THROMBODYNAMICS

HEMACORE
innovative diagnostics
of blood coagulation

Version 2.0

FIBRINOLYSIS EVALUATION
NEW FEATURE OF THROMBODYNAMICS ASSAY

Thrombodynamics and Fibrinolysis

Thrombodynamics Analyser allows visualization of the fibrinolysis process in presence of plasmin activators. Clot formation is activated by the immobilized TF in the cuvette, and then the lysis can be observed. An example of clot growth and further lysis is shown on the figure below:

| 2min | 10min | 30min | 60min |

Time-lapse images of clot growth and lysis in presence of 1nM of tPA
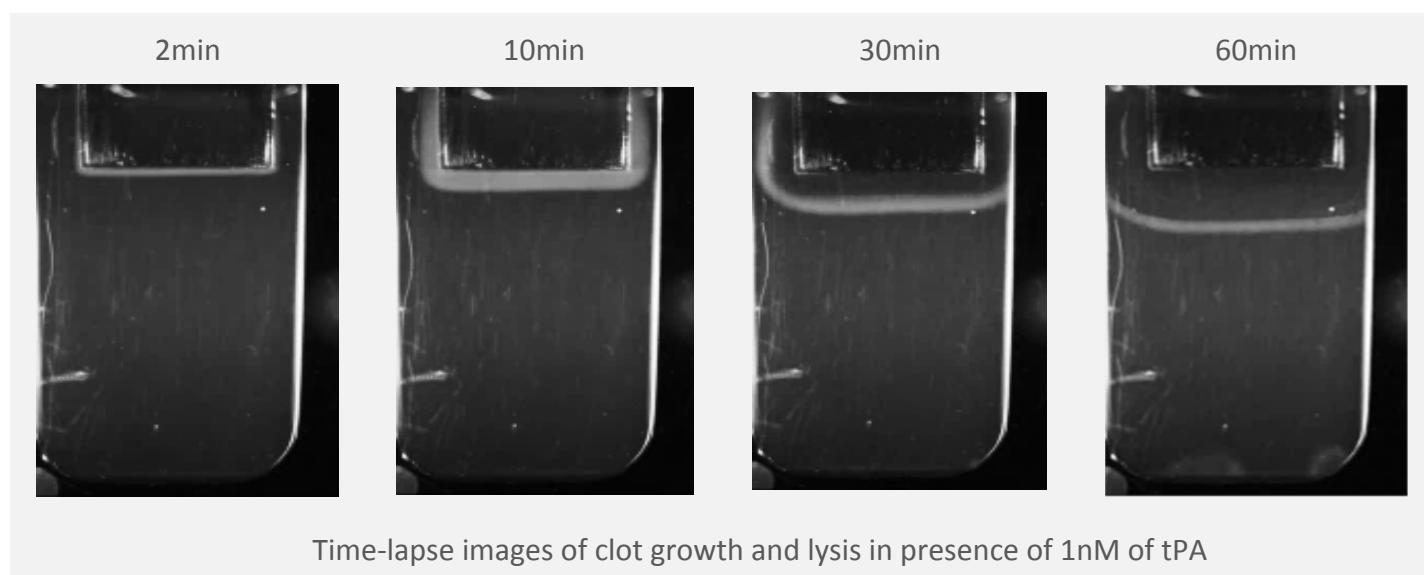
The initiation phase of clot formation occurs similarly to the basic Thrombodynamics setup. Further coagulation propagation can be observed simultaneously with the lysis process.

To quantify this process we developed an improved image processing algorithm. The new software version in addition to the fibrin clot growth allows quantifying the clot lysis process when plasminogen activators (tPA or uPA) are added to plasma before the measurement. Other activators potentially can be also used if they produce similar pattern of clot lysis. The main parameters that are measured in the updated software version are the time of lysis initiation and the lysis rate. More detailed information about the algorithm, the new parameters, can be found in the Section 1.

Thrombodynamics Software also provides a tool for manual calculation of fibrinolysis parameters if the automatic one is not suitable for any reason. The description is provided in the Section 2.

Examples of the lysis process in different situations are presented in the Section 3. The example of the results report can be found in the Section 4. A short guide for running the fibrinolysis measurement in the updated Software version can be found in the Section 5.

