because the last deposited layer of the PC sensor chip is SiO_2, it is easy to clean the sample after a previous measurement. One sensor chip can be used for a number of measurements (hundreds or thousands, until the chip would be mechanically damaged).

Clean the sensor chip with soft paper or cloth napkins dampened with ethanol and acetone to remove the oil completely. Following the initial cleaning, the sensor chip should be sonicated once in acetone and twice in ethanol (20-30 min each).
A plasma cleaner (such as Harrick Plasma PDC-32G) or UV cleaner (such as UV-185 from PCbiosensors – sold separately) can be used to increase the speed and improve the quality of sample preparation. Before the measurement, the sample and the flow cell can be treated with the plasma cleaner for 1 min on medium power, with air pressure of 400 mTorr.

WARNING!

Do not use the high power setting of the plasma cleaner to clean the sample and the flow cell. This can decrease the last layer of the PC structure.

If the UV cleaner is used instead, the sensor chip should be irradiated in UV-185 for 15-20 minutes or more (there is no risk of reducing the thickness of the last layer here).

After plasma or UV treatment, the SiO_2 surface of the PC chip becomes hydrophilic with a very low contact angle and is ready for surface modification.

Sensor chip installation

For good optical coupling between the prism and the sensor chip, a matching oil should be used.

The following method is recommended for applying the matching oil:

1) Remove the dust from the PC sensor chip (both sides of the chip should be clean).
2) Place one *small* drop of the matching oil on the opposite side of the sensor chip (with two white stripes) and press it into contact with the glass prism in the Biosensor's optical unit (see **Figure 5**). ***Note:*** *The white stripes may be horizontally aligned to fit the flow cell size.* An oil with high viscosity is preferable for this purpose. For example, the following oil is recommended:

Cargille laboratories, Cedar Grove, NJ 07009 (973)239-6633 cargille.com :

BK-7 matching liquid, n=1.5167 *(λ=5893 A),* viscosity: 1450cSt – 1916cP *(Temp.=25°C).*

3) Place the magnetic holder *with the flow cell installed* (as shown in **Figure 3**) to the prism *with the sensor chip attached (through the oil viscosity)* and turn the lever on the left side of the Biosensor's optical unit counter-clockwise. ***Note:*** *Be sure that the sensor chip encloses the flow cell completely and will not leak.* Due to magnetic forces, the flow cell will be pressed against the sensor chip, forming a flow cell with the PC sensor chip surface as one wall.

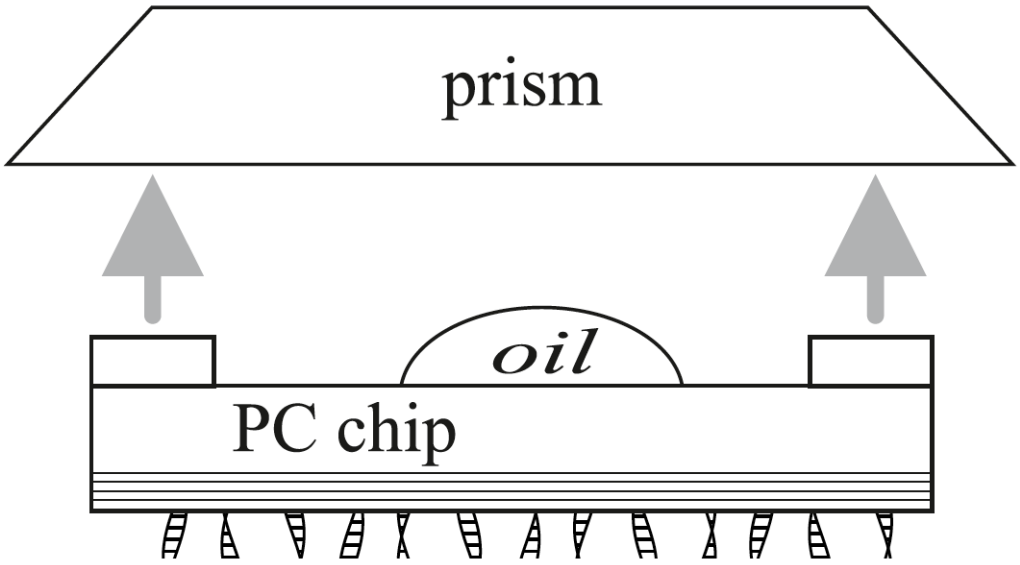

Figure 5. *Attaching the sensor chip to the prism (with a drop of high-viscosity oil).*

Sensor chip removal

Turn the lever on the left side of the Biosensor's optical unit clockwise. Remove the magnetic holder with the flow cell from the prism. Move the sensor chip vertically along the prism. Clean the sensor chip with a soft paper or cloth napkin dampened with ethanol and acetone to remove the oil completely. Following this, the sensor chip should be sonicated once in acetone and twice in ethanol (20-30 min each).

Clean the prism and the flow cell with a soft paper or cloth napkin dampened with ethanol and acetone.

Clean or replace the injection tubes after the measurement.

To clean a tube, connect it to the tube of the peristaltic pump using the standard metallic connector (enclosed). Set the end of the tube into the liquid tank filled with ethanol. Use the high flow rate (40-60) of the peristaltic pump for 3-5 min. Dry tubes thoroughly before reuse to avoid air bubbles in the system.

Flow cells

The flow cells are made from a quartz glass or plastic plate with two holes, through which metallic tube connectors are fitted to serve as inlet and outlet, respectively. The inlet tube is connected to a small tank filled with the solution under investigation. The flow rate is controlled by a peristaltic pump (e.g., Ismatec Reglo Digital). A typical depth is 5 μm to 50 μm, and corresponding flow cell volume is 0.5 μL to 5 μL.

Liquid system

The peristaltic pump, for example the *Ismatec Reglo Digital*, can be used for liquid handling from the liquid container to the flow cell. Set the inlet and outlet tubing into the liquid reservoirs. The tube from the reservoir is connected to the inlet of the flow cell. The outlet from the flow cell is connected to the inlet of the pump. The outlet from the pump is connected to the same or another reservoir.

If (at the very beginning of operation) the peristaltic pump cannot supply liquid to the system in pump down mode (when the pump is behind the liquid cell), reverse the direction of rotation of the pump and fill the liquid cell from the pump side. After filling the system with liquid, the direction of movement of the liquid can be changed as required.

When a 50 μm deep flow cell is used and the flow rate of the peristaltic pump is equal to 10, the liquid in the system moves at a rate of ~ 250μL/min. This volume flow rate corresponds to a linear flow rate through the flow cell of ~ 5mm/s.

The dead volume between the reservoir and the flow cell is dependent upon the length and diameter of the tube. It is equal to 2μL for each 1cm length of the tube, with ID=0.51mm (as used in this Biosensor). This gives a time delay in data of about 4 seconds when using a tube with a length of 10 cm (sufficient for connection with the standard reservoir). The dead volume between the liquid container and the flow cell should be kept as small as possible.

Injecting the sample

If a very tiny modification of biomolecular complexes on a photonic crystal surface should be measured, we advise do not change a flow direction and inject the sample under study into the inlet reservoir. In this case a continuous flow through the flow cell will not be interrupted or changed.

If the inlet reservoir should be exchanged, we advise to use the following procedure to avoid the formation of air bubbles in the system:

(a) change the direction of flow with the aid of the peristaltic pump;

(b) remove the inlet tube from the old reservoir and place it into a new reservoir with a new sample under study;

(c) change the direction of the flow again with the aid of the peristaltic pump.

Possible instrument's artifacts

Spikes

Spikes in the data may be caused by:

- Small air bubbles in the flow cell.
- A change in the direction of flow.

Flush the flow pump at a moderate flow rate (not more than 15). In the event of problems with air bubbles, use a high flow rate (25-35) for several seconds. The high flow rate helps to remove air bubbles that may be trapped in the system.