1. Numerical parameters of the fibrinolysis process

In addition to regular Thrombodynamics clot growth parameters the new software version allows quantifying fibrinolysis parameters in presence of specific activators such as tPA and uPA. These substances lead to dissolution of the fibrin clot after its formation with a specific pattern (Figure 1-1, A).
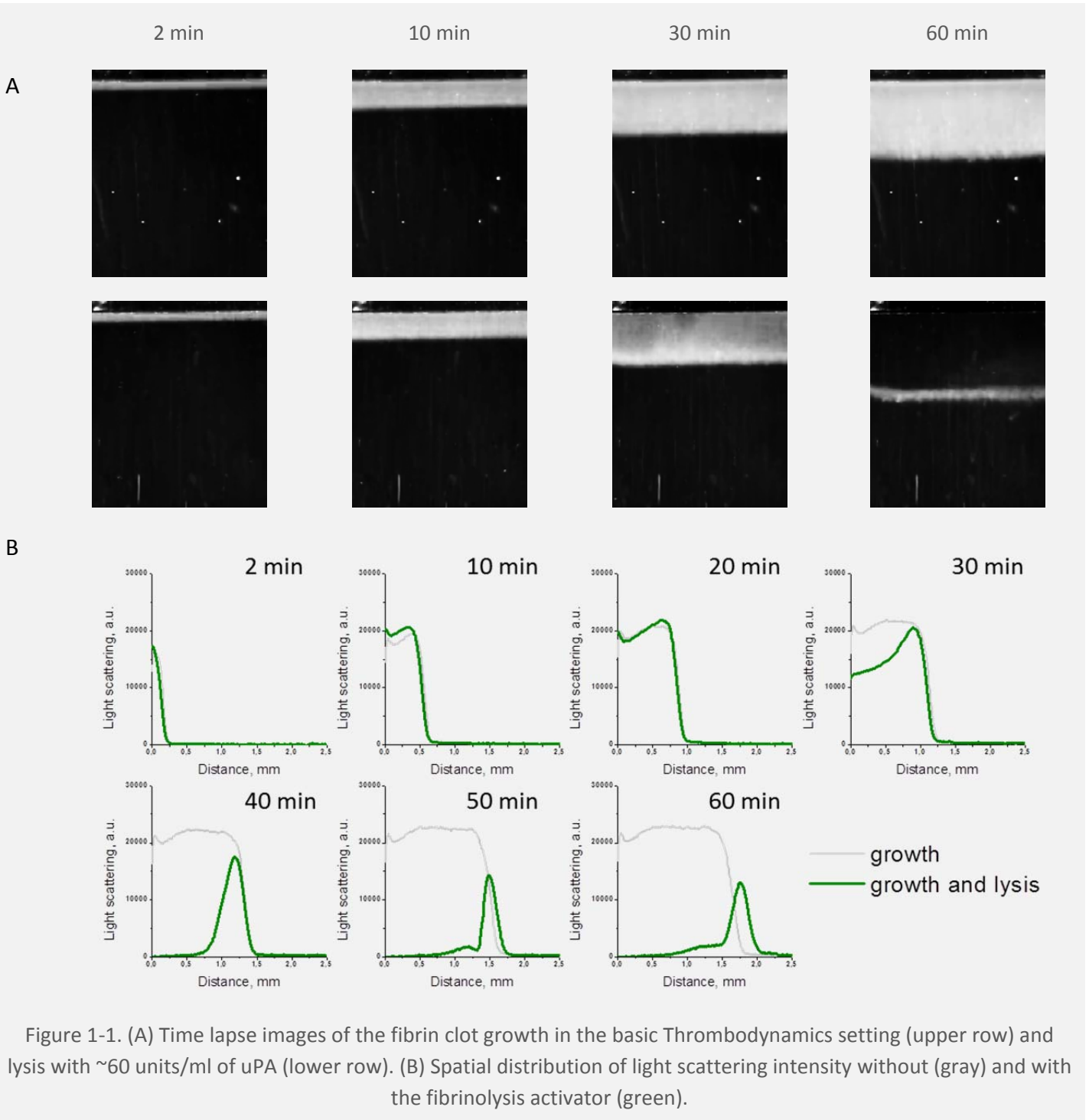

Figure 1-1. (A) Time lapse images of the fibrin clot growth in the basic Thrombodynamics setting (upper row) and lysis with ~60 units/ml of uPA (lower row). (B) Spatial distribution of light scattering intensity without (gray) and with the fibrinolysis activator (green).

Lysis process starts inside the clot which is already formed, while clotting process at the edge of the fibrin clot occurs normally. Then the lysis process starts following the clot propagation that looks like a moving line of fibrin. The lag-time of lysis initiation depends on the activator activity.

To describe the lysis process quantitatively the following procedure is applied. Activator position is determined automatically, and then light scattering spatial distribution is retrieved from each image (Figure 1-1 (B)). Then the light scattering profiles are analyzed to determine the lysis initiation point on the intensity profiles. This point is defined as a point of the minimal light scattering intensity inside the clot (at least 0.3mm far from the activator and not further than the clot edge) on the first profile that has a segment with the intensity below the threshold value of 70% of the initial clot brightness (Figure 1-2).
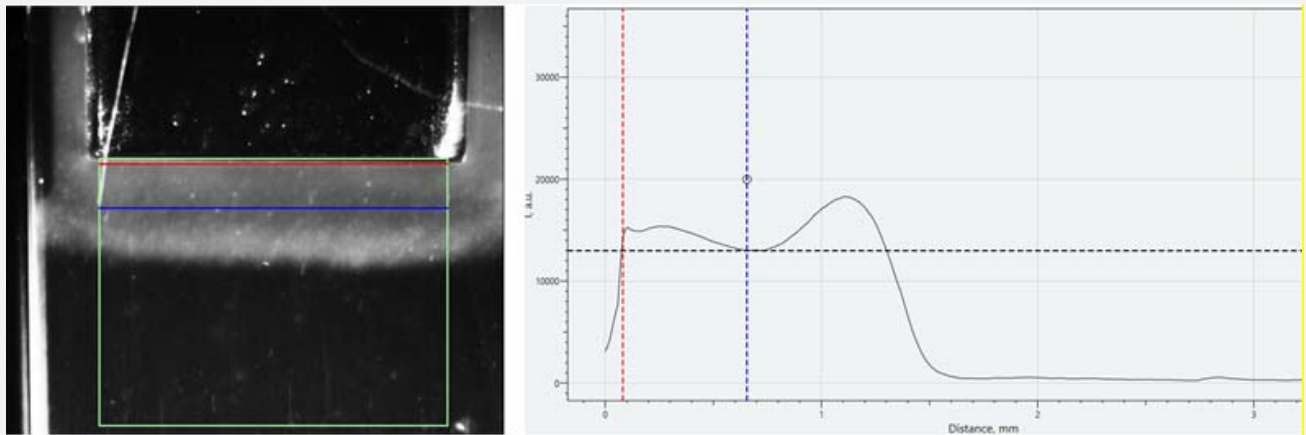

Figure 1-2. Description of the calculation algorithm. Image of the growing clot and the corresponding light scattering distribution. The point of lysis initiation is shown with the blue line. Red line shows the position of the activator. Black line corresponds to the threshold intensity.

Lysis onset time (**LOT, min**) is defined as a moment of time, when the light scattering intensity in this point inside the clot decreases 30% from the initial value.

The lysis progression (**LP, %/min**) is the linear rate of the light scattering intensity decrease as the percentage of the initial value in the following 5 min [LOT, LOT+5 min].

The position of the front edge of the clot is also detected automatically and can be used for the calculation of fibrin propagation rate both with and without the fibrinolysis activator.

The new algorithm was adjusted to the lysis onset times >10 min that approximately corresponds to < 200 units/ml of uPA or < 5nM of tPA. Other concentrations can also be used, but this may require use of manual calculation mode (see Section 2).