If the positioning of the sensor chip is unsatisfactory (air bubbles seen between the prism and the chip or in the flow cell), remove the sensor chip and carefully wash the prism surface, flow cell and sensor chip. Clean or replace the injection tube.

To reduce the probability of spikes, use deionized water, and clean the sensor chip and the flow system carefully.

Air bubbles on the sensor chip

When the flow cell is filled with liquid but the picture on the monitor is not analogous with what is shown in **Figure 4**, and there are many dark spots, either the sensor chip is not clean or there are small air bubbles in the flow cell. The presence of air bubbles on the surface of the sensor chip is clearly visible on the image from the color camera as dark spots. If they corrupt the spots under investigation, discard the data from these areas or remove the sensor chip, rinse and dry it thoroughly.

4. Operation

A sketch in **Figure 6** shows the principle of operation of the label-free Biosensor EVA 3.0.

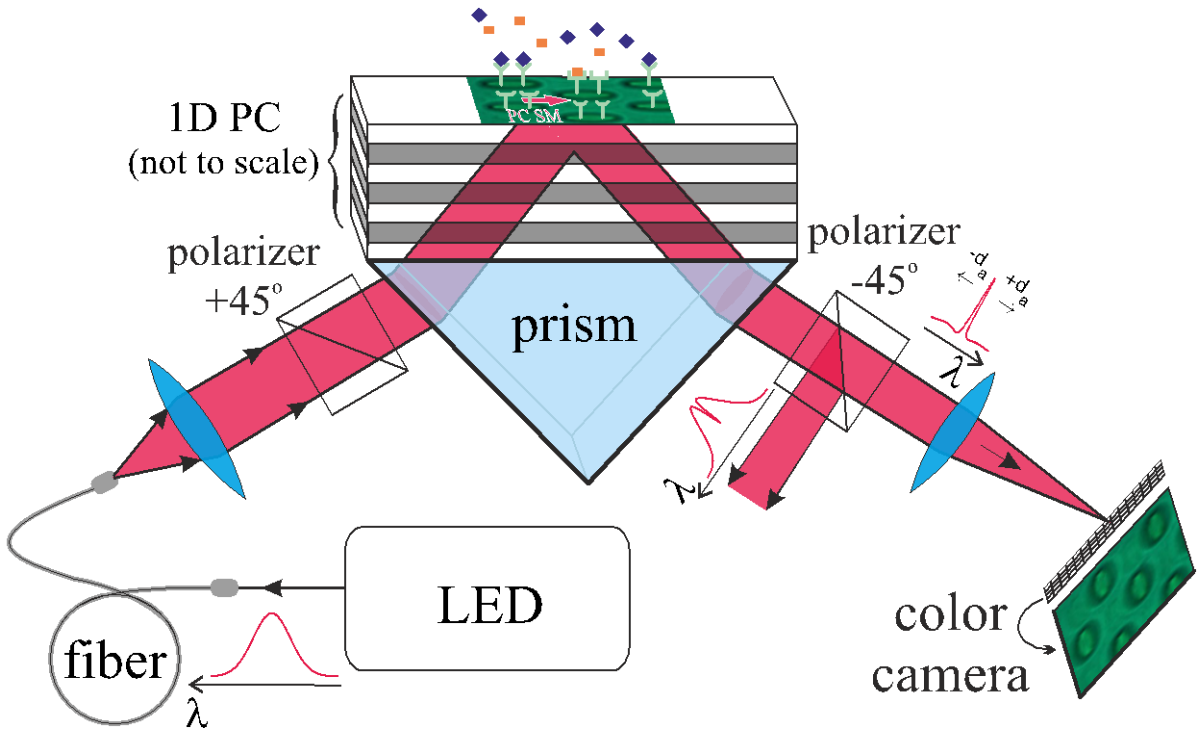

Figure 6. *Schematic of the PC SMi biosensor. Typical spectra of light as it passes from the LED to the color camera are shown.*

Data acquired by a color camera was processed and presented using EVA 3.0 software. The shift of the PC SM resonance wavelength was determined from the next normalized differential value:

$$z(x,y)=\frac{B(x,y)-G(x,y)}{B(x,y)+G(x,y)}, \quad (1)$$

where $B(x,y)$ and $G(x,y)$ are the intensities of the blue and green components in each color pixel (x,y) of the camera. To convert $z(x,y)$ into $\lambda(x,y)$, the responsivities of the color camera and a procedure described in [*Sensors&Actuators B*, (2018) v.276, 271–278] were used. Use *PCbiosensors3.exe* program for control and data acquisition from the Biosensor EVA 3.0 (see next section).

5. Software Overview

Software for EVA 3.0 includes two programs – the first is for device control and data acquisition (*PCbiosensors3.exe*), and the second is for data load, analyzing and data fitting (*PCbioViewer3.exe*).

Device control

After turning on the LED and the camera of the EVA 3.0 device (by two corresponding tumblers), you can launch the *PCbiosensors3.exe* program. Three windows (A, B, C), as displayed in **Figure 7**, will appear.

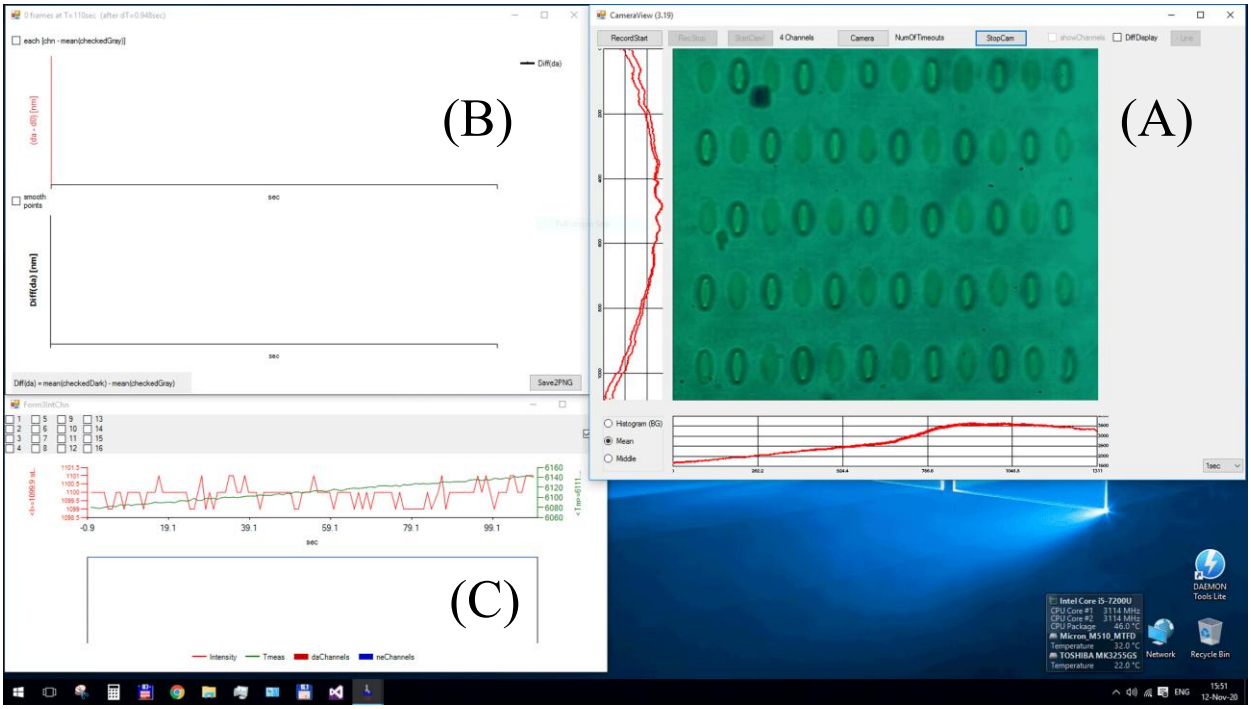

Figure 7. *Three windows will appear on the screen after the PCbiosensors3 program launch.*

The CameraView window (A) contains a picture from the CMOS camera, its x- and y-projections, and main controls. The quality of a sample can be easily estimated from this picture.

Images from the camera in **Figure 7** (A) are shown in their natural color. The biosensor detects a slight color change at each point of the camera using normalized difference (1) and recalculates it as a $\Delta\lambda$ shift. Thus, the device determines λ for each camera pixel to obtain the spatial distribution $\lambda(x,y)$ across the camera that reflects the spatial distribution of the waveguide resonance shifts across the sample.

✓ ***Hint***

In the CameraView window, you can make ***righMouseClick*** *on the image to obtain a $\lambda(x,y)$ value in this particular point and select Recording area (inside the image) by* ***Shift + leftMouseClick & drag****. Other options may be found by pressing* ***F1*** *in this window (*see **Figure 8***).*

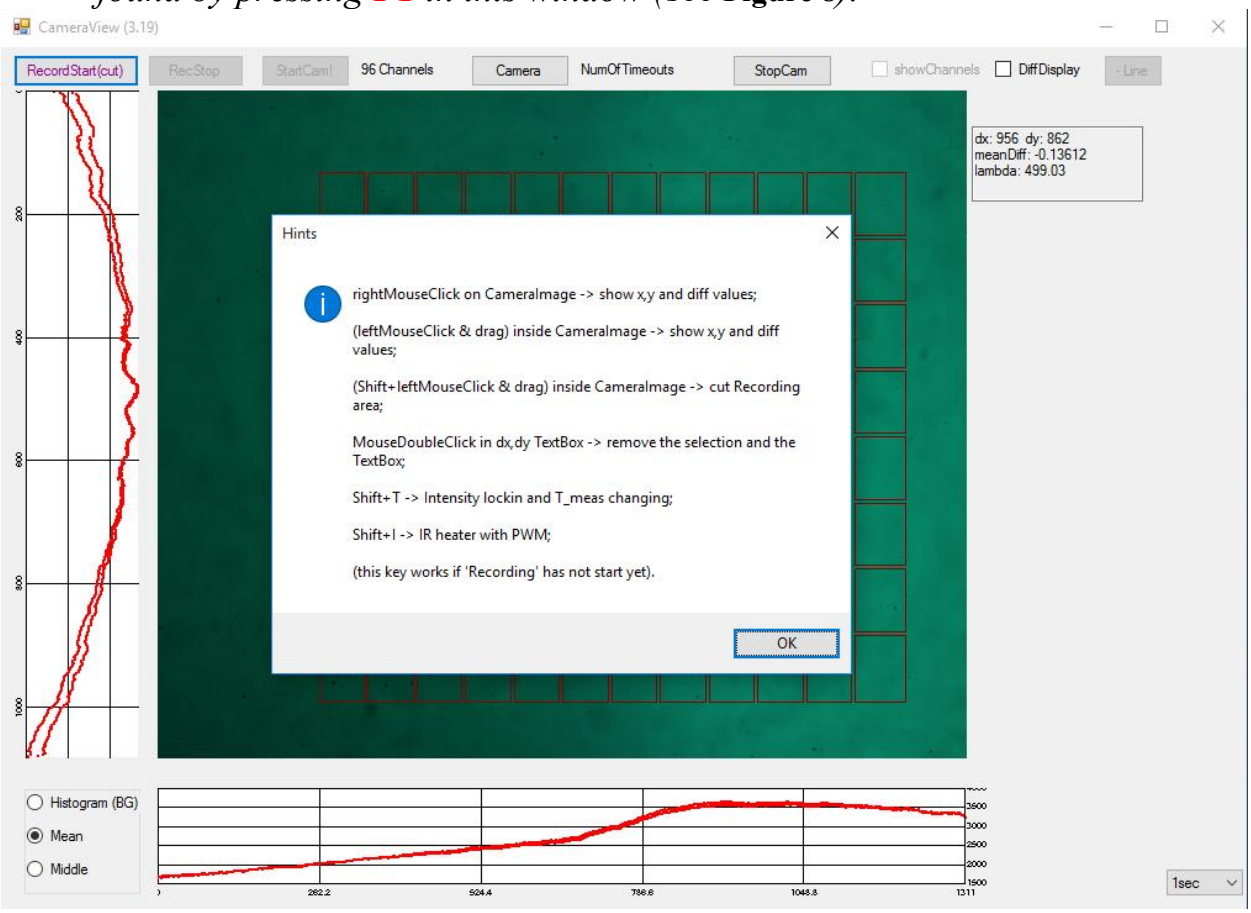

Figure 8. *Shortcuts hints (F1) in CameraView window.*

To increase the visible contrast of the images, one can use a pseudo-color (based on Eq. (1)) instead of the natural color. This does not change the kinetic curves and other data, whose registration is based on Eq. (1) in any case, but it does make any spatial changes in the images more noticeable to the naked eye and may help to divide the sample surface into an appropriate number of registration spots. The '*DiffDisplay*' checkbox is used to switch on this differential display. While checked, it converts an ordinary color images to pseudo-color images with a variable enhancement factor. For positive enhancement factor, a sinusoidal enhancement without plane subtraction is used. If a negative enhancement factor is set (or the '*-Line*' button is pressed), linear enhancement is performed with subtracting the zero,

first, and second order planes within the selected square (or within full images by default).

Increasing the contrast can be useful for selecting the Recording area on the image (while holding down the ***Shift*** key, see above). After selection, press the '*RecordStart(cut)*' button to open the RecordStart subwindow. This subwindow is necessary to select the number and location of registration channels inside the Recording area.

In the appearing RecordStart subwindow (see **Figure 9**), you may choose a number of channels and their spatial position, as well as the name of the data-saving directory. If you have already made chip splitting with the same spot pattern before, you can select this pattern in IniRef.dat file from that previous directory and load the old pattern by pressing '*old channels from IniRef.dat*'.