2. Image processing in the manual mode

If the default image processing algorithm seems to be not suitable for any reason, one can analyze the experimental results in the manual mode. Advanced manual calculation allows measuring the time course of clot formation and lysis process at any point inside the clot and changing the time interval of the lysis rate calculation (Figure 2-1).
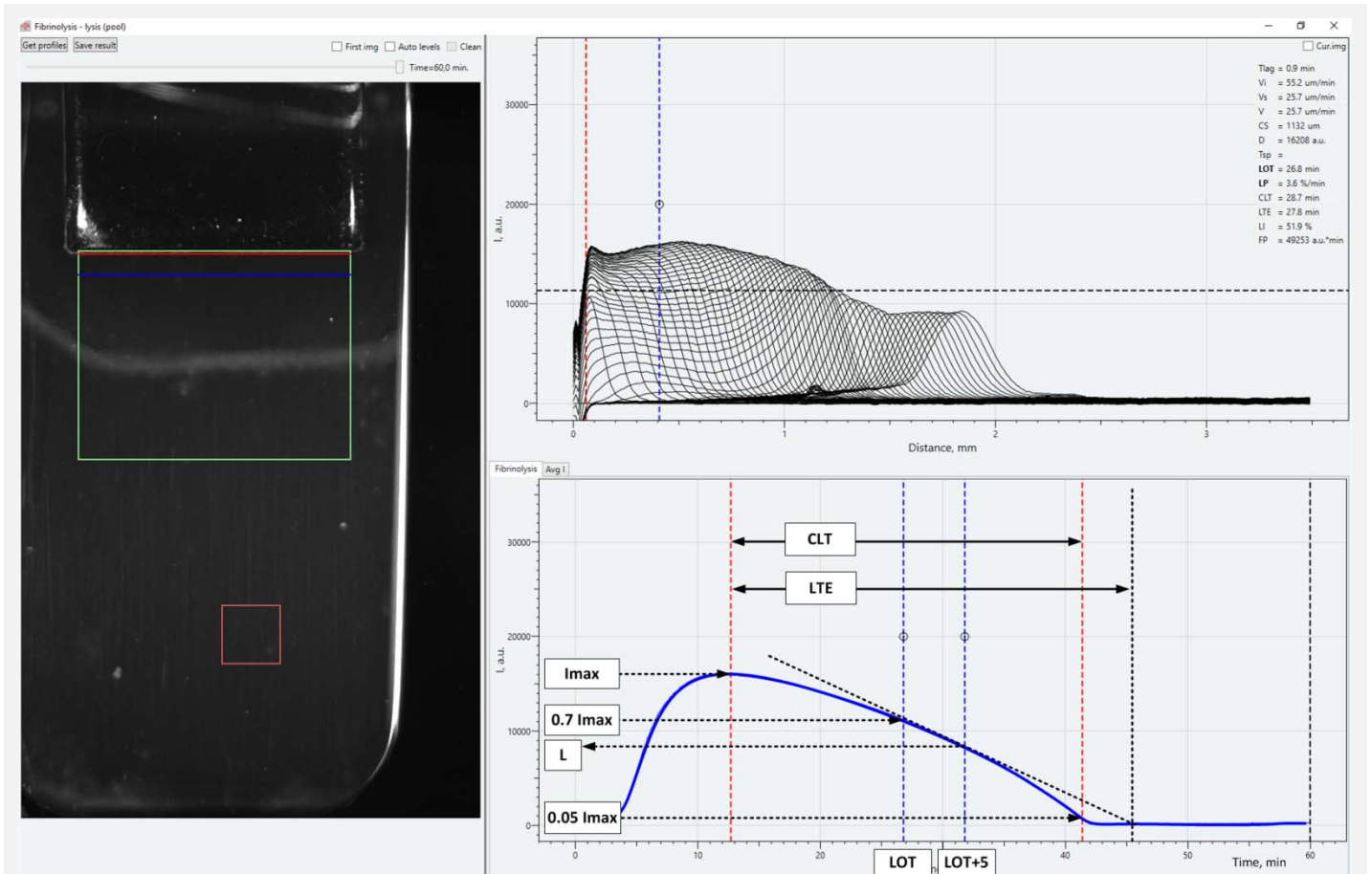

Figure 2-1. Manual data processing mode of Thrombodynamics Software. This mode allows measuring the light scattering change over time inside the fibrin clot or in the plasma volume.

In addition to main fibrinolysis parameters several other parameters are calculated in manual mode (marked on lower curve on Figure 2-1):

Clot lysis time (**CLT, min**) is defined as a time span between the moment when clot density starts to decrease from its maximum value *Imax* and time moment when clot density reaches *0.05*Imax*.

Lysis time estimation (**LTE, min**) is defined as a time span between the moment when clot density starts to decrease from its maximum value Imax and projected time moment (based on calculated lysis progression value) when clot density reaches zero value.

Lysis index (**LI, %**) is the relation of clot density *L* at time moment *LOT+5 min* to the maximum clot density *Imax*: *LI=L/Imax*100%*. It is the % of remaining clot density.