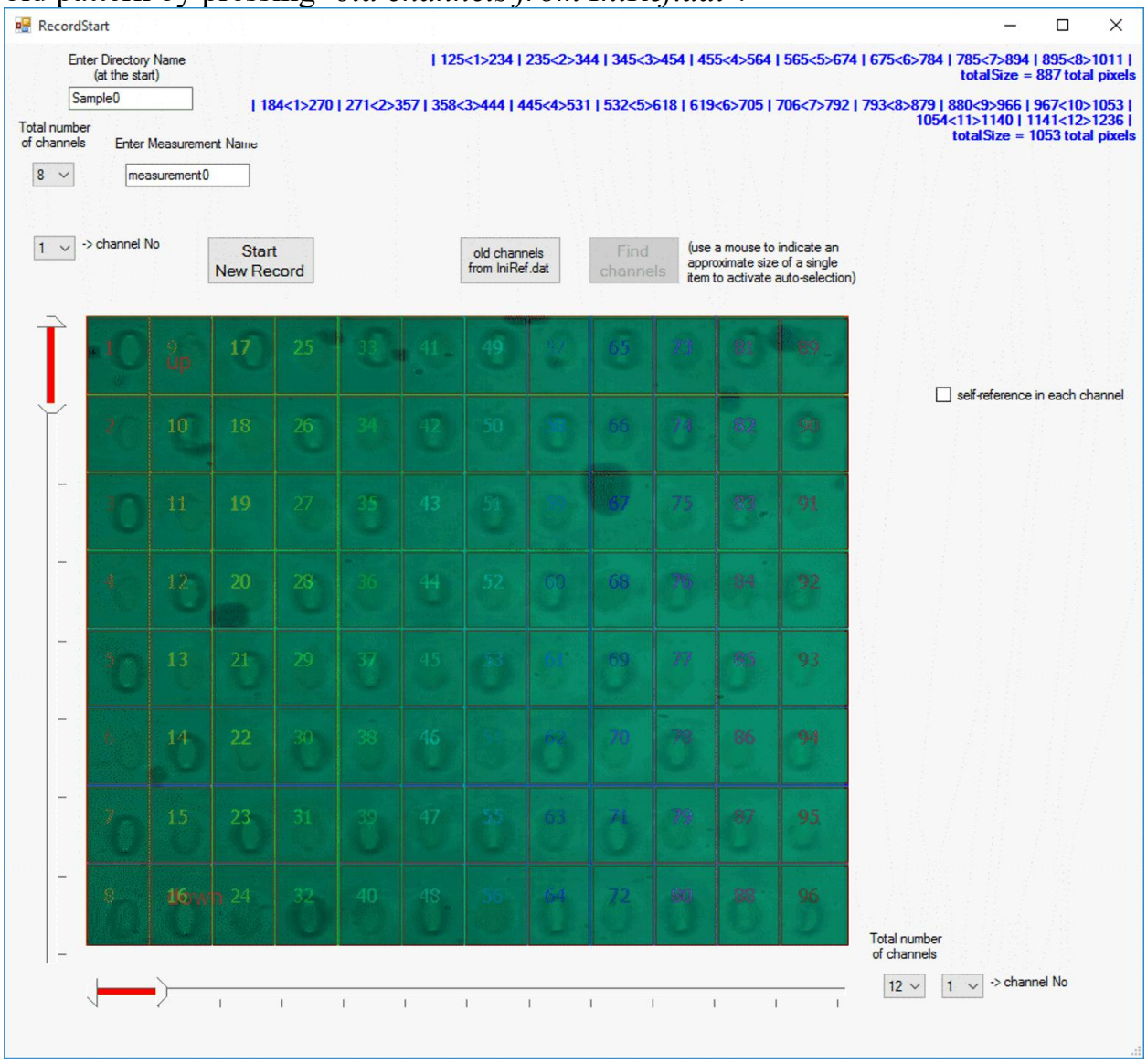

Figure 9. *RecordStart window, where the number of channels, their spatial position, and the name of the data-saving directory may be chosen.*

✓ ***Hint***

You can adjust the position of the old pattern template along the X and Y axes by ***Ctrl + Shift +X****. Other options may be found by pressing* ***F1*** *in this window (*see ***Figure 10****).*

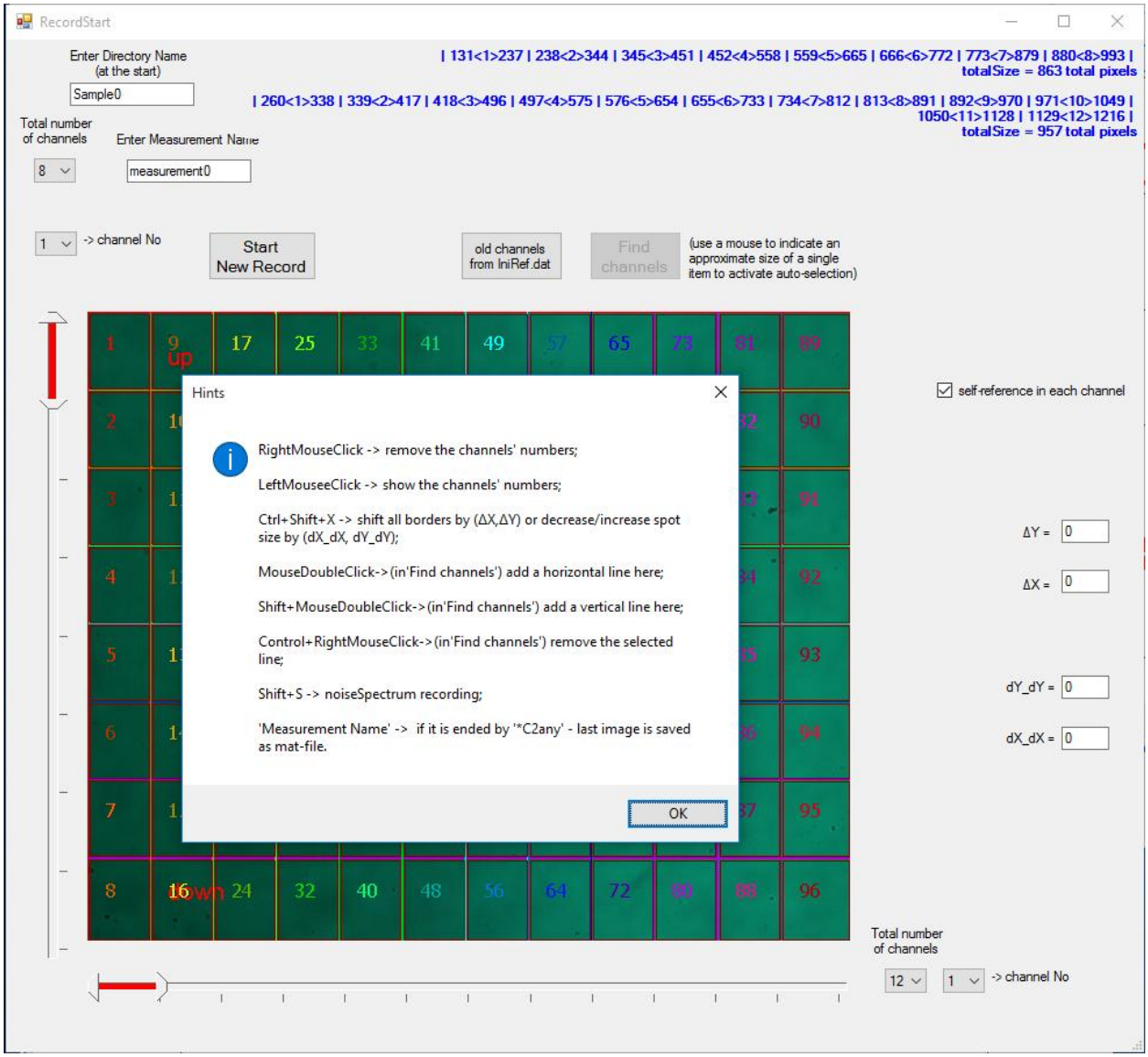

Figure 10. *Shortcuts hints (F1) in RecordStart window.*

The main choice that should be made in the 'RecordStart' subwindow before starting registration – it is the choice of the type of reference channels in the current sample:

1) **'*self-reference in each channel*' unchecked:**

If (on the current chip) there are separate empty spots that will later serve as reference spots, you should remember the numbering of these spots and subtract the data from these spots from the data from the registration channels. If there are no reference spots at all, and you do not plan to use reference spots during the current measurements – also leave the box 'self-reference in each channel' unchecked.

2) ‘***self-reference in each channel***’ **checked:**
If there are no specific reference spots on the current chip, but you want to use the free space around each registration channel as a reference area for that specific channel – check the box ‘self-reference in each channel'.

When the positioning of the registration spots is complete, you can start recording by pressing the ‘*Start New Record*’ button. After pressing the ‘*Start New Record*’ button, a new measurement begins, and its results are displayed in the data window (see **Figure 7**, item B).