3. Examples of measurements

Experimental conditions	Time-lapse images			Clot growth and fibrinolysis parameters
	5 min	**20 min**	**45 min**	
Pooled normal plasma tPA 25 nM				LOT = 14 min LP=23.5 %/min Vs= 25 um/min
Pooled normal plasma uPA 75 units/ml				LOT = 29 min LP= 2.9 %/min Vs= 28 um/min
Donor 1 uPA 60 units/ml				LOT = 50 min LP= 1.8 %/min Vs=26 um/min
Donor 2 uPA 60 units/ml				LOT = 30 min LP= 6.4 %/min Vs=28 um/min Tsp=38 min
Donor 3 uPA 60 units/ml				LOT = 28 min LP= 4.8 %/min Vs=24 um/min

Thrombodynamics Analyser

Fibrinolysis assay

Patient: Age: , History N:		13.06.2017 12:12	
Measurement title: lysis Plasma type: Fresh PFP Sample code: pool Physician: Medical Institution/Department: Diagnosis: Prescribed medications:		**TDL**	

Fibrin dynamics parameters	Unit	Parameter	Value	Norm.
Rate of clot growth	um/min	V	25,7	
Lag time	min	Tlag	0,9	
Initial rate of clot growth	um/min	Vi	55,2	
Stationary rate of clot growth	um/min	Vst	25,7	
Clot size at 30 min	um	CS	1132	
Clot density	a.u.	D	16208	
Spontaneous clotting	min	Tsp		

Fibrinolysis parameters	Unit	Parameter	Value	Norm.
Lysis onset time	min	LOT	26,8	
Lysis progression	%/min	LP	3,6	