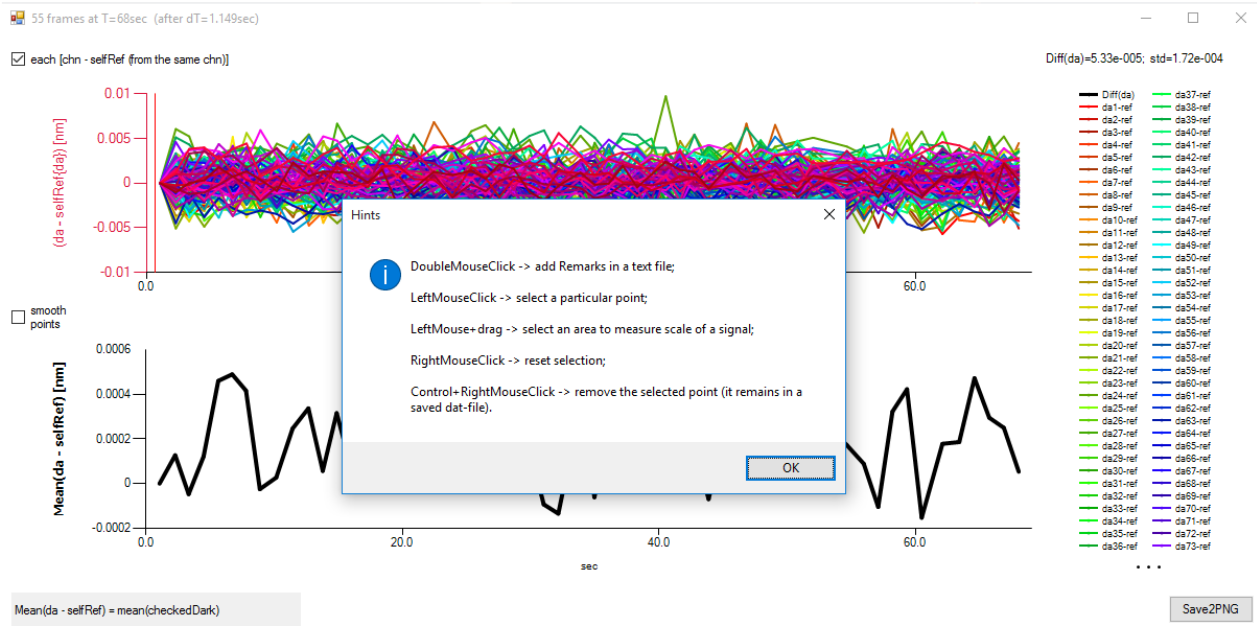

Figure 11. *Shortcuts hints (F1) in DataView window.*

The DataView window (**Figure 11**) contains two graphs. One of them displays adlayer thickness changes for each channel separately (in different colors), while another displays (in black) their combination in accordance with the formula:
‘*mean (of Plus channels) – mean (of Minus channels)*’
or
‘*Mean (da – selfRef)=mean(checkedDark)*’
(if the box ‘*self-reference in each channel*’ was checked in ‘RecordStart’ subwindow). By default, all channels marked as ‘Plus channels’ and, therefore, an initial black graph display a mean value of all da[nm]. Checked or unchecked channels for visualization is possible in window ‘Form3IntChn’ (see **Figure 7**, item C or **Figure 12**).

All measurement results for all channels are saved in a file, despite the fact that the channel is checked or not, therefore, these checkboxes are intended only for presentation view in the DataView window.

✓ ***Hint***

You can keep log journals and memorize some key points by double-clicking ***LeftMouseButton*** *at a specific point on the graph. An annotation mark will appear on the screen, and Notepad will be launched so that you can*

record your remarks about the current measurement. Save and close the Notepad window after remark typing and continue the measurement.

To save a screen picture of some characteristic data curves, you may press the '*save2PNG*' button. The picture will be saved in the current data directory.

✓ ***Hint***

Occasionally some spikes may occur in the data (e.g., due to small air bubbles in the flow cell) and the graph scale may change to an inconvenient value. You can either wait data updating (it takes <128 sec) or you can remove the spike point from the screen manually by pointing to the spike and pressing ***Ctrl + RightMouseButton****.*

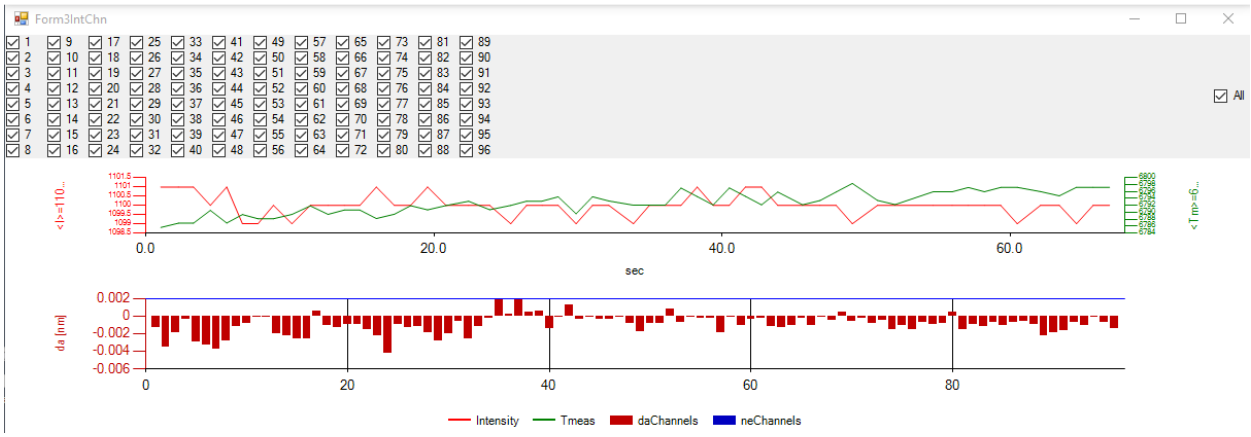

Figure 12. *Form3IntChn auxiliary window.*

The graphs in Form3IntChn window (see **Figure 12**) are auxiliary graphs, and it is rarely used in routing measurements. An upper graph displays mean intensity on the CMOS camera and a measurement time, which is required to retain this mean intensity. The mean intensity value (in this case <I> = 1100) is assigned at the beginning of the program with the first number following the name of the exe file (for example, *PCbiosensors3.exe 1100 32 0.5* – where the second number is a number of integration in one second, and the third number is a scale factor of the matrix in CameraWindow).

✓ ***Hint***

In the CameraView window **Figure** *(A), you can press* ***Shift T*** *to control the current Time values, switch on and off the lock-in of the Intensity value, and so on.*

WARNING!

As a rule, there is no need to change default settings in ordinary measurements.

To intermit a measurement, one should press the '*RecStop*' button. Later, one can continue data acquisition from the stopping point in the same directory with a different measurement name.

To stop the current set of measurements entirely, one should press the '*StopCam*' button and close the CameraView window. The program and all windows will be closed. The next measurement will be started from the initial values and will be saved in a different directory.

6. Data loading and analysis

To load, analyze and fit previously saved data, one should launch the program PCbioViewer. A window, as displayed in **Figure 13**, will appear.

The first button ('*Remarks and Curves Preview*') loads all remarks (inserted by double-clicking during measurements) and all pictures (made by '*save2PNG*' button during measurements) from the chosen data directory. This is an auxiliary button, and it serves to recall in which directory some particular data have been saved.

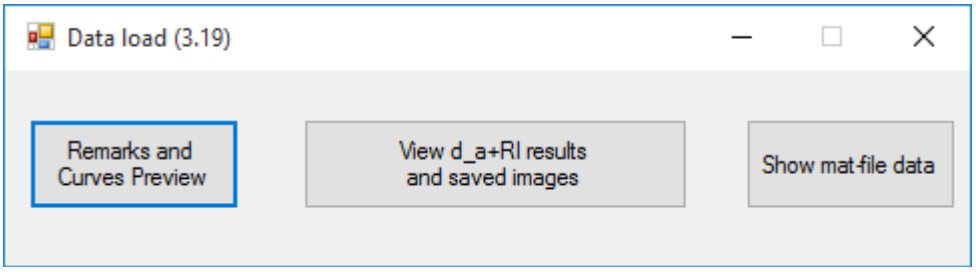

Figure 13. *Data load window.*

The second button ('*View d_a and RI results and saved images*') is the main button, and it loads the daResults.dat file from a chosen subdirectory of the main data directory PCbiosensors_data. The third button is again an auxiliary button, and it serves to view mat-file data.

Data fitting

The window 'ResultsData' (which appears after pressing the second button) permits one to select and scale a desirable section of data simply by pressing and holding the left mouse button. To choose a particular data interval for future fit, one can employ the following hint:

✓ ***Hint***

In the ResultsData window, one can select a section of data for future fitting in the 'Diff(da) [nm]' graph by pressing ***Shift + RightMouseButton*** *(see* **Figure 14** *for illustration). The same selection in the 'Diff(n) [RIU]' graph may be made by pressing the* ***Alt + RightMouseButton****.*

The selected portion of data points will be opened in a separate window, 'FormFitResults' (see **Figure 15**), where a fitting method may be chosen. There are several fitting methods in the program. All of these are illustrated in **Figure 16**. The internal details of the fitting process may be found in the 'FittingOutput' window, presented in **Figure 17**.

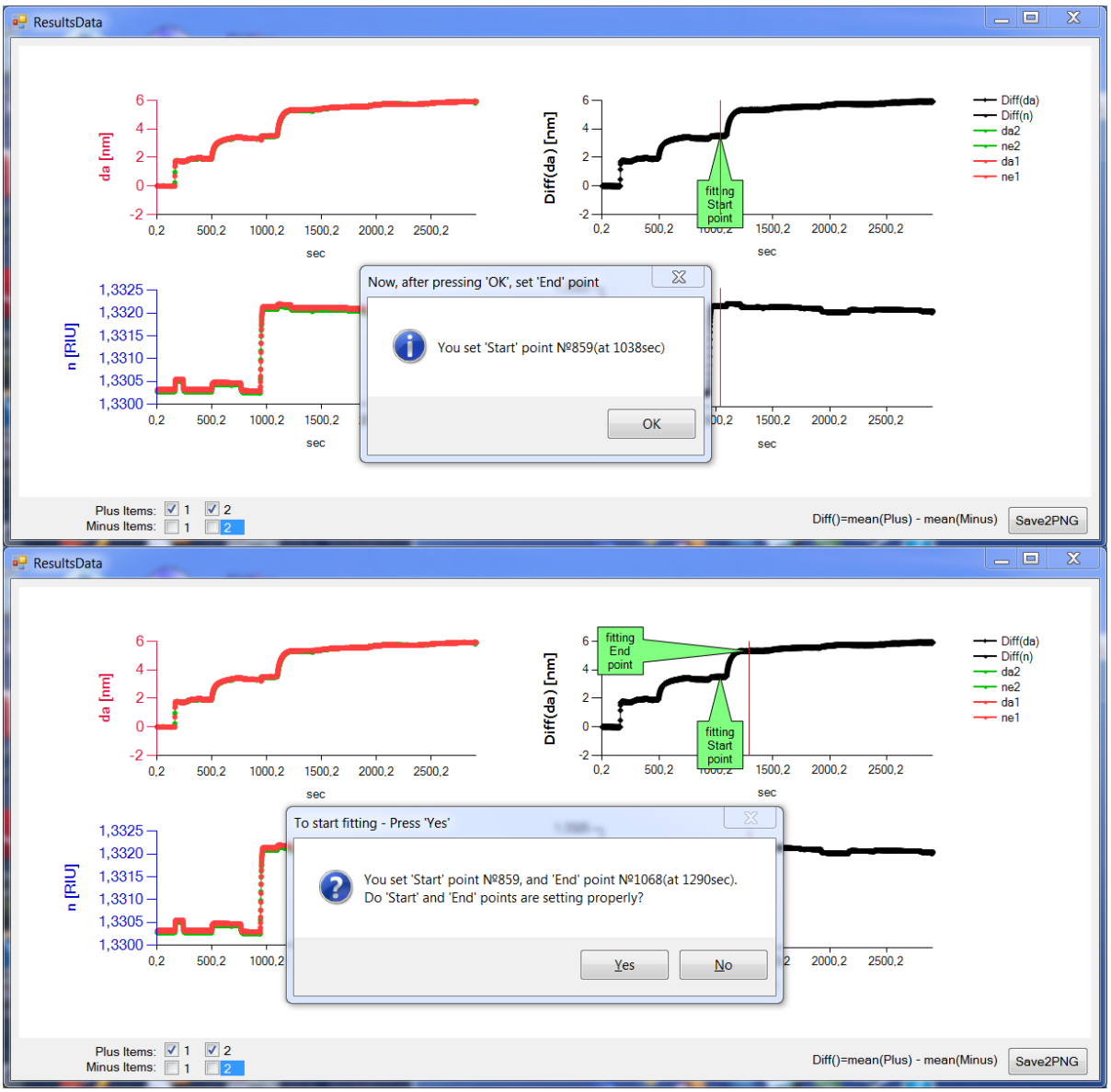

Figure 14. *Selection of 'Start' and 'End' points in data loaded from a file.*

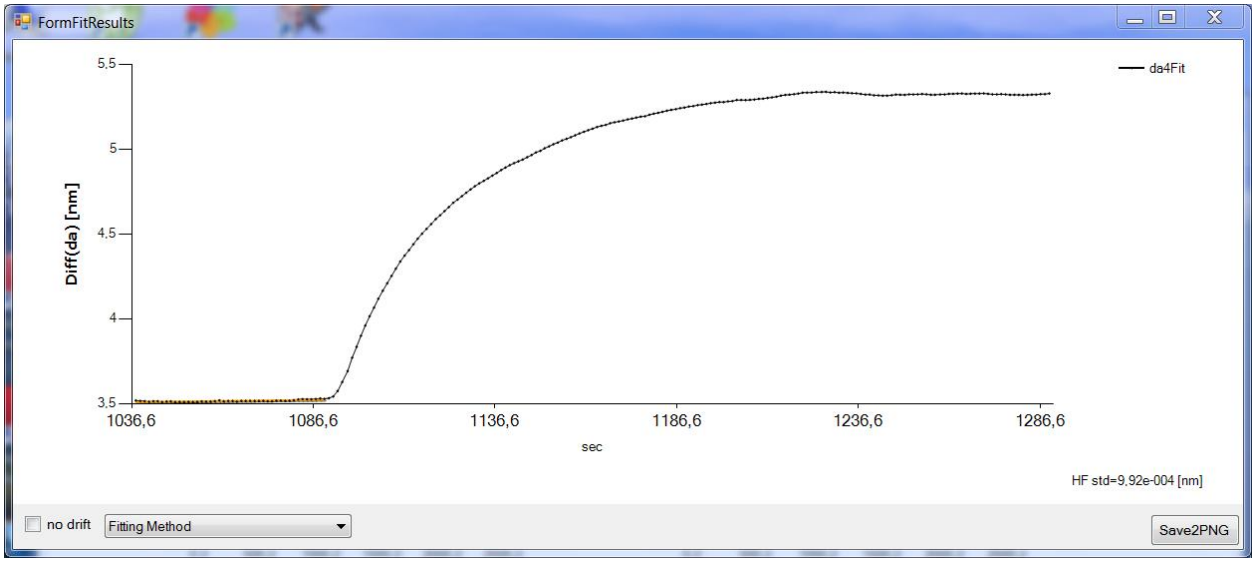

Figure 15*. Form for fitting selected part of data.*

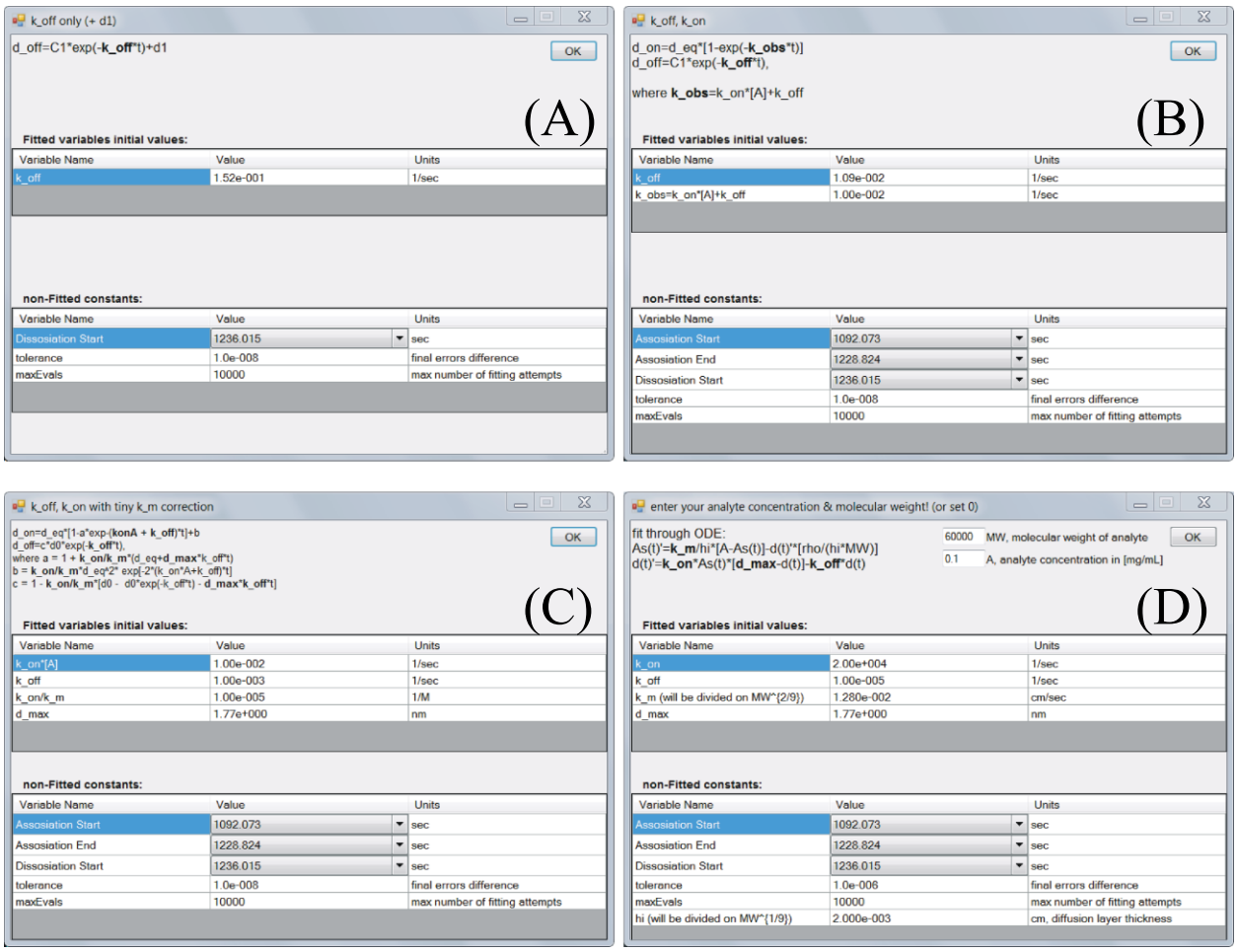
k_off only (+ d1)
d_off=C1*exp(-k_off*t)+d1
OK
(A)
Fitted variables initial values:
Variable Name | Value | Units
k_off | 1.52e-001 | 1/sec
non-Fitted constants:
Variable Name | Value | Units
Dissosiation Start | 1236.015 | sec
tolerance | 1.0e-008 | final errors difference
maxEvals | 10000 | max number of fitting attempts
k_off, k_on
d_on=d_eq*[1-exp(-k_obs*t)]
d_off=C1*exp(-k_off*t),
where k_obs=k_on*[A]+k_off
OK
(B)
Fitted variables initial values:
Variable Name | Value | Units
k_off | 1.09e-002 | 1/sec
k_obs=k_on*[A]+k_off | 1.00e-002 | 1/sec
non-Fitted constants:
Variable Name | Value | Units
Assosiation Start | 1092.073 | sec
Assosiation End | 1228.824 | sec
Dissosiation Start | 1236.015 | sec
tolerance | 1.0e-008 | final errors difference
maxEvals | 10000 | max number of fitting attempts
k_off, k_on with tiny k_m correction
d_on=d_eq*[1-a*exp-(konA + k_off)*t]+b
d_off=c*d0*exp(-k_off*t),
where a = 1 + k_on/k_m*(d_eq+d_max*k_off*t)