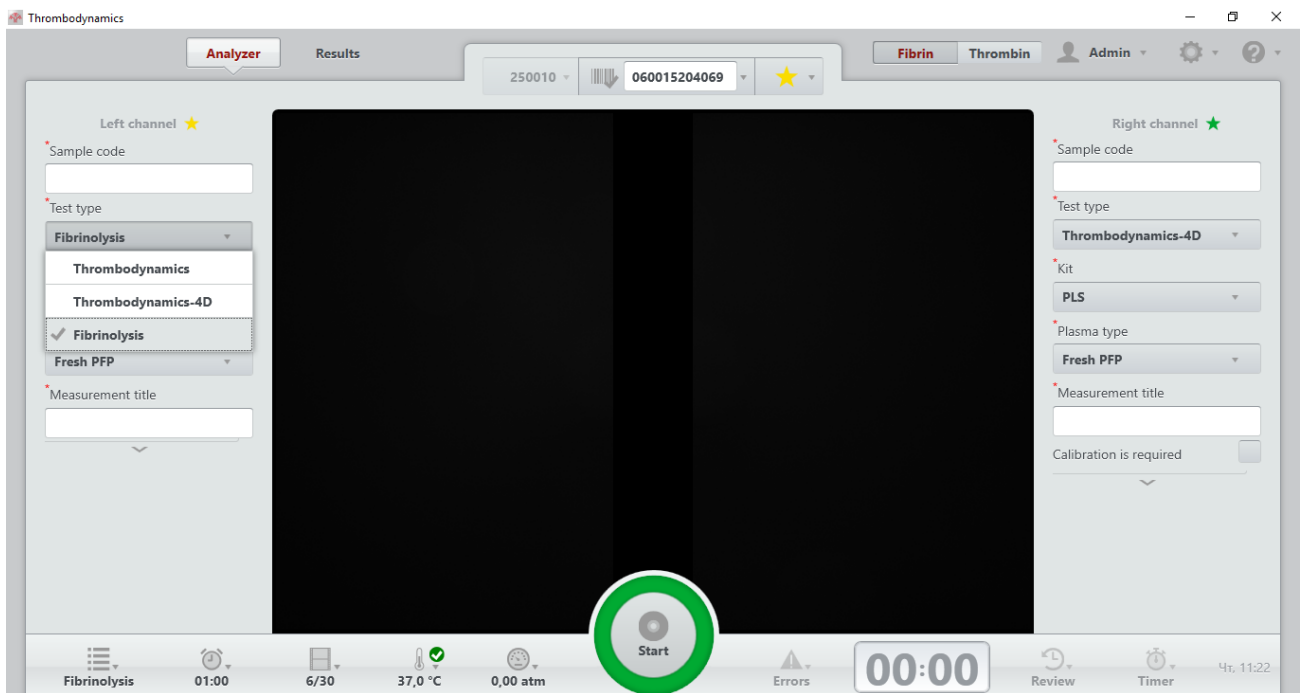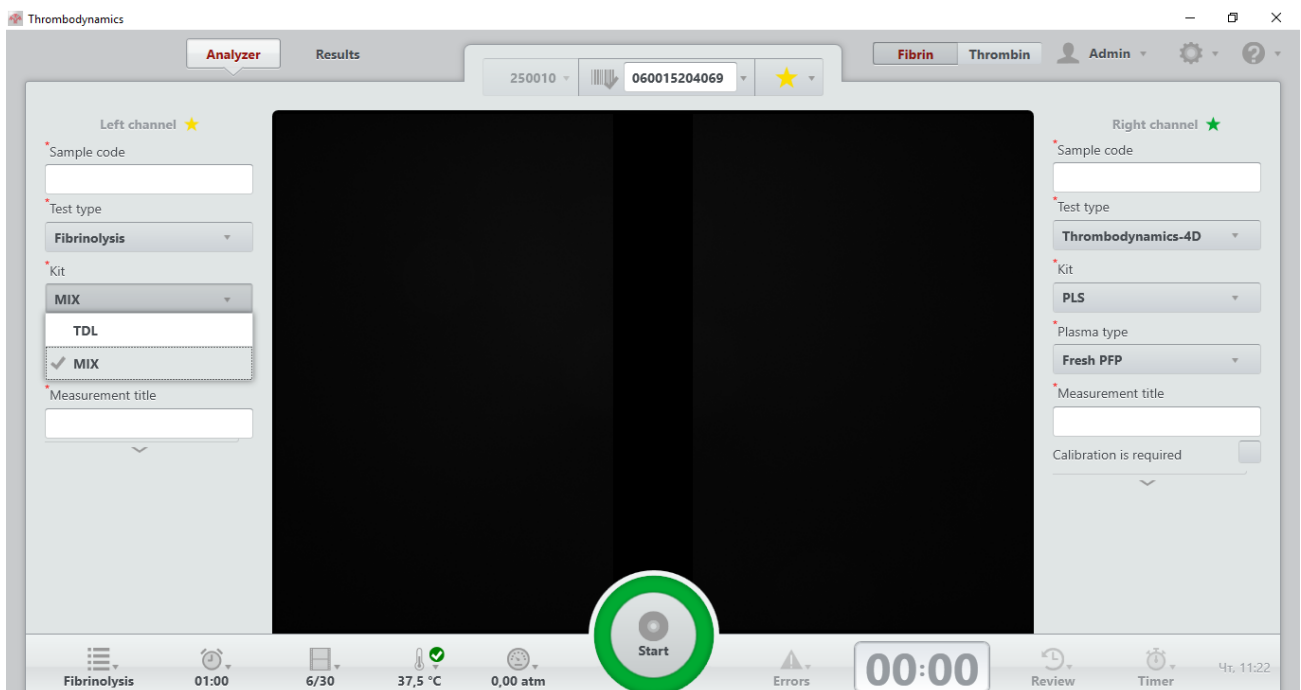| 5 min.	15 min.	30 min.	45 min.	60 min.
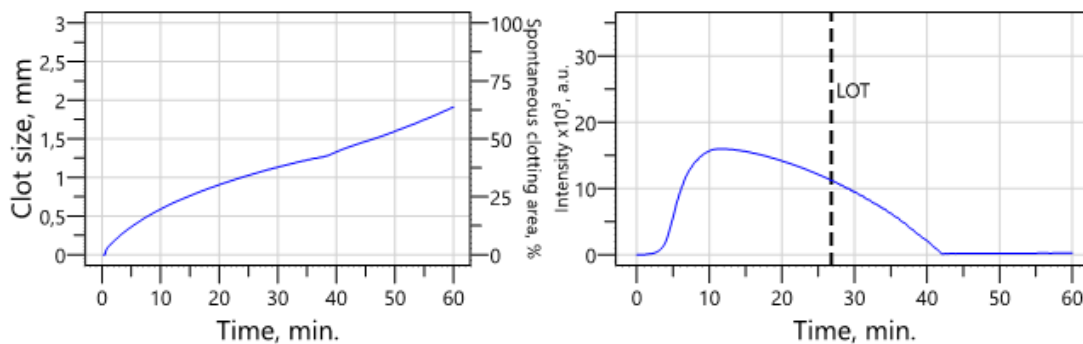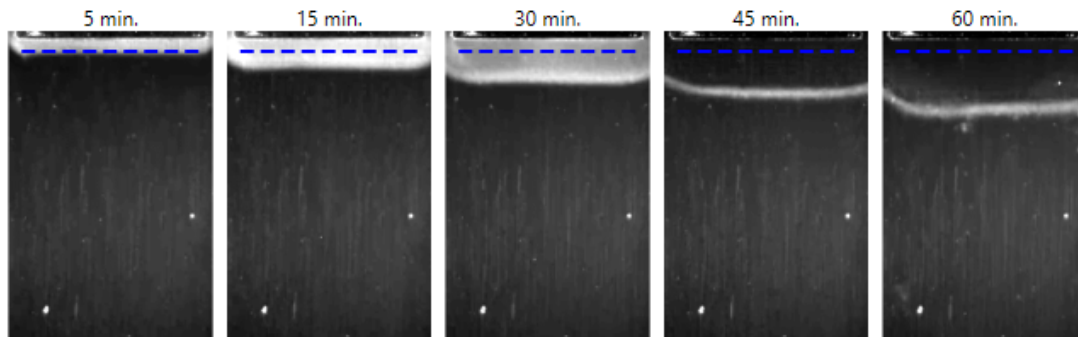
Tech info:
Comment:

ATTENTION: Research use only. Measurement results shouldn't be used for diagnostic purposes.
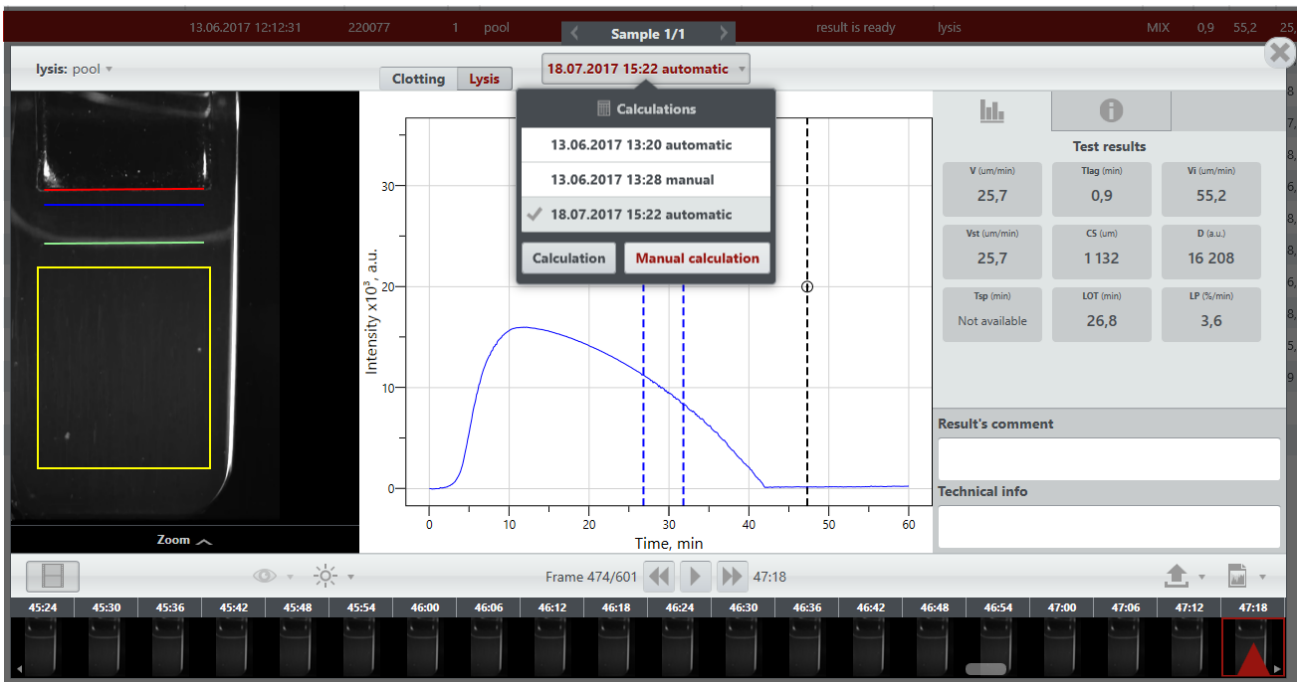S/N: 220077, Operator: , report created 18.07.2017 13:48

To run the fibrinolysis measurement choose the Fibrinolysis option from the Test type menu

Kit type MIX is a default option for a user-defined addition of a fibrinolysis activator. TDL kit is currently not available.