b = k_on/k_m*d_eq^2* exp[-2*(k_on*A+k_off)*t]
c = 1 - k_on/k_m*[d0 - d0*exp(-k_off*t) - d_max*k_off*t]
OK
(C)
Fitted variables initial values:
Variable Name | Value | Units
k_on*[A] | 1.00e-002 | 1/sec
k_off | 1.00e-003 | 1/sec
k_on/k_m | 1.00e-005 | 1/M
d_max | 1.77e+000 | nm
non-Fitted constants:
Variable Name | Value | Units
Assosiation Start | 1092.073 | sec
Assosiation End | 1228.824 | sec
Dissosiation Start | 1236.015 | sec
tolerance | 1.0e-008 | final errors difference
maxEvals | 10000 | max number of fitting attempts
enter your analyte concentration & molecular weight! (or set 0)
fit through ODE:
As(t)'=k_m/hi*[A-As(t)]-d(t)*[rho/(hi*MW)]
d(t)'=k_on*As(t)*[d_max-d(t)]-k_off*d(t)
60000 MW, molecular weight of analyte
0.1 A, analyte concentration in [mg/mL]
OK
(D)
Fitted variables initial values:
Variable Name | Value | Units
k_on | 2.00e+004 | 1/sec
k_off | 1.00e-005 | 1/sec
k_m (will be divided on MW^{2/9}) | 1.280e-002 | cm/sec
d_max | 1.77e+000 | nm
non-Fitted constants:
Variable Name | Value | Units
Assosiation Start | 1092.073 | sec
Assosiation End | 1228.824 | sec
Dissosiation Start | 1236.015 | sec
tolerance | 1.0e-006 | final errors difference
maxEvals | 10000 | max number of fitting attempts
hi (will be divided on MW^{1/9}) | 2.000e-003 | cm, diffusion layer thickness

Figure 16. *Methods for data fitting.*

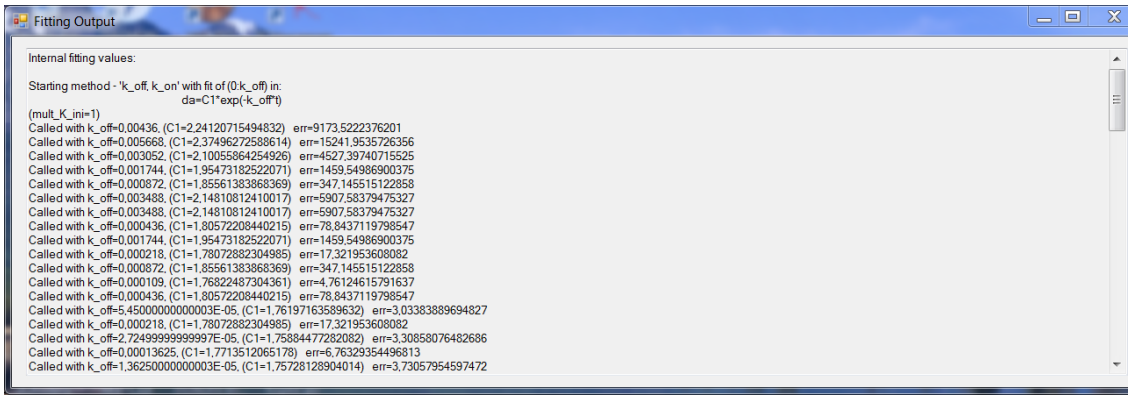

Figure 17. *Auxiliary window illustrating fitting process.*

Kinetic models

Several kinetic models for data fit may be chosen in the 'FormFitResults' window (**Figure 15**). In **Figure 16**, one can see available models (A-D) for fit. The main model is a Langmuir model (with and without drift – (B) in Figure 16). In this model (schematically illustrated in **Figure 18**):

$$\begin{aligned} d_{on} &= d_{eq} \cdot [1 - \exp(k_{obs} \cdot t)] \\ d_{off} &= C_1 \cdot \exp(k_{off} \cdot t) \end{aligned},$$

where

$$d_{eq} = \frac{k_{on} \cdot [A] \cdot d_{max}}{k_{on} \cdot [A] + k_{off}}$$

is an equilibrium adlayer thickness at analyte concentration [A], while

$$k_{obs} = k_{on} \cdot [A] + k_{off}$$

is an observable kinetic rate to the equilibrium.