Fibrinolysis parameters are calculated automatically after the measurement. Manual calculation mode can be activated from the results view window.
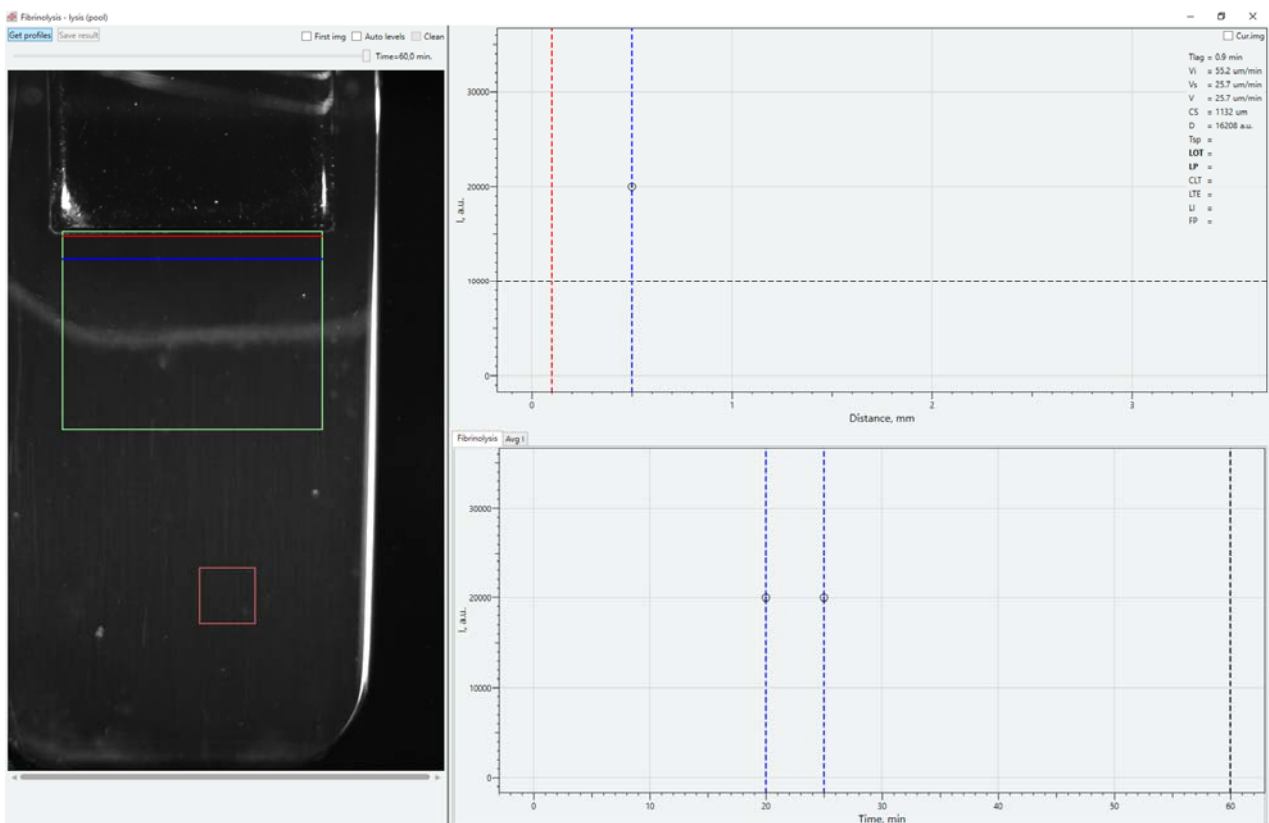
Press "Get profiles" button in the upper-left corner of the window to initiate image processing and get the light scattering distribution profiles.