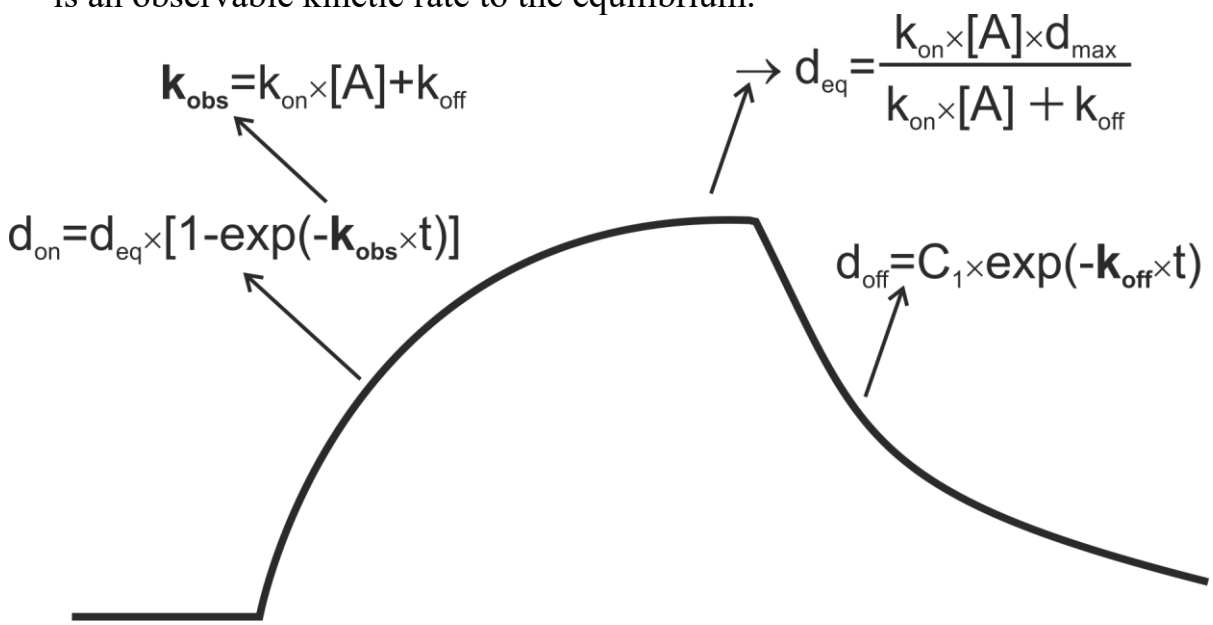

Figure 18. *A schematic kinetic curve showing association and dissociation phases.*

Additionally, one can fit the dissociation phase only with the assumption that some adlayer thickness d_1 persists at the exponential dissociation end (model (A) in **Figure 16**):

$$d_{off} = C_1 \cdot \exp(k_{off} \cdot t) + d_1 .$$

Two remaining models take into account effects of *mass transport limitation*. Model (D) in **Figure 16** fit experimental data using a system of two *ordinary differential equations* (ODE)

$$\frac{\partial A_s(t)}{\partial t} = \frac{k_m}{h_i} \cdot [A - A_s(t)] - \frac{\partial d(t)}{\partial t} \cdot \frac{\rho}{h_i \cdot MW}$$
$$\frac{\partial d(t)}{\partial t} = k_{on} \cdot A_s(t) \cdot [d_{max} - d(t)] - k_{off} \cdot d(t)$$

More details about this fit may be found in the following references:

[1] *"Extending the Range of Rate Constants Available from BIACORE: Interpreting Mass Transport-Influenced Binding Data,"* by D.G. Myszka et al. (Biophysical Journal, 1998, v.75, p.583–594).

[2] *"Kinetic and Thermodynamic Analysis of Ligand-Receptor Interactions: SPR Applications in Drug Development,"* by N.J. De Mol and M.J.E. Fischer (Handbook of Surface Plasmon Resonance, Chapter 5, pp. 123-172, Eds.: R.B.M. Schasfoort and A.J. Tudos, Cambridge: RSC Publishing, 2008).

The model (C) in **Figure 16** fit experimental data with the assumption that the *mass transport limitation* is weak and may be taken into account as a small perturbation in a standard Langmuir 1:1 model – with (k_{on}/k_m) as a small parameter:

$$\begin{aligned} d_{on} &= d_{eq} \cdot [1 - a \cdot \exp(-k_{obs} \cdot t)] + b \\ d_{off} &= c \cdot d_0 \cdot \exp(k_{off} \cdot t) \end{aligned} ,$$

where

$$a = 1 + \frac{k_{on}}{k_m} \cdot (d_{eq} + d_{max} \cdot k_{off} \cdot t)$$

$$b = \frac{k_{on}}{k_m} \cdot d_{eq}^2 \cdot \exp(-2 \cdot k_{obs} \cdot t)$$

$$c = 1 - \frac{k_{on}}{k_m} \cdot [d_0 - d_0 \cdot \exp(-k_{off} \cdot t) - d_{max} \cdot k_{off} \cdot t].$$

Results of various fitting models are illustrated in **Figure 19**.

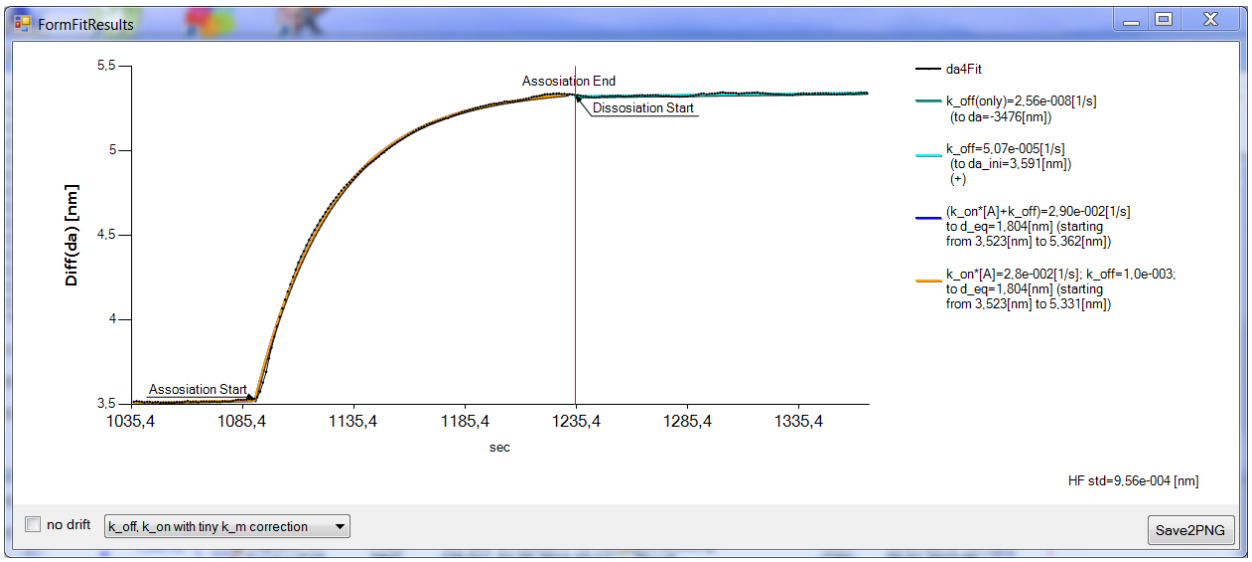

Figure 19. *Raw data and results of fitting.*